A survey of pole cell determinants in cytoplasm of *Drosophila* eggs (Insecta: Diptera)

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Preceding the blastoderm stage in *Drosophila* development, pole cells are formed from bulgings of posterior pole cytoplasm each including a nucleus. The pole cells have been attracting attention of developmental biologists for many years for their involvement of a cytoplasmic determinant, known as polar plasm or oosome, during their cellularization and determination on differentiation into primordial germ cells. Despite a great interest of developmental biologists, the biochemical analysis of the determinant was not feasible, until a method for assaying pole cell forming activity was established (Okada et al., 1974). Using the bioassay system, which involves microinjection of UV-irradiated eggs with material to be tested for the activity, one of the authors has shown that a subcellular fraction precipitated by a centrifugation at 27,000 g for 60 min from a homogenate of *Drosophila* embryos includes an activity of pole cell induction and that the activity is retained in a fraction held in 1.8 M sucrose when the 27,000 g fraction (called P-3 hereafter) was centrifuged on a discontinuous sucrose gradiant (Ueda & Okada, 1982).

We report here that RNA included in the P-3 fraction is responsible for pole cell formation.

Digestion of P-3 by Trypsin and RNAse

Trypsin-digested and RNAse-digested P-3 fraction showed a pole cell forming frequency of 32% and 2.5% on the bioassay, respectively. Intact P-3 fraction shows 30% of the frequence on the average. Apparently digestion by RNAse deprived pole cell forming activity of the P-3 fraction.

Pole Cell Forming Activity of RNA Extracted from P-3

Total RNA was extracted from P-3 according to a standard method. This crude RNA fraction exhibited a pole cell forming frequency of 11% (Togashi and Okada, 1982). Although the value is low, statistical test tells that this is significantly different from the value of the control, where embryos were irradiated but not injected.

The RNA fraction was applied on an oligo-dT column to obtain poly (A) + RNA and poly (A) − RNA fractions. Poly (A) + and poly (A) − RNA showed the pole cell forming frequency of 19% and 5% on the bioassay, respectively. Obviously the pole cell forming activity is retained in the poly (A) + RNA fraction.

Morphology of Pole Cells Formed by Injection of Poly (A) + RNA

Polar granules and nuclear bodies, reliable morphological markers of pole cells, are clearly observed in the pole cells induced by the poly (A) + RNA fraction in UV-irradiated eggs. However, these morphologically normal pole cells seemed unable to differentiate into germ cells.

Local Treatment of Eggs with Cycloheximide at the Posterior Pole

The treatment inhibited the pole cell formation. This indicates that the function of a poly (A) + RNA involves the translation.

Results suggest that a messenger type RNA present in the cytoplasm of *Drosophila* eggs may represent a function of polar plasm as a determinant, although we have not yet located the mRNA in the egg. Identification of translates and cloning of the gene coding for the mRNA are to be carried out.

References

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