Delays in Anaphase Initiation Occur in Individual Nuclei of the Syncytial *Drosophila* Embryo

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The syncytial divisions of the *Drosophila melanogaster* embryo lack some of the well established cell-cycle checkpoints. It has been suggested that without these checkpoints the divisions would display a reduced fidelity. To test this idea, we examined division error frequencies in individuals bearing an abnormally long and rearranged second chromosome, designated *C(2)EN*. Relative to a normal chromosome, this chromosome imposes additional structural demands on the mitotic apparatus in both the early syncytial embryonic divisions and the later somatic divisions. We demonstrate that the *C(2)EN* chromosome does not increase the error frequency of the late larva neuroblast divisions. However, in the syncytial embryonic nuclear divisions, the *C(2)EN* chromosome produces a 10-fold increase in division errors relative to embryos with a normal karyotype. During late anaphase of the neuroblast divisions, the sister *C(2)EN* chromosomes cleanly separate from one another. In contrast, during late anaphase of the syncytial divisions in *C(2)EN*-bearing nuclei, large amounts of chromatin often lag on the metaphase plate. Live analysis of *C(2)EN*-bearing embryos demonstrates that individual nuclei in the syncytial population of dividing nuclei often delay in their initiation of anaphase. These delays frequently lead to division errors. Eventually the products of the nuclei delayed in anaphase sink inward and are removed from the dividing population of syncytial nuclei. These results suggest that the *Drosophila* embryo may be equipped with mechanisms that monitor the fidelity of the syncytial nuclear divisions. Unlike checkpoints that rely on cell cycle delays to identify and correct division errors, these embryonic mechanisms rely on cell cycle delays to identify and discard the products of division errors.

INTRODUCTION

The somatic cell cycle consists of a series of dependent relationships in which the initiation of one event is dependent on the proper completion of a previous event (Hartwell, 1978; Hartwell and Weinert, 1989). For instance, in many systems the initiation of mitosis is dependent on the proper completion of DNA synthesis (Rao and Johnson, 1970; Tobey, 1975). This dependency usually is not the result of an intrinsic substrate-product relationship but is established by adding feedback controls to the basic mechanism driving the cell cycle (for

review see Pines, 1992). These extrinsic controls are called checkpoints (Weinert and Hartwell, 1988). Null mutations that eliminate a well established dependent relationship in the cell cycle provide the most convincing demonstration of checkpoints. In yeast, for instance, the *rad 9* mutation allows cells that have not properly completed DNA replication to enter mitosis (Weinert and Hartwell, 1988). Recently, checkpoints have been identified at other points in the cell cycle (Li and Murray, 1991; Hoyt *et al.*, 1991, reviewed in Murray, 1992).

In higher eukaryotes, many of the established checkpoints are not present during the initial embryonic divisions (Hartwell and Weinert, 1989). For example, inhibition of DNA replication during the initial *Drosophila* nuclear divisions does not inhibit the centrosome cycles (Raff and Glover, 1988). The mitotic divisions of the

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early *Drosophila* embryo are synchronous, occurring in waves that initiate near the poles of the embryo. These divisions occur without cell growth or cytokinesis, and the G1 and G2 stages of the cell cycle are greatly reduced (Rabinowitz, 1941; Sonnenblick, 1950; Turner and Mahowald, 1976; Zalokar and Erk, 1976; Foe and Alberts, 1983; Minden *et al.*, 1989). It has been suggested that the delays induced by checkpoints would not be compatible with these rapid synchronous divisions. The lack of checkpoints would be expected to result in a reduced division fidelity. The syncytial embryonic divisions may employ an alternate strategy for coping with division errors; rather than correcting the errors, the products of the abnormal divisions are eliminated (Hartwell and Weinert, 1989; Sullivan *et al.*, 1990, 1993).

In this paper, we have examined these ideas concerning embryonic checkpoints by comparing the fidelity of division in the syncytial embryonic and later somatic divisions of *Drosophila melanogaster*. We examined individuals with a normal karyotype and individuals with an abnormally long second chromosome called *C(2)EN*. Cytological studies and high adult survival rates indicate that this compound chromosome stock is euploid (Novitski, 1976; Novitski *et al.*, 1981). We chose the *C(2)EN* stock to impose additional structural demands on both the embryonic and later somatic division machinery. We demonstrate that the presence of the *C(2)EN* chromosome does not affect the error rate of the somatic divisions, but it greatly increases the error rate of the embryonic divisions. Unlike normal chromosomes, the sister *C(2)EN* chromosomes do not cleanly separate from one another during anaphase of the syncytial embryonic divisions. During the later neuroblast divisions, however, the sister *C(2)EN* chromosomes cleanly separate from one another at anaphase. Analysis of living *C(2)EN*-bearing embryos reveals that individual nuclei in the syncytial population of dividing nuclei often delay slightly in their initiation of anaphase, and the daughter nuclei they produce lose division synchrony with their neighboring nuclei and eventually sink into the interior of the embryo. We discuss these results with regard to current ideas concerning mechanisms that monitor the fidelity of the division cycle.

MATERIALS AND METHODS

Stocks

The compound chromosome two stock, designated *C(2)EN* (Lindsley and Zimm, 1992), described in this paper was kindly provided to us by the Indiana stock center (Bloomington, IN). We made use of three *C(2)EN* chromosomes: *C(2)EN*, *C(2)EN bw, sp*, and *C(2)EN bw, pr*. The synthesis of the compound chromosome is described by Novitski (1976) and Ashburner (1989). Control embryos were obtained from the wild-type Oregon-R stock (gift of T. Kornberg).

Cellular Analysis

Embryos were formaldehyde fixed by a modification of the Mitchison and Sedat (1983) protocol. This method is described in detail elsewhere

(Theurkauf, 1992). Nuclei were detected using a monoclonal anti-histone antibody (Chemicon, Temecula, CA) and the immunofluorescent staining protocol described by Karr and Alberts (1986). The embryonic expression of the *Krüppel* protein was detected with an anti-*Krüppel* antibody kindly provided by Sean Carroll (University of Wisconsin at Madison). Immunofluorescent localization of centrosomes in the *Drosophila* embryo was performed according to the procedure described by Sullivan *et al.* (1990).

Microscopy was performed using an Olympus IMT2 inverted photomicroscope (Scientific Instrument, Sunnyvale, CA) equipped with a Biorad MRC 600 laser confocal imaging system (Richmond, CA). The lenses used included the Olympus S Plan Apo 60X, Oil and the Olympus D Plan Apo 20X, UV. Oil.

In Vivo Fluorescence Analysis

The in vivo analysis of the syncytial embryonic divisions was accomplished through a procedure in which fluorescently-labeled histones were microinjected into embryos (Kellogg *et al.*, 1988; Minden *et al.*, 1989; Sullivan *et al.*, 1990). Observations and time lapse recordings were made on an Olympus IMT2 inverted microscope equipped with a Biorad MRC600 laser confocal imaging system.

Larval Neuroblast Cytology

The neuroblast cytology was performed according to the procedure described in Gatti and Goldberg (1991). The metaphase spreads were prepared by incubating neuroblast cells from third instar for 2 h in a saline solution (0.7% NaCl in distilled water) containing 10^{-4} M colchicine. This was followed by placing them in a 1% citrate hypotonic solution for 10 min and then fixing in a 1:1 mixture of acetic acid and methanol. For anaphase preparations, the dissected brains were immediately fixed without any previous colchicine or hypotonic treatment. The fixed preparations were stained with acetic-orcein.

RESULTS

The Cytology of the Compound Chromosome *C(2)EN*

The synthesis of *C(2)EN* by Novitski (1976) resulted in a chromosome in which both second chromosome homologues share a single centromere. Starting with a compound 2L chromosome (2L/centromere/2L), the multistep synthesis relied on translocations, recombination, and heterochromatic Y-linker elements to produce the following chromosome: 2R2L/centromere/2L2R. Micrographs of neuroblast cells depict the cytology of a normal and *C(2)EN*-bearing karyotype (Figure 1, a and b). It is clear that the *C(2)EN* individuals possess only a single chromosome 2 that is approximately twice the length of the normal chromosome 2. Figure 1c represents a more detailed view of the *C(2)EN*-bearing chromosome. The intervening Y heterochromatic linkers are clearly visible in the middle of each arm (see arrows). In this construction none of the euchromatic material was lost and *C(2)EN*-bearing individuals are euploid, viable, and fertile. Figure 1d depicts the *C(2)EN* chromosome during anaphase in which its additional length is clearly visible with respect to the other chromosomes.

Error Frequency of the Compound Chromosome *C(2)EN* During the Embryonic Divisions

During the syncytial cortical divisions of the early *Drosophila* embryo, the products of abnormal nuclear di-

Figure 1. Cytology of the compound chromosome, *C(2)EN*. (a) Metaphase of a normal female. (b) Metaphase of a female carrying the *C(2)EN* chromosome. (c) A detailed view of the *C(2)EN* chromosome. The c denotes the centromere, and the arrows indicate the brightly staining Y heterochromatic linker elements. (d) Anaphase configuration of the *C(2)EN*-bearing karyotype. The arrow indicates the overlap of the sister *C(2)EN* chromosomes.

visions sink into the interior of the embryo leaving a nuclear free area on the surface (Minden *et al.*, 1989; Sullivan *et al.*, 1990, 1993). We have used this phenomenon to estimate the frequency of nuclear division errors during the syncytial blastoderm divisions. Figure 2 shows a wild-type embryo and an embryo derived from the *C(2)EN*-bearing stock. Each embryo has been formaldehyde fixed and stained with an anti-histone antibody to reveal the location of the nuclei. Syncytial embryos derived from the *C(2)EN*-bearing stock have a much higher frequency of regions on the surface devoid of nuclei compared to normal embryos (Figure 2, A and C, see arrow). This interpretation is supported by images of the same embryos taken 5–10 μ beneath the surface.

In the normal embryo, no nuclei are present in this plane (Figure 2B). However, the *C(2)EN*-bearing embryo contains a high frequency of nuclei in the cortex beneath the normal surface monolayer of nuclei (Figure 2D, see arrow). These nuclei represent the products of the abnormal surface cortical divisions sinking into the interior of the embryo. When these nuclei sink inward, they leave their centrosomes behind on the surface (Figure 2, E and F).

The *C(2)EN* stock from which these embryos were derived produce embryos of three possible genotypes: *C(2)EN/C(2)EN*, *C(2)EN/0*, and *0/0*. We were only interested in determining the error rate of the euploid class, *C(2)EN/0* (this is the only class that survives to adulthood). To distinguish between these three genotypes, we scored the error frequency of embryos fixed in anaphase during the late syncytial divisions. At anaphase, we are able to cytologically identify embryos bearing a single *C(2)EN* chromosome (Figure 2H). As Table 1 indicates, 90 of 2676 anaphase nuclei from 6 *C(2)EN*-bearing embryos were scored as abnormal. This yields a 3.3% error rate compared to the 0.3% found for wild-type embryos.

To further establish that the increased error rate during the syncytial nuclear divisions was caused by the *C(2)EN* chromosome, we crossed *C(2)EN*-bearing females to normal males. This cross is sterile, producing two classes of aneuploid progeny with respect to chromosome two: *C(2)EN/2* individuals and *2/0* individuals. Because there is little gene expression during the initial embryonic divisions (McKnight and Miller, 1976; Zallouk, 1976; Anderson and Lengyel, 1981; Edgar and Schubiger, 1986; Merrill *et al.*, 1988; Wieschaus and Sweeton, 1988), these aneuploid individuals can develop relatively normally through the syncytial divisions. We could distinguish between these two classes during the syncytial divisions because *2/0* embryos and *C(2)EN/2* embryos possess one and three copies of the *Krüppel* gene, respectively. This gene is located on the second chromosome and is transcribed and translated from a central band of nuclei during the late cortical divisions (Gaul *et al.*, 1987). Consequently, with immunofluorescence analysis using the anti-*Krüppel* antibody, *2/0* embryos produce a light band, and *C(2)EN/2* embryos produce a dark band at nuclear cycle 14 (Figure 3). To quantify the nuclear errors observed in each, we analyzed midsection views of cycle 14 embryos double-stained for their *Krüppel* expression and nuclear distribution. In these views, abnormal nuclei that are sinking into the interior are readily detected in the cortical region just beneath the surface monolayer of nuclei (within 10 μ) (see arrows in Figure 3, C and D). Examination of 14 wild-type embryos derived from the standard Oregon-R stock produces a range of 0–4 such nuclei per embryo with an average of 1.5 nuclei per embryo. Analysis of the nine darkly stained *C(2)EN/2* embryos produced a range of 5–31 nuclei per embryo

Figure 2. The cortical nuclear distribution in normal and *C(2)EN*-bearing embryos. Surface views of embryos formaldehyde-fixed and stained with an anti-histone antibody are depicted (Oregon-R stock [A] and the *C(2)EN*-bearing stock [C]). B and D represent views 5–10 μ beneath the surface of the same Oregon-R and *C(2)EN* embryos. In the *C(2)EN*-bearing embryos, there are nuclear free areas on the surface (C, arrow) and also a large number of nuclei just beneath the surface monolayer (D, arrow). These nuclei are the result of the products of abnormal cortical divisions sinking inward. E and F depict a *C(2)EN*-bearing embryo doubly stained for its chromatin and centrosomes. In both figures, large numbers of free centrosomes are present (arrows). When the abnormal nuclei sink inward, their centrosomes remain behind on the surface. G depicts the anaphase configuration of a normal embryo during the cortical nuclear divisions. H depicts the anaphase configuration of a *C(2)EN*-bearing embryo at a similar stage. The *C(2)EN*-bearing embryo has a more diffuse staining pattern, and there is a large amount of chromosome material lagging on the metaphase plate. Scale bar, 100 μ in panels A, B, C, and D and 35 μ , 18 μ , 10 μ , and 10 μ in panels E, F, G, and H, respectively.

with an average of 14.7 nuclei per embryo. Analysis of nine lightly stained 2/0 embryos produced a range of 0–6 nuclei per embryo with an average of 2.0 nuclei per embryo. Even though both *C(2)EN/2* and 2/0 embryos are aneuploid and derived from the same females, only the embryos bearing the *C(2)EN* chromosome have a consistently large number of sunken nuclei. This suggests that only the *C(2)EN*-bearing embryos have a dramatically increased error rate during their cortical divisions.

Loss of Synchrony of the Nuclear Divisions Is Frequently Observed in the *C(2)EN*-bearing Embryos

To better understand the nature of the increased division errors, live *C(2)EN*-bearing embryos were injected with fluorescently labeled histones, and the syncytial divisions were imaged and recorded at 24–30-s intervals. Figure 4 depicts a series of images from a syncytial blas-

toderm embryo derived from a normal stock. Figure 4A depicts a field of ~15 nuclei in late prophase. Figure 4, B and C follow these nuclei into metaphase and into a state of maximum compaction just before they separate at anaphase. The nuclei are synchronous with respect to compaction; all reach a maximally compacted state simultaneously. Figure 4, D and E depict their synchronous separation at anaphase, and finally Figure 4, F–H follow the nuclei through telophase and into prophase of the next division cycle. These images highlight the synchrony observed at all stages of the division cycle between neighboring syncytial nuclei.

A similar analysis of living *C(2)EN*-bearing embryos reveals that the syncytial nuclear division synchrony is often lost. Figure 5 depicts a series of images of a *C(2)EN*-bearing embryo injected with fluorescently labeled histones and recorded over 30-s intervals. Figure 5A depicts nuclei in prophase and follows them to the point of maximum compaction on the metaphase plate in Figure 5,

C2/O × 2/2

Figure 3. The nuclear phenotype of *C(2)EN/2* and 2/0 embryos. *C(2)EN/2* and 2/0 are the two genotypes produced when *C(2)EN/0* individuals are crossed to normal 2/2 individuals. These two classes can be identified in the embryonic stages by the intensity of the anti-*Krüppel* antibody staining. *C(2)EN/2* embryos (A) will stain much darker than 2/0 embryos (B) (see text for explanation). The darker anti-*Krüppel*-stained *C(2)EN/2* embryos have a much larger amount of internal nuclei (C) than the lighter staining 2/0 embryos (D). This is the result of abnormal surface nuclei sinking into the interior of the embryo (see arrow). Scale bar, 50 μ .

Table 1. Frequency of somatic and embryonic division errors

A. Embryonic nuclear division errors				
Karyotype of the stock	Number of embryos scored	Number of nuclei scored	Number of abnormal or missing nuclei	Error frequency
Wild-type (total)	31	21 207	59	0.3%
C2/0 (by anaphase)	6	2 676	90	3.3%
B. Somatic larval neuroblast division errors				
Karyotype of the stock	Frequency of abnormal metaphase		Frequency of abnormal anaphase	
Wild-type	1/1 000 (0.1%)		0/100 (0%)	
C2/0	2/1 000 (0.2%)		1/200 (0.5%)	

H and I. The nucleus marked with the arrow is delayed in its compaction on the metaphase plate (see arrow Figure 5, C and D). The chromosomes in this nucleus are in a much more extended conformation than those found in its neighbors. However, just before anaphase, it appears as densely packed as its neighbors (Figure 5I). In Figure 5, J–L, it can be seen that anaphase is delayed. In Figure 5L, anaphase is just beginning in this nucleus, whereas the neighboring nuclei are well into telophase. The two sister products of this late anaphase division are usually removed from the cortex during the next division cycle. Of 637 divisions in C2-bearing embryos, 19 were delayed initiating anaphase and 25 failed to divide. Of these 25, 16 were in pairs and probably represented sister nuclei. As described below, these 16 are likely to be the result of a delayed anaphase in the previous division. Therefore ~3% of the divisions exhibit a delay in the initiation of anaphase. The extent of the delay ranges from <30 s to ~1 min in the 12–15-min nuclear cycles.

In some cases, we have followed a C(2)EN-bearing embryo for multiple cycles (Figure 6). The arrow follows a nucleus that is slightly delayed in initiating anaphase (Figure 6D). Consequently, the two sister anaphase products are slightly delayed in entering telophase (Figure 6, E and F). In the next division cycle, both of these nuclei are severely delayed in initiating anaphase (Figure 6H). They are not properly compacted and fail to successfully complete anaphase (Figure 6, I and J). These nuclei will eventually fall into the interior. In this example, a slight delay becomes exaggerated in the following cycle eventually resulting in loss of the sister nuclei from the dividing population. The abnormal divisions that often follow a delayed anaphase are of three types: 1) the nucleus fails to divide, 2) the division is unequal, or 3) the products of the division collide with neighboring nuclei.

In seven recordings we were able to follow the fate of nuclei delayed in anaphase initiation. Of 11 nuclei delayed in anaphase initiation, eight either failed to di-

vide or underwent a severely abnormal division. In 7 of these same 11 nuclei, either one or both of their products fell into the interior before the next anaphase. As demonstrated in Figure 6, it is likely that many of the delayed division products remaining in the cortex will fall into the interior in subsequent cycles.

During late telophase, the C(2)EN-bearing sister nuclei are frequently in an arc-like configuration of two opposing tear drops (Figure 6E). This structure is not seen in the syncytial divisions of normal embryos. This configuration probably results from the long C(2)EN chromosome remaining extended in late anaphase so that the nuclear membrane encompasses a tear-drop rather than a circular configuration of chromatin.

Error Frequency of the C(2)EN Chromosome in the Somatic Divisions

To determine the mitotic behavior of the compound chromosome in the larval divisions, we examined larval neuroblast preparations. This analysis is based on previous studies of *Drosophila* neuroblast cell division errors (Gatti *et al.*, 1974; Ripoll *et al.*, 1985; Gatti and Baker, 1989; Gatti and Goldberg, 1991). Division errors that produce chromosome aberrations can be detected by screening for chromosome fragments. Figure 7 depicts a series of anaphase preparations from the C(2)EN-bearing stock. In each instance, the long compound chromosome separates cleanly at anaphase, and no fragments are seen. Examination of large numbers of neuroblast preparations reveals that there are no differences in error frequency between the C(2)EN-bearing and the wild-type stocks (Table 1). We scored 1000 metaphases in 10 colchicine-treated neuroblast cells from both the C(2)EN-bearing larva and wild-type larva. We found two abnormal metaphases in the C(2)EN-bearing neuroblasts and one in the wild-type neuroblasts. We also scored 200 anaphases in six neuroblasts derived from normal larva and 200 anaphases in six neuroblasts derived from C(2)EN-bearing larva (these

Figure 4. Images of a syncytial Oregon-R embryo injected with fluorescently labeled histones. A depicts chromosome condensation during late prophase. The chromosomes begin to line up on the metaphase plate (B) and reach a point of maximum compaction (C). The sister chromosomes synchronously initiate anaphase (D). By late anaphase, the sister chromosomes have fully separated from one another (E). These nuclei synchronously enter telophase and begin prophase of the next nuclear cycle (F, G, and H). Images were selected from a series collected at 24-s intervals. Time elapsed from image A to H is 13.5 min. Scale bar, 10 μ .

Figure 5. Images of a syncytial *C(2)EN*-bearing embryo injected with fluorescently labeled histones. A through L follow a group of nuclei as they progress through prophase, metaphase, and into late telophase. The arrow marks a nucleus in which the chromosomes are delayed in compacting on the metaphase plate (D). As a result, this nucleus is late in initiating anaphase. When its neighbors are completing telophase, it is just beginning anaphase (J–L). The products of this late anaphase will sink into the interior of the embryo. Images selected from a series collected at 30-s intervals. Time elapsed from image A to L is 6.5 min. Scale bar, 10 μ .

Figure 6. A *C(2)EN*-bearing embryo is followed for multiple nuclear cycles. Images of a syncytial *C(2)EN*-bearing embryo injected with fluorescently labeled histone are shown. The arrow denotes a nucleus that is only slightly delayed in anaphase at the first cycle (D) and completes anaphase and telophase to produce two relatively normal looking mitotic products in the next interphase and prophase (G). However when these products are followed into the next mitotic cycle (H-J) they are extensively delayed (H) and fail to complete anaphase (I and J). These abnormal nuclei also sink into the interior of the embryo. Images selected from a series collected at 30-s intervals. Time elapsed from image A to J is 19 min. Scale bar, 10 μ .

Figure 7. The metaphase and anaphase configurations of larva neuroblast cells bearing the *C(2)EN* chromosome. a depicts a male metaphase spread and b and c depict male midanaphase spreads. d depicts a spread later in anaphase. e and f show neighboring cells in the same neuroblast in mid and late anaphase, respectively. Arrows indicate spindle poles. Sister *C(2)EN* chromosomes separate relatively normally during anaphase (see arrows).

anaphase preparations were not treated with colchicine). Only a single abnormal anaphase was observed in the *C(2)EN*-bearing neuroblasts, and none were observed in the normal neuroblasts. These values are not significantly different.

DISCUSSION

We have examined the effects of an abnormally long and rearranged compound second chromosome on both the rapid syncytial divisions of the embryo and the later slower divisions of the larval neuroblast cells. These studies demonstrate that the presence of the compound chromosome dramatically increases the error rate in the syncytial embryonic divisions but has little effect on the error rate in the later larval neuroblast divisions.

The embryonic nuclear division error rate is estimated by determining the number of nuclei that have dropped from the surface monolayer of nuclei during the syncytial cortical divisions. Previous studies have shown that the products of abnormal nuclear divisions sink inward (Minden *et al.*, 1989; Sullivan *et al.*, 1990, 1993). Embryos bearing the *C(2)EN* chromosome exhibit a nuclear-division error frequency some 10-fold higher than embryos with a normal karyotype.

We also crossed *C2/0* females to normal males (*2/2*) to eliminate the possibility of maternal effects causing the increased error rate found in *C(2)EN*-bearing embryos. This cross produces two classes of embryos: *C(2)EN/2* (trisomic for chromosome 2) and *2/0* (monosomic for chromosome 2). Even though both classes are aneuploid and derived from the same mother, the *C(2)EN/2* class exhibited over a sevenfold higher error rate than the sibling *2/0* class. In our studies, the increased error rate segregates with the *C(2)EN* chromosome.

To determine the effect of the *C(2)EN* chromosome on the division error rate in the later larval divisions, we scored the frequency of chromosome aberrations. Although this assay differs from the embryonic assay for division errors, it proves to be a reliable measure of a broad array of errors occurring during the neuroblast divisions (Gatti and Goldberg, 1991). In these studies, we found no difference in division error rates between the normal and *C(2)EN*-bearing larva.

Our observations suggest that the machinery of the larval neuroblast divisions is better equipped to cope with the compound chromosome than the syncytial embryonic divisions. Correlated with these results are cytological differences. In the neuroblast cells, the sister compound chromosomes are observed cleanly separating from one another during late anaphase. In contrast, during the syncytial divisions large amounts of chromosomal material often lag on the metaphase plate in nuclei bearing the compound chromosome. This is not observed in syncytial nuclei with the normal karyotype. Unlike the syncytial embryonic divisions, the neuroblast divisions are able to accommodate the increased length and rearrangements of the compound chromosome. An explanation that accounts for this difference is that the spindles of the neuroblast cells are longer than the spindles associated with the embryonic syncytial nuclei. Consequently, the long sister compound chromosomes could separate more readily during anaphase. Because our initial measurements have revealed variability in the spindle lengths, resolving this issue will require much more extensive analysis.

Following the syncytial divisions in living *C(2)EN*-bearing embryos reveals that a single nucleus in a population of dividing nuclei is capable of delaying its initiation of anaphase. Often, we observe that nuclei delayed in anaphase exhibit chromosomes in more extended conformations during the preceding prophase

to metaphase transition (Figure 5C). Difficulties of the long chromosome in achieving a proper metaphase configuration may be the cause of the anaphase delays.

Although the syncytial divisions of *Drosophila* do not exhibit oscillations in their cyclin levels as dramatic as those observed for other division cycles (Lehner and O'Farrell, 1989, 1990), it has been demonstrated that during the late *Drosophila* syncytial divisions cyclin levels reach a peak during metaphase and rapidly decrease by late anaphase (Edgar and O'Farrell, personal communication). In other systems, it has been shown that cyclin degradation is required for the initiation of anaphase (Murray *et al.*, 1989). Our observations of delays in the initiation of anaphase by individual nuclei raise the issue of whether cyclin degradation occurs globally or at the level of individual nuclei. If the degradation of cyclin is global, the observed delays in anaphase are the result of individual nuclei either overriding or being unable to respond to the initiation signal. If the degradation of cyclin occurs at the level of single nuclei, anaphase delay is likely to be the result of mechanisms inhibiting cyclin degradation. Subtle local oscillations in *Drosophila* cyclin A levels during the syncytial divisions provide support for this latter proposal (Lehner and O'Farrell, 1989). Whether these delays are a result of feedback controls or substrate/product relationships is not known.

In some cases of nuclei delayed in anaphase, it appears that their chromosomes have not achieved a normal state of compaction before the mitotic wave. Although the initiation of anaphase is delayed with respect to the syncytial population, anaphase appears to occur prematurely with respect to the state of chromosome configuration. Consequently abnormal division products are produced. In these nuclei in which the chromosomes are delayed in anaphase initiation, the centrosome/spindle cycle may be progressing normally with the mitotic wave. This would create an asynchrony between the chromosome and centrosome cycle and possibly lead to the more extensive anaphase delays and divisions failures observed in subsequent cycles. The *Drosophila* maternal-effect mutation *gnu* is an extreme example of the loss of coordination between the chromosome and centrosome cycles (Freeman *et al.*, 1986, Freeman and Glover, 1987). In *gnu*-derived embryos, centrosomes replicate independently of the DNA, and nuclear division never occurs. It may be that the nuclear division failure is a direct consequence of the asynchrony between the centrosome and chromosome cycles.

Our studies demonstrate that often the products of a delay in anaphase lead to severely abnormal divisions or nuclei completely failing to divide. These nuclei eventually drop into the interior of the embryo. This observation helps explain previous studies demonstrating that the products of a broad range of division abnormalities such as fusions at telophase, unequal divisions, and complete division failures are removed from

the cortex. Our analysis suggests that in many instances one of the first manifestations of each of these abnormalities is a delay in anaphase. We also observe, at a lower frequency, this anaphase delay and subsequent loss in normal embryos and believe it is a general phenomenon.

Cell cycle delays are not intrinsically harmful. In fact, delay is an essential component of the somatic checkpoints that maintain cell cycle fidelity (Hartwell and Weinert, 1989). In the syncytial embryo, however, delay frequently leads to division errors and removal of the abnormal division products from the dividing population. This indicates that mechanisms exist in the *Drosophila* embryo that link delays in the nuclear division cycle to nuclear loss. This process is beneficial to the organism as it effectively culls abnormal nuclei from the dividing population of cortical nuclei. Somatic checkpoints link delays in the cell cycle with repair to increase the division fidelity of individual cells. Our studies have outlined a mechanism operating during the cortical divisions of the syncytial *Drosophila* embryo that links delays in nuclear division to nuclear loss that ultimately leads to increased fidelity of the population of dividing nuclei.

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