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Centriolar cycle of fused cells

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SUMMARY

Ultrastructure of centrioles of fused cells containing heterophasic interphase nuclei, premature chromosome condensation, and a telophase-like nucleus were studied. The study indicated that the heterophasic cellular environments contributed by the different cell partners exert a mutually opposite effect on the structure of centrioles. The G_1-cell partner suppresses replication of S-centrioles. Asynchronous replications of G_1 and S-stage centrioles were observed in some G_1-S, G_1-G_2 and S-G_2 fused cells. In interphase-mitotic fused cells, centrioles of interphase stages underwent mitotic activation when their nuclei were induced to premature chromosome condensation. Daughter centrioles of G_1-, S-, and G_2-stages were also capable of mitotic activation independently if they were separated from their mother centriole. Inactive centrioles were observed in some cells containing G_1-premature chromosome condensation. When mitotic nuclei were induced to telophase-like nucleus formation, their centrioles were also inactivated. Concomitant events of induced nuclear and centriolar changes suggest that they might have been controlled by the heterophasic cytoplasmic factors through similar pathways.

Key words: centriole, centriolar cycle, cell cycle, fused cell

INTRODUCTION

Polykaryons obtained by random fusion of an asynchronous cell population represent unique cellular systems since such cells include nucleus-centriolar sets belonging to different periods of the cell cycle. In fused interphase cells, the heterophasic interphase nuclei achieve synchronization in their activities either by inducing early DNA synthesis in G_1-nuclei (Rao and Johnson, 1970, 1972), or by prolonging duration of G_2-nuclei (Rao and Johnson, 1970; Ghosh and Paweletz, 1984). In fused cells containing interphase and mitotic nuclei either interphase nuclei are induced to premature chromosome condensation (PCC) (Johnson and Rao, 1970) or mitotic chromosomes are induced to formation of a telophase-like nucleus (TLN) (Ikeuchi et al., 1970). Development towards PCC or TLN depends upon the balance of effects from the side of the mitotic cell and the interphase cell. The overwhelming effect of mitotic cell partner causes the interphase nucleus to form PCC and vice versa (Ikeuchi et al., 1971). A study of centrioles in such cellular systems would elucidate how the cellular environment belonging to different periods of the cell cycle would have an effect in the cyclic events of centrioles thus unraveling important aspects of interrelationship between nuclear and centriolar cycles. In this present work we studied the ultrastructure of centrioles of fused cells containing various combinations of interphase nuclei or mitotic figures whose cell cycle stages were identified with double labeling radioautography.

MATERIALS AND METHODS

Cell culture

A culture of embryonic pig kidney cell (PE) was maintained in medium 199 with 10% bovine serum. For the experiment, monolayer cells were grown on coverslips by plating 10^5 cells per ml and incubating for 2 days.

Radioautography

To identify G_1-, S-, and G_2-stage nuclei, the double labeling radioautography method was followed (Onishchenko et al., 1978). The first pulse labeling was done with [^{3}H]thymidine, and after 4.5 hours incubation in non-radioactive medium, a second pulse labeling was done with [^{14}C]thymidine. With this method G_1/G_0-nuclei would be unlabeled, early S-nuclei ^{14}C-labeled, late S-nuclei ^{14}C- + ^{3}H-labeled and G_2-nuclei would be ^{3}H-labeled. Cell fusion was done immediately after [^{14}C]thymidine labeling.

Cell fusion

Monolayer cells were fused with a modified method using PEG, DMSO and serum. Assessment of this method is described elsewhere (Manandhar et al., 1993). The cells were rinsed 3-times with 15% DMSO in serum, then treated with 50% PEG (molecular mass 4,000, pH 8) for 1 minute. After 1 minute the PEG solution was diluted 2-times by adding equal volumes of medium 199. The mixture was swirled for 15 seconds, and poured off. The cells were again washed 3-times with 15% DMSO in serum, and then rinsed with medium for 3-changes and incubated in the previous medium. The cells were fixed after 1.5 hours. We chose the 1.5 hour incubation period after fusion treatments for ultrastructural study so that the centrioles would be sufficiently exposed to the effect of the new cytoplasmic environment, and in the mean time attainment of synchronization would be minimum. Polykaryons obtained by this method show highest asynchronous mitotic activity at the 1.5 hour period (Manandhar et al., 1993).

Electron microscopy (EM)

For EM, cells were fixed with 2.5% glutaraldehyde post-fixed with 1% OsO_4 and embedded in Epon-812 with the standard method. After

embedding the cells, the coverslips were removed by immersing them in liquid nitrogen. The epoxy discs with cells on the surface were coated with nuclear photographic emulsion and exposed for 7 days. Fused cells with suitably labeled nuclei or mitotic figures were selected under a phase contrast microscope, photographed and marked with metal scribe. Serial sections (gold color) were cut in an LKB Ultratome, stained with uranil acetate and lead citrate (Reynolds, 1963), and observed under Hitachi-IIB, Hitachi-12, and JEM-100B microscopes.

RESULTS

Interphase-interphase fused cells

We studied complete serial sections of 17 fused cells, mainly dikaryons, containing different combinations of heterophasic nuclei (Table 1). The number of centrioles in polykaryons fully complies with the total number as it can be expected from the cell cycle periods of the fusing cells. In G_1-S dikaryons, centrioles from the S-partner were either replicated (cell nos 1,2,5 in Table 1) or unreplicated (cell nos 3,6). The S-centrioles were found to be replicated when the fusing S-cell partner belonged to the late stage. This observation corresponds to the earlier finding that in PE cells centriole replication starts not at the beginning of S-stage, but at the middle of it (Vorobjev and Chentsov, 1982). In one G_1-S fused cell

Table 1. Centrioles of fused cells containing various combinations of interphase nuclei

Cell no.	Nuclei	No. of solitary centrioles	No. of centrioles with procentriole	No. of diplosomes
1	$1G_1$-1S (L)	$1^{s}+1$	$1+1^{sa}$	—
2	$1G_1$-1S (L)	2	$1+1^{s}$	—
3	$1G_1$-1S (E)	$2+1^{sa}+1^{sav}$	—	—
4	$1G_1$-1S (E)	$1+2^{sa}$	1	—
5	$1G_1$-1S (L)	2	$1+1^{s}$	—
6	$1G_1$-1S (E)	$2+1^{sa}+1^{s}$	—	—
7	$1G_1 1G_2$	$5+1^{sa}$	—	—
8	$1G_1$-$1G_2$	$1+1^{a}$	—	$1+1^{a}$
9	$1G_1$-$1G_2$	1^{s}	1	$1+1^{a}$
10	$1G_1$-$1G_2$	—	$1+1^{sa}$	2
11	$1G_1$-$1G_2$	$5+1^{sa}$	—	—
12	$2G_1$-$1G_2$	6	—	1
13	1S (E) - $1G_2$	1^{sa}	3	—
14	1S (L) - $1G_2$	$3+1^{a}$	2	—
15	1S (E) - $1G_2$	1	1^{sav}	2
16	1S (L) - $1G_2$	$1+1^{a}$	2	1
17	2S (E-E) - $2G_2$	4	6	—

Incubation, 1.5 h after fusion treatment.

s, satellite; a, appendages; v, vacuole, Enclosed by brackets: E, early S-stage nucleus having ^{14}C-labelling; L, late S-stage nucleus having ^{14}C- + ^{3}H-labeling (see Materials and Methods).

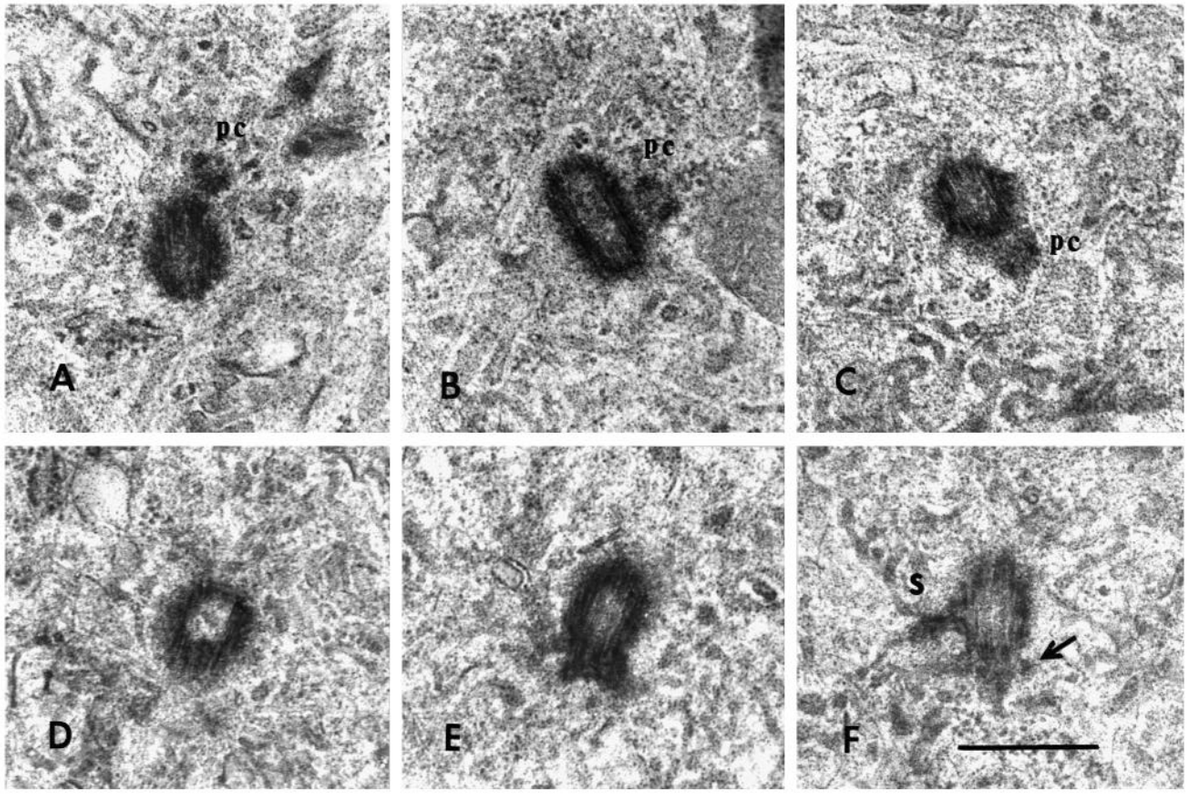

Fig. 1. Asynchronous replication of early S-centriole in S-G_2 fused cell (cell no. 13 of Table 1). Among four centrioles found in the cell, three possessed procentrioles (A,B and C). The fourth centriole, shown in 3 serial sections (D,E and F) lacked procentriole. pc, procentriole; s, satellite; arrow, appendages. Bar, 0.5 µm.

Table 2. Centrioles of fused cells containing various types of PCC

Cell no.	PCC system	No. of mitotically active: Diplosomes	No. of mitotically active: Solitary centrioles	No. of mitotically inactive centrioles
1	G_1	2	2	—
2	G_1	2	1	1
3	G_1	2	2	—
4	S (L)	4	—	—
5	S (L)	4	—	—
6	S (E)	1	6*	—
7	S (E)	4	—	—
8	S (L)	4	—	—
9	G_2	4	—	—
10	G_2	4	—	—
11	G_2	2	4	—
12	G_2	—	8	—
13	G_1-G_2	2	6	—
14	G_1-G_2	3	3	1
15	G_1-G_2	3	3	1
16	G_1-G_2	—	9	1

Incubation, 1.5 h after fusion treatment

*4 centrioles + 2 short daughter centrioles as shown in Fig. 6. Enclosed by brackets: E, early S-stage nucleus having ^{14}C-labeling; L, late S-stage nucleus having ^{14}C- + ^{3}H-labeling (see Materials and Methods).

Table 3. Centrioles of fused cells in which TLN is induced

Cell no.	Nuclei	No. of solitary centrioles	No. of centrioles with procentriole	No. of diplosomes
1	$1G_1$-1S(E)	$5+1^{sa}+1^{s}+1^{a}$	—	—
2	$1G_1$-$1G_2$	$1+1^{a}$	—	4
3	1S(L)-$1G_2$	$5+1^{av}$	2	1
4	$2G_2$	$3+1^{a}$	—	$2+2^{a}$
5	$3G_1$-1S(L)-$1G_2$	$6+1^{sav}+2^{sa}+1^{s}$	$1+1^{sa}$	2

Incubation, 1.5 h after fusion treatment.

s, satellite; a, appendages; v, vacuole. Enclosed by brackets: E, early S-stage nucleus having ^{14}C-labeling; L, late S-stage nucleus having ^{14}C- + ^{3}H-labeling (see Materials and Methods).

(cell no. 4) out of four centrioles, only one centriole possessed a procentriole. This means that out of two S-centrioles only one was replicated.

In some G_1-G_2 fused cells G_1-centrioles were precociously replicated, which took place asynchronously (cell no. 9), or synchronously (cell no. 10). In two S-G_2 cells whose S-nuclei were at an early stage, asynchronous replication of S-centrioles was observed (cell nos 13, 15). Out of four centrioles of the cell, three possessed procentriole or daughter centrioles, and one was solitary (Fig. 1). Presumably, out of three replicate centrioles, two came from G_2-cell and the third one from the S-cell partner.

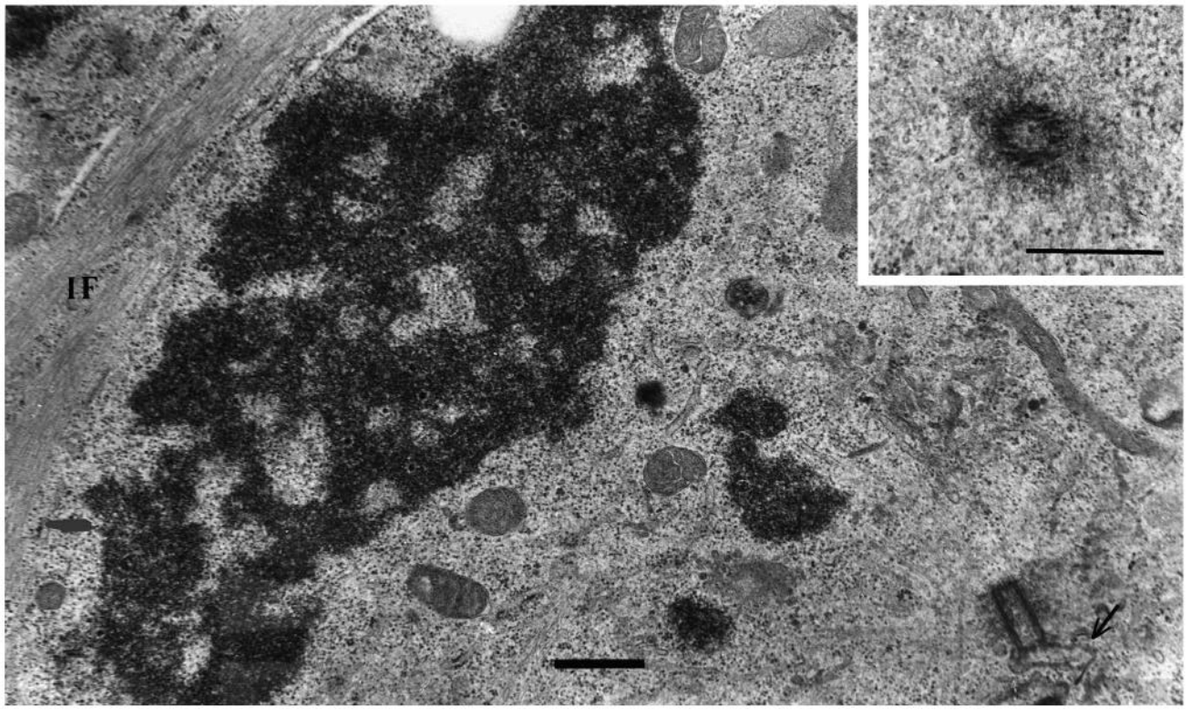

Fig. 2. Mitotically active G_1-centrioles forming a half spindle towards G_1-PCC. The centriole is visibly the mother centriole of G_1-cell partner because it possesses a vacuole at its distal end (see text). Another mitotically active centriole (inset), found lying close to this centriole, could be the daughter centriole of the G_1-cell. The polykaryon contained G_1-G_2 PCC and one metaphase plate (cell no. 13, Table 2). IF, intermediate filaments; arrow, vacuole. Bar, 0.5 µm.

Interphase-mitotic fused cells

We have analyzed complete serial sections of 16 fused cells containing mitosis-PCC (Table 2) and 5 fused cells containing interphase-TLN (Table 3).

By identifying the cell cycle stage of PCC with the help of double labeling radioautography and then studying the same cell under an electron microscope, we obtained direct evidences that centrioles of G_1-, S-, and G_2-stages undergo mitotic activation when their nuclei are induced to PCC. Induced mitotic activation of centrioles does not depend upon their structural maturity or replication. In cell nos 1, 3, 13 (Table 2), both mother and daughter G_1-centrioles possessed a fibrillar halo and formed poles of mitotic spindles. The mother centriole of the G_1-cell partner in cell no. 13 is distinguishable due to the presence of a remnant of the vacuole at its distal end (Fig. 2). As illustrated in the work of Tucker et al. (1979) and Albrecht-Buehler and Bushnell (1980), the mother centriole of the G_1/G_0 cells possessed a vacuole. Likewise, in all fused cells containing S-PCC, S-stage centrioles formed half spindles (cell nos 4-8, Table 2). Even the short daughter centrioles separated from S-stage mother centrioles were found to be mitotically active (Fig. 3). They acquired fibrillar halos and organized half spindles toward mitotic chromosomes independently. Consistently, centrioles of G_2-cell partners also undergo mitotic activation when their nuclei are induced to PCC (cell nos 9-12, Table 2). The daughter centrioles of the G_2-diplosomes formed spindle poles independently when they were separated from their mother centrioles (cell nos 11,12).

Mitotically inactive centrioles were observed in some fused cells containing G_1- or G_1-G_2 PCC (cell nos 2, 14-16). Such centrioles were devoid of fibrillar halos and did not radiate spindle microtubules even though they were lying close to the spindle pole of condensed chromosomes (data not shown).

When TLN is induced in mitotic chromosomes, their centrioles also lose mitotic activity; the mitotic spindles totally disorganize. The fibrillar halo disappears, and centrioles of the mitotic diplosomes lose orthogonal orientation. They develop features of interphase period such as satellites and appendages (Fig. 4). One disoriented centriole, possibly belonging to TLN in the fused cell, containing G_2- and S-interphase partners, possessed a vacuole showing quiescent features (cell no. 3, Table 3).

DISCUSSION

The centriolar cycle of normal PE cells consists of dissociation of mother and daughter centrioles from orthogonal arrangement in G_1-stage, formation of procentriole in the middle of S-stage, maturation of procentrioles into daughter centrioles in G_2-prometaphase, and separation of duplicated centriolar pairs (diplosomes) in dividing stages when the mother centriole of each diplosome acquires a fibrillar halo and forms a spindle pole. Details of the variation of centriolar morphology of PE cells at different stages of the cell cycle has been described in the original work (Vorobjev and Chentsov, 1982). In our study

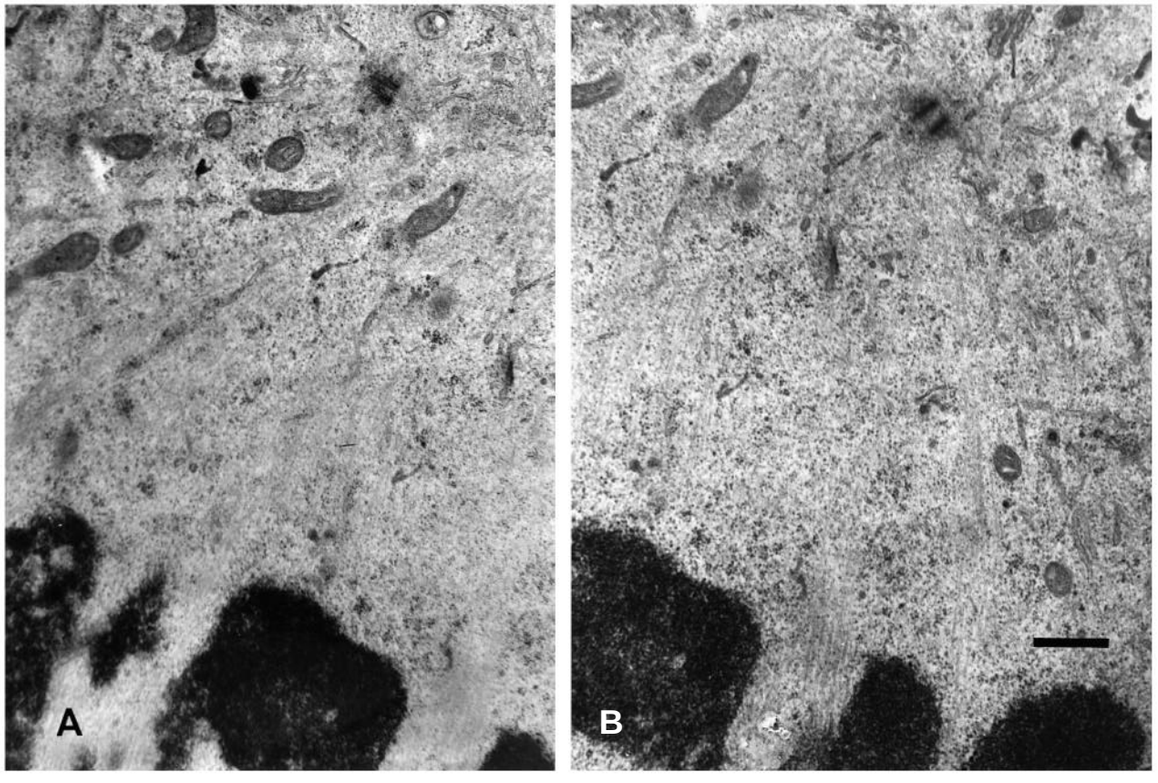

Fig. 3. (A,B) Mitotically active short daughter centrioles of the fused cells containing S-PCC and one metaphase plate (cell no. 6, Table 2). The short daughter centrioles have been separated from their mother centrioles which belong to the S-cell partner. Bar, 0.5 μm.

of the centriolar profile of fused cells, main attention was paid to the principal events of the centriolar cycle such as duplication, spindle pole formation, and disintegration.

In G_1-S fused cells, the G_1-cell partner seems to pose a dominant effect on the centriolar cycle by suppressing the replication of S-centrioles. The presence of an asynchronously replicated S-centriole in the G_1-S fused cell suggests suppression of centriole replication in such cells. In normally dividing cells replication of two S-stage centrioles is thought to be synchronous (Alvey, 1985; Vorobjev and Chentsov, 1982), but, if very early S-stage cells are chosen to analyze the mode of centriolar replication, an asynchronous beginning can be observed (Tucker et al., 1981). In such cells, factors for centriole replication would be insufficient for two centrioles to replicate together. Analogously, in G_1-S fused cells, factors for centriolar replication would be redistributed in the fused cytoplasm and thus would be diluted and limitedly available to the replication of centrioles. Formation of procentriole on early S-centriole may appear to be due to positive induction, but this interpretation is less likely because the other cell partner of the fused cell belongs to the G_1-stage during which centrioles do not replicate in PE cells (Vorobjev and Chentsov, 1982) or in other types of cells (for review see Vorobjev and Nadezhdina, 1987). Synthesis of precursor material for procentriole formation would not have begun in the G_1-cell and hence cannot induce premature replication of centrioles of the fusing cell partner.

The G_2-cell partner, however, induced early centriole replication in the G_1- and S-centrioles. It may appear that the G_1-centriole of the G_1-G_2 fused cells could have passed to S-stage during the 1.5 hour incubation period and begun replication, but the study of G_1-S fused cells has shown that centrioles do not replicate in early S-stage (discussed above). Therefore, precocious replication of the G_1-centriole in the G_1-G_2 fused cells should have been positively induced by the G_2-cell partner. Presence of asynchronously replicated S-centrioles in S-G_2 fused cells also indicates that the G_2-cell partner promotes procentriole formation in yet unreplicated S-centrioles. Quite possibly during the time of cell fusion, the S-stage cell may have an unreplicated centriole. After fusion with the G_2-cell, centriole replication is promoted under the influence of the G_2-cell partner. The asynchronous mode pursued in some cells is due to factor constrain, as discussed earlier.

The observations that induction of PCC of interphase nuclei accompanied by mitotic activation of their centrioles leads to the conclusion that the factor which induces PCC also causes mitotic activation of their centrioles. The possible factor involved could be maturation promoting factor (MPF) which is activated in dividing cells by combining with cyclins (Draetta et al., 1989). Elevated levels of active MPF during mitotic entry are shown to be responsible for nuclear membrane breakdown and chromosome condensation (Sunkara et al., 1979; Miake-Lye et al., 1983; Newport and Kirschner, 1984). Induction of PCC by mitotic factors present in the cytoplasm of mitotic cells was shown in the experiments in which mitoplasts (mitotic cells from which chromosomes have been removed) were fused with interphase cells (Sunkara et al., 1980; Rao et al., 1982). Regarding the mitotic activation of centrioles by MPF, evidence is provided by recent studies of isolated centrosome activities in *Xenopus* egg extract. Cen-

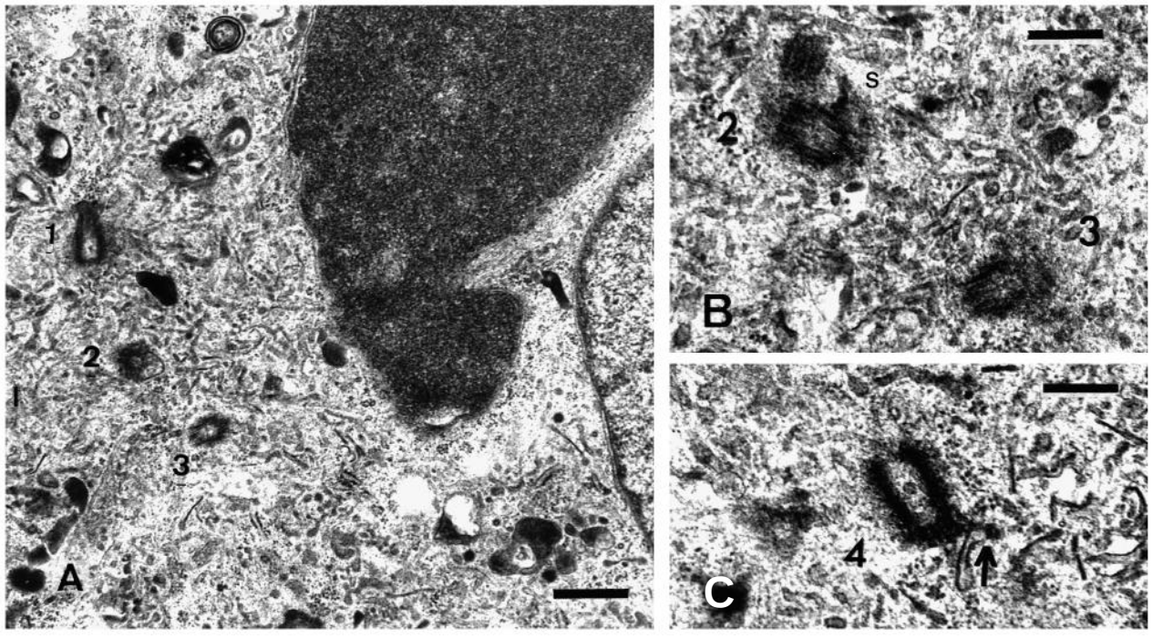

Fig. 4. (A-C) Induction of interphase characteristics on mitotic centrioles of the fused cell containing TLN and G-S stage interphase nuclei (cell no. 1, Table 3). The four centrioles (1,2,3,4) belong to the mitotic nucleus which has been induced to form TLN. The dense body lying near the centrioles of cell no. 2 in (B) is not a daughter centriole or procentriole because, in the consecutive sections, only an homogeneous dense structure was seen which was not differentiated into peripheral microtubular structure and central lighter zone as were observed in Fig. 2A or Fig. 3A,B,C. s, satellite; arrow, appendage. Bars: (A), 0.5 μm; (B,C), 0.25 μm.

trosomes (centrioles) form spindle structures when incubated in egg extract which has been stimulated to the mitotic state by addition of active cdc 2 kinase (Verde et al., 1990) or cyclin (Belmont et al., 1990). Paradoxically in heterokaryon systems consisting of fused thymocyte cell with dividing state mouse oocyte, thymocyte nuclei showed PCC formation, but their centrioles were not involved in spindle formation (Szollosi et al., 1986). Mitotically inactive centrioles lying outside the spindle pole in dividing polykaryons were also reported in some earlier works (Peterson and Berns, 1979; Dey et al., 1989). It should be noted that in the experiments of Peterson and Berns (1979), and Szollosi et al. (1986), nonproliferating cells (G_0) were used in cell fusion. The possibility that some centrioles (mother or daughter) are insensitive to the factor causing mitotic activation cannot be excluded. Quite possibly, some of the cells which we identified as G_1 might actually belong to G_0-stage whose centrioles remained inactive in fused cells. In our study of polykaryons containing only S- or G_2-PCC, all centrioles were mitotically active.

In fused cells, formation of TLN is the fundamentally opposite process of PCC. Nuclear events of TLN formation are very similar to telophase-G_1 transition (Matsui et al., 1972). In the normal mitotic process, telophase is proceeded by the fall of active MPF level (Gerhart et al., 1984), caused by degradation of cyclins (Minshull et al., 1989; Murray and Kirschner, 1989). Presumably, TLN formation in fused cells could be caused by rapid and precocious fall of active MPF which is shown to take place under the influence of interphase cell extract, particularly of G_1- and early S-stages (Adlakha et al., 1983). Loss of mitotic activity of centrioles and disorganization of spindles during TLN induction might be correlated to degradation of active MPF.

The present study shows that centrioles of the fused cells tend to synchronize in a similar manner to heterophasic nuclei. Therefore, the question may arise whether the structural and functional changes of centrioles are directly controlled by nuclei. Some experiments on egg and embryo cells involving enucleation (Sluder et al., 1986) or blocking DNA synthesis (Raff and Glover, 1989) or protein synthesis (Sluder et al., 1990; Gard et al., 1990) indicated that the centriole cycle is independent of the nucleus, but the egg cell and embryo cells are highly specialized and contain reserve pool of centriolar precursors. In dividing somatic cells it seems natural that centriolar precursor materials would deplete after completion of replication and a fresh supply would be required in each cell cycle to commence new replication. This is why in culture cells inhibition of RNA or protein synthesis remarkably suppresses procentriole formation (Stubblefield and DeFoor, 1972; DeFoor and Stubblefield, 1974; Phillips and Rattner, 1976) while inhibition of DNA synthesis does not suppress procentriole formation, but instead hinders their elongation (Rattner and Phillips, 1973). Thus, the nucleus would indirectly influence the centriolar cycle by controlling the synthesis of centriolar materials. When cells fuse, cellular environments or specific factors of different cell partners mix up. In such cells centriolar replication is likely to be controlled by the cytoplasmic pool of precursor materials contributed by the fusing cell partners. Likewise, the functional state of the centriole of fused interphase-mitotic cells is also determined by the mixed cytoplasmic environment which comprises a delicate balance between ‘interphase’ factor and ‘mitotic’ factor. The cytoplasmic factor seems to control the functional state of centrioles through the same pathway as it controls the mitotic state of the nucleus.

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