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DB 77

BIPOLAR ORGANIZATION OF CORTICAL ACTIN IN THE *TUBIFEX* EGG.

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Organization of cortical actin in *Tubifex* eggs from fertilization through the second polar body formation (PBF) has been examined in isolated cortices and whole mounts stained with rhodamine phalloidin. When eggs are fertilized, they exhibit intense fluorescence, as a result of microvillar elongation, all over the surface except a circular zone (30 μm in diameter) which appears as a 'black hole' due to much weaker fluorescence. Thereafter cortical actin gradually decreases in amount; only tiny aggregates of actin are seen in the cortex. During the first PBF, cortical actin appears to be localized at the animal pole and at grooves formed by the deformation movement. Shortly after the PBF, however, no regional difference is detected in the density of cortical actin. It is not until the second PBF that cortical actin is organized bipolarly. That is, cortical actin is distributed as a gradient increasing from the equator to the pole region in the animal and vegetal hemispheres of the egg. When eggs begin the second deformation movement accompanying the second PBF, streaks of actin bundles form in the cortex of the equator of the egg. They run meridionally and link actin sheets of the animal and vegetal hemispheres, suggesting their involvement in groove formation at the equator of the egg. Polarized distribution of cortical actin is retained even after the end of the deformation, while equatorial actin streaks disappear this time. These observations suggest that bipolar organization of cortical actin is generated shortly before the second PBF.

DB 78

UNEVEN DISTRIBUTION OF MITOCHONDRIAL ACTIVITY IN DROSOPHILA EARLY EMBRYOS.

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Vital staining of mitochondria with the fluorescent dye rhodamine 123 was performed in Drosophila early embryo. Rhodamine 123 accumulates specifically in mitochondria of living cells. This accumulation depends on the high transmembrane potential characteristic of functional mitochondria. We found that the fluorescence of rhodamine 123 was unevenly distributed and that the distribution pattern showed developmental changes.

Laser confocal scanning microscopy demonstrated in early cleavage embryos that the fluorescence intensity in the posterior pole plasm was 2.7-fold stronger than in the other periplasmic region. In late cleavage and pole bud stage embryos, no regional difference was observed in the fluorescence. In syncytial blastoderm, the fluorescence in pole cells was 2.4-fold stronger than in the other periplasmic region. In cellular blastoderm, the fluorescence in pole cells was 2.2-fold stronger than in somatic cells.

Transmission electron microscopy of early embryos revealed that mitochondria were not concentrated in the region with strong rhodamine 123 fluorescence. This suggests that mitochondria with a relatively high respiratory activity are distributed in the region stained with a rhodamine 123 strongly.

DB 79

INTERSPECIFIC TRANSPLANT OF CYTOPLASMIC FACTORS DETERMINING ANTEROPOSTERIOR POLARITY IN DROSOPHILA MELANOGASTER

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The phenotypes of D.melanogaster embryos with mutations in genes representing the 3 gene classes (anterior,posterior & terminal) which are required for the determination of anteroposterior (A-P) polarity can be rescued by the transplant of cytoplasm from wildtype embryos. As the first step in learning to what extent the A-P polarity mechanism known in D.melanogaster is conserved through evolution, we transplanted cytoplasm (0.2nl = 2% of D.m. egg volume) from Drosophila virilis wildtype embryos into such D. melanogaster mutant embryos. So far, rescue of anterior gene (bicoid, 19/32=59%) and posterior gene (nanos, 36/44=82%) mutant phenotypes, but not that of the terminal gene phenotype (torso, 0/97=0%) have been observed. Cytoplasm (0.05-0.15nl) from embryos of a chironomid midge (Paratanytarsus parthenogeneticus), although causing severe developmental defects rescued the nanos phenotype (9/32= 28%), whereas no positive results have yet been obtained with bicoid (0/32= 0%) and torso (0/39=0%). The data suggest that while the determination of A-P polarity is functionally conserved to some extent within the order Diptera, certain differences may exist even at the Drosophila genus level.

DB 80

SPLICING OF THE P-ELEMENT IN POLE CELLS DURING EARLY EMBRYOGENESIS OF DROSOPHILA MELANOGASTER

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It has been suggested that the third intron of the P-element is excised only in germ line cells. To test directly whether splicing occurrs in pole cells, the progenitor of germ line, during early embryogenesis, we designed a histochemical assays to measure the splicing activity. The lacZ gene, encoding β-gal, and the heat shock promoter of the hsp 70 gene were inserted into the downstream of the third intron and into the promoter region of the P-element, respectively. Flies were transformed with the fusion gene. In the transformant, the cells where the third intron is spliced out should be visualized with the staining for β-gal activity. When early syncytial blastoderms were heat-treated, β-gal activity was detected only in part of pole cells. Furthermore, we showed that the pole cells with the β-gal activity penetrated embryonic gonads and to differentiate into primordial germ cells. Taking into account that not all pole cells can differentiate into germ cells and that most PGCs in embryonic gonads showed β -gal activity, the results suggest the possibility that the splicing machinery of P-element has an important role in the developmental process of pole cells to germ cells.

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