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CELL SIGNALLING

Budding GPCRs

G protein-coupled receptors (GPCRs) are seven transmembrane cell surface receptors that regulate cell physiological processes in response to various ligands. It is well-established that their prolonged stimulation causes them to be internalized through endocytosis. Now, Soetedjo and Jin describe an additional mode of GPCR trafficking and signal regulation, whereby the GPCR VPAC2 (also known as vasoactive intestinal polypeptide receptor 2) is shed from primary cilia (sensory organelles that project from the cell surface of most eukaryotic cells) into the extracellular milieu.

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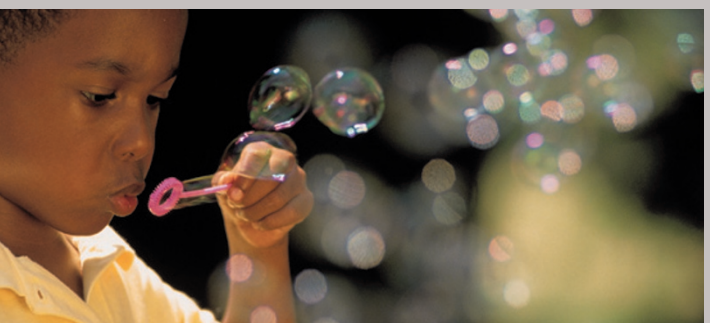

PATHFINDER

The authors showed that treatment of rat suprachiasmatic nuclei (SCN) cells with the ligand pituitary adenylate cyclase-activating peptide 27 (PACAP27) resulted in the loss of endogenous VPAC2 from primary cilia, while the numbers of cilia remained the same. Interestingly, using live cell imaging, they observed that, instead of being internalized, the receptors were dispersed in puncta outside the cell in the culture medium. Fractionation assays and electron microscopy revealed that VPAC2 is shed in ectosomes (plasma membrane-derived vesicles). Moreover, the removal of VPAC2 from the ciliary surface was highly selective, as other ciliary membrane proteins were not detected in the culture medium of ligand-treated cells.

Next, knockdown of the ESCRT (endosomal sorting complexes required for transport) components ESCRT-III, VPS4 (vacuolar protein sorting-associated protein 4) and LIP5 (LYST-interacting protein 5) resulted in decreased VPAC2 shedding, which suggests that they facilitate budding of the ligand-activated receptor from the membrane. In addition, VPS4 and LIP5 transiently accumulated in cilia in a ligand- and VPAC2-dependent manner. The authors therefore propose that ligand-activated VPAC2 itself mediates the generation of ectosomes through a mechanism that involves the translocation of VPS4 and LIP5 to cilia.

Finally, prolonged ligand treatment led to decreased phosphorylation of ERK, the downstream target of VPAC2; however, ERK phosphorylation was not abolished in cells with decreased VPS4 levels, which suggests that receptor shedding and not ligand treatment per se inhibits downstream signalling. In sum, this study shows that ligand-induced shedding of GPCR-containing ectosomes reduces the number of receptors on the cell surface to fine-tune signalling. It will be interesting to see whether other GPCRs are regulated by a similar mechanism.

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ORIGINAL RESEARCH PAPER Soetedjo, L. & Jin, H. Agonist-induced GPCR shedding from the ciliary surface is dependent on ESCRT-III and VPS4. *Current Biol.* http://dx.doi.org/10.1016/j.cub.2014.01.010 (2014)