Chapter 17
Sti1/Hop Plays a Pivotal Role in Hsp90 Regulation Beyond Bridging Hsp70

Michael Reidy

Abstract Since its initial characterization, Hop (Hsp90/Hsp70 organizing protein), known as Sti1 in yeast (stress inducible) is mostly understood to serve as a bridge that facilitates transfer of substrate "client" proteins from Hsp70 to Hsp90. Recent work has shown that Sti1 regulates Hsp90 in a manner distinct from its role as a bridge to Hsp70. This second function of Sti1 seems to be to position Hsp90 for subsequent steps of the client maturation cycle, after the client has been transferred from Hsp70. Thus, Sti1/Hop occupies a central gatekeeper role in the Hsp90 reaction cycle, by first facilitating client access to Hsp90 and then promoting the next steps of the cycle.

Keywords Chaperone · Co-chaperone · Hop · Hsp · Hsp70 · Hsp90 · Sti1

Abbreviations

3D	Three dimensional
AR	Androgen receptor
ATP	Adenosine triphosphate
cryoEM	Cryoelectron microscopy
DP	Aspartate/proline-rich motif
EM	Electron microscopy
FOA	5′Fluoro-orotic acid
GR	Glucocorticoid receptor
Hop	Hsp90/Hsp70 organizing protein
Hsp	Heat shock protein
MAP	Mitogen-activated protein

M. Reidy (✉)
Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA
e-mail: michael.reidy@nih.gov

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SdC	Sti1-dependent carboxy-terminal proximal
SdN	Sti1-dependent amino-terminal proximal
TPR	Tetratricopeptide repeat

17.1 Introduction

In order to discuss the role of Sti1 in Hsp90 regulation, it is necessary to first discuss Hsp90. Hsp90 is an evolutionarily conserved, essential (in eukaryotes) homodimer that regulates a diverse and expanding list of substrate proteins, known as clients (Karagoz and Rudiger 2015; Prodromou 2016). Through an open-close client binding mechanism, Fig. 17.1, Hsp90 regulates the activities and stabilities of client proteins such as kinases, transcription factors and metabolic enzymes as well as glucocorticoid and androgen receptors (GR and AR, respectively) in mammals. A large and diverse cohort of Hsp90 co-chaperones assist in the regulation of the eukaryotic Hsp90 reaction cycle. Table 17.1 lists the major co-chaperones, their names in yeast and mammalian systems and their known role or function in Hsp90 regulation.

Hsp90 is a modular protein with an amino-terminal (N) ATPase domain connected to a middle (M) domain via a charged linker, and a carboxy-terminal (C) dimerization domain immediately adjacent to the M-domain. Hsp90 exists as a homodimer joined at the C-terminal domain, and in the apo state the N-terminal domains are positioned the furthest possible from each other, called the "open

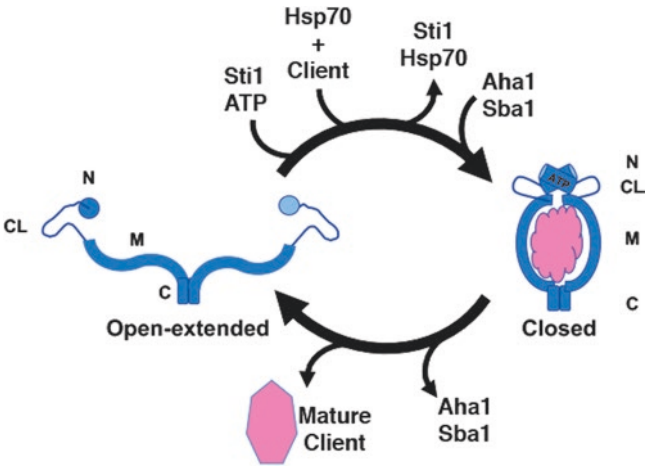

Fig. 17.1 A simplified view of the Hsp90 chaperone cycle. Hsp90 (blue) regulates the activity and stability of client proteins (pink) via an open-close mechanism that is assisted by various co-chaperones such as Hsp70, Sti1, Aha1 and Sba1 (see Table 17.1). The relative location of the N-terminal (N), charged linker (CL), middle (M) and C-terminal (C) domains are shown in the open (left) and closed (right) conformations. (See text for more details)

Table 17.1 The human and yeast names and functions of the major Hsp90 co-chaperones

Human	Yeast	Role in Hsp90 regulation
Hsc70, Hsp70	Ssa1–4	Delivery of clients
Hop	Sti1	Bridging Hsp90 and Hsp70, formation of client loading conformation, has three TPR domains
Aha	Aha1	Stimulator of Hsp90 ATP hydrolysis
p23	Sba1	Stabilizes ATP-bound, N-terminally dimerized conformation
Cdc37	Cdc37	Required for kinase clients
FKBP51, FKBP52, CyP-40	Cpr6, 7	Cyclophilin (peptidyl-prolyl cis-trans isomerase), has TPR domains

extended" conformation (Krukenberg et al. 2009; Schopf et al. 2017). In the ATP state, the N-terminal domains of both protomers make physical contact. In order to do so they must first rotate inwards and dock to the N-terminal portion of the M-domain. Hydrolysis only occurs when the N-terminal domains come into contact with each other in the "closed" conformation. Clients are bound in the interior space formed by the two M-domains in the closed conformation (Verba et al. 2016). Interaction with Hsp90 modifies the activity of the client in a specific manner. For example, GR and AR can only bind to their hormone ligands when in complex with Hsp90 and other co-chaperones (Pratt et al. 2006), while in the case of MAP kinases Hsp90 interaction is required for the phosphorylation and activation of target transcription factors (Pratt et al. 2006). After ATP hydrolysis the N-terminal domains dissociate and Hsp90 returns to the open extended conformation, releasing the mature client and resetting the cycle.

Different co-chaperones bind to distinct conformations of Hsp90. Since the position of the N-terminal domains relative to each other changes dramatically depending on the nucleotide state, several possible conformations theoretically exist between open-extended and closed. For example, Sba1 (p23 in humans) binds only the ATP-bound, fully closed conformation of Hsp90 (Ali et al. 2006). Presence of Sba1 in Hsp90 pull-downs from cell lysates treated with non-hydrolysable ATP analogs is generally regarded as a useful sensor for the closed conformation of Hsp90 (Zuehlke and Johnson 2012). In addition to the conformation of Hsp90 influencing the interaction of co-chaperones, the binding of some co-chaperones directly influences the conformation of Hsp90. For example, using cryoEM to construct 3D structures of complexes formed from purified proteins, the Agard lab showed that Hsp90 adopted a "client loading" conformation when bound to Hop (Southworth and Agard 2011). This conformation was partially closed relative to the open-extended and Sba1-bound conformations. Hydrophobic residues in the interior of the Hsp90 dimer M-domains aligned into patches when bound to Hop. These patches were postulated to be the sites of client interaction. The degree of conformational requirements for co-chaperone interaction reflect the tight regulation these co-chaperones exert on the Hsp90 reaction cycle.

17.1.1 STI1/Hop Structure/Function

Sti1/Hop is a 66 kilodalton protein containing three tetratricopeptide repeat (TPR) domains, termed 1, 2A and 2B, and two aspartate/proline motif (DP) domains, see Fig. 17.2. While Sti1 is not essential for *Saccharomyces cerevisiae*, cells lacking Sti1 are hypersensitive to Hsp90 specific inhibitors such as radicicol and fail to grow under sub-optimal conditions (Chang et al. 1997). Early co-crystallographic studies demonstrated that carboxylate clamps in Hop TPR1 and TPR2A specifically interacted with the C-terminal EEVD motifs present in Hsp70 (GPTIVEEVD) and Hsp90 (MEEVD), respectively (Scheufler et al. 2000). From these studies a straightforward model of Sti1/Hop bridging Hsp90 and Hsp70 was developed: TPR1 bound to Hsp70 and TPR2A bound to Hsp90, forming a tripartite complex that allowed the client bound to Hsp70 to be transferred to Hsp90. However, subsequent studies that explored the interaction between Hsp70 and Hop suggested that this model was inadequate (Carrigan et al. 2004), since Hsp70 proteins lacking the C-terminal EEVD motif bound Hop like wild type. Also, the authors found that mutations in TPR2A, TPR2B and DP2 negatively impacted Hsp70 binding, which was not predicted based on the contemporary model.

It was then shown using yeast genetics that Sti1 regulated Hsp70 and Hsp90 independently on some level. By studying the effects of mutations in Hsp70 on the stability of self-templating amyloids, called prions, that propagated in some strains of yeast in a chaperone-dependent manner, it was found that destabilization of the [*PSI*$^+$] prion by the dominant *SSA1-21* allele required the C-terminal GPTIVEED motif of Hsp70 that had been shown previously to be important for TPR interaction. The authors then showed that Sti1 was required for the *SSA1-21* effect on prion stability (Jones et al. 2004). In a follow-up study it was shown that mutations in Sti1 that mediated the *SSA1-21* effect did not affect the cells' sensitivity to radicicol. In this way the regulation of Hsp70 by Sti1 could be differentiated from that of Hsp90, since radicicol treatment had no effect on [*PSI*$^+$] prion stability (Song and Masison 2005). This suggested Sti1 could regulate Hsp70 separately from Hsp90. However, mutations in Sti1 that affected either prion stability or radicicol sensitivity negatively impacted maturation of exogenously expressed mammalian GR in yeast, a pathway that was known to require both Hsp90 and Hsp70. These findings supported the bridging model. Thus it was possible that Sti1 performed an important role in Hsp90 regulation in addition to bridging Hsp90 and Hsp70. Another notable finding from this study was the implication that Hsp90 was a ligand for Sti1 TPR2B. The mystery surrounding the ligand for TPR2B deepened when it was

Fig. 17.2 Domain structure of Hop/Sti1. Hop/Sti1 contains three tetratricopeptide (TPR) domains and two aspartate/proline motif domains. TPR1 and DP1 are separated from the rest of the molecule by a flexible linker. TPR2A, 2B and DP2 comprise a rigid module that makes extensive contacts with Hsp90. Not to scale

shown that mutations in both TPR1 and TPR2B were necessary to abolish physical and genetic interactions with Hsp70 (Flom et al. 2006, 2007), contradicting some results from the studies described above. Importantly, these reports also found that the Sti1 TPR2A domain alone was not sufficient to interact with Hsp90 but required the presence of the TPR2B domain as well, which did agree with findings from those same earlier studies (Song and Masison 2005).

Using negative staining EM and cryo-EM of stabilized Hop:Hsp90 complexes to construct high-resolution structures, the Agard group established a model for client transfer from Hsp70 to Hsp90 bridged by Hop (Southworth and Agard 2011). In their reconstructed models, two molecules of Hop bound on opposite sides of one V-shaped Hsp90 dimer. The orientation of the M-domain relative to the C-domain in each protomer resulted in a more closed Hsp90 dimer compared to Hsp90 without Hop, under similar conditions. The only structures of Hsp90 N-terminal domain that fit the density they observed were from the ATP-bound, fully closed Hsp90 crystal structure. This was remarkable because in the closed structure, the N-terminal domains make extensive contacts whereas in the Hop-bound reconstructions they were measured to be on average 80 Å apart. Thus, the conformation that Hsp90 adopted when bound to Hop represented an unobserved conformation they termed the "client-loading" conformation.

In the cryo-EM structures of Hop:Hsp90, the authors noted that only two of Hop's three TPR domains were clearly represented as electron densities that spanned across and out from the Hsp90 dimer (Southworth and Agard 2011). The missing TPR domain in the structures was probably due its flexibility. Some density was observed below the C-terminal domain that could represent the position of the third TPR domain. Unfortunately, this meant that the authors could not confidently predict the orientation of Hop relative to the Hsp90 dimer, because it was unclear whether the observed densities were TPR1 and 2A or TPR2A and 2B. To solve this the authors used gold particle labeling of the His6-tag on the N-terminus of Hop. From these images, the authors concluded that they were observing TPR1 and TPR2A, however they could not rule out the possibility of the alternative (TPR2A and 2B). This orientation, with TPR1 positioned adjacent to the client binding region of Hsp90, fit well with the idea that Hop facilitated client transfer from Hsp70 to Hsp90.

When the authors added Hsp70 to the reaction mixtures, they observed very little change in the Hop:Hsp90 complexes themselves, indicating that the client loading conformation is independent of Hsp70 (Southworth and Agard 2011). Interestingly, a single Hop:Hsp90 complex bound a single molecule of Hsp70, in agreement with the bridging model of Hop/Sti1 function. The authors concluded that binding of Hop/Sti1 to Hsp90 was probably performing a more active role in Hsp90 regulation than merely serving as a bridge between Hsp90 and Hsp70.

Two reports published the next year 1 month apart, one from the Mayer lab and the other from the Buchner lab demonstrated that Sti1 TPR2B interacted physically with Hsp90 using biochemical, biophysical and mass-spectrometry methods (Lee et al. 2012; Schmid et al. 2012). Both studies reaffirmed that TPR2A bound the Hsp90 C-terminal MEEVD motif and identified the N-terminal portion of the

M-domain of Hsp90 as the site of interaction with TPR2B, in agreement with the Agard model. Also, both the Mayer and Buchner models agreed that the TPR1 domain of Sti1 swings freely via flexible linker to allow interaction with Hsp70. Thus, both models positioned Hop/Sti1 in an orientation opposite to that suggested by the Agard model. The Mayer and Buchner models differed, however, in two important ways. First, the model put forth by the Mayer lab had the TPR2A of Sti1 bound to the C-terminal MEEVD of one Hsp90 protomer while the TPR2A and 2B domains of the same Sti1 molecule made contacts with the M-domain of the other Hsp90 protomer. The Buchner model also identified contacts between Sti1 TPR2A and 2B and the M-domain of Hsp90, but on the same protomer that bound TPR2A via the MEEVD motif.

The second big difference in the two studies was that the Buchner lab went one step further and found that both TPR1 and TPR2B bound to Hsp70. The finding that Sti1 TPR2B made contacts with both Hsp90 and Hsp70 clarified confusion arising from differing results from the earlier studies (Flom et al. 2007; Song and Masison 2005). In the Buchner model, Sti1 TPR2A binds the Hsp90 MEEVD motif while TPR2B makes contacts in the M-domain in such a way as to make the carboxylate clamp accessible. TPR1 binds to Hsp70 and through the flexibility provided by the linker positions Hsp70 to make contact with and transfer to TPR2B. Thus, the client bound to Hsp70 is oriented in such a way to facilitate its transfer to Hsp90. The major difference in the Agard and Buchner models, the orientation of Hop/Sti1 with respect to the Hsp90 dimer, may actually be resolved by allowing that the densities that were assigned to TPR1 are actually TPR2B, and it is TPR1 that is not present in their reconstructions because of the flexible linker. The subsequent finding that TPR2A and 2B form a rigid module that is separated from TPR1 by a long flexible linker reinforces this notion (Rohl et al. 2015). In a further refinement to the Buchner model, it was shown that Hsp90 influences the affinity of Sti1 for Hsp70. In the absence of Hsp90, Sti1 binds Hsp70 via the TPR2B domain, but when Sti1 is bound to Hsp90, TPR1 is the preferred binding site for Hsp70 (Rohl et al. 2015). These findings provide a possible mechanism for the separate regulation of Hsp70 and Hsp90 observed previously (Jones et al. 2004; Song and Masison 2005).

17.1.2 Hsp90 and Hsp70 Interact Independently of STI1/HOP

As we have seen, a host of biochemical, biophysical and structural studies have established a reasonable model for the molecular mechanism by which Sti1/Hop physically bridges Hsp70 and Hsp90. Yet, as mentioned above, loss of Sti1/Hop is not lethal under optimal growth conditions in yeast (Chang et al. 1997) or *C. elegans* (Gaiser et al. 2009; Song et al. 2009). On the other hand, Sti1/Hop does become essential when yeast cells are stressed by temperature changes or inhibition of Hsp90 ATPase (Chang et al. 1997). In *sti1*Δ yeast cells exogenously expressed mammalian glucocorticoid receptor fails to mature properly when exposed to hormone (Chang et al. 1997). While *C. elegans* lacking Sti1/Hop appear to have little

phenotype under optimal conditions, they do have a reduced lifespan, developmental defects and weakened resistance to stress (Gaiser et al. 2009; Song et al. 2009). And in mice, loss of Sti1/Hop is embryonically lethal (Beraldo et al. 2013). Thus the role Sti1/Hop plays must be important for Hsp90-regulated processes that are more complex than merely maintaining viability under optimal conditions. Indeed, Hsp70 and Hsp90 interact directly (Genest et al. 2013; Kravats et al. 2018), and this Sti1/Hop independent binding is conserved from prokaryotes to humans.

The observation that yeast cells lacking Sti1 were viable under optimal conditions but temperature sensitive was reported over 20 years ago (Chang et al. 1997). Ten years later a report from the Johnson lab identified mutations in yeast Hsp90 that were synthetically lethal with *sti1*Δ (Flom et al. 2007). In cells expressing Sti1, these Hsp90 mutants co-purified with less Hsp70 than wild type Hsp90. They also observed that Hsp70 co-purified with Hsp90 in cells lacking Sti1. The authors noted that one of their mutants, G309S (in the *HSC82* isoform of yeast Hsp90), relied on Sti1 to interact with Hsp70. Taken together with results of earlier in vitro studies that found Hsp90 and Hsp70 could only co-purify in the presence of Hop, findings that were refuted by later studies (see below) (Chen et al. 1996; Johnson et al. 1996), the authors concluded that there may be another unknown factor that mediated the interaction of Hsp70 and Hsp90 in the absence of Sti1. Later studies showed that the interaction between Hsp70 and Hsp90 was in fact direct (Genest et al. 2013; Kravats et al. 2018).

Several studies from the laboratory of Sue Wickner have demonstrated unequivocally that Hsp70 and Hsp90 interact directly. Working primarily with the *E. coli* Hsp90 paralog HtpG, Genest and co-workers showed that prokaryotic Hsp90 and Hsp70 directly interacted with each other, which was expected since prokaryotes lack a Hop/Sti1 paralog (as well other Hsp90 co-chaperones) (Genest et al. 2013). Remarkably, this study also identified a putative Hsp70 binding site on *E. coli* Hsp90. In a follow-up study in collaboration with the Johnson and Masison labs, the Wickner group reported that the direct interaction between Hsp90 and Hsp70 was evolutionarily conserved in yeast (Kravats et al. 2018). The mutations in bacterial Hsp90 that affected direct interaction with Hsp70 had an identical affect when the analogous mutations were made in yeast Hsp90, and the most severe of these mutants were synthetically lethal with loss of Sti1. Remarkably, these residues (K394 and K399 in *HSP82*) were very close on the 3D structure of Hsp90 to G309, the residue identified as important for Hsp70 interaction reported earlier (Flom et al. 2007) (see above). Using purified proteins, the authors went on to show that yeast Hsp90 and Hsp70 physically interacted in vitro, although weakly, and the association of the mutant Hsp90s and Hsp70 was strengthened by Sti1. Together, these findings provided strong biochemical and genetic evidence that the Hsp70 binding site on Hsp90 was conserved from bacteria to yeast.

Our recent paper investigated the nature of mutations in Hsp90 that were lethal only in cells lacking Sti1 (discussed in more detail below), it was shown that the human paralogs of Hsp90 and Hsp70 directly interacted, demonstrating conservation of this interaction to humans (Reidy et al. 2018). Human Hsp90β is able to support viability in yeast cells as the only source of Hsp90, but only when Sti1 is

present (Scheufler et al. 2000). We showed through genetics and biochemistry that a forward mutation that enhanced direct interaction with yeast Hsp70 (see below) or co-expression of a human Hsp70 isoform overcame the dependence of human Hsp90β on Sti1. These results provided strong evidence for the idea that the direct Hsp90-Hsp70 interaction was conserved in humans. Taken together, these studies support the idea that basic cellular functions are maintained by a direct Hsp90-Hsp70 interaction, and provide strong evidence that this interaction is, in eukaryotes, essential.

17.1.3 Beyond the Bridge: A Second Function for STI1/HOP?

Through various collaborations studying different mutations in yeast Hsp90 that affected aspects such as Hsp70 or client interaction, as well as through reports by other labs, we observed that point mutations in Hsp90 that were synthetically lethal with deletion of *STI1* clustered into two distinct regions on the 3D structure of Hsp90, see Fig. 17.3. Mutations in the first cluster such as G309S (Flom et al. 2007), V391E (originally described as important for Aha1 interaction in vitro, it was observed to be Sti1 dependent in vivo) (Retzlaff et al. 2010), and K399C (Kravats et al. 2018) were located in or near the Hsp70 binding site identified by Sue

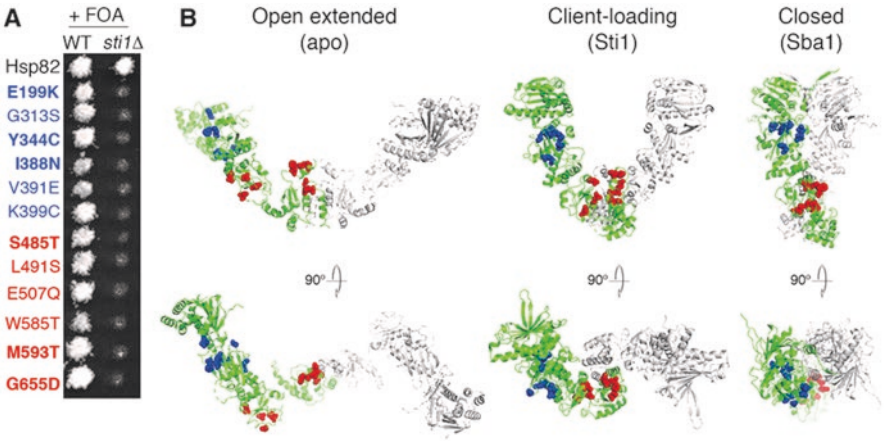

Fig. 17.3 Sti1-dependent mutants in Hsp90 lie in two distinct regions on the Hsp90 crystal structure. (**a**) Wild type (*STI1*$^+$) and *sti1*Δ cells expressing the indicated point mutations in *HSP82* (an isoform of yeast Hsp90) were grown on media lacking FOA then replica-plated to media containing FOA (shown here). The absence of growth of the mutants in *sti1*Δ cells (right column) demonstrates the inability of the indicated mutation to support viability as the only source of Hsp90. All mutants supported viability in *STI1*$^+$ cells (left column). (**b**) The mutants from A indicated as spheres on various conformations of Hsp90. Blue are SdN mutants and red are SdC mutants (see text for more detail). (Adapted from Reidy et al. 2018. *Genetics* 2018. Used by permission of the authors)

Wickner's lab. The dependence on Sti1 conferred by mutations in this area, which we labeled "SdN" (Sti1-dependent N-terminal proximal) made sense, since they were deficient in binding Hsp70 directly and thus should need Sti1 to serve as a bridge between Hsp90 and Hsp70. Less clear, however, was the functional requirement provided by Sti1 in the case of the second cluster of Sti1 dependent mutations, such as S481Y, L487S and M589A in the *HSC82* isoform (Flom et al. 2007) and E507Q and W585T in the *HSP82* isoform (Genest et al. 2013) (the Hsp82 mutants' Sti1 dependence was at the time unpublished). This second cluster of Sti1-dependent mutations was located at the junction of the M- and C-terminal domains, in a region identified as important for client interaction (Genest et al. 2013; Zuehlke et al. 2017) and was termed "SdC" (Sti1-dependent C-terminal proximal).

In order to convince ourselves that point mutations in Hsp90 that were Sti1 dependent were confined to two distinct regions on the Hsp90 structure, we conducted an unbiased screen to find new Sti1 dependent mutations. A pool of a *LEU2*-marked plasmid containing randomly mutagenized *hsp82* alleles was introduced via transformation into a strain that was deleted for *STI1* and both chromosomal copies of yeast Hsp90. In this parent strain viability is maintained by a copy of *HSP82* on a *URA3*-marked plasmid. Leu^+ transformants expressing mutant *hsp82* alleles that function like wild type under optimal conditions allowed for loss of the parental *URA3* plasmid when grown non-selectively and were thus able to grow on media containing 5′-fluoro-orotic acid (FOA), which is toxic to cells expressing *URA3*. Alternately, mutations that inactivated Hsp90 function were not able to lose the parental *URA3* plasmid and were sensitive to FOA. The FOA sensitive colonies from the first transformation that became FOA resistant upon introduction of a *STI1*-expressing *TRP1* plasmid in a second round of transformations were scored as Sti1 dependent. The mutant *hsp82* allele on the *LEU2* plasmid was then recovered and sequenced. After screening ~10,000 initial Leu^+ transformants in this way, we identified 6 new Sti1 dependent Hsp90 mutations. Remarkably, three of them (E199K, Y344C and I388N) mapped to the SdN region, and the other three (S485T, M593T and G655D) mapped to the SdC region (Reidy et al. 2018), see Fig. 17.3. Two of the SdC mutants identified were different substitutions in the same residue identified by the Johnson lab, but in the other isoform of yeast Hsp90. In total 12 Sti1-dependent mutants were identified, six from each class, Fig. 17.3. Two from each class were chosen to study further. One of the two chosen from each class had a more severe phenotype in *STI1* cells than the other, displaying radicicol sensitivity and a sorbitol-suppressed high temperature growth defect. This latter phenotype likely is due to a cell wall defect downstream of Hsp90, regulated by Hsp90 client MAP kinases such as Slt2 (Millson et al. 2005; Piper et al. 2006).

Any combination of individual SdN and SdC mutations in the same allele resulted in loss of viability in *STI1* containing cells under optimal conditions, strong genetic evidence that the different mutations affected different parts of a vital pathway. As expected, purified versions of the SdN mutant proteins bound much less Hsp70 compared to wild type Hsp82. However, the SdC mutations had no effect on Hsp70 binding, suggesting these mutations rely on Sti1 for something other than mediating interaction with Hsp70. One of the advantages of the yeast system is the

ability to rapidly perform forward genetics such as second-site suppressor screens. In this type of analysis, the phenotype conferred by the mutation-of-interest is suppressed by additional mutations, either targeted or generated randomly, in the same allele. We used this approach combined with other yeast genetic techniques and biochemistry to help understand why the SdN and SdC mutations depended on Sti1.

In the first screen we identified a second site suppressor of the SdN mutation K399C's Sti1 dependency. The isolated suppressor, E402K, was located right next to K399C, and we found that restoring positive charge in this region was responsible for the suppressive effect. Because E402R worked slightly better, it was chosen for further study. The SdN suppressor E402R dramatically increased direct binding to Hsp70 alone and when combined with K399C restored direct Hsp70 interaction to wild type levels. This result was exactly what could be predicted based on the knowledge of Sti1/Hop functions in Hsp90-Hsp70 bridging. However, combining the Hsp70-binding-enhancing mutation E402R with either SdC mutation failed to relieve Sti1 dependence. Furthermore, when expressed in cells containing Sti1, E402R made the SdC radicicol sensitivity and high temperature growth defects worse. Thus, increasing Hsp70 interaction in the context of the client-binding defective SdC mutation was deleterious to proper functioning of Hsp90. These results were strong evidence that Hsp90 SdC mutants required a function of Sti1 that was not its ability to mediate interaction with Hsp70. What was this second function of Sti1? The answer to this question was also obtained using a second-site suppressor screen. Several suppressor mutations in residue A107 (P, G, and T) in the N-terminal domain, and A577V on the other end of the molecule in the SdC region, were identified through their ability to rescue SdC mutant growth defects in *STI1* cells. The mutation A107N was already known to promote N-terminal dimerization (Millson et al. 2010; Prodromou et al. 2000; Vaughan et al. 2009). As a result A107N increased the intrinsic ATPase rate compared to wild type. The Neckers lab reported that A107N suppressed the growth defects of W585T (a severe SdC mutant) in *STI1*-containing cells by promoting closure of the dimer to compensate for the weakened client interaction of W585T (Zuehlke et al. 2017). We found that A107N also rescued SdC mutant Sti1 dependency (Reidy et al. 2018), which was not addressed in Zuehlke et al. (2017).

The second SdC suppressor, A577V, was located in the SdC region itself. In addition to its ability to suppress SdC phenotypes in cells that have Sti1, A577V also suppressed Sti1 dependency of SdC mutants (Reidy et al. 2018). Remarkably, residue A577 had also been independently studied previously. The Buchner, Virkhiver and Colombo labs reported that residue A577 was part of a long-range signaling network within Hsp90 that relayed information about the client bound state to the N-terminal domain (Morra et al. 2009; Retzlaff et al. 2009). Both A107N or A577V alone increased intrinsic ATPase rate in vitro and bound more Sba1 in vivo, demonstrating that both of these SdC suppressor mutations promoted N-terminal dimer closure (Reidy et al. 2018). Thus, SdC mutants were defective in proper dimer closure and required Sti1 to fulfill this crucial step in Hsp90 regulation.

In direct agreement with the idea that the two Sti1-dependent regions of Hsp90 relied on Sti1 for two different functions, the SdC suppressors (A107N or A577V) were unable to relieve the Sti1-dependence of the SdN mutations or rescue the growth defects of the severe SdN mutation expressed in *STI1* cells. Finally, combining the SdC suppressor A107N with the SdN suppressor E402R rescued the temperature growth defect of *sti1*Δ cells, while either alone only partially rescued. In other words, the two forward mutations imparted into Hsp90 itself the two functions of Sti1. Taken together, these findings are very strong evidence that Sti1 performs two roles in regulating Hsp90: mediating Hsp90 interaction with Hsp70 and assisting in proper N-terminal dimerization, presumably after client transfer. Figure 17.4 is a proposed model showing the effects of loss of Sti1/Hop functions on Hsp90 client capture. These two functions probably arise from the same interaction between Sti1/Hop and Hsp90. Furthermore, this study provides important in vivo validation for a large number of biochemical, biophysical and computational studies, and supports the idea first advanced by the Agard group that Sti1/Hop must be taking a more active role in Hsp90 regulation than merely serving as a bridge (Southworth and Agard 2011).

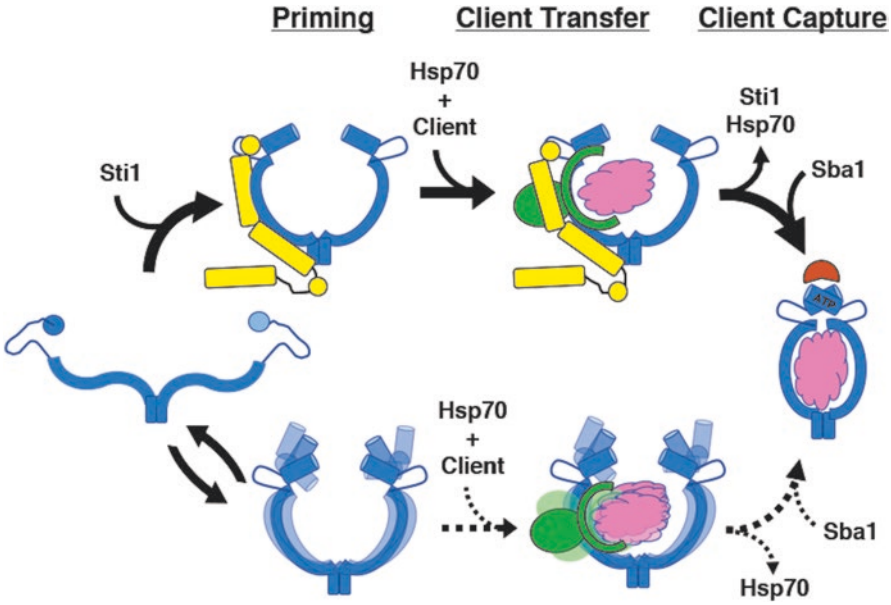

Fig. 17.4 A model for the dual roles of Hop/Sti1 in Hsp90 cycle regulation. Binding of Hop/Sti1 forces Hsp90 into the client-loading conformation, keeping Hsp90 open to accept the client from Hsp70 and then assisting Hsp90 N-terminal domain closure once the client has been successfully transferred

17.2 Conclusions

If, as described above, Sti1/Hop's role goes beyond bridging Hsp90 and Hsp70, several questions arise. First, if Sti1/Hop is so important for Hsp90 regulation, then why is it not essential? The answer to this partly lies in the fact that Hsp90 and Hsp70 can interact directly to provide enough function for cells to thrive under optimal conditions. However, *STI1* does become essential when cells are stressed. It is possible that the detrimental effects of loss of Sti1/Hop function are buffered under optimal conditions by the sheer abundance of Hsp90 in the cell. It has been shown that some point mutations in Hsp90 only give rise to a phenotype when their expression level is lowered (Jiang et al. 2013). It is reasonable to speculate that a similar phenomenon hides the full effect of loss of Sti1/Hop function. Evidence in support of this idea is the fact that *sti1*Δ cells are unable to grow when Hsp90 activity is reduced by normally sub-lethal concentrations of radicicol. Anecdotal evidence that *sti1*Δ yeast cells express even more Hsp90 than wild type cells (in which Hsp90 already comprises 1–2% of all cytosolic proteins) when grown under optimal conditions (our unpublished observations) perhaps supports the notion that loss of Sti1/Hop function is buffered by Hsp90 abundance. The fact that Sti1 functions could be bypassed by forward mutations in Hsp90 itself may also be interpreted in support of this idea (Reidy et al. 2018).

As mentioned above, residues in the SdC region were previously implicated in an interdomain signaling network (Morra et al. 2009; Retzlaff et al. 2009). Presumably this network sent information about the client state to the N-terminal ATPase domains. Mutations in this area that caused cells to depend on Sti1 were rescued by secondary mutations that increased the propensity of the N-terminal domains to dimerize. Thus this region of Hsp90 needs Sti1 to help it close. However, it remains unclear just exactly why SdC mutations need assistance in closing. It could be structural, in the sense that SdC mutations confer a mechanical impediment to N-terminal dimerization. The fact that the SdC region is located in the hinge between the M- and C-domains may support this argument. Alternatively, SdC mutant Hsp90 dimers may be unable to properly sense that the client has been delivered, and they need Sti1 to facilitate transfer of the information to the N-terminal domains. Of course, a third explanation is the combination of the first two: that a proper conformation is necessary for the signal to transmit, and SdC mutants have difficulty attaining this conformation without Sti1.

How is successful transfer of the client sensed? Does Sti1/Hop play an active role in the sensing mechanism? If so, what role to post-translational modifications play? It is interesting to wonder whether Sti1/Hop actively dissociates Hsp70 from Hsp90 after client transfer, and what happens to Sti1/Hop after the transfer is complete? The questions go on. The finding that Sti1/Hop plays a role in Hsp90 regulation beyond the bridge is an exciting development that hopefully will increase our understanding of this important cellular chaperone machine.

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