E106 Characterization of a New SUMO-1-Specific Proteases, Mouse UlpB and Human SUSP2

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SUMO-1 is a ubiquitin-like protein, and its conjugation to cellular proteins (SUMOylation) has been implicated to play important roles in multiple vital cellular processes, such as oncogenesis, cell cycle control, apoptosis and response to virus infection. Since Ulp1, several SUMO-1-specific proteases have been identified from various species (SUSP1, SENP1, Ulp2, SMT3IP1 and SMT3IP2). We cloned two new SUMO-1-specific proteases, named UlpB and SUSP2, from mouse brain and human brain by 5'-RACE method. They are cysteine proteases containing the well-conserved His/Asp/Cys catalytic triad. In addition, confocal microscopy showed that UlpB and SUSP2 are exclusively localized to the nucleus of NIH3T3 cells. Co-transfection of SUMO-1 with SUSPs results in release of SUMO-1 from the SUMO-1-conjugated proteins. These results suggest that UlpB and SUSP2 may play a role in regulation of many SUMO-1-mediated cellular functions.

E107 Cloning and Properties of the Homologs of a Ubiquitin Specific Protease, HAUSP

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Previously, we have purified and characterized an ubiquitin specific protease, UBP-8. Using the degenerate probes for the partial amino acid sequence of UBP-8, we isolated three cDNA clones. Since two of them encode proteins with an identical size of 75 kDa and the other does a 135-kDa protein, they were named as UBP75a, UBP75b, and UBP135, respectively. Of these, the deduced amino acid sequence of UBP135 was identical to that of HAUSP. The amino acid sequences of UBP75a and UBP75b were identical to the corresponding N-terminal region of UBP135, but UBP75a had a short C-terminal extension with distinct sequence while UBP75b lacked the C-terminal domain. Thus, it appears that the UBPs are generated by alternative splicing of their primary transcript. All the UBP enzymes were partially purified and found to be capable of generating free ubiquitin (Ub) from Ub-CEP80 and poly-Ub chains via isopeptide bonds, but not from linear Ub-polymer. Northern analysis showed that the mRNAs for all enzymes were the most abundant in the brain. Both UBP75a and UBP75b were localized to the nucleus as speckles, similar to HAUSP, which promotes the localization of PML into nuclear bodies. Thus, UBP75a and UBP75b may also play a role in the regulation of PML nuclear distribution.

E108 Binding motif analysis of proacrosin and the proacrosin binding protein

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When spermatozoa were extracted at pH 4.0, acrosomal protease, proacrosin, is extracted as a complex with the proacrosin binding protein. This complex dissociates, however, upon SDS treatment. In the present study, we have attempted to analyze the binding motifs of proacrosin and the proacrosin binding protein complex. For this experiment, various deletion mutants of proacrosin and the proacrosin binding protein cDNA clones were constructed. The protein products of these cDNAs were obtained by expressing these recombinant clones in E. coli and in vitro

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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