

Plant Immunity: The Origami of Receptor Activation

Dispatch

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Mutations in plant cytosolic *HSP90* genes have been found to impair the immune responses triggered by host pathogen receptors. *HSP90* links the plant receptors to other components essential for receptor function. The new findings suggest mechanistic parallels with steroid receptor regulation in animals.

Like animals, plants have evolved an elaborate surveillance system to protect them against pathogen infections. The receptors in plant immunity, called resistance (R) proteins, broadly fall into two classes with either extracellular or intracellular leucine-rich repeat (LRR) regions. The largest class in *Arabidopsis* are intracellular receptors, with a central nucleotide-binding (NB) domain and carboxy-terminal LRRs. Each may recognize one or a very few strain-specific pathogen effectors. Until now, most attempts to show that an R protein interacts directly with its cognate pathogen effector failed; in some cases the associations were found to be indirect and to involve other host proteins [1–3]. This led to the idea that R proteins might ‘guard’ a limited number of host proteins that are targets of pathogen effectors during pathogenesis [4]. So far, genetic approaches have identified relatively few components required for R gene function in plants [5]. A highly conserved protein class, the heat-shock proteins, has now been added to the list and work on these proteins promises to provide fresh insights to the molecular mechanics of the pathogen recognition process [6–9].

Heat-shock proteins are well known for their roles in the maturation of protein complexes and degradation of damaged or misfolded peptides, and for regulating the activity of many signal transduction proteins [10,11]. The new findings indicate that a particular subclass of heat-shock proteins, the cytosolic heat shock protein 90 (HSP90) family, have a general role in R-protein-triggered immunity in plants; the evidence suggests that HSP90s act physically close to R proteins, connecting to other components previously shown to be critical for R protein function. A common feature of HSP90s is that they bind their target proteins — often referred to as their ‘clients’ — in nearly mature conformations, retaining and releasing them in an activity cycle driven by ATP hydrolysis and regulated by binding of co-chaperones. HSP90s act as dimers and consist of an amino-terminal intrinsic ATPase domain, a central client-binding region, and a carboxy-terminal dimerization domain.

Hubert *et al.* [7] used a genetic approach which revealed cytosolic HSP90s as critical components in immunity triggered by an NB-LRR type R protein. Rare

specific mutations in one of four *Arabidopsis* genes encoding cytosolic HSP90 isoforms were identified in a large-scale conditional screen for mutants impaired in resistance triggered by the R protein RPM1 upon recognition of the *Pseudomonas syringae* effector AvrRpm1 [7]. Analysis of the recovered mutant alleles showed that all three were generated by single amino acid substitutions in the ATPase domain of HSP90.2, drastically reducing the steady-state levels of RPM1 in non-challenged plants but not interfering with the resistance reactions triggered by seven other tested R genes.

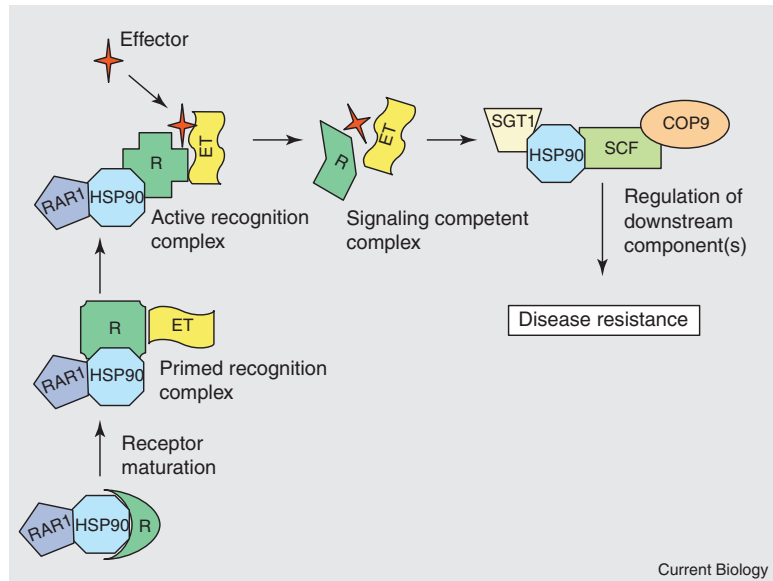
One of the HSP90.2 substitutions is equivalent to that creating a mutant form of yeast HSP90 that lacks the ATPase activity required for client turnover, yet retains the ability to dimerize. Paradoxically, an insertion allele of AtHSP90.2, presumably a null mutation, had no discernible effect on RPM1 function. Furthermore, the *Athsp90.2* mutant alleles with ATP binding site substitutions revealed a baroque genetic condition known as ‘non-allelic non-complementation’. In such cases, F₁ heterozygous products of a cross between plants bearing two unlinked recessive mutations are phenotypically similar to either homozygous single mutant. In this particular case, F₁ plants containing wild-type and mutant alleles of both RPM1 and HSP90.2 were susceptible to *P. syringae* expressing *avrRpm1*.

Non-allelic non-complementation provides clear evidence for dosage-sensitivity, and also suggests close physical associations — either direct physical interaction between RPM1 and HSP90.2 or an association in the same complex. Indeed, co-immunoprecipitation of RPM1 and HSP90 from plant extracts supported the conclusion that the proteins associate *in vivo*, although it remains to be shown whether the interaction is really direct and preferentially involves HSP90.2 and/or any of the other three closely related cytosolic HSP90 isoforms. One way of rationalizing the findings invokes functional redundancy in the HSP90 family, which could prevent inactivation of RPM1 in the *Athsp90.2* null mutant, whereas mutations of the HSP90.2 ATPase domain might prohibit functional redundancy by causing persistent, but non-productive, client binding.

In this scenario, non-productive binding might force incompletely folded RPM1 to enter a default degradation pathway. Hubert *et al.* [7] thus concluded that HSP90.2 has a role in folding RPM1 into a stable conformation or a signalling competent RPM1-containing complex. Preliminary evidence for the latter was obtained by co-immunoprecipitation experiments involving the *Arabidopsis* RIN4 protein. RIN4 is another component essential for RPM1 function and is known to interact directly with both RPM1 and the cognate *Pseudomonas* effector AvrRpm1 on the cytoplasmic face of plant plasma membranes [2]. In co-immunoprecipitation experiments, RIN4 did not appear to interact with HSP90, suggesting the existence of at least two pools of RPM1. This may also indicate that HSP90 acts transiently in the assembly of an RPM1-containing

Figure 1. Multiple conformational switches might accompany the assembly of an R protein complex and initiation of signal transduction triggered by effector perception.

Regulation of downstream signalling might involve SCF/COP9 complexes and could lead to removal of negative and/or activation of positive immune response regulators. R, intracellular NB-LRR protein; ET, effector target; SCF, E3 ubiquitin ligase complex containing CUL1, RBX1, SKP1 and an F-box protein; COP9, a protein complex similar to the lid subcomplex of the proteasome.



recognition complex which becomes competent for AvrRpm1 perception.

Pharmacological and genetic evidence supports the view that HSP90s also play a critical part in disease resistance mediated by another *Arabidopsis* NB-LRR protein, RPS2, upon recognition of its corresponding *P. syringae* effector, AvrRpt2 [8]. Either treatment with the HSP90 inhibitor geldanamycin or null mutation of *AtHSP90.1* impaired an efficient resistance response to *P. syringae* expressing *avrRpt2* and had weak effects on RPM1-dependent immunity. Although these data potentially conflict with the apparent functional redundancy of the cytosolic HSP90s (see above), it may be relevant that *AtHSP90.1* is the sole family member exhibiting pathogen-responsive expression and that RPM1 activates resistance about 10 hours earlier than does RPS2. Thus, one might speculate that assembly of a signalling-competent RPS2 recognition complex requires a higher HSP90 input (dosage) than assembly of the RPM1 complex.

Lu *et al.* [9] developed a large-scale virus-induced gene silencing (VIGS) method to randomly target several thousand *Nicotiana benthamiana* genes in order to assess their contribution to R protein function. This revealed genes encoding HSP90s as critical components in immune responses triggered by the NB-LRR proteins RX, N and PRF: RX and N recognize viral effectors derived from potato virus X (PVX) and tobacco mosaic virus (TMV), respectively; and PRF recognises the *P. syringae* effector AvrPto. The identified HSP90 VIGS clones targeted at least four closely related *NbHSP90* genes encoding cytosolic HSP90s. VIGS clones targeted at more divergent 3' untranslated regions of cytosolic *HSP90* mRNAs did not cause significant loss of disease resistance, perhaps because of functional redundancy in the HSP90 family. Plants expressing VIGS clones that targeted all closely related *NbHSP90* genes showed additional growth and developmental abnormalities. This is consistent with HSP90 having other functions besides immunity, as one would expect. It is noteworthy that RX protein levels were

markedly reduced in *NbHSP90*-silenced plants in the absence of viral effector. This is reminiscent of the drastic reductions of *Arabidopsis* RPM1 steady state-levels in *hsp90.2* mutants, indicating that HSP90 might have a more general role in the accumulation of NB-LRR proteins.

Kanzaki *et al.* [6] also used VIGS in *N. benthamiana* to assess the role of NbHSP90 and NbHSP70 in plant defence. They found that silencing either class of heat-shock proteins compromised cell death responses triggered by the INF1 effector of the oomycete *Phytophthora infestans* or by *Pseudomonas cichorii*, for which *N. benthamiana* exhibits 'non-host resistance', as it is not a natural host for this bacterial pathogen. Cell death is a frequent, though notoriously non-specific, marker of plant immunity, a notion reinforced in the study of Lu *et al.* [9], in which the vast majority of VIGS clones impairing R-protein-associated cell death did not cause increased pathogen growth. So it remains to be shown whether compromised cell death in response to the INF1 effector or *P. cichorii* challenge is the result of impaired (and unknown) R protein function and/or impaired activities of other HSP90 and HSP70 clients.

Takahashi *et al.* [8] homed in on HSP90 by starting from two conserved eukaryotic proteins, RAR1 and SGT1, known to have essential roles in many R protein-triggered immune responses to diverse plant pathogens and capable of binding each other [16]. Their binding requires the presence of a carboxy-terminal Zn²⁺-binding domain in RAR1, CHORD-II, and the middle region of SGT1 containing a 'CS' motif. Takahashi *et al.* [8] have shown that these proteins actually engage in a ménage à trois with HSP90. They showed that a second CHORD domain in RAR1, CHORD-I, interacts with HSP90's amino-terminal ATPase domain. SGT1, on the other hand, contacts the amino-terminal ATPase domain of HSP90 and the binding requires the presence of the carboxy-terminal dimerization domain. But the binding of HSP90 to RAR1 and HSP90 to SGT1 in plants is not mutually exclusive [7], indicating the potential for

disparate RAR1 and SGT1 activities. Animal RAR1 homologs and SGT1 share structural features with other co-chaperones previously shown to bind HSP90 [12,13]. This, and the capacity of RAR1 and SGT1 to bind HSP90, are strongly indicative of a co-chaperone-like activity in plant immunity.

Like many other co-chaperones, SGT1 most likely regulates folding processes of diverse protein complexes. For example, *Arabidopsis* SGT1b was shown to have a critical role in auxin signalling that depends on the SCF complex ubiquitin ligase [14]. And in budding yeast, SGT1 has been shown to have a role in activation/assembly of the CBF3 kinetochore complex and to physically associate with the LRR-containing adenylyl cyclase CYR1/CDC35 [13]. Although RAR1 appears to play a more specialized role in plant immunity, the human RAR1 homolog, melusin, functions in stress adaptation to mechanical stimuli in heart muscles [15]. It would not be surprising if melusin regulates folding of yet further protein complexes.

It remains to be shown whether RAR1 and/or SGT1 associate together with HSP90 and R proteins in a single complex and/or simultaneously co-regulate other complexes required for potential 'downstream' signalling events, such as ubiquitination-dependent processes [16–18]. An indication that the former might be true is the observation that RPM1 steady-state levels are greatly decreased or undetectable in non-challenged *rar1* or *hsp90.2* mutant backgrounds [7,19]. The role of SGT1 in R protein function is likely to be different, because RX protein accumulation was impaired following *NbHSP90* silencing but unaffected upon *NbSGT1* silencing [9].

Hubert *et al.* [7] point to a potential blueprint for R protein function that comes from extensive studies of animal steroid receptor regulation (reviewed in [11]). An intracellular hetero-complex consisting of several heat-shock proteins — including HSP90, HSP70 and HSP40 — as well as co-chaperones forces an opening of the steroid-binding cleft, driven by heat-shock protein ATPase activity, such that the binding pocket can be accessed by a steroid ligand. Assembly experiments revealed a multi-step activation process, each driven by ATP hydrolysis, producing first a 'primed complex' and then a steroid-binding competent complex.

Bearing in mind this precedent, the current data do not rule out the possibility that R proteins and their effectors directly interact at some point in R protein complex maturation, possibly only after multiple folding switches have taken place. An activated complex containing both R protein and effector might be the least stable form and just have escaped detection. Indirect experimental evidence suggests that RX undergoes substantial intramolecular conformational switches as a consequence of viral effector perception, perhaps a first indication that ligand-induced folding processes are critical for initiation of signal transduction [20].

Experiments in which the R protein complex is systematically reconstructed, either in yeast or *in vitro*, and attempts to trap R protein complex intermediates by genetic means, may help us gain further insights into the mechanics of effector recognition. Finally, another potential facet in the encounter between R proteins and

HSP90s is noteworthy: R proteins are highly polymorphic in natural populations and their LRRs are subject to diversifying selection, possibly at the cost of often becoming structurally unstable. Thus HSP90 may have been co-opted for R protein function by buffering intrinsic structural instability and assisting R proteins to fold into active configurations [10].

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