Quantitative imaging of chromatin decompaction in living cells

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Abbreviations:

- TSA – trichostatin A
- ORF – open reading frame
- qPCR – quantitative PCR
- SE – standard error
- SPB – spindle pole body
- GST – Glutathione S transferase
- smFISH – single molecule fluorescence in situ hybridization
- Pol II – RNA polymerase II
Abstract

Chromatin organization is highly dynamic and regulates transcription. Upon transcriptional activation, chromatin is remodeled and referred to as “open”, but quantitative and dynamic data of this decompaction process are lacking. Here, we have developed a quantitative superresolution microscopy assay in living yeast cells to visualize chromatin decompaction using the GAL7-10-1 locus as a model system. Upon transcriptional activation of these three clustered genes, we detect an increase of the mean distance across this locus by ~100 nm. This decompaction is linked to active transcription but is not sensitive to the histone deacetylase inhibitor trichostatin A or to deletion of the histone acetyl transferase Gcn5. By contrast, the deletion of SNF2 (encoding the ATPase of the SWI/SNF chromatin remodeling complex) or the deactivation of the histone chaperone complex FACT lead to a strongly reduced decompaction. Our assay quantifies and visualizes for the first time the nucleosome remodeling and eviction activities are major contributors to chromatin reorganization.

Introduction

DNA in the cell nucleus is present as chromatin in a tight complex with histones and other proteins. This complex is central to spatially organize the DNA strand by balancing the negative charges of the phosphate backbone and is also crucial for gene regulation. The three-dimensional chromatin conformation is highly dynamic and remodeled continuously as cells change their physiological state or their transcriptional programs. This remodeling is orchestrated by histone modifiers and chromatin remodelers, which change the interaction between nucleosomes as well as the interaction between histones, DNA and the protein complement present at a chromatin site, thereby affecting the spatial packing of nucleosomes, their location, mobility and density.

Activation of transcription typically leads to a change in chromatin conformation manifested in higher accessibility of the DNA to digestion or transposon integration (Tsompana and Buck, 2014). Although the changes in histone occupancy and accessibility have been studied extensively, the quantitative structural changes of chromatin on a single cell level remain poorly understood, and it is still largely unclear how the activities of chromatin remodeling complexes are spatially and temporally integrated in living cells. The chromatin ‘opening’ associated with transcriptional activation consists of two distinct changes in chromatin structure: spatial decompaction by changes in nucleosome-nucleosome interactions, and linear decompaction by changes in nucleosome density (Even-Faitelson et al., 2016). Both of these processes are thought to be tightly linked to the posttranslational modifications of histones, which characterize transcriptionally active and transcriptionally silent chromatin. For example, histone tail acetylation is generally associated with active chromatin (Li et al., 2007). In vitro, specific histone modifications directly influence the spatial organization of chromatin by mediating or restricting nucleosome-nucleosome interactions. For example, the acetylation of histone H4 at lysine 16 prevents the interaction between the acetylated tail and a neighboring nucleosome, lowering the propensity to form a compact 30 nm fiber (Shogren-Knaak et al., 2006). However, no effect on linear chromatin compaction was observed on autosomes in mammalian cells and C. elegans upon loss of H4K16 modification during differentiation (Taylor et al., 2013; Lau et al., 2017). In addition, evidence is accumulating that extended regular higher order chromatin structures like a 30 nm fiber do not form in vivo, and that chromatin fibers are present mostly in dispersed states in living cells (Fussner et al., 2012; Hsieh et al., 2015; Chen et al., 2016). This is corroborated by recent visualizations of chromatin chains in intact mammalian nuclei, showing that the locally appearance of the chromatin fiber is similar in regions of eu- and heterochromatin (Ou et al., 2017). It is therefore unclear how local chromatin folding is influenced by histone modifications in vivo.
In addition to their potential to mediate inter- and intrachromosomal interactions and thus to shape the longer-range organization of the chromatin fiber, nucleosomes constitute direct obstacles to the passage of RNA Polymerase II (Pol II) during transcription. Histones and entire nucleosomes are therefore evicted from the DNA during transcription by the concerted action of chromatin remodelers like the SWI/SNF and SWR1 complexes and histone chaperones like Asf1 and FACT (reviewed in Venkatesh and Workman, 2015). This nucleosome eviction (leading to a lower nucleosome density) would be expected to cause an increase in the effective length of the chromatin fiber, but this has not been investigated in vivo.

In order to study chromatin dynamics and organization in vivo, we took advantage of the budding yeast GAL locus, a highly regulated gene cluster which has served as a paradigm for inducible gene expression. The GAL locus comprises the GAL7, GAL10 and GAL1 genes located next to each other on chromosome II. These three genes, encoding enzymes required for the metabolism of galactose, are highly regulated depending on the carbon sources present in the growth medium. The genes are repressed in the presence of glucose, active in the presence of galactose (but the absence of glucose) and ‘derepressed’ in the absence of both glucose and galactose (e.g. in raffinose). Intricate regulation of carbon metabolic genes allows S. cerevisiae to adapt to and successfully compete with other organisms for various sugars present in the environment (New et al., 2014).

GAL gene activation involves the recruitment of several histone modifying enzymes (Carrozza et al., 2002; Wang et al., 2002; Govind et al., 2007) and the nucleosome occupancy at the locus is dramatically reduced (Schwabish and Struhl, 2004; Govind et al., 2007; Bryant et al., 2008). How this affects the chromatin conformation in vivo and to which extent chromatin decompaction and transcriptional activation are interdependent remains unclear. To address these important questions, we developed an assay to visualize chromatin compaction in living cells. Monitoring the distance between two chromosomal loci on either side of the GAL locus revealed a drastic linear decompaction upon activation of the GAL gene cluster. This decompaction was tightly coupled to transcriptional activity. Furthermore, the observed opening was not regulated by histone acetylation but depended on the activity of nucleosome-evicting chromatin remodelers.

Results

An assay to quantitatively analyze transcription-induced chromatin decompaction in living cells

To probe chromatin decompaction during transcription in a quantitative manner in living cells, we developed a microscopy-based assay to follow chromatin conformation in S. cerevisiae over time. We chose the GAL7-GAL10-GAL1 gene cluster as a model system, since it is very well studied and the presence of three co-regulated genes spanning ~ 5.8 kb is expected to give a clear decompaction response. LacO and TetO repeats were introduced on either side of the GAL gene cluster or in a control region and visualized with LacI-GFP and TetR-mCherry (Figure 1A). Bright green and red dots were readily detected in all cells (Figure 1A), and their distance was analyzed in cells grown in glucose (repressed), raffinose (derepressed) or galactose (active). In a control region adjacent to but not spanning the GAL locus (18 kb between LacO- and TetO-repeat insertion sites), no change in the distribution of measured distances was observed between the different carbon sources (Figure 1B). In contrast, in strains with a distance of 14 or 31 kb between the insertion points of the repeats across the GAL locus, the distribution clearly shifted towards larger distances in activated cultures (Figure 1B and C) increasing from a mean distance of 255±13 nm in glucose to 310±7 nm in galactose in the 14 kb reporter strain or from 327±12 nm to 391±14 nm in the 31 kb reporter strain (2D distances measured on single imaging plates; values are means of three biological replicates with standard error of the mean). As expected, the deletion of the transcriptional activator Gal4 abolished
the decompaction response, while deletion of the transcriptional repressor Gal80 led to
decompaction already in raffinose-grown cells (Supplemental Figure 1). Thus, we have developed a
microscopy assay that can readily detect chromatin decompaction upon transcriptional activation in
living cells.

To analyze the dynamics of decompaction, we acutely induced the GAL genes by adding galactose to
cells growing in raffinose. This led to an increase in the population mean distance over the course of
20 minutes (Figure 1D) mimicking the kinetics of transcriptional activation as seen e.g. by
quantitative PCR (qPCR) analysis (Green et al., 2012). Similarly, the addition of glucose to cells
growing in galactose resulted in fast compaction kinetics (Figure 1E) corresponding to the rapid shut-
down of transcription due to glucose-induced repression. Decompaction could also be induced with
faster kinetic within less than five minutes if cells were pregrown in galactose and then repressed
with glucose for only 1 h before re-induction with galactose (Figure 1F). In this case, the presence of
elevated levels of Gal1 and Gal3 proteins induce a ‘transcriptional memory’ and lead to faster
activation (Kundu and Peterson, 2010). Thus, the kinetics of chromatin compaction closely follows
transcriptional activity.

Active transcription correlates with chromatin decompaction in single cells

To directly correlate transcriptional activity with distance measurements on a single cell level, we
applied single molecule fluorescent in situ hybridization (smFISH) to visualize transcripts as they are
produced from the GAL locus in our distance reporter strains. We simultaneously used FISH probes
for all three GAL genes GAL1, GAL10 and GAL7, all labeled with the same fluorophore (Qasar670).
While cytoplasmic mRNAs are visible as individual spots, or hazy signal due to the large number of
molecules in induced cells, the transcription site is visible as a bright focus in the nucleus close to the
repeat-marked gene loci (Figure 2A). Transcription spots and cytoplasmic mRNA foci are absent in
cells grown in raffinose (Figure 2A, first panel). Upon induction with galactose, the fraction of cells
with transcription spots increased over the course of 30 min paralleling the increase in the mean
distance in the population after induction (Figure 2B). After 60 minutes or longer, a transcription
spot can be seen in virtually every cell (Figure 2A), but since our automatic detection routine does
not detect all transcription spots, the maximum measured percentage of cells with transcription spot
is ~ 80 % (Figure 2B).

To correlate transcription with distance, we analyzed early time points after induction, when only a
fraction of the cells shows a transcription spot (Figure 2B). We separately determined the distance
between the LacO- and TetO-repeat markers in cells with or without a detectable transcription spot.
Importantly, distances were measured in 3D on image stacks to provide additional resolution, since
fixation and FISH procedures led to an overall shrinkage of the sample. While only few cells with a
clear transcription spot were observed after 5 min and decompaction was still minimal, after 10 and
30 min the distances in cells with a transcription spots were significantly larger than in cells without a
transcription spot (Figure 2C and D). Thus, decompaction correlates with transcription also on a
single cell level.

Histone acetylation is dispensable for chromatin decompaction at the GAL locus

The increased distance distribution observed in our assay, which is indicative of decompaction of
chromatin during transcription, could result from reduced internucleosomal interactions, from the
eviction of nucleosomes, or both. Internucleosomal interactions are often mediated by histone tails
and are thought to be regulated by posttranslational modification. Histones H3 and H4 contribute
most to direct internucleosomal interactions but are also required for the recruitment of
transcriptional activators. Acetylation of H3 in vivo occurs predominantly through Gcn5 (Grant et al.,
1997). This histone acetyl transferase is a component of the SAGA complex, which is recruited to the
GAL locus by the transcriptional activator Gal4 (Carrozza et al., 2002; Govind et al., 2007). However, the deletion of neither the SAGA histone acetyl transferase Gcn5 nor its activating subunit Ada2 influenced chromatin decompaction across the GAL locus (Figure 3A). These mutations also do not affect pre-initiation complex formation at the GAL promoter (Bhaumik and Green, 2001) or mRNA production (Stafford and Morse, 2001; Green et al., 2012), suggesting that acetylation is dispensable for transcriptional activation and chromatin decompaction induced by Gal4. While dispensable for activation, Gcn5 activity is crucial for the association of the GAL locus with the nuclear periphery upon activation (Dultz et al., 2016). Therefore, our results rule out that the observed decompaction response is a consequence of relocalization of the GAL locus to the nuclear pore complex, which might, for example, generate pulling forces that could lead to stretching of the chromatin fiber.

We also tested whether the inhibition of histone deacetylases using trichostatin A (TSA) would affect chromatin decompaction. TSA treatment neither changed the steady state distance distributions (Figure 3B) nor did it affect induction or repression kinetics (Figure 3C). Since both the deletion of GCN5 and the inhibition of histone deacetylases targeted by TSA have been shown to globally affect acetylation levels of histones (Vogelauer et al., 2000), these results indicate that the global acetylation state of histones per se does not play a major role in regulating chromatin compaction at the GAL locus.

Mutants that affect transcription also affect decompaction
Although Gcn5 was dispensable for chromatin decompaction, deletion of the SAGA component Spt20, which is required for the stability of the complex (Grant et al., 1997) and for its recruitment by Gal4 (Bhaumik and Green, 2001; Larschan and Winston, 2001; Bryant and Ptashne, 2003), led to strongly reduced decompaction of the GAL locus (Figure 4 A and C). As previously reported (Bhaumik and Green, 2001), Spt20 mutants also show strongly reduced GAL gene transcription (Figure 4B).

Whereas SAGA is thought to be required to stabilize the general transcriptional coactivator Mediator at the GAL promoter, Gal4 is also able to directly recruit this complex (Traven et al., 2006). Indeed, cells with a deletion of GAL11, a Mediator component that mediates direct interaction with Gal4, or expressing a temperature sensitive mutant of the Mediator protein Med7 (med7-163) phenocopied sp t20Δ cells by exhibiting reduced overall decompaction in galactose as well as slowed decompaction kinetics (Figure 4 A and C and Supplemental Figure 2).

To analyze the effect of SPT20 or GAL11 deletions on a single cell level, we carried out single molecule FISH. After overnight growth in galactose, most cells displayed cytoplasmic mRNA signals, but the signal was strongly reduced compared to wildtype cells (Figure 4D). In addition, the intensity of transcription spots, which were still present in most cells, were reduced on average to ~ 30 % of the wildtype intensity (Figure 4D and E). Thus, also in the mutant cells, reduced transcription correlates with reduced decompaction of the GAL locus. Our results reveal a close linkage between transcriptional activation and decompaction, but they are not sufficient to establish a causality or temporal order between the two processes.

Nucleosome remodelers are required for decompaction
Next, we examined the function of nucleosome remodelers in our decompaction assay. As demonstrated by chromatin immunoprecipitation experiments, histone occupancy in the promoter of transcribed genes – but also in the open reading frame (ORF) of highly transcribed Pol II genes including the GAL genes – is reduced several fold upon activation (Lee et al., 2004; Schwabish and Struhl, 2004). Nucleosome eviction is mediated by the SWI/SNF chromatin remodeling complex and the histone chaperones Asf1 (Antisilencing function 1) and FACT (FAcilitates Chromatin Transcription), a heterodimeric complex consisting of Pob3 and Spt16 (Schwabish and Struhl, 2006, 2007; Venkatesh and Workman, 2015). If the observed decompaction across the GAL locus is due to
nucleosome eviction, depletion of either of these factors should lead to a reduction of
decomposition.

SWI/SNF is a chromatin remodeling complex with nucleosome sliding and nucleosome eviction
activities. At the GAL locus, it is required for rapid Pol II recruitment and activation (Kundu and
Peterson, 2010). We found that deletion of SNF2, encoding the ATPase subunit of the complex,
slowed down the kinetics of decompaction and strongly reduced the degree of final decompaction as
well (Figure 4A and C) consistent with a reduction in nucleosome eviction across the locus
(Schwabish and Struhl, 2006). Although inactivation of SWI/SNF has previously been reported not to
alter steady state GAL gene expression (Lemieux and Gaudreau, 2004), we observed strongly reduced
mRNA levels both by qPCR and sm FISH in snf2Δ cells (Figure 4B and D). In contrast to the SWI/SNF
complex, the chromatin remodeling complexes INO80 (represented by arp6Δ) and SWR1
(represented by swr1Δ) were not required for chromatin decompaction at the GAL locus (Figure 4A).

Histone chaperones play an important role in nucleosome eviction and deposition. Therefore, we
tested the involvement of the histone chaperones Asf1 and FACT, which have both been implicated
in the regulation of the GAL genes (Schwabish and Struhl, 2006; Xin et al., 2009). Deletion of ASF1 did
not prevent chromatin decompaction in our assay (Figure 5A), which is consistent with previous
fndings that showed only a small decrease in histone H3 (but not H2B) removal at GAL promoters
and ORFs (Schwabish and Struhl, 2006). In contrast, cells carrying temperature sensitive alleles for
either component of the FACT complex (pob3-7 or spt16ts) did not show decompaction one hour
after a shift to activating conditions at the restrictive temperature (Figure 5B). Together, these
findings indicate that the eviction of nucleosomes mediated by SWI/SNF and FACT is the major
source of decompaction observed in our assay.

Decomposition of the GAL locus is transcription dependent
If the observed decompaction is due to the removal of nucleosomes at the promoter and the ORF,
the degree of decompaction will be expected to scale with the length of the ORF. To test this
prediction, we introduced GAL promoter driven reporter genes at a different genomic site ~ 10 kb
telomeric of the native GAL locus and measured changes in chromatin distance in the presence or
absence of galactose (Figure 6A). The GAL promoter immediately followed by a terminator sequence
did not exhibit a significant decompaction response. In contrast, robust decompaction was observed
in a strain where the entire GAL1 gene including promoter and downstream sequences was inserted.
Decomposition, although to a weaker extent than for the entire GAL1 gene, was also observed in a
strain where we introduced a GAL1 promoter-driven ORF coding for Glutathione S transferase (GST).
Since the ORF of a single GST is shorter than the one of GAL1 (700 bp versus 1 kb), we also examined
the response in a reporter strain carrying two consecutive copies of the GST ORF (2xGST). However,
the presence of a second GST ORF only slightly increased the decompaction, indicating that the GAL1
ORF or 3’UTR have specific properties that enhance the observed decompaction response. However,
the 2xGST construct also contained MS2 stem loop sequences in the S’UTR that might affect
transcription (Heinrich et al., 2017) and thus decompaction.

Decomposition leads to an untypical open chromatin state at the GAL locus
Our data provide the first quantitative measurement of transcription-dependent decompaction in
living cells. To understand the changes in chromatin structure underlying the observed decompaction
response, we more closely analyzed the distance distributions of the populations. The 3D distance of
genomic loci on the same chromosome has been shown to scale with the distance in base pairs (van
den Engh et al., 1992). This is true also on chromosome II of budding yeast in both glucose and
galactose grown cells as shown previously by us (Dultz et al., 2016 and Figure 6B grey and black
datapoints). One explanation for the observed changes in compaction at the GAL locus could be that
the GAL locus – compared to bulk chromatin – is hyper-compacted in the repressed state. However, this is not the case: in the repressed or derepressed state (glucose or raffinose growth), the median distances of TetO and LacO marker pairs across the GAL locus (Figure 6B open red circles, data for raffinose grown cells not shown) are in line with the distances of other pairs of loci on chromosome II. This indicates that the compaction state of the locus is comparable to other regions on the same chromosome. In contrast, in galactose-grown cells, the median distance increased far above the distance expected from the linear distance on the chromosome (Figure 6B, filled red circles), showing that the GAL locus is strongly hypo-compacted in its active state. This is consistent with the interpretation that linear decompaction at the GAL locus upon transcriptional activation is due to the eviction of nucleosomes, and that the high transcriptional activity over three clustered genes at the GAL locus leads to a very low histone density and thus hypo-compaction.

Since our data provide quantitative measurements of this hypo-compaction, we applied computational modeling to predict the level of histone loss that could lead to such a distance increase. To this end, we adapted a previously developed polymer model of the yeast genome, which accurately recapitulates several experimentally determined features (Tjong et al., 2012; Dultz et al., 2016), by increasing the resolution of the model in the proximity of the GAL locus. In this model, the compaction of the chromatin fiber in the GAL locus had to be reduced by ~60% in order to recapitulate the observed increase in distances (Figure 6C and D), which corresponds to a 1.7-fold increase in linear extension. Since the wrapping of DNA on nucleosomes leads to an approximately 6-fold compaction, this suggests that about 30% of the nucleosomes at the GAL locus are evicted upon transcriptional activation. This is in accordance with nucleosome occupancy measurements (Schwabish and Struhl, 2006) and, at a density of 162bp/nucleosome (Horz and Zachau, 1980), corresponds to the eviction of approximately 11 of 36 nucleosomes across the locus.

Discussion

Recently, the development of chromosome conformation capture techniques has revolutionized the characterization of three-dimensional interaction landscapes of chromatin and has led to many important discoveries and new models of chromatin organization (reviewed in Pombo and Dillon, 2015; Bonev and Cavalli, 2016). However, these techniques require the fixation of cells and are usually applied to cell populations. Furthermore, it is not possible to directly convert the interactions detected by chromosome conformation capture into 3D distances in the cell nucleus. In fact, a direct comparison of chromosome capture analyses with DNA fluorescence in situ hybridisation results has revealed that the two techniques do not always result in congruent findings (Williamson et al., 2014). It is therefore crucial to analyze chromatin conformation in situ and also in vivo.

Chromatin decompaction upon transcriptional activation has been visualized in mammalian cells using large repeat arrays (Tumbar et al., 1999; Dietzel et al., 2004; Verschure et al., 2005; Hu et al., 2009). FISH analysis has also been employed to obtain distance measurements at activated loci (Chambeyron and Bickmore, 2004; Chambeyron et al., 2005; Morey et al., 2007). Although making important contributions to our understanding of changes in chromatin conformation upon transcriptional activation, quantitative interpretation is limited because fixation and DNA FISH protocols introduce modifications to the cell ultrastructure and repeat arrays may themselves influence the local chromatin environment. Therefore, we have used the budding yeast *Saccharomyces cerevisiae* to generate a system where live cell analysis is possible in a quantitative manner at an endogenous gene locus. Although we initially use a 3-gene system to establish our assay, we also show that this assay is sensitive enough to visualize decompaction of a single activated gene. Furthermore, the use of budding yeast allows us to study the basic level of chromatin decompaction in the absence of confounding changes in chromatin conformation like enhancer-promoter looping or looping out from a chromosome territory. Thus, our data yield quantitative...
measurements of chromatin decompaction upon transcriptional activation and allow us to model the changes in chromatin properties at an activated locus.

The role of acetylation

Interestingly, we found that deletion of the lysine acetyl transferase gene GCN5 or inhibition of lysine deacetylation by trichostatin A had no observable effect on the distance distribution in our compaction assay. In contrast, the treatment of mammalian cells with TSA was previously reported to affect chromatin organization globally and to lead to decompaction of heterochromatic regions (Toth et al., 2004; Lleres et al., 2009). However, these studies used global histone signals (intensity or FRET-FLIM) to assess compaction levels. These analyses thus do not give sequence-specific readout and report on spatial rather than on linear compaction. In agreement with our findings, DNA FISH analysis in mouse cells, which also tested linear compaction between two loci, showed decompaction in polycomb silenced regions but not elsewhere (Eskeland et al., 2010). Thus, very low acetylation levels may be crucial for the formation of certain subnuclear compartments in heterochromatic regions of the nucleus but may play a less prominent role in linear decompaction in the relatively open bulk yeast chromatin. Furthermore, acetylation could facilitate the binding of factors to specific DNA sequences by rendering the interactions between histones and DNA less tight. This could allow nucleosomes to slide more easily or create binding sites for bromodomain-containing chromatin effector proteins (Filippakopoulos and Knapp, 2014). In contrast to the GAL genes, other budding yeast genes were shown to fully depend on the HAT activity of Gcn5 for transcriptional activation. For example, this is true for a heterologous β-estradiol responsive transcriptional activator based on the viral VP16 transactivation domain (Stafford and Morse, 2001). Intriguingly, we found that upon activation via a β-estradiol-inducible VP16 fusion protein (Louvion et al., 1993), Gcn5 is essential for decompaction (Supplemental Figure 3) and neither gcn5Δ nor spt20Δ cells were able to decompact the GAL locus upon addition of β-estradiol (Supplemental Figure 3). This observation also corroborates the conclusion that transcriptional activation precedes decompaction.

The interplay of transcription and decompaction

Our mutant analysis shows that transcriptional activation and chromatin decompaction are tightly coupled. To directly address the role of Pol II in decompaction, we carried out experiments with cells expressing a temperature-sensitive mutant allele of the largest subunit of RNA Pol II, rpb1-1. rpb1-1 cells were induced with galactose one hour after a shift to the restrictive temperature. Both expression of GAL10 mRNA and decompaction were reduced but not abolished (Supplemental Figure 4A and B). Thus, it appears that induction at this locus is so strong that the temperature-sensitive rpb1-1 mutant is not fully effective to suppress decompaction and transcription. Nevertheless, together with the observation that an ectopic GAL promoter in the absence of an ORF is not sufficient to confer decompaction (Figure 6A), our results suggest that ORF transcription by Pol II is required for decompaction of the chromatin at the GAL locus. This is consistent with the observation in mammalian cells that only initial decompaction is mediated by transcriptional activation but for complete decompaction elongation by RNA polymerase is necessary (Hu et al., 2009). We hypothesize that the removal of nucleosomes at the promoter presents a first stage of decompaction, but elongation and the concomitant eviction of nucleosomes along the ORF constitutes the major contribution to chromatin opening. This interpretation is in line with the observation that opening of the GAL locus is not detected when both glucose and galactose are present (Figure 4C Galactose+Glucose condition), a condition where SWI/SNF activity has been reported to evict nucleosomes at the promoter (Bryant et al., 2008). However, in addition to the length of the ORF, the strength of activation and possibly the composition of the 3’ and 5’ UTRs may...
also contribute to the level of nucleosome eviction and thus decompaction. Importantly, the absence of a decompaction response from the promoter alone also indicates that longer range interactions mediated by the promoter, for example promoter-enhancer loops, do not play a role in our system allowing us to focus on the local changes in chromatin organization. Such effects or the release from higher spatial compaction states may have contributed to the transcription-independent decompaction that were observed previously (Hu et al., 2009).

After transcriptional repression by the addition of glucose, the signal of GAL mRNAs in the cytoplasm quickly diminishes. Although the nuclear FISH signal at the site of transcription is reduced as well, a weak spot can still be detected in many cells even 60 minutes after glucose addition (Supplemental Figure 5A and C). This most likely corresponds to polyadenylated GAL mRNAs previously reported to persist at the GAL genes upon transcriptional shut-off (Abruzzi et al., 2006). Interestingly, cells with a brighter RNA dots showed a distance distribution shifted to longer distances compared to cells with a weaker or absent RNA dot signal (Supplemental Figure 5B). This could indicate that transcription is still ongoing in those cells and thus the brighter RNA signal would stem from nascent transcripts. Alternatively, long-lived, chromatin-associated RNA dots might contribute to keeping chromatin in a partially decompacted state.

The role of nucleosome eviction

Histones are evicted during transcriptional elongation but repositioned after the passage of Pol II. Both eviction and deposition require the activity of histone chaperones like FACT. In highly transcribed genes like the GAL genes, individual transcription events follow closely after each other (Lenstra et al., 2015). In this case, deposition of histones between rapid rounds of transcription may not be possible, and thus a net loss of histones and nucleosomes occurs. Such a response would explain the dramatic unfolding of chromatin across the GAL locus observed in our assay. This is consistent with our observation that mutants with reduced transcriptional activity display a reduced decompaction. Here, initiation and polymerase II progression occurs at a lower frequency, which could allow for more time to deposit histones after the passage of Pol II. This interpretation is also in agreement with the observed correlation between ORF length and decompaction. However, for the moment it remains unclear why the GAL1 ORF and its native 3’UTR confer higher levels of decompaction compared to a GST ORF terminated by an ADH1 terminator. Of note, the presence of MS2 stem loops in our 2xGST construct may present a serious obstacle to Pol II (Heinrich et al., 2017) and therefore could lead to reduced Pol II density on the body of the gene.

Outlook

Transcription initiation displays large cell-to-cell variability, and the same can be expected also for decompaction. Unfortunately, we were not able to reliably measure decompaction kinetics on a single cell level, due to the large fluctuations in distances measured over time even for cells in steady state growth conditions. Therefore, further developments of reporter systems with reduced biological noise in combination with improved superresolution microscopy approaches will be needed to enable the analysis of decompaction kinetics on the single cell level. For example, the contribution of DNA sequences that are not involved in the observed response could be minimized by placing fluorescent reporters directly adjacent to the gene of interest. Adoptions of our system thus have the potential to shed further light on the dynamics of chromatin decompaction by allowing to study this process quantitatively in single cells under physiological conditions. The data from live cell microscopy experiments are highly complementary to data obtained from chromosome capture techniques and are very valuable to better understand chromatin organization and its dynamics in vivo. Furthermore, using CRISPR/Cas technologies, our assay could be readily transferred to higher eukaryotic systems as well. Optimized cassettes for the integration of LacO repeats by CRISPR/Cas have recently been created (Tasan et al., 2017) and the targeting of labelled CRISPR/Cas complexes.
Chen et al., 2013; Chen and Huang, 2014; Ma et al., 2016) will in the future provide a powerful and flexible tool to dissect the contribution of individual players to chromatin compaction in various model systems.

Methods

Plasmid construction

Plasmid were constructed using standard molecular biology techniques. pKW1008 was constructed by ligating annealed oligos UC586/867 into pFA6-GFP(s65t)-kan (Longtine et al., 1998) using PacI/AscI to replace GFP with a FLAG-tag. pKW2695, pKW2704 and pKW3264 were constructed by ligating PCR products of primers CH4505/CH4506, CH4513/CH4514 or CH6/CH106 on genomic DNA into pKW1689 (Green et al., 2012) after cutting vector and insert with XhoI and SacI. pKW3035 was constructed from a PCR product of UC5681/UC5682 on pKW3010 (Backlund et al., 2014) cut with DraI and ligated into pAFS135 (Straight et al., 1996) also cut with DraI. pKW3681 and pKW3682 were constructed by inserting fragments generated by primers CH1211/CH1213 or CH1211/CH1177 on genomic DNA into the pFA6a backbone containing a NatMx resistance cassette (cut with PacI/Xhol). pKW3683 was constructed by inserting into the same vector a stitched PCR product of the GAL1 promoter (CH1211/CH1218) and GST (CH1214/CH1215) with PacI/Xhol. pKW3999 was constructed in multiple steps. The final construct contains homology regions for a region on chromosome II which were amplified from genomic DNA using primers CH1795/CH1796 and CH1797/CH1798. Between the homology sites, the GAL1 promoter amplified by CH1211/CH2043 drives a construct with 24 MS2 stem loops (Grunwald and Singer, 2010) in the 5’ UTR and encoding 2xGST (CH1789/CH1794 and CH1791/CH1794) tagged with V5. It also contains the NatNT2 resistance cassette for selection and can be integrated after cutting NotI/Ascl. The Gal4DBD-ER-VP16 activation construct was transferred from the construct published in (Louvion et al., 1993) into a Ylplac204 backbone with an additional NatMx cassette added for selection in TRP+ strains (pKW3504). All plasmids used in this study are listed in Supplementary Table 1. Primers are listed in Supplementary Table 2.

Yeast strain construction

S. cerevisiae strains were constructed in the background of BY4741 and BY4742 (Brachmann et al., 1998) using standard yeast genetic techniques either by transformation of a linearized plasmid or of a PCR amplification product with homology to the target site (Baudin et al., 1993). The mutants spt20Δ, gal80Δ, gcn5Δ, asf1Δ, snf2Δ, gol11Δ, pob3-7, spt16ts, med7-163, rpb1-1, gal4Δ, swr1Δ and arp6Δ were constructed by mating of the wildtype strains with the corresponding strains from the MATa, MATα deletion collections (Winzeler et al., 1999) or the MATα temperature sensitive collection (Li et al., 2011) followed by sporulation and tetrad dissection. ADA2 was deleted by PCR-directed mutagenesis using primers CH395/CH396 on pKW1008 to generate KWY5104. Reporter strains for the experiments shown in Figure 6A were generated by transformation of KFY4067 with the PCR product of CH1272/CH1273 on pKW3681 (KWY6245), pKW3682 (KWY6247) or pKW3683 (KWY6252) respectively. Genotypes were confirmed by PCR. All yeast strains used in this study are listed in Supplementary Table 3.

Yeast culture conditions

Yeast cells were cultured in complete synthetic medium with 2 % of either glucose, galactose or raffinose as indicated at 30 °C. Temperature sensitive mutants were grown at 25 °C. Some kinetic experiments with mutants showing significant growth defects were also carried out at 25 °C. All experiments were carried out with cells in exponential growth phase. For microscopy experiments, cells were usually inoculated from saturated cultures into fresh medium and grown overnight to OD 0.5-0.8 and then imaged. Alternatively, cells were diluted in the morning and grown for additional 2-
3 cell cycles before the start of the experiment. Trichostatin A (Sigma Catalog #:T8552) was used at 50 µM.

Microscopy

Cells were pregrown in raffinose-containing medium and inoculated in 1 ml in 24 well plates overnight so that they reached an OD of 0.5-0.8 in the morning. Cells were then transferred to concanavalin A coated 384 well plates (Matrical) and imaged on a temperature controlled Nikon Ti Eclipse equipped with a Apochromat VC 100x objective NA 1.4 (Nikon) (filters: Spectra emission filters 475/28 & 542/27 and DAPI/FITC/Cy3/Cy5 Quad HC Filterset with 410/504/669 HC Quad dichroic and a 440/521/607/700 HC quad band filter (Semrock)) with exposure times of 50-200 ms. For timelapse experiments, induction with different sugars or trichostatin A was carried out on stage after the first imaging timepoint by mixing with an equal volume of trichostatin or sugar containing medium. Different fields of view were imaged at each timepoint to prevent negative effects of phototoxicity or bleaching on the analysis. 3D microscopy of living cells was carried out on a SpinningDisk microscope (Yokogawa Confocal Scanner Unit CSU-W1-T2) built on a Nikon TiE body and controlled with the VisiVIEW software using dual camera acquisition mode and a 100x NA 1.49 CFI Apo TIRF objective. Cameras were Orca Flash 4.0 V2, excitation lasers were a DPSS 488 nm (200mW) and a diode 561 nm (200 mW) laser. Z scanning was performed in streaming mode with a LUDL BioPrecision2 Piezo Stage using 2-by-2 binning with 100 ms exposure times per frame. Filters were: Dichroic quad-band DAPI/GFP/RFP/CY5, splitting filter to camera ports: 561LP, emission filters GFP/ET525/50 and mCherry ET630/75 respectively.

Single molecule fluorescence in situ hybridization

Single molecule fluorescence in situ hybridization was carried out according to (Mugler et al., 2016) with slight adaptations. The indicated strains were inoculated in synthetic medium containing 2 % raffinose and grown overnight to saturation. The next day, cells were diluted into fresh synthetic complete media containing 2 % raffinose or 2 % galactose and grown overnight at 30 °C to exponential growth phase (OD600 = 0.6-0.8). Cells were then induced by addition of glucose or galactose to final concentration of 2 % and fixed after the indicated timepoints for 15 min at 30 °C and for 15 min at 25 °C with 4 % paraformaldehyde (EM grade 32 % paraformaldehyde aqueous solution electron Microscopy Sciences 15714), washed with buffer B (1.2 M sorbitol, 100 mM KHPO₄ at pH 7.5, 4 °C) and stored at 4 °C overnight. Cells were then spheroplasted for 20 min using 1 % 20T zymolyase in 1.2 M sorbitol, 100 mM KHPO₄ at pH 7.5, 20 mM vanadyl ribonuclease complex and 20 µM β-mercaptoethanol, washed with buffer B to stop the spheroplasting reaction and then washed into 10 % formamide (Merck Millipore S4117) in 2x SSC.

Mixtures of DNA probes coupled to Quasar670 (Stellaris, LGC Biosearch, Novato, CA; probes were synthesized by BioCat, Heidelberg, Germany) were used for smFISH, targeting the GAL1, GAL7 and GAL10 ORFs (Supplementary Table 4). Per sample, 0.5 µl of each probe mix (stock 25 uM) was mixed with 2 µl of salmon-sperm DNA (10 mg ml⁻¹, Life Technologies, 15632-011) and 2 µl yeast transfer RNA (10 mg/ml, Life Technologies, AM7119). The probe mix was denatured in 50 µl per sample of hybridization buffer F (20 % formamide, 10 mM NaHPO₄ at pH 7.0) for 3 min at 95 °C and then mixed with 50 µl per sample hybridization buffer H (4x SSC, 4 mg/ml BSA (acyetylated) and 20 mM vanadyl ribonuclease complex). Cells (approximately corresponding to pellet of 5 ml initial culture) were resuspended in the hybridization mix and incubated for 8-12 hours at 37 °C. After four washing steps (10 % formamide/2x SSC; 0.1 % Triton/2x SSC; 2x SSC/DAPI; 2x SSC), cells were stored at 4 °C. Cells were imaged in concanavaline A coated 384 wells. Microscopy was performed with an inverted epifluorescence microscope (Nikon Ti) equipped with a Spectra X LED light source and a Hamamatsu Flash 4.0 sCMOS camera using a PlanApo 100 x NA 1.4 oil-immersion objective and the NIS Elements software. 31 z planes were acquired. The stack of the Qasar670 channel was acquired first due to
significant bleaching. mCherry and GFP channels were acquired plane by plane to minimize shift between channels.

Image analysis
Images were processed using FIJI (NIH ImageJ 1.51p and previous) and Diatrack (Vallotton et al., 2017). For 2D analysis of distances, position of chromosome location marked with LacI or TetR were detected by a 2D Gaussian fit using custom written scripts in Matlab (Dultz et al., 2016). Distances between detected spots were calculated. Due to negligible shift between the two channels in these datasets, correction was not required. For 3D analysis of distances (data acquired on dual camera spinning disk microscope), positions of chromosome location marked with LacI or TetR were detected in Diatrack in 3D using across-color tracking to pair corresponding spots from the same cell (parameters used: ‘exclude blurred’ 0.07-0.09, ‘exclude dim’ 20-200, full width half maximum for Gaussian fitting 2.5). Subsequently, positions were corrected for shift between the two channels using bead images (1 µm TetraSpeck fluorescent microspheres, ThermoFisher Scientific) acquired under identical imaging conditions. For shift correction, the field of view was subdivided into 150x150 pixel large squares and the mean shift for beads acquired in this region was applied to correct the positions of chromosome locations acquired in this region of the camera. The distances between corrected positions were calculated and plotted in Matlab as boxplots. For FISH analysis, position of chromosome location marked with LacI or TetR were detected in Diatrack in 3D using across-color tracking to pair corresponding spots from the same cell. Positions of transcriptions spots were detected on maximum intensity projections of the Qasar670 channel in Diatrack and used to categorize cells as having or not having a transcription spot for separate analysis of the two populations. For analysis of transcription spot intensities, transcription spots were detected on 3D stacks in Diatrack and intensity values were obtained from the Diatrack session file. Only the intensity of those transcription spots for which corresponding chromosome locations marked with LacI or TetR could be detected in Diatrack were included in the analysis. Bootstrapping was used to obtain 95% confidence intervals of the medians and mean. Typically 200-400 cells (but at least 90) were analyzed per condition in each experiment.

Quantitative real-time PCR
qPCR was performed as described previously (Dultz et al., 2016): 1 ml of cells at OD600 0.8-1 was harvested by centrifugation and snap frozen in liquid nitrogen. RNA was extracted using the RNeasy kit from Qiagen via mechanical disruption. 300 ng of total extracted RNA was used for reverse transcription. The RNA was first treated with DNase I using the DNA-free kit from Ambion according to the protocol of the manufacturer. Reverse transcription was performed according to the protocol of the manufacturer using Superscript II reverse transcriptase (Invitrogen) with random hexamer primers. Quantitative Real Time PCR was performed on a StepOnePlus Instrument (Invitrogen) using Absolute Blue QPCR Mix with SYBR Green and ROX (ThermoFisher Scientific). All experiments were carried out in three technical replicates and three biological replicates. Data was analyzed by the comparative CT method using ACT1 as endogenous control. Primers used for qPCR are listed in Supplementary Table 2.

Computational modeling
Average spatial distances were calculated from ensembles containing 1000 structure models of the entire haploid yeast genome. Ensembles were generated for each of the two GAL locus activation states (active/galactose and inactive/glucose), as described in (Tjong et al., 2012; Dultz et al., 2016). All chromosomes were modeled as chains of connecting beads subject to a number of spatial restraints. In particular, all chromosomes were confined inside a nucleus with a radius of 1 µm; the nucleolus and spindle pole body (SPB) are placed on opposite sides of the nucleus along the central nuclear axis; all non-ribosomal-DNA gene regions are excluded from the nucleolar volume;
Centromeric regions are proximal to the SPB, whereas telomeric regions are tethered to the nuclear envelope (allowing a maximal distance between telomeres and the nuclear envelope of 50 nm). Each bead accommodated ~3.2kb of genome sequence as described in (Tjong et al., 2012).

To reproduce the experimental data, we modeled a gradual decrease in chromatin compaction in the proximity of the **GAL** locus. For the 60 kb region starting at the position of the **GAL** locus, the compaction ratio was set to 1.6 kb per bead. We then determined the optimal chromatin compaction for the 6.2 kb **GAL** locus so that the models reproduce most closely the experimentally observed 3D fluorophore distances in both activation states. The **GAL** locus compaction in the inactive glucose state was found to be ~375 bp/bead whereas in the active galactose state it was set to ~200 bp/bead.

Each ensemble of genome structures was generated from 1000 independent simulations, each starting from random configurations. The optimization procedure consisted of a simulated annealing Molecular Dynamics run followed by Conjugate Gradients score minimization, both performed using IMP (Russel et al., 2012).

**Author contributions**

Conception and design of the work: ED and KW; Data collection: ED and RM; Data analysis and interpretation: ED and RM; development of code for image analysis: ED and PV; modelling: GP & FA

Drafting the article: ED; critical revision of the article: ED, RM, PV, GP, FA & KW.

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**Figure legends**

**Figure 1:** Measuring transcription-dependent chromatin decompaction *in vivo*. (A) Chromosome labeling scheme with LacO and TetO repeats on either side of the GAL7-10-1 locus and example image of cells expressing the reporter constructs. (B) Distribution of distances between LacO and TetO repeats in cell populations constitutively grown in media with glucose, galactose or raffinose as a carbon source. Boxes indicate 25 and 75 percentiles, blue line indicates the median of the distribution. Outliers are shown as grey crosses. (C) Histogram of distance distribution for TetR and LacI dots for 31 kb reporter strain in constitutive growth in glucose (black) or galactose (red). (D) Mean distance of cell population during addition of galactose to cells grown in raffinose (or continuous growth in either galactose or raffinose). Shaded areas show the standard error (SE) of four independent experiments. (E) Mean distance of cell population during addition of glucose to cells grown in galactose (or continuous growth in either galactose or raffinose). Shaded areas show the SE of four independent experiments. (F) Mean distance of cell populations (number of cells per time point approx. 200) during medium switch from glucose to galactose. Cells were pre-induced by overnight growth in galactose and then grown in glucose for one hour. Black curve shows fluorescence of Dextran-Alexa Fluor 680 (3000 MW, anionic) present in the glucose medium to monitor the medium switch in the microfluidics setup.

**Figure 2:** Opening correlates with transcription on single cell level. (A) Individual slices of microscope images showing single molecule FISH signal (bottom) in cells expressing genomic markers (green and red) and stained with DAPI (blue) grown in raffinose (0 min) or after different times of induction with galactose. The cells were hybridized with probes against GAL1, GAL7 and GAL10 mRNAs simultaneously, all labeled with the far-red dye Qasar670. In the image of the 10 min time point, two transcription spots in cells with little cytoplasmic mRNA signal are indicated by white arrowheads. A cell with many cytoplasmic mRNA foci is indicated with a red arrow. (B) Quantification of percentage of cells in which a bright transcription spot could be detected (Mean of three biological replicates, error bars indicate standard deviation). (C) Distribution of distances across the GAL locus for all cells (dark grey) and cells without (light grey) or with (green) transcription spot. Stars indicate that median interval of the median of the distances in cells classified as not carrying a transcription spot and vice versa. n: number of cells analyzed. Histograms below show comparison of distance distributions at 10 min and 30 min after induction. Shown is the data of one representative experiment of three biological replicates. (D) Means of median distances in cells classified for absence or presence of a transcription spot from three biological replicates (Error bars represent standard deviation).

**Figure 3:** The role of acetylation in chromatin opening. (A) Boxplot showing the distance distribution in wildtype cells or the indicated mutants. Cells were grown in raffinose to exponential phase and then glucose, galactose or raffinose were added to a final concentration of 2 % (raffinose 2 %+ 2 %) and cells imaged one hour after sugar treatment. Blue lines represent medians. Dashed lines show median distance in the wildtype in glucose (grey) and galactose (green) with the shaded area representing the 95 % confidence interval as determined by bootstrapping. Outliers are indicated by grey crosses. The boxes extend to the 25th and 75th quartile. Data shown is of one representative experiment of at least three biological replicates. (B) Boxplot showing the distance distribution in cells treated with TSA or DMSO as solvent control for 16 hours. (C) Opening and closing kinetics after treatment with TSA or DMSO for 16 hours. Mean of three biological replicates is shown, error bars represent standard error of the mean.

**Figure 4:** Decompaition correlates with transcriptional activation. (A) 3D distance distributions in the indicated mutants measured one hour after addition of the indicated sugars. Blue lines indicate median of the distribution, grey crosses represent outliers. Dashed lines show median distance in the...
wildtype in glucose (grey) and galactose (green) with the shaded area representing the 95 %
confidence interval as determined by bootstrapping. Each distribution is from one representative
experiment of at least three biological replicates and encompasses > 200 cells. (B) Fold expression of
GAL10 mRNA in different mutants relative to the wildtype determined by quantitative PCR
(normalized for ACT1 mRNA as endogenous control). Means of three or more biological replicates are
shown. Error bars represent standard deviations. (C) Kinetics of chromatin opening and closing in
wildtype and mutants cells. Cells were grown overnight in medium with 2 % raffinose or 2 %
galactose. Galactose or glucose respectively were added or not added to 2 % final concentration at
time point 0. Shown are the means of three or more biological replicates with the standard error
represented by the shaded areas. (D) smFISH for GAL1, GAL7 and GAL10 in the indicated strains
growing at steady state in galactose. Insets show enhanced contrast for indicated cells. Images are
maximum intensity projections of z stacks encompassing the entire cells. (E) Boxplot showing the
intensities of transcriptions spots in the different strains (distribution from one representative
experiment of at least three biological replicates).

Figure 5: Nucleosome chaperones are crucial for chromatin opening. (A) Boxplots showing the
distance distribution in wildtype and asf1Δ cells grown in raffinose and induced with the indicated
carbon sources for one hour. (B) Boxplots showing the distance distribution in wildtype cells and cells
carrying temperature sensitive mutations of the FACT components Pob3 or Spt16. Cells were grown
in raffinose at 25 °C, shifted to 37 °C for 1 hour and then induced with the indicated carbon sources
for one hour at 37 °C. On all boxplots, blue lines indicate the median of the distribution, grey crosses
represent outliers. Dashed lines show median distance in the wildtype in glucose (grey) and galactose
(green) with the shaded area representing the 95 % confidence interval as determined by
bootstrapping. Each distribution is from a representative experiment of at least three biological
replicates and encompasses > 90 cells.

Figure 6: Dependence of 3D distance on ORF length and linear distance on chromosome. (A) Distance
distributions in cells harboring the indicated inducible reporter constructs (pro: GAL1-10 promoter
followed directly by the ADH1 terminator). Stars indicate that median of distribution in galactose is
outside of the 95 % CI interval of the median in raffinose and vice versa (95 % CI determined by
bootstrapping). Representative experiment is shown. (B) Experimentally determined mean 3D
distance in various strains carrying the chromatin location markers TetO and LacO at different
distances from each other on chromosome II. Each datapoint is the mean of three or more medians
from different biological replicates. Grey and black datapoints are plotted from data published in
Dultz et al. 2016 Figure 4B. (C) Average 3D distance vs. genomic distance curves obtained from
simulated structures at two different values of compaction for the GAL locus. The markers
correspond to the experimental averages in the active and inactive conditions. In order to reproduce
the experimentally observed 3D distances, the compaction of GAL locus was set to 200 and 375
bp/bead respectively in the active and inactive conditions. (D) Two randomly selected snapshots of
the simulated GAL locus in the inactive (Gluc, left panel) and active (Gal, right panel) conditions.

Supplemental Figure 1: Transcription activation mutants also affect opening of the GAL locus. 3D
distance distribution of indicated strains. Strains were grown to exponential phase in raffinose
containing medium. The indicated sugars were added to a concentration of 2 % final and
measurements were taken after 1h of incubation.

Supplemental Figure 2: Kinetics of opening in med7-163 temperature sensitive mutant at restrictive
temperatures. Cells were grown overnight in medium with 2 % raffinose or 2 % galactose at 25 °C
and shifted to 37 °C one hour before timepoint 0. Galactose was added or not added to 2 % final
concentration at timepoint 0. Shown are the means of three biological replicates with the standard  
error represented by the shaded areas.

Supplemental Figure 3: Decompaction mediated by VP16 depends on SAGA’s HAT activity. (A)  
Schematic representation of the VP16 induction system. The Gal4 DNA binding domain (Gal4DBD) is  
fused to the activation domain of VP16 linked by the hormone binding domain of human estrogen  
receptor. Upon addition of β-estradiol to the cells, the fusion protein is released from its interaction  
with a heat shock protein and can activate transcription from promoters containing a Gal4  
recognition motif. (B-E) Decompaction kinetics in response to activation via galactose or β-estradiol.  
Cells were grown to exponential phase in medium with 2 % raffinose. Induction was carried out on  
the microscope stage at timepoint 0 with galactose (2 % final concentration) or β-estradiol (1 mM  
final concentration). Shown are the means of three biological replicates with the standard error  
represented by the error bars. (B) Wildtype. (C) Wildtype expressing β-estradiol responsive VP16  
activator. (D) gcn5:: expressing β-estradiol responsive VP16 activator. (E) spt20:: expressing β-  
estradiol responsive VP16 activator.

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rpb1-1 temperature sensitive mutant at permissive and restrictive temperatures. Cells were grown  
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Supplemental Figure 5: RNA dot persists after glucose repression and correlates with more open  
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