Heat Shock Protein 90.1 Plays a Role in *Agrobacterium*-Mediated Plant Transformation

Dear Editor,

Manybacterial proteins are involved in Agrobacteriummediated plant transformation. By contrast, relatively little is known about plant proteins that play key roles in transformation. Some of these host proteins interact with Virulence effector proteins, including VirE2, that are transferred from Agrobacterium to plants (Gelvin, 2010; Pitzschke and Hirt, 2010). A recent study indicated that the plant protein SUPPRESSOR OF G2 ALLELE OF SKP1 (SGT1), a co-chaperone of heat shock protein 90 (HSP90), is required for Agrobacterium-mediated transformation (Anand et al., 2012). These studies suggested the involvement of HSP90 in Agrobacterium-mediated transformation.

We investigated whether HSP90.1, a co-chaperone of SGT1, may also be important for Agrobacterium-mediated transformation. We assayed an Arabidopsis hsp90.1 T-DNA insertion mutant and 35S:Myc-HSP90.1-overexpression plants (Supplemental Figure 1A and 1B) for stable root transformation. Compared to controls, the hsp90.1-2 mutant was 1.7-fold less susceptible, whereas 35S:Myc-HSP90.1 plants were twice as susceptible to transformation (Figure 1A). Thus, decreased or increased HSP90.1 expression resulted in altered transformation susceptibility. Genomic DNA blots showed that the amount of uidA DNA, a transgene on the T-DNA, integrated into the hsp90.1-2 mutant genome was four-fold less than that of control plants. However, uidA integration into HSP90.1overexpression plants was 10.8-fold greater than that of control plants (Supplemental Figure 1C). The greater increase in T-DNA integration than that of stable root transformation may result from silencing of some integrated genes because of epigenetic effects such as DNA methylation (Park et al., manuscript in preparation).

VIP1 (VirE2 interacting protein 1) and VBF (VIP1 F-box binding protein) are host proteins that may be important for T-DNA subcellular trafficking and integration (Tzfira et al., 2001; Djamei et al., 2007; Gelvin, 2010; Zaltsman et al., 2010). We reasoned that VIP1 and/or VBF could interact with HSP90.1. To test this hypothesis, we conducted a Bimolecular Fluorescence Complementation (BiFC) assay in which two *A. tumefaciens* strains harboring in their T-DNAs genes encoding protein fusions with nVenus or cCFP were co-infiltrated into *Nicotiana benthamiana* leaves. The results showed an interaction between VIP1 and HSP90.1 (nVenus–VIP1 and cCFP-HSP90.1) as a yellow fluorescence signal in both the cytoplasm and the nucleus (Figure 1B), compared to empty vector combinations (nVenus–VIP1 + cCFP, and nVenus + cCFP-HSP90.1) showing no yellow fluorescence signals (Supplemental Figure 2A). Our BiFC assay did not detect interaction of HSP90.1 with VBF (Supplemental Figure 2A, lower panel), the latter of which recognizes and targets VIP1 and its bound VirE2 for degradation (Zaltsman et al., 2010). These data indicate that HSP90.1 interacts in leaves with VIP1 but not with VBF.

We next conducted co-immunoprecipitation (co-IP) studies. Three Agrobacterium strains individually harboring in their T-DNA regions 35S: Myc-HSP90.1, 35S: YFP-VIP1, or 35S: YFP-SGT1b were co-infiltrated into N. benthamiana leaves. Input proteins were observed using anti-GFP and anti-Myc antibodies, whereas interacting proteins were detected by anti-GFP antibodies (Figure 1C, left panel). The in vivo interaction between Myc-HSP90.1 (83kDa) and YFP-VIP1 (70kDa) is strong. YFP-VIP1 alone served as a negative control and did not react with anti-Myc conjugated beads. Our co-IP analysis also detected interaction between VIP1 and SGT1b in N. benthamiana leaves infiltrated with two Agrobacterium strains harboring in their T-DNA regions 35S:Myc-VIP1 or 35S-YFP-SGT1b (Figure 1C, right panel). These results suggest that SGT1b may work in concert with HSP90.1 to protect VIP1. Finally, we conducted yeast two-hybrid (Y2H) analyses to substantiate further the interaction between HSP90.1 and VIP1 (Supplemental Figure 2B). We chose VIP1 as the prey in our assays because VIP1 is a transcription factor and, therefore, VIP1 fused to the gal4 DNA binding domain (BD) was able to auto-activate expression of the reporter genes without interacting with a protein containing an acidic activation domain (AD). Yeast strain AH109 transformed with the BD-HSP90.1 and AD-VIP1 plasmids grew on triple dropout plates (SD/-Leu/-Trp/-His), indicating interaction of the two tested proteins (Supplemental Figure 2B, left four columns). We also detected the expression of the MEL1 reporter gene using an X-α-gal (5-bromo-4-chloro-3-indolyl α -D-galactopyranoside) assay, confirming the interaction between HSP90.1 and VIP1 (Supplemental Figure 2B, last column). Negative control strains containing BD-HSP90.1 and AD-empty vector plasmids, or BD-empty and AD-VIP1

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Figure 1 HSP90.1 Plays a Role in Agrobacterium-Mediated Plant Transformation.

(A) Tumorigenesis assay of Col-0, *hsp90.1–2*, and *355:Myc-HSP90.1* root segments inoculated with *Agrobacterium tumefaciens* A208 at 10⁵ cfu ml⁻¹. Lanes 1–3: Col-0, *hsp90.1–2*, and *355-Myc-HSP90.1*, respectively.

(B) HSP90.1 interacts with VIP1 by Bimolecular Fluorescence Complementation. Bar is 20 µm. mCherry (red) marks both the nucleus and cytoplasm. White arrow indicates the nucleus.

plasmids, did not grow on this medium. Thus, Y2H results further supported the HSP90.1–VIP1 interaction.

The interaction of HSP90.1 with VIP1 prompted us to investigate whether HSP90.1 could prevent VIP1 aggregation in leaves. We compared the pattern of VIP1-YFP fluorescence in infiltrated leaves of wild-type and hsp90.1-2 mutant plants. Large aggregates of YFP-VIP1 appeared in the cytoplasm of hsp90.1-2 mutant leaves, but no such large aggregates were seen in control samples (Figure 1D). Western blots showed similar levels of YFP-VIP1 protein in infiltrated Col-0 and hsp90.1-2 leaves, indicating that the intense YFP fluorescence in hsp90.1-2 leaves did not result from higher amounts of VIP1 protein (Supplemental Figure 3A). A key function of cytosolic HSP90 is to maintain the stability and prevent the aggregation of its client proteins. Geldanamycin (GDA), a specific inhibitor of HSP90, can decrease the stability of HSP90 client proteins, resulting in their aggregation and possible degradation (Theodoraki et al., 2012). Treatment of leaves with GDA resulted in YFP-VIP1 aggregation (Figure 1E, lower panel, and Supplemental Figure 3B). No such aggregates were detected in untreated infiltrated leaves (Figure 1E, top panel). Western blots showed a similar level of YFP-VIP1 in the absence or presence of GDA, suggesting YFP-VIP1 aggregation but not degradation (Supplemental Figure 3C).

VirE2 protein is important for Agrobacteriummediated transformation (Citovsky et al., 1992). GDA treatment of leaves causes VIP1 aggregation and, because VIP1 interacts with VirE2, we investigated the effect of GDA treatment on VirE2 solubility. Treatment of leaves with GDA caused YFP–VirE2 aggregation in the cytoplasm of infiltrated *N. benthamiana* leaves (Figure 1F, lower panel; in these cells, the cytoplasm is appressed to the cellular periphery by the large central vacuole), suggesting that HSP90 activity is important for maintaining VirE2 solubility and function, either directly or indirectly by preventing aggregation of the VirE2 interacting protein VIP1. We have shown that HSP90.1 is important for *Agrobacterium*-mediated plant transformation. We propose that HSP90.1 functions as a VIP1 molecular chaperone and facilitates transformation through stabilizing VIP1, VirE2, and/or other proteins important for transformation. This new role for a member of the HSP90 family had not previously been described. Gurel et al. (2009) reported that plant transformation susceptibility may be increased by heat treatment. Although heat treatment affects expression of many genes and regulatory pathways, increased expression of HSP90 could thus augment transformation efficiency.

Recently, Shi et al. (2014) indicated that VIP1 does not play an important role in *Agrobacterium*-mediated transformation. Data presented in that study, along with those presented here, suggest that HSP90.1 plays a role in transformation beyond that of influencing VIP1 function.

SUPPLEMENTARY DATA

Supplementary Data are available at *Molecular Plant Online.*

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(C) HSP90.1 interacts with VIP1 or SGT1b in pull-down assays. Left panel shows HS90.1 interacts with VIP1. Agrobacterium strains containing binary vectors encoding 35S:Myc-HSP90.1 or 35S:YFP-VIP1 genes were separately or co-infiltrated into N. benthamiana leaves. Transiently expressed YFP-VIP1 does not react with anti-Myc antibody conjugated beads. YFP-VIP1 (*) was detected by Western blots using anti-GFP antibodies. Right panel shows SGT1b interaction with VIP1. Agrobacterium strains containing binary vectors encoding 35S:Myc-VIP1 or 35S:YFP-SGT1b genes were separately or co-infiltrated into N. benthamiana leaves. In vivo protein expression was verified in infiltrated leaves 3 d after infiltration, and proteins were immuno-absorbed using anti-Myc (Myc-HSP90.1, 83 kDa or Myc-VIP1, 39 kDa) or anti-GFP (YFP-VIP1 and YFP-SGT1b; 70 kDa) antibodies.

(D) Fluorescence patterns of YFP–VIP1 in wild-type *Arabidopsis* and *HSP90.1* mutant plants. White bars indicate 25 µm (left panel) and 5 µm (right panel). Right panel is enlargement of the boxed region of the left panel. Yellow and white arrows indicate the nucleus and large YFP–VIP1 aggregates, respectively.

(E) Aggregation of YFP–VIP1 in *N. benthamiana* leaves treated with GDA. *Agrobacterium* harboring YFP–VIP1 constructs in the T-DNA were infiltrated into *N. benthamiana* leaves in the absence (top panel) or presence (lower panel) of 1 µM GDA.

(F) GDA treatment of leaves causes VirE2 cytoplasmic aggregation. *Agrobacterium* harboring *YFP–VirE2* constructs in the T-DNA were infiltrated into *N. benthamiana* leaves in the absence (top panel) or presence (lower panel) of 5 µM GDA. White bars are 20 µm. Orange arrow points to VirE2 aggregation in the cytoplasm, white arrows point to nuclei.

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