Complex formation, promiscuity and multi-functionality: protein interactions in disease-resistance pathways

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Accumulating evidence indicates that plant disease-resistance (R) proteins assemble in hetero-multimeric protein complexes in the absence of pathogens. Such complexes might enable the indirect recognition of pathogen effector molecules during attempted pathogen invasion. RAR1 and SGT1 are required for the function of most known R proteins. They interact with each other and with diverse protein complexes, which might explain their multi-functionality. The promiscuous behavior of RAR1 and SGT1 might be crucial for the formation and activation of R protein-containing recognition complexes as well as for regulating downstream signaling processes.

The ability of plants to combat pathogens is often conferred by disease-resistance (*R*) loci. These *R* genes encode proteins that recognize, directly or indirectly, pathogen effector molecules (encoded by *Avr* genes) [1]. Although different types of intruders, including fungal, bacterial, nematode and viral pathogens, can be detected by R proteins, subsequent defense responses are remarkably similar. These so-called hypersensitive responses (HR) include rapid ion fluxes, production of reactive oxygen species (ROI), accumulation of antimicrobial compounds, and are normally accompanied by a localized cell death (often called HR cell death) [2].

Most characterized R proteins are intercellular and contain a nucleotide binding domain (NB) and leucine-rich repeats (LRRs), which are hypothesized to confer recognition specificity [3]. Intracellular R proteins can be divided into subfamilies with members that have either a coiled-coil structure (CC–NB–LRR) or a motif related to the *Drosophila* Toll and human interleukin 1 receptor (TIR–NB–LRR) at their N-terminal end (Box 1). Transmembrane R proteins consist of extracellular LRRs and, in the case of rice Xa21, an intracellular serine/threonine kinase module [4]. Because R proteins are structurally related and their resistance responses are similar, it is thought that common signaling pathways are used against different pathogens.

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Box 1. Abbreviations and definitions of terms

Proteins

HSP90: heat shock protein 90, a chaperone.
MLA: mildew-resistance locus A.
RAR1: required for Mla12 resistance.
RIN4: RPM1-interacting protein 4.
RPM1: resistance to *Pseudomonas maculicola*.
RPP: resistance to *Peronspora parasitica*.
RPS2: resistance to *Pseudomonas syringae*.
SGT1: suppressor of G-two allele of skp1.
SKP1: suppressor of kinetochore protein.

Domains

CC: coiled-coil motif.
CHORD: cystein and histidine-rich domain.
CS: CHORD and SGT1 motif.
LRR: leucine-rich repeat.
NB: nucleotide binding site.
SGS: SGT1 specific domain, also called AC (adenylyl cyclase)-association domain.
TIR: Toll and interleukin receptor motif.
TPR: tetratrico peptide repeat.

Complexes

CBF3: centromere binding factor 3, a kinetochore complex.
COP9: An evolutionarily conserved protein complex similar to the lid subcomplex of proteasomes. It functions as a metalloprotease to cleave off a ubiquitin-like protein, NEDD8/RUB1 from CUL1, regulating the SCF complex activity.
SCF: A RING-type E3 ubiquitin ligase complex that contains CUL1, RBX1, SKP1 and F-box protein. F-box proteins serve as substrate determining factors in this complex.

Note: For simplicity, the *Arabidopsis* gene nomenclature rule is used irrespective of the origin of genes; capital letters in italics designate all eukaryotic wild-type genes (e.g. *RAR1*). Lower case letters in italics designate mutant genes (e.g. *rar1*). Eukaryotic proteins are indicated by capital letters (e.g. RAR1).

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R protein complexes

Although several R-AVR pairs have been isolated, the molecular mechanisms of AVR recognition by R proteins and activation of downstream signaling is poorly understood. The simplest model is that R proteins are receptors for the corresponding AVR molecules. Indeed, rice PI-TA, a CC–NB–LRR protein, directly interacts *in vitro* with AVR-PITA from *Magnaporthe grisea* [5]. However, direct interactions between R-AVR partners have rarely been demonstrated, indicating that the receptor-ligand model might be oversimplified [6]. Recently, several R proteins have been shown to form a complex with host proteins in the absence of pathogens [6–10], and it is likely that R proteins indirectly recognize *AVR* products through such preformed complexes [3,6].

Recent studies of a complex containing the *Arabidopsis* R protein RPM1 and an RPM1-interacting protein (RIN4, Box 1) have provided a model of how R proteins might recognize corresponding AVR determinants [6]. RPM1 is a CC–NB–LRR protein that confers resistance against *Pseudomonas syringae* expressing either of two distinct bacterial effector proteins, AvrRpm1 or AvrB. In a yeast two-hybrid screen to identify *Arabidopsis* proteins that bind AvrB, David Mackey *et al.* isolated RIN4 [6]. Notably RIN4 also interacts with AvrRpm1, which shares no sequence relatedness with AvrB. RIN4 seems to be required for RPM1 function because plants depleted for RIN4 exhibit loss of RPM1-dependent induction of HR cell death and disease resistance. Both AvrRpm1 and AvrB induced multi-phosphorylation of RIN4 in an RPM1-independent manner, suggesting that RIN4 is the target of the bacterial effectors and that RPM1 might detect conformational changes in RIN4 induced by phosphorylation upon pathogen infection.

Remarkably, RIN4 also associates with RPS2, a CC–NB–LRR-type R protein that recognizes yet another *P. syringae* effector protein, AvrRpt2 [11,12]. RIN4 overexpression inhibits RPS2-mediated HR cell death and disease resistance. By contrast, plants depleted for RIN4 become resistant against normally virulent pathogens and this phenotype is probably RPS2 dependent. Furthermore, delivery of AvrRpt2 by the bacteria induces RIN4 to ‘disappear’ and this phenomenon is not RPS2 dependent. These data suggest that RPS2 does not directly bind AvrRpt2 but instead detects AvrRpt2-mediated disappearance of RIN4. Thus, elimination of RIN4 might serve as a trigger to initiate RPS2-dependent hypersensitive cell death. Consistent with this hypothesis, *rin4* knockout mutants are lethal in the RPS2 background but appear normal in plants lacking RPS2.

Why do so many bacterial effectors appear to target RIN4? One possibility is that RIN4 is a regulator of basal defense responses and that these effectors might target RIN4 to create a ‘hospitable’ environment for bacterial growth [6]. If this were the case, then one would expect *rps2 rin4* double mutants to exhibit super-susceptibility to virulent bacteria. Nevertheless, obvious targets for pathogen effectors are defense regulators and this indeed seems to be the case for AvrPtoB, a *P. syringae* effector protein that blocks HR cell death triggered by distinct *R* genes [13]. Thus, finding targets for pathogen effectors might in turn lead to the identification of new plant defense components.

RAR1–SGT1 complex in *R* gene-triggered resistance

Mutations in barley *RAR1* suppress resistance against the powdery mildew fungus specified by the CC–NB–LRR gene *MLA12*, one of many resistance specificities encoded at the *MLA* disease-resistance locus [14]. Subsequent work has shown that *RAR1* is also essential for the function of a subset of *MLA*-encoded *R* specificities and of other unlinked powdery mildew *R* loci [15,16]. A conserved role of RAR1 in *R* gene-specified resistance against oomycete, bacterial and viral pathogens is now well documented through genetic analyses of the *Arabidopsis* homolog, *RAR1*, and by virus-induced gene silencing of *RAR1* in *Nicotiana benthamiana* [17–19]. These data demonstrate RAR1 use for the function of members of TIR–NB–LRR and CC–NB–LRR structural subtypes. This contrasts with the known preference of TIR–NB–LRR or CC–NB–LRR subtypes to engage either lipase-like EDS1 or membrane-associated NDR1, two other components in *R* gene-triggered resistance [20]. In addition, unlike EDS1, which is known to have a function in basal defense, limiting the growth of virulent pathogens [21], RAR1 appears to be specifically recruited for *R* gene-triggered resistance.

The RAR1 protein, which is highly conserved in most eukaryotic organisms, contains a pair of tandemly duplicated 60 amino acid sequence-related domains designated CHORD-I and CHORD-II (cysteine- and histidine-rich domains), each probably forming a novel zinc-finger structure [22]. Metazoan RAR1 homologs possess an additional C-terminal domain, the CS motif, which is also found in another conserved eukaryotic protein, SGT1 [22,23]. Such fusions, in which two domains are found in a single protein in one species and in two proteins in another species, are often indicative of physical interactions between the two domains [24]. Indeed, yeast two-hybrid analysis, *in vitro* binding assays and co-immunoprecipitation experiments of plant protein extracts have provided strong evidence for a direct physical interaction between RAR1 and SGT1 proteins [25,26].

Recent genetic evidence supports the widespread use of SGT1 in *R* gene-triggered resistance in plants. Gene silencing of the single-copy barley *SGT1* compromised a subset of powdery mildew *R* gene specificities at the *MLA* locus [25]. Similarly, silencing of *N. benthamiana SGT1* compromised the functions of potato *RX*, conferring resistance to potato virus X (PVX) and tobacco *N* against the tobacco mosaic virus [26,27]. Mutations in one of two closely related *Arabidopsis SGT1* genes, *SGT1b*, compromised a subset of *R* genes to the oomycete *Peronospora parasitica* [25,28,29]. Thus, SGT1 appears to exert, like RAR1, an important role in mediating resistance to different pathogen classes and both proteins are used by either of the two major structural subtypes of intracellular R proteins, TIR–NB–LRR and CC–NB–LRR members. Moreover, SGT1 is also required for certain ‘non-host resistance’ responses that render all genetic variants of a plant species immune to attack by all isolates of a pathogen species [27]. This suggests that *R* gene-mediated

Table 1. Requirement of RAR1 and SGT1 for resistance triggered by R proteins

R protein	Pathogen	RAR1	SGT1	Refs
Barley				
MLA1 (CC–NB–LRR)	Powdery mildew (*Blumeria graminis*)	No[a,b]	No[b]	[15,25]
MLA6 (CC–NB–LRR)	Powdery mildew	Yes[a,b]	Yes[b]	[15,25]
MLA12 (CC–NB –LRR)	Powdery mildew	Yes[a,b]	Yes[b]	[15,30]
Potato				
RX (CC–NB–LRR)	Potato virus X	NT	Yes[c,d]/No[e]	[27]
Tobacco				
N (TIR–NB–LRR)	Tobacco mosaic virus	Yes	Yes[c]	[19,26,27]
Tomato				
PTO (S/T kinase)	*Pseudomonas syringae*	NT	Yes[c,d]	[27]
CF-4 (extracellular LRR)	*Cladosporium fulvum*	NT	Yes[c,d]	[27]
CF-9 (extracellular LRR)	*Cladosporium fulvum*	NT	Yes[c,d]	[27]
Arabidopsis				
RPM1 (CC–NB–LRR)	*Pseudomonas syringae*	Yes[a]	No[e]	[17,18,28]
RPS2 (CC–NB–LRR)	*Pseudomonas syringae*	Yes[a]	No[e]/Yes[d]	[17,18,28]
RPS4 (TIR–NB–LRR)	*Pseudomonas syringae*	Yes/No[f]	No[e]	[17,18,28]
RPS5 (CC–NB–LRR)	*Pseudomonas syringae*	Yes[a]	Yes[a] (SGT1b)	[17,18]
RPP1A (TIR–NB–LRR)	*Peronospora parasitica*	No[a]	No[e]	[17,28]
RPP2 (TIR–NB–LRR)	*Peronospora parasitica*	No[a]	Yes[a] (SGT1b)	[17,18,28,29]
RPP4 (TIR–NB–LRR)	*Peronospora parasitica*	Yes[a]	Yes[a] (SGT1b)	[17,28,29]
RPP5 (TIR–NB–LRR)	*Peronospora parasitica*	Yes[a]	Yes[a] (SGT1b)	[17,28]
RPP8 (CC–NB–LRR)	*Peronospora parasitica*	No[a]	No[e]	[17,28]
RPP7 (unknown)	*Peronospora parasitica*	Yes[a]	Yes[a] (SGT1b)	[17,18,29]
RPW8 (CC-membrane)	Powdery mildew (*Erysiphe cichoracearum*)	NT	Yes[c,d]	[27]

Abbreviations: CC, coiled-coil motif; LRR, leucine-rich repeat; NB, nucleotide binding site; NT, not tested; TIR, Toll and interleukin receptor motif.
[a]Tested by mutant analysis.
[b]Tested by single-cell gene silencing in barley.
[c]Tested by virus-inducing gene silencing (VIGS) in *Nicotiana benthamiana*.
[d]Hypersensitive response test only.
[e]Tested only in *sgt1b*. It is still possible that *SGT1a* has a redundant function.
[f]Ecotype dependent.

resistance and non-host resistance share at least one common component.

Although RAR1 and SGT1 form a complex, the genetic dependence of an individual *R* gene function can vary (Table 1). For example, MLA6, N and RPP5 strongly depend on RAR1 and SGT1 proteins. MLA1 functions independently of RAR1 and, based on silencing experiments in barley, the requirement for SGT1 is weak, if any. Other cases such as RPM1 and RPS2 require only RAR1 but not SGT1b in *Arabidopsis*. In this case, SGT1a, a closely related homolog of SGT1b, might complement loss of SGT1b for these R protein functions. Both SGT1a and SGT1b can interact with RAR1 and complement yeast *sgt1* mutations, supporting this idea. RAR1 is not required for RPP2 function, whereas SGT1b is essential for establishing full resistance against *P. parasitica*. Such differential engagement of RAR1 or SGT1 for some *R* gene functions suggests both common and separate functions for the proteins.

Analysis of chimeric genes made from *MLA1* and *MLA6* has revealed further insights into the role of RAR1 and SGT1 in disease resistance [30]. *MLA* genes appear to be variants of a single gene at this complex *R* locus and encode intracellular CC–NB–LRR proteins that contain an extra C-terminal non-LRR (CT) region (CC–NB–LRR–CT architecture) [30,31]. At least the 108 kDa MLA1 protein appears to assemble in an ~700–800 kDa recognition complex in non-infected leaf cells (S. Mauch and P. Schulze-Lefert, unpublished). *MLA1*, *MLA6* and *MLA12* encode proteins that are ~97% sequence identical within the CC–NB domains and ~87% in the LRR–CT region [30]. In spite of this high level of sequence similarity, only MLA6 and MLA12 require RAR1 and SGT1 for an effective resistance response. Analysis of chimeric *MLA* genes generated by reciprocal domain swaps between *MLA1* and *MLA6* shows that the LRRs and CT domains are involved in recognition of cognate avirulence determinants [30]. However, RAR1 and SGT1 dependence appears to be modulated by CC–NB and LRR–CT sequences. Surprisingly, one chimera with only a third of the LRRs and the CT domain of MLA6 still recognizes AVRMLA6 and requires neither RAR1 nor SGT1. Thus, subtle differences of MLA protein sequence can dramatically alter RAR1 and SGT1 use without changing recognition specificity.

Possible operation points of the RAR1–SGT1 complex in disease-resistance pathways

Uncoupling of AVR recognition from RAR1 and SGT1 dependence strongly suggest that RAR1 and SGT1 are not involved in 'upstream' events such as processing or transport of AVR effectors (e.g. AVRMLA6). This is further supported by the finding that AvrRpt2-mediated disappearance of RIN4 occurs independently of RAR1 [12]. These data indicate that RAR1 and SGT1 might function in *R* gene-dependent resistance during: (1) formation of R protein complexes, (2) activation of assembled R protein complexes upon effector recognition, and (3) regulation of downstream signaling such as removal of negative

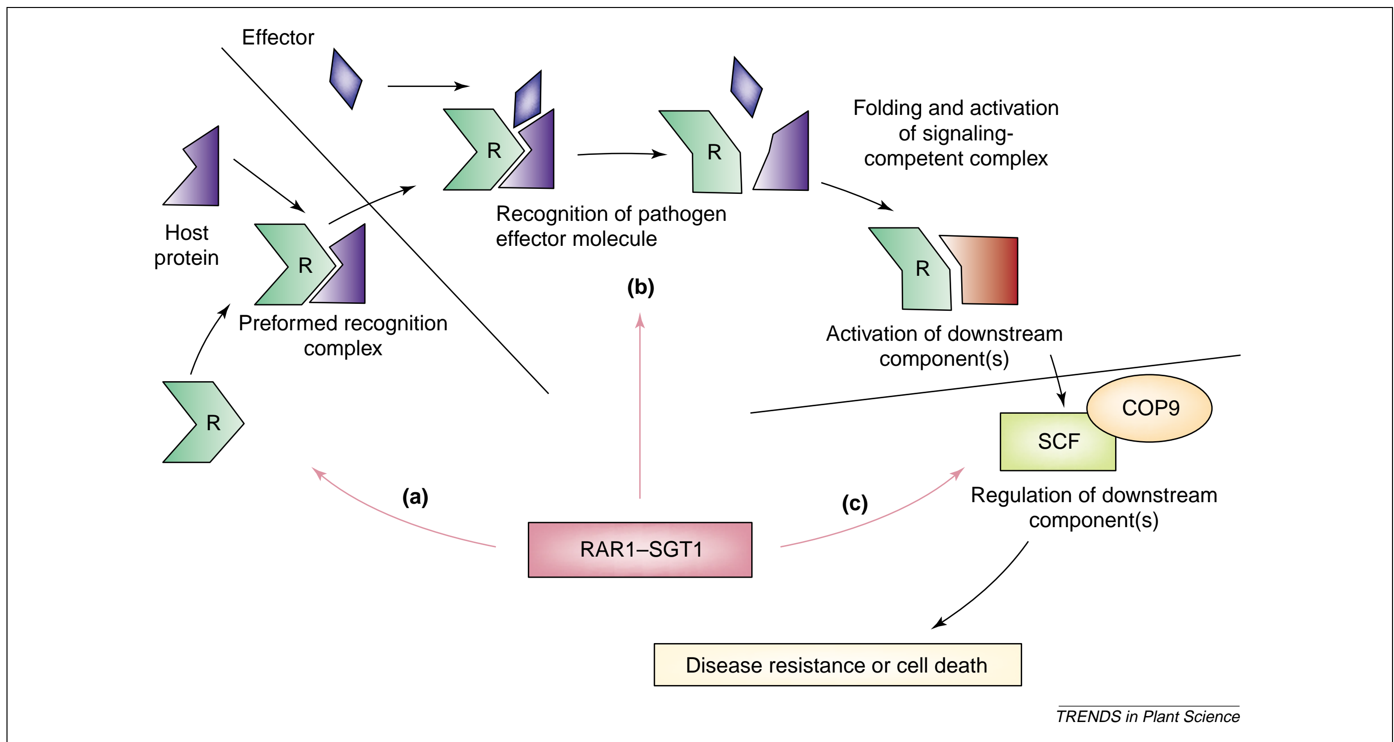

Fig. 1. Possible action points for RAR1–SGT1 in disease-resistance signaling. (a) Formation of resistance (R) protein complex with host protein(s). (b) Effector molecules are recognized by the assembled R protein complex and subsequent conformational changes activate downstream components. (c) Regulation of downstream signaling can occur by removing negative regulators and/or activating positive regulators by SCF and COP9 complexes.

regulators or activation of positive regulators (e.g. kinase or transcription factors) (Fig. 1).

R proteins appear to form hetero-multimeric recognition complexes. Misassembled or free R proteins might be useless or even be detrimental by auto-activating HR cell death. Thus proper assembly of R proteins might require a tight quality-control mechanism. We postulate a chaperone-like function for RAR1 and SGT1 in complex assembly of a subset of R proteins (see below). This proposed role might explain why RPM1 appears to be unstable in *rar1* mutant plants [18]. The differential requirements for RAR1 and SGT1 by different MLA proteins might reflect an intrinsic ability of some MLA variants to assemble in recognition complexes whereas other variants might need RAR1 and SGT1 assistance.

It is also possible that RAR1 and SGT1 are not involved in R protein complex formation but instead assist conformational changes of recognition complexes following recognition of cognate effector molecules. The significance of intramolecular interactions and conformational changes during the activation was revealed in studies involving RX, a CC–NB–LRR protein. Elegant experiments using co-expression of various RX parts as separate domains in *N. benthamiana* showed that physical interactions occur between the LRR and CC–NB domains as well as between CC and NB–LRR domains [32]. These intramolecular interactions between the RX domains were disrupted in the presence of the PVX coat protein, indicating that extensive conformational changes accompany RX activation. Recently, several mutants of RX have been identified that constitutively activate HR cell death in the absence of coat protein [33]. These mutants contain single amino acid substitutions in the NB or LRR domain and might indicate an inhibitory role of the intramolecular interactions. Because SGT1 gene silencing compromised cell death triggered by the auto-active RX variants but did not affect the stability of the proteins, SGT1 might either assist the folding of an auto-active state and/or might act downstream of activated RX [33].

In an alternative scenario, SGT1 and/or RAR1 might exert a role downstream of R protein complex formation and activation. For example, RAR1 and SGT1 might participate in the removal of negative regulators of resistance. Conceptually, disease resistance might be triggered by R protein containing recognition complexes and R protein-emitted signals might induce removal of checkpoints, whose function it is to prevent inadvertent defense and/or cellular suicide. Such negative regulators might be proteins that, when inactivated or mutated, confer constitutive or potentiated defense responses. Candidate negative regulators have been isolated, including *Arabidopsis* LSD1, MAPK4, EDR1 and barley MLO, and mutants of the corresponding genes exhibit heightened responsiveness for the onset of plant defense and cell death [34–37]. However, altered stability of these candidate negative regulators in the *rar1* or *sgt1* mutant background has not been reported. Alternatively, RAR1 and SGT1 might be required to activate positive regulators of disease resistance. Candidate activators of resistance include components of MAPK cascades [38], calcium-dependent protein kinase (CDPK) [39] or NADPH oxidase regulators [40]. Experiments using the *rar1* and *sgt1* mutant and silenced plants should help to resolve these possibilities in the future.

Possible biochemical functions of RAR1 and SGT1 complexes

Analysis of *SGT1* functions in yeast promises to provide leads for *SGT1* functions in plants because either of the two *Arabidopsis SGT1* homologs, *SGT1a* and *SGT1b*, can complement yeast *sgt1* mutant strains [25]. *Saccharomyces cerevisiae* SGT1 protein was originally shown to associate with SKP1 protein and to be required for assembly of the centromere-binding factor 3 (CBF3) kinetochore complex [23]. SKP1 is also a core component of SCF (SKP1/CULLIN/F-box protein) E3 ubiquitin ligases. SCF complexes play a broad role in regulating the stability and activity of many proteins in diverse physiological processes. E3 ubiquitin ligases recruit specific substrates and catalyze their ubiquitylation, often targeting them for degradation by the proteasome. Indeed, yeast SGT1 was found to associate with SKP1 in SCF complexes and is required for SCF-dependent cell-cycle control in *S. cerevisiae* [23]. At least the molecular associations of SGT1 in SCF complexes appear to be conserved in animals and plants. Mouse SGT1 was found to associate with the $\mathrm{SCF}^{\mathrm{SKP2}}$ complex [41]. In *N. benthamiana* and barley, SGT1 interacts with SKP1 and was found to co-immunoprecipitate another integral SCF component, CULLIN homologs [25,26]. Genetic evidence for a role of SCF complexes in *R* gene-triggered resistance has been obtained by virus-induced gene silencing of *N. benthamiana SKP1* genes, which resulted in compromised *N* gene-mediated resistance to tobacco mosaic virus (TMV) [26]. Taken together, plant SGT1 might have a role in targeting resistance-regulating proteins for destruction by the proteasome via specific SCF complexes [25,26].

Further evidence potentially linking *R* gene-mediated resistance to ubiquitylation processes has been obtained by the presence of COP9 (also known as CSN) signalosome subunits in RAR1 and SGT1 immunoprecipitates [25,26]. The multi-subunit COP9 signalosome is highly conserved in eukaryotic evolution, possesses significant structural similarity to the 19S regulatory lid of the proteasome, and functions at the interface between signal transduction and ubiquitin-dependent proteolysis [41,42]. Both the 19S regulatory lid and COP9 signalosome possess a metalloprotease activity to cleave off ubiquitin and ubiquitin-like NEDD8 protein from the targets, respectively [43,44]. By removing NEDD8, or RUB1 in *Arabidopsis*, from CULLINs in SCF complexes, the COP9 signalosome is thought to regulate SCF activity [41,45]. Genetic evidence of a role for the COP9 signalosome in *R* gene-mediated disease resistance was obtained by gene-silencing experiments of two COP9 subunits in *N. benthamiana*, *CSN3* and *CSN8*, leading in each case to compromised *N*-mediated resistance to TMV [26]. These data further support the involvement of ubiquitylation in disease-resistance signaling.

What could be the function of SGT1 in SKP1-containing complexes? In yeast, SGT1 was present only at a sub-stoichiometric level in the SCF complex, suggesting that it might not be an integral component of the complex [23]. Furthermore, although SGT1 is essential for CBF3 complex assembly in yeast, it seems that SGT1 is not required for SCF complex formation *per se*. For example, *E. coli*-produced human SCF components can be assembled *in vitro* without SGT1 [46]. In addition, the sgt1-3 mutant protein abolishes the interaction with SKP1 and leads to compromised CBF3 complex assembly, whereas ubiquitylation of SCF target proteins remained unaltered in this yeast mutant [23]. This might indicate that the SKP1–SGT1 interaction is not required for efficient ubiquitylation. By contrast, the sgt1-5 mutant protein leads to compromised SCF function but retains its ability to interact with SKP1 and also retains CBF3 function. This strongly suggests allele-specific perturbations of distinct SGT1 functions. One speculative model to explain these complex observations predicts that the association between SGT1 and SKP1 is crucial to bring SKP1 to CTF13, in turn triggering CBF3 complex formation. A second pool of SGT1 might transiently associate with SCF complexes, possibly assisting the ubiquitylation process once the SCF substrate is bound. Alternatively, SGT1 might facilitate substrate binding on assembled SCF complexes. It is possible that the regulatory role of SGT1 for SCF function is not mediated through direct interactions with SKP1 but via as yet unknown interactions with other SCF components, including SCF substrates.

Recent experiments using *S. cerevisiae* and *Schizosaccharomyces pombe sgt1/git7* mutant strains corroborate the multi-functionality of SGT1 because it was found to interact, genetically and physically, with yet another regulatory protein, LRR-containing adenylyl cyclase CYR1/CDC35 [47,48]. A mutation in SGT1 that abolished the interaction with CYR1 and thereby impaired cyclic AMP signaling, affected neither SCF nor CBF3 function, suggesting that the role of SGT1 in cAMP signaling occurs independently of SKP1. Similar to associations between the SGT1 and the SCF complex, only substoichiometric quantities of SGT1 were present in the adenylyl cyclase complex, suggesting that it might not be an integral but a transiently associated component.

What could explain the apparent promiscuity of SGT1 to enable it to interact with different regulatory protein complexes (Fig. 2)? SGT1 proteins contain a TPR domain that is an HSP90-binding domain in co-chaperones [25,49,50]. In addition, the CS motif in SGT1 probably adopts a fold similar to that of the p23 co-chaperone, which is known to interact with HSP90 and participates in the folding of different regulatory proteins [48,51]. Through its TPR and CS domains, SGT1 proteins might associate with HSP90, playing a chaperone-like role in the assembly or the conformational regulation of diverse multiprotein complexes [48,49]. Consistent with this hypothesis, the mouse $\mathrm{SCF}^{\mathrm{SKP2}}$ complex contains SGT1 and HSP90 [41]. Likewise, formation of active CBF3 requires the presence of HSP90 in yeast [50]. Furthermore, yeast two-hybrid analysis has revealed that plant SGT1 and RAR1 interact with HSP90 (A. Takahashi and K. Shirasu, unpublished).

In plant disease resistance, the postulated chaperone-like activity of SGT1 can fit in either of the three operation points discussed above. For instance, R protein complex formation probably requires a fine-tuned chaperone machinery. Likewise, activation of R protein complexes

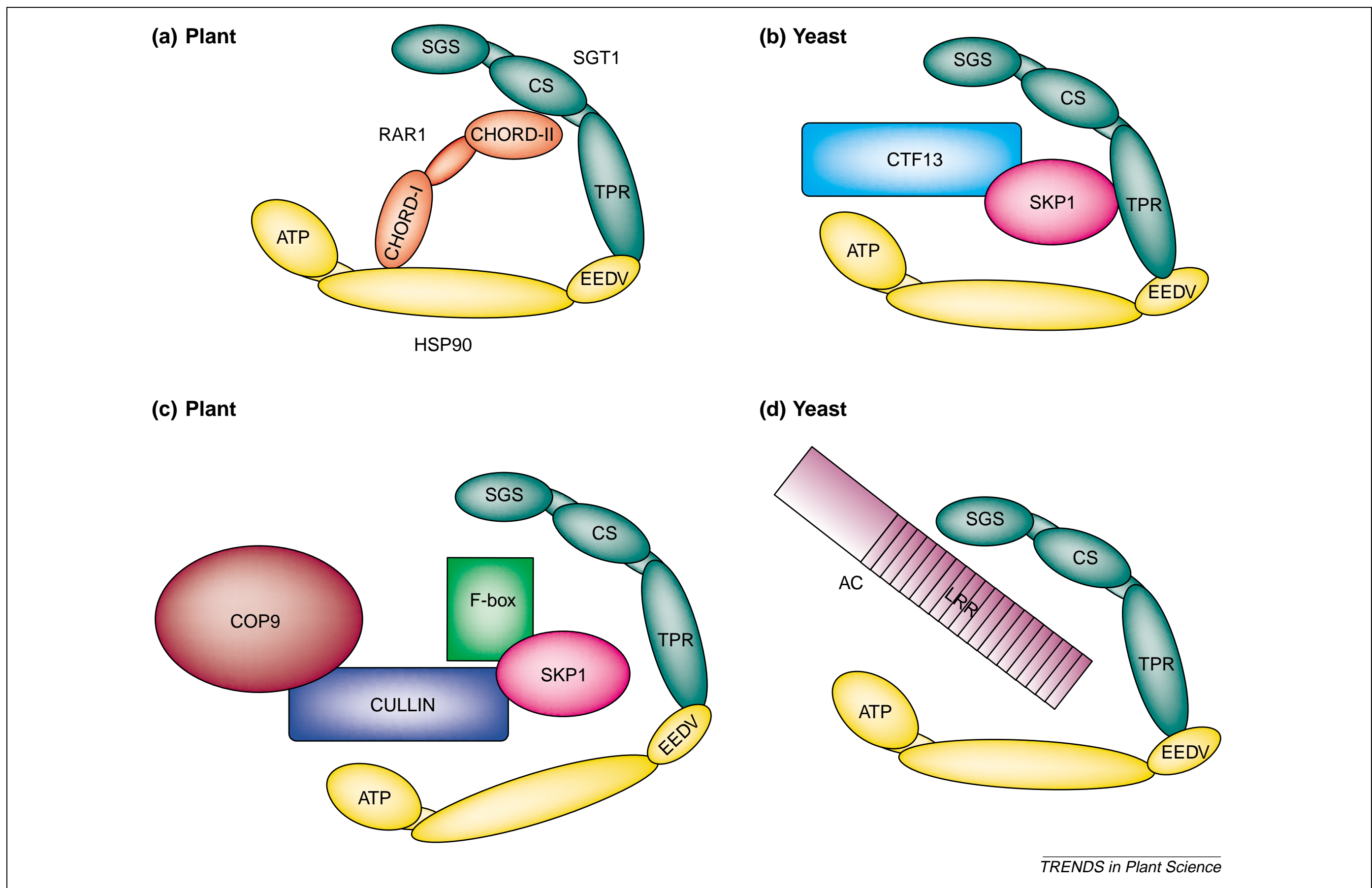

Fig. 2. Possible SGT1-containing complexes. (a) RAR1–SGT1–HSP90 complex, (b) SGT1–SKP1–CTF13 complex, (c) SGT1–SCF complex, (d) SGT1–AC CYR1 complex. Proteins are color coded: AC (purple), CTF13 (light blue), COP9 (dark red), CULLIN (dark blue), F-box (light green), HSP90 (yellow), RAR1 (red), SGT1 (dark green), SKP1 (pink). Abbreviations: AC, adenylyl cyclase; ATP, ATP binding site; LRR, leucine-rich repeat.

upon pathogen infection might occur in a similar manner to that of steroid hormone receptors, which are often accompanied by HSP90 and various co-chaperones [52]. In this context, it is interesting that SGT1 interacts with several LRR-containing proteins including CYR1 in yeast [48]. Mutational analyses revealed that the association of SGT1 with CYR1 was mediated by the SGS domain of SGT1 and the LRR domain of CYR1. Thus, plant SGT1 might also associate with some plant LRR-containing proteins, such as NB–LRR-type R proteins. In analogy to the multi-functionality of SGT1 in yeast, *Arabidopsis* SGT1b was recently shown to be involved in biological functions other than disease resistance. The *sgt1b* mutant compromised SCF^{TIR1}-mediated degradation of Aux/IAA target proteins without affecting auxin-stimulated *in planta* assembly of SCF^{TIR1} complexes [53]. Because the F-box protein TIR1 also contains several LRRs, it is possible that, analogous to the role of SGT1 in yeast SCF function, SGT1b assists the ubiquitylation process of Aux/IAA target proteins at assembled SCF^{TIR1} complexes. Further indirect evidence pointing to SGT1 multi-functionality in plants comes from the observation that SGT1 exists in at least two pools, one containing SCF E3 ubiquitination ligase(s) and another containing RAR1 [25]. Future structural and in depth biochemical studies combined with genetic analysis are needed to illuminate the dynamics of protein complexes for activation and signaling of disease-resistance reactions.

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