New and Notable

Signaling at Crossroads: The Dialogue between PDEs and PKA is Spoken in Multiple Languages

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One of the primary mechanisms to regulate the effective concentration of cyclic AMP (cAMP) and cyclic GMP (cGMP) is through the 3′:5′-phosphate bond hydrolysis, which is catalyzed by cyclic nucleotide phosphodiesterases (PDEs). Hence, PDEs control the amount of cAMP and cGMP second messengers locally available for the activation of a multitude of downstream signaling pathways and, in turn, for the control of a wide array of intracellular responses to extracellular stimuli (1–4). Because specific subtypes or isoforms within the large PDE superfamily can be selectively inhibited, PDEs are also valuable drug targets. PDE inhibition provides a means to locally activate cAMP and/or cGMP-dependent signaling within particular subcellular microdomains, with a selectivity that would be challenging to match through direct activation of downstream cyclic nucleotide receptors.

Among the eukaryotic cyclic nucleotide receptors, protein kinase A (PKA) serves as a primary relay of the cAMP signal and the interplay between PDEs and PKAs is central to the tight regulation of signaling in eukaryotes. The PDE:PKA crosstalk occurs at multiple levels. It has been known for over a decade that PDE and PKA are not only part of coupled feedback loop networks, but they are also colocalized to the same subcellular compartments through A-kinase anchoring proteins (AKAPs) (4). The combined action of feedback loops and AKAP-mediated subcellular compartmentalization involving specific PDE and PKA isoforms results in an exquisitely fine-tuned spatiotemporal control of the cAMP signal, tailored to specific cellular demands (1–6).

In this issue of the *Biophysical Journal*, Krishnamurthy et al. (7) add a new potential dimension in the complex PDE:PKA interplay by exploring the possibility of direct, AKAP-independent PDE/PKA interactions as a complement to AKAP-mediated tethering. Krishnamurthy et al. (7) focused on the Iα isoform of the regulatory subunit of PKA (RIα), which is known to bind cAMP with high affinity (K_d ~ nM), and address a long outstanding question in cAMP signaling: how is the cAMP signal terminated by PDEs after cAMP binds RIα and activates PKA?

When cAMP is sequestered within PKA R, it is inaccessible to PDEs and the approximately nM affinity of RIα for cAMP suggests that, in the absence of direct interactions between R and PDEs, signal termination through PDEs would be kinetically limited by the very slow off-rate for the dissociation of cAMP from RIα. Krishnamurthy et al. (7) propose a brilliant solution to this paradox by hypothesizing that PDEs can bind R, facilitate the dissociation of cAMP from RIα, and channel the cyclic nucleotide substrate directly from the phosphate-binding cassette of RIα to the active site of the PDE. This hypothesis was inspired by prior work from the same group on PKA and RegA in *Dictyostelium discoideum* (8) and it is extended here to mammalian PDEs. For this purpose, Krishnamurthy et al. (7) screened several mammalian PDE isoforms for RIα binding, utilizing fluorescence polarization measurements and found that RIα binds PDE8A, a close cAMP-selective PDE homolog of RegA. Although PKA RIα is likely to directly bind other PDE isoforms, the PDE8A:RIα complex was selected for further biophysical studies. The PDE8A/RIα interface was mapped using a combination of peptide arrays, hydrogen/deuterium exchange mass spectrometry, and computational docking (7).

Although the work of Krishnamurthy et al. (7) opens new perspectives on cAMP signal termination by PDEs, it also generates additional questions that must be addressed through further investigations.

First, it will be critical to fully probe the existence and functional relevance of endogenous AKAP-independent R:PDE interactions in vivo using, for instance, coimmunoprecipitation methods. Although potential competition between PDEs and the endogenous PKA C-subunit for binding to PKA R is a possible confounding complication, these or other in vivo approaches are also expected to reveal which R isoforms (Iα, IIα, Iβ, and IIβ) bind which PDE subtypes/isoforms. The PDE-versus-PKA R selectivity patterns are anticipated to provide additional means to rationalize the complex crosstalk between PKA and PDE. In this respect, we expect that the contribution of Krishnamurthy et al. (7) could be an example in which biophysics prompts further investigations in cell biology, rather than the other way around, as often observed. If confirmed in vivo, the work by Krishnamurthy et al. (7) will reveal a new function of PDEs as additional anchor proteins for PKA and it will be interesting to further dissect the interplay between the AKAP-mediated and the AKAP-independent PDE:PKA cross-talk. For instance, the PKA R:PDE interaction may also occur in the context of AKAP complexes. Thus, the anchoring protein may provide the subcellular

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location and limit the range of action of the PKA R:PDE subcomplex.

Second, whereas the docking approach taken by Krishnamurthy et al. (7) was useful to show that the proposed model for the PDE8A:RIα complex is structurally viable, accurate modeling of the PDE:R interactions is challenging due to the inherent dynamic nature of R (3,9), which may change in conformation upon PDE binding. Once the PDE8A:RIα interface is accurately mapped, it may provide a structural rationalization for the selectivity exhibited by RIα for different PDE isoforms. Further structural studies on the PDE8A:RIα complex will also clarify how these interactions involving PKA affect other known PDE8A partners, including the Raf-1 kinase that is also bound to and regulated by PDE8A (10).

Third, it should be considered that, although PDE-assisted weakening of the interactions of cAMP with RIα is a central part of the model proposed by Krishnamurthy et al. (7), the mechanism underlying the reduction of cAMP affinity is still not fully understood. In this respect, it will be critical to reassess the PDE8A:RIα interactions in the context of the allosteric thermodynamic cycle for cAMP-dependent control of PKA (9). This cycle arises from the coupling of the inhibitory and binding equilibria affecting apo RIα. The apo RIα form is often neglected because it is only minimally and transiently populated in vivo, inasmuch as PKA R is primarily bound to either cAMP or the catalytic subunit of PKA (C). However, the dynamics of apo RIα is believed to be a key determinant of the affinities of RIα for cAMP and C, as well as of PKA activation (9). Apo RIα samples a dynamic equilibrium of inactive and active conformations (9). In the case of the RIα cAMP-binding domain-A, the free energy landscape for the apo inhibitory equilibrium is nearly degenerate, resulting in comparable populations of inactive and active states and in maximal sensitivity to state-selective interactions, such as those with cAMP or C (9). Whereas cAMP selectively stabilizes the active conformation of RIα, C preferentially binds the inactive state of RIα.

One hypothesis to explain how PDE8A reduces the affinity of cAMP for RIα is to assume that PDE8A, similarly to C, selectively binds the inactive conformation of RIα, thus reducing the effective population of the active state, which binds cAMP with high affinity. As of this writing, this hypothesis is purely speculative, and may not capture the full complexity of PDE:PKA interactions, which may also cause currently uncharacterized structural perturbations. Overall, the work by Krishnamurthy et al. (7) is an important contribution to the field of cAMP signaling and opens new perspectives to understand how PDEs terminate and modulate the cAMP signal in time and space, both in an AKAP-dependent and independent manner.

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