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Molecular machinery mediating vesicle budding, docking and fusion

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Abstract. A general machinery buds and fuses transport vesicles which connect intracellular compartments with each other and allow communication with the extracellular environment. Cytoplasmic coat proteins deform membranes to bud vesicles and interact directly or indirectly with cargo molecules. Compartment-specific SNAREs (SNAP receptors) on vesicles and target membranes dock vesicles and provide a scaffolding for the general fusion machinery to initiate lipid bilayer fusion.

Key words. Vesicle budding and fusion; ARF; coat proteins; cargo receptor; NSF; SNAP; SNARE.

Abbreviations. NSF = *N*-ethylmaleimide-sensitive fusion protein; SNAP = soluble NSF attachment protein, SNARE = SNAP receptor; ER = endoplasmic reticulum; ARF = ADP-ribosylation factor, NEM = *N*-ethylmaleimide, GAP = GTPase activating protein.

Introduction

Each compartment in eukaryotic cells is characterized by a set of specific proteins and a distinct lipid composition, which together define its unique functional properties. The compartments along the major transport routes termed secretion, endocytosis and transcytosis exchange components with others by means of transport vesicles [1]. Each route is composed of a series of sequential vesicular transport steps. At each step transport vesicles form at a donor compartment and fuse with a specific acceptor compartment. To maintain the identity of the individual organelles, cargo has to be selected during vesicle budding and has to be specifically delivered to its correct destination. Recent data have provided insight into the underlying mechanisms of these processes, thereby expanding our understanding of how cells maintain and control their temporal and spatial organization and how they communicate with their environment. The core machinery mediating these processes will be outlined in the following sections.

Vesicle budding

Shuttling of cargo between organelles requires its packaging into distinct transport containers defined by their distinct size and, to a certain degree, specialized protein and lipid composition. In every instance, the generation of defined transport units is a prerequisite to control and balance import in and export from a distinct organelle. To ensure the generation of such transport units, coat proteins derived from the cytoplasm assemble at budding sites on the donor membrane. This process is triggered by nucleotide exchange on a class of small soluble GTP (guanosine 5′-triphosphate)-binding proteins, their membrane recruitment and the subsequent binding of coat proteins. The assembly of coat proteins deforms the membrane, resulting in the generation of coated buds which finally pinch off to release coated vesicles. Following their uncoating, the vesicles dock to and fuse with the target membrane (Figure 1A).

In case of the Golgi, the vesicular protein coat is termed COPI (*co*at *p*rotein) and assembles from the cytoplasmic coatomer complexes (*coat* prot*omer*) consisting of seven subunits [2, 3]. The binding of coatomer to the donor membrane and the subsequent assembly process occur after *A*DP (adenosine 5′-diphosphate)-*r*ibosylation *f*actor (ARF) in its GTP-bound form interacts with the membrane [4]. A GDP/GTP exchange factor and an ARF receptor mediating these processes have been postulated, but still await purification. Recent data also suggest the involvement of polyphosphoinositides and phosphatidic acid in the budding step [5–7]. These lipids seem to facilitate nucleotide exchange on ARF and might enhance the interaction of coatomer with the membrane. The vesicle fission step has been shown to require an additional lipophilic component, fatty acyl-coenzyme A [8]. Neither the target nor the exact function of the fatty acyl requirement are known. After the vesicle has pinched off, ARF hydrolyses its bound GTP [9], the coat disassembles and dissociates from the vesicle membrane, and an uncoated vesicle is generated which goes on to dock and fuse with the target membrane. GTP hydrolysis seems to be regulated by ARF-GAP (*G*TPase *a*ctivating *p*rotein) a cytosolic protein which is recruited to Golgi membranes and activated by a still unknown mechanism [10]. Again, polyphosphoinositides have been implicated to function at this step [11].

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Vesicle formation may require proper cargo selection, or in some cases cargo could be transported by default at its bulk concentration in a process termed bulk flow [12]. Cargo selection is accomplished by a protein's (or lipid's) enrichment in or exclusion from transport vesicles, which requires that cargo molecules carry transport signals or that resident components contain retention signals [13]. For example, the amino acid motifs KKXX and XXRR at the carboxy-and amino-termini, respectively, of integral membrane proteins act as transport signals for retrieval of resident proteins which have escaped from the endoplasmic reticulum (ER) [14–16]. KKXX or related peptides have been shown to interact with COPI coats, illustrating how a signal contained in a cargo molecule can be coupled to the transport machinery and thus ensure cargo packaging [17, 18]. However, since COPI-coated vesicles also carry cargo in the anterograde direction, coatomer should not interact with the KKXX motif at the ER membrane. Possible mechanisms could be that COPI coats exist in different conformational states, binding cargo either for anterograde or retrograde transport, or that post-translational modifications or interactions with additional components alter the binding properties of coatomer for KKXX [19].

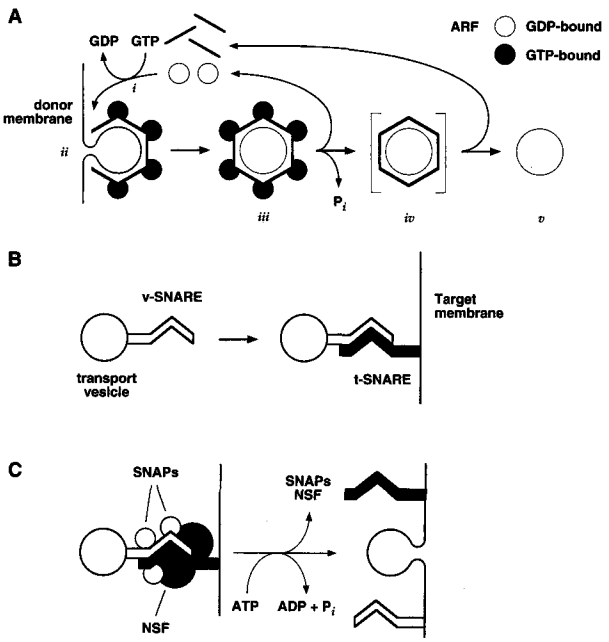

Figure 1. The molecular machinery mediating vesicle budding and fusion (A) Transport vesicle formation. (*i*) Vesicle budding is initiated when GDP on ARF is exchanged against GTP. The resulting ARF [GTP] binds to the membrane and recruits coat proteins. The assembly of coat proteins on the membrane incrementally deforms the corresponding area of the lipid bilayer, resulting in a bud. (*ii*) The bud pinches off in a reaction requiring fatty acyl coenzyme A. (*iii*) GTP hydrolysis results in dissociating of ARF yielding presumably a still-coated vesicle (*iv*), whose coat then disassembles and dissociates from the membrane (*v*). (B) Targeting of vesicles. Uncoated vesicles interact via their address signal, termed v-SNARE, with their cognate receptor on the target membrane, termed t-SNARE. (C) Initiation of vesicle fusion. SNAPs bind to assembled SNARE complexes at the attachment site of the vesicles to the target membrane, thereby allowing the subsequent binding of NSF. ATP hydrolysis by NSF disrupts the SNARE complex and initiates membrane fusion. It is not known whether additional components are required for the lipid bilayer fusion. (Reprinted with permission from Science 272: 227–234 (1996). Copyright 1996 American Association for the Advancement of Science.)

Lumenal proteins, which cannot possibly interact with cytosolic coat proteins in a direct way, likely contain other types of transport signals, which might be recognized by cargo receptors, integral membrane proteins which function as adapters to mediate the interaction with the vesicle coats. A family of integral membrane proteins, major constituents of both COPI- and COPII-coated vesicles, has been recently identified, whose members fulfill the criteria for such cargo receptors. P24 proteins have a variable amino-terminal domain, which might interact with cargo, and a conserved carboxy-terminal domain, binding coat proteins [20] (unpublished observation). Deletion of individual family members does not affect cell viability but does alter the transport efficiency of some but not all cargo molecules [21].

Vesicle docking and fusion

Transport from the donor compartment to the acceptor compartment is in some instances facilitated by vesicle-associated motors interacting with the cytoskeleton [22, 23], but this is not a prerequisite for transport and hence cannot be critical for targeting transport vesicles selectively to their destination. Thus, signals on the vesicles must exist that are recognized by a receptor on the intended target membrane. According to the SNARE hypothesis, this process is mediated – at least in part – by the unique pairing of *SNAP receptors* localized on vesicles (v-SNAREs) with their cognate t-SNAREs on target membranes [24] (Figure 1B). Most SNAREs are type II membrane proteins, with short lumenal or extracellular regions and comparably large cytosolic domains. The cytoplasmic domains are predicted to form coiled coils and to mediate the unique interactions between the distinct members of the v/t-SNARE families [69]. The system is best characterized in the yeast *Saccharomyces cerevisiae*. T-SNAREs (which should provide a functional definition of compartment borders) have been localized to the endoplasmic reticulum (Ufe1) [25], the cis Golgi network (Sed5) [26], the vacuole (Pep12) [27] and the plasma membrane (Sso1, 2) [28].

Physiological evidence for the role of SNARE proteins is provided by the fact that inactivation of t-SNAREs causes the accumulation of transport vesicles, which are no longer able to dock to the correct compartment. Massive overexpression of t-SNAREs has been shown to perturb cellular morphology, presumably by altering

the t-SNARE-containing compartment [29]. A t-SNARE localized to a compartment in which vesicular transport routes from several directions converge might serve several purposes in providing a docking site for vesicles originating from different organelles. For example, coimmunopreciptation experiments with an antibody directed against Sed5, a t-SNARE localized to the early Golgi, revealed the presence of several Sed5 interacting v-SNAREs, some of them mediating the docking of ER-derived vesicles, others presumably allowing the binding of vesicles derived from downstream Golgi compartments and returning to the early Golgi [30, 31]. V-SNAREs implicated in Golgi to plasma membrane transport were, however, absent in the SNARE complexes containing Sed5 [31]. They form a distinct complex with the plasma membrane t-SNAREs, thereby illustrating the high specificity of v-SNARE–t-SNARE interactions [32, 33]. Physiological evidence for the role of v-SNAREs in yeast is provided by the fact that some of them were originally identified in secretion-deficient mutants and that inactivation of these proteins results in accumulation of transport vesicles in vivo [34–36].

While studies in yeast can establish the basic mechanisms of vesicular targeting and fusion, studies in higher eukaryotes reveal an additional level of complexity. This is due to the complex organization of multicellular organisms with specialized cell types, organized in different tissues, each fulfilling specific tasks, and the establishment of elaborate intercellular communication systems. For example, in the rat four different t-SNAREs localized to the plasma membrane (syntaxins 1–4) have been identified, some of them showing tissue specificity [37]. Another example is the neuronal synapse, which is specialized in neurotransmitter release and used as a model system to study regulated secretion. Regulated secretion involves additional proteins that temporarily lock the fusion machinery in place until an appropriate signal releases the constraint [38–41]. Recent data indicate that the synaptic vesicle protein, synaptotagmin, plays a dual role as a specialized v-SNARE [42], on the one hand, and as a calcium sensor, on the other, and thereby links the signal for neurotransmitter release to the vesicle fusion machinery. The presence of two v-SNAREs on synaptic vesicles VAMP (vesicle-associated membrane protein) and synaptotagmin) and a cognate t-SNARE pair on the presynaptic plasma membrane (syntaxin and SNAP-25 (synaptosomal associated protein of 25 kD)) provides a multivalent vesicle binding site, explaining why neither the proteolytic cleavage of VAMP by tetanus toxin and certain serotypes of botulinum toxins nor the deletion of syntaxin prevents the accumulation of synaptic vesicles in the active zone [43–49].

Additional proteins regulating SNARE activity have been identified and are likely to ensure that SNAREs are only active in their proper intracellular localization. Members of the Sec1 protein family have been shown to interact with t-SNAREs and control their exposure to the cognate v-SNARE [50, 51]. Rab proteins, small GTP-binding proteins, are required for the assembly of SNARE complexes [31]. Both rab proteins and Sec1 family members are cytoplasmic proteins which are recruited to the membrane and exhibit a certain organelle specificity, indicating that these proteins likely add an additional layer of specificity to vesicular transport, maybe by a proofreading mechanism. More specialized interactions of distinct SNAREs with other components have been demonstrated. For example VAMP has been shown to interact with the synaptic vesicle protein synaptophysin in a way that is mutually exclusive with the binding to its t-SNAREs [52]. The t-SNARE syntaxin1 has been found to be associated with N-type calcium channels – yet another instance of close coupling between the vesicle docking and fusion machinery and that carrying the signal for exocytosis [46, 53, 54].

Assembled SNARE complexes provide a scaffolding for binding of general fusion components such as the *N*-ethylmaleimide-sensitive fusion protein (NSF) and the soluble NSF attachment proteins (SNAPs) [55–58]. The general fusion proteins are cytoplasmic proteins which function at many sites [59–61]. Inactivation of NSF by NEM causes accumulation of docked uncoated vesicles [62], indicating that NSF is required for consumption of vesicles docked via SNAREs. Additional evidence for the physiological role of NSF and for its general function in a variety of vesicular transport processes was provided by NSF mutants. The NSF mutant in *Drosophila*, comatose, causes paralysis of the flies, clearly establishing NSF's function in neurotransmission [63]. Similarly, the NSF mutant in yeast, sec18, causes an accumulation of transport vesicles and assembled SNARE complexes [31, 64, 65]. SNAPs mediate the interaction of NSF with membranes, as has been demonstrated using Golgi membranes [57, 66]. Three to six SNAP proteins bind to an assembled v-SNARE–t-SNARE complex [67, 68] (Figure 1C). In the neuronal synapse, the brain-specific form of SNAP (β-SNAP), but not the ubiquitous SNAP (α-SNAP), interacts with the specialized v-SNARE synaptotagmin [42]. ATP hydrolysis by NSF provides the energy to disrupt the complex, likely by causing conformational changes in SNAPs and one or several of the SNARE proteins [67]. The actual lipid bilayer fusion process is not understood; it cannot be excluded that additional components are necessary, but it seems likely that after ATP hydrolysis by NSF, one or several of the above-mentioned components are in an activated state to initiate fusion.

In summary, the rational scheme underlying transport between intracellular compartments seems to be established. But although the identification and isolation of the core machinery mediating these processes has proceeded during the past years, we are far from having a

complete understanding of mechanistic details. On the one hand, studying the machinery in its cellular background will help us to confirm its physiological functions; on the other, reconstitution of the purified components in vitro will provide the biophysical details about the reaction mechanisms. The completion of the yeast genome project will greatly facilitate the isolation of additional components and should give us pointers to the minimal set of proteins required for vesicular transport.

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