Epigenetic dynamics across the cell cycle

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Abstract

Progression of the mammalian cell cycle depends on correct timing and co-ordination of a series of events, which are managed by the cellular transcriptional machinery and epigenetic mechanisms governing genome accessibility. Epigenetic chromatin modifications are dynamic across the cell cycle, and are shown to influence and be influenced by cell-cycle progression. Chromatin modifiers regulate cell-cycle progression locally by controlling the expression of individual genes and globally by controlling chromatin condensation and chromosome segregation. The cell cycle, on the other hand, ensures a correct inheritance of epigenetic chromatin modifications to daughter cells. In this chapter, we summarize the current knowledge on the dynamics of epigenetic chromatin modifications during progression of the cell cycle.

Introduction

The cell cycle constitutes a chain of interconnected events including accurate duplication of DNA and segregation of chromosomes into two genetically identical daughter cells. These events outline the two critical and major phases of the cell cycle, the synthetic phase (S-phase), where DNA replication takes place, and the mitotic phase (M-phase), characterized by chromosomal

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segregation followed by cellular division. These two major phases are preceded by two gap phases, \( G_1 \)- and \( G_2 \)-phase respectively, allowing for cellular growth and checkpoint controls (see [1] for a review on the cell cycle).

Fidelity and reliability of the cell cycle is dependent on correct timing and co-ordination of its events, and the process is controlled by a highly orchestrated gene-expression programme. Gene expression is the result of an interplay between the available subset of transcription factors and the accessibility of a defined part of the genome. Genome accessibility is in turn managed by epigenetic regulators shaping the genome into open and repressed chromatin structures, predominantly acting on the basic building block of chromatin, the nucleosome. The nucleosome is a repeating unit consisting of approx. 146 bp of DNA wrapped around a histone octamer core constituted of two each of histones H2A, H2B, H3 and H4 (Figure 1). The majority of epigenetic regulators act by adding or removing of post-translational modifications on the genomic DNA or on the protruding N-terminal tails of the core histones. Genomic DNA modification consists in mammals of the methylation of cytosine bases within CpG dinucleotides. The addition of methyl groups by DNMTs (DNA methyltransferases) attracts complexes of methyl-DNA-binding proteins, resulting in DNA methylation.

**Figure 1. Genome compaction and chromatin regulation**

Genomic DNA is compacted into chromatin in the cell nucleus. Around 146 bp of DNA is wrapped around an octamer of core histones forming the nucleosome (yellow). Nucleosome core histones consist of two each of histones H2A, H2B, H3 and H4. Multiple layers of epigenetic modifications influence gene expression, including DNA methylation of cytosine bases within CpG dinucleotides (black lollipops), and histone post-translational modifications predominantly on the protruding N-terminal histone tails. Histone post-translational modifications include acetylation, methylation, phosphorylation and ubiquitination.
mostly in repression of gene activity [2]. Post-translational histone modifications include acetylation, methylation, phosphorylation, SUMOylation and ubiquitination, predominantly of residues on the N-terminal tails (Figure 1 and Table 1). Modified histone residues contribute a code for recognition by different trans-factors, which govern nucleosome structure and remodelling and hence regulate accessibility to the underlying DNA. Histone acetylation is generally linked to gene activation, partly because of the electrostatic repulsion induced by the acetyl groups and partly because acetylated histones constitute binding scaffolds for bromodomain-containing protein complexes [3]. Histone methylations, on the other hand, are correlated with either gene activity or repression depending on the residue modified. For instance, trimethylation of Lys9 and Lys27 on histone H3 (H4K9me3 and H3K27me3 respectively) and

<p>| Table 1. Major identified histone post-translational modifications and their function |
|---------------------------------|---------------------------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Histone post-translational modification</th>
<th>Enzyme</th>
<th>Function</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H3T3p</td>
<td>Haspin</td>
<td>Centromeric mitotic spindle function</td>
<td>[40,41]</td>
</tr>
<tr>
<td>H3K4me3</td>
<td>MLL2</td>
<td>Transcriptional activation</td>
<td>[42]</td>
</tr>
<tr>
<td>H3K9me2</td>
<td>G9a</td>
<td>Transcriptional repression</td>
<td>[43]</td>
</tr>
<tr>
<td>H3K9me3</td>
<td>SUV39H1</td>
<td>pRb-mediated silencing</td>
<td>[14]</td>
</tr>
<tr>
<td>H3S10p</td>
<td>Aurora B</td>
<td>Transcriptional up-regulation and chromatin condensation</td>
<td>[44]</td>
</tr>
<tr>
<td>H3T11p</td>
<td>Dlk/ZIP</td>
<td>Mitotic-specific phosphorylation</td>
<td>[45]</td>
</tr>
<tr>
<td>H3K14Ac</td>
<td>CBP/p300</td>
<td>Transcriptional activation</td>
<td>[46,47]</td>
</tr>
<tr>
<td>H3S28p</td>
<td>Aurora B/MSK1</td>
<td>Mitotic chromosome condensation</td>
<td>[48]</td>
</tr>
<tr>
<td>H3K36me3</td>
<td>SMYD2</td>
<td>Transcriptional repression, transcriptional elongation</td>
<td>[49]</td>
</tr>
<tr>
<td>H4K5Ac</td>
<td>Hat1, CBP/p300</td>
<td>Histone deposition, transcriptional activation</td>
<td>[50]</td>
</tr>
<tr>
<td>H4K12Ac</td>
<td>Hat1</td>
<td>Histone deposition</td>
<td>[51]</td>
</tr>
<tr>
<td>H4K16Ac</td>
<td>ATF2</td>
<td>Cell-cycle-dependent acetylation</td>
<td>[52]</td>
</tr>
<tr>
<td>H4K20me3</td>
<td>Pr-SET7</td>
<td>Transcriptional silencing, mitotic condensation</td>
<td>[16]</td>
</tr>
</tbody>
</table>
Lys20 on histone H4 (H4K20me3) are associated with gene repression, whereas trimethylation of Lys4 of histone H3 (H3K4me3) is associated with gene activity (for an in-depth review, see [4]).

Evidence indicates that epigenetic modulators regulate gene expression throughout the cell cycle, which in turn ensures correct inheritance of the epigenetic marks to the new daughter cells. However, the mechanisms underlying the inheritance of epigenetic marks are still poorly understood. Chromatin dynamics across the cell cycle is evident at two levels: on a global level where the dynamic change ensures ‘breathing’ of the genome, such as chromatin relaxation, compaction, chromosome alignment and segregation, and on individual genes where dynamic changes regulate specific gene expression, such as activation and repression of cell-cycle-regulated genes.

In this chapter, we summarize the current knowledge of the dynamics of epigenetic chromatin modifications. Although we focus on mammalian cells, similar dynamics are also observed in other organisms [5].

**G1-phase (Gap-1 phase) and the G1–S transition**

By far the longest phase of the mammalian cell cycle, G1 is also called the growth phase owing to cell and organelle growth and expansion. It is the stage of the cell cycle where cells are able to conduct their normal biosynthetic activities after a mitotic slow down. A rapid transcriptional activation of a large set of genes is observed, and a great amount of proteins and enzymes are being synthesized. This large transcriptional activity has been linked to a decrease in DNMT1 and DNMT3b levels [6], causing a decrease in global DNA methylation levels [7]. These findings were validated further by an increase in expression level of the DNMT1 disruptor AUF1 [ARE (AU-rich element)/poly(U)-binding/degradation factor 1] during G1-phase [8]. The global decrease in DNA methylation is accompanied by global acetylation of nucleosomes, which facilitates the activation of different sets of genes, including the E2F family of transcription factors known as vital players in cell-stage-dependent activation of gene expression during the mammalian cell cycle [9,10]. Several studies have shown that E2F4 forms a complex with the Rb (retinoblastoma) family member p130 and HDACs (histone deacetylases) in quiescent and early-G1 cells, which binds to promoters containing E2F sites. This mediates local de-acetylation of H3 and H4 and hence gene repression [11]. The E2F4–p130 complex is released from these promoters in mid-to-late-G1 and is replaced by a complex encompassing E2F1–E2F3 and the Tip60 HAT (histone acetyltransferase), inducing hyperacetylation of H3 and H4 on these promoters and hence gene activation [11,12]. In particular, H3K9Ac levels dramatically increase in late-G1 phase compared with low levels during G2/M [13]. Promoter-specific studies on CDC6 and E2F1 promoters identified them to be hypoacetylated in cells in quiescence and early-G1 [11]. This acetylation level increases in mid- and late-G1 and decreases again upon entry into S-phase, consistent with expression of the associated genes [11]. Another
layer of regulation comes from pRb (retinoblastoma protein), a main repressor of E2F-mediated transcriptional activity, which forms a complex with several members of the HDAC family along with the HMT (histone methyltransferase) SUV39H1 and recruits them to E2F-site-containing promoters [i.e. thymidine kinase, cyclin A, cyclin E, CDK (cyclin-dependent kinase) 2 and CDC2]. Gene repression is hence caused by deacetylation and H3 Lys9 methylation of targeted promoters [11,14]. This repression is relieved due to the disruption of the HDAC–pRb–SUV39H1 complex by the phosphorylation of cyclin D–CDK4/CDK6 at the end of the G1-phase [9]. As an example, ChIP (chromatin immunoprecipitation) studies on cell-cycle promoters revealed that the active marks H3K36me3, H3K79me2 and H3K4me3 occupy the active promoter of PCNA (proliferating-cell nuclear antigen) and are completely absent from the inactive promoter of cyclin B, whereas the repressive mark H4K20me3, on the other hand, showed an opposite occupational profile [15]. These findings were fortified further by the co-localization of H4K20me3 with its methyltransferase KMT5B/SUV4-20H, and H3K4me3 with menin, a component of the MLL complex responsible for the deposition of the H3K4me mark [15].

S-phase (synthesis phase)

The synthesis phase is a crucial stage of the cell cycle where histones and DNA modifications must be deposited correctly to the newly synthesized chromatin. With the exception of histone production, the S-phase is characterized by a very low rate of RNA transcription and protein synthesis. This is illustrated by an increase in global DNA methylation levels and in DNMT1 expression [6,7]. Moreover, DNMT1 knockdown leads to an arrest of firing of the origin of replication and an intra-S-phase arrest of DNA replication [8]. On the other hand, overexpression of DNMT1 induced by AUF1 depletion causes an increase in S-phase and G2/M-phase compared with controls [8].

Global methylation levels of the histone H3 and histone H4 tails have been shown to increase in late-G1- and S-phase [11] in agreement with data showing the highest histone H3 and H4 methyltransferase activity detected during this phase [16]. In contrast with repressive marks, multiple lysine acetylations on both the histone H3 and histone H4 tails have been linked to newly deposited histones in S-phase, and acetylation of newly synthesized histone H4 at Lys5 and Lys12 is highly conserved among species (reviewed in [17]). Histone acetylation during S-phase is interpreted to favour repair and reactivation of stalled replication forks; however, this hypothesis is still under question [17]. Others argue that increased histone acetylation could be the result of a high deposition rate of acetylated histones relative to the methylated ones [18]. For instance, H3K9Ac, which peaks during G1-phase, has been reported to remain high during S-phase [13], and the same goes for H4K16Ac [16]. In contrast, H4K20 and H3K79 methylations were not detected on newly deposited histones until later time points in the cell cycle [18]. On the other hand, H3K27 is rapidly monomethylated on newly synthesized histones, which is
probably due to the localization of EZH2 (the H3K27 methyltransferase) to sites of replication [18]. H4K20me3 was detected at its lowest levels in S-phase, in agreement with the predicted negative correlation with H4K16Ac [19].

On a local level, early-firing origins of replication are associated with high levels of activation marks, such as H3K9/H3K14 acetylation and H3K4me3, but low levels of H3K9me3 [20]. Late-firing origins, on the other hand, are associated with a compact chromatin conformation until their activation, and thus marked with low levels of H3K9Ac/H3K14Ac and H3K4me3 and increased H3K9me3 marks [20].

It is important to note that, although histone dynamics during S-phase are well documented (as reviewed by Probst et al. [21]), the comparison with other phases of the cell cycle is in need of further investigation, mainly due to the dilution of the marks during S-phase when new histones are being synthesized (Figure 2).

**Figure 2. Histone dynamics across the cell cycle**
Histone marks and chromatin-associated proteins summarized by up- (red) and down- (blue) regulation during each phase of the cell cycle. Dynamics within the cell cycle are represented in the graph.
G2-phase (Gap-2 phase) and M-phase (mitotic phase)

The G2-phase of the cell cycle is a short phase marked by significant protein synthesis, mainly involving production of microtubules. However, because of its short duration and the difficulty of synchronizing cells at this stage, documentation of post-translational histone modifications often merge the G2-phase with the following M-phase. The process of mitosis is complex and highly regulated. During mitosis, cell growth is halted and cellular energy is focused on the division into two daughter cells. Post-translational histone modifications are well documented during this phase in several cell types, mainly because of their importance for chromatin condensation and chromosomal segregation. Histone H3 phosphorylation is low until late interphase and spreads throughout the genome until prophase, where it constitutes a mitotic hallmark [22]. It is initiated in defined chromosomal domains during the G2-phase and peaks in mitosis in concert with a condensed chromatin state. The link between histone phosphorylation and chromatin condensation has been supported further by a premature chromosomal condensation in cultured cells following treatment with staurosporine, an inhibitor of the fostriecin phosphatase, which causes early H3 phosphorylation (reviewed in [23]). In particular, phosphorylation of Ser10 and Ser28 on the histone H3 tail (H3S10p and H3S28p) have been demonstrated to act together for chromosomal condensation and segregation during mitosis [13]. This is in agreement with the co-localization of the Aurora B kinase with histone H3 during mitosis and the aberrant chromosome segregation observed upon transient Aurora B inhibition [24]. Other histone phosphorylations have also been shown to be up-regulated during M-phase and linked to chromatin condensation, such as H3T3p, H3T11p, H3.3S31p [25,26], H2AS1p, H4S1p [25,27], as well as the H3T3 kinase VRK1 (vaccinia-related kinase 1), which also localizes to chromatin during mitosis [26].

Transcription is believed to be turned off during mitosis, which is in accordance with an observed global deacetylation of histones. A dramatic decrease in acetylation has been reported on histones H3K9, H3K18, H3K23, H4K5, H4K8, H4K12 and H4K16 during this stage [13,16,25,28,29]. Lysine deacetylation has been also observed on histone H2A and H2B, in particular H2AK5, H2BK12, H2BK15 and H2BK20 [25]. This global deacetylation phenomenon, in addition to its role in transcriptional repression, is thought to ensure a correct packaging of nucleosomes into metaphase chromosomes. This is supported by the observed destabilization of the chromatin fibre and the decondensation of the chromatin upon the induction of multiple acetylations on the histone tails. Shogren-Knaak et al. [29] showed that H4K16Ac, in particular, is responsible for chromatin decondensation. In addition, SirT2, an HDAC with preference for H4K16, has been shown to be highly expressed and associated with chromatin exclusively during mitosis [28]. Moreover, inhibition of HDAC3 causes a block of the cell cycle at the G2/M-phase as a result of mitotic defects [30]. Interestingly, Li et al. [30] showed that HDAC3 forms
a complex with Aurora B and is targeted to mitotic chromosomes, correlating with the dramatic deacetylation accompanying histone phosphorylation during mitosis.

The low levels of transcriptional activity during the mitotic phase is also evident from the histone methylation patterns. Lys35 methylation on histone H4 is generally increased, together with an expression peak of its HMT PR-Set7 [16,25]. The high level of H4K20 methylation correlates with the deacetylation of H4K5, H4K8, H4K12 and, in particular, H4K16 observed during M-phase [16,25]. Moreover, PR-Set7 has also been shown to be associated with mitotic chromosomes [16]. Other notable methylation dynamics during G2/M-phase occur on H3K17, H3K79 and H4R3 [16,25,31]. Global H3K9me3 is also increased during mitosis and returns to basal levels upon entry into G1-phase, and double depletion of the HMTs SUV39H1 and SUV39H2 results in low levels of H3K9me3 during mitosis and consequently in mitotic defects [32].

It is not known whether the increase in histone lysine methylations is a mitotic phase hallmark or the effect of slow kinetics of the methylation mark deposition on newly synthesized histones [18]. Recently, Pesavento et al. [31] showed that newly synthesized histone H4 tails remain unmethylated during S-phase and become progressively mono- and di-methylated during mitosis. These findings were supported further by a failure in cell-cycle progression upon PR-Set7 depletion [31]. However, that does not explain the slight decrease observed in H3K27 and H3K36 methylations [25]. Regardless of whether the appearance of H4K20 methylation during M-phase is an effect of its deposition kinetics, it appears to be independent of its fluctuation on G2/M active or repressed promoters. For instance, H4K20 methylation is absent from the active cyclin B1 promoter during G2/M-phase, as is the associated methyltransferase KMT5B, and is replaced by the activating H3K4me and H3K4me3 marks [15,33]. On the other hand, the silent PCNA promoter is enriched with KMT5B, H4K20me3 and lacks H3K36me3, H3K79me2, H3K4me, H3K4me3 and menin [15].

Furthermore, histone ubiquitination is highly dynamic. Joo et al. [34] showed that the ubiquitination levels of H2A decrease and reach their lowest level during mitosis, to recover only upon exit from M-phase and entry into the G1-phase. They also demonstrated that knockdown of the H2A-specific deubiquitinase Ubp-M in HeLa cells induced a slow down in proliferation and defects in the M-phase [34]. Furthermore, their analysis revealed that the observed inverse correlation between H2Aub and H3S10p is mainly caused by the disruption of the association of Aurora B kinase with the nucleosome facilitated by histone H2A ubiquitination. Hence, H2A deubiquitination by Ubp-M is required for subsequent mitotic H3S10p and chromosomal segregation [34].
**Quiescent/senescent state or G₀-phase (Gap-0 phase)**

In mature mammals, most cells reside in a prolonged non-dividing state called G₀-phase. In culture, cells usually enter G₀-phase under stress conditions, such as contact inhibition factor or serum starvation, and the loss of ability to maintain the G₀ stage is linked to carcinogenesis. Entry into G₀ is characterized by a shut-down of cyclin genes and an inactivation of specific gene programmes depending on cellular function. This is mainly linked to the E2F-dependent transcriptional machinery [35,36]. E2F6 has been shown to form complexes with different chromatin modifiers, such as Eu-HMT1 (euchromatic HMT1) and NG36/G9a, leading to H3K9 methylation of E2F responsive promoters and hence their repression [35]. In addition, PHC3 (polyhomeotic-like 3), a member of the polycomb complex, has also been shown to co-localize and form a complex with E2F6 in confluent and differentiated cells but not in cycling cells [36]. pRb is thought to form a complex with polycomb members and its ablation leads to a decrease of the H3K27me2/H3K27me3 mark on silent cyclin promoters after myogenic differentiation and thereby their activation [37].

On a global level, a general hypoacetylation is observed on histones H3 and H4 in quiescent cells compared with late-G₁- and S-phase, an outcome of E2F4–p130 complex formation [11]. On the DNA level, a general decrease in DNA methylation is observed in serum-starved cells as compared with cycling cells [6,8]. Levels of DNMT1 were also low in G₀, an outcome likely resulting from over-representation of its mRNA destabilizer AUF1. DNMT1 and DNA methylation levels are restored upon re-entry into the cell cycle, particularly upon S-phase entry [8]. A recent study by Sen et al. [38] also indicated that DNMT1 is down-regulated during differentiation, correlating with a decrease in DNA methylation on genes involved in differentiation. De novo methylation, however, also occurs on silent genes during differentiation [38].

**Conclusions and perspectives**

The currently available data show a clear and robust correlation between cell-cycle progression, transcriptional regulation and chromatin modifications. Although extensive studies have charted and linked different chromatin modifications to different phases of the cell cycle, as summarized in Table 2, it is worth noting that the datasets are often based on chemical arrest-and-release strategies, which are associated with unbalanced cell growth and severe perturbation of metabolic processes. Moreover, most data are based on immortalized or transformed cell lines, which are known to have different chromatin patterns from those of normal cells [39]. An important task for future studies will therefore be to analyse the global and local dynamics in primary cells in the absence of cell-cycle inhibitors.
Table 2. Histone post-translational modification dynamics across the cell cycle

<table>
<thead>
<tr>
<th>Phase</th>
<th>Global alteration in histone post-translational modifications</th>
<th>Alteration in chromatin-associated proteins</th>
<th>Functional consequences</th>
<th>Reference(s)</th>
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<tbody>
<tr>
<td>$G_0$</td>
<td>Increase</td>
<td>AUF1</td>
<td></td>
<td>[6–8,11,12]</td>
</tr>
<tr>
<td></td>
<td>Decrease</td>
<td>Global DNA methylation, acetylation of H3 and H4</td>
<td>DNMT1</td>
<td></td>
</tr>
<tr>
<td>$G_1$</td>
<td>Increase</td>
<td>H3 acetylation, H3K9Ac, H4 acetylation</td>
<td>AUF1</td>
<td>Gene expression</td>
</tr>
<tr>
<td>$G_1$</td>
<td>Decrease</td>
<td>Global methylation</td>
<td>DNMT1 and DNMT3b, VRK1</td>
<td>Gene expression</td>
</tr>
<tr>
<td>$S$</td>
<td>Increase</td>
<td>Global DNA methylation</td>
<td>DNMT1</td>
<td>Bona fide gene repression</td>
</tr>
<tr>
<td>$S$</td>
<td>Decrease</td>
<td></td>
<td></td>
<td>Bona fide gene repression</td>
</tr>
<tr>
<td>$G_{2/M}$</td>
<td>Increase</td>
<td>H2AS1p, H3p, H3T3p, H3K9me3, H3S10p, H3T11p, H3K17me, H3S28p, H3K79me, H3S31p, H4S1p, H4R3me, H4K20me</td>
<td>SirT2, PR-Set7, VRK1</td>
<td>Chromosome condensation and segregation</td>
</tr>
<tr>
<td>$G_{2/M}$</td>
<td>Decrease</td>
<td>H2AK5Ac, H2AUb, H2BK12Ac, H2BK15Ac, H2BK20Ac, H3K18Ac, H3K23Ac, H3K27me, H3K36me, H4K5Ac, H4K8Ac, H4K12Ac, H4K16Ac</td>
<td></td>
<td>Chromosome condensation and segregation</td>
</tr>
</tbody>
</table>
Summary

- Epigenetic modulators regulate gene expression throughout the cell cycle.
- The cell cycle ensures correct inheritance of epigenetic marks to new daughter cells.
- Chromatin dynamics across the cell cycle is evident at global levels and on individual genes.
- A general decrease in DNA methylation levels and an increase in histone acetylation levels is observed during G1-phase.
- A general increase in DNA methylation levels and histone acetylation levels is observed during S-phase.
- A general increase in histone phosphorylation levels and a decrease in histone acetylation and ubiquitination levels is observed during mitosis.
- A general decrease in DNA methylation and histone acetylation levels is observed in quiescent cells.

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