Modular exchange of tether factors differentiates the routes of protein trafficking from endosomes

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The endo-lysosomal system forms a complex network of interconnected organelles. How proteins migrate between these compartments is poorly understood. Schindler et al. have added a new piece to the puzzle by identifying a novel complex of tether factors that operates in the recycling of endosomal proteins towards the cell surface.

A multitude of endosomal subtypes exist that participate in endocytosis, recycling to the cell surface, exocytosis and in protein degradation [1] (Fig. 1). An analysis of the mechanisms of protein transfer between these organelles is complicated by the fact that endosomes and lysosomes are very dynamic and motile organelles that can fuse with each other and fragment. Furthermore, a single endosome can be organized in membrane domains that are continuous with each other but host distinct sets of proteins, e.g. different Rab-proteins [2]. Endosomes can also gradually change their character, as exemplified by the transformation of early into late endosomes. This "maturation" coincides with an exchange of the Rab-GTPases and of Rab-effector proteins on this organelle [3].

An important class of Rab-effectors consists of the multi-subunit tethering complexes (MTCs), which promote docking of two compartments, SNARE complex assembly and the

subsequent membrane fusion process [4-7]. Two independent studies by groups of Juan Bonifacino and Sean Munro have now discovered a novel MTC that they termed EARP (Endosome-Associated Recycling Protein) or GARPII, respectively. EARP/GARPII is structurally similar to the previously described GARP (Golgi-Associated Retrograde Protein) complex, sharing three of the four subunits of this MTC: Vps51/Ang2, Vps52, and Vps53 (Fig. 2A). In EARP, the fourth subunit Vps54 is replaced by a novel factor termed syndetin or Vps54L. This subunit exchange alters the localization of the complex. Vps54 localizes GARP to the trans-Golgi network while syndetin localizes EARP to Rab4-positive recycling endosomes. Syndetin could thus offer a novel tool to specifically interfere with fusion reactions on recycling endosomes. This may help to elucidate by which means endosomes exchange proteins for recycling them to the cell surface.

The mechanisms behind protein trafficking within the endosomal system are poorly understood. On the one hand, endosomes appear to mature by specific addition and/or subtraction of proteins from them, which implies vesicular trafficking reactions. This is exemplified by the maturation of early into late endosomes during which Rab5 is exchanged against Rab7 and the tether complex CORVET is transformed into the related HOPS complex [8, 9]. Transient fusion followed by re-fission of two organelles ("kiss-and-run") can also be observed in the endo-lysosomal system [10]. On the other hand, endosomes can be organized into distinct but continuous domains, characterized by the enrichment of specific Rab proteins [2]. A Rab domain may separate from the rest of the endosome by a fission event, as observed by Gruenberg and Stenmark for Rab7-containing structures that departed from Rab5-positive endosomes [11]. These endosomal carrier vesicles (ECVs) were proposed to serve as transporters of cargo to a stable late endosomal compartment. It is poorly understood how such carriers are detached, how cargo might be sorted into them and finally delivered. Similar events might take place during the transport of endocytosed proteins from early to recycling endosomes. One can assume that fusion of similar carriers with the recycling endosomes might depend on EARP. Inactivation of syndetin might then be a means to accumulate them, which should help to characterize these structures and elucidate their mechanism of formation.

Another interesting aspect highlighted by the work of the Bonifacino and Munro labs is the apparent modularity of multi-subunit tethering complexes. GARP and EARP share a set of core subunits and differ by only one protein that recruits the complexes to different

compartments and allows them to interact with different Rab proteins (Fig. 2A). This mode of organization recapitulates a paradigm revealed by studies of the endosomal CORVET and the HOPS complexes, which both consist of six subunits (Fig. 2B). The core of both complexes consists of four common subunits, Vps11, Vps18, Vps16 and Vps33. This core binds to two alternative sets of further subunits that define the specificity of the complex. In HOPS, the addition of the Vps39 and Vps41 subunits allows the complex to function at the interface between late endosomes and lysosomal/vacuoles membrane through Rab7/Ypt7 binding, whereas addition of Vps3 and Vps8 (in the place of Vps39 and Vps41) allows CORVET to function with Rab5/Vps21 on early endosomal membranes [12]. Similarly, another MTC, TRAPP, exists in three forms. They all interact with the same Rab-GTPase (Ypt1/Rab1) but function with it at distinct sites in ER-Golgi traffic, intra-Golgi-traffic, endosome-Golgi-traffic and autophagy [5]. Thus, modular variation of MTCs could be a general principle for organizing vesicular traffic. It could help to resolve the large discrepancy between the number of Rab-GTPases, of which 60 are known in metazoans, and the number of MTCs, which count only 10 so far. Rab-GTPases are assumed to be specific for a given trafficking route and to interact in this function with an assorted tethering complex. If true we should expect that many more MTCs should exist. Modularity might provide an economical way of using a limited number of subunits to generate a multitude of MTCs with specificities for distinct Rab proteins. Alternatively, an individual MTC might operate with several different Rab-proteins in different trafficking reactions. Since MTCs enhance the specificity of SNARE complex formation [6], modular MTCs could also explain how the many SNARE proteins in the endo-lysosomal system can be recruited into a number of distinct complexes that show an overlapping but non-identical composition of SNARE subunits. Then, we should expect that numerous MTC modules may have remained undetected so far, perhaps because their sequence diverges much more than their structure. Their great divergence at the primary sequence level has initially also prevented the community to recognize the similarities between different MTCs, which only became apparent once their structures had been solved [4].

The complexities of the endo-lysosomal system are puzzling and we remain far from understanding how the identity of these compartments is defined and maintained and how the exchange of material between them is organized on a molecular level. Further identification of trafficking components determining the recruitment of cargo and the directionality of the trafficking routes between them will help to eventually resolve these

questions and gain comprehensive insight into the functioning of this family of compartments.

References

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Figure legends

Fig. 1: Distribution of known multisubunit tether complexes (MTCs) over organelles in the endo- and exocytic pathways.

Fig. 2. Modularity of MTCs.
Subunit composition and Rab-protein interactions of A) GARP, EARP and B) CORVET and HOPS

Figure 2

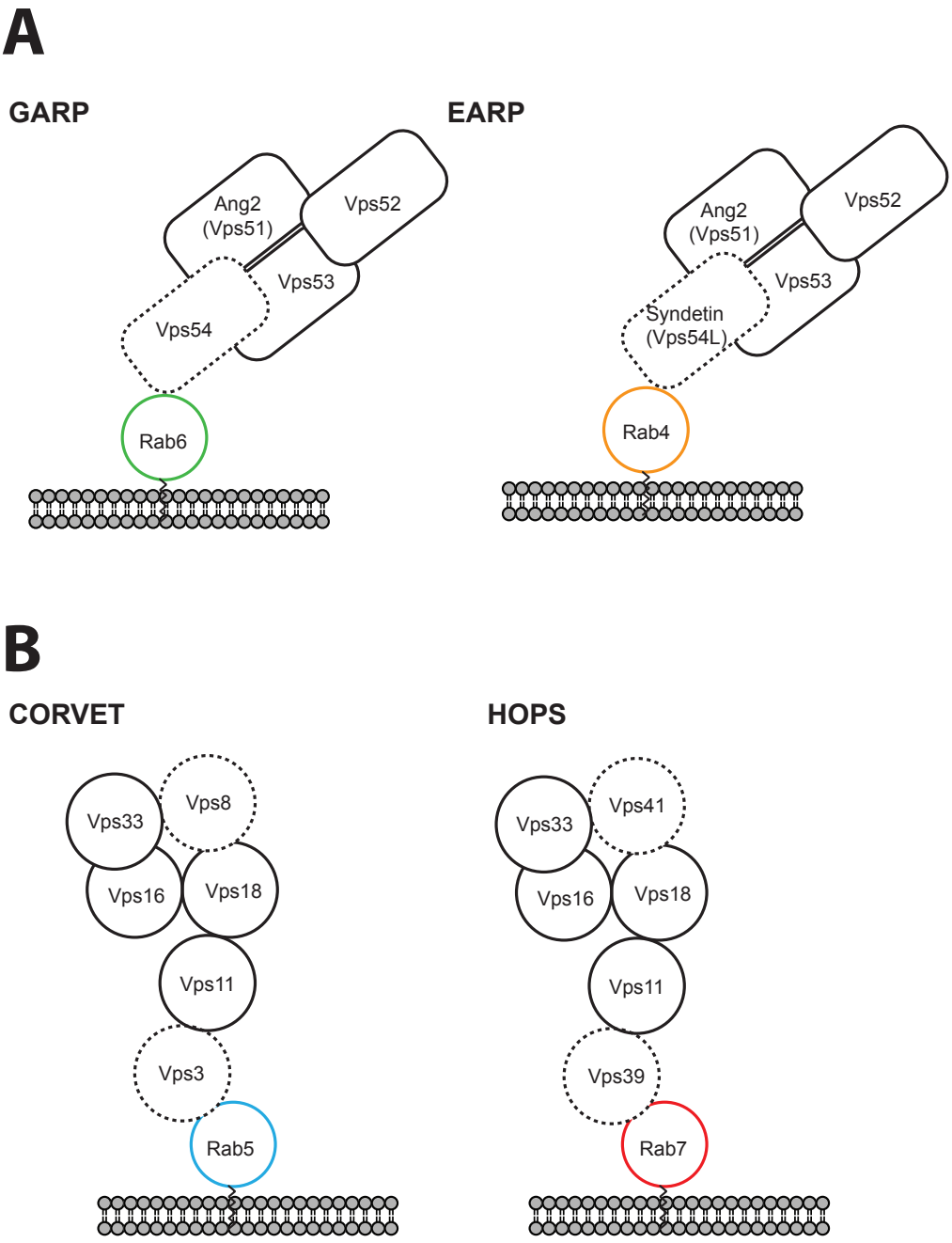
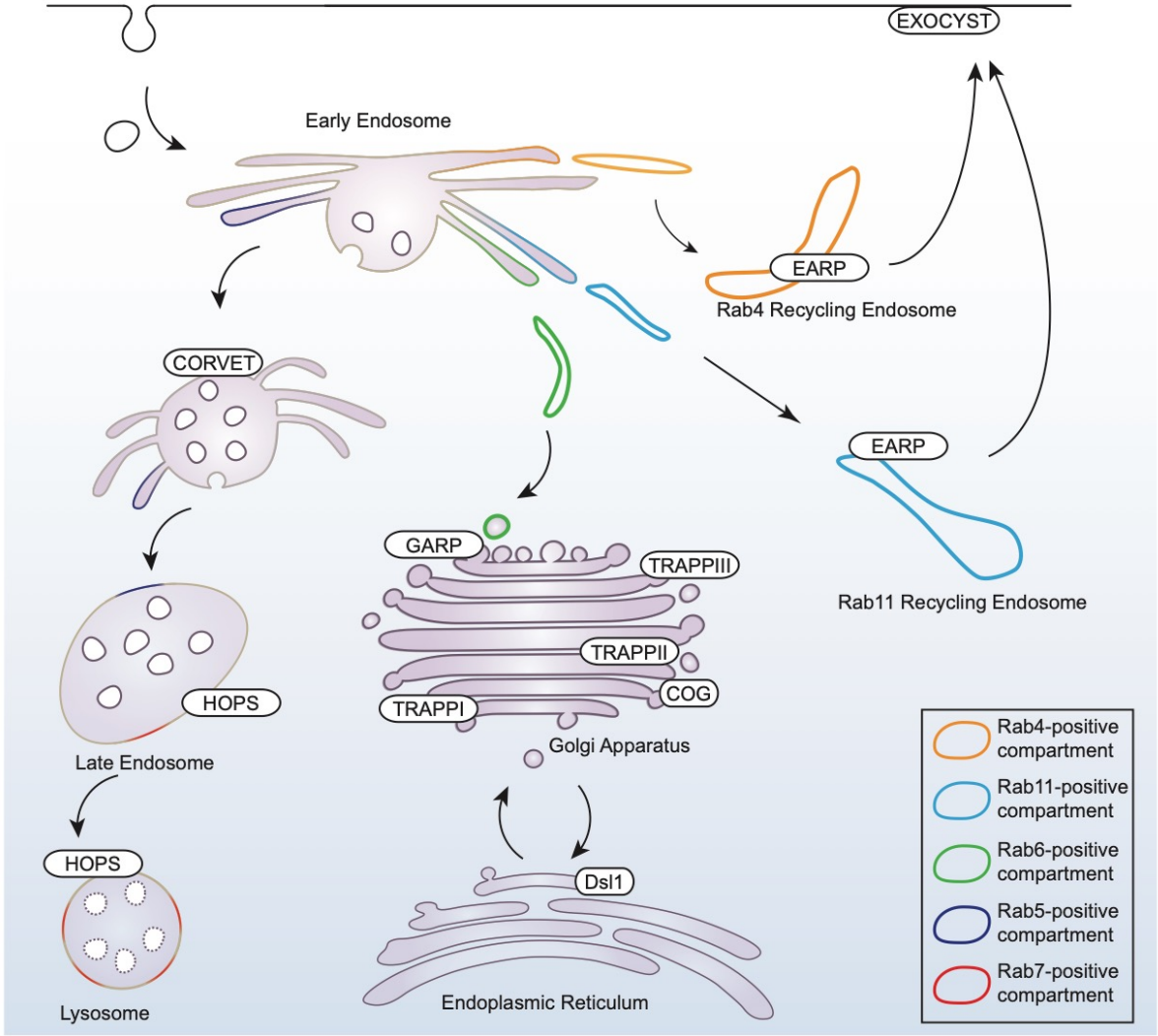

A
GARP
Ang2
(Vps51)
Vps52
Vps53
Vps54
Rab6
EARP
Ang2
(Vps51)
Vps52
Vps53
Syndetin
(Vps54L)
Rab4
B
CORVET
Vps33
Vps8
Vps16
Vps18
Vps11
Vps3
Rab5
HOPS
Vps33
Vps41
Vps16
Vps18
Vps11
Vps39
Rab7

Figure 1


EXOCYST
Early Endosome
EARP
Rab4 Recycling Endosome
CORVET
EARP
Rab11 Recycling Endosome
GARP
TRAPPIII
TRAPPII
COG
TRAPPI
Golgi Apparatus
HOPS
Late Endosome
HOPS
Lysosome
Dsl1
Endoplasmic Reticulum
Rab4-positive compartment
Rab11-positive compartment
Rab6-positive compartment
Rab5-positive compartment
Rab7-positive compartment