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REVIEW

The HSP90-SGT1-RAR1 Molecular Chaperone Complex: a Core Modulator in Plant Immunity

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The HSP90 (heat shock protein 90), SGT1 (suppressor of G-two allele of *Skp1*), and RAR1 (required for *Mla12* resistance) proteins in plants form a molecular chaperone complex which is involved in diverse biological signaling including development and disease resistance. The three components of this complex interact via specific protein binding motifs and recruit client proteins to initiate a specific signaling cascade in response to cellular or environmental cues. Although the functions of this chaperone complex during development/growth have not been well characterized, the HSP90 chaperone and SGT1 and RAR1 co-chaperones have been demonstrated to be essential signaling components of plant immune responses. These three proteins also play important roles in activation of the mammalian Nod genes, which possess a structurally conserved plant resistance (R) protein motif, NB-LRR (nucleotide binding site-leucine rich repeat). In this review, we summarize the structures and functions of these molecular chaperones, and discuss their putative modes of action in plant immune responses.

Keywords: chaperone, HSP90, plant immunity, RAR1, SGT1

HSP90, SGT1, AND RAR1 ARE COMPONENTS OF MOLECULAR CHAPERONE COMPLEXES THAT ARE CONSERVED ACROSS THE PLANT AND ANIMAL KINGDOMS

Molecular chaperones participate in not only the folding of newly synthesized proteins but also in several biological and cellular processes such as cell growth, development and signal transduction (Helmbrecht et al., 2000; Pavithra et al., 2007). The chaperone families of stress proteins including HSP40/70/90/100, and the small HSP proteins, are highly conserved in most organisms from bacteria to higher eukaryotes. In particular, the cytosolically abundant HSP90 protein functions in the diverse cellular processing of proteins such as folding, localization, and proteolysis (Pearl and Prodromou, 2006; Brown et al., 2007). Another identified function of HSP90 is as a buffer of genetic variation in developmental processes (Queitsch et al., 2002).

HSP90 plays a key role as a core component of various protein complexes that associate with other co-chaperones. The largest class of co-chaperones includes proteins such as Hop (HSP70- and HSP90-organizing protein) and Cyp40, which harbor one or more tetratricopeptide repeat (TPR) domains. A number of other TPR-containing co-chaperones include E3/E4 ubiquitin ligase from Cos-7 cells, protein phosphatase 5 (PP5) from mouse and plant, and prolyl isomerases from yeast which convey their own catalytic activities (Dolinski et al., 1997; Silverstein et al., 1997; Jiang et al., 2001; de la Fuente van Bentem et al., 2005). Moreover, HPS90 also interacts with non-TPR-type co-chaperones, such as p23 (Sba1 in yeast), in an MEEVD (a pentapeptide motif)-independent manner. Hence, it is possible that HSP90 is associated with both TPR-type and non-TPR-type co-chaperones (Takahashi et al., 2003).

Through its association with co-chaperones, HSP90 activates and catalyzes more than 100 clients to process cell cycle, developmental, and signaling events (Pearl and Prodromou, 2006; Pavithra et al., 2007). These substrates include telomerase (Holt et al., 1999), nitric oxide synthase (Lei et al., 2007), nuclear hormone receptors (Pratt and Toft, 2003), and protein kinases (Pearl, 2005), suggesting that this chaperone has essential functions in the activation of a variety of biological functions. In particular, protein kinases comprise the most prevalent group of HSP90 clients and are delivered to the HSP90 complex via an interaction with the co-chaperone Cdc37 (Shao et al., 2003). Bound kinases are stabilized and become active upon stimulation by the appropriate signals (Pearl, 2005).

In plant species, HSP90 isoforms are required for disease resistance against invading pathogens. For example, the *AtHSP90.1* and *AtHSP90.2* genes in *Arabidopsis* are required for the *RPS2*-mediated resistance against *Pseudomonas syringae* expressing *AvrRpt2* and for *RPM1*-mediated resistance to *P. syringae* expressing *AvrRPM1*, respectively (Fig. 1; Hubert et al., 2003; Takahashi et al., 2003). *HSP90* is also essential for *Rx*-mediated resistance to *Potato virus X* (*PVX*), *N*-mediated resistance to *Tobacco mosaic virus*, and *Pto*-mediated resistance to *P. syringae* expressing *AvrPto* (Lu et al. 2003; Liu et al. 2004). In contrast, the *hsp90.2-3* mutant with a point mutation in the ATP-binding domain of AtHSP90.2, known to be more sensitive to biotrophic pathogens, is more resistant to the herbivore *Trichoplusia ni* (Fig. 1; Sangster et al., 2007). These findings demonstrate that HSP90 plays an important role in the appropriate integration of diverse disease resistance signaling in higher plants.

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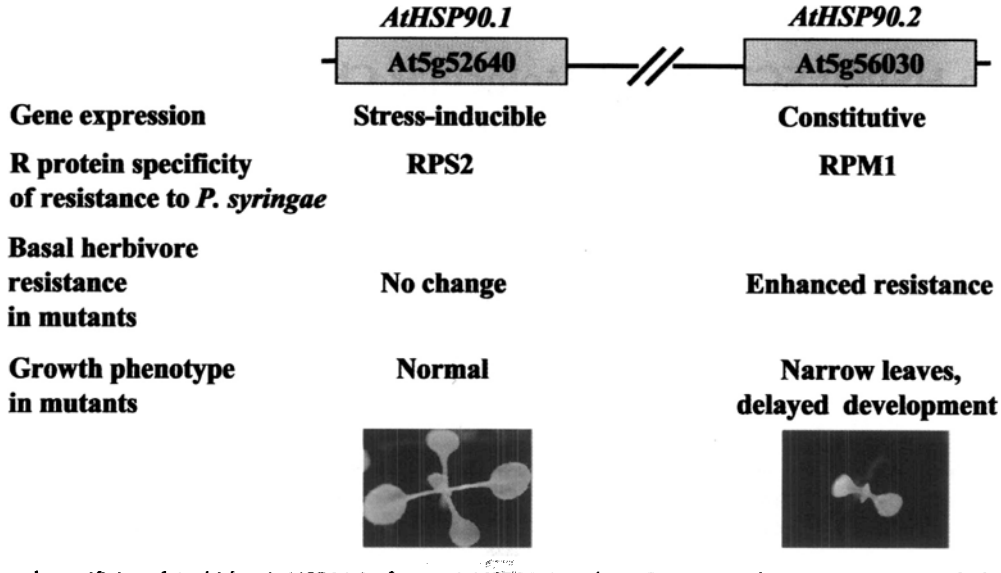

Figure 1. Functional specificity of *Arabidopsis* HSP90 isoforms, AtHSP90.1 and AtHSP90.2, in disease resistance and plant development. *AtHSP90.1* is highly stress-inducible, whereas *AtHSP90.2* is constitutively expressed. Inhibition of AtHSP90.1 and AtHSP90.2 result in the attenuation of RPS2- and RPM1-mediated resistance to *P. syringae* isolates, respectively. In contrast, an *AtHSP90.2* mutant, *hsp90.2-3*, is more resistant to the herbivore *T. ni*. In addition, mutations in *AtHSP90.2* yield a highly significant over-representation of seedlings with narrow leaves and delayed development (Sangster et al., 2007).

SGT1, a TPR-type co-chaperone of HSP90, functions in diverse processes such as immunity, CBF3 (centromere binding factor 3) kinetochore assembly (comprising SKP1, CTF13, NDC10 and CEP3), SCF (SKP1-Cullin/CDC53-F box) ubiquitin ligase complexes and cyclic AMP signaling (Kitagawa et al., 1999; Dubacq et al., 2002). Kinetochores assemble on centromeric DNA and thereby mediate the interaction of chromosomes with the mitotic spindle (Cleveland et al., 2003). SGT1 physically interacts with SKP1, a component of the CBF3 kinetochore assembly and of SCF ubiquitin ligase complexes. Hence, SGT1 is essential for cell cycle progression at the G1/S and G2/M phases (Kitagawa et al., 1999) and for SCF-mediated ubiquitination activity. In higher plants, ubiquitination is known to be involved in phytohormone, light, sucrose, immunity and developmental pathways (Callis and Vierstra, 2000). HSP70 is also a target of SGT1 (Spiechowicz et al., 2007). The fact that HSP70 contacts SGT1 and facilitates its transfer to HSP90 indicates that SGT1 is a component of multi-protein chaperone complexes. Notably, the *Arabidopsis SGT1* (*AtSGT1b*) gene has been identified in mutational analysis for loss of *RPP5*- and *RPP7*-mediated resistance (Austin et al., 2002; Tor et al., 2002), indicating that SGT1 also plays an important role in disease resistance signaling in higher plants.

The non-TPR-type co-chaperone RAR1 is an essential component of the R protein-mediated resistance responses in both monocot and dicot plant species. For example, in barley (*Hordeum vulgare*), a monocot, RAR1 was identified due to its requirement in the *Mla12*-mediated resistance to powdery mildew (*Blumeria graminis* f. sp. *hordei*) (Shirasu et al., 1999). In the dicot *Arabidopsis*, *rar1* mutants fail to perform R protein-mediated resistance in response to pathogenic *P. syringae* and *Peronospora parasitica* (Austin et al., 2002; Tornero et al., 2002).

A growing body of evidence now suggests that HSP90, SGT1, and RAR1 functionally co-operate as a molecular chaperone complex to transduce plant immune responses. Interestingly, the mammalian Nod family also requires HSP90/SGT1/RAR1 to activate and mediate innate immune responses, indicating that HSP90, SGT1 and RAR1 play similar roles in the immune response in both plant and animal species (Hahn, 2005; da Silva Correia et al., 2007a).

STRUCTURES AND PHYSICAL INTERACTION MOTIFS OF HSP90, SGT1, AND RAR1

HSP90

Structural analyses of HSP90 through its crystallization or through introduced mutations have revealed that this protein harbors an N-terminal domain with the capacity to bind nucleotides and chemical agents, a middle segment containing a catalytic loop and motifs for binding client proteins, and a C-terminal domain that is essential for dimerization. The HSP90 N-terminal pocket contains a binding site for ATP as has been revealed by experiments using competitive inhibitors of ATP binding, such as geldanamycin and radicicol (Stebbins et al., 1997). A number of mutagenesis studies have also implicated the middle segment of this chaperone as a major binding site for client proteins (Sato et al., 2000). The C-terminal domain of HSP90 is of particular importance also as it contains the MEEVD motif which is implicated in the binding of co-chaperones with TPR domains such as SGT1 (Chen et al., 1998; Prodromou et al., 1999).

SGT1

SGT1 has three known domains: a tetratricopeptide repeat (TPR), a cysteine- and histidine-rich domain (CHORD) and SGT1 (CS), and an SGT1-specific (SGS) motif. Two variable regions (VR1 and VR2) are inter-spaced between the TPR and CS, and between the CS and SGS motifs, respec-

tively. These three domains appear to have distinct protein-protein motifs. The TPR domain of yeast SGT1 binds Skp1, a protein component of the SCF ubiquitin ligase complex (Kitagawa et al., 1999). It has also been shown that the TPR domain mediates inter-protein associations (Cliff et al., 2005; Cortajarena and Regan, 2006). In addition, the dimerization of TPR-mediated SGT1 has been demonstrated in barley and is ionic-strength-dependent (Nyarko et al., 2007). The SGS domain of the human SGT1 has also been shown to associate with calcyclin (Nowotny et al., 2003).

RAR1

RAR1 is comprised of highly similar but distinct cysteine- and histidine-rich zinc-binding domains (CHORDs), an N-terminal CHORDI and a C-terminal CHORDII. The central region of this protein also contains a cysteine- and histidine-containing motif (CCCH motif). Although the function of the CCCH motif is currently unknown, CHORD-containing proteins have been shown to have important biological functions including a role in plant immunity (Shirasu et al., 1999), maintenance of the diploid state in *Aspergillus nidulans* (Sadanandom et al., 2004), and embryogenesis in *Caenorhabditis elegans* (Shirasu et al., 1999). RAR1 homologs are also present in eukaryotes, except for yeast (*Saccharomyces cerevisiae*) (Shirasu et al., 1999). Notably, metazoan RAR1 homologs possess the CS motif found at the C terminus of SGT1 (Shirasu et al., 1999; Kitagawa et al., 1999). Such fusions, in which two domains are found in a single protein in one species, are often indicative of physical interactions between the two domains that are present in two separate proteins in another species (Marcotte et al., 1999). Indeed, AtSGT1 has been shown to interact with *Arabidopsis* RAR1 (AtRAR1) in yeast (Azevedo et al., 2002).

Physical interaction of HSP90, SGT1, RAR1, and R (or NOD) proteins

It has been reported that HSP90 has many different sets of co-chaperones (Picard, 2002), whereas few physical interactors have so far been identified for SGT1 and RAR1. The MEEVD motif of HSP90 interacts with the TPR interaction domain of co-chaperones such as Hop (Sti1 in yeast) and PP5. The CS domain of SGT1 has a similar structural motif to p23, an HSP90 co-chaperone. Hence, the CS domain of SGT1 is also capable of interacting with HSP90 in human, yeast, and plants (Takahashi et al., 2003; Lee et al., 2004; Catlett and Kaplan, 2006; Botër et al., 2007). RAR1 interacts with SGT1 via the CHORDII domain of RAR1 and the CS domain of SGT1 (Azevedo et al., 2002; Botër et al., 2007; Wang et al., 2008). The CHORDI domain of RAR1 is also known to interact with the N-terminal half of HSP90, which contains the ATPase domain of this protein (Takahashi et al., 2003; Botër et al., 2007).

HSP90-SGT1-RAR1 and N, a resistance protein from tobacco, exist in a single complex in *N. benthamiana* plants. HSP90 directly interacts with the LRR domain of N in tobacco (Liu et al., 2004). A pair-wise immunoprecipitation experiment demonstrated interactions between not only HSP90 and RPM1 (an R protein) but also between RAR1 and SGT1 (Hubert et al., 2003). In this particular experiment, the authors reported that the HSP90 interaction with RAR1 does not require SGT1, nor does the HSP90 interaction with SGT1 require RAR1. These data suggest that RPM1 is an HSP90 client, and that RAR1 and SGT1 function independently as HSP90 cofactors. SGT1 has also been shown to interact with plant R proteins (Bieri et al., 2004; Leister et al., 2005). These findings suggest that the HSP90-SGT1-RAR1 chaperone complex interacts with plant R proteins.

Chp-1, a mammalian homologue of plant RAR1, interacts with the TPR domain of PP5 and the ATPase domain of HSP90 via the CHORD I and II domains, respectively

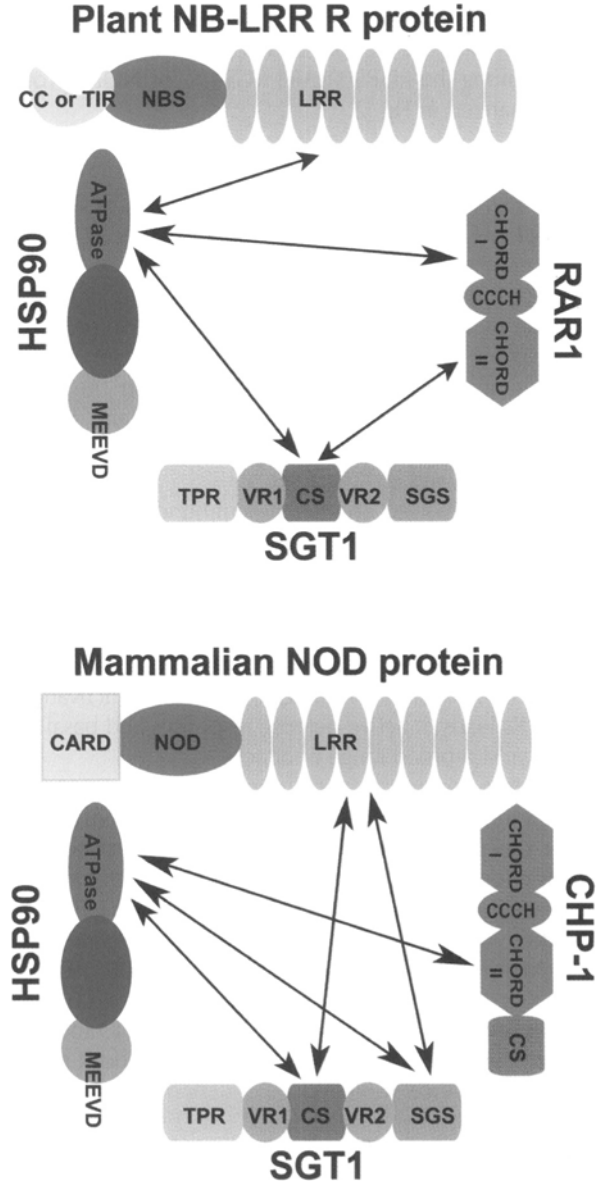

Figure 2. Physical interactions between HSP90-SGT1-RAR1 and plant NB-LRR R and mammalian Nod proteins. Motifs critical for protein-protein interactions are indicated. CHP-1 is an animal RAR1 homologue (Hahn, 2005). CC, coiled-coil; CCCH, cysteine- and histidine containing domain; CHORD, cysteine- and histidine-rich domain; CS, CHORD and SGT1 motif; SGS, SGT1 specific motif; LRR, leucine-rich-repeat; NB, nucleotide binding site; TIR, Toll-interleukin-1-receptor; TPR, tetratricopeptide repeat; VR, variable region. Arrows indicate protein-protein interactions.

(Hahn, 2005). The mammalian CS and SGS domains of SGT1 are required for the interaction of this protein with LRR domains of NALP3 (Nod-like receptor) and Nod1, which are structurally related to the plant NB-LRR resistance proteins (da Silva Correia et al., 2007a; Mayor et al., 2007). This indicates that the CS and/or SGS domain of SGT1 may associate with plant R proteins. In addition, the CS and SGS domains of SGT1 have been shown to be associated with HSP90 (Mayor et al., 2007). Nod1, which harbors a common structural feature with the plant NB-LRR proteins, is an intracellular sensor of bacterial peptidoglycan and also associates with the HSP90 complex (Hahn, 2005). HSP90 and SGT1 both contribute to the stability and activation of Nod1 (Hahn, 2005; da silva Correia et al., 2007a) and HPS90 is also crucial for Nod2 activity (Mayor et al., 2007). The interactions among the HSP90/RAR1/SGT1/R (or NOD) proteins are summarized in Fig. 2.

MULTI-FUNCTIONALITY OF THE HSP90, SGT1 AND RAR1 MOLECULAR CHAPERONE COMPLEXES

The accumulated evidence to date indicates that complex formation by HSP90, SGT1 and RAR1 with diverse proteins may explain their multi-functionality in plant immune responses against invading pathogens and in the cellular processes required for proper plant growth and development. Here the current view of their involvement in disease resistance pathways is discussed.

Plants have evolved an effective immune system to resist attack by microbial pathogens. This defense mechanism is primarily dependent upon sophisticated responses via the recognition of pathogen associated molecules (often called MAMPs or PAMPs) by pattern (or pathogen) recognition receptors (PRRs) (Dardick and Ronald, 2006; Jones and Dangl, 2006; Bittel and Robatzek, 2007). The activation of these PRRs leads to active defense responses and basal resistance against a broad range of attacks.

Plants also possess R protein-mediated resistance, governed by resistance (*R*) genes, many of which encode NB-LRR or receptor kinase proteins. R protein-mediated resistance is often associated with a hypersensitive response (HR) and is triggered upon recognition of pathogen effector or avirulence (Avr) proteins (Hammond-Kosack and Jones, 1997; Martin et al., 2003; Nimchuk et al., 2003; Lee and Lee, 2005; Jones and Dangl, 2006; Lee et al., 2006; Bent and Mackey, 2007). Significantly, HSP90, SGT1, and RAR1 have been shown to play a role in both basal and R protein-mediated resistance in plants.

Basal defense

Basal defense does not lead to strong levels of disease resistance in plants, but provides a first line of defense against pathogenic invaders. It is known that mutations in *rar1* enhance the susceptibility of both *Arabidopsis* and barley to virulent pathogens (Holt et al., 2005; Jarosch et al., 2005). In *Arabidopsis*, *rar1* mutations in different genetic backgrounds allow the enhanced growth of the virulent bacterial strain *P. syringae* pv. *tomato* (*Pst*) DC3000 (Holt et al., 2005). In barley, *RAR1* contributes to resistance in the epidermis and mesophyll during the differentiation stages of infection of the fungus *Magnaporthe grisea*, and this is dependent on the *MLO/mlo*-5 status. The loss of *RAR1* promotes susceptibility in the *mlo*-5 background to a compatible *M. grisea* isolate (Jarosch et al., 2005). These data demonstrate the essential role of *RAR1* in the basal resistance mechanism that limits pathogen growth in susceptible plants.

Consistently, the overexpression of the rice ortholog *OsRAR1* significantly increases basal resistance to a virulent bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain PXO99. These transgenic rice plants also show enhanced resistance to virulent blast fungal *M. grisea* races (Wang et al., 2008). In the same study, the rice *SGT1* (*OsSGT1*) gene was also found to enhance the basal resistance to the virulent *Xoo* and *M. grisea* races, suggesting that SGT1 is also possibly involved in basal resistance in plants. In contrast, mutations in *HSP90* do not affect the plant basal resistance to the virulent pathogen *Pst* DC3000 (Hubert et al., 2003; Takahashi et al., 2003).

Notably, the *Arabidopsis RAR1* gene is targeted by the *P. syringae* effector AvrB which suppresses MAMP-triggered host immunity. When *AvrB* is expressed in plants lacking the cognate resistance gene *RPM1*, this causes a suppression of the cell wall defense system induced by a well known flagellar peptide MAMP flg22 (Shang et al., 2006). Furthermore, co-immunoprecipitation experiments have indicated that RAR1 and AvrB interact in the plant. It is also well known that RAR1 is required for the function of multiple resistance proteins (see *R protein-mediated disease resistance*). Hence, it is possible that R proteins are recruited to a protein complex containing RAR1 to monitor effectors that suppress basal resistance. This would suggest a role of RAR1 as a molecular link between effector virulence function and effector-triggered immunity.

R protein-mediated disease resistance

HSP90, SGT1, and RAR1 associate with R proteins and initiate a signaling cascade in plant immune responses (Shirasu and Schulze-Lefert, 2003). The functions of these chaperone proteins in disease resistance responses in many monocot and dicot plant species have also been extensively investigated by mutant analyses (Shirasu et al., 1999; Austin et al., 2002; Hubert el al., 2003; Lu et al., 2003; Takahashi et al., 2003; Chandra-Shekara et al., 2004) and by virus induced gene silencing (VIGS)-mediated functional analyses (Liu et al., 2004; de la Fuente van Bentem et al., 2005; Leister et al., 2005; Scofield et al., 2005; Bhattarai et al., 2007) (Table 1). In particular, components of the molecular chaperone complexes are well studied in the *Arabidopsis* R protein-mediated immune responses to two different pathogens, *P. syringae* and *P. parasitica*. HSP90 and RAR1 are required for RPM1, RPS2, and RPS4, which are well characterized R proteins against *P. syringae* isolates (Austin et al., 2002; Hubert et al., 2003; Takahashi et al., 2003), but SGT1 is not required by these R proteins. Similarly, *P. parasitica* resistance proteins, such as RPP2, RPP4, and RPP8, employ differential components of HSP90, SGT1, and RAR1 during the plant immune response. RPP2 requires SGT1 but does not require RAR1. In the case of RPP4-mediated resistance,

however, both SGT1 and RAR1 are essential, whereas RPP8 does not require either of these proteins for a disease resistance response (Austin et al., 2002).

Such differing signal specificities have also been identified in interactions of the barley MLA resistance proteins and the pathogen powdery mildew. For example, SGT1 and RAR1 are required for MLA6- and MLA12-, but not MLA1-, mediated resistance (Azevedo et al., 2002). Consistently, tobacco N, a *Tobacco mosaic virus* resistance protein, and wheat Lr21, a *Puccinia triticina* resistance protein, require each of the HSP90, SGT1, and RAR1 proteins in plant immune responses against their target pathogens (Peart et al., 2002; Lu et al., 2003; Liu et al., 2004; Scofield et al., 2005). Furthermore, tomato Mi-mediated resistance was recently demonstrated to require HSP90 and SGT1 for insect and nematode resistance (Bhattarai et al., 2007). In summary, HSP90 is intimately involved in many of the examined R protein-mediated disease resistance pathways, whereas RAR1 and SGT1 show differential contributions to each of the R proteins (Table 1).

In contrast to the essential role of SGT1 in R protein-mediated disease resistance against biotrophic pathogens, it is noteworthy that in *Nicotiana benthamiana*, SGT1 is involved in symptom development during disease susceptibility to a necrotrophic fungus *Botrytis cinerea* (Oirdi and Bouarab, 2007). SGT1 also has a role as a positive regulator of HR mediated by some R proteins such as RPP4, RPP31, and RPS5 (Zhang et al. 2004; Holt et al. 2005). Given that HR is important for the virulence of *B. cinerea* (Govrin and Levine, 2000), these data suggest that *B. cinerea* uses the HR-controlling gene SGT1 to establish disease.

HSP90 and SGT1 functions in plant development

HSP90 is essential for normal growth and development in *N. benthamiana* and *Arabidopsis* (Queitsch et al., 2002; Liu et al., 2004; Sangster and Queitsch, 2005; Sangster et al., 2007). In experiments using a *Tobacco rattle virus* (TRV)-based VIGS system, HSP90-silenced *N. benthamiana* plants show meristem death and a severely stunted growth phenotype with chlorotic leaves (Liu et al., 2004). HSP90-dependent phenotypes have also been extensively studied in *Arabidopsis* treated with the specific HSP90 inhibitor geldanamycin or harboring a silenced HSP90 gene family (Queitsch et al., 2002; Sangster and Queitsch, 2005; Sangster et al., 2007). In these studies, a lack of HSP90 caused a variety of phenotypes such as alterations in flowering time, morphological features, and total seed set. Moreover, the phenotypic changes induced by HSP90 reduction were found to be dependent on the environmental temperature, suggesting that HSP90 functions at the interface between developmental and environmental cues.

SGT1 is required for SCF^{TIR1}-mediated auxin responses in *Arabidopsis* (Gray et al., 2003) which include auxin-related processes such as the inhibition of root growth, lateral root development, and hypocotyl elongation in temperature dependent manner. In addition, the roots of *OsSGT1*-overexpressing rice plants show less sensitivity to 2,4-D in comparison with wild type plants (Wang et al., 2008), which suggests that OsSGT1 is also involved in auxin-mediated signaling.

OsSGT1 also interacts with a ubiquitin-conjugating enzyme, Rad6, in yeast (Yamamoto et al., 2004). Rad6 is

Table 1. Requirement of HSP90, SGT1, and RAR1 in R protein-mediated disease resistance.

Host	R protein	Pathogen or pest	HSP90	SGT1	RAR1	References
Arabidopsis	RPM1 (CNL)[a]	*Pseudomonas syringae*	Yes[b]	No[b]	Yes	Austin et al., 2002; Hubert et al., 2003
Arabidopsis	RPS2 (CNL)	*Pseudomonas syringae*	Yes	No	Yes	Austin et al., 2002; Takahashi et al., 2003
Arabidopsis	RPS4 (TNL)	*Pseudomonas syringae*	Yes	No	Yes	Austin et al., 2002
Arabidopsis	RPP2 (TNL)[a]	*Peronospora parasitica*	NT[b]	Yes	No	Austin et al., 2002
Arabidopsis	RPP4 (TNL)	*Peronospora parasitica*	NT	Yes	Yes	Austin et al., 2002
Arabidopsis	RPP8 (CNL)	*Peronospora parasitica*	NT	No	No	Austin et al., 2002
Arabidopsis	RPW8 (CC)	*Erysiphe cichoracearum*	NT	Yes	NT	Peart et al, 2002
Arabidopsis	HRT (CNL)	*Turnip crinkle virus*	NT	No	No	Chandra-Shekara et al., 2004
Barley	MLA1 (CNL)	*Blumeria gaminis*	NT	No	No	Azevedo et al. 2002
Barley	MLA6 (CNL)	*Blumeria gaminis*	NT	Yes	Yes	Azevedo et al., 2002
Barley	MLA12 (CNL)	*Blumeria gaminis*	NT	Yes	Yes	Shirasu et al., 1999
Pepper	Bs2 (CNL)	*Xanthomonas campestris*	NT	Yes	No	Leister et al., 2005
Potato	Rx (CNL)	*Potato virus* X	Yes	Yes	NT	Peart et al, 2002; Lu et al., 2003
Tobacco	N (TNL)	*Tobacco mosaic virus*	Yes	Yes	Yes	Peart et al, 2002; Liu et al., 2004
Tomato	CF4 (LRR)	*Cladosporium fulvum*	NT	Yes	NT	Peart et al, 2002
Tomato	CF9 (LRR)	*Cladosporium fulvum*	NT	Yes	NT	Peart et al, 2002
Tomato	I-2 (CNL)	*Fusarium oxysporum*	Yes	NT	NT	de la Fuente van Bentem et al., 2005
Tomato	Mi (CNL)	*Meloidogyne* spp. *Macrosiphum euphorbiae*	Yes	Yes	No	Bhattarai et al., 2007
Tomato	PTO (kinase)	*Pseudomonas syringae*	Yes	Yes	NT	Peart et al, 2002; Lu et al., 2003
Wheat	Lr21 (CNL)	*Puccinia triticina*	Yes	Yes	Yes	Scofield et al, 2005

[a]CNL, CC-NBS-LRR. TNL, TIR-NBS-LRR.
[b]Yes, dependent. No, not dependent. NT, not tested.

known to play a central role in the post-replication repair pathway (Xiao et al., 2000), and its interaction with OsSGT1 suggests an involvement of this co-chaperone in DNA repair, possibly by degrading repair-related proteins. This is supported by the finding that the expression of both genes is induced by exposure to DNA-damaging agents such as UV and H_2O_2 (Yamamoto et al., 2004).

POSSIBLE MECHANISMS UNDERLYING THE ACTION OF THE HSP90-SGT1-RAR1 MOLECULAR CHAPERONE COMPLEXES IN PLANT IMMUNITY

As discussed above and shown in Table 1, HSP90-SGT1-RAR1 complexes are critical for resistance to diverse plant pathogens and pests (Table 1). Although the mechanism underlying the role of these complexes is unclear, it is likely that the HSP90-SGT1-RAR1 complex is involved in stabilizing R proteins ([1] in Fig. 3A). Recent studies also suggest that R proteins exist in multi-protein complexes and thereby require chaperones to maintain their function (Hubert et al., 2003; Muskett and Parker, 2003; Shirasu and Schulze-Lefert, 2003; Liu et al. 2004; Holt et al., 2005; Azevedo et al., 2006; Botër et al., 2007). Miss-assembled R proteins may be non-functional or detrimental to the plant cell. In such cases, the HSP90-SGT1-RAR1 chaperone complex likely contributes to the stability of its substrates. This concept is supported by the fact that HSP90-mediated signaling substrates become unstable when HSP90 activity is inhibited (Picard, 2002). In other words, these chaperone complexes bind R proteins and modulate their stability.

In this context, the fact that a subset of R proteins appears to be affected by *rar1* mutations can be explained by a "threshold model". When destabilized in an *rar1* mutant background, RAR1-independent R proteins accumulate at relatively high steady-state levels that are above the threshold required for efficient defense responses (Bieri et al., 2004). In contrast, RAR1-dependent R proteins are present at relatively lower levels than this critical threshold in *rar1* mutants. Interestingly, a previous report has shown that the impaired resistance of some *Arabidopsis* R proteins, such as RPS5, in the *rar1* mutant background is recovered in an *rar1/sgt1b* double mutant (Holt et al., 2005). This study demonstrated that while RPS5 accumulates to only 13% of the wild type levels in the *rar1* mutant, the accumulation of R protein was restored to about 60% of wild type levels in the *rar1/sgt1b* mutant. This finding suggests that AtSGT1b antagonizes the RAR1- and HSP90-dependent accumulation of R proteins, and that AtSGT1b assists in the degradation of these proteins.

It is known that *Arabidopsis* contains two SGT1 isoforms, AtSGT1a and AtSGT1b, which are highly conserved in terms of their TPR-CS-SGS domain structures. When AtSGT1a is expressed above a certain level, some NB-LRR R proteins such as RPS5 in an *sgt1b* mutant background are stabilized (Azevedo et al., 2006). It is therefore possible that an R protein deficiency, including that of RPS5, is recovered by lower AtSGT1a levels in the *rar1/sgtlb* mutant as this allows the assembly of competent chaperone complexes.

The HSP90 chaperone complexes may regulate conformational changes in the R proteins ([2] in Fig. 3A). In the absence of pathogens, R proteins are functionally silenced by intra-molecular interactions (Moffett et al., 2002; Belkhadir et al., 2004). Structure-function studies of the potato NB-LRR protein, Rx, have demonstrated that physical interactions occur in vivo between the NB-LRR domain and the amino-terminal CC motif, and also between the LRR and the CC-NBS domains (Moffett et al., 2002). Interestingly,

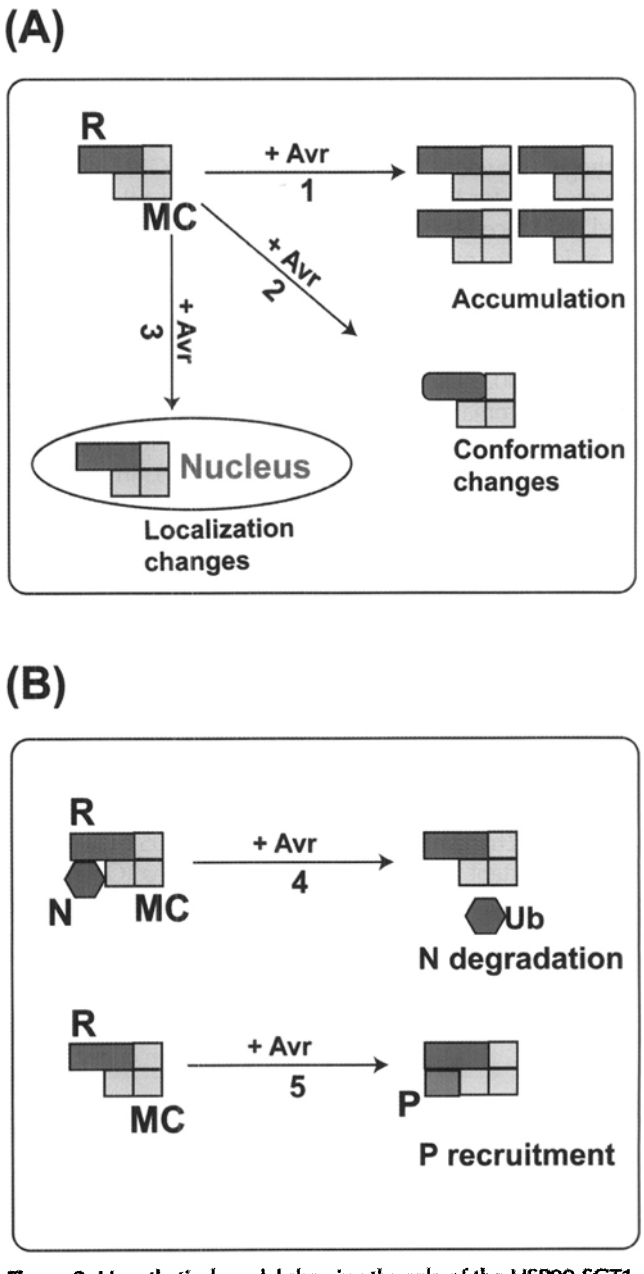

Figure 3. Hypothetical model showing the role of the HSP90-SGT1-RAR1 complex in R protein-mediated disease resistance. (A) Effects on R protein: The HSP90-SGT1-RAR1 (molecular chaperone, MC; yellow) complex is directly involved in regulating R protein (orange) by changing accumulation levels of the R protein (1), R protein conformation (2), and/or R protein localization (3). (B) Effects on regulators involved in the downstream signaling events: The HSP90-SGT1-RAR1 complex removes a negative (N; red) regulator via a ubiquitination (Ub)-mediated degradation process (4) or recruits a positive (P; green) regulator as a substrate (5). Avr, avirulence factor.

these interactions are disrupted in the presence of the Avr protein, *PVX* coat protein (CP), leading to an activated unstable form of Rx, which the NB domain that mediates downstream signaling is exposed (Moffett et al., 2002; Belkhadir et al., 2004). CP can convene or relieve molecular components that induce conformational changes in Rx. The HSP90-SGT1-RAR1 molecular chaperone complex is associated with R proteins (Hubert et al., 2003; Muskett and Parker, 2003; Shirasu and Schulze-Lefert, 2003; Liu et al. 2004; Holt et al., 2005; Azevedo et al., 2006), suggesting that it facilitates a fine-tuning of their conformation that can either lead to signal competent forms that mediate rapid activation of defense responses, or prevent inappropriate activation of the plant defense response that could cause a decreased cellular viability.

Chaperone complexes may also modulate the localization and trafficking of R proteins ([3] in Fig. 3A). Recent findings indicate that nucleo-cytoplasmic partitioning and nuclear activity are crucial for the function of several immune sensors (Shen et al., 2007; Shen and Schulze-Lefert, 2007). Notably, OsSGT1 and its interaction complexes are ubiquitously localized in the cytoplasm and nucleus (Wang et al., 2008), indicating that SGT1 likely shuttles between the cytoplasm and nucleus. It is thus possible that intra-molecular disulfide bonds among the conserved cysteines in SGT1 prevent TPR-mediated self-association, which in turn induces a dominant monomeric form of this protein. This form of TPR might play role in disease resistance signaling as a cellular sensor.

A similar mechanism of action is observed in NPR1-mediated plant defense responses. NPR1 is an essential regulator of systemic acquired resistance (SAR) in plants, which regulates defense responses to a broad range of pathogens. Activation of NPR1 is dependent on its monomeric versus oligomeric form. A reduced monomeric NPR1 accumulates in the nucleus and activates the expression of pathogenesis-related (*PR*) genes, whereas the oligomeric form is retained in the cytoplasm (Mou et al., 2003).

It is also possible that the molecular chaperone complex is involved in recruiting positive or removing negative regulators involved in the downstream signaling events during resistance responses ([4] and [5] in Fig. 3B). In this context, SGT1 may play an important role in the SCF-ubiquitination-mediated degradation of negative regulators of the defense response. SCF complexes are one of the RING-type ubiquitin E3 ligases that attach ubiquitin to target proteins, which are then eventually degraded by the 26S proteasome (Deshaies, 1999). In support of this notion, RING-type ubiquitin E3 ligases have been identified as critical components of the plant defense response (Salinas-Mondragon et al., 1999; Durrant et al., 2000; Wang et al., 2006). Importantly, the RAR1-SGT1 complex interacts with *Arabidopsis* CSN4 and CSN5, two COP9 signalosome components (Azevedo et al., 2002). Moreover, the silencing of genes encoding SKP1 and subunits of the COP9 signalosome causes the loss of *R* gene-mediated resistance in *N. benthamiana* (Liu et al., 2002). In animal immunity, Nod1 also interacts with the COP9 complex (da Silva Correia et al., 2007b), which is consistent with the finding that plant R proteins bind this complex. The association of N and Nod1 with SGT1 and the COP9 complex suggests that SGT1 is involved in ubiquitination-mediated immune responses in plants and mammals. Thus, SGT1 SGT1 likely plays a role in targeting resistance-regulating proteins for degradation by the 26S proteasome via a specific SCF complex (da Silva Correia et al., 2007b). Consistently, a previous report has shown that the *Arabidopsis* SGT1b protein has an RAR1-independent function that regulates programmed cell death HR during pathogen infection (Holt et al., 2005). In this study, SGT1b was found to be required for HR mediated by some *R* genes including *RPS5*-, *RPP4*- and *RPP31* (Holt et al. 2005). This also suggests that SGT1b may eliminate unidentified negative regulators.

As mentioned above, possible hypotheses for the mechanistic action of the HSP90 chaperone complexes are outlined in Fig. 3. In summary, a balanced activity of RAR1 and SGT1, in concert with HSP90, can modulate the stability or conformation changes of R proteins, as well as their signaling competence.

CONCLUDING REMARKS

The molecular chaperone complex HSP90-SGT1-RAR1 has diverse biological and cellular functions in plants. In the plant immune system, cytosolic HSP90 is a chaperone protein that maintains the steady-state accumulation of R proteins. SGT1 forms a complex with SCF ubiquitin ligase components and can both positively and negatively regulate NB-LRR protein accumulation, depending on the genetic background. RAR1 plays a generic role in maintaining the R protein levels. In addition, HSP90 can employ SGT1 and RAR1 as co-chaperones either to recruit clients that are involved in positive signaling or to remove negative signals. The HSP90-SGT1-RAR1 complex thus coordinately contributes to the stability and activation of R proteins and is therefore a critical component of the plant immune responses.

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