INTRODUCTION

A major goal in the field of developmental biology is to elucidate how a complex multicellular body pattern is established from a unicellular fertilized egg. Based on many studies using the techniques of cytology, histology, genetics and molecular biology, it has been generally accepted that a basic body plan is established during early development by the functions of maternal as well as zygotic gene products. In the present study, I focused my investigation on the roles of maternal genes in early development. Maternal genes are expressed during oogenesis and their products accumulate in the ooplasm. One of the most effective methodologies to understand the functions of maternal genes in the early development is isolation and characterization of maternal-effect mutations that have defects in early development. A fruit fly, Drosophila melanogaster, is a useful material for this purpose because of an accumulation of genetic information and of outstanding development in cellular and molecular basis technology in this species. Indeed, maternal-effect mutations affecting the early development have scarcely been isolated in any other organisms, with only exception of the nematode, Caenorhabditis elegans.

Early development of Drosophila has been histologically described in detail by several workers (Sonnenblick, 1950; Zalokar and Erk, 1976; Foe and Alberts, 1983; Campos-Ortega and Hartenstein, 1985). After fertilization, a zygotic

nucleus divides every 9 min without cytokinesis and the resulting nuclei migrate away from each other to approach the egg surface during the first eight synchronous intravitelline mitoses. At the completion of the 8th mitosis (256-nucleus stage or cycle 9, Foe and Albert, 1983), nuclei reach the subcortical cytoplasm in the somatic region while they penetrate the cortex or periplasm at the posterior pole region, where the nuclei are to contribute to form pole cells (presumptive primordial germ cells). The stage from fertilization through cycle 9 is called cleavage stage or intravitelline mitosis stage. Immediately after the 9th division, about 350 nuclei penetrate the periplasm in the somatic region. Nuclei in the periplasm divide without cytokinesis four more times every 15 min, and then plasma membrane infolds to isolate every syncytial blastoderm nucleus into a blastodermal cell. After completion of the cellular blastoderm, which is about 3.5 hours after egg laying at 25°C, the first morphogenetic movement starts.

Cytological studies on early Drosophila embryos have shown that the distribution pattern of cytoskeletal elements is characteristic of the stage and the area of embryos (Warn and Magrath, 1983; Warn et al. 1984; Warn et al. 1985; Miller et al. 1985; Warn, 1986; Warn and Warn, 1986; Karr and Alberts, 1986). From these results, it has been suggested that the cytoskeletal elements play key roles in some critical events in the early development, such as nuclear penetration of the periplasm, nuclear cap formation, pole

cell formation and blastodermal cell formation. However, these studies have not been extended to the genes that regulate stage- and region-specific distributions and functions of cytoskeletal elements. Among zygotic mutants, the earliest stage at which some developmental defects are detectable is the gastrulation stage. In contrast, many maternal-effect mutants affecting the development during cleavage stage or syncytial blastoderm stage are known. In addition, it has been demonstrated that incorporation of [^{3}H]uridine into mRNA is undetectable until the start of blastodermal cellularization (Zalokar, 1976). These results imply that most of zygotic genes are not expressed until syncytial blastoderm stage in Drosophila embryos. Thus stage- and region-specific distributions and functions of cytoskeletal elements before cellular blastoderm stage are presumably controlled by maternal gene products accumulating in the ooplasm.

On the other hand, many maternal-effect mutations affecting early development of Drosophila embryos have been isolated (Gans et al. 1975; Rice and Garen, 1975; Mohler, 1977; Komitopoulou et al. 1983). The phenotypes of these mutants were reported to include the cessation of development due to failure in various important processes during the early development (Zalokar et al. 1975; Rice and Garen, 1975). For example, fertilization is not completed in X^{1528} and X^{1578}; nuclear division is arrested during early cleavage stage in X^{1010}, X^{1042}, X^{1242} and X^{1526}; abnormal syncytial

blastoderm is formed in X^{572}, X^{1122} (renamed as paralog), X^{1140}, X^{1162}, X^{1371}; cellularization of blastoderm is not completed topically in mat(3)3 and mat(3)6, and totally in X^{1459} and mat(3)1; abnormal gastrulations occur in X^{573}, X^{1497}, X^{1509}. Some other maternal-effect mutations were characterized as grandchildless due to their inability to form pole cells and consequent germ cells (Thierry-Mieg, 1976; Niki and Okada, 1981; Thierry-Mieg, 1982). Although detailed cytological analyses have been made in a maternal-effect mutant giant nuclei (Freeman et al. 1986) and in several zygotic mutants (Wieschaus and Sweeton, 1988; Merrill et al. 1988), most of the maternal-effect mutations that affect critical events in early development have remained to be cytologically analysed in detail. Elucidation of spacio-temporal changes in the cytoskeletal organization in mutants with disordered cytological events will shed light on the mechanism underlying the strictly regulated organizations and functions of cytoskeletal elements during the early development. The aim of the present study is to elucidate how each cytological event is genetically controlled. For this purpose, I detailed cytological investigation of several maternal-effect mutations isolated so far for the phenotype of developmental disorder in the cleavage stage and in the syncytial blastoderm stage.

Firstly, I chose three maternal-effect mutations, gs(1)N26, gs(1)N441 and paralog (abbreviated as N26, N441 and par, respectively) for the cytological study aiming at the

role of cytoskeletal organization in nuclear migration during the cleavage stage. Although N26 and N441 were originally isolated as grandchildless-class mutations (Niki and Okada, 1981), N26 has been reported to have an abnormal pattern of nuclear arrival in the periplasm (Niki, 1984), and my preliminary observation showed that N441 initiated its abnormality in the nuclear migration at an early cleavage stage. par was isolated as a female-sterile mutant (Gans et al. 1975), and its disorder in blastoderm formation, which was very similar to that observed in N441, was reported afterwards (Zalokar et al. 1975). In the present study, I showed that a group of mutations including N441, N26 and par shares a common phenotype, in which both F-actin organization and nuclear migration are affected in the cleavage stage.

In the next step, I searched other maternal-effect mutations affecting the distribution of F-actin during the early development from previously isolated mutations. In this study, I found out that another group of mutations, including aurora and early D, affected F-actin organization and separation of cortical nuclei only during the syncytial blastoderm stage. This phenotype is complementary to that of the group including N441, N26 and par. Thus I identified two different groups of maternal genes that regulate respective stage-specific organization of F-actin during the early development of Drosophila.

In the last step, I examined cytological mechanism of pole cell formation using a posterior group mutant, tudor

(abbreviated as tud), which is deficient in pole cell formation. Based on the observation of tud embryogenesis, I proposed that primary nuclear penetration of the posterior pole region is locally controlled by unknown cytoskeletal machinery independent of the activity to form pole cells. On the contrary, observation of F-actin in tud embryos suggests that the F-actin organization is locally controlled depending on the activity to form pole cells.

In the present study, I have obtained several lines of evidence indicating the presence of the several sets of maternal genes that regulate stage- and region-specific organization of cytoskeletal elements, particularly of F-actin, in Drosophila early development. I here present a scheme of the mechanism to enable Drosophila early embryos to undergo a series of cytological events stably and reproducibly through developmental changes in the organization of F-actin.