MEMBRANE TRAFFICKING

Throttles and Dampers: Controlling the Engine of Membrane Fusion

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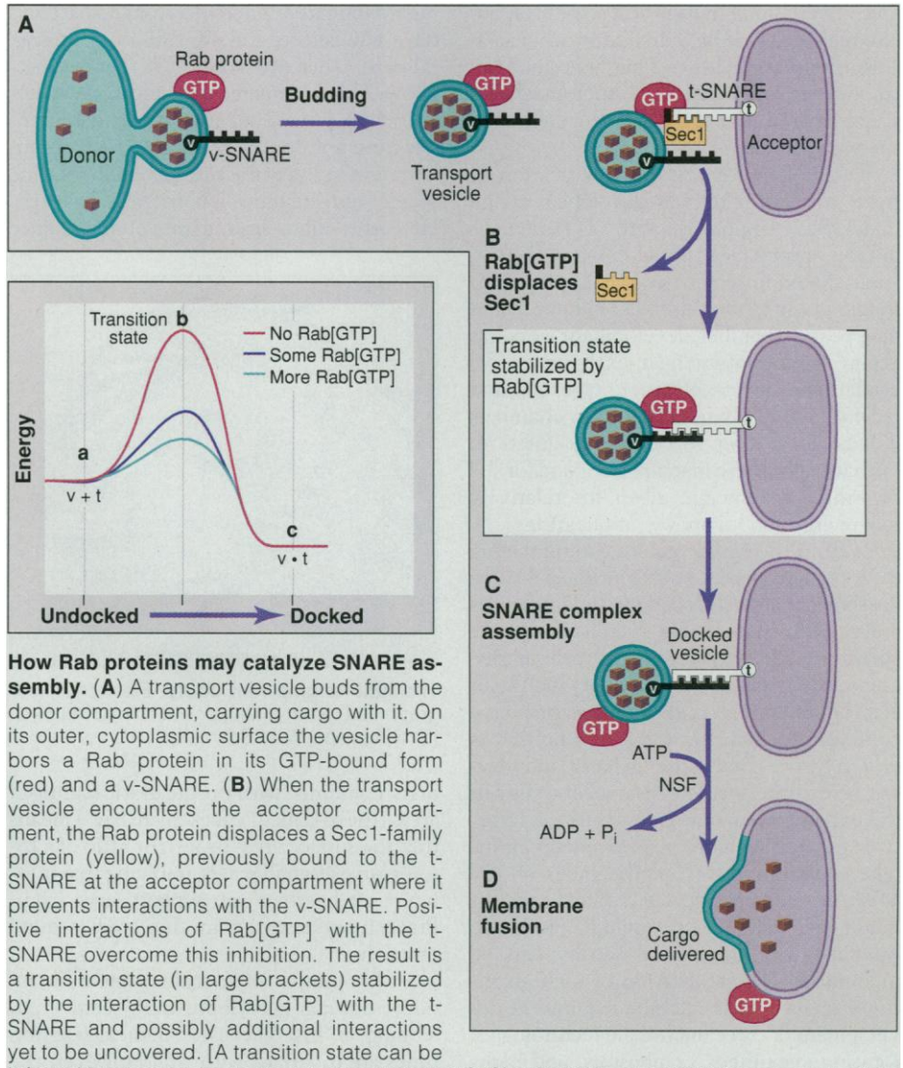
Eukaryotic cells use vesicle shuttles to maintain and propagate their compartmental organization. Experiments reported by Lupashin and Waters on page 1255 of this issue (*1*) illustrate how the core transport machinery that directs vesicles in space is controlled in a fourth dimension—time—by a family of guanosine triphosphatases (GTPases) called Rab proteins. In the process, they clarify the decade-old mystery of precisely what these ubiquitous proteins do.

As in containerized shipping, cells load membrane-enclosed transport vesicles with diverse protein cargo, all having a common destination. Similar vesicles store packets of enzymes or intercellular signaling molecules (like hormones and neurotransmitters) for rapid release from cells as physiology demands. A transport vesicle departs from one compartment loaded with cargo and moves through the cytoplasm until it reaches its destination. This occurs when the vesicle's v-SNAREs zip up with their cognate t-SNAREs located at the intended destination (*2*). Now docked at the correct location, the vesicle delivers its cargo by membrane fusion, a process akin to the coalescence of soap bubbles, in which the membrane of the vesicle becomes incorporated into the membrane of the target compartment.

The connection of GTPases to docking and fusion came when Novick and co-workers (*3*) discovered that the yeast *SEC4* gene is a distant relative of the mammalian proto-oncogene *ras*. *SEC4* mutants accumulate transport vesicles that bud from the Golgi but fail to dock with the outer cell membrane. Subsequently, another gene encoding a different Ras-related GTPase (Ypt1p) was found to be required for docking of endoplasmic reticulum (ER)–derived vesicles at the Golgi (*4*). Soon, many more Rab proteins were found, each required at just one transport step.

Because the Rab proteins travel with transport vesicles (*3*, *5*), it was natural to hypothesize that these GTPases are the core machinery that instructs vesicles where to dock. But this once-attractive idea has become increasingly untenable. First, whereas Rab proteins are essential for life in yeast, deletions in a Rab gene can nonetheless be bypassed in any of a number of ways, including simply increasing the cellular concentration of v-SNAREs required at the same transport step (*6*). This implies that Rab proteins are regulatory rather than core transport machinery. Furthermore, a single hybrid Rab protein containing elements of both the



How Rab proteins may catalyze SNARE assembly. (**A**) A transport vesicle buds from the donor compartment, carrying cargo with it. On its outer, cytoplasmic surface the vesicle harbors a Rab protein in its GTP-bound form (red) and a v-SNARE. (**B**) When the transport vesicle encounters the acceptor compartment, the Rab protein displaces a Sec1-family protein (yellow), previously bound to the t-SNARE at the acceptor compartment where it prevents interactions with the v-SNARE. Positive interactions of Rab[GTP] with the t-SNARE overcome this inhibition. The result is a transition state (in large brackets) stabilized by the interaction of Rab[GTP] with the t-SNARE and possibly additional interactions yet to be uncovered. [A transition state can be inferred but has not been directly demonstrated.] (**C**) v-SNAREs and t-SNAREs assemble from this transition state to form SNARE complexes that stably dock the vesicle to the target membrane. (**D**) Membrane fusion is initiated when NSF hydrolyzes ATP (with the help of SNAP proteins), disrupting the SNARE complex and initiating the fusion of the lipid bilayer of the vesicle with the lipid bilayer of the acceptor compartment. The cargo is thereby delivered. (**Inset**) The Rab protein acts as a catalyst, accelerating an otherwise spontaneous but slow reaction (assembly of v-SNARE with t-SNARE). When vesicles have budded but not yet docked with the target membrane, v- and t-SNAREs are separate (**a**). When the vesicle is firmly docked by an assembled SNARE complex (**c**), the system is at a lower energy level, reflecting the spontaneous nature of the assembly of the SNARE complex. The transition state (**b**) is of higher energy. As the amount of Rab[GTP] on the transport vesicle increases, the concentration of the transition state will increase correspondingly. Thus, Rab[GTP] will lower the activation energy for docking, speeding this process. The concentration of vesicles and target membranes in the transition state will also be increased by a rise in the concentration of v-SNAREs or other reactants, accounting for the multiple ways to bypass deletion of Rab (discussed in the text).

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ER-Golgi Rab protein (Ypt1p) and the Golgi–cell surface Rab protein (Sec4p) can fulfill Rab function at several steps of secretory pathway in the absence of both normal Rab proteins (*7*). This provides compelling evidence that Rab proteins do not accomplish vesicle targeting. Other insights into Rab proteins have also emerged, including the identification of critical proteins that can modulate GTP binding or hydrolysis by Rab proteins or that control attachment of Rab proteins to membranes (*8*).

Meanwhile, the SNARE protein family was discovered, and its central role in compartment-specific docking was established (*2*). With this framework in place, evidence has gradually accumulated during the last few years for a different view of Rab proteins, in which they somehow control the speed (but not the intrinsic specificity) of membrane fusion by regulating the rate of assembly of SNARE complexes. Complexes of v- and t-SNAREs are inherently stable and can assemble spontaneously in vitro in the absence of Rab proteins. Yet, Rab proteins act as upstream regulators of SNARE complex assembly in living cells because mutations in them prevent assembly of SNARE complexes (*9*, *10*). And recently, in an important advance, Zerial and colleagues (*11*) have shown directly that the level of Rab[GTP] determines the overall rate of membrane fusion. GTP hydrolysis is not only independent of fusion but actually is irrelevant to fusion per se, which occurs perfectly well with mutant Rab proteins that cannot hydrolyze GTP, also ruling out various kinetic proofreading models for Rab action (*12*). What is relevant to the rate of fusion is the absolute level of GTP-bound Rab, which is set by a balance that is modulated by various protein regulators and probably many unknown factors. Rab[GTP], produced from Rab[GDP], is the throttle that sets the pace of membrane fusion, nicely in keeping with the general role of GTP-binding proteins (G proteins) in biology, which turn on signaling pathways as they are switched into their GTP-bound states.

The engine of membrane fusion also has dampers in the form of a family of proteins (*13*) related to the yeast *SEC1* gene whose members bind to one or another t-SNARE (*9*, *14*). These proteins are required for vesicular transport but act as negative regulators of SNARE complex assembly, as first shown by Scheller and colleagues (*15*), who found that a Sec1-family protein prevents its t-SNARE from binding a v-SNARE. The physiological importance of damping fusion by this mechanism is clear. Certain mutations in a Sec1-family member (Sly1p) allow cells to live happily without the local Rab protein, Ypt1p (*6*). Rab proteins and Sec1-family proteins are therefore pitted against each other in a tug-of-war, the balance of which must somehow control the rate of fusion at the level of SNAREs.

Lupashin and Waters (*1*) now provide a molecular mechanism that explains how this works. Three new important observations establish the first concrete link between the Rab, Sec1p, and SNARE families of proteins. First, they report that the ER-Golgi Rab protein Ypt1p, normally present on transport vesicles budding from the ER, interacts with the free Golgi t-SNARE (Sed5p), but does not do so when this same t-SNARE is complexed with cognate v-SNAREs. Second, the local Sec1-family member (Sly1p) is bound to the same t-SNARE before, but not after, assembly with cognate v-SNAREs. Third, the amount of Sec1-family member bound to its t-SNARE systematically decreases as the level of the local Rab protein is increased in living cells.

Together, these new findings imply that Rab and Sec1-family proteins are, respectively, throttles and dampers of membrane fusion, directly opposing each other by allowing or preventing v-SNAREs access to t-SNAREs. The fusion engine, burning ATP as SNAREs are activated for fusion by the ATPase NSF, will be throttled up when the level of Rab[GTP] on transport vesicles is increased, displacing the Sec1-family dampers from t-SNAREs. Formally, Rab[GTP] acts as a catalyst of SNARE complex assembly (*9*), because it is neither generated nor consumed as it accelerates the docking reaction (see figure). In addition, Sec1-family proteins may improve the reactivity of cognate t-SNAREs toward Rab[GTP] (*1*).

We are probably only beginning to perceive a sophisticated regulatory and signal-transducing system that controls the speed of what have long been regarded as constitutive transport pathways. Because each transport segment has a distinct set of Rab proteins, any one segment can, in principle, be throttled up or down in speed independently of the others, allowing local flow patterns to be fine-tuned to momentary physiological needs.

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PLANETARY ATMOSPHERES

Warming Early Earth and Mars

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Sagan and Chyba (*1*), in their article on page 1217 of this issue, have revived an old debate about how liquid water was maintained on early Earth and Mars despite a solar luminosity 25 to 30% lower than that at present. A theory that has been popular for some time (*2*) is that greatly elevated concentrations of atmospheric CO_2, produced by the action of the carbonate-silicate cycle, provided enough of a greenhouse effect to warm early Earth. However, Rye *et al.* (*3*) have placed geochemical constraints on early atmospheric CO_2 abundances that fall well below the levels needed to warm the surface. These constraints are based on the absence of siderite ($FeCO_3$) in ancient soil profiles—a negative and, hence, rather weak form of evidence—and apply to the time period 2.2 to 2.8 billion years ago, when Earth was already middle aged. Nonetheless, the soil data provide some indication that atmospheric CO_2 levels may have been lower than previously thought. An even more serious problem arises if one tries to keep early Mars warm with CO_2. Model calculations predict that CO_2 clouds would form on Mars in the upper troposphere, reducing the lapse rate and severely limiting the amount of surface warming (*4*). A suggestion that CO_2 clouds may have warmed

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