University of Lodz

The Proceedings of the Conference on
The Challenges of Contemporary Cell Biology

Molecular Genetics, System Biology, Bioinformatics

April 20 – 21, 2009

The Conference to Honor
Professor Maria J. Olszewska
on Her Jubilee

Łódź University Press, 2009

Sponsors of the Conference

Perlan Technologies

PRECOPTIC Co.

SIGMA-ALDRICH®

WYDAWNICTWO NAUKOWE PWN

ZEISS

Syngen

Nikon

POLYGEN

prospecta Sp. z o.o.

Prospecta Sp. z o.o.
ul. Barburki 8
04-511 Warszawa
tel.: +48 (22) 812 02 90; 613 29 28
fax: +48 (22) 812 07 78
e-mail: t.reczajski@prospecta.pl
Internet: www.prospecta.pl

SYMBIOS
www.symbios.com.pl

BIO-RAD

Lab-JOT

WITKO®

fmk
Filip Maciej Konka
komputery, oprogramowanie, sieci
GSM +48 693 931 727

Please, visit us at our website:
www.wydawnictwo.uni.lodz.pl

Patronage:

Rector of the University of Lodz – Professor Włodzimierz Nykiel, Ph.D.

Conference supported by Polish Ministry of Science and Higher Education

Conference Organizers:

The Committee on Cell Biology of Polish Academy of Sciences
Institute of Physiology, Cytology, and Cytogenetics, University of Lodz
The Lodz Branch of the Polish Academy of Sciences

Organizing Committee:

Chairman: Andrzej K. Kononowicz – University of Lodz
Vice-Chairman: Elżbieta Wyroba – The Committee on Cell Biology of Polish Academy of Sciences

Members:
Maria Kwiatkowska – University of Lodz
Jerzy Kawiak – Editor of *Advances in Cell Biology* (*Postępy Biologii Komórki*)
Barbara Gabara – University of Lodz
Mirosław Godlewski – University of Lodz
Jacek Jurczakowski – The Lodz Branch of the Polish Academy of Sciences
Kazimierz Marciniak – University of Lodz
Janusz Maszewski – University of Lodz
Maria Skłodowska – University of Lodz

Scientific Committee:

Maria Kwiatkowska – University of Lodz
Jerzy Kawiak – Editor of *Advances in Cell Biology* (*Postępy Biologii Komórki*)
Andrzej K. Kononowicz – University of Lodz
Janusz Maszewski – University of Lodz

Conference Office:

Ewa Mikołajczyk-Zając – University of Lodz
Violetta Macioszek – University of Lodz
Katarzyna Hnatuszko-Konka – University of Lodz (*Book cover and Conference website design*)
Tomasz Kowalczyk – University of Lodz

Department of Genetics and Plant Molecular Biology and Biotechnology
Faculty of Biology and Environmental Protection
University of Lodz
S. Banacha 12/16, 90-237 Lodz,
Tel, Fax: 042 635 44 23
Conference e-mail: konfwwbk@biol.uni.lodz.pl

Editors of The Proceedings:
Andrzej K. Kononowicz, Ewa Mikołajczyk Zając, Janusz Maszewski

CONTENTS

PROFESSOR MARIA J. OLSZEWSKA – HER SCIENTIFIC AND ACADEMIC LIFE AND ACHIEVEMENTS (*IN POLISH*) **5**

CONFERENCE TIMETABLE **9**

CONFERENCE PROGRAM **10**

ABSTRACTS OF INVITED LECTURES **12**

Andrzej B. Legocki – *Evolution of symbiotic systems* **13**

Stefan Malepszy – *Cucumbers secrets disclosed: the investigation on nuclear genome sequence completed* **13**

Krzysztof Pawłowski – *Data, knowledge, and errors in biological databases - genes, genomes, relationship networks* **14**

Stanisław Bielecki – *Biotechnological nanomaterials* **14**

Jan Szopa – *The new generation of plant raw materials based on flax for medical and industrial use* **15**

Andrzej Jerzmanowski – *Plant linker histones – universal mediators in ABA dependent transcriptional control?* **16**

Zofia Szweykowska-Kulińska – *MicroRNAs biogenesis and their role in plant gene expression* **17**

Mariusz Jaskólski – *PR-10 proteins: a reservoir of hydrophobic ligands in plant cells?* **18**

Jerzy Silberring – *Clinical proteomics trends, pitfalls, and problems* **19**

Jerzy Kawiak – *Adult stem cells in biology and medicine* **20**

Elżbieta Kuta – *Plant holocentric chromosomes structure - evidence from molecular cytogenetics of Luzula species* **21**

Przemysław Wojtaszek – *Known unknowns. New functions of plant cell walls and their components* **22**

Elżbieta Wyroba – *Isoforms and isotypes of Rab proteins in plant and animal cells* **22**

Andrzej K. Kononowicz – *New vistas in plant bioreaktor technologies* **23**

ABSTRACTS OF POSTER PRESENTATIONS – SECTION I **25**

The Authors are kindly requested to be at their posters

ABSTRACTS OF POSTER PRESENTATIONS – SECTION II **76**

The Authors are kindly requested to be at their posters

AUTHOR'S INDEX **110**

NOTES **115**

PROFESSOR MARIA J. OLSZEWSKA

Maria Joanna Olszewska, cell biology, molecular cytogenetics, Born Apr. 21, 1929, Dąbrówka Polska, Poland. Education: High Sch. of Agriculture - Eng. (1949); University of Łódź – M.Sc. (1950), PhD (1956), Dr. Sci. (1960). Rockefeller Foundation fellowship for post-doctoral studies in prof. Brachet's Laboratory (1959-1960, Brussels, Belgium). Associate professor (1969), full professor (1978). University of Łódź: assistant, senior assistant, docent (1950-1971); head of the Laboratory of Cytochemistry (1961-1971), head of the Department of Plant Cytology and Cytochemistry (1971-1999). Since 1999 retired, affiliated with the Dept. of General Genetics, Plant Molecular Biology and Biotechnology, University of Łódź, Research concerns the role of cell nucleus, synthesis of nuclear DNA and RNA, cell cycle in plants and molecular cytogenetics. Corresponding member of the Polish Academy of Sciences (1989), full member (2005). President of the Łódź Branch of the Polish Academy of Sciences (1993-1998), member of the Praesidium of the PAS (1993-2006). Author and co-author, editor and co-editor of five handbooks (in Polish). Co-editor of a journal "Advances in Cell Biology" (in Polish – Postępy Biologii Komórki, articles concerning genetics and plant cell biology). Honours: Polonia Resituta Crosses III, II and I Class; Honorary member of the Soc. Sci. Lodzensis, Polish Botanical Soc., Polish Histo- and Cytochemical Soc., Polish Soc. of Cell Biology.

Selected publications:

Olszewska M.J., Brachet J. 1960. *Influence of localized ultra-violet irradiation on the incorporation of adenine-8-^{14}C and D,L-methionine^{35}S in Acetabularia mediterranea*. Nature **187:** 954-955.

Olszewska M.J., Wroński M., Fortak W. 1967. *A histochemical method for revealing disulphide bonds by means of hydrogen selenide*. Folia Histochem. Cytochem. **5:** 7-14.

Olszewska M.J., Godlewski M. 1972. *Autoradiographic study of the synthesis of nucleic acids and protein during the cell cycle of synchronously dividing antheridial filaments in Chara vulgaris* L. Folia Histochem Cytochem. **10:** 245-256.

Olszewska M.J. 1978. *Chromatin condensation in late G2 phase as a factor related to the earlier initiation of mitosis in successive generations of antheridial filaments of Chara vulgaris*. Microsc. Acta **80:** 115-126.

Olszewska M.J., Damsz B., Rabęda E., 1983. *DNA endoreplication and increase in number of chloroplasts during leaf differentiation in five monocotyledonous species with different 2C DNA content*. Protoplasma **116:** 41-50.

Olszewska M.J., Osiecka R. 1983. *The relationship between 2C DNA content, life cycle type, systematic position and the dynamics of DNA replication in parenchyma nuclei during growth and*

differentiation of roots in some dicotyledonous herbaceaous species. Biochem. Physiol. Pflanzen **178:** 581-599.

Olszewska M.J., Damsz B., Kuran H., Marciniak K. 1984. *Enhanced rRNA transport from nucleoli into cytoplasm at G1/S, S/G2 and G2/M transitory points of the cell cycle in root meristem of Helianthus annuus* L. Eur. J. Cell Biol. **33:** 1-6.

Olszewska M.J., Kononowicz A.K., Maszewski J., Mądrzak C., Konieczny A., Legocki A.B. 1989. *Changes in DNA content during rhizobial nodule development in Lupinus luteus L. II. In situ hybridization of cDNA coding for leghemoglobin*. Biol. Zent. Bl. **108:** 231-239.

Olszewska M.J., Marciniak K., Kuran H. 1990. *The timing of synthesis of proteins required for mitotic spindle and phragmoplast in partially synchronized root meristems of Vicia faba* L. Eur. J. Cell Biol. **53:** 89-92.

Olszewska M.J., Karłowski W., Legocki A.B. 1994. *In situ hybridization of two nodule-specific gene products from yellow lupin*. Folia Histochem. Cytobiol. **32:** 205-208.

Olszewska M.J., Gernand D., Godlewski M., Kunachowicz A. 1997. *DNA methylation during antheridial filament development and spermiogenesis in Chara vulgaris L. (Charophyceae) analyzed by in situ nick-translation*. Eur. J. Phycol. **32:** 287-291. Sakowicz T.,

Olszewska M.J. 1997. *DNA content, interphase AgNOR-area, number of* ^{3}*HrDNA hybridization signals and the methylation level in coding rDNA sequence in different organs of Lupinus luteus* L. Genetica **99:** 67-72. Łuchniak P., Małuszynska J.,

Olszewska M.J. 2000. *Different DNA methylation pattern in A and B chromosome of Crepis capillaris detected by in situ nick-translation. Comparison with molecular methods.* Folia Histochem. Cytobiol. **40:** 325-330.

Prof. zw. dr hab. Maria Joanna Olszewska

HER SCIENTIFIC AND ACADEMIC LIFE ACHIEVEMENTS

(in Polish)

Prof. zw. dr hab. Maria Joanna Olszewska, studia wyższe inżynierskie ukończyła na Wydziale Ogrodniczym Wyższej Szkoły Gospodarstwa Wiejskiego w Łodzi (1946-49), a tytuł magistra uzyskała w 1950 r. na Uniwersytecie Łódzkim. Stopień doktora (kandydata nauk) Profesor Olszewska uzyskała w 1956 r., stopień doktora habilitowanego – 1960 r., tytuł profesora nadzwyczajnego – 1969 r. a tytuł profesora zwyczajnego – 1976 r. Wyrazem uznania dla wybitnych osiągnięć naukowych prof. dr hab. Marii J. Olszewskiej było wybranie Jej w roku 1989 r. na członka-korespondenta Polskiej Akademii Nauk, a w roku 2004 na członka rzeczywistego PAN.

W latach 1961-70 Prof. dr hab. Maria J. Olszewska była kierownikiem Zakładu Cytochemii na Uniwersytecie Łódzkim, a w latach 1970-1999 – kierownikiem Katedry Cytologii i Cytochemii Roślin w UŁ. Dzięki wprowadzaniu nowoczesnych metod badawczych z zakresu cytochemii i cytogenetyki molekularnej, w Katedrze tej powstała szkoła naukowa, w ramach której wiele osób uzyskało stopnie doktora i doktora habilitowanego, a także tytuły profesora. Konsekwencją tego było powołanie na Uniwersytecie Łódzkim w 1993 r. Katedry Cytofizjologii; jej twórczynią była prof. dr hab. Maria Kwiatkowska (obecnie emerytowany profesor UŁ). W 1999 r. z Katedry Cytologii i Cytochemii Roślin został wyodrębniony samodzielny Zakład Cytogenetyki i Biologii Molekularnej Roślin, którego kierownikiem została początkowo dr hab. Regina Osiecka, prof. nadzw. UŁ, a od roku 2005 prof. dr hab. Andrzej K. Kononowicz. Zakład ten w 2008 r. został

przekształcony w Katedrę Genetyki Ogólnej, Biologii Molekularnej i Biotechnologii Roślin – jej twórcą był prof. dr hab. Andrzej K. Kononowicz. Po reorganizacji, która miała miejsce w październiku 2008 r. stan obecny jednostek wyłonionych z Katedry Cytologii i Cytochemii Roślin jest następujący: 1 – Katedra Cytologii i Cytochemii Roślin, kierownik – dr hab. Mirosław Godlewski, prof. nadzw. UŁ; ponadto zatrudniony jest w tej Katedrze profesor nadzw. UŁ dr hab. Kazimierz Marciniak; 2 – Katedra Cytofizjologii, kierownik – prof. dr hab. Janusz Maszewski, drugim samodzielnym pracownikiem jest dr hab. Andrzej Kaźmierczak, prof. nadzw. UŁ; 3 – Katedra Genetyki Ogólnej, Biologii Molekularnej i Biotechnologii Roślin, kierownik – prof. dr hab. Andrzej K. Kononowicz; w Katedrze są zatrudnieni ponadto 3 profesorowie nadzw. UŁ – dr hab. Regina Osiecka, dr hab. Alina Błaszczyk i dr hab. Tomasz Sakowicz. W ramach Katedry utworzona została Pracownia Cytogenetyki, a obowiązki jej kierownika pełni dr hab. Regina Osiecka, prof. nadzw. UŁ. Należy dodać, że Zakład Cytologii i Cytochemii Roślin kierowanych do niedawna przez prof. dr hab. Barbarę Gabarę został w październiku 2008 roku przekształcony w samodzielną Pracownię Mikroskopii Elektronowej, a jej kierownikiem została dr Sława Glińska. Warto przypomnieć w tym miejscu, że na przełomie lat 60' i 70', dwie osoby – wychowankowie Katedry Cytologii i Cytochemii Roślin zasilili dwa polskie uniwersytety: prof. dr hab. Alicja Górska-Brylass – Uniwersytet im. Mikołaja Kopernika w Toruniu a prof. dr hab. Bohdan Rodkiewicz – Uniwersytet im. Marii Curie-Skłodowskiej w Lublinie.

Bezpośrednio po przejściu na emeryturę, tj. od 1.10.1999 r. do 30.09.2001 r. Profesor Olszewska była zatrudniona na ¼ etatu w Zakładzie Cytogenetyki i Biologii Molekularnej Roślin, wydzielonym z Katedry Cytologii i Cytochemii Roślin. Obecnie Profesor Olszewska jest afiliowana przy Katedrze Genetyki Ogólnej, Biologii Molekularnej i Biotechnologii Roślin. W latach 1961-65 była kierownikiem Katedry Anatomii i Cytologii Roślin w Uniwersytecie Marii Curie-Skłodowskiej w Lublinie. Objęcie tego stanowiska, wymuszone przez ówczesne Ministerstwo nadzorujące szkolnictwo wyższe, było warunkiem przyznania etatu docenta w Uniwersytecie Łódzkim.

Praca badawcza. Od początku kariery naukowej głównym obiektem badań Profesor Olszewskiej było jądro komórkowe i cykl komórkowy. Z tego względu, jako miejsce pierwszego stażu podoktorskiego (1957 r., stypendium ówczesnego Ministerstwa Szkolnictwa Wyższego) wybrała słynny ośrodek kierowany przez prof. Jeana Bracheta (Université libre de Bruxelles), gdzie nauczyła się m.in. autoradiografii na poziomie komórkowym (zainicjowała tę technikę w Polsce), a następnie, w latach 1959-1960 (stypendium Fundacji Rockefellera) prowadziła badania na modelowym glonie jednokomórkowym *Acetabularia mediterranea*. Na tym obiekcie wykazała, że RNA cytoplazmatyczny jest syntetyzowany w jądrze komórkowym. W dalszej pracy badawczej, m. in. zainspirowana przez książkę prof. Bracheta „*Biochemical Cytology*" (*Academic Press*, 1957), Profesor Olszewska z powodzeniem adaptowała liczne metody biochemiczne do badań *in situ*. W poszukiwaniu dogodnego obiektu do badań cyklu komórkowego wprowadziła do nauki (eksploatowany do dziś) model – nici spermatogeniczne glonu *Chara vulgaris*. Wykazała m.in., że komórki nici dzielą się synchronicznie, ale cykl pozbawiony jest fazy G1; że po kolejnych podziałach następuje stopniowe ograniczanie wzrostu komórek, transkrypcji i translacji; a wreszcie, że po ostatnim podziale komórki przekształcają się w plemniki.

W toku dalszych badań, Profesor Olszewska opracowała metodykę synchronizacji cykli komórkowych w merystemach korzeni roślin wyższych (cykl G1 + S + G2 + M), opartą nie na toksycznych oddziaływaniach chemicznych, lecz na wpływie czynników fizycznych (światło, temperatura). W toku intensywnych prac eksperymentalnych z wykorzystaniem nowych modeli wykazano m.in., że:

- przejście przez punkty kontrolne G1/S, S/G2 i G2/M jest poprzedzone przez wzmożony transport rRNA z jąderka do cytoplazmy,
- 2 h przed inicjacją mitozy następuje synteza tubulin do puli niezbędnej dla budowy funkcjonalnego wrzeciona podziałowego i fragmoplastu,
- nasilenie endoreplikacji DNA podczas różnicowania komórek miękiszu korzeni nie zależy od rozmiarów genomu (2C DNA), ale typu cyklu życiowego: jest wyższe u gatunków jednorocznych niż bylin w obrębie tego samego rodzaju,
- w liściach roślin jednoliściennych endoreplikacja zachodzi tylko w pochwie okołowiązkowej do poziomu podobnego w miękiszu korzeni u tego samego gatunku,
- endoreplikacji towarzyszy proporcjonalna replikacja rDNA, ale jego ekspresja ulega znacznemu ograniczeniu wskutek kondensacji rDNA.

We współpracy z Profesorem Andrzejem B. Legockim i Jego Zespołem, w wyniku dwóch projektów badawczych dotyczących rozwoju brodawek korzeniowych z łubinu żółtego, wykazano drogą hybrydyzacji *in situ* cDNA kodującego leghemoglobinę, że liczba sygnałów w komórkach brodawek jest znacznie wyższa niż oczekiwana na podstawie zawartości DNA oraz, również *in situ* z użyciem mRNA, zróżnicowaną ekspresję podczas rozwoju brodawek genów kodujących dwie noduliny. Po wykazaniu, że nick-translacja *in situ* z zastosowaniem enzymów restrykcyjnych wrażliwych na metylację jest równie wiarygodna, jak metody molekularne, metodą tą wykazano m.in. postępującą metylację DNA podczas spermatogenezy u *Chara vulgaris* i wyższy poziom metylacji chromosomu B niż chromosomów A u *Crepis capillaris*.

Profesor Maria J. Olszewska jest autorką lub współautorką 117 prac doświadczalnych i 20 artykułów przeglądowych. Jest jedyną autorką lub współautorką 5 podręczników akademickich, spośród których dwa miały 2 wydania, a jeden – 6 wydań; wśród nich jest redaktorem i współredaktorem dwóch podręczników.

Kształcenie kadry. W okresie 1961-1999 r. Profesor Olszewska wykształciła ok. 100 magistrów, była promotorem w 14 zakończonych przewodach doktorskich i sprawowała opiekę nad 6 zatwierdzonymi przewodami habilitacyjnymi.

Praca dydaktyczna. Po habilitacji Profesor Olszewska prowadziła wykłady kursowe, monograficzne, seminaria magisterskie i doktoranckie. Była współinicjatorką i współorganizatorką powstania specjalizacji z genetyki w Uniwersytecie Łódzkim. Specjalizacja ta cieszyła się i nadal cieszy olbrzymim zainteresowaniem studentów, a po ukończeniu tych studiów; wielu magistrów genetyki było i jest przyjmowanych na Studium Doktoranckie Cytogenetyki, Genetyki Molekularnej i Radiobiologii na Uniwersytecie Łódzkim (którego członkiem Rady Naukowej Profesor Olszewska była od powstania Studium, tj. od 1988 r.).

Praca organizacyjna. Na przebogatą działalność organizacyjną Profesor Olszewskiej składają się liczne pełnione przez Nią funkcje: prodziekan (1966-69) i dziekan (1969-72) Wydziału Biologii i Nauk o Ziemi Uniwersytetu Łódzkiego; członek Zespołu Ekspertów Nauk Przyrodniczych (1972 - do dnia dzisiejszego) i jego przewodnicząca (1985-89 i 1991-93) przy kolejnych ministrach, którym podlegały uniwersytety; członek Centralnej Komisji ds. Tytułu i Stopni Naukowych (1994-96); w Polskiej Akademii Nauk: przewodnicząca/prezes Oddziału Łódzkiego (1993-98), członek Prezydium PAN 1993-2006; w okresie 1993-1998 z urzędu jako przewodnicząca/prezes Oddziału Łódzkiego PAN (z wyboru), następnie, przez dwie kadencje – z wyboru Zgromadzenia Ogólnego PAN; członek (4 kadencje) i przewodnicząca (1 kadencja) Komisji Nagród przy II Wydz. PAN; członek i recenzent (od początku istnienia) Komisji Nagród Prezydium OŁ PAN i Konferencji

Rektorów Państwowych Wyższych Uczelni Łodzi dla młodych pracowników nauki w dziale nauk biologiczno-medycznych.

W okresie pełnienia funkcji przewodniczącej OŁ PAN, z inicjatywy ówczesnego Prezydenta m. Łodzi, dr. Marka Czekalskiego, Profesor Olszewska doprowadziła wraz z Przewodniczącym Konferencji Rektorów Państwowych Uczelni Łodzi, prof. dr. hab. Michałem Seweryńskim, do sformułowania i podpisania w 1996 roku Porozumienia, w myśl którego Urząd m. Łodzi zobowiązał się do dofinansowywania badań naukowych łódzkich uczonych. Porozumienie to, unikatowe w skali Kraju, sprawnie funkcjonuje do dziś.

Funkcje społeczne. Łódzkie Towarzystwo Naukowe – sekretarz (1963-72) i przewodnicząca (1972-75) Wydziału III; Polskie Towarzystwo Histochemików i Cytochemików – przewodnicząca Oddziału Łódzkiego (1964-67), wiceprzewodnicząca (1967-76) i członek (1976-89) Zarządu Głównego. Członkostwo Rad Naukowych: Instytut Genetyki Roślin PAN (od 1981 r.), Instytut Chemii Bioorganicznej PAN (1992-2002), Centrum Mikrobiologii i Wirusologii PAN (1996-2002, przewodnicząca 2001-2002), Centrum Ekohydrologii PAN (2003-2006), Centrum Biologii Medycznej PAN (przewodnicząca Rady Naukowej 2004-2007), przekształconym w 2008 roku na Instytut Biologii Medycznej PAN (przewodnicząca Rady Naukowej 2008-2011). Współredaktor Postępów Biologii Komórki (od 1972 r., działy: komórka roślinna i informacja genetyczna), członek Komitetów Redakcyjnych czasopism z LF: *Acta Societatis Botanicorum Poloniae* (od 1975 r.), *Folia Histochemica et Cytobiologica* (od 1975 r.), *Acta Biologica Cracoviensis, Series Botanica* (od 1998 r.).

Nagrody. M. in. zespołowe – 2 w wyniku konkursu PAN (1958, 1967), 2 – Polskie Towarzystwo Histochemików i Cytochemików (1965, 1967), Polskie Towarzystwo Genetyczne (1983), indywidualna Łódzkiego Towarzystwa Naukowego (1981), zespołowa (1967) i indywidualna (1972) Ministra Oświaty i Szkolnictwa Wyższego.

Wyróżnienia: Doctor honoris causa Akademii Medycznej w Łodzi (1999); członek honorowy Polskiego Towarzystwa Histochemików i Cytochemików (1995), Polskiego Towarzystwa Botanicznego (1998), Łódzkiego Towarzystwa Naukowego (2000), Polskiego Towarzystwa Biologii Komórki (2008),. Za wyróżnienie można uznać zaproszenie Profesor Olszewskiej, jako honorowej wiceprzewodniczącej III Międzynarodowego Kongresu Histo- i Cytochemii (Nowy Jork, 1968) z pełnym pokryciem kosztów podróży w obie strony i pobytu w USA.

Odznaczenia państwowe: Krzyż Kawalerski (1972), Oficerski (1990) i Komandorski (1999) Orderu Odrodzenia Polski.

CONFERENCE TIMETABLE

9:00		**Andrzej Jerzmanowski** – *Plant linker histones – universal mediators in ABA dependent transcriptional control?*
9:30		**Zofia Szweykowska-Kulińska** – *MicroRNAs biogenesis and their role in plant gene expression.*
10:00		**Mariusz Jaskólski –** *PR-10 proteins: a reservoir of hydrophobic ligands in plant cells?*
10:30		**COFFEE BREAK**
11:00		**Jerzy Silberring** – *Clinical proteomics trends, pitfalls, and problems.*
11:30		**Jerzy Kawiak** – *Adult stem cells in biology and medicine.*
12:00		**POSTER SESSION** *The Authors are kindly requested to be at their posters*
13:00		**LUNCH**
14:00		**Elżbieta Kuta –** *Plant holocentric chromosomes structure - evidence from molecular cytogenetics of Luzula species.*
14:15		**Przemysław Wojtaszek –** *Known unknowns. New functions of plant cell walls and their components.*
14:45		**Elżbieta Wyroba –** *Isoforms and isotypes of Rab proteins in plant and animal cells.*
15:00	**OPENING CEREMONY**	
15:15		**Andrzej K. Kononowicz –** *New vistas in plant bioreactor technologies*
15:30	**Andrzej B. Legocki –** *Evolution of symbiotic systems.*	
16:00	**Stefan Malepszy –** *Cucumber's secrets disclosed: the investigation into nuclear genome sequence completed.*	**CLOSING OF THE CONFERENCE**
16:15	**Krzysztof Pawłowski –** *Data, knowledge, and errors in biological databases - genes, genomes, relationship networks.*	
16:45	**COFFEE BREAK**	
17:15	**Stanisław Bielecki –** *Biotechnological nanomaterials.*	
17:45	**Jan Szopa –** *The new generation of plant raw materials based on flax for medical and industrial use.*	
18:30	**DINNER PARTY WITH PROFESSOR OLSZEWSKA**	

MONDAY – APRIL 20, 2009

15:00 **CONFERENCE OPENING**

15:30 **SESSION I**
Chairman: Czesław S. Cierniewski

15:30 **Andrzej B. Legocki –** *Evolution of symbiotic systems*
16:00 **Stefan Malepszy –** *Cucumber's secrets disclosed: the investigation into nuclear genome sequence completed*
16:15 **Krzysztof Pawłowski –** *Data, knowledge, and errors in biological databases - genes, genomes, relationship networks*

16:45 **COFFEE BREAK**

17:15 **SESSION II**
Chairman: Adam Jaworski

17:15 **Stanisław Bielecki –** *Biotechnological nanomaterials*
17:45 **Jan Szopa –** *The new generation of plant raw materials based on flax for medical and industrial use*

18:15 **BREAK**

18:30 **DINNER PARTY WITH PROFESSOR MARIA J. OLSZEWSKA**

TUESDAY – APRIL 21, 2009

9:00 **SESSION III**
Chairman: Kazimierz Strzałka

9:00 **Andrzej Jerzmanowski –** *Plant linker histones – universal mediators in ABA dependent transcriptional control?*
9:30 **Zofia Szweykowska-Kulińska –** *MicroRNAs biogenesis and their role in plant gene expression*
10:00 **Mariusz Jaskólski –** *PR-10 proteins: a reservoir of hydrophobic ligands in plant cells?*

10:30 **COFFEE BREAK**

11:00 **SESSION IV**
Chairman: Wanda M. Krajewska

11:00 **Jerzy Silberring** – *Clinical proteomics trends, pitfalls, and problems*
11:30 **Jerzy Kawiak** – *Adult stem cells in biology and medicine*

12:00 **POSTER SESSION**

13:00 **LUNCH**

14:00 **SESSION V**
Chairman: Maria Kwiatkowska

14:00 **Elżbieta Kuta –** *Plant holocentric chromosomes structure - evidence from molecular cytogenetics of Luzula species*
14:15 **Przemysław Wojtaszek –** *Known unknowns. New functions of plant cell walls and their components*
14:45 **Elżbieta Wyroba –** *Isoforms and isotypes of Rab proteins in plant and animal cells*
15:15 **Andrzej K. Kononowicz –** *New vistas in plant bioreactor technologies*

16:00 **CLOSING OF THE CONFERENCE**

(This Book contains not verified copies of abstracts supplied by Authors)

ABSTRACTS OF INVITED LECTURES

Evolution of symbiotic systems

Monday – 15:30

Andrzej B. Legocki

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, PL

Author's e-mail: legocki@ibch.poznan.pl

In natural ecosystems the most successful beneficial interaction between genetically distant organisms are associations of higher plants with microorganisms. The best examples of such associations are ubiquitous symbiosis of large majority of land plants with *Glomeromycota* fungi forming arbuscular mycorrhiza and more specialized endosymbiosis of legumes with *Rhizobium* species. These interactions result from coordinated developmental programs adapted by both partners.

Fossil records and recent phylogenic estimations suggest that mycorrhiza is as old as the earliest land plants. Therefore, it may be postulated that colonization of the lands was in evolution dependent on fungal symbiosis.

A crucial role in the molecular dialogue between higher plants and microorganisms play chemical signals, which initiate complex recognition mechanisms in which participates large number of regulatory proteins. It has recently been documented that plant hormons cytokinins operating in legumes both locally and systematically are actively involved in development of symbiotic association as well as in morphogenesis of new organ – root nodule.

Cucumber's secrets disclosed: the investigation into nuclear genome sequence completed

Monday – 16:00

Stefan Malepszy, Rafał Wóycicki, Zbigniew Przybecki,

Department of Plant Genetics, Breeding and Biotechnology
Warsaw University of Life Sciences – SGGW, Nowoursynowska 166,
02-776 Warszawa, Poland

Author's e-mail: stefan_malepszy@sggw.pl

Complete genomic sequences are valuable source of new knowledge and opens up new scientific horizons for molecular genetics and functional genomics. In the last decade, these new scientific fields grow up expansively due to development of new generations of WGS sequenators. These machines are able to read longer and longer fragments of a DNA sequence with simultaneous reduction of costs of bp. As a result of this technical progress a new initiative, such as Arabidopsis 1001 genomes (http://1001.genomes.org) appeared.

In our approach for cucumber genome sequence determination we used BAC library of 32640 clones. Sequencing of ends of these BAC clones generated 442768885 nt of STC, representing ca.12.06% of the whole cucumber genome with coding sequence density of ca.24.76%. Subsequently, we sequenced the whole genome 8-times with help of a brand new GS FLX Titanium sequenator, that result of 8 millions fragments of average length of 375 nt. This method was also used for sequencing of so call "pared ends" that give additional 4-times cover of the whole genome sequence. After all we did genome annotation. We will present some selected and characteristic features of cucumber genome sequence and various research activities due to sequence disclosure.

Data, knowledge, and errors in biological databases: genes, genomes, relationship networks

Monday – 16:45

Krzysztof Pawłowski

Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland and Warsaw University of Life Sciences, Warsaw, Poland

Author's e-mail: k.pawlowski@nencki.gov.pl

High throughput 'omics' studies, like gene expression microarray or proteomics experiments, rely heavily on biological databases describing biological function and its various aspects. Many resources and tools for functional annotation exist, but only some of them focus on knowledge building, preferably in an iterative manner, as opposed to data gathering. Recent trends, including attempts at "wikipedisation" of scientific databases, are discussed.

An important problem is the presence of errors and misannotations in the realm of biological databases. The reasons for appearance of errors, along with their propagation, are addressed, together with approaches to prevent and remedy the misannotation problem.

Also, the persistence of identified errors and misannotations and obsolete data is mentioned. Lastly, use of function prediction methods is advocated for better interpretation of the biological data, and discovery of true novelty.

Biotechnological nanomaterials

Monday – 17:15

Stanisław Bielecki

Institute of Technical Chemistry, Technical University of Lodz, B. Stefanowskiego 4/10, 90-924 Lodz, Poland

Author's e-mail: stanb@p.lodz.pl

Nanomaterials have one or more dimensions in the range of 1 to 100 nm. Due to the greatly increased surface area they display differences from their large bulk forms that create enormous opportunities for new applications of them. They have become very important in the materials and medicine fields. Some nanomaterials, e.g. bacterial cellulose (BC) are available from microbial sources. The most efficient BC producers are *G. xylinus* strains.

Bacterial cellulose is the nanocrystalline, highly elastic and mechanically durable, non-pyrogenic and athrombogenic polymer. It is biocompatible with tissues and displays the substantial water-binding capacity. Our studies showed that BC was an excellent wound dressing that speeded up healing of various types of wounds. Binding of proangiogenic compounds [like lysophosphatidic acid (LPA)] to BC matrix gave rise to the new class of wound dressings that accelerated the wound healing process due to positive changes in transcriptomes of endothelial cells. The microbial cellulose is also applicable to internal medicine as a biomaterial for mesh in herniotherapy and for hollow tubes formation. It can be also applied as artificial blood vessels, for reconstruction of nerves or as an immunoprotective barrier for construction of artificial hybrid organs and as an artificial cartilage or in a plastic surgery. Some of these biotechnological products have been already used in medicine and some other ones have been subjected to intensive clinical tests and investigations. In this lecture some molecular and technological aspects of BC biosynthesis as well as modifications of microbial cellulose for different application is discussed

The new generation of plant raw materials based on flax for medical and industrial use.

Monday – 17:45

Jan Szopa, Anna Kulma, Magdalena Żuk

Faculty of Biotechnology, Wrocław University, Wroclaw, Poland

Author's e-mail: szopa@ibmb.uni.wroc.pl

The primary aim of the study is to give the well-known and valuable farmland plant that is *Linum* (flax) only such properties which allow for its restoration in agriculture in Poland and will stimulate its use as an indigenous raw material in processing industry as well as renew its tradition of cultivation. The basis of these actions is two types of crops produced through biotechnology and the generation of raw materials having entirely new quality in comparison to the current value of raw flax.

Oxylen, flax with antioxidants. This new type of flax, which fibres and seeds naturally synthesise strong antioxidants, is intended for biomedical and cosmetic uses. Flaxseed oil, value because of the presence of the fatty acids ω3 and ω6, needed in the human diet, pressed from the seeds of these plants does not require additional protection against oxidation of unsaturated fatty acids (for longer-term sustainability of oil) and can be directly used for consumption as well as in cosmetics and medicine to assist the treatment of inflammatory states.Residues from new flax seeds contain a lot of antioxidant (secoizolariciresinol from the group of lignans) that can be easily obtained with water solutions and which has a protective role in various types and stages of cancer (such as prostate cancer), autoimmune disease and both types of diabetes.Flax fibres of new generation are bioactive, therefore having superiority over currently used cotton fibres. Dressings from new generation flax will be certainly competitive when compared to biologically inert cotton and viscose dressings.

Recent pilot studies carried out on a group of patients with so-called injuries with difficulties in healing to which the medicine is powerless, inform that a new generation of dressings based on flax does not cause the allergy of wounds and seems to contribute positively in the healing process through visible reducing of seepage, cleaning wounds from pus secretions and mass of fibrin that results in the appearance of granulation tissue which is the first step in healing of ulcers.

Plastic flax. New flax is equipped with biodegradable, plastic fibres (polyhydroxybutyrate - PHB) and uses for biomedical and technical applications. The aim is to use the hitherto unknown bio-plastic fibres in the manufacture of new generation packaging and biomedical supplies. Bio-plastic fibres are the form of cellulose polymers chemically linked with polymers of polyhydroxybutyrate (bio-plastic) produced during the growth of plant using new methods of plant biotechnology.The goal is to produce composite bio-plastic fibres from polypropylene. The presence of hydrophobic polyhydroxybutyrate in the textile of bio-plastics guarantees a good and proven adhesion with hydrophobic polypropylene and following biodegradation. It was found that replacing 20% of polypropylene in products by the flax fibres from bio-plastics does not change their mechanical characteristics compared with a pure polypropylene. In global terms, this means that there will be about 28 million tonnes of polypropylene per year less in the environment. Moreover, limiting the use of polypropylene creates the possibility of a partial independence of the Polish economics from synthetic materials derived from the recast of crude oil. Application of bio-composites in medicine may be multidimensional from the surgical threads to the implants. In preliminary studies it was found that the composite of polypropylene together with bio-plastic fibres does not cause the aggregation of blood platelets in contrast to pure polypropylene and its composite with natural fibres that do not have bio-plastic. *In vitro* studies have also found that flax fibres with bio-plastic possess bacteriostatic properties. Therefore, bio-composites of flax fibres with bio-plastic open a new, hitherto unknown way of applying them in orthopaedic implants, tissue engineering and other areas of medicine.

Tuesday – 9:00

Plant linker histones:
universal mediators in ABA dependent transcriptional control?

Joanna Puzio[2], Marcin Puzio[1], Kinga Rutowicz[2], Łukasz Kniżewski[3], Krzysztof Ginalski[3] , Krzysztof Kokoszka[2], Paweł Siedlecki[1], and Andrzej Jerzmanowski[1,2]

[1]University of Warsaw, Laboratory of Plant Molecular Biology, Pawinskiego 5A, 02-106 Warsaw, Poland;
[2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland;
[3]Laboratory of Bioinformatics and Bioengineering, Interdisciplinary Center for Mathematical and Computational Modelling, University of Warsaw, Pawinskiego 5A, 02-106 Warsaw, Poland;

Author's e-mail: andyj@ibb.waw.pl

H1 or linker histones are abundant components of chromatin fibre. In animals, including mammals, numerous cell-type and stage-specific isoforms or variants of H1, each encoded by a separate gene, have been described. This complexity poses major technical problems in studies of the functional significance of H1 variability. Plants offer a much simpler model in which to study the function of linker histones. *Arabidopsis* has three H1 variants, two closely related somatic variants (H1-1 and H1-2) and a stress-inducible variant (H1-3) (1). Stress-inducible H1 variants are evolutionarily conserved in flowering plants and have been shown to be up-regulated during stress and upon ABA treatment (2). Structural characteristics of plant H1 proteins reveals differences between somatic- and stress-inducible variants, in both the central globular domain (GH1) and unstructured N- and C-terminal tails. We will discuss the results of experiments aimed at elucidating the importance of these differences for the potential role of H1 variability in plants.

References

1. Wierzbicki AT,Jerzmanowski, A (2005) Suppression of histone H1 genes in Arabidopsis thaliana results in heritable developmental defects and stochastic changes in DNA methylation. Genetics **16**, 997-1008.
2. Jerzmanowski A, Przewłoka, M, Grasser, K (2000) Linker histones and HMG1 proteins of higher plants. Plant Biology **2**, 586-597.

This research was supported by Polish Ministry of Science and Higher Education grant N301 158735 for A.J. and EMBO Installation and FNP (FOCUS) grants for K.G.

MicroRNAs biogenesis and their role in plant gene expression Tuesday – 9:30

Bogna Szarzyńska[1], Łukasz Sobkowiak[1], Bikram Datt Pant[2], Salma Balazadeh[2], Wolf-Rüdiger Scheible[2], Bernd Mueller-Roeber[2,3], Artur Jarmołowski[1], and Zofia Szweykowska-Kulińska[1]

[1]Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland
[2] Max-Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany
[3] University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Potsdam-Golm, Germany

Author's e-mail: zofszwey@amu.edu.pl

MicroRNAs represent short RNA molecules that control gene expression by targeting the cleavage of cognate complementary mRNAs or by inhibiting their translation. They act at the post-transcriptional level. In *Arabidopsis thaliana*, miRNAs are produced from RNA polymerase II transcripts in a multi-step process in which at least four proteins are involved: DCL1 (DICER-LIKE 1), HYL1 (HYPONASTIC LEAVES 1), SE (SERRATE), and HEN1 (HUA ENHANCER 1). Using *A.thaliana* homozygous *hyl1* mutant line, in which both alleles of *HYL1* gene are inactive, we studied the biogenesis of pri-miRNAs that require this protein for their proper maturation. *A.thaliana* HYL1 is a nuclear double stranded RNA-binding protein.

A quantitative real-time PCR platform for parallel quantification of 176 pri-miRNAs was used to reveal strong accumulation of 57 miRNA precursors in the *hyl1* mutant that completely lacks HYL1 protein. This approach enabled us for the first time to pinpoint particular members of *MIRNA* family genes that require HYL1 activity for efficient maturation of their precursors. Moreover, the accumulation of miRNA precursors in the *hyl1* mutant gave us the opportunity to carry out 3' and 5' RACE experiments which revealed that some of these precursors are of unexpected length. The alignment of HYL1-dependent miRNA precursors to *A. thaliana* genomic sequences indicated the presence of introns in 13 out of 21 genes studied. Some of the characterized intron-containing pri-miRNAs undergo alternative splicing such as exon skipping or usage of alternative 5' splice sites suggesting that this process plays a role in the regulation of miRNA biogenesis. In the *hyl1* mutant intron-containing pri-miRNAs accumulate alongside spliced pri-miRNAs suggesting the recruitment of HYL1 into the miRNA precursor maturation pathway before their splicing occurs. Careful bioinformatic analysis of long pri-miRNA precursors reveled that they contain additional, new miRNAs. The possible role of these new miRNAs in plant gene expression regulation is now under investigation.

Tuesday – 10:00

PR-10 proteins:
a reservoir of hydrophobic ligands in plant cells?

Mariusz Jaskólski

Center for Biocrystallographic Research, Institute of Bioorganic Chemistry of Polish Academy of Sciences, and Department of Crystallography, Faculty of Chemistry, A. Mickiewicz University, Poznan, Poland

Author's e-mail: mariuszj@amu.edu.pl

Although pathogenesis-related class 10 (PR-10) proteins are ubiquitous in plants, their precise role is unknown. They have been implicated in plant development and in stress-response reactions but the molecular aspects of these mechanisms remain obscure. In contrast, the PR-10 structural canon is very well defined. It consists of a large seven-stranded antiparellel β-sheet, wrapped around a long C-terminal α-helix. Together with two additional α-helices, this structural motif encloses a huge internal cavity, with hydrophobic interior and a volume of up to 4000 $Å^3$. Our structural studies of PR-10 proteins have concentrated on their ability to bind cytokinins. Cytokinins are potent plant hormones, also implicated in development and stress control. In addition to natural cytokinins, which are adenine derivatives such as *trans*-zeatin, there are also artificial, urea-derived molecules such as *N,N′*-diphenylurea, with the same physiological effects. A search for a cytokinin-specific binding protein (CSBP) in *Vigna radiata* had revealed a molecule tentatively classified in the PR-10 family. Our atomic-resolution crystallographic study of the CSBP protein in complex with *trans*-zeatin has confirmed this structural classification and revealed a perplexing view of the hormone binding mode. The hydrophobic cavity of the protein can accommodate either one or two *trans*-zeatin molecules with variable orientation, but with perfect definition in electron density maps. The same hormone (*trans*-zeatin) has been crystallized with a classic PR-10 protein form *Lupinus luteus*, and the crystal structure, again determined at near-atomic resolution, has revealed a yet different binding mode, with three *trans*-zeatin molecules in the binding cavity. Finally, the same PR-10 protein has been crystallized with *N,N′*-diphenylurea. In this case, the binding cavity is occupied by four ligand molecules, which have rather poor definition in electron density maps and almost no specific interactions with the protein. All these results taken together seem to suggest a new aspect of molecular recognition, challenge our view of specificity in hormone-protein interactions, and hint at a possible role for PR-10 proteins as a reservoir of hydrophobic molecules in the aqueous environment of the plant cell.

Clinical proteomics trends, pitfalls, and problems

Tuesday – 11:00

Jerzy Silberring

Department of Neurobiochemistry, Faculty of Chemistry, Jagiellonian University, Cracow, Poland

Author's e-mail: silber@chemia.uj.edu.pl

Clinical proteomics is an analytical strategy functioning at the edge between chemistry, biochemistry, and medicine. This approach utilizes modern and ultrasensitive techniques, such as microextraction and separation of complex biological samples, identification with the aid of mass spectrometry, and advanced bioinformatics. Nowadays proteomics is based on basic research and clinical investigations. Between those disciplines, there is a constant exchange of information (translation) and this process is part of the systems biology, i.e. understanding how human organism works.

In reality, i.e. in a daily laboratory practice this assumption, however ambitious, meets many methodological ambiguities. The major issue is a lack of the commonly accepted, standard (or optimal) analytical strategy to handle biological material, and to monitor specific diseases. There is also an urgent need to evaluate quality criteria for this multi-task approach. Without such widespread norms, data comparison between various laboratories is out of question.

Many ambiguities already appear during material collection phase. This is associated with the overall dynamics of such complex biological system as living organism, influence of age, medications, environment, a way the sample is collected and stored, etc. on the final result of the entire analysis. Additionally, several proteins comprise 96% of the protein content in plasma and the dynamic range of molecules reaches nearly 10^{10}, which obscures less abundant components having important biological impact. Thus, simultaneous analysis of a huge amount of raw data is far beyond capabilities of modern instrumentation and software, and, even more important, cannot be fully interpreted by operators and translated to a common biological sense. One spectacular example may here be cited, where authors honestly reported analysis of a mixture of 6 standard proteins, which resulted in a printout containing "identification" of ca. 800 proteins! How the above contributes to the reliable and reproducible analysis? Presentation aims to overview trends, problems, and pitfalls of clinical proteomics.

Adult stem cells in biology and medicine

Tuesday – 11:30

Jerzy Kawiak

Medical Centre of Postgraduate Education, Warsaw, Poland

Author's e-mail: jkawiak@cmkp.edu.pl

There are estimations on the number of cells building human adult body on 10^{16}. Life span of most cells is regulated and part of the cells is dying, and replaced by new, differentiating cells starting from the stem cells. The well known example is the hematopoietic system in the bone marrow. The stem cells are localized at different sites of the adult organizm e.g. in the liver, in the epithelium of the digestive tract, in the skin epithelium or skeletal muscles.

Tissue specific stem cells are descendants of embryonic stem cells. The cells forming morula are totipotent, however at the blastula stage they are starting to direct to different sites: to inner cell mass (ICM) known as pluripotential embryonic stem cells and trophoectoderm. These cells differentiate further, and part of these cells remains in the organs and tissues as tissue-specific stem cells.

Stem cells in the adult organizm may be recognized by markers present on both embryonic stem cells and tissue-specific stem cells. Examples of these markers are proteins Oct3/Oct4, CXCR4, NANOS, CD133, CD117, CD34, CD90. The stem cells has some common physiological functions (properties): 1) are self-renewal, may replicate forminng a new stem cells, 2) may differentiate to heterogenous tissue and organ functional cells, 3) are homeostatic, regulating differentiation and self-renewal in organs and tissues, and 4) they are self-protected against toxic and radiation stress.

The stem cells populate within some specific sites of the organism, where they remain due to chemokine systems. Well known chemokine system working in the bone marrow is ligand SDF-1 (stroma derived factor-1) and stem-cells membrane receptor CXCR4. Several stress factors may induce decrease in SDF-1 level in the bone marrow surrouding, resulting in translocation of the stem cells to the circulation and a new niche. The other known translocating system is WNT(ligand)-/ Frizzled LRP (receptor complex at the cell surface). Constant circulation of the low level of stem cells may be observed as well.

The first direct evidence for the existence of cancer stem cells came from observations of the acute myeloblastic leukemia. It started the attempts of isolation and description of the cancer stem cells from some tumors as well. The cancer stem cells represent less than 1% of tumor cells in the mouse models. It is assumed, that the mutations within normal stem cells may lead to appearance of cancer stem cells. Their long interphase period favour accumulation of the mutations within the cells and self-protection against toxic factors protect them from drugs action (chemotherapy).

Tuesday – 14:00

Plant holocentric chromosomes structure: evidence from molecular cytogenetics of Luzula species

M. Bożek[2], A. R. Leitch[1], Y. K. Lim, T. Haizel[3], G. Moore[3], E. Kuta[2]

[1]School of Biological and Chemical Sciences, University of London, London, UK
[2]Department of Plant Cytology and Embryology, Jagiellonian University, Cracow, Poland
[3]John Innes Centre, Norfolk, UK

Authors' e-mail: m.bozek@iphils.uj.edu.pl
e.kuta@iphils.uj.edu.pl

Holocentric (holokinetic) chromosomes contrary to monocentric have no primary construction instead centromeric DNA and kinetochore proteins are scattered along the entire chromatid. This chromosome type occurs in not closely related groups of both animal and plant species therefore is considered as evolutionary parallelism. Not localized kinetochore causes chromosome alteration (fussions, fissions) with no negative effect on cell divisions. Chromosome fragmentation and/or fusion as commonly occurring structural chromosome mutations substantially influence karyotype structure. The object of our long term studies are monophyletic *Luzula* D.C. genus with all species investigated so far with holocentric chromosomes. To analyze holocentric chromosome structure and karyotype alteration, species representing different subgenera and sections and also chromosome number were selected, including diploids with 12 standard chromosomes (*L. alpinopilosa*, *L. nivea*, *L. sylvatica*), with 6 very long chromosomes (*L. elegans* and *L. purpureosplendens*), with 48 very small chromosomes (*L. sudetica*) and hexaploid with 36 standard chromosomes (*L. multiflora*). AFLP markers were used to establish phylogenetic relationships between analyzed species. The resulting unrooted dendrogram places *L. multiflora, L. sudetica, L. rufa* form a clade group with high bootstrap support value of 78.

Chromosome features (size and number) nicely correspond with nuclear DNA content which in diploids with 12 standard chromosomes ranged 0.82 pg-1.14 pg while in diploids with 6 very long chromosomes reached more than 8 pg and in diploid with smallest 48 chromosomes was relatively low (0.80 pg) and similar to diploids with standard chromosomes. Hexaploid origin of *L. multiflora* was supported by DNA content of 2C=2.54 pg, equivalent to $2C_x$=0.85 pg (DNA content per single parental genome).

C-banding and fluorescent *in situ* hybridisation (FISH) with selected DNA sequences (centromeric repeats-LCS1, plant telomeric TTTAGGG, ribosomal DNA, retroelements) revealed unique pattern of heterochromatin and centromeric sequences distribution not similar to monocentric chromosomes. In all investigated species there were multiple (3-7) chromosomal sites containing Giemsa-stained heterochromatin in terminal, subterminal locations and scattered along the chromosome. LCS1 repeats distribution showed a uniform pattern along the whole chromatids with up to 4 major LCS1 clusters visible in some species. Retroelements Ty1/*copia* signals were scattered all along each chromatid with a tendency to form bands by clustering of small signals. Ty1/*copia* retroelements tree created on the base of PCR sequence amplification with primers against reverse transcriptase domain, PCR products cloning, sequencing and the sequence relationship analysis showed Ty1/*copia* heterogeneity within and between species.

Based on FISH and relative quantity analysis we can conclude that centromeric repeats LCS1 and Ty1/*copia* retroelements are major contributors to *Luzula* genome.

Tuesday – 14:15

Known unknowns. New functions of plant cell walls and their components

Przemysław Wojtaszek

Department of Molecular and Cellular Biology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

Author's e-mail: przemow@amu.edu.pl

Cell walls constitute an integral component of plant cells undergoing dynamic changes in response to internal and external signals. They form a major barrier between protoplasts and their surroundings. They also form a structural and functional continuum within the whole plant. Thus, cell walls could be defined both in relation to the protoplasts that produce them, and in relation to the whole plant.

Many functions have been ascribed to the walls. Some of them stem from the specific physicochemical, mainly mechanical, properties of the walls, other are the consequence of the chemical composition of various wall domains, yet another are resultants of both wall characteristics.

Recent years brought several very interesting observations demonstrating that our view of the walls should be to some extent modified. Here, three major aspects will be presented and discussed. First, cell walls should no longer be considered as an invariant structure around protoplast. They undergo intensive recycling and remodelling, enabling precise regulation of mechanical and chemical properties of various wall domains. Second, cell walls are deeply involved in signalling processes, among them those that affect the fate of individual cell as well as those that influence the growth and development of the whole plant. Third, the walls are not operating separately from the protoplast. Together with the plasma membrane and the cytoskeleton they are integrated into the structural and functional continuum spanning the whole cell. This enables them to control the cell's shape and, to some extent, function in response to diverse array of signals, first of all of mechanical nature. All these aspects will be illustrated with data coming from the work in the author's lab.

I would like to acknowledge the funding by the Ministry of Science and Higher Education grants PBZ-MNiI-2/1/2005, N302 028 32/2243, PBZ-MNiSW-2/3/2006, N303 294434, N301 164435, N301 157035 and N303 360735.

Tuesday – 14:45

Isoforms and isotypes of Rab proteins in plant and animal cells

M. Osińska, J. Wiejak, E. Wypych, E. Wyroba

Department of Cell Biology, Nencki Institute of Experimental Biology, Warsaw, Poland

Author's e-mail: e.wyroba@nencki.gov.pl

Rab proteins are the largest family of small monomeric GTPases in eukaryotic cells. A great expansion of Rabs is correlated with diversification of trafficking machinery (1-3). Many Rab isoforms and Rab isotypes (products of paralogous genes deriving from duplication events) are involved in endo- and exocytosis performing different functions in vesicle fusion and transport as well as in signaling, proliferation and differentiation (4).

It has been a challenging task to elucidate the function of particular isoform/isotype of Rab GTPases present on endomembranes of plant and animal cells. Isoforms of some Rab proteins such

as those of Rab5 proteins were found to cooperate in the regulation of endocytosis in mammalian cells (5), whereas Rab5 isoforms in *Trypanosoma brucei* have distinct functions in the endosomal system (6). Changes in isoform/isotype ratio and location were observed in different cell types as in the case of products of closely related *Paramecium* genes *rab7a* and *rab7b*. 3D modeling showed the overlapping structure of these two proteins that may be superimposed over human Rab7 model contrary to that of *Trypanosoma* counterpart possessing an insertion.

Striking differences in the structure of some Rab proteins in the protozoan parasite *Entamoeba histolytica* such as higher proportion of introns, atypical carboxyl terminus, and lack of switch I /switch II region were found. There are 39 Rab proteins (out of 91 identified) presumed to be unique to this parasite (7).

Interestingly, distinct functions of the particular Rab proteins were found in plants in comparison with mammalian cells. This is especially evident for Rab7 proteins known to play a role in late endocytic trafficking, biogenesis of phagosomes and directing the cargo to degradation pathway via lysosomal compartment (7-9). However, in plants Rab7 is involved in the developing of the tolerance to dehydration and salinity (10). As concerns Rab 11, known to act in recycling pathway in animals (8), the new data indicate that in plant cells Rab11 (RabA) protein is essential in transport to the cell membrane and cell wall synthesis and modification (11). The recent data point also to a new function of Rab3 reported to be involved in endosymbiosis in the tropical sea anemone (12).The problem of distinct effector proteins for different Rab GTPases will be also discussed by us in this review.

References:

1. Pereira-Leal JB & Seabra MC. *J Mol Biol*, ***313**, 889, 2001*
2. Pfeffer S & Aivazian D. *Nat Rev Mol Cell Biol* **5**, 886, 2004
3. Stenmark H & Olkkonen VM**.** *Genome Biol **2,** Reviews 3007.1, 2001*
4. Schwartz SL et al. *J. Cell Sci**. 120**, 3905, 2008*
5. Bucci C et al. *FEBS Lett. **366**, 65, 1995*
6. Pal A et al. *Biochem J. **374,** 443, 2003*
7. Saito-Nakano Y et al. *Exp Parasitol. **110**, 244, 2005*
8. Zerial M & McBride H. *Nat Rev Mol Cell Biol, **2**, 107, 2001*
9. Wyroba E et al. *Eur J Histochem, **51**, 163, 2007*
10. Nahm MY et al. *Plant Cell Physiol*, ***44**, 1341, 2003*
11. Lycett G. *J Exp Bot. **59**, 406, 2008*
12. Hong MC et al. *Comp Biochem Physiol B Biochem Mol Biol. **152**, 249, 2009*

New vistas in plant bioreactor technology development* Tuesday – 15:15

Andrzej K. Kononowicz[1], Piotr Łuchniak[1], Katarzyna Hnatuszko-Konka[1], Aneta Gerszberg[1], Tomasz Kowalczyk[1], Aneta Wiktorek-Smagur[2]

[1]Department of Genetics and Plant Molecular Biology and Biotechnology, University of Lodz, S. Banacha 12/16, 90-237 Lodz, Poland
[2] Nofer Institute of Occupational Medicine, Teresy 8, Lodz, Poland

Author's e-mail: akononow@biol.uni.lodz.pl

Progress made in the past decades in molecular biology and genetics as well as biochemistry and genetic engineering resulted in the development of technologies which enable the construction of plants characterized by novel, desirable traits. With the advent of biotechnology and gene transfer technology, a broad and extensive research brought about different expression systems for the production of recombinant proteins of industrial and pharmaceutical value. Since mid-1980, plants have been widely considered as important expression systems of numerous recombinant proteins as they undergo acetylating, phosphorylation, and glycosylation, as well as other post-translational modifications required for the biological activity of many eukaryotic proteins. Today, the plant

expression system is recognized as a new platform useful for the production of a large amount of proteins that can be either accumulated in plant tissues or, after rhizosecretion, isolated from root exudates of hydroponic cultures, and have been called plant bioreactors. However, it has to be emphasized, that engineering a plant to be a bioreactor for recombinant protein production may result in substantial remodeling of plant cell and/or tissue structural and functional organization. Cells, tissues, and/or entire plants have to cope with effects of overexpression and either accumulation or secretion of heterologous proteins. Although today the large-scale production of recombinant proteins in plants is limited by relatively low yields and difficulties in extraction and purification, the high potential of the plant system cannot be neglected, since it can be used for the production of a broad range of proteins – from small, monomeric proteins of prokaryotic origin and vaccine to very complex, multimeric antibodies. These issues as well as the recent developments and perspectives of the future application of the production of recombinant proteins utilizing plant bioreactors will be discussed.

* *We dedicate this work to Professor Maria J. Olszewska, the Great Scientist and Academic.*

This research was supported by Polish Ministry of Science and Higher Education (2 P04B 029 29) and University of Lodz (grant No 506/040996).

ABSTRACTS OF POSTER PRESENTATIONS SECTION I

The Authors are kindly requested to be at their posters

1

Flow cytometric estimation of nuclear DNA content in selected species of trees and shrubs belonging to the families Ulmaceae, Fabaceae and Rosaceae

A. Sadowska, I. Jędrzejczyk

Department of Genetics and Plant Breeding, University of Technology and Life Sciences, Kaliskiego 7, 85-789 Bydgoszcz, Poland

Author's e-mail: jedrzej@utp.edu.pl

Genome size affects certain phenotypic features of plants such as the duration of the cell cycle and cell division, the size of nuclei, tissues and organs, the length of the life cycle and seeds mass. Flow cytometry was used to estimate the genome size in leaves and seeds of 10 species of trees and shrubs belonging to the families Ulmaceae, Fabaceae and Rosaceae. In addition, seed mass and size was established. Plant material was collected from Botanical Garden of Kazimierz Wielki University in Bydgoszcz. For all species, before genome size estimation, leaves and seeds were tested for the presence of staining inhibitors. Nuclear DNA content was measured using CyFlow SL flow cytometer. Plant material were chopped simultaneously with the internal standard (*Petunia hybrida*; 2.85 pg/-2C or *Zea mays*; 5.43 pg/-2C) in nuclei isolation buffer supplemented with propidium iodide and ribonuclease A. For species containing polyphenols in the leaf cytosol an antioxidant PVP-10 was added. Obtained results showed that studied species of trees and shrubs had very small and small genomes; the content of 2C DNA ranged from 1.15 pg/2C in *Prunus padus* to 3.61 pg/2C in *Amorpha fruticosa*. For nine species out of ten no statistically significant differences between flow cytometrically estimated amount of DNA in the nuclei of leaves and of seeds were observed. This confirms that seeds are a suitable material for the estimation of genome size. There was no correlation between the 2C DNA content and the seed mass.

2

Connection between the formation of protein bodies and the level of nuclear DNA endoreduplication during *Pisum sativum* L. seeds development

K. Marciniak, A. Chmielnicka

Department of Plant Cytology and Cytochemistry, University of Lodz, Banacha 12/16, PL-90-237 Lodz, Poland;

Author's e-mail: kazmar@biol.uni.lodz.pl

Endoreduplication (DNA replication cycles without mitosis, endocycles) can accompany cell differentiation in many plant species. There are at least some interpretations concerning significance of this phenomenon. It is interesting that cells which transduce or store nutrient materials achieve relatively high endoreduplication levels. It is known that seeds of *Fabaceae* accumulate high levels of storage proteins in protein bodies.

The aim of this study was to determine the relation between the formation of protein bodies and the level of nuclear DNA endoreduplication during successive stages of *Pisum sativum* L. (cv. Paloma) seed development. Feulgen cytophotometric method was used to measure nuclear DNA content. The number and size of protein bodies were estimated in Feulgen-stained preparations in which proteins were stained with 0.02% Coomassie Brillant Blue R 250. Measurements were performed in the suspensor, endosperm and embryo.

In *P. sativum* suspensor is a big structure which consists of four multinuclear cells. It develops and grows intensively pushing an embryo properly into the endosperm. During seed development, endoreduplication process first starts in this structure. Dynamics of this process is higher in a spherical part of the suspensor where nuclei reach 256C DNA level. Accumulation of protein bodies was not observed in the suspensor. This structure degenerates in seeds, in which the size of embryos is about 2.5 mm. In *P. sativum* endosperm is of nuclear type. At first triploidal nuclei divide synchronously. Together with endosperm growth (embryo 0.5 mm in diameter) synchrony of divisions is replaced by mitotic waves. On the chalazal pole endoreduplication processes predominate. Nuclear DNA reaches 192C level in the endosperm surrounding embryos bigger than 2 mm in length. During endosperm development protein bodies appear early when the embryo is at the globular stage of development. Small amounts of storage proteins are present throughout endosperm development. They disappear during endosperm tissue hydrolysis. In the embryo significant accumulation of protein bodies appeared mainly in cotyledons while in the embryo axis only small protein bodies were observed. Accumulation of these bodies started at cotyledon stage when the embryo was 4 mm in size. It was preceded by nuclear DNA endoreduplication. First protein bodies appeared in the cells whose nuclei reached 8-16C DNA levels. No correlation was observed between the ploidy of cells and the diameter of protein bodies. The analysis of storage parenchyma cells from basal, apical, abaxial and adaxial zones from the cotyledons 4 mm in diameter showed that first protein bodies appeared in the basal zone. It was evidenced by the fact that nearly 50% of protein bodies in this zone were 1.25 μm in diameter while in the adaxial and central zones about 50% of them were 0.625 in diameter. Subsequently nuclear bodies appeared in the adaxial zone and then in the central one. In the apical and abaxial cotyledon zones protein bodies appeared in the embryos 6 mm in size while in the root and shoot meristems when they reached 7 mm. Diameters of protein bodies in the cotyledons 4 and 5 mm in size ranged between 0.625 μm and 3.75 μm while in the mature cotyledons in the central zone reached about 20 μm in diameter.

The performed investigation indicates that formation of protein bodies in the storage tissue of developing *P. sativum* seeds is connected with multiplication of nuclear DNA content. It is interesting that the proteins originally accumulated in endosperm, after hydrolyzation of this tissue, are probably used for accumulation of stored proteins in cotyledons.

3

Cytological analysis of BC_1 generation of wheat hybrids with genes of *T. monococcum* and *T. tauschii*

M. Majewska, W. Sodkiewicz, T. Sodkiewicz

Institute of Plant Genetics Polish Academy of Science, Strzeszynska 34, 60-479 Poznan, Poland

Author's e-mail: mmaj@igr.poznan.pl

The genetic barriers limited highly the possibilities of direct genes introgression from diploid into hexaploid wheat. Most often the difficulties were overcome on the way of bridge-crosses between diploid and tetraploid wheat, and further crosses of the obtained triploid hybrids with hexaploid wheat. The aim of the study was: (*1*) to obtain intergenera and interspecies wheat hybrids with introduced A^m-genome of *T. monococcum* (2n = 2x = 14) and D-genome of *T. tauschii* (2n = 2x = 14) and (*2*) to investigate the formation of unreduced female gametes in F_1 hybrids.

The plant material included F_1 hybrids (2n = 4x) of *T. tauschii* (acc. D1, D2, D51, D98) × *T. aestivum* (cv. Igna, Omega, Tercja) and F_1 hybrids (2n = 4x) of *T. aestivum* (cv. Igna, Tercja) × *T. monoccoccum* var. *nigricultum* and *T. sinskajae*. These allotetraploid hybrids were used to develop BC_1 generation in the process of backcrossing with parental hexaploid wheat.

Cytological analysis performed on BC_1 plants showed that two dominant types of gametes existed – completely unreduced gametes and partially reduced gametes. Hybrids of *T. tauschii* × *T. aestivum* were the source of the highest number of functional female gametes. The frequency of gamete production in that cross combination was on the level of 1.2% and was mainly influenced by the hexaploid genotype. BC_1 *T. tauschii* × *T. aestivum* plants gave female gametes with different chromosome numbers. The range was between 35 – 49 chromosomes. Distribution of somatic chromosome numbers in BC_1 progeny showed that the most numerous type of gametes was a group of partially reduced gametes with 20-, 21- or 22-chromosomes (28.3%). The frequency of gamete production in *T. aestivum* × *T. monoccoccum* hybrids was shown to be very low and resulted in 0.3%. In case of F_1 *T. aestivum* × *T. monoccoccum* var. *nigricultum* and *T. sinskajae* hybrids completely unreduced gametes with 28 chromosomes were produced. The main part of that type of gametes derived from the hybrids with *T. sinskajae*.

4

Molecular cytogenetics study of genome organization in the *Brachypodium* genus

Dominika Idziak[1], Elżbieta Wolny[1], Glyn Jenkins[2], and Robert Hasterok[1]

[1]*Department of Plant Anatomy and Cytology, Faculty of Biology and Environmental Protection, University of Silesia, Jagiellonska 28, 40-032 Katowice, Poland*
[2] *Institute of Biological, Environmental and Rural Sciences, Edward Llwyd Building, Aberystwyth University, Penglais, Aberystwyth, Ceredigion SY23 3DA, Wales, UK*

Author's e-mail: didziak@us.edu.pl

The genus *Brachypodium* comprises a few temperate grass species of wide geographical distribution and unclear phylogenetic relations. A member of this genus, annual weed *Brachypodium distachyon* has been proposed as a model system for grass crop genomics research. The features that promoted *B. distachyon* as a species of choice for comparative genomic studies are: small genome size (~350 Mb), low repetitive DNA content and close phylogenetic relationship with agronomically important cereals and forage grasses. Genetic and molecular tools for *B. distachyon* have been recently developed. The currently available resources include a set of diploid inbred lines, ESTs as well as BAC libraries and protocols for rapid and efficient transformation and mutagenesis. Draft genome sequence of a model line Bd21 has been already announced by the US Department of Energy Joint Genome Institute (www.brachypodium.org) and more annotated sequence is expected to be released soon.

An important part of developing *B. distachyon* as a model grass species is molecular cytogenetic analysis of its genomes as well as the genomes of its relatives. In this study we present the results of landing of large supercontigs from *B. distachyon* BAC libraries onto its chromosomes. This is a part of an international programme that aims to integrate physical and genetic maps for *B. distachyon*. We also present the recent progress on comparative cytological analyses of other members of the genus. Additionally we demonstrate initial results of our study on nucleolar dominance, the epigenetic phenomenon that occurs in some of the *Brachypodium* allotetraploids.

The authors acknowledge financial support from the Polish Ministry of Science and Higher Education (grant 2 PO4C 012 30). D.I. was the beneficent of a British Council funded YSP fellowship in 2008 and is the recipient of a scholarship from the UPGOW project co-financed by the European Social Fund. Thanks are due to Prof. Mike Bevan (JIC, Norwich, UK) for providing BAC clones used in supercontig mapping.

5

Histone demethylation

Joanna Wawszczyk, Jan Pałyga

Departament of Biochemistry and Genetics, Jan Kochanowski University, Kielce, Poland

Author`s e-mail: joannawawszczyk@wp.pl

Nucleosomes, the basic units of chromatin, are composed of 147 bp of DNA wrapped around a histone octamer that consists of two copies of H2A, H2B, H3 and H4. The N-terminal histone tails, especially those for histone H3 and H4, are targets to multiple posttranslational modifications such as phosphorylation, acetylation and methylation. These covalent modifications, most of which is potentially reversible, play fundamental roles in the regulation of chromatin structure and function. Unlike histone acetylation and phosphorylation, the histone methylation has been previously considered to be enzymatically irreversible. This notion was however, challenged by detection of histone demethylases, confirming a hypothesis about a reversibility of the histone methylation process. A discovery of the first histone lysine demethylase, LSD1 (lysine specific demethylase 1) five years ago was very soon followed by the detection of several enzymes specific for histones methylated at particular sites. The exploration of these demethylases advanced our understanding of the nature of the methylation/demethylation itself as well as the recruited protein complexes that regulate the chromatin structure and function. Histone demethylases play an important role in many biological processes that employ epigenetic regulation including X-chromosome inactivation, imprinting, cell cycle regulation as well as development of some human diseases.

6

Gradients of spatio-temporal chromatin states during aberrant cell division cycles in Allium

A. Żabka, J. Maszewski

Department of Cytophysiology, Institute of Physiology, Cytology and Cytogenetics, University of Lodz, Lodz, Poland

Author's e-mail: zabkka@poczta.onet.pl

Plant cells, when exposed to drugs that affect their advance throughout S phase or mitosis, either completely stop cell cycle progression and ultimately die, or increase their ploidies via multinucleate or endopolyploid states. We present evidence that prolonged incubations of *Allium cepa* root meristems with propyzamide (an antimitotic herbicide), or alternate treatments with hydroxyurea and caffeine give rise to extremely large and highly elongated polykaryotic cells with atypical images of mitotic divisions. The various types of abnormalities include both internuclear and interchromosomal asynchrony manifested by an uneven condensation of chromosomes or a series of successive mitotic stages. Asynchronous nuclear divisions and the sequences of structural changes observed among different groups of chromosomes may be thought of as the result of an uneven distribution of cell cycle regulators across the extremely large cells which had been formed throughout an extended period of experimental treatment. Another type of asynchrony that cannot be accounted for by an increased length of cells was observed following long-term incubation of roots with hydroxyurea. This kind of treatment revealed spatio-temporal gradients of chromatin states characteristic for various periods of S-phase (evidenced using immunocytochemical localization of BrdUrd), together with with an uncommon form of mitotic abnormality, manifested

in a gradual condensation of chromatin (spanning from interphase to prometaphase). Cells showing intrachromosomal asynchrony implicated both continuing changes in nucleosomal, nucleoskeletal and higher-order chromatin folding and the escape from a number of conserved cell cycle control mechanisms. Immunocytochemical study of polykaryotic and HU-treated cells using anti-β tubulin antibodies revealed severe perturbations in the microtubular organization of preprophase bands.

This work was supported by the grant 505/376 from the University of Lodz.

7

The dependence of premature chromosome condensation (PCC) on transcription in root meristem cells of *Vicia faba*.

A. Tomaszewska, J. Maszewski

Department of Cytophysiology, Institute of Physiology, Cytology and Cytogenetics, University of Lodz, Pilarskiego 14, 90-237 Lodz, Poland

Author's e-mail: agatomaszew@gmail.com

Transformation of interphase chromatin into condensed mitotic chromosomes is stringently regulated at the molecular level by a vast number of interrelated biochemical pathways termed to as cell cycle checkpoints. An overall role of mechanisms governed by these checkpoints is to detect abnormally structured or damaged DNA, to arrange spatio-temporal progression of the key cell-cycle events and to coordinate them with DNA repair processes. As a rule, cell-cycle checkpoints slow down or arrest progression of interphase and the onset of mitotic condensation of chromatin, thereby allowing time for appropriate repair mechanisms to correct genetic lesions before replicated DNA molecules are passed on to the next generation of daughter cells. The extreme complexity of signaling networks triggered in response to damaging factors makes the cell cycle checkpoints susceptible to various insults, which - in turn - may cause the shift of the cell towards aberrant mitotic division (PCC; premature chromosome condensation) irrespective of incomplete replication or/and DNA lesions. Similar effects can be observed during cell fusion experiments, after ectopic overexpression of CDK-cyclin B complexes, or following administration of specific drugs. The main event that brings about chemically induced PCC relies on putting a barrier on checkpoint functions by disrupting one or few of its elements needed to successfully transmit an inhibitory signal and to prevent the cells from entering mitosis. Hence, induction of an unscheduled chromosome condensation and a consequent nuclear division may proceed following application of inhibitors, which affect: (1) sensor protein kinases ATM/ATR (activated by DNA lesions or stalled replication forks and inhibited by caffeine), (2) effector protein kinases (Chk1/Chk2), or protein phosphatases PP1 and PP2A.

The main goal of our study was to determine whether induction of PCC in root meristem cells of *V. faba* depends on the *de novo* transcription of nuclear DNA. To answer this question, 4-d seedlings were incubated in 2.5 mM hydroxyurea (HU; a competitive inhibitor of ribonucleotide reductase, which depletes the intracellular dNTP pools and prevents cells from progressing through S-phase of the cell cycle), and an inhibitor of RNA polymerase, actinomycin D (Dactinomycin, ActD) was used during caffeine-mediated abrogation of the HU-activated intra-S-phase checkpoint. Comparisons of PCC indices (S-PCC and G2-PCC) and quantitative analyses of micronuclei revealed considerable decrease of prematurely condensed chromosomes in ActD-treated plants.

This work was supported by the grant 506/820 from the University of Lodz.

8

Double DNA breaks are necessary for proper condensed chromatin rearrangement and exchange of nuclear proteins in *Chara vulgaris* spermatids.

A. Wojtczak , K. Popłońska, M. Kwiatkowska

Department of Cytophysiology, University of Lodz, Pilarskiego 14, 90-231 Lodz, Poland

Author's e-mail: wojag@biol.uni.lodz.pl

In animal spermiogenesis numerous biochemical and structural modifications of chromatin take place due to intensive transcription going on in spermatids during their early differentiation. However, the most important process of spermiogenesis is chromatin remodeling during which its nucleosomal structure, characteristic of somatic cells becomes fibrillar resulting in silencing of the genome of mature spermatozoids. This process leads to extreme chromatin condensation and is the effect of the exchange of the histones into protamines or other strongly basic proteins.

Transient DNA breaks are necessery for proper rearrangement of spermatid chromatin to occur since a significant number of nucleosomal DNA supercoils is eliminated from mature spermatids. One of the earliest reactions of eukaryotic cells to DNA double-strend breaks in the chromosomal DNA is phosphorylation of the H2A histones, which is a very conservative process and was observed in different organisms (e.g. in yeast - γ-H2A, in human - γ-H2AX). Phosphorylation of H2AX histone results not only from DNA damage (caused by ionizing radiation, UV or chemical substances e.g. hydroxyurea) but also regularly takes place during spermiogenesis enabling correct chromatin remodeling.

The ultrastructural, cytochemical and immunofluorescence studies of *Chara* spermatid showed many similarities to spermiogenesis in mammals. Due to the fact that consecutive stages of the transformation of the nuclear structure accompanying nuclear protein exchange in *Chara* are known, we decided it was the suitable model for the research on double DNA breaks. This kind of research has not been conducted before during spermiogenesis in plants.

Immunocytochemical analyses with the use of antibodies against H2AX histone phosphorylated at serine 139 revealed endogenous double DNA breaks in *Chara vulgaris* spermatids in mid-spermiogenesis (stages V, VI, VII), when protamine-type proteins appeared in a nucleus, when the amount of histones decreased and that of protamine-type proteins increased. DNA breaks were not observed in early (stages I-IV) and late spermiogenesis stages (VIII-X), after the exchange of histones into protamine-type proteins finished. Similar fenomenon exists in animals. A hypothesis has been put forward that DNA breaks at stage V, when condensed chromatin adhere to nuclear envelope and then transforms into net-like structure at stage VI, may enable the chromosome repositioning to proper territories in mature spermatozoid. However, at stages VI and VII DNA breaks are necessary for the transformation of the nucleosomal structure into fibrillar one and finally into extremely condensed status of sleeping genes at stage X.

We also conduct immunocytochemical and ultrastructural studies to check what is the influence of topoisomerase II inhibitors on chromatin remodeling during spermiogenesis.

Our present and earlier results prove great similarity of the mechanisms regulating spermiogenesis in animals and in *Chara*. Thus we believe that *Chara* may be treated as a model object and our results may inspire the scientists interested in animal and human spermiogenesis.

This work was supported by the grant 505/390 from the University of Lodz.

9

Hybrids of *Salix viminalis* x *Populus* in morphological, cytoembryological and molecular investigations.

M. Zenkteler, M. K. Wojciechowicz, A. Bagniewska-Zadworna, E. Zenkteler

Department of General Botany, Institute of Experimental Biology, A. Mickiewicz University, 61-614 Poznan, Umultowska 89, Poland

Author's email: maczen@amu.edu.pl

Intergeneric hybrids of *Salix viminalis* L. with six *Populus* species: *P. alba* L., *P. simonii* Carr., *P. tremula* L. *P. tomentosa, P. trichocarpa* Torr et Gray and *P. violascens* Dode. have been obtained by using the *in vitro* stigmas pollination followed by embryo rescue technique. Identification of hybrid origin of plants was confirmed by morphological, embryological and molecular analysis.

Cytological observations under Fluorescent Microscope (FM) with aniline blue staining showed that pollen grains of selected *Populus* species started to germinate on stigmas, 24 h after pollination. During the next 12 h pollen tubes penetrated the style. Percentage of poplar pollen germination on willow stigmas was 38.9±2%. The entrance of pollen tubes inside the ovule micropyles occurred between 36-48 h after pollination. On microscopic slides the globular embryos were visible at 5-8 days after cross pollination. The endosperm was poor or any endosperm nuclei could be detected. In Transmission Electron Microscope (TEM) the hybrid embryos with cytoplasm-rich cells and embryos with shrinking protoplasts were observed. The protoplast shrinkage of the cells could indicate apoptic-like death of intergeneric hybrid embryos. The embryos which continued the development up to the cotyledonary stage were isolated from the ovules (18 – 20 days after pollination) and placed on modified ½ MS medium supplemented with 3% sucrose. Plantlets after acclimatization in pots were transferred to the experimental field in the Botanical Garden. Finally, in the field 3 plants of the cross of *S. viminalis* x *P. alba*; 52 plants of the cross *S. viminalis* x *P. tremula*, 6 plants of the cross *S. viminalis* x *P. violascens*, 4 plants of the crosses *S. viminalis* x *P. simonii* and *S. viminalis* x *P. trichocarpa* are growing.

Selected two years old plants were verified as hybrids by morphological characters and molecular analysis. Scanning Electron Microscope (SEM) observations of leaf abaxial side showed many differences between willow and poplars trichome arrangement and density. In the hybrid plants of *S. viminalis* x *P. alba* trichomes had intermediate characters and were more dense than on the willow leaf surface. Moreover leaf blades of *S. viminalis* and *P. alba* were wider and their edge was not as sharp as in the female parent leaves. In the cross of *S. viminalis* x *P. violascens* and *P. tremula* morphological differences were not noticed.

Molecular analysis for hybrid confirmation was based on RAPD primers. Fifty RAPD primers were used for screening parental plants and hybrids and the bands appearance and intensity were analyzed.

The investigations were supported by Grant R12 06 103.

10

Au SINEs elements in *Fabaceae* genomes

Marek Gadzalski, Tomasz Sakowicz

Department of General Genetics, Plant Molecular Biology and Biotechnology, University of Lodz, Lodz, Poland

Author's e-mail: mgadzal@biol.uni.lodz.pl

SINEs (short interspersed elements) are present in vast majority of eukaryotic genomes. They were found in plants, animals, fungi and other eukaryotes. SINEs can be the major components of the genomes (e.g. they constitute more than 13% of the genome of humans). These nonautonomous retroposons are 80-500 bp long, they do not encode any proteins and require the enzymatic machinery of an autonomous LINE partner for retroposition. Most of SINEs elements derive from tRNA genes and are transcribed by RNA polymerase III. They consist of three parts: 5' tRNA-related, central tRNA-unrelated and 3' polyA or adenine-rich tail. It was earlier considered to be "junk DNA" but recent studies indicate that SINEs have a significant role in gene evolution and affect gene structure and expression level. The distribution of these elements has been implicated in some genetic diseases and cancers. SINEs are successfully utilized as phylogenetic markers. Au SINE is a short interspersed element first identified in *Aegilops umbellulata*, a close relative of wheat. The Au elements were identified also in many *Gramineae, Solanaceae*, and *Fabaceae* species (however, could not be identified in *O. sativa* and *A. thaliana* genomes). Phylogenetic studies suggest that Au SINE originated before the divergence of monocots and eudicots. We searched several species of *Fabaceae* (*M. truncatula, L. japonicus* and *G.max*) for the presence of Au elements by database search. Au SINEs is the most widespread plant family of this retroposons, therefore they are very valuable genetic markers.

Fabaceae is widely distributed family of plants. Many species within this family are cultivated, making *Fabaceae* one of most important plant family in agriculture. Furthermore several species are well presented in genomic databases what allows to make thorough analyses.

We performed BLAST searches with Au SINEs sequences on NCBI website in order to survey *Fabceae* family species. Hits under given threshold values were manually verified by aligning with known Au SINEs elements. Our goal was to find relationship of Au SINEs within species and then within whole *Fabaceae* family.

This work was supported by University of Lodz grant No 506/040996

11

Identification of SSR markers in F_2 generation and in parental forms of rye

A. Wolska-Sobczak, H. Kubicka, A. Pyza

Botanical Garden – Center for Biological Diversity Conservation of the Polish Academy of Sciences, Prawdziwka 2, 02-973 Warsaw, Poland.

Author's e-mail: anetawolska27@o2.pl

The progress in the genetic breeding research on rye, depends, to a large extent, on the access to clean genetic initial materials of the required phenotypes, for example inbred lines. The next step

bases on the new, efficient and cheaper methods enabling the analysis of the genotype. Amongst the most frequently used methods are molecular techniques, which enable significantly shorten the breeding cycle, revealing structures of different alleles at the DNA sequence level. Microsatellites are very convenient nuclear markers (SSR, ISSR) for detection of the genetic variety of individual plants in the population.

The aim of this work was establishing the way of inheritance of white, vertical stripes of the same width on leaves and stems in the inbred lines of rye (chph), chosen from a unique collection of the Botanical Garden as well as the assessment of genetic variety on the basis of the SSR technique. Anatomical and physiological methods were used for detection and analysis of quantitative and qualitative changes in the chloroplast structure. There were found: a reduction of chloroplast number in mesophyll cells (50-90%), a reduction of the number of grana (50%) and thylakoids in granum (75 %) as well as the reduction of chlorophyll content and the ability of CO_2 binding of plants of this line in comparison with control plant material - a uniform, dark green color leaf.

On the basis of the segregation in F_1 and F_2 generations, it was established that the features of vertical stripes of the same width (chph), running from the base of the leaf to the top is determined by a single, recessive gene. Single-gene inheritance of this feature was confirmed in the back-crosses, where the segregation of green leaves of individual plants was close to the predicted 1:1 ratio. The mode of inheritance of the chlorophyll change of traits in leaves was checked using X2 test.

At the next stage of studies, DNA was isolated from plant material (parental forms and 120 plants of the F_2 generation). After establishing the genetic purity of the line, the polymorphism between parental forms (inbred line of vertical stripes and dark green leaves) was analyzed by SSR technique utilizing primers used earlier in research on rye. One hundred wtentyeight airs of primers (117 derived from the work of Hackauff and Wehling (2002) and 11 from the work of Saal and Wricke (1999), amongst which 5 pairs of them were obtained, giving polymorphic product amplification. They were used in further analyses on offspring of the F_2 generation to locate SSR linkage markers with traits of white vertical stripes and identical width.

12

Assigning linkage groups to *Lupinus angustifolius* chromosomes

Karolina Leśniewska, Barbara Naganowska

Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan

Author's e-mail: kles@igr.poznan.pl

Lupinus angustifolius (narrow-leafed lupin), a legume species with valuable agricultural traits, has been the subject of detailed genome analysis. The present work is focused on assigning linkage groups to appropriate chromosomes. Clones from a bacterial artificial chromosome (BAC) library of the *L. angustifolius* genome have been used as probes in fluorescence *in situ* hybridization (BAC-FISH) for physical mapping in mitotic metaphase chromosomes. BAC clones were selected by hybridization of the library with probes designed on the basis of sequences of MFLP genetic markers and of one of the nodulation genes. Some of them were randomly chosen. BAC clones were isolated from single *E. coli* colonies by a miniprep method and digested with *Not*I restriction enzyme. The quality and size of inserts were verified by pulse gel electrophoresis (PFGE). BAC DNA was labeled by nick translation (with digoxygenin-11-dUTP or tetramethyl-rhodamine-5-dUTP) and used for BAC-FISH reaction.

As a result of cytogenetic mapping, many BACs gave signals dispersed throughout numerous sites in chromosomes; this was probably caused by the presence of repetitive sequences within the

clones. From BACs, that generated unique, single-locus FISH signals, a range of cytogenetic markers for particular chromosomes were developed. The ends of these BAC clones were sequenced and used as a source of information for generating genetic markers. The Australian mapping population of 89 F_8 individuals was used for marker testing. Most of the analyzed markers were monomorphic. However five polymorphic STS markers (three present/absent types and two of the SNP type) were also obtained and could be localized within linkage groups of the *L. angustifolius* genetic map previously published by Australian researchers.

BAC-FISH mapping generated 12 chromosome-specific cytogenetic markers. Five markers based on BAC-ends sequences, mapped on the genetic map, allowed us to assign linkage groups to individual chromosomes. This work provides the foundation for integrating the genetic and physical maps of the narrow-leafed lupin.

13

Sequence identification, characterization and chromosomal localization of genes encoding chalcone isomerase in *Lupinus angustifolius.*

Ł. Przysiecka[1], C.J. Mądrzak[2], B. Wolko[1]

[1] *Laboratory of Structural Genomics, Institute of Plant Genetics Polish Academy of Sciences, Poznan, Poland*
[2] *Department of Biochemistry and Biotechnology, Poznan University of Life Sciences, Poland*

Author's e-mail: lprz@igr.poznan.pl

Chalcone isomerase (CHI) is one of the main enzymes in the phenylopropanoid biosynthesis pathway which catalyzes the cyclization of chalcone into (2S)-naringenin. Based on the EST sequence of chalcone isomerase from *Lupinus albus* specific primers generating product for *Lupinus angustifolius* were designed as probe for hybridization with the BAC library of the *L. angustifolius* nuclear genome. Suitable clones were selected and verified by PCR amplification using BAC insert DNA as a target and sequencing of the amplified fragment. The size of DNA inserts was determined by the PFGE. The total number of 10 selected BAC clones was end-sequenced and digested using restriction enzymes *Eco*130I and *Hind* III. On the basis of BAC fingerprints two contigs were constructed. One of them consists of six and the other of two BAC clones. Two remaining clones occurred as singletons.

Sequencing of two selected BAC clones belonging to two different contigs has been started using the chromosome walking method. It enabled to establish the nucleotide sequence of the whole chalcone isomerase genes present in both clones. Sequence analysis has shown that they are made up of 4 exons but their sequences differ from each other significantly.

The BAC-FISH method was performed in order to localize and identify the number of CHI gene loci on mitotic chromosomes of *L. angustifolius*. Eight clones belonging for both contigs were used as molecular probes. Different combinations of two clones as probes in the same FISH reaction were tested. The BAC clones which came from the same contig gave a single-locus signal on one pair of homologous chromosomes, but these from different contigs showed two distinct signals localized on two different pairs of chromosomes.

The further research will be directed on determination of the chalcone isomerase expression level in different organs of lupin plant using real-time RT-PCR.

14

Reverse transcriptase domain sequences from *Chenopodium quinoa* LTR retrotransposons

B. Kolano, E. Bednara, J. Małuszyńska

Department of Plant Anatomy and Cytology, University of Silesia, Poland

Author's e-mail: bozena.kolano@us.edu.pl

Chenopodium quinoa is a grain originated from Andean region of South America. It is a tetraploids species (2n=4x=36) with small genome (2.92 ±0.06 pg/2C). Because of its potential as an alternative crop and its nutritive value, in recent years interest has grown in understanding the genetics of quinoa. Retrotransposon and their remnants represent a major fraction of repetitive DNA in plant species and it is generally accepted that they play important role in plant genome evolution. Knowing the sequence and organization of retrotransposons seems to be necessary for understanding *C. quinoa* genome structure.

The conserved domains of reverse transcriptase (RT) genes of *Ty1-copy* and *Ty3-gypsy* groups of long terminal repeat (LTR) retrotransposones were amplified from *C. quinoa* genome using degenerated primers, cloned and sequenced. Among these 50% and 65% of respective clones of *Ty1-copia* and *Ty3-gypsy* RT sequences possessed stop codons or frame-shifts or both. The RT sequences corresponding to both the groups exhibit significant level of heterogeneity. Dot blot hybridization indicated that only one clone in case of *Ty3-gypsy* and two clones of *Ty1-copia* elements are present in *C. quinoa* genome in abundant number of copy. Chromosomal localization of the elements was indicated using fluorescent *in situ* hybridization.

15

Mass spectrometry analysis for the ATM, ATR, Chk2 and Chk1 protein kinases in root meristem cells of *Vicia faba*

D. Rybaczek[1], J. Dębski[2], A. Tomaszewska[1]

[1] *Department of Cytophysiology, University of Lodz, Lodz, Poland*
[2] *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland*

Author's e-mail: doryb@biol.uni.lodz.pl

Ataxia telangiectasia mutated kinase (ATM) and ataxia telangiectasia and Rad3-related kinase (ATR), belong to the phosphoinositide 3-kinase-related protein kinase (PIKK) family. They are key components of the intra-S-phase checkpoint pathway. ATM and ATR are also involved in initiation and progression of S phase in unstressed cells. ATM is a key player in response to radiation-induced DNA double strand breaks. Activated ATM activates Chk2 protein kinase (an SQ/TQ cluster domain) by its phosphorylation. Chk2 is phosphorylated at Thr68 and lead to Chk2 oligomerization and autophosphorylation. ATR responses primarily to UV damage and stalled replication forks and – in response – phosphorylate Chk1 protein kinase. Chk1 and Chk2 function at the G1-S, S-G2 and G2-M cell cycle transitions to phosphorylate and thereby inactivate Cdc25C and Cdc25A phosphatases, respectively. This allows cell cycle arrest and allows time for DNA repair processes. When Chk1/Chk2 are inhibited, Cdc25 phosphatase remains unphosphorylated,

mitosis is promoted even if the DNA damage is not repaired and – as consequence – chromosomal aberrations and apoptosis can occur.

Here we report on protein kinase identification by mass spectrometric analysis. After 2-D PAGE, the protein spots were excised, subjected to distaining, reduction and alkylation, prior to digestion with trypsin. In case of solution digests, protein mixture is directly applied to reduction, alkylation and digestion. Peptide mixture is resolved on HPLC system coupled to mass spectrometer, where two distinct kinds of measurements are performed: MS, where the peptide mass is obtained, and MS/MS where peptide is fragmented through collision with neutral gas (argon, helium). Comparative analysis of these fragments with the database allows for protein identification.

Here, we report the use of proteomic approach to identify protein kinase activity of the *Vicia faba* checkpoint kinases: ATM, ATR, Chk2, and Chk1 by analyzing total kinases and they phosphorylation sites. Here we examine: (1) autophosphorylation of ATM at Ser1981; (2) phosphorylated form of ATR (Ser428); (3) total form of Chk2 protein kinase; (4) Chk2 phosphorylated on Thr68; (5) total form of Chk1 protein kinase; and (6) Chk1 kinase phosphorylated at serine residues (Ser317 and Ser345). Examination of the dependency of each phosphorylation on ATM, ATR and Chk2 or Chk1, combined with immunocyto-chemical and biochemical analysis (immunoprecipitation and Western blots), revealed that these phosphorylations play a role in the regulation of intra-S-phase checkpoint pathways in *Vicia faba* root meristem cells (suggesting as well that a wide range of those cellular processes tightly connected with DNA replication in *Vicia faba* is likely regulated by these kinases).

Future studies, including DELFIA system, will hopefully provide further insight into the kinetics of action of these protein kinases.

This work was supported by grants: 2P04C 044 27 (N N303 355935) from Polish Ministry of Science and Higher Education and 505/392 from University of Lodz.

16

Functional genomic analysis of the proteins involved in interactions between the components of the cell wall, plasma membrane, and cytoskeleton in *Arabidopsis thaliana*

M. Maruniewicz, P. Wojtaszek

Department of Molecular and Cellular Biology, Adam Mickiewicz University, Poznan

Author's e-mail: michasia@amu.edu.pl

Structural and functional continuum between cell wall, plasma membrane and cytoskeleton (WMC) is indispensable for proper functioning of plant cells. It is involved in many developmental processes, like maintenance of the cell shape, and also in plant cell reactions to external and internal stimuli. At the present state of knowledge, it is assumed that proteins, and polysaccharides, fulfill the role of elements connecting various parts of the WMC continuum. However, the true knowledge about such elements is rather scarce.

Based on bioinformatics analyses, four proteins have been selected as potential linkers anchoring cytoskeleton and plasma membrane in the walls. These were analyzed with respect to their structure, presence of a signal peptide, transmembrane helix(-ces; TMH) or in order to confirm the predicted domain architecture. Identification of homologous proteins from other sources was also done as they can indicate possible function(-s).

Two proteins are defined by the presence of EGF motifs, often found in extracellular proteins, suspected to be involved in intermolecular interactions. One of them contains two EGF motifs, and the exostosin domain, responsible for glycosyltransferase activity (GT). Sequence analysis showed

that this protein belongs to family 47 of glycosyltransferases. Arabidopsis contains 39 genes coding for such GTs but only a few of plant GTs has been characterized so far. All known GTs are thought to act in the lumen of Golgi, and thus it would be extremely interesting to find out if protein with such activity could also be located in the extracellular compartment. The second of those proteins contains an EGF motif, and the metalloproteinase domain. Homology search analysis revealed the highest similarity to gp63 (leishmanolysin) from *Trypanosoma cruzi* which acts as a zinc dependent protease. Detailed sequence analysis and homology modeling showed that it belongs to metazincin family of proteases, very similar to leishmanolysin, and is probably active. Its function is as yet unknown.

Two remaining proteins belong to the formin family (formin 6 and formin 8) and are defined by the presence of formin homology domain 2. In animal cells, they act as actin nucleation and elongation factors. Not much is known about their functioning in plants. Both selected formins belong to class I, defined by the additional TMH and a proline-rich motif which probably extends into extracellular space, anchoring the proteins in cell wall. Analysis of *A. thaliana* insertion mutants revealed a significant decrease in the root length compared to wild type plants. The difference was greater in the case of plants grown on basal medium compared to plants grown under salt and osmotic stress conditions. However, preliminary visualization of the actin cytoskeleton arrangement showed no significant differences between mutants and wild type plants.

This work is funded by a Polish Ministry of Science and Higher Education grant N N303 360735 to P.W.

17

Functional analysis of intracellular proteins interacting with membranes within the cell wall-plasma membrane-cytoskeleton continuum in plant cells

M. Wierzchowiecka, P. Wojtaszek

Department of Molecular and Cellular Biology, Adam Mickiewicz University, Poznan

Author's e-mail: m.wierzchowiecka@wp.pl

The continuum consisting of the cell wall, plasma membrane and cytoskeleton (WMC) is one of the main structures of plant cell, responsible for its proper organization and functioning. Very important, yet still poorly understood, element of the WMC continuum is its internal part. Here, the cytoskeleton, anchored in the walls, is responsible for many processes, among them endo- and exocytosis, organelle positioning, cytokinesis or membrane trafficking. For a long time the existence of endocytosis in plant cells was neglected as the membrane trafficking against turgor pressure was considered impossible. However, recent numerous reports indicated clearly the dynamics of endocytic activities in plant cells, although the full knowledge about this process in plants is still far from complete.

For this research, four *Arabidopsis thaliana* proteins (At4g32160, At3g15920, At1g15240, At2g15900) were selected, which are suspected to play a role in cytoskeleton-dependent membrane trafficking. To predict the most probable function of those proteins, several bioinformatics analyses were performed.

Two selected proteins, At4g32160 and At3g15920, contain two important domains: PX and SPEC/Coil-Coiled, and this suggests that they are able to simultaneously interact with membranes and with cytoskeleton. Judging from the data on animals and yeasts cells, we assume that they might be responsible for endocytic vesicle mobility driven by actin polymerization. PX motif is a domain implicated in diverse functions such as cell signaling, vesicular targeting, membrane protein trafficking, and lipid modification. PX binds phosphatidylinositols, like PI(3)P or PI(4,5)P2. It

seems that PX domains present in At4g32160 and At3g15920 have all the required amino acids to fulfill such function. At that moment we can only suppose that these domains have also the ability to bind P(3)P, which is typical for the endocytic vesicles membranes. On the other hand, two domains: SPEC in At4g32160 and coiled-coil region in At3g15920, are suspected to interact with the actin cytoskeleton. However, this might only be confirmed through functionality assays.

The modular structures of two other proteins: At1g15240 and At2g15900 include four domains: transmembrane domain, PXA, PX, and Nexin_C. The presence of Nexin_C domain suggests that these proteins belong to a family of sorting nexins, and are responsible for sorting of the endosomes. On the other hand, the existence of transmembrane domain and PX suggests that they might also be connected with or anchored to other membranes.

On the basis of bioinformatics analyses we plan to perform a series of experiments with the use of functional genomics approaches, to confirm and investigate further the functions of these four proteins.

This work is funded by a Polish Ministry of Science and Higher Education grant N N303 360735 to P.W.

18

Lipoxygenase in the cell walls of cells of different plant organs

Ewa Szczuka[1], Aleksandra Seta[1], Waldemar Maksymiec[2], Ewa Skórzyńska-Polit[3], Irena Giełwanowska[4]

[1]*Department of Plant Anatomy and Cytology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland*
[2]*Department of Plant Physiology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland*
[3]*Department of Plant Physiology and Biotechnology, John Paul II Catholic University of Lublin, Krasnicka Av. 102, 20-718 Lublin, Poland*
[4]*Department of Plant Physiology and Biotechnology, University of Warmia and Mazury, Oczapowskiego 1A, 10-719 Olsztyn, Poland*

Author's e-mail: aszczuka@hektor.umcs.lublin.pl

For precise localization of the enzyme lipoxygenase (LOX; EC 1.13.11.12) in cell walls, the immunogold labelling technique was used. The observations were performed in the cells of leaves of *Phaseolus coccineus* (L.), *Arabidopsis thaliana* (L.) Heynh., *Gagea lutea* (L.) Ker.-Gaw., in the cells of the anther wall and pollen grains of *Gagea lutea*, and the cells of the developing ovule of *Larix kaempferi* (Lamb.) Carr. The following parts of the ovule were examined with an electron microscope: the integument, the nucellus, the dividing megaspore mother cell, and the female gametophyte at successive stages of its development.

The immunogold labeling method demonstrated that LOX occured mainly in the cytosol (most often, the immunogold particles were distributed randomly in the cytoplasm) and vacuoles of the cells of all the investigated ovule parts. The immunogold particles which revealed the presence of the enzyme were found to be associated with the plasma membrane and were also discovered in the close vicinity of mitochondria and near plastids. LOX was detected near short endoplasmic reticulum cisternae – mainly RER (rough endoplasmic reticulum). Some single immunogold particles were observed at or in the area of the nuclei of the cells. Immunogold particles, which indicate the presence of the enzyme LOX, were found in the cell walls of cells of all the investigated plant organs. Generally, in comparison with the inner part of the cells, the immunogold reaction in the cell walls was less intensive. In most cases, only single immunogold particles were

detected in in the cell wall area. The particles were also visible at the edges of the cell wall and in the close vicinity of the cell wall near the plasmalemma. Sometimes they were visible in plasmodesmata. Among all the investigated plant tissues, the most numerous immunogold particles were observed in the cell walls of degenerated cells of the anther wall and in the sporoderm of pollen grains of *Gagea lutea*, the fewest in the young, developing ovule of *Larix kaempferi*.

In order to determine the degree of specificity of the immunogold reaction, a control reaction including all the procedures was carried out. The control reaction was conducted by omitting the incubation with the primary antibody. Only single gold particles (a few per one nickel grid) were found in the specimens. In most grid meshes no gold particles were present.Immunolocalization of lipoxygenase in the electron microscope indicates a functioning „lipoxygenase pathway" in walls of all cells of the investigated ovule parts. The intensity of the immunogold reaction may indirectly indicate a weaker activity of the enzyme in the cell walls of the cells of the particular parts of the plants. The presence of LOX in the cell walls may be connected with the taking a part of the cell wall in signaling.

19

Allelopathic effect of *m*-tyrosine on expansin gene expression in tomato roots

K. Szafrański[1,2], A. Wiśniewska[2], A. Gniazdowska[2], R. Bogatek[2]

[1]*Department of Biotechnology, Warsaw University of Life Sciences - SGGW, Warsaw, Poland*
[2]*Department of Plant Physiology, Warsaw University of Life Sciences - SGGW, Warsaw, Poland*

Author's e-mail: km.szafranski@gmail.com

Allelopathy is one of the components of plant/plant interaction and is usually defined as the adverse effect of one plant species on another one, due to the production of phytotoxins. Allelochemicals can exhibit diverse modes of action in plants. The delay and reduction of seed germination and/or inhibition of root and shoot growth are the first, visible symptoms of allelopathy stress. *m*-Tyrosine is an allelopatic compound identified in root exudates of fine fescue grasses. Bertin et al. (2007) demonstrated that, this nonprotein amino acid inhibits root elongation growth of *Arabidopsis thaliana* plants. Expansin is considered as one of a primary agent in cell elongation, since this is a protein that catalyzes long-term expansion of cell walls, exhibiting cell-wall loosening activity. Expansin mediate nonenzymatic, pH-dependent extension of the plant cell wall, participate in the cell growth, and in many processes requiring wall modification. The aim of our work was to investigate the effect of *m*-tyrosine on tomato (*Lycopersicon esculentum Mill.cv.* Ożarowski Malinowy) root elongation by analysing expression of expansin genes in roots. We determined expression pattern of 7 expansin genes (*LeEXPA1, 2, 4, 5, 8, 9* and *18*). Tomato seeds were germinated in distilled water at 20°C in dark. Three-days-old equally germinated seedlings were transferred to *m*-tyrosine water solution (0.025 – 0.50 mM). Control seeds were transferred to water. Culture was prolonged up to 3 days in growing chamber in the darkness. Length of hypocotyls and roots of seedling were determined after 1, 2, and 3 days exposition to *m*-tyrosine. For gene expression analysis, total RNA was isolated from tomato roots after 1, 2 and 3 days of m-tyrosine treatment by phenol extraction method. The RNA concentration was determined and equilibrated to 100 ng μl^{-1} on spectrophotometer.

m-Tyrosine inhibited tomato root growth in dose dependent manner, while hypocotyls showed insensitivity to this allelochemical. Based on semi-quantitative reverse transcription – polymerase chain reaction, we observed no significant differences in expression of *LeEXPA1*, *LeEXPA2, LeEXPA4, LeEXPA5, LeEXPA9, LeEXPA18*. The most interesting result was noticed for *LeEXPA8*. The expression of *LeEXPA8* was down-regulated in roots of allelopathy stressed seedlings as *m*-

tyrosine concentration increased. Such expression pattern suggests that inhibition in tomato root elongation growth after *m*-tyrosine treatment may be due to modification in *LeEXPA8* expression.

References:
Bertin C., Weston L.A., Huang T., Jander G., Owens T., Meinwald J., Schroeder FC. 2007. Grass roots chemistry: *meta*-tyrosine, an herbicidal nonprotein amino acid. *PNAS* 104 (43): 16964-16969.

20

Diversity of *Arabidopsis thaliana* miRNA primary transcripts

Bogna Szarzyńska, Łukasz Sobkowiak, Halina Pietrykowska, Artur Jarmołowski, Zofia Szweykowska-Kulińska

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland

Author's e-mail: bogsza@amu.edu.pl

Taking the advantage that pri-miRNAs that require HYL1 action for their proper biogenesis accumulate in *hyl1* genetic background, we performed 5' and 3' RACE experiments for primary transcripts of 26 chosen *A. thaliana MIR* genes. It was interesting to find that the length of analyzed transcripts vary from almost 400 nt to over 4000 nt. We have found that pri-miRNAs typically contain multiple polyadenylation sites resulting in a great heterogeneity of their 3' ends. For some of characterized pri-miRNAs we identified more than one 5' end. In addition, the alignement of obtained sequences to *A. thaliana* genomic sequences revealed the presence of introns in 13 out of 26 analyzed *MIR* genes. We have also shown that transcripts of two characterized intron-containing *MIR* genes undergo alternative splicing such as exon skipping and 5' alternative splice site. The established lengths of miRNA primary transcripts taking together with their complex splicing pattern seem very intriguing. One of the possible explanations is that pri-miRNAs contain additional, yet uncharacterized small RNAs embedded in their sequence.

Our analysis concernig miRNA primary transcripts in the *hyl1* mutant background shed also new light on the HYL1 protein function. We have shown that the HYL1 depletion leads to the accumulation of both intron-containing and spliced pri-miRNA precursors, whereas it seems to have no influence on the accumulation of intron-containing pre-mRNAs. It suggests that the HYL1 protein is recruited into the miRNA precursor maturation pathway before their splicing occurs and that HYL1 couples pri-miRNA splicing and further steps of miRNA maturation.

21

Application of artificial microRNAs for silencing *CBP20* and *CBP80* gene in potato to obtain plants with enhanced drought tolerance.

M. Pieczyński[1], D. Bielewicz[1], J. Dolata[1], K. Floras[2], J. Hennig[2], D. Czyżewska[3], W. Marczewski[3], A. Jarmołowski[1], Z. Szweykowska-Kulińska[1]

[1] *Department of Gene Expression, Adam Mickiewicz University, Poznan, Poland*
[2] *Laboratory of Plant Pathogenesis, Institute of Biochemistry and Biophysics, Warsaw, Poland*
[3] *Plant Breeding and Acclimatization Institute, Mlochow, Poland*

Author's e-mail: marcin-pieczynski@wp.pl

CBP20 and CBP80 proteins bind to mRNA 5' cap structure. Together these proteins create the Cap Binding Complex (CBC) and play a role in splicing and translation. In our previous investigations we have shown that *Arabidopsis thaliana* plants with silenced *CBP20* or *CBP80* genes are drought tolerant in comparison to wild type plants. In this project we are carrying similar experiments with the aim of obtaining new lines of potato plants with higher drought tolerance. We obtained and sequenced *CBP20* and *CBP80* cDNAs for all four alleles of two potato cultivars Sante and Désirée. We found highly conservative fragments in each cDNA sequence and decided to use them as targets for gene silencing. We prepared silencing constructs based on two different approaches. The first one is based on RNAi pathway. We have used the pHannibal vector which carries the CaMV 35S promoter and pART27 bicistronic vectors. We expect to obtain the expression of a RNA hairpin in plant cells. Second approach, based on the RS300 vector, provides expression of artificial microRNA in plant to silence the target gene. Potato plants transformation was carried out using *Agrobacterium tumefaciens* system. Selected transformants were tested for the presence of the transgene in the genome. Using RT-PCR we analyzed the changes in the expression level of *CBP80* gene in transformed potato lines in comparison to the wild type plants. As an endogenous control of mRNA amount in RNA probes actin mRNA level was investigated. Our results show that *CBP80* gene is silenced in transformed potato lines up to 99%. Tests for the establishment of drought tolerance in transformed plants are now in progress.

22

Representational difference analysis reveals distinct gene expression pattern in male and female individuals in dioecious liverwort *Pellia endiviifolia* subspecies B

Izabela Pasternak, Aleksandra Rojek, Zofia Szweykowska-Kulińska

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland

Author's e-mail: izapaste@amu.edu.pl

The alternation between a diploid sporophytic generation and a haploid gametophytic generation is distinctive feature to the life cycle of plants. In higher plants, male and female gametophyte is reduced to few cells. The formation of the gametophyte from the sporophyte is the result of two sequential processes, sporogenesis and gametogenesis. In recent years, molecular and genetical studies have begun to reveal mutations and, in some cases, the corresponding genes that control

ovule (female gametophyte) and pollen (male gametophyte) development. Several excellent model systems for bisexual floral development (*Arabidopsis thaliana*), monoecy (*Oryza sativa*) and dioecy (*Silene latifolia*) are available for such analyses.

In lower plants that produce no flower, sex determination is manifested in the gametophyte generation with the production of egg- and sperm-forming gametangia on separate individual gametophytes. Among these plants there is considerably less information about mechanisms determining sexual reproduction. One of few exceptions is *Marchantia polymorpha*, a dioecious liverwort belonging to subclass *Marchantiidae.* The sex of each haploid gametophyte is determined cytologically and genetically by distinct sex chromosomes. However genes responsible for the sex determination are not known.

Differences in chromosome content are also observed in dioecious liverwort, *Pellia endiviifolia* subspecies B, belonging to subclass *Jungermaniidae*. In *Pellia endiviifolia* ssp B nine chromosomes have been described, but in male individual the karyotype was assigned as 8+m with regard to small size of the ninth chromosome comparing to that from female gamethophyte.

In order to examine differences in sex-linked gene expression pattern between male and female gametophytes of *Pellia endiviifolia* ssp B we performed representational difference analysis of cDNA (RDA-cDNA) using male cDNA library as tested sequences. Based on succesive rounds of subtractive hybridization followed by PCR, in this technique differentially expressed mRNAs have been isolated while all nondifferentially expressed - removed.

We conducted four rounds of subtractive hybridization using male cDNA fragments as the tester. We obtained different PCR products ranging from ~200bp to ~300bp which we cloned and sequenced. As a result of RT-PCR experiment we identified eight cDNA fragments that are specifically expressed only in male gametophytes producing antheridia in comparison to female thalli. The nucleotide sequences of five RDA-cDNA derived fragments showed no significant similarities to known sequences registered in the public databases. Two sequences indicated similarity to light-harvesting chlorophyll a/b binding proteins of photosystem II from *Pinus palustris, Pseudotsuga menziesii* and *Silene latifolia*. One cDNA fragment showed identity to Ras-related protein Rab11 from *Oryza sativa, Triticum aestivum* and *Physcomitrella patens subsp. Patens*.

23

Regulation of alternative splicing in plants under stress conditions

Weronika Walczak, Łukasz Szewc, Bogna Szarzyńska, Halina Pietrykowska, Zofia Szweykowska-Kulińska, Artur Jarmołowski

Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland

Author's e-mail: werwalcz@amu.edu.pl

Pre-mRNA splicing is an important step in the regulation of gene expression in all eukaryotes. During this process introns are removed from pre-mRNA and exons are joined to generate mRNA. Alternative splicing is a process by which multiple mRNAs can be generated from a single gene by selection of alternative splice sites in a pre-mRNA. It was reported that various stresses influence alternative splicing of pre-mRNAs, including those that encode splicing regulators. This may allow plants to quickly alter their transcriptomes in response to changing environmental conditions.

In our laboratory, we use *Arabidopsis thaliana suspension*-cultured cells as a biological model to analyse a response of the regulation system to stresses at the transcriptome level. Using Real Time PCR we analyzed the expression level of 1880 genes of transcription factors and 175 pri-

micro RNAs in cells stressed with 250 mM NaCl. We observed that the expression of some genes changes in a response to stress. We also carried out preliminary experiments for the influence of salinity stress on the level of mRNA isoforms that undergo alternative splicing. We analyzed in detail transcripts for 53 transcription factors. In 7 cases we observed essential changes in the level of mRNA isoforms.

24

Plant m-AAA proteases form homo-oligomeric and hetero-ligomeric complexes, which are associated with prohibitins

M. Kolodziejczak, J. Piechota, I. Juszczak, H. Jańska

Department of Biotechnology, University of Wroclaw, Poland

Author's e-mail: martak@ibmb.uni.wroc.pl

AAA proteases (also called FtsH proteases) are bifunctional enzymes which combine proteolytic and chaperon-like activities. They have been found in eubacteria as well as in chloroplasts and mitochondria. A notable feature of FtsH proteases is their membrane integration and the formation of high molecular weight complexes. One type of mitochondrial AAA proteases are inner membrane proteins which expose their catalytic side to the matrix (m-AAA). It has been shown that yeast's m-AAA proteases complex (1MDa) interacts with complex of prohibitins (1MDa) forming a 2 MDa supercomplex, which is supposed to act in quality control of membrane proteins.

Arabidopsis possess two mitochondrial m-AAA proteases: AtFtsH3 and AtFtsH10. However, the level of *AtFtsH3* transcript and protein is several times higher than AtFtsH10 in leaf tissue. Our studies using Blue Native gel electrophoresis have showed that both *Arabidopsis* m-AAA proteases form a high-molecular complex with a native mass of approximately 2MDa. Immunodepletion and co-immunopercipitation experiments have revealed that not only homo-oligomeric complexes but also hetero-oligomeric assembles containing both *Arabidopsis* m-AAA protease (AtFtsH3/AtFtsH10) can be found in leaf mitochondria. Moreover, we found that both types of complexes contain prohibitins. These results confirm close interactions between prohibitins and plant m-AAA proteases.

In order to examine the consequences of losing AtFtsH3 we have used mutants carrying a T-DNA insertion in the gene encoding this protein. We have observed that lack of AtFtsH3 protein is associated with highly increased AtFtsH10 protein level. This compensation occurs posttranscriptionally, because the level of *AtFtsH10* transcript does not increase. Real-time PCR analysis using both the total and polysomal RNA indicate that translational activity of the *AtFtsH10* transcript is higher in *ftsh3* than in WT plant.

25

Two-step processing of AtFtsH4, mitochondrial metalloprotease in *Arabidopsis thaliana*

M. Lech, B. Kmieć-Wiśniewska, H. Jańska

Department of Biotechnology, University of Wroclaw, Przybyszewskiego 63, 51-148 Wroclaw, Poland

Author's e-mail: Hanna.Janska@ibmb.uni.wroc.pl

Arabidopsis thaliana FtsH4 protease (AtFtsH4) is synthesised in cytosol and posttranslationally imported into mitochondria. The mature enzyme resides in the inner membrane with its catalytic site exposed towards the intermembrane space. This topology is typical for *i*-AAA proteases, which span the membrane only once.

Our previous experiments showed, that precursor of AtFtsH4 is processed in two steps. Both cleavages take place at the N-terminus of the precursor, and each of them results in a mass drop of about 3kDa. The aim of this work was to identify the exact cleavage sites using site directed mutagenesis and find proteases that take part in the processing.

Based on bioinformatic analysis we selected several potential sites of the processing and introduced R→G point mutations in arginines preceding the predicted cleavage site. We found that changing arginine 28 and 53 to glycine completely abolished the first and the second cleavage, respectively. Both of the processing sites represent motifs recognized by mitochondrial processing peptidase (MPP). To check involvment of MPP in the maturation of AtFtsH4, we incubated the precursor with MPP purified by blue native electrophoresis (BN-PAGE). As control we used AOX, a well define substrate of plant MPP. Incubation of purified MPP (bc1/MPP complex, supercomplex consisting of complex I and dimeric bc1/MPP supercomplex) with AtFtsH4 resulted in both cleavage events. In contrast, no processing was observed when the AtFtsH4 precursor was incubated with other complexes purified by BN-PAGE. We also checked influence of different classes of inhibitors on the cleavages. Both cleavages were inhibited by EDTA, known inhibitor of MPP. Surprisingly, the second cleavage was also stopped in the presence of Pefabloc – inhibitor of both serine proteases and serine/threonine phospahtases. Influence of phoshorylation/dephosphorylation processes on maturation of AtFtsH4 is further analyzed. Taken together our results indicate that MPP takes part in both steps of processing of AtFtsH4 precursor.

26

Does mitochondrial AtFtsH4 protease may determine *A. thaliana* vitality under continual moderate heat stress condition?

M. Kicia, E. Smakowska, Ł. Pogorzelec, H. Jańska

Department of Biotechnology, University of Wroclaw, Wroclaw, Poland

Author's e-mail: marta.paprocka@ibmb.uni.wroc.pl

AtFtsH4 is one of the inner-membrane-bound mitochondrial ATP-dependent metalloproteases in *A. thaliana*, whose catalytic site is exposed to the intermembrane space. It was shown that AtFtsH4 is involved in the biogenesis of oxidative phosphorylation system. The present results suggest that this protease may also play a crucial role in protecting mitochondria from damages caused by prolonged

moderate heat stress. We found, that a loss of AtFtsH4 protease did not significantly affect *Arabidopsis* growth at 22°C, however, developmental and morphological abnormalities occurred when plants were continuously growing at 30°C. The emergence of leaves was slightly delayed in *ftsh4* mutants, compared with wild-type plants. We also noticed that a lack of AtFtsH4 affects adult leaf shape. In the *ftsh4* plants continuously growing at 30°C, the bolting phase was abolished or delayed. Furthermore, the flower development was impaired and, as a consequence, *ftsh4* were unable to produce seeds. At the molecular level, we found the elevated level of ROS in the *ftsh4* adult leaves. Surprisingly, no ROS difference was observed in juvenile leaves. We also noticed accumulation of molecular chaperones (prohibitin, Hsp70) in all growth stages of *ftsh4* growing at 30°C. We postulate that the AtFtsH4 protease is an important regulator in the plant antioxidant defense system under continual moderate heat stress conditions.

27

Arabidopsis mutants *eds4-1* and *npr1-1/ndr1-1* and phytohormone-dependent defense response to the necrotrophic fungus *Alternaria brassicicola*

Szymon Dąbrowski, Violetta K. Macioszek, Andrzej K. Kononowicz

Department of Genetics and Plant Molecular Biology and Biotechnology, University of Lodz, S. Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: szymon.dabrowski@gmail.com

Alternaria brassicicola is a necrotrophic fungal pathogen causing economically important black spot disease of many *Brassicaceae* species. However, *Arabidopsis thaliana* shows resistance to infection by this fungus, which is associated mainly with the production of camalexin, as well as with the accumulation of phenolic compounds and callose deposition at the infection site. Interestingly, *eds4-1* (*enhanced disease susceptibility 4-1*) mutant is impaired in ethylene and jasmonic acid-dependent signaling pathways and shows decreased expression of pathogenesis-related gene *PDF1.2* after treatment with these phytohormones. NPR1 (NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES 1) protein is a pivotal regulatory factor that functions downstream of SA-dependent signaling pathway. Inactive NPR1 monomers form a polymeric complex joined together by disulfide bonds, which are reduced after a change in cellular redox state caused by activation of SA signaling pathway. This results in release of NPR1 monomers, which activate TGA1 transcription factor, thus inducing expression of PR genes. NDR1 (NON-RACE SPECIFIC DISEASE REISTANCE 1) is a small protein localized in plasma membrane, which is activated by pathogens, specifically by CC-NBS-LRR class of resistance proteins. It has been shown to be a key player in resistance of Arabidopsis plants to an avirulent strain of bacterial pathogen *Pseudomonas syringae* and biotrophic oomycete *Peronospora parasitica* following *gene-for-gene* interaction. *Npr1/ndr1* double mutant is affected in salicylic acid signaling and expression of pathogenesis-related gene *PR-1*.

Here we report on dynamic biochemical changes in *A. thaliana* leaf tissue in response to *A. brassicicola* during first 72 hours post inoculation. Differences in defense responses between wild-type *A. thaliana* Col-0 plants and phytohormone signaling pathway affected mutants, *eds4-1* and *npr-1/ndr1* were observed. We have tested oxidative burst outcome (superoxide anions and hydrogen peroxide generation), callose accumulation and expression of two pathogenesis-related proteins: PR-1 and PDF1.2. We also observed formation of appressoria by fungus at the sites of attempted leaf tissue penetration.

This work was supported by grant of Polish Ministry of Science and Higher Education N302 318833 (V.K.M.) and by University of Lodz grant No 505/40397 (A.K.K.).

28

Resistance and susceptibility of Arabidopsis mutants *pad3-1* and *jar1-1* to necrotrophic fungus *Alternaria brassicicola*

Sylwester Głowacki, Violetta K. Macioszek, Andrzej K. Kononowicz

Department of Genetics, Plant Molecular Biology and Biotechnology, University of Lodz, S. Banacha Street 12/16, 90-237 Lodz, Poland

Author`s e-mail: glowacki.sylwek@gmail.com

Necrotrophic fungus *Alternaria brassicicola*, which causes black spot disease of many *Brassica* species, is widely used as a model organism for studying plant-pathogens interactions. Infection of the wild-type Arabidopsis plants leads to incompatible reaction and results in formation of lesions limited in size. During the infection process, the fungus develops appressoria in order to penetrate the host tissue.

The aim of this experimentation was to study the mechanisms of defense response of two Arabidopsis mutants: *pad3-1 (phytoalexin deficient 3-1)* and *jar1-1 (jasmonate resistant 1-1)* to *Alternaria brassicicola*. *PAD3* encodes a cytochrome P450 monooxygenase (CYP71B15) that is required for synthesis of *Arabidopsis* indole alkaloid phytoalexin – camalexin (3-thiazol-2-yl-indole). Camalexin is thought to play the essential role to develop resistance of Arabidopsis to necrotrophic fungi. PAD3 catalyzes the last step of camalexin biosynthesis, i.e. conversion of dihydrocamalexic acid to camalexin. Arabidopsis *pad3* mutant is impaired in camalexin production and is susceptible to *A. brassicicola* infection. Contrary to wild-type plants, lesions caused by the fungus continue to spread throughout the plant and fungal growth is continued. On the other hand, *JAR3* encodes an amino acid synthetase that is involved in jasmonic acid (JA) synthesis. This enzyme belongs to the firefly luciferase superfamily and it catalyzes the synthesis of several JA-amido conjugates. It was reported earlier, that JA-dependent signaling pathway is an important factor in plant defense strategy against many different pathogens.

This study focuses on numerous aspects of response of *A. thaliana* Col-0 plants and two mutants *pad3-1* and *jar1-1* to *A. brassicicola* attack. Deposition of callose (visualized by aniline blue staining), accumulation of hydrogen peroxide (H_2O_2) and superoxide anions (O_2^-) (visualized by DAB and NBT staining respectively), expression of two pathogenesis-related proteins: PR-1 and PDF1.2 (RT-PCR) as well as description of subsequent stages of fungal development, including appressoria and anastomoses formation will be shown.

This work was supported by grant of Polish Ministry of Science and Higher Education N302 318833 (V.K.M.) and by University of Lodz grant No 505/40397 (A.K.K.).

29

Fallen into the fight for survival: interactions between accelerated cell death *Arabidopsis* mutants (*acd1* and *acd2*) and *Alternaria brassicicola*

Joanna Łaźniewska, Violetta K. Macioszek, Andrzej K. Kononowicz

Department of Genetics, Plant Molecular Biology and Biotechnology, University of Lodz, S. Banacha 12/16, 90-237 Lodz, Poland

Author`s e-mail: joanna.lazniewska@gmail.com

Arabidopsis thaliana accelerated cell death lesion mimic mutants, *acd1-20* and *acd2-2*, are characterized by developing spontaneous lesions resembling those produced during hypersensitive response (HR). These mutants also show rapid and unrestricted programmed cell death (PCD) upon pathogen invasion. *Acd1* and *acd2* are impaired in chlorophyll catabolism; they accumulate pheophorbide *a* (pheide *a*) and red chlorophyll catabolite (RCC) respectively, which cause runaway cell death phenotypes of these mutants. Pheide *a* has an ability to absorb the light, that may lead to production of singlet oxygen and subsequent activation of PCD. Similarly, RCC possesses a phototoxic activity that possibly causes cell death. *Alternaria brassicicola* is a necrotrophic fungus which actively kills host plant tissues by producing toxic compounds in order to obtain nutrients. It is an incompatible pathogen of *A. thaliana*, and thus infection of wild type plants results in lesions restricted to the inoculation vicinity. On the contrary, *acd1* and *acd2* mutants are unable to defend themselves effectively, which leads to plant disease and rapid fungal development.

Here, we present the course of several defense reactions in *A. thaliana* wild type plants in comparison to *acd1* and *acd2* mutants. *Arabidopsis* defense responses were analyzed quantitatively and qualitatively on both histochemical and molecular levels. These responses include oxidative burst, consisting in superoxide anions (nitroblue tetrazolium staining) and hydrogen peroxide (3,3`-diaminobenzidine staining) generation, callose deposition at the site of attempted fungal penetration (aniline blue staining) as well as the expression of genes encoding pathogenesis-related proteins, *PR-1* and *PDF1.2* (RT-PCR). Moreover, we examined some aspects of fungal development, focusing on the extent of spore germination and appressoria formation at particular time (8, 12, 24 and 48 hours) after inoculation.

This work was supported by grant of Polish Ministry of Science and Higher Education N302 318833 (V.K.M.) and by University of Lodz grant No 505/40397 (A.K.K.).

30

Effects of SAR inducers on nitric oxide generation in *Phytophthora infestans*

Ł. Janus, D. Abramowski, J. Floryszak-Wieczorek

Department of Plant Physiology, Poznan University of Life Sciences, Poland

Author's e-mail: l.janus@up.poznan.pl

So far, data collected by us strongly suggested the importance of nitric oxide in triggering the defense against pathogens. Short nitric oxide burst seems to be one of the earliest signals, which is needed to establish potato resistance to *Phytophthora infestans*. Moreover, it has been shown by us that nitric oxide is also generated by the fungus - *P. infestans* in potato - pathogen interaction. Using

DAF-2DA fluorochrome (specific for nitric oxide) we found that the pathogen colonizing susceptible potato cultivar also acquired the ability to produce considerable amounts of NO in host tissues, visible as strong green fluorescence.

The aim of presented study was to investigate whether and to what extent three types of SAR (systemic acquired resistance) inducers, e.g. BABA (β-amino-n-butyric acid), GABA (γ-amino-n-butyric acid) and Laminarine (storage glucan found in brown algae) could affect the nitric oxide generation in fungus - *Phytophthora infestans* and modify the germination of isolated spores or the infection of potato tissue. The choice of agents was based on prior observations that mentioned inducers were effective in systemic reduction of late blight disease symptoms of potato leaves.

The effect of sodium nitroprusside (SNP) as NO donor and SAR inducers on pathogen were estimated by spore germination assay and measure of mycelial growth of *P. infestans* on pea-agar medium. GABA and applied NO light increased fungal growth and its sporulation while BABA and Laminarine decreased number of germinated spores and mycelial growth. Then, analyzing nitric oxide generation by fungal hyphae and spores of *P. infestans* we observed enhanced green fluorescence of DAF-2T in response to BABA and GABA treatment, in contrast to Laminarine which did not affect fluorescence intensity of pathogen in comparison to the untreated one.

Obtained results suggest that used SAR inducers have not been very effective in elicitation nitrosative stress in *P. infestans*, probably because they stimulate disease resistance in potato, thus finally acting against the pathogen.

31

Reactive oxygen and nitrogen species in response of tomato cell suspension cultures to *Botrytis cinerea.*

E. Kaczkowska, U. Małolepsza

Department of Plant Physiology and Biochemistry, University of Lodz, Poland

Author's e-mail: ulmal@biol.uni.lodz.pl

Investigations were concentrated on tomato resistance to *Botrytis cinerea*. This necrotrophic pathogen causes gray mold, one of the most destructive tomato diseases, and is responsible for significant pre- and post-harvest yield loss. Till now no tomato cultivars resistant to *B. cinerea* are available. To recognize the rudiments of tomato resistance to *B. cinerea,* the physiological-biochemical changes were compared in suspension cultures of two tomato cultivars differing in susceptibility to the fungus: less susceptible – "Perkoz" (P) and more susceptible – "Corindo" (C). Cell suspension culture seems to be a good experimental model to study disease resistance reactions in plants because it makes recognition of mechanisms functioning at the cell level easier. Production of reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) during so-called "oxidative burst" has been considered a central event in activation of disease resistance. They may be directly involved in pathogen killing and strengthening of plant cell walls, as well as in triggering hypersensitive cell death and in systemic resistance signaling. Nitric oxide (NO) is considered to be required, together with ROS, to activate of disease resistance reactions of plant to infection with pathogen, however, ROS and NO cooperation in this process is still controversial, especially regarding their contribution to the defense responses to necrotrophic pathogens. Tomato cell suspension cultures were grown in Chandler medium supplemented with BAP (0.2 mg/cm^3) and 2,4-D (1mg/cm^3), in the dark. Three-day old cell suspensions were taken to experiments; some of them were inoculated with *B. cinerea* conidia. The rates of infection development were compared in more (C) and less (P) susceptible suspension cultures in parallel with changes in ROS: superoxide anion, hydrogen peroxide and nitric oxide concentrations.

Changes in superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities were also studied in uninoculated and *B.cinerea* inoculated cultures. The obtained results show that *B. cinerea* infection proceeded slower in the suspension culture of cv. "Perkoz" than in the suspension cultures of cv. "Corindio" and suggest that the mechanisms of resistance to the pathogen function at a single cell level. Constitutive ROS and NO concentrations were higher in the less susceptible cultivar (P) than in more susceptible one (C). Both kinds of tomato suspension cultures responded to inoculation with *B. cinerea* with enhanced synthesis of hydrogen peroxide, superoxide and nitric oxide but these reactions were faster and stronger in cv. "Perkoz" than in cv. "Corindo". Studies of the enzymes, which contribute to ROS generation (SOD) and destruction (CAT, APX), showed that SOD activity in the control cell suspension of cv. "Perkoz" was higher than in cv. "Corindo" whereas catalase and ascorbate peroxidase activites showed higher constitutive activity in the cell suspension of cv. "Corindo". APX and CAT activities decreased as a result of inoculation of tomato suspensions with the pathogen. The results indicate that inoculation of tomato suspension cultures with *B. cinerea* mobilize disease resistance reactions, which function at a single cell level and provide evidence that enhanced ROS and NO production strongly participate in these reactions.

32

Identification of *Phaseolus vulagris* lines resistant to the common bacterial blight and construction of molecular marker to determine internal polymorphism of the common bean Andean gene pool

M. Kwaśniak[1], M. Wołoszyńska[1], M. Gibała[1], H. Jańska[1], T. Kotlińska[2], E. Horodecka[3]

[1]*Institute of Biochemistry and Molecular Biology, Department of Cell Molecular Biology, University of Wroclaw, Poland*
[2]*Institute of Market Gardening, Laboratory of Genetic Resources, Skierniewice, Poland*
[3]*Polish Breeding and Seed Production Horticultural Plants, IWARZ-PNOS, Michalowice, Poland*

Author's e-mail: gosiakwasniak@op.pl

Common bacterial blight (CBB) is one of the most important bacterial diseases of *Phaseolus vulgaris*. The most effective and environmentally safe strategy to control this disease is breeding of cultivars that possess genetic resistance to CBB. We used four molecular markers which are linked to the CBB resistance to screen 15 genotypes originating from Europe, North and South America and 33 Polish cultivars. In the former group of genotypes all but one contained at least one marker (most commonly SAP6), in 3 genotypes two markers (SAP6 and BAC6) were detected. Among Polish cultivars only the SAP6 marker was found. All 22 analyzed yellow pod beans and only 1 green pod bean genotype possessed SAP6 marker. Bacteriological tests were applied to evaluate the CBB resistance in all genotypes included in our studies. All 15 European and American cultivars showed full resistance to CBB. None of the Polish cultivars of *P. vulgaris* presented similar level of resistance. However, 16 yellow pod genotypes showed relatively low susceptibility to CBB. The other yellow pod beans and some green pod beans presented middle susceptibility, whereas remaining green pod beans were highly susceptible. Hence, we conclude that the presence of the SAP6 marker is not enough to ensure the CBB resistance in common bean, but we can use the SAP6 detection as initial stage of selection of resistant cultivars.

Progress in bean breeding requires the exploitation of genetic variation that is present inside the gene pools of *Phaseolus vulgaris*. The Andean gene pool seems to have a narrow genetic base but it presents valuable breeding traits. Comparing to the Mesoamerican gene pool, Andean beans are more resistant to low temperature and have larger average seed size. Based on the FISH data presented in the literature, we constructed the molecular marker allowing to determine the internal

polymorphism of the Andean gene pool. The results of FISH experiments indicated that, contrary to Mesoamerican gene pool, Andean beans show a large diversity of the 45S rDNA loci number. We designed and optimized the real-time PCR assay to evaluate the number of 45S rDNA loci in common bean. As a reference gene we used the actin gene. So far we tested 24 cultivars from both gene pools (9 Mesoamerican and 15 Andean cultivars). As expected, we observed more diverse 45S rDNA copy numbers within Andean than Mesoamerican gene pool.

33

Efficacy and possible mechanisms of action of beneficial bacteria against fire blight (*Erwinia amylovora*)

A. Mikiciński., P. Sobiczewski, J. Puławska, S. Berczyński

Research Institute of Pomology and Floriculture, Department of Plant Pathology, Pomologiczna 18, Skierniewice, Poland

Author's e-mail: amikic@insad.pl

Fire blight is a very destructive bacterial disease of apple, pear and many other plant species, especially of *Rosaceae* family. Its control consists of integration of various methods to protect plants against infection, elimination of infection source and decrease plants susceptibility. One of the possibilities of plant protection is the use of biopreparations based on bacteria and yeasts. Till now over 10 such products were developed and introduced into market of various countries.

In our study of over 150 bacterial isolates originating from apple phyllosphere and soil environment six (3M, L16, 35M, B90, 43M, 48M) appeared to be highly effective in protection of pear fruitlets and blossoms and terminal shoots on apple trees. Some of them showed even higher efficacy than those of reference strains C9-1 (*Pantoea agglomerans*) and A 506 (*Pseudomonas fluorescens*) used as active ingredients in commercial biopreparations developed in USA. An experiment on eradication of *E. amylovora* in apparently healthy apple shoots protected with highly effective isolate 35M confirmed its very high antagonistic activity; very low amount of pathogen was detected using PCR method in less than 25% samples.

Research on biotic relationship between tested isolates and *E. amylovora* on 5 microbiological media (Nutrient Agar with sucrose, Nutrient Agar with glycerol, King B, LB and PYGA) showed that L16, 3M and 35M inhibited growth of pathogen on almost all of them while 43M, 48M and B90 did not show such activity. Production of N-acyl homoserine lactones (AHL) by bacteria was tested with AHL-indicator strain *Chromobacterium violaceum* CV026. Three isolates: 3M, 35M and 48M gave positive reaction, what indicates their ability to act on other bacteria. Moreover, isolates L16, 35M and 3M produced siderofores on medium containing complex: CAS - Fe^{3+} - HDTMA. It was also found that isolate 3M posses *prn*D gene involved in synthesis of pyrrolnitrin and 35M gave a characteristic length product with primers complementary to *plt*B gene coding for synthesis of pyoluteorin. However, the sequence of the obtained product showed only 79% homology to known *plt*B genes, but it was 100% complementary to gene coding for beta-ketoacyl synthase which is also known as a component in the biosynthesis of polyketide antibiotics. Isolates 3M and 35M gave also positive reaction in PCR with primers complementary to *gac*A gene – the response regulator gene, which influences the production of several secondary metabolites including antibiotics. Phenotypic characterization and sequence analysis of the 800 bp-long fragment of the 5' end of 16S rRNA gene allowed to classify isolate 35M to *Pseudomonas syringae*, L16 to *P. fluoresens*, 43M to *Citrobacter farmeri* (or *Pantoea agglomerans*), 48M and B90 to *P. agglomerans* and 3M to *Pseudomonas chlororaphis*.

34

Comparison of phenotypic characteristic and MP PCR based phylogeny of *Pseudomonas syringae* isolates originating from stone fruit trees

M. Sulikowska, J. Puławska, P. Sobiczewski

Research Institute of Pomology and Floriculture, Department of Plant Pathology, Pomologiczna 18, Skierniewice, Poland

Author's e-mail: msuliko@insad.pl

Of thirty fluorescent *Pseudomonas* isolates originating from symptomatic tissues of various organs of sweet (*Prunus avium*) and sour cherries (*Prunus cerasus*), plums (*Prunus domestica*), peaches (*Prunus persica*) and apricots (*Prunus armeniaca*), 23 were identified as *P. syringae* using LOPAT tests. Further characterization of those isolates by GATT'a and L-lactate utilization tests showed that 10 of them belonged to race 1 of *P. syringae* pv. *morsprunorum* (*Psm*) and six to race 2 of this pathovar. Seven other isolates were identified as pathovar *syringae* (*Pss*). Phenotypic and genetic determination of studied isolates to produce toxins revealed that 6 *Pss* isolates produced syringomycin, 3 *Psm* race 1 produced coronatine and all 6 *Psm* race 2 isolates produced yersiniabactin.

Determination of genetic diversity of all isolates using the melting profile PCR (MP PCR) method showed that obtained patterns strongly correlated to phenotypically distinguished pathovars. Separate clusters formed isolates of *Psm* races 1 and 2, but *Pss* were more heterogeneous. The reference strains LMG 2222 (*Psm* race 1), LMG 1247 (*Pss*), 2905 (*Pss*), CFBP 3800 (*Psm* race 2) were placed exactly in created clusters according to identified pathovars and races. A relationship between obtained MP PCR patterns and host plant for *Psm* race 1 isolates was also found. All *Psm* race 1 originating from sweet cherry were distinctly separated from plum's isolates of that race. Bacteria identified as *Pss* and *Psm* showed two types of symptoms on immature cherry fruits which corresponded to determined pathovars and races.

35

Profiling of isoflavonoids in seedlings of *Lupinus angustifolius* infected with *Colletotrihum lupini* by LC/UV/MS

D. Muth[1], M. Stobiecki[1], P. Kachlicki[2]

[1] *Institute of Bioorganic Chemistry PAS, Noskowskiego 12/14, 61-704 Poznan, Poland*
[2] *Institute of Plant Genetics PAS, Strzeszynska 34, 60-479 Poznan, Poland*

Author's e-mail: dorotus@ibch.poznan.pl

Isolavonoids consist the group of phenolic compounds broadly occurring in nature. This class of secondary metabolites is characterized by a great number of isomeric forms which are present in plants. Various modifications of basic squeleton C_6-C_3-C_6 like hydroxylation, methylation or prenylation at different positions make the differentiation of these compounds difficult. Moreover, flavonoids are usually accumulated in plant tissues as glycosides, frequently acylated with organic acids (e.g. malonic acid). The analysis of such complex extracts needs the separation step before

identification of particular compounds. We used LC/UV/MS/MS system which allowed us the differentiation and quantitation of isoflavonoid aglicones and their conjugates. We monitored the changes in profiles of isoflavonoids in leaves of narrow leaf lupine (*Lupinus angustifolius*) seedlings infected with pathogenic fungus (*Colletotrichum lupini*). This fungus causes anthracnose resulting in serious loses in crop yields of lupine worldwide. Our investigations were performed on inoculated and control seedlings of several dozen lines of *Lupinus angustifolius* to compare their resistance to the pathogen attack. Statistical analysis of quantitative changes in LC profiles demonstrated the role of prenylated isoflavones luteone and wighteone in the defense reaction. The correlation between concentration of isoflavone aglycones and infection symptoms was observed. The level of malonylated conjugates of isoflavonoids was also analyzed in order to determine the role of malonylation process in the plant response to the attack of pathogen.

36

Agrobacterium rhizogenes mediated transformation – this is how we do it.

K. Hnatuszko-Konka[1], P. Łuchniak[1], T. Kowalczyk[1], A. Gerszberg[1], A. Wiktorek-Smagur[2], A. K. Kononowicz[1]

[1] *Department of Genetics and Plant Molecular Biology and Biotechnology, University of Lodz, S. Banacha 12/16, Lodz, Poland*
[2] *Nofer Institute of Occupational Medicine, 8 Teresy, Lodz, Poland*

Author's e-mail: kath@biol.uni.lodz.pl

In recent years, simple and efficient procedures for obtaining transformed roots have been developed. *Agrobacterium rhizogenes*, the causative agent of hairy root disease in plants, alike *Agrobacterium tumefaciens*, transfers T-DNA fragment from its root-inducing (Ri) plasmid to plant cells, where it is stably integrated and expressed. It has to be emphasized, that while usually it takes a relatively short period of time to produce transformed roots following *A. rhizogenes*-mediated transformation, at least 3 months are required to regenerate transgenic plants when *A. tumefaciens* carrying Ti plasmid is used. Moreover, since each transgenic root represents an independent transformation event, therefore high number of transformed root lines can be produced and analyzed in only a few weeks. Here we report on efficient protocol for the production of *A. rhizogenes* transformed roots optimized in the Department of Genetics and Plant Molecular Biology and Biotechnology. Several A4 transformant variants of *A. rhizogenes* and two different protocols were used to assess efficiency of hairy root induction for *Nicotiana tabacum*, *Brassica rapa subsp. pekinensis* (Lour.) Hanelt, *Raphanus sativus var. radicula*, *Raphanus sativus* L. var. *niger* plants. Application of all experimental approaches resulted in co-transformed root formation in explant cultures of all investigated plant species. The hairy roots were grown under hygromycin selection pressure, which constituted co-transformant preselection step. Transgenic character of the root cultures was confirmed, by X-gluc histochemical assay (*GUSPlus* gene was used as a reporter gene).

This work was supported by Polish Ministry of Science and Higher Education grant No 2 P04B 029 29 (A.K.K.) and University of Lodz grant No 505/040397 (A.K.K.).

37

Genetic transformation of *Arabidopsis thaliana* by the *floral dip* method

A. Wiktorek-Smagur[1], K. Hnatuszko-Konka[2], A. Geszberg[2], P. Łuchniak[2], A. K. Kononowicz[2]

[1] Nofer Institute of Occupational Medicine, Teresy 8, Lodz, Poland
[2] Department of Genetics and Plant Molecular Biology and Biotechnology, University of Lodz, S. Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: kath@biol.uni.lodz.pl

The small flowering weed plant, *Arabidopsis thaliana*, is the best-studied model organism in plant biology. *Arabidopsis* has become a major model system for study molecular, genetic and biochemical processes in plants. The features of *Arabidopsis* that suit it for laboratory work are: small size of the mature plant and small, numerous seeds and short developmental cycle of *Arabidopsis* plants (ranges from 5 to 6 weeks). The small size of the plants and their ability to grow well in constant fluorescent light at room temperatures, rather in simple soil mixes or in sterile, defined media, make indoor maintenance of large populations of *Arabidopsis* plants inexpensive and easy.

Recently it has become possible to transform *Arabidopsis thaliana in planta* using *Agrobacterium* and vacuum infiltration or floral dip transformation method. *Arabidopsis* floral dip transformation is notable for several reasons. It requires minimal labor and no plant tissue culture methodology involved, thus a somaclonal variation is avoided. Flowering plants are simply dipped in or sprayed with an appropriate strain of *Agrobacterium tumefaciens* and then the seeds collected from these T_0 plants are germinated under selection pressure to identify transgenic T_1 individuals. A transformation frequency of at least 1% can be routinely obtained and minimum of several hundred independent transgenic lines. Here we describe the protocol routinely used in our laboratory for the floral dip method for *Arabidopsis* transformation. Transgenic *Arabidopsis* plants can be obtained in approximately 3 months.

This research was supported by Polish Ministry of Science and Higher Education grant No 2 P04B 029 29 (A.K.K.) and University of Lodz grant No 505/040397 (A.K.K.).

38

Increase of bioactive compounds in flax plants overexpressing enzymes of flavonoid synthesis pathway.

Magdalena Żuk, Jan Szopa

Faculty of Biotechnology, University of Wrocław, Poland.

Author's e-mail: mzuk@ibmb.uni.wroc.pl

Flavonoids are the group of secondary plant metabolites important for plant grown and development. They are also well known as very effective antioxidants with great significance for human health. We obtained flavonoid-rich plants using two strategies. The first was simultaneous overexpression of three genes of flavonoid synthesis (chalcone synthase, chalcone isomerase and

dihydroflavonol reductase) and the second overexpression of broad substrate specificity glucose transferase.

Both groups of these plants have increased level of, important for antioxidant potential phenylopropanoid compounds, like kaempferol, quercetin, phenolic acids, poli-unsaturated fatty acids and lignans. Because the constitutive promoter (CaMV 35S) was used for modification, the positive changes has been observed in whole plant body – green parts and seeds.

Flax seeds are very good source of plant lignan precursor secoisolariciresinol diglucoside (SDG), which is metabolised by bacteria in colon to produce the mammalian lignans enterolacton and enterodiol. Lignans inhibit cell proliferation and growth, making them potential anticancer agents (hormon-sensitive cancers). In our transgenic plants the significant increase of SDG level (up to 30-fold) was noticed. Dramatic increase of ferulic acid level (about 40 fold) was also observed. The high level of lignans was also found in seedcakes -matter left after pressing of oil from linseed of transgenic plants. That is the reason, why seedcake obtained from flavonoid-rich plants should be use as a very good source of bioactive compounds.

Also significantly higher level of antioxidants was detected in oil from transgenic plants. What is necessary to notice such obtained oil is rich of poli-unsaturated fatty acids and the ratio of ω6/ω3 was about this recommended by FAO/WHO for optimal human diet. Presence of antioxidants in this oil also resulted in increased unsaturated acids stability.

Flax fibers obtained from transgenic plants on semi- technical scale have increased content of many bioactive phenylopropanoids such as a phenolic acids and lignans. In fibers we also identified canabidiol –compounds which can reduce pain. That's the reason why is postulated to use the fabric produced from this fibers as a bandage.

39

In vitro propagation of 'Louise odier' - the vintage Bourbon rose.

K. Tracz[1], A. Kubicka[1], K. Kapruziak[1], R. Śnitko[1], B. Pietrzak[1], K. Bergier[2]

[1] *Section of Plant Physiology and Ecobiotechnology, Student's Scientific Circle of Biologists, Faculty of Biology and Environmental Protection, University of Lodz, Poland*
[2] *Department of Plant Physiology and Biochemistry, University of Lodz, Poland*

Author's e-mail: lizparcer@interia.pl

'Louise odier' is a valuable ornamental and fragrant rose bred by Margottin in 1851. It belongs to a group of Bourbon roses, hybrids originating from a chance cross between a form of 'Autumn Damask' and the 'Old Blush' China rose.

The rapid technique for *in vitro* multiplication of 'Louise odier' has been developed for commercial production of this cultivar. As explants, we used nodal fragments from mature plants. Single-nodal explants were surface sterilized using 70% ethanol for 10 min and 25% chlorox for 15 min and inoculated to modified Murashige and Skoog (MS) medium containing 2 mg/l benzyladenine (BA), 0.7 mg/l indole-3-acetic acid (IAA) and gibberellic acid (GA_3) to induce sprouting of buds followed by multiple shoot formation. Then shoots were multiplied on MS medium supplemented with various concentrations of BA, separately or in combination with (GA_3) and IAA. Combination of 1 mg/l BA with 0.5 mg/l GA_3 and 0.35 mg/l IAA was the most suitable treatment for shoot proliferation. *In vitro* derived shoots were subcultured on the fresh medium with identical composition within a four-week period. For root initiation, individual shoots (more than 2.5 cm long) were subcultured on the different media such as MS, ½ MS and ¼ MS supplemented with various concentrations of naphthalene acetic acid (NAA) or indole-3-butyric acid (IBA) (0.1 - 0.5 mg/l). Maximum rooting (70% of explants) was observed within 14 days of subculturing on the

medium with 0.5 mg/l IBA. The rooted plants were acclimatized in perlite and successfully transferred to soil after two weeks. Then the plants were grown in a growth chamber under controlled conditions of temperature, light and humidity. We showed the simple culture procedure for enhanced multiple shoot production and recovery of complete plants of the vintage Bourbon rose 'Louis odier'. We also hope that this protocol will be a universally applicable in large-scale propagation of this cultivar.

40

Isolated microspores as a tool for genetic studies and biotechnology

S. Oleszczuk, J. Zimny

Departament of Biotechnology and Plant Cytogenetics,
Plant Breeding and Acclimatization Institute, Radzikow, 05-870 Blonie, Poland

Author's e-mail: s.oleszczuk@ihar.edu.pl

Formation of embryos from microspores is an unusual event. *In vivo,* microspores undergo an asymmetric nucleus division and develop into the male gametophyte - a pollen grain. Under specific *in vitro* conditions, microspores can be induced to divert their development pathway from gametophytic to sporophytic. Microscopic observations of embryo formation in microspore cultures represent a unique opportunity to study plant embryogenesis. The ability of microspores to switch developmental pathways from pollen to embryo is usually dependent on some stress treatment (low or high temperature, starvation). The stress induced changes in gene expression cause a developmental switch. Using this system, biochemical and molecular changes during this alternate pathway of microspore development can be studied. Microspores isolated in large quantities represent populations of single cells capable of synchronous development. Due to these properties, microspores constitute a potential tool for various biotechnological applications such as the *in vitro* selection and mutagenesis. They may also be used to generate transgenic plants with desirable properties and to stabilize transformed lines. Moreover, microspore cultures can be a source of protoplasts, cell suspensions and "nurse" cultures for other cells. The production of doubled haploids via isolated microspore cultures shortens the production cycle of homozygous lines to one generation compared to six-seven generations in traditional breeding methods.

41

In vitro culture of isolated microspores of wheat

Andrzej Z. Czaplicki, Sylwia Oleszczuk, Janusz Zimny

Plant Breeding and Acclimatization Institute, Department of Biotechnology and Cytogenetic,
Radzikow, 05-870 Blonie, Poland

Author's e-mail: a.czaplicki@ihar.edu.pl

Potentially higher efficiency of regeneration of haploid plants can be achieved in isolated microspores cultures than in anther cultures. Progress in the development of techniques used in cultures of isolated microspores of cereals in recent years is large. In breeding practice, a culture of isolated microspores increasingly replaces the anther culture (rape, barley). Obtaining higher than in

anther cultures efficiency in haploid plant regeneration is possible due to the highly embryogenic nature of microspores and a short path of the culture. In addition, the presence of microspores in the form of individual cells allows the precise genetic manipulation, easy use of selection and mutation factors. Development of embryos from single cells allows tracking the process of regulating the formation of embryos at the molecular level.

Methods used to culture isolated microspores are therefore, more complex requiring the gradual overcoming of many obstacles in order to achieve positive results. High efficiency production of wheat embryos and regeneration of plants can be obtained only for certain genotypes. Because of that more research on factors promoting a high level of androgenesis from the isolated microspores are required. The subject of this study was isolated microspores of wheat spring variety Pitic 62.

The purpose of the experiments was to induce androgenic development of microspores isolated using blender, to produce embryos and to regenerate plants. Microspores were isolated using the method described by Zheng (Zheng *et al.* 2001 and 2002) and then grown in the dark at a temperature of 26°C. As a nurse culture, isolated ovaries of spring variety Orofen were used. Microspores divisions and the further embryos development were observed. As a result of experiment five albino plants were regenerated so far.

References:

Zheng M. Y., Liu W., Weng Y., Polle E., Konzak C.F. 2001. Culture of freshly isolated wheat (*Triticum aestivum* L.) microspores treated with inducer chemicals. Plant Cell Rep. 20: 685-690.

Zheng M. Y, Weng Y., Liu W., Konzak C. F. 2002. The effect of ovaryconditioned medium on microspore embryogenesis in common wheat (*Triticum aestivum* L.). Plant Cell Rep. 20: 802–807.

42

Adaptation of tobacco BY-2 cells to growth in the presence of ionic and nonionic factors

A. Kasprowicz[1], R. Janczara[1], P. Wojtaszek[1,2]

[1] *Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Collegium Biologicum, Umultowska 89, 61-614 Poznan, Poland*

[2] *Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland*

Author's e-mail:akas@amu.edu.pl

The very special case of the cell's response to stress is the adaptation to conditions that are lethal to non-adapted cells. We have applied a stepwise approach (18 months in total) to adapt the suspension-cultured tobacco BY-2 cells to extreme osmotic conditions evoked by high levels of ionic (NaCl, KCl) and nonionic (mannitol, sorbitol, polyethylene glycol) agents. Nonionic and ionic osmotica act in different manner and evoke specific responses of adapted cells. Ionic agents increase adhesive properties of the cells, and formation of cell aggregates, whereas nonionic agents stimulate strictly positioned cell divisions and thus induce formation of cell files. Proteins ionically bound to the walls or secreted into culture medium were isolated from adapted cells and cells exposed to short-term osmotic stress, and analyzed with 1- and 2D electrophoresis and further with MALDI-TOF mass spectrometry. Protein profiles from adapted/exposed cells were highly specific to the osmotic ionic/nonionic agent applied. Several cell wall modifying enzymes (xyloglucanases, endo-ß-1,3-glucanases) were overexpressed exclusively in adapted cells. Using an immunoelectrophoretic assay, we noticed that level of arabinogalactan proteins (AGPs) in cells adapted to grow in presence of nonionic osmotica increased substantially. The AGPs are differentially distributed in cell walls of control cells and in cells adapted to grow in presence of

different osmotica. In addition, the polysaccharide composition and distribution of callose and pectins in adapted cell lines is changed. Surprisingly, analyses of actin and tubulin cytoskeletons in adapted and non-adapted, unstressed cells revealed no significant changes. However, BY-2 cells exposed to short-term osmotic stress could cope with it in a cytoskeleton-dependent manner. Such cells revealed disruption of fine networks of cortical microfilaments and microtubules, and formation of thicker cables. It seems that upon prolonged exposure to osmotic stress conditions adaptive alterations in cell wall composition will occur. This will probably change anchoring of the cytoskeleton in the walls and modify functioning of the whole cell wall-plasma membrane-cytoskeleton continuum. In that way, cell's mechanical balance restoration will be ensured and cell will be able to resist osmotic pressure.

This research was founded by the Polish Ministry of Science and Higher Education grants: PBZ-KBN-110/P04/2004 and 2944/B/P01/2008/34 to PW and grant of Dean of Biology Faculty for best PhD students and scholarship from European Social Fund Programme for PhD students from Wielkopolska to AK.

43

Microtubules surround lipid bodies forming lipotubuloids as well as those singularly localized in the cytoplasm

M. Kwiatkowska, D. Stępiński, K. Popłońska, A. Wojtczak, J. T. Polit

Department of Cytophysiology, Pilarskiego 14, 90-231 Lodz, Poland

Author's e-mail: kwiat@biol.uni.lodz.pl

Ultrastructural studies of *Ornithogalum umbellatum* ovary epidermis showed big aggregations of lipid bodies entwined with microtubules running in various directions, which connect them. These aggregations have been named lipotubuloids due to the presence of lipids and microtubules in them (Kwiatkowska 1971, Acta Soc Bot Pol 41: 451-465). Recently it has been revealed that lipotubuloids are present also in *Haemanthus albiflos.* They contain numerous lipid bodies connected with microtubules, few mitochondria, dictyosomes and microbodies as well as autolytic vacuoles similarly as in *O. umbellatum*. Contrary to *O. umbellatum* lipotubuloids in which microtubules are stable (they can be fixed with OsO_4 without prefixation in glutaraldehyde and are not destroyed by colchicine) those in *H. albiflos* are labile. They are seen only when the fixation procedure is preceded by treatment with a microtubule stabilizer - taxol. This may be connected with posttranslational modifications of tubulin leading to incerase or decrease in microtubules stability, which is observed in various organisms.

The aim of our studies was to see whether 1) lipotubuloids are present in other plants, 2) microtubule are connected with singularly localized lipid bodies as well? The object of current studies were so called "elaioplasts" of *Vanilla planifolia* firstly described and named by Wakker (1888), "elaioplasts" of *Funkia Sieboldiana* and root meristematic cell lipid bodies of *Althea rosea* and *Arabidopsis thaliana*.

"Elaioplasts" of *Vanilla planifolia* are not plastids as the name suggests but they are aggregations of many lipid bodies localized in specific cytoplasm domain, with numerous ribosomes and some ER cisternae, surrounded with tonoplast at the vacuole side. Mitochondria, dictyosomes, microbodies and autolytic vacuoles are occasionaly seen. Longitudinal and cross sections of microtubules are present at lipid bodies. So "elaioplasts" of *V. planifolia* could be named lipotubuloids. "Elaioplasts" of *Funkia Sieboldiana*, similarly as the above ones, are aggregates of a vast number of lipid bodies, however mitochondria and dictyosomes are localized mainly at their peripheria. EM analysis of lipid bodies shows that microtubules, which are not so clear visible as in

O. umbellatum, arround them. However they resemble microtubules which can be seen in lipotubuloids of *H. albiflos* and *V. planifolia*. Thus *F. Sieboldiana* "elaioplasts" can be treated as lipotubuloids. We think that among "elaioplasts" which were named so according to Wakker and which are not plastids more lipotubuloids will be revealed. Lipid bodies of *Althea rosea* present in the cytoplasm of root meristematic cells are surrounded with weakly contrasted microtubules in the preparations treated with taxol and fixed with the mixture of glutaraldehyde and OsO_4.

In the case of *Arabidopsis thaliana* lipid bodies the situation is analogous as in the case of *A. rosea*. Thus, a hypothesis can be put forward that coexistence of lipid bodies with microtubules in plants is a common phenomenon, which may reflect structural correlation between these organelles.

This work was supported by the grant from Polish Ministry of Science and Higher Education: N N303 359035

44

Role of protein phosphatases PP1/2A in sucrose uptake by root meristem cells of *V. faba*

J. T. Polit

Departament of Cytophysiology, University of Lodz, Pilarskiego 14, 90-231 Lodz, Poland

Author's e-mail: justpoli@poczta.onet.pl

The sucrose induced resumption of replication and mitotic activity in the meristematic cells, blocked by carbohydrate starvation in two principal control points PCP1/G1 and PCP2/G2, requires efficient coordination of sucrose sensors and transporters, metabolic processes as well as mechanisms that control cell cycle progression. Sucrose which is taken by cells serves not only to reconstruct structural or storage polysaccharides, consumed during carbohydrate starvation, but it is also a signalling and regulatory molecule. Signalling network of which sucrose is a member has not been precisely identified yet. It is known that the processes of protein phosphorylation and dephosphorylation, catalyzed by protein kinases and phosphatases respectively, are its essential elements. These enzymes constitute the basic components of signal transduction pathways correlated both with the metabolism and the cell cycle. Previous research showed that between the moment of sucrose application to the cells stopped in PCP1 or PCP2 and the moment of replication and mitotic activity resumption, respectively there was a period of metabolic regeneration which, among others, comprised: starch grain biosynthesis in plastids, cell wall matrix polysaccharide biosynthesis in the Golgi apparatus, as well as increased activity of hexokinase regarded as one of the main enzymes of sucrose signal transduction pathway. For initiation and progress of the above processes, phosphatases PP1/2A are required because okadaic acid (OA), the specific protein phosphatase PP1/2A inhibitor, blocks them completely thus making cell cycle resumption impossible.

The aim of these studies was: firstly – to establish whether the cells take up sucrose with the comparable intensity at the beginning of the metabolic regeneration period, in the time of increased metabolic processes, and at the end of the metabolic regeneration period, when the reactions connected with the activation of replication and mitosis are more intensive; secondly – to establish whether OA, blocking reactions connected with sucrose metabolism also interferes with the process of its uptake from the medium. Label of [^{3}H]sucrose uptake was measured in whole root meristems of *Vicia faba* by liquid scintillation counting.

The results show that the intensity of [^{3}H]sucrose uptake by the cells deprived of nutritious substances is nearly 2-fold smaller during the initial period of metabolic regeneration than in the

period directly preceding the cell cycle resumption. It is probably connected with the significantly weakened metabolic activity in the cells stopped in PCP1 and PCP2 (for example decrease in respiration rate). Moreover, it was demonstrated that OA inhibited the sucrose uptake implicating PP1/2A involvement in dephosphorylation either in direct activation of sucrose transporters or sensors located in plasma membrane or indirect stimulation of proteins participating in their activation.

45

The influence of sucrose depletion or excess on tobacco BY-2 cell culture growth and protease activity

A. Domańska, A. Kobylińska, M. Godlewski

Department of Plant Cytology and Cytochemistry, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: adomansk@biol.uni.lodz.pl

In plants sugars are energetic resources and structural constituents of cells and they also act as important regulators of various processes associated with plant growth and development. The optimal, 2-4%, sucrose concentration in the medium of *in vitro* cultures provides both basic energy source and proper osmotic conditions controlling optimal growth and multiplication. Deprivation of sugar sources causes inhibition of cell proliferation and growth while plant reaction to high sucrose concentration can be species-dependent. In *Saccharomyces cerevisiae* sugars induce apoptosis when there is lack of other nutrients to support growth.

Apoptosis is programmed cell death in response to various stimuli including biotic and abiotic stresses. Cells undergoing programmed death activate the cascade of proteolytic processes such as enzymatic digestion by caspases. On the other hand, during nutrient starvation cells also undergo cellular protein degradation via autophagy. Therefore, proteolytic enzymes are initiated during cell death activation. On the contrary, plants can exude, particularly by roots, many various substances to the environment, among others the proteolytic enzymes, probably as a way to obtain a nitrogen source from the proteins in the medium.

The aim of our work was to compare growth rates of tobacco BY-2 cell cultures on the sucrose-deprived medium or on that with increased sucrose concentration and to check if under such conditions activity of proteases is induced in the cells or in the medium. Experiments: 3-ml samples of the 7-day cell suspension of *Nicotiana tabacum* BY-2 cell culture at stationary growth phase were transferred to 50 ml fresh LS medium containing 0% (sucrose starvation; -suc), 3% (control medium) or 6% (sucrose excess; +suc) (w/v) of sucrose. The cultures were grown in the flasks in darkness, at 25 ^{o}C with the rotation of 100 rpm. The number of cells, their vitality and fresh weight, proteolytic activity in the cell homogenates and in the medium fluids as well as medium pH changes were analyzed for 10 consecutive days.

The obtained results have revealed that: i) tobacco BY-2 cell culture growth on –suc medium was nearly blocked but on +suc was almost so intensive as in the control; ii) in comparison with the control cells significant morphologic changes were observed in the cells already after day 1st on +suc medium and during culture on –suc; iii) during the experiment the medium pH level changed from acid to neutral; iv) the proteolytic activity in the cells was increasing starting from the first day of the culture growth with the highest activity on 4th day and later it was rather low however, activities of proteases in the cells cultured on –suc or +suc media were several times higher than in the control which may be connected with the cell reaction to sugar stress conditions; v) the activity of proteases in the control culture medium appeared at the end of the logarithmic growth phase and

increased during the stationary phase, but in the sugar modified media (-suc, +suc) it was detected at the beginning of logarithmic growth phase.

46

Sugar metabolism in *Arabidopsis thaliana* L. during phosphate deficiency

Edyta Łukaszuk, Agnieszka Żak, Agnieszka Wędołowska, Iwona Ciereszko

Department of Plant Physiology, Uniwersytet of Bialystok,
Swierkowa 20b, 15-950 Bialystok, Poland

Author's e-mail: edytaluk@uwb.edu.pl

Phosphorus is an essential nutrient element, involved in several key plant functions. It is associated e.g. with energy transfer, transformation of sugars, nucleotides, phospholipids (Rychter and Rao 2005). Phosphate deficiency led to changes in plant growth and morphology (e.g. shoot reduction or proliferation of root hairs), metabolism and physiology (e.g. decrease of photosynthesis or respiration, changes in carbohydrate metabolism, mobilization of internal pool of phosphorus) (Ciereszko and Barbachowska 2000, Ciereszko et al. 2001, Rychter and Rao 2005). Such changes are often preceded by induction or repression of genes encoding various enzymes involved in metabolic pathways (Hammond et al. 2004).

The influence of phosphate deficiency on the sugar metabolism in *Arabidopsis thaliana* L. *vs* Columbia (wild type and *pho* mutants) was investigated. Wild type plants were grown for 2-7 weeks in phosphate deficient (-P) and complete nutrient (+P) solution; mutants: *pho1* (the mutant with Pi deficit in leaves) and *pho2* (mutant, that accumulate Pi in leaves) were grown 2-7 weeks in complete nutrient solution. Pi starvation caused reduced Pi content in wt and *pho1* leaves and roots but not in mutant *pho2*. Pi deficit led to an increase in the overall content of glucose and starch in leaves and roots in wild type and *pho* mutants, but had no significant influence on sucrose content in leaves and roots. Phosphate starvation increases activity of enzymes, which play important role in carbohydrate metabolism in plants, like sucrose synthase (EC 2.4.1.13), UDP-glucose pyrophosphorylase (EC 2.7.7.9), acid and neutral invertases (EC 3.2.1.26), ADP-glucose pyrophosphorylase (EC 2.7.7.27).

The data suggest, that plant respond to Pi deficiency by increasing activity of proteins involved in carbohydrate metabolism and sugar concentration in plant tissues. These modifications probably allow *A. thaliana* to acclimate to environmental changes, like low-Pi stress.

References:

Ciereszko I, Barbachowska A. 2000 Sucrose metabolism in leaves and roots of bean (Phaseolus vulgaris L.) during phosphate deficiency. *J. Plant Physiol*. 156: 640-644

Ciereszko I, Johansson H, Hurry V, Kleczkowski LA. 2001. Phosphate status affects the gene expression, protein content and enzymatic activity of UDP-glucose pyrophosphorylase in wild-type and *pho* mutants of *Arabidopsis*. *Planta* 212: 598-605

Hammond JP, Broadley MR, White PJ. 2004. Genetic responses to phosphorus deficiency. *Ann. Bot.* 94: 323-332

Rychter AM, Rao IM. 2005. Role of phosphorus in photosynthetic carbon metabolism. pp. 123-148 in: M. Pessarakli ed. Handbook of Photosynthesis, second edition, Marcel Dekker, Inc., New York

47

Differential response of oat cultivars to phosphate deficiency: acid phosphatase activities and growth

Ewa Żebrowska, Iwona Ciereszko

Department of Plant Physiology, Institute of Biology, University of Bialystok, Swierkowa 20b, 15-950 Bialystok, Poland

Author's e-mail: ewaw@uwb.edu.pl

The deficiency of phosphorus forms that are available to plants is a common feature in most cultivated soils. Plants have developed many strategies to enhance phosphate availability including secretion of organic acid or acid phosphatase (APase). Acid phosphatases (EC 3.1.3.2) nonspecifically hydrolyze monoester soil organic phosphorus at low pH, thereby increasing phosphate (Pi) availability (Duff et al.1994). The aim of this study was to compare 3 different oat varieties *(Avena sativa* L. *vs. Arab, Szakal* and *Polar)* in terms of their response to phosphate deficiency. Therefore, seedlings were cultured for 1-3 weeks in complete nutrient medium (+P, control) and without phosphate (-P). Extracellular APase activity was determined in root exudates, as described by (Ciereszko et al. 2002) and by *in vivo* stain (Gilbert 1999). Intracellular APase activity and inorganic phosphate content in plant tissues were also investigated (Ciereszko et al. 2002). Protein extracts from both root and shoot tissues were run on native discontinuous PAGE to determine which isoform(s) may be affected by phosphate deficiency, according to (Tomscha 2001). Phosphate starvation changed growth characteristics of all studied plants: shoots growth was reduced but elongation of roots was stimulated; root/shoot fresh weight increased about 4-6-times in comparison to the control. Pi deficit had a greater impact on growth of Polar than other oat varieties (Arab and Szakal). Pi content decreased in the leaves and roots of all the studied plants grown in the –P media (up to 5-10% of the control). Phosphate deficiency significantly affected the activity of extracellular APase which increased in comparison to the control plants. The APase secretion was especially intensive from young, growing zones of roots of -P plants, whereas in the mature root parts the activity of extracellular phosphatases was lower. Intracellular APase activity in shoot was significantly higher only for Polar cultivar grown on -P medium, whereas intracellular phosphatase activity in the roots was highest for Szakal variety. Three major APase isoforms were detected in shoots and roots of all plants under study but only one (about 20 kDa) was significantly affected by Pi deficit under all experimental conditions. The results indicate an important role of secreted acid phosphatases in the acclimation of oat to Pi deficiency. The results suggest that the studied oat varieties use different pools of acid phosphatases to acquire Pi from external or internal sources.

References:

Ciereszko I, Janonis A, Kociakowska M (2002) J Plant Nutr 25: 1115 - 1127
Duff SM, Sarath G, Plaxton WC (1994) Plant Physiol 90: 791 - 800
Gilbert G.A., Knight J.D., Vance C.P., Allan D.L. 1999. Plant Cell Environ. 22: 801-810.
Tomscha JL (2001) A PhD Thesis in Plant Physiology. The Pennsylvania State University

This work was supported by Grant from the Polish Ministry of Science and Higher Education, (2007-2010). We wish to thank PODR (Szepietowo, Poland) for oat seeds used in the experiments.

48

Plant roots secrete proteases. Utilization of medium proteins as nitrogen source

B. Adamczyk, M. Godlewski

Department of Plant Cytology and Cytochemistry, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: mgodlews@biol.uni.lodz.pl

Traditionally, it was assumed that plants can take up only the inorganic forms of nitrogen (IN). Now it is well known that also organic nitrogen (especially amino acids) could be an important and even preferred N source in nitrogen plant nutrition. It is assumed that soil proteins and protein-like substances are digested by proteolytic enzymes secreted by soil microorganisms. The liberated amino acids can be used by plants, however, it is well known, that plants compete with soil microbes for these nutrients and moreover accessibility of amino acids can be decreased because soil free amino acid pool is small, they diffuse slowly, and they have short half-life in the soil. If plants secreted proteases by roots, it would increase the amounts of free amino acids in the rhizosphere i.e. close to the root surface and in that way, this kind of nutrients would be more accessible for plants. Our earlier investigations showed such possibility. We discovered and documented the phenomenon of plants ability to secrete proteases by roots (Godlewski and Adamczyk, 2007. Plant Physiology and Biochemistry 45: 657-664). We also showed that the amounts of proteases secreted to medium are species-specific.

The aim of this study was to determine the role of proteases secreted by plant roots in nitrogen plant nutrition. In sterile, hydroponic seedling cultures IN was replaced with protein (kasein), which was the sole N source. Seedling growth and activity of proteases in the medium were compared for two plant species that secrete relatively large amounts of proteases (*Triticum aestivum* cv. Tacher and *Allium porrum* cv. Bartek) and one that secretes barely detectable amount of these enzymes (*Lactuca sativa var. capitata* cv. Ewelina). The seedlings of all three plant species were grown on the following media: a) deionized water, b) Murashige Skoog medium (MS), c) MS without IN, and d) MS without IN, but supplemented with various casein concentrations (0.01 %, 0.1 %, 1 %).

We showed that protein added to the medium of wheat and leek cultures fully compensated the lack of IN in the medium. The weight of seedlings cultivated without IN but with casein was higher than of those cultivated in MS medium. This effect was accompanied by increase in proteolytic activity of the medium and this activity was positively correlated with the concentration of casein in the medium and with growth rate of the seedlings. Contrary to the effects obtained with wheat and leek, with lettuce, which secreted very little proteases, we did not observe similar effects – casein added to the medium did not compensate the lack of IN and showed little effect on proteolytic activity in the medium.

Presence of proteolytic activity in the culture medium of wheat and leek cultivated without protein (deionized water, MS medium) indicates that secretion of proteases is not induced by protein presence in the medium, however addition of protein to the medium increases growth rate of plants and can probably stimulate secretion of proteases as a feedback reaction.

In conclusion, plant species which secrete significant levels of proteases to the medium (e.g. wheat and leek) can use proteins as a nitrogen source, without their digestion and/or their mineralization by soil microbes. However, plants which secrete very little amounts of proteases (e.g. lettuce) do not have this ability.

49

Isoflavone synthase in yellow lupin (*Lupinus luteus* L.)

D. Narożna[1], J. Paś[2], C. J. Mądrzak[1]

[1]Department of Biochemistry and Biotechnology, Poznan University of Life Sciences
Wolynska 35, 60-637 Poznan, Poland
[2]BioInfoBank Institute, Limanowskiego 24A, 60-744 Poznan, Poland

Author's e-mail: dorna@go2.pl

Isoflavonoids are synthesized in plants in the phenylpropanoid biosynthesis pathway. Isoflavone synthase (IFS) catalyses the conversion of naringenin into genistein or liquirtigenin into daidzein as the first enzyme of the isoflavonoid branch of this pathway. In many plant species this enzyme is encoded by small multigene families.

Two full copy cDNA sequences encoding isoflavone synthase (IFS) were selected from a yellow lupin (*Lupinus luteus* L.) root and nodule cDNA library. Sequence alignments and phylogenetic studies on the DNA and protein level of these clones, as compared to the sequences of isoflavone synthase from other plant species, were done. Based on the sequence of the IFS1 clone (FJ539089) the protein modeling of the yellow lupin IFS was performed.

50

Effects of ethylene perception and synthesis of inhibitors on antheridiogenesis and 1-aminocyclopropane-1-carboxylic acid content in *Anemia phyllitidis* gametophytes

A. Kaźmierczak[1], J. M. Kaźmierczak[2]

[1]Department of Cytophysiology, University of Lodz, Lodz, Poland
[2]School of Polish for Foreigners at the University of Lodz, Lodz, Poland

Author's e-mail: kazma@interia.pl

The ethylene perception inhibitors (silver ions, Ag^+; 2,5-norbornadiene, NBD) exerted opposite effects on GA_3-induced antheridia formation and on the 1-aminocyclopropane-1-carboxylic acid (ACC) content in *Anemia phyllitidis* gametophytes. Ag^+ enhanced while NBD inhibited GA_3-induced antheridiogenesis and each inhibitor modulated the level of ACC in a different manner. Cobalt ions (Co^{2+}) and aminooxyacetic acid (AOA), the ethylene synthesis inhibitors, also modulated the level of GA_3-induced ACC content differently. These results strongly confirm the suggestion that ethylene plays an important role of a modulator during "induction" and "expression" phases in GA_3-induced antheridiogenesis. The 3rd h of the "induction" phase is the time when elevation of ACC content induced while in the 6th h inhibited antheridiogenesis. Changes in ACC content and morphogenetic effects of GA_3-induced antheridiogenesis in *A. phyllitidis* gametophytes allowed to indicate that AOA together with NBD could participate in one while Co^{2+} and Ag^+ in another ethylene synthesis and signaling pathway.

51

ROS and RNS involvement in deep dormancy alleviation of apple embryos

U. Krasuska, K. Czajkowska, A. Gniazdowska, R. Bogatek

Department of Plant Physiology, Warsaw University of Life Sciences - SGGW, Warsaw, Poland.

Author's e-mail: u.krasuska@gmail.com

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in regulation of various physiological processes in plants. They play the role in plant responses to exogenous stimuli and are emerging as regulating factors in plant growth and development. Dormancy alleviation and promotion of seed germination is associated with generation of free radicals. Accumulation of ROS and RNS during early phase of germination point to their signaling role in this process.

Mature seeds of apple (*Mallus domestica* Borkh. cv. Antonówka) are dormant and do not germinate unless their dormancy is removed by several weeks of cold stratification. It was demonstrated before that short time pre-treatment by HCN or NO stimulates apple embryo germination and eliminates morphological abnormalities of young seedlings (e.g. asymmetric growth and greening of cotyledons) (Bogatek and Gniazdowska 2006).

The aim of the presented work was to characterize the signaling role of ROS in HCN- and NO-mediated apple embryo dormancy alleviation and ROS role in transition from germination to seedling growth.

Apple embryos were isolated from dormant seeds, and shortly pre-treated by gaseous HCN (6 h), vapors of acidified nitrite or sodium nitroprusside (SNP) (3 h), afterwards embryos were transferred on water. Embryos were cultured in 12/12 h (light/dark) fotoperiod, temperature 20/25 ºC for 7-8 days in growing chamber. The production of ROS (H_2O_2 and $O_2^{-\cdot}$) was measured and localized in the embryos immediately after treatment and 7 days after sowing.

The beneficial effect of HCN and NO on germination of dormant apple embryos was associated with transient accumulation of $O_2^{-\cdot}$ and H_2O_2 in embryonic axes just after HCN and NO pre-treatment. Undisturbed growth of seedlings developed from pre-treated embryos correlated well with enhanced ROS production in cotyledons. The presented data suggest that induction of oxidative stress during early germination implicates successful germination and is necessary for development of young seedling.

Reference:

Bogatek R., Gniazdowska A. 2006. Nitric oxide and HCN reduce deep dormancy of apple seeds. Acta Physiol. Plant. 28: 281-287.

52

Different composition of cell wall thickenings appearing in *Funaria hygrometrica* protonemata in response to Pb^{2+}, from the tip cell wall in control.

Magdalena Krzesłowska[1], Ewa Mellerowicz[2], Marta Lenartowska[3], Sławomir Samardakiewicz[4], Adam Woźny[1]

[1]*Laboratory of General Botany, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61- 614 Poznan, Poland*
[2]*Ůmea Plant Science Center, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, SE-90183 Umea, Sweden*
[3]*Laboratory of Developmental Biology, Institute of General and Molecular Biology, Nicolaus Copernicus University, 87-100 Torun, Poland*
[4]*Laboratory of Electron and Confocal Microscopy, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland*

Autor's e-mail: magdak@amu.edu.pl

Funaria hygrometrica protonemata cultured *in vitro* were treated for 48 h with 4μM Pb applied as an aqueous solution of $PbCl_2$. After that time, cell wall thickenings (CWTs) commonly appeared in the apical cells of protonemata. They were localized in the very tip of the cell (the main place of lead uptake). The thickenings had various shapes and sizes. Their composition was markedly different and more complex than tip cell wall (CW) of the apical cell in control protonemata that contained predominantly the JIM7 and LM6 pectin epitopes. One of the main differences was the high amount of low-esterified (mainly JIM5 epitope) and unesterified pectins (PAM1 epitope) in the CWT. These pectin epitopes were absent from the tip CW of control protonemata and are generally absent from apical CW of tip-growing cells (Li et al., 1994). To our knowledge, this is the first report about low-esterified and unesterified pectin accumulation in response to lead. It is especially interesting because they are able to bind Pb^{2+}, which restricts the entry of these metal ions into the protoplast. Moreover, within CWTs we detected JIM7, LM6 and LM5 epitopes, xylogucans, callose and at least 2 kinds of lipid compounds. Callose and lipids often formed regular layers within the thickenings and therefore could physically block the diffusion of lead ions. Such composition of CWTs could not be the only result of the accumulation of CW compounds delivered from GA to the growing tip. CWTs composition suggested important intracellular alterations within the protoplast caused by lead. One of the main reasons of pectins accumulation in CWTs, in particular JIM5 and PAM1 epitopes, was probably intensification of endocytosis observed in lead treated material. It might be involved in plant cell signaling in stress conditions and its resistance response to this metal. Processes that lead to appearance of other detected compounds are unknown presently.

Nevertheless, the high levels of low- and unestrified pectins and the pattern of callose and lipid distribution in the thickening, strongly suggests that the CWT was functioning in protonema cell as a barrier limiting the amount of lead ions entering the protoplast. Such conclusion is supported by the fact that CWT occurred mainly on the protonema tip, the main site of lead uptake.

This work was supported by grant PBZ KBN-110/PO4/2004. We are grateful to Professor J.P. Knox (University of Leeds, UK) for generous gift of monoclonal antibodies JIM5, JIM7, LM5, LM6, LM7, PAM1, and CCRCM1.

53

Comparative analysis of EDTA and EDDS effects on lead uptake and its distribution in *Pisum sativum* L.

Sylwia Michlewska, Sława Glińska

Laboratory of Electron Microscopy, Faculty of Biology and Environmental Protection
University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: sylwiam@biol.uni.lodz.pl

EDTA and especially biodegradable EDDS are recommended in chelate-enhanced phytoremediation. In order to minimize the phytotoxicity and environmental problems associated with the use of chelators the research should be continued to clarify the ways of metals and their chelated complexes transport and their localization in plant tissues and cell compartments. Therefore the aim of this study was to compare the lead uptake and distribution in the six-day-old *Pisum sativum* cv. Iłówiecki seedlings treated for 1-3 days with aqueous solutions of $Pb(NO_3)_2$ at the concentration of 100 μM or equimolar mixture of lead nitrate and EDTA or EDDS. Plants growing in distilled water were the control.

After 1 day of the experiment the sodium rhodizonate staining revealed Pb presence only in the material growing in lead nitrate solution - it was located mainly in the apical zones of both main and lateral roots. The roots of control and metal-chelate treated plants as well as the shoots in all experimental series were not stained. After 3 days of incubation Pb was detected also in the roots incubated in the mixture of Pb and EDDS, but they contained significantly less metal than those treated only with lead.

The ICP sequential spectrometry revealed that the seedlings growing 1 day in the presence of $Pb(NO_3)_2$ accumulated 10.169 g Pb / kg DW and after 3 days as much as 36.454 g Pb / kg DW. About 99 % of taken up Pb was located in the roots. Both examined chelators decreased Pb concentration in plants but to a different extend. After 3 days of incubation EDTA reduced Pb uptake 40-fold when EDDS only 7-fold. Moreover, EDTA more intensively enhanced the shoot/root ratio of Pb concentration; however, the amount of the metal in the aboveground part of plants was slightly lower than in the material treated only with lead.

The ultrastructural analysis of root meristematic cells treated 1 day with lead nitrate revealed the presence of numerous electron-dense Pb deposits in cell walls and intercellular spaces, few in vacuoles and cytoplasm and sporadically very small ones in cell organelles. Presence of the tested chelators diminished the number of Pb deposits in cell compartments except the cytoplasm in Pb+EDTA treated roots where their number slightly increased. Moreover, when Pb was applied with EDTA the large electron-dense circular structures 0.3-1 μm in diameter appeared in vacuoles while in the case of EDDS – smaller ones (about 0.1 μm) in the cell walls.

The obtained results indicate that EDTA reduced Pb uptake by pea seedlings more efficiently than EDDS but it markedly stimulated the translocation of the metal from roots to shoots. The examined chelators differently affected Pb localization in the root meristem cells. More detailed studies are needed to explain different mechanisms of its action.

54

Cadmium transport across tonoplast occurs on both: the ATP-dependent and proton gradient-dependent ways.

A. Papierniak, E. Kosatka, M. Migocka, G. Kłobus

Department of Plant Physiology, University of Wroclaw, Kanonia 6/8, 50-328 Wroclaw, Poland

Author's email: anna.papiernia@biol.uni.wroc.pl

Contamination of the environment with toxic heavy metals due to anthropogenic activities is a major environmental and human health problem. One of the most hazardous metal pollutant, cadmium, enters the environment mainly from industrial processes and phosphate fertilizers, as well as from the exhaust gases of automobiles. It's concentration in heavily polluted soil can reach 35 μM, even exceed 100 μM (Sanita di Toppi and Gabbrielli,1999 Knight et al., 1997). The excess of heavy metals can drastically affect the growth and development of plants, so these sessile organisms must have evolved different strategies of adaptation and resistance to toxic metals, particularly to Cd. They comprise Cd deposition in cell walls, subcellular compartmentalization in vacuole, and Cd chelation (Zhang et al., 1999). Many data indicate that cadmium accumulates in vacuole of plant cells, but the mechanism driving its transport across the tonoplast is not fully understood. Evidence on energy dependent Cd translocation into vacuole has been growing and even some proteins have been proposed as candidates involved in these processes. These proteins belong to CAX, CDF and ABC families of putative metal transporters.

Here we present evidence on the active cadmium transport into tonoplast vesicles isolated from *Cucumis sativus* root cells. We have tested the accumulation of cadmium ions in highly purified vacuole membranes. Cd accumulation in the vesicles was measured with use of two different sources of energy: ATP or artificially imposed transmembrane pH gradient.

Our results have demonstrated that Cd^{2+} transport across the tonoplast of cucumber root cells occurs by way of two mechanisms. First mechanism is coupled to the activity of V-type ATPase *via* ˙pH (a pH gradient) in a manner similar as the Ca^{2+}/H^{+} and Cd^{2+}/H^{+} antiport operating in the tonoplast of oat root cells (Salt and Wagner, 1993, V. Koren'kov at al, 2007). The kinetic properties of Cd^{2+}/H^{+} transport activity measured as a function of Cd^{2+} concentration was saturable and stimulated by the exogenous supplementation of nutrient solutions with Cd.

The results show also the presence of primary Cd transporter in tonoplast of cucumber root cells, which is directly dependent on ATP. To our knowledge, this work represents the first direct demonstration of a MgATP-energized "heavy metal" active transport system in plant vacuoles.

55

Cadmium influence on antioxidative enzymes activities and H_2O_2 localization in tissues of *Pisum sativum* L. var. Pegaz

K. Głowacka[1], M. Łojko[2], A. Źróbek-Sokolnik[3], A. Okorski[2], R. J. Górecki[1]

[1]Department of Plant Physiology and Biotechnology, Faculty of Biology, University of Warmia and Mazury, Oczapowskiego 1a, 10-719 Olsztyn, Poland
[2]Department of Diagnostics and Plant Pathophysiology, Faculty of Environmental Management & Agriculture, University of Warmia and Mazury, Plac Lodzki 5, 10-727 Olsztyn, Poland
[3]Department of Botany and Plant Protection, Faculty of Biology, University of Warmia and Mazury, Plac Lodzki 1, 10-727 Olsztyn, Poland

Author's e-mail: katarzyna.glowacka@uwm.edu.pl

Cadmium (Cd) is a highly toxic heavy metal, which concentration in air, soil and waters of the earth is continuously increasing due to industrial and urban activities and agricultural practices. This has become a serious world-wide environmental problem for plants, on which the heavy metal exerts a very strong phytotoxicity, but consequently also for livestock and human health, because the cadmium taken up by plants moves throughout the food chain.

In this work, cadmium influence on antioxidative enzymes activity (catalase-CAT, guaiacol peroxidase-GPX, ascorbate peroxidase-APX) and H_2O_2 localization in leaves, stems and roots of *Pisum sativum* L. var. Pegaz was analyzed. Four weeks old plants were treated with water (control), 10, 50, 100 and 200 μM $CdSO_4$. The enzymes activities were measured after 24 hours and one week of cadmium application in leaves and stems, and roots extracts. It has been shown that Cd decreases activities of almost all analyzed enzymes, both after 24 hours and one week of treatment. This effect was observed mostly in roots; only GPX activity in leaves and stems was on the same level as in control plants. Moreover, there has been no statistical correlation between analyzed enzymes activities and cadmium concentrations used at experiment.

The H_2O_2 localization was performed by DAB-uptake method (Thordal-Christensen *et al.* 1997) in leaves, stems and roots, after 24 hours and one week of Cd application. The most intensive cadmium effect was observed in roots after one week of treatment. The microscopical analysis showed the strongest H_2O_2-DAB dependent reaction after application of 100 and 200 μM $CdSO_4$. The H_2O_2 has been observed mostly in rhyzodermis, endodermis and in some cortex cells. The H_2O_2-DAB dependent reaction has been noticed also in the xylem cell walls (in the control plants too). However, the highest level of H_2O_2 was localized in the endodermis close to the xylem cells.

The results obtained in this work suggest that cadmium concentration used in the presented study probably inactivates analyzed antioxidative enzymes (CAT, GPX and APX). This has been noticed mostly in roots of treated plants. Moreover, characteristic H_2O_2 localization in the roots endodermis cells the nearest to the xylem may implicate the very intensive toxication of those cells by cadmium.

56

Exogenous nitric oxide modulates hydrogen peroxide levels in lupine (*Lupinus luteus*) leaves exposed to cadmium and lead

S. Pawlak, J. Chmielowska, A. Mulcan, M. Arasimowicz-Jelonek

Department of Plant Ecophysiology, Faculty of Biology A. Mickiewicz University, Poznan, Poland

Autor's e-mail: pawlaks@amu.edu.pl

Excess of heavy metals catalyses the formation of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide. Elevated levels of ROS induce oxidative damage of important macromolecules in plant cells. This toxic effect may be alleviated by several antioxidative systems. Differences in plant responses to heavy metals seem to depend not only on its concentration but also on the plant antioxidative abilities. Recently, it has been showed that nitric oxide (NO) can alleviate the oxidative stress caused by various stress factors. This effect could be contributed at least partly to the fact that exogenous NO enhances the activity of antioxidant enzymes. It has been proved that plants treated with NO donors such as sodium nitroprusside (SNP) exhibit lower H_2O_2 accumulation under exposure to abiotic stress factors or dysplay only periodical increase of the H_2O_2 level in response to biotic stress. Maintenance of high H_2O_2 concentration can cause membrane damage, protein peroxidation and DNA alteration.

The aim of the current work was to characterize changes in the level of hydrogen peroxide in leaves of yellow lupine (*Lupinus luteus v. Juno)* plants treated with Pb^{2+} and Cd^{2+} ions. Moreover, the role of NO in relation to H_2O_2 accumulation was analyzed.

To examine weather NO modulates H_2O_2 level in lupine leaves, ten days old plants were pretreated with 100µM SNP for 12 h and then were transferred for 48h, 72h and 96h to cadmium ($CdCl_2$) and lead ($PbCl_2$) solutions. Two concentrations of both metals were used: 10mg/l and 25mg/l in case of cadmium and 150 mg/l and 350 mg/l in case of lead. H_2O_2 was assayed with the use of cytochemical detection procedure by a color reaction with 3,3′-diaminobenzidine (DAB), according to Thordal-Christensen *et al*. (1997). The appearance of a red-brown stain on the leaf indicated the presence of H_2O_2.

Leaves treated with increasing concentration of Cd^{2+} and Pb^{2+} ions showed enhanced H_2O_2 accumulation during the first 48 h of stress duration. However, the most intensive staining indicating high level of H2O2 was noted in Pb-stressed (350 mg/l) lupine leaves. Application of exogenous NO to lupine plants resulted in a strong H_2O_2 accumulation early, during first 24 h, after heavy metal treatment. In turn, the considerable reduction of H2O2 amounts was observed under prolonged stress starting from 48 h of Cd^{2+} and Pb^{2+} treatment.

We conclude that the observed early burst of H_2O_2 caused by exogenous NO may induce specific signaling pathway towards protective plant responses to heavy metal stress. Moreover, histochemical detection of H_2O_2 suggests that NO might diminish oxidative stress induced by prolonged exposure to heavy metals.

57

Tissue specific DHN24 dehydrin is associated with acclimate to cold in *Solanum* species

Bartosz Szabała, Tadeusz Rorat

Institute of Plant Genetics, PAS, Strzeszynska 34, 60-479 Poznan

Author's e-mail: tror@igr.poznan.pl

Abundance of a dehydrin designated as DHN24 was investigated in *Solanum sogarandinum* and *Solanum tuberosum* plants differing in ability to acclimate to cold during development and in response to various environmental constraints. The DHN24 belongs to a KS_n-type dehydrins. Under control conditions, noticeable level of the DHN24 protein was observed in stems, tubers, and roots of *Solanum* species. No protein was detected in leaves. During low temperature treatment the DHN24 protein level substantially increased in tubers, in transporting organs and in apical parts, and only a small increase was observed in leaves. The increase in protein abundance was only observed in the plants able to cold acclimate and was found to parallel the acclimation capacity. Upon drought stress, the DHN24 level decreased in stems and in leaves, but increased in apical parts. Immunohistochemical localization of the DHN24 protein demonstrated that under control growth conditions DHN24 was only present in the phloem cells of vascular tissue of roots, stem and leaves. In plants subjected to low temperature the protein localized to the same tissue, however, it the protein was observed in more cells of the phloem tissue. Subcellular localization of the DHN24 protein was analyzed in the isolated nuclei, chloroplasts and mitochondria and in the soluble and appoplast protein fractions. The dehydrin was present in the cytosolic fraction and in the mitochondrial matrix. These results suggest that Dhn24 expression is regulated by organ specific factors in the absence of stress and by factors related to cold acclimation processes during low temperature treatment in collaboration with organ-specific factors. The SK3-type DHN24 dehydrin functions in phloem tissues during plant growth and acclimation to low temperatures.

58

Identification of leaf proteins differentially accumulated during cold acclimation between *Festuca pratensis* plants with distinct levels of frost tolerance

A. Kosmala[1], A. Bocian[1], M. Rapacz[2], B. Jurczyk[2], Z. Zwierzykowski[1]

[1]*Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland*
[2]*University of Agriculture in Kraków, Faculty of Agriculture and Economics, Department of Plant Physiology, Podluzna 3, 30-239 Cracow, Poland*

Author's e-mail: akos@igr.poznan.pl

Festuca pratensis (meadow fescue), the most frost tolerant species within the *Lolium-Festuca* complex, was used as a model for research aimed at identifying the cellular components involved in cold acclimation (CA) of forage grasses. Individual *F. pratensis* plants with contrasting levels of frost tolerance, high frost tolerant (HFT) and low frost tolerant (LFT) plant, were selected for comparative proteomic research. The work focused on the analyses of leaf protein accumulation before CA and after 2, 8, 26 hours, and 3, 5, 7, 14 and 21 days of CA, using a high-throughput two-

dimensional electrophoresis, and on the identification of proteins which were accumulated differentially between the selected plants by the application of mass spectrometry. The analyses of approximately 800 protein profiles revealed a total of 41 (5.1%) proteins that showed at a minimum of 1.5-fold differences in abundance, at a minimum of at one time point of CA for HFT and LFT individuals. It was shown that significant differences in profiles of protein accumulation between the analysed plants appeared relatively early during cold acclimation, most often after 26 hours of CA and a half of all the differentially accumulated proteins were the parts of photosynthetic apparatus. Several proteins identified herein have been reported to be differentially expressed during cold condition in plants for the first time in the current paper. The functions of the selected proteins in plant cells and their probable influence on the level of frost tolerance in *F. pratensis* are discussed. To the best of our knowledge, this work is the first comprehensive proteomic research on CA in monocotyledonous species which have the capacity to cold acclimate and develop frost tolerance.

The research was carried out in the frame of the project of Polish Ministry of Science and Higher Education (No 2 P06A 044 30).

59

Analysis of PsbO gene expression in *Festuca arundinacea* and *F. pratensis* plants exposed to drought and cold treatment

I. Pawłowicz, A. Kosmala

Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland

Author's e-mail: ipaw@igr.poznan.pl

Festuca arundinacea (*Fa*) and *F. pratensis* (*Fp*) are commonly used as models for the research on drought (*Fa*) and frost (*Fp*) tolerance within a group of forage grasses. These species are widely studied in terms of genetic and molecular adaptations to environmental stimuli, including functional analysis of particular genes. Under cell dehydration, induced by water deficit, salinity or low temperature, photosynthesis efficiency is decreased and photoinhibition enhanced. Photoinhibition is determined by the balance between the rate of photodamage of PSII, pigment-protein complex embedded in thylacoid membranes, and the rate of its repair. 33 kDa protein (PsbO) is the component of PSII reaction centre and is involved in generating the redox potential required to drive highly oxidizing water splitting reaction. Analysis of PsbO gene expression at transcript and protein level in *Fa* and *Fp* plants exposed, respectively, to drought and cold treatment was performed. Two *Fp* genotypes – one with the highest and the other with the lowest level of frost tolerance were selected from *Fp* population. Similarly, two *Fa* genotypes – one with the highest and the other with the lowest level of drought tolerance were selected from *Fa* population. *Fa* plants were then exposed to water deficit and *Fp* plants subjected to cold acclimation at 4°C. Plant material was collected at four different time points of stress treatment (1, 4, 7 and 14 day of treatment) and one day before the drought and cold conditions was initiated (as controls). Transcription profiles of PsbO gene by real time RT-PCR and protein abundance by Western blot method during stress treatment were obtained for each analyzed *Fa* and *Fp* genotype. Under water stress a significant decrease in PsbO protein abundance was observed in the most drought-resistant *Fa* genotype, whereas in the most drought-sensitive *Fa* plant remained unchanged. During cold acclimation of both *Fp* genotypes distinct in their level of frost tolerance a slight decrease in the abundance of PsbO protein was observed. The research regarding transcription profiles analysis is ongoing.

60

Preliminary analysis of changes in embryo axis mitochondria during salt stress.

Ł. Wojtyła[1], M. Grabsztunowicz[1], A. Kosmala[2], M. Garnczarska[1]

[1] *Department of Plant Physiology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland*
[2] *Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland*

Author's e-mail: wojtylal@amu.edu.pl

From all abiotic stresses, salt stress is the most severe environmental stress. Knowing mechanisms of plant response to salt stress is very important because acres of land are severely affected by salt. High concentration of salt in soil can affect plants in two ways: by limiting water amount which could be absorbed from soil and by toxic action of salt ions within plant cell. These effects lead to reduced plant growth and development, affect biomass production and overall plant productivity. The root is an organ which has direct contact with salt-contaminated soil. The mechanisms of perception and tolerance of high salinity by roots are important for entire plant. Proper functioning of mitochondrial electron transport chain is essential to provide adequate energy supply. Moreover mitochondria are the main site of production of reactive oxygen species (ROS) in non-photosynthetic plant tissues. The level of ROS increases in stress conditions and may lead to severe damages. That is why we have chosen mitochondrion as a particular organelle to investigation effect of salt stress. In this study we monitored changes in mitochondria from isolated lupine embryo axes growing on modified Heller medium with or without addition of 0,1M sodium chloride. Embryo axes were treated with salt for 24, 48 and 72 hours. Changes in peptide profile were analyzed using 2D-IEF-PAGE electrophoresis. We also analyzed profile of antioxidant enzymes by native electrophoresis. Ultrastructure analysis was carried using TEM-1200 Ex JEOL.

After 24 hours of salt treatment we observed decrease of water content in embryo roots to 68% of fresh weight in comparison to 90% in non- treated roots. In the next hours the differences between treated and non-treated roots were not statistically significant. Native electrophoresis of mitochondrial proteins and staining for catalase activity revealed more intense band in salt treated roots. In both NaCl-treated and control roots maximum CAT activity was observed 48 hours after isolation from seeds. Only one form of superperoxide dismutase, identified as manganese isoform, was observed in mitochondria and no significant differences between control and salt-treated organs could be distinguished. Two-dimension gel electrophoresis of mitochondrial proteins revealed that after 24 hours 38% of proteins had higher and 27% lower level of expression in salt treated roots. After 48 and 72 hours approximately 33% of proteins were both less and more abundant in salt stressed axes as compared to control. Ultrastructure analysis of mitochondria in salt stressed embryo axes and in organs grown without salt revealed more and greater spaces between two membranes in stressed one.

This work was partially supported by Dean of Biology Faculty, Adam Mickiewicz University; grant N PBWB-902/2008.

61

Cytotoxic effects of cyanamide on onion root growth

D. Sołtys [1], A. Rudzińska-Langwald[2], W. Kurek[2], E. Śliwińska[3], A. Gniazdowska[1], R. Bogatek[1]

[1]*Department of Plant Physiology, Warsaw University of Life Sciences - SGGW, Warsaw, Poland*
[2]*Department of Botany, Warsaw University of Life Science – SGGW, Warsaw, Poland*
[3]*Department of Genetics and Plant Breading,*
University of Technology and Life Sciences in Bydgoszcz, Bydgoszcz, Poland

Author's e-mail: kluska10@onet.eu

Allelopathy is defined as interaction between plant-plant, plant – microorganism and microorganisms-microorganisms as well. Positive and negative impact on plants is observed after released of allelopathic compounds into environment.

Cyanamide (CA) is one of the allelochemicals reported as a natural product of hairy vetch, which is utilized as a winter cover crop in orchards and fields. Although CA is well-known as synthetic fertilizer since 1989, its mode of action in plants is still unknown.

In this work the impact of CA on onion (*Alium cepa* L.) root growth was investigated. Onion bulbs were grown in water for 2 days and then transferred to CA water solution (2-10 mM). Bulbs were cultured on CA for additional 6 days in controlled environment. The recovery effect of CA phytotoxicity was also investigated. After one-day treatment by CA solution bulbs were transferred into water and their culture in water was prolonged for 5 days. Lengths, fresh and dry weight of roots were measured. Cell division in root tips was observed using light microscope. We also studied cell cycle in root tip cells using flow cytometric analyses of nuclear DNA content and immunodetection of α–tubulin.

Cyanamide inhibited root growth in dose dependent manner. The strongest inhibition of root growth was detected after exposure to 10 mM CA solution. The inhibition in roots growth was accompanied by disturbances in cell division of root tips. Ultrastructure observation suggests that CA suppressed mitotic cell division, especially methaphases and telophases and inhibited caryokinesis in root tip cells. No mitotic figures were observed after root treatment by 10 mM CA. The lower concentration of allelochemical induced aberrant mitotic figures with highly condensed chromosomes spread over the entire cell.

The presented data suggest that CA provokes fast disturbances in cell division of root tips: drops frequencies of mitosis, suppresses mitoses by abortion the microtubule formation leading to incomplete cell division, however, only slightly modifies cell cycle in root tip cells. Finally it results in root growth arresting.

62

Development of new methods for GMO detection: the role of national reference laboratory.

M. Żurawska-Zajfert, K. Grelewska, A. Linkiewicz, S. Sowa

GMO Controlling laboratory, Plant Breeding and Acclimatization Institute, Radzikow, Poland

Author's e-mail: gmo@ihar.edu.pl

Placing new GMO products on the market requires development of detection and quantification methods according to EU legislation. Moreover methods for GMO unauthorized in EU are needed for monitoring and controlling of our market. Therefore specific methods are essential to meet the requirements of EU regulations concerning labeling and traceability of GMO containing products in the supply chain.

GMO Controlling Laboratory at Plant Breeding and Acclimatization Institute is since 2004 a member of European Network of GMO Laboratories (ENGL) and acts as a national reference laboratory according to Regulation 1981/2006. ENGL was set up to support the Community Reference Laboratory (CRL) in harmonization and standardization of methods for detection and quantification of genetically modified organisms in plant derived products. Supporting ENGL we participate, among others, in method validation studies. The laboratory provides also scientific and technical support to national inspections that act as national enforcement laboratories. Our research projects concentrate on the development of qualitative and quantitative methods for GMO analysis, the impact of genetically modified plants on non-target organisms and the problem of coexistence of conventional, organic and biotech farming. The laboratory organizes training courses and workshops on GMO analysis. Our aim is to provide information and raise public awareness on issues concerning GMOs.

ABSTRACTS OF POSTER PRESENTATIONS SECTION II

The Authors are kindly requested to be at their posters

63

Unusual DNA structures as endogenous mutagen or the source of genetic instability in mycobacterial genome

Ewelina Rychta[1, 2], Anna Brzostek[1], Albino Bacolla[3], Paweł Mackiewicz[4], Jarosław Dziadek[1]

[1]*Institute for Medical Biology, Polish Academy of Science, Lodz, Poland*
[2]*University of Lodz, Lodz, Poland*
[3]*Texas A&M University System Health Science Center, Texas Medical Center, Houston, USA*
[4]*University of Wroclaw, Wroclaw, Poland*

Author's e-mail: erychta@interia.pl

Several sequences of DNA fragments are polymorphic and are able to exist as the normal, commonly known, right-handed, antiparallel, double helix, generally referred to as B-DNA or adopt alternative conformations that differ dramatically from B-DNA. This state is a function of sequence, topology (supercoil density), ionic conditions, protein binding, methylation, carcinogen binding, and other factors. Non-B conformations include hairpins, cruciforms, triplexes, tetraplexes, Z-DNA, bent DNA, nodule DNA, flexible, writhed DNA, slipped structures and sticky DNA. Non-B conformations promote genetic instabilities and could lead to many serious biological effects.

The main goal of this study was identification of unusual DNA structures in mycobacterial genome and the evaluation of their influence on genetic instability.

We constructed vectors carrying unusual, non-B conformations accompanied by marker genes essential to evaluate the vectors stability. The engineered vectors were constructed on the base of self-replicating shuttle plasmid pMV206 and integration plasmid pMV306. Both systems carried synthetic oligosequences cloned in orientation prone to form cruciform or slipped structures. Constructed vectors were tested in *E. coli rec* A^+ and *rec* A^- strains and then introduced into wild type and DNA repair defective strains of *M. smegmatis*.

Cruciform and slipped structures could be composed within constructed vectors. The stability of non-B conformations forming within investigated sequences introduced into *E. coli* depends on the presence of Rec A. Investigated sequences prone to form cruciform structure are less stable in *E. coli* rec A^+ than rec A^- strains, what shows that in *E. coli* HR process is involved in instabilities of DNA regions reach in this sequences.

Wild type and mutated *M. smegmatis* strains possessing integrated pMV306 vectors carrying sequences potentially prone to form slipped structure could lose Gm resistance gene with low frequency and at the same time could reconstruct Hyg resistance gene in HR independent process.

64

Streptomyces coelicolor Ku-proteins and their interactions

Małgorzata Guzek[1, 2], Anna Brzostek[1], Katarzyna Ginda[3], Dagmara Jakimowicz[3], Jarosław Dziadek[1]

[1]Institute for Medical Biology, Polish Academy of Science, Lodz, Poland;
[2]University of Lodz, Lodz, Poland
[3]Institute of Immunology and Experimental Therapy, Polish Academy of Science, Wroclaw, Poland

Author's e-mail: malgorzata.guzek@gmail.com

Double Strand Breaks (DSBs) are the most dangerous form of DNA damage caused by a variety of endogenous and exogenous agents and or reactive oxigen species, IR, desiccation or several chemotherapeutic agents. Left unrepaired, DSBs can lead to cell death, while incorrect repair can lead to gross chromosomal rearrangements. Cells have developed two major pathways to repair DSBs: homologous recombination (HR) and non-homologous and joining (NHEJ). The key factor of NHEJ is Ku protein. The eukaryotic Ku is a heterodimer Ku70/80 acting by binding DNA ends at DSBs with high affinity and aligning them, protecting from unspecific nucleolytic degradation. Eukaryotic Ku is also responsible for recruiting ligase IV/XRCC4 complex, consequently stimulating its ligation activity. Moreover, Ku protein in mammalian cells is engaged in cell signaling. Unlike their eukaryotic counterparts bacteria contain a single gene encoding a small Ku- like protein that exhibits homology with the core domain found in Eukaryotes. Mt-Ku binds to free linear DNA ends in a sequence-independent manner and interacts directly with bacterial DNA ligase to promote end joining. On the other hand genome sequence of *Streptomyces coelicolor* has revealed two *ku* genes encoding Ku-like proteins.

In this study we focus on identification of Ku-1_{St} and Ku-2_{St} interactions and/or selfbinding capabilities and screen the library of *S.coelicolor* looking for other proteins interacting with Ku-like proteins in this organism. To investigate the interactions of *Streptomyces* Ku proteins we used the Bacterial Two-Hybrid System based on reconstitution of the adenylate cyclase activity in *Eschericha coli.*

Using that method we confirmed the selfbinding of *S.coelicolor* Ku-1 protein and direct interactions between Ku-1 and Ku-2 proteins. Moreover, screening of the library led to the identification of other proteins interacting with Ku. The role of identified the putative *S.coelicolor* proteins binding to Ku-1 and/or Ku-2 proteins in NHEJ pathway will be evaluated by further examination.

65

Positive expression correlation of 5' overlapping genes

Joanna Ciomborowska, Natalia Lewandowska, Izabela Makałowska

Institute of Molecular Biology and Biotechnology, Adam Mickiewicz Universit,, Umultowska 89, 61-614 Poznan, Poland

Author's e-mail: joannac@amu.edu.pl

Although complete human genome is known since 2002 we keep being surprised by phenomena and genomic novelties. Examples of such are overlapping genes, for long time being considered to

be common only in prokaryotic genomes. Number of reports showed that overlapping genes are frequent in eukaryotic genomes. About 13% of protein coding genes in human genome share locus with gene on opposite strand. Despite number of studies, we still cannott answer why so many genes do overlap while big chunks of genomic space remain deserted. Some findings suggest that natural antisense transcripts play an important role in the gene regulation. However, the interaction between antisense and corresponding sense transcript does not follow any unified pattern and it is not clear whether antisense regulation is a common or an exceptional event in eukaryotic genomes. Few models have been proposed for the regulation of gene expression by *cis*- antitranscripts. Several studies showed that sense-antisense transcripts tend to have lower expression level, to be co-expressed and/or inversely expressed more frequently than expected by chance.

We performed expression analysis of human genes overlapping at 5' ends. We identified 146 such gene pairs in human genome. Analysis of expression pattern of identified genes was done based on ESTs data and SymGen microarray data. We analyzed the level of expression, measured by the number of ESTs, of human 5' overlapping genes and compared it with an expression level of randomly selected reference set. Both sets did not differ significantly in the distribution of ESTs representing these genes (Mann-Whitney U-test $z=0.04$, $p=0.97$). Due to the data availability, the expression correlation analysis was possible only for 86 pairs of human genes. For each pair we calculated Pearson correlation coefficient and identified 24 pairs of 5' overlapping gene showing significant expression correlation ($p < 0.05$). In all cases, the correlation was positive and there was no instance of significant negative correlation. Positive expression correlation was observed in majority for pairs of ubiquitous expression pattern. Our results contradict previously published analyses and suggest that a mechanism of overlapping genes expression regulation is not uniform and may depend on the overlap pattern.

66

An introduction to study of relations between eukaryotic gene structure and protein transmebrane topology. Application to 7TM receptor genes present in selected eukaryotic genomes.

Konrad Józef Dębski [1], Krzysztof Pawłowski[2,3]

[1] Laboratory of Genetics, Department of Potato Genetics and Parental Lines, Mlochow Research Center, Plant Breeding and Acclimatization Institute, Mlochow, Poland
[2] Laboratory of Bioinformatics and Systems Biology, Department of Molecular and Cellular Neurobiology, Nencki Institute of Experimental Biology Warsaw, Poland
[3] Department of Experimental Design and Bioinformatics, Warsaw University of Life Sciences, Warsaw, Poland

Author's e-mail: konraddebski@gmail.com

The organization of genetic information is one of the most important questions for modern genetics and bioinformatics. Eukaryotic genes have in their structure both coding (exons) and non-coding (introns) sequences. The complex structure of genes was discovered in 1970s. The domain structure of proteins was discovered in 1970s and 1980s. It was hypothesized that every exon coded an autonomic functional and structural unit of a protein. However, more recent newer investigations showed that this hypothesis was not completely true. It happens that introns disrupt gene fragments coding functional units of the protein. But it was also showed that introns very rarely disrupt gene fragments coding alfa-helices or beta-sheets.

The knowledge about structures coded by exons is very useful for the understanding of the evolution of gene and protein structures. To date, relationship between protein transmembrane domains and the exon structure of the corresponding gene have not been analysed. For preliminary

investigations, seven transmembrane (7TM) receptors (also known as G protein-coupled receptors, GPCRs) were chosen. The 7TM group is a very good material to investigate gene structure and its evolution. This super-family of receptors is a large and diverse group. These receptors have a common structure pattern and they are present in many organisms, including most eukaryotes. Comparison of gene structure with transmembrane topology of 7TM receptors (exactly studying correlation between positions of exon borders and transmembrane domain borders) should help us to verify hypothesis about relationship between exons and structural domains of proteins. However, in this study, the distributions of exon positions in relation to transmembrane regions do not differ in a statistically significant manner from a random distribution. We can only see some diversity in exon distribution in relation to protein secondary structure elements.

We suspect that lack of statistically significant differences between control and test groups is related to the way of producing control groups. We generate random structures of the 7TM receptors using the distribution of real gene structures and we think that this limited the randomness. Our next step is to investigate many different transmembrane receptors but this time we want to use as control groups random exon structures.

67

CAG repeat polymorphism in androgen receptor gene and breast cancer

E. Forma[1], M. Bryś[1], H. Romanowicz-Makowska[2], W. M. Krajewska[1]

[1]*Department of Cytobiochemistry, University of Lodz, Lodz, Poland*
[2]*Department of Pathology, Research Institute of Polish Mother's Memorial Hospital, Lodz, Poland*

Author's e-mail: forma24@o2.pl

Although the androgen receptor (AR) has been found to be expressed in female breast tumors, its role in breast cancer remains yet unclear. The *AR* gene is localized on chromosome X and its exon 1 characterized by CAG repeats encodes tract of 9-32 glutamines. The length of the CAG repeats varies among individuals and this polymorphism is believed to be related to AR transcriptional activity. We conducted study to determine whether variation in CAG repeat length within *AR* is a risk factor for breast cancer in the polish population.

Samples of 67 primary breast ductal carcinomas were obtained from patients (age range 32-78 years) undergoing surgery for breast neoplasm. The pathological evaluation report was obtained for each patient. Size of *AR* CAG alleles according to fluorescent-labeled polymerase chain reaction was determined by using a 377 DNA Sequencer running Genescan and Genotyper software. Of the 67 patients with primary breast cancer, 61 were heterozygous and 6 were homozygous. The mean CAG repeat number for homozygous women was 22, while for heterozygous women the mean repeat number was 20 for the short allele and 24 for the long allele. The length of CAG repeats either in one allele or in both alleles was inversely correlated with the histological grade of breast cancer. An association between positive lymph nodes and shorter CAG repeats in both alleles was also found.

Our results suggest that the CAG repeats may play a role in female breast cancer progression.

68

Nuclear events in apoptosis of leukemic cells induced by purine analogs combined with cyclophosphamide

A. Kaźmierczuk[1], J. D. Żołnierczyk[1], J. Z. Błoński[2], M. Rogalińska[1], B. Cebula[2], P. Smolewski[2], J. Węsierska-Gądek[3], T. Robak[2], Z. M. Kiliańska[1]

[1] *Department of Cytobiochemistry, University of Lodz, Lodz, Poland*
[2] *Department of Hematology, Medical University of Lodz, Lodz, Poland*
[3] *Department of Medicine I, Medical University of Vienna, Vienna, Austria*

Author's e-mail: zkilian@biol.uni.lodz.pl

B-cell chronic lymphocytic leukemia (B-CLL) is thought to be caused by an imbalance between birth and death of B-lymphocytes mainly due to their apoptosis and deregulation of cell cycle. Virtually all circulating B-CLL lymphocytes are arrested in the G_o/G_1 phase of cell cycle; however, a proliferating compartment is present in specialized structures located in lymph nodes and bone marrow of patients.

We have investigated the effect of combined treatment with purine analogs plus alkylator – cyclophosphamide, i.e. CC (cladribine + cyclophosphamide) or FC (fludarabine + cyclophosphamide) on some apoptotic events in primary B-CLL cell *in vivo* and *in vitro*. Freshly isolated leukemic cells from blood samples of untreated and drug-treated patients were fractionated into nuclear and postnuclear (cytoplasmic) fractions. Expression level of some nuclear proteins and cytochrome c involved in apoptosis of examined cells were determined by Western blot technique.

In this study, we observed that in primary leukemic cells histone H1.2 was released from nuclei of model cells to the cytoplasm after CC and FC treatment. The release of histone H1.2 is accompanied by an appearance of cytochrome c in the cytoplasmic fraction. Additionally, the proteolysis of nuclear proteins – poly(ADP-ribose)polymerase (PARP-1) and lamin B, what confirmes caspase-3/-7 activity, was shown.

In the *in vitro* study we have evaluated the chemosensitivity of leukemic cells to cladribine, fludarabine, R-roscovitine, mafosfamide, as well as to their combinations with mafosfamide (an active form of cyclophosphamide *in vitro*). R-roscovitine, a very promising anticancer agent is a small molecular weight inhibitor of several cyclin-dependent kinases.

The results from the *in vitro* experiments of B-CLL cells exposed to individual agents and their combinations revealed a good agreement between the reduction of viable cell levels and the induction of cell death as reflected by evaluation of the hallmarks of apoptosis such as PARP-1 and lamin B cleavage, fragmentation of DNA, and the increase in sub-G1 DNA content. The obtained data indicate very high activity of R-roscovitine in the killing of B-CLL cells and in rapid induction of their apoptosis.

In conclusion, obtained data suggest that among innovative, therapeutic approaches, use of R-roscovitine might be effective in achieving good results in B-CLL cells apoptosis induction and its application in clinical practice.

69

Changes of the level of thiol groups in blood platelet and plasma proteins obtained from patients with breast cancer

M. Kędzierska[1], B. Olas[1], B. Wachowicz[1], A. Jeziorski[2], J. Piekarski[2]

[1] *Department of General Biochemistry, University of Lodz, Poland,*
[2] *Department of Oncological Surgery, Medical University of Lodz, Poland*

Introduction: Breast cancer is the most popular malignant cancer towards women in Poland and in the whole world. This multistage process of cancer formation and development is associated by the lose of redox balance in the cell, overproduction of reactive oxygen and nitrogen forms, inhibition of antioxidant enzymes such as: superoxide dismutase, glutathione peroxidase, glutathione S-transferase, and catalase. All of these factors are responsible for phenomenon consolidation - oxidative stress, reinforced by operative procedure in case of patients with breast cancer. Both plasma and blood platelets as very active metabolism structures are highly sensitive to negative oxidative stress influence and are repeatedly ahead of hematological and nonhematological toxicity phenomenon in patients with breast cancer.
The aim: The aim of the our experiments was to research the changes of the level of thiol groups in blood platelet and plasma proteins from patients with benign breast diseases, patients with invasive breast cancer and healthy volunteers.
Research material: will be constituted by plasma and blood platelets separated from freshly drawn blood, taken from healthy volunteers aged and patients with benign and invasive breast cancer. The protocol was accepted by the Committee for Research on Human Subjects of the Medical University of Lodz number RNN/252/07/KB.
Research methods: The amount of free thiol groups in platelet and plasma proteins was estimated with 5,5'-dithio-bis(2-nitro-benzoic acid) (DTNB).
Results: Our studies demonstrated that the concentration of thiol groups in platelet proteins from patients with breast cancer differs from the concentration of thiol groups in platelet proteins obtained from healthy volunteers. The concentration of thiol groups in platelet proteins from patients with breast cancer was significantly lower (about 50,6%) than in blood platelets obtained from healthy volunteers. We have observed the same process in plasma proteins - the concentration of thiol groups was significantly lower (about 33,4%) than in plasma proteins obtained from healthy volunteers. In conclusion, the consequence of the oxidation of thiol groups in platelet or plasma proteins may be the alteration in protein structure associated with changes of hemostatic functions of blood platelets and plasma in breast cancer.

This work was supported by grant 505/373 from University of Lodz.

70

Association between of the c.73G>A (Val25Met) polymorphism of the *UBC9* gene and breast cancer

E. Synowiec[1], R. Krupa[1], A. Sobczuk[2], M. Pawlak[3], J. Błasiak[1], K. Woźniak[1]

[1] *Department of Molecular Genetics, University of Lodz, Lodz, Poland*
[2] *Polish Mother's Memorial Hospital, Lodz, Poland*
[3] *Department of Surgical Oncology, N. Copernicus Hospital, Lodz, Poland*

Author's e-mail: ewelinas@biol.uni.lodz.pl

Breast cancer is one of the primary female cancers globally and is the second leading cause of cancer death. DNA double-strand breaks (DSBs) are the most severe type of DNA damage and several studies suggest a link between deficient repair of DSBs and genetic predisposition to breast cancer. RAD51 and RAD52 are key components of the eukaryotic homologous recombination (HR) machinery and directly interact with UBC9. UBC9 (E2) SUMO conjugating enzyme play an important role in the DNA repair pathway via HR and genome integrity. We performed a case-control study to test the association between polymorphic variants of the *UBC9* gene (Val25Met, rs11553473) and breast cancer risk. Genotypes were determined in DNA from peripheral blood lymphocytes of 119 breast cancer patients and 153 healthy individuals by allele-specific PCR (ASO-PCR).

Breast cancer occurrence was strongly associated with the *UBC9* heterozygous polymorphic variant G/A (OR 7.61; 95% CI 4.06-14.30), whereas homozygous variant G/G was associated with significant reduction in breast cancer risk (OR 0.14; 95% CI 0.07-0.25).

We also correlated polymorphic variants of the *UBC9* gene with clinical characteristics of breast cancer patients: hormone receptors (estrogen and progesterone receptors), epidermal growth factor receptor (HER2) expression, and lymph node metastasis. We observed a strong association between the G/A genotype of the *UBC9* – c.73G>A polymorphism and the expression of the estrogen (OR 3.43; 95% CI 1.25-9.44) progesterone (OR 2.33; 95% CI 1.06-5.12) and HER2 (OR 2.50; 95% CI 1.01-6.15) receptors. We did not observe any association between studied polymorphism and breast cancer progression evaluated by lymph node metastasis.

The present study suggests a link between the *UBC9* gene polymorphism (c. 73G>A) and breast cancer risk among Polish women. This polymorphism may play a role in breast cancer development.

71

Prooxidant activity of pyrroline and pyrrolidine nitroxides in MCF-7 breast cancer cells: interaction with doxorubicin

Karolina Matczak[1], Aneta Koceva-Chyła[1], Krzysztof Gwoździński[2], Zofia Jóźwiak[1]

[1]*Department of Thermobiology,* [2]*Department of Molecular Biophysics, University of Lodz, Lodz, Poland*

Doxorubicin (DOX) is an effective anthracycline antibiotic, which is widely used as an important component of treatments for breast cancer. Higher doses of (DOX) ultimately cause dilated cardiomyopathy (DCM) and congestive heart failure (CHF) as serious side effects in treated patients. Thus, the accepted dose of DOX is limited to less than required for killing cancer cells. It

is commonly accepted that DOX cardiotoxicity is caused by mitochondrial oxygen species (ROS), generated in the redox reactions of drug during its biotransformation inside the cell. The heart is a very sensitive tissue to oxidative stress because of its highly oxidative metabolism and a lower level of antioxidant defense compared with other organs, e.g. the liver. One of strategies to reduce an oxidative stress in normal cells is combined treatment with antioxidants. Considerable efforts with different results have been made in using antioxidants and metal ion chelators to decrease the side effects of DOX administration and to enhance its chemosensitivity. At the same time such antioxidants are expected to retain antitumor effectiveness of DOX.

The aim of our study was to examine the effect of pyrroline and pyrrolidine nitroxides Pirolid (PD) and Pirolin (PL) on reactive oxygen species generated by doxorubicin in MCF-7 in breast cancer cells. Oxidative stress, which is responsible for oxidative damage to DNA, cell plasma membrane and induction of apoptosis, is an important mechanism of DOX anticancer activity. The nitroxides are stable organic nitric oxygen species, which contain nitroxyl functional group. They provide protection against oxidative stress via various mechanisms including SOD-mimic activity and detoxification of carbon-, oxygen-, and nitrogen-centered radicals, as well as oxidation of reduced transition metals. In contrast to the common concept, according to which the nitroxides' protective effect takes place via inhibition of the Fenton reaction, there are observations suggesting the opposite.

In our experiments MCF-7 cells were incubated with IC_{50} concentration of doxorubicin (3 μM) for 2 h and with 50 μM concentration of nitroxides for 3 h. When combination of both compounds was used the cells were pretreated with Pirolin or Pirolid for 1 h before the addition of DOX. H_2DCFDA fluorescent probe was employed to measure intracellular peroxide formation directly in cell monolayer. Kinetics of ROS formation was monitored over 0-180 min time period under cell culture conditions using fluorescent microplate reader.

We observed appreciable level of deacetylation and oxidation of DCFH-DA to fluorescent DCF not only in cells treated with DOX but also in those treated with nitroxides. In all cases a considerable and progressive increase in ROS production over time was found. It should be emphasized that in our experimental model Pirolin and Pirolid generated larger amount of reactive oxygen species than DOX. Moreover, these nitroxides enhanced intracellular peroxide formation by doxorubicin.

Our results demonstrate that pyrroline and pyrrolidine nitroxides do not reduce oxidative stress induced by DOX in breast cancer cells. Moreover, in certain conditions these antioxidants display prooxidant activity and augment oxidative stress induced by doxorubicin. These properties of pyrroline and pyrrolidine nitroxides and their ability to promote peroxide formation in cancer cells make them good candidates for eventual use in anticancer adjunctive therapy with doxorubicin.

This work was supported in part by Grant N 401 2337 33 of Polish Ministry of Science and High Education.

72

The interaction of paclitaxel and doxorubicin in human breast cancer cells

Karolina Matczak[1], Aneta Koceva-Chyła[1], Krzysztof Gwożdziński[2], Zofia Jóźwiak[1]

[1]Department of Thermobiology, [2]Department of Molecular Biophysics, University of Lodz, Lodz, Poland

Doxorubicin, a quinone-containing anthracycline antibiotic, is an important agent against a wide spectrum of human neoplasms. However, its high toxicity limits usage in cancer chemotherapy. In most patients doxorubicin (DOX) dose escalation does not improve disease-free or overall survival but do substantially increased cardiotoxicity, hematologic toxicity, and stomatitis. In contrast, the

addition of paclitaxel to DOX chemotherapy added only modest toxicity and led to a 17% and 18% decrease in the risk of recurrence and death, respectively.

Paclitaxel (Taxol) is an anticancer drug isolated from the bark of *Taxus brevifolia* and first taxane to show activity in breast cancer. The introduction of paclitaxel (PTX) into chemotherapy of metastatic breast cancer (MBC) as single agent or in combination with other anticancer drugs has yielded very encouraging results. In numerous phases II trials of MBC clinical response rates (cRR) between 56% and 62% were achieved. Paclitaxel has specific and unique cytotoxic mechanism of promoting assembly and preventing disassembly of microtubules. This anticancer drug arrests and subsequently kills cells in the G2 and M phases of the cell cycle due to disruption of microtubule dynamics. PTX has been shown to be partially non-cross resistant with many other anticancer drugs, including anthracyclines. It has been demonstrated to be an active drug in patients with breast cancer heavily pre-treated with doxorubicin (DOX). Currently PTX is mainly used for breast and ovarian cancer treatment, especially in patients resistant to doxorubicin. Paclitaxel given after standard chemotherapy has been shown to improve significantly outcomes in early breast cancer too. Because of its clinical importance, there has been interest in the combination of paclitaxel and doxorubicin.

The aim of our study was to elucidate the cytotoxic effects of combination of PTX and DOX given at various schedule on human breast cancer cells and to evaluate the schedule-dependent interaction of doxorubicin and paclitaxel in order to determine the schedule best suited to provide the basis for improved therapeutic benefit in combination chemotherapy.

Paclitaxel at IC_{50} concentration (0.4 μM) was administered in conjunction with DOX (0,5 – 7 μM) to human breast cancer cells MCF-7. The cells were exposed sequentially or simultaneously to the two drugs for 2 h and then incubated in drug-free medium for 72 h. In sequential treatment the cells were first incubated for 2 h with DOX, then PTX was added and incubation was continued for additional 2 h. The cell growth inhibition was determined by the MTT assay. The cytotoxic interaction of both drugs was determined at the IC50 level estimated on the basis of the survival curves. Comparable level of cytotoxicity was found for the single DOX treatment (IC_{50}DOX=3 μM) and for the DOX and PTX combination on sequential exposure to DOX followed by PTX (IC_{50} DOX=2.6 μM). About 30-fold increase in cytotoxicity was observed for the combined DOX and PTX treatment on simultaneous administration of both drugs (IC_{50}DOX=0.9 μM). These results suggest that combination of DOX with PTX and simultaneous administration of the two drugs may be better schedule for effective kill of human breast cancer cells than single DOX treatment or sequential administration of DOX followed by PTX. Further studies are required to evaluate the interaction of both drugs and their antitumor effect against different type of tumor cells in order to find the most suitable sequence to be tested for their administration in clinical practice.

73

The role of angiotensin II type 1 and 2 receptors blockers in the modulating cell proliferation of hormone-dependent prostate cancer line

K. Domińska, A. Piastowaska, A. Lachowicz-Ochędalska

Department of Comparative Endocrinology, Medical University of Lodz, Poland.

Author's e-mail: kamila.dominska@umed.lodz.pl

Angiotensin II is the most active multifunctional hormone of the local renin-angiotensin system. Apart from its classical actions, Ang II are involved in the regulation of cell growth, proliferation, cell migration, apoptosis, inflammation, differentiation, angiogenesis, which suggests that the peptides might also play role in a cancer. It is common knowledge that this peptide interacts with at least two types of receptors: AT1 and AT2. It has been generally accepted, that Ang II induces cell

proliferation by activating its AT1-receptor but stimulation of the AT2-receptor inhibits cell growth in different cell types. Although, there are some notes that both receptors can generated the signal for proliferation and apoptosis.

We examined influence of varied concentrations (10^{-11} - 10^{-9} M) of angiotensin II on cell proliferation in hormone-dependent prostate cancer lines. Secondary, we tested potential association of the effects of Ang II with two type of angiotensin receptor through applied the selective and nonpeptidic antagonists of the AT1 (losartan) and AT2 (PD 123319). The cell growth was measured non-isotopic immunoassay. The method analyzes the proliferation of cells by utilizing bromodeoxyuridine (BrdU) as an analog of the DNA nucleotide, thymidine, which incorporates into the synthesized DNA of actively dividing cells. The extent of BrdU incorporation is reflected in the intensity of absorbance of the final reaction.

Our dates clearly indicate that Ang II significantly increased the cell proliferation in androgen-dependent prostate cancer cell line. Expectedly the levels of stimulation were concentration-dependent and time-dependent. The exposure of LNCaP cells to Ang II together with 10^{-6}M losartan, decreased cell proliferation below basic values. Additionally, losartan alone significantly decreased the cell proliferation, too. In contrast, the specific receptor blocker AT2 stimulated the proliferation of LNCaP cells when was added to the culture medium together with Ang II. Nevertheless, the increased proliferation after exposure LNCaP in combination with PD123319 and Ang II in comparison to Ang II alone was poorer.

In conclusion, the results demonstrated that Ang II could play an important role in cell proliferation in hormone-dependent cell line. The Ang II exerted growth-stimulatory effects in LNCaP cells by AT1 receptor. Therefore, AT1 receptor blockers could be useful anticancer drugs for early stage, androgen-dependent prostate cancer. The clinical potential of manipulating the angiotensin system *seems promising* in the *treatment* prostate cancer.

Supported by MNISW Grant NN 403 355 533

74

The effect of hydrogen peroxide induced oxidative stress on *GABA-shunt* enzymes activity in PC12 and GH3 cell lines.

A. Kowalski, L. Żylińska, T. Boczek, E. Rębas

Department of Molecular Neurochemistry, Medical University of Lodz, Poland

Author's e-mail: akwlsk@gmail.com

GABA-shunt is highly important metabolic mechanism of maintaining the balance between glutamate and γ-aminobutyric acid – the major neurotransmitters in the central nervous system. Three enzymes involved in this process are glutamate decarboxylase (GAD), GABA-transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH). Due to the fact that the final product of *GABA-shunt* is Krebs cycle intermediate – succinate, we can consider GABA as a potential, alternative energetic substrate in the cell. Also, in consequence of this process ATP is generated from glutamate without production of ammonia. Moreover, excess of glutamate, toxic because of increasing Ca^{2+} inflow to the cell by NMDA receptors, can be eliminated by *GABA-shunt* pathway.

The aim of our work was to examine how the oxidative stress induced by hydrogen peroxide affects the activity of *GABA-shunt* enzymes in *Rattus norvegicus* cancer cell lines – PC12 and GH3. Both of examined lines possess the complete set of *GABA-shunt* enzymes. Additionally, we examined the aforementioned effect under disordered calcium homeostasis conditions, in stably

transfected PC12 cell line with suppressed expression of plasma membrane calcium ATPase isoform 3 (PMCA3).

Cells were cultured for 4 days in standard medium and then for 24, 48 and 72 hours in medium containing 200 μM hydrogen peroxide. The activity of glutamate decarboxylase was assayed in postnuclear fraction, using spectrofluorimetric method based on detection of GABA condensated with ninhydrin. GABA-transaminase and succinic semialdehyde dehydrogenase activities were estimated in mitochondrial fraction by spectrophotometric measurement of NADH formation.

Our results show that H_2O_2 in time-dependent manner changes the activity of tested enzymes. The greatest effect (increase in GAD activity) was observed after 72 hours of application. The influence of H_2O_2 on *GABA-shunt* enzymes activity was similar in both examined PC12 cell lines, but more significant in line with suppressed PMCA3. In GH3 cells we observed gradual increase in GAD activity during H_2O_2 treatment. Changes in GABA-T and SSADH activities were dependent on time of H_2O_2 exposure. The results may suggest that protective mechanism of *GABA-shunt* can be different in examined types of cells and can depend on calcium homeostasis.

This work was supported by the Medical University of Lodz grants 502-16-810, 502-16-809 and 503-6086-2.

75

Suppression of PMCA2 or PMCA3 affects the differentiation process in PC12 cells.

T. Boczek, A. Kowalski, L. Żylińska

Department of Molecular Neurochemistry, Medical University, 92-215 Lodz, Poland

Author's e-mail: tb9v@virginia.edu

Ca^{2+} plays an unique role in the proper functioning of neuronal cells and fluctuations in its concentration are one of the factors involved in signal transduction mechanisms. Keeping the cytosolic Ca^{2+} concentration in the nanomolar range and its return to the basal level after neuronal hyperpolarization is ensured by Ca^{2+}-ATPase (PMCA). This enzyme posesses four isoforms with PMCA2 and PMCA3 being unique for neuronal tissue.

Pheochromocytoma cell line (PC12) is a widely used and well defined model for studying neuronal processes. One of its unique feature is the ability to differentiate into pseudoneuronal phenotype after stimulation with growth factors but the similar effects can be mimic by dibutyryl-cAMP (db-cAMP).

Our previous research showed that either transient or stable transfection of the non-differentiated cells with antisense mRNA directed to neuro-specific isoforms of PMCA induced morphological changes similar to the early stages of neurogenesis. Here, we report the effect of diminished expression of PMCA2 or PMCA3 on the differentiation process in PC12 cells induced by db-cAMP. Cell lines with suppressed PMCA2 or 3, as well as a control line, were threated with 1mM of db-cAMP for 72h. All examined lines exhibited the tendency to form numerous neurites arranged in a compact net of synaptic connections. The differentiation process altered also a cellular morphology with the changes being the most visible in the somatic part. The total amount of PMCA detected with 5F10 monoclonal antibodies after 48h of stimulation, was decreased by 10% in a line with suppressed PMCA2 and by 30% in a line with decreased PMCA3, respectively. Moreover, in all transfected lines the amount of blocked isoforms was highly decreased. This may suggest the rearrangement in a bilayer density of PMCA in both transfected lines. The changes in PMCA amounts were accompanied with increased in sarco/endoplasmic Ca^{2+}-ATPase isoforms (SERCA).

These changes may indicate the potential compensatory mechanism being a trial for adaptation to disordered Ca^{2+} homeostasis.

The differentiation process influenced the cellular energy metabolism. The time-dependent increase in intracellular ATP concentration, measured by luminescence method, was observed in the line with suppressed PMCA2. In relation to the starting point (time 0 h) the concentration reached a value of 5 times higher after 72 h. In the line with suppressed PMCA3, ATP concentration measured after 72 h reached 120% of starting value and in a control line was decreased to 80%. In non-differentiated lines, populated in parallel to differentiation, the ATP level was fairly stable. Because ATP is necessary for functioning of ATP-dependent calcium pumps, the alteration in both PMCA and SERCA in transfected lines suggests the reciprocal interaction between Ca^{2+}, ATP concentration and expression level of calcium pumps.

Supported by grants: 502-16-809, 502-16-810 and 503-6086-2 from the Medical University of Lodz

76

Comparison of the influence of photodynamic therapy on the Me45 and MEWO cell lines *in vitro*

A. Wawrzuta[1], J. Saczko[1], J. Kulbacka[1], A. Chwiłkowska[1], M. Majkowski[2]

[1] *Department of Medical Biochemistry, Wroclaw Medical University, Wroclaw, Poland*
[2] *Biotechnology Faculty, Cytobiochemisry, University of Wroclaw, Poland*

Author's e-mail: awawrzuta@gmail.com

Melanoma is the most severe of all skin neoplasm as it may grow rapidly and metastasize. The application of photodynamic therapy (PDT) opens new perspectives in treatment of this tumor. PDT is an effective local cancer treatment that induces cytotoxicity through intercellular generation of reactive oxygen species. The disintegration of cellular structures and modulation of genetic information induced by PDT can direct cancer cells to a death pathway. This combined treatment is based on the specific photosensitizer accumulation in the tumour tissue, followed by irradiation with visible light. The hydrophobic photosensitizer tends to localize in the plasma and subcellular membranes, making these structures especially sensitive to the photooxidative damage. The photochemical interactions of the photosensitizer, light and molecular oxygen produce singlet oxygen and other forms of active oxygen. The oxidative stress is a factor, which initiates the cell death in PDT.

The aim of this study was to examine photodynamic therapy which induces oxidative stress in the human melanoma cell lines in vitro. We compared cells viability and the photosensitizer localization in the primary and recurrent cell line. Photofrin® (Ph), a photosensitizer that has been accepted for clinical studies, was applied in this study. Mito-Tracker Green was used as molecule marker to assign the location of mitochondria. As a recurrent cell line we used Me45 cell line, derived from a lymph node metastasis of skin melanoma in a 35-year-old male. Primary cell line was MEWO cell line (granular fibroblasts), derived from a human melanoma.

Viability studies have shown that there are significant differences between cells after PDT and untreated cells without irradiation. According our results we conclude that Ph is cytotoxic for both primary and recurrence melanoma cells. In cells without irradiation, viability was much higher. After 24 hours incubation with Ph (c_{Ph}=20 μg/ml) and with irradiation survived less then 20 % of cells. In the same combination, but without irradiation survived 65 % of cells (Me45) and above

80 % (MEWO). Moreover we demonstrated that Ph accumulates mainly in the mitochondrial membranes. This can lead to disturbances of mitochondrial transmembrane potential and finally to apoptosis.

77

Genotoxic agents responses of imatinib resistant BCR/ABL cells.

Joanna Żołnierowicz[1], Grażyna Hoser[1], Danuta Wasilewska[1], Mateusz Koptyra[2], Tomasz Skórski[1,2], Jerzy Kawiak[1]

[1] *Department of Clinical Cytology, Medical Center of Postgraduate Education, Warsaw, Poland*
[2] *Department of Microbiology and Immunology, Temple University, Philadelphia, USA*

Author's e-mail: jkulesza@cmkp.edu.pl

The progression of chronic myelogenous leukemia (CML) is related to the appearance of chromosomal translocation (9;22). As a result of this translocation Philadelphia (Ph) chromosome arises coding for constitutively active protein tyrosine kinase BCR/ABL. Specific tyrosine kinase inhibitor imatinib has been a breakthrough in CML treatment. Imatinib inhibits activity of BCR/ABL kinase and some other kinases. Occurrence of resistant leukemia cell clones during imatinib therapy becomes an important problem. One of the reasons of resistance to imatinib is development of clones expressing mutated forms of BCR/ABL. The negative effect of imatinib on cells, resulting from inhibition of cellular c-ABL involved in apoptosis after DNA damage, may be a reason of BCR/ABL mutated cells advantage. There is a doubt, whether during preparation of patients for allogenic transplant by use of radio- or chemotherapy do continue imatinib treatment aiming for depletion of remaining imatinib sensitive CML cells, or not.

The aim of this work was to compare sensitivity of murine myeloid Il-3 dependent 32D cell line, transfected with BCR/ABL gene with or without an inserted T315I or Y253H mutations conferring imatinib resistance, to genotoxic agents with or without imatinib treatment. Cell viability was assessed by MTT test. Cell proliferation analyses were also performed.

Imatinib does not affect apoptosis level and proliferation index of cells expressing mutated BCR/ABL kinase when incubated with genotoxic agents. Imatinib treatment of cells expressing BCR/ABL kinase, in the presence of a growth factor - IL-3, made them much more sensitive to genotoxic stress.

78

Oxidative stress assessment in red blood cells and plasma after gamma irradiation of blood to prevent transfusion-associated graft-versus host disease

H.M. Żbikowska, M. Gapik, A. Rogójska, J. Rywaniak

Department of General Biochemistry, University of Lodz, Lodz, Poland

Author's e-mail: zbikow@biol.uni.lodz.pl

Background: Transfusion-associated graft-versus host disease (TA-GVHD) is a rare but serious adverse effect of blood transfusion that is mediated by the engraftment of allogenic T-lymphocytes. Prevention of the TA-GVHD is routinely accomplished by irradiation of the blood components before transfusion with a recommended dose ranging from 20 to 50 Gy. Doses of this order of magnitude cause only minor changes in the essential constituents of red blood cells (RBCs). However, they promote the leakage of potassium from RBCs. The mechanism of this event remains unknown. The effect of gamma irradiation on other blood components has also been elucidated only incompletely. Gamma irradiation may result in the production of

reactive oxygen species. Thus, we investigated the effects of irradiation on the oxidation of proteins and lipids in the RBC membranes and in plasma.
Materials and methods: Plasma and RBC concentrates (or isolated erythrocyte membranes) from normal donors were exposed to gamma irradiation (20-50Gy) from the ^{60}Co source. The erythrocyte membranes were prepared from washed cells according to Dodge et al. Oxidative damage was assessed by the extent of protein oxidation (measured by the concentration of protein carbonyl groups and the protein thiols) and lipid peroxidation (measured by thiobarbituric acid-reactive substances, TBARS).
Results: Irradiation of the isolated erythrocyte membranes resulted both in the generation of carbonyl groups (by 15 and 30% at the dose of 30-40 and 50Gy, respectively) and the dose-dependent increase of the membrane protein –SH groups. When the membranes were prepared from the irradiated RBCs any significant protein carbonylation has not been noticed but the concentration of the membrane protein –SH groups were found to be elevated. In plasma the level of carbonyl groups has raised only after irradiation with the highest dose (50Gy), whereas, all tested doses of radiation (20-50Gy) decreased the concentration of plasma protein thiols. Gamma irradiation also induced TBARS formation, in RBCs in a dose-dependent manner and to the less extent in plasma.
Conclusions: Gamma irradiation at clinically applied doses fairly increased lipid peroxidation and caused a moderate protein oxidation in the blood components. Moreover, the RBC membrane proteins appeared to be vulnerable to partially fragmentation of the polypeptide chain and/or denaturation (increase of the -SH groups). A study on the potential functional/biological consequences of the blood component oxidative damage is in progress.

This work was supported by grant 506/810 from the University of Lodz.

79

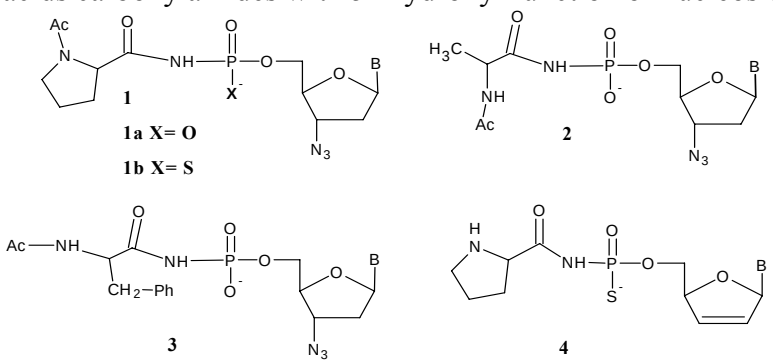
Nucleoside 5'-(N-acyl)phosphoroamido(thio)ates as substrates and inhibitors of DNA polymerases and Hint1 phosphoramidase

Aleksandra Pietkiewicz, Magdalena Ozga, Katarzyna Kulik, Ewa Radzikowska, Renata Kaczmarek, Janina Baraniak, Barbara Nawrot, Wojciech J. Stec

Centre of Molecular and Macromolecular Studies of the Polish Academy of Science, Department of Bioorganic Chemistry, Sienkiewicza 112, 90-363 Lodz, Poland

Author's e-mail: olap@cbmm.lodz.pl

AZT and d4T are analogues of nucleosides exhibiting strong antiviral effect against HIV-1. These drugs have to be intracellulary phosphorylated to mono-, di- and, finally, to triphosphates, which are active DNA polymerase inhibitors and terminators of DNA elongation. In the present studies we designed several derivatives of AZT and d4T with N-acylphosphoramido(thio)yl function, and tested these compounds as potential prodrugs in anti- HIV-1 therapy. Compounds **1-4** were synthesized by ring opening condensation of N-[2-thio-1,3,2- oxathiaphospholane] derivatives of corresponding amino acids carboxy amides with 5'-hydroxyl function of nucleosides (1).



B= Thy

Derivatives of AZT (**1a, 1b, 2, 3**) and d4T (**4**) were tested in *Single Base Extension* (SBE) experiment as substrates and inhibitors of Klenow DNA Polymerases: with or without exonucleolytic activity (KF^+ and KF^-, respectively), and Therminator Polymerase. All these compounds are good KF^- inhibitors, and none of them serves as KF^+ substrate. In contrary, preliminary experiments indicate that Therminator polymerase accepts compound **4** as a substrate (50% yield for the SBE reaction held at 75□C for 1 h) and gives the single nucleotide extended product of the expected molecular weight (MALDI-TOF MS). Under these conditions, in the control reaction carried out in the presence of TTP the longer products were formed.

In another set of experiments we checked whether the compounds **1-4** are substrates for Hint1 phosphoramidase, the enzyme which is able to hydrolyze the P-N bond in 5'-nucleoside phosphoamidates (2). It occurred that these derivatives are not recognised by Hint1 enzyme, and cannot be activated by this enzyme in cellular systems and *in vivo*. In conclusion, we demonstrate that 5'-(N-acyl)phosphoroamido(thio)ate derivatives of AZT and d4T are not promising candidates as prodrugs in anti HIV-1 therapy.

References:

(1) Baraniak J. et al., Tetrahedron Letters, 2004, 45, 4269-4272
(2) Krakowiak et al., JBC, 2004, 279,18,1811-1816

80

The influence of non-hydrolyzable analog of diadenosine tetraphosphate (Ap_4A) on survival rate of Fhit positive and negative cells

R. Pęcherzewska, A. D. Krakowiak, A. Tomaszewska, R. Kaczmarek, J. Baraniak, B. Nawrot, W. J. Stec

Centre of Molecular and Macromolecular Studies of the Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland

Author's e-mail: rozapech@bilbo.cbmm.lodz.pl

The FHIT (Fragile Histidine Triad) protein hydrolyzes diadenosine tri- and tetraphosphate (Ap_3A and Ap_4A) [1]. It is also known that FHIT is a tumor suppressor protein. There are data indicating that its overexpression and forming of a FHIT-substrate complex induces cell death by apoptosis [2]. The enzymatic activity of FHIT protein is not required to exert its suppression function in cancer cells [3].

We have synthesized a non-hydrolysable analog of FHIT substrate (Fig 1) of the structure ApsxpsA, where *ps* is a thiophosphate moiety and *x* is a glycerol residue. The earlier studies demonstrated that ApsxpsA is a very good inhibitor of FHIT hydrolaze activity [4]. The complex of this compound with FHIT protein has prolonged life time in comparison to the natural FHIT-Ap_4A complex. To examine whether the enhanced stability of the FHIT- ApsxpsA complex may enhance suppression function of FHIT, we have used this inhibitor to transfect selected types of FHIT positive and negative cells (HEK293T, A549, HUVEC) and showed the decreased cell viability in FHIT positive HEK293T line after treatment with ApsxpsA. The survival rate was determined by the MTT assay as mitochondrial metabolic function after 24, 48 and 72 h.

To check whether the decrease of cell viability was a result of cell apoptosis we have determined a caspase-3 activity in cells upon incubation with ApsxpsA using caspase-3,7 assay. Cell apoptosis was assessed as amount of fluorescent product released from profluorescent substrate by the caspase-3(7) action. We have observed ca. 10 - 20 % increase of activity of a caspase-3(7) in HEK293T cells after 6 h incubation with ApsxpsA. Our results may indicate that this non-hydrolysable analog of Ap_4A may induce caspase-dependent apoptosis in FHIT positive cells.

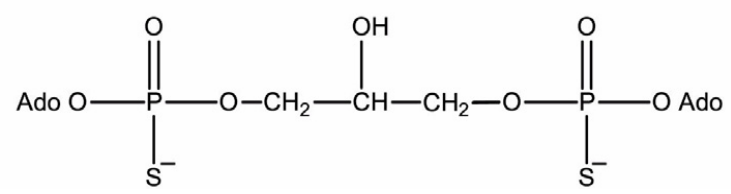


Fig 1. Structure of a non-hydrolysable analog of Ap_4A

References:
1. Barnes LD (1996) *Biochemistry* **35:** 11529–11535
2. Trapasso (2003) *Proc. Natl. Acad. Sci. U S A,* **100**: 1592-1597
3. Brenner C (2002) *Biochemistry* **41**: 9003-9014
4. Varnum J.M. (2001) *BMC Chem Biol.***1:**3

81

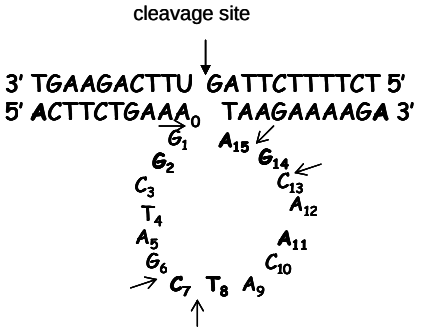
Deoxyribozymes 10-23 with extended catalytic activity and cellular stability

K. Majchrzak, B. Nawrot

Department of Bioorganic Chemistry, Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland

Author's e-mail: kinga@bio.cbmm.lodz.pl

Deoxyribozyme 10-23 is the most investigated metalloenzyme able to catalyze the cleavage of phosphodiester bond in a complementary RNA strand. We designed the DNA-enzyme 10-23 targeting 5'– GU-3'and consisting of two substrate binding arms complementary to the selected single stranded region of mRNA of aspartyl protease Asp2 (BACE1). We have extensively modified this DNA-zyme in the catalytic core as well in the recognition arms. 2'-OMe modifications were introduced in positions 2, 7, 8, 11, 14 i 15, which according to published data do not affect enzyme's catalytic activity (1). Moreover, phosphorothioate bonds introduced at positions P1 or P8, enhanced the catalytic efficiency of the enzyme. The same modifications at positions P7, P14 and P15 did not influence enzyme activity but might improve its nucleolytic stability (2). The series of modified DNAzymes was tested in *in vitro* conditions (single-turnover conditions, 3 mM and 25 μM Mg^{2+}, Tris-NaCl buffer pH 7.4) for their catalytic activity. The most active was the



Scheme of DNAzyme 10-23. 2'-OMe modified nucleotides are shown in bold and PS-bonds are indicated

enzyme possessing five phosphorothioate bonds at positions indicated by arrows in the scheme. Stability of modified enzymes was evaluated in the presence of cellular extracts obtained by lysis of

HeLa cells. The studies confirmed that both, PS-internucleotide bonds and 2'-OMe modifications improve DNAzyme stability.

References:
(1) Schubert S, Gül DC, Grunert H-P, Zeichhardt H, Erdmann VA, Kurreck J, *Nucleic Acids Res*, 2003, 31, 5982
(2) B Nawrot, K Widera, M Wójcik, B Rębowska, G Nowak, WJ Stec, *FEBS J*, 2007, 274, 1062-1072

82

Immunocytochemical and hybrydocytochemical detection of the *β*-catenin in livers from patients with chronic hepatitis C

A. Kasprzak[1], A. Adamek[2], W. Przybyszewska[1], K. Olejniczak[1], W. Biczysko[3], I. Mozer-Lisewska[2], M. Zabel[1]

[1]*Department of Histology and Embryology, University of Medical Sciences, Poznan, Poland*
[2]*Department of Infectious Diseases, University of Medical Sciences, Poznan, Poland*
[3]*Department of Clinical Pathomorphology, University of Medical Sciences, Poznan, Poland*

Author's e-mail: akasprza@ump.edu.pl

Beta-catenin, a key component of the Wnt patway, plays an important role in unregulated liver growth in liver tumors, in regulated growth during liver regeneration, and in *ex vivo* embryonic liver cultures. β-catenin is thought to transactivate mostly unknown target genes, which may stimulate cell proliferation or inhibit apoptosis. Aberrations of β-catenin may promote hepatocellular carcinoma (HCC) development in the course of chronic hepatitis, including chronic hepatitis C. In the course of liver regeneration following partial hepatectomy the protein undergoes translocation to cell nucleus. It remains unclear how far cellular expression of β-catenin *in vivo* may represent an index of progression of the disease and of proliferative activity in the liver in chronic hepatitis C virus (HCV) infection. Aim of the studies included detection of β-catenin (protein and mRNA for β-catenin) in liver biopsies from adults with chronic, long-lasting hepatitis C as related to hepatocyte proliferative activity, histopathological lesions and selected clinical data.

Liver biopsies from patients (n=30) with long-lasting chronic hepatitis C (mean duration of HCV infection approximately 20 years) were analysed by immunocytochemistry [avidin biotin-peroxidase complex method (ABC) and the ImmunoMax technique] and hybridization *in situ* method using digoxygenin-labelled probes. The control group I samples were obtained from livers of serologically HCV-, HBV-, HCMV- and EBV-negative organ donors and normal livers from tissue microarray panel (n=13). The control group II involved the livers with HCC patients and HCC samples from tissue microarray panel (n=18). Results of the immunocytochemical tests were compared to histological alterations in liver biopsies, proliferation index (antigen Ki-67 expression) and with selected clinical data in the patients.

In all chronically HCV-infected patients, only cytoplasmic and/or membrane localization of β-catenin was seen in hepatocytes and in the proliferating bile ducts. Cytoplasmatic and/or nuclear localization of β-catenin was seen focally only in a few HCC samples. Predominantly membrane localization was observed in control group I livers. mRNA for β-catenin was demonstrated in all β-catenin-immunopositive liver specimens. Transcript for β-catenin was located mostly in hepatocytes, but also in some bile ducts and liver sinusoidal cells. The reaction product was detected in the cell cytoplasm. A significantly higher total expression of β-catenin was noted in HCV-infected patients as compared to both control groups. No direct relationships could be demonstrated between expressions of β-catenin and of Ki-67 antigen in chronic hepatitis C group. No correlations could also be demonstrated between cellular expression of β-catenin on one hand

and *grading* or *staging*, alanine transferase (ALT), serum concentration of HCV RNA or alpha-fetoprotein (AFP) on the other.

Alterations of β-catenin expression, as indicated by its cytoplasmic and/or nuclear localization, appear to be frequent in chronic long-lasting hepatitis C and patients with HCC.

83

Role of follistatin in regulation of bone metabolism

A. Gajos-Michniewicz[1], A. Piastowska[1], E. Pawłowska[2], T. Ochędalski[1]

[1] *Department of Comparative Endocrinology, Medical University of Lodz, Poland*
[2] *Department of Pediatric Dentistry, Medical University of Lodz, Poland*

Author's e-mail: ania.gajos@interia.pl

Introduction: Follistatin (FS) is a single-chain, monomeric glycoprotein, distributed in a wide range of tissues. FS has no structural similarity but is functionally associated with the members of TGF-β superfamily. Follistatin has the ability to regulate the activity of various members of TGF-β superfamily, what indicates its wide range of action. Members of TGF-β superfamily have attracted attention for their involvement in bone metabolism. They play important role in bone physiology, influencing bone growth, turnover, bone formation and cartilage induction. Probably members of TGF-β superfamily cooperate each other in regulation of bone formation during fracture healing. As follistatin is considered to be the antagonist of TGF-β superfamily members, it play an important role in bone metabolism and development.
Aim of study: The aim of the study was to characterize the skeletal phenotype of mice with overexpression of follistatin.
Materials and methods: The experiment was performed on mice genetically modified in the Institute of Cell Biology, Swiss Federal Institute of Technology, Zürich, Switzerland. As the control, wild type mice of the strain C57BL/6J were used. Growth hormone level was determined with Rat/Mouse Growth Hormone ELISA Kit (LINCO Research). Bone strength was assesed in three-point bendig test. Femur bone and samples of the diets were mineralized and then determined by flame atomic absorption spectrophotometry. Transgenic and wild type mice were grouped into four age sets comprising both male and female individuals.
Results and discussion: Overexpression of follistatin results in various phenotypic changes, e.g. decreased body weight, skin alteration, abnormal teeth, bone fragility and skeletal alteration. Mice were examined in terms of amount of growth hormone and associated body weight, bone strength and bone mineral content. It was found that overexpression of follistatin results in decreased amount of growth hormone in the group of the youngest mice (10 days) in comparison with the control group. The overexpression of FS may affect the pituitary, what results in differences in growth hormone level. There are also visible differences in bone strength and bone mineral content between genetically modified and wild type mice. FS alters bone metabolism what results with decreased bone strength of transgenic individuals, whereas mineral analyses detected decreased amount of zinc in transgenic animals.
Conclusions: There is no skeletal phenotype that has been described for mice with overexpression of follistatin. Because of the lack of detailed analysis of bone tissue of these transgenic mice, the further experiments examining e.g. bone mineral content, bone microarchitecture and bone strength are required.

The study was supported by the Grants: no. 50219681 - from the Medical University of Lodz and grant no. N N404107733 from the Polish Ministry of Science and Higher Education.

84

The novel platinum(II) complexes differentially modulate DNA damage evoked by irinotecan in human cell lines *in vitro*

R. Kontek[1], B. Łukasiewicz[1], M. Śliwiński[1], A. Błaszczyk[1], B. Kontek[2], R. Osiecka[1]

[1] *Laboratory of Cytogenetics, Department of Genetics and Plant Molecular Biology and Biotechnology, University of Lodz, Lodz, Poland*
[2] *Department of General Biochemistry, University of Lodz, Lodz, Poland*

Author's e-mail: renkon@biol.uni.lodz.pl

In a recent time chemotherapeutics derived from plant compounds, like camptothecin and its derivatives, aimed at the inhibition of type I topoisomerase enzymes through DNA binding in its minor groove, has became popular. Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin; synonyms: CPT-11), is water-soluble, it exists in a prodrug form which is metabolized *in vivo* to the active, 100 - 1000 times more cytotoxic, metabolite SN-38. After binding the open topoisomerase I complex, SN-38 stabilizes it, forming the spatial obstacle for replication fork leading the synthesis of new DNA strand and for RNA polymerase in the region undergoing transcription at that moment. This leads to the formation of irreversible double-strand DNA breaks (DSB) which, while not repaired, can lead to the programmed cell death. Due to its different mechanism of action, irinotecan is used in polychemotherapy. Combination chemotherapy has become the mainstay treatment regimen for the vast majority of clinical cancers. Such combinations have been reported to be synergistically active against many tumor cell lines, including colorectal, leukemia, ovarian, squamous-carcinoma, small cell lung cancer and non-small cell lung cancer (NSCLC).

The presence of *cis*-[$PtCl_2$(4-pmOpe)$_2$] and *trans*-[$PtCl_2$(4-pmOpe)$_2$] caused the enhancement of the genotoxic effect evoked by irinotecan in A549 cancer cells (*non-small cell lung cancer line*). The presence of novel *cis*-DDP analogues in incubation solutions had a synergistic effect, increasing the level of DNA damage in the cells caused by the material exposition on different CPT-11 concentrations. After incubation of normal human lymphocytes in the tested complexes in that experimental series a slight increase in DNA damage level was noted. Especially, in case of the presence of *cis*-[$PtCl_2$(4-pmOpe)$_2$] and *trans*-[$PtCl_2$(4-pmOpe)$_2$] during the incubation of the A549 cells with CPT-11 the level of DNA lesions significantly outstripped that obtained in the series in case of the use of normal human lymphocytes. This points to the fact that *cis*-DDP analogues exhibit important anticancer activity and highlights the more pronounced effects of combination chemotherapy in cancer treatment. The compounds induced DNA damage at different levels depending on the cell types, which show differences in the sensitivity of those lines to the tested complexes.

85

The role of the DNA repair in therapy childhood acute lymphoblastic leukemia with methotrexate and 6-mercaptopurine

M. Stańczyk[1], M. Cuchra[1], J. Trelińska[2], A. Bieniek[3], A. Bielecka-Kowalska[4], M. Kowalski[4], W. Młynarski[2], I. Majsterek[1,4]

[1]Department of Molecular Genetics, University of Lodz, Lodz, Poland
[2]Department of Pediatrics, Medical University of Lodz, Lodz, Poland
[3]Medical Care Lodz-Baluty, Smugowa, 691-433 Lodz, Poland
[4]Department of Chronopharmacology, Medical University of Lodz, Lodz, Poland

Author's e-mail: malgosia.stanczyk@yahoo.pl

Acute lymphoblastic leukemia (ALL) is a disease characterized by the abnormal proliferation of immature lymphoid cells and their accumulation in the bone marrow and peripheral blood. It is the most common malignant disease in childhood with a peak at 2 ± 5 years of age. Although in the last decades, treatment outcome of childhood acute lymphoblastic leukemia has improved considerably, almost 20 % of the children either relapse or do not respond to treatment. The drugs most commonly used in the treatment of newly diagnosed childhood ALL are methotrexate (MTX), anticancer agent that belongs to antifolate metabolites, and 6-mercaptopurine (6MP).

The aim of our study was to evaluate the influence of the treatment with 6MP and MTX on DNA damage in peripheral blood lymphocytes from patients with ALL. To examine the misincorporation of uracil into DNA, the modified comet assay with uracil DNA glycosylase (Udg) was used. Additionally, we evaluated the effect of hydrogen peroxide (H_2O_2) at the concentration of 10 μM on the oxidative DNA damage and the effectiveness of DNA repair in lymphocytes from patients with ALL using the modified comet assay with Nth (Endonculease III) enzyme. The kinetic of DNA repair was assessed after 30, 60 and 120 min of incubation.

Our findings indicate that the level of uracil misincorporation was higher in lymphoblasts isolated during treatment with 6MP and MTX in comparison to lymphblasts before and after treatment. Moreover, it was found that the effectiveness of DNA repair was lower in lymphoblasts isolated during and after treatment with 6MP and MTX. The lymphoblasts isolated before treatment were able to recover within 120 min, in contrast to the lymphoblasts isolated during and after treatment. These results suggest that the treatment with antileukemic drugs, 6MP and MTX, decrease capacity of DNA repair in lymphoblast from patients with ALL.

86

The role of NER pathway in DNA recovery of head and neck squamous cell carcinomas

Łukasz Markiewicz[1], Tomasz Śliwiński[1], Pawel Rusin[1], Jurek Olszewski[2], Alina Morawiec-Sztandera[3], Krzysztof Kuśmierczyk[2], Dariusz Kaczmarczyk[3], Janusz Szemraj[4], Ireneusz Majsterek[1,5]

[1]*Department of Molecular Genetics, University of Lodz, Lodz, Poland*
[2]*Department of Otolaryngology and Oncology, Medical University of Lodz, Lodz, Poland*
[3]*Department of Head and Neck Cancer, Medical University of Lodz, Lodz, Poland*
[4]*Department of Medical Biochemistry, Medical University of Lodz, Lodz, Poland*
[5]*Department of Chronopharmacology, Medical University of Lodz, Lodz, Poland*

Author's e-mail: markiewicz77@gmail.com

Head and neck squamous cell carcinomas (HNSCC) comprise about 6% of all malignant neoplasms. One of the major risk factor of HNSCC is tobacco smoking. There are approximately 4 thousand chemical compounds identified in tobacco smoke. Many of them like polycyclic aromatic hydrocarbons (PAHs) and aromatic amins (AA) are well known carcinogens which form adducts with DNA. Cells remove those adducts mainly by using nucleotide excision repair (NER). We examined DNA repair efficiency in peripheral blood lymphocytes obtained from HNSCC patients and healthy subjects as well as HTB-43 larynx cancer cell line. NER activity was assessed in the cell extracts by use of an UV-irradiated plasmid as a substrate. After *in vitro* repair the level of [gamma ^{32}P]-dAMP incorporation during strand resynthesis as equivalent of NER efficiency was quantified by densitometry. Results obtained from these experiments indicate that there is decrease in the efficacy of NER in HTB-43 cells and HNSCC lymphocytes compared to healthy controls. In conclusion, we suggest that NER pathway is critical for DNA recovery of HNSCC cells what in turn may be responsible for higher susceptibility to mutagenesis and cancer transformation.

This work was supported by grant N301 099 32/3581 from Polish Ministry of Science and Higher Education.

87

Role of HRR and NHEJ repair in head and neck cancer cells susceptibility to genotoxic treatment

P. Rusin[1], A. Walczak[1], L. Markiewicz[1], W. Pietruszewska[2], J. Olszewski[3], A. Morawiec-Sztandera[4], W. Mlynarski[5], I. Majsterek[1,6]

[1] Department of Molecular Genetics, University of Lodz, Lodz, Poland
[2] Department of Otolaryngology, Medical University of Lodz, Lodz, Poland
[3] Department of Otolaryngology and Oncology, Medical University of Lodz, Lodz, Poland
[4] Department of Head and Neck Cancer, Medical University of Lodz, Lodz, Poland
[5] Department of Paediatrics, Medical University of Lodz, Lodz, Poland
[6] Department of Chronopharmacology, Medical University of Lodz, Lodz, Poland

Author's e-mail: pawelrusin@gmail.com

DNA repair might be critical for an effective anticancer therapy. We investigated the efficacy of double strand breaks (DSBs) repair by homologous recombination (HRR) and non-homologous end-joining (NHEJ) repair pathways in head and neck squamous cell carcinoma (HNSCC). We examined lymphocytes from HNSCC patients and lymphocytes obtained from control healthy subjects, as well HTB43 larynx cancer cell line. Extrachromosomal assay for DNA double-strand break repair (TAK assay) with DSBs plasmid subtracts was used to estimate an efficiency of HRR and NHEJ. In our study, there were not significant changes in NHEJ activity; however we found statistically significant decrease of HRR pathway in patients as compared to healthy controls. Our results suggest that DSBs recovery by HRR-dependent pathway may be critical for an effective head and neck cancer treatment with genotoxic drugs.

This work was supported by grant N301 099 32/3581 from Polish Ministry of Science and Higher Education.

88

RAD51 and *XRCC3* single nucleotide polymorphism association with risk of head and neck squamous cell carcinoma

A. Walczak[1], T. Śliwiński[1], P. Rusin[1], W. Pietruszewska[2], J. Olszewski[3], A. Morawiec-Sztandera[4], W. Młynarski[5], I. Majsterek[1,6]

[1] Department of Molecular Genetics, University of Lodz, Lodz, Poland
[2] Department of Otolaryngology, Medical University of Lodz, Lodz, Poland
[3] Department of Otolaryngology and Oncology, Medical University of Lodz, Lodz, Poland
[4] Department of Head and Neck Cancer, Medical University of Lodz, Lodz, Poland
[5] Department of Paediatrics, Medical University of Lodz, Lodz, Poland
[6] Department of Chronopharmacology, Medical University of Lodz, Lodz, Poland

Author's e-mail: walczak.anna@yahoo.com

Double strand breaks (DSBs) are the most lethal form of DNA damage repaired by homologous recombination (HRR) and non-homologous end joining (NHEJ). Inefficient repair may result in accumulation of DNA damages, genome instabilities and cancer transformation. It is reported that polymorphisms of genes involved in DSBs repair pathways may increase cancer risk occurrence. The aim of our research was to evaluate the association between head and neck squamous cell

carcinoma (HNSCC) and two single nucleotide polymorphisms (SNPs) of HRR genes: *XRCC3* Thr241Met and *RAD51* 135G>C. In case-control study we genotyped 288 HNSCC patients and 353 controls (taken from Polish population) for both polymorphisms. Analysis was performed by PCR-restriction fragment length polymorphism (PCR-RFLP). The results show that both of analyzed polymorphisms were associated with increase of HNSCC risk. We have found that Thr/Met genotype of Thr241Met *XRCC3* polymorphism is connected with higher risk of premalignant lesions occurrence. Moreover, Met241Met homozygote significantly increases risk of HNSCC as well as precancerous lesions (OR 2.92, 95%CI 1.92-4.44 and OR 2.18, 95%CI 1.28-3.71 respectively). Similar relationship was observed in case of *RAD51* 135G>C variant, where the positive association was found in both groups mentioned above (OR 3.86, 95%CI 2.6-5.71 and OR 6.43, 95%CI 3.96-10.47). What's more, we have found that combination of *XRCC3* Met241Met and *RAD51* 135G>C variants increases risk of HNSCC (OR 2.38, 95%CI 1.31-4.32) and precancerous lesions (OR 2.67, 95%CI 2.6-5.71). Also *XRCC3* Thr241Met and *RAD51* 135G>C combined genotypes are associated with higher risk of premalignant lesions. In conclusion, our results suggest that *XRCC3* Thr241Met and *RAD51* 135G>C polymorphisms may be associated with higher risk of precancerous lesions and HNSCC in Polish population.

This work was supported by grant N301 099 32/3581 from Polish Ministry of Science and Higher Education.

89

Changes of apoptotic events in stored blood platelets

J. Rywaniak, B. Wachowicz

Department of General Biochemistry, University of Lodz, Lodz, Poland

Author's e-mail: joanna_z_r@wp.pl

Objective: Blood platelets are the smallest anucleated blood cells derived from nucleated megakaryocytes. Their activation caused by agonists is essential for primary haemostasis. The studies of recent years have shown that they also may develop apoptotic events upon activation by agonists or during storage under standard blood bank condition. The aim of our study *in vitro* was to determine, whether storage of gel-filtrated platelets in Tyrode's buffer may cause some changes in the level of the apoptotic markers in platelets.
Materials and Methods: The monitoring of platelets apoptotic events was carried out in gel-filtrated platelets (3 x 10^7 plt/ml) suspended in Tyrode's buffer and stored for 3 and 6 hours after isolation. The apoptotic markers, such as exposure of phosphatidylserine (PS) on the platelets surface, platelet mitochondrial membrane potential ($DiOC_6$ and JC-1 staining), activation of caspase-3, the platelet microparticle (PMP) formation and expression of P-selectin were measured by flow cytometry (LSR II Flow Cytometer, Becton-Dickinson).
Results: Storage of platelets in suspension of Tyrode's buffer for 3 and 6 hours after isolation resulted in a distinct increase of phosphatidylserine exposure, the platelet microparticle formation, caspase-3 activation and mitochondrial membrane depolarization. Furthermore, storage of gel-filtrated platelets significantly increased expression of P-selectin on platelets membrane surface. The changes observed in stored platelets were time-depended.
Conclusions: Based on above findings, it seems that storage of gel-filtrated platelets (22° C, 3 and 6 h) in Tyrode's buffer induces apoptotic events in blood platelets and partly platelet activation (expression of P-selectin on blood platelets surface).

90

The effect of acetylsalicylic acid on apoptosis in blood platelets

J. Rywaniak[1], M. Bijak[2], M. Bobrowski[2], B. Wachowicz[1]

[1]Department of General Biochemistry, University of Lodz, Lodz, Poland
[2]Biological Scientific Student Society, University of Lodz, Lodz, Poland

Author's e-mail: joanna_z_r@wp.pl

Objective: Blood platelets play essential role in primary haemostasis. Thrombin as a strong platelet agonist induces platelet activation with stimulation of arachidonate pathway and production of prostanoids. It is also responsible for apoptotic events in blood platelets, however mechanisms of this process is still unknown. The aim of our study was to determine, whether acetylsalicylic acid (ASA) may prevent against activation of platelet apoptosis after thrombin stimulation, *via* its inhibitory effect on activity of cyclooxygenase-1 (COX) and inhibition of arachidonic acid pathway.
Materials and Methods: The monitoring of platelets apoptotic events was carried out in gel-filtrated platelets (3 x 10^7 plt/ml) preincubated with ASA (1.0, 5.0 and 10.0 mM) and then activated by thrombin (0.1 and 1.0 U/ml). Expression of P-selectin (marker of platelets activation), exposure of phosphatidylserine, platelet mitochondrial membrane potential ($DiOC_6$ and JC-1 staining), activation of caspase-3 and the platelet microparticle (PMP) formation were measured by flow cytometry using LSR II Flow Cytometer, Becton-Dickinson.
Results: Thrombin (0.1 and 1.0 U/ml) induced platelets activation (P-selectin expression), increased of phosphatidylserine exposure and platelets microparticle formation; only highest concentration of thrombin triggered also caspase-3 activation and mitochondrial potential depolarization. Preincubation of platelets with ASA (1.0, 5.0 and 10.0 mM) reduced platelets activation induced by 0.1 U/ml thrombin, but did not prevent against platelets apoptosis and activation caused by 1.0 U/ml thrombin concentration.
Conclusions: Our study demonstrates that in mechanisms of thrombin-trrigered platelet apoptosis arachidonic acid pathway is not involved.

91

Toxicity effect of homocysteine and its thiolactone on selected haemostatic proteins *in vitro*

J. Malinowska, A. Konowalczyk, P. Nowak, J. Kołodziejczyk, B. Olas

Department of General Biochemistry, University of Lodz, 90-237 Lodz, Poland

Author's e-mail: joanna_malinowska1@vp.pl

Objective: Homocysteine (Hcys) is non-protein sulfur-containing amino acid, an intermediate product of methionine metabolism. It is well known, that elevated plasma homocysteine is associated with an increased risk of atherosclerosis and thrombosis. Studies performed during the last two decades suggest that the atherogenic effect of homocysteine may be accounted for by homocysteine thiolactone (HTL). The modifications of haemostatic proteins (N-homocysteinylated or S-homocysteinylated proteins) induced by Hcys or its thiolactone seem to be the main reason of biotoxicity of homocysteine in cardiovascular diseases. Fibrinogen, plasminogen and other plasma proteins can be covalently modified by Hcys and HTL. Therefore, the aim of our study was to

examine the changes of biological activity of fibrinogen and plasminogen induced by homocysteine and its thiolactone.

Material and Methods: Plasma was isolated from citrate human blood. Human plasma was incubated with homocysteine (10 μM) and thiolactone homocysteine (1 μM) at 37º C for 30 minutes. Thrombin-catalyzed polymerization and tissue plasminogen activator (t-PA)- catalyzed lysis was monitored for 50 minutes as the change in turbidity at 415 nm. Plasminogen was activated by streptokinase and then its amidolytic activity by using a microplate reader for 80 minutes at 415 nm was estimated.

Results: Coagulation of human plasma incubated with Hcys and HTL was accelerated in comparison with control. Clots formed from Hcys- and HTL-treated human plasma was lysed more slowly than clots from untreated control plasma. Both Hcys and HTL modified plasminogen in human plasma and inhibited its activity in comparison with control samples. We also found that HTL effectively modified the activity of plasminogen at much lower concentration in comparison with Hcys.

Conclusions: Our studies confirmed that Hcys and HTL are able to disturb haemostasis. Detrimental effects of elevated plasma homocysteine on clot resistance to lysis in humans are consistent with a mechanism involving fibrinogen modification by homocysteine thiolactone. Homocysteinylation of fibrinogen can lead to abnormal resistance of fibrin clots to lysis. Modification of plasminogen and fibrinogen in human plasma may cause pathophysiological consequences, such as modulation of haemostasis system, which may in turn contribute to cardiovascular diseases.

This work was supported by the grant 506/810 from the University of Lodz.

92

Does garcinol effectively protect human blood platelets against oxidative stress induced by peroxynitrite?

Anna Reczyńska, Joanna Kołodziejczyk, Beata Olas

Department of General Biochemistry, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: anna.reczynska@interia.pl

Overproduction of oxidative and nitrative agents, such as peroxynitrite is a potential threat for cellular biomolecules and may lead to changes in their structure and functions. Blood platelet activation plays a crucial role not only in homeostasis but also in pathomechanisms of cardiovascular diseases, tumor progression, allergic inflammation and nonallergic responses. Blood platelets produce reactive oxygen and nitrogen species, such as superoxide anion and nitrogen oxide during the activation. Moreover, the availability of both superoxide anion and nitric oxide raises the possibility, that another strong nitrative agent, peroxynitrite, may be formed in platelets. Modifications of platelet proteins induced by peroxynitrite are responsible for altered response of these cells and may be an important factor in the pathogenesis of various diseases. Thus, the defense mechanisms against peroxynitrite are crucial for normal cellular function.

Garcinol, a natural biologically active compound is a polyisoprenylated benzophenone derivative, isolated from *Garcinia indica*. Recently, garcinol has been widely investigated because of its beneficial health properties, including antibiotic action, anticancerogenic properties and even antioxidative activity. However, antioxidant mechanism of garcinol and its biological significance remain unclear. Therefore, the aim of this study was to test the antioxidative activity of garcinol against peroxynitrite action on platelet proteins and lipids.

Blood was collected into ACD solution (citric acid/citrate–dextrose; 5:1; v/v). Suspensions of human platelets were preincubated with garcinol (used in a range 0.1 – 25.0 μM) and then $ONOO^-$ solution was added (to the final concentration 100 μM).

Peroxynitrite is a reactive diffusible molecule, able to penetrate the platelet membrane and the incubation of blood platelets with this agent results in the increase of thiobarbituric acid-reactive substances (TBARS), carbonyl groups and 3-nitrotyrosine level. In our studies, we estimated the level of 3-nitrotyrosine by a competition-ELISA method and we have not observed any protective action of garcinol on these damages to platelet proteins in comparison to negative control samples ($ONOO^-$ treatment without garcinol). However, another ELISA test performed to estimate proteins carbonylation showed that garcinol manages to decrease the carbonyl group generation in blood platelet proteins. We also examined the defence properties of garcinol against peroxynitrite-induced lipid peroxidation, measured as TBARS, a marker of lipid peroxidation. These experiments showed that garcinol partially inhibited the effect of peroxynitrite on platelet lipid peroxidation.

Our studies demonstrated that garcinol may partially contribute to cellular defence against the cytotoxicity of $ONOO^-$.

This work was supported by the grant 505/373 from the University of Lodz.

93

Beta-glucan as a protector of human blood platelets against oxidative stress induced by peroxynitrite

Karolina Królewska, Monika Smolarek, Joanna Kołodziejczyk, Joanna Saluk-Juszczak

Department of General Biochemistry, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: k_krolewska@o2.pl

Objective: The activation process, crucial for platelet function, plays an important role in the physiological and pathological processes of haemostasis. Hyperactivity of blood platelet can lead to thrombosis, atherosclerosis, cardiovascular and cerebrovascular disorders. Platelets generate reactive oxygen species (ROS) that can be involved in regulation of platelet activation and modulation of platelet activities. Regulation of platelets function by ROS is due to decreased bioavailability of nitric oxide (NO) generated in vessel wall, since NAD(P)H oxidase-generated ROS (mainly superoxide anion $O_2^{-\bullet}$) scavenge endothelial and platelet-derived NO in a fast reaction generating peroxynitrite ($ONOO^-$) as an end product. Peroxynitrite, a highly reactive agent may modulate platelet activation and function. Modifications (oxidation and nitration) of platelet proteins induced by peroxynitrite are responsible for altered response of these cells and may be an important factor in the pathogenesis of various diseases. Thus, the defence mechanisms against peroxynitrite are very important for biological activities of blood platelets. The aim of our study was to investigate the antioxidative activity of β-glucan against platelet proteins and lipids damages, induced by peroxynitrite. The β-glukans are naturally occurring polysaccharides. These glucose polymers are the constituents of the cell wall of certain pathogenic bacteria and fungi. β -glucans have the ability to stimulate the immune system and are classified as biological response modifiers. They can increase the host immune defense mainly by activation of complement system and enhancing macrophages and natural killer cell activity. There is little data about antioxidative activity of β-glucan.

Materials and methods: Human blood was collected into ACD solution (citric acid/citrate–dextrose; 5:1; v/v). After isolation, platelet suspensions were preincubated for 5 min.; at $37^{O}C$ with β-glucan (in a range 1-4 μg/ml) and then treated with $ONOO^-$ (0.1 mM).

Results: The incubation of blood platelets with $ONOO^-$ leads to oxidative/nitrative stress in these cells. It results in the increase of thiobarbituric acid-reactive substances (TBARS), carbonyl groups and 3-nitrotyrosine level. We measured the level of TBARS, as a marker of lipid peroxidation. Our studies showed that β-glucan possesses significant defence properties against peroxynitrite-induced lipid peroxidation. The level of TBARS was decreased even by 80% at the highest dose of β-glucan. The level of 3-nitrotyrosine measured by a competition-ELISA method, was also diminished. It indicates the protective effect of β-glucan (about 50%) on these platelet protein damages. Another ELISA test used to estimate proteins carbonylation caused by $ONOO^-$ action, also showed that β-glucan prevented against blood platelets modifications. Preincubation of blood platelets with β-glucan decreased the level of carbonyl groups in the dose dependent manner (by 20%-50%).
Conclusions: The obtained results showed the protective effects of β-glucan on blood platelets against the cytotoxicity of $ONOO^-$.

This work was supported by the grant 505/374 from the University of Lodz.

94

The anthocyanin-rich extract (from red cabbage leaves) action against hydroperoxide induced oxidative stress in human blood platelets

Monika Smolarek, Karolina Królewska, Joanna Saluk-Juszczak

Department of General Biochemistry, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: monikasmolarekl@wp.pl

Objective: Blood platelets, anucleate megacaryocyte-derived cells are one of the key elements of physiological and pathological processes of haemostasis. The excessive activation of platelets is responsible in part for thrombosis, atherosclerosis, ardiovascular and cerebrovascular disorders. Platelets generate reactive oxygen species (ROS) that can be involved in regulation of platelet activation and modulation of platelet activity. Induced by ROS modifications of platelet action may be an important factor in the pathogenesis of platelet-related diseases. Thus, the defence mechanisms against ROS are crucial for normal cellular function. Recently, there is an increasing interest in screening natural products present in diet and herbals for possible antioxidative agents. The aim of our studies was to investigate antioxidant effects of the anthocyanin-rich extract from red cabbage leaves (*Brassica oleracea rubrum*). Anthocyanins are generally accepted as the largest and most important group of water-soluble pigments in nature. They are considered to contribute to the healthiness of fruits and vegetables (e.g. red cabbage) for their antioxidant, anti-carcinogenic, anti-inflammatory, and anti-angiogenic properties. However, there is no data about their antioxidative activity on blood platelets. We studied the antioxidative action of anthocyanin-rich extract against platelet protein and lipid damages, induced by hydroperoxide.
Materials and methods: Human blood was collected into ACD solution (citric acid/citrate–dextrose; 5:1; v/v) and then the platelets were isolated. Platelet suspensions were preincubated for 5 min.; at $37^{O}C$ with anthocyanin-rich extract (used in a range 5-15 μM) and then treated with H_2O_2 (2 mM).
Results: The incubation of blood platelets with H_2O_2 leads to oxidative modifications in these cells. It resulted in an increase of the level of carbonyl groups. In our studies we estimated the level of carbonyl groups by ELISA test. In the presence of tested extract, a distinct decrease (even more than 50% for the highest concentration) of carbonyl group formation was detected. As a marker of lipid peroxidation we measured the level of TBARS. The increase of thiobarbituric acid-reactive substances (TBARS) after exposure of platelets to H_2O_2 was observed. In the presence of cabbage leave extract TBARS level was decreased in the dose dependent manner, even by 80% at the highest dose of extract.

Conclusions: Our studies showed that anthocyanins derived from red cabbage leaves possess significant antioxidative properties and prevent platelets against oxidation of lipids and proteins.

This work was supported by the grant 505/374 from the University of Lodz.

95

The protective effects of polyphenol-rich extract from berries of *Aronia melanocarpa* against peroxynitrite-induced changes in human fibrinogen

M. Borowiecka[1], M. Bobrowski[2], M. Bijak[2], P. Nowak[1]

[1]*Department of General Biochemistry, University of Lodz, Lodz, Poland*
[2]*Biological Scientific Student Society, University of Lodz, Lodz, Poland*

Author's e-mail: biochogl@biol.uni.lodz.pl

Objective: Increasing evidence indicates that oxidative and nitrative modification of plasma proteins involved in blood coagulation may lead to alternation of hemostatic process. Among plasma proteins fibrinogen (Fg) seems to be highly susceptible to oxidant attack. Peroxynitrite ($ONOO^-$) is a powerful physiological oxidant, nitrating species and inflammatory mediator formed *in vivo* in the reaction between superoxide anion and nitric oxide. Exposure of proteins to peroxynitrite results in modification of different amino acid residues and change of their function. Recently, there has been an increased interest in the screening of natural products present in fruits, vegetables and herbs for possible antioxidative agents. Therefore, the aim of our study was to assess if the extract from berries of *Aronia melanocarpa* containing: anthocyanidines, phenolic acids and quercetine glycosides, may protect plasma fibrinogen against nitrative and oxidative damage induced by $ONOO^-$.
Materials and Methods: Fibrinogen was isolated from citrated human plasma by cold ethanol precipitation technique, according to Doolittle (1968). Peroxynitrite was synthesized according to Pryor and Squadrio`s method (1995). Fibrinogen after peroxynitrite treatment was analyzed by Western blotting with anti-nitrotyrosine and anti-DNPH antibodies. Thrombin-catalyzed polymerization of fibrinogen was monitored for 20 min as the change in turbidity at 415 nm.
Results: The reaction of peroxynitrite (1-100 μM) with fibrinogen (6 μM) resulted in both structural modifications and clotting changes of this glycoprotein. Western blot analysis of peroxynitrite-treated fibrinogen with anti-nitrotyrosine and anti-DNPH antibodies showed nitration of tyrosine residues and formation of carbonyl groups and dityrosine formation between Aγ subunits of Fg. We also found that with the increasing $ONOO^-$ concentration, the ability of Fg to undergo clotting was diminished. The extract from *A. melanocarpa* (0.5-50 μg/mL) added to Fg 10 s before peroxynitrite (100 μM) significantly inhibited both, the formation of the HMW aggregates and nitration of Fg. The extract also decreased the amount of carbonyl groups in peroxynitrite-treated fibrinogen and abolished peroxynitrite-induced inhibition of fibrinogen polymerization (by 90% at 50 μg/mL)
Conclusions: The obtained results indicate that the natural extract from berries of *Aronia melanocarpa* has protective effects against peroxynitrite-induced oxidative/nitrative damage of plasma fibrinogen, and therefore may contribute to preventing of peroxynitrite-related cardiovascular or inflammatory diseases.

This work was supported by grant 505/373 from the University of Lodz.

96

Fluidity of liposome membranes doped with vanadium complex: ESR study

Dariusz Man[1], Marian Podolak[1], Aleksandra Man[2]

[1] *Institute of Physics, Opole University, Oleska 48, 45-052 Opole, Poland*
[2] *Institute of Biotechnology and Molecular Biology, Opole University, Kominka 4, 45-033 Opole, Poland*

Author's e-mail: dariusz.man@uni.opole.pl

Changes in membrane fluidity of vanadium-doped liposomes have been investigated to assess the kinetics of the fluidization process. vanadium complex [VO(Salaba)] were used as dopant. Liposomes were obtained by sonication of hen egg yolk lecithin (EYL). Electron paramagnetic resonance was applied (ESR) using spin probes TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) and 16-DOXYL-stearic acid (2-ethyl-2-(15-methoxy-15-oxopentadecyl)-4,4-dimethyl-3-oxazolidinyl-oxyl) localized at different sites within the membrane, to determine the spectroscopic parameters: partition (F) and rotation correlation time (τ), related to the membrane's fluidity.

It was found, that vanadium complex do considerably fluidize the membranes and the dynamics of this process depends on the concentration of the compound used and the membrane's area surveyed by the probes. Variations in fluidity observed after the vanadium complex had been introduced into the liposome were found to stabilize inside the double-layer and within the surface layer after ca. 20-25 and 50-60 h, most probably due to hydration of the hydrophilic part of the membrane.

97

Correlation of GAP-43 posttranslational lipid modification with restructuring of plasma membrane microdomains

K. Tułodziecka, N. Kulikova, K. A. Nałęcz

Nencki Institute of Experimental Biology, Warsaw, Poland

Author's e-mail: k.nalecz@nencki.gov.pl

Microdomains of plasma membrane which are resistant to detergent extraction (rafts, caveolae) contain large multiprotein assemblies consisting mainly of proteins involved in processes of signal transduction. Many proteins interacting transiently with these microdomains can be anchored due to posttranslational lipid modifications, out of which palmitoylation seems to play the most important role. Growth associated protein – GAP-43, the expression of which is upregulated in differentiating neural cells, is known to be palmitoylated at Cys^3 and Cys^4 and its palmitoylation in neuroblastoma NB-2a cells was observed to increase after treatment with palmitoylcarnitine (*Nałęcz et al., FEBS Lett. 2007, 581: 3950-3954*). Since GAP-43 is also known to regulate G_o activity, as well as to bind calmodulin, which points to its important role at the cross-road of different signaling pathways, the present study was focused on possible influence of palmitoylcarnitine on localization of GAP-43 in plasma membrane microdomains, as well as on GAP-43 interaction with lipids and proteins concentrated within these domains.

Under control conditions caveolin-1, a protein typical for caveolae and known to bind cholesterol, was observed to co-localize with cholesterol in the intracellular compartments, while GAP-43 was visible in the cell membrane. Palmitoylcarnitine treatment resulted in differentiation of NB-2a cells. Under these conditions, GAP-43 alone was detected in neuronal processes, while the

regions of co-localization with caveolin-1 and cholesterol were visible in the plasma membrane of the cell bodies. Caveolin alone could be also detected within the cell bodies, which implies a different mechanism of caveolin and cholesterol transfer to the cell surface.

GAP-43 can bind as well phosphatidylinositol 4,5-bisphosphate - $PIP(4,5)_2$, therefore immunocytochemitry experiments were performed in order to verify, if GAP-43 can co-localize with $PIP(4,5)_2$ microdomains. Several dot-like domains containing $PIP(4,5)_2$ were detected in NB-2a cells under control conditions. In differentiated cells (upon palmitoylcarnitine treatment) the amount of $PIP(4,5)_2$ microdomains augmented and they were present both in neurites as well as in plasma membrane of cell bodies. Few single spots of co-localization of GAP-43 with $PIP(4,5)_2$ were observed, as well as few sites containing $PIP(4,5)_2$, cholesterol and GAP-43, all of them in neural processes, while the main interaction occured between GAP-43 and cholesterol, found in plasma membrane of cell bodies.

The obtained results suggest the preferential interaction of palmitoylated GAP-43 with cholesterol-containing regions of plasma membrane, independently of caveolin-1 sorting and $PIP(4,5)_2$ microdomains.

98

Nucleo-cytoplasmic shuttling of human cohesins SA1 and SA2 expressed in yeast *Saccharomyces cerevisiae*

L. J. Tarnowski, P. Kowalec, A. Kurlandzka

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Author's e-mail: ania218@ibb.waw.pl

The cohesin complex ensures accurate separation of sister chromatids into two daughter cells. Several models for the cohesin complex have been proposed, but the one-ring embrace model is currently the most popular. The complex comprises four core protein subunits, essential for cell viability, that are conserved from yeast to vertebrates. In *Saccharomyces cerevisiae* mitotic cells, the cohesin complex consists of Scc1/Mcd1, Smc1, Smc3, and Irr1/Scc3. In human mitotic cells, the cohesin complex is composed of Rad21, Smc1, Smc3, and two Irr1 orthologues, SA1 and SA2. Each complex can contain only one of the two SA proteins and it is unknown whether the composition of the complex is stable or whether SA1 and SA2 are exchangeable.

We initially expressed human SA1 and SA2 proteins in yeast mutants to check whether they can replace Irr1p. Although neither of the SA proteins substituted for Irr1p function, we found that SA1p expressed in yeast has a nuclear localization, while the majority of SA2p is present in the cytoplasm. Analysis of the amino acid sequence of SA2 allowed us to identify putative nuclear export signals (NES) characteristic for Crm1p karyopherin – dependent export. Using yeast mutants defective in the *CRM1* gene or sensitive to Leptomycin B (Crm1p inhibitor) we found that SA2 is exported from the nucleus in a Crm1p-dependent manner. We assume that such export can be an element of SA1-SA2 exchange in the cohesin complex. At present, the putative NES-es are tested to identify those which are indeed functional. Moreover, although SA proteins can not replace Irr1p we found that their presence in yeast cells provoked phenotypic changes, which indicates that some functions are interchangeable between yeast and human cohesins.

99

Microbial degradation 4-n-nonylphenol by *Streptomyces mirabilis* IM P102: response to xenobiotic exposure.

M. Krupiński, J. Długoński

Department of Industrial Microbiology and Biotechnology, University of Lodz, Lodz, Poland

Author's e-mail: mkrupi@biol.uni.lodz.pl

The natural environment always includes different kinds of stress, for example xenobiotics stress. Microorganisms can often adapt to use xenobiotic chemicals as novel growth and energy substrates via various genetic mechanisms that subsequently determine the evolution of functional degradative pathways. Many anthropogenic organic compounds are suspected or known to be endocrine disruptors, which can influence the endocrine system and alter hormonal functions. 4-Nonylphenol is commercially important surfactant, which is used as detergent, emulsifier and in the formulation of pesticides or cosmetics. During the last decade, this xenobiotic has gained a lot of interest, since it has been designates as an endocrine disruptor.

Some microorganisms are being described as able to degrade 4-nonylphenol from environment of growth. During caring out research of microbiological degradation of 4-NP, there has been found bacterial strain, which effectively eliminate this compound. The aim of the present study was to identify analyzed strain.We used a 16S ribosomal RNA gene sequencing-based strategy to identify these bacteria. On the basis of our results we suggest that strain belong to *Streptomyces mirabilis.* We also investigated elimination kinetics of 4-n-nonylphenol and involvement intercellular enzymes with monooxygenase activity in decrease of the 4-n-NP concentration in cultures.

100

Tolerance to heavy metals and genetic characterisation of emulsifier producing fungus *Curvularia lunata* transformed with pAN7-1

K. Paraszkiewicz[1]*, J. Szemraj[2], J. Długoński[1]

[1] *Department of Industrial Microbiology and Biotechnology, University of Lodz, S. Banacha 12/16, 90-237 Lodz, Poland;*
[2] *Department of Biochemistry, University of Medicine, Mazowiecka 6/9, 90-131 Lodz, Poland*

Author's e-mail: ola@biol.uni.lodz.pl

Various physiological and genetic parameters of *Curvularia lunata* wild strain and its 7 pAN7-1 transformants were investigated. Preliminarily, tolerance of the wild strain to 5 selected heavy metals was determined. This fungus appeared to be most sensitive to copper as well as nickel and less sensitive to zinc, cadmium and lead. Lower concentrations of all examined metals were required to completely inhibit the growth of colonies in comparison to the mycelial growth in the stirred cultures. The effects of nickel and cadmium on the growth and accumulation properties of all studied *C. lunata* strains were compared. The results indicated that microbial growth and heavy metal ions uptake were significantly affected by initial metal concentrations. The most tolerant to nickel and cadmium strain (transformant XXII/13) exhibited the lowest heavy metals accumulation ability.

Southern blot chemiluminescent detection was used instead of radioactive system for the investigation of plasmid pAN7-1 integration with fungal genome. The molecular analysis showed a presence of hybridisation signals in all the investigated transformants. Inverse PCR method was used for the analysis of sequences flanking the *hph* gene. For the two transformants studied the sequences have no homology either with pAN7-1 or any known eucaryotic gene. Among all the examined *C. lunata* transformants only the strain XXII/13 distinctly differed in the intensity of the colony growth, sensitivity to heavy metals,

Last minute registration

101

The effect of lead on nucleolus in meristematic cells of *Allium sativum* L. roots

Łucja Balcerzak

Laboratory of Electron Microscopy, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

Author's e-mail: villussion@yahoo.com

Lead is an environmental pollutant with a wide range of toxic effects that were examined on many plants in a broad spectrum of parameters. However, there are only a few reports concerning the effect of lead on nucleoli, particularly on their ultrastructure. Therefore, the aim of this study was to investigate the influence of lead on morphology and ultrastructure of nucleolus as well as Ag-NOR proteins localization.

The three-day-old adventurous roots of *Allium sativum* L. were treated with aqueous solution of $Pb(NO_3)_2$ at the concentration of 10^{-4} M for 2 h. The control plants were hydroponically grown in distilled water.

In the control and lead treated root meristematic cells stained with $AgNO_3$ 1 to 4 nucleoli were observed in nucleus. The presence of lead caused a drop in the number of nuclei with 1 nucleolus accompanied by an increase in the number of nuclei with 2 - 4 nucleoli. Moreover, the light microscopy observations revealed the presence of silver-stained particles in the nucleoplasm and cytoplasm. After lead treatment the frequency of cells with silver-stained particles as well as the number of these particles in the cell significantly increased.

The electron microscopy analysis confirmed a typical ultrastructure of nucleolus in the control root cells, i.e. fibrillar centres (FCs) surrounded by the dense fibrillar component (DFC) and the granular component (GC) at the periphery, but after lead treatment these components were hardly distinguished. Additionally, in the control and lead treated material 1 - 4 oval to round shape structures attached to the nucleolus, in the close association with nuclear chromatin or free in the nucleoplasm even nearby the nuclear envelope were observed. They were built of the densely packed fibrils. Lead presence increased dimensions as well as the number of fibrillar structures. Moreover these structures were more frequently attached to the nucleolus and sporadically occurred in the cytoplasm.

To examine the localization of Ag-NOR proteins the silver-staining was applied at the electron microscopy level. In nucleoli of the control material the accumulation of silver grains was the most pronounced in the DFC, less - in the GC while FCs were not silver-stained. In the presence of lead only the GC was slightly silver-stained. In the fibrillar structures four types of silver-staining pattern were distinguished: I) strongly stained at the whole surface, II) moderately or slightly stained at the whole surface, III) strongly stained at the periphery and IV) not silver-stained. In lead

presence the second and the third type dominated in the nucleoplasm while the fourth type was observed in the cytoplasm.

The obtained data showed that lead enhanced the appearance of fibrillar structures composed mainly of Ag-NOR proteins outside the nucleolus. These particles were probably removed from nucleolus into the nucleus and even into the cytoplasm as a result of disturbance in the nucleologenesis.

102

Sequencing of the cucumber (*Cucumis sativus* L.) genome

Rafał Wóycicki , Wojciech Gutman , Magdalena Pawełkowicz , Wojciech Pląder , Anna Seroczyńska , Ewa Siedlecka , Mieczysław Śmiech , Justyna Witkowicz , Stefan Malepszy, Zbigniew Przybecki

Dept. of Plant Genetics, Breeding & Biotechnology,
Warsaw University of Life Sciences - SGGW,
Nowoursynowska 159, 02-606 Warsaw, Poland

Author's email: rafalwoycicki@gmail.com

Cucumber is an edible fruit and the plant belongs to the family Cucurbitaceae. The genus Cucumis contains more than 30 species, of which two are economically important crops, namely cucumber (*C. sativus* L) and melon (*C. melo* L). As a vegetable crop cucumber has big agriculture value, however the economic importance varies with the region. It is a model plant in studying sex determination and several molecular marker maps with the help of BAC libraries and STCs should work very well to achieve this aim by positional cloning. In the past two years the new generation of sequencing methods make even possible to sequence whole genome of desired organism. Our group has several achievements in cucumber genetics and biotechnology. Utilizing molecular markers we have found 1 536 sequences (deposited in NCBI database) and use them as markers for positional isolation of sex genes. After sequencing of 65 280 BAC ENDs (STCs - parts of them deposited in NCBI database), library fingerprinting was then planning to get the physical map as a base for gene isolation. Instead of fingerprinting we have chosen to sequence the whole genome by 454 Titanium (at Agencourt Bioscience Corporation, MA, USA). The genome was sequenced with 8x coverage and assembled in contigs. Currently we are working on joining them together using previously mentioned sequences, additional 4x coverage of 3 Kbp paired end reads and available genetic maps of this crop.

AUTHOR'S INDEX

A

Abramowski D., 48
Adamczyk B., 63
Adamek A., 93
Arasimowicz-Jelonek M., 70

B

Bacolla A., 77
Bagniewska-Zadworna A., 32
Balazadeh S., 17
Balcerzak Ł., 108
Baraniak J., 90, 91
Bednara E., 36
Berczyński S., 51
Bergier K., 55
Biczysko W., 93
Bielecka-Kowalska A., 96
Bielecki S., 14
Bielewicz D., 42
Bieniek A., 96
Bijak M., 100, 104
Błasiak J., 83
Błaszczyk A., 95
Błoński J. Z., 81
Bobrowski M., 100, 104
Bocian A., 71
Boczek T., 86, 87
Bogatek R., 40, 65,74
Borowiecka M., 104
Bożek M., 21
Bryś M., 80
Brzostek A., 77, 78

C

Cebula B., 81
Chmielnicka A., 26
Chmielowska J., 70
Chwiłkowska A., 88
Ciereszko I., 61, 62
Ciomborowska J., 78
Cuchra M., 96
Czajkowska K., 65
Czaplicki A. Z., 56
Czyżewska D., 42

D

Dąbrowski Sz., 46
Dębski J., 36
Dębski K. J., 79
Długoński J., 107
Dolata J., 42
Domańska A., 60
Domińska K., 85
Dziadek J., 77, 78

F

Floras K., 42
Floryszak-Wieczorek J., 48
Forma E., 80

G

Gadzalski M., 33
Gajos-Michniewicz A., 94
Gapik M., 89
Garnczarska M., 73
Gerszberg A., 23, 53, 54
Gibała M., 50
Giełwanowska I., 39
Ginalski K., 16
Ginda K., 78
Glińska S., 67
Głowacka K., 69
Głowacki S., 47
Gniazdowska A., 40, 65, 74
Godlewski M., 60, 63
Górecki R. J., 69
Grabsztunowicz M., 73
Grelewska K., 75
Guzek M., 78
Gwoździński K., 83, 84

H

Haizel T., 21
Hasterok R., 28
Hennig J., 42
Hnatuszko-Konka K., 23, 53, 54
Horodecka E., 50
Hoser G., 89

I

Idziak D., 28

J

Jakimowicz D., 78
Janczara R., 57
Janus Ł., 48
Jańska H., 44, 45, 50
Jarmołowski A., 17, 41, 42, 43
Jaskolski M., 18
Jenkins G., 28
Jerzmanowski A., 16
Jeziorski A., 82
Jędrzejczyk I., 26
Jóźwiak Z., 83, 84
Jurczyk B., 71
Juszczak I., 44

K

Kachlicki P., 52
Kaczkowska E., 49
Kaczmarczyk D., 97
Kaczmarek R., 90, 91
Kapruziak K., 55
Kasprowicz A., 57
Kasprzak A., 93
Kawiak J., 20, 89
Kaźmierczak A., 64
Kaźmierczak J. M., 64
Kaźmierczuk A., 81
Kędzierska M., 82
Kicia M., 45
Kiliańska Z. M., 81
Kłobus G., 68
Kmieć-Wiśniewska B., 45
Kniżewski Ł., 16
Kobylińska A., 60
Koceva-Chyła A., 83, 84
Kokoszka K., 16
Kolano B., 36
Kolodziejczak M., 44
Kołodziejczyk J., 100, 101, 102
Kononowicz A. K., 23, 46, 47, 48, 53, 54
Konowalczyk A., 100
Kontek B., 95
Kontek R., 95
Koptyra M., 89
Kosatka E., 68
Kosmala A., 71, 72, 73
Kotlińska T., 50
Kowalczyk T., 23, 53
Kowalec P., 106
Kowalski A., 86, 87
Kowalski M., 96
Krajewska W. M., 80
Krakowiak A.D., 91
Krasuska U., 65
Królewska K., 102, 103
Krupa R., 83
Krupiński M., 107
Krzesłowska M., 66
Kubicka A., 55
Kubicka H., 33
Kulbacka J., 88
Kulik K., 90
Kulikova N., 105
Kulma A., 15
Kurek W., 74
Kurlandzka A., 106
Kuśmierczyk K., 97
Kuta E., 21
Kwaśniak M., 50
Kwiatkowska M., 31, 58

L

Lachowicz-Ochędalska A., 85
Lech M., 45
Legocki A. B., 13
Leitch A. R., 21
Lenartowska M., 66
Leśniewska K., 34
Lewandowska N., 78
Lim Y. K., 21
Linkiewicz A., 75

Ł

Łaźniewska J., 48
Łojko M., 69
Łuchniak P., 23, 53, 54
Łukasiewicz B., 95
Łukaszuk E., 61

M

Macioszek V. K., 46, 47, 48
Mackiewicz P., 77
Majchrzak K., 92
Majewska M., 27
Majkowski M., 88
Majsterek I., 96, 97, 98
Makałowska I., 78
Maksymiec W., 39

Malepszy S., 13
Malinowska J., 100
Małolepsza U., 49
Małuszyńska J., 36
Man A., 105
Man D., 105
Marciniak K., 26
Marczewski W., 42
Markiewicz L., 97, 98
Maruniewicz M., 37
Maszewski J., 29, 30
Matczak K., 83, 84
Mądrzak C. J., 35, 64
Mellerowicz E., 66
Michlewska S., 67
Migocka M., 68
Mikiciński A., 51
Mlynarski W., 96, 98
Moore G., 21
Morawiec-Sztandera A. , 97, 98
Mozer-Lisewska I., 93
Mueller-Roeber B., 17
Mulcan A., 70
Muth D., 52

N

Naganowska B., 34
Nałęcz K. A., 105
Narożna D., 64
Nawrot B., 90, 91, 92
Nowak P., 100, 104

O

Ochędalski T., 94
Okorski A., 69
Olas B., 82, 100, 101
Olejniczak K., 93
Oleszczuk S., 56
Olszewski J., 97, 98
Osiecka R., 95
Osińska M., 22
Ozga M., 90

P

Pałyga J., 29
Pant Bikram D., 17
Papierniak A., 68
Paraszkiewicz K., 107
Pasternak I., 42
Paś J., 64
Pawlak M., 83
Pawlak S., 70
Pawłowicz I., 72
Pawłowska E., 94
Pawłowski K., 14, 79
Pęcherzewska R., 91
Piastowska A., 85, 94
Piechota J., 44
Pieczyński M., 42
Piekarski J., 82
Pietkiewicz A., 90
Pietruszewska W., 98
Pietrykowska H., 41, 43
Pietrzak B., 55
Podolak M., 105
Pogorzelec Ł., 45
Polit J. T., 58, 59
Popłońska K., 31, 58
Przybecki Z., 13
Przybyszewska W., 93
Przysiecka Ł., 35
Puławska J., 51, 52
Puzio J., 16
Puzio M., 16
Pyza A., 33

R

Radzikowska E., 90
Rapacz M., 71
Reczyńska A., 101
Rębas E., 86
Robak T., 81
Rogalińska M., 81
Rogójska A., 89
Rojek A., 42
Romanowicz-Makowska H., 80
Rorat T., 71
Rudzińska-Langwald A., 74
Rusin P., 97, 98
Rutowicz K., 16
Rybaczek D., 36
Rychta E., 77
Rywaniak J., 89, 99, 100

S

Saczko J., 88
Sadowska A., 26
Sakowicz T., 33

Saluk-Juszczak J., 102, 103
Samardakiewicz S., 66
Scheible W.-R., 17
Seta A., 39
Siedlecki P., 16
Silberring J., 19
Skórski T., 89
Skórzyńska-Polit E., 39
Sliwinski T., 97, 98
Smakowska E., 45
Smolarek M., 102, 103
Smolewski P., 81
Sobczuk A., 83
Sobiczewski P., 51, 52
Sobkowiak Ł., 17, 41
Sodkiewicz T., 27
Sodkiewicz W., 27
Sołtys D., 74
Sowa S., 75
Stańczyk M., 96
Stec W. J., 90, 91
Stępiński D., 58
Stobiecki M., 52
Sulikowska M., 52
Synowiec E., 83
Szabała B., 71
Szafrański K., 40
Szarzyńska B., 17, 41, 43
Szczuka E., 39
Szemraj J., 97, 107
Szewc Ł., 43
Szopa J., 15, 54
Szweykowska-Kulinska Z., 17, 41, 42, 43

Ś

Śliwińska E., 74
Śliwiński M., 95
Śnitko R., 55

T

Tarnowski L. J., 106
Tomaszewska A., 30, 36, 91
Tracz K., 55
Trelińska J., 96
Tułodziecka K., 105

W

Wachowicz B., 82, 99, 100
Walczak A., 98
Walczak W., 43
Wasilewska D., 89
Wawrzuta A., 88
Wawszczyk J., 29
Wędołowska A., 61
Węsierska-Gądek J., 81
Wiejak J., 22
Wierzchowiecka M., 38
Wiktorek-Smagur A., 23, 53, 54
Wiśniewska A., 40
Wojciechowicz M. K., 32
Wojtaszek P., 22, 37, 38, 57
Wojtczak A., 31, 58
Wojtyła Ł., 73
Wolko B., 35
Wolny E., 28
Wolska-Sobczak A., 33
Wołoszyńska M., 50
Woźniak K., 83
Woźny A., 66
Wóycicki R., 13
Wypych E., 22
Wyroba E., 22

Z

Zabel M., 93
Zenkteler E., 32
Zenkteler M., 32
Zimny J., 56
Zwierzykowski Z., 71

Ź

Źróbek-Sokolnik A., 69

Ż

Żabka A., 29
Żak A., 61
Żbikowska H.M., 89
Żebrowska E., 62
Żołnierczyk J. D., 81
Żołnierowicz J., 89
Żuk M., 15, 54
Żurawska-Zajfert M., 75
Żylińska L., 86, 87

NOTES