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α-Globin sequences are located in a region of early-replicating DNA in murine erythroleukemia cells*

(cell cycle/elutriation/Friend cells/cell synchrony)

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ABSTRACT **The time of replication in the S phase of regions of the mouse genome including the α-globin genes was determined in the murine erythroleukemia cell line transformed by Friend virus. Cells grown for short times in the presence of BrdUrd were fractionated into synchronous populations by centrifugal elutriation. The DNA was cleaved by restriction endonucleases, and fragments containing bromouracil (BrU-DNA) were isolated in density gradients of Cs_2SO_4. BrU-DNA fractions replicated during selected S-phase intervals were subjected to electrophoresis in agarose gels, transferred to diazobenzyloxymethyl-paper, and hybridized to an α-globin probe. Reconstruction experiments using a cloned mouse *Eco*RI fragment including one α-globin gene demonstrated that the extent of hybridization provides an accurate measurement of the concentrations of specific fragments in a DNA sample. The α-globin fragments were detected primarily in the BrU-DNA replicated during early S phase (approximately the first quarter of S). This result was confirmed in other synchrony experiments and by C_0t analysis. (C_0t is the initial concentration of DNA in mol of nucleotide per liter multiplied by the time in sec.) The temporal replication of mouse satellite sequences, already known from previous studies, was used as an internal control for cell synchrony. To show that the globin sequences were not lost from the cells in late S phase during isolation of the DNA, we quantitated the α-globin fragments in BrU-DNA prepared from a mixture of cells in early and late S phase. The results demonstrate that the α-globin gene regions in these cells are replicated during early S phase.**

DNA sequences in eukaryotes appear to be replicated in a defined temporal order that is maintained from one cell cycle to the next (reviewed in ref. 1). However, little is known about the mechanism by which this temporal control of replication is achieved. There is little information concerning the temporal replication of nonreiterated sequences, nor is it known if the order of replication differs in different cell types.

The globin genes have been studied extensively in many organisms (2–5). Several α-globin-like genes in mammalian genomes and their close linkage in humans have been described (2). In Southern transfer experiments with mouse DNAs cleaved by the *Eco*RI restriction enzyme, which does not cut within these genes, three prominent bands are detected upon hybridization to a probe homologous to α-globin mRNA (3). The band at 10 kilobases (kb) is a doublet, containing the two genes encoding the adult α-globin chains: the α-1 gene, on a 9.7-kb fragment (4), and the α-2 gene, on an 11.8-kb fragment (5). We refer to the sequences on these fragments as the α-globin gene regions. Two smaller fragments are also detected that contain α-globin sequences not expressed in adult mice (5).

We present evidence here that in a Friend erythroleukemia cell (Friend cell) line, α-globin gene sequences are located in a region of DNA that replicates during early S phase. Synchronous populations of Friend cells were isolated by centrifugal elutriation after growth for short intervals in the presence of BrdUrd. Samples of bromouracil-substituted DNA (BrU-DNA), replicated during selected S-phase intervals, were cleaved to completion with *Eco*RI, subjected to electrophoresis on agarose gels, and transferred to diazobenzyloxymethyl (DBM)-paper (6). The relative concentrations of the α-globin fragments were determined by hybridization to an α-globin probe. The results demonstrate that the replication of at least one nonreiterated mammalian DNA sequence is confined to a defined interval of S. Similarly, simian virus 40 DNA present in the Chinese hamster genome in approximately one viral equivalent replicates during a defined interval of S (M. Marchionni and D. Roufa, personal communication). The α-1 globin region is of particular interest due to its location in a set of well-characterized genes which represents a promising system for identification of an early-activated origin of replication.

METHODS AND MATERIALS

Cells and Synchronization. Cell line DS19, derived from clone 745A of C. Friend, and the conditions for cell culture, growth in medium containing BrdUrd (20 μg/ml), synchrony by elutriation, and flow microfluorometry have been described (7). Karyotypes obtained at the time these studies were carried out indicated a mean of 38 chromosomes with a range of 37–40. Two copies each of chromosomes 7 and 11, on which the globin genes reside, were identified by G-banding. The duration of the S phase in these cells is 7 hr, and the doubling time is 10.5 hr.

DNA Isolation and Restriction Enzyme Cleavage. DNA was prepared by a modification of the method of Walker and McLaren (8). The length of the double-stranded molecules obtained by this procedure was approximately 100 kb, as determined by electrophoresis in 0.3% agarose gels. DNA concentrations were determined by absorbance at 260 nm, by fluorescence with Hoechst 33258 with a DNA standard, and by the diphenylamine reaction with DNA and deoxyadenosine standards. The results of these methods agreed closely. Quantities of restriction endonucleases (New England Biolabs) required for complete cleavage were determined by titration assays by gel electrophoresis, and a 4- to 8-fold excess of enzyme was used to cleave mammalian DNAs. Phage λgtWES-Mα1 (3), kindly provided by P. Leder, contains the 9.7-kb α-globin *Eco*RI fragment used in the reconstruction experiments. The α-globin fragment was purified from agarose gels. Concentration of the fragment used as a standard in the transfer experiments was determined as above, and dilutions were monitored by addition of a radioactive tracer.

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Abbreviations: kb, kilobases; DBM, diazobenzyloxymethyl; FMF analysis, flow microfluorometric analysis.

*This paper is no. 7 in a series "DNA replication in synchronous cultured mammalian cells." Paper no. 6 is ref. 17.

RESULTS

Isolation of DNA Replicated During Selected Intervals of S Phase. Synchronous Friend-cell populations were obtained without chemical treatment by centrifugal elutriation, which separates these cells on the basis of size into populations in different stages of the cell cycle (7). Populations of exponentially growing Friend cells were separated into 17 fractions which we denote by the numbers 72–90. Two cell-synchrony protocols were used to obtain DNA replicated during selected S-phase intervals. BrdUrd was added to the growth medium of exponentially growing Friend cells for 2.5 hr either before fractionation by elutriation (retroactive synchrony) or afterwards (progressive synchrony). Molecules of newly replicating DNA may possess unusual structures which could result in their selective loss during the DNA isolation procedure. To prevent this, the period of growth in BrdUrd was followed by a 30-min chase in the presence of 10 μM thymidine. The interval of S during which the BrU-DNA had been replicated was determined by flow microfluorometric (FMF) analysis. This analysis also showed that the rate of DNA synthesis in the presence of BrdUrd did not vary appreciably throughout S. Appropriate cell fractions were pooled after elutriation, and DNA was isolated and separated into its BrdUrd-substituted and unsubstituted components by preparative isopycnic centrifugation in CsCl or Cs_2SO_4. Analytical ultracentrifugation showed less than 5% contaminating light molecules in the BrU-DNA.

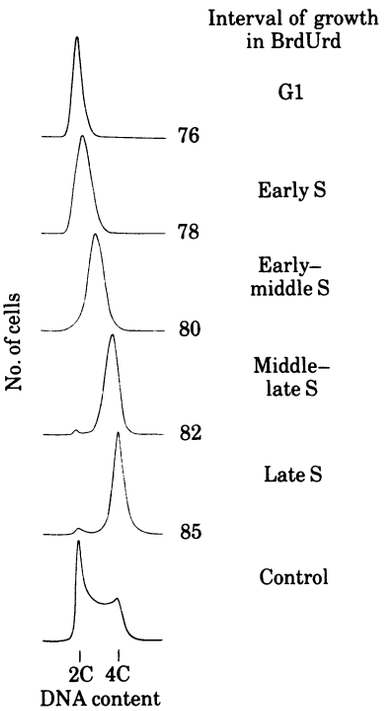

FIG. 1. Distribution of cellular DNA content in synchronous populations. Exponentially growing cells were separated on the basis of size by centrifugal elutriation after growth in medium containing BrdUrd (retroactive synchrony). FMF analysis shows the DNA content of cells from the elutriated fractions; C is the haploid DNA content of Friend cells in G1. The coefficients of variation (ratio of SD to mean cellular DNA content) for these distributions varied between 6% and 16%, with an average of 10%. BrU-DNA isolated from each cell fraction had been replicated during the cell-cycle interval indicated on the right. Fractions 72–76 contained cells that had been in G2, M, and G1 during growth in BrdUrd. Cells in S were separated into six fractions (77–82) with regularly increasing cellular DNA content, and mitotic and G2 cells were collected in fractions 83–85. The last fractions to be collected (86–90) included cells with two or more nuclei and polydisperse DNA distributions (not shown). These cells were discarded. There were fewer than 1% multinucleate cells in the fractions preceding 86. Fractions were pooled to obtain BrU-DNA replicated during the four S-phase intervals shown (see text). The control is Friend cells in exponential growth.

For retroactive synchrony, BrU-DNA replicated predominantly during the first hour of S (early S BrU-DNA) was recovered from fractions 77 and 78, which contained cells that had entered S during the interval of incorporation (Fig. 1). These cells had increased their DNA content by an average of 10% over the G1 value. Fewer than 3% had progressed beyond mid-S, and 78% had replicated less than 25% of their DNA at the time of elutriation. Fractions 79 and 80 contained cells that had incorporated BrdUrd into sequences replicated during most of the first half of S (early–middle S BrU-DNA). Two-thirds of the cells in these fractions had replicated less than 50% of their DNA, and many had initiated DNA synthesis during growth in BrdUrd. Fractions 81 and 82 contained cells that had incorporated BrdUrd predominantly during the middle third of S (middle–late S BrU-DNA). Cells in fractions 83–85 contained four haploid genome equivalents of DNA and included a high proportion of mitotic cells. The BrU-DNA in these cells was synthesized during the last third of the S phase (late S BrU-DNA).

As an additional assessment of the degree of synchrony, the relative levels of mouse satellite sequences in each of the BrU-DNA fractions were determined by C_0t analysis and by analytical density-gradient centrifugation. (C_0t is the initial concentration of DNA in mol of nucleotide per liter multiplied by the time in sec.) In agreement with the results of previous studies (reviewed in ref. 9), we observed the replication of mouse satellite DNA during the latter half of the S phase (not shown).

Quantitation of an α-Globin Restriction Fragment in Friend-Cell DNA Transferred to DBM-Paper. A reconstruction experiment was performed to demonstrate that gel-transfer experiments can be used to determine the concentration of the α-globin fragments in samples of *Eco*RI-cleaved Friend-cell DNA. The 9.7-kb fragment containing the α-1 gene was added to DNA from Friend cells or from salmon testes. To quantitate hybridization to the DNA of the 10-kb band, we analyzed the autoradiogram by densitometry. The area of each peak in the densitometric tracing is proportional to the amount of radiation incident upon the film. The peak area is plotted in Fig. 2 as a function of the amount of the cloned fragment added to each DNA sample. The points on the lower line represent the addition of the fragment to salmon testes DNA. Peak area increased linearly in this range with the amount of α-globin fragment electrophoresed, and the line intersected the origin as expected. The results shown by the upper line were obtained when the fragment was added to DNA prepared from Friend cells in the late logarithmic phase of growth. Extrapolation to zero concentration of added fragment gave 45 pg for the amount of α-globin fragment present in 5 μg of Friend-cell DNA. The quantity found in four independent determinations corresponds to the range 2.1 $\pm$ 0.6 copies per haploid genome equivalent. Thus, transfer experiments provide a simple procedure for precise titration of specific restriction fragments.

Quantitation of α-Globin *Eco*RI Fragments in Fractions of DNA Replicated During Selected Intervals of S. Aliquots of each *Eco*RI-cleaved BrU-DNA fraction obtained by retroactive synchrony were subjected to electrophoresis in neighboring lanes of an agarose gel and transferred to DBM-paper. To ensure that equal amounts of each DNA sample were electrophoresed, we determined concentrations by three independent methods (see *Methods and Materials*). In addition to BrU-DNA from elutriated cells, DNA from exponentially growing Friend

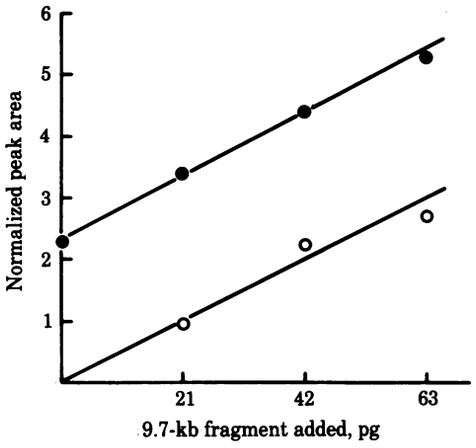

FIG. 2. Quantitation of α-globin fragments in Friend-cell DNA cleaved by *Eco*RI. Increasing quantities of the 9.7-kb α-globin *Eco*RI fragment (purified from recombinant λ phage) were added to 5 μg of *Eco*RI-digested Friend-cell DNA. These mixtures were subjected to electrophoresis in 0.8% agarose gels and partially cleaved *in situ* by sequential treatment with 0.15 M HCl and with 0.5 M NaOH/1 M NaCl. The DNA was transferred, covalently linked to DBM-paper, and hybridized in the presence of 10% (wt/vol) dextran sulfate (6). The 3-kb *Sac* I fragment of λgtWES-Mα1, which includes the α-globin gene, was labeled with [α-^{32}P]dCTP by nick-translation and used as a probe in the hybridizations. This probe hybridizes predominantly to the two fragments in the 10-kb band. Minor bands are also detected at 4.7 and 2.4 kb. Kodak X-Omat R film was exposed to the paper at −60°C with a Dupont Lightning Plus intensifying screen. A series of exposures was analyzed for each transfer to verify that the film response was linear. The developed film was scanned with a Joyce–Loebl MK III double-beam recording microdensitometer. Peak area is shown as a function of the amount of the cloned fragment added to each Friend-cell DNA sample (●). As a standard, increasing quantities of the cloned fragment were also added to 5 μg of DNA from salmon testes and transferred to the same paper (○); a straight line drawn through the points intersected the origin. If one copy of the 9.7-kb fragment is present per haploid Friend-cell genome, there must be 21 pg of this DNA in 5 μg of DNA from diploid Friend cells. The peak area resulting from addition of 21 pg of the cloned fragment to DNA from salmon testes was therefore set at 1, so that the scale of the ordinate corresponds to the number of copies of the fragment per haploid genome equivalent.

cells and from the cloned 9.7-kb fragment and BrU-DNA from unsynchronized cells, containing sequences replicated throughout S, were included in separate lanes of each transfer as a control. When autoradiograms from separate experiments were analyzed by densitometry, the ratios of the peak areas measured for each of these controls were reproducible to within 20%.

The temporal replication of the α-globin fragments was determined by hybridizing the S-phase BrU-DNA fractions with the α-globin probe after transfer to DBM-paper. In experiments with BrU-DNA fractions isolated by CsCl density-gradient centrifugation before restriction enzyme cleavage, the α-globin fragments were detected predominantly in early S BrU-DNA (Fig. 3). This indicated that the α-globin regions are replicated preferentially near the beginning of S. However, it is possible that these sequences are replicated at a different time than sequences surrounding them in the genome. If, during density-gradient centrifugation, the unsubstituted α-globin genes remained covalently attached to sequences that had incorporated BrdUrd, they might be carried into the BrU-DNA band even though they had not been replicated during that interval. To minimize this possibility, we cleaved samples of DNA from the elutriated cell fractions by *Eco*RI prior to density-gradient centrifugation. Although these restriction fragments formed broad bands in CsCl, they were resolved into separate components in the steeper Cs_2SO_4 gradients. Equal amounts

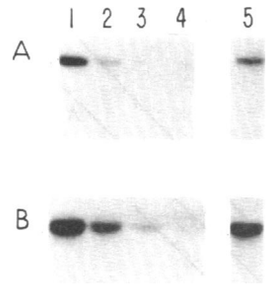

FIG. 3. Hybridization of the α-globin probe to BrU-DNA replicated during selected intervals of S. Four micrograms of each *Eco*RI-cleaved BrU-DNA fraction was subjected to electrophoresis, transferred to DBM-paper, and hybridized (see Fig. 2). Lane 1, early S BrU-DNA; lane 2, early–middle S BrU-DNA; lane 3, middle–late S BrU-DNA; lane 4, late S BrU-DNA; lane 5, BrU-DNA from unsynchronized cells. (*A*) BrU-DNA isolated in Cs_2SO_4 gradients after *Eco*RI cleavage; (*B*) BrU-DNA isolated in CsCl gradients before *Eco*RI cleavage.

of the four BrU-DNA samples were hybridized to the α-globin probe after electrophoresis and transfer to paper (Fig. 3). The probe again hybridized predominantly to the early S BrU-DNA (Table 1), confirming that sequences on the α-globin fragments are replicated during early S.

The relative concentrations of the α-globin fragments in each of the four S-phase BrU-DNA fractions are summarized in Table 1. More than twice as much probe hybridized to the early S fraction as to an equal amount of BrU-DNA from the unsynchronized control cells. To a lesser extent, the α-globin fragments were detected in the early–middle S BrU-DNA, which also includes sequences replicated at the beginning of S phase. The middle–late and late S BrU-DNA fractions contained the lowest concentrations of α-globin fragments. The limited hybridization that does occur to these fractions can be attributed to contamination during the synchrony procedure with sequences replicated during early S. The relative concentrations of the α-globin fragments in the four BrU-DNA fractions were also determined in a reconstruction experiment (Fig. 4). These results also demonstrated that the α-globin fragments were found in highest concentration in early S BrU-DNA. The results are therefore consistent with the replication of the α-globin genes occurring during an interval corresponding approximately to the first quarter of S.

Table 1. Relative concentrations of α-globin fragments in BrU-DNA fractions

Interval of growth in BrdUrd	Progressive synchrony	Retroactive synchrony	
	DBM-paper	DBM-paper	Solution*
Early S	2.4	2.0	3.6
Early–middle S	1.6	0.4	
Middle S			0.86
Middle–late S	<0.05	0.1	
Late S	0.6†	<0.05	0.57

Cells were synchronized, and BrU-DNA replicated during each S-phase interval was isolated by isopycnic centrifugation in CsCl and then sheared for hybridization in solution or it was isolated by Cs_2SO_4 density-gradient centrifugation after cleavage by *Eco*RI and hybridized on DBM-paper. The relative concentrations shown represent the amount of α-globin fragment detected in each fraction divided by the amount detected in an equal quantity of BrU-DNA from unsynchronized cells. Using these values, we calculated that 70–120% of the α-globin fragments replicated during the 2.5-hr interval of incorporation was present in the four BrU-DNA fractions from the elutriated cells.

* Cells were pooled to divide the S phase approximately into thirds.

† FMF analysis after growth in BrdUrd indicated that some cells had divided and reentered early S.

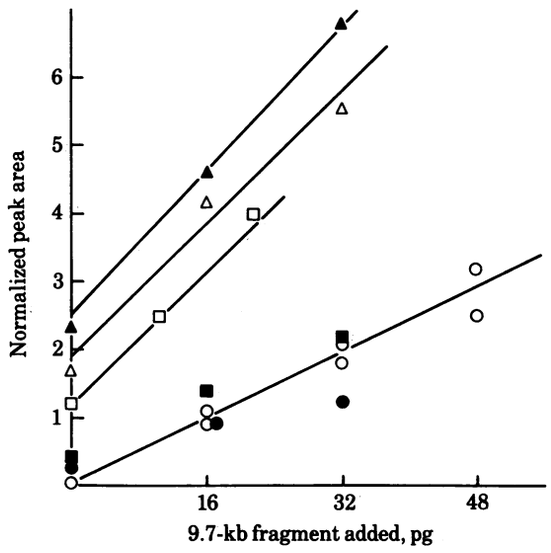

FIG. 4. Quantitation of α-globin fragments in BrU-DNA replicated during selected intervals of S. Increasing quantities of the cloned 9.7-kb α-globin fragment were added to 4 μg of *Eco*RI-cleaved BrU-DNA isolated from each pooled, elutriated cell fraction. Samples were subjected to electrophoresis on two different gels. A set of standards consisting of the cloned fragment added to DNA from salmon testes was included in lanes of each gel. The mixtures were analyzed and the data were normalized as described for Fig. 2. The amount of the 9.7-kb fragment expected in 4 μg of Friend-cell DNA is 16.8 pg. BrU-DNA replicated during early S (▲), early–middle S (△), middle–late S (■), and late S (●). BrU-DNA from unsynchronized cells (□); DNA from salmon testes (○).

We considered the possibility that the α-globin regions might be replicated during later S-phase intervals, but not detected. This might occur if, late in the cell cycle, cells contain constituents such as proteins that cause preferential loss of the α-globin regions during the isolation of BrU-DNA. To test for the presence of such constituents, we performed a mixing experiment. BrU-DNA, isolated from a mixture of cells in early S and an excess of cells from the late BrdUrd incorporation interval, was analyzed in a transfer experiment. To determine the proportion of this BrU-DNA contributed by each cell type, we examined DNA isolated separately from each cell fraction in the analytical ultracentrifuge to determine the average cellular content of BrU-DNA. The cells from the late interval contained a greater amount of BrU-DNA than the cells from early S. If the yield were not lowered by the presence of the cells from the late interval, we calculated that the concentration of α-globin fragments detected in BrU-DNA from the cell mixture should be 26% of that detected in BrU-DNA obtained from the early S-phase cells alone. In fact, the level measured was 33% of that for cells in early S. Thus, the recovery of the α-globin fragments was clearly not diminished by mixing with cells in late S and G2.

For progressive synchrony, four fractions of BrU-DNA were isolated, representing sequences replicated during S-phase intervals similar, but not identical, to those described above. This synchrony protocol permits direct determination by FMF analysis of the cell-cycle distribution of the elutriated cells at the start of the interval of incorporation. Early S BrU-DNA, replicated during the first 2 hr of S, was obtained from cells in fraction 76, which were in G1 at the time of elutriation. Fewer than 20% had progressed beyond the midpoint of DNA replication by the end of the interval of incorporation. Early–middle S BrU-DNA, replicated during the first half of S, was obtained from cell fractions 77 and 78. Both of these BrU-DNA fractions contained high levels of sequences replicated during early S, and both hybridized extensively to the α-globin probe (Table 1). The middle–late S BrU-DNA obtained by progressive synchrony from fractions 79 and 80 corresponds to the period 3–6 hr into S. We estimate from the FMF analysis that less than 15% of this BrU-DNA was replicated during the first quarter of S, and that only if the α-globin gene regions were replicated during this early interval would they be below the limit of detection in the middle–late S fraction. Because the α-globin gene regions were not detected in the middle–late S BrU-DNA, these sequences must be replicated during early S. The cells in fractions 81 and 82 were elutriated while in late S, with a small proportion in G2+M. FMF analysis indicated that 20–25% of these cells had divided, traversed G1, and again entered the S phase before the end of the interval of incorporation. The small amounts of α-globin fragments detected in the late S BrU-DNA fractions obtained by progressive synchrony can be attributed to cells that divided during the interval of incorporation and were synthesizing DNA during early S.

Temporal Replication of α-Globin Fragments Determined by Hybridization in Solution. An independent method of quantitation was also used to determine the time of replication of the α-globin regions. The cloned 9.7-kb fragment, labeled with ^{32}P by nick-translation, was denatured and allowed to reassociate in solution in the presence of BrU-DNA replicated during early, middle, or late S (Fig. 5). As a control, the labeled probe was also hybridized to BrU-DNA from an unsynchronized cell culture. The concentration of sequences homologous to the probe was almost 4 times greater in early S BrU-DNA than in BrU-DNA from unsynchronized cells (Table 1). In contrast, the concentration of these sequences in middle and late S BrU-DNA was less than that observed in the control. Thus, in agreement with the results obtained by gel transfer, these experiments indicate that the α-globin gene regions are replicated early in S phase.

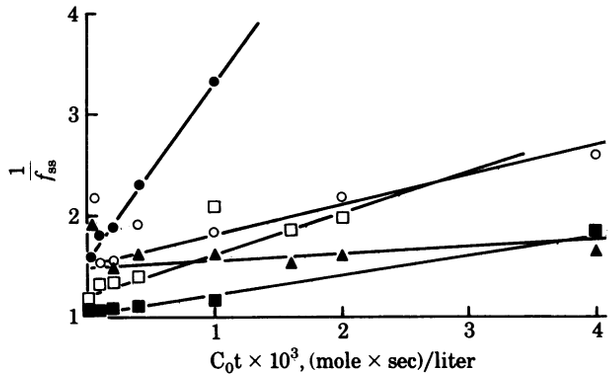

FIG. 5. Kinetics of reassociation of the 9.7-kb fragment containing the α-1 gene in the presence of BrU-DNA replicated during selected intervals of S. The 9.7-kb fragment, labeled with ^{32}P by nick-translation, was denatured and allowed to reassociate in the presence of sheared, denatured BrU-DNA from Friend cells replicated during selected intervals of S or from unsynchronized cells. The fragment was also allowed to reassociate in the presence of salmon testes DNA (no addition). C_0t values were determined from the initial concentration of the labeled fragment (C_0) and the time of incubation. The reaction was followed by susceptibility to nuclease S1. The reciprocal of f_{ss}, the fraction of bases in single-stranded form, increased to at least 4 for all fractions except the late S BrU-DNA, which did not contain a significant concentration of sequences capable of accelerating the reassociation of the probe. The slopes of the curves are proportional to the total concentration of α-globin sequences (labeled plus unlabeled) in each reaction. ▲, No addition. □, BrU-DNA from unsynchronized cells. BrU-DNA replicated during early S (●), middle S (○), and late S (■).

DISCUSSION

We have presented results indicating that the α-globin gene regions in Friend cells are replicated during the first quarter of S phase. Results in agreement with this conclusion were obtained in several experiments by using different methods for cell synchrony, for isolation of the substituted DNA, and for hybridization. A previous report indicating that the globin genes are replicated during middle-to-late S in a different Friend cell line relied upon a single method of chemically induced synchrony and a cDNA hybridization probe containing a mixture of α- and β-globin sequences (10).

Two genes for the adult α-globin polypeptides have been identified in BALB/c mice: α-1, residing on a 9.7-kb *Eco*RI fragment, and α-2, residing on a fragment of 11.8 kb (see *Introduction*). Although two bands were not resolved in the 0.8% agarose gels used in our quantitative transfer experiments, our data are consistent with hybridization by the probe to two separate fragments in the 10-kb band. The level of hybridization to the 10-kb band corresponds to about two copies per haploid genome (Fig. 2). In addition, two fragments of approximately 10 kb were resolved as bands of nearly equal intensity in transfers from 0.5% agarose gels, and the two adult α-globin genes were detected as separate bands in gel-transfer experiments with genomic DNA cleaved by restriction endonuclease *Sac* I (not shown). These studies indicate that both α-globin gene regions, which are closely linked (11, 12), are replicated during early S. This conclusion is based on comparisons of hybridization to BrU-DNA samples replicated during four defined intervals of the S phase. These samples were equally substituted with BrdUrd and were of similar molecular weights prior to cleavage by *Eco*RI.

The coding sequences of the α-1 gene encompass a region of approximately 1 kb near the middle of the 9.7-kb fragment (3). It is possible that the gene for α-globin is replicated at a different time than the sequences surrounding it on the fragment, but this would require the existence of an extremely small replicon. The replicons of mammalian DNAs are 50–300 kb long, and clusters of adjacent replicons often replicate at the same time (for review see ref. 13). BrU-DNA hybridizing to a genomic α-globin probe was recovered primarily from cells grown in the presence of BrdUrd during early S by isopycnic centrifugation of either large (≈100 kb) molecules or small (9.7 kb) *Eco*RI fragments. These results demonstrate that this gene must incorporate BrdUrd during early S if it is replicated as part of a replicon as large as those described above. We cannot rule out the possibility that the gene, or part of it, is replicated as part of a class of very small replicons that have not been detected. This question will only be resolved by an extensive analysis of the fine structure of the replicating region.

Previous studies have suggested that early-replicating DNA may be richer than late-replicating DNA in genes coding for cellular functions (14). Incorporation of BrdUrd into DNA replicated during the first half of the S phase has been shown to reduce cloning efficiency (14, 15) and, in one case involving a differentiated function, to inhibit the induction of hemoglobin synthesis in Friend cells grown in the presence of dimethyl sulfoxide (7). These studies have left open the question of whether the effects observed were due to incorporation of BrdUrd into structural genes or into sequences exerting a regulatory influence. Many nontranscribed sequences appear to be late replicating. It is possible that the time of replication and the transcriptional state of DNA sequences are related; for example, the configuration of the chromatin required for transcription may also be required for replication during early S. The present study has shown that at least one mammalian gene is located among early-replicating sequences in a cell line in which it appears to be in an active conformation by the criterion of sensitivity to DNase I (16) and in which it is expressed after commitment to differentiation.

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