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# Absence of a measurable $G_2$ phase in two Chinese hamster cell lines

(cell cycle/V79 cells/autoradiography)

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ABSTRACT **Evidence is presented that demonstrates the absence of a measurable $G_2$ phase in the cell cycles of two sublines of the Chinese hamster lung fibroblast V79. One of the sublines, in addition, lacks a detectable $G_1$ phase, thereby possessing a cell cycle comprised of simply two phases, DNA synthesis (S) and mitosis (M).**

The cell life cycle of most mammalian cells can be described in terms of four phases (1): mitosis (M), a period between mitosis and the initiation of DNA replication ($G_1$), DNA replication (S), and a period between the termination of DNA replication and the beginning of prophase ($G_2$). The lengths of S, $G_2$, and M are, in general, found to be relatively constant, while the length of $G_1$ can vary greatly among different cell types (2–4). In fact, Robbins and Scharff found that the $G_1$ phase is absent altogether in one rapidly growing subline of Chinese hamster V79 fibroblasts (5). I report here that this line also appears to lack a detectable $G_2$ period; that is, this cell line apparently possesses a cell cycle comprised of only two measurable phases, DNA synthesis (S) and mitosis (M). Furthermore, another V79 subline also appears to lack a measurable $G_2$ period. The possibility that other lines of Chinese hamster cells may also lack a $G_2$ or have a $G_2$ period significantly shorter than previously reported is also discussed.

## MATERIALS AND METHODS

**Cell Lines and Culture Conditions.** The Chinese hamster V79 cells used in this study were of two kinds. V79-743 is a line obtained from Dr. E. H. Y. Chu and is apparently a direct descendant of the original V79 line isolated by Ford and Yerganian (6). The V79 line lacking $G_1$, V79-8, on which most of the experiments reported here were performed, was obtained from Dr. R. Klevecz and is a descendant of the V79 subline first reported to lack a $G_1$ by Robbins and Scharff (5). Robbins and Scharff obtained their V79 cells from Elkind and Sutton (7), who isolated this fast growing line from the original parental line of Ford and Yerganian.

V79 cells were grown in monolayer on Falcon plastic flasks in Dulbecco's modified Eagle's medium supplemented with 15% fetal calf serum (Flow Labs) and buffered with 25 mM Tricine at pH 7.4.

**Cell Cycle Analysis.** Doubling times of the lines were determined by counting cells of duplicate parallel cultures over a 4-day period of logarithmic growth. The lengths of S and $G_2$ were determined by pulse-labeling a series of flasks of logarithmically growing cells with 4 $\mu$Ci of [$^3$H]thymidine per ml (specific activity 40–50 Ci/mmol, Amersham/Searle) for 15 min followed by a chase in complete medium plus 0.1 mM unlabeled thymidine. Samples for autoradiography were then taken at 0 min, 15 min, 30 min, 1 hr, 2 hr, and 3 hr after the pulse. The cells were trypsinized, treated with hypotonic solution (10 mM sodium citrate/30 mM potassium chloride) for 20 min followed by three 10-min cycles of fixation in methanol/glacial acetic acid (3:1), and dropped onto slides that had been dipped in $H_2O$ and either air- or flame-dried. The slides were coated with NTB2 emulsion, dried, exposed for 7–28 days, developed, and stained with crystal violet or Giemsa. The slides were then scored for % labeled nuclei and % labeled metaphases.

An estimation of the length of the mitotic stages in V79-8 was obtained by examining living cells as they grew at 37° in flasks placed on the stage of an inverted phase contrast microscope. The first visible change in nuclear morphology (i.e., nuclear condensation as evidenced by a grainy appearance of the nucleoplasm) was considered the beginning of prophase. These cells were then monitored and their nuclear morphologies noted and recorded as a function of time. Observations of eight cells were used for estimating the mean length and range of prophase (i.e., initial condensation to the appearance of well-defined chromosomal bodies) and the total length of mitosis (i.e., from the onset of prophase until the apparent end of cytokinesis).

## RESULTS

**Determination of Length of the $G_2$ Phase.** The strategy for determining the length of $G_2$ for the two V79 cell lines was to subtract the length of prophase (measured by *in situ* observation) from the length of $G_2$ + prophase. The average length of $G_2$ + prophase was determined by pulse-labeling logarithmically growing cells with [$^3$H]thymidine and preparing chromosome at intervals after the pulse. The time at which 50% of the metaphase cells are seen to be labeled was taken to be the average length of $G_2$ + prophase (8). Results in Fig. 1 show that for each of the V79 cell lines $G_2$ + prophase is no more than 15 min. Representative labeled mitotic chromosomes of V79-8 cells taken at 15 min, 30 min, and 2 hr after the pulse can be seen in

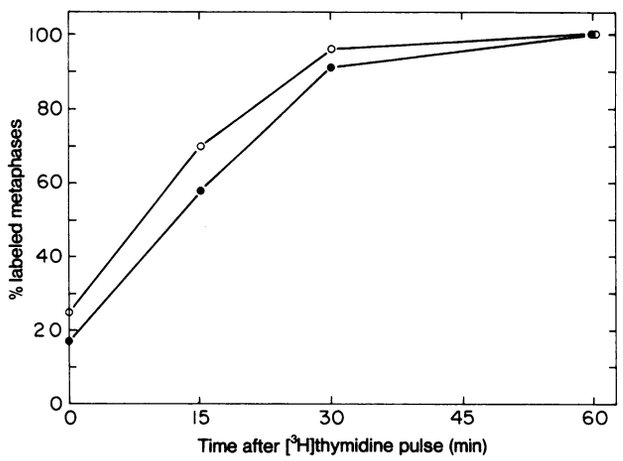


FIG. 1. Kinetics of appearance of labeled metaphases after a 15-min pulse-label with [$^3$H]thymidine administered to logarithmically growing cultures. (○) V79-8; (●) V79-743. Slides were exposed for 28 days under Kodak NTB2 emulsion.

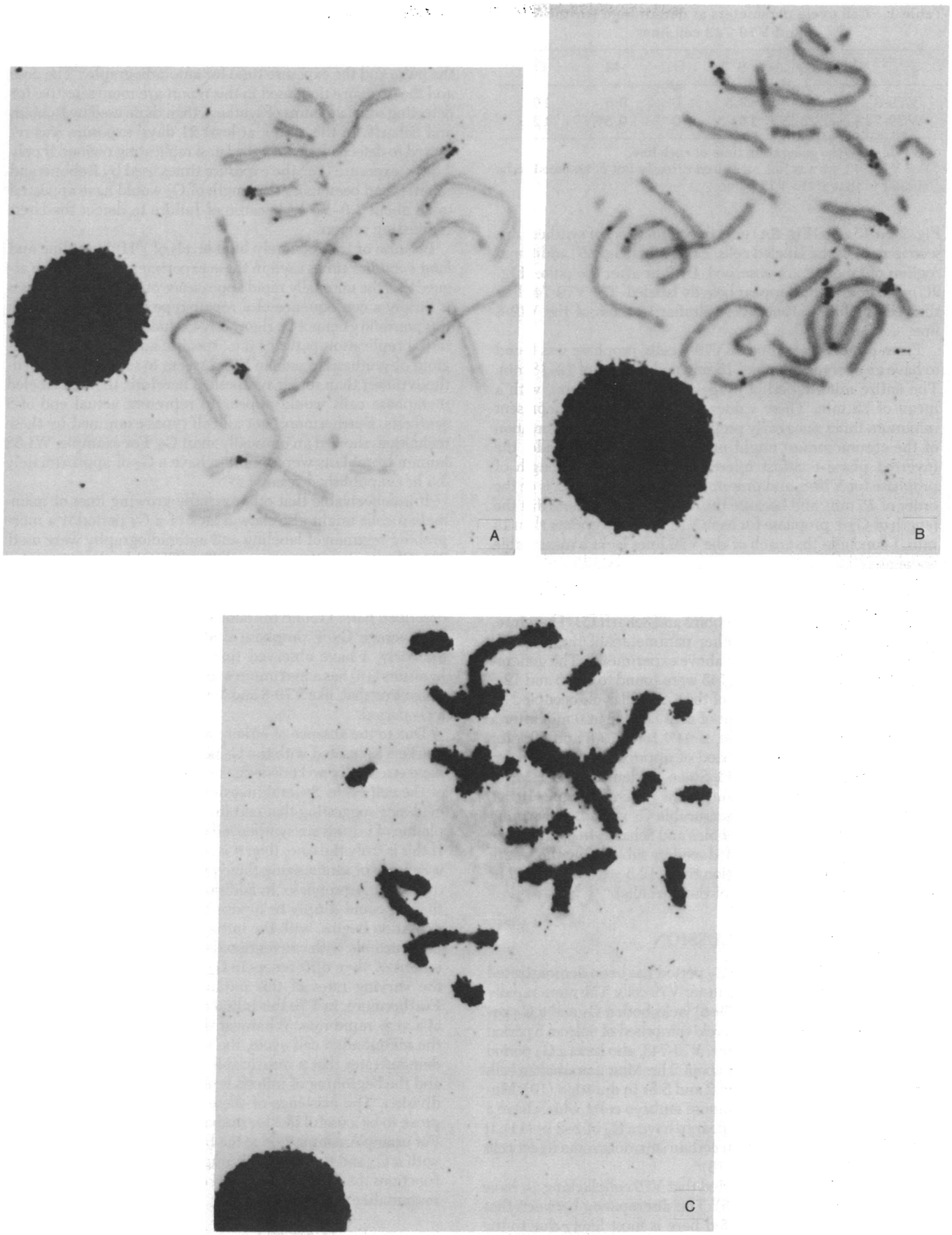


FIG. 2. Representative late-S labeling patterns of V79-8 cells seen at (*A*) 15 min, (*B*) 30 min, and (*C*) 2 hr after a 15-min pulse of [$^3$H]thymidine. Slides were exposed for 28 days under Kodak NTB2 emulsion.

Table 1. Cell cycle parameters as determined for the V79-8 and V79-743 cell lines

| | $G_1$ | S | $G_2$ | M | GT |
|---|---|---|---|---|---|
| V79-8 | 0 | 9.5 | 0 | 0.5 | 10 |
| V79-743 | 2 | 10 | 0 | 0.5* | 12.5 |

GT denotes the generation time of each line.
*For V79-743, M was not measured directly but is assumed to be similar to that of the V79-8 line.

Fig. 2. At 15 min (Fig. 2A) only two to four foci of synthesis are seen in most of the labeled cells. At 30 min (Fig. 2B) additional regions of labeling are observed. By 2 hr after the pulse (Fig. 2C) all chromosomes appear heavily labeled. The V79-743 line showed labeling patterns very similar to those of the V79-8 line.

From direct observation of V79-8 cells, prophase was found to have an average length of 15 min and a range of 12–18 min. The entire mitotic period ranges from 23 to 30 min, with a mean of 25 min. These values for V79-8 possibly represent minimum times since early prophase (i.e., the first condensation of the chromosomes) might not be readily visible under the inverted phase contrast microscope. Because the length of prophase for V79-8, and presumably also for V79-743, is on the order of 15 min, and because the results in Fig. 1 show that the length of $G_2$ + prophase for each V79 line is no greater than 15 min, I conclude that each of the V79 lines lacks a measurable $G_2$ phase.

**Lengths of S, $G_2$, and Generation Time.** It was important to ask whether the V79-8 cell line studied here actually did lack a $G_1$, as earlier reported by Robbins and Scharff (5). Therefore, additional information on other parameters of the V79 cell cycles was obtained from the above experiments. The generation times of V79-8 and V79-743 were found to be 10 and 12.5 hr, respectively. The percent of the total cell cycle occupied by S, as measured by the percent of cells labeled at 0 min after a pulse with [$^3$H]thymidine (9), is 94% for V79-8 and 80% for V79-743, indicating an S period of approximately 9.5 hr for V79-8 and 10 hr for V79-743. Since the length of S + M for V79-8 is 10 hr, which is the same as the generation time (10 hr), the V79-8 cell line has no measurable $G_1$ period, confirming the earlier observations of Robbins and Scharff. In contrast, the length of $G_1$ of V79-743, as deduced by subtracting the length of S plus M from the generation time (12.5 − 10 − 0.5), is 2 hr (see Table 1 for a summary of these results).

## DISCUSSION

The absence of a detectable $G_2$ period has been demonstrated for two sublines of Chinese hamster V79 cells. The more rapidly growing of the two lines (V79-8) lacks both a $G_1$ and a $G_2$ period, and therefore has a cell cycle comprised of only an S period and mitosis. The other V79 line, V79-743, also lacks a $G_2$ period but, interestingly, has a $G_1$ of about 2 hr. Most mammalian cells in culture have a $G_2$ between 2 and 5 hr in duration (10). Mukhergee showed that early mouse embryo cells, which have a generation time of 10 hr, appear to have a $G_2$ of 1–2 hr (11). It does appear, however, that in certain situations some insect cells fail to show a $G_2$ phase (12, 13).

Robbins and Scharff reported that V79 cells lacking $G_1$ have a $G_2$ period of about 2 hr (5). The discrepancy between that report and the data presented here is most likely due to the different labeling and autoradiography procedures used. Because the very late-labeling regions of V79 cells seen in this study appear to represent the synthesis of only a small fraction of the total DNA (see Fig. 2), detection of the very end of S is unusually dependent on the dose of [$^3$H]thymidine used during the pulse and the exposure time for autoradiography. The dose and the exposure time used in this report are more sensitive for detecting small amounts of synthesis than those used by Robbins and Scharff. In this study, at least 21 days' exposure was required to detect adequately the latest replicating regions. If only 7 days' exposure (i.e., the exposure times used by Robbins and Scharff) had been used, the length of $G_2$ would have appeared to be about 1.0–1.5 hr because of failure to detect the latest replicating regions.

Because of the relatively high levels of [$^3$H]thymidine and long exposure times used in these experiments, it could be argued that the unusually rapid appearance of labeled metaphases is simply a consequence of a repair-type synthesis. Although this possibility cannot be rigorously excluded, the nature of the initial replication patterns (i.e., specific and reproducible regions of synthesis as seen in Fig. 2) seem to support late-S synthesis rather than repair synthesis. Therefore, the first labeled metaphase cells would appear to represent actual end-of-S synthesis. Furthermore, not all cell types examined by these techniques showed an unusually short $G_2$. For example, WI-38 human fibroblasts were found to have a $G_2$ of approximately 3.5 hr (unpublished results).

It is conceivable that other rapidly growing lines of mammalian cells might also show a lack of a $G_2$ period if a more probing regimen of labeling and autoradiography were used. The Chinese hamster ovary cell line (CHO) has been reported to have an average $G_2$ + prophase time of 2 hr (14, 15). In contrast, using the conditions of labeling and exposure times described here, I found (unpublished results) that in CHO cells the average $G_2$ + prophase is a maximum of only 0.75 hr. Similarly, I have observed that the CHL-1 line of Chinese hamsters (16) has a maximum average $G_2$ + prophase of 0.5 hr, suggesting that, like V79-8 and V79-743, this line probably lacks a $G_2$ period.

Due to the absence of either specific genetic or biochemical markers associated with the $G_2$ period in mammalian cells, we have essentially no knowledge of events unique to this portion of the cell cycle. Several investigators have obtained indirect evidence suggesting that certain proteins crucial for the completion of mitosis are synthesized during the $G_2$ period (17, 18). If this is truly the case, then it is reasonable to assume that V79 is capable of synthesizing these proteins during the S period or during early prophase. In addition, Prescott (4) has speculated that $G_2$ could simply be a period in which chromosome condensation begins, with the initial steps of condensation being undetectable with conventional light microscopy. If this view is correct, then differences in $G_2$ lengths might be due only to the varying rates of this initial chromosome condensation. Furthermore, in V79 this initial condensation must take place at a very rapid rate. Whatever the nature of the $G_2$ period in the mammalian cell cycle, the $G_2$-less condition of V79 cells demonstrates that a measurable period between the end of S and the beginning of mitosis is not absolutely essential for cell division. The existence of these cell lines that lack $G_2$ could prove to be a useful tool for studying the nature of the $G_2$ phase. For example, comparison at the biochemical level between cells with a $G_2$ and those without might aid in identifying cellular functions that are restricted and crucial to the $G_2$ period of the mammalian cell cycle.

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