Neuroscience 190 (2011) 12–20

REVIEW

THE DISCOVERY OF THE SOLUBLE *N*-ETHYLMALEIMIDE-SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTOR COMPLEX AND THE MOLECULAR REGULATION OF SYNAPTIC VESICLE TRANSMITTER RELEASE: THE 2010 KAVLI PRIZE IN NEUROSCIENCE

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Abstract—Brain function depends on a crucial feature: The ability of individual neurons to share packets of information, known as quantal transmission. Given the sheer number of tasks the brain has to deal with, this information sharing must be extremely rapid. Synapses are specialized points of contact between neurons, where fast transmission takes place. Though the basic elements and functions of the synapse had been established since the 1950s, the molecular basis for regulation of fast synaptic transmitter release was not known 20 years ago. However, around 1990, crucial discoveries were made by Richard Scheller, James Rothman, and Thomas Südhof, leading a few years later to the formulation of the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) hypothesis and a new understanding of the molecular events controlling vesicular release of transmitter in synapses. The 2010 Kavli Prize in neuroscience was awarded to these three researchers, "for their work to reveal the precise molecular basis of the transfer of signals between nerve cells in the brain."

Key words: synapse, SNARE, syntaxin, SNAP-25, VAMP/synaptobrevin, synaptotagmin.

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Abbreviations: NSF, *N*-ethylmaleimide-sensitive factor; SNAP, soluble NSF attachment protein; SNAP-25, 25-kDa synaptosome-associated protein; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; t-SNARE, target-membrane SNARE; VAMP, vesicle-associated membrane protein; v-SNARE, vesicle-membrane SNARE.

One of the most intriguing and important scientific questions of the last couple of centuries has been: What is the biological foundation for our ability to think? Though the question is not yet answered fully, we have accumulated a vast amount of detailed information on the topic. Central to our current understanding is the neuron doctrine (His, 1886; Nansen, 1886; Forel, 1887; Waldeyer, 1891), entailing in its established form that information does not flow freely in the brain through a syncytium of cells, but rather, that it is passed on, from one distinct cell to the next, through points of inter-neuronal contact called synapses (Sherrington, 1897). The chemical nature of signal transmission from nerve cells was first established in the autonomous nervous system (Loewi, 1921; Dale, 1934). Later, Katz described the quantal nature of chemical transmission, leading to the hypothesis that the chemical transmitter was released from vesicles in the nerve endings (Del Castillo and Katz, 1954). Independently, such vesicles were anatomically demonstrated in parallel with Katz' physiological work (De Robertis and Bennett, 1955; Palay, 1956). From around 1970, a series of electron microscopic studies by John Heuser gave final proof of the vesicular nature of synaptic transmitter release (Heuser, 1989). However, 20 years later, the molecular link between the eletrophysiological events in the axon terminal and the vesicular release of the chemical transmitter, was still unresolved.

A series of impressive molecular biological and biochemical studies throughout the 1990s by Richard Scheller, James Rothman, and Thomas Südhof, then at Stanford, Sloan Kettering, and University of Texas, respectively, established the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) hypothesis and unraveled the basics of the molecular machinery and protein interactions serving to execute and tightly regulate the vesicle exocytosis of neurotransmitter in brain synapses. Except for a landmark paper in Cell in 1993 by Scheller and Rothman (Söllner et al., 1993a) and a review in Science in 2009 (Südhof and Rothman, 2009), the three researchers have not collaborated, but still their scientific paths have often intertwined closely, both in competition and in reciprocal stimulation. They have had considerable impact on our understanding of a host of proteins serving to regulate vesicle and synaptic functions, for example, rab proteins, rab3a-interacting molecule (RIM), neuronal Sec1/

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.05.057

murine18a (nSec1/Munc18), complexins, the exocyst, coatomers, tomosyn, synuclein, and ADP-ribosylation factor (ARF); in the present review, however, we will focus on the SNARE complex and its interacting partner synaptotagmin.

ROTHMAN DISCOVERS CYTOSOLIC PROTEINS NECESSARY FOR MEMBRANE FUSION

Rothman was not a neuroscientist by training, but a biochemist with a great interest in intracellular transport between organelles. The general cell biological questions that captivated his interest were as made for synaptic studies. The question of which molecular mechanisms serve to attach a transport vesicle to the next organelle and not to one that is too early or too late in the vesicle transport pathway was precisely one that would prove effective in elucidating the mechanisms of synaptic vesicular release.

In 1988, Rothman and his co-workers purified what would later prove to be a key player also in synaptic vesicle membrane fusion: *N*-ethylmaleimide-sensitive protein (NSF), a tetramer of 75 kDa (Block et al., 1988). They observed that uncoated transport vesicles accumulated when NSF was withheld from incubation of Golgi stacks with cytosol and ATP, indicating that NSF is needed for membrane fusion (Malhotra et al., 1988). A year later, Rothman discovered two other components that together bind NSF to Golgi membranes: an integral, heat sensitive membrane receptor; and a cytosolic factor, which they coined soluble NSF attachment protein (SNAP) (Weidman et al., 1989). Rothman concluded that these new factors, while allowing NSF to bind to the membrane, are also part of the fusion machinery.

While the original description of NSF related it to the Golgi organelle, Rothman subsequently suggested that NSF is a general component of the membrane fusion machinery at multiple stages of the secretory pathway. He used monoclonal antibodies against NSF to inhibit transport between endoplasmic reticulum and the Golgi stack in semi-intact cells. Addition of highly purified NSF restored this transport process (Beckers et al., 1989). Rothman then cloned and sequenced the NSF gene from Chinese hamster ovary cells. He compared this gene with the SEC 18 gene product of the yeast *Saccharomyces cerevisiae*, known to be essential for vesicle-mediated transport from the endoplasmatic reticulum to the Golgi apparatus. The two proteins were equivalent, suggesting that the mechanism of vesicular fusion is highly conserved, both between species and at different stages of transport (Wilson et al., 1992). The following year, Rothman's group purified three new and likely components of the membrane fusion machinery, termed alpha-SNAP, beta-SNAP, and gamma-SNAP (Clary et al., 1990).

A couple of years later, Rothman demonstrated a direct interaction between NSF, SNAPs, and an unknown integral membrane component in a detergent solubilized system (Wilson et al., 1992). NSF only bound to SNAPs in the presence of the integral receptor. Binding between these three components resulted in the formation of a multisubunit protein complex, which he called the 20S complex. This complex was able to disassemble in a process coupled to the hydrolysis of ATP. Later the same year he reported the existence of distinct alpha/beta-SNAP binding sites in Golgi membranes that appear to be part of the same receptor complex (Whiteheart et al., 1992). They identified an integral membrane protein of between 30 and 40 kDa to serve as an alpha-SNAP binding component of the multi-SNAP receptor complex. SNAPs would be activated to serve as adaptors for the targeting of NSF, by binding to a multi-SNAP-membrane complex. Cycles of assembly and disassembly could help confer specificity to the generalized NSF-dependent fusion apparatus. However, these articles still gave no hint that the authors grasped the possibility that the proteins could play a crucial role in synaptic transmission.

SCHELLER AND SÜDHOF SINGLE OUT SYNAPTIC VESICLE MEMBRANE PROTEINS ESSENTIAL FOR NEUROTRANSMITTER RELEASE

In parallel with Rothmans groundbreaking research, Scheller and Südhof (the latter often in collaboration with Reinhardt Jahn) characterized most of the membrane proteins that would prove to be crucial for understanding the membrane fusion machinery.

VAMP/synaptobrevin

In June 1988, Scheller reported that his laboratory had used a polyclonal antibody raised against purified cholinergic synaptic vesicles from Torpedo and discovered what he called vesicle-associated membrane protein 1 (VAMP-1) (Trimble et al., 1988). At this early stage, he suggested that VAMP-1 plays an important role in packaging, transport and release of neurotransmitters. Eleven months later, Südhof cloned the same protein, or homolog, from mammalian synaptic vesicles, calling it synaptobrevin. He also showed that it was conserved in a third species: Drosophila (Südhof et al., 1989). He concluded that VAMP-1/synaptobrevin is conserved from mammals to Drosophila and plays a central role in neurotransmission.

In July 1989, Scheller published his second paper on VAMP, using the Torpedo gene to isolate two independent classes of VAMP cDNA clones from rat brain (Elferink et al., 1989). Expressions of both VAMP transcripts were differentially expressed in the rat central nervous system. VAMP-1 was expressed at a higher level in the spinal cord, while VAMP-2 was highly expressed in the whole brain. In 1990, he followed up these data and investigated the expression patterns of the two isoforms in the brain. VAMP-1 expression was localized to a limited number of brain nuclei, primarily those involved in modulating somatomotor functions. VAMP-2 expression was more ubiquitous (Trimble et al., 1990).

But still, the specific function of these vesicle-associated proteins was far from clear. An important discovery was made by Montecoccu's laboratory at Padua University