

# The Intercentriolar Linkage Is Critical for the Ability of Heterologous Centrosomes to Induce Parthenogenesis in *Xenopus*

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**Abstract.** Centrosomes isolated from various sources, including human cells, have the capacity to induce parthenogenetic development when injected into unfertilized amphibian eggs. We recently isolated calf thymus centrosomes and showed that they differ structurally and functionally from previously isolated centrosomes of KE37 cells, in that the two centrioles in calf thymocytes are linearly associated by their proximal ends through a mass of electron dense material and nucleate few microtubules from their distal ends (Komesli, S., F. Tournier, M. Paintrand, R. Margolis, D. Job, and M. Bornens. 1989. *J. Cell Biol.* 109:2869–2878). We report here that these centrosomes are also unable to induce egg cleavage and examine the various possibilities which could account for this lack of competence. The results show that: (a) the kinetics of microtubule assembly on calf thymus centrosomes in *Xenopus* extracts are comparable to those of KE37 centrosomes;

(b) centrosomes isolated from thymus of calves raised under controlled conditions (without anabolic agents) also lack competence; (c) centrosomes isolated from bovine cells of other tissues are competent; (d) centrosomes isolated from thymus of three other species (rat, mouse, and human) are competent. Since the lack of activity of calf thymus centrosomes apparently was not linked to species or tissue differences, we compared the ultrastructure of the centrosomes in the various centrosome preparations. The results show a strict correlation between the linear arrangement of centrioles and the lack of activity of the centrosomes. They suggest that the centrosome cycle can be blocked when the centrioles are prevented from separating into a nonlinear configuration, a step which might be critical for the initiation of procentriole budding. They also indicate that the centrosome may be involved in the G0–G1 transition.

THE centrosome acts as the microtubule-organizing center in most animal cells. A striking feature of this organelle is its duplication cycle which parallels the chromosome duplication cycle during cell growth. The centrosome duplication process continues throughout the cell cycle and several distinct steps in the process that occur at specific times in the cell cycle have been described in somatic cells. These include loss of orthogonal orientation of centrioles in early G1 phase, initiation of orthogonal procentriole budding in late G1 phase, elongation of procentrioles during S and G2 phases, and separation of the two centriole pairs in prophase of mitosis (Kuriyama and Borisy, 1981; Rieder and Borisy, 1982; Vorobjev and Chentsov, 1982; Kochanski and Borisy, 1990).

However, these events in the centrosome cycle have yet to be described in molecular terms, and some of their aspects, such as the orthogonal budding of procentrioles, point to an unprecedented mechanism of duplication. The loss of the orthogonal orientation of centrioles in early G1 phase probably corresponds to the time of establishment of the intercentriolar

link between both parental centrioles (Bornens et al., 1987), and precedes the orthogonal budding of procentrioles. Although the latter are first observed at the G1–S border, when seen they may already represent a stage in elongation rather than the initial step in budding. It is likely in fact that the critical event that triggers centriole duplication cannot be observed directly. However, it is important to know whether that event is also critical for cell commitment to divide. We also do not know whether or not the redistribution of pericentriolar material in G2 phase onto the two duplicated centrosomes is preceded by a duplication mechanism of some kind. The centrosome duplication occurs only once in each cell cycle. How the nuclear and centrosomal cycles are coupled is a key issue. Recent reports have shown that centrosome duplication in early embryos can occur in the absence of the nucleus (Freeman et al., 1986; Sluder et al., 1986; Picard et al., 1988) and independently of the mitotic clock (Gard et al., 1990; Sluder et al., 1990). This suggests that there is an independent control mechanism for centrosome duplication. Nevertheless, mutual feedback controls

between the centrosome cycle and the cytoplasmic oscillator which controls the cell cycle have to be considered (Bailly et al., 1989).

Different strategies have evolved between species to restart the centrosome cycle during early development (for a review, see Bornens et al., 1990). The amphibian egg is a favorable system in which to study the initiation of centrosome duplication since it apparently lacks a functional centrosome. It can be activated by pricking. Cell cycling then resumes as judged by periodic contraction waves in phase with normal cleavages, cycles of DNA synthesis, or cycles of activation-inactivation of the maturation promoting factor (Hara et al., 1980). No cleavage, however, occurs after such activation of the egg. In contrast, cleavages are induced by injecting isolated centrosomes into the egg at the pricking step. These organelles thus appear to be powerful and exclusive parthenogenetic agents in amphibians (Maller et al., 1976). A striking feature of this system is that apparently no species specificity is required for the centrosomes: heterologous centrosomes isolated from sea urchin (Maller et al., 1976), mouse (Karsenti et al., 1984), and man (Bornens et al., 1987) are active in inducing cleavage. Isolated centrosomes are able to interact with the egg cytoplasm and nuclear material to form the first mitotic spindle. The capacity of centrosome to induce egg cleavage was shown to be independent of the stage in cell cycle that the cells were in at the time of centrosome isolation: centrosomes isolated from human cells synchronized at G1 or G2 phases, or centrosomes from peripheral human lymphocytes (G0) were shown to possess similar parthenogenetic activity (Tournier et al., 1989). Therefore, apparently a complete centrosome duplication cycle including the initiation step is triggered in *Xenopus* eggs. Recent experiments on centrosome-induced parthenogenesis have led to the possibility that the whole centrosome acts as a structural template to start the centrosome duplication cycle in *Xenopus* eggs, since no active soluble fraction could be isolated from the centrosomes (Klotz et al., 1990). It was shown that the parthenogenetic activity did not require a centrosome-associated nucleic acid, but was highly sensitive to proteases. Finally, the disorganization of the centriolar triplets was found to correlate with the loss of parthenogenetic activity.

Here we show that centrosomes isolated from calf thymocytes are unable to induce a parthenogenetic development when injected into metaphase-arrested *Xenopus* eggs. Centrosomes isolated from cells of other bovine tissues and from thymocytes of other species were also tested and shown to be competent. Measurements at the optical level of the inter-centriolar distances and examination at EM level of the various centrosome preparations showed that in contrast to the inactive calf thymus centrosomes that have centrioles in a colinear orientation, the centrioles in all the active centrosomes were not oriented linearly. This study establishes that there is a strict relationship between the linear structure of calf thymus centrosome and its lack of parthenogenetic activity.

## Materials and Methods

### Cell Culture

The KE37 human cell line of T lymphoblastic origin (Bornens et al., 1987) was cultivated in RPMI1640 (Eurobio Laboratories, Les Ulis, France) containing 7% calf serum at 37°C, 5% CO<sub>2</sub>.

### Isolation of Bovine Peripheral Lymphocytes

10 liter of bovine blood ( $5 \cdot 10^{-3}$  M EDTA) were diluted with 10 liter H<sub>2</sub>O and mixed for 2 min. NaCl was added to give a 0.9% final concentration and the mixture was centrifuged at 550 g for 13 min (4°C). The pellet was suspended in PBS (1.5 liter final volume) and then diluted with 3 liters H<sub>2</sub>O (4°C). The mixture was allowed to stand for 0.5 min, NaCl was then added to give a 0.9% final concentration. It was then centrifuged at 550 g for 13 min (4°C). The pellet obtained was resuspended in PBS, overlaid on a Percoll gradient (35 ml Percoll, 2 ml MOPS [pH 7.4], NaCl 0.9% q.s.p. 100 ml, divided among five tubes [Falcon Labware, Oxnard, CA]) and centrifuged at 1,400 g for 15 min (4°C). The supernatant was removed and the ring containing lymphocytes was collected with a Pasteur pipette.

### Preparation of Centrosomes

KE37 centrosomes were prepared as described in Bornens et al. (1987). Calf thymocyte centrosomes were prepared as described in Komesli et al. (1989). For centrosomes from thymocytes of other species, the procedure was miniaturized. Human thymocytes were obtained from a piece of thymus that had to be removed during surgery to gain access to the heart of a 4-yr-old child suffering cardiac disease. Six thymuses from 5-wk-old mice, two thymuses from 10-wk-old rats, and 20 g of human thymus obtained were mechanically dissociated in 40 ml PBS (80 ml for human thymus). The cell suspension contained  $\sim 10^9$  thymocytes ( $2 \cdot 10^9$  cells for human thymus). Further steps in the isolation were described in Komesli et al. (1989).  $5 \cdot 10^8$  of the bovine lymphocytes collected were pretreated for 1 h with  $2 \cdot 10^{-7}$  M of nocodazole and 1  $\mu$ g/ml of cytochalasin D. The centrosomes were isolated as described in Komesli et al. (1989), except that the procedure was miniaturized. However, the yield for these centrosomes was always low.

### Preparation of *Xenopus* Eggs Extracts

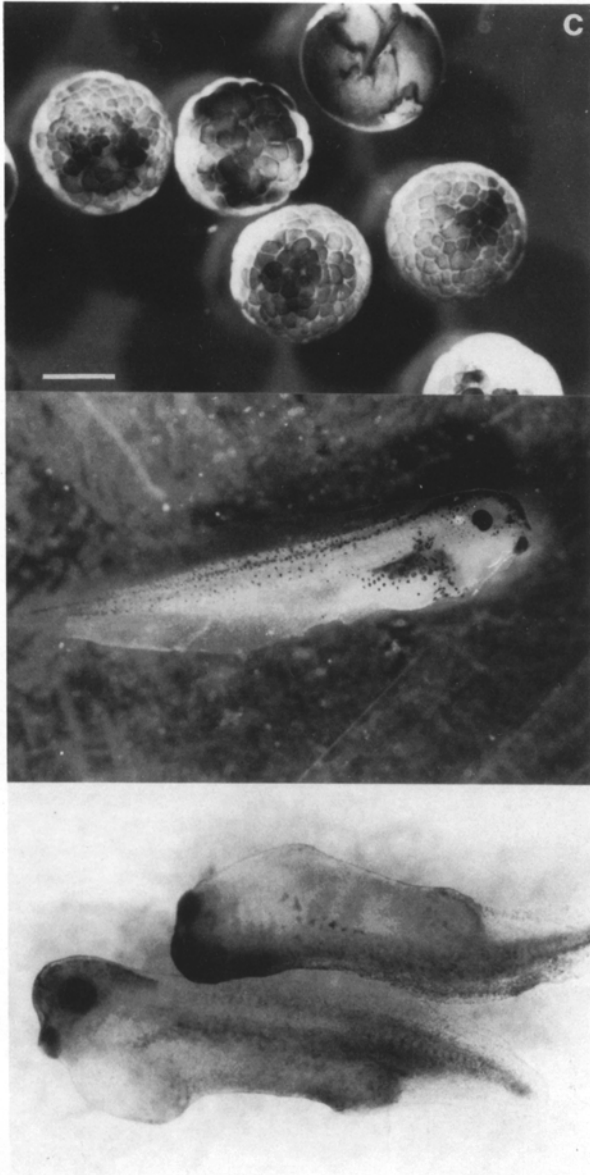
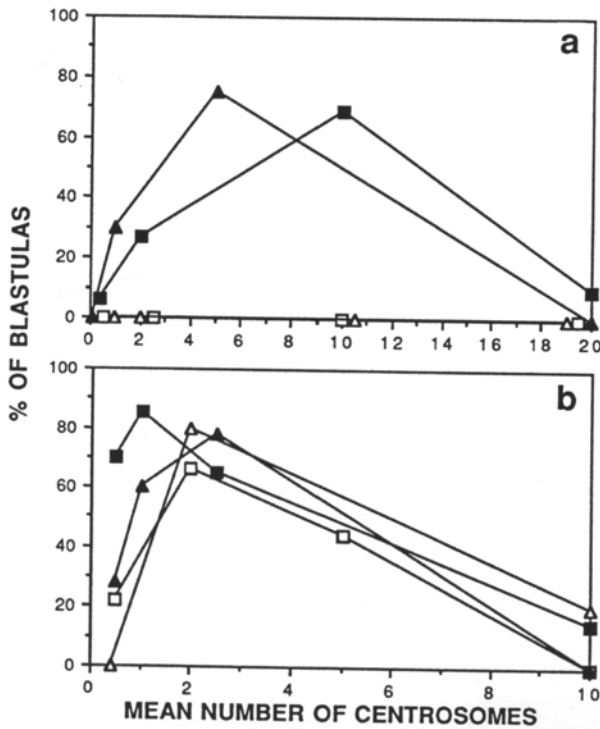
The eggs were dejellied in 2% cystein (pH 7.8), washed three times in 0.25 MMR, activated by an electric shock (2 s, 12 V) and incubated at 20°C in the same buffer for 40 min. Eggs were then washed in acetate buffer (100 mM K acetate, 2.5 mM Mg acetate, and 60 mM EGTA, pH 7.2) and transferred to 5 ml SW 50.1 tubes (Beckman Instruments, Inc., Palo Alto, CA) filled with acetate buffer containing 10  $\mu$ g/ml cytochalasin D and 1 mM DTT. Excess buffer was removed and eggs were centrifuged at 10,000 g for 10 min at 4°C. Each tube was punctured just above the yolk pellet and the supernatant was aspirated. After addition of the ATP regenerating system (1 mM ATP, 10 mM creatine phosphate, 80  $\mu$ g/ml creatine phosphokinase) the lysate was centrifuged at 100,000 g for 60 min at 4°C. The clear and turbid supernatants together were divided into 100- $\mu$ l aliquots at 4°C, frozen in liquid nitrogen, and kept at  $-80^\circ\text{C}$ .

### Assay for Microtubule Nucleation Activity in Interphasic Extracts

Microtubule nucleation on centrosomes in *Xenopus* extracts was assayed according to Tournier et al. (1989). 5  $\mu$ l of centrosomes ( $10^8$  centrosomes/ml) was mixed with 50  $\mu$ l of the extract at 4°C. The mixture was divided into four tubes (17  $\mu$ l per tube) and incubated for various times (1, 5, and 15 min) at room temperature and then fixed by the addition of 0.25% glutaraldehyde to each tube. The fixed material was loaded on 5 ml of 25% glycerol in RG2 buffer (80 mM Pipes/KOH, 1 mM MgCl<sub>2</sub>, 1 mM EGTA, pH 6.8) in 15 ml modified Corex tubes containing a 12-mm round coverslip (Evans et al., 1985). The asters were spun down at 300 g for 15 min. 1 ml of the supernatant was aspirated and the top was washed twice with 1 ml of 1% Triton X-100 in RG2. The coverslips were removed and fixed in  $-20^\circ\text{C}$  methanol for 5 min. Microtubules were visualized by immunofluorescence using a monoclonal anti- $\alpha$ tubulin antibody.

### Measurement of Intercentriolar Distances

An antitubulin antibody (Amersham, Les Ulis, France) was used to decorate the centrioles of the different preparations. Images were recorded on the same TMX 400 Kodak film for all preparations. TMX negatives (24  $\times$  36 mm, magnification 225) were projected on a screen (further magnification 44.4). Intercentriolar distances were measured between the centers of the tubulin-containing dots. The mean and the coefficient of variation of the intercentriolar distance in different isolates were calculated on 100 centrosomes from each source.



### Parthenogenetic Assay

Activity of a given centrosome isolate was assayed as described in Tournier et al. (1989). For each centrosome preparation, 8–12 eggs were injected at four different centrosome dilutions. The centrosome concentration was independently determined by immunofluorescence as described by Mitchison and Kirschner (1984).

### Electron Microscopy

Isolated centrosomes from “biological” calf thymocytes (bCT)<sup>1</sup> were sedimented onto coverslips and processed as described elsewhere (Bornens et al., 1987), except that they were treated for 20 min with 0.5% tannic acid (Simionescu and Simionescu, 1976) between glutaraldehyde and osmic acid fixation. Centrosomes in situ were examined in bovine lymphocytes (BL), rat thymocytes (RT), and mouse thymocytes (MT) as described in Komesli et al., (1989). The number of centrosomes observed in each case and the orientation of their centrioles was recorded.

In addition, centrosomes isolated from human thymocytes (HT) were observed in frozen-hydrated preparations according to the procedure described by Wade et al. (1990).

### Results

#### Calf Thymus Centrosomes Are Incompetent to Induce a Parthenogenetic Development in *Xenopus* Eggs

The parthenogenetic activity of calf thymus centrosome preparations was assayed by injecting increasing numbers of centrosomes into unfertilized *Xenopus* eggs. The centrosome concentration in each preparation was determined by double immunofluorescent staining. At none of the different concentrations of centrosomes injected (from <1 to >50 centrosomes per egg) were the calf thymus centrosomes able to induce egg cleavage (Fig. 1 a), whereas KE37 centrosomes injected as controls were maximally active in the same experiments for a mean number of five centrosomes injected. We were concerned by the possibility that calves from slaughter houses had been treated with anabolic agents to accelerate their growth (a practice that is legal up to a certain dosage), since thymus could be one of their targets. Centrosomes were therefore also prepared from untreated thymuses of two calves raised under controlled conditions. These thy-

1. Abbreviations used in this paper: bCT, biological calf thymus; BL, bovine lymphocytes; CT, calf thymus; CV, coefficient of variation; HT, human thymocytes; MT, mouse thymocytes; RT, rat thymocytes.

**Figure 1.** (a) Comparison of the parthenogenetic activity of centrosomes isolated from calf thymus and from bovine lymphocytes. CT ( $\Delta$ ) and bCT centrosomes ( $\square$ ) were unable to induce cleavage at any of the dilutions used, up to an average of 100 centrosomes (not shown). Centrosomes isolated from BL- ( $\blacksquare$ ) induced egg cleavage. The maximum number of blastulas were obtained with an average of 10 centrosomes injected per egg. ( $\blacktriangle$ ) KE37 centrosomes. (b) Parthenogenetic activity of centrosomes isolated from thymocytes of different species. Rat ( $\square$ ), mouse ( $\blacksquare$ ), and human ( $\Delta$ ) thymus centrosomes were active in inducing egg cleavage. Optimal results were obtained with an average of one to three injected centrosomes. ( $\blacktriangle$ ) KE37 centrosomes. For a and b, purified centrosomes were injected into unfertilized *Xenopus* eggs at various concentrations. 6 to 13 eggs were injected for each point. The number of blastulas was recorded 4 h after the injection. KE37 centrosomes were injected as a positive control, in all experiments. (c) Four blastulas and one tadpole obtained after injection of an egg with an average of one centrosome from MT. Two tadpoles obtained after injection of two eggs with an average of one centrosome each from human lymphoid cell (KE37). Bar, 0.6 mm.

muses were significantly less sclerotic and yielded a significantly higher number of thymocytes per gram of tissue. However, the centrosomes isolated from these thymuses (bCT) likewise did not induce parthenogenetic development (Fig. 1 a).

#### **Centrosomes Isolated from Other Bovine Cell Types Are Competent**

The possibility that this result was specific for the bovine species seemed unlikely since centriole-containing fractions from sea urchin eggs (Maller et al., 1976), and centrosomes from rodent (Karsenti et al., 1984) and human cells (Bornens et al., 1987; Tournier et al., 1989; Klotz et al., 1990) were shown to be active. To confirm this we examined centrosomes isolated from BL (Fig. 1 a) and cells from the bovine cell line LB96 (not shown), and found that both were able to induce parthenogenetic development.

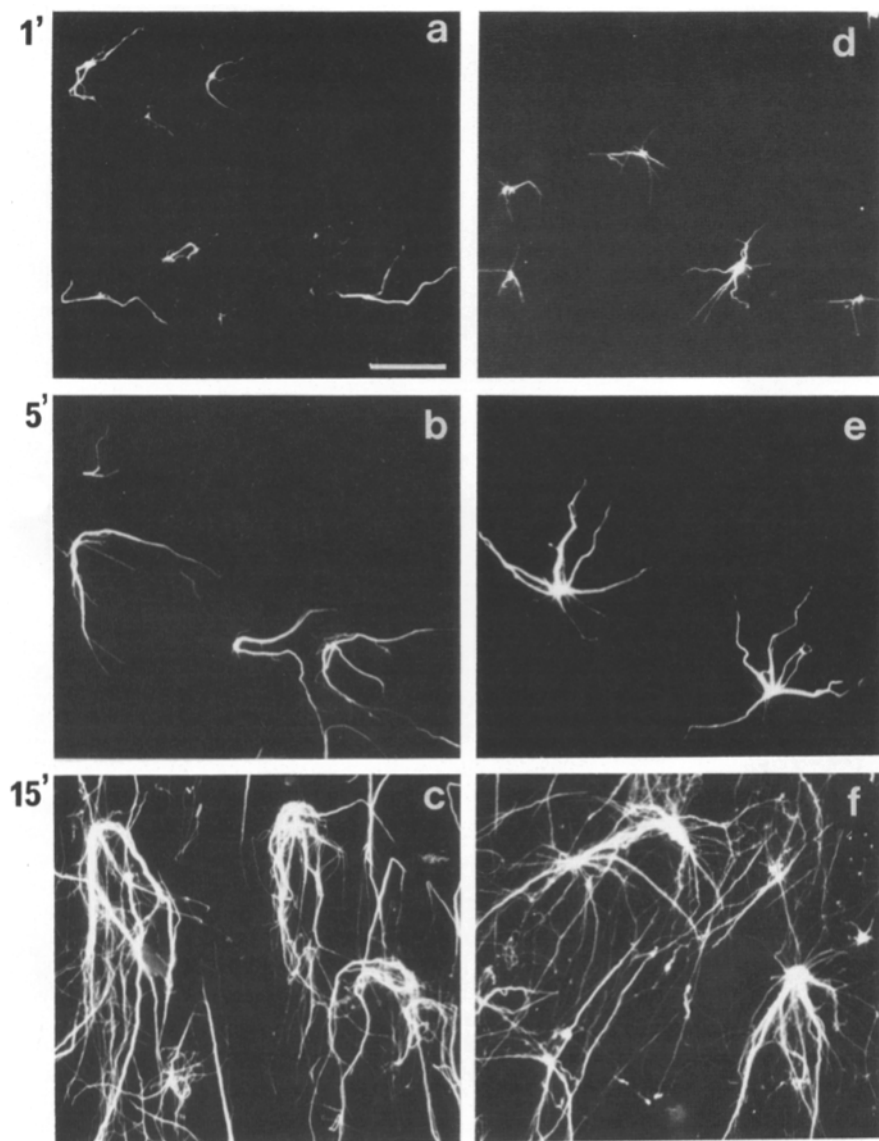
#### **Thymocyte Centrosomes from Other Species Are Competent**

The inactivity of calf thymocyte centrosomes could reveal a

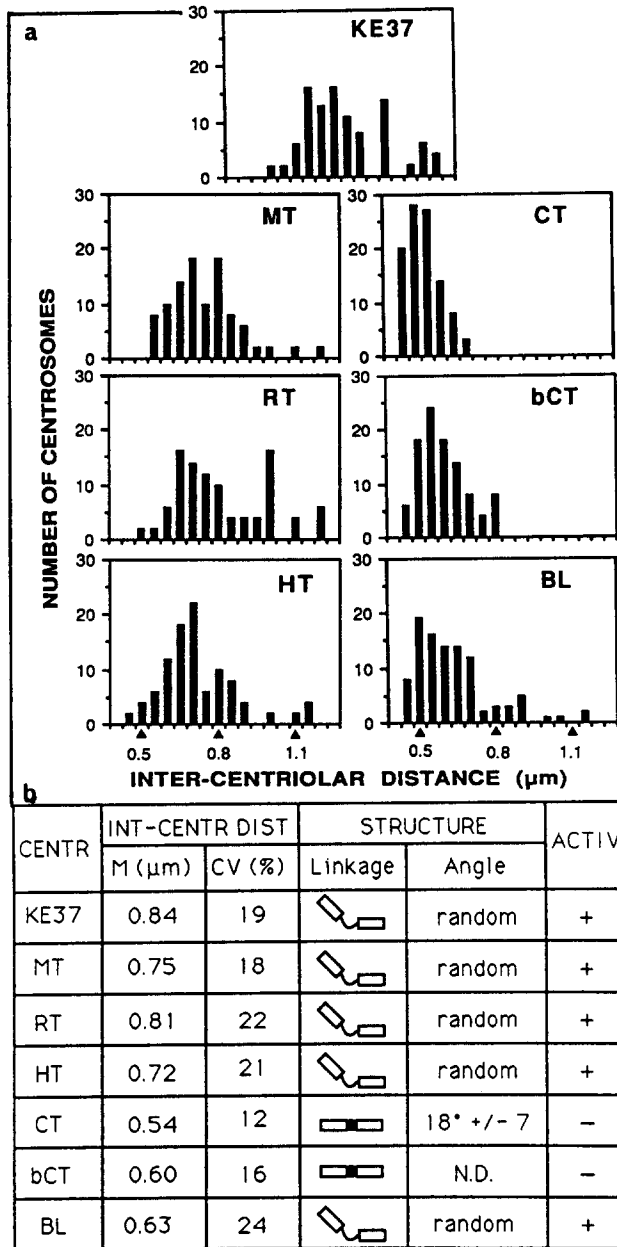
specific feature of thymic differentiation. We therefore isolated centrosomes from MT, RT, and HT, in which >85% of the cells were in the G0/G1 state (not shown), and assayed them for their parthenogenetic activity. The centrosomes from thymocytes of all three species were active (Fig. 1, b and c). We conclude therefore that the inactivity of the calf thymus centrosomes was not correlated with their thymic origin.

#### **Calf Thymus Centrosomes Form Microtubule Asters in Interphasic *Xenopus* Extracts**

We studied the microtubule nucleating activity of calf thymus centrosomes in frog extracts since parthenogenetic development induced by centrosomes implies that the latter assemble microtubules from the egg tubulin and interact with the egg pronucleus. Calf thymus centrosomes nucleated significantly fewer microtubules than centrosomes from human lymphoid cell line (KE37); the rate of microtubule elongation however was similar in both cases (Fig. 2), although the process did show a directional bias, in agreement with previous observations of in vitro nucleation (Komesli et al., 1989).



**Figure 2.** Kinetics of tubulin assembly on centrosomes isolated from calf thymus and from KE37 cells in interphasic extracts from *Xenopus* eggs. Centrosomes were incubated in the extracts at room temperature from 1 to 15 min. (a-c) Calf thymus centrosomes. (d-f) KE37 centrosomes. After 1 (a and d) and 5 min (b and e) of incubation, calf thymus centrosomes nucleated significantly fewer microtubules than KE37 centrosomes. The rate of microtubule elongation however was similar. After 15-min incubation (c and f), large asters with numerous microtubules were observed in both cases, suggesting that by this time the nucleating activity of calf thymus centrosomes had been complemented by egg nucleating material. Bar, 10  $\mu$ m.



**Figure 3.** (a) Histograms of the intercentriolar distances in centrosomes isolated from different cell types (see Materials and Methods). Note the differences in the distributions between calf thymus centrosomes (*CT* and *bCT*) and others. *KE37*, centrosomes from the human lymphoid cell line *KE37*; *MT*, mouse thymus centrosomes; *RT*, rat thymus centrosomes; *HT*, human thymocyte centrosomes; *BL*, bovine peripheral lymphocyte centrosomes. (b) Relationship between the intercentriolar distance, the centrosomal structure and their parthenogenetic activity. The mean of the intercentriolar distance (*M*, μm) and its coefficient of variation (*CV*, %) in the centrosomes of different isolates are indicated. Note that the *CV* of the intercentriolar distance in bovine peripheral lymphocyte centrosomes indicates a distribution that is quite different from that in calf thymus centrosomes. An ultrastructural study of the centrosomes either isolated or in situ showed that the calf thymus centrosomes previously described (Komesli et al., 1989), and *bCT* centrosomes had a colinear organization of their two centrioles, whereas in all other centrosomes they did not have this orientation (see column linkage). For calf thymocyte centrosomes, either in situ or isolated, the centrioles were basically colinear, associated through a filled electron dense material. In situ, the average angle

However, these differences were only detectable during the first 5 min. After 15-min incubation, large asters with numerous microtubules were observed in both cases, suggesting that the nucleating activity of calf thymus centrosomes had been complemented by nucleating material from the egg. We therefore provisionally concluded that microtubule nucleation was not the limiting factor responsible for their incompetence in the parthenogenetic assay.

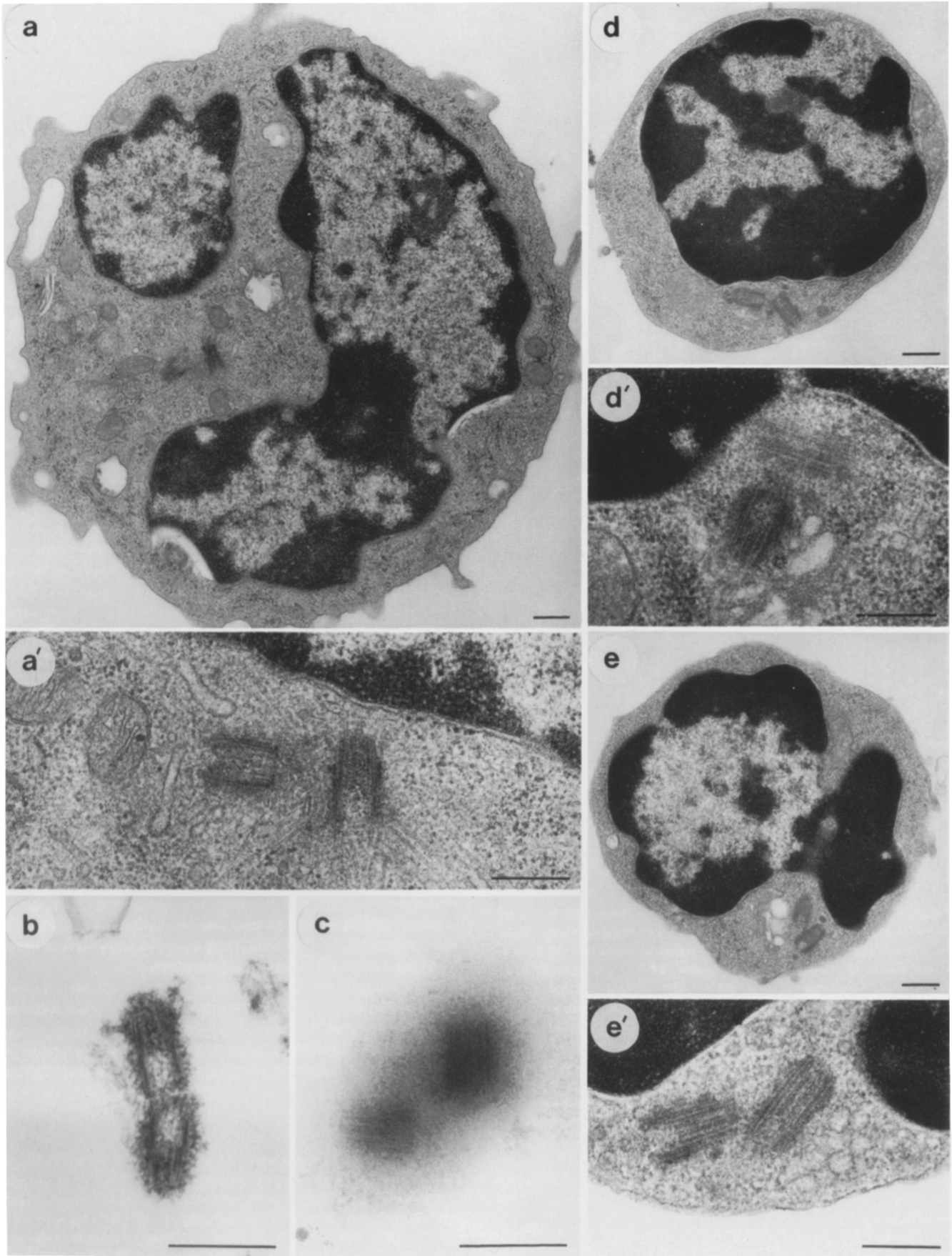
### Structure-activity Relationship of Isolated Centrosomes

A major characteristic of calf thymus centrosomes is the linear association of their two centrioles through a mass of electron-dense material. Since the inability of calf thymus centrosomes to induce cleavage was apparently not linked to a particular species or tissue, we envisaged the possibility that the inactivity could be due to their inability to duplicate. It was therefore of interest to study the structure of the different centrosome preparations we had tested in *Xenopus* eggs. Two independent approaches were then used to investigate the possible relationship between the centrosome structure and their parthenogenetic activity.

The first approach was to quantitatively analyze the intercentriolar distance at the optical level in centrosomes isolated from the different species and cell types. We have shown elsewhere (Komesli et al., 1989) that this approach can distinguish centrosomes from different sources. An antitubulin antibody was used to decorate the centrioles of the different preparations. Intercentriolar distances were measured between the centers of the tubulin-containing dots. The mean and the coefficient of variation of the intercentriolar distance in different isolates were calculated on 100 centrosomes from each source. Moreover, the angle between the centriolar axes of calf thymocyte centrosomes was calculated from pictures of both in situ and isolated centrosomes. From the results obtained, we were able to distinguish two different distributions: one, in which the mean and the coefficient of variation were small, as in *CT* and *bCT* centrosomes, and another in which the mean of the intercentriolar distance was significantly larger and the coefficient of variation indicated a larger range (Fig. 3). Only the centrosomes isolated from peripheral *BL* did not belong to either category: the mean of their intercentriolar distance was small (0.63 μm) as in centrosomes from calf thymus but the coefficient of variation was large (*CV* = 24%) indicating a distribution significantly different from calf thymus centrosomes.

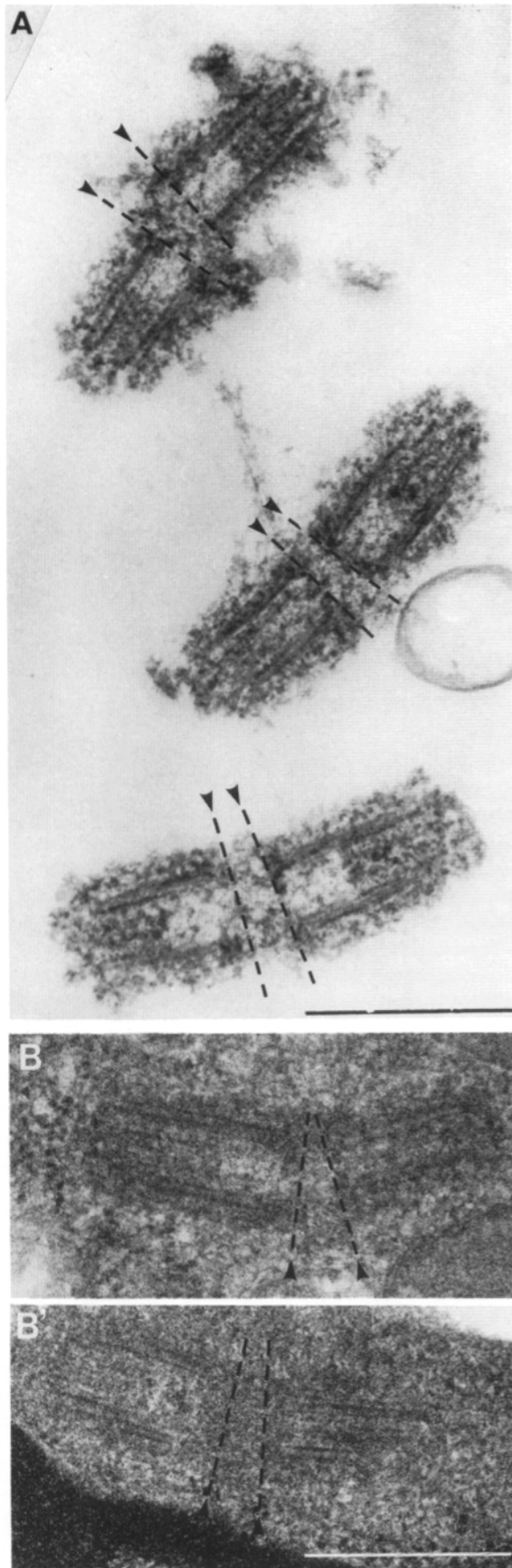
Even though the intercentriolar distance of *BL* centrosomes is similar to that of *CT* centrosomes, it is possible that the centrioles in the *BL* centrosomes rather than being colinear have a different orientation. To determine if this is the case, *BL* centrosomes were examined in more detail by

between the two centriole axes was 18° ± 7 (*n* = 6). In isolated centrosomes, the angle was comparable (13° ± 3; *n* = 7). Larger angles were rarely observed in both cases, up to 45° for isolated centrosomes and 48° for one centrosome in situ. All other centrosomes (*KE37*, *MT*, *RT*, *HT*, *BL*) had nonlinear centrioles with angles at random. Centrosomes active (+) in the parthenogenetic assay all have nonlinear centrioles, whereas colinear centrioles are inactive (-).



**Figure 4.** Structural organization of centrioles in centrosomes in situ (*a, a'*, bovine lymphocytes; *d, d'*, rat thymocytes; *e, e'*, mouse thymocytes), and in centrosomes isolated from bCT (*b*), and from human thymocytes (*c*). All centrosomes display a nonlinear arrangement of centrioles, except for bCT centrosomes (*b*) in which tightly associated centrioles show a linear organization. Bars, 0.5 μm.





**Figure 5.** Calf thymocyte centrosomes have a basically colinear orientation of their two centrioles both in situ and after isolation.

electron microscopy and compared to those in the other cell types either after their isolation or in situ. Centrosomes from rat and mouse thymocytes, and from peripheral bovine lymphocytes were examined in situ, and sections of cells showing either the two centrioles or longitudinal sections of one centriole were recorded (Fig. 4, *a*, *d*, and *e*). A total of 12 centrosomes from BL, as well as 15 from RT and 7 from MT, were examined, and none of them showed a colinear orientation of their two centrioles. In addition, in the 20 sections of BL, as well as in the 16 RT and 4 MT centrosomes, in which only one centriole cut longitudinally was present, no evidence of the other centriole was found, suggesting that their alignment is not colinear. As already reported, the linear configuration of CT centrosomes did not result from the isolation procedure as it could also be observed in situ (Komesli et al., 1989 and Fig. 5).

To determine if centrosomes from bCT were similar or different from those of calf thymocytes in their alignment of centrioles, the isolated centrosomes from this source were examined (Fig. 4 *b*). In bCT, the percentage of centrosomes that had the two centrioles in a linear configuration was ~85% and was comparable to that previously described for calf thymus centrosomes (Komesli et al., 1989). In contrast, in the 22 human thymocyte centrosomes examined, colinear arrangement of centrioles was never found (Fig. 4 *c*). The structure of KE37 centrosomes has been already described (Bornens et al., 1987; Klotz et al., 1990).

When the structure of centrosomes in the different isolates was compared with their parthenogenetic activity (Fig. 3 *b*), a correlation between structure and activity of the centrosome was evident. The centrioles associated through a mass of electron dense material in a linear configuration were inactive, whereas those which were not linearly aligned were active.

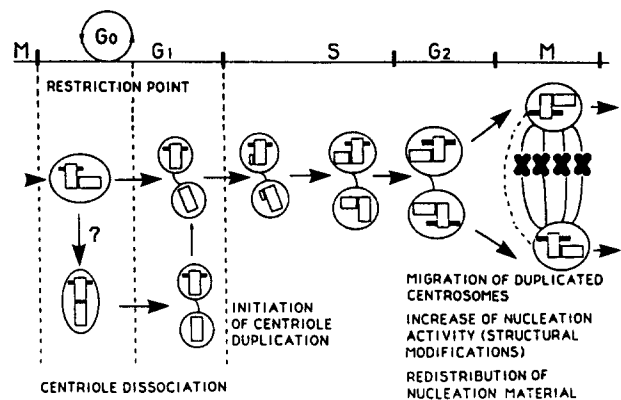
### Discussion

We have recently achieved mass isolation of centrosomes from calf thymocytes and have observed that the centrosomes obtained show linear biased growth of microtubules from the distal ends of centrioles. These centrosomes have linearly arranged centrioles. We showed that this association was not an artifact of isolation, as calf thymocyte centrosomes in situ had the same centriolar configuration (Komesli et al., 1989 and Fig. 5). However, calf thymocyte centrioles were not directly jointed side by side, but associated through a mass of electron-dense material that could correspond to a folded form of the inter-centriolar link observed in other cell types (Bornens et al., 1987; Komesli et al., 1989). To investigate the function of these centrosomes, we have assayed their capacity to induce cleavage in metaphase-arrested *Xenopus* eggs. We find that these centrosomes are unable to induce parthenogenetic development, and represent the first example of centrosomes isolated from metazoa incompetent in this respect. Only basal bodies isolated from *Tetrahymena* and *Chlamydomonas* were previously demonstrated to be inactive in the parthenogenetic test (Heideman and

(A) Isolated centrosomes. (B, B') centrosomes in situ. The broken lines and arrowheads show that, in both cases the two centrioles are associated through a mass of electron-dense material. Bars, 0.5  $\mu$ m.

Kirschner, 1975). We tested the different hypotheses that could explain the inactivity of these isolates. It seems that neither the species specificity nor their thymic origin are apparently involved in this peculiarity: since centrosomes isolated from peripheral bovine lymphocytes and centrosomes from mouse, rat, or human thymocytes were active (Fig. 1). Direct biochemical comparisons of active and inactive centrosomes is unlikely to be straightforward since previous studies suggested many differences in their protein composition (Komesli et al., 1989).

A likely requirement for centrosome-induced parthenogenesis is the duplication of the foreign centrosome (Tournier et al., 1989). We therefore considered the possibility that the lack of parthenogenetic activity of calf thymus centrosomes reflects their inability to duplicate, a possibility that could be related to the fact that most of the cells in thymus are in the G<sub>0</sub>-G<sub>1</sub> state (Rothenberg and Lugo, 1985). The pathways that lead from the activation of a quiescent cell to cell growth remain largely unknown. It has been suggested however that the centrosome could be one of the elements involved in growth control or cell commitment (Tucker et al., 1979; Sherline and Mascardo, 1982). A situation is known in yeast where the initiation of spindle pole body duplication takes place at START, a point at which other crucial cell cycle functions are also initiated (Byers and Goetsch, 1975). However, we previously showed that centrosomes isolated from human peripheral lymphocytes, another example of quiescent cells, were able to induce egg cleavage (Tournier et al., 1989). Therefore, the relationship, if any, between centrosome activity in the parthenogenetic assay and the quiescence of the cells from which they come is not simple. The mechanisms involved in the duplication of centrosomes are not understood. Centrosomes double in number in each cell cycle by orthogonal budding from the wall of the parent centrioles by a mechanism which is totally unclear. It has been recently confirmed that centriole distribution to daughter cells occurs by a semi-conservative process (Kochanski and Borisy, 1990). A pair of centrioles begins the cycle in an orthogonal orientation, i.e., at this stage the mother and daughter centrioles are still associated in the budding configuration. They dissociate and thereby lose their orthogonal relationship in early G<sub>1</sub> phase, the resultant single centrioles becoming the parental ones in the next generation (Kuriyama and Borisy, 1981; Kochanski and Borisy, 1990). Procentrioles assemble orthogonally to the parental centrioles in late G<sub>1</sub> phase, and then elongate during S and G<sub>2</sub> phases. A simple working hypothesis (see scheme in Fig. 6), which fits a large body of observations, is that the initiation of procentriole budding cannot occur as long as the two centrioles remain associated with each other. Accordingly the dissociation event may be important in the control of centrosome duplication. As long as the centrioles remain orthogonally associated, they cannot initiate a new round of duplication (Fig. 6). The centrosome cycle would overlap segments of two successive cell cycles. This possibility has already been suggested for other cell cycle functions: G<sub>1</sub> activities, for example, may begin during the previous cell cycle, concurrently with G<sub>2</sub> and mitotic events (Pardee, 1989). The overlap would ensure that the centrosome duplication occurs only once in each cell cycle, an absolute requirement for cell survival. In the linearly associated centrioles in calf thymus centrosomes, an intermediary step leading towards dissociation/disorientation of



**Figure 6.** Scheme for centrosome duplication during the cell cycle. We propose that initiation of procentriole budding cannot occur as long as the two parent centrioles remain closely associated with each other. Their dissociation would be necessary but not sufficient for the initiation of the budding of new centrioles. In calf thymus, the linear organization of centrioles and their close association would represent a block at an intermediary step in the dissociation of the two parent centrioles and the initiation of centrosome duplication. The curved broken line between the poles in mitosis represents our ignorance of the fate of the link between the two centriole pairs during early steps of mitosis. The black squares coming off the centriole's sides represent the distal arms of the pericentriolar material. Note that centrioles are represented as being associated through a mass of electron-dense material.

the centrioles may be blocked and the centrioles may therefore be unable to act as parental centrioles. In contrast, in centrosomes from G<sub>0</sub> cells such as human peripheral lymphocytes (Tournier et al., 1989) the centrioles are dissociated and are able to initiate procentriole budding (Fig. 6).

The G<sub>0</sub>-G<sub>1</sub> transition is a complex process in which some authors distinguish at least four subphases: competence, entry, progression, and assembly. Progression depends on the effects of limiting growth factors, nutrients or inhibitors, while assembly is a subphase in which neither protein synthesis nor serum seem to be required (Pardee, 1989). Our results suggest that a discrete structural event at the centrosome could be one of the landmarks in the progression. Since only a single centrosome is present in each cell, the events controlling the initiation of procentriole budding might well be important in determining the point at which the cell becomes committed to divide.

We are now trying to correlate the thymus centrosome activity with the stages in the thymic ontogenesis. It is possible that the contrasting results obtained with thymus centrosomes from various species might be related to differences in the differentiation schedule of their thymuses (a 3-mo-old calf thymus might not be comparable to that of a 5-wk-old mouse). A precise description of the steps in the duplication of the injected centrosome is also necessary, since it is essential for the formation of a functional mitotic spindle. In vitro assays for centrosome duplication should allow us to identify the factors controlling the initiation of procentriole budding and to test the capacity of the various centrosome preparations to duplicate. Recent results (Tournier, F., M. Cyrklaff, E. Karsenti, and M. Bornens, submitted for publication) show that this goal is feasible and that calf thymus centrosomes are indeed unable to initiate centriole budding under



conditions in which other centrosomes, competent to induce egg cleavage, are able to do so.

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