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Perspectives

PERSPECTIVES

The Love Story between Orai Calcium Entry Channels and Adenylyl Cyclases Gets even more Complicated

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A Perspective on "AKAP79 Orchestrates a Cyclic AMP Signalosome Adjacent to Orai1 Ca^{2+} Channels"

Those of us who are devotees of physiology (and hence loyal readers of *Function*) are likely from time to time to sit back, take a broader view, and simply marvel at the astounding complexity of living organisms. The way in which cellular signaling systems are able to seamlessly integrate so many different biochemical activities is particularly remarkable, and, moreover, absolutely crucial for the ability of the organism to maintain normal homeostasis and respond to stressors. Nuanced interactions between different signal transduction pathways, for example, second messenger systems like Ca^{2+} and cyclic AMP,[1] are at the heart of generating the mind-boggling diversity needed to simultaneously control so many cellular functions in so many different cell types.

In this issue, Kar and colleagues have revisited one such interaction, namely the intimate connection between store-operated Ca^{2+} entry via plasma membrane-localized Orai channels and adenylyl cyclases (ACs) situated adjacent to these channels.[2] This privileged spatial arrangement is important because of the potential for calcium ions to regulate cAMP production by ACs.[3] Conversely, cAMP has reported effects on Ca^{2+} influx via Orai channels,[4] potentially creating a tidy local feedback loop between the two messengers.

Such interactions can be difficult to dissect because of the multitude of ways in which Ca^{2+} and cAMP signals reciprocally regulate one another. Investigators must contend with the incredible isoform diversity presented by each of these signaling pathways. This is especially true for the cAMP pathway. There are 10 distinct ACs in mammals. These include nine transmembrane isoforms of adenylyl cyclase (AC1–AC9) and one "soluble" isoform (AC10 or "sAC). AC1, AC8, and sAC are activated by Ca^{2+} (via calmodulin or CaM in the case of AC1 and AC8), whereas AC5 and AC6 are directly inhibited by Ca^{2+}. There are also over 50 different PDEs and notably, members of the PDE1 family are stimulated by Ca^{2+}/CaM. Among the cAMP effectors, there are three types of Protein Kinase A (PKA) catalytic subunits (encoded by PRKACA, PRKACB, and PRKACG) and four types of PKA regulatory subunits (PRKAR1A, PRKAR1B, PRKAR2A, and PRKAR2B). Cyclic AMP signals are also mediated to a lesser extent by Epac proteins (exchange proteins directly activated by cAMP) and other emerging cAMP effectors (e.g., "CRIS," "POPDC"). There also exist multiple types of AKAPs (A-kinase anchoring proteins) that coordinate signaling proteins such as PKA regulatory subunits and their targets. A given cell type has its own "fingerprint" of isoform expression, so it is rarely possible to generalize findings in one cell type to all others.

Adding to the confusion is the fact that certain elements in signaling complexes can dynamically relocate, creating the potential for high levels of complexity in this crosstalk. For example, ER Ca^{2+} store depletion, which leads to the opening of Orai channels through interactions with ER-transmembrane STIM1 proteins clustered in membrane contact sites under the plasma membrane, also promotes recruitment and binding of AKAP79 to Orai. In this setting, AKAP79 serves as a coordinating center for multiple signaling proteins (calcineurin, ACs, PKA holoenzyme, PDEs, CaM, and the transcription factor NFAT). The importance of this arrangement is now well established: Ca^{2+}-dependent effectors are assembled and poised to "see" a high Ca^{2+} concentration near the pore of a Ca^{2+} channel, thereby enhancing the efficiency and fidelity of information transfer.[4–9]

The Ca^{2+}-activated adenylyl cyclase, AC8, is among the established targets of this type of regulation. Heterologous expression of AC8 in HEK293 cells was shown by the Cooper lab to directly interact with Orai1,[3,6] where it was subjected to markedly elevated local Ca^{2+} concentrations during Ca^{2+} influx

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(as assessed using genetically encoded Ca^{2+} reporters conjugated to AC8). AC8 tagged with FRET-based cAMP biosensors further revealed high concentrations of cAMP in this microdomain following Ca^{2+} entry. It's worth noting that local cAMP signals generated in this way can completely bypass the conventional mode of cAMP signaling through G-protein coupled receptors (GPCRs).

A recent paper by Zhang et al. proposed that endogenous AC8 in HEK 293 cells responded to Ca^{2+} entry through activated Orai1 channels to produce cAMP that locally stimulated PKA situated near Orai1, brought together by AKAP79.[4,10] PKA phosphorylation promoted rapid Ca^{2+}-dependent inactivation of Orai gating ("fast CDI") to put the brakes on Ca^{2+} entry. The group clearly showed that PKA had actions on Orai inactivation kinetics, and this translated into downstream effects on patterns of Ca^{2+} oscillations and nuclear translocation of NFAT4. However, work by Kar et al. in this issue of *Function* now calls into question the expression of an endogenous AC8 isoform in HEK293 cells.[2] Mass spec and qPCR showed no evidence for AC8 and indicated issues with the commercial AC8 antibody used by Zhang et al. Critically, using a sensitive FRET-based cAMP biosensor fused to AKAP79 ("AKAP79-CUTie"), the group also did not find evidence for the production of cAMP driven by store-operated Ca^{2+} entry through Orai channels in HEK 293 cells. Their Co-IP evidence suggests that a complex consisting of PKA, calcineurin and NFAT moves close to Orai channels after store depletion. A major new finding of the group is that PDE4, a Ca^{2+}-insensitive PDE, is also part of the complex when it becomes clustered adjacent to activated Ca^{2+} entry channels. The authors propose that this arrangement should improve the insulated nature of the cAMP/PKA microdomain near the pore of the channel.[2]

Reminiscent of the superstructure in HEK-293 cells, Brzezinska et al. very recently published a report of a complex in human arterial smooth muscle cells (HASMC) containing Orai1, AKAP79, and PKA, but which also incorporated the Ca^{2+}-sensitive PDE isoform, PDE1C.[8] Evidence for the possible presence of AC8 within this signalosome was also obtained. Inhibiting PDE1C was shown to reduce store-operated Ca^{2+} entry and selectively regulate the formation of leading-edge protrusions of migrating HASMCs.

In light of the results of Kar et al., the findings of Zhang and colleagues are even more intriguing. Why is PKA is situated in the Orai/AKAP79 signalsome? Kar et al. note that NFAT is a substrate for PKA and is negatively regulated by PKA phosphorylation, which prevents dephosphorylation by calcium-dependent calcineurin. In real life (not in the lab setting), cAMP is typically generated through the activation of G(alpha)s-coupled GPCRs. Kar et al. speculate that Ca^{2+}-inhibitable AC6 (the main AC isoform in HEK cells) could be a constituent of the complex coordinated by AKAP79. In this scenario, Ca^{2+} entry would keep AC6 in an inhibited state, reduce the chance of PKA phosphorylation of NFAT, and thereby not generate conflicting signals with respect to NFAT activation during Ca^{2+} entry. Future experiments on this topic will surely prove interesting.

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