Essays in Biochemistry (2023) EBC20220224
https://doi.org/10.1042/EBC20220224

PORTLAND PRESS

Review Article

Saccharomyces cerevisiae as a tool for deciphering Hsp90 molecular chaperone function

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Yeast is a valuable model organism for their ease of genetic manipulation, rapid growth rate, and relative similarity to higher eukaryotes. Historically, *Saccharomyces cerevisiae* has played a major role in discovering the function of complex proteins and pathways that are important for human health and disease. Heat shock protein 90 (Hsp90) is a molecular chaperone responsible for the stabilization and activation of hundreds of integral members of the cellular signaling network. Much important structural and functional work, including many seminal discoveries in Hsp90 biology are the direct result of work carried out in *S. cerevisiae*. Here, we have provided a brief overview of the *S. cerevisiae* model system and described how this eukaryotic model organism has been successfully applied to the study of Hsp90 chaperone function.

Introduction

Yeast as a model system has long been a valuable research tool for characterizing cellular function. Specifically, the budding yeast *Saccharomyces cerevisiae* has been used to study many of the most important fundamental regulatory processes in eukaryotes, including gene expression, signal transduction, cell cycle regulation, metabolism, stress response and apoptosis, and has also been successfully used to model neurodegenerative diseases [1–4]. The utility of *S. cerevisiae* as a model lies primarily in the ease of genetic manipulation, and there are comprehensive libraries of deletion, overexpression, and GFP-tagged strains [5–9]. These tools have made it possible to perform high-throughput screens and drug discovery studies as well as thoroughly study the function of specific pathways and proteins.

Yeast and mammalian model systems however have inherent differences that impact protein function and pathway regulation. Tissue-specificity in mammals [3] and the presence of a cell wall in yeast [10,11] are clear examples, while entire classes of proteins have evolved to cope with the increased complexity found in multicellular life. For example, while yeast only have one endogenous tyrosine kinase, humans have 90, demonstrating additional elements of signaling and regulatory networks that cannot be found or effectively replicated in yeast [12–14]. Similarly, the relationship between orthologous yeast and human proteins must be carefully validated, as proteins with high sequence similarity may have unique structural or functional properties [15].

Despite these limitations, several thousand human genes possess identifiable orthologs in yeast, making *S. cerevisiae* a powerful tool for carrying out protein function studies. Molecular chaperones promote the maturation of their substrate proteins in eukaryotes; therefore, *S. cerevisiae* has been used to study the impact of molecular chaperones on substrate protein function in a highly orthologous system. Here, we will discuss the impact of *S. cerevisiae* on unraveling the function and regulation of the molecular chaperone heat shock protein 90 (Hsp90).

Received: 21 December 2022
Revised: 01 February 2023
Accepted: 03 February 2023

Version of Record published:
13 March 2023

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Essays in Biochemistry (2023) EBC20220224
https://doi.org/10.1042/EBC20220224

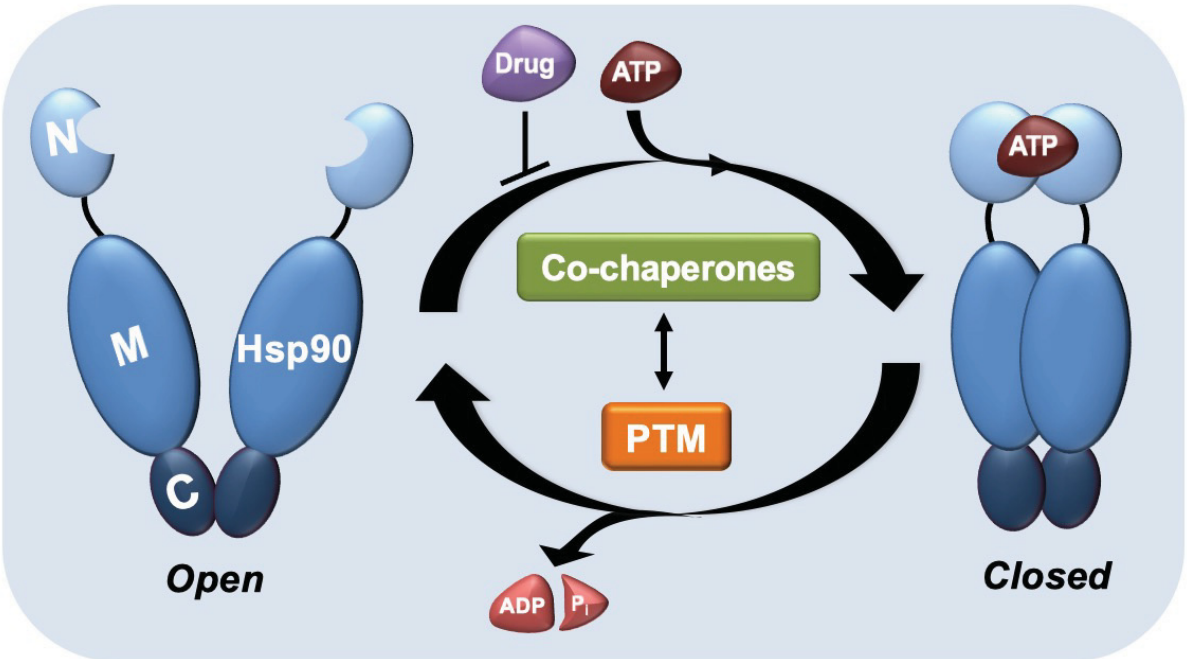

Figure 1. Hsp90 chaperone cycle

The Hsp90 molecular chaperone is composed of three functional domains: the N-terminal domain containing the ATP-binding site, the middle (M)-domain, which is the primary site of client interaction, and the C-terminal domain containing the tetratricopeptide repeat (TPR)-binding motif necessary for interaction with TPR-containing co-chaperones. Hsp90 undergoes a series of conformational changes coupled to the energy of ATP hydrolysis. The cycle of 'opening' and 'closing' promotes the maturation of Hsp90 substrate proteins, or clients. This process is tightly regulated by co-chaperones and post-translational modifications (PTM).

Characterizing Hsp90 chaperone function in yeast

Hsp90 is an essential molecular chaperone in eukaryotes. The functional unit of Hsp90 is a dimer and Hsp90 activity is driven by ATP. Hydrolysis of ATP induces a series of conformational changes that promote the folding, stability, and activation of dependent proteins, termed clients [16]. This 'chaperone cycle' is controlled by a diverse class of protein regulators termed 'co-chaperones' as well as post-translational modifications (PTM; Figure 1). As Hsp90 counts several hundred proteins as clients, describing Hsp90 chaperone function is crucial to a complete understanding of cellular signaling networks. The two mammalian cytosolic isoforms of Hsp90, Hsp90α and Hsp90β, share 62% and 63% similarity with the *S. cerevisiae* isoforms Hsp82 and Hsc82, respectively (Figure 2). While Hsp90α (Hsp82) and Hsp90β (Hsc82) isoforms have many overlapping functions and attributes, there are also key differences, many of which were characterized in yeast [17–26]. The essential functions of Hsp90 allow for rapid evaluation of the effect of Hsp90 mutations on chaperone activity. Mutations that abrogate Hsp90 function cause specific phenotypes, such as lethality and temperature sensitivity [27]. Further, many client-specific assays have been developed and characterized to assay Hsp90 function, such as firefly luciferase refolding [28] and β-galactosidase reporters for steroid hormone receptors [29–33] (Table 1).

Hsp90 structure and chaperone cycle

One aspect of chaperone biology that has been extensively studied using *S. cerevisiae* is the relationship between Hsp90 structure and its chaperone cycle. In fact, a detailed understanding of ATP binding to Hsp90 was first characterized using yeast [34], and the first structure of a full-length Hsp90 dimer was solved using Hsp82 in complex with the co-chaperone Sba1 (p23) [35]. Hsp82 is an ideal protein to perform biophysical analysis of the Hsp90 structure and chaperone cycle as Hsp82 does not contain any cysteine residues, allowing easy incorporation of fluorescent protein pairs. Several groups have taken advantage of Hsp82 to understand the *in vitro* dynamics of nucleotide exchange and impact of Hsp90 inhibitors [36–43], function of the charged linker region that connects the middle and

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(A)

Sequence Identity (%)	Hsp82	Hsc82	Hsp90α	Hsp90β
Hsp82		97.73	62.23	62.98
Hsc82	97.73		62.16	63.02
Hsp90α	62.23	62.16		86.74
Hsp90β	62.98	63.02	86.74	

(B)

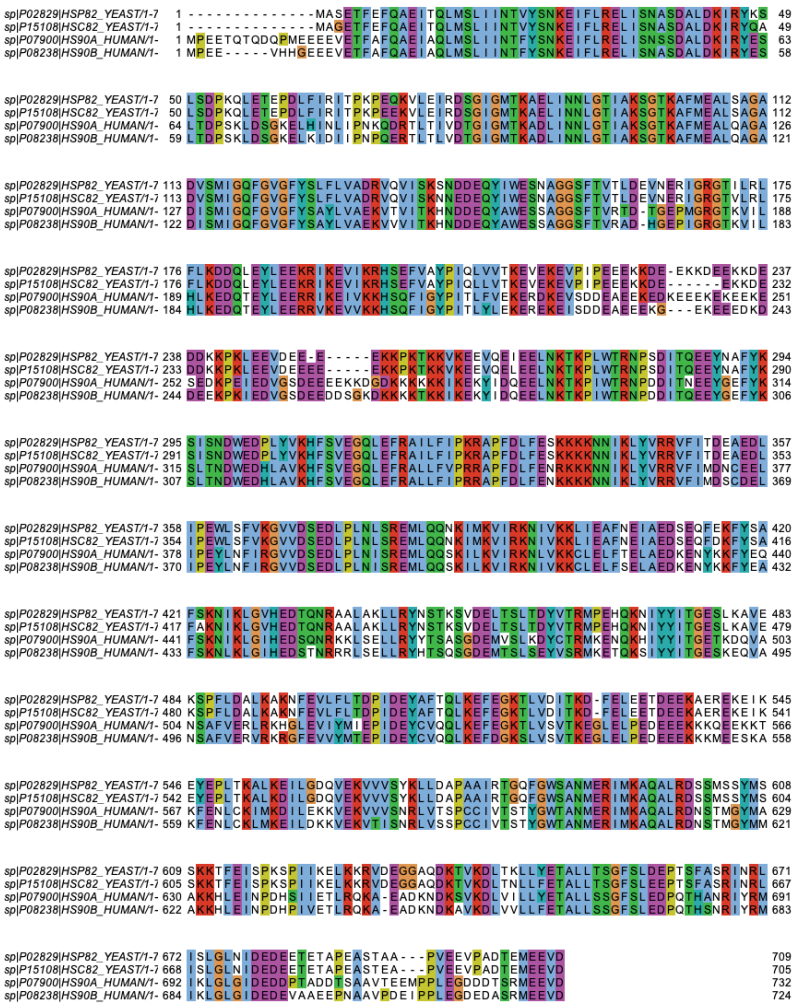

Figure 2. Sequence alignment of yeast and human Hsp90s

(**A**) Identity matrix of Hsp90 isoforms from yeast and human. *S. cerevisiae* Hsp82 (P02829) and Hsc82 (P15108) amino acid sequences and human Hsp90α (P07900) and Hsp90β (P08238) amino acid sequences were aligned using EMBL-EBI Clustal Omega tool. Percent identity between genes was determined by Clustal Omega. (**B**) Alignment of amino acid sequences of yeast and human Hsp90 isoforms. Jalview v2.11.2.5 was used to visualize the amino acid sequence alignment from Clustal Omega. Colors indicate conserved amino acid properties between genes: blue: hydrophobic (A,L,I,M,F,W,V,C); red: positive charge (K,R); magenta: negative charge (E, D); green: polar (N, Q, S, T); orange: glycine (G); yellow: proline (P); teal: aromatic (H, Y); white: unconserved.

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Essays in Biochemistry (2023) EBC20220224
https://doi.org/10.1042/EBC20220224

Table 1 Common assays for determination of Hsp90 chaperone activity

Yeast protein	Human protein	Assay	Function	Reference
Ste11	**Raf-1**	Western blot	Kinase activity	[118]
Slt2	**MAPK7**	Rlm1-*lacZ*	Kinase activity	[119]
v-Src	**c-Src**	Western blot	Kinase activity	[120]
N/A	**GR**	GRE-β-Galactosidase	Transcription	[121]
N/A	**ER**	ERE-β-Galactosidase	Transcription	[28]
N/A	**AR**	ARE-β-Galactosidase	Transcription	[79]
Hsf1	**Hsf1**	HSE-*lacZ*	Transcription	[23]
Firefly luciferase	**N/A**	Luminescence	Protein folding	[122]
N/A	**CFTR**	Western blot	Protein folding	[123]

N-terminal domains [44–50], interaction with co-chaperones and clients [39,42,43,51–53], and impact of mutations chaperone function [52,54].

Hsp90 dimerization and conformational rearrangement is essential for its ATP hydrolysis activity and therefore client maturation [55]. Truncation of the C-terminal domain disrupts Hsp90 dimerization and yeast solely expressing this truncation are not viable [56]. Similarly, the requirement for Hsp90 ATP hydrolysis in its chaperone activity was determined in yeast [57,58], and further confirmed using the ATP binding-deficient mutant E33A, which failed to support yeast viability [59,60]. Notably, Reidy and Masison found that human Hsp90α does not complement yeast growth as robustly as Hsp90β [61]. They further showed that Hsp90α primarily adopts a closed position, which can be reversed by a single point mutation in the N-domain (A166V). Hsp90α-A166V shifts to a more open position, similar to Hsp82, and restores yeast to a normal growth pattern [61]. This is consistent with work by Buchner's group demonstrating that Hsp90 dwell time in different conformational states was more important for supporting cell survival than the rate of ATP hydrolysis in Hsp90 mutants [42,59,62,63]. Interestingly, another study found that inhibition of Hsp82 N-domain rotation by deletion of the charged linker had a strong negative impact on Hsp90 ATPase activity yet had minimal effect on yeast viability [50]. Introducing mutations to Hsp90 can alter interaction with co-chaperone proteins, which could explain the conflicting results between these studies. Indeed, one study found that mutations of Hsp82 that impact interactions with co-chaperones subsequently alters Hsp90 conformational dwell time causing differing impacts on yeast viability and client maturation [64].

Hsp90 inhibitors

Hsp90 is often over-expressed or dysregulated in human cancers [65], prompting decades of research into developing therapeutic Hsp90 inhibitors [66]. *S. cerevisiae* has been a fundamental tool for evaluation of changes in Hsp90 inhibitor sensitivity in response to different cellular conditions [20,21]. Interestingly, expression of human Hsp90α or Hsp90β isoforms in yeast results in different sensitivities to the ATP-competitive inhibitor radicicol [20].

A drawback of many Hsp90 inhibitors in the induction of a heat-shock response which leads to increased production of Hsp90 and other chaperone proteins by the transcription factor Heat shock factor 1 (Hsf1). In an effort to combat this side-effect, Millson and Piper investigated whether rapamycin could inhibit Hsf1 in yeast to prevent the heat-shock response secondary to Hsp90 inhibitors [64,67]. While the authors found little synergy between rapamycin and the Hsp90 inhibitor radicicol, they did notice that presence or absence of co-chaperones, specifically Ppt1 (PP5) and Fbr1 (FKBP12) greatly affected yeast sensitivity to radicicol and rapamycin. This is consistent with studies done in mammalian cells showing that depletion or overexpression of Hsp90 co-chaperones can modulate the sensitivity of cells to Hsp90 inhibitors through changes in Hsp90 inhibitor affinity or drug uptake [15,21,68–75]. This important topic has recently been reviewed in more detail [76].

The role of Hsp90 in nuclear processes

Hsp90 interacts with ∼20% of the yeast proteome [77,78] and is therefore involved in regulating numerous cellular processes. The role of Hsp90 in regulating these processes has often been studied using *S. cerevisiae*. While Hsp90 is traditionally considered a cytosolic protein, increasing reports of Hsp90 governing nuclear processes suggest there is more to be uncovered about Hsp90 nuclear function. Hsp90 has long been known to regulate transcription through chaperoning of steroid hormone receptors (SHR) [27,28,32,79]. *S. cerevisiae* is well-suited to understanding the specifics of SHR regulation as SHR stability, ligand binding, and transcriptional activity can be easily evaluated in

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Table 2 Hsp90 co-chaperone proteins with functional characterization in yeast

Yeast Gene	Human gene	References
AHA1	**AHSA1**	[44,51,72,73,102,103,107,112,124–129]
Hch1	**N/A**	[73,102,103,113,125–128,130]
CDC37	**p50/CDC37**	[22,102,103,107,124,131–134]
Sba1	**p23/PTGES3**	[102,103,124,135–137]
Sti1	**HOP**	[102,103,124,138]
N/A	**FKBP4, FKBP5**	[139–141]
Cpr6 & Cpr7	**PPID**	[45,102,103,135,138,142–145]
Ppt1	**PP5**	[102,103,115,117,146,147]
Cns1	**TTC4**	[102,103,142,148]
She4	**UNC45A/B**	[149–151]
Tah1, Pih1 (R2TP complex)	**RPAP3, PIH1D1 (R2TP complex)**	[102,103,152,153]
SGT2	**SGTA**	[154,155]
SGT1	**SUGT1**	[102,111,156–159]
N/A	**FNIP1, FNIP2**	[15,69,100]
N/A	**Tsc1**	[70,100]
IDS2	**N/A**	[108,160]

yeast [49]. Hsp90 also indirectly controls transcription through chaperoning of Hsf1 and ERK5 [23,67,81–84]. Recently, Hsp90 was found to regulate activation of some genes by chaperoning nuclear myosin [85]. Other nuclear processes in which Hsp90 has been implicated include chromosome segregation [86], DNA repair [46,87,88], chromatin organization [85,89] and assembly of transcriptional complexes [90].

S. cerevisiae as a tool to study the Hsp90 chaperone code

The chaperone code is broadly defined as the collection of co-chaperones and post-translational modifications that provide multi-level regulation of Hsp90 chaperone function. *S. cerevisiae* has been used to study the impact of co-chaperones and PTM on Hsp90 function using client reporter assays, temperature sensitivity and drug sensitivity [14,15,69,91–100]. While many co-chaperones and PTM are conserved between yeast and man, *S. cerevisiae* is of particular value to study the effect co-chaperones that exist only in higher eukaryotes. Employing well-characterized techniques, co-chaperones that do not exist in yeast can be genetically introduced, allowing interpretation of results without any confounding effect from endogenous levels of the protein.

S. cerevisiae as a tool to study co-chaperones

At least 30 co-chaperones of mammalian Hsp90 have been identified to date, many of which are conserved between yeast and human [101]. Twenty of these co-chaperones have been identified and studied initially in *S. cerevisiae*, with a comprehensive comparison of co-chaperone deletion in yeast done by Buchner's group [102,103] (Table 2). Here, we will focus on discussion of using yeast to study non-native co-chaperones such as, FNIP1, FNIP2 and Tsc1. FNIP1/2 and Tsc1 are examples of co-chaperones that exist only in higher eukaryotes, yet have been functionally characterized in yeast [15,70]. FNIP1 and FNIP2 were known to stabilize the tumor suppressor folliculin (FLCN). FLCN was identified as an Hsp90 client, leading to the discovery that the FNIPs are Hsp90 co-chaperones. Interestingly, when expressed in yeast, FNIP1 and FNIP2 are lethal in cells expressing human Hsp90α, but not in cells expressing Hsp82, perhaps due to the inhibitory effect of FNIPs on Hsp90 ATPase activity [15]. Interestingly, Lst4 was thought to be the yeast ortholog of the FNIPs; however, Lst4 did not interact with yeast Hsp90 or inhibit its ATPase activity, affirming that Lst4 is not a true ortholog of FNIP1/2 [15].

Similar to the FNIPs, Tuberous sclerosis complex protein 1 (Tsc1) is an Hsp90 co-chaperone which does not have an ortholog in *S. cerevisiae* [70]. In fact, there are many parallels between FNIPs and Tsc1 co-chaperone functions. Both FNIPs and Tsc1 were identified as co-chaperones because of their role in stabilizing the tumor suppressors FLCN and Tsc2, respectively; however, they also maintain the activity of other Hsp90 clients including kinases such as Src and Raf, and non-kinases such as GR [15,70]. Additionally, FNIPs and Tsc1 proteins were known to exist as homodimers in cells [70,104–106], and FNIP1:FNIP2 heterodimers were shown to interact comparably with Hsp90 compared with FNIP1 and FNIP2 homodimers [15]. In light of their striking similarities, a recent study sought to evaluate the interplay between the FNIPs and Tsc1 co-chaperones. Notably, Tsc1 was found to interact with FNIP1

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but not FNIP2 [100]. To dissect the impact of FNIPs and Tsc1 hetero-co-chaperone complexes on Hsp90 chaperone function, *S. cerevisiae* was used to express each co-chaperone alone or in pairs [100]. When expressed in yeast in different combinations, FNIP1, FNIP2 and Tsc1 differentially activated Hsp90 clients such as v-Src and steroid hormone receptors. Notably, the presence of these co-chaperones led to an increase in GR and ER affinity for their ligands when expressed in yeast, with co-expression of different pairs of co-chaperones leading to the highest increase in affinity. Together, these results suggest that in mammalian cells subpopulations of Hsp90 in complex with different co-chaperones prime SHRs to respond to varying ligand levels.

Post-translational modification of co-chaperones can also be studied in *S. cerevisiae*. PTM of Hsp90 co-chaperones such as Cdc37 [107], FNIP1 [69], Ids2 [108], and Sgt1 [109–111] has recently been reviewed in great detail [76]. Sager et al. identified a series of serine residues of FNIP1 which are phosphorylated by CK2 in a sequential manner. The impact of relay phosphorylation of FNIP1 on its co-chaperone function was partially studied in yeast expressing human Hsp90α using client reporter assays. Expression of FNIP1 phospho-null mutant in yeast abrogated the activity of clients such as v-Src, Ste11, Slt2, Hsf1 and GR. By increasing the number of serine residues available for phosphorylation, client activity could be gradually restored, with maximal activity in the presence of FNIP1-WT. This study highlights the utility of *S. cerevisiae* client activity assays for evaluating co-chaperone function; however, such assays can also be used to investigate the impact of PTM on Hsp90 chaperone function itself, and will be discussed in the next section.

S. cerevisiae as a tool to study Hsp90 post-translational modifications

Post-translational regulation of Hsp90 is critical to direct its chaperone function. PTM are often studied by introducing point mutations to mimic the modification, or substitution to an amino acid that cannot be modified. As discussed above, *S. cerevisiae* can easily be genetically manipulated to study point mutations of Hsp90, making it an ideal system to study Hsp90 PTM. Due to the high sequence similarity between Hsp82 and human Hsp90α, *S. cerevisiae* also allows examination of the conservation of PTM in eukaryotes. While many components of the Hsp90 chaperone system are conserved between yeast and man, the co-chaperone Hch1 exists only in yeast. Hch1 functions similarly to Aha1 in that it stimulates Hsp90 ATPase activity [112]. Importantly, Zuehkle et al. found that Hch1 is functionally replaced in humans by phosphorylation of Hsp90α-Y627 [113]. Specifically, the authors found that phosphorylation of this residue contributed to Hsp90 interdomain communication and resulted in impaired N-domain dimerization, similar to the observed impact of Hch1 [113]. In contrast with this important finding, many PTM are conserved from yeast to humans, several of which have been characterized in detail and will be discussed below, as well as summarized in Table 3.

Phosphorylation is the most well-characterized Hsp90 PTM in terms of its impact on Hsp90 ATPase activity, co-chaperone and client binding, and regulation of cellular processes [114]. Phosphomimetic mutation of serine residues in the Hsp82 M- and C- domains have dramatic impacts on Hsp82 structure and function [115]. Specifically, residues that were identified as phosphorylated in yeast had varying effects on Hsp82 conformation, ATPase activity, client chaperoning, and ability to support viability in yeast. Notably, the phosphatase co-chaperone Ppt1 was found to dephosphorylate Hsp82-S485 and Hsp82-S604 in cells; however, the kinase(s) responsible for phosphorylating the reported residues have yet to be identified [115]. Phosphorylation of Hsp82-T22 (Hsp90α-T36) by the conserved and constitutively active CK2 disrupted Hsp90 interaction with co-chaperones Aha1 and Cdc37, and client proteins, both in yeast and mammalian cells [92]. Notably, phosphomimetic Hsp82-T22E had a dramatically reduced ATP hydrolysis rate [92] and further work by this group showed that expressing Hsp82-T22E as the sole copy of Hsp90 hypersensitized yeast to Hsp90 inhibitors GA, RD, SNX2112 and STA-9090 [91]. Similarly, phosphorylation of another N-domain residue, Hsp82-T101 (Hsp90α-T115) by Mps1 greatly reduced Hsp90 ATPase activity, and overexpression of Mps1 or expression of phosphomimetic Hsp82-T101E provided increased sensitivity to Hsp90 inhibitors [116]. Using yeast expressing the non-phosphorylatable Hsp82-T101A or the phosphomimetic T101E, the authors found that this phosphorylation broadly affected the Hsp82 interactome and identified cell cycle components as key Hsp82 interactors. In fact, they found that Mps1-mediated phosphorylation of T101 fluctuated throughout the cell cycle and was important for mitotic arrest [116]. Interestingly, T101 is not the only PTM that is linked to the cell cycle. Phosphorylation of Hsp82-Y24 (Hsp90α-Y38) by the only tyrosine kinase in *S. cerevisiae*, Swe1 (Wee1) occurs primarily during the S-phase of the cell cycle [14]. Interestingly, Hsp82-Y24 phosphorylation led to ubiquitination and degradation of Hsp82, perhaps explaining why the phosphomimetic Hsp82-Y24E was unable to support yeast growth when expressed as the sole copy of Hsp90 [14].

S. cerevisiae has also been used to study Hsp90 function in response to differing nutrient conditions. Chen et al. reported Ids2 as a co-chaperone of Hsc82 which regulates Hsc82 ATPase activity and chaperone function [108]. They

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Table 3 Amino acid residues with site-specific functional information in yeast

Hsp82	Hsp90α	Reference
N/A	**T7A**	[161]
T22	**T36**	[27,92]
Y24	**Y38**	[14]
S25	**S39**	[126]
E33	**E47**	[162]
A41	**A55**	[27]
R46	**R60**	[64]
D79	**D93**	[162]
G81	**G95**	[27]
A97	**A111**	[163]
T101	**T115**	[27]
K102	**K116**	[64]
A107	**A121**	[164]
A131	**A145**	[61]
A152	**A166**	[61]
G170	**G183**	[27]
K178	**K191**	[97]
E199	**Q212**	[165]
P281	**P301**	[166]
W300	**W320**	[19]
G313	**G333**	[27]
F329	**F349**	[167]
D330	**D350**	[19]
F332	**F352**	[19]
Y344	**Y364**	[165]
R346	**R366**	[59]
F349	**F369**	[19]
N377	**N397**	[19]
S379	**S399**	[115]
R380	**R400**	[19]
E381	**E401**	[27]
Q384	**Q404**	[19]
S485	**S505**	[115]
I388	**I408**	[165]
V391	**V411**	[165]
K394	**K414**	[166]
K399	**K419**	[166]
G428	**G448**	[64]
E431	**E451**	[168]
S485	**S505**	[163]
L491	**L511**	[167]
E507	**E527**	[169]
Y508	**Y528**	[169]
T511	**Q531**	[169]
T521	**T541**	[163]
Q582	**T603**	[170]
F583	**Y604**	[170]
G584	**G605**	[170]
W585	**W606**	[169]
S586	**T607**	[171]
A587	**A608**	[27]
N588	**N609**	[170]
M589	**M610**	[169]
E590	**E611**	[170]
I592	**I613**	[167]
M593	**M614**	[167]

Continued over

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Essays in Biochemistry (2023) EBC20220224
https://doi.org/10.1042/EBC20220224

Table 3 Amino acid residues with site-specific functional information in yeast (Continued)

Hsp82	Hsp90α	Reference
S602	**N622**	[115]
S604	**S623**	[115]
Y606	**Y627**	[113]
L651	**L671**	[167]
L652	**L672**	[167]
G655	**G675**	[165]
F664	**H684**	[167]

further showed that glucose intake led to phosphorylation of Ids2, which disrupted interaction with Hsc82 and its chaperone function. The authors posit that under caloric restriction Ids2 is not phosphorylated, enhancing its interaction with Hsc82 and promoting its chaperone activity to augment cell survival [108]. Congruently, phosphorylation of Hsp82-S485 is important for Hsp82 nuclear translocation during starvation. Preventing phosphorylation by expressing Hsp82-S485A in yeast led to decreased survival during nutrient deprivation, highlighting the importance of Hsp82 phosphorylation as a cellular level event [117].

In addition to phosphorylation, *S. cerevisiae* has been used to study Hsp90 SUMOylation and acetylation. SUMOylation of Hsp82-K178 (Hsp90α-K191) was the first modification to be reported as an asymmetric PTM (occurring on only one protomer of an Hsp90 dimer) [97]. Comparable to phosphorylation, SUMOylation impacted yeast and human Hsp90 binding to co-chaperones, and specifically SUMOylation recruited Aha1 and enhanced Hsp90 binding to its inhibitors [97]. Taken together, these studies emphasize the diverse applications of *S. cerevisiae* for studying PTM of Hsp90.

Conclusions and future of *S. cerevisiae* research

For more than 30 years, the budding yeast *S. cerevisiae* has served as an ideal model system to study Hsp90 molecular chaperone structure and function. Many facets of Hsp90 regulation, including the impact of co-chaperones and PTMs, have been easily and thoroughly characterized, providing concepts that can be directly translated to higher eukaryotes for further study.

Moving forward, several outstanding questions in Hsp90 research can explored using *S. cerevisiae*: (1) How can Hsp90 inhibitor treatment strategies be improved for successful clinical application? Identification of biomarkers and validation of secondary targets for inhibition in combination with Hsp90 are necessary for Hsp90 inhibitor development. Here, we have reviewed the use of yeast in the characterization of Hsp90 regulation and downstream impact on Hsp90-dependent signaling networks. Going forward, yeast will be instrumental to the continued development Hsp90 inhibitors, and as previous work has shown, *S. cerevisiae* is an efficient and translatable model organism that can be used for much of the foundational work. (2) Can the complex Hsp90 regulatory network be utilized to further our understanding of Hsp90 function in cancer? Differential PTM state and co-chaperone functionality in cancer modulate Hsp90 activity, substrate interactions, and druggability. Assessing the impact of these differential interactions and providing a cellular context for the effect on Hsp90 in a well-characterized model organism such as yeast is essential to a holistic understanding of Hsp90 regulation. *S. cerevisiae* will continue to provide fundamental information as we move toward a complete description of the functional implications of the chaperone code.

Summary

- Hsp90 is an essential molecular chaperone that functions as a hub of cellular signaling networks.
- The budding yeast *Saccharomyces cerevisiae* has been used to thoroughly characterize Hsp90 structure, function and regulation.
- Further work in both yeast and human cells is required to fully articulate the complex regulatory architecture known as the ‘chaperone code’.

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was partly supported by Upstate Foundation (to M.M.) and Carol M. Baldwin Breast Cancer Fund (to M.M.). This work was also supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number (R35GM139584) (to M.M.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Abbreviations

FLCN, folliculin; Hsf1, Heat shock factor 1; Hsp90, Heat shock protein 90; PTM, post-translational modifications; SHR, steroid hormone receptors; TPR, tetratricopeptide repeat; Tsc1, Tuberous sclerosis complex protein 1.

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