MSCBMP2840 - Regulation of Membrane Traffic
2014 Course Syllabus

Organizers:

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Focus of the class:

While the focus of this course is to analyze membrane/protein traffic along both the biosynthetic and endocytic pathways, our general goal is to teach students how to read and interpret the literature. In particular, we emphasize the conclusions of the assigned papers, examine the experimental basis of these conclusions, and discuss their validity. The course is updated each year to include topics in which new and interesting developments have occurred. Emphasis is placed on how membrane traffic is regulated and where applicable how it is disrupted or subverted during disease processes. The course is of general interest to students, fellows, and faculty interested in cell biology, immunology, neurobiology, pharmacology, and virology.

Meeting times and place:

Students are required to attend the Local Traffic Symposium on Membrane Traffic on Thursday the 1st of May (Frick Fine Arts Building Auditorium). You must register using the online application at: https://apps.bio.cmu.edu/localtraffic/.

Class will meet each Thursday from 1:30 PM – 3:30 PM in the Department of Cell Biology Conference Room (South BST Room 362).

The organizational session for the class will be held on Thursday, May 8th from 2:00 – 3:00 PM in the Cell Biology Conference room.

The first class session will be Thursday, May 15th 2014. Your attendance is required at every session.

Structure of the course and how you will be evaluated:

There will be no formal test. Instead, your letter grade will depend on your participation (50%), and an ~30 minute mini-lecture (50%) that you will prepare as an introduction for one of the sessions described below.

Each session begins with a mini-lecture. In your mini-lecture you will provide background information on a designated subject so that any reasonable person can understand *the subject and its significance.* You will *prepare a handout* that your classmates can treasure and use as reference in their future studies. In this handout *include a bibliography citing any reference material* that you found useful in preparing your lecture. The lecture should be *no longer than 30 minutes*. The best way to assess the length of your presentation is to practice it, out loud, before a mock audience. Leave a few minutes for the plethora of questions you will receive. *After reading the papers you can consult with the faculty about topics that should be included in your mini-lecture.*

Following the mini-lecture we begin the discussion of the assigned papers. We always start the session by answering questions about unfamiliar techniques or ideas. No question is stupid, and any question you may have is likely one that other students have as well. We then examine each paper. *Particular emphasis is placed on the hypothesis or questions being answered, the conclusions of the papers, and the experiments that support these conclusions*. We spend time discussing proposed models, and alternative models. Finally, we also discuss ways in which you could test these alternative models.

Participation in these sessions is assessed as follows. We will try our hardest to promote exciting discussion and an open exchange of ideas. In return, we expect that you will be dynamic, excited, and gung-ho about the lectures, presenters, and papers. These qualities will be reflected in the numerous questions you will ask of the other participants. There is no expectation that you will know or understand everything. Participation means that you ask questions when you don't understand or when you want to know more. N.B. Your organizers will ensure that everyone participates.

Class sessions:

Local Traffic Meeting (Thursday May 1st 2014)

This is the annual membrane traffic seminar and students are required to attend this meeting as part of their course requirement. The meeting will take place in the Frick Fine Arts Building on the Pitt campus (across the street from the Carnegie Library). You must register online using the following link:https://apps.bio.cmu.edu/localtraffic/

Organization Session (Thursday, May 8th 2014)

This will be a short session where we discuss course requirements and assign mini-lecture topics.

Session I (May 15th 2014). Protein translocation into the ER. (Dr. Brodsky)

Assigned papers:

Forte GM, Pool MR, Stirling CJ. N-terminal acetylation inhibits protein targeting to the endoplasmic reticulum. PLoS Biol. 2011 May;9(5):e1001073.

Ast T, Cohen G, Schuldiner M. A network of cytosolic factors targets SRP-independent proteins to the endoplasmic reticulum. Cell. 2013 Feb 28;152(5):1134-45.

Reviews:

Mandon EC, Trueman SF, Gilmore R. Protein translocation across the rough endoplasmic reticulum. Cold Spring Harb Perspect Biol. 2013 Feb 1;5(2). pii: a013342.

Hegde RS, Kang SW. The concept of translocational regulation. J Cell Biol. 2008 Jul 28;182(2):225-32.

Topics to include in mini-lecture:

What is a signal sequence, and what roles do they play in translocation? How are signal sequence-containing proteins targeted to the ER membrane? How was SRP discovered and what is its generally accepted mechanism of action in pre-protein targeting? How do SRP-independent substrates translocate into the ER? What is the molecular machinery at the ER membrane that receives and translocates pre-proteins across the ER membrane in yeast and mammals?

Session II (May 22nd 2014). Protein folding and quality control (Dr. Brodsky)

Assigned papers:

Xie W, Kanehara K, Sayeed A, Ng DT. Intrinsic conformational determinants signal protein misfolding to the Hrd1/Htm1 endoplasmic reticulum-associated degradation system. Mol Biol Cell. 2009 Jul;20(14):3317-29.

Mehnert M, Sommer T, Jarosch E. Der1 promotes movement of misfolded proteins through the endoplasmic reticulum membrane. Nat Cell Biol. 2014 Jan;16(1):77-86.

Reviews:

Braakman I, Hebert DN. Protein folding in the endoplasmic reticulum. Cold Spring Harb Perspect Biol. 2013 May 1;5(5):a013201.

Brodsky JL. Just a trim, please: refining ER degradation through deubiquitination. Cell. 2013 Aug 1;154(3):479-81.

Topics to include in mini-lecture:

What are molecular chaperones? Which chaperones and enzymes promote protein folding in the ER lumen? How do they facilitate folding? How does protein glycosylation provide a signal that indicates whether proteins are properly folded? How are misfolded proteins identified and routed for destruction?

Session III (May 29th 2014). Exit from the ER (Dr. Aridor)

Assigned papers:

1. Sedlin controls the ER export of procollagen by regulating the Sar1 cycle. Venditti R1, Scanu T, Santoro M, Di Tulio G, Spaar A, Gaibisso R, Beznoussenko GV, Mironov AA, Mironov A Jr, Zelante L, Piemontese MR, Notarangelo A, Malhotra V, Vertel BM, Wilson C, De matteis MA. Science. 2012 Sep 28;337(6102):1668-72.

2. ER cargo properties specify a requirement for COPII coat rigidity mediated by Sec13p. Copic A1, Latham CF, Horlbeck MA, D'Arcangelo JG, Miller EA. Science. 2012 Mar 16;335(6074):1359-62.

Reviews:

1. COPII and the regulation of protein sorting in mammals. Zanetti G1, Pahuja KB, Studer S, Shim S, Schekman R. Nat Cell Biol. 2011 Dec 22;14(1):20-8.

2. Vesicle-mediated export from the ER: COPII coat function and regulation. D'Arcangelo JG1, Stahmer KR, Miller EA. Biochim Biophys Acta. 2013 Nov;1833(11):2464-72.

Topics to include in mini-lecture:

What are coat proteins? How were they identified and described? What are the budding sites between the ER and the Golgi? What is the composition of COPII? How is COPII assembled? How is cargo being selected? What are export motifs? How is luminal cargo such as procollagen selected? What is the role of the GTPase activity of Sar1 in coat assembly and cargo selection? How is the GTPase activity regulated? How is COPII compared to other coats?

Session IV (June 5th 2014). Vesicle targeting and fusion (Dr. Aridor)

Assigned papers:

1. SNARE proteins: one to fuse and three to keep the nascent fusion pore open. Shi L1, Shen QT, Kiel A, Wang J, Wang HW, Melia TJ, Rotherman JE, Pincet F. Science. 2012 mar 16;335(6074):1355-9.

2. Complexin cross-links prefusion SNAREs into a zigzag array. Kümmel D1, Krishnakumar SS, Radoff DT, Li F, Giraudo CG, Pincet F, Rothman JE, Reinisch KM. Nat Struct Mol Biol. 2011 July 24;18(8):927-33.

Reviews:

1. Ungar, D. and Hughson, F.M. 2003. SNARE protein structure and function. Annu. Rev. Cell Dev. Biol. 19:493-517.

2. Südhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM Proteins. Science. 323(5913):474-7, 2009.

Topics to include in mini-lecture:

What are SNARE proteins and how are they organized within the cell? What is the role of NSF and α-SNAP proteins? What is the SNARE motif? How do SNARE proteins assemble and what is the structure of the SNARE core complex? What are the requirements for membrane fusion? How do viral proteins promote membrane fusion? How would SNARE proteins cause membrane fusion? What are clostridial toxins? How do we know that SNAREs mediate membrane fusion in biological systems? Can SNARE proteins account for the specificity of membrane fusion? How is SNARE mediated fusion regulated?

Session V (June 12th 2014). Biogenesis of ERGIC & Golgi (Dr. Linstedt)

Assigned papers:

Kurokawa et al (2014). Contact of cis-Golgi with ER exit sites executes cargo capture and delivery from the ER. Nature Commun. 5:3653

Rizzo et al. (2013). The dynamics of engineered resident proteins in the mammalian Golgi complex relies on cisternal maturation. JCB 201:1032

Reviews:

Brandizzi and Barlowe (2013) Organization of the ER-Golgi interface for membrane traffic. Nature reviews 14:382.

Glick and Luini (2011). Models for Golgi Traffic: A critical assessment. Cold Spring Harbor Perspective. http://cshperspectives.cshlp.org/cgi/doi/10.1101/cshperspect.a005215.

Topics to include in mini-lecture:

What are the ERGIC (aka VTCs) and Golgi apparatus: their key functions, their key structural features? Compare and contrast cisternal maturation and vesicle transport models. What key testable hypotheses does each model suggest and how do these stack up against the data that has accumulated? What are the underlying mechanisms that establish and maintain these compartments? That is, for sorting: which coats and what are their cargo specificities? For membrane fusion: which SNAREs and what are their localizations?

Session VI (June 19th 2014). Golgi structure/function (Dr. Linstedt)

Assigned papers:

Farber-Katz et al. (2014) DNA Damage Triggers Golgi Dispersal via DNA-PK and GOLPH3. Cell 156:413.

D'Angelo et al. (2013). Vesicular and non-vesicular transport feed distinct glycosylation pathways in the Golgi. Nature 501:116.

Reviews:

Cancino and Luini (2013) Signaling Circuits on the Golgi Complex. Traffic 14:121.

De Matteis et al. (2014). Phosphatidylinositol-4-phosphate: The Golgi and beyond. BioEssays. 35-612.

Topics to include in mini-lecture:

Review Golgi structural features. Review Golgi functions including glycosylation and roles in various signaling pathways. Relate Golgi structural features to its various functions.

Session VII (June 26th 2014). Clathrin-independent endocytosis (Dr. Apodaca)

Assigned papers:

Kumari S and Mayor S (2008). ARF1 is directly involved in dynamin-independent endocytosis. Nature Cell Biology 10:30-41.

Prosser DC, Drivas TG, Maldonado-Báez L, and Wendland B (2011). Existence of a novel clathrin-independent endocytic pathway in yeast that depends on Rho1 and formin. Journal of Cell Biology 195:657-671.

Reviews:

Howes MT, Mayor S, and Parton RG (2010). Molecules, mechanisms, and cellular roles of clathrin-independent endocytosis. Current Opinion in Cell Biology 22:519-527.

Maldonado-Báez L, Williamson C, and Donaldson J.G. (2013). Clathrin-independent endocytosis: a cargo-centric view. Experimental Cell Research 319:2759-2769.

Topics to include in mini-lecture:

What is clathrin-independent endocytosis? How many forms of clathrin-independent endocytosis have been described and how are they defined? What molecules are associated with clathrin-independent endocytosis? What is known about the mechanisms by which clathrin-independent carriers are generated? What are the functions of clathrin-independent endocytosis? What is the fate of cargoes internalized by clathrin-independent endocytosis?

Session VIII (July 3rd 2014). Compensatory endocytosis (Dr. Apodaca)

Assigned papers:

Watanabe S., Rost BR, Camacho-Pérez M, Davis, M.W., Söhl-Kielczynski B, Rosenmund C, and Jorgensen EM (2013). Ultrafast endocytosis at mouse hippocampal synapses. Nature 504:242-247.

Yao CK, Lin YQ, Ly CV, Ohyama T, Haueter CM et al. A synaptic vesicle-associated Ca^{2+} channel promotes endocytosis and couples exocytosis to endocytosis. Cell 138:947-960.

Reviews:

Wu L-G, Hamid E, Shin W, and Chiang H-C (2014). Exocytosis and endocytosis: modes, functions, and coupling mechanisms. Annual Review of Physiology 76:301-331.

Topics to include in mini-lecture:

What is compensatory endocytosis (CE) and why is it important? By which pathways is membrane recovered during CE? What are the functions of CE? How is CE regulated? By what mechanisms is CE coupled to exocytosis?

Session IX (July 10th 2014). Clathrin-mediated endocytosis (Dr. Sorkin)

Assigned papers:

Doyon JB, Zeitler B, Cheng J, Cheng AT, Cherone JM, Santiago Y, Lee AH et al (2011) Rapid and efficient clathrin-mediated endocytosis revealed in genome-edited mammalian cells. Nat Cell Biol 13(3):331–337

Taylor MJ, Perrais D, Merrifield CJ (2011) A high precision survey of the molecular dynamics of mammalian clathrin-mediated endocytosis. PLoS Biol 9(3):e1000604

Reviews:

McMahon HT, Boucrot E (2011) Molecular mechanism and physiological functions of clathrin- mediated endocytosis. Nat Rev Mol Cell Biol 12(8):517–533

Topics to include in mini-lecture:

What is the structure of clathrin-coated pit and vesicle? What machinery does a

clathrin-coated vesicle need to package to assure proper downstream functioning? What is the kinetics of clathrin-dependent endocytosis? What are the molecular determinants that promote cargo recruitment into clathrin coated pits?

Session X (July 17th 2014). BAR-domain proteins and endocytosis (Dr. Sorkin)

Assigned papers:

McKanna JA, Haigler HT, Cohen S. Hormone receptor topology and dynamics: morphological analysis using ferritin-labeled epidermal growth factor. Proc Natl Acad Sci U S A. 1979;76(11):5689-93.

Stringer DK & Piper RC (2011) A single ubiquitin is sufficient for cargo protein entry into MVBs in the absence of ESCRT ubiquitination. J Cell Biol 192(2):229-242.

Review:

Wollert T, Hurley JH. Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. Nature. 2010;464(7290):864-9. Epub 2010/03/23.

Henne WM, Buchkovich NJ, Emr SD. The ESCRT pathway. Developmental cell. 2011;21(1):77-91. Epub 2011/07/19.

Topics to include in mini-lecture:

What is multi-vesicular bodies (MVB) or endosomes? How morphological analysis of endocytosis led to dissection of the molecular mechanisms of MVB sorting? What is the role of ubiquitination in endosomal sorting? What is the physiological role of MVB sorting?

Session XI (July 24th 2014). Glycosylation and cell function (Dr. Weisz)

Assigned papers:

Cha, S-K et al. 2008. Removal of sialic acid involving Klotho causes cell-surface retention of TRPV5 channel via binding to galectin-1. Proc. Natl. Acad. Sci. USA. 105:9805-9810.

Leunissen, EHP et al. 2013 The epithelial calcium channel TRPV5 is regulated differentially by Klotho and sialidase. J. Biol. Chem. 288:29238-29246.

Review:

Dennis, J.W. et al. 2010. Adaptive regulation at the cell surface by N-glycosylation. Traffic. 1569-1578.

Topics to include in mini-lecture:

Describe the pathways for assembling N- and O-linked glycans. What are the known functions of glycans? Describe examples of how glycan structures are known to change during development or differentiation. What are galectins? What is Klotho?

Session XII (July 31, 2014). Membrane remodeling and actin dynamics during cell migration (Dr. Kwiatkowski)

Assigned papers:

Exo70 generates membrane curvature for morphogenesis and cell migration.
Zhao Y, Liu J, Yang C, Capraro BR, Baumgart T, Bradley RP, Ramakrishnan N, Xu X, Radhakrishnan R, Svitkina T, Guo W.
Dev Cell. 2013 Aug 12;26(3):266-78.

Exo70 stimulates the Arp2/3 complex for lamellipodia formation and directional cell migration.
Liu J, Zhao Y, Sun Y, He B, Yang C, Svitkina T, Goldman YE, Guo W.
Curr Biol. 2012 Aug 21;22(16):1510-5.

Reviews:

Exorcising the exocyst complex.
Heider MR, Munson M.
Traffic. 2012 Jul;13(7):898-907.

New insights into the regulation and cellular functions of the ARP2/3 complex.
Rotty JD, Wu C, Bear JE.
Nat Rev Mol Cell Biol. 2013 Jan;14(1):7-12.

The Interdependence of Membrane Shape and Cellular Signal Processing.
Schmick M, Bastiaens PI.
Cell. 2014 Mar 13;156(6):1132-1138.

Topics to include in mini-lecture:

What are the basic principles of cell migration? What is the function of the Arp2/3 complex in actin network assembly, and how is Arp2/3 activity regulated (WAVE complex/WASP, Rho-family GTPases, etc.)? What is the exocyst complex? What is its role in membrane remodeling, and why is remodeling critical for cellular functions? How does membrane shape regulate signaling? Why are membrane remodeling and cytoskeletal dynamics linked?