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Review

Role of chromatin during herpesvirus infections

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ABSTRACT

DNA viruses have long served as model systems to elucidate various aspects of eukaryotic gene regulation, due to their ease of manipulation and relatively low complexity of their genomes. In some cases, these viruses have revealed mechanisms that are subsequently recognized to apply also to cellular genes. In other cases, viruses adopt mechanisms that prove to be exceptions to the more general rules. The double-stranded DNA viruses that replicate in the eukaryotic nucleus typically utilize the host cell RNA polymerase II (RNAP II) for viral gene expression. As a consequence, these viruses must reckon with the impact of chromatin on active transcription and replication. Unlike the small DNA tumor viruses, such as polyomaviruses and papillomaviruses, the relatively large genomes of herpesviruses are not assembled into nucleosomes in the virion and stay predominantly free of histones during lytic infection. In contrast, during latency, the herpesvirus genomes associate with histones and become nucleosomal, suggesting that regulation of chromatin *per se* may play a role in the switch between the two stages of infection, a long-standing puzzle in the biology of herpesviruses. In this review we will focus on how chromatin formation on the herpes simplex type-1 (HSV-1) genome is regulated, citing evidence supporting the hypothesis that the switch between the lytic and latent stages of HSV-1 infection might be determined by the chromatin state of the HSV-1.

The last two decades have witnessed exciting developments in our knowledge of how eukaryotic gene transcription is regulated. Early developments focused on the *cis*-regulatory elements associated with specific gene promoters and on the *trans*-acting factors that bind to these elements. More recent progress has revealed dynamic aspects of chromatin structure and the mechanisms whereby chromatin and its modifications influence gene expression.

DNA viruses have long served as model systems to elucidate various aspects of eukaryotic gene regulation, due to their ease of manipulation and relatively low complexity of their genomes. In some cases, these viruses have revealed mechanisms that are subsequently recognized to apply also to cellular genes. In other cases, viruses adopt mechanisms that prove to be exceptions to the more general rules. The double-stranded DNA viruses that replicate in the eukaryotic nucleus typically utilize the host cell RNA polymerase II (RNAP II) for viral gene expression. As a consequence, these viruses must reckon with the impact of chromatin on active transcription and replication. Unlike the small DNA tumor viruses, such as polyomaviruses and papillomaviruses, the relatively large genomes of herpesviruses are not assembled into nucleosomes in the virion and stay predominantly free of histones during lytic infection. In contrast, during latency, the herpesvirus genomes associate with histones and become nucleosomal, suggesting that regulation of chromatin *per se* may play a role in the switch between the two stages of infection, a long-standing puzzle in the biology of herpesviruses.

In this review we will focus on how chromatin formation on the herpes simplex type-1 (HSV-1) genome is regulated, citing evidence supporting the hypothesis that the switch between the lytic and latent stages of HSV-1 infection correlates with changes in the chromatin state of the HSV-1. Before going into the details of HSV-1, we will briefly summarize some of the recent advancements in regulation of chromatin and transcription by RNAP II as it pertains to the rest of this review.

1. Transcription in eukaryotes

Eukaryotic DNA is packaged in the form of nucleosomes, whereby approximately 147 bp of DNA is wrapped around a protein octamer that consists of two copies of each core histone (H2A, H2B, H3 and H4). Further compaction of nucleosomes is mediated by the linker histone H1 and other non-histone proteins. Although RNAP II can transcribe efficiently *in vitro* from naked DNA templates, the packaging of DNA into nucleosomes inhibits transcription. The past few decades have witnessed great progress in our understanding of how the inhibitory effect of chromatin on transcription can be overcome. Four principal mechanisms include the covalent modification of histone tails and globular domains, remodeling of nucleosomes, incorporation of histone variants, and removal or disruption of nucleosomes at actively transcribed genes. These four

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general mechanisms will be described briefly before turning to the role of chromatin and its modification during herpesvirus infections.

1.1. Histone modifications and transcription

Covalent or post-translational modifications of the amino-terminal tails of core histones have been extensively characterized [84], although the globular domains can also be modified [124,141,178,183,186]. The most prominent covalent histone modifications include acetylation, methylation, ubiquitinylation, phosphorylation, and prolyl isomerization [84]. In many cases, specific modifications on specific residues of particular histones have been correlated as either positive or negative markers of transcriptional activity [84]. Genome-wide studies that employed chromatin immunoprecipitation (ChIP) assays coupled with DNA microarrays or high-throughput sequencing have shown that particular modifications are predominantly localized to distinct regions of target genes, such as the upstream regulatory regions, core promoters, or the 5′ and 3′ portions of the transcribed regions [144]. For instance, histone H3 acetylated on lysines 9 and 14 (H3K9/K14ac) localizes to the promoter and 5′ ends of actively transcribed genes. Methylation of histone H3 can be an indicator of either active or inactive transcription, depending on which lysine residue is modified. Histone H3 methylation also follows a distinct pattern of localization through the body of a gene; for example, H3K4me3 is mainly present around the transcriptional start site, whereas H3K36me3 is localized towards the middle and 3′ ends of actively transcribed genes. Other H3 methylation marks, such as H3K9me3 or H3K27me3, are strictly associated with inactive transcription and are observed over broad regions of silenced genes.

In parallel with the identification of covalent histone tail modifications has come the discovery of the corresponding enzymes that catalyze these reactions. For instance, histone methyltransferases are rather specific for the target lysine or arginine residue. Histone acetylation is somewhat less specific: a given histone acetyltransferase (HAT) might modify several residues, and several different HATs might have overlapping substrate specificities. For instance, the HATs p300, CBP, and PCAF can all acetylate H3K14 [105,115,158]. As a rule, the covalent marks are reversible by enzymes such as histone deacetylases (HDACs) and lysine demethylases, indicative of the highly dynamic nature of chromatin modifications and multiple potential levels of transcriptional regulation [84].

Covalent modifications of histones are thought to have two principal consequences. The first is the direct impact of modification on higher-order chromatin structure. For instance, the loss of positive charge on lysines upon acetylation is associated with relaxed chromatin structure [84]. The second potential outcome is the recognition of specific histone modifications by other proteins that function as transcription factors or coactivators. Two examples of such mechanism are proteins containing bromodomains, which bind to acetylated lysines, and proteins containing chromodomains, which bind to methylated lysines [25,161]. Since a number of bromodomain- or chromodomain-containing proteins are themselves chromatin-modifying enzymes, this recognition enables the propagation or cooperativity of histone modifications and chromatin remodeling [25,161].

1.2. Chromatin remodeling and transcription

The second major class of chromatin-modifying factors comprises protein complexes that utilize ATP hydrolysis to induce changes in the positions of nucleosomes on DNA and hence are called chromatin-remodeling complexes. Chromatin remodeling may result in sliding of the nucleosomes on DNA, DNA looping on the nucleosome particle, or histone octamer transfer *in trans* [40,154].

Several families of chromatin-remodeling complexes have been identified. The prototypes of these families include SWI/SNF, ISWI, INO80, and NURD/Mi-2/CHD, all of which contain an ATPase subunit and have both similar and distinct functions. For instance, the ISWI and NURD/Mi-2/CHD families are both involved in transcriptional repression, yet a separate function of the ISWI family is to induce ordered chromatin assembly. The SWI/SNF family, on the other hand, is primarily associated with active transcription. In mammals, remodelers of the SWI/SNF family are represented by two separate complexes that have hBRM and BRG1 as their ATPase subunits. In addition to their role in transcription, hBRM and BRG1 remodeling complexes in mammals are involved in processes such as cancer progression, differentiation, and development [154].

1.3. Incorporation of histone variants and role of histone chaperones in transcription

The third mechanism that influences the impact of chromatin on gene regulation is the incorporation of histone variants. Whereas the canonical core histones are each encoded by multiple genes that are expressed predominantly in the S phase of the cell cycle, histone variants are encoded by single-copy genes that are expressed independent of DNA replication. Histone variants are thought to exert their actions mainly by influencing the stability of nucleosomes or higher-order chromatin structure, but not by differential covalent modifications, as in most cases the sites for covalent histone modifications are conserved among the variants. Another theory postulates that exposure of different surface residues in histone variants may serve as binding sites for other proteins [70,72].

Although H1, H2A, and H3 have multiple variants, no histone variants have been identified for H2B and H4. The H2A variants in humans include H2A.X, H2A.Z, H2A-Bbd, and macroH2A, each of which has a distinct localization pattern and function [70,72]. For instance, macroH2A is localized to the inactive X chromosome, where it is thought to contribute to heterochromatin formation. In contrast, H2A-Bbd is excluded from the inactive X chromosome and accumulates at actively transcribed genes. H2A-Bbd is an exceptional histone variant in that it shares only 48% sequence identity with histone H2A and lacks a number of structural features characteristic of histone H2A family. As such, this histone variant is thought to participate in destabilization of nucleosomes, which then facilitates the recruitment of transcription factors and coactivators that facilitate active transcription [47]. Although in yeast H2A.Z prevents the spread of heterochromatin, in higher eukaryotes it might also function in the formation of heterochromatin. The principal function of H2A.X is not in transcription but in DNA repair: phosphorylated H2A.X (γ-H2A.X) marks the regions of double-stranded DNA breaks and thus aids in recruiting the DNA repair machinery. Among the two major H3 variants, CENP-A is localized exclusively to the centromeres and contributes to formation of kinetochore and chromosome segregation. The other histone H3 variant, H3.3, differs from the canonical H3 by only a few amino acid substitutions but is expressed throughout the cell cycle and is present in transcriptionally active regions. A large number of histone H1 variants, which share a conserved core domain yet have more divergent N- and C-termini, have been identified in humans. Although histone H1 variants were initially thought to have redundant functions, recent findings indicate that they may also have specific roles in gene regulation [67].

The assembly of histones and histone variants into nucleosomes requires the activities of a number of proteins and protein complexes [59,156]. CAF1 and HIRA are two such assembly factors that incorporate H3.1 (canonical histone H3) and H3.3 into nucleosomes in a replication-dependent and -independent manner, respectively. Another histone chaperone, Asf1a, interacts with both CAF1 and HIRA, and as such it is involved in both replication-dependent and -independent histone assembly. Assembly of other histone variants might also be mediated by specific protein complexes, most of which are yet to be identified. For instance the SWR1 complex, which