REGULATION OF DNA REPLICATION ON SUBCHROMOSOMAL UNITS OF MAMMALIAN CELLS

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ABSTRACT

The regulation of DNA replication at a subchromosomal level in mammalian cells has been investigated. DNA fiber autoradiographs were prepared from mouse L-929 cells pulse labeled with [^{3}H]thymidine. Initiation events and subsequent chain growth occurring over short stretches (up to three replication units in length) of chromosomal DNA were analyzed. The results show that adjacent units usually initiate replication synchronously and that this synchrony is related to the proximity of initiation sites. In addition, adjacent units are of similar size and the rates of replication fork progression within units and on adjacent units are similar. The rate of fork progression increases with increasing replication unit size. Finally, no evidence for fixed termination sites for the units has been found. These observations suggest that despite large variations in size of replication units, timing of initiation events, and rates of fork progression found in chromosomal DNA as a whole, these processes are closely regulated within subchromosomal clusters of active replication units.

The chromosomal DNA of mammals as well as of other eukaryotes is organized for the purposes of replication into multiple discrete units (4, 11). These linear units, of varying size, each contain a centrally placed initiation point. The replication forks proceed bidirectionally from this point until they meet with forks from adjacent units at which time the newly synthesized DNA chains fuse, producing the long strands characteristic of eukaryotic chromosomal DNA. It has been proposed that these are the basic units of control in the initiation of replication, each being composed of a pair of replication sections and two replication forks (11). The replication units are of varying size (4, 9, 11, 14) as measured by the distance between activated initiation sites; such sites are located at irregular intervals of up to 400 μm along the chromosome (9, 14). The mean rate of chain growth or fork progression[1] is somewhat less than 1 μm/min, with as much as a fivefold variation in the same cell type (4, 7, 8, 11, 13). The mechanisms that regulate initiation and chain growth at subchromosomal levels, and thus are responsible for these variations, are unknown.

Evidence has been presented that in amphibian and insect cells the size of replication units varies with the stage of development (2, 4) and that in avian cells neighboring units tend to be similar in

[1] The terms *rate of DNA chain growth* and *rate of replication fork progression* refer to the same parameter when measured as distance per unit time (i.e., micrometers per minute) and will be used interchangeably in this paper.

length (14). Also, there are data indicating that mammalian cells in early S phase have slower rates of chain growth than do those in mid S phase (15).

Much of this information has been accumulated by the technique of DNA fiber autoradiography, which is particularly suitable for the analysis of the events of replication at subchromosomal levels. In the present work, I have used this technique to answer several questions on the regulation of DNA replication over small lengths of the mammalian chromosome involving one or several replication units. Specifically, I have asked (*a*) Do adjacent units initiate replication synchronously? (*b*) What is the effect of proximity of initiation sites on initiation synchrony? (*c*) Are the units in localized regions of the chromosome of similar size? (*d*) How closely regulated is the rate of fork progression in replication sections within the same unit and in adjacent units? (*e*) Does the rate of fork progression vary with the size of the units? and (*f*) Is there any evidence for fixed termination sites?

MATERIALS AND METHODS

Procedures used in this study are generally similar to those in previous studies (7–9). Modifications will be detailed with the individual experiments.

RESULTS

Do Adjacent Units Initiate Replication Simultaneously?

With appropriate pulse-labeling protocols using [^{3}H]thymidine to label cellular DNA for fiber autoradiography, two different types of autoradiographic patterns representing ongoing replication in individual units are obtained. These indicate whether initiation of replication took place before or after the beginning of the radioactive pulse on a particular unit (Fig. 1). The degree of synchrony of initiation events on adjacent units can be determined to an extent by analyzing the frequency with which each of these characteristic patterns appears on DNA fiber autoradiograms containing two adjacent active replication units.

Mouse L-929 cells in monolayer cultures were exposed to [^{3}H]thymidine and their DNA was processed for fiber autoradiography as indicated in the legend to Fig. 1. On the developed autoradiographs, the majority of patterns produced by pulse labeling DNA sequentially with [^{3}H]thymidine of high specific activity (hot pulse) and then of low specific activity (warm pulse) were of two types: (*a*) those produced by units in which initiation occurred *after* the beginning of the pulse showed linear arrays of heavy grain density flanked on either side by arrays of lighter grain density (Fig. 1 *a*) representing DNA replicated during the hot pulse and then during the warm pulse (postpulse initiation patterns); and (*b*) those produced by the units in which initiation occurred before the beginning of the pulse showed a short clear area indicating DNA replicated before the start of the hot pulse, flanked by arrays of heavy grain density proceeding to lighter grain density arrays (Fig. 1 *b*) indicating DNA replicated during the hot and warm pulses with [^{3}H]thymidine (prepulse initiation patterns).

If initiation events occur randomly rather than synchronously, then along any DNA autoradiogram containing two replication units the probability of obtaining two patterns of the same type (either postpulse or prepulse, Fig. 1 *a*, *b*) or of different types (Fig. 1*c*) can be calculated from the observed frequencies of both patterns according to the distribution $(p + q)^2 = 1$. If initiation is synchronous over short stretches of the chromosome, then the proportion of autoradiograms with two patterns of the same type should be significantly higher than the expected proportion. To examine this, autoradiograms containing two adjacent replication units were scored to determine the proportion having two initiation patterns of the same type. In an experiment in which DNA was labeled with [^{3}H]thymidine during a 30-min hot pulse followed by a 30-min warm pulse, only 0.15 of the autoradiograms showed a mixture of the two patterns, whereas 0.85 of the pairs contained either two postpulse or two prepulse patterns. A similar experiment utilizing a 10-min hot pulse followed by a 2-h warm pulse showed that 0.20 of autoradiograms contained both patterns, whereas 0.80 showed initiation patterns of one type only. These data are shown in Table I as exp 1 and 2. The observed proportions are significantly different from those expected if initiation were random ($P < 0.005$ for both exp 1 and 2).

Thus, initiation on adjacent units appears to occur nonrandomly. Synchrony was not absolute: in up to one-fifth of pairs, initiation occurred clearly at different points in time, either before or after the beginning of the hot pulse. The slightly higher proportion of autoradiograms showing both

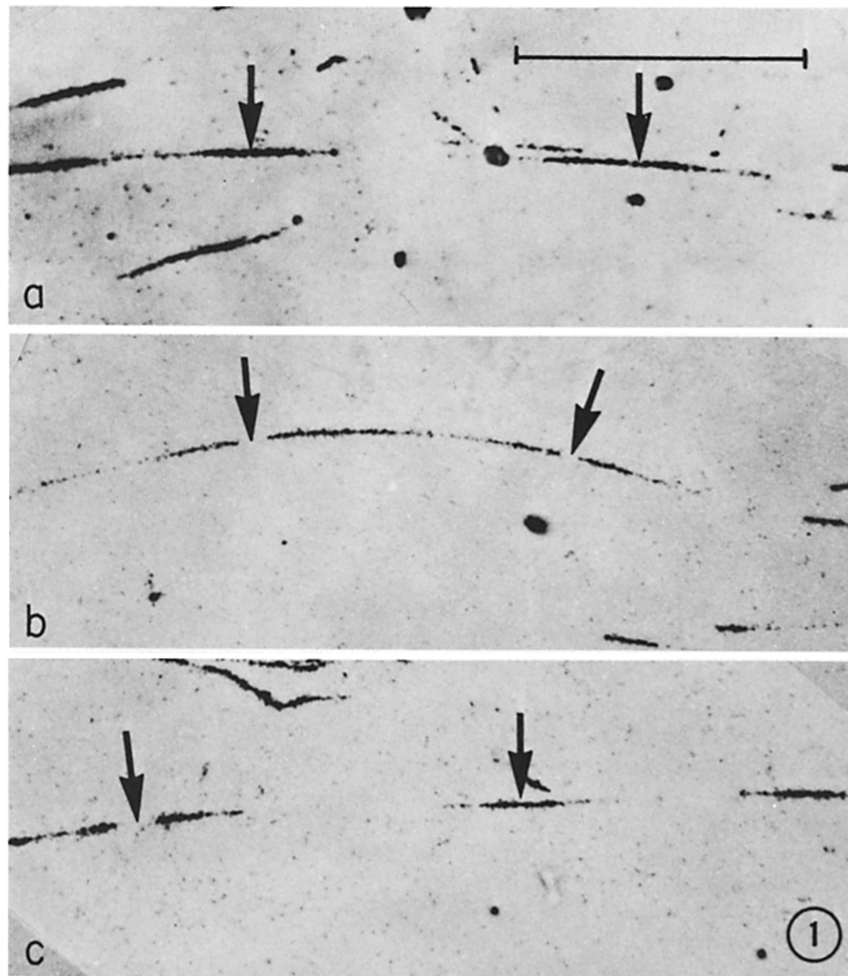

FIGURE 1 Autoradiographic patterns on adjacent replication units. 30 min before the beginning of a pulse-labeling period with [^{3}H]thymidine, monolayers of L-929 cells in logarithmic growth were exposed to fluorodeoxyuridine (2×10^{-6} M) to exhaust their endogenous thymidylate pools. It was kept in the medium during the pulse to increase the incorporation of exogenous thymidine. The cells were then exposed to [^{3}H]thymidine of high specific activity at 50 Ci/mmol, 5×10^{-6} M for 30 min (the hot pulse), then for an additional 30 min to [^{3}H]thymidine of lower specific activity at 5 Ci/mmol, 5.5×10^{-5} M (the warm pulse). After the pulse-labeling period, the cells were processed for DNA fiber autoradiography by gently lysing them on glass slides and spreading the labeled DNA with a glass rod. After being coated with nuclear track emulsion NTB-2 (Eastman Kodak Co., Rochester, N.Y.), the slides were exposed for 6 or 8 mo and then developed. The details of the pulse-labeling and autoradiographic techniques have been published (7, 8). The arrows in the photograph indicate the centers of units where initiation of DNA replication occurred. The bar represents 100 μm. All three micrographs were taken at the same magnification. (*a*) Two adjacent units in which DNA replication initiated after the onset of the hot pulse at the sites indicated by the arrows, and chain growth then proceeded bidirectionally during the remainder of the hot pulse (high-grain density arrays) and into the warm pulse (low-grain density arrays). These are postpulse initiation patterns. (*b*) Two adjacent units in which DNA replication started before the onset of the pulse. Initiation presumably occurred at the center of the clear areas indicated by the arrows, then proceeded bidirectionally. DNA chain growth proceeded through the entire hot pulse (high-grain density arrays) and into the warm pulse (low-grain density arrays). These are prepulse initiation patterns. (*c*) Two adjacent replication units, one showing a prepulse initiation pattern (left-hand arrow) and one a postpulse (right-hand arrow).

patterns (0.20) observed with the shorter hot pulse (10 min), although not significant statistically, is readily explainable. Since synchrony is not perfect, slightly higher proportions should be observed with shorter hot pulses when this method of analysis is used.

What Is the Effect of Proximity of Initiation Sites on Synchrony?

Synchrony of initiation events might be demonstrably related to the proximity of initiation sites if the conditions serving to begin replication on one unit do so on an adjacent unit. An agent inhibiting initiation might decrease the synchrony of initiation events along the DNA fiber. Reovirus, a cytoplasmic RNA virus, inhibits the frequency of DNA initiation in that initiation points occur at more widely spaced intervals in infected cells than they do in noninfected controls (9). If synchrony is determined in part by the proximity of adjacent sites, then adjacent replication units that are farther apart should show less synchrony. I therefore determined the proportion of adjacent replica-

TABLE I

DNA Fibers Containing Two Adjacent Replication Units with Similar or Different Initiation Patterns

	Proportion				
		Observed§			
Types of initiation patterns*	Expected‡	Exp 1‖	Exp 2	Exp 3	Exp 4
Postpulse + postpulse	0.226	0.40	0.37	0.40	0.31
Prepulse + prepulse	0.276	0.45	0.43	0.31	0.32
Postpulse + prepulse	0.499	0.15	0.20	0.29	0.37

* As illustrated in Fig. 1 and defined in its legend.

‡ Calculated from the observed frequency of both patterns in exp 1 according to the distribution $(p + q)^2 = 1$, where the proportion of postpulse initiation figures $= p = .475$ and the proportion of prepulse initiation figures $= q = .525$. The sum of the expected probabilities is 1.001 rather than 1.000 because of rounding error. The proportions are very similar to those observed earlier in a much larger sample from a 30-min pulse with [^{3}H]thymidine of high specific activity (9), and are also similar to those observed in exp 2 in which the high specific activity pulse was 10 min.

§ L-929 cells were pulse labeled with [^{3}H]thymidine and processed for autoradiography as in the legend to Fig. 1. The high specific activity (hot) and low specific activity (warm) pulses were for 30 min each (exp 1 and 3) or for 10 min and 120 min, respectively (exp 2 and 4). In exp 3 and 4, the cells were infected with reovirus 8 h before the [^{3}H]thymidine pulse. This produced a 63.2% reduction in incorporation of [^{3}H]thymidine as compared to that in uninfected cells, determined in replicate cultures pulse labeled with [^{3}H]thymidine (0.5 μCi/ml) for 10 min at 8-h postinfection. All four experiments were performed concomitantly, and the controls were sham infected 8 h before the pulse, as previously described (9). The developed autoradiographs were examined by light microscopy. To insure randomness of selection, preselected microscopic fields were examined as outlined previously (8), and no more than one appropriate autoradiogram was scored from one microscopic field at a magnification of 200–250. Internal autoradiograms containing two adjacent replication units were scored for two postpulse patterns, two prepulse, or one of each. 100 such autoradiograms were scored in each experiment. Statistical significance (presented in the text) was determined by applying the chi-square test for goodness of fit to expected and observed frequencies or for independence of variables, using contingency tables.

‖ The observed frequencies of postpulse and prepulse initiation figures are nearly equal in both exp 1 and 2. This would be expected if the amount of chain growth occurring during the hot pulse was small compared to the total length of the replication section, as in exp 2. In exp 1 the frequency of postpulse initiation figures should be higher because of exclusion from scoring of prepulse initiation figures which terminated replication before the beginning of the warm pulse. The explanation for the frequencies observed in exp 1 is not clear, but may be related to such factors as slowing of chain growth as the replication fork approaches termination or initiation events occurring during the 30-min block with fluorodeoxyuridine. These factors are under investigation presently.

tion units showing similar initiation patterns in reovirus-infected cells.

Cells were infected with reovirus at a multiplicity of 200 plaque-forming units per cell 8 h before the beginning of the [^{3}H]thymidine pulse. [^{3}H]thymidine incorporation was inhibited 63.2% in infected cells at the time of the radioactive pulse. Autoradiograms containing two adjacent pairs of replication units were scored. In infected cells exposed to a 30-min hot pulse followed by a 30-min warm pulse, 0.29 of autoradiograms showed a mixture of the two types of patterns, whereas 0.71 of pairs showed two initiation figures of the same type. In similar monolayers of infected cells, when the cells were labeled during a 10-min hot pulse followed by a 2-h warm pulse starting 8 h after infection, 0.37 of autoradiograms showed a mixture of the two types of patterns, whereas 0.63 of pairs showed two initiation figures of the same type. These data are shown in Table I as exp 3 and 4.

The observed proportions are again significantly different from those expected if initiation were random ($P < 0.005$). Thus, reovirus does not completely eliminate the synchrony of initiation. However, the observed proportions in infected cells are significantly different from those in uninfected cells pulsed in similar fashion ($P < 0.025$ for exp 1 vs. exp 3; $P < 0.01$ for exp 2 vs. exp 4). Therefore, it can be concluded that the synchrony of initiation is decreased by reovirus infection. The distances between initiation sites were greater in infected cells compared to control cells in agreement with earlier findings (9). The mean interinitiation interval was 55.2 μm in exp 1 vs. 73.5 μm in exp 3, and 57.2 μm in exp 2 vs. 91.2 μm in exp 4. The longer mean interval in exp 4 resulted from scoring of well-separated adjacent initiation sites which were connected by newly replicated chains that fused during the long 2-h warm pulse. These data support the proposition that synchrony is related to the proximity of initiation sites. Again, a slightly higher proportion of autoradiograms showed both patterns (0.37) with the shorter hot pulse (exp 3 vs. exp 4).

Are Adjacent Replication Units of Similar Size?

It has been determined previously that activated initiation sites occur at irregular intervals along the chromosome in eukaryotic cells (4, 9, 10, 11) and that the distance between sites can vary in different cells from the same organism (2, 4). Despite this variability, neighboring (i.e. adjacent) initiation intervals, and therefore neighboring replication units, tend to be similar in length in avian cells (14). I have determined whether adjacent replication units in mammalian cells tend to be of like size. L-929 cells were pulse labeled and processed for autoradiography as indicated in the legend to Fig. 1. 50 internal autoradiograms containing at least 3 initiation sites were traced and the intervals between sites measured. The left-hand interinitiation length was compared with the right-hand length. The correlation coefficient of the two variables was 0.39. This is significant statistically at the 0.05 level and shows that neighboring units tend to be of like size. An analysis of variance of the data similar to that carried out by McFarlane and Callan (14) for avian cells leads to the same conclusion, in that like initiation intervals tend to occur alongside one another and that there is no special correspondence among the left-hand lengths nor among the right-hand lengths.

Regulation of Rate of Chain Growth at the Level of Replication Units

Earlier studies have shown that the average rate of chain growth in nonsynchronized cells is between 0.6 and 0.7 μm/min/fork (8). There is a fairly wide range of rate, however—from as low as 0.4 to over 1.0 μm/min. It was of interest to determine how closely regulated the rate of chain growth is on the two forks of a replication unit and on the forks of neighboring units, and thus whether some of the variations seen in nonsynchronized cells actually exist within a single cell over a short length of chromosome at a particular stage of S. The rate of chain growth was investigated within individual units by comparing the rate of the left-hand fork with that of the right-hand fork; and on neighboring units by comparing fork movement along the four replication sections of a pair of units. 50 autoradiograms of the type depicted in Fig. 2 were traced. The rate of chain growth on each of the four sections (*a–d*, Fig. 2) was determined. The rates of the two forks within each unit and the four forks among the two units were correlated. The data are shown in Table II. The correlation coefficients for each of the comparisons were significant statistically at the level of 0.01. In addition, 50–60% of all pairs in each series measured were within 1 mm of each other at a

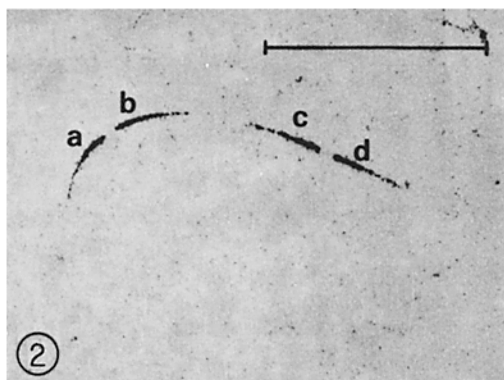

FIGURE 2 Adjacent prepulse autoradiographic patterns used for determinations of chain growth on individual replication sections. The DNA was labeled with [^{3}H]thymidine and prepared for autoradiography as indicated in the legend to Fig. 1. The bar represents 100 μm. The letters "*a*"–"*d*" mark the four individual replication sections in the two units and indicate the sections compared in the experiment in Table II.

TABLE II
Comparison of DNA Chain Growth on Individual Replication Sections of Two Adjacent Replication Units

Chain growth on different sections*	Correlation coefficient
Within units	
a vs. b *and* c vs. d	0.692
Between units	
a vs. c	0.527
a vs. d	0.498
b vs. c	0.619
b vs. d	0.593

* Using the autoradiographic slides from exp 1 in Table 1, 50 internal autoradiograms of the type depicted in Fig. 2 were traced. The amount of chain growth that took place on each of the four replication sections during the 30-min hot pulse was determined by measuring the lengths of the arrays of high grain density. Comparisons were made of the lengths of each of the four replication sections and correlation coefficients calculated. The letters "a" to "d" refer to the replication sections so labeled in Fig. 2. For the comparisons of sections within replication units (a vs. b *and* c vs. d), n = 100; for the comparisons of sections among the two units (a vs. c, a vs. d, b vs. c, b vs. d), n = 50. All correlation coefficients in the table are statistically significant as determined by critical values. In addition, the five can be considered homogeneous ($0.1 > P > 0.5$) and thus representative of samples from a population exhibiting a common correlation among the variables.

magnification of 500. On the basis of previous experiments, 1 mm is the limit of error of such measurements. The data indicate that there is a tendency for neighboring forks either sharing the same replication unit or on adjacent units to travel at the same rate. Therefore, over short stretches of chromosome replicating during a particular stage of S, rate of chain growth is rather closely regulated.

Does the Rate of Fork Progression Vary with the Size of the Replication Unit?

The size of the replication units varies at different stages of development in amphibian (4) and insect cells (2). However, in both cases there is little variation in the rate of chain growth on these rather different-sized units. The mean size of replication units in mammalian tissue culture cells is quite variable (9). I wished to determine whether there was a correlation between unit size and rate of chain growth to gain some insight into whether the two processes, initiation and chain growth, might be jointly regulated in a mammalian cell.

With the use of tracings from experiments just described on fork progression, the length of the enclosed interinitiation distance on each of the 50 autoradiograms was compared with the mean rate of chain growth on the four sections of the autoradiogram (mean of *a*, *b*, *c*, and *d*—Fig. 2). The scattergram from these comparisons is shown in Fig. 3. The divergency in replication unit size and in the rate of chain growth is similar to that observed previously (7, 9). The correlation coefficients between the two variables is 0.60, which is significant at the 0.01 level. Correlation between the interinitiation site length and the rate of chain growth on each of the four individual sections of the autoradiogram was from 0.45 to 0.57. These values are also significant at the 0.01 level. Thus replication units of greater size seem to have faster rates of chain growth.

Do Fixed Termination Sites Exist?

The nature of termination sites for DNA replication is not known. The presence or absence of fixed termination sites would have important implications for the organization of replicating units. I reasoned that if the sites were fixed at intervals along the mammalian chromosome, then certain types of autoradiographic patterns should be seen at a frequency high enough to be scored.

These are of at least two types and they are shown diagrammatically in Fig. 4. The sudden cutoff of the track in the center patterns could be interpreted as indicating that the replication fork had reached a fixed termination site. The most convincing pattern would then be that indicated in the

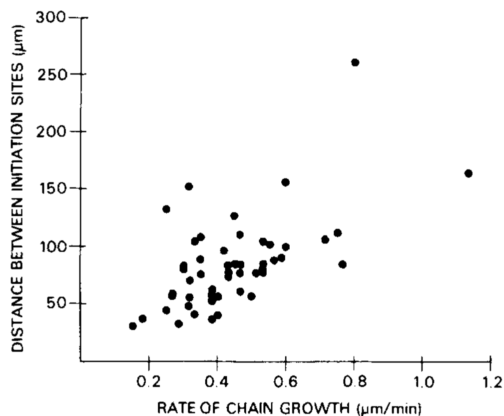

FIGURE 3 Scattergram comparing interinitiation distance with rate of chain growth over the same region of chromosomal DNA. 50 autoradiograms of the type depicted in Fig. 2 were traced. The distance between the two initiation sites (the center-to-center length, "*ab*" to "*cd*") was compared to the mean rate of chain growth calculated from the lengths of heavy grain density on all four replication sections ("*a*"–"*d*").

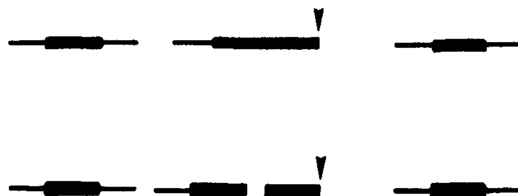

FIGURE 4 Diagrammatic representation of two autoradiograms that could indicate the presence of fixed termination sites. The heavy lines represent that DNA replicated during a hot pulse; the lighter lines, during a warm pulse. The arrowheads indicate points at which replication would stop if a fixed site were reached. In the upper diagram, replication was initiated at the center of the middle pattern after the beginning of the [^{3}H]thymidine pulse and proceeded bidirectionally. Chain growth continued on the left-hand fork of this unit through the hot and the warm pulses. On the right-hand fork, chain growth was stopped during the hot pulse by the putative termination point. The lower section is the same except that replication began at the center of the middle pattern before the beginning of the pulse.

lower part of the figure, where initiation on the center unit occurred before the onset of the pulse and continued on the right-hand fork of this unit to a point where it reached a putative termination site and then stopped. The center pattern in the upper part of the figure would represent a similar situation, except that initiation occurred after the beginning of the hot pulse and terminated on the right-hand side before the onset of the warm pulse. The upper diagram, however, is also compatible with unidirectional replication, a situation which is not common in eukaryotic cells but seems to occur with a low but measurable frequency (8, 12). Autoradiographic slides prepared from L-929 cells pulse labeled and processed as described in the legend to Fig. 1 were examined. Autoradiograms containing three initiation sites were noted and those corresponding to the diagrams in Fig. 4 were scored.

A long search of many slides from several experiments failed to reveal any internal autoradiograms of the type indicated by the diagram in the lower part of Fig. 4. 12% of internal autoradiograms were of the type indicated in the upper part of Fig. 4, and two of these are shown in Fig. 5. If such figures represent unidirectional replication, 12% compares quite favorably with the 10–20% of units involved in unidirectional replication found by us and by others in earlier studies (8, 12). The inability to locate the more diagnostic autoradiogram diagrammed in the lower part of Fig. 4 suggests that the autoradiograms shown in Fig. 5 most probably represent isolated examples of unidirectional replication rather than fixed termination sites.

DISCUSSION

There are two components to ongoing DNA replication in mammalian cells: initiation of new DNA chain synthesis and chain growth. The present findings show that both these components are closely regulated within subchromosomal clusters of active replication units.

Adjacent replication units appear to be of similar size. This is shown in the present work for mammalian cells and concurs with the findings in other studies on avian cells (14). Since there is a large variation in the size of these units within the cell (4, 9, 11), there may be classes of DNA within the chromosome, each characterized by units of similar size. Additional evidence for this has been

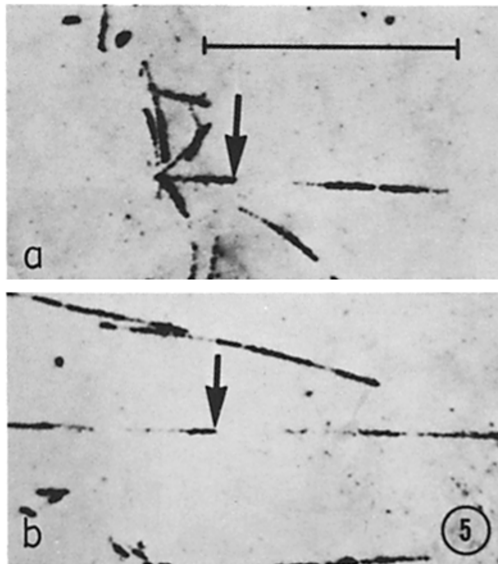

FIGURE 5 Autoradiograms corresponding to the replication pattern shown diagrammatically in the upper part of Fig. 4. The bar represents 100 μm. The arrows indicate points at which replication forks could have hit fixed termination sites or points that might have served for the initiation of unidirectional replication. Note that the ends of the high-grain density tracks indicated by the arrows face low-grain density tracks across a short clear area. Thus, the high-density track ends are probably within continuous DNA fibers and do not represent breaks in the DNA chain.

presented by Hori and Lark[2], who have demonstrated a class of short units activated late in the S phase of cells of the kangaroo rat, *Dipodomys ordii*, which contain 60% late-replicating satellite DNA (3). They have suggested that the short units represent satellite DNA. These short units occur in clusters over localized regions of the chromosome. Thus, the eukaryotic chromosome may be characterized by replication units of uniform size within subchromosomal clusters but with marked variation in unit size among clusters.

Within these clusters, the initiation of DNA replication appears to occur synchronously (9, 10). The findings presented here show that it is usually synchronous on adjacent replication units. This synchrony is in part determined by the distance between initiation sites, because reovirus, an agent that causes an increase in the distance between active sites within clusters (9), causes a decay in the synchrony of initiation events on adjacent units.

DNA chain growth within clusters is also uniform. The rates of movement of forks within a replication unit as well as the forks on adjacent units show a high degree of positive correlation. Painter and Schaeffer showed by the use of equilibrium sedimentation techniques that HeLa cells in mid S phase have a faster rate of chain growth than do early S phase cells (15). This variation thus may represent clusters of units with uniform rates of growth being activated at different stages of S—the units activated in mid S having the faster rates of growth.

A further correlation shown in the present work is that the rate of chain elongation varies with the size of replication units. Thus, there may be joint regulation of the two component processes of DNA replication, initiation and chain growth, at the level of subchromosomal clusters, at least in heteroploid tissue culture cells in which the duration of S phase is relatively constant from generation to generation.

The picture emerges of eukaryotic DNA replication occurring in bursts over various regions of the chromosome. Each burst activates a battery of uniformly sized units within a cluster in a synchronous fashion, and chain growth then proceeds at uniform rates on all units within the cluster. These bursts are not necessarily sequential; probably a number of clusters are active at any one time on a single chromosome. Within a cluster, individual replication units may initiate later than the majority (10).

Not all initiation points within a cluster need be active during one S phase. The size of units varies in the same organism within different types of cells and different stages of development (2, 4). In addition, the distance between initiation points in mammalian cells is increased during infection with reovirus, an inhibitor of cellular DNA synthesis (9). Callan offered two possible explanations for inactivity of sites. (*a*) There are qualitatively different types, and specific enzyme molecules responsible for starting DNA synthesis recognize specific and different base-pair sequences on the DNA; or (*b*) there is one class, but successful initiation depends on exposure of the site, which may be controlled by the macromolecular packag-

[2] Hori, T.-a., and K. G. Lark. 1974. Autoradiographic studies of the replication of satellite DNA in the kangaroo rat. Autoradiographs of satellite DNA. *J. Mol. Biol.* **88**:221–232.

ing of the DNA-histone complex (5). Blumethal et al. postulated a mechanism for controlling the duration of S phase in somatic cells, suggesting that the structure of heterochromatic regions of the chromosome can be varied, and the resulting changes in the topography of compacted and noncompacted regions occlude or open up initiation sites (2). Such a mechanism could serve to control the activation of initiation sites during an S phase, and prevent reactivation of a site after its unit has completed replication. In this regard, Amaldi et al. made the very interesting observation that at least a small proportion of sites within the same mammalian cell are reproducible from generation to generation (1). These sites might represent a class consistently recognized or exposed during each cell generation. A function for such units might be to serve as "triggers" for activating replication within a cluster.

The characteristics of termination sites of DNA replication have not been thoroughly studied. In a negative sense, no evidence for fixed sites has been found (2, 3, 14). In experiments in which the frequency of initiation events has been reduced, evidence has been presented to show that chain growth continues beyond what would have been the site of termination in noninhibited cells (6). In the present study, patterns that would be diagnostic for fixed points could not be found. Other types of patterns showing a sharp cutoff of grain density are equally compatible with unidirectional replication (as illustrated in Fig. 5) or with fusion of adjacent prepulse autoradiograms (not illustrated). A reasonable alternative to fixed sites is termination occurring when two adjacent replication forks meet at a point determined by the rate of fork progression.

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