MOLECULAR AND CELLULAR BIOLOGY, May 1988, p. 2149–2158
0270-7306/88/052149-10$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 8, No. 5

Replication Program of Active and Inactive Multigene Families in Mammalian Cells†

K. S. HATTON, V. DHAR, E. H. BROWN, M. A. IQBAL, S. STUART, V. T. DIDAMO, AND C. L. SCHILDKRAUT*

Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461

Received 29 September 1987/Accepted 27 January 1988

In a comprehensive study, the temporal replication of tissue-specific genes and flanking sequences was compared in nine cell lines exhibiting different tissue-specific functions. Some of the rules we have determined for the replication of these tissue specific genes include the following. (i) Actively transcribed genes usually replicate during the first quarter of the S phase. (ii) Some immunoglobulin genes replicate during the first half of S phase even when no transcriptional activity is detected but appear to replicate even earlier in cell lines where they are transcribed. (iii) Nontranscribed genes can replicate during any interval of S phase. (iv) Multigene families arranged in clusters of 250 kilobases or less define a temporal compartment comprising approximately one-quarter of S phase. While these rules, and others that are discussed, apply to the tissue-specific genes studied here, all tissue-specific genes may not follow this pattern. In addition, housekeeping genes did not follow some of these rules. These results provide the first molecular evidence that the coordinate timing of replication of contiguous sequences within a multigene family is a general property of the mammalian genome. The relationship between replication very early during S phase and the transcriptional activity within a chromosomal domain is discussed.

Early observations in many laboratories suggested a relationship among chromosome condensation, late DNA replication, and gene inactivity (for review, see reference 4). Examples include the DNA of the inactive X chromosome in female mammals or genes showing variegated patterns of inactivity in *Drosophila melanogaster* when they are transposed from their normal position to one proximal to centromeric late replicating heterochromatin. Several observations have been reported indicating that, during the S phase, the genome of higher eucaryotes replicates in a bipartite manner separated by a pause (27, 37 and references therein). It has been suggested that, after this pause, the genome could replicate in a manner that renders it transcriptionally incompetent (21). It has also been suggested that there are distinct early and late compartments for replication of genes for specialized functions (21, 51 and references therein).

To study the relationship between replication and gene expression on a molecular basis, we carried out initial studies on the temporal replication of single-copy genes in mammalian cells (18). The α-globin gene replicates very early during S phase in the Friend virus-transformed murine erythroleukemia (MEL) cell line in which it is transcribed. In subsequent studies, we showed that several immunoglobulin heavy-chain constant-region (IgC_H) and variable-region (IgV_H) genes also replicate very early during S phase when they are transcribed (10). Here we examine the temporal order of replication of genes for specialized functions in several types of differentiated cells in which they either are transcribed or are transcriptionally silent. Among the findings presented here is the first demonstration that, even when their transcription is not detectable, some genes replicate during the first half or even near the beginning of S phase. Thus, we present a detailed description of the relationship between transcriptional activity and temporal replication which does not provide evidence (in the cell lines we have studied) for distinct early and late compartments in which active and inactive genes must replicate. Further evidence that S phase is not divided into two distinct temporal compartments is derived from a detailed study of the rate of DNA replication in the MEL cell line in which we did not observe a significant pause during DNA replication (9).

The approach we have developed is comprehensive in that we examined tissue-specific genes expressed in nine differentiated cell lines. In most instances, we measured the presence or absence of steady-state or nascent transcripts for these tissue-specific functions. We divided the S phase into four time compartments by centrifugal elutriation, which fractionates cells according to size and avoids the use of potentially toxic compounds. This allowed us to distinguish events that occurred in the first and second quarters of S phase.

We have shown (10) that one factor that plays a role in determining the temporal order of gene replication is chromosomal position. In the MPC11 cell line, for example, there are two copies of the Cγ2b gene. The productively rearranged, transcribed copy is very early replicating. The other allele, on another chromosome, is located about 10 kilobases (kb) from the rearranged portion of c-*myc* exon 1. This copy of Cγ2b replicates very late during S phase. Thus, two apparently identical genes and their flanking sequences in the same cell can replicate at very different times during S phase depending on their chromosomal location.

In the present study, we examine several multigene families and begin to define the size of the regions that determine the relationship between chromosomal location and temporal replication. We present evidence that sequences located within a 250-kb region replicate during the same quarter of S phase. These results include both early and late replicating

* Corresponding author.

† Dedicated to Arthur Kornberg on the occasion of his 70th birthday.

gene clusters. We have not yet detected an early replicating gene within a late replicating cluster or a late replicating gene within an early replicating cluster.

MATERIALS AND METHODS

Cell lines, culture conditions, BUdR labeling, and centrifugal elutriation. Sources, culture conditions, 5-bromodeoxyuridine (BUdR) labeling, and centrifugal elutriation were as described previously for MEL, S107, MPC11, and L60T cell lines (10) and RL♂11, 22D6, and 300-19P cell lines (2, 9). BALB/c 3T3 and L60T cells and a hepatoma cell line, Hepa 1.6 (14), were grown in monolayers in Dulbecco modified Eagle medium containing 10% fetal calf serum and labeled with BUdR (20 μg/ml) for 2 h.

Isolation and processing of DNA. The preparation, restriction endonuclease digestion with *Eco*RI, and isolation of newly replicated DNA by Cs_2SO_4 centrifugation, fractionation according to size by agarose gel electrophoresis, and transfer to diazobenzyloxymethyl paper (52) of bromouracil-labeled DNA (BU-DNA) were performed as described previously (7).

Determination of nuclear DNA content at the time a gene replicates. The assignment of C values for nuclear DNA content at which a gene replicates was determined from the relative concentrations of *Eco*RI segments in BU-DNA from different intervals of the S phase as described previously (9).

In vitro nuclear transcription and RNA preparation. Cells were grown either in suspension (MEL, MPC11, and S107) or attached (Hepa 1.6) in Dulbecco modified Eagle medium containing 10% fetal bovine serum. When attached, cells were rinsed twice with ice-cold phosphate-buffered saline, scraped from tissue culture flasks, and collected by centrifugation for 5 min at 500 × *g*. Nuclei from 1×10^8 to 2×10^8 cells were isolated by the Triton lysis method (24).

In vitro nuclear transcription, RNA isolation, and hybridization were performed by the method of McKnight and Palmiter (35). Briefly, nuclei (5×10^7) were incubated in duplicate for 25 min at 26°C in 200 μl of 16% (vol/vol) glycerol–20 mM Tris hydrochloride (pH 8.0)–5 mM $MgCl_2$–150 mM KCl–0.4 mM each ATP, GTP, and CTP–200 μCi of [α-^{32}P]UTP (4,000 Ci/mmol). Nuclear RNA was precipitated with trichloroacetic acid and then reprecipitated with 2 volumes of ethanol and 0.3 M sodium acetate (pH 4.5). An aliquot was precipitated with trichloroacetic acid, and total incorporated radioactivity was determined. Labeled RNA (2×10^7 to 5×10^7 cpm) was hybridized to genomic DNA or cDNA clones immobilized on nitrocellulose filters (5 μg per slot) for 3 to 4 days at 43°C. Filters were washed in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% sodium dodecyl sulfate at 65°C for 2 h and treated for 30 min at 37°C with pancreatic RNase A (10 μg/ml in 1× SSC). Filters were washed again at 55°C in 0.1× SSC–0.1% sodium dodecyl sulfate for 30 min, dried, and autoradiographed for 1 to 5 days, using Kodak XAR film at −70°C. Signals were quantitated by densitometric scanning.

DNA probes. Probes for the murine immunoglobulin heavy-chain constant region, including p3′α, M67-19, γ2b [γ2b(11)[7] and pBR1.4], γ1(MEP10), and J_H (pJ11), were described previously (9). The derivation of the α-globin, λgtWES-Mα1 (18), β-globin, pCR1-βG9 (7), and V_H T15pCV1 (10) probes has been described in earlier studies. The kappa variable-region probe Vκ19, provided by R. Perry, is a genomic clone from the 5′-flanking region to the middle of the variable region (31). The BmB2 (43) factor B cDNA clone, the BmS10 clone, and C2M1, a C2 cDNA clone (unpublished data), were provided by A. S. Whitehead and H. Colten. BmS10 is a 0.9-kb cDNA clone spanning the α and γ chain of C4 described by Sackstein and Colten (42). Due to the homology between C4 and Slp, BmS10 also cross-hybridizes to the latter gene (41). Two different probes were used to detect the 21-hydroxylase (21-OH) gene in blots. One contains a 3.7-kb *Bam*HI genomic segment (from cosmid E-36 of reference 11) which spans 90% of the 21-OH B gene and was provided by D. Chaplin. A second 21-OH clone, pDS101, is a 12-kb *Hin*dIII genomic fragment provided by D. Robins. The cloned 1.4-kb *Eco*RI-*Sal*I (ES 1.4) and 3.7-kb *Sal*I-*Hin*dIII (SH 3.7) genomic segments are derived from the 3′ and 5′ ends, respectively, of the murine tyrosine aminotransferase gene (46). A Thy-1 928-base-pair *Pst*I genomic clone (MTGC2322) was constructed by Teng et al. (M. Teng, R. Basch, and J. Buxbaum, manuscript in preparation). A genomic clone (4.75-kb *Eco*RI fragment) of α-fetoprotein (AFP; pAFP7B), described by Gorin and Tilghman (22), and the cloned 5.8-kb *Eco*RI fragment from the albumin gene (29) were provided by S. Tilghman. The genomic clones from the amylase gene cluster, *Eco*RI-*Hin*dIII segments of pG2-3L3 (0.5 kb) and p47a (0.75 kb), and an *Eco*RI segment, p23-1.8 (1.8 kb), are located in the upstream flanking regions of Amy-1 and Amy-2.1 and in a pseudogene, respectively (provided by M. Meisler and D. Gumucio) (54). α_1-Antitrypsin (c-liv-3) and major urinary protein (MUP) cDNA clones were provided by K. Krauter (15). pECκ, a 6.6-kb *Eco*RI-*Bam*HI genomic fragment which encompasses the Jκ-Cκ region (12), was provided by R. Perry. pMg 3 (5.1-kb *Eco*RI/*Pst*I fragment) includes the first two introns of the dihydrofolate reductase (DHFR) gene ligated to a fragment of a cDNA clone to give an entire coding region (19). The human cDNA clone containing the T-cell receptor gene β-chain constant (Tcβ) region which hybridizes to murine $C_T\beta1$ and $C_T\beta2$ was purchased from Oncor, Inc., Gaithersburg, Md. (for a map, see reference 34). The murine T-cell receptor β1 (pUCVβ1) variable-region probe, provided by Lee Hood, is a 300-base-pair *Eco*RI/*Xba*I fragment (32). $pM1XH_{1.0}$ (provided by R. Palmiter) contains a 1-kb unique *Xba*I/*Hin*dIII fragment of the mouse metallothionein-1 gene (for a restriction map, see reference 16). pMH2a.1, provided by W. Marzluff, is a 1.0- to 1.2-kb genomic insert containing the H2a gene and flanking sequences (48).

RESULTS

Our strategy was to determine whether there was a coordinate temporal order of replication for geographically close genes and their flanking sequences. Eight clusters of tissue-specific genes in different chromosomal locations were studied based on the availability of probes spanning a significant portion of the cluster. Nine different cell lines were examined, each of which exhibited one or more of some of the tissue-specific functions encoded by these genes.

The cell lines studied and their tissue of origin are listed in Table 1. Cells grown in the presence of BUdR for two h were fractionated by centrifugal elutriation into four size classes which corresponded to different stages of the S phase. Thus, we obtained cell populations which had incorporated BUdR into DNA replicated at different stages of the S phase (18). BU-DNA that replicated during four selected intervals of S phase was prepared, digested with *Eco*RI, separated by density gradient centrifugation in Cs_2SO_4, size fractionated

TABLE 1. Transcribed genes that replicate very early during S phase[a]

Cell line	Mouse strain	Tissue of origin	Transcripts detected	Transcripts not detected[b]
MEL	DBA/2J	Erythroid, Friend virus transformed	α and β globin	AFP, albumin, amylase, factor B, MUP
S107	BALB/c	B-cell plasmacytoma	Cκ, Cα, V_HT15	AFP, albumin, amylase, α-globin, β-globin, factor B, MUP
MPC11	BALB/c	B-cell plasmacytoma	Cκ, Cγ2b, Vκ19	AFP, albumin, amylase, MUP, α-globin, β-globin, factor B
22D6	BALB/c	Pre-B Abelson murine leukemia virus transformed	Cμ,[c] N-*myc*[d]	AFP, albumin, amylase, α-globin
300-19P	NIH/Swiss	Pre-B Abelson murine leukemia virus transformed	Cμ,[c] N-*myc*[d]	
RLδ11	BALB/c	T-cell leukemia	Tcβ, Thy-1	Albumin, amylase, α-globin, factor B, MUP
L-cell (L60T)	C3H	Connective tissue	C2	Albumin, amylase, factor B, MUP
3T3	BALB/c	Embryo	c-*myc*[d]	Albumin, amylase, α-globin, factor B, MUP
Hepa 1.6	C57L/J	Hepatoma	AFP, albumin, C2, amylase	α-Globin, β-globin, factor B, MUP

[a] Steady-state transcripts from the genes listed in column 4 were detected at high levels in each of the murine cell lines indicated. Abbreviations: V_H, immunoglobulin heavy-chain, variable region; Tcβ, constant region of the β chain of the T-cell receptor. $C_κ$, Constant region of the kappa light-chain locus. For the cell lines shown, total RNA (20 μg) was fractionated through formaldehyde-agarose gels and transferred to nitrocellulose paper. After hybridization to probes specific for the genes shown, the presence or absence of a particular transcript was determined by autoradiography. X-ray films were exposed overnight and for 1 to 2 months. Transcripts either were observed or were below the level of detection. (For additional details, see Fig. 1 and 2.) RNA prepared from specific tissues served as controls for tissue-specific genes. RNA prepared from thymus served as a control for Tcβ and Thy-1. Liver RNA was used for the detection of MUP, $α_1$-antitrypsin, AFP, and albumin. Rat adrenal gland RNA was used as a control for 21-hydroxylase.

[b] In addition, the following also were not detected in any of the cell lines, except where indicated: 21-hydroxylase, C4, Thy-1, C2, and Tcβ. Tyrosine aminotransferase and $α_1$-antitrypsin were assayed in all of the cell lines except 300-19P and were not detected.

[c] Alt et al. (3).

[d] Iqbal et al. (30 and references therein).

by agarose gel electrophoresis, and transferred to diazobenzyloxymethyl paper. The relative concentrations of *Eco*RI segments containing a particular gene or flanking sequence were determined as described previously (see reference 10 and references therein). These concentrations were used (as previously described in detail in reference 9) to determine, in a particular cell line, the value most closely corresponding to the DNA content (C value) of the nucleus at the time the gene replicated. Total RNA prepared from each cell line was used to detect the different steady-state transcripts for tissue-specific genes.

Clustered DNA segments replicate during the same interval of S phase. Many of the genes and flanking sequences whose temporal replication we have measured are located in clusters as part of multigene families. Genes within a cluster encompassing 15 to 250 kb replicate within the same interval (about 2.5 h) of S phase. We have not observed a gene that replicates during the first quarter of S phase to be closer than 250 kb to a gene that replicates during the last quarter. Thus, DNA sequences are spatially organized with respect to temporal replication.

We have examined in detail eight clusters in nine murine cell lines. The segments have been mapped in seven of these clusters. (i) We have examined an *Eco*RI segment containing five exons of the AFP gene and another *Eco*RI segment containing five exons of the albumin gene. These two segments, which are about 33 kb apart, replicate at approximately the same time in S phase (Table 2). (ii) The β-major and β-minor globin genes, which are about 15 kb apart, replicate at approximately the same time in S phase (Table 2). (iii) The approximately 450-kb (32) T-cell receptor β-chain gene cluster containing the two constant-region genes ($C_Tβ1$ and $C_Tβ2$) and the β1 variable-region gene replicate during the same quarter of the S phase in a particular cell line (Table 2). (iv) Sequences of the Vκ19 family were detected in four to five *Eco*RI segments, using a genomic probe. Although these segments have not been physically linked, we expect them to encompass at least 30 kb from the sizes of *Eco*RI segments detected with this probe. In each cell line examined, all of the members of this variable-region gene family that we detected replicated at very similar times during the S phase (Table 2). (v) The T15 family of immunoglobulin heavy-chain variable-region genes has been estimated to encompass at least 70 kb (G. Siu, R. Perlmutter, and L. Hood, personal communication). In each cell line examined, four members of this variable-region gene family that we detected replicated during one particular quarter of the S phase (Table 2). (vi) We have studied the temporal replication of the cluster containing the IgC_H genes and the J and D segments in several murine cell lines (7, 9). In several non-lymphoid cell lines, 16 *Eco*RI segments spanning an approximately 300-kb region, which includes the IgC_H, J, and D genes and their flanking sequences, replicated during the first half of S phase, although these genes

TABLE 2. Genes that are early replicating when expressed or located within an expressed multigene family[a]

Gene	C value in cell line:								
	MEL	S107	MPC11	L60T	3T3	22D6	300-19P	RLδ11	Hepa 1.6
AFP	2.6	3.5	3.1		2.7			3.3	2.0[b]
Albumin	2.6	3.4	3.2	3.0	2.7	2.5	2.6	3.2	2.0[b]
β-Globin									
β Major	2.1	4.0	3.8	3.9	3.3	3.1		2.6	3.4[c]
β Minor	2.1	3.9	3.8	3.9	3.3	3.0		2.6	
T-cell β chain									
Constant region									
$C_T\beta 1$	2.5	3.6	3.4		3.4			2.0[b]	3.2
$C_T\beta 2$	2.6	3.5	3.4		3.4			2.0[b]	3.0
Variable region									
β1	2.4							2.0	
K variable region, Vκ19									
16.0 kb	3.0	3.3	2.0[d]						3.3
8.5 kb	3.5	3.3	2.0[d]						3.3
6.2 kb	3.4	3.3	2.0[d]						3.2
Heavy-chain variable, V_HT15									
V1	3.4	2.0[b]	3.6	3.5	3.2	2.0[d]			
V3	3.4	2.2[d]	3.6	3.5	3.6	2.1[d]			
V11	3.3	2.0[d]	4.0	3.5	3.4	2.0[d]			
V13	3.3	2.1[d]	4.0	3.4	3.6	2.0[d]			

[a] These genes replicate significantly later or during the second half of S phase when they are not expressed. Values are DNA contents (C value, where C is the haploid DNA content of cells in G1) of the cell nucleus at the time of gene replication. The DNA contents were determined as described by Brown et al. (9) from the flow microfluorimetric profiles of the cell fractions obtained by centrifugal elutriation and used to prepare BU-DNA replicated during four selected intervals of the S phase. Autoradiographs on the linear range of exposure were used to determine the relative concentrations of a particular *Eco*RI segment in the BU-DNA that replicated during each of four intervals of S phase. The concentrations were expressed as percentages normalized to 100, and the maximum percent error was determined as described previously (30). This error was either <10% or between 10 and 15% for >75% of the C values listed.

[b] Transcribed gene.

[c] Hepa 1.6 is derived from mouse strain C57L/J, which has two β-globin genes (βt and βs) instead of β major and β minor. Both genes are located on 10-kb *Eco*RI segments (53) which were indistinguishable on agarose gels in this study.

[d] Located within a multigene cluster containing a transcribed gene in either this cell line or a subclone. One of the genes in the V_HT15 family (V1) is expressed in S107 cells (13). In 22D6, transcripts of V_H558 were detected at high levels (56); however, the exact molecular distance between the V_H558 and the V_HT15 families is not known.

were transcriptionally inactive. Furthermore, at least 200 kb of this locus replicated during the same quarter of S phase. In contrast, in all of the lymphoid cell lines examined, the IgC_H gene cluster replicated very early during S phase, when the nuclear DNA content was near a C value of 2.0. No difference in temporal replication is detected between the various segments examined in this region (Table 3). (vii) Five genes located in a cluster of about 200 kb of the S region of the murine *H-2* complex replicated during the first quarter of S phase in all of the cell lines studied here. The functionally unrelated 21-hydroxylase gene located in this cluster also replicated during the same quarter of S phase (see Table 5). (viii) We have measured the temporal replication of several segments containing exons of the salivary (Amy-1) and pancreatic (Amy-2) mouse α-amylase genes. About 12 genomic exon segments (detected with the cDNA probe pMSa104 [45]) encompassing about 50 kb replicated during the last quarter of S phase in several cell lines in which they were not expressed (data not shown). Two segments that are about 40 kb apart and flank the Amy-1 gene also replicated during this same quarter of S phase (see Table 6).

For a particular cluster of genes and flanking sequences, each of the *Eco*RI segments we examined replicated during the same quarter of the S phase. The three largest clusters examined, the IgC_H region, the β-chain of the T-cell receptor, and the S region of the major histocompatibility complex, allowed us to define the maximum length (approximately 250 kb) of a cluster of nontranscribed DNA that could replicate during one-quarter of the S phase. As described below, the time (early or late) during the S phase at which the cluster replicates appears to depend on the transcriptional activity within or near the cluster.

Genes that usually replicated late in cells in which transcripts are not detected and replicated very early in S phase in cells in which they are expressed. We observed that DNA segments containing the genes for albumin and AFP, β-globin, the T-cell receptor β-chain constant region (Tcβ), Vκ19, and the T15 IgV_H gene were very early replicating (Table 2) in the Hepa 1.6, MEL, RLδ11, MPC11, and the S107 cell lines, respectively, in which high-steady-state levels of transcripts were observed from these genes. In other cell lines in which their transcripts were not detected, these genes replicated significantly later or during the second half of the S phase, and in many of these cell lines they replicated during the last third of the S phase (Table 2). We determined that there were no detectable steady-state levels of transcripts for these genes (in the cell lines examined [Table 1]) in which they replicated during the second half of

TABLE 3. Genes that replicate during first half of S phase when not expressed and are usually earlier replicating when expressed

Gene	C value in cell line:								
	MEL	S107	MPC11	L60T	3T3	22D6	300-19P	RLδ11	Hepa 1.6
α-Globin	2.1[b]	2.3	2.3	2.4		2.4	2.4	2.2	
Kappa, constant region (Cκ-Jκ)	2.4	2.0[b]	2.0[b]			2.0	2.0[c]		2.9
Cγ2b	2.5		2.0[b]	2.5	2.5	2.0[c]	2.3[c]	2.5[c]	2.4
γ1	2.5					2.0[c]	2.0[c]	2.4[c]	
J_H	2.8	2.0[b]	2.0[b]	2.9	3.0		2.0[b]	2.8	
Cμ	2.7			3.0		2.0[b]	2.0[b]		
Cα	2.3	2.0[b]	2.0[c]	2.4	2.7	2.0[c]	2.0[c]	2.2[c]	2.3

[a] For method of C-value determination, see Table 2, footnote *a*. Transcripts containing Cμ sequences have been detected in 22D6 (3). A subclone of 22D6 produces $D_{sp}J_H$ transcripts (38). A shortened μ transcript (Dμ) is detected in 300-19P (38, 39). In RLδ11, a transcript containing Cμ sequences was also detected (3). Cκ is expressed in a subclone of 300-19 P (39).

[b] Transcribed gene.

[c] Located within a multigene cluster containing a transcribed gene in this cell line or a subclone.

the S phase. A typical example is shown for AFP, in which we demonstrate that less than one transcript per cell could be detected (Fig. 1).

Genes that replicated during the first third of the S phase in cells in which transcripts of these genes were not detected, but replicated earlier in cells in which they are expressed. Several immunoglobulin genes, including Cα, Cγ2b, Cγ1, Cμ, J region, and Cκ, replicated during the first third to first half of S phase in all cell lines analyzed, irrespective of their transcriptional activity. In those cell lines in which we detected the expression of these genes, they are among the earliest replicating genes we measured, and we calculated the DNA content of the nucleus at the time they replicate to be close to a C value of 2.0. We caution that, since the determination of absolute C values is subject to error, relative comparisons of replication times of DNA sequences within a particular cell line are much more reliable than comparisons between two different cell lines. Thus, from these calculations, with this reservation, in the MPC11 cell line, the gene for Cγ2b which is expressed in this B-cell plasmacytoma appears to replicate earlier in S phase than in any of the nonlymphoid murine cell lines in which transcripts of this gene were not detected. Similarly, in the B-cell plasmacytomas (MPC11 and S107) which express Cκ and in the pre-B-cell line (300-19) which can express Cκ upon further subcloning (39), the Cκ gene appeared to be earlier replicating than in other murine cell lines, such as MEL, in which it is not expressed (Table 3). In the MEL cell line, we found no detectable steady-state levels of Cγ2b or Cκ transcripts. We estimated that we could have detected less than one copy of Cγ2b or Cκ mRNA per cell (Fig. 2) in the MEL cell line. This was demonstrated by reconstruction experiments with RNA from MPC11 cells which have been reported to have about 3×10^4 to 4×10^4 molecules of Cγ2b and Cκ per cell (44).

We also prepared nascent RNA from isolated nuclei of MEL cells and Hepa 1.6 cells to show that there were no detectable rapidly turning over Cγ2b or Cκ transcripts in cell lines in which these genes replicated during the first third of S phase. Purified plasmid DNAs containing either Cγ2b or Cκ sequences were applied to nitrocellulose filters in 1-cm slots. Transcription was carried out in nuclei isolated from MEL, Hepa 1.6, MPC11, and S107 cells, and RNA was

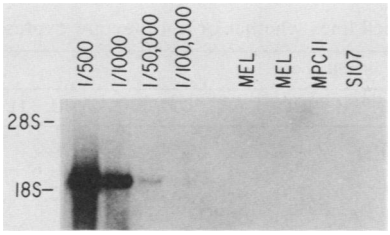

FIG. 1. Limit of detection of steady-state transcripts. Steady-state levels of AFP transcripts were not observed under conditions (autoradiography for about 60 days) in which as few as one transcript per cell could be detected. Total RNA from fetal liver cells was diluted serially as indicated in total RNA from MEL cells. After electrophoresis in formaldehyde-agarose gels, transcripts were not detected in RNA prepared from MEL, S107, and MPC11 cells.

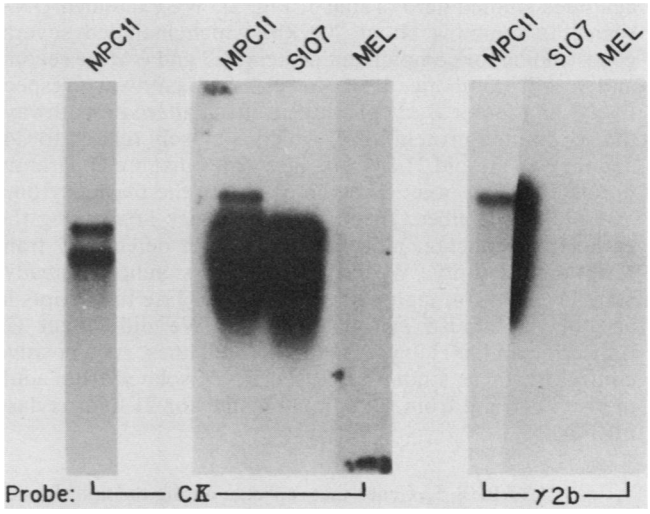

FIG. 2. Absence of poly(A)$^+$ transcripts. Steady-state levels of transcripts for the Cκ and Cγ2b genes are not detected in the MEL cell line in which these genes replicate during the first third of the S phase. Poly(A)$^+$ RNA from MEL cells and control poly(A)$^+$ RNA from the MPC11 and S107 plasmacytoma cell lines were fractionated in formaldehyde-agarose gels, transferred to nitrocellulose paper, and hybridized to insert DNA from pECκ and Cγ2b(11)[7] cDNA probes. As a control, RNA from the MPC11 and S107 plasmacytoma cell lines was present in separate lanes. To have sufficient sensitivity to detect the presence of less than 10 transcripts per cell, the autoradiographs were exposed for about 60 days, with the control RNA lanes from the plasmacytoma cell lines shielded from the X-ray film by a strip of Lucite. The lane at the left is from a separate 1-day exposure autoradiograph showing the normal and truncated kappa mRNA transcripts present in MPC11 cells.

TABLE 4. In vitro nuclear transcription of early and late replicating genes[a]

Gene	Amt of [^{32}P]RNA bound in each cell line[b]			
	MEL	MPC11	S107	Hepa 1.6
Albumin	1.7			250
α_1-antitrypsin	0.8			0.2
Cκ	1.7	189	168	0.2
Cγ2b	0.7	135		

[a] Cloned genomic sequences or cDNA sequences in pBR322 were immobilized on nitrocellulose filters and hybridized to ^{32}P-labeled RNA synthesized in isolated nuclei. The filters were washed and analyzed by autoradiography.
[b] Numbers are optical densities which reflect relative transcription rates of the genes analyzed in a particular cell line. Optical densities (corrected for pBR322 background) of the resulting signals on X-ray films were obtained by analysis with a Quantimet 920 image analyzer.

hybridized to the filters containing DNA from the Cγ2b and Cκ plasmids. Compared with the plasmacytoma cell nuclei, the level of hybridization of the ^{32}P-labeled RNA from the MEL or Hepa 1.6 nuclei was barely detectable (slightly above the background level for a control pBR322 plasmid DNA) (Table 4). As a positive control, we detected significant transcriptional activity of the Cκ genes in MPC11 and S107 cells. The Cγ2b genes are transcriptionally active in MPC11 cells but not in MEL and Hepa 1.6 cells. Both Cκ and Cγ2b represent a class of genes which replicate during the first third of S phase even when they are not transcribed at detectable levels.

Genes that replicated during the first third of S phase whether or not they were expressed. DNA segments from the S region of the murine major histocompatibility complex (*H-2*) replicated during the first third of S phase in all of the cell lines studied here (Table 5; Fig. 3). We examined DNA segments spanning about 200 kb, which included several genes coding for complement proteins C2 and C4 (the second and fourth components of the classical pathway, respectively) and factor B, a constituent of the alternate pathway; the sex-limited protein (Slp), which is closely related to C4; and the two steroid 21-hydroxylase genes that are apparently unrelated to the other S-region genes. In the plasmacytoma and pre-B cell lines, these S-region genes are among the earliest to replicate, although we did not detect any transcriptional activity. We did not detect significant steady-state levels of C4, factor B, or 21-hydroxylase transcripts in any of the mouse cell lines tested. We did detect C2 transcripts in the Hepa 1.6 and L60T cell lines. As a positive control for these studies, we used RNA isolated from adult mouse liver and from rat adrenal gland (for 21-hydroxylase mRNA).

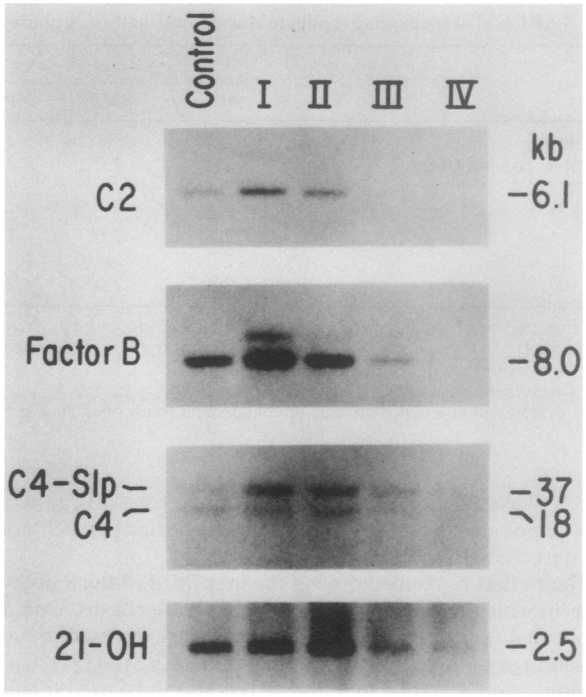

FIG. 3. Replication of the sequences of the murine S region of the major histocompatibility complex in MEL cells. Diazobenzyloxymethyl transfers were prepared from BU-DNA that replicated during four distinct intervals (I to IV) of S phase and hybridized sequentially to the probes indicated. The control is DNA from exponential MEL cells. The results shown for factor B and 21-hydroxylase are from overexposed films chosen for reproduction. All calculations were made from films that were on the linear exposure range.

Genes that replicated during the second half of S phase and were not expressed. The gene coding for α_1-antitrypsin replicated during the middle or second half of S phase in all cell lines examined (Table 6). We did not detect steady-state levels of α_1-antitrypsin transcripts in any of the cell lines examined. We did detect a high level of transcripts in adult liver. We were not able to detect the synthesis of α_1-antitrypsin transcripts in isolated nuclei from two different cell lines analyzed (Table 4). The MUPs also replicated in the later half of S phase in all cell lines examined (Table 6). Transcripts of this gene were not detected in any of the cell lines examined but were detected in male adult liver, in which a high level of expression was observed.

Housekeeping genes can replicate during any interval of S

TABLE 5. Genes that replicate during the first third of S phase in most cell lines whether or not they are expressed

Gene	C value[a] in cell line:								
	MEL	S107	MPC11	L60T	3T3	22D6	300-19P	RLδ11	Hepa 1.6
Factor B	2.2	2.0	2.0	2.2	2.6	2.1	2.0	2.5	2.2
21-Hydroxylase	2.5	2.1	2.1	2.4		2.0		2.6	2.3
C2	2.2	2.0	2.0	2.0[b]	2.5	2.0	2.0	2.0	2.1[b]
C4	2.3	2.0	2.0	2.2					
C4 Slp	2.4	2.0	2.0	2.4					
Thy-1	2.3		2.0	2.5	2.4	2.1		2.0[b]	2.4
Tyrosine amino-transferase									
3′	2.6	2.4	2.3	2.9	3.0	2.5		2.2	2.3
5′	2.4	2.3	2.3			2.3			

[a] Values are DNA contents of the cell nucleus at the time of gene replication. For method of C-value determination, see Table 2, footnote *a*.
[b] Transcribed gene.

TABLE 6. Nontranscribed genes that replicate during the second half of S phase in most cell lines

Gene	C value[a] in cell line:								
	MEL	S107	MPC11	L60T	3T3	22D6	300-19P	RLδ11	Hepa 1.6
α_1-Antitrypsin	2.9	3.9	3.6	3.2			3.0	3.3	2.8
MUP	3.1	3.7	3.6		3.4	3.6	3.6	3.5	3.5
Amylase									
5′ Amy-1	3.5	3.7	4.0	4.0				3.6	
5′ Amy-2.1	3.4	3.8						3.4	

[a] Values are DNA contents of the cell nucleus at the time of replication. For method of C-value determination, see Table 2, footnote *a*.

phase. We have measured the temporal replication of the genes coding for rRNA, H2A histone, and DHFR. These studies were carried out in eight of the mouse cell lines described above. The temporal replication of the rRNA genes was determined (in MEL, S107, and MPC11 cell lines) to take place throughout the S phase but predominantly during the first half (data not shown). This is consistent with multiple copies on different chromosomes replicating at different times during S phase. The genes coding for H2A histone and DHFR replicated at different times during S phase depending on the cell line examined (Table 7). In the mouse L60T cell line, the DHFR and one of the H2A genes replicated during the second half of S phase. The H2A histone gene that replicates predominantly during the second half of the S phase is transcribed in this cell line (W. Marzluff, personal communication). Thus, unlike the tissue-specific genes, some transcribed housekeeping genes can replicate late during S phase.

DISCUSSION

We have carried out a quantitative study of the temporal replication of genes and flanking sequences, using centrifugal elutriation techniques which avoid potentially toxic exogenous agents. The temporal replication of tissue-specific genes was compared in cell lines in which there are significant differences in the level of their transcriptional activity. Nine murine cell lines examined exhibited differentiated functions characteristic of liver, lymphoid (pre-B, B, and T cells), erythroid, and fibroblast tissues from which they were derived. Based on the results of this study, we determined the rules for the replication during S phase of these tissue-specific genes and of several housekeeping genes. For tissue-specific genes, the relationship between replication timing and transcription falls into several categories. (i) When these genes are actively transcribed, they usually replicate during the first quarter of the S phase. (ii) Some of these genes replicate late in the S phase in cell lines in which they are not expressed. (iii) Other genes replicate during the first half of S phase even when we do not detect transcriptional activity; however, they replicate even earlier in cell lines in which they are expressed. (iv) Some genes replicate early regardless of their expression. (v) Genes that are not transcribed can replicate during any interval of S phase and need not replicate late during S phase. (vi) Genes can replicate late during S phase in a cell line in which they are not transcribed even if this cell line is derived from a tissue in which these genes are normally transcribed. Two examples are the liver-specific genes MUP and α_1-antitrypsin, which are not expressed and are late replicating in all cell lines examined, including the liver-derived cell line Hepa 1.6. (vii) Many of these sequences are organized in multigene families spanning about 15 to 250 kb. Clusters of 250 kb or less define a temporal compartment comprising approximately one-quarter of S phase. (viii) We have not yet observed an early replicating gene in a late replicating cluster or a late replicating gene in an early replicating cluster. (ix) Housekeeping genes can replicate during any interval of S phase. Two housekeeping genes which appear to be expressed in the L60T cell line replicated during the second half of S phase in this cell line. Some of these rules are shown diagramatically in Fig. 4. While these rules apply to the genes we have studied here, all tissue-specific genes may not follow this pattern. One tissue-specific gene that does not appear to replicate exclusively during the first half of S phase is amylase in the Hepa 1.6 cell line in which some expression is detected (data not shown).

We conclude that the temporal order of replication of tissue-specific genes depends on the spatial organization of these genes and their flanking sequences. Earlier studies that used fiber autoradiography indicated that at least some clusters of replicons initiate at approximately the same time during the S phase. It is not known, however, whether the replicons observed may have represented a specific class, for example, replicons for repeated-sequence DNA. The observation that segments within the gene clusters we have examined replicate at similar times during S phase is striking since many of these segments are far enough apart (≥50 to 100 kb) to be on different replicons. The results of the present study provide the first molecular evidence that the coordinate timing of replication of contiguous sequences within a multigene family is a general property of the mammalian genome. In addition, our studies also suggest that the interval of S phase during which these chromosomal

TABLE 7. Housekeeping genes

Gene	C value[a] in cell line:							
	MEL	S107	MPC11	L60T	22D6	300-19P	RLδ11	Hepa 1.6
DHFR		2.5	2.8	3.2	2.0	2.0	2.8	2.9
H2A	2.5	2.6	2.3	3.3				
Metallothionein	2.4	2.0	2.1	2.2	2.1		2.5	2.4

[a] Values are DNA contents of the cell nucleus at the time of gene replication. For method of C-value determination, see Table 2, footnote *a*.

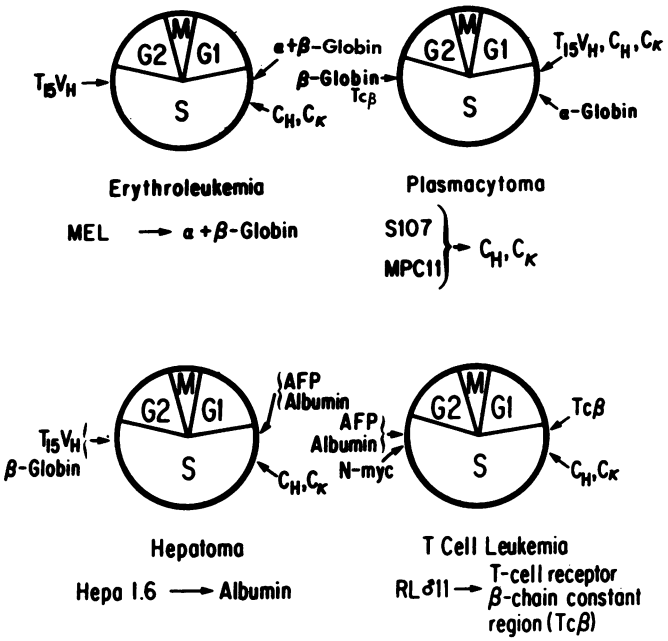

FIG. 4. Tissue-specific genes replicate earlier in cell lines in which they are transcribed than in those in which transcriptional activity was not detected (for abbreviations, see Table 1, footnote *a*). Schematic diagrams show the cell cycle for four cell lines. An arrow indicates the approximate time of replication during S phase for each gene. The transcribed genes in each cell line are presented in Table 1.

regions replicate is related to the transcriptional activity within chromosomal domains.

There could be several advantages to genes for specialized functions being located in regions of chromatin that differ in their accessibility to transcription factors and possibly to replication enzymes. Since the mammalian genome is several orders of magnitude more complex than that of *Escherichia coli*, the specificity of recognition proteins required for the regulation of gene expression need not be correspondingly increased if auxiliary mechanisms can be used to simplify the recognition process. Compartmentalization into active and inactive chromatin could be used as a coarse regulatory control mechanism for certain genes encoding specialized functions. The simultaneous modulation of several genes by this mechanism could be more effective when these genes are organized into domains of multigene families.

One mechanism by which even a small change in the temporal order of DNA replication could play a role in gene expression is through the selective binding of transcription factors to genes. For those transcription factors present in the cell in limiting amounts, the first of a set of binding sites to replicate would sequester all of the available transcription factor (8). The order of replication of certain genes could thus provide an additional selective advantage for the regulation of their transcriptional activity. A model has been presented (23, 55) in which the somatic 5S rRNA genes replicate earlier in S phase than the oocyte genes and thus derive a competitive advantage for binding the transcription factor TFIIIA when it is present in limiting amounts in somatic cells. Evidence consistent with this model has been obtained for the 5S RNA genes in *Xenopus laevis* (20, 25, 26). These authors have shown that the expressed somatic 5S genes replicate earlier than the nonexpressed oocyte 5S genes in cultured somatic cells.

Previous studies showed that many transcribed genes for tissue-specific functions replicate early during S phase. The observations reported here allow us to reach several new conclusions concerning more subtle rules for temporal replication. Our studies provide a resolution sufficient to detect relatively small differences in the temporal replication of single-copy tissue-specific genes and to determine the value that most closely corresponds to the nuclear DNA content at the time that a gene replicates in a particular cell line. For some genes we observed large differences in their time of replication (i.e., early versus late S phase) in cell lines in which they are expressed compared with those lines in which they are silent. For other genes such as those in the IgC_H and immunoglobulin kappa constant-region ($IgC\kappa$) loci, we observed small differences (within the first third or the first half of S phase). We can reliably measure small differences when we compare the temporal order of replication of genes in a particular cell line. We are more cautious in our interpretation when we compare the temporal order of replication of genes in different cell lines, since we estimate that the absolute C values can be subject to errors of 10 to 20%. Relatively small differences in the replication time of the genes mentioned above were observed in cell lines in which these genes were expressed or silent. For example, the $C\kappa$ gene replicates near the beginning of S phase in three cell lines where it is expressed and replicates later but still before the middle of S phase in four other cell lines which do not express $C\kappa$. For several C_H genes, the same general phenomenon was observed. In four B-cell-derived lines, the heavy-chain constant-region genes replicate near the beginning of S phase, while in five other non-B-cell lines these genes replicate later but still during the first half of S phase. Although we exercise caution in comparing two or three cell lines, the same pattern of earlier replication in as many as nine cell lines strongly suggests that, when differences in the temporal order of replication are observed for these immunoglobulin genes, they replicate earlier in cell lines in which they are transcribed than in those in which transcriptional activity is not detected.

All of the immunoglobulin genes examined here replicated at the beginning of the S phase in pre-B and B-cell lines. In each of these cell lines, transcripts have been detected from at least one of the genes in each immunoglobulin multigene family. This includes the $V\kappa 19$ light-chain family in MPC11, the T15 heavy-chain variable-region family in S107, and the heavy-chain constant-region family in both cell lines. In these pre-B and B-cell lines, all of the members of these multigene families we have analyzed replicate at the beginning of S phase, regardless of their transcriptional activity. All of the IgC_H genes are present in the two pre-B cell lines we have studied, while transcription has been reported only for the $C\mu$ gene. We have observed that several constant-region genes also replicate just as early during S phase as the transcribed $C\mu$ gene in these pre-B cell lines. These results suggest that early replication of a transcribed immunoglobulin gene is associated with the early replication of surrounding DNA sequences. We speculate that this early replicating region represents a chromatin domain in which all of the DNA sequences replicate near the beginning of S phase. Studies are in progress to determine whether this observation also applies to families of genes other than those coding for immunoglobulin molecules. Further studies will be required to determine whether transcripts of particular genes play a role in the initiation of DNA replication early in S

phase. Several early replicating genes for which we do not detect transcriptional activity are located in clusters in which all of the members studied are very early replicating. We speculate that these genes may be located near an expressed gene or in an open region of chromatin.

One way that the cell could control the timing and site of replication would be to use sequence-specific origins for DNA replication. Different origins may be used depending on the chromatin structure, which in turn will depend on the state of differentiation of the cell. There are already many precedents for the use of alternative origins in a particular stretch of DNA. For example, when the primary origin of replication is deleted, an alternative origin is used in the replication of T7 bacteriophage DNA (40). The number of origins of replication has been shown to change during development in *D. melanogaster* (6). Evidence has also been presented to show that the mammalian genome contains many potential origins of replication (50) of which only a subset may be used in a given cell type. The results of an earlier study (9) suggest that origins which are active in the IgC_H cluster in B cells may be silent in non-B cells.

Evidence has been presented that the three-dimensional configuration of the genome differs in different cell types (summarized in reference 5). In some instances nuclear organization appears to reflect the transcriptional activity of blocks of chromatin (28). We have shown that a chromosomal domain consisting of active tissue-specific genes and flanking nontranscribed sequences can replicate early in S phase. The same domain, when inactive, may replicate later in S phase in a different cell type, perhaps due to a different three-dimensional configuration. We suggest that each differentiated cell type can use different subsets of the many potential origins of replication in the mammalian genome. Use of an upstream origin may be required to initiate transcription of active genes for differentiated functions (33, 47, 49). The choice of which of these replication origins are activated could be determined by the nuclear location and three-dimensional organization of the genome in a particular cell type.

Some functions in the interphase nucleus could be controlled by chromatin condensation and accessibility. One level at which gene expression is regulated by chromatin organization could be the accessibility to a multienzyme complex of the type described by Alberts (1) that carries out DNA replication or a complex that carries out transcription as well as DNA replication as has been suggested by Reddy and Pardee (36). The temporal order of replication of DNA sequences may reflect the accessibility to such a complex and should provide information about their nuclear compartmentalization and topological organization and associations.

ACKNOWLEDGMENTS

These studies were supported by grants to C.L.S. from the Public Health Service National Institutes of Health (NIH) (GM 22332) and the American Cancer Society (CD-312). E.H.B. is a Special Fellow of the Leukemia Society of America. K.S.H. was supported by an NIH Postdoctoral Fellowship (F32 CA07744). V.D. was supported by an NIH Training Grant (5 T32 CA09060). Support was also provided by Cancer Center Core Grant NIH/NCI P30-CA13330.

LITERATURE CITED

1. **Alberts, B. M.** 1984. The DNA enzymology of protein machines. Cold Spring Harbor Symp. Quant. Biol. **49**:1–12.
2. **Alt, F., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore.** 1981. Organization and reorganization of immunoglobulin genes in A-MuLV-transformed cells: rearrangement of heavy but not light chain genes. Cell **27**:381–390.
3. **Alt, F. W., N. Rosenberg, V. Enea, E. Siden, and D. Baltimore.** 1982. Multiple immunoglobulin heavy-chain gene transcripts in Abelson murine leukemia virus-transformed lymphoid cell lines. Mol. Cell. Biol. **2**:386–400.
4. **Balazs, I., E. H. Brown, and C. L. Schildkraut.** 1974. The temporal order of replication of some DNA cistrons. Cold Spring Harbor Symp. Quant. Biol. **38**:239–245.
5. **Blobel, G.** 1985. Gene gating: a hypothesis. Proc. Natl. Acad. Sci. USA **82**:8527–8529.
6. **Blumenthal, A. B., H. J. Kriegstein, and D. Hogness.** 1974. The units of DNA replication in *Drosophila melanogaster* chromosomes. Cold Spring Harbor Symp. Quant. Biol. **38**:205–223.
7. **Braunstein, J. D., D. Schulze, T. DelGiudice, A. Furst, and C. L. Schildkraut.** 1982. The temporal order of replication of murine immunoglobulin heavy chain constant region sequences corresponds to their linear order in the genome. Nucleic Acids Res. **10**:6887–6901.
8. **Brown, D. D.** 1984. The role of stable complexes that repress and activate eucaryotic genes. Cell **37**:359–365.
9. **Brown, E. H., M. A. Iqbal, S. Stuart, K. S. Hatton, and C. L. Schildkraut.** 1987. Rate of replication of the murine immunoglobulin heavy-chain locus: evidence that the region is part of a single replicon. Mol. Cell. Biol. **7**:450–457.
10. **Calza, R. E., L. Eckhardt, T. DelGiudice, and C. L. Schildkraut.** 1984. Changes in gene position are accompanied by a change in time of replication. Cell **36**:689–696.
11. **Chaplin, D. D., L. J. Galbraith, J. G. Seidman, P. C. White, and K. L. Parker.** 1986. Nucleotide sequence analysis of murine 21-hydroxylase genes: mutations affecting gene expression. Proc. Natl. Acad. Sci. USA **83**:9601–9605.
12. **Coleclough, C., R. P. Perry, K. Karjalainen, and M. Weigert.** 1981. Aberrant rearrangements contribute significantly to the allelic exclusion of immunoglobulin gene expression. Nature (London) **290**:372–378.
13. **Crews, S., J. Griffin, H. Huang, K. Calame, and L. Hood.** 1981. A single V_H gene segment encodes the immune response to phosphorycholine: somatic mutation is correlated with the class of the antibody. Cell **25**:59–66.
14. **Darlington, G. J., H. P. Bernhard, R. A. Miller, and F. H. Ruddle.** 1980. Expression of liver phenotypes in cultured mouse hepatoma cells. J. Natl. Cancer Inst. **64**:809–819.
15. **Derman, E., K. Krauter, L. Walling, C. Weinberger, M. Ray, and J. E. Darnell.** 1981. Transcriptional control in the production of liver specific mRNAs. Cell **23**:731–739.
16. **Durnam, D. M., F. Perrin, F. Gannon, and R. D. Palmiter.** 1980. Isolation and characterization of the mouse metallothionein-I gene. Proc. Natl. Acad. Sci. USA **77**:6511–6515.
17. **Edenberg, H. J., and J. A. Huberman.** 1975. Eukaryotic chromosome replication. Annu. Rev. Genet. **9**:245–284.
18. **Furst, A., E. H. Brown, J. D. Braunstein, and C. L. Schildkraut.** 1981. α-Globin sequences are located in a region of early-replicating DNA in murine erythroleukemia cells. Proc. Natl. Acad. Sci. USA **78**:1023–1027.
19. **Gasser, C. S., C. C. Simonsen, J. W. Schilling, and R. T. Schimke.** 1982. Expression of abbreviated mouse dihydrofolate reductase genes in cultured hamster cells. Proc. Natl. Acad. Sci. USA **79**:6522–6526.
20. **Gilbert, D. M.** 1986. Temporal order of replication of *Xenopus laevis* 5S ribosomal RNA genes in somatic cells. Proc. Natl. Acad. Sci. USA **83**:2924–2928.
21. **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.
22. **Gorin, M. B., and S. Tilghman.** 1980. Structure of the α-fetoprotein gene in the mouse. Proc. Natl. Acad. Sci. USA **77**: 1351–1355.
23. **Gottesfeld, J., and L. S. Bloomer.** 1982. Assembly of transcriptionally active 5S RNA gene chromatin *in vitro*. Cell **28**:781–791.
24. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (London) **311**:433–438.
25. **Guinta, D. R., and L. J. Korn.** 1986. Differential order of replication of *Xenopus laevis* 5S RNA genes. Mol. Cell. Biol.

6:2536–2542.
26. **Guinta, D. R., J. Yun Tso, S. Narayanswami, B. A. 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.
27. **Holmquist, G. P.** 1987. Role of replication time in the control of tissue-specific gene expression. Am. J. Hum. Genet. **40**:151–173.
28. **Hutchison, N., and H. Weintraub.** 1985. Localization of DNAase I-sensitive sequences to specific regions of interphase nuclei. Cell **43**:471–482.
29. **Ingram, R. S., R. W. Scott, and S. Tilghman.** 1981. α-Fetoprotein and albumin genes are in tandem in the mouse genome. Proc. Natl. Acad. Sci. USA **78**:4694–4698.
30. **Iqbal, M. A., J. Chinsky, V. Didamo, and C. L. Schildkraut.** 1987. Replication of proto-oncogenes in mammalian cell lines. Nucleic Acids Res. **15**:87–103.
31. **Kelley, D. E., C. Coleclough, and R. P. Perry.** 1982. Functional significance and evolutionary development of the 5′-terminal regions of immunoglobulin variable-region genes. Cell **29**:681–689.
32. **Lai, E., R. K. Barth, and L. Hood.** 1987. Genomic organization of the mouse T-cell receptor β-chain gene family. Proc. Natl. Acad. Sci. USA **84**:3846–3850.
33. **Laskey, R. A., and R. M. Harland.** 1981. Replication origins in the eukaryotic chromosome. Cell **24**:283–284.
34. **Malissen, M., K. Minard, S. Mjoisness, M. Kroneberg, J. Goverman, T. Hunkapiller, M. B. Prystowsky, Y. Yoshikai, F. Fitch, T. W. Mak, and L. Hood.** 1984. Mouse T cell antigen receptor: structure and organization of constant and joining gene segments encoding the β polypeptide. Cell **37**:1101–1110.
35. **McKnight, G. S., and R. D. Palmiter.** 1979. Transcriptional regulation of the ovalbumin and conalbumin genes by steroid hormones in chick oviduct. J. Biol. Chem. **256**:2621–2624.
36. **Reddy, P., and A. B. Pardee.** 1980. Multienzyme complex for metabolic channeling in mammalian DNA replication. Proc. Natl. Acad. Sci. USA **77**:3312–3316.
37. **Remington, J. A., and R. A. Flickinger.** 1971. The time of DNA replication in relation to RNA synthesis in frog embryos. J. Cell. Physiol. **77**:411–422.
38. **Reth, M. G., and F. W. Alt.** 1984. Novel immunoglobulin heavy chains are produced from DJ_H gene segment rearrangements in lymphoid cells. Nature (London) **312**:418–423.
39. **Reth, M. G., P. Ammirati, S. Jackson, and F. W. Alt.** 1985. Regulated progression of a cultured pre-B-cell line to the B-cell stage. Nature (London) **317**:353–355.
40. **Richardson, C. C.** 1983. Bacteriophage T7: minimal requirements for the replication of a duplex DNA molecule. Cell **33**: 315–317.
41. **Robins, D. M., M. Malissen, L. Hood, A. Ferriera, D. Walthall, and M. Mitchell.** 1986. Multiple C4/Slp genes distinguished by expression after transfection. Mol. Cell. Biol. **6**:134–141.
42. **Sackstein, R., and H. R. Colten.** 1984. Molecular regulation of MHC Class III (C4 and factor B) gene expression in mouse peritoneal macrophages. J. Immunol. **133**:1618–1625.
43. **Sackstein, R., H. R. Colten, and D. E. Woods.** 1983. Phylogenetic conservation of a Class III major histocompatibility complex antigen, factor B. J. Biol. Chem. **258**:14693–14697.
44. **Schibler, U., K. B. Marcu, and R. P. Perry.** 1978. The synthesis and processing of the messenger RNAs specifying heavy and light chain immunoglobulins in MPC-11 cells. Cell **15**:1495–1509.
45. **Schibler, U., M. Tosi, A. C. Pittet, L. Fabiani, and P. K. Wellauer.** 1980. Tissue-specific expression of mouse α-amylase genes. J. Mol. Biol. **142**:93–116.
46. **Schmid, W., G. Muller, G. Schutz, and S. Glueckson-Waelsch.** 1985. Deletions near the albino locus on chromosome 7 of the mouse affect the level of tyrosine amino-transferase mRNA. Proc. Natl. Acad. Sci. USA **82**:2866–2869.
47. **Seidman, M. M., A. J. Levine, and H. Weintraub.** 1979. The asymmetric segregation of parental nucleosomes during chromosome replication. Cell **18**:439–449.
48. **Sittman, D. B., R. A. Graves, and W. F. Marzluff.** 1983. Structure of a cluster of mouse histone genes. Nucleic Acids Res. **11**:6679–6697.
49. **Smithies, O.** 1982. The control of globin and other eukaryotic genes. J. Cell Physiol. Suppl. **1**:137–143.
50. **Taylor, H. J.** 1977. Increase in DNA replication sites in cells held at the beginning of S phase. Chromosoma **62**:291–300.
51. **Taylor, H. J.** 1984. Origins of replication and gene regulation. Mol. Cell. Biochem. **61**:99–109.
52. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.
53. **Weaver, S., M. B. Comer, C. L. Jahn, C. A. Hutchison III, and M. H. Edgell.** 1981. The adult β-globin genes of the "Single" type mouse C57BL. Cell **24**:403–411.
54. **Wiebauer, K., D. L. Gumucio, J. M. Jones, R. M. Caldwell, H. T. Hartle, and M. H. Meisler.** 1985. A 78-kilobase region of mouse chromosome 3 contains salivary and pancreatic amylase genes and a pseudogene. Proc. Natl. Acad. Sci. USA **82**:5446–5449.
55. **Wormington, W. M., and D. D. Brown.** 1983. Onset of 5S RNA gene regulation during *Xenopus* embryogenesis. Dev. Biol. **99**: 248–257.
56. **Yancopoulos, G. D., and F. W. Alt.** 1985. Developmentally controlled and tissue-specific expression of unrearranged V_H gene segments. Cell **40**:271–281.