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Pak Activity Regulates Calpain-dependent Degradation of E3b1

Susan Chi[P], Soohee Chang[1], Dongeun Park[1]

School of Biological Sciences, Seoul National University, Seoul 151-742

E3b1, a binding partner of Eps8 plays a critical role in the receptor tyrosine kinase-mediated Rac activation by facilitating the interaction of Eps8 with Sos-1 and the consequent activation of the Rac-specific GEF activity of Sos-1. Here we present evidence that E3b1 protein levels are regulated by the Ca2+-activated protease calpain whose activity is in turn regulated by the activity of Pak, a key effector molecule of E3b1-Eps8-Sos-1 tricomplex. Serum starvation of Rat2 or COS7 cells resulted in rapid loss of E3b1 that was reversible by calpain inhibitors. This degradation of E3b1 was blocked by expression of active Pak1 mutant, Pak1H83, 86L. Activation of endogenous Pak by expression of active Rac1 mutant, Rac1G12V, also inhibited the degradation of E3b1 upon serum starvation. In contrast, inhibition of endogenous Pak activity by expression of Pak autoinhibitory domain (AID) elicited the degradation of E3b1 even in the presence of serum. Taken together, these findings indicate that E3b1 can be down-regulated by calpain activation and stabilized by Pak activation. The regulation of E3b1 level through integration of various signals that affect on the activity of calpain or Pak may provide a fine tuning mechanism for receptor tyrosine kinase-mediated Rac activation.

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Interaction between Epithin and Filamin Is Essential for the Release of Epithin by PMA

Yongcheol Cho[P], Chanhee Kang[1], Chungho Kim[1], Hyoseon Lee[1], Dongeun Park[1]

School of Biological Sciences, Seoul National University, Seoul 151-747

To analyze the signaling mechanism of epithin release, we treated mouse thymic epithelial cell line, with phorbol ester (PMA, phorbol 12-myristate 13-acetate). PMA treatment induced the release of epithin into culture medium and also translocation of epithin to cell-cell contacts where epithin may be released by autoactivation. PMA-induced release and translocation were inhibited by nonspecific protein kinase inhibitor, staurosporin and also by the p38 MAPK inhibitor, SB203580, but not by the Erk MAPK inhibitor, PD98059. Surprisingly, the translocation of epithin was accompanied by the rearrangement of actin microfilaments from stress fibers into cortical actin filaments, and this rearrangement of actin microfilaments was inhibited by SB203580. In addition, an actin disruption agent, cytochalasin D, inhibited PMA-induced release and translocation of epithin. These results suggest that p38 MAPK pathway may regulate the release of epithin through rearrangement of actin microfilaments. Next, to identify the linker mocule between Epithin and actin filament, we search the epithin cytoplasmic domain binding protein using yeast two hybrid system. As one of interesting clone, actin cross-linking protein, filamin B was identified as a epithin cytoplasmic binding protein and this interaction was further confirmed by pull-down and co-immunoprecipitation assay. Interestingly, PMA induced the translocation of filamin to cortical region and also increased the interaction of epthin with filamin. These results suggest that filamin could be a linker molecule between epithin and cortical actin, and the actin microfilaments dependent-translocation of epithin to cell-cell contact could be mediated by filamin.

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Activation of Thyroid Stimulating Hormone β-subunit Gene by Transcription Factor LIM Homeodomain (Lhx2)

Kee Kwang Kim[P], Jae Hoon Lee[1], Jin Su Park[1], Jee Yeon Park[1], Kyoon Eon Kim[C]

Department of Biochemistry, Institute of Biotechnology, Chungnam National University, Taejon 305-764

Previous studies showed that pLIM (Lhx3) functionally cooperates with Pit1 to stimulate transcription of the TSH (Thyrotropin Stimulating Hormone) subunit gene. However the molecular basis of the TSH subunit gene regulation by Lhx2 (LIM Homeobox 2) in anterior pituitary thyrotropes are not well understood thus far. In this study, deletion analysis was employed to delineate the sequences of the TSH subunit gene which are involved in Lhx2 regulation. Transient transfection experiment demonstrated that the -170 / -79 region of the TSH subunit gene was necessary and sufficient for the activation of this gene by Lhx2. Also, gel mobility shift assay also showed that homeodomain of Lhx2 binds to the -170 / -79 region of the TSH subunit promoter. In addition, DNase I protection analysis demonstrated differences in DNA digestion patterns in between 101 to 73 region, where the repeated sequences of 5'- GCAATT-3' were identified. Mutant studies revealed that this region is critical for Lhx2 effect. Real-time PCR was employed to show that cAMP elevated the intracellular Lhx2 mRNA level. All these data being taken together, we concluded that Lhx2 is required for full activity of the TSH subunit gene expression in the thyrotrope cells. Furthermore, we propose a model, in which various physiological signals elevate intracellular cAMP levels in thyrotrope cells, which in turn stimulates Lhx2 gene expression and thus TSH subunit gene expression.

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Role of cAMP Regulation by cAMP-specific Phosphodiesterase (apPDE) in Synaptic Plasticity in *Aplysia*

Min-Jeong Kim[P], Jin-A Lee[1], Changhoon Lee[1], Deok-Jin Chang[1], Seung-Hee Lee[1], Hyoung Kim[1], Yong-Seok Lee[1], Bong-Kiun Kaang[C]

National Research Laboratory of Neurobiology, Institute of Molecular Biology and Genetics, School of Biological Sciences, Seoul National University, Seoul 151-742

Phosphodiesterase (PDE) plays an important role in synaptic plasticity via cAMP regulation in various organisms. However, the role of PDE was not studied in the synaptic plasticity of *Aplysia*. Thus, we cloned a novel *Aplysia* PDE (apPDE) and characterized its function. The apPDE protein exhibited cAMP-specific PDE activity which was specifically inhibited by rolipram and IBMX. In situ hybridization analysis showed that apPDE mRNA was located in bag cells, sensory cells etc. subcellular localization. We characterized the role of apPDEin synaptic plasticity by overexpression and RNA interference of apPDE gene. Overexpressed apPDE reduced membrane excitability induced by 5-HT treatment. In addition, overexpression or inhibition of apPDE gene blocked both short-term and long-term facilitation. However, apPDE overexpression did not block synaptic facilitation at depressed synapses. These results indicate that cAMP regulation by apPDE plays an important role in synaptic plasticity of *Aplysia* in a specific manner.