THE FUNCTION OF NUCLEAR ARCHITECTURE:
A Genetic Approach

Angela Taddei, Florence Hediger, Frank R. Neumann, and Susan M. Gasser
University of Geneva, Department of Molecular Biology, CH-1211 Geneva 4, Switzerland; email: susan.gasser@molbio.unige.ch

Key Words  nuclear organization, telomeres, heterochromatin, lamins, DNA replication

Abstract  Eukaryotic genomes are distributed on linear chromosomes that are grouped together in the nucleus, an organelle separated from the cytoplasm by a characteristic double membrane studded with large proteinaceous pores. The chromatin within chromosomes has an as yet poorly characterized higher-order structure, but in addition to this, chromosomes and specific subchromosomal domains are nonrandomly positioned in nuclei. This review examines functional implications of the long-range organization of the genome in interphase nuclei.

A rigorous test of the physiological importance of nuclear architecture is achieved by introducing mutations that compromise both structure and function. Focussing on such genetic approaches, we address general concepts of interphase nuclear order, the role of the nuclear envelope (NE) and lamins, and finally the importance of spatial organization for DNA replication and heritable gene expression.

CONTENTS

NUCLEAR ORGANIZATION .................................................. 306
Function-Based Compartments ........................................... 306
Global Chromosome Arrangements ................................... 308
Long-Range Pairing Mediated by Repetitive Sequences ............ 311
Role of Long-Range Organization in Differentiated Gene Expression .... 316
NUCLEAR LAMINS AND CHROMATIN ANCHORING ................. 317
NE-Associated Proteins .................................................... 318
LEM Domain Proteins Bind BAF ......................................... 321
The Nuclear Lamina and Gene Expression ............................. 322
Linking Nuclear and Cellular Architecture ............................ 324
DNA REPLICATION AND NUCLEAR STRUCTURE ................. 324

1All authors contributed equally to this chapter. Address after December 2004: Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland.

0066-4197/04/1215-0305$14.00
NUCLEAR ORGANIZATION

It is useful to distinguish three types of subnuclear organization: the first reflects an order imposed by enzymatic functions inherent to the nucleus, often the result of interactions between large multicomponent complexes. The second is a global order that reflects whole chromosome positioning. The third is based on aspects of primary DNA sequence and its secondary folding properties, or interactions of these in trans, which can create subcompartments based, for example, on repetitive sequence motifs. Experimental support for these models is discussed below.

Function-Based Compartments

Biosynthetic activities abound in the nucleus and include DNA replication, repair, RNA transcription, splicing, RNA and DNA modification, and perhaps a limited amount of protein synthesis, often controlled by regulated systems of protein modification and degradation. Many activities are concentrated in subnuclear foci called nuclear bodies, which include nuclear speckles, Cajal bodies, and PML bodies (reviewed in 136, 227). Nuclear bodies are not surrounded by lipid bilayers and are usually characterized by specific antigens that are generally in rapid exchange with nonbound species. For most foci, the targeting or recruitment of proteins can be attributed to motifs responsible for protein-protein or protein-nucleic acid interactions (92, 146, 149, 263). This suggests that function-based foci can arise through the self-assembly of essential components without an underlying template or scaffold. The proposed functions of nuclear bodies and speckles, either for storage, assembly, or as sites of specific enzymatic activity, are covered in recent reviews (136, 227).

The most dominant function-based nuclear compartment is the nucleolus, the site of RNA Pol I-mediated rDNA transcription and ribosome subunit assembly. Although its morphology varies among cell types, the nucleolus generally consists of three domains (reviewed in 202, 217). Innermost is the fibrillar center (FC), which contains both inactive and active rDNA genes. The processing and assembly of rRNA take place in the surrounding dense fibrillar component (DFC), while the latter steps of ribosome maturation occur in the outermost granular component (GC). In budding yeast the nucleolus is a crescent-shaped structure abutting the nuclear envelope, occupying roughly one third of the nucleus. Plant and animal nuclei contain multiple nucleoli, often adjacent to heterochromatin. In all cases nucleolar morphology is influenced by the growth rate of the cell.
How is this elaborate nuclear substructure formed? Genetic studies in budding yeast have elegantly addressed the mechanisms underlying nucleolar biogenesis. Strains were created bearing deletions of the endogenous rDNA gene cluster, and its absence was complemented by multicopy plasmids expressing rRNA from either a Pol I or a Pol II promoter (186, 244). When expressed from the Pol I promoter, the plasmid-borne rDNA genes and the nucleolar marker Ssb1 colocalize to multiple foci adjacent to the NE, as opposed to forming the normal crescent-shaped structure (186). When transcribed by RNA Pol II, the rDNA genes and Ssb1 formed 1 to 2 foci that did not associate with the NE. Despite the differences in spatial organization, the internal structure of these nucleolar structures was conserved (244). Because transcripts and processing were the same in both cases, one can attribute the differences in subnuclear position to the RNA polymerase involved.

This model was tested by introducing a mutation that disrupts RNA Pol I complex formation (rpa12Δ). In this mutant, Ssb1 was redistributed from the periphery to internal sites that resemble the foci of Pol II-transcribed rDNA genes (186). These authors conclude that the formation of contiguous, crescent-shaped nucleoli requires the genomic array of tandemly repeated rDNA genes (150–200 copies), and that Pol I-mediated transcription itself targets nucleolar components to the NE. Moreover, the nucleolar infrastructure forms even in fragmented, mislocalized nucleoli, indicating that this compartment is self-organizing. Surprisingly, these manipulated strains have few other defects apart from a 20% to 40% decrease in growth rate.

In human cells, rDNA genes are found on five different pairs of chromosomes and cluster to form intranuclear nucleoli. The trans-association of nucleolar organizer regions (NORs) occurs in an asynchronous manner shortly after telophase (208), independently of rDNA transcription and prior to chromosome decondensation (50). Intriguingly, pre-rRNAs synthesized in late G2 of the previous cell cycle appear to be recruited to NORs together with the transcription machinery, and all subsequent steps of assembly require RNA Pol I activity (50, 217). It is still unclear what mediates NOR association and fusion, yet ~1 h after this event, both active nucleoli and silent NORs move to more central intranuclear positions coincident with a major repositioning of chromosomes (208, 234).

In yeast, RNA Pol I and III transcription are temporally and spatially coordinated at least partly because the 5S RNA, a Pol III transcript, lies adjacent to the 35S rRNA coding region (126). Surprisingly, however, tRNA transcripts, which are also Pol III dependent, accumulate in the nucleolus even though the 9 to 16 copies of each tRNA gene are scattered throughout the yeast genome (13). Moreover, the active tRNA genes themselves cluster and colocalize with the nucleolar 5S gene signal (242). This association is dependent on both the transcriptional status of the tRNA genes and Pol I-mediated transcription, which, as explained above, is necessary for nucleolar integrity. The grouping of expressed tRNA genes at the nucleolus restricts the volume within which Pol III transcription factors function, yet requires that the yeast nucleolus is accessible to all 16 chromosomes.
In contrast to the highly localized transcription of rDNA by RNA Pol I, Cook and colleagues have shown that there are thousands of RNA Pol II “transcription factories” dispersed throughout the nucleoplasm of mammalian cultured cells, each comprising around 5 to 8 RNA Pol II complexes (119, 122). It is unclear whether coordinately regulated RNA Pol II genes are grouped together in an expression site, or whether these foci simply reflect the random juxtaposition of neighboring transcription units. Given that a typical RNA Pol II gene is only transiently associated with a polymerase [i.e., roughly 5–10 min/h (124, 212)], active Pol II transcription is unlikely to constitute a stable element of subnuclear organization.

In contrast, eukaryotic DNA replication is also organized into foci, which appear in recognizable patterns that persist for hours during S phase in mammalian cells [(49, 182, 187) and references therein]. Their importance for genomic replication is discussed below.

Global Chromosome Arrangements

Besides this function-based organization, whole chromosomes are thought to adopt reproducible positions within the nucleus. There may be fundamental differences in the spatial distribution of small and large genomes, and two configurations are commonly recognized. The first is one in which most chromosomes are polarized within the nucleus, an arrangement exemplified by the Rabl configuration (198). Here centromeres and telomeres are at opposite ends of a nucleus, a distribution that arises naturally as the anaphase spindle pulls on kinetochore-containing chromosomes. The Rabl configuration is readily observed in rapidly dividing embryonic cells and is well-described for Drosophila (73, 110, 112). Nonetheless, no mutations have been found that disrupt it, including those that compromise telomere integrity such as UbcD1 (30). In Drosophila and related insects (Diptera), the Rabl configuration is accompanied by homologue pairing, which ensures that homologous chromosomes in a diploid somatic cell assume nearly identical positions. Although homologue pairing in flies is developmentally regulated (111), there is no compelling evidence for homologue pairing in most plants, mammalian cells, or in vegetatively growing yeast (153). Moreover, because yeast and many plants maintain the Rabl configuration throughout interphase without homologue pairing, there is clearly no obligatory link between these two phenomena (reviewed in 216, 227). To date no mutations have been reported that disrupt homologue pairing in flies, not even zeste, a mutation that compromises transvection effects (81).

The second type of global nuclear organization is based on the hypothesis that individual chromosomes maintain discrete “territories,” each defining a compartment within the nucleus (20). This non-overlapping distribution is readily observed in vertebrate cells in early prophase as chromosomes begin to condense, yet was also detected in G1- and S-phase cells by whole chromosome FISH (fluorescence in situ hybridization) (41). The relative positioning of chromosomes is not determined by a species-specific floorplan, however, because no reproducible pattern of chromosomal territories (CTs) persists in all nuclei of a given multicellular organism. Moreover, FISH and real-time imaging have both documented extensions of chromatin from one CT into a neighboring one (68, 157, 252, 260), an event
correlated with active transcription in mammalian cells. This does not invalidate the notion of CTs, but allows for extensive chromosomal cross-talk.

Some principles of CT organization have been proposed. For instance, in several mammalian cell types a radial distribution is observed such that gene-poor chromosomes are enriched at the nuclear periphery (42, 94, 239, 240), an arrangement that is not found in proliferating fibroblasts (40) or quiescent cells (23). Certain chromosomes showed a reproducible juxtaposition in quiescent human fibroblasts (180) and mouse splenocytes (191), whereas other groups found random distributions (38, 94). Until the proteins are identified that position CTs or mutations are found that disrupt nuclear organization, the only question is whether a given organization is heritable. Below we examine experiments that address this question.

**IS RELATIVE CHROMOSOME POSITION HERITABLE?** To study the mitotic inheritance of a cell-specific chromosome distribution, photobleaching techniques were used to impose patterns in the chromatin of nuclei that were expressing histone H2B forms fused to GFP derivatives. By comparing pre- and post-mitotic patterns of bleached zones, one can observe a gross similarity of chromatin distribution in some daughter cells (83, 257). Quantitation by Walter and colleagues suggested that pattern perpetuation occurs at low frequency, however, and that positioning is often lost due to chromatin movements soon after cytokinesis. Indeed, chromatin repositioning is most pronounced at the beginning of G1 phase (218, 243, 257). Intriguingly, the post-mitotic persistence of a heritable chromosomal distribution was lost when cells continued to divide or were treated with Hoechst (83), an intercalating dye that displaces proteins from the narrow minor groove of DNA (257). Thus, although some degree of heritable order was detected, perhaps reflecting the intrinsic symmetry of cytokinesis, this cellular memory seems short-lived, at least in tissue-culture cells.

The Bickmore laboratory took a more rigorous approach to this question, scoring the positions of specific GFP-tagged chromosomal loci relative to other nuclear structures in early G1-phase daughter cells of a human fibrosarcoma line (243). Positions were mapped relative to nucleoli or the NE, and results were compared with distributions that would occur stochastically. The majority of daughter cell pairs showed dissimilar locus positioning, arguing for a nonsymmetrical distribution of homologous loci following mitotic division. Although in apparent disagreement with the above studies, these authors did not actually monitor global CT position, which may have remained grossly similar between daughter cells. In summary, we propose that some aspects of CT position may be maintained through mitosis in immortalized cells lines, yet specific loci are unlikely to be restored to specific sites. Stochastic interactions of chromatin with chromatin, or chromatin with nuclear structures in early G1 phase, are likely to move chromosomal domains to different locations or reinforce their original position. Indeed, Dimitrova & Gilbert (49) demonstrated a significant movement of heterochromatin and appropriately tagged early and late-replicating regions, until ∼2 h after telophase in CHO cells. Missing are studies of mutants that generate an aberrant positioning of chromosomes.
LOCAL AND LONG-RANGE CHROMATIN DYNAMICS  In order for chromosomes to
change position there must also be an underlying movement of chromatin per se.
Indeed, one of the more surprising findings of recent years, documented through
rapid time-lapse imaging of green fluorescent protein (GFP)-tagged chromosomal
loci (166), is how mobile chromatin can be. Improved high-resolution fluorescence
tracking has allowed a detailed quantitation of chromatin dynamics in interphase
nuclei, revealing high rates of local chromatin motion in species ranging from
yeast to human.

Quantitation of the movement of specific chromosomal loci shows that most are
engaged in continual, albeit spatially constrained, movement. The diffusion coef-
ficient of different loci ranges from $1 \times 10^{-4}$ to $1 \times 10^{-3} \, \mu m^2/s$ and for nonrepeti-
tive open chromatin domains the radii of constraint were similar for budding yeast,
*Drosophila*, and human [$r = 0.5-0.7 \, \mu m$ (32, 108, 166)]. When time-lapse imaging
is adequately rapid, two types of motion can be distinguished: smaller, saltatory
movements <$0.2 \, \mu m$ that occur every 1–2 s, and larger, more rapid movements
[i.e. >0.5 \mu m in a 10.5 sec interval (108)]. In budding yeast, the smaller move-
ments are observed for euchromatic as well as silent regions, whereas the larger
movements are characteristic for euchromatic loci but are nearly undetectable for
telomeres (105). Changes in cellular energy levels (through depletion of glucose
or addition of protonophores, which reduce intracellular ATP levels) induce a de-
crease or complete abolition of large movements (108). Thus, although resembling
Brownian motion, chromatin mobility seems to be influenced by energy-requiring
enzymatic events.

The extent of spatial constraints imposed by the environment on a given locus
can be calculated by plotting the mean squared displacement (MSD) over fixed
intervals of time (166). These MSD plots reach a plateau at time intervals of 60
to 100 s from which the radius of constraint of a given site can be derived, and
compared with the movement of other loci or other cell types. The movement
of euchromatic loci in flies, human, and yeast is confined to volumes of ~0.5–
0.7 \mu m in radius, which constitutes a large fraction of a yeast nucleus ($r = 1 \, \mu m$),
but not of a mammalian one [$r \geq 10 \, \mu m$ (32, 108, 247)]. This movement would com-
promise an organization with spatially distinct CTs in yeast, but not in mammalian
cells.

The degree of spatial constraint imposed on a locus depends on two addi-
tional variables: cell cycle stage and the nature of the locus observed. In G2-phase
*Drosophila* spermatocytes, there is both random, locally constrained movement
and a long-range directional movement, which occurs over a much longer time
scale. A decrease in step size for the rapid movements and termination of the
long-range migration were observed in late G2 spermatocytes just before they
enter meiosis (247). This drop in chromatin mobility correlates with a clear de-
velopmental change characterized by a nuclear reorganization during which bulk
chromatin moves from a central region to three distinct perinuclear masses (29). In
budding yeast, on the other hand, different genomic loci show a general decrease
in mobility during S phase (108). The reduced mobility does not correlate with
the passage of a replication fork, and instead might reflect either a reduction in ribonucleotide pools that accompanies S phase, or replication fork clustering into foci, a result of trans-interactions between distant genomic loci.

In both yeast and human cells, some loci have constraints imposed on their mobility due to interactions with a nuclear structure [NE, lamina, spindle pole body, or the nucleolus (32, 108)]. Importantly, higher mobility is restored when the association with the nuclear structure is lost. Disruption of the yeast \textit{yku70} gene leads to the release of telomere 6R from the NE and a significantly higher mobility of the locus (105). In mammalian cells, treatment with the transcriptional inhibitor DRB, which disrupts nucleolar structure, leads to an increase in the local dynamics of a locus integrated near rDNA repeats (32). Finally, poorly characterized stochastic events may also influence the dynamics of tagged loci. For instance, although most human telomeres move little, occasional rapid transitions occur, as if a telomere were momentarily liberated from constraints (177). Spontaneous movement of yeast telomeres away from the NE is also observed (105), possibly reflecting sporadic transcription or chromatin remodeling events.

Besides spatial constraints imposed by nuclear substructure, a given locus is also restricted in its mobility by the contiguity of the chromatin fiber itself. In budding yeast, chromatin can be excised from the chromosome as an intact ring that maintains its nucleosomal status (79). The resulting episome moves without detectable constraint throughout the nucleoplasm, indicating that once freed of flanking DNA, there is no restriction on its movement. This shows that even actively transcribed chromosomes restrain mobility (M.R. Gartenberg, F.R.N. & S.M.G., personal communication). These freely diffusing chromatin loci, the nondirectional diffusion monitored for mRNA (124), and the rapid diffusion-based exchange rates detected by FRAP (fluorescence recovery after photobleaching; reviewed in 173) for most chromatin-bound factors all indicate that it is unnecessary to postulate interchromatin channels to account for the mobility of macromolecules in interphase nuclei (41). The documented chromatin mobility may well itself facilitate factor/binding site searches or homology searches during recombination.

**Long-Range Pairing Mediated by Repetitive Sequences**

Stable somatic homologue pairing is, as far as we know, only manifest in \textit{Diptera}, yet associations between physically distant loci are widely observed for genomic regions that contain stretches of repetitive elements. Simple-repeat elements found at telomeres or centromeres are obvious candidates for long-range pairing, which, in turn, will undoubtedly contribute to the global interphase positioning of chromosomes. As such, long-range repeat interactions constitute a third element determining interphase nuclear organization.

**CENTROMERIC REPEAT INTERACTIONS**

Centromeres generally consist of simple DNA repeats packaged into heterochromatin and a limited number of genes uniquely designed for transcription in a heterochromatic environment (256). Centromeric DNA repeats cluster to form either the chromocenter or, as occurs more
frequently in mammalian cells, several foci of pericentric heterochromatin. These associate occasionally with other repetitive DNA sequences or position themselves around nucleoli (93, 109). Chromocenters stain intensely with DNA intercalating agents, due to their sequence bias, and are enriched for heterochromatic markers such as Heterochromatin Protein 1 (HP1). Chromocenter organization depends on underacetylated histone tails, which then later are methylated and bound by HP1 (237). Importantly, however, conditions that delocalize HP1 from heterochromatin do not induce a loss of chromocenter organization (160, 195), indicating that HP1 does not itself promote centromere clustering.

In *Schizosaccharomyces pombe*, centromeric regions are also highly repetitive, consisting of two distinct elements: the *otr* region, which is bound by the fission yeast HP1 (Swi6), and a central core region that contains the histone H3 variant Cnp1 (CENP-A) and an unusual nucleosomal structure (231). In mitotically dividing fission yeast, centromeres cluster near the SPB as well as with each other (72). In interphase, mutants affecting the central core structure induce a loss of centromere clustering, whereas the loss of rik1, a WD-40 repeat protein that is required for stable pericentric heterochromatin, does not (7). Although no longer clustered, the centromeres remain near the NE, suggesting the existence of a kinetochore-independent mechanism for the NE-anchoring of heterochromatin, like the Sir4-Esc1 pathway described for silent chromatin in budding yeast [see below and (236)].

**TELOMERE-TELOMERE INTERACTIONS** Whereas telomere clustering is a universally conserved feature of the pre-pachytene “bouquet” stage of meiosis, in mitotically growing cells these interactions are well-documented only in yeast, trypanosomes, and *Plasmodia*. In these cases, telomere clustering is often accompanied by anchoring at the NE, thus contributing to the global positioning of interphase chromosomes. The clustering of telomeres in budding yeast contributes to the repression of subtelomeric chromatin, conferring a telomere position effect (TPE), which resembles the position-effect variegation (PEV) nucleated by centromeric repeats in flies. Indeed, it is thought that the mechanisms that bring fission and budding yeast telomeres together are likely to provide paradigms for other repeat-dependent interactions.

In fission yeast, the telomeric repeat is bound by Taz1, a Myb-domain DNA-binding protein, that recruits spRap1 and spRif1. The subtelomeric regions contain the heterochromatin factors Swi6, Clr4, and Rik1, which also participate in heterochromatin at centromeric repeats and the mating-type loci. Clr4 is the fission yeast equivalent of the Su(var)3-9 histone methyltransferase, which modifies histone H3 on lysine 9 to allow recruitment and binding of the HP1 equivalent, Swi6. The Taz1-Rap1-Rif1 and the Swi6-Clr4-Rik1 complexes both induce compact chromatin structures at telomeres, albeit with different properties. Importantly, TPE in fission yeast depends primarily on Taz1 and SpRap1, and only to a minor extent on Swi6, Clr4, and Rik1 (2, 36, 127).

During interphase, the six fission yeast telomeres cluster into 1 to 2 foci near the NE (72), an organization that is, surprisingly, affected by mutations in the
key factors of the RNAi machinery [ago1, dcr1, and rdp1 (95)]. In these mutants, telomeres fail to cluster but remain perinuclear. The fission yeast RNAi machinery is required for epigenetic gene silencing at centromeres or mating type loci, being linked to H3 K4 methylation and Swi6 binding (96, 251). It is not required, however, to any significant extent, for telomere-linked silencing. Indeed, the loss of RNAi leads to a loss of histone H3 K9 methylation, Swi6 mislocalization (251) and reduced telomere clustering, but does not disrupt TPE. Conversely, these same mutations do impair centromere repression, although centromere clustering persists. In summary, the RNAi pathway promotes telomere-telomere interactions in S. pombe [as do, to lesser extents, Rik1 and Clr4 (55)], but is dispensable for telomere length regulation, TPE, and telomere-NE association (95).

Superficially, the S. cerevisiae telomeric organization resembles that of fission yeast, in that 32 telomeres cluster in 4 to 7 perinuclear foci (88), yet the mechanisms that tether telomeres are quite different. In contrast to most other organisms, budding yeast lacks HP1, methylation on histone H3 K9, and the RNAi machinery. Replacing this mechanism for silent chromatin formation is the Sir2-3-4 complex, a heterotrimer with NAD-dependent histone deacetylase activity and histone tail-binding capacity (103). At telomeres Sir proteins are recruited by interaction with the ScRap1 C terminus, which, like Taz1, is a Myb-domain containing repeat binding factor. Budding yeast telomeres also bind the heterodimeric complex Ku70/80, which cooperates with Rap1 to recruit and nucleate the Sir complex. The Sir complex then spreads along histone tails to repress nearby genes. Sir proteins are present in limiting amounts in the yeast nucleus, and are sequestered at telomeric foci (88, 159).

In budding yeast, two parallel pathways, one dependent on the Ku heterodimer and the second on the silencing factor Sir4, mediate telomere-NE association (105, 236). Both proteins mediate anchorage in the absence of silencing, although the presence of TPE enhances tethering efficiency (105, 236). The bifunctional role of Sir4 as a mediator of repression and an anchor for silent chromatin underscores the close relationship of the two functions. In vivo a non-silent telomere can be localized at the NE through interaction with Ku; and being thus placed in a zone enriched for Sir proteins, this telomere is thought to have a better chance to become repressed (4). Once the Sir-dependent chromatin is established, silencing itself will reinforce the perinuclear localization through the Sir4 anchoring pathway (Figure 1). In this way, the formation of silent chromatin is self-reinforcing and contributes to the spatial positioning of telomeres.

Given the absence of nuclear lamina, a meshwork of intermediate filament proteins that underlies the NE of most eukaryotic cells (86), it was unclear what might anchor Ku and Sir4 at the yeast NE. The pore-associated myosin-like factors, Mlp1 and 2, have been proposed to bind Ku (77), yet detailed studies revealed no defect in telomere localization or silencing in double mlp1 Δmlp2Δ strains (5, 104, 105). Moreover, telomeres and Sir foci do not colocalize with pores by FISH, IF, or live imaging, being enriched instead in spaces between pore complexes where a large acidic NE-associated protein called Esc1 provides a Sir4 anchoring site (5, 236; M.R. Gartenberg & S.M.G., personal communication). Esc1 binds Sir4 in
two-hybrid assays (5) and localizes at the nuclear envelope independently of Ku and Sir4 (236). Although the physiological anchor for telomere-bound yKu is not known, in a yku-deficient strain, Esc1 becomes essential for telomere anchoring. Conversely, if the yKu anchor is intact, esc1 has only minor effects on telomere localization, confirming the redundancy of anchoring pathways. Surprisingly, esc1 mutants, unlike yku mutants, show only a minor loss of TPE.

Mammalian telomeres seem to be randomly positioned throughout the cell nucleus (14, 155) and at some points of the cell cycle are preferentially internal (62, 253). Nonetheless, trans-associations between 2 to 3 telomeres occur, as demonstrated by live imaging of human fibroblasts (177), or FISH on U2OS cells (181). Intriguingly, the association is more frequently observed in noncycling cells, much like the yeast telomeric foci, which are more pronounced under conditions of glucose depletion (T. Cheutin, A.T., T. Misteli & S.M.G., unpublished observations).

**BIOLOGICAL FUNCTIONS OF TELOMERE CLUSTERING**  
Insight into the function of telomere clustering is provided by pathogens such as *Plasmodium falciparum*, *Trypanosoma brucei*, or *Candida glabrata*. In all three species, telomeres are grouped at the NE, as they are in budding yeast (33, 69). In addition, orthologues of yeast SIR proteins have been identified in *Plasmodia* (210) and *Candida* (45), increasing the likelihood for conserved anchoring and clustering pathways. In these pathogens, telomere clustering is clearly linked to the organism’s virulence (45, 106), which in *Trypanosoma* requires a sequential activation of variant-specific surface glycoprotein genes (*vsg* or *var*) following translocation into a subtelomeric expression site, and in *Plasmodia* involves an epigenetic mechanism of allelic exclusion. Coat protein variation allows the parasites to escape the host immune system. The clustering of different *var* genes in foci, corresponding to telomeric foci, is thought to enhance recombination efficiency among such genes, thereby increasing the parasites’ virulence. Although anchoring proteins are not yet characterized, Figueiredo and colleagues showed that the clustering of *Plasmodia* telomeres depends on repetitive subtelomeric elements (64).

In the fungal pathogen *Candida glabrata*, virulence depends on adherence of the parasite to the intestinal epithelia through the lectin EPA-1 (37). Indeed, EPA-1 is a member of a family of related *Candida* genes found in subtelomeric clusters, most of which are not expressed at any one time. Their repression resembles the reversible position effect found at budding yeast telomeres, and is mediated by homologues of the ScSir3 and ScRap1 proteins (45). Also regulating cell adherence and a developmental transition to a filamentous form are the budding yeast *FLO* genes. While the *FLO11* gene is non-subtelomeric and expressed, all other members of the *FLO* gene family are repressed in a variegated manner owing to their physical proximity to telomeres (97). Repression is reversible, heritable and involves a subset of the Sir proteins, but does not reflect an extension of TPE per se (97).

In summary, unicellular organisms use the clustering of telomeres at the nuclear periphery and its associated recombination and repression phenotypes to regulate genes crucial for their survival. Unusual recombination behavior also characterizes
budding yeast telomeres, which recombine efficiently among themselves, but poorly with internal chromosomal sites (197). This recombination behavior is thought to reflect constraints imposed on telomeres by the NE, since telomere recombination rates increase in strains lacking the telomere anchor, yKu (197).

PAIRING-DIRECTED SILENCING IN DROSOPHILA  In Drosophila, plants, and humans, the presence of a transgene sequence can influence the transcriptional state of the endogenous gene. This phenomenon was called homology-dependent gene silencing (HDGS) in plants or transvection in Drosophila (reviewed in 241, 262). While some of this repression is posttranscriptional, transgene repeats inserted at distant positions on the same or different chromosomes in wheat and Arabidopsis were observed to cluster together (1, 68), suggesting that physical interaction might be involved in gene silencing. Some models for transvection also argue for a physical interaction of the two homologous sequences, although it is documented in only a few cases.

One example of pairing directed silencing is the brown\textsuperscript{Dominant} (bw\textsuperscript{D}) allele in Drosophila, a phenotype caused by the insertion of a heterochromatic block into the coding region of the brown gene, which is located on the right end of Chr2. In heterozygotes, this insertion causes variegated expression of the second wild-type copy of brown, producing variegated eye color. By monitoring the subnuclear position of the bw\textsuperscript{D} allele at different stages of fly development, Dernburg and colleagues found that the mutant locus was often juxtaposed to the centromeric heterochromatin of Chr2 (Cen2) in larval, although not in embryonic, cells (47). Somatic pairing of chromosomes was not altered by the bw\textsuperscript{D} mutation, and this alignment therefore positioned both the mutant and wild-type alleles close to Cen2 repeats. Seeking a situation in which this triple association was disrupted, Csink and colleagues found that the Cen2-bw\textsuperscript{D} allele association could be partially suppressed by mutations affecting PEV, e.g., Su(var)205, which encodes the Drosophila HP1 (43). To test whether the association of bw\textsuperscript{D} with Cen2 requires the large stretch of homologous sequence in \textit{cis}, the bw\textsuperscript{D}-Cen association was analyzed in cells carrying translocations of the bw\textsuperscript{D} region. By shifting the bw\textsuperscript{D} from the end of Chr2 to Chr3 or Chr4, it was observed that bw\textsuperscript{D} no longer associates with Cen2. Moreover, when present on Chr3, bw\textsuperscript{D} had a slight but significant tendency to associate with Cen3, despite its lack of homology with the bw\textsuperscript{D} insert (206). These observations led to the idea that heterochromatin clustering is not strictly dependent on sequence homology but rather on heterochromatin-bound proteins, and that intrachromosomal interactions may be favored by proximity.

A recent study argues that the link between chromosome architecture and transcriptional repression is widespread in Drosophila. A screen from the Karpen group showed that out of a series of Mod(var) proteins (suppressors or enhancers of PEV), half affect native chromosome morphology and cell cycle progression (138), including Su(var)2-10, a suppressor of PEV that colocalizes with the nuclear lamin in interphase (101). Mutant su(var)2-10 nuclei show disorganized and improperly condensed chromosomes, as well as defects in telomere clustering.
and telomere-NE associations. Thus, Su(var)2-10, like the yeast SIR proteins or fly PcG, may represent a class of factors involved in both transcription and long-range chromosomal interactions.

INSULATOR FUNCTIONS AND NUCLEAR STRUCTURE  
The insulator provides a further example of a chromatin function that has been correlated with tethering or sequestering of sequences. The term insulator refers to two different activities that can be distinguished experimentally. The first is an enhancer-blocking activity, which shields a promoter from the action of a distant enhancer. The second is a silencing barrier activity, which protects genes from invasion by neighboring heterochromatin (reviewed in 25). Although within genomes these activities are sometimes linked, proteins involved in the process often confer only one of the two activities. Both functions have been proposed to involve specific configurations of chromatin, such as a topologically defined loop, tethered to nuclear pores (16, 82, 121). Several examples are described below.

In Drosophila, the element Fab-7 contains an insulator flanked by a Polycomb group (PcG) response element that recruits PcG proteins. This element physically associates in trans with an integrated copy of the same element located on another chromosome (9). The association is lost in the absence of PcG protein, which also compromises a correlated silencing function. The Drosophila gypsy insulator has also been studied in detail. Its insertion into the genome causes the surrounding DNA to move to the nuclear periphery in a Su(Hw)-dependent manner (82). Similar to Fab-7 and scs boundary elements, two gypsy elements inserted at distant sites were reported to associate (16, 26, 27). However, Xu and colleagues later showed that the preferential peripheral association of gypsy-containing loci can be genetically separated from the minimal 340-bp insulator element that is sufficient to bind Su(Hw). Moreover, the Su(Hw) insulator can function in the nuclear interior (264). Thus gypsy-mediated anchoring seems not to be a prerequisite for Su(Hw) insulator function.

In vertebrates, a conserved sequence-specific factor CTCF is implicated in the function of a broad range of insulators, and it has been demonstrated to tether a transgene containing the CTCF insulator near the nucleolus (269). Again, it is argued that its insulator activity may reflect a higher-order level of genome organization. This is reminiscent of activities isolated in a fusion protein screen in yeast, which can confer barrier activity by tethering to nuclear pores (121). Missing to date is genetic evidence that disruption of the spatial positioning ablates native insulator function.

Role of Long-Range Organization in Differentiated Gene Expression

Despite the positive evidence of the bwD allele, there are both examples and counterexamples in mammalian cells, in which a locus moves (or does not) relative to a heterochromatin domain, in response to a change in its transcriptional state.
One must conclude that movement away from heterochromatin is neither absolutely required nor sufficient for transcriptional activation (24, 158). Indeed, several experiments suggest that the impact of large-scale repositioning needs to be considered on a longer timescale as a change necessary in cycling cells undergoing non-transient changes in transcription. For instance, in a study of the position of several differentiation-regulated genes in immature or mature B cells, Brown and colleagues showed that inactive genes associate with heterochromatic domains (24), yet this association is only seen in cycling cells. In quiescent cells, association of repressed loci with pericentric heterochromatin is lost, being re-established only after mitogenic activation (24). Spatial reorganization was also reported for genes repressed during T-lymphocyte activation (Rag-1 and TdT). The relocalization of silenced loci to pericentric heterochromatin was observed only in normal differentiating thymocytes, and not in an immature T cell line in which Rag-1 and TdT are repressed in a transient manner.

In quiescent or senescent fibroblasts, the human gene-poor Chr 18 adopts a random position within the nucleus as opposed to its preferential peripheral location detected in proliferating cells (23). The restoration of Chr 18 association with the nuclear periphery requires passage through S phase and mitosis and was still not complete in the subsequent G1 phase, again suggesting that chromosome organization is less tightly regulated in nonproliferating than in proliferating cells.

Why would the stable spatial reorganization of mammalian nuclear architecture require proliferation? We propose that nuclear compartments become increasingly pronounced and functionally important as cells differentiate. It is well documented that eukaryotic replication is spatially and temporally regulated, as is its link with epigenetic determination (168). Genomic loci found in the same compartment during S phase are likely to be replicated at the same time and to come in contact with the same chromatin factors right after replication. Histone variants, modifiers, or chromatin binding factors that influence factor accessibility are likely to act coordinately on newly replicated loci, providing a means to re-establish a given transcriptional and/or spatial pattern of organization in daughter cells, determined by chromatin marks deposited during replication. As such, subnuclear compartments may not be critical for immediate biological events yet would provide a mechanism for the accurate inheritance of transcription patterns. If replication provides an opportunity to reinforce epigenetic marks, it follows that dividing and differentiating cells would tend to have a more robust nuclear organization.

NUCLEAR LAMINS AND CHROMATIN ANCHORING

One element of nuclear architecture implicated primarily in differentiated cell integrity is the nuclear lamina, a perinuclear meshwork of intermediate filament (IF) proteins that associates with an increasing number of inner NE proteins. Lamins are the oldest of the IF family members (35, 71), consisting of a central rod of \( \alpha \)-helical heptad repeats flanked by head and tail domains, which are critical for lamin polymerization (233). Lamins are also dispersed throughout the nucleoplasm,
possibly forming a thin fibrillar network (11, 117). Whereas the perinuclear lamin polymer resists solubilization by salt and chaotropic agents, internal lamins do not.

Lamins are generally classified as A-type or B-type (86). Mammalian A-type lamins include four developmentally regulated variants of the \textit{LMNA} gene (lamins A, C, AΔ10, and C2), expressed in a wide variety of differentiated cells. B-type lamins (lamins B1, B2, and B3 encoded by the essential \textit{LMNB1} and \textit{LMNB2} genes) are present in both embryonic and differentiated cells. To date, no homologues have been detected in plants (203) or in unicellular organisms such as yeast, although they are conserved in flies and worms (34). Nonetheless, several lamin paralogues have been proposed to exist in plants, based on either partial peptide sequence homology to IFs, immunological cross-reaction, or a perinuclear localization (17). These include the 134-kDa NMCP1 protein, which participates in an insoluble perinuclear structure (167). In contrast, a salt-resistant perinuclear framework is missing altogether in yeast (28), perhaps owing to its small genome and closed mitosis.

NE-Associated Proteins

Metazoan cells express many proteins that interact specifically with the lamins. These can be divided into two groups, one comprising integral proteins of the inner nuclear membrane and including several isoforms of lamin-associated protein 2 (LAP2), LAP1, emerin, MAN1, Unc84, and the lamin B receptor [LBR; see (34, 84)]. Figure 2 summarizes interactions involving these and other integral nuclear envelope (INE) proteins. The second group includes proteins that link these components to chromatin. This ever-expanding group of proteins includes germ cell-less (GCL), young arrest (YA), transcriptional repressors MOK2, and Retinoblastoma protein (Rb), sterol regulatory element binding protein (SREBP) factors 1a and 1b, and a 10-KDa, ubiquitous dsDNA-binding factor called Barrier to Autointegration Factor or BAF (141, 143, 230). BAF is widely conserved and binds a 40-aa motif called LEM, a domain shared by several major INE proteins [Figure 2; (27, 274)]. A global BLAST search for LEM domain proteins identified three proteins in \textit{Caenorhabditis elegans}, at least six in \textit{Drosophila}, and many additional LEM proteins in human (34). Some, but not all, LEM domains may share overlapping or redundant roles in nuclear organization.

This redundancy is illustrated by the RNAi-mediated depletion in \textit{C. elegans} of two LEM proteins, emerin and Man1. Efficient depletion of emerin produced no detectable phenotype, whereas the depletion of the Man1 protein to \(\sim 90\%\) was lethal to approximately 15\% of embryos (90, 151). By antagonizing both LEM proteins, however, even an incomplete depletion of Man1 killed all embryos by the 100-cell stage. Such cells showed impaired chromosome segregation and anaphase bridges leading to aneuploidy and death (151). The authors conclude that the worm emerin and Man1 proteins share at least one as yet unidentified function essential for cell division. Functional redundancy may account for the fact that emerin RNAi
FUNCTIONAL NUCLEAR ARCHITECTURE

in HeLa cells was non-lethal (100) and that chromosomal positioning at the NE was unaltered in emerin-deficient cells (21).

A further NE member of the LEM domain family is LAP2, which is present in multiple isoforms in mammalian cells (12, 75, 102), zebrafish, and Xenopus (78, 137, 213), although flies and worms lack LAP2 (34). The major LAP2 forms are \( \beta \), which binds lamin B1 directly (67), and \( \alpha \), which lacks a transmembrane anchor and binds lamin A (46, 249). The LAP2 proteins are unique in having both a LEM and LEM-like domain, which allows LAP2 to bind both DNA or chromatin as well as BAF (27, 74, 75). Since BAF can itself simultaneously bind DNA and LAP2 (221), the LAP2-chromatin association may be regulated in multiple ways. Injection of the BAF-binding LAP2\( \beta \) N-terminal domain into G1-phase HeLa nuclei, or its addition to a cell-free system, has a strong dominant negative effect on nuclear assembly, growth, and replication (78, 265). The effect of LAP2\( \beta \) deletions or point mutations have not yet been reported.

Novel NE components have been proposed by two recent biochemical/proteomic screens that identified, respectively, 19 and 67 new integral NE components, as well as detecting a number of known NE markers (51, 211). Of the new components, 12 have predicted enzymatic activities, 2 (Syne-1 and Syne-2) are related to a known NE-associated component, Nesprin, and another resembles the lamin-binding Unc-84 from C. elegans, which is implicated in nuclear migration and anchoring (51, 142, 162, 211). Whether any of these mediates chromatin anchoring is unknown.

GENETIC ANALYSES OF LAMIN FUNCTION To address the cellular function of lamins, genetic approaches were pursued in worms and flies. C. elegans has only one lamin gene (lmn-1), which is expressed in all cells except mature sperm (150), while Drosophila has both a B-type (Dm0) and A/C-type lamin (C). Lamin Dm0 is expressed ubiquitously, whereas lamin C is found in late-embryonic and differentiated cells (233). Reduced Dm0 expression inhibits nuclear membrane assembly and induces the formation of annulate lamellae, which are cytoplasmic stacks of membrane containing incomplete nuclear pores (145). A mutant Dm0 allele also profoundly affects nuclear morphology and impairs Drosophila development, again owing to alterations in cytoplasmic organization (91). Lamin depletion by RNAi in C. elegans produces pleiotropic phenotypes including rapid fluctuations in nuclear shape, nuclear pore clustering, and abnormal chromosome segregation during mitosis (90, 150). Some embryos escape lethality due to residual levels of lmn-1, yet these have a high incidence of sterility and reduced germ cell count (150). Although improper chromosome segregation appears to be the lethal downstream event of lamin depletion in both flies and worms, the critical execution point for lamin function may well be DNA anchoring during replication or gene expression.

In mammalian cells, RNAi studies demonstrate the different roles of A-type and B-type lamins: B-type lamins are essential for individual cell growth but A-type lamins are not (56, 100). Both proteins associate with decondensing chromosomes,
yet the roles of the two proteins are clearly different. The A-type lamin associates in a complex with BAF and emerin, while lamin B binds earlier, independently, and at a different site (46, 99, 176). If lamin B1 integration into the NE is blocked by mistargeting protein phosphatase I, cells undergo apoptosis (229), an event that does not occur when lamin A assembly fails.

Confirming the RNAi results, homozygous laminA/C knockout mice appear normal at birth. However, their growth rate slows within 2 to 3 weeks; they incur muscle wasting, a loss of white fat, and death by week 8 (235). Histological examination reveals skeletal and cardiac muscle abnormalities that correlate with changes in cardiomyocyte nuclear size and shape, and very significant disorganization of the subnuclear distribution of heterochromatin (184, 235). Cultured Lmna−/− fibroblasts or myocytes accumulate abnormal nuclear blebs with reduced levels of lamins, LAP2, and nuclear pore components in the blebbed regions (235). Centromeric heterochromatin becomes fragmented and relocates to the nuclear interior (184), and in both A/C-silenced HeLa cells and Lmna−/− mice, the LEM-protein emerin shifts from the NE to the endoplasmic reticulum (100, 235).

The general cardiac dysfunction of Lmna−/− mice is proposed to reflect a lack of cytoskeletal tension, possibly due to a loss of a nuclear-cytoskeletal interface (184). This correlates with an increased sensitivity of Lmna−/− cells to mechanical stress and a corresponding loss of mechanotransduction signaling (135), underscoring a role for lamins in nucleo-cyto-skeletal interactions (see below).

An interesting feature of Lmna−/− mice is the similarity of their symptoms to those of Emery Dreifuss Muscular Dystrophy patients (EDMD; 58). Indeed, in humans, Lmna mutations cause both the autosomal dominant (18) and autosomal recessive forms of EDMD (199), while mutations in a lamin A ligand, emerin, are responsible for X-linked EDMD (15). Numerous mutations throughout the human LMNA gene have been linked to eight further diseases, collectively called laminopathies. The laminopathies are generally late-onset diseases with skeletal or muscular atrophy, lipodystrophy, or neuropathy, reflecting structural degeneration of highly differentiated tissues (reviewed in 179).

ANCHORING CHROMATIN DIRECTLY AND THROUGH BRIDGING FACTORS  Early studies correlating the nuclear lamina with chromatin organization were based on the isolation of insoluble nuclear structures from HeLa cells. When histones and soluble proteins were extracted from intact nuclei, genomic DNA remained bound in torsionally constrained loops. DNA loops were much larger in structures that contained the nuclear lamina-pore complex material only (type II matrices), whereas type I matrices preserved an RNAse-insensitive internal network of protein that could also anchor genomic DNA and restrict loop size (139, 140). Subsequent DNA-binding studies confirmed the existence of sequence-independent lamin-DNA interactions but suggested that the physiological interactions with the nuclear lamina were likely to be mediated by histones and other bridging proteins (reviewed in 248). Although the number of candidates that may bridge between
FUNCTIONAL NUCLEAR ARCHITECTURE

Lamins and chromatin is ever-expanding, we present here but a few examples, with a focus on interactions between LBR and HP1, lamin Dm0 and YA, and insights into LEM domain protein-BAF interaction (Figure 2).

In vertebrates, the integral NE lamin B-binding factor LBR has sterol C14 reductase activity, yet independently may also mediate NE-heterochromatin association. Physiological evidence supporting LBR-chromatin interactions comes from a study of HL-60 cells induced to differentiate by retinoic acid (188). During differentiation to a granulocytic cell form, cellular LBR levels increase markedly, lamin levels drop, and the nucleus becomes blebbed, accumulating sheets of chromatin anchored at the NE (188). It is proposed that the loss of lamins increases nuclear envelope plasticity while the upregulation of LBR increases chromatin-NE interactions. When the same cells are induced to form macrophages by phorbol esters, lamin expression increases, emerin is localized to the NE, and there is no nuclear blebbing (188). Importantly, in patients suffering from the Pelger-Huet Anomaly (PHA), a genetic disease caused by a mutation in LBR (113), one sees similar phenotypes: Granulocytes have reduced LBR levels, abnormal chromatin organization, and misshapen nuclei.

An N-terminal LBR domain mediates a sequence-independent, yet saturable and high-affinity ($K_D \sim 4$ nM) interaction with linker DNA (53) and associates with HP1 in various binding assays (266). A complex of LBR, HP1, and the H3/H4 dimer could be isolated (196), suggesting a mechanism through which HP1 might anchor heterochromatin at the NE. HP1 binds tightly to tri-methylated K9 of histone H3 (134) and could thereby recruit H3 methyl-K9 marked chromatin to the NE-associated LBR. Indeed, a GST-LBR N-terminal fusion precipitated such chromatin from “nuclear ghosts” (161). Problematic with this model is the fact that in vivo analyses do not show HP1 is particularly enriched at the NE. Moreover, RNAi-mediated depletion of the Drosophila LBR homologue did not severely affect nuclear organization in either cultured cells or early embryos, although LBR binds tightly to Dm0 lamin and chromatin (255). The DmLBR may bind heterochromatin uniquely in differentiated cells and may require proteins other than HP1. A further potential stage-specific linker between chromatin and lamin in Drosophila is the maternally encoded component Young Arrest [YA; (85)]. YA mutant phenotypes are consistent with a role in proper chromatin decondensation in very early embryogenesis (267). The protein binds tightly to and colocalizes with lamin Dm0, as well as showing affinity for histone H2B and chromatin, albeit at fairly low affinities in vitro [$K_D = 1.1 \mu M$ (268)].

LEM Domain Proteins Bind BAF

Probably the most universal candidate for providing a link between LEM domain proteins (LAP2α, LAP2β, emerin, MAN1, otefin, Bocksbeutel) and chromatin is the small, highly conserved DNA-binding protein called BAF (74, 141, 143, 254). Biochemical studies have shown that BAF binds dsDNA as a multimer in a molar ratio of 2:1 (274), and immunostaining shows BAF throughout the nucleus (74, 99, 220). Its presence at the NE thus results from its affinity for LEM proteins, not DNA.
FRAP, FLIP (fluorescence loss in photobleaching), and FRET (fluorescence resonance energy transfer) analyses in HeLa cells have recently monitored the stability of the BAF-NE interaction, comparing its turnover with that of other NE components (220). Nucleoplasmic GFP-BAF has an extremely rapid recovery after photobleaching ($t_{1/2} = 47\,\text{ms}$), close to that of free GFP ($t_{1/2} = 30\,\text{ms}$), while the NE-bound GFP-BAF population was about three times less mobile. This turnover rate is still much higher than that recorded for GFP-emerin, GFP-LAP2β, or GFP-MAN1, which have recovery half times of $\sim 60\,\text{s}$. Although artefacts due to overexpression of a GFP-fusion cannot be entirely excluded, these results suggest that BAF molecules exchange rapidly at their LEM binding sites. Similarly rapid turnover rates were also monitored for HP1 at its binding sites (31, 63); thus it is possible to contribute to long-range chromatin structure without being integrated into higher-order complexes that impair exchange.

Reminiscent of the double Man1/emerin RNAi results, depletion of BAF in worms and flies resulted in aberrant chromosome segregation (274) and other phenotypes typical for impaired mitosis, i.e., lethality at the fly larval-pupal stage, small brains, and missing imaginal disks. Electron and light microscopy confirmed multiple distortions in interphase nuclear structure including abnormal lamin distribution, large clumps of chromatin and nuclear blebbing (76), possibly resulting from impaired chromatin reassembly with the NE following mitosis. Indeed, in several species BAF plays an important role during chromatin decondensation in late telophase (215). BAF is responsible for the dominant negative effect of the Lap2β N-terminal domain (221), and a dominant BAF mutation displaces emerin, LAP2β, and lamin A (but not lamin B) from reforming nuclei. It appears, therefore, that as nuclei assemble the LAP2β-BAF complex recruits emerin to chromatin, which in turn promotes lamin A binding (99, 215).

The Nuclear Lamina and Gene Expression

In addition to the interactions necessary for nuclear assembly, the NE-association of chromatin is correlated with gene repression. In one model, the nuclear lamina (e.g., lamins, LBR, or LEM proteins) sequesters folded chromatin from transcription, whereas in a second, the NE would play a more active role by stabilizing repressive complexes. Alternatively, lamins might directly interfere with transcription.

The interference of lamin with RNA pol II activity has been examined using dominant negative alleles of lamin A, which makes it difficult to distinguish direct from indirect effects. Microinjection of an N-terminal deletion of lamin A, lmaΔN, leads in vitro to the formation of lamin aggregates and alters the distribution of splicing factors, without influencing chromatin distribution or nuclear transport (133, 225). Under these conditions, there is a dramatic decrease in RNA Pol II-mediated transcription and a delocalization of TBP to lamin aggregates. Conversely, the reversible inhibition of RNA Pol II causes a coordinated reorganization of lamin A/C and splicing factors (133). Given that lamins are not integral components of the basal transcriptional machinery and behave in this assay in a
FUNCTIONAL NUCLEAR ARCHITECTURE

manner coordinated with splicing factors (133), interpretation of lmna dominant negative effects is not straightforward. Feedback inhibition due to splicing defects is possible.

More convincing is the involvement of lamins in transcription through transcriptional regulators. Again, the simplest scenario is one in which either the peripheral NE or intranuclear lamin A/Lap2α complexes stabilize a repressive complex on DNA, such as the Rb-E2F complex, preventing gene activation. Blot overlay, immunoprecipitation, and extraction experiments have shown a reproducible interaction between hypophosphorylated Rb and nuclear lamin A (163, 190), an interaction that in vivo appears to require a co-complex with LAP2α (165). The hypophosphorylated Rb represses the transcription of E2F-regulated genes by binding E2F. Upon cell growth stimulation, Rb is phosphorylated and releases both lamins and E2F-DP, which presumably remains bound to its target promoter. In quiescent primary skin fibroblasts, the expression of LAP2α and phosphorylated Rb drop below detectable levels but are restored upon re-entry into the cell cycle (165). Given that LAP2α and lamin A are not exclusively perinuclear, this Rb-E2F repression mechanism could also function internally.

Like Rb, the conserved and ubiquitously expressed transcription regulator Germ cell less (GCL) also binds E2F-DP3α and thereby represses E2F-dependent gene transcription (44, 131, 185). Interestingly, GCL also binds a conserved domain common to LAP2β and emerin and localizes in speckles at the NE (44, 114, 185). GCL also mediates the localization of E2F-DP3α to these sites (44). Overexpression of either GCL or LAP2β partially represses E2F target genes, while complete repression required overexpression of both (185). This additive effect may indicate a function for LAP2β that is independent of GCL, perhaps acting through BAF. BAF and GCL bind in a mutually exclusive manner to emerin in vitro, suggesting that the two factors may regulate each other’s interaction (114). Whether BAF and GCL compete as well for LAP2β is not known.

The binding of Rb to lamin A and LAP2α, and of GCL to LAP2β and emerin, implicates NE components in the repression of E2F-DP-regulated genes. Most transcription assays testing this repression were performed with reporter constructs and overexpressed transgenes. However, a recent study shows that mgcl1−/− mice have abnormal sperm development, altered testis-specific gene expression patterns, and highly distorted nuclei in the differentiated tissues that normally have high Gcl1 levels, i.e., exocrine pancreas, liver, and testes (131). This mouse study confirms that a NE-mediated regulation of gene expression can occur under physiological conditions in differentiated tissues.

Other developmentally expressed factors that colocalize with or bind NE proteins (Figure 2) include a key regulator of cholesterol metabolism, SREBP-1 (152), Oct-1 (120), and the death-promoting repressor Btf, which binds the same domains in emerin as GCL (98, 128). The transcriptional repressor MOK2, which binds to lamins A/C in vitro, competes for the binding of the Drosophila cone-rod homeobox protein, CRX, at its target promoters (258). In other assays, fly BAF was shown to bind to and decrease the transcriptional activity of CRX, which
regulates photoreceptor differentiation (52). Finally, during *Xenopus* embryogenesis the NE protein XMAN1 antagonizes bone morphogenic protein signaling and neural induction by interacting with the coactivator Smad1 (189). We propose that tissue-specific repressors, as well as growth repressors like Rb, use the NE to help promote repression, profiting from a stable nuclear subcompartment that may sequester chromatin remodelers and histone deacetylases. As shown for yeast telomeres, such subcompartments can facilitate the establishment and maintenance of transcriptional repression.

**Linking Nuclear and Cellular Architecture**

A final role for NE proteins in differentiated tissues is that illustrated by the nesprins (nuclear envelope spectrin repeat, a.k.a., Syne, Myne, or NUANCE), which bind both lamin A and emerin (171, 172). These large integral NE proteins have varying numbers of spectrin repeats (SR), usually a C-terminal membrane-spanning domain and an actin-binding domain of claponin-homology (172, 273). In general, all known SR family members share one basic function, that of linking membranes to the cytoskeleton. They thereby fulfill a specialized role in establishing and maintaining the 3-D architecture of the cell. In structured tissues such as skeletal and cardiac smooth muscle, nuclei have distinct subcellular positions, which requires the binding of nesprin-1α to both the nuclear lamina and cytoplasmic actin (6, 273).

Genetic studies in flies and worms reinforce the idea that nesprin serves the tissue-specific function of coordinating the nucleus with the cytoskeleton, particularly in structured tissues. Loss of the *Drosophila* homologue MSP-300 (272) or the *C. elegans* nesprin ANC-1 [nuclear anchorage defective (228)] severely affects nuclear positioning, as does overexpression of the CeANC-1 actin-binding domain. Flies that bear mutations in MSP-300 die with muscular deficiencies, underscoring the importance of proper nuclear positioning in differentiated tissues (204).

Another fly member of the nesprin family called Klarsicht (Klar) diverges by having no cytoplasmic actin-binding domain. Nonetheless, in *Drosophila* eye development, Klar functions to ensure the movement of photoreceptor nuclei by linking the microtubule organizing center (MTOC) to the nuclear lamina (65, 194). In this system, both the lamin Dm0 and Klar are necessary for proper nuclear migration, providing genetic evidence for critical cytoskeletal-NE connections. Such studies provide examples of how the loss of lamin and nesprin integrity might lead to cytoskeleton-based dystrophies.

**DNA REPLICATION AND NUCLEAR STRUCTURE**

Eukaryotic genomes initiate DNA replication at multiple sites (origins) dispersed along each linear chromosomal arm. In budding yeast, origins of replication are short *cis*-acting sequences called ARS elements (Autonomously Replicating
Sequences) that support both autonomous plasmid replication and genomic initiation events. In most other organisms, genomic origins are less strictly sequence-defined and far more dependent on chromatin structure (reviewed in 54).

Replication Foci

In higher eukaryotes, S-phase DNA synthesis occurs in discrete foci (Figure 3A), which change in number and distribution as S phase progresses [(49, 182, 187) and references therein]. By counting foci and estimating the number of replication origins, it was proposed that between 5 and 10 bidirectional forks cluster in an average replication focus in cultured HeLa cells (123), and on average, ~10 origins cluster at 20 to 40 foci in yeast (144, 193). In cultured cells, a combination of pulse-labeling with BrdUTP and DNA combing showed that neighboring origins tend to fire synchronously (123), and double-pulse labeling experiments showed coordinate progression of replication forks within a focus (156, 164, 238). Neighboring replication foci colocalize in metaphase chromosomes, and the propensity of given domains to associate in S phase seems to be mitotically heritable (123). Taken together, these observations reinforce the hypothesis that replication foci are sites at which polymerases remain stationary, and through which template DNA is pulled as it replicates (Figure 3A). In mammalian cells, not only polymerases but chromatin assembly factors and modifying enzymes colocalize to sites of replication (132, 205), suggesting a spatial coordination of DNA replication and the duplication of chromatin structure (reviewed in 168).

The progressive appearance of discrete patterns of replication foci raises the question whether replication complexes disassemble and reform, or move as S phase progresses. This was addressed with time-lapse imaging of GFP-PCNA in mammalian cells. Replication foci do not move directionally, merge, or divide, but instead focal pattern changes seem to reflect coordinated, but asynchronous, assembly and disassembly events (147). The reproducible appearance of recognizable focal patterns may also be linked to an organism’s developmental stage. In early Xenopus and Drosophila embryos, S phase is extremely short, owing to initiation events every 5 kb. At the mid-blastula transition (MBT), a developmental switch leads to the selection of preferred initiation sites, coincident with a major remodeling of chromatin structure and transcriptional competence (118, 207). Thereafter, the amount of late-replicating DNA increases as cells differentiate (66).

Patterns of replication origin firing differ between primary and transformed mammalian cells. Kennedy and colleagues showed that in primary culture cells, replication in early S phase initiates at a small number of perinucleolar foci, a pattern not observed in immortalized cell lines (129). The subsequent focal patterns are similar in transformed and primary culture cells, but the pattern changes again in the final 3 or 4 cell divisions prior to exit from the cell cycle (10). The final rounds of DNA synthesis occur exclusively in the perinucleolar replication foci that typify
early S phase in primary cells, such that all genomic sequences are replicated at these foci only. These authors suggest that the spatio-temporal organization of DNA replication can be regulated by cell-cell contact or signaling pathways that regulate cell growth. The change in replication patterns may also help prepare the cell for quiescence.

**DNA Replication, Lamins, and Matrix**

Intranuclear lamins have also been correlated with sites of replication. B-type lamins localize to late S-phase heterochromatic replication sites in mouse NIH 3T3 cells (174), whereas lamin A/C was found at the earliest sites of DNA synthesis in human WI38 cells by immunostaining (129). Biochemical support for lamin function comes from the immunodepletion of lamin B3 (XLB3) from interphase *Xenopus* egg extracts. This immunodepletion impairs replication in reconstituted nuclei (169, 183), although the effects could be indirect, reflecting destabilization of the NE or nuclear transport mechanisms. More compelling are experiments in which dominant negative forms of lamin A are added after nuclear assembly. These did not impair the initiation of DNA replication, but instead blocked elongation, while NE integrity and protein import were unaffected (57, 175, 226). When DNA synthesis is blocked by lmnaΔN, elongation factors such as PCNA and RFC became dispersed, suggesting that lamins may help stabilize replication foci. For this function, the redundancy of lamins B and A/C needs to be tested, since the complete absence of lamin A/C clearly does not impair replication during early mouse development.

Various differential extraction procedures have shown that DNA replication foci are recovered with a nucleoskeleton or matrix, and that mammalian replication intermediates can be significantly enriched by isolating a matrix fraction (116, 192, 246). Indeed, in many species origins of replication colocalize with genomic S/MAR sequences (3, 48, 125). To test the notion that the S/MAR is essential for initiation in CHO cells, one such element, situated ∼10 kb from the bidirectional DHFR origin, was cleanly excised from its genomic location (170). The deletion provoked loss of the matrix-binding activity but had no effect on either the efficiency or timing of initiation. The MAR-free origin did, however, have a delayed separation of replicated sisters, as detected by the delayed appearance of doublet FISH signals. At face value, this suggests that the AT-rich MAR normally facilitates homologue separation, something achieved either by recruiting DNA topoisomerases or displacing/inhibiting cohesin. Perhaps reflecting the same mechanism, a mammalian MAR has recently been shown to improve the mitotic distribution of an episome but not to influence its replication per se (209).

**Determining the Timing of Origin Firing**

One of the more intriguing links between nuclear organization and replication is the correlation of specific replication patterns with the temporal progression of S phase. Individual budding yeast origins fire reproducibly in early, mid-, or late-S phase (60), and genomic approaches have confirmed regulated origin firing
along entire chromosomes in yeast, fly, and human cells (201, 214, 259, 261). In budding yeast, origin activation in early S phase appears to be the default state for an extrachromosomal ARS, and cis-acting chromosomal elements, such as telomeres, delay origin firing until late-S phase (61). Late activation, which is controlled by chromosomal context, is also observed at internal origins, although sequence analysis failed to identify specific motifs responsible for late firing (70). Recently, it was noted that the two ends of each of the 16 yeast chromosomes fire synchronously (201). Surprisingly, left and right telomeres do not reproducibly share sequence homology yet can be juxtaposed to each other, which may implicate nuclear organization in regulating origin firing.

For *S. cerevisiae* origins, the late-firing status is established between mitosis and START (200) and correlates with a transient, but nonetheless preferential, localization at the NE in early G1 phase (107). However, the excision of a late-firing origin after this time point allowed the chromatin ring to diffuse freely away from the telomere while remaining late replicating (107, 200). This suggests that an origin’s position in S phase is less critical than its localization in early G1. The early G1-phase factors that determine late firing may be either perinuclear enzymes that induce characteristic changes in chromatin or factors that are loaded onto the origin itself, in a NE-facilitated manner.

Is perinuclear positioning sufficient to confer the late-firing status? This possibility was excluded by a study in which an origin was anchored to the NE through targeted interaction with an integral membrane protein called Yif1 (270). Anchoring was not sufficient to delay the firing of an early origin, although the nucleation of silent chromatin, which also confers anchoring, is. In brief, the timing of initiation of a subtelomeric origin is selectively advanced 10 min in silencing-deficient yeast strains (232), as well as in strains lacking the end-binding complex yKu (39). Since telomeres in *sir*-deficient strains remain peripherally anchored by the yKu complex (105), these results suggest that both chromatin structure and position regulate yeast origin timing. Finally, the Raghuraman-Heun excision experiment suggests that whatever is contributed by NE-proximity in G1 phase becomes an intrinsic component of the origin’s structure, leading to late initiation despite subsequent delocalization.

In mammalian cells, the temporal pattern of origin firing is also established during G1 phase at the Temporal Decision Point or TDP, which is established in CHO nuclei ~1 to 2 h after metaphase (49). Studies that compare the distribution of differentially labeled early and late-replicating domains in a subsequent S phase (induced by incubation of isolated nuclei in a *Xenopus* egg extract) suggest that the establishment of timing may correlate with the resumption of distinct chromatin positions, either at the NE or near the nucleolus, in interphase nuclei (49). By looking at specific origins, it was shown that the late-replication character of the silent β-globin locus is established in early G1 phase, coincident with its repositioning near peripheral heterochromatin (148), and a similar correlation could be documented for the mouse-imprinted Igf2-H19 (89). In this case, the two parental alleles were found in different subnuclear compartments in fetal liver cells, with the early replicating allele more internal than the late-replicating imprinted allele.
Surprisingly, in nonexpressing embryonic stem cells, the positions were switched: The late-replicating locus was more internal than the earlier one. Still, paternal and maternal alleles were clearly restricted to different subnuclear compartments and in both cases correlated with differential replication timing (89).

Further studies show that, as in yeast, position alone cannot determine the time at which a mammalian origin fires. The clearest example of this is given by silent leukocyte-specific genes that are associated in trans with centric heterochromatin in mouse lymphocytes, yet replicate early (8). In contrast, the integration of transgenes directly into this heterochromatin domain renders them late replicating, a status lost when the repressed transgene shows variegated expression. This argues that neither juxtaposition in trans to heterochromatin nor transcriptional repression per se is sufficient to ensure late replication in mice. Nonetheless, expression status can impact replication timing, even though its effects can be mitigated by cis-acting elements or chromatin structures. Indeed, for the developmentally regulated replication timing patterns of the human β-globin locus, the Igf2-H19-, and the SNRPN-imprinted loci, cis-acting enhancers or locus-control elements clearly influence replication timing, independently of their impact on transcription (89, 219, 222).

TRANSCRIPTION AND REPLICATION TIMING  The most widely accepted generalization about replication and transcription was based on studies of mammalian chromosomes: At a gross chromosomal level, late-replicating Giemsa-dark bands contain tissue-specific genes, whereas the early replicating Giemsa-light or R-bands contain ubiquitously expressed housekeeping genes (115). More recently, an increasing number of exceptions to the correlation between constitutive expression and early replication has forced its re-evaluation: G- and R-bands may have features other than transcription that influence replication timing (66).

Genome-wide replication profiling in yeast and flies have examined rigorously the relationship between transcription and replication timing (201, 214). In S. cerevisiae, no correlation between transcription and timing of DNA replication was observed and early firing seems to be a default state (201). On the other hand, in Drosophila Kc cells replication timing and expression data were examined for 5077 genes; a good but not absolute correlation was found, linking early replication with expressed genes (214). Exceptions to this are numerous, however, particularly in vertebrates. Examples include the leukocyte-specific genes discussed above (8), the silent XIST gene on the active X chromosome, which replicates before the expressed allele on the inactive X (80), and asynchronous replication of imprinted or monoallelically expressed genes, a pattern that actually precedes the establishment of differential expression (87).

There are also exceptions among simple repeat DNAs: The centromeric bovine satellite I repeat, alpha-satellite sequences in human cells, pericentric heterochromatin and centromeres in mice, some human telomeres, the Drosophila inner centromeric sequences, and β-heterochromatin all replicate in early or mid-S phase and not late-S phase like other simple repeat sequences [(130, 187, 214) and references therein]. Even in fission yeast, the heterochromatic centromeres and silent mating-type cassettes replicate in early S phase (130). This last result is
particularly striking because, unlike budding yeast, fission yeast pericentric heterochromatin has the same histone components and modifications as centric heterochromatin has in higher eukaryotes. In conclusion, it seems that some heterochromatin structures lead to late replication whereas others do not. This rules out a strong causal relationship between the two, and we suggest instead that transcription and replication are linked through a more subtle third characteristic, an aspect of chromatin structure that influences both.

ORIGIN TIMING AND CHROMATIN STRUCTURE Besides the delay imposed by SIR-mediated silencing on initiation (232), the histone tail deacetylase Rpd3 also contributes to replication timing in budding yeast cells (250). More generally, Turner and colleagues correlated late replication with underacetylated histone tails in several organisms (245). Linker histones may also contribute to temporal programming in some cells. For example, the lack of H1 in the Xenopus early embryo correlates with the absence of origin specificity and temporal organization, and its addition to a Xenopus egg extract reduces the frequency of initiation events (154). Moreover, the progressive accumulation of H1 after the MBT coincides with the accumulation of late-replicating DNA (66).

If the local composition of chromatin is a major determinant for origin timing, then late replication could also be responsible for maintaining chromatin structure, since some histone deacetylases and remodeling enzymes are specifically associated with late-replicating foci [see below (168)]. Consistent with this view is the fact that differential replication timing is a feature of all monoallelic expressed loci, a state that is established very early in development before differential gene expression is manifest (87). Moreover, the asynchronous replication of imprinted genes can occur independently of either gene expression or DNA methylation (89): The imprinted locus Igf2-H19 is still asynchronously replicated in Dnmt1- and Dnmt3L-deficient stem cells, which lack differential DNA methylation and imprinted gene expression (89). In this case, rather than resulting from transcription, replication timing is more likely to participate in the establishment of the different transcription states.

A further example of this is the phenomenon of allelic exclusion, which regulates selective antigen receptor expression during differentiation of the mammalian immune system (178). Mostoslavsky et al. showed that asynchronous replication is established randomly in pre-lymphocytes early in development and is maintained thereafter by clonal growth. This pattern may represent an epigenetic mark for allelic exclusion, since the early replicating allele is almost always initially selected to undergo rearrangement in B cells. FISH analyses of both human and mouse genes expressed in a random monoallelic fashion showed chromosome-specific timing for the replication of such genes, as if all imprinted genes on either the maternal or paternal homologue are regulated coordinately. Thus homologue nonequivalence is not restricted to the X chromosome (59, 223), and the selection of an allele as early or late-replicating seems to be chromosome, not gene, specific. Since chromosomes are found in different territories, these phenomena may be spatially controlled.
Epigenetic marks not only have to be established but also propagated through cell division, and the problem boils down to a question of how local chromatin structure is duplicated. We propose below that both subnuclear localization and replication timing provide means for the establishment and perpetuation of epigenetic features.

Establishment and Maintenance of Epigenetic States

The replication fork provides an opportune situation for both creating and propagating an epigenetic state of chromatin. Parental histones are distributed randomly to the daughter strands of replicated DNA (224), yet newly synthesized histones are also incorporated. Whether a specific chromatin modification is reinforced or removed may result from the time within S phase at which replication occurs, particularly if the chromatin component or modifier itself shows S-phase regulation (reviewed in 168). One of the best arguments in favor of this hypothesis is based on the microinjection of reporter plasmids into Rat1 cells at different times during S phase (271). The exogenous DNA was assembled into transcriptionally active, hyperacetylated chromatin if injected in early S phase, while it was assembled into transcriptionally inert, hypoacetylated chromatin when injected late. Once microinjected DNA assumed a particular replication profile, its transcriptional state was preserved through cell division. That replication timing correlates with the type of chromatin assembled can be explained by the fact that a histone deacetylase, HDAC2, and a chromatin remodeling complex, WICH, seem to be specifically localized to late-replicating foci (22, 205). Modulating the impact of replication timing are specific cis-acting sequences or regulatory factors like Rb, whose presence may modulate the chromatin of Rb-regulated early replicating genes (19, 129).

We propose further that the spatial organization of DNA synthesis contributes in a manner similar to temporal factors: If different types of chromatin are juxtaposed, they form a subcompartment of the nucleus that can provide a local concentration of chromatin modifying factors (Figure 3B). As discussed above (236), chromatin composition can also, in turn, determine sequence localization (Figure 1). Indeed, although long-term inactivation does not always correlate with late replication, it does correlate with relocation to a subnuclear domain in which heterochromatin factors such as HP1 concentrate (8). The enrichment of HP1, a mark for heterochromatin, persists during DNA replication (238), allowing a rapid reassociation with replicated sequences and subsequent re-packaging into a heterochromatic state.

Finally, changes in the spatial positioning of replication can be used to switch transcriptional states. This may play a role, for example, in monoallelic exclusion events, and the change in expression that correlates with a cell’s withdrawal from the cell cycle (10). In this latter case, the sequence of replication patterns was modified several cell divisions before quiescence, with the result that the entire genome then passed through perinucleolar foci for replication, possibly permitting a global remodeling of chromatin. This may be aided by the binding of Rb, as well
as HDAC1 and HDAC2, which colocalize with these perinucleolar replication sites. If this can be shown to lead directly to a modification of the transcriptional status within the nucleus and be modulated by specific mutations, we will have come a significant way toward demonstrating the synergistic impact that nuclear architecture and \textit{cis}-acting regulators can have on differentiated gene expression.

**PERSPECTIVES**

The spatial positioning of chromosomes and subchromosomal domains in the interphase nucleus is a control mechanism that acts not in place of, but together with, tissue-specific transcription factors and gene promoter controls to facilitate the heritable patterning of gene expression. Genetics tells us that the disruption of nuclear order compromises the integrity of highly differentiated cells. The nuclear envelope and the clustering of simple repeat DNA concentrates general chromatin repressors, making sure that they act efficiently on sequences in their proximity and not promiscuously throughout the nucleoplasm. Redundancy in anchoring mechanisms is a recurrent theme. The resulting nuclear compartments allow an organism to be economical with mechanisms for gene repression, using general chromatin repression systems for different sets of genes in different tissues. Future studies will elucidate how these domains are chosen and maintained through organismal development.

**ACKNOWLEDGMENTS**

We apologize to all colleagues we could not cite due to space limitations. We thank the members of the Gasser laboratory for tolerance during this writing endeavor, and thank Dr. K. Bystricky for reading the text. A.T. acknowledges EMBO for a long-term fellowship and S.M.G. the Swiss National Science Foundation and Swiss Cancer League for continued support.

The \textit{Annual Review of Genetics} is online at http://genet.annualreviews.org

**LITERATURE CITED**


Global chromosome positions are transmitted through mitosis in mammalian cells. Cell 112:751–64


149. Li H, Bingham PM. 1991. Arginine/serine-rich domains of the su(wa) and tra RNA processing regulators target proteins to a subnuclear compartment implicated in splicing. *Cell* 67:335–42


197. Pryde FE, Gorham HC, Louis EJ. 1997. Chromosome ends: all the same under


factor interacts with the cone-rod homebox and represses its transactivation function. J. Biol. Chem. 277:43288–300
Figure 1  Self-perpetuating mechanisms for the anchoring of silent loci in yeast. 1. yKu70/80 or Sir4 can be recruited independently of silencing to unanchored telomeric loci or silencers. yKu binds the ends of chromosomes, and Sir4 interacts with both the Rap1 C-terminal domain and internal silencers. 2. Binding of yKu and Sir4 will bring the loci to the NE through interactions between Sir4-Esc1, and yKu bridges to an uncharacterized NE factor. This will bring these loci close to other silent chromatin, such as telomeres and mating-type loci. 3. The clustering of silent loci creates a high local concentration of silencing factors, which will increase the probability of assembling silent chromatin and of Sir complex spreading along the chromatin fiber. 4. Sir complex binding will create additional NE anchorage sites through Sir4-Esc1 interactions, reinforcing the peripheral localization of the silent locus.
Interactions between proteins and chromatin at the nuclear envelope. Proteins are classified as integral nuclear envelope proteins (yellow), lamins (gray), or chromatin binding transcription regulators (red). Simple connecting lines indicate interactions that have been shown with biochemical assays, and bold lines indicate multiple lines of evidence. The numbers near the connecting lines correspond to relevant references, and interactions with the NE are indicated above the corresponding protein. For clarity, interactions between inner NE proteins [for LBR: (53, 113, 161, 196); for LAP2: (67, 75, 185), or lamins (150, 184)] and DNA/chromatin are not shown. Due to space limitations, we are unable to cite all relevant references, particularly with respect to DNA/chromatin interactions with NE components. The proteins hatched in gray are specific to Drosophila.
A

S phase

0-6 h
6-9 h
9-11 h
11-12 h

B

Temporal organization

S phase

Spatial organization

Euchromatic/heterochromatic

Early/Late:
specific factors available
Specific chromatin structure
Specific timing of replication

Parental nucleosomes

Concentration of specific factors
Specific chromatin structure
Specific localization
New nucleosomes

See legend on next page
Figure 3  Genomic replication is organized in recognizable and sequential focal patterns. (a) CHOC 400 cells were synchronized at the G1/S border and released into S phase. At various intervals thereafter, cells were pulse labeled for 5 min with BrdU and stained with anti-BrdU antibodies. Shown are examples of successive temporal patterns of replication (49). We model replication within one replication focus (large oval), containing origins (small circles) of three chromatin loops attached to polymerizing sites (small ovals). As replication occurs, daughter strands are extruded in loops and the parental strand slides through the fixed sites. In the bottom panel, isolated BrdU-labeled DNA was spread as a linear fiber and visualized by fluorescent detection of the newly synthesized DNA (123). Each panel contains three regions of newly replicated DNA along one fiber of 375 kb. Images supplied by D.A. Jackson. (b) Model for epigenetic propagation of a chromatin state through replication position and timing. During DNA replication, parental histones are randomly segregated between the two-daughter strands (224), and newly synthesized histones are incorporated as well. Restricted availability of specific factors (e.g., histone modifiers, chromatin remodelers, or other factors) either in time (left) or space (right) could help propagate specific chromatin structures, which would in turn determine their specific replication timing or localization within the nucleus.