ASYNCHRONOUS REPLICATION OF HELA S3 CHROMOSOMAL DEOXYRIBONUCLEIC ACID

ROBERT B. PAINTER. From the Battelle Memorial Institute, Columbus, Ohio

Asynchronous replication of chromosomal deoxyribonucleic acid (DNA) of the Chinese hamster has been reported by Taylor (1), and Lima-de-Faria (2) published somewhat similar findings in the grasshopper, *Melanoplus*. More recently Wimber (3) has reported asynchrony in chromosomal replication in *Tradescantia*, and Woodard *et al.* (4) have observed the same phenomenon in *Vicia*. In some cases at least part of the replication of the sex chromosomes occurred at times different from that of the autosomes. Previously we reported (5) that the replication of the chromosomes in the HeLa S3 cell occurred for the most part at the same time, but that either the onset or the termination, or both, did not occur simultaneously in all the chromosomes. However, the experimental design did not allow results which would yield information concerning the time parameters of replication, and the grain densities were not heavy enough to allow statements about the participation of single chromosomes in replication.

In this note we shall present some information on the time sequence of chromosomal replication in HeLa S3, illustrated with autoradiograms demonstrating clearly the activity of individual chromosomes.

MATERIALS AND METHODS

Tritiated thymidine (H^3TDR), at a specific activity of 3.0 c/mM, was added directly to the medium of coverslip cultures in Leighton tubes. In some experiments 0.1 μg was used for 30 minutes, in others 1.0 μg for 10 minutes, after which the medium was removed, the cells were washed twice with Hanks' solution, and a medium containing unlabeled thymidine (10 μg/ml) was added. We have previously shown (6, 7) that this treatment completely removes all H^3TDR for subsequent labeling and that no increase in percentage of labeled cells or in grain count per cell occurs after this treatment. Colchicine was added to give a final concentration of 0.2 μg/ml 1 hour before removal of cultures for sacrifice at 0, 2, 4, 6, 12, and 24 hours after media change. All cultures were washed with phosphate-buffered saline (PBS) and treated for 10 to 30 minutes with quarter strength PBS, in order to spread the chromosomes before fixing with acetic acid–ethanol (1:3). The coverslips were removed, stained by the Feulgen method, and mounted (cell side up) on microscope slides, and stripping film autoradiograms were prepared. After exposure of the film (varying from 2 weeks to 3 months) the preparations were developed, and scanned for the presence of labeled and unlabeled metaphases and for labeled metaphases showing label over only a part of the total chromosome set.

RESULTS

Among the first labeled metaphases to appear (at 2 hours after H^3-thymidine incubation), only a few of the chromosomes were labeled, but at later times a progressively increasing fraction of the labeled metaphases showed grains over a greater and greater fraction of the chromosomes. After 6 hours, by far the greater fraction of the labeled metaphases had grains over every chromosome. We have previously shown that the variations in grain distribution which occur over HeLa S3 cells grown on glass are not detectably affected by overlying cytoplasm (5). Figs. 1 to 3 show examples of the asynchrony of chromosomal replication which occurs. Fig. 1 shows a cell in which only a few chromosomes are labeled. Metaphases showing label over only one, two, or three chromosomes are relatively frequent 2 to 4 hours after H^3TDR incubation. Fig. 2 shows a cell where about one-half the chromosomes are labeled. Metaphases with 25 per cent or more labeled chromosomes become increasingly frequent with time in preparations fixed 2 to 5 hours after incubation with the tracer. Fig. 3 shows a cell that has all the chromosomes labeled except one. This kind of labeling is rare and occurs later than the kinds shown in Figs. 1 and 2. Fig. 4 shows the appearance of a metaphase in which all the chromosomes are heavily labeled, the rule for metaphases appearing at 6 hours and later,

although very rarely a partially labeled metaphase is seen, as in Fig. 3.

Another interesting observation has been the finding of partially labeled individual chromosomes, examples of which are found in Figs. 5 and 6. Such labeling is much more prevalent at the early times (2 to 6 hours) after labeling. Although the localization is difficult to identify on these small chromosomes, the grains often seem to be restricted to a position near the centromere, but other parts of the chromosomes are sometimes exclusively labeled. This phenomenon is restricted to the early times after labeling, and variations in cytoplasmic shielding cannot account for it (see Discussion). Moreover, the grain counts over the chromosomes are so heavy (20 or more per chromosome) that unlabeled areas cannot arise as often as is observed by chance alone.

DISCUSSIONS

The first labeled division figures to appear after a "burst" labeling with H^3TDR are those which were near the end of the DNA synthesis phase at the time of incubation with the tracer (6). The fact that the majority of the labeled metaphases appearing soon after tracer incubation have some unlabeled chromosomes shows that the time when different chromosomes finish replication varies to a great extent. Such a difference in labeling over individual chromosomes cannot arise by variability in shielding by cytoplasm or cell membrane, since such a variability would be independent of time after tracer incubation. The tendency for heavier, but still incomplete, labeling of the metaphases up to 5 hours after the tracer incubation indicates that some chromosomes must complete replication comparatively early, and others finish at different times thereafter. Thus the time of cessation of replication varies rather widely, probably as much as 2 or more hours, among the chromosomes.

On the other hand, the homogeneously labeled metaphases which are found at later times, with no recurrence of a time of marked heterogeneous labeling, make it appear that the onset of replication of the chromosomes is almost simultaneous. The occasional appearance of a metaphase with

FIGURE 1

Autoradiogram of HeLa S3 colchicine-treated metaphase of a kind often found 2 to 4 hours after short incubation with tritiated thymidine. About six chromosomes have clusters of grains lying above them. × 1500.

FIGURE 2

Autoradiogram of HeLa S3 colchicine-treated metaphase, typical of those found frequently at times from 2 to 6 hours after short incubation with tritiated thymidine. Completely unlabeled chromosomes lie among chromosomes with varying numbers of grains overlying them. × 1500.

FIGURE 3

Autoradiogram of HeLa S3 metaphase at 12 hours after tracer incubation. One chromosome (arrow) is unlabeled. This type of labeling is very rare. × 1300.

FIGURE 4

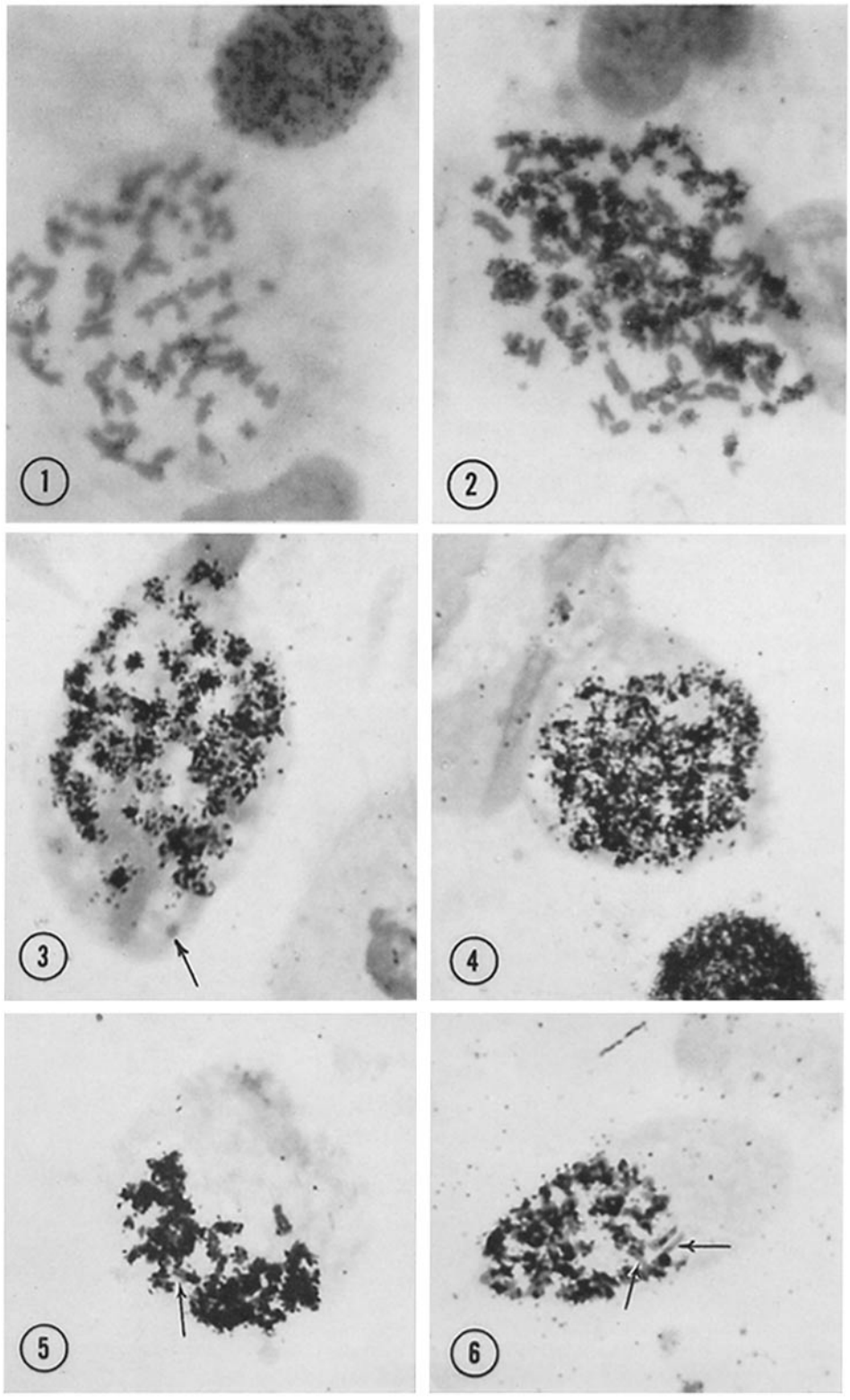
Autoradiogram typical of metaphases found at 6 hours and later following tracer incubation. All chromosomes are heavily labeled. × 1300.

FIGURE 5

Autoradiogram showing partial labeling of chromosome at 3 hours after labeling. Arrow points to unlabeled part of a chromosome that has grains lying only over the area of the centromere. × 1300.

FIGURE 6

Another example of metaphase with two partially labeled chromosomes (arrows) at 3 hours after labeling. × 1300.



one to a few unlabeled chromosomes at these times indicates that the synchrony in onset of replication is not quite perfect. However, such patterns are so rare at the later times, as compared with their great preponderance in earlier labeled metaphases, that it appears that the onset of replication of all the chromosomes must occur within a few minutes. These results are quite similar to those reported for *Tradescantia* by Wimber (3).

The asynchrony of chromosomal replication in the HeLa S3 cell therefore appears to be almost exclusively due to differences in the times when the individual chromosomes finish duplication, the onset being practically simultaneous. This differs somewhat from the pattern in Chinese hamster cultures, where the sex chromosomes evidently start and finish much later than the autosomes. However, the HeLa strain has been in culture for many years, and it is possible that the replication pattern has been altered during the long sustained *in vitro* conditions.

Under any circumstances it is not surprising that asynchrony in chromosomal replication of the type observed in HeLa does occur. Since the onset of replication takes place at the same time in chromosomes of greatly varying size, and, presumably, of varying DNA content, the cessation of replication would have to occur at different times in different chromosomes if the rate of synthesis along each chromosome is relatively constant. We are studying the distribution of label in more detail to see if the asynchrony in replication is regularly reproducible and, if so, to determine more exactly the pattern of replication of individual chromosomes. We hope to compare these results with those in primary cultures from humans.

The observation that parts of individual chromosomes are heavily and exclusively labeled is similar to that found in the Chinese hamster (1), in rye (2), and in *Tradescantia* (3). Such observations are of importance for attempts at building "models" of the chromosome. It seems to us that, considering the secondary coiling required, the hypothesis that chromosomal DNA is one continuous strand is unlikely, in the light of these examples of highly localized labeling. Even assuming that replication of DNA can begin simultaneously at several points within the hypothesized single molecule, it is extremely difficult to conceive a situation wherein these simultaneously duplicated sites, formed relatively far from one another in the uncoiled condition during interphase, would coil up into the same chromosomal area at mitosis. A more logical chromosomal model, compatible with the labeling results, would be one consisting of many molecules of DNA, arranged in such a manner that areas close to one another during replication in interphase are similarly situated during mitosis.

This work was aided by Grant CY-5222 from the National Institutes of Health.

Received for publication, May 3, 1961.

REFERENCES

1. TAYLOR, J. H., *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 455.
2. LIMA-DE-FARIA, A., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 457.
3. WIMBER, D. E., *Exp. Cell Research*, 1961, **3**, 402.
4. WOODARD, J., RASCH, E., and SWIFT, H., *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 445.
5. PAINTER, R. B., DREW, R. M., and GIAUQUE, B. G., *Exp. Cell Research*, 1960, **21**, 98.
6. PAINTER, R. B., and DREW, R. M., *Lab. Invest.*, 1959, **8**, 278.
7. FEINENDEGEN, L. E., BOND, V. P., and PAINTER, R. B., *Exp. Cell Research*, 1961, **23**, 381.