

RESULTS

1. Division and migration of nuclei at cleavage stages

1.1. Egg contraction in N441, N26 and par embryos

Deficiency in egg contraction was the first abnormality recognized in the development of N441, N26 and par embryos that had been derived from their respective homozygous mothers. In the wild type, the ooplasm contracts during the early cleavage stage resulting in forming a space under the vitelline membrane at the posterior pole region (Fig. 1A). So-called pole buds protrude into this space to form pole cells. In contrast, mutant embryos had hardly any space under the vitelline membrane throughout the cleavage stage, provided that the embryos were derived from homozygous females raised at a restrictive temperature (25.5°C) (Figs. 1B-1D).

1.2. Division and migration of nuclei

Nuclear arrival in the posterior pole periplasm has been reported in N26 as delaying 30 min from the time of nuclear arriving in the other regions (Okada, 1982). Observations of living embryos with a time-lapse video confirmed this and revealed similar delay also in N441 (*cf.* Fig. 7B) and par.

This delay can be caused either by elongated mitotic cycle in the posterior half of the embryo, or by retarded

nuclear migration toward the posterior. To test these possibilities, I observed more than a hundred fixed cleavage embryos from each of N441, N26 and par strains for recording the number, the distribution pattern, and the mitotic phase of nuclei in every single embryo.

The number of nuclei was ascertained to increase by a power of two even in mutant embryos, no matter a temperature condition in which their mothers were raised. However, the distribution pattern of nuclei was different between the mutant and wild-type embryos. The difference was most conspicuous at late cleavage stages (Figs. 2B-2D). In wild-type embryos at cycle 8 (128-nucleus stage; Foe and Alberts, 1983), nuclei were distributed equidistantly from the cortex and from each other, causing the nuclei to line up on the subcortical plane (Fig. 2A). In cycle-8 mutant embryos, however, nuclei (except those destined for yolk nuclei) have migrated for an approximately equal distance from their original position, where karyogamy occurred, resulting in a somewhat off-spherical nuclear distribution (Figs. 2B-2D). This indicates that posteriorward and anteriorward migration of nuclei is affected by the mutations.

The mitotic cycle of nuclei was almost completely synchronized even in mutant embryos until cycle 6. At cycle 7 through 9, a mitotic wave was observed in mutant embryos, although a shift in the mitotic phase in a wave never exceeded one mitotic cycle. It occurred most frequently in embryos with a weak phenotype, occasionally in ones produced

by homozygous mutant females raised under a permissive condition (Fig. 3A). This weak mitotic wave drifted always from posterior to anterior, parallel to the anteroposterior axis and also to the gradient of local nuclear density. In mutant embryos deriving from homozygous females raised under a restrictive condition, prominent cortical mitotic waves were constantly observed throughout the syncytial blastoderm stage (Fig. 3B). On the other hand, weak mitotic waves starting from both poles to meet at the middle region of an embryo were observed even in wild-type embryos at the syncytial blastoderm stage (cf. Fig. 7A). The direction of a mitotic wave was consistent with the gradient of local nuclear density in wild-type as well as in mutant embryos. It may be a general rule that the lower the local nuclear density is the faster a mitotic cycle proceeds in a syncytium. From all these results, it is concluded that nuclear division itself is not directly affected in N441, N26 and par embryos.

In wild-type embryos, nuclei penetrate the cortex in the posterior pole region at cycle 9 (256-nucleus stage), and in the other region at cycle 10. In mutant embryos, however, nuclei prematurely penetrated the cortex in a region around 70% egg length (posterior pole as 0% egg length) at cycle 8 or 9 (Figs. 2C, 2D).

1.3. Quantitative analysis of nuclear migration toward the poles in wild-type and mutant embryos

To understand the effects of mutations on nuclear migration in the cleavage stage, the most anterior and the most posterior nuclei in each embryo were recorded at every cleavage cell cycle for their positions on the anteroposterior axis. The results were compared between the wild type and the mutants, N441, N26 and par.

In the mutants as well as in the wild type, the positions of both the most anterior and the most posterior nuclei were approximately constant in embryos at a given nuclear cycle under a restrictive condition (Table 1; Fig. 4). At cycle 1 (1-nucleus stage), the zygotic nucleus was localized at 70-75% egg length level (0% egg length represents the posterior pole) with no significant difference among all strains I examined.

In wild-type embryos, the range of nuclear distribution shifts toward the center of the embryos during the first four nuclear cycles, causing the most anterior nucleus to stay at approximately similar position during this period. Afterwards the migration of the most anterior and the most posterior nuclei toward the respective poles continued by cycle 9 (256-nucleus stage) (Fig. 4). During this, the nuclear migration toward the lateral cortex also occurred (Foe and Alberts, 1983; Fig. 2A). In the posterior pole region, a few nuclei penetrated the periplasm shortly after the 8th nuclear division, and in the anterior pole region, several nuclei penetrated the periplasm shortly after the 9th nuclear division, which confirmed the previous reports

(Zalokar and Erk, 1976; Foe and Alberts, 1983; Warn et al. 1985).

In N441, N26 and par embryos produced by females homozygous for respective mutations raised under a restrictive condition, the enlargement of the range of nuclear distribution accompanying cleavage mitoses proceeded slower than in wild-type embryos, besides the shift of the population of nuclei as a whole toward the central region of the embryos hardly occurred (Table 1, Fig. 4). This causes the mutant phenotype, in which an anteriorly clustered spherical nuclear distribution lasts until a late cleavage stage (Figs. 2B-2D).

In embryos derived from homozygous mutant females raised at a permissive temperature (18°C), nuclear distribution was basically of that in wild-type embryos (Table 2; Fig. 5). In addition, in embryos derived from heterozygous mutant females raised at a restrictive temperature, nuclear distribution was identical to that in wild-type embryos throughout the cleavage stage (Table 3; Fig. 6).

1.4. Effects of N441, N26 and par on the development after cycle 9

A time-lapse video analysis of living embryos showed that the tardy posteriorward nuclear migration in N441, N26 and par caused nuclear penetration of the posterior pole cortex to be 20-30 min later than that in the normal developmental schedule of the wild type (Fig. 7).

Subsequently, nuclei arrived in the other regions of the cortex, and it was at cycle 12-13 when the whole egg cortex was penetrated by nuclei (Figs. 7B, 8). At a late syncytial blastoderm stage, prominent cortical mitotic waves and an extra (i.e. the 14th) cortical mitosis in the posterior region were observed in mutant embryos (Fig. 7B; cf. Figs. 3B, 23D, 24A, 24C). The former may be caused by a gradient of local nuclear density as mentioned above. The latter can be explained by a putative rule in which cortical mitotic cycles continue until when the local nuclear density exceeds a certain value, and once it exceeds the value, cytokinesis of somatic cells is initiated. In N441 embryos, the minimum nuclear density in a region where cytokinesis was initiated after the 13th mitosis was approximately 10 nuclei / 400 μm^2 . In addition, the maximum nuclear density in a region where cytokinesis occurred after the 14th mitosis was approximately 20 nuclei / 400 μm^2 . Thus it can be postulated that when a local nuclear density is less than 10 nuclei / 400 μm^2 the nuclei enter the next mitotic cycle before cellularization of blastoderm. Similar results have been obtained in N26 (Okada, unpublished results).

In the cortex of posterior pole region, the polar plasm is localized. The polar plasm has been reported to include factors essential for pole cell formation and subsequent germ cell differentiation (Okada et al. 1974; Illmensee and Mahowald, 1974, 1976). The retarded nuclear penetration of the polar plasm 30 min later than that in the normal

developmental schedule of wild-type embryos has been reported to cause a defect in pole cell formation in temporally ligated embryos (Okada, 1982). Moreover, during mitoses of cortical nuclei at an early syncytial blastoderm stage, at which no nucleus had penetrated the posterior pole cortex in mutant embryos, abnormal cytoplasmic flows were observed around the posterior pole region. The flows caused dislocation and diffusion of polar granules, which are organelles localized only in the polar plasm, from their original position (Fig. 9A). Thus defect in pole cell formation in the mutants might be caused by such a disturbance of the polar plasm during unusual cytoplasmic flows before the interaction between cleavage nuclei and the polar plasm.

In N441 cellular blastoderm embryos from mothers raised under a permissive condition, a group of cells possessing nuclear bodies (which are electron-dense structures localized in the nuclei of pole cells in the wild type) and polar granules were observed among the blastodermal somatic cells (Niki and Okada, 1981). Histological analysis showed that these "blastodermal pole cells" were isolated from the blastodermal cell layer during gastrulation and migrated following the normal route to reach gonadal rudiments. However, these cells were formed not by budding off as in the wild type but by infolding of egg plasma membrane (Fig. 9B). Multivesicular bodies (which are thought to compensate for excess or insufficient plasma membrane) were frequently

observed near infolding plasma membrane (Fig. 9B, white arrows), suggesting the possibility that they have a role in supplying new plasma membrane during cytokinesis of the "blastodermal pole cells".

In a part of mutant embryos from mothers raised under a restrictive condition, fusions or deletions of body segments were observed (Figs. 10B-10D). The cause of these defects in segmentation will be discussed later.

1.5. Effects of cytoskeletal inhibitors on nuclear migration

To elucidate whether treatment of wild-type embryos with a cytoskeletal inhibitor induces a nuclear distribution type similar to that in the mutants or not, effects of cytoskeletal inhibitors on nuclear migration were examined.

When wild-type embryos were treated at or before cycle 3 with 1-10 $\mu\text{g/ml}$ cytochalasin B or 0.5-10 $\mu\text{g/ml}$ cytochalasin D for 5 min, the embryos exhibited the mutant type nuclear distribution (Fig. 11B). When wild-type embryos were treated at a later stage (cycle 4-6) with the same concentration of cytochalasins as above, or when wild-type embryos were treated at cycle-3 with a slightly lower concentration of cytochalasins, the embryos showed a delay in nuclear migration toward both poles. This confirmed the report by Zalokar and Erk (1976). The treatment with cytochalasin at a concentration of 0.01 $\mu\text{g/ml}$ or less had no visible effect on nuclear migration, and normal larvae hatched from the treated

embryos. These results suggest that the three mutations here studied may have some defects in a microfilament system that is involved in machinery working at early cleavage stages for the migration of nuclei toward the poles.

Colchicine had a quite different effect on nuclear migration. Treatment of wild-type embryos before cycle 4 with 0.1-0.5 $\mu\text{g/ml}$ colchicine for 5 min did not alter cleavage mitotic cycles and scarcely hinder the nuclear migration toward both poles. However, nuclear migration toward the lateral cortex was heavily inhibited. Consequently, colchicine resulted in an extremely elongated ellipsoidal distribution of nuclei in cycle-7 embryos (Fig. 11C). This is completely different from the mutant phenotypes. A treatment with 0.01 $\mu\text{g/ml}$ colchicine or 10 $\mu\text{g/ml}$ lumicolchicine (Fig. 12D) had no visible effect on nuclear migration. It can thus be concluded that the three mutations here described have no effect on the microtubule system in cleavage embryos.

1.6. Nuclear behavior in RW630 embryos

Observing a stock of maternal-effect mutations for the distribution of nuclei in the cleavage embryos, I noticed that at late cleavage stages, a mutation, 1(3)c21R^{RW630} (referred to as RW630), showed a similar nuclear distribution to that of embryos treated with a low concentration of colchicine (Figs. 12B, 12C). In RW630 embryos, migration of nuclei toward the lateral cortex was retarded through late

cleavage stages (Fig. 13). In addition, during syncytial blastoderm stages, RW630 embryos showed a characteristic "paired nuclei blastoderm" phenotype similar to that in embryos treated with a low concentration of colchicine (Figs. 14B, 14C). This was different from syncytial blastoderm embryos treated with cytochalasins, in which nuclei were not paired but irregularly distributed in the blastoderm caused by frequent collisions of nuclei after a mitosis (Fig. 14D). Thus the RW630 phenotype may be caused by a mutation affecting the organization or functions of microtubules, although distribution of microtubules in this mutant has not been examined.

1.7. Quantitative analysis of nuclear migration between cycles 1 and 2

As shown above, in N441, N26 and par embryos at late cleavage stages, a characteristic phenotype of an anteriorly clustered nuclei with spherical distribution and precocious nuclear penetration of the cortex around 70% egg length level were observed. From these results, I postulated a working hypothesis that there is a mechanism, which functions normally in wild-type embryos and has some defects in the mutants, to keep nuclei separate from each other above a certain distance away and also from the egg cortex throughout the cleavage stage. This mechanism is also needed (1) for initial posteriorward migration of "nuclear sphere" (a virtual sphere within which cleavage nuclei are distributed)

during the early cleavage stage, (2) for change of the "nuclear sphere" into ellipsoid at cycle 5 (16-nucleus stage), and (3) for synchronized and gradual approach of nuclei to egg cortex during the late cleavage stage. If this mechanism functions from the first mitotic cycle, it may be possible to detect some differences between wild-type and mutant embryos in the behavior of cleavage nuclei during the first mitotic cycle.

1.7.1. Determination of three-dimensional positions of nuclei in cycle-1 and -2 embryos

Whole-mount preparations of wild-type and mutant embryos were observed with a compound microscope (Nikon), and the position of nuclei were recorded with the help of two ocular micrometers (a 100-division linear and a 20x20 grating type) and the micrometer on the fine focal adjustment knob of the microscope, the scale of which had been calibrated in advance. To minimize the effect of shrinkage or swelling of embryos during preparation, each measured value was normalized by egg length (EL).

In the measurement to locate the nuclei in cycle-2 embryos, I neglected the possible slight displacement of mounted embryos: the anteroposterior axis of each embryo was presumed to be precisely vertical to the light axis of the microscope. The dorsal midline of an embryo was defined as the anteroposterior line through the point on the dorsal

surface at an equal distance from two (or three when recognizable) polar body nuclei.

Actual distance between two nuclei (" d ") at cycle 2 was calculated from d_o and d_z by the formula (1) from the values measured on the lateral perspective (Figs. 15B, 15C; for symbols see the legend for Fig. 15).

$$d = (d_o^2 + d_z^2)^{1/2} \quad (\% \text{ EL}) \quad (1)$$

In addition, orientation of two sister nuclei in cycle-2 embryos was three-dimensionally determined with respect to the anteroposterior and dorsoventral axes of an embryo, on the assumption that the orientation is limited by distances between two nuclei and between a nucleus and the cortex. An angle A (" A " in Fig. 15B) formed where the line linking two nuclei and the anteroposterior axis cross each other was calculated by the following formula (2).

$$A = \cos^{-1}(d_y/d) \quad (0^\circ \leq A \leq 90^\circ) \quad (2)$$

In the same way, an angle B (" B " in Fig. 15C) formed between the plane including two nuclei and being parallel to the anteroposterior axis and the one including dorsal midline and the midpoint of two nuclei was calculated by the following formula (3) based on the values measured on the perspective from the posterior in Fig. 15C.

$$B = \tan^{-1}(q/p) + \tan^{-1}(d_x/d_z) \quad (0^\circ \leq B \leq 90^\circ) \quad (3)$$

Furthermore, an anti-clockwise angle \underline{C} (" \underline{C} " in Fig. 15C) formed between the plane including the dorsal midline and the midpoint of two nuclei and the one including the anterior nucleus and the midpoint of two nuclei and being parallel to the anteroposterior axis was determined from angle " \underline{B} " in the above formula ($0^\circ \leq \underline{C} < 360^\circ$).

1.7.2. Shift of nuclei as a whole along the anteroposterior axis during the period between cycles 1 and 2

The migration of two daughter nuclei along the anteroposterior axis was expressed by a difference between nuclear position at cycle 1 (" \underline{X} " in Fig. 15A) and the position of the midpoint of two nuclei at cycle 2 (" \underline{Y} " in Fig. 15B).

As mentioned above, in wild-type as well as in N441 and N26, the position of nucleus at cycle 1 (" \underline{X} ") was approximately 72-75% EL level (Table 4) and showed no significant difference among the strains.

The position of the midpoint of two nuclei at cycle 2 (" \underline{Y} ") was significantly displaced in wild-type embryos from the nuclear position at cycle 1 (" \underline{X} ") toward the posterior (Table 4), indicating that the daughter nuclei from the first cleavage move as a whole toward the posterior some time in the period between cycles 1 and 2. Although I tried to

determine the nuclear phase when this posteriorward migration occurred, the results were not conclusive.

In N441 and N26, however, no significant difference between "X" and "Y" was detectable (Table 4), indicating that these mutations affect the embryos as early as the first nuclear division. As a result, posteriorward nuclear migration may be extremely reduced.

1.7.3. Three-dimensional distance between two nuclei at cycle 2

In wild-type embryos, the distance between two nuclei was approximately 11% EL (approximately 55 μ m) throughout cycle 2 (Table 5). In N441 and N26, however, it was 7-9% EL (Table 5). No difference in egg length was detectable among the strains I examined. Statistic tests showed that the differences were significant. Thus the results indicate that the distance between two daughter nuclei from the first nuclear division is larger in the wild type than in the mutants. This was ascertained in all nuclear phases at cycle 2, except metaphase in N441 (Table 5).

1.7.4. Anteroposterior orientation of two nuclei at cycle 2

The angle "A" calculated by formula (2) includes an error, although it was less than 5 degrees in most cases. Thus I tested the significance of the difference in the frequencies from random orientation after categorization of

angle "A" into two classes, i.e., $0^\circ \leq A < 45^\circ$ and $45^\circ \leq A \leq 90^\circ$. If the two nuclei are randomly oriented, the frequencies of both classes are expected proportional to respective three-dimensional angles, which are $(2-2^{1/2})\pi$ and $2^{1/2}\pi$, respectively.

In wild-type embryos, orientation of two nuclei at cycle 2 was not random but more anteroposteriorly positioned (Table 6). In mutant embryos, however, the frequencies were indistinguishable from those expected for random orientation (Table 6).

1.7.5. Dorsoventral orientation of two nuclei at cycle 2

The values of angle "B" calculated by formula (3) have a rather consistent error of about 5-6 degrees (the error was calculated from the measurement errors of d_x , d_z , p and q). However, when the two nuclei were oriented nearly in anteroposterior direction, the error exceeded 10 degrees. The cases in which the error exceeded 10 degrees were omitted from data. The number of embryos that were omitted from the data was 9, 3 and 0 in the wild type, N441 and N26, respectively.

I categorized angle "B" into two classes: dorsoventral (D-V) orientation where $0^\circ \leq B < 45^\circ$, and right-left (R-L) orientation where $45^\circ \leq B \leq 90^\circ$. If the two nuclei are randomly oriented, the frequency of either class should be 0.5.

In the wild type, N441 and N26, the frequency of D-V and R-L orientation was indistinguishable from that expected from random orientation (Table 7).

In addition, the position of the anterior nucleus with respect to the dorsoventral axis was random in the mutants at cycle 2. In wild-type embryos, however, the anterior nucleus was more frequently localized in the right than in the left hemisphere (Table 8).

2. F-actin distribution during the cleavage stage

2.1. F-actin distribution in wild-type embryos

Induction of a mutant phenocopy in nuclear migration by treating wild-type embryos with cytochalasins suggests that this group of mutants has a defect in function or organization of microfilament system. To determine whether the abnormal nuclear migration in N441, N26 and par embryos is caused by defects in the microfilament system, I compared the distribution of F-actin during the cleavage stage between wild-type and the mutant embryos, using double fluorescent staining with DAPI and rhodaminyl phalloidin as a DNA specific dye and an F-actin specific probe, respectively (Figs. 16A, 16B), according to Warn et al. (1984). Neither autofluorescence nor non-specific binding of rhodaminyl phalloidin was detectable (Figs. 16C, 16D).

In wild-type cleavage embryos and unfertilized eggs, F-actin was densely distributed throughout the cortex. The

cortical F-actin layer was relatively uniform with numerous microprocesses and very small F-actin aggregates were abundant just beneath the cortex throughout the cleavage stage (typically seen in the marginal region in Fig. 17C), as previously reported (Warn, 1986; Karr and Alberts, 1986). Since the strong cortical signal intervenes a signal from inner locations, cryostat sections as well as whole mount specimens were observed for detecting F-actin localized deeply in the yolk. A laser confocal microscope was found to be more reliable on the observation of the deeply localized F-actin. Besides, it was effective on disclosing a loose 30 μ m-thick F-actin-enriched layer (Fig. 17E, between arrows) lining the cortical F-actin layer.

The F-actin distribution pattern in the yolk mass changed with development. At cycle 2 (2-nucleus stage), a very weak signal of F-actin was recognizable in midway between two nuclei (Fig. 17A), while no other stable F-actin signal was detected in embryos except in the cortex. In unfertilized eggs and cycle 1 embryos, such a signal was undetectable, suggesting that it was formed during or shortly after the first mitosis. The F-actin signal persisted up to cycle 4 at the geometrical center of a "nuclear sphere", a virtual sphere within which cleavage nuclei are distributed (Figs. 17C, 17E). Confocal images showed several small aggregates of F-actin in this region (Fig. 17E, arrowheads). I refer to this accumulated F-actin at the center of the nuclear sphere as a central domain of F-actin. The central

domain continued to grow during cycle 2 through 4 and became more easily detectable, although it was much weaker than cortical F-actin even at cycle 4.

At cycle 5 (16-nucleus stage), the pattern of F-actin distribution in the yolk mass was variable among embryos. In some cases, the central domain of F-actin was weaker than that of cycle-3 or -4 embryos, was elongated anteroposteriorly and was subdivided into several fragments, all of which avoided nuclei (Fig. 18A). In other cases, the central domain was very weak or hardly detectable, and F-actin was weakly detectable in energids; this F-actin domain will be referred to as energid domain. As soon as the energid domains appeared, the central domain rapidly declined to disappear. The central domain was no longer detectable at cycle 6. Based on these observations, I conclude that the transition of F-actin distribution from the central to the energid domain takes place at cycle 5 in wild-type embryos.

At cycle 6 through 9, energid domains of F-actin were observed as areas of generally brighter phalloidin staining with many small aggregates (Figs. 18C, 19A). A strong staining of the nuclear islands with rhodaminyl phalloidin at cycle 9 has been shown by Warn (1986). When the nuclei in energids were at stages from metaphase to anaphase, energid domains of F-actin were all dumbbell-shaped with their long axis in accordance with the axis of the mitotic spindles (Fig. 19).

After nuclear arrival in the cortex, the distribution pattern of cortical F-actin has been reported to change dramatically (Warn et al. 1984; Warn et al. 1985; Warn, 1986). Although they did not describe the energid domain, it is obvious that their description includes the changes in energid domains that have almost arrived to the cortex. In addition, a deeper layer of F-actin aggregates surrounding the contracting central yolk mass appeared at cycle 10 and persisted through the syncytial blastoderm stage (Fig. 20, arrows).

2.2. F-actin distribution in mutant embryos and phenocopies

During the early cleavage stage, mutant embryos had many F-actin aggregates scattered in the cortical F-actin layer (Figs. 21B-21D, 22B-22E). Although small F-actin aggregates were abundant just beneath the cortical F-actin layer in wild-type embryos as mentioned above, the aggregates in the mutants were larger in diameter (maximally about 5 μm), fewer in number, and more irregular in shape with an open center in some aggregates (Fig. 22B, middle of the three arrowheads; Fig. 22E). The density of the aggregates in the mutants was not always constant among embryos or regions in a single embryo, although they were most remarkable in N26 among the three mutants I observed. These aggregates were not detectable during oogenesis in any of the mutants. In embryos derived from mutant females raised under a permissive

condition or from heterozygous females, the cortical F-actin layer was indistinguishable from that in wild-type embryos.

F-actin aggregates in the cortex were still prominent at cycle 6 and they gradually disappeared afterwards. The disappearance of the aggregates was initiated in the area at around 70% egg length level (the posterior pole as 0%), where nuclei prematurely penetrate the cortex in mutant embryos at cycle 8-9. Then the area without the aggregates widened toward the poles (Fig. 23A). At around cycle 12, when whole cortex of mutant embryos had been nucleated, the cortex restored its basically normal, smooth distribution of F-actin, although mitotic waves drifting from posterior to anterior were still prominent at this stage (Figs. 23C, 23D, 24). Thus the effects of mutations on the organization of cortical F-actin is probably limited to the cleavage stage.

At cycle 2, the central domain of F-actin was observable only on rare occasions in mutant embryos. At or after cycle 3 the central domain became detectable, being gradually enlarged afterwards (Figs. 25A, 25C, 25E, 26). The transition of F-actin distribution from the central to energid domain started later in the mutants than in wild-type embryos, and slowly proceeded in the mutants during cycle 7 through 9 (Fig. 27). In addition, the loose F-actin-enriched layer lining the cortical F-actin in the mutants was about 5 to 10 μm thick (Fig. 26) and was generally less-developed than that in wild-type embryos in which it was about 30 μm thick (Fig. 17E). Changes in the distribution

patterns of nuclei and F-actin during the cleavage stages in wild-type and mutant embryos are schematically summarized in Fig. 28.

In a phenocopy induced by treating wild-type embryos with 1 µg/ml cytochalasin B, the cortical F-actin lost its smooth distribution and was drastically fragmented (Fig. 29A). Another difference of the phenocopy from the mutants was that restoration of a smooth cortical F-actin layer after the nucleation of the cortex never occurred in phenocopies (Fig. 29B). Cortical nuclei in cytochalasin-treated embryos frequently showed irregular distribution, resulting from collisions between adjacent nuclei after their divisions (Zalokar and Erk, 1976; Fig. 29C). In addition, the cellularization of blastoderm was inhibited in the phenocopies.

2.3. Effects of aur and e1D on F-actin distribution

To find out other maternal-effect mutations that have a defect in F-actin organization, I carefully examined the phenotypes and F-actin distribution of embryos in a group of mutants in which cortical mitotic waves during syncytial blastoderm stages are prominent, or defects in blastodermal cellularization or pole cell formation are observed. As a result, I found that aurora (aur) and early D (e1D) shared a complementary phenotype to that of N441, N26 and par.

Throughout the cleavage stage, nuclear migration and F-actin distribution in aur and e1D embryos could not be

distinguished from the wild type. Pole cells were formed normally in these mutant embryos. After nuclear penetration of the cortex, a prominent reorganization of the cortical F-actin layer corresponding to cortical nuclei occurred to show a periodic pattern of F-actin distribution in wild-type embryos (Fig. 30B) as previously reported (Warn et al. 1984). However, in the mutants, cortical F-actin showed a somewhat diffuse distribution in the periplasm except for nuclei. The distribution was rather rough with numerous tiny aggregates (Figs. 30D, 30F). At a late cleavage stage, cortical F-actin in the mutant still persisted the original features (Figs. 31D, 31F), and cortical nuclei collided with their neighbors during mitosis, resulting in an irregular nuclear distribution (Figs. 31C, 31E) like in a wild-type embryo treated with cytochalasin B (Fig. 31G).

Subsequently, aur and elD embryos showed a strong defect in cellularization of blastoderm, and the development was arrested at this stage.

3. Genetical analysis of N441, N26 and par

3.1. Cytological mapping of N26 and N441

Cytological map positions of N26 and N441 were determined by crossing mutant males with strains with deficiencies covering their respective loci on the X-chromosome. N26 was complemented by Df(1)RA2, Df(1)KA14, Df(1)C52, Df(1)v^{-L15}, Df(1)ras-v-17Cc8, Df(1)N71, Df(1)HA85

and Df(1)KA6 but not by Df(1)RA37 (Fig. 32). N441 was complemented by Df(1)N71, Df(1)HA85, Df(1)KA6, Df(1)KA10 and Df(1)C246 but not by Df(1)N105, Df(1)JA26 and Df(1)HF368 (Fig. 33). From these results, cytological map positions of N26 and N441 were located at 10A6-B3 and 11A7-B9, respectively. These loci do not coincide with any reported genetic loci of cytoskeletal proteins. par is located at 3B3 (Thierry-Mieg, 1982), at which no cytoskeletal protein gene is known to be located either.

To elucidate whether actin in N441 and N26 eggs is different in amount from that in wild-type eggs or not, proteins in ovaries or cleavage embryos were analysed by two-dimensional gel electrophoresis (Fig. 34). There was no detectable difference in relative content of actin in ovaries and also in embryos among the wild type, N441 and N26 (arrowheads (a) in Fig. 34 indicate the spots of actin, the identification of which was according to Savoini *et al.* 1981). I found out reproducible differences in some spots among the strains (spots (b)-(e) in Fig. 34). However, from a comparative analysis of ovarian proteins among female flies with different genotypes (Table 9), it was concluded that differences in spots (b) and (c) were caused by a genetic background and not by the loci I focused here. For spots (d) and (e), which were not detectable in N26 ovaries, I could not determine whether these spots are involved in a function of N26⁺ gene or not.

3.2. Hemizygous phenotypes of mutants

Embryos derived from N441/Df(1)N105 females were completely identical in the phenotype to ones derived from N441/N441 females. On the other hand, embryos derived from Df(1)N105/+ and N441/+ females showed a complete wild-type phenotype. Thus the N441 mutation probably behaves as an amorphic allele under a restrictive condition.

About a half (32 out of 62) of the embryos derived from Df(1)RA37/+ females terminated their development at an early cleavage stage. This is probably due to the lack of the hfs locus in Df(1)RA37, which has been reported to cause haplo-insufficient female sterility (Lefevre, 1969; Zimulev *et al.* 1981; Eberl and Hilliker, 1988). N26 complemented hfs^{EH326}. Embryos that derived from Df(1)RA37/+ females and survived early cleavage stage skipped egg contraction (79%, 11 out of 14) and showed slight retardation in nuclear migration and in the transition of the central domain of F-actin to the energid domain. The central domain was observed in some cases as late as cycle 6. On the other hand, in 86% (69 out of 80) of embryos derived from N26/Df(1)RA37 females, the development was arrested at an early cleavage stage or before the first cleavage. The nuclear migration and the transition of F-actin distribution in these embryos were similar to those in embryos derived from N26/N26 females. Affected by extreme regional differences in the timing of blastodermal cellularization along the anteroposterior axis, embryos derived from N26/Df(1)RA37 females showed strong disorder in

morphogenesis and hardly hatched out even if they had wild-type fathers. Embryos derived from N26/+ females could not be distinguished from wild-type embryos. Thus N26 is regarded as a hypomorphic mutation of a gene causing haplo-insufficient female sterility, and is independent of hfs although located in the 10A-B region. Embryos derived from par/Df(1)w²⁵⁸⁻⁴⁵ females were principally the same as those derived from par/par females concerning nuclear migration and the distribution of F-actin. None of the three mutations I examined showed detectable zygotic lethality in hemizygous females.

3.3. Double mutation analysis

To test a functional interaction between N441 and N26 mutations, phenotypes of their double mutant strains were analysed.

Eight double mutant strains were screened from 268 recombinants between N441 and N26 strains by respective complementation tests. All double mutant strains showed an extremely strong phenotype in nuclear migration and F-actin distribution. Embryos deriving from N26 N441 / N26 N441 mothers raised under a restrictive condition showed an almost completely spherical nuclear distribution at cycle 7 (Fig. 38A). It is just like a wild-type embryo treated with 5-10 µg/ml cytochalasin B for 5 min at a very early cleavage stage. Many irregular aggregates of F-actin were scattered

in the cytoplasm of the cycle-7 embryo and no central or energid domain of F-actin was detectable (Fig. 38B).

4. Cytological analysis of tud embryos

4.1. Nuclear penetration of polar plasm in tud embryos

As reported previously (Zalokar and Erk, 1976; Foe and Alberts, 1983; Warn et al. 1985), nuclei penetrate the egg cortex at cycle 9 in the posterior pole region and at cycle 10 in the other regions. Therefore, nuclear penetration of the posterior polar cortex, where pole cells (presumptive primordial germ cells) are formed, is specifically regulated to occur one nuclear cycle earlier than in the somatic regions.

To examine whether this specific local regulation of nuclear migration is caused by posterior polar plasm or not, I examined the timing of nuclear penetration of posterior polar cortex in a maternal-effect mutant, tudor (abbreviated as tud). tud is a member of so-called "posterior group" mutants which have been reported to have defects in formation of pole cells (cf. Fig. 44B) and abdominal segments (Boswell and Mahowald, 1985; Nüsslein-Volhard et al. 1987). Cytoplasmic transplantation experiments have shown that both of the activities to form pole cells and abdominal segments are localized in the posterior polar plasm (Illmensee and Mahowald, 1974, 1976; Frohnhöfer et al. 1986). Electron

microscopic observations showed that polar granules, which are cytoplasmic organelles localized only in the polar plasm (cf. Fig. 39A, arrowheads), could not be found out in the posterior pole region of tud embryos (Fig. 39B), confirming the previous report (Boswell and Mahowald, 1985). Nevertheless, a time-lapse video analysis on the development of living embryos and observation of fixed embryos showed that nuclei penetrate the posterior polar cortex normally at cycle 9 in tud embryos (Fig. 40B). Thus cytoskeletal machinery responsible for nucleation in the posterior pole region one mitotic cycle in advance of the somatic regions is probably of normal function even in tud embryo. This function may be independent of the activity of polar granules.

4.2. F-actin distribution in tud pole buds

After the nuclear penetration of the cortex, cytokinesis occurs at first only in the posterior pole region to form pole cells. To elucidate how F-actin is locally organized during pole cell formation, I compared the distribution of F-actin between wild-type and tud embryos at stages from cycle 9 to 14.

Before nuclear penetration of the posterior cortex (this occurred at cycle-9 interphase in both strains as mentioned above), a thin but prominent F-actin layer was observed in the whole egg cortex both in wild-type and tud embryos.

In wild-type embryos, pole buds protrude at cycle-10 interphase. From cycle-10 interphase through cycle-10 telophase, protruding pole buds continued to have a dense F-actin layer in the cortex (Figs. 41A, 42A, 43A), which confirmed the previous report (Warn et al. 1985). At cycle-11 interphase, several pole cells were isolated from the syncytium. The isolated pole cells also had a dense F-actin layer in the whole cortex (Fig. 44A).

Before and at the interphase of cycle 10, no difference was detectable in F-actin distribution between tud and wild-type embryos. However, at cycle-10 metaphase, cortical F-actin of "pole buds" became reduced in tud embryos, especially in a region around the tip of "pole buds" (Fig. 41B). Subsequently, "pole buds" in tud embryos diminished in size and became flattened in contrast to those in the wild type at cycle-10 metaphase (Fig. 42B). From cycle-10 anaphase to cycle-10 telophase, "pole buds" in tud embryos remained to be flattened, and their cortical F-actin to be hardly detectable (Figs. 42B, 43B). At cycle-11 interphase, cortical F-actin layer reappeared on the "pole buds" of tud embryos. Subsequently, in tud embryos, these cyclic changes in the shape of "pole buds" and in the distribution pattern of F-actin in the cortex of "pole buds" repeated through syncytial blastoderm stages until initiation of the cellularization of blastoderm, resulting in a cellular blastoderm without pole cells (Fig. 44B).