E127 βPix binds dynamin GTPase though SH3 domain : A role in the regulation of the function of dynamin by phosphorylation

Hyun-Jung Park*, Seung-Hye Lee and Dongeun Park
School of Biological Science, Seoul National University, Seoul, Korea

βPix is a SH3 domain-containing protein that is highly concentrated in nerve terminals where it colocalized with proteins involved in synaptic vesicle recycling. Here we show that SH3 domain of βPix bound to dynamin that plays an essential role in the regulation of receptor-mediated endocytosis. We also have examined the subcellular localization of overexpressed βPix and dynamin in COS7 and neuroblastoma cells. βPix and dynamin were detected in endosome-like structure that colocalized with a marker for fluid-phase uptake. In co-transfected cells, vesicle structures were larger and aggregated than in non-transfected cells. In addition we observed that the level of dynamin phosphorylation upregulated by βPix co-expression. Dynamin phosphoryl-ation was reduced by src kinase inhibitor. Our data demonstrate that βPix functionally interacts with dynamin and localizes to endosomal compartment where βPix regulates the function of dynamin by protein phosphorylations.

E128 βPix-b_L, a novel isoform of βPix, generated by alternative translation, induces macropinocytosis

Sangmyung Rhee*, Sujung Yang, Seung Joon Lee and Dongeun Park
School of Biological Sciences, Seoul National University

βPix, a Pak-interacting exchange factor, is known to be involved in the regulation of Rho family small GTPases. Here we characterize a novel 105-kDa βPix isoform, βPix-b_L, which expression is regulated by internal ribosome entry site in the 5'UTR of βPix-b. The extra N-terminus contains a putative calponin homology (CH) domain. To investigate its cellular function, we transiently expressed wild type or mutant cDNA of βPix-b_L in COS7 cells. Interestingly, expression of βPix-b_L results in an induced macropinocytosis and this event is completely blocked by the DH mutant of βPix-b_L. These results indicate that βPix-b_L enhances Rac activity, and then inducing pinocytosis through activated Rac. However, inhibition of pinocyotosis by DH mutant cannot be recovered by co-expression of dominant active Rac or Pak, suggesting Rac or Pak activation by βPix-b_L may be necessary but not sufficient. Meanwhile, biochemical analysis and immunofluorescent microscopy reveal that the CH domain of βPix-b_L is able to interact with tubulin and actin. Taken together, precise positioning of βPix-b_L along microtubule or actin is responsible to induce micropinocytosis through Rac and Pak activation.

E129 Molecular Cloning and Characterization of a Novel Mouse βPix Isoform

Taeho Kim* and Dongeun Park
School of Biological Sciences, Seoul National University, Seoul, Republic of Korea

βPix, a Pak-interacting guanine nucleotide exchange factor is known to be involved in the regulation of Cdc42/Rac GTPases and Pak kinase activity. Currently, three βPix isoforms, βPix-a, -b, and c have been reported. In this study, the partial cDNA of a novel βPix splice variant was isolated from a mouse brain cDNA library and the full cDNA of this splice variant was found using 5'-RACE. The cloned βPix isoform, named βPix-d, lacks leucine zipper domain that is present in other βPix isoforms and essential for βPix dimerlizaion, and has a 11 amino acid addition at carboxyl terminus and novel 3'-UTR. *In situ* hybridization studies with the βPix-d specific probes in

the rat embryo show that βPix-d isoform is expressed mainly in the central nervous system. In contrast to other βPix isoforms, βPix-mediated membrane ruffles are not detected and the cellular localization of βPix-d is mainly in nucleus in NIH3T3 fibroblast. NLS sequences in GIT1 binding domain of βPix-d are critical for nuclear localization of βPix-d. These finding imply that βPix-d might have novel function in nucleus.

E130 Calcineurin-Dependent Dephosphorylation of Ryanodine Receptor Down-Regulates Activity of the Ca2+ Release Channel in Skeletal Muscle

Dong Wook Shin* and Do Han Kim
Department of Life Science, Kwangju Institute of Science and Technology

Calcineurin is a Ca^{2+} and calmodulin-dependent protein phosphatase with diverse cellular functions. Here we examined the physical and functional interactions between calcineurin and RyR/Ca^{2+} release channel in skeletal C2C12 myotubes. Co-immunoprecipitation experiments revealed that the association between RyR and calcineurin exhibits a strong Ca^{2+} dependence. This association involves a Ca^{2+} dependent interaction between calcineurin and FK506-binding protein (FKBP12), an accessory subunit of RyR. Pretreatment with cyclosporin A (CsA), an inhibitor of calcineurin, enhanced the caffeine-induced Ca2+ release (CICR) in C2C12 cells. Overexpression of a constitutively active form of calcineurin in C2C12 cells, △CnA(deletion of 391-521 a. a), resulted in a decrease in CICR. This decrease in CICR activity was partially recovered by pretreatment with CsA. Furthermore, overexpression of an endogenous calcineurin inhibitor (cain) or an inactive form of calcineurin (△CnA(H101Q)) resulted in upregulation of CICR. Taken together, our data suggest that. calcineurin-mediated dephosphorylation of RyR through FKBP12 may play an important role in the Ca^{2+} signaling of muscle contraction and relaxation.

E131 The Physiological Role of Asp-Rich Region of Calsequestrin in the Regulation of Ca^{2+} Homeostasis of Skeletal Muscle

Dong Wook Shin*, Jae Man Lee and Do Han Kim
Department of Life Science Kwangju Institute of Science and Technology

Calsequestrin (CSQ) is a high capacity Ca^{2+} binding protein in the junctional sarcoplasmic reticulum (SR) of striated muscles, and has been shown to regulate the RyR / Ca^{2+} release channel through triadin and junctin. We previously reported that *asp*-rich region (352-367 a.a) of CSQ binds to triadin as well as Ca^{2+} (Shin, D et al., 2000). Here, we investigated the physiological role of this region on the channel activity of RyR by measuring cytoplasmic Ca^{2+} concentration using C2C12 skeletal myotubes. Overexpression of wt CSQ in C2C12 cells enhanced caffeine-induced Ca^{2+} release, whereas overexpression of *asp*-rich region deleted CSQ (△*asp*-CSQ; deletion of 352-367 a.a) reduced the caffeine-induced Ca^{2+} release. In addition, overexpression of △*asp*-CSQ recovered the peak amplitude of depolarization-induced Ca^{2+} release which was down-regulated by overexpressed wt CSQ. Furthermore, overexpression of △*asp*-CSQ restored thapsigargin-induced Ca^{2+} and Mn^{2+} influxes which was markedly diminished in wt CSQ-overexpressed myotubes. Taken together, these findings suggest that the *asp*-rich region is essential for function of CSQ and Ca^{2+} homeostasis of skeletal muscle.

E132 NF-kB Attenuates 3-Hydroxykynurenine-Induced Neuronal Cell Death

Hyun Jung Lee[1], Myoung Woo Lee[2]*, Hee Sun Chae[1], Jae Hyung Bach[1], Myung Ju Shin[2] and Soon Cheol Park[2]
[1]Department of Anatomy, College of Medicine and [2]Department of Life Science, College of Natural