Molecular Mechanisms of Membrane Trafficking

May 17 - 19, 2018

Seoul National University

Organizers

Tae-Young Yoon
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Registration:
mmmt.snu.ac.kr

Invited Speakers

Axel Brunger
Stanford University

Phyllis Hanson
Washington University in St. Louis

Reinhard Jahn
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Cong Ma
Huazhong Univ. of Sci. & Tech.

Alex Merz
University of Washington

Fred Meunier
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Liz Miller
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Joji Mima
Osaka University

Suzanne Pfeffer
Stanford University

Sandy Schmid
University of Texas Southwestern

Jingshi Shen
University of Colorado

Lukas Tamm
University of Virginia

Yongli Zhang
Yale University

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May 17, 2018 (Thursday)

10:00-10:10 am **Opening Remarks**

Session I: **Neuronal Exocytosis**
Session Chair: Yongli Zhang

10:10 - 10:40 am Axel T. Brunger (Stanford University, USA)
"Molecular mechanisms of synaptic vesicle priming and fusion"

10:40 - 11:10 am Lukas Tamm (University of Virginia, USA)
"The Calcium Trigger of Neuronal Exocytosis"

11:10 - 11:30 am **Coffee / Tea break**

Session II: **Regulation of membrane fusion**
Session Chair: Lukas Tamm

11:30 - 12:00 pm Reinhard Jahn (Max-Planck Institute for Biophysical Chemistry, Germany)
"Membrane targeting and fusion – lessons from functional reconstitution"

12:00 - 12:30 pm Tae-Young Yoon (Seoul National University, South Korea)
"Focused clamping of the neuronal SNARE complex by complexin under high mechanical tension"

12:30 - 13:30 pm **Lunch (Lunch Box)**

Short programs (for who are interested):
1. Hiking to the Gwanak Mountain (SNU is located in the Gwanak Mountain)
2. Campus tour

16:30 - 18:00 pm **Poster session I**

18:00 – 19:30 pm **Symposium Banquet**
(Faculty House: Walking 10 min from the symposium hall)

Session III: **Rabs and Tethers**
Session Chair: Sandy Schmid

19:45 – 20:15 pm Suzanne R. Pfeffer (Stanford University, USA)
"Rab GTPase phosphorylation in Parkinson's Disease"

20:15 - 20:45 pm Joji Mima (Osaka University, Japan)
"Reconstituted membrane tethering mediated by human Rab-family small GTPases in a chemically defined system"

20:45 - 21:15 pm Mary Munson (University of Massachusetts Medical School, Worcester, USA)
"Molecular Architecture and Function of the Exocyst Complex in Vesicle Trafficking"

21:30 pm - **Beer Happy Hour** at the Hoam Faculty House
(where the speakers are staying)

May 18, 2018 (Friday)

Session IV: **Regulation of fusion by SNAREs/SMs/Sec17 (I)**

Session Chair: Suzanne Pfeffer

09:00 - 09:30 am Alexey J. Merz (University of Washington, Seattle, USA)
"Dynamic interactions among SNARES, SMs, and disassembly chaperones control membrane fusion"

09:30 - 10:00 am Hongki Song (Geisel School of Medicine at Dartmouth, USA)
"Sec17 and Sec18 have novel, ATP-independent functions in the stimulation of fusion."

10:00 – 10:30 am Frederic A. Meunier (University of Queensland, Australia)
"Dynamic nanoscale reorganization of priming proteins during neuroexocytosis"

10:30 - 10:50 am **Coffee / Tea break**

Session V: **Membrane Trafficking**

Session Chair: Alex Merz

10:50 - 11:20 am Liz Miller (MRC Laboratory of Molecular Biology, UK)
"Protein quality control in vesicular traffic: cargo occupancy influences trafficking specificity."

11:20 - 11:50 am Jingshi Shen (University of Colorado, Boulder, USA)
"Genome-scale dissection of mammalian membrane trafficking – new players and unexpected mechanisms"

11:50 - 12:20 am Phyllis Hanson (Washington University, St. Louis, MO, USA)
"Expanding roles for the ESCRT machinery"

12:20 - 13:20 pm **Lunch** (Lunch Box)

13:00 - **Excursion and Dinner** (in a place in the Gangnam area)

May 19, 2018 (Saturday)

Session VI: **Regulation of fusion by SNAREs/SMs/Sec17 (II)**

Session Chair: Joji Mima

08:30 - 09:00 am Yongli Zhang (Yale University, USA)
"Munc18-1 and Vps33 catalyze directional SNARE assembly by templating SNARE association"

09:00 - 09:30 am Cong Ma (Huazhong University of Science and Technology, China)
"Mechanism of Munc13s in synaptic vesicle priming"

09:30 – 10:00 am Sanghwa Lee (Gwangju Institute of Science and Technology, South Korea)
"Single-molecule dissection of the regulation of neuronal SNARE assembly by Munc18-1"

10:00 - 11:00 am **Coffee/Tea break with snacks and Poster Session II**

Session VII: **Dynamin and Dynamin-like GTPases**

Session Chair: Fred Meunier

11:00 - 11:30 pm	Youngsoo Jun (Gwangju Institute of Science and Technology, South Korea) "SNAREs support atlastin-mediated ER membrane fusion"
11:30 - 12:00 pm	Marijn G. J. Ford (University of Pittsburgh, PA, USA) "Not all Dynamin-Related Proteins are alike: The unique architecture of Vps1"
12:00 - 12:30pm	Sandra L. Schmid (UT Southwestern Medical Center, TX, USA) "Dynamin-1 as a nexus between signaling and endocytosis"
12:30 – 12:50 pm	**Poster awards and Concluding remarks**
12:50 pm	**Lunch** (Lunch box)

Speakers:

Molecular mechanisms of synaptic vesicle priming and fusion

Axel T. Brunger[1]

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The central nervous system relies on electrical signals traveling along neurons at high speeds. Signals are also transmitted between two neurons, or from a neuron to a muscle fiber through synaptic junctions. Synaptic transmission relies on the release of neurotransmitter molecules into the synaptic cleft. This release in turn depends on a process called membrane fusion to ensure that the neurotransmitter molecules that are contained in synaptic vesicles are released into the synaptic cleft as quickly as possible. Membrane fusion is an important process in many areas of biology, including intracellular transport and hormone release, but it occurs much faster (< 1 millisecond) for synaptic vesicle fusion than for these other processes. Moreover, it is precisely calcium regulated. Recent structural and biophysical studies of priming and calcium triggering of synaptic vesicle fusion will be presented.

References

[1] Q. Zhou, P. Zhou, A.L. Wang, D. Wu, M. Zhao, T.C. Südhof, A.T. Brunger. The primed SNARE-complexin-synaptotagmin complex for neuronal exocytosis. *Nature* **548**, 420-425 (2017).
[2] Y. Lai, U.B. Choi, J. Leitz, H.J. Rhee, C. Lee, B. Altas, M. Zhao, R.A. Pfuetzner, A.L. Wang, N. Brose, J. Rhee, A.T. Brunger. Molecular mechanisms of synaptic vesicle priming by Munc13 and Munc18. *Neuron* **95**, 591–607 (2017).
[3] P. Gipson, Y. Fukuda, R. Danev, Y. Lai, D.-H. Chen, W. Baumeister, A.T. Brunger. Morphologies of synaptic protein membrane fusion interfaces. *Proc. Natl. Acad. Sci. USA* **114**, 9110-9115.

The Calcium Trigger of Neuronal Exocytosis

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Regulated exocytosis is a process by which neurotransmitters, hormones, and secretory proteins are released from the cell in response to elevated levels of calcium. In cells, secretory vesicles are targeted to the plasma membrane, where they dock, undergo priming, and then fuse with the plasma membrane in response to calcium. The specific roles of essential proteins and how calcium regulates progression through these sequential steps currently are incompletely resolved. Here we have used purified neuroendocrine dense core vesicles and artificial membranes to reconstruct *in vitro* the serial events that mimic SNARE-dependent membrane docking and fusion during exocytosis. Calcium recruits these vesicles to the target membrane aided by the protein CAPS while synaptotagmin catalyzes calcium-dependent fusion; both these processes are dependent on $PI4,5P_2$. The soluble proteins Munc18 and complexin-1 are necessary to arrest vesicles in a docked state in the absence of calcium, while CAPS and/or Munc13 are involved in priming the system for an efficient fusion reaction. This experimental approach allowed us to discover the precise molecular mechanism of calcium-synaptotagmin triggering of the SNARE-mediated fusion reaction in this physiological reconstituted system.

Reference

[1] Science Advances 3: e1603208 (2017) and unpublished results.

Membrane targeting and fusion – lessons from functional reconstitution

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Docking and fusion in the secretory pathway involves members of conserved protein families such as Rab, SM-, CATCHR and SNARE proteins. Although the molecular machinery is unique for each trafficking step, there are common principles that probably reflect common molecular mechanisms. In our own work we try to shed light on the underlying mechanisms using functional reconstitution of purified SNARE proteins in artificial membranes. Here I will focus mostly on the endosomal system and discuss recent results concerning the specificity of SNARE-mediated targeting and the mechanism of membrane fusion.

Focused clamping of the neuronal SNARE complex by complexin under high mechanical tension

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Neuronal SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) catalyse the fusion of synaptic vesicles with presynaptic membranes through the formation of SNARE complexes. Complexin (Cpx) is the only presynaptic protein that tightly binds to the neuronal SNARE complex and therefore regulates synaptic vesicle fusion. However, it remains unclear how Cpx modulates the energy landscape involved in the SNARE complex assembly, especially when mechanical tension was loaded on the SNARE complex. Using magnetic tweezers, we studied how Cpx interacts with a single neuronal SNARE complex, and found that the molecular effects of Cpx manifested only under high mechanical tensions above 13 pN. We found that Cpx mechanically stabilized the central four-helix bundle composed of the SNARE motifs. At the same time, Cpx prevented the zippering of SNARE complexes from reaching completion by inhibiting assembly of the linker domains. These results suggest that Cpx generates a focused clamp for the neuronal SNARE complex in a linker-open conformation. Our results provide a hint as to how Cpx cooperates with neuronal SNAREs to prime synaptic vesicles in preparation for synchronous neurotransmitter release.

Rab GTPase phosphorylation in Parkinson's Disease

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Parkinson's disease-associated LRRK2 kinase phosphorylates a subset of Rab GTPases on a conserved residue in their switch-II domains which interferes with effector binding, GEF activation and GDI retrieval. Martin Steger and Matthias Mann systematically analyzed the Rab protein family and found 14 of them (Rab3A/B/C/D, Rab5A/B/C, Rab8A/B, Rab10, Rab12, Rab29, Rab35 and Rab43) can be specifically phosphorylated by LRRK2 in vitro, with evidence for endogenous phosphorylation for ten of them (Rab3A/B/C/D, Rab8A/B, Rab10, Rab12, Rab35 and Rab43) [1]. Affinity enrichment mass spectrometry revealed that the primary ciliogenesis regulators, RILPL1 and RILPL2 specifically interact with the LRRK2-*phosphorylated* forms of Rab8A, Rab10, and Rab12. Together, we have shown that primary cilia formation is blocked in fibroblasts derived from pathogenic LRRK2-R1441G knock-in mice. These results implicate LRRK2 in primary ciliogenesis and suggest that Rab-mediated protein transport and/or signaling defects at cilia may contribute to LRRK2-dependent pathologies [1]. Previous work indicated that Rab29, located within the PARK16 locus that is often mutated in Parkinson's disease patients, operates in a common pathway with LRRK2. In collaboration with the lab of Dario Alessi, we have shown that Rab29 recruits LRRK2 to the trans Golgi network and greatly stimulates its kinase activity [2]. Pathogenic LRRK2 R1441G/C and Y1699C mutants that promote GTP binding to LRRK2 are more readily recruited to the Golgi and activated by Rab29 than wild-type LRRK2. We identified conserved residues within the LRRK2 ankyrin domain that are required for Rab29-mediated Golgi recruitment and kinase activation. Our data reveal that Rab29 is a master regulator of LRRK2, controlling its activation and localization. Work is in progress to understand how a Rab cascade involving Rab29 activation of Rab8 and Rab10 phosphorylation interferes with ciliation via specific Rab effector proteins.

References

[1] M. Steger et al., Systematic proteomic analysis of LRRK2-mediated Rab GTPase phosphorylation establishes a connection to ciliogenesis. Elife. 2017 Nov 10;6. pii: e31012 (2017).
[2] E. Purlyte et al., Rab29 activation of the Parkinson's disease-associated LRRK2 kinase. EMBO J. 37, 1-18(2018).

Reconstituted membrane tethering mediated by human Rab-family small GTPases in a chemically defined system

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Membrane tethering, the initial contact between transport carriers (e.g., secretory vesicles, endocytic vesicles, etc.) and their target membrane compartments (e.g., subcellular organelles, the plasma membrane, etc.), is a critical step to determine and control the spatiotemporal specificity of membrane trafficking in all the eukaryotic endomembrane systems. Based on a large body of prior genetic, cell biological, biochemical, and structural studies, Rab (Ras related in brain) family small GTPases and a number of Rab-interacting proteins (termed Rab effectors; e.g., coiled-coil tethering proteins, multisubunit tethering complexes, etc.) are thought to be responsible for driving the membrane tethering events. However, indeed whether and how Rab GTPases and their specific Rab effectors directly act upon membrane tethering still remains enigmatic. Using the chemically defined reconstitution system with purified proteins of eleven representative human Rab-family GTPases (Rab1a, 3a, 4a, 5a, 6a, 7a, 9a, 11a, 14, 27a, and 33b) and synthetic liposomal membranes bearing physiologically-mimicking complex lipid compositions, our recent reconstitution studies have revealed the intrinsic potency of human Rab GTPases to physically and specifically tether two distinct membranes *via* homotypic and heterotypic *trans*-assemblies of Rab proteins on the membranes (Tamura & Mima, 2014; Inoshita & Mima, 2017; Mima, 2018).

Reference

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Molecular Architecture and Function of the Exocyst Complex in Vesicle Trafficking

Dante Lepore[1], Michael Feyder[1], Chanwoo Lee[2], Sang-Hyun Rah[2], Lillian Kenner[3], Tae-Young Yoon[2], Adam Frost[3], and Mary Munson[1]

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The regulation of vesicular traffic to precise intracellular compartments is essential for cell growth, homeostasis, signaling, cell division, and development. Membrane fusion between vesicles and their target membrane is carried out via SNARE proteins; however, additional regulatory control immediately prior to fusion is essential. The exocyst is a large, multisubunit protein complex implicated in tethering and regulation of the fusion of post-Golgi secretory vesicles with the plasma membrane, but its mechanism of action is poorly understood. We take a multidisciplinary approach to elucidate its structure and function. Our groundbreaking purification method enabled us to characterize the intact yeast exocyst complexes, plus derived subcomplexes and mutants. Our genetic, biochemical and negative stain EM studies of the architecture of the yeast exocyst appear to be consistent with a newly determined cryoEM structure of the intact complex. We are currently using a combination of EM, crosslinking and mass spectrometry, and integrative modeling, to map binding of subunits with partner proteins, as well as single molecule biophysical analyses of SNARE-mediated liposome fusion, to elucidate the function of exocyst in vesicle tethering and fusion.

Dynamic interactions among SNARES, SMs, and disassembly chaperones control membrane fusion

Braden T. Lobingier[1], Mengtong (Tom) Duan[1], Anika Burrell[1], Daniel P. Nickerson[1], Cortney G. Angers[1], Matthew L. Schwartz[1], Rachael L. Plemel[1], William T. Wickner[3], Michael Zick[3], Alexey J. Merz[1,2]*

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Tethers and SM proteins bring two membranes into proximity, and promote the "zippering" of unpaired SNARE proteins on opposite membranes to assemble a pre-fusion *trans*-SNARE complex. Recent work shows that the zippering is itself catalyzed by proteins of the Sec1/Munc18 (SM) family. Further zippering does the mechanical work of bringing the membranes into close apposition, triggering the onset of lipid mixing and then full fusion. Following fusion, the spent *cis*-SNARE complex is disassembled and re-energized for subsequent rounds of fusion. SNARE disassembly is powered by an ATPase, Sec18 (NSF) and its adapter, Sec17 (alpha-SNAP). In 2009, we first reported that Sec17 has an unexpected ability to directly promote SNARE-mediated fusion [1,2]. In this talk I will describe how: Sec17 co-assembles with SM proteins on SNARE complexes [3]; Sec18 can augment the fusogenic function of Sec17, *without* ATP hydrolysis [4]; Sec17 penetration of the lipid bilayer promotes its fusogenic function [4,5]; and (iv) Sec17 can either inhibit or promote fusion, with the outcome of the Sec17-SNARE interaction being gated by the SM [5]. Together these dynamics lead to a general model for SM function which explains why SM deletion annihilates SNARE-mediated fusion *in vivo*.

References

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Sec17 and Sec18 have novel, ATP-independent functions in the stimulation of fusion

Hongki Song and William Wickner

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Yeast vacuole fusion has been reconstituted with liposomes of defined lipids and pure recombinant proteins: 4 SNAREs, a hexameric tethering complex HOPS, the Rab Ypt7, and SNARE chaperones Sec17 and Sec18. Reconstitution allowing in-depth study of fusion mechanism [1]. HOPS and Ypt7 mediate tethering, then HOPS catalyzes assembly of the 4 SNAREs into a trans-complex anchored in each membrane, and Sec17 and Sec18 associate with the SNARE complex [2, 3]. This allows the rearrangement of non-bilayer prone, fluid lipids to give fusion [4]. My recent studies reveal a crucial role of a small domain of the Qa-SNARE which is immediately upstream of the canonical SNARE domain [5]. In addition, I have shown that Sec17 association with HOPS-assembled trans-SNARE complexes promotes fusion, likely by driving completion of SNARE zippering. Strikingly, Sec18 protein promotes fusion independently [6]; since it associates the only the N-terminal region of the SNARE domains, this may entail a novel and still unknown mechanism. Currently I am assaying whether the SM function of HOPS is required to be physically associated with tethering, or whether these two can be separated.

Reference

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Dynamic nanoscale reorganization of priming proteins during neuroexocytosis

Frederic A. Meunier[1], Bademosi[1] A.T., Kasula[1] R. and Padmanabhan[1] P.[2]

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Communication between neurons relies on a process known as neuroexocytosis during which synaptic vesicles containing neurotransmitters dock, are primed and then fuse with the presynaptic plasma membrane, thereby releasing their content post-synaptically. The proteins involved in this mechanism are constantly subjected to Brownian motion and hence must be organised in functional nanoclusters to optimise the speed of the fusion process. How the inherent mobility of these proteins on the presynaptic membrane is compatible with docking, priming and ultimately fusion, is still subject to numerous investigations. In this talk, I will highlight key changes in the nanoscale organization of the SM-protein Munc18-11-3 and the SNARE protein syntaxin1A2-6, that are critical for neuroexocytosis. Based on key differences in diffusive behaviour which we have observed in neurosecretory cells and at the Drosophila neuromuscular junction, I will provide evidence for a model of syntaxin1A and Munc18-1 dynamic nanocluster organization. These differences are likely to reflect key roles of these priming proteins in ensuring both the speed and efficiency of neuroexocytosis.

References

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Protein quality control in vesicular traffic: cargo occupancy influences trafficking specificity

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ER-derived COPII vesicles largely exclude ER resident proteins and misfolded nascent proteins. We're interested in understanding how this selectivity is achieved. We screened ~4500 yeast strains for defects in ER retention. A large proportion of screen hits encode proteins involved in biogenesis of cell wall proteins. We propose that the absence of these abundant highly glycosylated proteins creates space in COPII vesicles that permits stochastic capture of ER residents by bulk flow. We confirmed that lax quality control correlates with vesicle occupancy, vesicle size and vesicle number, consistent with our model. Together, our data suggest a stochastic model for ER export based on cargo-coat affinities to drive cargo selectivity and steric crowding to exclude inappropriate cargo. We propose that cells must balance the efficiency of vesicle production with cargo occupancy of vesicles to prevent inappropriate leakage of nascent proteins and ER residents.

Genome-scale dissection of mammalian membrane trafficking – new players and unexpected mechanisms

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Cargo proteins moving between organelles are transported by membrane-enclosed vesicles. The core engines mediating vesicle trafficking are now well established. However, we are only beginning to understand the regulatory networks superimposed upon the core engines to adjust the rate and direction of membrane transport according to physiological demands. The advent of the revolutionary CRISPR-Cas9 genome editing system enabled us to systematically identify new components of the regulatory networks. We developed new screening platforms and performed unbiased genome-wide CRISPR genetic screens to dissect the exocytosis and endocytosis of cell surface transporters, fundamental processes in cell physiology. Our screens identified known regulators but most of the hits were not previously known to regulate the pathways. I will focus on the unexpected mechanisms of RABIF/MSS4 in exocytosis and AAGAB in endocytosis. I will also discuss how the principles uncovered in our studies shed light on vesicle trafficking in general.

Expanding roles for the ESCRT machinery

Phyllis Hanson

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The ESCRT-III machinery comprises a collection of proteins that form polymeric filaments involved in membrane remodeling and fission in numerous contexts, notably during the formation of multivesicular endosomes. Recent studies highlight additional roles for ESCRT proteins in an increasing range of membrane remodeling reactions that may not be limited to the topology traditionally associated with ESCRT pathway function. I will discuss new insights into ESCRT-III function on endolysosomal membranes, with a particular focus on their involvement in repair of transiently damaged endolysosomes.

Munc18-1 and Vps33 catalyze directional SNARE assembly by templating SNARE association

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Sec1/Munc18-family (SM) proteins are required for SNARE-mediated membrane fusion, but their roles and mechanisms of action are poorly understood. Using single-molecule force spectroscopy, we found that the SM protein Munc18-1 catalyzes step-wise assembly of three synaptic SNAREs (syntaxin, VAMP2, and SNAP-25) into a four-helix bundle. Munc18-1 targets syntaxin N-terminal domain and juxtaposes the SNARE motifs of syntaxin and VAMP2 on its surface. Next, SNAP-25 quickly binds the templated SNAREs to form a partially-zippered SNARE complex. Finally, full zippering displaces Munc18-1. Munc18-1 enhances the rate, specificity, and accuracy of SNARE assembly, all of which are crucial for exocytosis. Phosphorylation of residues in the SNARE binding surface of Munc18-1 stabilizes or destabilizes the template complex in a position-dependent manner, consistent with their roles in synaptic vesicle fusion and neurotransmission. A second SM protein, Vps33, chaperones vacuolar SNARE assembly in an analogous manner, suggesting that SM protein mechanism is conserved. Thus, our findings reveal a conserved intermediate that are pivotal for regulating SNARE assembly and membrane fusion, providing new insights into the working mechanism of the SNARE-SM fusion machinery.

References

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Mechanism of Munc13s in synaptic vesicle priming

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Munc13s have a central function in synaptic vesicle priming, yet the underlying mechanism remains unclear. In our work, we demonstrated that Munc13s MUN domain catalyzes the transition from the Munc18-1/Syntaxin-1 complex to the SNARE complex. We identified an interaction between the NF hydrophobic pocket located at the midpoint of the MUN domain and the RI sequence in the syntaxin-1 linker region that is essential for MUN domain catalysis and priming function. Our data revealed that the MUN domain does not dissociate the closed Munc18-1/Syntaxin-1 complex, but rather induces a conformational change in the syntaxin-1 linker region, thus enabling syntaxin-1 to transit into the ternary SNARE complex once synaptobrevin-2 and SNAP-25 are added. These data suggest that Munc18-1/syntaxin-1/Munc13s network of interactions allows exquisite regulation of synaptic vesicle priming.

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Single-molecule dissection of the regulation of neuronal SNARE assembly by Munc18-1

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Neurotransmitter release by synaptic vesicle fusion critically depends on N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) and SM protein Munc18-1. Recent in vitro assays have shown that Munc18-1 play essential roles in neuronal SNARE assembly, but the underlying mechanisms are still unclear. In this work, we used single-molecule fluorescence resonance energy transfer (FRET) imaging to investigate the regulation mechanism of SNARE assembly by Munc18-1. Here, we determined that Munc18-1 induced closed syntaxin-1 conformation both in the presence and absence of SNAP-25. This result may give insight into a role of Munc18-1 as a template for proper alignment of SNARE complex. At the end of this talk, our expectations about future developments of single-molecule FRET assays for this study will be briefly discussed.

SNAREs support atlastin-mediated ER membrane fusion

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Dynamin-like GTPases of the atlastin family are thought to mediate homotypic endoplasmic reticulum (ER) membrane fusion; however, the underlying mechanism and regulatory factors remain largely uncharacterized. To study the molecular mechanism underlying the yeast atlastin Sey1p-dependent ER membrane fusion, we developed a simple and quantitative in vitro assay using isolated yeast ER microsomes. Using this assay, we found that ER-associated SNAREs and sterols play a critical role in Sey1p-mediated ER fusion. In this talk, I will present our recent data suggesting that ER SNAREs and ergosterol interact with Sey1p to support homotypic ER membrane fusion in the budding yeast *Saccharomyces cerevisiae*.

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Not all Dynamin-Related Proteins are alike: The unique architecture of Vps1

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The dynamin-related proteins (DRPs) are large multi-domain GTPases required for diverse membrane remodeling events. DRPs self-assemble into helical structures but how these structures are tailored to their cellular targets remains unclear. We demonstrate that the fungal DRP Vps1 primarily localizes to and functions at the endosomal compartment. We present crystal structures of a Vps1 GTPase-BSE fusion in different nucleotide states to capture GTP hydrolysis intermediates and concomitant conformational changes. Using cryoEM, we determined the structure of full-length GMPPCP-bound Vps1. The Vps1 helix is more open and flexible than that of dynamin. This is due to further opening of the BSEs away from the GTPase domains. A novel interface between adjacent GTPase domains forms in Vps1, instead of the contacts between the BSE and adjacent stalks and GTPase domains as seen in dynamin. This configuration highlights a unique helical architecture for Vps1 and illustrates inherent structural flexibilities of DRP self-assembly.

Dynamin-1 as a nexus between signaling and endocytosis

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Clathrin-mediated endocytosis (CME) is the major endocytic pathway in mammalian cells. It is responsible for the uptake of transmembrane receptors and transporters, for remodeling plasma membrane composition in response to environmental changes, and for regulating cell surface signaling. CME occurs via the assembly and maturation of clathrin-coated pits (CCPs) that concentrate cargo, invaginate and pinch-off forming clathrin-coated vesicles. The large GTPase dynamin is an essential component of the CME machinery. Dynamin is best known for its role as the prototypical fission GTPase that assembles into helical structures around the narrow necks of invaginated CCPs and catalyzes membrane fission. Recent studies have shown that dynamin is also a key regulator of early steps in CME. Here we report that dynamin-1 (Dyn1), previously assumed to be a neuron-specific isoform, is activated in non-neuronal cells downstream of Akt/GSK3β signaling to accelerate CME by increasing the rates CCP initiation and maturation. Dyn1 establishes a positive feedback loop that involves APPL-positive early endosomes and the reciprocal crosstalk between signaling and endocytosis. This feedback loop is often upregulated in cancer cells to enhance cancer cell proliferation, survival and metastasis.