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REVIEW ARTICLE

Transcription factor retention on mitotic chromosomes: regulatory mechanisms and impact on cell fate decisions

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During mitosis, gene transcription stops, and the bulk of DNA-binding proteins are excluded from condensed chromosomes. While most gene-specific transcription factors are largely evicted from mitotic chromosomes, a subset remains bound to specific and non-specific DNA sites. Here, we review the current knowledge on the mechanisms leading to the retention of a subset of transcription factors on mitotic chromosomes and discuss the implications in gene expression regulation and their potential as an epigenetic mechanism controlling stem cell self-renewal and differentiation.

Keywords: cell fate; M-G1 transition; mitotic bookmarking; mitotic chromosomes; non-specific DNA binding; transcription factors

During mitosis, chromosomes undergo major structural reorganization, resulting in their highly condensed aspect at the microscopic scale [1]. While this observation was made over a century ago, the biochemical composition and the structural organization of mitotic chromosomes remain incompletely understood. Intuitively, one could reason that the highly condensed mitotic chromatin environment should by itself result in the exclusion of DNA-binding proteins. However, since the size of most individual proteins is in the nanometer range, the apparent condensation at the micro-scale may not necessarily lead to an impairment of dynamic exchanges of proteins within the mitotic chromatin environment. Current estimations report a mere two- to three-fold reduction in the volume occupied by chromatin during mitosis [2–4], and DNase I hypersensitivity and ATAC-seq experiments performed on mitotic cells showed that mitotic chromatin displays relatively unchanged accessibility profiles as compared to interphase DNA [5–7]. Therefore, there is currently no solid basis for physical hindrance of DNA accessibility during mitosis by chromatin compaction. Nevertheless, gene transcription is globally interrupted at the onset of chromosome condensation and resumes only at the mitosis to G1 transition. Furthermore, the three-dimensional organization of the genome is largely disrupted during mitosis. This raises the question of how daughter cells faithfully restore the gene expression program to preserve their phenotypic state. While the passive distribution of trans-regulating elements to daughter cells arguably plays a role in the transmission of gene expression patterns after cell division, mounting evidence suggests that epigenetic marks that are retained on specific genomic loci during mitosis play an important role in regulating post-mitotic transcriptional reactivation. DNA methylation is essentially preserved throughout mitosis, and many histone marks are to some extent also maintained [8], some of them being directly involved in the transmission of epigenetic information impacting on transcriptional memory. Methylation of H3K4 was shown to be required for the inheritance of

Abbreviations
ChIP-seq, chromatin immunoprecipitation followed by high throughput sequencing; FACS, fluorescence-activated cell sorting; FRAP, fluorescence recovery after photobleaching; NLS, nuclear localization signal; TBP, TATA-box-binding protein; TFs, transcription factors.

transcriptional states through cell division [9] and H4K5 acetylation was also shown to mark active loci during mitosis and allows the binding of Brd4, a chromatin reader enhancing transcriptional reactivation of previously active genes [10]. Finally, poly-ADP-ribose polymerase 1 (PARP1), which is able to attach poly-ADP chains to histones, remains attached to mitotic chromosomes and plays a role in transcriptional reactivation after mitotic exit [11]. Therefore, there is accumulating evidence that covalent modifications of histones and their corresponding interacting proteins play an important role in transmission of gene expression programs through mitosis.

The presence of trans-acting factors that are involved in transcription and retained on mitotic chromosomes was suggested 20 years ago using S1 nuclease sensitivity assays. These experiments showed that promoters that were transcriptionally active in interphase have a perturbed configuration in mitosis, in contrast to those that were transcriptionally silent [12], thus hinting at the presence of trans-acting factors that may stay bound to mitotic chromosomes. Subsequently, both immunofluorescence imaging and the use of fluorescently tagged proteins allowed to unravel a number of DNA-binding proteins [13] and transcription factors (TFs) that associate with mitotic chromosomes [6,14–18]. Most notably, TATA-box-binding protein (TBP) binds mitotic chromosomes [19] and associates with promoters that are transcribed during interphase, therefore marking these loci for transcription re-establishment after mitotic exit [20]. Finally, a subset of gene-specific TFs was shown to be retained on sequence-specific sites on mitotic chromosomes, which led to speculations on their potential role in maintaining cell-type-specific gene expression programs during cell division. Here, we review the current knowledge on specific TFs that are retained on mitotic chromosomes, the techniques that are used to assess mitotic DNA binding, and discuss potential regulatory mechanisms and functional implications of mitotic chromosome binding on gene expression and cell fate decisions.

Mitotic chromosome binding and mitotic bookmarking

Mitotic chromosome binding versus bookmarking

Mitotic chromosome binding refers to the general association of a protein to mitotic chromatin and is often determined by colocalization of immunolabeled or fluorescently tagged proteins with DNA by fluorescence microscopy. In metazoans, mitotic chromosomes are not delimited by a nuclear membrane, and thus, it is often assumed that this colocalization is mediated by direct physical interaction. An important consequence of the sequestration of a protein within the subcellular volume that contains mitotic chromosomes is the increased local concentration that enhances the probability of molecular contacts with DNA by the law of mass action (potential implications will be discussed in Section 3).

Mitotic bookmarking refers to the presence of a chromatin mark or to the physical interaction of a DNA-binding protein with specific sites in the genome. The number of sites bound by TFs on mitotic chromosomes has been consistently estimated to be significantly lower than in interphase, ranging between a few hundred to a few thousand sites [14,15,21–23], although potential methodological limitations could also contribute to these observations (discussed more in detail in Section 2). Nevertheless, this suggests that only a relatively small number of sequence-specific binding sites are bound by TFs during mitosis. Since these numbers are unlikely to give rise to a signal in fluorescence microscopy that is strong enough to be discriminated from background autofluorescence [24], there is to date no evidence that mitotic bookmarking can be observed by microscopy. Conversely, there is no direct evidence that mitotic chromosome binding implies mitotic bookmarking. Finally, most studies remain speculative on the functional role of bookmarking, which is difficult to address unambiguously as there is currently no method to alter histone marks or binding of TFs specifically during mitosis. The development of new molecular tools will be required to evaluate the functional consequences of both sequence-specific and non-specific binding of TFs during mitosis.

Transcription factors bound to mitotic chromosomes

Tissue-specific transcription factors have long been thought to be largely stripped off DNA during mitosis [7], but a minority of them was shown to associate with mitotic chromosomes. Interestingly, most of the ones identified so far are involved in cell fate regulation. GATA1, a major hematopoiesis regulator, remains partially bound to mitotic chromosomes and bookmarks genes involved in cell fate regulation [17]. Target site-specific bindings were also demonstrated for FoxA1, a pioneer TF involved in liver differentiation [16]; Runx2, involved in osteoblast lineage [18,25]; RBPJ, a major Notch effector [26]; and CTCF, a

DNA-binding protein orchestrating the 3D conformation of the genome and allowing regulatory insulation of genomic regions from each other [27]. Recently, several key pluripotency regulators have been reported to bind to mitotic chromosomes, namely SOX2 [6,14,15], OCT4 [6,14,15], ESRRB [23] and Klf4 [15], and were confirmed for mitotic bookmarking activity. For most of these examples, mitotic chromatin binding was shown to be mediated by the DNA-binding domain, thus suggesting a direct sequence-specific interaction with mitotic DNA. This raises the question of whether certain classes of DNA-binding domains are particularly prone to bind mitotic chromosomes. However, at present, the relatively small numbers of TFs that have been assessed for this property are largely insufficient to substantiate this hypothesis.

Methods to study mitotic chromosome binding and mitotic bookmarking

Mitotic chromosome binding of TFs is generally based on the observed colocalization of a protein with fluorescent histones or fluorescent DNA labels in mitotic cells. Immunofluorescence has been broadly used to label DNA-binding proteins, since it allows probing the localization of unmodified, endogenous proteins. However, the most widely used fixation method based on formaldehyde has long been misleading in the field, since it was shown to rapidly disrupt the interactions of TFs with mitotic chromosomes [6,13]. The reduction of TF binding upon formaldehyde fixation of mitotic cells might be explained by an alteration of the mitotic chromatin structure [13] and/or by the progressive fixation of cytoplasmic TF molecules [6,13]. Since sequence specifically bound TFs have longer DNA residence times, they might be more likely to be cross-linked to DNA before losing their DNA binding ability as compared with unspecifically bound or freely diffusing TFs. Alternative fixation protocols, such as methanol-based fixation, can be used to solve this issue [13,14,28], but many antibodies do not work optimally in these conditions. An attractive alternative is the use of fluorescent/luminescent proteins or tags such as SNAP- or Halo-tags as fusion partners, allowing for the monitoring of subcellular localization of DNA-binding proteins in living cells. The use of different tags fused in either N- or C-terminal positions by knock-in or overexpressed at moderate levels has shown to produce very consistent results [6,14,15,23], and is also amenable to quantitative measurements of mitotic DNA binding by comparing fluorescence intensities on mitotic chromatin to the cytoplasm.

Proteomics approaches can be used to obtain a more comprehensive assessment of proteins binding to mitotic chromosomes [29–31]. By using quantitative proteomics combined with advanced bioinformatics, Ohta *et al*. were able to identify ~ 4000 proteins associated with mitotic chromosomes, among which 255 TFs [31]. A considerable advantage of these approaches is to directly identify mitotic chromosome binding of hundreds of endogenously expressed proteins, without relying on overexpressed or fluorescently tagged proteins. However, it requires a large amount of material and relies on drugs to synchronize cells in mitosis, and is thus not immune to artifacts stemming from drug toxicity. It is also less amenable to the extraction of truly quantitative measurements to compare the extent of mitotic sequestration for different TFs.

Both imaging and proteomics methods provide information on mitotic chromosome binding but do not interrogate mitotic bookmarking properties, which require the assessment of sequence-specific binding events. The most widely used method to identify sequence-specific binding of TFs on mitotic DNA is chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq). Typical TFs bind thousands to tens of thousands of sites as assessed by ChIP-seq in unsynchronized cells. These binding sites are identified based on the lysis of millions of cells, and, since most TFs [32,33] have typically short residence times on DNA, most of these sites are likely to be transiently occupied, which is also corroborated by the sparse number of peaks found in single-cell ChIP-seq experiments [34]. Since the typical ChIP-seq signal obtained from mitotic cells is generally low as compared with unsynchronized cell populations, it is particularly crucial to obtain pure mitotic cell populations to avoid signal contamination by interphase cells. Drug synchronization protocols do not always provide sufficiently pure mitotic cell populations [35,36], thus these can be combined with subsequent fluorescence-activated cell sorting (FACS) using mitotic-specific antibodies against either H3S10P [35] or MDM2 [37]. Alternatively, mitotic shake-off can be used to purify mitotic cells after chemical synchronization [15,23]. Importantly, ChIP-seq invariably uses formaldehyde-based fixation to covalently but reversibly attach TFs to their interacting locations in the genome. While the limitations of this fixation method may contribute to the low number of peaks that are generally observed after ChIP-seq [14,16,17], it is also possible that formaldehyde mainly disrupts non-specific mitotic DNA interactions, which would explain the large loss of mitotic chromosome binding while maintaining a minority of specifically bound molecules.

Single molecule imaging has recently been used to determine the presence of specific DNA-binding events on mitotic chromatin. In contrast to ChIP-seq, it does not allow to directly demonstrate that the observed events occur on known DNA consensus motifs; however, the similar residence time on DNA that has been observed for SOX2 and OCT4 [6,14] on specific binding sites *in vitro* versus interphase or mitotic cells suggests that these long-lived interactions (in the second range) generally reflect specific DNA-binding interactions. Furthermore, single molecule imaging has the advantage of providing single live cell data and is not subject to fixation artifacts.

Potential mechanisms involved in binding to mitotic chromosomes

Specific versus non-specific DNA binding

TFs generally interact with DNA through both sequence-specific and non-sequence-specific interactions (Fig. 1). Single molecule [32] and fluorescence recovery after photobleaching (FRAP) experiments [38] suggest that a minority of TF molecules are specifically bound to DNA in interphase cells. Furthermore, the global retention of some transcription factors on mitotic chromosomes despite the small number of peaks observed by mitotic ChIP-seq makes it highly unlikely for specific DNA-binding events to lead to observable mitotic chromosome binding. A striking example is SOX2, for which we reported a limited number of sequence-specific binding events assessed by ChIP-Seq despite its strong enrichment on mitotic chromosomes [14], although as mentioned previously, technical issues are also likely to contribute to the reduced number of mitotic peaks since divergent results have been

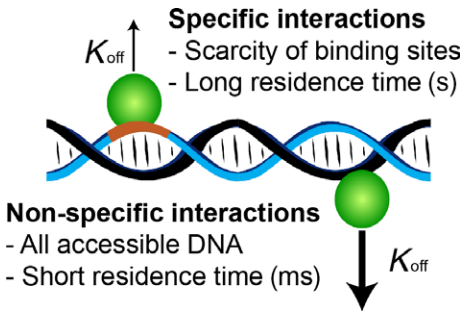

Fig. 1. Specific and non-specific binding to DNA. Binding of transcription factors on specific binding sites leads to stronger and longer interactions as compared with non-specific DNA, but the number of sites for specific interactions is much smaller than for non-specific interactions.

reported in the literature [14,15,23]. Cirillo *et al.* observed that FOXA1 could bind both specifically and non-specifically to nucleosomal DNA via distinct parts of the molecule [39] and a mutant of FOXA1 in which amino acids interacting with specific bases on DNA were substituted could still interact with mitotic chromosomes [16]. Consistently, an ESRRB protein in which three specific DNA-interacting amino acids were mutated was shown to remain bound to mitotic chromosomes, albeit to a lesser extent than wild-type ESRRB [23]. Therefore, while converging evidences point at non-sequence-specific DNA binding as largely responsible for the observed colocalization of some TFs with mitotic chromosomes, its relative contribution to mitotic chromosome binding and functional significance will need to be rigorously assessed in the future.

Mitotic chromosome binding and pioneer activity

Pioneer activity refers to the ability of certain TFs to bind to highly condensed, developmentally silenced regions and recruit histone modifiers and chromatin remodellers to open these regions [40]. Many TFs with pioneer activity are also retained on mitotic chromosomes, such as FOXA1, SOX2, and OCT4. Since mitotic chromatin is more condensed than interphase chromatin, it has been proposed that pioneer activity may also allow mitotic chromatin binding because of their high affinity for nucleosomal DNA. Interestingly, SOX2 has been shown to have a higher affinity for non-sequence-specific nucleosomal DNA than OCT4 [41], which is also less markedly sequestered on mitotic chromosomes [14]. However, to which extent the fraction of DNA wrapped around nucleosomes increases during mitosis in unclear. As mentioned above, mitotic DNA is far from being generally inaccessible, and mitotic chromatin is biochemically distinct from heterochromatin found in interphase [42] and targeted by pioneer TFs. Finally, there is at least one example of TF (KLF4) that has been reported to have pioneer activity [43] for which there is conflicting evidence about its retention on mitotic chromosomes [6,14,17]. Therefore, while there is an evidence and a theoretical basis arguing for a common basis to these two properties, broader characterization of pioneer and mitotic bookmarking properties will be required to understand how these are related.

Role of nuclear import sequences

While small TFs (>40kD) can in principle diffuse freely through the membrane with nuclear pores

during interphase, larger transcription factors require active nuclear import. In the classical import pathway, most nuclear localization signal (NLS)-bearing proteins are imported by importins, which follow a nucleus/cytoplasm gradient of Ran-GDP/Ran-GTP and release their cargo in the nucleus [44]. Ran-GTP is generated from Ran-GDP by its nuclear guanine nucleotide exchange factor, RCC1 [45], bound on chromatin through interactions of its DNA-binding domain and loop-forming domains with the histones H2A and H2B and nucleosomal DNA [46,47]. During mitosis, the nuclear membrane breaks down, leading to a fusion of nuclear and cytoplasmic compartments, allowing DNA-binding proteins devoid of an active NLS to access chromosomes. Interestingly, the gradient of Ran-GTP allowing for nuclear import in interphase is maintained during mitosis, since the retention of RCC1 on mitotic chromosomes sustains a high local concentration of Ran-GTP in the vicinity of chromatin [48] (Fig. 2). Therefore, proteins harboring an active NLS such as mitotic spindle elements are still directed toward mitotic chromosomes [48]. Since transcription factors generally carry NLS sequences, this machinery could contribute to mitotic chromosome localization of some of them. We have recently shown that the DNA-binding domain of SOX2, which alone is sufficient to bind mitotic chromatin, displays a less marked colocalization with mitotic chromosomes upon deletion of its NLS sequence [14]. Consistently, mutations of all positively charged amino acids within the two SOX2 NLS lead to its exclusion from mitotic chromosomes [6]. The decreased mitotic chromosome binding of an OCT4 mutant devoid of its PouH DNA-binding domain can be rescued by the addition of a nuclear localization signal [14]. Strikingly, despite their absence of intrinsic DNA-binding activity, Halo-Tag or mCherry can be partially targeted to mitotic chromosomes by tagging it with an NLS sequence [6]. Finally, importazole (an importin-β inhibitor) decreases the ability of a mutant of the transcription factor HNF1β to bind mitotic chromosomes upon cold shock [49], but does not affect the mitotic chromosome-binding activity of the wild-type protein. Importantly, beside the potential role for active processes in conferring NLS with the ability to bind mitotic chromosomes, their high density in positively charged amino acids could also play a role by facilitating the interaction with negatively charged DNA on mitotic chromatin (Fig. 2). More systematic experiments will be required to

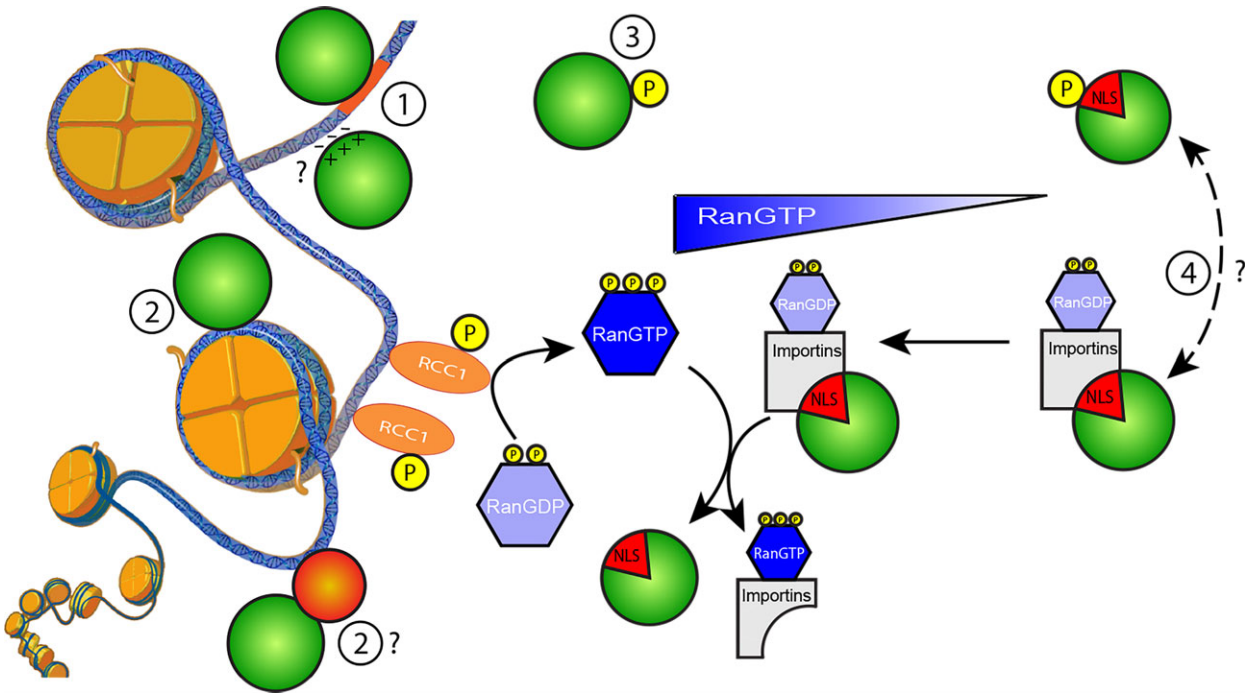

Fig. 2. Known and potential mechanisms that affect mitotic chromosome binding. 1) Direct interactions with DNA through specific binding or electrostatic interactions. 2) Interactions with histones and other proteins bound to mitotic chromosomes. Those might be influenced by histones modifications. 3) Post-translational modifications such as phosphorylation. 4) Deposition on mitotic chromosomes by the nuclear import machinery.

disentangle the contribution of active versus purely electrostatic mechanisms in the ability of NLS sequences to confer or enhance mitotic chromosome binding.

Role of post-translational modifications

M phase-specific phosphorylation affects a large number of nuclear proteins and can alter nuclear import signals by CDK1-dependent NLS phosphorylation [50]. Some transcription factors, such as the POU homeodomain transcription factor Oct1, have also been shown to be phosphorylated on their DNA-binding domain during mitosis, leading to a loss of DNA-binding capacity [51,52]. Consistently, mitotic-specific phosphorylation of CTCF reduces its DNA-binding affinity [53], and phosphorylation was proposed as a mechanism to inhibit DNA-binding activity of C2H2 zinc finger proteins [54,55]. Taken together, this suggests that cell-cycle-dependent phosphorylation could play a regulatory role as a mechanism to actively evict TFs from mitotic chromosomes (Fig. 2), and raises the possibility that at least in some cases, mitotic chromosome binding could be a 'default' state, resulting from the absence of negative regulation by mitotic phosphorylation.

Functional significance of mitotic chromosome binding by TFs

Role in transcriptional reactivation

As mentioned above, mitotic bookmarking by histone post-translational modifications and chromatin readers/writers has been shown to regulate the speed of transcriptional reactivation of bookmarked genes after mitotic exit. This led to the hypothesis that bookmarking of sequence-specific TFs on specific genes could also lead to rapid reactivation of expression programs upon mitotic exit [17,22]. GATA1 was initially reported as accelerating the transcriptional reactivation of some bookmarked genes [17], but a further study suggested that it might not be the case for the majority of mitotically bound GATA1 genes [56]. In the case of FOXA1, target genes were shown to be rapidly reactivated upon mitotic exit independently of their bookmarking status, arguing against a role for sequence-specific binding events during mitosis in transcriptional reactivation, but suggesting that local concentration of FOXA1 on mitotic chromosomes by non-sequence-specific DNA binding could allow rapid transcriptional reactivation of all FOXA1 target genes [16]. Finally, SOX2 target genes, independently of their bookmarked status, did also not undergo particularly rapid transcriptional reactivation [57], and live cell imaging of a SOX2/OCT4 luciferase reporter showed similar reactivation kinetics than a luciferase reporter [57] driven by STAT3, which is excluded from mitotic chromosomes [14]). Therefore, the role of mitotic chromosome binding in the kinetics of gene reactivation remains largely unclear.

Role in cell fate decisions

Many TFs reported for mitotic chromosome binding/bookmarking are involved in regulating cell fate decisions. This led to the hypothesis that mitotic chromatin binding could be essential for these factors to regulate the phenotypic state of daughter cells after mitotic exit, by regaining rapid control over gene expression in early G1. This is also in line with evidence that both M and G1 phase of the cell cycle are permissive windows for cell fate decisions [58,59]. Consistent with this hypothesis, several factors, including MLL [22], GATA1 [17], and RBPJ [26], were reported to be enriched on cell-type-specific genes. ESRRB was also shown to be enriched on pluripotency regulator genes during mitosis, and its dowregulation led to altered expression of many cell fate regulators in early G1. However, these studies did not address the functional role of these TFs at the Mitosis to G1 (M-G1) transition in cell fate regulation. Recently, it was reported that the presence of SOX2 and OCT4 at the M-G1 transition is required for robust pluripotency maintenance [14,15]. Furthermore, SOX2 overexpression throughout the cell cycle except at the M-G1 transition completely failed to enhance neuroectodermal commitment, suggesting an essential role of SOX2 in differentiation during this time window [14]. Importantly, the average expression level of control cell lines was carefully matched to levels of the mitotically degraded form of SOX2 that were thus slightly higher outside of this M-G1 transition. Therefore, these results suggest that the role of SOX2 at the M-G1 transition cannot be compensated during other cell-cycle phases. While it is tempting to speculate that the rapid regain of control of SOX2 on its target genes in early G1 is essential for cell fate regulation, only few genes were shown to be delayed in their transcriptional reactivation after mitotic exit in the absence of SOX2 at the M-G1 transition [57]. Therefore, the molecular mechanisms underlying cell fate control in this temporal window remain unclear, and the sequence of events initiated by SOX2 during mitotic exit remains to be clarified. Finally, the importance of M-G1 transition in the function of

other TFs and the correlation with their retention on mitotic chromatin need to be addressed.

Conclusions and perspectives

It is still assumed that most TFs are excluded from mitotic chromatin; however, only a small fraction of them has been investigated for this property. In the future, larger-scale assessment of TFs binding to mitotic chromosomes could not only allow to broadly expand the identification of mitotic chromosome binders but also shed light on whether particular TF properties could be predictive of mitotic chromosome binding. For example, while it is now well established that TF DNA-binding domains are essential for this property, there is not enough data to assess whether certain types of DNA-binding domains are more prone to mitotic chromosome binding than others. There is also relatively sparse evidence for the contribution of properties such as the ability to bind heterochromatin or nuclear localization sequences in regulating mitotic chromosome binding. Could simple physicochemical properties of TFs such as charge and size also play a role? Since DNA is negatively charged, and unspecific DNA interactions are largely responsible for the sequestration of some TFs on mitotic chromosomes, this raises the possibility that the number and spatial positioning of positive charges within the DNA-binding domain of TFs could contribute to their non-sequence-specific retention on DNA. In this regard, the enhancement of mitotic chromosome binding by NLS sequences [6,14] described in Section 2 could be explained by their positive charges. Finally, some TFs could also be partially or completely retained on mitotic chromosomes through indirect interactions with DNA. For example, we have recently shown that SOX2 expression can enhance the localization of OCT4 on mitotic chromatin, and this is dependent on both DNA-binding and OCT4-interacting domains of SOX2, suggesting a direct interaction on mitotic chromosomes [14]. Finally, Kadauke *et al.* [17] suggested that the presence of different cofactors and/or peculiarities of the mitotic environment could be responsible for shifting GATA1 from its interphase occupancy sites toward (GATA)n repeats in intergenic regions.

The functional significance of TF retention on mitotic chromatin and mitotic bookmarking remains poorly understood. While many of these TFs have also been identified as pioneer factors and cell fate regulators, the role of mitotic chromatin binding in their function remains unclear. We have recently demonstrated that the presence of SOX2 at the mitosis-G1 (M-G1) transition regulates pluripotency maintenance and neuroectodermal differentiation, suggesting that retention on mitotic chromosomes may be involved in controlling the reactivation of transcriptional programs in early G1 [9] (Fig. 3). In the future, the development of tools allowing to selectively abrogate mitotic chromosome binding without affecting interphase DNA interactions would allow to more specifically interrogate the contribution of mitotic chromosome retention to these findings. This should also allow for assessing whether mitotic binding simply serves to increase local concentration of TFs at the M-G1 transition or also plays a regulatory role on the local chromatin environment during M phase. While there is emerging evidences that the M-G1 transition may be critical for the function of SOX2 and OCT4 in cell fate decisions, the underlying molecular mechanisms remain unclear. While covalent modifications of histones [10] and their binding to chromatin readers [22] have been shown to allow for rapid transcriptional reactivation upon mitotic exit, this notion has remained less clear for mitotic bookmarking TFs. An unresolved question in the field is whether the dynamics of histone modifications during mitosis are comparable to the rapid exchange of TFs on specific DNA sites [32]. In principle, the covalent nature of histone modifications may allow for more permanent marking of specific genomic loci; however, this remains to be addressed quantitatively.

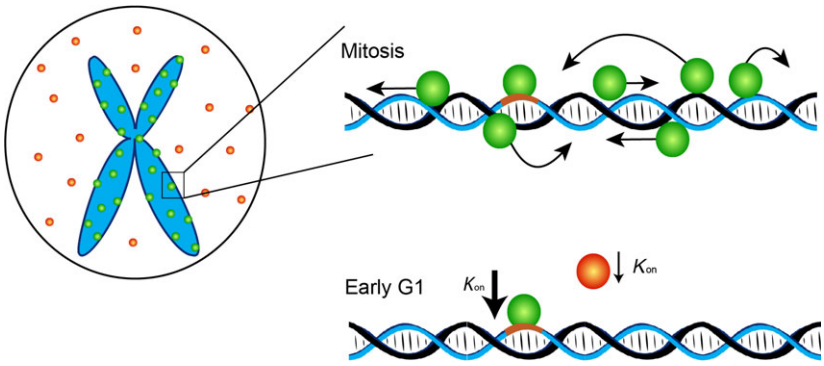

Fig. 3. Consequences on transcription factor binding in early G1. The local concentration of a transcription factor on chromosomes upon mitotic exit is influenced by its propensity to bind mitotic chromosomes. The search time for specific sites may be reduced for transcription factors retained on mitotic chromosomes, therefore increasing the on-rate (Kon) and enhancing specific binding sites occupancy in early G1.

It also remains unclear whether sequence-specific DNA-binding events occurring during mitosis are functionally relevant in shaping the local chromatin environment, or simply a consequence of the preferential accessibility of a subset of binding sites. The Zaret laboratory recently proposed a role of non-specific mitotic DNA binding in rapid gene reactivation [16,57], by virtue of a high local concentration leading to an increased on-rate in early G1 (Fig. 3). In accordance with this hypothesis, they reported that genes regulated by FoxA1 were reactivated faster after mitotic exit, independently of their bookmarking status. Importantly, the dominance over potentially competing TFs during mitotic exit conferred by an increased on-rate does not necessarily imply a faster transcriptional reactivation in early G1, but rather a dominance in taking over the regulatory control of these genes [14]. Furthermore, it was recently shown that the three-dimensional organization of the genome [60] and the promoter-enhancer contacts are completely disrupted during mitosis [56]. Since OCT4 and SOX2 have been shown to play an important role in genome organization by their involvement into long-range contacts [61–63], it is tempting to speculate that their presence at the M-G1 transition could be crucial for the prompt re-establishment of these interactions. In the future, it will be important to explore how mitotic chromatin binding alters the gene regulatory landscape in early G1 beyond the mere assessment of pre-mRNA synthesis or RNA Pol II loading timing, to understand at which steps in preparing genes for their re-expression (or the maintenance of their repression) do mitotic chromosome-binding TFs play a role.

In conclusion, our understanding of underlying mechanisms, molecular and phenotypic consequences of mitotic chromosome binding by TFs is still in its infancy. However, the broad diversity of behaviors among different TFs suggests that this property is highly regulated and may have important implications on the regulation of gene expression programs and cell fate in dividing cells. This is particularly relevant to the context of stem cells, which need to undergo numerous cell divisions while maintaining their pluripotent/multipotent state. Future studies aiming to clarify the molecular basis and consequences of mitotic chromosome binding will shed light on its impact in controlling cell fate decisions.

References

1 Flemming W (1878) Zur Kenntnis der Zelle und ihrer Teilung-Erscheinungen. *Schr Naturwiss Ver Schlesw-Holst* **3**, 23–27.

2 Vagnarelli P (2012) Mitotic chromosome condensation in vertebrates. *Exp Cell Res* **318**, 1435–1441.

3 Booth DG, Beckett AJ, Molina O, Samejima I, Masumoto H, Kouprina N, Larionov V, Prior IA and Earnshaw WC (2016) 3D-CLEM reveals that a major portion of mitotic chromosomes is not chromatin. *Mol Cell* **64**, 790–802.

4 Martin RM and Cardoso MC (2010) Chromatin condensation modulates access and binding of nuclear proteins. *FASEB J* **24**, 1066–1072.

5 Hsiung CC, Morrissey CS, Udugama M, Frank CL, Keller CA, Baek S, Giardine B, Crawford GE, Sung MH, Hardison RC *et al.* (2015) Genome accessibility is widely preserved and locally modulated during mitosis. *Genome Res* **25**, 213–225.

6 Teves SS, An L, Hansen AS, Xie L, Darzacq X and Tjian R (2016) A dynamic mode of mitotic bookmarking by transcription factors. *Elife* **5**, e22280.

7 Martinez-Balbas MA, Dey A, Rabindran SK, Ozato K and Wu C (1995) Displacement of sequence-specific transcription factors from mitotic chromatin. *Cell* **83**, 29–38.

8 Terrenoire E, McRonald F, Halsall JA, Page P, Illingworth RS, Taylor AM, Davison V, O'Neill LP and Turner BM (2010) Immunostaining of modified histones defines high-level features of the human metaphase epigenome. *Genome Biol* **11**, R110.

9 Muramoto T, Muller I, Thomas G, Melvin A and Chubb JR (2010) Methylation of H3K4 Is required for inheritance of active transcriptional states. *Curr Biol* **20**, 397–406.

10 Zhao R, Nakamura T, Fu Y, Lazar Z and Spector DL (2011) Gene bookmarking accelerates the kinetics of post-mitotic transcriptional re-activation. *Nat Cell Biol* **13**, 1295–1304.

11 Lodhi N, Kossenkov AV and Tulin AV (2014) Bookmarking promoters in mitotic chromatin: poly (ADP-ribose)polymerase-1 as an epigenetic mark. *Nucleic Acids Res* **42**, 7028–7038.

12 Michelotti EF, Sanford S and Levens D (1997) Marking of active genes on mitotic chromosomes. *Nature* **388**, 895–899.

13 Pallier C, Scaffidi P, Chopineau-Proust S, Agresti A, Nordmann P, Bianchi ME and Marechal V (2003) Association of chromatin proteins high mobility group box (HMGB) 1 and HMGB2 with mitotic chromosomes. *Mol Biol Cell* **14**, 3414–3426.

14 Deluz C, Friman ET, Strebinger D, Benke A, Raccaud M, Callegari A, Leleu M, Manley S and Suter DM (2016) A role for mitotic bookmarking of SOX2 in pluripotency and differentiation. *Genes Dev* **30**, 2538–2550.

15 Liu Y, Pelham-Webb B, Di Giammartino DC, Li J, Kim D, Kita K, Saiz N, Garg V, Doane A, Giannakakou P *et al.* (2017) Widespread mitotic

bookmarking by histone marks and transcription factors in pluripotent stem cells. *Cell Rep* **19**, 1283–1293.
16 Caravaca JM, Donahue G, Becker JS, He X, Vinson C and Zaret KS (2013) Bookmarking by specific and nonspecific binding of FoxA1 pioneer factor to mitotic chromosomes. *Genes Dev* **27**, 251–260.
17 Kadauke S, Udugama MI, Pawlicki JM, Achtman JC, Jain DP, Cheng Y, Hardison RC and Blobel GA (2012) Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1. *Cell* **150**, 725–737.
18 Young DW, Hassan MQ, Yang XQ, Galindo M, Javed A, Zaidi SK, Furcinitti P, Lapointe D, Montecino M, Lian JB *et al*. (2007) Mitotic retention of gene expression patterns by the cell fate-determining transcription factor Runx2. *Proc Natl Acad Sci USA* **104**, 3189–3194.
19 Christova R and Oelgeschlager T (2002) Association of human TFIID-promoter complexes with silenced mitotic chromatin in vivo. *Nat Cell Biol* **4**, 79–82.
20 Chen D, Dundr M, Wang C, Leung A, Lamond A, Misteli T and Huang S (2005) Condensed mitotic chromatin is accessible to transcription factors and chromatin structural proteins. *J Cell Biol* **168**, 41–54.
21 Zaret KS, Lerner J and Iwafuchi-Doi M (2016) Chromatin scanning by dynamic binding of pioneer factors. *Mol Cell* **62**, 665–667.
22 Blobel GA, Kadauke S, Wang E, Lau AW, Zuber J, Chou MM and Vakoc CR (2009) A reconfigured pattern of MLL occupancy within mitotic chromatin promotes rapid transcriptional reactivation following mitotic exit. *Mol Cell* **36**, 970–983.
23 Festuccia N, Dubois A, Vandormael-Pournin S, Gallego Tejeda E, Mouren A, Bessonnard S, Mueller F, Proux C, Cohen-Tannoudji M and Navarro P (2016) Mitotic binding of Esrrb marks key regulatory regions of the pluripotency network. *Nat Cell Biol* **18**, 1139–1148.
24 Newman RH, Fosbrink MD and Zhang J (2011) Genetically encodable fluorescent biosensors for tracking signaling dynamics in living cells. *Chem Rev* **111**, 3614–3666.
25 Zaidi SK, Young DW, Pockwinse SM, Javed A, Lian JB, Stein JL, van Wijnen AJ and Stein GS (2003) Mitotic partitioning and selective reorganization of tissue-specific transcription factors in progeny cells. *Proc Natl Acad Sci USA* **100**, 14852–14857.
26 Lake RJ, Tsai PF, Choi I, Won KJ and Fan HY (2014) RBPJ, the major transcriptional effector of Notch signaling, remains associated with chromatin throughout mitosis, suggesting a role in mitotic bookmarking. *PLoS Genet* **10**, e1004204.
27 Burke LJ, Zhang R, Bartkuhn M, Tiwari VK, Tavoosidana G, Kurukuti S, Weth C, Leers J, Galjart N, Ohlsson R *et al*. (2005) CTCF binding and higher order chromatin structure of the H19 locus are maintained in mitotic chromatin. *EMBO J* **24**, 3291–3300.
28 Kumar S, Chaturvedi NK, Kumar S and Tyagi RK (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.
29 Morrison C, Henzing AJ, Jensen ON, Osheroff N, Dodson H, Kandels-Lewis SE, Adams RR and Earnshaw WC (2002) Proteomic analysis of human metaphase chromosomes reveals topoisomerase II alpha as an Aurora B substrate. *Nucleic Acids Res* **30**, 5318–5327.
30 Gassmann R, Henzing AJ and Earnshaw WC (2005) Novel components of human mitotic chromosomes identified by proteomic analysis of the chromosome scaffold fraction. *Chromosoma* **113**, 385–397.
31 Ohta S, Bukowski-Wills JC, Sanchez-Pulido L, Alves Fde L, Wood L, Chen ZA, Platani M, Fischer L, Hudson DF, Ponting CP *et al*. (2010) The protein composition of mitotic chromosomes determined using multiclassifier combinatorial proteomics. *Cell* **142**, 810–821.
32 Gebhardt JC, Suter DM, Roy R, Zhao ZW, Chapman AR, Basu S, Maniatis T and Xie XS (2013) Single-molecule imaging of transcription factor binding to DNA in live mammalian cells. *Nat Methods* **10**, 421–426.
33 McNally JG, Muller WG, Walker D, Wolford R and Hager GL (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science* **287**, 1262–1265.
34 Rotem A, Ram O, Shoresh N, Sperling RA, Goren A, Weitz DA and Bernstein BE (2015) Single-cell ChIP-seq reveals cell subpopulations defined by chromatin state. *Nat Biotechnol* **33**, 1165–1172.
35 Loomis RJ, Naoe Y, Parker JB, Savic V, Bozovsky MR, Macfarlan T, Manley JL and Chakravarti D (2009) Chromatin binding of SRp20 and ASF/SF2 and dissociation from mitotic chromosomes is modulated by histone H3 serine 10 phosphorylation. *Mol Cell* **33**, 450–461.
36 Fox MH, Read RA and Bedford JS (1987) Comparison of synchronized Chinese hamster ovary cells obtained by mitotic shake-off, hydroxyurea, aphidicolin, or methotrexate. *Cytometry* **8**, 315–320.
37 Campbell AE, Hsiung CC and Blobel GA (2014) Comparative analysis of mitosis-specific antibodies for bulk purification of mitotic populations by fluorescence-activated cell sorting. *Biotechniques* **56**, 90–91. 93-4.
38 Voss TC and Hager GL (2008) Visualizing chromatin dynamics in intact cells. *Biochim Biophys Acta* **1783**, 2044–2051.

39 Cirillo LA, Lin FR, Cuesta I, Friedman D, Jarnik M and Zaret KS (2002) Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol Cell* **9**, 279–289.
40 Zaret KS and Carroll JS (2011) Pioneer transcription factors: establishing competence for gene expression. *Genes Dev* **25**, 2227–2241.
41 Soufi A, Garcia MF, Jaroszewicz A, Osman N, Pellegrini M and Zaret KS (2015) Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming. *Cell* **161**, 555–568.
42 Probst AV, Dunleavy E and Almouzni G (2009) Epigenetic inheritance during the cell cycle. *Nat Rev Mol Cell Biol* **10**, 192–206.
43 Soufi A, Donahue G and Zaret KS (2012) Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. *Cell* **151**, 994–1004.
44 Stewart M (2007) Molecular mechanism of the nuclear protein import cycle. *Nat Rev Mol Cell Biol* **8**, 195–208.
45 Cole CN and Hammell CM (1998) Nucleocytoplasmic transport: driving and directing transport. *Curr Biol* **8**, R368–R372.
46 Nemergut ME and Macara IG (2000) Nuclear import of the ran exchange factor, RCC1, is mediated by at least two distinct mechanisms. *J Cell Biol* **149**, 835–850.
47 Makde RD, England JR, Yennawar HP and Tan S (2010) Structure of RCC1 chromatin factor bound to the nucleosome core particle. *Nature* **467**, 562–566.
48 Li HY and Zheng Y (2004) Phosphorylation of RCC1 in mitosis is essential for producing a high RanGTP concentration on chromosomes and for spindle assembly in mammalian cells. *Genes Dev* **18**, 512–527.
49 Lerner J, Bagattin A, Verdeguer F, Makinistoglu MP, Garbay S, Felix T, Heidet L and Pontoglio M (2016) Human mutations affect the epigenetic/bookmarking function of HNF1B. *Nucleic Acids Res* **44**, 8097–8111.
50 Rona G, Borsos M, Ellis JJ, Mehdi AM, Christie M, Kornyei Z, Neubrandt M, Toth J, Bozoky Z, Buday L *et al.* (2014) Dynamics of re-constitution of the human nuclear proteome after cell division is regulated by NLS-adjacent phosphorylation. *Cell Cycle* **13**, 3551–3564.
51 Roberts SB, Segil N and Heintz N (1991) Differential phosphorylation of the transcription factor Oct1 during the cell cycle. *Science* **253**, 1022–1026.
52 Segil N, Roberts SB and Heintz N (1991) Mitotic phosphorylation of the Oct-1 homeodomain and regulation of Oct-1 DNA binding activity. *Science* **254**, 1814–1816.
53 Sekiya T, Murano K, Kato K, Kawaguchi A and Nagata K (2017) Mitotic phosphorylation of CCCTC-binding factor (CTCF) reduces its DNA binding activity. *FEBS Open Bio* **7**, 397–404.
54 Dovat S, Ronni T, Russell D, Ferrini R, Cobb BS and Smale ST (2002) A common mechanism for mitotic inactivation of C2H2 zinc finger DNA-binding domains. *Genes Dev* **16**, 2985–2990.
55 Rizkallah R, Alexander KE and Hurt MM (2011) Global mitotic phosphorylation of C2H2 zinc finger protein linker peptides. *Cell Cycle* **10**, 3327–3336.
56 Hsiung CC, Bartman CR, Huang P, Ginart P, Stonestrom AJ, Keller CA, Face C, Jahn KS, Evans P, Sankaranarayanan L *et al.* (2016) A hyperactive transcriptional state marks genome reactivation at the mitosis-G1 transition. *Genes Dev* **30**, 1423–1439.
57 Deluz C, Strebinger D, Friman ET and Suter DM (2017) The elusive role of mitotic bookmarking in transcriptional regulation: insights from Sox2. *Cell Cycle* **16**, 601–606.
58 Halley-Stott RP, Jullien J, Pasque V and Gurdon J (2014) Mitosis gives a brief window of opportunity for a change in gene transcription. *PLoS Biol* **12**, e1001914.
59 Pauklin S and Vallier L (2013) The cell-cycle state of stem cells determines cell fate propensity. *Cell* **155**, 135–147.
60 Naumova N, Imakaev M, Fudenberg G, Zhan Y, Lajoie BR, Mirny LA and Dekker J (2013) Organization of the mitotic chromosome. *Science* **342**, 948–953.
61 Schoenfelder S, Furlan-Magaril M, Mifsud B, Tavares-Cadete F, Sugar R, Javierre BM, Nagano T, Katsman Y, Sakthidevi M, Wingett SW *et al.* (2015) The pluripotent regulatory circuitry connecting promoters to their long-range interacting elements. *Genome Res* **25**, 582–597.
62 Denholtz M, Bonora G, Chronis C, Splinter E, de Laat W, Ernst J, Pellegrini M and Plath K (2013) Long-range chromatin contacts in embryonic stem cells reveal a role for pluripotency factors and polycomb proteins in genome organization. *Cell Stem Cell* **13**, 602–616.
63 de Wit E, Bouwman BA, Zhu Y, Klous P, Splinter E, Verstegen MJ, Krijger PH, Festuccia N, Nora EP, Welling M *et al.* (2013) The pluripotent genome in three dimensions is shaped around pluripotency factors. *Nature* **501**, 227–231.