Evidence for interaction between Hsp90 and the ER membrane complex

Tambudzai Kudze¹ · Carlos Mendez-Dorantes¹ · Chernoh Sallieu Jalloh¹ · Amie J. McClellan¹

Received: 16 March 2018 / Revised: 24 April 2018 / Accepted: 27 May 2018 / Published online: 28 May 2018
© Cell Stress Society International 2018

Abstract

Numerous putative heat shock protein 90 (Hsp90)-interacting proteins, which could represent novel folding clients or co-chaperones, have been identified in recent years. Two separate high-throughput screens in yeast uncovered genetic effects between Hsp90 and components of the ER membrane complex (EMC), which is required for tolerance to unfolded protein response stress in yeast. Herein, we provide the first experimental evidence supporting that there is a genuine interaction of Hsp90 with the EMC. We demonstrate genetic interactions between *EMC2* and the known Hsp90 co-chaperone encoded by *STH1*, as well as Hsp90 point mutant allele-specific differences in inherent growth and Hsp90 inhibitor tolerance in the absence and presence of *EMC2*. In co-precipitation experiments, Hsp90 interacts with Emc2p, whether or not Emc2p contains amino acid sequences designated as a tetratricopeptide repeat motif. Yeast with multiple EMC gene deletions exhibit increased sensitivity to Hsp90 inhibitor as well as defective folding of the well-established Hsp90 folding client, the glucocorticoid receptor. Altogether, our evidence of physical, genetic, and functional interaction of Hsp90 with the EMC, as well as bioinformatic analysis of shared interactors, supports that there is a legitimate interaction between them in vivo.

Keywords Heat shock protein 90 · ER membrane complex · Hsp90 · EMC · Endoplasmic reticulum · NVP-AUY922

Introduction

The heat shock protein 90 (Hsp90) molecular chaperone is a ubiquitous, abundant, and essential component of eukaryotic cells (Schopf et al. 2017). Hsp90, in collaboration with a host of co-chaperones, plays important roles not only in the “housekeeping” functions of cells under normal conditions, but it is also further upregulated under conditions of cellular stress affecting the stability of its folding “clients.” First noted for its participation in the folding and activation of a small number of client proteins, namely steroid hormone receptors and kinases, the folding and functional repertoire of Hsp90 has vastly expanded in the past decade and a half. This is due in large part to high-throughput, in some cases genome-wide, approaches in *Saccharomyces cerevisiae* aimed at

further defining its scope of cellular functions and interactors (Gong et al. 2009; Gopinath et al. 2014; McClellan et al. 2007; Millson et al. 2005; Zhao et al. 2005). An in-depth understanding of Hsp90’s cellular activities is increasingly important in light of the fact that Hsp90, by virtue of enabling the activity of oncogenic versions of its folding clients, is being investigated as a tractable target for therapeutic intervention in cancers (Chatterjee and Burns 2017; Jhaveri et al. 2012). Hsp90 and its co-chaperones are also culpable in the replication of viral proteins critical for infectivity and are meaningfully implicated in neurogenerative disorders, particularly tauopathies (Dickey et al. 2007; Geller et al. 2012; Geller et al. 2007; Luo et al. 2007; Pratt and Toft 2003; Shelton et al. 2017; Wang et al. 2017).

At present, from the results of both targeted experimental and high-throughput approaches, there are 1789 unique *S. cerevisiae* Hsp90 interactors curated in the online database BioGRID (Stark et al. 2006), which has served as one public resource used toward building a complete picture of Hsp90 interactions in the cell (Echeverria et al. 2011). These interactors may represent not only folding clients but also co-chaperone proteins that regulate and define the mechanism and client interactions of Hsp90 (Cox and Johnson 2018; Eckl and Richter 2013). While Hsp90 co-chaperones interact with

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12192-018-0908-z>) contains supplementary material, which is available to authorized users.

✉ Amie J. McClellan
amcclellan@bennington.edu

¹ Division of Science and Mathematics, Bennington College, Bennington, VT, USA

different regions of Hsp90 utilizing various types of protein motifs, one of the most common interaction domains shared by Hsp90 co-chaperones is comprised of the tetratricopeptide repeat (TPR) motif; co-chaperones that interact via their TPR domain bind to highly conserved residues at the extreme C-terminus of Hsp90 (MEEVD) (Scheufler et al. 2000).

Two different genome-wide screens in yeast identified the open reading frame YJR088C as a potential Hsp90 interactor (McClellan et al. 2007; Zhao et al. 2005). This is of interest as YJR088C is predicted to contain TPRs, suggesting that it could be a novel Hsp90 co-chaperone. Subsequently, this ORF was named *EMC2* when it was identified as a member of the evolutionarily conserved ER membrane complex (EMC) whose components contribute to the successful tolerance of ER stress, namely the unfolded protein response (UPR) (Jonikas et al. 2009). While the essential cellular roles of the EMC have remained somewhat elusive, in yeast, it has been linked not only to ER stress, but it has also been predicted to act as a tethering complex that facilitates the transfer of ER-synthesized phospholipids to mitochondria (Lahiri et al. 2014). In mammalian cells, EMC components have been connected to autophagy (Li et al. 2013; Shen et al. 2016), shown to protect against West Nile virus-induced cell death (Ma et al. 2015), and most recently, demonstrated to facilitate the insertion of newly synthesized transmembrane domain-bearing proteins into the ER membrane (Guna et al. 2018). The latter is in keeping with the EMC's occurrence in the genetic network that attends to a misfolded ABC transporter in yeast and from work in *Caenorhabditis elegans*, in which the EMC assists the synthesis of acetylcholine receptors (Louie et al. 2012; Richard et al. 2013).

Herein, we investigate whether the occurrence of *EMC2* as a putative interaction partner of Hsp90 is borne out by experimental validation. In addition, as two other members of the EMC, *EMC5* and *EMC6*, were also enriched in Hsp90 target screens (McClellan et al. 2007), we extend our analysis to the larger EMC. Our findings demonstrate genetic, physical, and functional interactions between Hsp90 and the EMC, suggesting that there is indeed a bona fide relationship between them in cells.

Materials and methods

Yeast media, manipulations, strains, and plasmids

Standard yeast media recipes for rich growth medium (YPD), synthetic complete (SC), and synthetic complete dropout media (e.g., SC-Ura) were followed (Adams 1997). Standard lithium acetate transformation was used to introduce plasmids into yeast (Adams 1997).

Strains herein referred to as WT90, G170D, E381K, T101I, and G313S were derived from a W303 background and have the following genotype: *MATa ade2-1 leu2-3,112 his3-11,15*

trp1-1 ura3-1 can1-100 hsc82::LEU2 hsp82::LEU2 HIS3::GPD-HSP82 (WT90) or *hsp82^{G170D}, hsp82^{E381K}, hsp82^{T101I}, and hsp82^{G313S}* (Nathan and Lindquist 1995). All other strains are BY4743 diploid (*MATa/α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*) or BY4741 haploid (*MATa his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*) strains from the yeast deletion collection, with singly deleted genes replaced by the *KANMX4* cassette (Giaever 2002). The multiple *EMC* deletion strains (Lahiri et al. 2014) are in BY4741 with the following specific modifications: YSL4 (*emc1::HIS5 emc5::kanMX4*), YSL5 (*emc3::HIS5 emc6::kanMX4*), YSL6 (*emc1::HIS5 emc2::hygMX4 emc5::kanMX4*), YSL9 (*emc1::HIS5 emc2::hygMX4 emc3::HIS5 emc5::kanMX4 emc6::kanMX4*), YSL27 (*emc1::HIS5 emc2::hygMX4 emc3::HIS5 emc6::kanMX4*), and YSL57 (*emc2::hygMX4 emc5::kanMX4 emc6::kanMX4*) and were kindly provided by William Prinz (Cell and Molecular Biology, NIDDK, NIH).

To generate the $\Delta emc2\Delta sti1$ double-deletion strain, the following primer pair was used to amplify the *HIS3* gene and append the *STI1* sequences (lowercase) to both ends:

```
5' attcctcactgtagctactaaaacaacctatacgaagaagATGA
CAGAGCAGAAAGCCCTAG 3'
5' gtaaaaaagaattcaagataataaagtattatctgattattCTAC
ATAAGAACACCTTTGGTGG 3'
```

The purified PCR fragment was introduced to BY4741 $\Delta emc2$ yeast using standard lithium acetate transformation and recovery in YPD prior to being plated on selective media (SC-HIS) and incubated at 30 °C for several days. Resulting colonies were screened further to confirm successful homologous recombination via PCR using primers internal to the *STI1* gene and by immunoblotting protein extracts with anti-Sti1p antisera.

To delete *EMC2* in WT and mutant Hsp90 strains, the following primer pair was used to amplify the kanamycin resistance cassette from pFA6a-3xHA-kanMX6 (Longtine et al. 1998) and append the *EMC2* sequences (lowercase) to both ends:

```
5' ttgacaattatgaataccaaagCGTACGCTGCAGGTCGAC
3'
5' gcggtaatcttattcttattgtagacttcgcATCGATGAATTCGA
GCTCG 3'
```

The purified PCR fragment was introduced to yeast using standard lithium acetate transformation and recovery in YPD prior to being plated on selective media (YPD + G418) and incubated at 30 °C for several days. Resulting colonies were screened via PCR using primers internal to *EMC2* to confirm successful deletion.

Molecular cloning

The following primer pair was used to amplify the *EMC2* gene and append a *Bam*HI site (underlined) and FLAG tag (MDYKDDDDK; coding sequence in bold) to the 5' end and an *Xho*I site (underlined) to the 3' end:

5' GAGGATCCATGGATTACAAGGATGACGA
CGATAAGATCTTGAAGGATTTGGTTAGAG 3'
5' TGCTCGAGTCATATCTTATTCAAATTAATTC 3'

All subsequent cloning manipulations of *EMC2* utilized *Bam*HI and *Xho*I restriction sites in target vectors.

The following primers were used, in conjunction with the above primers, to amplify *EMC2* sequences preceding and following the nucleotides encoding amino acids 163–196 (first predicted TPR repeat motif):

5' GCGAATTCATCAAGCGGGAATTT 3' (used with
*Bam*HI primer above to amplify pre-TPR fragment;
*Eco*RI site underlined)
5' TTAGAATTCTACGCTTGTGTTTGGTAGG 3' (used
with *Xho*I primer above to amplify post-TPR fragment;
*Eco*RI site underlined)

The resulting PCR products were purified, digested with *Eco*RI and either *Bam*HI (pre-TPR fragment) or *Xho*I (post-TPR fragment), and ligated in the presence of a cloning vector cleaved with *Bam*HI and *Xho*I. The resulting *emc2Δtpr* sequence encodes a protein lacking endogenous amino acids 163–196, but with two introduced amino acids, a glutamic acid and a phenylalanine, between the pre- and post-TPR fragments (as a consequence of the *Eco*RI sequence in the primers). All subsequent cloning manipulations of *emc2Δtpr* utilized *Bam*HI and *Xho*I restriction sites in target vectors.

Serial dilution growth assays

Yeast strains were inoculated into 2 mL of appropriate media and grown overnight in a 30 °C shaking incubator. The next day, the optical density at 600 nm (OD₆₀₀) of each strain was determined with a Genesys™ 20 visible spectrophotometer (Thermo Scientific). Each strain was then diluted to an OD₆₀₀ of 0.01, from which subsequent tenfold dilutions were generated (0.001, 0.0001, and 0.0001). Five microliters of each dilution was spotted onto solid media. Plates were incubated at 30 or 37 °C, as indicated, for 2–4 days.

Liquid growth curve assays

Yeast strains were inoculated into 2 mL of appropriate media and grown overnight in a 30 °C shaking incubator. The next

day, cultures were diluted to similar starting OD₆₀₀ values (~0.1) and loaded, in triplicate for each condition (no drug, 50 μM NVP-AUY922, or 150 μM NVP-AUY922) into a 96-well plate. The plate was incubated at either 30 or 37 °C, as indicated in figure legends, with shaking, in a Tecan Infinite F200 plate reader (Tecan Group, Ltd.). OD₅₉₅ readings were taken every 90 min for 12 h.

Affinity pulldowns

Yeast expressing histidine-tagged Hsp90 as the sole version of Hsp90 (GPD-Hsp82^{FP}) (Chang and Lindquist 1994) were transformed with an empty vector, as well as with *URA3*-marked plasmids for the GPD-driven expression of Flag-tagged full-length Emc2, *emc2Δtpr*, and His3 (intended as a negative control Flag-tagged protein; it expresses at similar level and is of comparable size). Positive transformants, confirmed by anti-Flag immunoblot, were grown overnight in a 30 °C shaking incubator in 50 mL SC-Ura. Cells were harvested in mid/late log phase (OD₆₀₀ readings at the time of harvest ranged from 0.92 to 1.5), washed once with cold sterile water, and frozen in liquid nitrogen for storage at –80 °C. Subsequent affinity pulldowns were carried out essentially as described (Franzosa et al. 2011), except using Flag agarose (Anti-FLAG M2 affinity gel; Sigma-Aldrich) in place of glutathione agarose. Total protein extracts and Flag agarose-bound proteins were resolved by 12% SDS-PAGE, transferred to nitrocellulose, and analyzed with anti-(His)₆ antisera (to detect Hsp90) and anti-Flag antisera.

Beta-galactosidase assays

Yeast were co-transformed with plasmids (kindly provided by Jill Johnson, Department of Biological Sciences, University of Idaho) encoding mammalian glucocorticoid receptor (GR) and encoding beta-galactosidase under the transcriptional regulation of the glucocorticoid response element (GRE-lacZ). Induction of GR folding and subsequent beta-galactosidase assays were conducted as previously described (Liu et al. 1999).

Results

EMC2 exhibits genetic interactions with the Hsp90 chaperone machinery

Three of the six components originally identified as the EMC in yeast (Jonikas et al. 2009) were identified as Hsp90 interactors in large-scale screens: *EMC2*, *EMC5*, and *EMC6* (McClellan et al. 2007; Zhao et al. 2005). We chose to focus our initial efforts on *EMC2* for two reasons: first, *EMC2* encodes the only soluble, cytoplasmic EMC protein, and second,

EMC2 is predicted to encode a TPR domain, an interaction motif of both well-studied and emerging Hsp90 co-chaperones (Cox and Johnson 2018; Eckl and Richter 2013). The best established Hsp90 co-chaperone, Sti1p/p60/Hop, interacts with conserved residues in the extreme C-terminus of Hsp90 via its C-terminal TPR domain (Chen et al. 1996; Chen and Smith 1998). To investigate whether *STI1* and *EMC2* exhibit any genetic interaction, we created a $\Delta sti1\Delta emc2$ double-deletion strain to explore whether growth was exacerbated on media containing the Hsp90 inhibitor NVP-AUY922, herein referred to as NVP, which has previously been shown to be effective in yeast (Armstrong et al. 2012; Brough et al. 2008). As shown in Fig. 1, tested yeast strains were largely insensitive to NVP at 30 °C but exhibited varying degrees of growth inhibition at 37 °C (compare Fig. 1a to Fig. 1b). While yeast lacking *EMC2* grew similarly to wild-type (WT) yeast at the lower concentration of NVP (75 μ M), $\Delta emc2$ yeast grew more poorly than WT at the higher concentration (200 μ M); $\Delta sti1$ yeast exhibit temperature sensitivity at 37 °C, even in the absence of NVP, and this growth defect is significantly exacerbated by NVP. Of note, the $\Delta sti1\Delta emc2$ double-deletion strain grew noticeably worse than $\Delta sti1$ both in the absence and presence of 75 μ M NVP (Fig. 1b). Neither strain grew at 37 °C at the 200 μ M concentration. The increased sensitivity of $\Delta sti1\Delta emc2$ yeast to NVP was also observed in liquid growth experiments conducted at 37 °C (Fig. 1c). Importantly, quantifiable and statistically significant growth rate differences ($p < 0.001$) between $\Delta sti1$ yeast or $\Delta emc2$ yeast and the $\Delta sti1\Delta emc2$ double-deletion strain were observed even at a lower concentration of NVP than was utilized in the serial dilution experiments. Of note, there was also a significant difference ($p < 0.01$) in the

doubling time of WT yeast and the $\Delta sti1\Delta emc2$ strain in the absence of Hsp90 inhibitor that was not observed between WT and either single deletion strain.

To explore whether the presence or absence of Emc2p generates detectable effects on the inherent or drug-challenged growth of Hsp90 mutant yeast, we next conducted growth experiments using yeast strains containing deletions of both endogenous copies of Hsp90 (*HSP82* and *HSC82*) and expressing a single allele of either WT *HSP82* (WT90) or various *hsp82* point mutants (G170D, E381K, T101I, G313S) (Nathan and Lindquist 1995). WT and mutant Hsp90 strains with and without the additional deletion of *EMC2* were grown as triplicate samples in the absence or presence of increasing concentrations of the Hsp90 inhibitor NVP-AUY922. The results, shown in Fig. 2, demonstrate obvious condition-dependent differences in the growth profiles. For example, while *EMC2* deletion had little to no effect on WT90 yeast grown in the absence of drug, the G170D $\Delta emc2$ strain clearly exhibited slowed growth (compare Fig. 2a to Fig. 2b). In other cases, such as in the presence of the lower concentration of NVP (50 μ M), point mutant yeast lacking *EMC2* demonstrated improved tolerance to Hsp90 inhibitor (compare T101I to T101I $\Delta emc2$; Fig. 2c, d). These data are consistent with previous studies in which co-chaperones differentially affected the behavior of strains expressing Hsp90 alleles with point mutations in different structural domains of the protein (i.e., N-terminal nucleotide-binding domain, middle domain, or C-terminal dimerization domain), including having positive or negative effects on inherent viability as well as on Hsp90 inhibitor sensitivity (Armstrong et al. 2012; Flom et al. 2012; Johnson et al. 2007; Tenge et al. 2015).

Fig. 1 Yeast lacking *STI1*, *EMC2*, or both display increased sensitivity to Hsp90-inhibiting drug at 37 °C. **a** Tenfold serial dilutions of BY4741 wild-type (WT) or deletion (Δ) strains on plates lacking (DMSO) or containing indicated concentrations of NVP-AUY922 (NVP); plates were incubated at 30 °C for 2 days. **b** Same as **a**, except plates were incubated at 37 °C for 2 days (DMSO only) or 3 days (NVP-containing). **c** The indicated yeast strains were grown in liquid media for 12 h at 37 °C in the absence or presence of 50 μ M NVP. Data

shown are mean $\pm$ standard deviation of triplicate samples. Statistically significant differences ($p < 0.001$) as determined by Student's *t* test are indicated with brackets and double asterisks (**). Additional statistically significant differences ($p < 0.01$) were found for the following pairwise comparisons: WT (0)/ $\Delta sti1\Delta emc2$ (0), $\Delta sti1$ (0)/ $\Delta sti1\Delta emc2$ (0), $\Delta sti1\Delta emc2$ (0)/ $\Delta sti1\Delta emc2$ (50 μ M), and $\Delta emc2$ (0)/ $\Delta sti1\Delta emc2$ (0). A statistically significant difference ($p < 0.05$) was also observed between $\Delta sti1$ (0) and $\Delta sti1$ (50 μ M).

The above experiments demonstrate a genetic interaction between *EMC2* and an established Hsp90 co-chaperone (*STI1*), and between *EMC2* and various Hsp90 alleles, but yield no information as to what the nature of that interaction may be. To address this, we next examined whether known Hsp90 co-chaperones with established roles as part of the Hsp90 machinery differentially affect the growth of yeast containing a single copy of *HSP82* that do (WT90) or do not (WT90 Δ *emc2*) contain *EMC2*. Of the seven tested co-chaperones, only the expression of *STI1* and *TAH1* yielded differential results; while *TAH1* expression permitted the growth of both *HSP82* and *HSP82* Δ *emc2* strains at 37 °C in the presence of NVP, *STI1* expression rescued the ability of WT90 yeast to grow in the presence of NVP at 37 °C only if *EMC2* was present (Fig. 3). This observation, with allele-specific variation, was also made with mutant Hsp90 strains containing or lacking *EMC2*. Specifically, we observe better growth rescue attributable to *STI1* expression in G313S yeast only when *EMC2* is present (Fig. 4a), and while *TAH1* expression assists both T101I and T101I Δ *emc2* growth in the presence of NVP, *STI1* expression robustly rescues T101I growth, but not T101I Δ *emc2* growth, under the same conditions (Fig. 4b). While TPR domain-containing co-chaperones such as Sti1p and Tah1p interact with the same region of Hsp90 (the extreme C-terminal amino acid MEEVD), they each facilitate interaction or scaffolding with different protein clients or complexes (see “Discussion”). Our findings, therefore, indicate that there are likely to be both *EMC2*-dependent and *EMC2*-independent functions of Hsp90 in the cell. Furthermore, the data suggest that there is at least partial overlap between *EMC2* dependence and *STI1* dependence.

Collectively, these various growth experiments support that there is an authentic genetic interaction between *EMC2* and the Hsp90 chaperone machinery in yeast, experimentally validating the identification of *EMC2* as an Hsp90 interactor in multiple high-throughput screens (McClellan et al. 2007; Zhao et al. 2005).

EMC2 physically interacts with Hsp90

We next tested whether Emc2p and Hsp90 physically interact. Numerous predictive algorithms were applied to the amino acid sequence of Emc2p in order to identify any sequence(s) likely to interact with Hsp90, specifically those predicted to be TPR motifs. While some programs, including ELM (Dinkel et al. 2016) and SMART (Letunic and Bork 2018), identified no potential TPR motifs in EMC2p, InterPro (Finn et al. 2017), PROSITE (Sigrist et al. 2013), and CDD/SPARCLE (Marchler-Bauer et al. 2017) all predict Emc2p amino acids 163–196 as a TPR motif. We thus generated *emc2* Δ *tpr*, which lacks the coding sequence for amino acids 163–196 and which no longer encodes any predicted conserved domains, TPR, or

otherwise (Marchler-Bauer et al. 2017). As shown in Fig. 5, affinity isolation of Flag-tagged Emc2p (lane 3), but not of a control protein (Flag-His3p; lane 2), co-isolated Hsp90. Of note, the deletion of the predicted TPR motif did not diminish the interaction of Emc2p with Hsp90 (lane 4). Thus, while this experiment supports that Hsp90 and Emc2p physically interact, it also suggests that a TPR domain, commonly found in Hsp90 co-chaperones, is not the means of interaction. Interestingly, while CDD/SPARCLE no longer predicts any TPR motifs with the removal of amino acids 163–196, analysis of the full-length *EMC2* sequence predicts additional TPR motifs (amino acids 196–236 and 241–269), which could theoretically facilitate the interaction between Hsp90 and Emc2p. Of note, however, these additional putative TPR motifs were not identified by InterPro or PROSITE.

The EMC exhibits genetic and functional interactions with Hsp90

As mentioned above, other members of the EMC have been identified as Hsp90 interactors in high-throughput screens, namely *EMC5* and *EMC6* (McClellan et al. 2007; Zhao et al. 2005). We thus extended our analysis from Emc2p to the larger EMC by utilizing yeast strains harboring deletions of multiple EMC components (Lahiri et al. 2014). Strains lacking two (YSL4: Δ *emc1* Δ *emc5* and YSL5: Δ *emc3* Δ *emc6*), three (YSL6: Δ *emc1* Δ *emc2* Δ *emc5* and YSL57: Δ *emc2* Δ *emc5* Δ *emc6*), four (YSL27: Δ *emc1* Δ *emc2* Δ *emc3* Δ *emc6*), or five (YSL9: Δ *emc1* Δ *emc2* Δ *emc3* Δ *emc5* Δ *emc6*) members of the EMC were first tested for sensitivity to NVP by serial dilution growth assay. While all of the strains tested were unaffected even by a high concentration of NVP at 30 °C (200 μ M), YSL9, YSL27, and YSL57 exhibited clear growth defects at 37 °C in the presence of only 75 μ M NVP (Fig. 6) and failed to grow at all in the presence of 200 μ M NVP (data not shown).

We next tested whether the ability of Hsp90 to fold the well-established client protein GR was affected by EMC component deletion. As expected, WT yeast robustly fold GR while *sti1* Δ yeast exhibit severely compromised GR folding (Fig. 7) (Chang et al. 1997). Furthermore, two of the multiple EMC deletion strains, YSL9 and YSL27, lacking five or four components of the EMC, respectively, were severely hampered with regard to GR folding ($p < 0.001$). Of note, however, the conditional expression of exogenous (v-src) or endogenous (Sti1p) kinases, which is toxic to yeast in an Hsp90-dependent manner (Brugge et al. 1987; Louvion et al. 1998; Xu and Lindquist 1993), did not result in diminished growth of multiple EMC deletion strains (data not shown). This suggests that the loss of EMC-Hsp90 interaction does not globally affect the folding of Hsp90 clients.

a No Drug **b** No Drug **c** 50 μ M NVP **d** 50 μ M NVP **e** 150 μ M NVP **f** 150 μ M NVP

Fig. 2 Yeast expressing point mutant Hsp90 alleles exhibit differential sensitivity to both Hsp90-inhibiting drug and *EMC2* deletion. The diagram at the top of the figure indicates the location of point mutations within the primary structure of Hsp90. Yeast containing (a, c, e) or lacking (b, d, f) *EMC2* were grown in liquid media in the absence (a, b) or presence of 50 μ M (c, d) or 150 μ M (e, f) NVP-AUY922 for 12 h at 37 °C. Data shown are mean $\pm$ standard deviation of triplicate samples. Embedded legend for a applies also to c and e; embedded legend for b applies also to d and f

These experiments utilizing yeast lacking multiple components of the EMC support that there is not only a larger genetic but also a functional interaction between Hsp90 and the EMC. In order to clarify the nature of EMC-Hsp90 interaction, we looked for common genetic and physical interactors of EMCs 1–6 and Hsp90 using published data curated in BioGRID (Stark et al. 2006). First, we analyzed whether and what Gene Ontology biological process classifications are enriched amongst genes/proteins that interact with one or more components of the EMC. The top five statistically significant categories include transcriptional regulation, cellular transport, and cell cycle (Table 1). When instead applying the same analysis to genes/proteins that interact with HSP82 and/or HSC82

(collectively referred to as Hsp90 interactors in this analysis), a completely different set of GO classifications is enriched, most notably ones pertaining to the ribosome and protein translation. We next focused on genes/proteins common to multiple individual components of the EMC. As shown in Table 1, there are 23 common interactors amongst *EMC2*, *EMC5*, and *EMC6*, the three EMCs published to interact with Hsp90 and deleted in the NVP-sensitive strain YSL57 (Fig. 6b). When this analysis is extended to also include interactors shared with Hsp90, the number of common interactors drops to 12; however, the most significantly enriched GO classification, protein insertion into the ER membrane, does not change. When interactions amongst EMCs 1, 2, 3, and 6 (proxy for NVP-sensitive strain YSL27) and Hsp90 are compared, nine common interactors remain. Interestingly, these nine interactors persist when EMCs 1, 2, 3, 5, and 6 (as deleted in NVP-sensitive strain YSL9) and Hsp90 are compared, as well as when all six EMCs and Hsp90 are compared, there is additionally no change in the identity of enriched GO biological process classifications from comparing only EMCs 2, 5, and 6 and Hsp90. Of note, these nine shared interactors are also the only ones common to EMCs 1–6 when compared amongst themselves

Fig. 3 Yeast expressing wild-type Hsp90 respond differently to co-chaperone expression in the absence or presence of *EMC2*. **a** Tenfold serial dilutions of yeast (WT90) transformed with either empty vector

(EV) or various Hsp90 co-chaperone expression plasmids (as indicated) on media lacking (DMSO) or containing 150 μ M NVP-AUY922. **b** Same as **a**, except that yeast additionally lack *EMC2* (WT90 Δ emc2)

Fig. 4 Point mutant Hsp90 strains $\pm$ EMC2 demonstrate variability in response to *STI1* and *TAH1* expression in the presence of Hsp90 inhibitor. Tenfold serial dilutions of yeast expressing either G313S (**a**) or T101I (**b**) point mutant Hsp90 transformed with empty vector (EV) or

plasmids constitutively expressing Hsp90 co-chaperones *Sti1p* or *Tah1p*, as indicated. Plates were incubated at 30 °C for 2 days (DMSO and 25 μ M NVP) or 4 days (75 μ M NVP)

(and not to Hsp90 interactors). Thus, there is a robust core of shared EMC/Hsp90 interactors, and of GO assignments

ascribed to those interactors, that may represent biological processes in which Hsp90 and the EMC collaborate.

Fig. 5 Full-length Emc2p and Δ tp-emc2p co-precipitate Hsp90. Native protein extracts from yeast containing GPD-driven His-tagged Hsp82 as the only copy of Hsp90 (GPD-Hsp82^{FP}; Chang and Lindquist 1994) were transformed with vectors expressing no Flag-tagged protein (empty vector), a control Flag-tagged protein (Flag-His3p), Flag-Emc2p, or Flag-emc2 Δ tp. Equal amounts of lysate (10% input shown in the top gel

panel) were subjected to Flag agarose pulldowns (IP), SDS-PAGE, and immunoblot (IB) analysis with anti-His and anti-Flag antisera to detect Hsp90 and Flag-tagged proteins, respectively. The bottom gel (IP:Flag and Co-IP) was separated into two pieces (indicated by dashed line) following transfer to nitrocellulose. The arrow indicates the anti-Flag antibody light chain

Fig. 6 Yeast strains lacking multiple components of the EMC are hypersensitive to Hsp90 inhibitor. **a** Tenfold serial dilutions of BY4741 wild-type (WT) or multiple *emc* deletion strains (YSL; Lahiri et al. 2014) on media lacking (DMSO) or containing 200 μ M NVP-AUY922 (NVP); plates were incubated at 30 $^{\circ}$ C for 2 days. **b** Same as **a**, except plates were incubated at 37 $^{\circ}$ C for 2 days (DMSO) or 3 days (75 μ M NVP). YSL4 = $\Delta emc1 \Delta emc5$, YSL5 = $\Delta emc3 \Delta emc6$, YSL6 = $\Delta emc1 \Delta emc2 \Delta emc5$, YSL9 = $\Delta emc1 \Delta emc2 \Delta emc3 \Delta emc5$, YSL27 = $\Delta emc1 \Delta emc2 \Delta emc3 \Delta emc6$, YSL57 = $\Delta emc2 \Delta emc5 \Delta emc6$

Extension of findings to recently named members of the yeast EMC

A recently published bioinformatic analysis identified two likely additional components of the yeast EMC, *EMC7* (known as *SOP4*) and *EMC10* (YDR056C), previously thought to be conserved only in metazoans (Wideman 2015). In order to include them in our analysis, we conducted growth assays of yeast strains individually deleted for each of the eight EMC members. Figure 8 shows that all individual deletion strains, including $\Delta emc7/sop4$ and $\Delta emc10/ydr056c$, exhibit some sensitivity to the inclusion of NVP in the media, supporting that they, as do the previously identified EMC components, interact genetically with Hsp90.

Discussion

This study represents an effort to determine whether the occurrence of *EMC2*, *EMC5*, and *EMC6* in Hsp90 interactor datasets from large-scale genetic screens is indicative of a genuine biological interaction (McClellan et al. 2007; Zhao et al. 2005). Herein, we provide substantial experimental

evidence in support of in vivo interaction between the Hsp90 chaperone machinery and the EMC. *EMC2*, which encodes a soluble, cytosolic component of the EMC, as well as a potential TPR domain that could indicate a means of interaction with Hsp90, was investigated first. We found that yeast with deletions of both *EMC2* and the well-known Hsp90 co-chaperone *STI1* exhibited increased sensitivity to the Hsp90 inhibitor NVP-AUY922, as assessed by growth on solid or in liquid media (Fig. 1). We next utilized yeast containing various point mutant *HSP82* alleles as the sole cellular source of Hsp90; these strains behaved differently when exposed to NVP, for example appearing largely unaffected by the concentrations used (T101I), exhibiting clear dose-dependent growth defects (G313S), or even showing slight growth improvement (E381K; Fig. 2a, c, e). The specific effect that *EMC2* deletion had on WT and point mutant Hsp90 strains also varied significantly in liquid growth experiments in the absence or presence of NVP. If there is indeed a genuine interaction between Hsp90 and the EMC, this result is expected since each point mutation likely affects the inherent function(s) and interaction(s) of Hsp90 differently, a supposition supported by previous work utilizing these and other point mutant Hsp90 alleles in yeast (Armstrong et al. 2012; Chen

Fig. 7 Glucocorticoid activity is compromised in yeast lacking multiple EMC components. Mid-log phase cultures of the indicated yeast strains, all constitutively expressing glucocorticoid receptor (GR) and bearing a glucocorticoid response element (GRE) upstream of the beta-galactosidase gene, were grown in the absence (–) or presence (+) of steroid hormone (10 μ M deoxycorticosterone, DOC) at 30 °C for 1 h. Subsequent cell extracts were subjected to beta-galactosidase activity assays and arbitrary units of activity calculated (see “Materials and methods”). Data represent the mean $\pm$ standard deviation from a minimum of four experimental replicates. Highly statistically significant differences as determined by Student’s *t* test are indicated with brackets and single (* p < 0.01) or double (** p < 0.001) asterisks (differences for Δ sti1 were also p < 0.001, brackets not shown). YSL4 = Δ emc1 Δ emc5, YSL5 = Δ emc3 Δ emc6, YSL6 = Δ emc1 Δ emc2 Δ emc5, YSL9 = Δ emc1 Δ emc2 Δ emc3 Δ emc5, YSL27 = Δ emc1 Δ emc2 Δ emc3 Δ emc6, YSL57 = Δ emc2 Δ emc5 Δ emc6

et al. 1998; Flom et al. 2012; Johnson et al. 2007; Louvion et al. 1998). Further genetic evidence of an interaction is provided by experiments in which established Hsp90 co-chaperones are overexpressed in WT *HSP82* yeast (Fig. 3) or point mutant *hsp82* yeast (Fig. 4) that contain or lack *EMC2*. Most notably, the ability of Sti1p to improve the growth of strains on media containing NVP is abolished when *EMC2* is deleted. The additional observation that *TAH1* overexpression could rescue the Hsp90 inhibitor sensitivity of yeast whether or not *EMC2* was present suggests that while some cellular functions of Hsp90 involve *EMC2*, others do not. Sti1p localizes primarily to the cytoplasm and has the ability to bind simultaneously to both Hsp70 and Hsp90, thus facilitating the delivery of a subset of client proteins to Hsp90. Tah1, on the other hand, serves to bridge Hsp90 to the larger R2TP complex (Kakihara and Houry 2012), which is implicated in the assembly of complexes in the nucleus (snoRNP biogenesis, for example) and the cytosolic assembly of complexes that are important for functions in the nucleus (i.e.,

RNA polymerase II). Perhaps Sti1p additionally functions with Hsp90 in secretory pathway-related activities (discussed below), and it is those activities that are also EMC-dependent but do not require Tah1p.

Altogether, these experiments establish a robust genetic interaction between Hsp90 and *EMC2*. This relationship extends to a physical interaction as well (Fig. 5), although the precise region of Emc2p that facilitates interaction with Hsp90, as well as whether binding is direct or indirect, remains unknown. The finding that deletion of amino acids critical for predicted TPR domain formation does not affect the ability of Emc2p to bind Hsp90 does not necessarily mean that it is not acting as a co-chaperone; there are both numerous examples of Hsp90 co-chaperones that lack TPR domains as well as at least one example of an Hsp90 co-chaperone that has a TPR domain but does not use it for interaction with Hsp90 (Lee et al. 2004). As the latter case suggests that the TPR domain may instead be used to bridge the binding of Hsp90 to other proteins, such as in the context of a multi-protein complex, we next extended our analysis to include the larger EMC. Yeast lacking multiple components of the EMC exhibit increased sensitivity to Hsp90 inhibitor in growth assays (Fig. 6) and, interestingly, display a lack of glucocorticoid receptor folding (Fig. 7). Collectively, our experimental evidence supports a genetic, physical, and functional interaction of the Hsp90 chaperone machinery and the EMC.

Gene Ontology analysis of published interactions ascribed to either Hsp90 or combined individual EMC components yields distinct results; the top five most significantly enriched GO biological process assignments for each have none in common (Table 1). When instead comparing only interactors shared amongst EMCs 1–6, the results are no different when Hsp90 interactors are also included. Thus, the only published interactors shared amongst EMCs 1–6 are also published Hsp90 interactors, suggesting that the enriched GO categories may represent the cellular processes in which Hsp90 and the EMC interact. It is therefore worth noting that the top significantly enriched category is that of “protein insertion into the ER membrane,” which has been recently described as a function of the EMC in mammalian cells (Guna et al. 2018).

There is currently only indirect evidence that Hsp90 has some role to play in this process. Yeast with deletions of each of the five members of the “guided entry of tail-anchored proteins” complex (Jonikas et al. 2009; Schuldiner et al. 2008), GET1, GET2, GET3, GET4, and GET5 (aka MDY2), exhibit synthetic growth defects in combination either with chemical inhibition of Hsp90 or with a temperature-sensitive point mutant allele of Hsp90, *hsp82*^{G170D} (McClellan et al. 2007; Nathan and Lindquist 1995; Zhao et al. 2005). The requisite GET pathway co-

Table 1 Significantly enriched GO biological process assignments ascribed to EMC components and Hsp90

Gene: number of unique BioGRID interactors	Interactions compared	Interactors in common	Top 5 enriched GO biological process assignments (category: <i>p</i> value)
EMCs 1–6: 573	EMCs 1–6	n/a	Regulation of transcription from RNA polymerase II promoter [GO:0006357]: 6.869e-07 Protein transport [GO:0015031]: 1.976e-06 Transport [GO:0006810]: 2.718e-06 ER-associated protein catabolic process [GO:0030433]: 6.946e-06 Cell cycle [GO:0007049]: 1.509e-05
HSP90: 1789	Hsp90 (Hsc82 and/or Hsp82)	n/a	Translation [GO:0006412]: < 1e-14 Translational elongation [GO:0006414]: 9.996e-08 Ribosome biogenesis [GO:0042254]: 1.121e-06 Protein phosphorylation [GO:0006468]: 1.198e-06 Protein folding [GO:0006457]: 2.149e-06
EMC2: 146 EMC5: 187 EMC6: 154	EMCs 2, 5, and 6	23	Protein insertion into ER membrane [GO:0045048]: 0.0001153 De novo protein folding [GO:0006458]: 0.0001726 Cellular protein localization [GO:0034613]: 0.0003209 SRP-dependent co-translational protein targeting to membrane [GO:0006614]: 0.0005135 Response to unfolded protein [GO:0006986]: 0.0006263
As above	EMCs 2, 5, and 6 and Hsp90	12	Protein insertion into ER membrane [GO:0045048]: 3.019e-05 Cellular protein localization [GO:0034613]: 8.427e-05 Response to unfolded protein [GO:0006986]: 0.000165 Retrograde vesicle-mediated transport, Golgi to ER [GO:0006890]: 0.001115 Positive regulation of DNA repair [GO:0045739]: 0.001817
EMC1: 147 EMC3: 108 EMC4: 82 And as above	EMCs 1, 2, 3, and 6 and Hsp90 or EMCs 1, 2, 3, 5, and 6 and Hsp90 or EMCs 1, 2, 3, 4, 5, and 6, ± Hsp90	9	Protein insertion into ER membrane [GO:0045048]: 1.648e-05 Cellular protein localization [GO:0034613]: 4.605e-05 Response to unfolded protein [GO:0006986]: 9.026e-05 Retrograde vesicle-mediated transport, Golgi to ER [GO:0006890]: 0.0006129 Positive regulation of DNA repair [GO:0045739]: 0.001363

Unique interactor lists for EMC1, EMC2, EMC3, EMC4, EMC5, EMC6, and Hsp90 were determined using data from BioGRID (<https://thebiogrid.org>; Stark et al. 2006) files downloaded on January 9, 2018. GO biological process assignments were generated using Funspec (<http://funspec.med.utoronto.ca/>; Robinson et al. 2002). Self-interactions were removed from each individual list of unique interactors. See Table S1 for identities of shared interactors

chaperone SGT2, which contains a TPR motif, also has reported interactions with Hsp90 (Chartron et al. 2011; Franzosa et al. 2011; Krysztofinska et al. 2017; Liou et al. 2007; McClellan et al. 2007; Wang et al. 2010). Additionally, there are numerous established interactions between EMC components and members of the GET pathway (Costanzo et al. 2010; Costanzo et al. 2016; Hoppins et al. 2011; Jonikas et al. 2009; Pan et al. 2006; Schuldiner et al. 2005; Schuldiner et al. 2008; Surma et al. 2013). These interaction-based speculations aside, Hsp90 has been suggested previously to assist both the trafficking and membrane insertion of v-src (Xu and Lindquist 1993), Hsp90 inhibition diminished the ability of the Rab-specific GDP dissociation inhibitor (α GDI) to extract Rab3a from synaptic membranes (Sakisaka et al. 2002), and Hsp90 collaborates with rab11 in the membrane insertion and/or retrieval of α -synuclein (Liu et al. 2009); thus, a role for Hsp90 in facilitating protein insertion into cellular membranes is not without precedent.

Recent work in mammalian cells suggests that while the GET complex is more suited for handling target proteins with highly hydrophobic transmembrane domains, the EMC instead assists proteins whose less hydrophobic tails are shielded by calmodulin prior to ER membrane insertion (Guna et al. 2018). It is interesting to note that there are reported interactions in yeast of calmodulin with both Hsp90 and GET2, but not with the EMC (Costanzo et al. 2016). In yeast, the GET pathway definitively interacts with the tail-anchored protein Sec22p, which has a transmembrane helix hydrophobicity of ~87% (Wang et al. 2010). SEC22 also has reported interactions with both the EMC and Hsp90 (Costanzo et al. 2016; Jonikas et al. 2009; Zhao et al. 2005), raising the possibility that Hsp90 may facilitate triage of ER-destined tail-anchored membrane proteins to one, the other, or both pathways. This could perhaps be managed in conjunction with other cellular chaperones and co-chaperones that act upstream of Hsp90; intriguingly, there are numerous examples of such that

Fig. 8 Single- Δemc deletion strains display sensitivity to NVP-AUY922. Tenfold serial dilutions of BY4743 wild-type (WT) or deletion (Δ) strains on solid media lacking (DMSO) or containing 75 μ M NVP-AUY922 (NVP); plates were incubated at 37 °C for 2 days (DMSO) or 4 days (NVP)

are known interactors of only Hsp90 and GET pathway members (Ssa1/2, Sse1/2, Hsp104, Hsp42, Sis1, Scj1, Ydj1, She4, and Cdc37), and of only Hsp90 and EMC components (Hsp31, Caj1, and Hlj1), or that interact with all three systems (Ssb2, Ssc1, Lhs1, Cpr7, and Cdc48; for interaction references, see <https://www.thebiogrid.org>). Some of these proteins are themselves tail-anchored ER membrane proteins, for example Hlj1 (Beilharz et al. 2003), and could instead (or additionally) represent targets of a proposed Hsp90/EMC sorting pathway; of note, the membrane domain of Hlj1 has a hydrophobicity of only ~71%, within the established range of proteins expected to transit via the EMC (transmembrane domain hydrophobicity < 72%) versus the GET complex (transmembrane domain hydrophobicity > 73%) (Guna et al. 2018). Taken all together, it seems that it would be worthwhile to directly investigate a possible role for Hsp90 in ER membrane protein targeting or insertion.

A remaining possibility is that the substantial connections between Hsp90, the EMC, and the GET complex are instead, perhaps, the consequence of Hsp90's participation in the assembly or maintenance of cellular protein complexes. The results of large-scale Hsp90 target screens have a demonstrated enrichment for highly interactive network hubs, which may reflect the significant presence of members of multi-protein complexes in these datasets (McClellan 2007; Gopinath 2014). Most notable, perhaps, is the enrichment of secretory pathway complex components including two or more members of the COG, AP-1, AP-3, TRAPP, ESCRT I, ESCRT II, ESCRT III, COPI, and Retromer complexes (McClellan 2007). The EMC would also have been included in these findings but had not yet been identified at that time. It is perhaps worth speculating

that the essential function of Hsp90 in eukaryotic cells has to do with the pleiotropic functions of Hsp90 in the assembly, disassembly, or maintenance of the multitude of protein complexes throughout the endomembrane system. Hsp90 has roles in other critical cellular functions; for example, it has demonstrated ribosomal interactions in both eukaryotes and prokaryotes but yet is not essential for viability in the latter (Babu et al. 2014; Butland et al. 2005; Franzosa et al. 2011; Kim et al. 2006; Tenge et al. 2015). That Hsp90 essentiality may have co-evolved with the endomembrane system, and multicellular eukaryotes, aligns well with the presence, and essential nature, of only cytosolic Hsp90 in the single-celled eukaryote *Saccharomyces cerevisiae* and the subsequent arisal of compartment-specific Hsp90s in higher eukaryotes, which are found in the mitochondria, the endoplasmic reticulum, and even the extracellular matrix.

Overall, our results support that Hsp90 and the EMC interact, consistent with a possible role for Hsp90 in either independently assisting the conformational maintenance/assembly of the EMC, and other protein complexes involved in protein targeting and trafficking, or in playing an important role in assisting or determining the pathway that a protein destined for a certain fate, such as membrane insertion, may take. One could envision both the former and the latter being facilitated by interactions with specific co-chaperones that direct Hsp90 or its clients to alternate paths.

Acknowledgements We are grateful to Jill Johnson for Hsp90 co-chaperone expression plasmids, as well as v-src, Ste11, and glucocorticoid receptor folding assay plasmids, and to William Prinz for supplying the multiple EMC deletion strains. We additionally thank David Norman and Gerry Bolgos for their general laboratory assistance. A.J.M. thanks Jeff Brodsky, John Christianson, Jill Johnson, Paul LaPointe, and Liz

Miller for their helpful suggestions and discussions throughout this work and Lars Ellgaard for critical reading of the manuscript.

Funding information A.J.M. acknowledges the support of NIH R15 AREA Grant GM087654 and internal faculty grants from Bennington College.

References

- Adams A, Gottschling DE, Kaiser CA, Stearns T (1997) Methods in yeast genetics: a Cold Spring Harbor Laboratory course manual. Cold Spring Harbor Laboratory, Plainview
- Armstrong H, Wolmarans A, Mercier R, Mai B, LaPointe P (2012) The co-chaperone Hch1 regulates Hsp90 function differently than its homologue Aha1 and confers sensitivity to yeast to the Hsp90 inhibitor NVP-AUY922. *PLoS One* 7:e49322 doi:<https://doi.org/10.1371/journal.pone.0049322>
- Babu M, Arnold R, Bundalovic-Torma C, Gagarinova A, Wong KS, Kumar A, Stewart G, Samanfar B, Aoki H, Wagih O, Vlasblom J, Phanse S, Lad K, Yeou Hsiung Yu A, Graham C, Jin K, Brown E, Golshani A, Kim P, Moreno-Hagelsieb G, Greenblatt J, Houry WA, Parkinson J, Emili A (2014) Quantitative genome-wide genetic interaction screens reveal global epistatic relationships of protein complexes in *Escherichia coli*. *PLoS Genet* 10:e1004120. <https://doi.org/10.1371/journal.pgen.1004120>
- Beilharz T, Egan B, Silver PA, Hofmann K, Lithgow T (2003) Bipartite signals mediate subcellular targeting of tail-anchored membrane proteins in *Saccharomyces cerevisiae*. *J Biol Chem* 278:8219–8223. <https://doi.org/10.1074/jbc.M212725200>
- Brough PA et al. (2008) 4,5-Diarylisoazole Hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer *J Med Chem* 51:196–218 doi:<https://doi.org/10.1021/jm701018h>
- Brugge JS, Jarosik G, Andersen J, Queral-Lustig A, Fedor-Chaiken M, Broach JR (1987) Expression of Rous sarcoma virus transforming protein pp60v-src in *Saccharomyces cerevisiae* cells. *Mol Cell Biol* 7:2180–2187
- Butland G et al. (2005) Interaction network containing conserved and essential protein complexes in *Escherichia coli* *Nature* 433:531–537 doi:<https://doi.org/10.1038/nature03239>
- Chang H-CJ, Lindquist S (1994) Conservation of Hsp90 macromolecular complexes in *Saccharomyces cerevisiae*. *J Biol Chem* 269:24983–24988
- Chang HC, Nathan DF, Lindquist S (1997) In vivo analysis of the Hsp90 cochaperone Sti1 (p60). *Mol Cell Biol* 17:318–325
- Chartron JW, Gonzalez GM, Clemons WM, Jr. (2011) A structural model of the Sgt2 protein and its interactions with chaperones and the Get4/Get5 complex *J Biol Chem* 286:34325–34334 doi:<https://doi.org/10.1074/jbc.M111.277798>
- Chatterjee S, Burns TF (2017) Targeting heat shock proteins in cancer: a promising therapeutic approach *Int J Mol Sci* 18 doi:<https://doi.org/10.3390/ijms18091978>
- Chen S, Smith DF (1998) Hop as an adaptor in the heat shock protein 70 (Hsp70) and hsp90 chaperone machinery. *J Biol Chem* 273:35194–35200
- Chen S, Prapapanich V, Rimerman RA, Honore B, Smith DF (1996) Interactions of p60, a mediator of progesterone receptor assembly, with heat shock proteins hsp90 and hsp70 *Mol Endocrinol* 10:682–693 doi:<https://doi.org/10.1210/mend.10.6.8776728>
- Chen S, Sullivan WP, Toft DO, Smith DF (1998) Differential interactions of p23 and the TPR-containing proteins Hop, Cyp40, FKBP52 and FKBP51 with Hsp90 mutants *Cell Stress Chaperones* 3:118–129
- Costanzo M et al. (2010) The genetic landscape of a cell *Science* 327:425–431 doi:<https://doi.org/10.1126/science.1180823>
- Costanzo M, VanderSluis B, Koch EN, Baryshnikova A, Pons C, Tan G, Wang W, Usaj M, Hanchard J, Lee SD, Pelechano V, Styles EB, Billmann M, van Leeuwen J, van Dyk N, Lin ZY, Kuzmin E, Nelson J, Piotrowski JS, Srikumar T, Bahr S, Chen Y, Deshpande R, Kurat CF, Li SC, Li Z, Usaj MM, Okada H, Pascoe N, San Luis BJ, Sharifpoor S, Shuteriqi E, Simpkins SW, Snider J, Suresh HG, Tan Y, Zhu H, Malod-Dognin N, Janjic V, Przulj N, Troyanskaya OG, Stagljar I, Xia T, Ohya Y, Gingras AC, Raught B, Boutros M, Steinmetz LM, Moore CL, Rosebrock AP, Caudy AA, Myers CL, Andrews B, Boone C (2016) A global genetic interaction network maps a wiring diagram of cellular function *Science* 353 doi:<https://doi.org/10.1126/science.aaf1420>
- Cox MB, Johnson JL (2018) Evidence for Hsp90 co-chaperones in regulating Hsp90 function and promoting client protein folding *Methods Mol Biol* 1709:397–422 doi:https://doi.org/10.1007/978-1-4939-7477-1_28
- Dickey CA et al. (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins *J Clin Invest* 117:648–658 doi:<https://doi.org/10.1172/JCI29715>
- Dinkel H et al. (2016) ELM 2016—data update and new functionality of the eukaryotic linear motif resource *Nucleic Acids Res* 44:D294–300 doi:<https://doi.org/10.1093/nar/gkv1291>
- Echeverria PC, Bernthaler A, Dupuis P, Mayer B, Picard D (2011) An interaction network predicted from public data as a discovery tool: application to the Hsp90 molecular chaperone machine *PLoS One* 6:e26044 doi:<https://doi.org/10.1371/journal.pone.0026044>
- Eckl JM, Richter K (2013) Functions of the Hsp90 chaperone system: lifting client proteins to new heights *Int J Biochem Mol Biol* 4:157–165
- Finn RD et al. (2017) InterPro in 2017—beyond protein family and domain annotations *Nucleic Acids Res* 45:D190–D199 doi:<https://doi.org/10.1093/nar/gkw1107>
- Flom GA, Langner E, Johnson JL (2012) Identification of an Hsp90 mutation that selectively disrupts cAMP/PKA signaling in *Saccharomyces cerevisiae* *Curr Genet* 58:149–163 doi:<https://doi.org/10.1007/s00294-012-0373-7>
- Franzosa EA, Albanese V, Frydman J, Xia Y, McClellan AJ (2011) Heterozygous yeast deletion collection screens reveal essential targets of Hsp90 *PLoS One* 6:e28211 doi:<https://doi.org/10.1371/journal.pone.0028211>
- Geller R, Vignuzzi M, Andino R, Frydman J (2007) Evolutionary constraints on chaperone-mediated folding provide an antiviral approach refractory to development of drug resistance. *Genes & Dev* 21:195–205. <https://doi.org/10.1101/gad.1505307>
- Geller R, Taguwa S, Frydman J (2012) Broad action of Hsp90 as a host chaperone required for viral replication *Biochim Biophys Acta* 1823:698–706 doi:<https://doi.org/10.1016/j.bbamer.2011.11.007>
- Giaever G, A. M. Chu, L. Ni, C. Connelly, Riles, L. et al. (2002) Functional profiling of the *Saccharomyces cerevisiae* genome *Nature* 418:387–391
- Gong Y, Kakiyama Y, Krogan N, Greenblatt J, Emili A, Zhang Z, Houry WA (2009) An atlas of chaperone-protein interactions in *Saccharomyces cerevisiae*: implications to protein folding pathways in the cell. *Mol Syst Biol* 5:275. <https://doi.org/10.1038/msb.2009.26>
- Gopinath RK, You ST, Chien KY, Swamy KB, Yu JS, Schuyler SC, Leu JY (2014) The Hsp90-dependent proteome is conserved and enriched for hub proteins with high levels of protein-protein connectivity *Genome Biol Evol* 6:2851–2865 doi:<https://doi.org/10.1093/gbe/evu226>
- Guna A, Volkmar N, Christianson JC, Hegde RS (2018) The ER membrane protein complex is a transmembrane domain insertase. *Science* 359:470–473. <https://doi.org/10.1126/science.aao3099>
- Hoppins S et al. (2011) A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane

- organization in mitochondria *The J Cell Biol* 195:323–340 doi:<https://doi.org/10.1083/jcb.201107053>
- Jhaveri K, Taldone T, Modi S, Chiosis G (2012) Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers *Biochim Biophys Acta* 1823:742–755 doi:<https://doi.org/10.1016/j.bbamcr.2011.10.008>
- Johnson JL, Halas A, Flom G (2007) Nucleotide-dependent interaction of *Saccharomyces cerevisiae* Hsp90 with the cochaperone proteins Sti1, Cpr6, and Sba1 *Mol Cell Biol* 27:768–776 doi:<https://doi.org/10.1128/MCB.01034-06>
- Jonikas MC et al. (2009) Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum *Science* 323:1693–1697 doi:<https://doi.org/10.1126/science.1167983>
- Kakihara Y, Houry WA (2012) The R2TP complex: discovery and functions *Biochimica et Biophysica Acta* 1823:101–107 doi:<https://doi.org/10.1016/j.bbamcr.2011.08.016>
- Kim TS, Jang CY, Kim HD, Lee JY, Ahn BY, Kim J (2006) Interaction of Hsp90 with ribosomal proteins protects from ubiquitination and proteasome-dependent degradation *Mol Biol Cell* 17:824–833 doi:<https://doi.org/10.1091/mbc.E05-08-0713>
- Krysztofinska EM, Evans NJ, Thapaliya A, Murray JW, Morgan RML, Martinez-Lumbreras S, Isaacson RL (2017) Structure and interactions of the TPR domain of Sgt2 with yeast chaperones and Ybr137wp *Front Mol Biosci* 4:68 doi:<https://doi.org/10.3389/fmolb.2017.00068>
- Lahiri S, Chao JT, Tavassoli S, Wong AKO, Choudhary V, Young BP, Loewen CJR, Prinz WA (2014) A conserved endoplasmic reticulum membrane protein complex (EMC) facilitates phospholipid transfer from the ER to mitochondria. *PLoS Biol* 12:e1001969. doi:<https://doi.org/10.1371/journal.pbio.1001969>
- Lee YT, Jacob J, Michowski W, Nowotny M, Kuznicki J, Chazin WJ (2004) Human Sgt1 binds HSP90 through the CHORD-Sgt1 domain and not the tetratricopeptide repeat domain. *J Biol Chem* 279:16511–16517. doi:<https://doi.org/10.1074/jbc.M400215200>
- Letunic I, Bork P (2018) 20 years of the SMART protein domain annotation resource *Nucleic Acids Res* 46:D493–D496 doi:<https://doi.org/10.1093/nar/gkx922>
- Li Y et al. (2013) A novel ER-localized transmembrane protein, EMC6, interacts with RAB5A and regulates cell autophagy *Autophagy* 9:150–163 doi:<https://doi.org/10.4161/auto.22742>
- Liou ST, Cheng MY, Wang C (2007) SGT2 and MDY2 interact with molecular chaperone YDJ1 in *Saccharomyces cerevisiae* *Cell Stress Chaperones* 12:59–70
- Liu XD, Morano KA, Thiele DJ (1999) The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. *J Biol Chem* 274:26654–26660
- Liu J et al. (2009) Rab11a and HSP90 regulate recycling of extracellular alpha-synuclein *J Neurosci* 29:1480–1485 doi:<https://doi.org/10.1523/JNEUROSCI.6202-08.2009>
- Longtine MS et al. (1998) Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae* *Yeast* 14:953–961 doi:[https://doi.org/10.1002/\(SICI\)1097-0061\(199807\)14:10<953::AID-YEA293>3.0.CO;2-U](https://doi.org/10.1002/(SICI)1097-0061(199807)14:10<953::AID-YEA293>3.0.CO;2-U)
- Louie RJ et al. (2012) A yeast phenomic model for the gene interaction network modulating CFTR-DeltaF508 protein biogenesis *Genome Med* 4:103 doi:<https://doi.org/10.1186/gm404>
- Louvion JF, Abbas-Terki T, Picard D (1998) Hsp90 is required for pheromone signaling in yeast *Mol Biol Cell* 9:3071–3083
- Luo W, Dou F, Rodina A, Chip S, Kim J, Zhao Q, Moulick K, Aguirre J, Wu N, Greengard P, Chiosis G (2007) Roles of heat-shock protein 90 in maintaining and facilitating the neurodegenerative phenotype in tauopathies. *Proc Natl Acad Sci USA* 104:9511–9516. doi:<https://doi.org/10.1073/pnas.0701055104>
- Ma H, Dang Y, Wu Y, Jia G, Anaya E, Zhang J, Abraham S, Choi JG, Shi G, Qi L, Manjunath N, Wu H (2015) A CRISPR-based screen identifies genes essential for West-Nile-virus-induced cell death. *Cell Rep* 12:673–683. doi:<https://doi.org/10.1016/j.celrep.2015.06.049>
- Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY, Bryant SH (2017) CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Res* 45:D200–D203. doi:<https://doi.org/10.1093/nar/gkw1129>
- McClellan AJ, Xia Y, Deutschbauer AM, Davis RW, Gerstein M, Frydman J (2007) Diverse cellular functions of the Hsp90 molecular chaperone uncovered using systems approaches. *Cell* 131:121–135. doi:<https://doi.org/10.1016/j.cell.2007.07.036>
- Millson SH, Truman AW, King V, Prodromou C, Pearl LH, Piper PW (2005) 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). *Eukaryot Cell* 4:849–860. doi:<https://doi.org/10.1128/EC.4.5.849-860.2005>
- Nathan DL, Lindquist S (1995) Mutational analysis of Hsp90 function: interactions with a steroid receptor and a protein kinase. *Mol Cell Biol* 15:3917–3925
- Pan X, Ye P, Yuan DS, Wang X, Bader JS, Boeke JD (2006) A DNA integrity network in the yeast *Saccharomyces cerevisiae*. *Cell* 124:1069–1081. doi:<https://doi.org/10.1016/j.cell.2005.12.036>
- Pratt WB, Toft DO (2003) Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med* 228:111–133
- Richard M, Boulin T, Robert VJ, Richmond JE, Bessereau JL (2013) Biosynthesis of ionotropic acetylcholine receptors requires the evolutionarily conserved ER membrane complex. *Proc Natl Acad Sci USA* 110:E1055–E1063. doi:<https://doi.org/10.1073/pnas.1216154110>
- Sakisaka T, Meerlo T, Matteson J, Plutner H, Balch WE (2002) Rab-alphaGDI activity is regulated by a Hsp90 chaperone complex. *EMBO J* 21:6125–6135
- Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, Hartl FU, Moarefi I (2000) Structure of TPR domain–peptide complexes. *Cell* 101:199–210. doi:[https://doi.org/10.1016/S0092-8674\(00\)80830-2](https://doi.org/10.1016/S0092-8674(00)80830-2)
- Schopf FH, Biebl MM, Buchner J (2017) The HSP90 chaperone machinery. *Nat Rev Mol Cell Biol* 18:345–360. doi:<https://doi.org/10.1038/nrm.2017.20>
- Schuldiner M, Collins SR, Thompson NJ, Denic V, Bhamidipati A, Punna T, Ihmels J, Andrews B, Boone C, Greenblatt JF, Weissman JS, Krogan NJ (2005) Exploration of the function and organization of the yeast early secretory pathway through an epistatic miniarray profile. *Cell* 123:507–519. doi:<https://doi.org/10.1016/j.cell.2005.08.031>
- Schuldiner M, Metz J, Schmid V, Denic V, Rakwalska M, Schmitt HD, Schwappach B, Weissman JS (2008) The GET complex mediates insertion of tail-anchored proteins into the ER membrane. *Cell* 134:634–645. doi:<https://doi.org/10.1016/j.cell.2008.06.025>
- Shelton LB, Baker JD, Zheng D, Sullivan LE, Solanki PK, Webster JM, Sun Z, Sabbagh JJ, Nordhues BA, Koren J III, Ghosh S, Blagg BSJ, Blair LJ, Dickey CA (2017) Hsp90 activator Aha1 drives production of pathological tau aggregates. *Proc Natl Acad Sci USA*
- Shen X, Kan S, Hu J, Li M, Lu G, Zhang M, Zhang S, Hou Y, Chen Y, Bai Y (2016) EMC6/TMEM93 suppresses glioblastoma proliferation by modulating autophagy. *Cell Death Dis* 7:e2043. doi:<https://doi.org/10.1038/cddis.2015.408>
- Sigrist CJ et al (2013) New and continuing developments at PROSITE. *Nucleic Acids Res* 41:D344–D347. doi:<https://doi.org/10.1093/nar/gks1067>
- Stark C, Breitkreutz BJ, Reguly T, Boucher L, Breitkreutz A, Tyers M (2006) BioGRID: a general repository for interaction datasets. *Nucleic Acids Res* 34:D535–D539. doi:<https://doi.org/10.1093/nar/gkj109>

- Surma MA, Klose C, Peng D, Shales M, Mrejen C, Stefanko A, Braberg H, Gordon DE, Vorkel D, Ejsing CS, Farese R Jr, Simons K, Krogan NJ, Ernst R (2013) A lipid E-MAP identifies Ubx2 as a critical regulator of lipid saturation and lipid bilayer stress. *Mol Cell* 51: 519–530. <https://doi.org/10.1016/j.molcel.2013.06.014>
- Tenge VR, Zuehlke AD, Shrestha N, Johnson JL (2015) The Hsp90 cochaperones Cpr6, Cpr7, and Cns1 interact with the intact ribosome. *Eukaryot Cell* 14:55–63. <https://doi.org/10.1128/EC.00170-14>
- Wang F, Brown EC, Mak G, Zhuang J, Denic V (2010) A chaperone cascade sorts proteins for posttranslational membrane insertion into the endoplasmic reticulum. *Mol Cell* 40:159–171. <https://doi.org/10.1016/j.molcel.2010.08.038>
- Wang Y, Jin F, Wang R, Li F, Wu Y, Kitazato K, Wang Y (2017) HSP90: a promising broad-spectrum antiviral drug target. *Arch Virol* 162: 3269–3282. <https://doi.org/10.1007/s00705-017-3511-1>
- Wideman JG (2015) The ubiquitous and ancient ER membrane protein complex (EMC): tether or not? *F1000Research* 4:624. <https://doi.org/10.12688/f1000research.6944.2>
- Xu Y, Lindquist S (1993) Heat-shock protein hsp90 governs the activity of pp60v-src kinase *Proc Natl Acad Sci USA* 90:7074–7078
- Zhao R, Davey M, Hsu YC, Kaplanek P, Tong A, Parsons AB, Krogan N, Cagney G, Mai D, Greenblatt J, Boone C, Emili A, Houry WA (2005) Navigating the chaperone network: an integrative map of physical and genetic interactions mediated by the hsp90 chaperone. *Cell* 120:715–727. <https://doi.org/10.1016/j.cell.2004.12.024>