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Minireview

SNAREs and targeted membrane fusion

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Abstract Intracellular vesicular transport involves targeting of vesicles to their correct destination and subsequent fusion with the target membrane. These processes employ unique compartment-specific proteins in combination with general components which act at several transport steps. This review will summarize recent progress made in identifying such a machinery whose assembly and activity is modulated by a variety of regulatory proteins.

Key words: Secretion; Vesicle; Fusion; NSF; SNAP; SNARE

1. Introduction

Intracellular transport of cargo between compartments is mediated by vesicles [1]. Vesicles bud and pinch off at a donor compartment and move to and fuse with their target compartment. In striking contrast to the unidirectional transport of cargo, transport machinery (including membrane lipids) has to be recycled, creating a backward flow of material which again, in all likelihood, requires the same transport mechanisms. The processes of targeting and fusion, both topics of this review, have to occur with high precision to maintain the integrity of the cell. Fusion will be defined as the set of events that follows docking, up to and including lipid bilayer intermixing of vesicle and target membranes.

2. NSF and SNAPs

Since vesicular transport connects multiple intracellular compartments [1], at every instance recapitulating the same mechanistic themes, it seems only natural that the executing machinery should employ common components. Such general components should exhibit a widespread distribution on several compartments, most likely recruited from a common cytosolic pool. The first component to be identified which fulfills these criteria is the *N*-ethylmaleimide sensitive fusion protein (NSF) [2]. Inactivation of NSF by *N*-ethylmaleimide, an alkylating agent, results in accumulation of transport vesicles, which are docked to the target compartment [3]. NSF is involved in transport from the endoplasmic reticulum to the Golgi [4], intra-Golgi transport [3], neurotransmission [5], endosome-to-endosome fusion [6,7] and transcytosis [8]. Mutation of the yeast equivalent of NSF, Sec18, [9] causes transport vesicles to accumulate at several stages along the secretory pathway [10], clearly arguing for a role of NSF/Sec18 in vesicle consumption.

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NSF is a trimeric cytosolic protein, with each monomer composed of three domains: an amino-terminal domain followed by two domains, D1 and D2, each of which possesses a consensus ATP-binding site [11,12]. ATP hydrolysis by domain D1 is required for transport and domain D2 supports trimerization of monomers [12]. The amino-terminal domain has been speculated to bind the soluble NSF attachment proteins (SNAPs), cytosolic protein adapters tethering NSF to membranes. NSF does not interact with cytosolic SNAPs but only with SNAPs already bound to membranes. It is thought that binding of a SNAP to its receptor induces a conformational change in SNAP which exposes an NSF binding site [13]. Binding of NSF to SNAPs increases the ATPase activity of NSF [14], suggesting that NSF function relies on converting the chemical energy stored in ATP into conformational changes transmitted to SNAPs and SNAP receptors.

Three different SNAPs have been identified: α, β and γ, each capable of mediating binding of NSF to membranes and supporting intra-Golgi transport in vitro [15]. α-SNAP and β-SNAP are closely related (83% sequence identity), but in contrast to α-SNAP, which shows a general distribution, β-SNAP is selectively localized to the brain [16]. γ-SNAP is more distantly related to α/β-SNAP (25%/23% sequence identity respectively) [16]. Compelling in vivo and in vitro evidence exists for a function of α-SNAP in regulated exocytosis. Addition of α-SNAP stimulates facilitated neurotransmitter release in the synapse [17] and Ca^{2+} regulated exocytosis in permeabilized adrenal chromaffine cells [18]. Distinct SNAP-peptides block neurotransmission in the giant squid axon efficiently and reversibly [17]. Sec17 [19] is the yeast homologue of α-SNAP [20]. The sec17 phenotype is the accumulation of transport [21] vesicles again underlining the physiological function of SNAPs in fusion. A yeast homologue of γ-SNAP has not yet been found.

3. SNAREs

Both NSF and SNAPs are cytosolic proteins that act at several transport steps. Therefore, membrane components should exist that are specific for distinct organelles and that are able to recruit SNAPs and NSF to these membranes. Indeed, SNAP receptors (SNAREs) are compartment specific and, surprisingly, it has been shown that SNAP receptors are complexes composed of proteins originating from compartments connected by direct vesicular transport [5]. In each case one partner originates from the donor compartment and is found in transport vesicles, while the other one is localized in the acceptor compartment (Table 1).

The first SNARE complex discovered originated from the

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neuronal synapse [5] and was composed of the synaptic vesicle-associated protein (VAMP) [22,23], the synaptosome-associated protein of 25 kDa (SNAP-25) [24], and syntaxin1 [25,26]. Both of the latter are localized to the presynaptic plasma membrane. Each of these proteins is the target of a class of neurotoxins which are derived from *Clostridium tetanus* or *Cl. botulinum* [27]. These toxins are site-specific proteases for the individual synaptic SNAREs, thereby blocking neurotransmitter release and thus clearly establishing the physiological relevance of these SNAREs. Due to their distinct locations, VAMP was defined to be a vesicle-associated SNARE (v-SNARE) and syntaxin/SNAP-25 as target-associated SNAREs (t-SNAREs) [5].

The SNARE hypothesis [5] generalizes these observations: it states that each transport vesicle carries a specific v-SNARE which pairs in a unique match with its cognate t-SNARE, found only at the intended target membrane. This hypothesis was confirmed by the isolation of a SNARE complex involved in ER to Golgi transport from yeast [28]. This SNARE complex consists of at least 7 proteins: Sed5, Bos1, Bet1, Sec22, P28, P26 and P14. Bos1, and Sec 22 have been localized to transport vesicles derived from the ER [29–32], and P26 shows significant homology to Sec22; therefore, these proteins may be classified as v-SNAREs. Sed5 and its mammalian homologue syntaxin 5 have been localized to the *cis*-Golgi network [33,34] and therefore act as t-SNAREs. The exact function and classification of P28 and P14 are unclear due to the lack of sequence information. The existence of at least three different v-SNAREs which all interact with the same t-SNARE, i.e. Sed5, could argue for a combinatorial code to ensure the fidelity of vesicular transport. Alternatively, different transport vesicles, each characterized by a subset of v-SNAREs and presumably moving either in an anterograde or a retrograde direction might dock to the same t-SNARE.

Most SNAREs are type II membrane proteins. They contain a short luminal or extracellular carboxy-terminal domain, span the membrane once, and expose the bulk of their mass in the cytosol. The cytosolic domains form the basis for specific interactions of cognate SNARE pairs, perhaps via coiled-coil structures [35]. These interactions of paired SNAREs provide the scaffold for binding of SNAPs [36,37] and subsequently of NSF (Fig. 1).

A stable complex of SNAREs, SNAPs and NSF can be isolated from a detergent extract and migrates with a sedimentation constant of 20 Svedberg in velocity centrifugation [13]. This complex is only stable in the absence of hydrolyzable ATP (e.g. ATPγS or ATP in absence of Mg^{2+}). In the presence of Mg^{2+}/ATP, NSF will dissociate the SNARE complex, resulting in separation of v-SNAREs and t-SNAREs [36] (Fig. 1).

That this may be part of NSF's role in vivo is suggested by studies in yeast. To isolate the ER/Golgi SNARE complex mentioned above in a stable form, it was necessary to accumulate the complex in cells harboring a temperature sensitive Sec18 (NSF) allele at the restrictive temperature [28]. Apparently, after vesicles have been docked via SNAREs, SNAPs and NSF now prime the vesicles for, or execute, the fusion event by disassembling the docking partners (see Fig. 1). Despite the role of SNAPs and NSF in disassembly of SNAREs after vesicle docking, they might have additional functions before or during vesicle docking. For example, NSF has been found to be associated with synaptic vesicles but in a mode in which it can not be released by Mg^{2+}/ATP [38]. Further analysis will be required to identify proteins which mediate this type of membrane association of NSF.

SNAREs thus can define, in a functional manner, the borders of intracellular transport units. Isolation of SNAREs from different intracellular compartments, especially from the Golgi should provide important insights about the functional organization of these organelles and thus confirm or revise models based mainly on morphology. If SNAREs indeed delineate compartment borders then it is essential that SNAREs are sorted to their correct destination in an inactive form and are only activated upon arrival. Each SNARE should thus contain, in addition to the domain conferring the correct interaction with its partner SNARE, a domain ensuring its correct intracellular localization and a module to allow switching between active and inactive forms. Therefore SNAREs should be ideal model proteins to study intracellular protein targeting signals.

Indeed studies of this kind have demonstrated the importance of both the membrane spanning and cytoplasmic domain [34]. Due to their topology (type II membrane proteins) and the absence of a signal sequence, SNAREs do not follow the cotranslational transport pathway across the ER membrane [39]. VAMP, for instance, inserts posttranslationally into the ER membrane, in a process that is independent of the translocation machinery needed for proteins with signal sequences [40]. How this occurs and how VAMP passes through the secretory pathway in an inactive state before reaching the synaptic vesicles needs further investigation. In contrast to most SNAREs, P26 and SNAP-25 do not have membrane spanning regions; they seem to be membrane anchored via lipid moieties. P26 possesses a carboxy-terminal CAAX box [28], and SNAP-25 possesses a cluster of cysteines, which seem to be the target

Table 1
Individual members of the SNARE, Sec1 and rab protein families function at distinct transport steps

		ER-Golgi	post Golgi
Yeast	v-SNAREs	Bos1 Sec22 P26 Bet1?	Snc1 Snc2
	t-SNAREs	Sed5	Sso1 Sso2 Sec9
	Sec1 family	Sly1	Sec1
	rab family	Ypt1	Sec4
Mammalian	v-SNAREs	?	VAMP cellubrevin
	t-SNAREs	syntaxin5	syntaxins1–4 SNAP-25
	Sec1 family	?	Sec1 homologue
	rab family	rab1 rab2	rab3 rab6 rab8

Abbreviations: ER, endoplasmic reticulum; t-SNAREs, target-associated SNAP receptors; v-SNAREs, vesicle-associated SNAP receptors; VAMP, vesicle-associated membrane protein; SNAP-25, synaptosome-associated protein 25.

of palmitoylation [41]. How these lipid modifications play a role in targeting or other events like vesicle docking and fusion needs to be tested.

After the vesicles have fused with their target membrane, the v-SNAREs now reside in the acceptor compartment and have to be recycled to their compartment of origin. Whether the v-SNARE plays an active or a passive role in this is unclear. Nevertheless the activity of v-SNAREs which have reached the acceptor compartment needs to be modulated to avoid futile cycles of budding from and fusion with the same compartment.

4. Components modulating the accessibility of SNAREs

SNAREs interact with several proteins in addition to their cognate SNAREs and SNAPs (Table 1).

(i) Members of the Sec1 protein family [42,43] show both genetic and physical interactions with t-SNAREs [28,44]. Individual members in yeast act at distinct transport steps: Sly1 [30] in ER–Golgi transport, Slp1 [45] in transport to the vacuole and Sec1 [19] in Golgi-plasma membrane transport. The mammalian Sec1 homologue [44,46–48] blocks the assembly of the synaptic SNAREs into a complex in vitro [49] and overexpression in *Drosophila* blocks neurotransmitter release in vivo [48], clearly demonstrating a regulatory role.

(ii) Genetically linked to the Sec1 family is another protein family: that of rab proteins a class of small GTP binding proteins. Individual members of this family exhibit a certain degree of transport step specificity [50]. Genetic interactions between rabs and SNAREs have been established [29] and biochemical studies have demonstrated that rab proteins play a role in assembly of specific SNARE complexes [28,51]. These interactions clearly link the transport step specific protein families of SNAREs, Sec1 and rab with each other. Formation of SNARE complexes seems to be lightly regulated through inclusion of several check points to ensure the overall fidelity of vesicular transport.

(iii) Regulated secretion requires additional mechanisms to allow triggered cargo release. Signal sensors, acting as a clamp (or mediating their effects via clamps), are necessary to keep an otherwise constitutively active fusion machinery in an 'off' state. Ideally such regulatory components should be associated with or be an integral part of secretory vesicles, thus ensuring that only those vesicles harboring such components are controlled by a triggering event. A candidate component, synaptotagmin, fulfilling all criteria expected for a calcium sensor [52,53], has been identified on synaptic vesicles. Synaptotagmin has also been shown to interact with SNAREs, to share common binding sites with SNAPs, and to be associated with ω-conotoxin sensitive Ca^{2+} channels on the presynaptic plasma membrane, thus placing it in the correct vicinity to control neurotransmitter release. Homologues of synaptotagmin, localized to non neuronal tissues that exhibit regulated secretion, have been identified [54]. Therefore, it seems that tagmins fulfill a role as signal sensors, but it is not clear whether they act as the Ca^{2+} sensor for fast exocytosis or if they play a role in vesicle mobilization or docking.

(iv) On synaptic vesicles the accessibility of the v-SNARE VAMP for its partner t-SNARE seems to be regulated by its interaction with synaptophysin [55,56], another synaptic vesicle protein. It would be not surprising if similar mechanisms apply to constitutive secretion, since v-SNAREs on the donor compartment should be in an inaccessible state to avoid direct fusion between two closely opposed organelles.

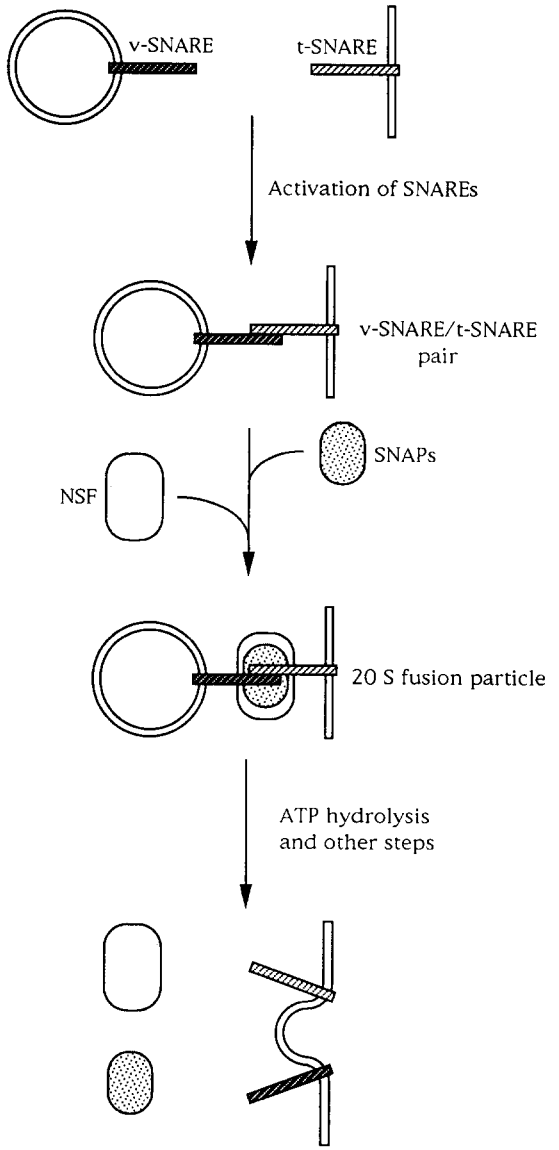

Fig. 1. Hypothetical model of the action of SNAREs, SNAPs and NSF during vesicle docking and fusion. After activation, a v-SNARE binds to its cognate t-SNARE. The activation process might involve association or dissociation of proteins to or from the SNAREs and could – in case of the v-SNARE – already occur during vesicle budding. The rab and Sec 1 protein families are likely to be involved in this process. In the presence of SNAPs and NSF, which both bind to the assembled SNAREs, fusion is initiated and ATP hydrolysis results in a conformational switch in the SNARE complex. *Abbreviations:* v-SNARE, vesicle-associated SNAP receptor; t-SNARE, target-associated SNAP receptor; SNAP, soluble NSF attachment protein; NSF, *N*-ethylmaleimide-sensitive fusion protein.

5. Concluding remarks

SNAREs are likely to guide transport vesicles through the secretory pathway of the cell, and SNAPs and NSF seem to initiate vesicular fusion. How these processes occur mechanistically, how the act of membrane fusion is actually mediated in

biophysical terms, and which components catalyze this event will be topics for future studies. Furthermore, we are just beginning to get an idea of how regulatory components modulate vesicle docking and fusion. A cast of characters in the form of additional transport factors are waiting to be included in the script.

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References

[1] Palade, G.E. (1975) Science 189, 347–358.
[2] Block, M.R., Glick, B.S., Wilcox, C.A., Wieland, F.T. and Rothman, J.E. (1988) Proc. Natl. Acad. Sci. USA 85, 7852–7856.
[3] Malhotra, V., Orci, L., Glick, B.S., Block, M.R. and Rothman, J.E. (1988) Cell 54, 221–227.
[4] Beckers, C.J.M., Block, M.R., Blick, B.S., Rothman, J.E. and Balch, W.E. (1989) Nature 339, 397–400.
[5] Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P. and Rothman, J.E. (1993) Nature 362, 318–324.
[6] Diaz, R.T., Mayorga, L.S., Weidman, P.J., Rothman, J.E. and Stahl, P.D. (1989) Nature 339, 398–400.
[7] Rodriguez, L., Stirling, C.J. and Woodman, P.G. (1994) Mol. Biol. Cell. 5, 773–783.
[8] Sztul, E., Colombo, M., Stahl, P. and Samanta, R. (1993) J. Biol. Chem. 268, 1876–1885.
[9] Wilson, D.W., Wilcox, C.A., Flynn, G.C., Chen, E., Kuang, W.-J., Henzel, W.J., Block, M.R., Ullrich, A. and Rothman J.E. (1993) Nature 339, 355–359.
[10] Graham, T.R. and Emr, S.D. (1991) J. Cell Biol. 114, 207–218.
[11] Tagaya, M., Wilson, D.W., Brunner, M., Arango, N. and Rothman, J.E. (1993) J. Biol. Chem. 268, 2662–2666.
[12] Whiteheart, S.W., Rossnagel, K., Buhrow, S.A., Brunner, M., Jaenicke, R. and Rothman, J.E. (1994) J. Cell Biol. 126, 945–954.
[13] Wilson, D.W., Whiteheart, S.W., Wiedmann, M., Brunner, M. and Rothman, J.E. (1992) J. Cell Biol. 117, 531–538.
[14] Morgan, A., Dimaline, R. and Burgoyne, R.D. (1994) J. Biol. Chem. 269, 29347–29350.
[15] Clary, D.O. and Rothman, J.E. (1990) J. Biol. Chem. 265, 10109–10117.
[16] Whiteheart, S.W., Griff, I.C., Brunner, M., Clary, D.O., Mayer, T., Buhrow, S.A. and Rothman, J.E. (1993) Nature 362, 353–355
[17] DeBello, W.M., O'Connor, V., Dresbach, Whiteheart, S.W., Wang, S.S.-h., Schweizer, F.E., Betz, H., Rothman, J.E. and Augustine, G.J. (1995) Nature 373, 626–630.
[18] Morgan, A. and Burgoyne, R.D. (1995) EMBO J. 14, 232–239.
[19] Novick, P., Field, C. and Schekman, R. (1980) Cell 21, 205–215.
[20] Griff, I.C., Schekman, R., Rothman, J.E. and Kaiser, C.A. (1992) J. Biol. Chem. 267, 12106–12115.
[21] Kaiser, C.A. and Schekman, R. (1990) Cell 61, 723–733.
[22] Trimble, W.S., Cowan, D.M. and Scheller, R.H. (1990) Proc. Natl. Acad. Sci. USA 85, 4538–4542.
[23] Baumert, M., Maycox, P.R., Navone, F., De Camilli, P. and Jahn, R. (1989) EMBO J. 8, 379–384.
[24] Oyler, G.A., Higgins, G.A., Hart, E.R., Battenberg, E., Billingsley, M., Bloom, F.E. and Wilson, M.C. (1989) J. Cell Biol. 109, 3039–3052.
[25] Bennett, M.K., Calakos, N. and Scheller, R.H. (1992) Science 257, 255–259.
[26] Inoue, A., Obata, K. and Akagawa, K. (1992) J. Biol. Chem. 267, 10613–10619.
[27] Niemann, H., Blasi, J. and Jahn, R. (1994) Trends Cell Biol. 4, 179–185.
[28] Søgaard M., Tani, K., Ye, R.R., Geromanos, S., Tempst, P., Kirchhausen, T., Rothman, J.E. and Söllner, T. (1994) Cell 78, 937–948.
[29] Shim, J., Newmann, A.P. and Ferro-Novick, S. (1991) J. Cell Biol. 113, 55–64.
[30] Dascher, C., Ossig, R., Gallwitz, D. and Schmitt, H.D. (1991) Mol. Cell. Biol. 11, 872–885.
[31] Newmann, A.P., Shim, J. and Ferro-Novick, S. (1990) Mol. Cell. Biol. 10, 3405–3414.
[32] Lian, J.P. and Ferro-Novick, S. (1993) Cell 73, 735–745.
[33] Hardwick, K.G. and Pelham, H.R.B. (1992) J. Cell Biol. 119, 513–521.
[34] Banfield, D.K., Lewis, M.J., Rabouille, C., Warren, G. and Pelham, H.R.B. (1994) J. Cell Biol. 127, 357–371.
[35] Hayashi T., McMahon, H., Yamasaki, S., Binz, T., Hata, Y., Südhof, T.C. and Niemann, H. (1994) EMBO J. 13, 5051–5061.
[36] Söllner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H. and Rothman, J.E. (1993) Cell 75, 409–418.
[37] McMahon, H.T. and Südhof, T.C. (1995) J. Biol. Chem. 270, 2213–2217.
[38] Hong, R.M., Mori, H., Fukui, T., Moriyama, Y., Futai, M., Yamamoto, A., Tashiro, Y. and Tagaya, M. (1994) FEBS Lett. 350, 253–257.
[39] Kutay, U., Hartmann, E. and Rapoport, T.A. (1993). Trends Cell Biol. 3, 72–75.
[40] Kutay, U., Ahnert-Hilger, G., Hartmann, E., Wiedenmann, B. and Rapoport (1995) EMBO J. 14, 217–223.
[41] Hess, D.T., Slater, T.M., Wilson, M.C., and Skene, J.H.P. (1992) J. Neurosci. 12, 4634–4641.
[42] Aalto, M.K., Keränen, S. and Ronne, H. (1992) Cell 68, 181–182.
[43] Bennett, M.K. and Scheller, R.H. (1993). Proc. Natl. Acad. Sci. USA 90, 2559–2563.
[44] Hata, Y., Slaughter, C.A. and Südhof, T.C. (1993) Nature 366, 347–351.
[45] Wada, Y., Kitamoto, K., Kanabe, T., Tanaka, K. and Anraku, Y. (1990) Mol Cell. Biol. 10, 2214–2223.
[46] Pevsner, J., Hsu, S-C. and Scheller, R.H. (1994) Proc. Natl. Acad. Sci. USA 91, 1445–1449.
[47] Garcia, E.P., Gatti, E., Butler, M., Burton, J. and De Camilli, P. (1994) Proc. Natl. Acad. Sci. USA 91, 2003–2007.
[48] Schulze, K.L., Littleton, J.T., Salzberg, A., Halachmi, N., Stern, M., Lev, Z. and Bellen, H.J. (1994) Neuron 13, 1099–1108.
[49] Pevsner, J., Hsu S.C., Braun, J.E., Calakas, N., Ting, A.E., Bennett, M.K. and Scheller., R.H. (1994) Neuron 13, 353–361.
[50] Simons, K. and Zerial, M. (1993) Neuron 11, 789–799
[51] Lian, J.P., Stone, S., Jiang, V., Lyons, P. and Ferro-Novick, S. (1994) Nature 15, 698–701.
[52] Littleton, J.T. and Bellen, H.J. (1995) Trends Neurosci. 18, 177–183.
[53] Kelly, R.B. (1995) Curr. Biol. 5, 257–259.
[54] Jacobson, G., Bean, A.J., Scheller, R.H., Juntti-Berggeren, L., Deeney, J.T., Berggeren, P.-O. and Meister, B. (1994) Proc. Natl. Acad. Sci. USA 91, 12487–12491.
[55] Calakos, N., and Scheller, R.H. (1994) J. Biol. Chem. 269, 24534–24537.
[56] Edelmann, L., Hanson, P.I., Chapman, E.R. and Jahn, R. (1995). EMBO J. 14, 224–231.