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SPOTLIGHT

Drive, filter, and stick: A protein sorting conspiracy in photoreceptors

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The sorting of proteins into different functional compartments is a fundamental cellular task. In this issue, Maza et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201906024) demonstrate that distinct protein populations are dynamically generated in specialized regions of photoreceptors via an interplay of protein-membrane affinity, impeded diffusion, and driven transport.

Eukaryotic cells are characterized by complex spatial organization, with the cell divided into functionally distinct compartments that require the selective delivery and maintenance of a specific complement of proteins. The problem of protein sorting—delivering the right set of proteins to each compartment—is fundamental to cell biology. For many proteins it remains unclear whether delivery is a one-way journey, with permanent retention at the destination, or a matter of rapid interchange between different regions with a bias toward localization at the target.

One-way protein delivery can be achieved by packaging proteins into vesicles carried by molecular motors, followed by tight binding or rapid processing (e.g., sorting of synaptic proteins between neuronal terminals [1]). Even when the transport process itself is diffusive, tight binding or barriers to escape can make sorting effectively unidirectional. For instance, mitochondrial and nuclear protein import relies on strong membrane binding followed by insertion through pores (2, 3). Cellular regions not enclosed by a membrane can also allow for essentially one-way delivery, given sufficiently high binding strength as to severely limit protein escape.

In contrast, many protein sorting tasks are accomplished via mechanisms that involve only weak binding to target structures, allowing for rapid dynamic interchange between cellular regions. Enhanced localization is then achieved by either active transport mechanisms that bias protein motion (e.g., advection-driven polarization in early embryos [4]) or multivalent interactions enabling the formation of dynamic yet well-localized puncta (e.g., rapid protein interchange in P granules [5]). In this issue, Maza et al. demonstrate that photoreceptor cells couple short-scale directed transport with weak binding to ensure robust accumulation of proteins in their outer segment (OS) while enabling rapid exchange with other compartments (6).

Photoreceptor rod neurons exemplify highly specialized cells with morphologically and functionally distinct compartments linked by a continuous cytoplasm yet exhibiting very different protein populations. The rod OS region (Fig. 1) is an expanded and specialized primary cilium, packed with membranous discs studded with many rhodopsin proteins and associated peripheral membrane proteins (PMPs) such as rhodopsin kinase (7). The traditional picture of protein delivery to primary cilia relies on a combination of a leaky size-dependent diffusion barrier at the cilium base (8), association with motor-driven intraflagellar transport complexes (IFT trains; 9), and localized binding to the ciliary membrane to enable retention. At the opposite end of the rod cell body lies the presynaptic spherule, which requires its own complement of signaling proteins, conventionally assumed to be delivered by motor-driven vesicles (10). Given their long narrow structure and morphologically distinct compartments, rod cells provide an ideal model system for investigation of protein sorting. However, the mechanisms of how different complements of proteins are maintained in the cytoplasmically connected regions have remained mysterious.

Maza et al. demonstrate that the combination of small lipid tags and protein charge form a "compartmentalization code" that directs PMPs in photoreceptor neurons to their target regions (6). Using modified fluorescent protein probes, they show that positively charged prenylated probes are depleted from the OS while acylated probes are enriched there. A natural hypothesis, often assumed upon identification of sorting tags, is that these features dictate strong membrane affinity and consequent localization whenever the protein encounters certain cellular regions. Indeed, a simple model of diffusive exploration and selective binding is sufficient to quantitatively reproduce the localization of positively charged prenylated probes to the presynaptic region (Fig. 1, bottom).

In contrast, the dynamics and distribution of PMPs localized to the OS cannot be explained by membrane binding affinity alone. Binding tightly to photoreceptor disc membranes inevitably comes at the cost of restricting protein mobility. Yet, when redistribution timescales were measured by fluorescence recovery after photobleaching, OS-enriched probes moved quite freely throughout the OS. Such rapid equilibration indicates weak binding to the disc membranes, which is insufficient to explain the probe enrichment.

Maza et al. (6) also show, by tracking the flux of photoactivated protein out of the OS, that the diffusivity of OS-enriched probes is substantially hindered in the narrow connecting cilium linking the cell body to the OS. This observation highlights the existence of a leaky diffusive barrier that appears to be a ubiquitous feature in ciliary protein sorting (8). However, such a

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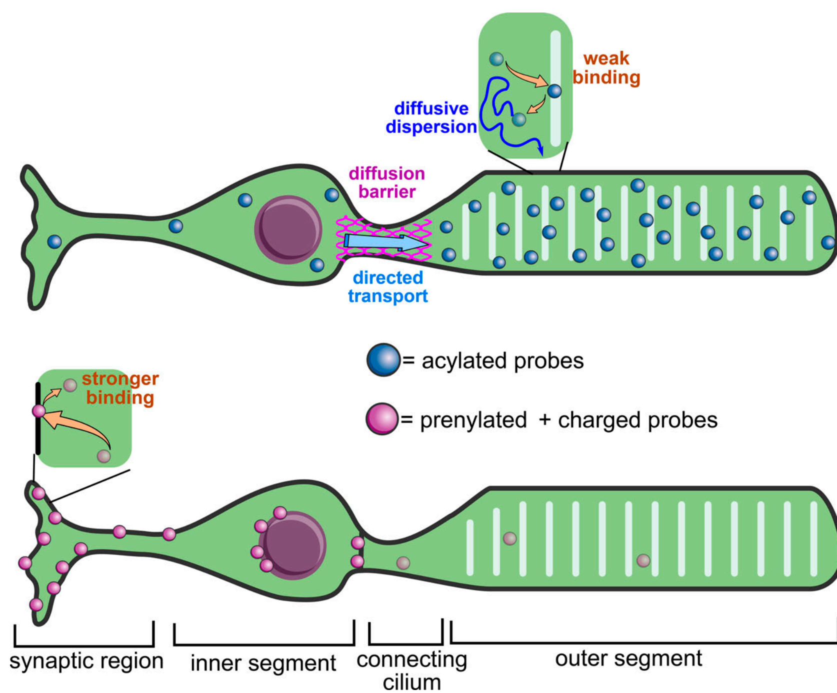

Figure 1. **Selective binding, directed transport, and a diffusion barrier conspire to achieve protein sorting between distinct compartments of photoreceptor cells.** Top: Acylated probes are enriched in the OS through directed transport supported by hindered diffusion in the connecting cilium. Their weak membrane binding enables them to diffuse rapidly throughout the OS. Bottom: Enrichment of prenylated, charged probes in the synaptic region is consistent with strong membrane binding and simple diffusive transport. Illustration produced using SMART (Servier Medical Art; https://smart.servier.com) in accordance with a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

barrier is expected to be bidirectional, limiting protein entry into as well as exit from the OS. With diffusive transport alone, such passive barriers cannot enrich proteins in any region. The contradiction between robust localization yet dynamic redistribution within the OS thus indicates the existence of an alternate, nondiffusive transport mechanism.

As no such mechanism has previously been described in photoreceptors, Maza et al. (6) turn to quantitative modeling to demonstrate that directed transport in the connecting cilium, with a modest velocity, is sufficient to reconcile the experimental observations. This flow combines with weak binding in the OS and a diffusive barrier that slows protein leakage out of the OS, to enable simultaneous enrichment yet dynamic equilibration of tagged proteins in the OS (Fig. 1, top). Thus, protein sorting in photoreceptors is not a one-way journey to a permanent destination but rather a highly dynamic process of constant protein recycling between different cellular regions.

These results highlight an important general principle in cell biology—that tight binding to cellular membranes is unnecessary for robust protein sorting and that quite weak binding can be sufficient when coupled with directed transport mechanisms. Notably, such transport could operate in both directions in a selective fashion, pushing proteins with certain tags toward the OS while depleting other proteins from this region. The nature of these transport mechanisms remains rather mysterious at present. A likely possibility is the involvement of the BBsome adaptor complex and the IFT trains, which have been postulated to deliver proteins into and out of primary cilia structures in general (9). Another possibility noted by Maza et al. (6) is an electrophoretic driving force arising from the voltage gradient known to exist in photoreceptor cells, where the OS tip has a positive voltage relative to other cellular regions (11). This gradient may also contribute to the preferential motion of positively charged probes away and negatively charged probes toward the OS, though the importance of this effect remains to be established.

The study by Maza et al. (6) elucidates the interplay between small modulations of protein binding affinities, directed transport, and local diffusion barriers in robustly localizing proteins to specific cellular regions. This work highlights the ability of cells to maintain well-defined compartments without the need for complete separation from the pervading cytoplasm and without sacrificing the dynamic interchange of components crucial to the design of a highly responsive active system.

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1. Tas, R.P. 2017. *Neuron.* https://doi.org/10.1016/j.neuron.2017.11.018
2. Stojanovski, D., et al. 2012. *Cold Spring Harb. Perspect. Biol.* https://doi.org/10.1101/cshperspect.a011320
3. Wente, S.R., and M.P. Rout. 2010. *Cold Spring Harb. Perspect. Biol.* https://doi.org/10.1101/cshperspect.a000562
4. Goehring, N.W. 2011. *Science.* https://doi.org/10.1126/science.1208619
5. Brangwynne, C.P. 2009. *Science.* https://doi.org/10.1126/science.1172046
6. Maza, N.A., et al. 2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201906024
7. Besharse, J.C. 2012. Photosensitive membrane turnover: differentiated membrane domains and cell-cell interaction, Part I. *In* The retina: A model for cell biological studies. Elsevier.
8. Hu, Q., and W.J. Nelson. 2011. *Cytoskeleton (Hoboken).* https://doi.org/10.1002/cm.20514
9. Lechtreck, K.F. 2015. *Trends Biochem. Sci.* https://doi.org/10.1016/j.tibs.2015.09.003
10. Schlager, M.A., and C.C. Hoogenraad. 2009. *Mol. Brain.* https://doi.org/10.1186/1756-6606-2-25
11. Hagins, W.A., et al. 1970. *Biophys. J.* https://doi.org/10.1016/S0006-3495(70)86308-1