

Emerging Roles of Presynaptic Proteins in Ca^{++} -Triggered Exocytosis

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Molecules involved in late steps of neurotransmitter release at the synapse can be examined by noting the two speeds of the components of the exocytotic burst that are triggered by an increase in free Ca^{++} . From studies of Ca^{++} -dependent exocytosis of large dense-core vesicles in chromaffin cells, it seems that initiation of the SNARE complex is the molecular event underlying the priming process and that Munc13 acts as a priming factor by opening syntaxin. If synaptic mechanisms are similar, much could be learned from the molecular and kinetic studies that can be performed in chromaffin cells. The twinning of techniques from biophysics and molecular biology has led to remarkable progress in understanding the molecular mechanisms of synaptic transmission. Here we review the current picture of Ca^{++} -triggered exocytosis, which has emerged from studies of a simple cellular model, the adrenal chromaffin cell. We discuss the molecular players that have been assigned a specific role in a particular step of this process and give a brief outlook on what these insights might tell us about mechanisms of short-term plasticity at classical synapses.

Transport vesicles, pinching off from donor compartments and fusing with target membranes, are a common theme in several subfields of cell biology and the neurosciences (1). Correspondingly, many classes of proteins such as SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors), Muncs, and Secs have been found in most of the cellular compartments involved in such membrane trafficking (1, 2), and it is generally held that similar mechanisms underlie the processes of endocytosis and membrane fusion in the different systems. However, when one considers the details, namely, how researchers in the different fields view the sequence of molecular events, major differences become apparent. In neurotransmitter release, for instance, overwhelming evidence has accumulated over the last few years that the SNARE proteins syntaxin, synaptobrevin, and SNAP-25 are intimately involved in the final steps of membrane fusion. In fact, a physically plausible mechanism for fusion is one in which formation of a tight coiled-coil complex between these proteins pulls the participating membranes together (Fig. 1A) (3, 4). In this view, the energy liberated by complex formation is made available for overcoming the energy barrier of lipid rearrangements during fusion. However, work on homotypic vacuolar fusion in yeast assigned a relatively early role to SNAREs (5). Molecules, such as phosphatases (6), calmodulin, and V_0 [the integral

domain of vacuolar H^+ -ATPase (7)], are believed to be essential for the final stages of membrane fusion. Likewise, an essential late role of SNAREs in neurotransmitter release has been questioned on the basis of knockout studies in *Drosophila* (8) and mice (9).

Here, we review the evidence regarding molecules involved in late steps of neurotransmitter release by focusing on one particular property of this Ca^{++} -triggered process—its speed. Release of neurotransmitters and hormones is known to proceed in fractions of seconds or even milliseconds after the free Ca^{++} concentration, $[\text{Ca}^{++}]$, rises—a process that has been trimmed by evolution for speed. On the other hand, it is clear that vesicles have to undergo a number of steps (translocation, docking, priming) before they are ready to fuse in such a rapid manner. These steps are likely to be slower, such that one would expect to observe a typical sequence of events, when $[\text{Ca}^{++}]$ is suddenly increased from low values into a range where rapid release occurs. First, a rapid burst of exocytosis, by vesicles that had been in a release-competent state at the time of the Ca^{++} -increase. This would be followed by a much slower, sustained response (at sustained high $[\text{Ca}^{++}]$) by vesicles that have to undergo preceding, rate-limiting reactions before they can fuse under those conditions. This expectation forms the basis for a possible kinetic distinction between manipulations that affect late-acting molecules and those that are important during earlier steps. For instance, one would expect the $[\text{Ca}^{++}]$ during a stimulus to affect the time course of the exocytotic burst. Likewise, manipulations with the Ca^{++} sensor should alter the timing. Priming factors, on the other hand, would

primarily influence the sustained component and/or the amplitude of the exocytotic burst, that is, the number of release-competent vesicles immediately before the stimulus.

Demonstrating such expectations at synapses is not straightforward. Only few neuronal preparations allow one to control the Ca^{++} -stimulus sufficiently well in space and time for a quantitative analysis. In fact, examples of seemingly contradictory or complicating evidence are numerous, which led to suggestions about additional release constraints (10), facilitating mechanisms that modulate the release apparatus (11, 12), and adaptation/refractoriness (13). However, there are some neurosecretory systems where the expectations, sketched above, can be nicely verified, with just one complication: Detailed kinetic analysis of data from adrenal chromaffin cells revealed that the exocytotic burst, after flash-induced liberation of $[\text{Ca}^{++}]$ from “caged” Ca , is not homogeneous. Rather, one has to assume that there are two populations of vesicles that release at largely different rates. Heterogeneous release probability, however, is also a property of many types of synaptic vesicles.

The Flash Experiment

Rapid release of signaling compounds from caged precursors by ultraviolet illumination, has been used as a rapid stimulus in many fields of modern biology (14, 15), including neuroscience (16). When this approach was first applied to neurosecretory cells (pituitary cells) (17), the response pattern described above was observed. It was soon extended to adrenal chromaffin cells (18) and pancreatic beta cells (19). In most cases, the caged compound was infused into the cell interior in the whole-cell patch-clamp mode, and exocytosis was monitored by measuring membrane capacitance and/or by using carbon fiber amperometry (20). Such an experiment, performed on bovine adrenal chromaffin cells, is shown in Fig. 1B. Here, a flash of ultraviolet light rapidly elevated $[\text{Ca}^{++}]$ from a basal level of about 300 nM to about 20 μM . As a consequence, membrane capacitance increased rapidly by about 600 fF. This increase signals the release of about 240 vesicles, because each vesicle fusing with the plasma membrane enlarges its area, contributing about 2.5 fF (21). Subsequently, capacitance keeps increasing at a rate of about 50 fF/s, which corresponds to an ongoing rate of exocytosis of about 20 vesicles per second. In the light of the above discussion, these would be vesicles

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that undergo a priming reaction and release immediately (at high $[Ca^{++}]$) when they reach the release-competent state. We will present evidence below that this priming reaction is intimately linked to the initiation of SNARE complex formation.

Close inspection of traces like that of Fig. 1B shows that the exocytotic burst is, indeed, composed of two components. These are termed the “slowly releasable pool” (SRP) and the “rapidly releasable pool” (RRP); they can be fitted by two time constants. Many studies have shown that these time constants are strongly dependent on the concentration of Ca^{++} reached after the flash (17, 22, 23), confirming that the burst represents Ca^{++} -dependent triggering of release. Voets has shown that the amplitude of the burst is independent of post-flash $[Ca^{++}]$, as expected (23). However, it increases when the concentration of Ca^{++} preceding the flash is elevated, a finding that confirms “ Ca^{++} -dependent priming” of chromaffin granules, as observed in studies on permeabilized cells (24). This Ca^{++} -dependent priming occurs on the time scale of tens of seconds, and it can enlarge the size of the releasable pool by up to a factor of 5 (25). Likewise a reduction of preflash $[Ca^{++}]$ can reduce the size of the release-ready pool of vesicles within a few seconds (26). The distinction between Ca^{++} -dependent priming and Ca^{++} -dependent triggering requires a time resolution of at least a second. Because such resolution is very often not obtained in studies on permeabilized cell suspensions, many features of Ca^{++} dependence attributed in such studies to Ca^{++} triggering (27) may be indeed consequences of Ca^{++} -dependent priming.

Provided that the

exocytotic burst represents Ca^{++} action on (partially?) preassembled SNARE complexes, the sustained component should represent early stages of SNARE complex formation. Two components of the burst are observed (the SRP and RRP of Fig. 1C), which should indicate the presence of two forms of such complexes. These might represent two types of vesicles, different isoforms of SNARE proteins or different states of interaction with other proteins. However, additional points of evidence restrict such options: Both components involve the release of catecholamines (20, 25). Therefore, the idea that one component might represent large dense-core vesicle exocytosis, and the other one, small clear vesicles containing a fast neurotransmitter (28) can be excluded for adrenal chromaffin cells. Also, it has been demonstrat-

ed that the two forms of release competence interconvert on the time scale of a few seconds (29), which precludes major changes in their release machineries. For instance, it seems unlikely that different isoforms of SNAREs exchange for each other on a time scale of seconds. Rather, different degrees of tightness of the SNARE complexes, possibly stabilized to different extents by interacting partners (such as synaptotagmin or complexin), may cause the kinetic differences in the responses to rapid Ca^{++} elevation. In fact, complexin dissociates from the SNARE complex on exactly the same time scale as the observed transitions between RRP and SRP (30). This scenario depends on the assumption that the starting point for the Ca^{++} -dependent triggering reaction is one of the two states mentioned above, both of which

may be trans-SNARE complexes, zippered to different degrees. They may be dynamic structures—contrary to *in vitro* *cis* complexes—that can complete the zippering reaction only after membrane fusion, which allows the membrane anchors to align. This final rearrangement of lipids may need a Ca^{++} -dependent squeeze provided by synaptotagmin or other accessory proteins.

The Molecular Players

In order to shed some light on the molecular players involved in Ca^{++} -dependent exocytosis of large dense-core vesicles in chromaffin cells, a large number of experiments have been performed over the past few years. In these studies, a protein thought to be involved in the final steps preceding exocytosis has been either knocked out or overexpressed. In addition, mutant forms of these proteins have been overexpressed to gain insight into which region of the protein is involved in which step of Ca^{++} -depen-

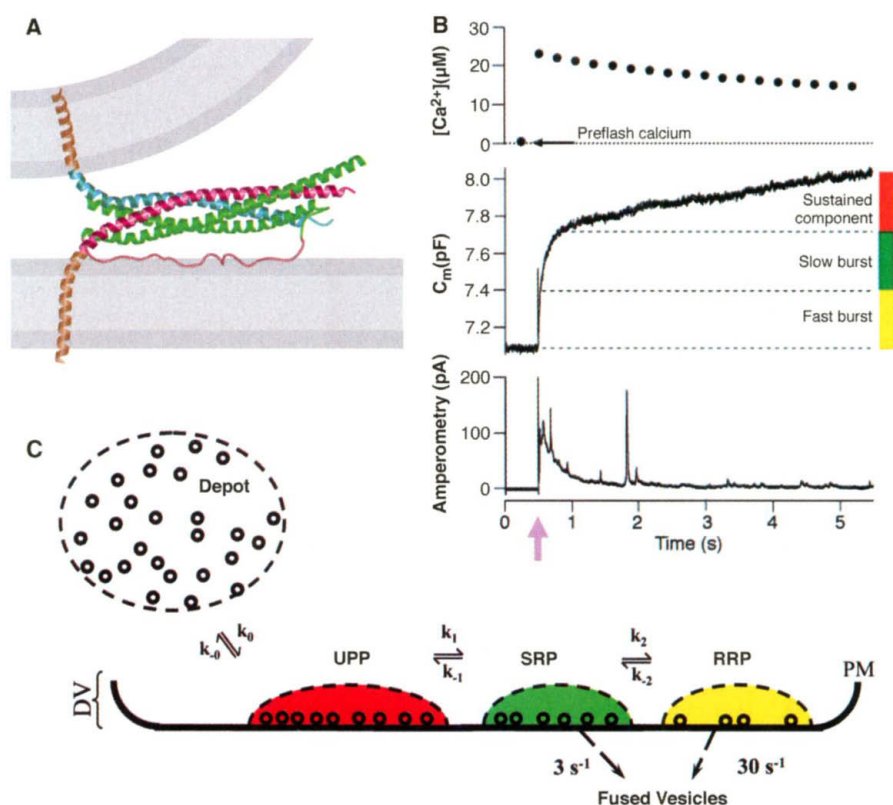


Fig. 1. Vesicle pools and the flash response. (A) A cartoon depicting a SNARE complex (37). (B) A typical response, observed by flash photolysis when a flash (see arrow) causes a step increase of cytosolic $[Ca^{++}]$ from a basal level of about 300 nM to 20 μ M (upper trace). The ensuing increase in membrane capacitance (middle trace) and the amperometric signal, as measured by a carbon fiber microelectrode (lower trace), are shown. Membrane capacitance increases in proportion to the number of chromaffin granules that fuse with the plasma membrane. The color code assigns parts of the trace to the fusion of vesicles from the RRP (yellow) or SRP (green) or to slow priming (with release immediately following) of previously unprimed vesicles (red). The carbon fiber signal (lower trace) measures oxidation of released catecholamines. Some scattered spikes represent the release of catecholamine packets from single granules, which happen to be released very close to the carbon fiber. Catecholamine released more remotely shows up as a slower, smoothed-out wave. (C) Vesicles are depicted in four different states. Vesicles of the depot pool (about 2000) enter the unprimed pool, UPP, when they dock at the membrane. A total of 850 morphologically docked vesicles (as observed in electron micrographs) are subdivided into the UPP (about 650 vesicles) and a primed pool, with the latter again subdivided into the slowly releasable pool, SRP, and rapidly releasable pool, RRP (each about 100 vesicles). Priming is defined functionally in the sense that all vesicles are termed “primed,” if they can release within about a second of elevation of $[Ca^{++}]$. Primed vesicles are believed to be in one of the two states when they have at least partially assembled SNARE complexes.

dent exocytosis. One major basis for the design of these experiments was the crystallization of the so-called core complex, which consists of the cytoplasmic regions of syntaxin, SNAP-25, and synaptobrevin (31). The crystal revealed a four-helix bundle with each of the four helices containing a leucine zipper-like layer of 15 hydrophobic residues facing each other (Fig. 1A). This structural feature most likely accounts for the unusual thermal stability and detergent resistance of the assembled SNARE complex. Surprisingly, the crystal structure also revealed the existence of an ionic layer ("zero layer") composed of three glutamine residues and one arginine embedded within the four-helix bundle. Overexpression of SNAP-25 Q174L in adrenal chromaffin cells led to a selective reduction in the sustained component in the flash experiment, whereas the kinetic rates of the exocytotic burst remained unaffected (32). The zero layer is thus important for the supply of release-competent vesicles which supports the above-mentioned hypothesis that the initiation of SNARE complex assembly is the molecular basis for the priming reaction. If this assumption holds true, the accessibility of the SNARE complex components might be one rate-limiting factor for the priming reaction. This might be particularly the case for syntaxin, which also forms a highly stable complex with Munc18. Crystallization of that complex revealed that syntaxin is held by Munc18 in a closed conformation (33). In this conformation, the NH₂-terminus of syntaxin is folded back onto the COOH-terminal helix, thereby preventing this helix from participating in formation of the core complex. Thus, syntaxin has to be opened up in order to make it available for SNARE complex formation, and any molecule that could perform such function would be by definition a priming factor. Recent evidence from knockout studies in *Caenorhabditis elegans*, *Drosophila*, and mice has assigned such a role to the protein Munc13 (34–40). When overexpressed in chromaffin cells, Munc13-1 leads to a threefold increase in secretion (41). This increase is due to a threefold larger size of both RRP and SRP and a threefold increase in the sustained component, nicely meeting the above-mentioned expectations for a priming factor. Therefore, Munc13-1, in terms of the vesicle pools of Fig. 1C, transfers vesicles from the UPP to the SRP, most likely by converting syntaxin to its open conformation (42). Support of this notion came from a recent study in *C. elegans* (43), where it was demonstrated that overexpression of syntaxin L166A/E167A, a mutant that does not bind Munc18 and thus has its COOH-terminus available for SNARE complex formation, could entirely rescue the severe unc13 phenotype.

From the data above, it seems evident that initiation of the SNARE complex is the molecular event underlying the priming process and that Munc13 acts as a priming factor by

opening syntaxin. However, several questions about the priming process still remain open. One concerns the fact that the known Munc13 isoforms are not expressed in all synapses. Thus, it seems likely that other priming factors that fulfill a similar function, must exist. One potential candidate is the so-called "calcium-dependent activator protein for secretion" [CAPS (44)]. Dialysis of an antibody against CAPS blocks, to a large degree, secretion in melanotrophs and chromaffin cells (45, 46), and knockout of *CAPS* in *Drosophila* leads to a 50% block of glutamatergic transmission and a threefold accumulation of synaptic vesicles at active zones (47). However, neither knockout nor overexpression of *CAPS* has any detectable effect on Ca⁺⁺-dependent exocytosis in chromaffin cells (48). Another open question concerns the Ca⁺⁺ dependence of the priming process. As mentioned above, the filling state of SRP and RRP, that is, the priming reaction, is strongly dependent on the preflash [Ca⁺⁺] (23). Although Munc13 has several, putatively Ca⁺⁺-binding C2 domains, it remains to be shown whether these sites actually bind calcium *in vivo* and consequently accelerate the priming rate. It seems equally plausible that the Ca⁺⁺ dependence of priming is mediated by other priming factors, e.g., protein kinase C (49).

According to the scenario outlined above, vesicles are in a release-competent state once the formation of the SNARE complex has been correctly initiated. What distinguishes vesicles residing in the SRP from those vesicles residing in the RRP? A first glimpse of knowledge arose from experiments with tetanus toxin and different botulinum neurotoxins. These endoproteases display a remarkable specificity as they cleave only members of the SNARE complex at a single site. Dialysis of botulinum neurotoxin E, D, or C1 and tetanus toxin into chromaffin cells led to a complete loss of secretion, further highlighting the importance of intact SNARE complexes for Ca⁺⁺-dependent exocytosis. Interestingly, dialysis of botulinum neurotoxin A resulted in a selective loss of the fast-burst component, that is, the RRP (20). Botulinum neurotoxin A cleaves SNAP-25 between amino acids 197 and 198, thereby cutting off the last nine COOH-terminal residues. In intact SNARE complexes, these amino acids form the most membrane-proximal part in one helix of the four-helix bundle. Thus, these data indicate that residues of the trans-SNARE complex, which are in close proximity to the membrane anchors, play an instrumental role in mediating fast, Ca⁺⁺-dependent exocytosis from the RRP. These findings were confirmed by overexpression of SNAP-25Δ9 in intact chromaffin cells (32, 50). In PC12 cells, the inhibition of secretion by botulinum neurotoxin A is paralleled by a

shift in the Ca⁺⁺-dependent binding of synaptotagmin to SNAP-25 toward higher Ca⁺⁺ concentrations (51). Synaptotagmin, with its two C2 domains, has widely been held as at least one Ca⁺⁺ sensor for exocytosis (52). In molecular terms, this finding might provide an explanation for the botulinum neurotoxin A data, if one assumes that cleavage of the COOH-terminal end of SNAP-25 weakens its interaction with the Ca⁺⁺ sensor of exocytosis. Further support for this view comes from a study of chromaffin cells derived from synaptotagmin-knockout mice. Similar to cells treated with botulinum neurotoxin A, secretion in these cells selectively lacks the fast-burst component mediated by vesicles fusing from the RRP, whereas the slow-burst component and the sustained component are hardly affected (53). Furthermore, the recent finding that neutralization of negative amino acids in the COOH-terminus of SNAP-25 interferes with the Ca⁺⁺-triggering step of exocytosis might well be explained by a reduced interaction of the SNARE complex with synaptotagmin (54). The picture emerging from these data seems to support the model originally proposed by Pelham and co-workers (3). Vesicle fusion from the RRP would require the zippering of the four-helix bundle of the trans-SNARE complex up to the membrane anchors. Synaptotagmin as the Ca⁺⁺ sensor would probably provide the final impetus to overcome the energy barrier of lipid rearrangement during the fusion process, either by interaction with the SNARE complex or by perturbing lipids in its immediate vicinity. Accessory proteins like complexin and snapin, which increase the binding affinity of synaptotagmin to the SNARE complex or stabilize the complex, would facilitate or enhance the transition from the SRP to the RRP (55, 56).

If the full zippering of the complex is required for fusion from the RRP, one should also be able to eliminate the fast-burst component by interfering with the zippering process. This has indeed been shown by using a monoclonal antibody against SNAP-25 (57). The epitope of this antibody consists of amino acids 20 to 40 of SNAP-25 (58), which are part of the NH₂-terminal helix contributing to the four-helix bundle of the SNARE complex. *In vitro*, this antibody prevents SNARE complex formation. Dialysis of the Fab fragment into chromaffin cells resulted in a largely reduced sustained component and in a selective loss of the fast-burst component. The reduced sustained component is the expected result of inhibition of SNARE complex formation, whereas the loss of the fast-burst component signals a destabilization of the RRP in its equilibrium with the SRP. These findings are in excellent agreement with the molecular picture on priming and interconversion between SRP and RRP

drawn above. One would conclude that the antibody binds both to monomeric SNAP25 and to partially assembled SNARE complexes of the SRP, thereby inhibiting the formation of new SNARE complexes and preventing the transition of partially assembled ones from SRP to RRP. However, the exact molecular composition of vesicles residing in the SRP remains unclear, as well as the nature of the Ca^{++} sensor that mediates fusion of vesicles from the SRP.

Vesicle Pools and Short-Term Synaptic Plasticity

The work discussed so far has brought up new features that have to be considered for a better understanding of Ca^{++} -triggered fusion. First, dense-core vesicles seem to exist in two different states with heterogeneous release probability (at a given $[\text{Ca}^{++}]$) and second, priming factors, such as Munc13, regulate the recruitment of new vesicles during sustained stimulation. The question then arises whether similar phenomena are seen in neurons and whether insights into the molecular mechanisms of dense-core granule release might help to understand neurotransmitter release and short-term synaptic plasticity. The answer to the first question is a clear "Yes." In fact, evidence for heterogeneous release probability at synapses exceeds that described above and derives from various types of experiments (59–62). Likewise, the role of Munc13 as priming factor has been shown in neuromuscular junctions of *C. elegans* (39) and *Drosophila* (37) and hippocampal neurons of mice (38). Furthermore, two isoforms of Munc13 produce two distinct forms of short-term plasticity, one (Munc13-1) characterized by short-term depression at moderate stimulation frequencies and another one (Munc13-2) by a Ca^{++} - and stimulation-dependent augmentation (40). Unfortunately, a direct kinetic separation of Ca^{++} -dependent triggering and priming reactions, as in the case of the flash experiment, is not possible in most nerve terminals. However, in hippocampal cells, vesicle pool sizes at any given moment can be determined as the cumulative release induced by a short sucrose application (63). This measurement allows the study of the recovery of vesicle pools after strong stimuli and estimation of the release probability by relating a given response to the pool size. In this way, vesicle recruitment or priming can be distinguished from Ca^{++} triggering.

In addition, caged Ca^{++} experiments can also be performed at a particularly large presynaptic terminal—the calyx of Held (64, 65). They reveal a relationship between release rates and $[\text{Ca}^{++}]$. They do allow one to obtain a relationship between release rates and $[\text{Ca}^{++}]$ that is steeper than the one for dense-core granules and reaches higher values at high $[\text{Ca}^{++}]$. Unfortunately, however, technical difficulties

so far preclude a clear answer to the question whether there are two well-defined kinetic components to the exocytotic burst, as there are in chromaffin cells. However, calyces can also be whole-cell voltage-clamped and, therefore, Ca^{++} currents of nearly the shape of a rectangular pulse can be applied (66, 67). Analyzing responses to such stimuli, it turns out that they also consist of two components. Under physiological conditions, the two components merge; however, by including a small amount of EGTA in the patch pipette, the components can be well separated (68). The two components differ in their characteristic time constant by a factor of about 10. After a strong stimulus, which depletes both components, the rapidly releasing one recovers slowly with a time constant of several seconds, whereas the slowly releasing one is halfway recovered after a pause as short as 50 ms. The steady-state release measured during a prolonged stimulus is compatible with this rapid recovery time constant (69). Thus, we are again confronted with a system in which at least two vesicle pools and two late steps in the release process can be distinguished. Do similar molecular mechanisms underlie the two cases? More specifically, are the two components of release—as postulated for the case of the chromaffin cell—a result of intrinsic differences in the release machinery or are they due to different efficiency in coupling between Ca influx and release? The latter might be the case, if rapidly releasing vesicles were better integrated into active zones and thereby more tightly coupled to Ca^{++} channels. The answer to this question bears on a number of interesting issues. Are the two components responsible for synchronous and asynchronous release, respectively? What is the reason for their very different speed of recovery? Why is the recovery of one component (the rapidly releasing and slowly recovering one) regulated by cAMP and Ca^{++} -calmodulin (68, 70) and the other one not? What are the consequences of differential regulation of the two pools for short-term depression and the behavior of neuronal networks?

If the underlying mechanisms were similar, one could learn a lot about synapses from the molecular and kinetic studies that can be performed in chromaffin cells. However, there are some indications that the two systems behave differently, and even the differences may be interesting: Overexpression of priming factors in chromaffin cells leads to large parallel changes in pool sizes. No such prominent changes have been observed in neurons (71); rather, manipulations that modulate pools and their dynamics either caused the specific increase of one pool (70) or else left the steady-state pool size unaltered (68). Like-

wise, the rapidly recovering pool of vesicles stayed constant in size during experiments in which the other one recovered slowly at the calyx of Held (68). In similar experiments performed on chromaffin cells one of the pools seemed to "consume" the other one while it recovered (29). These differences may be consequences of the fact that presynaptic terminals have well-defined active zones, in which the release machinery is linked to a large macromolecular complex (72), whereas in chromaffin cells the molecular context may be different, even if the basic release machinery is conserved. In any case, comparisons between the two systems combined with molecular manipulations and well-resolved kinetic measurements promises major advances in our understanding of synaptic mechanisms in the near future.

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VIEWPOINT

Neural and Immunological Synaptic Relations

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A synapse is a stable adhesive junction between two cells across which information is relayed by directed secretion. The nervous system and immune system utilize these specialized cell surface contacts to directly convey and transduce highly controlled secretory signals between their constituent cell populations. Each of these synaptic types is built around a microdomain structure comprising central active zones of exocytosis and endocytosis encircled by adhesion domains. Surface molecules that may be incorporated into and around the active zones contribute to modulation of the functional state of the synapse.

Although at present there is no direct connection between immunological specificity and specificity in the nervous system, some fruitful ideas may be generated by comparing the two biological systems. For example, in both systems there is specific recognition of a wide range of structures, and also storage of information acquired.

—G. Edelman, 1968. (1)

There is the notion that we should be able to make meaningful comparisons between the nervous system and the immune system. Both systems utilize specific molecular recognition events between discrete cells, cell:cell adhesion, positional stability, and directed secretion for communication to fulfill their respective functions. Both systems have evolved highly sophis-

ticated forms of information storage. A focal point for this comparison has become the concept of the synapse. The high degree of functional organization of synapses makes them ideal models for general understanding of cell-cell communication.

The concept of the synapse as a nexus of communication between neurons is now well over 100 years old. It is only recently, however, that the immunological counterpart has been identified. In the immune system, the synapse functions to provide specificity to the action of otherwise non-specific soluble agents through confinement to the synaptic cleft and to coordinate cell migration and antigen recognition during induction of the immune response. The comparison of these two synaptic junctions is useful in that they appear to share common features, but more importantly the two synapses have been approached from such different and complementary angles that some fruitful ideas should be generated by the comparison. We will argue that the immunological synapse is a valid concept by many criteria, but we must first get past some major differences.

Differences in Neural and Immune Architecture

A critical difference in the functional context of the neural and immunological synapses is in the basic “wiring” of the systems. The central nervous system (CNS) is to a great extent hard-wired and retains precise connectivity patterns throughout adult life, with neurons projecting long axonal processes that form synapses on complex dendritic trees of other neurons that may be quite distant from the cell nucleus. Whereas CNS synapses may be formed and pruned back in the adult, the long dendritic and axonal processes anchor the cell bodies and prevent cell migration. Thus, the CNS synapse is an “action at a distance” junction, in relation to the nucleus where transcription takes place. Therefore, most functions of CNS synapses may be considered “postnuclear” in that they are carried out without a requirement for immediate transcriptional regulation, although synaptic stimulation can lead to transcriptional changes in the long term. CNS synapses can alter their efficacy by processes such as receptor clustering by scaffold proteins (2). Synapse-forming neurons are terminally differentiated.

In contrast, the immune system operates through rapidly migrating T cells and their partners, the dendritic cells (DCs), that congregate in tissues like lymph nodes. This is essential to the operation of the immune system, because each T cell expresses a different antigen specificity and the point at which an antigen will enter the body to become associated with a DC is not predictable. So it is essential that T cells and DCs congregate and make many random contacts to possibly find

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