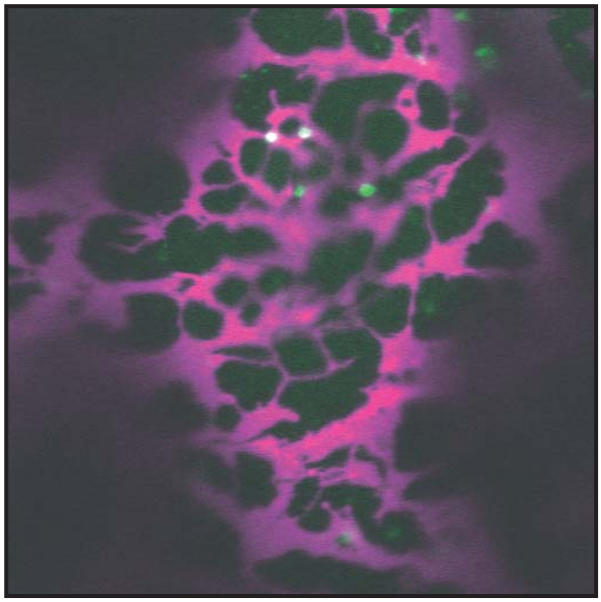
Proceedings of

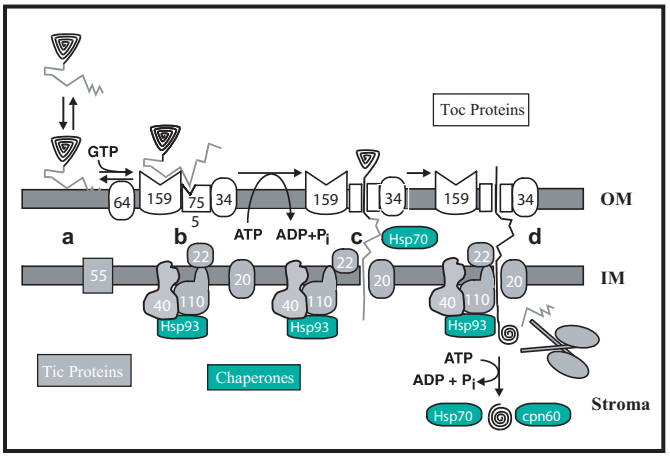
The Canadian Society of Plant Physiologists 2005 Eastern Regional Meeting

Wilfrid Laurier University, Waterloo, ON
December 16-17, 2005

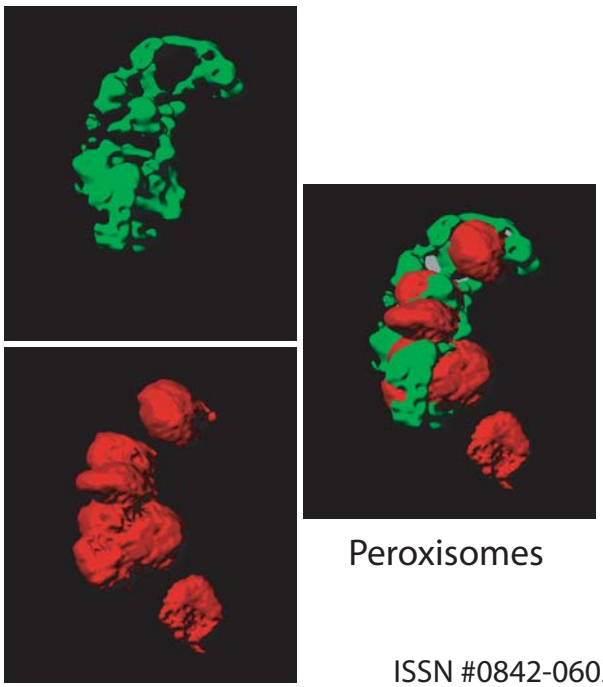
Organelle Biogenesis and Dynamics



Secretory pathway



Protein import into chloroplasts



Peroxisomes

Délibérations de

La Société Canadienne De Physiologie Végétale Congrès Régional de l'Est 2005

L'université Wilfrid Laurier, Waterloo, ON
16-17 décembre, 2005

Biogénèse et Dynamique des Organites

ISSN #0842-0602
Vol. 49 #1

Cover Illustrations:

Left: A pea (*Pisum sativum* cv Sparkle) seedling, 9 days after planting (photo provided by B.J. Ferguson and F.C. Guinel).

Top right: a Golgi marker fused to the yellow fluorescent protein (pseudocoloured magenta) re-absorbed into the ER in the presence on the inactive mutant of the GTPase ARF1 encoded in a double-cistronic vector expressing a soluble peroxisomal marker fused to the cyan fluorescent protein, CFP-SKL (pseudocoloured green). This marker allows the identification of cells expressing untagged effector molecules thanks to the visualization of the peroxisomes (provided by F. Brandizzi).

Centre right: Schematic representation of protein import into chloroplasts (provided by J. Froehlich)

Bottom right: Three-dimensional computer-rendered reconstruction of an aggregate of peroxisomes (green) and chloroplasts (red) in a mesophyll cell of a GFP-SKL-transformed tobacco (*N. benthamiana*) plant infected with the tomato bushy stunt virus (provided by R. Mullen).

Organizing Committee

Frédérique C. Guinel, Department of Biology, Wilfrid Laurier University
Matthew D. Smith, Department of Biology, Wilfrid Laurier University

The organizers would like to thank the following for their generous financial support:

Dr. Jane Rutherford, Chair, Department of Biology, Wilfrid Laurier University
Dr. Arthur Szabo, Dean, Faculty of Science, Wilfrid Laurier University
Dr. David Peirson, Dean, Graduate Studies and Research, Wilfrid Laurier University
Laurier Lecture Series

In addition, the organizers would like to thank the following for their organizational assistance:

Ms. Debbie Currie
Ms. Kristen Porritt
Mrs. Elma Schweigert
Biology Students for help with moving the furniture.

Canadian Society of Plant Physiologists

Société Canadienne de Physiologie Végétale

WILFRID LAURIER UNIVERSITY

VERITAS OMNIA VINCIT

WLU BIOLOGY

Schedule at a Glance

Friday, December 16

7:00-10:00 p.m.	Social/mixer and Registration	Recital Hall Foyer

Saturday, December 17

8:30-9:00 a.m.	Registration, refreshments, and Poster setup	Senate & Board Chambers (SBC) Foyer
9:00-9:10 a.m.	Welcoming Remarks Dr. Art Szabo, Dean of Science, WLU	Senate & Board Chambers
Plenary Session	***Organelle biogenesis and dynamics***	Senate & Board Chambers
9:10-9:55 a.m.	Federica Brandizzi, University of Saskatchewan	
10:00-10:45 a.m.	Robert Mullen, University of Guelph	
10:45-11:05 a.m.	Coffee and refreshments	SBC Foyer
11:05-11:50 a.m.	John Froehlich, Michigan State University	
11:50-1:30 p.m.	Lunch and Posters	SBC Foyer
1:30-3:00 p.m.	Concurrent Oral Sessions A & B	Paul Martin Centre and Senate & Board Chambers
3:00-3:45 p.m.	Coffee, refreshments and Posters	SBC Foyer
3:45-5:30 p.m.	Concurrent Oral Sessions C & D	Paul Martin Centre and Senate & Board Chambers
5:15 - 6.00 p.m.	Jury deliberations for Awards	Paul Martin Centre
5:30 p.m.	Posters and light reception	SBC Foyer
6:00 p.m.	Closing Remarks and Award presentations	Senate & Board Chambers
6:15 p.m.	Brown bag snack pick-up	SBC Foyer

Detailed Schedule

Plenary session **Senate & Board Chambers**

Chair: Dr. Greg Vanlerberghe, University of Toronto at Scarborough

Organelle biogenesis and dynamics

PP1 9:10 - 9.55 a.m.
Protein secretion in plant cells: looking for a way out.
Federica Brandizzi
Department of Biology, University of Saskatchewan, Saskatoon

PP2 10:00 -10.45 a.m.
Peroxisome biogenesis and the hijacking of peroxisomes by tumboviruses.
A.W. McCartney[1], J.S. Greenwood[1], M.R. Fabian[2], K.A. White[2], Robert T. Mullen[1]
[1] Department of Molecular and Cellular Biology, University of Guelph
[2] Department of Biology, York University

10:45 – 11:05 am Coffee and refreshments **SBC Foyer**

PP3 11:05 – 11:50 am
Pushing and pulling: Examining the process of protein translocation into chloroplasts by using two novel cross-linking approaches.
John E. Froehlich
DOE-MSU Plant Research Laboratory, Michigan State University, East Lansing

1:30 - 3:00 pm Concurrent sessions A and B

Session A - Enzymology Paul Martin Centre

Chair: Dr. Barbara Moffatt, University of Waterloo

A1. 1:30 - 1.45 pm
Water movement through seed coats and into embryos of soybean: a comparison of permeable and impermeable seeds.
Chris Meyer, E. Steudle, and C.A. Peterson.

A2. 1:45 - 2:00 pm
Characterization of K^+-dependent and -independent L-asparaginases from Arabidopsis.
Frédéric Marsolais, L. Bruneau, and R. Chapman.

A3. 2:00 - 2:15 pm
Towards identifying the *in vivo* roles of NAD^+ kinases in *Arabidopsis thaliana*.
Jeffrey C. Waller, U. Schumann, R.T. Mullen, and W.A. Snedden.

A4. 2:15 - 2:30 pm
Investigating the *cis*-elements of winter rye glucanase promoters.
Shauna A. McCabe, M.Griffith, and B.A. Moffatt.

A5. 2:30 - 2:45 pm
Characterization of a recombinant *Arabidopsis* gamma-aminobutyrate transaminase.
Shawn M. Clark, and B. Shelp.

A6. 2:45 - 3:00 pm
Plant cytosolic nucleoside diphosphate kinase: autophosphorylation on a conserved Ser residue and protein trans-phosphorylation activity.
S. Dorion, F. Dumas, and Jean Rivoal.

1:30 - 3:00 pm Concurrent sessions A and B

Session B – Molecular/Cell Senate & Board Chamber

Chair: Dr. Michael Wang, University of Waterloo

B1. 1:30 - 1:45 pm
The chimeric cyclic nucleotide-gated ion channel AtCNGC11/12 induces programmed cell death.
William Urquhart, A. Gunawardena, W. Moeder , and K. Yoshioka.

B2. 1:45 - 2:00 pm
Alternative splicing and chromosome dynamics at the *MADS-AFFECTING FLOWERING* locus of *Arabidopsis thaliana*.
Sarah Rosloski, and V. Grbic.

B3. 2:00 - 2:15 pm
Tung (*Vernicia fordii*) diacylglycerol acetyltransferase 1 and 2 localize to distinct subdomains of the endoplasmic reticulum.
Satinder Gidda, J. Shockey, S.J. Rothstein, J. Dyer, and R. Mullen.

B4. 2:15 - 2:30 pm
Does the tomato bushy stunt virus hijack ESCRT in the formation of peroxisomal multivesicular bodies?
Andrew McCartney, A. Howard, G. Smith, S. Gidda, and R. Mullen.

B5. 2:30 - 2:45 pm
Localization of DIR1 – a component of the long-distance systemic acquired resistance signaling complex.
Marc J. Champigny, H. Shearer, A. Mohammad, M. Neumann, K. Haines, Z. Zhao, and R.K. Cameron.

B6. 2:45 - 3:00 pm
Enhancing resistance to pathogen ingress in tomato by down-regulating its eIF5A-3.
Fengshan Ma, Z. Liu, T-W. Wang, L. McNamara, and J. Thompson.

3:45 - 5:30 pm Concurrent sessions C and D

Session C – Biochemistry/Engineering Paul Martin Centre

Chair: Dr. Ian Tetlow, University of Guelph

C1. 3:45 - 4:00 pm
Identification and characterization of candidate tail-anchored integral membrane proteins in plant, yeast and mammalian proteomes.
Priya K. Dhanoa, M. Mistry, D. Wickrama, X. Yang, M.P. Henderson, D.W. Andrews, and R.T. Mullen.

C2. 4:00 - 4:15 pm
Enhancement of salinity tolerance by engineering chloride volatilization into plants.
Simendeep Kaur, S. Babayeva, P. Koonjul, and H. Saini.

C3. 4:15 - 4:30 PM
A new plant-derived marker for efficient selection of transgenic plants.
Priyum Koonjul, S. Babayeva, and H. Saini.

C4. 4:30 - 4:45 pm
Generating Arabidopsis PDT proteins in a yeast expression system.
Oliver Corea and Susanne Kohalmi.

C5. 4:45 - 5:00 pm
Starches and their functional properties.
Ian J. Tetlow.

C6. 5:00 - 5:15 pm
Regulation of starch synthesis by protein complex formation.
Fushan Liu, I. Tetlow, and M.Emes.

C7. 5:15 - 5:30 pm
Characterisation of disproportionating enzyme from wheat.
Nicole Bresolin, Z. Li, B. Kosar-Hashemi, I. Tetlow, M.Chatterjee, S. Rahman, M.K. Morell, C.A. Howitt, and M.J. Emes.

3:45 - 5:30 pm Concurrent sessions C and D

Session D – Structure/Physiology/Biochemistry Senate & Board Chamber

Chair: Dr. Neil Emery, Trent University

D1. 3:45 - 400 pm
The effect of vesicular-arbuscular mycorrhizae on root anatomy as it impacts on acquisition in tomato.
Jeff Taylor, and A. Waltenbaugh.

D2. 4:00 - 4:15 pm
Characterisation of the mechanical properties of the pollen tube during apical growth.
Rabah Zerzour and A. Geitman.

D3. 4:15 - 4:30 pm
The physiological basis for photoperiodic injury in tomato: a role for circadian rhythms and nitrite toxicity.
Lorraine I. D'Silva, M.C. Micallef, V. Coneva, and B.J. Micallef.

D4. 4:30 - 4:45 pm
Acclimation of the psychrophilic strain (*UWO 241*) and the mesophilic strain (*SAG 49.72*) of *Chlamydomonas raudensis* to temperature and irradiance.
Beth Szyszka, A. G. Ivanov, N.P.A. Hüner.

D5. 4:45 -5:00 pm
Cytokinins in tissues and phloem sap explain reduced apical dominance of *Lupinus angustifolius* transformed with an isopentenyl transferase (IPT) and a flower specific promoter (TP12).
Neil Emery, P. Smith, and C. Atkins.

D6. 5:00 - 5:15 pm
Light-harvesting mutants of *Chlamydomonas reinhardtii*.
Kenneth Wilson and L.Gray.

D7. 5:15 - 5:30 pm
Photoinhibitory responses in contrasting ecotypes of *Thellungiella*.
Jillian N. Kriger, Heather M. Baerr, Anna T. Hanson, and Gordon R. Gray.

Plenary session: 9:10-9:55 am **Senate & Board Chambers**

PP1. Protein secretion in plant cells: looking for a way out

Federica Brandizzi
Department of Biology, University of Saskatchewan, Saskatoon, Canada
email: federica.brandizzi@usask.ca

Cells built and package secretory material in a series of compartments, named the secretory pathway. The endoplasmic reticulum (ER) and Golgi apparatus form the early secretory pathway.

To provide further understanding on mechanisms that regulate protein export from the ER to the Golgi apparatus, we have explored the dynamics of protein trafficking between the ER and Golgi, and their spatial and functional relationship with the cytoskeleton using live cell imaging techniques.

It appears that plant cells contain multiple mobile Golgi stacks distributed over the entire cytoplasm. These stacks move with the ER by means of actin-myosin motors[1]. The domains of the ER dedicated to the export of proteins, the ER export sites (ERES) form secretory units that move along the surface of the ER together with the Golgi[2]. We also found that the integrity of Golgi and ERES is regulated by the activity of specific GTPases, such as Sar1 and Arf1.

We have also explored the requirements for proteins to leave the ER and move towards the Golgi. Our results indicate a stringent signal-regulated mechanisms for ER export of multispanning, type I and type II membrane proteins[3]. For example, we found that mutations of a specific di-acidic motif (DXE) in the cytosolic tail of proteins such as CASP, a Golgi matrix protein with a type II membrane topology[4], cause a reduction of the export of this protein from the ER (Figure 1). Similar finding have been found for type I and multispanning membrane proteins[3].

Our results indicate that in plant cells the ER and Golgi form a dynamic membrane system whose components continuously cycle through the ER via a regulated membrane trafficking pathway.

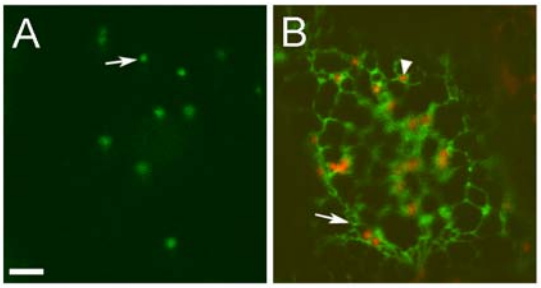


Figure 1 – Signal-driven ER export of plant Golgi membrane proteins.
A – A GFP fusion to CASP, a membrane-associated Golgi matrix protein, targets the Golgi apparatus in tobacco leaf epidermal cells (arrow). **B –** When a di-acidic signal of the cytosolic tail of this protein is mutated to non-charged amino acids, the export of the protein to the Golgi apparatus (arrowhead) is drastically reduced and the CASP mutant is visible in the ER network (arrow). A yellow fluorescent protein fusion to ERD2[5] is used as a Golgi marker. Scale bar = 5 μm.

References

1. Brandizzi, F., Snapp, E.L., Roberts, A.G., Lippincott-Schwartz, J., and Hawes, C. (2002). Membrane protein transport between the endoplasmic reticulum and the Golgi in tobacco leaves is energy dependent but cytoskeleton independent: evidence from selective photobleaching. Plant Cell **14,** 1293-1309.

2. daSilva, L.L., Taylor, J.P., Hadlington, J.L., Hanton, S.L., Snowden, C.J., Fox, S.J., Foresti, O., Brandizzi, F., and Denecke, J. (2005). Receptor salvage from the prevacuolar compartment is essential for efficient vacuolar protein targeting. Plant Cell **17,** 132-148.

3. Hanton, S.L., Renna, L., Bortolotti, L.E., Chatre, L., Stefano, G., and Brandizzi, F. (2005). Diacidic motifs influence the export of transmembrane proteins from the endoplasmic reticulum in plant cells. Plant Cell **17,** 3081-3093.

4. Renna, L., Hanton, S.L., Stefano, G., Bortolotti, L., Misra, V., and Brandizzi, F. (2005). Identification and characterization of AtCASP, a plant transmembrane Golgi matrix protein. Plant Mol Biol **58,** 109-122.

5. Brandizzi, F., Frangne, N., Marc-Martin, S., Hawes, C., Neuhaus, J.M., and Paris, N. (2002). The destination for single-pass membrane proteins is influenced markedly by the length of the hydrophobic domain. Plant Cell **14,** 1077-1092.

Plenary session: 10:00-10:45 am **Senate & Board Chambers**

PP2. Peroxisome biogenesis and the hijacking of peroxisomes by tombusviruses

Andrew W. McCartney[1], John S. Greenwood[1], Marc R. Fabian[2], K. Andy White[2] Robert T. Mullen[1]
[1] Department of Molecular and Cellular Biology, University of Guelph
[2] Department of Biology, York University
email: rtmullen@uogueph.ca

Peroxisomes are highly dynamic organelles with regard to their metabolic functions, shapes, distribution, movements and biogenesis. They are also important as sites for the development of some viral pathogens. It has long been known that certain members of the tombusvirus family (e.g., tomato bushy stunt virus [TBSV]) recruit peroxisomes for viral RNA replication and that this process is accompanied by extensive inward vesiculations of the peroxisomal boundary membrane leading to the formation of a peroxisomal multivesicular body (pMVB). While pMVBs are known to contain the protein components of the virus' membrane-bound RNA replication complex, the molecular mechanisms by which these proteins are targeted to peroxisome membranes and participate in pMVB biogenesis are not well understood. Recently, we showed that the TBSV 33-kDa replication protein (p33), expressed on its own, targets initially to peroxisomes where it causes their progressive aggregation and eventually the formation of peroxisomal "ghosts". These altered peroxisomes are distinct from pMVBs since they lack internal vesicles and, instead, are surrounded by novel cytosolic vesicles that contain p33 and that appear to be derived from evaginations of the peroxisomal boundary membrane. Concomitant with these dramatic changes in peroxisomes, p33 and resident peroxisomal membrane proteins are relocalized in an Arf1-dependent manner to peroxisomal ER (pER), a specialized subdomain of the ER where normally nascent peroxisomes appear to be derived. Mutational analysis of p33 revealed that its intracellular sorting is mediated by several targeting signals including three peroxisomal targeting elements that function in a cooperative manner and a pER targeting signal that resembles an arginine-based motif responsible for the vesicle-mediated retrieval of escaped ER membrane proteins from the Golgi. The implications of these results with respect to our overall understanding of pMVB biogenesis and the biogenetic link between peroxisomes and pER via vesicle transport are discussed.

Plenary session: 11:05-11:50 am **Senate & Board Chambers**

PP3. Pushing and Pulling: Examining the Process of Protein Translocation into Chloroplasts by Using Two Novel Cross-linking Approaches

John E Froehlich
DOE-MSU Plant Research Laboratory, Michigan State University, East Lansing MI 48823
email: froehli5@msu.edu

A significant unsolved problem in cell biology is understanding how cytoplasmically synthesized proteins are targeted to various compartments within eukaryotic cells. In plants, chloroplasts offer an excellent system for studying this problem. Most chloroplastic proteins are encoded in the nucleus, synthesized in the cytoplasm as higher molecular weight precursors, and imported posttranslationally across the two envelope membranes (see Fig. 1). The long-term goal of our research program is to understand the molecular details of protein import into chloroplasts.

Recent progress in the identification and characterization of components of the transport apparatus has allowed formulation of specific hypotheses regarding the details of protein transport into chloroplasts (see Fig. 1). We propose to test a specific hypothesis that derives from earlier work in this lab, i.e. that a stromal molecular chaperone from the HSP 100 family (Hsp93) forms a chaperone complex with other translocation components, i.e., Tic110 and Tic40, to provide the driving force for the transport of precursor proteins into chloroplasts (Fig. 1). Despite the evidence that a portion of the chloroplastic Hsp93 is present in translocation complexes, little experimental evidence is currently available to support the attractive hypothesis that Hsp93 functions, possibly in concert with other translocation components, to provide the driving force for protein import. We wish to test this hypothesis by developing and employing two novel cross-linking strategies to investigate precursor interactions with the chloroplastic Tic complex and with stromal molecular chaperones.

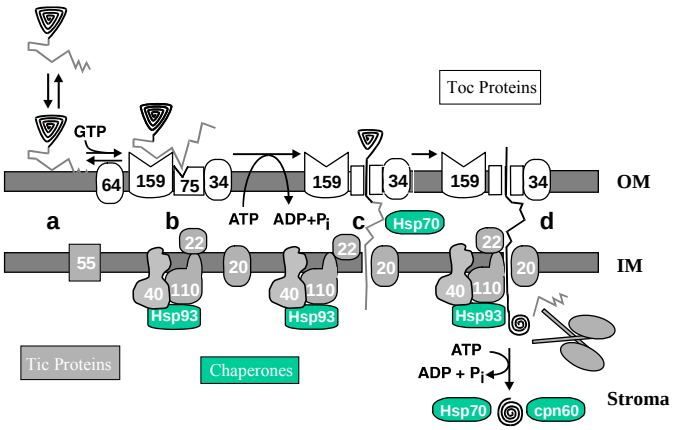


Figure 1. Schematic representation of protein import into chloroplasts

References

Bedard J, Jarvis P. Recognition and envelope translocation of chloroplast preproteins. J Exp Bot. (2005) 419:2287-320.

Constan D, Froehlich JE, Rangarajan S, Keegstra K. A stromal Hsp100 protein is required for normal chloroplast development and function in Arabidopsis. Plant Physiol. (2004) 3:3605-15.

Presentation A1: 1:30-1:45 pm **Paul Martin Centre**

Water movement through seed coats and into embryos of soybean: a comparison of permeable and impermeable seeds.

Chris J. Meyer[1], Ernst Steudle[2], and Carol A. Peterson[1]
[1]Department of Biology, University of Waterloo, Waterloo, ON, Canada, N2L 3G1
[2]Department of Plant Ecology, University of Bayreuth, D-95440 Bayreuth, Germany
email: cj_meyer1@yahoo.ca

Usually, when soybean seeds are immersed in water, they soon begin to imbibe, soften and swell. Stone seeds, however, can remain dry and hard for long periods of time. Previously, a correlation was noted between the structure of the outer cuticle of the seed and its capacity for water uptake: the cuticle of permeable seeds had small cracks whereas that of stone seeds was continuous. The present investigation confirmed that the stone seeded trait is a feature of the seed coat, since isolated embryos from both stone and permeable seeds took up water at equal rates. When hydrated, seed coats of stone seeds were permeable to water but their hydraulic conductivity was smaller than that of coats from permeable seeds by a factor of five. Whole, permeable seeds take up water initially through the dorsal side of the seed coat and later through the hilum. The sites of initial water uptake by the cotyledons correlate with externally visible changes (i.e. wrinkling) in the coat. Some circumferential movement of water through the coat occurs, presumably through the air spaces of the osteosclereid layer. Imbibition by whole seeds is a two-phase process, the first dominated by hydration of the seed coat and the second by hydration of the embryo.

Presentation A2: 1:45-2:00 pm **Paul Martin Centre**

Characterization of K^+-dependent and –independent L-asparaginases from *Arabidopsis*.

Frédéric Marsolais, Luanne Bruneau, and Ralph Chapman
Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Centre
email: marsolaisf@agr.gc.ca

L-asparaginases (EC 3.5.1.1) are hypothesized to play an important role in the reallocation of transported N to sink tissues, especially in legume developing seeds. Two plant L-asparaginase subtypes have been previously identified according to their K^+-dependence for catalytic activity. An L-asparaginase homologous to *Lupinus* K^+-independent enzymes with activity towards β-aspartyl dipeptides, At5g08100, has been previously characterized as a member of the N-terminal nucleophile amidohydrolase superfamily in *Arabidopsis*. In this study, a K^+-dependent L-asparaginase from *Arabidopsis*, At3g16150, was characterized. Recombinant At3g16150 and At5g08100 share a similar subunit structure and conserved auto-proteolytic cleavage site exposing a catalytic Thr nucleophile, as determined by ESI-MS. The catalytic activity of At3g16150 was enhanced approximately 10-fold in the presence of K^+. The order of monovalent cation preference was $K^+ > Na^+ > Rb^+$. At3g16150 was strictly specific for L-Asn and had no activity towards β-aspartyl dipeptides. At3g16150 also had an approximately 55-fold higher catalytic efficiency with L-Asn relative to At5g08100. Among β-aspartyl dipeptides tested, At5g08100 had a preference for β-aspartyl-His, with a catalytic efficiency comparable to that determined with L-Asn. Asn-His and Asp-His constitute preferential sites of spontaneous isoaspartyl formation in proteins under physiological conditions. Phylogenetic analysis confirmed that At3g16150 and At5g08100 belong to two distinct subfamilies. Transcript levels of *At3g16150* and *At5g08100* were highest in sink tissues, especially in flowers and siliques early in development, as determined by quantitative RT-PCR. The overlapping spatial patterns of expression argue for a partially redundant function of the enzymes. However, the higher catalytic efficiency of K^+-dependent enzymes suggests that they may metabolize L-Asn more efficiently under conditions of high metabolic demand for N.

Presentation A3: 2:00-2:15 pm **Paul Martin Centre**

Towards identifying the *in vivo* roles of NAD^+ Kinases *in Arabidopsis thaliana.*

Jeffrey C. Waller[1], Uwe Schumann[2], R. T. Mullen[2], and Wayne A. Snedden[1].
[1] Queen's University, Department of Biology, Kingston, ON, K7L 3N6
[2] University of Guelph, Department of Molecular and Cellular Biology, Guelph, ON
email: wallerj@biology.queensu.ca

In the model higher plant *Arabidopsis thaliana*, the *de novo* biosynthesis of the vital triphosphopyridine nucleotide NADP is catalyzed solely by two NAD^+ kinases (NADKs) and one NADH kinase (NADHK) that use Mg^{2+}-ATP to phosphorylate either oxidized or reduced NAD respectively. Calmodulin, a ubiquitous eukaryotic Ca^{2+}-binding protein, responds to intracellular $[Ca^{2+}]_{cytosolic}$ fluxes or "signals" by activating numerous target enzymes. Intriguingly, plants possess a Ca2+ and calmodulin (CaM)-dependent NADK that has been implicated in stress response and pathogen defence. We have previously shown that the CaM-binding NADK isoform is localized exclusively to the chloroplast stroma and that the CaM-nonbinding NADK isoform is localized exclusively to the cytosol. In addition, we have identified a novel NADHK isoform and shown that it is localized exclusively to the peroxisomal matrix by a novel PTS1 targeting signal. Furthermore, we have determined the tissue distribution of each of the three NAD(H)K isoforms. The chloroplastic NADK is localized mainly to photosynthetic tissue while the cytosolic NADK is localized mainly to the root tissue. The peroxisomal NADHK was found to localize to the reproductive tissue and to the plant secondary and tertiary vasculature. However, the roles of each isoform remain cryptic, especially without the identification of the Ca^{2+}- and CaM-binding and –activated NADK isoform. In order to investigate the in vivo roles and regulation of plant NAD(H)Ks, we have developed homozygous transgenic plants that constitutively express the *NADK*'s transcript in the sense and antisense orientation as well as T-DNA insertion plants in order to identify the Ca^{2+}- and CaM-binding and –activated NADK isoform. We have also created a truncated version of the stromal NADK lacking the putative chloroplast transit peptide for biochemical studies. Preliminary findings and possible roles of NAD(H)Ks in higher plants are presented.

Presentation A4: 2:15-2:30 pm **Paul Martin Centre**

Investigating the *cis*-elements of winter rye glucanase promoters.

Shauna A. McCabe, Marilyn Griffith, and Barbara A. Moffatt.
Department of Biology, University of Waterloo
email: samccabe@gmail.com

Cold-acclimated (CA) winter rye (*Secale cereale L*) produces two apoplastic glucanases with both antifreeze and pathogenesis-related activities. These enzymes are distinct from the glucanases that are present in non-acclimated (NA) winter rye, which lack antifreeze activity. In order to investigate the basis for differential expression between these glucanases, nested-PCRs and genomic walking techniques have been used to obtain the promoters of β-1,3-endoglucanases from NA and CA winter rye. To date, the promoter of one β-1,3-endoglucanase with antifreeze activity, designated *glu1*, has been recovered along with promoter sequences of 4 otherβ-1,3-endoglucanases, designated *glu*2 through *glu*5. Comparison of the *cis*-acting elements in the promoters of these glucanases will be presented, along with an overview of future experiments designed to relate these *cis*-elements with the expression of cold related transcription factors.

Presentation A5: 2:30-2:45 pm **Paul Martin Centre**

Characterization of a recombinant *Arabidopsis* gamma-aminobutyrate transaminase.

Shawn M. Clark and Barry J. Shelp
Department of Plant Agriculture, University of Guelph
email: sclark02@uoguelph.ca

Gamma-aminobutyrate (GABA) is a ubiquitous non-protein amino acid that has been implicated in stress metabolism and signaling in plants. GABA transaminase (GABA-T) catalyses the catabolic breakdown of GABA to succinic semialdehyde, and both pyruvate- and 2-oxoglutarate-dependent activities are reported in the literature. In this report, we further characterized an *Arabidopsis* pyruvate-dependent GABA-T, which also exhibited irreversible glyoxylate-dependent GABA-T activity, using enzyme-linked assays to determine the formation or disappearance of reducing equivalents, and HPLC to determine the formation of amino acid products. Removal of the hydrophobic mitochondrial signaling peptide improved the total recovery of recombinant enzyme and increased specific activity by 2-fold without altering specificity. The recombinant enzyme exhibited maximal activity at pH 9, and did not utilize 2-oxoglutarate as an amino acceptor or serine, asparagine and glutamate as amino donours. Kinetic parameters for the glyoxylate- and pyruvate-dependent reactions were similar, with physiologically-relevant affinities. The catalytic lysine residue, lysine 327, was identified as site-directed mutations that prevented Schiff base formation and removed all detectable enzymatic activity, with no impact on the protein as determined by equilibrium denaturation analysis. Implications of glyoxylate-dependent GABA-T activity to photorespiration will be discussed.

Presentation A6: 2:45-3:00 pm **Paul Martin Centre**

Plant cytosolic nucleoside diphosphate kinase: autophosphorylation on a conserved Ser residue and protein trans-phosphorylation activity.

Sonia Dorion[1], France Dumas[2] and Jean Rivoal[1].
[1]IRBV, Université de Montréal, 4101 rue Sherbrooke est, Montréal, QC, H1X 2B2, Canada. [2]Biotechnology Research Institute, 6100 avenue Royalmount, Montréal, QC H4P 2R2, Canada.
email: jean.rivoal@umontreal.ca

Nucleoside diphosphate kinase (NDPK) is a ubiquitous enzyme catalyzing the transfer of the γ-phosphate from ATP (or other NTPs) to acceptor NDPs. This activity depends on a catalytic His residue, which autophosphorylates during the transfer of the phosphate group. In addition to this reaction, some animal NDPKs have the capacity to phosphorylate other proteins. As part of our ongoing effort to understand the function of plant cytosolic NDPK (NDPK1), we have expressed in *E. coli* the wild type (WT) as well as mutated forms of a cDNA encoding *Solanum chacoense* NDPK1 and purified the corresponding 6xHis-tagged proteins. After incubation of WT NDPK1 with [γ-^{32}P]ATP, the protein was radioactively labeled. This label was sensitive to acid and alkaline treatments, suggesting phosphorylation on His and Ser residues. Tryptic digestion followed by Edman sequencing of NDPK1 demonstrated autophosphorylation of the protein on the conserved Ser117 residue. Ser117 autophosphorylation was dependent on the catalytic His115 residue. Mutants of Ser117 were generated to evaluate the possible function of this phosphorylation. Mutants S117A and S117D incorporated less ^{32}P in autophosphorylation assays, consistent with the fact that they lack the Ser117 phosphorylation site. Kinetic analysis of the pseudo-phosphorylated S117D mutant demonstrated that the presence of a negative charge at position 117 dramatically lowered catalytic efficiency of the enzyme. Thus, autophosphorylation of Ser117 could have a regulatory function on NDPK1 activity. In addition to this autophosphorylating activity, we found that NDPK1 is able to phosphorylate *in vitro* proteins present in crude potato cell extracts. The His115 residue is responsible for this activity, because a H115A mutant displayed no protein phosphotransferase activity. Cytosolic GAPDH was among the 4 proteins that were identified as phosphorylated targets of NDPK1. These data suggest that NDPK1 could function to regulate plant glycolysis through post translational modification of a key enzyme in this pathway.

Presentation B1: 1:30-1:45 pm **Senate & Board Chambers**

The chimeric cyclic nucleotide-gated ion channel AtCNGC11/12 induces programmed cell death.

W. Urquhart, A. Gunawardena, W. Moeder and K. Yoshioka
University of Toronto, Department of Botany
email: wurquhart@hotmail.com

A hypersensitive response (HR) is a controlled death of cells that develops in plants in response to an attack by an avirulent pathogen. A number of mutants have been identified which spontaneously develop HR like cell death. These mutants fall into the lesion mimic mutant group of which *cpr22* is a member. The *cpr22* mutant constitutively expresses pathogen response genes as well as having elevated levels of salicylic acid. The *cpr22* mutant has a 3 kb deletion within a cluster of cyclic nucleotide gated ion channel (CNGC) genes which has resulted in the creation of a novel chimeric CGNC gene (*AtCNGC11/12*). Transient expression of *AtCNGC11/12* in *N. benthamiana* leaves corresponds directly with the developing cell death in the region. Through confocal microscopy with GFP fusion protein, we have demonstrated that this novel CNGC is localized in the plasmamembrane and it is from here that *AtCNGC11/12* causes the cell to undergo programmed cell death (PGC). In cells expressing *AtCNGC11/12*, plasma membrane shrinkage, membrane blebbing, and intact organelles are all observed. These observed characteristics are hallmarks of PCD and are absent in necrotic cells strongly indicating that the lesion formation in *cpr22* is the result of HR development. By the use of site directed mutagenesis, it appears that there are at least two sites in AtCNGC11/12 that are necessary for the development of HR in *N. benthamiana* leaves. These two positions are at the amino terminal region and the cytosolic portion of the 5th transmembrane domain. Thus the cell death that develops in the *cpr22* mutant is in fact ordered programmed cell death and there are at least two critical areas of AtCNGC11/12 that allow it to induce HR like cell death.

Presentation B2: 1:45-2:00 pm **Senate & Board Chambers**

Alternative splicing and chromosome dynamics at the *MADS-AFFECTING FLOWERING* locus of *Arabidopsis thaliana.*

Sarah Rosloski and Vojislava Grbic
Department of Biology, University of Western Ontario
email: sroslos@uwo.ca

Evidence suggests that the four MADS-box homologues at the *MADS-AFFECTING FLOWERING* locus of *Arabidopsis thaliana* are floral repressors associated with the vernalization response. All four genes are known to be alternatively spliced although the functional consequences of the variant forms are poorly understood. My work has shown the prevalence of certain splice variants at *MAF2* and *MAF3* is sensitive to change of temperature. At 4^{o}C a splice variant that is predicted to produce the full length repressor is preferentially increased for both genes. When plants are transferred from 4^{o}C to 21^{o}C, a rapid transition (hours) to the increased production or stabilization of variants with a premature stop codon is observed. When transferred from 21^{o}C to 4^{o}C, a gradual predominance of the full-length form is observed over days. The study of temperature effects on splice variation at *MAF2* and *MAF3* was complemented by the discovery of a mutant of *MAF2* in the lab. Although the mutant has a large insertion in the alternatively spliced region of *MAF2* it continues to be cold-sensitive. The inserted region is a copy of the alternatively-spliced region of *MAF3*. The insertion has characteristics that suggest this region of *MAF3* may be capable of moving in a transposition-like fashion such as a target-site duplication, filler DNA, proximal terminal inverted repeats and hyper-active homologous recombination in adjacent sequences. Preliminary analysis of 22 ecotypes suggests the movement of the alternatively spliced region of *MAF3* into the last exon of *MAF2* may have occurred multiple times in Arabidopsis populations globally. cDNA sequence analysis suggests that the insertion allele may create a protein of comparable size to the WT with features that are both contrasting and comparable to the WT protein. Re-occurence and conservation of the insertion allele in lines from distant locations may signal it has functional significance to flowering time in Arabidopsis. Future research will expand the search for novel occurrences of the hybrid allele and will consider the commonalities of alternative splicing and transposition.

Presentation B3: 2:00-2:15 pm **Senate & Board Chambers**

Tung (*Vernicia fordii*) diacylglycerol acyltransferase 1 and 2 localize to distinct subdomains of the endoplasmic reticulum.

Satinder Gidda[1], Jay Shockey[2], Steven J. Rothstein[1], John Dyer[2] and Robert Mullen[1].
[1]Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada; [2]Southern Regional Research Center, USDA-ARS, New Orleans, LA, USA.
email: sgidda@uoguelph.ca

The seeds from tung tree (*Vernicia fordii*) produce large quantities of triacylglycerols (TAGs) that contain ~80% eleostearic acid, a novel conjugated fatty acid that is used in numerous industrial applications. To date, intensive biochemical and molecular studies have provided significant insights into the details of the metabolic pathways involved in TAG biosynthesis in tung and other oilseeds, and more recently, many of the genes involved in this process have been cloned. However, despite these advances, little is known about the subcellular organization and regulation of oil biosynthetic enzymes. Here, we report the subcellular localization and the molecular targeting signals for two different diacylglycerol acyltransferase enzymes (DGAT1 and DGAT2) expressed in developing tung seeds during biosynthesis of tung oil. Using confocal laser-scanning immunofluorescence microscopy and tobacco BY-2 suspension cells as model system to study protein sorting in plant cells in *vivo*, we showed that both tung DGAT1 and DGAT2 localized to specific regions or subdomains of the endoplasmic reticulum (ER). Interestingly, these regions of the ER are distinct between the two DGAT enzymes. Amino acid sequence alignment of all known plant DGAT1 and DGAT2s revealed the presence of a sequence-specific motif at their C termini consisting of ϕ-X-X-K/R/D/E-ϕ- (where ϕ are large hydrophobic amino acid residues) and similar to a motif identified previously in *Arabidopsis fatty acid* desaturase 2 (FAD2). Using the tomato *Cladosporium fulvum*-9 (Cf9) disease resistance gene product fused to GFP as a type 1 membrane reporter protein, we confirmed that the cytosolic-exposed C-terminal residues -YYHDL- in DGAT1 and -LKLEI- in DGAT2 function in ER localization, although their position relative to the C terminus of the protein is different than that of *Arabidopsis* FAD2. Taken together, these findings raise the possibility of spatial separation of the two DGATs for production of different pools of triacylglycerols within the cell, despite utilization of similar ER retrieval mechanisms for each protein.

Presentation B4: 2:15-2:30 pm **Senate & Board Chambers**

Does the tomato bushy stunt virus hijack ESCRT in the formation of peroxisomal multivesicular bodies?

Andrew McCartney, Alex Howard, Graham Smith, Satinder Gidda and Robert Mullen
Department of Molecular and Cellular Biology, University of Guelph
email: amccar01@uoguelph.ca

A hallmark of single-stranded RNA viruses is their ability to recruit and modify host-cell organelle membranes as the sites of viral RNA replication. Although the molecular mechanisms underlying these novel membrane rearrangements are unknown, they are presumed to be mediated by the membrane-bound virus replication proteins. We and others have shown previously that the tomato bushy stunt virus (TBSV) recruits and modifies peroxisomes into structures termed peroxisomal multivesicular bodies (pMVBs). pMVBs are characterized by the presence of numerous small internal vesicles/spherules that are formed by the inward vesiculation of the organelle's boundary membrane. Interestingly, pMVBs are structurally reminiscent to endosomal MVBs (also referred to as late endosomes) found in almost all eukaryotic cells. This morphological similarity suggests that, during pMVB biogenesis, TBSV exploits the host-cell machinery normally responsible for the formation of late endosomes, seventeen soluble class E Vps proteins that collectively are known as ESCRT (endosomal sorting complex required for transport). Indeed, it is now well established that certain other viruses, namely the retroviruses HIV and Ebola, hijack ESCRT to facilitate their outward budding from an infected animal cell. To begin to investigate the potential role of ESCRT in pMVB biogenesis in plant cells, we identified several putative Arabidopsis ESCRT homologs (i.e., Arabidopsis class E Vps proteins) through comparisons of established yeast and human ESCRT proteins. Using the yeast two-hybrid system, we then determined that many of these putative Arabidopsis ESCRT homologs displayed protein-protein interactions that were consistent with the protein interaction networks reported for their human and/or yeast counterparts. In addition, at least three of the putative plant ESCRT proteins tested thus far interacted also with one or more of the TBSV replication proteins and/or portions thereof. These latter data suggest that, similar to some retroviruses, TBSV exploits ESCRT in the formation of pMVBs.

Presentation B5: 2:30-2:45 pm **Senate & Board Chambers**

Localization of DIR1, a component of the long-distance systemic acquired resistance signaling complex.

M.J. Champigny. H. Shearer, A. Mohammad, M. Neumann, K. Haines, Z. Zhao, and R.K. Cameron
Department of Biology, McMaster University
email: champimj@mcmaster.ca

Plants employ sophisticated strategies to defend themselves against microbial pathogens. In addition to basal and *R* gene-mediated resistance that is expressed locally after primary pathogen exposure, infection with certain necrosis-causing pathogens ("immunization") induces resistance in distant tissues to subsequent attack by a broad range of pathogens (Systemic Acquired Resistance, SAR). A key feature of SAR is the requirement for a mobile, long distance signal capable of inducing defense responses in distal tissues. The Arabidopsis *defective* in *induced resistance* (*dir1-1*) mutant exhibits normal local resistance but fails to develop SAR in distant leaves. *dir1-1* can perceive the SAR signal present in petiole exudates from wild type immunized leaves, but *dir1-1* exudates do not contain this signal, indicating that DIR1, a putative lipid transfer protein, is required either for the production or transmission but not the perception of a mobile signal. We examined the expression and localization of DIR1 during the development of SAR in wild type and transgenic lines. DIR1: DIR1-GUS expression analysis indicated that DIR1 is expressed in the mesophyll and xylem and phloem cells of the vasculature. DIR1 contains a functional signal sequence as demonstrated by detection of DIR1 in intercellular washing fluids. Western analysis demonstrated that DIR1 protein is present in petiole exudates (enriched for phloem sap) collected from immunized, but not mock-inoculated leaves, suggesting that DIR1 protein is able to access the phloem after SAR induction. These results indicate that DIR1 is in the right place (the phloem) at the right time (after SAR induction) to act as the SAR long distance signal or a component of the long distance signal complex.

Presentation B6: 2:45-3:00 pm **Senate & Board Chambers**

Enhancing resistance to pathogen ingress in tomato by down-regulating its eIF5A-3.

Fengshan Ma, Zhongda (Chris) Liu, Tzann-Wei (Mike) Wang, Linda McNamara, and John E. Thompson
Department of Biology, University of Waterloo, Waterloo, Ontario
email: f3ma@uwaterloo.ca

The eukaryotic translation initiation factor 5A (eIF5A), which is a small protein (~18 kDa), is thought to exist ubiquitously in all eukaryotes (eIF5A) and Archaea (aIF5A). eIF5A is post-translationally modified by converting a conserved lysine residue near its N-terminus to hypusine, which is found only in eIF5A. There is evidence that eIF-5A may facilitate translation by shuttling specific subsets of mRNA from the nucleus to the cytoplasm. In each of the few plant species examined, eIF5A exists in three (*Arabidopsis*) or four isoforms (other plants) that are functionally different. For example, one of the four eIF5A isoforms, eIF5A-3, in tomato regulates its responses to wounding or pathogen attack. Earlier, we documented that eIF5A-3 is up-regulated in wounded tissues. It seems quite possible to enhance the resistance of tomato plants to pathogens by down-regulating *eIF5A-3* expression. Three strategies were taken. In one study, we generated tomato lines that constitutively over-express the 3' UTR of *eIF5A-3* in the antisense orientation. These plants exhibited tolerance against *Pseudomonas syringea* to various degrees, and there is a correlation between the extent of eIF5A down-regulation and tolerance to the pathogen. But, this feature is accompanied by changed morphology of the plants and their wound-healing process. These undesirable effects probably result from down-regulation of other eIF5A isoforms, albeit to a lower extent than the down-regulation of eIF5A-3. To down-regulate eIF5A-3 with higher specificity, we have taken RNAi and siRNA approaches by targeting short regions of the 3' UTR of the eIF5A-3 gene. Transgenic lines have been established and detailed phenotyping and pathological studies will be carried out.

Presentation C1: 3:45-4:00 pm **Paul Martin Centre**

Identification and characterization of candidate tail-anchored integral membrane proteins in plant, yeast and mammalian proteomes.

Priya K. Dhanoa[1], Meeta Mistry[2], Denil Wickrama[2], Xuemei Yang[2], Matthew P. Henderson[2], David W. Andrews[2] and Robert T. Mullen[1].
[1]Department of Molecular and Cellular Biology, University of Guelph.
[2]Department of Biochemistry and Biomedical Sciences, McMaster University
email: pdhanoa@uoguelph.ca

Tail-anchored (TA) (N_{out}-C_{in}) integral membrane proteins are found in essentially all cellular membranes where they carry out a variety of essential functions, including the regulation of apoptosis, oxidation or reduction reactions, and vectorial transport of vesicles through the secretory system. While the intracellular trafficking of some TA proteins has been well characterized, our understanding of TA proteins biogenesis in general is limited because only a relatively few TA proteins have been identified in evolutionarily diverse organisms. In this study, we employed a computer algorithm called TAMP (Tail-Anchored Membrane Proteins) to identify all of the TA proteins in the *Arabidopsis thaliana*, *Saccharomyces cerevisiae* and *Homo sapiens* proteomes. Specifically, we challenged TAMP to identify within all three proteomes open reading frames lacking an amino-terminal signal sequence and/or an internal TMD(s), but possessing a carboxyl-terminal TMD(s). In total, 653, 171 and 981 candidate TA proteins were identified in the *A. thaliana*, *S. cerevisiae* and *H. sapiens* proteomes, respectively, representing approximately 3% of the total proteins in each proteome. While many of these candidate TA proteins have been functionally annotated using Gene Ontology, ~ 45% of the proteins identified in each proteome are of unknown function(s). Results obtained from preliminary localization experiments revealed that several of the plant TA proteins of unknown function(s) localize to a wide variety of subcellular compartments including endoplasmic reticulum, plastids, mitochondria, plasma membrane and the nucleus. Further, our initial characterization of selected plastid-localized TA proteins with known or unknown functions indicate that they are sorted from their sites of syntheses in the cytosol to the plastid either by a prototypic N-terminal transit peptide or by targeting information located in their C termini. The implications of these and other results in terms of our understanding of the general mechanisms responsible for the proper sorting of TA proteins will be discussed.

Presentation C2: 4:00-4:15 pm **Paul Martin Centre**

Enhancement of salinity tolerance by engineering chloride volatilization into plants.

Simendeep Kaur*, Sima Babayeva, Priyum Koonjul and Hargurdeep Saini
Institut de recherche en biologie végétale, Université de Montréal, 4101, rue sherbrooke est, Montreal, QC, H1X2B2
email: simendeep.kaur@umontreal.ca

Several organisms possess enzymes that can convert chloride ions to chloromethane gas through one-step enzymatic methylation of the former. Presence of this enzyme activity in certain organisms that live in saline habitats has been interpreted as a possible mechanism for chloride detoxification via its volatilization. This possibility has never been experimentally tested. Our research group has cloned a cabbage gene encoding a thiol methyltransferase (TMT) enzyme that can methylate a variety of substrate including chloride. Although the latter is not its natural function in cabbage, we engineered this gene into a number of species, which otherwise lack the enzyme as well as its usual metabolic context. All the engineered species acquired an ability to efficiently transform chloride ions to chloromethane. Parallel with this, the transformed plants developed a high degree of tolerance to NaCl salinity, which was toxic to the untransformed counterparts. The results convincingly demonstrate that volatilization of chloride is a detoxification event that can contribute to the plant's ability to withstand salinity stress. This ability can, therefore, be used to engineer crop species with enhanced salt tolerance.

Presentation C3: 4:15-4:30 pm **Paul Martin Centre**

A new plant-derived marker for efficient selection of transgenic plants.

Priyum Koonjul, Sima Babayeva and Hargurdeep Saini
Institut de recherche en biologie végétale, Université de Montréal, 4101 rue Sherbrooke est, Montreal, QC H1X2B2
email: priyumk@yahoo.ca

Genetic transformation is a random, low frequency process. Therefore, identification of transformants in a population requires the use of selectable markers that can flag the cells or organisms into which a targeted gene has been successfully integrated. There exist approximately about 50 marker genes that are currently being used as selectable markers. The most common among these is the gene conferring resistance to the antibiotic kanamycin. Antibiotic-resistant genes have, however, attracted much criticism because of concerns about spreading antibiotic resistance to human beings and animals. We present a new marker gene, *tmt-1*, encoding a thiol methyltransferase enzyme that is postulated to detoxify thiocyanate ($^{-}$SCN) by methylating it to volatile CH_3SCN. This property of TMT allows the gene encoding this enzyme to be used as a selectable marker where transformants growing in the presence of $^{-}$SCN survive, whereas, the non-transformants lacking this enzyme die or suffer serious growth inhibition. The marker gene, isolated from cabbage, has been successfully tested for the selection of tobacco and *Arabidopsis thaliana* plants and in root cultures of a number of species. It allows selection with high fidelity regardless of whether it is naturally present or absent in a species.

Presentation C4: 4:30-4:45 pm **Paul Martin Centre**

Generating *Arabidopsis* PDT proteins in a yeast expression system.

Oliver Corea, Mark Bernards and Susanne Kohalmi
University of Western Ontario, Dept. of Biology, London, ON
email: orcorea@uwo.ca

Phenylalanine is one of three aromatic amino acids that are essential for protein synthesis in all living organisms. In plants these amino acids are also precursors for a wide range of secondary metabolites including flavonoids and lignins. As such they are essential for plant survival, providing structural support and protection. Extensive studies have been performed to analyze phenylalanine biosynthesis in bacteria. These studies show that synthesis occurs predominantly via the classical pathway where the precursor prephenate is converted to phenylalanine through a phenylpyruvate intermediate. In contrast, biochemical data suggests that higher plants use the arogenate pathway where phenylalanine is synthesized from prephenate via arogenate. Support for this theory comes from biochemical detection of arogenate dehydratase (ADT) activities in plant cell extracts. These enzymes catalyze the decarboxylation and dehydration reaction of arogenate to form phenylalanine. In *Arabidopsis thaliana* a family of six genes encode proteins with a high sequence similarity to prephenate dehydratase (PDT) proteins of bacteria. However, these genes are thought to encode ADTs according to biochemical evidence for plants. There has been no clear link between biochemical and molecular data to demonstrate specific enzymatic functions of these proteins. A yeast expression system is being used to synthesize two of the six *Arabidopsis* ADTs for molecular and biochemical analysis such as substrate specificity and regulatory interactions. Currently we have data demonstrating that the ADT proteins for *Arabidopsis* can be expressed and isolated from yeast.

Presentation C5: 4:45-5:00 pm **Paul Martin Centre**

Starches and their functional properties.

Ian J. Tetlow
Molecular and Cellular Biology, College of Biological Sciences, University of Guelph, Guelph, Ontario, N1G 2W1, Canada
email: itetlow@uoguelph.ca

Starch is a major component of the human diet and is also used in a wide variety of industrial and non-food applications. Variations in polymer composition and fine structure underpin many of these end-uses. The structural properties of starches exploited in certain industrial and food applications will be discussed, with particular emphasis on the human health benefits associated with resistant starches in the diet.

Presentation C6: 5:00-5:15 pm **Paul Martin Centre**

Regulation of starch synthesis by protein complex formation.

Fushan Liu, Ian Tetlow, and Mike Emes
Department of Molecular and Cellular Biology, University of Guelph
email: fliu@uoguelph.ca

The pathway of starch biosynthesis involves a number of enzyme activities: ADP glucose pyrophosphorylase (AGPase) is responsible for synthesizing the precursor of starch biosynthesis (ADPglucose), starch synthases (SSs) add the glucose moiety from ADPglucose to a pre-existing glucan chain, thus elongating glucan chains, branching enzymes (SBEs) create branches within amylopectin, and debranching enzymes (DBEs) are involved in trimming the growing starch granule. The formation of protein complexes between key enzymes of starch biosynthesis has been shown by gel filtration chromatography, co-immunoprecipitation experiments and cross-linking experiments, and it is supposed that these protein complexes play an important role in the formation of the starch granule. Preliminary characterization of protein complex(es) comprising SSs and SBEs is described. Data from gel retardation experiments showed SS/SBE protein complexes display a higher affinity for both starch and amylopectin than their respective monomers, suggesting a more efficient means of catalysis produced by protein complexes. Treatment of isolated complex with alkaline phosphatase caused dissociation of the proteins into monomers, suggesting the formation of protein complexes is phosphorylation dependant.

Presentation C7: 5:15-5:30 pm **Paul Martin Centre**

Characterisation of disproportionating enzyme from wheat.

Nicole Bresolin, Zhongyi Li, Behjat Kosar-Hashemi, Ian Tetlow, Manash Chatterjee, Sadequr Rahman, Matthew K Morell, Crispin A. Howitt and Michael J. Emes.
Department of Molecular and Cellular Biology, University of Guelph
email: nbresoli@uoguelph.ca

Disproportionating enzyme or D-enzyme (EC 2.4.1.25) is an α-1,4 glucanotransferase which catalyses cleavage and transfer reactions involving α-1,4 linked glucans altering ("disproportionating") the chain length distribution of pools of oligosaccharides. While D-enzyme has been well characterised in some plants, e.g. potato and Arabidopsis, very little is known about its abundance and function in cereals which constitute the major source of starch worldwide. To address this we have investigated D-enzyme in wheat (*Triticum aestivum*). Two putative D-enzyme cDNA clones have been isolated from tissue specific cDNA libraries. TaDPE1-e, from an endosperm cDNA library, encodes a putative polypeptide of 575 amino acid residues including a predicted transit peptide of 41 amino acids. The second cDNA clone, TaDPE1-l, from an *Aegilops taushii* leaf cDNA library, encodes a putative polypeptide of 579 amino acids including a predicted transit peptide of 45 amino acids. The mature polypeptides TaDPE1-e and TaDPE1-l were calculated to be 59 and 60 kDa respectively and had 96% identity. The putative polypeptides had significant identity with deduced D-enzyme sequences from corn and rice, and all the expected conserved residues were present. Protein analysis revealed that D-enzyme is present in the amyloplast of developing endosperm and in germinating seeds. D-enzyme was partially purified from wheat endosperm and shown to exhibit disproportionating activity in vitro by cleaving maltotriose to produce glucose as well as being able to add maltoheptaose chains to the outer chains of glycogen and amylopectin.

Presentation D1: 3:34-4:00 pm **Senate & Board Chambers**

The effect of vesicular-arbuscular mycorrhizae on root anatomy as it impacts ion acquisition in tomato.

Jeff H. Taylor and Adriann Waltenbaugh
Department of Biology, Slippery Rock University of Pennsylvania
email: jeffrey.taylor@sru.edu

Mycorrhizae are a symbiotic association between plants and fungi, in which the fungus aids the plant in obtaining mineral nutrients from the soil. Vesicular-arbucular mycorrhizae (VAM) are a subtype of the symbiosis common to most herbaceous plants. It has been noted that VAM-associated plants often have a reduced root system compared to plants lacking VAM. This is primarily thought to be due to the fungus reducing the need for more metabolically expensive roots by absorbing nutrients via the extraradical hyphae. A previous study of ectomycorrhizal roots established that the symbiosis also impacted root anatomy at the cellular level, resulting in the mycorrhizal roots having a greatly reduced plasmalemma surface area available for ion uptake. In this study, a similar analysis of VAM roots was conducted, with the goal of ascertaining the impact of VAMs on potential absorbing plasmalemma surface area (PAPS) in tomato roots. Inoculated and non-inoculated tomatoes were established in a growth chamber and maintained for seven weeks post germination. Trypan blue analysis established that the inoculated plants were mycorrhizal, while the non-inoculated plants were not found to have VAM associations. As would be expected, the inoculated plants had a greatly reduced averaged root length compared with the non-inoculated plants. However, when the roots were analyzed at the cellular level, it was found the PAPS was not significantly different between the mycorrhizal and non-mycorrhizal plants. Further investigations of additional herbaceous plants are underway.

Presentation D2: 4:00-4:15 pm **Senate & Board Chambers**

Characterisation of the mechanical properties of the pollen tube during apical growth.

Rabah Zerzour and Anja Geitmann
Institut de recherche en biologie végétale, Université de Montréal
email: rabah.zerzour@umontreal.ca

The pollen tube is specialized in the delivery of the male gametes to the ovule. In order to do so an extremely long tubular structure is built quickly by anisotropic expansion. The growth process at the extreme apex of the pollen tube is characterized by a cyclic process involving vesicle transport directed toward apex, calcium triggered exocytosis, cell wall assembly and expansion driven by turgor pressure. This process is finely regulated and relies on several structures whose precise functioning and coordination in this context are still elusive: the cytosqueleton, cytoplasmic ion gradients, ion fluxes, and the cell wall pliability. The mechanical properties of the pollen tube cell wall are crucial in this oscillating growth process since they are likely to be the triggering mechanical oscillator. To understand this mechanism we use specific inhibitors of cytoskeletal and metabolic functioning and we monitor how growth rate oscillations and cellular stiffness are modified. Using cytochalasin B, a drug interfering with actin polymerization, we were able to show that reduction of apical cellular stiffness is a prerequisite for growth. These experiments also confirmed our initial hypothesis that cellular stiffness exhibits an oscillatory pattern that accompanies growth fluctuations.

Presentation D3: 4:15-4:30 pm **Senate & Board Chambers**

The physiological basis for photoperiodic injury in tomato: a role for circadian rhythms and nitrite toxicity.

Lorraine I. D'Silva, Malgre C. Micallef, Viktoriya Coneva, and Barry J. Micallef
Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1
email: ldsilva@uoguelph.ca

Photoperiodic injury of vegetative tissues (i.e. leaf chlorosis and necrosis) can occur under either extended photoperiods or non-24 h light/dark cycles in a number of plants. Although documentated nearly 80 years ago by Garner and Allard (1927), the physiological basis for this injury is unknown. The involvement of circadian rhythms has previously been proposed. Processes involved in N metabolism are known to show diel or circadian patterns, and two toxic compounds including nitrite and ammonium are produced during nitrate reduction. We tested two hypotheses in this study: (1) differences in the genetic predisposition for photoperiodic injury will also be associated with differences in circadian rhythm patterns; and (2) nitrite or ammonia toxicity is associated with photoperiodic injury. Tomato was used since it is particularly susceptible to this injury, and tomato cultivars that demonstrate variation in susceptibility to photoperiodic injury have been identified. 'Micro-Tom' showed no injury under all conditions tested, whereas 'BasketVee' displayed the most severe injury symptoms. Patterns of cotyledon movement and stem elongation were tested in four cultivars under either a 12 or 24 h photoperiod, and a correlation was found between susceptibility to photoperiodic injury and the ability to maintain normal circadian rhythms under a 24 h photoperiod. Leaf nitrite levels elevated significantly prior to the development of photoperiodic injury in susceptible cultivars but not in 'Micro-Tom', although nitrate and ammonium levels either remained the same or decreased. Photoperiodic injury symptoms could be induced using the nitrate-analogue chlorate, which is converted to the nitrite-analogue chlorite. We propose that the physiological basis for photoperiodic injury in plants is nitrite toxicity resulting from an altered coordination between nitrate and nitrite reduction activities under photoperiodic cycles favouring the injury. The significance of these findings in relation to the function of circadian rhythms in plants will also be discussed.

Presentation D4: 4:30-4:45 pm **Senate & Board Chambers**

Acclimation of the psychrophilic strain (*UWO 241*) and the mesophilic strain (*SAG 49.72*) of *Chlamydomonas raudensis* to temperature and irradiance.

Beth Szyszka, Ivanov, Alex G., and Hüner, Norman P.A.
University of Western Ontario, Dept. of Biology, London, ON N6A 5B8
e-mail: bszyszka@uwo.ca

Chlamydomonas raudensis Ettl. (UWO 241) was isolated from Antarctica's permanently ice-covered Lake Bonney. Recently, this psychrophilic green alga was identified as a strain of *C. raudensis Ettl. (SAG 49.72)*, a mesophile. Previous studies have shown that *UWO 241* is unable to redistribute light energy through state I – state II transitions. In contrast to short-term state-transitions, long-term steady state growth was used to examine the capacity of the two strains of *Chlamydomonas raudensis* to acclimate to varying temperature and light regimes. In response to higher growth light conditions, both strains exhibited a gradual decrease in the abundance of the PSI reaction center polypeptide, PsaA, relative to the D1 protein of PSII. This decrease in PSI also occurred in *UWO 241* in response to increased growth temperature. The psychrophile's ability to adjust photosystem stoichiometry suggests that a functional state transition mechanism is not required for long-term redistribution of absorbed excitation energy. However, differences in the phosphorylation profiles of thylakoid proteins were observed among the two strains. *UWO 241* phosphorylated only serine residues, whereas *SAG 49.72* phosphorylated both threonine and serine residues. PAM chlorophyll fluorescence measurements performed on both strains indicated that the parameters of qP, NPQ, ΦII and ΦNO showed similar trends in response to growth under varying conditions. Both strains of *C. raudensis* increased non-regulated quenching (ΦNO) with increasing growth light intensity, however, *UWO 241* directed a higher proportion of light energy towards this pathway compared to *SAG 49.72*. We conclude that although the Antarctic strain, *UWO 241*, has adapted to an extremely stable environment of Lake Bonney, it has retained the capacity to acclimate to various growth light and temperature regimes.

Presentation D5: 4:45-5:00 pm **Senate & Board Chambers**

Cytokinins in tissues and phloem sap explain reduced apical dominance of *Lupinus angustifolius* transformed with an isopentenyl transferase (IPT) and a flower specific promoter (TP12).

Neil Emery[1], Penelope Smith[2], and Craig Atkins[2]
[1]Department of Biology, Trent University, Peterborough ON, K9J 7B8
[2]Faculty of Natural and Agricultural Sciences, University of Western Australia, Crawley, Western Australia
email: nemery@trentu.ca

Pod set in lupin can be dramatically improved with exogenous application of cytokinin strategically applied to the pedicels of flowers. Thus, in an effort to stabilize grain yield, *Lupinus angustifolius* was transformed using the disarmed Ti plasmid from *Agrobacterium tumefaciens* containing the IPT gene from *Agrobacterium*. Expression of the IPT gene was driven by the TP12 promoter from tobacco. Yield changes varied among transformed lines, but most exhibited reduced apical dominance with vigorous growth in basal and first order apical branches. This latter phenotype was consistent with changed CK concentrations determined by GC-MS. Compared to untransformed parent lines, total CK was higher in the IPT-transformed plants, with the most obvious increase in the branch meristems (mainly iP, DZ, [9R]iP). CK were also increased in the flowers (mainly iP and [9R]DZ), and in the phloem (mainly [9R]iP). For other organs, the CK concentrations of IPT transformed plants were either not different (young fruits, filling seeds) or even lower (leaves) than those of controls. Northern analysis showed that transgene expression was highest in the flowers, but there was also expression in the vegetative apex. The data are consistent with the idea that increased branch growth is promoted, at least in part, by high CK synthesis in flowers, which may be translocated in the phloem to branch meristems.

Acknowledgements: Grains Research and Development Corporation (Australia) for funding and Anna Klyne and Natalie Fletcher and Simone Chapple for technical help with lupin transformation.

Presentation D6: 5:00-5:15 pm **Senate & Board Chambers**

Light-harvesting mutants of *Chlamydomonas reinhardtii*.

Kenneth Wilson and Lindsay Gray
Biology Department, University of Saskatchewan
email: ken.wilson@usask.ca

The unicellular green alga *Chlamydomonas reinhardtii* is an ideal model organism for the genetic dissection of photosynthetic processes. It is easily transformed, non-photosynthetic mutants can be grown on a reduced carbon source, and all three genomes are sequenced. The light-harvesting complex is a pigment-protein structure which not only increases the light absorbing capacity of photosynthetic organisms, but ironically, it also aids in protection of the chloroplast from damage due to excess light. Of the 28 light-harvesting complex genes in the *C. reinhardtii* genome, 9 are thought to be associated with PSI and 11 with PSII. The absolute stoichiometry of the individual subunits varies greatly in response to the growth environment. In an attempt to understand the assembly of these numerous polypeptides into a functional complex, and to further our understanding of how the LHC can protect the cell from excess light, we commenced a genetic screen for strains which would suppress the high-light lethal phenotype of a ΔpsaF mutant. The suppressor mutants that we obtained can be divided into 2 groups based on pigmentation: yellow (low LHC abundance) or green (normal LHC abundance). Our current studies focus on explaining the suppressor phenotypes in terms of their photosynthetic functionality, while genetic mapping is being carried out to identify the mutation at the genomic level. Initial findings on three suppressor strains will be presented.

Presentation D7: 5:15-5:30 pm **Senate & Board Chambers**

Photoinhibitory responses in contrasting ecotypes of *Thellungiella*.

Jillian N. Kriger, Heather M. Baerr, Anna T. Hanson and Gordon R. Gray.
Department of Plant Sciences, University of Saskatchewan
email: gr.gray@usask.ca

Thellungiella salsuginea is an emerging model species for the examination of abiotic stress tolerance. *Thellungiella* displays a high sequence similarity to that of *Arabidopsis* and shares many other characteristics of a good genetic model system. While this has resulted in numerous molecular studies, detailed physiological examinations of his plant are limited. There are two ecotypes of *Thellungiella* currently being examined. The Shandong ecotype grows in the warm-temperate, semi-humid coastal areas of eastern China with temperatures ranging from -5°C to 31°C. In contrast, the Yukon ecotype thrives in the sub-arctic and semi-arid regions of the Yukon Territories in Canada, with temperatures ranging from -18°C to 14°C. It is well established, in cereals and *Arabidopsis*, that development at low temperature results in an increased tolerance to photoinhibition of photosynthesis. In part, this is due to a re-programming of photosynthetic carbon metabolism. Much of that knowledge has been gained from the examination of spring and winter cultivars of wheat (*Triticum aestivum* L.) which differ in their ability to withstand freezing. We have initiated photosynthetic studies with *Thellungiella* in these ecotypes from contrasting environments to determine if the mechanisms which result in photosynthetic acclimation to low temperature are similar. While development at low temperature increased tolerance to photoinhibition of photosynthesis in both ecotypes, this was much more pronounced for the Yukon ecotype in comparison to the Shandong ecotype. This difference was not observed between the ecotypes when development occurred under standard growth conditions. Furthermore, our preliminary data indicate that the observed increase in tolerance to photoinhibition is not attributable to a non-photochemical mechanism.

Poster Session Abstracts **SBC Foyer**

P1. The diel availability of sucrose can affect circadian growth patterns and branching in Micro-Tom tomato.

Rosa L. Aiello, Malgre C. Micallef, and Barry J. Micallef
Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1
email: bmicalle@uoguelph.ca

Growth of plant organs including main and lateral stems show characteristic circadian rhythm patterns, and thus the sink activities of plant organs do vary in a diel (i.e. diurnal + nocturnal) manner. There is also evidence that leaf C export patterns (i.e. sink activity) can show diel variations. Therefore, interactions between diel source and sink activities may play a role in plant development. We tested the following hypotheses in the present study: (1) the diel availability of sucrose can affect plant development; and (2) alterations in the diel availability of sucrose can affect circadian growth patterns. The diel availability of sucrose was altered in 'Micro-Tom' tomato using exogenous sucrose fed through the roots for one hour daily starting at one of three times: (1) one hour after lights on (AM); (2) 3 h after lights on (Noon); (3) 2 h before lights off (PM). Vegetative and reproductive growth was monitored, sucrose depletion from the media was quantified, and diel growth patterns for main and lateral stems were measured using rotary motion sensors. Significant increases in side branch growth, flowering, and fruiting were found for all sucrose treatments, although sucrose uptake was only 2-4% of the daily net C exchange rate. Controls showed that osmotic and microbial effects were not responsible for the alterations in development. The greatest effect on side branch growth, flowering, and fruiting was found for the PM treatment followed by the AM and then Noon treatment, and these responses correlated with circadian stem extension patterns. In response to sucrose, lateral branching was enhanced and the relative diel stem extension patterns for main stem and side branch growth became less superimposed on each other. The significance of these findings in relation to apical dominance in plants is discussed.

P2. Is gamma-hydroxybutyrate production involved in cellular redox homeostasis?

Wendy L. Allan and Barry J. Shelp
Plant Agriculture Department, University of Guelph
email: wallan@uoguelph.ca

Under a variety of stresses, glutamate decarboxylation is stimulated, resulting in □-aminobutyrate (GABA) accumulation. Subsequently, GABA is catabolized to succinic semialdehyde (SSA) via GABA transaminase and then SSA is either oxidized to succinate via SSA dehydrogenase using NAD^+ as the preferred cofactor, or reduced to □-hydroxybutyrate (GHB) via SSA reductase using NADPH as the preferred cofactor. Therefore, we hypothesized that stress-induced NADPH accumulation stimulates GHB production. In this study, GHB accumulated over time in leaves of *Arabidopsis* and tobacco plants under low oxygen stress (up to 9 h in dark), and in shoots of *Arabidopsis* plants under drought (up to 9 d under light:dark cycles) or salt stress (up to 9 h in light), and preceded a rise in NADPH levels. If tobacco was allowed to recover after 3 h of low

oxygen stress, GHB concentrations declined, as did the NADPH/$NADP^+$ ratio. These data suggest that flux through the GABA shunt during stress contributes to cellular redox homeostasis.

P3. Inorganic carbon acquisition by the chrysophyte *Mallomonas papillosa*.

Shabana Bhatti and Brian Colman
Department of Biology, York University
email: shabana@yorku.ca

It is now widely established that many microalgae express CO_2 concentrating mechanisms (CCM) which consists in most species examined of the active uptake of either, or both, bicarbonate and CO_2 with an accessory enzyme carbonic anhydrase. Little is known about the mechanism of inorganic carbon (Ci) uptake of chrysophyte algae. Photosynthetic characteristics of the chrysophyte alga *Mallomonas papillosa* were investigated to determine whether this species has some form of CCM. The photosynthetic oxygen evolution rate of air-grown cells demonstrated an optimum in the pH range of 5.0 to 7.0. This species lacks external carbonic anhydrase, the cells have no capacity for direct bicarbonate uptake and have a low affinity for dissolved Ci. The overall internal pH was determined by distribution between the cells and medium, of ^{14}C-benzoic acid over the pH range 5.5 to 6.0, and [2-^{14}C]-5,5-dimethyloxazolidine-2,4-dione over the pH range 6.5 to 7.0. As the external pH was lowered from 7.0 to 5.5, the internal pH of the cells decreased from 8.31 to 7.75. The ΔpH was great enough to allow the uptake of Ci by diffusion. Direct measurements of the intracellular Ci pool of *Mallomonas* in the light were determined over an external pH range of 6.5 to 8.0. Preliminary results indicate that the concentration of the internal pool of unfixed Ci is lower than the external concentration and is proportional to the ΔpH between the cell interior and the external medium. This is clear evidence for the uptake of CO_2 by diffusion in this species. The K_m [CO_2] Rubisco was calculated to be 19.5 μM. The $K_{1/2}(CO_2)$ of the cells is higher than the $K_m(CO_2)$ of Rubisco and therefore the affinity of the cell for CO_2 appears to be determined by the $K_m(CO_2)$ of Rubisco. Therefore, the chrysophyte *Mallomonas papillosa* has no CCM.

P4. Sclereid facilitated survival of *Eriophorum vaginatum* L. growing in metal contaminated areas.

Sarah J. Bogart and Ewa Cholewa.
Department of Biology, Nipissing University
email: saharabogarti@hotmail.com

Eriophorum vaginatum, a tussock forming sedge, thrives in cold, nutrient poor environments and metal contaminated industrial areas. The survival of *E. vaginatum* might be correlated with the presence of lignified sclerenchyma cells, or sclereids. Sclereids present in *E. vaginatum*'s corm are alive at maturity and associated with

vascular bundles. Previous study implied that these sclereids may be involved in internal nutrient recycling. It has also been demonstrated that *E. vaginatum* accumulates and stores metals (e.g. ^{137}Cs, Pb, Cu, and As). We are determining whether or not sclereids are metal detoxification centers within the corm of *E. vaginatum*. Moreover, sclereids were discovered in the closely related, but non-tussock forming, *E. viridicarinatum* Engelm. (Fern.). *E. viridicarinatum* was also found in nutrient challenging and contaminated sites. Sclereids differ in this species from those of *E. vaginatum* in colour and potentially in composition yet, position, size and morphology are similar. Sclereids in both species are localized on the adaxial side of vascular bundles in the corm which implies a function in addition to mechanical support. We are currently examining the metal composition of sclereids from *E. vaginatum* collected from contaminated sites near Sudbury, ON, employing ICP-MS. This analysis will determine metal content of sclereids and may shed light on the role of sclereids in the mechanism of plant survival in contaminated environments. Results from this study may lead to the use of *E. vaginatum* as a bioremediative plant throughout the northern hemisphere.

P5. Analysis of *HUA2* Gene Family in *Arabidopsis thaliana*

Sathya Challa, Nicholas Watkins and Vojislava Grbic
University of Western Ontario, London, Canada, N6A 5B7
email: schalla@uwo.ca

Flowering, the transition from vegetative to reproductive growth is a major developmental switch in plants life cycle. Proper timing of this transition is crucial to ensure reproductive success, and is regulated by environmental cues and endogenous signals. The importance of this timing mechanism has become increasingly clear from genetic studies, which have revealed a large number of genes involved in regulation of flowering. *HUA2* is one of the genes that are identified from *Arabidopsis*, and acts as a floral repressor (Doyle *et al*., 2005; Wang *et al*., manuscript in preparation). The *HUA2* gene in *Arabidopsis* acts as a repressor of floral transition and belongs to a family of four genes, named as *HUA2 L5* (At5g08230), *HUA2 L2* (At2g48160), and *HUA2 L3* (At3g63070). The *HUA2* gene, and members of the gene family have following putative motifs; PWWP, RPR, proline-rich region containing PPLP, and nuclear localization signals. HUA2-like proteins have not been identified in animal species, indicating that this family of proteins may play roles unique to the plant development. My hypothesis is that *HUA2* homologues in *Arabidopsis* have redundant functions to *HUA2* in plant development. The overall objective of my project is to investigate the functions of *HUA2* gene family in plant development. As a first step to study the functions of the *HUA2* gene family, I investigated the developmental and tissue specific expression patterns in vegetative and reproductive tissues. Expression patterns of members of *HUA2* gene family are largely overlapping, suggesting possible functional redundancy. To test the function of each member of the *HUA2* family we are characterizing the T-DNA insertion lines in *HUA2 L5, HUA2 L2,* and *HUA2 L3*. So far we have characterized abolished mRNA expression in *HUA2 L2* and *HUA2 L3*, but not in *HUA2 L5*. Promoter::GUS analysis is currently underway to determine the tissue specific expression pattern of each homologue. Creation of double, triple and multiple order

mutants between *HUA2* and *HUA2* homologues will ultimately test functional redundancy among these genes.

P6. Identification and Characterization of the Arabidopsis Exocyst Complex

Yolanda T. Chong and Daphne R. Goring
Department of Botany, University of Toronto
email: yolanda.chong@utoronto.ca

Vesicle trafficking is an important activity in plants that is central to a number of processes such as cell growth/expansion, pollen tube elongation and gravitropic response. Vesicles can travel intracellularly between compartments within the cell; vesicle trafficking can also include endocytosis, where cargo is taken in for processing from outside of the cell, and exocytosis, where products from inside the cell are brought to the plasma membrane or secreted to the cell exterior. Molecular mechanisms of exocytosis have been extensively studied in yeast and mammals resulting in the identification of a multi-subunit complex called the exocyst. This complex is involved in the docking of exocytotic vesicles during polarized secretion. The yeast exocyst is composed of eight proteins: Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p and Exo84p. Through bioinformatic analysis of the Arabidopsis genome, putative homologues for the exocyst complex have been identified. AtSec6, AtSec8, AtSec10 and AtExo84 appear to be single copy genes whereas putative AtSec3, AtSec5 and AtSec15 may be encoded by two genes. However, Exo70 in Arabidopsis appears to have diverged into a family of 23 members. Our primary focus is on AtExo70s. Analysis of microarray databases revealed that AtExo70s is widely expressed in Arabidopsis. Two independent T-DNA insertion lines have been identified for AtExo70s, and homozygotes from both lines show a similar phenotype. Knockout plants appear to be sterile and produce less pollen. To investigate the role of AtExo70s in vesicle trafficking, the full length protein was fused to GFP and transiently expressed in tobacco BY-2 suspension cells. Preliminary results show that the fusion protein localized to distinct regions in the cell.

P7. Cloning, expression, purification and properties of a putative plasma membrane hexokinase from *Solanum chacoense.*

Éric Claeyssen, Owen Wally, Daniel P Matton, David Morse and Jean Rivoal.
IRBV, Université de Montréal, 4101 rue Sherbrooke est, Montréal, QC H1X 2B2 Canada.
email: jean.rivoal@umontreal.ca

A full-length hexokinase cDNA was cloned from *Solanum chacoense*, a wild relative of the cultivated potato. Analysis of the predicted primary sequence suggested that the protein product, ScHK2, contains a membrane-anchor domain at the N-terminus. This domain would direct targeting of ScHK2 to the plant secretory pathway and the protein is predicted to be inserted into the plasma membrane, facing the cytosol. Using N-terminal sequence alignments and several programs for subcellular localization prediction, we

found that the majority of hexokinases from Solanaceae and from *Arabidopsis* may also be targeted to the secretory pathway. This possibility was tested directly. The cDNA encoding for ScHK2 was overexpressed in transgenic potato roots. In accordance with the predicted subcellular localization of ScHK2, plasma membrane fractions prepared from roots overexpressing the construct contained much higher hexokinase protein levels than control roots transformed with an empty vector. ScHK2 was expressed as a (6xHis)-tagged protein in *Escherichia coli*. The recombinant protein was purified by Ni-NTA chromatography followed by anion exchange chromatography on Fractogel EMD DEAE-650 (S). The purified recombinant protein was electrophoretically homogeneous and had a specific activity of 5.3 μmol/min/mg protein. The kinetic properties of the purified enzyme were examined with various substrates. The k_{cat}/K_m ratios were higher with glucose and mannose compared to fructose, indicating a strong preference of the enzyme for the first 2 substrates. Among NTPs, ATP was the preferred substrate. (6xHis)-tagged ScHK2 was highly sensitive to pH variations between 7.7 and 8.7. The enzyme was strongly inhibited by ADP and insensitive to glucose-6-phosphate. These findings constitute the first kinetic characterization of a homogeneous plant hexokinase preparation. The subcellular localization data together with the kinetic properties of the enzyme lead us to hypothesize that ScHK2 could be involved in the energization of glucose at the plasma membrane level in order to fuel glycolysis.

P8. Comparison of inorganic carbon acquisition mechanisms in two *Monoraphidium* species.

Gareth A. Corrigan, and Brian Colman
Department of Biology, York University.
e-mail: colman@yorku.ca

Algal species which grow in natural alkaline waters have evolved mechanisms for the efficient uptake of substrate for photosynthesis from a CO_2-limited environment. These can consist of active CO_2 and bicarbonate transport systems and the expression of an external carbonic anhydrase (CA). Inorganic carbon uptake systems in two related species, *Monoraphidium braunii* and *M. contortum* have been investigated. External CA was detected potentiometrically in both species but was found to be repressed by growth on high [CO_2] and its activity blocked by acetazolamide (AZA), a specific CA inhibitor. Since the expression of external CA usually occurs only in algal species with active CO_2 uptake and is repressed by growth on high [CO_2], it can be assumed that both species possess active CO_2 uptake systems. Bicarbonate transport was demonstrated in both species by comparing the rate of photo-synthetic O_2 evolution, of cells treated with AZA, with the maximum spontaneous CO_2 supply rate at pH 8.0 and a known bicarbonate concentration. Bicarbonate transport in *M. contortum* was found to be repressed by growth on high [CO_2], an effect common in micro-algae, but was not repressed in *M. braunii* under the same conditions. The constitutive expression of bicarbonate transport in *M. braunii* indicates a lack of control, at the molecular level, of the regulation of this transporter by the external conditions.

P9. Developmental profiles of DNA methylation in early-flowering lines of flax (*Linum usitatissimum* L.).

M.M. De Decker[1,2], J.C.L. Brown[1], and M.A. Fieldes[1]
[1]Department of Biology, Wilfrid Laurier University
[2]Department of Biology, University of Waterloo
email: mdedecker@wlu.ca

DNA methylation is an epigenetic trait that is passed on from generation to generation. It is thought to regulate gene expression, through site specific changes and through its role in chromatin modelling. Although patterns of methylation can change with development, they are thought to be reset between generations. Nevertheless, treatments that demethylate plant genomes can produce altered levels of methylation that are heritable, and can induce a range of morphological and developmental abnormalities. In 1990, seeds from two inbred lines of flax, Large (L) and Royal (R), were treated with a demethylating agent, 5-azacytidine, and self-pollination and selection over 2-3 subsequent generations produced four pure-breeding, early-flowering lines. These lines differ from their controls in a number of phenotypic characteristics and it has been demonstrated that the early-flowering phenotype is controlled by at least two loci. In addition, DNA from young shoot tips of the early-flowering lines is hypomethylated and, in the progeny generations of outcrosses, the hypomethylation cosegregates with the early-flowering phenotype. The studies reported here provide data, from two early-flowering lines (RE1 and RE2) and their control line (RC), that illustrate the changes in methylation level that occur during development, from the beginning of early vegetative growth to the onset of flowering. Together with information on the concentration of DNA recovery, tissue weights and, in some cases, chlorophyll content, these data tested two hypotheses: (a) that developmental profiles for methylation levels in flax would reflect tissue age and differentiation and (b) that DNA from the early-flowering lines, RE1 and RE2, would be hypomethylated, relative to RC, in all tissue and throughout development. The results provided support for the first hypothesis but only partial support for the second hypothesis. Unexpectedly, DNA from older or more differentiated tissues of the early flowering lines was not always hypomethylated.

P10. Molecular cloning and characterization of isoflavonoid specific glucosyltransferase and malonyltransferase from soybean.

Mana Farhangkhoee, Ralph Chapman and Sangeeta Dhaubhadel
Southern Crop Protection and Food Research Center, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON, N5V 4T3
email: dhaubhadels@agr.gc.ca

Isoflavonoids are a diverse group of biologically active natural products that accumulate in soybean seeds during development. They serve important roles in symbiotic nitrogen fixation, as well as defence against various biotic and abiotic stress. In soybean, isoflavone aglycones are converted to their glyco-conjugates by glucosyltransferases (GTs) and malonyl-glyco-conjugates by malonyltransferases (MTs). The conjugation step

confers stability and solubility to isoflavone aglycones enabling their compartmentalization to vacuoles or transport to the site of accumulation. We used functional genomic approach to identify isoflavonoid specific GT and MT from soybean seeds. TIGR Soybean Gene Index (http://www.tigr.org/tigr-cripts/tgi/T_index.cgi?species=soybean) was searched by key words to make a list of candidate genes. From the group of candidate ESTs for GTs and MTs, the full length cDNAs for 6 of the GTs and 10 of the MTs were obtained using 5' and 3' RACE. The full length genes were cloned into an expression vector for the production of recombinant enzymes in a heterologous system. *In vitro* enzymatic activity assays were conducted for GTs and MTs using ^{14}C-UDP-glucose and ^{14}C-malonyl CoA, respectively, as donors with several substrates. Thin layer chromatography was used to separate the labelled products that co-migrated with the known standards. HPLC and LC-ES/MS were performed to further confirm the reaction products. Among several recombinant enzymes, GT6-1 showed glycosylation activity towards all three soybean isoflavone aglycones and MT7 exhibited malonylation activity towards isoflavone glucosides. The highest level of expression of *GT6-1* transcript was observed in pods, flower buds, flower and mature embryos whereas *MT7* was expressed at higher level in embryos. Increased accumulation of both *GT6-1* and *MT7* transcripts were observed in response to stress.

P11. Functional genomics of the 5GT-like gene family of *Vitis labrusca.*

Dawn Hall and Vincenzo De Luca
Department of Biological Sciences, Brock University
email: dh00aa@brocku.ca

Concord grape (*Vitis labrusca*) accumulates mono- and di-*O*-glucosylated anthocyanin pigments in the skin following the onset of veraison. Glucosylation at the 3-*O* position stabilizes the reactive anthocyanin aglycone and is catalyzed by the enzyme UDP-glucose anthocyanin 3-*O* glucosyltransferase (3gt). Subsequent glucosylation at the 5-*O* position is catalyzed by the enzyme UDP-glucose: anthocyanin 5-*O* glucosyltransferase (5gt). Members of this gene family have been previously cloned and functionally characterized from 8 different species of plants that share high degrees of nucleotide sequence similarity and that group together on a neighbour-joining phylogenetic tree. Based on the reasonable assumption that similar 5GTs may catalyze this reaction in grapevine, homology-based cloning was used to isolate 3 full-length and 2 partial 5gt-like genes from *V. labrusca* skin. However, purification of this enzyme to homogeneity combined with protein sequencing of the 5GT from *V. labrusca* grape skin permitted the cloning a sixth glucosyltransferase that belonged to a separate clade of this growing gene family. The risks of making assumptions from phylogenetic analyses and the importance of proteomic approaches in identifying the appropriate candidate gene to target will be highlighted.

P12. **Gene function discovery in activation tagged poplar lines.**
Edward Harrison[1], Bush, M.[1], McPhee, D.[2], Vitez, R.[2], Sharma, V.[2], O'Malley, B.[2], Seguin, A.[3], Mackay, J.[3] and Regan, S.[1]
[1]Biology Department, Queen's University, Kingston, Ontario, CANADA, K7L 3N6
[2]Biology Department, Carleton University, Ottawa, Ontario, K1S 5B6
[3]Centre de Recherche en Biologie Forestière, Université Laval, Québec, CANADA G1K 7P4, URL: www.arborea.ulaval.ca

We have recently produced a large population of mutant poplars that we expect will accelerate our understanding of tree growth and development. Activation tagging is an insertional mutagenesis method which we have used as part of a Genome Canada funded project to create a population of over 2500 lines in the model tree *Populus*. Activation tagging is well suited for mutant production in trees because it involves the random insertion of a multimerized CaMV 35S enhancer into the host plant genome, thereby causing transcriptional upregulation of a nearby gene (Weigel *et al*, 2000). This generates a dominant, gain-of-function phenotype that can be identified in the primary transformant, without the need for homozygous lines. We have begun to analyze these lines and have identified 25 distinct morphological mutants that are of interest. Using a PCR based approach called "Genome Walking", we are identifying the insertion site of the T-DNA vector within the genome for many of these mutants. The task of identifying the insertion sites is made possible by the availability of the *Populus* genome that was released by the Joint Genomics Institute in 2005. Once we identify the insertion site, the adjacent genes are tested for unusually high gene expression that could be attributed to the insertion of the enhancer. For the more interesting mutants, we will perform transcript profiling using the poplar microarray generated by the Genome Canada project. Ultimately, these lines will provide valuable information about tree growth and development, and will eventually help us identify superior trees in the forest.

P13. The carboxyl terminus of cytochrome b_5 functions as a negative regulatory sequence for conferring endoplasmic reticulum specificity.
Yeen Ting Hwang,[1], Henderson, M.P.[2], Dyer, J.M.[3], Andrews, D.W.[2] and Mullen, R.T.[1]
[1] Department of Molecular and Cellular Biology, University of Guelph
[2] Department of Biochemistry and Biomedical Sciences, McMaster University
[3] USDA-ARS Southern Regional Research Center
email: hwangy@uoguelph.ca

Tail-anchored (TA) membrane proteins are a small, but growing class of proteins that are targeted and integrated post-translationally into their respective organelles via unique targeting signals located at their C termini. The molecular mechanisms responsible for the specificity of TA protein targeting, however, are not well defined. Previously, we described the biogenesis of four closely related tung (*Vernicia fordii*) cytochrome b_5 isoforms (Cb_5-A, -B, -C and -D) that play an essential role in lipid biosynthesis (Hwang et al. 2004. Plant Cell 16: 3002). Specifically, we identified the sequence-specific and physicochemical properties of the targeting signal motifs for the endoplasmic reticulum (ER)-localized

isoforms Cb_5-A, -B and -C and the mitochondrial-localized isoform Cb_5-D. We showed also that the ER and mitochondrial isoforms of Cb_5 retained their targeting specificity both *in vivo* in BY-2 cells and *in vitro* with purified ER and mitochondria derived from mammalian cells. Here, we address the question of how the ER targeting specificity of Cb_5 is achieved. Using a cell-free targeting assay, our results indicate that removal of the short C-terminal sequences (CTSs) from Cb_5-A and -B did not disrupt their ER targeting; however, it did result in a loss in selectivity with some aberrant binding of the truncated proteins to mitochondria. The CTS of Cb_5A was also demonstrated to be sufficient in conveying ER targeting specificity since when this sequence was used to replace the corresponding sequence in Bcl-2, a TA protein that is normally targeted to both ER and mitochondria, targeting to mitochondria was inhibited. Taken together, these data and those from other experiments with liposomes and trypsin-treated membranes suggest that the CTS of Cb_5A acts as a negative regulatory element that facilitates ER specific targeting by preventing spontaneous insertion of the protein's single TMD into other (incorrect) subcellular membranes.

P14. Water pathways in injured leaves of *Codiaeum variegatum.*

Jennifer Janusz and Ewa Cholewa.
Department of Biology, Nipissing University.
email: ewac@nipissingu.ca

Brochidodromous (closed) venation pattern in leaves is redundant and allows for large amounts of water dispersion. Recent studies have suggested the midrib may not be crucial to net leaf water distribution. The purpose of this study is to determine how leaves compensate when major water pathway of water transport through the midrib is lost. Groups of six *Codiaeum variegatum* leaves were used in this experiment: one control group and two experimental groups whose midribs were cut. The first experimental group's midribs were cut before the first set of secondary veins branched from the midvein (approx. 1 cm from the petiole), and the second experimental group's midribs were cut after the first set of secondary veins branched from the midrib (approx 2.5 cm from petiole). All leaves were placed in a 1% Safranin O solution and after four days were observed for predominate water pathways. Both experimental groups displayed different solute distribution patterns from the control group. Dye was present in 100% of minor veins sampled within the lower portion of both experimental groups' leaf laminas. In comparison, control group leaves displayed dye in only 33% to 50% of minor veins within similarly sampled areas. In control leaves, predominate water pathway included the midrib. In experimental leaves water was transported through secondary, tertiary and quaternary veins, bypassing the site of injury to the midrib. Experimental injured leaves continued to transport large amounts of water despite the damage and maintained normal leaf functions (continuing to function normally a year after damaged occurred within some leaves). These results suggest the midrib's predominant role may be a mechanical support due to heavily lignified tracheary elements than a compulsory part of water distribution.

P15. Purification and characterization of 16OH tabersonine O-methyltransferase (16-OMT) from *Catharanthus roseus.*

Dylan Levac
Department of Biological Sciences, Brock University
email: dylanlevac@hotmail.com

The monoterpene indole alkaloid (MIA) Vindoline is an essential moiety of the antineoplastic agents vinblastine and vincristine and is formed from tabersonine through six sequential enzymatic steps that may involve at least three different cell types. O-methylation of 16-hydroxytabersonine is catalyzed by an S-adenosylmethionine (SAM) dependant O-methyltransferase (OMT) that has yet to be purified to homogeniety. Studies using laser capture microdissection (LCM) and carborundum abrasion (CA) techniques have shown that 16-OMT is expressed specifically in epidermal leaf cells of *Catharanthus* (Murata, Plant J, 2005, 44:581). We show in this report that CA technique can be used for large scale extraction of leaf epidermis enriched proteins. This extraction procedure facilitated the purification to homogeneity of 16-OMT by size exclusion, adenosine agarose affinity and anion exchange chromatography to yield 400ng of a 55 KDa protein. Sequencing of 16-OMT yielded 6 peptide sequences that were similar but not identical to functionally characterized Flavonoid –O-Methyltransferases from *Catharanthus roseus* (Schroder, Phytochemistry, 2003, 62:127). This information is presently being used to obtain the gene encoding 16-OMT from Catharanthus leaf epidermis.

P16. Metabolic biodiversity of monoterpenoid indole alkaloid biosynthesis in different flowering cultivars of *Catharanthus roseus*.

Mary Magnotta, Jun Murata and Vincenzo De Luca
Department of Biology, Brock University
email: mm04zk@brocku.ca

Madagascar periwinkle, *Catharanthus roseus* (L) G. Don, a medicinally important plant, produces anticancer dimeric alkaloids, vinblastine and vincristine in the leaves. This plant is cultivated with an ornamental aim to produce plants with different growth habits, more flower colors and bigger flowers. In order to investigate whether these morphological variations have led to modifications in alkaloid yield, 56 different flowering cultivars, including our own research cultivar *C. roseus* cv. Little Delicata, were studied. The expression profiles of monoterpenoid indole alkaloids, namely vindoline produced a single cultivar that accumulated low levels of vindoline compared to Little Delicata. Additional expression profiling studies with enzymes involved in vindoline biosynthesis clearly identified the biochemical bottleneck responsible for the low vindoline phenotype that was observed in this line. In summary this study revealed that the selection methods used to select for *Catharanthus* flower cultivars have not also generated large variations in alkaloid profiles and it remains to be investigated if breeding for alkaloid content is possible.

P17. Origins and evolutionary history of alternative oxidase and plastid (plastoquinol) terminal oxidase.

Allison E. McDonald and Greg C. Vanlerberghe.
Departments of Botany and Life Sciences, University of Toronto at Scarborough
email: allison@utsc.utoronto.ca

Alternative oxidase (AOX) and plastid (plastoquinol) terminal oxidase (PTOX) are related membrane-bound di-iron carboxylate proteins that act as quinol oxidases in the respiratory and photosynthetic electron transport chains of mitochondria and chloroplasts, respectively. The recent discovery of a functional AOX in bacteria and animals, as well as the presence of PTOX in cyanobacteria and photosynthetic protists have challenged the paradigm that these proteins are limited to a select few eukaryotic organisms and instead demonstrates that they are widespread in the prokaryotic and eukaryotic domains. Based upon their sequence similarity and distribution in prokaryotes, it is likely that a common ancestral protein diversified into AOX within the proteobacteria and PTOX within the cyanobacteria. The most parsimonious explanation for the wide distribution of AOX and PTOX in eukaryotes is that these proteins entered the eukaryotic lineage via the primary endosymbiotic events that led to the creation of mitochondria (AOX) and chloroplasts (PTOX) respectively. We suggest that AOX and PTOX have subsequently spread throughout the eukaryotic domain by vertical inheritance and in the case of some organisms by secondary and tertiary endosymbiotic events. In support of this hypothesis, organisms whose predecessors have harbored mitochondria, but have never possessed plastids (i.e. animals, fungi, choanoflagellates, and some protists) may contain AOX but always lack PTOX. Alternatively, organisms whose predecessors have harbored both mitochondria and plastids (primary, secondary, or tertiary) in the past (i.e. algae, plants, haptophytes, heterokonts, cryptophytes, dinoflagellates) may contain both AOX and PTOX. AOX and PTOX may play analogous roles within their respective bioenergetic systems.

P18. Analysis of the regulation of recombinant protein fate in plants

Rima Menassa and Angelo Kaldis
Agriculture and Agri-Food Canada, London, Ontario
Email: menassar@agr.gc.ca

During the past few years, a wide variety of recombinant proteins have been expressed in plants. Some proteins such as the reporter proteins GUS and GFP accumulate to high levels in plants, while others accumulate to very low levels. Inherent properties of these proteins, protein turnover rates, as well as their subcellular localisation affect their accumulation levels.
The human interleukin-10 protein (IL-10) is a labile protein with a half-life *in vivo* of 30 minutes. Furthermore, IL-10 requires post-translational modifications and assembly, and is a good representative of other therapeutic proteins. However, IL-10 does not accumulate in plants to the levels that are desirable for therapeutics production. The purpose of this study is to develop an understanding of the regulation of recombinant

protein fate in plants, particularly identifying the step that limits the accumulation of recombinant proteins. We used GUS as a long-lived, high accumulating protein example, and IL-10 as a low accumulating protein example, determined the half-life of these proteins, and looked at the effect of proteasome inhibitors, lysosome inhibitors, and protease inhibitors on the levels of recombinant proteins in extracts. We found that the half-life of IL-10 is approximately 24 hours, and that the proteasome plays a definite role in the degradation of IL-10. Indeed, the proteasome inhibitors MG132 and MG115 protect the IL-10 protein. Because both these inhibitors have a calpain inhibiting property as well, we tested ALLN, a calpain inhibitor to verify if it was this activity that protected IL-10. Calpain inhibitor had a protective effect on IL-10, but much less than MG132 or MG115. None of the inhibitors tested had any effect on GUS accumulation levels in the 24 hour treatment period.

P19. Localization of tabersonine 16-hydroxylase and 16-OH tabersonine-16-O-methyltransferase to leaf epidermal cells defines them as a major site of precursor biosynthesis in the vindoline pathway in *Catharanthus roseus*.

Jun Murata[1], Dorothee Bienzle[2] and Vincenzo De Luca[1]
[1]Biological Sciences, Brock University, St. Catharines, Ontario L2S3A1
[2]Ontario Veterinary College, University of Guelph, Guelph, Ontario, N1G2W1
email: jmurata@brocku.ca

The Madagascar periwinkle (*Catharanthus roseus*) produces the well known and remarkably complex anticancer dimeric alkaloids vinblastine and vincristine, which are derived from the coupling of vindoline and catharanthine monomers. Recent data from *in situ* RNA hybridization and immunolocalization suggest that combinatorial cell factories within the leaf are involved in vindoline biosynthesis. In this study, the cell types responsible for vindoline biosynthesis were identified by laser-capture microdissection/RNA isolation/RT–PCR to show that *geraniol hydroxylase*, *secologanin synthase*, *tryptophan decarboxylase*, *strictosidine synthase*, *strictosidine ß-glucosidase* and *tabersonine 16-hydroxylase* can be detected preferentially in epidermal cells. A new and complementary application of the carborundum abrasion (CA) technique was developed to obtain epidermis-enriched leaf extracts that can be used to measure alkaloid metabolite levels, enzyme activities and gene expression. The CA technique showed that tabersonine and 16-methoxytabersonine, together with 16-hydroxytabersonine-16-*O*-methyltransferase, are found predominantly in *Catharanthus* leaf epidermis, in contrast to vindoline, catharanthine and later enzymatic steps in vindoline biosynthesis. The results show that leaf epidermal cells are biosynthetically competent to produce tryptamine and secologanin precursors that are converted via many enzymatic transformations to make 16-methoxytabersonine. This alkaloid or its 2,3 dihydro-derivative is then transported to cells (mesophyll/idioblast/laticifer) within *Catharanthus* leaves to complete the last three or four enzymatic transformations to manufacture vindoline.

P20. *Lotus japonicus RED1* and *HIT* define two consecutive stages for the rhizobial symbiosis.

Jeremy D. Murray, Bogumil Karas, and Krzysztof Szczyglowski
Southern Crop Protection and Food Research Centre, AAFC, London, ON
email: murrayj@agr.gc.ca

The invasion of *Lotus japonicus* roots by the dinitrogen fixing symbiotic bacteria *Mesorhizobium loti* is characterized by a) entry of bacteria into the epidermal cell via an infection thread b) the progression of the infection thread through multiple cortical cell layers towards the nodule primordium c) the uptake of the bacteria into plant host cells via endocytosis. A mutant screen in the *har1* hypernodulating background yielded, amongst others, two mutants that help define the first two stages of the rhizobial infection. *RED1* (*RHIZOBIAL ENTRY DENIED*) mutants are characterized by bacterial binding to the root hairs, normal root hair curling, the formation of oversized microcolonies, and a failure to gain entry into the epidermal cells. In the *har1-1*/*har1-1* hypernodulation background an occasional wild-type nodule is formed. The presence of many small white bumps on infected RED1 mutant roots indicates that Nod factor signalling to the cortex is intact. *HIT* (*HYPERINFECTED*) mutants are competent for the initial stage of infection and form an excessive number of infection threads through root hairs. However, a failure to properly direct the growing infection threads through the cortical cells leads to a delay in nodule formation and a reduced number of nodules as compared to wild-type. The nodules that are formed are misshaped but appear to efficiently fix nitrogen. Therefore, both *RED1* and *HIT* specify genetic functions that are required for efficient colonization of the *L. japonicus* root by *M. loti*.

P21. Altered root development of the pea nodulation mutant R50 (*sym16*).

Alicia N. Pepper and Frédérique C. Guinel.
Department of Biology, Wilfrid Laurier University
email: pepp5940@wlu.ca

R50 is a pea mutant displaying short and thick basal internodes, decreased epicotyl height, and fewer and shorter lateral roots (LR). It has elevated levels of cytokinins (CK) because of deficient activity and/or altered expression of the enzyme CK oxidase/dehydrogenase. To characterize the R50 root phenotype, we studied the development of R50 root vasculature and compared it to that of Sparkle, the parent from which R50 was derived. Two sets of 18 plants were harvested at 4 different ages, and cross-sections of primary root (PR) were made by hand at three different locations. Two LR were also sectioned 1cm distal from their site of branching. Toluidine blue-stained sections were viewed by light microscopy and the number of xylem poles counted. Sparkle PR possessed 3 xylem poles, whereas the number of poles in R50 varied from 3 to 5 and often increased from tip to base. However, as R50 aged, more plants displayed the triarch vasculature. Surprisingly, LR vasculature differed from that of PR; Sparkle LR had 3 to 4 poles but those of R50 had 2 to 3. The diameters of root and stele were recorded in another set of 18 plants and possible correlations were drawn. There was a

consistent root/stele diameter ratio in Sparkle; however, the ratio was more variable in R50. No correlation was found between number of poles, stele diameter and root diameter. Finally, the numbers of initiated and emerged LR were determined in cleared root systems. There were less LR initiated and emerged in R50 than in Sparkle. Here, we have demonstrated that R50 root development is significantly different from that of Sparkle. Whereas some of our findings can be explained by the high CK levels of R50, others could be evidence for an auxin involvement in the R50 root phenotype.

P22. Role of anaerobic respiratory enzymes in low temperature sweetening tolerance of potato.

Reena Grittle Pinhero, Leslie Copp, Clara Lopez Amaya, Alejandro G. Marangoni and Rickey Y Yada
Department of Food Science, University of Guelph
e-mail: rpinhero@uoguelph.ca

Low temperature sweetening (LTS) of potato occurs when tubers are stored at temperatures below 9-10°C and is characterized by the accumulation of reducing sugars such as sucrose, glucose and fructose. LTS cause dark-brown colored chips and fries which are not acceptable by consumers. However, low temperature storage offers certain advantages such as prevention of microbial growth, decreased sprouting, lower respiratory losses as well as reducing/eliminating the use of chemical sprout inhibitors. Certain potato varieties are more tolerant to LTS than others. The objective of this study was to understand the genetic basis of LTS tolerance in relation to certain anaerobic respiratory enzymes. In this study, we compared the isozyme profiles of alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH) by native PAGE and the gene expression levels of ADH, LDH and pyruvate decarboxylase (PDC) by relative quantitative RT-PCR in two potato varieties such as Monona (LTS-sensitive) and ND860-2 (LTS-tolerant) at two different temperatures, 10°C and 4°C. Our study showed changes in isozyme profiles as well as gene expression levels of ADH, LDH and PDC between the varieties and temperature treatments. Two ADH isozymes were observed in ND860-2, whereas only one was evident in Monona. Eight LDH isozymes were observed in ND860-2, while Monona possessed only six isozymes. ND860-2 had two unique low molecular weight isozymes. AC glacier chip, a moderately LTS tolerant variety possessed seven LDH isozymes. In general, the gene expression levels of PDC, ADH and LDH were increased at 4°C compared to their levels at 10°C. However, ND860-2 showed greater increases in PDC and LDH expression levels compared to Monona. This study shows that ADH, LDH and PDC may play a role in conferring LTS tolerance in ND 860-2.

P23. Assembly of distinct Toc complexes in *Arabidopsis thaliana*.

Lynn Richardson and Matthew D. Smith
Department of Biology, Wilfrid Laurier University
email: rich4050@wlu.ca

Plastid differentiation and chloroplast biogenesis in particular, are important events in plant development. Chloroplast differentiation relies on successful import of many nuclear-encoded proteins. Initial steps of import involve preprotein recognition and translocation across the outer membrane, which are mediated by components of the Toc complex (translocon at the outer membrane of chloroplasts). These processes involve two families of homologous receptors, the Toc159 and Toc34 GTPases. In *Arabidopsis*, members of the Toc159 receptor family (atToc159, atToc132, and atToc120) are thought to exist in free soluble and membrane-inserted forms, suggesting a possible model for protein targeting to the outer membrane. According to this model a Toc159 receptor recognizes preproteins and targets them to the outer membrane via a GTP-dependent interaction with Toc34 family members (atToc33 and atToc34). This study aims to provide further evidence for preferential interaction between specific members of the Toc159 and Toc34 families of GTPases. This will involve using chloroplasts isolated from two *Arabidopsis* mutants, the atToc33 knockout mutant *ppi1*, and atToc34 knockout mutant *ppi3*, with *in vitro* translated, radiolabelled Toc159 family members for *in vitro* targeting assays. Evidence exists indicating a preferential interaction between atToc159 and atToc33, and also between atToc132/120 and atToc34. Thus, it is hypothesized that the targeting efficiency of atToc159 will be reduced in *ppi1* chloroplasts relative to wild type chloroplasts, while the targeting efficiency of atToc132/120 will be reduced in *ppi3* chloroplasts compared to wild type chloroplasts. This study will potentially provide further evidence for the preferential interaction between specific members of the Toc159 and Toc33 families in *Arabidopsis*, supporting the hypothesis that distinct Toc complexes exist for import of specialized groups of proteins. We will present our most current data on the preferential targeting of Toc159 family members to distinct Toc complexes.

P24. A simple, inexpensive and consistent method of growing Arabidopsis hydroponically.

Mary Robison, Matthew Smid and David Wolyn
Dept. of Plant Agriculture, Bovey Bldg.,
University of Guelph, Guelph, ON N1G 2W1
email: mrobison@uoguelph.ca

Several methods of growing *Arabidopsis thaliana* hydroponically have been published, but in our hands, most methods are complicated, expensive, waste seed or simply do not work. We present a simple and consistent method of growing Arabidopsis hydroponically that uses easy-to-obtain and inexpensive materials, produces highly homogeneous material, is easy to set up and maintain, does not waste seed, prevents algal growth and disease, and accommodates all life stages of the plant.

P25. Here, there and everywhere: housekeeping enzymes localizing to multiple subcellular compartments.

Sarah Schoor[1], Sanghyun Lee[1], Matt Smith[2] and Barbara Moffatt[1]
[1]Department of Biology, University of Waterloo;
[2]Department of Biology, Wilfrid Laurier University.
email: sschoor@gmail.com

S-adenosylhomocysteine (SAH) is a by-product of SAM-dependent methylation reactions as well as an inhibitor of the methyltransferases (MTs) involved. By ensuring the breakdown of SAH, SAH hydrolase and adenosine kinase (ADK) are required in maintaining the hundreds of MT activities required for plant growth. Since transmethylation occurs in all subcellular compartments, SAH is also produced throughout the cell. Surprisingly both SAH hydrolase and ADK are proposed to reside only in the cytosol, so it remains a mystery as to how the SAH produced in other compartments is catabolized. This project has used immunogold labeling and β-glucuronidase (GUS) translational fusion lines to establish the localization of each isoform of SAH hydrolase and ADK of *Arabidopsis thaliana*. Leaf and meristem tissues of wild-type plants, and mutants deficient in either ADK1 or ADK2 were labeled using antibodies specific for ADK or SAH hydrolase. ADK and SAHH cDNA GUS translational fusion were expressed under the control of a constitutive promoter or their natural promoters. Analysis of both the immunogold labelling results and the GUS fusion lines revealed all ADK and SAH hydrolase isoforms localize to the cytosol, chloroplast and nucleus. Ongoing studies are designed to elucidate the metabolic and developmental signals directing the subcellular targeting of these enzymes.

P26. Dormancy of *Chara vulgaris* oospores.

Joana Sederias and Brian Colman.
Department of Biology, York University.
e-mail: sederias@yorku.ca

Germination and dormancy-breaking requirements of *Chara vulgaris* oospores have been investigated in two populations. Oospores harvested from surface sediments and from the plants of 2 lakes, Schumacher (Sch) and Little Pearl Lake (LPL), in Timmins, Ontario, exhibited contrasting germination rates of 45% vs. 18% for sedimentary; and 6% vs 4% for fresh oospores. Sedimentary oospores exhibit a dormancy, readily broken by gibberellic acid, or stratification and red light, which in turn increases sensitivity to GA_3, whereas fresh oospores exhibit dormancy which is less sensitive to such treatments. ABA inhibited the GA-enhanced germination, but had more effect on sedimentary oospores than the fresh. These results indicate a clear difference in primary and secondary dormancy in *Chara* oospores, which exhibit primary dormancy when first dehisced from the mother plant, broken primarily by a low temperature treatment, followed by secondary dormancy which is broken by other environmental conditions. Starch hydrolysis was greatly increased in stratified, sedimentary oospores when treated with GA_3 and red light. Activity of α-amylase was induced by GA3, stratification and red light in both fresh and

sedimentary oospores, which provides evidence of an interaction between the phytochrome mechanism and hormonal regulation, not common in any algal group. Oospores from both populations responded similarly to the dormancy-breaking treatments, but their overall viabilities were significantly different.

P27. Biochemical characterization of a recombinant *Arabidopisis* succinate semialdehyde/glyoxylate reductase.

Barry J. Shelp[1], Gordon J. Hoover[1], A. Rod Merrill[2], and Shawn M. Clark[1]
Departments of Plant Agriculture[1] and Molecular and Cell Biology[2], University of Guelph, Guelph, ON N1G 2W1
email: bshelp@uoguelph.ca

Gamma-hydroxybutyrate is synthesized *in vivo* from gamma-aminobutyrate by transamination to succinate semialdehyde (SSA), and subsequent reduction of the aldehyde group. In this report, a putative succinate semialdehyde reductase *Arabidopsis* cDNA was functionally expressed in *Escherichia coli*, and the recombinant protein purified 2.3-fold to homogeneity based on SDS gel electrophoresis. Native gel electrophoresis and the temperature dependence of enzymatic activity indicated that the subunits assembled into multiple molecular species. Equilibrium denaturation analysis revealed that enzymatic activity could be dramatically stabilized by the addition of 20% sorbitol; in this environment, maximum activity at 22^{o}C was found near pH 7.8. Kinetic analysis revealed that the purified protein effectively catalyzed the conversion of physiological concentrations of SSA to gamma hydroxybutyrate via an essentially irreversible, NADPH-based mechanism; saturation kinetics for SSA (K_m = 1.7 mM) and NADPH (K_m = 27 µM) displayed hyperbolic and sigmoidal patterns, respectively. The protein also catalyzed the conversion of glyoxylate to glycolate, with saturation kinetics for glyoxylate (K_m = 69 µM) and NADPH (K_m = 22 µM) displaying sigmoidal and hyperbolic patterns, respectively. Based on the performance constants k_{cat}/K_m or $k_{cat}/K_{0.5,}$ the enzyme had about a 50-fold higher preference for glyoxylate than SSA. Various other aldehydes and dicarbonyl compounds, including both small and bulky molecules, served as inefficient substrates. These characteristics indicate that the enzyme should be designated as a NADPH-dependent succinate semialdehyde/glyoxylate reductase, and further suggest that it could act as a regulatory step in plant aldehyde metabolism.

P28. Early intracellular response profiling in plants using targeted fluorescent protein probes.

Alison Sinclair and Jaideep Mathur
Laboratory of Molecular Cell Biology, Department of Plant Agriculture, University of Guelph. 50 Stone Road. Guelph. N1G 2W1.
email: sinclaia@uoguelph.ca

Approaches towards understanding plants and their inner workings is undergoing a rapid transformation as we rely increasingly on “live imaging” technology for visualizing,

tracking and quantifying gene products in living plant cells. Fluorescent proteins such as the *Aequorea victoria* derived Green Fluorescent Protein and its colour variants have played a major role in this revolution. Today, a large number of transgenic plants expressing fluorescent probes targeted to different sub-cellular structures and compartments are available to plant scientists. Our 'live-cell' visualization approach is aimed at generating an exhaustive cell-biological profile, backed by a dissection of molecular-genetic mechanisms underlying early subcellular responses of plants to diverse biotic and abiotic stimuli. Our experimental approach is as follows:

1. Creating transgenic lines carrying multiple targeted fluorescent markers for simultaneous multi-colour live visualization of sub-cellular components in *Arabidopsis* and tobacco,
2. Carrying out baseline observations involving organelle localization and behaviour within several model cell types that are developing under normal growth conditions using 4D-imaging and tracking techniques.
3. Carrying out parallel treatments and observations using wild-type and mutant (*Arabidopsis* plants carrying mutations in cytoskeleton related genes like ARP2/3-complex subunits/ ROP-GTPases/ MAPs) transgenic marker lines. Treatments will include challenging plants with well-characterized cytoskeletal-drugs, and different abiotic (eg. temperature, salt, osmotic) and biotic (eg. wounding, bacterial / fungal infestation) stimuli.

Our studies would result in the first comprehensive documentation of early sub-cellular response of plant cells to diverse stimuli. They would help in identifying common molecular mechanisms that might be triggered in response to completely different environmental stimuli. Finally, our results would pave the way for understanding the basic laws that must be involved in the "creation of order from apparent chaos"; a phrase that aptly sums up and links sub-cellular behaviour to a precise cellular response.

P29. The Toc159 family of chloroplast protein import receptors in *Arabidopsis thaliana.*

Matthew D. Smith[1], C.M. Rounds[2], F. Wang[2], Y.I. Ivanova[2], and D.J. Schnell[2]
[1]Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada
[2]Department of Biochem. & Mol. Biol., University of Massachusetts, Amherst, MA, U.S.A.
email: msmith@wlu.ca

The Toc (translocon at the outer envelope membrane of chloroplasts) complex is of critical importance to plants, as it is responsible for recognizing and initiating the import of many essential plastid preproteins. This complex acts coordinately with the Tic (translocon at the inner envelope membrane of chloroplasts) complex to complete translocation of preproteins into the stroma. The core of the Toc complex consists of Toc159, Toc75 and Toc33. Toc75 forms at least part of the channel through the outer membrane, whereas Toc159 and Toc33 are related GTPases thought to be involved in preprotein recognition and regulation of import. In *Arabidopsis thaliana*, these GTPases are represented by small gene families. There are four Toc159 family members (Toc159, Toc132, Toc120 and Toc90) and two members of the Toc33 family (Toc34 and Toc33).

We have established that Toc159 interacts directly and specifically with transit peptides of photosynthetic preproteins destined for the chloroplast, through a binding site that is comprised at least in part by the GTPase domain of the receptor. In addition, we have demonstrated that Toc159 and Toc132/Toc120 differentially associate with Toc33 and Toc34, and therefore constitute structurally distinct Toc complexes. Toc132 and Toc120 also appear to specifically recognize and bind non-photosynthetic preproteins. These findings are consistent with the observation that *Arabidopsis* null mutants lacking one of Toc132 or Toc120 have no discernible phenotype, whereas double knockouts lacking both Toc132 and Toc120 are lethal. Collectively, these data have led to the hypothesis that members of the Toc159 family are transit peptide receptors that represent distinct routes for targeting preproteins to plastids, and that these separate pathways are required to ensure balanced import of proteins that are essential to the many biochemical pathways housed within plastids.

P30. Confocal imaging and three - dimensional reconstruction of endodermal cell walls, and visualizing endodermal cell files along the onion root axis.

Ishari Waduwara and Carol A. Peterson
Department of Biology, University of Waterloo
e-mail: ciwaduwa@sciborg.uwaterloo.ca

The endodermis is one of the “physiological sheaths” in plant roots that play a significant role in fundamental root function. In most plant species, suberin lamellae are eventually deposited as secondary walls in the endodermis. Some cells of this layer, however, do not develop these lamellae and are called “passage cells”. These are usually situated near the xylem poles as seen in root cross sections. A longitudinal view is needed to determine whether the passage cells are in continuous files, scattered, or more numerous in regions where lateral roots will be produced. Techniques are available to stain Casparian bands and suberin lamellae in cleared, whole roots. The present work is the first to provide a CLSM (confocal laser scanning microscope) procedure for suberin lamellae, allowing passage cells to be distinguished by their lack of staining. The endodermis was exposed by treating roots with pectinase for 24 h, after which the central cortex and epidermis could be peeled away from the endodermis and stele. Further isolation of endodermal layer was achieved by removing the xylem strands. The suberin lamellae in the endodermis were stained with 0.01% Nile red dissolved in lactic acid. This solvent served to clear the tissue, resulting in a better visualization of the lamellae. A series of longitudinal optical sections of the endodermal cell walls were obtained using an LSM 510 META CLSM, and the wall was reconstructed three-dimensionally. This enabled passage cell walls to be distinguished from the endodermal cell walls with suberin lamellae. Imaging the endodermal cell files made it evident that the passage cells were scattered within the endodermis and were not concentrated in any one region. The physiological significance of this pattern is that water and ions such as calcium and magnesium (that are impeded by suberin lamellae) will be transferred to a wide area of the stele.

List of Participants

Name	Affiliation	Abstract Number
Al-daoud, Fadi	McMaster University	
Aldea, May	University of Toronto	
Allan, Wendy	University of Guelph	P2
Bender, Kyle	Nipissing University	
Bhatti, Shabana	York University	P3
Bogart, Sarah	Nipissing University	P4
Brandizzi, Federica	Department of Biology University of Saskatchewan	PP1
Brauer, Liz	University of Guelph	
Bresolin, Nicole	University of Guelph	C7
Busch, Florian	University of Western Ontario	
Bush, Michael	Queen's University	P12
Cameron, Robin	McMaster University	B5
Challa, Sathya	University of Western Ontario	P5
Champigny, Marc	McMaster University	B5
Chlup, Michael	Wilfrid Laurier University and University of Waterloo	
Cholewa, Ewa	Nipissing University	P4, P14
Chong, Yolanda	University of Toronto	P6
Clark, Shawn	University of Guelph	A5, P27
Colman, Brian	York University	P3, P8, P26
Corea, Oliver	University of Western Ontario	C4

Costea, Mihai	Wilfrid Laurier University	
Czarny, Jennifer	University of Waterloo	
DeDecker, Michelle	Wilfrid Laurier University and University of Waterloo	P9
Dhanoa, Priya	University of Guelph	C1
Dhaubhadel, Sangeeta	Agriculture & Agri-Food Canada	P10
D'Silva, Lorraine	University of Guelph	D3
Duan, Jin	University of Waterloo	
Emery, Neil	Trent University	D5
Emes, Michael	University of Guelph	C6, C7
Enstone, Daryl	University of Waterloo	
Fang, Xingxiao	University of Waterloo	
Farhangkhoee, Mana	Agriculture Canada	P10
Fieldes, Mary-Ann	Wilfrid Laurier University	P9
Froehlich, John	DOE Plant Research Lab Michigan State University	PP3
Gallardo, Jorge	University of Concepcion	
Geisler, Matt	University of Western Ontario	
Geisler-Lee, Jane	University of Western Ontario	
Gidda, Satinder	University of Guelph	B3, B4
Gong, Xuemei	University of Guelph	
Gong, Yujie	University of Guelph	
Gray, Gordon	University of Saskatchewan	D7
Guinel, Frédérique	Wilfrid Laurier University	P21

Guy, Robert	University of British Columbia	
Hall, Dawn	Brock University	P11
Hao, Jouai	University of Waterloo	
Harrison, Edward	Queen's University	P12
Hood, Rebecca	University of Western Ontario	
Howard, Alex	University of Guelph	B4
Humphrey, Tania	University of Toronto	
Hwang, Yeen Ting	University of Guelph	P13
Kalala, Bob		
Kalinina, Anna	Agriculture & Agri-Food Canada	
Kaur, Simendeep	Université de Montréal	C2
Ko, Kenton	Queen's University	
Kohalmi, Susanne	University of Western Ontario	C4
Koonjul, Priyum	Université de Montréal	C2, C3
Lapointe, Line	Université Laval	
Lee, Sanghyun	University of Waterloo	P25
Levac, Dylan	Brock University	P15
Liu, Fushan	University of Guelph	C6
Ma, Fengshan	University of Waterloo	B6
Machado, Cibele	University of Guelph	
Magnotta, Mary	Brock University	P16
Marsolais, Frédéric	Agriculture & Agri-Food Canada	A2
Mathur, Jaideep	University of Guelph	P28
McCabe, Shauna	University of Waterloo	A4

McCartney, Andrew	University of Guelph	B4, PP2
McDonald, Allison	University of Toronto at Scarborough	P17
McIntyre, Bonnie	Wilfrid Laurier University	
McNaughton, Amy	Wilfrid Laurier University	
Menassa, Rima	Agriculture & Agri-Food Canada	P18
Meyer, Chris	University of Waterloo	A1
Micallef, Barry	University of Guelph	D3, P1
Moffatt, Barb	University of Waterloo	A4, P25
Mullen, Robert	Molecular and Cellular Biology University of Guelph	PP2, A3, B3 B4, C1, P13
Murata, Jun	Brock University	P16, P19
Murray, Jeremy	Agriculture & Agri-Food Canada	P20
Park, Eunmi	University of Waterloo	
Payne, Chris	University of Western Ontario	
Pepper, Alicia	Wilfrid Laurier University	P21
Peterson, Carol	University of Waterloo	A1, P30
Pinhero, Reena	University of Guelph	P22
Possmayer, Marc	University of Western Ontario	
Reid, Alexandra	Agriculture & Agri-Food Canada	
Renton, Jeff	Nipissing University	
Richardson, Lynn	Wilfrid Laurier University	P23
Rivoal, Jean	Université de Montréal	A6, P7
Robison, Mary	University of Guelph	P24
Rosloski, Sarah	University of Western Ontario	B2

Saini, Hargurdeep	Université de Montréal	C2, C3
Salt, Jennifer	University of Toronto	
Schoor, Sarah	University of Waterloo	P25
Sederias, Joana	York University	P26
Shelp, Barry	University of Guelph	A5, P2, P27
Sinclair, Alison	University of Guelph	P28
Slater, Susan	University of Guelph	
Smith, Graham	University of Guelph	B4
Smith, Matt	Wilfrid Laurier University	P23, P25, P29
Szyszka, Beth	University of Western Ontario	D4
Taylor, Jeff	Slippery Rock University, PA	D1
Taylor, Marston	Wilfrid Laurier University	
Tetlow, Ian	University of Guelph	C5, C6, C7
Todorvic, Biljana	University of Waterloo	
Urquhart, William	University of Toronto	B1
Vanlerberghe, Greg	University of Toronto at Scarborough	P17
Waduwara, Ishari	University of Waterloo	P30
Waller, Jeff	Queen's University	A3
Wang, Tzann-Wei	University of Waterloo	B6
Wilson, Ken	University of Saskatchewan	D6
Wolyn, David	University of Gueph	P24
Yee, Donna	University of Toronto	
Zerzour, Rabah	IRBV, Montreal	D2