Nuclear and Cytoplasmic Mitotic Cycles Continue in Drosophila Embryos in which DNA Synthesis Is Inhibited with Aphidicolin

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Abstract. We have microinjected aphidicolin, a specific inhibitor of DNA polymerase α, into syncytial *Drosophila* embryos. This treatment inhibits DNA synthesis and, as a consequence, nuclear replication. We demonstrate that under these conditions several cycles of both centrosome replication and cortical budding continue, although the cycles have a longer periodicity than is normally found. As in uninjected embryos, when the cortical buds are present, the embryos have nuclei containing decondensed chromatin surrounded by nuclear membranes as judged by bright annular staining with an anti-lamin antibody. As the buds recede, the unreplicated chromatin condenses and lamin staining becomes weak and diffuse. Thus, both cytoplasmic and nuclear aspects of the mitotic cycle continue following the inhibition of DNA replication in the *Drosophila* embryo.

THE mitotic divisions in early embryos of many insects, echinoderms, molluscs, and amphibians consist of rapid successions of M and S phases with no discernible G1 or G2 phases as found at later stages of development. The ability of some embryos to proceed through various aspects of the cell cycle in the absence of a nucleus has led to the idea that there is a fundamental cell cycle oscillator that dictates the timing of the early mitoses in these embryos, and that this oscillator can operate independently of the nucleus (for review see Kirschner et al., 1985). For example, periodic surface contractions continue in enucleated *Xenopus* embryos (Hara et al., 1980), and centrosomes can continue to divide in enucleated sea urchin embryos (Sluder et al., 1986). More recently it has been shown that cycles of histone kinase activity, and M phase or maturation-promoting factor (MPF)[1] activity continue in activated *Xenopus* eggs in the absence of any nuclear components (Dabauvalle et al., 1988).

MPF was first described as a factor(s) that induces G2-arrested *Xenopus* oocytes to mature by completing the second meiotic division. Subsequently, MPF activity was shown to oscillate during the cell cycle, peaking in each M phase (Wasserman and Smith, 1978; Gerhart et al., 1984). When partially purified, MPF is either injected into G2-arrested oocytes or added to cell-free extracts, it induces nuclear envelope breakdown, chromosome condensation, and mitotic spindle formation, supporting the hypothesis that oscillating cytoplasmic signals can drive the nuclear cycle. In

the early embryos of several organisms a class of proteins called cyclins are synthesized and then degraded during each cell cycle (Rosenthal et al., 1980; Evans et al., 1983; Standart et al., 1987). The relationship between MPF and the cyclins is not yet clear.

Experiments using aphidicolin, a specific inhibitor of DNA polymerase α, to inhibit DNA synthesis have demonstrated that aspects of the cytoplasmic cycle can also continue in the presence of an unreplicated nucleus. For example, centrosomes continue to divide in both sea urchin (Sluder and Lewis, 1987) and starfish embryos (Nagano et al., 1981) treated with aphidicolin, and surface contraction waves continue in aphidicolin-treated *Xenopus* embryos (Kimelman et al., 1987). The behavior of the unreplicated nucleus in aphidicolin-treated embryos has only been examined in sea urchins (Sluder and Lewis, 1987). In this case the nuclear envelope does not always break down, and when it does, the chromatin can be seen as an amorphous mass of fibers. The chromosomes do not condense, and the nuclear membrane does not reform, even though centrosome division continues and the formation of a cleavage furrow is initiated. Thus, it seems that although some parts of the cytoplasmic cycle continue in aphidicolin-treated sea urchin embryos, the cycles of chromatin condensation and nuclear envelope breakdown are dramatically affected by the inhibition of DNA synthesis, suggesting that unreplicated DNA may interfere with these aspects of the nuclear cycle.

In the present study, we have examined the effect of aphidicolin on various aspects of the cell cycle in *Drosophila* embryos. The *Drosophila* embryo is a syncytium that undergoes 13 rapid nuclear divisions during the first two and a half

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1. *Abbreviations used in this paper:* DIC, differential interference contrast; MPF, maturation promoting factor.

hours of development. The first eight rounds of mitosis occur in the interior of the embryo. At telophase of nuclear cycle nine the majority of the nuclei migate to the cortex, where they undergo a further four rounds of mitosis before cellularization occurs at interphase of cycle 14 (Zalokar and Erk, 1976; Foe and Alberts, 1983). We have previously shown that embryos laid by *Drosophila* females homozygous for the mutation *gnu*, undergo DNA synthesis in the absence of nuclear division, and yet centrosomes continue to divide (Freeman et al., 1986; Freeman and Glover, 1987).

Here we report that cytoplasmic cycles of centrosome division and cortical budding continue in *Drosophila* embryos in which DNA synthesis is inhibited with aphidicolin, although the cycle time is slowed. More importantly, nuclear cycles of chromatin condensation/decondensation and of lamin disassembly/assembly continue in such embryos, in contrast to the situation reported in aphidicolin-treated sea urchin embryos.

Materials and Methods

Injection of Embryos

Oregon R flies were kept in population cages at 24°C. Embryos were collected on grape juice agar plates supplemented with a small amount of live yeast suspension. The first two collections of the day were discarded and subsequent collections were made at 30-min intervals. The embryos were dechorionated by hand on a piece of Scotch double-sided sticky tape and were placed on another piece of Sellotape double-sided sticky tape on a No. 1 coverslip. (We found that if embryos were dechorionated with 60% domestic bleach and then left under halocarbon oil for a few hours they were more resistant to subsequent fixation.) The embryos were left to desiccate for 10 minutes at 18°C (the temperature of our injection room) before they were covered with Voltalef oil (type 10S; Atochem (UK) Ltd.). They were allowed to develop at 24°C until they reached the required developmental stage and were injected (in the middle of the embryo) with injection buffer (5 mM KCl, 0.1 mM sodium phosphate, pH 6.8) containing 100 µg/ml aphidicolin (diluted from a 1 mg/ml stock in DMSO). Control embryos were injected with injection buffer containing 10% DMSO alone. We estimate that the injection volume was ∼3–5% of the embryo volume (Okada et al., 1980), giving an internal concentration of aphidicolin of ∼5 µg/ml. After injection the embryos were allowed to develop for varying amounts of time at 24°C before fixation.

Fixation of Embryos

The coverslip holding the injected embryos was placed in a watch glass containing heptane. The heptane was gently pipetted over the embryos until they detached from the sticky tape. The embryos were then fixed in formaldehyde as described previously (Freeman et al., 1986). Because the fixation process usually failed to remove the vitelline membrane completely, all of the embryos were finally devitellinized by hand on Scotch double-sided sticky tape, partially covered in 0.1% Triton X-100 in buffer A (15 mM Tris-HCl, pH 7.4, 60 mM KCl, 15 mM NaCl, 1.5 mM spermine, 0.5 mM spermidine) to avoid dehydration. For staining with antibodies against tubulin, embryos were treated with taxol for 30 s before they were fixed as described previously (Freeman et al., 1986). At the concentration used (0.5 µM) taxol seems to stabilize only preexisting microtubule structures (Karr and Alberts, 1986; Freeman et al., 1986).

Antibody Staining of Fixed Material

Embryos were stained as described by Freeman et al. (1986), except that the staining was done in small watch glasses. After staining, the embryos were washed twice in buffer A for a total of 30 min, and then once in buffer A containing 1 µg/ml Hoechst 33258 (Riedal De Haen AG, Hannover, FRG) for 20 min. The embryos were mounted in 85% glycerol containing 2.5% n-propyl gallate. Centrosomes were stained with Rb188 (1:500), a rabbit anti-serum that recognizes a centrosome-associated antigen (Whitfield et al., 1988), followed by goat anti-rabbit IgG coupled to either fluoroscein

or rhodamine. Microtubules were stained with YL1/2 (Kilmartin et al., 1982), a rat monoclonal antibody that recognizes α tubulin followed by mouse anti-rat IgG coupled to fluorescein. Lamins were stained with T47, a mouse monoclonal antibody (kindly donated by Dr. H. Saumweber), followed by goat anti-mouse IgG coupled to rhodamine. All second antibodies were obtained from Jackson Immuno Research Laboratories Inc. (Avondale, PA) and used at dilutions of between 1:100 and 1:500, as appropriate. Fluorescence microscopy was carried out on a Nikon Microphot-FX microscope and pictures were taken on TP 135 film and developed in D-19 developer (both Eastman Kodak, Rochester NY).

Observation of Living Embryos

The adhesive of standard Sellotape was dissolved in heptane. The heptane was painted in a stripe on a coverslip and allowed to dry, leaving behind a transparent adhesive stripe onto which embryos were placed for injection. The embryos were injected as described above, and the coverslip was inverted on two coverslips stuck to a slide to allow oxygen exchange. The embryos were observed using Reichart-Jung Nomarski differential interference contrast (DIC) optics and photographed with TP 135 film which was developed in HC110 developer (both Eastman Kodak).

[³H]Thymidine Incorporation

Embryos were injected with injection buffer containing 1 µCi/µl [³H]thymidine with or without aphidicolin. They were allowed to develop for varying amounts of time and then removed from the sticky tape as described above. The embryos were then homogenized in 200 µl of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. 10 µl of the homogenate was placed on a GF/A filter (Whatman Inc., Clifton, NJ) and the rest was precipitated with 2 vol of 10% TCA. The acid precipitate was washed onto a GF/A filter, and the filter was washed three times with 96% ethanol. 3 ml of Aquasol (New England Nuclear, Boston, MA) was added to the filters, which were then counted in a scintillation counter.

Results

Aphidicolin Inhibits DNA Synthesis and Nuclear Replication

To examine the effects of aphidicolin on nuclear division in *Drosophila* embryos, it was necessary to inject the drug at defined stages of development. As the first nine nuclear divisions take place within the interior of the syncitium it is difficult to stage embryos during this period. At nuclear cycle nine, pole buds begin to appear at the posterior end of the embryo and pole cells are formed at the interphase of cycle 10. We therefore injected aphidicolin during two developmental periods: (a) after a 30-min egg collection, in which case all of the embryos should be at about nuclear cycle three or earlier, and (b) when pole cells are first seen at nuclear cycle 10. After injection, the embryos were left to develop for up to four hours. They were then fixed, stained with the DNA-binding fluorochrome Hoechst 33258, and examined by fluorescence microscopy. the number of nuclei seen per embryo was consistent with our expectation that nuclear division was inhibited at the time of aphidicolin injection (not shown). Embryos injected with injection buffer alone developed normally over the time course of the experiments and showed no unusual cytoskeletal or nuclear structures when stained by indirect immunofluorescence with antibodies against tubulin, lamin, or centrosomes (not shown).

Aphidicolin has been shown specifically to inhibit DNA-polymerase α–dependent DNA synthesis in many systems. We tested its effectiveness in *Drosophila* embryos by coinjecting [³H]thymidine and aphidicolin. In each of three experiments, aphidicolin inhibited [³H]thymidine incorporation by 88–92% relative to control embryos injected with
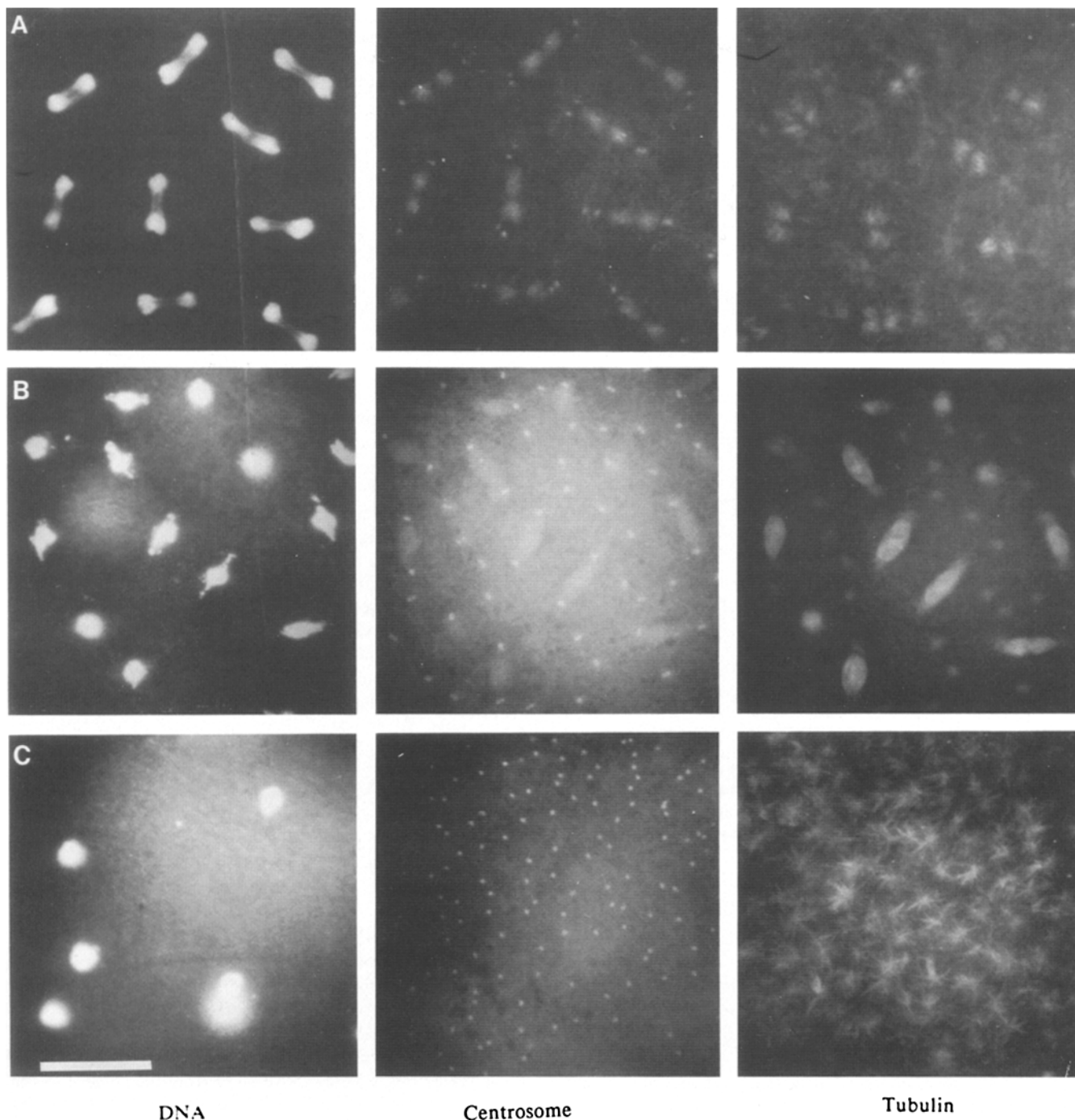
DNA   Centrosome   Tubulin

Figure 1. The distribution of DNA, centrosomes, and microtubules in aphidicolin-injected embryos at various times after injection. Embryos were injected with aphidicolin at about the time of pole cell formation (nuclear cycle 9, see text). They were allowed to develop for various lengths of time before fixation and staining with Rb188 (anti-centrosome) and Yl1/2 (anti-tubulin) followed by rhodamine-coupled goat anti–rabbit and fluorescein-coupled mouse anti–rat antibodies. The embryos were finally incubated with Hoechst 33258 (see Materials and Methods). The figure shows fields from typical embryos fixed 10 (*A*), 45 (*B*), and 90 (*C*) min after injection of the drug. Bar, 20 μm.

[³H]thymidine alone (see Materials and Methods). This is comparable to the inhibition achieved in other systems (e.g., Ikegami et al., 1979; Ikegami et al., 1978; Nagano et al., 1981). The residual 10% incorporation could be due either to DNA repair or to DNA-polymerase β– and γ–dependent DNA synthesis.

Centrosomes Continue to Divide in Aphidicolin-injected Embryos

In all subsequent experiments, aphidicolin was injected at about nuclear cycle nine, the time of pole cell formation. After injection, embryos were allowed to develop for varying lengths of time before they were fixed and stained by indirect

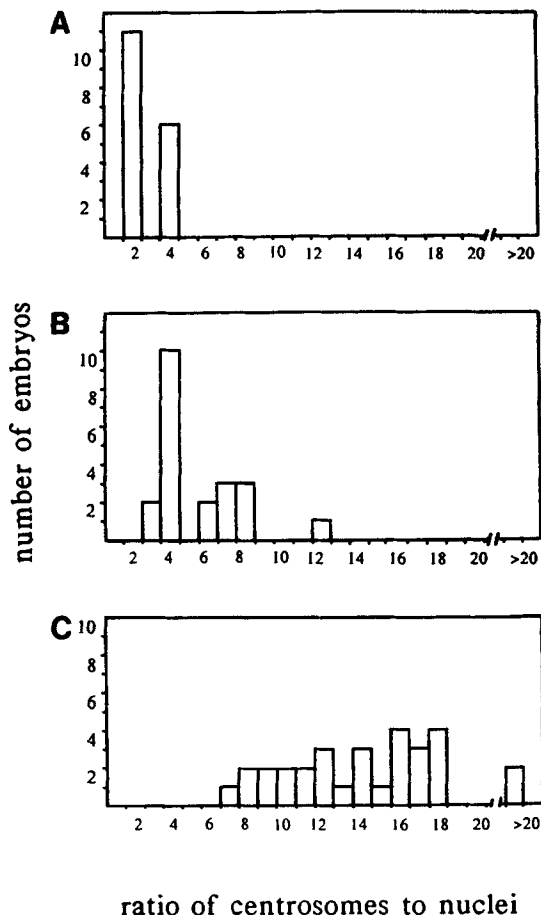ratio of centrosomes to nuclei

Figure 2. The ratio of centrosomes to nuclei after the injection of aphidicolin into *Drosophila* embryos. Embryos were injected with aphidicolin and allowed to develop for varying lengths of time before fixation and staining as described in the legend to Fig. 1. The ratios were obtained by counting the number of nuclei and centrosomes in photographs of embryos that had been allowed to develop for 10 (*A*), 45 (*B*), and 90 (*C*) min after injection of aphidicolin.

immunofluorescence. Fig. 1 *A* shows a field of nuclei in an embryo that was allowed to develop for ∼10 min after injection of the drug. The embryo is at the late telophase stage of mitosis as judged by the decondensing chromatin and the presence of the midbody, a structure characteristic of the telophase spindle. The centrosomes appear to have just divided, as often occurs at late telophase in *Drosophila* embryos. Normally, the chromosomes would be well-separated by the telophase stage of mitosis, but in this case there is still a chromatin bridge linking the two daughter nuclei. This presumably reflects the inability of the spindle to separate DNA that has not been fully replicated. The effect of aphidicolin on nuclear division presumably depends on the proportion of the genome that has replicated at the time the drug was injected: if the genome was almost fully replicated, mitosis might proceed quite normally to produce two daughter nuclei each with two centrosomes; if the genome had just begun to replicate, mitosis might fail to separate the daughter nuclei, resulting in a single nucleus with four centrosomes. We observed that all of the embryos fixed 10 min after injection had a ratio of centrosomes to nuclei of two or four (Fig.

2) and all of the centrosomes were closely associated with their corresponding nucleus.

Fields from two embryos fixed 45 and 90 min after injection with aphidicolin are shown in Fig. 1, *B* and *C*, respectively. In these particular fields the ratio of centrosomes to nuclei is ∼7:1 (Fig. 1 *B*) and 24:1 (Fig. 1 *C*), demonstrating that centrosomes continued to divide after the injection of aphidicolin. The extra centrosomes did not remain associated with the nuclei. The ratios of centrosomes to nuclei in all of the embryos examined in these experiments are shown in Fig. 2. While the ratios tended to increase with time, they did not increase in a strict geometric progression as might be expected if all of the centrosomes divided in a coordinated fashion. We could not be certain however, that we were counting all of the centrosomes in these experiments; although the majority were at the surface of the embryo, additional centrosomes were observed at different planes of focus throughout the embryo (data not shown). It is likely that centrosome replication did occur in a coordinated fashion in aphidicolin-treated embryos since we observed many examples of embryos in which all the centrosomes appeared to be in the process of dividing synchronously. This can be seen in Fig. 1 *B* and Fig. 6 *B*, where almost all the centrosomes are closely paired.

As can be seen in Fig. 1, the centrosomes in aphidicolin-treated embryos were functional with respect to microtubule nucleation. The microtubular structures observed in treated embryos were similar to those seen in untreated embryos. Fig. 1 *B*, for example, shows condensed chromatin organizing bipolar spindles, each with one or two centrosomes at either pole. In addition, there are asters of microtubules nucleated by extra centrosomes that are not associated with chromatin. In Fig. 1 *C*, the majority of the centrosomes are at the surface of the embryo and are nucleating asters. The nuclei are below the surface and do not interact with the asters on the surface, although some of these nuclei have centrosomes associated with them (not shown).

Cycles of Cytoplasmic Budding Also Continue

Previous studies on *Drosophila* embryos using DIC microscopy have shown that between nuclear cycle 10 to 14 the interphase portion of the cell cycle is associated with a cortical budding at the embryo surface. An interphase nucleus is associated with each bud (Foe and Alberts, 1983). As the nuclei enter mitosis they move away from the surface of the embryo and the cortical budding recedes. Fig. 3 shows the budding cycles in an untreated embryo studied by DIC microscopy. Alternate frames show successive minimal and maximal stages of budding. As illustrated, the number of buds increased progressively with the number of nuclei (frames at 4, 11, 21, 31, and 54 min) and the buds did not completely disappear at mitosis (frames at 8, 14, 24, and 37 min).

Embryos injected with aphidicolin at about nuclear cycle 10 also showed cycles of cortical budding, but the buds were much less pronounced than those in uninjected controls. Fig. 4 shows one such embryo: budding was maximal at 12, 22, 56, and 77 min after injection, and the number of buds in any given area of the embryo appeared to double with each cycle. Cycles of cortical budding were observed in all 13 embryos followed in this way; the timing and length of each cy-
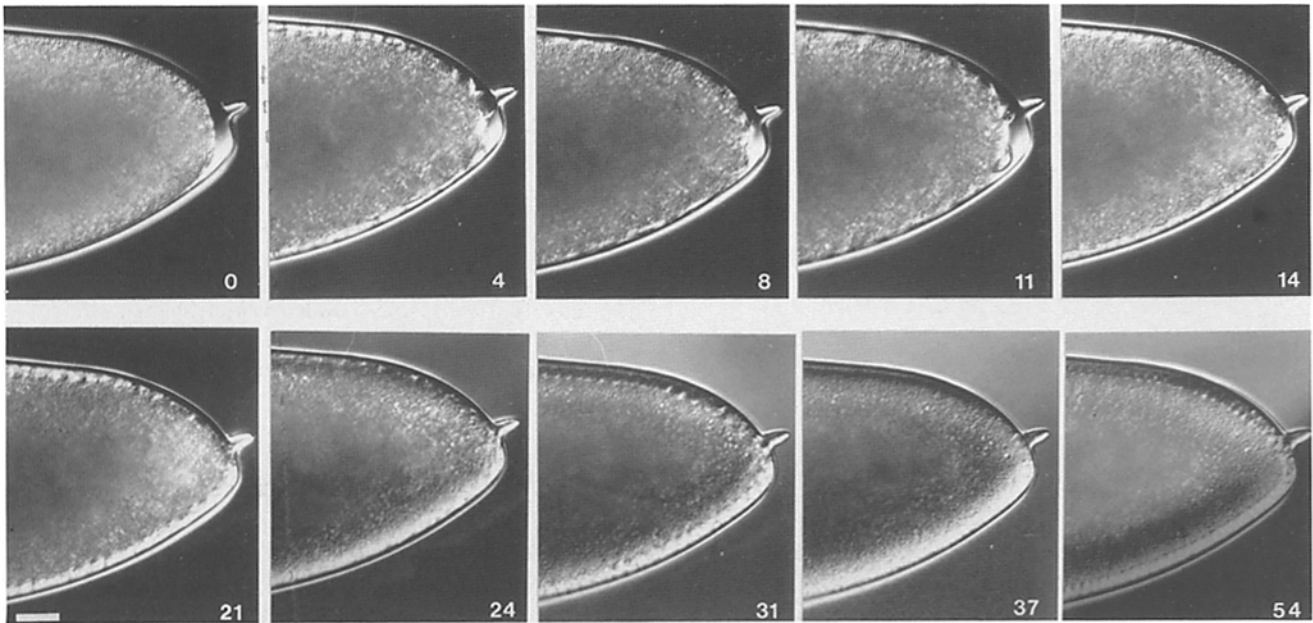
Figure 3. Cortical budding cycles in an untreated embryo. The developing embryo was observed with DIC optics as described in Materials and Methods. Zero time represents the point at which cortical budding was first observed. This is one minute into interphase of cycle 10 (Foe and Alberts, 1983). Budding is maximal at 4, 11, 21, and 31 min (as shown), and at 41 min (not shown). The nuclei in the last frame at 54 min are in the process of cellularization. The timing of successive cycles in eight embryos injected with buffer is shown in Table I. Bar, 40 μm.

cle, however, was more variable than in untreated controls (see Table I).

All of the embryos followed with DIC optics were fixed directly after observation, immunostained to reveal centro-somes, and counterstained with Hoechst 33258. This confirmed that nuclear division had not occurred whereas centrosome division had continued. Fig. 5 shows the results of staining the embryo illustrated in Fig. 4. The ratio of centro-
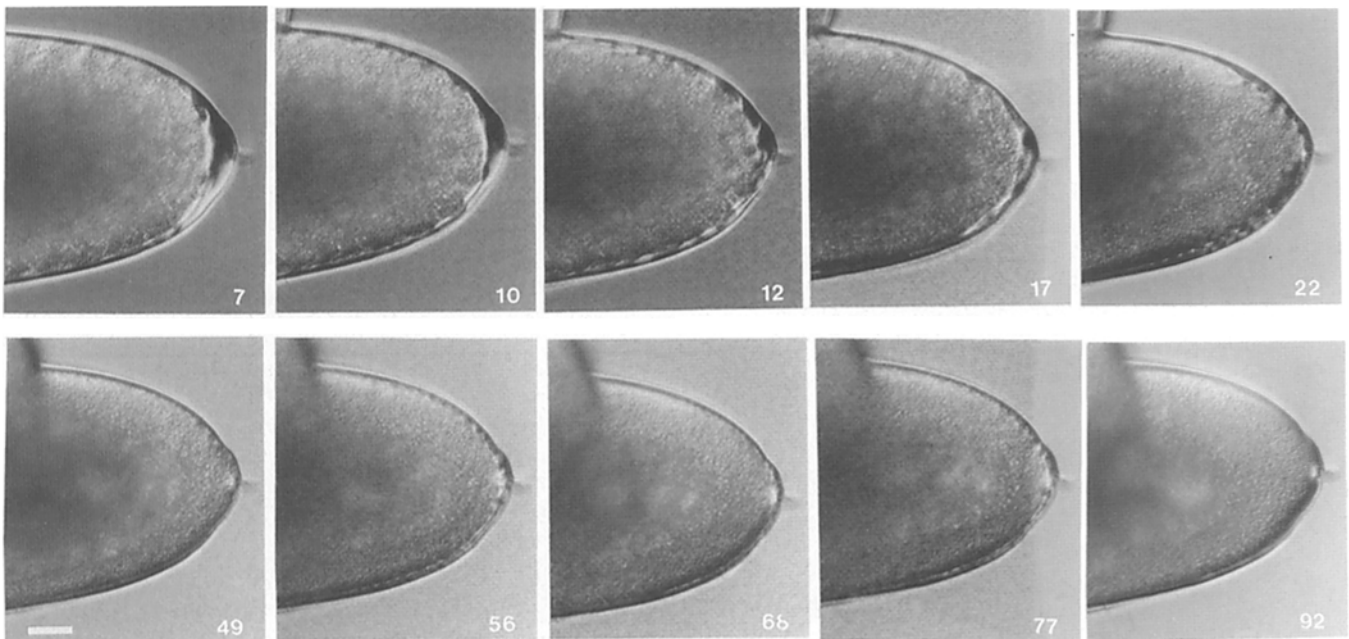

Figure 4. Cortical budding cycles in an embryo injected with aphidicolin. The embryo was injected with aphidicolin and observed with DIC optics as described in Materials and Methods. Zero time is the point at which this embryo was injected with aphidicolin. As in Fig. 3, this is one minute into interphase of cycle 10. Because of the time needed to prepare the embryo for observation, the first picture was taken after 7 min when the cortical budding associated with interphase of nuclear cycle 10 was starting to fade. Budding was maximal at 12, 22, 56, and 77 min. The timing of successive cycles in embryos that were observed in this way is shown in Table I. Bar, 40 μm.

Table I. Budding Cycle Times of Aphidicolin-injected Embryos

Nuclear cycle	Length of time (min) when buds apparent				Length of time (min) when buds not apparent			
	aphidicolin*		control‡		aphidicolin*		control‡	
	mean	range	mean	range	mean	range	mean	range
10	6.2	(6–7)	5.7	(5–7)	3.4	(3–4)	3.8	(3–4)
11	7.2	(6–15)	6.8	(5–7)	7.2	(3–21)	3.3	(3–5)
12	8.0	(4–10)	8.2	(6–9)	16.1	(8–30)	4.1	(3–5)
13	9.0	(5–15)	14.0	(13–15)	12.6	(6–15)	5.8	(5–7)

* 10 embryos were injected with aphidicolin and timed accurately through cycle 10; 13 were observed for cycle 11 and 12; and 9 for cycle 13.
‡ Eight embryos were injected with buffer and observed through cycles 10–14.

DNA

Centrosome
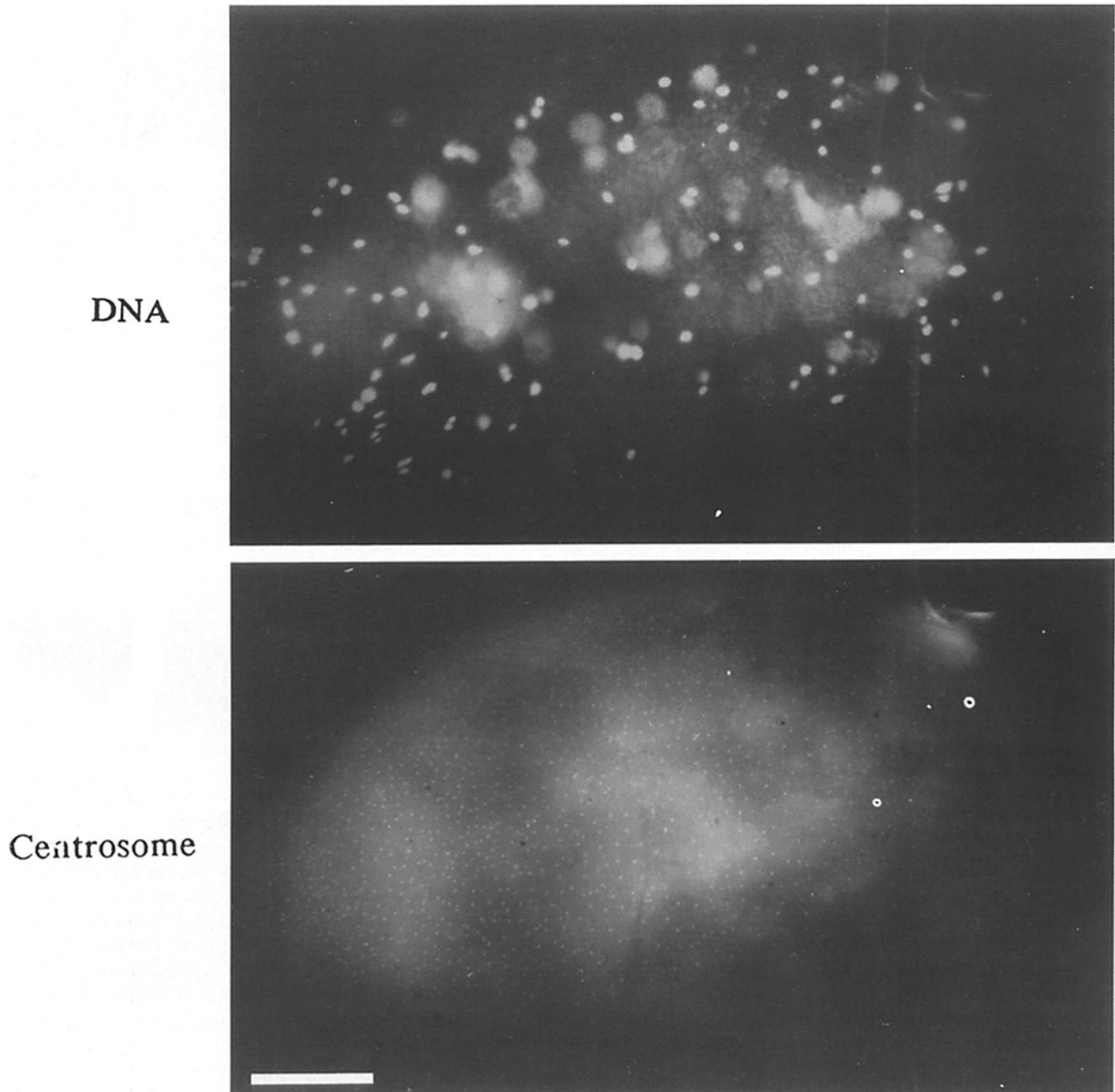
Figure 5. Fixed preparation of the embryo illustrated in Fig. 4. After the observation of the cortical budding cycles, the embryo shown in Fig. 4 was fixed and stained sequentially with Rb188, rhodamine-conjugated goat anti–rabbit, and Hoechst 33258. Bar, 50 μm.
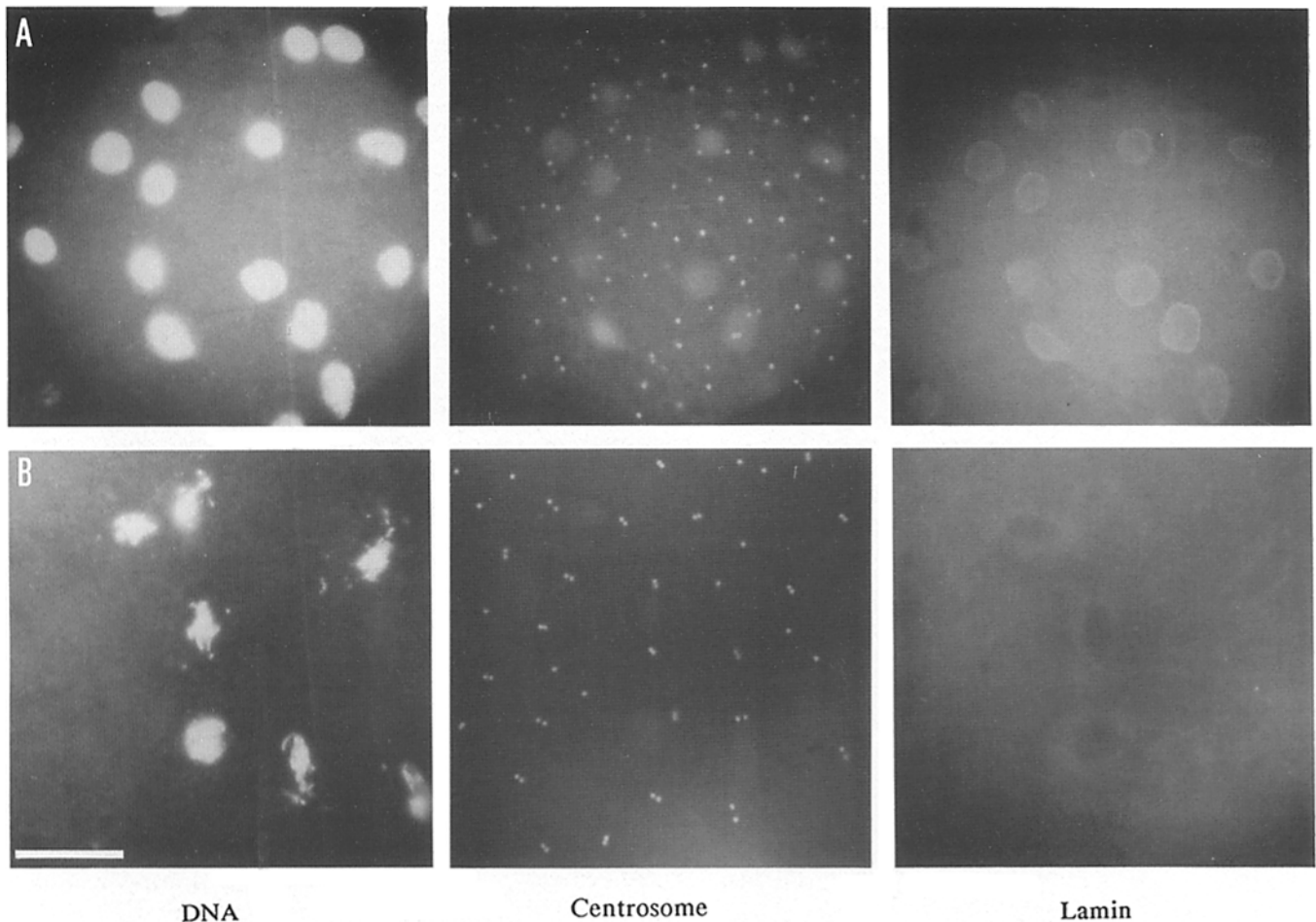
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somes to nuclei is approximately 18:1, suggesting that the centrosomes have gone through three to four rounds of division in the absence of nuclear division. We had observed by DIC microscopy that this embryo went through four rounds of cortical budding after aphidicolin injection (Fig. 4). This approximate correlation between the number of budding cycles and the number of centrosome divisions in the absence of nuclear replication was observed in all embryos that were followed in this way.

Nuclear Cycles of Chromatin Condensation and Lamin Disassembly Continue in Aphidicolin-treated Embryos

Although the cortical budding cycle continued in aphidicolin-treated embryos, the nuclei appeared to fall away slightly from the surface of the embryo and so nuclei were rarely visible within the cortical buds. Presumably this is also the reason why the budding was less pronounced than in untreated embryos. Because the nuclei were not visible at the surface of the treated embryos, it was not possible to see by DIC microscopy whether the nuclei were cycling in unison

with the cytoplasmic budding cycles, as would be the case in an untreated embryo. We were able to obtain evidence, however, that this was indeed the case.

Different embryos injected with aphidicolin and then fixed and stained with Hoechst after one or two hours had nuclei in different states of condensation, suggesting that the chromatin might be proceeding through cycles of condensation/decondensation even though DNA synthesis was inhibited. Fig. 6 for example, shows fields of nuclei in two embryos fixed 90 min after injection and stained by indirect immunofluorescence using anti-centrosome and anti-lamin antibodies. In both cases there are extra centrosomes around the nuclei. In Fig. 6 *A*, however, the nuclei have the appearance of normal interphase nuclei with decondensed chromatin, whereas in Fig. 6 *B* the chromatin is condensed and some chromatin structure is visible. The abnormal tangled appearance of the chromatin in Fig. 6 *B* might reflect the inability of the spindle to separate unreplicated chromatin. The staining with the anti-lamin antibody indicated that the degree of chromatin condensation correlated with the state of the nuclear lamina. The lamins, which are major components of the nuclear envelope, are phosphorylated during nuclear
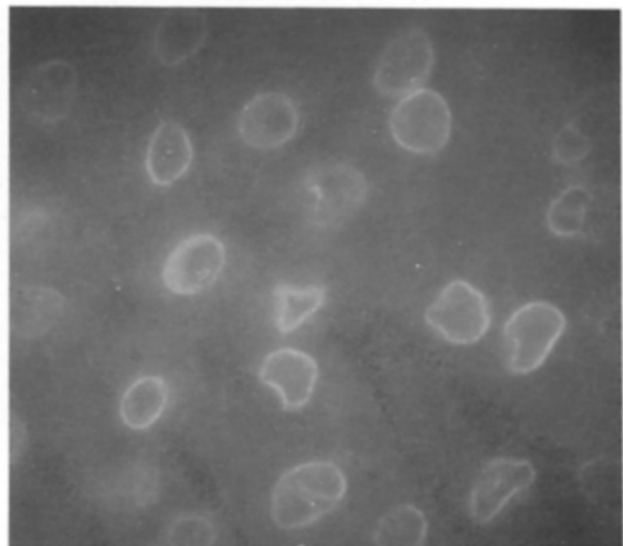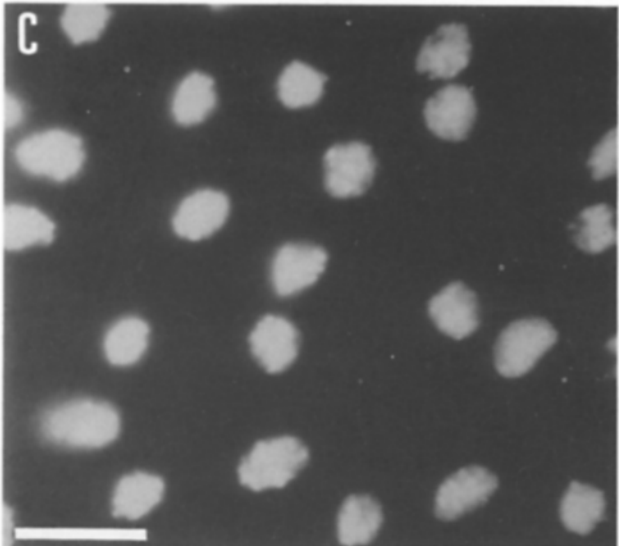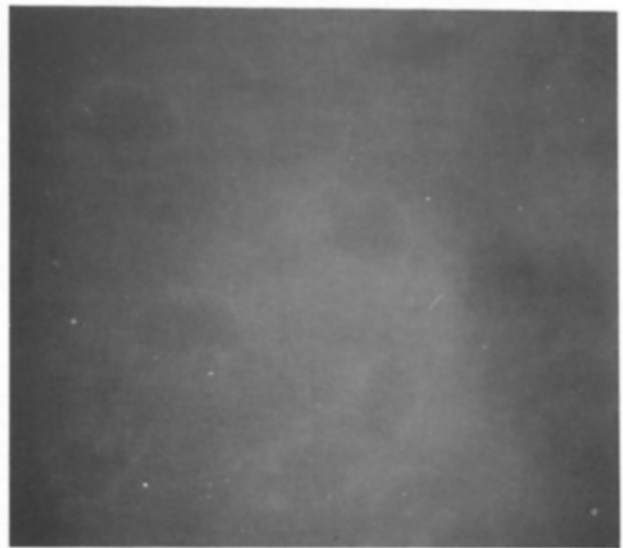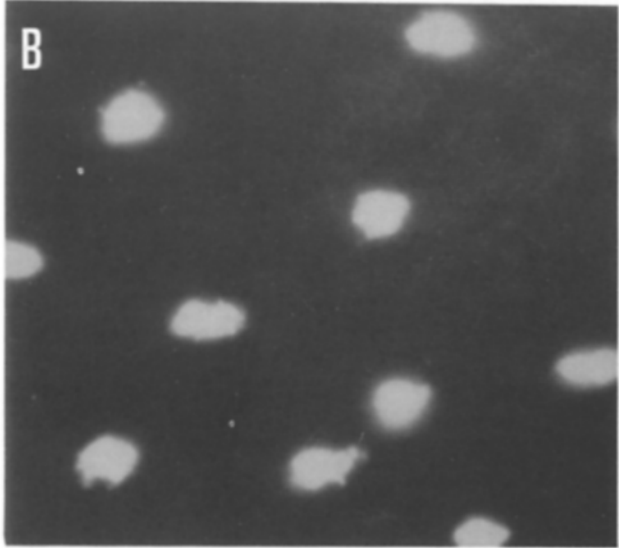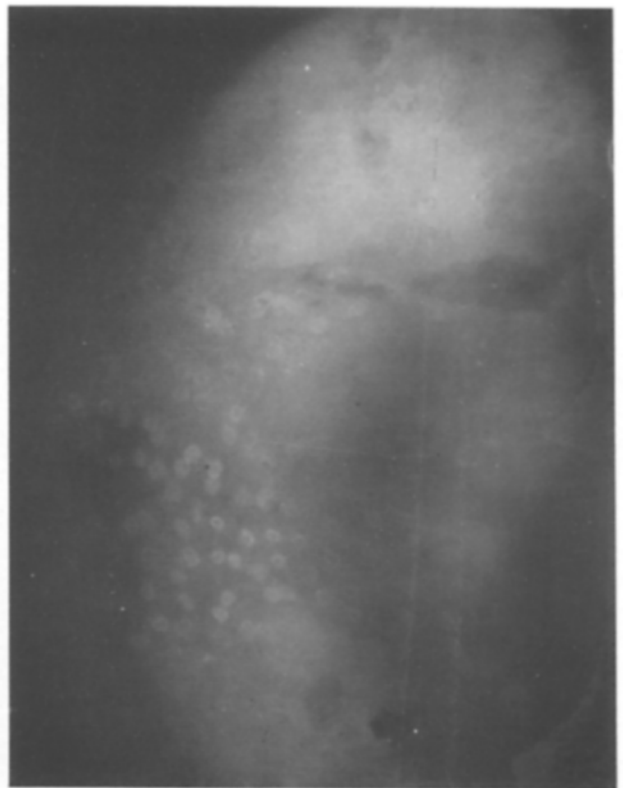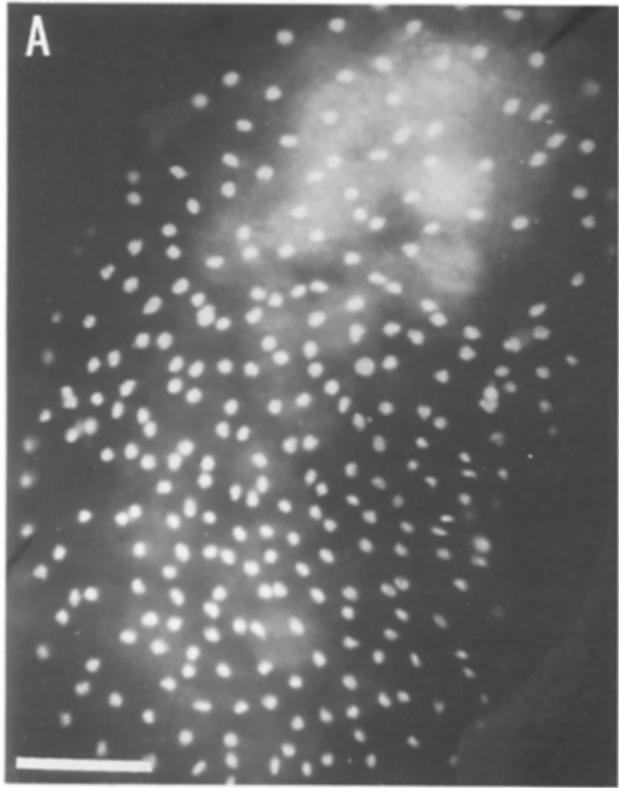
DNA   Lamin

envelope breakdown as the embryo enters M phase (for review see Gerace, 1986). This change in nuclear envelope structure can be followed with anti-lamin antibodies, which stain the nuclear envelope of interphase nuclei, but give a weaker, more diffuse staining in the rest of the mitotic cycle (Fuchs et al., 1983). Both staining patterns were seen in aphidicolin-treated embryos: the decondensed interphase-like nuclei were surrounded by bright annular lamin staining, whereas the staining around condensed chromatin was weaker and more diffuse (Fig. 6).

A number of aphidicolin-treated embryos were observed in those clearly defined areas containing nuclei in one or other of these two states, as might be expected if mitotic waves were still passing through the embryos. Normally the nuclei in a *Drosophila* embryo enter mitosis in waves that usually originate from both poles (Foe and Alberts, 1983). These waves are also apparent in the cortical budding cycles of the embryo. In the aphidicolin-treated embryos the budding cycles also usually occurred in waves, although they often took longer to traverse the embryo (3–10 min) compared with the waves in control embryos (0.5–2.0 min). To test whether we could use the cortical budding cycle to predict the state of the chromatin, we allowed an aphidicolin-treated embryo to proceed through two cycles of budding and then fixed it just as the cortical buds were starting to fade from both poles. As predicted, the nuclei at both poles had lost their surrounding lamins while the nuclei in the middle had not (Fig. 7 *A*). Fig. 7 *B* shows two fields from this embryo that illustrate this point more clearly.

In all of the embryos that were fixed when buds had faded (seven in total) most of the chromatin was condensed and had a diffuse anti-lamin antibody staining. In all of the embryos fixed when buds were present (six in total), most of the chromatin was decondensed and was surrounded by a nuclear membrane, as judged by the anti-lamin staining. The finding that we could use the cortical budding cycle to predict the state of the chromatin strongly suggests that cycles of chromatin condensation and lamin disassembly continued in synchrony with the cortical budding cycles in aphidicolin-treated embryos.

Mitotic Cycles Are Slowed in Aphidicolin-injected Embryos

The cycle times of the embryos followed in real time are shown in Table I. Although the time of the first division cycle after injection was relatively unaffected, all of the subsequent cycle times in the aphidicolin-injected embryos were more variable and noticeably longer than in uninjected embryos. In most of the treated embryos the M phase of the cell cycle, the period where the buds were not apparent was the most dramatically affected. The length of the S phase equivalent, where buds were present, was variable but was often comparable to or shorter than the S phase of untreated embryos.

These real time observations show that the cell cycle was slowed in aphidicolin-treated embryos and suggest that the major delay occurred in M phase.

These findings are supported by our observations on fixed embryos. 90 min after injection of aphidicolin into a batch of embryos, the ratio of centrosomes to nuclei was variable, some embryos had ratios as low as 7:1, while others had ratios of >20:1 (Fig. 2). This implies that in some embryos the centrosomes divided only once or twice during the 90 min, while in others they have divided at least three or four times. In untreated embryos, on the other hand, the four rounds of mitosis that precede cellularization always take place in under 1 h. Our observations on fixed embryos suggest that aphidicolin-treatment resulted in a delay to the cycle mainly in M phase. Of the aphidicolin-treated embryos, ∼55–65% had the majority of their chromatin in a condensed state (not shown). This is in contrast to noninjected embryos in which only 30–40% have condensed chromatin.

Discussion

We have previously reported that embryos laid by *Drosophila* females homozygous for the mutation *gnu* develop a small number of giant nuclei (Freeman et al., 1986; Freeman and Glover, 1987). The centrosomes of these embryos continue to divide many times, however, demonstrating that centrosome replication and nuclear division can be uncoupled. By injecting aphidicolin into wild-type *Drosophila* embryos, we now demonstrate that centrosomes can proceed through multiple rounds of division in the absence of DNA replication. Similar experiments examining centrosome replication in aphidicolin-treated sea urchin embryos have yielded conflicting results (Nishioka et al., 1984; Brachet and De Petrocellis, 1981; Sluder and Lewis, 1987). Our findings are consistent with the demonstrations that centrosome division continues when DNA replication is inhibited in both sea urchin (Sluder and Lewis, 1987) and starfish embryos (Nagano et al., 1981). It seems, however, that protein synthesis is required for the centrosome cycle, since an injection of cycloheximide into *Drosophila* embryos blocks both nuclear and centrosome replication (Raff, J. W., unpublished observations). This is perhaps to be expected since studies on *Xenopus* have shown that protein synthesis is required for maturation and cleavage, and for the cyclical appearance of MPF activity (Gerhart et al., 1984).

Cortical budding cycles also continue in aphidicolin-injected *Drosophila* embryos. As in untreated embryos, the cortical budding occurs in waves, usually spreading from both poles. The number of buds at the cortex of the treated embryos roughly doubles with each new budding cycle, even though the number of nuclei remain constant. This suggests that some cytoplasmic component, not associated with the nucleus, is doubling every cell cycle, and this component is

Figure 7. An aphidicolin-injected embryo fixed as the cortical budding receded from both poles. The embryo was injected 4 min into interphase of cycle 10, and was observed with DIC microscopy through the cortical budding cycles equivalent to nuclear cycles 10 and 11. It was fixed as the buds associated with interphase of cycle 12 started to recede from the poles of the embryo. (*A*) A view of the whole embryo in which only the nuclei in the middle region are surrounded by a bright annular lamin staining. (*B*) A high power view of the anterior end of the embryo showing condensing chromatin and diffuse lamin staining. (*C*) A high power view of the middle of the embryo showing decondensed chromatin surrounded by a bright lamin staining. Bars: (*A*) 50 µm; (*B* and *C*) 10 µm.

capable of organizing the cortical buds. An obvious candidate for this component is the centrosome, and we are currently investigating the relationship between centrosomes and the cytoskeletal network that forms the cortical buds (Warn et al., 1984, 1987; Karr and Alberts, 1986). In aphidicolin-injected embryos that are fixed when cortical buds are apparent at the surface, the majority of nuclei are in an interphase-like state with decondensed chromatin surrounded by a nuclear envelope, as judged by the bright annular staining with an anti-lamin antibody. In treated embryos that are fixed when the cortical budding has receded, the majority of the chromatin is condensed and not enveloped by lamins. Treated embryos that are fixed during the progression of the budding-waves show nuclear structures consistent with the spreading of a mitotic state through the embryo. It seems, therefore, that in both treated and untreated embryos, the disappearance of the cortical buds correlates with the transition of nuclei into the M phase of the cell cycle. This suggests that the signals dictating the condensation state of the chromatin and the phosphorylation state of the lamins continue to cycle in aphidicolin-treated embryos, and that nuclei in which DNA replication has been inhibited are able to respond to these signals.

It has recently been demonstrated that cycles of MPF activity can occur in activated *Xenopus* oocytes devoid of any nuclear components (Dabauvalle et al., 1988), suggesting that the signals that drive the nuclear cycle are produced in the cytoplasm and oscillate in the absence of a nucleus. Our findings suggest that in *Drosophila* embryos, such signals are produced in the presence of unreplicated nuclei, and that these nuclei are capable of responding to them. The Drosophila embryo appears to differ in this respect from the sea urchin embryo. In the aphidicolin-treated sea urchin embryos, nuclear envelope breakdown is variable from one embryo to another and in some embryos it never occurs, even though the centrosomes proceed through multiple rounds of division (Sluder and Lewis, 1987). In embryos where the nuclear envelopes do break down, the chromatin can be seen as an amorphous mass of fibers; it does not condense properly, and nuclear envelopes do not reform. This result suggests that, in sea urchins, nuclei in which DNA synthesis has been inhibited may be unable to respond to oscillating cytoplasmic signals. Alternatively, a subset of cytoplasmic signals that drive the nuclear cycle might not be produced in aphidicolin-treated sea urchin embryos while other aspects of the cycle continue. While it is not clear why *Drosophila* and sea urchin embryos should differ in this respect, it is possible that the cellular organization of the sea urchin embryo imposes an additional set of constraints upon the mitotic cycle that are not found in the syncytial *Drosophila* embryo.

In common with aphidicolin-treated sea urchin, *Xenopus*, and starfish embryos, aphidicolin-treated *Drosophila* embryos show a considerable delay in the cell cycle. The reason for the delay is not known. Although aphidicolin is a specific inhibitor of DNA polymerase α (Ikegami et al., 1978), it could have nonspecific effects that slow the cell cycle. Another possibility is that the cell cycle oscillator might be coupled to DNA replication, but this coupling may be overridden after a certain delay. Thus, the inhibition of DNA replication would only cause a delay in the cell cycle rather than a complete block. In its simplest form, this model would predict that the cell cycle would be delayed in S phase while

the oscillator waited for some DNA synthesis–dependent signal. We find, however, that the delay in the cell cycle occurs mainly in M phase. Perhaps the most likely explanation is that the disorganized state of the aphidicolin-treated embryo in some way delays the cell cycle. There is good evidence that in sea urchin embryos the spatial organization of tubulin has a role in the timing of mitotic events. Under conditions where tubulin polymerization is inhibited (Sluder, 1979), or the mitotic spindle is rearranged (Sluder and Begg, 1983), the embryo spends much longer in M phase while the S phase of the cell cycle is unaffected. In the aphidicolin-injected *Drosophila* embryo, the presence of extra centrosomes could affect tubulin kinetics. This, together with the presence of unreplicated nuclei that cannot be separated by the spindle, could cause a delay in M phase.

It is particularly striking that rounds of DNA replication are not required for cycles of chromatin condensation/decondensation and nuclear envelope breakdown/reformation. This is in contrast to results obtained in yeasts and cells in culture where blocking DNA synthesis effectively blocks the cell cycle. In the early *Drosophila* embryo there seems, therefore, to be no absolute requirement for the correct completion of S phase for both the nuclear and cytoplasmic events of M phase to take place. This is not to say that some critical aspect of S phase is not completed, and if indeed aphidicolin has its only effect on DNA polymerase α, this may well be the case. Nevertheless, DNA synthesis is dramatically inhibited and chromosome replication, a major objective of the cell cycle, does not occur.

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