MAP1A Light Chain 2 Interacts with Exchange Protein Activated by Cyclic AMP 1 (EPAC1) to Enhance Rap1 GTPase Activity and Cell Adhesion*

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We have recently demonstrated that light chain 2 (LC2) of the microtubule-associated protein MAP1A interacts with the cyclic AMP (cAMP)-binding domain of exchange protein directly activated by cyclic AMP 1 (EPAC1). In the present study we used a simultaneous expression system and found that LC2 enhances both basal and 8-(4-chloro-phenylthio)-2′-O-methyladenosine-3′,5′-cytidine monophosphate (8-CPT-2Me-cAMP)-stimulated Rap1 activation by EPAC1. LC2 is known to stabilize microtubules; therefore we examined whether microtubules enhanced Rap1 activation by LC2. Nocodazole inhibited Rap1 activity in cells transfected with EPAC1 alone but had little effect on Rap1 activity in cells transfected with both EPAC1 and LC2. This indicates that part of the actions of LC2 in enhancing EPAC1 activity may be through stabilization of microtubules. We also found that in cells transfected with LC2, Rap1 was more sensitive to activation by 8-CPT-2Me-cAMP. Moreover, LC2 enhanced the ability of transfected and endogenous EPAC1 to interact with cyclic AMP-agarose, indicating that LC2 elicits conformational changes in the cAMP domain of EPAC1, enhancing its ability to be activated by cyclic AMP. We also found that disruption of the interaction of endogenous EPAC1 and LC2 with antibodies to the cAMP domain of EPAC1 abolished Rap1 activity in PC12 cell lysates, demonstrating the importance of LC2 for EPAC1 activation in these cells. Consistent with a role of EPAC1 in controlling integrin activity, we found that cell adhesion to laminin was enhanced in LC2- and EPAC1-transfected cells stimulated with 8-CPT-2Me-cAMP. LC2 is therefore a biological enhancer of EPAC1 activity toward Rap1 and associated downstream signaling mechanisms.

Cyclic AMP is a ubiquitous second messenger the synthesis of which is coupled to the activation of many G-protein-coupled receptors to regulate a wide variety of cellular responses (1). Activation of heterotrimeric G-proteins stimulates production of cyclic AMP by one or more isoforms of adenyl cyclase. Cyclic AMP phosphodiesterases degrade cyclic AMP to 5′AMP and thereby regulate intracellular concentrations of cyclic AMP (2). Cyclic AMP exerts its effects via cyclic AMP-dependent protein kinase (PKA), a cyclic AMP-gated ion channels, and two isoforms of exchange protein directly activated by cyclic AMP (EPAC) (2). EPAC proteins, otherwise known as cyclic AMP guanine nucleotide exchange factors, represent a new mechanism for determining signaling specificity within the cyclic AMP signaling pathway (3–6). Prior to the discovery of EPACs it was presumed that the principal intracellular receptor for cyclic AMP was PKA in many cell types (7).

EPACs are specific guanine nucleotide exchange factors for the Ras GTPase homologues, Rap1 and Rap2, which EPACs activate independently of PKA (4, 5). Rap GTPases cycle between inactive GDP- and active GTP-bound forms. Specific guanine nucleotide exchange factors like EPAC1 convert Rap proteins to the active form, whereas GTPase-activating proteins complete the cycle by converting Rap to the inactive form (8). Rap1 appears to function as an antagonist of Ras signaling by trapping Ras effectors (such as Raf-1) in an inactive complex (8). However, numerous reports indicate that Rap1 signaling independent of Ras is also important in controlling cellular processes (8). One of the most consistent findings is the involvement of Rap1 in integrin-mediated cell adhesion (8). The cyclic AMP-EPAC-Rap1 pathway has been implicated in the control of cell spreading and cell adhesion through the α3β1 integrin (9), whereas the cyclic AMP-EPAC-Rap2B pathway has been shown to control the activity of phospolipase Cε (10).

We have recently discovered that EPACs can physically interact with light chain 2 (LC2) of the microtubule-associated protein 1A (MAP1A) (11). MAP1A and LC2 are generated as a single mRNA in the same open reading frame and are translated as a pre-MAP1A/LC2 polypeptide. The pre-MAP1A/LC2 protein is then proteolyzed to yield the 249-amino acid LC2 polypeptide and 2556-amino acid MAP1A protein (12). LC2 has been shown to interact with the actin and microtubular cytoskeleton and has been shown to catalyze the polymerization of microtubules (13). Our identification of EPAC as a protein-binding partner for LC2 suggests that interaction with microtubules might be functionally important for EPAC activity. Indeed, this seems to be plausible in light of recent results from members of the Cheng laboratory (14), who used immunolocalization to demonstrate that EPAC1 associates with the mitotic spindle during the M-phase of the COS7 cell cycle. LC2 may therefore serve as an “adaptor” protein promoting the interaction of EPAC with the microtubular cytoskeleton, perhaps to facilitate activation of Rap1 (13, 15–18). In the present study...
we investigate the role of LC2 and the microtubule cytoskeleton in regulating the cyclic AMP-EPAC-Rap1 pathway.

**EXPERIMENTAL PROCEDURES**

**Materials—** Anti-LC2 polyclonal antibody and rat LC2 cDNA were gifts from Professor Friedrich Propst, University of Vienna. Cyclic AMP-agarose was purchased from Sigma.

**Antibodies—** Specific polyclonal antibodies against EPAC1 were generated and affinity-purified using the synthetic EPAC1 peptide spanning residues 41–60, (CD)FSESLEQASTVLRAG, by Alpha Diagnostic International Inc. (San Antonio, TX). Polynomial antibody elution fractions were stored at −70 °C. Polyclonal antibodies against the DEP and cAMP domains of EPAC1 were generated by Diagnostics Scotland (Carluke, Scotland) using fusion protein immunogens formed between glutathione S-transferase (GST) and the individual PCR-amplified domains as described (11).

**Generation of Expression Vectors—** For simultaneous expression of Myc-tagged EPAC1 and LC2 in mammalian cells we used the pBUDCE4.1 (Invitrogen) vector. pBUD-EPAC1Myc/LC2 was generated by subcloning the full open reading frames for LC2 and EPAC1 into the EF-1α and cytemagalovirus multiple cloning sites of pBUDCE4.1, respectively. LC2 was inserted at the XhoI/KpnI site in the EF-1α multiple cloning site, and EPAC1 was inserted at the HindIII/Xhali site in the cytemagalovirus multiple cloning site.

**Growth of Cell Lines—** HEK293 and COS1 cells were grown in Dulbecco’s modified Eagle’s medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (Sigma), 2 mM l-glutamine (Sigma), and a 2% solution of penicillin-streptomycin. HEK293 and LC2 were transiently transfected with either pBUD-EPAC1Myc or pBUD-EPAC1Myc/LC2 and then lysed in 55 mM Tris-HCl, pH 7.4, 132 mM NaCl, 22 mM NaF, 11 mM sodium pyrophosphate, 10 mM magnesium chloride, 1% (v/v) Triton X-100 containing complete protease inhibitor mixture (Roche Applied Science). Cell extracts were incubated with 10 μg of immobilized GST-RalGDS-RBD for 60 min at 4 °C and then with 50 μL of GST for 1 h. The beads were then washed four times in lysis buffer followed by SDS-PAGE and immunoblotting with anti-Rap1 antibody.

**Cyclic AMP-Agarose Pull-down Assay—** HEK293 cells were transiently transfected with either pBUD-EPAC1Myc or pBUD-EPAC1Myc/LC2 and then lysed in 55 mM Tris-HCl, pH 7.4, 132 mM NaCl, 22 mM sodium fluoride, 11 mM sodium pyrophosphate, 1.1 mM EDTA, and 1% Triton X-100 containing complete protease inhibitor mixture (Roche Applied Science). A lysis buffer containing 5% glacial acetic acid, 1% Triton X-100. The lysates were clarified by centrifugation at 10,000 × g for 1 min at 4 °C. After equalizing the protein concentration using the Bradford assay, cell lysates were serially diluted at a ratio of 1:2 and then incubated with 50 μL of GST-cyclic AMP-agarose beads on a rotator for 1 h at 4 °C. The beads were then washed three times with lysis buffer, resuspended in sample buffer, and separated by SDS-PAGE.

**Protein concentrations were measured by the method of Bradford,** using bovine serum albumin as a standard (20).

**RESULTS**

**LC2 Enhances EPAC-activated Rap1—** We recently identified by yeast two-hybrid screens LC2 as a protein interaction partner for EPAC (11). To test the functional consequences of this interaction in cells we generated pBUD expression vectors that allowed the expression of LC2 (pBUD-LC2) and Myc-tagged EPAC1 (pBUD-EPAC1Myc) individually or synchronously (pBUD-EPAC1Myc/LC2) in mammalian HEK293 cells (Fig. 1A). LC2 has been reported to interact with microtubules in vivo and in vitro, and it induces rapid polymerization of tubulin (13). Treatment of HEK293 cells with the microtubule depolymerizing agent nocodazole led to disruption of the microtubule network throughout the cell, resulting in a dispersed cytoplasmic staining pattern throughout the cytoplasm (Fig. 1B).

In contrast treatment of cells with taxol appeared to stabilize microtubules forming aggregated structures in cells (Fig. 1C). In cells expressing LC2, microtubule integrity was maintained despite treatment of cells with nocodazole (Fig. 1B). This is consistent with a previous report that LC2 is necessary for stabilization of microtubules (13). Expression of LC2 had little apparent effect on taxol-treated cells (Fig. 1B). Quantification of these experiments revealed that around 30% of cells expressing LC2 were protected against the depolymerizing effects of nocodazole. This equates to the degree of expression of LC2 in the culture population.

We next tested the involvement of microtubule stability and LC2 expression on basal Rap1 activation by EPAC1. Cells were transfected with LC2, EPAC1, or a combination of the two, and Rap1 activity was measured using the RalGDS-RBD-GST pull-down assay described previously (19). Interestingly we found that transfection with LC2 alone caused a small activation of Rap1 (Fig. 1, C and D). Given the ability of LC2 to stabilize microtubules, this suggests that Rap1 activity may be sensitive to microtubule stability. Transfection with EPAC1 gave a greater activation of Rap1 than LC2 (Fig. 1, C and D). The ability of EPAC1 to activate Rap1 independently of activation is probably due to a subpopulation of transfected EPAC1 being activated by endogenous cyclic AMP levels. The largest increase in basal Rap1 activation was observed following cotransfection of cells with both EPAC1 and LC2 (Fig. 1, C and D). This suggests that LC2 may enhance EPAC1 activity toward Rap1. To test an involvement of microtubules in the enhancement of EPAC1 activity by LC2, we pretreated cells with nocodazole, and this was found to abolish EPAC1-activated Rap1 but did not affect LC2-activated Rap1 (Fig. 1, C and D). Nocodazole only partially inhibited Rap1 activation by the combination of LC2 and EPAC1 (Fig. 1, C and D). Treatment with taxol had little effect on Rap1 activation (Fig. 1, C and D). This indicates that EPAC1 can increase basal Rap1 levels in a mechanism that involves polymerized microtubules. In addition, LC2 is capable of enhancing EPAC1 activity toward Rap1 and protecting EPAC-activated Rap1 from nocodazole treatment, probably through its ability to stabilize the cellular microtubule network (Fig. 1B).
LC2 Enhances Activation of the EPAC1-Rap1 Pathway by 8-CPT-2Me-cAMP—

We next sought to examine whether LC2 affects activation of Rap1 by the EPAC-specific cyclic AMP analogue, 8-CPT-2Me-cAMP (21). We found that transfection of cells with pBUD-EPAC1Myc facilitated a small, 2–3-fold activation of Rap1 by 8-CPT-2Me-cAMP (Fig. 2, A and B). However, in cells transfected with a combination of EPAC1 and LC2 (pBUD-EPAC1Myc/LC2) we witnessed a very large, 100-fold activation of Rap1, equivalent to the levels achieved with the non-hydrolysable GTP analogue GTP$_\gamma$S (Fig. 2, A and B). This demonstrates that LC2 cooperates with EPAC1 to enhance Rap1 activation by 8-CPT-2Me-cAMP. We tested this further by examining the effect of different concentrations of 8-CPT-2Me-cAMP on Rap1 activation (Fig. 2C). We found that in EPAC1-transfected cells a small activation of Rap1 occurred at 1 $\mu$m 8-CPT-2Me-cAMP and was maximal at 100 $\mu$m (Fig. 2C).
In contrast, in cells transfected with both EPAC1 and LC2, Rap1 activation was observed at lower concentrations of 8-CPT-2Me-cAMP (0.1 μM), and at maximal concentrations (100 μM) Rap1 activation was greater than that observed in cells transfected with EPAC1 alone (Fig. 2C). This suggests that LC2 not only increases the sensitivity of EPAC1 to activation by 8-CPT-2Me-cAMP but that it also increases maximal activation by this cyclic AMP analogue. We found that nocoda-
zole had little effect on 8-CPT-2Me-cAMP-stimulated levels of Rap1 in pBUD-EPAC1Myc/LC2-transfected cells; however, it did disrupt stimulated Rap1 levels in pBUD-EPAC1Myc-transfected cells (Fig. 2D). This suggests that in addition to enhancing cyclic AMP-stimulated Rap1 levels, LC2 protects Rap1 activity against microtubule disruption. This is probably because of the ability of LC2 to stabilize the microtubular network (Fig. 1B).

**LC2 Enhances Interaction of EPAC1 with Cyclic AMP**—We demonstrated previously that the interaction between EPAC1 and LC2 occurs through the cAMP-binding domain of EPAC1 (11). This led us to speculate that the enhancement of EPAC1 sensitivity to activation by 8-CPT-2Me-cAMP (Fig. 2C) elicited by LC2 might occur through modification of the conformation of the EPAC1 cAMP domain. To examine this we tested the ability of various concentrations of EPAC1 to interact in pull-down assays with cyclic AMP-agarose beads (Fig. 3A). HEK293 cells were transfected with pBUD-EPAC1Myc or pBUD-EPAC1Myc/LC2, and various concentrations of cell lysates were precipitated with cyclic AMP-agarose (Fig. 3A). Cell lysates were also probed with anti-Myc antibody to show that co-transfection with LC2 did not affect the expression levels of endogenous EPAC1. Experiments were carried out three times with similar results. D) Immunoblots from the experiment described in Fig. 3C were scanned densitometrically, and the values were plotted as means ± S.E. for three separate experiments.

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**FIG. 3.** LC2 enhances the ability of EPAC1 to interact with cyclic AMP. A, cell lysates were prepared from HEK293 cells that had been transfected with pBUD-EPAC1Myc or pBUD-EPAC1Myc/LC2 and then incubated with cyclic AMP-agarose beads. EPAC1 bound to the beads was detected by immunoblotting with an anti-Myc antibody (lower panel). In the upper panel, cell lysates were probed with anti-Myc antibody to demonstrate that co-transfection with LC2 did not affect the expression of EPAC1Myc. Experiments were carried out three times with similar results. B, densitometric results from the experiment in A were plotted as means ± S.E. for three separate experiments. C, cell lysates were prepared from HEK293 cells that had been transfected with pBUD-LC2 and then incubated with cyclic AMP-agarose beads. Endogenous EPAC1 bound to the beads was detected by immunoblotting with an anti-EPAC1 antibody (lower panel). In the upper panel, cell lysates were probed with anti-Myc antibody to demonstrate that transfection with LC2 did not affect the expression levels of endogenous EPAC1. Experiments were carried out three times with similar results. D, immunoblots from the experiment described in Fig. 3C were scanned densitometrically, and the values were plotted as means ± S.E. for three separate experiments.
expression with LC2 did not significantly affect the expression levels of EPAC1Myc (Fig. 3A). We found that half-maximal binding of EPAC1 to cyclic AMP was increased by approximately 1 order of magnitude in cells transfected with EPAC1 and LC2 compared with cells transfected with EPAC1 alone (Fig. 3, A and B). This suggests that LC2 can modify EPAC1 to enhance its interaction with cyclic AMP, which might explain why EPAC1 co-transfected with LC2 is activated at lower concentrations of 8-CPT-2Me-cAMP (Fig. 2C).

We next tested whether expression of LC2 could affect the ability of EPAC1 endogenously expressed in COS1 cells to interact with cyclic AMP (Fig. 3, C and D). Expression of LC2 in COS1 cells did not affect the expression levels of endogenous EPAC1 as determined by immunoblotting cell lysates with a specific anti-peptide EPAC1 antibody (Fig. 3C). However, expression of LC2 in cells dramatically increased the ability of endogenous EPAC1 to interact with cyclic AMP (Fig. 3, C and D). Consistent with our EPAC1/LC2 co-expression experiments, LC2 enhanced the half-maximal binding of endogenous EPAC1 with cyclic AMP by approximately 1 order of magnitude (Fig. 3D). Together these results suggest that the ability of LC2 to enhance EPAC1-activated Rap1 may be through sensitizing EPAC1 to activation by cyclic AMP.

Interaction between Endogenous EPAC1 and LC2 Is Required for Rap1 Activation—Thus far we had used co-transfection to demonstrate enhancement of EPAC1-activated Rap1 by LC2. We next decided to test the significance of interaction between endogenous EPAC1 and LC2. Because LC2 interacts with EPAC1 through the EPAC1 cAMP domain we generated anti-cAMP polyclonal antibody and tested its ability to inhibit interaction between EPAC1 and LC2 in COS1 cell lysates (Fig. 4A). Cell lysates were prepared from PC12 cells, which express both EPAC1 and LC2 (Fig. 4A), and then incubated with the anti-cAMP antibody or an anti-DEP domain antibody as a control (Fig. 4A). These antibodies had previously been found to recognize EPAC1 by immunoblotting but were unable to immunoprecipitate endogenous EPAC1 (results not shown). Next,
cell lysates were immunoprecipitated with an anti-LC2 antibody, and immunoprecipitates were resolved by SDS-PAGE and immunoblotted with anti-EPAC1 and anti-LC2 antibodies (Fig. 4A). Immunoblots revealed that endogenous EPAC1 co-precipitated with LC2; however, incubation with the anti-cAMP antibody blocked the interaction between EPAC1 and LC2 (Fig. 4A). This is consistent with the cAMP domain being responsible for the interaction between LC2 and EPAC1 (11). In contrast the anti-DEP antibody did not affect the interaction between EPAC1 and LC2 (Fig. 4A).

We next tested whether antibody competition for LC2 binding to EPAC1 could affect Rap1 activation. Cell lysates were prepared from PC12 cells, which express both EPAC1 and LC2 (Fig. 4A), or COS1 cells, which express EPAC1 (Fig. 3C) but not LC2 (results not shown). These were then incubated with anti-cAMP or anti-DEP antibodies and then stimulated with diluent or 8-CPT-2Me-cAMP (Fig. 4, B and C). Rap1 activity was then measured using the RalGDS-RBP-GST pull-down assay (19). Stimulation with 8-CPT-2Me-cAMP prompted an ~2-fold activation of Rap1 in PC12 cell lysates; however, Rap1 activity in COS1 cell lysates was hardly affected (Fig. 4, B and C). Incubation of PC12 cell lysates with the anti-cAMP antibody, which inhibits interaction between EPAC1 and LC2 (Fig. 4A), also completely abolished basal Rap1 levels and prevented further activation by 8-CPT-2Me-cAMP (Fig. 4, B and C). Pretreatment of PC12 cell lysates with the anti-DEP antibody, which did not affect the interaction between EPAC1 and LC2 (Fig. 4A), had no effect on basal or stimulated Rap1 levels. In contrast, treatment of COS1 cell lysates, which do not express LC2, with the anti-cAMP antibody did not affect basal or stimulated Rap1 levels. These results demonstrate that inhibition of endogenous LC3 interaction with endogenous EPAC1 prevents the ability of EPAC1 to activate Rap1. Together with the previous co-transfection experiments this suggests that LC2 is an important enhancer of EPAC1 activity toward Rap1.

EPAC and LC2 Cooperate to Enhance Cell Adhesion to Laminin—It has been reported recently that the cyclic AMP-EPAC-Rap1 pathway regulates cell adhesion to laminin through the α3β1 integrin (9). Given that LC2 appears to enhance EPAC1-activated Rap1 (Fig. 2C) we examined whether this is translated into effects on cell adhesion. Consistent with our observations of Rap1 activation, we found that maximal cell adhesion was seen in HEK293 cells stimulated with 8-CPT-2Me-cAMP and co-transfected with both LC2 and EPAC1 (Fig. 5A). Approximately 30% more cell adhesion was seen in these cells compared with cells transfected with EPAC1 alone (Fig. 5A). We also tested the effects of nocadazole and taxol in regulating cell adhesion. We found again, consistent with our observations of Rap1 activation, that nocadazole, but not taxol, blunted cell adhesion in EPAC1-transfected cells but not in cells transfected with LC2 alone or LC2 plus EPAC1 (Fig. 5A). This effect is probably because of the ability of LC2 to stabilize microtubules in the presence of nocadazole (Fig. 1B) and suggests that an intact microtubular cytoskeleton is required for proper cell adhesion.
adhesion. We also found in cells transfected with the pBUD vector alone that 8-CPT-2Me-cAMP stimulation approximately doubled the amount of cell adhesion to laminin, and this was severely inhibited by nocadazole (Fig. 5A). We were surprised by this result because we had previously found that there was no detectable Rap1 activation in pBUD-transfected HEK293 cells (Fig. 2D). This suggests either that 8-CPT-2Me-cAMP can promote cell adhesion independently of Rap1 or that very low levels of Rap1 activity, below the level of sensitivity of our activation assay, are required to promote cell adhesion.

To confirm that Rap1 controls cell adhesion in these cells we transfected cells with constitutively active Rap1V12 and dominant negative Rap1N17 (22). Transfection with Rap1V12 was found to enhance cell adhesion to laminin in the presence or absence of 8-CPT-2Me-cAMP, whereas Rap1N17 dramatically inhibited cell adhesion (Fig. 5B). Importantly Rap1N17 inhibited the promotion of cell adhesion promoted by treatment of cells with 8-CPT-2Me-cAMP (Fig. 5B). Together with our Rap1 activity data (Fig. 2D) this supports the idea that activation of very low levels of endogenous Rap1 is sufficient to promote cell adhesion. Together these results demonstrated that Rap1 is a key determinant in the regulation of cell adhesion in these cells. Moreover, the enhancement of signal transduction through the cyclic AMP-EPAC-Rap1 pathway by LC2 has functional consequences for cell behavior.

**DISCUSSION**

We demonstrated previously that LC2 interacts directly with the cyclic AMP-binding domain of EPAC1 (11). In the present study we have found that co-transfection of cells with LC2 and EPAC1 enhances the ability of 8-CPT-2Me-cAMP to activate Rap1 in a dose-dependent manner (Fig. 2C). Moreover, its reported ability to stabilize microtubules (13), LC2 appears to protect Rap1 activation from the disruptive effects of nocadazole treatment (Fig. 1, C and D). Our results suggest that microtubule stability is critical for EPAC1 to be able to activate Rap1. The ability of LC2 to protect EPAC1-activated Rap1 activity may be important in cells where differences in spatial stability and dynamics of the microtubular cytoskeleton may be essential for critical cell processes, for example, in neuron development. In addition, LC2 may also play a role in maintaining EPAC1 activity during mitosis because EPAC1 has been reported to associate with the microtubules of the mitotic spindle (14).

In addition to its effects on microtubule stability LC2 appears to have direct effects on the ability of EPAC1 to activate Rap1. This appears to be through direct interaction with EPAC1 because antibody competition for the binding of endogenous LC2 to the cyclic AMP-binding domain of endogenous EPAC1 was shown to completely ablate basal and 8-CPT-2Me-cAMP-stimulated Rap1 levels in PC12 cell lysates (Fig. 4, A and B). Furthermore, our data suggest that the amplification of stimulated Rap1 levels by LC2 may be through as yet undetermined conformational changes in the cyclic AMP-binding domain of EPAC1. This is based on two lines of evidence. First, co-transfection of LC2 with EPAC1 lowers the minimal dose of EPAC-specific 8-CPT-2Me-cAMP required to activate Rap1 (Fig. 2B). Second, LC2 appears to enhance the ability of the cyclic AMP-binding domain of EPAC1 to directly interact with immobilized cyclic AMP (Fig. 2C). LC2 therefore appears to be an enhancer of EPAC1 function by increasing sensitivity to activation by cyclic AMP. In this respect the cyclic AMP-binding domain of EPAC1 is thought to have a lower sensitivity to cyclic AMP than PKA in vitro (23). Half-maximal activation of PKA occurs in vitro at around 1 μM cyclic AMP, whereas half-maximal activation of EPAC1 occurs at 40 μM (23). It could be envisaged that through direct protein interaction with suitable binding partners like LC2, the activation of EPAC1 by cyclic AMP could be “tuned” to sensitivities approaching those of PKA. This could occur in a tissue-specific manner, thereby adapting the cyclic AMP-EPAC1-Rap1 pathway to the requirements of particular cell types. Careful structural analysis of the LC2/EPAC1 interaction domain will have to be carried out to determine the mechanisms of LC2 enhancement of EPAC1 function.

**REFERENCES**

1. Beavo, J. A., and Brunton, L. L. (2002) *Nat. Rev. Mol. Cell Biol.* 3, 710–718
2. Houslay, M. D. (1998) *Semin. Cell Dev. Biol.* 9, 161–167
3. Rehmann, H., Rueppel, A., Bos, J. L., and Wittinghofer, A. (2003) *J. Biol. Chem.* 278, 23508–23514
4. Kawasaki, H., Springett, G. M., Mochizuki, N., Toki, S., Nakaya, M., Matsuda, M., Housman, D. E., and Grayshb, A. M. (1998) *Science* 282, 2275–2279
5. de Rooij, J., Zwartkruis, F. J., Verheijen, M. H., Cool, R. H., Nijman, S. M., Wittinghofer, A., and Bos, J. L. (1998) *Nature* 396, 474–477
6. Rehmann, H., Prakash, B., Wolf, E., Rueppel, A., de Rooij, J., Bos, J. L., and Wittinghofer, A. (2003) *Nat. Struct. Biol.* 10, 26–32
7. Engh, R. A., and Bossemeyer, D. (2001) *Adv. Enzyme Regul.* 41, 121–149
8. Bos, J. L., de Rooij, J., and Reedquist, K. A. (2001) *Nat. Rev. Mol. Cell Biol.* 2, 369–377
9. Enserink, J. M., Price, L. S., Methi, T., Mahic, M., Sonnenberg, A., Bos, J. L., and Tasken, K. (2004) *J. Biol. Chem.* 279, 44889–44896
10. Schmidt, M., Evelin, S., Weerink, P. A., van Dorp, F., Rehmann, H., Lomasney, J. W., and Jakobs, K. H. (2001) *Nat. Cell Biol.* 3, 1020–1024
11. Magiera, M. M., Gupta, M., Rundell, C. J., Satish, N., Ernens, I., and Yarwood, S. J. (2000) *Biochem. J.* 382, 803–810
12. Langkopf, A., Hammarback, J. A., Muller, R., Valerie, R. B., and Garner, C. C. (1992) *J. Biol. Chem.* 267, 16561–16566
13. Noiges, R., Eichinger, R., Kutschera, W., Fischer, I., Nemeth, Z., Wichte, G., and Preuß, F. (2002) *J. Neurosci.* 22, 2106–2114
14. Qiao, J., Mei, F. C., Popov, V. L., Vergara, L. A., and Cheng, X. (2002) *J. Biol. Chem.* 277, 26581–26586
15. Arzuma, Y., and Dasso, M. (2000) *Curr. Opin. Cell Biol.* 12, 302–307
16. Kahana, J. A., and Cleveland, D. W. (1999) *J. Cell Biol.* 146, 1205–1210
17. Kalab, P., Ru, P. R., and Dasso, M. (1999) *Curr. Biol.* 9, 481–484
18. Nishimoto, T. (2000) *J. Biol. Chem.* 275, 14657–14662
19. McPhee, I., Houslay, M. D., and Yarwood, S. J. (2000) *FEBS Lett.* 477, 213–218
20. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254
21. Enserink, J. M., Christiansen, A. E., de Rooij, J., van Trigt, M., Schwede, F., Geneser, H. G., Doskeland, S. O., Blank, J. L., and Bos, J. L. (2002) *Nat. Cell Biol.* 4, 901–906
22. Cook, S. J., Rubinfeld, B., Albert, I., and McCormick, F. (1993) *EMBO J.* 12, 3475–3485
23. Bos, J. L. (2003) *Nat. Rev. Mol. Cell Biol.* 4, 733–738
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