

# 5 | Molecular mechanisms of membrane fusion in the endocytic pathway

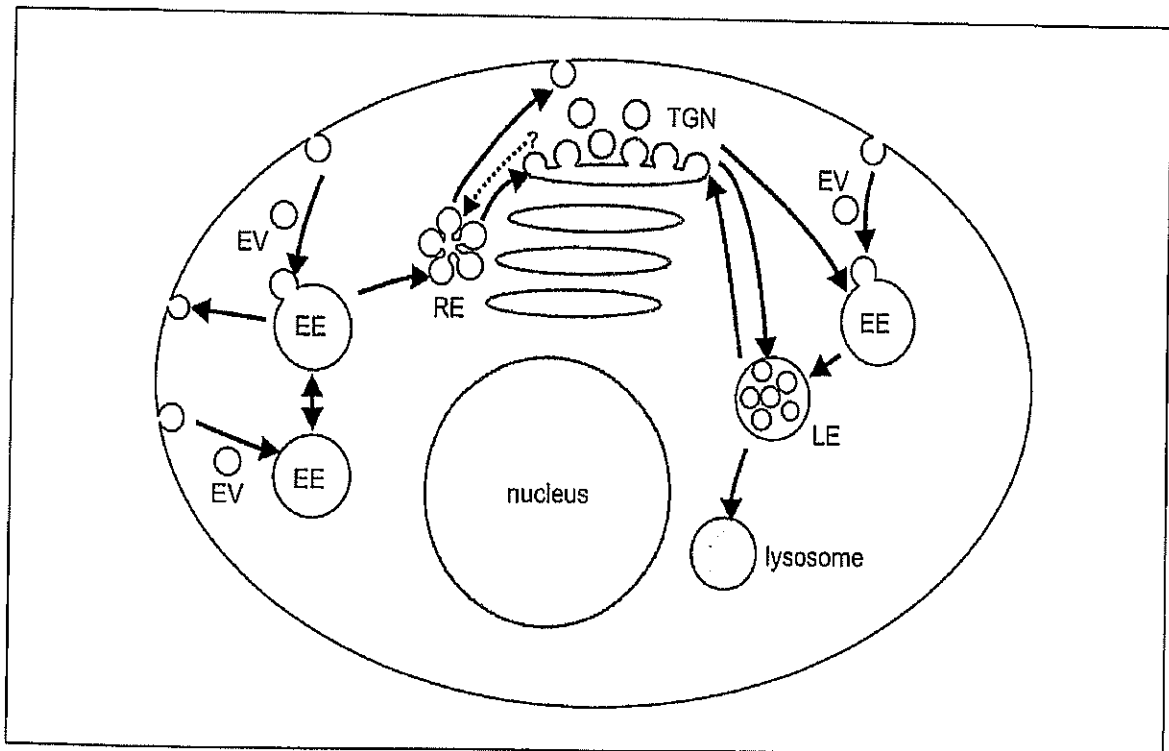
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## 1. Introduction

The endocytic pathway of mammalian cells consists of distinct membrane-bound compartments that receive internalized molecules from the plasma membrane and recycle them back to the surface (early endosomes and recycling endosomes) or sort them to degradative compartments (late endosomes and lysosomes) (see Fig. 1). Endocytic membrane traffic between these organelles is a dynamic process that involves membrane budding and fusion reactions as well as the movement of endocytic vesicles and endosomes. The complexity of the pathway and the extensive membrane exchange between compartments necessitate a tight regulation of membrane fusion events, so that the overall organization of the endomembrane system remains intact during membrane turnover. Since the mechanisms of formation of endocytic vesicles at the plasma membrane are covered elsewhere (see Chapters 1–4), in this review we will first give a brief overview of how endocytic membrane docking and fusion are being studied, and then discuss our current understanding of the molecular machinery underlying this process, with special emphasis on what has been learnt from the fusion between early endosomes in mammalian cells.

## 2. Experimental systems to study endocytic membrane docking and fusion

Microscopy of living cells has revealed the plasticity and dynamics of endocytic organelles, which undergo continuous fusion and fission reactions (1–4). Our current view of the underlying molecular mechanisms is the result of the convergence of several independent experimental approaches. Like with studies of secretion, yeast genetics has proven to be a powerful tool to identify the various constituents of the molecular apparatus involved (5, 6). Genetic screens, such as those for *vps* (vacuolar



**Fig. 1** Endocytic membrane traffic. In the left half of the schematized cell, endocytosis, homotypic early endosome fusion, and recycling (which can either take place from early endosomes or from recycling endosomes), are illustrated. In the right half, transport from early endosomes to late endosomes and lysosomes, and trafficking between the TGN and endosomes, is illustrated. Abbreviations: EE, early endosome; EV, endocytic vesicle; LE, late endosome; RE, recycling endosome; TGN, *trans*-Golgi network.

protein sorting), *end* (endocytosis defective), and *pep* (peptidase deficient) mutants have yielded several candidate molecules for the regulation of endocytic membrane fusion (see Chapter 10). From yeast genetics alone it is difficult to pin-point the precise function of a given gene product. However, the phenotypic analysis of yeast mutants has often been informative as to the possible role of a protein in membrane docking and fusion. This is the case with proteins whose functional inactivation leads to endocytic transport defects as well as the accumulation of transport intermediates (as verified by EM). Vps class D proteins are good examples (see Section 7). In mammalian cells, the expression of dominant inhibitory or stimulatory mutants of small GTPases has yielded important information, as exemplified by Rab5 (Section 4).

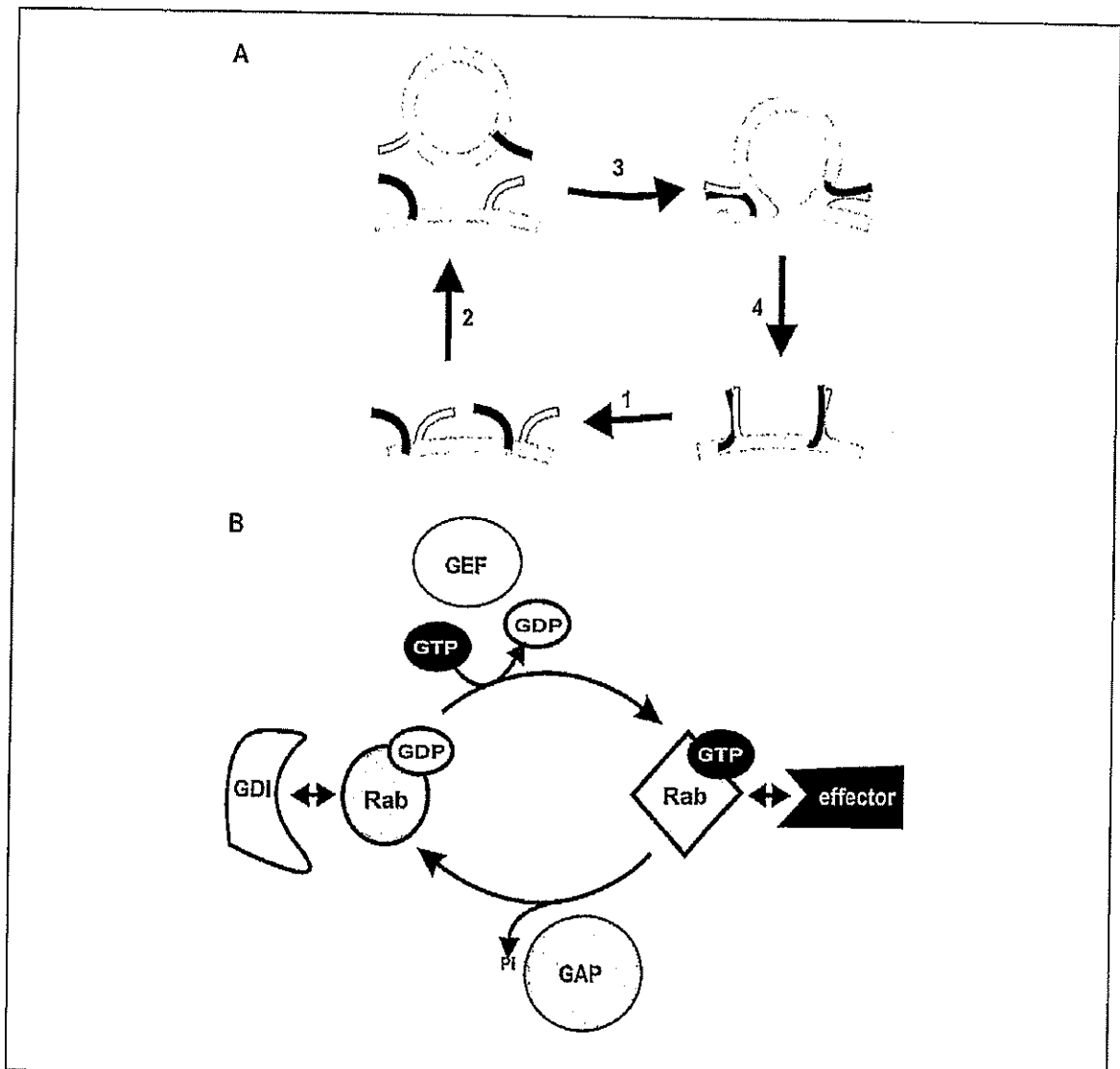
Because genetic studies and expression of dominant interfering mutants can only provide indirect evidence for a role in membrane fusion, more direct approaches have been developed. One powerful assay in mammalian cells reconstitutes the homotypic fusion between early endosomes (7, 8). In this assay, two early endosome fractions are loaded with distinct markers that can form a complex if they meet (e.g. antigen-antibody, biotin-avidin), and are mixed in the presence of cytosol and ATP. If fusion occurs, the two markers will react to form complexes, and these can subsequently be retrieved (for instance, on antibody-coated magnetic beads) and quantified. In a similar manner, the heterotypic fusion between endocytic vesicles and early endosomes can be studied (9, 10). Likewise, several related assays have been em-

ployed to reconstitute the fusion between endocytic carrier vesicles (ECVs) and late endosomes (11), and between late endosomes and lysosomes (12). While endosome fusion has been difficult to reconstitute in yeast, another homotypic fusion event, that of vacuoles (the yeast equivalent of lysosomes), has successfully been reconstituted. The experimental set-up is equivalent to that of homotypic early endosome fusion, except that the vacuole fusion assay measures a biochemical reaction that takes place when vacuoles from two appropriately genetically engineered yeast strains fuse. This assay was originally established to measure vacuole inheritance from mother to daughter cells (13), but it has proven useful for studies of the general molecular mechanisms involved in membrane fusion.

The introduction of the green fluorescent protein (GFP) in cell biology has recently provided a novel tool for the studies of membrane fusion. Now it is possible to follow membrane fusion in living cells by video microscopy, upon the expression of GFP fusion proteins that are targeted to endocytic organelles. Such studies have yielded information about the dynamics of endosome fusion (2, 3) as well as endosome motility (4).

### 3. Rab GTPases and SNAREs in membrane docking and fusion

The main players in membrane docking and fusion have been identified through a combination of biochemical, genetic, and molecular biological approaches. A hexameric ATPase, NSF (*N*-ethyl maleimide-sensitive factor), is required for membrane traffic in yeast and mammalian cells (14). The ATPase activity of NSF is regulated by another essential protein, SNAP (soluble NSF attachment protein) (15), and the NSF/SNAP complex binds to SNAP receptors known as SNAREs (16). SNAREs are divided into two subfamilies. One subfamily of SNAREs was originally found enriched on vesicles (*v*-SNAREs), whereas the other subfamily was associated with target membranes (*t*-SNAREs). As will be further discussed below, such a strict segregation of SNAREs between vesicles and organelles does not exist and this terminology is perhaps misleading. Therefore, the alternative names of R- and Q-SNAREs (after conserved arginine and glutamine residues that distinguish the two subfamilies) have been proposed (17). Nevertheless, for simplicity, we will use here the original terminology. Results from the *in vitro* vacuole fusion assay (see Section 2) have revealed that fusion requires the formation of *trans*-SNARE complexes, that is, complexes between *v*-SNAREs on the one membrane and *t*-SNAREs on the other one (18). X-ray crystallography and NMR have unravelled the core structure of the SNARE complex involved in the fusion of synaptic vesicles with the presynaptic plasma membrane (19, 20). These studies reveal that the two *t*-SNAREs, SNAP-25 and syntaxin1, form a tight coiled-coil complex with the *v*-SNARE VAMP-2. Interestingly, the four  $\alpha$ -helices involved in the complex formation (two from SNAP-25 and one each from syntaxin1 and VAMP-2) are all oriented in a parallel manner. This suggests that their zipper-like complex formation may release sufficient energy to drive the fusion of



**Fig. 2** SNARE and Rab proteins in membrane docking and fusion. (A) *trans*-SNARE complexes in membrane docking/fusion. 1 Pre-existing SNARE complexes are dissociated ('primed') through the activity of NSF/SNAP. 2 Primed SNAREs on a vesicle interact with primed SNAREs on a target membrane. 3 The SNAREs form tight complexes through parallel coiled-coil interactions, and the energy released may power membrane fusion. 4 After fusion, the SNARE complexes reside in the target membrane. The 't-SNAREs' are indicated in black and 'v-SNAREs' in grey. (B) the Rab GTPase cycle. The Rab protein switches between two different conformations depending on whether GDP or GTP is bound. GDP/GTP exchange is catalysed by a GDP/GTP exchange factor (GEF), whereas a GTPase activating protein (GAP) stimulates the GTPase activity of the Rab protein. The GDP-bound form of the Rab protein is recognized by GDP dissociation inhibitor (GDI), which regulates its membrane association (not illustrated here). The GTP-bound form of the Rab protein interacts with effector molecules.

the two opposing bilayers (Fig. 2A). Indeed, *trans*-SNARE complex formation is sufficient to cause the fusion of lipid/detergent micelles *in vitro*, albeit with low efficiency (21–23). NSF has similarity to heat shock ATPases involved in protein folding, and the role of NSF/SNAP may be to disassemble pre-existing SNARE complexes, in order for SNAREs to be reused for a new round of vesicular transport.

The original SNARE hypothesis implicates SNAREs in vesicle targeting as well as in fusion (16). However, while recent work has indeed confirmed the role of SNAREs

in the fusion of lipid bilayers, their role as the sole molecular determinants in vesicle targeting and fusion seems implausible. First, although distinct SNAREs may show different intracellular localizations, SNAREs are generally found on multiple organelles. For instance, syntaxin1 is found on synaptic vesicles, endosomes, and on the plasma membrane, and syntaxin6 is found both in the TGN and on early endosomes (24, 25). Taking into account that SNAREs are integral membrane proteins and consequently need to be recycled by membrane trafficking, this comes as no surprise. Secondly, v- and t-SNAREs form complexes in a rather unspecific manner *in vitro* (26). Thirdly, the function of NSF in post-mitotic Golgi vesicle docking appears to be independent of its ability to prime SNAREs (27). Fourthly, a wealth of yeast genetic data indicate that SNAREs do not determine fusion specificity (28). It thus seems unlikely that these molecules by themselves would play the leading role in vesicle targeting. There is indeed an additional layer of regulation provided by another group of proteins, the Rab GTPases (29, 30).

Rab proteins belong to a family of about 40 members, and just like SNAREs, different Rab GTPases are localized to distinct compartments and regulate distinct trafficking steps. In contrast to SNAREs, Rab GTPases are reversibly attached to membranes, thus enabling their efficient recycling from acceptor to donor membranes. This is accomplished through the association of lipophilic geranylgeranyl groups, attached to C-terminal cysteine residues, with the lipid bilayer. Geranylgeranylated Rab GTPases are presented to, and removed from membranes by, an essential protein, Rab GDP dissociation inhibitor (GDI) (31, 32). Like most other GTPases, Rab GTPases switch their conformation depending on whether GDP or GTP is bound, and this determines their ability to interact with effector molecules (which specifically occurs with the GTP-bound form) (see Fig. 2B). Recent studies indicate that one Rab GTPase may have numerous effectors, some of which may mediate vesicle 'tethering' prior to SNARE complex formation (see Section 4). The latter is in agreement with both yeast genetic studies and biochemical studies in vacuole or endosome fusion assays (Section 2), which indicate that Rab GTPases act prior to SNAREs in membrane docking and fusion. It thus appears that Rab GTPases and their effectors cause transport vesicles to tether to their correct target membranes, whereas SNAREs then take over to trigger the actual membrane fusion process. However, as discussed in Section 6, it is still possible that not only SNAREs, but also Rab effectors, may participate in the fusion reaction.

#### 4. Rab5 and its effectors in early-endosome fusion

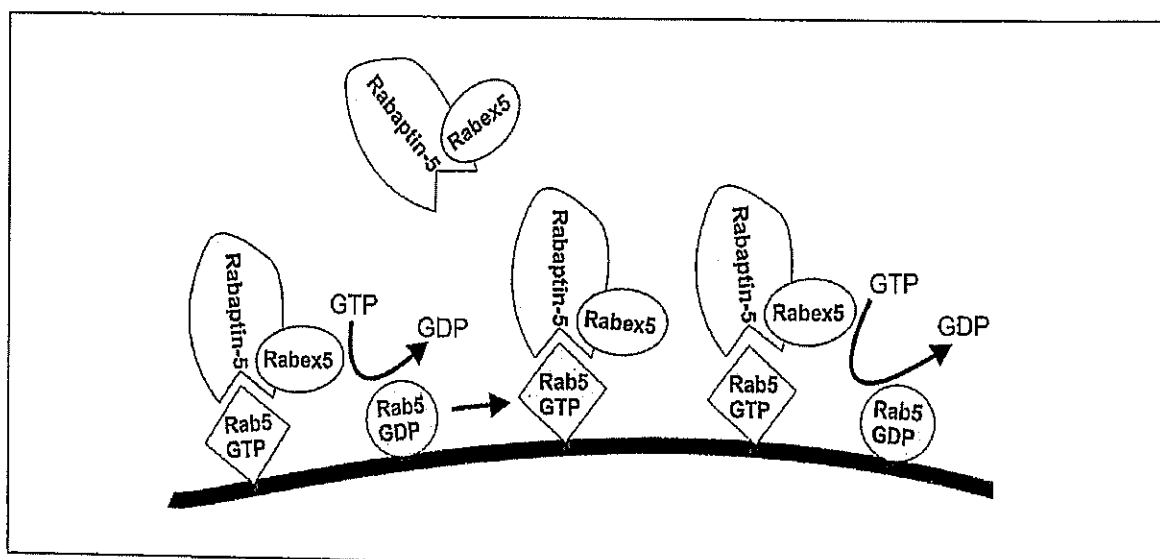
Several Rab GTPases and SNARE proteins are thought to function in endocytic membrane traffic (33, 34). Among the Rab GTPases, Rab5 has been most studied, and much of our knowledge about Rab function in general derives from studies of this GTPase. Rab5 is found on the cytosolic side of early endosomes, endocytic vesicles, and the plasma membrane (35, 36). Expression of a GTPase-deficient mutant (Rab5<sup>Q79L</sup>) causes an increased rate of endocytosis and the formation of giant early endosomes. Conversely, the expression of a mutant with preferential affinity for GDP (Rab5<sup>S34N</sup>)

inhibits endocytosis and causes the formation of very small early-endocytic profiles (37). Rab5 is essential for homotypic early-endosome fusion *in vitro* (8), and such fusion is stimulated by Rab5<sup>Q79L</sup> and inhibited by Rab5<sup>S34N</sup> (37). Moreover, a mutant (Rab5<sup>D136N</sup>) that binds xanthine instead of guanine nucleotides stimulates endosome fusion in the presence of the non-hydrolysable XTP $\gamma$ S, and inhibits fusion in the presence of XDP (38). These results indicate that Rab5, in its GTP-bound form, stimulates early-endosome fusion. With Rab5<sup>D136N</sup>, XTP hydrolysis occurs even when endosome fusion is inhibited, indicating that nucleoside triphosphate hydrolysis is not required for membrane fusion but rather exerts a regulatory function on this process.

The search for Rab5 effectors, first with the yeast two-hybrid system and more recently through affinity chromatography, has yielded a surprisingly high number of Rab5:GTP-interacting molecules. In fact, no less than 22 cytosolic proteins could be eluted from a Rab5:GTP $\gamma$ S affinity column (39). Even though not all these proteins may bind directly to Rab5:GTP, this illustrates that Rab proteins, contrary to early belief, regulate multiple effectors and control more than one biochemical event. The latter has been demonstrated in the case of Rab5, which, in addition to its role in endocytic membrane fusion, also plays a role in the formation of endocytic vesicles (10), and in the motility of endocytic vesicles along microtubules (4).

Which then are the effectors of Rab5 in endocytic membrane fusion? The first one to be identified was Rabaptin-5 (40), a dimeric coiled-coil protein that is found in a complex with Rabex-5, a GDP/GTP exchange factor (GEF) for Rab5 (10). Rabaptin-5 is recruited to early endosomes in a Rab5- and GTP-dependent manner, and its immunodepletion from cytosol strongly inhibits early endosome fusion *in vitro* (40). Upon such immunodepletion, endosome fusion can be restored by the addition of the Rabaptin-5/Rabex5 complex, but not by Rabaptin-5 alone. The presence of a GEF in a complex with a Rab5 effector suggests that this protein is needed in order to generate sufficient amounts of Rab5:GTP on the endosome membrane (10). The association of Rabex5 with a Rab5 effector suggests the possibility that a Rab5 signal may be locally amplified at the membrane: The binding of Rabaptin-5 to Rab5:GTP may bring Rabex-5 in to a position to convert a neighbouring Rab5:GDP into Rab5:GTP. This Rab5:GTP may then recruit another Rabaptin-5/Rabex5 complex, and so on, resulting in a patch of Rab5:GTP in the membrane (Fig. 3). This, together with biochemical studies indicating that Rab5 effectors are present in large oligomers on the membrane of early endosomes (3), may explain the 'hot spots' of Rab5/effector complexes observed on endosomes (2, 3).

While the C-terminus of Rabaptin-5 contains a Rab5-binding domain, a Rab4-binding domain has been detected at its N-terminus (41). Like Rab5, Rab4 is found on early endosomes, but this GTPase appears to control membrane recycling rather than incoming endocytic traffic (42, 43). This raises the possibility that Rabaptin-5 may serve to co-ordinate endocytic membrane transport with recycling. Interestingly, cleavage of Rabaptin-5 by caspase-3, thus separating the Rab4- and Rab5-binding domains, seems to provide the molecular explanation for the inhibition of endosome fusion observed during programmed cell death (apoptosis) (44, 45).



**Fig. 3** Recruitment of Rabaptin-5/Rabex5 to endosomes by Rab5. Interaction of Rabaptin-5 with Rab5:GTP brings Rabex5 in position to promote GDP/GTP exchange of a neighbouring Rab5:GDP. The resulting Rab5:GTP may then recruit another Rabaptin-5/Rabex5 complex, which may activate another Rab5:GDP, and so on. This results in a local amplification of Rab5 activation.

Even though the Rabaptin-5/Rabex5 complex is essential for both homotypic early-endosome fusion and for the fusion between endocytic vesicles and early endosomes (10), it is not sufficient for these transport reactions. Endocytic membrane fusion requires several other Rab5 effectors (39). Strikingly, another Rab5 effector, EEA1 (early endosome antigen 1) can alone support membrane fusion under special experimental conditions. In fact, if sufficient amounts of EEA1 are added to early endosomes, their fusion is stimulated even in the absence of cytosol (32). EEA1 thus appears to be a core component of the membrane docking and fusion machinery, whereas the main role of Rabaptin-5/Rabex5 might be to maintain a high local level of endosomal Rab5:GTP, so that EEA1 may be efficiently recruited to endosomes. Surprisingly, however, in the presence of excess EEA1, endosome fusion can occur even when the endosomes have been treated with GDI in order to remove Rab5. This suggests that Rab5-mediated recruitment is dispensable at high EEA1 concentrations, and that EEA1 may interact with additional membrane molecules (39). Indeed, EEA1 has been found to interact both with a membrane lipid (see Section 5) and with endosomal SNARE molecules (see Section 6). Rab5 is symmetrically distributed between endocytic vesicles and early endosomes (46), and its presence on both membranes is required both in heterotypic (endocytic vesicle to endosome) and homotypic (endosome to endosome) fusion (47, 48). In contrast, EEA1 is only found on early endosomes, and the finding that it nevertheless is required for heterotypic membrane fusion makes EEA1 a good candidate for conferring directionality to vesicular traffic from the plasma membrane to early endosomes (46). The multiplicity of Rab5 effectors, however, suggests that EEA1 is only one component of the early endosome transport machinery and other molecules are likely to co-operate with EEA1 and the Rabaptin-5 complex in endocytic membrane docking and fusion.

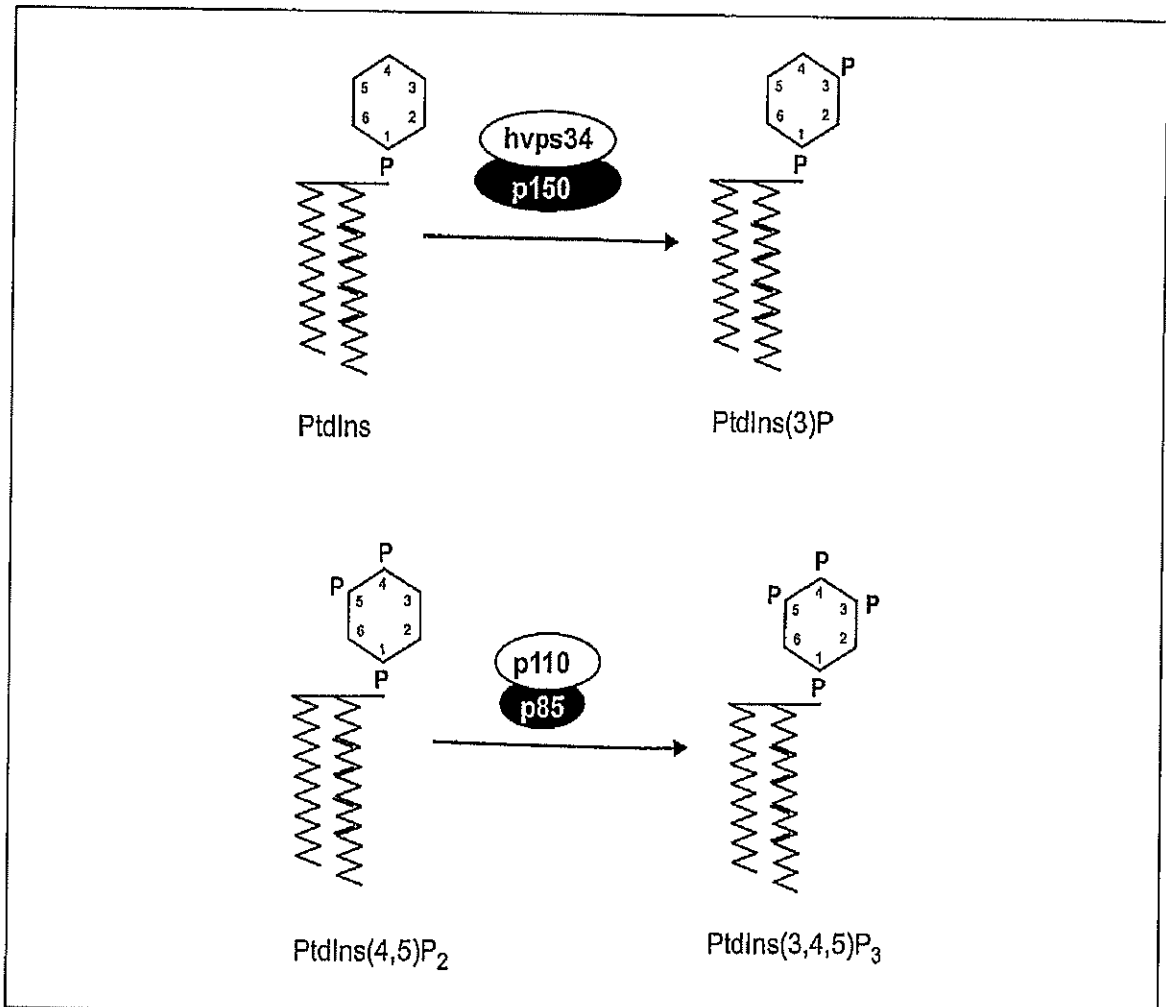


Fig. 4 Generation of 3'-phosphoinositides by PI 3-kinases.

## 5. PI 3-kinase and endocytic membrane fusion

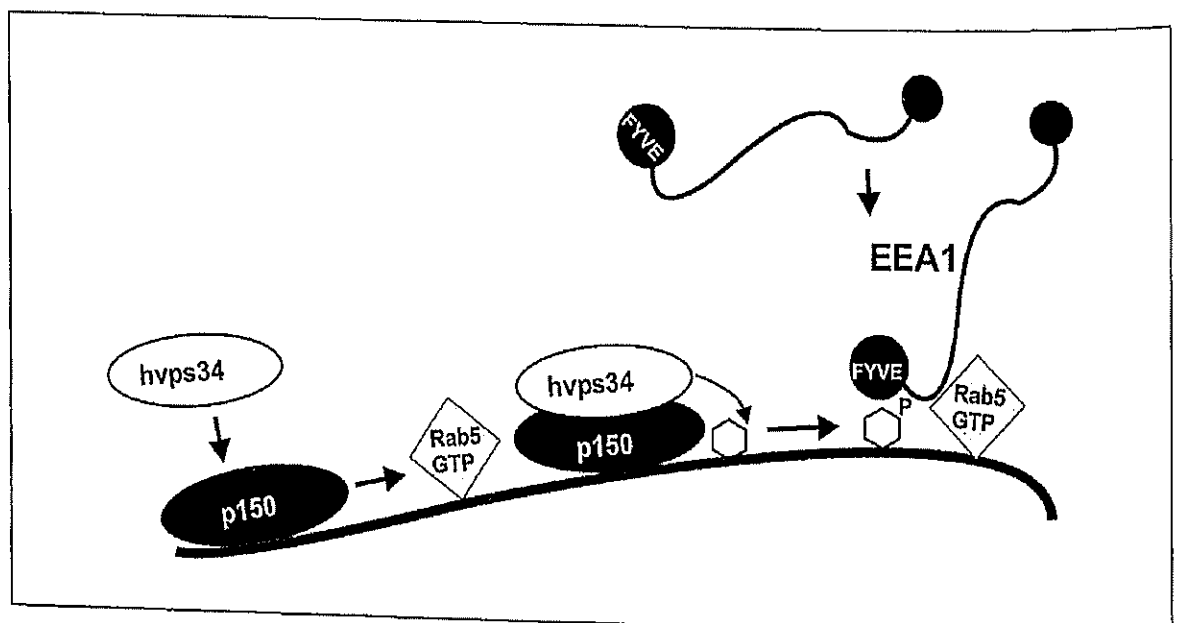
Phosphatidylinositol 3-kinases (PI 3-kinases) phosphorylate phosphatidylinositol (PtdIns) and its derivatives PtdIns-4-phosphate (PtdIns(4)P) and PtdIns-4,5-bisphosphate (PtdIns(4,5)P<sub>2</sub>) to yield the 3'-phosphoinositides PtdIns(3)P, PtdIns(3,4)P<sub>2</sub>, and PtdIns(3,4,5)P<sub>3</sub>, respectively (see Fig. 4) (49, 50). The finding that PI 3-kinase activity is essential for vacuolar protein sorting in yeast (see later) (51), prompted several studies of the involvement of this kinase in mammalian membrane trafficking. These studies revealed that, at least in some cell types, PI 3-kinase is essential for endocytosis as well as for endocytic recycling and late endocytic trafficking (52–57). The studies of how wortmannin, a specific PI 3-kinase inhibitor, blocks endosome fusion (58–60) have contributed to shed light on one of the mechanisms involved: The PI 3-kinase product, PtdIns(3)P binds to proteins containing FYVE zinc finger domains (61), including EEA1 (34, 62, 63). EEA1 contains Rab5-binding domains at its N- and C-termini, and the C-terminal Rab5-binding domain is adjacent to a FYVE finger. The C-terminus of EEA1 (comprising the Rab5-binding domain and the FYVE finger) is necessary and sufficient for the targeting of EEA1 to early endosomes (64),



and both Rab5-binding (through the Rab5-binding domain) and PtdIns(3)P-binding (through the FYVE finger) are required for the efficient recruitment of EEA1 to endosomes *in vivo* (65). This ensures that EEA1 is only recruited to membranes that contain both Rab5:GTP and PtdIns(3)P and may explain how EEA1 becomes specifically localized to early endosomes.

The above model implies that early endosomes are enriched in PtdIns(3)P, and this has recently been verified by the use of a PtdIns(3)P-specific probe (H. Stenmark and R. Parton, unpublished). What directs the localized production of PtdIns(3)P on early endosome membranes? A clue has emerged through the finding that hvps34, the PI 3-kinase that appears to be the principle enzyme responsible for PtdIns(3)P production in mammalian cells (66), is an effector of Rab5 (67). It binds to Rab5:GTP together with its regulatory subunit, p150, and this binding probably ensures the 3'-phosphorylation of PtdIns on early endosomes. Rab5 thus plays a dual role in the recruitment of EEA1 to membranes: first, through a direct binding to EEA1, and secondly, through the stimulation of PtdIns(3)P production on endosome membranes (see Fig. 5).

It is intriguing that a second PI 3-kinase, p110- $\beta$ , has also been identified as a Rab5 effector (67). This PI 3-kinase is thought to mainly phosphorylate PtdIns(4,5)P<sub>2</sub> *in vivo*, thus yielding PtdIns(3,4,5)P<sub>3</sub>, and, unlike hvps34, whose activity is constitutive, p110- $\beta$  is activated via the binding of various agonists to their receptors (66). Unlike hvps34, this PI 3-kinase neither seems to function at the level of Rab5-dependent early endosome fusion, nor in endosome motility along microtubules and it is not yet known if p110- $\beta$  and PtdIns(3,4,5)P<sub>3</sub> play a role in the other Rab5-regulated functions, such as vesicle formation.

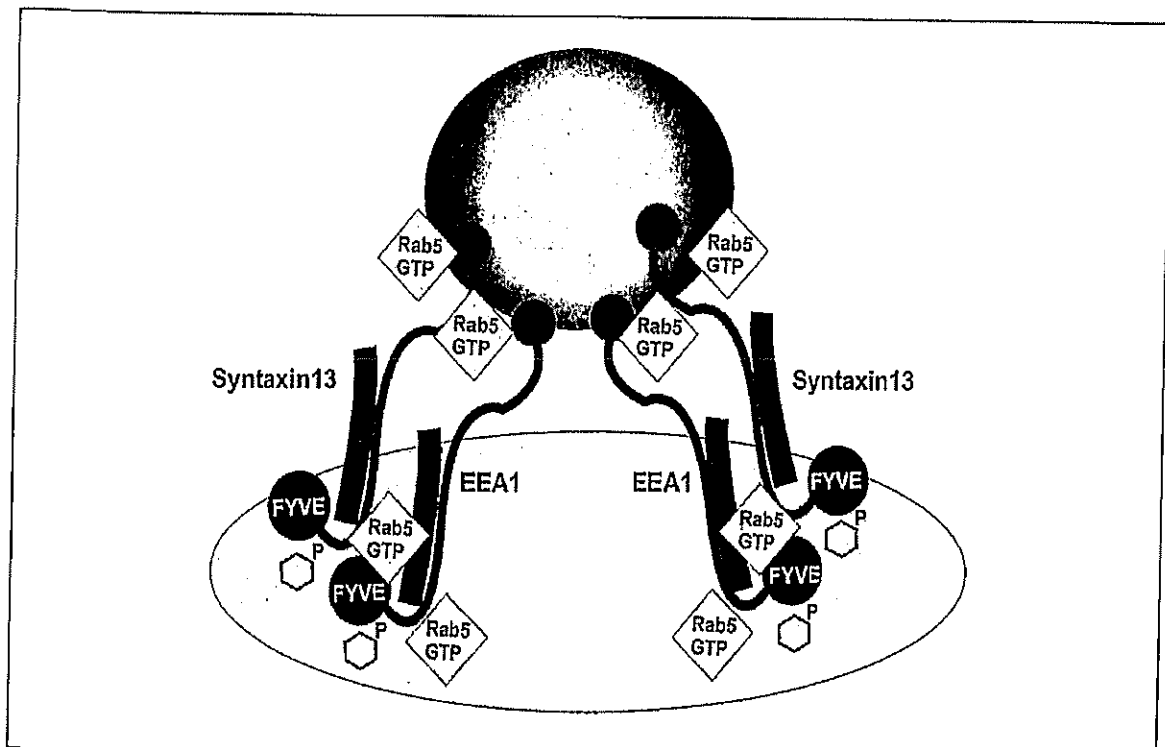


**Fig. 5** Recruitment of EEA1 to endosome membranes via Rab5 and PtdIns(3)P. Rab5 serves a dual role in EEA1 recruitment. First, to recruit locally the PI 3-kinase hvps34 (by interacting with its membrane adaptor p150), thus locally stimulating the phosphorylation of PtdIns into PtdIns(3)P. Secondly, by interacting with the Rab5 binding domain of EEA1. This interaction is of low affinity, and efficient EEA1 recruitment relies on the additional binding of the FYVE domain to PtdIns(3)P.

## 6. EEA1 and SNARE complex formation

As mentioned in Section 4, early-endosome fusion *in vitro* can proceed in the apparent absence of Rab5, provided that excess EEA1 is present. Excess EEA1 even obviates the need for PI 3-kinase activity (39), suggesting that both Rab5:GTP and PtdIns(3)P play synergistic rather than obligatory roles in the recruitment of EEA1 to endosomes. This further suggests that other EEA1-binding molecules exist on early endosome membranes. Indeed, EEA1 has been found to interact with the t-SNAREs syntaxin6 and syntaxin13 (3, 68), which are both present on early endosomes (syntaxin6 is abundant in the *trans*-Golgi network (TGN) as well). Rab GTPases and their effectors are thought to operate prior to SNARE complex formation in membrane fusion (see Section 3), and the EEA1–syntaxin interaction may provide insight into the molecular dynamics of this process.

While the functional role of the EEA1–syntaxin6 interaction is not known (the potential regulation of TGN to endosome traffic by EEA1 has not been investigated), several results link EEA1 to syntaxin13 function (3). Recombinant syntaxin13 that lacks the transmembrane domain inhibits early-endosome fusion *in vitro*, and the same is the case with anti-syntaxin13 antibodies. Furthermore, a synthetic FYVE peptide that inhibits the interaction of syntaxin13 with EEA1 inhibits early-endosome fusion with similar concentration dependence. Finally, EEA1, NSF, Rabaptin-5, Rabex5,



**Fig. 6** The function of EEA1 in endocytic membrane docking and fusion. EEA1 may tether two Rab5-positive structures, such as an endocytic vesicle and an early endosome, by virtue of its two Rab5-binding sites. On the membranes, EEA1 assembles into oligomeric complexes that contain Rabaptin-5/Rabex5 (not shown), NSF (not shown), and syntaxin13. This ensures a spatially controlled SNARE priming. Membrane fusion following this tethering step conceivably involves the complex formation between syntaxin13 and a v-SNARE (not shown), as well as rearrangements of EEA1 oligomers.

and syntaxin13 (but not a number of other t-SNAREs) can be found together in high molecular weight complexes that are stabilized in the presence of a C-terminal EEA1 construct that appears to block endosome fusion at a post-docking stage. Interestingly, these membrane-associated complexes are regulated by the ATPase activity of NSF, suggesting that NSF may serve as a chaperone for Rab effector complexes as well as for SNAREs. The association of NSF with the high molecular weight EEA1 complexes suggests that EEA1 may recruit NSF to prime SNAREs locally (Fig. 6). The exact nature and function of the high molecular weight EEA1 complexes that are formed transiently during endosome fusion is not known. Conceivably, this system would make it possible that the SNAREs are active only in the site where membrane docking occurs and ensure that promiscuous SNARE complex formation would not lead to uncontrolled membrane fusion. Moreover, since it has been postulated that viral proteins form fusion pores through the formation of oligomeric complexes, in an analogous manner EEA1 might co-operate with syntaxin13 to form a fusion pore (3). Clearly, further work is required in order to establish whether EEA1 functions only in vesicle tethering or whether it also participates directly in membrane fusion. Such studies will ultimately require the development of assays that reconstitute endocytic docking and fusion using purified components (21).

## 7. Conservation of the endocytic membrane fusion machinery

The general membrane trafficking regulators NSF, SNAP, and Rab GDI are all structurally and functionally conserved between mammals and yeast (69). The same is the case with Rab5 (70), whose yeast homologue, Vps21p/Ypt51p, is involved in vacuolar protein sorting and endocytic membrane traffic (71-73). Interestingly, Vps21p is found in the class D subclass of Vps proteins, which include the Rabex5 homologue, Vps9p (74), the PI 3-kinase, Vps34p (51), and the possible EEA1 homologue, Vac1p/Vps19p (71). Members of this subclass are thought to regulate the docking of Golgi-derived or endocytic vesicles with endosomes. Moreover, the FYVE finger of Vac1p/Vps19p is essential for its function, and this protein interacts directly with Vps21p (75, 76). Vac1p/Vps19p can also be found in a complex with the possible syntaxin13 homologue, Pep12p (77). It thus appears that endocytic membrane fusion in yeast and mammals employs similar molecular machinery. However, so far, early endocytic membrane fusion has not been studied in detail in yeast, and there may still be important differences between yeast and mammalian endosome fusion. For instance, no Rabaptin-5 homologue has been found in the yeast *Saccharomyces cerevisiae* and, interestingly, this yeast also lacks a Rab4 homologue. Furthermore, while Vps21p is involved in Golgi to endosome traffic, it remains to be established whether Rab5 regulates such traffic. Given that syntaxin6, which interacts with EEA1, has been implicated in *trans*-Golgi network to endosome trafficking (25), the latter issue deserves further experimentation.

The fact that the machinery of early-endocytic membrane fusion appears to be

fairly well conserved from yeast to humans raises the question: How general are the mechanisms involved? Studies of homotypic vacuole fusion in yeast (see Section 2) have provided important clues. First, vacuole membrane docking requires a Rab GTPase, Ypt7, whose presence on both membranes is essential for fusion (78, 79). Secondly, homologues of NSF and SNAP are required at a pre-fusion step (80). Thirdly, *trans*-SNARE-pairing is required for fusion (18, 81). Similarly, Rab7 and SNARE proteins have been found to be essential for late endocytic trafficking in mammalian cells (82, 83). This conservation is by no means limited to the endocytic pathway. It is well established that exocytic membrane fusion and intra-Golgi traffic also require the participation of both Rab and SNARE proteins as well as a complex of cytosolic proteins, many of which contain coiled-coil domains (84–86). Thus, general principles emerge from the studies of intracellular transport in various steps (biosynthetic and endocytic pathways) and experimental systems, namely that distinct trafficking routes are based on common molecular mechanisms for membrane docking and fusion.

## 8. Conclusion

Current evidence indicates that membrane fusion in the early endocytic pathway is regulated by a protein complex whose recruitment to specific membrane domains is ensured by the GTPase Rab5 and the 3'-phosphoinositide, PtdIns(3)P. EEA1, which is central in this complex, mediates membrane tethering and possibly plays a role in membrane fusion in concert with syntaxin13 and NSF/SNAP. This conserved molecular machinery confers a spatial and temporal control of early endocytic membrane docking and fusion.

The molecular machinery that causes early-endocytic membrane fusion consists of general components, such as NSF and SNAP, as well as unique endocytic components such as Rab5, syntaxin13, and EEA1. While the specific recruitment of EEA1 to early endosomes is ensured by its binding to Rab5:GTP and PtdIns(3)P, prior to its interaction with syntaxin13, it is not known how Rab5 and syntaxin13 become targeted to early endosomes. The identification of the molecules involved remains a major task. It also remains to be established which v-SNARE(s) interact(s) with syntaxin13 on early endosomes.

Even though we know most of the key molecules in early-endocytic membrane fusion, the exact mechanism of the fusion process remains enigmatic. Further progress will depend on the successful reconstitution of endocytic membrane fusion *in vitro*, starting from purified components.

## Acknowledgements

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