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Membrane fusion in cells: molecular machinery and mechanisms

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Abstract

Membrane fusion is a *sine qua non* process for cell physiology. It is critical for membrane biogenesis, intracellular traffic, and cell secretion. Although investigated for over a century, only in the last 15 years, the molecular machinery and mechanism of membrane fusion has been deciphered. The membrane fusion event elicits essentially three actors on stage: anionic phospholipids - phosphatidylinositols, phosphatidyl serines, specific membrane proteins, and the calcium ions, all participating in a well orchestrated symphony. Three soluble N-ethylmaleimide-sensitive factor (NSF)-attachment protein receptors (SNAREs) have been implicated in membrane fusion. Target membrane proteins, SNAP-25 and syntaxin (t-SNARE) and secretory vesicle-associated membrane protein (v-SNARE) or VAMP were discovered in the 1990's and suggested to be the minimal fusion machinery. Subsequently, the molecular mechanism of SNARE-induced membrane fusion was discovered. It was demonstrated that when t-SNARE-associated lipid membrane is exposed to v-SNARE-associated vesicles in the presence of Ca^{2+}, the SNARE proteins interact in a circular array to form conducting channels, thus establishing continuity between the opposing bilayers. Further it was proved that SNAREs bring opposing bilayers close to within a distance of 2–3 Å, allowing Ca^{2+} to bridge them. The bridging of bilayers by Ca^{2+} then leads to the expulsion of water between the bilayers at the contact site, allowing lipid mixing and membrane fusion. Calcium bridging of opposing bilayers leads to the release of water, both from the water shell of hydrated Ca^{2+} ions, as well as the displacement of loosely coordinated water at the phosphate head groups in the lipid membrane. These discoveries provided for the first time, the molecular mechanism of SNARE-induced membrane fusion in cells. Some of the seminal discoveries are briefly discussed in this minireview.

Keywords: membrane fusion • SNAREs • membrane proteins • phospholipids • calcium • porosome / fusion pore • cell secretion

Introduction

The process of membrane fusion is as old as life itself. The very birth of the unit of life, the cell, may well have taken place with the encapsulation of a primodial life-soup within a membranous sac, and the subsequent fission and fusion of membranes, ultimately giving birth to the various functionally specialized sub-cellular organelles as we know them today. Whatever may be the case, membrane fusion in cells is an essential and fundamental requirement, occurring in all life forms – from yeast to humans. The pro-

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cess is so fundamental, that without it, life essentially would cease to exist. Therefore, the quest to understand membrane fusion in cells has been on for more than a century. Only in the last 15 years, the molecular machinery and mechanism of the process has been brought to light with the pioneering discoveries of Bhanu P. Jena and James E. Rothman, in the field.

Discovery of SNAREs: proteins that bring opposing bilayers close to each other

During the 1970's, extensive studies by D. Papahadjopoulos and his research team [1], and later throughout the 1980's, studies from various laboratories including that of S. Ohki [2, 3], clearly demonstrated the requirement of divalent cations, especially Ca^{2+} on fusion of opposing phospholipid membranes. Then, in the 1990's [4, 5], James E. Rothman and his research team demonstrated that three soluble *N*-ethylmaleimide-sensitive factor (NSF)-attachment protein receptors (SNAREs), are universally involved in membrane fusion in mammalian cells [4, 5]. Target membrane proteins SNAP-25 and syntaxin, collectively termed t-SNAREs or target SNAREs, and secretory vesicle-associated membrane protein: VAMP or v-SNARE, are part of the conserved protein complex involved in fusion of opposing bilayers in cells. The discovery of SNARE proteins, and determination of their involvement in membrane fusion in cells, was truly a breakthrough, since for the first time, it provided an explanation for specificity and regulation of membrane fusion in cells. With the discovery of SNAREs, the requirement of Ca^{2+} on fusion of opposing phospholipid membranes was completely overlooked, so much so that SNAREs came to be recognized as the "minimal fusion machinery" [5]. Nonetheless, the next major question *i.e.*, the molecular mechanism of SNARE-induced fusion of opposing bilayers, remained unsolved.

SNARE complex: structure, assembly, and disassembly

To understand the molecular mechanism of SNARE-induced membrane fusion, an understanding of the structure of the SNARE complex was essential. Using truncated soluble t- and v-SNAREs, the structure of the resultant complex formed, termed the core domain, was determined at 2.4 Å using x-ray crystallography [6]. As it became clear from subsequent studies [7], the x-ray crystallographic study [6] nuanced the importance of membrane-associated SNAREs. As with most membrane proteins, the interaction and resultant arrangement of the t-/v-SNARE complex were found to be quite different when t-/v-SNAREs are in solution as opposed in association with membrane [7]. Using atomic force microscopy and bilayer electrophysiological assays, Bhanu P. Jena and his research team demonstrated for the first time that full length t-SNARE and v-SNARE in opposing bilayers interact in a circular array (forming ring-like channels), to form conducting channels in the presence of calcium [7, 8]. On the contrary, when the same full length v- and t-SNAREs are in solution (in absence of membrane), or even when one of the SNAREs is in solution, t-/v-SNARE interactions fail to form such conducting channels [7]. These studies by Jena [7, 8] were a major breakthrough in our understanding of SNARE-induced membrane fusion. They demonstrated that t-SNAREs and v-SNARE should reside in opposing membranes to allow appropriate t-/v-SNARE interactions, leading to membrane fusion in the presence of calcium. They further prove that in the physiological state in cells, both SNAREs and Ca^{2+} operate as the minimal fusion machinery [8]. Using SNARE-reconstituted liposomes and bilayers [8], Jena showed that *(i)* there is a low fusion rate (τ=16 min) between t- and v-SNARE-reconstituted liposomes in the absence of Ca^{2+}; however, *(ii)* exposure of t-/v-SNARE liposomes to Ca^{2+}, drives vesicle fusion on a near physiological relevant time-scale (τ ~10s), establishing an essential role of Ca^{2+} in membrane fusion. Since the Ca^{2+} effect on membrane fusion in SNARE-reconstituted liposomes was found to be downstream of SNAREs, it suggests a regulatory role for Ca^{2+}-binding proteins in membrane fusion in the physiological state in cells [8]. These studies clearly reveal that not just SNAREs, but both ***SNAREs and Ca^{2+} are the minimal fusion machinery in cells*** [7, 8]. Native and synthetic vesicles exhibit a significant negative surface charge primarily due to the polar phosphate head groups. These polar head groups produce a repulsive force, preventing aggregation and fusion of apposing vesicles.

In their pioneering studies [8], Jena and his research team further demonstrate that SNAREs bring opposing bilayers closer to within a distance of 2–3 Å, thus allowing Ca^{2+} to interact with the phospholipid head groups, and bridging them. The bound Ca^{2+} then leads to the exclusion of water between the bilayers at the bridging site, allowing lipid mixing and membrane fusion. Hence SNAREs, besides bringing opposing bilayers closer, dictate the site and size of the fusion area during cell secretion [8]. The size of the t-/v-SNARE complex forming the pore is dictated by the curvature of the opposing membranes, hence depends on the size of t-/v-SNARE-reconstituted vesicles. The smaller the vesicles, the smaller the pores formed [9]. The assembly and disassembly of the SNARE complex, has also been determined by Jena and his research team [10].

Molecular mechanism of SNARE-induced membrane fusion

At the atomic level, the participation of calcium in SNARE-induced membrane fusion has further been determined by Jena and his research team [11]. Calcium ion is essential to life's processes, and participates in diverse cellular and physiological functions. Although calcium is present in abundance within cells, it is well sequestered and is available only on demand [11]. Upon certain cellular stimulus, Ca^{2+} concentration at specific nano environments in a cell becomes elevated by several orders of magnitude within a brief period (< 1ms) [11]. This prompt mobilization of Ca^{2+} is essential for many physiological functions, such as the release of neurotransmitters. Not surprisingly, calcium ion channels have been found in direct association with t-SNARE (SNAP-23), at the base of fusion pores or porosomes (permanent supramolecular structures at the cell plasma membrane [12–19]), where secretory vesicles dock and fuse to release their contents [20]. Calcium ion [Ca^{2+}] exists in its hydrated state within cells [11]. Since hydrated calcium $[Ca(H_2O)_n]^{2+}$ with its first hydration shell possess six water molecules, and measure >6 Å, it would be impossible to squeeze between the 2.8–3 Å space established by t-/v-SNAREs, between the apposing vesicle bilayers [8, 11]. The answer is simple, as elucidated by Jena and his research team [11]. When t- and v-SNARE vesicles are allowed to mix in a calcium-free buffer, prior to the addition of calcium, no fusion occurs. On the contrary, when t- and v-SNARE vesicles are allowed to mix in a calcium-buffer, vesicles aggregate and fuse [11]. When NSF-ATP (for SNARE complex disassembly) [10] is present in the assay buffer containing calcium, a significant inhibition in aggregation and fusion of proteoliposomes is observed. NSF, in the absence of ATP, has no effect. These results demonstrate that NSF-ATP disassembles the SNARE complex, thereby reducing the number of interacting vesicles in solution. In addition, disassembly of trans-SNARE complex will then leave apposed bilayers widely separated, out of reach for the formation of Ca^{2+}-phosphate bridges, preventing membrane fusion. Similarly, if the restricted area between adjacent bilayers delineated by the circular arrangement of the t-/v-SNARE complex is formed, then hydrated Ca^{2+} ions are too large to be accommodated between the bilayers, and hence subsequent addition of Ca^{2+} would have no effect. However, when t-SNARE vesicles interact with v-SNARE vesicles in the presence of Ca^{2+}, the t-/v-SNARE complex formed allow formation of calcium-phosphate bridges between opposing bilayers, leading to the expulsion of water around the Ca^{2+} ion to enable lipid mixing and membrane fusion [11]. The calcium bridging of apposing bilayers allows for the release of water from the hydrated Ca^{2+} ion, leading to bilayers destabilization and membrane fusion. In addition, the binding of calcium to the phosphate head groups of the apposing bilayers, may also displace the loosely coordinated water at the phosphate groups, further contributing to the destabilization of the lipid bilayer, leading to membrane fusion [11]. Following determination of the molecular mechanism of calcium on SNARE-induced membrane fusion by Jena and his research team [7, 8, 11], the important role of calcium in cell secretion was further confirmed by Rothman in collaboration with another group [21]. These discoveries by Jena and Rothman, have finally determined the molecular machinery and mechanism of membrane fusion in cells. The main steps in membrane fusion, porosome opening and cell secretion of SNARE-induced membrane fusion during secretion, are highly controlled and regulated events (Fig. 1): 1) vesicles dock at the base of porosomes by tethering to porosome-associated t-SNAREs by

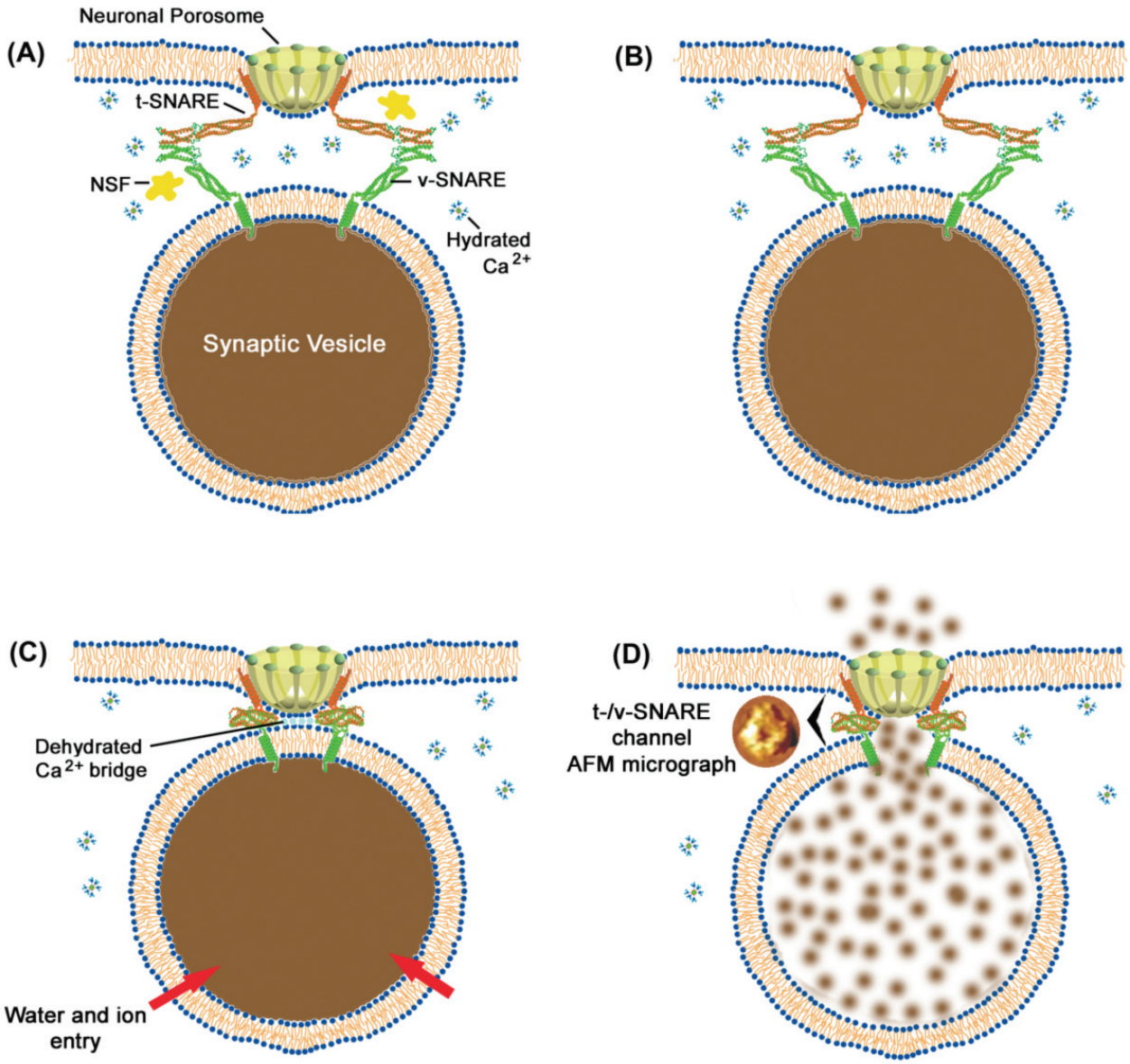

Fig. 1 Schematic diagram depicting synaptic vesicle fusion at the base of porosome, leading to neurotransmitter release at the nerve terminal. (**A**) Note, in presence of NSF, t-SNARE and v-SNARE fail to form a complex. (**B**) In the absence of active NSF, t-SNAREs and v-SNARE interact in a circular array to form a channel, bringing the opposing bilayers within a distance of 2.8–3 Å. (**C**) If the t-/v-SNARE channel is formed in the presence of calcium, the hydrated calcium ion looses its water shell by bridging with the opposing phospholipid head groups, resulting in lipid mixing and the establishment of a conducting t-/v-SNARE channel. (**D**) Secretory vesicles, with a build-up of intra vesicular pressure, can then pump out the vesicular contents through the SNARE channel (actual atomic force micrograph of neuronal t-/v-SNARE ring channel is shown) and out the porosome opening.

vesicle-associated v-SNARE; 2) the t-/v-SNARE interact in a circular array to form a ring complex, pulling the opposing bilayers into close proximity (2.8–3 Å) complex; 3) during this process, the hydrated Ca^{2+} present between the opposing bilayers (*i.e.*, the base of porosome and the secretory vesicle membrane), is able to bridge with the phospholipid head groups, allowing the release of the water shell from the hydrated Ca^{2+} ion; 4) this leads to the destabilization of the lipid bilayers within the

t-/v-SNARE channel complex, leading to lipid mixing and membrane fusion; 5) the porosomes opening then dilates; 6) secretory vesicles swell, generating intravesicular pressure, which drive the expulsion (partially or completely, depending on the intravesicular pressure); 7) following secretion, vesicle reseals and detaches from the membrane (empty or partially empty) due to SNARE complexes disassembly by NSF-ATP.

Finally, after almost a century of studies to understand the molecular mechanism of membrane fusion in cells, the subject has been greatly elucidated. With further developments of technology and tools, we may one day in the future be able to understand the rearrangement and interactions of individual atoms in real time (<femtosecond), during membrane fusion. Membrane fusion, like any other biochemical reaction in the living cells, approached at a spatial resolution of single atom and temporal resolution at femtosecond, will bring about the next breakthrough. Nonetheless, the current discovery has brought us several orders of magnitude closer to this reality.

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