



Published in final edited form as:

Mol Cell. 2016 April 7; 62(1): 63–78. doi:10.1016/j.molcel.2016.02.025.

Mammalian Heat Shock Response And Mechanisms Underlying Its Genome-wide Transcriptional Regulation

Dig B. Mahat¹, H. Hans Salamanca^{1,2}, Fabiana M. Duarte¹, Charles G. Danko³, and John T. Lis¹

¹Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York, USA

²Present address: College of Medicine, Upstate Medical University, Syracuse, New York, USA

³Baker Institute for Animal Health, Cornell University, Ithaca, New York, USA

Summary

The Heat Shock Response (HSR) is critical for survival of all organisms. However, its scope, extent, and the molecular mechanism of regulation is poorly understood. Here we show the genome-wide transcriptional response to heat-shock in mammals is rapid, dynamic, and results in induction of several hundred and repression of several thousand genes. Heat Shock Factor-1 (HSF1), ‘the master regulator’ of the HSR, controls only a fraction of heat-shock induced genes, and does so by increasing RNA polymerase II release from promoter-proximal pause. Notably, HSF2 does not compensate for the lack of HSF1; however, Serum Response Factor appears to transiently induce cytoskeletal genes independently of HSF1. The pervasive repression of transcription is predominantly HSF1-independent, and is mediated through reduction of RNA polymerase II pause-release. Overall, mammalian cells orchestrate rapid, dynamic, and extensive changes in transcription upon heat-shock that are largely modulated at pause-release, and HSF1 plays a limited and specialized role.

eTOC Blurbs

Using PRO-seq, Mahat et al. identify hundreds of genes that are upregulated and thousands that are downregulated transcriptionally in mammals during heat-shock. A majority of this regulation is independent of HSF1 and HSF2, and many rapidly-and-transiently-upregulated cytoskeletal genes depend on SRF. Both upregulation and downregulation are modulated at promoter-proximal pause-release.

Correspondence: jtl10@cornell.edu.

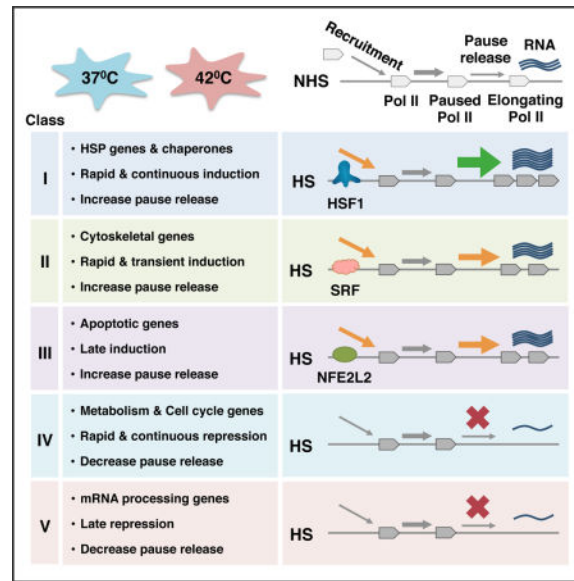
Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author contributions

D.B.M., H.H.S. and J.T.L. conceived and designed the study. D.B.M. performed the heat-shock experiments and prepared PRO-seq and ChIP-seq libraries. D.B.M. analyzed the data and D.B.M. and J.T.L. interpreted the results. C.G.H performed transcription wave calling analysis using hidden Markov model. D.B.M. and J.T.L. wrote the manuscript and H.H.S., F.M.D., C.G.D. helped in editing the manuscript.

Accession Numbers:

Raw and processed PRO-seq and ChIP-seq data are deposited in GEO (GSE71708).



Introduction

The evolutionarily conserved heat shock response (HSR) protects cells from the proteotoxic environment of heat stress. Elevated temperature and other stresses triggers HSR leading to rapid and robust induction of heat shock protein genes (*Hsps*) (Lindquist and Craig, 1988). HSPs are molecular chaperones responsible for maintaining protein homeostasis and are critical for survival during stress (Lindquist and Craig, 1988).

The HSR is orchestrated at the level of transcription by heat shock transcription factor (HSF) (Parker and Topol, 1984). Vertebrates have four *Hsf* genes, *Hsf1-4*. HSF1 is considered the master regulator of the HSR and is the ortholog of the sole *Hsf* gene in invertebrates. HSF2 is the ubiquitously expressed HSF1 paralog that interplays with HSF1 during HSR and is involved in developmental pathways (Sarge et al., 1991). HSF3 and HSF4 show tissue-restricted expression and their roles in HSR remain to be explored (Akerfelt et al., 2010). HSF1 is constitutively expressed as an inactive monomer, but upon HS, it trimerizes and binds to the inverted repeats of nGAAn pentamers known as heat shock element (HSE) in the promoter of *Hsp70* (Perisic et al., 1989; Westwood et al., 1991). HSF1 then recruits co-factors that dramatically increases the transcription of *Hsps* (Akerfelt et al., 2010).

HSF1 also plays an important role in aging and longevity (Morley and Morimoto, 2004), protects organisms from obesity by regulating energy expenditure (Ma et al., 2015), and reduces susceptibility to stress in elderly hearts (Locke and Tanguay, 1996). More importantly, cancer cells co-opt HSF1 to support malignancy (Dai et al., 2007; Mendillo et al., 2012), making its reduction in level or activity a potentially better target for cancer therapy than the several small inhibitory molecules against HSPs that are in ongoing clinical trials. In contrast, enhanced chaperone expression by activation of HSF1 can improve prognosis of protein aggregates-related neurodegenerative disorders (Neef et al., 2011). This dichotomy in the roles of HSF1 in cancer and neurological diseases requires a deeper

understanding of the precise molecular mechanism of HSF1 driven gene regulation before clinical application of HSF1-based therapeutic tools.

Understanding the HSR-regulated networks of genes is also important to decipher how healthy cells maintain proteostasis. Earlier genome-wide studies using microarray and RNA-sequencing indicated that additional genes besides *Hsps* are regulated during the HSR (Brown et al., 2014; Trinklein et al., 2004). These assays measure stable mRNA and lack the temporal resolution to reveal the first-order transcriptional regulatory mechanisms. Transcription regulation consists of several steps any one of which might regulate gene expression, including RNA polymerase II (Pol II) recruitment to the promoter, promoter-proximal pausing, release from the pause, and Pol II elongation rates (Fuda et al., 2009). Despite many efforts, the breadth of transcriptional regulation during HSR, the precise step(s) modulated, and the kinetics and dynamics of the regulation remain to be fully understood.

Here, we examine the HSR at the transcriptional level using precision nuclear run-on sequencing (PRO-seq) (Kwak et al., 2013) - an assay that maps transcriptionally engaged Pol II at nucleotide resolution by nascent RNA sequencing. PRO-seq measurements in mouse embryonic fibroblasts (MEFs) derived from HSF1 knockout (*Hsf1*^{-/-}) mouse, HSF1 & HSF2 double knockout (*Hsf1&2*^{-/-}) mouse, and their wild type (WT) littermate (Lecomte et al., 2010; McMillan et al., 2002; 1998) were compared to each other and to HSF1 ChIP-seq data to identify the genome-wide targets of HSF1 and its role in HSR. A time course of PRO-seq and HSF1 ChIP-seq during HS revealed both primary and secondary transcriptional responses. We find HSF1 to be critical for induction of *Hsps*, other chaperones, and over 200 additional genes; however, the activation and repression of transcription during HSR are remarkably extensive and the majority of these changes are HSF1-independent. Among these, a collection of cytoskeletal genes is transiently induced by a novel regulator of the HSR. Our analyses decipher the mechanistic step in transcription where HSF1 acts to induce transcription, as well as the HSF1-independent mechanism of global repression. Together, these comprehensive and highly sensitive analyses indicate that HSR is much more elaborate than previously appreciated, and regulators in addition to HSF1 are mobilized.

RESULTS

HS triggers rapid, robust, and diverse changes in transcription

To characterize the global changes in transcription associated with HSR and to understand the role of HSF1, we performed genome-wide PRO-seq assays on WT and *Hsf1*^{-/-} MEFs. We prepared two biological replicates of PRO-seq libraries at 37°C (NHS) and 2.5, 12, and 60 minutes after an instantaneous HS at 42°C (Figure 1A). The libraries were sequenced to high depth (Table S1) and mapped to the mouse genome (mm10). The biological replicates correlated well (Figure S1A and Table S2), and as expected, the *Hsf1*^{-/-} MEFs produced no HSF1 protein (Figure 1B), nor any PRO-seq reads in the deleted region of the *Hsf1* gene (Figure S1B).

Normalization of genomic libraries by conventional methods such as total mapped reads or ribosomal RNA reads are inadequate when dealing with significant changes in total transcription. Therefore, we devised a novel approach for normalizing the PRO-seq libraries using PRO-seq reads from the 3' end of very long genes (>400 kb), the regions beyond the advancing or receding wave of Pol II even at the longest HS time point (60'HS) (Figures S1C and S1D). This normalization approach was validated using three different tests. First, the PRO-seq density after normalization in the 3' ends of significantly upregulated and downregulated genes at 12'HS is unchanged, while the 5' ends show the expected change (Figures S1E – note this includes a larger collection of genes than used for normalization and excludes the genes used for normalization). Second, a set of genes identified as unaffected during HSR in MEFs using microarrays (Trinklein et al., 2004) showed no changes in PRO-seq density between NHS and HS conditions after normalization (Figures S1F). Third, a previously defined group of housekeeping genes (la Grange et al., 2005) also showed no systematic deviation between HS and NHS conditions (Figure S1G). After normalization, genes that could be falsely detected in differential expression analysis due to a) transcription running past the 3' end of upstream genes and b) internal TSS or intronic enhancers were eliminated using dREG (Danko et al., 2015) (Figure S2A) (see methods).

A substantial fraction of the transcriptome changes upon HS, and the number of genes detected and the levels of change progressively increase with time (Figures 1C and S2B). Moreover, the kinetics and dynamics of change in transcription is remarkably diverse (Figure 1D). First, many *Hsps* are robustly and persistently induced in an HSF1-dependent manner (such as *Hsph1* with ~ 60 fold induction). Second, many genes are immediately and transiently induced (like *Vcl*), where the advancing wave of newly transcribing Pol II is particularly noticeable. This induction is independent of HSF1. Third, many genes show late induction (such as *Ptprm*) and the majority of these are independent of HSF1. Fourth, a large fraction of the expressed genes (like *Kif14*) are significantly downregulated, and nearly all are independent of HSF1. Overall, DESeq2 identifies significant upregulation of 10% and downregulation of 55% of all active genes (Figure 1E). For the majority of these genes, the change in transcription measured by PRO-seq is recapitulated at the mRNA level measured by RNA-seq (Shalgi et al., 2014), despite the fundamental difference between the two assays and mRNA stability being a part of RNA-seq measurement (Figure S2C). Detecting a change in RNA-seq requires a higher change in transcription than required for PRO-seq due to the higher level of steady-state mRNA level compared to nascent RNA, leading to a diminished change in levels seen by RNA-seq relative to PRO-seq (Figures S2C and S2D). Thus, our results indicate that the gene regulation in response to HS occurs at the level of transcription and consists of multiple distinct regulatory programs that are captured here with high spatiotemporal resolution and sensitivity afforded by the PRO-seq assay.

Majority of the HS-regulated genes are HSF1-independent

The numbers of genes showing similar regulation in both WT and *Hsf1*^{-/-} MEFs is unexpectedly large (Figure 1E & 1F). The overlap reported in Figure 1F is likely an underestimation as many of the uniquely upregulated genes in WT MEFs are upregulated in *Hsf1*^{-/-} MEFs and vice versa, but don't meet the DESeq2 threshold (Figure S2E). The downregulated genes show even more overlap between WT and *Hsf1*^{-/-} MEFs indicating

that transcriptional repression occurs by mechanisms that are largely HSF1-independent. Gene ontology (GO) analysis using DAVID (Huang et al., 2009) show, as expected, that genes upregulated only in WT MEFs are enriched for chaperones involved in protein folding and stress response (Figure S2F). Genes upregulated only in *Hsf1*^{-/-} MEFs are enriched for ATPase and protein kinases, while those upregulated in both cell types are enriched for transcription factors (TFs) and protein kinases involved in developmental processes. Overall, more than 87% of genes regulated at 60'HS in WT are similarly regulated in *Hsf1*^{-/-} MEFs, indicating that HSF1-independent mechanisms mediate much of the widespread change in transcription upon HS.

HSF1 binds to promoters of a small fraction of HS-induced genes

To understand the role of HSF1 binding during HSR, we performed HSF1 ChIP-seq in WT and *Hsf1*^{-/-} MEFs at NHS, 12'HS, and 60'HS (Figure 2A). We optimized ChIP-seq parameters such as sonication (Figure S3A), crosslinkers and cross-linking duration (Figure S3B), and antibody concentrations (Figure S3C), and used two different antibodies (Table S4) recognizing different parts of HSF1 to maximize detection of HSF binding sites and to minimize false-positive peaks (Chen et al., 2012). Biological replicates correlated well (Figure S3D) and were combined, and ChIP-seq peaks were called using MACS. As expected, we find prominent HSF1 peaks in the promoters of classical *Hsps* (for example, *Hsph1*), and the two antibodies generated similar ChIP-seq profiles (Figure 2B). HSF1 peaks identified here are highly specific, 89% of the HSF1 bound sites contain canonical HSE (p-value < 0.00001) (Figures S3E and S3F), and the fold enrichment of HSF1 peaks correlates with the motif match score of the HSE beneath the peaks (Figure S3G). While HSF1 occupies some sites prior to HS, most sites are detectably bound only after HS (Figure 2C). The majority of HSF1 peaks are located far from the nearest TSSs (Figure 2D); however, all the classical inducible *Hsps* have HSF1 binding within 1 kb upstream of their TSS (Figure S3H). Therefore, we defined a region 1kb upstream and 500 bp downstream of the TSS as the promoter and examined the distribution of HSF1 binding on promoter, intragenic, and intergenic regions. The density of HSF1 peaks is highest in promoters; however, higher incidences of absolute binding events occur in intragenic and intergenic regions (Figure 2E).

PRO-seq reveals that 17% of genes with HSF1 bound to their promoters undergo HSF1-dependent transcription induction upon HS (Figures 2F, magenta bars); however, 53% and 4% of genes with HSF1 bound on their promoters are repressed or unchanged upon HS independently of HSF1 (blue bars and purple bars respectively). This demonstrates that promoter bound HSF1 does not always induce transcription and may require a promoter to have additional features that it can collaborate with. Moreover, nearly all of the HSF1-promoter-bound repressed genes are also repressed in the *Hsf1*^{-/-} MEFs, indicating that promoter-bound HSF1 is not responsible for repression. Intriguingly, 13% of the HSF1-promoter-bound genes induced upon HS are also induced in *Hsf1*^{-/-} MEFs (green bar) indicating that some HSF1-bound genes do not require HSF1 for their induction.

Similarly, promoter binding of HSF1 is not always necessary even for genes showing HSF1-dependent transcription. Only ~35% of genes that depend on HSF1 for induction upon HS have HSF1 bound at their promoters (Figure 2G). This indicates that HSF1 can exert its

influence from a distance, presumably from an enhancer. Together, these observations show that the promoter-bound HSF1 is not responsible for the induction or repression of the majority of HS-regulated genes.

HSF1 binding to gene-body is not the mechanism of transcription repression

Past studies have proposed that HSF1 bound in the gene-body creates an obstacle to transcribing Pol II that leads to repression of transcription (Guertin and Lis, 2010; Westwood et al., 1991). Here, we find that transcription of genes that have HSF1 bound in the gene-body is mostly regulated in an HSF1-independent manner (Figures S3I). Approximately 50% of these genes are repressed upon HS; however, this repression occurs in *Hsf1*^{-/-} MEFs as well. To assess if the gene-body bound HSF1 creates steric hindrance to transcribing Pol II, we examined PRO-seq density around HSF1-intragenic-bound sites at 60'HS. We detect divergent transcription at these sites, a signature of enhancers (Core et al., 2014), which is reduced in *Hsf1*^{-/-} (Figure 2H). PRO-seq levels upstream of the HSF1 sites reveal no significant Pol II accumulation that would be expected from the steric hindrance created by the gene-body bound HSF1. Thus, the HSF1 bound in the body of a gene is not an obstacle to transcription and does not contribute to repression during HS.

Cytoskeleton genes are induced extremely early independent of HSF1

The very early kinetics of induction of genes upon HS beyond the classic *Drosophila Hsps* (O'Brien and Lis, 1991) has not been examined to date. Here, we find that many genes in MEFs are significantly induced by 2.5'HS, and the majority of these early-induced (EI) genes are HSF1-independent (Figure 3A). Induction of these genes continues to 12'HS; after which, transcription declines to below basal levels (Figure 3B).

Gene ontology analysis revealed that many of these EI genes, especially the HSF1-independent ones, encode proteins with biological function related to cytoskeletal structure and function (Figure 3C). Dynamic rearrangement of cytoskeleton in the cells has been previously documented during HSR (Laszlo, 1992), and cytoskeleton proteins are critical for survival during heat-stress (Baird et al., 2014). Proteomics analysis also showed an increase in the level of some cytoskeletal proteins in nuclear extract after two hours of HS (Raychaudhuri et al., 2014). However, the extremely rapid and transient induction of selective cytoskeleton genes during HS has not been detected before.

Because induction of these genes is predominantly HSF1-independent, we searched for TF-binding motifs in their promoters. Among the 1200 TF-binding motifs examined, serum response factor (SRF) binding motif is the most highly enriched (Figures 3D & S4A). SRF is known to induce a class of genes known as immediate-early genes, which are rapidly and transiently induced in response to various extracellular stimuli (Schratt et al., 2001). SRF is also a known effector of MAP kinase pathway, which is often implicated in stress. Therefore, we examined the SRF binding during HS in WT MEFs by ChIP-seq (Table S4). We found that the transiently induced genes upon HS that contain SRF binding motif in their promoters are bound by SRF in a transient manner mirroring the kinetics of transcription induction upon HS (Figures 3E & 3F). This finding strongly implicates SRF as a novel regulator of cytoskeletal genes during HSR.

Cytoskeleton genes and *Hsps* show similar kinetics of induction

One striking feature of PRO-seq data in induced genes is a distinct wave of elongating Pol II that are in the midst of transcription (Figure 3G & S4B). Here, we used a three-state Hidden Markov Model (HMM) (Danko et al., 2013) to calculate the distance traveled by waves of newly released Pol II in EI genes. Our approach calculates the difference in PRO-seq density between time points in 50 bp windows throughout the gene and identifies the region (wave) with a difference in transcription (Figures S4C & S4D). PRO-seq wave measurements using this approach indicate that Pol II release from the pause region of EI genes occurs on average within the first minute and a half! The median distance travelled by PRO-seq wave at 2.5'HS is 2.7 kb (Figure S4E) and the average elongation rate of Pol II in EI genes is 2.6 kb/min, calculated using the length of PRO-seq wave at 2.5'HS and 12'HS in genes induced at both time points (Figure 3H). Moreover, the length of PRO-seq waves at 2.5'HS in cytoskeleton genes and classical *Hsps* are very similar (Figure 3I), and the induction kinetics of HSF1-dependent and HSF1-independent genes are also highly analogous (Figure S4E). These findings demonstrate that transcription is induced very early during HSR and the kinetics of induction is similar between HSF1-dependent (*Hsps*) and HSF1-independent (cytoskeleton) genes.

Inhibition of pause release causes massive downregulation of transcription

Historically, the transcriptionally induced genes have been the focus of HS studies. However, many more genes are transcriptionally repressed than induced upon HS and this global repression is independent of HSF1 (Figure 4A). Genes undergoing repression display two distinct kinetics: some genes are gradually and consistently repressed over the course of HS (*Pcdh18*), but the majority are repressed only after 12'HS (*Cdkal1*) (Figures 4B & 4C).

The PRO-seq pattern following HS provides clues to how repression is mediated. While the PRO-seq density decreases on the gene-body, it increases on the 5' end of repressed genes (Figures 4D & S4F). This accumulation of Pol II in the first 100bp downstream of TSS (Figure 4E) indicates that the transcriptional repression in the majority of the downregulated genes is a result of reduced paused Pol II release into productive elongation. This extensive downregulation could in principle be a result of thermal-induced increase in transcription rate of elongating Pol II that would decrease Pol II density in the gene-body. We calculated the Pol II elongation rate by comparing positions of the clearing wave of Pol II in downregulated genes at 12'HS and 60'HS. We find the average elongation rate of Pol II during HS to be 2.1 kb/min (Figure S4G), which is comparable to the elongation rate of Pol II under normal temperature in mouse embryonic stem cells (1.8 – 2.4 kb/min) (Jonkers et al., 2014). Furthermore, the downregulation of transcription observed by PRO-seq is nicely reflected at the mRNA level measured by RNA-seq (Figure S2C, right panel). Thus, the decrease in gene-body PRO-seq reads is not the result of an increase in elongation rate, but rather results from a genuine downregulation in the frequency with which Pol II transitions from the pause to productive elongation.

We performed GO analyses to examine the enriched functional classes in downregulated genes. Genes involved in metabolism, cell cycle, and mitosis are represented in the early-repressed class, while genes in the late-repressed class are enriched for mRNA splicing,

mRNA processing and nuclear transport functions (Figure 4F). Conceivably, cells may enter into a low metabolic profile upon HS by immediately shutting down genes involved in cell cycle and metabolism. However, mRNA processing and efficient splicing could be critical in the early phase of HSR when *Hsps* and IE genes are robustly induced. The two kinetic classes of downregulated genes that have distinct functions imply that repression of transcription is a highly-regulated process, rather than a previously suggested non-discriminatory HS-induced global repression of transcription (Teves and Henikoff, 2011).

HS-regulated *Hsps* are dependent on HSF1

The ~75 genes in the broad HSP family (Kampinga et al., 2009) are not all induced upon HS. While classical *Hsps* show HSF1-dependent induction, more than half of *Hsps* are transcriptionally repressed (Figures 5A and S5A). One notable example is *Hsp90b1*, an endoplasmic reticulum associated chaperone involved in unfolded protein response, which is repressed upon HS independently of HSF1, while other *Hsp90s* are robustly induced in an HSF1-dependent manner (Figure S5D). Strikingly, almost all transcriptionally induced *Hsps* have prominent HSF1 peaks in their promoters (Figures 5B and S5B), accompanied by the presence of HSEs underneath the peaks (Figures 5C and S5C). Overall, our results show that some *Hsps* are rapidly and robustly induced upon HS; however, many genes in the HSP family are not bound by HSF1 and are not transcriptionally induced in response to HS, suggesting the role of post-transcriptional (Theodorakis and Morimoto, 1987) and translational regulation (Zhou et al., 2015) in the increased gene expression of some *Hsps* upon HS or the likely specialization of different HSPs in different tissues or in different stress responses.

HSF1 induces transcription by increasing promoter-proximal pause release

The PRO-seq profiles across the promoter proximal and gene-body regions provide insight to Pol II's progress through the distinct steps in the transcription cycle during HS. Moreover, contrasting these profiles in WT and *Hsf1*^{-/-} MEFs reveals the role of HSF1 in the changes observed. We first examined the genes that exhibit a significant increase in PRO-seq density in their gene-body by 60'HS in WT but not in *Hsf1*^{-/-} MEFs (n=102) (Figure 5D, middle panel) and also have HSF1 bound in their promoter region (Figure 5D, left panel). A simple interpretation of these profiles is that HSF1 acts to increase the rate of Pol II release from the pause, a step that is accelerated by P-TEFb kinase, which is known to be recruited to HS loci in an HSF1-dependent manner (Lis et al., 2000). P-TEFb phosphorylates components of the paused Pol II complex and enables Pol II to embark into productive elongation (Renner et al., 2001). An alternative mechanism to explain these profiles is that HS-induces an HSF1-dependent decrease in early termination that leads to higher gene-body Pol II density. However, this anti-termination model was ruled out, at least for *Hsp70*, by our previous study showing the termination rate of promoter-proximal paused Pol II is similar in HS and NHS cells and could not account for the HS-induced increase in gene-body Pol II density (Buckley et al., 2014). A second alternative explanation for these profiles is that elongation rates are slowed during HS in an HSF1-dependent manner leading to increase in gene-body Pol II density. However, such a decrease in elongation rate is neither consistent with the increases in the mRNA levels of these genes (Figure S2C), nor supported by the direct measurement of elongation rates (Figures 3G–I) (Ardehali and Lis, 2009). Thus, these 102

HSF1-promoter-bound and transcriptionally induced genes appear to be regulated by HSF1 upon HS in an HSF1-dependent manner at the step of pause Pol II release into productive elongation, likely through the known HSF1-dependent recruitment of the P-TEFb kinase (Lis et al., 2000).

Interestingly, this class of 102 genes also shows an increase in PRO-seq density in their pause region upon HS (Figure 5D, right panel). In contrast to the gene-body, this increase is independent of HSF1. We hypothesize that this increase is simply a consequence of the massive HSF1-independent downregulation of genes (Figures 1E, 1F, & 4A) upon HS, which increases the cellular pool of Pol II and thereby drives HSF1-independent Pol II loading on promoters by mass action. Because the global downregulation of transcription is also HSF1-independent, this increase availability of Pol II occurs in both WT and *Hsf1*^{-/-} MEFs.

HSF1 is also capable of acting from a distal enhancer, at least when bound to a site composed of an array of HSEs (Bienz and Pelham, 1986). We identified many genes that show HSF1-dependent induction upon HS but don't have HSF1 bound in their promoters (n=150) (Figure 2G) suggesting that these genes could be regulated by HSF1 acting from distal enhancers. Indeed, these genes show the same changes in PRO-seq profile in both gene bodies and pause regions as the class of 102 HSF1-promoter-bound, HSF1-dependent induced genes (Figure 5E). Therefore, HSF1 bound at distal enhancers likely regulates genes by increasing the release of paused Pol II, while the loading of Pol II on promoters is HSF1-independent, indicating the promoter-proximal pause release is the primary step regulated in all HSF1-mediated transcription induction during HSR.

Enhancers bound by HSF1 would be expected to have the divergent transcription profile characteristic of active enhancers (Core et al., 2014; Kim et al., 2010). PRO-seq profiles around the intergenic and intragenic HSF1-bound regions show that the divergent transcription appears upon HS (Figures 5F & S5E), suggesting that these distal HSF1 bound regions become active enhancers during the HSR. This increase in divergent transcription around the distal HSF1-bound sites is unlikely to be an effect of HSF1 directly stimulating the recruitment of Pol II, because the initiation of divergent transcription is detectable only at 60'HS even though the sites are occupied by HSF1 prior to 60'HS (Figures 5F & S5E - left and mid panels). The delayed recruitment of Pol II is more consistent with an indirect role of HSF1 on Pol II recruitment to enhancers, perhaps a consequence of opening of enhancer regions that allows the binding of other TFs and Pol II made available during the massive downregulation of thousands of genes.

HSF2 does not compensate for the loss of HSF1 during HSR

HSF2, like HSF1, is ubiquitously expressed in mammals (Akerfelt et al., 2010). The DNA binding domains of HSF1 and HSF2 are highly similar and bind to identical HSEs as homo- or hetero-trimers (Sandqvist et al., 2009). To test whether HSF2 is compensating for the lack of HSF1 in *Hsf1*^{-/-} MEFs, we performed PRO-seq in nuclei isolated from *Hsf1*&2^{-/-} MEFs at 37°C (NHS) and 2.5, 12, and 60 minutes after an instantaneous HS at 42°C and processed these data like WT and *Hsf1*^{-/-} MEFs (Figure S6A and Tables S5, S6, and S7).

HS induces significant changes in PRO-seq density of many genes in *Hsf1&2*^{-/-} (Figure 6A), and most genes that are regulated upon HS in WT and *Hsf1*^{-/-} are also regulated in *Hsf1&2*^{-/-} (Figures 6B & S6B). The number of significantly upregulated genes upon HS in *Hsf1&2*^{-/-} are similar to *Hsf1*^{-/-} MEFs (Figures 6C vs. 1E), as is their degree of upregulation (Figure 6D, left panel), indicating that HSR mediated transcription induction in *Hsf1*^{-/-} is not mediated by HSF2.

The number of significantly downregulated genes is somewhat lower in *Hsf1&2*^{-/-} relative to the *Hsf1*^{-/-} MEFs (Figures 6C vs. 1E) and the extent of downregulation is also comparatively low (Figure 6D, right panel). This observation that some genes downregulated in *Hsf1*^{-/-} are not downregulated in *Hsf1&2*^{-/-} raises the possibility that HSF2 mediates repression of at least some genes. For instance, the constitutive transcription in *Fgf7* and *Mark1* is repressed in *Hsf1*^{-/-} MEFs but is restored in *Hsf1&2*^{-/-}, suggesting that HSF1 normally prevents HSF2 from repressing these genes (Figures 6E & S6C). HSF2 may also play a dominant role over HSF1 in repression of genes like *Prrc2b* during HS, as its downregulation detected at 60'HS in both WT and *Hsf1*^{-/-} MEFs is not observed in *Hsf1&2*^{-/-} (Figure 6E). The fewer downregulated genes in *Hsf1&2*^{-/-} compared to *Hsf1*^{-/-} MEFs imply that HSF2 could be directly or indirectly working as a repressor. HSF2 is known to repress HSR in mitotic cells, (Elsing et al., 2014), but the HSF2 mediated constitutive repression in absence of HSF1 as well as the dominant repression of some genes during HS has not been reported before in interphase cells.

Downregulated genes in *Hsf1&2*^{-/-} are also regulated by inhibition of promoter proximal pause release, similar to WT and *Hsf1*^{-/-} MEFs (Figure 6F). The similarity in the regulation of *Hsps* in *Hsf1*^{-/-} and *Hsf1&2*^{-/-} further indicates that HSF2 does not compensate for the lack of HSF1 (compare Figures 5A & S5A to S6D & S6E). Thus, while HSF2 may mediate downregulation of a subset of genes, most of the changes, especially the transcription induction upon HS, observed in *Hsf1*^{-/-} MEFs are not mediated by HSF2.

HS-regulated genes have different kinetics, dynamics, chromatin marks, and functions

Our results demonstrate an elaborate network of transcriptional regulation in response to heat stress; hundreds of genes are induced and thousands of others are repressed. This coordinated regulation exhibits temporal precision and selectivity for functional gene groups. We broadly divided the kinetics and dynamics of transcription regulation during HS in WT MEFs in five unique classes (Figure 7A).

Class I represents the HS-inducible genes including *Hsps* that are induced throughout the duration of HS in this study. Upon HS, many of these genes are bound by HSF1 at their promoters. The AP-1 binding motif is enriched within the HSF1 peaks in the promoter of these genes (Figure S7A), suggesting a likely co-operation between HSF1 and AP-1. We show that the HSF1 induces transcription in HSF1-dependent genes of this class by increasing the release of paused Pol II into productive elongation. In addition to HSF1, the binding motif of SIX4 is also enriched in the promoters of some genes in this class (Figure S7B).

Class II comprises EI genes described in Figure 3. HS rapidly and transiently induces transcription of these genes. Many genes in this class belong to cytoskeleton family and are characterized by a strong enrichment of SRF binding motif in their promoters. The metagene profile of PRO-seq density in the pause region of these genes also shows a comparable net increase of paused Pol II at 2.5'HS and 12'HS that decrease at 60'HS (Figure S7C). Thus, both Pol II pause release and recruitment appear rapidly and transiently regulated during HSR, and SRF and its coregulators are strong candidates for mediating this response.

Class III represents genes that are induced during the late phase of the HSR. Most of these genes are regulated independently of HSF1 and are likely targets of TFs that are induced in the earlier phase of HSR. Promoters of genes in this class are enriched in antioxidant binding elements where Nuclear Factor, Erythroid 2-Like 2 (NFE2L2) is known to bind and induce oxidative stress response genes (Li and Kong, 2009). Some of these genes have a role in apoptosis. Unlike class II genes, PRO-seq density in the pause regions of these genes increases over the course of HS (Figure S7D), while increase in the gene-body only occurs at 60'HS.

Class IV comprises genes that are downregulated immediately upon HS. These genes are characterized by progressive reduction in PRO-seq density over the course of HS due to the inhibition of Pol II release into productive elongation from the promoter-proximal paused state. This class is enriched for genes related to metabolism, cell cycle, and protein synthesis.

Class V consists genes that are unaffected during the early phase of the HSR but are downregulated in the late phase. Similar to the class IV, these genes are also regulated at the level of pause release. Interestingly, genes in this class are highly enriched for processes like splicing, mRNA processing, and nuclear transport.

To distinguish the characteristic features of five kinetic classes, we probed for all published and ENCODE-deposited genome-wide data in MEFs. We found that the kinetic classes are differentially enriched for several histone marks and Pol II prior to HS (Figure 7B and S7E). The class II genes are primed with higher levels of paused Pol II and higher active histone modifications compared to both class I and class III. In contrast, we did not find significant differences in histone modifications between the downregulated classes (class IV and class V). In general, active histone marks positively correlate and inactive histone marks negatively correlate with Pol II levels in the promoter region. Overall, the differences in the kinetics and dynamics of regulation, in the associated TFs, Pol II, and histone marks, and in the specific biological processes targeted by each gene class indicate a highly regulated and carefully calibrated response mechanism that cells have evolved to cope with heat stress.

Discussion

The PRO-seq assay used here provides a kinetic series of snapshots of the changes in transcribing RNA polymerases in the immediate response to an instantaneous heat stress. The properties of this assay - high sensitivity, large dynamic range ($>10^5$), genome-wide scope and base-pair resolution - provide a fine grain panorama of the changes in

transcription across the genome and show that the extent of the HSR is much more pervasive than previously appreciated, with significant upregulation of 10% and downregulation of 55% of all active genes. This regulation is extremely rapid, inducing changes in transcriptional patterns in as little as a minute and half. Notably, the response is far from monolithic, and based on the kinetics and dynamics of transcription changes, HS-regulated genes fall into five distinct classes. Interestingly, these classes are also distinct in terms of the transcription factors binding motifs and chromatin marks in their promoters and the broad functions of the regulated genes.

HSF1 is generally considered to be the master regulator of the HSR. Our analyses in both *Hsf1*^{-/-} and WT MEFs reveal that HSF1 accounts for only a fraction of the transcriptionally-induced genes upon HS and it has no detectable role in genome-wide repression. Analysis of the *Hsf1&2*^{-/-} MEFs shows that HSF2 does not compensate for the absence of HSF1 for induction of upregulated genes, indicating non-redundant roles of this ubiquitously-expressed HSF1 paralog.

This study identifies over 900 genes that are induced upon HS in HSF1-independent manner, which are not mediated by HSF2, and identifies additional TFs as potential activators of HSR. One such TF that appears to play a large role in mediating the HSR is SRF. Arsenite-mediated stress is known to activate SRF via MAPK pathway, (Heidenreich et al., 1999); however, SRF's role in HSR was completely uncharacterized until now. SRF transiently binds the promoters of transiently-induced IE genes during HSR, the majority of which are cytoskeletal genes. We hypothesize that the dramatic cytoskeletal changes at the onset of HSR, such as formation of stress fibers comprised of actin filaments, is the trigger that selectively induce transcription of genes in cytoskeleton family via the action of SRF. SRF activity depends on the MAL cofactor, which binds to monomeric actin, and this actin binding sequesters MAL to the cytoplasm (Vartiainen et al., 2007). In this model, the actin polymerization caused by HS reduces the level of monomeric actin allowing MAL to translocate into the nucleus and form a complex with SRF and induce SRF-responsive genes. The transient induction of these genes could be mediated by the known feed-back loop where monomeric actin expression driven by SRF inhibits MAL association with SRF and its potential to induce transcription (Sotiropoulos et al., 1999).

The escape of paused Pol II into productive elongation can be a rate-limiting and regulated step in transcription elongation (Adelman and Lis, 2012). Comparative analyses of PRO-seq density in paused region and body of genes in WT and *Hsf1*^{-/-} MEFs supports the model that HSF1 induces transcription in HSF1-dependent upregulated genes by increasing the release of paused Pol II into productive elongation. The details of the mechanism remain to be resolved, but HSF1 binding to major HS loci is known to be required for the recruitment of P-TEFb (Lis et al., 2000), whose kinase activity is in turn required for progression of paused Pol II into productive elongation (Jonkers et al., 2014; Price, 2000; Takahashi et al., 2011).

In addition to promoters, HSF1 binds to many intragenic and intergenic sites. Intragenic HSF1 bound sites were previously proposed to repress transcription by creating a barrier to transcription. This hypothesis does not explain the downregulation observed in our study, as

we find the intragenic HSF1 has very little influence on transcriptional downregulation of the bound genes. The HSF1-intragenic-bound genes that show transcriptional repression in WT are also repressed in *Hsf1*^{-/-} MEFs with comparable magnitude and kinetics. We further show that the density of Pol II upstream of the intragenic-bound HSF1 sites does not increase, indicating gene-body bound HSF1 does not create appreciable impediment to elongating Pol II.

Intergenic-bound HSF1 is difficult to assign to a target gene without a high-resolution chromatin interaction map. Nevertheless, the divergent PRO-seq profile around the intergenic-bound HSF1 sites and the presence of 150 HSF1-dependent genes without promoter-bound HSF1 indicates that at least some of the intergenic-bound HSF1 functions as enhancers. The transcription induction of these 150 genes also occurs by an HSF1-dependent increase in pause release. This further strengthens the role of HSF1 in HSR as a factor that increases the release of paused Pol II into productive elongation. Finally, the insights into the mechanism of HSF1 action and its primary gene targets will hopefully prove useful in fine-tuning the ongoing development of HSF1-based therapeutics for cancer and neurodegenerative disorders.

EXPERIMENTAL PROCEDURES

Cell lines and heat shock

Immortalized MEFs generated from *Hsf1*^{-/-}, *Hsf1&2*^{-/-}, and its wild type littermate mice were grown in DMEM supplemented with 10% heat inactivated FBS (v/v) and 1% Penicillin Streptomycin (v/v) at 37°C with 5% CO₂ and 90% humidity.

Instantaneous HS was performed using ~80% confluent cells by adding pre-heated (42°C) conditioned media collected from identically growing cells and by incubating the cell plates at 42°C for the desired time. NHS cells grown at 37°C and the NHS and HS cells were harvested identically (see Supplemental Experimental Procedures).

PRO-seq library preparation

Nuclei were isolated as described previously (Core et al., 2008), and nuclear run-on experiments and PRO-seq library preparation were performed as described previously (Kwak et al., 2013) with some modifications (see Supplemental Experimental Procedures).

HSF1 and SRF ChIP-seq library preparation

Chromatin immunoprecipitation and ChIP-seq library preparation were performed as described previously (Guertin and Lis, 2010) with some modifications (see Supplemental Experimental Procedures).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Ivor Benjamin and Yves Le Drian for providing MEFs, Richard Morimoto for HSF1 antibody, and Reut Shalgi for RNA-seq data in 3T3 cells. We thank Peter Schweitzer of the Cornell genomic facility for his help in sequencing. We also thank current and past members of the Lis lab, especially Abdullah Ozer, Leighton Core, and Nicholas Fuda for their suggestions in manuscript preparation. Research reported in this publication was supported by NIH grant R01GM25232. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

- Adelman K, Lis JT. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nat Rev Genet.* 2012; 13:720–731. [PubMed: 22986266]
- Akerfelt M, Morimoto RI, Sistonen L. Heat shock factors: integrators of cell stress, development and lifespan. *Nat Rev Mol Cell Biol.* 2010; 11:545–555. [PubMed: 20628411]
- Ardehali MB, Lis JT. Tracking rates of transcription and splicing in vivo. *Nat Struct Mol Biol.* 2009; 16:1123–1124. [PubMed: 19888309]
- Baird NA, Douglas PM, Simic MS, Grant AR, Moresco JJ, Wolff SC, Yates JR, Manning G, Dillin A. HSF-1-mediated cytoskeletal integrity determines thermotolerance and life span. *Science.* 2014; 346:360–363. [PubMed: 25324391]
- Bienz M, Pelham HR. Heat shock regulatory elements function as an inducible enhancer in the *Xenopus hsp70* gene and when linked to a heterologous promoter. *Cell.* 1986; 45:753–760. [PubMed: 3085957]
- Brown JB, Boley N, Eisman R, May GE, Stoiber MH, Duff MO, Booth BW, Wen J, Park S, Suzuki AM, et al. Diversity and dynamics of the *Drosophila* transcriptome. *Nature.* 2014; 512:393–399. [PubMed: 24670639]
- Buckley MS, Kwak H, Zipfel WR, Lis JT. Kinetics of promoter Pol II on Hsp70 reveal stable pausing and key insights into its regulation. *Genes & Development.* 2014; 28:14–19. [PubMed: 24395245]
- Chen Y, Negre N, Li Q, Mieczkowska JO, Slattery M, Liu T, Zhang Y, Kim T-K, He HH, Zieba J, et al. Systematic evaluation of factors influencing ChIP-seq fidelity. *Nature Publishing Group.* 2012; 9:609–614.
- Core LJ, Martins AL, Danko CG, Waters CT, Siepel A, Lis JT. Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. *Nat Genet.* 2014; 46:1311–1320. [PubMed: 25383968]
- Dai C, Whitesell L, Rogers AB, Lindquist S. Heat shock factor 1 is a powerful multifaceted modifier of carcinogenesis. *Cell.* 2007; 130:1005–1018. [PubMed: 17889646]
- Danko CG, Hah N, Luo X, Martins AL, Core L, Lis JT, Siepel A, Kraus WL. Signaling pathways differentially affect RNA polymerase II initiation, pausing, and elongation rate in cells. *Molecular Cell.* 2013; 50:212–222. [PubMed: 23523369]
- Danko CG, Hyland SL, Core LJ, Martins AL, Waters CT, Lee HW, Cheung VG, Kraus WL, Lis JT, Siepel A. Identification of active transcriptional regulatory elements from GRO-seq data. *Nature Publishing Group.* 2015; 12:433–438.
- Elsing AN, Aspelin C, Björk JK, Bergman HA, Himanen SV, Kallio MJ, Roos-Mattjus P, Sistonen L. Expression of HSF2 decreases in mitosis to enable stress-inducible transcription and cell survival. *The Journal of Cell Biology.* 2014; 206:735–749. [PubMed: 25202032]
- Esnault C, Stewart A, Gualdrini F, East P, Horswell S, Matthews N, Treisman R. Rho-actin signaling to the MRTF coactivators dominates the immediate transcriptional response to serum in fibroblasts. *Genes & Development.* 2014; 28:943–958. [PubMed: 24732378]
- Fuda NJ, Ardehali MB, Lis JT. Defining mechanisms that regulate RNA polymerase II transcription in vivo. *Nature.* 2009; 461:186–192. [PubMed: 19741698]
- Guertin MJ, Lis JT. Chromatin landscape dictates HSF binding to target DNA elements. *PLoS Genet.* 2010; 6
- Heidenreich O, Neininger A, Schraft G, Zinck R, Cahill MA, Engel K, Kotlyarov A, Kraft R, Kostka S, Gaestel M, et al. MAPKAP kinase 2 phosphorylates serum response factor in vitro and in vivo. *J Biol Chem.* 1999; 274:14434–14443. [PubMed: 10318869]

- Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc.* 2009; 4:44–57. [PubMed: 19131956]
- Jonkers I, Kwak H, Lis JT. Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. *Elife.* 2014; 3:e02407. [PubMed: 24843027]
- Kampinga HH, Hageman J, Vos MJ, Kubota H, Tanguay RM, Bruford EA, Cheetham ME, Chen B, Hightower LE. Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones.* 2009; 14:105–111. [PubMed: 18663603]
- Kim T-K, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, Harmin DA, Laptevich M, Barbara-Haley K, Kuersten S, et al. Widespread transcription at neuronal activity-regulated enhancers. *Nature.* 2010; 465:182–187. [PubMed: 20393465]
- Kwak H, Fuda NJ, Core LJ, Lis JT. Precise maps of RNA polymerase reveal how promoters direct initiation and pausing. *Science.* 2013; 339:950–953. [PubMed: 23430654]
- de la Grange P, Dutertre M, Martin N, Auboeuf D. FAST DB: a website resource for the study of the expression regulation of human gene products. *Nucleic Acids Research.* 2005; 33:4276–4284. [PubMed: 16052034]
- Laszlo A. The effects of hyperthermia on mammalian cell structure and function. *Cell Prolif.* 1992; 25:59–87. [PubMed: 1554820]
- Lecomte S, Desmots F, Le Masson F, Le Goff P, Michel D, Christians ES, Le Dréan Y. Roles of heat shock factor 1 and 2 in response to proteasome inhibition: consequence on p53 stability. *Oncogene.* 2010; 29:4216–4224. [PubMed: 20498630]
- Li W, Kong A-N. Molecular mechanisms of Nrf2-mediated antioxidant response. *Mol Carcinog.* 2009; 48:91–104. [PubMed: 18618599]
- Lindquist S. The heat-shock response. *Annu Rev Biochem.* 1986; 55:1151–1191. [PubMed: 2427013]
- Lindquist S, Craig EA. The heat-shock proteins. *Annu Rev Genet.* 1988; 22:631–677. [PubMed: 2853609]
- Lis JT, Mason P, Peng J, Price DH, Werner J. P-TEFb kinase recruitment and function at heat shock loci. *Genes & Development.* 2000; 14:792–803. [PubMed: 10766736]
- Locke M, Tanguay RM. Diminished heat shock response in the aged myocardium. *Cell Stress Chaperones.* 1996; 1:251–260. [PubMed: 9222610]
- Ma X, Xu L, Alberobello AT, Gavrilova O, Bagattin A, Skarulis M, Liu J, Finkel T, Mueller E. Celastrol Protects against Obesity and Metabolic Dysfunction through Activation of a HSF1-PGC1 α Transcriptional Axis. *Cell Metab.* 2015
- McMillan DR, Christians E, Forster M, Xiao X, Connell P, Plumier JC, Zuo X, Richardson J, Morgan S, Benjamin IJ. Heat Shock Transcription Factor 2 Is Not Essential for Embryonic Development, Fertility, or Adult Cognitive and Psychomotor Function in Mice. *Mol Cell Biol.* 2002; 22:8005–8014. [PubMed: 12391166]
- McMillan DR, Xiao X, Shao L, Graves K, Benjamin IJ. Targeted disruption of heat shock transcription factor 1 abolishes thermotolerance and protection against heat-inducible apoptosis. *J Biol Chem.* 1998; 273:7523–7528. [PubMed: 9516453]
- Mendillo ML, Santagata S, Koeva M, Bell GW, Hu R, Tamimi RM, Fraenkel E, Ince TA, Whitesell L, Lindquist S. HSF1 Drives a Transcriptional Program Distinct from Heat Shock to Support Highly Malignant Human Cancers. *Cell.* 2012; 150:549–562. [PubMed: 22863008]
- Morley JF, Morimoto RI. Regulation of longevity in *Caenorhabditis elegans* by heat shock factor and molecular chaperones. *Mol Biol Cell.* 2004; 15:657–664. [PubMed: 14668486]
- Neef DW, Jaeger AM, Thiele DJ. Heat shock transcription factor 1 as a therapeutic target in neurodegenerative diseases. *Nat Rev Drug Discov.* 2011; 10:930–944. [PubMed: 22129991]
- O'Brien T, Lis JT. RNA polymerase II pauses at the 5' end of the transcriptionally induced *Drosophila* hsp70 gene. *Mol Cell Biol.* 1991; 11:5285–5290. [PubMed: 1922045]
- Parker CS, Topol J. A *Drosophila* RNA polymerase II transcription factor binds to the regulatory site of an hsp 70 gene. *Cell.* 1984; 37:273–283. [PubMed: 6722872]
- Perisic O, Xiao H, Lis JT. Stable binding of *Drosophila* heat shock factor to head-to-head and tail-to-tail repeats of a conserved 5 bp recognition unit. *Cell.* 1989; 59:797–806. [PubMed: 2590940]

- Price DH. P-TEFb, a cyclin-dependent kinase controlling elongation by RNA polymerase II. *Mol Cell Biol.* 2000
- Raychaudhuri S, Loew C, Körner R, Pinkert S, Theis M, Hayer-Hartl M, Buchholz F, Hartl FU. Interplay of Acetyltransferase EP300 and the Proteasome System in Regulating Heat Shock Transcription Factor 1. *Cell.* 2014; 156:975–985. [PubMed: 24581496]
- Renner DB, Yamaguchi Y, Wada T, Handa H, Price DH. A highly purified RNA polymerase II elongation control system. *J Biol Chem.* 2001; 276:42601–42609. [PubMed: 11553615]
- Sandqvist A, Björk JK, Akerfelt M, Chitikova Z, Grichine A, Vourc'h C, Jolly C, Salminen TA, Nymalm Y, Sistonen L. Heterotrimerization of heat-shock factors 1 and 2 provides a transcriptional switch in response to distinct stimuli. *Mol Biol Cell.* 2009; 20:1340–1347. [PubMed: 19129477]
- Sarge KD, Zimarino V, Holm K, Wu C, Morimoto RI. Cloning and characterization of two mouse heat shock factors with distinct inducible and constitutive DNA-binding ability. *Genes & Development.* 1991; 5:1902–1911. [PubMed: 1717345]
- Schratt G, Weinhold B, Lundberg AS, Schuck S, Berger J, Schwarz H, Weinberg RA, Rüther U, Nordheim A. Serum response factor is required for immediate-early gene activation yet is dispensable for proliferation of embryonic stem cells. *Mol Cell Biol.* 2001; 21:2933–2943. [PubMed: 11283270]
- Shalgi R, Hurt JA, Lindquist S, Burge CB. Widespread Inhibition of Posttranscriptional Splicing Shapes the Cellular Transcriptome following Heat Shock. *Cell Rep.* 2014; 7:1362–1370. [PubMed: 24857664]
- Sotiropoulos A, Gineitis D, Copeland J, Treisman R. Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. *Cell.* 1999; 98:159–169. [PubMed: 10428028]
- Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CAS, Kong SE, Szutorisz H, Swanson SK, Martin-Brown S, Washburn MP, et al. Human mediator subunit MED26 functions as a docking site for transcription elongation factors. *Cell.* 2011; 146:92–104. [PubMed: 21729782]
- Teves SS, Henikoff S. Heat shock reduces stalled RNA polymerase II and nucleosome turnover genome-wide. *Genes & Development.* 2011; 25:2387–2397. [PubMed: 22085965]
- Theodorakis NG, Morimoto RI. Posttranscriptional regulation of hsp70 expression in human cells: effects of heat shock, inhibition of protein synthesis, and adenovirus infection on translation and mRNA stability. *Mol Cell Biol.* 1987; 7:4357–4368. [PubMed: 3437893]
- Trinklein ND, Murray JI, Hartman SJ, Botstein D, Myers RM. The role of heat shock transcription factor 1 in the genome-wide regulation of the mammalian heat shock response. *Mol Biol Cell.* 2004; 15:1254–1261. [PubMed: 14668476]
- Vartiainen MK, Guettler S, Larijani B, Treisman R. Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL. *Science.* 2007; 316:1749–1752. [PubMed: 17588931]
- Westwood JT, Clos J, Wu C. Stress-induced oligomerization and chromosomal relocalization of heat-shock factor. *Nature.* 1991; 353:822–827. [PubMed: 1944557]
- Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR, Qian S-B. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. *Nature.* 2015

Highlights

~1500 genes are up- and ~8000 are down-regulated during the 1st hour of heat shock

HSF1 induces *heat shock protein* genes by increasing promoter-proximal pause-release

Upon heat shock, SRF transiently induces immediate-early cytoskeletal genes

Broad repression during heat-shock in mammals occurs by inhibiting pause-release

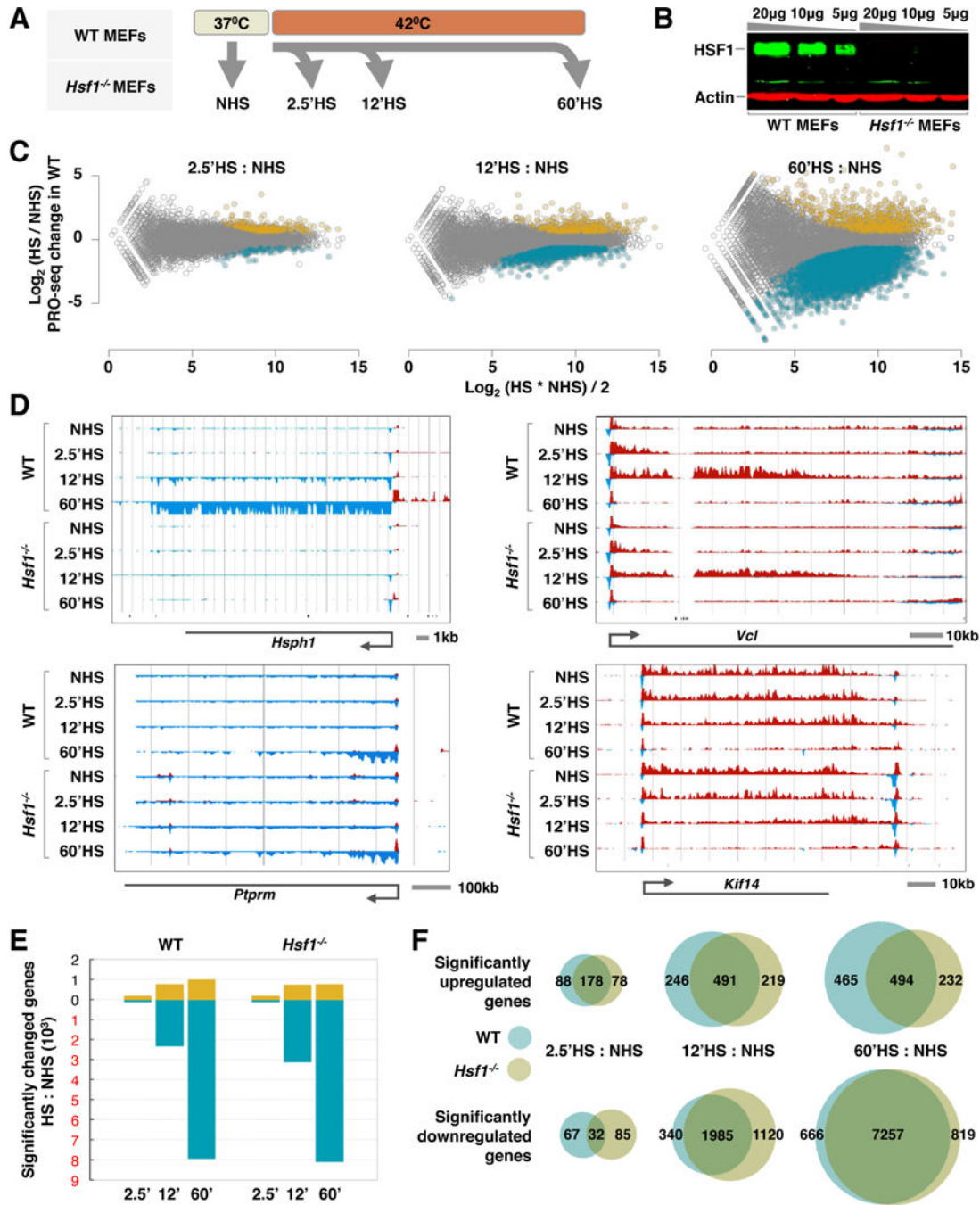


Figure 1. HS induces rapid, dynamic, and extensive changes in transcription in a mostly HSF1-independent manner
 (A) Experimental set-up, PRO-seq assay was performed in nuclei isolated from WT and *Hsf1*^{-/-} MEFs at NHS, 2.5'HS, 12'HS, and 60'HS.
 (B) Quantitative Western Blot analysis of HSF1 in WT and *Hsf1*^{-/-} MEFs. Actin is used as the loading control.
 (C) 'Minus-average' (MA) plots represent PRO-seq density change in the gene-body of all genes (n=23460) between NHS and 2.5'HS (left panel), 12'HS (mid panel), and 60'HS (right panel).

panel) in WT MEFs. Significantly upregulated genes (p-value < 0.001 in DESeq2) are shown in gold and significantly downregulated genes are shown in blue.

(D) Screenshots of four genes with different kinetics and dynamics of regulation. PRO-seq density in sense and antisense direction is shown in red and blue respectively. Small vertical black bars at the bottom of PRO-seq tracks represent the genomic regions that do not map uniquely at 36bp resolution.

(E) Number of significantly changed genes upon HS. Upregulated genes are shown in gold and downregulated genes are shown in blue.

(F) Venn diagram of significantly upregulated and downregulated genes between WT and *Hsf1*^{-/-} MEFs.

See also Figures S1, S2 and Tables S1, S2, S3

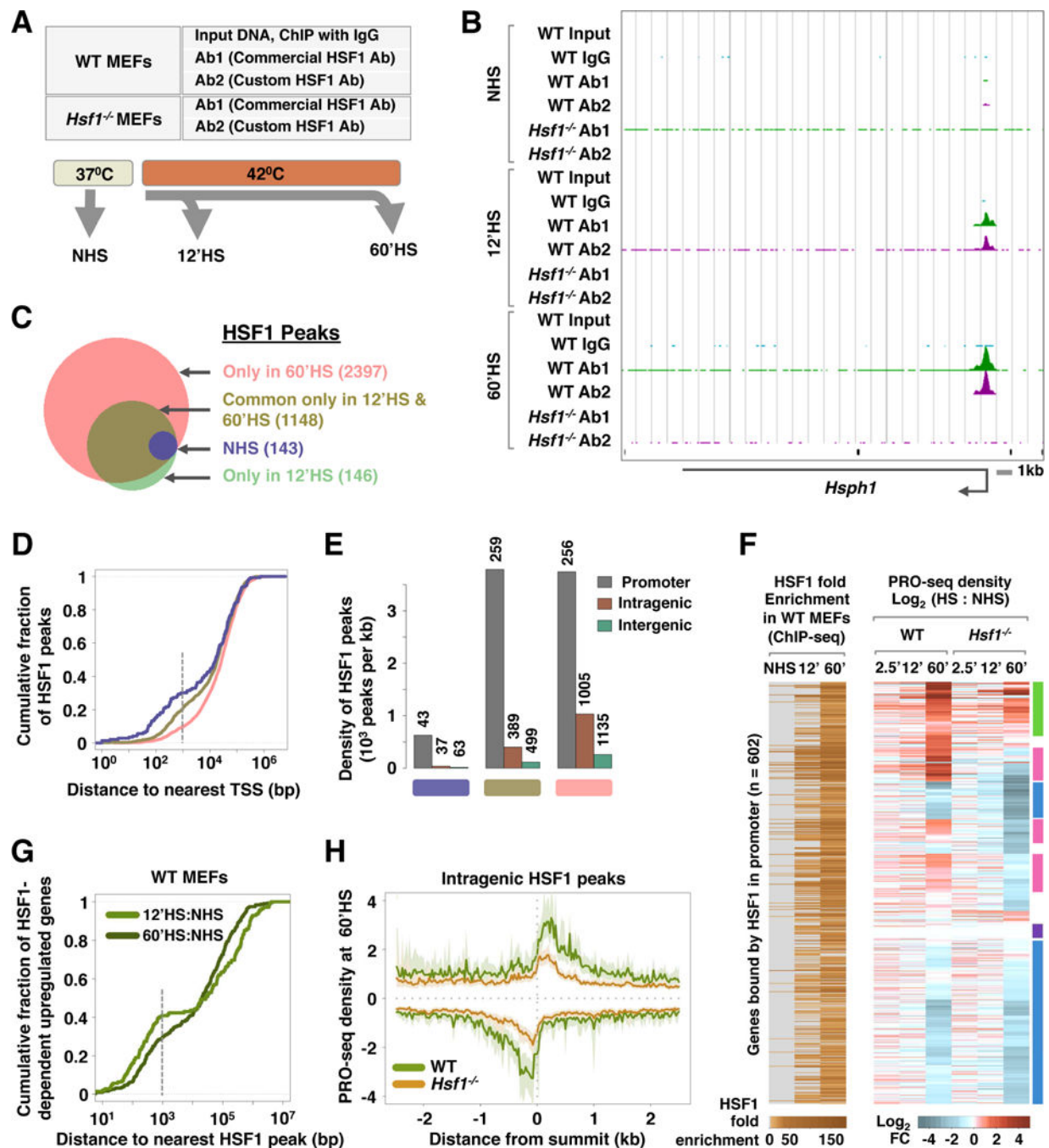


Figure 2. HSF1 binds and regulates a small fraction of HS induced genes

(A) Experimental set-up, ChIP-seq libraries in WT MEFs were made in duplicates with Input DNA, chromatin immunoprecipitated with non-specific IgG and two different HSF1 specific antibodies – Ab1 and Ab2 - at NHS, 12'HS, and 60'HS. Additionally, ChIP-seq libraries were also made with both HSF1 specific antibodies in *Hsf1*^{-/-} MEFs.

(B) Screenshot of *Hsp1* gene shows ChIP-seq read density in WT and *Hsf1*^{-/-} MEFs. The scale on the y-axis represents the number of ChIP-seq tags under the peaks.

(C) Venn diagram of the number of HSF1 peaks identified by MACS at different time points.

(D) Cumulative fraction of HSF1 peaks from the nearest annotated TSS. Colors of the cumulative distribution functions correspond to the different classes represented in the venn diagram in panel C. The dotted line represents 1 kb and the x-axis is in \log_{10} scale.

(E) Density of HSF1 peaks in discrete genomic regions. The absolute numbers of HSF1 peaks are shown over the bars. Colors below the sets of three genomic regions correspond to the different classes represented in the venn diagram in panel C.

(F) Heatmap of HSF1 binding by ChIP-seq (left, grey to brown) and change in PRO-seq density (right, blue to red) before and after HS in all HSF1-promoter-bound genes. Purple bar represents genes induced in both cell types, magenta bars represent HSF1-dependent induced genes, and blue bars represent HSF1-independent repressed genes.

(G) Cumulative fraction of HSF1-dependent upregulated genes in WT MEFs from the nearest HSF1 peaks. The dotted line represents 1 kb and x-axis is in \log_{10} scale.

(H) PRO-seq density (with 95% confidence interval in light shades) at 60'HS around the center of intragenic-bound HSF1 sites. Upstream region of the intragenic-bound HSF1 sites lack HSF1-dependent accumulation of PRO-seq density.

See also Figure S3 and Table S4

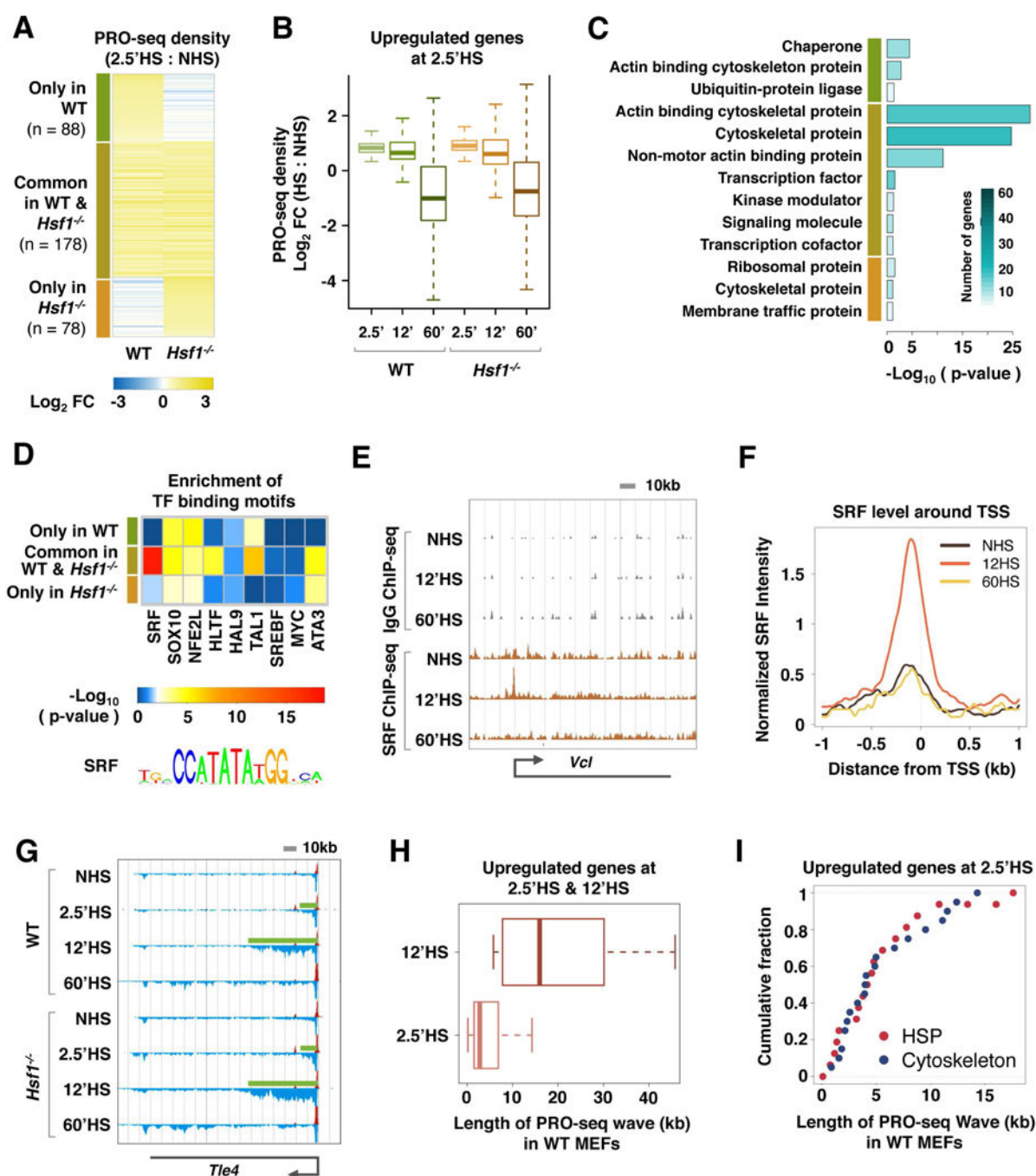


Figure 3. Early induced genes are primarily cytoskeleton genes, which are induced as early as *Hsps* in HSF1-independent manner

(A) Heatmap of PRO-seq density fold change in significantly upregulated genes at 2.5'HS (EI genes) in WT only (green), common in both WT & *Hsf1*^{-/-} (olive), and *Hsf1*^{-/-} only (orange).

(B) Change in PRO-seq density of EI genes at all time points.

(C) GO analysis of the three classes of EI genes (same color scheme as in A). The heatmap denotes the number of genes in each GO class and the length of the bar (x-axis) denotes the p-value.

(D) Significantly enriched TF motifs out of 1200 scanned in the promoter of genes in the three classes of EI genes. P-value of motif enrichment over 3rd order Markov model is represented in the heatmap. Sequence logo of DNA binding motif of the most significantly enriched TF is shown.

(E) Screenshot of *Vcl* gene shows the SRF ChIP-seq density. SRF binding is significantly enriched on the promoter of the gene at 12'HS and returns to level similar to NHS by 60'HS.

(F) Composite profile of SRF ChIP-seq density around the TSS of transiently induced genes upon HS that contain SRF binding element on their promoters.

(G) Screenshots of *Tle4* gene with a distinctive PRO-seq wave. Green bars represent the distance traversed by new waves of Pol II calculated by three-state HMM.

(H) Distribution of length of PRO-seq waves at 2.5'HS and 12'HS in EI genes that are significantly upregulated at 12'HS as well.

(I) Cumulative fraction of cytoskeleton genes and *Hsps* and their length of PRO-seq waves at 2.5'HS.

See also Figure S4

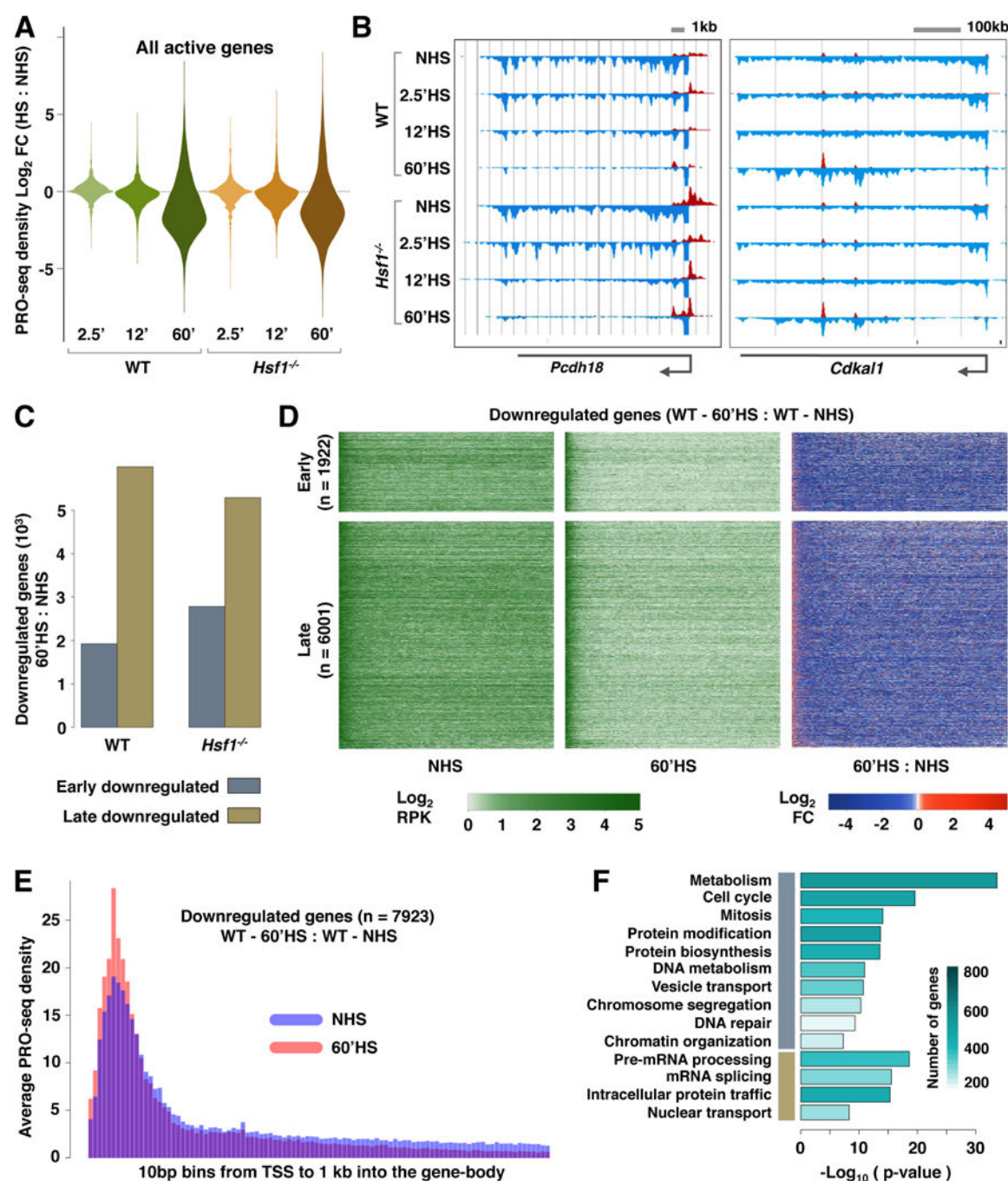


Figure 4. Transcription is repressed upon HS in majority of the active genes

(A) Center-of-mass plot shows the status of PRO-seq density change in all active genes (n = 15893).

(B) Screenshots of two downregulated genes with different kinetics of transcription repression upon HS (left is early- downregulated and right is late- downregulated).

(C) Breakdown of significantly downregulated genes at 60'HS into two classes - early and late.

(D) Heatmap of PRO-seq density before HS (left), after 60'HS (mid), and fold change from 60'HS to NHS (right) for significantly downregulated genes at 60'HS in WT MEFs. Each row represents a gene, scaled to same length and divided into 100 bins, from TSS up to polyA site for genes shorter than 24 kb and up to 24 kb for genes longer than 24kb.

(E) Histogram of average PRO-seq density in 10 bp bins from TSS to 1 kb into the gene-body for significantly downregulated genes at 60'HS (n= 7923).

(F) GO analysis of the significantly downregulated genes using DAVID. GO terms enriched in early and late downregulated classes are represented by color scheme in C.

The heatmap denotes the number of genes in each GO class.

See also Figure S4

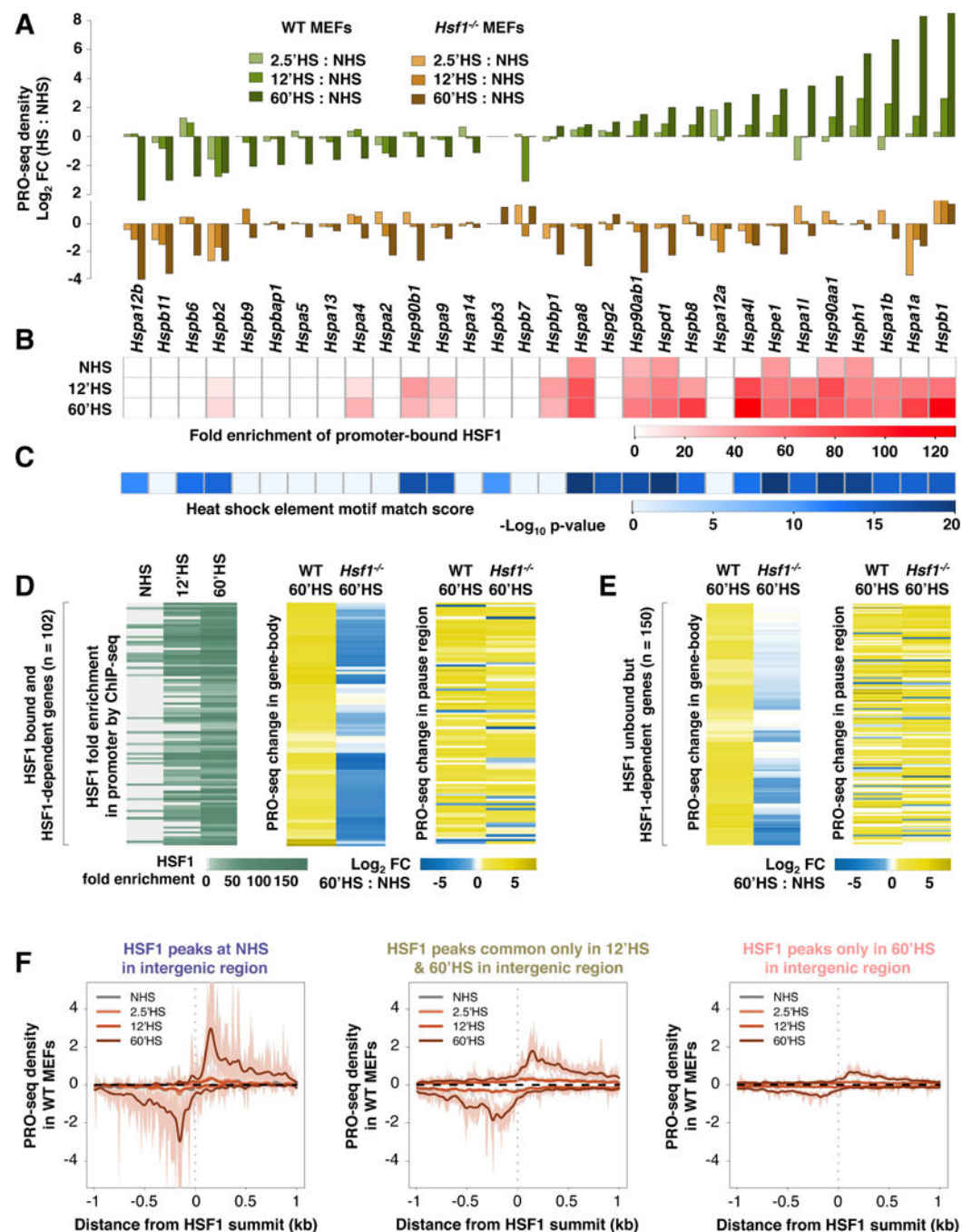


Figure 5. HSF1 is required for induction of classical *Hsps* upon HS and works by increasing pause release

(A) PRO-seq density change upon HS in 30 *Hsps* in WT (top) and *Hsf1*^{-/-} MEFs (bottom) ordered by increasing fold change at WT 60'HS.

(B) HSF1 fold enrichment in the promoter of the corresponding 30 *Hsps* in WT MEFs.

(C) HSE in the promoter of the corresponding 30 *Hsps*.

(D) Heatmap of HSF1 binding (left), change in PRO-seq density in gene-body (center) and pause region (right) of the 102 genes that are bound by HSF1 in the promoter and show HSF1-dependent transcription induction at 60'HS.

(E) Change in PRO-seq density in gene-body (left) and pause region (right) of the 150 genes that are not bound by HSF1 in the promoter but show HSF1-dependent transcription induction at 60'HS.

(F) PRO-seq density (with 95% confidence interval in light shades) before and at time points after HS in WT MEFs around the center of HSF1-intergenic-bound sites at NHS (left), common only in 12'HS & 60'HS (center), and only in 60'HS (right). The color of text in the titles refers to the three classes represented by the same colors in Figure 2C.

See also Figure S5

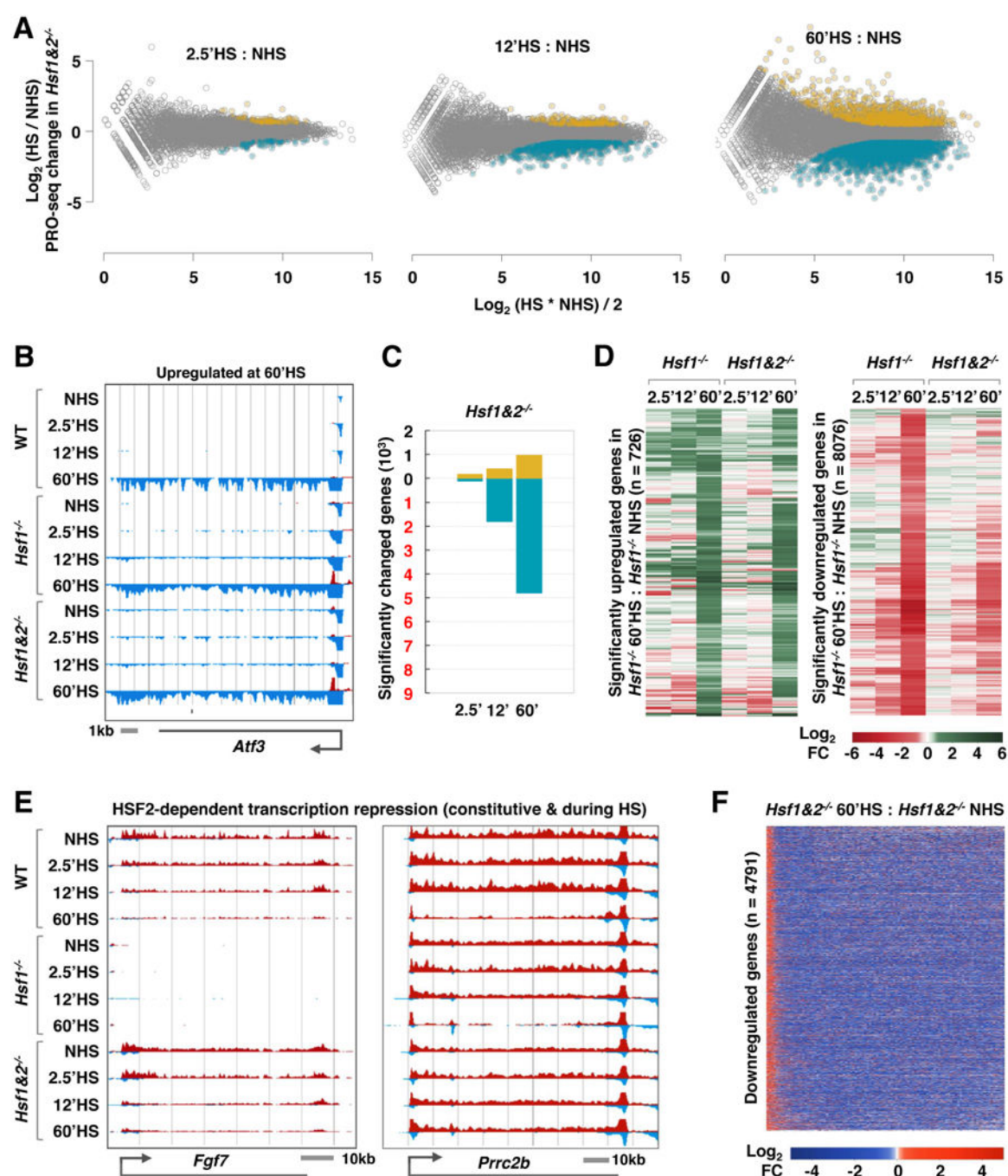


Figure 6. HSF2 is not responsible for transcriptional changes upon HS in *Hsf1*^{-/-} MEFs
 (A) MA plots represent PRO-seq density change in the gene-body of all genes ($n=23460$) between NHS and 2.5'HS (left panel), 12'HS (mid panel), and 60'HS (right panel) in *Hsf1&2*^{-/-} MEFs. Significantly upregulated genes are shown in gold and significantly downregulated genes are shown in blue.
 (B) Screenshots of *Atf3* (significantly upregulated at 60'HS) showing similar kinetics of PRO-seq density change upon HS in WT, *Hsf1*^{-/-}, and *Hsf1&2*^{-/-} MEFs.

(C) Number of significantly changed genes upon HS in *Hsf1&2*^{-/-} MEFs. Upregulated genes are shown in gold and downregulated genes are shown in blue.

(D) Transcriptional status in *Hsf1&2*^{-/-} MEFs upon HS for significantly upregulated genes (left) and significantly downregulated genes (right) in *Hsf1*^{-/-} MEFs.

(E) Screenshots of genes that could be repressed by HSF2 in absence of HSF1 (*Fgf7*) or during HS even in presence of HSF1 (*Prrc2b*).

(F) Heatmap of PRO-seq density fold-change at 60'HS relative to NHS for significantly downregulated genes at 60'HS in *Hsf1&2*^{-/-} MEFs. Each row represents a gene, scaled to same length and divided into 100 bins, from TSS up to polyA site for genes shorter than 24 kb and up to 24 kb for genes longer than 24kb.

See also Figure S6 and Tables S5, S6, S7

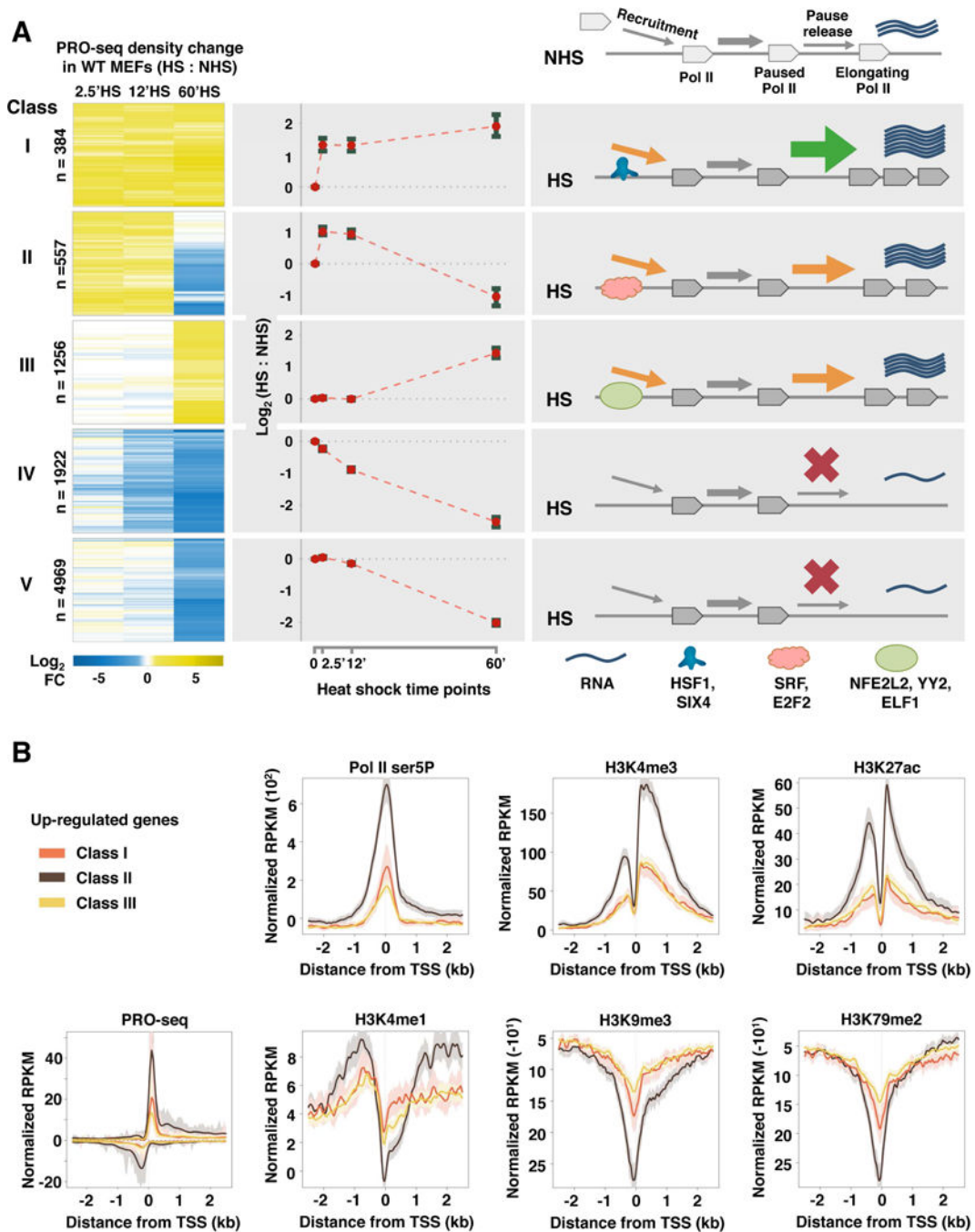


Figure 7. Kinetics and dynamics of transcription induction and repression during HSR
(A) Kinetics, dynamics, and the proposed mechanism of the five major classes of HS-regulated genes in WT MEFs. Heatmap on the left panel shows the gene-body PRO-seq density change upon HS for all genes in each class. The mid panels show the average change in gene-body PRO-seq density - the red points represent the average log₂ fold change for all genes and the green error bars represent 95% confidence interval. The right panels show the step(s) in transcription being regulated. Green arrow indicates positive regulation, the orange arrow represents likely but uncertain regulation, red cross indicates negative regulation, and

thickness of the arrows indicates the extent of regulation. The most highly enriched TF motifs in different classes are shown at the bottom.

(B) Level of different histone marks and Ser5 Pol II (with 95% confidence interval in light shades) prior to HS around the TSS of three kinetic classes of transcriptionally-induced genes upon HS. PRO-seq density around the TSS for those genes is also shown.

See also Figure S7