

New and Notable

The Role of Allostery in the Termination of Second Messenger Signaling

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Signaling is required for function; cells need to perceive and efficiently respond to their microenvironment. Signals can propagate in many ways, including changes in pH, diffusion, chemical reactions, or physical binding; they can also take place through second messengers (1). Signals are received via allosteric events that perturb the molecular conformation of a target macromolecule. Second messenger binding initiates intracellular signal transduction cascades that trigger physiological changes; these often couple to kinase cascades that amplify the breadth of the first extracellular signal. Cyclic AMP (2,3) and Ca^{2+} (4) are among the commonly used second messengers in eukaryotic signaling systems. Cyclic AMP (cAMP) is synthesized by adenylyl cyclase after stimulation by a hormone-activated G protein-coupled receptor. While cellular responses are largely the outcome of long range allosteric propagation, insight into signaling at the conformational level must be obtained by probing allosteric effects on a single-molecule level (5).

Time-dependent signaling bursts must both initiate and terminate, but most of the detailed studies focus on

the initiation events. In this issue of the *Biophysical Journal*, Krishnamurthy et al. (6) provide a unique, in-depth view of the termination step for cAMP signaling by the cytosolic cAMP receptor, protein kinase A (PKA) regulatory subunit, carried out by phosphodiesterase (PDE). PKA regulatory subunit and PDE from *Dictyostelium discoideum* (R_D and RegA, respectively) were used as a model system. On its own, cAMP-dependent protein kinase A (PKA) exists as an inactive complex of the regulatory and catalytic subunits. Signaling initiates upon binding of cAMP to the regulatory subunit (R_D), which elicits conformational changes that result in the release of the active catalytic subunit. Signaling terminates through the action of cAMP PDE, which forms direct interactions with the cyclic nucleotide binding (CNB) domain of PKA regulatory subunit and hydrolyzes cAMP to 5'AMP. Importantly, the high local concentrations of cAMP in the cell preclude reassociation of the catalytic and regulatory domains of PKA. Consequently, signal termination occurs only when the cAMP population is drained by phosphodiesterase hydrolysis.

Earlier (3), the authors proposed a mechanism for termination of signaling through the activity of a PDE bound near the second cAMP binding site on R_D (CNB:B), whereby substrate channeling between cAMP sites in R_D delivers cAMP to the active site of PDE for hydrolysis. This channeling proposition aimed to address the puzzling question of how cAMP, which binds with high affinity (nM K_d) at R_D 's CNB:A, can be hydrolyzed at PDE's active site. Krishnamurthy et al. (3) argued that without direct interactions between R_D and PDE, PDE's cAMP hydrolysis activity and thus signal termination would be limited by the very slow off-rate for the dissociation of cAMP from R_D . Thus the authors hypothesized that PDEs bind R_D , eliciting a conformational change that promotes cAMP dissociation from CNB:A. cAMP in

solution is then channeled through CNB:B to the active site of the PDE.

In this issue of the *Biophysical Journal*, Krishnamurthy et al. (6) focus on the significant question of how R_D :PDE complex formation and dissociation take place, illustrating for the first time, to our knowledge, a mechanism through which cAMP signaling can be terminated in a timely manner through allostery. Amide hydrogen/deuterium exchange mass spectrometry (HDXMS) (7,8) is a powerful conformational probe tool for mapping allosteric signaling, identifying flexible and disordered regions. Here HDXMS is exploited to monitor slow conformational transitions in R_D . Krishnamurthy et al. (3) show that at high cAMP concentrations, R_D and PDE form a stable complex, with PDE interacting at R_D 's CNB:B. This validates the formation of the proposed stable ternary complex in cAMP signal termination. The authors propose that such a complex permits substrate channeling, in accord with the model for coordinated cAMP hydrolysis discussed above. The channel model is in agreement with all their experimental data and leads the authors to postulate that signaling will persist for as long as cAMP channeling persists. Once the cAMP pool is depleted, signaling will terminate. In the absence of cAMP, the destabilized complex will dissociate, and R_D will reassociate with the catalytic subunit of PKA, with the departing PDE allosterically priming R_D for its autoinhibition role.

According to this scenario, allostery plays two important roles in the termination of cAMP signaling. First, PDE binding allosterically promotes the release of the tightly bound cAMP from R_D 's CNB:A, kinetically increasing the local concentration of cAMP. Second, channeling of cAMP via R_D 's CNB:B to PDE's active site helps PDE efficiently hydrolyze cAMP. This implies that the temporally regulated

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expression level of PDE corresponds to a precisely regulated termination of PKA activation through allostery.

This remarkable scenario fits well the HDXMS data (6). Importantly, the substrate channeling model of Krishnamurthy et al. (3,6) implies that PDE alone cannot efficiently hydrolyze cAMP. Measurements of the catalytic k_{cat} and K_m of PDE (9) in the presence and absence of R_D could verify the critical role of allostery in cAMP hydrolysis. Kinetic data might indicate the possibility of an alternative allosteric activation taking place through a conformational change in PDE elicited by R_D binding. The data in Krishnamurthy et al. (6) clearly illustrate that in the absence of cAMP, PDE does not bind R_D . This fact tells us that one function of the allosteric interaction between PDE and R_D binding is to ensure that PDE (even if it is already expressed in the cell) is precisely activated only when the cAMP level is high.

Finally, it is noteworthy that Krishnamurthy et al. (6) observe that at short timescales, the conformational flexibility at CNB:A decreases upon cAMP binding, whereas at long time-

scales, it increases. This suggests broader ensembles including conformational changes far away, as well as possible perturbations upon ligand dissociation. This significant observation argues that probing binding site flexibility without accounting for time regimes may not capture accurately the conformational attributes upon binding.

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REFERENCES

1. Langeberg, L. K., and J. D. Scott. 2015. Signalling scaffolds and local organization

of cellular behaviour. *Nat. Rev. Mol. Cell Biol.* 16:232–244.

2. Moleschi, K., and G. Melacini. 2014. Signaling at crossroads: the dialogue between PDEs and PKA is spoken in multiple languages. *Biophys. J.* 107:1259–1260.
3. Krishnamurthy, S., B. S. Moorthy, ..., G. S. Anand. 2014. Active site coupling in PDE:PKA complexes promotes resetting of mammalian cAMP signaling. *Biophys. J.* 107:1426–1440.
4. Nussinov, R., S. Muratcioglu, ..., O. Keskin. 2015. The key role of calmodulin in KRAS-driven adenocarcinomas. *Mol. Cancer Res. molcanres.0165.2015.*
5. Tsai, C. -J., and R. Nussinov. 2014. A unified view of “how allostery works”. *PLOS Comput. Biol.* 10:e1003394.
6. Krishnamurthy, S., N. K. Tulsian, ..., G. S. Anand. 2015. Parallel allosteric relays mediated by cAMP and PDE coordinate activation and termination phases in cAMP signaling. *Biophys. J.* 109:1251–1263.
7. Kaveti, S., and J. R. Engen. 2006. Protein interactions probed with mass spectrometry. *Methods Mol. Biol.* 316:179–197.
8. Hoofnagle, A. N., K. A. Resing, and N. G. Ahn. 2003. Protein analysis by hydrogen exchange mass spectrometry. *Annu. Rev. Biochem. Biomol. Struct.* 32:1–25.
9. Wang, H., Y. Liu, ..., H. Ke. 2007. Structural insight into substrate specificity of phosphodiesterase 10. *Proc. Natl. Acad. Sci. USA.* 104:5782–5787.