

translation. The binding motifs of each recombinant protein were analyzed by GST pull down assay and further confirmed by BIAcore. The results demonstrated that the proacrosin SIII domain interacted with the leucine-rich domain of the proacrosin binding protein. In addition, there is another interaction between SII domain of proacrosin and the acidic amino acid domain of the proacrosin binding protein.

E109 Is the 32kd sperminogen produced from proacrosin posttranslational modification mechanism?

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The potential mechanism to produce 32 kd sperminogen has been investigated by northern and western blot analyses. Since the lowest molecular mass sperminogen, 32 kd sperminogen, was characterized as a part of proacrosin/acrosin system from peptide sequence analysis, the potential mechanism to produce 32 kd spermiogen was studied. We have attempted northern blot analysis of the boar testicular mRNA with the 4 different DNA probes, each of which corresponds to the exon of boar proacrosin genomic sequence. Northern blot analysis demonstrated that all 4 probes detected only one species of mRNA, proacrosin mRNA, signifying that 32 kd sperminogen is not produced by differential splicing of the precursor to proacrosin mRNA. Next, to test the possibility that 32 kd sperminogen might be produced by the random breakdown of proacrosin, intact proacrosin was purified and allowed to decay in the test tube. When this sample was analyzed by western blot, the 32 kd sperminogen antibody did not detect any protein band which corresponds to 32 kd sperminogen, implying that 32 kd sperminogen is produced by specific posttranslational modification mechanism in the male germ cells during the formation of spermatozoa.

E110 The Involvement of Clock-Bmal1 Heterodimers in Serum-responsive Induction of mPer1 Expression

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Serum-responsive induction of mammalian *period1* (*Per1*) gene expression is supposed to be critical in the circadian oscillation of clock gene expression in mammalian cells, but the molecular mechanism underlying this event is poorly understood. Regarding the fact that a rapid mouse *Per1* (*mPer1*) induction is controlled at the transcriptional level and does not involve synthesis of new proteins, we postulated the involvement of Clock-Bmal1 heterodimer, a well-known positive regulator of *mPer1* transcription, in rapid induction of *mPer1* transcription. *mPer1* promoter fused with luciferase open reading frame was transiently transfected into NIH-3T3 cells with or without Clock19 expression vector, a dominant negative form of Clock. Twenty four hours after serum starvation, serum shock was given and relative luciferase activities were measured. Clock19 overexpression partially blocked the *mPer1* induction by serum shock. We also examined the E-box binding activities of Clock-Bmal1 heterodimer by gel shift assay. Serum shock increased E-box binding activities of Clock-Bmal1 heterodimer within 30 minutes. Taken together, these results suggest that Clock-Bmal1 heterodimer is involved in the rapid induction of *mPer1* by serum shock.

E111 The 31kDa Fragment, Phosphorylation-dependent Cleavage Product of p130Cas, is an Apoptotic Mediator in Rat-1 Fibroblast Cell

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During apoptosis, p130Cas is cleaved by caspase-3 in phosphorylation-dependent manner and the 31kDa fragment is generated. The phosphorylated p130Cas by LPA appears to be resistant to cleavage by caspase-3 and the dephosphorylation of p130Cas by CIP, PP1 and LAR enhanced the production of 31kDa fragments. Moreover, double mutations substituting the Glu of S743 and Y751 at a cleavage site was also resistant to caspase-3 cleavage, suggesting that production of the 31kDa fragment is regulated by phosphorylation. The 31kDa fragment, which contains a divergent helix-loop-helix(HLH) motif, is translocated into nucleus during etoposide-induced apoptosis and is likely to interact with E47 via HLH domain. In addition, the overexpression of GFP-fused 31kDa fragment induced the morphological changes characteristic of apoptosis, suggesting that the 31kDa fragment may be translocated into nucleus and thereby regulate the onset of apoptosis through interaction with the transcriptional regulatory HLH proteins, in which the complex may lead to induction of cell death gene associated with the induction of apoptosis.

E112 Role of Cloned ApCAM-Associated Protein (CAMAP) on Long-Term Facilitation in Aplysia

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The growth of synaptic connections that accompanies 5-HT-induced long-term facilitation (LTF) in *Aplysia* is associated with the internalization of apCAM at the surface membrane of the sensory neuron. In this study, to gain more insight into apCAM function, we searched for binding proteins of apCAM by the yeast two-hybrid

method by using as bait the cytoplasmic tail of apCAM. Sequence analysis revealed that one cDNA clone, we obtained, has some homology to a microtubule associated protein. This apCAM-associated protein (CAMAP) was coimmunoprecipitated with apCAM from transfected HEK293T cells. Confocal microscopic analysis also showed that CAMAP colocalized with apCAM at the cytoplasmic face of plasma membrane in cultured *Aplysia* neurons. In sensori-motor coculture, overexpression of CAMAP in *Aplysia* sensory neurons induced LTF by application of a single pulse of 5-HT that normally induced only short-term facilitation (STF). In addition, microinjection of antisense CAMAP into the sensory neurons completely blocked the LTF induced by 5-HT, without affecting STF. These results suggest that CAMAP plays a crucial role in LTF presumably by interacting with apCAM to induce internalization and synaptic growth associated with LTF in *Aplysia*.

E113 Presence of Specific Receptor for Dendroaspis Natriuretic Peptide in the Freshwater Turtle Kidney

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Dendroaspis natriuretic peptide (DNP), a 38-amino acid residue peptide, was isolated from the venom of the green mamba snake (*Dendroaspis angusticeps*), and shared functionally important sequence homologies with ANP, BNP and CNP. Although it has been designated as a new member of natriuretic peptide family, the natriuretic peptide receptor for DNP is still not clear. The present study was undertaken to investigate the presence of DNP-dependent receptor in the kidney of freshwater turtle. By quantitative in vitro receptor autoradiography, specific ¹²⁵I-DNP binding sites were localized in glomerulus and renal tubules. In the presence of excess