Development 120, 2131-2142 (1994)
Printed in Great Britain © The Company of Biologists Limited 1994

The *Drosophila* maternal-effect mutation *grapes* causes a metaphase arrest at nuclear cycle 13

Patrick Fogarty*, Robert F. Kalpin and William Sullivan

Sinsheimer Laboratories, Department of Biology, University of California, Santa Cruz, CA 95064, USA

*Author for correspondence

SUMMARY

***grapes* (*grp*) is a second chromosome (36A-B) maternal-effect lethal mutation in *Drosophila melanogaster*. We demonstrate that the syncytial nuclear divisions of *grp*-derived embryos are normal through metaphase of nuclear cycle 12. However, as the embryos progress into telophase of cycle 12, the microtubule structures rapidly deteriorate and midbodies never form. Immediately following the failure of midbody formation, sister telophase products collide and form large tetraploid nuclei. These observations suggest that the function of the midbody in the syncytial embryo is to maintain separation of sister nuclei during telophase of the cortical divisions. After an abbreviated nuclear cycle 13 interphase, these polyploid nuclei progress through prophase and arrest in metaphase. The spindles associated with the arrested nuclei are stable for hours even though the microtubules are rapidly turning over. The nuclear cycle 13 anaphase separation of sister chromatids never occurs and the chromosomes, still encompassed by spindles, assume a telophase conformation. Eventually neighboring arrested spindles begin to associate and form large clusters of spindles and nuclei. To determine whether this arrest was the result of a disruption in normal developmental events that occur at this time, both *grp*-derived and wild-type embryos were exposed to X-irradiation. Syncytial wild-type embryos exhibit a high division error rate, but not a nuclear-cycle arrest after exposure to low doses of X-irradiation. In contrast, *grp*-derived embryos exhibit a metaphase arrest in response to equivalent doses of X-irradiation. This arrest can be induced even in the early syncytial divisions prior to nuclear migration. These results suggest that the nuclear cycle 13 metaphase arrest of unexposed *grp*-derived embryos is independent of the division-cycle transitions that also occur at this stage. Instead, it may be the result of a previously unidentified feedback mechanism.**

Key words: *Drosophila melanogaster*, embryogenesis, cell cycle, checkpoint, cytoskeleton, microtubule, midbody

INTRODUCTION

In *Drosophila*, and many other higher eukaryotes, the initial embryonic divisions are synchronous, exhibit highly abbreviated G1 and G2 stages, and occur without cell growth. *Drosophila* has proved to be an excellent system for elucidating the structural and regulatory mechanisms governing these initial divisions because it is amenable to molecular, cellular, and genetic approaches (Foe and Alberts, 1983; O'Farrell et al., 1989; Fyrberg and Goldstein, 1990; Ripoll et al., 1992; Schejter and Wieschaus, 1993).

The first thirteen divisions of the *Drosophila* embryo are rapid and occur in meta-synchronous waves without accompanying cytokinesis. These divisions have been thoroughly described using both fixed and live embryos (Rabinowitz, 1941; Sonnenblick, 1950; Zalokar and Erk, 1976; Turner and Mahowald, 1977; Foe and Alberts, 1983; Stafstrom and Staehelin, 1984; Minden et al., 1989). Nuclear cycles 1-7 occur in the interior of the embryo. During nuclear cycles 8 and 9, the majority of the nuclei migrate to the cortex where they continue to divide synchronously and finally cellularize during interphase of nuclear cycle 14. The nuclei that remain in the interior of the embryo undergo two more rounds of synchronous division and become polyploid. During nuclear cycle 9, a few nuclei migrate to the cortex ahead of the main body of nuclei and form pole cells, the precursors to the germline.

The dynamics of the cortical cytoskeleton has also been well described (Warn et al., 1984, 1985, 1987; Karr and Alberts, 1986; Warn and Warn, 1986; Kellogg et al., 1988). The cortical cytoskeleton is particularly rich in actin, which is present as a homogeneous network prior to nuclear migration. Once the nuclei reach the cortex during interphase of cycle 10, the actin forms caps between the nuclei and the plasma membrane. As the nuclei enter metaphase and spindles form, the actin along with its closely associated plasma membrane redistributes to form furrows surrounding each of the metaphase spindles. This process repeats itself until cellularization during interphase of nuclear cycle 14.

Many of the dependent relationships and feedback controls found in more conventional somatic cell cycles are not present during these syncytial divisions (Hartwell and Weinert, 1989; Foe et al., 1993). For instance, mutations in *S. cerevisiae* that

disrupt DNA synthesis prevent entry into mitosis and the continuation of the centrosome cycle (Pringle and Hartwell, 1981). Null mutations that relieve the dependency of mitosis on proper DNA synthesis indicate that the dependency is a consequence of extrinsic controls (checkpoints) rather than intrinsic substrate/product relationships (Hartwell and Weinert, 1989). In contrast, drug studies and mutational analysis of *Drosophila* demonstrate that improper DNA replication during the syncytial divisions neither prevents entry into mitosis nor does it inhibit centrosome replication (Freeman et al., 1986; Freeman and Glover, 1987; Raff and Glover, 1988; Shamanski and Orr-Weaver, 1991). The reduced number of dependency relationships in the syncytial *Drosophila* embryo also explains why very few of the many maternal-effect mutations that disrupt these divisions arrest the embryo at a specific nuclear cycle or a specific stage in the nuclear cycle.

Although reduced, dependency relationships and feedback controls are present during the syncytial divisions in the *Drosophila* embryo. Embryos treated with microtubule disrupting agents arrest with their chromatin in a metaphase-like configuration (Zalokar and Erk, 1976; Foe and Alberts, 1983). This suggests the presence of a feedback mechanism from the microtubules to the chromosomes; an improperly formed spindle inhibits the continuation of the nuclear cycle. Feedback controls and dependency relationships are also revealed by delays as well as arrests (Hartwell and Weinert, 1989). In some cells, if a chromosome is slow to align on the metaphase plate, anaphase is delayed (Hartwell and Weinert, 1989; McIntosh, 1991). Similarly, in the syncytial *Drosophila* embryo, abnormal chromosome compaction during metaphase may cause single nuclei to delay anaphase initiation (Sullivan et al., 1993b). These delayed nuclei are eventually removed from the dividing population. This suggests that feedback controls operate during the metaphase to anaphase transition in syncytial *Drosophila* embryos. In this paper, we describe a maternal-effect mutation, *grapes (grp),* which produces embryos that arrest in metaphase of nuclear cycle 13. This phenotype may be a consequence of feedback controls operating in the syncytial embryo.

grp was initially identified as a member of a set of maternal-effect mutations in which the affected embryos undergo abnormal divisions only after the nuclei have migrated to the cortex (Sullivan et al., 1990; Postner et al., 1992; Sullivan et al., 1993a). Each of these mutations uniquely and specifically disrupts the cortical cytoskeleton and has proved useful in elucidating the interactions between the nuclei and the cortical cytoskeleton. During anaphase and telophase of nuclear cycle 12 in *grp*-derived embryos, the spindle deteriorates and the usually pronounced midbody never forms. Without the midbody, sister telophase nuclei snap-back and fuse. These observations suggest that the midbody is responsible for maintaining separation of sister nuclei during telophase of the cortical divisions. The fused telophase nuclei undergo an abbreviated nuclear cycle 13 interphase, fail to properly condense their chromosomes during prophase, and arrest in metaphase. Anaphase separation of chromatids never occurs and the nuclei eventually assume a telophase configuration. These arrested nuclei are encompassed by spindles with a pair of centrosomes at each pole. The *grp*-derived embryos remain in this arrested state for hours.

X-irradiation experiments demonstrate that the *grp* induced arrest is not a direct consequence of processes specific to nuclear cycle 13. In normal embryos, X-irradiation induces a high error rate but no arrest. In contrast, X-irradiation of *grp*-derived embryos results in a metaphase arrest even during the pre-migration and initial cortical nuclear cycles. These results are discussed in the context of embryonic cell cycle dependency relationships and feedback controls.

MATERIALS AND METHODS

Stocks

The laboratory of Y. N. Jan and L.Y. Jan (U.C. San Francisco) generously provided us with the *grp* mutation (Bier et al., 1989). Df(2L)H20, a deficiency that uncovers *grp* (kindly supplied to us by the Indiana stock center, Bloomington, Indiana), was also used in the phenotypic analysis. Oregon-R served as the wild-type control stock. Other mutations and chromosomes are described by Lindsley and Zimm (1992). The stocks were maintained on a standard corn meal/molasses medium at 25°C.

Fixation and immunofluorescence

Embryos were formaldehyde fixed by a modification of the Mitchison and Sedat procedure (1983). This method is described in detail elsewhere (Theurkauf, 1992). Immunofluorescence labeling was performed as described by Karr and Alberts (1986). The DMAP190 anti-centrosomal antibody was a gift from Douglas Kellogg (Kellogg et al., 1989). Both propidium iodide (Sigma, St. Louis, MO) (Fig. 6) and anti-histone antibody (Chemicon, Temecula, CA) (Figs 1, 5) were used to observe the nuclei. The propidium iodide staining was accomplished by gradually rehydrating the fixed embryos followed by an extensive rinse in PBS (0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3). RNase was added to a concentration of 10 mg/ml, and incubated at 37°C for 2 hours. The embryos were rinsed in PBS and mounted in a 50% glycerol, PBS solution containing 1 mg/ml N-N-1-4-phenylenediamine (Sigma, St. Louis, MO) and 1 μg/ml propidium iodide.

Microscopy was performed using an Olympus IMT2 inverted photoscope equipped with a Biorad 600 laser confocal imaging system. The lenses used included the Olympus S Plan Apo 60×, Oil and the Olympus D Plan Apo 20×, UV, Oil. The cortical nuclear cycle was determined by employing the Biorad imaging software to estimate the surface nuclear density.

In vivo fluorescence analysis

The in vivo analysis of the chromosome and tubulin behavior during the syncytial cortical divisions was accomplished through a procedure in which fluorescently labeled histone or tubulin were microinjected into embryos and time-lapse images taken using a fluorescence microscope (Kellogg et al., 1988; Minden et al., 1989). The embryos were prepared for microinjection by hand dechorionation and mounting on a coverslip with a thin film of glue (prepared by dissolving double sided tape in heptane). The proteins, labeled with 5-(and 6)-carboxytetramethylrhodamine, succinimidyl ester (NHSR), were microinjected into the embryos. Observations were made on the confocal equipped Olympus IMT2 inverted microscope described above.

X-irradiation studies

0.5- to 2.5-hour wild-type and *grp*-derived embryos were exposed to 0.3 kR of X-irradiation with a Torrex120D X-ray generator (Astrophysics Research, Long Beach, CA). The X-irradiated embryos were formaldehyde fixed and double stained to observe their chromosomes and spindles after 60 minutes of development. Each embryo was

scored with respect to the cell cycle number and phase at the time of fixation. This analysis allowed us to determine mitotic indexes for wild-type and *grp*-derived embryos. The mitotic index is defined here as the percentage of embryos in metaphase between nuclear cycle 2 and 12.

In a second study, living wild-type and *grp*-derived embryos, injected with NHSR-labeled histone, were exposed to 0.3 kR of X-irradiation during interphase of nuclear cycle 10. Confocal microscopy allowed us to follow the nuclear behavior in these living embryos after X-irradiation. We followed 3 embryos each from wild-type and *grp*-derived females and found that *grp*-derived embryos respond by arresting in metaphase while no arrest was seen in wild-type.

RESULTS

Embryos derived from females homozygous for *grp* develop normally through interphase of nuclear cycle 12

The nuclear morphology and distribution through interphase of cycle 12 *grp*-derived embryos does not differ significantly from wild-type embryos at the equivalent stage (Fig. 1, compare A (wild-type) and C (*grp*-derived)). After this stage, however, the *grp*-derived embryos begin to exhibit an uneven nuclear distribution and irregular shaped nuclei. This phenotype becomes more extreme as the nuclei eventually form distinct clusters (Fig. 1D). Cellularization does not occur and the nuclei remain in this clustered state for hours. Unlike other maternal-effect mutations that exhibit nuclear abnormalities during the cortical divisions, the abnormal nuclei in *grp*-derived embryos do not recede into the interior (Sullivan et al., 1993a,b; Schejter and Wieschaus, 1993).

To quantify these observations, we scored embryos as abnormal if greater than 2% of the nuclei were fused, dramatically out of division synchrony, or beneath the monolayer of nuclei during the cortical divisions. During nuclear cycles 5-10, the percentage of abnormal embryos derived from either wild-type, *grp*/*grp*, or *grp*/*Df* females are not significantly different (Table 1). The percentage of abnormal cycle 11 *grp*-derived embryos was slightly higher than found for wild-type embryos. However, during nuclear cycles 12-14, greater than

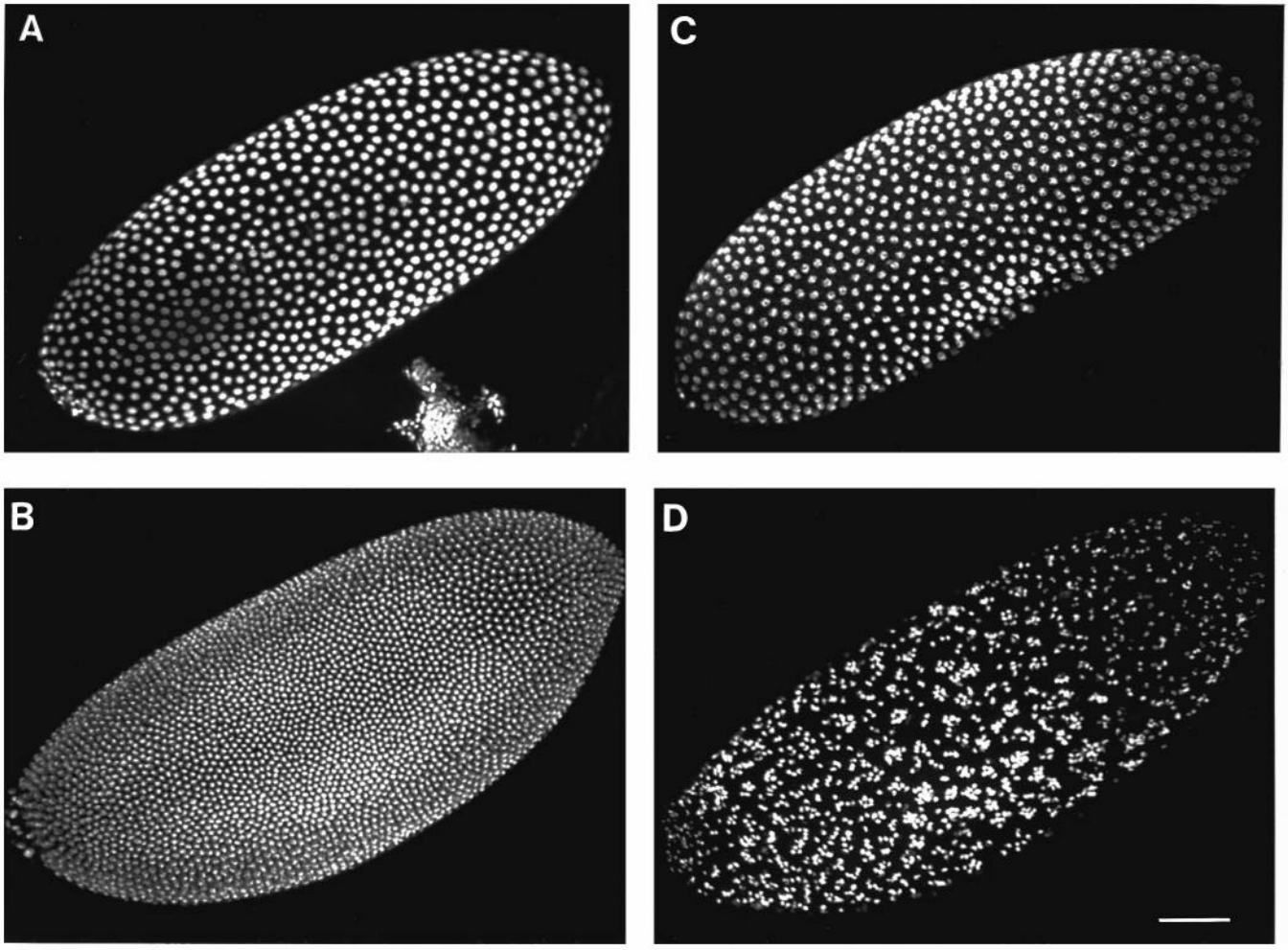


Fig. 1. The nuclear distribution in embryos derived from wild-type females (A,B) and homozygous *grp* females (C,D) is shown. Wild-type and *grp*-derived embryos exhibit a similar nuclear distribution at interphase of nuclear cycle 12 (A and C, respectively). After nuclear cycle 12, the *grp*-derived embryos exhibit abnormal nuclear spacing and morphology. B and D depict the nuclei in a cycle 14 wild-type embryo and a *grp*-derived embryo at the equivalent time in development, respectively. *grp*-derived embryos arrest prior to cellularization with the cortical nuclei distributed in grape-like clusters (D). Images represent a projection of 10, 1 μm optical sections. Scale bar, 17 μm.

Table 1. Embryos derived from wild-type, homozygous *grp*, and hemizygous *grp* females, between cycles 5 and 14, were scored for nuclear abnormalities

	Nuclear cycle (per cent normal embryos)				
Mother's genotype	5-9	10	11	12	13-14
wild-type	97.5 (80)	96.0 (25)	100 (26)	96.5 (29)	96.3 (27)
grp1/grp1	98.8 (90)	90.7 (54)	80.0 (20)	9.1 (22)	2.8 (71)
grp1/Df(2L)H20	100 (6)	100 (15)	81.3 (16)	9.5 (21)	0.0 (50)

If greater than 2% of the nuclei were fused, displayed asynchronous divisions, or were beneath the monolayer of cortical nuclei, the embryo was scored as abnormal. The frequency of normal *grp*-derived embryos dramatically decreases after nuclear cycle 11. The percentage of normal embryos is shown with the number scored in parentheses.

90% of the *grp*-derived embryos exhibited extensive irregularities in nuclear distribution and morphology. Of the hundreds of *grp*-derived embryos scored, no normal cycle 13 or any 14 embryos were observed.

Fusion of sister nuclei occurs during telophase of nuclear cycle 12

The behavior of the cortical nuclear divisions was analyzed in living *grp*-derived embryos by injecting these embryos with fluorescently labeled histone. In accordance with the analysis of fixed embryos, the nuclear distribution, division synchrony, and nuclear morphology are normal during nuclear cycles 10 and 11 in *grp*-derived embryos (data not shown). Fig. 2A-E follows the nuclei in a *grp*-derived embryo from interphase to telophase of nuclear cycle 12. These nuclei proceed normally through metaphase. Even though some asynchrony in the initiation of anaphase is observed, most nuclei successfully separate their sister chromatids into two distinct products. However, during telophase of cycle 12, a high proportion of sister nuclei collide to form abnormally large nuclei (Fig. 2E-H). Of 548 cycle 12 anaphases, fusion of sister telophase nuclei occurred in 386 (70%; 11 embryos scored). The fusion products undergo an abbreviated nuclear cycle 13 interphase and progress into metaphase with their chromosomes condensed but improperly aligned on the plate (Fig. 2I-K). The nuclei appear to arrest in metaphase; anaphase separation of sister chromosomes never occurs and spindles are present (see below). However, over time the distinct chromosome structure is lost and the nuclei display a telophase-like configuration (Fig. 2L).

Improper midbody formation during telophase of nuclear cycle 12 is observed in *grp*-derived embryos

The microtubule dynamics of the cortical nuclear divisions were analyzed by injecting living *grp*-derived embryos with fluorescently labeled tubulin. Fig. 3A-E follow the microtubule dynamics of a group of nuclei in a *grp*-derived embryo from metaphase of cycle 10 to interphase of cycle 11. Fig. 3F-J follow microtubule dynamics of their daughter nuclei (metaphase of cycle 12 to interphase of cycle 13). Microtubule dynamics and morphology are normal through metaphase of nuclear cycle 12. As observed in normal embryos, a distinct midbody is present between sister telophase nuclei (Fig. 3C,D, see arrows). Normal cycle 12 metaphase spindles of the *grp*-derived embryo are shown in Fig. 3F. As anaphase progresses, the microtubule structures rapidly deteriorate. By measuring distances between centrosomes (data not shown), anaphase B is found to be impaired in 40-50% of the divisions and the normally pronounced midbody never forms (compare Fig. 3C with 3H).

These recordings also demonstrate that immediately after failed midbody formation sister telophase nuclei fuse. In the tubulin-injected embryos, the nuclei appear as non-labeled (black) images because the labeled tubulin is excluded when the nuclear envelope reforms during each telophase (Fig. 3D,E). In *grp*-derived embryos, during late anaphase/early telophase the sister nuclei fuse to create abnormally large nuclei (Fig. 3I,J). These tubulin recordings correspond well with those of the cortical nuclear behavior in *grp*-derived embryos determined in the fixed and live material (histone injected).

Immunofluorescent analysis of the microtubule pattern in fixed *grp*-derived embryos also reveals improper midbody formation during telophase of nuclear cycle 12. Fig. 6A (see arrow) depicts the midbody observed in late anaphase and early telophase nuclei during the cortical divisions in a wild-type embryo. No midbody is present between sister anaphase and early telophase nuclei in *grp*-derived embryos (Fig. 6C, arrow).

grp-derived embryos arrest in nuclear cycle 13 with their chromosomes encompassed by spindles

In *grp*-derived embryos, after nuclear fusion at telophase of cycle 12 and an abbreviated cycle 13 interphase, metaphase spindles form. Two centrosomes, rather than a single centrosome, are present at each pole of these cycle 13 spindles (Fig. 4). In wild-type syncytial divisions, centrosome duplication occurs during late anaphase/early telophase and each telophase nucleus possesses a single pair of closely associated centrosomes. Therefore, the fusion of two telophase nuclei, in *grp*-derived embryos, produces a large interphase nucleus with a pair of centrosomes at each pole rather than a single pair of centrosomes at one pole (see Fig. 8 for a schematic diagram). Centrosome migration seldom occurs and robust spindles develop between the opposing centrosome pairs (Fig. 4C). The *grp*-derived embryos arrest at this point; metaphase of cycle 13 with a pair of centrosomes at each spindle pole. Although these spindles remain intact for hours, they are quite dynamic. Photobleaching studies reveal that the microtubules in these spindles are rapidly turning over (data not shown). In addition, neighboring spindles interact and, with time, become clustered (Fig. 5A,C). After an hour and a half the nuclei begin to degrade, but the spindle complexes remain intact (Fig. 5B,D).

X-irradiation induces a metaphase arrest at all syncytial cycles in *grp*-derived but not wild-type embryos

Interphase of nuclear cycle 13 represents a transition point in the regulation of the syncytial nuclear cycles (Edgar and O'Farrell, 1989). To determine whether the nuclear cycle 13 metaphase arrest observed in *grp*-derived embryos is a consequence of these transition events, we examined the response of wild-type and *grp*-derived embryos to X-irradiation. In many cells, X-irradiation causes cell cycle delays allowing time for DNA repair before the cell cycle resumes (Hartwell and

Weinert, 1989). 0.5- to 2.5-hour embryos derived from *grp* and wild-type females were exposed to 0.3 kR of X-irradiation and the mitotic index of pre-cycle 12 embryos determined at 60 minutes after X-irradiation. The unirradiated controls exhibited a mitotic index of 29% (89 embryos scored) and 40% (55 embryos scored) for wild-type and *grp*-derived embryos,

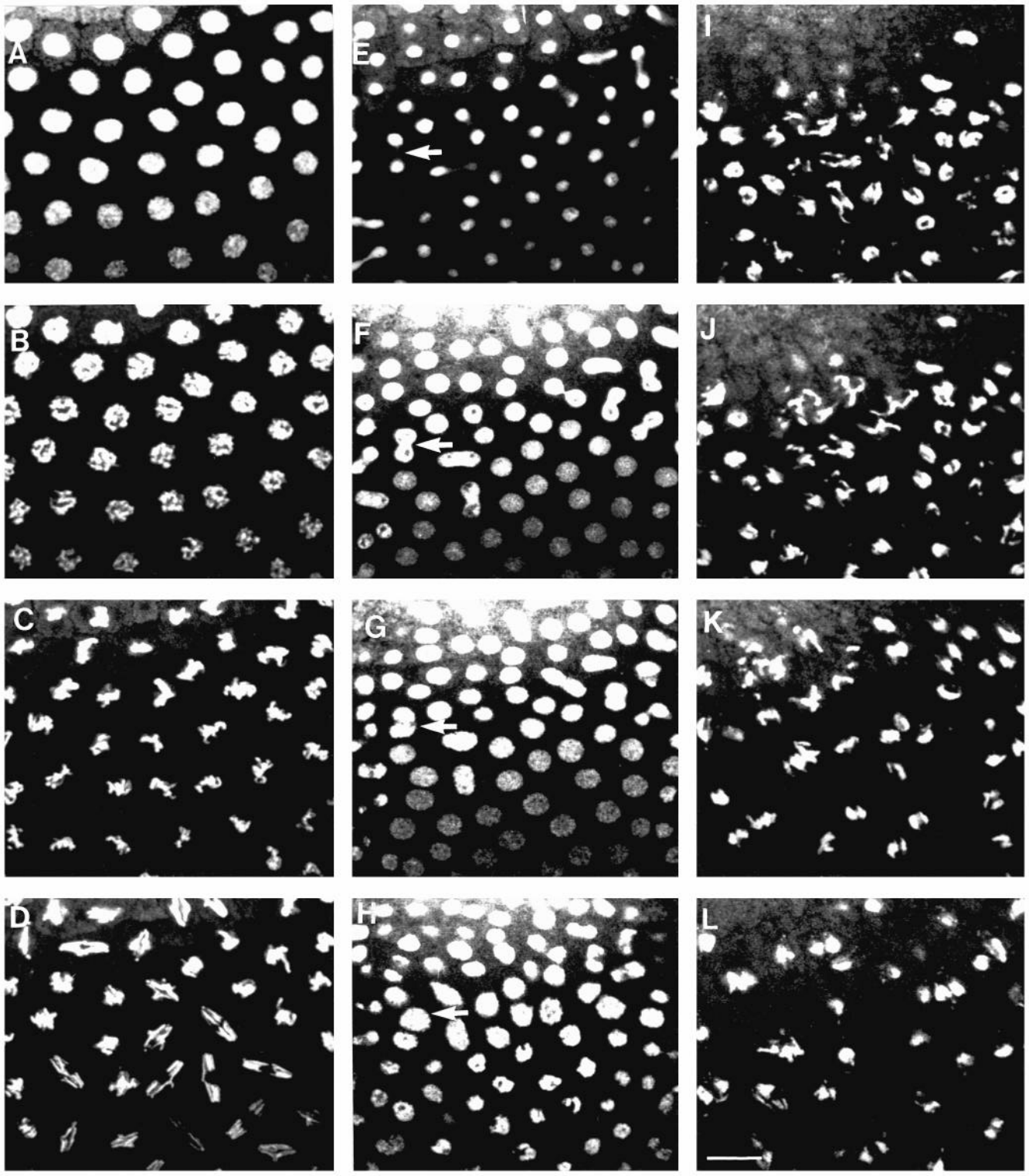
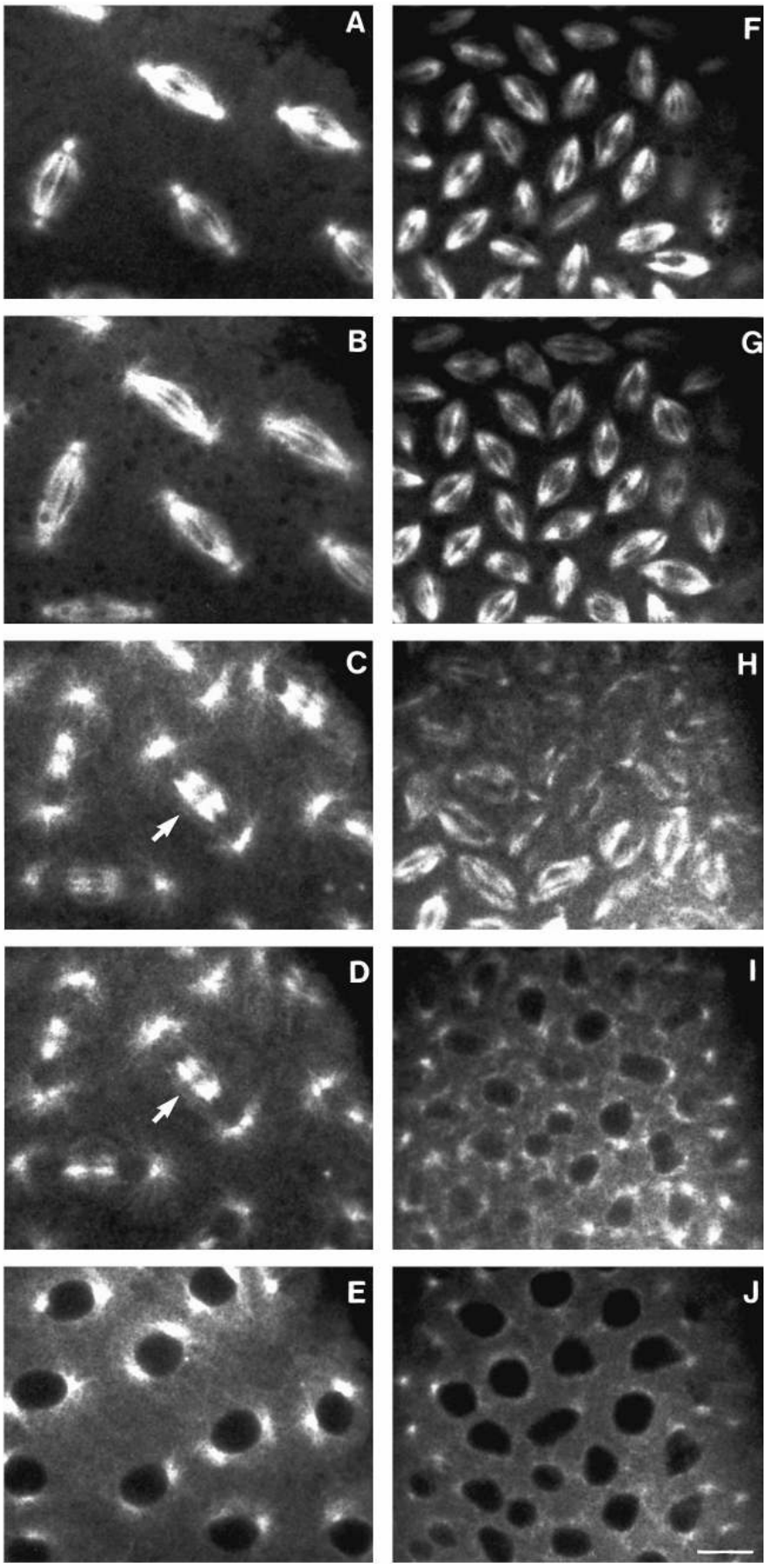


Fig. 2. Through fluorescently labeled histone injections, the nuclear dynamics of a living *grp*-derived embryo are followed from interphase of nuclear cycle 12 to its metaphase arrest at nuclear cycle 13. During telophase of nuclear cycle 12, a high percentage of the sister nuclei fuse (arrows in E-H). The chromosomes of these fused nuclei condense (I,J), enter metaphase (I-K), and eventually assume a telophase-like configuration (L). The elapsed time for A-L is 0' 0", 1' 31", 3' 38", 5' 20", 5' 50", 8' 34", 10' 43", 13' 40", 16' 15", 18' 20", 21' 47", 26' 53", respectively. Scale bar, 4 μm.

respectively. If the embryos were incubated for 60 minutes after exposure to the X-irradiation, the wild-type embryos exhibit a mitotic index of 27% (86 embryos scored) and *grp*-derived embryos exhibit a mitotic index of 83% (101 embryos scored). The nuclear (blue) and microtubule (red) arrangements of wild-type and *grp*-derived embryos after X-irradiation are depicted in Fig. 6B and D, respectively. In addition, 0.5- to 2.5-hour *grp*-derived embryo collections fixed 60 minutes after X-irradiation yielded a much younger age distribution than equivalently treated wild-type embryos. In fact, we observed a number of *grp*-derived embryos as early as metaphase of nuclear cycle 2. These data suggest that *grp*-derived, but not wild-type embryos, respond to X-irradiation by arresting in metaphase.

We also examined live embryos to monitor directly the response of wild-type and *grp*-derived embryos to X-irradiation. Wild-type embryos, injected with NHSR-labeled histone, were exposed to 0.3 kR of X-irradiation during interphase of nuclear cycle 10 and were examined immediately with time-lapse confocal microscopy. These embryos progress through nuclear cycle 10 and into interphase of nuclear cycle 11 with some division errors (data not shown). During metaphase and anaphase of nuclear cycle 11, a dramatic increase in division errors is observed (Fig. 7A-F). Irregularly shaped nuclei and nuclei below the surface monolayer (in the process of receding into the interior) are observed by telophase of nuclear cycle 11 (Fig. 7E,F). These embryos exhibit extensive errors during the subsequent cortical divisions, but do not arrest.

A *grp*-derived embryo X-irradiated during interphase of nuclear cycle 10 also progresses to interphase of nuclear cycle 11 (data not shown). However, the cycle 11 nuclei progress through prophase and arrest with their chromosomes in a condensed metaphase-like state (Fig. 7G-L). Fig. 7L demonstrates that the X-irradiated embryos maintain this metaphase arrest for at least 30 minutes.



DISCUSSION

The *grp* mutation was initially identified as a member of a class of mutations in

which the nuclei undergo abnormal divisions only after they migrate to the cortex. The other members of this class include *daughterless-abo-like*, *nuclear-fallout*, *scrambled,* and *sponge* (Sullivan et al., 1990; Postner et al., 1992; Sullivan et al., 1993a). Two additional mutations in this class have been identified in more recent screens of maternal-effect mutations (W. Sullivan and W. Theurkauf unpublished observations). Because proper cortical nuclear division is dependent on the dynamics of the cortical cytoskeleton, we believe some of the mutations in this class disrupt genes that encode products that are involved in processes or components specific to the cortical cytoskeleton. For example, *scrambled*, *nuclear-fallout,* and *sponge* specifically disrupt the actin dynamics during the cortical divisions (Postner et al., 1992; Sullivan et al., 1993a).

The cortical specific nuclear defect of *grp*-derived embryos is more difficult to understand. Microtubule structures and midbody formation are specifically disrupted during anaphase and telophase of nuclear cycle 12 even though these structures are common to all mitotic divisions. The nuclear cycle specificity of the defect and deficiency analysis argue that this phenotype is not a result of a hypomorphic allele. The *grp* mutation may only slightly compromise the integrity of the microtubules and the midbody; therefore, division defects would be manifest only during the later cortical divisions when crowding places additional structural demands on the mitotic apparatus.

Since midbody failure during nuclear cycle 12 is the most dramatic initial defect in *grp*-derived embryos, this suggests that the *grp* gene may encode a product involved either in the formation or stability of the midbody. A number of proteins that localize to the midbody have been identified (Nislow et al., 1990; Earnshaw and Bernat, 1991; Rattner, 1992; Nislow et al., 1992; Margolis and Andreassen, 1993). In addition, some of these proteins have been shown to interact directly with microtubules and one exhibited a kinesin-like microtubule-based motor activity (Nislow et al., 1992). However, the *in vivo* function of most of these proteins remains unclear. Whether the *grp* product localizes to the midbody and is a microtubule associated protein must await molecular characterization.

The consequence of failed midbody formation during anaphase and telophase of nuclear cycle 12 is that sister nuclei collide to form a single tetraploid nucleus. It appears that an intact midbody is essential for maintaining the separation of sister telophase nuclei. In contrast to divisions that undergo conventional cytokinesis, no actin-based membrane separates the two telophase products during the cortical divisions in the *Drosophila* embryo. It may be that the unusually pronounced midbody found during the syncytial divisions has evolved as an alternative means of maintaining separation of sister telophase nuclei (Fig. 8). Previous studies have demonstrated that the specialized pseudocleavage furrows, which encompass each metaphase spindle, serve to maintain proper spindle and nuclear distribution during the syncytial cortical divisions (Sullivan et al., 1990; Postner et al., 1992; Sullivan et al., 1993a). Pronounced midbodies and pseudocleavage furrows appear to be specialized adaptations of the *Drosophila* embryo

Fig. 3. Through fluorescently labeled tubulin injections, the microtubule dynamics of a living *grp*-derived embryo are followed from metaphase of nuclear cycle 10 to telophase of nuclear cycle 12. A-E follow a *grp*-derived embryo from metaphase of nuclear cycle 10 to interphase of nuclear cycle 11. A pronounced midbody spans sister nuclei during late anaphase and into telophase (arrows C,D). The microtubule dynamics in *grp*-derived embryos is normal through metaphase of nuclear cycle 12 (F). Although cycle 12 anaphase appears to initiate normally (G), a distinct midbody never forms (compare H with C). Consequently, sister telophase nuclei fuse and form abnormally large nuclei (I,J). The elapsed time in A-J is: 0' 0", 1' 7", 1' 59", 2' 20", 4' 32", 20' 49", 23' 22", 24' 49", 26' 32", 29' 0", respectively. Scale bar, 2.8 μm.

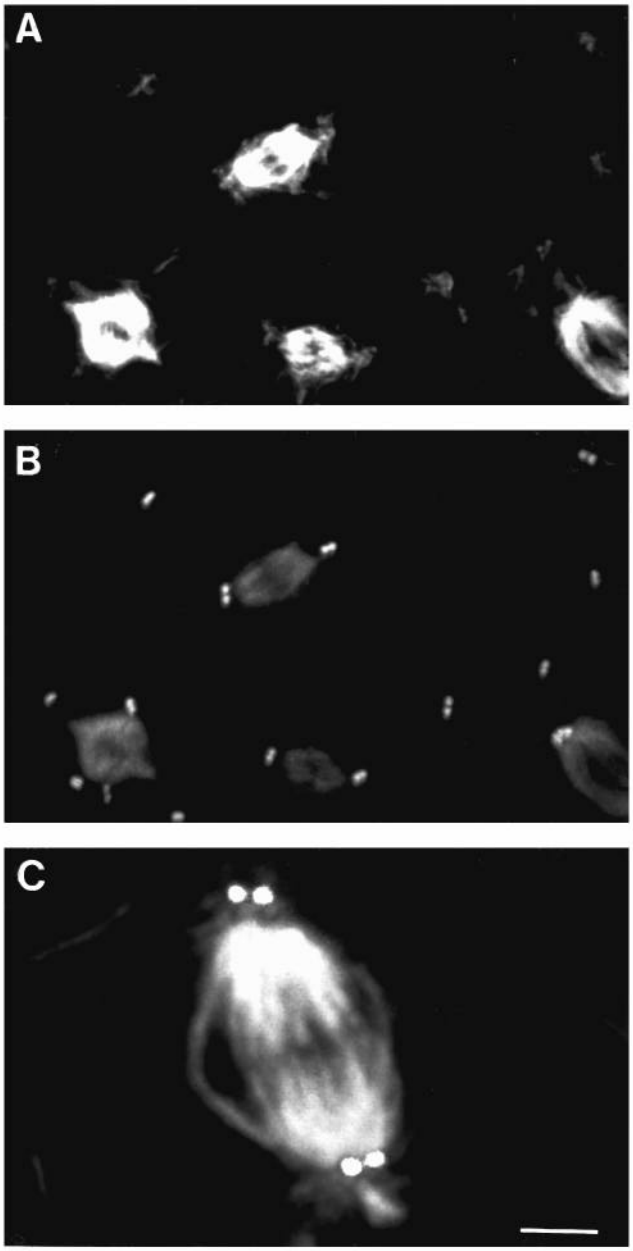


Fig. 4. The arrest phenotype of *grp*-derived embryos double stained for the microtubules (A) and centrosomes (B). The poles of the arrested spindles are occupied by a pair of centrosomes. Even disassociated centrosomes exist in pairs. C depicts a merged image of the centrosomes on the arrested spindles in a *grp*-derived embryo. Images represent a projection of 4, 1 μm optical sections. Scale bar, (A,B) 4 μm; (C) 1 μm.

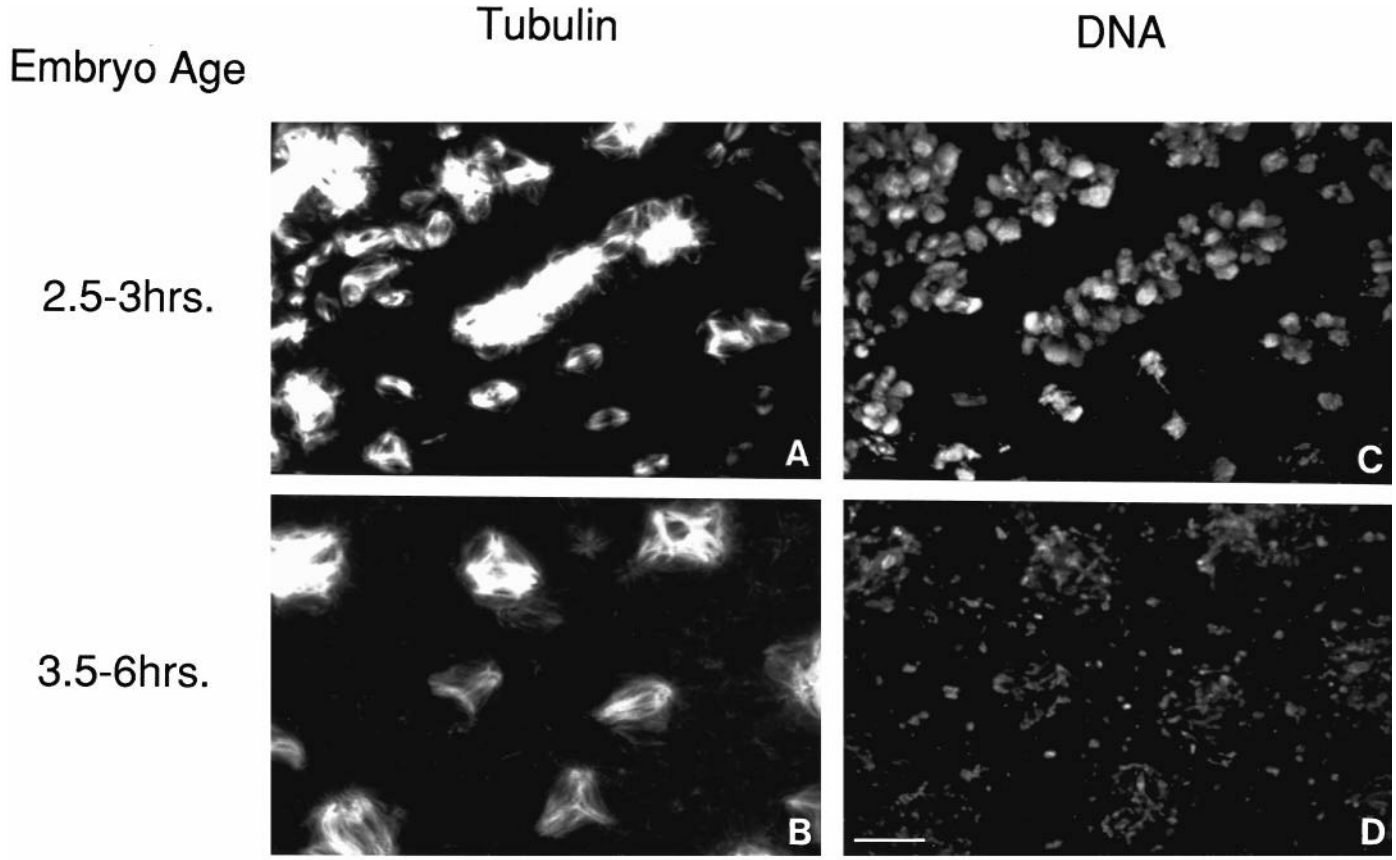


Fig. 5. *grp*-derived embryos stained for both their microtubules (A,B) and nuclei (C,D). These embryos arrest with clusters of cycle 13 telophase-like nuclei (C) encompassed by spindles (A). After more than an hour and a half in the arrested state, the chromatin degrades (D), but the spindles remain (B). Scale bar, 4 μm.

to maintain orderly nuclear divisions in the absence of cytokinesis.

In *wild-type* embryos, the centrosomes duplicate during telophase and migrate to opposite poles during interphase and prophase. The fusion of sister nuclei during telophase of cycle 12 in *grp*-derived embryos produces a tetraploid nucleus with two pairs of diametrically opposed centrosomes (Fig. 8). Separation of sister centrosomes seldom occurs and these nuclei form a spindle with a pair of centrosomes at each pole. Possibly, the presence of centrosomes at the opposing pole inhibits their separation.

The *grp* mutation is unique because it produces a syncytial blastoderm arrest. After nuclei fuse during telophase of cycle 12, they undergo an abbreviated cycle 13 interphase. These nuclei enter metaphase, but never progress into anaphase; separation of sister chromatids never occurs. However, over time the distinct chromosome structure is lost and the nuclei display a telophase-like configuration. These arrested nuclei are encompassed by mature metaphase spindles and remain in this state for hours. There are a number of possible explanations for this arrest. Previous studies indicate that metaphase of nuclear cycle 13 represents a transition point in the regulation of the syncytial nuclear cycles. At this time, the maternally derived *string* is rapidly degraded and progression of the cell into mitosis relies on zygotically supplied *string* (Edgar and O'Farrell, 1989). The *grp* product may provide a component intimately involved in this transition. Another possibility is that these embryos arrest because of mechanistic considerations. Polyploid nuclei with two centrosomes at each pole is a situation unique to *grp*-derived embryos. This configuration of centrosomes, nuclei, and spindles may stall the cell cycle at metaphase. That is, if this configuration could be phenocopied in a wild-type embryo a metaphase arrest would also occur. A third possibility is that the *grp* mutation may be revealing a previously unidentified syncytial division feedback mechanism.

To distinguish among these alternatives, we examined the response of *grp*-derived and wild-type embryos to low doses of X-irradiation. X-irradiation produces both single and double-stranded breaks and illicits a specific feedback response in the cell cycle of yeast and other organisms (Hartwell and Weinert, 1989, Powell and McMillan, 1990; Game, 1993; Price, 1993; Olive and Banath, 1993). We found that wild-type *Drosophila* embryos X-irradiated in interphase of nuclear cycle 10 will progress through metaphase of nuclear cycle 10 with some division errors. In the following cycle, the frequency of division errors increases dramatically. However, the syncytial nuclei do not undergo a cell-cycle arrest in response to X-irradiation. In contrast, if a *grp*-derived embryo is X-irradiated in interphase of nuclear cycle 10, the nuclei will progress through the first metaphase, but will arrest in the metaphase of the following cycle (nuclear cycle 11). These

arrested nuclei possess fully formed spindles. This metaphase arrest of *grp*-derived embryos can be induced prior to nuclear cycle 13 and even prior to cortical migration.

These X-irradiation studies suggest that the cycle 13 metaphase arrest observed in untreated *grp*-derived embryos is not a result of a disruption of the normal developmental transitions that occur at nuclear cycle 13. They also indicate that the *grp*-induced arrest is not dependent on the specific nuclear, centrosomal, and spindle configuration found in the cycle 13 metaphase *grp*-derived embryos. The *grp* mutation may be revealing a previously unidentified feedback response of the syncytial *Drosophila* embryo.

A model that is consistent with both the cycle 13 metaphase arrest and the earlier X-irradiation induced arrest of *grp*-derived embryos is that the syncytial embryo possesses a feedback mechanism sensitive to a combination of both spindle and nuclear cues. Previous studies have shown that wild-type embryos possess a spindle feedback; disrupting the spindle with microtubule depolymerizing drugs elicits a metaphase arrest (Zalokar and Erk, 1976; Foe et al., 1993). The primary

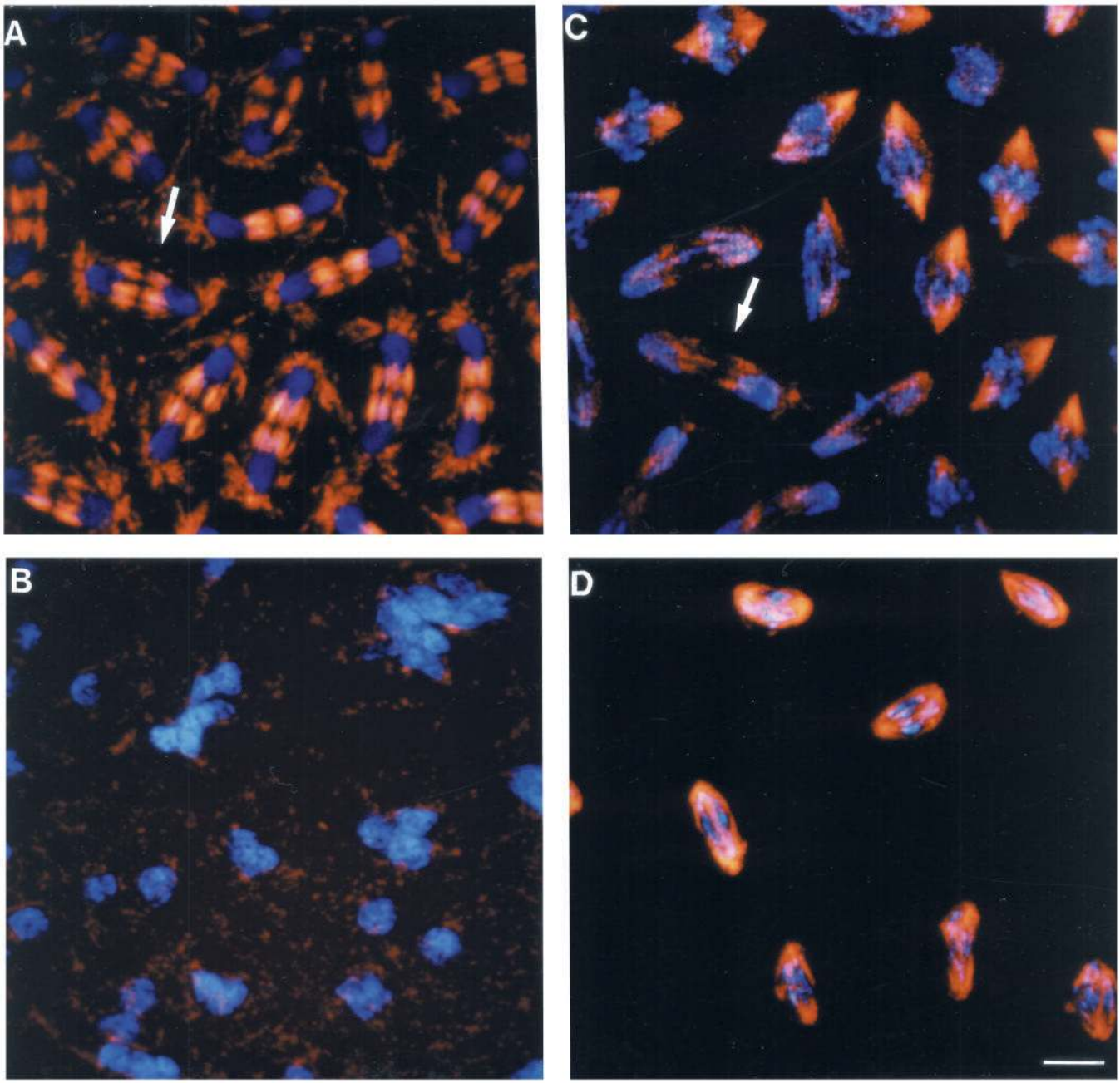


Fig. 6. Immunofluorescent images of the microtubules (red) and nuclei (blue) in wild-type embryos (A,B) and *grp*-derived embryos (C,D). The arrow in A points to the midbody in a wild-type embryo at anaphase/telophase of cycle 12. The arrow in C points to the equivalent structure in a *grp*-derived embryo at anaphase/telophase of nuclear cycle 12. B and D depict wild-type and *grp*-derived embryos, respectively, fixed 60 minutes after exposure to 0.3 kR of X-irradiation. Scale bar, 4 μm.

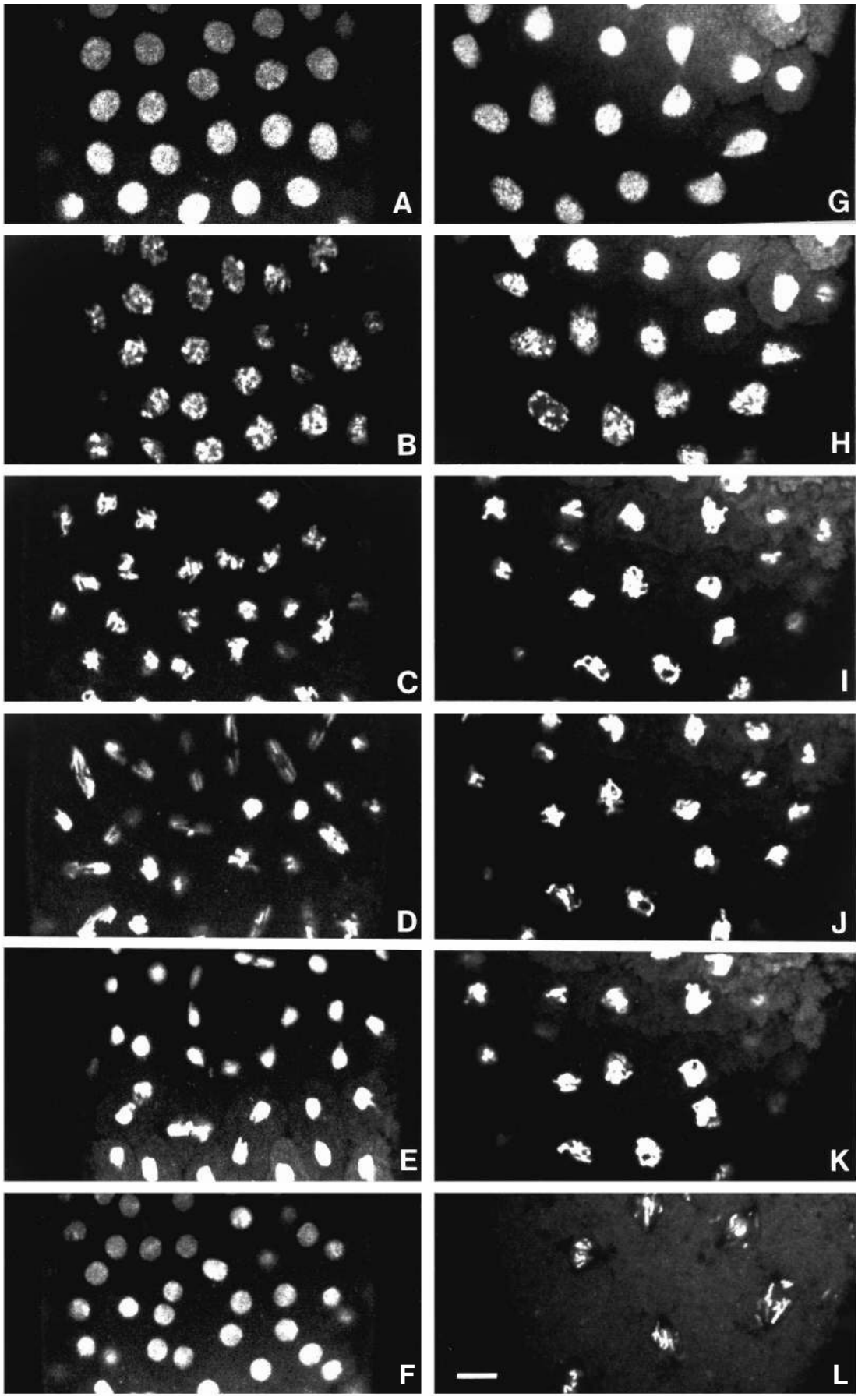


Fig. 7. The response of wild-type (A-F) and *grp*-derived embryos (G-L) to X-irradiation. Embryos injected with fluorescently labeled histone were exposed to 0.3 kR of X-irradiation during interphase of nuclear cycle 10. Both *grp*-derived and wild-type embryos progress to interphase of nuclear cycle 11 with some nuclear defects. A-F follow an X-irradiated wild-type embryo from interphase of nuclear cycle 11 to interphase of nuclear cycle 12. During metaphase and anaphase of nuclear cycle 11, division synchrony is lost and extensive abnormalities are observed (C,D). The nuclei continue to divide without arrest. G-K depict an equivalent set of images of an X-irradiated *grp*-derived embryo. Unlike the X-irradiated wild-type embryos, the *grp*-derived embryos arrest at metaphase of nuclear cycle 11 (H-L). K and L depict the nuclei 17 and 47 minutes after X-irradiation, respectively. Scale bar, 4 μm.

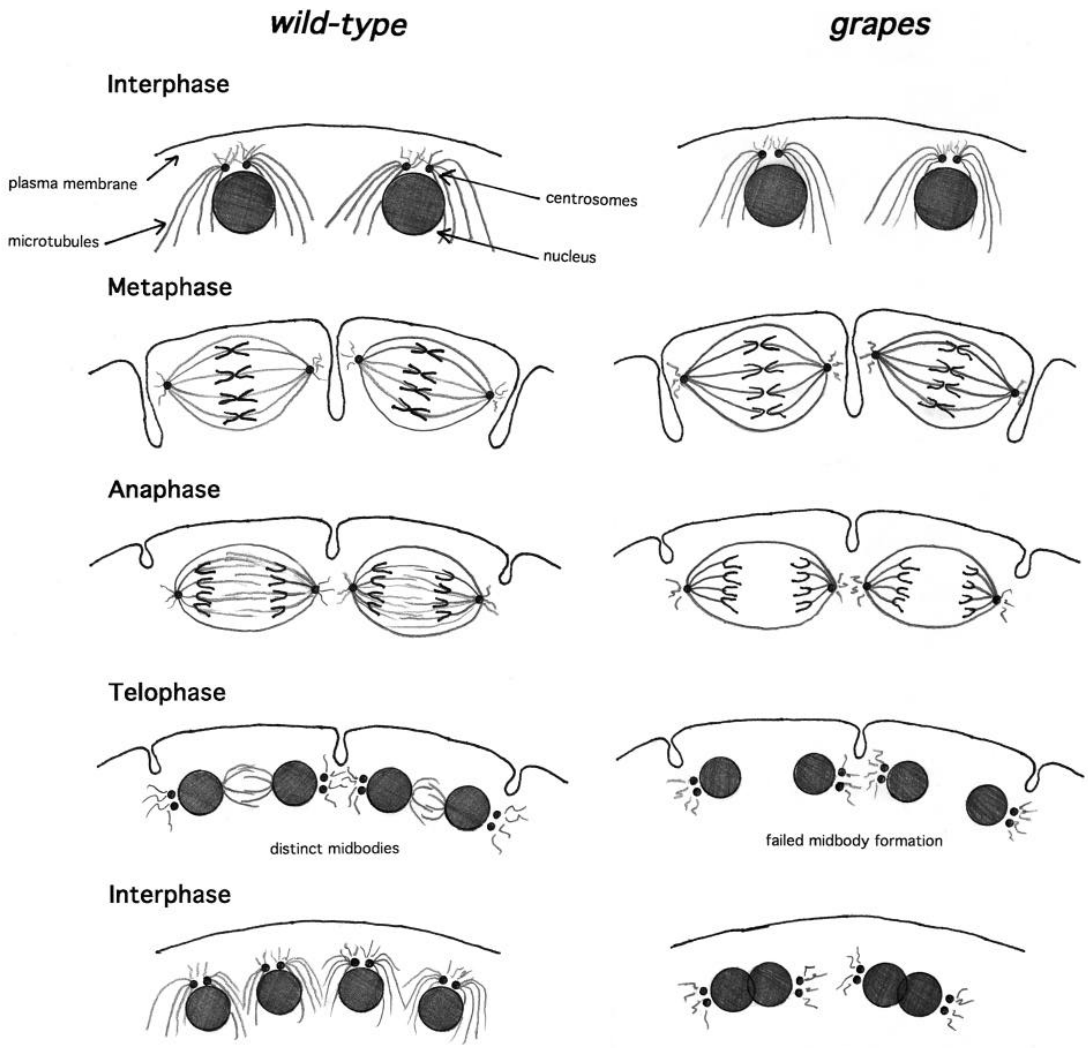


Fig. 8. Schematic illustration of the consequences of midbody failure in *grp*-derived embryos. Failed midbody formation during the cortical divisions results in fusion of sister telophase nuclei. Because centrosome duplication occurs during telophase, the fusion products possess two opposing pairs of centrosomes. This phenotype suggests that the midbody is responsible for maintaining separation of sister nuclei during the cortical divisions.

defect in *grp*-derived embryos may be a slight defect in microtubule function. This defect is not sufficient to elicit the spindle feedback response. However, the microtubule defect leads to failed midbody formation during telophase of nuclear cycle 12 and consequently fusion of sister telophase nuclei. These abnormal nuclei arrest in the next metaphase. Possibly the combination of defective microtubules and abnormal nuclei elicits this response. Abnormal nuclei or slightly damaged spindles alone is not sufficient to elicit a feedback response, but together they elicit a response leading to a metaphase arrest. X-irradiation of *grp*-derived embryos produces abnormal nuclei at an earlier cycle and consequently the metaphase arrest occurs at an earlier cycle. X-irradiation of wild-type embryos does not produce an arrest because the spindles are not defective; abnormal nuclei alone are not sufficient to induce the arrest response.

Another explanation of the *grp* phenotype, is that the normal *grp* product may override feedback controls which monitor the state of the chromatin. In the absence of the *grp*-product, these feedback controls are active and prevent exit from mitosis if the chromatin is abnormal. In our studies, both X-irradiation damage and fusion of sister telophase nuclei were sufficient to elicit the feedback response. The difficulty with this explanation is that it does not readily explain failed midbody formation in *grp*-derived embryos. One would have to postulate that the *grp* product also plays a role in microtubule dynamics and stability. A number of lethal null alleles of checkpoint genes in yeast have been identified (Li and Murray, 1991). The product of one of these genes plays diverse roles in providing both an essential cellular function and also acting as a cell cycle checkpoint (Li et al., 1993). Currently, we are extending our cellular analysis and analyzing *grp* molecularly to determine if either of these explanations is correct.

We would like to thank Margaret Fuller and Douglas Kellogg for their critical reading of the manuscript. We would also like to thank Andrew Murray, William Theurkauf, Douglas Kellogg, Ted Weinert, and Margaret Fuller for helpful discussions. This work was supported by grants to W. Sullivan from the National Institutes of Health (R29 GM46409-01) and the American Cancer Society (JFRA-366).

REFERENCES

Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, S., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., Jan, L. Y., and Jan, Y.

N. (1989). Searching for pattern and mutation in the *Drosophila* genome with a P-lacZ vector. *Genes Dev.* **3**, 1273-1287.

Earnshaw, W. C. and Bernat, R. L. (1991). Chromosomal passengers: toward an integrated view of mitosis. *Chromosoma* **100**, 139-146.

Edgar, B. A. and O'Farrell, P. H. (1989). Genetic control of cell division patterns in the *Drosophila* embryo. *Cell* **57**, 177-187.

Foe, V. E. and Alberts, B. M. (1983). Studies of nuclear and cytoplasmic behavior during the five mitotic cycles that precede gastrulation in *Drosophila* embryogenesis. *J. Cell Sci.* **61**, 31-70.

Foe, V. E., Odell, G. and Edgar, B. A. (1993). Mitosis and Morphogenesis in the *Drosophila* Embryo: Point and Counterpoint. In *The Development of Drosophila melanogaster*. (ed. Michael Bate and Alfonso Martinez Arias), Plainview, NY: Cold Spring Harbor Laboratory Press.

Freeman, M., Nusslein-Volhard, C. and Glover, D. M. (1986). The dissociation of nuclear and centrosomal division in gnu, a mutation causing giant nuclei in *Drosophila*. *Cell* **46**, 457-468.

Freeman, M. and Glover, D. M. (1987). The gnu mutation of *Drosophila* causes inappropriate DNA synthesis in unfertilized and fertilized eggs. *Genes Dev.* **1**, 924-930.

Fyrberg, E. A. and Goldstein, L. S. B. (1990). The *Drosophila* cytoskeleton. *A. Rev. Cell Biol.* **6**, 559-596.

Game, J. C. (1993). DNA double-strand breaks and the RAD 50 - RAD 57 genes in Saccharomyces. *Sem. Cancer Biol.* **4**, 73-83.

Hartwell, L. H. (1978). Cell division from a genetic perspective. *J. Cell Biol.* **77**, 627-637.

Hartwell, L. H. and Weinert, T. A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science* **246**, 629-634.

Karr, T. L. and Alberts, B. M. (1986). Organization of the cytoskeleton in early *Drosophila* embryos. *J. Cell Biol.* **102**, 1494-1509.

Kellogg, D. R., Mitchison, T. J. and Alberts, B. M. (1988). Behavior of microtubules and actin filaments in living *Drosophila* embryos. *Development* **103**, 675-686.

Kellogg, D. R., Field, C. M. and Alberts, B. M. (1989). Identification of microtubule-associated proteins in the centrosome, spindle, and kinetochore of the early *Drosophila* embryo. *J. Cell Biol.* **109**, 2977-2991.

Li, R. and Murray, A. W. (1991). Feedback control of mitosis in budding yeast. *Cell* **66**, 519-531.

Li, R., Havel, C., Watson, J. A. and Murray, A. W. (1993). The mitotic feedback control gene MAD2 encodes the alpha-subunit of a prenyltransferase. *Nature* **366**, 82-84.

Lindsley, D. L. and Zimm, G. G. (1992). *The Genome of Drosophila melanogaster*. San Diego: Academic Press.

Margolis, R. L. and Andreassen, P. R. (1993). The telophase disc: its possible role in mammalian cell cleavage. *Bioessays* **15**, 201-207.

McIntosh, J. R. (1991). Structural and mechanical control of mitotic progression. *Cold Spring Harbor Symp. Quant. Biol.* **56**, 613-619.

Minden, J. S., Agard, D. A., Sedat, J. W. and Alberts B. M. (1989). Direct cell lineage analysis in *Drosophila melanogaster* by time lapse three dimensional optical microscopy of living embryos. *J. Cell Biol.* **109**, 505-516.

Mitchison, T. J. and Sedat, J. W. (1983). Localization of antigenic determinants in whole *Drosophila* embryos. *Dev. Biol.* **99**, 261-264.

Nislow, C., Sellitto, C., Kuriyama, R. and McIntosh, J. R. (1990). A monoclonal antibody to a microtubule-associated protein blocks mitotic progression. *J. Cell Biol.* **111**, 511-522.

Nislow, C., Lombrillo, V. A., Kuriyama, R. and McIntosh, J. R. (1992). A plus-end directed motor enzyme that moves antiparallel microtubules in vitro localizes to the interzone of mitotic spindles. *Nature* **359**, 543-547.

O'Farrell, P. H., Edgar, B. A., Lakich, D. and Lehner, C. F. (1989). Directing cell division during development. *Science* **246**, 635-640.

Olive, P. L. and Banath, J. P. (1993). Induction and rejoining of radiation-induced DNA single-strand breaks: "tail moment" as a function of position in the cell cycle. *Mut. Res.* **294**, 275-283.

Postner, M. A., Miller, K. G. and Wieschaus, E. (1992). Maternal-effect mutations of the *sponge* locus affect cytoskeletal rearrangements in *Drosophila melanogaster* embryos. *J. Cell Biol.* **119**, 11205-1218.

Powell, S. and McMillan, T. J. (1990). DNA damage and repair following treatment with ionizing radiation. *Radiotherapy Oncol.* **19**, 95-108.

Price, A. (1993). The repair of ionising radiation-induced damage to DNA. *Sem. Cancer Biol.* **4**, 61-71.

Pringle, J. R. and Hartwell, L. H. (1981). The Saccharomyces cerevisiae cell cycle. In *Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance* (ed. Jeffrey N. Strathern, Elizabeth W. Jones and James R. Broach), Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Rabinowitz, M. (1941). Studies on the cytology and early embryology of the egg of *Drosophila melanogaster. J. Morphol.* . **69**, 1-49.

Raff, J. W. and Glover, D. M. (1988). Nuclear and cytoplasmic mitotic cycles continue in *Drosophila* embryos in which DNA synthesis is inhibited with aphidicolin. *J. Cell Biol.* **107**, 2009-2019.

Rattner, J. B. (1992). Mapping the mammalian intercellular bridge. *Cell mot. cytoskel.* **23**, 231-235.

Ripoll, P., Carmen, M. and Molina, I. (1992). Genetic analysis of cell division in *Drosophila*. *Curr. Dev. Biol.* **27**, 275-307.

Schejter, E. D. and Wieschaus, E. (1993). Functional elements of the cytoskeleton in the early *Drosophila* embryo. *A. Rev. Cell Biol.* **9**, 67-99.

Shamanski, F. L. and Orr-Weaver, T. L. (1991). The *Drosophila plutonium* and *pan-gu* genes regulate entry into S phase at fertilization. *Cell* **66**, 1289-1300.

Sonnenblick, B. P. (1950). The early embryology of *Drosophila melanogaster*. In *Biology of Drosophila* (ed. M. Demerec), pp. 62-167. New York: John Wiley and Sons. Reprinted, 1965, New York and London: Hafner.

Stafstrom, J. P. and Staehelin, L. A. (1984). Dynamics of the nuclear envelope and nuclear pore complexes during mitosis in the *Drosophila* embryo. *Eur. J. Cell Biol.* **34**, 179-189.

Sullivan, W., Minden, J. M. and Alberts, B. M. (1990). *daughterless-abo-like*, a *Drosophila* maternal-effect mutation that exhibits abnormal centrosome separation during the late blastoderm divisions. *Development* **110**, 311-323.

Sullivan, W., Fogarty, P. and Theurkauf, W. (1993a). Mutations affecting the cytoskeletal organization of syncytial *Drosophila* embryos. *Development* **118**, 1245-1254.

Sullivan, W., Daily, D. R., Fogarty, P., Yook, K. J. and Pimpinelli, S. (1993b). Delays in anaphase initiation occur in individual nuclei of the syncytial *Drosophila* embryo. *Mol. Biol. Cell* **4**, 885-896.

Theurkauf, W. E. (1992). Behavior of structurally divergent alpha-tubulin isotypes during *Drosophila* embryogenesis: evidence for post-translational regulation of isotype abundance. *Dev. Biol.* **154**, 205-217.

Turner, R. R. and Mahowald, A. P. (1977). Scanning electron microscopy of *Drosophila melanogaster* embryogenesis. I. The structure of the egg envelope and the formation of the cellular blastoderm. *Dev. Biol.* **50**, 95-108.

Warn, R. M., Magrath R. and Webb, S. (1984). Distribution of F-actin during cleavage of the *Drosophila* syncytial blastoderm. *J. Cell Biol.* **98**, 156-162.

Warn, R. M., Smith, L. and Warn, A. (1985). Three distinct distributions of F-actin occur during the divisions of polar surface caps to produce pole cells in *Drosophila* embryos. *J. Cell Biol.* **100**, 1010-1015.

Warn, R. M. and Warn, A. (1986). Microtubule arrays present during the syncytial and cellular blastoderm stages of the early *Drosophila* embryo. *Expl. Cell Res.* **163**, 201-210.

Warn, R. N., Flegg, L. and Warn, A. (1987). An investigation of microtubule organization and functions in living *Drosophila* embryos by injection of a fluorescently labeled antibody against tyrosinated tubulin. *J. Cell Biol.* **105**, 1721-1730.

Zalokar, M. and Erk, I. (1976). Division and migration of nuclei during early embryogenesis of *Drosophila melanogaster. J. Micro. Cell* **25**, 97-106.

(Accepted 16 May 1994)