

## THE ABSENCE OF A DETECTABLE G<sub>1</sub> PHASE IN A CULTURED STRAIN OF CHINESE HAMSTER LUNG CELL

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The life cycle of actively growing mammalian cells has been divided into four phases (1) which include the following: mitosis (M); the period between mitosis and DNA synthesis (G<sub>1</sub>); DNA synthesis (S); and the period from S to mitosis (G<sub>2</sub>). While S and G<sub>2</sub> apparently bear a relatively constant relationship to each other, G<sub>1</sub> is highly variable (2) and is undetectable in cells from some lower organisms (3-7). On the other hand, all cultured mammalian cells thus far studied have a detectable G<sub>1</sub>(2). There are a few *in vivo* studies carried out on ascites tumor cells and ameloblasts in which G<sub>1</sub> was purportedly absent since the total generation, or doubling time equalled the sum of G<sub>2</sub>, mitosis and S (8-10). Because of the approximations inherent in the determination of G<sub>2</sub> and S by radioautographic techniques, this calculation is necessarily an approximation also. It thus is useful to report studies on a strain of Chinese hamster lung cells which can be synchronized by selective detachment of mitotic cells (11) and in which G<sub>1</sub> can be shown to be absent by direct measurement.

### MATERIALS AND METHODS

The Chinese hamster lung cells used in these studies were kindly supplied by Dr. M. Elkind, and were derived from a strain isolated by Ford and Yerganian (12). They were maintained in Eagle's medium (13) supplemented with 10% fetal calf serum and 0.2% lactalbumin hydrolysate. Pure populations of mitotic cells were obtained as described previously by the method of selective detachment (11), and the resulting synchronized cultures were grown in suspension. The length of G<sub>1</sub> and S was determined by incubating 1 ml aliquots of the synchronized culture with thymidine-<sup>14</sup>C (0.1 μc/ml) for 20 min beginning immediately after harvesting and continuing for one life-cycle. Protein and RNA syntheses were measured concomitantly on separate 1 ml aliquots using amino acids-<sup>14</sup>C (1 μc/ml NEC-445 amino-acid mixture) and uridine-<sup>14</sup>C (0.1 μc/ml), respectively (11). As an independent assay, G<sub>1</sub> was also studied by thymidine-<sup>3</sup>H radioautography of cells during and immediately after mitosis. Mitotic cells were selectively detached into medium containing the isotope (5 μc/ml) and samples were removed at 10-min inter-

vals thereafter. Cells were washed twice in cold, balanced salt solution, resuspended in 2% ethanol/acetic acid (3:1) for 5 min, centrifuged, resuspended in 100% ethanol/acetic acid, and air-dried on microscope slides. The slides were coated with NTB-3 liquid emulsion, were exposed 3-5 days and, after development by standard techniques, were stained through the emulsion with 0.25% toluidine blue (14). The length of G<sub>2</sub> was determined by pulse labeling logarithmically growing cells on coverslips for 15 min with thymidine-<sup>3</sup>H (5 μc/ml), washing with medium containing unlabeled thymidine (0.01 mM), and fixing for radioautography at 30-min intervals thereafter. The time between the pulse and the earliest appearance of labeled mitotic cells was taken as G<sub>2</sub>.

### RESULTS AND DISCUSSION

Chinese hamster lung cells selectively detached from monolayers yielded pure populations of mitotic cells consisting of about 60% metaphase and 40% anaphase or telophase cells. When these cells were incubated with thymidine-<sup>14</sup>C for 15 min significant amounts of isotope were incorporated into acid-precipitable, KOH-resistant, and DNase-sensitive material (Fig. 1). By this time, about 40% of the cells had completed mitosis and had passed into interphase. The rate of incorporation increased to a maximum 4 hr after synchronization and then declined. DNA synthesis thus starts as the cells enter interphase and increases in rate without detectable lag. RNA and protein syntheses also rise rapidly immediately after mitosis and begin to plateau by mid-cycle, a pattern similar to that previously found in HeLa cells (11). Although synchrony is lost to a significant extent after one life-cycle, the shape of the thymidine-<sup>14</sup>C incorporation curve indicates an S period of about 6-7 hr.

While the immediate onset of DNA synthesis in a population of synchronized cells suggested the absence of G<sub>1</sub>, this was established by direct examination of individual cells. Mitotic cells were selectively detached into thymidine-<sup>3</sup>H and pulsed for 15 min. By the end of the pulse, 46% of the cells had completed mitosis and, of these, 47%

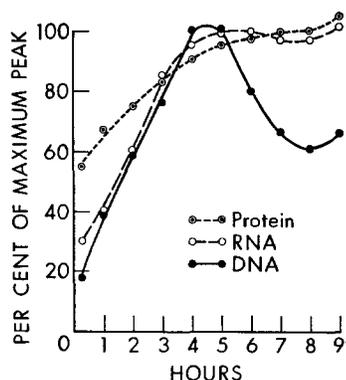


FIGURE 1 Macromolecular synthesis in synchronized hamster cells. Cells were prepared as described in Materials and Methods and incubated with the appropriate precursors. Counts per minute were plotted as per cent of the point of maximum incorporation during the first cycle (0-8 hr). Incorporation of uridine into DNA was always less than 10% and has not been corrected for in the figure.

showed nuclear incorporation of isotope by radioautography. Clearly there was no detectable  $G_1$  in a significant fraction of these cells. The lack of label in about one-half of the interphase cells may be due to heterogeneity of the population or the metabolic state of the cells at the time of collection. We have, in fact, noted that monolayer overgrowth before synchronization induces a lag in the onset of S which simulates  $G_1$ .

Radioautography was also useful in more accurately establishing the length of  $G_2$  in these cells. In randomly growing cells pulse-labeled with thymidine- $^3\text{H}$  and "chased" in medium containing "cold" thymidine plus colchicine, the first labeled mitotic cells arrested in metaphase appeared  $1\frac{1}{2}$ -2 hr after the pulse (Fig. 2). Since these initial cells must have been in S at the time of the pulse, the span of  $G_2$  is thus established as  $1\frac{1}{2}$ -2 hr. The duration of S may also be estimated from Fig. 2 as the time between the initial appearance of labeled mitotic cells and the point at which they no longer accumulate, i.e., the slope of the curve approaches zero.

In this report, direct evidence for the absence of a detectable  $G_1$  period in a cultured mammalian cell line has been obtained with synchronized cells. These observations, taken together with the variable length of  $G_1$  in many cell types, demonstrate that an interval between the end of mitosis and the onset of DNA synthesis is not essential for the replication of DNA in mammalian cells. In fact,

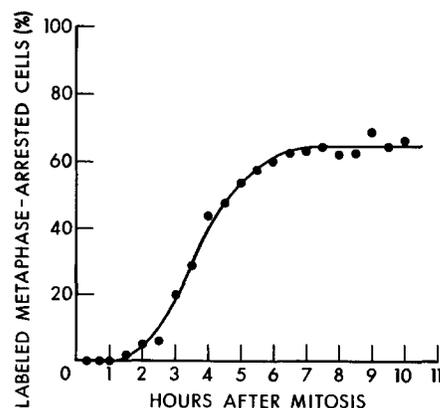


FIGURE 2 Random cells were labeled with thymidine- $^3\text{H}$  and treated with colchicine; the per cent of labeled metaphase arrested cells was determined as described in the text.

some of the enzymes partaking in the synthesis for DNA appear at the onset of S (15), and similar findings have been reported for certain chromosomal proteins as well as their sites of synthesis (16). None of these studies rule out the possibility that cells lacking  $G_1$  make some kind of preparation for DNA synthesis during the preceding  $G_2$  but, if one considers the radical metabolic and morphologic transitions which occur during mitosis, this seems unlikely.

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