

Distribution of apoptosis-like cells in sea urchin early embryogenesis

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It has been reported that the number of cells per embryo increases from the cleavage stage to the pluteus stage. Also, it has been reported that the number of cells per embryo from the early gastrula stage to the mid-gastrula stage increases very slightly (Mizoguchi, 1999). A detailed analysis of cell proliferation during this period would thus seem to be necessary.

On the other hand, Roccheri *et al.* (1997) reported spontaneous apoptosis at the early pluteus stage, especially in the regions of arm and intestine. However, it is unknown whether apoptosis occurs before the pluteus stage.

Using the Tumor, Neuro and/or Cardio TACS *in situ* apoptosis detection kit (terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling: TUNEL method, Trevigen, USA), the Comet assay kit (Trevigen, USA) and DAPI (4,6-diamidino-2-phenylindole dihydrochloride) staining, we investigated the distribution of apoptosis-like cells in sea urchins during early embryogenesis to clarify the relationship between cell death and morphogenesis.

Materials and methods

The embryos of the sea urchin *Hemicentrotus pulcherrimus* were used in the present study. Three methods of detection of apoptosis signals in the embryos were performed: (1) defects in chromosomes and punctured nuclear envelopes of cells were detected by DAPI staining; (2) apoptosis-like cells were stained a brown colour by the TUNEL method; (3) some clear tails which detected the fragmentation of DNA were found by the Comet assay. These signals indicate apoptosis-like cells.

Results

No apoptosis-like cells were found in embryos by the Comet assay at the morula stage. About 20 apoptosis-like cells were found in the animal and vegetal hemispheres and near the blastopore by the TUNEL method

at the swimming blastula stage. Some clear tails were found by the Comet assay at the mesenchyme blastula stage. The signals of DAPI staining and the TUNEL method were found in almost the same part of the embryo. About 10 apoptosis-like cells were found in embryos by the TUNEL method at the early gastrula stage. In the mid-gastrula and late gastrula stages apoptosis-like cells were found in embryos by the TUNEL method. Apoptosis-like cells were found in the structure of the archenteron by the TUNEL method at the late gastrula stage.

Discussion

Using the TUNEL method, the Comet assay kit and DAPI staining, we found apoptosis-like cells of sea urchins during early embryogenesis. No apoptosis-like cells were found in the embryos before hatching. At the blastula and gastrula stages, apoptosis-like cells were found in the animal and vegetal hemispheres.

It has been reported that gastrulation is accompanied by the rearrangement of invaginating epithelial cells (Ettensohn, 1985) and that cells are added to the archenteron during and following secondary invagination (Martins *et al.*, 1998). It is probable that apoptosis-like cells are triggers for these cell movements and/or rearrangement of embryonic cells.

Taking these reports together it seems that apoptosis-like cells found at the blastula and gastrula stages may play a role in morphogenesis, especially in archenteron formation and/or regulation of cell number in the sea urchin embryo.

References

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