Cell Research (2006) 16:S131-S157

www.nature.com/cr

APOCB2006-09-001

Large-scale identification of novel M-phase phosphoproteins

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Large collection of phosphopreoteins is essential for understanding cellular signaling pathways and ultimately allows insights into numerous disease pathologies, since protein phosphorylation plays an important role in cell cycle regulation. Monoclonal antibody MPM-2 recognizes a large set of mitotic-specific phosphoproteins in a phosphorylation-dependent manner and therefore provided a specific tool for investigating significance of phosphorylation in cell cycle process. However, only minority of the MPM-2 antigens have been identified so far. Here we described an application of existing techniques for large-scale identification of phosphorproteins recognized by MPM-2. Mitotic extracts were resolved by two-dimensional gel electrophoresis and the separated spots were excised from 2D gels and subjected directly to western blotting to screen for MPM-2 binding. The spots which were reactive with MPM-2 were selected, and the corresponding proteins were collected from a couple of parallel 2D gels and pooled together for SDS-electrophoresis. After once more Western blot with MPM2 antibody, the counterpart of positive band was cut from a parallel gel for MS identification. Using this strategy, 100 protein spots were screened and 22 proteins containing potential MPM-2 epitope were identified in addition to Topoiosmerase II, MAP-4 and HSP70 as known MPM-2 antigens. These results were further validated by cross-immunoreaction, co-immunoprecipitation and immunofluorescence. This unprecedented large collection of new MPM-2 phosphoproteins permitted a detailed accounting of cellular regulation pathways managed by phosphorylation.

***Keywords*:** MPM-2 antigens; phosphoprotein; cell cycle

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APOCB2006-09-002

The comparison on structure between extracellular polypeptide signals of plant and human cytokines

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Extracellular polypeptide signals of plant has been proved that they are important signals in cell transduction as extracellular polypeptide signals of animals. A lot of informations can be found from extracellular polypeptide signals of animals, such as cytokines and cell growth factors of human, But only few kinds of extracellular polypeptide signals of plant and their receptor have been found , which are systemin, CLV3, ENDO40, PSK and SCR. The common character that very correlative amino acid composition of four kinds of extracellular polypeptide including extracellar polypeptide signals of Microbe, extracellar polypeptide signals of plant, cykokines of human and cell growth factors of human have been reported. Through this common character of amino acid composition, some maybe new extracellular polypeptides of plant had been found. Using tools of bioinformatics, amino acid sequences of those extracellular polypeptide signals from plants , animals were studied. Some interesting results are found that there are common characters about extracellular polypeptide signals from different species, which is the very correlative antigenicity and hydrophobicity of extracellular polypeptides signals. All those can prove that there are same characters in 3D structure about extracellular polypeptide signals from different species. By the software on the Internet, we compare the structure of calmodulin and cytokines of human. Using the results and findings from dabases of Families of Structurally Similar Proteins (FSSP), we give a 3D structure of receptor of calmodulin, which may be an important new extracellular polypeptide signals of plant. What we have found would help find new plant extracellular polypeptide signals and their receptors.

***Keywords*:** plant; cytokine; structure; bioinformatics; apoplast

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APOCB2006-09-003

Improper control of gap junctions by dysfunctional protein kinase C gamma leads to cell death

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The passage of apoptotic signals through open gap junctions is linked to oxidative stress-induced neurodegeneration, a pathogenesis of various neurodegenerative disorders. Protein kinase C gamma (PKCgamma) responds to

oxidative activation by H_2O_2 and subsequently decreases cell-to-cell gap junctional communication. Missense mutations in PKCgamma cause the spinocerebellar ataxia type 14 (SCA-14), a late onset, neurodegenerative disorder. To elucidate the mechanism of PKCgamma SCA-14 mutations on neurodegeneration, we reproduced these SCA-14 mutations H101Y, S119P, and/or G128D in neural HT22, RGC-5, lens epithelial N/N1003A cells, and in mice. Expression of the SCA-14 PKCgamma mutations had the following effects: 1) PKCgamma SCA-14 mutations caused a loss of cell-to-cell gap junctional communication control. 2) The cells with PKCgamma SCA-14 mutations had higher active caspase-3 levels which were further elevated by H_2O_2, and this resulted in increased cell apoptosis. 3) Inhibition of gap junctions by 18-alpha-glycyrrhetinic acid abolished the apoptotic effects of SCA-14 mutations and/or H_2O_2. 4) PKCgamma mutants had similar (H101Y) or lower (S119P, G128D) basal enzyme activities, and were not activated by H_2O_2. 5) Overexpression of the PKCgamma mutations diminished endogenous PKCγ enzyme activity, PKCγ autophosphorylation on Thr514, and PKCgamma membrane translocation. 6) A C1B1 synthetic peptide can restore the responses of SCA-14 mutants to H_2O_2. 7) extensive purkinje neuron degeneration and retinal ganglion cell layer damage occur in PKCgamma H101Y transgenic mice. Our results conclusively demonstrate that the effect of PKCgamma SCA-14 mutations on gap junctions may result in propagation of injury signals to neural cells through open gap junctions resulting in neural cell death. Thus, improper control of gap junctions by PKCγ SCA-14 mutations during oxidative stress may contribute to neurodegenerative disorders such as SCA-14.

Keywords**:** protein kinase C gamma; gap junctions; cell death; apoptosis; neurodegeneration

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APOCB2006-09-004

c-Abl is required for the F-actin assembly triggered by L-selectin crosslinking

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Recruitment of leukocytes from the blood into tissues is controlled by a variety of adhesion molecules on the surface of the endothelium and circulating leukocytes. L-selectn is one of these molecules. It constitutively expresses on leukocytes and mediates the initial capture and subsequent rolling of leukocytes along ligands expressed on endothelial cells. In addition to its role in adhesion, an intracellular signaling role for L-selectin has been recognized. Crosslinking of human L-selectin with monoclonal antibody mobilized intracellular Ca^{2+}, increased tumor necrosis factor α and interleukin 8 mRNA expression, induced O^{2-} generation, and enhanced some proteins phosphorylation. Its cytoplasmic domain is involved in signal transduction following L-selectin crosslinking and in the relation of receptor binding activity in response to intracellular signals. In our present work, we demonstrated that L-selectin crosslinking led to F-actin polymerization and redistribution in human neutrophils. Using immuno-fluorescence microscopy, we observed that F-actin redistribution spatiotemporally related to the polarization of L-selectin. STI571, a specific inhibitor for cytoplasmic tyrosine kinase c-Abl, can inhibit F-actin polymerization and c-Abl redistribution in the activated neutrophils. c-Abl is a nonreceptor tyrosine kinase ubiquitously expressed and contains a catalytic domain, polyproline rich regions, and SH2 and SH3 domains that are involved in protein-protein interactions and may also regulate the kinase activity. c-Abl could regulate actin cytoskeleton in different activated cell lines. In our work, we showed that the kinase activity of c-Abl was greatly increased and it was spatially reclined to the region where F-actin and L-selectin concentrated in the activated neutrophils. Furthermore, we also observed that c-Abl was co-immunoprecipitated with L-selectin. The association between L-selectin and c-Abl was reduced by cytochalasin B. These results suggested that c-Abl was involved in the F-actin alteration triggered by L-selectin crosslinking in human neutrophils.

Keywords**:** L-selectin; c-Abl kinase; F-actin assembly; protein association

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APOCB2006-09-005

In vitro* and *in vivo* stimulation of extracellular signal-regulated kinase (ERK) by prothoracicotropic hormone in prothoracic gland cells and its developmental regulation in the silkworm, *Bombyx mori

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The present study investigated the activation of extracellular signal-regulated kinase (ERK) by prothoracicotropic hormone (PTTH) in prothoracic gland cells of the silkworm, *Bombyx mori*. The results showed that PTTH stimulated ERK phosphorylation in a time-dependent manner and ecdysteroidogenic activity *in vitro*. The ERK

phosphorylation inhibitors PD 98059 and U0 126 blocked both basal and PTTH-stimulated ERK phosphorylation and ecdysteroidogenesis. In addition, activation of glandual ERK phosphorylation by PTTH appears to be developmentally regulated with refractoriness of gland cells to PTTH occurring during the later stages of both the 4th and last larval instars. Moreover, *in vitro* activation of ERK phosphorylation of prothoracic glands by PTTH was also verified by *in vivo* experiments: injection of PTTH into day 6 last instar larvae greatly increased activity of glandular ERK phosphorylation and ecdysteroidogenesis. From these results, it is suggested that development-specific changes in ERK phosphorylation may play a role in PTTH stimulation of ecdysteroidogenesis.

***Keywords*:** ERK phosphorylation; signal transduction; development; ecdysteroidogenesis; prothoracic gland cells

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APOCB2006-09-006

Beta1-integrin orientates epithelial polarity through Rho GTPases and laminin assembly

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The kidney is primarily comprised of epithelial cells. Epithelial cells are highly polarized and their plasma membrane is divided into discrete domains. The apical surface face the lumen, and the basolateral surface interact with other cells and underlying extra-cellular matrix. Establishment and maintenance of polarity is critical to the functioning of epithelial cells. Most work on epithelial polarization has used cells grown on artificial filter support. We have used a three dimensional culture system in which is closer *in vivo* to study how beta1integrin and Rho Gtpases coordinate to control the epithelial polarization. Individual Madin-Darby canine kidney(MDCK) cells grown in collagen gel from cyst spherical cysts comprising a monolayer of cells surrounding a hollow lumen. The cells are polarized. Addition of beta1integrin function-blocking antibody AIIB2 give rise to cysts with inverted polarity. We showed that normal polarity is restored by either expression of constitutively active Rac1or addition of exogenous laminin. We also found inhibition of ROCK, a major effector of RhoA, or myosin not only revert 1integrin blockage induced phenotype, but also rescue the phenotypes induced by expression of dominant-negative Rac. These findings indicate that beta1integrin orientates polarity through Rho GTPases and laminin assembly.

***Keywords*:** beta1-integrin; laminin assembly; Rho GTPases; polarity

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APOCB2006-09-007

JWA regulates HeLa cell migration via MAPK cascades and F-actin rearrangement

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Mitogen activated protein kinase (MAPK) cascades are thought to mediate diverse biological functions such as cell growth, differentiation and migration. Here we show that JWA, a novel microtubule-associated protein (MAP) is indispensable for the rearrangement of actin cytoskeleton and activation of MAPK cascades induced by arsenic trioxide (As_2O_3) and phobol ester (PMA). JWA deficiency blocked anti-migratory effect of As_2O_3 but enhanced the migratory effect of PMA in HeLa cells. JWA SDR-SLR motifs are not only necessary for MEK–ERK activation, but for cell migration. Further studies found that JWA differentially regulates cell migration via ERK downstream effectors focal adhesion kinase (FAK) and cyclooxygenase-2 (COX-2). Our data first time provide unexpected role of JWA on HeLa cell migration behaviors.

***Keywords*:** MAPK; Factin; cell migration; Jwa gene ; signal transduction

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APOCB2006-09-008

Early myocardial cell biological changes in acute myocardium ischemia of rat

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To find a sensitive way to check the earlier changes of ischemia myocardial biological activity that can be applied in clinic induced myocardial infarction by ligation of the left anterior descending artery, the adult SD rats suffered from experimental myocardial infarction were divided into four groups: 0 min (control), 5 min, 20 min, and 45 min. The alteration of ATP content, the expression of glucose regulated protein 75 (grp 75) gene and hypoxia-inducible factor 1α (HIF1α) gene, the release of cytochrome C and the degradation of Poly (ADP-ribose) polymerase (PARP) were examined in the infarction area, the margin of the in-

farction area and the "normal" area of the myocardial tissue respectively. The results revealed that (1) the content of the ATP in the infarction area and the margin of the infarction area increased remarkably in 20 min and 45 min (2) The expression of grp 75 gene and HIF1α gene were almost unchanged. (3) The immunohistochemistry showed that the release of cytochrome C and the degradation of PARP took place within 5 min in single cells of the infarction area after ligation and expanded to large areas within 45 min. We conclude that the release of cytochrome C and the degradation of PARP in infarction area occur early in the process of the apoptosis after acute myocardial infarction and can be a sensitive way to check the early ischemia myocardial biological activity in clinic.

***Keywords*:** myocardial infarction; Grp 75; HIF1; cytochrome C; PARP

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APOCB2006-09-009

Feedback control of MKP-1 expression by p38

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Mitogen-activated Protein (MAP) kinases play a critical role in innate immune responses to microbial infection through eliciting the biosynthesis of proinflammatory cytokines. MAP phosphatases (MKP)-1 is an archetypical member of the dual-specificity phosphatase family that deactivates MAP kinases. Induction of MKP-1 has been implicated in attenuating the lipopolysaccharide (LPS) and Peptidoglycan (PGN) responses, but how the expression of the MKP-1 is regulated is still not fully understood. Here, we show that inhibition of p38 MAP kinase by specific inhibitor SB 203580 or RNA interference (RNAi) markedly reduced the expression of MKP-1 in LPS or PGN-treated macrophages, which is correlated with prolonged activation of p38 and JNK. Depletion of MAPKAP kinase 2 (MK2), a downstream substrate of p38, by RNAi also inhibited the expression of MKP-1. The mRNA level of MKP-1 is not affected by inhibition of p38, but the expression of MKP-1 is inhibited by treatment of cycoheximide. Thus, p38 MAPK plays a critical role in mediating expression of MKP-1 at post-transcriptional level. Furthermore, inhibition of p38 by SB 203580 prevented the expression of MKP-1 in LPS-tolerized macrophages and restored the activation of MAP kinases after LPS restimulation. These results indicated a critical role of p38-MK2-dependent induction of MKP-1 in innate immune responses.

***Keywords*:** MKP-1; p38; TLRs; PGN; LPS tolerance; innate immunity

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APOCB2006-09-010

Par-4 and transcription-dependent apoptosis in a model of Alzheimer's disease

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Gamma-secretase generated beta-amyloid precursor protein (APP) intracellular domain (AICD) binds to other proteins and translocates to the nucleus wherein it regulates gene expression and promotes apoptosis. AICD may therefore be responsible for contributing to signal transduction pathways that predispose to transcription-dependent apoptotic cell death in Alzheimer's disease (AD). Two major forms of AICD (as a result of alternative gamma-secretase cleavages in APP) have been reported: CTF59 and CTF57. Prostate apoptosis response-4 (Par-4) is a leucine zipper protein associated with neuronal degeneration and aberrant APP processing in AD. Structure-function analysis indicated that apoptosis induced by Par-4 is dependent on Par-4 translocation to the nucleus via a bipartite nuclear localization sequence. Thus, both AICD and Par-4 may induce cell death that was dependent on nuclear translocation. We now report that Par-4 specifically interacts with an APP C-terminal fragment (CTF) peptide corresponding to the amino acid sequence of CTF28-52, and co-expression of Par-4 significantly exacerbated apoptosis induced by CTF57. In addition, RNAi-mediated silencing of Par-4 expression protects against apoptotic cell death induced by CTF57. These results suggest that Par-4 is directly involved in regulation of cell signaling mediated by AICD. Further studies are underway to examine if Par-4 regulates AICD activity by altering nuclear translocation of AICD and/or AICD-dependent pro-apoptotic transcriptional activity. Identification of Par-4 as a critical regulatory factor of AICD activity will help to reconcile the amyloid and apoptosis hypotheses of AD, and provide valuable insight in cellular and molecular mechanisms underlying the pathogenesis of the disease.

***Keywords*:** Par-4; beta-amyloid precursor protein; tran-

scription; apoptosis; alzheimer's disease

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APOCB2006-09-011

The endogenous reactive oxygen species stimulate TNFα-induced NF-κB activation by targeting on activation of NF-κB-inducing kinase in oral squamous carcinoma cells

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Reactive oxygen species (ROS) could stimulate or inhibit nuclear factor-κB (NF-κB) signaling pathway. However researches have been done largely in settings with the exogenous ROS, and the target molecules of ROS has not been clear. In the present study a pair of oral squamous cell carcinoma (OSC) lines that with a mild difference in the endogenous ROS level was used, thus allowing for investigation of the mechanisms by which the subtle fluctuation of the endogenous ROS regulate NF-κB and the possible target. In this OSC cell model the higher inherent ROS was constituted by stable expression of antisense Mn-SOD, and confirmed by western blot for antisense SOD expression and flow cytometric detection for the basal ROS level. The endogenous ROS was slightly higher and simultaneously NF-κB activation upon TNFα stimulation was slightly more potent in cells expressing antisense SOD, namely OSC-AS-SOD, than in wild-typed (OSC-WT) cells. The difference in NF-κB activation between two cell types was enhanced by overexpression of NIK, and abrogated by overexpression of its kinase-negative mutant. Although IKKα and IKKβ, downstream of NIK, were both involved in TNFα-mediated activation of NF-κB, and overexpression of these DNA respectively promoted NF-κB activation, the difference in NF-κB activation between two cell types was not abolished by overexpressing the kinase-negative mutants of IKKα or IKKβ. Consistently, IkBα degradation pattern showed that IkBα was degraded more rapidly in response to TNFα in OSC-AS-SOD cells than in OSC-WT cells after overexpression of NIK, but this difference between two cell types almost disappeared after overexpression of NIK mutants. These data suggested that NIK might be a direct and critical target molecule of the endogenous ROS. We then evaluated whether NIK-controlled IKKα and IKKβ activation in response to TNFα stimulation differed between two cell types. The phosphorylations of IKKα and IKKβ responded to TNFα *in vivo* were more substantial in OSC-AS-SOD cells than in OSC-WT cells after overexpression of NIK. The NIK mutant delayed the phosphorylations of IKKα and IKKβ in these two cell types. Meanwhile, in vitro kinase assays of NIK showed that NIK significantly phosphorylated IKKα and IKKβ in OSC-AS-SOD cells within 10 min post-TNFα stimulation, but no phosphorylations of IKKα and IKKβ were observed in OSC-WT cells under the same conditions. The present studies demonstrated that NIK is an upstream target molecule of ROS regulation in NF-κB signaling pathway. A slight elevation of endogenous ROS promotes TNFα-stimulated NF-κB activation through facilitating the activation of NIK and subsequent phosphorylation of IKKα and IKKβ. The mechanisms by which the endogenous ROS regulate NF-κB pathway evidence that ROS may function as physiological signaling modulators on NF-κB signaling cascades through its ability to facilitate the activity of the critical target NIK. Since NF-κB transactivation controls multiple cell activities, including enhanced proliferation, resistance to apoptosis, insensitivity to cytotoxic drugs and *etc*, these findings also strongly suggest that the altered intracellular microenvironment related to redox state may influence biological behaviors of cancer cells, and therefore manipulation of redox state and NF-κB activation may interfere cancer treatment.

***Keywords*:** OSC cells; endogenous ROS; NIK (NF-κB-inducing kinase); NF-κB

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APOCB2006-09-012

Pharmacological concentration of estrogen induces growth arrest

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High doses of estrogen are believed to be present at the time of ovulation in the ovary, it is also an extremely important hormone widely used in the clinic for the prevention and treatment of postmenopausal symptoms and as contraceptives. In the present study, we examined the effect of pharmacological doses of 17 beta-estradiol (E2) on the growth of ovarian cancer HO-8910 cells and its mechanism. The growth inhibitory activity of E2 was assessed using the MTT assay, and the apoptosis of HO-8910 cells was analyzed using flow cytometry with annexin V/PI staining. The expression of estrogen receptors and the phosphorylation of p38 were examined by Western blot. We found that pharmacological doses of E2 significantly

inhibited the growth of ovarian cancer HO-8910 cells to 30% of control after 72 h of treatment and this effect was associated with a concordant increase in the number of apoptotic cells. Membrane impermeable E2-BSA had no such effect on HO-8910 cells. ER-beta, but not ER-alpha was detected in HO-8910 ovarian cancer cells, and pure antiestrogen, ICI 182.780 could not reverse the effect of E2. We also found that high dose of E2 induced a quickly but persistent activation of p38/MAPK in HO-8910 cells, and the p38/MAPK inhibitor SB203580 partially antagonized E2-induced anti-growth effects on HO-8910 cells. These data suggest that high concentrations of E2 play antiproliferative and proapoptotic roles in HO-8910 human ovarian cancer cells via non-genomic pathway and p38/MAPK signaling pathway may involved in this process.

Keywords**:** ovarian cancer; estrogen; estrogen receptor; apoptosis; p38/MAPK

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APOCB2006-09-013

A key role of Pim kinases in v-Abl oncogene-mediated cellular transformation

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Previous studies from our laboratory have demonstrated that v-Abl oncoprotein activates Jak-STAT signaling which then transcriptionally induces SOCS-1 expression (Danial *et al*., 1995, Science **269**:1875-1877; Limnander *et al*., 2004, Molecular Cell **15**: 329-341). SOCS-1 protein functions in a classical feedback loop to inhibit Jak kinases. The precise mechanism by which Abl oncogene bypasses SOCS-1 negative regulation has not been fully understood. Here we have examined the role for Pim kinases in v-Abl-mediated transformation of pre-B cells. We show that expression of Pim-1 and Pim-2, but not Pim-3 is Abl kinase dependent in v-Abl transformants and Pim kinase deficiency has profound effects on the v-Abl transformation. Bone marrow cells derived from either Pim-1-/- Pim-2-/- or Pim-1-/- Pim-2-/- Pim-3-/- mice show dramatic decrease in v-Abl transformation efficiency whereas ectopic co-expression of either Pim-1 or Pim-2 with v-Abl allows the normal transformation of Pim-1-/- Pim-2-/- pre-B cells. We have found that there are pools of Pim-1 and Pim-2 that are associated with SOCS-1 in v-Abl transformants. Interestingly, triple knockout of Pim-1, Pim-2 and SOCS-1 results in partial restoration of v-Abl transformation efficiency. Furthermore, both protein levels and phosphorylation levels of SOCS-1 are significantly reduced in Pim-1-/- Pim-2-/- Pim-3-/- v-Abl- transformed pre-B cells as compared with wild-type v-Abl transformants. These data suggest that Pim kinases play an important role in the v-Abl transformation most likely via participating in SOCS-1 modification and modulating SOCS-1 protein levels. Based on these findings, we propose a model for how Abl oncogene could overcome SOCS-1 inhibition to constitutively activate Jak-STAT signaling.

Keywords**:** Pim; Abl oncogene; SOCS-1; Jak-STAT; pre-B cell

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APOCB2006-09-014

Signaling by alpha2beta1 integrin: the role of receptor clustering and cross talk between alpha2-I and beta1-I domains

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Integrin alpha2beta1 in an abundant collagen receptor expressed on many epithelial and mesenchymal cells and on platelets. Integrins can mediate signals through the plasma membrane in the both directions. First, the activity of the integrins can be regulated by intracellular signals ("inside-out" signaling). Second, the binding of an extracellular ligand to an integrin may activate intracellular signaling pathways ("outside-in" signaling). Here, the structural requirements of signaling by alpha2beta1 integrin has been studied. The results stress the role of both receptor clustering and conformational regulation. To study the "inside-out" signaling mechanisms, we used 12-O-tetradecanoylphorbol-13-acetate (TPA) to activate protein kinase C (PKC). TPA induced a ligand independent macroaggregation of alpha2beta1 integrin. Concomitantly, TPA increased the avidity of alpha2beta1 for collagen and the number of conformationally activated alpha2beta1 integrins. The structural change was shown using monoclonal antibody 12F1, which recognizes the "open" (active) conformation of the "inserted" domain (alpha2-I) in the alpha2 subunit. E336 in alpha2-I domain regulates a switch between "open" and "closed" conformation, since mutation alpha2-E336A inhibited the TPA related increase in the number of 12F1 positive, activated integrins. Mutation E336A also reduced basic cell adhesion to collagen, but without reducing mAb 12F1 binding. E336A prevented neither the TPA related increase in adhesion to collagen nor alpha2beta1 aggrega-

tion. Thus alpha2beta1 integrin avidity is regulated by two synergistic mechanisms. First, an alpha2-E336 dependent switch to the "open" alpha2-I conformation, and second an alpha2-E336 independent mechanism associated with receptor aggregation. Culturing cells within a lattice of collagen fibrils is a classic method used to study the function of collagen receptors. Here, alpha2beta1 integrin dependent phosphorylation of p38 MAPK was used to explore the structural requirements of "outside-in" signaling within the collagen lattice. Based on molecular modeling, we propose that collagen fibrils, unlike monomers, act as multivalent ligands and induce the clustering of collagen receptors. alpha2 integrin antibodies, used as non-ligand aggregators, also activated p38, thus linking integrin activation to receptor clustering and direct signaling, rather than to an indirect integrin mechanoreceptor function. This result also raised the question, whether any conformational changes participate in signaling. Our molecular models indicate that in alpha2beta1, alpha2-E336 can act as an "intrinsic" ligand for the beta1-I domain. The corresponding residue in the beta2 integrins is known to serve as a critical switch mediating conformational responses. Here, the E336A mutation prevented p38 activation after alpha2beta1 clustering. Thus, "outside-in" signaling by alpha2beta1 requires both receptor clustering and communication between alpha and beta subunits.

Keywords**:** cell adhesion; integrins; collagen; signaling; receptor

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APOCB2006-09-015

The ubiquitin-proteasome pathway and its role in the regulation of apoptosis

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The ubiquitin-proteasome pathway regulates almost all the cellular activities by catalyzing the degradation of most proteins in eukaryotic cells. Ubiquitin ligases (E3s) determine the specificity and timing of ubiquitination. We characterized a novel E3, Nrdp1, and discovered that Nrdp1 downregulates ErbB3 (a member of the EGF receptor family), BRUCE/Apollon (an Inhibitor of Apoptosis Protein and a ubiquitin ligase), and NF-kB pathways by catalyzing ubiquitination and proteasomal degradation. Our results further demonstrate that BRUCE is essential to inhibit apoptosis in certain types of cells by antagonizing both the precursor and mature forms of the pro-apoptotic factors, Smac and caspase-9. Therefore, we found that the Nrdp1-mediated degradation of critical anti-apoptotic proteins is a novel pathway for triggering apoptosis. In addition, we developed affinity methods to rapidly purify 26S proteasomes from mammalian cells. By this approach, we discovered a novel subunit of the proteasome, and found that this subunit is important for the binding of the proteasomal deubiquitinating enzyme, UCH37, to the proteasome and for efficient proteolysis. These findings can be important for the understanding of the ubiquitin-mediated proteolysis and for the treatment of various related diseases.

Keywords**:** ubiquitin; proteasome; apoptosis; Nrdp1; BRUCE/ apollon

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APOCB2006-09-016

Insufficiency of MAPK pathway and excess of oxidative phosphorylation pathway are the two main features of cold syndrome

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We collected a potential Cold Syndrome (CS) family by a questionnaire of cold feelings and confirmed it by a 40-items scoring table based on certain theories of Traditional Chinese Medicine (TCM). Then the transcriptome of CS were tested by microarray technique and validated by RT-PCR. Moreover, we assayed the enzyme activities of two key members (e.g. MEK1, ERK1/2) within the signal pathway of Ras/MAPK by means of ELISA. Simultaneously, we recruited 10 healthy volunteers as control. A typical CS family was obtained, of which 15 were evaluated as CS patients and 6 non-CS. Results of the cDNA microarray revealed that there were 479 genes (or ESTs) differentially expressed in the CS transcriptome, which were involved in energy metabolism mainly, and as well as signal transduction, transport, development, cell cycle and immunity. The rather high ratio (12%) of the abnormally expressed genes found on more than two microchips compared with those of the above 479 genes suggested the facilitation to study complex disorders such as CS by the classic method, pedigree. Pathway analysis demonstrated the most interesting discovery in this paper is, the low level of energy output in CS can principally attributed to the inferior function of MAPK, a signal transduction pathway regulating the energy metabolism. The excessive expression of major components in response to the oxidative phosphorylation

pathway and glycolysis/gluconeogenesis pathway may be considered as kind of feedback or complementary mechanism prompted by the low level of energy production in CS disorders. Results of RT-PCR confirmed those of the microarray approach. The significant low level of the enzyme activities of MEK1 and ERK1/2 in MAPK pathway examined by means of ELISA reconfirmed the inferior condition of MAPK pathway in CS patients. Taken together, the most important discovery in this paper was the insufficient MAPK pathway which might be a central monitor attributing to the abnormal low level of energy output in CS; whereas the major pathways in response to the production of energy, the oxidative phosphorylation pathway and Glycolysis/gluconeogenesis pathway were significantly over-expressed. CS patients undergoing overall damage in the mass-energy-information-carry networks; the abnormal low energy output can limit all kinds of other functions—just like the engine to a car— and precisely tallied with the results of our transcriptome assays.

Keywords**:** cold syndrome (CS); traditional Chinese medicine (TCM); pedigree; transcriptome; mitogen actived protein kinase (MAPK)

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APOCB2006-09-017

Depressing integrin β4 level and SOD activity and elevating ROS level and NADPH oxidase activity induced apoptosis in neurons by safrole oxide

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The apoptosis of neural cells is a very important event in the development of central nervous system (CNS). This study was designed to find a useful chemical small molecule that can induce neural cell apoptosis, so as to understand the mechanisms of neuron survival and apoptosis. We first investigated the effects of safrole oxide on the survival of primary culture mouse neurons, then examined expression of integrin β4, the level of intracellular reactive oxygen species (ROS), Manganese-dependent superoxide dismutase (MnSOD) and NADPH oxidase activity in the neurons treated with safrole oxide. We discovered that safrole oxide could induce neuron apoptosis. In the apoptosis process, the expression of integrin β4 was suppressed, the level of intracellular reactive oxygen species (ROS) was elevated remarkably, Manganese-dependent superoxide dismutase (MnSOD) activity decreased significantly, and the activity of NADPH oxidase was increased dramatically. Our data suggested that safrole oxide was an effective inducer of neuron apoptosis. Both integrin β4 and ROS were implicated in the process of neuron apoptosis. It was likely that the level of ROS was mediated by MnSOD and NADPH oxidase in this process. The findings provide us a powerful chemical probe for investigating neuron apoptosis mediated by integrin β4 and ROS.

Keywords**:** neurons; apoptosis; integrin β4; reactive oxygen species; safrole oxide

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APOCB2006-09-018

PI-PLC and PC-PLC performed their contrary functions by affecting Akt phosphorylation, P53 expression and cell cycle in apoptosis of vascular endothelial cells

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In our previous studies, we found that phosphatidylcholine-specific phospholipase C (PC-PLC) and phosphatidylinositol-specific phospholipase C (PI-PLC) played contrary roles in the apoptosis of vascular endothelial cells (VECs), but the mechanism underlying the phenomenon remains unclear. To address this question, in this study, we investigated the changes of cell cycle distribution, the expression of P53 and the phosphorylation of Akt, when PI-PLC was inhibited by its specific inhibitor compound 48/80, and we also examined the phosphorylation of Akt when VEC apoptosis was inhibited by D609, a specific inhibitor of PC-PLC. The results showed that suppression of PI-PLC promoted VEC apoptosis by inhibiting Akt phosphorylation, elevating P53 expression and affecting the cell cycle distribution. Contrarily, suppression of PC-PLC promoted the phosphorylation of Akt. The data suggested that PI-PLC and PC-PLC might control the apoptosis by jointly regulating Akt phosphorylation, P53 expression and affecting cell cycle in VEC.

Keywords**:** Akt; PI-PLC; PC-PLC; p53; vascular endothelial cells

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APOCB2006-09-019

Lactoferrin induces interferon-alpha gene expression through a pathway involving IRF-3 and IRF-1 activation

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Interferon (IFN) plays a major role in the antiviral activity of human cells and there is a large number of viral IFN genes in the human include 13 INF-alpha, 1 IFN-beta and 1 IFN-ω. In previous study, we found bovine lactoferrin (LF) stimulated cells showed antiviral activity against dengue and enterovirus. However, the precise mechanism is still unclear. Therefore, we focused in this study to understand whether LF can induce human lung epithelial cell line A549 and monocyte (THP1) to produce IFN. A549 cells and THP1 cells were incubated with different doses of LF for 24 h. Results from RT-PCR demonstrated that mRNA of INF-alpha 4, but not INF-alpha 1, INF-alpha 5 nor INF-beta was increased in the presence of LF. The production of IFN was further supported by immunofluorescent stain showing nuclear translocation of interferon regulatory factor (IRF)-1 and IRF-3, which are important factors in the regulation of IFN-alpha gene promoter. In addition, nuclear translocation of signal transducer and activator of transcription (STAT)-1 indicated INFα4 may be secreted by LF-stimulated cells and act autocrinally or paracrinally to induce antiviral responses of cells. Therefore, the antiviral antivity induced by LF may partly explain by IFN production of LF-stimulated cells.

Keywords**:** lactoferrin; interferon-alpha; IFN regulatory factor

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APOCB2006-09-020

Bilayer gating mechanism of the stretch-activated potassium channel in atrial myocytes of the adult rat

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Mechanical stimuli affect cardiac electric activity that is termed mechano-electrical feedback. Stretch-Activated Potassium Channels (SACs) may play a sensor role in this signal transduction. The purpose of this study was to establish a mechanical microenvironment without the cortical F-actin for SAC and to determine the dependence of channel activation on cortical skeleton. The giant patch method of treating myocytes with hypotonic solution was introduced in this study. Double staining technique was used to label the membrane and F-actin to confirm that F-actin did not exist beneath the bleb membrane. One kind of stretch-activated potassium channel was registered on the bleb devoid of cortical F-actin. The mean conductance was 70±5 pS at +30 mV and 65±8 pS at -30 mV. We presume that tension may activate SAKC in cardiac myocytes directly through the lipid molecule of the bilayer and the cortical actin is not the necessary condition for the activation of SAKC.

Keywords**:** stretch-activated ion channels; F-actin; sarcolemma; atrial myocytes; rat

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APOCB2006-09-021

Mechanisms of regulation of telomerase activity

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Telomerase, a ribonucleoprotein complex, maintains chromosome ends (telomeres) for genomic stability and cell proliferation. While telomerase activity is required for stem cells and cancer cells, how telomerase activity is regulated remains to be established. Studies were undertaken to investigate molecular mechanisms in the regulation of telomerase catalytic subunit gene expression and protein interaction. The sex hormone estrogen has been shown to up-regulate telomerase activity in cultured cancer cells. Using an estrogen deficient mouse model, telomerase activity was analysed in different tissues. We found high telomerase activity in the adrenal gland of wild-type female mice and that estrogen-deficiency correlated with significantly reduced telomerase activity. Treatment with estrogen for three weeks restored telomerase activity to levels similar to that observed in wild-type mice. Since the adrenal gland is critically involved in the maintenance of homeostasis and the ability to adapt to stressful conditions, these results suggest that a decline in estrogen production may contribute to the aging process by acting on the gene of telomerase in the adrenal gland. Furthermore, using *in vitro* binding assays, we found that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was implicated in telomere maintenance. GAPDH bound single-stranded telomeric DNA repeats in a promiscuous manner. However, the binding was dependent on phosphorylation of GAPDH. These findings suggest that GAPDH preferentially interact with single-stranded telomere 3' and thereby regulating telomerase accessibility. Phosphorylation of GAPDH may be a key mechanism regulating the action(s) of GAPDH

for telomere maintenance, chromosome integrity and cell proliferative potential.

***Keywords*:** telomerase; gene expression; protein interaction

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APOCB2006-09-022

Cloning of expressed gene fragments down-regulated by MAP kinase in *Setosphaeria turcica* with MEK-specific inhibitor

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MAPK signal transduction pathway is known as one of the universal regulation systems on pathogenicity of fungal pathogens, but its function in *Setosphaeria turcica*, an important foliar pathogen of corn, was remaining unclear until we reported that the MEK-specific inhibitor, U0126, could significantly decrease the abilities of the fungus to produce appressoria and infect susceptible corn leaves. In this paper, we aim to clone the expressed gene fragments in the downstream of MAPK pathways for the purpose of further cloning of genes functioned in the fungal pathogenicity. DMSO-dissolved U0126 was proportionally added into the modified Fris medium before fungal inoculation. Addition of the same quantity of DMSO without U0126 was served as blank treatment. After fungal incubation, RNA isolation, DNase I digestion and cDNA synthesis, 26 ten-nucleotide primers were served to run DDRT-PCR with 3 poly(A) anchor primers respectively. The PCR products were separated by denatured PAGE and the cDNA fragments in differential bands between U0126 and blank treatments were purified from the gel. 157 fragments were recovered from the visualized 169 differential fragments and subjected to reverse Northern blotting. Only 94 fragments exhibited positive signals indicating that about 40.1% fragments were falsely amplified in DDRT-PCR. The 94 fragments could be divided into three groups. Group A included 23 fragments which were shown in both U0126 and blank treatments. Group B included 28 fragments which showed only in blank treatments, indicating of genes specifically inhibited by U0126. The rest 43 fragments belonged to group C which were genes expressed only when U0126 existed. After the 94 fragments were secondly amplified with same primer pairs, 115 PCR products were recovered from the agarose gel and shown positive signals in the second reverse Northern blotting. More than one PCR products acquired in second PCR was possibly because of more than one cDNA fragments in some PAGE bands. Cloning, sequencing and homologous analysis of four fragments in Group C showed that the 296bp fragment was homogenous to genes coding glyceraldehyde-3-phosphate dehydrogenase in *Pseudomonas putida* (81.32% Identity), the 333bp fragment was homogenous to genes coding cation transport ATPase in *Magnetospirillum magnetotacticum* (30.77% Identity), the 479bp fragment was homogenous to gene coding LINE-1 reverse transcriptase homolog in *Homo sapiens* (83.33% Identity), and the 601bp fragment was homogenous to gene coding nuclease in *Agrobacterium tumefaciens* (53.49% Identity). The above results indicated that U0126 possibly functioned by increasing the physical metabolism, signal transmembrane transport, gene transcription and nucleotide digestion. Although the definite mechanisms could not be elucidated so far, the successive cloning and sequencing of other fragments and further cDNA genes would doubtlessly uncover more secrets in pathogenicity regulation of fungal pathogens.

***Keywords*:** *Setosphaeria turcica*; MAPK; U0126; DDRT-PCR

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APOCB2006-09-023

The influence of pH value in AS buffer on the stomatal aperture and root hair formation of maize seedlings

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In this study, we investigated stomatal aperture and root hair of maize seedlings under AS (artificial solution) buffer using culture system and found that pH had a strong influence on stomatal aperture and root hair formation. Our study showed that compared with the PEG treatment, the stomatal aperture increased slightly under pH+PEG treatment, except pH4.5; the stomatal aperture under –0.2MPa+pH were larger than that under –0.5MPa+pH. We also found that root hairs were significantly different under AS buffer of different pH. The amount of root hairs was more under pH4.5, 5.0, 5.5, 6.5 treatments, but less under pH7.5 and pH8.0. Furthermore, the root hairs were slim and long under pH4.5 and 5.0, while they were short and strong under pH5.5 and 6.5. Among all treatments, the richest root hair was the observed under pH5.0. Under PEG+AS buffer treatment, the obvious change was the amount reduction of root hairs, but no significant difference was observed among pH treatments. The shape of root hairs was short

and strong under –0.5MPa+pH than –0.2MPa+pH.

***Keywords*:** stomatal aperture; root hair; pH; maize; scanning electron microscopic

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APOCB2006-09-024

Wnt/β-catenin signals disperse AChR clusters at the neuromuscular junction

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Neuromuscular synapses are formed between motor neurons and skeletal muscle fibers. The dynamic interaction between positive and negative signals are necessary for remodeling or sculpturing to better match the nerve terminals and to form perforated or pretzel-like structures. Nerve-derived protein agrin activates muscle-specific kinase (MuSK), leading to the clustering of acetylcholine receptors (AChRs) through the protein rapsyn, whereas negative signals remain not well defined. Here we report a role of Wnt/β-catenin signaling in regulating AChR clustering. We found that Wnt3 treatment accelerated dispersion of AChR clusters, this effect was prevented by soluble Frizzled 8 or DKK1, two Wnt antagonists. Furthermore, treatment of C_2C1_2 myotubes with MG132 or LiCl, both of which stabilize β-catenin, also promoted dispersion of AChR clusters. Interestingly, Wnt, MG132, or LiCl treatment resulted in a decrease of rapsyn level. In contrast, knockdown of β-catenin with siRNA stabilized AChR clusters and increased rapsyn promoter activity in reporter assays. AChR clusters are larger in mice deficient for β-catenin in skeletal muscles. An abnormal presynaptic phenotype was also observed in β-catenin mutant mice, whereby nerve trunks were distributed along muscle margins but not endplates and the width of endplates became bigger. Thus, Wnt/β-catenin signaling plays a dispersing role for AChR clusters at the neuromuscular junction by regulating the expression of synaptic proteins, such as rapsyn.

***Keywords*:** AChR; Wnt; β-catenin; rapsyn

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APOCB2006-09-025

Activation of geranylgeranyltransferase I by neuronal activity and BDNF promotes dendritic morphogenesis

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Proper dendritic arborization is essential for the establishment of neuronal circuitry. Dendrite growth is regulated by neuronal activity, neurotrophins, and Rho family GTPases. However, the mechanisms that converge these signals remains poorly understood. Here we report a role of geranylgeranyltransferase I (GGT), a prenyltransferase which tethers proteins to plasma membrane, in dendritic morphogenesis. KCl depolarization or BDNF treatment activated GGT and promoted membrane association of Rac1 in cultured hippocampal neurons. Overexpression of GGTβ-subunit enhanced dendritic arborization. Conversely, KCl- or BDNF-dependent dendrite formation was attenuated by down-regulation or inhibition of GGT. In addition, KCl- or BDNF-induced membrane association of Rac was decreased when GGT was inhibited. Furthermore, overexpression of Myr-Rac, which tethers Rac to plasma membrane independent of protein prenylation, prevented the loss of dendrite arborization induced by GGT down-regulation. These results identify GGT as an important regulator for activity or BDNF-induced dendritic growth.

***Keywords*:** GGT; dendritic arborization; neuronal depolarization; BDNF; Rac

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APOCB2006-09-026

Quantum dots light up cell: probing the possible receptor of GABA in plants

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Although γ-Aminobutyric Acid (GABA) is ubiquitous in all prokaryotic and eukaryotic organisms, its role in plants is poorly understood. Since the discovery that the level of GABA is involved in pollen tube growth and guidance (Palanivelu *et al*., 2003; Yang, 2003; Ma, 2003), much more attention has been paid to this enigmatic neurotransmitter. In addition to the traditional concept that the level of GABA accumulation in plants is an osmotic adaptation to various abiotic and biotic stresses, more and more evidence showed that it functioned as an extracellular and intracellular signaling molecule to regulate the physiological processes. Such as it could stimulate ethylene biosynthesis in sunflower involving in the senescence process (Kathiresan *et al*., 1997); It could specifically up-regulate the uptake

of nitrate and the expression of the nitrate transporter gene (BnNrt 2) in *Brassica napus* (Beuve *et al*., 2004) and regulate the expression of members of the 14-3-3s gene family in *Arabidopsis* seedlings (Lancien *et al*., 2006). All these data greatly broadened our understanding of the role of GABA. We speculate that the mechanism underlying this is based on GABA receptors similar to that in animals. Whereas, nothing is known about this putative receptor in plants up to now. To determine whether GABA receptors are also present on the membrane of pollen protoplasts, a promising fluorescence probe of quantum dots (QDs) was constructed and applied (Yu *et al*., 2006). The water soluble CdSe-ZnS (core-shell) QDs were synthesized and verified to possess good optical properties. GABA was then bioconjugated to the QDs in the presence of 1-ethyl-3-(3)-dimethylaminopropyl carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to make the fluorescence probe. Using the probe, GABA-binding sites were detected on the protoplast membrane of both pollen and somatic cells. Both the fluorescent signals on the surface of the protoplasts and the Ca^{2+} oscillation assayed via the Ca^{2+} probe Fluo-3/AM inside the protoplasts provided evidences that the potential GABAB receptors are present on plant protoplast membrane. [Reference: Yu GH, Liang JG, He ZK, Sun MX. Quantum dot-mediated detection of γ-aminobutyric acid binding sites on the surface of living pollen protoplasts in tobacco. Chem Biol 2006; **13**:723-731.]

Keywords**:** quantum dots; γ-aminobutyric acid; fertilization; pollen protoplast; tobacco

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APOCB2006-09-027

CUG-initiated FGF-2 specifies is cardioprotective against myocardial ischemia/reperfusion injury: involvement of PKC translocation and activation of Akt and p70S6K

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FGF-2 is a multifunctional heparin-binding protein, existing in low molecular weight, "lmw-FGF-2", translated from AUG start codons, or high molecular weight, "hmw-FGF-2", translated from CUG start codons, isoforms. In the adult heart, the lmw-FGF-2 isoform exerts beneficial effects such as increased resistance to ischemic injury and lasting cardioprotection due to its potent angiogenic activity. It is not known as yet whether the different types of FGF-2 isoforms exert different biological effects in the adult heart in regards to resistance against myocardial injury induced by ischemia. Therefore, effect of CUG-initiated, high molecular weight FGF-2 on myocardial ischemia/reperfusion was investigated. Ex vivo rat hearts were subjected to 30 min ischemia and 60 min reperfusion. Normal saline, high molecular weight FGF-2 (hmw-FGF-2) or low molecular weight FGF-2 (lmw-FGF-2) (10 mg in 10 ml normal saline) was perfused with perfusate into the heart during the first 10 min of reperfusion. Distribution of exogenous FGF-2, changes of vinculin, Akt, p70S6K and cytochrome C in the heart, translocation of PKC a,e and z subtypes as well as cardiac mechanic functions were examined. Results showed that exogenous 'hmw' or 'lmw'FGF-2 was broadly distributed into the myocardium during 1 h reperfusion. Intensive and intact cytoskeletal protein vinculin staining were seen in both 'lmw' and 'hmw'FGF-2, but not normal saline, administered hearts. Cardiac functions including systolic pressure, ±dp/dtmax and developed pressure were significantly improved in 'hmw' and 'lmw'FGF-2 groups compared to saline controls (P<0.05 or 0.01). There were less cytochrome C release and higher Akt, p70S6K activities and more translocation of PKC subtypes to myocardial particulate fraction in both FGF-2 treated hearts than in saline treated controls (P<0.05 or 0.01). We conclude that CUG-intiated FGF-2, like its low molecular weight isoform, is cardioprotective against myocardial ischemia/reperfusion injury, which may involve PKC translocation and activation of Akt and p70S6K.

Keywords**:** CUG-initiated FGF-2; myocardial ischemia/reperfusion; apoptosis; signal transduction

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APOCB2006-09-028

Inhibition of basophil cell line KU812 degranulation by siRNA targeted PLCγ1 silencing

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Degranulation of Mast cells or basophils is mediated by release of cellular calcium and plays a central role in allergic disease. The release of intracellular calcium is modulated by the second messenger IP3 which is produced from membrane metabolism catalyzed by PLCγ1. To investigate the prevention effect of PLCγ1 inhibition on degranulation we designed a targeted PLCγ1 silencing by siRNA using human basophil cell line KU812. Through selection of stable expression cells introduced with PLCγ1 targeted siRNA

expression system we observed that the histamine release of KU812 cell was significantly decreased and the expression of PLCγ1 down-regulated. This observation indicates that the silencing of allergic signaling may have a potential application value in treatment of allergic disease.

***Keywords*:** basophil; degranulation; siRNA; silencing; allergy

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APOCB2006-09-029

Regulation of epidermal growth factor receptor endocytosis by its intramolecular region of C-terminus

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It was proven that ligand (epidermal growth factor, EGF) binding is essential for the rapid internalization of epidermal growth factor receptor (EGFR), the events induced by ligand binding probably contribute to the regulation of EGFR internalization. These events include receptor dimerization, activation of intrinsic tyrosine kinase activity and autophosphorylation. Whereas the initial results are controversial regarding the role of EGFR kinase activity in EGFR internalization, more recent data suggest that EGFR kinase activation is essential for EGFR internalization. However, our previous investigation demonstrated that inhibition of EGFR kinase activation by mutation or by chemical inhibitors did not block EGF-induced EGFR internalization. Instead, proper EGFR dimerization is necessary and sufficient to stimulate EGFR internalization. What's more, the EGFR internalization is also regulated by its C-terminal intermolecular codes. Here, by EGFR mutants with one or more motifs deleted at its intracellular domain we have shown that the deletion mutant from 1014 amino acid residue of EGFR C-terminal could not be internalized after EGF binding by using immunofluorescence and flow cytometry analysis. So the code determining EGFR internalization may be located in this region. We conclude that EGFR internalization is regulated by EGFR intermolecular code, rather than EGFR kinase activation. Our results also define a new role for EGFR dimerization: by itself it can drive EGFR internalization, independent of its role in the activation of EGFR kinase.

***Keywords*:** EGFR; dimerization; endocytosis

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APOCB2006-09-030

Immunoelectron microscopy and RNAi analysis of the function of eukaryotic class II release factor (eRF3) in *Ciliates euplotes*

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Translation termination of protein biosynthesis is controlled by two major components: the mRNA stop codon at the ribosome A site and the polypeptide chain release factors (RFs). In eukaryotes, translation termination is mediated by two interacting release factors, eRF1 and eRF3. eRF1 decodes all three stop codons and triggers the release of the nascent polypeptide. eRF3 is a GTPase that stimulates eRF1 activity in a GTP-dependent manner. GTPase activity of eRF3 requires the presence of both eRF1 and ribosomes. It was confirmed that the eRF3 GTP-bound form interact with eRF1 *in vitro* and *in vivo* to constitute the actively translation termination complex by using ciliates Euplotes octocarinatus in our group. eRF3 has a complex domain structure. Only its C-terminal is responsible for termination of translation. The N and M regions of eRF3 proteins are not conserved even though some common features can be found. Many facts have been discovered in yeast and Drosophila melanogaster to support the hypothesis of a role of eRF3 in connection with the cytoskeleton, the mitotic spindle, the nuclear division and the chromosome segregation. Our previous study showed eRF3 was specifically localized in basal bodies of Euplote cells, suggesting that eRF3 may be involved in tubulin activity. Whereas above data, we presume that eRF3 is likely a moonlighting protein that plays variant roles in different compartment of the cells. In order to understand the roles of eRF3, we prepared antibodies against eRF3, and performed localization studies by immunoelectron microscopy. Our results indicate that eRF3 is present in the macronucleus and micronucleus of the ciliate *E. octocarinatus*. The micronucleus serves as a germinal nucleus and is transcriptional inactive during vegetative reproduction. It divides by mitosis. The macronucleus is a somatic nucleus. It is transcriptional active and divides by amitosis. The evidence for amitosis is that macronuclear divisions occur without spindle formation. Interestingly, the macronuclear division appears to be carried out by macronuclear microtubules and by cytoplasmic microtubules in a cooperative manner. This, however, means that there must take place microtubule assembly in the macronucleus. The possible functions of eRF3 in these nuclei were assayed by RNAi interference methods. The results show that the nuclei are destructed in eRF3 gene interfered Euplotes cells, suggesting that eRF3 is probably

involved in the organization of nuclei by microtubules. The investigation provides data at a subcellular and molecular level for a multifunction of eRF3 in eukaryotic cells. It also raises the question whether protein synthesis occurs in nuclei in addition to the one occurring in the cytoplasm. These data suggest the possible couple of transcription and translation in nuclei of eukaryotic cells. This process was observed in nuclei of human cell.

***Keywords*:** polypeptide release factor; immuno-localization; RNAi; eRF3; multifunction

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APOCB2006-09-031

Intracellular M-CSF modulate AP-1 activity and the cell cycle through Jab1

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Cytokines are multifunctional mediators that classically act by receptor-mediated pathways. Macrophage colony-stimulating factor (M-CSF) is a cytokine that is involved in the genesis and progress of several diseases. The non-receptor-dependenttargets of intracellular M-CSF action have so far remained unclear. Here we show that M-CSF speciacally interacts with an intracellular protein, Jab1, which is a coactivator of AP-1 transcription. that also promotes degradation of the cyclin-dependent kinase inhibitor p27Kip1. M-CSF colocalizes with Jab1 in the cytosol, and both endogenous and exogenously added M-CSF following endocytosis bind Jab1. M-CSF increases Jab1-enhanced AP-1 activity, but does not interfere with the induction of the transcription factor NFKB. Jab1 activates c-Jun amino-terminal kinase (JNK) activity and enhances endogenous phospho-c-Jun levels, and M-CSF enhances these effects. M-CSF also stimulates Jab1-dependent cell-cycle regulation by inhibiting p27Kip1 expression through degradation of p27Kip1 protein. We conclude that M-CSF may act broadly to positively regulate Jab1-controlled pathways and that the M-CSF-Jab1 interaction may provide a molecular basis for key activities of M-CSF.

***Keywords*:** macrophage colony-stimulating factor; trascriptional factor; cell cycle; coactivator

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APOCB2006-09-032

Neuroprotection of gypenosides against glutamate-induced apoptosis in cultured cerebral cortex cells: involvement of CytC/Capase3 signal pathway

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Gynostemma pentaphyllum is widely used in traditional Chinese medicine. Preliminary studies indicate antioxidative role of gypenosides (GPs), the active component of Gynostemma. Excessive extracellular glutamate causes neuronal damage and degeneration in brain disorders. *In vitro* studies have identified two major mechanisms of glutamate cytotoxicity: excitotoxicity and oxidative stress. Excitotoxicity in mature neurons is mediated through N-methyl-D-aspartate and other glutamate receptors. In immature neural cells, glutamate cytotoxicity is known to be involved in oxidative stress by piling free radicals up. It was therefore of interest to investigate whether GPs protect cortical cells against glutamate oxidative injury through increasing activities of antioxidant enzymes and the clearance of free radicals. Primary cultures of cortical cells were prepared from 13.5-day-old fetal rats. Dose and time effect of GPs protection against glutamate neurotoxicity were determined by using the method of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide reduction. Cortical cells were incubated with GPs for 12h before exposure to 2 mM glutamate for 12h (treatment throughout) then intracellular component activity and level were measured. We found that pretreatment with GPs (200 μg/ml) significantly protected cells from glutamate-induced (2 mM for 12h) cell death. Results illustrate that GPs significantly enhance activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) as well as Na^+–K^+ ATPase and decrease level of H_2O_2. Furthermore GPs down-regulate mitochondrial membrane potential and release of Cytochrome C. GPs were also found to inhibit activation of caspase-3. Thus, We conclude that i) GPs protect cortical cells by multiple oxidative actions via activating SOD, GSH-Px and Na^+–K^+ ATPase, suppressing glutamate-induced intracellular H_2O_2 elevation. ii) GPs function in the transduction pathway involving the change of mitochondrial potential and release of cytochrome c thereupon to prevent the entry of neurons into a caspase-dependent cell death. Findings in this present study imply novel role of GPs and their remarkable preventative and therapeutic potential in treatment of neurological diseases involving glutamate and oxidative stress.

***Keywords*:** gypenosides; glutamate; cerebral cortex cells;

cytochrome C; Caspase 3

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APOCB2006-09-033

Smad7 antagonizes TGF-β signaling in the nucleus by interfering with functional Smad-DNA complex formation

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Smad7 plays an essential role in the negative feedback regulation of TGF-β signaling. It has been shown to inhibit TGF-β signaling at the receptor level by its stable interaction with the active type I receptors. Smad7 can interfere with the binding and thus activation of R-Smads to type I receptors, or recruiting E3 ubiquitin ligases Smurf1 and Smurf2 to receptors and thus targeting them for degradation in the ubiquitin-dependent manner. Here, we showed that Smad7 was predominantly located in the nucleus of Hep3B cells. The targeted expression of Smad7 in the nucleus conferred a superior inhibitory activity on TGF-β signaling as determined by reporter assay in mammalian cells and by its effect on zebrafish embryogenesis. Furthermore, Smad7 greatly repressed Smad3/4 and Smad1/4- enhanced the reporter genes expression in TGF-β type I receptor TβRI-deficient R1B/L17 cells and Hep3B cells, indicating that Smad7 can function independently of type I receptor. Oligonucleotide precipitation assay revealed that Smad7 can specifically bind to the Smad responsive element via its MH2 domain, and the DNA-binding activity was further confirmed in vivo with the promoter of PAI-1, a TGF-β target gene, by chromatin immunoprecipitation. Finally, we provided evidence that Smad7 disrupts the formation of the TGF-β-induced functional Smad-DNA complex. Our findings suggest that Smad7 inhibits TGF-β signaling in the nucleus in a novel mechanism.

***Keywords*:** Smad7; TGF-β; DNA-binding; antagonist

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APOCB2006-09-034

Borneol depresses P-glycoprotein expression in brain microvessel endothelial cells by a NF-κB signalling mediated mechanism

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The multi-drug transporter, P-glycoprotein (P-gp) on brain microvessel endothelium that form the blood brain barrier, influences movement of lipophilic substances in and out of the brain. In this study, we examined the effect of borneol on the expression and activity of P-gp in rat brian microvessel endothelial cells (rBMECs), and the signaling passways regulating P-gp expression. Borneol increased intracellar accumulation of rhodamine 123, also depressed P-gp expression determined by immunoblotting and mdr1a, mdr1b mRNA levels determined by real time RT-PCR. Borneol could activate Akt, ERK1/2, and its downstream transcription factor, c-Jun, nuclear factor-κB(NF-κB). Blockade of Akt activation with LY294002, of ERK1/2 with U0126 and of c-Jun with SP600125 did not abolish the borneol-induced P-gp decreases. Inhibition of NF-κB with MG132 obscured the borneol-induced P-gp decreases. These data suggested that borneol depressed P-gp expression in brain microvessel endothelial cells may be by increasing NF-κB signaling .

***Keywords*:** borneol; P-glycoprotein; brain microvessel endothelial cells; NF-κB; blood-brain barrier

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APOCB2006-09-035

MCP-1 mediates TGF-β promoted angiogenesis by stimulating vascular smooth muscle cell migration

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Transforming growth factor-β (TGF-β) and its signaling mediators play crucial roles in vascular formation. Our previous microarray analysis has identified monocyte chemoattractant protein-1 (MCP-1) as a TGF-β target gene in endothelial cells (ECs). Here, we report that MCP-1 mediates the angiogenic effect of TGF-β by recruiting vascular smooth muscle cells (VSMCs) and mesenchymal cells to-

wards ECs. By employing chick chorioallantoic membrane assay, we showed that TGF-β promotes the formation of new blood vessels and this promotion is attenuated when MCP-1 activity is blocked by its neutralizing antibody. Wound healing and transwell assays established that MCP-1 functions as a chemoattractant to stimulate migration of VSMCs and mesenchymal 10T1/2 cells toward ECs. Furthermore, the conditioned media from TGF-β-treated ECs stimulate VSMC migration, and inhibition of MCP-1 activity attenuates TGF-β-induced VSMC migration toward ECs. Finally, we found that MCP-1 is a direct gene target of TGF-β via Smad3/4. Taken together, our findings suggest that MCP-1 mediates TGF-β-stimulated angiogenesis by enhancing migration of mural cells towards ECs and thus promoting the maturation of new blood vessels.

Keywords**:** angiogenesis; TGF-β; MCP-1; endothelial cells; VSMC migration

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APOCB2006-09-036

Single-molecule imaging reveals transforming growth factor receptor monomers

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Transforming growth factor-b (TGF-β) and related growth factors control many important cellular processes such as cell proliferation, differentiation and apoptosis. TGF-β signals through two transmembrane serine/threonine kinase receptors, type I (TbRI) and type II (TbRII) receptors. It is generally believed that receptor dimerization is essential for kinase activation of recepters which are tyrosine kinases. However, very little is known about the activation mechanism of transmembrane serine/threonine kinase receptors such as TGF-β. Transforming growth factor-b has been suggested to signal by cooperating pre-existing homodimer of its type I and type II receptors to form heterotetrameric receptor complexes by overexpression method and immunoprecipitation analyses. Using a newly developed method of observing individual GFP-tagged receptor molecules in living cells with single-molecule fluorescence microscopy, we found that the majority of the receptors were actually monomers in resting cells and dimerized upon TGF-β1 stimulation. This indicates that like receptor tyrosine kinases, serine/threonine kinase receptors also follow the general model of monomer dimerization during signal transduction.

Keywords**:** single molecule imaging; living cell; transforming growth factor receptor; monomer

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APOCB2006-09-037

Ca^{2+}-binding membrane protein, AtNCL may involve in Ca^{2+} and Na^{+} ion homestasis of *Arabidopsis thaliania* under salt stress

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When cells meet stresses, its cytoplasm membrane is the first one to contact with stimulations. So the transporters, especially the ions transport proteins, accelerating or delaying the exchange of material and energy between the cell and the environment would respond to the stresses, Ultimately, the relation between the plant body and the environment come to relative balance as soon as possible. Because the calcium has very important functions in the plant signal transduction and response to the stress, we pay much more attention to the calcium ion transport proteins. By analysis of bioinformatics method, we found a supposed calcium binding protein in Arabidopsis thaliana. We named it AtNCL, which was presumed to have the conservative structure region similar to the NCX (Na^{+}/Ca^{2+} exchanger) domain of ions transportation. And it had signal peptide sequence and mutli-transmembrane domains just like the NCX protein. So we presumed that it was a membrane-binding protein taking charge of ions transmembrane transportation. First of all, we identified that the analytical signal peptide sequence has the putative secretory function by the method of the YSST. We expressed its assumed EF-hand calcium binding domains in *E.coli*. The result of SDS-PAGE electrophoresis indicated that AtNCL has the ability to bind calcium. Then we cloned the whole cDNA and the promoter sequence, constructed a series of expression vectors, and finished the relative transgenic works. Using these transgenic lines, we studied the tissue expression and the subcellular location of AtNCL. The results suggested that AtNCL had universal tissue expressive characteristic. Its expression quantity was comparatively high in the whole level, especially in the renascent tissue. But it was lower in the mature and senescent tissue. The result of subcelluar location indicated that this protein was preferentially located at the cytoplasm membrane. The structure and location information also indicated its functional characteristic. Furthermore, atncl-1, one T-DNA insert mutant homozygous line, was identified. In the same

time, the RNAi transgentic transgenic T1 heterozygous lines were got. Then we used these materials to study the response of AtNCL when it met different stresses. The purpose was to explore the biological function of AtNCL .The results showed that expressive quantity of AtNCL would have changes in different degree during a period when it met a certain concentration of ions stress, temperature and hormone stimulations. Otherwise, depending on the physiological state of the seedlings after being treated with stresses, we could found that AtNCL surely do some thing in the reaction of ion and hormone stimulations. By the method of AAS (atomic absorption spectroscopy), we mensurated the ions' content of mutant, wild type and over expression line. The results demonstrated that the mutant had less Na^+ and more Ca^{2+} than wild type, while the over expression line was just on the contrary. And when the plants met salt stress, the mutant has the different change of the content of Ca^{2+}. Then we supposed that: AtNCL is a membrane protein that had the ability of transporting Na^+ and Ca^{2+}.

Keywords**:** arabidopsis thaliania; Ca^{2+}; ion homestasis; stress

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APOCB2006-09-038

The regulation of GABA(A) receptor endocytosis via PRIP, a phospholipase C-related but catalytically inactive protein

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Efficacy of synaptic inhibition depends on number of cell surface expressed GABA(A) receptors. It is important to elucidate the mechanisms involved in regulation of the receptor number. Our recent studies revealed that PRIP (phospholipase C-related but catalytically inactive protein) regulates GABA(A) receptor signaling as assessed by PRIP knockout (KO) mice analysis. In the present study, we studied a possible involvement of PRIP in the modulation of postsynaptic GABA(A) receptor number by brain-derived neurotrophic factor (BDNF), which rapidly down-regulates GABA(A) receptor surface number. The exposure to BDNF reduced the GABA-evoked inhibitory current in cultured hippocampal neuron of wild type mice, whereas a little potentiation was observed in the PRIP-KO mice, corresponding to the surface expression of GABA(A) receptor number. We found that PRIP bound to beta subunits of GABA(A) receptor, and therefore we mapped the region in PRIP responsible for the interaction with the beta subunits. The peptide corresponding to the region blocked the attenuation of GABA-evoked inhibitory current in wild type hippocanpal neurons in response to BDNF application. Since clathrin/AP2 protein complex responsible for the receptor endocytosis was co-immunoprecipitated with PRIP, PRIP might be involved in the clathrin/AP2-mediated GABA(A) receptor endocytosis. These results indicate that the direct interaction between the beta subunits and PRIP plays a key role in the process of GABA(A) receptors endocytosis.

Keywords**:** GABA(A) receptor; endocytosis; BDNF; PRIP; knockout mice

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APOCB2006-09-039

Involvement of the transcriptional repressor DREAM in the regulation of calcitonin gene expression

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Calcitonin (CT), whose secretion from thyroid glands is regulated by increases in the concentration of extracellular Ca^{2+}, is a well-known polypeptide hormone to regulate calcium homeostasis through the effects on target tissues such as bone and kidney. However, the molecular mechanisms underlying the gene expression dependent on Ca^{2+} have not been clarified. The downstream regulatory element (DRE) antagonist modulator (DREAM) was recently identified as a Ca^{2+}-dependent transcriptional repressor. This transcription factor is highly expressed not only in the brain but also in the thyroid glands in human tissues. In this study, we have investigated the possible involvement of DREAM in the regulation of human CT gene expression and secretion. Luciferase assay using TT cells, a medullary thyroid carcinoma cell line, showed that a particular region in the CT gene promoter repressed the promoter activity under basal conditions, but induced the activity when the Ca^{2+} concentration was increased. We found two DRE core sequences in a region located upstream from the transcription start site of CT gene. Gel mobility shift assay confirmed the binding of DREAM to the CT-DRE and also indicated that DREAM bound to the DRE in a Ca^{2+} dependent manner. We generated stable transfectants of TT cells with wild type or mutant DREAM, which lacks the responsiveness to Ca^{2+} changes. In contrast to the wild type, overexpression of the mutant DREAM inhibited the increase in CT secretion induced by a calcium ionophore. The addition of forskolin to increase adenosine 3, 5-cyclic monophosphate (cAMP) activated the CT promoter, probably by the interaction of DREAM with cAMP responsive element binding proteins, independent of the activation by

Ca^{2+}. Together, these results suggest that DREAM plays an important role in human CT gene expression in a Ca^{2+} and cAMP dependent manner.

***Keywords*:** calcitonin; DREAM; calcium; cAMP

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APOCB2006-09-040

Cell surface expression of GABA(A) receptor is regulated by PRIP (phospholipase C-related inactive protein)

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GABA(A) receptors are a family of ligand-gated ion channels that are pentamer composed predominantly of alpha, beta, and gamma subunits. They are the major target of the endogenous inhibitory neurotransmitter (GABA) and have been implicated in a variety of brain functions including sedation, hypnosis, anxiety, learning and memory. The heterologous subunit composition of the GABA(A) receptor is known to be associated with the distinct pharmacological and physiological properties. In the present study, we have elucidated that PRIP (phospholipase C-related inactive protein), a novel Ins (1, 4, 5) P3 binding protein, regulates the GABA signaling via the receptors by analyzing PRIP knockout (KO) mice; the sensitivity to diazepam was reduced as assessed by biochemical, electrophysiological and behavioral analyses of PRIP KO mice, suggesting the dysfunction of the gamma2 subunit-containing GABA(A) receptors, a target of benzodiazepine drug, diazepam. We then examined the mechanisms by which PRIP molecule regulates cell-surface expression of gamma2 subunit-containing GABA(A) receptor. Disruption of the direct interaction between PRIP and the beta subunit of GABA(A) receptors by PRIP-binding peptide inhibited cell-surface expression of gamma2 subunit-containing GABA(A) receptors, while the expression of alpha and beta subunits were not altered by the peptide in GH3 and HEK293 cells. Collectively, PRIP molecules are involved in trafficking of gamma2 subunit containing GABA(A) receptors to cell-surface membrane, probably by facilitating the function of GABA(A) receptor-associated proteins including GABA(A) receptor associated protein.

***Keywords*:** GABA receptor; trafficking; diazepam

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APOCB2006-09-041

Chlorophyllin induces apoptosis in human breast cancer MCF-7 cells with regulations of antiapoptotic Mcl-1 and proapoptotic Bim expressions

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Chlorophyllin (CHL) is a mixture of sodium copper salts of chlorophyll derivatives and is marketed as an over-the-counter drug for controlling body, fecal and urinary odor in geriatric and osteomy patients and as an accelerant in wound healing. We have reported previously that CHL inhibits proliferation and induces apoptosis in the human breast carcinoma MCF-7 cells. This study was conducted to further investigate the mechanisms by which CHL induces apoptosis in the breast cancer cells. After 24 h of incubation with the IC(50) of CHL, i.e. 123.5 μg/ml, dissipation of mitochondrial membrane potential in the breast cancer cells was demonstrated by flow cytometry, accompanied by alterations of the apoptosis-modulatory Mcl-1 and Bim but not Bcl-2, Bcl-xL and Bad expressions in the immunoblot studies. Cleavage of PARP was also observed in meanwhile, implying the participation of caspases in the cell death process. Levels of procaspase-9 and -6 were decreased, suggesting that the caspases might be cleaved and thus became activated in the cell death process. Applications of caspase-9 (Z-LEHD-FMK) or caspase-6 (Ac-VEID-CHO) inhibitor partially inhibited the DNA fragmentation and thus confirmed the roles of the caspases in the CHL-induced apoptosis. However, application of the general caspase inhibitor (Z-VAD-FMK) failed to completely retard the apoptosis, demonstrating that the cell death process might also be mediated by some caspase-independent events in addition. Collectively, the results indicate that the signaling cascades initiated by CHL possibly involve regulations of Mcl-1 and Bim so that the signals are integrated at the level of mitochondrial dysfunction and both the caspase-dependent and caspase-independent death pathways are triggered in the breast cancer cells.

***Keywords*:** chlorophyllin; apoptosis; MCF-7; Mcl-1; Bim

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APOCB2006-09-042

Baicalein induces apoptosis in human melanoma A375 cells through both the extrinsic and intrinsic apoptotic pathways

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Traditional Chinese medicine (TCM) has been used for hundreds of years as medicinal remedies. One of the widely used Chinese medicinal plants is *Scutelleria baicalensis* Gorgi (Huang Qin). *S. baicalensis* is one of the important Chinese herbal medicines with anti-inflammatory and anti-cancer activities, which has also been reported to be rich in flavonoids and other useful phytochemicals. Baicalein is a flavone isolated from the dried root of the plant. The flavone has been widely reported for its therapeutic effects, for instance, antiproliferative effects on hepatoma cells and prostate cancer cells. Nevertheless, the underlying mechanisms of antiproliferative effects of baicalein are poorly understood. Human melanoma is hardly curable. One of the reasons would be its notorious resistance to a wide range of anticancer drugs. We therefore investigated if baicalein could exhibit similar antiproliferative effect on human malignant melanoma A375 cells and the signal transduction pathways involved in the growth inhibition. Treatment with baicalein for 48 h resulted in growth inhibition in a dose-dependent manner, with IC(50) of 37.5 mM. Baicalein induced DNA fragmentation, a hallmark of apoptosis, in A375 cells after S-phase arrest. The apoptotic mode of cell death was further evidenced by the cleavage of poly-(ADP-ribose) polymerase (PARP) after the baicalein treatment. The baicalein-induced apoptosis in the melanoma cells is caspase-dependent since cleavage of caspase-8 and caspase- 3 were observed after 24 and 48 h of treatment, respectively. This was subsequently counter-proved by the inhibition of PARP cleavage after the administration of caspase-8 inhibitor (Z-IE(OMe)TD(OMe)-FMK) with the baicalein treatment. The temporal difference between caspase-8 and caspase-3 activation suggested that the caspase-dependent apoptotic cascade was sequentially activated. This was further substantiated by the inhibition of caspase-3 activation after the co-administration of caspase-8 inhibitor with baicalein. Activation of caspase-8 was observed as early as 24 h of treatment, revealing that caspase-8 would probably be the initiator of apoptosis, via the extrinsic death receptor signaling pathway. One of the death receptors, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) was up-regulated by baicalein from 24 h treatment onwards, which was coherent with the activation of caspase-8. Mitochondrial membrane depolarization was also observed after 48 h, accompanied by cytochrome c release into the cytosol. Consequently, caspase-9 activation was observed after 60 h incubation. Pro-apoptotic Bax was up-regulated but no significant change of anti-apoptotic Bcl-2 was found. Either caspase-8 or caspase-3 inhibitor (Z-D(OMe)QMD(OMe)-FMK) could inhibit formation of the partially activated 37-kD cleaved caspase-9 fragment, but not the fully activated 35-kD caspase-9. This suggested that activated caspase-8 and -3 might play a role in the caspase-9 activation. Findings from this study will help us understand more the cell death mechanisms induced by baicalein. This may also help in exploring new alternative drugs in combating human melanoma, which has been a big impediment in the current war against cancer.

***Keywords*:** baicalein; apoptosis; A375; TRAIL; caspase

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APOCB2006-09-043

Fatty acid synthase inhibitors induce apoptosis in human melanoma A375 cells with downregulation of antiapoptotic Bcl-2 and survivin expressions

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Fatty acid synthase (FAS) synthesizes fatty acids for body uses by catalyzing the synthesis of 16-carbon palmitate from condensation of malonyl-CoA and acetyl-CoA, playing a crucial role in energy homeostasis in mammals. FAS over-expression and hyperactivity have been recently characterized in a wide spectrum of human malignancies, including carcinoma of breast, ovary, oral tongue, colorectum, lung, prostate, oesophagus, stomach, skin and bladder, with low expression levels in normal tissues. We investigated in this study the mechanism of growth inhibition in human melanoma A375 cells by FAS inhibitors. MTT assay revealed that FAS inhibitors cerulenin, C75 and triclosan significantly reduced cell proliferation and viability dose-dependently by 20.8 % to 87.1 % of the control level from 20 to 160 mM at 24 and 48 h treatment. Immunoblotting studies showed that both cerulenin and C75 induced poly(ADP-ribose) polymerase (PARP) cleavage in A375 cells dose-dependently at 24 h of treatment, suggesting that the FAS inhibitors might have induced apoptosis in the melanoma cells. The proapoptotic effect of the FAS inhibitors were further determined by DNA flow cytometry. Both cerulenin and C75 significantly elevated subG1 or apoptotic cells by 7.8- and 9.4-fold respectively at 24 h treatment. Preceding the apoptosis induction, both the FAS inhibitors induced G2 arrest so that the G2/M cells elevated by 2.4- and 5.5-fold, respectively. Executioner procaspases 3, 6 and 7 were found to be cleaved, indicating that the cerulenin-

and C75-induced apoptosis involved caspase activations. Initiator procaspase 9 started to be cleaved at 20 mM, with committant release of cytochrome c from mitochondria into the cytosol. On the other hand, cleavages of extrinsic procaspases 8 and 10 were observed at 80 mM of either inhibitor. Anti-apoptotic Bcl-2 protein was downregulated with elevated level of its phosphorylated form. Proapoptotic Bim was also elevated by C75, with cleavage into smaller isoforms Bim(S) and Bim(L). Survivin, which binds to and inhibits caspase 3, was downregulated after either cerulenin or C75 treatment. However, no significant regulation of other Bcl-2 proteins, including Bcl-X(L), Bax and Bmf, was found. Bid, the crosstalk protein between the extrinsic and intrinsic apoptotic pathways, was cleaved, indicating that it might behave as the linkage between the two pathways to elicit the executioner caspase activations. Although it had been proposed that the FAS-inhibition-induced apoptosis in breast cancer cells was triggered by substrate accumulation of malonyl-CoA, 5-(tetradecyloxy)-2-furoic acid, an inhibitor of acetyl-CoA carboxylation, induced and enhanced the cerulenin- and C75-induced PARP and caspase-3 cleavage in the melanoma cells. This suggested that malonyl-CoA accumulation was not the main factor in the cerulenin- and C75-induced apoptosis.

***Keywords*:** fatty acid synthase; cerulenin; C75; apoptosis; A375

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APOCB2006-09-044

Novel role of Akt in regulating Clk/STY, serine/arginine-rich proteins function and pre-mRNA splicing

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The regulation of pre-mRNA splice sites utilization provides a versatile mechanism for determining gene expression and proteome diversity. Accumulating evidence have attributed the causes of various human disease onset to missplicing events, suggestting the importance of alternative splicing. Clk (Cdc2-like kinase, also named Sty) proteins phosphorylate serine/arginine-rich (SR) proteins that carry out pre-mRNA splicing. Clk/STY proteins are phosphorylated on their serine/threonine residues both *in vitro* and *in vivo*, however, the upstream signaling events that control Clk/Sty kinase function have not been clearly addressed. Here we show that the phosphorylation and activation of Clk/STY is up-regulated in a PI3-Kinase/Akt-dependent manner. Akt phosphorylates Clk/STY in their Akt substrate motifs, leading to modulation of SR proteins function and regulation of alternative splicing events induced by insulin, such as that of protein kinase CβII. Inhibition of Clk by a Clk siRNA or Clk-specific inhibitor, or inhibition of Akt by either siRNA or LY294002 suppressed the phosphorylation of both Clk/STY, and SR proteins, as well as alternative splicing. In addition, Akt directly modulates SR proteins activation via phosphorylating the Akt substrate motifs in SR proteins. Furthermore, these signaling events have been confirmed in the muscle, liver and brain tissues of Akt2-null mice. This newly identified PI3-Kinase/Akt→Clk→SR proteins→alternative splicing cascade demonstrates a novel regulation of gene expression by PI3-Kinase/Akt.

***Keywords*:** Akt; PI3-Kinase splicing ; Clk/STY; SR protein; splicing

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APOCB2006-09-045

Gypenosids-mediated neuroprotection from oxidative toxicity requires suppression of extracellular signal-regulated kinase activation

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Oxidative toxicity induced by glutamate causes degeneration in brain disorder. More extracellular glutamate inhibit a glutamate/cystine antiporter, which results in depletion of intracellular cysteine and blockade of glutathione synthesis. We have previously found that Gypenosides(GPs) can protect primary cortical cells via enhancing intracellular glutathione(GSH), blocking accumulation of reactive oxygen species. At present, pharmacological and biochemical approaches were used to elucidate the effects of GPS on the involvement of growth factor signaling pathways mediating neuroprotection in primary cortical cells after glutamate oxidative toxicity. We found that the activation of extracellular signal-regulated kinase1/2(ERK1/2) signaling pathway was essential for neuronal cell death in glutamate toxicity and GPs inhibited the activation of ERK1/2 caused by glutamate. Inhibition of ERK1/2 signaling with PD98059 could not abolish the protective effect of GPs while it enhanced the protective ability of GPs. Further studies revealed that by inhibition of the activation of ERK1/2, GPs depressed the expression level of c-fos gene and blocked the other following downstream elements responsible for the transduction of death signal exerted by glutamate. Furthermore GPs were also found to reduce the release of Cytochrome

C through ERK1/2 independent pathway which routinely follows glutamate oxidative toxicity. Thus, We conclude that 1)ERK1/2 activity is required for some initial steps of a cell death program that folds when neurons are subjected to oxidative stress. 2) GPs protected cortical cells from glutamate-induced oxidative toxicity and this neuroprotective effect was mediated by ERK1/2 signal pathway. 3)However, the protective effects of GPs do not appear to be decided independently by ERK1/2 signal pathway, but rather it appear to be interconnected.

***Keywords*:** gypenosides; oxidativetoxicity; signal pathway; neuroprotection

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APOCB2006-09-046

Recombinant HuZP3 induces the acrosome reaction in human sperm mediatied by Gi protein

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Recombinant human zona pellucida 3 peptides huZP3a22~176 and huZP3b177～348 was expressed in E.coli. and purified，because recombinant human zona pellucida 3 may be served as an alternatvie tool of native ZP3 to diagnose male sterility and study immune contraceptive.We examined whether huZP3a22~176 and huZP3b177~348 may trigger acrosome reaction of human spermatozoa and explored possible mechanism which mediated acrosome reaction. By chlortetracycline staining, assessment of acrosome reaction was performed. Intracellular free calcium concentration $[Ca^{2+}]i$ in Fura-2/AM-loaded human sperm was monitored with spectrofluorophotometer. We found that the peptides huZP3a22~176 and huZP3b177~348 were significantly capable of eliciting acrosome reaction in a dose-dependent manner, respectively. With an addition of the peptide, $[Ca^{2+}]i$ level was raised like a peak and plateau. The acrosome reaction could be inhibited by Gi protein sensitivity pertussis toxin (PTX), EGTA and a T-type calcium channel blocker pimozide, whereas verapamil was less effective. This study suggests the peptides huZP3a22~176 and huZP3b177~348 have the role similar to human ZP, mechanism in respond to the peptides may involve in influx of calcium, Gi protein pathway and T-type calcium channel.

***Keywords*:** human sperm; acrosome reaction; Zona pellucida 3; Gi protein; calcium channel

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APOCB2006-09-047

Study cellular functions using RabMAbs that specific recognize phosphorylation of signaling proteins

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Phosphorylation is one of the most intensely studied post–translational modifications (PTM). This PTM plays critical roles in the regulation of many cellular processes including: cell cycle, growth, apoptosis and differentiation. The identification and characterization of phosphorylation sites is crucial for the understanding of various signaling events and pathogenesis of many diseases associated. High quality antibodies against various phosphorylation sites are essential tools for monitoring these phosphorylation events. Rabbit monoclonal antibody technology is known to have significant advantage over mouse antibody technology in making phospho-specific antibodies due to rabbit's large antibody repertoire and improved immune response to small-size epitopes. Rabbit monoclonal antibodies generally have higher specificity and affinity than mouse antibodies. Over the past few years, we have established a robust technology platform to develop rabbit monoclonal antibodies including phospho-specific antibodies with consistent high quality and high success rate. Epitomics is currently supplying to the scientific community more than 60 phospho-specific antibodies against different phosphorylation sites of a variety of targets and over 300 phospho- specific peptides are under development. The phospho-specificity of antibodies is characterized by Western blot, ELISA, dot blot, flow cytometory, immunohistochemical analysis and fluorescent immunostaining. These phospho-specific antibodies are powerful tolls for cellular functions profiling and compound profiling for drug screening.

***Keywords*:** phospho antibody; RabMAb; Cell signal

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APOCB2006-09-048

Differential regulation of inflammatory signal transduction by endocytic mechanisms

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It is well established that degradation of cell surface receptors through endocytosis is a common mechanism of down-regulating signaling receptors. We have recently explored this link by studying the endocytic regulator CHMP5. CHMP5 is a coiled-coil protein homologous to the yeast Vps60/Mos10 gene and other ESCRT-III complex members, although its precise function in either yeast or mammalian cells was unknown. We deleted the CHMP5 gene in mice, with the resulting phenotype being early embryonic lethality, reflecting defective late endosome function and dysregulation of signal transduction. Chmp5$^{-/-}$ cells exhibit enlarged late endosomal compartments containing abundant internal vesicles expressing proteins characteristic of late endosomes and lysosomes. This is in contrast to ESCRT-III mutants in yeast, which are defective in multivesicular body (MVB) formation. The degradative capacity of Chmp5$^{-/-}$ cells was reduced and undigested proteins from multiple pathways accumulated in enlarged MVBs that failed to traffic their cargo to lysosomes. Therefore, CHMP5 regulates late endosome function downstream of MVB formation and loss of CHMP5 enhances signal transduction by inhibiting lysosomal degradation of activated receptors. Interestingly we have subsequently found that a number of endocytic regulators (including CHMP5) associate specifically to certain intermediates of specific signal-transduction pathways, but not to others, thereby controlling which signaling pathways are subject to receptor-mediated endocytosis. This raises the possibility that distinct signal transduction pathways emanating from the same receptor (e.g. TNF-mediated activation of NF-κB, AP-1 or apoptosis), can be selectively and differentially regulated by endocytic mechanisms.

***Keywords*:** ESCRT; CHMP; NF-kappa B; TNF; endocytosis

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APOCB2006-09-049

The role and co-receptor requirements of Nogo-66 receptor homologue 2 (NgR2) in neurite growth inhibition

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The Nogo-66 receptor (NgR) is the neuronal receptor for myelin-associated inhibitors (MAIs), such as Nogo-A, oligodendrocyte protein (OMgp) and myelin-associated glycoprotein (MAG). Together with the GPI-linked NgR, transmembrane coreceptors molecules LINGO-1, p75 and/or TROY, NgR transduces neurite growth inhibitory signals that limit axon regeneration both *in vitro* and *in vivo*. Two NgR homologues, NgR2 (NgRH1/NgRL3) and NgR3 (NgRH2/NgRL2), have been identified. Specific rabbit polyclonal antibodies were generated against NgR and NgR2, and surveys of their expression in different parts and developmental stages of the mouse brain were performed. NgR and NgR2 expression overlaps to a certain degree, but are not identical, and the latter appears to be enriched in cerebral cortex and cerebellum purkinjie cells in adult mice. Neuro-2a neuroblastoma cells expresses NgR, but have low levels of NgR2. Transient over-expression of either NgR2 or LINGO, but not NgR itself, shortens neurite length of mouse cerebral cortical neurons in culture. Furthermore, over-expression of NgR2 diminished immunofluorescent labeling of microtubules and microtubule associated proteins. Co-immunoprecipitation experiments suggest that NgR2 interacts with LINGO-1. The role and co-receptor requirements of NgR2 in transducing a neurite growth inhibition signal are currently being investigated in both primary cortical neurons as well as Neuro-2a cells.

***Keywords*:** Nogo-66 receptor; NgR2; neuronal regeneration

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APOCB2006-09-050

Ethanol inhibits neural precursor cell proliferation through disrupting muscarinic-activated signaling pathways

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Ethanol is a teratogenic factor interfering with neural precursor cell proliferation and survival in the developing central nervous system. We have generated a model neural precursor cell to study the interaction of ethanol with both growth factor and transmitter-stimulated proliferation. Neural precursor cells were dissociated from the embryonic rat telencephalon and maintained in serum-free defined medium containing basic fibroblast growth factor (bFGF) and Epidermal Growth Factor (EGF). These cultured neural precursor cells were sensitive to low concentrations of ethanol (25-50 mM), which are comparable to those found in the blood of moderate to heavy drinkers. Ethanol blocked proliferation stimulated by growth factors(bFGF and EGF) and by carbachol, an agonist at muscarinic acetylcholine receptors, in a dose-dependent manner. In addition, ethanol attenuated autonomous expansion of neural precursor cells

occurred following withdrawal of bFGF and EGF. The latter effect was associated with an increase in the number of apoptotic cells indentified by staining of propidium iodide (PI) and Ho.33342. Furthermore, we also investigated the ethanol's disruption effect of muscarinic acetylcholine receptors (mAChR)-mediated signaling pathway, which is critical to its proliferative effects in our in vitro model. We found that 25 -100 mM ethanol significantly inhibited mAChR-stimulated cytosolic Ca^{2+} signaling increase, as well as Akt phosphorylations in a dose-dependent manner. These result indicated that ethanol's suppression of proliferation induced by growth factors(bFGF and EGF) and carbachol in this model likely involves PLC and PI3K signaling pathways. These effects in vitro may help to explain the devastating effects of prenatal ethanol exposure *in vivo*, which contribute to the fetal alcohol syndrome.

Keywords**:** neural precursor cells; ethanol; proliferation; Ca^{2+} signaling; Akt phosphorylations

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APOCB2006-09-051

Phenotypic analysis of OsCR4 gene function in rice

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OsCR4 gene was identified by searching rice (*Oryza sativa* L.) genome using ZmCR4 amino acid sequences as entry. It has 87% amino acid identity and 90% similarity with ZmCR4 and belongs to the representative TNFR receptor-like kinase. OsCR4 was expressed in all detected tissues, including callus, stem, leaf, panicle, except for root. While the OsCR4 transcripts were reduced distinctly with the maturation of the panicle, as revealed by semi-quantitative RT-PCR and GUS staining. A fragment in the extracellular domain of OsCR4 with antigenic index was expressed as GST fusion protein and anti-OsCR4 antibody was produced, which recognized OsCR4 only in microsome of rice. Both RNAi and over-expression transgenic lines were generated for functional analysis of OsCR4 *in vivo*. We found that the increased or decreased transcription level of OsCR4 actually resulted in similar phenotypes including abnormal glumes color, abnormal seed coat color, reduction of grains per panicle, high proportion of non-plump seeds, plant dwarfism, crinkly or rolled or fusion leaves due to the irregular morphology of epidermis cell, especially the bulliform cells, and the partial cuticle defects. However the OsCR4 protein level in both RNAi and over-expression transgenic plants by western blot with anti-OsCR4 antibody was too low to be detectable, indicating that the expression of OsCR4 in rice was regulated at translational or post-translational level by an unknown mechanism. Real-time PCR showed that the expression of OsCR4 did not change much before and after salt stress. All the results mentioned above revealed that OsCR4, similar with ZmCR4, is an important gene that functions in regulation of growth and development in rice. This enriches the study of CR4 family in monocot.

Keywords**:** receptor like kinase; RNA interference; OsCR4

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APOCB2006-09-052

Modulation of TGF-signaling in the cytoplasm and the nucleus

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TGF-β signaling plays pivotal roles in embryogenesis and cancer development, and this signaling pathway is tightly regulated. Dappers are newly identified proteins and have implicated to regulate Wnt signaling in low vertebrates. In the present study, we present evidence that Dapper2 may regulate TGF-β/Nodal signaling by influencing intracellular sorting and thus degradation of the endocytosed TGF-β/Nodal receptors to fine-tune signal output. The activity of Dapper2 in regulating TGF-β signaling is also evolutionally conserved in fish and human. Smad7 plays an essential role in the negative feedback regulation of TGF-β signaling by inhibiting TGF-β signaling at the receptor level. Our data show that Smad7 is predominantly localized in the nucleus of Hep3B cells. The targeted expression of Smad7 in the nucleus conferred a superior inhibitory activity on TGF-β signaling. Furthermore, Smad7 repressed Smad3/4- and Smad1/4-enhanced the reporter gene expression, indicating that Smad7 can function independently of type I receptors. Smad7 can specifically bind to the Smad responsive element and disrupt the formation of the TGF-β-induced functional Smad-DNA complex. Our findings suggest that Smad7 inhibits TGF-β signaling in the nucleus in a novel mechanism.

Keyword**:** TGF-β; Dapper; Smad7

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APOCB2006-09-053

Extracellular calmodulin: existence, functions, and transmembrane mechanism

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Calmodulin (CaM), traditionally a well known intracellular calcium sensor that mediates many signaling pathway, has been found in the extracellular (apoplast) of many species of plants, and functions of extracellular CaM in accelerating cell proliferation, protoplast cell wall regeneration, and in promoting pollen germination and tube growth, have also been described physiologically in our previous studies. To provide further genetic evidence for the existence and functions of extracellular CaM to support our hypothesis that apoplastic CaM might be used as a polypeptide signal in plant development, we first verified cell wall localization of CaM in living cells by visualizing fluorescence of GFP fusion protein with soybean CaM (SCAM), and interestingly found that highly conserved isoform SCaM-1, -2, -3, but not divergent isoform SCaM-4, -5, all of which are stably expressed in tobacco cells are preferably secreted. Next we confirmed the promotion effect of extracellular CaM on pollen germination and tube growth by phenotypic analyzing *Arabidopsis* wild type pollen, transgenic pollen over-expressing extracellular AtCaM-2, a conserved isoform in *Arabidopsis*, and transgenic pollen with apoplastic CaM attenuated by over- expressing extracellular CaM-binding peptide.To understand transmembrane mechanism of extracellular CaM function, we did radio-ligand binding assay with ^{35}S-labeled AtCaM-2, and disclosed the specific, reversible, and saturable calmodulin binding sites on the surface of both *A. thaliana* suspension-cultured cells and its protoplasts. Chemical crosslinking of 35S-labeled AtCaM-2 further revealed 117- and 41-kDa plasma membrane proteins, which might be promising candidates for receptor-like protein specifically bound to extracellular CaM. Besides, phospholipase C activity, cytosolic Ca^{2+} concentration were also found to be increased specifically in response to exogenous CaM when applied outside of pollen protoplast. Furthermore, heterotrimeric Gα protein regulated pollen plasma membrane Ca^{2+} channel is involved in mediating extracellular CaM signal. All our findings suggest that apoplastic CaM may be a polypeptide signal in plants development, and its signaling pathway is different from that of intracellular CaM.

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APOCB2006-09-054

Site-specific sumoylation of SIRT1 regulates its deacetylase activity and cell's response to genotoxic stress

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Members of the Silent Information Regulator 2 (SIR2) family of proteins are NAD+-dependent deacetylase highly conserved among the genomes of organisms ranging from yeast to human. They play critical roles in diverse biological processes such as transcriptional silencing, rDNA recombination, and aging, *etc*. Similar to yeast SIR2, Sirturin-1 (SIRT1), the closest homolog of yeast SIR2, has been suggested to regulate the life-span of mammals in response to calorie restriction or nutrient starvation. Despite the significant role of SIR2 family in multiple essential biological processes, little is known about the molecular events that regulate the activity of these important enzymes. In the present study, we show that human SIRT1 is sumoylated at Lys-734, which is required for its optimal ability to deacetylate p53. Further investigations identify SENP1 as the desumoylase and HDAC4 as a positive regulator for SIRT1 sumoylation. Acute genotoxic stress with ultraviolet light or hydrogen peroxide induced the interaction between SIRT1 and SENP1, decreased SIRT1 sumoylation and suppressed its ability to deacetylate p53. More importantly, increased SIRT1 sumoylation through the silencing of SENP1 with siRNA is associated with cell's resistance to the toxic effect of ultraviolet light and hydrogen peroxide. The study is the first demonstration for a significant role of SIRT1 sumoylation in controlling its deacetylase activity and identifies SIRT1 desumoylation through SENP1 as an important regulator of the cell's response to acute DNA damage and oxidative stress. The findings carry important implications in SIRT1-regulated processes such as aging, longevity, and tumnorigenesis.

***Keywords*:** SIR2; SIRT1; p53; stress; sumoylation

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APOCB2006-09-055

Essential role of cyclin-dependent kinases in apoptosis progression

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The mammalian cell cycle is rdriven by the sequential activation of a series of cyclin-dependent kinases. Accumulating evidence suggests that cyclin-dependent kinase might contribute to apoptosis process in some experiment condition. Here we using three distinct apoptosis system including membrane receptor-mediated apoptosis induced by TRAIL, G2/M phase arrest associated apoptosis induced by paclitaxel, G1/S phase arrest associated apoptosis induced by etoposide, to investigate the role of cyclin-dependent kinase in apoptosis progression. The Cyclin-dependent kinase 2, cdk2 activity is obviously up-regulated in all three apoptosis system. Forced inhibition of Cdk2 activity effectively suppressed the loss of mitochondrial membrane potential, cytochrome c release, and caspase activation, while, forced activation of Cdk2 activity dramatically promoted these events. Importantly, forced regulation of Cdk2 activity do not interfere with the dawnstream events of cytochrome c release induced by Bax protein. Thus, we propose that the up-regulation of Cdk2 activity is essential for the upstream of mitochondrial events but not for that occur after the release of mitochondrial cytochrome c release.

Keywords**:** apoptosis; cyclin A-Cdk2; cytochrome c; caspase; mitochondrial membrane potential

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APOCB2006-09-056

Activation of protein kinase C up-regulates HERG protein expression by inhibition of proteasome function

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HERG/IKr K^+ channels are responsible for controlling action potential duration. Mutation or blockade of these channels may produce long QT syndrome (LQTs). Although HERG currents regulated by protein kinase C (PKC) have been studied, little is known about its effect on channel proteins involved in trafficking and expression. In the present study, we found that stimulation of both PKC with phorbol 12-myristate 13-acetate (PMA), a PKC activator, and α1A-adrenergic receptor with phenylephrine (PE), a selective alpha1A-adrenergic receptor agonist, up-regulated HERG protein expression dose-dependently in HEK293 which stably expressed HERG K channel and α1A-adrenergic receptor. Blockade of PKC with chelerythrine abolished these effects. Treatment of other transfected ion channels such as KvLQT1, Kir2.1, Kv1.4 and Kv3.4 with the same CMV promoter with PE for 24 hours did not change their abundances, suggesting that the effect of PE is specific to the HERG channel protein. The action of PE was not affected by geldanamycin, a specific heat shock protein (Hsp) 90 inhibitor. Moreover, PE did not alter the protein abundance of Hsp90 and Hsp70 and the maturation of HERG protein, suggesting that the effect of PE was not via altering trafficking of HERG protein. To determine whether the effect of PE depends on *de novo* protein synthesis or not, we investigated whether the new synthesized nuclear factors are required for the effect of PE on the transcription of HERG gene. Treatment with cycloheximide or anisomycin, two protein synthesis inhibitor did not abolish the effect of PE on HERG abundance. Blockade of lysosome function with NH4Cl, bafilomycin and lysosome protease inhibitor cocktails failed to affect the effect of PE. These data suggest that the effect of PE was neither via enhancing channel synthesis nor via inhibiting lysosome-mediated protein degradation. To determine the involvement of proteosome, N-acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN) and MG132, two proteosome inhibitors, were used. Both of these two drugs abolished the effect of PE, suggesting that activation of alpha1A-adrenergic receptor may inhibit proteosome function and therefore increase HERG channel protein expression. So in summary, in the present study we demonstrated for the first time that alpha1A-adrenergic receptor/PKC activity is a potential post-translational regulator of cardiac HERG expression.

Keywords**:** protein kinase c; protein degradation; proteasome; trafficking

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APOCB2006-09-057

The search for molecular mechanisms for malaria parasite cell cycle—melatonin induces transcript level changes in the parasite

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Malaria is the most devastating parasitic disease in humans, afflicting 300-500 million people worldwide and being responsible for 1.5-2.7 million deaths annually. A better understanding of molecular details of parasite cell biology is urgently required for development of new drugs against malaria.Our lab and others have reported that the parasite sense the environment. Plasmodium is able to subvert the host endocrinal system and uses melatonin to control its cell cycle (Hotta *et al*, Nature Cell Biology 2000; **2**:466-468). The signaling transduction pathways involved in the control of the parasite cell-cycle by melatonin requires a highly complex cross-talk between the Ca^{2+} and cAMP (Gazarini *et al,* J Cell Biol 2003, **161**:103-110; Beraldo *et*

al, J Cell Biology 2005, **170**:551). In addition it has been demonstrated that the action of molecules derived from the host/vector that can be recognized by the malaria parasite signaling machinery are tryptophan-derived metabolites such as tryptamin, N-acetylserotonin (NAS) and serotonin. On the other hand, the upstream signaling pathway components, i.e. membrane receptors, of *Plasmodium* still remain to activate its signaling machinery for the control of their growth and differentiation. Although serpentine receptors are present in such evolutionary distant organisms as bacteria, fungi, plants and metazoans, up to now their presence in malaria parasites are largely unexplored.We are currently testing four serpentine receptors in malaria parasites as constitute novel members of the largest class of membrane receptors widespread in living organisms, the class of G protein-coupled receptors (GPCRs), more generally called serpentine or heptahelical receptors. Plasmodium synthetic genes were transfected to HEK293T cells for functional assays. Finally, we are investigating for transcript level changes after *Plasmodium falciparum* parasites exposition to the host hormone in culture. Parasites (24 hours after invasion) were incubated for 3, 6 or 12 h with melatonin or solvent control and RNA extracted. These samples were submited to real time analysis, where the relative transcript levels of 43 genes were determined. Our data shows that the transcript levels of 10 genes significantly changed (>2 fold) after either the 6 or 12 hours incubations. Nevertheless, the identification of GPCRs in *Plasmodium* represents a breakthrough and may open new avenues for chemotherapy in malaria.

***Keywords*:** cell cycle; malaria parasite; signal transduction; calcium; transcriptional changes

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APOCB2006-09-058

Hsp70 inhibits lipopolysaccharide-induced NF-κB activation by interacting with TRAF6

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Inducible heat shock protein 70 (Hsp70) is one of the most important HSPs for maintenance of cell integrity during normal cellular growth as well as pathophysiological conditions. Tumor necrosis factor receptor-associated factor 6 (TRAF6) is a crucial signaling transducer that regulates a diverse array of physiological and pathological processes and is essential for activating NF-kappaB signaling pathway in response to bacterial lipopolysaccharide (LPS). Here we report a novel mechanism of Hsp70 for preventing LPS-induced NF-kappaB activation in RAW264.7 macrophage-like cells. Our results show that Hsp70 can associate with TRAF6 physically in the TRAF-C domain and prevent TRAF6 ubiquitination. The stimulation of LPS dissociates the binding of Hsp70 and TRAF6 in a time-dependent manner. Hsp70 inhibits LPS-induced NF-kappaB signaling cascade activation in heat-shock treated as well as Hsp70 stable transfected RAW264.7 cells and subsequently decreases iNOS and COX-2 expression. Two Hsp70 mutants, Hsp70DeltaC(1-428aa) with N-terminal ATPase domain and Hsp70C(428-642aa) with C-terminal domain, lack the ability to influence TRAF6 ubiquitination and TRAF6-triggered NF-kappaB activation. Taken together, these findings indicate that Hsp70 inhibits LPS-induced NF-kappaB activation by binding TRAF6 and preventing its ubiquitination, and results in inhibition of inflammatory mediator production, which provides a new insight for analyzing the effects of Hsp70 on LPS-triggered inflammatory signal transduction pathways.

Keywords: Heat shock protein 70; tumor necrosis factor receptor-associated factor; Lipopolysaccharide; NF-kB; ubiquitination

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APOCB2006-09-059

Edaravone regulated calcium-independent 5-lipoxygenase pathways and its protective effect on oxygen hlucose feprivation injury

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Background: Inflammation is one important pathological change after cerebral ischemia. The eicosanoid mediators (mainly including prostaglandins and leukotrienes) have been reported to be involved in inflammation after cerebral ischemia. 5-lipoxygenase (5-LOX, EC 1.13.11.34) and its products, leukotrienes play a key role in cerebral ischemic injury. There results were obtained from autopsy samples of human brain or *in vivo* animal experiment, but here not been studied at the cellular lever. One of the events of 5-LOX activation has been observed in the proinflammation cells, such as rat basophilic leukemia cells, alveolar macrophage and human polymorphonuclear leukocytes. It has also been reported that N-methyl-D-aspartate (NMDA), one of the exogenously excitatory amino acids, induces AA release in

primary culture of cerebellar neurons, and NMDA receptor antagonist MK-801 reduces leukotriene C4 (one of the 5-LOX metabolites) production in rat ischemic brain. Moreover, glutamate receptor stimulation by kainate increases 5-LOX expression in the brain. Therefore, excitotoxicity may initiate post-ischemic inflammation after brain injury via 5-LOX activation and induce cell apoptosis. However, no direct evidence is available for 5-LOX activation induced by excitotoxicity in neural cells. Recently, we have reported that edaravone have the effects of anti-apoptosis on PC12 cells after NMDA reperfusion injury. In this study, we were first to investigate edaravone regulated 5-LOX pathways and its protective effect on NMDA excitotoxicity reperfusion injury. Results: Viability of PC12 cells decreased in time-dependent (1-6 h) after NMDA-reperfusion by MTT analysis, but edaravone increased the viability significantly. Most of PC12 cells were apoptosis character after NMDA-reperfusion detected by flow cytometer analysis, electron microscope and Hoechst 33258/PI staining. Immunohistochemical analysis showed that NMDA induced 5-LOX translocation to the nuclear membrane after 1-6 h exposure, which was confirmed by Western blotting, indicating that 5-LOX was activated. Edaravone inhibited 5-LOX translocation after NMDA-reperfusion injury and protect PC12 cells from NMDA-reperfusion injury. Furthermore, We then determined intracellular calcium ($[Ca^{2+}]i$) level to observe whether edaravone affect $[Ca^{2+}]i$ elevation and the relation of $[Ca^{2+}]i$ and 5-LOX pathway activation. Conclusion: In summary, We first found that NMDA induces 5-LOX activation and that edaravone inhibits NMDA activated 5-LOX. These findings suggest that neuroprotective effects of edaravone on ischemic or other brain injury may be partly mediated by inhibiting 5-LOX activation. Furthermore this neuroprtector effect is independent on $[Ca^{2+}]i$ concentration.

Keywords**:** 5-lipoxygenase pathways; oxygen glucose deprivation; edaravone; calcium-independent; PC12 cells

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APOCB2006-09-060

Changes in dye coupling of root epidermis and cortex cells of *Arabidopsis thaliana* demonstrated by a caged probe

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Plasmodesmata is a special highly regulated cytoplasmic channels that swan the wall of two cells in plant, which let water, ion, and molecular signals transport among cells, provide cell-to-cell communication and coordinate growth and development in plant tissues. Caged probe is novel tool to study cell-to-cell communication. In this paper, caged probe was loaded into the cells of root in the seedlings with 6 to 9 true leaves by changing temperature during loading dye in bid to widening plasmodesmata aperture in *Arabidopsis*. The direction and degree of uncaged probe from the region (cell) of interest (Roi) of the root at different region were determined and assessed by time series and quantitative tool, respectively. Uncaged dye moved very rapidly at all directions from the uncaged cell in the epidermis cells of root tip at division zone. It was clearly that more uncaged dye spread in the cells near root cap, which showed more plasmodesmata distributes between the uncaged cell and the cell near root cap. The pattern of uncaged probe displaying in cortex cells at the division zone was similar to the epidermis cell in the same zone. When the cell of root cap was uncaged by UV laser, the uncaged dye was diffuse among the cells of root cap and also to the cells of epidermis cells. When the epidermis cell at elongation zone was uncaged, more dye transported to the longitudinal cells than to the transverse cells, and less dye spread to the cells near root hair zone than to the cell far from the same zone. The epidermis cells developed into two kinds of cells, i.e. the cell with hair and the cell without hair. As the cell with hair was uncaged, the uncaged dye just diffuse into the same cell but not to the cells around the uncaged cell, which indicated that the cell with hair was isolated. The uncaged probe did not flow to other cells but just diffuse in the uncaged cell without root hair at root hair zone, suggesting that the plasmodesmata had been closed in the cell. The relative speed of uncaged dye spreading to neighboring cells was assayed in different cells with different zone by the slope at the time when intensity of fluorescence reached half of highest level. Among them, the epidermis cells in division zone got the highest speed, and the cells near root hair zone got the lowest. Changing in direction and speed of dye spreading among different cells reflected a correlation between symplastic communication and the development of epidermis cells in root of *Arabidopsis*.

Keywords: *Arabidopsis thaliana*; root; caged fluorescein; laser scanning confocal microscopy (CLSM)

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