PERSPECTIVES: CELL BIOLOGY

Fusion Without SNAREs?

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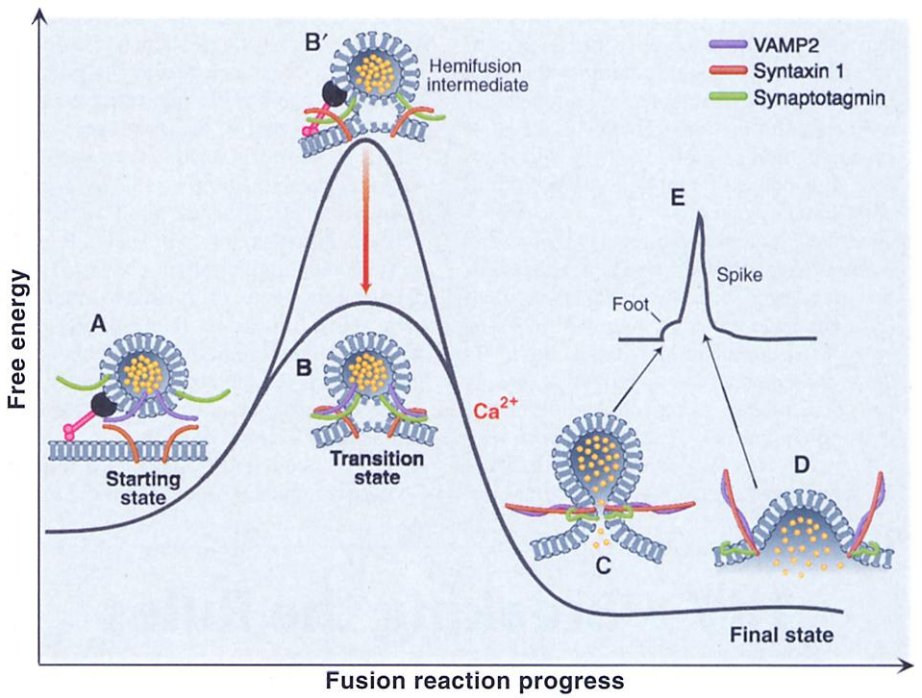
The organization of cellular organelles depends on regulating the mixing of specific membrane compartments through modulation of membrane fusion. Nowhere is membrane fusion more exquisitely regulated than during synaptic transmission between neurons—neurotransmitter-laden vesicles fuse with the plasma membrane of the presynaptic neuron upon receipt of a calcium signal. All intracellular membrane fusion events are governed by the formation of a specific complex of proteins called SNAREs (soluble *N*-ethylmaleimide–sensitive factor–attachment protein receptors) that bridge the two membranes and pull them together to facilitate the mixing of membrane lipids (*1*) (see the figure). Neurotransmitter release is likely to be regulated by a vesicle membrane protein called synaptotagmin. This protein interacts with the SNARE complex as well as with phospholipids in a calcium-dependent manner, suggesting that it is a key player in membrane fusion (*1*). In this issue, the molecular components of the fusion machinery are scrutinized in two high-resolution electrophysiology studies by Schoch *et al.* (*2*) on page 1117 and Wang *et al.* (*3*) on page 1111.

There is compelling evidence that SNARE complexes are essential for the fusion of membranes (*1*). However, surprisingly, fusion is not abolished in yeast, flies, and worms engineered to lack a synaptic vesicle SNARE called VAMP2 (synaptobrevin), or when VAMP2 is cleaved by neurotoxins (*4*–*7*). In their study, Schoch *et al.* (*2*) confirm these results in primary embryonic hippocampal neuronal cultures from mice lacking VAMP2. They show that calcium-stimulated release of neurotransmitter is decreased by a factor of 100, and that only about 10% of spontaneous release (miniature excitatory currents, or minis) and osmotic shock–induced release (assumed to stimulate the same pool of vesicles as calcium but in a calcium-independent manner) persists [compare with (*5*)]. Schoch *et al.* conclude that these remaining neurotransmitter-release events result from SNARE-independent fusion because the closest VAMP2 isoforms, VAMPs 1 and 3, are absent and thus cannot substitute for VAMP2 activity. However, there are at least 35 SNARE genes expressed in mammalian species, so it remains possible that another family member compensates for the eliminated protein. For example, VAMP7—which can replace VAMP2 in SNARE complexes in vitro (*8*) and mediates constitutive fusion with the plasma membrane during neurite extension in developing hippocampal cells (*9*)—may substitute for VAMP2 with reduced efficiency. In addition, target membrane SNAREs (t-SNAREs) found on the vesicle membrane (*10*) may allow complexes consisting exclusively of t-SNAREs (that is, SNAP-25 and syntaxin 1) to mediate this residual fusion. This hypothesis could be easily tested by examining whether the residual fusion in the VAMP2-deficient cells is resistant to cleavage of these t-SNAREs by botulinum neurotoxin C.

Pure lipids can be fused under appropriate ionic conditions, and a number of proteins other than SNAREs can promote membrane fusion (*11*). Thus, SNAREs themselves are not essential for fusion, but instead can be viewed as catalyzing the process by stabilizing a transition state—perhaps the hemifusion intermediate or the fusion pore (see the figure). The observation that calcium-stimulated neurotrans-



Membranes in motion. Energy profile of membrane fusion and the part played in this process by SNAREs and synaptotagmin (vesicle membrane proteins). After synaptic vesicle docking, which involves rab (black) and rab effector proteins (pink), the synaptic vesicle–associated membrane protein VAMP2 (synaptobrevin) specifically forms complexes with the target membrane SNAREs (t-SNAREs), syntaxin 1 and SNAP-25, at the plasma membrane. (**A**) The SNARE complex is composed of a four-helical bundle, with VAMP2 (dark blue) and syntaxin 1 (red) each contributing one helix, and SNAP-25 contributing two helices (not shown). The full formation of the trans-SNARE complex (where VAMP and syntaxin are in opposite membranes) forces the two membranes together and induces curvature such that the proximal membrane leaflets intermingle (**B**). This produces an energetically unstable hemifusion intermediate. In the absence of VAMP (**B′**), the SNARE complex cannot form and stabilize the transition state (red arrow). It is not yet clear at which stage the SNARE complex is arrested before the arrival of the calcium trigger—it could be before or during the hemifusion state. It is also uncertain at which stage synaptotagmin interacts with the SNARE complex. In one scenario, receipt of the calcium trigger (Ca^{2+}) would prompt synaptotagmin (green) to promote the rearrangement of the transmembrane domains of syntaxin and VAMP. This would enable the formation of a very stable cis-SNARE complex (with all SNAREs in the same membrane), causing the mixing of distal leaflets and the creation of a small hole, the fusion pore (**C**), detected as the foot of an amperometric current spike (**E**). (Overexpression of synaptotagmin could thus control fusion pore size through the SNARE complex.) The fusion pore then dilates to relax the membrane and neurotransmitter is released (**D**), creating the main spike of the electrophysiology recording (**E**).

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mitter release is more affected than spontaneous release by the absence of VAMP2 is consistent with the idea that the SNARE complex is already at least partially assembled and awaits the arrival of the calcium trigger to facilitate full assembly and the completion of membrane fusion (*12*, *13*). In contrast, spontaneous release (minis) and sucrose-evoked release, reflecting non– (or low–) calcium-dependent fusion events, are less severely affected by the loss of SNARE catalysis. Notably, 90% of this fusion was eliminated in the absence of VAMP2, indicating that the preferred mechanism of fusion does actually depend on the SNARE complex. Whether calcium ions directly affect the SNARE complex or instead work through a calcium-sensing protein remains to be determined.

The strongest candidate for the calcium-sensing protein is synaptotagmin. This protein was initially dubbed a "fusion clamp" because it was thought to bind to SNAREs, preventing their fusion activity until the arrival of calcium ions. However, because synaptotagmin also oligomerizes and interacts with plasma membrane phospholipids upon binding calcium ions, it could act alternatively as a positive regulator of fusion by becoming inserted into the membrane or by interacting with the fusion pore itself (*14*). The latter model is supported by Wang *et al.*'s (*3*) amperometry experiments. In these experiments, the investigators directly measured release of catecholamine neurotransmitters from PC12 cell dense-core vesicles as a current formed by the oxidation of the neurotransmitter on a carbon-fiber electrode. Their analysis centers on the small initial signal, or "foot," seen at the leading edge of the main spike of neurotransmitter-induced current (see the figure). The foot most likely reflects the flux of a tiny amount of neurotransmitter from the vesicle through the fusion pore as soon as the lipids fully merge, whereas the main spike reflects the expulsion of the remaining neurotransmitter when the fusion pore dilates. Overexpression of synaptotagmin I increases foot duration, whereas overexpression of synaptotagmin IV decreases foot duration. One interpretation of these data is that synaptotagmins directly interact with the fusion pore and regulate its formation and expansion. It remains unclear how this might occur mechanistically and why the two synaptotagmin isoforms have opposite effects. Thus, although the precise physiological function of synaptotagmin is still not understood, the Wang *et al.* study suggests that it should no longer be viewed as a fusion clamp but instead more like a valve, regulating the diameter and open time of the fusion pore by stabilizing the pore lipids. However, like VAMP2, synaptotagmin is unlikely to be absolutely essential because rapid calcium-dependent fusion is not completely abolished in synaptotagmin-deficient cells (*15*). Biochemical mysteries surrounding synaptotagmin also abound, as it is not yet clear whether synaptotagmin acts by becoming inserted into the membrane upon receipt of the calcium trigger, or through altered interactions with SNARE proteins.

It has been widely assumed that the SNARE complex is essential for the merger of membranes and thus might control the rate of a fusion event. In contrast, synaptotagmin is a favorite candidate for the calcium sensor. But it may be that VAMP2 along with the other SNAREs is critical for efficient membrane fusion, particularly in response to calcium ions, whereas the rate of a fusion event itself could be controlled by the synaptotagmins. These data support earlier biochemical studies (*14*) showing that the job of synaptotagmin is intimately tied to that of the SNAREs. Molecular elucidation of the nature of the fusion pore will no doubt shed more light on the individual parts played by the proteins involved. New physical methods, including biochemically reduced preparations that accurately reflect in vivo events, need to be developed in order to address these complex physiological processes in molecular detail (*16*).

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PERSPECTIVES: VIROLOGY

HIV—Breaking the Rules for Nuclear Entry

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The human immunodeficiency virus (HIV) gains access to human chromosomes by sabotaging the nuclear structure of the host cell. This clever ploy may explain how HIV can infect nondividing host cells such as macrophages (*1*). In nondividing cells, access to the nucleus is limited to proteins or protein complexes that (i) have a nuclear localization signal, and (ii) do not exceed the size limit (~25-nm diameter) of the nuclear pores (*2*). The preintegration complex (PIC) of HIV, which contains a double-stranded DNA copy of the viral genome, forms in the cytoplasm and must enter the nucleus so that the viral DNA can be inserted into a host cell chromosome. However, with a diameter of ~56 nm (*3*), the intact PIC is much too large to pass through the pores in the nuclear membrane by any known mechanism.

Vpr is one of three HIV proteins thought to mediate nuclear entry of the PIC. Vpr is a small protein (11.7 kD, 96 residues) that shuttles between the cytoplasm and nucleus of the host cell (*4*) and can arrest proliferating cells in late G_2 phase of the cell cycle (*5*). On page 1105 of this issue, de Noronha and colleagues (*6*) report that the expression of wild-type Vpr protein in HeLa cells is sufficient to induce transient herniations in the nuclear envelope of these cells. Sporadically and spectacularly, these herniations burst, releasing soluble nuclear proteins into the cytoplasm and presumably also allowing cytoplasmic proteins to freely enter the nucleus (see the figure). Equally remarkable, the broken sections of the nuclear envelope apparently reseal within minutes. De Noronha *et al.* on page 1105 (*6*) propose that these transient ruptures of the nuclear envelope may provide an unconventional route for nuclear entry that bypasses the size-restricted nuclear pore complexes.

Bursting of the Vpr-induced nuclear herniations releases key cell cycle regulators—which include the kinase Wee1, the phosphatase Cdc25C, and cyclin B—into the cytoplasm of the host cell. These proteins regulate the transition from G_2 to mitosis (*7*), and their repeated release into the cytoplasm might explain how Vpr causes G_2 arrest. Consistent with this idea, Vpr mutants that fail to cause herniations also fail to cause G_2 arrest (*6*).

It is remarkable that a single protein, Vpr, can so profoundly disrupt nuclear en-

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