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Chromatin Structure and DNA Replication: Implications for Transcriptional Activity

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The period in the cell cycle when the genome is replicated (S phase) is crucially important for the establishment and maintenance of programs of differential gene activity. Not only must DNA be replicated, but the chromosome itself must be duplicated. The majority of genes in the proliferating cell of a defined type retain the same states of transcriptional activity through cell division. This requires the duplication of the precise nucleoprotein complexes directing gene transcription or repression on the nascent DNA templates. The maintenance of these specific regulatory complexes through replication reflects the commitment of a defined cell type or line to a particular state of determination. Preexisting chromosomal structures are transiently disrupted by transit through the replication elongation complex. Most of these structures are faithfully reassembled following replication through mechanisms discussed in this chapter. However, the transient disruption of these structures also offers a window of opportunity for modifying regulatory nucleoprotein complexes. These alterations can either activate genes through the disruption of repressed states, or direct the repression of previously active genes. Thus, cell division offers a molecular mechanism to redirect the commitment of a cell toward a particular determined state. A consideration of the processes occurring at the eukaryotic replication fork suggests how this important development process might be accomplished.

IMPLICATIONS OF DNA REPLICATION FOR STABLE STATES OF TRANSCRIPTIONAL ACTIVITY

Active and Repressed States of Eukaryotic Genes

The local nucleoprotein complexes required to maintain a eukaryotic gene in an active or repressed state have been defined in some detail (Tjian and Maniatis 1994; Wolffe 1994a). Transcriptional activity for a given gene depends on a number of sequence-specific transcription factors (e.g., SP1), structural proteins (e.g., HMGI/Y), and non-DNA-binding proteins associated with the promoter interacting to recruit general transcription factors (e.g., TFIIA, TFIIB) together with the TFIID complex (containing TBP [*TATA binding protein*] and the TAFs [*TATA associated factors*]). The assembly of this large nucleoprotein complex is initiated through the association of DNA-binding proteins and requires many intermediate steps leading to the recruitment of RNA polymerase II and, eventually, to transcription itself. Conversely, several features may determine a gene to be transcriptionally inactive. A common mechanism appears to be a deficiency in an essential component required for the assembly of the active complex. If this component is a DNA-binding protein, the cognate DNA sequence might become associated with the histone proteins. Specific nucleosomal structures assembled by the core histones (H2A, H2B, H3, and H4) might restrict the subsequent association of either sequence-specific DNA-binding proteins or the basal transcriptional machinery (Simpson 1991; Wolffe 1994b). Other proteins may stabilize repressive higher-order chromatin structures dependent on prior association of the core histones (Hansen and Wolffe 1994); these include linker histone variants (Khochbin and Wolffe 1994) or the chromodomain (*chromatin modification organizer*) proteins such as HP1 and Polycomb (James and Elgin 1986; Paro and Hogness 1991).

Generally, the assemblies of nucleosomes or transcription complexes on the promoter of a eukaryotic gene are mutually exclusive. The prior assembly of nucleosomes can prevent transcription factors from binding to DNA and, conversely, the prior assembly of a transcription complex prevents nucleosome formation from repressing transcription (Fig. 1). Although these results provide an excellent molecular basis for the maintenance of stable states of gene expression in a terminally differentiated nondividing cell, they do not explain why either transcriptionally active or inactive states are assembled onto DNA in the first place, nor do they explain how such states can be propagated through cell division. Clearly, because both nucleoprotein structures can incorporate the same DNA molecule, the possibility exists of a competition occurring between the assembly of the two structures. This competition, in fact, occurs dur-

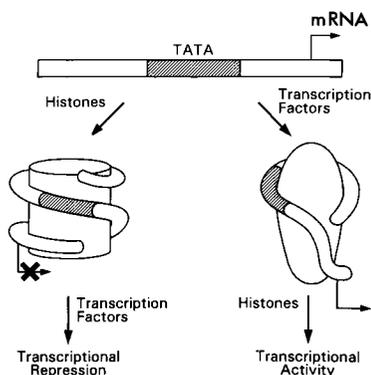


Figure 1 Nucleosome assembly and transcription complex assembly are often mutually exclusive. Two alternate pathways are shown for the association of DNA-binding proteins with a promoter containing a TATA homology. The start site of transcription of mRNA is indicated by the bent arrow.

ing the staged assembly of either active or repressed states following replication (see Almouzni et al. 1990a; Aparicio and Gottschling 1994). Molecular mechanisms that influence the outcome of this competition direct the commitment of a cell to a particular state of determination or facilitate developmentally regulated switches in cell fate. However, to appreciate how this competition occurs, we must first discuss the consequences of DNA replication for preexisting chromatin structures.

Impact of DNA Replication on Preexisting Chromatin Structures

Chromatin consists of long arrays of nucleosomal DNA interspersed with specific regulatory nucleoprotein complexes. The replication fork moves through chromatin without apparent impediment. Replication fork progression disrupts preexisting nucleosomes; however, the fate of regulatory nucleoprotein complexes depends on the particular structure examined.

Nucleosomes

Major considerations for preexisting nucleosomes during the replication process are whether the histones present in the nucleosome stay together on nascent DNA, and whether nucleosomes are randomly or conservatively segregated to daughter DNA strands. DNA replication requires the transient unwinding of duplex parental DNA into two single-stranded regions. Although histones associate with single-stranded DNA (Al-

mouzni et al. 1990b), they do not assemble nucleosomes. This property, coupled with the competing protein-DNA interactions involved in DNA synthesis at the replication fork, probably accounts for nucleosome disruption. Histones released from the parental chromatin during replication *in vitro* can be easily sequestered onto competitor DNA (Gruss et al. 1993). However, *in vivo* these histones are sequestered onto daughter DNA molecules close to the replication fork (see Fig. 4) (Sogo et al. 1986; Perry et al. 1993).

A nucleosome contains an octamer consisting of two molecules each of the four core histones (H2A, H2B, H3, and H4) and a single molecule of a fifth linker histone (H1). The four core histones and the linker histone have very selective interactions with each other (Fig. 2A) (Arents et al. 1991; Arents and Moudrianakis 1993). Our most detailed understanding of nucleosomal architecture and construction has relied on *in vitro* experiments that have attempted to reconstruct nucleosomes with purified histones. These experiments have been informative, although they involve dialysis from high salt to low salt concentrations and do not employ the molecular chaperones used *in vivo* (see below). The central "kernel" of the nucleosome is made up of two heterodimers of histones H3 and H4. Only when this "tetramer" is bound to DNA can two heterodimers of H2A and H2B bind to complete assembly of the histone octamer (Hayes et al. 1991). One heterodimer of H2A and H2B binds to either side of the histone tetramer in an interaction dependent on both protein-protein and protein-DNA contacts. Only when the complete octamer of core histones has assembled on DNA can a single molecule of linker histone be stably bound (Fig. 2B) (Hayes and Wolffe 1993). The exact position of the linker histone within the nucleosome is currently the subject of controversy (Pruss et al. 1995); however, in the one case in which it has been mapped within a specific nucleosome, it occupies an asymmetric position within the nucleosome (Fig. 2C) (Hayes et al. 1994).

In vivo a comparable assembly of the nucleosome occurs. A tetramer (H3, H4)₂ and a dimer (H2A, H2B) are stable at physiological ionic strengths (Eickbusch and Moudrianakis 1978). However, they will not associate together in the absence of DNA under physiological conditions. The tetramer (H3, H4)₂ must again associate with DNA on newly replicated DNA before H2A and H2B can complete the nucleosome core (Almouzni et al. 1990a). Histone H1 is the last protein to be stably sequestered, completing the nucleosome (Fig. 3) (Worcel et al. 1978). I expand on the details of replication-coupled chromatin assembly later in this chapter.

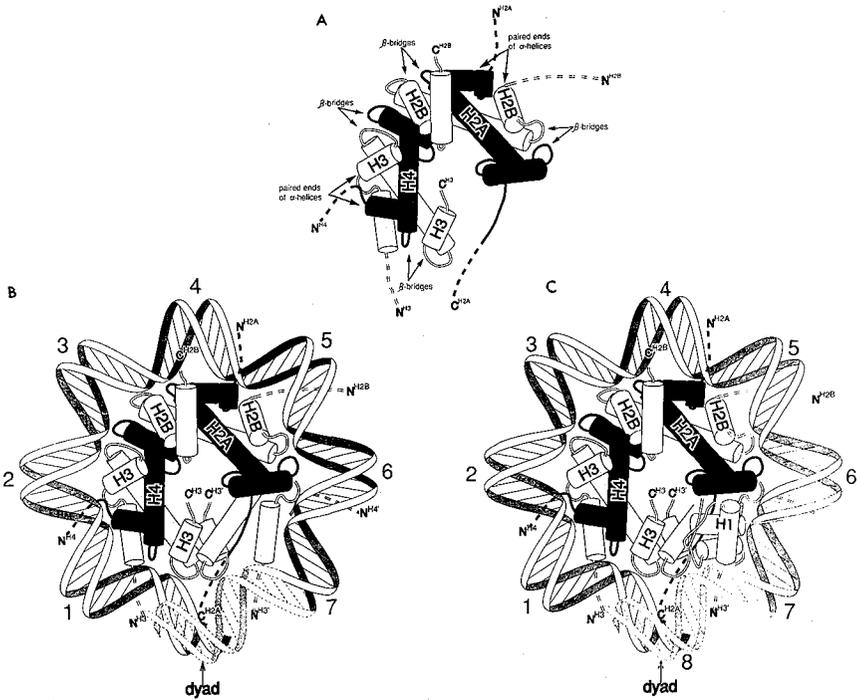


Figure 2 Nucleosomal architecture. (A) The histone fold and DNA-binding motifs. The relative juxtaposition of the two histone heterodimers as viewed "from the top" (i.e., along the superhelical axis of the DNA) is shown. The approximate positions of the flexible histone tails are shown by broken lines. Note the six regularly spaced domains (*double arrows*) predicted to be involved in DNA binding. (B) A view down the superhelical axis of the nucleosome core. The helical turns of DNA are numbered relative to the dyad axis (0). (C) One potential position for the linker histone H1 globular domain within the nucleosome (Pruss et al. 1995).

Jackson (1987, 1990) determined that a substantial fraction of the preexisting octamers associated with DNA within the chromosome *in vivo* fell apart following replication into dimers (H2A, H2B) and tetramers (H3, H4)₂. Tetramers from these preexisting nucleosomes rapidly reassociate with daughter DNA duplexes (Gruss et al. 1993). Newly synthesized dimers (H2A, H2B) can then be sequestered to complete the octamer, mixing old and new histones into a single structure (see Fig. 4). The disruption of preexisting nucleosomal structure at the replication fork, coupled to dissociation of the histones from DNA,

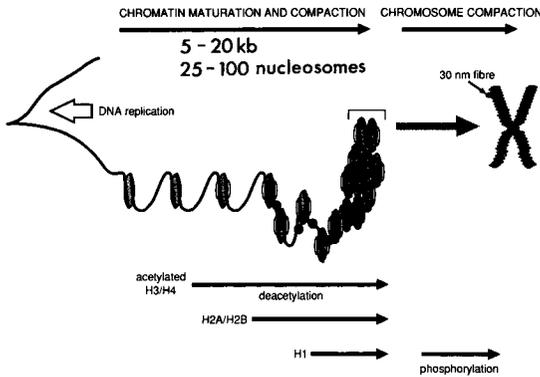


Figure 3 An in vivo pathway for de novo chromatin assembly coupled to replication. Chromatin assembly on nascent DNA. Acetylated histones H3 and H4 (*stippled ellipsoids*) are sequestered by the DNA first, histones H2A and H2B (*open ellipsoids*) follow, and, finally, histone H1 (*dark circle*) binds, stabilizing chromatin folding within the irregular 30-nm fiber. During this progressive assembly of chromatin, DNA is compacted, nucleosome formation leads to a sevenfold compaction of DNA, and the subsequent formation of the 30-nm fiber contributes a further sevenfold compaction. These compactations represent the major topological constraints of DNA in a eukaryotic nucleus. Mature chromatin is predominantly deacetylated. During mitosis, histone H1 is phosphorylated and 30-nm fibers pack together to assemble the mitotic chromosome. As much as 5–20 kb of nascent DNA, including 25–100 nucleosomes, may be present as "immature" chromatin associated with the replication fork at various levels of compaction (see text for details).

strongly suggests that the dispersive segregation of these histones to both daughter DNA duplexes occurs during replication (Cusick et al. 1984; Sogo et al. 1986; Burhans et al. 1991; Krude and Knippers 1991; Randall and Kelly 1992; Sugawara et al. 1992). Importantly, the incorporation of preexisting histone tetramers (H3, H4)₂ into nascent chromatin provides a means of maintaining and propagating a stable state of gene activity. The old H3 and H4 present in the nascent chromatin retain their preexisting posttranslational modification state (Perry et al. 1993). This differs from that of newly synthesized H3 and H4 and can potentially influence subsequent transcription of the associated DNA (see below). The dispersive segregation of "old" histones coupled to maintenance of their preexisting states of modification provides a molecular mechanism whereby an epigenetic imprint might be propagated through replication (Fig. 4, see below).

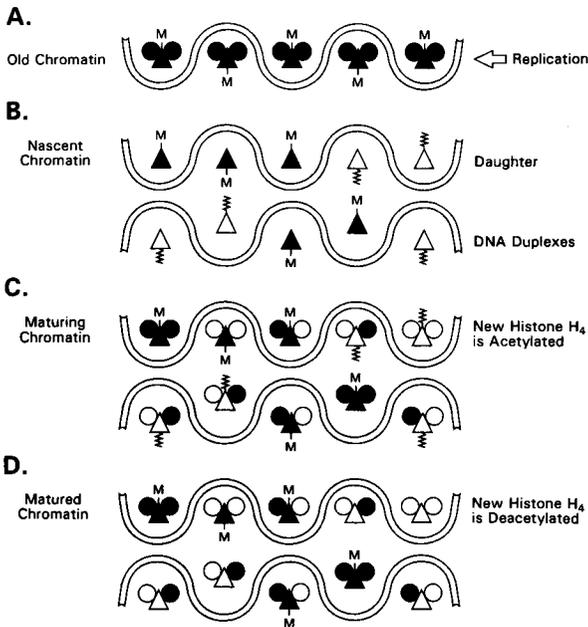


Figure 4 Nucleosome disruption during replication and reassembly following replication. (A) Old chromatin consisting of preexisting nucleosomes (histone octamer plus DNA) containing a tetramer (H3, H4)₂ (filled triangle) and two dimers (H2A, H2B) (filled circles). The histones in the tetramer are modified (M). Replication displaces these histones from DNA; the octamer can fall apart into tetramers and dimers. (B) Nascent chromatin. Old tetramers associate with both daughter DNA duplexes. Newly synthesized tetramers (open triangles) containing diacetylated histone H4 (zigzag line) also associate with daughter DNA in a process facilitated by CAF-1. (C) Maturing chromatin. Old and new dimers (open circles) bind to the tetramers. (D) Matured chromatin. New tetramers are deacetylated.

Regulatory Complexes

A special case for a regulatory nucleoprotein complex maintaining association with DNA throughout the cell cycle is the protein assembly that regulates use of an origin of replication itself. Stable association of proteins with an origin through the cell cycle has been established through *in vivo* footprinting methodologies on the replication origin of Epstein-Barr virus (Hsieh et al. 1993) and on a yeast chromosomal ARS element (Diffley and Cocker 1992). Implicit in the maintenance of these regulatory complexes through S phase is the concept that they duplicate themselves. An attractive mechanism for the maintenance of regulatory complexes through replication requires multiple copies of a given *trans-*

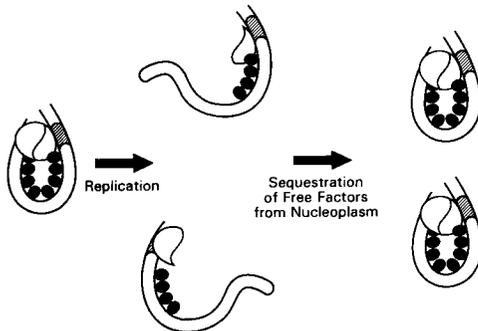


Figure 5 A regulatory nucleoprotein complex could make use of multiple protein-DNA interactions to maintain integrity through replication. Following replication, proteins partition to daughter DNA duplexes. Free factors are then sequestered from the nucleoplasm to reassemble two daughter complexes.

acting factor to bind to the regulatory DNA sequences (Fig. 5) (Brown 1984). This could be determined by sequence or structural selectivity. If the preexisting multimeric protein complex is split during replication, copies of the *trans*-acting factors could be segregated to both daughter DNA duplexes. These *trans*-acting factors could then either directly sequester other factors from the nucleoplasm making use of protein-protein interactions, or they could maintain the regulatory DNA sequences accessible in the face of ongoing chromatin assembly, such that when other factors became available they could bind to DNA. Structurally driven protein association is consistent with the maintenance of DNA distortion throughout the cell cycle at the Epstein-Barr viral origin (Hsieh et al. 1993).

In contrast to origin complexes, the basal transcriptional machinery appears to be removed from promoter elements by the passage of a replication fork (Wolffe and Brown 1986). Replication is found to be dominant to the transcription process, and a direct consequence of replication fork progression through an active 5S rRNA gene is the displacement of transcription factors. Several correlations from *in vivo* work support the generality of this observation. There is a clear antagonism between transcription and replication on efficiently replicating SV40 DNA molecules (Lebkowski et al. 1985; Lewis and Manley 1985). Replication forks invade the transcriptionally active ribosomal RNA genes in yeast (Saffer and Miller 1986; Lucchini and Sogo 1994). Thus, replication apparently resets the transcriptional status of a chromosome to "ground zero." The component protein molecules that

determine transcriptional activity have to reassemble regulatory complexes de novo on the daughter DNA duplexes. This reassembly occurs not on naked DNA, but on a nascent chromatin template.

Chromatin Assembly Has Replication-dependent and -independent Pathways

Replication-independent Pathways

Early work on physiological chromatin assembly pathways made use of cell-free preparations from *Xenopus* oocytes and eggs (Laskey et al. 1978; Glikin et al. 1984). More recently, extracts of *Drosophila* embryos have been used with similar results (Becker and Wu 1992). For both systems, chromatin assembly on nonreplicating DNA is relatively slow, taking several hours to assemble nucleosomes to a physiological density (one nucleosome per 180–200 bp). This contrasts with the rapid assembly of chromatin in vivo during early embryogenesis in *Xenopus* and *Drosophila*, where entire cell cycles take only 30 minutes and 10 minutes, respectively. Thus, the molecular mechanisms that mediate chromatin assembly in the absence of DNA replication have questionable physiological relevance. Nevertheless, these systems have provided useful information on the biochemistry of the assembly process.

In *Xenopus* oocytes, histones are synthesized under the control of distinct regulatory mechanisms that operate outside of S phase. Tetramers (H3, H4)₂ are stored in a complex with the molecular chaperone N1/N2 (Kleinschmidt et al. 1986). Dimers (H2A, H2B) are stored in a complex with the chaperone nucleoplasmin (Dilworth et al. 1987). Both chaperones exchange histones onto DNA at physiological ionic strength. N1/N2 must function before nucleoplasmin to assemble a nucleosome (Kleinschmidt et al. 1990). During normal development, nucleoplasmin has a specialized role in the remodeling of *Xenopus* sperm chromatin, where it facilitates the exchange of sperm-specific basic proteins for histones H2A and H2B (Philpott and Leno 1992). Nucleoplasmin and N1/N2 allow large amounts of histones to be stably sequestered in the *Xenopus* oocyte and egg; however, a role for these proteins in directly mediating chromatin assembly during early embryogenesis remains to be established.

Replication-dependent Pathways

In vivo in normal somatic cells, the vast bulk of the histone proteins are synthesized during S phase. These histones are immediately assembled onto nascent DNA at the replication fork (Ruiz-Carrillo et al. 1975; Jack-

son et al. 1976). Stillman (1986) discovered that the chromatin assembly process is coupled to replication. The molecular chaperone mediating the process is chromatin assembly factor 1 (CAF-1), which requires ongoing DNA replication to function (Smith and Stillman 1989). CAF-1 directs the association of the tetramer (H3, H4)₂ with replicating DNA. Dimers (H2A/H2B) then bind in a CAF-1-independent process to complete the histone octamer (Smith and Stillman 1991; see also Fotedar and Roberts 1989). CAF-1 requires a modified tetramer (H3, H4)₂ from the cytosol of human cells in order to function (Kaufman and Botchan 1994). This is potentially a key regulatory event in distinguishing the biochemistry of replication-dependent and -independent chromatin assembly pathways. It is possible that the phosphorylation and diacetylation of histone H4 coupled to its synthesis (Ruiz-Carrillo et al. 1975; Jackson et al. 1976; Dimitrov et al. 1994) may be necessary for chromatin assembly. Whether CAF-1 has specific interactions either with highly modified H4 and/or with the replication machinery itself are important questions yet to be resolved.

Almouzni and colleagues (Almouzni and Méchali 1988a,b; Almouzni et al. 1990b, 1991) established that replication-coupled pathways of chromatin assembly also exist in *Xenopus*. However, the molecular chaperones that couple replication to chromatin assembly, such as the CAF-1 found in somatic cells, remain to be defined. These replication-dependent pathways direct the efficient assembly of nucleosomes both in vitro and in vivo with kinetics that could easily accommodate a cell cycle duration of 30 minutes (Almouzni and Méchali 1988a; Almouzni et al. 1990b; Almouzni and Wolffe 1993). The mechanism of enhanced assembly involves both the rapid deposition of the histone tetramer (H3, H4)₂ and facilitation of the subsequent deposition of dimers (H2A, H2B) (Almouzni et al. 1990b). Similar results consistent with a facilitated two-step assembly of chromatin have been obtained in mammalian systems (Gruss et al. 1990).

The de novo assembly of chromatin on replicating templates in vitro provides a useful independent confirmation of earlier work on the staged assembly of chromatin during S phase in vivo. As discussed earlier, DNA replication disrupts preexisting nucleoprotein structures within the chromosome. Histones that are displaced during replication reassociate with newly synthesized DNA, but do so randomly on both daughter DNA duplexes. A consequence of this segregation is that nascent chromatin has a 50% enrichment of preexisting histones. The remainder of the histones incorporated into chromatin are newly synthesized. Radiolabeling of these newly synthesized histones has allowed the

kinetics of their incorporation into chromatin and subsequent modification to be determined.

Newly synthesized and preexisting histone tetramers (H3, H4)₂ associate with nascent DNA (Worcel et al. 1978; Jackson 1987, 1990); this is followed over the space of several minutes by the sequestration of both preexisting and newly synthesized histone dimers (H2A, H2B). Thus, the majority of nucleosomes behind a replication fork are hybrids of both old and new core histones. Finally, a mixture of newly synthesized and preexisting histone H1 stably associates with the nascent chromatin.

The overall process of chromatin maturation as assayed by nuclease sensitivity requires as long as 10–20 minutes in a rapidly proliferating mammalian cell (Cusick et al. 1983). Assuming a rate of replication fork movement of 0.5–1 kb of DNA per minute, this implies that 25–100 nucleosomes are present on both of the nascent DNA duplexes as "immature" chromatin during S phase (see Fig. 3). The initial rapid deposition of old and new histones H3 and H4 on newly synthesized DNA reflects the nuclease-sensitive stage, whereas the subsequent deposition of histone dimers (H2A, H2B) and histone H1 correlates with the appearance of regular nucleosomal arrays and nuclease resistance (Smith et al. 1984). The sequential sequestration of histones is clearly once again related to the structure of the nucleosome, since the tetramer (H3, H4)₂ forms the core of the structure, whereas histones H2A and H2B bind at the periphery of the nucleosome, and histone H1 can only associate in its proper place after two turns of DNA are wrapped around the core histones (Fig. 2) (Hayes et al. 1991; Hayes and Wolffe 1993).

Newly synthesized histone H4 is phosphorylated and acetylated in the amino-terminal tail domain (Ruiz-Carillo et al. 1975; Jackson et al. 1976). Approximately 30 minutes after deposition during chromatin assembly, the diacetylated H4 is deacetylated to its mature form. If H4 deacetylation is inhibited, chromatin never achieves the nuclease resistance of bulk chromatin, indicative of the formation of stable higher-order structures. Histone H1 may be less efficiently incorporated into chromatin containing acetylated H4 (Perry and Annunziato 1989; but see Ura et al. 1994). Thus, histone diacetylation is likely to maintain nascent chromatin in a structure that is more accessible to other DNA-binding proteins.

In summary, chromatin assembly *in vivo* is coupled to replication, most probably through the activity of specific molecular chaperones such as CAF-1. Nucleosome assembly occurs in stages and involves transient posttranslational modifications of core histones synthesized during S phase (Figs. 3, 4).

Epigenetic Mechanisms: The Assembly of Active and Repressed Transcriptional States

In vivo experiments using *Saccharomyces cerevisiae* suggest that replication disassembles repressed chromatin states and facilitates the access of *trans*-acting factors to DNA (Aparicio and Gottschling 1994). Other experiments using yeast suggest that replication has an essential role in facilitating the repression of specific genes (Miller and Nasmyth 1984). We have discussed how biochemical experiments indicate that replication introduces a dynamic aspect to chromosomal structure, both directing the disassembly of preexisting structures and facilitating the assembly of nucleosomes. A central issue in gene regulation is how the assembly of nucleoprotein structures following replication can maintain or alter states of potential transcriptional activity.

Repression

Replication and transcription are most clearly seen to be linked in yeast. Components of the yeast origin recognition complex (ORC) regulate both the initiation of replication within the chromosome and the repression of transcription within the same chromosomal domain (Bell et al. 1993). The molecular mechanisms responsible for the repression of transcription directed by ORC are unknown. Two possible explanations are (1) that the ORC compartmentalizes adjacent chromatin into a transcriptionally incompetent environment within the nucleus, or (2) that the ORC influences the type of chromatin assembled adjacent to it. The ORC complex may be a greatly streamlined version of the replication factories of larger eukaryotes (Cook 1991). These replication factories represent special nuclear compartments at which proteins involved in the replication process are sequestered. It is possible that a gene adjacent to the origin is directed by the ORC to reside in a replication-competent but transcriptionally incompetent environment. Alternatively, if replication itself is essential for transcriptional repression (Miller and Nasmyth 1984; Laurensen and Rine 1992), then the coupling of chromatin assembly to the replication process could contribute to repression. Preexisting transcriptionally active complexes would be displaced by the replication fork. *trans*-Acting factors would then have to compete for assembly against the deposition of histones. In vivo and in vitro experiments in *Xenopus* demonstrate that the coupling of nucleosome assembly to replication can very effectively repress basal transcription (Almouzni et al. 1990a; Almouzni and Wolffe 1993). As discussed earlier, the ORC complex provides one biological example of the maintenance of sequence-

specific or structure-dependent protein-DNA interactions through the replication process. However, since the ORC also serves to initiate the replication process, maintenance of the ORC may occur under circumstances distinct from the transcription complexes or chromatin structures that are exposed to the fully assembled replication-elongation complex.

We have discussed how the histones already on the template during replication are segregated randomly to the daughter DNA duplexes, but within the context of small groups of nucleosomes. This maintenance of histone modification states potentially influences *trans*-acting factor access to DNA. Moreover, if proteins that modify the subsequent folding of nucleosomal arrays or that modify histones themselves, for example, by acetylation or deacetylation, are also partitioned in this way, the properties of a chromatin domain might be stably propagated. For example, histone H4 acetylation may interfere with the association of histone H1 with chromatin (Perry and Annunziato 1989). Histone H1 is known to repress specific genes *in vivo* (Bouvet et al. 1994). Other proteins that might recognize properties of the "old" histones within nascent chromatin include the chromodomain proteins that initiate the formation of heterochromatin. These are also good candidates for propagating pre-existing states of chromatin-mediated transcriptional repression (Fig. 6).

Activation

In vitro experiments using cell-free preparations of *Xenopus* eggs indicate that stable states of gene activity can be propagated in a nuclear environment (Wolffe 1993; Barton and Emerson 1994). How might this occur?

The simplest situation leading to continued gene activity would be the case in which a superabundance of transcription factors specific for a given gene was available within the nucleus throughout the cell cycle, including S phase. The factors would always be able to bind to their regulatory elements should they become accessible, recruiting the basal transcriptional machinery to the nascent promoter DNA, and thereby preventing histones or other proteins from binding to the TATA homology. Several features of nascent chromatin facilitate the association of transcription factors (Wolffe 1991). For example, the complex of the 5S rRNA gene with the tetramer (H3, H4)₂ is not repressive to transcription (Wolffe 1989; Tremethick et al. 1990; Almouzni et al. 1991), whereas the complete octamer of core histones (H2A, H2B, H3, H4)₂ is repressive at high densities of octamers bound to DNA (Clark and Wolffe 1991; Hayes and Wolffe 1992). Moreover, acetylation of the core

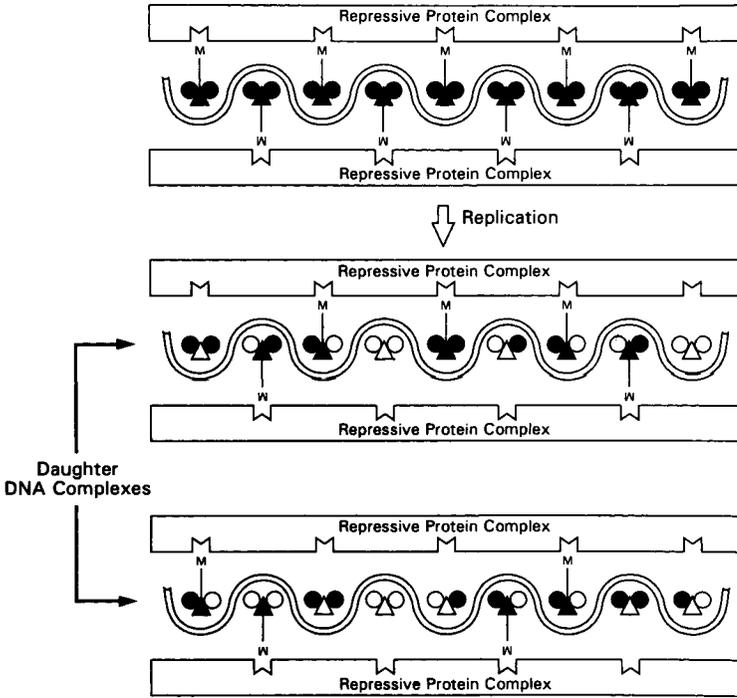


Figure 6 Preexisting histone modifications could provide an epigenetic imprint. A repressive protein complex (e.g., containing chromodomain proteins) recognizes a histone modification (M). Following replication, modified histones are segregated to both daughter DNA duplexes sufficient to sustain interaction with the repressive protein complex.

histones facilitates transcription factor access to DNA even when the complete octamer is bound (Lee et al. 1993). The histone tetramer (H3, H4)₂ recognizes the DNA sequences that position the nucleosome containing the 5S rRNA gene (Hayes et al. 1991), hence it is probable that the formation of a specific chromatin structure also has a role in allowing transcription factors access to the template. Thus, following replication, it is probable that sequence-specific DNA-binding proteins have an opportunity to reassociate with daughter DNA molecules (Fig. 7). Replication might under certain circumstances facilitate gene activation (Enver et al. 1988; Wilson and Patient 1993; Aparicio and Gottschling 1994).

The regulated activity of a transcription factor, such that it becomes able to bind to DNA or to function during S phase, could lead to transcription activation in a way that is replication-dependent. In a developmental context, this event might be coupled to a particular embryonic

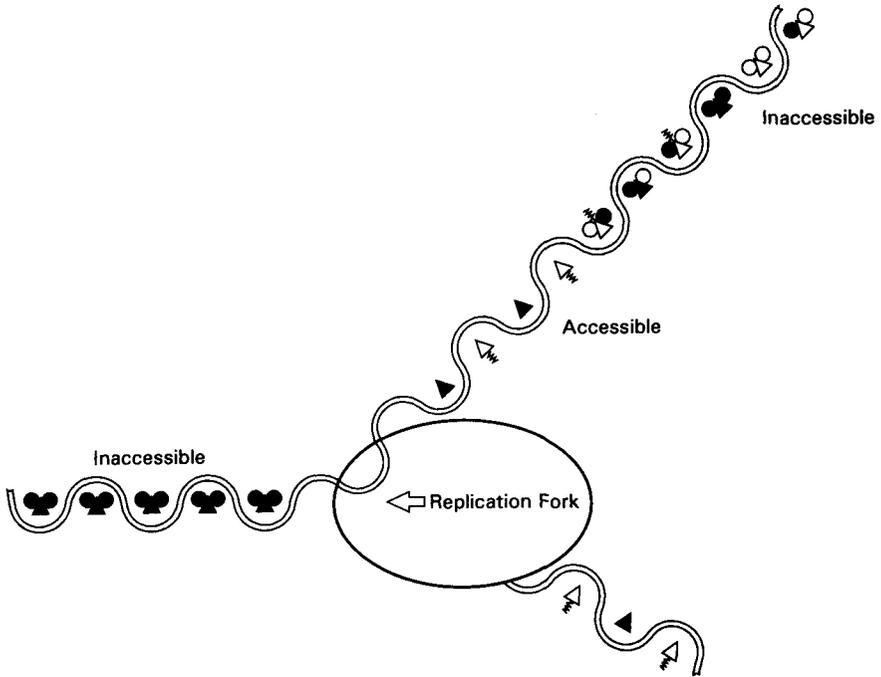


Figure 7 Replicative disruption of preexisting chromatin structures provides a window of opportunity for transcription factors to program genes. Nucleosome assembly is represented as in Fig. 4. The accessibility of nascent, maturing, and mature chromatin to *trans*-acting factors is indicated.

cleavage cycle or to a regulated period of cell division. For example, in *Caenorhabditis elegans* and the sea urchin, replication events are correlated with changes in the commitment of cells to a particular developmental fate (Mita-Miyazawa et al. 1985; Edgar and McGhee 1988). Similar changes can occur in differentiated cells that express one set of specialized genes and that can switch to another program of gene expression only after one or more cell divisions (e.g., Wolffian regeneration of the lens; Takata et al. 1964). However, replication events are not necessarily essential for changing gene expression within a particular cell (Chiu and Blau 1984; Blau et al. 1985). This is not surprising, since chromatin structure is not completely inert *in vivo*. Histones H2A, H2B, and H1 are known to exchange with a pool of free histones in a cell (Louters and Chalkley 1985). Complexes of DNA with only histones H3 and H4 therefore exist for a limited amount of time. However, a comparison of the rate and efficiency of gene activation in the presence or absence of cell proliferation has not yet been made.

The maintenance of specific transcription factor-DNA interactions through replication, as discussed earlier for the ORC, might be facilitated by considering the promoter, the enhancer, and locus control regions not as separate entities, but as contributory components to a single structure (Wolffe 1990). This could be achieved through protein-protein interactions between the distinct nucleoprotein complexes assembled at each regulatory element. One reason for the separation of these regulatory elements over extensive distances may be that any single structure might be independently disrupted by DNA replication, while the other would remain intact. If protein binding to one sequence element influences the binding of proteins to the other, then the intact nucleoprotein complex might facilitate the re-formation of the disrupted one.

Replication Timing

Chromatin organization outside the ORC may also have significance for the initiation of replication and the timing of this initiation in S phase. If replication disrupts both active and repressed chromatin structures, then the entire nucleus has to be remodeled after each replication event. I have suggested a means of accomplishing this remodeling; however, the re-formation of nuclear structures has other implications. If there are limiting transcription factors available in a cell, then a gene that is replicated early in S phase has more opportunity for the assembly of an active transcription complex than a gene that replicates late. This is simply because the gene that replicates early is available for transcription factors to bind before all of the early-replicating portion of the genome has sequestered these factors. A late-replicating gene therefore experiences a relative deficiency in factor availability (Gottesfeld and Bloomer 1982; Wormington et al. 1982). Conversely, it is also possible that the type of chromatin assembled early in S phase is more accessible to transcription factors than chromatin assembled late in S phase. For example, early-replicating chromatin may sequester histones that are more highly acetylated and, consequently, more accessible to the transcription factors that maintain continued transcription activity. Transcriptionally active genes replicate early in S phase (Goldman et al. 1984; Gilbert 1986; Wolffe 1993). The reason for this early replication is unknown, but possibilities include the local disruption of chromatin structure by transcription complexes, such that the DNA within those chromatin domains becomes more accessible to the replication machinery (Wolffe and Brown 1988). Many transcription factors may also be replication factors (DePamphilis 1988, 1993); consequently, local concentrations of transcription factors may favor the assembly of replication initiation complexes.

The issue very much is one of which came first: the chicken or the egg, or both? It is possible to argue that active transcription complexes open chromatin to admit replication factors, or, alternatively, that these sites are replicated first and are thus more accessible to transcription factors. Whether either or both of the much discussed mechanisms operate *in vivo* remains to be established.

CURRENT PROBLEMS AND FUTURE PROSPECTS

Chromatin structure is now realized to reflect a dynamic interaction between the many protein complexes that both organize DNA and fulfill regulatory roles. A much simplified picture suggests that replication disrupts local chromatin structures that preexist on the chromosome before replication. The subsequent reassembly of the nucleosome necessitates a staged process using modified histones that might be more accessible to transcription factors. This would provide a window of opportunity for reestablishing particular states of transcriptional activity. On a more global scale (>1–2 kb), chromatin proteins that retain a particular modification (e.g., acetylation) or that cooperatively influence chromosome structure toward activation or repression could provide an imprint on chromatin activity through DNA replication and chromosome duplication. Replication is established as having a major impact on preexisting nucleoprotein structures and a major role in their reassembly. Although significant attention has been given to the enzymology of the duplication of DNA, relatively little progress has been made concerning the enzymology of chromosomal duplication. The molecular mechanisms of chromatin assembly are not defined in any detail. The definition of molecular chaperones such as CAF-1 is a major advance; however, how CAF-1 functions is unknown. Does CAF-1 have a catalytic or structural role? Does it interact with the elongation complex? What are the special features of the histones that allow CAF-1 to utilize them for nucleosome assembly? On a more mundane level, we do not know the precise sequences or structures of the histone proteins necessary for chromatin assembly. The enzymes that transform nascent chromatin into a mature structure are yet to be defined at the molecular level. How mature chromatin is recognized by other proteins that influence states of gene repression, such as the chromodomain proteins, is unknown.

At this time, only the simple 5S rRNA genes of *Xenopus* have been extensively analyzed with respect to the significance of intermediates in chromatin assembly for the capacity to program genes for future transcription. This work is greatly in need of extension to a broader spectrum

of eukaryotic genes. Preliminary work in *Drosophila* is consistent with a transition from programmable to stable repressed states as chromatin matures (Kamakaka et al. 1993). Caution must be used with many of the interpretations concerning the general impact of chromatin structure on transcription, since *in vitro* chromatin assembly systems currently make use of oocyte or embryonic extracts. These systems contain histone variants or modifications not found in normal somatic cells (Dimitrov et al. 1994). It is to be hoped that chromatin assembly systems coupled to replication that employ biochemically defined histones from normal somatic cells will be developed.

The impact of DNA replication on gene expression is readily analyzed through yeast genetics (Miller and Nasmyth 1984; Aparicio and Gottschling 1994; Laurenson and Rine 1992); however, homologous biochemical systems are currently lacking to test the many hypotheses proposed to explain the phenomena observed. Much progress in the biochemistry of yeast replication can be anticipated. The further reconstruction of determinative events in development will require continued consideration of the fate of regulatory nucleoprotein complexes during replication (Diffley and Cocker 1992). This is an important focus for future research. At a biochemical level, *in vitro* systems capable of maintaining states of gene expression through replication offer considerable promise (Wolffe 1993; Barton and Emerson 1994). The future clearly has the exciting prospect of understanding and thus reconstructing chromosomal duplication in all its complexity at a molecular level.

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