

AN INVESTIGATION OF THE CENTRIOLE CYCLE USING 3T3 AND CHO CELLS

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SUMMARY

An investigation of the centriole cycle was based on serial-section electron microscopy of the centrioles of 790 3T3 cells and 40 CHO cells (incomplete sets of sections were excluded from the analysis). Time-course studies indicated that procentrioles first appear 4 h after the start of *S*-phase; evidence was obtained to suggest that they elongate progressively during the subsequent stages of the cell cycle, and that they start to separate from their parents during mitosis. An association of the structural features of the centriole cycle with the Two-transition Model of the Cell Cycle was ruled out by the finding of two centrioles in most quiescent cells, and the appearance of procentrioles in *S*-phase in transiently stimulated cells.

INTRODUCTION

The centriole has fascinated cell biologists for over a century, with its precise geometrical structure, its location at the poles of the mitotic spindle, and its unique method of replication. New centrioles are assembled at the bases of existing ones (Bernard & De Harven, 1960) and the process is co-ordinated with the cell cycle, ensuring that successive generations of cells have the same number of centrioles.

This led to the hypothesis that the centriole is involved in the control of cell division via the Two-transition Model of the Cell Cycle (Brooks, Bennett & Smith, 1980). Such a role is compatible with what is known of the centriole cycle, but complete assessment of the hypothesis would require more precise data on the timing of the events of the centriole cycle than those published hitherto.

Three events are seen in the centriole cycle: the appearance of daughter centrioles (procentrioles) beside the proximal end of each adult centriole, their elongation to adult length, and their separation from their parents. These events are thought to occur in mid *S*-phase, mitosis and early *G*₁, respectively (Robbins, Jentzsch & Micali, 1968; Erlandson & De Harven, 1971; Rattner & Phillips, 1973; Copenhaver, 1975; Phillips & Rattner, 1976; Vorobiev & Chentsov, 1978, 1982). However, critical examination of the data reveals considerable latitude in the estimates of the timing of each of the events, so a quantitative electron microscopic study was undertaken to define the centriole cycle more precisely.

Key words: centriole, procentriole, cell cycle.

MATERIALS AND METHODS

Cell culture

Swiss 3T3 cells (clone 4A C5), were obtained from Dr R. F. Brooks (Imperial Cancer Research Fund), 3T3 K cells from Dr C. H. O'Neill (ICRF) and CHO cells from Flow Laboratories. They were cultured in Dulbecco's modification of Eagle's medium (E4), supplemented with calf serum (CS) or foetal calf serum (FCS), in a humidified incubator in the presence of 10 % CO₂. Confluent cultures (on 3 cm Melinex discs) were fixed, processed and sectioned *in situ*. Subconfluent cultures (in 15 cm plastic Petri dishes) were removed with trypsin before fixation, and made into a pellet in agar (Gowans, 1973).

Quiescence was induced in 3T3 cells by serum deprivation (2 % FCS for 5 days or 0.5 % FCS for 2–3 days), by allowing the cells to become confluent in a large volume of medium containing 5 % serum, or by a combination of both methods.

Cell-cycle experiments were performed by assessing the incidence of procentrioles at intervals after stimulation of quiescent cultures of 3T3 cells with 20 % FCS.

Exponentially growing cultures of 3T3 cells were prepared in 15 cm Petri dishes using E4 medium supplemented with 10 % FCS. The medium was changed daily until the cells were almost confluent, when they were removed from the dishes with trypsin, fixed immediately and processed as a pellet in agar.

Cultures for mitotic cell preparations were grown in 2.5 l Winchester bottles in 200 ml of E4 medium containing 10 % FCS (25 % in the final 24 h). Debris and dead cells were removed 1 h before the final shake, after which the cells were poured straight into an equal volume of 5 % glutaraldehyde in Sørensen's buffer. The bulk of the resulting suspension was reduced by centrifugation and resuspension in progressively smaller volumes of 2.5 % glutaraldehyde and the cells were made into a pellet for processing. CHO cells were used initially, because of anticipated difficulties in dislodging sufficient 3T3 cells, but a pellet of mitotic 3T3 cells was subsequently made by pooling the cells from four Winchester bottles.

Electron microscopy

Cells were fixed in 2.5 % glutaraldehyde in Sørensen's phosphate buffer (pH 7.4, 1 h), rinsed three times in Sørensen's buffer, post-fixed in 1 % osmium tetroxide (1 h), dehydrated in a graded methanol series (including *en bloc* staining with 0.5 % uranyl acetate in 70 % methanol for 30 min), rinsed in acetone and embedded in Spurr Epoxy resin.

Serial sections in the gold interference range were cut using a diamond knife, and mounted on copper slot grids covered with a Formvar film. Records were made of the sequence numbers of the sections located successfully over the apertures of the grids: it was often possible to obtain long and complete sequences of serial sections, even though they were mounted on different grids.

Cells grown on Melinex were sectioned parallel to their bases, after the Melinex had been peeled off. Approximately the first 50 sections were cut from the bottom of each block and mounted on two or three grids as appropriate. No selection was made of the areas to be sectioned, but the confluent nature of the cultures ensured a reasonable yield of centrioles.

Sections were stained with lead citrate (Reynolds, 1963) and viewed in a Philips 301 electron microscope (accelerating voltage, 100 kV; objective aperture, 20 μ m).

Double-label electron-microscope autoradiography

The double-label autoradiographic technique (Alvey, 1981), was used on confluent cultures of 3T3 cells (on Melinex discs) with a sequence of: [¹⁴C]thymidine (0.5 μ Ci/ml) for 1 h, no label for 2 h, [³H]thymidine (10 μ Ci/ml) for 1 h, followed by immediate fixation. After peeling away the Melinex, the under surface of the embedded cells was coated with Ilford Nuclear Research emulsion (type L4), which was developed after 7 days, and the whole preparation was re-embedded in more Epoxy resin. Vertical serial sections were cut to include the cells and adjacent emulsion. The labelling pattern of each isotope could be distinguished in the electron microscope, allowing the stage of each cell in the cell cycle to be deduced.

Light-microscope autoradiography

Autoradiography was performed on a sample of cells from each culture. Confluent cultures were exposed to tritiated thymidine before fixation and a piece was cut from the Melinex disc at the dehydration stage. With subconfluent cultures, one or more 1-cm Melinex squares were placed in the 15-cm dishes before the cells were seeded and removed at appropriate times during the culture period. They were pulse-labelled with [^3H]thymidine ($0.7 \mu\text{Ci/ml}$) in separate 3-cm Petri dishes, fixed in 95 % (v/v) acetic acid in ethanol (20 min) and rinsed in 100 % ethanol. The dehydrated cells from both methods were coated with Ilford Nuclear Research emulsion (type K5; exposed for 1 week at 4°C), stained with 10 % Giemsa solution and embedded in a thin layer of Araldite on a glass slide; after polymerization the Melinex was peeled off.

Estimation of procentriole incidence

A centriole can be classified as lacking a procentriole only if all of the possible procentriole sites are seen to be vacant. One empty section may sometimes be seen between a longitudinally sectioned adult centriole and its transversely sectioned procentriole (Fig. 1), so a procentriole was scored as absent only when two clear sections were seen at each end of a series containing a centriole sectioned longitudinally or transversely (one clear section with centrioles sectioned obliquely).

The same criteria were also applied to centrioles with visible procentrioles, to avoid bias in the analysis. If all the possible procentriole sites were not included in the sections, the centriole was classified as 'not assessable' for procentriole incidence, regardless of the existence of any fortuitously situated procentriole.

Estimation of procentriole length

An approximate classification of procentriole length was used in view of the wide variety of directions in which they were sectioned. Procentrioles sectioned transversely (TS) or obliquely (OS) were classified according to the number of sections in which they were seen. Short: 1 section (TS), 1 or 2 sections (OS); medium: 2 sections (TS), 3 sections (OS); long: more than 2 sections (TS), more than 3 sections (OS). Procentrioles sectioned longitudinally were classified as short if their length was less than $\frac{1}{3}$ of the adult centriole length or less than half the adult width, medium if between $\frac{1}{3}$ and $\frac{2}{3}$ adult length or between half and full width and long if greater than $\frac{2}{3}$ adult length or greater than adult width.

RESULTS

Validity of observations on single centrioles

Only one centriole was found in many of the cells because the whole of each cell was rarely included in the available serial sections. However, 347 3T3 cells were seen with two centrioles assessable for procentrioles, and in 345 of these both centrioles were at the same visible stage of duplication. One of the exceptions had a medium-length and a short procentriole, whereas the other had one centriole with a short procentriole and one with dense pericentriolar material at its proximal end, but no clear-cut procentriole. The low incidence of discordance between the centrioles of a pair made it reasonable to assume that whenever a cell was seen with only one centriole, its unseen partner (if any) would also be at a similar stage in the centriole cycle.

Incidence of aberrant cells

In early experiments the frequencies of 3T3 cells with more than two centrioles or more than one nucleus were: 2/42, 12/46 and 5/46. In later experiments aberrant cells were ignored at the time of microscopy, but the overall incidence never appeared

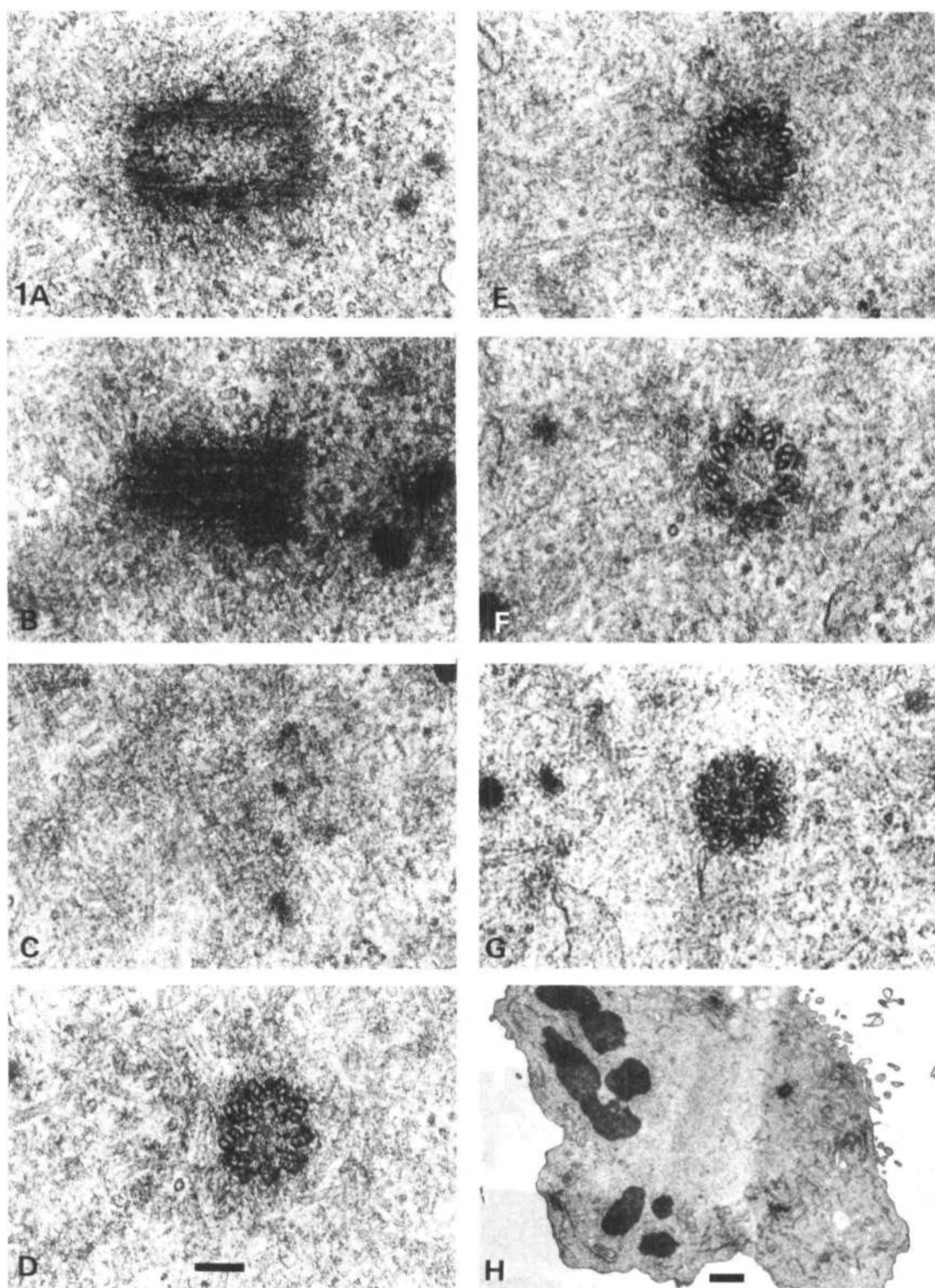


Fig. 1. Serial sections from a CHO cell in metaphase. Alignment of the negatives (aided by cell surface landmarks) indicated that the structure seen in near-longitudinal section is the parent centriole, and the transversely sectioned structure is its pro-centriole lying adjacent to one of its ends. It is clear from these sections that the separation between a centriole and pro-centriole may be as much as the thickness of one section. A-G: bar, $0.1\ \mu\text{m}$; $\times 80,000$. H: bar, $1\ \mu\text{m}$; $\times 5\,500$.

Table 1. *Procentriole incidence in the early period following serum stimulation*

	Length of stimulation period (h)	Labelling index (%)	Procentriole incidence in assessable cells
3T3 K cells	0	2	
	16	34	0/33
3T3 C5 cells	0	<1	0/16
	1	<1	0/29
	20	18	0/33

3T3 K cells were plated (1.5×10^6 cells/dish) into a 15 cm Petri dish containing three Melinex squares and grown in E4 + 2 % FCS for 48 h, then E4 + 0.5 % FCS for 60 h, after which they were stimulated with 20 % FCS and fixed 16 h later. Light-microscope autoradiography was performed on Melinex squares removed at the beginning and end of the stimulation period.

3T3 C5 cells were plated (2×10^6 cells/dish) into 3 cm Petri dishes, each containing a 3 cm Melinex disc, and allowed to become confluent in E4 + 2 % FCS over 5 days. Replicate cultures were stimulated with 14 % FCS for various periods before fixation.

In each case the procentriole incidence is lower than would be expected if procentrioles appear at or before the start of S-phase, and such a null hypothesis can be rejected. C5: χ^2 (1 d.f.) = 5.896, $P < 0.02$; K: χ^2 (1 d.f.) = 15.42, $P < 0.001$.

to be much greater than 10 %, a level consistent with the findings of other workers (Albrecht-Buehler & Bushnell, 1979).

Timing of procentriole appearance

Four experiments indicated that procentrioles appear after the start of S-phase in 3T3 cells.

The procentriole incidence in exponentially growing 3T3 cells was 38 % (15/40; 95 % confidence limits: 23 % and 54 %), indicating that procentrioles appear in the third quarter of the average cell cycle under these experimental conditions.

Cells sectioned early after serum stimulation contained no procentrioles, even though some cells had already entered S-phase (Table 1).

A time-course study of procentriole appearance and entry into S-phase showed a rise in procentriole incidence 4 h after the initial rise in labelling index (Fig. 2). This evidence is particularly strong because, at each time point, the two types of investigation were performed on the same population of cells after the Melinex disc had been cut in half.

A double-label autoradiographic technique (Alvey, 1981) was used to identify cells at specific stages in the cell cycle (Table 2). No recognizable procentrioles were seen in two of 11 cells that must have been in S-phase for at least 3 h, and the other nine had only short procentrioles (Fig. 3).

Rate of procentriole elongation

Crude assessment of procentriole lengths suggested that short, medium and long procentrioles are present at successive stages in interphase (Table 3, Fig. 4), and that procentriole elongation may be virtually complete by the time of mitosis, at least in

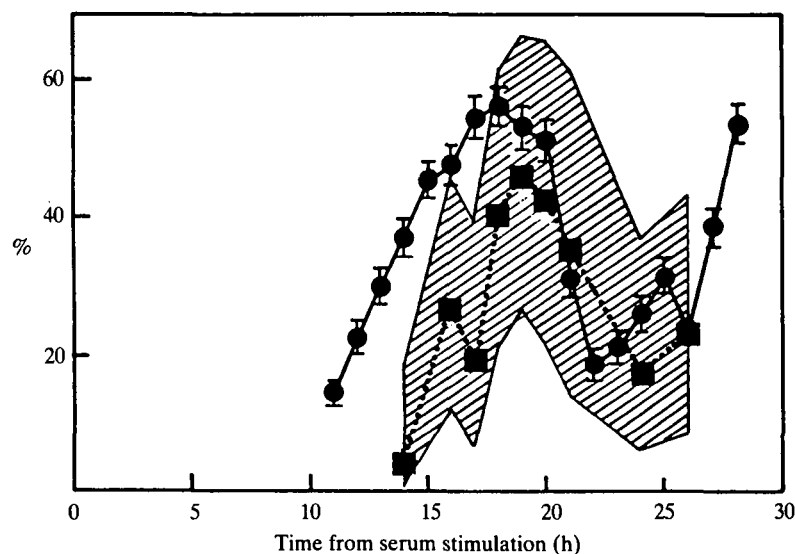


Fig. 2. The time course of procentriole appearance (■---■) and entry in S-phase. 3T3 C5 cells were plated (6.7×10^5 cells/dish) into 3 cm Petri dishes (containing a 3 cm Melinex disc) and allowed to become confluent (E4 + 2 % FCS for 4 days). Replicate cultures were stimulated (20 % FCS) for various periods before fixation. Each Melinex disc was cut in half: one half being processed for light microscope autoradiography, the other for electron microscopy. Procentriole incidence was assessed on a minimum sample size of 17 assessable cells, and the 95 % confidence limits for each proportion are shown: vertical bars for the labelling index (●—●); hatched zone for the procentriole incidence.

some cells. However, accurate measurements were thwarted by the wide range of orientations in which procentrioles were sectioned and the small number of observations do not permit firm conclusions.

Timing of procentriole separation

In a preparation of mitotic CHO cells, one cell was found with a chromatin pattern that suggested that it was undergoing the transition from telophase to G_1 . The procentriole in this cell had already separated from its parent, raising the possibility that this process occurs in mitosis, not in early G_1 as is widely believed. Accordingly, a closer examination was made of the 32 CHO cells and 22 3T3 cells seen in mitotic-shake experiments. Of those in which the orientation could be assessed, the majority had procentrioles still adjacent to their parents, but with an angle between them that was no longer a right angle (Table 4, Fig. 5). In interphase cells, the loss of the orthogonal relationship between a centriole and its procentriole was seen infrequently.

In a significant proportion of the mitotic cells the procentrioles appear to have started breaking away from their parents, in many cases by a kind of twisting action. Compression during sectioning might cause distortion of the angle, but the 'hinged' appearance in many of the micrographs could not be due to this mechanism alone.

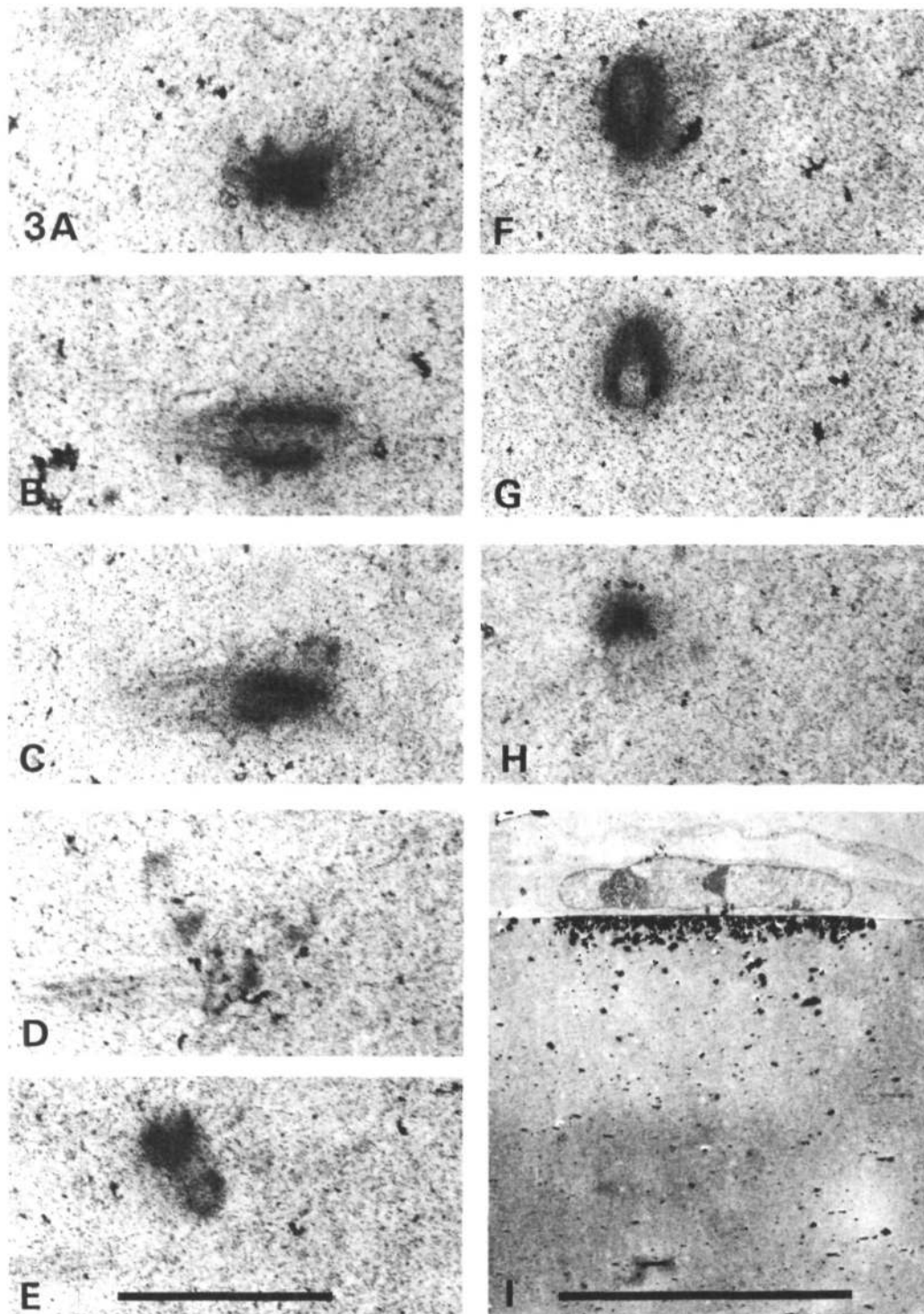


Fig. 3. Serial sections of a 3T3 cell processed by the double-label autoradiographic technique. **i** is a low-power view showing the labelling pattern of both ^3H and ^{14}C , indicating that the stage in the cell cycle is at least 3 h from the start of *S*-phase. Both centrioles have short procentrioles. The quality of the micrographs is impaired by contamination of the Formvar used to coat the slot grids; this was only discovered after many 'precious' ribbons of sections had been mounted. **A-H**: bar, $1\ \mu\text{m}$; $\times 35\ 000$. **i**: bar, $10\ \mu\text{m}$; $\times 4\ 800$.

Table 2. *Incidence of procentrioles in cells at different stages of the cell cycle, as determined by the double-label autoradiographic technique*

Stage in cell cycle	Labelling pattern	No. of cells found	Procentriole incidence	Procentriole length
G ₁	Unlabelled	4	0/4	—
Early S-phase	³ H only	0	—	—
Mid S-phase to early G ₂	³ H + ¹⁴ C	7	6/7	All short
Mid S-phase to late G ₂	¹⁴ C present	4	3/4	All short
Metaphase	³ H, n/a*	1	1/1	Long

3T3 K cells were plated (3×10^6 cells/dish) into 5 cm Petri dishes (containing a 3 cm Melinex disc and a 1 cm Melinex square) and allowed to reach confluence in E4 + 5 % FCS. Replicate cultures were stimulated for various periods with E4 + 20 % FCS, and in the final 4 h each culture was exposed to two types of labelled thymidine in the sequence: [¹⁴C]thymidine (0.5 μ Ci/ml) for 1 h, no label for 2 h, [³H]thymidine (10 μ Ci/ml) for 1 h. Light-microscope autoradiography was performed on the Melinex squares, and the cultures with the highest labelling indices (18 h and 21 h) were processed by the double-label autoradiographic technique (Alvey, 1981). The stage in the cell cycle could be deduced from the labelling sequence and the pattern of grains beside each cell: ³H produced a narrow dense zone of grains close to the nucleus; ¹⁴C tracks extended deeper into the emulsion layer.

*³H-labelling could not be assessed in some cells because they overlapped their neighbours, thereby elevating their bases from the culture surface.

Table 3. *Distribution of the lengths of procentrioles observed in all the experiments of this investigation*

Experiment	Interval from stimulation to fixation (h)	Proportion of assessable cells with procentrioles (%)		No. of procentrioles found in each length category		
				Short	Medium	Long
Time-course exp. (Fig. 2)	14	4	1/28	1	0	0
	16	27	8/30	7	0	1
	17	19	5/26	5	0	0
	18	40	10/25	8	2	0
	19	46	12/26	7	4	1
	20	43	9/21	5	4	0
	21	35	6/17	4	2	0
	24	18	5/28	2	3	0
	26	23	6/26	2	2	2
Exponential culture		38	15/40	5	9	1
Transient stimulation exp. (Fig. 6)	24 continuous	71	27/38	14	12	1
	24 transient	54	21/39	16	5	0

The lengths of procentrioles were estimated using the criteria described in Materials and Methods.

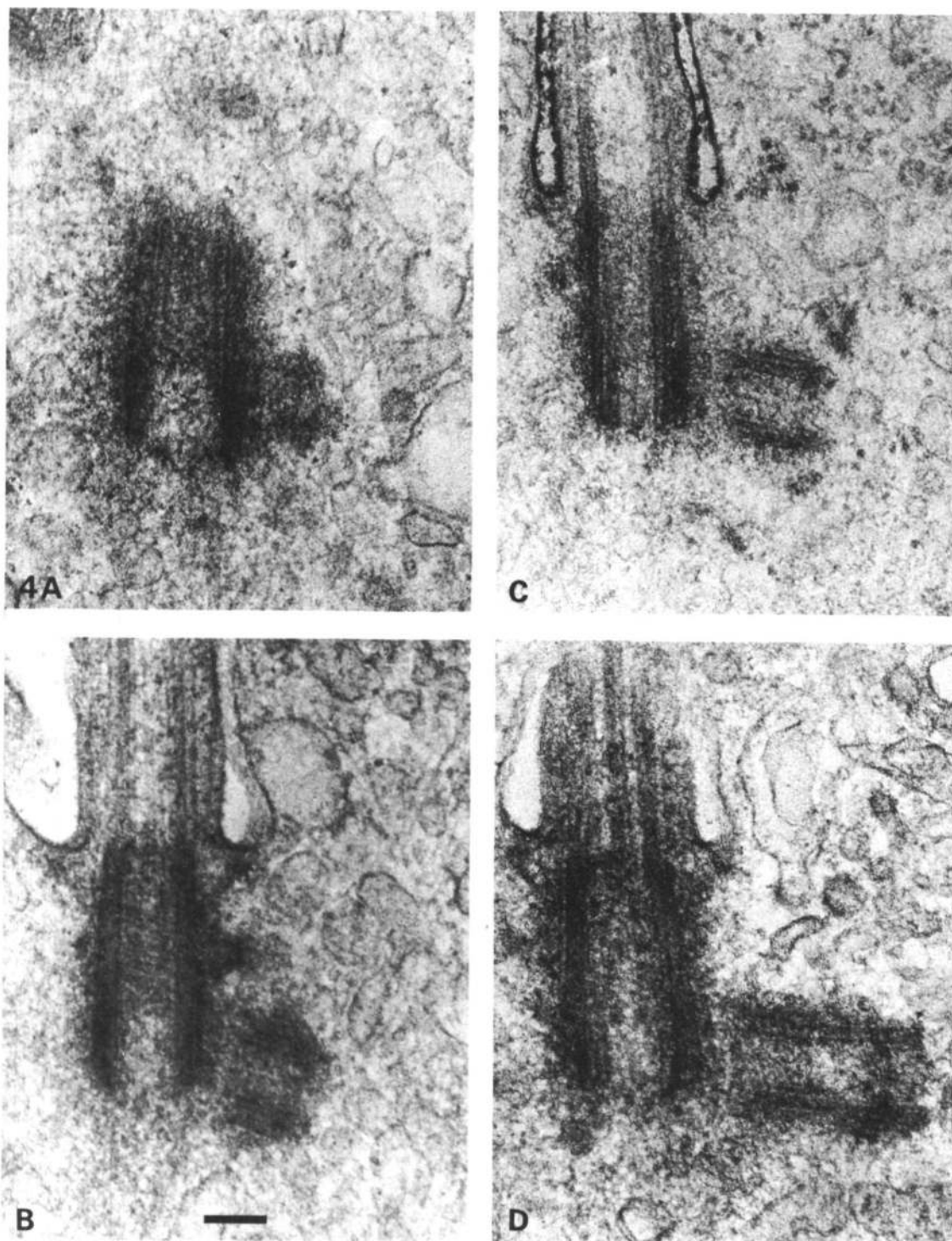


Fig. 4. Sections from four different interphase 3T3 cells illustrating the range of pro-centriole lengths seen. These favourably orientated sections are not representative of the whole study. Only five of 790 3T3 cells had a centriole and pro-centriole both sectioned longitudinally; the vast majority were sectioned with some degree of obliquity. Bar, $0.1 \mu\text{m}$; $\times 100\,000$.

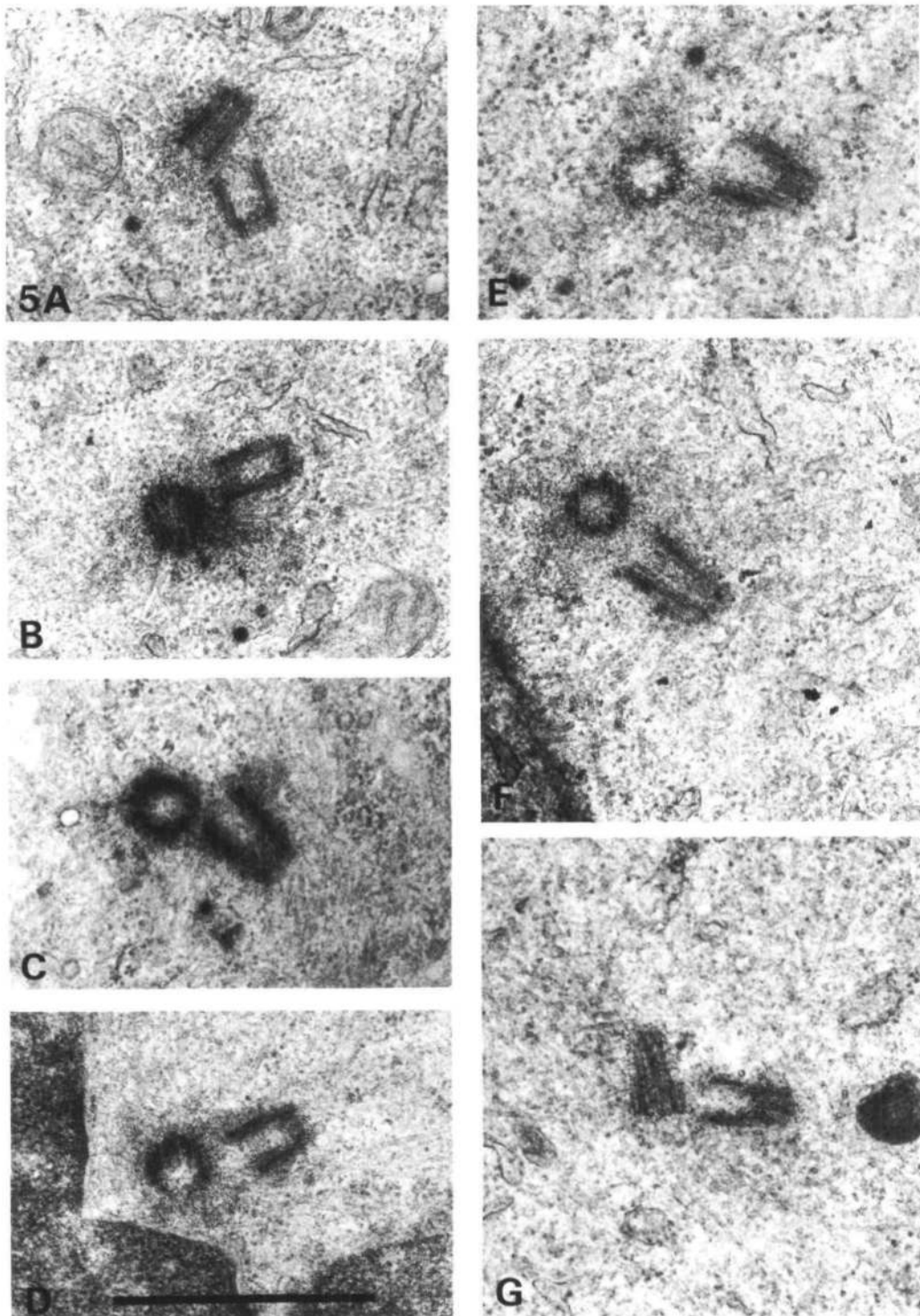


Fig. 5. Individual sections from seven different cells in mitosis. The strict orthogonal orientation of the pro-centrioles has been partially disrupted. This suggests that they are starting to break away from their parents; the unusual appearances cannot all be explained by the obliqueness of the plane of section or by compression during sectioning. A-E, CHO cells; F, G, 3T3 cells. Bar, 1 μ m; $\times 43\,000$.

Table 4. *Incidence of procentrioles with a non-orthogonal relationship to their parent centriole*

Orientation of procentrioles	3T3 cells		CHO cells
	Interphase	Mitosis	Mitosis
Apparently orthogonal	26	1	0
Not orthogonal	2	5	11
Not assessable	121	11	21

The numbers of mitotic cells with orthogonal or non-orthogonal procentrioles, as judged by visual assessment of the serial sections. A group of interphase 3T3 cells from other experiments is also included for comparison.

Table 5. *Frequencies of 3T3 cells observed with one or two centrioles*

Cell type	Method of inducing quiescence	Labelling index (%)	Mode of fixation	Observed number of cells with each centriole complement	
				Solitary centriole	A pair of centrioles
3T3 C5	Low serum, confluence	2.6	Monolayer	12	28
3T3 C5	Low serum, confluence	<1	Monolayer	9	9
3T3 K	Low serum	<1	Pellet	15	33
3T3 K	Confluence	<1	Pellet	7	17
3T3 K	Confluence	<1	Monolayer	0	26
Totals				43	113

Results from all quiescent cultures are summarized; binucleate cells and those with more than two centrioles have been excluded. The 3T3 K cells were extremely flat and in the culture sectioned as a monolayer virtually the whole of each cell was included in the sections. In the other groups, the available sections rarely included the whole of any particular cell, so those apparently containing a solitary centriole could well have had another centriole in the unseen sections.

Relationship of the centriole cycle to the cell cycle

If the centriole mediates the events of the two-transition model (Brooks *et al.* 1980), two conditions would have to be met: that most cells in quiescent cultures have only one centriole, and that procentrioles appear during *S*-phase only in cells committed to the next round of division (i.e. most cells in rapidly growing populations).

However, two centrioles were seen in the majority of 3T3 cells in quiescent cultures (Table 5) and the proportion was highest in the groups with the greatest degree of sampling from each cell. Thus, there is no support whatsoever for the idea that quiescent cells normally contain just one centriole.

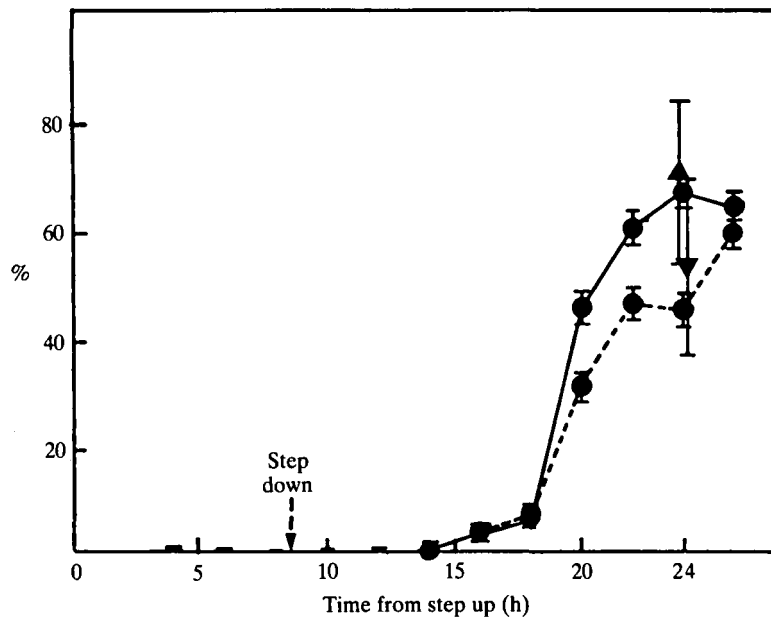


Fig. 6. The time of appearance of pro-centrioles in relation to commitment to the next cell cycle. 3T3 K cells were plated (7×10^6 cells/dish) into 15 cm Petri dishes (containing three 1 cm Melinex squares) and after 2 days in E4 + 0.5 % CS replicate cultures were stimulated (20 % CS) for various periods. Duplicate cultures were used for each of the stimulation periods of 10 h or more: one remained in high serum for the whole of the stimulation period ((●—●); (▲) pro-centriole index), the other was returned to 0.5 % CS after 8.5 h ((●---●); (▼) pro-centriole index). Light-microscope autoradiography was performed on each culture before fixation; serial sections were cut from the pair of cultures fixed at 24 h. The 95 % confidence limits for each proportion are indicated by vertical bars and the points at 24 h have been separated horizontally slightly for clarity.

Similarly, a link between the appearance of pro-centrioles and commitment to the next cell cycle was discounted by comparing a culture of 3T3 cells stimulated continuously for 24 h, with one stimulated for only the first 8.5 h of a 24 h period (Fig. 6). In both cases the incidence of pro-centrioles was similar to the labelling index in the same culture, whereas one would expect only a minority of the transiently stimulated cells to be committed to the next cell cycle under these conditions (Bürk, 1970). Thus pro-centriole appearance must be related to events in the current cell cycle.

DISCUSSION

Timing of pro-centriole appearance

The appearance of pro-centrioles 4 h after the start of S-phase is a consistent finding and it confirms the conclusions of some previous studies (Erlandson & De Harven, 1971; Copenhagen, 1975; Vorobiev & Chentsov, 1978, 1982). The latter is the

most comprehensive of these, using serial sections of individual cells pre-selected by autoradiography, and the findings are in exact agreement with the present work.

However, in two earlier investigations cultures timed from a mitotic selection were used and it was suggested that procentrioles appear in G_1 (Robbins *et al.* 1968; Rattner & Phillips, 1973). The different findings may be due to simple variation between cell lines; alternatively, it is possible that the physical and chemical disturbances of the mitotic selection may have had different effects on the rate of progress through the cell cycle and centriole cycle, respectively. Rattner & Phillips (1973) commented that the doubling time of their cells was 2 h longer after mitotic selection than in populations of the same cells growing exponentially: if progress through the centriole cycle was not similarly slowed down, procentrioles would appear earlier in the cell cycle than in undisturbed cells.

Rate of procentriole elongation

Accurate measurements of the rate of procentriole elongation are hampered by the fact that procentrioles may be sectioned in any three-dimensional orientation. No previous investigator has successfully overcome this problem, yet merely ignoring it can lead only to unwarranted conclusions.

Rattner & Phillips (1973) suggested that procentrioles are nearly half of the adult length when they first appear and remain that length until late prophase, when they rapidly grow to full length, but this hypothesis is based on a relatively small number of observations on random sections. Vorobiev & Chentsov (1978, 1982) measured a progressive increase in procentriole length throughout S -phase, G_2 and prophase, but they do not state the accuracy of their measurements. Kuriyama & Borisy (1981) measured procentriole lengths in whole-mount preparations of nuclear-centrosome complexes, in CHO cells. Their frequency distribution from exponentially growing cultures does not show a preponderance for any category of procentriole length, consistent with a uniform rate of procentriole elongation, but it is unclear whether the measurement errors and sampling errors are sufficiently low to rule out alternative hypotheses.

Few other workers have commented on the rate of procentriole elongation, but procentrioles of various lengths can be seen in the many published micrographs of centrioles in interphase cells. The present study conforms to that pattern, and favours a uniform rate of elongation. However, despite the availability of serial sections of 7903T3 cells, the current data are insufficient for more elaborate conclusions. The more one sees of the problem the harder it seems to find an authentic solution.

Timing of procentriole separation

In mitosis procentrioles are seen adjacent to their parents, but in the subsequent interphase the two components usually appear to be separated. In view of the brief duration of mitosis it seems reasonable to assume that separation occurs in early G_1 , and this is often asserted (e.g. see Erlandson & De Harven, 1971). However, no experimental work has ever defined the stage in G_1 at which the separation occurs: indeed such would be a formidable task.

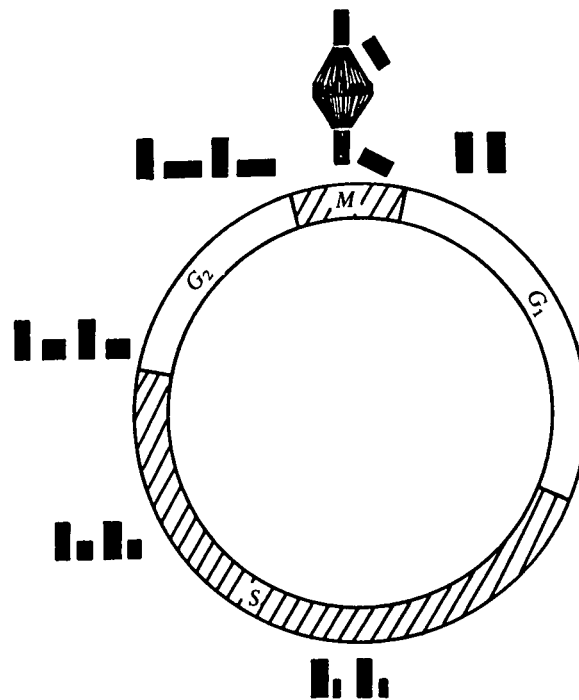


Fig. 7. The centriole cycle as suggested by the findings of this investigation. Procentrioles appear in mid *S*-phase and elongate progressively during the subsequent stages of the cell cycle. They begin to separate from their parents at a time that may vary and may be as early as the start of mitosis.

The present work indicates that procentrioles may start to separate from their parents during mitosis – a suggestion that has not been made previously. However, the loss of the orthogonal orientation of a centriole and its procentriole can also be seen in the micrographs illustrating other studies, although its significance appears to have been unnoticed (see fig. 6 of Stubblefield, 1968; fig. 20 of Stubblefield & Brinkley, 1967; figs 2, 15 of Rieder, 1979; fig. 23*b* of Roos, 1973; figs 8, 9, 22 of Vorobiev & Chentsov, 1982).

Even stronger corroboration comes from the study of Zeligs & Wollman (1979), which was primarily concerned with the differences in the amount of pericentriolar amorphous material surrounding adult centrioles and procentrioles. They found four telophase cells in which the procentriole and its parent were so far apart that they had great difficulty in determining which was which. For the purposes of their investigation those cells could not be classified, but in the present context they add considerable weight to the suggestion that procentriole separation may start before the mother cell has actually divided.

Indeed, similar observations were made with the light microscope by Pollister (1933), who wrote: "In the early telophase, just as the nuclear membrane is developing, it can first be seen that the centrioles are doubled, and by late telophase a sphere is reorganized around the two centrioles in each of the sister cells". It is unlikely that

the centriole and procentriole of his amphibian leucocytes could have been resolved as separate structures unless they had actually moved apart, so the suggestion that procentrioles begin to separate from their parents in mitosis is extremely plausible.

Two-transition model of the cell cycle

An association between the two-transition model of the cell cycle and the structural aspects of the centriole cycle is completely ruled out by the finding of two centrioles in quiescent cells and procentrioles in *S*-phase of transiently stimulated cells. However, a link with the functional aspects is not excluded. The experiments of Mazia, Harris & Bibring (1960) on sea-urchin eggs established that inhibition of the process of mitosis does not also inhibit the functional maturation of the mitotic centres. Transient exposure to mercaptoethanol resulted in quadripolar mitoses in a proportion of cells and the proportion increased in relation to the duration of the mitotic block, indicating that the mitotic centres become functionally mature at different times after mitosis. Similar observations have been made in mammalian cells using desacetylcolchicine, a reversible colchicine analogue (J. A. Smith, unpublished observations), and the kinetics of the phenomenon show a strong similarity to the features of the two-transition model – a similarity that is still unexplained. The present study has disproved a link with centriole structure but further investigation will be required before the functional status of the centriole or pericentriolar material can be completely ruled out as the vehicle of the two-transition model.

The centriole cycle in 3T3 cells

The view of the centriole cycle emerging from this study differs from the one that was prevalent before. It seems that centrioles replicate by developing procentrioles that appear 4 h after the start of *S*-phase, elongate progressively and begin to separate from their parents during mitosis (Fig. 7).

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