The Hsp90 Molecular Chaperone Regulates the Transcription Factor Network Controlling Chromatin Accessibility

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Abstract

Genomic events including gene regulation and chromatin status are controlled by transcription factors. Here we report that the Hsp90 molecular chaperone broadly regulates the transcription factor protein family. Our studies identified a biphasic use of Hsp90 in which early inactivation (15 min) of the chaperone triggered a wide reduction of DNA binding events along the genome with concurrent changes to chromatin structure. Long-term loss (6 h) of Hsp90 resulted in a decline of a divergent yet overlaying pool of transcription factors that produced a distinct chromatin pattern. Although both phases involve protein folding, the early point correlated with Hsp90 acting in a late folding step that is critical for DNA binding function, whereas prolonged Hsp90 inactivation led to a significant decrease in the steady-state transcription factor protein levels. Intriguingly, despite the broad chaperone impact on a variety of transcription factors, the operational influence of Hsp90 was at the level of chromatin with only a mild effect on gene regulation. Thus, Hsp90 selectively governs the transcription factor process overseeing local chromatin structure.

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Introduction

Chromatin is an amalgam of DNA, RNA, and protein that serves to condense genomes into suitable packages to fit within nuclei, protect the DNA from damage, yet still provide ready access to the underlying genomic information [1]. At its foundation, chromatin is built with nucleosomes where the DNA is wrapped around histone octamers. The placement, density, and configuration of nucleosomes are dictated by the vast array of transcription factors that are expressed within a given cell [2]. Transcription factors are advantageous for governing large multifarious chromatin structures requiring exact assemblage because transcription factors selectively recognize cognate DNA motifs (driving precision) and are able to interact with a broad array of cofactors including numerous chromatin modifying complexes (providing a breadth of outcomes). The core principle enabling the prominent nuclear role played by transcription factors in both gene regulation and chromatin architecture is a select yet dynamic DNA binding function that enables effective responses to ever fluctuating physiological conditions encountered by cells.

Historically, transcription factors were considered any protein capable of influencing gene expression levels [3]. In general, a transcription factor exacts control over a gene by binding to a cognate DNA motif (consensus element) found at or near the locus start site. Once DNA bound, the transcription factor nucleates a series of cofactors (*e.g.*, histone acetyltransferases, chromatin remodelers, mediator) to generate the needed changes to the transcription rate of a given target. In addition to gene regulation, it is now evident that transcription factors also perform other duties along the genome including the regulation of chromatin structure. Paralleling gene regulatory events, transcription factors influence the status of chromatin by recognizing cognate DNA elements and recruiting a series of cofactors to influence both the local and global architecture [2]. Although it is unclear what determines whether a transcription factor mediates gene- and/or chromatin-regulatory events at a given DNA site, it is apparent that

transcription factors are the keystone components of genomic pathways. Despite the pivotal roles played by transcription factors, the cellular process enabling the functionality of the heterogeneous transcription factor protein family is poorly understood.

Although transcription factors have the common ability to modulate gene promoter activities, the protein family does not share a common domain-structure or even a conserved amino acid motif. Rather, numerous polypeptide folds are used to oversee cellular gene programs. For instance, DNA binding domains (DBDs) use a variety of forms including basic helix-loop-helix (bHLH), zinc finger, Myb-like, homeodomain, basic leucine zipper (bZIP), as well as other types [3]. The complexity of the transcription factor family is expanded by variances within the amino acid sequences of the different folds. For example, single residue changes within an alpha helix of a bHLH domain can account for the recognition of vastly different cognate DNA elements [3]. Even with these differences the functional goal of any DBD is the same—to selectively bind DNA. How such a structurally diverse protein family is effectively managed to reach a singular end point is unclear.

We believe the Hsp90 molecular chaperone oversees the sizeable transcription factor protein family. Hsp90 was initially identified as a stable component of various steroid aporeceptor transcription factor complexes [4]. Early work implied the interaction was required to maintain the solubility and functionality of the hormone binding domain, whereas more recent work suggests Hsp90 modulates the DNA binding activity of steroid receptors although direct evidence is lacking [5]. However, Hsp90 has been shown to support the DNA binding activities of certain bHLH factors, including MyoD and the Dioxin receptor, along with the Greek-key β-sandwich DBD of p53 [6–10]. Whether Hsp90 influences other types of DBD folds is not clear. Perhaps notably, Hsp90 has long been regarded as an epigenetic factor and a broad regulatory role with DNA binding proteins might explain why Hsp90 impairment has been linked to enigmatic genomic events including aneuploidy and long-term phenotypic variation [11–13]. Here, we explored the influence of Hsp90 on local chromatin structure as well as the DNA binding factors working along the genome.

Results

Chromatin accessibility displays temporal changes after loss of Hsp90

To investigate the dependence of chromatin structure on Hsp90, we exploited budding yeast engineered to express either the wild-type (WT) or the temperature-sensitive (*ts*) variant G170D as its sole source of Hsp90 in conjunction with high-throughput sequencing of DNase I treated nuclei (DNase-Seq) [14,15]. Use of the *ts* allele avoids off-pathway complications associated with Hsp90 inhibitors and DNase-Seq permits an assessment of DNA binding activities (DNA footprints) in addition to evaluating local chromatin architecture [16]. We previously used this tactic to discover that Hsp90 regulates the RSC (remodel the structure of chromatin) nucleosome-remodeling complex [17]. In our prior work, we probed the local chromatin structure after a short-term (15 min) inactivation of Hsp90 [17]. Under these conditions, DNase I cleavage levels were enhanced at RSC-dependent sites because Hsp90 terminates RSC action (*i.e.*, dissociates the nucleosome-bound complex) [17]. However, further analysis of this DNase-Seq dataset revealed that at RSC-independent sites there was a general reduction in the status of open chromatin with an ~15% decrease in the number of DNase I hypersensitive sites (DHSs) contributing to an ~13% decline in the total length of open chromatin across the genome (Fig. 1A; Table S1). Hence, the main feature triggered by the loss of Hsp90 is a reduction in DNA access (*i.e.*, chromatin openness) indicating that Hsp90 contributes to other chromatin-associated activities besides RSC.

To expand our evaluation, we checked the chromatin structure after a prolonged inactivation of Hsp90 (6 h). We selected the 6 h point because it was the longest Hsp90-inactivation time at which the growth of G170D yeast continued and was fully recovered after a shift back to a permissive temperature (30°C) indicating that no permanent damage to cell viability had occurred (Fig. S1A). Overall, the chromatin displayed a further reduction in accessibility relative to the 15-min treatment as there was an ~17% reduction in the total length of open chromatin after 6 h at 37°C in G170D expressing yeast compared with WT Hsp90 (Fig. 1B and Table S1). In contrast to the 15-min time point, sites where DNA accessibility significantly increased (*e.g.*, RSC-associated sites) were not common after 6 h of Hsp90 loss (Fig. 1A and C). Yet, when comparing cells expressing WT Hsp90 *vs.* G170D, the total number of DHSs increased slightly as G170D had ~9% less DHSs than WT Hsp90 at 6 h, whereas the decline was ~13% at 15 min (Table S1). However, the rise in the number of DHSs mainly stemmed from the "splitting" of peaks (*i.e.*, one large peak in WT deteriorated into two or more smaller peaks) as well as a general loss of weak DHSs in both WT and G170D cells incubated at 37°C for 6 h rather than from the emergence of de novo open chromatin sites.

As nucleosome density/positioning typically dictates DNA accessibility, we checked the nucleosome status at two DHSs found to be impacted after

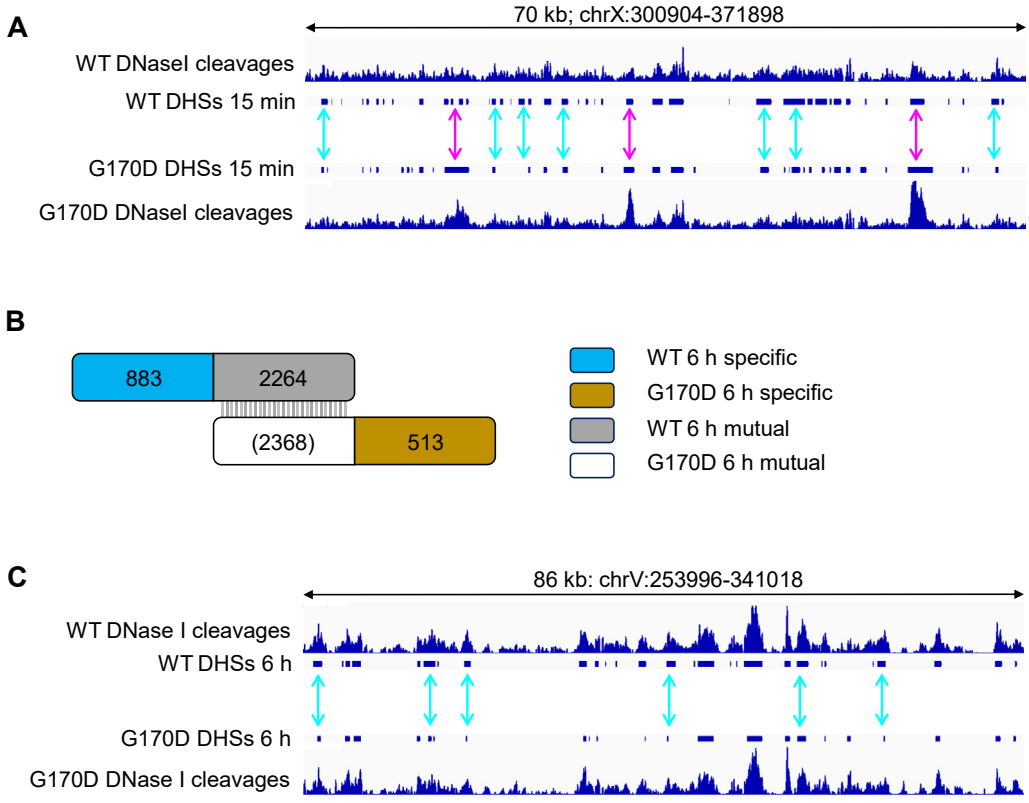

Fig. 1. Chromatin architecture is Hsp90-dependent. (**A**) Chromatin access was probed by DNase I cleavage in yeast expressing Hsp90 wild type (WT) or the temperature sensitive allele G170D after incubation at 37°C for 15 min [17]. A representative section of the genome is shown with the pink arrows demarking DHSs with increased hypersensitivity and blue arrows marking reduced DHSs after loss of Hsp90 in the G170D strain. (**B**) The pattern of DHSs changed after long-term (6 h) inactivation of Hsp90. The blue bar represents DHSs unique to WT, the gray/white bars are the overlapping DHSs, and the bronze bars are the DHSs unique to G170D after 6 h at 37°C. (**C**) The local chromatin structure was distinct after long-term (6 h) Hsp90 inactivation. A representative section of the genome is shown with the blue arrows marking DHSs with reduced length and/or hypersensitivity after 6 h at 37°C in G170D.

6 h of Hsp90 inactivation by DNase-Seq (Fig. 2C). Here, we used the small molecule inhibitor Radicicol as an alternative means to inactivate Hsp90 and used an established PCR-based MNase mapping assay [17] to assess the nucleosome conditions at these loci. At both sites the apparent nucleosome density was increased thereby justifying why DNA accessibility declined (Fig. 2C and Fig. S1B).

Of note, the previously described RSC-associated DHSs with increased hypersensitivity at 15 min of Hsp90-inactivation were no longer detected after 6 h at 37°C (Fig. S2A) [17]. Whether the loss is Hsp90-dependent, however, is not clear because these DHSs also dissipated in the WT background after 6 h at 37°C (Fig. S2A). Besides the RSC sites, we observed other chromatin changes linked to the prolonged incubation at 37°C including a rise in DHSs at subtelomeric regions, tRNA genes, and long terminal repeats (LTRs) (Fig. S2B and C; Table S2). These chromatin alterations are consistent with prior studies showing that environmental changes including increased temperature reduce subtelomeric gene silencing and increase transcription at LTR retrotransposons [18,19]. Nevertheless, the general trend following Hsp90 inactivation is a decline in chromatin accessibility.

Chromatin changes correlate with declined transcription factor DNA occupancies

In addition to revealing local chromatin structure, DNase-Seq is useful for detecting short protected DNA sites within DNase I hypersensitivity sites (*i.e.*, DNA footprints). In conjunction with a DNA motif scan the transcription factors bound within DHSs can be identified based upon homology to established cognate binding elements (*i.e.*, consensus sequences) [16,20]. A genome-wide digital footprint analysis of the 15 min and 6 h datasets showed that the reduction in open chromatin after Hsp90 inactivation correlated with a decline in DNA-bound proteins (Figs. 2 and S3). Although the total number

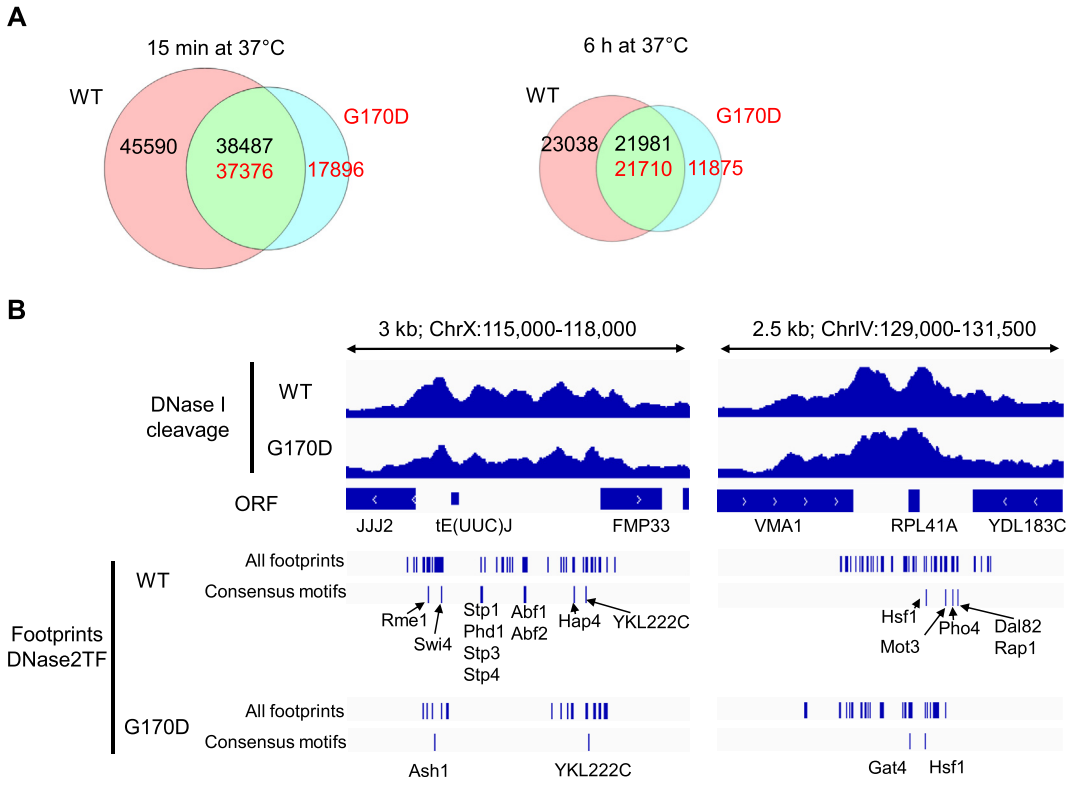

Fig. 2. Loss of Hsp90 correlates with a decline in DNA footprints. (**A**) The total number of DNA footprints in yeast expressing WT or G170D was determined after 15 min or 6 h incubation at 37°C, as marked. The Venn diagrams indicate the absolute overlap in the number of DNA motif types independent of exact location (numbers in black are for WT, whereas those in red are for G170D). DNA footprints were detected within computed DHSs using the DNase2TF (fdr 0.01) algorithm (20). (**B**) The DNA footprint pattern altered significantly after loss of Hsp90. The DNase I cleavage levels at representative sites, either chromosome X 115,000–118,000 bp or chromosome IV 129,000–131,500 bp are shown along with the detected DNA footprints. DNA motifs corresponding to known consensus elements were identified with RSA-tools using frequency matrices obtained from JASPAR that are marked accordingly as well as the open reading frames (ORFs) [21,22].

of identified DNA footprints varied depending upon the computational program used, the trends were consistent with ~33% less occupied DNA motifs after 15 min of Hsp90 inactivation and ~25% less after 6 h (Figs. 2 and S3). Briefly, the three programs we used vary the footprint detection algorithms and computing depth thereby leading to different numbers of occupied DNA footprints [16,20,23]. Nevertheless, the trends and overlap (~85%) in the computed footprints with respect to the founding digital footprinting program [16] indicated a consistent decline in bound transcription factors after the loss of the Hsp90 (Fig. S3).

To investigate the impact of Hsp90 on DNA binding events, we focused on the computational findings revealed using the DNase2TF program with a 1% false discovery rate (0.01 fdr) because this tactic produced the highest potential coverage of DNA footprints [20]. With these criteria, 84077 footprints were detected in yeast expressing WT Hsp90 grown for 15 min at 37°C and 55272 in yeast with G170D, whereas 45019 footprints were identified in WT after 6 h at 37°C but only 33585 were found in G170D (Fig. 2A). Paralleling the chromatin affects, an influence of prolonged incubation at 37°C was apparent even in yeast expressing WT Hsp90. Yet at both time points a significant Hsp90-dependent effect was apparent because there was a ~35% decline in footprints at 15 min and ~26% after 6 h. When a more in-depth evaluation was performed where motif locations were followed, it was evident that ~55% of the WT footprints were lost after Hsp90 inactivation at either time point but that the emergence of new footprints in G170D buffered the decline in total numbers (Fig. 2A). For example, 17896 footprints arise along the genome in G170D after 15 min at 37°C, and these G170D-specific binding events essentially veil the apparent decline in total footprints (Fig. 2A and Table S3).

To illustrate these convoluted changes, we show discreet areas of chromosome X and IV that displayed typical alterations in the DNA footprint

patterns occurring after 6 h of Hsp90 loss (Fig. 2B). At both sections it is evident that all footprints, ones corresponding to identified cognate motifs as well sites bound by unidentified factors, were reduced in the G170D background and that the occupied consensus elements changed. Importantly, these were not isolated examples. If we evaluate the detected DNA footprints along a larger tract of DNA (chromosome I between bases 120,000 and 180,000), we found that a distinct set of DNA binding activities are apparent under each experimental condition with Hsp90 loss drove a decline of DNA footprints at either 15 min or 6 h (Figs. 3A and S4A; Table S4). Within this region the total number of occupied consensus elements was 327 for WT 15 min, 201 for G170D 15 min, 265 for WT 6 h, and 160 for G170D 6 h, and each time point displayed a distinct set of DNA binding activities (Fig. S4A). Of note, the pattern of occupied DNA sites varied between the 15 min and 6 h time points suggesting that the impact of Hsp90 inactivation shifted with time.

Extending our analysis to a genome-wide view revealed that the occupancy of 32 of 164 consensus motifs declined (count in G170D was 75% or less relative to WT Hsp90) after 15 min of Hsp90 inactivation and that 61 were down after 6 h (Figs. 3B and S4B; Table S4). As before, there were considerable changes in the pattern of transcription factor binding, which resulted in a low overlap with only ~7% (5660 of the 84077) of the sites found in the WT 15 min being occupied in the other three datasets (Table S5). Regardless of whether a cognate binding partner was known for a DNA motif or not, Hsp90 inactivation reduced the occupancy of all DNA binding activities (Figs. 3B and S4B). A two-tailed statistical test on the 6 h datasets indicated a significant difference (P value = 0.0493) across all sites. The 15 min data were not as straightforward because not all sites show a change, yet if the top 96 motifs (164 total) were considered a difference was apparent (P value = 0.0094). Given that only a small percent (~18%) of the observed DNA footprints align with the known consensus motifs (Fig. S4B) [22], these observations are notable because it indicates that Hsp90 has a general role in promoting all cellular DNA binding factors.

Hsp90 supports transcription factor protein stability and DNA binding

Hsp90 is a central component of the cellular molecular chaperone system and is known to maintain the stability of labile proteins [4]. As such, a probable mechanism driving the broad reach of

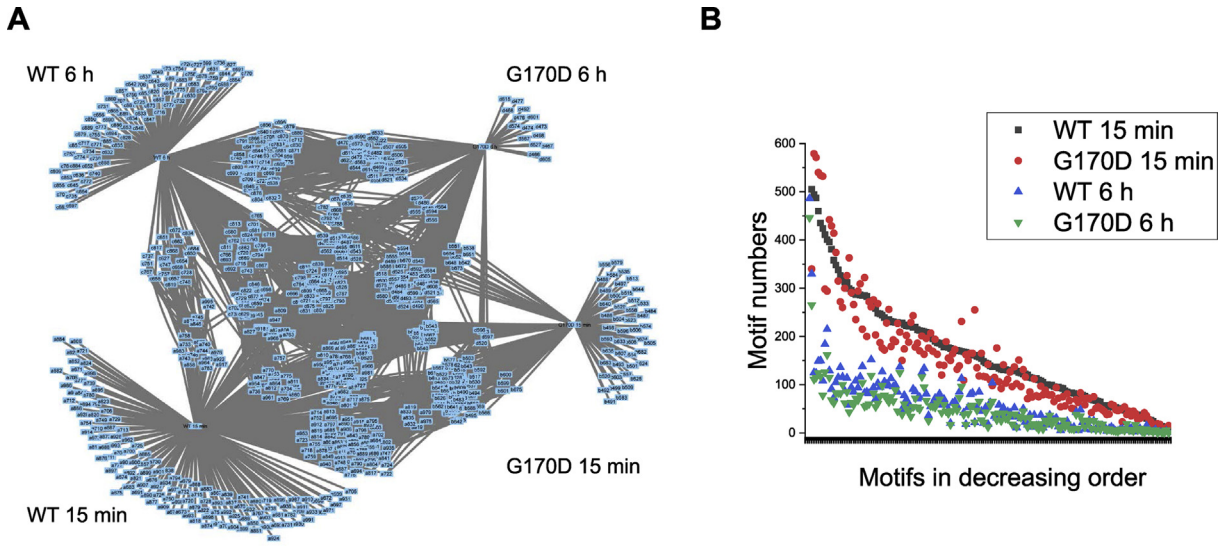

Fig. 3. Protein-DNA binding patterns shift at 37°C with an Hsp90-dependency. DNA footprints were detected using DNase-Seq and RSA-tools in yeast expressing WT or G170D Hsp90 incubated 15 min or 6 h at 37°C. (**A**) DNA footprints on chromosome I between 120,000−180,000 bp were determined by DNase-Seq using DNase2TF (fdr 0.01) from yeast expressing WT or G170D Hsp90 incubated at 37°C for 15 min or 6 h. The total number of footprints in this region was 327 for WT 15 min, 201 for G170D 15 min, 265 for WT 6 h, and 160 for G170D 6 h. The pattern of overlap is shown in (**A**) using Cytoscape. The actual identified DNA footprints are listed in Table S4 indexed as a-series for WT 15 min, b-series for G170D 15 min, c-series for WT 6 h, and d-series for G170D 6 h at 37°C. (**B**) The genome-wide occupancies of consensus motifs generally declined in the absence of Hsp90 activity (*i.e.*, G170D background). For illustrative purposes, all consensus motifs were graphed in order of binding usage observed in the WT 15 min sample and the actual numbers and motifs are shown in Table S3.

Hsp90 in DNA binding events is the maintenance of steady-state protein levels. To check this possibility, we followed transcription factors displaying reduced DNA footprints at 15 min (Cbf1), at 6 h (Abf1, Reb1, Rsc3), or at both 15 min and 6 h (Ecm22, Ino2, Ino4, Mcm1, Rap1). Interestingly, only Mcm1 showed a reduction in protein levels at the 15 min time point, but all except Cbf1 showed reduced protein amounts by 6 h of Hsp90 loss (Fig. 4A). We validated these findings using the Hsp90 inhibitor Radicicol and the transcription factors Abf1, Ino2/4, Rap1 (Fig. 4B). Hence, Hsp90 is broadly used to support long-term (6 h) transcription factor protein stability in budding yeast (Fig. 4A and B).

We checked whether the ability of Hsp90 to protect transcription factors is conserved. We treated 3T3 mouse fibroblast cells with the Hsp90 inhibitor Radicicol and monitored the levels of c-Myc, HSF1, and GATA5. In the presence of Radicicol, the levels of all the transcription factors declined (Fig. 4C). Although the conserved ability of Hsp90 to maintain protein stability can explain why DNA binding events were reduced after 6 h of Hsp90 inactivation, why DNA occupancies declined after only 15 min of Hsp90 loss was not apparent because steady-state levels remain constant at the early time point.

Prior studies showed that Hsp90 can "activate" the DNA binding activities of the muscle-specific

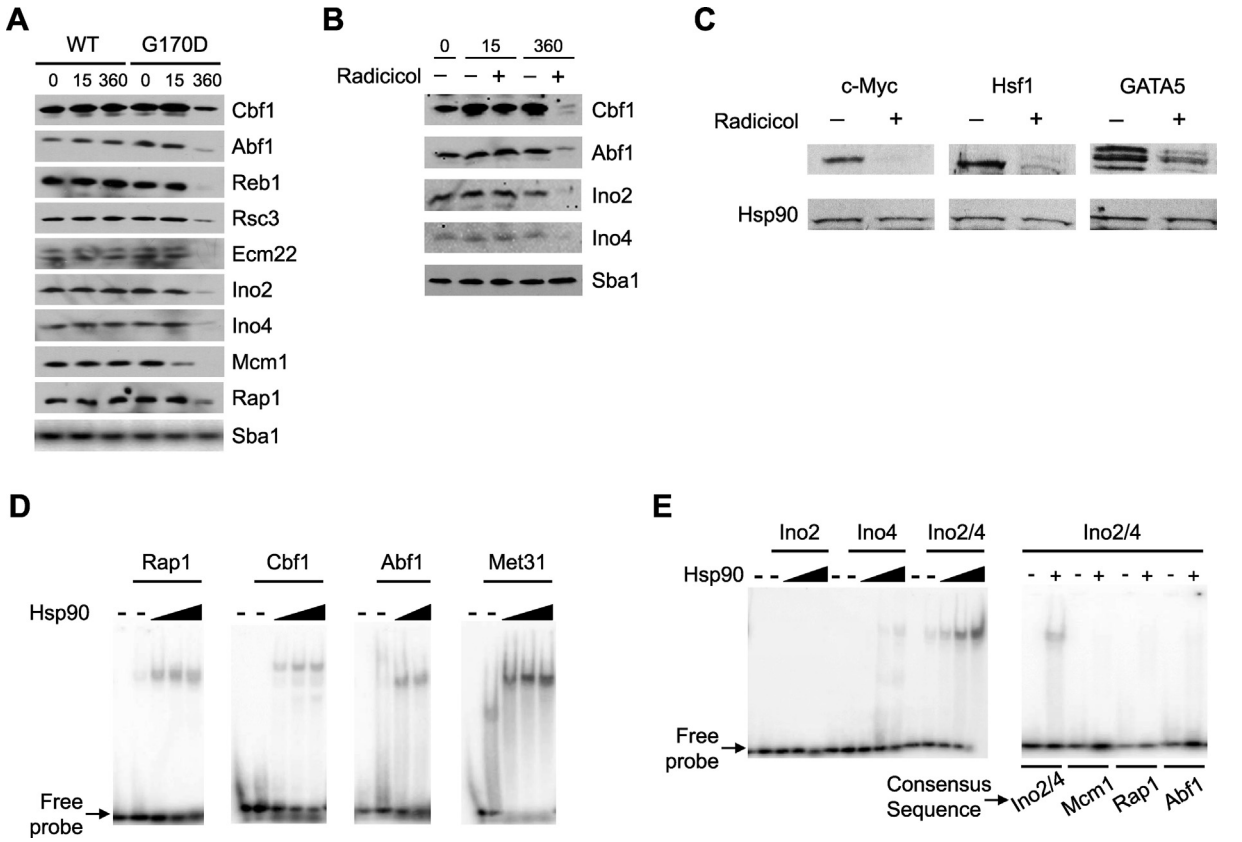

Fig. 4. Transcription factor protein stability and activation of DNA binding activity are Hsp90-dependent. (**A**) The steady-state protein levels of the indicated transcription factors were visualized by immunoblot analysis from yeast expressing WT or G170D Hsp90 grown at 30°C (0) or 37°C for 15 min or 6 h, as marked. All transcription factors were TAP-tag fusions detected using an anti-TAP antibody. (**B**) The steady-state protein levels of the indicated TAP-tagged transcription factors were visualized by immunoblot analysis from yeast untreated or treated for 15 or 360 min with either DMSO or Radicicol (20 μM), as indicated. (**C**) The steady-state protein levels of the c-Myc, Hsf1, and GATA5 transcription factors in the mouse fibroblast cell line 3T3 grown in the absence or presence of Radicicol (10 μM) were determined by immunoblot analysis using antibodies selected for each factor. (**D**) The DNA binding activities of recombinant Rap1 (250 pM), Cbf1 (1 nM), Abf1 (100 nM), and Met31 (1 μM) were determined by EMSA using radiolabeled cognate consensus element for each factor alone or in the presence of increasing amounts of recombinant yeast Hsp90 (4, 12, and 34 μM), and total protein amounts were balanced with BSA. (**E**) The DNA binding activities of Ino2 (1.25 μM), Ino4 (1.25 μM), and Ino2/Ino4 (300 nM) were determined by EMSA using a radiolabeled consensus element alone or in the presence of increasing amounts of recombinant yeast Hsp90 (4, 12, and 34 μM) with total protein amounts being balanced with BSA. The specificity of Ino2/Ino4 was assessed using radiolabeled probes representing consensus elements for Ino2/4, Mcm1, Rap1, or Abf1, as marked.

bHLH transcription factors MyoD and E12 [6,8]. We investigated whether Hsp90 might broadly influence the DNA binding functions of transcription factors irrespective of the type of DBD fold. We selected nine potential targets that displayed reduced DNA footprints after Hsp90 inactivation (Table S3). These transcription factors contain bHLH (Cbf1, Ino2, Ino4), zinc finger (Abf1, Met31, Rsc3, Rsc30), or Myb-like (Rap1, Reb1) DBDs.

We purified each from *Escherichia coli* as SUMO fusion proteins, thereby avoiding exposure to a eukaryotic Hsp90 homolog and then tested the capacity of each to bind to their cognate DNA element alone or in the presence of increasing levels of yeast Hsp90 (Figs. 4D, E, and S5). With the exception of Reb1 (Fig. S5B), all of the transcription factors required exposure to yeast Hsp90 in order to bind their consensus DNA motifs. Addition of Radicicol to the preformed transcription factor-DNA complexes had no apparent effect suggesting Hsp90 is only transiently needed to establish the DNA binding activities (Fig. S5C). Of note, Ino2 and Ino4 work as a heterodimer and the Hsp90 effect was most apparent when both Ino2/Ino4 were present in the reaction along with a consensus DNA element (Fig. 4E). Hence, Hsp90 serves a broad spectrum of transcription factors with varying types of DBDs. Likely, the capacity of Hsp90 to "activate" DNA binding proteins is indicative of Hsp90's ability to mediate a late step in a protein folding pathway (*i.e.*, these factors were isolated as soluble folded proteins from *E. coli*).

Hsp90-dependent gene expression changes

The wide influence of Hsp90 on both transcription factors and DNase hypersensitivity sites prompted the logical speculation that Hsp90 inactivation would trigger significant changes in gene expression. To investigate this point, we isolated RNA from cells expressing wild-type or G170D Hsp90 grown at 37°C for 6 h and performed RNA-Seq. Computational analysis indicated that only 113 genes were upregulated (GFold $\log_2 \geq 1.5$) and 152 genes were downregulated (GFold $\log_2 \leq -1.5$), demonstrating an unexpectedly mild influence of Hsp90 loss on gene expression (Table S6). The majority of the genes with altered expression also displayed chromatin changes by the 6 h treatment time point with the activated genes having increased chromatin accessibility and the repressed loci displaying declined access (Fig. 5 and Table S7).

Thus, the primary outcome of Hsp90's influence over transcription factor stability/activity was altered chromatin accessibility (Fig. 1). Likely, the inherent specificity of the transcription process (*i.e.*, higher affinity DNA binding elements being used to regulate gene activity) limits the impact on gene regulation, whereas the flexibility of chromatin maintenance allows Hsp90-dependent fluctuations to be tolerated. It is plausible that continued inactivation of Hsp90 eventually leads to a full collapse of the transcription factor network, which would help explain why the chaperone is essential. However, validating this concept is unlikely, given the wide client network served by Hsp90 and the likely considerable pleotropic effects on homeostasis that occur once cells are no longer able to recover from the inactivation of this chaperone, which we have avoided (Fig. S1A).

Discussion

Transcription factors are keystone components dictating central biological processes including chromatin architecture and gene regulation [2,3]. Despite decades of intensive research on transcription factors, little is understood on how these critical proteins are established and maintained. Here, we provide evidence that the Hsp90 molecular chaperone broadly oversees the transcription factor protein

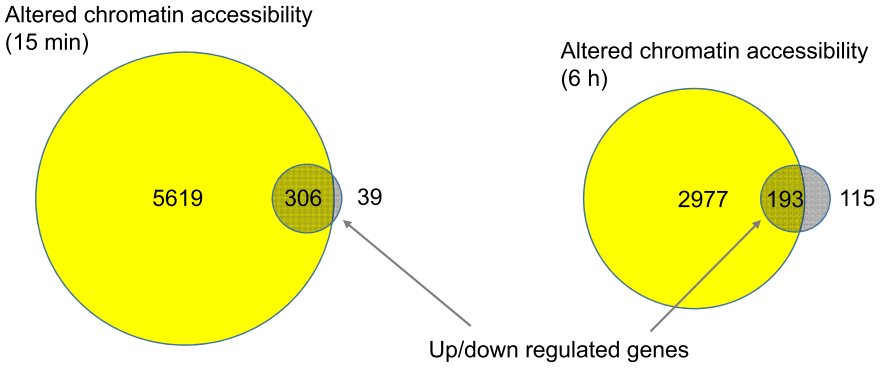

Fig. 5. Loci displaying changes in gene expression also have altered chromatin accessibility after Hsp90 inactivation. Venn diagrams depicting the overlap between sites with altered chromatin structure (gain or loss of DHS size) at 15 min or 6 h and changed gene expression (GFold $\log_2$1.5) [24] is shown.

network. Our data support a model in which Hsp90 is required for both late folding events maturing transcription factors into active DNA binding units as well as fostering steady-state polypeptide levels. Recent studies have delineated the mechanistic contributions of both Hsp90 and Hsp70 to the folding events of the p53 transcription factor [25,26]. Our study expands the influence of Hsp90 to include the majority of a yeast cell's DNA binding factors, as the pattern of DNA occupancy shifted significantly after Hsp90 inactivation (Fig. 2).

Historically, Hsp90 was considered a strict cytosolic molecular chaperone [29]. Yet, a growing body of evidence indicates that not only does Hsp90 have a presence in the nucleoplasm but also that numerous nuclear pathways are dependent upon Hsp90 [30]. One of the more notable phenotypes associated with Hsp90 is its ability to work as a capacitor of morphological evolution [31]. Hsp90 silences widespread variance in morphogenic pathways, and transient impairment of Hsp90 reveals these traits [11]. Notably, the Hsp90-buffered phenotypes map to both coding loci as well as *cis*-regulatory sites of the genome suggesting a role in transcription factor DNA binding [32].

Although the mechanism(s) driving Hsp90's capacitor activity is yet to be delineated, several lines of evidence implicate epigenetic-/genetic-related pathways [33]. An early report showed that Hsp90 inactivation triggered a heritable altered chromatin state noting it to be comparable with mutations in the epigenetic factor Trithorax, which was later shown to be an Hsp90-client [34,35]. Other mechanisms mediating the capacitor function have been proposed including roles for Hsp90 in preventing transposon-based mutagenesis, instability of repetitive DNA elements, chromosome rearrangements, aneuploidy, and other types of DNA damage [27,28,33]. Whether one or all of these pathways contribute to the evolutionary capacitor function is not clear. Nevertheless, each pathway is reliant upon DNA binding proteins to operate. Hence, the broad influence of Hsp90 on DNA binding factors might explain why Hsp90 has such a wide-ranging influence on genomic events including its enigmatic ability to work as an evolutionary capacitor [33].

Minimally, the ability of Hsp90 to modulate numerous transcription factors can account for the extensive physical and functional distribution of the chaperone across a genome. In *Drosophila melanogaster*, Hsp90 localizes at or near the transcription start site of approximately one-third of all genes, as shown using the chromatin immunoprecipitation assay [36]. In addition, Hsp90 has been observed by immunofluorescence at select DNA loci in *Drosophila* salivary glands where multiple copies of the genome are aligned thereby facilitating visualization. Under heat shock conditions, Hsp90 localizes to the classic 93D heat shock puff in *D. melanogaster*, the 48B puff in *Drosophila hydei*, and the Balbiani ring puffs in *Chironomus thummi* [37]. Inhibition of transcription but not protein synthesis blocked nucleation of Hsp90 to these puffs, implying a chaperone role in transcription events [37]. Our demonstration that Hsp90 regulates most DNA binding proteins provides a mechanistic rationale for why Hsp90 is widely linked to transcription-associated sites.

Besides physical interactions with numerous genomic loci, Hsp90 also has been shown to have functional influences over diverse nuclear pathways [30]. For instance, inhibition of Hsp90 can trigger aneuploidy, and aneuploidy cells show impaired Hsp90 function [12,13]. While these phenotypes have been ascribed, in part, to Hsp90's role in kinetochore assembly, other defects likely contribute because the chromosome duplications are not always closely linked to centromeres [38,39]. Certainly a decline in any DNA binding proteins overseeing chromosome stability/counting would negatively impact ploidy. Other DNA damage response pathways are influenced by Hsp90's capacity to maintain steady-state levels of select DNA binding proteins including BRCA1/2 and the MRE11/RAD50/NBN complex [40–42]. Whether Hsp90-dependent changes in DNA binding by these proteins influences phenotypic variation is yet to be resolved.

Despite the variety of nuclear pathways that Hsp90 modulates, our presented work raises an apparent question: if most transcription factors are affected, why is the outcome mainly on chromatin architecture as opposed to gene regulation? One potential is the direct role that Hsp90 has with the chromatin remodeling complex RSC in which the chaperone fosters the transition of the remodeler between nucleosomal targets and prolonged Hsp90 inactivation, which leads to loss of the RSC DNA binding subunit Rsc3 (Fig. 4A) [17]. Perhaps notably, Hsp90 shares genetic and/or physical interactions with six of eight yeast chromatin remodelers [43,44]. Alternatively, the Hsp90-dependent influence on chromatin might be more apparent because chromatin is inherently more flexible than transcription. For instance, multiple transcription factors can coordinate the same local chromatin architecture but gene promoter control is typically transcription factor-specific [45]. Thus, an additional plausible mechanism guarding against changes in the gene program, as the availability of transcription factors declines, is the veritable funneling of the existing proteins to the high-affinity motifs typically used to regulate promoter activities.

The ability of Hsp90 to govern the heterogeneous DNA binding protein family reveals a new level of chaperone influence on homeostasis. Although it has been well appreciated that Hsp90 regulates the protein kinase network [4], our presented work

demonstrates that Hsp90 has an equally wide impact on proteins controlling the chromatin system. Minimally, Hsp90 fosters steady-state levels of kinases as well as maintaining these proteins in "activatable" states, which would compare with the DNA binding end point. Distinguishing these two large protein families would be how Hsp90 recognizes each. Many kinases are bound through a common electrostatic surface found within the amino-terminal lobe of the kinases [46], whereas the transcription factors share few structural features. How Hsp90 manipulates the more physically diverse DNA binding protein family and why impairment of Hsp90 leads to such radical changes in the pattern of DNA binding factors is yet to be determined. Perhaps changes to kinase signaling are impacting transcription factors' interactions, thereby altering where the proteins occupy the genome. Regardless of the additional inputs prompting the change in DNA binding patterns, our work provides new insights into the physiological relevance of Hsp90 that likely have a direct bearing on how Hsp90 functions as an evolutionary capacitor and is a driver of numerous cancers [33,47].

Experimental Procedures

Yeast

The used *Saccharomyces cerevisiae* strains were previously described [14,48]. TAP-tagged variants were produced by standard recombinant methods by introducing the fusion tag into yeast expressing either WT or G170D Hsp90 as a sole source of the chaperone [49].

DNase-Seq analysis

The DNase-Seq protocol was adapted from an established method [16] as described previously [15]. DNase I hypersensitive sites were identified with hotspot [50] and DNA footprints with published programs [16,20,23].

Protein purification

Recombinant Abf1, Cbf1, Ino2, Ino4, Met31, Rap1, Reb1, Rsc3, and Rsc30 were purified as His_6-SUMO fusion proteins [51]. Yeast Hsp90 was purified as previously described [52].

EMSAs

DNA binding assays used radiolabeled probes representing the indicated cognate DNA elements (20 nM) and incubated at 37°C for 30 min in the described binding buffer [53]. Probe sequences were GAAGATCACTTCTA ACCAAAG (Abf1); GCGCACGTGACTACAACTGT GGCTG (Cbf1); TTAATTCACATGGAGCAGA (Ino2); TGCGGCATGTGAAAAGTATT (Ino4); GCGCACGTGAC TACAACTGTGGCTG (Met31); CACACACCCACACAC CACA (Rap1); GGGGAAGCGGGTAAGCTGCC (Reb1); and ACGCGCGCGCGGCCGGGCCA (Rsc3/30). Reactions were resolved on 4% native polyacrylamide 1xGTG gels, dried, and imaged using a PhosphorImager (Molecular Dynamics).

Tissue culture

The mouse fibroblast vSRC 3T3 cell line was a kind gift of Dr. Leonard Neckers (NCI). The cells were cultured in Dulbecco's modification of Eagle's Medium with 4.5 g/L glucose, L-glutamine, and sodium pyruvate supplemented with 10% heat-inactivated fetal bovine serum. Where indicated, Radicicol (10 μM) or DMSO was added to 70% confluent cells, which were then incubated for 24 h.

RNA-Seq

Total RNA was isolated from yeast expressing WT or G170D Hsp90 grown in YPD medium at 37°C for 6 h ($OD_{595} = 0.7$) using Yeast RiboPure isolation kit (Ambion). The RNA-Seq libraries were prepared with Illumina's TruSeq Stranded mRNA Sample Prep kit, sequenced in one lane for 101 cycles with Illumina HiSeq2000, and processed with Casava 1.8.2. The reads were trimmed with trimmomatic [54], aligned to the sacCer3 reference genome (UCSC, April 2011) with STAR [55], and differential gene expression was computed with GFold (V1.1.0) [24].

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Author Contributions

ZG contributed experimental, theoretical, and writing effort; LDB and JK added experimental and writing work; BCF contributed experimental, theoretical, and writing effort.

Conflicts of Interest

The authors have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmb.2019.09.007.

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