"A-kinase" regulator runs amok to provide a paradigm shift in cAMP signaling

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The activity of the archetypal protein kinase A (PKA) is typically thought of in regards to the catalytic subunit, which is inhibited by the regulatory subunits in the absence of cAMP. However, it is now reported that one of the regulatory subunit isoforms (PKA-RIα) takes on a function of its own upon binding to cAMP, acting independently of this canonical cAMP signaling mechanism. PKA-RIα instead binds to and stimulates the catalytic activity of a guanine nucleotide exchange factor (P-REX1) that itself promotes Rac1 GTPase activation. This newly discovered function of PKA-RIα adds an additional layer of complexity to our understanding of cAMP signal transduction.

The second messenger cyclic AMP (cAMP) acts in part by stimulating cAMP-dependent protein kinase (PKA).[2] In the inactive PKA holoenzyme, the intrinsic kinase activities of the catalytic subunits are inhibited by their association with regulatory subunits. Binding of cAMP to these regulatory subunits releases activated catalytic subunits that phosphorylate diverse cellular proteins to elicit a broad range of cellular responses. Thus, in this canonical model, the regulatory subunits are appropriately named, with catalytic subunits adopting the active role in downstream signaling. A new paper by Adame-García *et al.* (1) questions this paradigm by showing that the regulatory subunit isoform PKA-RIα acts independently of the catalytic subunit to directly activate a guanine nucleotide exchange factor (GEF) designated as P-REX1.

P-REX1 is the phosphatidylinositol 3,4,5-trisphosphate (PIP_3) -dependent Rac exchange factor 1 (2). Its catalytic GEF activity activates Rho GTPases, which are small G proteins that include RhoA, CDC42, and Rac1, to regulate cytoskeletal remodeling, cell migration, cell adhesion, and mitosis. Although P-REX1 GEF activity is known to be inhibited by PKA-catalyzed phosphorylation (2), Adame-García *et al.* now report (1) that PKA-RIα interacts directly with P-REX1 to increase its GEF activity, an effect that is independent of PKA catalytic subunit activity. This surprising finding is an outgrowth of the same laboratory's prior study, which used a yeast 2-hybrid screen to demonstrate that a fragment of P-REX1 containing two PDZ domains (P-REX1–PDZ1–PDZ2) acted as "bait" to capture its "prey," which corresponded to a C-terminal fragment of PKA-RIα (3). At that time, the P-REX1 interaction site on PKA-RIα was established to be cyclic nucleotide-binding domain B (CNBD-B), and this site recognized the P-REX1–PDZ1–PDZ2 domains (Fig. 1) (3). However, the functional significance of this interaction, if any, was unknown.

The P-REX1–PKA-RIα interaction is now further evaluated by Adame-García *et al.* (1) so that its modulation by cAMP is revealed. The authors use cAMP analogs that selectively activate PKA holoenzymes containing type I but not type II (PKA-RII) regulatory subunits. Treatment of MCF-7 breast cancer cells with these PKA-RIα–selective analogs activates both P-REX1 and Rac1 (1). Furthermore, siRNA knockdown of PKA-RIα blocks cAMP-dependent activation of P-REX1, whereas siRNA knockdown of P-REX1 blocks cAMP-dependent activation of Rac1 (1). The actions of these cAMP analogs are unaffected by siRNA knockdown of PKA catalytic subunit isozyme α (Cα), thereby demonstrating that PKA-RIα exerts novel PKA catalytic subunit–independent actions to control cellular function (1).

In vitro pulldown assays using natural cAMP immobilized to agarose beads demonstrate co-purification of P-REX1 and PKA-RIα from MCF-7 cell lysates, thereby indicating a direct interaction of P-REX1 and PKA-RIα (1). However, this is not the case for pull-downs performed using beads to which the cAMP antagonist (R_p)-8-AHA–cAMPS is immobilized. Importantly, (R_p)-8-AHA–cAMPS binds PKA-RIα assembled within the PKA holoenzyme, but fails to reproduce the action of natural cAMP to liberate PKA-RIα upon holoenzyme dissociation. Thus, P-REX1 most likely binds to free PKA-RIα generated as a consequence of cAMP-induced holoenzyme dissociation. This conclusion is substantiated using purified P-REX1 and PKA-RIα (1).

Additional studies of Adame-García *et al.* (1) investigate how binding of cAMP to PKA-RIα influences P-REX1 GEF activity. Using HEK293 cells transfected with a CNBD-B–R335K PKA-RIα mutant that is insensitive to cAMP, it is reported that unlike wild-type PKA-RIα, this mutant interacts with P-REX1 in a pull-down assay using P-REX1–PDZ1–PDZ2 as the bait (1). Because this assay is performed using cells not treated with cAMP, the

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[2] The abbreviations used are: PKA, protein kinase A; P-REX1, phosphatidylinositol 3,4,5-trisphosphate-dependent Rac exchange factor 1; C, catalytic; R, regulatory; CNB-B, cyclic nucleotide-binding domain B; ACRO, acrodysostosis; PIP_3, phosphatidylinositol 3,4,5-trisphosphate.

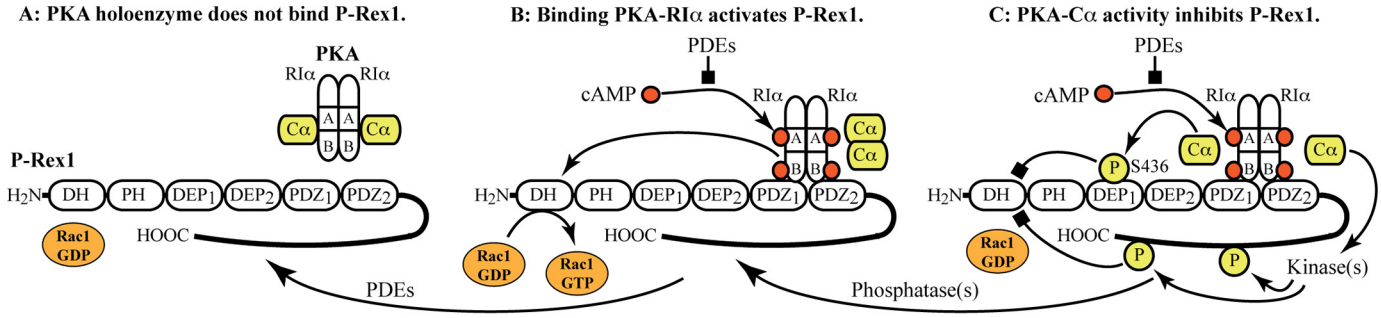

Figure 1. Effects of PKA on P-REX1 activity. *A*, at low levels of cAMP, PKA exists mainly as a holoenzyme consisting of regulatory (RIα) and catalytic (Cα) subunits that do not bind to or phosphorylate P-REX1. *B*, when levels of cAMP rise, the A and B binding sites on RIα are occupied by cAMP, thereby stimulating PKA holoenzyme dissociation. RIα then binds to PDZ domains of P-REX1 to stimulate the GEF activity of the DH domain, thereby activating Rac1. *C*, activated PKA Cα subunits phosphorylate P-REX1 at Ser-436 of the DEP1 domain, so that P-REX1 GEF activity is inhibited. Phosphatases maintain P-REX1 in the unphosphorylated state so that it can be activated by RIα, whereas phosphorylated P-REX1 cannot be activated by RIα. These actions of cAMP are counteracted by cyclic nucleotide phosphodiesterases (*PDEs*) that hydrolyze cAMP. Thus, the balance of P-REX1 cycling between nonphospho and phospho states dictates up- or down-regulation of P-REX1 GEF activity in response to cAMP.

CNBD-B–R335K mutation might result in a binding gain-of-function that supports a cAMP-independent interaction of P-REX1 and PKA-RIα. However, additional functional assays demonstrate that the mutant PKA-RIα does not support P-REX1 activation in the absence of added cAMP (1). Therefore, for wild-type PKA-RIα, it seems likely that binding of cAMP to CNBD-B is in fact necessary for stimulated P-REX1 GEF activity.

Findings obtained using mutant Δ366–379 PKA-RIα that is missing 14 amino acids in the C-tail of CNBD-B further illuminate the role that cAMP plays as a determinant of P-REX1 GEF activity. This mutant is cAMP-insensitive, and it is designated as ACRO because it is found in patients with acrodysostosis (ACRDYS) skeletal dysplasia. For HEK293 cells not treated with cAMP, the ACRO mutant binds strongly to P-REX1–PDZ1–PDZ2 (1), as might be expected if it too acquires a binding gain-of-function not found in wild-type PKA-RIα. However, unlike assays performed using the R335K PKA-RIα mutant, there are high levels of active P-REX1 in MCF-7 cells transfected with the ACRO mutant but not treated with cAMP (1). The author's explanation for these findings is that the Δ366–379 truncation of CNBD-B alters its C-tail structure so that it binds to and constitutively activates P-REX1 (1). Thus, the overall findings provide a new paradigm by which cAMP exerts a nonconventional action to control cellular function.

How is it possible to explain the seemingly paradoxical opposing actions of PKA to either enhance GEF activity through direct binding of PKA-RIα to P-REX1 or to instead down-regulate GEF activity through phosphorylation of P-REX1? Adame-García *et al.* (1) propose that a discrete pool of P-REX1 is exclusively activated by its direct binding to PKA-RIα if and only if P-REX-1 is not previously phosphorylated by PKA (Fig. 1). This would mean that opposing PKA catalytic subunit activity phosphorylates P-REX1 so that it is sequestered in an inactive state that must be dephosphorylated before it can be directly activated by PKA-RIα (Fig. 1). Thus, there is cycling of P-REX1 between nonphospho and phospho states so that the balance of this cycling dictates up- or down-regulation of P-REX1 GEF activity in response to increased levels of cAMP (1). How would this pool of unphosphorylated P-REX1 be established? Potentially, this pool of P-REX1 is under tight regulatory control by Ser/Thr protein phosphatases localized within cytosolic microdomains that maintain P-REX1 in its unphosphorylated state so that it can be directly activated by PKA-RIα (Fig. 1).

A critically important question that remains to be determined is whether this new and nonconventional model of PKA-RIα action can generally be applied to other forms of cAMP signaling that are PKA-mediated. More speculatively, there may exist intricate signal transduction cross-talk in which G protein–coupled receptors and/or growth factor receptors modulate cAMP-dependent control of P-REX1. In fact, P-REX1 GEF activity is already established to be under the control of G protein βγ subunits, PIP_3, and mTOR signaling pathways (2), thereby providing exciting new opportunities to explore what we thought to be a well-understood process.

References

1. Adame-García, S. R., Cervantes-Villagrana, R. D., Orduña-Castillo, L. B., del Rio, J. C., Gutkind, J. S., Reyes-Cruz, G., Taylor, S. S., and Vázquez-Prado, J. (2019) cAMP-dependent activation of the Rac guanine exchange factor P-REX1 by type I protein kinase A (PKA) regulatory subunits. *J. Biol. Chem.* **294,** 2232–2246 CrossRef Medline
2. Welch, H. C. (2015) Regulation and function of P-Rex family Rac-GEFs. *Small GTPases* **6,** 49–70 CrossRef Medline
3. Chávez-Vargas, L., Adame-García, S. R., Cervantes-Villagrana, R. D., Castillo-Kauil, A., Bruystens, J. G., Fukuhara, S., Taylor, S. S., Mochizuki, N., Reyes-Cruz, G., and Vázquez-Prado, J. (2016) Protein kinase A (PKA) type I interacts with P-Rex1, a Rac guanine nucleotide exchange factor: Effect on PKA localization and P-Rex1 signaling. *J. Biol. Chem.* **291,** 6182–6199 CrossRef Medline