

# AP-1B: polarized sorting at the endosome

Linton M. Traub and Gerard Apodaca

**Polarized epithelia require selective protein trafficking to establish and preserve distinct apical and basolateral surfaces. Recent work indicates that in polarized cells, the AP-1B clathrin adaptor recognizes certain basolateral targeting signals in an endosomal compartment where it seems to not only capture cargo but also promote recruitment of targeting and fusion machinery, ensuring accurate polarized sorting.**

Polarized epithelial cells are characterized by physically separate apical and basolateral surfaces, each with distinct lipid and protein composition. Once established, cell polarization is maintained by dedicated protein sorting pathways that promote proper delivery of newly synthesized membrane components from the *trans*-Golgi network (TGN) or recycling of internalized proteins back to the appropriate cell surface. Basolateral targeting is often governed by tyrosine- or dileucine-based sorting signals within the cytosolic portion of integral membrane proteins. At least a subset of these basolateral sorting signals are decoded by the epithelial-specific adaptor protein complex AP-1B. New work published in the *Journal of Cell Biology* begins to lift the veil on how AP-1B operates and challenges the idea that export to the basolateral plasma membrane occurs solely from the TGN<sup>1,2</sup>.

AP-1B and the closely related AP-1A complex are members of a heterotetrameric adaptor family devoted to sorting cargo at the plasma membrane and within the cell. Each complex consists of two large subunits ( $\beta 1$  and  $\gamma$ ), a medium subunit ( $\mu 1$ ) and a small ( $\sigma 1$ ) subunit. The  $\beta 1$  subunit is responsible for the interaction with clathrin, whereas the  $\mu 1$  subunit binds to select cargo molecules. Importantly, AP-1A and AP-1B complexes differ only in the  $\mu 1$  subunit, with  $\mu 1B$  roughly 80% identical to  $\mu 1A$ <sup>3</sup>.

Evidence for a discrete role of AP-1B in polarized sorting came from the discovery that LLC-PK1 cells, a polarized kidney cell line that does not express  $\mu 1B$ <sup>3</sup>, mis-sort the low-density lipoprotein receptor (LDLR) to the apical surface but that stable reintroduction of  $\mu 1B$  restores proper sorting of the

LDLR to the basolateral surface<sup>4</sup>. The simplest interpretation of these results is that AP-1B participates in the assembly of clathrin-coated vesicles that transport select cargo directly from the TGN to the basolateral surface. In contrast, the AP-1A complex manages the sorting of newly synthesized lysosomal hydrolases in non-polarized cells. AP-1-containing clathrin-coated structures have long been known to form at the TGN, but because AP-1A and AP-1B are nearly identical it is difficult to envisage spatial segregation of vesicle populations carrying the respective adaptor complexes.

Now, however, Fölsch *et al.* demonstrate that biochemically isolated AP-1B-containing clathrin-coated vesicles show no evidence of AP-1A and *vice versa*<sup>1</sup>. This suggests that the two vesicle populations may assemble on distinct intracellular compartments, an idea supported by the clear segregation of AP-1A from AP-1B in LLC-PK1 cells<sup>1,5</sup>. Using immunoelectron microscopy, Fölsch *et al.* find that AP-1B-containing clathrin coats appear to be spatially separated from the stacked Golgi cisternae and associated AP-1A-coated buds and vesicles. In fact,  $\mu 1B$  colocalizes better with endocytosed transferrin in juxtannuclear recycling endosomes than with authentic TGN markers<sup>6</sup>, and isolated AP-1B-coated vesicles contain the transferrin receptor (TfR)<sup>1</sup>, indicating that AP-1B might operate on transferrin-positive recycling endosomes, a possibility suggested previously<sup>6</sup>.

Accurate basolateral targeting and fusion requires the exocyst, a multiprotein tethering complex composed of eight subunits, including Sec6, Sec8 and Exo70. In polarized epithelia, a pool of the exocyst is found on the lateral plasma membrane, in close proximity to the tight junctions (demarcating sites for vesicle fusion with the basolateral surface). Now, Fölsch *et al.* report that the exocyst components Sec8 and Exo70 also cluster with AP-1B in perinuclear, transferrin-positive structures<sup>1</sup>. AP-1B is needed to generate exocyst docking sites at this perinuclear location, because the exocyst is not similarly distributed in LLC-PK1 cells lacking AP-1B but expressing AP-1A. Intriguingly, exocyst components also cofractionate with AP-1B-containing clathrin-coated

vesicles, raising the possibility that the exocyst may be necessary for the proper deposition of cargo at the basolateral surface. The connection to the exocyst is strengthened in a companion study where Rab8, a small GTPase believed to promote membrane targeting of the exocyst complex, also partially localizes with AP-1B to a transferrin-positive perinuclear endocytic compartment<sup>2</sup>. Overexpression of an activated Rab8 mutant (Rab8<sup>Q67L</sup>) in polarized MDCK cells results in selective mis-sorting of AP-1B cargo (LDLR and the vesicular stomatitis virus G-protein (VSV-G)) and perturbs the intracellular distribution of AP-1B, while leaving AP-1A adaptors unaffected<sup>2</sup>.

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The localization of AP-1B to recycling endosomes or a potentially related compartment has broad implications for sorting of newly synthesized cargo in polarized epithelial cells. Consistent with their findings, and with earlier reports<sup>7,8</sup>, Fölsch *et al.* propose that newly-synthesized AP-1B-dependent cargo such as VSV-G move rapidly and directly from the TGN to recycling endosomes and, subsequently, interact with AP-1B and associated basolateral targeting machinery such as the exocyst (Fig. 1a)<sup>1</sup>. However, an important experimental caveat that remains to be addressed is whether VSV-G transits directly from the TGN to the recycling endosome, rather than after delivery to and internalization from the plasma membrane. The function of Rab8 in the delivery process is unknown, but it could regulate the

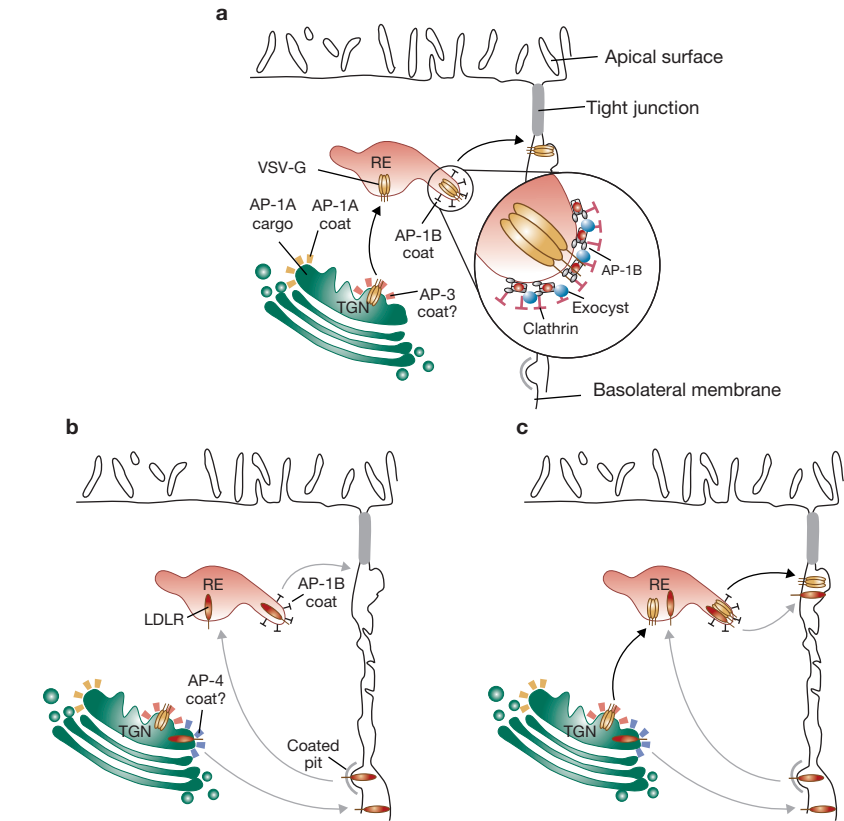
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association of the exocyst with forming basolateral vesicles. It is also unknown whether transit of cargo from the TGN to recycling endosomes occurs by default or is signal mediated, or whether all exocytic cargo (that is, both apical and basolateral) is directed to recycling endosomes for ultimate signal decoding. The latter would be a significant paradigm shift from the current model that many, but not all, epithelial cells use direct TGN-to-cell-surface pathways to maintain cell surface polarity of newly synthesized proteins. In addition, it would indicate that as in hepatocytes, most membrane protein sorting occurs within the endosome.

A different model has been proposed previously by Gan *et al.* (Fig. 1b)<sup>6</sup>, who made the surprising observation that in LLC-PK1 cells, newly synthesized LDLRs are transported directly from the TGN to the basolateral surface and that this transport is not dependent on AP-1B. Instead, their data indicate that AP-1B functions primarily by driving recycling from endosomes. A third possibility to account for these disparate results is a hybrid model in which both direct and indirect pathways occur, depending on the cargo molecule involved (Fig. 1c). Although seemingly non-parsimonious, there are multiple pathways for delivery to both lysosomes and the TGN, and it is therefore possible that different cargo could be targeted to recycling endosomes via different pathways. Notwithstanding these differences, it is now clear that AP-1B operates at an endosomal site but, ultimately, what is the biological utility of sorting from the recycling endosome? For those basolateral membrane proteins that are endocytosed, only a small fraction of their life is spent moving from the TGN to the cell surface. The majority of time is spent cycling between the cell surface and recycling endosomes. Localizing AP-1B to these endosomes ensures the steady-state maintenance of cell surface polarity.

The data from Gan *et al.* also suggests that a sorting complex(s) other than AP-1B functions at the TGN to specify basolateral sorting. Other mechanisms for targeting basolaterally destined proteins most probably exist, as hepatocytes sort essentially all membrane proteins to the basolateral surface but do not express  $\mu 1B^3$ , and proteins that contain dileucine-like targeting motifs are properly sorted to the basolateral surface of LLC-PK1 cells<sup>9</sup>. Intriguingly, the AP-4 complex is localized to the TGN and endosomes and can bind to isolated basolateral sorting signals<sup>10</sup>. Attenuating AP-4 expression disrupts basolateral localization of several proteins<sup>10</sup>. Finally, AP-3 may be required for exit of the basolateral marker VSV-G from the TGN of non-polarized HeLa



**Figure 1** Potential trafficking pathways for polarized sorting of AP-1B-dependent cargo. (a) Direct model for AP-1B sorting. Newly synthesized AP-1B cargo (for example, VSV-G) transits directly from the TGN to recycling endosomes (RE). In this model, it is unknown whether exit of cargo from the TGN occurs by default or is dependent on specific coat protein complexes. In non-polarized cells there is evidence that AP-3 may be required for VSV-G export from the TGN<sup>11</sup>. At the point of entry into recycling endosomes, VSV-G enters clathrin-coated regions containing AP-1B, thus sorting it away from molecules targeted for other cellular destinations. Vesicle components could also include the exocyst complex and other regulatory proteins such as Rab GTPases (not shown), to ensure correct targeting of the cargo to the basolateral membrane. (b) Indirect model for AP-1B-dependent sorting. Newly synthesized AP-1B cargo (for example, LDLR) is transported directly from the TGN to the basolateral plasma membrane. After endocytosis, the LDLR is transported to recycling endosomes where AP-1B-dependent sorting and formation of basolateral transport vesicles occurs. In this model the newly synthesized LDLR is sorted first in the biosynthetic pathway, possibly by the AP-4 coat complex<sup>10</sup>. The normally basolateral steady-state distribution of this protein depends on AP-1B-dependent sorting events that subsequently occur in recycling endosomes. (c) Hybrid model showing that cargo may take either direct or indirect routes to recycling endosomes, where AP-1B-dependent sorting occurs.

cells<sup>11</sup>, possibly indicating that this adaptor might also be involved in polarized sorting.

The new work also raises several questions about AP-1B function and localization. Given the close homology between  $\mu 1A$  and  $\mu 1B$ , one pressing question concerns how the specificity of AP-1B for basolateral cargo is achieved. The simplest model is that similarly to the  $\mu 2$  subunit of the plasma-membrane-associated AP-2 complex,  $\mu 1B$  physically binds short linear sorting determinants within the cytosolic domain of associated cargo. The

conundrum is that basolateral sorting signals are widely divergent. The basolateral sorting signal of VSV-G closely mimics the YXX $\emptyset$  motifs (where X is any amino acid and  $\emptyset$  represents a residue with a bulky hydrophobic side chain)<sup>12</sup> found in endocytosed cargo. However, other AP-1B cargo (such as the LDLR) contain sorting signals with only superficial resemblance to YXX $\emptyset$  motif, and the sorting signal in the TfR (also a basolaterally-targeted protein) is completely unrelated to YXX $\emptyset$ . An obvious possibility is that signal

recognition is mediated by more than one surface of  $\mu 1B$ . Alternatively, as there is no evidence at present for binding of AP-1B to basolateral sorting signals directly, intermediary cargo-recognition proteins may exist which, in turn, bind to AP-1B.

A related question concerns how AP-1A and AP-1B are recruited to distinct membranes given that they are virtually identical. In the case of AP-1A, translocation onto membranes is regulated by phosphoinositides and the small GTPase ARF1, although ARF1 probably drives membrane recruitment of AP-1B too. Apart from cargo, the only specific binding partners of the  $\mu$  subunits known

are the synaptotagmins, so it is conceivable that differentially positioned synaptotagmin isoforms underlie compartmental segregation of AP-1A and AP-1B. Alternatively,  $\mu 1B$  may differentially bind membrane lipids at the target organelle. Future experiments will undoubtedly provide answers to many of the questions raised, including how all basolateral sorting signals are decoded by protein complexes such as AP-1B. □

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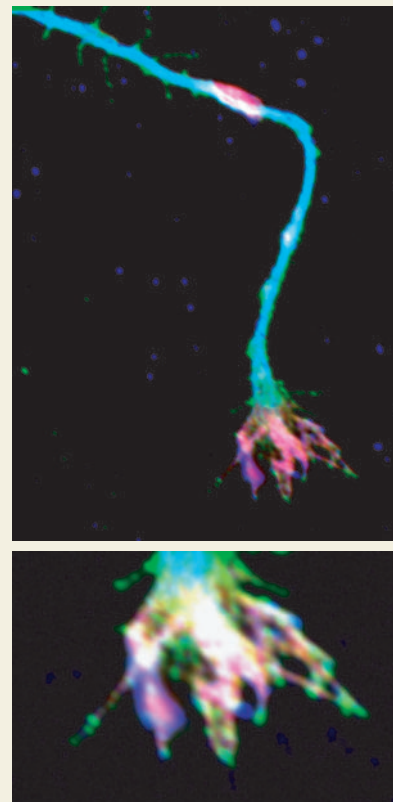
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## A filopodial synapse?

The growth cone tip of a developing axon is a motile structure, rich in finger-like filopodial protrusions and specialized for sensing the local environment during axon pathfinding. Now, a study by Sabo and McAllister (*Nature Neurosci.* **6**, 1264–1269 (2003)) reveals that growth cone filopodia of cortical neurons may also be localized sources of diffusible signals, including neurotransmitters. Using live imaging of a green fluorescent protein (GFP)-tagged version of the synaptic vesicle protein VAMP2, the authors find that filopodia contain motile vesicles (green punctae in accompanying image) which resemble synaptic vesicles by their protein content. Motility of these vesicles, which is likely to be tubulin-based (shown in blue), is observed in both directions within filopodia, and some vesicles are seen to change directions rapidly throughout the length of the filopodia.

Localized stimulation of the axon results in release of the vesicle contents by fusion with the filopodial plasma membrane, as assayed by the vesicle marker dye, FM 4-64. This is also likely to result in release of the neurotransmitter, glutamate, as the vesicles contain the necessary glutamate transporter. Given this seems to be an early form of synaptic release that most probably occurs before the assembly of complete presynaptic specializations, it could be an important step in early synaptogenesis. Glutamate is known to have effects on the motility of both dendritic and axonal filopodia; thus, filopodia could self-regulate their own motility and that of neighbouring dendrites. Another intriguing possibility is that activity-induced release of vesicles could change the molecular make-up of the filopodial membrane.

Whether these processes are involved in axon guidance or early synapse formation remains to be shown. At mature synapses, the presynaptic specializations that mediate vesicle release are a complex network of protein complexes. Growth cone filopodia are relatively short-lived (on the scale of minutes), and may function as a good model for the early steps of presynaptic specialization and synaptogenesis. Finding ways to disrupt this prototype of synaptic release will be the first step in investigating its functional relevance for development of the nervous system.



An immunofluorescence microscopy image of the growth cone of a cortical neuron axon triple labelled for VAMP2 (green), tubulin (blue) and actin (red). Filopodial vesicles containing VAMP2 can be seen as green punctae in the close-up of the growth cone shown in the bottom panel.

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