POLAR LOBE FUNCTION FOLLOWING EQUAL FIRST CLEAVAGE IN ILYANASSA OBSOLETA EMBRYOS

JoAnn Render
Biology Department, Hamilton College, Clinton, NY 13323

In many embryos that form polar lobes it has been possible to prevent polar lobe formation, leading to the equal distribution of lobe (vegetal pole) material during first cleavage, usually by compressing the embryo. Such equalization of cleavage often leads to the duplication of lobe-dependent structures in resulting larvae, although all previous attempts with Ilyanassa obsoleta embryos have been unsuccessful, with the development of equalized embryos becoming arrested during subsequent cleavages. I set out to investigate this discrepancy using alternative methods of equalization.

Cleavage in I. obsoleta embryos can also be equalized using the microfilament disruptor cytochalasin B (CB) because the microfilament band mediating polar lobe formation is more sensitive to the drug than the microfilament band mediating cleavage (Conrad, G. and Williams, D.C. 1974. Dev. Biol. 36: 363-378). I. obsoleta (Nassarius obsoletus) were collected from intertidal mudflats on the east side of Thompson Island. Embryos were exposed to 1-3µg/ml CB (1 mg in 1 ml DMSO stock diluted with sterile seawater) from the completion of meiosis until controls had just begun to cleave. Control embryos exposed to equivalent DMSO concentrations developed normally. Equalized embryos do not form a polar lobe, but complete cleavage, resulting in two equal cells. At second cleavage each cell forms a polar lobe, then divides unequally, resulting in two large and two small cells, although the large cells could either be opposite each other (CDCD configuration) or adjacent (CCDD configuration). Equalized embryos were cultured for 7 days to the larval stage, then analyzed for duplications of shell, foot, operculum, statocysts and eyes. Duplication of at least one of these structures was observed in 93% of the larvae from equalized embryos, while 64% exhibited duplication of two or more structures. Using an appropriate method of cleavage equalization, therefore, I. obsoleta larvae do possess duplicated lobe-dependent structures.

Although duplication of shell was observed following equalization, it was much less frequent than duplication of other lobe-dependent structures. Single shells in larvae from equalized embryos were often large and irregularly shaped. To test the possibility that shell duplications were sometimes masked due to the fusion of shell material produced by two shell glands, equal blastomeres were separated at the two cell stage with a fine glass needle. When such isolated cells were cultured to the larval stage and analyzed as pairs, shell duplication was more than three times as frequent, supporting the idea that many cases of shell duplication are masked in larvae from whole equalized embryos. Duplication in the case of the pairs meant that each half larva formed a shell, demonstrating that each half, which was derived from a cell containing half of the original polar lobe material, was capable of differentiating lobe-dependent structures. Foot and operculum duplications were also more frequent in pairs of half larvae than in whole larvae. Two or more duplicated structures were observed in 81% of the pairs.

In summary, this study has demonstrated that duplications of lobe-dependent structures do arise in I. obsoleta larvae following equalization of first cleavage. Data from pairs of half embryos show that each half of the embryo is capable of forming lobe-dependent structures if polar lobe material is included at first cleavage. Pair data also reveal that lobe-dependent structure duplications can be masked in whole larvae. Supported by a Markey Fellowship from the MDIBL, a Bristol-Myers Company Grant of Research Corporation and a Faculty Research Award from Hamilton College.