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Mitotic Partitioning of Transcription Factors

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ABSTRACT

Mitosis is a highly orchestrated process involving numerous protein kinases and phosphatases. At the onset of mitosis, the chromatin condensation into metaphase chromosomes is correlated with global phosphorylation of histone H3. The bulk of transcription is silenced while many of the transcription-associated proteins, including transcription and chromatin remodeling factors, are excluded from chromatin, typically as a consequence of their phosphorylation. Components of the transcription machinery and regulatory proteins are recycled and equally partitioned between newly divided cells by mechanisms that may involve microtubules, microfilaments or intermediate filaments. However, as demonstrated in the case of Runx2, a subset of transcription factors involved in lineage-specific control, likely remain associated with their target genes to direct the deposition or removal of epigenetic marks. The displacement and re-entry into daughter cells of transcription and chromatin remodeling factors are temporally defined and regulated. Reformation of daughter nuclei is a critical time to re-establish the proper gene expression pattern. The mechanisms involved in the marking and re-establishment of gene expression has been elucidated for few genes. The elucidation of how the memory of a programmed expression profile is transmitted to daughter cells represents a challenge. J. Cell. Biochem. 105: 1–8, 2008.

KEY WORDS: MITOSIS; GENE EXPRESSION; EPIGENETIC MARKS; TRANSCRIPTION FACTORS; CHROMATIN REMODELING; PHOSPHORYLATION

At the onset of mitosis, while chromosome condensation takes place to facilitate segregation of sister chromatids between daughter cells, the nuclear envelope ruptures and the bulk of transcription activity ceases. The entry into mitosis is also characterized by extensive protein phosphorylation. The condensation of interphase chromatin into mitotic chromosomes, restricting the accessibility of DNA to transcription factors and RNA polymerases, has been put forward as the most evident mechanism of transcription shut-down [Gottesfeld and Forbes, 1997]. However, on and off exchanges of transcription factors and nucleosome components were observed from condensed mitotic chromosomes, suggesting that transcription arrest is due to reasons other than chromatin condensation itself [Chen et al., 2005]. Alternative mechanisms include inactivation by phosphorylation and/or displacement of RNA polymerases, chromatin remodeling complexes and transcription factors from mitotic chromosomes [Gottesfeld and Forbes, 1997; John and Workman, 1998]. When cells exit mitosis, specific gene expression patterns must be re-established. It has been proposed that a subset of factors act as molecular bookmarks by binding to mitotic chromatin, as genes poised for reactivation are marked by protein-dependent structural distortions [Michelotti et al., 1997]. Several studies providing evidence to support this hypothesis are reviewed here. Furthermore, the elucidation of the spatial and temporal mitotic reorganization of regulatory proteins has been informative about the role of these proteins in preserving epigenetic marks during mitosis and restoring lineage-specific gene expression in progeny cells.

EPIGENETIC MARKS

In recent years, an explosion of knowledge has pointed to the importance of histone post-translational modifications (PTMs) in the recruitment of regulatory proteins and regulation of gene expression. The preservation of the gene expression profile through cell division includes the preservation of histone PTMs. Thus, a brief overview of histone PTMs is presented here.

Nuclear DNA is packaged into nucleosomes, the basic repeating structural units in chromatin. The nucleosome consists of a histone octamer, two of each H2A, H2B, H3, and H4, around which 147 bp DNA is wrapped. Core histones undergo an array of reversible PTMs, including acetylation (ac), methylation (me), ubiquitination (ub), and phosphorylation (ph). Some PTMs (active marks) are associated with transcriptionally active chromatin regions, while others (repressive marks) correlate with silent regions. Histone acetylation

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usually marks active genes as does di- or trimethylation of K4 of H3 (H3K4me2/3) whereas dimethylation of K9 of H3 (H3K9me2) constitutes a repressive mark [Peterson and Laniel, 2004; Sims and Reinberg, 2006]. There is still much to learn about the role of histone PTMs in chromatin structure and function. Histone PTMs function to disrupt chromatin structure and/or provide a ''code'' for recruitment or occlusion of nonhistone chromosomal proteins to chromatin. These recruited proteins are referred to as ''readers''. In this reader group are ''effectors'', which have activities for further histone PTMs or ATP-dependent chromatin remodeling [Ruthenburg et al., 2007]. Enzymes catalyzing reversible histone PTMs are referred to as writers. Note that a new nomenclature has been accepted for the chromatin modifying enzymes [Allis et al., 2007].

SPATIAL MITOTIC REORGANIZATION

EXCLUSION OF TRANSCRIPTION-ASSOCIATED PROTEINS FROM CHROMATIN AND ROLE OF PROTEIN PHOSPHORYLATION

During interphase, transcription-associated proteins generally exhibit a punctate distribution throughout the mammalian nucleus [Grande et al., 1997; He et al., 2005]. At the onset of mitosis, many of these proteins are excluded from condensing chromosomes and are dispersed throughout the cell. This is the case with RNA polymerase II [Parsons and Spencer, 1997; Christova and Oelgeschlager, 2002] and many transcription factors, for example, Oct-1, Oct-2, Ets-1, c-Fos, B-Myb, E2F-1, Bcl-6, HSF1, C/EBP, Sp1 [Martinez-Balbas et al., 1995; Boyd et al., 2003], Ikaros [Brown et al., 1997], Sp3 [He and Davie, 2006], GATA-1 [Xin et al., 2007], and PAX3 [Corry et al., 2008]. The general transcription factor TFIIB, which is part of the RNA polymerase II pre-initiation complex, is also displaced [Chen et al., 2002; Prasanth et al., 2003], as well as factors associated with the U6 gene transcribed by RNA polymerase III [Komura and Ono, 2005]. Similarly, the chromatin remodeling factors lysine acetyl-transferases (KATs) and histone deacetylases (HDACs) are excluded from condensing chromosomes [Kruhlak et al., 2001; Kouskouti and Talianidis, 2005]. However, in a conflicting report, a subpopulation of the p300 KAT has been found associated with chromatin throughout mitosis [Zaidi et al., 2003]. Binding of the TATA binding protein TBP to its target sequences in c-*myc* and c-*fos* promoters was not detected during mitosis [Komura et al., 2007], although a good proportion, if not all of TBP protein, was found associated with condensed chromosomes in other studies [Chen et al., 2002; Christova and Oelgeschlager, 2002; Prasanth et al., 2003]. Tissue-specific transcription factors, like Runx2 in osteogenic cells (rat osteosarcoma ROS 17/2.8)[Zaidi et al., 2003] and NF-E2 in murine erythroleukemia cells [Xin et al., 2007], are predominantly redistributed to an extra-chromosomal space in metaphase and anaphase, except for a subset of foci which remain associated with chromatin throughout mitosis. In interphase HeLa cells, cofactor NC2 is associated with the promoter of active genes, including *hsp70*, *ets-2*, histone H2B and histone H4 genes. Entry into mitosis decreased NC2 promoter occupancy at *hsp70* and *ets-2* promoters, but not significantly at histone H2B and histone H4 promoters [Christova and Oelgeschlager, 2002].

For proteins hBrm and Brg-1, components of the human SWI/SNF chromatin remodeling complex, the mitotic displacement from HeLa cells chromosomes is concurrent with their phosphorylation. Moreover, while the level of Brg-1 protein remains constant in the mitotic phase, the level of hBrm is strongly reduced [Muchardt et al., 1996]. Thus, mitotic phosphorylation and degradation in the case of the hBrm-based SWI/SNF complex are thought to contribute to mitotic transcription silencing [Muchardt et al., 1996; Sif et al., 1998]. Mitotic phosphorylation of transcriptional corepressors of the Groucho/transducin-like Enhancer of split (Gro/TLE) family is also correlated with a decreased nuclear interaction [Nuthall et al., 2002].

C_2H_2 zinc finger proteins constitute a predominant family among mammalian transcription factors, and the linkers separating the finger motifs in the DNA-binding domain are highly conserved, as they are likely to contain the consensus amino acid sequence TGEKP. Among this family, Ikaros and Sp1 are not only excluded from chromatin in early mitosis, but it has also been shown that the three linkers of Ikaros and at least one of the Sp1 linkers are phosphorylated [Dovat et al., 2002]. Similarly, the DNA-binding ability of Oct-1 and GHF-1, two members of the POU family, is lost upon mitotic phosphorylation of the same conserved amino acid of the POU domain [Segil et al., 1991; Caelles et al., 1995]. These findings imply that mitotic inactivation of transcription factors through phosphorylation may be a mechanism common to every family of transcription factors. It may also pertain to histone deacetylases, as phosphorylation of HDAC2 has been observed in mitotic cells [Galasinski et al., 2002].

RECYCLING AND EQUAL PARTITIONING OF TRANSCRIPTION FACTORS

Transcription factors Sp1 and Sp3 are expressed in all mammalian cells and participate in the regulation of genes involved in most cellular processes [Davie et al., 2008]. In human breast cancer cells MCF-7 cells, Sp1 and Sp3 levels, as well as HDAC2 and estrogen receptor α (ERα) levels, remain constant during cell division [He and Davie, 2006]. This implies that these regulatory proteins are recycled into daughter nuclei, as it has been established for transcription factor Runx2 [Zaidi et al., 2003] and RNA polymerase II transcription factors TFIIB and TBP; although in the case of the RNA polymerase II transcription factors, a low turnover rate was observed [Prasanth et al., 2003].

The mitotic partition and reformation in G1 phase of nuclear bodies has also been the subject of recent studies. Nuclear speckles or interchromatin granule clusters (IGCs) contain proteins for pre-mRNA processing. At the onset of mitosis, these proteins become diffused through the cytoplasm and during metaphase reassemble into mitotic interchromatin granules (MIGs), structurally analogous to IGCs. It was proposed that MIGs play a role in recycling splicing factors during mitosis, and participate in the re-formation of nuclear speckles by releasing their components for ensuing entry into daughter nuclei [Prasanth et al., 2003]. A similar role was attributed to mitotic accumulation of PML bodies protein (MAPP) for the recycling of PML bodies, involved in transcription regulation among other cellular processes. However, MAPPs are structurally and biochemically different from PML bodies, having lost some PML bodies components such as SUMO-1, Sp100 and Daxx. Moreover, a subset of MAPPs is associated with condensed chromosomes throughout mitosis, possibly providing a nucleation site for the

reformation of PML bodies in G1 phase [Dellaire et al., 2006]. Current information suggests that the bulk of regulatory proteins are recycled and equally partitioned into progeny nuclei.

A recent study has revealed a link between mitotic phosphorylation and protein stability, demonstrating that Sp1 is phosphorylated at residues Thr278 and Thr739 by the c-Jun NH2-terminal kinase (JNK) 1 in the mitotic phase of epithelial tumor and glioma cell lines. These phosphorylation events protect Sp1 from mitotic ubiquitin-dependent degradation in tumor cell lines. In sharp contrast, JNK1 is inactivated during mitosis in normal cells, and the level of Sp1 is significantly reduced during mitosis. Hence, mitotic JNK1-induced phosphorylation of Sp1 increases its stability, resulting in an accumulation of Sp1 in cancer cells. Thus, mitotic stability of Sp1 may be a therapeutic target in the treatment of some cancers [Chuang et al., 2008]. It is possible that the maintenance of Sp1 levels in mitotic cancer cells gives the cancer cells an advantage by enabling them to faster reset transcription at mitosis exit. Of significant importance for research in general, this report warrants a cautionary note regarding the biological relevance of data resulting from the use of cancer cell lines, as many of these data may not apply to primary cells. Most of the studies mentioned here were done on cancer (e.g., HeLa) cell lines. In view of the above report, it would be interesting to examine the mitotic stability of transcription factors in primary cells.

MITOTIC RELOCATION OF REGULATORY PROTEINS

Despite their binding to the same DNA sites, Sp1 and Sp3 have different functional roles. Unlike Sp1 which is usually an activator of transcription, Sp3 functions as an activator or a repressor. Their binding to a target site is reciprocally exclusive. Moreover, Sp1 and Sp3 occupy different subnuclear sites, and these subnuclear sites are associated with the nuclear matrix. It has been proposed that the spatial position of a promoter next to a Sp1 or Sp3 domain influences which of the two factors occupies the Sp binding site and affects gene regulation [He et al., 2005]. Thus, it is important that the distinct distribution of Sp1 and Sp3 foci is maintained and transferred to the nuclear matrix of progeny cells. It was shown that Sp1 and Sp3 keep their distinct punctate distribution during mitosis, while they get entirely displaced from the condensed chromosomes and are temporarily localized with the microfilaments [He and Davie, 2006] (Fig. 1). This observation suggests that F-actin may participate in the mitotic shuttle of Sp1 and Sp3 foci. Sp1 and Sp3 foci may maintain their separate identities throughout mitosis as a result of indirect interactions with F-actin mediated by different proteins. It is possible that a differential association of Sp1 and Sp3 with the microfilaments supports their subsequent differential association with the nuclear matrix in interphase nuclei.

A subset of the Runx2 transcription factor was reported to colocalize with the microtubules during mitosis [Young et al., 2007b], suggesting that the microtubules may be important to the subcellular location of a subset of Runx2 during mitosis. The insulator protein CTCF is associated with mitotic chromosomes, except for a subpopulation which colocalizes with centrosomes in a microtubule-independent manner from metaphase to anaphase, and relocalizes with midbodies at telophase and in the reformed nuclei [Zhang et al., 2004; Burke et al., 2005].

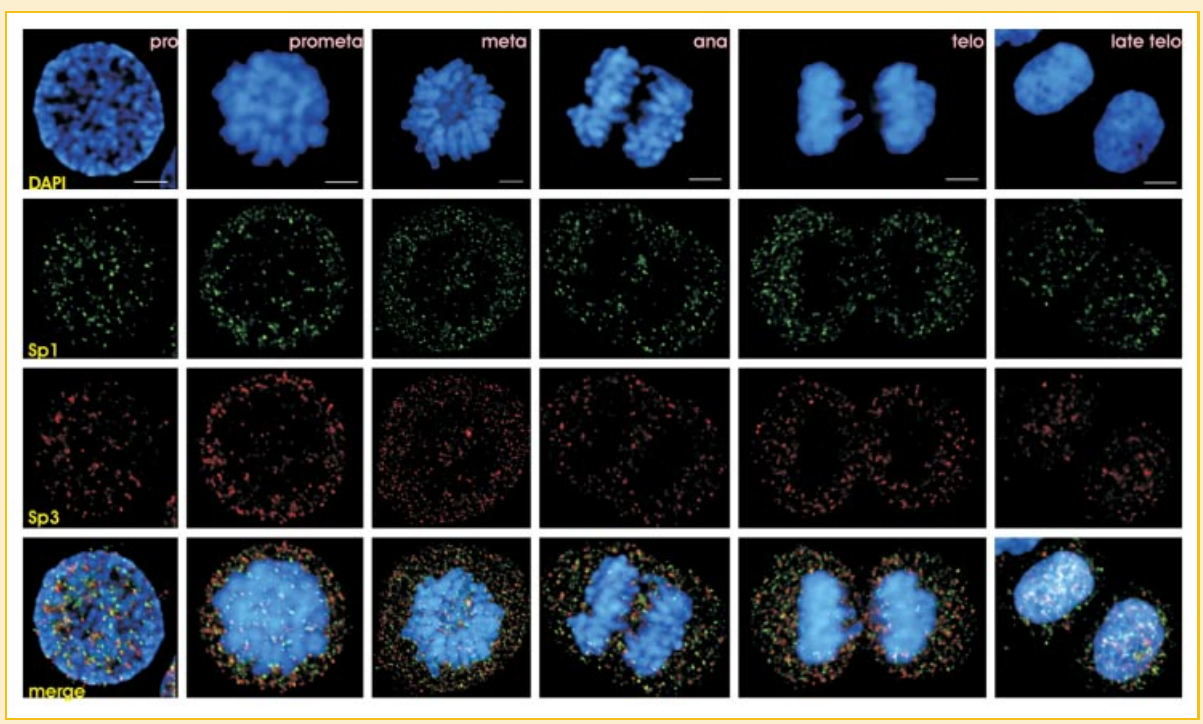

Fig. 1. Distribution of Sp1 and Sp3 throughout the mitosis stages. MCF-7 cells were grown on coverslips in estrogen complete medium, fixed, and double labeled with anti-Sp1 and anti-Sp3 antibodies. DNA was stained by DAPI. Sp1 and Sp3 distributions were visualized by fluorescence microscopy and image deconvolution as described in He and Davie [2006]. Single optical sections are shown. Yellow in the merge images signifies colocalization. Bar, 5 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Activation of the Ras-MAPK or p38 stress kinase pathways results in the transient phosphorylation of histone H3 at S10 or S28, parallel with the transcriptional activation of specific genes including intermediate early genes and HDAC1 gene. Phospho-serine adaptor 14-3-3 ε and ζ proteins are recruited to H3S10ph and H3S28ph, with their association to S10ph being more stable if phosphorylated H3 is also acetylated at K9 or K14. 14-3-3 ε and ζ proteins are readers of the H3 phospho marks, and are required for transcriptional induction [Macdonald et al., 2005; Winter et al., 2008]. However, their role in the context of transcription is not known. On the other hand, global phosphorylation of H3 at S10 as well as S28 by Aurora B kinase is a hallmark of mitosis and correlates with chromatin condensation. Nonetheless, 14-3-3 ε and ζ are dissociated from mitotic chromosomes. It has been suggested that this exclusion is due to phosphorylation of 14-3-3 proteins and their subsequent binding to intermediate filament proteins including cytokeratins [Winter et al., 2008], an event which has been named "14-3-3 sink," and is thought to contribute to the activation of the mitotic activator Cdc25 [Margolis et al., 2006].

ROLE OF TRANSCRIPTION FACTORS ASSOCIATED WITH MITOTIC CHROMOSOMES

Although many transcription-associated proteins are dispersed through the mitotic cell, some factors stay associated with the condensed metaphase chromosomes, for example, $p67^{SRF}$ [Gauthier-Rouviere et al., 1991], AP-2 [Martinez-Balbas et al., 1995], and FoxI1 [Yan et al., 2006].

HP1 proteins are involved in heterochromatin organization and position effect variegation, and are recruited to chromatin by methylation of H3 K9. They exist as three isoforms in mammalian cells: HP1α and HP1β colocalizing with heterochromatin in interphase and HP1γ associated with both heterochromatin and euchromatin. In mitotic HeLa cells, HP1α stays bound primarily at heterochromatic sites, while HP1β and HP1γ are partly bound to chromosomes and partly dispersed in cytoplasm [Minc et al., 2001]. In a subsequent study, HP1β was shown to be mostly released from HeLa and 10T1/2 mitotic chromosomes, and the release of HP1β from chromatin was dependent on the Aurora B-mediated phosphorylation of H3 S10 [Fischle et al., 2005]. However, this study disagrees with a previous report stating that phosphoacetylation of H3, resulting in H3K9meS10phK14ac, is responsible for the displacement of HP1α from mitotic chromosomes [Mateescu et al., 2004]. The later proposal is not supported by a study analyzing H3 phosphorylation and acetylation through the mammalian cell cycle. Although an increase in H3S10phK14ac levels is observed during mitosis, it does not happen in the same time frame as the reported dissociation of HP1α from mitotic chromosomes [McManus and Hendzel, 2006].

Polycomb group (PcG) complexes are repressors associated to heterochromatin, which play a crucial role in the regulation of developmental genes. They bind to specific DNA sequences and silence gene expression by modifying histones. PcG complexes consist of three classes, PRC1, PRC2, and PhoRC. Their recruitment to chromatin is very intricate [Schuettengruber et al., 2007]. In a simplified version, PCR1 is the reader of the H3K27me3 mark deposited by PRC2, whereas PCR2 is recruited to chromatin via PhoRC which includes a sequence specific DNA binding protein as well as a protein binding specifically to mono- and dimethylated H3K9 and H4K20. In turn, PRC1 is an effector which directs H2A ubiquitination, contributing to silencing by unidentified mechanisms. In human fibrosarcoma and osteosarcoma cells, PRC1 was shown to maintain its structural integrity and colocalize with condensed chromosomes throughout mitosis. PRC1 complexes are also evenly distributed between separating chromatids [Saurin et al., 1998].

The Brd4 protein is a member of the BET family which carries two tandem bromodomains and binds to acetylated H3 and H4, exhibiting more affinity for diacetylated or higher-acetylated H3 and H4, including H3K14ac (although monoacetylated), H3K9acK14ac, H4K5acK12ac, and H4K5acK8acK12acK16ac. Even though a marked deacetylation of core histones occurs when cells enter mitosis, some H3 and H4 conserve their acetylation modifications to which Brd4 remains bound throughout mitosis. This behavior of Brd4 differs from that of other bromodomain proteins (Brg1, hBrm, PCAF, GCN5, P300/CBP, and $TAF_{II}250$) which are displaced from chromatin. It is possible that the interaction of Brd4 with the subset of H3 and H4 histones that have escaped the global mitotic deacetylation contributes to the transfer of active marks to daughter nuclei [Dey et al., 2003]. Likewise, mitotic preservation of H3K4me2/3, H3K79me2 as well as H3 and H4 acetylation were reported at the promoter and 5′coding region of active genes. It was proposed that these histone modifications contribute to the conveyance of active chromatin configuration to progeny cells [Kouskouti and Talianidis, 2005]. However, the readers of these modifications have not yet been identified.

The zinc-finger protein CTCF has insulator activity and is thought to demarcate nuclear matrix-dependent points of transition in chromatin, thereby forming topologically independent chromatin loops that may support gene silencing. In contrast to other zinc finger proteins, CTCF is associated in a sequence-specific fashion to its target sites in mitotic chromosomes. Although the CTCF interaction with the enhancer/promoter at the Igf2/H19 locus is lost during mitosis, the long-range DNA interactions established by CTCF at the same locus are maintained. It was postulated that, even though the nuclear matrix is dissociated during mitosis, the chromatin domain delimited by the matrix attachment regions to which CTCF binds could be stabilized by CTCF binding the two separate sequences [Burke et al., 2005]. However, an association of CTCF with mitotic c-*myc* or DM1 genes could not be detected in another study [Komura et al., 2007].

HSF2 is a transcription factor recognizing the heat shock elements of the *hsp70i* promoter. Unlike the related HSF1 factor, HSF2 is not involved in the stress-induced transcription of *hsp70i* and is not excluded from mitotic chromosomes. It was recently demonstrated that in mitotic cells, HSF2 bound to *hsp70i* promoter recruits the phosphatase 2A to promote the dephosphorylation and inactivation of condensin in the surrounding area, thus hindering local chromatin compaction and bookmarking the *hsp70i* promoter. The biological significance of this bookmarking was verified when reduced levels of HSF2 resulting from RNA interference led to a

decreased heat-induced level of hsp70i protein in G1 phase and a corresponding decrease in cell viability [Xing et al., 2005].

The Runx2 transcription factor, with a Runt DNA-binding domain, is essential for osteoblastic differentiation and skeletal morphogenesis and is a scaffolding protein recruiting coactivators or repressors and chromatin modifying enzymes to promoters. Runx2 acts as a repressor in the regulation of rRNA transcription, interacting with rRNA gene promoters in complexes with RNA polymerase I transcription factors UBF1 and SL1. As mitotic occupancy by Runx2 of rRNA genes has been demonstrated, Runx2 may ensure inheritance of lineage-specific regulation of rRNA gene expression to daughter cells [Young et al., 2007a]. Additionally, Runx2 is sequence-specifically associated with its polymerase II-transcribed target genes in mitotic chromosomes, and bookmarks these genes as transcriptionally poised by preserving active marks such as H4 acetylation and H3K4me2, thus transmitting epigenetic memory to progeny cells [Young et al., 2007b].

TEMPORAL MITOTIC REORGANIZATION

The displacement from chromatin of transcription-associated proteins generally occurs in prophase and the restoration to colocalization with chromatin takes place mostly in telophase [Martinez-Balbas et al., 1995; Kruhlak et al., 2001; Prasanth et al., 2003; Zaidi et al., 2003]. However, the existence of a more precise temporal regulation controlling the spatial mitotic reorganization has been revealed in several studies.

The onset of mitosis promotes the formation of the DNA topoisomerase IIα phosphoepitope named MPM-2 site, since it is recognized by the mitotic protein monoclonal 2 antibody. However, this mitosis-specific phosphorylation event is not performed by a mitosis-specific kinase but by CK2, a constitutively active kinase. It has been found that this CK2 specificity is due to a temporal regulation. In interphase, CK2 and protein phosphatase 2A (PP2A) colocalize and compete with each other with the net result that the DNA topoisomerase IIα MPM-2 phosphoepitope site is kept unphosphorylated. During mitosis, PP2A is displaced from the nucleus in prophase, while protein kinase CK2 is released later in prometaphase. This temporal progression explains how CK2, a constitutively active kinase, is responsible for the mitosis-specific phosphorylation of the DNA topoisomerase IIα MPM-2 site [Escargueil and Larsen, 2007]. This study provides evidence of the importance of a temporal order at mitotic entry. It is possible that other MPM-2 phosphoepitopes on other proteins result from the same strategy.

Temporal changes can also play a mechanistic role in the progression of mitosis. Histone methylation has been considered as a stable and transmissible epigenetic mark. However, changes in levels of histone H3 trimethylated at K9 (H3K9me3) have been detected during mitosis. Levels of H3K9me3 rapidly increase during early mitosis until metaphase, and then rapidly decrease to return to interphase levels by early G1 phase [McManus et al., 2006]. This is consistent with the recruitment of the KMT1A (formerly Suv39h1) K-methyltransferase to the pericentromeric heterochromatin, as cells enter mitosis [Aagaard et al., 2000]. The loss of H3K9me3 between anaphase and cytokenesis is attributed to the strong activity of an unidentified K-demethylase. This dynamic methylation of H3 during mitosis is thought to have a role in the congression and segregation of chromosomes [McManus et al., 2006].

Dynamic mitotic changes of other H3 PTMs have also been reported. H3 S10 and S28 mitotic phosphorylation is initiated in late G2 phase, reaches its peak in metaphase, and then decreases until cytokinesis when it returns to its interphase level. However, during the course of mitosis, phosphorylation of S10 increases faster and decreases slower than phosphorylation of S28. Thus, S10 and S28 phosphorylation events are governed by differential temporal dynamics, which could be achieved by spatial reorganization or post-translational modifications of Aurora B or phosphatases [McManus and Hendzel, 2006]. Moreover, a marked decrease in H3K9ac is observed in mitosis whereas H3K14ac levels remain unchanged. Levels of H3K9acK14ac, H3K9acS10ph or H3S10phK14ac also fluctuate throughout mitosis. These observations suggest differential and temporal regulation patterns of the different involved KATs and HDACs [McManus and Hendzel, 2006]. It must be assumed that a subset of KATs and HDACs remain associated to the condensed chromosomes even though the bulk of these modifying enzymes have been shown to be displaced [Kruhlak et al., 2001].

A sequential re-entry of regulatory proteins into daughter nuclei has been described in several studies. Components of the transcriptional machinery re-enter daughter nuclei in an ordered process, after the nuclear envelope and lamina are assembled. First the initiation competent form of RNA polymerase II and the general transcription factors materialize in the nucleus, then the pre-mRNA processing factors (components of nuclear speckles), and last the elongation competent form of RNA polymerase II in M/G1 phase [Prasanth et al., 2003]. In agreement with this study, Runx2 complete colocalization with chromatin is restored, while RNA processing factor SC35 still exhibits a dispersed cytosol distribution [Zaidi et al., 2003]. MAPPs, providing a source of PML bodies for the newly formed daughter nuclei, also persist in the cytoplasm of cells in G1 phase [Dellaire et al., 2006]. In contrast, both Sp1 and Sp3 re-enter daughter nuclei while the lamina is reforming, earlier than the initiation competent form of RNA polymerase II and the general transcription factors [He and Davie, 2006]. It is possible that the Sp1 and Sp3 slightly earlier re-entry in the nucleus is due to their association with nuclear matrix components, assuming that the nuclear matrix re-assembly occurs prior to or simultaneously with the lamina and nuclear envelope re-assembly. Moreover, Sp3 regroups into daughter nuclei before Sp1, thus, has the opportunity to bind to Sp sites before Sp1 and, depending on promoter context, can act as an activator or a repressor [He and Davie, 2006] (Fig. 2). Yet, HDAC2 reorganization has been shown to precede that of Sp3, with HDAC2 returning to colocalization with DNA in early telophase [He and Davie, 2006]. It has been suggested that once the HDACs re-bind to chromatin, these enzymes re-establish chromosome territories and/or compartments [Kruhlak et al., 2001]. Of course, the reformation of the nuclear envelope initiating in anaphase is also a temporally regulated process [Salina et al., 2001; Stewart et al., 2007].

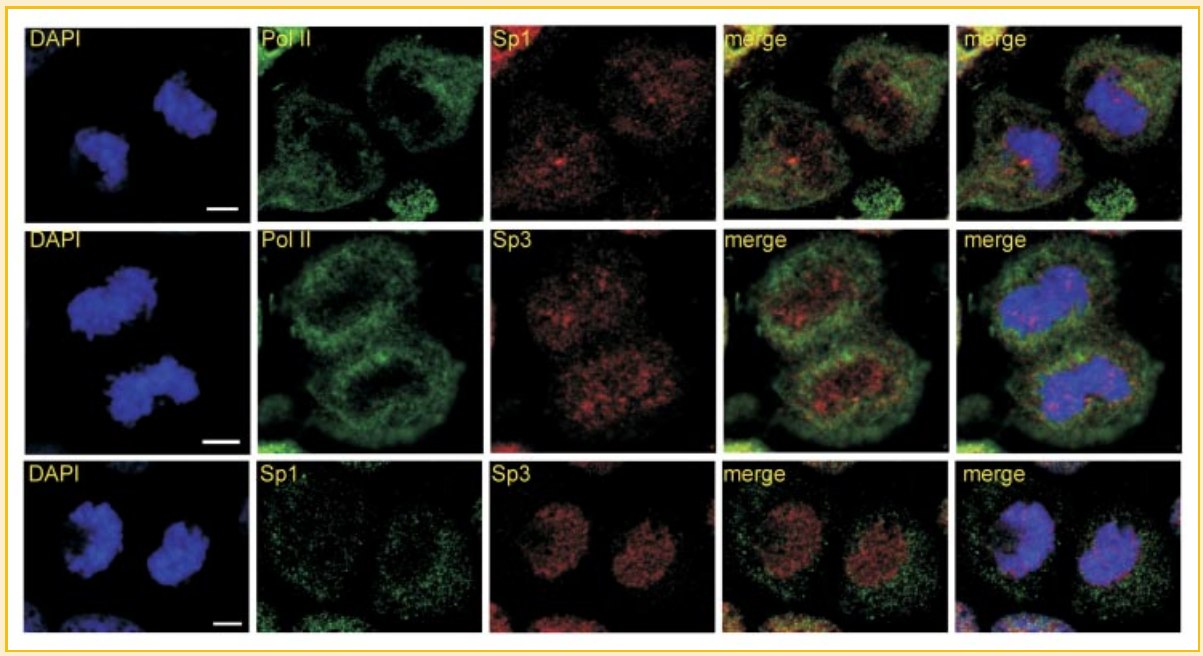

Fig. 2. Sequential re-entry of Sp1, Sp3 and RNAP II into daughter nuclei. MCF-7 cells were grown on coverslips in estrogen complete medium, fixed, and double labeled with anti-Sp1 or anti-Sp3 and anti-RNAP II antibodies or with anti-Sp1 and anti-Sp3 antibodies. DNA was stained by DAPI as described in He and Davie [2006]. Bar, 5 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

Many transcription and chromatin remodeling factors are excluded from chromatin during mitosis. In some instances, the histone mark is maintained but the reader is modified, mostly by phosphorylation. In other instances, the generation of an additional mark causes the displacement of the reader from chromatin. Several discrepancies occur in the literature about the extent of displacement of some regulatory proteins or about the nature of the mark(s) causing the displacement. These inconsistencies deserve a cautionary note regarding the quality and specificity of the antibodies used in these studies. For example, an antibody raised against a specific mark (e.g., anti-H3K9acK14ac) may be prevented to bind to this antigen if another mark is present on the histone tail (e.g., S10ph). Such occurrence has been documented as occlusion [Clayton et al., 2006], although it could also be considered as a specificity issue. In contrast to most transcription factors, ''lineage-specific master regulators'' like Runx2 seem to maintain occupancy of their target gene promoters during mitosis and have multiple key roles in the control of gene expression in progeny cells, thus coordinating cell fate, proliferation and growth control.

There is much more to learn about the temporal order of transcription and chromatin remodeling factors displacement and re-entry into daughter cells. The latter is an important time to re-establish or alter the epigenetic program. In cancer cells, the increased stability of transcription factors like Sp1 may facilitate a rapid re-establishment of epigenetic programs. Sp1 is able to bind to DNA as multimers, and therefore may act as an architectural protein generating long-range DNA interactions and creating functional chromatin domains. In support of this potential role, Sp1 re-associates with post-mitotic chromatin before the gene expression machinery. Further, in cancer cells, levels and spectrum of transcription and chromatin remodeling factors change. Hence mitosis is the perfect window of opportunity to re-shuffle the deck to execute changes in the epigenetic programming guiding gene expression.

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