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Possible Involvement of a Cell Cycle Control System Dependent on Nuclear Activities in Establishment of the Cell Division Interval in Early *Xenopus* Embryos

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ABSTRACT—In early *Xenopus* embryos, continuous exposure of embryos to aphidicolin and inactivation of the nucleus by ultraviolet-irradiation induce prolongation of the cell division interval. The extent of prolongation of the cell division interval appears to depend on how heavily DNA replication is suppressed by the treatments. Embryos showing significantly prolonged cell division intervals tend to fail normal cell divisions, often forming abortive furrows. There appears to be a critical point for the extent of prolongation of the cell division interval at 30% that divides the success and failure of normal cell division. This percentage, 30%, coincides with that for the prolongation of the interval of oscillatory activities seen in enucleated eggs. The presence of an intact nucleus that can undergo DNA replication rescues normal cell divisions with normal intervals and a normal furrow. Histologically, the nucleus in embryos showing more than 30% prolongation of cell division intervals appears to fail DNA replication and remain unduplicated with a round morphology like the interphase nucleus. A number of unduplicated asters lacking chromosomes are found in these embryos. We conclude from these results that early *Xenopus* embryos, like late embryos, have a cell cycle control system that is affected by DNA replication and is involved in establishing the cell division interval.

INTRODUCTION

Early *Xenopus* embryos, like other early animal embryos, undergo cycles of cell divisions at rather short, species-specific, constant intervals. Studies in early 80s (Hara *et al*., 1980; Sakai and Kubota, 1981; Shinagawa, 1983) revealed that many of the oscillatory activities associated with cell divisions, such as cyclic changes in egg's rigidity and cyclic generation of the surface contraction waves (SCWs), are seen without cell division itself in enucleated *Xenopus* eggs. From this finding they concluded that the unique intervals of cell divisions in early *Xenopus* embryos must be controlled fundamentally by oscillatory activities present in the cytoplasm. Kirschner and his co-workers (Kirschner *et al*., 1981; Murray and Kirschner, 1989; Murray *et al*., 1989; Nurse, 1990), from their own studies, advanced this conclusion and proposed that early *Xenopus* embryos have a master oscillator in the cytoplasm, which consists of the M-phase-promoting factor (MPF), and drives all activities essential to the occurrence and progression of cell division cycle in early embryos.

Their proposal is so simple and fascinating that it has been broadly accepted. However, Shinagawa (1983, 1985, 1986, 1992) has pointed out a possibility that the process of establishment of cell division cycle in early *Xenopus* embryos is not so simple. In fact, Shinagawa (1983, 1985) has showed that the duration of the cycle of oscillatory activities in enucleated eggs is not exactly the same as, but about 30%, longer than, that of the corresponding cycle of cell divisions in nucleated eggs. This finding implies that systems or activities affected by nuclear activities should be involved in the process of establishing the cell division interval, shortening the intervals of the fundamental oscillatory activities. Shinagawa (1983, 1985) further has demonstrated that *Xenopus* eggs prevented from forming the mitotic apparatus with colchicine show 10-20% longer intervals of cytoplasmic oscillatory activities than that of cell division cycle in normal eggs. Shinagawa (1983, 1985) has concluded from this result that formation of the mitotic apparatus is involved in shortening the intervals of fundamental cytoplasmic oscillatory activities to establish normal intervals of cell divisions. On the basis of a preliminary experiment on the periodicity of oscillatory activities in eggs exposed to aphidicolin, Shinagawa (1992) has suggested possible involvement of a system affected by the state of DNA replication in the process of shortening the longer intervals of cytoplasmic oscillatory activities.

In this relation, we have paid attention to Newport and co-workers' studies (Dasso and Newport, 1990; Smythe and Newport, 1992), which demonstrated that cells of late *Xenopus* embryos and cell free extracts mimicking the cytochemical state of late embryos have a feedback cell cycle control

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system that monitors unreplicated DNA. The system reportedly blocks cell's entry into the mitotic phase until the nucleus in the cell or cell free extract completes DNA replication successfully. According to them, the activation of MPF via the modulation of a tyrosine kinase, which normally leads to entry into the mitotic phase, is suppressed by a S-phase/M-phase transition checkpoint in the late *Xenopus* embryonic cells and cell free extracts as long as the nucleus has not yet completed DNA replication. A large number of studies (reviewed by Hartwell and Weinert, 1989; Murray, 1992) so far have provided evidence for the presence of a feedback cell cycle control system that is affected by the replication of DNA in a wide variety of somatic eukaryote cells.

By contrast, there is little evidence that suggests the presence of such a cell cycle control system in early animal cells. Rather, it has been thought that early animal cells, including early *Xenopus* embryonic cells, have no cell cycle control system affected by DNA replication because Newport and Dasso (1989) demonstrated that early *Xenopus* embryos showed normal cell division at normal intervals even though they were continuously exposed to a DNA replication inhibitor, aphidicolin, from shortly after fertilization. Only one exception is the study by Clute and Masui (1997). They reported that blastomeres dissociated from early *Xenopus* embryos showed cell divisions at slightly longer intervals than those of control blastomeres when exposed continuously to aphidicolin from 2-cell stage. This finding suggests that early *Xenopus* embryos may have a cell cycle control system affected by DNA replication. In the present study, we confirm the possible presence of such a system and its involvement in the process of establishment of the cell division interval in early *Xenopus* embryos.

MATERIALS AND METHODS

Preparation and handling of eggs

Adult females of *Xenopus laevis* were injected with 400 IU of human chorionic gonadotropin and kept in a bath at 22-23°C overnight to induce ovulation. Eggs were stripped into a plastic Petri dish and fertilized with 1 ml of 100% modified de Boer's saline (100% MDB: 110 mM NaCl, 1.3 mM KCl, 0.44 mM $CaCl_2$ and 3.0 mM Hepes at pH 7.3) containing finely macerated testis. After activation contraction occurred, the jelly coat was removed by pipetting eggs in 1% sodium thioglycolate in 50% modified Steinberg's saline (100% MSS: 58.2 mM NaCl, 0.67 mM KCl, 0.34 mM $Ca(NO_3)_2$, 0.83 mM $MgSO_4$ and 3.0 mM Hepes at pH 7.3) at pH 8.5. Fertilized eggs were incubated at 20-21°C.

Exposure to aphidicolin

Aphidicolin (a DNA polymerase α inhibitor; Wako Pure Chemical Product Inc., Osaka, Japan) was dissolved in 100% dimethylsulfoxide (DMSO) at a concentration of 20 mg/ml and kept as a stock solution at 4°C. Aphidicolin solution for exposure of eggs was prepared by diluting a stock solution in 50% MSS to a final concentration of 15-90 μg/ml. The final concentration of DMSO in the solution is expected to be 0.075-0.45%, which concentrations of DMSO have no or very little effect on cell cycle events in early *Xenopus* embryonic cells. Fertilized eggs were continuously exposed to the aphidicolin solution (15-90 μg/ml) from within 10 min of fertilization.

Ultraviolet-irradiation of sperm nuclei

Sperm was irradiated with ultraviolet (u.v.) light following procedures of Pogany (1971, 1973) and Briedis and Elinson (1982). One piece of testis was finely macerated and suspended in 5 ml of 100% MDB. The suspension was spread at less than 2 mm deep in a glass Petri dish coated with egg albumin/glycerol. The dish was placed under a u.v. generator (UVGL-25; UVP Inc., San Gabriel, California, USA) 5 cm away from the generator and irradiated with 254-nm light for 50 sec. During irradiation the suspension was continuously agitated so that all sperms should be equally irradiated. By this irradiation, sperm is expected to receive an energy of 1,600 erg/mm^2, which is reportedly sufficient for complete inactivation of the nucleus (Pogany, 1971, 1973, 1976). After irradiation, the sperm suspension was transferred to a centrifugation tube, and sperm was precipitated by centrifugation at 1,000 × g for 6 min. Collected sperm was resuspended to 0.5 ml of 100% MDB and used for fertilization.

Removal of the female nucleus

The female nucleus were removed following the procedure of Shinagawa (1992). After dejellying the fertilization membrane was removed manually with watchmaker's forceps. Denuded eggs was handled on a 3% agar sheet under 50% MSS. The female nucleus, which resides immediately beneath the first polar body, was withdrawn along with a small amount of cytoplasm with a micropipette within 15 min of fertilization. By this procedure, the nuclei are successfully removed at a rate of more than 95%.

Preparation of eggs containing only the u.v.-irradiated nucleus

Eggs were fertilized with sperm irradiated with ultraviolet light. After activation contraction occurred, the female nucleus was withdrawn with a micropipette. When more than one nucleus was found by histological examination with a DNA staining dye after experiments, data were discarded because it indicates the failure of either the process of inactivation of the sperm nucleus with u.v.-irradiation or the process of removal of the female nucleus.

Examination of DNA replication

DNA replication was examined by the following two procedures: (1) Embryos were slightly fixed in 0.1 ml of 50% MSS containing 4% formaldehyde at 7 hr after fertilization. After 5 μl of 10 μg/ml Hoechest dye 33258 was added to the solution, the embryos were crashed between a glass slide and a cover slip, and were examined with an epifluorecent microscope. (2) Eggs were injected with 40-50 nl of 300 μM biotin-11-dUTP at 10 min before the initiation time of the S-phase of individual cell cycles, which is expected from the standard time tables for early *Xenopus* cell cycle events by Graham and Morgan (1966) and Huntchison *et al.* (1989). Eggs were incubated until the next M-phase at 20-21°C. Eggs were then transferred to 100% methanol containing 1% formaldehyde and fixed in it at −20°C overnight. Following dehydration in 100% ethanol, eggs were embedded in paraffin wax (m.p. 54-56°C) and cut at 8 μm into serial sections. Sections were hydrated through an ethanol series and washed in phosphate buffer saline (PBS: 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.0 mM K_2HPO_4 at pH 7.3) 3 times for 5 min each. They were then treated for 20 min with Texas Red-conjugated streptavidin (Amersham Co., Arlington Heights, IL, USA) diluted at 1/100 in PBS and washed in PBS 3 times for 5 min each. Sections were stained with 5 μg/ml 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI; Molecular Probe Inc., La Jolla, CA, USA) and washed in PBS 3 times for 5 min each. They were mounted in PBS/glycerol (50:50) solution containing 0.1% p-phenylenediamine and examined with an epifluorescent microscope.

Examination of asters and the mitotic apparatus

Asters and the mitotic apparatus were examined following the procedure of Houliston and Elinson (1991) with modifications. Embryos were fixed at −20°C overnight in 100% methanol containing 1% formaldehyde. Following hydration through a series of methanol/

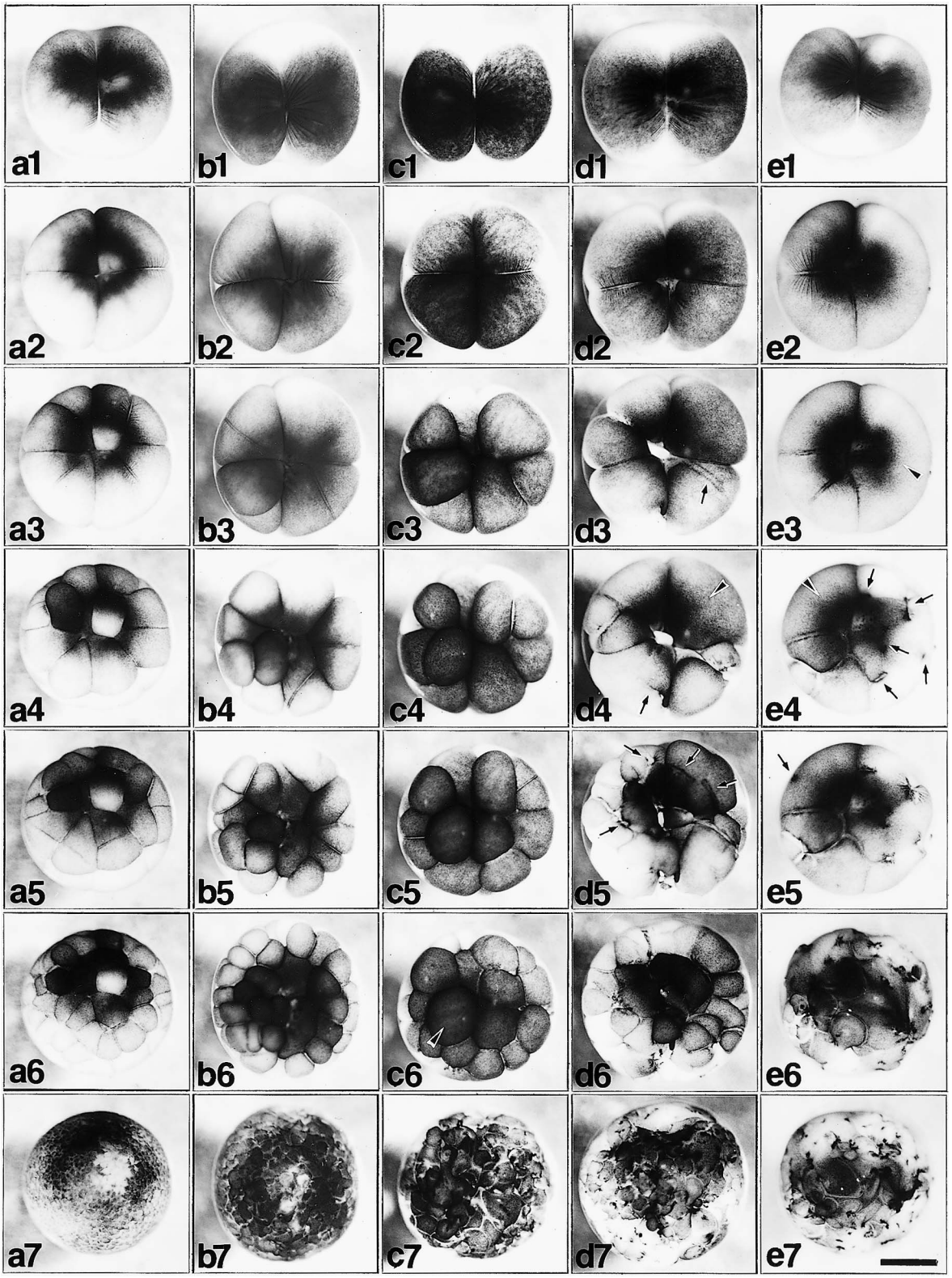

Fig. 1. Top view at 7 stages of a normal embryo (**a1**-**a7**), embryos exposed to 15, 30 or 90 μg/ml aphidicolin (**b1**-**b7**, **c1**-**c7**, **d1**-**d7**, respectively) and an embryo subjected to u.v.-inactivation of the nucleus (**e1**-**e7**). Photos of embryos at individual stages are aligned according to their stage, not the absolute time after fertilization, except photos of embryos in **a7**-**e7** at 7 hr after fertilization. Embryos exposed to high concentrations of aphidicolin or subjected to u.v.-inactivation of the nucleus develop slower than normal embryos and those exposed to low concentrations of aphidicolin. Note that embryos exposed to 30 or 90 μg/ml aphidicolin and those subjected to u.v.-inactivation of the nucleus often came to fail normal cell division and formed abortive furrows after several cell divisions. Arrows and arrowheads indicate the sites of abortive furrows and those at which a furrow was expected to form but failed, respectively. Scale bar equals 0.5 mm.

PBS solutions, eggs were extracted with PBS containing 0.25% Triton-X 100 for 30 min, washed in PBS 2 times for 15 min each and dehydrated through ethanol/PBS series. Embryos were embedded in paraffin wax (m.p. 52-54°C) and cut at 8 μm. Sections were hydrated in an ethanol series, washed 3 times in PBS for 5 min each and blocked at room temperature for 60 min with PBS containing 0.1% Triton-X 100, 30 mg/ml skim milk and 10 mg/ml BSA. After 3 washes in PBS for 5 min each, sections were reacted overnight at 4°C with anti-β-tubulin mouse IgG (Amersham Japan Inc. Tokyo, Japan) diluted at 1/500 in PBS containing 0.5 mg/ml sodium azide. After 3 washes in PBS for 5 min each, sections were treated with PBS containing 0.1% Tween 20 and 10 mg/ml BSA for 30 min, and reacted at room temperature for 2 hr with fluorescein-conjugated anti-mouse-IgG goat antiserum diluted at 1/100 in PBS containing 0.5 mg/ml sodium azide. After 3 washes in PBS for 5 min each, sections were treated with 5 μg/ml DAPI and washed in PBS 3 times for 5 min each. They were mounted in PBS/glycerol (50:50) solution containing 0.1% p-phenylenediamine and examined with an epifluorescent microscope.

Monitoring of cell division interval

Eggs to be compared for cell division intervals were placed closely in a single plastic Petri dish (3 cm in diameter) or adjacent wells of a single microplate to avoid unexpected temperature variation between them. Formation of a cell division furrow was carefully observed. Observation was usually started at about 10 min before the expected initiation time of individual cell divisions and continued at 1-min interval until the cell division completed. The time of the start of furrow formation was taken as the time of cell division.

RESULTS

Effect of exposure to low concentrations of aphidicolin

Previous studies (Huntchison *et al.*, 1989; Minshull *et al.*, 1989; Newport and Dasso, 1989; Dasso and Newport, 1990) reported that early *Xenopus* embryos underwent apparently normal cell divisions with normal division intervals when exposed to 10-20 μg/ml aphidicolin, a DNA polymerase α inhibitor. Their reports imply that early *Xenopus* embryos, unlike our expectation, do not have a cell cycle control system that is affected by the state of DNA replication. To test if their reports are correct, we first repeated their studies with embryos continuously exposed to 15-20 μg/ml aphidicolin from shortly after fertilization and examined carefully cell division intervals in them.

As they reported (Huntchison *et al.*, 1989; Minshull *et al.*, 1989; Newport and Dasso, 1989; Dasso and Newport, 1990), embryos exposed to 15 or 20 μg/ml aphidicolin underwent nearly normal cell divisions without cell division arrest, extreme delay of cell divisions or significant disturbance of formation of cell division furrows and developed to apparently normal blastulae (Fig. 1b). However, we found that the cell division interval in those embryos, unlike their reports, did have a tend to extend gradually but steadily up to 20% during the first 6 cell cycles (Fig. 2a, b and Table 1). This result agrees with a report by Clute and Masui (1997). Thus, exposure to relatively low concentrations of aphidicolin does have an effect to expand cell division intervals and delay cell divisions in early *Xenopus* embryos. This suggests that early *Xenopus* embryos can have a sort of cell cycle control system that is affected by the state of DNA replication.

Histological examination revealed that, in agreement with the report by Newport and Dasso (1989), embryos exposed to 15-20 μg/ml aphidicolin contained quite a few nuclei at the blastula stage although the number was far small as compared with the expected number for a normal embryo at the same stage (Fig. 3a, b). A majority of the cells of those embryos contained asters lacking chromosomes (Fig. 3b, b'). It is evident that exposure to 15-20 μg/ml aphidicolin does not completely suppress DNA replication in early *Xenopus* embryos during initial several cell cycles, as suggested by Newport and Dasso (1989). This suggests that even low concentrations of aphidicolin that can not suppress DNA replication completely could have an effect to expand the cell division interval up to 20%.

Effect of exposure to high concentrations of aphidicolin

To know the effect of exposure to aphidicolin at concentrations high enough to suppress DNA replication completely, we examined cell division intervals in embryos exposed to aphidicolin at 30-90 μg/ml. Unlike embryos exposed to15-20 μg/ml aphidicolin, those exposed to 30-90 μg/ml aphidicolin displayed 30-50% prolongation of the cell division interval at as early as the 2-4th cell division, with the extent of prolongation depending on the concentration of aphidicolin applied (Fig. 2c-f). In particular, the cell division interval was prolonged to 130-150% of the normal interval at as early as the 2nd or 3rd cell division when embryos were exposed to 90 μg/ml aphidicolin (Fig. 2f). Thus, it is evident that exposure to 30-90 μg/ml aphidicolin has an effect to extend cell division intervals 30-50%.

Even those embryos, however, did not show complete arrest of cell division unlike late embryos or somatic cells exposed to aphidicolin. Instead, they often failed normal cell division with a normal furrow and formed abortive division furrows (Fig. 1c, d). Those embryos often developed to partially or superficially cellularized, blastula-like embryos (Fig. 1c, d).

Histologically, the nucleus often suffered from a syndrome of fragmentation at early stages in embryos exposed to 30-90 μg/ml aphidicolin. No duplicated nuclei were detected in those embryos at later stages such as the blastula-like stage (Fig. 3c, d). In those embryos, by contrast, a rather large number of asters lacking chromosomes were detected, which suggests duplication of asters independent of duplication of the nucleus (Fig. 3c', d'). Direct examination with biotin-11-dUTP/streptavidin of DNA replication revealed that DNA replication was nearly completely suppressed at the third cell cycle in embryos exposed to 90 μg/ml aphidicolin (Fig. 4g-i) whereas it was not significantly suppressed at the same cycle in those exposed to 30 μg/ml aphidicolin (Fig. 4d-f).

Correlation of more than 30% extension of the cell division interval and failure of normal cell division

In the course of the above examinations, we realized that there is a correlation between more than 30% prolongation of the cell division interval and failure of normal cell division. We found that while embryos showing less than 30% prolongation of the cell division interval successfully underwent nor-

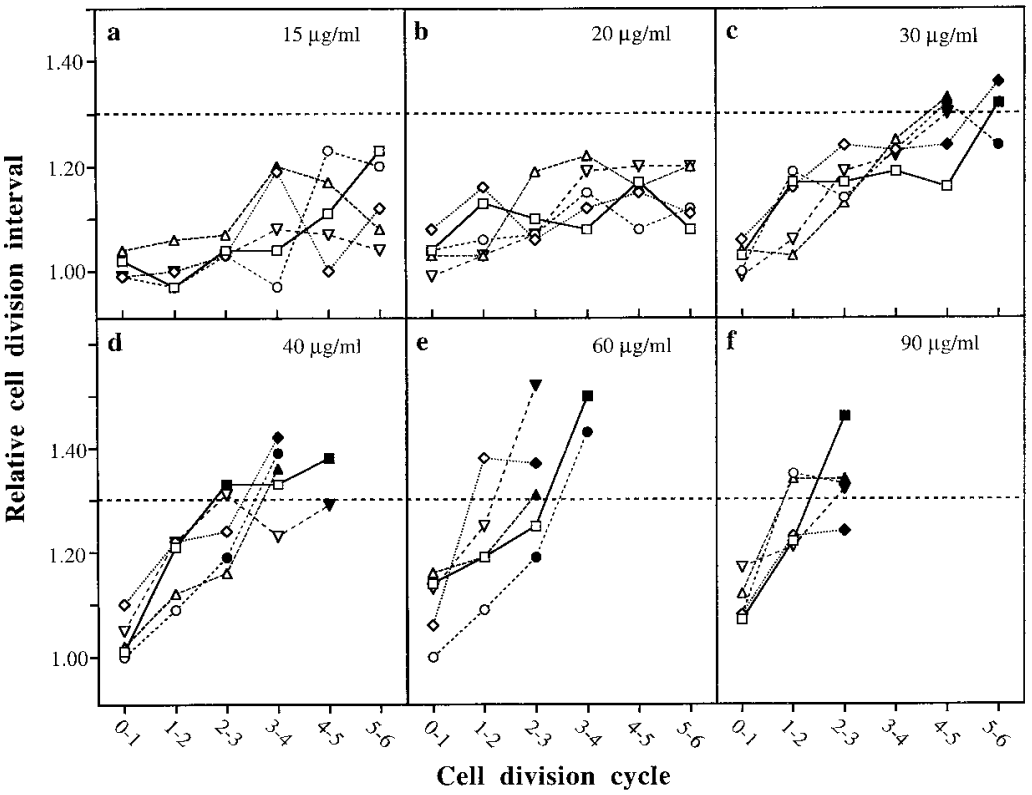

Fig. 2. Changes in cell division intervals in embryos subjected to 15-90 μg/ml aphidicolin relative to the corresponding intervals in intact sibling embryos from the same females. Three to five examples are provided for each experiment. The concentration of aphidicolin applied to embryos is shown on top. Open and closed symbols indicate the relative time of occurrence for cell division with a normal furrow and that with abortive furrows, respectively. Note that embryos usually fail normal cell division, instead forming abortive division furrows, when the interval is prolonged 30% or more.

Table 1. Average relative length of the cell division interval in embryos exposed to different concentrations of aphidicolin (Mean ± s.d.)

Concentration of aphidicolin	n	Interval					
		0-1	1-2	2-3	3-4	4-5	5-6
15 μg/ml	12	1.00 ± 0.03	1.02 ± 0.06	1.03 ± 0.04	1.08 ± 0.08	1.10 ± 0.08	1.09 ± 0.08
20 μg/ml	12	1.04 ± 0.04	1.06 ± 0.06	1.09 ± 0.05	1.16 ± 0.06	1.11 ± 0.06	1.10 ± 0.05
30 μg/ml	12	1.03 ± 0.02	1.15 ± 0.06	1.19 ± 0.05	1.24 ± 0.07	1.28 ± 0.06	1.29 ± 0.05
40 μg/ml	12	1.05 ± 0.03	1.19 ± 0.06	1.24 ± 0.08	1.34 ± 0.10	1.32 ± 0.10	
60 μg/ml	12	1.08 ± 0.05	1.24 ± 0.09	1.32 ± 0.12	1.39 ± 0.12		
90 μg/ml	11	1.12 ± 0.07	1.27 ± 0.10	1.35 ± 0.08			

mal cell divisions with a normal division furrow (Figs. 1b and 2a, b), those showing more than 30% prolongation of the cell division interval due to exposure to 30-90 μg/ml aphidicolin usually failed normal cell division, forming abortive division furrows (Figs. 1c, d and 2c-f). There seems to be a critical point for the extent of prolongation of the cell division interval at 30% that divides the success or failure of normal cell division with a normal division furrow.

Histological examination revealed that there is also a correlation between more than 30% extension of the cell division interval and complete suppression of DNA replication in embryos exposed to aphidicolin (Figs. 2 and 4). While em-

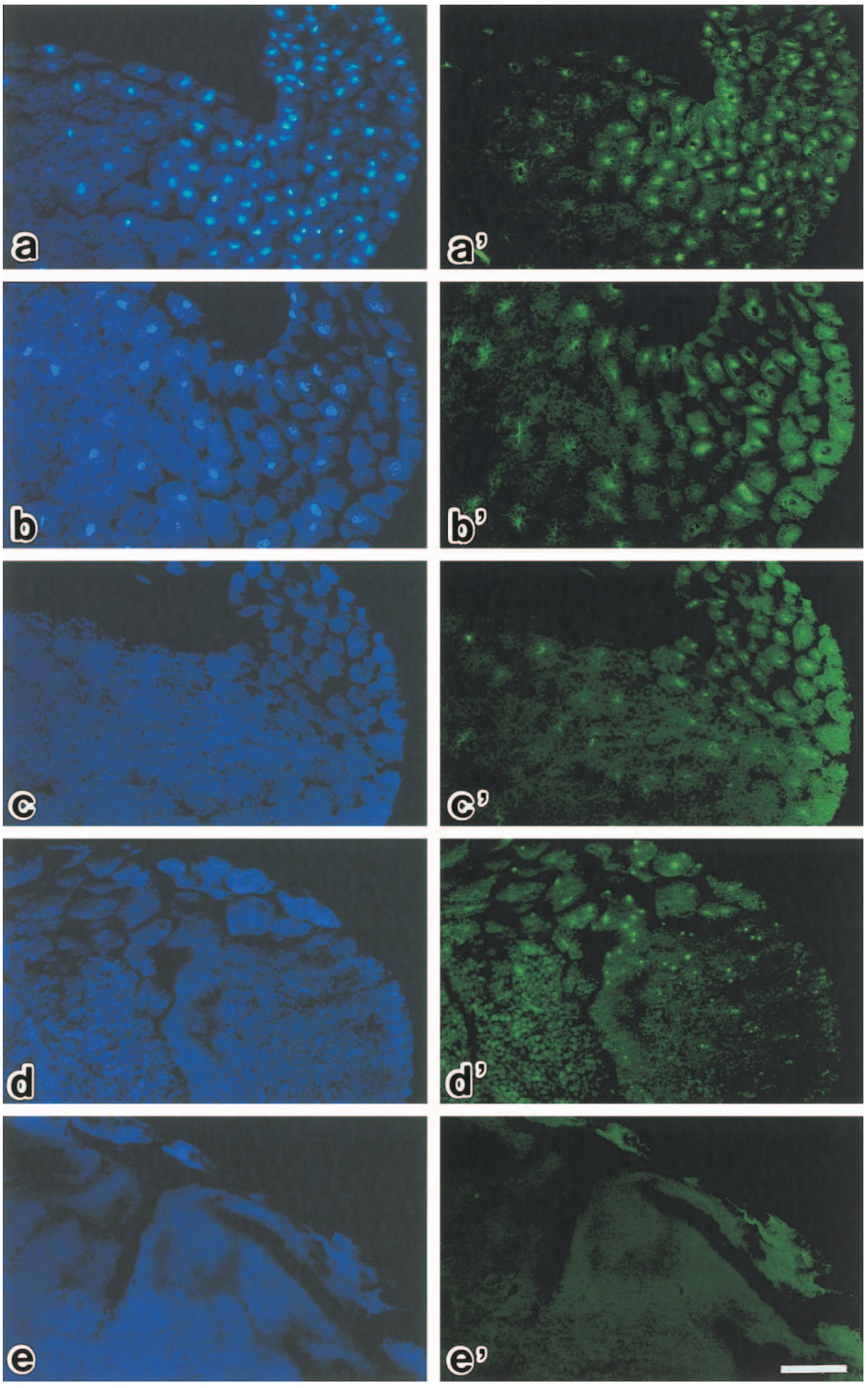

Fig. 3. DAPI (**a**-**e**) and anti-β-tubulin immunofluorescence (**a'**-**e'**) staining of sections of embryos fixed at 9 (**a**-**d**, **a'**-**d'**) and 7 (**e**, **e'**) hr after fertilization. Embryos were exposed to 0 (**a** and **a'**), 15 (**b** and **b'**), 30 (**c** and **c'**) or 90 (**d** and **d'**) μg/ml aphidicolin or subjected to u.v.-inactivation of the nucleus (**e** and **e'**). Note that although the nucleus has duplicated rather significantly in embryos exposed to 15 μg/ml aphidicolin, it has not duplicated in those exposed to 30-90 μg/ml aphidicolin though asters has duplicated in all embryos exposed to 15-90 μg/ml aphidicolin. Scale bar equals 0.1 mm.

bryos showing only 20% extension of the cell division interval at the second cell division underwent DNA replication rather successfully (Fig. 4d-f, d'-f'), those showing a 35% prolongation of the cell division interval failed DNA replication almost completely (Fig. 4g-i, g'-i').

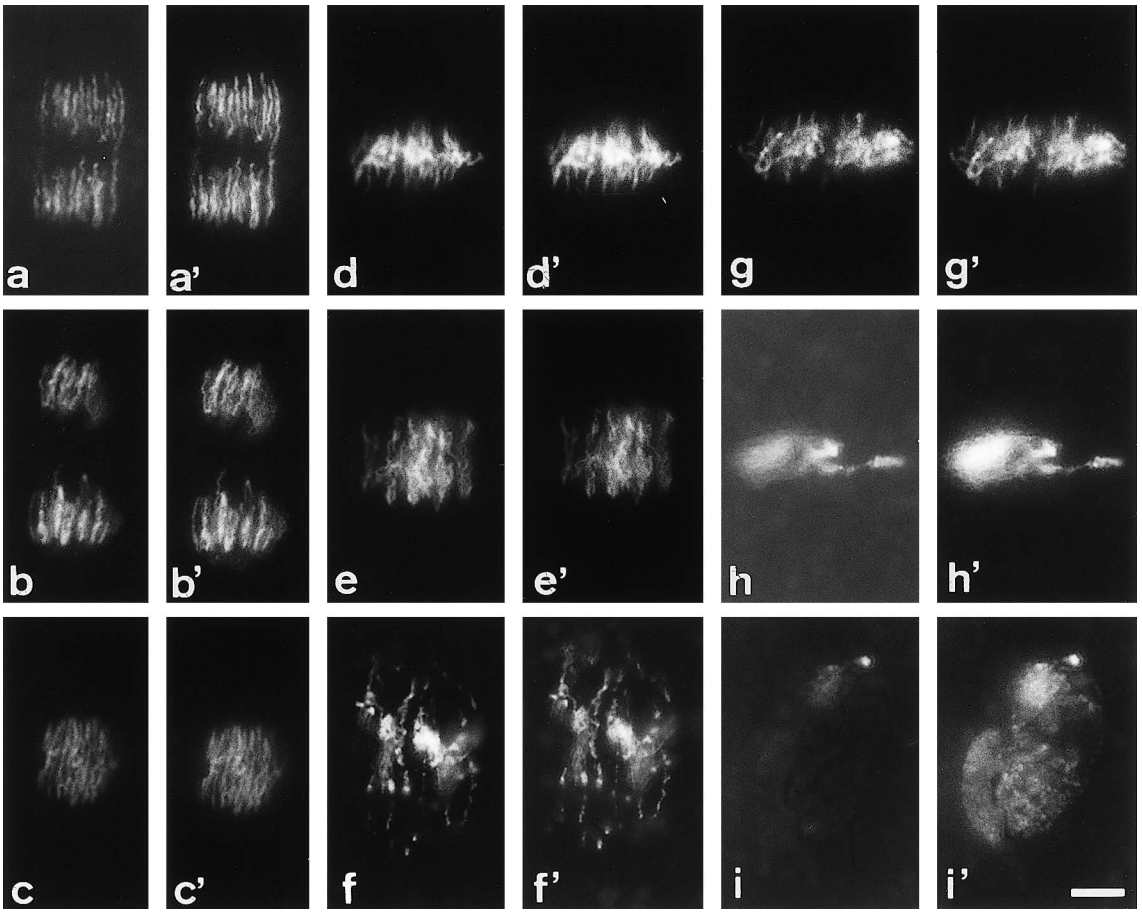

Fig. 4. Images of nuclei or chromosomes stained with Texas red conjugated streptavidin (TRSA, **a**-**i**) and DAPI (**a'**-**i'**). The two stainings react with biotin-dUTP incorporated into newly replicated DNA and DNA itself, respectively. Nuclei and chromosomes in normal embryos (**a**-**c** and **a'**-**c'**) and embryos exposed to 30 μg/ml (**d**-**f** and **d'**-**f'**) or 90 μg/ml (**g**-**i** and **g'**-**i'**) aphidicolin are shown. Each pair of TRSA and DAPI images (**a** and **a'**, **b** and **b'** etc.) are of the same section. Each embryo was fixed at 5 min before the expected times of 1st (**a**, **a'**, **d**, **d'**, **g** and **g'**), 2nd (**b**, **b'**, **e**, **e'**, **h** and **h'**) and 3rd (**c**, **c'**, **f**, **f'**, **i** and **i'**) cell divisions. Scale bar equals 10 μm.

Effect of u.v.-inactivation of the nucleus

We further tested the effect on cell division interval and the morphology of cell divisions of suppression of DNA replication by u.v.-irradiation instead of exposure to aphidicolin. To do this, we obtained eggs that contained only a nucleus inactivated by u.v.-irradiation by fertilizing eggs with u.v.-irradiated sperm and removing the female nucleus (see Materials and Methods).

Embryos thus obtained often showed a 30-50% prolongation of the cell division interval at as early as the 2nd cell division and failed normal cell divisions like those exposed to 90 μg/ml aphidicolin (compare Fig. 5a with Fig. 2f). Despite the failure of normal cell divisions, they often developed to superficially cellularized embryos when sibling embryos reached the midblastula stage (Figs. 1e and 3e, e'). The correlation between more than 30% extension of cell division interval and failure of normal cell division is similarly seen in these embryos, suggesting that such a correlation is related to the state of suppression of DNA replication (Fig. 5a).

Histological examination revealed that those embryos contained only one nucleus with round shape like the interphase nucleus (Fig. 6d'). This indicates that u.v.-irradiation of sperm nucleus successfully suppressed DNA replication. Interestingly, the nucleus was often found along, but not associated, with mitotic asters in the same cell (Fig. 6d and d'). In those embryos individual cells contained more than one pair of asters lacking the spindle containing chromosomes, suggesting duplication of asters independent of DNA replication (Fig. 6a-d, a'-d').

Rescue of normal cell division by the normal nucleus

We further examined that the effect on cell division of the presence of two nuclei, one can and the other cannot undergo DNA replication, in the same cell. As shown in Fig. 5b and Table 2, embryos that contained nuclei, one from u.v.-irradiated sperm and the other from intact female nucleus, did show quite normal cell divisions without any prolongation of cell division intervals or formation of abortive division furrows (Table 2). They usually developed to apparently normal blastulae (data not shown). Obviously, cell divisions with division intervals and furrows were rescued by the presence of a nucleus capable of undergoing DNA replication.

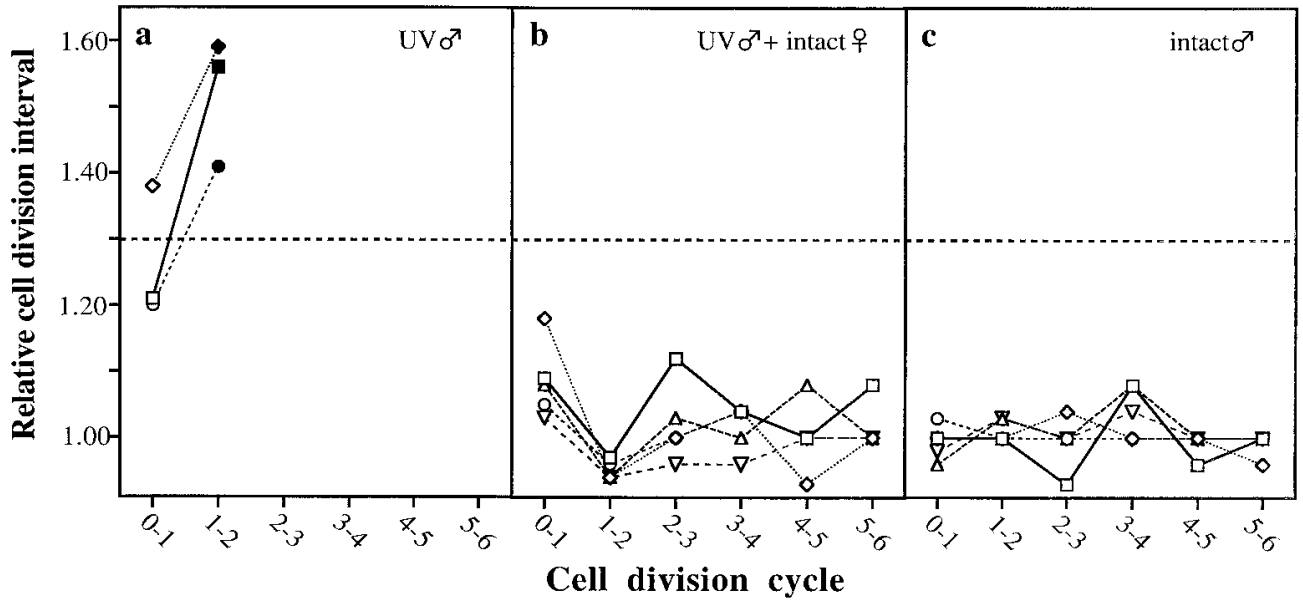

Fig. 5. Changes in cell division intervals in embryos that contained only an u.v.-inactivated male (**a**), both an intact female and an u.v.-inactivated male (**b**) or only an intact male nucleus (**c**) relative to the corresponding intervals in intact sibling embryos from the same females. Three to five examples are provided for each experiment. Open and closed symbols indicate the relative time of occurrence for cell division with a normal furrow and that with abortive furrows, respectively. Note that both significant prolongation of the cell division interval and formation of abortive furrows were avoided by allowing embryos to retain an intact female nucleus in addition to an u.v.-inactivated male nucleus. It is obvious that the syndromes seen in embryos containing only a u.v.-irradiated nucleus are not ascribed to the absence of the female nucleus because those containing only an male nucleus that is not u.v.-irradiated showed normal cell divisions at normal division intervals in the absence of the female nucleus.

DISCUSSION

Early *Xenopus* embryos have a cell cycle control system affected by DNA replication

We showed that exposure to aphidicolin at 15-90 μg/ml lead to 20-50% prolongation of the cell division interval in early *Xenopus* embryos. The rate and ultimate extent of prolongation of the cell division interval depended on the concentration of aphidicolin applied. In those embryos, both nuclear duplication and DNA replication appeared to be suppressed more significantly when they were exposed to high concentrations of aphidicolin than when they were exposed to low concentrations of aphidicolin. Direct inactivation of the nucleus by u.v.-irradiation resulted in 30-50% prolongation of the cell division interval like exposure to 90 μg/ml aphidicolin. These results indicate that there is a cell cycle control system or process affected by the state of DNA replication in early *Xenopus* embryos.

Possible process of establishment of the cell division intervals in early *Xenopus* embryos

As shown above, there was a critical point for the extent of prolongation of the cell division interval at 30% that divides the success and failure of normal cell division with a normal division furrow. This percentage coincides with the average percentage for the prolongation of the interval of oscillatory activities seen in enucleated *Xenopus* and *Cynops* eggs (Shinagawa, 1983, 1985, 1986). This coincidence implies possible involvement in establishing cell cycle intervals in early *Xenopus* embryos of a cell cycle control system that is affected by DNA replication. Taking these findings together, we presume the process of establishment of cell division intervals in early *Xenopus* embryos as well as the roles of the above system and the master oscillator as follows.

Every cell of early *Xenopus* embryos has both the master oscillator, which drives cytoplasmic activities essential to the progression of cell cycle, and a system that is affected by the state of DNA replication in the nucleus. In the normal cell cycle, on the completion of DNA replication in the nucleus the DNA replication-sensitive system activates the master oscillator to signal the cell to enter the M-phase and form the mitotic apparatus. When the completion of DNA replication is delayed by some causes for 30% time of one normal cell cycle or less, the system still activates the master oscillator to prompt the cell to enter the M-phase and form a functional mitotic apparatus. When the completion of DNA replication is delayed for more than 30% time of one normal cell cycle, the master oscillator spontaneously activates the cell to enter M-phase and form a mitotic asters without the signal from the DNA replication-sensitive system. This spontaneous cytoplasmic S/M transition without the completion of DNA replication disrupts the coordination of mitotic aster formation and chromosome condensation and interfere with the formation of a functional mitotic apparatus. Failure of formation of a functional mitotic apparatus causes the failure of the formation of a cell division furrow.

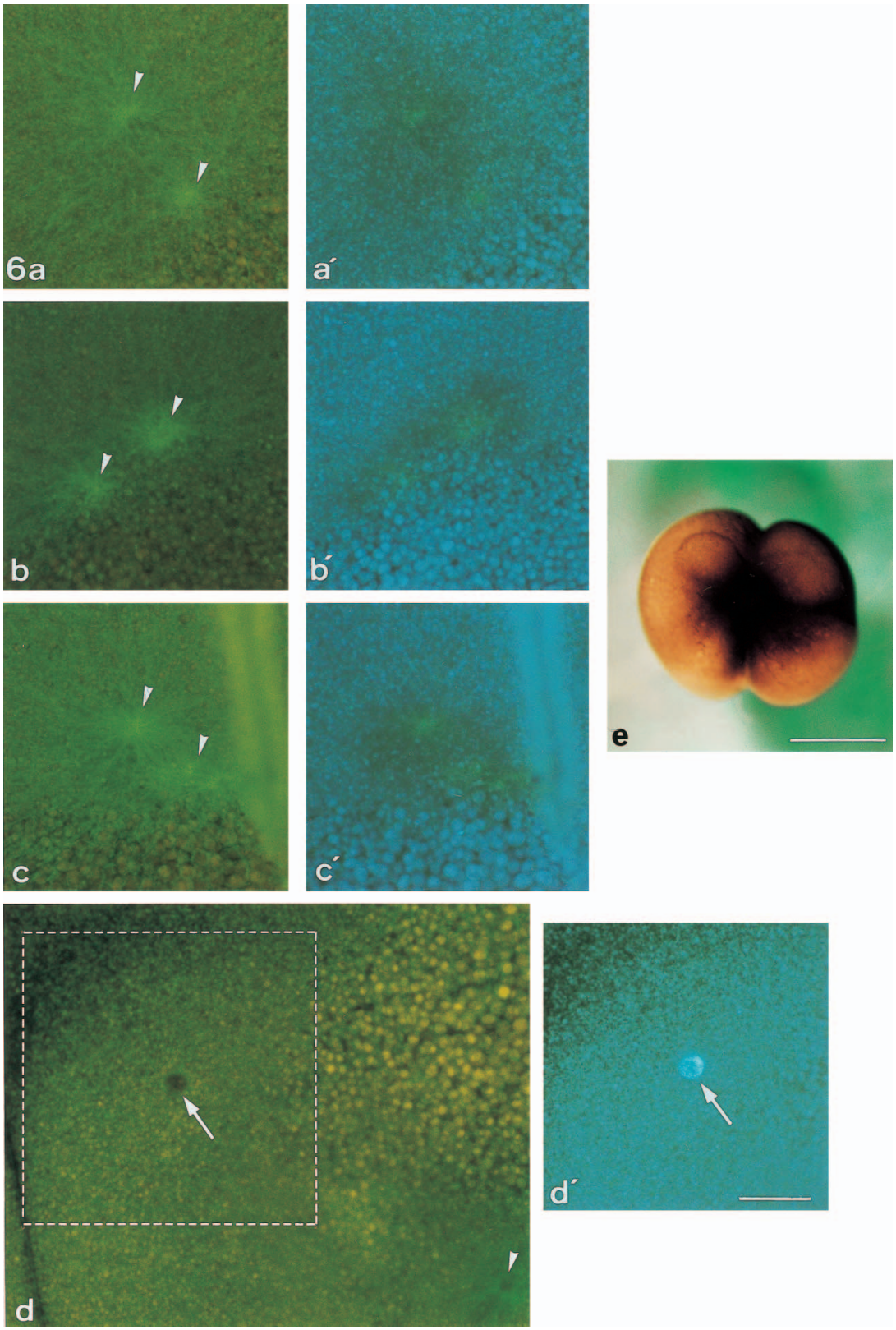

Fig. 6. Anti-β-tubulin immunofluorescence (**a**-**d**) and DAPI (**a'**-**d'**) staining of sections of an embryo and its top view (**e**). The embryo was subjected to u.v.-inactivation of the nucleus and fixed at 2.5 hr after fertilization. Asters in **a** and **b** were found in the left cell of the embryo and those in **c** were found in the upper right cell of the embryo. Asters and a nucleus in **d** were found in the lower right cell. The square surrounded by broken lines in **d** indicates the same portion as that shown by **d'**. Note that only one nucleus in spherical form is seen in the entire embryo (**d** and **d'**). Arrows and arrowheads depict a nucleus and asters, respectively. Scale bar equals 50 μm for **a**-**d** and **a'**-**d'** and 0.5 mm for **e**.

Table 2. Average relative length of the cell division interval in embryos containing different sets of nuclei (Mean ± s.d.)

Nuclear type	n	Interval					
		0-1	1-2	2-3	3-4	4-5	5-6
U.v.-ed ♂	13	1.28 ± 0.08	1.72 ± 0.40				
U.v-ed ♂ + intact ♀	18	1.08 ± 0.09	1.01 ± 0.06	0.99 ± 0.06	1.03 ± 0.06	0.99 ± 0.04	1.01 ± 0.05
Intact ♂	20	1.00 ± 0.04	1.00 ± 0.04	0.99 ± 0.04	1.04 ± 0.06	1.00 ± 0.05	1.00 ± 0.03

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