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Regulative potential to form an amniotic cavity in mesomeres of a direct developing echinoid, *Peronella japonica*

Chisato Kitazawa[1] *and Shonan Amemiya*[1,2]

[1]Department of Biological Sciences, Graduate School of Science, University of Tokyo, Japan

[2]Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo, Japan

Peronella japonica, a direct developer, exhibits certain peculiar features during development, particularly heterochrony, a change in the relative timing of expression among tissues and organs. One of the important heterochronical changes in the species was found in the development of the amniotic cavity, a component of an adult rudiment. In indirect developers the amniotic cavity is formed on the left side of the larval body in the late pluteus stage. In *P. japonica* the organ is formed at the gastrula stage in the region located on the midline of the larval body.

In the present study, the ability of partial embryos isolated from 8- or 16-cell stage embryos of *P. japonica* to differentiate an amniotic cavity was investigated to assess the regulative potential of a direct developer.

The embryos were dissected at 8-cell stage with a glass needle to obtain half embryos. Some of the half embryos were further divided into four blastomeres to obtain mesomere pairs. Each half embryo and blastomere that did not form micromeres but divided equally during the next cleavage was identified as an animal cap and presumptive mesomere pair. Isolated animal caps and mesomere pairs were cultured, and differentiation of the amniotic cavity was examined at 24 and 48 h after fertilisation, when the organ in the normal embryos had already completed differentiation.

A total of 69 animal caps were produced. Among them, 39 (57%) formed an amniotic cavity, and one specimen formed two amniotic cavities. Twenty-three specimens (33%) failed to form the organ. An indentation found in 6 specimens (10%) could not be definitely identified as an amniotic cavity. Among a total of 109 mesomere pairs, 79 (72%) formed an amniotic cavity, and 30 specimens (28%) did not.

The 16-cell stage embryos were composed of eight mesomeres, four macromeres, and four micromeres. The animal cap composed of eight mesomeres was obtained by dissecting the embryo in the equatorial plane. Some of the isolated animal caps were further divided into mesomere pairs. Among a total of 102 animal caps, 98 (96%) formed an amniotic cavity, and 4 (4%) did not. The ability to form an amniotic cavity was greater in the embryos derived from the animal cap isolated at the 16-cell stage than at the 8-cell stage. The results suggest that the fate of the animal cap for amniotic cavity differentiation in normal embryos is determined by the 16-cell stage. Sixty-seven (49%) of 136 embryos derived from the mesomere pairs formed an amniotic cavity, but 41 (30%) did not. An indentation formed in 28 specimens (21%) could not be definitely identified as an amniotic cavity. There are two possible reasons why the ability to form the amniotic cavity of the mesomere pairs isolated from the 16-cell stage embryos was lower than that of the presumptive mesomere pairs isolated from the 8-cell stage embryos. One is that the regulative potential to form the amniotic cavity of each mesomere pair decreased with progression of determination of the fate to form an organ. Another is that every pair of mesomeres isolated from a 16-cell stage embryo did not always consist of the daughter cells of one blastomere at the 8-cell stage of the embryo, but one mesomeres in each pair of mesomeres might be derived from daughter cells of one blastomere and the other from those of adjoining blastomere. None of the single mesomeres isolated from the 16-cell stage embryo could form an amniotic cavity, suggesting that a minimum cell volume is required for its formation.

Ten of the 13 reaggregated embryos produced by four mesomere pairs isolated from a 16-cell stage embryo formed an amniotic cavity, but the other 3 specimens did not. The embryos derived from the animal caps and the mesomere pairs isolated from both the 8- and 16-cell stage embryos had completed formation of the amniotic cavity by 24 h after fertilisation. The amniotic cavities that had formed in the partial embryos were no longer seen by 48 h after fertilisation. These partial embryos never formed endodermal and/or mesodermal organs. All four mesomere pairs derived from 8- or 16-cell stage embryos had the ability to form an amniotic cavity, suggesting that formation of the amniotic cavity in the normal embryo is regulated by a signal from laterally adjoining blastomeres.