New and Notable

Seeing Is Believing: The Stalk Intermediate

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Membrane biophysicists have sought the molecular nature of biomembrane fusion for more than 30 years. In this time, two conflicting hypotheses have evolved to explain very different observations. One, based on electrophysiological measurements on patch-clamped cells (1), is the *proteinacious pore hypothesis*. This model, popular among neurobiologists, contends that the initial fusion pore consists of a single proteinacious channel that later opens into a full fusion pore due to dissipation of the initial protein pore components into lipid bilayer. Recent mutational studies of the *trans*-membrane domain of the synaptic fusion protein syntaxin claim to support this hypothesis and propose that several syntaxin *trans*-membrane domain's may form the initial pore (2). The alternative hypothesis is the *lipidic pore hypothesis*. It contends that the fusion pore derives from non-lamellar lipid structures and proceeds through an initial partially fused structure in which lipids mix although aqueous compartments do not. The partially fused state is termed hemifusion. A large number of observations offer support for this view for viral, exocytotic, and model systems (3–8). Despite the preponderance of evidence for the lipidic pore hypothesis, no one has until now actually observed in a biological system a lipidic pore or one of the presumed lipidic intermediates thought to precede the lipidic pore. An article in this issue of the *Biophysical Journal* (9) dramatically alters this situation.

Submitted July 13, 2006, and accepted for publication July 24, 2006.

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0006-3495/06/10/2747/02 $2.00

The lipidic pore hypothesis is most often expressed in terms of the ''stalk model''. This proposes specific structures for the intermediates that lead to a fusion pore. After examining bilayers supported on mica sheets (10), the stalk structure was proposed to consist of merged contacting or *trans* monolayers and unfused *cis* monolayers (11), as illustrated in Fig. 1 of Zampighi et al. (9). Siegel proposed a ''modified stalk model'' in which another type of intermediate structure, the *trans*-membrane contact (TMC), might also be energetically possible (12). Kinetic studies of PEG-mediated vesicle fusion established two intermediate states (13). Simple calculations based either on a macroscopic materials model (14) or on a field theoretical treatment with course-grained models of lipid and water molecules (15) showed that both the stalk and TMC were candidate structures for these intermediates.

The first structural evidence for the lipidic pore model came from x-ray scattering studies by Yang and Huang of pure lipid systems in which under extreme dehydration and elevated temperature, a stable rhombohedral lipid phase consisting of hexagonally packed stalk structures was documented (16). Although this demonstrates that stalk-like structures are stable under certain conditions, it does not demonstrate these structures in fusing biomembranes. Zampighi et al. (9) report in this issue of the *Biophysical Journal* conical thin sectioning and electron tomography analysis of samples of rat cortical synapse ''active zones'' that provides the first evidence for a stalk or TMC structure at the point of contact of fusing membranes. They observe both ''docked'' (within 15 nm of the presynaptic membrane active zone) and undocked synaptic vesicles, with docked vesicles existing in three possible states: 1), distinct (i.e., with a membrane clearly distinct from that of the presynaptic membrane, ~15%), 2), hemifused (having a region where contacting membrane leaflets could not be detected, ~75%), and 3), fused (having a pore evident in single membrane present in the hemifused region, ~10%). This is consistent with the observation of different functional pools of synaptic vesicles (17) and with the suggestion that the fast-release pool could consist of hemifused vesicles (18). The resolution of these micrographs (~4 nm) and the diameter of the hemifused structures (~8 nm; see Fig. 1 in Zampighi et al. (9)) made it difficult to determine whether stalks or TMC were present in hemifused regions, although the conclusion of a hemifused structure seems irrefutable. Similarly, it is difficult, if not impossible, to discern the location of pores (edge or center of hemifused structures) or to be certain about the location of proteins associated with these structures. Zampighi et al. point out filamentous structures near to but not in the hemifusion structures. Nonetheless, the very small size of the hemifused structures makes it unlikely that there is significant protein material intimately associated with them. Although this uncertainty means that the elegant reconstructed images of Zampighi et al. will not end the controversy between the lipidic- and proteinacious-pore camps, they do provide a quantum jump in structural support for the lipidic pore model.

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