Review

The Effects of Replication Stress on S Phase Histone Management and Epigenetic Memory

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Abstract

When a cell divides, it must not only accurately duplicate its genome but also recapitulate its programme of gene expression. A significant body of evidence suggests that an important fraction of the information specifying the transcriptional programme of vertebrate cells is carried epigenetically by post-translational modifications of histone proteins. For such a system to operate, propagation of key histone marks must be coupled to replication such that they remain correctly associated with the underlying DNA sequence, despite the huge disruption to chromatin structure generated by unwinding the parental DNA strands. Focusing on vertebrate cells but drawing on experimental evidence from a wide range of systems, we will examine the evidence that histone mark propagation through replication contributes to transcriptional stability. We then discuss the emerging molecular mechanisms that ensure that histone recycling is tightly coupled to DNA replication, focusing on how parental histone proteins are chaperoned around the replication fork, and the strategies that ensure that this process is not disrupted by impediments to replication.

Introduction

Chromatin represents an elegant solution to the complex problem of compacting and protecting extremely long and charged DNA molecules within the eukaryotic nucleus while, at the same time, allowing access to the genetic code. The basic subunit of chromatin, the nucleosome, comprises eight histone proteins, a tetramer of $(H3/H4)_2$ bound by two H2A/H2B dimers. Around this core, approximately 146 bp of double-stranded DNA is wrapped in 1.65 helical turns. Nucleosomes are spaced at approximately 200 bp intervals with the intervening DNA bound by the linker histone, H1 (reviewed in Ref. [1]). Nucleosomes are intrinsically inhibitory to transcription [2,3], and thus, efficient gene expression requires that the DNA is made accessible by nucleosome modification or displacement. Conversely, gene repression can be enforced by the formation of heterochromatin, a compact and higher-order chromatin structure that renders the DNA inaccessible to transcription factors and RNA polymerase.

The observation that histone modifications could influence RNA synthesis from chromatin templates [4,5] suggested that histones might provide a basis for regulating gene expression. In turn, this developed into the notion that cells might propagate chromatin structures able to act as an "epigenetic code" determining the transcriptional competence of individual genes [6]. The subsequent elucidation of the general structure of chromatin [7–9] and the nucleosome itself [10,11], coupled with a growing appreciation of the potential role of histone modifications in regulating transcription, led to the suggestion that these modifications might not just be effectors of chromatin accessibility but that they also carry epigenetic information [12].

A vast array of histone post-translational modifications have been described [13]. They are concentrated in the unstructured N-terminal tails of the histone proteins, which protrude through the gyres of the DNA in the nucleosome structure [11] but are also found in the globular core where they can directly influence electrostatic interactions between the histones and the DNA [14]. Furthermore,

numerous studies have revealed correlations between these "chromatin marks" and the transcriptional state of the underlying DNA [15–17]. Thus, for example, transcriptionally active chromatin is linked with the trimethylation of lysine 4 and the acetylation of lysines 9 and 14 of histone H3, or H3K4me3 and H3K9/14ac for short. Conversely, transcriptionally inactive regions of the genome feature H3K9me3 or H3K27me3.

However, whether any of this myriad of histone modifications are truly vectors of epigenetic memory, rather than simply being effectors of chromatin function, or indeed mere epiphenomena, remains contentious [18–20]. Indeed, there is evidence for multiple mechanisms contributing to the robustness of transcriptional states through replication including transcription factor segregation, RNA interference, and DNA methylation, and we have previously considered how these mechanisms may interact with histone-modification-based epigenetic memory [21]. Here, we will focus on the role of histone modifications and their propagation in the maintenance of epigenetic memory over repeated cell divisions. For a histone modification or group of modifications to be deemed to carry epigenetic information through DNA replication, three criteria must be met:

(1) The histone and modification should be sufficiently stable during interphase that any epigenetic information carried is not lost before the next round of replication.

(2) During replication, the modification must be propagated from the parental chromatin to that formed on the daughter strands while remaining in register with the underlying DNA sequence.

(3) Once present on the daughter chromatin, the modification should be capable of promoting its own installation on nearby, newly synthesised histones that are incorporated into the nascent daughter strands in order to maintain nucleosome density.

If these criteria are met, and the propagated histone modifications are necessary to specify a stable transcriptional state, then an important prediction is that the interruption of histone flow during replication should result in failure to maintain the transcriptional state associated with the modification.

In the four sections of this review, we will examine the extent to which these criteria are met and then discuss the prediction by exploring the epigenetic consequences of failure to maintain processive replication.

Global Turnover of Histones and Their Modifications

The ability of histone post-translational modifications to make a viable contribution to epigenetic memory, that is, to act as epigenetic marks, will be influenced by the overall stability of the modification and the host histones. H2A/B dimers can be removed from the nucleosome without the loss of DNA wrapping [22] and are turned over rapidly, particularly during transcription [23–25]. In contrast, H3 and H4 are turned over less frequently, making them more attractive as vectors of mitotic epigenetic information. Nonetheless, the idea that H3 and H4 are sufficiently stable to transmit epigenetic information across cell divisions has been challenged by pulse-labelling experiments in *Drosophila* cells, suggesting that the time for a histone protein to turn over is, on average, less than a cell cycle [26]. However, even the maximum observed turnover rate is less than that caused by S phase when parental nucleosomes are diluted by 50%, a situation from which the levels of many key histone modifications recover during the ensuing G1 phase [27]. Thus, the potential for histone turnover to disrupt the propagation of epigenetic information will also be determined by the ability of adjacent nucleosomes to "reeducate" newly incorporated molecules [28], a point we return to below.

In terms of histone modifications, the half-life of histone methylations is generally significantly longer than that of histone acetylations [29–33], consistent with the suggestion that histone acetylations are generally more likely to be short-term effectors of histone function [19]. Thus, the H3/4 tetramer and its methylation modifications will form the focus of our discussion as the most likely histone modifications to participate in epigenetic memory.

Propagation of Marked Parental Histones through DNA Replication

The recycling of parental histone proteins displaced ahead of the replication fork not only constitutes a ready supply of histones close to the fork but also provides a potential vector for carrying epigenetic information. The general rules governing histone recycling were largely worked out in classic biochemical experiments during the 1970s and 80s and still provide a framework for understanding how histones could transmit epigenetic information. During S phase, the reestablishment of chromatin is closely coupled to replication in both human and yeast cells [34–38]. Both parental histone proteins (those present prior to replication and displaced ahead of the replicative helicase) and newly synthesised histones contribute to the formation of chromatin on the daughter DNA strands. Behind the replication fork, $(H3/4)_2$ tetramers are deposited on both leading and lagging strands as soon as sufficient double-stranded DNA is synthesised [39–43]. This histone recycling takes places largely without the mixing of old and new H3/4 dimers [44–46], although increased tetramer splitting has been noted in transcribed regions in both yeast [47] and human cells [46]. Nucleosome density is maintained by interspersing newly synthesised H3/4 between the parental tetramers, such that on average the old:new histone ratio on each new strand is 50:50. In contrast, the association of H2A and H2B with the newly deposited