CELL SIGNALLING

Putting the brakes on sonic hedgehog

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is mediated by
... GPR161
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The organization of cellular differentiation during development requires the sensing of graded morphogen signals, such as sonic hedgehog (SHH). A recent study by Mukhopadhyay *et al.* describes a novel signalling receptor pathway in which the G protein-coupled receptor (GPCR) GPR161 mediates the basal repression of SHH-dependent gene transcription and can be downregulated when SHH is activated.

SHH signals are transduced by primary cilia in neuroepithelial cells to direct patterning of the vertebrate neural tube. Mutations that disrupt ciliary assembly, such as those affecting intraflagellar transport (IFT), result in a lack of SHH-dependent ventral cell types in the developing neural tube. Earlier work had shown, surprisingly, that IFT-A complex subunits (unlike other IFT proteins) can antagonize the SHH pathway; this suggests that IFT-A might have a role in SHH signalling independent

of its role in the formation of cilia. This group had previously shown that IFT-A recruits the tubby-like protein TULP3 to cilia, which in turn recruits GPCRs. The authors therefore proposed that a GPCR could be responsible for IFT-A–TULP3-mediated SHH antagonism during vertebrate neural tube development. This study shows that GPR161 satisfies the required criteria for such a GPCR.

First, GPR161 is expressed early in mouse embryonic development and can be detected in the cilia of various ciliated cells. Second, the ciliary localization of GPR161 was markedly decreased in cultured mouse cells in the absence of TULP3 or IFT-A. Third, GPR161 is highly conserved among vertebrates, and the authors identified a conserved sequence motif in the third intracellular loop of GPR161 that is required for ciliary localization. Fourth, GPR161 is expressed throughout the neural tube during embryonic development in mice.

Moreover, the authors identified GPR161 as a negative regulator of the SHH pathway. *Gpr161*-knockout mouse embryos had increased levels of SHH-dependent gene transcripts, and the expression area for these transcripts was extended from ventral to dorsal regions of the neural tube. This resulted in a ‘ventralization’ phenotype, in which ventral cell types were specified at the expense of lateral and dorsal cell types in the neural tube of knockout embryos.

Normally, in the absence of SHH, GLI transcription factors, such as GLI3, are proteolytically processed to repressor forms (GLIR). This processing is mediated by cAMP-dependent protein kinase A (PKA), but until now the signals that mediate activation of PKA were unknown. Here, the authors showed that the extent of PKA-mediated GLI3 processing to GLI3R, which is inhibited by SHH, was also strongly decreased in *Gpr161*-knockout embryos. Together, the results are indicative of increased SHH signalling in the absence of GPR161. Demonstrating that the effect was direct, overexpression of GPR161 resulted in increased cAMP production — a process requiring the canonical Gas heterotrimeric G protein that activates adenylyl cyclase — and therefore could lead to PKA activation.

The authors conclude that PKA-dependent basal repression of SHH signalling is mediated by IFT-A–TULP3-regulated GPR161. Treatment with SHH resulted in complete loss of endogenous GPR161 from cilia by internalization into the recycling endocytic compartment. Thus, a positive feedback loop limits GPR161 action to sustain active SHH signalling when required.

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ORIGINAL RESEARCH PAPER Mukhopadhyay, S. *et al.* The ciliary G-protein-coupled receptor Gpr161 negatively regulates the sonic hedgehog pathway via cAMP signaling. *Cell* **152**, 210–223 (2013)

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