ABSTRACT

In early development of *Drosophila* embryos, a series of characteristic cytological events proceed in a highly reproducible and coordinated manner. Among them, important ones are fertilization, nuclear divisions, nuclear migration, pole cell formation, and cellularization of blastodermal cells. Although many maternal-effect mutations affecting the early embryonic development have been isolated in this species, little is known about the molecules these mutations affect. To elucidate how these cytological events in the early development are controlled by maternal genes, maternal-effect mutations isolated so far were cytologically analysed in detail, especially paying attention to nuclear migration and F-actin organization in the mutants. In the present study, I characterized four groups of mutations and proposed stage-specific regulation of F-actin organization by different groups of genes.

(1) The first group of mutations including *gs(1)N441*, *gs(1)N26* and *paralog* was found to share a common maternal-effect phenotype. Mutant embryos were deficient in egg contraction that occurs at an early cleavage stage in wild-type embryos, besides the mutants exhibited retarded nuclear migration while synchronous nuclear divisions were unaffected. The retarded migration caused nuclei to remain in the anterior part of the embryo retaining their spherical distribution even in a late cleavage stage, at which nuclei

are lined up in the subcortex in the wild type. This consequently resulted in an extreme delay in nuclear arrival in the posterior periplasm, in defective pole cell formation, in prominent mitotic waves during syncytial blastoderm stage, and in fusion and deletion of body segments.

More precise analysis of nuclear migration between cycle 1 and 2 was carried out by determining the three-dimensional positions of nuclei at cycle 2. This showed in this group of mutants that total posteriorward nuclear migration did not occur, that the two sister nuclei were less-separated from each other, and that orientation of the two nuclei with regard to the anteroposterior axis was random. These differences in the mode of nuclear migration between the mutants and the wild type can simply be explained by a hypothesis that the mutants have some defects in machinery needed to separate cleavage nuclei from each other and from the egg cortex. The anteriorly clustered spherical nuclear distribution and precocious nuclear penetration of an anterior cortex during a late cleavage stage in the mutants are also consistent with this hypothesis.

A mutant phenocopy was induced in wild-type embryos that were treated with cytochalasin B or D at a very early cleavage stage. Remarkable differences were noticed in the organization of cortical F-actin between the mutants and the wild type throughout the cleavage stage. Obvious irregular-sized aggregates of F-actin were dispersed in the cortex of mutant embryos, in contrast to wild-type embryos in which the

cortical F-actin layer was smooth and underlying F-actin aggregates were smaller than those of the mutants in size. The transition of the distribution pattern of F-actin in the yolk mass, from the centralized type (referred to as the central domain) to the fragmented type (referred to as energid domains), occurred later in the mutants than in the wild type.

After nuclear penetration of the egg cortex, essentially normal F-actin organization was recovered in mutant embryos. These results suggest that this group of mutations affects the mechanism underlying establishment and transition of F-actin organization required for normal egg contraction and nuclear migration in the cleavage embryos.

(2) The second group of mutations including $\underline{l(3)c21R}^{RW630}$ showed retarded nuclear migration toward the sides to approach the egg cortex during late cleavage stages, and less distance between sister nuclei in the cortex than in the wild type during syncytial blastoderm stages. A phenocopy of the mutant was induced in wild-type embryos that were treated with a low concentration of colchicine. This mutant may probably be caused by a defective organization or a malfunction of microtubule system that operates in nuclear penetration of the egg cortex and separation of sister nuclei in the cortex.

(3) The third group of mutations, including aurora and early D, showed a complementary phenotype to that of the first group. During cleavage stages, nuclear migration and

F-actin organization in the mutants could not be distinguished from those in the wild type. However, after nuclear arrival in the cortex, cortical F-actin layer remained unchanged in the mutants, in contrast to the wild type in which F-actin showed a periodical pattern in distribution corresponding to nuclear cycle and to local nuclear density. In a late cleavage stage, cortical nuclei often collided with each other during syncytial blastodermal mitoses. This resulted in an irregular nuclear distribution in the cortex. Consequently, cellularization of blastoderm was inhibited. A phenocopy of the mutants was induced by treating wild-type embryos with cytochalasin B or D at the stage immediately prior to nuclear penetration of the cortex. Thus this group of mutations showed a defect in the cortical F-actin organization only in the syncytial blastoderm stage. The cortical F-actin in the syncytial blastoderm stage may play a role in preventing the cortical nuclei from collision. This suggests an essentially same function of F-actin is required in the blastoderm stages as that in the cleavage stages.

(4) A posterior group mutant, <u>tudor</u>, has been reported to have defects in the assembly or localization of the polar plasm, resulting in deficiency in pole cell formation and in deletion of abdominal segments. Unexpectedly, nuclei penetrated the cortex in the normal developmental schedule in <u>tudor</u> embryos; that occurred at cycle 9 in the posterior pole region, and at cycle 10 in the somatic regions. This

suggests that the nuclear penetration of posterior cortex in advance of the somatic regions depends on unknown localized cytoskeletal machinery but not on the localized activities of the polar plasm. On the other hand, protruding pole buds had a dense cortical F-actin layer that remained throughout pole cell formation in wild-type embryos, whereas in *tudor* embryos, cortical F-actin in "pole buds" was hardly observable and the shape of "pole buds" became temporally flat during mitosis. These results suggest that the organization of cortical F-actin during pole cell formation is locally regulated by the posterior polar plasm in wild-type embryos.

In conclusion, I showed that four groups of maternal genes regulate stage- and region-specific organizations and functions of cytoskeletal elements, especially of microfilament system, in the early development of *Drosophila* embryos. Identification of these genes and characterization of their roles may be an important step to elucidate molecular mechanisms underlying developmental processes in the stages from cleavage to blastoderm formation. In addition, the present study shed light on the contribution of major cytoskeletal elements, i.e., the microfilament and microtubule systems, to nuclear migration and pole cell formation, and further on the importance of the normal process of nuclear migration for the normal segment pattern formation.