MSCBMP2840 - Regulation of Membrane Traffic, 2022

The specific focus of this 2-credit graduate course is to analyze the mechanisms underlying membrane/protein traffic along both the biosynthetic and endocytic pathways. In particular, we emphasize the conclusions of assigned papers, examine the experimental basis of these conclusions, and discuss their validity. The course is updated to include topics in which new and exciting developments have occurred. Emphasis is placed on how membrane traffic is regulated and where applicable how it is disrupted or subverted in disease.

Specific Topics include: Protein translocation into the ER, Protein folding and quality control, ER dynamics, Unconventional protein secretion, Exit from the ER and Golgi delivery, Membrane tension and exocytosis, Clathrin-mediated endocytosis, Targeting of endocytosed proteins to MVBs, Membrane traffic in disease, Membrane contact sites, Regulation of endocytosis and protein degradation, and Clathrin-independent endocytosis, Lysosome-dependent degradation

Meeting times and place:

The class will meet each **Tuesday from 1:30-3:30** in the Department of Cell Biology Conference Room (BST South, Room 362) from **MAY 3rd – JULY 26th** (Students are permitted to miss one session)

If needed due to an emergency, illness, or travel, please coordinate a Zoom link with the specific instructor on a given day.

We also strongly encourage students to attend the "Local Traffic Symposium on Membrane Traffic" on Thursday the 5th of May (Frick Fine Arts Building Auditorium). To register, please go to http://www.cbp.pitt.edu/localtraffic/

Course Administrator: **Sarah Biancardi – S362 South BST – 412-624-3177-SAB181@pitt.edu**

Organizer: **Jeffrey Brodsky, Ph.D.** – A320 Langley Hall – 661-2527 – jbrodsky@pitt.edu

Participating Faculty:

Gerard Apodaca, Ph.D. – 383-8893 – gla6@pitt.edu
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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 two ~30 minute mini-lectures (50%), which you will prepare as an introduction for 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 PARTICIPANTS:

Samantha Bryce	sbryce2@andrew.cmu.edu
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Session I (May 3, 2022). Protein translocation into the ER. (Dr. Brodsky)

Assigned papers:

Defining the physiological role of SRP in protein-targeting efficiency and specificity.
Costa EA, Subramanian K, Nunnari J, Weissman J**.** Science. 2018 Feb 9;359(6376):689-692. doi: 10.1126/science.aar3607.

The Protease Ste24 Clears Clogged Translocons.
Ast T, Michaelis S, Schuldiner M.Cell. 2016 Jan 14;164(1-2):103-114.

Reviews:

Mechanisms of Sec61/SecY-mediated **protein translocation** across membranes.
Park E, Rapoport TA. Annu Rev Biophys. 2012;41:21-40.

Topics for discussion:

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 does SRP function? 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 10, 2022). Protein exit from the ER. (Dr. Aridor) ***Presenter: Julia***

Assigned papers:

ER-to-Golgi protein delivery through an interwoven, tubular network extending from ER.
Weigel AV, Chang CL, Shtengel G, Xu CS, Hoffman DP, Freeman M, Iyer N, Aaron J, Khuon S, Bogovic J, Qiu W, Hess HF, Lippincott-Schwartz J.
Cell. 2021 Apr 29;184(9):2412-2429.

TANGO1 membrane helices create a lipid diffusion barrier at curved membranes.
Raote I, Ernst AM, Campelo F, Rothman JE, Pincet F, Malhotra V.
Elife. 2020 May 26;9:e57822.

Reviews:

A tango for coats and membranes: New insights into ER-to-Golgi traffic.
Aridor M. Cell Rep. 2022 Jan 18;38(3):110258.

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

Topics to include in mini-lecture:

Topics to include in the mini lecture: What are the cytosolic components of the COPII coat? How are these recruited to membranes? How do coat components interact with each other and how these function in cargo selection, sorting and vesicle formation and release? What is the molecular architecture of the ER exit sites (ERES) and how do

resident ERES proteins (cTAGE5/TANGO1/Sec16) interact with the COPII coat? How do ERES-COPII interactions regulate coat activities? What are cargo receptors? How can cargo regulate vesicle formation? How is COPII recruitment coupled to COPI recruitment and what are functional roles for this coupling? What are Brefeldin A and H89 and how to these drugs regulate traffic?

Session III (May 17, 2022). Unconventional protein secretion. (Dr. Linstedt) ***Presenter: Aidan***

Assigned papers:

Steringer JP, Lange S, Čujová S, Šachl R, Poojari C, Lolicato F, Beutel O, Müller HM, Unger S, Coskun Ü, Honigmann A, Vattulainen I, Hof M, Freund C, Nickel W. Key steps in unconventional secretion of fibroblast growth factor 2 reconstituted with purified components. Elife. 2017 Jul 19;6. pii: e28985. PMCID: PMC5601999.

Dimou E, Cosentino K, Platonova E, Ros U, Sadeghi M, Kashyap P, Katsinelos T, Wegehingel S, Noé F, García-Sáez AJ, Ewers H, Nickel W. Single event visualization of unconventional secretion of FGF2. J Cell Biol. 2019 Feb 4;218(2):683-699. PMCID: PMC6363455.

Reviews:

Rabouille C. Pathways of Unconventional Protein Secretion. Trends Cell Biol. 2017 Mar;27(3):230-240. PMID: 27989656.

Cruz-Garcia D, Malhotra V, Curwin AJ. Unconventional protein secretion triggered by nutrient starvation. Semin Cell Dev Biol. 2018 Nov;83:22-28. PMID: 29486236.

Gee HY, Kim J, Lee MG. Unconventional secretion of transmembrane proteins. Semin Cell Dev Biol. 2018 Nov;83:59-66. PubMed PMID: 29580969.

Topics to include in mini-lecture:

General overview of UPS with focus on types. How does UPS contrast with conventional? What are the categories of UPS? What are the reasons for UPS (i.e. what advantages might it confer for secretion of its cargo)? What is the relationship of cellular stress and UPS? What are the cargoes and mechanisms for type 1? What is FG2: its function, its structure? What are the cargoes and mechanisms for types 3&4 of UPS? Functional significance? Role of stress?

Session IV (May 24, 2022). Membrane tension and exocytosis. (Dr. Apodaca) ***Presenter: Kathryn***

Assigned papers:

Gauthier NC, Fardin MA, Roca-Cusachs P, Sheetz MP. Temporary increase in plasma membrane tension coordinates the activation of exocytosis and contraction during cell spreading. Proc Natl Acad Sci U S A. 2011 Aug 30;108(35):14467-72. doi: 10.1073/pnas.1105845108. Epub 2011 Aug 1. PMID: 21808040; PMCID: PMC3167546.

Lemière J, Ren Y, Berro J. Rapid adaptation of endocytosis, exocytosis, and eisosomes after an acute increase in membrane tension in yeast cells. Elife. 2021 May 13;10:e62084. doi: 10.7554/eLife.62084. PMID: 33983119; PMCID: PMC9045820.

Reviews:

Le Roux AL, Quiroga X, Walani N, Arroyo M, Roca-Cusachs P. The plasma membrane as a mechanochemical transducer. Philos Trans R Soc Lond B Biol Sci. 2019 Aug 19;374(1779):20180221. doi: 10.1098/rstb.2018.0221. Epub 2019 Jul 1. PMID: 31431176; PMCID: PMC6627014.

Pontes B, Monzo P, Gauthier NC. Membrane tension: A challenging but universal physical parameter in cell biology. Semin Cell Dev Biol. 2017 Nov;71:30-41. doi: 10.1016/j.semcdb.2017.08.030. Epub 2017 Aug 26. PMID: 28851599.

Topics to include in mini-lecture:
What is exocytosis? What is regulated vs constitutive exocytosis? What is membrane tension and how is it regulated? What impact does tension have on exocytosis and endocytosis? How do you measure membrane tension?

Session V (May 31, 2022). Protein folding and quality control in the secretory pathway. (Dr. Brodsky) ***Presenter: Julia, Samantha, and Aidan***

Assigned papers:

Structural basis of ER-associated protein degradation mediated by the **Hrd1** ubiquitin ligase complex.
Wu X, Siggel M, Ovchinnikov S, Mi W, Svetlov V, Nudler E, Liao M, Hummer G, Rapoport TA.Science. 2020 Apr 24;368(6489):eaaz2449.

Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER proteostasis environments.
Shoulders MD, Ryno LM, Genereux JC, Moresco JJ, Tu PG, Wu C, Yates JR 3rd, Su AI, Kelly JW, Wiseman RL. Cell Rep. 2013 Apr 25;3(4):1279-92.

Reviews:

Protein quality control in the secretory pathway. Sun Z, Brodsky JL.J Cell Biol. 2019 Oct 7;218(10):3171-3187.

Topics to include in mini-lecture:

What are some key molecular chaperones in the ER and how do they function? Which chaperones and enzymes promote protein folding in the ER lumen? How are misfolded proteins identified and routed for destruction (i.e., what is the ERAD pathway)? What is the Unfolded Protein Response (UPR), and how is it activated? What does the UPR do? How might ERAD and the UPR be targeted to treat various diseases?

Session VI (June 7, 2022). Membrane contact sites. (Dr. Hammond) ***Presenter: Alexa***

Assigned papers

Sohn M, Korzeniowski M, Zewe JP, Wills RC, Hammond GRV, Humpolickova J *et al.* PI(4,5)P2 controls plasma membrane PI4P and PS levels via ORP5/8 recruitment to ER–PM contact sitesRegulation of PM PI(4,5)P2 levels via ORP5/8. *J Cell Biology* 2018; 217: 1797–1813.

Ghai R, Du X, Wang H, Dong J, Ferguson C, Brown AJ *et al.* ORP5 and ORP8 bind phosphatidylinositol-4, 5-biphosphate (PtdIns(4,5)P2) and regulate its level at the plasma membrane. *Nat Commun* 2017; 8: 757.

Reviews:

Prinz WA, Toulmay A, Balla T. The functional universe of membrane contact sites. Nat Rev Mol Cell Bio 2020; 21: 7–24.

Topics to include in mini-lecture:

What are membrane contact sites (MCS)? What common features are found in proteins that form them? What functions are associated with MCS? What is a lipid transfer protein? What determines the direction of lipid transport? Why does lipid transfer occur at MCS?

NOTE: On June 14th, there is no class, but we strongly urge you to attend the Department seminar in Cell Biology at 11 AM in the 5th Floor Board Room in the Eye and Ear Institute. Details to follow…

Session VII (June 21, 2022). Clathrin independent exocytosis. (Dr. Apodaca) ***Presenter: Sofya***

Assigned papers:

Watanabe S, Mamer LE, Raychaudhuri S, Luvsanjav D, Eisen J, Trimbuch T, Söhl-Kielczynski B, Fenske P, Milosevic I, Rosenmund C, Jorgensen EM. Synaptojanin and Endophilin Mediate Neck Formation during Ultrafast Endocytosis. Neuron. 2018 Jun 27;98(6):1184-1197.e6. PMCID: PMC6086574

López-Hernández T, Takenaka KI, Mori Y, Kongpracha P, Nagamori S, Haucke V, Takamori S. Clathrin-independent endocytic retrieval of SV proteins mediated by the clathrin adaptor AP-2 at mammalian central synapses. Elife. 2022 Jan 11;11:e71198. doi: 10.7554/eLife.71198. PMID: 35014951; PMCID: PMC8752090.

Reviews:

Ferreira APA, Boucrot E. Mechanisms of Carrier Formation during Clathrin- Independent Endocytosis. Trends Cell Biol. 2018 Mar;28(3):188-200. doi: 10.1016/j.tcb.2017.11.004. Epub 2017 Dec 11. PMID: 29241687.

Renard HF, Boucrot E. Unconventional endocytic mechanisms. Curr Opin Cell Biol. 2021 Aug;71:120-129. doi: 10.1016/j.ceb.2021.03.001. Epub 2021 Apr 13. PMID: 33862329.

Optional review: Watanabe S, Boucrot E. Fast and ultrafast endocytosis. Curr Opin Cell Biol. 2017 Aug;47:64-71. doi: 10.1016/j.ceb.2017.02.013. Epub 2017 Apr 6. Review. PubMed PMID: 28391090.

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 dynamin and what are synaptojanin and endophilin? 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 (June 28, 2022). Membrane traffic in disease. (Dr. Weisz) ***Presenter: Samantha***

Assigned papers:

Issler N et al. (2022) A founder mutation in EHD1 presents with tubular proteinuria and deafness. *J. Amer. Soc. Nephrol*. 33: 732–745.
https://doi.org/10.1681/ASN.2021101312

Reviews:

Nielsen R et al. (2016) Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. *Kidney Intl*. 89: 58–67.
http://dx.doi.org/10.1016/j.kint.2015.11.007

Topics to include in mini-lecture:

Describe the basic structure of the kidney nephron and the functions of the proximal tubule and the inner ear. Explain the difference between glomerular vs tubular proteinuria and possible causes for each. Review our current understanding of the organization and regulation of the apical endocytic pathway in the proximal tubule. Describe the structure, distribution, and function of EHD1 and related family members. What are the unique challenges of studying endocytic trafficking in kidney cells in vivo and in vitro? What are the biggest open questions that remain unresolved?

Session IX (July 5, 2022). ER dynamics. (Dr. Lee) ***Presenter: Crystal***

Assigned papers:

Zheng et al, ER proteins decipher the tubulin code to regulate organelle distribution, 2021, Nature 601(7891):132-138. DOI: 10.1038/s41586-021-04204-9

A role for endoplasmic reticulum dynamics in the cellular distribution of microtubules, 2022, PNAS 119(15) DOI: 10.1073/pnas.2104309119

Reviews:

Westrate et al, Form follows function: The importance of endoplasmic reticulum shape, 2015, AnnRevBiochem 84:791-811. DOI: 10.1146/annurev-biochem-072711-163501

Topics to include in mini-lecture:

What are some essential ER functions? What is the structure of the ER? What are the structural and functional differences between peripheral ER sheets and tubules? What are some of the proteins implicated in ER structuring and what are the current models for how ER sheets, tubules and 3-way junctions are generated and maintained? What types of dynamic behavior does the ER network exhibit? What are the basics of microtubule (MT) polymerization and organization in mammalian cells? What roles do MTs play in ER network structure and distribution?

Session X (July 12, 2022). Clathrin mediated endocytosis. (Dr. Sorkin) ***Presenter: Elif***

Assigned papers:

Sochacki KA, Dickey AM, Strub MP, Taraska JW. Endocytic proteins are partitioned at the edge of the clathrin lattice in mammalian cells. Nat Cell Biol. 2017 Apr;19(4):352-361. doi: 10.1038/ncb3498.

Taylor MJ, Perrais D, Merrifield CJ. A high precision survey of the molecular dynamics of mammalian clathrin-mediated endocytosis. PLoS Biol. 2011 Mar;9(3):e1000604. doi: 10.1371/journal.pbio.1000604.

Reviews:

Kaksonen M, Roux A. Mechanisms of clathrin-mediated endocytosis. Nat Rev Mol Cell Biol. 2018 May;19(5):313-326. doi: 10.1038/nrm.2017.132

Topics to include in mini-lecture:

What is the structure of a clathrin-coated pit and vesicle? What molecule machinery is assembled on the membrane to assure proper clathrin-mediated endocytosis? What are the kinetics of clathrin-mediated endocytosis? What are the molecular determinants that promote cargo recruitment into clathrin coated pits?

Session XI (July 19, 2022). Targeting of endocytosed proteins to MVBs. (Dr. Sorkin)
Group Presentation: Kathryn, Alexa, Crystal

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 Nov;76(11):5689-93. doi: 10.1073/pnas.76.11.5689.

Quinney KB, Frankel EB, Shankar R, Kasberg W, Luong P, Audhya A. Growth factor stimulation promotes multivesicular endosome biogenesis by prolonging recruitment of the late-acting

ESCRT machinery. Proc Natl Acad Sci U S A. 2019 Apr 2;116(14):6858-6867. doi: 10.1073/pnas.1817898116. Epub 2019 Mar 20. PMID: 30894482

Reviews:

Henne WM, Buchkovich NJ, Emr SD. The ESCRT pathway. Dev Cell. 2011 Jul 19;21(1):77-91. doi: 10.1016/j.devcel.2011.05.015. PMID: 21763610.

Topics to include in mini-lecture:

What are multi-vesicular bodies/endosomes (MVB/MVE)? 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 XII (July 26, 2022). Autophagy (Dr. O'Donnell)
Group Presentation: Elif and Sofya

Assigned papers:

Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. Kazuhiko takeshige, Misuzu Baba, Shigeru Tsuboi, Takeshi Noda, and Yoshinori Ohsumi. The Journal of Cell Biology (1992) 119(2):301.
PMID: 1400575; PMC2289660; DOI: 10.1083/jcb.119.2.301

A protein conjugation system essential for autophagy. N Mizushima, T Noda, T Yoshimori, Y Tanaka, T Ishii, MD George, DJ Klionsky, M Ohsumi, and Y Ohsumi. *Nature* (1998) 395(6700):395.
PMID: 9759731; DOI: 10.1038/26506

An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis. Muriel Marin, Janice Griffith, ester Rieter, Lakshmi Krishnappa, Daniel J. Klionski, and Fluvio Reggiori. *Journal of Cell Biology* (2010) 190 (6): 1005-1022.
PMID: 20855505; PMC3101592; DOI: 10.1083/jcb.200912089

Reviews:

Dynamics and diversity in autophagy mechanisms: lessons from yeast. Hitoshi Nakatogawa, Kuninori Suzuki, Yoshiaki Kamada, and Yoshinori Ohsumi. *Nature Reviews: Molecular and Cell Biology* (2009) 10 (7):458.
PMID: 19491929; DOI: 10.1038/nrm2708

Autophagy in major human diseases. Klionsky, DJ., et al. EMBO J (2021) 40 (19):e108863.
PMID: 34459017; DOI: 10.15252/embj.2021108863

Topics to include in the mini-lecture:
How was autophagy discovered? What conditions induce activation of autophagy? What machinery helps form autophagosomes? Where does autophagic membrane come from? What is the broader physiological impact of defective autophagy induction in organisms?