58th Annual Meeting
58ème Conference Annuelle

Lipids: The Membrane and Beyond

June 14-17, 2015
Dalhousie University, Halifax, Nova Scotia

Keynote Speakers
Andrea Ballabio – Telethon Institute of Genetics and Medicine, Italy
Lukas K. Tamm – University of Virginia School of Medicine
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<td><strong>Sunday - June 14, 2015</strong></td>
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<td>Keynote Lecture : Andrea Ballabio</td>
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<td>6:00 - 7:00 pm</td>
<td>Session 1: Cellular Lipid Transport Chair: Roger McLeod</td>
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<td>7:00 - 9:30 pm</td>
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<td>8:30 - 11:45 am</td>
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<td>Session 7: Advanced Techniques Chair: Neale Ridgway</td>
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# 58th Annual Meeting

**Lipids: The Membrane and Beyond**

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We are delighted to welcome you to the 58th Annual Meeting of the Canadian Society for Molecular Biosciences (CSMB) on “Lipids: The Membrane and Beyond” from June 14-17, 2015 in the picturesque and bustling port city of Halifax, Nova Scotia on the Atlantic Ocean. For the many non-Haligonians in the crowd, thank you for taking time, effort and hard-earned grant dollars to travel to Halifax!

Our goal as we put our scientific program together was to cover a broad spectrum of topics from the wide field of lipids-oriented research, a field with a long history as a major research focus both here at Dalhousie and throughout Canada. We aim to encourage and foster discussions and collaborations among scientists from the diverse corners of lipids-oriented research, and at levels from undergraduate student through to PI. We hope that you will come away with both an appreciation of broader aspects of our shared field and exposure to some cool science from your own area of research.

Our scientific sessions have been assembled by individual members of the organizing committee – we are grateful for their hard work in this regard, and are delighted to have so many outstanding scientists willing to travel to Halifax and tell us about the latest work going on in their labs. Thanks also to all the organizing committee for their hours of work behind the scenes. Without this spirit of volunteerism, this meeting simply would not have taken place.

We would also like to encourage all presenting attendees to consider submitting a minireview or original research article for the special issue of *Biochemistry and Cell Biology (BCB)* that will be devoted to our meeting. These articles will undergo the full, rigorous peer-review of *BCB*. Space will be limited, so we would ask that research groups with multiple attendees consolidate their submissions. Further detail will be provided to all attendees by our guest editors, Drs. Barbara Karten and Neale Ridgway.

Finally, we are very grateful to the numerous sponsors, which include a variety of Dalhousie University sources, journals, granting agencies, vendors and the generous Suraj Manrao. All of this support has helped us to keep the meeting affordable! This has also allowed us to offer a number of trainee poster and travel awards, over and above those that the CSMB itself is able to provide. Stay tuned for our 2015 awards presentations at the conference banquet, an event that is sure to be a highlight, at historic Pier 21 overlooking the Halifax harbour.

Barbara Karten and Jan Rainey, Conference Co-Chairs

On behalf of the organizing committee:
Roy Duncan  Thomas Pulinilkunnil
Petra Kienesberger  Neale Ridgway
Roger McLeod  Aarnoud van der Spoel
Dear CSMB Meeting Attendees,

I am delighted to welcome you to Dalhousie University for the 58th Annual Meeting of the Canadian Society for Molecular Biosciences. It is our privilege to host this distinguished group of researchers from across Canada, the United States and Europe. Thank you for taking the time to travel to our medical campus in downtown Halifax. I hope you will agree with the sentiment that Halifax is Canada’s "best-kept secret" – we are proud to be both centrally located and to play a key role in the community of this bustling hub of Atlantic Canada.

As befits our status as Atlantic Canada’s most research-intensive university, with membership in the U15 Group of Canadian Research Universities, I am sure that your time here will be scientifically stimulating. Beyond the bounds of your meeting itself, please note that Dalhousie has a rich history as one of Canada’s oldest universities. I hope you will have a chance to wander around our historic, and expanding, campus. Beyond our campus, I hope you can enjoy the world’s longest harbor front boardwalk and see some of the natural beauty. Dalhousie is always looking for the brightest and the best, and we’d certainly love to welcome you back in future.

With warm wishes,

[Signature]

Richard Florizone
President and Vice-Chancellor
Canadian Society for Molecular Biosciences  
Société canadienne pour les Biosciences moléculaires

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Université de Montréal

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Dr. James Davie  
University of Manitoba

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Hospital for Sick Children

Mr. Mustapha Lhor  
Université Laval

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University of Toronto

Dr. Frances Sharom  
University of Guelph

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Ottawa, ON K1G 4S3  
Canada  
Tel.: 613 421 7229  
Fax.: 613 421 9811  
Email: contact@csmb-scbm.ca

Dear participant of the 58th Annual CSMB Conference,

In the name of the board I wish you a warm welcome to Halifax. I am convinced that you will enjoy the scientific and social program of this conference. I would like to thank the conference organizers Jan Rainey and Barbara Karten and their colleagues on the organizing committee for putting together such a strong program featuring leading Canadian researchers and international experts in the field of lipid and membrane biology.

We are grateful that you have decided to join us at this annual meeting helping us to fulfill the society’s mandate to advance and promote the molecular understanding of biology. The CSBM has advanced on several topics and the advocacy work continues to be our highest priority. We have also revised our website and the trainee members on the board do now play key roles for the operation of our society. This is most important, since training and encouraging the next generation of scientists remains one of our key mandates, especially in times when budgetary challenges are preoccupying for many of our members.

1. **Website and social media.** Our website has just been updated, giving it a much more appealing and contemporary look, check it out at http://csmb-scbm.ca/. We are now also well present in social media (Facebook, Twitter) hoping to provide value, rapid communication of information and opportunities for feedback to our members.

2. **Trainee involvement.** The newest additions to the board of the CSMB are representatives of postdocs and graduate students. Their initiatives aimed at increasing trainee implication take more and more place in our work, as they should. The board has also decided to increase its support for scientific and social events organized by graduate students and postdocs. These are very worthy investments into the advancement of the next generation of scientists who will hopefully get involved in the society and help us shape what we do for them.
3. Advocacy work. The next federal election is due in October 2015. All political parties are in the process of preparing their election platforms and the next federal budget will be presented soon. It is therefore most important that the society as well as individual scientists make their voices heard now in order to educate decision makers about the challenges faced by the scientific community. In December 2014, we wrote to the Prime Minister of Canada as well as to the Ministers of Industry and Health, to the leaders of the opposition parties, and to the spokespersons for Science and Technology of the NDP and the Liberal Party of Canada. The key propositions of the CSMB remain steady, 3% annual increases of CIHR and NSERC budgets targeted to open competitions, continuation of support for the CFI, reinstatement of the NSERC RTI grant program and a marked increase of the indirect cost program supporting research at institutions. We have received encouraging feedback and we had a very interesting conversation in person with Ted Hsu, the Liberal critic for Science and Technology. We have also received meaningful responses from the Office of the Prime Minister, the Minister of Industry James Moore, Minister of State (Science and technology) Ed Holder and from the leader of the opposition Thomas Mulcair (NDP).

It transpires from these interactions that politicians are simply not aware (enough) of the fact that there are problems concerning research funding in Canada. It is urgent to change this situation! We strongly encourage all our members, especially trainees who are the most impacted by this situation, to contact their members of parliament making them aware of the constraints of research funding leading to losses of jobs, research productivity and innovation. The full documentation of the exchanges with politicians as well as suggestions to our members are available on our website in the Advocacy section.

We have also initiated a dialogue with other scientific societies in Canada aimed at speaking with a coordinated voice in increasing numbers with similar messages. Our exchanges with the Canadian Society of Microbiologists, the Canadian Society of Immunology, the Canadian Society of Pharmacology and Therapeutics and the Canadian Council of University Biology Chairs are very encouraging. We should soon be able to speak up for scientists and trainees in Canada in a coordinated fashion.

4. International conference next year in Vancouver. Preparations and networking are intensifying for the international conference in Vancouver in 2016. We organize this conference conjointly with the International Union of Biochemistry and Molecular Biology (IUBMB) and the Pan-American Association for Biochemistry and Molecular Biology (PABMB). We expect more than 1000 participants at this conference! We hope that many of you will participate at this landmark event that will greatly increase the visibility of Canadian molecular biosciences at the international level.

I hope that our various activities continue to meet your interests and that they will convince you to renew your membership or join if you are not a member yet! We would appreciate it if you encourage and motivate your colleagues to join the CSMB. It would be equally important if you made your voice heard towards politicians; as citizens of this country, you have all the liberty to do so. Strength is in numbers, please use this opportunity to help putting funding for research in Canada on the political map! Thank you for your consideration and support for the CSMB.

Best regards,

Christian Baron
Université de Montréal
President of the Canadian Society for Molecular Biosciences
Conference sites:

Main conference:
Sir Charles Tupper Medical Building: 5850 College Street, Halifax, B3H 4R2

Monday night dinner:
Life Sciences Research Institute (LSRI): 1344 Summer Street, Halifax, B3H 4R2

Tuesday night banquet:
Canadian Museum of Immigration at Pier 21: 1055 Marginal Road, Halifax, B3H 4P7

Hotel and Residences:
Atlantica Hotel: 1980 Robie Street, Halifax, B3H 3G5
Howe Hall: 6230 Coburg Road, Halifax, B3H 4R2
LeMarchant Place: 1246 LeMarchant Street, Halifax, B3H 4R2
From the Atlantica Hotel to the Tupper Building:

From the Atlantica Hotel, just walk down Robie Street to the second traffic light at the major intersection of Robie Street and Spring Garden Road/Coburg Road (Halifax seems to like having several names for the same street). Cross Robie Street here. Walk further along Robie to the next street, which is College Street, and turn left. The Tupper Building is the tall building to your right.

The Atlantica also provides complimentary limousine service to Dalhousie in peak hours.
From the Dal Residences to the Tupper Building:

Monday night dinner:

The Monday night dinner is a buffet in the Atrium of the new Life Sciences Research Institute of Dalhousie University.

The LSRI is connected to the Tupper Building by pedway, but access limitations make it easier to walk around the building. Construction on College St. may make University Ave. a better bet for walking.
Tuesday night banquet:

The banquet on Tuesday night will take place in the Canadian Museum of Immigration at Pier 21.

The exhibits are open at 5.30 pm on Tuesday night. Museum staff will be present to answer questions, and there will be a short video about the history of Pier 21 for all who are interested. We will have a reception in the museum, and dinner in the adjacent banquet hall.

Your conference badge will give you free entrance to the museum throughout the conference and for another three days after the conference.

Walking directions to Pier 21 banquet (~1.8 km, 20 min): Exit from the Tupper Link towards University Avenue and turn left on University. University becomes Morris St. at South Park St. – continue on Morris until it ends at Lower Water St. Turn right onto Lower Water until it ends at Terminal Rd. (You can also continue towards the water onto the Harbourfront Boardwalk at this point and turn right on the boardwalk, ending up at the Market.) Turn left on Terminal Rd. You should see the Halifax Seaport Farmer’s Market as Terminal curves to become Marginal Rd. Walk past the Market and the Garrison Brewery and Pier 21 will be on your left at 1055 Marginal Rd.
Organizing Committee/Comité Organisateur

Scientific Committee:

Dr. Barbara Karten, Co-Chair, Dalhousie University
Dr. Jan Rainey, Co-Chair, Dalhousie University
Dr. Roy Duncan, Dalhousie University
Dr. Petra Kienesberger, Dalhousie University
Dr. Roger McLeod, Dalhousie University
Dr. Thomas Pulinilkunnil, Dalhousie University
Dr. Neale Ridgway, Dalhousie University
Dr. Aarnoud van der Spoel, Dalhousie University

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Dr. Philip Hieter
Dr. John Orlowski
Dr. Jan Rainey

Trainee Representatives Mr. Mustapha Lhor
Dr. Anastassia Voronova

Bulletin Editor/ Editeur du bulletin Dr. Frances Sharom
Dear Colleague,

We are very pleased to hear that you are attending the Canadian Society for Molecular Biosciences 58th Annual Meeting here in Halifax on “Lipids: The Membrane and Beyond”. As in the past, the conference will be accompanied by a special issue of the journal *Biochemistry and Cell Biology* published by NRC Research Press.

We invite you to submit a research manuscript or review article related to your presentation at this meeting for this special issue. Over the years the issues of *Biochemistry and Cell Biology* associated with the CSMB annual meetings have been highly cited and become a permanent record of the conference. We hope that you will accept our invitation to contribute. Manuscripts should be submitted by October 2016 following the standard submission guidelines of the journal which can be found at:

http://www.nrcresearchpress.com/journal/bcb

Submissions will undergo the standard rigorous peer review process of the journal.

We hope that you will be able to contribute an article. It would be helpful to hear from you as soon as possible to know whether you will participate, so that we can ensure that your manuscript is directed to the special issue.

Sincerely,

Neale D. Ridgway, Ph.D.
Barbara Karten, Ph.D.
Co-editors of BCB Special Issue “Lipids: The Membrane and Beyond”
Conference Supporters

The following organizations and companies have generously provided support for this conference and are thanked for their valuable contributions and their on going support. Les entreprises et organismes suivants ont généreusement contribué à la tenue de ce congrès. Nous désirons les remercier sincèrement de leur collaboration et de leur appui soutenu.

**Academic Sponsors**

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58th Annual Meeting CSMB
Lipids: The Membrane and Beyond
June 14 – 17, 2015
Theatre B, Sir Charles Tupper Medical Building, Dalhousie University
5850 College Street, Halifax, NS B3H 4R2

Sunday, June 14, 2015

3.00 pm CSMB Registration Opens – Medical Student Lounge, 2nd Floor, Tupper Link

Sunday, June 14, 2015, Theatre B, Tupper Link

5.50 pm Opening Remarks

6.00 pm Keynote Lecture:
Dr. Andrea Ballabio (Telethon Institute of Genetics and Medicine, Italy)
“The lysosome as a signaling hub”

7.00 pm – 9.30 pm
Session 1: Cellular Lipid Transport, Chair: Roger McLeod

7.00 pm Frederick Maxfield (Weill Cornell Medical College, New York)
“Intracellular Cholesterol Transport”

7.40 pm Cameron Scott (University of Geneva, Switzerland)
“Wnt directs the endosomal flux of LDL-derived cholesterol and lipid droplet homeostasis”

7.55 pm Greg Fairn (St. Michael’s Hospital, Toronto)
“Complementary probes reveal that phosphatidyserine is required for the proper transbilayer distribution of cholesterol”

8.35 pm Stacy Horner (Duke University, Durham)
“Proteomic analysis of mitochondrial-associated ER membranes during RNA virus infection reveals dynamic changes in protein and organelle trafficking”

8.50 pm Barbara Karten (Dalhousie University, Halifax)
“Endosomal cholesterol trafficking to mitochondria and mitochondrial function”

9.30 pm Reception – Medical Student Lounge, 2nd Floor, Tupper Link

Monday, June 15, 2015, Theatre B, Tupper Link

8.30 am - 11.30 am
Session 2: Lipid Metabolism, Chair: Aarnoud van der Spoel

8.30 am Tobias Hartmann (Saarland University, Germany)
“Alzheimer’s disease amyloid precursor protein (APP), a complex lipid regulator and lipid sensor system”

9.10 am Ryan Bradley (University of Waterloo, Waterloo)
“AGPAT4 is a mitochondrial lysophosphatidic acid acyltransferase that regulates mouse brain phosphatidylinositol levels and learning and memory”

9.25 am Jerry Chipuk (Mount Sinai Hospital, New York)
“Outer mitochondrial membrane composition and shape regulate commitment to apoptosis”
10.05 am  **Sabri Rial** (UQAM, Montréal)  
“The medium chain fatty acid hexanoate reduces the de novo synthesized fatty acids accumulation and improves insulin sensitivity of hepatocytes”

10.20 am  **Coffee Break - Tupper Link**

10.50 am  **Russell Bishop** (McMaster University, Hamilton)  
“Diverse roles for PagP in the palmitoylation of lipid A and phosphatidylglycerol”

11.30 am  **Louis Dacquay** (University of Ottawa, Ottawa)  
“The yeast lysine acetyltransferases NuA4 and Rtt109 inhibit the function of Osh4p”

11.45 am  **Presentation by NSERC**  
What’s new at NSERC, overview of the Discovery Grants Program, results/statistics from the most recent competition and other funding opportunities available at NSERC

12.25 pm  **Lunch - Medical Student Lounge, 2nd Floor, Tupper Link**

12.50 pm  **Poster Session and Exhibitors - Tupper Link**  
Even numbered posters present: 36-58 from 12.50-1.50 pm; 60-78 from 1.50-2.50 pm

2:15 pm  **Coffee – Tupper Link**

3.00 pm - 5.30 pm  **Session 3: Lipid Droplets, Chair: Barbara Karten**

3.00 pm  **Rudolf Zechner** (Medical University Graz, Austria)  
“The crossroads of lipolysis and lipid synthesis”

3.40 pm  **Christopher Choy** (Ryerson University, Toronto)  
“Phosphatidylinositol 3,5-bisphosphate regulates transcription factors that govern lysosomal gene expression”

3.55 pm  **Petra Kienesberger** (Dalhousie University, Saint John)  
“Novel adipokine related to metabolic dysfunction”

4.35 pm  **Louis Lapierre** (Brown University, Providence)  
“Autophagy-mediated longevity is modulated by lipoprotein biogenesis”

4.50 pm  **Robert Farese** (Harvard University, Cambridge)  
“Mechanisms of fat synthesis and storage in lipid droplets”

5.30 pm  **Dinner – LSRI Atrium**

7.30 pm - 10.00 pm  **Session 4: Membrane Fusion, Chair: Thomas Pulinilkunnal**

7.30 pm  **Leonid Chernomordik** (NIH, Bethesda)  
“Cell-cell fusion in development”

8.10 pm  **Christopher Brett** (Concordia University, Montréal)  
“Intermediates of native organelle membrane fusion accommodate two outcomes”

8.25 pm  **Jeffrey Lee** (University of Toronto, Toronto)  
“Structural analysis of viral-host membrane fusion”

9.05 pm  **Sebastian Fiedler** (University of Toronto, Toronto)  
“Vesicle leakage reflects lipid selectivity of antimicrobial lipopeptides from bacillus subtilis”

9.20 pm  **Roy Duncan** (Dalhousie University, Halifax)  
“Reovirus FAST proteins: small modular membrane fusion machines”
Tuesday, June 16, 2015, Theatre B, Tupper Link

8.30 am - 11.45 am  
**Session 5: Protein-Lipid Interactions, Chair: Jan Rainey**

8.30 am  **Roger McLeod** (Dalhousie University, Halifax)  
“Structural characterization of lipid-binding fragments of human apolipoprotein B100”

9.10 am  **Parthajit Mukherjee** (Brock University, Saint Catharines)  
“Lipid recognition and membrane binding by human wild-type oxysterol binding protein (OSBP)”

9.25 am  **Michael Oda** (Children’s Hospital Oakland Research Institute, Oakland)  
“High density lipoprotein for membrane catalyzed”

10.05 am  **Kyungsoo Shin** (Dalhousie University, Halifax)  
“Testing the potential for membrane catalyzed modification of proprotein processing specificity”

10.20 am  **Coffee Break – Tupper Link**

10.50 am  **Valerie Booth** (Memorial University, St. John’s)  
Biophysical studies of protein-lipid interactions in complex systems”

11.30 am  **Antreas Kalli** (University of Toronto, Toronto)  
Interaction of the human band 3 anion exchanger membrane protein with lipids: Insights from molecular dynamics simulations”

11.45 am  **GE Healthcare New Investigator Award Lecture**  
**Vincent Archambault** (IRIC/U de Montréal, Montréal)  
“Cell cycle control by the Greatwall-PP2A axis”

12.25 pm  **Lunch – Medical Student Lounge, 2nd Floor, Tupper Link**

12.50 pm  **Poster Session and Exhibitors – Tupper Link**  
Odd numbered posters present: 37-59 from 12.50-1.50 pm; 61-77 from 1.50-2.50 pm

2.15 pm  **Coffee – Tupper Link**

2.50 pm  **Keynote Lecture**  
**Lukas Tamm** (University of Virginia, Charlottesville)  
“Membrane fusion in neurotransmitter release and virus entry”

3.50 pm - 4.20 pm  
**Session 6: Selected Abstracts, Chair: Roy Duncan**

3.50 pm  **Costin Antonescu** (Ryerson University, Toronto)  
“The acyltransferase LYCAT regulates phosphoinositides and specific stages of endomembrane traffic”

4.05 pm  **Gerhard Multhaupt** (McGill University, Montréal)  
“Aβ-lipid interactions in mediating amyloid toxicity in Alzheimer disease”

4.20 pm  **Award Lecture: NRC Research Press Senior Investigator**  
**Michael James** (University of Alberta, Edmonton)  
“The structure of alpha-L-iduronidase and its role in mucopolysaccharidosis-I”
5.00 pm  Annual General Meeting of the CSMB - Theatre B
6.00 pm  Pier 21: Exhibits Open to Conference Attendees – Reception
7.00 pm  Pier 21: Nova Scotia-Influenced Banquet

Wednesday, June 17, 2015, Theatre B, Tupper Link

8.30 am – 11.45 am  Session 7: Advanced Techniques, Chair: Neale Ridgway

8.30 am  Steffany Bennett (University of Ottawa, Ottawa)
“Translational lipidomics: harmonizing ESI-LC-MS targeted lipidomic methodologies in clinical study of Alzheimer’s disease”

9.10 am  Phillipe-Pierre Robichaud (Université de Moncton, Moncton)
“The click chemistry probe 19-alkyne arachidonic acid appears to be a good analogue to study arachidonate-phospholipid metabolism but not eicosanoid metabolism”

9.25 am  Rosemary Cornell (Simon Fraser University, Burnaby)
“How a membrane-binding lipid compositional sensor regulates the activity of CCT”

10.05 am  Svetlana Baoukina (University of Calgary, Calgary)
“Simulation study of dynamic heterogeneity in lipid bilayers”

10.20 am  Coffee Break- Tupper Link

10.50 am  Mary Kraft (University of Illinois, Urbana)
“Investigating plasma membrane organization and its determinants with secondary ion mass spectrometry”

11.30 am  Gil Prive (University of Toronto, Toronto)
“Saposin picodiscs for lipid studies”

11.45 am  Award lecture: Young Scientist Award in Genetics
Luigi Bouchard (Université de Sherbrooke, Sherbrooke)
“Increasing evidence supporting epigenetic programming and regulation of HDL-cholesterol metabolism”

12.30  Closing remarks
Dr. Andrea Ballabio

Andrea Ballabio is the founder and director of the Telethon Institute of Genetics and Medicine (TIGEM) in Naples, Italy. He is also Professor of Medical Genetics at the Faculty of Medicine of the University of Naples “Federico II” and Visiting Professor at both Baylor College of Medicine in Houston, Texas, and at the University of Oxford, UK. Prof. Ballabio’s research interests are the elucidation of the biological mechanisms underlying genetic diseases and the development of innovative therapeutic approaches. Prof. Ballabio’s team identified numerous genes whose mutations cause human inherited diseases, leading to the discovery of their pathogenetic mechanisms. Prof. Ballabio’s current research focuses on the transcriptional regulation of lysosomal biogenesis and autophagy, on the role of the lysosome as a signaling hub, and on the mechanisms underlying lysosomal storage disorders and common neurodegenerative diseases. He has published over 290 papers in international scientific journals. Prof. Ballabio was the President of the European Society of Human Genetics and Council member of the European Molecular Biology Organization (EMBO). He is a recipient of an Advanced Investigator Grant of the European Research Council (ERC). He has received numerous national and international awards for research and culture, among which the 2007 Award of the European Society of Human Genetics. In 2007 he received the “Knighthood of the Italian Republic” by the President of Italy.

Lecture Topic:
The lysosome as a signaling hub

Recent evidence indicates that the importance of the lysosome in cell metabolism and organism physiology goes far beyond the simple disposal of cellular garbage. This dynamic organelle is situated at the crossroad of the most important cellular pathways and is involved in sensing, signaling and transcriptional mechanisms that respond to environmental cues, such as nutrients. Two main mediators of these lysosomal adaptation mechanisms are the mTORC1 kinase complex and the TFEB transcription factor. These two factors are linked in a lysosome-to-nucleus signaling pathway that provides the lysosome with the ability to adapt to extracellular cues and control its own biogenesis. Modulation of lysosomal function by acting on TFEB has a profound impact on cellular clearance and energy metabolism and is a promising therapeutic target for a large variety of disease conditions.

Dr. Lukas Tamm

Dr. Tamm is the Harrison Distinguished Professor in Molecular Physiology and Biological Physics, Vice-Chair of the Department of Molecular Physiology and Biological Physics, and Director of the Center for Membrane Biology at the University of Virginia School of Medicine. He received his Diploma in Molecular Biosciences from the University of Basel, Switzerland, in 1978, and his Ph.D. in Biophysics from the same institution in 1982 under the supervision of Joachim Seelig, using solid-state NMR techniques to characterize lipid-protein interactions. As a postdoctoral fellow in the laboratory of Harden McConnell at Stanford University (1982-1984), Dr. Tamm originated the development of supported bilayers as a new model membrane system and co-discovered lipid domains in monolayers at the air-water interface. Dr. Tamm returned to the University of Basel for six years as a Research Assistant Professor before moving to the University of Virginia (Charlottesville) in 1990. Dr. Tamm was one of the first to solve structures of integral membrane proteins by NMR and has continued to develop and exploit supported membrane systems, single molecule tracking, single vesicle
fusion technology, and NMR to explore membrane protein structures and peptide-membrane interactions. His major interests include structural dynamics of presynaptic membrane fusion, molecular mechanisms of viral membrane fusion, bacterial outer membrane protein structure and function, and the structure and dynamics of ordered lipid domains. In 2014, Dr. Tamm received the Humboldt Research Award in recognition of his fundamental discoveries and their significant impact on the field, in 2008 he was selected to join the Faculty of 1000 in Structural Biology, and in 2002 he received a MERIT award from the NIH.

Lecture Topic:
Membrane Fusion in Neurotransmitter Release and Virus Entry

Neurotransmitters are released from synaptic vesicles into the synaptic cleft between coupled neurons by highly regulated exocytosis in a matter of milliseconds or less. SNARE molecules residing in the vesicle and presynaptic target membranes catalyze membrane fusion between the two membranes after stimulation by rapid influx of calcium into the terminal of the secreting neuron. The molecular machine that drives this fast fusion reaction and its regulation by accessory proteins such as calcium binding synaptotagmin, complexin, and Munc18 will be discussed.

Abstract:
Cell cycle control by the Greatwall-PP2A axis

The eukaryotic cell cycle is regulated by reversible phosphorylation. Cyclin B-Cdk1 triggers the events of mitotic entry by the phosphorylation of multiple substrates. At mitotic exit, several of these substrates are dephosphorylated to allow the cells to return to interphase. While the role of Cyclin B-Cdk1 and its regulation in this process have been appreciated for decades, the implication of phosphatases and their regulation have come into light more recently. In animals, the Protein Phosphatase 2A in complex with its regulatory subunit B55 (PP2A-B55) plays an important role in the dephosphorylation of Cyclin B-Cdk1 substrates at mitotic exit. A loss of PP2A-B55 activity leads to defects in chromosome segregation, nuclear reassembly and cytokinesis. Work by several groups including ours has uncovered how PP2A-B55 is regulated in the cell cycle. Greatwall (Gwl) has been discovered in Drosophila as a kinase required for mitosis and meiosis. Genetic studies in flies and biochemical work in frog egg extracts have determined that Gwl is required to antagonize PP2A-B55 at mitotic entry by phosphorylating endosulfine, which then becomes a potent and specific inhibitor of PP2A-B55. A failure in this mechanism leads to mitotic collapse after nuclear envelope breakdown. While Gwl is nuclear in interphase, PP2A-B55 is mostly cytoplasmic. We have shown that Gwl suddenly relocates from the nucleus to the cytoplasm during mitotic entry, just before nuclear envelope breakdown, and that this strict control of Gwl localization is required for its function.
We are investigating the importance and the molecular mechanisms of the spatiotemporal regulation of the Gwl-PP2A module. We are also using genetic and proteomic approaches to identify the crucial substrates of PP2A-B55 that must be protected from its activity at mitotic entry and dephosphorylated by it at mitotic exit. Our work reveals fundamental molecular mechanisms regulating the cell division cycle. This knowledge serves as a basis to better understand aberrant cell division in cancer and envision new therapeutic avenues.

**NRC Research Press Senior Investigator Award**

Michael James, University of Alberta, Edmonton, AB

Dr. James is an Emeritus Distinguished University Professor in the Biochemistry Department, University of Alberta in Edmonton, Alberta, Canada. His research career extends over 47 years at the University of Alberta. He is one of the founding members of the longstanding, celebrated MRC (now CIHR) Group in Protein Structure and Function at the University of Alberta. Dr. James is a structural biologist who uses macromolecular X-ray crystallography as his primary research tool. His major areas of research interest currently are: proteolytic enzymes and their protein inhibitors; glycolytic hydrolases and the enzymic mechanisms of carbohydrate hydrolysis; and the development of antiviral agents. In addition, Dr. James’ group is involved in the Structural Genomics Consortium on Myobacterium tuberculosis. With the worldwide upsurge of antibiotic resistance to the isolates of this organism (MDR TB and XDR TB), the identification of new targets for antibiotic design against this diabolical organism is of paramount importance.

In the field of the glycosyl hydrolases, the James’ laboratory has turned their interest to lysosomal storage diseases. In particular the group has determined the structures of β- hexosaminidases A and B, the two enzymes in which mutations are behind the genetic diseases, Tay-Sachs disease and Sandhoff Disease, respectively. More recently the group has determined the structure of α-L- iduronidase the mutants of which are the cause of Mucopolysaccharidosis type I. Not only did this research determine the native structure, but also the structure of α-L-iduronidase in the presence of several different iduronyl derivatives has allowed for the determination of the enzymatic mechanism of α-L-iduronidase. Dr. James was elected a Fellow of the Royal Society of London in 1989 and a Fellow of the Royal Society of Canada in 1985. He earned his doctorate in 1966 from Oxford University where he studied under the guidance of the late Nobel Laureate Professor Dorothy Hodgkin, O.M., F.R.S.

**Abstract:**

**The Structure of Alpha-L-Iduronidase and it’s role in Mucopolysaccharidosis-I**

Lysosomal storage diseases (LSDs) are a broad class of devastating genetic diseases that collectively represent over 50 disorders. These diseases are progressive in nature as the affected individual is unable to degrade certain macromolecules. The mucopolysaccharidoses (MPSs) are a diverse group representing 11 separate genetic disorders each caused by a deficiency of a specific lysosomal hydrolase involved in the stepwise degradation of glycosaminoglycans (GAGs). As a consequence, GAGs accumulate within the lysosomes of all cells and ultimately, through mechanisms that are poorly understood, lead to progressive and debilitating symptoms. One of the MPSs, MPS I, is a rare (~1.05/100,000 births), autosomal, recessive lysosomal storage disease caused by a deficiency of lysosomal a-L-iduronidase (IDUA). The mutations in a-L-iduronidase that result in MPS I likely fall into the category of misfolding and as a result there is defective transport of IDUA out of the ER into the Golgi. Our laboratory, along with the labs of Dr. Allison Kermode at Simon Fraser University and that of Dr. Steve Withers at UBC have determined the 3D structure of human IDUA. We have also determined the structures of several inhibitors bound to hIDUA and from those data have been able to interpret a reasonable enzymatic mechanism for hIDUA. Our work has also shed light on many of the misfolding mutations that are the cause of the disease. Unexpectedly we have also observed that the glycan bound to Asn372 plays a major role in the catalytic mechanism of hIDUA.
Young Scientist Award in Genetics
Luigi Bouchard, University of Sherbrooke, Sherbrooke, QC

Dr. Bouchard holds the position of associate professor of genetics and epigenetics at the Department of Biochemistry, Faculty of Medicine and Health Sciences, Université de Sherbrooke and is head of the Department of Molecular Biology and Genetics at the university-affiliated Chicoutimi Hospital. After his Ph.D. studies in genetic epidemiology at Université Laval under the mentorship of Dr. Louis Pérusse, he completed postdoctoral fellowships in transcriptomics (Dr. Marie-Claude Volh, CHU de Québec) and epigenomics (Dr. Arturas Petronis, University of Toronto). From 2008 to 2010, he was assistant professor, Department of Medicine, Université de Montréal. Since 2009, he has been leading a research group dedicated to understanding how epigenetic mechanisms are involved in the development of obesity, diabetes and cardiovascular disease, and identifying causal epigenetically-modified genes. His group is at the forefront of this growing field of research.

With its analyses of specific obesity, diabetes and lipid candidate genes and the use of state-of-the-art analytical methods to survey a large fraction of the epigenome, this group was the first to report that maternal hyperglycemia and familial hypercholesterolemia are associated with DNA methylation changes (a central epigenetic mechanism) in several genes with many of them being involved in metabolic and cardiovascular disease pathways. He now has the goal to demonstrate that these epigenetic changes could explain why some children have an increased risk of developing obesity and diabetes, as per the Developmental Origin of Health and Disease (DOHaD) hypothesis, and to identify new markers for cardiovascular disease.

Abstract:
Increasing evidence supporting epigenetic programming and regulation of HDL-cholesterol metabolism

Atherosclerosis, the primary cause of cardiovascular disease (CVD), affects endothelial and smooth muscle cells of large arteries and is characterized by chronic low-grade inflammation. Its development is a long process sometimes beginning in early childhood with possible fetal origins. High-density lipoprotein-cholesterol (HDL-C) is known to have an athero-protective role according to its role in reverse cholesterol transport and its antioxidant, anti-inflammatory and antithrombotic properties. These all contribute to HDL’s ability to prevent coronary artery disease. However, recent clinical trials aiming to improve the CVD risk profile by increasing HDL-C levels have been unsuccessful, clearly underlying the need to better understand HDL-C metabolism and properties. Indeed, HDL-C levels in blood have a clear genetic component that remains difficult to explain with traditional genetic approaches. This has led to suggest that epigenetics could be involved in programming and regulating HDL-C metabolism.

Epigenetics refers to the regulation of DNA transcription that is independent of the DNA sequence. DNA methylation occurring at position 5’ of the cytosine pyrimidine ring is the more stable and best understood epigenetic phenomenon. DNA methylation is partially inherited but is also dynamic. More recently, new aspects of the structural complexity of HDL have been revealed with the discovery that HDL carries microRNAs with functional capabilities. MicroRNAs are small RNA molecules that bind specific messenger RNA (mRNA) to regulate gene expression and protein synthesis. They are implicated in the regulation of central metabolic pathways and considered by many as an epigenetic mechanism. Both DNA methylation and miRNAs variations have profound phenotypic effects. This conference will present examples of the most recent evidence supporting epigenetic programming and regulation of HDL-cholesterol metabolism and perspectives on the high potential of microRNAs.
Abstracts: Speakers

Session 1: Sunday, June 14, 2015  7:00 pm – 9:30 pm

Intracellular Cholesterol Transport


We have identified the sterol transport protein, StarD4, as a major cytoplasmic transporter of sterol among organelles. Using dehydroergosterol, a fluorescent sterol that mimics many of the properties of cholesterol, we have measured the rates of transport between the plasma membrane and the endocytic recycling compartment (ERC). We find that increased expression of StarD4 significantly increases the rate of sterol transport between these membranes. Knockdown of StarD4 expression by siRNA reduces sterol transport from the plasma membrane to the endoplasmic reticulum, but this also has dramatic effects on the overall lipid composition of the cells. We are using rapid inactivation of StarD4 to explore the short term effects of StarD4 depletion. We have also used an in vitro assay to measure transfer of DHE between liposomes based on FRET to dansylated lipids in acceptor liposomes. We find that the presence of certain phosphoinositides can accelerate StarD4-mediated transfer approximately 10-fold, suggesting that transfer in cells may occur selectively based on the phosphoinositide content of the organelles. In separate studies, we are looking at pharmacological correction of the dysfunctional egress of cholesterol from Late Endosomes and LysoSomes (LE/Ly) in Niemann-Pick C disease. We have found that treatment of cells with histone deacetylase inhibitors can correct the protein folding defect for many mutants of the NPC1 protein, and we are exploring the mechanistic basis for this while also participating in a clinical trial of an FDA approved histone deacetylase inhibitor in NPC1 patients. References: Maxfield, F.R. and Wüstner, D. (2012) Analysis of cholesterol trafficking with fluorescent probes. Methods in Cell Biology, 108: 367-393. PMID: 22325611. PMC3626500. Iaea, D.B. and Maxfield, F.R. (2015) Cholesterol trafficking and distribution. Essays in Biochemistry 15: 43-55. Mesmin, B., et al. (2011) STARD4 abundance regulates sterol transport and sensing. Mol Biol Cell, 22: 4004-4015. PMCID: PMC3204063Pipalia, N.H., et al. (2011) Histone deacetylase inhibitor treatment dramatically reduces cholesterol accumulation in Niemann-Pick Type C1 mutant human fibroblasts. Proc. Natl. Acad. Sci., USA, 108: 5620-5625. PMCID: PMC3078401

Wnt directs the endosomal flux of LDL-derived cholesterol and lipid homeostasis

Cameron C. Scott, Vossio, S., Vacca, F., Snijder, B., Larios, J., Schaad, O., Guex, N., Kuznetsov, D., Martin, O., Chambon, M., Turcatti, G., Pelkmans, L. and Gruenberg, J. ‘Department of Biochemistry, University of Geneva, ‘Faculty of Sciences, Institute of Molecular Life Sciences, University of Zurich, ‘Vital-IT group, Swiss Institute of Bioinformatics, University of Lausanne, ‘Biomolecular Screening Facility, SV-PTECH-PTCB, Swiss Federal Institute of Technology (EPFL)

The Wnt pathway, which controls crucial steps of the development and differentiation programs, has been proposed to influence lipid storage and homeostasis. In this paper, using an unbiased strategy based on high content genome-wide RNAi screens that monitored lipid distribution and amounts, we find that Wnt3a regulates cellular cholesterol. We show that Wnt3a stimulates the production of lipid droplets, and that this stimulation strictly depends on endocytosed, LDL-derived cholesterol and on functional early and late endosomes. We also show that Wnt signaling itself controls cholesterol endocytosis and flux along the endosomal pathway, which in turn modulates cellular lipid homeostasis. These results underscore the importance of endosome functions for LD formation and reveal a previously unknown regulatory mechanism of the cellular programs controlling lipid storage and endosome transport under the control of Wnt signaling.

Complementary probes reveal that phosphatidylserine is required for the proper transbilayer distribution of cholesterol

Greg Fainr, ‘Keenan Research Centre for Biomedical Science, St. Michael's Hospital, ‘Departments of Biochemistry and Surgery, University of Toronto

Cholesterol is an essential component of metazoan cellular membranes as it helps to maintain the structural integrity and fluidity of the plasma membrane. Recently, we have developed a cholesterol biosensor, termed D4H, based on the fourth domain of Clostridium perfringens theta-toxin, which recognizes cholesterol in the cytosolic leaflet of the plasma membrane and organelles. Predictably, the D4H probe disassociates from the plasma membrane upon cholesterol extraction and after perturbations in cellular cholesterol trafficking. When used in combination with a recombinant version of the biosensor, we show that plasmaemmal phosphatidylserine is essential for retaining cholesterol in the cytosolic leaflet of the plasma membrane. Our in vitro experiments reveal that 1-stearoy-2-oleoyl phosphatidylserine (SOPS) can induce phase separation in cholesterol-containing giant unilamellar vesicles and effectively shield cholesterol from cholesterol oxidase. These results, together with past findings, suggest that cholesterol and SOPs are enriched together in nanodomains such as caveolae. Current experiments involve characterizing the presence of cholesterol and phosphatidylserine enriched nanodomain in the inner leaflet of the plasma membrane using high
resolution immuno electron microscopy. Finally, we are investigating how sub-lethal concentrations of staurosporine and related small molecules cause the internalization of plasmalemmal cholesterol and phosphatidylinerse.

**Proteomic analysis of mitochondrial-associated ER membranes during RNA virus infection reveals dynamic changes in protein and organelle trafficking**

Stacy M. Horner\(^1\), Vazquez, C.\(^1\) and Beachboard, D.\(^1\) \(^1\)Department of Molecular Genetics and Microbiology, Duke University Medical Center, \(^2\)Department of Medicine, Duke University Medical Center

Antiviral innate immunity to RNA virus infection is organized between the ER and mitochondria on a subdomain of the ER called the mitochondrial-associated ER membrane (MAM). MAVS is the host protein that transmits this antiviral signaling, and it serves as a platform for signaling complexes assembling on the MAM in association with mitochondria and peroxisomes. To identify components that regulate the formation of these MAVS signaling complexes on the MAM, we characterized the proteome of MAM, ER, and cytosol from cells infected with either chronic (hepatitis C) or acute (Sendai) RNA virus infections, as well as mock-infected cells. We used comparative analysis of protein trafficking dynamics during both chronic and acute viral infection to identify the proteins and biochemical pathways recruited into or away from the MAM during antiviral signaling. In addition, by using this proteomics approach, we identified several new MAVS-interacting proteins and regulators of antiviral signaling. Further, our results reveal a dynamic cross-talk between subcellular compartments during both acute and chronic RNA virus infection. Overall, our results demonstrate the importance of the MAM as a central platform that coordinates innate immune signaling to initiate immunity against RNA virus infection.

**Endosomal cholesterol trafficking to mitochondria and mitochondrial function**

Barbara Karten\(^1\), Kennedy, BE.\(^1\), Madreiter, CT.\(^2\), Vishnu, N.\(^2\), Malli, R.\(^2\), Hundert, AS.\(^3\), Graier, WF.\(^2\) and Weaver, ICG.\(^3\) \(^1\)Department of Biochemistry and Molecular Biology, Dalhousie University, \(^2\)Institute of Molecular Biology and Biochemistry, Medical University Graz, \(^3\)Department of Psychology and Neuroscience, Dalhousie University

Mitochondria are relatively cholesterol-poor compared to other cellular membranes, and are, therefore, highly sensitive to changes in their cholesterol content. Changes in mitochondrial cholesterol are associated with alterations in fluidity and permeability of mitochondrial membranes, and with alterations in mitochondrial function. Elevated mitochondrial cholesterol levels have been observed in several pathological conditions, including certain neurodegenerative diseases. Niemann-Pick Type C disease is an autosomal recessive neurodegenerative disorder caused by loss-of-function mutations in Niemann-Pick Type C1 (NPC1) or NPC2. The late endosomal NPC1 and NPC2 proteins functionally interact to mediate cholesterol transport from endosomes to the endoplasmic reticulum and plasma membrane. Defects in either NPC1 or NPC2 cause accumulation of unesterified cholesterol in late endosomes and severe imbalances in cellular cholesterol homeostasis. In addition, loss of NPC1, but not of NPC2, leads to elevated mitochondrial cholesterol levels. We have used investigated endosome-to-mitochondria cholesterol trafficking and the consequences of altered mitochondrial cholesterol in cultured cells and characterized energy metabolism in brain tissue of NPC1-deficient mice. Our findings show that cholesterol transport from endosomes to mitochondria is mediated by NPC2 and the late endosomal transmembrane protein Stard3, while NPC1 is not required for this pathway. Increased mitochondrial cholesterol levels were associated with deficiencies in mitochondrial respiration and activation of antioxidant response systems. NPC1-deficient murine brain showed alterations in glucose and amino acid metabolism, increased antioxidant systems, as well as alterations in one-carbon metabolism and DNA methylation even before neuronal cell death. The mechanisms leading to these alterations and their role in NPC disease pathogenesis require further investigation, but may provide a basis for additional intervention strategies for NPC disease.
Alzheimer’s Disease Amyloid Precursor Protein (APP), a Complex Lipid Regulator and Lipid Sensor System

Tobias Hartmann, Deutsches Institut fuer Demenzpraevention, Saarland University, Germany

APP is infamous for its role in Alzheimer’s disease due to overproduction of a small proteolytic APP breakdown product the amyloid β (Aβ). In contrast to this pathological role, Aβ has a significant function in lipid homeostasis which, as recent clinical data suggest, might hold a clue for treatment of Alzheimer’s disease. Aβ and another APP breakdown product, the APP intracellular domain (AICD), function as very potent suppressors and activators of a range of key lipid enzymes, either by direct interaction or by transcriptional regulation. Knock-out of APP revealed that this activity represents an essential physiological function of APP, the only ubiquitous essential physiological function for Aβ known to date. APP turnover into Aβ and AICD chiefly depends on fatty acid chain length, unsaturation, positioning of double bonds (n-3/n-6, cis/trans), sterol and sphingolipid content in membranes – and especially in the brain. Especiﬁcally interesting in this context are gangliosides, because some of them show the strongest ever recorded stimulus in Aβ production, while others reduce Aβ levels. In response to lipid triggered alteration in Aβ and AICD levels, APP regulates in feedback control the activity of several main lipid metabolic pathways, including sterol, sphingomyelin, plasmalogens and gangliosides. Again, gangliosides are very interesting, as not only the total ganglioside synthesis, but the ratio of major brain gangliosides is controlled as well. For this, thea- and β-series homoeostasis is targeted via Aβ mediated inhibition and AICD controlled expression of the GD3-synthase, resulting in an efﬁcient shift in ganglioside composition. Taken together, following a lipid challenge, APP lipid homeostasis aims at adjusting membrane composition to achieve physiological lipid levels. One consequence of this is that Aβ levels should be reduced, but this fails in Alzheimer’s disease. Based on this knowledge the generation of lipid therapy in Alzheimer’s disease has been attempted. Suitable lipid based treatments strongly reduce Aβ production and increase neuroprotection in animal studies. The ﬁrst human clinical trials show promising results, including increased memory performance.

AGPAT4 is a mitochondrial lysophosphatidic acid acyltransferase that regulates mouse brain phosphatidylinositol levels and learning and memory

Ryan Bradley 1, Marvyn, P.M. 1, Aristizabal Henao, J.J. 1, Mardian, E.B. 1, Bloemberg, D.A. 1, George, S. 1, Aucoin, M.G. 1, Quadrilatero, J. 1, Stark, K.D. 2 and Duncan, R.E. 1 1Department of Kinesiology, University of Waterloo, 2Department of Chemical Engineering, University of Waterloo

The acylglycerophosphate acyltransferase/lysophosphatic acid acyltransferase (AGPAT/LPAAT) family is a group of homologous proteins that function in phospholipid synthesis and remodeling through the acyl-CoA-dependent acylation of lysophospholipids. Some utilize lysophosphatic acid (LPA) as a primary acyl acceptor, and therefore act as true AGPAT/LPAAT enzymes in the synthesis of phosphatidic acid (PA). Others have been reclassiﬁed based on substrate preference for alternative lysophospholipids. AGPAT4 has not yet been fully characterized with regards to subcellular localization, substrate speciﬁcity, or physiological function. In agreement with others, we found that AGPAT4 is most highly expressed in the brain. However, we discovered that AGPAT4 predominantly localizes to the outer mitochondrial membrane, and has in vitro activity with lysophosphatic acid as an acyl acceptor but not lysophosphatidylethanolamine, lysophosphatidylcholine, lysophosphatidylserine, lysophosphatidylinositol, lysophosphatidylglycerol, monosaccardiolipin, or dilsaccardiolipin, and therefore most accurately should remain classiﬁed as an AGPAT/LPAAT. We examined the effect of in vivo AGPAT4 overexpression in Sf9 insect cells and found, surprisingly, no signiﬁcant effect on phosphatidic acid levels, but a signiﬁcant increase in phosphatidylinositol PI). We found that in the brains of Agpat4-/- mice, there was no reduction of PA levels, but AGPAT4 gene ablation did signiﬁcantly reduce total brain PI levels by >50%. Behavioural testing of AGPAT4 mice in the Morris Water Maze indicated that Agpat4-/- mice had signiﬁcantly impaired memory and spatial learning ability compared to wildtype controls. This work indicates the importance of AGPAT4 in supporting synthesis of brain PI, which is known to be involved in neurotransmission and neuroplasticity.

Outer mitochondrial membrane composition and shape regulate commitment to apoptosis

Jerry Edward Chipuk Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai

The mitochondrial pathway of apoptosis is engaged following developmental cues, irreparable cellular stress, or exposure to chemotherapeutics. In order for apoptosis to initiate, pro-apoptotic BCL-2 proteins must converge upon the outer mitochondrial membrane (OMM) to induce mitochondrial outer membrane permeabilization (MOMP) and cytochrome c release. BAK and BAX are the key pro-apoptotic BCL-2 proteins that activate and oligomerize within the OMM to induce MOMP, but little is known about how mitochondrial membranes control BAK/BAX function. To better understand the role of membranes in BAK/BAX-dependent MOMP, my laboratory developed numerous biochemically-defined OMM and cellular systems that speciﬁcally examine BAK/BAX function. Using these models, my laboratory described an active role for mitochondrial - heterotypic membrane interactions in maintaining mitochondrial sensitivity to BAX/BAX-dependent apoptosis. We identiﬁed that neutral sphingomyelinases regulate mitochondrial sensitivity to pro-apoptotic BCL-2 proteins; and that sphingolipid metabolism contributes two hydrophobic species, sphingosine-1-PO4 and hexadecenal, which are directly required by BAK and BAX to permeabilize the OMM and initiate apoptosis, respectively. Furthermore, we observed for many years that increased membrane curvature negatively regulates BAX-dependent MOMP. Based on these observations, we developed a new research program examining the impact of mitochondrial size on conformation changes within BAX that are essential for MOMP. These investigations revealed that mitochondrial shape is
responsible for the stable release of alpha helix 9 from the core globular structure of BAX. Together, these data suggest that mitochondrial composition and shape directly govern BAK/BAX-dependent MOMP and apoptosis, and reveal novel pharmaceutical targets to regulate cellular sensitivity to apoptosis.

The medium chain fatty acid hexanoate reduces the de novo synthesized fatty acids accumulation and improves insulin sensitivity of hepatocytes
Sabri Ahmed Rial1,2, Vivier, S.1, Benchouchou, M.1, Rafrafi, S.1, Veilleux, H.1 and Mounier, C.1,2,1 Sciences biologiques, UQÂM, Canada, 1Biomed UQAM-UQTR-INRS/AF, Canada, 2Université de Lille 1, France

Objectives: Evaluate the impact of hexanoate (C6) on the hepatic lipid metabolism and insulin sensitivity. Our previous studies showed that C6 may inhibit de novo lipogenesis (DNL) by decreasing FAS expression, one of the key lipogenic enzymes. Methods: Effects on DNL: HepG2 cells and primary rat hepatocytes were incubated for 24h with insulin (I), triiodothyronine (T3) and C6 in presence of [14C]-acetate. 14C incorporation in TG was quantified by thin layer chromatography. Expression levels of FASN and ACACA genes were evaluated by RT-PCR. Effects on IS: Cells were incubated for 24h with palmitate and in presence of C6 for an additional 24h, before stimulation with insulin. The phosphorylation levels of Akt, mTOR and p70s6k were measured by Western blot while glycogen and phosphatidic acid (PA) syntheses rates were quantified using fluorometric kits. Results: C6 significantly reduces (2-fold) I and T3 induced TG synthesis in correlation with a decreased expression of FASN and ACACA. In lipotoxic conditions as well as in controls, C6 exerts a positive effect on IS. In both conditions, C6 significantly enhances the glycogen synthesis in response to I. Moreover C6 seems to induce a direct activation of the mTOR/p70s6k axis independently of PI3k/Akt. No change in PA (described as a potential activator of mTOR metabolite) levels has been associated to C6. Conclusions: Our study clearly shows that C6 inhibits I and T3-induced DNL and improves insulin sensitivity probably through a direct action on mTOR.

Diverse Roles for PagP in the Palmitoylation of Lipid A and Phosphatidylylglycerol
Russell E. Bishop Biochemistry, and M.G. deGroote Institute for Infectious Disease Research, McMaster University

PagP is an outer membrane phospholipid palmitoyltransferase of Gram-negative bacteria that functions to modulate outer membrane permeability and the host immune response to infections. E. coli PagP is a small beta-barrel enzyme with an interior palmitoyl-group binding pocket known as the hydrocarbon ruler. The phospholipid donor must migrate into the outer membrane external leaflet before diffusing laterally through the PagP beta-barrel wall at an opening known as the crenel. Crenel gating enforces regioselectivity for the phospholipid sn-1 acyl chain in order to exclude C18 acyl chains, which are primarily esterified at the sn-2 position. The main function of the hydrocarbon ruler is to exclude C14 acyl chains esterified at the sn-1 position, thus ensuring that only C16 palmitate is selected from the membrane phospholipid pool. The acceptor of the palmitate chain is lipid A, or endotoxin, an acylated and phosphorylated disaccharide of glucosamine. Lipid A binds opposite the crenel at another lateral opening in the PagP beta-barrel wall known as the embrasure. The enzyme and both substrates form a ternary complex with palmitoyltransfer catalyzed by His33 and Ser77 while Arg114 positions the phosphate head group of the phospholipid donor. Recently, we have observed that the glyceral head group of phosphatidylylglycerol (PG) can also function as a palmitate acceptor for PagP, which proportionately synthesizes both palmitoyl PG and palmitoyl lipid A in the outer membranes of S. typhimurium. A divergent homologue of PagP is present in P. aeruginosa, but this enzyme lacks the ability to palmitoylate PG and palmitoylates the distal glucosamine unit of lipid A whereas most enterobacteria palmitoylate the proximal glucosamine unit. Interestingly, some enterobacteria possess two PagP paralogues, both of which palmitoylate lipid A, but only one of which palmitoylates PG. When these bacteria palmitoylate lipid A, we speculate that palmitoyl PG fortifies the outer membrane permeability barrier, which might be either beneficial or deleterious under particular environmental conditions.

The yeast Lysine Acetyltransferases NuA4 and Rtt109 inhibit the function of Osh4p
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The yeast Oxysterol-Binding Protein (OSBP) homologue, Osh4p, is a highly conserved sterol/phosphatidylinositol-4-phosphate(PI4P)-binding protein which plays a role in lipid transport and in regulating Golgi-derived vesicle trafficking processes. These functions are driven through Osh4p lipid-binding affinities and stimulation of PI4P-phosphatases such as Sac1. However, the molecular mechanism(s) which regulate Osh4p-dependent activation of PI4P-phosphatases, and in turn vesicle trafficking, are not well understood. Evidence from high-throughput proteomic and genetic screens suggests that lysine acetylation, a reversible post-translation modification, may be involved in regulating Osh4p function. Importantly, a comparative analysis of Osh4p acetylation sites revealed the presence of acetylation on the highly conserved lysine residue, K109, which is essential for PI4P-binding and function. In this report we demonstrate that although Osh4p is acetylated on multiple residues, mutation of the highly conserved K109 residue to mimic acetylation is sufficient to prevent localization of Osh4p to the Golgi and to suppress OSH4-agonistic effect on the bypass phenotype of SEC14, an essential gene that regulates Golgi secretion function. To identify lysine acetyltransferases (KATs) involved in regulating Osh4p functions, we performed two complementary genetic screens of the entire subset of known non-essential KATs. We found that NuA4 and Rtt109 mutants exhibited synthetic dosage lethality with OSH4-overexpression as well as OSH4-dependent synthetic sickness with sec14. The result of both of these screens supports a model wherein the KAT activity of NuA4 and Rtt109 is required to inhibit the function of Osh4p. In conclusion, we postulate that lysine acetylation could be a conserved mechanism through which OSBP regulates PI4P phosphatases and subsequent vesicle
The crossroads of lipolysis and lipid synthesis

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Phosphatidylinositol 3,5-bisphosphate regulates transcription factors that govern lysosomal gene expression

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The phosphatidylinositol 3,5-bisphosphate [PtdIns(3,5)P2] is a lysosomal signaling lipid known for its role in modulating lysosomal size. Loss of PtdIns(3,5)P2 causes distinctive lysosomal swelling, a phenomenon whose exact mechanism remains incompletely understood. Here we investigate the possible role of this lysosomal biogenesis in PtdIns(3,5)P2 dependent lysosomal enlargement. The transcription factor EB (TFEB) is a master regulator of lysosomal biogenesis and is regulated by the mammalian target of rapamycin (mTORC1) in response to the cellular nutrient status. When nutrients are abundant, mTORC1 is active and phosphorylates TFEB to maintain cytosolic retention. Under cellular starvation, mTORC1 is inactive; TFEB becomes dephosphorylated and freely enters the nucleus. In turn, mTORC1 was shown to be localized and activated by PtdIns(3,5)P2. Thus, PtdIns(3,5)P2 may help repress TFEB function by maintaining an active mTORC1. To test this, we use apilimod to inhibit PIKfyve, the PtdIns(3,5)P2 synthesizing kinase. Depletion of PtdIns(3,5)P2 caused TFEB-GFP to translocate from the cytosol to the nucleus and enhanced transcription of various lysosomal genes, including cathepsin D. Additional members of the TFEB family including MITF and TFE3 also translocated to the nucleus in PIKfyve-suppressed cells. Apilimod treatment increased TFEB gel mobility similar to mTORC1 inhibition, suggesting a possible dephosphorylation of TFEB in the absence of PtdIns(3,5)P2. However, and most strikingly, mTORC1 remained active in apilimod-treated cells as reported by the phosphorylation of two mTORC1 effectors, S6K and ULK1. Thus, PtdIns(3,5)P2 appears to control TFEB-mediated lysosome gene expression, independently of mTORC1 activity. We are currently investigating how TFEB is regulated by PIKfyve and if TFEB-mediated gene expression contributes to the dramatic enlargement of lysosomes in PtdIns(3,5)P2-deficient cells.

Novel adipokine related to metabolic dysfunction

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Background: Obesity is characterized by excess adipose tissue and often leads to metabolic dysfunction, which may manifest itself in insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular disease. Maladaptive changes in adipose tissue lipid metabolism and inflammation underlie metabolic perturbations in obesity. Adipose tissue also secretes adipokines, which play an important role in the development of obesity-induced complications and/or serve as biomarkers of metabolic dysfunction. Autotaxin (ATX) is a novel adipokine that functions as lysophospholipase D, hydrolyzing circulating lysophosphatidylcholine to lysophosphatic acid (LPA), a potent bioactive signaling lipid. Although recently the ATX-LPA signaling pathway has been implicated in obesity and insulin resistance, the role of ATX in obesity and obesity-related comorbidities remains incompletely understood.

Objective: Our objective was to examine the relationship between ATX, obesity, and obesity-related metabolic dysfunction.

Results: Our data demonstrate that in humans, serum ATX positively correlated with measures of adiposity and markers of insulin resistance, including fasting glucose, insulin, and HOMA-IR. Serum levels of ATX were also increased with non-alcoholic fatty liver disease and independently associated with hepatic steatosis in obese, non-diabetic women. Consistent with these clinical data, serum ATX activity was increased in mouse models with diet-induced obesity and metabolic dysfunction when compared to lean controls and was lower in the fasted compared to the fed state. In 3T3-L1 adipocytes, ATX expression markedly increased during differentiation and secreted ATX activity was upregulated when cells were incubated in the presence of high insulin levels.

Conclusion: These findings suggest that ATX may play a role in obesity-related comorbidities and/or serve as clinically useful biomarker for obesity-induced metabolic dysfunction.

Autophagy-mediated longevity is modulated by lipoprotein biogenesis

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Autophagy-dependent longevity models in C. elegans display altered lipid metabolism, including a decrease in the intestinal production and secretion of the yolk lipoprotein vitellogenin and increased neutral lipid storage, but the contribution of intracellular lipid distribution in lifespan extension is not fully understood. Here we report that lipoprotein production, autophagy and lysosomal lipolysis are linked and modulate lifespan in a conserved fashion. We find that over-expression of vitellogenin reduces the lifespan of long-lived animals by impairing the induction of autophagic and lipophagic genes necessary for longevity. Increased vitellogenesis also results in intestinal lipid depletion in long-lived animals, demonstrating that elevated intestinal lipid storage found
in long-lived animals is primarily due to reduced vitellogenesis. Indeed, we find that silencing vitellogenin genes is sufficient to increase lifespan via autophagic and lipophagic gene induction and by redistributing lipid bound for secretion toward neutral lipid droplet storage, thereby increasing the amount of lipids available for lipophagy. Lifespan extension from reduced vitellogenesis requires transcription factors DAF-16/FOXO and HLH-30/TFEB, suggesting that the transcriptional regulation of autophagy is coordinated with lipoprotein biogenesis. Nuclear hormone receptors (NHR) NHR-49/PPARα and NHR-80/HNF4 are also required to extend lifespan under reduced vitellogenesis and enhanced lysosomal lipolysis, highlighting novel roles for these NHRs in lysosomal lipid signaling. In dietary-restricted worms and mice, the expression of vitellogenin and hepatic apolipoprotein B, respectively, are significantly reduced, suggesting a conserved link in lifespan modulation. Altogether, our study suggests that lipoprotein biogenesis is an important mechanism in aging by impairing the transcriptional induction of autophagy.

Mechanisms of Fat Synthesis and Storage in Lipid Droplets

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The synthesis and storage of neutral lipids, such as triglycerides, are crucial for both cellular and physiological energy homeostasis. When fuels are abundant, triglycerides are synthesized in the ER and subsequently stored in cytosolic organelles called lipid droplets. We are studying the molecular processes that govern the synthesis of energy storage lipids as well as their storage in and mobilization from lipid droplets. Studies on genetic factors controlling lipid droplet growth and utilization will be presented, as will relevant studies of the physiology of lipid storage.
Cell-Cell Fusion In Development

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We are most interested now in the mechanisms of cell-to-cell fusion in formation and regeneration of skeletal muscles and bones. Myoblast fusion into multinucleated myotubes is a crucial step in skeletal muscle development and regeneration. Macrophage fusion generates osteoclasts that play important role in bone remodeling. A key challenge in studying the fusion stage of multinucleated syncytium formation is to isolate the actual fusion event from processes that prepare the cells for fusion and to distinguish the proteins that are required for fusion from those required for pre-fusion stages. We accumulated the cells at the ready-to-fuse stage by blocking formation of early fusion intermediates with lysophosphatidylcholine. Lifting the block allowed us to explore a largely synchronized fusion between myoblasts and between macrophages and to identify annexins and dynamin as proteins involved in distinct and conserved fusion stages.

Our recent studies on fusion stage of dengue virus and HIV infections and on cell-to-cell fusion in muscles and bones emphasize the importance of interactions between proteins involved in fusion and different anionic lipids. Myoblast fusion into multinucleated myotubes is a crucial step in skeletal muscle development and regeneration. Macrophage fusion generates osteoclasts that play important role in bone remodeling. A key challenge in studying the fusion stage of multinucleated syncytium formation is to isolate the actual fusion event from processes that prepare the cells for fusion and to distinguish the proteins that are required for fusion from those required for pre-fusion stages. We accumulated the cells at the ready-to-fuse stage by blocking formation of early fusion intermediates with lysophosphatidylcholine. Lifting the block allowed us to explore a largely synchronized fusion between murine myoblasts and between murine macrophages. We found that initial merger of two cell membranes detected as lipid mixing involved extracellular annexins acting in a functionally redundant manner. Subsequent stages of myoblast fusion and macrophage fusion depend on dynamin activity and cell metabolism. Early and late fusion stages involved anionic lipids phosphatidylserine and PIP2. We have also studied fusion stage of cell entry by dengue virus and HIV and found that initiation of these fusion processes involve interactions between viral fusogens and anionic lipids (bis(monoacylglycerophosphate and phosphatidylserine).

Intermediates of Native Organelle Membrane Fusion Accommodate Two Outcomes

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Vaccular lysosome membrane fusion represents the last step of the autophagy and endocytosis pathways in all eukaryotic cells. It is required to expose membrane-encapsulated material to lumenal hydrolases for degradation. Results from computational modeling and reconstituted systems suggest hemifusion intermediates underlie this important bilayer fusion reaction. But these intermediates have not been resolved during fusion of native membranes. Here we imaged intermediates during homotypic vacuolar lysosome membrane fusion using light and electron microscopy. Prior to bilayer fusion apposing membranes are separated by 8 nm to accommodate trans-SNARE complex formation. Subsequent hemifusion intermediates – many of which have not been visualized during proteoliposome fusion – constitute two pathways with different outcomes controlling the quantity of membrane internalized into the lumen for degradation. We speculate that the intermediate pathway selected contributes to organelle morphology and membrane turnover.

Structural analysis of viral-host membrane fusion

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The entry of enveloped viruses requires the fusion of the viral and host membranes in order to release their genetic material into the cytoplasm for replication. This process is mediated by its surface displayed envelope glycoprotein (GP). Class I viral envelope GPs are typically comprised of two subunits: a receptor binding domain that is responsible for interactions with specific cellular receptors and a transmembrane-anchored fusion domain (TM) that drives the membrane fusion events. The TM undergoes a drastic conformational change into an energetically stable postfusion six-helix bundle to provide the energetics for fusion. While the TMs all adopt similar structures and utilize conserved mechanisms, these proteins proceed through various cellular environments. We provide a comprehensive structural analysis across multiple viral GPs that now identify general trends and signatures important in stabilizing the viral fusion process. Our studies identify new targets and provide templates for the design and development of antiviral inhibitors different than those currently in use.
Vesicle leakage reflects lipid selectivity of antimicrobial lipopeptides from Bacillus subtilis

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Cyclic lipopeptides act against a variety of pathogens and, thus, constitute highly efficient crop-protection agents. For example, commercially available pesticides contain mixtures of different Bacillus subtilis lipopeptide families, such as surfactins (SF), iturins (IT), and fengycins (FE). Besides other effects, the antimicrobial activity of these peptides is mainly mediated by permeabilizing cellular membranes. Avis and co-workers (1) correlated the minimum inhibitory concentrations (MICs) of fengycin A for several fungal phytopathogens with the respective fungal membrane compositions. They suggest that ergosterol (ERG) and anionic lipids (including phosphatidylglycerol, PG) might attenuate but phosphatidylethanolamine (PE) headgroups enhance the fungicidal activity of lipopeptides. We compare these results with those of a fluorescence-lifetime based leakage assay, varying these components one-by-one in model membranes. To evaluate the impact of FE and SF on mammalian model membranes, we also include cholesterol in our study. Our data, indeed, suggest that ergosterol as well as PG oppose FE activity, whereas SF activity is much less sensitive to ergosterol and activated by PG. This is in line with the overall selectivity for fungi of FE, and the relatively broad range of cells targeted by SF. Cholesterol strongly inhibits FE, in line with its low hemolytic activity. PE, even more so if combined with PG, inhibits FE in agreement with its overall low activity against bacteria, suggesting that PE content in fungal membranes does not relate to sensitivity against FE. In addition, we discuss the leakage mechanisms and the molecular interactions that may underlie these selectivity effects. (1) Wise, C., J. Falardeau, I. Hagberg, and T. J. Avis (2014) Phytopathology 104:1036–1041

Reovirus FAST proteins: small modular membrane fusion machines

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The reovirus fusion-associated small transmembrane (FAST) proteins are the smallest known membrane fusion proteins, and are the only example of viral membrane fusion proteins that evolved to mediate cell-cell rather than virus-cell membrane fusion. The FAST proteins do not form homotypic trans-acting complexes, but instead function from the donor membrane to drive cell-cell fusion. There are six distinct proteins in the FAST protein family, and all are bitopic membrane proteins with a very small (<40 residues) N-terminal ectodomain and a larger cytosolic endodomain separated from each other by a single transmembrane domain. Each of these domains plays an active role in the fusion process, and each has its own unique repertoire and arrangement of structural motifs. All three fusion modules contain dynamic structural motifs that alter the lamellar membrane structure to promote lipid mixing and pore formation. These motifs include novel fusion peptides, an unusual new type of lipid packing sensor, and an atypical transmembrane domain architecture. Our model of how these motifs function in a coordinated manner from both sides, and within, the membrane in which they reside to drive membrane fusion forms the basis of this presentation.
**Structural characterization of lipid-binding fragments of human apolipoprotein B100**

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Apolipoprotein (apo) B100 is a 550 kDa hydrophobic glycoprotein that forms the structural backbone for the assembly of triglyceride (TG)-rich lipoproteins. ApoB100 is predicted to contain 5 domains: an N-terminal globular 8a1 domain followed by alternating amphipathic ß-strand and a-helical regions (N-ßa1-ß1-a2-ß2-a3-C). However, the hydrophobicity and size of apoB100 have prevented experimental verification of this structural prediction. Functional studies in cell culture have shown that a region of apoB100, near the C-terminus of the ß1 domain is important in TG recruitment during VLDL assembly in hepatic cells, and that this region of apoB100 also triggers the proteasomal degradation of the protein when assembly of VLDL is inefficient. This region of apoB may mediate the expansion of the TG core of VLDL, and amino acids 1694-1880 (B1), within the ß1 domain, have been shown to bind avidly to TG in vitro. We have expressed and purified two 20 kDa fragments, B1 and B2 (amino acids 1881-2070) from near the junction between the ß1 and a2 domains in bacterial culture for structural characterization. Both proteins were modestly soluble in aqueous solution in the absence of lipid mimetics, but were soluble in the presence of lipid mimetics and lyso phospholipids. Under conditions ensuring no more than one apoB molecule per micelle, circular dichroism spectroscopy of B1 and B2 in dodecylphosphocholine (DPC) or micelles formed with three different lyso phospholipids indicated predominantly a-helical character. Moreover, preliminary NMR spectroscopy of 13C/15N-labeled B1 and B2 in DPC micelles also indicated an a-helical structure. Our studies provide the first atomic-level evidence for amphipathic a-helical structural elements in the TG-binding regions of apoB100.

**Lipid Recognition and Membrane Binding by Human Wild-type Oxysterol Binding Protein (OSBP)**

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Oxysterols are 27-carbon oxidized derivatives of cholesterol which act as major signaling molecules during the regulation of sterol metabolism. Oxysterol binding protein (OSBP) and related proteins constitute a large eukaryotic gene family which is characterized by a highly conserved C-terminal sterol-binding domain and N-terminal pleckstrin homology (PH) domain known for recognizing phosphatidylinositol 4-phosphates (PIPs) with high specificity. Recent studies using fluorescence-based in vitro transfer and binding assays have established that OSBP acts as a PIP-sterol exchanger by transporting oxysterols from ER to trans-Golgi while bringing PIP back to ER. This two-way trade is achieved by the unique ability of the sterol-binding domain to effectively bind oxysterols as well as PIP. This is further aided by ER and Golgi membrane tethering by FFAT motif and PH domain, respectively. Using Dual Polarization Interferometry (DPI), a label-free analytical approach for studying the binding kinetics of one biomolecule to another that is physically adsorbed to a surface, our group focuses on investigating the behavior of human wild-type OSBP on immobilized phospholipid bilayers. OSBP was observed to favor binding to membranes containing phosphatidylinositol-4-phosphate PI(4)P as opposed to phosphatidylcholine (PC). The lipid association profiles suggested that the protein promptly interacts with PI(4)P in a concentration-dependent manner before rapidly dissociating from the bilayer, arguably due to PI(4)P-extraction. Such an event was never observed in case of PH domain mutant of OSBP. Replacing PI(4)P with varying concentrations of cholesterol and 25-hydroxycholesterol in the adsorbed membrane showed increased binding but no signs of sterol-extraction. We are currently involved in building a kinetic model of the binding (and possible extraction) events which is crucial in developing a molecular-level description of OSBP's function.

**High Density Lipoprotein function in health and disease**

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Plasma levels of HDL cholesterol (HDL-C) are inversely associated with coronary artery disease (CAD). However, the frequency of CAD patients with normal to high HDL-C levels is sufficient to question the predictive power of HDL-C at the individual level. The anti-atherogenic nature of HDL is derived largely from its ability to efflux cholesterol from macrophage foam cells. Cholesterol efflux to HDL is wholly dependent upon the availability of lipid-poor apoA-I to initiate ABCA1-mediated cholesterol mobilization. Because the vast majority of apoA-I is already associated with HDL in plasma, the most likely source of lipid-poor apoA-I in the artery wall is the apoA-I that has disengaged from HDL. We have demonstrated that the rate of apoA-I HDL binding/displacement is an indirect measure of its cholesterol efflux capacity, as it is this reaction that generates the pool of apoA-I that serves as the primary cholesterol acceptor for cholesterol efflux within the arterial wall. We recently capitalized on this observation and developed a means of measuring the rate of apoA-I binding/displacement from HDL as a measure of HDL particle dynamic index (HDI), which is representative of HDL's plasticity. This approach is based on our understanding of the structure of lipid-free and HDL-associated apoA-I and employs electron paramagnetic resonance (EPR) spectroscopy, which is able to accurately detect spin labels in plasma and other complex fluids. By applying this approach to the evaluation of human samples, we’ve found that this measure is strongly inversely correlated with clinical states that are known risk factors from cardiovascular disease (CVD). More importantly, this measurement is independent of age and gender so presents a powerful approach to evaluating the maladaptive influence of CVD risk factors on HDL function. Through our recent work we’ve discovered that several CVD predisposing conditions – metabolic syndrome, diabetes, and chronic inflammation have substantial negative effects on HDL function.
Testing the potential for membrane catalyzed modification of proprotein processing specificity

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Apelin is a peptide ligand for a class A G-protein coupled receptor that regulates angiogenesis, the adipoinuslar axis, and cardiovascular function. Following signal peptide removal, a 55-residue proprotein (proapelin) is expected to be produced. The proprotein is further processed into the observed bioactive forms having 13, 17, or 36 residues, all of which share the proapelin C-terminal region. Dibasic residue motifs are present at each cleavage site, suggesting proprotein convertase subtilisin/kexin (PCSK)-dependent processing. In addition, 13- and 17-residue cleavage sites have been shown to be proximal to β-turns, a feature shared by many PCSK substrates. Interestingly, the manifestation of β-turns increases with the presence of lipids, suggesting that proprotein-membrane interactions may promote or modulate processing by PCSKs. By CD spectropolarimetry, proapelin exhibits a disordered conformation that changes with temperature but not with pH. Nuclear magnetic resonance (NMR) spectroscopy suggests a highly dynamic conformation in solution at both 37°C and 5°C, with some increased structuring at 5°C. In the presence of anionic lipids, but not zwitterionic lipids, CD and NMR shows increased β-turn character. This is indicative of preferential lipid interaction. Incubation of proapelin with PCSK3 produced apelin-13 preferentially and specifically across the pH range of the secretory pathway, while neither PCSK1 nor 7 could cleave proapelin. The accessibility of each dibasic site in proapelin to PCSK as a function of membrane environment and pH (corresponding to those observed in the secretory pathway) is being determined. This will allow for identification of other enzymes involved in apelin isoform production and test the hypothesis that membrane-mediated changes are responsible for processing to different apelin isoforms. This would represent the first direct evidence of membrane participation in proprotein processing.

Biophysical studies of protein-lipid interactions in complex systems

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"Biophysical" techniques, such as NMR, calorimetry and molecular dynamics simulations, are commonly used to unveil important aspects of protein-lipid interactions. However, such techniques are generally applied to well-behaved molecules in highly simplified systems and it can be difficult to interpret the results in the light of the actual, complex biological systems. In my research group, we try to bridge the gap between the two worlds. NMR studies with intact bacteria demonstrate that antimicrobial peptides (AMPs) are much less effective at disrupting lipid bilayers in bacteria than in model lipid systems. Differential scanning calorimetry studies of bacteria treated with the AMP MSI-78 reveal a novel mechanism of action for this peptide. Comparing NMR studies of model systems with studies of actual lung surfactant indicate similarities in lipid-protein interactions. Structural studies of the extremely hydrophobic protein SP-B are aided by the combination of molecular dynamics simulation and experiment.

Interaction of the human Band 3 anion exchanger membrane protein with lipids:
Insights from molecular dynamics simulations

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Human Band 3 is a major glycoprotein found in erythrocytes where it catalyzes the electro-neutral exchange of bicarbonate and chloride across the membrane. Band 3 is a dimer and each subunit is comprised of a cytosolic domain (residues 1-360) and a membrane domain (residues 361-911) that contains 14 transmembrane helices. Despite much available functional data for Band 3, the role of its diverse lipid environment in Band 3 function remains elusive in part due the complexity of the erythrocyte plasma membrane. The erythrocyte membrane has a very high concentration of cholesterol (~50%) in both leaflets with phosphatidylcholine (POPC) and sphingomyelin in the outer leaflet and POPC, phosphatidylethanolamine (POPE), and phosphatidylserine (POPS) in the inner leaflet. Molecular dynamic simulations of membrane proteins in lipid bilayers can provide a powerful tool for studying specific lipid-protein interactions and for building dynamic models of membrane proteins in a native milieu. In this study the dimeric membrane domain of Band 3 (structure kindly provide by Dr. So Iwata) was assembled into increasing complex bilayers that resemble the erythrocyte plasma membrane to study the interaction of Band 3 with its lipid environment. Negatively-charged phospholipids (POPS and PIP2) were found to strongly interact with Band 3 forming an anionic annulus around the protein in the inner bilayer leaflet. Cholesterol was also found to associate preferentially with Band 3, particularly at the dimer interface. The dynamic interaction of Band 3 with anionic lipids and cholesterol may regulate its stability, anion exchange activity and its interaction with other proteins.
The acyltransferase LYCAT regulates phosphoinositides and specific stages of endomembrane traffic
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Phosphoinositides (PIs) are signaling lipids that control organelle dynamics, proliferation, nutrient uptake, autophagy and apoptosis. There are seven species of PIs defined by the phosphorylation of their inositol headgroups, a process controlled by specific lipid kinases and phosphatases. While much as been learned about PI inositol headgroup phosphorylation, much less is known about the regulation and function of the incorporation of specific fatty acyl chains within PIs. Importantly, PIs exhibit unique specificity of composition of fatty acyl chains, such that the majority of PIs are 1-steroyl (18:0), 2-arachidonyl (20:4). This specific composition of fatty acyl within PIs is controlled in part by the PI acyltransferase LYCAT. How LYCAT, and in turn incorporation of specific fatty acids within PIs, controls the function of PIs is poorly understood. Perturbation of LYCAT by siRNA gene silencing resulted in alterations of the cellular localization and levels of phosphatidylinositol-(4,5)-bisphosphate (PIP2) and phosphatidylinositol-3-phosphate (PIP3), which control membrane traffic from the plasma membrane to early endosomes, but was without effect on other PI species examined. Consistent with this, silencing of LYCAT reduced cell surface levels of transferrin receptor (TfR), a membrane protein that undergoes constitutive internalization from the plasma membrane followed by recycling to the cell surface. LYCAT silencing delayed TfR transit to early endosomes as well as TfR recycling. Collectively, these results show that the PI acyltransferase LYCAT controls the function of specific species of PIs, and suggest that regulation of fatty acyl content of PIs is a novel dimension to the control of PI function.

Aβ-lipid interactions in mediating amyloid toxicity in Alzheimer disease
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Background: Significant changes in the brain lipid homeostasis have been detected during the progression of Alzheimer disease, including altered cholesterol metabolism or elevated levels of plasma ceramides and sphingomyelins. Within the transmembrane sequence of amyloid precursor protein (Aβ residues 29 to 49), our group has established that G33 is the critical residue that mediates amyloid-beta (Aβ) peptide oligomerization and toxicity.Methods: Electron Microscopy, in vivo Alzheimer model (Drosophila melanogaster), MALDI Mass Spectrometry, Organotypic Slice Cultures, Size-Exclusion Chromatography, Surface Plasmon ResonanceResults: Our systematic analysis of Aβ-lipid interactions revealed that residues 29 to 49 of neurotoxic Aβ42 binds more strongly to sphingomyelin, GM1, phosphatidylcholine, and a mixture of neuronal lipid membranes, as compared to its non-toxic counterparts Aβ40 and Aβ42 G33I. To attenuate the interaction between Aβ42 and lipids, we have characterized an eight amino acid peptide (so-called “Aβ-oligomer Interacting Peptide” (AIP) with alternating hydrophobic and hydrophilic amino acids) that can preferentially bind to highly toxic Aβ tetra/hexamers to render Aβ-AIP complexes non-toxic. Conclusions: The interaction of Aβ42 oligomers with sphingomyelin-rich membrane regions, such as “lipid rafts”, may play a crucial role in mediating amyloid toxicity in vivo. The development of therapeutics that interfere with Aβ-lipid interactions may provide a new, preventative strategy for Alzheimer disease.
Translational lipidomics: Harmonizing ESI-LC-MS targeted lipidomic methodologies in clinical study of Alzheimer's Disease

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The emerging field of neurolipidomics seeks to understand how dynamic changes in membrane composition regulate brain cell function and how these changes can be used to biomarkers to predict disease outcome and track disease fate. Commonly conceptualized as undulating fields of identical molecules, neuronal membranes are, in fact, made up of hundreds of chemically and molecularly diverse lipid species. For the first time, significant technological advances in high performance liquid chromatography (LC), electrospray ionization (ESI), and matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS) are enabling membrane composition to be proﬁled comprehensively at the molecular level. Coupled with subcellular fractionation and careful consideration of extraction protocols that enrich for different phospholipid families, species that vary by only one double bond, a single methylene group, or carbon chain linkage can now be quantified directly in synaptic preparations. These advances are allowing for discovery of novel biomarkers of disease transition, progression, and fate and new mechanistic insight into the determinative roles of lipid metabolism in neurodegenerative disease. Yet, as with imaging biomarkers, accuracy and reproducibility are fundamentally dependent on how lipid biomarkers are measured. Here, we ask whether changes in phosphocholine (PC) membrane predict transition from a pre-symptomatic to symptomatic state distinguish normal elderly from mild cognitive impairment (MCI) and AD and we describe challenges in harmonization of protocols, analyses, and lipid identification required for replication of biomarker results obtained in through neurolipidomic investigations.

The click chemistry probe 19-alkyne arachidonic acid appears to be a good analogue to study arachidonate-phospholipid metabolism but not eicosanoid metabolism

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Alkyne and azide analogues of naturally occurring compounds, which can be coupled to sensitive tags by click chemistry, are powerful emerging tools to study biological processes. Arachidonic acid (AA, 20:4 n-6) is a polyunsaturated fatty acid that can be transformed into eicosanoids, which are potent biologically-active compounds like leukotrienes, prostaglandins and lipoxins. AA-alkyne has been suggested to be a sensitive probe for the study of cellular arachidonic acid metabolism. In the current study, AA-alkyne phospholipid and eicosanoid metabolism was compared to that of AA. In pulse-label experiments in human Jurkat cells, the initial incorporation of AA-alkyne into phosphatidylcholine species and its subsequent remodelling into phosphatidylethanolamine species was similar to that of [3H]AA, characteristic of the CoA-independent arachidonate-phospholipid remodelling pathway. AA-alkyne was also metabolized into its 22:4 elongation product, similar to that measured with AA. The metabolism of AA-alkyne into bioactive lipid mediators was then measured in stimulated primary human neutrophils and platelets. AA-alkyne was transformed into 5-hydroxyeicosatetraenoic acid (5-HETE)-alkyne and leukotriene B4 (LTB4)-alkyne by stimulated human neutrophils. However, the measured 5-HETE-alkyne/LTB4-alkyne ratio was 10.2±0.97 whereas the 5-HETE/LTB4 ratio was 0.45±0.09 when AA was provided as substrate. This suggests that the second step of the 5-LO-catalysed reaction was less efficient with the alkyne substrate. Furthermore, LTB4-alkyne was a significantly poorer chemotactic agent for human neutrophils than LTB4 with EC50 values of 20nM (CI: 13nM-34nM) and 0.8nM (CI: 0.4nM-1.7nM), respectively. Stimulated human platelets metabolized exogenous AA-alkyne into the 12-lipoxygenase product 12-HETE-alkyne, but synthesized 6.4±2.6-fold less product than when exogenous AA was provided as substrate. Overall, these results suggest that AA-alkyne may be a good surrogate for studying cellular AA-phospholipid metabolism, but not for studies of bioactive lipid mediator production and activity. (Supported by the Canadian Institutes of Health Research and the New Brunswick Health Research Foundation).

How a membrane-binding lipid compositional sensor regulates the activity of CCT

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The key regulatory enzyme in the synthesis of phosphatidylcholine is CTP:phosphocholine cytidylyltransferase (CCT). CCT is responsible not only for the production of PC and PC-derived lipids, but contributes to phospholipid compositional homeostasis. CCT has a built-in lipid sensor which reversibly binds membranes deficient in PC. In the CCT soluble form this lipid sensor is an auto-inhibitory (AI) device. In the membrane-bound form it is folded into a surface-embedding amphipathic helix that triggers an activating switch, elevating catalytic efficiency by ~200-fold. Our structure-function analysis of CCT, a prototype for amphitropic enzymes, is revealing themes that can translate to the lipid regulation of other amphitropic enzymes: (i) Membrane binding domains can have context-specific functions (auto-inhibitory in the soluble form and activating in the membrane-bound form); (ii) Switching between forms is facilitated by a flexible linker between the regulatory AI motif and the active site; (iii) The linker may actively participate in regulation. We are using an array of chemical and biophysical techniques, e.g. site-directed fluorescence anisotropy, X-ray crystallography, ESI-mass spectroscopy, and molecular dynamics simulations to establish the allosteric pathway mediating communication between the membrane binding domain and the enzyme’s active site.
Simulation study of dynamic heterogeneity in lipid bilayers
Svetlana Baoukina, Rozmanov, D. and Tieleman, D. P. Biological Sciences, University of Calgary, Information Technologies, University of Calgary

Lipid bilayers constitute the basis of biological membranes. Understanding lipid mixing and phase behavior can provide important insights into membrane lateral organization (the raft hypothesis). Here we investigate model lipid bilayers below and above the miscibility transition temperatures. Molecular dynamics simulations with the MARTINI coarse-grained force field are employed to model bilayers on a length scale approaching 100 nm and a time scale of tens of microseconds. Using mixtures of saturated and unsaturated lipids, and cholesterol, we reproduce the coexistence of liquid-crystalline and gel, as well as liquid-ordered and liquid-disordered phases. By raising the temperature or adding hybrid lipids (with a saturated and an unsaturated chains), we induce a gradual transition from a two-phase to a one-phase state. We characterize the evolution of bilayer properties along this transition. Domains of coexisting phases change to dynamic heterogeneity with local ordering and compositional de-mixing. We analyze the structural and dynamic properties of domains, correlation times and lengths of composition fluctuations, and calculate the in-plane structure factors.

Investigating plasma membrane organization and its determinants with secondary ion mass spectrometry
Mary L Kraft School of Chemical Sciences, University of Illinois

Cell membrane organization is critical to healthy biological processes and those associated with disease. The abundances of sphingolipids and cholesterol within the plasma membrane influence many important cellular functions. Yet, the distributions of most lipid species within the plasma membrane are not well established due to a lack of suitable imaging techniques. To address this issue, we pioneered the use of high-resolution SIMS, performed with a Cameca NanoSIMS 50, for directly imaging metabolically incorporated, stable isotope-labeled lipids in actual cell membranes. The NanoSIMS 50 is a state-of-the-art secondary ion mass spectrometer that can image the elemental and isotopic composition at the surface (top few nm) of a sample with as high as 50-nm-lateral resolution. To permit visualizing the cholesterol and sphingolipids in the plasma membrane with a NanoSIMS, we use metabolic labeling with 15N-sphingolipid precursors (15N-sphingosine and 15N-sphinganine) and 18O-cholesterol to selectively incorporate distinct stable isotopes, 15N and 18O, into the cellular sphingolipids and cholesterol, respectively. Then the cells are prepared for SIMS analysis by chemically fixing them with a method that does not alter membrane lipid distribution. Finally, a Cameca NanoSIMS 50 instrument is used to map the lipid-specific isotope enrichments on the surfaces of the cells with better than 100-nm-lateral resolution. Using this approach, we have found that the metabolically incorporated 15N-sphingolipids are enriched within distinct domains in the plasma membranes of fibroblast cells. In contrast, cholesterol is fairly evenly distributed within the plasma membrane, and is not enriched in the sphingolipid domains. By imaging the effects of actin depolymerization on the 15N-sphingolipid distribution in the plasma membrane, we have determined that the sphingolipid domains are dependent on an intact cytoskeleton. Overall, our data supports a model in which lipid and protein organization within the plasma membrane is actively established by the actin cytoskeleton and its associated proteins.

Saposin picodiscs for lipid studies
Gil Privé, Leney, A.C., Klassen, J.S., Xiong, Z.J. and Popovic, K. Department of Medical Biophysics, University of Toronto, Princess Margaret Cancer Centre, Department of Chemistry, University of Alberta, Department of Biochemistry, University of Toronto

The breakdown of glycosphingolipids by hydrolase enzymes in lysosomes requires the participation of saposin “activator proteins”. The saposins are small, membrane-active proteins that can exist in either a soluble state or in a membrane-bound state. These proteins act as enzyme co-factors by forming complexes with various lipid substrates and present these in a reaction-compatible state to the respective active sites of the hydrolases. We have solved crystal structures of the human saposin proteins SapA, SapB, SapC and SapD in the closed “apo” or soluble state, as well as a complex consisting of an open-form SapA belt surrounding a small cluster of internally bound lipid/detergents [1]. These unusual SapA “picodiscs” have utility as non-detergent lipid-solubilizing system with applications in lipidomics and protein-lipid interactions [2]. A key feature of the saposin proteins is a hinge region that allows the proteins to open and expose their inner hydrophobic surfaces to lipids, much in the same way as with the exchangeable apolipoproteins. Based on data from atomic force microscopy, light scattering, gel filtration and x-ray crystallography, we find that the four homologous saposins use similar molecular mechanisms to produce different effects on lipids, ranging from aggregation to solubilization. [1] Structure of Saposin A Lipoprotein Discs. Popovic et al., PNAS (2012). [2] Picodiscs for Facile Protein-Glycolipid Interaction Analysis. Leney et al., Anal Chem. (2015).
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Nuclear-localized CTP:phosphocholine cytidylyltransferase alpha regulates phosphatidylcholine synthesis required for lipid droplet biogenesis

Adam J. Aitchison1,2, Arsenault, D.J.1,2 and Ridgway, N.D.1,2 1Dalhousie University, 2Atlantic Research Centre

The CDP-choline (Kennedy) pathway, under the control of the rate-limiting enzyme CTP:phosphocholine cytidylyltransferase (CCTa), provides phosphatidylcholine (PC) for cellular membranes as well as the phospholipid monolayer on lipid droplets (LD), specialized organelles that store neutral lipids. Here we report that CCTa expression and PC synthesis is induced during differentiation of 3T3-L1 cells and human preadipocytes. RNAi silencing of CCTa expression in 3T3-L1 cells resulted in increased LD size but did not affect triacylglycerol storage or adipogenesis. During 3T3-L1 cell differentiation, CCTa translocated from the nucleoplasm to the nuclear envelope and cytosol but did not associate with cytoplasmic LDs. During the differentiation of human preadipocytes, CCTa expression was also induced but the enzyme remained entirely in the nucleoplasm. Oleate treatment of non-adipocyte cell lines promoted LD biogenesis and caused CCTa to localize to the nuclear envelope and/or cytoplasm. In rat intestinal epithelial cells (IEC-18), RNAi silencing of CCTa increased LD size but decreased LD number and triacylglyceride deposition as a result of oleate-induced cytotoxicity. We conclude that a combination of increased expression, coupled with translocation to the nuclear envelope and export into the cytoplasm, drives the synthesis of PC necessary for triglyceride storage. Moreover, the results indicate the mechanism for activation of PC synthesis in mammalian adipocytes and oleate-treated cells does not involve CCTa translocation to lipid droplets.

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Bacterial lipid compositions are correlated to environmental variations in temperature, pH and salinity.

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Bacterial membrane polar lipid compositions vary significantly between species and phyla making them useful chemotaxonomic markers for microbial identification. These lipid content differences correlate to the respiration state and ecological niche of bacteria, indicating that specific lipid profiles are favoured in different growth environments. Previous studies examining lipid content changes in model organisms, like Escherichia coli, have indicated that specific phospholipids and fatty acids vary in response to changes in growth temperature, pH, and salinity. In this study, the lipid compositions of 380 diverse bacterial species were compared to their known temperature, pH, and salinity growth ranges to determine if all lipids are influenced similarly under these conditions. The polar lipid compositions from 380 taxonomically diverse bacteria species representing thermophiles, psychrophiles, acidophiles, alkaliphiles, halophiles and mesophiles were also statistically compared to their known the respiration state (aerobic, facultative and anaerobic). The results of this bioinformatic analysis identified unique patterns of key lipids from different phyla that correlated to each of the three growth conditions. Fatty acids that were correlated to variations in temperature, pH and salinity included saturated short (11-14C:0) and long (>18C:0) acyl chains, as well as for antesis- branched (a13-17:0) lipids. The significance of correlations between lipids and the three growth factors improved when the respiration state of the organism was considered. Opposing correlations were noted between polar lipids and each of the three growth conditions according to their Gram- type. In conclusion, this analysis identified that unique lipid patterns in various phyla were influenced by temperature, pH and salinity. It also provides evidence that supports lipid composition variation in bacteria exists to accommodate their diverse environments.
The intracellular localisation and phosphorylation profile of the human 5-lipoxygenase Δ-13 isoform differs from that of its wild-type counterpart

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5-lipoxygenase (5-LO) catalyzes leukotriene (LT) biosynthesis by a mechanism that involves interactions with 5-lipoxygenase activating protein (FLAP) and coacasin-like protein (CLP). 5-LO splice variants were recently identified in human myeloid and lymphoid cells, including the catalytically inactive ?-13 isoform whose transcript lacks exon 13. ?-13 inhibits 5-LO product biosynthesis when co-expressed with active wild-type (WT) 5-LO. The objective of this study was to investigate potential mechanisms by which ?-13 interferes with 5-LO product biosynthesis in transfected HEK293 cells. When co-expressed with WT 5-LO, ?-13 inhibited LT but not 5-HETE biosynthesis. This inhibition was independent of ?-13-FLAP interactions since it occurred in cells expressing FLAP or not. In cell-free assays CLP enhances 5-LO activity through interactions with tryptophan-102 of 5-LO. In the current study, the requirement for W102 was extended to whole cells, as cells expressing the 5-LO W102A mutant produced little 5-LO products. W102A mutants of ?-13 inhibited 5-LO product biosynthesis as effectively as ?-13 suggesting that inhibition is independent of interactions with CLP. Confocal microscopy showed that WT 5-LO was primarily in the nucleoplasm whereas W102A mutants showed a diffuse cellular expression. Despite the retention of known nuclear localisation sequences, ?-13 was cytosolic and concentrated in ER-rich perinuclear regions where its effect on LT biosynthesis may occur. W102A mutants of ?-13 showed the same pattern. Consistent with subcellular distribution patterns, ?-13 was hyper-phosphorylated on S523 and S273 compared to WT 5-LO. Together, these results reveal a role for W102 in nuclear targeting of 5-LO suggesting that interactions with CLP are required for nuclear localization of 5-LO, and are an initial characterisation of the ?-13 isoform whose inhibition of LT biosynthesis appears independent of interactions with CLP and FLAP. Better knowledge of the regulation and properties of alternative 5-LO isoforms will contribute to understanding the complex regulation of LT biosynthesis.

Oxysterol binding protein sequesters phosphatidylinositol 4-phosphate at an endoplasmic reticulum/Golgi interface when activated by 25-hydroxycholesterol

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Oxysterol binding protein (OSBP) exchanges cholesterol and phosphatidylinositol 4-phosphate (PI-4P) between the endoplasmic reticulum (ER) and Golgi apparatus membranes. 25-hydroxycholesterol (25OH) is reported to inhibit this exchange reaction and increase Golgi PI-4P due to reduced ER degradation by SAC1. 25OH-activated OSBP at Golgi/ER sites also recruits ceramide transfer protein and stimulates sphingomyelin (SM) synthesis by a mechanism involving increased PI-4 kinase IIa activity. Here we used PI-4P-specific probes and mass analysis to determine the effect of 25OH and OSBP on Golgi PI-4P, and how this is regulated by OSBP PI-4P-sterol binding activity. Contrary to expectations, detection of PI-4P in the trans-Golgi/trans Golgi network using a headgroup-specific antibody was inhibited by 25OH treatment of fibroblasts or CHO cells. Similarly, results with a SidM P4M domain probe in live cells suggested that 25OH activation of OSBP reduced Golgi PI-4P. However, phosphatidylinositol mono-phosphate (PIP) analysis by LC/MS/MS revealed that cellular PIPs were increased slightly or unaffected by 25OH and/or OSBP depletion. Expression of wild-type OSBP or a sterol-binding mutant, but not a PI-4P binding mutant, in CHO cells blocked antibody detection of PI-4P suggesting that OSBP ‘sequesters’ or ‘protects’ PI-4P from detection by headgroup probes. Addition of 25OH to these cells reduced PI-4P immunodetection. OSBP PI-4P- and sterol-binding mutants did not restore SM synthesis when expressed in OSBP-depleted cells, further evidence for a 25OH-PI-4P exchange

Functional and structural insight into the outer-membrane assembly of autotransporter proteins

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Background: Autotransporters constitute the largest family of secreted proteins in Gram-negative bacteria, and are considered important virulence factors involved in many pathogenic functions. However, the mechanisms driving the secretion and folding of this family of outer membrane proteins remains enigmatic. An operon encoding the TamA/TamB inner and outer membrane spanning complex has recently been reported as a translocation and assembly module (TAM) for the autotransporter family; although function of the TamB component has not been characterized, the TamA subunit was suggested to be the autotransporter translocon inserting autotransporters within the outer-membrane while it catalyzes the secretion of their passenger domain at the bacterial cell surface. Methods: Here, we initiated a complete investigation of these components combining systematic proteomic, genetic, and crystallographic approaches to assess the individual contribution of TamA and TamB in autotransporter biogenesis. Results & Conclusion: Our study reveals TamB as the essential component required to secrete a functional autotransporter to the cell surface and thus identifies TamB as a key chaperone factor required for the maturation of passenger domains. The crystal structure TamA confirms its striking resemblance to the OMP85 family of insertases (BamA) alluding to TamA’s role in OM biogenesis. While TamA is not essential for autotransporter secretion, the physical and functional associations we observed between Tam and Bam components suggest an interplay between these two biological systems and supports the notion that the Tam complex function as an auxiliary machinery to the OM biogenesis. Thus, the secretion mechanism of autotransporters requires TamB to nucleate proper folding within the periplasm and utilizes an OMP85 member for translocation to the bacterial surface.
Leucine inhibits myocardial palmitate oxidation at baseline and following ischemia reperfusion

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Background: Increases in serum branch chain amino acids, specifically leucine, are associated with obesity-induced whole body insulin resistance. Impairment in insulin signaling leads to the pathological remodeling of cardiac metabolism rendering the heart from obese rodents and humans susceptible to ischemic heart failure. To date, the direct effect of leucine on cardiac metabolism remains unclear.

Methods & Results: To examine the effect of leucine on cardiac metabolism, mouse hearts were perfused aerobically in the working mode with Krebs-Henseleit buffer containing 5 mM [U-14C]glucose and 1.2 mM [9,10-3H]palmitate in the presence or absence of 5 mM leucine for 30 min. Oxidation of glucose and palmitate was measured by quantitative collection of 14CO2 and 3H2O with concomitant analysis of cardiac function. During the aerobic period, leucine perfused hearts exhibited a significant increase in glucose oxidation and a significant decrease in palmitate oxidation in the absence of alterations in heart rate and cardiac power. Furthermore, in the leucine perfused hearts, glucose contribution to Krebs cycle acetyl CoA production was 98% versus 48% in vehicle perfused hearts signifying that leucine shifts the reliance of cardiac metabolism from fatty acids to glucose. Suppression of palmitate oxidation also coincided with decreases in coronary lipoprotein lipase activity, which is indicative of decreased delivery of fatty acids to the leucine perfused heart. To simulate ischemia reperfusion conditions ex vivo, hearts were subjected to 18 min of global no-flow ischemia followed by 40 min of aerobic reperfusion. Leucine perfused hearts continued to display inhibition of palmitate oxidation when compared to vehicle perfused hearts suggesting that cardiac substrate utilization is modified by branch chain amino acids during both normoxia and hypoxia. Significance: Chronic inhibition of fat oxidation by branch chain amino acids during obesity and diabetes could render the myocardium susceptible to metabolic maladaptation and ischemia reperfusion injury.

Effects of trans-Vaccenic and Elaidic Acids on Lipid and Lipoprotein Metabolism

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Consumption of diets high in trans fatty acids from chemically hydrogenated oils has been associated with an increased risk for the development of cardiovascular disease. However, much less is known about the health effects of naturally occurring trans fatty acids or of specific component fatty acids in chemically hydrogenated oils. The objective of this study was to compare the effects of cis- and trans- monounsaturated C18 fatty acids on lipid and lipoprotein metabolism in Syrian Golden hamsters and HepG2 cells. Thirty six male Syrian Golden hamsters, approximately 9 weeks of age, were given a high-fat diet (15%, w/w) supplemented (1%, w/w) with oleic acid (OA, C18:1, cis9), elaidic acid (EA, C18:1, trans7) or vaccenic acid (VA, C18:1, trans11) for 6 weeks. Plasma cholesterol and triglyceride levels increased from baseline after 2 weeks in all treatment groups and increases were not different among the groups at the end of the study. Poloxamer 407 injections and density gradient ultracentrifugation was used to examine hepatic VLDL secretion. Triglycerides secreted in VLDL were similar in all treatment groups. Hamsters receiving EA also had reduced levels of cholesterol in both the VLDL and LDL density fractions. Moreover, while OA supplementation significantly increased plasma apoB levels, EA and VA supplemented animals had apoB levels comparable to control hamsters. Radiolabeling studies in HepG2 cells indicated that apoB stability and secretion were unaffected by trans fatty acid supplementation. These
observations suggest that EA and VA have similar effects on hepatic lipoprotein production and that individual trans fatty acids alone, of chemical or biological origin, may not be responsible for an increased risk of cardiovascular disease.

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Examining E. coli and P. aeruginosa PagP structure-function relationships

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Many Gram-negative bacteria have the ability to structurally modify outer membrane lipids in response to specific environmental cues, including those encountered during the colonization of a host. The bacterial outer membrane enzyme PagP transfers a palmitate chain from a phospholipid to the lipid A (endotoxin) component of lipopolysaccharide when outer membrane lipid asymmetry is perturbed. P. aeruginosa isolated from the airways of chronically infected cystic fibrosis patients are found to possess constitutively palmitoylated lipid A. We recently identified a structural homologue of PagP, encoded by the P. aeruginosa PhoP-dependent gene Pa1343, which mediates palmitoylation of lipid A. This lipid A modification renders the bacterium more resistant to the killing action of certain antimicrobial peptides and stimulates cytokine production leading to inflammation. Despite the lack of detectable primary sequence similarity with known PagP enzymes, the beta-barrel tertiary structure with an interior hydrocarbon ruler is conserved. The P. aeruginosa homologue incorporates a palmitate chain into lipid A on the distal glucosamine sugar at the 3’ position, compared to the 2 position of the proximal sugar seen with the enterobacterial PagP. We are interested in identifying the molecular basis and functional significance of each palmitoylated lipid in controlling outer membrane asymmetry and thus provide a means to dissect the functional role of Regioselective lipid A palmitoylation modification. In a P. aeruginosa and E. coli heterologous PagP expression system, we isolate palmitoylated lipid A. These observations suggest that PagP’s regiospecificity for the 2 or 3’ positions of lipid A. Recent findings indicate that PagP homologues from E. coli and S. typhimurium also use phosphatidylglycerol (PG) as a palmitate acceptor to make palmitoyl-PG. However, unlike the enterobacterial PagP, the P. aeruginosa homologue does not utilize PG as a palmitate acceptor. In a P. aeruginosa and E. coli heterologous PagP expression system, we isolate palmitoyl-PG from the regioselective lipid A palmitoylation modification and thus provide a means to dissect the functional significance of each palmitoylated lipid in controlling outer membrane permeability, antimicrobial resistance, and the host immune response.

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Regulation of the adipocyte autotaxin by nutritional stimuli and insulin resistance

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A major complication of obesity is insulin resistance wherein metabolically relevant tissues including skeletal muscle, liver, and adipose tissue, show a decreased response to insulin. Bioactive adipokines secreted from adipocytes are believed to play a major role in obesity-induced insulin resistance. ATX is a novel adipokine that produces the signaling messenger, lysophosphatidic acid, and has recently been implicated in obesity-induced insulin resistance in mice and humans. The goal of this study was to examine the in vitro and in vivo regulation of ATX using 3T3-L1 adipocytes and C57Bl6 mice following nutritional alterations and impairment of insulin function. ATX mRNA and protein expression markedly increased during 3T3-L1 adipocyte differentiation, which was paralleled by an increase in ATX activity in the media. Incubation of 3T3-L1 adipocytes with high concentrations of glucose was sufficient to augment ATX activity in the media whereas fatty acids had no significant impact on ATX activity. Nutrient deficiency either via serum withdrawal in 3T3-L1 adipocytes or by fasting C57Bl6 mice decreased ATX activity in media and serum, respectively. Nutritional induction of obesity and insulin resistance in C57Bl6 mice either by using a high fat or high-fat-high sucrose diet resulted in a 1.5-2-fold increase in serum ATX activity in both fed and fasted states when compared to the Chow-fed controls. To examine whether insulin resistance influences ATX expression and activity in adipocytes, we subjected 3T3-L1 adipocytes to different stimuli that impair insulin signaling – Tnfα, hypoxia, Tnfα plus hypoxia, and hyperinsulinemia. Interestingly, while incubation with Tnfα led to a moderate upregulation of ATX, hypoxia blunted ATX expression and activity. Hyperinsulinemia had no effect on adipocyte ATX. Taken together, our data suggest that ATX in serum and adipocytes is nutritionally regulated in health and obesity-insulin resistance and that distinct modes of stimulating insulin resistance have divergent effects on adipocyte ATX.

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Steroid hormones induce stearoyl-CoA desaturase-1 in hormone-sensitive breast and prostate cancer cells

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Stearoyl-CoA desaturase-1 (SCD-1) catalyzes the production of monounsaturated fatty acids that are essential for membrane biogenesis, and is required for cell proliferation in many cancer cell types. Although steroid hormones are required for the proliferation of hormone-sensitive breast and prostate carcinomas, estrogen is a known repressor of SCD-1 expression in liver and adipose. This study addresses the impact of estrogen and androgen on SCD-1 expression in breast and prostate cancer carcinoma cells, respectively. MCF-7 mammary carcinoma cells and LNCaP prostate carcinoma cells were hormone-starved then treated or not with 17β-estradiol and with the synthetic androgen metribolone (R1881), respectively. 17β-estradiol and R1881 significantly induced cell proliferation that was accompanied by an increase in SCD-1 activity, measured by changes in cellular monounsaturated/saturated fatty acid ratios. We measured fold increases of 4.3±0.05 and 3.7±0.04 in 16:1n-7/16:0 ratios and of 1.6±0.1 and 1.7±0.1 in 18:1n-9/18:0 ratios in treated MCF-7 and LNCaP cells, respectively, compared to untreated controls. SCD-1 mRNA and protein expression were then measured by qPCR and western blot. 17β-estradiol (10nM) induced a 2.6±0.1 fold increase in SCD-1 mRNA and a 3.9±0.3 fold increase in SCD-1 protein levels in MCF-7 cells. R1881-treated LNCaP cells had an 8.2±1.2 fold increase in SCD-1 mRNA levels.
and a 6.7±1.5 fold increase in SCD-1 protein at low-dose R1881 (100 pM). At higher dose (100 nM R1881), treated cells had 20.3±0.01 fold and 10.2±2.7 fold increases in SCD-1 mRNA and protein, respectively. In MCF-7 mammary cells, treatment with the SCD-1 inhibitor A959372 reduced 17β-estradiol-induced cell proliferation by 48%, while siRNA silencing of SCD-1 reduced proliferation by 70%. In conclusion, estrogen and androgen induce SCD-1 expression and activity in breast and prostate carcinomas, respectively. These results support SCD-1 as a therapeutic target in hormone-sensitive cancers. (Supported by the Canadian Breast Cancer Foundation, CIHR and the NB Health Research Foundation).

Labrador tea (Rhododendron groenlandicum) attenuates insulin resistance in a diet-induced obesity mouse model.

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Using a diet-induced obesity (DIO) mouse model, we investigated the antidiabetic effect of Labrador tea [Rhododendron groenlandicum (Oeder) Kron and Judd], a beverage and medicinal tea used by the Cree Nations of northern Quebec. C57BL6 mice were divided into five groups and given standard chow (~4% of lipids) or high-fat diet (~35% of lipids) for 8 weeks until they became obese and insulin resistant. Treatment began by adding the plant extract at three doses (125, 250 and 500 mg/kg) to the high-fat diet for another 8 weeks. At the end of the study, insulin-sensitive tissues (liver, skeletal muscle, adipose tissue) were collected to investigate the plant's molecular mechanisms. Labrador tea significantly reduced blood glucose (13%), the response to an oral glucose tolerance test (18.2%) and plasma insulin (65%) while preventing hepatic steatosis (42% reduction in hepatic triglyceride levels) in DIO mice. It stimulated insulin-independent Akt pathway (55%) and increased the expression of GLUT4 (53%) in skeletal muscle. In the liver, Labrador tea stimulated the insulin-dependent Akt and the insulin-independent AMP-activated protein kinase pathways. The improvement in hepatic steatosis observed in DIO-treated mice was associated with a reduction in inflammation (through the IKK α/β) and a decrease in the hepatic content of SREBP-1 (39%). Labrador tea exerts potential antidiabetic action by improving insulin sensitivity and mitigating high-fat diet-induced obesity and hyperglycemia. They validate the safety and efficacy of this plant, a promising candidate for culturally relevant complementary treatment in Cree diabetics.

Biophysical analysis of a successful protocol to reconstitute tetramers of the M2 muscarinic receptor

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The human G protein-coupled receptor M2 muscarinic receptor has been functionally reconstituted in its tetramer state into mixed lipid bilayers (Redka et al. 2013). This is achieved by first solubilizing the receptor in mixed detergent micelles composed of digitonin and sodium cholate, then reconstituting it into vesicles composed of phosphatidylcholine, phosphatidylserine and cholesterol, followed by detergent removal. To understand how the individual detergent and lipid components used in this empirical protocol contribute to the stability and activity of the receptor, we used isothermal titration calorimetry (ITC) to study the self-assembly of the mixed surfactant system; differential scanning calorimetry and pressure perturbation calorimetry to probe the phase behavior of the membrane; ITC, fluorescence (time-resolved) leakage assays and dynamic light scattering to characterize detergent-lipid interactions.

Effect of acute chemical chaperone treatment on apoB metabolism in HepG2 cells

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Apolipoprotein B100 (apoB) is the essential structural protein component of very low (VLDL) and low density (LDL) lipoproteins. ApoB is thought to contain hydrophobic regions which are responsible for assembly of the protein with its cargo of triglycerides (TG) and cholesterol esters (CE). ApoB acquires its initial lipid cargo cotranslationally and this contributes to the stability of the protein. In the human hepatoma HepG2, VLDL assembly is inefficient, and lipid-poor apoB is degraded by the endoplasmic reticulum-associated degradation (ERAD) pathway, culminating in proteolysis in the cytosolic proteasome. Chemical chaperones, such as 4-phenyl butyric acid (PBA), have been shown to rescue several mutant proteins from ERAD and protect cells from the effects of pathological ER stress. We explored whether acute treatment of HepG2 cells with PBA could rescue apoB from ERAD and improve its secretion efficiency. PBA treatment reduced the accumulation of polyubiquitinated apoB in the cytosol, suggesting that apoB targeting for ERAD was reduced. Consistent with this, PBA also reduced the association of apoB with the cellular chaperone proteins Hsp70 and Bip. However, PBA only marginally improved total apoB secretion and the density gradient profile of the secreted apoB lipoproteins was unaffected by PBA treatment, suggesting that PBA only improved the secretion of lipid-poor apoB. However, when oleic acid was included in the medium, PBA enhanced the secretion of apoB in the VLDL density range. Pre-treatment with MEK/ERK inhibitor U0126 is known to enhance lipid mobilization for VLDL assembly in HepG2 cells. In the presence of U0126, PBA had little additional effect on apoB secretion or particle density. Taken together, these data suggest that PBA can rescue lipid-poor apoB from ERAD, perhaps by reducing the engagement of endogenous quality control processes. However, preventing apoB degradation alone is not sufficient to enhance VLDL assembly.
Recombinant apelin-36 as a tool to characterize apelin-receptor binding by NMR spectroscopy

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Apelin is a peptide hormone that signals through a class A G-protein coupled receptor (apelin receptor; AR) to control multiple physiological processes such as the cardiovascular system, energy metabolism, and angiogenesis. Apelin is expressed as a 77 residue long preproprotein that is subsequently processed via signal-peptide cleavage to a putative 55-residue proapelin form. This is further processed to bioactive apelin isoforms retaining the last 13, 17, or 36 C-terminal residues. These isoforms exhibit different receptor affinities, which lead to different downstream signaling, and are thought to control the apelinergic system through regulation of the AR. Characterizing individual apelin isoforms is important to understand ligand-receptor binding, conformational changes that occur, and subsequent receptor activation. Herein, we present the first high-resolution structural characterization of the longest bioactive isoform, apelin-36, using biophysical techniques. By circular dichroism (CD) spectropolarimetry, apelin-36 appears to be mostly random coil in solution. This correlates well to nuclear magnetic resonance (NMR) spectroscopy, which also shows a highly dynamic conformation in solution. Interestingly, both CD and NMR spectra of apelin-36 showed increased β-turn-like characteristics in the presence of anionic lipids, but not zwitterionic lipids, in a similar fashion to the shorter isoforms. This suggests preferential lipid interactions and the potential of membrane-catalyzed receptor binding. The conformational changes that occur upon interaction with AR are being determined by NMR, allowing full characterization of ligand-receptor binding. Recombinant apelin-36, in contrast to shorter isoforms, facilitates this through its higher receptor affinity and the ability to uniformly enrich the peptide with NMR-active carbon-13 and/or nitrogen-15 nuclei. Our goal is to identify the specific amino acids involved and the conformation of both the ligand and the receptor during interaction.

Functional Regulation of the TRP-type Channel (PKD2L1) by Lipids

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It was the drosophila transient receptor potential (trp) gene that led to the huge discovery of the TRP channels and their roles in health and disease. The TRP superfamly comprises seven families of cation channels. As a member of the TRP superfamly, polycystic kidney disease 2-like-1 (PKD2L1) is a non-selective Ca-regulated cation channel and is also called TRPP3. PKD2L1 regulates sour tasting in the tongue and Ca concentration in primary cilia. PKD2L1 is expressed in multiple tissues, including the retina, brain, kidneys, testis and heart. It was found in 1998 that the drosophila TRP channel is phosphorylated by the eye specific protein kinase C (ePKC) and that such phosphorylation is part of a negative feedback mechanism that regulates Ca influx. Here we studied roles of phosphorylation in PKD2L1 function. We wondered whether the common PKC activator and the diacylglycerol (DAG) mimic, phorbol 12-myristate 13-acetate (PMA), affects PKD2L1 channel function. Using Xenopus oocyte expression system, the two-microelectrode voltage-clamp (TMVC), immunofluorescence (IF), biotinylation and Western blotting we found that PMA, but not its biologically non-active analogue, 4a-phorbol 12,13-didecanoate (4aPDD), inhibits PKD2L1 channel function and reduces its plasma membrane localization. In comparison, we found that PKD2L1 mutant T338A has a substantially reduced response to the PMA treatment. These preliminary results indicate that membrane phospholipids regulate PKD2L1 channel function that may result in phosphorylating residue T338 or an adjacent site and thereby affecting its surface membrane density. Experiments aiming at providing further documentations are in progress. This study will constitute an important step towards understanding the molecular mechanism underlying the biological functions of PKD2L1 such as sour tasting. Supported by NSERC and CIHR (to XZG).

Sphingosine 1-phosphate induction of endothelial cytokine expression is differentially modulated by extracellular matrix proteins

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Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid regulator of numerous physiological processes, including angiogenesis. The angiogenic process in general, and S1P in particular has also been associated with inflammation. Bio-Plex analysis of a panel of 27 cytokines found that the proinflammatory, proangiogenic cytokines, IL-6, IL-8 and MCP-1 were induced in human umbilical vein endothelial cells with S1P stimulus. Parallel experiments with vascular endothelial growth factor (VEGF) demonstrated regulation of an overlapping set of cytokines. Induction of gene expression in cells grown on tissue culture plastic or a variety of different extracellular matrix proteins were tested. Compared to expression in cells grown on plastic, both the basal and induced expression were significantly upregulated in cells grown on collagen I, fibronectin and laminin I. Basal expression of all 3 cytokines was upregulated on collagen I and fibronectin, while laminin I induced expression of IL-6 and IL-8 alone. Treating the cells with either S1P or VEGF further upregulated expression of these cytokines. MCP-1 upregulation was more sensitive to S1P, while IL-6 and IL-8 expression were more strongly induced by VEGF. Collagen IV and Matrigel had little effect on expression levels, suggesting that the effect of matrix proteins on expression is not universal. Inhibitors demonstrated that NF-kappaB activation was essential for expression of all the cytokines. In contrast, IL-6 and MCP-1 expression is dependent on JAK1/2, STAT3 and STAT5 activation, whereas IL-8 expression was dependent on JAK1/2 and STAT5 activation. NF-kappaB and STAT activation were confirmed by
phosphoprotein analysis and luciferase-based promoter studies confirmed STAT regulated gene expression. These studies show that both S1P and VEGF induce expression of cytokines that are regulated by activation of the NF-kappaB and JAK-STAT pathways in endothelial cells. Extracellular matrix proteins modulate cytokine expression induced by both sphingolipid and protein growth factors, suggesting that the microenvironment plays a role.

Association of low-density lipoprotein (LDL) with PCSK9 in the regulation of LDL-cholesterol levels

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Background: Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is a secreted plasma protein that binds and promotes degradation of cell-surface low density lipoprotein (LDL) receptors in the liver, impairing clearance of LDL from the blood. The prodomain of PCSK9 has an autoinhibitory effect on LDL receptor binding. We have previously shown that PCSK9 binds to LDL in circulation, and that an N-terminal region of the autoinhibitory PCSK9 prodomain is necessary for this interaction. PCSK9 also exhibits decreased LDLR-degrading activity in the presence of LDL, indicating that this interaction could play a role in regulating LDL levels through equilibrium of inactive LDL-bound PCSK9 and active free circulating PCSK9. Purpose: We aimed to further characterize the role of the prodomain in the PCSK9-LDL interaction by looking at the importance of specific residues and regions in the PCSK9 prodomain to LDL binding. Methods: Targeted residues in the N-terminal region of the PCSK9 prodomain were altered or deleted, and the effect of these alterations on LDL binding was assessed using ultracentrifugation. LDL receptor-mediated cellular uptake of PCSK9 into HuH7 cells was used to investigate the ability of these PCSK9 variants to bind LDL receptors. Results: The hydrophobicity of a PCSK9 sulfation site at Y38 was found to be important to LDL-binding. Loss of LDL binding also resulted from deletion of a short acidic stretch of residues containing Y38 as well as replacement of an adjacent hydrophobic stretch of residues with a disordered linker. Helical wheel modeling of the prodomain N-terminal showed the potential for a lipid-ordered amphipathic helix to form, and replacing residues A44 and L41 with helix-disrupting prolines abolished LDL binding without affecting binding to LDL receptor. Wild-type PCSK9 was found to associate with liposomes. Conclusions: These results further pinpoint specific amino acid sequences in the PCSK9 prodomain that regulate the LDL-binding interaction. Our future studies will also determine the importance of these sequences in LDL receptor binding and may reveal novel target sites for inhibiting the PCSK9-LDLR interaction.

Structure-function correlation for the membrane-induced 8th helix of the apelin receptor

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The apelin receptor (AR or APJ) is a class A G protein-coupled receptor (GPCR) that is activated by various isoforms of the peptide hormone apelin and by another recently discovered hormone called ELABELA/TODDLER. AR is involved in cardiovascular and central nervous system regulation, as well as in adipoinusar axis function and regulation of fluid homeostasis. Literature suggests that a relatively short a-helical stretch just C-terminal to the heptahelical transmembrane (TM) domain, referred to as the “8th helix”, is necessary for plasma membrane localization and function of several other class A GPCRs. In an effort to understand the mechanism for anterograde transport of the receptor, we have expressed and purified a 71-amino acid long C-terminal fragment of the AR, referred to here as the AR C-tail. Sequence-based secondary structure prediction suggests the presence of an amphipathic 12-residue a-helix, with the remainder predicted to be disordered. This helical segment should be proximal to the membrane, as it is initiated almost immediately C-terminal to the putative 7th and final TM helix of AR. Circular dichroism (CD) spectroscopy shows that the AR C-tail acquires some degree of helical character when in the presence of anionic detergent micelles, with diffusion-ordered NMR (DOSY) demonstrating protein-micelle binding, validating these predictions. Structure determination of the micelle-bound state by NMR spectroscopy is currently being carried out, with resonance assignment underway. Site-directed mutagenesis is also being carried out to validate the structural significance of the a-helical region in the C-terminus of the AR.

Consumption of Ahiflower Oil Is Safe and Increases Tissue EPA Levels Compared to Flaxseed Oil – Results of a Phase 1 Clinical Trial

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Ahiflower oil is a dietary oil rich in stearidonic acid (SDA, 18:4 n-3) extracted from Buglossoides arvensis seeds. We report herein a randomized, double blind, placebo-controlled Phase I Clinical Trial. This is the first study of this novel dietary oil in humans. Healthy subjects (n=40) supplemented their diets (28 days, 9.8 g/d) with Ahiflower oil (46% α-linolenic acid (ALA), 20% SDA) or with flaxseed oil (59% ALA). Safety parameters were blood and urine chemistries, blood lipid profiles, hepatic and renal function tests and hematology. No clinically-significant safety-related values were measured in either group. Fasting plasma, RBC, polymorphonuclear (PMN) and mononuclear cells (MC) were collected at baseline, 14 and 28 days and fatty acids were measured by gas chromatography. Linear mixed models (repeated measures design) probed for differences in time and time x treatment interaction using gender, age and BMI as covariates. Amongst significant tissue fatty acid changes,
EPA (20:5 n-3) as % of total fatty acids increased from baseline to day 28 in both the Ahiflower (plasma, RBC, PMN and MC, respectively: 0.46 vs 1.34, 0.44 vs 0.74, 0.15 vs 0.24 and 0.20 vs 0.48; all p<0.05) and flaxseed (0.43 vs 0.76, 0.42 vs 0.55, 0.16 vs 0.19, 0.20 vs 0.27; all p<0.05) groups. Notably, EPA accrual in plasma and cells was greater in the Ahiflower group (time x treatment interactions, p<0.05) and the change in plasma EPA at day 28 was 2.5-times greater in the Ahiflower group. In conclusion, Ahiflower oil is a safe and sustainable seed oil for enrichment of tissues with n-3 PUFA and is more effective than flaxseed oil. (Funded by: CIHR, Atlantic Innovation Fund, Technology Crops Int'l).

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Phosphatidylinositol 4-phosphate dynamics during phagocytosis

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Phosphoinositides play a central role in phagocytosis, a critical pathway whereby particulate matter is internalized and degraded by cells. Phosphoinositide levels undergo acute changes during the course of phagosome formation and maturation. Upon particle engagement phosphatidylinositol 4,5-bisphosphate ([PtdIns(4,5)P2]) and phosphatidylinositol 3,4,5-trisphosphate undergo marked concentration changes that signal the cytoskeletal and membrane remodeling required for phagosome formation. Once the phagosome is internalized, phosphatidylinositol 3-phosphate (PtdIns3P) accumulates for approximately 10 minutes, directing the early stages of phagosome maturation. However, to date the fate of phosphatidylinositol 4-phosphate (PtdIns4P) has not been investigated.

Recently, a genetically encoded biosensor was developed to visualize PtdIns4P in live cells. A tandem version of the probe, called P4M2x, consists of two copies of the PtdIns4P-binding of SidM, a Legionella pneumophila effector, tagged with a fluorescent protein. Expression of P4M2x in RAW 264.7 macrophages revealed tri-phasic changes of the PtdIns4P during phagocytosis of IgG-coated particles. PtdIns4P, which is normally present in the plasma membrane, underwent a transient, moderate increase as the phagosome sealed, which coincided with the disappearance of PtdIns4P ([as reported by the biosensor PLCd(PH)]). This initial increase was followed by the virtual disappearance of PtdIns4P, which coincided with accumulation of PtdIns3P ([reported by the biosensor EEA1(2xFYVE)]). After approximately 10 minutes, at the time when PtdIns3P disappeared, a gradual reacquisition of PtdIns4P was observed, reaching levels in excess of those normally found at the plasma membrane. This reappearance of PtdIns4P was preceded by the sequential acquisition of Rab5 followed by Rab7 –early and late maturation indicators, respectively. The reacquisition of PtdIns4P was accompanied by the appearance of P4M2x-positive tubules that extended dynamically from phagosomes towards the Golgi. The mechanisms underlying these changes and their functional implications will be discussed.

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Characterization of visual Retinol dehydrogenases and their transmembrane domains

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Retinoids are playing major roles in many physiological functions, such as differentiation, growth, and vision. In the eye, retinoids are mainly implicated in the visual cycle, which is occurring both in photoreceptors and in the retinal pigment epithelium. Retinol dehydrogenases (RDHs), such as RDH8 and RDH11, are enzymes responsible for crucial steps (oxid-reduction reactions of retinoids) of this visual cycle. They belong to a large family of proteins designated as short-chain dehydrogenases/ reductases (SDRs), which thus share common features (sequence signature, Rossmann fold, etc.). RDHs have been postulated to be anchored to membranes by means of their C- (v.g. RDH8) or N-terminal segment (v.g. RDH11) to accomplish their functions. The structure of these RDHs and specifically their transmembrane domain has been predicted using computational tools. The secondary structure and membrane binding of these enzymes as well as their N- and/or C-terminal segment have been investigated using circular dichroism, infrared spectroscopy and pressure measurements. Langmuir monolayers have been used as a model membrane to study lipid-peptide or lipid-protein interactions. Furthermore, infrared spectroscopy in monolayers has allowed the observation of a different behaviour of the RDHs transmembrane domains depending on their length or the type of phospholipid used. Finally, truncated RDHs have been produced to determine the effect of the deletion of their transmembrane segment on the structure and function (activity and binding) of these RDHs.

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Roles of PI4P in secretory granule biogenesis

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Phosphatidylinositol 4-kinases (PI4Ks) are responsible for production of the lipid phosphatidylinositol 4-phosphate (PI4P), a crucial resident of Golgi membranes that regulates membrane trafficking events such as secretion. We have discovered that PI4KII but not fww function is required for normal development of the Drosophila larval salivary gland. In PI4KII mutants, mucin-containing glue granules were considerably smaller than in wild type. Furthermore, some of the glues were mistrafficked to enlarged late endosome and lysosomes. The glue granule associated SNARE SNAP24 was also found on enlarged vesicles in PI4KII mutants. To further examine the role of PI4P and endocytic machinery in glue granule biogenesis, I employed a reverse genetic screen to identify potential genetic interactions using publicly available transgenic RNAi lines. A selection of candidate
genes including Rab small GTPases, SNAREs, and various endocytic proteins were screened. 9 Rab GTPases resulted in either small or improper glue granule production after RNAi knock down. I am currently investigating the localization of these Rab GTPases in the larval salivary gland and I am overexpressing the dominant negative mutant proteins to see whether I could recapitulate the RNAi phenotype. I have also identified a number of endocytic and Golgi SNAREs that are important for proper glue granule formation. Interestingly, PI4KI was found on enlarged vesicles when Syntaxin-7 and -16 were knocked down, which further suggests co-regulation between PI4KI and these SNAREs. Moreover, another positive hit SNAP29 localized to glue granules when overexpressed in wild type and I am currently investigating the localization of SNAP29 in our PI4KII mutant. In conclusion, I have identified a number of potential regulators of secretory granule biogenesis and I plan to further examine these hits to determine whether they are PI4KII dependent and whether they are directly involved in the biogenesis of glue granules.

Effect of Oxysterol Binding Protein (OSBP) Phosphorylation on Membrane Binding and Lipid/Sterol Extraction

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OSBP likely acts as a phosphatidylinositol phosphate (PIP)-sterol exchanger and transports sterols from the endoplasmic reticulum (ER) to trans-Golgi while bringing PIP back to the ER. Our collaborator (Dr. Neale Ridgway, Dalhousie University) has demonstrated that OSBP regulates sterol binding and the activation of sphingomyelin synthesis (SM) depending on the phosphorylation status of the protein. Their study suggested that different phosphorylation states regulate the directionality and/or specificity of sterol transfer activity, along with ER-Golgi localization. Moreover, it appears that the phosphorylation of OSBP enhances sterol binding since a phosphomimic OSBP mutant was shown to have increased in vitro cholesterol and oxysterol binding capacity (Ridgway et al., 2012, Mol Biol of Cell. 23). We have investigated the role of human OSBP phosphorylation on lipid and sterol recognition using immobilized bilayers and Dual Polarization Interferometry (DPI). Using both the human OSBP and its phosphomimic (S5E), made by Ridgway's group, we have determined the ability of OSBP to bind to phospholipid bilayers and the variation in binding that occurs with incorporation in the bilayer of the Golgi-specific phosphatidylinositol-4-phosphate (PI(4)P), and/or sterols. Studies on the effect of OSBP phosphorylation on binding to membranes of differing phospholipid and varying sterol content are reported. These measurements are crucial for building a molecular mechanism of this sterol/lipid exchange/transfer protein.

The Role of Neuronal Cholesterol Biosynthesis

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Cholesterol is imperative for membrane structure and function, and can be acquired through endocytosis or synthesized intracellularly. The brain contains 25% of total body cholesterol, all of which is synthesized in situ by neurons and glial cells. In early development, neuronally-synthesized cholesterol is essential for neurite outgrowth and synapse formation. As neurons mature, they rely increasingly on cholesterol derived from glia. However, certain neuronal cell types including hippocampal neurons continue to produce cholesterol in the mature brain, yet the advantage of maintaining this energetically costly process remains unclear. Our goal is to determine the role of neuronal cholesterol biosynthesis in mature hippocampal neurons. Here we use RNAi-mediated gene silencing of late stage cholesterol biosynthetic enzymes, namely squaene synthase (FDFGT1) and lanosterol demethylase (CYP51), to inhibit neuronal cholesterol biosynthesis and concomitantly alter cholesterol precursor levels. Using viral constructs with a neuron-specific promoter and fluororescent reporters, we are assessing the effects of neuronal cholesterol depletion on mitochondrial dynamics and synapse formation. Analysis of fluorescent live-cell images revealed a reduction in mitochondrial length, possibly suggesting alterations with fusion and/or fission events, or alterations in mitophagy. Inhibition of neuronal cholesterol biosynthesis revealed an increase in dendritic spine density, suggesting possible alterations in synapse formation and synaptic plasticity. Future investigations will help to reveal how these morphological alterations affect neuronal function.

Lysosome transporter degradation by organelle membrane fusion

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Lysosomes, or vacuoles in yeast cells, are the terminal compartments of the endocytic and autophagy pathways, responsible for the breakdown of membrane-encapsulated biological material into its constituents for reuse by eukaryotic cells. Lysosome physiology relies on transporter protein activity, best signified by loss-of-function mutations linked to lysosomal storage diseases. These lysosomal transporters are critical for eukaryotic cell physiology as they regulate the luminal environment, export cellular nutrients, and contribute to cellular signaling. Despite their importance, little is known about the lifetime of these lysosomal transporters or how they are regulated. Using Saccharomyces cerevisiae as a model, we have discovered a novel, selective degradation pathway intrinsic to homotypic vacuole fusion required for transporter turnover. Using fluorescence microscopy, a pHluorin-based reporter assay and western blotting, we show that vacuolar transporters are selectively sorted into an areas apposing vacuole membranes that are internalized and degraded upon fusion. By studying multiple transporters, we find that these proteins have diverse lifetimes. We also demonstrate that substrate levels regulate transporter degradation and this pathway is important for protein quality control. Finally, we confirm that the multivesicular body and autophagy machinery are not required for observed protein degradation. Rather, the membrane fusion machinery seems to play a role in protein sorting as well as internalization. In all, we have characterized a new pathway responsible for vacuole transporter degradation that determines membrane protein expression levels responsible for organelle function and...
PagP molecular diversity: lipid A acylation regioselectivity and role for duplicated isoforms

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PagP is an enzyme of lipid metabolism that resides in the outer membrane (OM) of various Gram-negative bacteria. The E. coli PagP transfers a palmitate from the sn-1 position of a glycerophospholipid to the 2 position of lipid A, conferring resistance to some cationic antimicrobial peptides and attenuating the activation of host immune defenses. PagP is an eight-stranded β-barrel enzyme, with a ligand binding pocket interior, the hydrocarbon ruler. The conserved catalytic residues His33, Ser77 and Arg114 are located on the extracellular side of the enzyme. PagP is activated when the OM is perturbed, causing phospholipid migration to the outer leaflet of the OM. Phospholipids access the enzyme through a gate in the β-barrel wall known as the crenel. The crenel enforces phospholipid acyl chain regiospecificity by blocking C18 stearate acyl chains at the sn-2 position, while the hydrocarbon ruler excludes C14 myristate, thus selecting a C16 palmitate from the sn-1 position. Lipid A approaches the enzyme through another opening opposite the crenel, the embrasure. Details of the mechanism by which lipid A binds to the enzyme remain to be established. Recently it was observed that PagP also uses phosphatidylglycerol (PG) as a palmitoyl acceptor in Salmonella OMs. We theorize that palmitoyl-PG helps to fortify the permeability of the OM under specific environmental conditions. PagP homologues are distributed mainly between the Gamma and Beta-proteobacteria, and exhibit regiospecificity for the 2 and/or 3’ positions of lipid A. PagP’s lipid A regioselectivity seems to be dependent on the lipid A exhibited by the bacteria in addition to the genetic regulation afforded by bacterial lifestyle. Some enterobacteria species have two PagP isoforms. In vitro studies indicate that one isoform is able to palmitoylate PG, while the other does not. These two PagP isoforms will help us to dissect the functions of palmitoyl lipid A and palmitoyl PG during host-bacterium interactions.

4,4-Dimethyl-4-silapentane-1-sulfonate (DSS) exhibits concentration-dependent diffusion and chemical shift behaviour in both aqueous and micellar solution

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Sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) is the most widely accepted internal standard for aqueous protein NMR studies. Since its introduction as a chemical shift standard, however, concerns have been raised surrounding its propensity to interact with biological molecules through both hydrophobic and electrostatic interactions. While DSS has been shown to interact with nucleotides and proteins, to date no study has explicitly investigated potential interactions between DSS and lipids or detergents. We find that two membrane mimetic systems - sodium dodecyl sulfate (SDS) and dodecyl phosphocholine (DPC) micelles - noticeably decrease the diffusion coefficient of DSS observed in Diffusion Ordered NMR Spectroscopy, with substantial decrease in the case of DPC. This is accompanied by a change in the chemical shift of the 0 ppm-reference methyl peak of DSS. We also show that the diffusion coefficient of DSS decreases as a function of increasing DSS concentration without detergents, and that the peak shape and chemical shift of the 0 ppm-reference methyl peak of DSS relative to water vary with DSS concentration. Similar results were obtained for the alternative reference compound 4,4-dimethyl-4-silapentane-1-ammonium trifluoroacetate (DSA). Taken together, these findings show that DSS and DSA each interact with detergent micelles, and also have the previously unreported possibility of forming aggregates at high concentration. Chemical shift variation for these two molecules has clear implications for their use as reference standards.

Reoviruses FAST proteins recruit ESCRT machinery to generate fusogenic FAST-exosomes

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Almost all viruses encode at least one small membrane-associated protein, collectively referred to as viroporins (e.g. HIV vpu, influenza virus M2, hepatitis C virus p7, poliovirus 2B). This subclass of viral proteins are adept at remodelling cell membranes and reprogramming membrane biogenesis pathways to promote virus replication, assembly and release. The reovirus fusion-associated small transmembrane (FAST) proteins are a unique subgroup of viroporins that induce cell-cell fusion and syncytium formation, promoting virus dissemination and pathogenesis. A yeast two-hybrid screen identified components of the exosome pathway as genetic interaction partners of the FAST proteins. OptiPrep gradients, immunoblotting, biochemical assays, inhibitor studies and electron microscopy were further performed. We discovered that FAST proteins upregulate exosome biogenesis and the release of fusogenic FAST-protein exosomes. Results indicate the p14 FAST protein interacts with components of the ESCRT machinery (Tsg101 and Alix) and Rab-dependent vesicular trafficking pathways, and inhibitor studies revealed that both of these pathways are involved in the generation of FAST protein-exosomes. Most interestingly, reovirus particles are released as infectious vesicle-encapsulated nonenveloped virus (VENEV) particles. These results indicate FAST proteins promote reovirus dissemination and pathogenesis by inducing formation of both syncytia and infectious VENEVs.
Characterizing the structural and dynamic aspects of large fragments of the apelin receptor involved in trafficking and ligand binding.

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G-protein coupled receptors (GPCRs) represent, pharmacologically, the most important class of membrane proteins that modulate various signaling cascades that play a pivotal role in cellular functions. The apelin receptor (AR or APJ) is a class-A GPCR activated by various isoforms of the peptide hormones apelin and apela. The apelin-AR system has been proposed as a therapeutic target for cardiovascular disease, central nervous system disorders and various cancers. Because GPCRs are inherently dynamic, nuclear magnetic resonance (NMR) spectroscopy is well-suited to characterization of both the structural aspects and dynamic effects of ligand binding. NMR, however, requires milligram quantities of protein enriched with 15N, 13C, and or 2H isotopes. Full-length isotope-enriched GPCRs are very challenging to produce; therefore, we are employing the “divide and conquer” approach to study the AR in pieces. We have, for the first time, demonstrated the use of small gene tags to enhance the expression of the first three transmembrane segments with the N-terminal tail of the AR (TM1-3) in E. coli. Purified AR TM1-3 is now being studied in various membrane-mimetic environments. Currently, we are solving the 3D solution-state NMR structure in trifluoroethanol:water (1:1 v:v) and optimizing the conditions to study the receptor fragment in both micelles and bicelles. We have also expressed, isoipe-enriched and purified the 70 amino acid intracellular C-terminal tail of the AR to probe its structure and dynamics. Our biophysical data indicate the presence of a membrane-proximal 8th helix upon exposure to membranous environments. This helix is, putatively, common to class-A GPCRs but poorly characterized from a structure-function perspective. The correlation between structure, membrane-association and function in receptor trafficking for the 8th helix is being investigated.

Cargo Selection by Activated Rab11 Direct Interaction with a Novel Tribasic Golgi Export Signal Directs AP1-dependent Golgi-Plasma Membrane Trafficking

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Sorting and trafficking of integral membrane proteins to the plasma membrane is essential for cellular homeostasis. Our understanding of the pathways and sorting signals that regulate protein trafficking is far from complete, particularly as it relates to protein exit from the Golgi. The reovirus fusion-associated small transmembrane (FAST) proteins traffic through the ER-Golgi pathway to the plasma membrane where they cause cell-cell membrane fusion. We recently reported that the polybasic motif (PBM) in the cytosolic tail of reptilian reovirus p14 FAST protein is a novel tribasic autonomous Golgi export signal. In attempt to analyze cellular factors regulating Golgi export of p14, yeast two-hybrid analysis identified Rab11A as a genetic interaction partner of p14. Co-immunoprecipitation (co-IP) determined that p14 interacts preferentially with GTP-bound activated Rab11A in a PBM-dependent manner. Overexpression of dominant-negative Rab11A, but not Rab5, significantly reduced p14 surface expression. Fluorescence resonance energy transfer (FRET) microscopy indicated activated Rab11 directly interacts with p14 dependent on the PBM, the first example of activated Rab11 directly interacting with membrane cargo for Golgi-plasma membrane trafficking. Furthermore, RNA interference revealed that both Rab11 and adaptor protein 1 (AP1), but not AP3 or AP4, are required for efficient p14 trafficking from the trans-Golgi network (TGN) to the plasma membrane. This is also the first indication of Rabs regulating adaptor proteins at the TGN for anterograde vesicle traffic, and provides a clear indication that AP1 can mediate anterograde traffic from the Golgi to the plasma membrane.

Probing Membrane Catalyzed Apelin-Receptor Interactions by Fluorescence Spectroscopy

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Apelin is a peptidic hormone that activates the class A G-protein-coupled apelin receptor. Apelin is found in several bioactive isoforms in the body, ranging from 12 to 55 amino acids in length. Apelin-17 has previously been shown to bind to micelles of anionic detergents, undergoing a membrane-induced structural transition. It has been theorized that membrane-interaction and the induced conformational changes are necessary for peptide recognition by the receptor, while also serving to increase local concentration of the ligand in what is known as the membrane catalysis hypothesis. Here, we describe a method for conjugating both synthetic and recombinant apelin to fluorescent probes. Labelled peptides were shown to maintain bioactivity, as demonstrated by a phosphorylated extracellular signal-regulated kinase (pERK) assay in apelin receptor-transfected human embryonic kidney cells. These peptides were then used in fluorescence experiments to test for membrane-interaction, as required by the membrane catalysis model. Förster resonance energy transfer (FRET) experiments were performed with fluorescently labeled apelin isoforms to native tryptophan in fragments of the apelin receptor to both localize the ligand-receptor binding interface and to quantify the peptide-receptor interaction.
Determining the Role of Oxysterol Binding Protein Related Protein 4 (ORP4) in Cell Proliferation and Survival

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Oxysterol binding protein (OSBP) and related proteins (ORPs) constitute a 12-member family of mammalian lipid binding and transport proteins that are active at different organelles. ORP4 is expressed as a full-length protein (ORP4L) as well as two N-terminally truncated variants (ORP4M and ORP4S) that differ from ORP4L in the absence of an intact PH domain. Cholesterol, oxysterols and phosphatidylinositol 4-phosphate compete for binding to the ORP4 C-terminal lipid-binding domain. ORP4 is the only known ORP required for cell proliferation, but the lipid and protein partners that are required for this proliferative activity are unknown. Lentiviral short-hairpin RNA knockdown of ORP4 in HeLa, HEK 293, HCT116 and 40-16 cells induced growth arrest based on crystal violet staining and MTT viability assay. ORP4 knockdown increased p53 levels in HCT116 and 40-16 cell lines but p53 phosphorylation status was unchanged. However, ORP4 knockdown in 379.2 (p53-/-) and 8054 (p21-/-) cell lines had no significant effect on viability compared to control cells, suggesting that the p53/p21 pathway is not involved. Mass spectrometry was used to identify a putative MAPK phosphorylation site in the C-terminal lipid-binding domain of ORP4L (serines 762, 763, 766 and 768). An ORP4L phospho-mimetic aspartate mutant (ORP4L S762-768D) expressed in HeLa cells was associated with vimentin aggregates not observed in cells expressing the corresponding ORP4L alanine mutant (ORP4L S762-768A) or wild-type ORP4L. A potential role for ORP4L and ORP4M in genomic stability is indicated by their interaction with the centromere assembly protein Mis18B. These data suggest that ORP4L phosphorylation by MAPK may alter interaction with the cytoskeletal and/or genomic regulators to control cell cycle.

Critical Roles and Structural Features of Cell Membrane Fusion Protein Modules

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Cell-cell membrane fusion, orchestrated by protein fusogens, is crucial for numerous biological processes. The reovirus fusion-associated small transmembrane (FAST) proteins are the smallest known autonomous fusogens. All FAST proteins assume a bitopic topology in the plasma membrane, with a single transmembrane domain flanked by small N-terminal ectoplasmic and larger C-terminal endoplasmic domains. All three of these small domains function as fusion modules, and recent NMR studies are revealing critical structure-function relationships essential for FAST protein-mediated membrane fusion. The avian reovirus p10 FAST protein ectodomain (ARV p10_ECTO) contains a small, geometrically constrained, cystine noose that promotes aqueous exposure of hydrophobic residues and functions as a fusion peptide. The a-helical reptilian reovirus p14 FAST protein transmembrane domain (RRV p14_TMD) is inherently flexible due to a degree of curvature and exhibits unexpected interfacial positioning of the hydrophobic and hydrophilic residues, including essential N-terminal β-branched residues and a solvent-accessible cleft. A funnel-shaped TMD architecture, due to clustering of bulky aromatic residues toward the C-terminus, might also promote membrane curvature changes required for fusion pore formation. The baboon reovirus p15 FAST protein contains a cytoplasmic fusion-inducing lipid packing sensor (BRV p15_FLiPS) comprising a helix-loop-helix conformation that is functionally equivalent to, but structurally distinct from, classical amphipathic helices or amphipathic lipid packing sensors. This motif preferentially partitions into hydrophobic defects present in highly curved membranes, such as the rim of fusion pores. Together, these structural motifs function from both sides and within the membrane to mediate cell membrane fusion.
Interaction of the P-glycoprotein multidrug transporter (ABCB1) with sterols

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The ABC transporter P-glycoprotein (Pgp, ABCB1) actively exports structurally diverse substrates from within the lipid bilayer, leading to multidrug resistance. Many aspects of Pgp function are altered by the phospholipid environment, but its interactions with sterols remain enigmatic. We investigated the functional interaction between purified Pgp and various sterols in detergent solution and phospholipid proteoliposomes. Fluorescence studies showed that the fluorescent sterol derivatives, dehydroergosterol, cholestatrienol and NBD-cholesterol, interact intimately with Pgp, resulting in both quenching of protein Trp fluorescence and enhancement of sterol fluorescence. Kd values indicated binding affinities in the 3-9 microM range. Collisional quenching experiments suggested that Pgp-bound NBD-cholesterol was protected from the external milieu, and resonance energy transfer was observed between Pgp Trp residues and the sterol. Cholesterol hemisuccinate altered the thermal unfolding of Pgp, and greatly stabilized its basal ATPase activity in both detergent solution and reconstituted proteoliposomes of certain phospholipids. Other sterols, including dehydroergosterol, did not stabilize the basal ATPase activity of detergent-solubilized Pgp, which suggests that this is not a generalized sterol effect. The phospholipid composition and cholesterol hemisuccinate content of Pgp-proteoliposomes altered the basal ATPase and drug transport cycles differently. A search for CRAC/CARC motifs indicated that Pgp contains several putative consensus cholesterol-binding motifs located at different depths facing the lipid bilayer, some close to the gates of the substrate-binding pocket. These results indicate that a variety of different sterols interact with Pgp to produce significant modulatory effects on its structure, stability, enzymatic activity and transport function, possibly by binding to certain regions of the protein.

Hereditary Spastic Paraplegia: Modeling Mutations Found in the Glycosphingolipid-Metabolizing Enzyme β-glucosidase 2 (GBA2)

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Patients presenting with a combination of spastic paraplegia and cerebellar ataxia (SPastic Gait locus #46, SPG46) have recently been found to carry mutations in the GBA2 gene, which encodes β-glucosidase 2, an enzyme that degrades the membrane lipid glucosylceramide. SPG46 is a complex neurodegenerative/neurological disorder, with muscle weakness and spasticity in the upper and lower limbs, and cerebellar and cerebral atrophy, thin corpus callosum, peripheral neuropathy and cognitive impairment. The affected patients were first diagnosed early in life (median age 7, range 1-20). Currently, the cascade of events leading from mutations in the GBA2 gene to SPG46 is largely unexplored. Experimental Approach: We have generated cDNAs coding for five truncated and five missense GBA2 mutants found in SPG46 patients. COS-7 and HeLa cells were transfected with the mutant cDNAs, and analyzed for transfection efficiency, protein expression, enzyme activity, and subcellular localization. Results: All GBA2 truncated and point mutants were catalytically completely inactive. The lack of enzyme activity was not due to poor transfection efficiency or decreased protein expression, which were confirmed by luciferase reporter assay and western blotting, respectively. Immunofluorescence staining of the cells overexpressing the truncated mutants gave a punctate pattern of staining ranging from smaller to larger rounded structures, which are yet to be identified. Conclusion: The absence of enzyme activity is shared among ten GBA2 variants carrying mutations identified in SPG46 patients. Further analysis is required to understand the subcellular localization of the GBA2 mutants and consequences of these mutations for the development and progress of SPG46.

Glucolipotoxicity reduces lysosomal protein expression and impairs autophagy in the obese-diabetic mouse heart

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Background: Loss of insulin action in obese-diabetic hearts disrupts cardiac metabolism leading to glucotoxicity and lipotoxicity. Glucolipotoxicity induces cardiac injury by causing mitochondrial dysfunction and endoplasmic reticulum stress. Recent studies reported that hearts from obese-diabetic patients exhibit toxic accumulation of protein aggregates, indicating that lysosomal macroautophagy and/or chaperone mediated autophagy (CMA) are impaired. The central regulator of lysosomal function and macroautophagy is transcription factor EB (TFEB), and the rate limiting step for CMA is the binding of lysosomal associated membrane protein-type 2A (LAMP-2A) to proteins targeted for degradation. Whether glucolipotoxicity targets lysosomal proteins to impair proteolytic degradation remains to be examined. Hypothesis: Glucolipotoxicity down-regulates TFEB and LAMP-2A in the obese-diabetic heart to suppress lysosomal autophagy. Methods and Results: C57BL6 mice were fed either chow (4% kcal from fat) or high fat-high sucrose (HFHS) diet (45% kcal from fat) for 15 weeks. HFHS-fed mice exhibited increased fat mass, insulin resistance and hyperglycemia. Furthermore, systolic and diastolic function were depressed in hearts from HFHS-fed mice with concomitant increases in cardiac triacylglycerol, ceramides, and long chain acyl CoAs, indicators of lipotoxicity. Immunoblot analysis of heart lysates revealed significant down-regulation of LC3-BII, a marker for macroautophagy with concomitant reduction in TFEB, LAMP-2A. Hsc70 and Hsp90 protein expression signifying an impairment in lysosomal proteolysis. Notably, protein expression of activating phosphorylation of mammalian target of rapamycin (mTOR) and its downstream effectors S6 kinase and S6 were increased by 3-4 fold with concomitant increases in inactivating phosphorylation of TFEB in hearts from HFHS-fed mice, suggesting that TFEB inactivation and autophagy inhibition is secondary to mTOR activation.
Significance: In the obese-diabetic heart, glucolipotoxicity inhibits lysosomal protein expression to disrupt cardiomyocyte proteostasis, thereby causing cardiac injury.

Determining the role of phosphatidylinositol phosphate kinase homolog in phosphoinositide metabolism and endosomal transport

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The generation of phosphatidylinositol 4,5-bisphosphate (PI4,5P2) by phosphatidylinositol 4-phosphate 5-kinases (PIP5Ks) is essential for many of the functions of the plasma membrane. Recent evidence has demonstrated roles for this lipid in the endocytic pathway. The endosomal system consists of morphologically, compositionally and functionally distinct domains that are classified as ‘early’ and ‘late’ because they are sequentially accessed by tracers that are internalized by receptor-mediated or bulk-flow endocytosis. The mechanisms controlling the endosomal pool of PI4,5P2 remain poorly understood. Phosphatidylinositol phosphate kinase homolog (PIPKH) was identified based on sequence similarity to the PIP5K isoforms although it does not have intrinsic PIP5K activity itself. However, PIPKH co-precipitate with PIP5K isoforms suggesting that PIPKH might function to regulate the activity or subcellular distribution of the PIP5Ks. Transiently expressed mCherry-PIPKH localizes to membrane structures clustered around the nucleus. Overexpressing of mCherry-PIPKH led to the recruitment of GFP-PIP5K isoforms (alpha, beta and gamma) to these perinuclear structures. These structures were also positive for the type II phosphatidylinositol 4-kinas suggesting that they are competent for synthesizing both PI4P and PI4,5P2. Surprisingly, we find that the structures are positive for PI3,4,5P3 and not PI4P or PI,45P2. The PIPKH positive structures also contained Rab11, Rab5, and Rab7 markers of recycling, early and late endosomes. Transiently expressed GFP-RILP-C33, a marker of active Rab7, also localized to PIPKH structures. Currently, it is unclear if these are clustered endosomes remain distinct or if they comprise an aberrant hybrid endosomal compartment. Preliminary experiments suggest that PIPKH and/or the presence of PI3,4,5P3 on endosomes alters their identity of these organelles that either over-stimulates dynein mediated transport towards the microtubule organizing center or prevents the ability of kinesin motors to transport these endosomes away from the nucleus.

Examining the role of the outer membrane protein PagP in cell signaling in Escherichia coli.

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Bacterial antibiotic resistance defense mechanisms include outer membrane modification of lipopolysaccharide (LPS). PagP is an outer membrane protein in Escherichia coli that palmitoylates lipid A, the anchor for LPS at the external leaflet of the outer membrane. PagP transfers a palmitate group on the R-3-hydroxymyristate chain at position 2 of lipid A. Three amino acid residues (H31, S77 and R114) are involved in the palmitoyltransferase activity of the cell surface. In its periplasmic domain, PagP also exhibits a putative catalytic triad (D61, H67 and Y87), which has been implicated in cell signaling. PagP is dormant in wild type cells, but the lptD4213 mutant activates PagP by enabling phospholipid migration to the cell surface. Eight genetic constructs were previously prepared. The extracellular catalytic residue mutation S77A, the putative catalytic triad Y87F, wild type PagP and negative control Bad18 were transduced independently into ΔpagP and lptD4213/ΔpagP mutant backgrounds. To test cell signaling, two mutants and two control samples were submitted to RNA-sequencing. The differentially expressed genes were selected based on the Log2FoldChange > -2 and +2, and the p adjusted value ≤ 0.05. The results of the RNA sequencing confirmed upon comparing Y87F versus wild type that PagP's putative
periplasmic triad is indeed connected to processes that occur in the cytoplasm, in addition to other cellular compartments. Ten genes were down-regulated and their products are related to aerobic respiration. Forty genes were up-regulated, the majority of which are related to anaerobic respiration. In order to elucidate whether or not PagP can synthesize a molecule that could be used as a second messenger, the in vitro activity of PagP was explored in various detergent systems. We are presently examining how PagP modification of phospholipids correlates with biological signal transduction mechanisms.
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