The HSP90-SGT1 Chaperone Complex for NLR Immune Sensors

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Abstract

The nucleotide-binding domain and leucine-rich repeat-containing (NLR) proteins function as immune sensors in both plants and animals. NLR proteins recognize, directly or indirectly, pathogen-derived molecules and trigger immune responses. To function as a sensor, NLR proteins must be correctly folded and maintained in a recognition-competent state in the appropriate cellular location. Upon pathogen recognition, conformational changes and/or translocation of the sensors would activate the downstream immunity signaling pathways. Misfolded or used sensors are a threat to the cell and must be immediately inactivated and discarded to avoid inappropriate activation of downstream pathways. Such maintenance of NLR-type sensors requires the SGT1-HSP90 pair, a chaperone complex that is structurally and functionally conserved in eukaryotes. Deciphering how the chaperone machinery works would facilitate an understanding of the mechanisms of pathogen recognition and signal transduction by NLR proteins in both plants and animals.

Contents

INTRODUCTION

Plants and animals face microbial attacks as a hazard of everyday life, and have evolved innate immunity systems to defend against these threats. The initial step of the immunity signaling pathway is recognition of intra- or extracellular pathogen-derived molecules. Quite remarkably, both plants and animals utilize proteins with similar structures for this purpose. Externally oriented transmembrane-type proteins containing leucine-rich repeat (LRR) domains detect extracellular molecules, whereas cytoplasmic sensors possess nucleotide-binding (NB) and LRR domains (24, 52). The LRR domain serves as a pattern-recognition receptor to detect pathogen-derived molecules or host proteins that are targeted by pathogen peptides that have entered the cell, so-called effectors (107). In this review, these proteins are collectively referred to as immune sensors and, more specifically, proteins with an NB-LRR core architecture are referred to as NB and LRR-containing (NLR) immune sensors (120).

In plants, the most effective specific resistance to pathogens is conferred by resistance (*R*) genes (52). *R* genes have been widely used in breeding agriculturally important plants, and have greatly contributed to the genetic value of modern crop species. A number of *R* genes have been isolated and characterized over the past 15 years from a wide range of plant species, and most of them encode NLR proteins. Despite the intensive research focused on these proteins, the molecular mechanisms underlying the recognition of pathogens, activation of the NLR molecule, and signal transduction to downstream components have not yet been sufficiently explained to form a robust, unified model. Genetic screening for critical immune system genes has identified a few particular sets of genes. One set is composed of genes involved in the function of salicylate, a

LRR: leucine-rich repeat

NB: nucleotide binding

NLR protein: NB and LRR-containing protein

key immunity-regulating molecule [for more details see recent reviews (32, 130)]. The other set appears to function more closely with *R* gene products. This review focuses on this set of genes, namely *REQUIRED FOR MLA12 RESISTANCE 1* (*RAR1*), *SUPPRESSOR OF THE G2 ALLELE OF SKP1* (*SGT1*), and *HEAT SHOCK PROTEIN 90* (*HSP90*), on what is currently known about how these gene products function in plant immunity, and on a comparison with animal NLR-type immunity.

HSP: heat shock protein

NLR IMMUNE SENSORS

NLR Proteins as Sensors in Innate Immunity

Most *R* genes encode structurally similar proteins that contain three distinct core modules: an N-terminal variable region (VR), the NB domain, and the LRR domain (52) (**Figure 1**). In some cases there are additional domains at the N or C termini. Animals also have proteins with the core VR-NB-LRR ternary module architecture and, as in plants, these proteins are involved in sensing pathogen products and in the regulation of cell signaling and death. The family of these immune sensors is now called NLR (120). Twenty-one NLR proteins are known in humans, and they can be further classified into five subfamilies on the basis of VR sequences: NLRA, NLRB, NLRC, NLRP, and NLRX (120) (**Figure 1**). Two well-characterized NLRC members, nucleotide-binding oligomerization domain–containing 1 and 2 (NOD1 and 2), recognize peptidoglycan (PGN) derivatives from bacterial cell walls and trigger inflammatory gene expression via NF-κB, a transcriptional activator (22, 39, 40). In the plant kingdom, typical NLR proteins

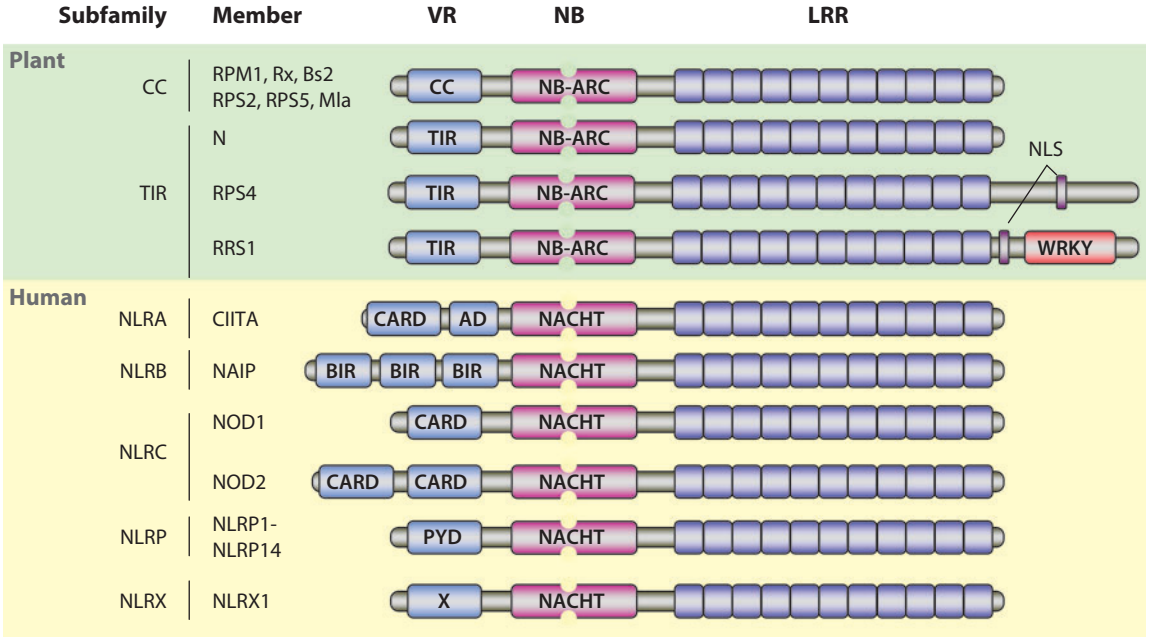


Figure 1

Schematic representation of NB and LRR-containing protein (NLR)-type immune sensors in plants and humans. VR, variable region; NB, nucleotide binding; NB-ARC, nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4 [InterPro ID (IPR): 002182]; LRR, leucine-rich repeat (IPR001611); CC, coiled coil; TIR, Toll/Interleukin-1 receptor (IPR000157); NLS, nuclear localization signal; WRKY, WRKY-containing DNA-binding domain (IPR003657); CARD, caspase activating and recruitment domain (IPR001315); AD, acidic domain; NACHT, domain present in NAIP, CIITA, HET-E, and TP1, (IPR007111), BIR, baculovirus inhibitor of apoptosis repeat (IPR001370); PYD, pyrin domain (IPR004020).

Pst: *Pseudomonas syringae* pv. *tomato*

can be found in mosses (2) and seed-bearing plants (61, 74), but not in unicellular green algae such as *Chlamydomonas* (72). Interestingly, PGN derivatives in *Candida* are recognized by an LRR-containing protein, adenylyl cyclase (Cyr1p), although this protein does not contain an NB domain (134).

Core Architecture of NLR Immune Sensors

The VR domains of plant NLR immune sensors have been assigned to subfamilies on the basis of secondary structure, although their amino acid sequences are quite distinct. The most common type of VR has a so-called coiled coil (CC) domain, and includes the well-studied *Arabidopsis* RPM1, RPS2, and RPS5; barley Mla alleles; and potato Rx. The Toll interleukin-1 receptor (IL-1R) (TIR) subfamily contains VR domains that are homologous to those of the human IL-1R, and includes tobacco N and *Arabidopsis* RPS4. The N-terminal VR region appears to bind to specific host proteins. For example, RPM1 binds to RIN4 (69), RPS5 binds to PBS1 (1), Mla binds to WRKY1 (106), and Rx binds to RanGap2 (94, 115) by their CC domains. In the case of RPM1, RIN4 is targeted by the corresponding pathogen effector, AvrRpm1 from *Pseudomonas syringae* pv. *tomato* (*Pst*). Similarly, *Pst* AvrPphB targets PBS1, the partner of RPS5. Modification of the RPM1 and RPS5 CC domain binding proteins triggers downstream immune responses. Thus, the CC domain of these NLR immune sensors confers specificity by binding to a particular pathogen target. Conversely, WRKY1, the CC domain binding protein of Mla alleles, appears to directly control immune responsive genes as a transcriptional repressor in response to powdery mildew effector Avr_{A10} (106), although it is not yet known if Avr_{A10} interacts directly with WRKY1 (see below). Thus, the VR region can serve as a binding site for a pathogen target or for downstream regulatory proteins.

The NB domain of plant NLR proteins is coupled to a distinct domain called ARC (found in the human apoptotic protease-activating factor APAF-1, R protein, and the nematode CED-4). This module is often referred to as the NB-ARC unit (**Figure 1**). The corresponding unit in vertebrate NLR proteins is called NACHT (domain present in NAIP, CIITA, HET-E, and TP1) (56). The NB-ARC and NACHT domains have ATPase activity and are thought to function as a switch for conformational changes (59, 116). Indeed, the crystal structure of the NB-ARC domain from APAF-1 reveals that the ADP-bound form is in a closed conformation and locks APAF-1 in an inactive state (91). The structural configuration predicts that any perturbation of the nucleotide-binding pocket, such as an exchange of nucleotides, may disrupt its packing conformation, resulting in an open and active state. Several autoactive or autoimmune mutants of plant NLR proteins contain mutations in this pocket (10, 108, 117). Because similar mutations in human NOD2 cause autoimmune phenotypes, this domain likely represents a conserved regulatory switch for NLR proteins (118).

The closed inactive state of the NB-ARC domain is often maintained by the adjacent LRR domain, because mutations in the LRR or in the region between NB-ARC and LRR result in the autoactivation of several plant NLR proteins (10, 108, 117), as does deletion of the LRR domain of both plant and animal NLR proteins (90, 118). The LRR motif is a pattern recognition domain that confers binding specificity on the NLR protein. Because of its binding specificity, the LRR domain has been proposed as a receiver domain for pathogen-derived molecules (35). If this is the case, a large number of specific receiver domains would have had to evolve during the evolutionary arms race against a wide variety of pathogens. Indeed, NLR proteins are the most polymorphic proteins found in *Arabidopsis*, and the polymorphisms are mainly located in the LRR domain (25). However, another class of NLR proteins is not very polymorphic. Members of this class, including RPM1, RPS2, and RPS5, are often found to interact with a host protein that is targeted by pathogens (5, 68, 104). In this case, the LRR would not be

expected to recognize pathogen determinants, but rather to detect conformational changes in the host protein. In either case, upon recognition of pathogen molecules, NLR proteins undergo a conformational change that allows self-oligomerization and/or interactions with other proteins to transduce the immunity signal (73, 90, 106, 107).

Localization of NLR Proteins

Unlike the transmembrane-type immune sensors, NLR proteins appear to recognize pathogen determinants in the cytoplasm. For example, major pools of plant NLR proteins, including RPM1, the Mla alleles, and RPS4, are membrane associated but have no obvious transmembrane domains (13, 16, 131). Conversely, N and Bs4 are soluble and mainly cytoplasmic (20, 101). However, some NLR proteins dynamically change location after recognition of pathogen determinants. For instance, *Arabidopsis* RRS1 directly recognizes the bacterial effector PopP2 and comigrates with its target into the nucleus (29). RRS1 may have a transcriptional regulatory function, because its C-terminal end contains the transcription factor domain WRKY, a well-studied module that activates or represses defense gene expression (36). Mutations in the RRS1-WRKY DNA-binding domain result in an autoimmune response, suggesting that the RRS1-WRKY DNA-binding domain normally has a negative regulatory function in immunity (82). Barley MLA, which confers resistance to powdery mildews, also interacts with WRKY transcriptional repressors in the nucleus (106). These interactions occur only after MLA recognizes an effector molecule from powdery mildew fungus and presumably inhibits WRKY repressor activity, resulting in defense gene induction. Thus, MLA directly links activation of NLR and downstream transcriptional regulation. This sort of direct link between a sensor and transcription factor (TF) may be a common theme, because other NLR proteins such as N or RPS4 require nuclear localization to elicit immune responses (20, 131).

CHORD-CONTAINING PROTEINS

Loss of RAR1 Function Leads to Impaired Immunity in Plants

Ha*: *Hyaloperonospora arabidopsis

Genetic screening for loss of resistance in plants identified several common components required for the function of NLR immune sensors. One of them, *RAR1*, was originally identified in a barley cultivar that contains an allele of *MLA* (121). *rar1* mutants are susceptible to a range of, but not all, powdery mildew isolates, suggesting that *RAR1* encodes a component specific to a particular set of *MLA* alleles (53, 88). *RAR2* was originally identified in the first screen but was later found to be another allele of *MLA12* (105). In total, this genetic screening identified 23 *MLA12* and 2 *RAR1* alleles, but no other mutations (53). This lack of other mutations, despite saturation of the screening, makes it apparent that the signaling pathway downstream of an *R* gene is rather short and/or many components are functionally redundant. Alternatively, the loss of additional signaling system components could be lethal. The short list of signaling components that could be isolated in the genetic screen would thus provide only a limited number of targets for disruption by pathogen attack. The conciseness of this system may in fact be one of its key self-protective mechanisms, and also indicates that *RAR1* may function in close association with the *R* gene product itself (see below). Because the loss of the specific immunity phenotype can be restored by an additional mutation in *ROM1*, it is unlikely that *RAR1* encodes the sensor (37).

The barley studies originally indicated that RAR1 may be a specific component for particular immune sensors, but the isolation of many *rar1* mutants in *Arabidopsis* proved otherwise. The *rar1* mutants were identified in three completely independent genetic screenings: one for loss of resistance conferred by *RPS5* against the bacterial pathogen *Pst* DC3000 containing *avrPphB* (127), one for loss of resistance against the oomycete pathogen *Hyaloperonospora arabidopsis* (*Ha*; formerly known as *Peronospora parasitica*) conferred by *RPP5* (78), and one

CHORD: cysteine- and histidine-rich domain

CHP: CHORD-containing protein

CS: CHORD-containing protein and SGT1

for loss of RPM1-dependent recognition of the corresponding *Pst* AvrRpm1 effector (123). Later, *RAR1* was also found to be required for *RLM1*- or *RLM2*-dependent resistance to *Leptosphaeria maculans*, a causal agent of blackleg disease (112). These data showed that *RAR1* is required for the function of multiple and distinct *R* genes that encode NLR immune sensors in both monocots and dicots. Interestingly, the susceptible phenotype of *rar1* mutants varies depending on the pathogen-ecotype combination. For example, *rar1-10* (Ler-0) is susceptible to *Pst-avrRps4*, but *rar1-21* (Col-0) and *rar1-1* (Ws-0) are at least partially resistant to the same pathogen (6, 49, 78, 123, 127). Because these mutations are likely to be null, the phenotypic difference is possibly due to an unidentified receptor(s) in Ws-0 and Col-0 that recognizes AvrRps4 but does not genetically require *RAR1* for its function (131).

The importance of *RAR1* in NLR-dependent resistance pathways was also shown by gene silencing methods in several plants. In *Nicotiana benthamiana*, an *R* gene encoding the NLR protein N against tobacco mosaic virus was shown to require *RAR1* (64). Similarly, *LR21*-dependent resistance against leaf rust in wheat is also mediated by *RAR1* (102). However, *RAR1* is not required by many other NLR-encoding *R* genes such as tomato *Mi* (12), potato *RB* (11), and pepper *Bs2* (58) and *Bs4* (100). Experiments in *RAR1*-silenced transgenic rice lines showed that *RAR1* is not essential for *Pib*, which encodes an NLR against rice blast fungus (119). In contrast, basal resistance to normally virulent races of rice blast fungus or bacterial blight is significantly reduced in *RAR1*-silenced lines. This result is consistent with earlier reports that *RAR1* is involved in basal resistance to virulent *Pst* in *Arabidopsis* or blast fungus in barley (49, 51). What these data might indicate is that basal resistance to virulent pathogens may also be conferred, at least partly, by RAR1-dependent NLR immune sensors. Virulent *Pst* strains can produce more than 30 effectors (41), so some of them could be recognized by NLR proteins, which would induce a weak basal defense response. Alternatively, RAR1 may be required for some transmembrane-type immune sensors that confer weak resistance to virulent pathogens. However, RAR1 is not required for the function of FLS2, a well-studied transmembrane-type immune sensor (140), and currently there is no report that RAR1 is required for any other known transmembrane sensors, such as EFR (139) or Xa21 (110).

RAR1 Encodes a CHORD-Containing Protein

RAR1 was originally cloned via the use of a map-based cloning method in barley (109). *RAR1* encodes a highly conserved eukaryotic protein that contains two similar but distinct domains termed cysteine- and histidine-rich domain 1 (CHORD1) and CHORD2 (**Figure 2*a***). In vitro biochemical studies indicate that CHORD1 and CHORD2 are novel modules that bind to two zinc atoms (48). *RAR1* is a single-copy gene in plants and *rar1* mutants have no detectable phenotype other than loss of disease resistance, indicating that *RAR1* is not essential for growth and development, but instead functions exclusively in immunity in higher plants (78, 109, 123). Interestingly, *RAR1* is not found in the *Chlamydomonas* genome, which lacks typical NLR-encoding genes, (72) further supporting the idea of a tight functional link between RAR1 and plant NLR proteins. By contrast, many other eukaryotes contain RAR1 homologs, but their module architecture is slightly different. For instance, *Phytophthora* and protozoan RAR1 homologs contain CHORD1 and CHORD2 but lack the CCCH domain that is highly conserved between the CHORD domains (109, 124). Metazoans and fungi (except the yeasts) produce CHORD-containing proteins (CHPs) that have a C-terminal extension called the CHORD-containing protein and SGT1 (CS) domain (109) (**Figure 2*a***). *Aspergillus nidulans* contains a single copy CHP-encoding gene (*chpA*), and its knockout is viable as a haploid but, strikingly, not as a diploid (95). Similarly, as a diploid organism, *Caenorhabditis elegans*

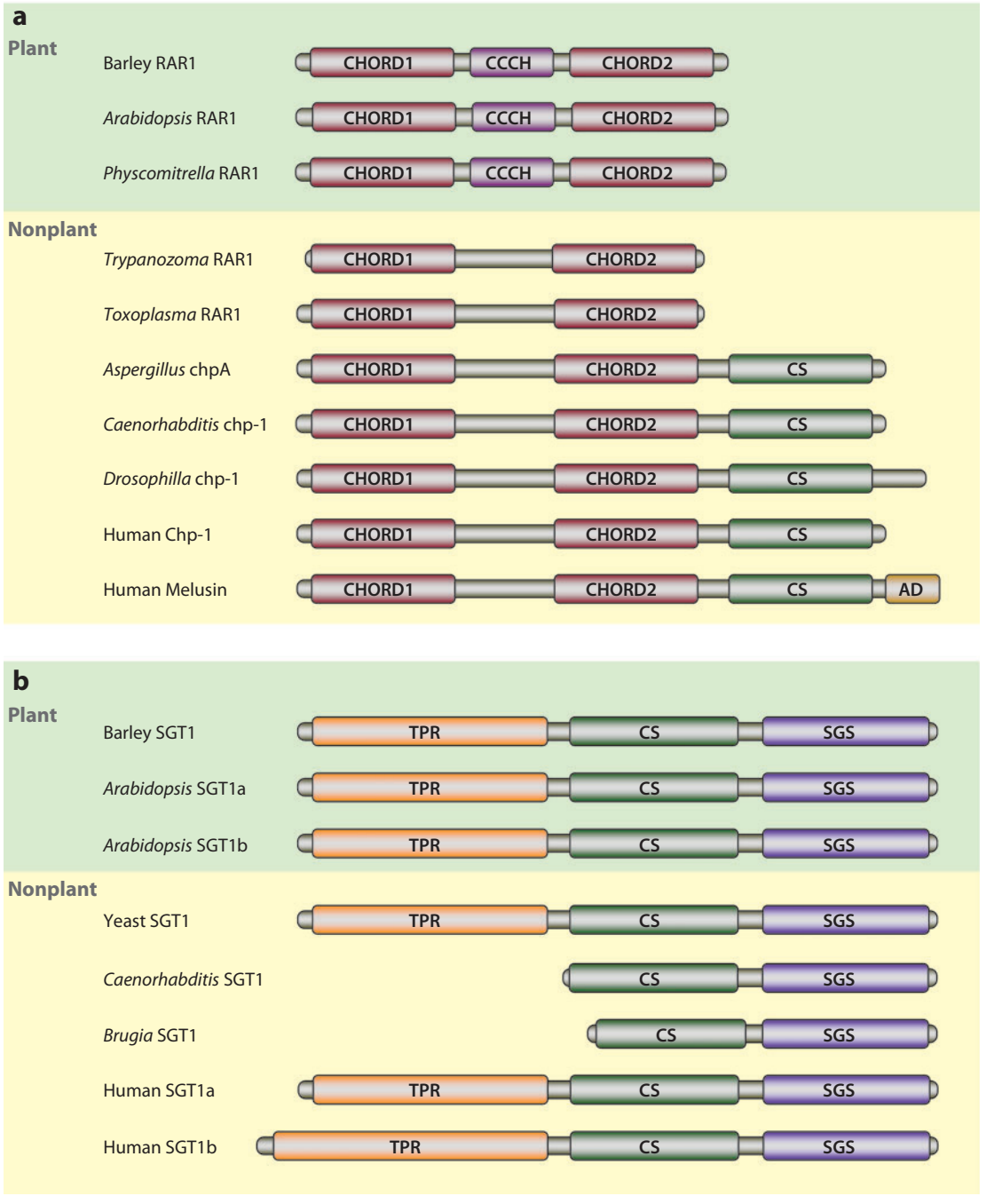


Figure 2

CHORD-containing proteins and SUPPRESSOR OF THE G2 ALLELE OF SKP1 (SGT1) proteins in eukaryotes. (*a*) CHORD-containing proteins. CHORD, cysteine- and histidine-rich domain [InterPro ID (IPR): 007051]; CCCH, CCCH-containing domain; CS, CHORD-containing protein and SGT1 (IPR007052). (*b*) SGT1 proteins. TPR, tetratricopeptide repeats (IPR013026); SGS, SGT1-specific domain (IPR007699).

requires its ortholog *chp-1* gene for viability (109, 141).

SGS domain: SGT1-specific domain

Unlike other organisms, mice and humans have two CHP-encoding genes: *Chp-1* and *Melusin* (19, 109). The silencing of *Chp-1* does not impair the function of human NLR proteins NOD1 or NOD2 in breast cancer cell lines, indicating that Chp-1 may not be involved in NLR function in vertebrates (26). Alternatively, the Chp-1 homolog Melusin may have a redundant function, although it is not known if *Melusin* is expressed in this cell line. However, *Melusin* is highly expressed in striated muscles (18). In mice, the loss of *Melusin* leads to reduced left ventricle hypertrophy (LVH) (thickening of the left lower chamber cardiac muscle) under stress conditions (17). Constitutive expression of *Melusin* in heart tissue results in sustained hypertrophy and prevents the changes associated with heart failure (28). Although Melusin was originally isolated as an interactor of β1-integrin, a membrane receptor that links extracellular matrix proteins to cytoskeletal elements, the biological importance of this interaction remains unclear (18). Melusin contains an extra C-terminal acidic domain that is required for Ca^{2+} binding (19), although its biological significance is not known. Similarly, Melusin has not yet been connected to immune responses.

SGT1 PROTEINS

SGT1 Is a RAR1 Binding Protein

Yeast two-hybrid screens using RAR1 as bait identified SGT1 as an RAR1 interactor (7, 62). The requirement of *SGT1* for immunity in plants is shown mostly by transient silencing of a number of NLR proteins, including MLA (7, 47), N (62, 87), Bs2 (58), Bs4 (100), Rx (87), RPS4 (137), Prf (77), Mi (12), I2 (125) R3a (14), and LR2 (102). In addition, SGT1 is also required for immune responses triggered by non-NLR-type sensors such as Cf4, Cf9, or RPW8 (87). This requirement indicates that either SGT1 function is not limited to the NLR sensors, or some unknown SGT1-dependent NLR proteins also operate downstream of non-NLR-type sensors. Similarly, SGT1 is also necessary for immunity responses triggered by the overexpression of a truncated form of calcium-dependent protein kinase (CDPK) (66). This particular form of CDPK may activate an SGT1-dependent NLR, or SGT1 could simply function downstream of CDPK without NLR involvement. *Arabidopsis* contains two SGT1 isoforms, SGT1a and SGT1b. The importance of SGT1b is demonstrated by the loss of *Ha* resistance in the *sgt1b* mutant that would otherwise be provided by RPP5 (4) or RPP7 (122). The *sgt1a/sgt1b* double mutant is embryo lethal in *Arabidopsis*, indicating that the SGT1 proteins are essential for growth and development (6). SGT1 is also involved in auxin and jasmonate responses (42), as well as in heat shock tolerance (81). This involvement is a marked difference from its interactor, RAR1, which appears to function only in immunity in plants.

Yeast SGT1 Mutant Phenotypes

As in the case of RAR1, SGT1 is highly conserved among eukaryotes. However, unlike RAR1, SGT1 is also found in yeast and *Chlamydomonas*. *SGT1* was originally isolated as a dosage suppressor of *skp1* in yeast, in which *SGT1* is an essential gene. SGT1 functions in several distinct biological processes, such as CBF3 kinetochore assembly, SCF ubiquitin ligase complex formation, and activation of the LRR-containing adenylyl cyclase, Cyr1p (31, 55). SGT1 has distinct tetratrico peptide repeat (TPR), CS, and SGT1-specific (SGS) domains, and its biological functions can be assigned to these domains (**Figure 2*b***). For example, mutations in the TPR domain arrest mitosis in the G2/M phase at the nonpermissive temperature because CBF3 kinetochore assembly is impaired (55, 60, 92). *sgs* mutants are defective in the activation of Cyr1p (31, 98) and halt at the G1 phase, because SCF complex formation is disturbed (55). It would also be interesting to know if the SGS domain of SGT1 is required for *Candida* Cyr1p recognition of bacterial PGN (134).

Human SGT1 Function

In humans, a single copy of *SGT1* encodes two isoforms, SGT1a and its splice variant SGT1b, which has 33 extra amino acids instead of Ser^{110} in the TPR domain (79, 142) (**Figure 2*b***). Although the functional importance of the splice variant is currently unclear, silencing of both *SGT1a* and *SGT1b* causes defects in kinetochore assembly similar to yeast (113), suggesting that there is functional conservation in eukaryotes. More importantly, *SGT1* knockdown by RNAi prevents an inflammatory response to bacterial PGN mediated by NLR protein NLRP3 (formerly called NALP3) in human cells (71). NOD1 and NOD2 also require SGT1 for responding to PGN derivatives (26). Thus, SGT1 is the first component known to be required for the function of both plant and human NLR proteins.

RAR1 AND SGT1 AS COCHAPERONES OF HSP90

HSP90 as a RAR1 and SGT1 Interactor, and Its Importance in Immunity

A second RAR1 interactor isolated in the yeast two-hybrid screen is the molecular chaperone HSP90 (63, 114), which is a highly conserved, essential protein involved in the assembly and stabilization of key signaling proteins such as protein kinases or receptors in eukaryotic cells (86). Interference with HSP90 expression or use of the specific inhibitor geldanamycin demonstrated the importance of HSP90 in immunity conferred by Mla (47), N (63), Prf (65), Mi (12), I2 (125), R3a (14), Lr21 (102), and RPS2 (114). The *Arabidopsis* case is more complicated, because it has four genes for cytoplasmic HSP90 (96). *Arabidopsis HSP90.1* is highly inducible by *Pst* infection, but *HSP90.2*, *HSP90.3*, and *HSP90.4* are expressed more or less constitutively (114). Loss of *HSP90.1* compromises *RPS2*-, *RLM1*-, and *RLM2*-dependent resistance, but has no effect on *RPM1* resistance (112, 114). However, point mutations in *HSP90.2* affect *RPM1* but not *RPS2* resistance (50). These point mutations are all located in the ATP-binding pocket in the N-terminal domain of HSP90.2. Surprisingly, a null mutant of *HSP90.2* is fully capable of RPM1-dependent immunity. Because all four HSP90 proteins are highly similar (97% identity), the isozymes are not expected to have distinct biochemical functions, which leaves temporal and spatial expression differences as the primary mechanism for their NLR protein specificity. HSP90.2 mutants may also possibly form nonfunctional heterodimers with other HSP90 isozymes.

The possibility of a functional link between HSP90 and RAR1 is further strengthened by the HSP90-SGT1 interaction. SGT1 contains TPR and CS domains that could be associated with HSP90 (**Figure 2*b***). The TPR domain is closely related to that of protein phosphatase 5, which binds to the C-terminal pentapeptide MEEVD of HSP90 (27, 93, 99), and the CS domain is structurally similar to p23, a cochaperone of HSP90 (15, 31, 38, 57) (**Figure 3**). Deletion analysis indicates that the CS domain of plant SGT1 is required and sufficient for its binding to HSP90, but the TPR domain is not needed (114). SGT1 homologs in several *Caenorhabditis* species and *Brugia malayi* do not have a TPR domain, further suggesting that the TPR domain is not essential for conserved SGT1 function per se (**Figure 2*b***). Furthermore, NMR structural analysis showed that the CS domain of human SGT1 directly binds to HSP90 (57). Large-scale mutagenesis and NMR analysis of plant SGT1 also confirmed that the CS domain is required and sufficient for HSP90 binding (15). However, in vitro studies of yeast SGT1 showed that deletion of the TPR domain greatly reduces its interaction with HSP90 (Hsc82), but the CS domain retains weak binding activity (21). Yeast two-hybrid analysis of yeast SGT1 and HSP90 also indicated that the TPR domain of yeast SGT1 is required for SGT1-HSP90 interaction. Similar to other TPR-containing cochaperones, the TPR domain may bind to the

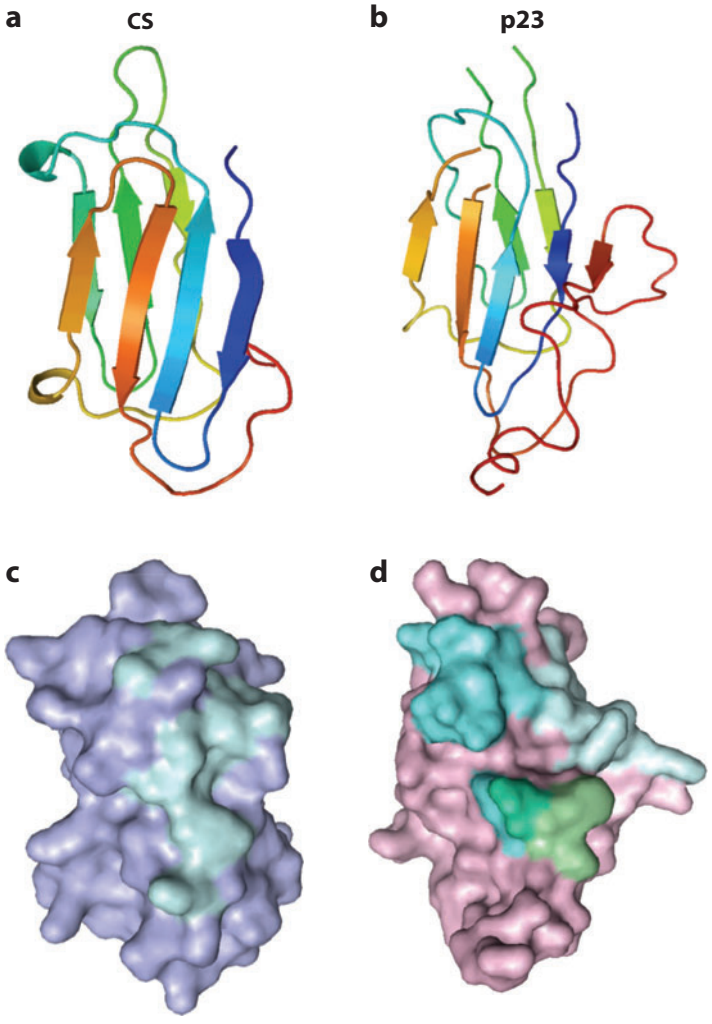


Figure 3

Comparison of the CHORD-containing protein and SGT1 (CS) domain and p23. The CS domain and p23 are structurally similar but have distinct binding sites for HEAT SHOCK PROTEIN 90 (HSP90). Backbone tracing of (*a*) the CS domain and (*b*) p23/Sba1 based on NMR and crystal/X-ray structural analyses (3, 15, 54). Molecular surfaces of (*c*) CSa and (*d*) p23/Sba1. The amino acids involved in HSP90 binding are colored to match **Figure 4**.

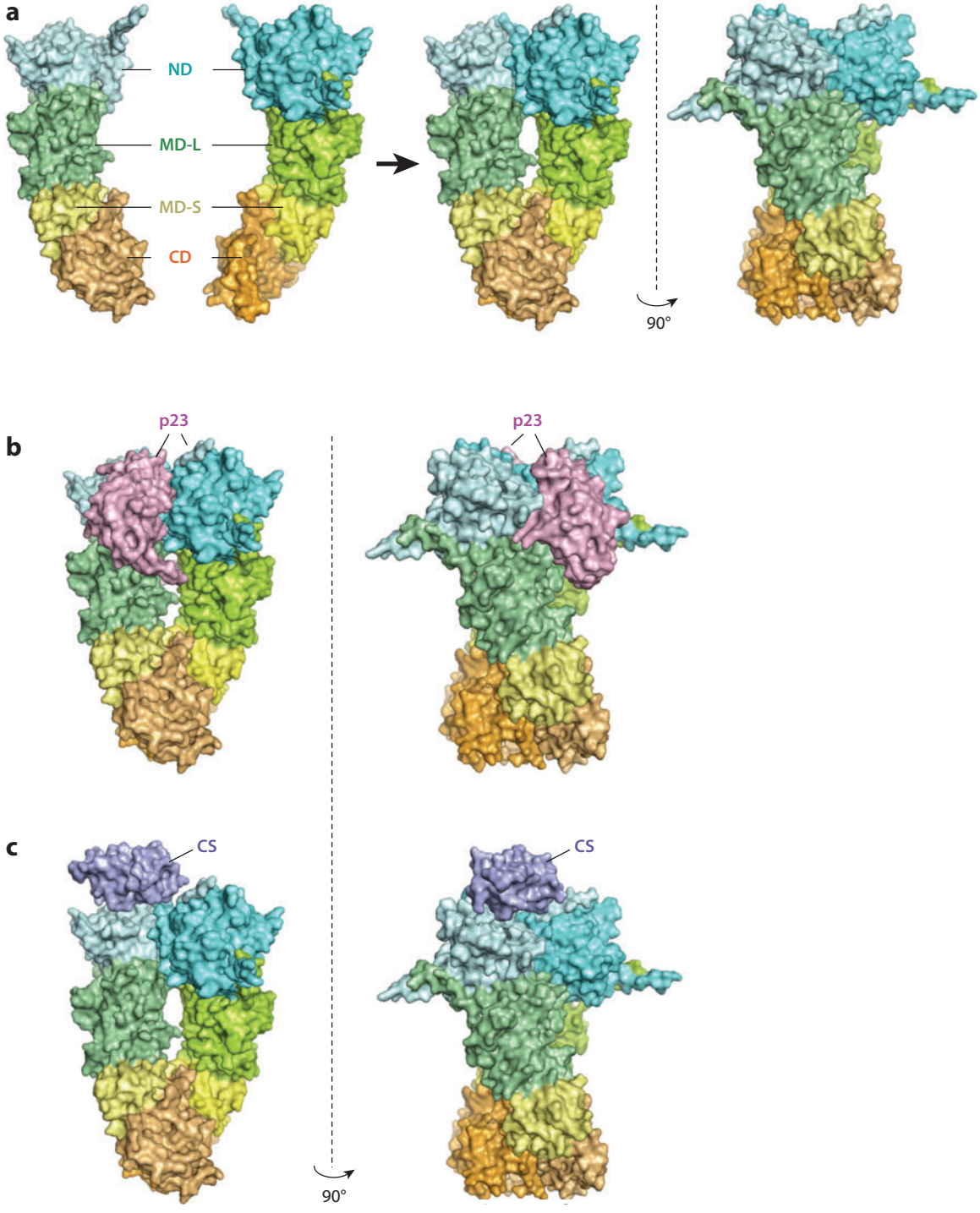
C-terminal end of HSP90 in yeast. Alternatively, because TPR mediates dimerization of SGT1 (84) and HSP90 functions as a dimer (86), the HSP90-SGT1 interaction may be stabilized by SGT1 dimerization, at least in yeast. Gel filtration experiments using cell extracts showed that SGT1 is eluted in fractions that contain proteins with apparent molecular mass ranges of approximately 80 kD, which is similar to the expected size of the SGT1 dimer (81). The largest SGT1 pool is likely to consist of dimers not in association with other large proteins such as HSP90 (84), or other interactions are too weak to be detected in cell extracts.

HSP90-RAR1-SGT1 Interaction Domains

Although the CS domain and p23 are structurally similar, major differences exist in how they bind to HSP90 (**Figure 3**). The HSP90 binding aspect of the CS domain is a four-stranded β-sheet, which is similar to the HSP90-binding side of p23, but does not have the C-terminal strand that is responsible for much of the p23-HSP90 interaction (3, 15). HSP90-p23 cocrystalization data elegantly demonstrated that p23 (Sba1 in yeast) forms a complex with the closed state of HSP90 in a 2:2 stoichiometry (3) (**Figure 4*a***). HSP90 consists of an ATP-binding N-terminal domain (ND, residues 1–216 in yeast), the large (residues 262–444) and small (residues 445–524) middle domains (MD) that mediate binding of substrate proteins, and the C-terminal constitutive dimerization domain (CD, residues 525–709) (3). When ATP binds to ND, the near lid segments (residues 94–125) rotate nearly

Figure 4

The molecular chaperone HEAT SHOCK PROTEIN 90 (HSP90) and its interaction with p23 and SGT1. Molecular surface of yeast HSP90 (*a*) monomers and a dimer as the ATP-bound closed form based on X-ray crystallography (3). ND, N-terminal domain (*blue and light blue*); MD-L, large middle domain (*green and light green*); MD-S, small middle domain (*yellow and light yellow*); CD, C-terminal domain (*orange and light orange*). (*b*) HSP90 interaction with p23. Backbone tracing of the $(HSP90)_2$-$(p23/Sba1)_2$ complex based on Ali et al. (3). (*c*) HSP90 interaction with the CHORD-containing protein and SGT1 (CS) domain. The $(HSP90)_2$-$(CS)_1$ complex model based on $(HSP90\text{-}ND)_1$-$(CS)_1$ X-ray crystallography and NMR superimposed on the HSP90 dimer structure. Note that because of a steric clash between the two CS domains they can not bind simultaneously to the closed form of HSP90 dimer. All molecular graphics were produced with the PyMOL program (**http://www.pymol.org**).



180° from the open position, stabilizing the ND association in a HSP90 dimer. Each p23 molecule associates with two NDs of a closed HSP90 dimer and with one large MD of a monomer (**Figure 4*b***). This three-point interaction likely stabilizes the closed conformation and extends the lifetime of the particular state that is essential for substrate activation. In contrast to p23, the CS domain binds to the ADP-bound form with the lid segment open in the CS:ND crystal (136). The CS binding regions end up far from the ATP binding pocket and lid segment, whereas p23 interacts with residues in the segment that are available only in the ATP-bound closed state (3, 54, 136). Notably, the CS domain also associates with residues in the N-terminal strand of HSP90, which moves significantly during the ATPase cycle (54, 136). Thus, the ATPase-driven conformational change within the HSP90 dimer would force dissociation of SGT1. Although SGT1 could also bind to the ATP-bound (closed) form of HSP90 and p23 binds to a distinct site, SGT1 and p23 do not associate with HSP90 at the same time (54). This finding also suggests that SGT1 and p23 have distinct functions in the modulation of HSP90 activity. The in vitro cochaperone activity of SGT1 in association with HSP90 via this different and apparently novel form of interaction (135) suggests that HSP90 may be able to bind with different cochaperones, each of which may provide tailored chaperone activity.

The CS domain binds to both HSP90 and RAR1. The CS-RAR1 interaction is mediated by the RAR1-CHORD2 domain, which is necessary and sufficient for binding to SGT1 (7, 103). NMR surface mapping and mutational analyses revealed that the ND of HSP90 and CHORD2 of RAR1 bind to the opposite sides of the CS domain (15). The CHORD2-CS interaction is of particular interest because metazoan CHP proteins contain these domains in tandem (**Figure 2*a***). This interaction is an excellent example of the Rosetta Stone model, which predicts functional and physical links between two domains in different proteins if these domains are found in a single peptide in other organisms (70). The locations of CHORD2 and HSP90 binding surfaces on opposite sides of the CS domain raise the possibility that CHORD2 and HSP90 simultaneously coassociate with SGT1. In fact, not only can a CHORD2-SGT1-HSP90 ternary complex be formed, but the addition of CHORD2 also stabilizes the SGT1-HSP90 interaction in vitro (15). Deletion analysis of RAR1 in the yeast two-hybrid system showed that CHORD1, but not the highly homologous CHORD2, is sufficient for binding to HSP90-ND (114). CHORD2 had weak in vitro HSP90 binding activity (15), but no binding activity was detected in yeast two-hybrid assays (114). CHORD2 may thus bind to HSP90, but only in the presence of SGT1, resulting in a stable ternary complex. In this context, then, it is noteworthy that both CHORD1 and CHORD2 of human CHP-1, as well as melusin, can clearly associate with HSP90 (43, 97, 133). Melusin also has a cochaperone function (97), indicating that the CHORD-containing proteins represent a new class of HSP90 cochaperones that act in conjunction with SGT1.

The Dynamic Nature of the RAR1-SGT1-HSP90 Complex

The dynamic nature of RAR1-SGT1-HSP90 complex formation may be inferred from observations that CHORD1 can interfere with the SGT1-HSP90 interaction, whereas CHORD2 can enhance it (15). The interference would occur because the CHORD1 interaction site overlaps with the CS binding region at the N-terminal domain of HSP90 (54). However, and rather paradoxically, RAR1 does not interfere with, but instead enhances, the SGT1-HSP90 interaction (15). CHORD1 and CS may each bind to a different HSP90 molecule in a dimer while CHORD2 stabilizes the CS-HSP90 interaction, creating an asymmetric complex (**Figure 5*a***, structure 4). Such asymmetric complex formation is likely to be transient, but may hold the HSP90 dimer in a state in which substrate can be loaded or released. In

this case, RAR1 would act as an enhancer of the SGT1-HSP90 chaperone machine.

Consistent with this idea, the loss of resistance phenotype of *rar1* and *sgt1* mutants is additive for some NLR proteins (4, 7). A curious case in *Arabidopsis*, however, is that the *rar1* phenotype is reversed in a *rar1/sgt1b* double mutant when tested for RPS5- or RPP8-based immunity (49). This antagonistic relationship is somehow specific to particular NLR pathways, because it is not found in immunity conferred by RPM1, RPS2, or RPS4 (49). Although the molecular mechanism underlying this peculiar phenotype is unclear, a fine balance between RAR1 and SGT1 interactions with HSP90 seems to be important for substrate folding and/or activation.

NLR as a Substrate of the RAR1-SGT1-HSP90 Chaperone

A potential target for the RAR1-SGT1-HSP90 chaperone complex is the immune sensor. This possibility was first suggested by the significant reduction of RPM1 protein levels in *rar1* mutant lines (78). Later, other NLR proteins such as MLA1, MLA6, and Rx (13), as well as RPS5 (49), were shown to require RAR1 for steady-state accumulation. In particular, *MLA1* does not genetically require *RAR1* for immunity against powdery mildew (88, 138), but MLA1 protein levels are reduced to only approximately a quarter of wild-type levels in the *rar1* mutant, which may be sufficient to trigger a defense response (13). MLA6 protein levels are also reduced by approximately the same percentage, but because normal MLA6 expression is so much lower than that of MLA1, the total amount of protein is much lower in the *rar1* mutant, and may be below the threshold needed to trigger a response. This difference in absolute levels in the *rar1* mutant may explain the genetic requirement for *RAR1* for the MLA6 NLR protein response. Thus, although genetic analyses suggest *RAR1* specificity for particular NLR pathways, biochemically most NLR proteins require RAR1 for stabilization. NLR stability is likely to be determined by the LRR domain, as shown in

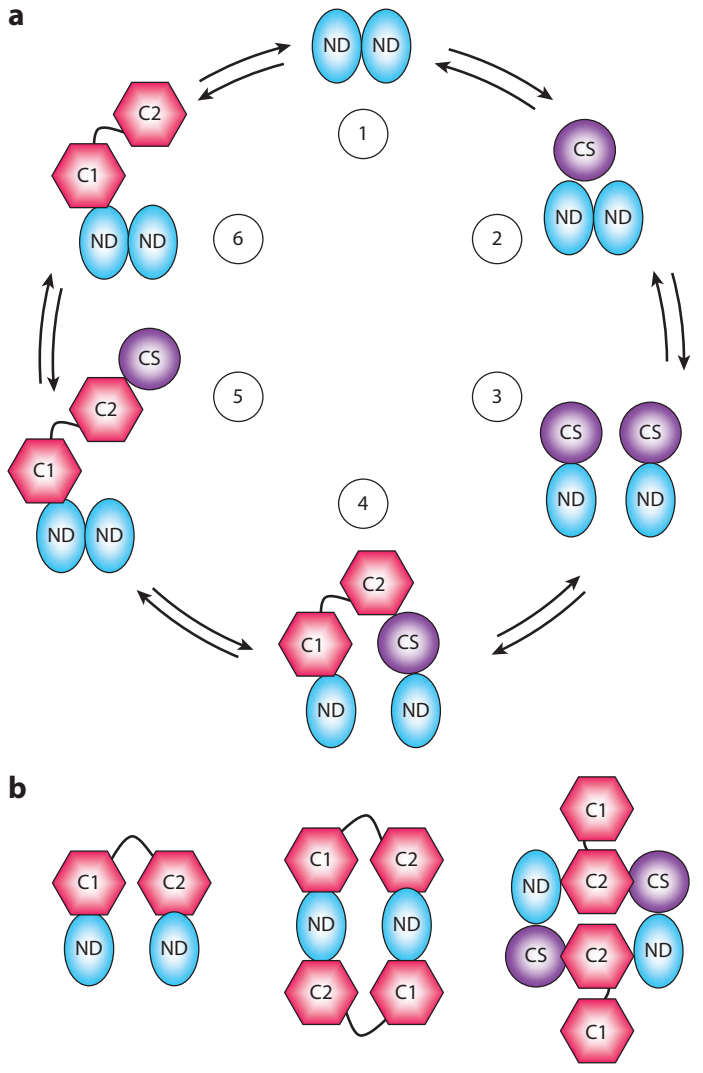


Figure 5

Proposed model for dynamic interactions of HEAT SHOCK PROTEIN 90 (HSP90), SUPPRESSOR OF THE G2 ALLELE OF SKP1 (SGT1), and REQUIRED FOR MLA12 RESISTANCE 1 (RAR1). (*a*) A model for dynamic interactions is proposed in which HSP90 is the central figure in a cycle that involves interacting domains as follows: N-terminal domain (ND) from (HSP90), CHORD-containing protein and SGT1 (CS) from SGT1, and cysteine- and histidine-rich domain 1 or 2, CHORD1 or 2 (C1 or C2) from RAR1. (1) ND dimerizes in the closed form of HSP90. (2) Only one CS can bind to the closed form. (3) Two CSs bind to the open form. (4) C1 binds to ND while C2 interacts with CS simultaneously. (5) CS dissociates in a closed form. (6) C1 also binds to the closed form. In this model, interactions occur sequentially in either the clockwise or counterclockwise direction. In the clockwise direction, RAR1 efficiently dissociates SGT1 from HSP90. The counterclockwise direction would indicate that RAR1 helps to bring SGT1 (possibly with an NLR protein as a substrate) into HSP90. (*b*) Other interactions that are possible if CHORD2 can also bind to HSP90.

barley MLA alleles in which RAR1 dependency is located in the LRR domain (44, 105).

As in the case of RAR1, SGT1 is also required for the steady-state accumulation of certain NLRs, such as Rx (6, 15) and N (73). The CS and SGS domains of SGT1 are essential for accumulation (15). Mutation, silencing, or inhibition of HSP90 likewise reduces the levels of Rx (65), RPM1 (49, 50), and RPS5 (49). Thus, many NLR proteins apparently require HSP90 for their stability, and possibly for maintaining their sensory signal-competent state. One intriguing observation is that RPM1 and RPS5 do not require HSP90 activity for their stabilization if SGT1b is missing (49). However, in this case it is unclear whether stabilized RPM1 and RPS5 are in the signal-competent conformational state or not. The stable and the signal-competent forms of an NLR may be be different. When NLR proteins or their domains are overexpressed, no reduction in stability due to *SGT1* silencing is detected (76). Presumably, cellular levels of the signal-competent form of NLRs are very low, and the massive expression of NLRs, under the control of a strong promoter and/or by transient expression, may produce nonfunctional proteins. These excess proteins could possibly accumulate in inclusion bodies.

The functional link between HSP90 and NLR proteins is likely mediated, at least partly, by SGT1 (**Figure 6**; **Table 1**). In yeast, SGT1 is required for the function of LRR-containing adenylyl cyclase Cyr1p, and a mutation in the SGS domain suppresses a temperature-sensitive allele in the LRR domain, strongly indicating a direct interaction between the SGS domain and the LRR domain. In a yeast two-hybrid screening using yeast SGT1 as bait, a number of LRR-containing proteins were consistently isolated (31). In plants, the LRR domains of Bs2 and MLA1 associate with SGT1 (13, 58). For MLA1, the SGS domain of SGT1 is sufficient for interaction with the LRR. Similarly, human SGT1 was identified from yeast two-hybrid screening that used the LRR domain of NLRP3 as bait (71). Furthermore, NLRP2, NLRP4, NLRP12, Nod1, Nod2, and

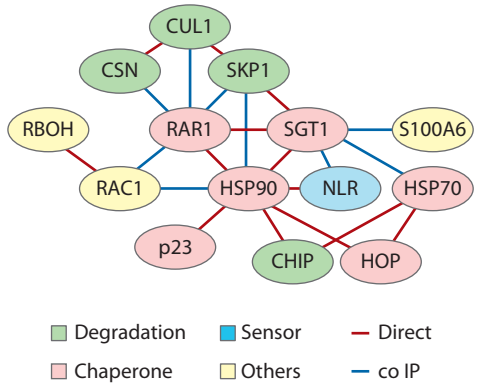


Figure 6

REQUIRED FOR MLA12 RESISTANCE (RAR1)-SUPPRESSOR OF THE G2 ALLELE OF SKP1 (SGT1)-HEAT SHOCK PROTEIN 90 (HSP90) interaction map. Red lines indicate confirmed direct interaction by in vitro experiments using purified proteins. Blue lines indicate associations detected by coimmunoprecipitation experiments. The detection of interactions is detailed in **Table 1**.

NLRC4 were all found to be associated with SGT1 (26, 71). Importantly, HSP90 was always detected in the immunoprecipitates along with the NLRs (71). Both CS and SGS domains are required for the interactions, suggesting that SGT1 can bind to HSP90 and an NLR protein simultaneously (26, 71). Notably, HSP90 can bind to the NACHT domain of NLRP3 without SGT1, but for binding to the LRR domain, SGT1 is always found together with HSP90 (71). In plants, HSP90 was found to associate with RPM1 (50), N (63), and I2 (125). As in the case of plant NLRs, human NLR proteins require HSP90 chaperone activity to maintain steady-state levels (26, 43, 71).

A remarkable difference between plants and humans is suggested by the observation that human NLRs require SGT1 for inflammasome activity but not for their accumulation (26, 71). However, these observations should be interpreted with caution because the experiments were conducted using transiently expressed NLR proteins in human culture cells. Antibodies against specific endogenous NLR proteins or lines expressing tagged NLR proteins at endogenous levels may be required for detecting

Table 1 Detection of proteins that interact with CHORD-containing proteins and SGT1

Protein pair		Organism	Method of detection	Reference
RAR1	SGT1*	Barley	Yeast two-hybrid (Y2H), co-immunoprecipitation (co-IP)	7
		Nicotiana benthamiana	Y2H, co-IP, in vitro	62
		Rice	Y2H, bimolecular fluorescence complementation (BiFC)	126
		Arabidopsis	Y2H, fluorescence resonance energy transfer–fluorescence lifetime imaging microscopy (FRET-FLIM)	7, 9
	HSP90	Barley	Y2H	114
		N. benthamiana	Y2H, co-IP, in vitro	63
		Rice	co-IP	119
		Arabidopsis	Y2H, co-IP	50, 114
	SKP1	*N. benthamiana*	co-IP	62
	CUL1	*N. benthamiana*	co-IP	62
	CSN4	*N. benthamiana*	co-IP	62
		Barley	co-IP	7
	CSN5	Barley	co-IP	7
	Rac1	Rice	co-IP	119
CHP-1	NOD1	Human	co-IP	43
	HSP90	Human	Y2H, in vitro	43
Melusin	SGT1*	Human	co-IP	97
	HSP90	Human	co-IP, in vitro	97
	β1-integrin	Human	Y2H, in vitro	18
SGT1*	HSP90	Barley	co-IP	114
		N. benthamiana	Y2H, co-IP, in vitro	63
		Arabidopsis	Y2H, co-IP	15, 50
		Yeast	Y2H, co-IP, in vitro	8, 21
		Human	co-IP, in vitro, NMR	57, 80
	HSP70	*Arabidopsis*	co-IP	81
		Yeast	co-IP	8
		Human	co-IP	111
	Bs2	*N. benthamiana*	co-IP	58
	MLA1	Barley	Y2H	13
	NLRP3	Human	Y2H, co-IP	71
	NLRP2	Human	co-IP	26, 71
	NLRP4	Human	co-IP	26, 71
	NLR12	Human	co-IP	71
	Nod1	Human	co-IP	26, 71
	Nod1	Human	co-IP	26, 71
	NLRC4	Human	co-IP	71

(*Continued*)

Table 1 (*Continued*)

Protein pair		Organism	Method of detection	Reference
	NLRC4	Human	co-IP	71
	SKP1	Barley	co-IP	7
		N. benthamiana	Y2H, co-IP, in vitro	62
		Yeast	Y2H, co-IP, in vitro	8, 55
	CUL1	*N. benthamiana*	co-IP	62
		Yeast	co-IP	55
	CSN4	*N. benthamiana*	co-IP	62
		Barley	co-IP	7
	CSN5	Barley	co-IP	7
	S100A6	Human	co-IP, in vitro	83

**Arabidopsis* and humans contain two copies of SGT1: SGT1a and SGT1b.

the stabilization activity of SGT1. In summary, HSP90 and SGT1 associate with NLR proteins, and chaperone activity is required for both plant and human NLR-dependent immune responses.

HSP90 cooperates with another chaperone, HSP70 [often called HSC70 (heat shock cognate 70), Ssa1 and Ssb1 in yeast], which also appears to associate with SGT1 (8, 81, 111). Unlike HSP90, HSP70 capture of newly synthesized proteins or unfolded polypeptides occurs under stress conditions (128). With the help of cochaperones Hop (Sti1 in yeast) and/or HSP40 (Ydj1 in yeast), the substrate of HSP70 is transferred to HSP90, which mediates the last step of protein maturation (129). Hop connects HSP90 and HSP70 by forming a multichaperone complex (128) (**Figure 6**; **Table 1**). As in the case of Hop, SGT1 associates with HSP90 and HSP70 (8, 81, 111). Hop and SGT1 can bind to HSP90 simultaneously (21), thus SGT1 and HSP70 interaction can be mediated by a HSP90-Hop complex. Although HSP70 is often found in coimmunoprecipitation or pull-down experiments, presumably because of nonspecific binding to unfolded peptide regions, the SGT1-HSP70 interaction seems to be rather specific. Firstly, the interaction is mediated at the SGS domain of SGT1 in both plants and humans (81, 111). Secondly, coexpression of the SGS binding protein S100A6 (calcyclin), a small calcium binding protein (83), reduces SGT1-HSP70 interaction in a Ca^{2+}-dependent manner in human cells (111). How HSP70 and S100A6 affect the SGT1-NLR interaction is not clear. It is plausible that the SGT1-NLR interaction is initially mediated by HSP70, followed by transfer to HSP90. Overexpression of HSP70 reduces NLR-dependent immunity in *Arabidopsis*, but it does not induce R protein instability (81). Thus, the precise function of HSP70 in immunity remains unknown.

Link to the Ubiquitin-Dependent Protein Degradation Pathway

The HSP90 chaperone machinery is often tightly associated with the ubiquitin-dependent degradation pathway leading to the 26S proteasome (75). This association is probably a part of a quality control mechanism that assures prompt degradation of unfolded or improperly folded sensors to avoid inappropriate activation of signal pathways. Several components involved in protein degradation pathways associate with a member of the RAR1-SGT1-HS90 chaperone complex (**Figure 6**; **Table 1**). In yeast, SGT1 directly binds to SKP1, a component of the SCF (SKP1, Cullin, F-box protein) ubiquitin ligase complex, acting as an adaptor to link HSP90 and SCF (21, 55). SKP1 and its associated protein CULLIN1 (CUL1) were also found in an SGT1 complex in plants (7, 62),

and the SKP1-SGT1 interaction was shown to be direct (62). *Arabidopsis* SGT1b is consistently required for SCF-mediated hormone responses (42). HSP90 can potentially link folding and degradation pathways, because it can simultaneously interact with SGT1 and CHIP (carboxy terminus of the Hsc70-interacting protein), which contains a ubiquitin ligase domain (136).

In plants, RAR1 and SGT1 also associate with the COP9 signalosome (CSN) (7, 62), which removes NEDD8 from CUL1, thereby inactivating the SCF complex (67). The interaction between RAR1/SGT1 and CSN may not be direct, because a yeast two-hybrid screening did not identify the pair (**Figure 6**; **Table 1**). The contribution of ubiquitin-dependent degradation to immunity signaling was shown by the fact that silencing SKP1 or the CSN components impairs *N* gene resistance against tobacco mosaic virus (62). However, the SKP1-SGT1 interaction may not be critical for immunity because another NLR protein, Rx, does not require an SGT1 TPR domain (15), which is the interaction domain for SKP1 in yeast (21). One possibility is that the SKP1/CSN-dependent ubiquitin pathway functions downstream of the immune sensors, and that the function of SGT1 in association with the ubiquitination machinery via SKP1 is to mediate the degradation of improperly folded NLR proteins.

RAR1, SGT1, and HSP90 Expression Profiles

Although RAR1 and SGT1 interact with each other, the transcriptional regulation of their encoding genes is different, perhaps reflecting their distinct functions. On the basis of publicly available microarray data for *Arabidopsis*, *RAR1* is expressed at a very low level and is not very responsive to pathogen infection, but *SGT1a* and *SGT1b* are highly inducible upon *Ha* inoculation or under various stress conditions (6, 81). However, no significant change at the protein level was observed upon infection (4). A similar result is obtained with *HSP90.1*, which is highly expressed upon *Pst* inoculation and stress conditions, but total protein levels are essentially unchanged (114). Newly synthesized SGT1 and HSP90 may be needed to cope with stress conditions. Conversely, *Melusin* is coexpressed with HSP90 and HSP70 in animals in response to mechanical stresses, suggesting a tight functional link under cellular duress (97).

Localization of the Chaperone Components

The major pool of HSP90 is in the cytoplasm, but HSP90 can be shuttled into the nucleus when it binds to substrates such as the glucocorticoid receptor (89). Fluorescently tagged versions of RAR1 and SGT1 were found both in the cytoplasm and nucleus (81, 126). Because a C-terminal tag rendered SGT1 nonfunctional, interpretation of the data using this version was validated by biochemical fractionation followed by antibody-based detection of native SGT1 (81). The fractionation experiment also revealed that the SGS domain of SGT1, which associates with HSP70 and NLR proteins, is required for its nuclear localization, suggesting that SGT1 may shuttle into the nucleus with its substrate (81). A bimolecular fluorescence complement assay showed that RAR1 and SGT1 can associate in both the cytoplasm and the nucleus (126), but a fluorescence resonance energy transfer–fluorescence lifetime imaging microscopy (FRET-FLIM) study detected the RAR1-SGT1 interaction only in the cytoplasm (9). A limitation of these studies is that the functionality of the fluorescently tagged proteins was not tested, and these proteins were overexpressed. Other information regarding localization comes from an interaction study in rice showing that RAR1, HSP90, and HSP70, but not SGT1, can be coimmunoprecipitated with the RAC1 small GTPase, a plasma membrane protein (119). Rice RAC1 is a critical, positive regulator of reactive oxygen species production by the RBOH-type NADPH oxidases that are activated upon infection (85), and the direct interaction of RAC1 with the EF hand–type Ca^{2+}-binding domain of RBOH leads to

activation of the oxidase domain (132). Because the NLR-dependent recognition of effectors leads to RBOH activation, RAR1, HSP90, and HSP70 may mediate the connection between NLR proteins and RAC1 in the cytoplasm.

The RAR1-SGT1-HSP90 Chaperone as a Target of Plant Pathogens

RAR1, SGT1, and HSP90 are key regulators of NLR immune sensors in plants, which makes these proteins a susceptible link in plant disease defense. Several cases have been reported in which pathogens require these components for virulence. For example, the *Pst* effector AvrB requires RAR1 (103) to induce chlorosis in *Arabidopsis*. RAR1 and AvrB likely act in close proximity, because the split luciferase complementation system is activated when its N- and C-terminal halves are fused to these proteins (23). The coimmunoprecipitation of RAR1 and AvrB has been proposed as an indication that RAR1 is a virulence target of AvrB (103). AvrB contains a myristoylation site and interacts directly with the membrane-associated protein RIN4, which associates with RPM1 and RPS2 (30). In addition, a recent report showed that AvrB-triggered chlorosis is caused by activation of TAO1, an NLR protein (33). Thus, a simpler explanation for AvrB-dependent chlorosis is that AvrB weakly activates TAO1, possibly via RIN4, and that TAO1 requires RAR1 for its stabilization. A similar case is found for the bacterial effector AvrPtoB from *Pst*. AvrPtoB suppresses immunity responses triggered by a bacterial pathogen-associated molecular pattern (PAMP), flg22, a component of flagellin (45, 46), and the AvrPtoB-dependent suppression requires SGT1 or RAR1 (45). One possibility is that, analogous to AvrB, AvrPtoB leads to weak activation of an NLR, which suppresses PAMP-dependent immunity responses. The third example is the case of *Botrytis cinerea*, a necrotrophic pathogen that requires host SGT1 for its virulence (34). Necrotrophs such as *B. cinerea* may activate NLR-dependent responses to trigger cell death and thus obtain nutrients from the dead cells. Whether *B. cinerea* directly targets the chaperone machinery remains unknown.

CONCLUSIONS AND PERSPECTIVES

Plants contain a large number of NLR proteins that confer immunity against a wide variety of pathogens. The core module architecture of NLR is shared by known animal immune system sensors, and the key chaperone system operates in a similar fashion in both plants and animals. With the isolation and characterization of these proteins, we are in a much better position to answer a number of long-standing questions: 1) How do NLR proteins biochemically sense pathogens and activate downstream signaling compounds, and how is the system shut down or limited after the pathogenic attack has been foiled? 2) How does the chaperone system selectively find NLR proteins and maintain its signal-competent state? 3) What criteria do the chaperone complexes use to select which NLR proteins to fold and which ones to degrade? More specifically, we may come to understand how RAR1 and SGT1 mechanistically regulate HSP90 by solving the structure of a RAR1-SGT1-HSP90 ternary crystal. We may also determine if the RAR1-SGT1-HSP90 chaperone complex functions in translocation or activation of NLR proteins upon recognition of pathogen-derived compounds by establishing a cellular assay system combined with in vitro reconstitution experiments.

SUMMARY POINTS

1. The nucleotide-binding (NB) and leucine-rich repeat (LRR)-containing (NLR) proteins function as immune sensors in both plants and animals.

2. NLR-type sensors are the substrates of a structurally and functionally conserved chaperone complex that consists of HEAT SHOCK PROTEIN 90 (HSP90) and its cochaperone SUPPRESSOR OF THE G2 ALLELE OF SKP1 (SGT1).
3. Cysteine- and histidine-rich domain (CHORD)-containing proteins represent a novel family of HSP90 cochaperones.
4. REQUIRED FOR MLA12 RESISTANCE 1 (RAR1), a CHORD-containing protein in plants, regulates the HSP90-SGT1 complex, resulting in the stabilization of NLR proteins.

FUTURE ISSUES

1. How do RAR1 and SGT1 mechanistically regulate HSP90?
2. Does the RAR1-SGT1-HSP90 chaperone complex also function in translocation or activation of NLR proteins upon recognition of pathogen-derived compounds?
3. How does the chaperone complex decide "to degrade or not to degrade"?
4. What is the recognition-competent state of an NLR protein?

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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