Z309 Ultrastructral Changes of Zona Pellucida Surface during Hatching in Mouse Embryos

전 일 경[1*], 한 성 원[1,3], 권 중 균[2], 정 호 삼[2], 김 문 규[1]
한양대학교 자연과학대학 생물학과[1], 의과대학 해부학교실[2]
을지의과학센터[3]

To investigate the roles of intrinsic embryonic factor and extrinsic uterine factor in hatching of mouse embryos, we treated benzamidine to blastocyst for the inhibition of trypsin-like enzyme activity and transferred the oocyte and 2-cell embryo to pseudopregnant female uterus at the time of hatching. The ultrastructural changes of zona pellucida (ZP) surface were examined by SEM. The inner surface of ZP of blastocyst developed *in vitro* appeared uneven and holes which resulted from the removal of cytoplasm processes disappeared, whereas that of benzamidine-treated group showed moderately smooth. The outer surface of ZP of the oocyte and 2-cell embryo transferred to uterus for 36hr was changed to be more smooth than that of *in vitro* group. Taken together, a trysin-like enzyme originated from embryo seemed to lyse the inner surface of ZP and uterine fluid may lyse the outer surface of ZP. Therefore, both of trypsin-like enzyme and uterine fluid induce the hatching of mouse embryo synergstically.

Z310 Functions of actin in the sequential formation of basal body, flagella, and cytoskeletal microtubule systems during *N. gruberi* differentiation.

Han, Ji Woong* and JooHun Lee
Department of Biology, Yonsei University

During the differentiation of *Naegleria gruberi* amebas into flagellates, microtubule-based organelles -basal body, flagella, and cytoskeletal microtubule systems- are sequentially formed *de novo* in less than 2 hrs. At 70 min after the initiation, most of the differentiating cells are in spherical shape and 50% of the cells have a pair of flagella. Within the additional 15 min, flagellates begin to elongate and cytoskeletal microtubule array begin to form from the base of flagella. To understand the mechanism of these sequential formation of microtubule organelles in the *N. gruberi* differentiation, we started to examine the distribution of actin in the differentiating cells and investigated their functions. We have found that an actin-based structure is formed de novo during differentiation. This structure began to be observed in 30 min cells. At 70 min, this structure is located near the base of flagella. As the differentiation further proceeds, 85 min, the actin structure begin to move toward the distal end of the flagellated cell and to be disorganized. At the end of differentiation, the actin structure was not evident. The timing of this structure formation and the pattern of its location are almost identical to the reported localization of the tubulin mRNAs. Cytochalasin treatment at the beginning of differentiation inhibited the formation of this structure and if the drug was added at late stages (~30 min, 65 min) disrupted the structure. This disruption of the actin structure caused the cells to form cytoskeletal microtubule before the formation of flagella. These results showed actin or their stable complex might regulate the order of microtubule organelles formation by blocking assembly of cytoskeletal microtubules before the formation of flagella in time-dependent manner