Z307 Effects of Aphidicolin and α-Amamitin on the Expression of E-cadherin and Cellular Flattening in the Preimplantation Mouse Embryos

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In this study, we inverstigated the effects of aphidicolin and α-amanitin on the expression of E-cadherin and the cellular flattening of mouse embryos. Firstly, the transcripts and protein of E-cadherin were detected in the oocytes and the preimplantation embryos and the immunoactivity of E-cadherin was concentrated in the cell-cell adhesion region onward 8-cell stage. Secondly, the relation between DNA and RNA synthesis and compaction in the early and late 2-cell embryos was asserted by the treatment of aphidicolin and α-amanitin. The exposure to α-amanitin resulted in the inhibitory effect on the cellular flattening of mouse embryos, but the exposure to aphidicolin did not. In addition to the morphological aspect, the occurrence of cellular flattening was confirmed by immunofluorescent dye. Thirdly, the effect of aphidicolin and α-amanitin on the transcription of E-cadherin gene was monitered by RT-PCR. Steady-state mRNA level for E-cadherin gene was not different in the aphidicolin-treated embryos, but significantly different in the α-amanitin-treated embryos compared with that of control. This result suggests that the intercellular flattening of mouse embryo seems to depend on the transcriptional events, but not depend on number of the cleavage, and takes place according to a biological clock.

Z401 Purification and Characterization of an Inducible Antibacterial Peptide from the Larvae of *Protaetia brevitarsis*

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Three types of antibacterial peptides(protaetins 1, 2 and 3) have been isolated and purified from the larval hemolymph of *P. brevitarsis* using gel permeation chromatography, preparative acid-urea PAGE and reverse-phase FPLC. Acid extracts of the cell-free hemolymph prepared by mixing with 10% acetic acid were used as starting materials for purification. Ultrasensitive radial diffusion and overlay assays were carried out to monitor the antibacterial fractions through purification steps. Of three antibacterial peptides, protaetin 1 has been successfully purified to homogeneity.

The N-terminal sequence of protaetin 1 was determined by gas-phase Edman degradation and the molecular mass was also measured to be 9283.95 kDa by MALDI-TOF mass spectrometry. From antibacterial radial diffusion assay, we could confirm that the antibacterial activities of protaetin 1 were effective against Gram positive and Gram negative bacteria. Although the other two antibacterial peptides(protaetin 2, 3) were not completely purified, the molecular masses of protaetin 2 and 3 were estimated as 7 and 12 kDa on 16.5% Tricine SDS-PAGE, respectively. Further works including purification of protaetin 2 and 3 and cDNA cloning for protaetin 1 are undertaking.