The 61st Symposium of the Society of General Physiologists
Membrane Biophysics of Fusion, Fission, and Rafts in Health and Disease

(organized by Frederic Cohen and Joshua Zimmerberg)

The Journal of General Physiology

The 61st meeting of the Society of General Physiologists took place in Woods Hole, MA on September 5–9, 2007. The theme of the featured symposium was "Membrane Biophysics of Fusion, Fission, and Rafts in Health and Disease." The symposium was organized by Fred Cohen from Rush University Medical Center in Chicago and Josh Zimmerberg of National Institutes of Health (NIH), Bethesda. The Symposium featured an unusually large number of speakers, 40, which reflected the wide diversity of issues discussed and the increasingly detailed understanding of the molecular mechanisms involved in these membrane processes. The meeting drew 150 participants from 15 countries. The symposium itself was divided into eight sessions with foci that ranged from viral mechanisms of fusion to lipid domain formation, with much lively discussion on many other topics in between. The weather was typical early September Woods Hole—perfect—and despite the rather crowded program, the participants had time to enjoy the unique atmosphere, including the marine environment during boat tours arranged by the organizers.

The meeting got underway with back-to-back keynote addresses that provided examples of the important role of basic research of membrane processes for understanding human diseases. In the first, Robert Brown (Harvard University) discussed dysferlin, membrane repair, and muscular dystrophy. Dysferlin is a large membrane protein that has been implicated in membrane repair processes. Mutations in dysferlin cause a variety of dystrophies without disrupting the dystrophin complex. Various mouse models are available that recapitulate the human disease phenotype. Dysferlin contains several C2 domains that may play a role in Ca^{2+}-dependent membrane localization, but the mechanism underlying dysferlin's normal function, and how mutations cause muscular dystrophies are still unclear. William Gall (NIH) provided an overview of disorders of lysosome-like organelles. As a model for such diseases, he focused on melanosome biogenesis, where diseases including Hermansky-Pudlak Syndrome, Griscelli Syndrome, and Chediak-Higashi Syndome highlight specific molecular defects in this process.

Session I, chaired by the coorganizer Josh Zimmerberg, had as its focus viral, synaptic, and intracellular fusion mechanisms. Stephen Harrison (Harvard University) pointed out that viruses have all of the molecular components for fusion built into themselves, but different strategies for cell invasion are employed by different types of viruses. Viruses such as HIV and influenza are trimeric precursors with a cleavage required to expose an N-terminal fusion peptide. On the other hand, flaviviruses and herpesviruses become trimers during a conformational transition that exposes an internal fusion loop. How many trimers participate, whether mechanochemical mechanisms suffice, and whether completion of the conformational changes facilitate fusion are questions that are still being investigated. Margaret Kielian (Albert Einstein College of Medicine) and Gregory Melikan (University of Maryland) elaborated on some of the structural features involved in viral fusion. Flavivirus, alphavirus, denguevirus, and Semliki virus may exist in a short-lived but stable prehairpin intermediate conformational state that may contain enough energy to drive conformational changes in the hairpin required for fusion. Retroviral envelope proteins adopt a prehairpin intermediate associated with receptor binding followed by a second prebundle intermediate that forms a pore, which is subsequently stabilized to form the fusion pore. Bill Wickner (Dartmouth College) shifted gears to yeast models of fusion, where genetics and the vacuole provided insights into a complex called HOPS that is required for rab-sensitive SNARE complex formation. Reinhard Jahn (Max-Planck) discussed the structural features of the four-helix bundle SNARE complex and, using proteoliposomal assays, showed that only specific combinations of v- and t-SNAREs promoted fusion. He also discussed the mechanism by which synaptotagmin confers Ca^{2+} sensitivity of the fusion process, demonstrating that synaptotagmin interacted specifically with neuronal SNAREs and required acidic phospholipids in the t-SNARE liposomes. Two speakers focused on Rab small GTP-binding proteins in exocytosis. Ed Steunkel (University of Michigan) reported on the role of Rab27a in insulin secretion. An effector for rab in this process is synaptotagmin-like protein 4a (slp4a), which interacts with other components of the SNARE complex proteins. Using fluorescence resonance energy transfer, he detailed studies that attempted to understand the role of the rab GTPase cycle in its molecular interactions with slp4a and the secretory vesicles. Christopher Brett (Washington) proposed that rabs played a role in sensing membrane tension that promoted the transition from a hemi-junction to a fusion pore.

J. Gen. Physiol. © The Rockefeller University Press $30.00
Volume 131 Number 2 February 2008 99–102
http://www.jgp.org/cgi/doi/10.1085/jgp.200709921

In Session II the focus shifted to synaptic vesicle fusion. Thomas Südhof (University of Texas, Southwestern Medical Center) described a number of biochemical and functional studies that exploited various mouse models to understand the role of synaptotagmin in vesicular exocytosis. He described complexin, a protein that binds in the assembled SNARE helical bundle complex, as a required step for synaptotagmin to function as a Ca^{2+} sensor for fusion. He proposed that fusion would involve synaptotagmin displacement of complexin in a Ca^{2+}-dependent manner. Moreover, of the synaptotagmins, only isoforms 1, 2, and 9 can promote Ca^{2+}-dependent exocytosis in transfected neurons, and they do it with distinct kinetics and Ca^{2+} affinities. A separate slow, synaptotagmin-independent high-affinity Ca^{2+}-sensitive mechanism also regulates exocytosis. A quantitative model with two different Ca^{2+} sensors could account for the kinetics of transmitter release. Ling-Gang Wu (NIH) described electrophysiological capacitance measurements at nerve terminals of the calyx of Held in which endocytosis kinetics were measured. He illustrated that both vesicular and bulk endocytosis mechanisms appeared to be clathrin mediated following exocytosis, particularly during strong stimulation, but found little evidence for the so-called kiss and run mechanism in the release step. Josep Rizo (University of Texas, Southwestern Medical Center) asked how the SNARE complex effected membrane bending. One suggestion was that such membrane buckling could be produced by the electrostatic field generated by synaptotagmin's C2 domains. However, a major question during the discussion was whether the proposed mechanism provides sufficient energy to drive fusion. Harvey McMahon (MRC) pointed out that the SNARE complexes would be expected to physically impede the interaction of lipids in the vesicle and plasma membrane, and suggested that there should be a mechanism to create a protein-free zone. He described a variety of studies that implicated synaptotagmin in promoting the membrane curvature necessary to enhance fusogenicity. Synaptotagmin binds to membranes in a Ca^{2+}- and phospholipid-dependent manner. Using a reconstitution system, he showed that synaptotagmin could promote membrane tubule formation in a Ca^{2+}-dependent manner, and that tubule formation was proportional to the amount of fusion observed. A lively discussion ensued regarding whether this mechanism could create the right curvature, or even whether such curvature was necessary.

Session III, chaired by the coorganizer Fred Cohen, focused on proteins and models of the fusion pore. Dixon J. Woodbury (Brigham Young University) discussed the importance of palmitoylation of SNAP25 for interacting with membranes. He detects fusion of native synaptic vesicles with planar lipid bilayers (BLMs) either by incorporating nystatin channels in synaptic vesicles and monitoring changes in bilayer conductance or by measuring release of dopamine by amperometry. For planar bilayers devoid of SNAP-25, fusion proceeds normally and mimics exocytosis. But SNAP-25 must be present in its native form (i.e., palmitoylated) in the vesicle for fusion to occur. Because SNAP-25 is a v-SNARE, but not a t-SNARE, in this system, it may have a more promiscuous and regulatory role in SNARE fusion than previously thought. Meyer Jackson (University of Wisconsin) described results of amperometry recordings of norepinephrine release from PC12 cells and posterior pituitary nerve terminals (see Zhang and Jackson on p. 117 of this issue). For PC12 cells, kinetic analysis was used to infer functional transitions in single fusion pores. For either wild-type or mutated synaptotagmin I, the Ca^{2+}-binding C2 domains influenced the kinetics of exocytosis at distinct sites in the mechanistic pathway. Mutations in SNARE proteins altered the kinetics of exocytosis in a manner consistent with some degree of SNARE complex disassembly at the moment of fusion pore opening. For posterior pituitary nerve terminals, it was found that synaptotagmin IV, a novel variant of synaptotagmin I with no known Ca^{2+}-binding activity, is very abundant. Comparisons of capacitance recordings from pituitary nerve terminals of wild-type and synaptotagmin IV knockout mice indicated that this isoform acts a negative regulator of exocytosis. Leonid Chernomordik (NIH) presented studies that identified fusion proteins in the development of *Caenorhabditis elegans*. Two proteins were explored by expressing them in heterologous cells. The expression of either of two proteins, each essential for distinct cell fusion reactions, led to formation of multinucleate cells. This identifies each as a fusion protein, the first identifications of developmental cell fusogens. In separate sets of experiments, baculovirus gp64 was expressed and cell–cell fusion was used to determine mechanisms of cell fusion from early fusion intermediates, including hemifusion, through expansion of the fusion pores that eventually culminates in complete disappearance of the membrane junctions between fusing cells. It was found that fusion pores formed at or near the openings of actin cortex and late stages of pore expansion were dependent on cell metabolism. Pharmacological experiments led to the conclusion that actin cytoskeleton does not promote pore expansion, but rather retards it. Following the formal talks, a point/counterpoint session discussed whether fusion proceeded through hemifusion as an intermediate or whether fusion was initiated by a proteinacious pore. Meyer Jackson briefly presented evidence for the latter, followed by Leonid Chernomordik arguing for the former. The most compelling evidence for a protein-lined fusion pore was obtained from experiments with the t-SNARE syntaxin. A variety of mutations in the membrane anchor of this protein, altering steric and/or electrostatic interaction, affect flux through a fusion pore. Mutations throughout the nonmembrane-spanning

domains of the SNARE proteins failed to alter fusion pore flux. Several lines of experiments favor hemifusion as an intermediate, but none do so conclusively. The evidence includes (a) demonstrations that hemifusion can occur for different fusion proteins, (b) replacing the transmembrane domains of viral fusion proteins with those of proteins unrelated to fusion or with a lipid anchor still supports the formation of fusion pores, and (c) the lipid dependence of fusion is conserved for diverse biological settings and fusion between lipid bilayers free of protein displays a very similar dependence. The attendees then participated in a lively discussion that considered alternate interpretations of experiments highlighted by the two advocates and proposed experiments that might be able to rule out each of the models.

In Session IV, the dynamics of physiological processes and biochemical events of vesicular trafficking were discussed. Ronald W. Holz (University of Michigan) discussed unexpected findings in primary chromaffin cells that show that increased motion and travel, rather than stable docking, characterizes the last moments of secretory granules before exocytosis. George Augustine (Duke University) presented a novel and powerful technique of uncaging peptides that interfere with specific steps in synaptic transmission in the squid axon. This technique can time the occurrence of specific steps in the pathway. Evidence was presented that NSF and complexin can regulate synaptic transmission within 300 ms of neurotransmitter release. Sanford M. Simon (Rockefeller University) discussed the dynamics of dynamin, clathrin, and epsin using TIRFM in functioning cells. Tomas Kirchausen (Harvard University) continued on this theme, focusing on the dynamics of the uncoating of the triskelion of recently endocytosed vesicles by HSC 70 and auxilin. Matt Ferguson (NIH) described small angle neutron scattering studies that demonstrated molecular flexibility of clathrin triskelia. Erik Jorgensen (University of Utah) described combined genetic, functional, and morphological approaches in *C. elegans* that illuminated the function of UNC13 and CAPS protein (UNC31) in synaptic transmission of vesicles and dense core granules, respectively. Both these proteins acted as if they open the closed conformation of syntaxin to allow its SNARE motif to function in exocytosis. Finally, John Heuser (Washington University) reviewed, in light of present understanding, aspects of the now classical work that he performed 35 years ago with Thomas Reese (NIH) in which rapid freezing of the stimulated frog neuromuscular junction revealed the cycle of synaptic vesicle exocytosis and endocytosis. He emphasized the need for renewed EM studies with even more refined techniques for further progress in understanding membrane dynamics in synaptic transmission. It was evident from the enthusiastic questions and comments from the audience and the responses of John Heuser that the classical studies from the 1970s are still relevant to current questions concerning synaptic transmission.

Session V explored the role of dynamin in the fission process of endocytosis. Sandra Schmid (Scripps Research Institute) discussed the regulation of dynamin–membrane interactions by nucleotides and effectors. Dynamin associates preferentially with $PI(4,5)P_2$ patches in membranes. This promotes its self assembly, which is regulated by nucleotides and interactions with proteins that sense membrane curvature, including endophilin and amphiphysin as well as sorting nexin 9. Jenny Hinshaw (NIH) discussed the role of various members of the dynamin family in membrane remodeling. Vadim Frolov (NIH) extended the discussion by describing elegant studies that measured lipid tube electrical conductance as a strategy to measure tube diameter. He then described experiments examining the effects of dynamin and nucleotides in order to better define the molecular requirements for both constriction and fission. Pietro de Camilli (Yale University) emphasized the role of BAR domains in the formation of curved membrane structures that promote tubulation. He also discussed some cell biological features observed in cells from dynamin 1 knockout mice.

Session VI was a point/counterpoint series of presentations regarding the kiss and run mechanism of synaptic vesicle exocytosis. Richard Tsien (Stanford University) took the "point" point of view, with Tim Ryan (Cornell University), Tom Schwartz (Harvard University), and Jurgen Klingauf (Max-Planck) providing the "counterpoint." Richard Tsien described experiments that exploited the pH sensitivity of the fluorescence of individual quantum dots that had been trapped in synaptic vesicles to conclude that kiss and run is the predominant method of exocytosis during the early phase of transmitter secretion. Tim Ryan exploited the pH sensitivity of VGlut1-pHluorin fluorescence to arrive at the opposite conclusion. VGlut1 is localized exclusively on synaptic vesicles. When fused to GFP-pHluorin, high sensitivity imaging revealed single vesicle fusion and retrieval events in cultured hippocampal neurons. Whereas he observed rapid events with kinetics consistent with kiss and run, such events were consistent with a single distribution with a time constant of many seconds, inconsistent with a major role for kiss and run. Tom Schwartz described studies of the *Drosophila* neuromuscular junction, in which he used genetic approaches to discover that synaptojanin and endophilin enhance the rate of classical endocytosis. In their absence, endocytosis still occurs, but at reduced rates. With fast stimulation, the excitatory postsynaptic potentials get bigger, indicating that bigger vesicles are formed, which is inconsistent with a kiss and run mechanism. Finally, Jürgen Klingauf raised a number of issues, including the possibility that the quantum dots in Tsien's experiments might

affect the properties of the vesicle and its normal fusion mechanism, and suggested that kiss and run cannot be a major mechanism.

Session VII was concerned with "protein recruitment, membrane dynamics, and membrane microdomains." Stuart McLaughlin (Stony Brook University) described how clusters of basic residues on proteins, such as syntaxin, produced a local positive electrostatic potential that can act as a basin of attraction for the polyvalent acidic lipid $PI(4,5)P_2$. Michael Kozlov (Tel Aviv University) described theoretical aspects of membrane shaping by proteins and provided a clear picture of the major concepts involved in membrane bending. Adam Frost (Yale University) continued the topic of membrane curvature by considering how F-Bar proteins bend bilayers to conform to their banana-like curvature. Jens Coorssen (University of Calgary) described how specific lipids may provide the critical negative curvature that enables Ca^{2+}-triggered fusion. Ken Jacobson (University of North Carolina) described how the raft concept may need to be altered to conform to the realities of biological membranes and how small rafts, produced by minor cross-linking of outer leaflet components, may be induced to transiently anchor to the cytoskeleton underlying the membrane. Sergey Akimov (Frumkin Institute) outlined a theory of domain formation in membranes as a process of wetting of proteins by lipids.

Finally, Session VIII, entitled "COMING FULL CIRCLE: Lipids in rafts, endocytosis, and fusion" featured two speakers, Kai Simons (Max Planck) and Ari Helenius (Swiss Federal Institute of Technology). Kai Simons' talk dealt with domain-induced budding in post-Golgi transport, focusing on the isolation of raft clusters from cells. He described recent work from his laboratory dealing with immunoisolation of raft carriers from yeast, as they make their way toward the plasma membrane. The so-called Fus-Mid vesicles are enriched in sphingolipids, and have low phosphatidylcholine content. Similar studies are in progress with MDCK cells. Ari Helenius discussed his recent work on the entry of Vaccinia virus in the host cell. Vaccinia appears to enter via macropinocytosis, after "surfing" along filopodia, and generating of blebs in the host cell membrane. The viral membrane is rich in phosphatidylserine, and this is essential for infectivity; the lipid activates a signaling pathway: Rae 1, PAK 1, actin, etc. Retraction of the bleb drives macropinocytosis. Macropinocytosis is the process by which remnants of apoptosed cells are taken up by surviving cells. Thus Vaccinia virus can be said to enter the host cells by "apoptotic mimicry."

This symposium was generously supported by the NIH Office for Rare Diseases, NICHD, the Jain Foundation, FEI, Chroma, Nikon, Genentech, Zeiss, Avanti, and Coherent.

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