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INVITED REVIEW

The heat shock response: A case study of chromatin dynamics in gene regulation[1]

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Abstract: Recent studies in transcriptional regulation using the *Drosophila* heat shock response system have elucidated many of the dynamic regulatory processes that govern transcriptional activation and repression. The classic view that the control of gene expression occurs at the point of RNA polymerase II (Pol II) recruitment is now giving way to a more complex outlook of gene regulation. Promoter chromatin dynamics coordinate with transcription factor binding to maintain the promoters of active genes accessible. For a large number of genes, the rate-limiting step in Pol II progression occurs during its initial elongation, where Pol II transcribes 30–50 bp and pauses for further signals. These paused genes have unique genic chromatin architecture and dynamics compared with genes where Pol II recruitment is rate limiting for expression. Further elongation of Pol II along the gene causes nucleosome turnover, a continuous process of eviction and replacement, which suggests a potential mechanism for Pol II transit along a nucleosomal template. In this review, we highlight recent insights into transcription regulation of the heat shock response and discuss how the dynamic regulatory processes involved at each transcriptional stage help to generate faithful yet highly responsive gene expression.

Key words: heat shock, epigenome, RNA polymerase II, nucleosome turnover.

Résumé : Des études récentes portant sur la régulation de la transcription réalisées avec le système de réponse au choc thermique de Drosophila ont élucidé plusieurs des processus dynamiques de régulation qui gèrent l'activation et la répression de la transcription. Le point de vue classique qui veut que le contrôle de l'expression génique survienne au point de recrutement de l'ARN polymérase II (Pol II) s'oriente maintenant vers une conception plus complexe. La dynamique de la chromatine d'un promoteur se coordonne avec la liaison des facteurs de transcription pour maintenir accessibles les promoteurs des gènes actifs. Pour un grand nombre de gènes, l'étape limitante de la progression de Pol II survient lors de son élongation initiale, où Pol II transcrit de 30 à 50 paires de bases et s'interrompt en attendant d'autres signaux. La chromatine de ces gènes « en pause » possède une architecture et une dynamique uniques comparativement aux gènes où le recrutement de Pol II est une étape limitante de l'expression. La progression de l'élongation de Pol II le long de ces gènes produit un turnover des nucléosomes, un processus continu d'éviction et de remplacement, ce qui suggère l'existence d'un mécanisme potentiel de transit de Pol II le long de la matrice nucléosomale. Dans cet article de revue, nous mettons en lumière les aperçus récents de la régulation transcriptionnelle de la réponse au choc thermique, et nous discutons de la façon dont les processus de régulation dynamiques impliqués dans chaque étape de la transcription aident à générer une expression génique fidèle et pourtant hautement réactive. [Traduit par la Rédaction]

Mots-clés : choc thermique, épigénome, ARN polymérase II, turnover des nucleosomes.

Introduction

Transcriptional regulation is central to development, environmental response, and disease progression and occurs at each major stage of the transcription process. In the initial stage, gene specific transcription factors recruit RNA polymerase II (Pol II) and general transcription factors to the promoter of the gene, forming the preinitiation complex (PIC), in response to signal cascades that link transcription with intra- and extracellular cues (Baumann et al. 2010). Promoter DNA is then melted, and Pol II becomes dependent on factors that prevent backtracking and arrest (Fish and Kane 2002). Promoter clearance occurs when Pol II transitions into productive initiation, but it can also pause 30–50 bp downstream of the transcription start site (TSS) (Li and Gilmour 2011). For a large percentage of the genes, this pause in elongation serves as the rate-limiting step in gene expression and provides an added layer of regulation (Levine 2011). Once pausing is relieved, Pol II enters productive elongation where it encounters an ordered chromatin template. After the whole gene is transcribed, Pol II dissociates from the template and can then be recycled to begin the process anew (Shandilya and Roberts 2012). At each stage, a multitude of factors associate with and regulate Pol II, primarily through the C-terminal domain (CTD) of its largest subunit. The CTD consists of tandem heptapeptide repeats of Y-S-P-T-S-P-S. Each individual residue can be modified, but phosphorylation of the serine residues is most critical to Pol II function. The hypophosphorylated Pol II is recruited to promoters to form the PIC, but the phosphorylation of the fifth serine residue (Ser5) transitions Pol II into productive initiation (Kim et al. 2010). Pausing after initiation is relieved when the second serine (Ser2) becomes phosphorylated (Kim et al. 2010). Aside from the factors that regulate Pol II itself, the CTD also acts as a docking region for many chromatin-related factors that modulate the Pol II template. Many of the mecha-

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nisms regulating Pol II, and its interaction with chromatin, have been discovered using model systems for transcription such as the heat shock response in *Drosophila*.

The heat shock response has long been a gold standard for studying gene regulation. An evolutionarily conserved defense mechanism, the heat shock response involves a rapid and global transcriptional response to protect the cell against many types of stressors, including heat, cold, oxidative stress, heavy metal, and alcohol poisoning (Akerfelt et al. 2010). Under normal conditions, the master heat shock transcription factor HSF exists as an inactive monomer. At the onset of stress, HSF trimerizes and binds to promoters of heat shock protein (*hsp*) genes. HSF binding signals the release into productive elongation of Pol II that is paused downstream of the TSS, resulting in synchronous activation of stress-inducible genes from 10–1000-fold induction within minutes (Lindquist 1986; Lis 1998). Concurrent with the activation of *hsp* genes is the downregulation of global transcription and a temporary halt in normal translation to prevent the accumulation of misfolded products (Lindquist 1981; McKenzie et al. 1975; Tissieres et al. 1974). The simultaneous presence of gene induction and repression occurring in a fast system makes the heat shock response ideal for probing the dynamic processes in chromatin that accompany transcriptional regulation.

The primary unit of chromatin is the nucleosome, 147 bp of DNA wrapped around an octameric histone complex, which is organized across the genome in a conserved fashion (Luger et al. 1997; Mavrich et al. 2008). Since the majority of eukaryotic DNA exists as nucleosomes, factors must necessarily counteract this packaging to allow Pol II access to the DNA at each stage of the transcription process. In fact, eukaryotes have evolved many redundant mechanisms to allow DNA accessibility during transcription that converge as major hubs of regulation. These include histone posttranslational modifications (PTMs), incorporation of histone variants, remodeling by ATP-dependent remodelers, and nucleosome eviction and replacement (Henikoff 2008). For example, promoters have evolved to restrict nucleosome occupancy through DNA sequences that are antinucleosomal (Iyer and Struhl 2012) and through preferential incorporation of variant histones that form inherently less stable nucleosomes (Jin and Felsenfeld 2007). This then allows specific transcription factors to access the promoter region to recruit Pol II and associated general transcription factors to form the PIC. During active elongation, many factors that modify chromatin associate with the traveling Pol II, including histone acetyltransferases (HATs) and histone methyltransferases (HMTs) that modify N-terminal tails of genic histones during transcription (Jenuwein and Allis 2001; Rando 2012). These PTMs are predicted to alter the conformation of the nucleosome, such as an opening of the nucleosome upon acetylation (Czarnota et al. 1997). Histone chaperones and remodelers also associate with Pol II during elongation to promote histone variant deposition that results in partial to full unwrapping of the nucleosome, hinting at a potential mechanism for Pol II transit through the nucleosomal template (Henikoff 2008).

In light of recent studies using the heat shock response as a model system for gene regulation, we review the dynamic interplay between Pol II and chromatin and highlight the regulatory mechanisms that govern the transcription process, both in gene activation and repression. We also examine the decades-old question of how Pol II transcribes through a nucleosome. We discuss how the physical properties of Pol II, such as elongation rate and density on a given gene, can affect the fate of transcribed nucleosomes. Finally, we consider how nucleosomes can regulate Pol II transit and, consequently, gene expression.

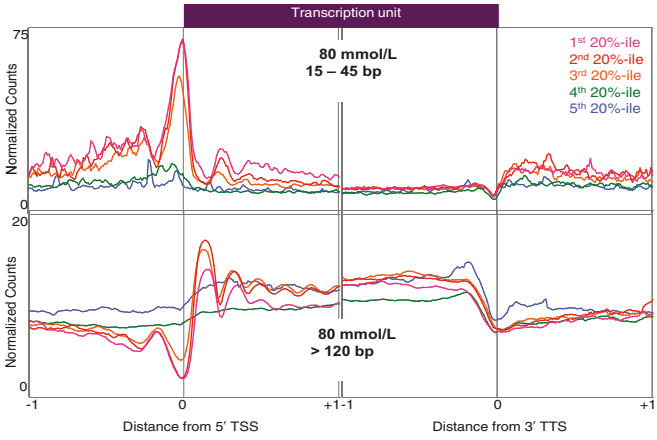
Gene activation and the heat shock response

Promoter chromatin dynamics

One of the earliest events in gene activation is the promoter binding of gene specific transcription factors, such as HSF, which serve as transcriptional effectors of intra- and extracellular signaling cascades. These factors must access specific sequences in the context of nucleosomal DNA. HSF rapidly binds to promoters of *hsp* genes upon heat shock, but under normal conditions, the chromatin at *hsp* gene promoters already exists in an accessible state. Early mapping of chromatin structure using DNase I, which under limiting conditions preferentially digests highly accessible DNA, revealed that the *hsp* promoters are hypersensitive to digestion (Costlow and Lis 1984; Wu 1980). Furthermore, the hypersensitive regions coincide with the binding sites of sequence-specific factors such as HSF and GAGA factor (Costlow and Lis 1984; Tsukiyama et al. 1994). DNase I hypersensitivity is independent of the HSF binding sites heat shock elements (HSEs), as its deletion or mutation does not significantly alter chromatin architecture on the *Hsp26* gene (Lu et al. 1993). In contrast, changes in the underlying sequence of the GAGA element, TSS, and the pause site for Pol II reciprocally influence HSF binding at its cognate sites in the *Drosophila Hsp70* gene (Lu et al. 1993; Shopland et al. 1995). The relationship between promoter chromatin dynamics and transcription factor binding has been interrogated genome-wide using HSF (Guertin and Lis 2010; Guertin et al. 2012). The presence of DNase I hypersensitive sites, along with hyperacetylation of histones, predict which binding sites will be bound by HSF or not, suggesting that promoter nucleosome dynamics participate in target selection and activation (Guertin and Lis 2010; Guertin et al. 2012). A comparison of other transcription factor binding and genome-wide DNase I hypersensitive sites suggests that the use of promoter chromatin context to differentiate among binding sites is general (Rhee and Pugh 2011).

Complementary to DNase I, micrococcal nuclease (MNase) digestion patterns can provide another perspective of promoter chromatin dynamics. MNase is an endonuclease that introduces nicks on exposed double-stranded DNA (Desai and Shankar 2003) and further nibbles ends until it encounters a block in the form of bound protein, such as the nucleosome (Henikoff et al. 2011; Kent et al. 2011). MNase mapping of nucleosomes coupled with genome-wide mapping technologies has revealed a canonical nucleosome organization that is conserved across eukaryotes (Mavrich et al. 2008). A prominent aspect of this organization is a nucleosome-depleted region near the TSS of most genes, followed by well-positioned nucleosomal arrays within the gene bodies. The nucleosome-depleted region results from a highly dynamic nucleosome structure at the TSS that is enriched for the H3.3 and H2A.Z histone variants (Jin and Felsenfeld 2007). Nucleosome depletion at the TSS is important for reliable gene expression (Bai et al. 2010). The enzymatic action of MNase, however, suggests that any protein will confer protection when bound to the DNA. When coupled with paired-end sequencing that can reveal the size of the MNase-digested fragment, MNase can also be used to map any DNA-binding protein, allowing for the visualization at high resolution of both the nucleosomal (~150 bp) and subnucleosomal (<90 bp) components of chromatin in yeast, where the latter consists primarily of transcription factors and chromatin remodelers (Henikoff et al. 2011). In *Drosophila* cells, the heat shock response system proved useful in revealing the dynamics of both nucleosomal and subnucleosomal chromatin components in response to transcriptional perturbation (Teves and Henikoff 2011). On the uninduced *Hsp70* promoter, short MNase-protected fragments mapped to previously identified DNase I hypersensitive sites (Costlow and Lis 1984) and binding sites for GAGA factor and TATA binding protein (Teves and Henikoff 2011). Genome-wide analyses of these subnucleosomal fragments showed that they localize primarily at the promoters and TSSs of most genes and

Fig. 1. The distribution of subnucleosomal and nucleosomal particles within the transcription unit is correlated with expression. All genes were grouped into quintiles by expression level, and the average normalized counts per 10 bp window was determined for each quintile in the 2-kb region flanking the transcription start site (TSS) and transcription termination site (TTS).

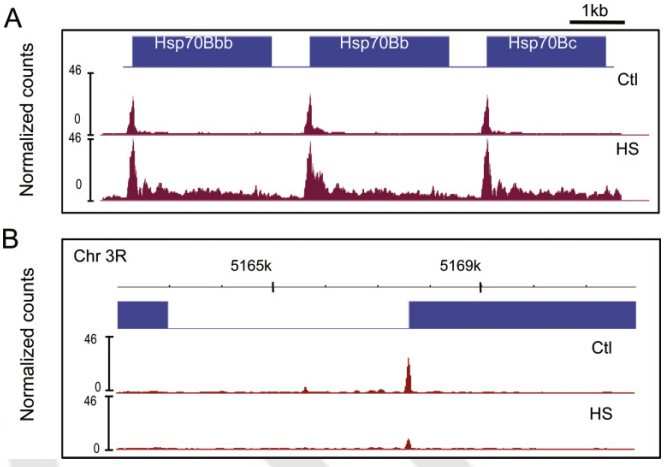


that their presence is highly correlated with gene expression (Fig. 1), further confirming that these fragments result from MNase protection of bound transcription factors. These subnucleosomal fragments also localize to regions of low nucleosomal occupancy, suggesting a dynamic equilibrium between nucleosomes and DNA-binding proteins. Upon heat shock induction, the subnucleosomal peaks at the *Hsp70* promoter decreased (Teves and Henikoff 2011), highlighting the dynamic nature of promoter chromatin during gene expression.

Paused RNA polymerase and chromatin

The *hsp* genes were among the first genes identified where the rate-limiting step for expression occurs at Pol II elongation (Gilmour and Lis 1986). For decades, the paradigm for gene expression posited that regulation occurs to modulate the recruitment/initiation of Pol II. That is, once Pol II is recruited to the gene, it fires uniformly to produce the transcript. The *hsp* genes, however, were known to contain Pol II on the gene body even under non-induced conditions. Furthermore, the Pol II is located about 30 bp downstream of the TSS and was shown to be transcriptionally competent, containing ~30 bp of nascent RNA chain (Rougvie and Lis 1988). This paused Pol II is stable, persisting for long periods of time, and associating with factors that promote pausing, such as the 5,6-dichlorobenzimidazole (DRB) -sensitivity inducing factor (DSIF), the negative elongation factor (NELF) (Missra and Gilmour, 2010; Wu et al. 2003), and components of the RNA-interference (RNAi) pathway (Cernilogar et al. 2011). Upon activated HSF binding, the kinase positive elongation factor P-TEFb is recruited to the paused Pol II and phosphorylates DSIF, NELF, and Pol II itself at Ser2 of the CTD to transform the paused complex into an actively elongating one (Peterlin and Price 2006). NELF dissociates from Pol II and the phosphorylated DSIF subsequently acts to promote elongation as it travels down the gene with Pol II (Yamada et al. 2006). In recent years, promoter-proximal Pol II pausing has emerged as a global mechanism of gene regulation. Genome-wide studies in *Drosophila*, mouse, and human cells have estimated that roughly 30% of the genome is regulated at the level of elongation (Levine 2011). This list is highly enriched for developmental genes. In one estimate, at least half of the *Drosophila* developmental control genes contain paused Pol II (Levine 2011). One proposal suggests that regulation at the elongation step has evolved to allow for rapid and synchronous activation of a set of genes to allow for precisely timed development within a population of cells (Levine 2011).

Fig. 2. RNA polymerase II (Pol II) from the low-salt-soluble chromatin represents the stalled species. The mapped reads for Pol II ChIP from low-salt-soluble input material are converted into normalized reads in the 87C *Hsp70* region (A), and a representative control region in chromosome 3R (B).



The initial observation that the MNase-digested, low-salt-soluble, active chromatin fraction of *Drosophila* S2 cells is enriched for short (~50 bp) DNA fragments that map to the TSSs of genes known to contain paused Pol II (Weber et al. 2010) suggested that these fragments result from paused Pol II protection of the pause site from MNase digestion. Indeed, when the low-salt-soluble fraction was used as input material for native chromatin immunoprecipitation (ChIP) with antibodies against Pol II, this hypothesis was confirmed (Teves and Henikoff 2011). At the *Hsp70* gene, low-salt-soluble Pol II localizes at 30 bp downstream of the TSS of the uninduced *Hsp70* gene, and heat shock induction further intensifies the Pol II signal at the pause site as the gene becomes highly expressed (Fig. 2A). In contrast, the low-salt-soluble Pol II is lost from the TSS of most nonheat shock responsive genes (Fig. 2B).

Several studies have shown that the chromatin architecture of paused genes is markedly different from nonpaused genes (Gilchrist et al. 2008, 2010; Weber et al. 2010). The canonical nucleosome organization is evident in nonpaused genes, containing an array of well-positioned nucleosomes within gene bodies. Interestingly, the nucleosomes within paused genes are less well positioned, lower in occupancy, and show increased nucleosome turnover (discussed below) (Teves and Henikoff 2011). Also, one study showed that when Pol II pausing is inhibited, paused genes gain a nucleosome at the TSS, suggesting that Pol II elongation and nucleosomes cooperate to maintain regulation of these genes (Gilchrist et al. 2010). However, the regulation occurring at the TSS of paused genes does not explain the disrupted nucleosome organization within their gene bodies. As stated above, one proposed role for regulating Pol II at the elongation step is to provide a mechanism for a fast yet uniform rate of expression. By preventing bursts of multiple Pol IIs at a given time, the organism can then generate synchronous expression of key genes across multiple cells (Levine 2011). In vitro, such a uniform rate of Pol II progression allows for the survival of the nucleosome, while bursts of multiple Pol IIs result in nucleosome eviction (Bintu et al. 2011; Jin et al. 2010; Kulaeva et al. 2010). Given this reasoning, the expectation is that paused genes would experience less nucleosome turnover, not more. An explanation for this discrepancy may be found in the mechanics of Pol II itself.

As Pol II elongates, it generates a wave of positive supercoils ahead and negative supercoils behind (Baranello et al. 2009; Liu and Wang 1987). One estimate using mathematical modeling based on experimentally determined physical properties of Pol II and chromatin during transcription suggests that a wave of posi-

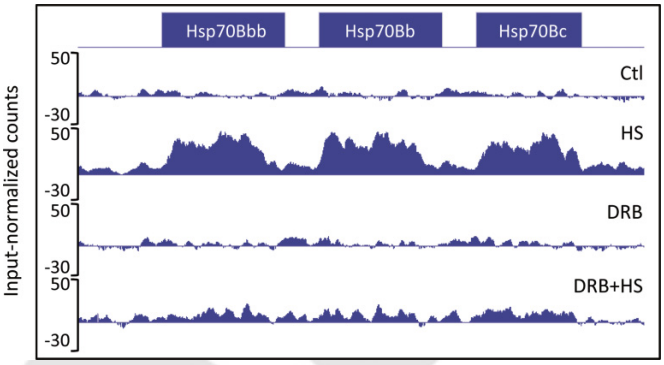
tive supercoils generated by transcription of 5 bp propagates through chromatin at a rate of 2 orders of magnitude faster than Pol II elongation (Becavin et al. 2010). In yeast, accumulation of unresolved positive supercoils inhibits transcription globally, affecting over 80% of yeast genes (Gartenberg and Wang 1992; Joshi et al. 2010). Topoisomerases relieve the resulting torsional tension caused by processive enzymes such as Pol II, thereby allowing for continuous and successive rounds of transcription. Interestingly, inhibition of topoisomerase II and release of torsional tension from DNA nicking lead to decreased DNase I hypersensitivity in the promoters of active β-globin genes (Villeponteau et al. 1984; Villeponteau and Martinson 1987), suggesting an intimate connection between DNA superhelicity and chromatin structure. Indeed, positive supercoiling of DNA templates has been shown to restrict nucleosome assembly (Gupta et al. 2009), while negative supercoiling promoted assembly (Hizume et al. 2004). When the *Hsp70* gene was shown to lose nucleosomes ahead of the initial transcribing Pol II upon heat shock (Petesch and Lis 2008), Zlatanova and Victor (2009) proposed that the Pol-II-generated wave of positive supercoils ahead of Pol II destabilizes downstream nucleosomes. Paradoxically, active elongation on the *Hsp70* gene does not seem necessary as DRB inhibition of Poll II still resulted in the loss of nucleosomes in the *Hsp70* gene body at heat shocked cells (Petesch and Lis 2008). However, DRB specifically inhibits the kinase activity of P-TEFb, which acts at the transition of initiation and elongation ~30 bp downstream of the TSS by catalyzing the phosphorylation of Ser2 on the CTD (Marshall et al. 1996; Marshall and Price 1995). If the estimate that transcription of 5 bp is sufficient to generate a wave of positive supercoils (Becavin et al. 2010) is accurate, it may actually predict that nucleosomes would still be lost at the *Hsp70* gene even under DRB inhibition of elongation. Perhaps the pausing of Pol II after transcription of ~30 bp maintains these genes under torsional stress to destabilize genic nucleosomes. In this way, once the signal for elongation is received, Pol II transits through the gene most efficiently. Probing the relationship among Pol II mechanics, DNA structure, and nucleosome organization will further our understanding not only of paused Pol II regulation, but also of the very basic mechanisms of the transcription process itself.

Nucleosome dynamics and Pol II elongation

Once Pol II enters productive elongation, it faces an array of ordered nucleosomes. Interestingly, gene bodies and exons have higher nucleosome occupancy than introns and intergenic regions (Chen et al. 2010). Yet, Pol II moves along chromatin in vivo at a rate comparable with its rate of movement along naked DNA templates, whereas a single nucleosome in vitro presents a formidable barrier to transcriptional elongation (Knezetic and Luse 1986; Luse and Studitsky 2011). The question of how Pol II moves through a nucleosome, and the resulting fate of the nucleosome after Pol II has passed through, has been the subject of debate for decades (Petesch and Lis 2012). Several in vitro models have been proposed for Pol II traversal through a nucleosome that ultimately centre on the resulting nucleosome and are as follows: complete survival of the octamer, partial survival where either one or both H2A/H2B dimers are lost while the H3/H4 tetramer is retained (Kireeva et al. 2002; Kuryan et al. 2012), and lastly, full dissociation of the octamer (Bintu et al. 2011; Jin et al. 2010). To potentially discern among these models, we must first understand nucleosome dynamics.

Most new nucleosomes are formed immediately behind the replication fork and consist of canonical histones. Histones can also be replaced outside of replication and result in the incorporation of special histone variants. Two universal histone variants are deposited during transcription, H2A.Z and H3.3. To replace the H2A/H2B dimer, the nucleosome has to partially unwrap (Ahmad 2005). However, replacement of the H3 in the central $(H3/H4)_2$ tetramer with the H3.3 variant results in unwrapping of the

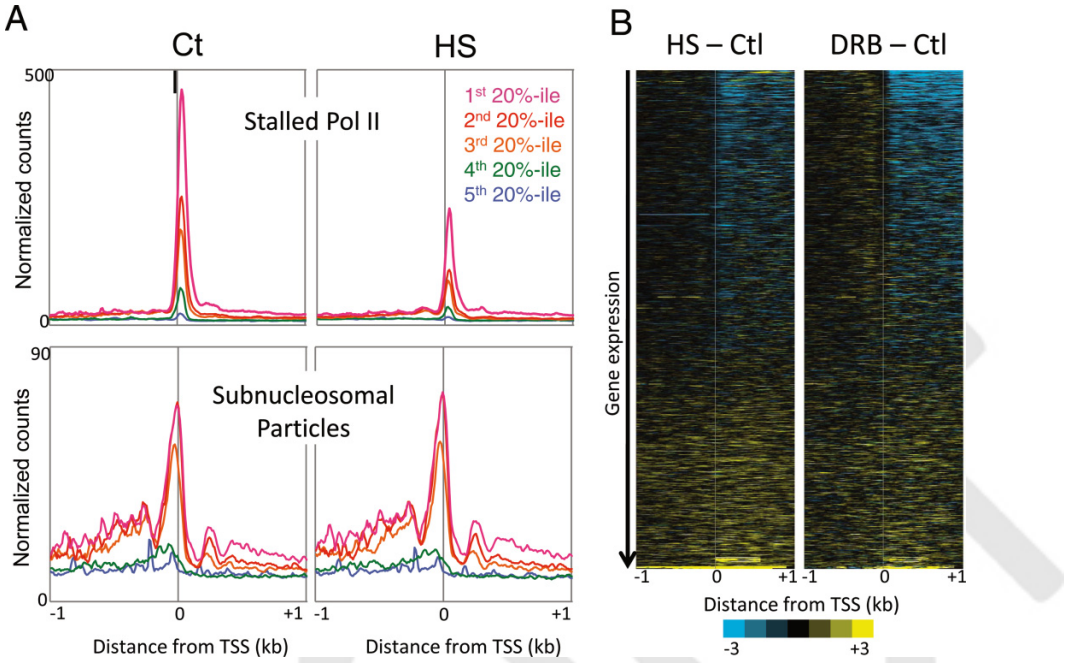
Fig. 3. Nucleosome turnover is dependent on active transcription. Covalent attachment of tags to capture histones and identify turnover signals before and after heat shock (HS) and with or without the RNA polymerase II (inhibitor 5,6-dichlorobenzimidazole (DRB) treatment are shown for the 87C *Hsp70* region.



nucleosome beyond the dyad axis and eviction (Ahmad 2005). Therefore, H3.3 incorporation marks nucleosome turnover events. Profiling of H3.3 in the *Drosophila* genome has revealed that the highest level of H3.3 deposition occurs within bodies of highly transcribed genes (Mito et al. 2005). The correlation between H3.3 variant deposition and transcription suggests that elongation results in disruption of transcribed nucleosomes. Because there are only 4 amino acids that differ between H3 and H3.3, these experiments were performed using exogenous transgenes that were induced for several days, reflecting steady-state levels of deposition. To further define nucleosome dynamics, a new method was developed adapting metabolic labeling of newly synthesized proteins for chromatin profiling (Deal et al. 2010). The methionine analog azidohomoalanine (Aha) is incorporated into newly synthesized proteins in the absence of methionine. The azide moiety of Aha can then react with an alkyne-adapted biotin linker through a copper catalyzed cycloaddition reaction, providing a biotin tag on all newly synthesized proteins. Isolation of nuclei followed by extraction of MNase-digested chromatin provides the input material for streptavidin pulldown to enrich for nucleosomes containing a newly synthesized histone, thus marking a recent turnover event. This technique is called covalent attachment of tags to capture histones and identify turnover (CATCH-IT). CATCH-IT signals correspond well with H3.3 incorporation, confirming that H3.3 marks sites of nucleosome turnover within bodies of transcribed genes. Brief Aha pulses allowed for nucleosome turnover times to be estimated. As expected from the H3.3 profiling experiments, the highest expressed genes experience the highest rate of nucleosome turnover, ~20 turnover events per nucleosome during each cell cycle (Deal et al. 2010). These experiments imply that the rate of Pol II elongation is directly related to the rate of nucleosome turnover in *Drosophila* cells, which brings up an important question. Are nucleosomes actively removed ahead of the transcribing Pol II to allow progression? As before, the heat shock response provides insight into this process.

As early as 40 years ago, scientists discovered that certain regions of the *Drosophila* polytene chromosomes undergo massive decondensation in response to heat, producing the heat shock puffs (Simon et al. 1985). These puffs are accompanied by changes in DNase I sensitivity and MNase digestion patterns, such that the gene body becomes more sensitive to nuclease digestion after induction and the ordered array of nucleosomes becomes disordered (Levy and Noll 1981; Wu et al. 1979). Furthermore, multiple ATP-dependent chromatin remodelers coordinate in regulating *hsp* genes in yeast (Erkina et al. 2010; Shivaswamy and Iyer 2008) as individual nucleosomes become remodeled throughout the ge-

Fig. 4. Effects of heat shock-mediated genome-wide repression on chromatin dynamics. The occupancy of stalled RNA polymerase II (Pol II) decreases during heat shock (HS; top), whereas the average occupancy of subnucleosomal particles shows no net change (bottom) (A). Nucleosome turnover decreases genome-wide during heat shock in a similar manner when Pol II is artificially inhibited using 5,6-dichlorobenzimidazole (DRB) (B). Ctl, control; and TSS, transcription start site.



nome in response to heat (Shivaswamy et al. 2008). At the *Hsp70* gene, Petesch and Lis (2008)discovered that nucleosomes are lost within seconds of heat shock, even before the first transcribing Pol II has passed through. CATCH-IT signals after 15 min of heat shock show prominent peaks of turnover (Fig. 3), suggesting that the initial loss of nucleosomes is followed by subsequent cycles of assembly and disassembly (Teves and Henikoff 2011). At least in the case of *Hsp70*, nucleosomes are disrupted ahead of the transcribing Pol II, but continue to associate within the activated *Hsp70* gene, despite the high density of Pol IIs.

Gene repression and chromatin

Activation of the *hsp* genes during heat shock occurs concurrent with a rapid and global reduction in transcription, although until recently, how this process occurs was unknown. In *Drosophila* salivary glands, Pol II is lost from transcriptionally active, developmentally regulated puff sites upon heat shock (Jamrich et al. 1977). In *Drosophila*, the low-salt-soluble, stalled Pol II is lost throughout the genome, particularly from the TSSs of most genes (Teves and Henikoff 2011) (Fig. 2B, Fig. 4A), and is dependent on an active RNAi machinery (Cernilogar et al. 2011). Interestingly, AGO2-associated small antisense RNAs are increased genome-wide upon heat shock, suggesting a role for siRNAs in the *Drosophila* heat shock response (Cernilogar et al. 2011). Similarly, in mammalian systems, global repression is mediated by the heat shock induced expression of noncoding RNAs that function to disrupt contacts between Pol II and promoter DNA (Yakovchuk et al. 2009), implying that loss of Pol II has coevolved with the heat shock response for efficient global repression. Interestingly, although the stalled Pol II is lost from most genes, the genome-wide subnucleosomal pattern at the TSS is maintained during heat shock (Fig. 4A), suggesting that most of the transcription factors remain associated with the promoters of most genes even as Pol II is lost (Teves and Henikoff 2011). Similarly, in yeast, most of the factors in the preinitiation complex remain bound to promoters after heat shock (Zanton and Pugh 2006). Such maintenance of the underlying transcriptional machinery may provide the mechanism for efficient recovery after the stress is removed. Concurrent with the loss of stalled Pol II, nucleosome turnover within bodies decreased in a similar manner as when Pol II elongation was artificially inhibited using a drug (Teves and Henikoff 2011) (Fig. 4B). This implies that Pol II elongation is a major cause of nucleosome turnover within gene bodies. However, nucleosome turnover was not completely eliminated, suggesting that there are transcription-independent mechanisms that maintain nucleosome dynamics.

The global transcriptional repression that occurs under heat shock is perhaps unique in mechanism because it is necessarily fast, global, and reversible. Upon removal of heat, cells more gradually return to their normal physiological state, including their normal transcription levels. It is possible that this particular mechanism for repression, the removal of Pol II followed by decreased nucleosome turnover while promoter chromatin architecture is maintained, is used for certain genes that require plastic regulation. For complete gene silencing, cells have evolved redundant mechanisms, many of which take advantage of the inhibitory properties of heterochromatin (Wutz 2011). It is likely that dynamic systems involved in gene expression, such as Pol II and chromatin dynamics, serve as feedback mechanisms for regulation. During activation, Pol-II-mediated disruption of chromatin, both at promoters and gene bodies, allows for increased DNA accessibility for future transcription events, while the decreased chromatin dynamics inhibit factor access to DNA and thus promoting repression. In this way, minor changes in chromatin dynamics can have important consequences in regulating gene expression levels.

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