

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

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β 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 β 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 β Pix and dynamin in COS7 and neuroblastoma cells. β 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 β Pix co-expression. Dynamin phosphorylation was reduced by src kinase inhibitor. Our data demonstrate that β Pix functionally interacts with dynamin and localizes to endosomal compartment where β Pix regulates the function of dynamin by protein phosphorylations.

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

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β 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 β Pix isoform, β Pix-b_L, which expression is regulated by

internal ribosome entry site in the 5'UTR of β 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 β Pix-b_L in COS7 cells. Interestingly, expression of β Pix-b_L results in an induced macropinocytosis and this event is completely blocked by the DH mutant of β Pix-b_L. These results indicate that β Pix-b_L 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 β Pix-b_L may be necessary but not sufficient. Meanwhile, biochemical analysis and immunofluorescent microscopy reveal that the CH domain of β Pix-b_L is able to interact with tubulin and actin. Taken together, precise positioning of β Pix-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 β Pix Isoform

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

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

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Calcineurin is a Ca²⁺ and calmodulin-dependent protein phosphatase with diverse cellular functions. Here we examined the physical and functional interactions between calcineurin and RyR/Ca²⁺ release channel in skeletal C2C12 myotubes. Co-immunoprecipitation experiments revealed that the association between RyR and calcineurin exhibits a strong Ca²⁺ dependence. This association involves a Ca²⁺ 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, Δ 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 (Δ 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 Ca²⁺ signaling of muscle contraction and relaxation.

E131 The Physiological Role of Asp-Rich Region of Calsequestrin in the Regulation of Ca²⁺ Homeostasis of Skeletal Muscle

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Calsequestrin (CSQ) is a high capacity Ca²⁺ binding protein in the junctional sarcoplasmic reticulum (SR) of striated muscles, and has been shown to regulate the RyR / Ca²⁺ 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 Ca²⁺ (Shin, D et al., 2000). Here, we investigated the physiological role of this region on the channel activity of RyR by measuring cytoplasmic Ca²⁺ concentration using C2C12 skeletal myotubes. Overexpression of wt CSQ in C2C12 cells enhanced caffeine-induced Ca²⁺ release, whereas overexpression of *asp*-rich region deleted CSQ (Δ *asp*-CSQ; deletion of 352-367 a.a) reduced the caffeine-induced Ca²⁺ release. In addition, overexpression of Δ *asp*-CSQ recovered the peak amplitude of depolarization-induced Ca²⁺ release which was down-regulated by overexpressed wt CSQ. Furthermore, overexpression of Δ *asp*-CSQ restored thapsigargin-induced Ca²⁺ and Mn²⁺ 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 Ca²⁺ homeostasis of skeletal muscle.

E132 NF- κ B Attenuates 3-Hydroxykynurenine-Induced Neuronal Cell Death

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