

**E127  $\beta$  Pix binds dynamin GTPase though SH3 domain : A role in the regulation of the function of dynamin by phosphorylation**

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$\beta$  Pix 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  $\beta$  Pix bound to dynamin that plays an essential role in the regulation of receptor-mediated endocytosis. We also have examined the subcellular localization of overexpressed  $\beta$  Pix and dynamin in COS7 and neuroblastoma cells.  $\beta$  Pix 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  $\beta$  Pix co-expression. Dynamin phosphorylation was reduced by src kinase inhibitor. Our data demonstrate that  $\beta$  Pix functionally interacts with dynamin and localizes to endosomal compartment where  $\beta$  Pix regulates the function of dynamin by protein phosphorylations.

**E128  $\beta$  Pix-b<sub>L</sub>, a novel isoform of  $\beta$  Pix, generated by alternative translation, induces macropinocytosis**

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$\beta$  Pix, 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  $\beta$  Pix isoform,  $\beta$  Pix-b<sub>L</sub>, which expression is regulated by

internal ribosome entry site in the 5'UTR of  $\beta$  Pix-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  $\beta$  Pix-b<sub>L</sub> in COS7 cells. Interestingly, expression of  $\beta$  Pix-b<sub>L</sub> results in an induced macropinocytosis and this event is completely blocked by the DH mutant of  $\beta$  Pix-b<sub>L</sub>. These results indicate that  $\beta$  Pix-b<sub>L</sub> enhances Rac activity, and then inducing pinocytosis through activated Rac. However, inhibition of pinocytosis by DH mutant cannot be recovered by co-expression of dominant active Rac or Pak, suggesting Rac or Pak activation by  $\beta$  Pix-b<sub>L</sub> may be necessary but not sufficient. Meanwhile, biochemical analysis and immunofluorescent microscopy reveal that the CH domain of  $\beta$  Pix-b<sub>L</sub> is able to interact with tubulin and actin. Taken together, precise positioning of  $\beta$  Pix-b<sub>L</sub> along microtubule or actin is responsible to induce micropinocytosis through Rac and Pak activation.

**E129 Molecular Cloning and Characterization of a Novel Mouse  $\beta$  Pix Isoform**

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$\beta$  Pix, 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  $\beta$  Pix isoforms,  $\beta$  Pix-a, -b, and c have been reported. In this study, the partial cDNA of a novel  $\beta$  Pix 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  $\beta$  Pix isoform, named  $\beta$  Pix-d, lacks leucine zipper domain that is present in other  $\beta$  Pix isoforms and essential for  $\beta$  Pix dimerization, and has a 11 amino acid addition at carboxyl terminus and novel 3'-UTR. *In situ* hybridization studies with the  $\beta$  Pix-d specific probes in

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

### **E130** Calcineurin-Dependent Dephosphorylation of Ryanodine Receptor Down-Regulates Activity of the $Ca^{2+}$ Release Channel in Skeletal Muscle

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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  $Ca^{2+}$  release (CICR) in C2C12 cells. Overexpression of a constitutively active form of calcineurin in C2C12 cells,  $\Delta$ 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 ( $\Delta$ 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

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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 ( $\Delta$ *asp*-CSQ; deletion of 352-367 a.a) reduced the caffeine-induced  $Ca^{2+}$  release. In addition, overexpression of  $\Delta$ *asp*-CSQ recovered the peak amplitude of depolarization-induced  $Ca^{2+}$  release which was down-regulated by overexpressed wt CSQ. Furthermore, overexpression of  $\Delta$ *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- $\kappa$ B Attenuates 3-Hydroxykynurenine-Induced Neuronal Cell Death

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