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Gene bookmarking accelerates the kinetics of post-mitotic transcriptional re-activation

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Abstract

Although transmission of the gene expression program from mother to daughter cells has been suggested to be mediated by gene bookmarking, the precise mechanism by which bookmarking mediates post-mitotic transcriptional re-activation has been unclear. Here, we used a real-time gene expression system to quantitatively demonstrate that transcriptional activation of the same genetic locus occurs with a significantly more rapid kinetics in post-mitotic cells versus interphase cells. RNA polymerase II large subunit (Pol II) and Bromodomain Protein 4 (BRD4) were recruited to the locus in a different sequential order upon interphase initiation versus post-mitotic re-activation resulting from the recognition by BRD4 of increased levels of histone H4 lysine 5 acetylation (H4K5Ac) on the previously activated locus. BRD4 accelerated the dynamics of mRNA synthesis by de-compacting chromatin and hence facilitating transcriptional reactivation. Together, using a real-time quantitative approach, we identified differences in the kinetics of transcriptional activation between interphase and post-mitotic cells that are mediated by a chromatin-based epigenetic mechanism.

Transcriptional induction is a dynamic process that involves the recruitment of a transcriptional activator, members of the chromatin remodeling and transcription machineries as well as proteins functioning in RNA processing, packaging, and export[1-3]. Most studies examining gene expression have utilized *in vitro* systems and/or analyzed fixed cells. However, over the past few years a number of groups have developed live cell

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Author Contributions R.Z., T.N., and D.L.S. designed the research; R.Z. and T.N. performed the experiments and analyzed data; Y.F. wrote the MatLab software for quantitative analysis and also analyzed data; Z.L. performed the super-resolution structured illumination microscopy and locus size analysis. R.Z. and D.L.S. wrote the paper.

Methods

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturecellbiology

imaging approaches to study various aspects of gene expression at high spatial/temporal resolution[4-9]. A thorough analysis of the dynamics of the gene expression machinery and its interactions with chromatin within the context of the cell nucleus is essential to advance our understanding of the regulation of gene expression.

Transcription is silenced during cell division; however, it must be re-activated upon exit from mitosis to maintain cell lineage[10-13] and cell cycle progression. Transmission of the expression status of genes from mother to daughter cells has been suggested to be mediated by gene bookmarking, which may involve transcription factors, histone modifications/variants, and/or DNA methylation[10-13]. However, the mechanism by which gene bookmarking mediates post-mitotic transcriptional re-activation has not been fully elucidated. While several recent studies have examined transcriptional activation in interphase cells[8,9,14-16], the kinetics of transcriptional activation of the same genetic locus in interphase versus post-mitotic cells has thus far not been investigated. In this study, we quantitatively identified differences in the kinetics of gene expression of the same genetic locus in interphase versus post-mitotic cells. Our findings revealed H4K5Ac as a chromatin-based epigenetic bookmark mediating the transmission of the active chromatin state. In addition, we identified the BRD4 protein, which recognizes this bookmark, to function in chromatin decompaction thus facilitating post-mitotic transcriptional reactivation.

Results

Differences in the kinetics of transcriptional activation of the same genetic locus in interphase and post-mitotic cells

In order to study the kinetics of transcriptional induction we developed a series of cell lines stably expressing different sets of proteins based upon the previously described doxycycline (Dox) inducible U2OS-2-6-3 cells[8,17] (Fig. 1a). These cell lines correctly recapitulate the dynamics of Pol II and nascent transcripts (MS2) during the cell cycle, coordinated with nuclear envelope integrity as monitored by LaminB1 labeling[18,19] (Supplementary Fig. S1).

U2OS-2-6-3 cells, stably expressing pTet-On, mCherry-Pol II and MS2-YFP, allowed real time imaging of the dynamics of Pol II recruitment and mRNA synthesis indicated by the MS2 signal (Fig. 1a). Upon Dox (1μg/ml) induction, Pol II was gradually recruited to the locus resulting in an increase of the MS2 signal, indicative of interphase transcriptional activation (Fig. 1b). Initial Pol II recruitment preceded the accumulation of MS2 by approximately 3 minutes consistent with previous findings[14] (Supplementary Movie S1). We established a quantitative approach to align the Pol II and MS2 signals to allow comparison of the kinetics of their recruitment during interphase transcriptional activation (Supplementary Fig. S2). Quantification of cells induced with Dox during interphase showed that Pol II and MS2 reached a plateau level in 208 min and 158 min, respectively (Fig. 1d, 1e, blue traces and bars), indicating a steady but slow increase in the activation of the genetic locus associated with interphase induction. During mitosis, both Pol II and MS2-YFP signals were cleared from the locus (Fig. 1c). However, upon exit from mitosis, Pol II rapidly re-accumulated at the locus and mRNA synthesis was first detected approximately 2-6 mins later (Fig. 1c; Supplementary Fig. S1, Supplementary Movie 2). Interestingly, in contrast to the relatively slow kinetics associated with interphase transcriptional activation,

both Pol II and MS2 reached a plateau level much more rapidly (16 min and 34 min, respectively) during post-mitotic transcriptional re-activation (Fig. 1d, e; red traces and bars). By comparing the rising time (see Supplementary Methods) during post-mitotic transcriptional re-activation versus interphase activation, we found that the kinetics of Pol II and MS2 recruitment was more than 13 times and 5 times faster, respectively. The rapid re-activation was not due to a primed activation as the Tet-on activator was also released during mitosis, nor the accessibility of Dox since higher concentration of Dox (10μg/ml) did not accelerate interphase initiation (Supplementary Fig. S3a, b). It is interesting to note that MS2 had a shorter signal rising time (Fig. 1e) than Pol II (Fig. 1d), which is likely due to differences in the dynamic nature of Pol II and MS2 recruited to the locus. While Pol II can reside at the locus in different states (i.e. engaged, paused, or escaped)[16], and gradually accumulates, the level of MS2 is strictly limited by the amount of actively transcribed mRNA, which leaves the locus upon being fully transcribed[20]. It has been calculated that only ~1% of the recruited Pol II is actively functioning at the transcription site during the steady-state transcriptional phase[16]. The late Pol II plateau that we observed may be the consequence of this very active transcription site creating a transcriptional microenvironment that acts as a seed to recruit excess free polymerase which rapidly exchanges on/off the locus.

Earlier studies examined post-mitotic transcription by evaluating global transcription from cell populations at limited time points[21-25]. The present study carried out with significantly higher temporal resolution in individual living cells, demonstrated a significantly increased kinetics of Pol II recruitment and mRNA synthesis in post-mitotic cells (Fig. 1). The differential kinetics observed here suggests that different mechanistic variations may be involved in these two themes of transcriptional activation.

Interphase transcriptional activation results in a transcriptional memory that is transmitted through mitosis

The faster kinetics of post-mitotic transcriptional re-activation could be mediated by one of two possible mechanisms. First, global chromatin de-condensation upon exit from mitosis could facilitate the transcription process for any gene activated during that time window. Second, a gene bookmark left by a previous transcriptional activation could account for the rapid re-activation of the same gene. To distinguish between these two possibilities, we performed an ‘induction during mitosis’ experiment. Cells containing an inactive locus were synchronized at metaphase by Nocodazole treatment (50μg/ml, 6hrs) and upon Nocodazole wash-out Dox was added to induce transcription (Fig. 2a). The kinetics of transcriptional activation paralleled that of interphase induction, rather than post-mitotic re-activation (Fig. 2b, c), indicating that the signal for rapid post-mitotic re-activation must be generated during the prior interphase. To exclude the possibility that Nocodazole treatment slowed down transcriptional kinetics, we induced transcription in interphase cells with Dox overnight and then synchronized the cells with Nocodazole. After releasing the cells from Nocodazole, we still detected the characteristic rapid recruitment of Pol II and MS2 in post-mitotic cells (Supplementary Fig. S3c). These results suggested that a gene bookmark left by prior interphase transcriptional activation was crucial for the rapid kinetics of post-mitotic

transcriptional re-activation, and that global changes in chromatin decondensation in post mitotic cells was not sufficient for accelerating transcriptional activation.

Next, we examined whether a gene that was previously activated in interphase would show increased kinetics upon a second interphase activation without passing through mitosis. Dox was added to interphase cells to induce transcription for 24hrs, and then Dox was washed out to abolish transcription at the locus. Dox was then re-added to induce a second interphase transcriptional activation (Fig. 2d). During the inactivation process of washing out Dox (~3h), both the Pol II and MS2 signals dissociated from the locus, and the locus re-condensed (Supplementary Fig. S3d). During the second interphase transcriptional activation, the kinetics of both Pol II and MS2 recruitment were not increased, but instead were comparable to the first interphase transcriptional activation (Fig. 2e, f). These results indicated that transcriptional memory can be preserved during mitotic transcriptional silencing, but not during interphase transcriptional inactivation. Together, we demonstrated that interphase transcriptional activation can induce transcriptional memory, which can be preserved during mitosis and is crucial for rapid post-mitotic transcriptional re-activation.

Interphase transcriptional activation results in a bookmark that is transmitted through mitosis

To decipher the mechanism of increased transcriptional kinetics of post-mitotic re-activation, we were interested in identifying the specific “bookmark” responsible for rapid gene activation in this system. Histone modifications have been recognized as one type of gene bookmark that can mediate transcriptional re-activation in daughter cells[11,12,22,24,26]. We performed chromatin immunoprecipitation (ChIP) utilizing antibodies against several histone modifications, including H3K4me3, H3K36me3, H4K5Ac, H4K8Ac, H4K12Ac, H4K16Ac, H3K9Ac and H3K14Ac[22,24,26-31], and different primer sets spanning the promoter region and downstream regions of the genetic locus (Fig. 3a). Following interphase Dox induction, the most dramatic increase was that of H4K5Ac at the promoter region of the genetic locus, whereas the other histone modifications did not show as significant association or change at this locus (Fig. 3b), consistent with a recent study[14]. If a histone modification serves as a gene bookmark for post-mitotic transcriptional re-activation, it should be preserved on the chromatin during mitosis. Indeed, when cells were synchronized at metaphase by Nocodazole, the increased H4K5Ac was well preserved at the promoter region, although transcription was fully inactivated during metaphase (Fig. 3c). These results suggest that gene bookmarking at this locus may be part of an ‘epigenetic memory’ carried out by the H4K5Ac mark. Interestingly, previous studies have indicated histone acetylation to be present through mitosis[25,31]. Importantly, a known mark for inactive loci, H3K9Me3 was found to be significantly decreased upon interphase Dox induction while the total H3 level was not changed (Supplementary Fig. S4b). To further confirm the association of acetylated H4 (H4Ac) on the previously activated genetic locus during mitosis, we performed immunofluorescence (IF) on metaphase cells using an antibody recognizing H4Ac (Fig. 3d, Supplementary Fig. S4c). Together, these results show that interphase transcriptional activation induces H4 acetylation, which is preserved during mitosis and may serve as an epigenetic gene bookmark recognized by the transcriptional

machinery in post-mitotic cells, thereby mediating rapid post-mitotic transcriptional re-activation.

BRD4 modulates post-mitotic transcriptional re-activation

In order to achieve the rapid kinetics associated with post-mitotic transcriptional re-activation, a gene bookmark must be recognized by the transcriptional machinery. It has been reported that BRD4, which binds acetylated histones[27,32], is involved in regulating post-mitotic gene expression[21,25]. Previous studies have indicated a role for BRD4 in the recruitment of the positive transcription elongation factor b (p-TEFb) complex[21,25,33], and BRD2 and BRD4 have been shown to be recruited to an interphase Dox induced locus[14]. To determine the precise role of BRD4 in post-mitotic transcriptional re-activation, we examined the recruitment of BRD4 paired with other members of the transcription machinery such as Pol II and CDK9, a member of the p-TEFb complex, by transiently expressing different pairs of these proteins (BRD4 and Pol II, CDK9 and Pol II, or BRD4 and CDK9). By IF labeling, we found the association of BRD4 on the induced locus during mitosis in ~50% of cells (Supplementary Fig. S5a). We found that Pol II recruitment preceded both BRD4 and CDK9 recruitment by approximately 3-6 min upon interphase induction (Fig. 4a, left panel; Supplementary Fig. S5b), while BRD4 and CDK9 showed no detectable time difference in recruitment under our imaging conditions (Supplementary Fig. S5d). However, during post-mitotic transcriptional re-activation, BRD4 was recruited to the locus prior to both Pol II and CDK9 by approximately 2-4 min (Fig. 4a, right panel; Quantification shown in Figure 4b; Supplementary Fig. S5c, S5e). The concomitant recruitment of BRD4 and CDK9 during interphase transcriptional activation likely results from BRD4 binding and recruiting CDK9 to form a functional transcription elongation complex[21,33,34]. However, recruitment of BRD4 to the post-mitotic locus prior to Pol II and the clear time lag between BRD4 and CDK9 recruitment suggests that BRD4 may recognize the H4K5Ac mark at the locus, and thus may be involved in additional functions during post-mitotic transcriptional re-activation.

Several studies have suggested that BRD4 is important for post-mitotic transcriptional re-activation, as decreased levels of mRNA transcripts or protein product were observed after knock-down of BRD4[21,25]. Thus, we were interested in determining if BRD4 knock-down would slow down the kinetics of post-mitotic transcriptional re-activation. We used BLOCK-iT™ Fluorescent Oligo (Invitrogen) to label BRD4 knock-down cells (Supplementary Fig. S6a, S6b). We then imaged mitotic cells treated with either siBRD4 or control siRNA. Cells treated with a control siRNA exhibited a decreased kinetics of mRNA synthesis during post mitotic re-activation likely due to stress induced via liposome mediated transfection as previously reported[35,36] and which can inhibit Pol II elongation[37]. We found that when BRD4 was knocked-down, cells proceeded to go through mitosis and, ultimately, significant mRNA production was observed in daughter cells (Fig. 4c). However, quantification showed that the kinetics of mRNA production during post-mitotic transcriptional re-activation was significantly reduced in siBRD4 cells, as compared to control siRNA cells (Fig. 4d).

A recent discovery identified a selective inhibitor of bromodomain and extra-terminal (BET) family members, named JQ1, which exhibited selective binding to the bromodomains of BET family members, with highest affinity toward BRD3 and BRD4[38]. JQ1 treatment significantly decreased the stability of BRD4 association at the active transcription site as tested by fluorescence recovery after photobleaching (FRAP) experiments (Supplementary Fig. S7). More interestingly, JQ1 treatment significantly slowed down the kinetics of post-mitotic mRNA synthesis (Fig. 4f), suggesting the stable binding between the bromodomain and acetylated histones is important for H4K5Ac-BRD4 mediated efficient post-mitotic transcriptional re-activation. Either siBRD4 or JQ1 treatment did not significantly slow down interphase initiation, suggesting a more prominent role of BRD4 in mediating efficient post-mitotic transcriptional re-activation (Supplementary Fig. S6c-e).

A role for BRD4 in chromatin decompaction

Previous studies reported BRD4 binding to mitotic chromosomes throughout mitosis in mouse cell lines but not in HeLa cells [21,25,39]. In human U2OS cells, when the locus was activated by Dox in interphase, BRD4 could be detected on the locus in ~50% of mitotic cells by IF, although there was no general association of BRD4 with mitotic chromosomes (Supplementary Fig. S5a). These results suggest that, while the majority of BRD4 seems to be excluded from the chromosomes during mitosis, the acetylated histones at some bookmarked promoters could retain a population of BRD4. Therefore, although it is difficult to detect BRD4 on mitotic chromosomes by IF, the presence of 200 copies of our locus likely amplified the fluorescent signal of BRD4 enough to allow us to detect the association of BRD4 at the mitotic locus. Interestingly, BRD4-associated mitotic loci are larger than those loci that were not previously activated by Dox induction (Fig. 4g, h). JQ1 treatment abolished the BRD4 association on the mitotic loci that were previously induced by Dox addition, and resulted in the size of the mitotic loci being comparable to uninduced loci (Fig. 4g, h). These findings point to a role for BRD4 and acetylated histones in chromatin decompaction. Interestingly, it has been reported that the architecture of the promoter region of formerly activated genes is less compact during mitosis[11,12,40,41], which might be due to the association of BRD4 on these promoters.

To further investigate whether BRD4 could influence chromatin compaction and if BRD4 is responsible for the accelerated transcriptional re-activation, we tethered BRD4 to the locus via the LacI fusion protein. Surprisingly, even in the absence of Dox induction, tethered BRD4 resulted in significant de-compaction of the locus with no detectable Pol II recruitment and mRNA synthesis (Fig. 5a). This de-compaction is not associated with changes in histone modification, i.e. increased acetylation or reduced H3K9me3, on the tethered locus (Fig. 5b). Upon inducing BRD4-de-compacted loci with Dox during interphase, we observed significantly accelerated kinetics of both Pol II recruitment and MS2 accumulation (Fig. 5c-d; the interphase induction plots from Fig. 1 (blue lines and boxes) are co-plotted for ease of comparison). To further understand the de-compaction function of BRD4, we examined 15 different deletion mutants of BRD4 that contain different protein domains (Fig. 6a). Surprisingly, among the two bromodomains (BD1 and BD2) of BRD4, only BD1 is critical for the locus de-compaction function, and tethering BD1 together with the short N-terminal domain (NBD1), rather than BD1 alone, was

sufficient to de-compact the locus (Fig. 6b), suggesting the N-terminal domain is required for proper folding of the BD1. Tethering this minimum domain (NBD1) indeed accelerated transcriptional initiation upon interphase induction, while tethering the mutant only lacking BD1 had no effect (Fig. 6c and compare with Fig. 5d). Upon JQ1 treatment, LacI-NBD1 was still able to decompact the locus suggesting that the decompaction function of NBD1 is independent of its binding to acetylated histones (Fig. 6bv, +JQ1 compared with −JQ1). These results suggest that the early recruitment of BRD4 and its chromatin de-compaction effect may be functionally responsible for mediating rapid post-mitotic transcriptional re-activation. This newly discovered role of BRD4 is not totally unexpected as chromatin acetylation is related to open chromatin architecture[6,42,43] and chromatin compaction has been found to directly affect transcription *in vivo*[44]. Together, these results demonstrate that BRD4 is a key mediator of the rapid kinetics of post-mitotic transcriptional re-activation, likely through stable binding with H4K5Ac and subsequent chromatin decompaction, thereby facilitating the recruitment of other members of the transcriptional initiation and elongation machineries.

Discussion

Taken together, our results suggest the following model (Fig. 6d). Upon transcriptional induction in interphase, the gene expression machinery is recruited to the locus. Transcriptional activation results in an increased level of H4K5Ac at the promoter region of the locus. Upon entry into mitosis, transcription is shut-down, however, the enhanced level of H4K5Ac is maintained and transmitted to daughter cells, serving as a gene bookmark for post-mitotic transcriptional re-activation. A residual amount of BRD4 is also associated with the locus through strong binding with H4K5Ac and maintains a relatively less compacted locus. Upon exit from mitosis, additional BRD4 is rapidly recruited to the daughter loci, presumably through recognition of the H4K5Ac marks at the promoter region, and this recruitment leads to rapid chromatin decompaction at the locus, which further facilitates the recruitment of other members of the transcription machinery, i.e. Pol II and CDK9. BRD4 and members of the transcription machinery then work cooperatively to achieve the rapid post-mitotic transcriptional re-activation of the daughter loci in early G1 cells.

Timely activation of bookmarked genes is essential for cell cycle progression and embryonic development. Previous studies showed that knock-down of BRD4 in NIH3T3 and mouse embryonic fibroblasts (MEFs) results in G1 arrest or a delay of G1 gene expression[21,45]. In addition, the promoter regions of M/G1 genes have been shown to be associated with both acetylated histones and BRD4 during mitosis[25]. Our observations of BRD4 recruitment to daughter nuclei and the loci prior to Pol II recruitment in post-mitotic cells suggest a more global role for BRD4 in post-mitotic gene activation. It remains to be determined what regulates the selection of BRD4 associated genes. Interestingly, not all bookmarks are directly correlated with previous interphase transcriptional status. A recent study[46] showed a vast reorganization of mixed lineage leukemia (MLL) protein occupancy during the cell-cycle. For example, some genes were occupied in both interphase and mitosis while other acquired the bookmark only during mitosis, yet the overall function of these MLL bookmarks was to modulate post-mitotic gene activation[46].

Our results also raise some very interesting questions and suggest new directions for future studies. For example, exactly how does BD1 decompact chromatin, why does BD2 not contribute to chromatin decompaction, and what is the structural benefit of the small N-terminal domain to BD1 in chromatin decompaction? Our results also suggest that it will be beneficial to systematically re-examine the function of other bromodomain containing proteins, in particular other BET family members. Indeed, BRD2 has also been shown to be associated with mitotic chromosomes by selectively binding to acetylated H4K5/K12[47-49]. Among all the bromodomains, the BD2 of BRD2 is most highly conserved with BD1 of BRD4[38].

In summary, we established a system to investigate gene bookmarking during post-mitotic transcriptional re-activation and its underlying mechanism with integrative approaches. Using a real-time imaging approach, we quantitatively revealed the significantly more rapid kinetics of post-mitotic transcriptional re-activation than interphase transcriptional activation at the same genetic locus in individual living cells. This study demonstrated that post-mitotic transcriptional re-activation is mediated by a distinct mechanism mediated by gene bookmarking. Furthermore, tethering experiments revealed a previously unrecognized function of BRD4 in chromatin de-compaction, which may be key to the mechanism mediating the rapid kinetics of transcriptional re-activation in post-mitotic cells. Together, our findings significantly advance the understanding of gene bookmarking and the mechanism of epigenetic memory in mediating transcriptional re-activation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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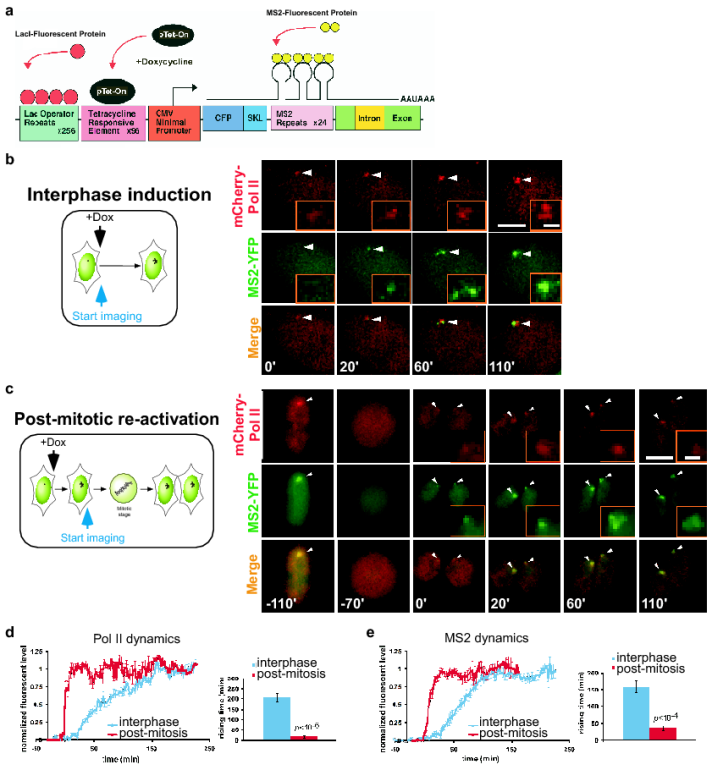

Figure 1.

Transcriptional activation of the same locus exhibits faster kinetics during post-mitotic activation than in the previous interphase. **(a)** Schematic diagram of the gene expression system (Modified from Janicki et al. 2004[8]). Binding of LacI-Fluorescent Protein to the lac operator repeats results in visualization of the gene locus. pTet-On expression in the presence of Dox induces gene expression driven by the minimal CMV promoter. MS2-Fluorescent Protein binds to the MS2 stem loop repeats, rendering visualization of the transcribed mRNA. 200 copies of the gene expression cassette are stably integrated as a transgene array at human 1p36 in U2OS 2-6-3 cells. The diagram is not drawn to scale. **(b)** Interphase induction: Upon Dox induction, mCherry-Pol II and MS2-YFP were recruited to the locus (arrowhead). Image stacks were acquired every 3mins. 0′ indicates the first time point where Pol II is detectable at the locus. Scale bars, 10μm, 2μm. **(c)** Post-mitotic re-activation: cells were induced with Dox overnight (O.N.) before imaging. Cells with an active locus were followed. Both Pol II and MS2-YFP were dissociated from the locus upon entry into mitosis. Upon exit from mitosis, both fusion proteins were recruited back to the daughter loci shortly after formation of daughter nuclei (arrowheads). Images were taken every 2mins. Scale bars, 10μm, 2μm. **(d)** Pol II recruitment was 13 times more rapid during post-mitotic re-activation, as shown by quantitative analysis of Pol II recruited to the locus during interphase induction and post-mitotic re-activation. The Pol II signal at the locus was quantified across the imaging session for each cell and the average curve was generated as described in Methods. The rising time of the Pol II signal for interphase induction and post-mitotic re-activation was plotted as mean±S.E.M (208.1±22.9min versus 16.5±6.0min) (n=17 for interphase induction, n=19 for post-mitotic re-activation). **(e)** mRNA production was 5 times more rapid during post-mitotic re-activation, as shown by quantitative analysis

of mRNA production at the locus during interphase induction and post-mitotic re-activation. MS2-YFP signal was quantified across the imaging session for each cell and the average curve was generated as described in Methods. The rising time of the MS2-YFP signal for interphase induction and post-mitotic re-activation was plotted as mean±S.E.M (157.6±19.0min versus 34.3±7.6min) (n=13 for interphase induction, n=25 for post-mitotic re-activation).

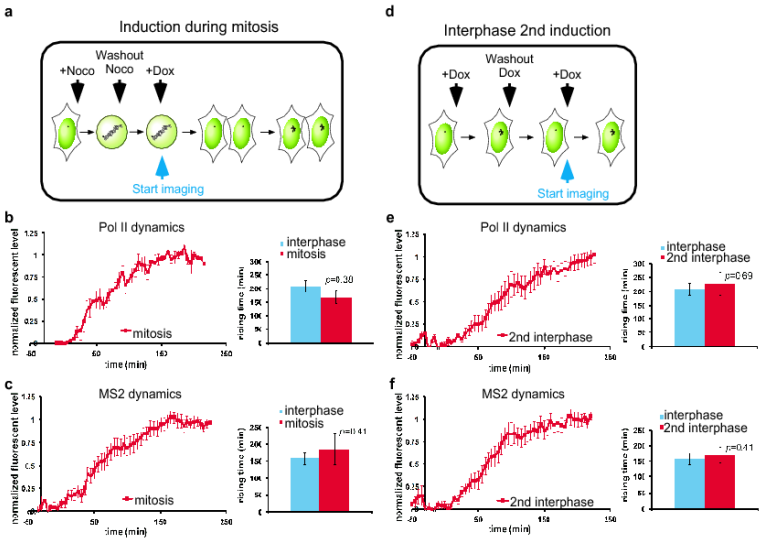

Figure 2.

Global chromatin decondensation or a second interphase induction is not sufficient to provide a more rapid transcriptional induction. **(a)** Schematic diagram of induction during mitosis. Cells in which the locus was inactive were synchronized with Nocodazole (50μg/ml) and, after wash-out, Dox was added and cells were allowed to progress through the cell cycle. Such cells will be referred to as mitosis induced cells. (**b**) Rapid chromatin decondensation upon exit from mitosis, in and of itself, is not sufficient for rapid transcriptional induction. The average curve of Pol II was generated as previously described. The rising time of Pol II signal for interphase induction (taken from Figure 1d, for ease of comparison) and post-mitotic induction was plotted as mean±S.E.M (208.1±22.9min versus 168±21.8min) (n=17 for interphase induction, n=7 for post-mitotic induction). (**c**) The average curve of MS2-YFP was generated as previously described. The rising time of MS2-YFP signal for interphase induction (taken from Figure 1e, for ease of comparison) and post-mitotic induction was plotted as mean±S.E.M (157.6±19.0min versus 185.2±45.5min) (n=13 for interphase induction, n=10 for post-mitotic induction). **(d)** Diagram of the experimental protocol for studying cells that have been transcriptionally induced in interphase twice. **(e)** Silencing of an active locus during interphase followed by a second transcriptional induction does not result in increased kinetics of transcriptional induction. The average curve of Pol II was generated as previously described. The rising time of Pol II signal for initial (taken from Figure 1d, for ease of comparison) and second interphase induction was plotted as mean ±S.E.M (208.1±22.9min versus 227±41min) (n=17 for interphase induction, n=9 for interphase 2nd induction). **(f)** The average curve of MS2-YFP was generated as previously described. The rising time of MS2-YFP signal for initial (taken from Figure 1e, for ease of comparison) and second interphase induction was plotted as mean±S.E.M (157.6±19.0min versus 169.2±49.6min) (n=13 for interphase induction, n=9 for interphase 2nd induction).

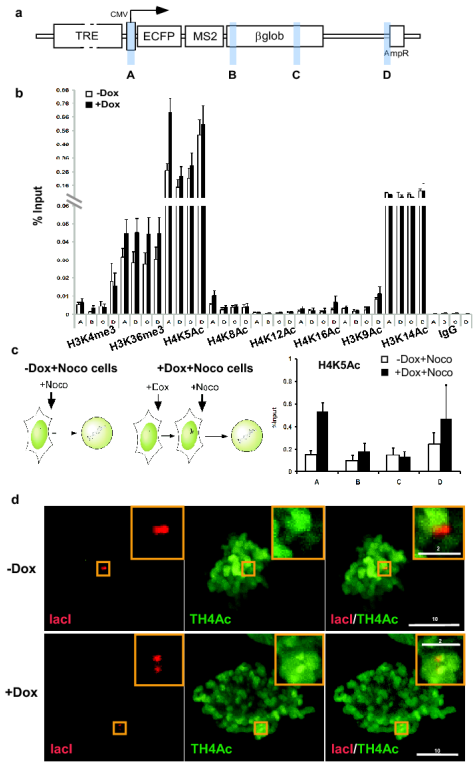

Figure 3.

Histone H4 lysine 5 acetylation (H4K5Ac) is a bookmark for active transcription in interphase and is maintained during mitosis**. (a)** Schematic diagram of primer sets used for chromatin immunoprecipitation experiments (ChIP). **(b)** H4K5Ac showed the largest increase on the promoter region after interphase transcriptional activation, whereas the other active histone modifications (H3K4me3, H3K36me3, H4K8Ac, H4K12Ac and H4K16Ac) did not show as large an increase. Cells stably expressing pTet-ON, were treated with or without Dox (1μg/ml) for 24hrs before being collected for ChIP experiments. Results were collected from 3 biologically independent experiments (mean±S.E.M.). **(c)** Increased H4K5Ac after interphase transcriptional activation was preserved in mitotic cells. U2OS 2-6-3 cells, stably expressing pTet-ON, were induced by Dox (1μg/ml) for 24hrs, followed by treatment with Nocodazole (50μg/ml) in the presence of Dox for another 16hrs for synchronization, before being collected by mechanical shake off for ChIP experiments. Results were collected from 3 biologically independent experiments. (mean±S.E.M.) **(d)** Association of Acetylated H4 (TH4Ac) at the locus during mitosis after Dox induction. Cells induced with Dox (1μg/ml, 24hrs) were fixed and immunolabeld with anti-H4Ac antibody (green). Association of TH4Ac with the locus (enlarged, +Dox) can be detected in mitotic cells. Scale bars, 10μm, 2 μm.

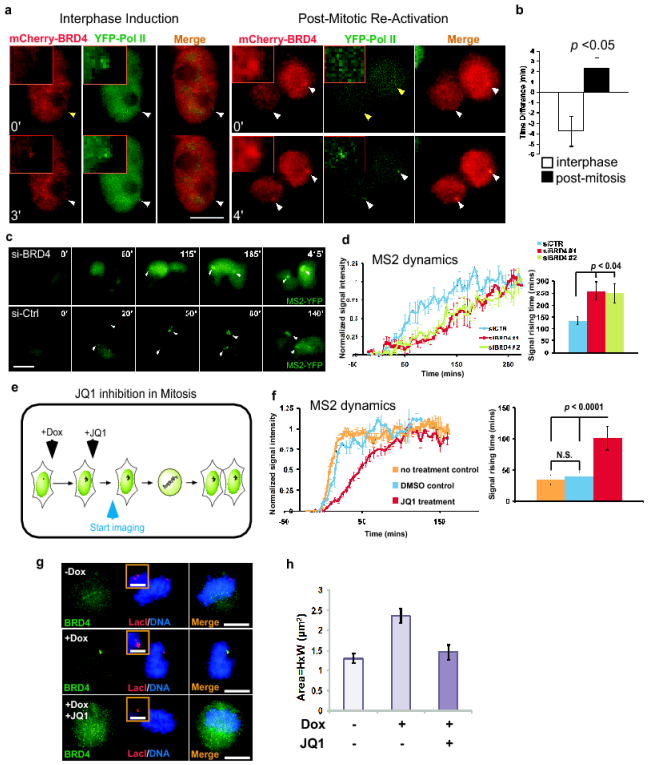

Figure 4.

BRD4 regulates efficient post-mitotic re-activation of transcription**. (a)** BRD4 and Pol II were recruited to the same genetic locus, with different sequential dynamics upon interphase induction vs. post-mitotic re-activation (daughter locus of cell on right is enlarged.) (white arrowheads indicate presence of signal; yellow arrowheads indicate absence of signal). Scale bar, 10μm. **(b)** Pol II was recruited prior to BRD4 upon interphase induction (n=7), while BRD4 was recruited before Pol II during post-mitotic re-activation (n=7) (mean±S.E.M.) **(c)** siRNA knockdown of BRD4 delayed post-mitotic transcriptional re-activation. Arrowheads indicate mRNA production revealed by MS2-YFP signal. Scale bar, 10μm. **(d)** BRD4 regulates efficient post-mitotic transcriptional re-activation, as shown by quantitative analysis of the rising time of mRNA production at the locus in post-mitotic cells treated with either siBRD4#1 (258.75±38.0min; n=5), or siBRD4#2 (249.17±40.0min; n=6), or a control siRNA (133.3±18.7min; n=6) (mean±S.E.M.) **(e)** Diagram of JQ1 experiments. **(f)** JQ1 treatment significantly slowed down the post-mitotic transcriptional activation, as shown by quantitative analysis of the rising time of mRNA production at the locus in post-mitotic cells treated with either DMSO control (41±6.2min; n=5) or JQ1 (100.91±8.1min; n=11). The no treatment control from Figure 1e is re-plotted, for ease of comparison. **(g)** Cells were transiently transfected with LacI-mCherry and were treated as indicated with Dox or Dox + JQ1 in interphase. Cells were fixed and immunolabeled for BRD4 and metaphase cells were examined. DNA was also stained. **(h)** Loci are indicated by LacI-mCherry, and the area of the loci was determined (n=27, 40, 34 for −Dox, +Dox and +JQ1 groups, respectively). Scale bar: 2μm for insert and 10μm for main panel.

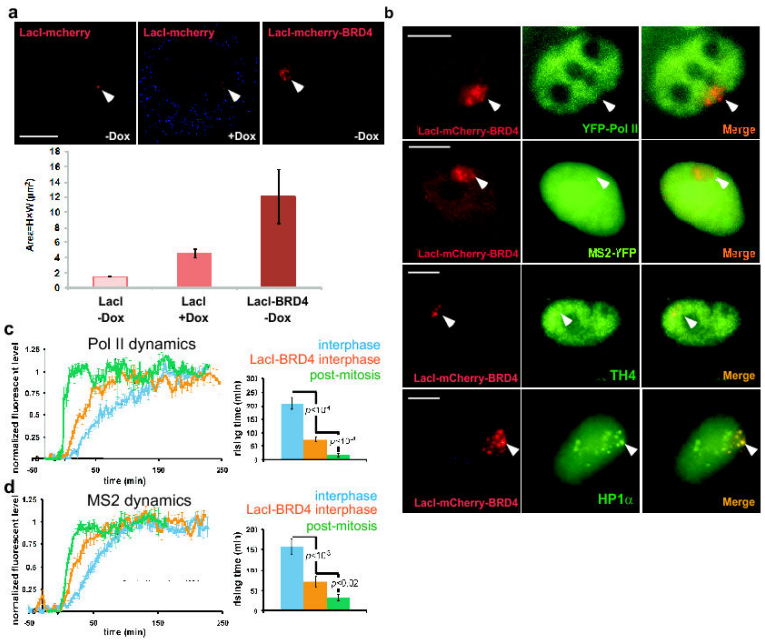

Figure 5.

BRD4 facilitates post-mitotic transcriptional re-activation through chromatin decompaction. **(a)** Locus size change under different treatments. The locus is compacted in the absence of Dox, and there was no CFP protein production. Upon Dox addition the locus was de-compacted, and there was CFP protein production. The locus was even more de-compacted with LacI-mCherry-BRD4 tethering in the absence of Dox, but no CFP was produced. (n=24, 27, 36 for LacI−Dox, LacI+Dox and LacI−BRD4−Dox respectively). **(b)** Tethering BRD4 protein, by fusing BRD4 with LacI-mCherry, to the locus in the absence of Dox de-compacted the locus without the recruitment of Pol II or mRNA production (arrowheads), in cells stably expressing YFP-Pol II or MS2-YFP. BRD4 tethering did not result in significant increase of histone acetylation on the locus, nor did it result in decreased association of heterochromatin marker HP1α. Cells were transiently transfected with LacI-mCherry-BRD4 and were fixed at interphase. Cells were immunostained for TH4 or HP1α. (Scale bar, 10μm) **(c)** Tethering BRD4 protein to the locus significantly accelerated Pol II recruitment during interphase induction (75.8±10.6min) as compared to control interphase induction (taken from Figure 1d, for ease of comparison) without BRD4 tethering (208.1±22.9min). However, Pol II kinetics was still significantly slower than that in post-mitotic re-activation (16.5±6.0min) (mean±S.E.M.). (n=17 for interphase induction, n=13 for LacI-BRD4 interphase induction, n=19 for post-mitotic re-activation). **(d)** Tethering BRD4 protein to the locus significantly accelerated mRNA synthesis during interphase induction (71.5±13.2min) as compared to control interphase induction (re-plotted from Figure 1d for ease of comparison) without BRD4 tethering (157.6±19.0min). Tethering BRD4 results in kinetics that are closer to that observed in post-mitotic re-activation (34.3±7.6 min). (mean±S.E.M.) (n=13 for interphase induction, n=17 for LacI-BRD4 interphase induction, n=25 for post-mitotic re-activation).

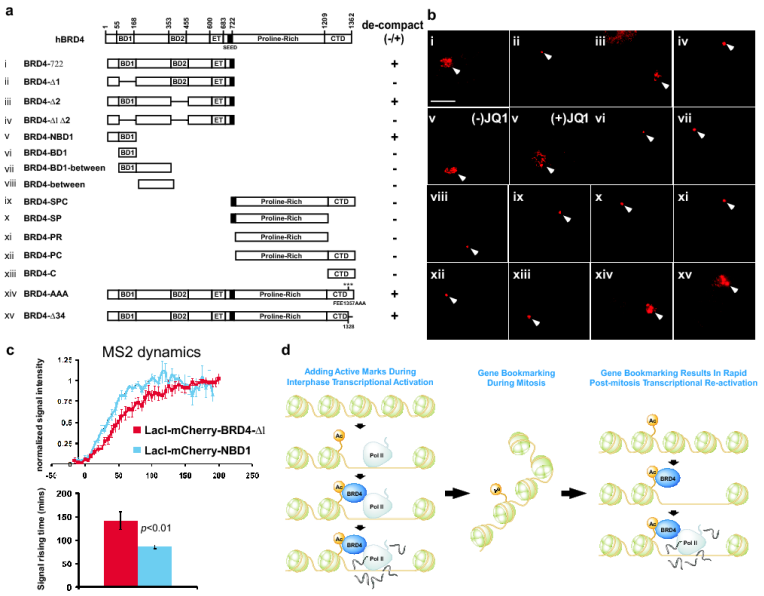

Figure 6.

(a) Diagram of the protein domains contained in different BRD4 deletion mutants. BD: bromodomain; ET: extraterminal[50]; SEED: Ser/Glu/Asp-rich region[51]; CTD: c-terminal domain[50]. **(b)** BD1 but not BD2 is critical for the de-compaction function of BRD4. Cells were transiently transfected with indicated LacI-mCherry-fused BRD4 mutants. Among these mutants, 72% of the cells transfected with BRD4-722 (n=106 cells), 80% of those transfected with BRD4-Δ2 (n=108 cells), and 79% of those transfected with BRD4-NBD1 (n=104 cells), demonstrated decompacted loci; while no decompacted locus could be identified in the cells transfected with the constructs indicated (−) in **(a)** (> 100 cells per construct). Tethering LacI-BRD4-NBD1 in the presence of JQ1 still resulted in decompact loci (v, +JQ1) compared with control (v, −JQ1). Cells were transiently transfected with LacI-mCherry-fused BRD4-NBD1 and were treated with JQ1 for 6hrs. **(c)** The minimum de-compaction mutant NBD1 was sufficient to accelerate interphase transcriptional induction. Analysis of the rising time of mRNA production in Dox induced interphase cells expressing either LacI-mCherry-BRD4-Δ1 (141.25±19.4min; n=12) or LacI-mCherry-NBD1 (85.77±4.7min; n=13). **(d)** A model of transcriptional induction in interphase, and transcriptional re-activation in post-mitotic cells.