SHORT-TERM AND LONG-TERM POTENTIATION OF EXOCYTOSIS AND EXOCYTOSIS-DEPENDENT CELL MEMBRANE REPAIR IN FIBROBLASTS

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Ca^{2+}-regulated exocytosis is apparently universally present in cells, and plays an essential role in maintaining the integrity of the cell membrane. If a cell experiences a membrane disruption in the micron diameter range, Ca^{2+} influx at the wound site triggers exocytosis that is essential for successful cell membrane repair. We demonstrated here that repeated membrane disruption revealed potentiation of Ca^{2+}-regulated exocytosis in 3T3 fibroblasts, which was also reflected in faster membrane resealing rates. This potentiation of exocytosis was PKA-dependent in the early stages: in the intermediate-term required protein synthesis, and for long-term depended on the activation of transcription factor CREB. These findings reveal the existence of short-term and long-term potentiation of Ca^{2+}-regulated exocytosis in a non-neuronal cell.

EFFECT OF PHENYLARSINE OXIDE (PAO) ON PKC ACTIVATION IN PORCINE POLYMORPHONUCLEAR LEUCOCYTES

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We examined the effect of phenylarsine oxide (PAO), which interacts specifically with vicinal dithiol in protein, on reguration of protein kinase C (PKC) activity. We previously reported that in PMA-treated porcine polymorphonuclear leucocytes (PMNs), PAO could not prevent translocation of PKC-beta2 to plasma membrane. However, electrophoretic mobility shift assay suggested that PAO may inhibit PKC-beta2 autophosphorylation. In the present study, we examined the effect of PAO on PKC activity. PMNs were PMA-stimulated, followed by preparation of lysate for SDS-PAGE. By Western blot analysis using anti-phosphoserine antiboby, we could not detect any effect of PAO on phosphorylation of celluar proteins.

Phosphorylation of starfish stem-loop binding protein in starfish oocyte extract

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Unlike all other metazoan mRNAs, histone mRNAs are the only cellular mRNAs that do not have a polyA tails, ending instead in a highly conserved stem-loop sequence. Because the histone genes do not have introns, the only processing reaction necessary for formation of the mature histone mRNA is an endonucleolytic cleavage to form the 3' end. The stem-loop interacts with a specific RNA-binding protein, termed the stem-loop binding protein (SLBP) or the hairpin binding protein (HBP), which participates in pre-mRNA processing and remains associated with the mature histone mRNA. The stem-loop is also essential for translation, presumably as a complex with SLBP.

We have previously cloned the gene encoding starfish SLBP (SSLBP) by screening the expression cDNA library. The complete SSLBP contains 435 amino acids with a predicted molecular mass of 49 kDa, significantly smaller than the 72 kDa estimated for the SSLBP by SDS-PAGE. Here, we report that SSLBP is phosphorylated in starfish cell-free extract from mature oocyte in a cdc2 kinase-dependent manner, but not in extract from immature oocyte.

PHOTOPERIODISM AND MORPHOLOGICAL CHANGES IN BROWN FATCELLS OF THE LAND LEECHES, *Haemadipsa zeylanica var. japonics*

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We reported that the brown fat cell of land leeches and oil droplets becamelarge at low temperatures. In this study, we observed that their swollen brownfat cells relate to temperature and photoperiodism. We collected them ata forest in Chiba maintained by the Univ. of Tokyo once a week from Nov. 2000 to Mar.2001. We used the weather observation facilities in Kiyosumi, the day lengthobservation at the National Observatory and electron microscopes. As a result,in mid-Nov. the fat cells, which accumulated many oil droplets, werespindle shaped. The cytoplasm was small and dark. At the end of Nov.the fat cells, which changed to the shape of swollen eggs, included very largeoil droplets. The cytoplasm, which increased in volume, became lighter incolor.There also were mitochondoria surrounding the nucleus. The average-temperature was 11.3°C in the middle of Nov., and 10.2°C fromNov. 20(daylength 10 h 10 m) to the end of Nov. (9 h 58 m on Nov. 30).It was guessed that the changes in the swollen brown fat cellswere more related to photoperiodism than to the effects of temperature.