ELECTROPHYSIOLOGICAL RECORDINGS OF SNARE “FUSION PORES” IN A RECONSTITUTED SYSTEM

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The membrane fusion machinery has evolved independently in both viruses and eukaryotic cells to fuse apposed bilayers. Viral fusion proteins (Types I and II) and eukaryotic SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors) show unique architectures, but each use their protein folding energy to merge membranes. In the SNARE proteins, cognate v- and t-SNAREs on vesicles and target membranes pair to form the core machinery for intracellular membrane fusion. Energy made available from the “zipping-up” of the SNARE complex is utilized to drive the fusion of lipid bilayers. Electrical measurements of exocytic cells and expressed viral proteins have shown that, in most cases, the “fusion pore” dilation consists of a slow and fast phase, with the initial pore diameter close in size to an ion channel. We have developed an experimental system to test the “fusion pore” kinetics of the SNARE proteins alone, based on the “flipped-SNARE” system. Here, cognate SNAREs which are ectopically expressed on two populations of mammalian cells (v- and t-cells), mediate cell:cell fusion. This system has the advantage of topologically isolating the SNAREs from cellular factors which may regulate their activity, making assignment of distinct kinetic processes more clear. The “flipped-SNARE” system has now been used to identify distinct SNARE folding states including hemifusion, “kiss and run”, and full fusion. In addition, we have made electrical recordings of hemifused cells demonstrating that no “non-enlarging” pores exist between partner cells. Detection of “fusion pore” lifetimes are underway, to measure the kinetics of the SNARE-alone pore, and subsequently the contributions of protein mutations, lipids, and regulator factors on pore lifetimes.