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THE β-MAJOR AND β-MINOR GLOBIN GENES IN MURINE ERYTHROLEUKEMIA CELLS REPLICATE DURING THE SAME EARLY INTERVAL OF THE S PHASE

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Summary: The time of replication in S phase was determined for the 7.3 kb EcoRI segment containing the β-major globin gene and the 14 kb EcoRI segment containing the β-minor globin gene in a murine Friend erythroleukemia virus transformed (MEL) cell line. Cells were obtained from different intervals of S phase by centrifugal elutriation to avoid artifacts of chemical synchronization. Newly synthesized DNA from different parts of S phase were obtained by isolation of 5-bromouracil (BU) labelled DNA from these cells. The BU-DNA synthesized during four different intervals of S was transferred to diazobenzyloxymethyl (DBM) paper and hybridized with a β-globin cDNA probe. Quantitation showed that both β-globin segments were replicated in the first quarter of the S phase with no significant difference in their time of replication in this MEL cell line.

Studies on the temporal order of replication of DNA from higher eukaryotes has shown that, as is the case in prokaryotes, DNA replicated in one temporal interval of the S phase is replicated in the same temporal interval of the next S phase (1). The interval during which multiple copy sequences replicate in mammalian cells has been studied in several laboratories (see e.g., 2).

The replication of nonrepetitive DNA sequences in eukaryotes also occurs during defined intervals of the S phase. In our previous studies of the replication of the genomic segments of Friend virus transformed murine erythroleukemia (MEL) cells containing two adult α-globin genes, replication of these segments was shown to be restricted predominantly to the first third of S phase (3). Others have reported that SV40 DNA

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sequences integrated at different unique sites, into the Chinese hamster genome, each replicated during a different defined interval of S (4).

Studies on the replication of closely mapping immunoglobulin heavy chain constant region gene segments have shown that the Cα, $C\gamma_{2b}$ and Cμ EcoRI segments spanning a distance of 180 kb, are replicated during the first half of S in the same order in which they are arranged in the genome (5). The Cμ segment, which is about 180 kb 5' of Cα gene, is replicated at a significantly later time than the Cα segment. More recently, we have shown that several EcoRI segments containing variable region genes located 5' of the Cμ segment replicate late during S in the MEL cell line (6). These segments which are located further (5') than the Cμ gene from the Cα gene replicate still later during S than the Cμ gene (6).

It has been shown that the β-major and β-minor globin genes are about 15 kb apart in the Balb/c genome (7). Previous studies on the β-globin genes indicated that these genes are replicated either in early S (8) or during the middle of S (9) but did not distinguish between the β-major and β-minor-genes. We have found that both the 7.3 kb β-major and 14 kb β-minor segments are replicated in the first quarter of S in this MEL cell line. No significant difference in the replication time of these two segments could be detected in our studies.

MATERIALS AND METHODS

Exponentially growing MEL cells (DS-19), with an S phase of approximately 7.5 hours and a doubling time of 11 hours were pulse labelled with 20 μg/ml of 5-bromodeoxyuridine (BUdR) for 2 hours. Cell fractrions containing BU-DNA replicated during four different intervals of S were then obtained by centrifugal elutriation as previously described (3,5). The distribution of DNA content per cell was determined for each fraction by flow microfluorometric (FMF) analysis using the propidium iodide hypotonic citrate technique (10). Appropriate sequentially elutriated cell fractions were pooled to contain cells in four successive intervals of S. High molecular weight bromouracil

(BU) substituted DNA, replicated during these four intervals of S was prepared. The DNA was cleaved exhaustively with EcoRI, and the BU labelled segments were isolated by ultracentrifugation in neutral Cs_2SO_4 density gradients. Equal amounts of this BU-DNA representing each of the four intervals of S were electrophoresed in adjacent lanes of an 0.8% agarose gel and then transferred to DMB paper as described (11).

The transfers of covalently attached BU-DNA were each hybridized with 1 X 10^6 cpm/lane of a[^{32}P] nick translated β-globin cDNA probe derived from the recombinant plasmid pCRI-βG9 (12). Briefly pCRI-βG9 was isolated from E. coli C600 cells using chloramphenicol amplification. The plasmid was then cleaved with Hha I and centrifuged on a 10-40% sucrose gradient at 54,000 rpm for 16 hours in a Beckman SW65 rotor. The gradients were fractionated to obtain the 2.3 kb fragment containing the β-globin insert which was used as a probe.

RESULTS

MEL cells were separated according to size by centrifugal elutriation. As previously described (3,5,6), FMF analysis was used to determine the modal DNA content of the cells in each fraction. Appropriate fractions were pooled to produce four populations of cells that contained BU-DNA replicated during four different intervals of S. The four DNA samples were digested to completion with EcoRI and after isolation, the BU-DNA was separated according to size by agarose gel electrophoresis and transferred to DBM paper. After hybridization with the β-globin cDNA probe, the relative concentrations of the detected β-globin EcoRI segments were determined from the resulting autoradiograph (Fig. 1) by densitometry.

The small differences in relative concentrations that are evident (Fig. 2) when the results of two independent elutriation experiments are compared or when the results for the β-major and β-minor genes are compared are most likely due to background hybridization to the DBM transfers, rather than to differences in the timing of gene replication.

The time of replication of the EcoRI genomic segments containing the β-major and β-minor globin genes was determined from their relative concentrations in the BU-DNA synthesized in

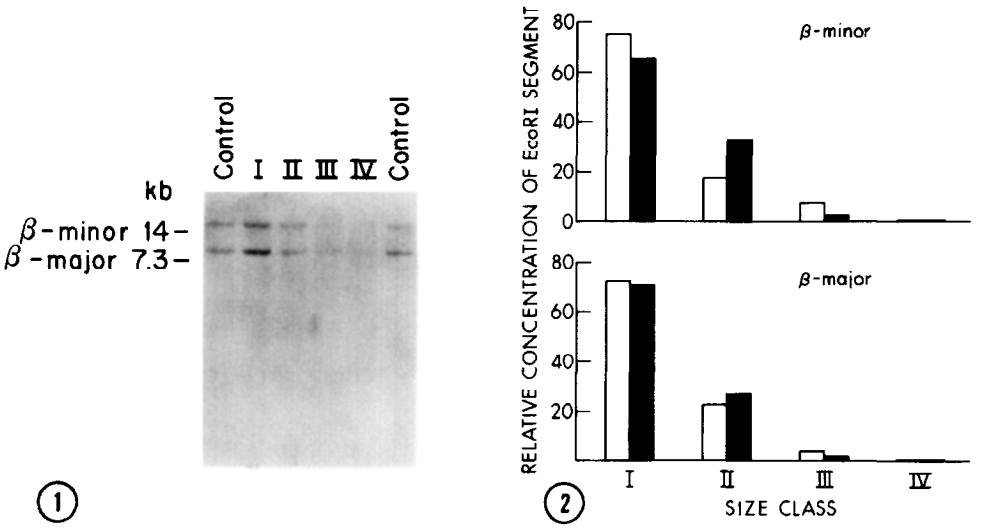

Figure 1. Autoradiogram of a 72 hour exposure of a DBM transfer of BU-DNA hybridized with a β-globin cDNA probe. The BU-DNA was isolated from MEL cells obtained from successive intervals of S phase by centrifugal elutriation. Four μg of BU-DNA from each interval of S phase was electrophoresed in adjacent lanes of a 0.8% agarose gel. The transfers were hybridized with 1 X 10^6 cpm/lane of a HhaI segment of pCR1-βG9 nick translated with [^{32}P] dCTP to a specific activity of 1 X 10^8 cpm/μg. The β-globin probe hybridizes to a 7.3 kb EcoRI segment containing the β-major globin gene and to a 14 kb segment containing the β-minor globin gene.

Figure 2. Relative concentrations of EcoRI segments containing β-major and β-minor globin genes synthesized during different intervals of S in MEL cells. Cells were fractionated according to position in the S phase. EcoRI segments of BU-DNA replicated during different intervals of S were fractionated on agarose gels. This DNA was transferred to DBM paper and after hybridization as described in Fig. 1., the autoradiograms were traced using a Joyce Loebl microdensitometer. The total area of the β-major or β-minor bands in the four intervals was normalized to 100. These normalized values are proportional to the relative concentrations of the EcoRI segments in each of the four intervals. The open and solid boxes represent the results of two independent elutriation experiments.

different parts of S phase. The relative concentrations of the β-minor globin segments in the BU-DNA replicated in each of four intervals of the S phase show that each of these segments is replicated in a non-random manner, primarily during the first quarter of the S phase. The average DNA content per cell at the time the β-globin segments are replicated was calculated as previously described (5). Under the growth conditions in these experiments the β-globin segments are replicated when the cellular DNA content is approximately 2.1 ± 0.2C.

DISCUSSION

The β-globin gene segments are about 15 kb apart in the mouse genome (7). We have previously determined the nuclear DNA content at the times that various immunoglobulin heavy chain constant region genes replicated (5). The rate of DNA replication observed was consistent with that previously observed (about 1.8 kb/min.) in mouse cells by autoradiography (13). At this replication rate, the difference in the concentration of the β-major and β-minor segments in the four S phase intervals would not be detected in this study. Thus, the results of Figure 2 are consistent with the previously observed rates of DNA replication.

We have previously observed (6) very similar temporal replication patterns for EcoRI segments that are located within clusters of about 25 kb. Included in one cluster we previously studied (6) were the functionally unrelated genes Cγ2b and c-myc. Another cluster studied contained both 3' and 5' flanking segments of the Cγ2b gene. In some of the cell lines in which the temporal replication of clusters of genes was examined, one of the genes was transcribed (6). There was no evidence that more than one gene was transcribed in each cluster. One example is the T15 VH constant region heavy chain family in S107 cells which contains a pseudogene located 16 kb from the transcribed VH gene. In other instances we studied the replication of clusters in which genes would be transcribed in some cells of the mouse, but were not transcribed in the particular cell line examined (5,6). In the studies presented here, the gene cluster is functionally different from those described above in that two closely linked genes are both transcribed in the MEL cell line that has been induced to differentiate along the erythroid pathway by growth in the presence of dimethylsulfoxide,

hexamethylene bisacetamide, butyric acid and other compounds (for review see 14). We have measured the temporal order of replication of the β-globin genes in the non-induced MEL cell line. In these cells, β-minor globin transcripts have been detected while β-major globin transcripts may be present in low amounts (15). Thus in the uninduced MEL cell line, the two β-globin genes are either both transcribed or have the capacity to be transcribed upon induction. The two EcoRI segments containing β-globin genes also follow the pattern described above in which segments separated by less than 30 kb replicate during the same interval of S. These results, along with those previously reported indicate that DNA sequences located within a cluster comprising about 30 kb replicate during the same quarter of the S phase.

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