

## Why endosomes recycle GPCRs

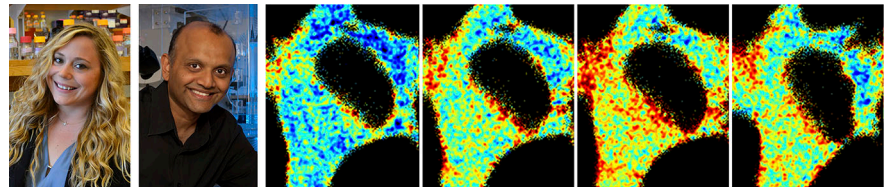
G protein–coupled receptor recycling pathways allow cells to modulate downstream signaling.

Bowman et al. provide a novel explanation for why activated G protein–coupled receptors (GPCRs) migrate from the cell surface to the endosomes (1). The study indicates that GPCRs that have moved to certain locations on endosomes activate specific genes—and thus presumably induce different cellular responses.

GPCRs help us perceive our surroundings, control our blood pressure, mobilize our immune cells, and perform a host of other essential tasks. When GPCRs are activated, they shuttle from the cell membrane to endosomes. If the receptors lack certain amino acid sequences, they proceed to the lysosome for destruction. But GPCRs that possess these sequences home in on tubular sections of the endosome that carry actin/sorting nexin/retromer (ASRT) domains (2). From there, the receptors return to the cell membrane. In contrast, other types of receptors that also travel to the endosomes after they bind their ligands, such as nutrient receptors, return to the cell membrane even if they lack the distinctive sequences, a mechanism called bulk recycling (3). During their time in the endosomes, these receptors localize to tubules that lack ASRT domains.

What do cells gain from this complex choreography? Researchers think that one benefit is that receptor relocation enables a cell to adjust the strength of its response to stimulation. But recent evidence shows that GPCRs can signal from the cell membrane and from endosomes, suggesting that the move could alter the effects of receptor activation (4).

To investigate this possibility, Bowman et al. stimulated  $\beta$ -2 adrenergic receptors (B2AR), a type of GPCR, in cultured cells. Within five minutes, most of the receptors had transferred to the ASRT domains of endosomes. But when the team inhibited the kinase PKA, which phosphorylates B2AR, the receptors were evenly distributed



(Left to right) Shanna Bowman, Manojkumar Puthenveedu, and Daniel Shiwarski (not pictured) investigated the consequences of GPCRs' relocation to tubular portions of endosomes that harbor ASRT domains. In this heatmap time series of a single kidney cell, red indicates high levels of cAMP, a measure of GPCR activation. Levels of cAMP are low when the cell is first stimulated (left), but they surge as GPCRs at the surface of the cell are activated. cAMP levels remain elevated as GPCRs are internalized and continue signaling (right).

PUTHENVEEDU PHOTO COURTESY OF TIM KAUIEN FOR CARNEGIE MELLON UNIVERSITY; BOWMAN PHOTO COURTESY OF BRIAN BOWMAN

between the ASRT and bulk recycling tubules. Removing two key phosphorylation sites in B2AR also eliminated the receptors' tubule preference, suggesting that phosphorylation by PKA helps steer B2AR to the ASRT domains.

Using a biosensor that detects activated B2AR, the scientists next determined that stimulated receptors are present in both types of tubules. But another biosensor that identifies activated  $G\alpha$  subunits, the portion of the G protein switched on by GPCRs, showed a different pattern. Activated G proteins only accumulated in the ASRT-containing tubules, suggesting that B2AR molecules in these locations are able to signal, whereas the receptors in the tubules that perform bulk recycling are not.

Activated G proteins stimulate the production of cyclic AMP (cAMP), which in turn boosts the expression of certain genes. Previous work suggested that cAMP produced from endosomes turns on different genes than cAMP generated at the cell surface. Bowman et al. tested whether the location of B2AR determines which genes it activates. They compared the expression of three genes that are turned on by endosomal cAMP to the expression of a reference gene, which isn't affected by endosomal cAMP. When the researchers

stimulated B2AR, they found that activity of the three cAMP-dependent genes increased between five and eight times as much as the activity of the reference gene.

The team then used three techniques to block endocytosis and another method to disrupt ASRT domains. In each case, expression of the three endosomal cAMP-dependent genes did not increase after activation of B2AR. The scientists also followed the activity of the genes in cells that carried the phosphorylation-resistant version of B2AR, which can spread to both types of tubules. In these cells, B2AR stimulation had no effect on gene expression.

These findings suggest another explanation for why cells direct activated GPCRs to the ASRT-containing tubules of endosomes. "The main reason might be to move the receptor to an active signaling domain, not just to change the number of receptors at the cell surface," says senior author Manojkumar Puthenveedu. Researchers still need to work out how the changes in gene expression triggered by GPCR trafficking modify the behavior and function of cells. A large fraction of drugs target GPCRs, and the study suggests that identifying molecules that relocate the receptors could fine-tune the effects of these medications.

1. Bowman, S.L., et al. 2016. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201512068>
2. Puthenveedu, M.A., et al. 2010. *Cell.* 143:761–773.
3. Maxfield, F.R., and T.E. McGraw. 2004. *Nat. Rev. Mol. Cell Biol.* 5:121–132.
4. Tsvetanova, N.G., et al. 2015. *J. Biol. Chem.* 290:6689–6696.

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