The EMBO Journal (2006) 25, 2007–2016 | © 2006 European Molecular Biology Organization | All Rights Reserved 0261-4189/06
www.embojournal.org

THE EMBO JOURNAL

Role of SGT1 in resistance protein accumulation in plant immunity

Cristina Azevedo[1,4], Shigeyuki Betsuyaku[2,4], Jack Peart[1], Akira Takahashi[1], Laurent Noël[2,3], Ari Sadanandom[1], Catarina Casais[1], Jane Parker[2,*] and Ken Shirasu[1,*]

[1]The Sainsbury Laboratory, John Innes Centre, Norwich, UK, [2]Max-Planck-Institut für Züchtungsforschung, Department of Plant Microbe Interactions, Köln, Germany and [3]Laboratoire de Biologie du Développement des Plantes, UMR6191 CNRS-CEA, Université Aix-Marseille II, CEN Cadarache, Saint Paul-lez-Durance Cedex, France

A highly conserved eukaryotic protein SGT1 binds specifically to the molecular chaperone, HSP90. In plants, SGT1 positively regulates disease resistance conferred by many Resistance (R) proteins and developmental responses to the phytohormone, auxin. We show that silencing of *SGT1* in *Nicotiana benthamiana* causes a reduction in steady-state levels of the R protein, Rx. These data support a role of SGT1 in R protein accumulation, possibly at the level of complex assembly. In *Arabidopsis*, two SGT1 proteins, AtSGT1a and AtSGT1b, are functionally redundant early in development. *AtSGT1a* and *AtSGT1b* are induced in leaves upon infection and either protein can function in resistance once a certain level is attained, depending on the R protein tested. In unchallenged tissues, steady-state AtSGT1b levels are at least four times greater than AtSGT1a. While the respective tetratricopeptide repeat (TPR) domains of SGT1a and SGT1b control protein accumulation, they are dispensable for intrinsic functions of SGT1 in resistance and auxin responses.

The EMBO Journal (2006) **25,** 2007–2016. doi:10.1038/sj.emboj.7601084; Published online 13 April 2006
Subject Categories: plant biology
Keywords: cell death; chaperone; CHORD; HSP90; TPR

Introduction

Most eukaryotic organisms are able to recognize potential pathogens and express appropriate defences to prevent disease. In plants, one of the most effective surveillance systems is controlled by Resistance (R) proteins that recognize, directly or indirectly, specific pathogen effectors, thereby triggering a rapid immune response (Belkhadir *et al*, 2004). The largest class of R proteins shares structural motifs with animal proteins involved in innate immunity, suggesting that related processes may operate in plants and animals (Inohara and Nunez, 2003). R protein recognition of different pathogens amplifies a common set of basal defences (Tao *et al*, 2003) and often involves massive localized accumulation of reactive oxygen species and programmed cell death known as the hypersensitive response (HR) (Shirasu and Schulze-Lefert, 2000).

To date, few components that regulate fundamental aspects of R protein triggered responses have been isolated and characterized in detail (Hammond-Kosack and Parker, 2003). Among those identified, *RAR1* (require for *Mla12* resistance), *HSP90* (heat shock protein 90) and *SGT1* (suppressor of the G2 allele of *skp1*) are required for resistance mediated by multiple R proteins recognizing viral, bacterial, oomycete or fungal pathogens (Shirasu and Schulze-Lefert, 2003; Schulze-Lefert, 2004). RAR1 contains two highly conserved zinc-binding domains called CHORD-I and CHORD-II (cysteine and histidine rich domain). The functions of CHORD containing proteins vary in different eukaryotic organisms. In mouse, a CHORD protein known as Melusin acts as a biomechanical sensor that prevents cardiac failure in response to pressure overload (Brancaccio *et al*, 2003). In *Aspergillus*, the CHORD-containing protein (CHP1) is essential for maintenance of diploidy (Sadanandom *et al*, 2004). *Caenorhabditis elegans* requires CHP for proper development while yeast does not have proteins with CHORD domains (Shirasu *et al*, 1999). Involvement of CHP proteins in immune responses in these organisms has not been demonstrated.

We showed previously that CHORD-I of RAR1 binds to the molecular chaperone HSP90 (Takahashi *et al*, 2003). Inhibitor treatments, partial silencing, or mutations of cytosolic HSP90 isoforms resulted in attenuated resistance responses, implying that RAR1 may function closely with HSP90 (Hubert *et al*, 2003; Lu *et al*, 2003; Takahashi *et al*, 2003). In support of this idea, both RAR1 and HSP90 are required to stabilize certain NB-LRR proteins. The *Arabidopsis* NB-LRR type R protein RPM1 did not accumulate in *Arabidopsis rar1* or *hsp90.2* mutants (Tornero *et al*, 2002; Hubert *et al*, 2003). Similarly, accumulation of potato Rx and barley MLA1 and MLA6 was reduced in backgrounds depleted in *RAR1* (Bieri *et al*, 2004). A current hypothesis is that direct association of RAR1 with HSP90 stabilizes R proteins in a restrained conformation that is competent to receive pathogen signals in a manner broadly similar to steroid receptor complexes in animals (Hubert *et al*, 2003; Schulze-Lefert, 2004).

Both RAR1 and HSP90 bind to SGT1 (Takahashi *et al*, 2003). SGT1 is also a conserved eukaryotic protein that functions in multiple biological processes through interaction with different protein complexes (Shirasu and Schulze-Lefert, 2003). SGT1 contains three distinct domains: a tetratricopeptide repeat domain (TPR), the CS motif (present in CHP and SGT1 proteins) and the SGS motif (SGT1 specific sequence). In yeast, the TPR domain of SGT1 is essential but not sufficient for binding to HSP90 (Bansal *et al*, 2004). By

*Corresponding authors: J Parker, Max-Planck-Institut für Züchtungsforschung, Department of Plant Microbe Interactions, Carl-von-Linné-Weg 10, 50829 Köln, Germany. Fax: +49 221 5062353; E-mail: parker@mpiz-koeln.mpg.de or K Shirasu, RIKEN Plant Science Center, 1-7-22 Suehiro, Tsurumi, Yokohama 230-0045, Japan. Tel.: +81 45503 9445; Fax: +81 45503 9573; E-mail: ken.shirasu@psc.riken.jp
[4]Equal first authors

Received: 16 August 2005; accepted: 16 March 2006; published online: 13 April 2006

contrast, the CS motif of barley SGT1 is sufficient to bind to the ATPase domain of HSP90 (Takahashi *et al*, 2003). The CS motif also interacts with the CHORD-II domain of RAR1. It is not clear whether the CS domain binds to RAR1 and HSP90 cooperatively or competitively. The SGS motif of yeast SGT1 mediates interaction with the LRR domains of CYR1/CDC35 (Dubacq *et al*, 2002). Similarly, barley SGT1 interacts with the LRR domain of MLA1 via its SGS domain in a yeast two-hybrid assay (Bieri *et al*, 2004).

Arabidopsis contains two SGT1 isoforms, AtSGT1a and AtSGT1b, which are highly conserved in their TPR–CS–SGS domain structures and 87% similar at the amino-acid level. Several genetic screens identified *AtSGT1b* as a component of certain *R* gene triggered resistance responses (Austin *et al*, 2002; Tör *et al*, 2002). Also, a mutation in *AtSGT1b* (*eta3*) was identified as a genetic enhancer of the *tir1-1* mutation that exhibits impaired responses to auxin (Gray *et al*, 2003). TIR1 is an F-box protein that interacts with AtCUL1, RBX1, and an SKP1-like protein to form an SCF complex (Gray *et al*, 2001). An auxin stimulus causes the SCF^{TIR1} complex to target negative regulators of the pathway, such as auxin/indoleacetic acid (Aux/IAA) family proteins, for ubiquitin-mediated degradation (Gray *et al*, 2001).

Mutations in *AtSGT1a* were not isolated in various genetic screens to identify components of either disease resistance or the auxin response, suggesting that AtSGT1b may be preferentially recruited to these pathways. In this study, we investigated the contribution of AtSGT1a to disease resistance and auxin hormone signalling. We establish that AtSGT1a is able to contribute positively to resistance triggered by the NB-LRR type R proteins Rx, N, and RPP5 and can complement for loss of AtSGT1b in auxin signalling. Consistent with these findings, *AtSGT1a* expression is induced by pathogen infection. However, a certain amount of AtSGT1a must be attained for resistance and this level depends on the R protein tested. AtSGT1a is inherently less stable than AtSGT1b in plant tissues, and we have identified two threonine residues in the TPR domain that are, at least in part, responsible for the difference in accumulation of these two SGT1 isoforms. Intriguingly, the TPR domain of SGT1 is dispensable in both resistance and auxin responses, suggesting that this domain acts primarily at the level of SGT1 stability. We demonstrate that SGT1 is required for accumulation of Rx, suggesting that SGT1 positively controls steady-state levels of preactivated R proteins. We propose that plant R proteins differ in the amounts of SGT1 needed to trigger effective resistance.

Results

SGT1 controls the abundance of Rx in Nicotiana benthamiana

Two SGT1 binding proteins, RAR1 and HSP90, are needed to stabilize R proteins. To test if SGT1 is also involved in stabilization of R proteins, we depleted levels of *N. benthamiana SGT1* (*NbSGT1*) by virus induced gene silencing (VIGS). In these experiments, we used a transgenic line of *N. benthamiana* that expresses an HA-tagged NB-LRR protein, Rx. Silencing of *NbSGT1* in these *N. benthamina* plants resulted in reduction of steady-state levels of Rx protein (Figure 1). This was not the case for silencing of *NbEDS1*, a plant defence component that is not essential for Rx resistance (Peart *et al*, 2002). Steady-state levels of HSP90 were unchanged in

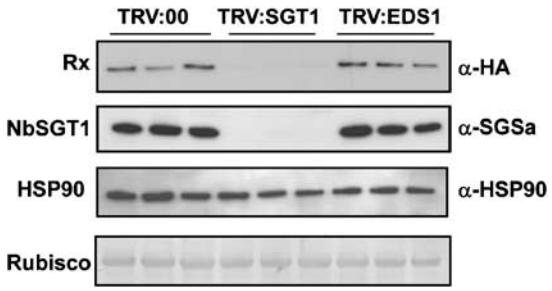

Figure 1 NbSGT1 positively controls steady-state levels of Rx. Western blot analysis of Rx, NbSGT1 and HSP90 levels using α-HA, α-SGSa and α-HSP90 antibodies, respectively. The Rx-4HA transgenic *N. benthamiana* plants were inoculated with TRV:00 (control), TRV:NbSGT1 or TRV:NbEDS1. Plants were sampled at 13–16 days postinoculation. Protein extracts were prepared as previously described (Bieri *et al*, 2004). Equal loading of total protein was checked by Ponceau S staining of Rubisco. For each treatment results, representative of three independent experiments are shown.

NbSGT1 silenced plants. We conclude that, similar to RAR1 and HSP90, NbSGT1 is essential for stabilizing the Rx resistance protein in its preactivation state.

AtSGT1a is dispensable for resistance to Pseudomonas syringae pv. tomato (Pst)

Recent studies showed that AtSGT1b antagonizes RAR1- and HSP90-dependent accumulation of an *Arabidopsis* NB-LRR protein, RPS5, suggesting that AtSGT1b assists RPS5 degradation rather than stabilization (Holt *et al*, 2005). To explore this apparent discrepancy with our data, we investigated possible involvement of the second *Arabidopsis SGT1* gene, *AtSGT1a* in disease resistance by isolating an *AtSGT1a* T-DNA insertion line, designated *sgt1a-1* (Figure 2A). For comparison, we also isolated an *AtRAR1* T-DNA insertion line in the same background (Ws-0), designated *rar1-1* (Figure 2A). Western blot analysis of these lines revealed that *sgt1a-1* and *rar1-1* are likely null mutations (Figure 2B). Inoculation of *rar1-1* leaves with different *Pst* DC3000 strains expressing *avrRpm1*, *avrRpt2*, *avrRps4* or *avrPphB* confirmed the genetic requirement for *AtRAR1* in *RPM1*, *RPS2*, and *RPS5* resistance and showed that *RAR1* is genetically dispensable for *RPS4* resistance in Ws-0 (Figure 2C–G). Growth of *Pst* harboring *avrRpm1*, *avrRpt2*, *avrRps4* or *avrPphB* in *sgt1a-1* was similar to that observed in wild-type Ws-0, indicating that *AtSGT1a* is not genetically required for these resistance responses (Figure 2D–G). An *sgt1a-1 rar1-1* double mutant also exhibited responses equivalent to *rar1-1* (Figure 2C–G).

An sgt1a-1 sgt1b-1 double mutant is embryo lethal

Since neither *sgt1a-1* (this study) nor *sgt1b* mutants (Muskett *et al*, 2002; Tör *et al*, 2002) were compromised in resistance to *Pst* strains, we reasoned that AtSGT1a and AtSGT1b may have redundant functions in these signalling pathways. To test this hypothesis, we attempted to create an *sgt1a-1 sgt1b-1* double mutant. As *AtSGT1a* and *ATSGT1b* are on the same chromosome IV, we first created parental lines homozygous for one allele and heterozygous for the other (*SGT1a sgt1a/sgt1b sgt1b* or *sgt1a sgt1a/SGT1b sgt1b*) and characterized their seeds. The double mutant was embryo lethal (Figure 2H and I, Supplementary Table 1). These results show that SGT1

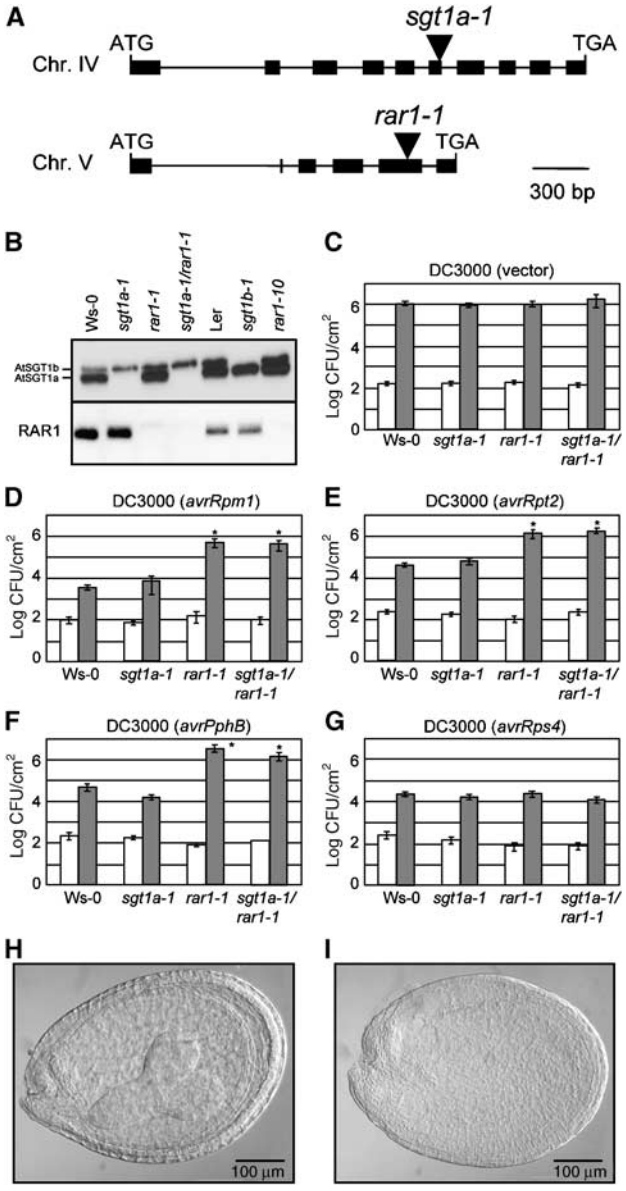

Figure 2 Analysis of of *Arabidopsis sgt1a-1* and *rar1-1* mutants. (**A**) Relative position of T-DNA insertions within the *AtSGT1a* and *AtRAR1* genes. Exons are indicated by the black boxes. (**B**) Protein expression of AtSGT1a, AtSGT1b and AtRAR1. Western blot analysis of total protein extracts from mutant and wild-type plants probed with α-SGSa or α-RAR1 antibody, as indicated. (**C**) Bacterial growth analysis of *Pst* DC3000 (vector) after infiltration of leaves of 6–7 week-old plants with bacterial suspensions (1×10^5 c.f.u./ml). Leaves were harvested at 0 (white column) and 3 days (grey column) after inoculation. (**D**) Same analysis for *Pst* DC3000 (*avrRpm1*), (**E**) *Pst* DC3000 (*avrRpt2*), (**F**) *Pst* DC3000 (*avrRphB*), (**G**) *Pst* DC3000 (*avrRps4*). Asterisks indicate that bacterial growth is significantly different ($P<0.05$) from the wild-type control. Experiments were repeated at least three times with similar results. (**H**) DIC image of cleared seed from wild-type plant. (**I**) DIC image of cleared seed from a self-pollinated mutant plant homozygous for *sgt1a-1* mutation and heterozygous for *sgt1b-1*.

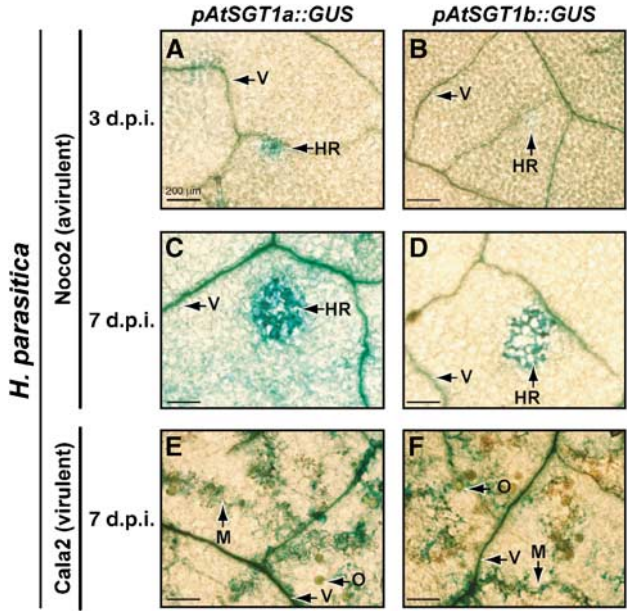

Figure 3 *AtSGT1a* and *AtSGT1b* expression is induced by pathogen infection. Induction of β-glucuronidase (GUS) activity in Ler transgenics expressing *pAtSGT1a*::*GUS* (**A**, **C**, **E**) or *pAtSGT1b*::*GUS* (**B**, **D**, **F**) was examined 3 and 7 days after inoculation (dpi) with avirulent (Noco2) or virulent (Cala2) *H. parasitica*, as indicated. GUS-stained leaves were viewed under a light microscope. Pictures are representative of three independent experiments using three independent transgenic lines for each construct. HR, hypersensitive response at pathogen infection foci; M, pathogen mycelium; O; pathogen oospores; V, vasculature. Bars represent 200 µm.

activity is essential for early development in *Arabidopsis* and that AtSGT1a and AtSGT1b are redundant in this process.

AtSGT1a is induced upon pathogen infection

We examined the modes of *AtSGT1a* and *AtSGT1b* expression upon pathogen infection by making stable transformants in which the promoters of *AtSGT1a* or *AtSGT1b* were fused to the β-*glucuronidase* (*GUS*) gene. Three independent transgenic lines for each construct were inoculated with *Hyaloperonospora parasitica* isolate Noco2 (avirulent; recognized by *RPP5*) or isolate Cala2 (virulent) and examined at different time points after inoculation. In the incompatible (Figure 3A–D) and compatible (Figure 3E and F) interactions, there was induction of GUS activity of both *pAtSGT1a*::*GUS* and *pAtSGT1b*::*GUS* around pathogen infection sites and the leaf vasculature. More intense GUS staining of *pAtSGT1a*::*GUS* than *pAtSGT1b*::*GUS* was consistently observed at infection foci and around the vascular tissues (Figure 3A–F). This may reflect higher basal levels of *AtSGT1a* expression in the leaves. However, data from gene expression microarrays of *Arabidopsis* leaves indicate similar levels of *AtSGT1a* and *AtSGT1b* mRNAs in healthy *Arabidopsis* leaves and early responsiveness (2 h) of *AtSGT1a* but not *AtSGT1b* transcripts to infiltration with *Pst* DC3000 expressing *avrRpm1* (Zimmermann *et al*, 2004). We concluded that *AtSGT1a* is responsive to multiple biotic stresses consistent with a role of *AtSGT1a* in defence, despite preferential genetic recruitment of *AtSGT1b* in *R* gene-triggered resistance.

AtSGT1a can function in R gene-mediated resistance

We explored whether differences in *AtSGT1a* and *AtSGT1b* expression levels in *Arabidopsis* may account for preferential genetic recruitment of *AtSGT1b* in certain *R* gene mediated responses. Since *RPP5* resistance is compromised in the *sgt1b-3* null mutant (Austin *et al*, 2002), we tested whether

overexpression of AtSGT1a would revert this phenotype. *sgt1b-3* plants were transformed with *AtSGT1a* expressed under the control of either the constitutive 35S or native *AtSGT1b* promoter (respectively denoted 35S::gAtSGT1a and pAtSGT1b::gAtSGT1a). Homozygous transgenic lines with different levels of transgene expression were selected on Western blots (Figure 4A; Supplementary Figure 1) and infected with *H. parasitica* isolate Noco2 (Figure 4B; Supplementary Table 2). Lines 7.1 and 8.5 with high AtSGT1a protein levels (⩾10 fold higher than in the *sgt1b-3* mutant; Figure 4A; Supplementary Figure 1) fully complemented the *sgt1b-3* defect, manifested by restoration of spatially restricted HR lesions after pathogen infection of leaves. Lower levels of AtSGT1a expression in lines 3.4, 3.6 and 8.10 (<8-fold higher than in *sgt1b-3*; Figure 4A; Supplementary Figure 1) only partially compensated for loss of *AtSGT1b* in *RPP5* resistance, as seen by the development of HR lesions together with trailing necrosis (TN) and occasional sporulation on leaves infected with Noco2 (Figure 4B; Supplementary Table 2). For comparison, *sgt1b-3* was also transformed with *AtSGT1b* controlled either by its own (pAtSGT1b::gAtSGT1b) or by the *AtSGT1a* promoter (pAtSGT1a::gAtSGT1b). As observed for AtSGT1a, we found a dose-dependent complementation of the *sgt1b-3* defect by AtSGT1b. For example, AtSGT1b expression in line 2.3 was not sufficient to fully complement *sgt1b-3* in *RPP5* resistance (Figure 4; Supplementary Figure 1 and Supplementary Table 2). In contrast, transgenic lines 5.1, 5.3 and 6.3 that expressed greater levels (⩾3-fold higher than in line 2.3) of AtSGT1b fully complemented *sgt1b-3* (Figure 4; Supplementary Figure 1 and Supplementary Table 2). These results demonstrate that AtSGT1a, like AtSGT1b, is able to function in *R* gene-mediated resistance and that a minimum level of either protein needs to be attained for full expression of disease resistance.

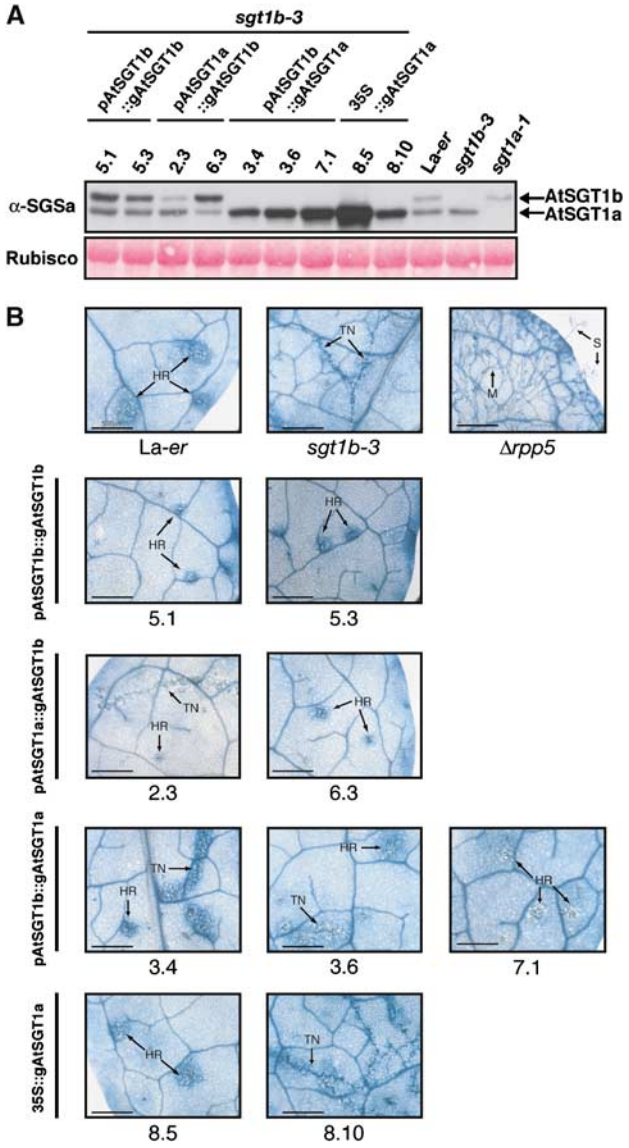

Figure 4 Expression of AtSGT1a or AtSGT1b in transgenic *Arabidopsis* complements *sgt1b-3* in *RPP5* resistance. (**A**) Western blot analysis of total leaf extracts from different *sgt1b-3* plants transformed with *AtSGT1a* or *AtSGT1b* under different promoters. SGT1 proteins were visualized by the α-SGSa antibody. (**B**) Lactophenol trypan blue staining of Noco-2 infected leaves 7 days after inoculation reveals necrotic plant cells and pathogen structures. HR, hypersensitive response; M, mycelium; TN, trailing plant cell necrosis; S, sporangiophore. Bars represent 500 μm.

To determine if AtSGT1a can participate in defence signalling pathways other than those tested in *Arabidopsis,* we developed a heterologous transient complementation assay in *N. benthamiana*. This assay was based on the fact that VIGS of *NbSGT1* leads to compromised *R* gene triggered resistance against several pathogens (Liu *et al*, 2002; Peart *et al*, 2002). If AtSGT1a is able to function in resistance, we expected it to complement the loss of *NbSGT1*. We monitored both hypersensitive plant cell death (HR) associated with transient expression of PVX coat protein (PVX-Tk) and resistance against green fluorescent protein (GFP)-tagged recombinant PVX conditioned by *Rx* (Peart *et al*, 2002). For transient assays, we used two types of *Agrobacterium* strain/plasmid sets, C58C1 (pBin19 based vector, 35S promoter) and GV3101 (pPM90RK, pPAM_MCS based vector, 35S promoter) for high and low expression, respectively. Analyses of AtSGT1a expression mediated by the two different strains showed that levels with C58C1 were two to five times higher than that observed for GV3101 (Figure 5A). As previously reported (Peart *et al*, 2002), no HR was observed in TRV:NbSGT1 plants co-infiltrated with *Agrobacterium* cells carrying the vector control and a plasmid expressing PVX-Tk (Figure 5B). By contrast, transient expression of either AtSGT1a or AtSGT1b using the low expression system restored the HR (Figure 5B). Similarly, there was a significant but incomplete reduction of PVX-GFP accumulation in TRV:NbSGT1 plants transiently expressing AtSGT1a or AtSGT1b while no strong reduction of PVX:GFP expression was observed with the control vector (Figure 5C). Expression of AtSGT1a or AtSGT1b in wild-type *N. benthamiana* TRV:00 plants had no effect on HR or PVX:GFP (Figure 5B and C). These data show that AtSGT1a has the capacity to function in resistance mediated by *Rx*.

We then investigated whether AtSGT1a and AtSGT1b can function in *N*-mediated resistance against GFP-tagged recombinant TMV (TMV:GFP; Peart *et al*, 2002). Using the low expression system, transient expression of AtSGT1b, but not AtSGT1a, suppressed GFP expression in TRV:NbSGT1 plants, indicating that AtSGT1a was unable to complement the loss of *NbSGT1* in *N* resistance (Figure 6A). However, when AtSGT1a was expressed using the high expression

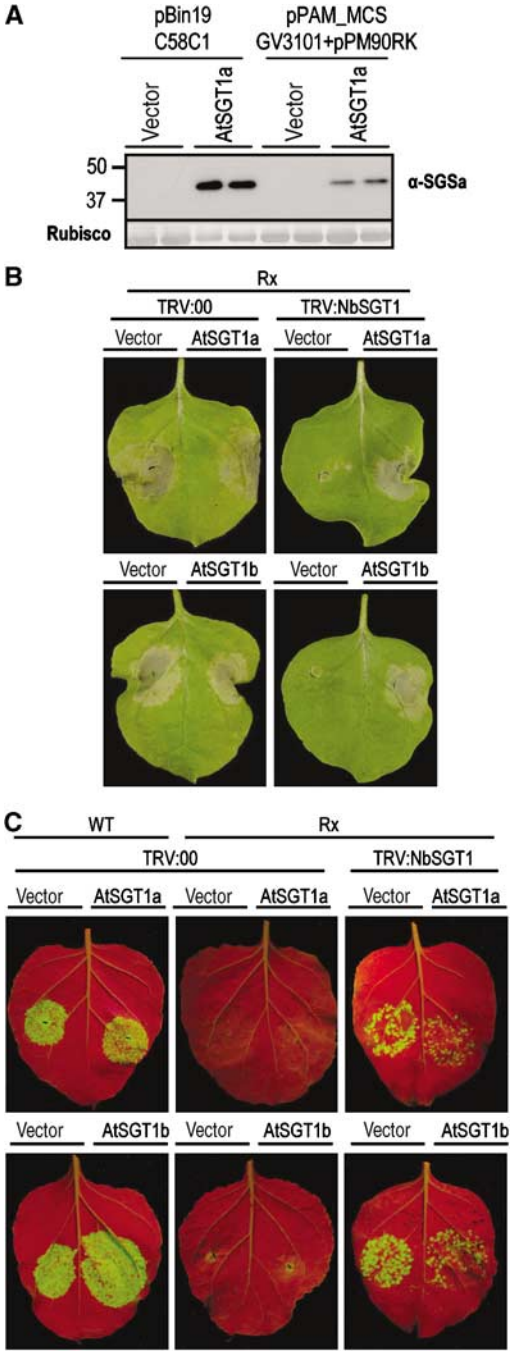

Figure 5 AtSGT1a and AtSGT1b are functional in *Rx* resistance. (**A**) Western blot analysis of differential AtSGT1a protein expression levels by *Agrobacterium* strains C58C1 with a pBIN61-based vector and GV3101 with a pPAM-MCS vector. Protein extracts from *N. benthamiana* leaves infiltrated with indicated strains were separated by SDS–PAGE and blotted on a membrane. AtSGT1a was visualized by α-SGSa antibody. (**B**) Appearance of HR elicited by co-infiltration of *Agrobacterium* expressing the PVX-Tk (PVX coat protein) and the test constructs. One week-old wild type and transgenic *N. benthamiana* plants (*Rx*, *N*) were inoculated with TRV:NbSGT1 or TRV:00 by *Agrobacterium* infiltration. Approximately 3 weeks later PVX elicitor was co-expressed with the test constructs AtSGT1a, AtSGT1b or empty vector by *Agrobacterium* infiltration. Plants were photographed 5 days post-inoculation under white light. (**C**) *Rx* resistance against PVX. Co-infiltration of *Agrobacterium* carrying PVX:GFP ($OD_{600} = 0.001$) and the test construct ($OD_{600} = 0.25$) 3 weeks after inoculation of TRV:00 or TRV:NbSGT1. Accumulation of PVX:GFP was monitored by GFP fluorescence under UV illumination 5 days post inoculation. Similar results were obtained in three independent experiments.

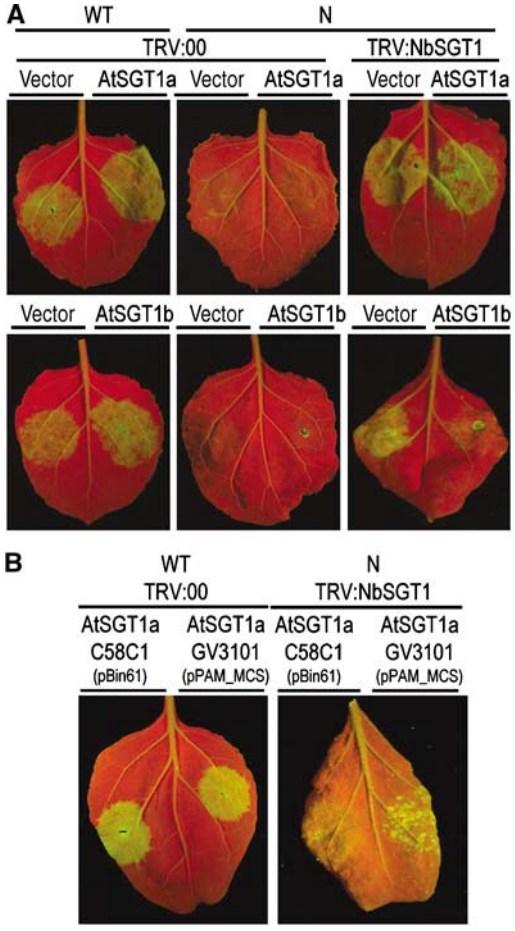

Figure 6 Dose-dependent recruitment of AtSGT1a in *N* resistance. (**A**) AtSGT1b but not AtSGT1a is functional in *N* resistance against TMV when expressed from the *Agrobacterium* strain GV3101 (pMP90RK) in a pPAM-MCS-based vector. Wild type and transgenic *N. benthamiana* plants were treated as described in Figure 5. Co-infiltration of *Agrobacterium* carrying TMV:GFP ($OD_{600} = 0.025$) and the test construct ($OD_{600} = 0.25$) as indicated. Accumulation of TMV:GFP was monitored by GFP fluorescence under UV illumination 6-7 days post inoculation. Similar results were obtained for three independent experiments. (**B**) Comparison of two *Agrobacterium* strains, C58C1 with a pBIN61-based vector and GV3101 with a pPAM-MCS vector for AtSGT1a functionality in *N* resistance.

system, full complementation of *N* resistance was observed in TRV:NbSGT1 transgenic plants (Figure 6B). These results show that AtSGT1a can function in *N* resistance when highly expressed. They also demonstrate that higher levels of AtSGT1a are required for resistance mediated by *N* compared to *Rx*.

Differential accumulation of AtSGT1b and AtSGT1a in planta is determined by the TPR domain

To investigate if there is an inherent difference between AtSGT1a and AtSGT1b accumulation in *Arabidopsis*, we generated multiple independent stable transgenic lines of *sgt1b-3* expressing AtSGT1a or AtSGT1b under their own promoters and C-terminally fused to a StrepII (SII) affinity purification tag (Witte *et al*, 2004). AtSGT1b was more highly expressed than AtSGT1a in three independent transformants, as shown in Western blot analysis of one representative line

for each construct (Figure 7A). Levels of AtSGT1b-SII were at least four times higher than AtSGT1a-SII when measured in a dilution series of leaf soluble extracts on a Western blot probed with anti-SII antibody (data not shown). Affinity purification of silver stainable amounts of both proteins after transient expression in *N. benthamiana* leaves established that the SII-tagged AtSGT1a and AtSGT1b were detected to the same efficiency by anti-SII antibody (Supplementary Figure 2). Additionally, we found that α-SGSa has higher affinity for AtSGT1a than for AtSGT1b since the difference in protein expression was not apparent using this antibody (Figure 7A, lanes 3 and 4).

To identify domains responsible for differential accumulation, we created chimeric constructs by swapping the TPR domains of AtSGT1a and AtSGT1b, designated AtSGT1a/b/b and AtSGT1b/a/a (Figure 7B). Western blot analyses revealed that AtSGT1b/a/a accumulated to higher levels than AtSGT1a wild-type protein after transient expression in *N. benthamiana* leaves. Conversely, AtSGT1a/b/b accumulated to lower levels than wild-type AtSGT1b. Further sequence comparison between AtSGT1a and other plant SGT1 proteins revealed the existence of three conserved amino acids in the N-terminal TPR domain of all plant SGT1 proteins except AtSGT1a (Supplementary Figure 3). In AtSGT1a, amino acids at positions 91, 100 and 118 in the TPR domain are threonines whereas all other plant SGT1 proteins contain alanines at these positions. Phosphorylation predictions indicated that Thr^{91} and Thr^{100} are strong candidates for phosphorylation (scoring of Thr^{91}-0.630 and Thr^{100}-0.828; based on NetPhos 2.0 server http://www.cbs.dtu.dk/services/NetPhos/). When both Thr^{91} and Thr^{100} in AtSGT1a were mutated to alanines ($AtSGT1a^{(T91A+T100A)}$) the protein accumulated to greater levels than wild-type AtSGT1a after transient expression in *N. benthamiana* leaves (Figure 7B). By contrast, exchange of the corresponding Ala^{91} and Ala^{100} to Thr in AtSGT1b ($AtSGT1b^{(A91T+A100T)}$) caused the protein to accumulate to lower levels than wild-type AtSGT1b (Figure 7B). From these data we concluded that the N-terminal TPR domain of AtSGT1a reduces the steady-state level of *Arabidopsis* SGT1 proteins whereas the same domain from AtSGT1b enhances SGT1 accumulation.

Next, we tested whether the AtSGT1a and AtSGT1b amino-acid exchange variants are functional in *N. benthamiana* pathology assays using the low expression system, GV3101. As expected, $AtSGT1a^{(T91A+T100A)}$ and $AtSGT1b^{(A91T+A100T)}$ were able to complement NbSGT1 for *Rx* resistance (data not shown). Similarly, both $AtSGT1a^{(T91A+T100A)}$ and $AtSGT1b^{(A91T+A100T)}$ complemented NbSGT1 for *N* resistance in TRV:NbSGT1 plants (Figure 7C). Therefore, an increase in protein stability observed in $AtSGT1a^{(T91A+T100A)}$ is sufficient to attain a level required for *N* resistance while the amount of $AtSGT1b^{(A91T+A100T)}$ was above the minimal level. Analysis of protein expression of SGT1 derivatives in yeast did not show significant differences in stability (data not shown), suggesting that instability due to the presence of the AtSGT1a TPR domain is a plant-specific phenomenon.

The TPR domain is dispensable for SGT1 resistance function and auxin signalling

A link between the nature of the TPR domain and stability of *Arabidopsis* SGT1 proteins led us to speculate that the TPR domain may have a regulatory role. We therefore transiently

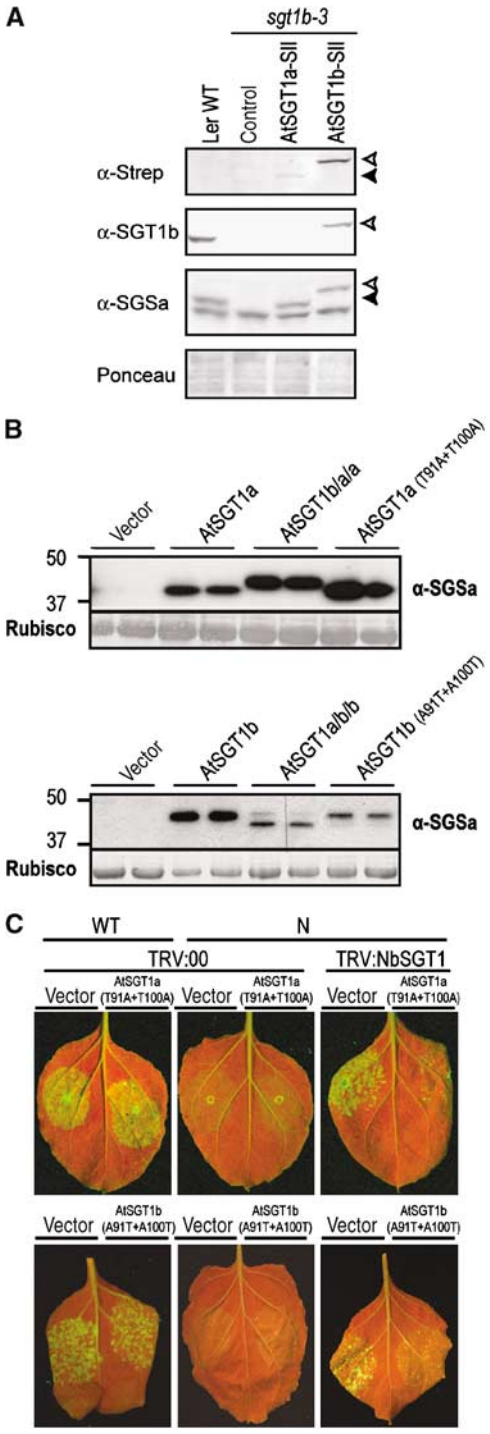

Figure 7 The TPR domain controls steady-state levels of *Arabidopsis* SGT1 proteins. (**A**) Western blot analysis of AtSGT1a-SII (indicated by black arrow) and AtSGT1b-SII (white arrow). (**B**) Relative abundance of different AtSGT1a and AtSGT1b constructs transiently expressed in TRV:NbSGT1 plants determined by Western blot analysis with the α-SGSa antibody. A chimeric construct AtSGT1a/b/b containing the TPR domain (M1-V163) from AtSGT1a and the remaining protein from AtSGT1b (V171-Y357) while the reciprocal chimera AtSGT1b/a/a contains M1-A170 from AtSGT1b fused to V164-I350 of AtSGT1a. (**C**) $AtSGT1a^{(T91A+T100A)}$ and $AtSGT1b^{(A91T+A100T)}$ contructs were transiently expressed in TRV:00 or TRV:NbSGT1 plants as indicated and analysis of *N* resistance performed as in Figure 6A. Accumulation of TMV:GFP was monitored by GFP fluorescence under UV illumination 6–7 days post inoculation.

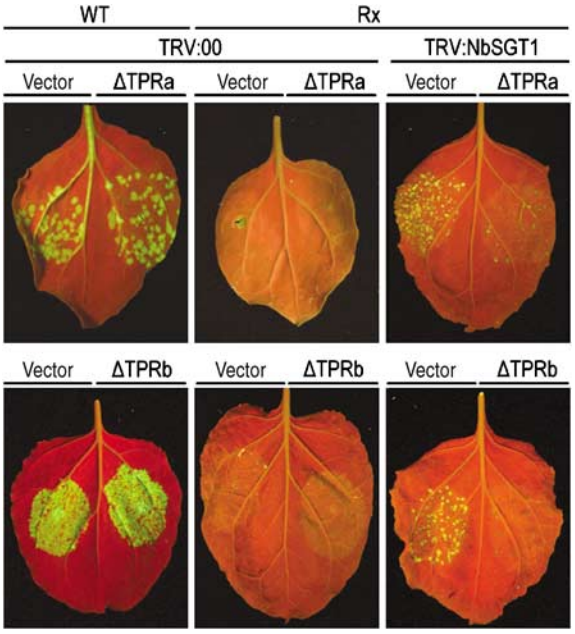

Figure 8 The TPR domain is dispensable for *Rx* resistance in *N. benthamiana*. ΔTPRa and ΔTPRb were expressed using the *Agrobacterium* strain GV3101 (pMP90RK) with a pPAM_MCS-based vector. Experiments were performed as described in Figure 5.

expressed AtSGT1a or AtSGT1b that lack their corresponding TPR domain (ΔTPRa or ΔTPRb) in TRV:NbSGT1 *N. benthamiana* transgenic plants and tested for restoration of *Rx* resistance. The ΔTPRa and ΔTPRb both complemented NbSGT1 in *Rx* mediated resistance as seen by the lack of GFP fluorescence after inoculation with PVX:GFP (Figure 8). These results demonstrate that the TPR domain of either AtSGT1a or AtSGT1b is not essential for resistance conditioned by *Rx*, at least when they are transiently expressed.

In order to assess the influence of the AtSGT1a and AtSGT1b TPR domains in *Arabidopsis*, we transformed the *sgt1b-1* null mutant with ΔTPRa and ΔTPRb and selected independent lines with different levels of transgene expression by Western blot analyses (Supplementary Figure 4). First, we tested whether constitutive expression of either ΔTPRa or ΔTPRb is able to complement the *sgt1b-1* defect in *RPP5* resistance to *H. parasitica* isolate Noco2. Complementation was observed in a dose-dependent manner by both ΔTPRa and ΔTPRb (Supplementary Figure 4). Lines with higher levels of ΔTPRa or ΔTPRb expression exhibited complete resistance whereas lines with lower levels of expression were partially resistant to Noco2 as seen by the appearance of a combination of discrete HR lesions and TN (Supplementary Figure 4). Next, we tested these lines for auxin sensitivity. We found that ΔTPRa or ΔTPRb transgenic lines also complemented *sgt1b-1* as seen by restoration of auxin sensitivity (Supplementary Figure 4). These results indicate that the TPR domain of *Arabidopsis* SGT1 is dispensable for resistance function and auxin signalling.

Discussion

AtSGT1a and AtSGT1b are functionally redundant

Arabidopsis contains two highly similar proteins, AtSGT1a and AtSGT1b, that bind to RAR1, a component required for the resistance response mediated by several R proteins. Despite the high similarity between *AtSGT1a* and *AtSGT1b*, genetic screens for loss of resistance identified mutations in *AtSGT1b* but not *AtSGT1a*. In this study, we show that both AtSGT1b and AtSGT1a mediate resistance in a dose-dependent manner and that there is functional redundancy between the two proteins during embryo development. These data are consistent with previous findings that all tested *R* genes were compromised in *NbSGT1* silenced *N. benthamiana* plants (Peart *et al*, 2002) and that both *Arabidopsis* SGT1 proteins are able to complement *sgt1* mutations in yeast (Azevedo *et al*, 2002). An explicit prediction from these results is that *Arabidopsis R* genes previously identified not to require *AtSGT1b* (Austin *et al*, 2002; Tör *et al*, 2002) utilize *AtSGT1a* in an *sgt1b* mutant. Supporting this idea, we found that *AtSGT1a* promoter activity was highly induced by pathogen infection (Figure 2). AtSGT1a may thus reach a level required for the full resistance triggered by certain R proteins in the absence of AtSGT1b. We did not observe loss of resistance phenotype in *sgt1a-1* against *Pst* strains (Figure 2C–G) probably because there is sufficient AtSGT1b protein for these R proteins to signal effectively.

R proteins differ in the levels of SGT1 needed for resistance

Our studies in *N. benthamiana* and *Arabidopsis* reveal that the levels of SGT1 proteins required for resistance depends on the R protein tested. Transient complementation using high and low expression vectors showed that Rx requires lower amounts of AtSGT1a than N does to function (Figures 5 and 6). Similarly, RPP5 resistance is restored in transgenic plants expressing high levels of AtSGT1a (Figure 4) even though endogenous AtSGT1a is insufficient to mediate resistance in the *sgt1b* mutant (Austin *et al*, 2002; Tör *et al*, 2002). Transgenic lines expressing lower amounts of AtSGT1b did not fully complement the *sgt1b-3* defect in *RPP5* resistance, suggesting that there is also a critical dose of AtSGT1b required for proper R protein function. In barley, transient single cell silencing of *HvSGT1* compromised resistance conferred by *MLA6* but not *MLA1* (Azevedo *et al*, 2002). In the light of our new data, we consider it likely that MLA6 requires higher levels of HvSGT1 than MLA1. Transient silencing may not have been complete, leaving sufficient HvSGT1 for MLA1 to operate. In yeast, HvSGT1 can associate with MLA1-LRR but not MLA6-LRR (Bieri *et al*, 2004). Perhaps, interaction between MLA6-LRR and HvSGT1 is weak or transient and not detectable in this assay and this may explain why MLA6 requires more HvSGT1 than MLA1 for resistance.

The TPR is a regulatory domain in Arabidopsis *SGT1 proteins*

The TPR domain of SGT1 has high similarity to that of the serine threonine protein phosphatase 5 (Pp5) (Takahashi *et al*, 2003). Recently, the TPR domain of Pp5 was shown to maintain an auto-inhibited conformation with a C-terminal domain suppressing the phosphatase activity (Yang *et al*, 2005). Here, we demonstrate that the TPR domain is at least in part responsible for the difference in AtSGT1a and AtSGT1b accumulation (Figure 7). In particular, amino acid residues Thr91 and Thr100 in the TPR domain of AtSGT1a confer reduced levels of this protein. It is possible that phosphorylation of Thr91 and Thr100 by a plant specific

kinase may cause a conformational change rendering AtSGT1a less stable. Consistent with this idea, we did not observe significant differences in the amounts of *Arabidopsis* SGT1 proteins expressed in yeast or *Escherichia coli* (Marta Boter and Ken Shirasu, unpublished).

AtSGT1b is required genetically for the auxin response mediated by SKP1-containing SCF^{TIR1} (Gray *et al*, 2003). Yeast SGT1 mediates CBF3 kinetochore assembly also through its interaction with SKP1 (Kitagawa *et al*, 1999). The TPR domain of SGT1 is necessary but not sufficient for the interaction (Bansal *et al*, 2004; Lingelbach and Kaplan, 2004). In contrast, *C. elegans* and *Drosophila* SGT1 proteins do not contain a TPR domain (Kitagawa *et al*, 1999). We show that although the TPR domain controls *Arabidopsis* SGT1 protein stability, it is not important for intrinsic disease resistance and auxin signalling functions of SGT1. We reason therefore that interaction of SKP1 and SGT1 via the TPR domain is not crucial to resistance and auxin signalling pathways. While association between SGT1 and SKP1 has been demonstrated in barley and tobacco co-IP experiments (Azevedo *et al*, 2002; Liu *et al*, 2002), we were unable to demonstrate interaction between *Arabidopsis* SGT1 proteins and SKP1 homologues either by yeast two hybrid assays or by co-IP experiments (data not shown). SGT1 has low affinity for SKP1 (Bansal *et al*, 2004; Lingelbach and Kaplan, 2004) and our current protocols may not detect such weak or transient interactions.

SGT1 functions in R protein accumulation in disease resistance

An outstanding question is how SGT1 proteins regulate plant R protein function. We show that silencing of *NbSGT1* results in reduced steady-state levels of Rx and conclude that SGT1, like RAR1 and HSP90, has a role in maintaining stability of preactivated R proteins. A previous study showed that silencing HSP90 resulted in greater reduction of Rx protein levels compared to those in *NbSGT1* silenced plants, suggesting that HSP90 may also affect other processes, such as translation of Rx (Lu *et al*, 2003). Holt *et al* provided evidence that AtSGT1b antagonizes RAR1- and HSP90-dependent accumulation of RPS5, suggesting that AtSGT1b assists RPS5 degradation. However, interpretation of SGT1 activities in *Arabidopsis* is complicated by the presence of two functional SGT1 proteins with different expression characteristics, as shown here. Our data lead us to consider an alternative model to explain the results of Holt *et al* (2005). In this model (Figure 9), RPS5 and other NB-LRR protein stabilization in *sgt1b* may be conferred by AtSGT1a when expressed above a certain level. In wild-type *Arabidopsis*, AtSGT1b provides the principle SGT1 activity due to its higher abundance and possibly also preferential HSP90 association (Hubert *et al*, 2003). In the absence of AtSGT1b, AtSGT1a activity may be invoked. We have demonstrated that the amounts of both SGT1 proteins required for resistance depends on the R protein tested. Therefore, for some R proteins, such as RPP5 (Austin *et al*, 2002), the amount of active AtSGT1a in the absence of AtSGT1b may not be sufficient for proper R protein function. If certain R proteins, such as RPS5, require lower AtSGT1a levels for assembly of competent complexes, this would explain recovery of RPS5 protein in *rar1 sgt1b* mutants observed by Holt *et al* (2005). By the same argument, instability of RPM1 in *rar1* is not recovered in *rar1 sgt1b* double mutants, possibly because RPM1 requires higher levels of AtSGT1a than does RPS5 to signal resistance. The balance of assembly and degradation of such complexes is likely to be tightly regulated, and we cannot exclude a role for SGT1 or other co-chaperones in degradatory processes once R proteins are activated (Figure 9).

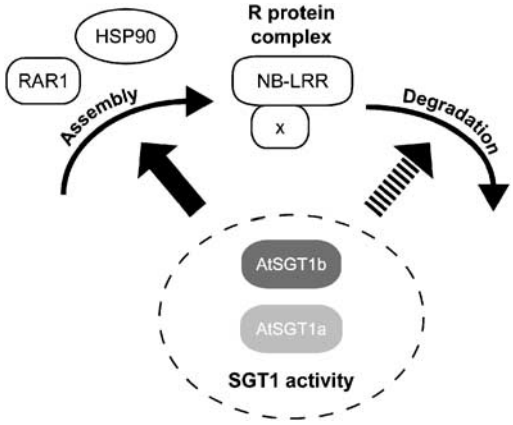

Figure 9 Model for SGT1 function in assembly of plant NB-LRR protein complexes. An NB-LRR type R protein is depicted as part of a 'preactivated' multiprotein complex with other plant protein(s) (shown as x), whose assembly requires the cooperative activities of the HSP90 chaperone and RAR1. Our data argue for a role of SGT1 in assembly (solid straight black arrow) of pre-existing NB-LRR proteins. In *Arabidopsis*, two active SGT1 isoforms, AtSGT1a and AtSGT1b, are expressed. The predominant SGT1 activity in leaves is exerted by AtSGT1b due to its higher accumulation (indicated by dark grey shading) than AtSGT1a (light grey shading) before pathogen challenge, and possibly also preferential HSP90 binding. In the absence of AtSGT1b and RAR1, AtSGT1a activity is sufficient for accumulation of some NB-LRR proteins, such as RPS5, but not others, such as RPP5 or RPM1. This function is supported by an intrinsic capability of AtSGT1a to complement the *sgt1b* mutant when expressed at a certain level and by differences in the amounts of SGT1 needed for resistance conferred by various R proteins tested. It is likely that a fine balance between NB-LRR protein assembly and degradation is mediated by chaperone and co-chaperone associations. While the model depicts a principle activity of SGT1 in R protein assembly, it does not preclude an SGT1 contribution to degradation of R proteins (broken straight black arrow) or downstream signalling components.

Holt *et al* (2005) reported that instability of RPM1 and RPS5 caused by the treatment with a specific HSP90 inhibitor, geldanamycin (GDA) in wild-type plants was not observed in *sgt1b* mutants. One explanation for this phenomenon is that AtSGT1b, but not AtSGT1a, requires HSP90 activity to stabilise R proteins. In this scenario, GDA is no longer able to inhibit the SGT1 activity in the absence of AtSGT1b, because AtSGT1a functions independently of HSP90 activity. A yeast two-hybrid analysis showing that AtSGT1b but not AtSGT1a interacts with full-length HSP90 (Cristina Azevedo and Ken Shirasu unpublished) is consistent with this hypothesis. In this sense, AtSGT1a may be an exceptional SGT1 protein since all other tested SGT1 proteins associate with HSP90 (Takahashi *et al*, 2003; Bansal *et al*, 2004; Lee *et al*, 2004).

In yeast, SGT1 and HSP90 cooperate in maintaining transient protein interactions essential for proper assembly of kinetochore complexes (Bansal *et al*, 2004; Lingelbach and Kaplan, 2004). Here, we showed that SGT1 positively controls the steady-state levels of an R protein, similar to RAR1 and HSP90 (Figure 9). It is tempting to speculate that SGT1

functions closely with HSP90 and RAR1 to mediate of R protein complex assembly in plants. SGT1 was shown to play a role in intramolecular interactions of the Bs2 R protein in transient *N. benthamiana* expression assays (Leister *et al*, 2005). Presumably, proper folding of Bs2 is important for complex formation and SGT1 may function through interaction with Bs2 via the SGS domain and interaction with HSP90 via CS domain. Since barley RAR1 and SGT1 also act additively on MLA6 resistance (Azevedo *et al*, 2002), the function of RAR1 may be to enhance the performance of SGT1–HSP90 complexes.

Materials and methods

Plant materials, bacteria and virus strains

The *sgt1a-1* and *rar1-1* mutants were identified by screening the Ws-0 Wisconsin T-DNA KO lines for insertions in the *AtSGT1a* or *AtRAR1* genes as described (Krysan *et al*, 1999). Transgenic *N. benthamiana* plants carrying *N* and *Rx* resistance genes, their growth conditions, VIGS of the *NbSGT1* using tobacco rattle virus vector were described previously (Peart *et al*, 2002). *Pst* DC3000 strains and *H. parasitica* isolates used in this study are described (Austin *et al*, 2002; Takahashi *et al*, 2003). The virus strains PVX:GFP and TMV:GFP were described previously (Peart *et al*, 2002). *Agrobacterium* carrying PVX-Tk was kindly provided by G Farnham. Agro-infiltration to transiently express genes in *N. benthamiana* was performed according to Peart *et al* (2002). Details of Agroinfiltration protocol are also provided in the Supplementary Material.

Plasmid constructs

The AtSGT1a and AtSGT1b derivatives used in agro-infiltration were amplified from existing clones (Azevedo *et al*, 2002) with primers containing specific restriction sites that allowed subcloning into different destination vectors. Details of construction schemes are described in Supplementary Material.

Whole-mount preparation of seed

Seeds were fixed in ethanol:acetic acid (3:1) for 1 h followed by washing in 70% ethanol twice. Clearing was obtained after 30 min in a derivative of Hoyer's medium (chloral hydrate:distilled water:glycerol [8:3:1 g]). Observations were performed using differential interference contrast (DIC) optics.

Western blot analysis

Rat anti-SGSa and rabbit anti-SGT1b antibodies used here are described by Azevedo *et al* (2002) and Austin *et al* (2002), respectively. Anti-RAR1 and anti-HSP90 antibodies were described previously (Azevedo *et al*, 2002; Takahashi *et al*, 2003). Rabbit anti-SGSa antibodies were raised against the SGSa domain and affinity purified. The protocol for StrepII tag detection using StrepTactin AP-conjugate is described by Witte *et al* (2004). Total protein extracts from agro-infiltrated *N. benthamiana* leaves were prepared by grinding 2 cm^2 of leaf tissue in 100 µl of ice-cold 1 × GTEN extraction buffer (10% glycerol, 25 mM Tris–HCl pH 7.5, 1 mM EDTA, 150 mM NaCl, 5 mM dithiothreitol (DTT) and 1% of plant protease inhibitor inhibitor cocktail (Sigma)) and 4 × of SDS–PAGE sample buffer. Samples were boiled for 5 min, quick spun and 5 µl run on a 12% SDS–PAGE gel, blotted onto PVDF membranes and visualized using antibodies described with ECL-Plus (Amersham). ImageJ software (http://rsb.info.nih.gov/ij, US National Institute of Health, Bethesda, MD) was used for quantitative comparison of SGT1 proteins on the blots (see Supplementary Figure 1 for details).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

Acknowledgements

We thank David Baulcombe for providing the *N. benthamiana* transgenic plants and VIGS constructs. This work was supported by grants from the Gatsby Charitable Organization (KS) and the UK Biotechnology and Biological Sciences Research Council (KS) and fellowships from Fundação para a Ciência e a Tecnologia to CA. Support to JP, SB and LN by a Deutsche Forschungsgemeinschaft 'SFB 635' Grant, The Alexander von Humboldt Foundation and The Max-Planck Society is gratefully acknowledged. SB was supported in part by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

References

Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JD, Parker JE (2002) Regulatory role of SGT1 in early R gene-mediated plant defenses. *Science* **295:** 2077–2080

Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K, Schulze-Lefert P (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science* **295:** 2073–2076

Bansal PK, Abdulle R, Kitagawa K (2004) Sgt1 associates with Hsp90: an initial step of assembly of the core kinetochore complex. *Mol Cell Biol* **24:** 8069–8079

Belkhadir Y, Subramaniam R, Dangl JL (2004) Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Curr Opin Plant Biol* **7:** 391–399

Bieri S, Mauch S, Shen QH, Peart J, Devoto A, Casais C, Ceron F, Schulze S, Steinbiss HH, Shirasu K, Schulze-Lefert P (2004) RAR1 positively controls steady state levels of barley MLA resistance proteins and enables sufficient MLA6 accumulation for effective resistance. *Plant Cell* **16:** 3480–3495

Brancaccio M, Fratta L, Notte A, Hirsch E, Poulet R, Guazzone S, De Acetis M, Vecchione C, Marino G, Altruda F, Silengo L, Tarone G, Lembo G (2003) Melusin, a muscle-specific integrin beta(1)-interacting protein, is required to prevent cardiac failure in response to chronic pressure overload. *Nat Med* **9:** 68–75

Dubacq C, Guerois R, Courbeyrette R, Kitagawa K, Mann C (2002) Sgt1p contributes to cyclic AMP pathway activity and physically interacts with the adenylyl cyclase Cyr1p/Cdc35p in budding yeast. *Eukaryot Cell* **1:** 568–582

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCFTIR1-dependent degradation of AUX/IAA proteins. *Nature* **414:** 271–276

Gray WM, Muskett PR, Chuang H-W, Parker JE (2003) The *Arabidopsis* SGT1b protein is required for SCFTIR1-mediated auxin response. *Plant Cell* **15:** 1310–1319

Hammond-Kosack KE, Parker J (2003) Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. *Curr Opin Biotechnol* **14:** 177–193

Holt BE, Belkhadir Y, Dangl JL (2005) Antagonistic control of disease resistance protein stability in the plant immune system. *Science* **309:** 929–932

Hubert DA, Tornero P, Belkhadir Y, Krishna P, Takahashi A, Shirasu K, Dangl JL (2003) Cytosolic HSP90 associates with and modulates the *Arabidopsis* RPM1 disease resistance protein. *EMBO J* **22:** 5679–5689

Inohara N, Nunez G (2003) NODs: Intracellular proteins involved in inflammation and apoptosis. *Nat Rev Immunol* **3:** 371–382

Kitagawa K, Skowyra D, Elledge SJ, Harper JW, Hieter P (1999) *SGT1* encodes an essential component of the yeast kinetochore assembly pathway and a novel subunit of the SCF ubiquitin ligase complex. *Mol Cell* **4:** 21–33

Krysan PJ, Young JC, Sussman MR (1999) T-DNA as an insertional mutagen in *Arabidopsis*. *Plant Cell* **11:** 2283–2290

Lee YT, Jacob J, Michowski W, Nowotny M, Kuznicki J, Chazin WJ (2004) Human Sgt1 binds HSP90 through the CHORD-Sgt1 domain and not the tetratricopeptide repeat domain. *J Biol Chem* **279:** 16511–16517

Leister RT, Dahlbeck D, Day B, Li Y, Chesnokova O, Staskawicz BJ (2005) Molecular genetic evidence for the role of SGT1 in the intramolecular complementation of Bs2 protein activity in *Nicotiana benthamiana*. *Plant Cell* **17:** 1268–1278

Lingelbach LB, Kaplan KB (2004) The interaction between Sgt1p and Skp1p is regulated by HSP90 chaperones and is required for proper CBF3 assembly. *Mol Cell Biol* **24:** 8938–8950

Liu Y, Schiff M, Serino G, Deng XW, Dinesh-Kumar SP (2002) Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene-mediated resistance response to Tobacco mosaic virus. *Plant Cell* **14:** 1483–1496

Lu R, Malcuit I, Moffett P, Ruiz T, Peart J, Wu A-J, Rathjen JP, Bendahmane A, Day D, Baulcombe DC (2003) High throughput virus-induced gene silencing identifies heat shock protein 90 as a cofactor of plant disease resistance. *EMBO J* **22:** 5690–5699

Muskett PR, Kahn K, Austin MJ, Moisan LJ, Sadanandom A, Shirasu K, Jones JDG, Parker JE (2002) *Arabidopsis* RAR1 exerts rate-limiting control of R gene-mediated defenses against multiple pathogens. *Plant Cell* **14:** 979–992

Peart JR, Lui R, Sadanandom A, Malcuit I, Moffett P, Brice DC, Schauser L, Jaggard DAW, Xiao S, Coleman MJ, Dow M, Jones JDG, Shirasu K, Baulcombe DC (2002) The ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proc Acad Natl Sci USA* **99:** 10865–10869

Sadanandom A, Findlay K, Doonan JH, Schulze-Lefert P, Shirasu K (2004) CHPA, a cysteine- and histidine-rich-domain-containing protein, contributes to maintenance of the diploid state in *Aspergillus nidulans*. *Eukaryot Cell* **3:** 984–991

Schulze-Lefert P (2004) Plant immunity: the origami of receptor activation. *Curr Biol* **14:** R22–R24

Shirasu K, Schulze-Lefert P (2000) Regulators of cell death in disease resistance. *Plant Mol Biol* **44:** 371–385

Shirasu K, Lahaye L, Tan M-W, Zhou F, Azevedo C, Schulze-Lefert P (1999) A novel class of eukaryotic zinc-binding protein is required for disease resistance signaling in barley and deveolpment in *C. elegans*. *Cell* **99:** 355–366

Shirasu K, Schulze-Lefert P (2003) Complex formation, promiscuity, and multi-functionality: protein interactions in disease resistance pathways. *Trends Plant Sci* **8:** 252–258

Takahashi A, Casais C, Ichimura K, Shirasu K (2003) HSP90 interacts with RAR1 and SGT1, and is essential for RPS2-mediated resistance in *Arabidopsis*. *Proc Natl Acad Sci USA* **100:** 11777–11782

Tao Y, Xie ZY, Chen WQ, Glazebrook J, Chang HS, Han B, Zhu T, Zou GZ, Katagiri F (2003) Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. *Plant Cell* **15:** 317–330

Tör M, Gordon P, Cuzick A, Eulgem T, Sinapidou E, Mert-Turk F, Can C, Dangl JL, Holub EB (2002) *Arabidopsis* SGT1b is required for defense signaling conferred by several downy mildew resistance genes. *Plant Cell* **14:** 993–1003

Tornero P, Merritt P, Sadanandom A, Shirasu K, Innes RW, Dangl JL (2002) RAR1 and NDR1 contribute quantitatively to disease resistance in *Arabidopsis*, and their relative contributions are dependent on the R gene assayed. *Plant Cell* **14:** 1005–1015

Witte CP, Noel LD, Gielbert J, Parker JE, Romeis T (2004) Rapid one-step protein purification from plant material using the eight-amino acid StrepII epitope. *Plant Mol Biol* **55:** 135–147

Yang J, Roe SM, Cliff MJ, Williams MA, Ladbury JE, Cohen PTW, Barford D (2005) Molecular basis for TPR domain-mediated regulation of protein phosphatase. *EMBO J* **24:** 1–10

Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W (2004) GENEVESTIGATOR. *Arabidopsis* microarray database and analysis toolbox. *Plant Physiol* **136:** 2621–2632

