Published in final edited form as:

Adv Cancer Res. 2016 ; 129: 89–106. doi:10.1016/bs.acr.2015.08.002.

HSP90 IN CANCER: TRANSCRIPTIONAL ROLES IN THE NUCLEUS

S. K. Calderwood¹, L. Neckers²

¹Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA02215,

²Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD20892.

Abstract

Hsp90 plays a key role in fostering metabolic pathways essential in tumorigenesis through its functions as a molecular chaperone. Multiple oncogenic factors in the membrane and cytoplasm are thus protected from degradation and destruction. Here we have considered Hsp90's role in transcription in the nucleus. Hsp90 functions both in regulating the activity of sequence specific transcription factors such as nuclear receptors and HSF1, as well as impacting more globally acting factors that act on chromatin and RNA polymerase II. Hsp90 influences transcription by modulating histone modification mediated by its clients SMYD3 and trithorax / MLL, as well as by regulating the processivity of RNA polymerase II through negative elongation factor (NELF). It is not currently clear how the transcriptional role of Hsp90 may be influenced by the cancer milieu although recently discovered posttranslational modification of the chaperone may be involved. Dysregulation of Hsp90 may thus influence malignant processes both by modulating the function of specific transcription factors and effects on more globally acting general components of the transcriptional machinery.

Keywords

Hsp90; cancer transcription; posttranslational; modification; RNA polymerase II; trithorax

Introduction

Hsp90 belongs to a family of proteins, initially named for their mobility on SDS-PAGE gels, now known to have at least five family members: these include two cytoplasmic members, an ER resident cousin and a mitochondrially located sibling (Felts et al., 2000; Lindquist and Craig, 1988). The current review deals with the proteins Hsp90 α and Hsp90 β found in the cytoplasm and nucleus that are encoded by, respectively, the *HSP90AA1* and *HSP90AB1* genes (Sreedhar et al., 2004). Hsp90 α and Hsp90 β are both molecular chaperones and take part in the later stages of protein folding, refining the structures of client proteins into functional conformations. Both proteins can associate with ATP and carry out molecular

chaperone functions that require ATP hydrolysis in the folding reaction. ATP hydrolysis by Hsp90 is, in itself, quite slow but it is increased to physiologically relevant rates by association with regulatory proteins known as co-chaperones that catalyze various steps in the chaperoning of client proteins into active conformations as well as determining substrate selection(Calderwood, 2013).

The duplication event that gave rise to two related Hsp90 isoforms is thought to have occurred about 500 million years ago, permitting the development of some divergent functions for the two proteins that have only recently been investigated with any intensity(Sreedhar et al., 2004). However many of the studies reviewed here did not take into account the potential for isoform-specific functions and the Hsp90 properties we discuss are largely the aggregate of the properties of the two isoforms. Hsp90 α is the more stress-inducible family member and contains arrays of heat inducible *cis*-acting elements (heat shock elements- HSE) in its promoter while Hsp90 β functions more as a “housekeeping chaperone”. HSE are binding sites for heat shock factor 1 (HSF1), a protein that responds to proteotoxic stress by oligomerizing and activating the transcription of genes whose promoters contain HSE(Wu, 1995). This response to stress is extremely rapid and robust. HSF1 can be observed associating with the *hsp70* gene within 30 seconds of heat shock(Bunch et al., 2014).

Hsp90 and Cancer

Expression of most HSPs, as well as HSF1, is increased in cancers with a wide range of morphologies (Ciocca and Calderwood, 2005). However, there is, as yet little solid data defining the mechanisms underlying this effect, although the ubiquity and magnitude of the changes observed suggest one or more rather generally occurring mechanisms for the increased levels of HSPs(Ciocca et al., 2013). A role for HSF1 in driving up levels of *HSP* transcription might be predicted *a priori* due to its prominence in activating *HSP* promoters. As the key responder to proteotoxic stress, one might envisage a mechanism whereby changes in the tumor cell proteome, including oncogene mutation and amplification, changes in ploidy and generally enhanced translation would lead to HSF1 activation(Santagata et al., 2013; Tang et al., 2005). Indeed exposure of cells to Hsp90 inhibitors leads to the depletion of a wide range of oncoproteins due to their unfolding when the chaperone is inactivated and subsequent degradation by the proteasome(Miyata et al., 2013). Such findings have led to the hypothesis that cancer cells become progressively “addicted to chaperones” and that HSP levels consequently become elevated to permit the expansion of an oncogenic proteome.

One anomaly in considering this hypothesis is that HSF1 appears to be active in cancer cells despite high levels of tumor cell Hsp90(Mendillo et al., 2012) (Fig. 1). Hsp90 has generally been considered the principal negative regulator of HSF1. Studies *in vitro* involving immunodepletion of Hsp90 have demonstrated activation of the factor(Zou et al., 1998). Consequently, triggering of the heat-shock response by proteotoxic stresses was considered to involve the sequestration of Hsp90 by an expanding population of unfolded proteins(Zou et al., 1998). In cancer then, elevated levels of Hsp90 would be predicted to repress HSF1 and limit increases in *HSP* gene transcription and HSP protein levels through the mechanism

of end-product inhibition. There appears however to be “something different” about Hsp90 in cancer cells; for instance the tumor proteome seems exquisitely sensitive to Hsp90-targeting drugs (Kamal et al., 2003). One intriguing explanation for this sensitive state might involve cancer-related alterations in the growing number of reported posttranslational modifications to Hsp90 that might influence its intracellular properties (Miyata, 2009; Mollapour and Neckers, 2012).

As mentioned, we still do not have a clearly defined mechanism to account for the increased levels of Hsp90 and other chaperones in most cancers. Rather than the chaperone titration model described above, direct modification of HSF1 itself could explain increases in its level and activation state during oncogenesis with consequent upregulation of Hsp90 expression. (Ciocca et al., 2013). It has, for instance been shown that HSF1 is repressed by glycogen synthase kinase 3 (GSK3), a kinase that phosphorylates HSF1 on serine 303 (Chu et al., 1996; Chu et al., 1998). Phospho-S303-HSF1 then becomes modified by SUMO (Small Ubiquitin-Like Modifier) at lysine 298, a potent repressive modification (Hietakangas et al., 2003). The SUMO moiety comes in two major varieties, encoded by the *SUMO1* and homologous *SUMO2 and 3* genes (Bettermann et al., 2012; Raman et al., 2013; Subramonian et al., 2014). SUMO2/3 appears to regulate many transcription factors involved in carcinogenesis; deSUMOylation of SUMO2/3-modified factors leads to activation (Subramonian et al., 2014). One might thus propose a mechanism involving deSUMOylation of HSF1 in cancer, although no experimental evidence exists to support this hypothesis currently. Hsp90 has also been shown to be a substrate for SUMOylation and this modification may likewise modulate the properties of Hsp90 in regulating transcription (Mollapour et al., 2014).

Hsp90 in the nucleus and regulation of transcription

---Regulation of sequence-specific transcription factors

Although Hsp90 plays significant roles in the nucleus, it is not clear that the chaperone possesses nuclear transport motifs. Indeed Hsp90 appears to possess sequences in the C-terminus that suppress nuclear uptake (Passinen et al., 2001). Nuclear localization may thus depend on co-chaperones or other associated proteins (Longshaw et al., 2004). The participation of Hsp90 in regulating transcription factor function was initially shown in studies of steroid hormone receptors (Dittmar and Pratt, 1997; Kirschke et al., 2014) (Fig. 2, top panel). Hsp90 was shown to assemble in complexes with a number of co-chaperones including p23, Hop, Hsp40, immunophilins FKBP1 and FKBP2 as well as Hsp70 in unliganded cytoplasmic glucocorticoid receptors (GR). Exposure to ligand then led to release of the GR from the chaperone complexes, recruitment of co-activators and activation of transcription. Early studies by Picard et al demonstrated mechanistic features of this process that have been seen repeatedly in subsequent investigations of Hsp90-client regulation (Picard et al., 1990). Binding to the chaperone complex appeared to retard *trans*-activation of gene expression mediated by GR, while disruption of such complexes inhibited hormone binding and receptor activation. Based on these and similar data, it has been suggested that the Hsp90 complex maintains the receptor in a stable inactive complex that is nonetheless poised for high affinity hormone binding and rapid activation (Picard et al.,

1990). A similar mechanism with some variation has been described for a number of other steroid hormone receptors as well as for HSF1 (Voellmy and Boellmann, 2007). Interestingly HSF1 appears to retain only a partial dependence on Hsp90 and although the chaperone can restrain transcriptional activity under uninduced conditions, free HSF1 appears to be stable in the absence of HSP binding (Zou et al., 1998).

--Regulation of general transcriptional machinery

In addition to effects on specific transcription factors, Hsp90 seems essential for proper function of the general transcription machinery because it can mediate Pol II assembly in the cytoplasm and nuclear import of the fully assembled holoenzyme (Boulon et al., 2010). Indeed, an increasing amount of evidence for Hsp90 activities in the nucleus is accumulating which seems to imply roles beyond conventional chaperoning activities (Figure 2, bottom panel). For instance, involvement of the chaperone in disassembly of chromatin-bound transcriptional complexes, in nucleosomal remodeling in yeast and in the activity of histone methyltransferases in mammalian cells have been reported (Bryant et al., 2008; Freeman and Yamamoto, 2002; Hamamoto et al., 2004).

There are at least two major checkpoints in transcriptional regulation that control RNA polymerase II (Pol II) activity in the nucleus and RNA synthesis. These include: (1) formation of the pre-initiation complex at the transcription start site (TSS) and promoter escape of Pol II and (2) promoter proximal pausing of Pol II close to the TSS of inducible genes followed by release of Pol II for processive elongation triggered by *trans*-activators. As in the cell cycle, each of these checkpoints is regulated by members of the cell division kinase (cdk) family (Egloff and Murphy, 2008). An important target for these kinases is a region in the C-Terminal Domain (CTD) of the primary subunit of Pol II (RPB1) that contains multiple tandem copies of a repeating 7 amino acid sequence (Y1-S2-P3-T4-S5-P6-S7). The Pol II CTD thus presents multiple potential phosphorylation sites enabling the "hyperphosphorylation" of Pol II in vivo (Egloff and Murphy, 2008). The CTD does not appear to have a major role in the enzymatic properties of Pol II but instead provides a highly regulated scaffold for binding regulators of transcription. At least two of these residues, phospho-S2 and phospho-S5 appear to play decisive roles in transcription. Pol II, when phosphorylated on residue S5 is found near the TSS. This modification is mediated by a cdk7-cyclin H complex and appears to control promoter clearance. Pol II S2-phospho, the result of cdk9-cyclin T complex (also known as the Positive Transcription Elongation Factor, P-TEF-b) - mediated phosphorylation is found more frequently in the polymerase when it is associated with the gene body and 3' regions of genes. Therefore occupancy of the gene body by P-TEF-b is a characteristic of elongating Pol II species (Adelman and Lis, 2012; Egloff and Murphy, 2008). P-TEF-b has been shown to overcome the promoter proximal pausing of Pol II and to lead to RNA elongation mediated in part by Pol II S2-phospho. Regulation of transcription by P-TEFb also involves inhibitory phosphorylation of negative regulators of elongation including the DRB Sensitivity Inducing Factor (DSIF) and Negative Elongation Factor (NELF), modifications that promote release of the Pol II from the paused state (Adelman and Lis, 2012).

Cdk-family molecules are amongst the most prominent clients of Hsp90/co-chaperone Cdc37 (p50) complexes (Gray et al., 2008; Stepanova et al., 1996), suggesting a potential role for such Cdk::chaperone complexes in regulating transcription. Cdk9-containing P-TEFb complexes were shown to be critical in regulating the *Drosophila* hsp70 gene (Lis et al., 2000). Hsp90 and Cdc37 were also shown to be essential for the assembly of the P-TEFb complex and elongation in HIV1 transcription (Fong and Zhou, 2000; O’Keeffe et al., 2000).

More recently it was shown, using high resolution mapping techniques that Hsp90 is found in association with chromatin near the transcriptional start sites of multiple genes (Sawarkar et al., 2012). Chromatin-associated Hsp90 appears to have a functional role, and its inhibition by two chemically distinct inhibitors (the natural products geldanamycin and radicicol) that both target the N-domain ATP binding site led to large increases in gene transcription, apparently due to relief of Hsp90-mediated inhibition (Sawarkar et al., 2012). Highly represented among these targets were genes shown previously to be, like Hsp70, regulated by promoter proximal pausing. These included targets such as c-Myc, p53, Delta and Notch that are well known for their dysregulation in many cancers (Sawarkar et al., 2012). As mentioned above, P-TEFb relieves pausing and permits elongation of RNAs through phosphorylation of the CTD of Pol II at the 2-position and through modification of DSIF and NELF, suggesting potential roles for Hsp90 in these mechanisms (Adelman and Lis, 2012). Sawarkar et al were able to show physical interactions between the NELF subunit NELF-E and Hsp90 by co-immunoprecipitation in *Drosophila* and human tissues as well as co-occupation of sites on chromatin by Hsp90 and NELF using ChIP analysis (Sawarkar et al., 2012). Hsp90 was shown to be essential for stabilizing NELF on chromatin, although not participating in NELF-Pol II interactions. Questions suggested by these data include the nature of the mechanism(s) by which Hsp90 is inactivated, and transcription permitted during pause release. One possible mechanism could include NELF phosphorylation by P-TEFb, a change that might alter Hsp90-NELF-E affinity or direct modifications to Hsp90 during transcription. Dysregulation of Hsp90 dynamics in cancer could therefore have significant effects on the levels of transcription, particularly of *HSP* genes as well as other rapidly inducible genes that utilize promoter proximal pausing, many of which participate in the cancer phenotype (Fig. 2, bottom panel).

It should also be noted that Pol II can also accumulate in an inactive form upstream of the TSS of a number of genes induced during development in *Caenorhabditis elegans* (*C. elegans*) and this accumulation may also permit rapid and synchronized rounds of transcription (as with release of pausing) (Hsu et al., 2015; Maxwell et al., 2014). This process, known as Pol II docking or Pol II poisoning appears to be different in nature to pausing, with the Pol II complex binding upstream of the TSS rather than downstream as in pausing. As many of the mechanisms involved in transcription are highly conserved during evolution one would predict a role for Pol II poisoning in mammalian transcription. However, little evidence has accrued regarding mechanisms that govern this process and thus a role for Hsp90 in this process is currently unknown.

--Regulation of chromatin modifications

Another key role for Hsp90 in the nucleus appears to be interaction with Trithorax (Trx) (Tariq et al., 2009). Trithorax is a member of the TrxG group with homology to the mammalian *mixed leukemia lineage* (MLL) family of histone methyltransferases that ensure the activity of target enhancers and promoters through maintenance of respectively, Histone3 Lysine4 Methyl (H3K4Me1) and Histone3 Lysine4 trimethyl (H3K4Me3) levels (Hu et al., 2013; Morgan and Shilatifard, 2013). During the development of differentiated cells from pluripotent stem cells, Trx functions in opposition to the Polycomb genes that repress target genes through the repressive histone mark Histone3 Lysine27 Trimethyl (H3K27Me3) (Xu and Rubin, 1993). Many developmental genes incorporate areas containing bivalent chromatin marks (both H3K4Me3 and H3K27Me3) and are considered to be in a poised state ready for either rapid induction or repression following environmental cues (Puri et al., 2015). Hsp90 functions to stabilize chromatin-associated Trx and thus influences the activity of a range of developmentally regulated genes as well as HSP genes through its histone methyltransferase activity directed towards H3K4Me3 (Puri et al., 2015; Tariq et al., 2009) (Smith et al., 2004). Loss of Hsp90 function, and inactivation of Trx would favor Polycomb mediated repression, permit demethylation of H3K4Me3 and lead to alterations of cell fate decisions as well as loss of stress protein transcription.

Translocations involving the Trx-related MLL gene occur in poor prognosis human leukemias and are thought to drive leukemogenesis by disrupting normal hematopoietic development. (Thirman et al., 1993). More recently it has been shown that members of the Trx / MLL family, particularly MLL2 and MLL3 are dysregulated in a large proportion of solid tumors (reviewed (Ford and Dingwall, 2015)). Thus Hsp90 could exert important effects on tumorigenesis via regulation of Trx / MLL factors and these interactions may have significant future implications for cancer treatment (Trepel et al., 2010).

Hsp90 contains a C-terminal domain that binds to tetratricopeptide (TPR) domain proteins, the most notable of which is the scaffold protein Hop (Calderwood, 2013). One such protein is TTC5 (tetratricopeptide5) that is capable of binding to transcriptional effectors including the protein acetyltransferase p300 and stimulating transcription under stress conditions (Davies et al., 2011; Demonacos et al., 2004). Future studies might investigate a role for interaction of Hsp90 with this nuclear protein that contains six TPR domains and is thus potentially capable of binding to Hsp90 and nucleating large transcriptional complexes (Calderwood, 2013).

Another transcriptionally active molecule linked to Hsp90 regulation is the ATP-dependent chromatin remodeling factor Rvb1 (RuvB like1), a key component of the Ino80 and SWR-C chromatin remodeling complexes (Ruden and Lu, 2008). In a yeast protein interactome screen, Hsp90 was shown to bind two novel co-chaperones, TPR-containing protein associated with Hsp90 (Tah 1p) and Pih 1p (Protein interacting with Hsp90), and these proteins mediated Hsp90 association with a Rvb1p/Rvbp2p complex (Zhao et al., 2005). Rvb family helicases are found in all eukaryotes and are important in chromatin remodeling / transcriptional activation, suggesting the potential significance of such Hsp90 / Rvb1 interactions in the regulation of mammalian gene expression (Gnatovskiy et al., 2013).

Regulation of Hsp90 function in the nucleus

What mechanisms might regulate Hsp90-mediated transcriptional control in the nucleus? Such regulation could involve the ATP-driven Hsp90 chaperone cycle and its influence by co-chaperones (Calderwood, 2013; Neckers and Ivy, 2003). Indeed, p23/Hsp90 complexes have been shown to localize to nuclear receptor sites in a hormone-dependent manner and to attenuate transcriptional activities, while Cdc37/Hsp90 complexes can be found associated with P-TEFb (Freeman and Yamamoto, 2002; Stepanova et al., 1996). Co-chaperones have various roles in cancer and their relative abundance and activities would likely influence Hsp90 activities in the nucleus, as recently reviewed (Calderwood, 2013).

Another exciting possibility for regulation of Hsp90 function in the nucleus is posttranslational modifications (PTMs) of the chaperone itself as well as its associated proteins (Fig. 3). Hsp90 contains multiple potential phosphorylation sites that are emerging as key regulatory residues that respond to cell signaling pathways (Mollapour and Neckers, 2012). For instance, Casein Kinase 2 (CK2) seems intimately linked to Hsp90 function: CK2 is one of the major Hsp90 binding proteins, requires chaperoning by this HSP, and can phosphorylate Hsp90 at multiple sites as well as the co-chaperones CDC37/p50 and FKBP52 and the transcription factor HSF1 (Miyata, 2009; Mollapour et al., 2011a; Mollapour et al., 2011b; Soncin et al., 2003). CK2 may be involved in regulating transcription factor activity/stability. For instance, phosphorylation of Hsp90 α on serine 231 by CK2 destabilized association of the chaperone with the aryl hydrocarbon receptor (a nuclear receptor family protein) and diminished transcription (Ogiso et al., 2004). CK2 has been shown to be active in the nucleus, is known to play a significant role in cancer signaling and its intimate involvement with Hsp90 suggests a potential role for Hsp90-CK2 interactions in cancer (Filhol and Cochet, 2009; Filhol et al., 2015; Miyata, 2009).

Although Hsp90 has been shown to interact with the Pol II complex at multiple promoter proximal paused genes through binding to NELF-E, the mechanisms involved in relief of Hsp90-associated pausing and resumption of productive elongation after transcriptional stimulus are not clear (Sawarkar et al., 2012). A potentially analogous situation has recently been reported showing that Tripartite Motif containing 28 (TRIM28) can stabilize Pol II pausing when bound to the ORFs of paused genes such as *hspa1b*, and then, on receipt of a stimulus such as heat shock can permit elongation (Bunch, 2015; Bunch et al., 2014). Conversion of TRIM28 to a factor permissive for transcriptional elongation involved the nuclear kinase DNA-dependent protein kinase (DNA-PK) which phosphorylates the protein and inactivates its *trans*-repressor function, normally transmitted through its polySUMOylated C-terminal region (Bunch, 2015). Interestingly, this is a mechanism “borrowed” from the DNA repair field as TRIM28 phosphorylation by DNA-PK, ATM or ATR plays a key role in repair of DNA double strand breaks through its interaction with chromatin (Lemaitre and Soutoglou, 2014).

There is considerable current interest in novel transcriptional regulatory pathways that incorporate some of the mechanisms characterized previously in DNA maintenance and repair: roles for topoisomerase I, topoisomerase II, Ku70, Ku80, DNA-PK have been reported (Ju and Rosenfeld, 2006; Puc et al., 2015). A number of years ago, Hsp90 α was

shown to be phosphorylated *in vitro* on two N-terminal sites (T5, T7) by DNA-PK (Lees-Miller and Anderson, 1989). A recent study found that DNA damage-induced phosphorylation of these residues in cells, and this phosphorylation was DNA-PK dependent (Solier et al., 2012). It would be intriguing to learn whether such modifications might be related to global roles for Hsp90 in transcription, such as its influence on the elongation pathway checkpoint by association with NELF or its effects on histone modification through interaction with Trx factors. Threonines 5 and 7 are found only in Hsp90 α , and Hsp90 α silencing affects DNA-PK stability and function that cannot be rescued by Hsp90 β (Solier et al., 2012), suggesting a possible Hsp90 isoform-specific role in transcriptional regulation. In contrast, both Hsp90 isoforms can be phosphorylated by Src, a modification that appears to enhance molecular chaperone activity (Duval et al., 2007). While one intuitively regards non-receptor tyrosine kinases such as Src as effectors of plasma membrane signaling events, these kinases play important roles in the nucleus. For instance triggering of DNA repair leads to phosphorylation of TRIM28 by Src on chromatin and reverses gene silencing events normally mediated through the factor Heterochromatin Protein 1 γ (HP1 γ) (Kubota et al., 2013).

In addition to phosphorylation, another PTM often encountered in transcriptional activators is modification by SUMO (small ubiquitin-like modifier), a modification that usually inhibits transcription due to recruitment of repressor complexes (Duval et al., 2007). Hsp90 has been shown to be SUMOylated in yeast and mammalian cells, an event that leads to recruitment of the co-chaperone Aha1 and reduction in its efficiency in chaperoning some clients (Mollapour et al., 2014).

Conclusions

Although traditionally considered a cytosolic chaperone, recent work is revealing intriguing new roles for Hsp90 in the nucleus and in regulation of transcription. These include facilitation of transcription initiated through the binding of sequence specific transcription factors including nuclear receptors, HSF1 and others. Regulation of nuclear client protein activity by Hsp90 involves a series of interactions (Picard et al., 1990; Sawarkar et al., 2012). In most cases, Hsp90 binds to clients in complexes containing co-chaperones that stabilize the client while maintaining it in an inactive state. Release of clients from such complexes leads to a burst of activity followed by loss of such activity, presumably due to unfolding and degradation of the free client protein. The participation of Hsp90 in transcription, however, been shown to extend beyond individual client transcription factors. Global roles for the chaperone are emerging in: (1) regulating elongation through NELF-E, (2) modulating epigenetic regulation through Trx/MLL binding, (3) modifying histone H3K4 methylation through association with SMYD3, and (4) affecting chromatin remodeling by associating with Rvb1/Rvb2 (Hamamoto et al., 2004; Ruden and Lu, 2008; Sawarkar et al., 2012; Tariq et al., 2009; Zhao et al., 2005) (Fig. 2, bottom panel). One can thus envisage a scenario in which these “nuclear” activities of Hsp90 could influence tumorigenesis through genome-wide effects on transcription.

In addition, Hsp90 appears to assume an “altered state” in many cancers, an as yet poorly understood condition in which the chaperone is more sensitive to ATP-competitive inhibitors

(Kamal et al., 2003; Moulick et al., 2011). Likely mechanisms for such altered drug sensitivity include PTMs of Hsp90 and its co-chaperones (Mollapour et al., 2014; Mollapour and Neckers, 2012) (Fig. 3). These and perhaps other PTMs may also influence the transcription-modifying activities of Hsp90 in cancer described here.

The investigation of Hsp90's role(s) in the nucleus is currently at an early stage and much effort will be required to establish the full significance of Hsp90 in transcription. Future studies that seem particularly compelling include the following:

- (1) Many of the studies reviewed here were carried out in model organisms including yeast, *C. elegans* and *Drosophila*. In most cases, it remains to be determined whether Hsp90 plays similar roles in mammalian cells in regulating processes such as Pol II pausing, chromatin modification by Trx and chromatin remodeling by Rvb1p/Rvbp2p containing complexes as discussed above. While the high level of conservation of many of the components of gene transcription would suggest similar functions will be seen in mammals, it will be crucial to learn whether such is really the case. If so, how important are they in mammalian biology and how do these functions become altered in cancers. In the course of this work, we anticipate discovery of other new and unexpected roles for Hsp90 in transcription.
- (2) A priority also should be placed on establishing the differential / shared roles of Hsp90 α and Hsp90 β in the globally acting pathways of transcriptional regulation mentioned above. This will enable us to understand the regulatory roles of these two molecules more precisely as well as help guide future development of Hsp90-targeted drugs.
- (3) Another major area of investigation, likely to be highly productive, will be determining the signal transduction pathways and PTMs that regulate Hsp90 activity in transcription in response to environmental cues. A number of important PTMs have been characterized, as reviewed above, and roles for these regulatory modifications in switching transcription on or off are anticipated.
- (4) Of major relevance for the current discussion are the questions, (a) What is the importance of transcriptional regulation by Hsp90 in cancer and how is it deregulated during tumor progression? – (b) Finally, could the Hsp90-dependent transcriptome be selectively targeted by clinically tolerable small molecules that might become useful anticancer drugs in the future.?

Acknowledgements

We thank the Department of Radiation Oncology, BIDMC for support and encouragement. We are particularly thankful to Jane Trepel for valuable discussions. In addition we thank Heeyoun Bunch, Tom Prince and Ayesha Murshid.

This work was supported in part by NIH research grants RO-1CA047407, R01CA119045 and RO-1CA094397 (SKC) and by funds from the Intramural Research Program of the National Cancer Institute (LN, projects Z01 BC011032-01 and Z01 SC010074-12).

References

- Adelman K, and Lis JT (2012). Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. *Nature reviews. Genetics* 13, 720–731.

- Bettermann K, Benesch M, Weis S, and Haybaeck J (2012). SUMOylation in carcinogenesis. *Cancer Lett* 316, 113–125. [PubMed: 22138131]
- Boulon S, Pradet-Balade B, Verheggen C, Molle D, Boireau S, Georgieva M, Azzag K, Robert MC, Ahmad Y, Neel H, et al. (2010). HSP90 and its R2TP/Prefoldin-like cochaperone are involved in the cytoplasmic assembly of RNA polymerase II. *Molecular cell* 39, 912–924. [PubMed: 20864038]
- Bryant GO, Prabhu V, Floer M, Wang X, Spagna D, Schreiber D, and Ptashne M (2008). Activator control of nucleosome occupancy in activation and repression of transcription. *PLoS Biol* 6, 2928–2939. [PubMed: 19108605]
- Bunch H, Calderwood SK (2015). TRIM28 as a Novel Transcriptional Elongation Factor. *BMC, molecular biology* (in press).
- Bunch H, Zheng X, Burkholder A, Dillon ST, Motola S, Birrane G, Ebmeier CC, Levine S, Fargo D, Hu G, et al. (2014). TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release. *Nature structural & molecular biology* 21, 876–883.
- Calderwood SK (2013). Molecular cochaperones: tumor growth and cancer treatment. *Scientifica* 2013, 217513. [PubMed: 24278769]
- Chu B, Soncin F, Price BD, Stevenson MA, and Calderwood SK (1996). Sequential phosphorylation by mitogen-activated protein kinase and glycogen synthase kinase 3 represses transcriptional activation by heat shock factor-1. *The Journal of biological chemistry* 271, 30847–30857. [PubMed: 8940068]
- Chu B, Zhong R, Soncin F, Stevenson MA, and Calderwood SK (1998). Transcriptional activity of heat shock factor 1 at 37 degrees C is repressed through phosphorylation on two distinct serine residues by glycogen synthase kinase 3 and protein kinases Calpha and Czeta. *The Journal of biological chemistry* 273, 18640–18646. [PubMed: 9660838]
- Ciocca DR, Arrigo AP, and Calderwood SK (2013). Heat shock proteins and heat shock factor 1 in carcinogenesis and tumor development: an update. *Archives of toxicology* 87, 19–48. [PubMed: 22885793]
- Ciocca DR, and Calderwood SK (2005). Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell stress & chaperones* 10, 86–103. [PubMed: 16038406]
- Davies L, Paraskevopoulou E, Sadeq M, Symeou C, Pantelidou C, Demonacos C, and Krstic-Demonacos M (2011). Regulation of glucocorticoid receptor activity by a stress responsive transcriptional cofactor. *Molecular endocrinology* 25, 58–71. [PubMed: 21147850]
- Demonacos C, Krstic-Demonacos M, Smith L, Xu D, O'Connor DP, Jansson M, and La Thangue NB (2004). A new effector pathway links ATM kinase with the DNA damage response. *Nature cell biology* 6, 968–976. [PubMed: 15448695]
- Dittmar KD, and Pratt WB (1997). Folding of the glucocorticoid receptor by the reconstituted Hsp90-based chaperone machinery. The initial hsp90.p60.hsp70-dependent step is sufficient for creating the steroid binding conformation. *The Journal of biological chemistry* 272, 13047–13054. [PubMed: 9148915]
- Duval M, Le Boeuf F, Huot J, and Gratton JP (2007). Src-mediated phosphorylation of Hsp90 in response to vascular endothelial growth factor (VEGF) is required for VEGF receptor-2 signaling to endothelial NO synthase. *Molecular biology of the cell* 18, 4659–4668. [PubMed: 17855507]
- Egloff S, and Murphy S (2008). Cracking the RNA polymerase II CTD code. *Trends in genetics : TIG* 24, 280–288. [PubMed: 18457900]
- Felts SJ, Owen BA, Nguyen P, Trepel J, Donner DB, and Toft DO (2000). The hsp90-related protein TRAP1 is a mitochondrial protein with distinct functional properties. *The Journal of biological chemistry* 275, 3305–3312. [PubMed: 10652318]
- Filhol O, and Cochet C (2009). Protein kinase CK2 in health and disease: Cellular functions of protein kinase CK2: a dynamic affair. *Cellular and molecular life sciences : CMLS* 66, 1830–1839. [PubMed: 19387551]
- Filhol O, Giacosa S, Wallez Y, and Cochet C (2015). Protein kinase CK2 in breast cancer: the CK2beta regulatory subunit takes center stage in epithelial plasticity. *Cellular and molecular life sciences : CMLS*.
- Fong YW, and Zhou Q (2000). Relief of two built-in autoinhibitory mechanisms in P-TEFb is required for assembly of a multicomponent transcription elongation complex at the human

- immunodeficiency virus type 1 promoter. *Molecular and cellular biology* 20, 5897–5907. [PubMed: 10913173]
- Ford DJ, and Dingwall AK (2015). The cancer COMPASS: navigating the functions of MLL complexes in cancer. *Cancer genetics*.
- Freeman BC, and Yamamoto KR (2002). Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science* 296, 2232–2235. [PubMed: 12077419]
- Gnatovskiy L, Mita P, and Levy DE (2013). The human RVB complex is required for efficient transcription of type I interferon-stimulated genes. *Molecular and cellular biology* 33, 3817–3825. [PubMed: 23878400]
- Gray PJ Jr., Prince T, Cheng J, Stevenson MA, and Calderwood SK (2008). Targeting the oncogene and kinome chaperone CDC37. *Nature reviews. Cancer* 8, 491–495. [PubMed: 18511936]
- Hamamoto R, Furukawa Y, Morita M, Imura Y, Silva FP, Li M, Yagyu R, and Nakamura Y (2004). SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells. *Nature cell biology* 6, 731–740. [PubMed: 15235609]
- Hietakangas V, Ahlskog JK, Jakobsson AM, Hellesuo M, Sahlberg NM, Holmberg CI, Mikhailov A, Palvimo JJ, Pirkkala L, and Sistonen L (2003). Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. *Molecular and cellular biology* 23, 2953–2968. [PubMed: 12665592]
- Hsu HT, Chen HM, Yang Z, Wang J, Lee NK, Burger A, Zaret K, Liu T, Levine E, and Mango SE (2015). TRANSCRIPTION. Recruitment of RNA polymerase II by the pioneer transcription factor PHA-4. *Science* 348, 1372–1376. [PubMed: 26089518]
- Hu D, Gao X, Morgan MA, Herz HM, Smith ER, and Shilatifard A (2013). The MLL3/MLL4 branches of the COMPASS family function as major histone H3K4 monomethylases at enhancers. *Molecular and cellular biology* 33, 4745–4754. [PubMed: 24081332]
- Ju BG, and Rosenfeld MG (2006). A breaking strategy for topoisomerase IIbeta/PARP-1-dependent regulated transcription. *Cell cycle* 5, 2557–2560. [PubMed: 17106262]
- Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, and Burrows FJ (2003). A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature* 425, 407–410. [PubMed: 14508491]
- Kirschke E, Goswami D, Southworth D, Griffin PR, and Agard DA (2014). Glucocorticoid receptor function regulated by coordinated action of the Hsp90 and Hsp70 chaperone cycles. *Cell* 157, 1685–1697. [PubMed: 24949977]
- Kubota S, Fukumoto Y, Aoyama K, Ishibashi K, Yuki R, Morinaga T, Honda T, Yamaguchi N, Kuga T, Tomonaga T, et al. (2013). Phosphorylation of KRAB-associated protein 1 (KAP1) at Tyr-449, Tyr-458, and Tyr-517 by nuclear tyrosine kinases inhibits the association of KAP1 and heterochromatin protein 1alpha (HP1alpha) with heterochromatin. *The Journal of biological chemistry* 288, 17871–17883. [PubMed: 23645696]
- Lees-Miller SP, and Anderson CW (1989). The human double-stranded DNA-activated protein kinase phosphorylates the 90-kDa heat-shock protein, hsp90 alpha at two NH2-terminal threonine residues. *The Journal of biological chemistry* 264, 17275–17280. [PubMed: 2507541]
- Lemaitre C, and Soutoglou E (2014). Double strand break (DSB) repair in heterochromatin and heterochromatin proteins in DSB repair. *DNA repair* 19, 163–168. [PubMed: 24754998]
- Lindquist S, and Craig EA (1988). The heat-shock proteins. *Annual review of genetics* 22, 631–677.
- Lis JT, Mason P, Peng J, Price DH, and Werner J (2000). P-TEFb kinase recruitment and function at heat shock loci. *Genes & development* 14, 792–803. [PubMed: 10766736]
- Longshaw VM, Chapple JP, Balda MS, Cheetham ME, and Blatch GL (2004). Nuclear translocation of the Hsp70/Hsp90 organizing protein mSTI1 is regulated by cell cycle kinases. *Journal of cell science* 117, 701–710. [PubMed: 14754904]
- Maxwell CS, Kruesi WS, Core LJ, Kurhanewicz N, Waters CT, Lewarch CL, Antoshechkin I, Lis JT, Meyer BJ, and Baugh LR (2014). Pol II docking and pausing at growth and stress genes in *C. elegans*. *Cell reports* 6, 455–466. [PubMed: 24485661]
- Mendillo ML, Santagata S, Koeva M, Bell GW, Hu R, Tamimi RM, Fraenkel E, Ince TA, Whitesell L, and Lindquist S (2012). HSF1 drives a transcriptional program distinct from heat shock to support highly malignant human cancers. *Cell* 150, 549–562. [PubMed: 22863008]

- Miyata Y (2009). Protein kinase CK2 in health and disease: CK2: the kinase controlling the Hsp90 chaperone machinery. *Cellular and molecular life sciences : CMLS* 66, 1840–1849. [PubMed: 19387550]
- Miyata Y, Nakamoto H, and Neckers L (2013). The therapeutic target Hsp90 and cancer hallmarks. *Current pharmaceutical design* 19, 347–365. [PubMed: 22920906]
- Mollapour M, Bourboulia D, Beebe K, Woodford MR, Polier S, Hoang A, Chelluri R, Li Y, Guo A, Lee MJ, et al. (2014). Asymmetric Hsp90 N domain SUMOylation recruits Aha1 and ATP-competitive inhibitors. *Molecular cell* 53, 317–329. [PubMed: 24462205]
- Mollapour M, and Neckers L (2012). Post-translational modifications of Hsp90 and their contributions to chaperone regulation. *Biochimica et biophysica acta* 1823, 648–655. [PubMed: 21856339]
- Mollapour M, Tsutsumi S, Kim YS, Trepel J, and Neckers L (2011a). Casein kinase 2 phosphorylation of Hsp90 threonine 22 modulates chaperone function and drug sensitivity. *Oncotarget* 2, 407–417. [PubMed: 21576760]
- Mollapour M, Tsutsumi S, Truman AW, Xu W, Vaughan CK, Beebe K, Konstantinova A, Vourganti S, Panaretou B, Piper PW, et al. (2011b). Threonine 22 phosphorylation attenuates Hsp90 interaction with cochaperones and affects its chaperone activity. *Molecular cell* 41, 672–681. [PubMed: 21419342]
- Morgan MA, and Shilatifard A (2013). Drosophila SETs its sights on cancer: Trr/MLL3/4 COMPASS-like complexes in development and disease. *Molecular and cellular biology* 33, 1698–1701. [PubMed: 23459940]
- Moulick K, Ahn JH, Zong H, Rodina A, Cerchietti L, Gomes DaGama EM, Caldas-Lopes E, Beebe K, Perna F, Hatzi K, et al. (2011). Affinity-based proteomics reveal cancer-specific networks coordinated by Hsp90. *Nature chemical biology* 7, 818–826. [PubMed: 21946277]
- Neckers L, and Ivy SP (2003). Heat shock protein 90. *Curr Opin Oncol* 15, 419–424. [PubMed: 14624223]
- O'Keeffe B, Fong Y, Chen D, Zhou S, and Zhou Q (2000). Requirement for a kinase-specific chaperone pathway in the production of a Cdk9/cyclin T1 heterodimer responsible for P-TEFb-mediated tat stimulation of HIV-1 transcription. *The Journal of biological chemistry* 275, 279–287. [PubMed: 10617616]
- Ogiso H, Kagi N, Matsumoto E, Nishimoto M, Arai R, Shirouzu M, Mimura J, Fujii-Kuriyama Y, and Yokoyama S (2004). Phosphorylation analysis of 90 kDa heat shock protein within the cytosolic arylhydrocarbon receptor complex. *Biochemistry* 43, 15510–15519. [PubMed: 15581363]
- Passinen S, Valkila J, Manninen T, Syvala H, and Ylikomi T (2001). The C-terminal half of Hsp90 is responsible for its cytoplasmic localization. *European journal of biochemistry / FEBS* 268, 5337–5342.
- Picard D, Khursheed B, Garabedian MJ, Fortin MG, Lindquist S, and Yamamoto KR (1990). Reduced levels of hsp90 compromise steroid receptor action in vivo. *Nature* 348, 166–168. [PubMed: 2234079]
- Puc J, Kozbial P, Li W, Tan Y, Liu Z, Suter T, Ohgi KA, Zhang J, Aggarwal AK, and Rosenfeld MG (2015). Ligand-dependent enhancer activation regulated by topoisomerase-I activity. *Cell* 160, 367–380. [PubMed: 25619691]
- Puri D, Gala H, Mishra R, and Dhawan J (2015). High-wire act: the poised genome and cellular memory. *The FEBS journal* 282, 1675–1691. [PubMed: 25440020]
- Raman N, Nayak A, and Muller S (2013). The SUMO system: a master organizer of nuclear protein assemblies. *Chromosoma* 122, 475–485. [PubMed: 23917899]
- Ruden DM, and Lu X (2008). Hsp90 affecting chromatin remodeling might explain transgenerational epigenetic inheritance in Drosophila. *Current genomics* 9, 500–508. [PubMed: 19506739]
- Santagata S, Mendillo ML, Tang YC, Subramanian A, Perley CC, Roche SP, Wong B, Narayan R, Kwon H, Koeva M, et al. (2013). Tight coordination of protein translation and HSF1 activation supports the anabolic malignant state. *Science* 341, 1238303. [PubMed: 23869022]
- Sawarkar R, Sievers C, and Paro R (2012). Hsp90 globally targets paused RNA polymerase to regulate gene expression in response to environmental stimuli. *Cell* 149, 807–818. [PubMed: 22579285]

- Smith ST, Petruk S, Sedkov Y, Cho E, Tillib S, Canaani E, and Mazo A (2004). Modulation of heat shock gene expression by the TAC1 chromatin-modifying complex. *Nature cell biology* 6, 162–167. [PubMed: 14730313]
- Solier S, Kohn KW, Scroggins B, Xu W, Trepel J, Neckers L, and Pommier Y (2012). Heat shock protein 90alpha (HSP90alpha), a substrate and chaperone of DNA-PK necessary for the apoptotic response. *Proceedings of the National Academy of Sciences of the United States of America* 109, 12866–12872. [PubMed: 22753480]
- Soncin F, Zhang X, Chu B, Wang X, Asea A, Ann Stevenson M, Sacks DB, and Calderwood SK (2003). Transcriptional activity and DNA binding of heat shock factor-1 involve phosphorylation on threonine 142 by CK2. *Biochemical and biophysical research communications* 303, 700–706. [PubMed: 12659875]
- Sreedhar AS, Kalmar E, Csermely P, and Shen YF (2004). Hsp90 isoforms: functions, expression and clinical importance. *FEBS letters* 562, 11–15. [PubMed: 15069952]
- Stepanova L, Leng X, Parker SB, and Harper JW (1996). Mammalian p50Cdc37 is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. *Genes Dev* 10, 1491–1502. [PubMed: 8666233]
- Subramonian D, Raghunayakula S, Olsen JV, Beningo KA, Paschen W, and Zhang XD (2014). Analysis of changes in SUMO-2/3 modification during breast cancer progression and metastasis. *J Proteome Res* 13, 3905–3918. [PubMed: 25072996]
- Tang D, Khaleque MA, Jones EL, Theriault JR, Li C, Wong WH, Stevenson MA, and Calderwood SK (2005). Expression of heat shock proteins and heat shock protein messenger ribonucleic acid in human prostate carcinoma in vitro and in tumors in vivo. *Cell stress & chaperones* 10, 46–58. [PubMed: 15832947]
- Tariq M, Nussbaumer U, Chen Y, Beisel C, and Paro R (2009). Trithorax requires Hsp90 for maintenance of active chromatin at sites of gene expression. *Proceedings of the National Academy of Sciences of the United States of America* 106, 1157–1162. [PubMed: 19144915]
- Thirman MJ, Gill HJ, Burnett RC, Mbangkollo D, McCabe NR, Kobayashi H, Ziemann-van der Poel S, Kaneko Y, Morgan R, Sandberg AA, et al. (1993). Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. *The New England journal of medicine* 329, 909–914. [PubMed: 8361504]
- Trepel J, Mollapour M, Giaccone G, and Neckers L (2010). Targeting the dynamic HSP90 complex in cancer. *Nature reviews. Cancer* 10, 537–549.
- Voellmy R, and Boellmann F (2007). Chaperone regulation of the heat shock protein response. *Advances in experimental medicine and biology* 594, 89–99. [PubMed: 17205678]
- Wu C (1995). Heat shock transcription factors: structure and regulation. *Annual review of cell and developmental biology* 11, 441–469.
- Xu T, and Rubin GM (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* 117, 1223–1237. [PubMed: 8404527]
- Zhao R, Davey M, Hsu YC, Kaplanek P, Tong A, Parsons AB, Krogan N, Cagney G, Mai D, Greenblatt J, et al. (2005). Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. *Cell* 120, 715–727. [PubMed: 15766533]
- Zou J, Guo Y, Guettouche T, Smith DF, and Voellmy R (1998). Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell* 94, 471–480. [PubMed: 9727490]

Figure 1. Hsp90 Interaction with HSF1 and HSP Synthesis in Stress and Cancer. HSF1 is depicted as being regulated by Hsp90 in a negative feedback loop. Escape from Hsp90 repression may involve modifications to HSF1 and Hsp90 itself and may include effects on the transcription factor or on more global factors including promoter proximal pausing and histone modifications.

Figure 2. Participation of Hsp90 in Transcription.

Upper part of figure depicts Hsp90 associated with a transcription factor (TF) in a chaperone-co-chaperone complex (p23, immunophilin (IF) complex -TPR protein Hop and Hsp70 and Hsp40). This type of regulation is found in nuclear receptors and HSF1.

In the lower part of the figure, we show Hsp90 participating in global effects on transcriptional activation mediated by SMYD3, Trx/MLL, Rvb and NELF involving chromatin remodeling, histone modification and Pol II regulation.

Figure 3. Posttranscriptional Modifications that may Influence Role of Hsp90 in Transcription. We depict Hsp90 phosphorylation by CK2, DNA-PK and Src as well as its SUMOylation as known PTMs that may modulate its impact on transcriptional events.