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A stress protein interface of innate immunity

What seems to be a 'mere' cofactor of a core factor to some, might be the main component to others; it is all a question of perspective and, probably quite often, pure semantics. SGT1 (SUPPRESSOR OF G2 ALLELE OF SKP1) was discovered genetically before it was recognized as being a cofactor of the molecular chaperone HSP90 (HEAT-SHOCK PROTEIN 90). The molecular details of this interaction and its physiological relevance are the subject of a paper from the Shirasu and Guerois groups, in which they combine structural biology, biochemistry and genetics (Kadota *et al*, 2008, this issue). The interaction surfaces were analysed by nuclear magnetic resonance (NMR) spectroscopy and by testing point mutants with pull-down experiments for interaction *in vitro* and by testing them for function in the resistance of plants against pathogens *in vivo*. If these approaches left any doubt that the protein–protein interactions are real, then the *in vivo* complementation by mutants with compensatory swaps of amino acids in the interface between the two partner proteins leaves no room for appeal. The purpose of this protein complex is to support crucial pathogen-sensor proteins of the plant immune system—the NB-LRR (NUCLEOTIDE BINDING AND LEUCINE-RICH REPEAT) proteins—of which *Arabidopsis* encodes approximately 125 (Jones & Dangl, 2006). Remarkably, both the SGT1–HSP90 molecular chaperone machine and its targets, the NB-LRR proteins, also have a function in the innate immune responses of animals (da Silva Correia *et al*, 2007; Mayor *et al*, 2007).

HSP90 is usually considered to be the central subunit of a molecular machine. It owes this prominent role to the fact that it is an abundant cytosolic protein, perhaps even the most abundant protein in unstressed cells (Lai *et al*, 1984), and that it has ATPase activity, suggesting that it might 'burn' ATP to function as a molecular chaperone (Picard, 2002; Pearl & Prodromou, 2006; Neckers, 2007; Wandinger *et al*, 2008). Proteins that depend on HSP90 for folding, stability, assembly and/or function are known as substrates or 'clients', and their number continues to increase (for an updated list, see http://www.picard.ch/downloads/Hsp90interactors.pdf). A characteristic of HSP90 substrates is that they are degraded by the proteasome when HSP90 function is inhibited pharmacologically or genetically (Whitesell & Lindquist, 2005; Pearl *et al*, 2008). However, exactly what HSP90 does to its substrates and how remains poorly understood and is the subject of intense research.

Over the past two decades, it has been recognized that HSP90 does not act alone. Cofactors—known as co-chaperones in this context—bind to HSP90 in a multitude of assortments to regulate its interactions with and effects on substrates, or its ATPase activity. So far, there are approximately 30 proteins in this category, which is far too many to have a clear picture of how they work together. Even the term 'co-chaperone' is not as clear as it might seem. The distinction between a substrate and a co-chaperone can be difficult to make; for example, the 'established' co-chaperones AHA1 (ACTIVATOR OF HSP90 ATPASE 1) and CPR6 (CYCLOSPORIN-SENSITIVE PROLINE ROTAMASE 6) stimulate the ATPase activity of HSP90 (Panaretou *et al*, 2002), but it seems that some substrates might also stimulate its activity (McLaughlin *et al*, 2002). Substrates, but not co-chaperones, are expected to be degraded on inhibition of HSP90 with a drug, but some are not degraded or even stabilized (see, for example, McClellan *et al*, 2005; Chen & Balch, 2006; He *et al*, 2007). The histone deacetylase HDAC6 is a regulator of HSP90 because it keeps it in the deacetylated active form and also has features of an HSP90 substrate (Rao *et al*, 2008).

The pejorative connotation of the 'co' in co-chaperones is unfair and inappropriate for a number of other reasons; several co-chaperones display HSP90-independent chaperone activities themselves (Bose *et al*, 1996; Freeman *et al*, 1996; Kimura *et al*, 1997). Thus, given the large number of HSP90 co-chaperones—and despite the abundance of HSP90—it is likely that some co-chaperones have independent functions. These might be completely HSP90-independent or inverted with HSP90 playing the role of the ATP-churning servant, the ATPase-driven conformational gymnastics of which (Bron *et al*, 2008; Krukenberg *et al*, 2008; Richter *et al*, 2008) serve the purposes of the 'co-'chaperone. Therefore, now that I turn to discuss SGT1, the reader will understand that I call it an HSP90 co-chaperone for lack of a better term... and out of ignorance of the breadth of SGT1 functions.

The gene encoding SGT1 was discovered in the budding yeast as a high-copy suppressor of a mutation in the gene for SKP1—a component of the SCF (Skp1–Cullin–F-box) ubiquitin ligase complex required for kinetochore function (Kitagawa *et al*, 1999). It was found to be linked to plant pathogen resistance when its plant orthologue was identified as an interactor of RAR1 (REQUIRED FOR MLA12 RESISTANCE) in a yeast two-hybrid screen (Azevedo *et al*, 2002), as RAR1 was known to be an essential factor for resistance mediated by NB-LRR genes (Shirasu *et al*, 1999). Shortly thereafter, it was recognized that HSP90 is also involved, both for kinetochore function (Stemmann *et al*, 2002) and for plant resistance (Hubert *et al*, 2003; Takahashi *et al*, 2003).

To bind to HSP90, RAR1 and SGT1 use a CHORD (cysteine- and histidine-rich domain) and a CS motif (present in metazoan CHORD and SGT1 proteins), respectively (Fig 1). The two domains structurally resemble those of the co-chaperone p23 (Dubacq *et al*, 2002; Garcia-Ranea *et al*, 2002), a well-characterized regulator of the HSP90 ATPase domain (Ali *et al*, 2006). In the case of SGT1, it came as a surprise that its TPR (tetratricopeptide repeat)

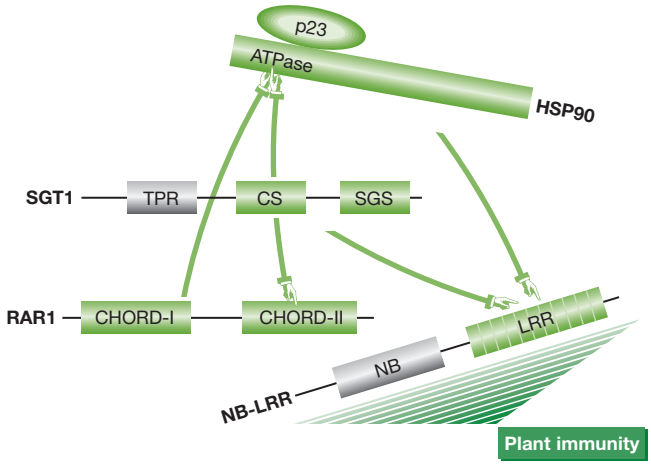

Fig 1 | Schematic representation of HSP90, SGT1, RAR1 and NB-LRR interactions. For clarity, the protein–protein interfaces are only indicated by arrows, as a dynamic three-dimensional representation would be necessary to do justice to all known interactions. Note that the lengths of proteins and structural motifs are not to scale. The signalling pathways downstream from the NB-LRR proteins that lead to immunity in plants are largely unknown. HSP90, HEAT-SHOCK PROTEIN 90; NB-LRR, NUCLEOTIDE BINDING AND LEUCINE-RICH REPEAT; RAR1, REQUIRED FOR MLA12 RESISTANCE; SGT1, SUPPRESSOR OF G2 ALLELE OF SKP1.

domain—a protein–protein interaction domain used by many other co-chaperones to dock to HSP90—is not involved in this interaction (Lee *et al*, 2004; Catlett & Kaplan, 2006; Botër *et al*, 2007; Zhang *et al*, 2008). Instead, the TPR domain—which is not essential for SGT1 function in plant immunity—might be important in regulating the relative protein levels of the isoforms SGT1a and SGT1b in *Arabidopsis* (Azevedo *et al*, 2006), possibly by allowing the HSP90–SGT1 complex to connect to SKP1 and its partners in the SCF ubiquitin ligase complex (Catlett & Kaplan, 2006). SGT1 also uses the CS domain to bind to RAR1 through a second CHORD domain (Azevedo *et al*, 2002). Previous work from the Shirasu and Guerois groups has shown that opposite sides of the CS domain are used to bind to HSP90 and RAR1 (Botër *et al*, 2007). The plot thickens with the new report (Kadota *et al*, 2008); here, the in-depth analysis of the interaction surfaces by NMR and mutagenesis reveals that SGT1 and RAR1, with the CS and CHORD-I domains, respectively, bind to overlapping surfaces on the ATPase domain of HSP90. The CHORD-I domain of RAR1 competes with SGT1 for HSP90 binding, but ternary complexes can nevertheless form (Botër *et al*, 2007). In any case, this is not a quiet threesome, as there are several other co-chaperones that bind to HSP90 nearby, notably p23. On the basis of their structural analysis of the CS–HSP90 complex, the authors had previously suggested that p23 binds to HSP90 differently, owing, in part, to an additional strand missing in the CS domain of SGT1 (Botër *et al*, 2007); they have now confirmed this experimentally. Despite the structural similarities between p23 and the CS domain, the interfaces on HSP90 do not overlap. The crystal structure of the HSP90–SGT1 CS complex, recently reported in a study to which the Shirasu group also contributed (Zhang *et al*, 2008), confirms the structure modelled on the NMR data. The fact that full-length SGT1 competes with p23 for HSP90 binding (Kadota *et al*, 2008) might indicate an indirect conformational effect or steric hindrance. The various binding modes of the co-chaperones correlate with different functional consequences for HSP90: p23 preferentially binds to the ATP-bound form of HSP90 and inhibits its ATPase activity, whereas SGT1 binds to the ADP-bound form and does not influence the ATPase (Catlett & Kaplan, 2006). In fact, the functional consequences of SGT1 binding to HSP90, other than preventing the access of some other co-chaperones, remain unknown.

What about the substrates of all this? Although there is no doubt that SGT1 and HSP90 are required for maintaining NB-LRR proteins in a state that allows them to respond to an infection, the details of the interactions of the NB-LRR proteins with SGT1 and/or HSP90 are far less well understood. The structural consequences of these interactions for the NB-LRR proteins are completely unknown. SGT1 uses the carboxy-terminal SGS (SGT1-specific) domain to interact directly or indirectly with the LRR domain of plant R (RESISTANCE) proteins (Dubacq *et al*, 2002; Bieri *et al*, 2004), whereas SGT1 apparently needs both the CS and SGS domains to bind to the LRR domain of animal NOD-like receptors; Mayor *et al*, 2007). HSP90 was shown to interact directly with part of the LRR domain of the I-2 protein of tomato (de la Fuente van Bentem *et al*, 2005), but a more extensive analysis in the animal system, albeit not with purified proteins, indicated an interaction with the nucleotide-binding and oligomerization domain (the NACHT domain) of the NOD-like receptors (Mayor *et al*, 2007). In this system, the interaction of HSP90 with the LRR domain of the NOD-like receptor NALP3 was found to be stimulated by SGT1, suggesting a far more complex interplay between substrate, HSP90 and SGT1.

I have alluded several times to other interactions in which SGT1 (and HSP90) can engage and there are others that could be discussed. This bewildering network calls for a more careful and detailed analysis, for which the paper by Kadota *et al* (2008) provides an excellent roadmap. Obtaining a more detailed view of the protein–protein interfaces will be the first step towards drawing a clearer picture of the higher order protein complexes and their dynamics. Whether other interfaces rely on salt bridges—as does the one between the CS domain of SGT1 and HSP90—remains to be seen. With such an understanding of the molecular contacts, it might also be possible to generate compensatory amino-acid changes across other types of interface. If this approach were applicable to the *in vivo* interactions between substrates such as an NB-LRR protein and the HSP90–SGT1 tandem, it would be an exciting quantum leap for the field.

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