MOLECULAR AND CELLULAR BIOLOGY, Apr. 1988, p. 1657–1663
0270-7306/88/041657-07$02.00/0

Vol. 8, No. 4

Opposite Replication Polarities of Transcribed and Nontranscribed Histone H5 Genes

JAMES P. TREMPE,† YOSHIE I. LINDSTROM, AND MICHAEL LEFFAK*
Department of Biochemistry, Wright State University, Dayton, Ohio 45435

Received 19 May 1987/Accepted 24 December 1987

We used an in vitro nuclear runoff replication assay to analyze the direction of replication of the active and inactive histone H5 genes in avian cells. In embryonic erythrocytes the transcribed histone H5 gene displayed sensitivity to endogenous nuclease cleavage. In contrast, this gene was insensitive to endogenous nuclease digestion under the same conditions in nuclei of the lymphoblastoid cell line MSB-1, and histone H5 gene transcripts were not detectable by dot-blot analysis of MSB-1 cell RNA. When nuclei were isolated from embryonic erythrocytes and incubated with bromodeoxyuridine triphosphate, runoff replication from endogenous nuclease cleavage sites led to a relative enrichment for fragments near the 3′ end of the histone H5 gene in the density-labeled DNA. In nuclei of MSB-1 cells or chicken embryo fibroblasts, however, runoff replication from restriction enzyme-cut sites (or induced endogenous nuclease-cut sites in MSB-1 nuclei) led to a relative enrichment for fragments near the 5′ end of the H5 gene in dense DNA. Based on the enhanced incorporation of bromodeoxyuridine into origin-distal regions of DNA during the in vitro runoff replication assay, we conclude that the active histone H5 gene in embryonic erythrocytes is preferentially replicated in the transcriptional direction from an origin in the 5′-flanking DNA, whereas its inactive counterparts in MSB-1 cells and chicken embryo fibroblasts are preferentially replicated in the opposite direction.

The replication of eucaryotic chromosomes is under both spatial and temporal controls, as evidenced by the firing of local clusters of replicons according to developmentally regulated programs and the replication of particular chromosome domains during similar intervals of the S phase in successive cell cycles (reviewed in reference 16). That the genomic location of an immunoglobulin gene sequence can influence its schedule of replication has been shown by Calza et al. (3).

A relationship between transcriptional activity and the timing of replication has been inferred based on the observation that genes are generally replicated earlier in the S phase in cells where they are transcribed than in cells where they are inactive (7, 13). In the case of the *Xenopus laevis* kidney cell line TrXo, translocation of oocyte-type 5S rDNA genes from their normal telomeric location to a pericentromeric location is correlated with both a shift from late to early S-phase replication and the accumulation of oocyte 5S transcripts (11, 15), supporting the suggestion that the earlier replication of somatic versus oocyte 5S genes in normal cells may contribute to the selective transcription of the somatic genes (14, 38).

A structural link between replication and transcription is suggested by the observations that under normal cell growth conditions the H1 histones and octamer cores of bulk chromatin are conservatively replicated (23–25, 34, 36), whereas the core histone octamers of transcribed chromatin and the HMG 14/17 nonhistone pair preferentially associated with transcribed nucleosomes (37) are replicated nonconservatively but nonrandomly (22, 34). Based on the nonrandom deposition and segregation of chromosomal proteins at replication, Smithies (31) has proposed that the transcriptional potential of a gene may be directly related to its replication polarity. According to this hypothesis active genes are replicated from upstream origins, whereas inactive genes are replicated from either upstream or downstream origins. In agreement with this model, we have shown previously that the active (and inactive) avian alpha-globin genes alpha-pi and alpha-D are replicated in the transcriptional direction (19). The organization of the simian virus 40 and polyomaviral genomes (33) and the extrachromosomal rDNA genes of *Tetrahymena thermophila* and *T. pyriformis* (4, 10, 29, 35) are also consistent with this proposal; however, the alternating convergent and divergent transcriptional polarities of the closely interspersed chicken histone genes (8) and the antiparallel arrangement of the nested *Drosophila melanogaster GART-PCP* gene pair (17) suggest that this model may not be generally applicable in its simplest form.

To investigate this question further, we used an in vitro runoff replication assay (IVR) to analyze the direction of replication through the active and inactive histone H5 (2) genes of chicken embryonic erythrocytes and fibroblasts and the MSB-1 lymphoblastoid cell line. When nuclei are incubated in vitro in the presence of bromodeoxyuridine (BrdUrd) triphosphate (BrdUTP), the endogenous DNA polymerases extend replication forks previously initiated in the intact cells, leading to the preferential density labeling of origin-distal DNA segments (19). Based on the graded incorporation of density label across the histone H5 gene locus, we conclude that the active histone H5 gene is replicated from an upstream origin in its 5′-flanking DNA, whereas the inactive histone H5 genes are replicated from downstream origins.

MATERIALS AND METHODS

Fibroblasts were obtained from the skins of 14-day chicken embryos and cultured in RPMI 1640 medium containing 10% tryptose phosphate broth, 4% fetal calf serum, 2% chicken serum, and 0.1% gentamycin at 35°C in a humidified 5% CO_2 atmosphere. Cells were used in the replication reactions at passage 5. The procedures for the

* Corresponding author.
† Present address: Laboratory of Molecular and Cellular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20205.

culture of MSB-1 cells, isolation of 5-day chicken embryo erythrocytes, and preparation of nuclei, cytosol, and replication cocktail were as described previously (19, 28). For in situ digestion with *Hin*dIII, 1 ml of nuclei (A_{260} of 1.5) in TME buffer (10 mM Tris chloride [pH 7.8], 4 mM $MgCl_2$, 1 mM disodium EDTA) was brought to 50 mM NaCl, and 5,000 to 10,000 U of restriction enzyme was added. Incubation was at 37°C for 40 min before the addition of cytosol fraction (1 to 2 cell equivalents) and replication cocktail. For endogenous nuclease (EN) digestion during the IVR, nuclei were suspended in TME–50 mM NaCl–1.5 mM $CaCl_2$ and incubated at 37°C for 40 min in the presence of 1 to 10 cell equivalents of cytosol fraction before the addition of replication cocktail. DNA was isolated by organic solvent extraction, digested with *Pst*I (10 U/μg of DNA) under conditions recommended by the supplier (Bethesda Research Laboratories, Inc.), and centrifuged to equilibrium on CsCl gradients. Light (1.69 to 1.70 g/ml) and heavy (1.74 to 1.76 g/ml) DNAs were desalted and electrophoresed as described previously (19). Each IVR experiment was performed independently at least three times, with similar results.

The H5 gene probe mentioned in these experiments was the 900-base-pair (bp) cDNA clone 10-52 generously provided by J. R. E. Wells (20, 21). The probe was labeled to 1 to 2 dpm/fg by replacement synthesis (18). RNA was isolated by the methods of Chirgwin et al. (5) and Glisin et al. (12). Electrophoresis, Southern hybridization (26, 32), and quantitation with the LKB 2202 Ultrascan laser densitometer and Gelscan 2190 program were as reported previously (19). Reconstruction controls demonstrating the linearity of densitometric signal response over the range of film exposures employed were presented previously (19) and were confirmed for these experiments by quantitative comparison of multiple film exposures.

RESULTS

IVR in erythrocyte nuclei. We reported that the in vivo direction of replication through a segment of DNA can be deduced after an IVR reaction in which endogenous DNA polymerase molecules are nominally limited to completing the replication of that segment in the presence of a density label (19). In this procedure nuclei are isolated from cells in logarithmic growth and incubated in vitro in the presence of a replication cocktail including nuclease and BrdUTP. The DNA is subsequently purified, digested with one or more additional restriction enzymes, and banded on neutral CsCl. An essential feature of this approach is the introduction of a barrier to the progress of replication forks during the in vitro replication reaction so that sequences immediately 3′ to this boundary are labeled by few polymerases, whereas sequences farther downstream are traversed by a relatively larger number of polymerases. Under these conditions the BrdUrd density label is preferentially incorporated into origin-distal regions of DNA. This can be detected after isopycnic centrifugation and blot hybridization as a change in the relative stoichiometry of restriction fragments across the density gradient. We showed previously that endonuclease cleavage sites or endogenous boundary sites could be used as replication barriers in determining the direction of replication through the avian alpha-globin locus (19). To analyze the replication of the histone H5 gene in embryonic erythrocytes, EN digestion was used to provide the replication barrier sites.

Under the IVR reaction conditions EN cleavages occurred ca. 0.5 kbp 5′ and 1.9 kbp 3′ to the internal *Pst*I target (Fig. 1); these regions are also hypersensitive to DNase I digestion in erythrocyte nuclei (J. P. Trempe, Ph.D. thesis, Wright State University, 1986). Five-day erythrocyte nuclei were incubated in the IVR cocktail containing BrdUTP; then the DNA was purified, digested with *Pst*I, banded to equilibrium on neutral CsCl, and electrophoresed on agarose. Hybridization of an H5 cDNA probe to a blot of this gel showed that the stoichiometry of the 5′ (1.6 kbp) and 3′ (2.6 and 3.2 kbp) *Pst*I-*Pst*I fragments, which were not cleaved by the endogenous nuclease, was similar in dense and light DNA; in the absence of a barrier to replication these fragments were uniformly density labeled. In contrast, the 0.5-kbp 5′ EN-*Pst*I fragment was strongly depleted in dense DNA, and the 1.9-kbp 3′ *Pst*I-EN fragment was enriched (Fig. 2). Thus, those fragments which resulted from endogenous nuclease cleavage during IVR displayed nonuniform labeling. The depletion of the 0.5-kbp fragment in dense DNA indicates that in the presence of a barrier to replication (the EN cleavage) few DNA polymerases are available to density label this fragment. The greater labeling of the 1.9-kbp fragment relative to the 0.5-kbp fragment is due to polymerases which run off from within the 0.5-kbp fragment. Therefore, in terms of replication, the depleted 0.5-kbp fragment is upstream relative to the 1.9-kbp fragment, and replication proceeds through the gene in the transcriptional direction. Were replication to pass through this region in the antitranscriptional direction, the 1.9-kbp fragment would have been depleted relative to the 0.5-kbp fragment in dense DNA.

Comparison of the total signal from the 5′ H5 gene fragments to that from the 3′ fragments eliminates the possible contribution of a transient, differential sensitivity to EN to these data and shows that the 5′ fragments are depleted in the incorporation of density label relative to the 3′ fragments. The same polarity of replication was deduced

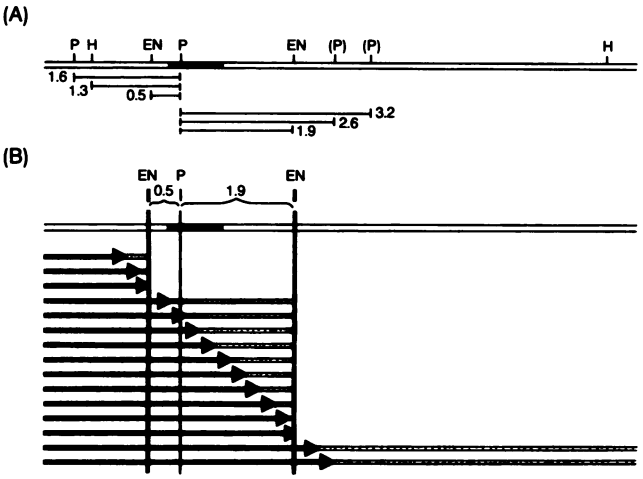

FIG. 1. (A) Endonuclease cleavage map of the chicken histone H5 gene region. The map has been modified from the data of reference 20. P, *Pst*I; (P), *Pst*I site polymorphism; H, *Hin*dIII. The filled bar represents the (intronless) histone H5 gene. Thin, bracketed lines denote endonucleases cleavage fragments discussed in the text. Sizes are in kilobase pairs. (B) IVR in the transcriptional direction through the H5 locus, when EN sites act as barriers to the progress of replication. Arrowheads indicate the position and direction of replication forks within the H5 region in an asynchronous population of nuclei when in vitro replication and incorporation of BrdUTP begins. Solid arrows, light DNA; stippled bars, dense DNA. DNA fragments generated by endonuclease cleavages and discussed in the text are indicated. See the text for further details.

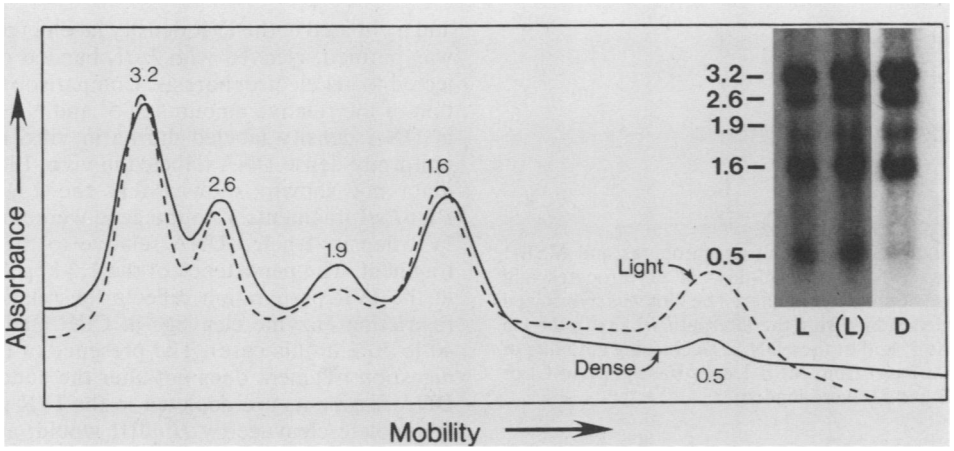

FIG. 2. IVR of 5-day chicken erythrocyte nuclei. Embryonic erythrocyte nuclei were submitted to replication in vitro in the presence of BrdUTP under conditions promoting EN cleavage (see Materials and Methods). DNA was isolated, digested to completion with *Pst*I, and banded on CsCl. Light and dense DNA was recovered, electrophoresed on a 0.8% agarose gel, blotted to nitrocellulose, and hybridized to a mixture of 5′ and 3′ H5 cDNA probes. DNA fragment sizes are indicated in kilobase pairs. Scans of light (lane L) and dense DNAs (lane D) were normalized to the signals from the 1.6-kbp bands. Lane (L) contains an overloaded light DNA sample for visualization of the 1.9-kbp band.

after erythrocyte nuclei were cleaved with EN plus *Hin*dIII during IVR, followed by *Pst*I digestion of the isolated DNA (data not shown; cf. Fig. 5). These results confirmed that replication of the H5 gene in embryonic erythrocytes occurs preferentially in the direction of transcription.

In vivo BrdUrd labeling of the H5 locus. To show that the in vitro incorporation of BrdUrd per se is not responsible for changing the relative proportion of H5 gene fragments in light versus BrdUrd-substituted DNAs, the H5 domain was uniformly density labeled by in vivo incubation of erythrocytes with BrdUrd for 5 to 6 h. DNA was isolated from these cells and separated into light and heavy fractions as described above. The ratio of 5′ (1.6-kbp) to 3′ (3.2-kbp, 2.6-kbp) H5 gene fragments was essentially the same in dense and light DNA (or bulk DNA; data not shown) after the gradient fractionation and blot hybridization procedure (Fig. 3). Therefore the change in the stoichiometry of 5′ to 3′ fragments during IVR must result from the disproportionate incorporation of density label across the H5 locus due to experimentally imposed barriers.

IVR in lymphocyte nuclei. To compare the replication polarity of an inactive histone H5 gene to that of the active erythrocyte H5 gene, we chose to examine the rapidly growing MSB-1 cells, a line of chicken lymphoblastoid cells transformed by Marek's disease virus. Histone H5 protein is not detectable in the nuclei or chromatin of these cells (22), nor does the H5 gene in isolated MSB-1 nuclei display the DNase I sensitivity observed in erythrocyte nuclei under the same conditions (Schmitz, Trempe and Leffak, manuscript preparation). To test directly the level of expression of the H5 gene in MSB-1 cells, an H5 cDNA probe was hybridized to a dot-blot containing RNA from 5-day erythrocytes and MSB-1 cells (Fig. 4). No hybridization signal was detected at the highest levels of MSB-1 RNA loaded, whereas erythrocyte RNA gave a detectable signal above background at 100-fold less RNA. Taken together, these observations imply that the H5 gene in MSB-1 nuclei is in an inactive state.

MSB-1 cell nuclei were incubated for IVR as above, after a preincubation with the restriction endonuclease *Hin*dIII. Under standard IVR conditions EN cleavages comparable to those observed in erythrocytes nuclei were suppressed (see below), and *Hin*dIII cut the H5 DNA approximately 1.3 kbp 5′ and 7 kbp 3′ to the internal *Pst*I site. The DNA was isolated, digested with *Pst*I, and analyzed as above. In

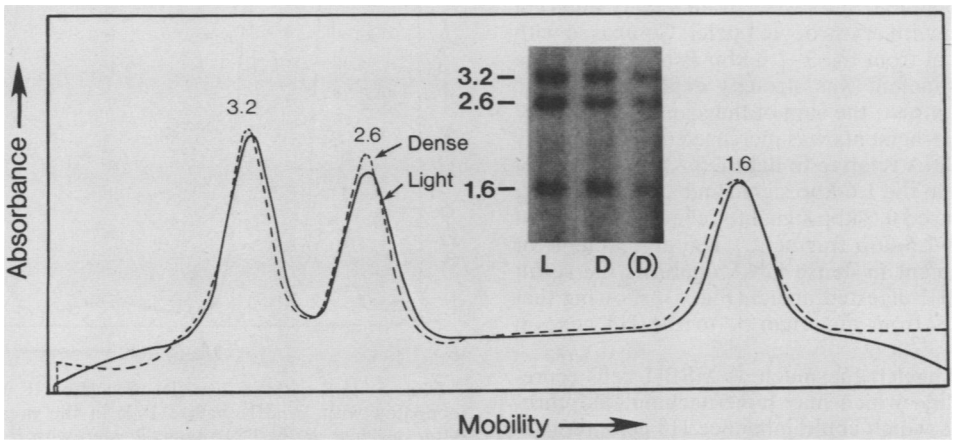

FIG. 3. In vivo density labeling of the H5 locus. Erythrocytes were isolated from 5-day-old chicken embryos and incubated in medium containing BrdUrd for 5 to 6 h. DNA was purified, digested with *Pst*I, and analyzed as in Fig. 2. Scans of light (lane L) and dense (lane D) DNA were normalized to the intensities of the 1.6-kbp bands. Lane (D) contains a decreased loading of dense DNA.

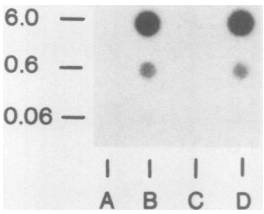

FIG. 4. H5 gene expression in 5-day erythrocytes and MSB-1 cells. Total RNA was isolated from 5-day-old erythrocytes and MSB-1 cells and spotted onto a nylon filter. The blot was hybridized to a 700-bp fragment extending from the internal *Pst*I site in the H5 cDNA sequence to the 3′ end of the cDNA. Decreasing amounts of RNA (6.0, 0.6, and 0.06 μg) from MSB-1 cells (lanes A and C) or erythrocytes (lanes B and D) were loaded.

contrast to the results of Fig. 3, runoff replication of the H5 genes in MSB-1 nuclei led to the depletion of the 3′ *Pst*I-*Pst*I fragments in dense DNA relative to the 5′ *Hin*dIII-*Pst*I fragments (Fig. 5), suggesting that the inactive H5 sequences are replicated from an origin in the 3′ flanking DNA. To demonstrate that the detection of differential labeling across the H5 locus relied on runoff replication from a defined boundary, MSB-1 nuclei were submitted to IVR as above, but omitting *Hin*dIII digestion. After DNA purification, *Pst*I digestion, isopycnic centrifugation, electrophoresis, and blot hybridization, there was no significant difference in the ratio of 5′ and 3′ *Pst*I fragments in light and dense DNA fractions (Fig. 6). This indicates that during IVR in the absence of imposed barrier sites there is no inherent bias detectable in the incorporation of BrdUTP (such as might result from the stalling of polymerases or a nonuniform distribution of replication forks) across the H5 locus, and that a sufficient majority of DNA polymerases completely traverses this region so that it appears uniformly labeled.

To confirm that the H5 gene in MSB-1 cells is replicated from a downstream origin, we wished to repeat the IVR reaction with an alternative set of initial endonuclease cleavages. We therefore took advantage of the observation that modification of the IVR reaction cocktail to include an increase in the amount of the postribosomal cytosol fraction from approximately 1 to 5 to 10 cell equivalents per nuclear equivalent exposes endogenous nuclease-hypersensitive cleavages in MSB-1 nuclei at sites similar to those observed in erythrocyte nuclei (Schmitz et al.,in preparation). When MSB-1 nuclei were allowed to replicate in vitro under these conditions, however, the results were dramatically different from those obtained with erythrocyte nuclei. Compared with the normalized signal from the 3′ 2.6-kbp *Pst*I fragment the 1.9-kbp EN-*Pst*I fragment was strongly depleted in dense DNA (Fig. 7). In contrast, the sum of the signals from the 5′ 0.5-kbp and 1.6-kbp fragments was increased (approximately twofold) in dense DNA relative to light DNA. (We interpret the slight reduction in the 1.6-kbp signal and a compensating portion of the increased 0.5-kbp signal to reflect the extent of EN activity on the 1.6-kbp fragment.) The enrichment for the 0.5-kbp 5′ fragment in dense DNA confirms the result obtained with *Hin*dIII-digested nuclei (Fig. 5), showing that replication proceeds from an origin 3′ to the H5 gene in MSB-1 cells.

IVR in fibroblast nuclei. Inasmuch as MSB-1 cells represent a continuous line which may have accumulated chromosomal alterations which could influence H5 gene replication, we tested the replication polarity of the inactive H5 gene in low-passage-number cultures of chicken embryo fibroblasts (CEF). CEF nuclei were digested with *Hin*dIII and incubated in the IVR density labeling cocktail. The DNA was purified, cleaved with *Pst*I, banded on CsCl, and subjected to gel electrophoresis. Comparison by blot hybridization of the relative amounts of 5′ and 3′ H5 gene fragments in DNA density labeled during in vitro replication and in uniformly dense DNA (labeled in vivo; Fig. 8) or light DNA (data not shown) showed that the 2.6-kbp and 3.2-kbp *Pst*I-*Pst*I fragments 3′ to the gene were both depleted in the IVR density-labeled DNA relative to the 1.3-kbp 5′ DNA fragment. The persistence of the 1.6-kbp *Pst*I-*Pst*I fragment in the IVR preparation reflects the relative inefficiency of restriction enzyme cleavage in CEF nuclei (approximately 40 to 50% in this case). The presence of the 1.6-kbp partial digestion fragment does not alter the conclusion that the 3′ DNA fragments are depleted in the IVR preparation, since incomplete cleavage by *Hin*dIII would, at worst, lead to a labeling pattern more similar to that of the in vivo-labeled DNA. Thus, as in MSB-1 cells, replication of the inactive H5 gene of normal CEF appears to initiate at a site 3′ to the gene.

DISCUSSION

The molecular mechanisms by which chromatin replication is involved in establishing and propagating structures which promote or preclude transcription are not known. The nonrandom deposition and segregation of histones and nonhistone proteins suggest that the structure of the nucleosome itself is likely to play a role in this process (14, 22–25, 27, 30, 34, 36, 38). In the present report we demonstrate a direct correlation between the transcriptionally active state of the histone H5 gene and the replication of this gene from an upstream origin. Our inference of replication polarity is based on the differential incorporation of density label across the histone H5 gene region. Control experiments show that the incorporation of the BrdUrd label either in vivo or in vitro in the absence of imposed blocks to DNA polymerase results in the uniform density labeling of this domain and that subsequent restriction endonuclease digestion, gradient centrifugation, fractionation, electrophoresis, and blot transfer-hybridization do not detectably alter the stoichiometry of newly replicated H5 DNA fragments relative to their representation in bulk DNA.

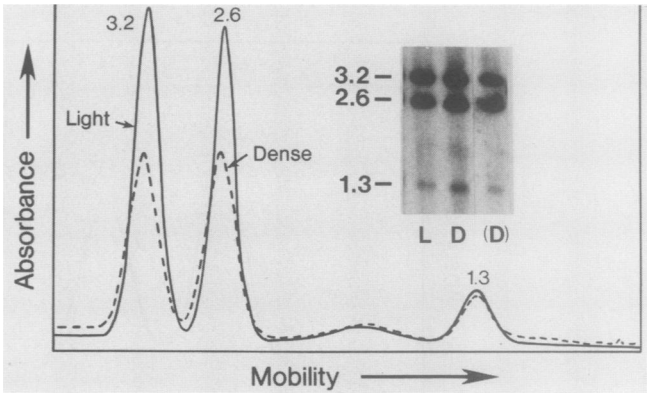

FIG. 5. IVR of MSB-1 nuclei with *Hin*dIII. MSB-1 nuclei were incubated with *Hin*dIII before IVR in the presence of BrdUTP. After purification the DNA was digested with *Pst*I and analyzed as in Fig. 3, except that the scans of light (lane L) and dense (lane D) DNA were normalized to the 1.3-kbp band signals. Lane (D) contains a decreased loading of dense DNA.

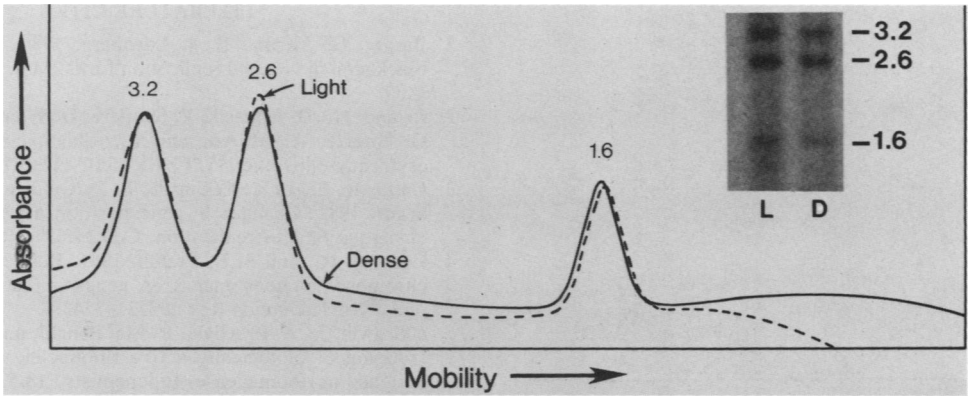

FIG. 6. Replication of MSB-1 nuclei without nuclease cleavage. MSB-1 nuclei were incubated as in Fig. 5, omitting restriction enzyme. DNA was purified, digested with *PstI*, and analyzed as in Fig. 2.

In contrast, when the chromatin is exposed to nuclease cleavages in situ, subsequent replication in the presence of BrdUTP leads to the nonuniform incorporation of density label. Preincubation of nuclei with various restriction nucleases decreases the extent and rate of IVR DNA synthesis in approximate proportion to the expected random frequency of occurrence of the enzyme target site in DNA (19). The simplest interpretation of these observations is that cuts introduced in the DNA template represent barriers to the progression of DNA polymerases (however, this has not been proven directly). Although this scenario predicts that few, if any, DNA polymerase molecules would sufficiently replicate sequences immediately 3′ to the barrier site to allow their representation in heavy DNA, we did observe fragments which bordered the presumptive boundary site in the newly replicated fraction of DNA. We attribute this to in situ nuclease digestion occurring after versus before the passage of the replication apparatus and to the breadth of DNA distributions across the CsCl gradients; however, we cannot eliminate the possibility that not every H5 gene copy is replicated in the same direction and that we detect only the preferred polarity of replication.

This discussion does not ignore the possible existence of other types of potential endogenous blocks to replication fork movement (e.g., matrix attachment sites, replication origins). At least in MSB-1 cells, however, the data of Fig. 6 (IVR without enzyme digestion) show that such boundaries must be sufficiently distant that the entire H5 locus appears uniformly labeled. Based on the distribution of *PstI* sites around the H5 gene and the sensitivity of the IVR assay (19), we estimate this distance to be greater than 8 to 10 kbp. Under conditions of in vitro replication erythrocyte nuclei display site-specific endogenous nuclease cleavages; therefore a similar minus-enzyme control reaction is not possible with these nuclei. Although the proximity of endogenous boundary sites in erythrocyte nuclei could quantitatively affect the ratio of 5′ and 3′ DNA fragments, it would not change the ratio from greater to less than unity. Therefore an unambiguous interpretation of replication direction is still possible.

We conclude that the preferred direction of replication of the active histone H5 genes of embryonic chicken erythrocytes is the transcriptional direction, whereas the inactive H5 genes in MSB-1 cells and CEF are replicated in the opposite direction. Recently, Dalton et al. (6) demonstrated that the active H5 gene in the ts34 erythroid cell line shows an attachment site to the residual nuclear protein matrix within a 780-bp *NaeI-SacI* restriction fragment spanning the 5′ EN site. In contrast, neither this fragment nor the 920-bp 3′-flanking fragment is bound to the matrix in a Marek's

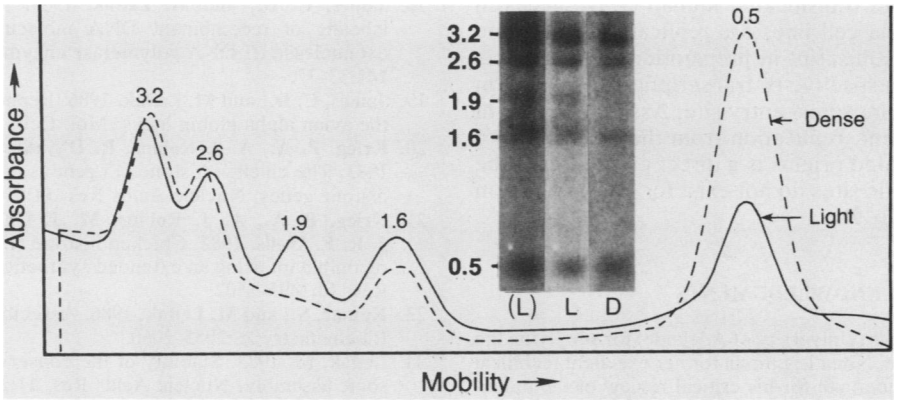

FIG. 7. IVR of MSB-1 nuclei with EN digestion. MSB-1 nuclei were incubated under conditions promoting cleavage by the EN (see Materials and Methods) before the addition of the IVR cocktail and BrdUTP. DNA was purified, digested with *PstI*, and analyzed as in Fig. 6. Scans of light (lane L) and dense (lane D) DNA were normalized to the signals from the 2.6-kbp fragment bands. Lane (L) shows an overexposure of lane L, emphasizing the 1.9-kbp band.

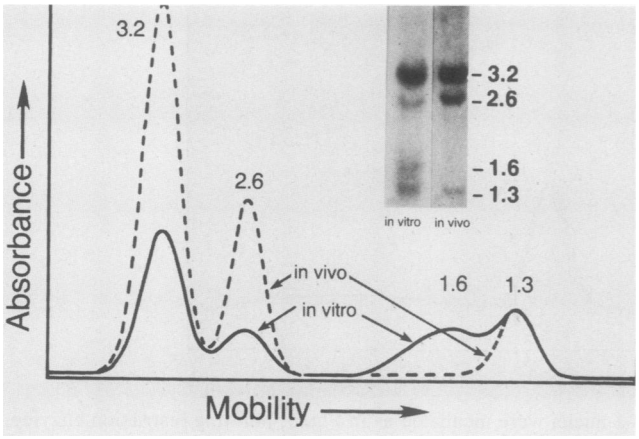

FIG. 8. IVR of CEF nuclei with *Hin*dIII digestion. CEF nuclei were incubated with *Hin*dIII before IVR in the presence of BrdUTP. After purification the DNA was digested with *Pst*I and banded on CsCl. The fragment pattern of in vitro-labeled dense DNA is compared with that of dense DNA which had been labeled in vivo, purified, digested to completion with *Hin*dIII and *Pst*I, and run on a parallel CsCl gradient. The scans of in vivo- and in vitro-labeled DNA were normalized to the signals of the 1.3-kbp band.

disease virus-transformed T-cell line (comparable to MSB-1 cells) in which the H5 gene is inactive.

We showed previously that replication proceeds from upstream (5′) origins through the alpha-globin locus irrespective of transcriptional activity in erythrocytes and MSB-1 cells (19). The alpha-globin and H5 gene IVR results are consistent with the generalization that active genes are replicated in the transcriptional direction and that cells may switch the replication polarity of genes in concert with gene activation or repression. However, these observations do not establish an obligate relationship between replication and transcription and may merely reflect the fact that the exposure of certain chromosomal sites can facilitate the entry of both DNA transcription and replication machinery. The replication in the early S phase of genes whose transcription is activated by *trans*-acting factors and/or chromosome translocation suggests that protein binding and chromosome position may also influence replication polarity (3, 7, 13). Consistent with the idea that chromosomal position may influence replication polarity, our recent data indicate that the germ line and translocated human c-*myc* genes in two Burkitt lymphoma cell lines are replicated in opposite directions (Leffak, manuscript in preparation). Thus, genes may be replicated irrespective of transcriptional orientation from the nearest chromosomal entry site. Assuming uniform rates of fork movement, replication from the nearer of two simultaneously activated origins is a direct prediction of the hypothesis that specific sites do not exist for the termination of DNA replication (1, 9).

ACKNOWLEDGMENTS

We thank J. R. E. Wells (University of Adelaide) for supplying the histone H5 cDNA probe, Nona Lightman for her excellent technical assistance, and Joel Huberman for his critical review of the manuscript. DNA sequence information was obtained through the BIONET resource.

This work was supported by Public Health Service grant GM-35877 from the National Institutes of Health to M.L.

LITERATURE CITED

1. **Berger, C. A., and H. J. Edenberg.** 1986. Pyrimidine dimers block simian virus 40 replication forks. Mol. Cell. Biol. **6**:3443–3450.
2. **Briand, G., D. Kmiecik, P. Sautiere, D. Wouters, O. Borie-Loy, G. Biserte, A. Mazen, and M. Champagne.** 1980. Chicken erythrocyte histone H5. FEBS Lett. **112**:147–151.
3. **Calza, R. E., L. A. Eckhardt, T. DelGuidice, and C. L. Schildkraut.** 1984. Changes in gene position are accompanied by a change in time of replication. Cell **36**:689–696.
4. **Cech, T. R., and S. L. Brehm.** 1981. Replication of the extrachromosomal ribosomal RNA genes of Tetrahymena thermophila. Nucleic Acids Res. **9**:3531–3543.
5. **Chirgwin, J., A. Przybyla, R. MacDonald, and W. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
6. **Dalton, S., H. B. Younghusband, and J. R. E. Wells.** 1986. Chicken histone genes retain nuclear matrix association throughout the cell cycle. Nucleic Acids Res. **14**:6507–6523.
7. **D'Andrea, A. D., U. Tantravahi, M. Lalande, M. A. Perle, and S. A. Latt.** 1983. High resolution analysis of the timing of replication of specific DNA sequences during S phase of mammalian cells. Nucleic Acids Res. **11**:4753–4774.
8. **D'Andrea, R. J., L. S. Coles, C. Lesnikowski, L. Tabe, and J. R. E. Wells.** 1985. Chromosomal organization of chicken histone genes: preferred associations and inverted duplications. Mol. Cell. Biol. **5**:3108–3115.
9. **Edenberg, H. J., and J. Huberman.** 1975. Eucaryotic chromosome replication. Annu. Rev. Genet. **9**:245–284.
10. **Engberg, J., N. Din, H. Saiga, and T. Higashinakagawa.** 1984. Nucleotide sequence of the 5′ terminal coding region for pre-mRNA and mature 17S rRNA in Tetrahymena thermophila rDNA. Nucleic Acids Res. **12**:959–972.
11. **Ford, P. J., and T. Mathiesen.** 1976. Control of 5S RNA synthesis in *Xenopus laevis*. Nature (London) **261**:433–435.
12. **Glisin, V., R. Crkvenjakov, and C. Byus.** 1974. Ribonucleic acid isolated by cesium chloride centrifugation. Biochemistry **13**: 2633–2637.
13. **Goldman, M. A., G. P. Holmquist, M. C. Gray, L. A. Caston, and A. Nag.** 1984. Replication timing of genes and middle repetitive sequences. Science **224**:686–692.
14. **Gottesfeld, J., and L. Bloomer.** 1982. Assembly of transcriptionally active 5S RNA gene chromatin in vitro. Cell **28**:781–791.
15. **Guinta, D. R., J. Y. Tso, S. Narayanswami, B. Hamkalo, and L. J. Korn.** 1986. Early replication and expression of oocyte-type 5S RNA genes in a *Xenopus* somatic cell line carrying a translocation. Proc. Natl. Acad. Sci. USA **83**:5150–5154.
16. **Hand, R.** 1978. Eucaryotic DNA: organization of the genome for replication. Cell **15**:317–325.
17. **Henikoff, S., M. A. Keene, K. Fetchel, and J. Fristrom.** 1986. Gene within a gene: nested Drosophila genes encode unrelated proteins on opposite DNA strands. Cell **44**:33–42.
18. **James, C. D., and M. Leffak.** 1984. Replacement synthesis labeling of recombinant DNA molecules using the *E. coli* exonuclease III/DNA polymerase enzyme pair. Anal. Biochem. **141**:33–37.
19. **James, C. D., and M. Leffak.** 1986. Replication polarity through the avian alpha-globin locus. Mol. Cell. Biol. **6**:976–984.
20. **Krieg, P. A., A. J. Robins, R. D'Andrea, and J. R. E. Wells.** 1983. The chicken histone H5 gene is unlinked to core and H1 histone genes. Nucleic Acids Res. **11**:619–627.
21. **Krieg, P. A., A. J. Robins, M. J. Gait, R. C. Titmas, and J. R. E. Wells.** 1982. Chicken histone H5: selection of a cDNA recombinant using an extended synthetic primer. Nucleic Acids Res. **10**:1495–1502.
22. **Kumar, S., and M. Leffak.** 1986. Assembly of active chromatin. Biochemistry **25**:2055–2060.
23. **Leffak, M.** 1983. Stability of the conservative mode of nucleosome assembly. Nucleic Acids Res. **11**:2717–2731.
24. **Leffak, M.** 1984. Conservative segregation of nucleosome core histones. Nature (London) **307**:82–85.
25. **Leffak, M., R. Grainger, and H. Weintraub.** 1977. Conservative assembly of nucleosomal histones. Cell **12**:837–845.

26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Manser, T., T. Thacker, and M. Rechsteiner.** 1980. Arginine-rich histones do not exchange between human and mouse chromosomes in hybrid cells. Cell **19**:993–1003.
28. **Muller, M. T., K. Kajiwara, and G. C. Mueller.** 1981. Role of cytosol proteins in DNA chain growth and chromatin replication in Friend erythroleukemia cell nuclei. Biochim. Biophys. Acta **653**:391–407.
29. **Niles, E. G., J. Sutiphong, and S. Haque.** 1981. Structure of the *Tetrahymena pyriformis* rRNA gene. Nucleotide sequence of the transcription initiation region. J. Biol. Chem. **256**:12849–12856.
30. **Prior, C. P., C. R. Cantor, E. V. Johnson, and V. G. Allfrey.** 1980. Incorporation of exogenous pyrene-labeled histone into *Physarum* chromatin: a system for studying changes in nucleosomes assembled in vivo. Cell **20**:597–608.
31. **Smithies, O.** 1982. The control of globin and other eucaryotic genes. J. Cell. Physiol. Suppl. **1**:137–143.
32. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
33. **Tooze, J.** 1980. DNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Trempe, J. P., and M. Leffak.** 1985. Histone H1 and HMG 14/17 are deposited nonrandomly in the nucleus. Nucleic Acids Res. **13**:4853–4869.
35. **Truett, M. A., and J. G. Gall.** 1977. The replication of ribosomal DNA in the macronucleus of Tetrahymena. Chromosoma **64**: 295–303.
36. **Weintraub, H., S. J. Flint, M. Leffak, M. Groudine, and R. Grainger.** 1977. The generation and propagation of variegated chromosome structures. Cold Spring Harbor Symp. Quant. Biol. **42**:401–416.
37. **Weisbrod, S.** 1982. Active chromatin. Nature (London) **297**:289–295.
38. **Wormington, W. M., M. Schlissel, and D. D. Brown.** 1983. Developmental regulation of *Xenopus* 5S RNA genes. Cold Spring Harbor Symp. Quant. Biol. **47**:879–884.