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Maintaining Transcriptional Specificity Through Mitosis

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Abstract

Virtually all cell types have the same DNA, yet each type exhibits its own cell-specific pattern of gene expression. During the brief period of mitosis, the chromosomes exhibit changes in protein composition and modifications, a marked condensation, and a consequent reduction in transcription. Yet as cells exit mitosis, they reactivate their cell-specific programs with high fidelity. Initially, the field focused on the subset of transcription factors that are selectively retained in, and hence bookmark, chromatin in mitosis. However, recent studies show that many transcription factors can be retained in mitotic chromatin and that, surprisingly, such retention can be due to nonspecific chromatin binding. Here, we review the latest studies focusing on low-level transcription via promoters, rather than enhancers, as contributing to mitotic memory, as well as new insights into chromosome structure dynamics, histone modifications, cell cycle signaling, and nuclear envelope proteins that together ensure the fidelity of gene expression through a round of mitosis.

INTRODUCTION

Two original observations indicated that the chromatin state and transcriptional activity of eukaryotic cells are profoundly altered during each mitotic phase of the cell cycle. The first observation, made by conventional light microscopy in the nineteenth century, was that the chromosomes condense markedly during the metaphase period of mitosis. This condensation facilitates the faithful segregation of sister chromatids to daughter cells during mitotic exit. The second observation, made by assessing bulk RNA polymerase activity, was that the rate of transcription declines dramatically as cells progress through metaphase (114). Transcription then ramps back up when segregated chromosomes decondense, during mitotic exit. Indeed, the genome was considered to be monotonically compacted and transcriptionally silent during mitosis, raising the question of how genes become reactivated in a cell-specific fashion for each physiological state and type of parent cell.

A neutral model held that upon chromosome decondensation, transcriptional regulatory factors in the cell could simply rebind their target sequences, and after mitosis, daughter cells would carry out their business. However, classic in vivo footprinting studies revealed that only a subset of binding sites for regulatory transcription factors remain occupied through mitosis (88, 90). These observations suggested a hierarchical model whereby the mitotic chromatin-binding proteins were bookmarking factors. Such factors would impart a memory of the premitotic transcriptional state and seed the reoccupancy of other transcription factors during chromosome decondensation and mitotic exit.

The advent of far more sensitive measures of protein occupancy, via live cell imaging, as well as more sensitive measures of chromatin structure and transcription, has led to unexpected views of how the genome is faithfully reactivated during mitotic exit. Indeed, it is now known that the transcription of many genes is not completely suppressed, that promoters (rather than enhancers) mark the activity of prior transcriptional states, that nonspecific binding of transcription factors is prevalent on mitotic chromatin, and that mitotic chromatin is not monotonically condensed, but rather has heterogeneous local structures, modifications, and motion dynamics. Together, these features contribute to, or are permissive of, mitotic memory. The field has been reviewed extensively (38, 104, 109), and here we focus on the latest advances and insights.

BASAL PROMOTER-BINDING FACTORS AND LOW-LEVEL TRANSCRIPTION CONFERRING MITOTIC MEMORY

Promoters, rather than enhancers, generally maintain their open state throughout mitosis (31, 36, 51, 132, 133) (**Figure 1**). General, promoter-binding transcription factors such as TBP, TFIIB, and TFIID are strongly retained on mitotic chromatin (15, 18, 36, 100, 132, 141). TBP interacts with WDR5, a chromatin-associated factor that binds methylated histone H3K4 at active promoters in interphase, while in mitosis, TBP and WDR5 recruit the anaphase-promoting complex (APC/C) to promoter regions, leading to histone H2B being modified on K11 and K48 with branched ubiquitin chains (100). The ubiquitinated H2B leads to histone degradation and contributes to the open chromatin state of promoters in mitosis (100). DNA topoisomerase I (TOP1) alters the topological states of DNA during transcription and is important for RNA polymerase II (RNAPII) elongation. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) for RNAPII and TOP1 with an alternative fixation method [formaldehyde and disuccinimidyl glutarate (DSG)] revealed that RNAPII and TOP1 partially dissociate from chromosomes as mitosis initiates and reassociate with chromatin in mitosis to promote low-level transcription (140). Impaired TOP1–RNAPII interactions or acute depletion of TOP1 impairs RNAPII rebinding to promoters and causes a cell cycle delay. TOP1 also interacts with TFIID (89, 124) and modulates higher-order

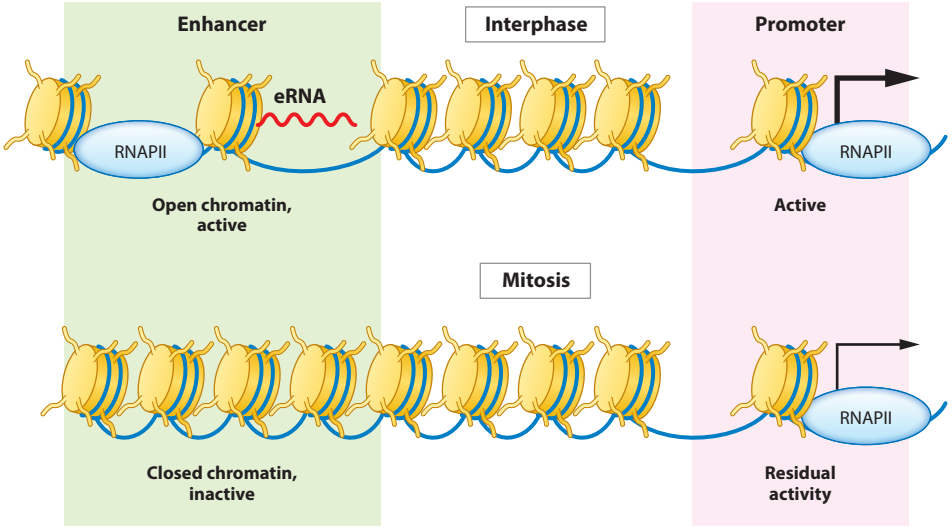

Figure 1

The chromatin structure of enhancers and promoters is denoted by the presence or absence of nucleosomes. Active states exhibit local (enhancers) or elongated (promoters) transcription, denoted by the blue ovals signifying RNAPII. Abbreviations: eRNA, enhancer RNA; RNAPII, RNA polymerase II.

genome folding (96), which could be impactful during mitosis. In summary, promoters remain busy during mitosis, while enhancers generally shut down.

In mitosis, transcription itself is modulated in various general ways. CDK1/cyclin B and its downstream kinases phosphorylate TFIID and TFIIH, which can inhibit transcription (2, 84, 123), and they translocate transcription termination factor 2 (TTF2) into the nucleus at the onset of mitosis (40, 58). The mitotic kinase Plk1 phosphorylates cyclin T1, inhibiting the cyclin T1/Cdk9 complex on phosphorylation of the C-terminal domain of RNAPII (56), and thereby inhibiting elongation. Cdc15, but not the Cdk7 and Cdk9 kinases that promote interphase RNAPII initiation and elongation, phosphorylates Ser5 and Ser2 of the C-terminal domain to promote transcription during mitosis in budding yeast (126). Although Cdc15 is present only in fungi and basal eukaryotes, and not in humans, Thr4 of the C-terminal domain is specifically phosphorylated in mitosis by mitotic kinase Plk1, causing that form of RNAPII to be associated with centrosomes (47). It remains to be determined whether Plk1 or other kinases induce low-level transcription by RNAPII in noncentromeric regions.

In the presence of an inhibitor that blocks promoter release, RNAPII accumulates at promoters in early mitosis. In untreated control conditions, RNAPII does not accumulate at promoters and is hard to detect on protein-coding regions by ChIP methods (75), presumably due to limitations of sensitivity. However, live cell imaging of individual, Halo-tagged RNAPII molecules reveals that they are retained on mitotic chromatin (132), as does immunostaining for RNAPII Ser2-P, an active form of polymerase (102, 105). Altogether, the latest data indicate that basal promoters retain binding factors, RNAPII, and their open state during mitosis, while enhancers are diminished in features such as H3K27ac and open chromatin that normally define an active state, and interphase loops between enhancers and promoters are lost (35, 95, 145).

Methods that measure nascent transcription typically require isolating nuclei and incubating them in nucleotide precursors of RNA synthesis, but mitotic cells undergo nuclear envelope breakdown, challenging such methods. To overcome this problem, a study used a highly

sensitive, cell-permeable substrate for 5-ethynyluridine RNA sequencing (EU-RNA-seq) to detect nascent transcripts (102, 103). This technology enabled the detection of low-level expression of approximately 8,000 genes in mammalian cell mitosis. The genomic data were confirmed by RNA fluorescence in situ hybridization (FISH) of nascent transcripts. A study that successfully used global nuclear run-on followed by RNA sequencing (GRO-seq) also detected significant transcription during early mitosis (82). To summarize, current data indicate that basal promoters retain significant activity during mitosis and, in the absence of enhancer function, likely account for the basal, low-level transcription seen at so many genes (**Figure 1**). Taken together, the recent data indicate that promoters represent a primary memory mechanism for conveying prior transcriptional states through mitosis.

CONSEQUENCES OF BASAL PROMOTER ACTIVITY IN MITOSIS

Recent studies uncovered that nascent pre-mRNA and noncoding RNA have a structural role for promoting open, dynamic chromatin structure (20, 42), condensate formation (45), and transcription (115). Thus, low-level transcription might keep the local chromatin open around promoters to enable robust and faithful gene reactivation during mitotic exit. Not surprisingly, the transcriptional activation of genes precedes the accumulation of corresponding mRNAs in each cell cycle (82). Thus, low-level transcription provides an initial pool of nascent RNA for mRNA and subsequent protein synthesis during mitotic exit. Furthermore, RNAPII is required for cohesin reloading to CTCF-bound sites and loop formation during mitotic exit (147) (**Figure 2*a***), thereby

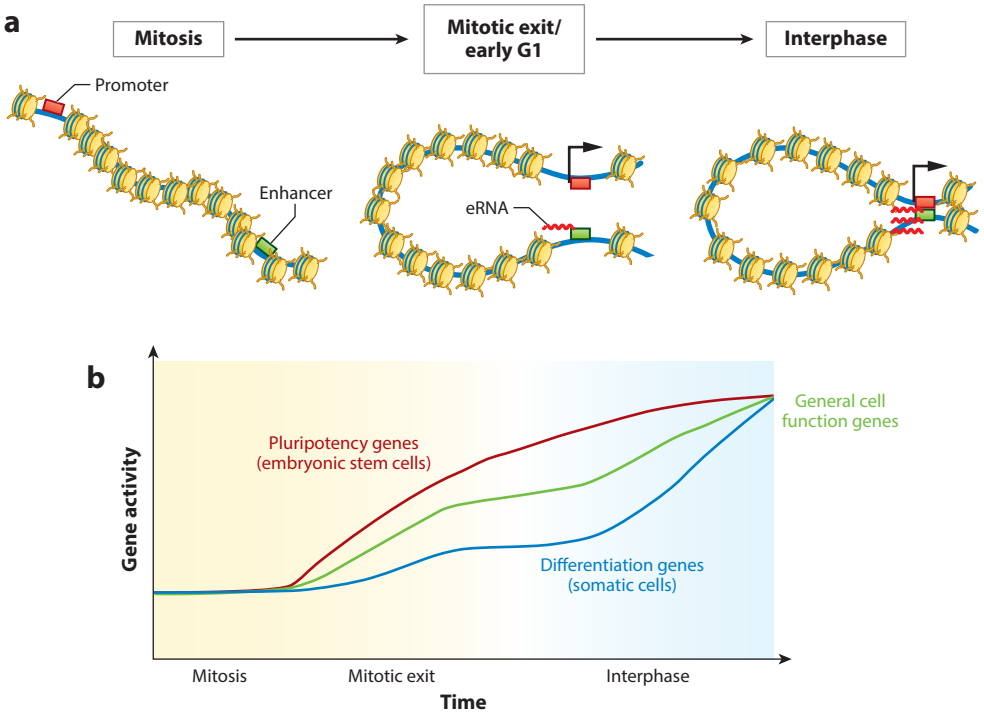

Figure 2

(*a*) Separation of enhancers and promoters in mitosis and their reengagement with one another during mitotic exit, to generate enhancer–promoter loops. (*b*) Cascade of gene reactivation from the low levels seen in mitotic cells. The timing of reactivation of different classes of genes is shown. Abbreviation: eRNA, enhancer RNA.

possibly promoting subsequent gene expression patterns in the context of reestablishing higher-order chromatin reorganization (111, 145), as discussed further below. Indeed, transcription of centromeric DNA is required for proper chromosome segregation (14, 78). We note that the latter structural impact of transcription makes it challenging to use RNAPII inhibitors to study gene reactivation at long time points after mitotic exit.

BOOKMARKING TRANSCRIPTION FACTORS ELICIT A CASCADE OF POSTMITOTIC GENE ACTIVATION

Careful analyses at early and later times of mitotic exit have revealed an initial burst of expression of many genes (50, 102, 110), presumably enabled by promoters being open and poised for transcription (75). After this initial burst, additional groups of genes are reactivated at different times of mitotic exit, with the initial genes relating more to rebuilding the cell and the later genes relating more to specialized functions in somatic cells (60, 102, 110), while pluripotency-associated genes are the first to be reactivated in embryonic stem cells (109) (**Figure 2*b***). Notably, studies have observed a correlation between the reactivation kinetics of enhancer RNAs (eRNAs) and the enhancers' target genes, and the activation of eRNAs also correlates with the reestablishment of enhancer–promoter loops (35, 95, 109, 145). Given that enhancers are activated by transcription factors and that there is a well-controlled cascade of gene reactivation during mitotic exit, enhancer reactivation underscores the importance of transcription factor dynamics in mitotic chromatin. The timing of reengagement of transcription factors in mitotic exit could reflect their functional hierarchy in development (144).

On the one hand, the initial in vivo footprinting data definitively established that only a subset of sequence-specific transcription factors remain bound to promoter sequence in mitosis, and hence the factors were called bookmarking factors (88, 90). This finding was concordant with many immunofluorescence studies on cells fixed with formaldehyde, showing that only a subset of transcription factors remain bound to mitotic chromosomes, although the sensitivity of the experiments or the antibodies could explain the results (30, 71, 80, 104, 128, 143). On the other hand, the apparent exclusion of proteins from mitotic chromosomes can be due to a formaldehyde-based cross-linking artifact (31, 132, 133). Thus, an increasing number of studies have avoided fixation and used live cell imaging with fluorescent or Halo tags that are genetically attached to the protein of interest. Using CRISPR-Cas9, one study engineered Halo tags into embryonic stem cell lines at the endogenous loci for key pluripotency factors, general transcription factors, and signaling response factors, providing natural control of the timing and levels of expression (133). The endogenous gene tagging strategy demonstrated that Sox2, Oct4, Esrrb, and TBP are retained on mitotic chromatin, while signal-responsive transcription factors, including Stat3 and Hsf1, are excluded from mitotic chromatin. To date, conventional, formaldehyde-based cross-linking for ChIP has shown that only a minority of interphase target sites remain occupied in mitotic chromatin by transcription factors that, otherwise, clearly remain bound overtly to mitotic chromosomes when assessed by immunofluorescence (13, 59, 116, 128). Thus, there may be bookmarking of only a subset of specific sites by such factors, or the method may be causing an underestimation of binding. To address this, DSG or glyoxal followed by formaldehyde was used to elicit higher ChIP signals and improve the ChIP signal-to-background ratio (31). In the future, it will be good to also explore the CUT&RUN (cleavage under targets and release using nuclease) method in mitosis, which does not employ fixation to map interactions between transcription factors and chromatin sites (127).

Extensive binding of transcription factors to mitotic chromosomes in living cells (as assessed by GFP or Halo tag fusions) without extensive target site occupancy (as assessed by ChIP) indicates that a substantial fraction of the transcription factors are bound nonspecifically to the chromatin

(13, 117). This point was firmly established by mutants of FoxA1 that abolished sequence-specific binding but allowed nonspecific DNA and nucleosome interactions and were quantitatively bound to mitotic chromosomes (13). Nonspecific retention of transcription factors increases their local concentration, facilitating access to target sequences during mitotic exit.

Small interfering RNA and auxin-inducible degron technologies have revealed the genetic importance of mitotic bookmarking factors for target gene reactivation during mitotic exit (8, 13, 59, 132). In one study, the use of a nuclear export signal–based, mitosis-specific, dominant-negative Brn2 showed that this neuronal bookmarking factor is important in reactivating its target gene, *Nestin* (128). However, as noted by the authors, and as is the case with other genetic approaches, it remains difficult to distinguish whether phenotypes are due to the impairment of bookmarking in mitosis or transcription factor rebinding to specific sites during early G1. A mathematical model–based study indicated that faster reactivation dynamics during mitotic exit correlate with the total number of factor binding sites within target gene promoters (120) and that bookmarking factors work by keeping an open chromatin state during mitosis rather than searching for new target sequences efficiently during mitotic exit. A different perspective emerged from a study of Prospero/Prox1, which is retained at H3K9me3-pericentromeric heterochromatin via liquid–liquid phase separation in *Drosophila* mitotic neural precursors (80). Liquid–liquid phase separation of Prospero depends on its intrinsically disordered regions, which, in mitotic exit, recruit heterochromatin protein 1a (HP1a) into phase-separated condensates, drive H3K9me3-heterochromatin expansion, and silence target genes. Clearly, mitotic control extends from activation to repression.

THE ROLE OF HISTONE MODIFICATIONS, PARTICULARLY REPRESSIVE ONES, IN MITOTIC INHERITANCE

There has been much work and speculation on the potential roles of active and repressive histone modifications in imparting a memory of a prior gene expression state through mitosis. Indeed, any modification that enables passage of an expression state through cell division could be considered to be truly epigenetic. From basic principles, it has been noted that the enzymes that elicit the major H3K9me3 and H3K27me3 repressive marks—namely, Suv39H1, Suv39H2, and SetDB1 for H3K9me3 and the PRC2 complex for H3K27me3—each contain domains that recognize the respective marks and hence have the feed-forward capacity to be epigenetic (118). Concordantly, various studies indicate that histone methylation can be globally retained on mitotic chromatin, whereas it remains controversial whether histone acetylation is so retained. Quantitative mass spectrometry revealed that global levels of H3K4me1, H3K4me3, H3K9me3, H3K27me3, and H3K36me3 are mostly similar between mitosis and interphase, but the global levels of H3K9ac, H3K14ac, H3K18ac, and H3K27ac are decreased (55), albeit by a small amount (**Figure 3*a***). Loss of H3K27ac in mitosis is observed around most enhancers but not around super-enhancers (83), while H3K27ac is retained around promoters of housekeeping genes and enhancers of cell-specific genes that are important for cellular identity maintenance (83). Inhibition of mitotic H3K27ac perturbs transcriptional reactivation kinetics (60, 110). Given the potential broad effects of enzyme inhibitors, the field has the most to learn from studies that take incisive, temporally regulated genetic approaches.

Other mass spectrometry studies have found that histone modifiers associated with activation, including HAT1, PRMT1, and MLL4, are depleted from mitotic chromatin, whereas modifiers associated with repression, such as G9A, Suv39h1, and DNMT1, are retained in mitotic chromatin (36) (**Figure 3*b***). The repressive modifiers Suv39h1/h2 and PRC1/2 components and the DNA methylation proteins Dnmt1/3a/3b and MeCP2 are also found in mitotic chromatin (26), as are the heterochromatin factors DBC1, ZNF326, and QSER1 and mitotic RNAPII-binding

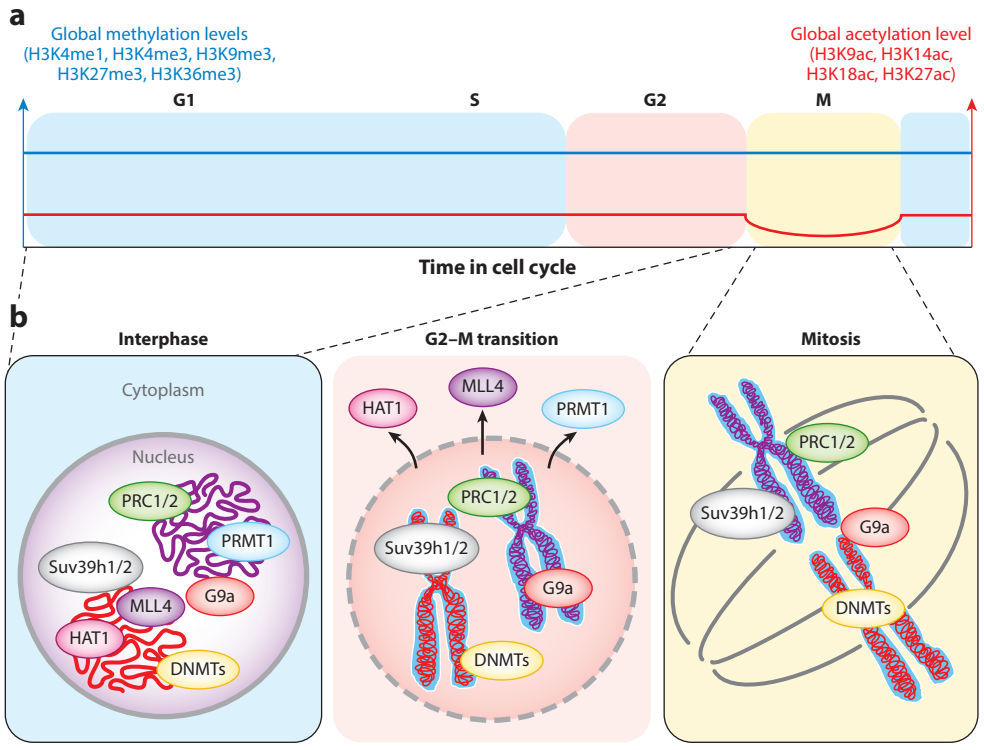

Figure 3

(*a*) Selective diminution of histone acetylation in mitosis (*red line*) versus maintenance of histone methylation (*blue line*). Global levels of histone methylations are mostly similar between mitosis and interphase, but the global levels of histone acetylations are decreased. (*b*) Chromosomal loss of a subset of chromatin-modifying enzymes in mitosis and retention of other chromatin-modifying enzymes in mitosis. The repressive histone modifiers tend to be retained in mitotic chromatin, whereas active histone modifiers tend to be evicted from mitotic chromatin.

proteins and general promoter factors (6, 25, 36, 74, 91). H3K9me2 is an evolutionarily conserved, nuclear peripheral heterochromatin mark and is retained through mitosis. A high-resolution, three-dimensional immuno-oligo-FISH-based study uncovered H3K9me2 in mitosis associating with the reassembling nuclear lamina in daughter cells during mitotic exit, which acted as a three-dimensional architectural guidepost (113). Yet while an earlier study demonstrated that lamina-associated domains are stochastically reshuffled through mitosis, it also found that the H3K9me2 methyltransferase G9a promotes lamina-associated domain–nuclear lamina contacts after mitosis (63). A histone demethylase for H3K9me3, KDM4C, is retained on mitotic chromatin (66). Recruitment of Jarid2-PRC2 (PRC2.2) during S and G2–M phases leads to more robust gene repression of target bivalent genes in pluripotent stem cells (5). In summary, there is substantial evidence that repressive histone modifications contribute to maintaining an initial, interphase off state of genes as they progress through mitosis.

CHANGES IN GLOBAL CHROMOSOME ARCHITECTURE IN MITOSIS: DRIVEN BY TRANSCRIPTION?

Features of higher-order chromatin organization, such as A/B compartments (the largest higher-order feature), topologically associating domains (TADs), and a subset of enhancer–promoter

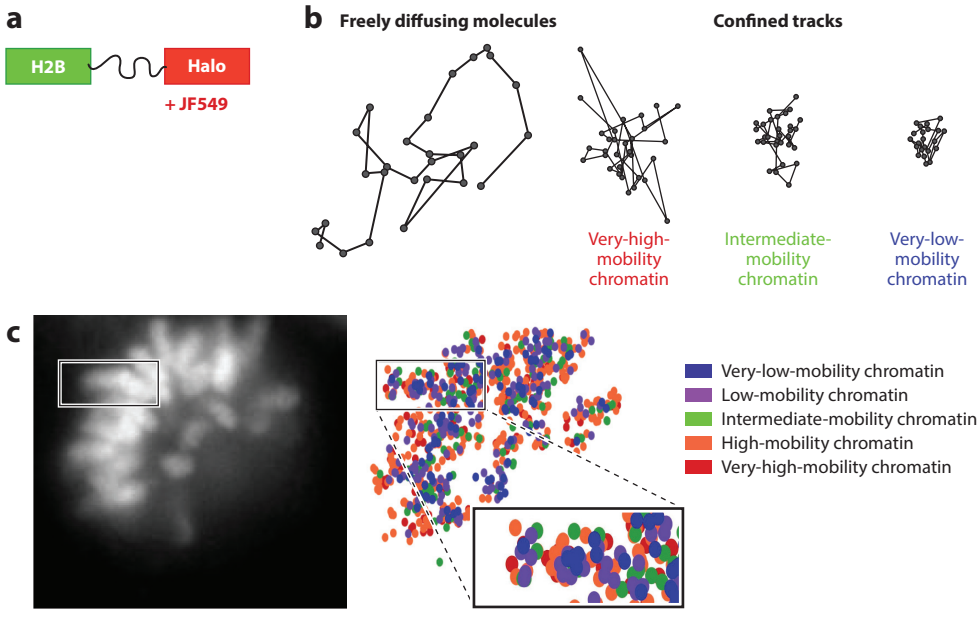

Figure 4

Chromatin states are heterogeneous across chromosomal arms in mitosis, reflecting inherent dynamics. (*a*) A histone H2B–Halo tag fusion construct, which binds and activates the JF549 dye to become highly fluorescent and is useful for single-molecule imaging in living cells. (*b*) Single-molecule tracking over time, which can reveal freely diffusing molecules and molecules with increasingly confined motion tracks (for details, see Reference 72). (*c*) Diverse chromatin mobility states along chromosome arms during mitosis of a living cell. The colored dots in the right subpanel indicate different chromatin mobility states across the chromosome arms visualized in the left subpanel. Diverse chromatin mobility states can be observed in mitotic chromatin of H235 cells. Data were taken from live cells at the time of imaging and reflect dynamic states even within compacted chromosome arms. Panel *c* adapted with permission from Reference 72.

chromatin loops, are rapidly disrupted in a condensin-dependent manner in prometaphase (35, 95, 145) (**Figure 2*a***). Restoration of interphase chromatin organization starts in anaphase and telophase, with the formation of A/B compartments and *cis*-regulatory loops between enhancers and promoters, and continues into G1 (24, 93, 95, 109). As the cell enters G1, the compartments continue to strengthen. However, the kinetics of chromatin loop re-formation during mitotic exit are more heterogeneous, and, indeed, various enhancer–promoter loops persist through mitosis (145). It is not until early G1 that TADs are re-formed, with sub-TADs forming first before merging into larger structures (110, 145). Unexpectedly, single-molecule tracking of Halo-tagged core histone H2B revealed diverse states of chromatin compaction and chromatin mobility along mitotic chromosome arms in living cells (72) (**Figure 4**). The rare, transient, and stochastic nature of long-range chromatin interactions, as recently assessed quantitatively by in situ hybridization and nuclear imaging, in comparison with the interactions captured by genomic methods, has led to questions about such interactions' causal role in gene activity (32). Given the reestablishment of long-range interactions in mitotic exit, specific genetic perturbations of the interactions in mitosis, along with careful assessments of the impact on gene reactivation, could provide an important test of functionality.

With regard to genomic correlations, compartments that harbor early reactivated enhancers and genes reach their final compartmentalization faster than late reactivated enhancers and genes

(110). TAD boundaries that overlap with at least one early gene or enhancer show faster and stronger insulation compared with boundaries with later reactivated genes or enhancers or with no transcriptional activity. Enhancer–promoter loops that are preferentially enriched in H3K27ac and TBP are reactivated earlier (110), and use of a p300 inhibitor showed that H3K27ac depletion in mitosis was associated with misregulation of transcriptional reactivation during mitotic exit (60, 110). However, higher-order chromatin reorganization was largely unaffected (110). RNAPII promotes the recruitment and activity of histone acetyltransferases (87), and acute degradation of RNAPII results in decreasing H3K27ac levels in asynchronous cells and cells reentering G1 (147). Thus, transcription, rather than H3K27ac per se, may be driving higher-order chromatin reorganization during mitotic exit. Yet inhibition of transcription by acute degradation of RNAPII or triptolide treatment during mitotic exit did not cause obvious changes in A/B compartments, TAD structure, or insulation (57, 146). On the other hand, a more recent study concluded that RNAPII is required for both compartment and loop establishment following mitosis by promoting cohesin reloading to chromatin (147). Also, one of the originally described bookmarking transcription factors, BRD4 (22), is an agonist for NIPBL to promote cohesin recruitment during mitotic exit (76). Disparities among the studies might be due to Hi-C technology detecting structural loops better than regulatory loops (9, 23, 49, 109) or the difference between inhibition and degradation times of RNAPII, as the authors of the recent study noted (147).

ROLES OF COHESIN-MEDIATED LOOP DYNAMICS IN MITOSIS

During mitosis entry, cohesin removal in prophase diminishes elongating polymerase II and nascent RNA from chromatin (111). In telophase, condensin-mediated loops are lost, and a transient folding intermediate, devoid of most loops, forms before cohesin-mediated CTCF–CTCF loops and TADs re-form (1). The accumulation of cohesin, but not CTCF, is rate limiting for structural loop re-formation during mitotic exit (145). Yet one study found that acute degradation of CTCF during the M to G1 phase influences chromatin structure reorganization at several levels (146). This study also demonstrated that a small fraction of genes are differentially expressed by CTCF depletion. For genes that are downregulated by CTCF depletion, CTCF functions mainly as a transcriptional activator, but for genes that are upregulated by CTCF depletion, it works as an insulator by shielding the genes from inappropriate enhancers (146). Given that CTCF depletion affects only part of chromatin reorganization and a limited number of genes' reactivation, a CTCF-independent feature of higher-order chromatin reorganization may exist. Indeed, CTCF-independent cohesin binding sites are cell type specific, whereas CTCF-dependent cohesin binding sites are the same across different cell types (121). Importantly, cohesin knockdown, but not CTCF knockdown, impairs cell cycle reentry (121). These results suggest that cohesin is more important for cellular identity inheritance through mitosis than CTCF. Specifically, for cell type–specific chromosomal interactions, cohesin might work with cell type–specific transcription factors, including mitotic bookmarking factors in mitosis, while for non-cell-specific chromosomal interactions, cohesin might work with CTCF to create structural boundaries.

MITOTIC KINASE MODULATION OF HETEROCHROMATIN PROTEINS, LINKER HISTONES, AND NUCLEOSOME REMODELERS IN MITOSIS

Mitotic kinases phosphorylate chromatin-related proteins and thereby modulate structure. The Aurora B kinase is activated in mitosis by a series of phosphorylation reactions that originate from Cdk1/cyclin B (61, 148). In turn, Aurora B phosphorylates Ser10 on histone H3, causing the dissociation of HP1α, HP1β, and HP1γ from mitotic chromatin, even though the

trimethylation level of H3K9me3 remains unchanged (33, 48). HP1s have multiple mitotic phosphorylation sites. The major phosphorylation site on HP1α is located in the hinge region and is reversibly regulated by Aurora B and phosphatases PP2A and PP2Cβ (98). Mitotically phosphorylated HP1α can still bind to H3K9me3 mononucleosomes, indicating that H3S10 phosphorylation might also be required to promote HP1α's dissociation from mitotic chromatin. Although recent studies showed that phosphorylation of the N-terminal extension of human HP1α increases its nucleosome-binding specificity and accelerates heterochromatic phase separation (68, 99), multiple mitotic phosphorylations on HP1α might have a synergistic effect on the reduction of its nucleosome association and thereby modulate its activity to bridge H3K9me3 nucleosomes (86), thus causing a chromatin structure change during mitosis.

Linker histone H1.4 is an H1 variant that is expressed in somatic cells, and the amino acid sequence around H1.4K26 is similar to that of H3K9. Thus, not surprisingly, like H3S10, H1.4S27 is phosphorylated by the Aurora B kinase in mitotic human cells (46). Given that H1.4K26 is methylated by G9a and EZH2 and that HP1 binds to H1.4K26me2/3 (21, 67, 135), H1.4S27 phosphorylation by Aurora B is an additional mechanism to dissociate HP1 from mitotic chromatin (21). H1.4S35 is phosphorylated in mitosis by another kinase, PKA (19). Interestingly, H1.4S35 phosphorylation dissociates H1.4 from mitotic chromatin, indicating that this phosphorylation is necessary for maintaining proper mitotic chromatin structure and progression (19). Although the genomic domains that H1.4 dissociates from are not known, H1.4S35 phosphorylation dissociation from promoters could keep the regions open in mitosis (27, 70).

Given that H1.4 is involved in heterochromatin establishment in interphase cells by interacting with SirT1, a class III histone deacetylase (HDAC) (sirtuin) (136), it is interesting to ask how mitotic H1.4 phosphorylation may affect interactions with HDACs or other chromatin-modifying proteins. Relatedly, HDAC3 is phosphorylated by mitotic casein kinase 2 (CK2), a downstream kinase of CDK1, and the phosphorylation promotes interactions between H1.3 and SMART and NCoR (108). Interestingly, the mitotic HDAC3–H1.3 complex has a deacetylation activity on H3K9ac but not on other histone acetylations, such as H4K5ac. We speculate that the mitotic HDAC3–H1.3 complex contributes to the closing of chromatin at enhancers during mitosis.

The ATP-dependent chromatin-remodeling complexes BRG1 and hBrm are phosphorylated during mitosis (92, 125). Mitotic phosphorylation of BRG1 leads to its inactivation of ATP-dependent remodeling activity and dissociation from mitotic chromatin, whereas mitotic phosphorylation of hBrm leads to its degradation (125). Interestingly, the activities are restored during mitotic exit by dephosphorylation, allowing them to reassociate with chromatin and exhibit remodeling activity (125). CK2 phosphorylates BRG1 in mitotic cells of different cell types and organisms, suggesting functional conservation across tissues and species (101). Since chromatin-remodeling complexes can preferentially bind to active enhancers during development and cancer progression (3, 4, 10, 94, 106), the phosphorylation of BRG1 and its removal from mitotic chromatin might contribute to the closing of enhancer chromatin until mitotic exit.

CONSEQUENCES OF NUCLEAR ENVELOPE BREAKDOWN AND REASSEMBLY DURING MITOSIS

The dynamics of nuclear envelope breakdown and the consequent reorganization of nuclear envelope proteins, nuclear pore complexes, the nuclear lamina, peripherally located genes, and the inner membrane itself are major factors for considering how genes may be regulated during mitosis. Mitotic kinases and phosphatases govern the disassembly and reassembly of many of the nuclear envelope's constituent proteins (41). The nuclear envelope is perforated by holes that

are occupied by nuclear pore complexes, which provide selective transport of macromolecules between the nucleus and cytoplasm. Phosphorylation of nucleoporins (NUPs) in nuclear pore complexes by mitotic kinases leads to pore complex disassembly (37, 77). A- and B-type nuclear lamins are intermediate filament proteins that constitute the nuclear lamina on the inner nuclear envelope. Mitotic kinases phosphorylate the lamins, causing depolymerization that facilitates nuclear envelope breakdown (43, 112, 139). During nuclear envelope breakdown, many NUPs are phosphorylated by CDK1, including the Nup107–160 and Nup53–93 subcomplexes, Nup98, NDC1, GP210, and others (7, 29, 37, 85), leading them to disperse, where they may remain as stable subcomplexes. In addition to nuclear pore complexes regulating nuclear transport, it has become appreciated that NUP constituents function in interphase by binding to regulatory sequences and directly affecting gene expression (107, 130). Considering that the genome sequentially contacts the nuclear periphery components during mitotic exit (63, 150), further work is needed to assess the role of nuclear pore complexes and NUPs in helping to maintain (or reestablish) cellular identity through mitosis.

In interphase cells, nuclear lamins interact with large heterochromatic regions called lamina-associated domains, which contain mostly transcriptionally inactive genes at the nuclear periphery (12, 79). In mitotic cells, the CDK–cyclin B complex predominantly phosphorylates Ser22 and Ser392 in lamin A/C, Ser23 and Ser393 in lamin B1, and Thr34, Ser37, and/or Ser405 in lamin B2, and these phosphorylations contribute to depolymerization of nuclear lamins and nuclear envelope breakdown (43, 79, 112). Conversely, dephosphorylation of these mitotic sites is considered to be required for nuclear lamin repolymerization and nuclear lamina reassembly during mitotic exit (97, 112, 134). Phosphorylated lamins are also observed in interphase cells (34). Unexpectedly, Ser22-phosphorylated lamin A/C is observed in the nuclear interior of interphase human fibroblast cells (54). Ser22-phosphorylated lamin A/C interacts with numerous genomic sites with features of active enhancers, and not lamina-associated domains, at locations co-bound by the transcriptional activator c-Jun and correlating with active transcription of apparent target genes (54). We speculate that the Ser22 phosphorylation of lamin A/C in interphase cells might remain from mitotic phosphorylation and function as transcriptional memory to recruit transcription factors, such as c-Jun, into active enhancer regions during mitotic exit. There are 18 phosphorylation sites in lamin A/C, including Ser22 and Ser392, that can be phosphorylated in interphase and mitotic cells (64, 79), making functional genetic tests challenging.

In interphase cells, cyclic GMP–AMP synthase (cGAS) detects DNA in the cytosol as a signal of invasion by microbial pathogens (131). The nuclear envelope functions as a nuclear–cytoplasmic barrier to prevent cGAS pathway activation by self-DNA in interphase cells. After nuclear envelope breakdown in mitotic cells, cGAS is tightly associated with mitotic chromatin (142, 149), which prevents oligomerization and thus cGAS pathway inactivation (73, 149). Thus, mitotic chromatin protects cellular identity from an otherwise destructive cGAS pathway, reflecting the diverse functions that must be accounted for during mitotic progression.

NEW TECHNOLOGIES TO OVERCOME EXPERIMENTAL LIMITATIONS IN THE FIELD

The field has come a long way from the neutral model that transcription factors just "sort it out" to reactivate genes during mitotic exit. On the other hand, many of the molecular perturbations of function that have been employed, such as transient gene knockdowns and addition of inhibitors, lack the temporal resolution to definitively assign a phenotype to the brief period when cycling cells undergo mitosis. Degron technology is a huge advance in this context (59), but the picky

among us can challenge whether the method to inactivate proteins is quick and selective enough to distinguish a true bookmarking function during metaphase from a deficiency in binding and/or reactivating the genome during mitotic exit. Perhaps engineering cells to express temperature-sensitive alleles (129) or using optical methods (16) would allow sufficient temporal resolution to dynamically control mitotic bookmarking proteins and other regulatory components during metaphase or at different times during mitotic exit.

Understanding exactly which proteins are interacting with each other in mitotic chromatin remains challenging and could be helped by proximity-based proteomics methods, such as BioID and TurboID (11, 119). These methods have been adapted to map protein–protein interactions at specific time points (17, 122), which would be applicable for careful time-course analyses during mitosis and exit. ChromID identifies proteins that are bound in the vicinity of particular chromatin marks (137), which again would be useful in time-course studies. A biotinylated, nuclease-deficient Cas9 protein and sequence-specific guide RNAs can be used to identify proteins that interact with a specific locus (81), which could be employed with a temporally regulated system. To summarize, new methods to map interactions among proteins, between proteins and histone modifications, and between proteins and target loci could provide far more resolution to our understanding of molecular dynamics during mitosis.

The function of nonspecific binding of bookmarking transcription factors to metaphase chromosomes is still not clear (13, 116), other than to say that it allows the factors first access to the genome during mitotic exit. Recent studies have employed single-molecule tracking with Halo-tagged proteins to study the dynamics of histones, transcription factors, and chromatin regulators in interphase cells (53, 62, 72). The single-cell technology could help resolve the respective activities of mitotic chromatin-binding proteins at different stages of mitosis and across different segments of mitotic chromosomes.

Most of the molecular studies of mitotic memory have been performed on cells in culture, which imparts a homogeneity that is not seen for cells in tissues, bathed by signals and structures in their environment. Genes and particularly their enhancers are often modulated by environmental signals (39, 44, 69). Given that, as described, signaling transcription factors seem not to be engaged in mitotic chromatin (133), during mitotic exit the factors could depend upon particular extracellular signals to engage the genome and faithfully reset transcriptional programs. Recently developed organoid culture technologies (52, 65, 138) may provide a step toward reproducing the complexity of in vivo tissue conditions, which can be coupled with advances in two-photon imaging of molecules within living tissue (28). Taken together, new technologies are poised to advance our understanding of how gene programs are accurately reestablished after the dramatic changes that allow the chromosomes to sort appropriately during cell division.

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