Just say NO: nitric oxide regulation of Hsp90

The 90 kDa heat-shock protein (Hsp90) was first identified as being expressed in response to elevated temperature. Since this initial observation, Hsp90 has been shown to be a molecular chaperone heavily involved in the regulation of signal transduction through its crucial role in folding and stabilizing hundreds of proteins that constitute nodal points in numerous signalling pathways (www.picard.ch/downloads/Hsp90interactors.pdf).

The chaperone activity of Hsp90 depends on ATP binding and hydrolysis, which is coupled to a conformational cycle that involves the opening and closing of a dimeric molecular clamp formed by the transient association of the amino-terminal domains of Hsp90 (Pearl & Prodromou, 2006). In solution, the protein exists as a dimer owing to the constitutive association of highly conserved motifs in its carboxy-terminal domain. ATP binds to the N-terminal domains of Hsp90, stabilizing their transient dimerization (Prodromou *et al*, 1997). In bacteria, Hsp90 conformational dynamics seem to be determined solely by nucleotide binding and hydrolysis (Graf *et al*, 2009). Hsp90 co-chaperones—which are found only in eukaryotes—provide an additional layer of regulation to the Hsp90 chaperone cycle by enhancing (in the case of Aha1) or slowing (for example, Sti1) the rate of Hsp90 ATPase activity (Fig 1). In multicellular organisms, certain cell-specific post-translational modifications of Hsp90 also affect its ATPase activity, as well as its interactions with co-chaperones and client proteins. This adds a third layer of regulation to the Hsp90 chaperone cycle that provides cells and tissues with the means to fine-tune Hsp90 function in direct response to unique local environmental conditions (Scroggins & Neckers, 2007).

An emerging example of such regulation is the S-nitrosylation of Hsp90 by its client protein, endothelial nitric oxide synthase (eNOS). In this issue of *EMBO reports*, the Buchner laboratory provides some mechanistic insight into the importance of this process (Retzlaff *et al*, 2009). As its name implies, eNOS is found in endothelial cells, and eNOS-dependent nitric oxide (NO) production is essential for cardiovascular homeostasis, vasodilation, and the growth and survival of vascular endothelial cells. Initial studies demonstrated that Hsp90 interacts with eNOS to positively regulate its activity (Garcia-Cardena *et al*, 1998).

S-nitrosylation is the reversible but covalent modification of reactive cysteine thiols in proteins by NO (Hess *et al*, 2005). Martinez-Ruiz and colleagues first reported that human HSP90 α was S-nitrosylated in endothelial cells, and showed that this modification occurred at Cys 597 (Martinez-Ruiz *et al*, 2005). The secondary structure surrounding Cys 597 is conserved in Hsp90 homologues from a variety of species, and a cysteine is found at this position in the Hsp90 proteins of most multicellular eukaryotes (Retzlaff *et al*, 2009). Cys 597 is located in a three-stranded β -sheet that is in close proximity to the C-terminal dimer interface and also to the region

of human HSP90 where eNOS is thought to interact (Fontana *et al*, 2002). As it is located at the opposite end of the protein from the ATPase domain, this residue would not be predicted to influence HSP90 ATPase activity. However, S-nitrosylation of Cys 597 was reported to inhibit HSP90 ATPase activity, reducing its ability to activate eNOS *in vitro* (Martinez-Ruiz *et al*, 2005). The authors proposed a feedback mechanism in which HSP90-dependent eNOS activation generates NO, which in turn reacts with Cys 597 to inhibit further eNOS activation. In this model, HSP90 acts as a molecular sensor of local NO concentration and adjusts NO production accordingly. Although this proposal was intriguing, how S-nitrosylation of Cys 597 might inhibit HSP90 ATPase activity was not explained. A recent study using molecular dynamics simulations suggested a possible explanation by showing that communication between the N-domain and C-domain of yeast Hsp90 is highly responsive to nucleotide binding (Morra *et al*, 2009). Specifically, the binding of ATP to the N-domain was shown to propagate long-range communication with a C-terminal segment encompassing residues 574–580 (Ala 577 is the equivalent residue in yeast Hsp90 to Cys 597 in the human homologue), followed by a coordinated conformational change throughout Hsp90 leading to an ATPase competent state.

In this issue of *EMBO reports*, Retzlaff and colleagues show the importance of Cys 597 in modulating Hsp90 ATPase activity (Retzlaff *et al*, 2009). Surprisingly, the replacement of Ala 577 with cysteine or isoleucine in yeast Hsp90 was found to increase the basal ATPase activity by several orders of magnitude compared with the wild-type protein. When the yHsp90A577C mutant was S-nitrosylated *in vitro*, its ATPase activity was reduced to the level of wild type. Furthermore, the substitution of asparagine—a mimic of nitrosylated cysteine—for Ala 577 diminished the activity twofold compared with wild type. Given the much lower basal ATPase activity of human HSP90 than the yeast protein, attempts to detect the impact of S-nitrosylation on the human wild-type protein were unsuccessful. However, the authors observed that S-nitrosylation caused a marked inhibition of Aha1-stimulated HSP90 ATPase activity. Furthermore, S-nitrosothiol-mimicking mutants of both yeast and human HSP90 displayed markedly reduced responsiveness to, and affinity for, Aha1.

To further characterize the role of S-nitrosylation in Hsp90 dynamics, the Buchner group also showed that C-terminal dimers of yHsp90A577I—which has high ATPase activity—had a longer half-life than wild type. By contrast, the half-life of subunit exchange of yHsp90A577N—which has low ATPase activity—was relatively brief. The strength of dimerization of the C-terminal domains of yHsp90A577I was also greater than that of wild type, whereas the strength of dimerization of the C-terminal domain of the A577N mutant was weaker. Nucleotide-dependent N-terminal dimerization of yHsp90A577N was also significantly impaired. The impact of S-nitrosylation- and S-nitrosothiol-mimicking mutants on Hsp90 ATPase activity, N-domain dimerization kinetics and C-terminal dissociation constants indicates that this modification has a profound impact on Hsp90 conformational

Fig 1 | Hsp90 conformational dynamics. (A) Hsp90 cycles between ATPase-competent and ATPase-incompetent states. ATP binding to the amino-terminal (N) domains of Hsp90 stabilizes their transient association, which is essential for subsequent ATP hydrolysis. Hsp90 is constitutively dimerized through motifs in the carboxy-terminal (C) domain of each protomer. (B) ATP binding to the N-terminal domain propagates a conformational signal to the C-terminal domain concurrent with acquisition of the ATPase-competent conformation. ATPase activity is enhanced—and Hsp90 cycling increased—by the binding of the co-chaperone Aha1 to the middle domain of Hsp90. S-nitrosylation of Cys 597 in the C-terminal domain disrupts ATP-induced signal propagation from N-terminal to C-terminal domains (and vice versa), inhibits ATPase activity by preventing the necessary conformational change and Aha1-mediated stimulation, and weakens C-terminal association of Hsp90 protomers.

dynamics, and favours an ATPase-incompetent state in which the N-terminal domains are not dimerized (Fig 1). The chaperone activity of such an Hsp90 conformation is compromised, as is the chaperone activity of S-nitrosylated and mutant Hsp90 proteins.

These findings—as useful experiments should do—raise many questions that are important to address. Exactly how does S-nitrosylation of Hsp90 affect its conformational dynamics? Is S-nitrosylation of Cys 597 in higher eukaryotes a client-specific regulatory mechanism or are other Hsp90 clients also regulated by modification of Cys 597? Does S-nitrosylation of Hsp90 affect its binding to co-chaperones and eNOS in a manner that is consistent with the impact of this modification on Hsp90 conformational dynamics? The nitric oxide synthases nNOS and iNOS are also activated by Hsp90 (Peng *et al*, 2009; Yoshida & Xia, 2003); can these and other physiological sources of NO promote S-nitrosylation of Hsp90? Why does Hsp90 in higher eukaryotes seem to be subject to a greater degree of fine-tuning than yeast Hsp90? Could an expanded set of post-translational modification sites have coevolved with a larger clientele in higher eukaryotes and, if so, to what end? Does S-nitrosylation of Cys 597 or mutation of Ala 577 affect the affinity of C-terminal-binding Hsp90 inhibitors such as novobiocin,

or of N-terminal inhibitors such as geldanamycin? Lastly, NO is emerging as a potential anticancer agent that can overcome tumour-cell resistance to conventional chemotherapy (Huerta *et al*, 2008). Hsp90 inhibitors share this property and are being developed clinically with this characteristic as a major focus. Could the antitumour activity of NO be mediated, at least in part, through its ability to S-nitrosylate and inactivate Hsp90 in cancer cells? Answering these questions will allow us to better understand the complex regulation of this essential molecular chaperone in higher eukaryotes.

REFERENCES

- Fontana J, Fulton D, Chen Y, Fairchild TA, McCabe TJ, Fujita N, Tsuruo T, Sessa WC (2002) Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. *Circ Res* **90**: 866–873
- Garcia-Cardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, Sessa WC (1998) Dynamic activation of endothelial nitric oxide synthase by Hsp90. *Nature* **392**: 821–824
- Graf C, Stankiewicz M, Kramer G, Mayer MP (2009) Spatially and kinetically resolved changes in the conformational dynamics of the Hsp90 chaperone machine. *EMBO J* **28**: 602–613
- Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS (2005) Protein S-nitrosylation: purview and parameters. *Nat Rev Mol Cell Biol* **6**: 150–166
- Huerta S, Chilka S, Bonavida B (2008) Nitric oxide donors: novel cancer therapeutics (review). *Int J Oncol* **33**: 909–927
- Martinez-Ruiz A, Villanueva L, Gonzalez de Orduna C, Lopez-Ferrer D, Higuera MA, Tarin C, Rodriguez-Crespo I, Vazquez J, Lamas S (2005) S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities. *Proc Natl Acad Sci USA* **102**: 8525–8530
- Morra G, Verkhivker G, Colombo G (2009) Modeling signal propagation mechanisms and ligand-based conformational dynamics of the Hsp90 molecular chaperone full-length dimer. *PLoS Comput Biol* **5**: e1000323
- Pearl LH, Prodromou C (2006) Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annu Rev Biochem* **75**: 271–294
- Peng HM, Morishima Y, Clapp KM, Lau M, Pratt WB, Osawa Y (2009) Dynamic cycling with Hsp90 stabilizes neuronal nitric oxide synthase through calmodulin-dependent inhibition of ubiquitination. *Biochemistry* [published online, 30 July] doi:10.1021/bi901058g
- Prodromou C, Roe SM, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1997) Identification and structural characterization of the ATP/ADP-binding site in the Hsp90 molecular chaperone. *Cell* **90**: 65–75
- Retzlaff M, Stahl M, Eberl HC, Lagleder S, Beck J, Kessler H, Buchner J (2009) Hsp90 is regulated by a switch point in the C-terminal domain. *EMBO Rep* **10**: 1147–1153
- Scroggins BT, Neckers L (2007) Post-translational modification of heat-shock protein 90: impact on chaperone function. *Expert Opin Drug Discov* **2**: 1403–1414
- Yoshida M, Xia Y (2003) Heat shock protein 90 as an endogenous protein enhancer of inducible nitric-oxide synthase. *J Biol Chem* **278**: 36953–36958

Bradley T. Scroggins and Len Neckers⁺ are at the Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, 9000 Rockville Pike, Hatfield Clinical Research Center, Room 1-5940, Bethesda, Maryland 20892, USA

⁺Corresponding author. Tel: +1 301 496 5899;

Fax: +1 301 402 0922;

E-mail: len@helix.nih.gov

Keywords: Hsp90; molecular chaperone; S-nitrosylation

Submitted 7 August 2009; accepted 25 August 2009;

published online 18 September 2009

EMBO reports (2009) **10**, 1093–1094. doi:10.1038/embo.2009.212