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66. Earlier proposals of a related nature were made by R. J. Goldacre, *Exp. Cell Res. Suppl.* **8**, 1 (1961); B. M. Shaffer, *Exp. Cell Res.* **37**, 12 (1965); *J. Theor. Biol.* **8**, 27 (1965).
67. The effects of drugs that affect the cytoskeleton are interesting: cytochalasin B inhibits the ruffling membrane of fibroblasts [S. B. Carter, *Nature (London)* **213**, 261 (1967)] and nerve growth cones [K. M. Yamada, B. S. Spooner, N. K. Wessells, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 1206 (1970)], whereas colchicine has no obvious effect [B. S. Spooner, K. Yamada, N. K. Wessells, *J. Cell Biol.* **49**, 595 (1971)]. Colchicine treatment does, however, lead to a loss of directed movement in fibroblasts, although the locomotory system still functions [J. M. Vasiliev *et al.*, *J. Embryol. Exp. Morphol.* **24**, 625 (1970)]. The effects of these drugs on the capping process agree well with their effects on ruffling, further suggesting that the two processes are related.
68. M. Abercrombie and J. E. M. Heaysman, *Exp. Cell Res.* **6**, 293 (1954).
69. M. Abercrombie and E. J. Ambrose, *ibid.* **15**, 332 (1958).
70. The transit time for the low-density lipoprotein (LDL) receptor on stationary human fibroblasts is very short—a fraction of a minute. I expect that the transit time for this receptor on motile cells would be greater, as would be the proportion of these receptors inside the cell. If this is so, it would tie cell motility to a biochemical measurement.
71. I thank my colleagues for helpful suggestions, especially G. Mitchison, F. H. C. Crick, R. D. Kornberg, B. Pearse, and H. C. Berg.

Replication Timing of Genes and Middle Repetitive Sequences

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Summary. DNA replication in mammals is temporally bimodal. "Housekeeping" genes, which are active in all cells, replicate during the first half of the S phase of cell growth. Tissue-specific genes replicate early in those cells in which they are potentially expressed, and they usually replicate late in tissues in which they are not expressed. Replication during the first half of the S phase is, therefore, a necessary but not sufficient condition for gene transcription. A change in the replication timing of a tissue-specific gene appears to reflect the commitment of that gene to transcriptional competence or to quiescence during ontogeny. Most families of middle repetitive sequences replicate either early or late. These data are consistent with a model in which two functionally distinct genomes coexist in the nucleus.

The biphasic nature of DNA replication in eukaryotic cells (*1*) allows us to speak of two classes of replication units or "replicons" (*2*), early and late (Fig. 1A). The replicons in each temporal class are found in clusters of about 20 (*2*), and these can be resolved into a longitudinal pattern of early and late replication bands by substitution with bromodeoxyuridine (BrdU) followed by fluorescence microscopy (Fig. 1B). The "replication banding" pattern coincides almost exactly with the trypsin-Giemsa banding pattern routinely used by mammalian cytogeneticists (Fig. 1B) (*3–6*). The AT-specific fluorochromes (A, adenine; T, thymidine), such as quinacrine or Hoechst 33258, also give the same euchromatic banding pattern because the early-replicating DNA is GC-rich (G, guanine; C, cytosine) and the late-replicating DNA is AT-rich (*4*).

Very late replication is traditionally equated with genetic inertness. Constitutive heterochromatin, such as blocks of satellite DNA, and facultative heterochromatin, such as the inactive X chromosome in mammalian females, replicate late and are genetically inert (*7*). Mueller *et al.* (*8*) first showed distinct early- and late-replicating DNA fractions in euchromatin (*9*) and subsequently postulated that only the early euchromatin had active genes (*10*). Many geneticists believe that all genes are confined to the early-replicating, Giemsa-light bands, and that the later-replicating, Giemsa-dark bands, although euchromatic in the strict sense, are devoid of genes and rich in middle repetitive sequences (*11, 12*).

A number of investigations have determined the time of replication of specific genes, all of which were early (included in Table 1). Others have shown that various mutagens are most effective when introduced during the first half of S phase (*10, 13*). Stambrook and Flickinger (*14*) presented cytological evidence that the time of replication of particular DNA sequences might change during development, and suggested that ". . . RNA molecules synthesized by one cell type and not another would be coded for by genes which would replicate early in the S period in the first case, and later in the S period in the second case" (*14*, p. 101; see also *15*). In spite of these concepts, critical data concerning the functional importance of early- as compared to late-replicating euchromatin are lacking. We have described a method for fractionating early- and late-replicating DNA's from V79-8 hamster cells and characterized these DNA fractions with respect to DNA reassociation kinetics, complementarity to total polyadenylated RNA, and chromatin sensitivity to deoxyribonuclease I (*4*). Early- and late-replicating DNA were similar in these respects. In this article, we describe specifically the occurrence of protein-coding and middle repetitive (MR) DNA sequences in early- as compared to late-replicating DNA in V79-8 and HeLa cells. We present evidence that genes which are potentially active in a given cell type replicate early in that cell type, and that genes which are permanently inactive replicate late. We suggest that the portion of the mammalian genome that contains tissue-spe-

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cific genes (*16*) is the Giemsa-dark-staining euchromatin. This portion has diverged functionally from the remainder of the genome, thereby resulting in the "coarse" silencing of genes, 97 percent of which are transcriptionally inactive in any one cell type.

Transcriptionally Competent Genes

Replicate Early

HeLa and V79-8 cells were synchronized (*17*) and grown in 5-bromodeoxyuridine–substituted medium either during the first half (S_E; Fig. 1A) or during the second half (S_L) of S phase. DNA substituted with BrdU was separated from the unsubstituted DNA by buoyant density on a cesium chloride (CsCl) gradient (Fig. 1C) (*4*).

Early- and late-replicating DNA was digested to completion with restriction endonucleases and fractionated on 0.8 percent agarose gels. Ethidium bromide–stained gels were photographed and evaluated to ensure correct quantitation and complete digestion of DNA. The DNA was denatured and transferred to nitrocellulose (*18*). Plasmids or inserts from well-characterized complementary DNA (cDNA) or genomic clones were labeled to high specific activity (about 10^8 counts per minute per microgram of DNA) with [α-^{32}P]dCTP (deoxycytidine triphosphate) by nick translation and hybridized to DNA blots (*19*).

When HeLa and V79 DNA were hybridized with the cDNA of CAD (carbamyl phosphate synthetase, aspartate transcarbamylase, and dihydroorotase) (*20*), the early-replicating DNA was preferentially hybridized (Fig. 2). Hybridization was not completely absent from the late-replicating DNA lanes. This may reflect (i) genuine cell-to-cell variation in the time of replication of the CAD gene, (ii) imperfect separation of the half-substituted from the unsubstituted DNA strands on CsCl gradients, or (iii) incomplete cell synchronization. We believe that incomplete cell synchronization is the most likely cause of this "spillover," and conclude that the CAD gene is an early-replicating gene in HeLa and V79 cells.

After the CAD probe was removed (*19*), the same DNA blot was allowed to hybridize with a human cDNA clone of phenylalanine hydroxylase (PH) (*21*). Hybridization was primarily to the late-replicating HeLa DNA, showing that PH replicates late (Fig. 3). This was the first indication that coding sequences could replicate late in S phase. Use of the same DNA blot with both the CAD and PH probes demonstrates that neither biased loading of the gel nor other technical artifacts are responsible for the differential hybridization observed.

The argininosuccinate synthetase (AS) probe (*22*) provides an example of a

Table 1. Time of replication of specific genes.

DNA probe*	Cell line†	Replication time‡§	Remarks‖
Adenine phosphoribosyl transferase, hamster (*59*)	HeLa	—	Constitutive
	V79	E	
Argininosucinnate synthetase, human (*22*)	HeLa	E	Constitutive L, E, E+L pseudogenes (*23*)
	V79	E¶	
CAD, hamster (*20*)	HeLa	E	Constitutive
	V79	E	
Dihydrofolate reductase, mouse	Mouse LB	E(*60*)	Constitutive
	CHO	E(*61*)	
Dihydrofolate reductase, human (*62*)	HeLa	E	
Glucose 6-phosphate dehydrogenase, human	HeLa	E(*63*)	Constitutive
	V79	—(*63*)	
Harvey c-*ras* (oncogene)	HeLa	E(*28*)	Constitutive (*64*)
	V79	E(*28*)	
Hypoxanthine–guanine phosphoribosyl transferase (HPRT), human	HeLa	E(*65*)	Constitutive L, E, E+L pseudogenes (*66*)
HPRT, hamster	V79	E(*4*)	
Metallothionein I, mouse	V79	E(*67*)	Constitutive
β-Tubulin, chicken (*68*)	HeLa	E	Constitutive: all pseudogenes E
	V79	E	
Tyrosine aminotransferase, rat (*69*)	HeLa	E	Constitutive
	V79	E	
Apolipoprotein A-I, human (*70*)	HeLa	E	Intestinal epithelium, macrophage, liver
γ-Interferon, human	HeLa	E+L(*71*)	Lymphoid cells
Immunoglobulin heavy chain constant region, mouse	MEL	E(*29*)	Lymphoid or hematopoietic
Growth hormone, placental lactogen, human (*72*)	HeLa	E	Pituitary (*73*)
	V79	—	
α-Globin, human	HeLa	E(*28*)	Erythroid
α-Globin, mouse	MEL	E(*74*)	
β-Globin, human	HeLa	L(*28*)	Erythroid
β-Globin, mouse	MEL	E(*27*)	
α_1-Antichymotrypsin, human (*75*)	HeLa	L	Liver, absent in HeLa (*76*)
	V79	—	
α_1-Antitrypsin, human (*77*)	HeLa	L	Liver, absent in HeLa (*76*)
	V79	—	
β-Casein, mouse (*78*)	HeLa	—	Mammary gland
	V79	L	
Phenylalanine hydroxylase, human (*21*)	HeLa	L	Liver
	V79	L¶	

*Organism from which the cDNA or genomic clone was prepared follows the name of the gene or the protein it codes. The notes indicate clone used, published description, and supplier if different from originator. †HeLa, human cervical carcinoma, probably epithelial; V79, Chinese hamster lung fibroblast, V79-8; CHO, Chinese hamster ovary fibroblast; MEL, murine erythroleukemia. Mouse lymphoblastoid (LB) cells had amplified dihydrofolate reductase genes. ‡E, early; L, late; E+L indicates equal hybridization to both early- and late-replicating DNA. Dash (—) denotes the absence of hybridization at high stringency. §The data on replication time are from our laboratory except for dihydrofolate reductase (*60, 61*), immunoglobulin gene (*29*), mouse α-globin (*74*), and mouse β-globin (*27*). ‖Presumed or known tissue specificity is indicated. "Constitutive" indicates genes presumed active in all cell types. Time of replication of pseudogenes is mentioned when applicable. ¶Hybridization carried out at low stringency (*19*).

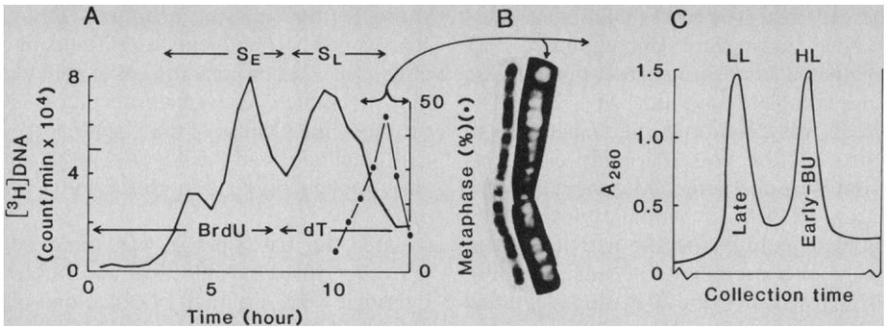

Fig. 1. Purification of early- and late-replicating V79-8 DNA. (A) Refrigerated hamster V79 metaphase cells were transferred to 37°C at time zero and the amount of ^{3}H-labeled thymidine incorporated into DNA (solid line) was measured at intervals during S phase. The two peaks of DNA synthesis correspond to the first (S_E) and second (S_L) halves of S phase. The mitotic index is indicated by the broken line. Another population of metaphase cells was grown in BrdU-substituted medium until the expected mid–S phase pause and released into thymidine. At the time of the expected metaphase burst, chromosome spreads and DNA were prepared. (B) A partial karyotype was prepared from a Hoechst 33258–stained slide photographed in the fluorescence microscope (right). The pattern of fluorescence is equivalent to the trypsin Giemsa-banding pattern (left). (C) DNA was fractionated on a CsCl density gradient. The absorbancy at 260 nm of the fractions was monitored in a flow cell, and heavy (*HL*) and light (*LL*) peak fractions were collected and used in subsequent experiments. [Courtesy of *Cell* (*4*)]

complex hybridization pattern in that some bands in the genomic blot hybridize primarily to late-replicating DNA, some to early-replicating DNA, and some equally to both fractions (Fig. 4). Beaudet *et al.* (*22, 23*) showed that the AS probe hybridizes to at least 19 genomic fragments in Eco RI–digested human genomic DNA. The 3.7-kilobase (kb) fragment is part of the expressed gene for argininosuccinate synthetase (*23, 24*), and replicates early. The remaining bands, which replicate early, late, or without temporal preference, represent processed pseudogenes (*25*).

Replication times of various cloned genes are summarized in Table 1. For most genes studied, all hybridized bands were clearly overrepresented either in the early-replicating fraction or in the late-replicating fraction. α_1-Antitrypsin, α_1-antichymotrypsin, and PH are liver-specific enzymes. We would not expect them to be expressed in HeLa (epithelial) cells. Similarly, the milk protein β-casein should not be expressed in V79 fibroblast cells. All of the late-replicating genes shown in Table 1 are known or likely to be inactive in the tissues in which they were studied (*26*). Genes expressed in all tissues ("housekeeping genes"), for example, CAD and dihydrofolate reductase, were found to be early replicating. The β-globin gene, a tissue-specific gene known to be active in the hematopoietic MEL (murine erythroleukemia) cell line, replicated early in MEL cells (*27*) but late in HeLa cells (*28*). On the basis of these data, we conclude that genes which can be transcribed replicate early.

We believe that early replication is a necessary but not sufficient condition for gene transcription. All expressed genes replicate early (data in Table 1), but a number of unexpressed tissue-specific genes also replicate early. The latter class of genes includes α-globin, apolipoprotein A-I and growth hormone genes in HeLa cells, and immunoglobulin heavy chain constant region genes in MEL cells (*29*). The immunoglobulin genes may be transcriptionally competent in all hematopoietic lineages, that is, its acquisition of transcriptional competence may precede the separation of erythroid and lymphoid cell lineages. Exceptional patterns of early replication in HeLa cells may reflect some of the peculiarities of this cell line. On the basis of some messenger RNA (mRNA) complexity studies, the number of expressed genes in HeLa cells is two to four times greater than the number of expressed genes in mouse fibroblasts or hen oviduct tissue (*30*). HeLa cells do, therefore, exhibit many gene products not characteristic of their tissue origin. A hypotriploid human breast tumor cell line replicates its homologous chromosomes asynchronously (*31*). The hypotriploid HeLa cell line may behave similarly, perhaps accounting for our finding of single-copy sequences in HeLa that seem to replicate both early and late (Table 1). Treisman and Maniatis (*32*) have found that endogenous α-globin transcripts accumulate in HeLa cells after heat shock, while β-globin transcripts could not be detected under the same conditions. Their finding supports our contention that unexpected early replication of HeLa genes reflects their unexpected transcriptional competence.

Five of eight tested housekeeping genes hybridized at high stringency to both hamster and HeLa DNA, while none of six tissue-specific genes tested did so. The implications of these data for sequence conservation in housekeeping as opposed to tissue-specific genes are discussed below.

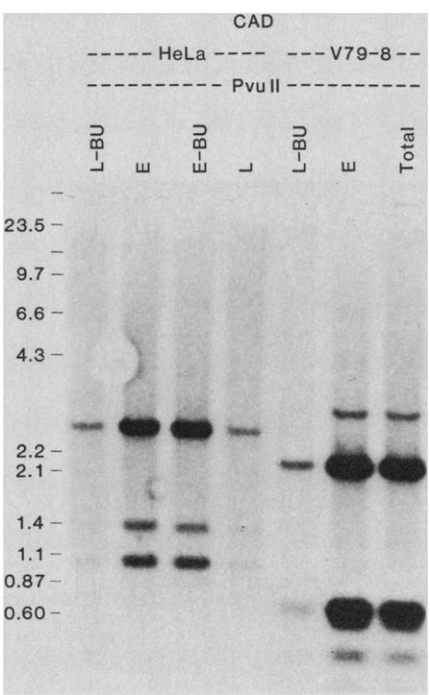

Fig. 2. Replication timing of the CAD gene in HeLa (lanes 1 to 4) and V79-8 hamster (lanes 5 to 7) cells. Early- or late-replicating DNA (5 μg) was digested with Pvu II and placed on an agarose gel for electrophoresis, Southern transfer, and hybridization to the $pCAD_{41}$ probe (*20*). Molecular markers in all figures are Hind III–digested lambda and Hae III–digested φX174 DNA. Fragment sizes are in kilobases. Autoradiogram shows preferential hybridization of CAD probe to early-replicating DNA (lanes 2, 3, and 6). This was independent of whether BrdU substitution was during S_E (lanes 3 and 4) or S_L (lanes 1 and 2, and 5 and 6).

Early and Late Middle Repetitive Sequence Families

Middle repetitive sequences are of unknown function, are dispersed throughout the genome, and account for 20 percent of the V79 cell's DNA (*4*). In situ hybridization with total MR DNA as a probe indicated that MR sequences are concentrated in Giemsa-dark (primarily late-replicating) bands (*12*); this was confirmed with the use of a single cloned MR sequence as a probe (*33*). In hamster cells (*34*) and frog embryos (*35*), MR DNA appears to replicate throughout the S phase. We reported that both the early

and late DNA fractions contain equal amounts of MR DNA in the V79 cell line, in which the genome is almost entirely euchromatic (*4*). Using cloned dispersed MR (10^2 to 10^5 copy) sequences, randomly chosen from a V79 genomic bank, we show that the members of any one MR family are largely confined to either the early or late DNA fractions.

A hamster genomic library (*36*) was screened with total nick-translated V79 DNA. Forty-eight positive clones, presumably containing inserts of hamster MR DNA, were prepared by the alkaline "mini-lysis" procedure (*37*), and plasmid DNA was spotted onto nitrocellulose paper (*38*). Portions of eight serial (1 to 5) dilutions were applied, starting with 0.4 μg per spot. Control spots contained 5 μg of intact CAD plasmid ($pCAD_{41}$) DNA. Duplicate filters were then probed with labeled early- or late-replicating V79 DNA. For each cloned plasmid, the number of complementary copies per genome present in the early- or late-replicating DNA fractions was crudely estimated by matching spots of the same absorbancy with the CAD spot being used as a single-copy gene control (*39*). Most MR clones preferentially hybridized to early- or late-replicating DNA (Table 2). The copy number estimated to be in the early- or late-replicating genome fractions are presented in Fig. 5 for selected clones.

Labeled inserts from 14 clones designated on the basis of spot blot experiments described above as representing MR families whose members predominantly replicate early (early MR's), late (late MR's), or without temporal preference (shared MR's) were hybridized to Southern blots of early- or late-replicating V79 DNA. Results qualitatively agreed with the spot blot designations. For example, the clones C5 and B4, according to spot blot data, represented presumptive early-replicating families. These clones showed stronger hybridization to Southern blots of early-replicating than to late-replicating DNA (Fig. 5). Similarly, clones B7 and C3, identified as late-replicating families by spot blot analysis, hybridized predominantly to late-replicating DNA, while presumptive shared MR's hybridized equally to early- and late-replicating DNA (Fig. 5). The extent of preferential hybridization to either the early- or late-replicating DNA was as great for many of the MR clones as it was for single-copy genes. Thus, most MR families consist predominantly of either early-replicating or late-replicating members.

To identify MR sequences that would hybridize interspecifically, nick-translated total HeLa DNA was hybridized at 30°C instead of 40°C (*19*) to a spot blot of the 34 MR clones (15 early MR, 11 shared MR, 8 late MR). Twelve MR clones (9 early MR, 2 shared MR, 1 late MR) showed interspecific hybridization (Table 2). Thus the early MR's are more likely to hybridize to both V79 and HeLa DNA than are late MR's. Six early MR clones, including B4 (Fig. 5), and one late MR (B7) were labeled and used to probe Southern transfers of HeLa DNA. All seven showed the same replication time bias in HeLa as they had in V79.

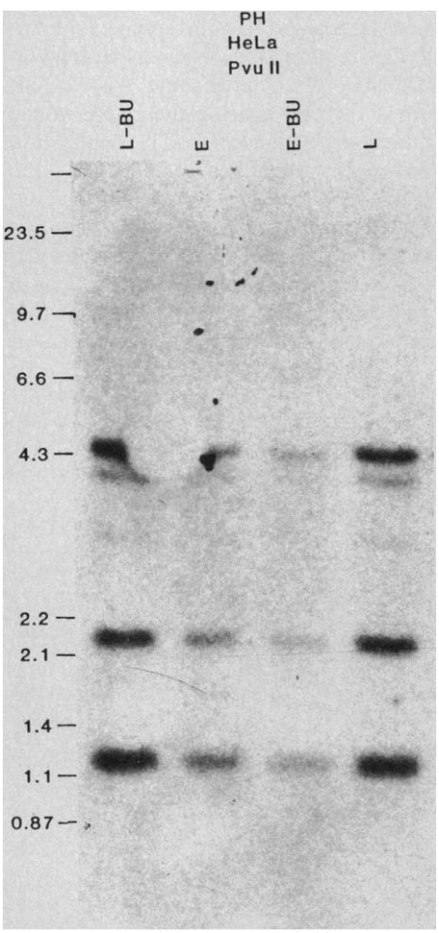

Fig. 3. Replication timing of phenylalanine hydroxylase in HeLa cells. Autoradiogram from the same filter shown in Fig. 2 demonstrates preferential hybridization of phPH72 probe (*21*) to late-replicating DNA (lanes 1 and 4). Hybridization to V79-8 hamster DNA (not shown) was not detected at high stringency.

Replication and Transcription

Mammalian genome replication occurs in clusters of early and late replicons (*2*) which are responsible for the banded appearance of the chromosomes (Fig. 1B) (*4*). Sampling the chromosome replication pattern by probing with 20 known protein-coding sequences suggested a relation between the time of replication of a gene in a given cell type and the functional state of that gene (Table 1). Processed pseudogenes, which are presumably nonfunctional, replicate either early or late. Active or transcriptionally competent genes always replicate early. Late-replicating genes are inactive in the cells studied. The β-globin gene is late-replicating in HeLa cells (Table 1) (*28*), where it is transcriptionally incompetent, and early-replicating in the erythropoietic MEL cell line in which it is active (Table 1) (*27*). In summary, housekeep-

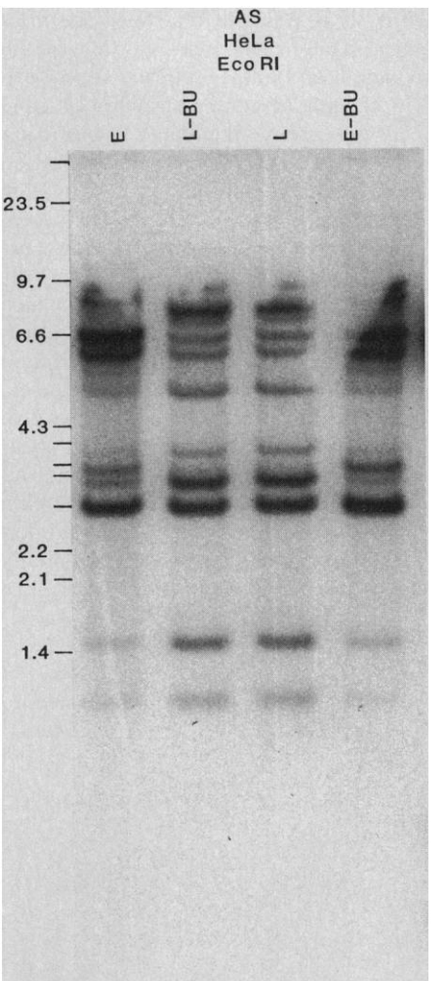

Fig. 4. Replication timing of HeLa sequences homologous to the argininosuccinate synthetase probe. Early- or late-replicating HeLa DNA (5 μg) was digested with Eco RI and fractionated on an agarose gel for Southern transfer and hybridization to pAS1 (*22*) probe. The 3.7-kb band (second mark below 4.3-kb marker) represented the coding sequences for argininosuccinate synthetase; hybridization was preferentially to early-replicating DNA (lanes 1 and 4). Pseudogene bands, however, hybridize either equally well to early- and late-replicating DNA (the 3.8-kb band; fourth notch below 4.3-kb marker) or preferentially to late-replicating DNA (for example, the 3.4-kb band; third mark below 4.3-kb marker).

ing genes are active in all cell types and replicate early. Tissue-specific genes are transcriptionally incompetent in most cell types and probably replicate late in those cells.

We believe that the replication time of a gene is related to its potential for transcription rather than to actual transcriptional activity for the following reasons. First, genes that are transcribed at high levels in differentiated cells are early replicating even in precursor cells that are not yet expressing the differentiated phenotype (*27*). Second, immunoglobulin genes for the heavy chain constant region replicate early in MEL cells (*29*). Although these genes are probably not actively transcribed in these erythroid cells, it is possible that they are transcriptionally competent, in view of the common and ontogenetically recent origin of immune-competent and erythroid cells (*40*). Third, the ability of particular DNA sequences to undergo transcription is reflected in chromatin conformation and composition (*41*). Active or potentially active genes occur in intermediate deoxyribonuclease I–sensitive "domains" about 100 kb in length (*42*) which are believed to indicate that transcription is possible rather than that transcription is actually occurring (*43*). DNA replication (*2*) and intermediate sensitivity to deoxyribonuclease I (*42, 44*) both occur in "units" (domains and replicons, respectively) of the order of 30 to 330 kb, suggesting that early replicons and intermediate deoxyribonuclease I sensitivity domains are equivalent structures, and that they are the units of transcriptional competence.

Table 2. Replication timing of V79-8 MR families in V79 and HeLa cells. Labeled early-replicating V79 DNA hybridized more strongly to 15 of the 34 spotted clones than did labeled late-replicating DNA. These 15 clones were provisionally classified as early MR sequences. Nine of these 15 clones also hybridized to HeLa DNA at high or reduced (10°C lower) stringencies. Clones hybridizing equally to early- and late-replicating DNA (shared MR's) and late MR's were similarly defined and challenged with labeled HeLa DNA.

Temporal class	Families (No.)	
	Total	Cross-hybridizing to HeLa DNA
Early MR	15	9
Late MR	9	1
Shared MR	10	2
Total	34	12

If, as we contend, transcriptionally competent genes replicate early, then tissue-specific genes must change their replication time at some point in development; the replication pattern at the molecular level would differ among different tissues. The euchromatic replication banding pattern evident by light microscopy (that is, at the replicon cluster level) appears to be the same in all tissues (*45*). Therefore, the switching of specific genes from a late to an early (or vice versa) replication regimen probably involves only a single replicon (deoxyribonuclease I–sensitive or supercoilable loop domain) (*46*), not an entire cluster. A tissue-specific gene might map to a Giemsa-dark band or late replicon cluster, but itself replicate early in certain tissues.

Transcription appears to be coupled to early replication in *Physarum* (slime mold). In this organism, only the early replicons contain transcription units visible by electron microscopy (*47*). Flickinger (*48*) has reviewed the evidence that, in cultured HeLa and hamster cells, the rates of total RNA and mRNA synthesis remain constant during G_1, increase during S_E, and remain constant during S_L and G_2. He also reviewed the evidence that S_L DNA, because of its smaller replicon size, faster rate of replication fork movement, and decreased chromatin sensitivity to S_1 nuclease, is more like transcriptionally inactive heterochromatin than it is like S_E DNA.

As to why S_E is functionally different from S_L, the bimodal S phase suggests that a given replicon replicates either entirely within S_E or entirely within S_L. We postulated that, after early replication is completed, the complex of enzymes required for DNA replication (*49*) probably dissociates from early replicons, thereby causing the pause in the middle of S phase (*4*). The replication complexes then reassociate with the DNA, initiating late replication, as suggested (*4*). The functional difference between S_E and S_L could be explained if any replicon initiated after the pause were replicated in some manner that renders it transcriptionally incompetent.

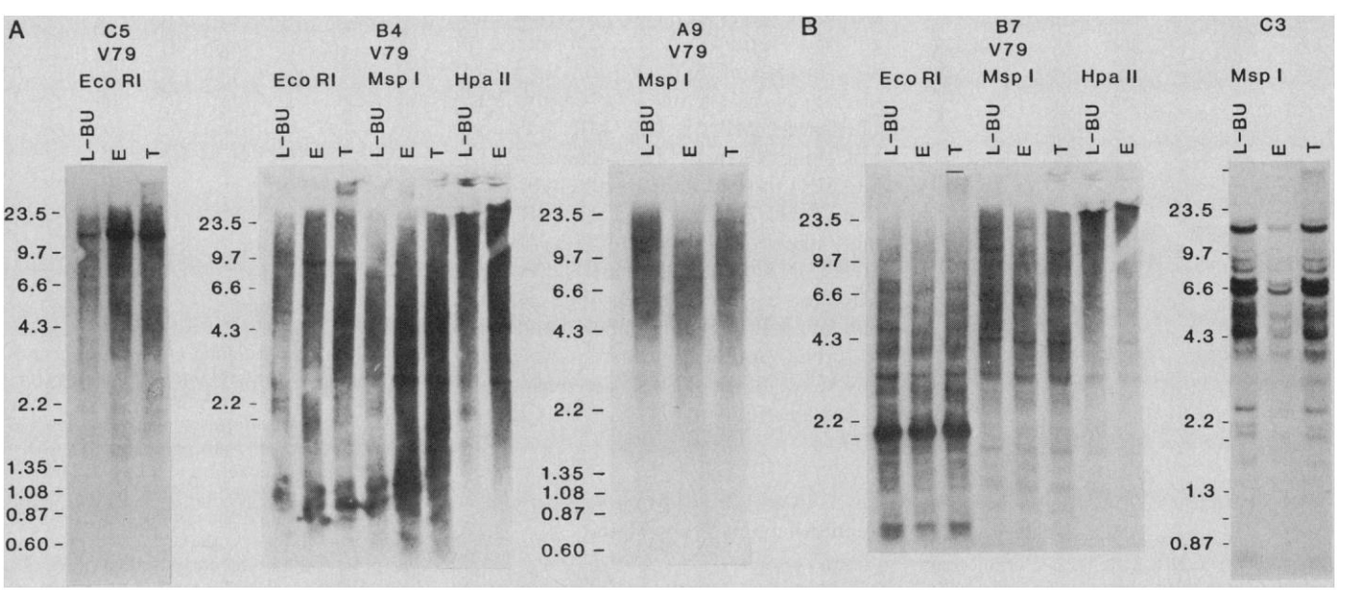

Fig. 5. Blotted total, late-, and early-replicating DNA from V79 hamster or HeLa cells were probed with nick-translated insert from cloned V79 DNA sequences provisionally classified as representing early, late, or shared MR families (Table 2). Estimated copy numbers early- (E) or late-replicating (L), based on spot blot data, follow each clone designation. C5 (1200 E/580 L) and B4 (4000 E/304 L) are early MR's; B7 (259 E/2101 L) and C3 (51 E/1103 L) are late MR's; and A9 (680 E/680 L) is a shared MR. The blot probed with B4 was subsequently probed with the late MR, B7.

The exact nature of this conformational change in the DNA is not known. Late replication of the inactive X chromosome can spread into an autosomal segment and is associated with evidence of gene inactivation both at the single gene and at the gross phenotypic level (*50*). Replication in S_L, then, indicates genetic inactivity.

Table 3. The two-genome model of chromosome organization.

Euchromatic genome		
Housekeeping		Ontogenetic
Constitutive genes		Tissue-specific genes
Giemsa-light bands		Giemsa-dark bands
Early replicating	<<<Commitment<<<	Late replicating
dG + dC rich*		dA + dT rich*
Similar in V79-8 and HeLa		Different in V79 and HeLa
Msp I sensitive†		Msp I resistant

*dG, Deoxyguanylate; dC, deoxycytidylate; dA, deoxyadenylate; dT, deoxythymidylate. †Early- and late-replicating DNA are terminally digested almost equally by Hpa II but not by Msp I (*39*).

The Two-Genome Model

Dispersed throughout the genome are many MR sequences (*51*) which together constitute about 20 percent of the V79 genome (*4*). In our study of 34 MR's, 15 MR families were predominantly early MR's, 8 were late MR's, and 11 were shared MR's. Many MR sequences are mobile elements (*51, 52*) and could theoretically insert anywhere in the genome. The existence of MR sequence families with a preponderance of copies replicating either late or early suggests that these temporal fractions of the genome exchange mobile MR sequences to a remarkably limited extent. Thus, these two fractions of the genome are quite distinct in the kinds of coding sequences they contain and, being far from equilibrium for mobile elements, seem to represent two mutually isolated genomes.

On the basis of our data and that reviewed in this article, we propose the two-genome model for the mammalian euchromatic genome shown in Table 3. We can speak of two physically separate genomes, a housekeeping genome and an ontogenetic genome, coexisting in the same nucleus and corresponding to the Giemsa-light and -dark bands described by cytogeneticists. The distinguishing features of the housekeeping genome are that it contains genes which are active in all cells, while the ontogenetic genome contains those genes which are tissue- or developmental stage–specific in their expression. The housekeeping genome always replicates during S_E. In most tissues, most of the ontogenetic genome replicates late, but when tissue-specific genes are committed to transcriptional competence, the replicons containing them become early replicating.

The silencing of DNA by late replication might provide an economical means to regulate a complex genome. If the mammalian genome contains 124,000 tissue-specific genes, only 4000 of which are active in any one cell type, then 97 percent of the ontogenetic genome must be permanently transcriptionally inactive (*53*). The problems of inducing and repressing transcription of large numbers of genes (*54*) would be reduced by rendering unneeded genes unavailable to the transcriptional apparatus through late replication. The regulatory machinery of the cell would then be available for fine-tuning the expression of a reasonable number of housekeeping and tissue-specific genes.

The bipartite nature of the genome proposed above permits several testable predictions concerning the structure and regulation of the mammalian genome to be made.

1) We hypothesized that genes in late replicon clusters should remain inactive and unavailable to the transcriptional apparatus until controlling events in development prod individual replicons into an early-replicating, transcriptionally competent state. Our data are consistent with this prediction. It should be possible to systematically test the replication time of the same gene in a number of tissues in which it is known to be active and a number in which it is inactive.

2) If MR families are somehow involved in the *cis*-acting control of gene activation during development (*55*), then all copies of well-defined MR families should be late-replicating in some tissues, and all copies early-replicating in others. Tissue-specific transcription of MR sequences has been reported in mouse brain (*56*), rat brain (*57*), and in *Dictyostelium* (*58*) and might profitably be studied with respect to replication timing. One would predict that copies of these sequences should be demonstrable in the same replicon as tissue-specific genes. Middle repetitive sequence copies may be origins of replication (*52*), a change in conformation of which allows early replication and transcriptional competence.

3) If many MR sequences are mobile elements (*51, 52*), then our observations that some families are represented almost entirely within one genome or the other suggest that these sequences are mobilized in such a way that additional copies occur preferentially in the genome from which they arose. If the genomes are indeed isolated, further research should reveal additional MR and multigene families confined or largely confined to one genome or the other.

4) We might further predict that housekeeping functions are fairly similar in different organisms, but that tissue-specific functions are subject to selection from the environment in which different species are found. Therefore, the housekeeping genomes should be more similar among different species than the ontogenetic genomes. Our data suggest that late MR's and tissue-specific genes hybridize interspecifically less frequently than do early MR's and housekeeping genes.

Note added in proof: While this article was in press, Calza *et al.* (*58a*) described the analysis of the replication timing of immunoglobulin, c-*myc*, and α-globin genes in several mouse hematopoietic cell lines. The results presented support our hypothesis that only early-replicating genes are capable of expression. They demonstrated a difference in the time of replication of immunoglobulin heavy chain variable region genes between those cells in which they were in embryonic configuration and those in which VDJ joining had occurred.

References and Notes

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