

SPOTLIGHT

Robustness and innovation along the endocytic route: Lessons from darkness

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What mechanisms ensure the loading of a SNARE into a nascent carrier? In this issue, Bowman et al. (2021. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202005173>) describe an unprecedented mechanism where two sorting complexes, AP-3 and BLOC-1, the latter bound to syntaxin 13, work as a fail-safe to recognize sorting signals in VAMP7, a membrane protein required for fusion to melanosomes. Their observations define one of the first examples of distributed robustness in membrane traffic mechanisms.

Eukaryotic cells are defined by a complex collection of membrane-bound organelles, each with their unique catalog of constituents, such as membrane proteins. A precise repertoire of membrane proteins is necessary for these organelles to function properly. Membrane proteins selectively populating either melanosomes (or any other endomembrane organelle) originate in the endoplasmic reticulum and travel to their final destination. This observation raised one of the most fundamental questions in cell biology: how do all membrane proteins reach their diverse destinations despite being born in the same place, the endoplasmic reticulum? The answer has been in the making for ~60 yr.

The prevailing model considers that membrane and soluble proteins in the lumen of organelles are selectively loaded at the donor membrane into membrane-bound carriers of vesicular or tubular nature. Selective cargo loading is accomplished by cytoplasmic protein complexes that sort these complexes into a nascent carrier (1). In this issue, Bowman et al. (2) focused on two sorting complexes, the adaptor complex adaptor protein-3 (AP-3) and biogenesis of lysosome-related organelles complex (BLOC)-1 complex (3). Once formed, membrane-bound carriers must fuse with their target organelle to deliver their content (1). The

fusion step is controlled by a complex machinery centered around fusogenic membrane proteins known as SNAREs. SNAREs must be sorted into a carrier (R-SNAREs) in order to be competent for fusion (1). Preventing an R-SNARE from loading into its carrier impairs carrier fusion with the target membrane and results in dramatic consequences for cells and organisms. For example, removal of the R-SNARE from carriers bound to melanosomes generates melanosomes that fail to produce pigment, a cellular phenotype used by Bowman et al. (2). In the case of synaptic vesicles, elimination of R-SNAREs from these vesicles prevents their fusion with the plasmalemma halting neurotransmission with overt manifestations such as paralysis (4). Thus, any carrier without an R-SNARE is a cellular and organismal catastrophe.

How does a carrier acquire its SNARE in order to deliver their content to a donor compartment? So far, the model has been one of binary interactions between a SNARE signal and a sorting complex that recognizes that signal (5). These binary interactions can be tested by either mutagenesis of the SNARE signal or the domain in the sorting complex that binds the SNARE signal. Either one of these experimental manipulations results in SNARE depletion from the target membrane and defective function of the

target organelle. But what happens when the disruption of a binary interaction does not reveal any of the expected phenotypes? Frequently, such outcomes are explained away by an unidentified, speculative, and unattractive redundancy within the system. Bowman et al. take the long and winding road to identify the source of the so-called “redundancy” in the delivery of an R-SNARE vesicle-associated membrane protein 7 (VAMP7) to melanosomes. VAMP7 travels from endosomes (the donor compartment) to the melanosome (the target compartment) via tubules. VAMP7 concentrates in these tubules by a tripartite process established by a super-complex made by AP-3, BLOC-1, and syntaxin 13. VAMP7 is loaded into these tubules either because AP-3 sorts VAMP7 by direct binding or because BLOC-1 sorts VAMP7 into the same tubule using the VAMP7-binding property of syntaxin 13. Here syntaxin 13, a target or Q-SNARE, moonlights in this mechanism as an “accessory adaptor” linking VAMP7 to BLOC-1. Importantly, there is no role for syntaxin 13 as a SNARE in the fusion of tubules to melanosomes (6). Bowman et al. expose defects in pigmentation and cargo delivery from endosomes to melanosomes (the catastrophe) only when the interactions of AP-3 with VAMP7 and BLOC-1 with syntaxin 13 are simultaneously

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abrogated by mutagenesis. These experiments elegantly reveal the identity of the fail-safe mechanism in VAMP7 sorting to melanosomes.

If carriers without R-SNARE lead to catastrophic failures, what are the fail-safe mechanisms built in the R-SNARE loading step? Redundancy of components can be seen as biology's engineering approach to build fail-safe systems. The capacity to withstand disruption defines a system's robustness (7, 8). If the loading of an R-SNARE into a vesicle is a critical step in any trafficking event, it is reasonable to ask how cells build a system that assures R-SNARE loading into carriers. One such approach comes in the form of several paralogs R-SNAREs loaded on a vesicle by their cognate sorting complex, such as in synaptic vesicles, coat protein complex II, or clathrin-coated vesicles (4, 9, 10, 11, 12). In these cases, different SNAREs bind to their dedicated sorting molecule following a binary mechanism. This represents a robustness built by copies of the same type of entities, much like the two-parachute fail-safe approach in skydiving. In contrast, Bowman et al. present the first example where the system's resilience is built by two dissimilar strategies acting on the same R-SNARE (2). This would be analogous to replacing one of the two parachutes with a jet pack during skydiving. Such systems where robustness is built by dissimilar strategies are known as systems with distributed robustness. While distributed robustness is well known in the organization of metabolic networks and developmental mechanisms, to the best of our knowledge this is a one-of-a-kind example in membrane traffic mechanisms (7, 8).

Robust systems tolerate transient variation, such as environmental variation, but more importantly, robust systems can buffer permanent modifications, including genetic variation, keeping the system away from catastrophe (7, 8). Buffered genetic variation could contribute to noncatastrophic phenotypic variation, opening the door for intermediate phenotypes to emerge in the short term (7, 8). For example, melanosomes contributing to degrees of tanning caused seasonal variations on skin UV light

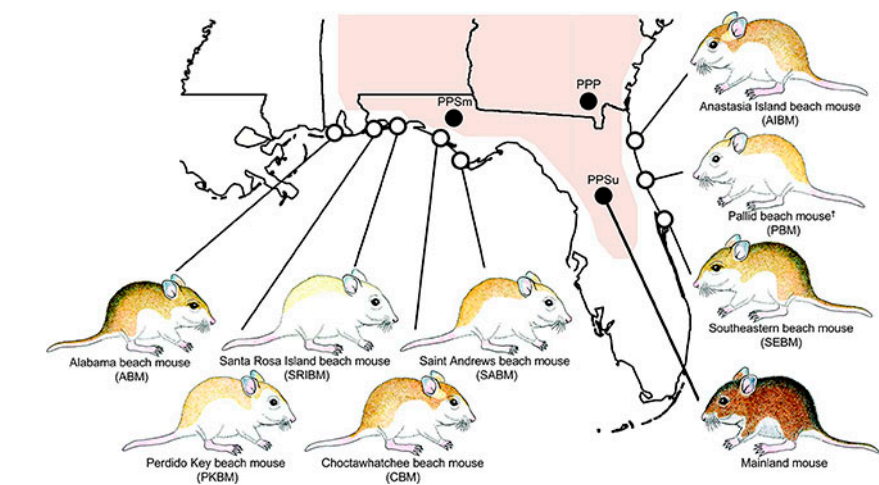


Figure 1. **Variation of fur color among beach mice.** Diagram shows the geographic distribution of the beach mouse *Peromyscus polionotus*. Brown shading represents the habitat of mainland subspecies. Color variation is attributed to allelic variation in one gene involved in melanosome pigmentation. The figure is reproduced from Steiner et al. (14) with permission of Oxford University Press, and abbreviations in parentheses designate subspecies as described by the authors. PPSm, *P. polionotus sumneri*; PPP, *P. p. polionotus*; PPSu, *P. p. subgriseus*.

exposure. However, buffered genetic variation could contribute to potential future phenotypic evolution in the long run (7, 8). This last contribution of robustness could open the door for the appearance of cell type-specific membrane traffic mechanisms and the emergence of novel organelles, such as the melanosome. In addition, a robust melanosome biogenesis system could be permissive for the evolution of fur colors selectable by the environment. This is the case of beach mice where allelic variation in one gene contributes to the emergence of adaptive beach mouse color patterns (Fig. 1; 13, 14). While these ideas are speculative, the excitement of Bowman et al.'s work is that they invite us to think beyond the immediacy of the process they studied. Their elegant findings suggest conceptual novelty in membrane traffic in the form of distributed robustness. The idea of distributed robustness may become a cell trafficking principle waiting to be revealed by à la Bowman experimentation.

Acknowledgments

This work was supported by grants from the National Institutes of Health (1R1AG060285

to V. Faundez and 4K00NS108539 to K.S. Singleton).

The authors declare no competing financial interests.

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