# Hsp90: from structure to phenotype

Sophie E Jackson, Christine Queitsch & David Toft

**A recent international conference focused on Hsp90, a molecular chaperone that plays a critical role in a diverse array of cellular processes including the assembly and maturation of some important 'client' proteins, many of which are involved in signal transduction.**

Hsp90 forms a central part of the cellular assembly (and, as recent work suggests, disassembly) machine[1]. This chaperone machine aids in the folding, assembly-disassembly and activation of a wide range of substrate or client proteins, including many kinases and transcription factors as well as other proteins (**Fig. 1**). As such, Hsp90 is central to many cellular processes including growth, cell cycling, apoptosis, cancer, stress response, endocrine function, plant immunity, development and even evolution[2]. Since Hsp90 was first shown to be the target of the ansamycin anti-tumor agent geldanamycin, there has also been increasing interest in Hsp90 as a therapeutic target[3].

Hsp90 is a large, homodimeric protein with three main structural domains[4]. The N-terminal domain contains the ATP- and geldanamycin-binding site, and is responsible for the weak intrinsic ATPase activity of Hsp90 . The middle domain, which is thought to be the major site of client protein binding, is connected to the N-terminal domain through a highly charged linker region. The C-terminal domain contains the dimerization interface and a conserved C-terminal MEEVD motif, which is responsible for binding TPR-containing cochaperones. Cytosolic Hsp90 binds to many other proteins (collectively termed cochaperones) that regulate its activity and form part of the Hsp90 assembly machine (**Fig. 1**). In contrast to many other molecular chaperones, Hsp90 acts on a specific set of client proteins.

*S.E.J. is in the Chemistry Department, Cambridge University, Lensfield Road, Cambridge, CB2 1EW, UK. C.Q. is at the Bauer Center for Genomics Research, Harvard University, Cambridge, Massachusetts 02138, USA. D.T. is in the Department of Biochemistry and Molecular Biology, Mayo Clinic, College of Medicine, 200 First Street SW, Rochester, Minnesota 55905, USA.*
*e-mail: sej13@cam.ac.uk, CQueitsch@CGR.harvard.edu and toft.david@mayo.edu*

The European Molecular Biology Organization Workshop and 2nd International Conference on the Hsp90 Chaperone Machine* was held in the Swiss village of Gwatt on the banks of Lake Thun, surrounded by the Bernese Alps, from 25 to 29 September 2004. The success of the conference was due, in large part, to the efforts of the organizers: Johannes Buchner (Technische Universität München) and Didier Picard (Université de Genève). They not only chose a superb venue, but put together an exciting scientific program.

The conference covered a wide range of topics addressing all aspects of Hsp90 structure and function, from biophysical, mechanistic and structural studies to the role of Hsp90 on an organismal level. Several reports on new cochaperones demonstrated the complexity and versatility of the Hsp90 machine. The expanding role of Hsp90 in a diverse array of cellular processes was illustrated in many talks, including those on the endoplasmic reticulum (ER) and *Escherichia coli* homologs Grp94 and HtpG. A session focused on drugs and diseases highlighted the importance of Hsp90 as a pharmaceutical target.

## New structural and mechanistic insights into Hsp90 function

Although Hsp90 interacts with a relatively small set of client proteins as compared with other general chaperones, these clients have quite different structures and functions. Understanding how Hsp90 specifically recognizes its clients,

therefore, represents a considerable challenge. Several talks addressed this important question by identifying the binding regions on client proteins as well as in Hsp90 itself. Ami Citri from the Weizmann Institute presented recent work on the erbB receptor family establishing a key sequence in erbB2 that mediates recognition by Hsp90. This sequence is located on a surface loop in the kinase domain of the receptor and is important in regulating dimerization and therefore the kinase activity.

Structural studies on Hsp90 and its homologs have also provided important information on how client proteins might be recognized and bound by Hsp90. David Agard (University of California San Francisco) presented recent work on the structure of the C-terminal domain of the *E. coli* homolog HtpG[5]. The structure shows an unusual solvent-exposed α-helix. When the structure is docked into a 3.5-Å molecular envelope of the full-length protein, these helices project toward what is thought to be the central, client protein–binding cavity. The sequence of these helices is remarkably similar to that of helix 12 of the steroid receptor family, leading to the suggestion that this helix may displace helix 12 on the receptors, thus facilitating ligand binding.

The ATPase activity of Hsp90 is known to be essential for its function and mutants with hyper- and hypo-ATPase activity have compromised function *in vivo*. The middle domain of Hsp90 is also known to play a key role in the binding of client proteins, and previous structural work showed that it can interact with and affect the activity of the N-terminal ATPase domain. The structural basis for the stimulation of ATPase activity by the cochaperone Aha1 was presented by Chris Prodromou (Institute of Cancer Research, London). Aha1 binding to the middle domain of Hsp90 repositions a loop in the middle domain such that it optimizes interactions with the N-terminal domain critical for ATP hydrolysis[6]. Johannes Buchner presented work showing that the cochaperone Sti1 inhibits the ATPase activity of Hsp90 but, in addition, stimulates the ATPase of Hsp70 (ref. 7). As Sti1 binds to both Hsp70 and Hsp90, it is thus critical in mediating the transfer of client proteins. Stimulation of the ATPase activity of Hsp70 promotes dissociation of the client protein from Hsp70, whereas inhibition of the ATPase activity of Hsp90 facilitates association of the client with Hsp90.

## The role of conformational changes in Hsp90 action

Conformational changes within and between domains are known to be critical to Hsp90 function. Conformational changes induced in Hsp90 upon nucleotide and inhibitor binding were addressed by several speakers. Chris Prodromou presented further evidence for an N-terminal dimerization model for yeast Hsp90, whereas Sophie Jackson (Cambridge University) presented an alternative model for human Hsp90. In this second model, ATP binding is followed by a conformational change, but this does not involve dimerization of the N-terminal domains[8]. Changes to the structure of Hsp90 upon nucleotide or inhibitor binding were nicely illustrated in two talks by David Agard and Dan Gewirth (Duke University Medical Center). Dan Gewirth showed that in the structure of the Grp94 N-terminal domain a hydrophobic patch is exposed, leading to the suggestion that this may be another potential cochaperone or client-protein binding site. Remarkably similar conformational changes were observed by David Agard when comparing the isolated N-terminal domain of HtpG with ADP and the full-length apo structure. David Agard presented very new and exciting results on the high-resolution structure of full-length HtpG in both apo and ADP-bound states, revealing dramatic alterations in domain relationships and suggesting a model for how ATPase is activated. Dan Gewirth presented several structures of the N-terminal domain of Grp94 in complex with nucleotides and inhibitors. There are substantial differences in the mechanism of action of Grp94 compared with that of Hsp90 (no cochaperones have yet been identified and there is no detectable ATPase activity), and subtle structural differences may account for the different modes of action. In particular, nucleotides and inhibitors induce different conformations of the lid: the inhibitors stabilize a closed conformation whereas nucleotides force the lid into an open conformation that allows N-domain dimerization[9]. The differences can be attributed to an insert of five amino acids in Grp94 that repositions a glycine in the ATP-binding site. From all the structural and mechanistic studies, it is becoming clear that Hsp90 can adopt numerous conformations and may be very flexible in solution in the absence of client protein or cochaperones.

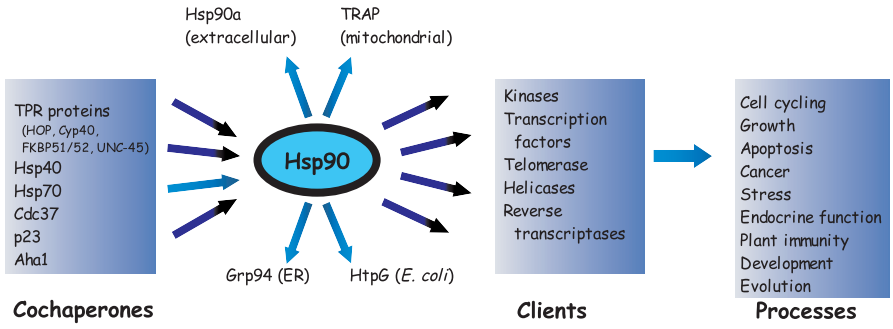


**Figure 1** Cytosolic Hsp90 and its homologs Grp94, TRAP and HTPG along with numerous cochaperones form the cellular assembly machine. They act on a range of client proteins and thereby control many cellular processes.

## Hsp90 as a therapeutic target

The fact that Hsp90 has become such a popular target is due, in part, to the intrinsic selectivity of tumor cells toward geldanamycin (GA) and its derivatives. The molecular basis for this has not been understood. Francis Burrows (Conforma Therapeutics) presented work addressing this important issue. He showed that Hsp90 in tumor cells is present in a highly active state in which it is in complex, has a high ATPase activity and high affinity for inhibitors such as GA[10]. Although more work is needed to characterize this state in detail, this result has provided a much-needed model for the differences in Hsp90 activity in normal and tumor cells. Paul Workman (Institute for Cancer Research) reported on the success of phase I clinical trials with the geldanamycin derivative 17-AAG, describing in detail the effects of this drug on the cellular levels of important biomarker proteins such as cdk4 and raf[3]. Preliminary trials using combinatorial approaches where 17-AAG is used in conjunction with other drugs such as the taxanes show much promise as an alternative therapeutic strategy. Combinatorial therapeutic approaches were also described by Len Neckers (National Cancer Institute, National Institutes of Health), in this case with inhibitors of Hsp90 and the proteasome. He also described recent experiments on the effects of Hsp90 inhibitors on cell migration and tumor invasiveness that have come up with surprising results. In these cases, even cell-impermeable inhibitors are active, leading to the discovery of an extracellular form of Hsp90, Hsp90α. Hsp90α seems to play a key role in the activity of matrix metalloproteases (MMPs), which are known to

control cell migration. However, caution was stressed in a study presented by John Price (St. Vincent's Institute, Melbourne, Australia) showing an enhancement of bone loss in response to 17-AAG treatment. In a mouse model, 17-AAG increased tumor-associated bone destruction by stimulating osteoclast formation.

## Cochaperones

The diverse activities of Hsp90 seem to be coordinated in part by numerous cochaperone proteins that can bind Hsp90 and contribute specialized functions to the chaperone machine[11]. The actual functions of most of these cochaperones are still unclear. They may modulate specific steps in the chaperone pathway, assist in recruiting or chaperoning client proteins, or direct trafficking of chaperone complexes. However, it now seems that some Hsp90 cochaperones have a variety of activities, including some that aren't necessarily linked to Hsp90.

This is the case for the small, deceptively simple cochaperone p23, which binds to Hsp90 complexes late in the chaperoning process. Brian Freeman (University of Illinois) has studied the activity of p23 in facilitating the assembly and disassembly of transcription complexes of steroid receptors at specific gene loci[12]. In a more global approach, the localization of p23 (Sba1p in yeast) to specific sites in the yeast genome revealed many sites of interaction, including five sites within telomeric regions that were studied in detail. p23 may also have a role in apoptosis. Gro Gausdal (Haukeland University Hospital) showed that treatment of leukemic cell lines with chemotherapeutic drugs caused cleavage in the C-terminal tail of p23 by caspases-3 and 7. In this setting, the Hsp90 inhibitor geldanamycin did not promote apoptosis, but it enhanced anthracyclin-induced caspase activation, p23 cleavage and cell death. These results suggest a role of p23, and perhaps Hsp90, in cell survival signaling.

Another quite surprising function of p23 is in prostaglandin synthesis. p23 has been shown to have prostaglandin $E_2$ synthase activity[13]. As reported by Yoshihito Nakatani (Showa University), this activity is independent of Hsp90 although it is enhanced by Hsp90 and also by phosphorylation of p23. In mice, a homozygous knockout of p23 prepared by Didier Picard's group was lethal at or just before birth. However, embryonic development seemed almost normal with noticeable defects in skin and lung development. Thus, many biological processes can proceed in the absence of p23 and its multiple functions.

The Hsp90 and Hsp70 cochaperone Hop (Sti1) is not essential in yeast but its deletion enhances the sensitivity of yeast to mutations in related proteins. This approach was used by Jill Johnson (University of Idaho) to identify new proteins that are functionally linked to Hop. In this way, the ATP-dependent helicase SSL2/RAD25 was identified as a novel client protein that may also be dependent on Hsp90 chaperoning for its activity.

In another study on biological dependency, knockout mice were prepared by David Smith's group (Mayo Clinic) for the cochaperone FKBP52. This is one of several cochaperones that bind Hsp90 through tetratricopeptide repeat (TPR) domains, but the functions of FKBP52 are not well understood[14]. In most respects, the mice seem quite normal in the absence of FKBP52; however, both male and female mice show reproductive failure, indicating a need for FKBP52 in the development of reproductive systems. These studies and additional reports on HARC, Aha1, cdc37, GC UNC-45 and several plant cochaperones attest to the variety and high level of research activity on cochaperones.

## Hsp90 in plant immunity

Beyond exploring Hsp90 structure, its ATP hydrolysis cycle, and cochaperones, several speakers addressed the role of the Hsp90 chaperone machinery in an organismal context across different kingdoms of life. As Todd Sangster of the Lindquist laboratory (Whitehead Institute for Biomedical Research) pointed out, plants have emerged as powerful complementary tools to fungi and mammalian models for investigating the multifaceted functions of this complex chaperone. Owing to their sessile life style and continuous development, plants are intricately linked to their environment, probably enhancing the importance of Hsp90-mediated chaperoning of signaling transduction cascades. Whereas much remains to be done to get Hsp90 plant research on even footing with other models, the comprehensive set of *Arabidopsis thaliana* lines reduced in the cytosolic Hsp90 isoforms that were presented by Sangster will considerably aid future efforts to elucidate Hsp90 function and degree of isoform redundancy.

Like animals, plants have evolved elaborate defense systems against pathogens. Ken Shirasu (The Sainsbury Laboratory) introduced resistance protein (R-protein)-triggered plant immunity and presented evidence for a role of plant Hsp90 and the proposed cochaperones RAR1 and the TPR-containing SGT1 in pathogen recognition and R-protein stability[15]. Shirasu presented evidence that SGT1 binds to Hsp90 through its middle region (CS), which is structurally similar to p23, and that several mutations in this region resulted in loss of Hsp90 binding and disease resistance. Both RAR1 and SGT1 can interact with Hsp90 independently of each other and it is unclear if and how they modulate Hsp90's proposed function in R-protein folding and/or stabilization.

Hsp90's ATPase activity is crucial for disease resistance. Several *hsp90.2* mutant alleles identified by David Hubert of the Dangl laboratory (University of North Carolina) are likely to disrupt ATP contacts[16]. In contrast to *Hsp90.2*-null alleles, these mutants show impaired disease resistance mediated by the R-protein RPM1 in plants. *hsp90.2* and *rar1* mutants can show constitutively decreased RPM1 protein. *RAR1*-dependent R-protein stability and or function, however, can hinge on subtle differences as reported by Ken Shirasu and by Stephan Mauch of the Schulze-Lefert laboratory[17] (Max-Planck-Institut für Züchtungsforschung). For example, the barley R-protein MLA6 requires *RAR1*, whereas resistance mediated by the 91% identical MLA1 is not affected. In another apparent analogy to the animal model, Adina Breiman (Tel Aviv University) reported that other TPR-domain-containing cochaperones, the plant FKPBs, may also affect plant immunity. It remains to be shown whether some of the mechanics of steroid hormone receptor maturation, including the sequential binding of different cochaperones and ATP hydrolysis, have parallels in the maturation of R-proteins.

## Hsp90 as a capacitor of phenotypic variation

Alternatively, but by no means mutually exclusively, the association of the plant Hsp90s and the R-proteins may indicate another facet of Hsp90 function. R-proteins are highly polymorphic, probably reflecting the dynamics of ongoing plant and pathogen interactions[18]. Specifically, the LRR domain of R-proteins, which is contacted by the Hsp90 complex, is under diversifying selection[19]. Hsp90 has been shown to 'hide' the phenotypic consequences of underlying polymorphisms in plants and fruit flies[20,21]. If Hsp90 function is challenged by environmental stress or mutation, such polymorphisms can be expressed, prompting the suggestion that Hsp90-mediated storage and release may influence evolutionary processes. It is an intriguing possibility that the interaction with Hsp90 may aid the diversification of R-proteins by buffering their intrinsic structural instability[22].

So far, neither the frequency nor the identity of Hsp90-buffered polymorphisms has been reported, parameters that are important to judge whether this phenomenon has the suggested implications for evolution. Christine Queitsch (Harvard University) outlined her strategy to elucidate the frequency and identity of Hsp90-buffered polymorphisms in

many phenotypic traits in naturally existing *Arabidopsis* populations. Using a quantitative genetics approach, she presented results of a pilot study on stem length that uncovered novel Hsp90-dependent genomic loci.

The protozoan parasite *Leishmania donovani* exploits Hsp90's environmental malleability to switch between its two life stages: the insect-specific promastigote stage and the mammalian-specific amastigote stage[23]. Joachim Clos (Bernhard Nocht Institute for Tropical Medicine) previously showed that inhibition of Hsp90 with geldanamycin is sufficient for differentiation from promastigote to amastigote. At the meeting, he reported the existence of higher-order Hsp90 complexes in the rapidly growing promastigotes that disappear in the slower growing amastigotes, along with results of proteome analysis for both life stages.

## Functioning without a cohort of cochaperones

Prokaryotic HtpG and the organelle-specific Hsp90s, GRP94 and TRAP1, seem to act without cochaperones. A cellular function for these 'lonely' chaperones is established only for GRP94, whereas we know little about the bacterial homolog HtpG, which lacks a marked mutant phenotype and is absent in many prokaryotes.

Thus far the only prokaryotic organism with an established function for HtpG is the cyanobacterium *Synechococcus* sp. PCC7942. HtpG is required for basal and acquired thermotolerance, acclimation to low-temperature stress, and oxidative stress[24–26]. Hitoshi Nakamoto (Saitama University) reported that *htpG* mutants show reduction of photosynthetic pigments under normal conditions. This reduction involves instability of linkers that connect hexamers of the pigment phycocyanin to assemble phycobilisomes, the primary light-harvesting complexes. A yeast two-hybrid approach identified a protein functioning in heme biosynthesis, the uroporphyrinogen decarboxylase HemE, as an HtpG-interacting protein, possibly representing a client.

Queitsch used phylogenetic profiling to determine genes whose presence or absence is correlated with *HtpG* presence across 63 sequenced organisms. Among the genes associated with Hsp90 presence are some that are involved in stress responses and motility as well as others implicated in DNA repair after UV treatment.

Based on his data on the regulation of GRP94-client interaction by ATP and ADP, Chris Nicchitta (Duke University Medical Center) proposed that levels of adenosine nucleotides may serve as a 'stress sensor' for the ER-specific Hsp90. Decreases in adenosine nucleotide levels would allow for derepression of GRP94, facilitating interaction with clients.

As Yair Argon (University of Pennsylvania) reported, loss of GRP94 function in mice causes embryonic lethality, owing to a failure to develop the mesoderm germ layer. Mutant embryonic stem (ES) cells give rise to a number of cell types with the exception of muscle cells and show inhibition of $Ca^{2+}$ homeostasis and hypersensitivity to serum deprivation. Both mutant animals and ES cells represent excellent models for studying mammalian *GRP94* function *in vivo*.

## Perspective

As amply demonstrated in Gwatt, the Hsp90 chaperone machine is moving forward and gaining momentum. We have made progress in understanding how Hsp90's structural features may determine its cellular function. Mutation screens and transgenic models have added new Hsp90 clients and shed light on cochaperone function *in vivo*. We have learned that cochaperones may function independently of Hsp90. Most importantly, Hsp90 has emerged as a promising drug target for cancer therapy. In short, we are looking forward to meeting again in two years to see how much farther the Hsp90 chaperone machine has taken us. ■

**The EMBO workshop and second international conference on the Hsp90 Chaperone Machine was held in Gwatt, Switzerland, 25–29 September, 2004. For other information about the conference, see http://www.hsp90.org.*


1. Wegele, H., Muller, L. & Buchner, J. *Rev. Physiol. Biochem. Pharmacol.* **151**, 1–44 (2004).
2. Picard, D. *Cell. Mol. Life Sci.* **59**, 1640–1648 (2002).
3. Workman, P. *Cancer Lett.* **206**, 149–157 (2004).
4. Pearl, L.H. & Prodromou, C. *Adv. Prot. Chem.* **59**, 157–186 (2002).
5. Harris, S.F., Shiau, A.K. & Agard, D.A. *Struct. Fold. Des.* **12**, 1087–1097 (2004).
6. Meyer, P. *et al. EMBO J.* **23**, 511–519 (2004).
7. Wegele, H., Haslbeck, M., Reinstein, J. & Buchner, J. *J. Biol. Chem.* **278**, 25970–25976 (2003).
8. McLaughlin, S.M., Ventouras, L.-A., Lobbezoo, B.G. & Jackson, S.E. *J. Mol. Biol.* **344**, 813–826 (2004).
9. Immormino, R.M. *et al. J. Biol. Chem.* **279**, 46162–46171 (2004).
10. Kamal, A. *et al. Nature* **425**, 407–410 (2003).
11. Caplan, A.J. *Cell Stress Chaperones* **8**, 105–107 (2003).
12. Freeman, B.C. & Yamamoto, K.R. *Science* **296**, 2232–2235 (2002).
13. Tanioka, T., Nakatani, Y., Wemmyo, N., Murakami, M. & Kudo, I. *J. Biol. Chem.* **275**, 32775–32782 (2000).
14. Smith, D.F. *Cell Stress Chaperones* **9**, 109–121 (2004).
15. Takahashi, A., Casais, C., Ichimura, K. & Shirasu, K. *Proc. Natl. Acad. Sci. USA* **100**, 11777–11782 (2003).
16. Hubert, D.A. *et al. EMBO J.* **22**, 5679–5689 (2003).
17. Shen, Q.H. *et al. Plant Cell* **15**, 732–744 (2003).
18. Stahl, E.A., Dwyer, G., Mauricio, R., Kreitman, M. & Bergelson, J. *Nature* **400**, 667–671 (1999).
19. Bergelson, J., Kreitman, M., Stahl, E.A. & Tian, D. *Science* **292**, 2281–2285 (2001).
20. Queitsch, C., Sangster, T.A. & Lindquist, S. *Nature* **417**, 618–624 (2002).
21. Rutherford, S.L. & Lindquist, S. *Nature* **396**, 336–342 (1998).
22. Schulze-Lefert, P. *Curr. Biol.* **14**, R22–R24 (2004).
23. Wiesgigl, M. & Clos, J. *Mol. Biol. Cell* **12**, 3307–3316 (2001).
24. Hossain, M.M. & Nakamoto, H. *Curr. Microbiol.* **46**, 70–76 (2003).
25. Hossain, M.M. & Nakamoto, H. *Curr. Microbiol.* **44**, 291–296 (2002).
26. Tanaka, N. & Nakamoto, H. *FEBS Lett.* **458**, 117–123 (1999).