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Correspondence

Response: The "Tail" of the Twin Adaptors

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In this issue of *Developmental Cell*, a Matters Arising by Juan Bonifacino's laboratory (Guo et al., 2013) addresses an emerging topic in epithelial cell biology, the complementary roles of the clathrin adaptors AP-1A and AP-1B in basolateral trafficking in epithelia. AP-1A and AP-1B are twin tetrameric clathrin adaptors; AP-1A is ubiquitous, whereas AP-1B is expressed only by epithelial cells. They share three subunits (β1, σ1, and γ) but differ in the possession of different (albeit 80% homologous) medium subunits (μ1A and μ1B). The role of AP-1B in basolateral trafficking was established over a decade ago (Fölsch et al., 1999), whereas the participation of AP-1A in basolateral trafficking was only demonstrated last year, in two collaborative papers between Bonifacino's laboratory and our laboratory, one of them in *Developmental Cell* (Carvajal-Gonzalez et al., 2012; Gravotta et al., 2012). Therefore, the details of how they complement each other in basolateral sorting are still unclear. Guo et al. now postulate a model in which the two adaptors are paralogs with identical localization and function that differ in the repertoire of proteins that they can sort.

Because of the extreme structural similarity of AP-1A and AP-1B, a vexing problem in the field has been obtaining reliable data on the localization of these adaptors. Guo et al. address this problem by attaching three Myc or HA tags or single copies of GFP or mCherry, separated by a flexible linker, to the C-terminal tails of μ1A and μ1B. This approach is not without risk, because the C termini are the exposed, cargo-binding regions of the medium subunits. Nonetheless, the tagged subunits were incorporated into AP-1 (as demonstrated by coprecipitation and colocalization with γ-adaptin), and transfection of tagged μ1B was able to restore basolateral localization of low density lipoprotein receptor (LDLR) in LLC-PK1 cells, an epithelial cell line that lacks AP-1B. Results in both fixed-cell and live-imaging assays clearly showed that C-terminally tagged μ1A and μ1B fully colocalize with each other, colocalize to the same extent with *trans*-Golgi network (TGN) and early endosomal markers, and bind to the membrane through the same ARF proteins. Interestingly, yeast two-hybrid assays with a panel of basolateral sorting signals showed that some basolateral proteins interact better with μ1B than with μ1A, some interact well with both adaptors, and one interacts better with μ1A. Basolateral proteins of the first group (an example is LDLR) are more prone to become depolarized in the absence of AP-1B. Because μ1B is expressed only by epithelial cells, whereas μ1A is ubiquitous, Guo et al. hypothesize that AP-1B confers epithelial cells with the evolutionarily advantageous capability to sort a larger number of basolateral proteins. The authors propose that, otherwise, the adaptors carry out similar sorting functions at both TGN and endosomes (Figure 1A).

Are the twin adaptors functionally identical? Some published evidence illustrates overlapping functions. An early study by Schu and coworkers indeed showed that transfection of μ1B into fibroblasts obtained from μ1A-knockout mice restored retrograde transport of mannose 6-phosphate receptor from early endosomes to TGN (Eskelinen et al., 2002). Furthermore, live-imaging assays in one of last year's papers demonstrated that LDLR exits the Golgi apparatus of Madin-Darby canine kidney (MDCK) cells (an epithelial cell line that expresses both adaptors) equally well upon RNA silencing of μ1A or μ1B, but its Golgi exit is blocked upon knockdown of both adaptors (Gravotta et al., 2012). However, there is also considerable evidence showing that AP-1A and AP-1B perform distinct functions. AP-1B cannot substitute for AP-1A in the retrieval of furin from endosomes to the TGN (Fölsch et al., 2001). Expression of AP-1B but not of AP-1A enhanced the recruitment of exocyst subunits to the perinuclear region of the cell (Fölsch et al., 2003). Furthermore, a variety of sorting assays in MDCK cells support the idea that the two adaptors carry out overlapping but distinct sorting functions in the biosynthetic and recycling routes of basolateral PM proteins. RNA silencing of AP-1B in MDCK cells did not disrupt the biosynthetic sorting of LDLR, transferrin receptor (TfR), or Coxsackie adenovirus receptor (CAR) but, rather, their postendocytic recycling (Diaz et al., 2009; Gravotta et al., 2012; and references therein). These results indicate that endogenous AP-1A cannot substitute for AP-1B in the recycling pathway of these proteins, even when TfR and CAR bind μ1A or μ1B equally well by yeast two-hybrid assay. On the other hand, the biosynthetic route of those proteins was disrupted by the simultaneous RNA silencing of both adaptors, suggesting that both AP-1A and AP-1B cooperate in that pathway. Importantly, knockdown of AP-1A in MDCK cells did not disrupt the polarity of six basolateral PM proteins, which contrasted with the strong depolarization caused by AP-1B knockdown (Gravotta et al., 2012). These results support a model in which AP-1A and AP-1B have asymmetric roles in basolateral sorting (Figure 1B). According to this model, AP-1B controls basolateral trafficking at TGN and recycling endosomes in both biosynthetic and recycling routes, whereas AP-1A operates an alternative biosynthetic route from TGN to the basolateral membrane.

The conclusion by Guo et al. that AP-1B expands the repertoire of basolateral signals recognized by epithelial cells is important. It suggests a possible explanation for the evolutionary appearance of this adaptor. Possession of two basolateral sorting adaptors confers epithelial cells with the flexibility to regulate the polarity of defined subsets of basolateral

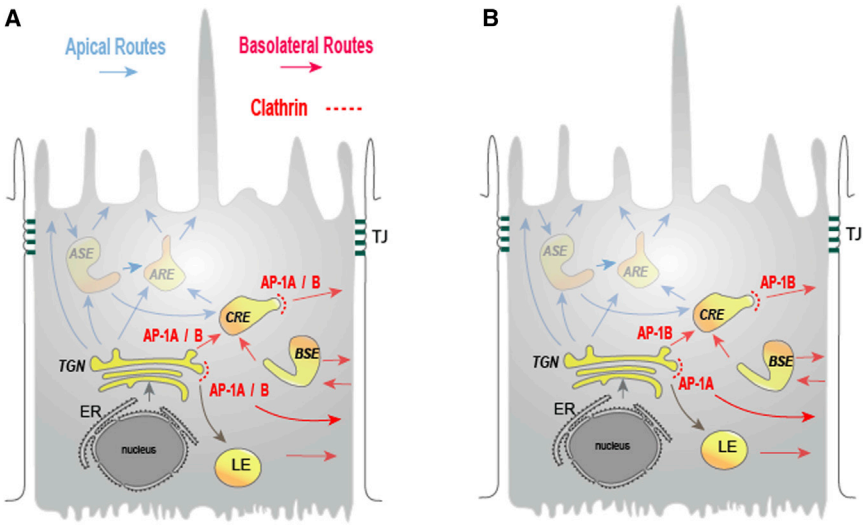

Figure 1. Complementary Roles of AP-1A and AP-1B in Basolateral Protein Sorting
(A) Based on colocalization, biochemical data, and yeast two-hybrid data, Guo et al. propose that AP-1A and AP-1B carry out similar basolateral sorting functions at both the TGN and common recycling endosome (CRE) and differ in their ability to interact with different sets of basolateral sorting signals.
(B) Sorting assays suggest a model in which the twin adaptors have distinct but partially overlapping sorting functions. AP-1B mediates the postendocytic recycling of basolateral PM proteins internalized via basal sorting endosomes (BSEs) and CREs, whereas both AP-1A and AP-1B cooperate in the biosynthetic route, mediating exit of basolateral proteins from the TGN probably into different routes to the plasma membrane. AP-1A also mediates transport of lysosomal hydrolases from TGN to late endosomes, likely in cooperation with GGAs.
RE, endoplasmic reticulum; TGN, *trans*-Golgi network; ASE, apical sorting endosomes; ARE, apical recycling endosomes; BSE, basal sorting endosomes; CRE, common recycling endosomes; LE, late endosomes.

proteins. Some native epithelia, e.g., retinal pigment epithelium (Diaz et al., 2009) and kidney proximal tubule (Schreiner et al., 2010), do not express AP-1B and therefore localize basolateral proteins at the apical membrane, where they perform important functions for the host organ. Analysis of the localization and intracellular sorting of a larger panel of basolateral proteins is required to definitively elucidate whether the sorting functions of AP-1B are identical or just overlapping, and to what extent. A note of caution, however, is that although the colocalization results by Guo et al. are very impressive, they were obtained with tagged and overexpressed proteins, which could lead to the loss of subtle differences in the localization of the adaptors. Published evidence suggests that the C terminus of μ1B is necessary for the recruitment of AP-1B to recycling endosomes through an amino acid patch not present in μ1A; recruitment of AP-1B can be blocked by PTEN, presumably through hydrolysis of its substrate $PI(3,4,5)P_3$, a phosphatidyl inositol lipid enriched at recycling endosomes and believed to be required for basolateral trafficking (Fields et al., 2010). As cell biologists further study these twin adaptors, they may be baffled by their sorting identity and localization like a spectator of Shakespeare's *Comedy of Errors* trying to figure out the confounding identities and locations of the twin brothers Antipholus, from Syracuse and Ephesus.

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