7.341 The Intracellular Sorting Machinery and Its Involvement in Pathogen Infections and Disease

Fall 2015. Tuesdays 11 am- 1 pm. (Class date and time are flexible.) Room 68-150.
(NOTE: time is flexible and subject to change in response to needs of students)
Instructor: Raïssa Eluère (raissae@mit.edu, 410-330-9115, laboratory of Chris Kaiser)

Course Abstract:

In eukaryotic cells, cellular functions are performed in specific compartments called organelles. For example, DNA is replicated and transcribed in the nucleus, membrane proteins are degraded in the lysosome, and ATP is generated in the mitochondria. This compartmentalization of activities optimizes cellular functions but requires more complex mechanisms of intracellular signaling and transport than used by prokaryotic organisms. For example, in neurons neurotransmitters are delivered to specific locations (synapses) at the ends of axons. How can different molecules have different specific localizations within and outside cells? How can different molecules be secreted at different times and different places? The intracellular sorting machinery, which consists of a large set of protein complexes and is attached to the membranes of vesicles as well as to the plasma membrane, controls the transport of vesicles between organelles and between organelles and the plasma membrane. These protein complexes are conserved from yeast to human and are specialized for different aspects of the transport process. These complexes can also be the targets of bacterial toxins and viruses, and mutations that affect these complexes can cause disease. For example, the SNARE (SNAP REceptor) proteins mediate the fusion of vesicles with their target membranes and are themselves the target of the botulinum and tetanus bacterial toxins. The ESCRT (Endosomal Sorting Complexes Required for Transport) machinery enables a unique mode of membrane remodeling that results in the budding of membranes away from the cytoplasm. This process is required for the formation of the MVB (MultiVesicular Body) precursor organelle of the lysosome and, interestingly, for the budding of the HIV from the infected host cells. In addition, the neurodegenerative disorder FTD (FrontoTemporal Dementia) can be caused by mutations in the *CHMP2B* gene, which encodes a component of ESCRT complex. The analysis of the intracellular transport system has resulted in four Nobel Prizes between 1970 and 2013 and remains an area of extremely active research. In this course we will discuss past and present experiments that have allowed researchers to discover the cell's sorting machineries, how they are used by pathogens and their involvement in disease. The main goal will be for students to learn how to read and critically interpret the primary scientific literature. This course will provide exposure to a broad range of scientific approaches, including genetics, biochemistry, cell biology and high-resolution microscopy, and their applications in studies of a broad variety of organisms, including yeast, *Drosophila*, mouse and human. Students will visit a research laboratory using advanced live cell imaging tools for the study of the cell's sorting machinery.

Course Format:

In this literature-based course, students will learn to read and critically evaluate primary research articles published in the field of intracellular sorting machinery and its involvement in pathogen infections and disease. Emphasis will be placed on learning how to analyze papers to extract key points, to design and critique experiments, and to understand the relevant techniques in the field.

During each session, we will discuss two scientific articles, with emphasis paced on the principles of experimental design, the use of control experiments and the interpretation of experimental data. At the end of each session, the instructor will present a 10-15 minute introduction to the topic of the following week's assigned papers.

Assignments:

Attendance and participation in weekly discussion:

Attendance is required at all sessions. Students are expected to have read the assigned papers thoroughly and to actively participate in the discussion of each of the assigned papers. The main goals of these discussions will be to determine together THE key figure/table of the paper and the key control(s) of the experiments and to determine if the authors reach the appropriate conclusions given their experimental results. Students are expected to send the instructor at least one question about each assigned paper each week before the day of the session. These questions should help students discuss the paper the next day during the session. These questions, which may be about techniques, vocabulary, foundational concepts, etc,. will be used to begin the discussion each week.

Written Assignment (Due before beginning of session 8: 11/10/15)

Students will be provided with a list of articles that will not be discussed in class. Before our 4th session (10/6/15), each student will select an article and write a 1000-word essay. This essay should include a brief summary of the results and focus on critically evaluating the experiment design, the author's use of appropriate controls, and the author's conclusions and whether alternative interpretations are plausible. Instructors will provide an example critique, which will be briefly discussed in class. The instructor will be available to meet with students to discuss their critique prior to the due date.

Oral presentation: Week 12 (12/08/15)

During the final session of class, each student will give a 15-20 minute oral presentation that should open a class discussion. Presentations will be based on a relevant research article chosen by each student during the semester; articles should be chosen and submitted to the instructor by 11/24/15. Students will use PubMed or a similar database to identify papers relevant to the topic and then select a paper for critical analysis. The instructor will approve paper selections. Students are expected to identify the key experiment and controls and to analyze the interpretations of the results and the overall rigor of the paper. The presentation should also include a discussion about how the paper

is relevant to its field. The instructor will be available to meet with the students to discuss their presentations prior the due date. PowerPoint presentations (no more than 10 slides) are recommended.

Grading:

Grading for this course is pass/fail and will depend on student attendance, preparedness, participation in class discussions, and satisfactory completion of the required assignments.

Week 1 (9/15/12): Course introduction

This first class will serve as an introduction to the course. After we get to know each other, the instructor will provide an overview of the intracellular sorting machinery to give students a basic knowledge of the field. We will also discuss strategies for searching and reading the primary scientific literature. Towards the end of the class, we will introduce the first two papers in preparation for the next class.

Week 2 (9/22/15): The first identification of the machinery of intracellular trafficking

The different organelles of the cell require a system of vesicles to allow the transport of molecules and proteins from one organelle to another. The best known of these pathways is the secretory pathway, in which secreted proteins synthesized in the endoplasmic reticulum (ER) are transferred to the Golgi and later to the plasma membrane for exocytosis. The different organelles are connected by vesicles that bud from the donor organelle and fuse to the recipient organelle or plasma membrane. In this session will see how genetics and *in vitro* biochemistry combined allowed researchers to begin to identify the different proteins responsible for the formation, transport and fusion of the vesicles connecting the different cellular compartments.

- Order of events in the yeast secretory pathway. Novick P, Ferro S, Schekman R. **Cell.** 1981 Aug;25(2):461-9.
- COPII-coated vesicle formation reconstituted with purified coat proteins and chemically defined liposomes. Matsuoka K., Orci L, Amherdt M, Bednarek SY, Hamamoto S, Schekman R, Yeung T. **Cell.** 1998 Apr 17;93(2):263-75.

Week 3 (9/29/15): The sorting signal of the cargo proteins

For vesicles to mediate selective transport of cargo proteins, the coat proteins of the vesicles must distinguish between cargos and resident proteins of the donor organelle. For cargo transmembrane proteins, sorting information is encoded in a cytoplasmic sorting signal that interacts with the coat proteins. Many such signal sequences have been identified for the different coat proteins COP I, II and clathrin. The first paper identifies signal sequences on the cytosolic part of membrane cargos required for the retention of protein in the ER. The second paper identifies a cluster of acid amino acids as a signal sequence for endocytosis and localization in the Golgi.

- Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. Jackson MR, Nilsson T, Peterson PA**. EMBO Journal**. 1990 Oct;9(10):3153-62.
- An acidic sequence within the cytoplasmic domain of furin functions as a determinant of trans-Golgi network localization and internalization from the cell surface.Voorhees P1, Deignan E, van Donselaar E, Humphrey J, Marks MS, Peters PJ, Bonifacino JS**. EMBO Journal**. 1995 Oct 16;14(20):4961-75.

Week 4 (10/6/15): Membrane fusion, the SNARE complex

SNARE (SNAP REceptor) proteins have been proposed to mediate all intracellular membrane fusion events. The first paper uses an artificial liposome system to show that SNARE proteins are both necessary and sufficient for membrane vesicle fusion. The second paper shows how macrophages have generated a novel Golgi-associated SNARE complex to accommodate their requirement for increased cytokine secretion.

- NAREpins: Minimal machinery for membrane fusion. Weber T, Zemelman BV, McNew JA, Westermann B, Gmachl M, Parlati F, Söllner TH, Rothman JE. **Cell**. 1998 Mar 20;92(6):759-72.

- Syntaxin 6 and Vti1b form a novel SNARE complex, which is up-regulated in activated macrophages to facilitate exocytosis of tumor necrosis factor-α. Murray RZ, Wylie FG, Khromykh T, Hume DA, Stow JL. **The Journal of Biological Chemistry**. 2005 Mar 18;280(11):10478-83. Epub 2005 Jan 7.

NO CLASS 10/13/15 (Monday schedule of classes to be held)

Week 5 (10/20/15) Botulinum and tetanus neurotoxins hydrolyze SNARE proteins

By 1993, researchers knew that the anaerobic bacterium *Clostridium* produces several related neurotoxins that block exocytosis of synaptic vesicles in neurons and that are responsible for the clinical manifestations of botulism and tetanus. The papers for this week support the hypothesis that synaptobrevin, syntaxin and SNAP-25 (SNARE proteins) have a direct function in synaptic vesicle exocytosis given the identification of these proteins as targets of clostridial neurotoxins. In the first paper researchers report that the inhibition of neurotransmitter release by clostridium neurotoxins is associated with hydrolysis of syntaxin, strongly supporting the view that syntaxin participates in exocytotic membrane fusion. From the second paper, we will see how the use of neurotoxins helped researchers understand that the three SNARE proteins form a tight complex.

- Botulinum neurotoxin C1 blocks neurotransmitter release by means of cleaving HPC-1/syntaxin. Blasi J, Chapman ER, Yamasaki S, Binz T, Niemann H, Jahn R. **EMBO Journal**. 1993 Dec;12(12):4821-8.

- Synaptic vesicle membrane fusion complex: action of clostridial neurotoxins on assembly. Hayashi T, McMahon H, Yamasaki S, Binz T, Hata Y, Südhof TC, Niemann H. **EMBO Journal**. 1994 Nov 1;13(21):5051-61.

Week 6 (10/27/15): Synaptotagmin: calcium sensor for synaptic vesicles release

In week four, we saw that the SNARE proteins allow vesicle fusion in *in vitro* reconstitution assays. However, vesicle fusion *in vitro* is slow and calcium-independent. These features sharply contrast with those of synaptic transmission, which occurs on a millisecond timescale and relies on Ca^{2+}. The identity of the Ca^{2+} sensor(s) that triggers vesicle fusion is still under investigation, but many studies point toward an essential function for synaptotagmin (syt) in coupling Ca^{2+} to SNARE-mediated fusion. In the first paper, the authors undertake an electrophysiological analysis of *Drosophilia syt* mutants disrupted in distinct functions of synatotagmin. Their data indicate that synaptotagmin is the major Ca^{2+} sensor to synchronize neurotransmitter release and that synaptotagmin integrates the Ca^{2+} signal through its interaction with the SNARE complexes, the target membrane and with itself. The second paper provides the first images of a calcium-dependent synaptotagmin oligomer assembled on phospholipid bilayers.

- Visualization of synapto I oligomers assembled into lipid monolayers. Wu Y, He Y, Bai J, Ji SR, Tucker WC, Chapman ER, Sui SF. **Proc Natl Acad Sci U S A (PNAS)**. 2003 Feb 18;100(4):2082-7. Epub 2003 Feb 10.

- Synaptotagmin I functions as calcium sensor to synchronize neurotransmitter release. Yoshihara M1, Littleton JT. **Neuron.** 2002 Dec 5;36(5):897-908.

Week 7 (11/03/15): Viral cell entry

As obligatory intracellular parasites with limited genetic capacity, viruses have evolved to hijack intrinsic cellular pathways to enter the cell and deliver their genomes to specific cellular locations for replication. The first paper analyzes the process of virus internalization by the clathrin-dependent endocytosis machinery and reveals the importance of the actin cytoskeleton for the invagination of these endocytosis vesicles. In the second paper, the authors use a four-dimensional visualization approach to follow the uptake and trafficking dynamics of single virus particles in live epithelial cells.

- Vesicular stomatitis virus enters cells through vesicles incompletely coated with clathrin that depend upon actin for internalization. Cureton DK, Massol RH, Saffarian S, Kirchhausen TL, Whelan SP. **PLoS Pathogens**. 2009 Apr;5(4).

- Similar uptake but different trafficking and escape routes of reovirus virions and infectious subvirion particles imaged in polarized Madin–Darby canine kidney cells. Boulant S, Stanifer M, Kural C, Cureton DK, Massol R, Nibert ML, Kirchhausen T. **Molecular Biology of the Cell.** 2013 Apr;24(8):1196-207.

Week 8 (11/10/15): Visit to the laboratory of Dr. Tomas Kirchhausen at Harvard Medical School

The research in the laboratory of Tomas Kirchhausen focuses on the processes that mediate and regulate the movement of membrane proteins throughout cells. In particular, they study the molecular mechanisms that underlie the cell's sorting machinery responsible for receptor-mediated endocytosis and for secretion and how this machinery can be highjacked by toxins, viruses and bacterial pathogens to enter cells. They also study how during cell division cells control their size and organelle architecture. To analyze these different complex molecular mechanisms, this laboratory has developed high-resolution imaging and live-cell imaging techniques. Their most famous 'molecular movie' follows in real time the formation of a vesicle coated by the protein clathrin. https://www.youtube.com/watch?v=o_EUHu4OJus

Week 9 (11/17/15): Parasitic infection

- Studies of host-pathogen interactions have revealed that virulence often requires the usurpation of existing cell signaling pathways or membrane traffic machinery of the host. The first paper addresses the molecular mechanism of entrance into the host cell by the protozoan parasite *Trypanosoma cruzi,* which causes Chagas disease. For its invasion of the host cell, *T. cruzi* induces a signaling cascade that recruits lysosome vesicles to the site of parasitic contact. These lysosomes are then induced to fuse with the plasma membrane, creating vacuoles that *T. cruzi* may enter. In this paper, the authors show that the fusion of these lysosome vesicles with the plasma membrane requires synaptotagmin VII (SytVII). Interestingly, the same year another team showed that the fusion of lysosomal vesicles at the plasma membrane, which is Ca^{2+} and SytVII dependent, is a physiological mechanism for the cell to repair its membrane. The subject of study of the second paper is the subversion of the phagosome of the host cell by the bacterium *Legionella pneumophila*. The authors show the existence of physical connections among the endoplasmic reticulum (ER) vesicles, the mitochondria and the *L. pneumophila* phagosome. The authors also report the decrease of thickness of the phagosomal membrane to resemble that of the attached ER vesicles. These morphological changes are believed to protect the *L. pneumophila* infected phagosome from fusing to the lysosome, making the bacteria safe from degradation.

- The exocytosis-regulatory protein synaptotagmin VII mediates cell invasion by *Trypanosoma cruzi.* Caler EV1, Chakrabarti S, Fowler KT, Rao S, Andrews NW. **The Journal of Experimental Medicine**. 2001 May 7;193(9):1097-104.

- How the parasitic bacterium *Legionella pneumophila* modifies its phagosome and transforms it into rough ER: implications for conversion of plasma membrane to

the ER membrane. Tilney LG, Harb OS, Connelly PS, Robinson CG, Roy CR. **Journal of Cell Science**. 2001 Dec;114(Pt 24):4637-50.

Week 10 (11/24/15): Viral hijacking of the ESCRT machinery for virion release

For a virus, the last step in a host cell is the release of the newly synthesized virions into the environment. This elaborate process is particularly complicated for enveloped viruses, which must be encapsulated in a lipid membrane derived from the infected cell. HIV is one of the many enveloped viruses, including Ebola, that use the cellular ESCRT (Endosomal Sorting Complexes Required for Transport) machinery for virion release. The first paper demonstrates a link between a viral protein and a key component of the cellular endosomal sorting complex required for transport (ESCRT) in the budding process. In the second paper, the authors reconstitute the recruitment of ESCRT proteins to HIV-1 budding sites in a completely defined *in vitro* system.

- Overexpression of the N-terminal domain of TSG101 inhibits HIV-1 budding by blocking late domain function. Demirov DG, Ono A, Orenstein JM, Freed EO. **Proc Natl Acad Sci U S A (PNAS)**. 2002 Jan 22;99 (2):955-60.

- *In vitro* reconstitution of the ordered assembly of the endosomal sorting complex required for transport at membrane-bound HIV-1 Gag clusters. Carlson LA, Hurley JH. **Proc Natl Acad Sci U S A (PNAS)**. 2012 Oct 16;109 (42):16928-33. doi: 10.1073/pnas**.**1211759109. Epub 2012 Oct 1.

Week 11 (12/01/15): Defects of intracellular trafficking in disease

Only a few mutations in genes encoding components of the secretory machinery have been associated with human diseases. In the first paper the authors show that the amino acid substitution F382L (substitution of phenylalanine to leucine at position 382) in the protein Sec23, a component of the COPII-coated vesicles that transport protein from the endoplasmic reticulum (ER) to the Golgi complex, leads to abnormal ER-Golgi traffic and causes Cranio-lenticulo-sutural dysplasia. This disease is an autosomal recessive disorder characterized by different symptoms including cataracts, facial dysmorphism and skeletal defects. The authors of the second paper try to understand the molecular mechanism associated with Parkinson's disease, a neurodegenerative disorder affecting 1% of people over the age of 60. Aggregated α-synuclein (α-syn) fibrils form Lewy bodies (LBs), the signature of lesions of Parkinson's disease (PD), but the pathogenesis and neurodegenerative effects of LBs remain enigmatic. The authors of this paper show that aggregation of α-syn induces the accumulation of cytoplasmic vesicles in *Saccharomyces cerevisiae*.

- Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. Boyadjiev SA, Fromme JC, Ben J, Chong SS, Nauta C, Hur DJ, Zhang G, Hamamoto S, Schekman R,

Ravazzola M, Orci L, Eyaid W. **Nature Genetics**. 2006 Oct;38(10):1192-7. Epub 2006 Sep 17.

- Alpha-synuclein-induced aggregation of cytoplasmic vesicles in *Saccharomyces cerevisiae.* Soper JH, Roy S, Stieber A, Lee E, Wilson RB, Trojanowski JQ, Burd CG, Lee VM. **Molecular Biology of the Cell.** 2008 Mar;19(3):1093-103. doi: 10.1091/mbc.E07-08-0827. Epub 2008 Jan 2.

Week 12 (12/08/15): Oral presentations

See "Course Format" for a description of what is expected.

This week we will also discuss the course overall -what worked, what could be changed and improved and the impact the course might have on what students envision for their futures.