E113 Cloning and Characterization of a cDNA for 45-kDa Matrix Protein of Delayed Fusing Lysosome in *Amoeba proteus*

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Previously we produced 2 monoclonal antibodies (mAbs), LCA45 for a matrix protein and LYA64 for a membrane protein of lysosomes in *A. proteus* and showed that amoeba have lysosomes in subpopulations [Mol & Cells 6: 316-324, 1996). Lysosomes staining with LCA45 antibody fused with phagosomes after 3 h from their formation and recycled to the lysosomes after cellular digestion of food. In this study we cloned a cDNA of 1.26 kb by screening a cDNA library of amoeba constructed in λZAP using LCA45 as a probe. The cDNA contained a poly-A tail and encoded a protein of 45 kDa. In a search for homologous gene from databases, the protein was found to be a noble protein showing partial homology to endoprotease of *Hydra attenuata*. In the deduced amino acid sequences, ^{9}AsnSerSer^{12} appeared to be the only one site for heavy mannose type glycosylation. In the analysis of amino acid sequences by PCGENE, the protein had C-terminal KDKKLK as an antigenic determinant.

E114 Characterization of a Monoclonal Antibody for Ubiquitinated Proteins and Detection of Heat-Shock Responsive Ub-Proteins in *Amoeba proteus*

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A monoclonal antibody (mAb) reacting with many different proteins (14 - 200 kDa) of *A. proteus* was characterized and used in the detection of ubiquitinated proteins on stress. By indirect immunofluorescence microscopy we found the antigens to be dispersed throughout the cytoplasm but were more concentrated in the nucleus. The mAb cross-reacted with proteins of *Tetrahymena*, *Xenopus* embryo and mouse macrophages. In an analysis for specificity, the mAb reacted with poly-Ub and Ub-fusion proteins larger than 14 kDa, but did not react with monomeric Ub. In amoebae about 20 Ub-proteins were found to increase or decrease during a 5-h heat shock at 33°C and the level of 16 Ub-proteins was changed during starvation for 8 days period. In two-dimensional analysis of immunoblot we could reveal more than 40 Ub-proteins. Further characterization of these heat-shock sensitive Ub-proteins will elucidate the cellular factors responsive to stresses in amoebae.