Membrane traffic: trans-Golgi tethers leave a surprisingly small GAP

Francis A. Barr

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU.

Email: francis.barr@bioch.ox.ac.uk

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Activation and inactivation of Rab GTPases by GEFs and GAPs promotes or terminates vesicle tethering to organelles, respectively. This simple model is challenged by new evidence that a catalytically inactive Rab GAP promotes rather than terminates vesicle tethering at the trans-Golgi.

In eukaryotic cells, membrane organelles are connected by selective vesicle-mediated transport. Once formed, these vesicles must identify and then fuse with the correct target organelle. The initial identification process is possible because organelle membranes are encoded with specific signals facing the cytoplasm [1, 2]. In the simplest model, these are phosphatidyl-inositol phosphates (PIPs) and Rab and Arl family GTPases (Figure 1a). Such signals are decoded by a heterologous group of protein complexes, generally termed tethering factors due to their role in linking different membrane surfaces [3]. In this way, tethers contribute to the specificity of vesicular transport (Figure 1b). It is commonly believed that form follows function. In the area of molecular and cellular biology, this is often taken to mean that conserved protein domains have conserved or related functions. Recent work on the mechanism of trans-Golgi tethering [4] shows how tenuous this assumption can be in reality. Munro and colleagues show that the Rab GAP TBC1D23 (Tre2-Bub2-Cdc16 domain containing family member 23), a member of a family of proteins that would be expected to negatively regulate tethering, in fact promotes vesicle tethering. This startling, yet certain, conclusion is supported by a number of lines of evidence that build on earlier work.

Previous studies have determined that golgin family tethers provide crucial determinants for the specificity of vesicle tethering to different subcompartments of the Golgi [5, 6]. For the golgin-97/245 subfamily of tethers, two key properties are necessary. First, a conserved C-terminal GRIP domain interacts with the active form

of the GTPase Arl1. This anchors the tether at the trans-Golgi [7, 8], defining the target organelle. Second, a conserved N-terminal short-linear motif F++L embedded in a basic lysine-rich sequence [9], defines the target vesicle. Shin et al [4] use this information to design a strategy to identify the vesicle population captured by golgin-97. Because vesicle tethering events are short-lived, simple biochemical pulldowns often fail to capture the transient functional interactions between proteins on the vesicle and target organelle surface. To circumvent this issue, Shin et al [4] use an in vivo biotinylation approach to tag proteins coming into close proximity of the golgin-97/245 F++L vesicle capture signal.

This method identified two key classes of proteins – transmembrane vesicle cargo proteins and cytoplasmic proteins that could contribute to tethering. The cargo molecules carboxypeptidase D (CPD) and the cation-independent mannose-6-phosphate receptor (CI-MPR) indicate that the target vesicle population is endosome-derived. In terms of potential tethering factors, the major validated partner was TBC1D23, a member of a large family of proteins containing a domain shown to have specific Rab GAP activity. However, as Shin et al note [4], the TBC1D23 GAP domain lacks key conserved residues implicated in GAP-promoted GTP hydrolysis [10]. This view is supported by other contemporaneous work reporting that TBC1D23 has no detectable GAP activity to a panel of 55 human Rabs [11]. Further complicating this picture, TBC1D23 has not one, but two potential enzyme domains. A TBC1 Rab GAP domain and a rhodanese homology domain associated with either sulphurtransferase or phosphatase activity. This latter domain does contain the putative active-site cysteine required for enzyme activity. However, the core DCRP motif is similar to that found in MAP-kinase phosphatases, where it forms part of an ERK2 binding domain rather than the catalytic centre.

Many enzymatic protein families have both active and inactive members often termed pseudo-enzymes [12]. A number of these pseudo-enzymes bind a signal related to the substrate seen by active family members, and thereby are proposed to act as regulators or modulators [13]. In this case, one might have assumed that a Rab GAP pseudo-enzyme is acting as an effector molecule binding to the active form of the GTPase, rather than inactivating it by promoting GTP hydrolysis. Remarkably, Shin et al identify a completely different mechanism, whereby the TBC1D23 TBC1-domain binds to the F++L signal at the N-terminus of golgin-97/245 [4]. Therefore, while the identification of a TBC1 Rab GAP domain does indicate a function in membrane traffic, the mechanism by which it acts appears to have nothing to do with this activity.

The authors then turned to the question of how TBC1D23 links to vesicles and extended their proteomics approach for this purpose. This revealed that a C-terminal region of TBC1D23 discrete from either the GAP or rhodanese homology domains interacts with the FAM21A subunit of the WASH (Wiskott-Aldrich Syndrome Protein and SCAR Homolog) complex [4]. Notably, this complex has been linked to the endosomal retromer sorting pathway [14]. Based on the membrane cargo proteins identified [4], this is consistent with the notion that the golgin-97 tethering pathway captures endosome derived vesicles (Figure 1c). This of course raises the question of how WASH is recruited to endosomal vesicles. More generally, this could be seen as a question about the nature of the recognition signal on endosomes or endosome-derived vesicles. As clearly summarised elsewhere, the WASH and retromer complexes form a sorting platform for transmembrane cargo proteins on endosomes [15]. In addition, retromer also interacts with the endolysosomal GTPase Rab7A [16]. This interaction is negatively regulated by the Rab7A GAP TBC1D5 as

one would expect in a canonical tethering cycle [17]. Whether or not WASH can interact with retromer and TBC1D23 simultaneously remains unclear. Both interact with the C-terminal region of FAM21A, so this is a point for further investigation. One possibility raised by these findings is that transmembrane cargo proteins and Rab7A together with retromer and the WASH complex form the landmark signal on endosomes recognised by the golgin-97/TBC1D23 tethering machinery~~, through a combined retromer and WASH assembly~~.

These ideas are not without issues. First, there is the problem of directionality in the system. Does this couple endosome derived vesicles to the trans-Golgi or trans-Golgi derived vesicles to the endosomes? This is a difficult question to answer and relates to precisely when the landmark signals seen by the tethering assembly are generated. For example, if Arl1 is activated on vesicles leaving the trans-Golgi, then the latter possibility would be more likely. Again, this highlights the need to understand the order of interactions between the trans-Golgi golgin-97/TBC1D23 tether and the presumed endosomal WASH/retromer assembly. Some evidence does favour idea that endosome-derived vesicles are being tethered to the Golgi. When golgin-97/245 and TBC1D23 were knocked out, the transmembrane cargo proteins showed altered distribution in vesicles that failed to cluster in the Golgi-region. However, a more critical examination of this evidence would note that if the vesicles cannot tether then they will of course be distributed throughout the cell rather than close to an organelle, be it endosome or trans-Golgi.

Shin et al have defined a new trans-Golgi tethering complex, widely conserved throughout the eukaryotic kingdom, that acts on a trafficking event crucial for transport between the endosomes and the trans-Golgi. Why then do the golgin-97/245 and TBC1D23 knockout cells show such minor defects? Steady state

distributions of some transmembrane proteins of the endocytic pathway are altered, and the levels of at least one, TGN46, is dramatically reduced. One explanation is that there are multiple routes connecting the trans-Golgi and endosomes. There is a known degree of redundancy or overlap in golgin-mediated tethering [6], and unravelling this will require further studies. Furthermore, analysis of SNARE complexes and Rab GTPases indicates that there are two distinct routes for trans-membrane cargo involving other golgins [18]. Again, this needs to be accounted for when ascribing the relationship between tethering complexes and other components of the vesicle transport machinery.

However, like all convenient answers, simple redundancy is likely to be too simplistic a view. Two other recent studies have identified homozygous truncating or splice-site mutations in TBC1D23 in the recessive developmental disorder of the brain, pontocerebellar hypoplasia (PCH) [11, 19]. PCH is typically associated with mutations in genes required for mitochondrial tRNA processing or mitochondrial tRNAs directly [20]. Both these studies provide clear evidence that a variety of mutations lead to loss of the TBC1D23 protein, and effectively a null phenotype [11, 19]. This results in defective neuron positioning and the loss of a subset of neurons and brain structures. Do these studies hint at a general role for catalytically inactive Rab GAPs as membrane tethers? As Marin-Valencia et al note [11], mutations in two other related proteins, TBC1D24 and TBC1DK, also cause brain developmental disorders. While only a correlation, it does suggest a direction for further studies of those proteins and their role in brain development.

Increasingly, we are confronted by problems like the one under discussion here, where even detailed cell biological knowledge of such components and their interactions fails to fully address the problem. Why does an apparently general

defect in the machinery of trafficking between the trans-Golgi and endosomes result in such a specific developmental disorder? Despite a huge leap in understanding, we are still left with important questions like this, about the functions of core cell biological pathways in the context of human development.

Figure Legend

Figure. Tethering complexes decode specific vesicle membrane and organelle surface signals. *a.* Switch-like activation and inactivation of Rab/Arf/Arl GTPases is catalysed by GDP-GTP Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs). The active form of the GTPase (T) promotes membrane tethering together with a second signal, often a specific PI-lipid such as PI3P at endosomes or PI4P at the Golgi. This cycle is thought to play a crucial role in both the timing and specificity of transient, long-range recognition events in vesicular transport. *b*. Canonical tethering complexes link specific vesicles and target organelles by recognition of defined surface signals. Action of the GAP promotes GTP-hydrolysis and returns the GTPase to an inactive state (D), terminating the cycle. *c*. Golgin-97/245 family tethers are recruited to the trans-Golgi by a specific GTPase, Arl1. Rather than terminating or opposing the tethering cycle by triggering GTP hydrolysis, the TBC1D23 Rab GAP makes physical links promoting vesicle tethering. The Rab GAP domain binds to an F++L signal in the golgins and a second C-terminal region interacts with the FAM21A subunit of the WASH complex on endosomal vesicles. Details of retromer and further interactions to RAB7A on the endosome are not shown.

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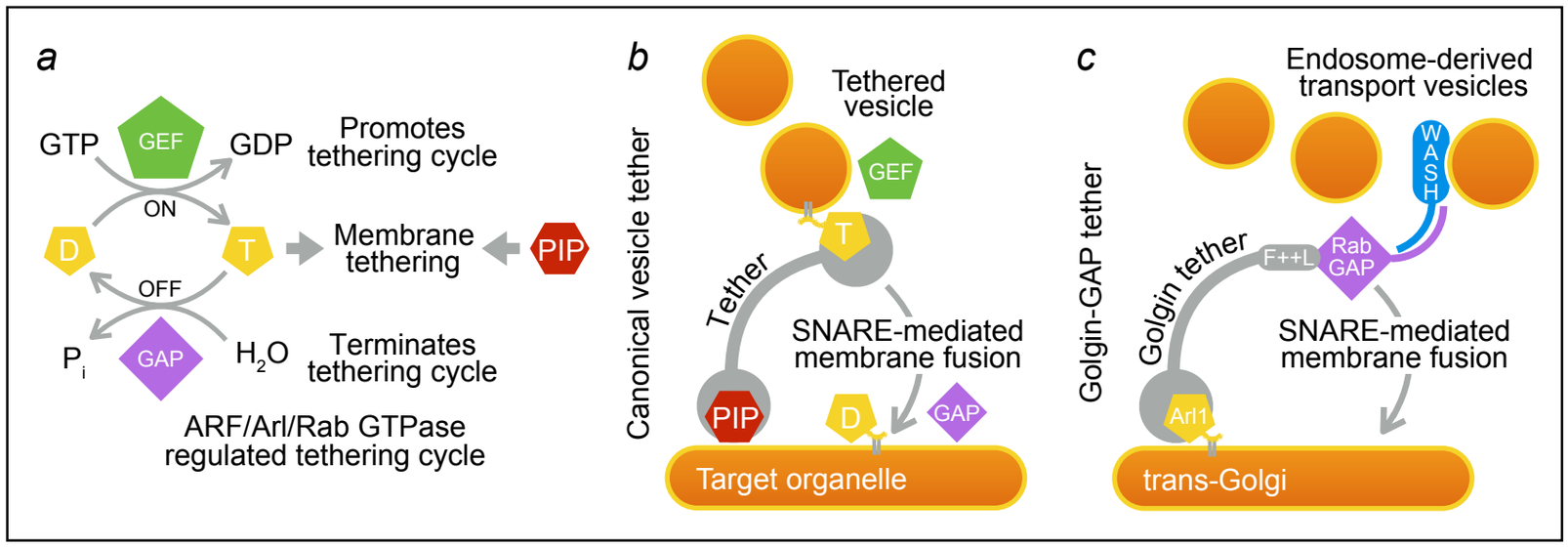
a
GTP
GEF
GDP
Promotes tethering cycle
ON
D
T
Membrane tethering
PIP
OFF
P_i
GAP
H_2O
Terminates tethering cycle
ARF/Arl/Rab GTPase regulated tethering cycle
b
Canonical vesicle tether
Tethered vesicle
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Tether
SNARE-mediated membrane fusion
PIP
D
GAP
Target organelle
c
Endosome-derived transport vesicles
WASH
Golgin tether
F++L
Rab GAP
SNARE-mediated membrane fusion
Golgin-GAP tether
Arl1
trans-Golgi