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HSP90 manages the ends

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Abstract

The telomere environment requires a means to efficiently assemble and disassemble a multitude of structures to operate correctly and to help achieve cellular homeostasis. Telomeres are challenged by a common binding specificity displayed many of the protein components for telomeric DNA, which could result in competitive DNA interactions, and also by a cell cycle-restricted timing of events, which enforces a narrow working period to perform numerous tasks. In this review, we discuss how the HSP90 molecular chaperone network averts these obstacles and facilitates an effective operation of the telomere system.

A dynamic cellular environment enables multi-step pathways

To achieve and maintain physiological balance a cell requires numerous biological pathways to harmoniously operate, including paths functioning at or near the DNA. The variety of processes working along the genome is considerable, yet the number of employed factors eclipses this total. Typically, the individual components assemble into distinct complexes that serially operate along a pathway to perform a cellular task. Adding to the complexity is a multitude of fluctuating internal and external signals that must be monitored to initiate, continue or halt a requisite action. Although cooperative interactions between proteins of the same process provide a mechanism for rapid and precise assembly, the inherent stability of such organized structures would interfere with the proper timing of biological systems [1]. Hence, cellular factors are needed to destabilize the various protein assemblies to efficiently transition between structures or terminate pathway activity [2]. We suggest that molecular chaperones have evolved, in part, to foster a general and continuous mobile protein environment within the cell—including events that occur along the genome [3].

To illustrate the potential complexities and obstacles faced by most, if not all, biological paths we will focus on the impact of one molecular chaperone network on a single cellular pathway. Specifically, we will delve into the potential points of influence that the Heat Shock Protein 90 (HSP90) chaperone machinery has on telomere protein biology. By coordinating the assembly and disassembly of numerous telomere complexes we speculate that HSP90 and its associated co-chaperones facilitate a precise and efficient working environment that would be highly beneficial for telomere function.

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The Telomere Protein System

Telomeres consist of a tract of G-rich DNA motifs culminating in a 3' single-stranded overhang (telomeric DNA) that is recognized by a collection of associated factors (telomere-binding proteins) that maintain the terminal DNA at an appropriate length to preserve genome stability and cell viability [4]. A multitude of events must occur at telomeres to achieve function and all of the activities are challenged by a need to work precisely and efficiently within a limited time frame. Notably, a single shortened telomeric end is sufficient to induce cellular senescence whereas an unregulated telomere system can lead to malignant growth [5,6]. Hence, telomere biology presents an ideal molecular model to understand the cellular mechanisms that are required to proficiently direct and drive a pathway, which effects homeostasis.

Multiple protein complexes assemble at telomeres to execute specific functions that are required for protection and extension of the terminal DNA, which consists of [4,7]. In general, the complexity of the telomere varies in accordance with the stage of the cell cycle; however, specific cellular needs can also direct molecular decisions. For instance, telomeric DNA is only extended in S-phase, yet not all telomeres are extended every cell cycle [8]. In a short temporal window (<15 minute) during late S-phase a decision is made based upon the length of the double-stranded telomeric tract (*i.e.*, the number of telomeric repeats are essentially counted) to either generate a telomerase-extendable state or form an unextendable structure [9].

If a telomeric end is approaching a critically shortened state, which would trigger cellular senescence if reduced further, then the 3' overhang will be lengthened [6]. Extension of the DNA requires several different S-phase specific activities including DNA resectioning, extending and fill-in replicating, which are all mediated by distinct protein complexes (Figure 1). Interestingly, components of each assemblage are capable of binding to the single-stranded G-rich overhang, which presents the opportunity for inhibitory competitive DNA interactions. Yet, all three events efficiently operate within the allotted narrow time period following DNA replication in S phase. If, however, the telomeric DNA tract is sufficiently long, then the structure will remain in an unextendable or capped state although the composition of the cap will vary as the cell cycle progresses (Figure 1) [4,7]. Hence, the telomere environment must be maintained in a dynamic state in order to both respond to the signals that dictate function and to perform the requisite work that is required to maintain the chromosomal termini. But what mechanism(s) are used to properly assemble (*i.e.*, avoid competitive binding) and disassemble (*i.e.*, transition between structures) the various telomere complexes?

Eukaryotic molecular chaperone network

Molecular chaperones comprise a diverse protein family that is primarily defined by an *in vitro* activity (*i.e.*, ability to suppress non-native protein aggregation) rather than by a conserved amino acid sequence or domain [10]. Chaperone family members generally display a promiscuous protein binding capacity with an affinity for short hydrophobic amino acid motifs that likely accounts for the shared ability to suppress non-native protein aggregation *in vitro* [11]. To offset the broad binding capacities and to avoid interfering with the activity of a target protein, chaperones typically have short-lived, low affinity interactions with a client. These two evolved characteristics make molecular chaperones ideal candidates to facilitate a functional dynamic environment comprised of a wide array of proteins.

The eukaryotic molecular chaperone system is primarily coordinated around the two highly abundant chaperones HSP90 and HSP70 along with their cognate co-chaperones [12,13]. Although many cellular activities have been identified for the HSP70 network, the general *in vivo* roles of HSP90 and its many co-chaperones (*e.g.*, p23, CDC37, AHA1, SGT1) remain elusive despite their conservation within eukaryotes. As the HSP90 chaperone complex was originally identified in stable association with signaling proteins (*i.e.*, kinases and steroid

receptors), it has been largely argued that this chaperone network serves to maintain metastable factors in forms that can be readily activated [14]. However, recent proteomic and genetic interaction studies suggest that signaling protein maintenance might be the tip of the iceberg for the duties of the eukaryotic HSP90 chaperone machinery [15–17].

Based on evolutionary considerations, cellular reliance on the HSP90 chaperone system has significantly increased. Prokaryotes typically contain a single non-essential HSP90 gene (*e.g.*, *HtpG* in *Escherichia coli*), yet eukaryotes often have multiple HSP90 genes, which are essential, and have acquired a collection of co-chaperone partner proteins that do not appear in prokaryotes [18]. By contrast, HSP70 proteins are largely regarded as one of the most conserved proteins across the three domains of life [13]. Yet the functional activities of HSP70 have apparently changed, as an involvement with DNA-associated events have lessened [19]. Although the workload shift in the chaperone system likely has numerous important physiological consequences, we suspect one key change has been an employment of the HSP90 machine to foster homeostasis by modulating protein–DNA dynamics.

Historically molecular chaperones have been recognized as factors that mediate the association and disassociation of protein complexes [20]. Recent studies indicated that certain chaperones serve comparable roles with protein–DNA structures [21–28]. For instance, several molecular chaperones including HSP90, HSP70 and p23 can promote protein–DNA dynamics of diverse targets including components involved in RNA transcription, telomere maintenance, DNA repair and DNA replication [3,29–31]. In this review, we will focus on the role of the HSP90 chaperone system in modulating the assembly and disassembly of telomere structures.

The HSP90 machine associates with the telomerase enzyme

The initial connection between HSP90 and telomere components was made while attempting to identify cellular factors required for telomerase holoenzyme function [32]. The authors discovered that HSP90 and the p23 cochaperone interact with the protein subunit of human telomerase (hTERT) through a yeast two-hybrid screen. Telomerase is a specialized reverse transcriptase (RT) in which the core enzyme comprises a protein module (TERT in humans and Est2 in yeast) and an RNA subunit (human TR and yeast TLC1) [4,7]. Telomerase utilizes the RNA as a template to extend the 3' chromosomal ends by 100 nucleotides or more per cell cycle by reiteratively appending a short (6–8 bases) telomeric DNA repeat during each binding event [9,33]. Whereas core human telomerase is sufficient for robust DNA extension activity *in vitro*, core yeast telomerase is not [34,35]. Nonetheless, both homologs rely on a multitude of additional proteins for proper control; HSP90 and p23 represent the first two proteins shown to directly interact with telomerase and contribute to its enzymatic activity [32].

The link between telomerase and molecular chaperones was extended by the finding that additional chaperones including HSP70, HSP40 and HOP (HSP organizing protein) associate with the core human enzyme, presumably to assemble the RT and RNA template *in vitro* [36]. This observation fit with the classic steroid receptor–chaperone model in which chaperones are required to generate and maintain the hormone binding state of receptors through a stable interaction that persists until the receptor is hormone-activated [14]. Surprisingly, HSP90 and p23 remain associated with telomerase even after extending a telomeric DNA substrate, suggesting that these chaperones have a role beyond telomerase protein folding [36]. Notably, HSP90 and p23 also function with DNA-associated steroid receptors to promote a dynamic DNA binding cycle that is required for proper gene regulation [22,24]. If HSP90 and p23 regulate both telomerase and receptors at or near DNA, then perhaps these chaperones have a general cellular function with diverse DNA-binding proteins.

Unpredictably, an early yeast genetic study showed that overexpression of either Hsp82 or Hsc82 (the two yeast HSP90 isoforms) led to telomeric DNA shortening, thus suggesting a

negative effect of Hsp82/Hsc82 in telomere DNA maintenance *in vivo* [37]. The cause of the telomere DNA length decline was not apparent. However, the potential for Hsp82 might affect telomere-binding proteins besides telomerase was revealed when *HSP82* was identified as a high-copy suppressor of two mutations (*cdc13-1* and *stn1-157*) known to alter telomere DNA length [37]. Hence, HSP90 proteins might affect multiple telomere-associated proteins.

The observation that telomeric DNA length was essentially normal in a strain expressing limited Hsp82 levels (~10% of normal) further supported the contention that Hsp82 does not have a positive effect on telomerase [37]. An Hsp82 protein reduction tactic was used because the eukaryotic HSP90 genes are essential and therefore it is not possible to test for *in vivo* effects in the absence of an HSP90 protein. It should be noted, however, that normal yeast have ~500,000 Hsp82 molecules per cell [38]. Thus, if telomerase is a high-affinity substrate for Hsp82, then a change in telomeric DNA length might not be expected in yeast expressing ~50,000 Hsp82 molecules.

At the very least, the two initial reports investigating the connection between HSP90 and telomere proteins suggested that the relationship would be complicated. Although the disconnect between the *in vitro* and *in vivo* data might be unsatisfying, it is not entirely surprising given the nature of the cellular chaperone network (*i.e.*, abundant and promiscuous proteins). For example, HSP70 is the fundamental nascent chain binding protein *in vivo* [39]. Yet, loss of the *E. coli* HSP70 gene *DnaK* results in only mild phenotypes [40]. However, in the absence of both *DnaK* and the Trigger Factor (TF) molecular chaperone, cells die owing to massive protein aggregation [41]. Nonetheless, based on *in vitro* assays, a role for *DnaK* in protein folding had been long accepted prior to the discovery of the synthetic lethal phenotype between *DnaK* and TF. Therefore, *in vitro* studies can identify important chaperone functions that are not readily apparent by typical *in vivo* work given the redundant and/or compensatory nature of the cellular molecular chaperone system.

To dissect the functional role of HSP90 with telomerase, suitable genetic conditions, in conjunction with a more detailed biochemical analysis, were necessary. To avoid potential complications that might occur in the presence of wild-type HSP90 proteins, Toogun *et al.* [27] screened yeast expressing select *hsp82* or *hsc82* alleles in an attempt to identify telomere-associated mutants. Notably, several derivatives_[SC3] displayed both a declined telomere DNA length *in vivo* and correlative decrease in telomerase DNA extension activity *in vitro*. Further mechanistic work revealed that human and yeast HSP90 proteins can promote DNA binding and nucleotide affinity for telomerase [27,42]. Hence, HSP90 chaperones positively contribute to telomere DNA length maintenance by supporting telomerase. Of note, yeast *Sba1* (the p23 ortholog) can dissociate telomerase DNA complexes, suggesting that Hsp82 and *Sba1* might coordinate a dynamic telomerase DNA binding cycle that parallels the chaperone receptor model [2,26]. Whether direct communication between Hsp82 and *Sba1* is required to mediate the dynamics or if the chaperones function autonomously is yet to be addressed. Nonetheless, if Hsp82 supports telomerase, then why does Hsp82 overexpression lead to telomere DNA shortening [37]?

Hsp82 intersects with numerous telomere proteins

Genetic studies suggested that Hsp82/Hsc82 affect other telomere proteins given that yeast expressing an Hsc82 S481Y mutant had shortened telomeric DNA length, but normal telomerase activity *in vitro* [27] and, more notably, that *HSP82* is a high-copy suppressor of two telomere defect mutants, *cdc13-1* and *stn1-157* [37]. Both *Cdc13* (Cell division cycle 13) and *Stn1* (Suppressor of *Cdc13*) are critical for telomere DNA maintenance: *Cdc13* is a central recruitment factor required for nucleating both extension (*i.e.*, telomerase) and protection telomere structures, and *Stn1* is an essential component of the protection (capping) complex

[43,44]. In addition to Cdc13 and Stn1, recent high-throughput studies have identified numerous telomere-associated factors as potential Hsp82 targets either through genetic or proteomic interactions [15–17] (Table 1).

The telomere protein system must carry out a variety of activities, which includes protecting, extending and replicating the terminal DNA, in order to maintain the chromosomal ends (Figure 1). Although telomeres from all eukaryotes perform similar functions, detailed studies in budding yeast make it a particularly useful model system. During each cell cycle the telomere protein composition transitions from one complex to the next depending upon phase and marked need. For example, at telomeres with relatively short telomeric DNA tracts the organization will fluctuate from a M-phase capping complex (minimally Cdc13–Stn1–Ten1) to a G1 capping structure that also includes YKu70–YKu80-stabilized telomerase (core subunits) to the DNA replication machinery in S-phase to a DNA resectioning assemblage (involving the MRX complex and the Exo1 and Sae1 nucleases) that degrades a segment of the C-strand in late S phase to a DNA extending structure (telomerase–Est3–Est1–Cdc13) for the G-rich 3'-overhang strand to a DNA polymerase α complex (Pol1, Pol12 and primase) that fills in the complementary C-strand during S/G2 and then returns to the capping structure found in M phase (Figure 1). In addition to this route, the telomere structure can also remain capped throughout the cell cycle. The decision to follow an extendable or unextendable path is determined, in part, through a counting mechanism involving the number of double-stranded telomeric DNA motifs (*i.e.*, length of terminal DNA), the Rap1–Rif1–Rif2 protein complex, and the telomere-associated kinases Tel1 and Mec1 [7]. Intriguingly, Hsp82 interacts with at least one operational component required for each of these telomere events (highlighted in red in Figure 1). But what functional role does Hsp82 have with these various factors?

The telomere system is confronted with difficulties that might be alleviated with the assistance of a molecular chaperone. Specifically, telomeres face significant protein motility challenges, as there is a need to transition between multiple structures, the individual telomere assemblies require mobility to function efficiently (*e.g.*, telomere lengthening requires telomerase repositioning) and the system must avoid potential competitive DNA binding events that would otherwise sterically interfere with function (*e.g.*, Cdc13, Est1 (Ever shorter telomeres), MRX, Stn1–Ten1 and telomerase are all single-stranded G-rich DNA binding proteins) [4,7]. Although there are a number of possible explanations to account for the various challenges, we favor a model in which the telomere environment is maintained in a dynamic state by the HSP90 machinery. If telomeres were governed by dynamic remodeling, then the individual components would exchange rapidly, fostering efficient transitions between complexes and avoiding competitive binding events through transient DNA interactions. Notably, a recent report showed that Hsp82 could solve these issues for at least one telomere component, Cdc13 [28].

Cdc13 plays a central role in yeast telomere biology, as it is required to nucleate both capping and extending complexes to the DNA-ends [43]. Genetic studies demonstrate that Cdc13 protects chromosomal termini along with Stn1–Ten1 and is also required to extend the telomeric DNA in conjunction with the telomerase holoenzyme [4,7]. To determine whether Hsp82 influences Cdc13-mediated events, an *in vitro* system was established in which Cdc13 alone could stimulate telomerase DNA extension activity, but an unextendable telomeric DNA structure formed in the co-presence of Stn1–Ten1 [28]. Significantly, addition of Hsp82 dissociated the Cdc13–Stn1–Ten1–DNA complex but did not impede Cdc13's ability to stimulate telomerase. Hence, Hsp82 permitted a transition between the capping and extending assemblies without interfering with the positive telomerase regulatory events provided by Cdc13. Whether this disassembly activity explains why *HSP82* is a high-copy suppressor of the *cdc13-1* and *stn1-157* mutations remains unknown. However, the ability of Hsp82 to promote DNA dissociation (*i.e.*, Cdc13) and association (*i.e.*, telomerase) of different telomere

proteins might account for the extensive interaction network between Hsp82 and telomere proteins, as it would create a self-organizing telomere environment (Box 1).

To illustrate, we will expand upon the conceivable events that occur in late S phase when telomeres are directed to either extendable or unextendable states [7]. Initial expectations posited that all telomeric DNA would be lengthened during the duplication of each chromosome [45,46]. However, direct testing showed that individual telomere size is not increased every cell cycle [9]. Furthermore, the extended telomeres displayed a sizeable variability in the amount of DNA added, as the interquartile range in extension length was 14–80 nucleotides. To account for the distribution in extension lengths, telomeres must be governed by a system that continuously reacts to regulatory signals and can control telomerase action before and after each added telomeric DNA repeat. To be able to respond to the incoming signals, we suspect that the HSP90 chaperone system maintains the telomere environment in a dynamic state (Figure 2). In our model, Hsp82 promotes the continual dissociation of the unextendable complex (Cdc13–Stn1–Ten1), which will reform unless the requisite components and signals for constructing the extendable structure are present. For example, assembly of the telomerase holoenzyme is restricted to S-phase since the essential Est1 subunit is only expressed at this point of the cell cycle [47]. In addition, the actions of certain kinases (*e.g.*, Cyclin-dependent kinase 1 (Cdk1) and Tel1) appear to guide telomeres towards the requisite state by minimally modifying Cdc13. Cdk1-mediated phosphorylation likely disfavors Cdc13 interactions with Stn1 and fosters association with Est1 [48]. In addition, the Tel1 kinase is required for telomere DNA maintenance, and its potential phosphorylation targets include Cdc13 and the MRX complex subunit Xrs2 [49,50]. However, the precise mechanistic contributions of Tel1 signaling remain incompletely understood.

After nucleation of the modified telomerase holoenzyme at a telomere, Hsp82 and its cochaperone Sba1 likely promote a cyclical telomerase action, as Hsp82 supports DNA binding by telomerase and Sba1 dissociates telomerase DNA complexes [26,27]. How Hsp82 promotes binding while Sba1 facilitates release is not clear though it might occur through a chaperone-mediated opening and closing of the DNA binding cleft. Nevertheless, rapid cycling of telomerase on and off of the telomeric DNA would be beneficial for effective extension of the chromosomal termini. Following sufficient telomeric DNA lengthening, Sba1-mediated dissociation of the telomerase complex and action by unidentified phosphatases (which would reverse the influence of the Cdk1 and Tel1 kinases), likely promote the reformation of the Cdc13–Stn1–Ten1 unextendable structure. Hence, by maintaining the telomere components in a dynamic state, the Hsp82 chaperone machine would permit the system to efficiently transition between different operative states, which are guided by post-translational modifications and the presence of the required subunits; the chaperone-mediated interchange would also foster effective action for complexes that require movement (*e.g.*, telomerase-mediated DNA extension).

HSP90, telomere proteins and cancer

The HSP90 chaperone network serves a wide variety of client proteins operating in a number of pathways required for cell viability including telomere maintenance, signal transduction, transcription, protein transport, chromatin remodeling, and protein stabilization [12,14]. The influence of the HSP90 system on these cellular functions is magnified during periods of stress [17]. Normally the HSP90 system aides cell survival; however, during periods of chronic physiological shock (*e.g.*, cancer) the beneficial influence of HSP90 might shift to a detrimental role as HSP90 appears to be central for supporting a broad range of cancers [51]. As might be anticipated, components of the HSP90 machinery, including HSP90 and p23, correlatively increase in expression with the development of metastatic growth [52–55]. In a comparable

manner, telomerase activity also appears to play a central role in general cancer progression [5].

Mammalian telomerase is only expressed in highly proliferative cell types such as germ and somatic stem cells [56]. Normal differentiated cells typically reach replicative senescence once the DNA length of even a single telomere is reduced to a critically shortened size [6]. In contrast to average cells, ~85–90% of all malignant tissues display telomerase activity, and tumors without telomerase are often diagnosed as benign [57]. Telomerase up-regulation minimally averts senescence and allows the altered cell to perpetuate and generate additional cancerous cells (*e.g.*, tumor formation). Intriguingly, the human protooncogene *c-Myc* can activate transcription of both *HSP90A* and *TERT* [58]. Although a rise in telomerase expression is a common route to increased activity, other mechanisms are apparent. For example, experiments using a prostate cancer model showed that increased telomerase activity correlates with a rise in HSP90 and p23 levels, and not in telomerase protein expression, as cells progressed towards malignancy [52]. Modulation of telomerase activity through HSP90-dependent mechanisms has been suggested to occur in numerous cancer types including hepatomas, adenomas, glioblastomas and melanomas [59–62]. Thus, telomerase activity and molecular chaperone levels correlate with cancer cell progression and these phenomena appear to be linked. The high prevalence of HSP90 and telomere defects in a broad range of cancers makes these ideal candidates for medicinal purposes and both have been pursued as molecular targets for anticancer therapeutics in clinical trials (Box 2).

Several reports exploiting cell culture models indicate that a combinatorial tactic incorporating HSP90 and telomere inhibitors might be rewarding. Given the reliance of telomerase on HSP90, it was anticipated that reducing HSP90 activity would foster telomerase inhibition. Indeed, the combination of the template antagonist GRN163L and 17-(Allylamino)-17-demethoxygeldanamycin (17AAG), and HSP90 inhibitor, greatly increased the induced death of myeloma cells [63]. As telomerase has not been shown to support HSP90 activity, it was perhaps surprising to note that reducing the basal levels of the telomerase protein subunit TERT by an RNAi strategy led to increased 17AAG potency in culture [64]. Furthermore, the application of the G-quartet compound RHPS4 in conjunction with 17AAG had a synergistic inhibitory effect on breast cancer cell growth [65]. Conceivably, the finding that HSP90 modulates telomere-capping components helps rationalize the observed synergy with the telomere uncapping drug [28]. Together, the inhibitor studies support the premise that combinatorial targeting of HSP90 and telomeres will have a beneficial medicinal outcome.

In addition to reducing or preventing tumor growth, mixing HSP90 and telomere compounds might prove advantageous for other reasons. As mentioned, telomerase activity is present in dividing cells, including stem and germ lines, and therefore a means to avoid these cell types would be beneficial. In general, cancer cells display an ~100-fold higher affinity for HSP90 inhibitors relative to normal tissue [66]. Hence, if telomere compounds were co-delivered with HSP90 inhibitors, then a selective advantage might be gained in targeting malignant tissues. Furthermore, as 17AAG can be labeled and traced within whole organisms, it might be possible to apply additional cytotoxic agents locally [67]. Hence, the co-use of HSP90 inhibitors with telomere drugs could provide a multi-faceted therapeutic benefit.

Concluding remarks

Telomere protein biology serves as an excellent molecular paradigm to understand how the efficiency of a single system can impact homeostasis, as telomere dysfunction can lead to either cellular senescence or uncontrolled growth (*i.e.*, cancer). Given the number of employed proteins with common binding specificities (Figure 1), a decline in telomere efficiency is highly plausible due to complex misassembly or impaired structure disassembly. The fundamental

properties of molecular chaperones (abundant proteins with promiscuous but weak binding activities) would help the telomere system avoid these challenges by cultivating a self-organizing environment (Box 1). In essence, the ability of the HSP90 chaperone network to foster telomere protein dynamics parallels the more established molecular chaperone roles in protein folding in which a chaperone does not dictate the final folded structure (path direction) but rather helps the nascent chain (system) avoid off-pathway energy barriers that commonly occur in protein folding (multi-step) energy landscapes. Given the large number of HSP90 interacting telomere targets (Figure 1), it will be interesting to learn whether future studies continue to reveal a role for the HSP90 network in creating a dynamic telomere environment.

Box 1

Molecular chaperone-mediated protein self-organization

By modulating the DNA-bound state of a broad array of telomere proteins, HSP90 could create a local telomere environment functioning through the principles of self-organization. Self-organizing systems operate by continuously breaking down into simpler components and either remaining in a more elementary state (termination of activity) or reassembling if the appropriate components and energy resources are available (transition between complexes) [68]. Essentially, if telomeres were maintained in a self-organizing state, then the system would readily shift between different functional states upon the integration of either additional proteins or signals. The capacity to rapidly assimilate information and generate the correct action is likely very relevant for telomeres given the cell cycle restricted timing of events and the number of factors and complexes that must be properly assembled and disassembled in order to function [4,7].

Box 2

HSP90 and telomere therapeutics

The most common HSP90 inhibitors are based upon natural benzoquinone ansamycins (*e.g.*, geldanamycin) that are bound in the ATP-binding pocket of HSP90 and inhibit both nucleotide hydrolysis and chaperone functions [69–71]. Geldanamycin derivatives including 17AAG and IPI504, which have increased hydrophilicity and lowered hepatotoxicity, currently are in Phase I-III trials [72]. Although the clinical trials for various HSP90 inhibitors (from benzoquinones to purine-scaffold compounds) are promising, the single compound studies appear to have limited efficacy [73,74]. Fortunately, the lack of significant toxicity and an ability to trace certain HSP90 inhibitors position these compounds as ideal agents for combinatorial treatments [75].

Telomere therapeutics has been approached from several angles, although the two most promising avenues have focused on the telomerase RNA template and the telomeric DNA. Mammalian telomeric DNA repeats can form G-quartet structures and small molecules designed to foster the four-stranded quartet fold cause telomeric DNA shortening by uncapping the termini, which eventually leads to cellular senescence [65,76]. Compounds targeting telomerase are often designed as RNA antagonists that hybridize with the template region and block DNA extension activity [77]. The most promising antagonist is GRN163L, which causes progressive telomere shortening and inhibits cell growth [78]. Unfortunately, telomere therapies typically have a substantial effective lag time, which depends on the initial telomeric DNA length in the treated cells, in order to induce replicative senescence and prevent tumor growth [65,79]. Hence, telomere disruption, either through deprotection of the ends or by blocking telomerase activity, is likely insufficient for cancer treatment given the time scale necessary for an effective response. However, given the long-term

potential of preventing tumor regrowth, the telomere inhibitors might prove advantageous in conjunction with other cytotoxic agents [80].

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References

1. Misteli T. The concept of self-organization in cellular architecture. *J Cell Biol* 2001;155:181–185. [PubMed: 11604416]
2. Freeman BC, Yamamoto KR. Continuous recycling: a mechanism for modulatory signal transduction. *Trends in Biochem Sci* 2001;26:285–290. [PubMed: 11343920]
3. DeZwaan DC, Freeman BC. HSP90: the Rosetta stone for protein dynamics? *Cell Cycle* 2008;7:1006–1012. [PubMed: 18414022]
4. Gilson E, Géli V. How telomeres are replicated. *Nat Rev Mol Cell Biol* 2007;8:825–838. [PubMed: 17885666]
5. Shay JW, Wright WE. Telomerase activity in human cancer. *Curr Opin Oncol* 1996;8:66–71. [PubMed: 8868103]
6. Hemann MT, et al. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 2001;107:67–77. [PubMed: 11595186]
7. Shore D, Bianchi A. Telomere length regulation: coupling DNA end processing to feedback regulation of telomerase. *EMBO J* 2009;28:2309–2322. [PubMed: 19629031]
8. Marcand S, et al. Cell cycle restriction of telomere elongation. *Curr Biol* 2000;10:487–490. [PubMed: 10801419]
9. Teixeira MT, et al. Telomere length homeostasis is achieved via a switch between telomerase extendible and -nonextendible states. *Cell* 2004;117:323–335. [PubMed: 15109493]
10. Ellis RJ, van der Vies SM. Molecular Chaperones. *Annu Rev Biochem* 1991;60:321–347. [PubMed: 1679318]
11. Hendrick JP, Hartl FU. Molecular chaperone functions of heat-shock proteins. *Annu Rev Biochem* 1993;62:349–384. [PubMed: 8102520]
12. Wegele H, et al. HSP70 and HSP90—a relay team for protein folding. *Rev Physiol Biochem Pharmacol* 2004;151:1–44. [PubMed: 14740253]
13. Mayer MP, Bukau B. HSP70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci* 2005;62:670–684. [PubMed: 15770419]
14. Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the HSP90/HSP70-based chaperone machinery. *Exp Biol Med* 2003;228:111–133.
15. Millson SH, et al. A two-hybrid screen of the yeast proteome for HSP90 interactors uncovers a novel HSP90 chaperone requirement in the activity of a stress-activated mitogen-activated protein kinase, Slt2p (Mpk1p). *Euk Cell* 2005;4:849–860.
16. Zhao R, et al. Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the HSP90 chaperone. *Cell* 2005;120:715–727. [PubMed: 15766533]
17. McClellan AJ, et al. Diverse cellular functions of the HSP90 molecular chaperone uncovered using systems approaches. *Cell* 2007;131:121–135. [PubMed: 17923092]
18. Johnson JL, Brown C. Plasticity of the HSP90 chaperone machine in divergent eukaryotic organisms. *Cell Stress Chaperones* 2009;14:83–94. [PubMed: 18636345]
19. Ziemieowicz A, et al. Calf thymus Hsc70 and Hsc40 can substitute for DnaK and DnaJ function in protein renaturation but not in bacteriophage DNA replication. *FEBS Lett* 2001;507:11–15. [PubMed: 11682050]
20. Ellis RJ. Protein misassembly: macromolecular crowding and molecular chaperones. *Adv Exp Med Biol* 2007;594:1–13. [PubMed: 17205670]

21. Shakhovich R, et al. Conformational activation of a basic helix-loop-helix protein (MyoD1) by the C-terminal region of murine HSP90 (HSP84). *Mol Cell Biol* 1992;12:5059–5068. [PubMed: 1406681]
22. Freeman BC, Yamamoto KR. Disassembly of transcriptional regulatory complexes by molecular chaperones. *Science* 2002;296:2232–2235. [PubMed: 12077419]
23. Müller L, et al. HSP90 regulates the activity of wild type p53 under physiological and elevated temperatures. *J Biol Chem* 2004;279:48846–48854. [PubMed: 15358771]
24. Stavreva DA, et al. Rapid glucocorticoid receptor exchange at a promoter is coupled to transcription and regulated by chaperones and proteasomes. *Mol Cell Biol* 2004;24:2682–2697. [PubMed: 15024059]
25. Walerych D, et al. HSP90 chaperones wild-type p53 tumor suppressor protein. *J Biol Chem* 2004;279:48836–48845. [PubMed: 15358769]
26. Toogun OA, et al. The p23 molecular chaperone promotes functional telomerase complexes through DNA dissociation. *Proc Natl Acad Sci USA* 2007;104:5765–5770. [PubMed: 17389357]
27. Toogun OA, et al. The HSP90 molecular chaperone modulates multiple telomerase activities. *Mol Cell Biol* 2008;28:457–467. [PubMed: 17954556]
28. DeZwaan DC, et al. The Hsp82 molecular chaperone promotes a switch between unextendable and extendable telomere states. *Nat Struct Mol Biol* 2009;16:711–716. [PubMed: 19525972]
29. Konieczny I, Zylicz M. Role of bacterial chaperones in DNA replication. *Genet Eng* 1999;21:95–111.
30. Richter K, et al. The cellular world according to HSP90. *Nat Struct Mol Biol* 2007;14:90–94. [PubMed: 17277798]
31. Hager GL, et al. Transcription Dynamics. *Mol Cell* 2009;35:741–753. [PubMed: 19782025]
32. Holt SE, et al. Functional requirement of p23 and HSP90 in telomerase complexes. *Genes Dev* 1999;13:817–826. [PubMed: 10197982]
33. Britt-Compton B, et al. Short telomeres are preferentially elongated by telomerase in human cells. *FEBS Lett* 2009;583:3076–3080. [PubMed: 19716824]
34. Cohn M, Blackburn EH. Telomerase in yeast. *Science* 1995;269:396–400. [PubMed: 7618104]
35. Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989;59:521–529. [PubMed: 2805070]
36. Forsythe HL, et al. Stable association of HSP90 and p23, but Not HSP70, with active human telomerase. *J Biol Chem* 2001;276:15571–15574. [PubMed: 11274138]
37. Grandin N, Charbonneau M. HSP90 levels affect telomere length in yeast. *Mol Genet Genomics* 2001;265:126–134. [PubMed: 11370858]
38. Ghaemmaghami S, et al. Global analysis of protein expression in yeast. *Nature* 2003;425:737–741. [PubMed: 14562106]
39. Beckmann RP, et al. Interaction of HSP70 with newly synthesized proteins: implications for protein folding and assembly. *Science* 1990;248:850–854. [PubMed: 2188360]
40. Bukau B, Walker GC. Cellular defects caused by deletion of the *Escherichia coli* dnaK gene indicate roles for heat shock protein in normal metabolism. *J Bacteriol* 1989;171:2337–2346. [PubMed: 2651398]
41. Deuring E, et al. Trigger factor and DnaK cooperate in folding of newly synthesized proteins. *Nature* 1999;400:693–696. [PubMed: 10458167]
42. Keppler BR, et al. The biochemical role of the heat shock protein 90 chaperone complex in establishing human telomerase activity. *J Biol Chem* 2006;281:19840–19848. [PubMed: 16714764]
43. Pennock E, et al. Cdc13 delivers separate complexes to the telomere for end protection and replication. *Cell* 2001;104:387–396. [PubMed: 11239396]
44. Grandin N, et al. Stn1, a new *Saccharomyces cerevisiae* protein, is implicated in telomere size regulation in association with Cdc13. *Genes Dev* 1997;11:512–527. [PubMed: 9042864]
45. Shampay J, Blackburn EH. Generation of telomere-length heterogeneity in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 1988;85:534–538. [PubMed: 3277178]
46. Blackburn EH, et al. Recognition and elongation of telomeres by telomerase. *Genome* 1989;31:553–560. [PubMed: 2698831]

47. Osterhage JL, et al. Proteasome-dependent degradation of Est1p regulates the cell cycle-restricted assembly of telomerase in *Saccharomyces cerevisiae*. *Nat Struct Mol Biol* 2006;13:720–728. [PubMed: 16862158]
48. Li S, et al. Cdk1-dependent phosphorylation of Cdc13 coordinates telomere elongation during cell-cycle progression. *Cell* 2009;136:50–61. [PubMed: 19135888]
49. Nakada D, et al. ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev* 2003;17:1957–1962. [PubMed: 12923051]
50. Tseng SF, et al. The telomerase-recruitment domain of the telomere binding protein Cdc13 is regulated by Mec1p/Tel1p-dependent phosphorylation. *Nucleic Acids Res* 2006;34:6327–6336. [PubMed: 17108359]
51. Whitesell L, Lindquist SL. HSP90 and the chaperoning of cancer. *Nat Rev Cancer* 2005;5:761–772. [PubMed: 16175177]
52. Akalin A, et al. A novel mechanism for chaperone-mediated telomerase regulation during prostate cancer progression. *Cancer Res* 2001;61:4791–4796. [PubMed: 11406554]
53. Oxelmark E, et al. The co-chaperone p23 differentially regulates estrogen receptor target genes and promotes tumor cell adhesion and invasion. *Mol Cell Biol* 2006;26:5205–5213. [PubMed: 16809759]
54. Elmore LW, et al. Overexpression of telomerase-associated chaperone proteins in prostatic intraepithelial neoplasia and carcinomas. *Oncol Rep* 2008;20:613–617. [PubMed: 18695913]
55. McDowell CL, et al. Expression of HSP90 chaperone [corrected] proteins in human tumor tissue. *Int J Biol Macromol* 2009;45:310–314. [PubMed: 19576239]
56. Flores I, et al. Telomerase regulation and stem cell behaviour. *Curr Opin Cell Biol* 2006;18:254–260. [PubMed: 16617011]
57. Cech TR. Beginning to understand the end of the chromosome. *Cell* 2004;116:273–279. [PubMed: 14744437]
58. Teng SC, et al. Direct activation of HSP90A transcription by c-Myc contributes to c-Myc-induced transformation. *J Biol Chem* 2004;279:14649–14655. [PubMed: 14724288]
59. Lee CL, et al. Strategic shotgun proteomics approach for efficient construction of an expression map of targeted protein families in hepatoma cell lines. *Proteomics* 3:2472–2486. [PubMed: 14673797]
60. Shervington A, et al. Glioma: what is the role of c-Myc, HSP90 and telomerase? *Mol Cell Biochem* 2006;283:1–9. [PubMed: 16444580]
61. Becker B, et al. Induction of HSP90 protein expression in malignant melanomas and melanoma metastases. *Exp Dermatol* 2004;13:27–32. [PubMed: 15009113]
62. Mitsiades CS, et al. Antimyeloma activity of heat shock protein-90 inhibition. *Blood* 2006;107:1092–1100. [PubMed: 16234364]
63. Shamma MA, et al. Telomerase inhibitor GRN163L inhibits myeloma cell growth in vitro and in vivo. *Leukemia* 2008;22:1410–1418. [PubMed: 18449204]
64. Villa R, et al. Inhibition of telomerase activity by geldanamycin and 17-allylamino, 17-demethoxygeldanamycin in human melanoma cells. *Carcinogenesis* 2003;24:851–859. [PubMed: 12771028]
65. Cookson JC, et al. Pharmacodynamics of the G-quadruplex-stabilizing telomerase inhibitor 3,11-difluoro-6,8,13-trimethyl-8H-quinto[4,3,2,k1]acridinium methosulfate (RHPS4) in vitro; activity in human tumor cells correlates with telomere length and can be enhanced, or antagonized, with cytotoxic agents. *Mol Pharmacol* 2005;68:1551–1558. [PubMed: 16150933]
66. Kamal A, et al. A high-affinity conformation of HSP90 confers tumour selectivity on HSP90 inhibitors. *Nature* 2003;425:407–410. [PubMed: 14508491]
67. Daozhen C, et al. Synthesis of (131)I-labeled-[(131)I]iodo-17-allylamino-17-demethoxy geldanamycin ([131I]iodo-17-AAG) and its biodistribution in mice. *Cancer Biother Radiopharm* 2007;22:607–612. [PubMed: 17979563]
68. Prigogine I, Nicolis G. Biological order, structure and instabilities. *Q Rev Biophys* 1971;4:107–148. [PubMed: 4257403]
69. Whitesell L, Cook P. Stable and specific binding of heat shock protein 90 by geldanamycin disrupts glucocorticoid receptor function in intact cells. *Mol Endocrinol* 1996;10:705–712. [PubMed: 8776730]

70. Prodromou C, et al. Identification and structural characterization of the ATP/ADP-binding site in the HSP90 molecular chaperone. *Cell* 1997;90:65–75. [PubMed: 9230303]
71. Wandinger SK, et al. The HSP90 chaperone machinery. *J Biol Chem* 2008;283:18473–18477. [PubMed: 18442971]
72. <http://clinicaltrials.gov/ct2/results?term=hsp90>
73. Kim YS, et al. Update on HSP90 inhibitors in clinical trial. *Curr Top Med Chem* 2009;9:1479–1492. [PubMed: 19860730]
74. Powers MV, Workman P. Inhibitors of the heat shock response: biology and pharmacology. *FEBS Lett* 2007;581:3758–3769. [PubMed: 17559840]
75. Hwang M, et al. HSP90 inhibitors: multi-targeted antitumor effects and novel combinatorial therapeutic approaches in cancer therapy. *Curr Med Chem* 2009;16:3081–3092. [PubMed: 19689285]
76. Burger AM, et al. The G-quadruplex interactive molecule BRACO19 inhibits tumour growth, consistent with telomere binding and interference with telomerase function. *Cancer Res* 2005;65:1489–1496. [PubMed: 15735037]
77. Chen H, et al. Strategies targeting telomerase inhibition. *Mol Biotechnol* 2009;41:194–199. [PubMed: 18956258]
78. Herbert BS, et al. Lipid modification of GRN163, an N3' → P5' thio-phosphoramidate oligonucleotide, enhances the potency of telomerase inhibition. *Oncogene* 2005;24:5262–5268. [PubMed: 15940257]
79. Dikmen ZG, et al. In vivo inhibition of lung cancer by GRN163L: a novel human telomerase inhibitor. *Cancer Res* 2005;65:7866–7873. [PubMed: 16140956]
80. Burger AM. Highlights in experimental therapeutics. *Cancer Lett* 2007;245:11–21. [PubMed: 16647200]

Figure 1. HSP90 intersects with numerous proteins that function in various phases of the telomere system

Interactions between HSP90 and yeast telomere proteins, which are highlighted in red, have been identified through genetic and proteomic tactics with many emerging in recent high-throughput studies [15–17]. To effectively operate, the telomere system nucleates a number of different protein complexes to chromosomal ends on a cell cycle and need basis [4]. In M phase, a telomere is in a capped, non-extendable state to prevent DNA damage and non-homologous end joining (i). Although the telomere remains non-extendable in G1, the complex is modified by the addition of the core telomerase subunits (ii). In S phase the DNA replication machinery duplicates the DNA with the exception of the extreme 5' terminus (iii). Following replication, the DNA resectioning machinery degrades a section of the 5' C-strand (iv). In late S phase the telomere can transition to an extendable state by assembling the telomerase holoenzyme to lengthen the 3' G-strand (v). In S/G2, the C-strand is filled in by the DNA polymerase α machinery and the telomere structure returns to a protective capping complex (vi). The identities of the individual components or complexes are indicated.

Figure 2. HSP90 maintains the telomere environment in a dynamic state that permits rapid and efficient reactions to incoming signals and components

In late S phase, telomeres are guided to either a telomerase extendable state or maintained in a protective unextendable form depending upon the length of the adjacent telomeric DNA [7]. In order to transition to a required structure during the narrow time frame afforded in late S phase, telomere structures are likely continuously disassembled and reassembled into various complexes depending upon the available components (*e.g.*, Stn1–Ten1 vs. telomerase–Est1–Est3) and local signals (*e.g.*, Cdk1 and Tel1 kinases). In yeast, Hsp82 fosters both dissociation (protection components) and association (extension subunits) of telomere structures. In conjunction with the Sba1 co-chaperone that destabilizes the extension complex, telomeres are able to efficiently transition between the different functional states in order to properly maintain the chromosomal termini.

Table 1

Potential Yeast Hsp90 Telomere Interacting Partners

Gene	Function	Refs
<i>SIR2</i>	Chromatin Silencing	15
<i>CTF4</i>	DNA Repair	16
<i>EXO1</i>	DNA Resectioning	
<i>MRE11</i>	DNA Resectioning	
<i>POL12</i>	Fill-in Replication	
<i>RAD52</i>	Telomere Maintenance	
<i>SLX8</i>	Telomere Maintenance	
<i>SUM1</i>	Chromatin Silencing	
<i>BUD32</i>	Telomere Maintenance	17
<i>CDC7</i>	Chromatin Silencing	
<i>CDC13</i>	Extending/Capping	
<i>EST1</i>	Extending	
<i>MEC1</i>	Extending	
<i>RAD51</i>	Telomere Maintenance	
<i>RAD59</i>	Telomere Maintenance	
<i>STN1</i>	Capping	
<i>SWD3</i>	Telomere Maintenance	
<i>YKU80</i>	Capping + Core	
<i>SBA1</i>	Extending	26
<i>EST2</i>	Extending	27