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Function of Rab3 GDP–GTP Exchange

Minireview

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A felicitous convergence of independent lines of research, biochemistry in mammals and genetics in C. elegans, has given us unexpected insight into the biology of rab3 (Iwasaki et al., 1997 [this issue of *Neuron*]; Wada et al., 1997). Rab3 is a member of a family of more than 30 low molecular weight GTP-binding proteins. It constitutes an abundant synaptic vesicle protein that regulates neurotransmitter release (reviewed by Südhof, 1995). The new results describe a protein that catalyzes GDP–GTP exchange specifically on rab3 and is essential for localizing rab3 to synaptic vesicles. These findings provide the first biological evidence for the targeted recruitment of rab3 to synaptic vesicles as a major mechanism in presynaptic function, and constitute the first demonstration for any rab protein that the reassociation of rab proteins with their target organelles is functionally essential.

The Rab3 Cycle in the Nerve Terminal

The presynaptic nerve terminal is filled with synaptic vesicles containing neurotransmitters (Figure 1, step A). Synaptic vesicles move to the active zone of the plasma membrane (B) where they firmly dock (C). After docking, vesicles undergo membrane fusion (exocytosis; D). Membrane fusion is triggered in a nerve terminal by Ca^{2+} influx during membrane depolarization by an action potential. Afterward, the empty vesicles are endocytosed (E) via clathrin-coated vesicles (F). Empty vesicles recycle (G) to be refilled with neurotransmitters and restart the pathway (A). Thus, synaptic vesicles cycle in the nerve terminal with a rate that is regulated by Ca^{2+} influx during action potentials.

In mammals, synaptic vesicles contain two very similar rab3 isoforms, rab3A and rab3C, which are differentially distributed. By contrast, there is evidence for only a single rab3 in C. elegans. Rab3 is present on all free and docked synaptic vesicles in resting nerve terminals but absent from the plasma membrane (Matteoli et al., 1991). Rab3, like other rab proteins, does not contain a transmembrane region but is attached to phospholipid bilayers by a C-terminal hydrophobic modification composed of two geranylgeranyl residues (Farnsworth et al., 1991; Johnston et al., 1991). On the vesicles, rab3 is present in the GTP-bound state and complexed with a putative effector protein called rabphilin-3 (Shirataki et al., 1993; Li et al., 1994). Rabphilin binds to GTP-rab3 but not to GDP-rab3. In isolated nerve terminals, strong stimulation of exocytosis leads to hydrolysis of GTP-rab3 to GDP-rab3 and loss of rabphilin and rab3 from synaptic vesicles (Fischer von Mollard et al., 1994; Stahl et al., 1994, 1995). This suggests that Ca^{2+}-triggered membrane fusion promotes the hydrolysis of GTP-rab3 to GDP-rab3, resulting in the release of rabphilin from vesicular rab3 and the subsequent dissociation of GDP-rab3 from synaptic vesicles (Figure 1). To complete the cycle, GDP-rab3 must then reassociate with free synaptic vesicles under GDP–GTP exchange.

Thus, in a nerve terminal, the rabphilin cycle is nested in the rab3 cycle, and the rab3 cycle in the synaptic vesicle cycle. Although these cycles as such are well established, many questions regarding the timing and mechanisms of individual steps remain unanswered. Three lines of evidence suggest that rabphilin and rab3 dissociation occur in conjunction with Ca^{2+}-triggered synaptic vesicle fusion, and that they occur after docking but before endocytosis of vesicles: 1. Tetanus and botulinum toxins inhibit neurotransmitter release by interfering with Ca^{2+}-triggered fusion (D in Figure 1) without affecting synaptic vesicle docking or Ca^{2+} influx. These toxins also prevent rab3 and rabphilin dissociation after membrane depolarization and Ca^{2+} influx, suggesting that docking and Ca^{2+} are not sufficient for dissociation (Stahl et al., 1995). 2. α-latrotoxin causes vesicle fusion in the absence of Ca^{2+} by a nonphysiological mechanism. However, vesicle fusion induced by α-latrotoxin in Ca^{2+}-free medium does not cause rabphilin and rab3 dissociation, suggesting that a normal Ca^{2+}-triggered fusion reaction is required for dissociation (Stahl et al., 1995). 3. Clathrin-coated vesicles from brain lack rab3, indicating that rab3 dissociates before endocytosis (Maycox et al., 1992). Since coated vesicles lack rab3 but most free vesicles contain rab3, it seems likely that rab3 reassociates with synaptic vesicles soon after decoating, but the exact point of reassociation is unclear.

A possible function of the rab3 cycle may be to attach rab3 reversibly to a synaptic vesicle only on the exocytic limb of the synaptic vesicle pathway. This would allow the GTP-dependent recruitment of effector proteins, such as rabphilin, to synaptic vesicles only during exocytosis. Support for this hypothesis was derived from studies with mice that lack rab3A, the major rab3 isoform, but still contain rab3C (Geppert et al., 1994). In the rab3A mutant mice, rabphilin is decreased by $>$70% only in neurons that do not contain rab3C. Rab3C is probably functionally redundant with rab3A but only expressed in subsets of neurons. In the absence of rab3, rabphilin is not transported to synapses but retained in the cell bodies where it is degraded (Li et al., 1994). Thus, at least in mice, rab3 is essential for proper positioning of rabphilin to the exocytic sites of the synapse.

Proteins Mediating the Rab3 Cycle

At least three types of protein activities are required for the rab3 cycle: a GTPase activating protein complex that triggers GTP-hydrolysis in conjunction with exocytosis, a protein that removes GDP-rab3 from vesicles after exocytosis, and an activity that reassociates GDP-rab3 with free synaptic vesicles under GDP–GTP exchange. Of these three activities, the reassociation of rab3 with synaptic vesicles is arguably the most interesting activity because this step involves the conversion of a soluble GDP-rab3 complex to a membrane-bound GTP-rab3 form and determines the specificity of the localization of rab3. Components of all of these activities were purified in recent years in Yoshimi Takai's laboratory in pioneering work, establishing a framework of activities to guide the rab3 cycle.

A GTPase-activating protein for rab3 that is composed of at least two subunits was recently described

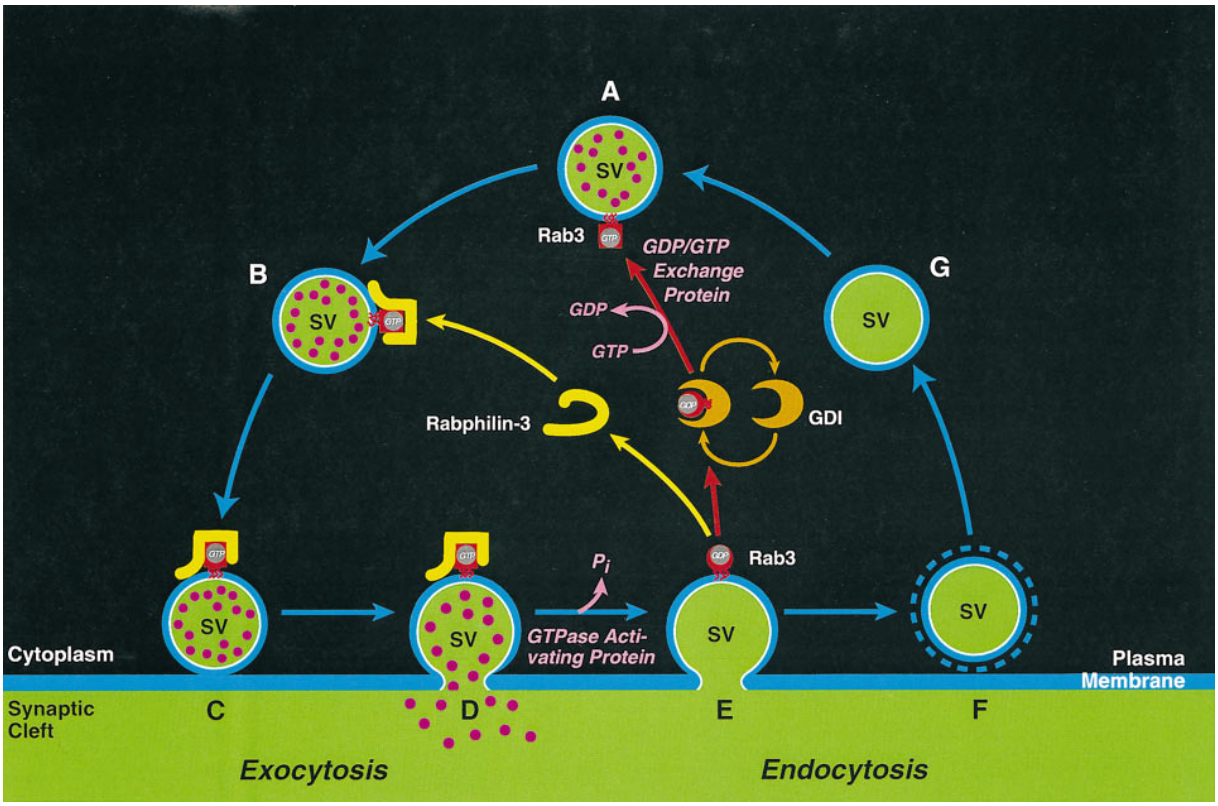

Figure 1. The Synaptic Vesicle (SV), Rab3, and Rabphilin Cycles

Synaptic vesicles containing rab3 in the GTP-bound form (A) bind rabphilin-3 (B) and move to the active zone of the synapse where they firmly dock (C). Docked vesicles undergo Ca^{2+}-triggered fusion (D) resulting in exocytosis, during or after which a GTPase activating protein triggers the hydrolysis of rab3-bound GTP to GDP, leading to rabphilin dissociation. GDI then acts on GDP-bound rab3 on the empty vesicles (E), after which the vesicles are endocytosed via clathrin-coated pits (F) and recycled (G). GDP-rab3 becomes a substrate for a set of proteins that include a GDP–GTP exchange protein, which catalyzes the reloading of rab3 with GTP and allows its reassociation with synaptic vesicles in A.

(Fukui et al., 1997). The biological role and specificity of this protein remains to be tested. The tissue distribution of one of the subunits does not match that of rab3, and it exhibits no homology to known rab GTPase-activating proteins from yeast. Nevertheless, it seems likely that it will participate in triggering GTP hydrolysis in the rab3 cycle (steps D–E in the vesicle cycle in Figure 1). After GDP-rab3 has been generated on the vesicles, it is probably removed from the vesicles by a protein called GDI (GDP Dissociation Inhibitor). GDI was initially identified by the Takai laboratory based on its ability to inhibit GDP-dissociation from rab3 but was then found to complex with all GDP-bound rab proteins tested, and to remove them from membranes by forming a soluble complex with them (Araki et al., 1990). GDI is a fascinating example of the functional economy of cells. It constitutes an evolutionarily conserved component of trafficking pathways that can act on innumerable rab proteins but nevertheless only reacts with GDP-bound rabs (Garrett et al., 1993). Although rabs are modified by bulky geranylgeranyl goups of great hydrophobicity, GDI can mask these hydrophobic groups completely to make the proteins soluble.

After GDP-rab3 has been solubilized from exocytosed synaptic vesicles by GDI (E in Figure 1), it has to reassociate with free vesicles in the backfield of the synapse in step A (Figure 1). The mechanisms involved in this step are unclear but have to include GDP–GTP exchange. Major progress in understanding this step was made recently with the discovery of a rab3-specific GDP–GTP exchange protein (Wada et al., 1997). Initial data bank searches with the sequence of the GDP–GTP exchange protein revealed no interesting homologies except for its identity with a random cDNA called DENN. The recombinant protein has a high GDP–GTP exchange activity in vitro, suggesting that it may function in the reassociation of rab3 to synaptic vesicles. Support for this hypothesis is unexpectedly derived from the experiments on *aex-3* reported in this issue of *Neuron*.

Mislocalization of Rab3 in* aex-3 *Mutants

aex-3 is a mutation originally isolated in C. elegans in a screen for altered defecation behavior (Thomas, 1990). Alleles for the same mutation were independently isolated in other screens directed toward nervous system functions, suggesting that the mutant gene encodes a protein involved in multiple neuronal functions. A careful

study of *aex-3* mutants revealed that they resist aldicarb, a cholinesterase inhibitor, and that the mutation genetically interacts with mutations in two presynaptically acting genes, syntaxin and CAPS (Iwasaki et al., 1997). Together, these results indicated a presynaptic defect. The most interesting result with the *aex-3* mutants, however, was the discovery that rab3 is not transported to synapses in these mutants but accumulates in cell bodies. This defect seems to be selective to rab3, since synaptotagmin, another synaptic marker, is transported to synapses. With *aex-3*, the first gene was discovered that is essential for the proper localization of a rab protein to its target organelle.

aex-3 *Probably Encodes a Rab3 GDP–GTP Exchange Protein*

Comparison of the *aex-3* sequence with that for the just-reported sequence of the rab3 GDP–GTP exchange protein (DENN; Wada et al., 1997) yields a surprise: the two proteins are highly homologous, suggesting that *aex-3* encodes a rab3 GDP–GTP exchange protein in C. elegans. This provides an immediate explanation for the phenotype. If no GDP–GTP exchange happens on rab3, the protein is presumably constitutively bound to GDI in a soluble complex and cannot be attached to synaptic vesicles. Thus, these data provide the first evidence that GDP–GTP exchange is essential for rab3 localization to synaptic vesicles. They also suggest that rab3 by itself is not synaptic but becomes synaptic only by virtue of its association with synaptic vesicles. These findings are not only important in highlighting the central role for the rab3 cycle in rab3 function but provide the first approach to study the specificity of the association of rab proteins with a target organelle.

Caveats

Although the high degree of sequence homology between aex-3 and the mammalian rab3 GDP–GTP exchange protein suggest a similarity in function, this hypothesis must be tested biochemically. In addition, it is not certain yet that aex-3 and the rab3 GDP–GTP exchange protein DENN are exact homologs because the mammalian EST data banks contain other sequences that are at least partially more homologous to aex-3 than to the exchange protein DENN. It is possible that multiple, evolutionarily related components are involved in the reassociation of rab3 with synaptic vesicles and that aex-3 corresponds to one of the components of the system. Another important unexplained observation is the finding, described in the paper by Iwasaki et al., that the *aex-3* mutants exhibit a more severe phenotype than *rab3* mutants in C. elegans. Among the conceivable explanations for this finding is that aex-3 is not specific for rab3 but also acts on other rab proteins or that C. elegans, like vertebrates, has additional isoforms of rab3. The most likely explanation, however, is that GDP-rab3 accumulates in the *aex-3* mutants and sequesters essential binding proteins, possibly GDI or other unidentified components of the molecular machinery that target rab proteins to their resident organelles.

Implications for Other Rab Proteins

More than 30 rab proteins are known in mammals, providing a rich hunting ground for cell biologists. Do these rab proteins undergo a cycle of dissociation and association as a function of membrane traffic similar to rab3? Are similar mechanisms involved? These questions are difficult to address. Unlike synaptic vesicles whose traffic can be uniquely activated by Ca^{2+}, it is difficult to determine whether the other rabs dissociate from their resident membranes as a function of fusion. However, several lines of evidence suggest that the findings for rab3 may be generally applicable: 1. GDI universally binds to all GDP-complexed rab proteins and is an essential component for yeast and Golgi trafficking reactions (Garrett et al., 1993; Elazar et al., 1994). 2. Studies of GDI-complexed rab5 and rab9 in vitro demonstrate that they can be rebound to their resident organelles under GDP–GTP exchange (corresponding to Figure 1, step A for rab3), indicating a similar reassociation with target organelles (Soldati et al., 1994; Ullrich et al., 1994). 3. Data bank analyses with the aex-3 and rab3 GDP–GTP exchange protein sequences suggest that additional proteins with similar domains may be present (e.g., the human ST5 gene; Lichy et al., 1996). This indicates that GDP–GTP nucleotide exchangers may exist for other rab proteins.

Thus, it seems likely that similar to rab3, all rab proteins will have cycles of association and dissociation that are catalyzed by related proteins (GTPase activating proteins and GDP–GTP exchangers) or the same proteins (GDI). It is less clear, however, whether the functions of rab proteins in docking and fusion and the nature of their interactions with putative effectors are also comparable. Nevertheless, with the description of *aex-3*, the first mutation in a specific rab cycle protein was analyzed, providing a significant step forward.

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