DEPENDENCE OF CENTRIOLE FORMATION ON PROTEIN SYNTHESIS

STEPHANIE GORDON PHILLIPS and J. B. RATTNER

From the Department of Anatomy, Washington University, St. Louis, Missouri 63130 and the Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92664. Dr. Phillip's present address is the Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, New York 10032. Dr. Rattner's present address is the C.N.R.S. Centre de Genetique Moleculaire, Gif-sur-Yvette, France 91190.

ABSTRACT

Centriole formation was studied after inhibition of protein synthesis for various portions of the cell cycle. Synchronous populations of mitotic L929 (mouse) cells were plated into petri dishes and the course of procentriole formation was monitored by electron microscope analysis. The frequency with which procentrioles were seen in association with mature centrioles normally increased steadily in the interval from 4 to 12 h after mitosis. The formation of procentrioles was abruptly inhibited by the addition of cycloheximide at any time from mitosis until 12 h postmitosis (S phase). This suggested that the formation of procentrioles was dependent upon protein synthesis immediately before their appearance. Prophase-associated elongation of procentrioles appeared to occur normally in cells treated with cycloheximide for up to 4 h before prophase, though the mitotic index in treated cultures decreased somewhat. Thus, protein synthesis did not appear to be essential for procentriolar elongation to the mature length.

The synthesis of centrioles is under tight control by the cell. Generally, a cell makes exactly two centrioles in the course of a cell cycle. Greater numbers of centrioles may be generated if needed as basal bodies when the cell is preparing to generate cilia (5, 10, 25). The time of centriole production has been worked out in proliferating cultured fibroblastic cells (15, 16; see reference 3 for review). It is found that each daughter cell of a division receives a pair of orthogonally arranged, full-length (about 0.55 μm long) centrioles. The two centrioles separate from each other in G_1 and, near the onset of S phase, one daughter procentriole appears adjacent and perpendicular to each mature centriole. The procentrioles are about half the length of a mature centriole and remain that length until prophase, when they elongate to the mature length (15, 17). Thus, the pair of centrioles at the pole of the metaphase spindle again consists of two full-length centrioles. The mechanism by which the cell maintains such tight control on the time of centriole duplication and the quantity and size of procentrioles is not known.

In an earlier study (15), we used an inhibitor of DNA synthesis to dissociate the event of nuclear DNA synthesis from procentriole production. It appeared that procentriole formation occurred independently of whether or not DNA synthesis was allowed to proceed. On the other hand, the process of elongation of procentrioles to their mature length appeared to be strictly correlated with the onset of prophase events. In this paper, we present the results of experiments which test the requirements for protein synthesis in procentriole

formation. Centrioles are probably composed principally of protein (20), though difficulties in purifying these small organelles has made it difficult to determine their precise composition. It has been suggested that they contain nucleic acid as well as protein (13, 23; see reference 9 for review). We hypothesized that there might be a time during the cell cycle when centriolar proteins were synthesized. Experiments were designed to inhibit protein synthesis at specific periods of the cell cycle and to analyze the effect on procentriole formation and elongation.

MATERIALS AND METHODS

Cell Maintenance and Synchronization

Experiments utilized a recently cloned derivative of L929 cells (an established fibroblastic mouse line) which were originally obtained from the American Type Cell Culture Collection, Rockville, Md. Cells were routinely maintained as monolayer cultures in glass bottles and were subcultured by trypsinization every 4 days. They were grown in Joklik-modified minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) with 10% fetal calf serum in the presence of 5% CO_2.

Experiments were performed in subconfluent cultures growing in Blake bottles in 40 ml of medium. Mitotic cells were harvested by mechanical selection (29). Debris was removed and the medium was replaced with fresh medium twice at 1-h intervals before beginning an experiment. Thereafter, mitotic cells were harvested at intervals of 75 min. Mitotic indexes of cells obtained in this manner were ascertained for every sample at the time of harvest by fixing an aliquot of the cells with three parts methanol: one part acetic acid, air drying the cells on slides, and staining them with Giemsa's. Mitotic indexes were generally about 90%. Cultures with mitotic indexes lower than 80% were discarded. Immediately after harvest, mitotic cells were centrifuged into a pellet, resuspended by pipetting, and plated into 35 × 10 mm Lux plastic petri dishes (Lux Scientific Corp., Thousand Oaks, Calif.).

Electron Microscope Analysis

Monolayer cultures growing on plastic were fixed *in situ* at intervals after mitosis with 3% glutaraldehyde in 0.1 M collidine buffer at pH 7.4. After 1 h, the cultures were washed twice with collidine buffer and postfixed for 1–2 h in 1% osmium tetroxide solution buffered as described above. The cultures were then dehydrated in a graded ethanol series and embedded in Epon 812 (2).

Epon disks obtained by the procedure described above were examined with a 100 × phase, oil immersion objective lens. Selected regions were scored with a diamond marker and cut from the Epon disk. Serial sections in the silver range were cut on an LKB ultramicrotome III (LKB Instruments, Inc., Rockville, Md.) and collected on copper-slotted grids coated with a solution of 1% nitrocellulose in amylacetate. They were examined in a Siemens Elmiskop I microscope operated at 60 kV.

Synchronized cultures fixed at the following times after mitosis were examined by electron microscopy: 6 h, 8 h, 9 h, 10 h, 10.5 h, 11 h, 12 h, 12.5 h, 13.5 h, 14.5 h, 15.5 h, and 16 h. Cycloheximide (Sigma Chemical Co., St. Louis, Mo.) at 25 μg/ml was added to experimental cultures for various lengths of time before fixation and was present until the time of fixation.

For quantitation of the effect of cycloheximide on procentriole formation, a system was devised for monitoring cells fixed at a given time postmitosis for the presence or absence of procentrioles. Random sections were taken from blocks of cells from various places in a petri dish culture. Every time a profile of a centriole was encountered, it was categorized according to its orientation and to whether a procentriole was also transected in the same section. Seven categories of centriole profiles were distinguished: (*a*) one mature (full-length) centriole in longitutinal section; (*b*) two mature, separated (nonperpendicular) centrioles in longitudinal section; (*c*) a cross section through one or two centrioles (actually, a very few cases were observed of cross sections through two centrioles in the same cell); (*d*) a longitudinal section through a mature centriole and a cross section through another centriole a little distance away; (*e*) a longitudinal section through two mature centrioles associated in orthogonal array; (*f*) one mature centriole (sectioned either longitudinally or in cross section) with a short procentriole impinging upon it perpendicularly; (*g*) two separated mature centrioles, each with a perpendicularly associated, short procentriole. Categories (*f*) and (*g*) were the two possible configurations in which it was known with assurance that procentriole formation had occurred. The cases of categories (*a*), (*b*), (*c*), (*d*), and (*e*) were ambiguous with respect to procentriole formation. Cross sections through mature centrioles were not distinguishable from cross sections through procentrioles. In addition, a given section could have missed procentrioles which were present elsewhere in the cell. Although no independent analysis was made as to what percent of the time procentrioles were detected when they were actually present, the data obtained in the above-described manner suggested that procentrioles were detected with roughly 40% of the frequency with which they occurred, since the maximum frequency with which they were observed tended to plateau at 30–50% at times when they were probably present in most cells. Regardless of the real frequency of cells containing procentrioles, the method of analysis was adequate to determine whether any given population of cells had a greatly different frequency of procentriole formation than another population.

Inhibition of Protein Synthesis

Cycloheximide was added to the medium in synchronized cultures in petri dishes to give a final concentration

of 25 μg/ml. Experiments were carried out to demonstrate that cycloheximide at this concentration was effective in inhibiting protein synthesis in L929 cells under our experimental conditions as follows: 10^6 cells in 2 ml of medium were plated into replicate 35-mm plastic petri dishes. 24 h later, [^{14}C]leucine, sp act 312 μCi/mmol, 0.25 μCi/ml was added to the culture. Cycloheximide, 25 μg/ml or 100 μg/ml, was simultaneoulsy added to experimental dishes. Incorporation was stopped after 15 min, 30 min, 1 h, 2 h, 4 h, or 8 h by withdrawing the medium, washing briefly with cold (4°C) Hanks' balanced salt solution, and adding cold 0.2 M perchloric acid for 2 h. Dishes were washed with cold 80% ethanol and dried. The bottoms were punched out and counted on a planchet counter. All dishes were set up in triplicate.

RESULTS

Inhibition of Protein Synthesis

Experiments were performed to determine a dosage of cycloheximide for inducing adequate inhibition of protein synthesis without producing generalized cell damage. It was observed that treatment of cells for 2 h with 600 μg/ml of cycloheximide was so detrimental to the cells that adverse effects were readily detectible by electron microscope examination. It was not necessary to use such high levels of cycloheximide, however, since 25 or 100 μg/ml decreased the rate of [^{14}C]leucine incorporation by about 95% as calculated from the slopes of the plots of determinations made 1–8 h after addition of the drug (Fig. 1). We decided to use the lower level of cycloheximide (25 μg/ml) in all experiments on centriole formation since the increment in level of inhibition was small in relation to the increase in dosage of cycloheximide, and we felt that a lower level of cycloheximide was less likely to cause generalized damage to the cell.

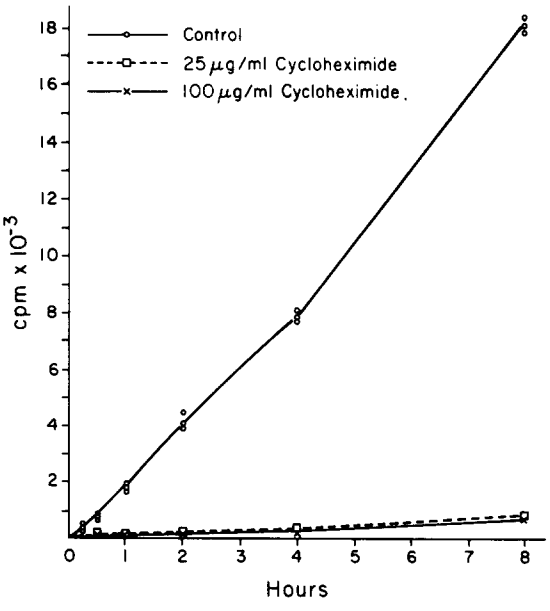


FIGURE 1 Perchloric acid-insoluble incorporation of [^{14}C]leucine in control cultures and in the presence of 25 μg/ml of cycloheximide or 100 μg/ml of cycloheximide.

Timing of Procentriolar Protein Synthesis

The cell cycle time of the clone of cells used in these experiments averaged about 19 h under the conditions used for synchronization (15). Half the cells began S phase by about 9 h after mitosis. Each cell entered G_1 with a pair of mature centrioles, and procentrioles appeared in association with each mature centriole at about the time the S phase started. These observations on the normal course of procentriole formation were quantitated in the present experiments (Table I). At 6–8 h after mitosis, about 2% of sections which passed through centrioles also transected adjoining procentrioles (Table I). By 12 h after mitosis, 30–50% of the sections through centrioles included adjoining procentrioles (Tables I, II, III, and IV), and the frequency with which procentrioles were found in association with mature centrioles remained between 30% and 50% in cells fixed at 13.5, 14.5, 15.5, and 16 h after mitosis (Tables III and IV). By about 14 h after mitosis, some cells were seen entering mitosis, and after 16 h, a significant proportion of cells were again in G_1 so that the percent of cells with procentrioles fell below 30% (Table IV). Therefore, it was not practical to continue experiments beyond this time. Generally, 50 sections through centrioles were scored for each experimental point; nevertheless, considerable variability occurred from one experiment to the next in the frequency with which procentrioles were seen. For instance, in six different cultures fixed between 12.5 and 13.5 h after mitosis, the average frequency with which procentrioles were seen was 37%, with a range from 22% to 52%. This variability reflects statistical variation due to the necessarily small sample sizes (50 centrioles) as well as differences in the growth rate of cells presumably due to variations in culture conditions from one experiment to the next. In spite of these variations, within any one experimental set the percent of cells with procen-

TABLE I

Inhibition of Procentriole Formation by Addition of Cycloheximide from 6–10 h after Mitosis

Time of cycloheximide addition: h post-mitosis	Time fixed:h post-mitosis	No. of cells scored	Synchrony: mitotic cells at time 0	Centriole profile observed: a \	b / \	c o (rarely oo)	d | o	e |⁻	f |⁻/o⁻	g |⁻ o⁻	Centriole profiles with procentriole*
			%								%
–	6	50	82	26	1	21	0	0	2	0	4
–	8	50	95	28	0	21	0	0	1	0	2
–	10	50	84	22	3	14	1	0	10	0	20
–	12	100	84	41	2	22	0	0	34	1	35
6	12	50	82	25	0	24	0	0	1	0	2
8	12	50	95	23	3	20	2	0	2	0	4
10	12	43	90	22	2	17	0	0	2	0	5
–‡	12	50	90	25	0	24	0	0	1	0	2

Each table represents one experiment.

* The last column is derived from adding together all profiles in which procentrioles were observed (configurations 6 and 7) and dividing by the number of cells scored (given in third column).

‡ *Colcemide* added at 10 h.

TABLE II

Inhibition of Procentriole Formation by Addition of Cycloheximide up to 8 h after Mitosis

Time of cycloheximide addition: h post-mitosis	Time fixed: h post-mitosis	No. of cells scored	Mitotic cells at time 0	Centriole profile observed: a \	b / \	c o	d | o	e |⁻	f |⁻/o⁻	g |⁻ o⁻	Centriole profiles with procentriole*
			%								%
–	9	50	97	20	8	14	0	0	0	8	16
–	10.5	50	100	16	2	15	0	0	14	3	34
–	12	50	88	10	0	11	3	0	22	4	52
0	12	50	98	33	2	15	0	0	0	0	0
4	12	30	82	21	0	9	0	0	0	0	0
8	12	50	97	27	7	16	0	0	0	0	0

* The last column is derived from adding together all profiles in which procentrioles were observed (configurations 6 and 7) and dividing by the number of cells scored (given in third column).

TABLE III

Effect of Cycloheximide Addition later than 11 h after Mitosis

Time of cycloheximide addition: h post-mitosis	Time fixed: h post-mitosis	No. of cells scored	Mitotic cells at time 0	Centriole profile observed: *a* \	*b* / \	*c* o (rarely oo)	*d* | o	*e* |–	*f* |–/o–	*g* |– o–	Centriole profiles with procentriole*
			%								%
–	11	50	95	17	1	17	3	3	8	1	18
–	12.5	61	91	22	1	19	1	1	15	2	28
–	13.5	50	99	16	0	15	1	1	15	2	34
–	14.5	50	94	18	0	17	0	0	15	0	30
–	15.5	30	92	9	0	10	1	0	9	1	33
11	13	58	92	26	0	20	0	0	7	5	21
12.5	14.5	50	94	20	0	15	0	0	14	1	30

* The last column is derived from adding together all profiles in which procentrioles were observed (configurations 6 and 7) and dividing by the number of cells scored (given in third column).

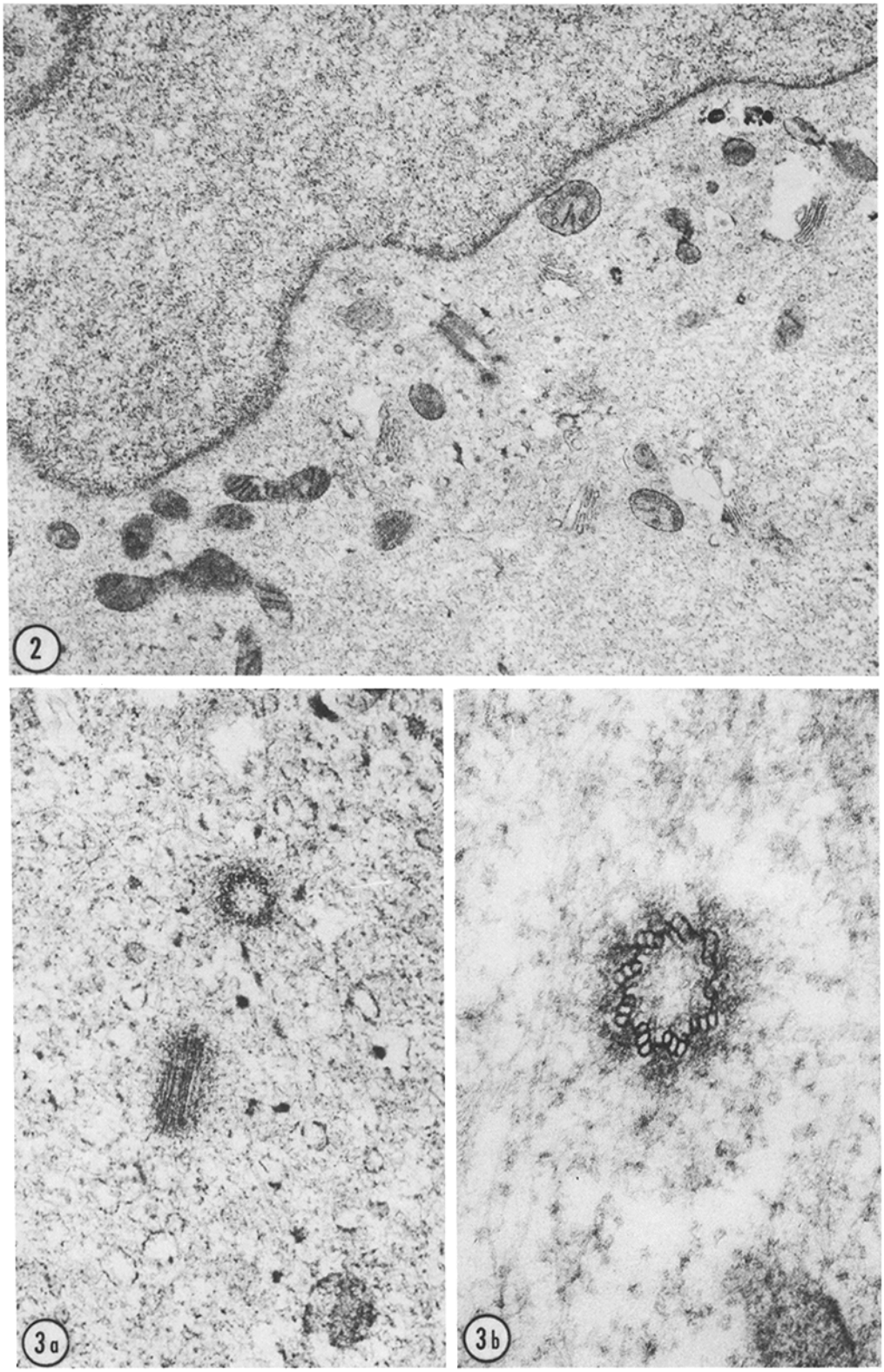
TABLE IV

Effect of Cycloheximide Addition after Completion of Most Procentriole Formation

Time of cycloheximide addition: h post-mitosis	Time fixed: h post-mitosis	No. of cells scored	Mitotic cells at time 0	Centriole profile observed: *a* \	*b* / \	*c* o	*d* | o	*e* |–	*f* |–/o–	*g* |– o–	Centriole profiles with procentriole*
			%								%
–	12	50	93	18	0	15	1	0	15	0	30
–	16	50	85	13	0	18	3	0	18	3	42
–	18‡	60	90	20	2	22	2	4	10	0	17
12	14	50	90	20	0	11	1	0	11	2	26

* The last column is derived from adding together all profiles in which procentrioles were observed (configurations 6 and 7) and dividing by the number of cells scored (given in third column).

‡ Many cells were presumed to be in G_1 of the next cell cycle by this time as many mitotic cells were seen in cultures by 16–18 h after mitotic selection.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3a
3b

trioles was found to increase steadily in the interval from 8 to 12 h after mitosis and then level off.

In experiments where cycloheximide was added to cultures at the time mitotic cells were collected and plated into petri dishes, the cells returned to interphase and attached to the plastic. When cycloheximide remained in the culture from mitosis until the time of fixation 12 h later, the cells had a normal appearance upon electron microscope examination (Fig. 2). As in untreated cells, the nucleus regained interphase morphology with a surrounding nuclear membrane, the chromatin decondensed and the nucleoli reformed, which indicates that these processes can occur under conditions where protein synthesis is severely inhibited. However, if cycloheximide was present from mitosis until fixation 12 h later, no procentrioles were seen (Table II). The mature centrioles appeared to have separated, as they normally do before procentriole formation (Fig. 3 *a*). The mature centrioles had normal morphology (Fig. 3 *b*). Thus, though cycloheximide did not detectably affect mature centrioles, procentrioles did not form when protein synthesis was inhibited for the first 12 h after mitosis.

We attempted to obtain a closer approximation to the time of synthesis of proteins essential to procentriole formation by waiting some time after mitosis before adding cycloheximide. When cycloheximide was added 4 h, 6 h, or 8 h after mitosis and cells were fixed 12 h after mitosis, no (or very few) procentrioles were found in association with the mature centrioles (Tables I and II). Thus, it appeared that procentriole formation was dependent upon *de novo* protein synthesis within a short time (less than 4 h) of the time of formation.

Further experiments demonstrated that the addition of cycloheximide brought additional procentriole formation to a stop rather quickly. When cycloheximide was added to cells at a time when procentriole formation was actively occurring (10 or 11 h after mitosis), fixation 2 h later revealed a halt in the increase in frequency with which procentrioles were seen, though the numbers of procentrioles continued to increase in control untreated cultures (Tables I and III). In some experiments, a decrease in frequency of procentrioles was seen in treated cultures, but this was not consistently the case. When cycloheximide was added at about 12 h after mitosis, the time when the peak number of procentrioles had been reached, no consistent difference was seen in frequency of procentrioles between cycloheximide-treated and control untreated cultures fixed at the same time after mitosis (Tables II, III, and IV).

Prophase Procentriolar Elongation

Centriole formation occurs in two phases. The first event, near the G_1-S boundary, is the appearance of a procentriole next to each parent. This procentriole is about half the length of the mature centriole. The second phase is elongation of the daughter centriole to its mature length, which occurs during the period from the onset of prophase through metaphase. Experiments were set up to examine the effect of cycloheximide on the mitotic events of daughter centriole elongation. In these experiments, cycloheximide was added to random cultures for various lengths of time before fixation, cultures were fixed, and mitotic cells were examined in the electron microscope. It was first determined that cells would continue to enter mitosis in the presence of cycloheximide at the concentration used. It was found that the mitotic index of random cultures fell gradually with increasingly longer times in the presence of cycloheximide, but some mitotic cells were still found after 4 h of cycloheximide treatment (Fig. 4). Mitotic cells initially accumulated in the presence of colcemide and cylcoheximide at about the same rate as with colcemide alone, but, between 3 and 4 h, cycloheximide-induced depression on net entry into mitosis became apparent. Nevertheless, the data suggested that some cells were still entering mito-

FIGURE 2 Normal interphase appearance in cell fixed 12 h after mitotic selection. Though cycloheximide was present in the culture for the full 12 h from the time of mitosis, no obvious abnormalities are seen in chromatin decondensation, nuclear membrane formation, centriole morphology, or general cytoplasmic substructure. × 13,000.

FIGURE 3 Cells from culture treated with cycloheximide from mitosis until the time of fixation 12 h later. Separation of mature centrioles has occurred, but no procentrioles are seen. Morphology of mature centrioles is normal. (*a*) × 13,500; (*b*) × 40, 500.

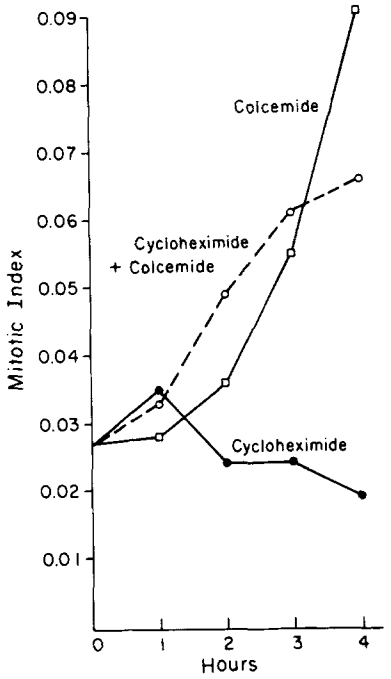


FIGURE 4 Fraction of mitotic cells in cultures treated with cycloheximide, colcemide (1 μg/ml) or cycloheximide (25 μg/ml) and colcemide (1 μg/ml) simultaneously. 3,000 cells, representing three cultures, were scored for each point. Cycloheximide treatment causes a decrease in the number of cells entering mitosis, but there are still cells entering mitosis after several hours in cycloheximide.

sis even after 3 h in cycloheximide. As can be seen in Figs. 5 and 6, daughter centriole elongation occurred normally during prophase even in cells which had been in cylcoheximide for the 3 h preceding prophase. Thus, concurrent protein synthesis did not appear to be necessary for daughter centriole elongation.

DISCUSSION

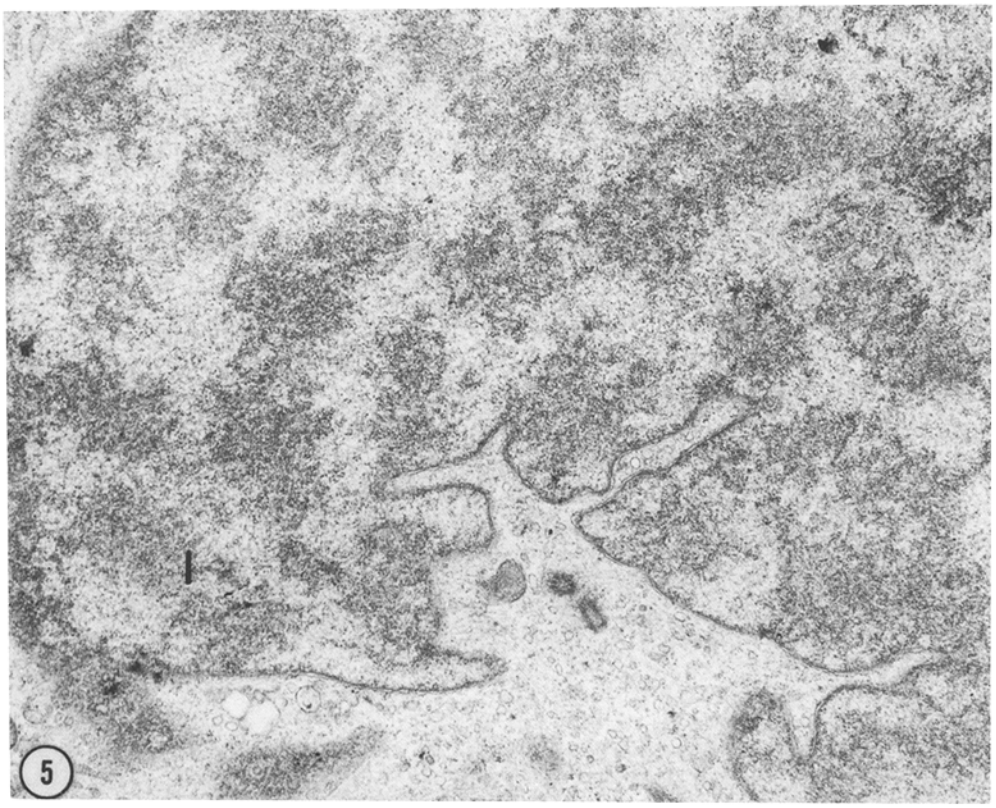
In this study, we found that procentrioles did not form when cycloheximide was present. Our data suggest that proteins necessary for procentriole formation are synthesized during, or just before, the time that procentrioles appear in the cell. Inhibition of RNA synthesis also appears to block procentriole formation (4). Inhibition of DNA synthesis does not seem to affect procentriole formation (4, 15). Thus, the duplication of centrioles proceeds within general constraints similar to those relating to the synthesis of many other cell proteins. That is, centriole formation is dependent upon RNA and protein synthesis but not DNA synthesis.

Our experiments showed that the elongation of daughter centrioles, which normally occurs during prophase in these cells (15), is not dependent upon *de novo* protein synthesis. This is consistent with the observation that protein synthesis is normally depressed at this time (11); thus, procentriole elongation normally proceeds at a time when protein synthesis is minimal. It seems likely that procentriole elongation utilizes previously synthesized precursors. Some of the protein of centrioles may be similar to or identical with protein found in microtubules. Centriolar microtubules resemble other microtubules structurally and have close spatial associations with other microtubules (24, 26). Some experimental observations suggest that centrioles are rich in tubulin (6), though this has not been rigorously proven as yet. Since the amount of microtubule protein per cell apparently doubles in G_2 (8), a pool of microtubule protein should be available at the time of prophase, and some of it could be used in procentriole elongation.

The role of *de novo* protein synthesis in the generation of flagellar microtubules has been examined in a number of systems. In some instances, the synthesis of new microtubule protein precedes flagellar growth (6, 12, 18, 28). In other cases, pre-existing tubulin pools may be utilized for microtubule assembly (14, 19, 27, 33). Thus, microtubule assembly may utilize either tubulin pools or *de novo* protein synthesis. In the case of centriole assembly, it appears that both of these means may be utilized, with new procentrioles arising from newly synthesized protein and the elongation of centrioles proceeding via pools. In studies of sys-

FIGURE 5 Prophase cell from a random culture fixed after 3 h of cycloheximide treatment. The daughter centriole of this pair no longer has the stubby appearance of a new procentriole, but has apparently elongated. × 9,000.

FIGURE 6 Anaphase cell from a random culture treated for 3 h with cycloheximide. The daughter centriole has elongated to the mature length. × 14,000



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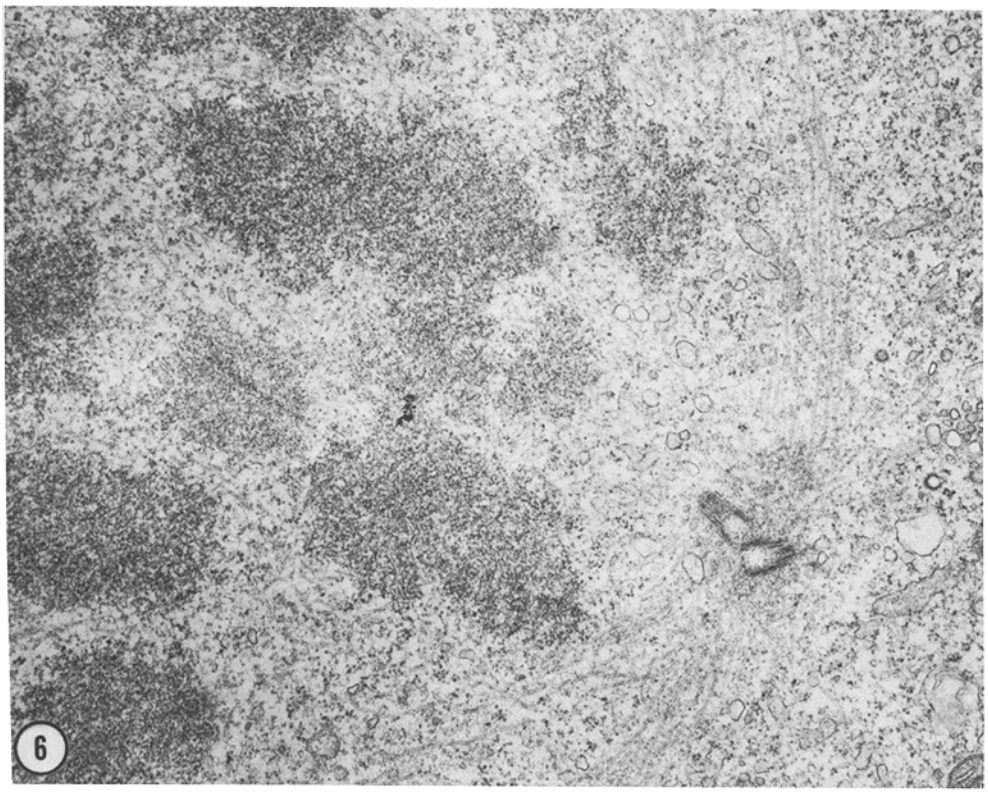

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tems where large numbers of basal bodies are synthesized within a short period of time, it was found that a burst of tubulin synthesis preceded the appearance of centrioles (5, 12). In *Stentor*, regeneration of oral membranellar band basal bodies was not dependent upon DNA synthesis but was sensitive to cycloheximide (34), suggesting that this centriole synthesis was also dependent on *de novo* protein synthesis.

We found that L cells continued to enter mitosis for at least 4 h in the presence of 25 μg/ml of cycloheximide, though the rate of cells entering mitosis gradually declined during that time. There have been many reports that inhibition of protein synthesis stops further mitosis in less than 90 min, but these studies have been done in cells with very short cell cycles (less than 12 h) such as Chinese hamster lines (1, 21, 30–32) and a fast-growing mouse leukemia line (7). In HeLa or human amnion cells, which have a long cell cycle time compared to L929 cells, *p*-fluorophenylalanine, an amino acid analog, must be applied for about 8 h to prevent further division (22). It seems possible that in cells with longer cell cycles, synthesis of proteins essential for division may be completed several hours before division.

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BIBLIOGRAPHY

1. Bacchetti, S., and W. K. Sinclair. 1970. The relation of protein synthesis and radiation-induced division delay in Chinese hamster cells. *Radiat. Res.* **44:**788–806.
2. Brinkley, B. R., E. Stubblefield, and T. C. Hsu. 1967. The effects of colcemid inhibition on the fine structure of the mitotic apparatus of Chinese hamster cells *in vitro*. *J. Ultrastruct. Res.* **19:**1–18.
3. Brinkley, B. R., and E. Stubblefield. 1970. Ultrastructure and interaction of the kinetochore and centriole in mitosis and meiosis. *In* Advances in Cell Biology. D. M. Prescott and L. Goldstein, editors. Appleton-Century-Crofts, Inc., New York.
4. De Foor, P. H., and E. Stubblefield. 1974. Effects of actinomycin D, amethopterin and 5-fluoro-2′-deoxyuridine on procentriole formation in Chinese hamster fibroblasts in culture. *Exp. Cell Res.* **85:**136–142.
5. Dirksen, E. R. 1968. Observations on centriole formation in the development of ciliated epithelium of mouse oviduct. *J. Cell Biol.* **39:**34.
6. Dirksen, E. R., and I. Staprans. 1975. Tubulin synthesis during ciliogenesis in the mouse oviduct. *Dev. Biol.* **46:**1–13.
7. Doida, Y., and S. Okada. 1969. Radiation-induced mitotic delay in cultured mammalian cells (L5178Y). *Radiat. Res.* **38:**513–525.
8. Forrest, G. L., and R. R. Klevecz. 1972. Synthesis and degradation of microtubule protein in synchronized Chinese hamster cells. *J. Biol. Chem.* **247:**3147.
9. Fulton, C. 1971. Centrioles. Origin and Continuity of Cell Organelles. Springer-Verlag New York Inc., New York. 170–221.
10. Gall, J. G. 1961. Centriole replication. A study of spermatogenesis in the snail *Viviparus*. *J. Biophys. Biochem. Cytol.* **10:**163–193.
11. Hodge, L. D., E. Robbins, and M. D. Scharff. 1969. Persistence of messenger RNA through mitosis in HeLa cells. *J. Cell Biol.* **40:**497–507.
12. Kowit, J. D., and C. Fulton. 1974. Programmed synthesis of tubulin for the flagella that develop during cell differentiation in *Naegleria gruberi*. *Proc. Natl. Acad. Sci. U.S.A.* **71:**2877.
13. Randall, J., and C. Disbrey. 1965. Evidence for the presence of DNA at basal body sites in *Tetrahymena pyriformis*. *Proc. Roy. Soc. Lond. B. Biol. Sci.* **162:**473–491.
14. Rannestad, J. 1974. The regeneration of cilia in partially deciliated *Tetrahymena*. *J. Cell Biol.* **63:**1009–1017.
15. Rattner, J. B., and S. G. Phillips. 1973. Independence of centriole formation and DNA synthesis. *J. Cell Biol.* **57:**359–372.
16. Robbins, E., G. Jentzsch, and A. Micali. 1968. The centriole cycle in synchronized HeLa cells. *J. Cell Biol.* **36:**329–339.
17. Roos, U.-P. 1973. Light and electron microscopy of rat kangaroo cells in mitosis. I. Formation and breakdown of the mitotic apparatus. *Chromosoma.* **40:**43–82.
18. Rosenbaum, J. L., and F. M. Child. 1967. Flagellar regeneration in protozoan flagellates. *J. Cell Biol.* **34:**345.
19. Rosenbaum, J. L., J. E. Moulder, and D. Ringo. 1969. Flagellar elongation and shortening in *Chlamydomonas*. *J. Cell Biol.* **41:**600–619.
20. Rubin, R. W., and W. P. Cunningham. 1973. Partial purification and phosphotungstate solubilization of basal bodies and kinetodesmal fibers from *Tetrahymena pyriformis*. *J. Cell Biol.* **57:**601–612.
21. Schneiderman, M. H., W. C. Dewey, D. B. Leeper, and H. Nagasawa. 1972. Use of mitotic selection procedure for cell cycle analysis. *Exp. Cell Res.* **74:**430–436.
22. Sisken, J. E., and E. Wilkes. 1967. The time of synthesis and the conservation of mitosis-related proteins in human amnion cells. *J. Cell Biol.* **34:**97–110.

23. SMITH-SONNEBORN, J., and W. PLAUT. 1967. Evidence of the presence of DNA in the pellicle of *Paramecium*. *J. Cell Sci.* **2**:225-234.
24. SOROKIN, S. 1962. Centrioles in the formation of rudimentary cilia by fibroblasts and smooth muscle cells. *J. Cell Biol.* **15**:363-377.
25. SOROKIN, S. P. 1968. Reconstruction of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.* **3**:207-230.
26. SOTELO, J. R., and O. TRUJILLO-CENÓZ. 1958. Electron microscopic study on the development of ciliary components of neural epithelium of the chick embryo. *Z. Zellforsch Mikrosk. Anat.* **49**:1-12.
27. STEPHENS, R. E. 1972. Studies on the development of the sea urchin *Stronglyocentrotus droebachiensis*. III. Embryonic synthesis of ciliary proteins. *Biol. Bull.* **142**:489-504.
28. STAPRANS, I., and E. R. DIRKSEN. 1974. Microtubule protein during ciliogenesis in the mouse oviduct. *J. Cell Biol.* **62**:164-174.
29. TERASIMA, T., and L. J. TOLMACH. 1963. Growth and nucleic acid synthesis in synchronously dividing populations of HeLa cells. *Exp. Cell Res.* **30**:344-362.
30. TOBEY, R. A., E. C. ANDERSON, and D. F. PETERSEN. 1966. RNA stability and protein synthesis in relation to the division of mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **56**:1520-1527.
31. TOBEY, R. A., D. F. PETERSEN, E. C. ANDERSON, and T. T. PUCK. 1966. Life cycle analysis of mammalian cells. III. The inhibition of division in Chinese hamster cells by puromycin and actinomycin. *Biophys. J.* **6**:567-581.
32. WALTERS, R. A., and D. F. PETERSEN. 1968. Radiosensitivity of mammalian cells. II. Radiation effects on macromolecular synthesis. *Biophys. J.* **8**: 1487-1504.
33. WILLIAMS, N. E., and E. M. NELSON. 1973. Relation of microtubules in *Tetrahymena*. II. Relation between turnover of microtubule protein and microtubule dissociation and assembly during oral replacement. *J. Cell Biol.* **56**:458-465.
34. YOUNGER, K. B., S. BANERJEE, J. K. KELLEHER, M. WINSTON, and L. MARGULIS. 1972. Evidence that synchronized production of new basal bodies is not associated with DNA synthesis in *Stentor coeruleus*. *J. Cell Sci.* **11**:621-637.