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SPOTLIGHT

BEACH domain proteins in membrane trafficking and disease

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Two recent papers by Szentgyörgyi et al. (http://doi.org/10.1083/jcb.202401167) and Pankiv et al. (http://doi.org/10.1083/jcb.202408173) provide new insights into the roles of BEACH domain proteins in membrane trafficking and cellular homeostasis. They explore which membranes they are recruited to, how they are recruited, and the potential coat-like functions of these proteins.

Beige and Chediak-Higashi (BEACH) domain proteins are a family of proteins containing the ~280 amino acid BEACH domain (1). BEACH domain proteins are highly conserved across eukaryotes and have been associated with various rare genetic diseases in humans. There are nine members in the human genome and the seven "typical" members are unusually large, in the top 1% of proteins by molecular weight (Fig. 1).

The large size of BEACH domain proteins makes them challenging to characterize, complicating efforts to clone, express, purify, tag, and visualize. Studies in mouse models, KO cell lines, and human patient tissue have revealed that they are essential for various subcellular processes, with membrane trafficking being a shared theme (1). Despite this clear link between the BEACH domain proteins with membrane trafficking and disease, the fundamental function of this family of proteins has remained elusive.

Among the typical BEACH domain proteins, LPS responsive beige-like anchor protein (LRBA) has garnered significant attention due to its crucial role in T-cell function and immune regulation. LRBA deficiency has been shown to cause T-cell dysfunction and mistargeting of the immune receptor CTLA-4 (4, 5). Intriguingly, loss of either CLTA-4 or LRBA exhibits a similar clinical phenotype. Thus, when an interaction between LRBA and the cytosolic tail of CTLA-4 was identified, the prevailing hypothesis was that LRBA has an essential function in regulating CTLA-4 trafficking (4). Defining the localization and function of LRBA is, therefore, essential for understanding the molecular mechanism of BEACH domain proteins and provide context for these human diseases.

Szentgyörgyi et al. used patient-derived cell lines to demonstrate, in support of previous findings, that LRBA deficiency leads to specific defects in the endolysosomal system (3). Interestingly, Szentgyörgyi and colleagues co-localize LRBA to the Golgi and a range of cytoplasmic endosomes, vesicles, and tubules (Fig. 1 A). Despite being previously associated with RAB11-positive endosomes, Szentgyörgyi et al. found only a modest co-localization with these compartments. Conversely, they refine the endosomal localization of LRBA to mostly RAB4-positive fast-recycling endosomes. This endosomal pool was sensitive to an ARF1/3 double-knockout, and AlphaFold modeling indicated a direct interaction between LRBA and ARFs. Mutation of the predicted binding interface still allows proper ARF1 recruitment to endosomes but impairs LRBA localization. Immunoprecipitation of tagged GTP-locked ARF1 co-immunoprecipitates LRBA. Taken together, this indicates that there are at least two pools of LRBA in the cell, a Golgi-localized and an endosome-localized, and the endosomal pool is recruited in an ARF1/3-dependent manner.

Thus, disruption of LRBA recruitment to endosomes affects normal endolysosomal function, leading to defects in cellular trafficking, including for proteins such as CTLA-4. Proper LRBA regulation is essential for maintaining endolysosome homeostasis, and defects in this pathway may contribute to disorders like immunodeficiency. In addition, this highlights how important it is to define the localization of each BEACH domain protein in the cell.

Through systematic co-localization analysis, Pankiv et al. assign seven BEACH domain family members to the endomembrane system of mammalian cells (2) (Fig. 1 A). Each of the BEACH domain proteins was found to localize to a unique set of endomembranes as defined by RAB and ARF small GTPases. The researchers identified NBEAL1 at the Golgi and in Golgi-to-plasma membrane anterograde and retrograde vesicles. LRBA and NBEA were found at the *trans*-Golgi network and in tubular sorting endosomes. ALFY/WDFY3 was localized to peripheral secretory and early endosomal vesicles, while NSMAF was found in early/sorting endosomes. LYST was present in late endosomes/lysosomes and NBEAL2 was associated with recycling endosomes. Interestingly, LRBA- and NBEA-

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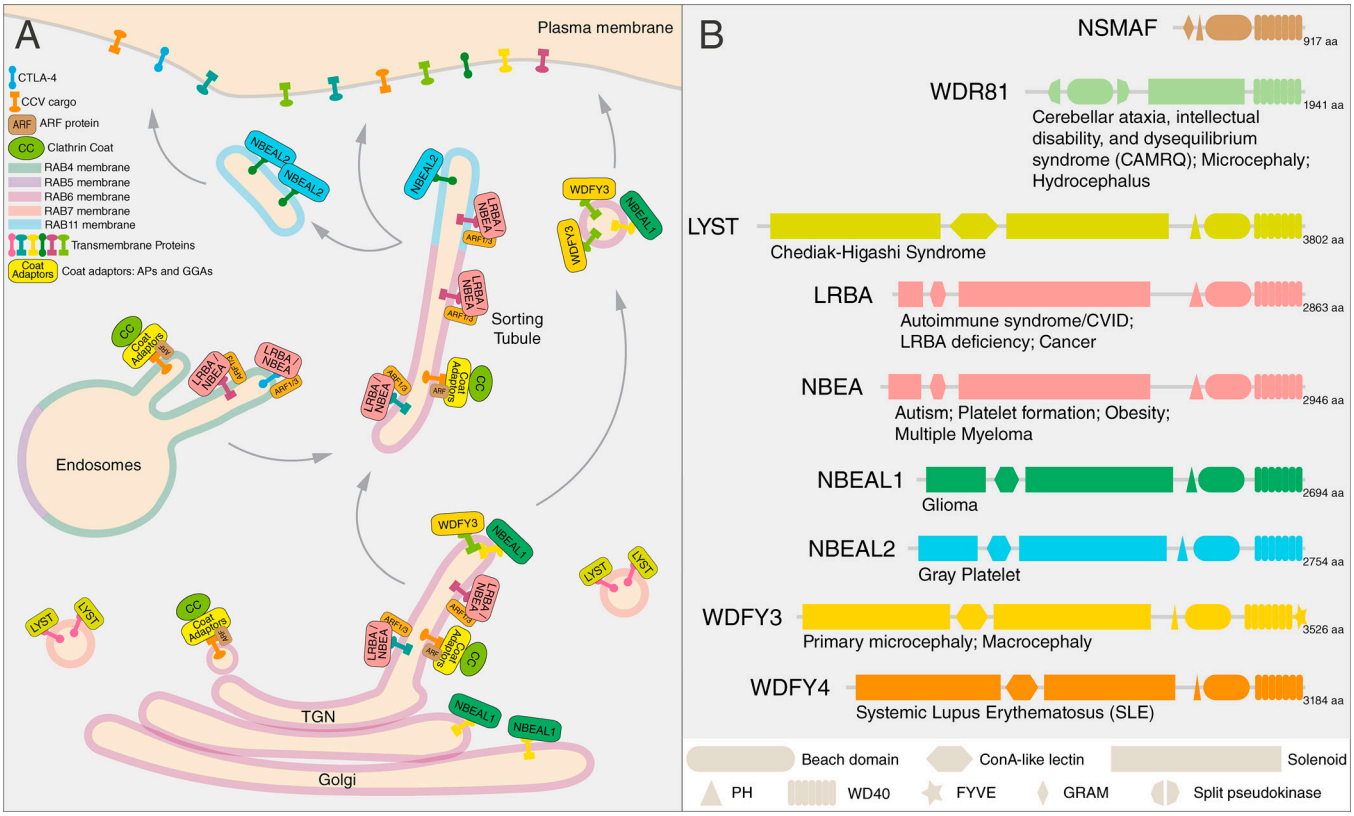

Figure 1. **Overview of localization and domain architecture of BEACH domain proteins. (A)** Model depicting the intracellular localization of BEACH domain proteins adapted from Pankiv et al. (2) and Szentgyörgyi et al. (3). Each family member localizes to a unique set of endomembranes as defined by RAB and ARF GTPases. Membrane domains containing these BEACH domain family members excluded clathrin/clathrin-adaptor proteins and vice versa. This suggests a shared functional role, likely through the BEACH domain, at the interface of several compartments in the secretory pathway. **(B)** Domain architecture of all nine BEACH domain-containing proteins. Most share a conserved C-terminus configuration. BEACH domain proteins were first linked to human disease through mutations in the *LYST* gene, causing Chediak-Higashi Syndrome. However, they have since been associated with multiple other rare genetic disorders and complex diseases, mostly involving neurologic and immune phenotypes. CCV = clathrin coated vesicle, AP = adaptor protein, GGA = Golgi-localized, γ-ear-containing, ARF (ADP-ribosylation factor)-binding protein, and FYVE = Fab1, YOTB, Vac1 and EEA1 domain, LYST = lysosomal trafficking regulator, NBEA = neurobeachin, NSMAF = neutral sphingomyelinase activation associated factor, COP = coat protein complex.

positive tubular structures were decorated with patches of clathrin adaptors and receive cargo from both secretory and endocytic recycling pathways, which implies they may be a protein sorting hub at the interface of the Golgi, endosomes, and cell surface. The localization to various endomembrane tubular structures suggests a shared functional role through the BEACH domain at the membrane compartments bridging the interface of the Golgi, the various endosomes, and the cell surface.

Using AlphaFold structural predictions to compare the proteins across the family of seven typical BEACH domain proteins, the researchers identified a shared domain organization that includes an α-solenoid/ConA-like domain with a PH-BEACH domain assembly and finally WD40-repeats. This α-solenoid/WD40 domain structure is similar to known coat proteins like the COP family (6), hinting that BEACH domain proteins may function as membrane coats (Fig. 1 B). This, in combination with the localization to unique endomembrane tubules, led the researchers to postulate that the typical BEACH domain proteins act as coat proteins. This hypothesis is particularly exciting as pleomorphic tubular carriers, budding from the *trans*-Golgi network, have long been observed and postulated to exist without a classical protein coat (7).

Protein coats have to fulfill two functions (8): (1) cargo selection, by recognizing and binding to specific cargo molecules such as transmembrane proteins, ensuring their concentration and packaging into the forming vesicle; (2) vesicle shaping, by assembling into a scaffold-like structure that curves the membrane to form a vesicle. They are often recruited by coincidence detection, allowing them to interact directly with cargo at the right place and time.

Pankiv et al. expand our understanding of the interaction between BEACH domain proteins and cargoes. They not only define the essential residues on CTLA-4 for BEACH domain binding but also demonstrate that multiple BEACH family members can bind to the cargo's cytoplasmic tail. Taking together, (1) the interaction between LRBA and ARFs characterized by Szentgyörgyi and colleagues, (2) the ability of BEACH domains to bind cargo, and (3) the coat-like domain structure, the model proposed by Pankiv and colleagues

emerges. In this model, BEACH domain proteins act as a coat, allowing coincidence detection and membrane specificity to sort proteins at the interfaces of the endomembrane system.

Moving forward, exploring whether BEACH domain proteins fulfill these key aspects of a functional coat will be crucial. Do they recognize a consensus motif for cargo binding such as YxxΦ or dileucine like other known clathrin adaptors (9)? Do they assemble into tightly packed coats that can bend membranes and facilitate vesicle fission or fusion with donor/acceptor compartments? Do BEACH domain proteins target any group of specific cargos? What defines their individual membrane specificity? Additionally, given the size and diversity of domains in these proteins, do BEACH domains and their proteins perform other, as-yet-unexplored functions beyond coat-like activities?

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