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Opinion Article

OPINION ARTICLE

Defining the Specialized Functions of cAMP Signals in an Organelle Formerly Deemed to Have No Function: The Primary Cilium

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The primary cilium is a decidedly "cute" organelle that first attracted the attention of electron microscopists in the early 1960s (**Figure 1**). A few prescient scientists of the last century recognized that the cilium was more than just an ultrastructural curiosity. But this was not the prevailing view; in fact, cell biology textbooks published only three decades ago discounted the cilium as a "vestigial" structure of "unknown function." Interest in this diminutive organelle began to seriously build around 20 yr ago with the discovery of its central role in several disease states. These included autosomal dominant polycystic kidney disease (ADPKD), and inherited "ciliopathies" such as Bardet–Biedl syndrome. The ciliary localization of signaling pathways such as hedgehog and its connection to glioblastoma accelerated further interest.[1]

Now, in 2023, the primary cilium has been launched from relative obscurity to the focus of exuberant scientific inquiry. Discoveries of new connections between ciliary biology and cellular functions (autophagy, neuronal migration, and necroptosis, to name a few) seem to emerge daily. Loss of proper function is implicated in an ever-expanding list of human diseases and conditions that include obesity and appetite control, cancer, cognitive decline, and diabetes.[1]

Among the most titillating of observations to come to light in the past years (for some of us, at least), was that cilia are enriched with a specific subset of G-protein coupled receptors (GPCRs) that are typically linked to cAMP signaling through Galpha(s) or Galpha(i).[2] Another early observation regarded the very exclusive sequestration of adenylyl cyclase 3 (AC3) in neuronal cilia, and its link to obesity and depression. Other AC isoforms (eg, AC5 and AC6) are now known to localize to cilia in different cell types, along with phosphodiesterases and beta-arrestins. The main effector of the cAMP signal, protein kinase A (PKA), is tethered within the organelle by A-kinase anchoring proteins (AKAPs), ready to phosphorylate a host of potential and confirmed ciliary PKA targets. Direct measurements by our lab using targeted cAMP biosensors showed that stimulation of GPCRs confined exclusively in the cilium produced local cAMP signals. (Interestingly, this response was greatly amplified during activation of the hedgehog pathway.)[3] Taken together, it appears everything is in place to operate a self-contained cAMP signaling circuit in the cilium. But what special purpose does it serve, especially considering that small molecules like cyclic nucleotides move freely between cytosol and cilium?

Recent studies now implicate ciliary GPCRs coupled to cAMP production in the control of several physiological functions. For example, Hilgendorf et al. reported that white adipose tissue expansion and differentiation required FFAR4, a ciliary GPCR that could be stimulated by omega-3 fatty acids to locally produce cAMP.[4] FFAR4 stimulation was shown to converge on the chromatin remodeling protein, CTFC, to turn on the adipogenic transcriptional program. Since FFAR4 was localized only to cilia, the presumption was that these effects were initiated by the ciliary cAMP signal.

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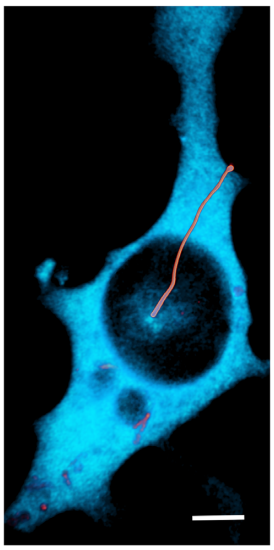

Figure 1. Primary cilia are present on most cell types and are typically 1–10 μm in length and 200 nm wide. Shown here is a confocal of a live mIMCD3 cell presenting a particularly long primary cilium highlighted by mCherry-tagged 5HT6 receptor (pink). The nonnuclear cytosol (depicted in blue) is labeled by an expressed fluorescent protein construct with a nuclear exclusion sequence (NES-Venus). Scale bar = 5 μm.

More recently, the Reiter group used a panel of cilium-targeted cAMP modifiers to provide definitive evidence for the importance of locally generated cAMP in regulating hedgehog transcription.[5] The participation of ciliary cAMP in the suppression of the hedgehog pathway was previously inferred in breakthrough studies reported a decade earlier by Mukhopadhyay and colleagues.[6] This group described Gpr161, an orphan GPCR in the cilium that constitutively produced cAMP to suppress resident Gli transcription factors through PKA phosphorylation. Of note, Gpr161 was later shown to function as an AKAP, tethering PKA directly to the receptor.[7] The Reiter team used targeted optogenetic tools such as light-activated adenylyl cyclase (bPAC), designer GPCRs (DREADDs) coupled to cAMP production, and dominant-negative PKA to selectively control signaling in cilium and cytosol. Hedgehog signaling preferentially responded to cAMP derived from the ciliary compartment, with profound developmental effects in a zebrafish model.[5]

Just last year the Wachten lab published a startling result indicating that cAMP in the cilium was responsible for cyst formation in an in vitro ADPKD model.[8] This group also used targeted bPAC constructs as well as stimulation of endogenous prostaglandin receptors (EP4) in cilia. Differential effects of chronic cAMP elevation in the cilium were observed, including transcriptional effects mediated by CREB shuttling between cilium and nucleus. A novel small-molecule activator of a specific phosphodiesterase (the long isoform of PDE4) reduced ciliary cAMP and counteracted the cystogenic effect.

Possibly more intriguing yet are the findings of Hsu-Hsien Sheu and colleagues, who described a synaptic structure that released serotonin onto ciliary 5-HT6 receptors of hippocampal neurons.[9] The 5-HT6 receptor is classically coupled to cAMP, but evidence of ciliary cAMP elevation was not reported in their model (perhaps similar to what we observed in cultured renal cells in the absence of hedgehog activation).[3] Rather they showed an alternate coupling of serotonin-activated receptors to a noncanonical Galpha(q/11)-Trio-RhoA pathway, ultimately resulting in chromatin remodeling and transcriptional changes.

The physiological importance of GPCR signaling in an organelle that was once deemed to have "no function" is now on the cusp of being understood. Two of the papers highlighted here directly demonstrate the functional importance of a dedicated ciliary cAMP microdomain. A next step will be to integrate these findings with emerging concepts of cAMP nanodomains, highly localized cAMP hotspots generated near activated GPCRs that probably represent the main mode for physiological cAMP/PKA signaling.[10] Direct measurements of nanodomains in cilia have not yet been reported but will likely explain the ability of the cell to maintain separate biological outcomes for cAMP signals generated by physiological agonist doses in cilia vs. cell body. However, as the results with the neuronal ciliary 5-HT6 receptor show, the signaling landscape in the cilium can be unpredictable. The future will see further dissection of the specific roles of ciliary cAMP in different cellular contexts, a challenging prospect considering the identity and distribution of ciliary GPCRs is incomplete. Whether these receptors even use cAMP or are rather preferentially coupled to other intermediates when in the ciliary milieu is also not known. Tools for measuring and manipulating second messengers and GPCR function selectively within the organelle will undoubtedly be at the forefront of addressing these questions.

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Conflict of Interest

A.M.H. holds the position of Editorial Board Member for *Function* and is blinded from reviewing or making decisions for the manuscript.

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