Biochemical Society Transactions (2024)
https://doi.org/10.1042/BST20231022

PORTLAND PRESS

Review Article

A dynamic role for transcription factors in restoring transcription through mitosis

Marek A. Budzyński*, Alexander K.L. Wong*, Armin Faghihi* and Sheila S. Teves

Department of Biochemistry and Molecular Biology, Life Sciences Institute, University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada

Correspondence: Sheila S. Teves (sheila.teves@ubc.ca)

Mitosis involves intricate steps, such as DNA condensation, nuclear membrane disassembly, and phosphorylation cascades that temporarily halt gene transcription. Despite this disruption, daughter cells remarkably retain the parent cell's gene expression pattern, allowing for efficient transcriptional memory after division. Early studies in mammalian cells suggested that transcription factors (TFs) mark genes for swift reactivation, a phenomenon termed 'mitotic bookmarking', but conflicting data emerged regarding TF presence on mitotic chromosomes. Recent advancements in live-cell imaging and fixation-free genomics challenge the conventional belief in universal formaldehyde fixation, revealing dynamic TF interactions during mitosis. Here, we review recent studies that provide examples of at least four modes of TF–DNA interaction during mitosis and the molecular mechanisms that govern these interactions. Additionally, we explore the impact of these interactions on transcription initiation post-mitosis. Taken together, these recent studies call for a paradigm shift toward a dynamic model of TF behavior during mitosis, underscoring the need for incorporating dynamics in mechanistic models for re-establishing transcription post-mitosis.Canadian Institutes for Health Research ProjectPJT-162289National Sciences and Engineering Research Council DiscoveryRGPIN-2020-06106Stem Cell Network Early Career Researcher Jump Start Awards ProgramECR-C4R1-11Canada Research Chairs CRC Tier II AwardCRC-RS 2021-00294Michael Smith Health Research BC Research Trainee Award

Introduction

Mitosis is a fundamental and highly regulated process in the life cycle of cells, ensuring the faithful transmission of genetic information to subsequent cell generations. This process is highly disruptive to the transcription of active genes as it inactivates the transcriptional machinery through phosphorylation cascades, condenses the DNA into mitotic chromosomes, and disassembles the nuclear membrane for mitotic spindle assembly [1–3]. Yet, daughter cells maintain the identity of the parent cell by reactivating the original pattern of gene expression after cell division. How is transcriptional memory propagated across mitosis?

The discovery of specific *cis*-regulatory regions that remain accessible in condensed chromosomes has led to the term coined 'mitotic bookmarking' (Figure 1A) [4]. It was hypothesized that these accessible regions result from transcription factors (TFs) binding to condensed DNA to 'mark' genes for swift reactivation in early G1 [4]. However, previous studies have observed that most TFs dissociate from mitotic chromosomes [5–7], leading to an apparent contradiction in the field of mitotic bookmarking: how do chromosome regions maintain accessibility without TF binding? A few exceptions to the TF-exclusion rule were found [8–11], suggesting that a select few TFs can act as mitotic bookmarkers (Figure 1B). However, several studies uncovered that formaldehyde fixation of mitotic cells leads to the exclusion of TFs from mitotic chromosomes [12,13]. Subsequent studies further showed that when examined under live-cell imaging, most TFs show varying degrees of enrichment on mitotic chromosomes (Figure 1C) [11,12,14–17]. Furthermore, through methodologies that

*Co-first Authorship

Received: 11 December 2023
Revised: 1 March 2024
Accepted: 6 March 2024

Version of Record published:
25 March 2024

Biochemical Society Transactions (2024)
https://doi.org/10.1042/BST20231022

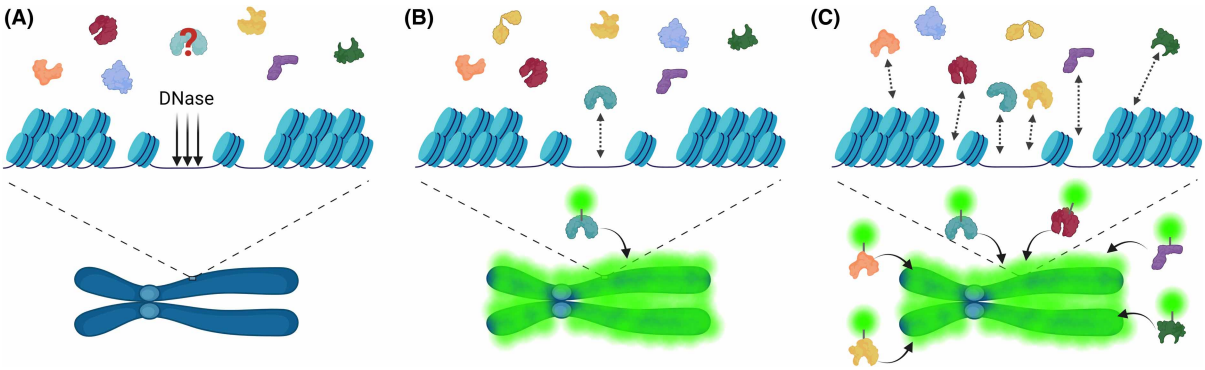

Figure 1. Stages in the discovery of mitotic bookmarking.

(**A**) DNase I footprinting showing hypersensitive (accessible) sites on mitotic chromosomes provided the first indication of potential mitotic bookmarkers, fueling the hunt for transcription factors (TFs) that bind to accessible regions on mitotic chromosomes. (**B**) Certain cell-type specifying TFs were identified as exceptions to the rule of TF exclusion during mitosis, implying that only specific TFs have the capability to function as mitotic bookmarkers. (**C**) Further investigations using live-cell imaging revealed that the majority of transcription factors exhibit diverse levels of enrichment on mitotic chromosomes.

examine TF dynamics, these TF-mitotic interactions are found to be more dynamic than previously observed [8,10,12,18], suggesting that TF dynamics may play an important role in reactivating transcription programs following mitosis.

In this mini-review, we build on recent studies and literature reviews that discuss the current state of the field of mitotic bookmarking by highlighting a shift toward a more dynamic system of TFs and their activity during mitosis. This shift is enabled by dynamics-centered imaging technologies and fixation-free genomics. Furthermore, we discuss at least four modes of interaction between TFs and mitotic DNA, ranging from exclusion to site-specific binding, and their connection to TF dynamics. Additionally, we explore the molecular determinants that govern TF dynamics on mitotic chromosomes. Lastly, we review recent studies on restarting transcription following mitosis, and speculate how a dynamic model for TF interactions might facilitate this process.

Mitotic bookmarking: a historical perspective

Previous efforts at identifying TFs as potential mitotic bookmarkers primarily relied on visualizing TF localization on mitotic chromosomes through antibody-based immunofluorescence (IF) [5–7], requires formaldehyde fixation to 'freeze' all processes [19]. Many studies using IF showed that dozens of TFs are localized outside of mitotic chromosomes [5,20,21], though antibody labeling could bias toward more 'accessible' epitopes on the periphery of the chromosomes. A review from 2013 stated that out of thousands of DNA-binding proteins in the mammalian genome, only 34 can bind to mitotic DNA, many of which are chromatin-interacting proteins [22]. These studies led to the belief that most TFs are excluded from mitotic chromosomes, fueling the question of how DNA-accessible regions are maintained in mitosis and how transcription is faithfully re-established.

Chromatin immunoprecipitation (ChIP) analysis has also been used to map protein binding across DNA, providing locus-specificity that IF-based approaches lack. ChIP also commonly uses formaldehyde fixation to freeze TF–DNA interactions [23]. Using this approach, studies have shown that most DNA-bound proteins lose site-specific binding during mitosis, confirming IF-based studies. For example, RNA Polymerase II (Pol II) has been shown to be localized away from mitotic chromosomes [24] and ChIP-PCR showed greatly decreased signal for Pol II binding in mitosis compared with asynchronous cells at various gene loci [25]. These results suggested that visual localization as measured by IF corresponded with DNA binding as measured by ChIP, and that only a few select TFs could act as mitotic bookmarkers.

Interest in the mitotic bookmarking theory increased with the use of live-cell imaging of TFs fused with fluorescent proteins (FPs) or fluorescently labeled tags. This approach led to the identification of several TFs as highly enriched on mitotic chromosomes, including FOXA1, GATA1, Oct4, and Sox2 [12,14,15], and other factors such as androgen receptor (AR) and Pregnane and Xenobiotic Receptor (PXR) [11,16,17]. In many cases, however, enrichment on mitotic chromosomes as measured by live-cell imaging did not correspond well

with site-specific binding as measured through ChIP-based assays. For instance, live-cell imaging of FoxA1, Sox2, and Oct4 indicated significant enrichment on mitotic chromosomes but ChIP-based assays revealed unexpectedly low binding signals [10,26]. These studies led to the first branching in TF behavior during mitosis — that TFs can 'coat' mitotic chromosomes, and/or bind site-specifically, and that these behaviors are not always congruent.

More recently, several studies challenged the underlying assumption that formaldehyde fixation provides an immediate and accurate snapshot, at least for the mitotic cell. In two independent studies, HNF1b-GFP and Halo-Sox2 both colocalize with mitotic DNA, but become excluded after fixation with formaldehyde [12,27]. A proposed model for this effect is that cross-linking starts as formaldehyde molecules enter the cell membrane and follows an inherent rate that moves toward the center of the cell, forming a gradient of cross-linking. TFs diffusing throughout the cytoplasm would be cross-linked first before TFs that are localized on mitotic chromosomes [12]. This model implies that (1) for TFs with k_{off} rates faster than the kinetics of the formaldehyde reaction, there is sufficient time to unbind DNA and be displaced during the fixation process, but (2) for TFs with k_{off} rates slower than the fixation kinetics, the DNA bound TFs would be fixed before they unbind. In support of this model, TBP and CTCF have been shown to have k_{off} rates of 1 min or longer and are able to be fixed on mitotic chromosomes [12,28,29]. In contrast, Sox2 with a k_{off} rate of ∼12 s exhibits formaldehyde-based exclusion [12]. Previous studies showing that paralogous proteins cross-linking differently during mitosis, for example, MLL1 cross-linked on mitotic chromosomes but not MLL2 [30], could be explained with this kinetic model if one paralog has much more dynamic DNA interaction than the other.

For the field of mitotic bookmarking, challenging the universality of formaldehyde fixation is groundbreaking. It brought to question all the previous data based on IF and ChIP assays. However, it also provided a potential rationale for the discrepancy between live-cell imaging and ChIP-based results by hypothesizing that TFs that are both enriched by live-cell imaging and binding site-specifically would exhibit more stable DNA-binding dynamics, whereas those that show enrichment but not site-specific binding would have very fast DNA-binding dynamics. It also led to a new appreciation for the role of dynamics in this process and for the technologies that have enabled these discoveries.

Uncovering TF dynamics in mitosis

Imaging-based approaches such as fluorescence recovery after photobleaching (FRAP) are well suited in measuring dynamic properties of TFs, which include diffusion rates of free molecules, the fraction of molecules bound to DNA versus unbound, and DNA-binding kinetic properties such as k_{on} and k_{off} rates [31–34]. In FRAP assays, an FP-tagged TF is bleached by a high-intensity laser beam to photobleach a defined region. The recovery of fluorescence in the region is then recorded over time. The amount and rate of fluorescence recovery are proportional to the fraction of immobile molecules (DNA-bound TFs) in that region and the dynamics of diffusing molecules (free TFs) throughout the cell, respectively. FRAP analysis of TFs such as Esrrb-GFP, GFP-FoxA1, and Halo-Sox2 in interphase cells suggested that each TF has inherent binding and diffusing dynamics, and, importantly, most TFs revealed higher dynamics during mitosis compared with interphase cells. For instance, FRAP recovery rates in mitosis vs interphase for GFP-FoxA1, Halo-Sox2, and Esrrb-GFP were two-fold, five-fold, and four-fold faster, respectively [8,10,12]. These results illustrate that TF behavior during mitosis is more nuanced than the 'bound/enriched' versus 'excluded' categories, and that the mitotic state likely contributes to the increase in TF dynamics.

Recent innovations have enabled an unprecedented increase in temporal and spatial resolution well beyond the diffraction limit, giving rise to various methods collectively known as 'super-resolution' microscopy [35]. One such technique that has been used to study TF dynamics is single-molecule localization microscopy coupled with single particle tracking analysis (SMLM-SPT) [36,37]. In this assay, fluorescently labeled TFs are localized individually and tracked over time, and varying image acquisition rates allow for kinetic measurements of DNA bound and freely diffusing molecules. For instance, coupling sparse labeling with slow acquisition rates (2–5 Hz, slow tracking) leads to diffusing TFs to 'blur' out, while DNA-interacting TFs appear as diffraction-limited spots whose length of time at one location is proportional to its residence time on DNA [38]. Alternatively, imaging at much higher acquisition rates (100–200 Hz, fast tracking) allows tracking of TF displacement pattern, and through mathematical modeling, the fraction of molecules bound versus freely diffusing can be extracted, along with the associated diffusion rates [39]. These techniques have been used to measure TF behavior during mitosis and confirm the increased dynamics in mitosis compared with interphase cells. For example, studies using slow tracking showed that the residence time of Sox2, HSF1, and HSF2 in

Biochemical Society Transactions (2024)
https://doi.org/10.1042/BST20231022

mitosis decreases by 40–50% compared with interphase [12,18]. Furthermore, studies using fast tracking showed that the DNA-bound fraction of Sox2 decreases from 30% in interphase to ∼20% during mitosis [12]. Taken together, the increased dynamics of TFs during mitosis may appear to be a common phenomenon.

To identify genomic sites specifically occupied by TFs during mitosis, several approaches have been developed to circumvent the formaldehyde fixation artifact. One approach relies on a double cross-linking ChIP assay, where cells are first cross-linked with disuccinimidyl glutarate (DSG) followed by formaldehyde [26]. It is presently unclear how DSG overcomes the cross-linking artifact. One possibility is that it has a much faster cross-linking kinetics than formaldehyde, though this remains to be tested. This approach has been used to detect site-specific binding of Esrrb, Oct4, Nanog, MYC, and MAX in mitosis. For both Esrrb and MAX, the number of bound sites is reduced in mitosis, representing 29.9% and 11.8%, respectively, of their interphase-bound sites, whereas MYC, Oct4, and Nanog showed near complete loss of binding in mitosis [8,40]. Although dual cross-linking allows the detection of site-specific binding in mitosis by some TFs, it remains to be determined how closely the dual cross-linking represents the native conditions of the mitotic cell.

An alternative approach avoids fixation altogether and instead uses enzymatic-based processing to query TF–DNA-binding under native conditions. These methods include the newly developed Cleavage Under Targets and Release Under the Nucleosome (CUT&RUN) and Cleavage Under Targets and Tagmentation (CUT&Tag) assays [41,42]. In these assays, whole cells or nuclei are immobilized on magnetic beads and permeabilized to allow for protein–DNA mapping using MNase (CUT&RUN) or Tn5 (CUT&Tag). CUT&RUN has been used to map CTCF, Sox2, and Esrrb during mitosis [43,44], while CUT&Tag has been used for Sox2, HSF1, and HSF2 [18]. In general, the number of TF-bound sites during mitosis is decreased compared with interphase cells. It ranges from near complete loss of binding (CTCF) to 50–85% reduction in site-specific binding (HSF1, Sox2, Esrrb) [43,44]. To date, the only exception is HSF2, where a modest increase of binding sites in mitosis was observed [18]. Although these approaches do not use formaldehyde cross-linking, how well the native conditions are preserved during the assay remains to be seen. Taken together, these studies show that current technologies are revealing much more nuance in TF–DNA interaction during mitosis than the simple bound versus excluded dichotomy.

Modes of TF–DNA interaction during mitosis and their molecular determinants

Applications of the above technologies have expanded our understanding of how TFs interact with mitotic DNA along two axes: enrichment on chromosomes as measured by imaging (coating) and site-specific binding as measured by genomics (binding). Combinations of these properties result in at least four distinct categories (Figure 2). The first is the exclusion mode (group I), where the TF is neither coating nor binding. Examples of TFs in group I include Sox13 and Nanog. A second mode is considered the classical bookmarking (group II), where TFs are both coating and binding. Examples of TFs belonging to group II include Sox2, ESRRB, PXR, and the vitamin D receptor (VDR) [45,46]. A third mode is characterized by coating but not binding (group III). This rare mode of interaction has been observed for a mutant FoxA1, in which a mutation in the DNA-binding domain leads to decreased site-specific binding but not coating. More recently, we have shown examples of a fourth type of TF interaction with mitotic DNA (group IV) in which a TF binds but does not coat. TFs in this category include HSF1 and HSF2.

What determines how a TF interacts with DNA during mitosis? The minimal components of a TF include a structurally folded DNA-binding domain (DBD) that recognizes specific DNA sequences, and a trans-activating domain (TAD) that enables protein–protein interactions for transcription activation [47]. From there, other domains may be included, such as a nuclear localization signal (NLS), a nuclear exclusion signal (NES), dimerization/trimerization regions, and ligand-binding domains [48,49]. The DBD plays a pivotal role in determining TF behavior during mitosis. For instance, removal of the Sox2 DBD or mutations of the specific DNA-binding residues completely abolishes its coating of mitotic DNA [12]. Furthermore, replacing the DBD of Sox13 (group I) with that of Sox2 (group II) causes the hybrid protein to exhibit coating behavior [18]. In some cases, the DBD also allows for non-specific DNA binding of TFs through an affinity for the DNA backbone. Mutations in FoxA1 that decrease its non-specific binding lead to decreased coating without affecting site-specific binding, whereas mutations in FoxA1 DNA-binding residues lead to continued coating, presumably through non-specific interactions, but loss of site-specific binding [10]. These studies provide extensive support for the importance of the DBD in determining TF behavior during mitosis.

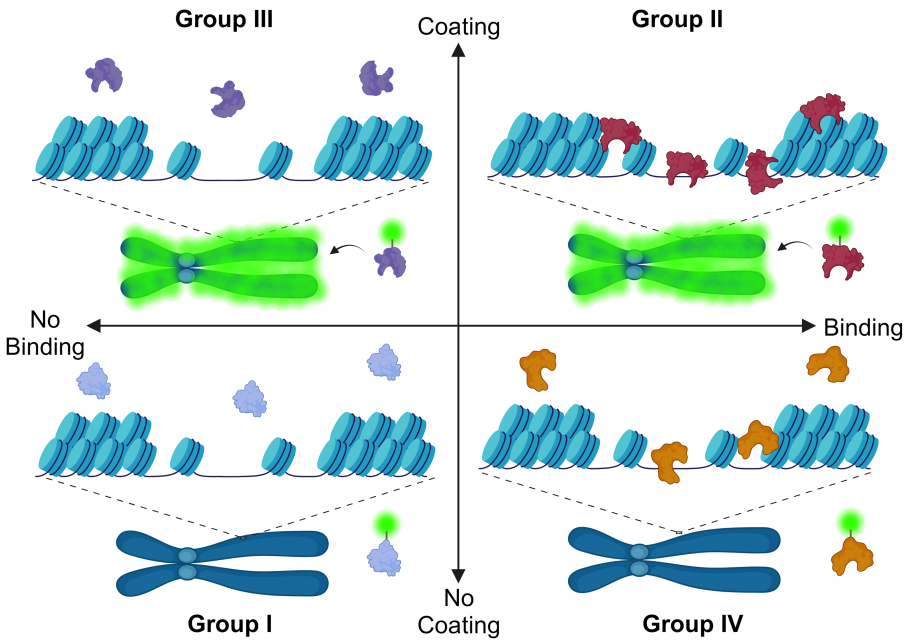

Figure 2. Four modes of TF-mitotic DNA interactions.

Combinations of two characteristic axes, mitotic chromosome coating, and site-specific binding, lead to at least four modes of TF interactions. Group I: no binding and no coating; Group II: coating and binding; Group III: coating and no binding; and Group IV: binding but no coating.

How do other TF domains affect its behavior during mitosis? Previous studies showed that a functional NLS is required for establishing interaction with mitotic DNA. For instance, mutations in the NLS of Sox2 abolished its ability to coat mitotic DNA, and fusing a strong NLS to the non-DNA-binding HaloTag enabled coating behavior [12]. However, the NLS was not always sufficient to confer coating behavior, since fusing a strong NLS to HSF2, which does not coat chromatin during mitosis, did not result in coating behavior [18]. This result suggested that, for some TFs, other regulatory domains determine their behavior during mitosis. For instance, HSF2 has been shown to be one of the rare TFs that exhibit binding but not coating behavior (group IV). In addition to a DBD, HSF2 contains domains for trimerization, regulation, and trans-activation [50,51]. The HSF2 DBD on its own showed coating and binding capabilities, but the addition of the trimerization domain to the DBD led to the loss of coating [18], suggesting that the inherent capability of the HSF2 DBD to coat mitotic chromosomes is counteracted by the trimerization domain. Therefore, although the behavior of TFs during mitosis is primarily dictated by its DBD, other domains can influence, and sometimes completely reverse, its behavior, which may have implications in how TFs promote transcription initiation after mitosis.

Transcription regulation in mitosis

Previous studies have established that transcription is greatly reduced during mitosis through the phosphorylation of general TFs and hyperphosphorylation of Pol II heptad repeats in the C-terminal domain [52,53]. However, recent studies are shedding new mechanistic insight into the role of chromatin and its structural regulators in mitotic transcription inhibition. During the onset of mitosis, chromatin undergoes massive reorganization, leading to the loss of chromatin loops and topologically associated domains that predominate interphase chromatin organization [1,54]. Factors that contribute to organizing interphase chromatin, including Topoisomerases, CTCF, and Cohesin, show altered behavior during mitosis, with Topoisomerases I and II (TOP1 and 2) remaining integral to the structure of mitotic chromosomes [50], and overall loss of binding by CTCF and Cohesin [44,55], though some studies have also shown CTCF binding at specific loci during mitosis [56]. Intriguingly, the removal of Cohesin from mitotic chromosomes seems critical for Pol II eviction during mitosis, as knockdown of the Cohesin removal factor WAPL led to the retention of transcriptionally engaged Pol II on mitotic chromosomes [57]. Similarly, the activity of TOP1 is required for clearing Pol II from the

Biochemical Society Transactions (2024)
https://doi.org/10.1042/BST20231022

chromosomes at the onset of mitosis, as loss of TOP1 results in the retention of Pol II on the DNA during mitosis [58]. These results suggest that chromatin condensation is intricately connected to the overall transcription repression during mitosis. More importantly, in both cases, retention of Pol II on mitotic chromosomes caused delays in cell cycle progression and improper reactivation of transcription following mitosis, emphasizing the importance of clearing Pol II during mitosis.

That said, recent studies have also challenged the belief that transcription is repressed during mitosis. Despite the phosphorylation cascade that inactivates the Pol II machinery, transcription occurs at centromeric DNA during mitosis [59]. Furthermore, recent studies report that some transcription outside of centromeres occurs during mitosis [60]. Using pulse-chase labeling with 5-ethynyluridine (5-EU) of newly transcribed RNA, Palazola et al. showed that most genes (~8000) exhibited 200-fold decreased 5-EU incorporation in mitosis compared with interphase cells. In contrast, 484 genes showed 100-fold increase in 5-EU labeling in mitosis, and these genes are enriched for those involved in extracellular structure. The magnitude and significance of gene-specific transcription during mitosis remain to be further studied.

The first wave of transcriptional reactivation occurs before cytokinesis is even completed and the levels of activity surpass those of interphase cells, suggesting a hyperactive transcription state following mitosis [59,60,61,62] that could be influenced by TF-mitotic bookmarking [63]. Furthermore, the group of genes that are first reactivated post-mitosis vary depending on cell type. For instance, in human hepatoma (HUH7) and osteosarcoma (U2OS) cells, the first to be reactivated are 'housekeeping genes' involved in basic cellular functions, while cell-type-specific genes are re-expressed at later time points [60,62]. However, in mouse embryonic stem cells, pluripotency genes are re-expressed first along with housekeeping genes [63]. Why do stem cells not follow hierarchical reactivation observed in different cell types? While more studies are needed, concurrent expression of stem cell-specific and housekeeping genes is likely related to their short G1 phase that lacks a G1/S checkpoint, and to their need for self-renewal capacity that is strictly dependent on pluripotency TFs [64–66].

Mechanisms of transcriptional memory and future prospects

How do cells reactivate transcription after mitosis? From the onset of the mitotic bookmarking field, this question has remained a challenge to address. The difficulty arises from the temporal nature of the phenomenon, specifically, separating the function of TF activity during interphase versus mitosis. Fusion with mitosis-specific or drug-inducible degrons has been employed to degrade TFs specifically during mitosis followed by transcriptional analysis, including for sequence-specific TFs like Sox2 [14], OCT4 [67], GATA1 [9], GATA2 [68], for nuclear receptors like ESRRB and NR5A2 [69], for the general TF TBP [28], and for the structural chromatin regulator CTCF [55,70]. In some cases, mitosis-specific degradation led to altered transcription reactivation for target genes [28,69] or deficient cell identity maintenance [14,67,68], but others had limited to no effects [55,70], highlighting the importance of functional analyses of mitotic bookmarking. Another functional approach has been the use of small molecule inhibitors. For example, the chromatin regulator BRD4, part of the BET (Bromodomain and Extra-Terminal motif) family of proteins, has been shown previously to bind mitotic chromosomes extensively [71], but BRD4 displacement through small molecule inhibitors showed little effect on transcription reactivation after mitosis [72]. These studies suggest that binding on mitotic chromosomes may not always equate with a functional role in maintaining transcriptional memory through mitosis.

Although our review has focused on the role of TFs in this process, chromatin also plays a major role. Studies have shown that certain histone marks are largely retained during mitosis at the gene promoters and enhancers, including most of H3 methylation [54,61,62] and acetylation [72]. For example, H3K9 acetylation (H3K9Ac), a hallmark of active promoters, and H3K27 acetylation (H3K27Ac), a hallmark of active enhancers, are retained at specific loci on mitotic chromosomes [67,73,74]. Importantly, loss of H3K27Ac during mitosis caused by pharmacological inhibition of the p300/CBP acetyltransferase resulted in dysregulation of almost half of the post-mitotic reactivated genes, with cell-type-specific genes being the most affected [62]. These results suggest that histone PTMs play an important role in marking genes that are reactivated after cell division.

The discovery that the vast majority of TFs and other transcription regulators can interact with mitotic chromatin, and that these interactions are highly dynamic, begs the question of how these dynamic interactions lead to gene reactivation. For certain TFs, a clear link between mitotic binding and gene reactivation has been established. For instance, MAX binding during mitosis leads to recruitment of MYC immediately following mitosis, allowing for rapid re-expression of target genes [40], and rapid degradation of Sox2 and Oct4 during

mitosis impairs pluripotency maintenance [14,67]. Given that most TFs interact with mitotic chromosomes in a variety of ways, it remains to be seen whether an overarching model for how TFs promote transcriptional memory can be discerned. It is likely that each TF, with its own distinct properties, will have a specific contribution that collectively leads to the complex pattern of gene reactivation that is unique to each cell type. Importantly, as the field of mitotic bookmarking matures, a challenge presents itself in assessing the functional effects of the different modes of TF bookmarking in terms of cellular memory. Such functional tests would also need to consider the roles that protein and mRNA stability may play in this process. As new technologies provide increased sensitivity, resolution, and precision in measuring TF binding and transcription reactivation, we are well-positioned to more fully define the dynamic roles of TFs in promoting transcriptional memory following cell division.

Perspectives

- Transcriptional memory through mitosis is important for maintaining cell identity.
- TFs exhibit a dynamic mode of interacting with mitotic chromosomes.
- How dynamic TF interactions promote transcriptional memory remains to be determined.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
This work was supported by the Canadian Institutes for Health Research Project Grant award to S.S.T. (PJT-162289), the National Sciences and Engineering Research Council Discovery Grant award to S.S.T. (RGPIN-2020-06106), the Stem Cell Network Early Career Researcher Jump Start Awards Program to S.S.T. (ECR-C4R1-11), the Canada Research Chairs CRC Tier II Award to S.S.T. (CRC-RS 2021-00294), and the Michael Smith Health Research BC Research Trainee Award to M.A.B.

Author Contribution
Conceptualization: M.A.B., A.K.L.W., A.F., S.S.T. Visualization: A.K.L.W., A.F., S.S.T. Funding acquisition: M.A.B., S.S.T. Supervision: S.S.T. Writing—original draft: M.A.B., A.K.L.W., A.F., S.S.T. Writing—review and editing: M.A.B., A.K.L.W., A.F., S.S.T.

Open Access
Open access for this article was enabled by the participation of the University of British Columbia in an all-inclusive *Read & Publish* agreement with Portland Press and the Biochemical Society.

Acknowledgements
For insightful comments on the manuscript, we thank Jennifer Mitchell. S.S.T. is a Michael Smith Foundation for Health Research Scholar.

Abbreviations
ChIP, chromatin immunoprecipitation; DSG, disuccinimidyl glutarate; FPs, fluorescent proteins; IF, immunofluorescence; NLS, nuclear localization signal; Pol II, Polymerase II; TFs, transcription factors.

References
1 Gibcus, J.H., Samejima, K., Goloborodko, A., Samejima, I., Naumova, N., Nuebler, J. et al. (2018) A pathway for mitotic chromosome formation. *Science* **359**, eaao6135 https://doi.org/10.1126/science.aao6135
2 Smoyer, C.J. and Jaspersen, S.L. (2014) Breaking down the wall: the nuclear envelope during mitosis. *Curr. Opin. Cell Biol.* **26**, 1–9 https://doi.org/10.1016/j.ceb.2013.08.002

3 Gottesfeld, J.M. and Forbes, D.J. (1997) Mitotic repression of the transcriptional machinery. *Trends Biochem. Sci.* **22**, 197–202 https://doi.org/10.1016/s0968-0004(97)01045-1
4 Michelotti, E.F., Sanford, S. and Levens, D. (1997) Marking of active genes on mitotic chromosomes. *Nature* **388**, 895–899 https://doi.org/10.1038/42282
5 Martínez-Balbás, M.A., Dey, A., Rabindran, S.K., Ozato, K. and Wu, C. (1995) Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell* **83**, 29–38 https://doi.org/10.1016/0092-8674(95)90231-7
6 John, S. and Workman, J.L. (1998) Bookmarking genes for activation in condensed mitotic chromosomes. *BioEssays* **20**, 275–279 https://doi.org/10.1002/(SICI)1521-1878(199804)20:4<275::AID-BIES1>3.0.CO;2-P
7 Sarge, K.D. and Park-Sarge, O.-K. (2009) Mitotic bookmarking of formerly active genes: keeping epigenetic memories from fading. *Cell Cycle* **8**, 818–823 https://doi.org/10.4161/cc.8.6.7849
8 Festuccia, N., Dubois, A., Vandormael-Pournin, S., Gallego Tejeda, E., Mouren, A., Bessonnard, S. et al. (2016) Mitotic binding of Esrrb marks key regulatory regions of the pluripotency network. *Nat. Cell Biol.* **18**, 1139–1148 https://doi.org/10.1038/ncb3418
9 Kadauke, S., Udugama, M.I., Pawlicki, J.M., Achtman, J.C., Jain, D.P., Cheng, Y. et al. (2012) Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1. *Cell* **150**, 725–737 https://doi.org/10.1016/j.cell.2012.06.038
10 Caravaca, J.M., Donahue, G., Becker, J.S., He, X., Vinson, C. and Zaret, K.S. (2013) Bookmarking by specific and nonspecific binding of FoxA1 pioneer factor to mitotic chromosomes. *Genes Dev.* **27**, 251–260 https://doi.org/10.1101/gad.206458.112
11 Rizvi, S., Chhabra, A., Tripathi, A. and Tyagi, R.K. (2023) Mitotic genome-bookmarking by nuclear hormone receptors: a novel dimension in epigenetic reprogramming and disease assessment. *Mol. Cell. Endocrinol.* **578**, 112069 https://doi.org/10.1016/j.mce.2023.112069
12 Teves, S.S., An, L., Hansen, A.S., Xie, L., Darzacq, X. and Tjian, R. (2016) A dynamic mode of mitotic bookmarking by transcription factors. *eLife* **5**, e22280 https://doi.org/10.7554/eLife.22280
13 Raccaud, M. and Suter, D.M. (2018) Transcription factor retention on mitotic chromosomes: regulatory mechanisms and impact on cell fate decisions. *FEBS Lett.* **592**, 878–887 https://doi.org/10.1002/1873-3468.12828
14 Deluz, C., Friman, E.T., Strebinger, D., Benke, A., Raccaud, M., Callegari, A. et al. (2016) A role for mitotic bookmarking of SOX2 in pluripotency and differentiation. *Genes Dev.* **30**, 2538–2550 https://doi.org/10.1101/gad.289256.116
15 Blythe, S.A. and Wieschaus, E.F. (2016) Establishment and maintenance of heritable chromatin structure during early *Drosophila* embryogenesis. *eLife* **5**, e20148 https://doi.org/10.7554/eLife.20148
16 Saradhi, M., Sengupta, A., Mukhopadhyay, G. and Tyagi, R.K. (2005) Pregnane and Xenobiotic Receptor (PXR/SXR) resides predominantly in the nuclear compartment of the interphase cell and associates with the condensed chromosomes during mitosis. *Biochim. Biophys. Acta* **1746**, 85–94 https://doi.org/10.1016/j.bbamcr.2005.10.004
17 Kumar, S., Chaturvedi, N.K., Kumar, S. and Tyagi, R.K. (2008) Agonist-mediated docking of androgen receptor onto the mitotic chromatin platform discriminates intrinsic mode of action of prostate cancer drugs. *Biochim. Biophys. Acta* **1783**, 59–73 https://doi.org/10.1016/j.bbamcr.2007.11.002
18 Price, R.M., Budzyński, M.A., Shen, J., Mitchell, J.E., Kwan, J.Z.J., Teves, S.S. (2023) Heat shock transcription factors demonstrate a distinct mode of interaction with mitotic chromosomes. *Nucleic Acids Res.* **51**, 5040–5055. https://doi.org/10.1093/nar/gkad304
19 Im, K., Mareninov, S., Diaz, M.F.P. and Yong, W.H. (2019) An introduction to performing immunofluorescence staining. *Methods Mol. Biol.* **1897**, 299–311 https://doi.org/10.1007/978-1-4939-8935-5_26
20 He, S. and Davie, J.R. (2006) Sp1 and Sp3 foci distribution throughout mitosis. *J. Cell Sci.* **119**, 1063–1070 https://doi.org/10.1242/jcs.02829
21 Adamson, A.L. (2005) Epstein-Barr virus BZLF1 protein binds to mitotic chromosomes. *J. Virol.* **79**, 7899–7904 https://doi.org/10.1128/JVI.79.12.7899-7904.2005
22 Kadauke, S. and Blobel, G.A. (2013) Mitotic bookmarking by transcription factors. *Epigenetics Chromatin* **6**, 6 https://doi.org/10.1186/1756-8935-6-6
23 Rodríguez-Ubreva, J. and Ballestar, E. (2014) Chromatin immunoprecipitation. *Methods Mol. Biol.* **1094**, 309–318 https://doi.org/10.1007/978-1-62703-706-8_24
24 Dirks, R.W. and Snaar, S. (1999) Dynamics of RNA polymerase II localization during the cell cycle. *Histochem. Cell Biol.* **111**, 405–410 https://doi.org/10.1007/s004180050374
25 Christova, R. and Oelgeschläger, T. (2002) Association of human TFIID–promoter complexes with silenced mitotic chromatin in vivo. *Nat. Cell Biol.* **4**, 79–82 https://doi.org/10.1038/ncb733
26 Festuccia, N., Owens, N., Papadopoulou, T., Gonzalez, I., Tachtsidi, A., Vandoermel-Pournin, S. et al. (2019) Transcription factor activity and nucleosome organization in mitosis. *Genome Res.* **29**, 250–260 https://doi.org/10.1101/gr.243048.118
27 Lerner, J., Bagattin, A., Verdeguer, F., Makinistoglu, M.P., Garbay, S., Felix, T. et al. (2016) Human mutations affect the epigenetic/bookmarking function of HNF1B. *Nucleic Acids Res.* **44**, 8097–8111 https://doi.org/10.1093/nar/gkw467
28 Teves, S.S., An, L., Bhargava-Shah, A., Xie, L., Darzacq, X. and Tjian, R. (2018) A stable mode of bookmarking by TBP recruits RNA polymerase II to mitotic chromosomes. *eLife* **7**, e35621 https://doi.org/10.7554/eLife.35621
29 Hansen, A.S., Pustova, I., Cattoglio, C., Tjian, R. and Darzacq, X. (2017) CTCF and cohesin regulate chromatin loop stability with distinct dynamics. *eLife* **6**, e25776 https://doi.org/10.7554/eLife.25776
30 Blobel, G.A., Kadauke, S., Wang, E., Lau, A.W., Zuber, J., Chou, M.M. et al. (2009) A reconfigured pattern of MLL occupancy within mitotic chromatin promotes rapid transcriptional reactivation following mitotic exit. *Mol. Cell* **36**, 970–983 https://doi.org/10.1016/j.molcel.2009.12.001
31 Carisey, A., Stroud, M., Tsang, R. and Ballestrem, C. (2011) Fluorescence recovery after photobleaching. *Methods Mol. Biol.* **769**, 387–402 https://doi.org/10.1007/978-1-61779-207-6_26
32 McNally, J.G., Müller, W.G., Walker, D., Wolford, R. and Hager, G.L. (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science* **287**, 1262–1265 https://doi.org/10.1126/science.287.5456.1262
33 Verneri, P., Romero, J.J., De Rossi, M.C., Alvarez, Y., Oses, C., Guberman, A. et al. (2018) Imaging transcription factors dynamics with advanced fluorescence microscopy methods. *Mech. Dev.* **154**, 60–63 https://doi.org/10.1016/j.mod.2018.05.003
34 Mueller, F., Wach, P. and McNally, J.G. (2008) Evidence for a common mode of transcription factor interaction with chromatin as revealed by improved quantitative fluorescence recovery after photobleaching. *Biophys. J.* **94**, 3323–3339 https://doi.org/10.1529/biophysj.107.123182

35 Schermelleh, L., Ferrand, A., Huser, T., Eggeling, C., Sauer, M., Biehlmaier, O. et al. (2019) Super-resolution microscopy demystified. *Nat. Cell Biol.* **21**, 72–84 https://doi.org/10.1038/s41556-018-0251-8
36 Liu, C., Liu, Y.-L., Perillo, E.P., Dunn, A.K. and Yeh, H.-C. (2016) Single-molecule tracking and its application in biomolecular binding detection. *IEEE J. Sel. Top. Quantum Electron.* **22**, 6804013 https://doi.org/10.1109/JSTQE.2016.2568160
37 Hugelier, S., Colosi, P.L. and Lakadamyali, M. (2023) Quantitative single-molecule localization microscopy. *Annu. Rev. Biophys.* **52**, 139–160 https://doi.org/10.1146/annurev-biophys-111622-091212
38 Izeddin, I., Récamier, V., Bosanac, L., Cissé, I.I., Boudarene, L., Dugast-Darzacq, C. et al. (2014) Single-molecule tracking in live cells reveals distinct target-search strategies of transcription factors in the nucleus. *eLife* **3**, e02230 https://doi.org/10.7554/eLife.02230
39 Hansen, A.S., Woringer, M., Grimm, J.B., Lavis, L.D., Tjian, R. and Darzacq, X. (2018) Robust model-based analysis of single-particle tracking experiments with Spot-On. *eLife* **7**, e33125 https://doi.org/10.7554/eLife.33125
40 Gonzalez, I., Chervova, A., Escoll, P., Altamirano-Pacheco, L., Mueller, F., Dubois, A. et al. (2023) MYC and MAX drive the reactivation of the genome after mitosis. *bioRxiv* 2023.08.13.553120. https://doi.org/10.1101/2023.08.13.553120
41 Skene, P.J. and Henikoff, S. (2017) An efficient targeted nuclease strategy for high-resolution mapping of DNA binding sites. *eLife* **6**, e21856 https://doi.org/10.7554/eLife.21856
42 Kaya-Okur, H.S., Wu, S.J., Codomo, C.A., Pledger, E.S., Bryson, T.D., Henikoff, J.G. et al. (2019) CUT&tag for efficient epigenomic profiling of small samples and single cells. *Nat. Commun.* **10**, 1930 https://doi.org/10.1038/s41467-019-09982-5
43 Zhu, Z., Chen, X., Guo, A., Manzano, T., Walsh, P.J., Wills, K.M. et al. (2023) Mitotic bookmarking by SWI/SNF subunits. *Nature* **618**, 180–187 https://doi.org/10.1038/s41586-023-06085-6
44 Oomen, M.E., Hansen, A.S., Liu, Y., Darzacq, X. and Dekker, J. (2019) CTCF sites display cell cycle-dependent dynamics in factor binding and nucleosome positioning. *Genome Res.* **29**, 236–249 https://doi.org/10.1101/gr.241547.118
45 Rana, M., Dash, A.K., Ponnusamy, K. and Tyagi, R.K. (2018) Nuclear localization signal region in nuclear receptor PXR governs the receptor association with mitotic chromatin. *Chromosome Res.* **26**, 255–276 https://doi.org/10.1007/s10577-018-9583-2
46 Pike, J.W. and Meyer, M.B. (2010) The vitamin D receptor: new paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D3. *Endocrinol. Metab. Clin. North Am.* **39**, 255–269 https://doi.org/10.1016/j.ecl.2010.02.007
47 Frankel, A.D. and Kim, P.S. (1991) Modular structure of transcription factors: implications for gene regulation. *Cell* **65**, 717–719 https://doi.org/10.1016/0092-8674(91)90378-c
48 Frietze, S. and Farnham, P.J. (2011) Transcription factor effector domains. *Subcell. Biochem.* **52**, 261–277 https://doi.org/10.1007/978-90-481-9069-0_12
49 Soto, L.F., Li, Z., Santoso, C.S., Berenson, A., Ho, I., Shen, V.X. et al. (2022) Compendium of human transcription factor effector domains. *Mol. Cell* **82**, 514–526 https://doi.org/10.1016/j.molcel.2021.11.007
50 Jaeger, A.M., Pemble, C.W., Sistonen, L. and Thiele, D.J. (2016) Structures of HSF2 reveal mechanisms for differential regulation of human heat-shock factors. *Nat. Struct. Mol. Biol.* **23**, 147–154 https://doi.org/10.1038/nsmb.3150
51 Westerheide, S.D., Raynes, R., Powell, C., Xue, B. and Uversky, V.N. (2012) HSF transcription factor family, heat shock response, and protein intrinsic disorder. *Curr. Protein Pept. Sci.* **13**, 86–103 https://doi.org/10.2174/138920312799277956
52 Segil, N., Guermah, M., Hoffmann, A., Roeder, R.G. and Heintz, N. (1996) Mitotic regulation of TFIID: inhibition of activator-dependent transcription and changes in subcellular localization. *Genes Dev.* **10**, 2389–2400 https://doi.org/10.1101/gad.10.19.2389
53 Gebara, M.M., Sayre, M.H. and Corden, J.L. (1997) Phosphorylation of the carboxy-terminal repeat domain in RNA polymerase II by cyclin-dependent kinases is sufficient to inhibit transcription. *J. Cell. Biochem.* **64**, 390–402 https://doi.org/10.1002/(sici)1097-4644(19970301)64:3<390::aid-jcb6>3.0.co;2-q
54 Ghosh, R.P. and Meyer, B.J. (2021) Spatial organization of chromatin: emergence of chromatin structure during development. *Annu. Rev. Cell Dev. Biol.* **37**, 199–232 https://doi.org/10.1146/annurev-cellbio-032321-035734
55 Owens, N., Papadopoulou, T., Festuccia, N., Tachtsidi, A., Gonzalez, I., Dubois, A. et al. (2019) CTCF confers local nucleosome resiliency after DNA replication and during mitosis. *eLife* **8**, e47898 https://doi.org/10.7554/eLife.47898
56 Burke, L.J., Zhang, R., Bartkuhn, M., Tiwari, V.K., Tavoosidana, G., Kurukuti, S. et al. (2005) CTCF binding and higher order chromatin structure of the H19 locus are maintained in mitotic chromatin. *EMBO J.* **24**, 3291–3300 https://doi.org/10.1038/sj.emboj.7600793
57 Perea-Resa, C., Bury, L., Cheeseman, I.M. and Blower, M.D. (2020) Cohesin removal reprograms gene expression upon mitotic entry. *Mol. Cell* **78**, 127–140.e7 https://doi.org/10.1016/j.molcel.2020.01.023
58 Wiegard, A., Kuzin, V., Cameron, D.P., Grosser, J., Ceribelli, M., Mehmood, R. et al. (2021) Topoisomerase 1 activity during mitotic transcription favors the transition from mitosis to G1. *Mol. Cell* **81**, 5007–5024.e9 https://doi.org/10.1016/j.molcel.2021.10.015
59 Chan, F.L. and Wong, L.H. (2012) Transcription in the maintenance of centromere chromatin identity. *Nucleic Acids Res.* **40**, 11178–11188 https://doi.org/10.1093/nar/gks921
60 Palozola, K.C., Donahue, G., Liu, H., Grant, G.R., Becker, J.S., Cote, A. et al. (2017) Mitotic transcription and waves of gene reactivation during mitotic exit. *Science* **358**, 119–122 https://doi.org/10.1126/science.aal4671
61 Hsiung, C.C.-S., Bartman, C.R., Huang, P., Ginart, P., Stonestrom, A.J., Keller, C.A. et al. (2016) A hyperactive transcriptional state marks genome reactivation at the mitosis-G1 transition. *Genes Dev.* **30**, 1423–1439 https://doi.org/10.1101/gad.280859.116
62 Kang, H., Shokhirev, M.N., Xu, Z., Chandran, S., Dixon, J.R. and Hetzer, M.W. (2020) Dynamic regulation of histone modifications and long-range chromosomal interactions during postmitotic transcriptional reactivation. *Genes Dev.* **34**, 913–930 https://doi.org/10.1101/gad.335794.119
63 Chervova, A., Festuccia, N., Altamirano-Pacheco, L., Dubois, A. and Navarro, P. (2023) A gene subset requires CTCF bookmarking during the fast post-mitotic reactivation of mouse ES cells. *EMBO Rep.* **24**, e56075 https://doi.org/10.15252/embr.202256075
64 Ahuja, A.K., Jodkowska, K., Teloni, F., Bizard, A.H., Zellweger, R., Herrador, R. et al. (2016) A short G1 phase imposes constitutive replication stress and fork remodelling in mouse embryonic stem cells. *Nat. Commun.* **7**, 10660 https://doi.org/10.1038/ncomms10660
65 Li, V.C., Ballabeni, A. and Kirschner, M.W. (2012) Gap 1 phase length and mouse embryonic stem cell self-renewal. *Proc. Natl Acad. Sci. U.S.A.* **109**, 12550–12555 https://doi.org/10.1073/pnas.1206740109

66 Aladjem, M.I., Spike, B.T., Rodewald, L.W., Hope, T.J., Klemm, M., Jaenisch, R. et al. (1998) ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. *Curr. Biol.* **8**, 145–155 https://doi.org/10.1016/S0960-9822(98)70061-2

67 Liu, Y., Pelham-Webb, B., Di Giammartino, D.C., Li, J., Kim, D., Kita, K. et al. (2017) Widespread mitotic bookmarking by histone marks and transcription factors in pluripotent stem cells. *Cell Rep.* **19**, 1283–1293 https://doi.org/10.1016/j.celrep.2017.04.067

68 Silvério-Alves, R., Kurochkin, I., Rydström, A., Vazquez Echegaray, C., Haider, J., Nicholls, M. et al. (2023) GATA2 mitotic bookmarking is required for definitive haematopoiesis. *Nat. Commun.* **14**, 4645 https://doi.org/10.1038/s41467-023-40391-x

69 Chervova, A., Molliex, A., Baymaz, H.I., Coux, R.-X., Papadopoulou, T., Mueller, F. et al. (2024) Mitotic bookmarking redundancy by nuclear receptors in pluripotent cells. *Nat. Struct. Mol. Biol.* **31**, 1–10. https://doi.org/10.1038/s41594-023-01195-1

70 Zhang, H., Lam, J., Zhang, D., Lan, Y., Vermunt, M.W., Keller, C.A. et al. (2021) CTCF and transcription influence chromatin structure re-configuration after mitosis. *Nat. Commun.* **12**, 5157 https://doi.org/10.1038/s41467-021-25418-5

71 Dey, A., Ellenberg, J., Farina, A., Coleman, A.E., Maruyama, T., Sciortino, S. et al. (2000) A bromodomain protein, MCAP, associates with mitotic chromosomes and affects G(2)-to-M transition. *Mol. Cell. Biol.* **20**, 6537–6549 https://doi.org/10.1128/MCB.20.17.6537-6549.2000

72 Behera, V., Stonestrom, A.J., Hamagami, N., Hsiung, C.C., Keller, C.A., Giardine, B. et al. (2019) Interrogating histone acetylation and BRD4 as mitotic bookmarks of transcription. *Cell Rep.* **27**, 400–415.e5 https://doi.org/10.1016/j.celrep.2019.03.057

73 Javasky, E., Shamir, I., Gandhi, S., Egri, S., Sandler, O., Rothbart, S.B. et al. (2018) Study of mitotic chromatin supports a model of bookmarking by histone modifications and reveals nucleosome deposition patterns. *Genome Res.* **28**, 1455–1466 https://doi.org/10.1101/gr.230300.117

74 Pelham-Webb, B., Polyzos, A., Wojenski, L., Kloetgen, A., Li, J., Di Giammartino, D.C. et al. (2021) H3k27ac bookmarking promotes rapid post-mitotic activation of the pluripotent stem cell program without impacting 3D chromatin reorganization. *Mol. Cell* **81**, 1732–1748.e8 https://doi.org/10.1016/j.molcel.2021.02.032