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ARTICLE ADDENDUM

HSP90 stabilizes auxin receptor TIR1 and ensures plasticity of auxin responses

Etsuko Watanabe[a,*], Shoji Mano[b,c], Ikuko Hara-Nishimura[d,**], Mikio Nishimura[a], and Kenji Yamada[a,d,e]

[a]Department of Cell Biology, National Institute for Basic Biology, Okazaki, Japan; [b]Department of Evolutionary Biology and Biodiversity, National Institute for Basic Biology, Okazaki, Japan; [c]Department of Basic Biology, School of Life Science, SOKENDAI (The Graduate University for Advanced Studies), Okazaki, Japan; [d]Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan; [e]Malopolska Center of Biotechnology, Jagiellonian University, Krakow, Poland

ABSTRACT

Heat shock protein 90 (HSP90) is a highly conserved molecular chaperone that facilitates the maturation of target proteins. Here, we report that the auxin receptor TIR1 is a target of cytosolic HSP90 and that HSP90 and TIR1 form a complex. Inhibition of HSP90 compromised the nuclear localization of TIR1, and abrogated plant responses to the hormone auxin. Our findings suggest that HSP90 positively regulates auxin receptor function. We also propose that HSP90 buffers or hides phenotypic variations in animals and plants by masking mutations in some of its target proteins. Support for this proposal comes from the *tir1-1* mutant of Arabidopsis, which showed a root growth defect that was only seen after inhibition of HSP90. We have developed a model in which cytosolic HSP90 works like a capacitor for auxin-related phenotypic variation via regulation of the auxin receptor in response to environmentally and genetically induced perturbations.

Abbreviations: AFB, auxin signaling F-box; ARF, auxin-response factor; BiFC, bimolecular fluorescence complementation; GFP, green fluorescent protein; GUS, β-glucuronidase; HSP, heat-shock protein; IAA, indole acetic acid; DEX, dexamethasone; SCF/Skp/Cullin, F-box–containing complex; TIR, transport inhibitor response

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Heat shock protein 90 (HSP90) aids the folding of nascent proteins into their mature forms. The targets of this molecular chaperone include transcription factors, kinases, phosphatases, and receptors that play important roles in signal transduction pathways in plants, animals, and yeasts.[1-3] HSP90 co-operates with co-chaperones such as SGT1 to achieve these effects.[4] In addition, HSP90 is involved in the heat shock response by regulating the state of heat shock transcription factors. However, to date, relatively few of the target proteins of HSP90 have been identified in plants compared to animals and yeast. Identifying new target proteins, such as those engaged in important signaling pathways mediated by plant hormones such as auxin, will undoubtedly contribute to our understanding of the functional mechanisms of these proteins.

Auxin has a central role in plant growth and development[5] by promoting the degradation of Aux/IAA transcriptional repressors, thereby allowing auxin response factors (ARFs) to activate the transcription of auxin-responsive genes.[6] In this process, auxin receptors (TIR1/AFB proteins), which are components of an SCF ubiquitin ligase complex, guide Aux/IAA proteins to the ubiquitin-dependent degradation system.[7,8] In our recent publication, we identified the first example of a mutation in the auxin receptor TIR1 that was "buffered" by HSP90. [9]

In order to increase our understanding of the role of HSP90 in plant growth, we examined plants with defects in HSP90 chaperone activity. Four genes encoding cytosolic HSP90 homologues (*HSP90.1*, *HSP90.2*, *HSP90.3*, and *HSP90.4*) are located together on chromosome 5 in Arabidopsis and it is difficult to generate multiple HSP90-knockout mutants. We successfully introduced a D80N and E303K mutation into *HSP90.2* (HSP90.2^{D80N} and HSP90.2^{E303K}); these mutations correspond to the D79N mutation in yeast HSP82 and the E313K mutation in Drosophila HSP83, respectively, and both cause dominant-negative effects.[10,11] These mutant forms of HSP90.2 are exogenously expressed in Arabidopsis under the control of a dexamethasone (DEX)-inducible system.[9] Root growth of transgenic plants expressing HSP90^{E303K} was reduced in the presence of DEX suggesting that inhibition of HSP90 activity severely disturbed plant growth. The number of lateral roots was lower in the mutants than in wild type, and this phenotype was similar to that found in auxin receptor deficient mutants. We therefore speculated that HSP90.2 was probably involved in the auxin response. Treatment of

CONTACT Kenji Yamada kenji.yamada@uj.edu.pl Plant Molecular Biology Laboratory, Malopolska Center of Biotechnology, Jagiellonian University, Krakow 30–387, Poland.
*Present address: Cryobiofrontier Research Center, Iwate University, Morioka 020–8550, Japan.
**Present address: Faculty of Science and Engineering, Konan University, Kobe 658-0073, Japan.

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Arabidopsis with the HSP90 inhibitors geldanamycin (GDA) or radicicol (RAD) reduced β-*glucuronidase* (*GUS*) expression in *Arabidopsis* harboring the auxin-responsive marker gene, *DR5:GUS*. In addition, expression of the *IAA5* gene was reduced in plants expressing HSP90.2^{E303K}. These results indicated that HSP90 was required for the expression of auxin-responsive genes.

Auxin promotes the degradation of Aux/IAA transcriptional repressors, thereby allowing auxin response factors (ARFs) to activate the transcription of auxin-responsive genes.[6] We analyzed IAA7-GFP degradation in response to auxin and found that treatment with RAD inhibited the degradation of IAA7-GFP. This suggests that HSP90 induced the degradation of IAA proteins and induced the expression of auxin-responsive genes during the auxin response. During Aux/IAA degradation, the auxin receptor proteins TIR1/AFB guide Aux/IAA proteins to the ubiquitin-dependent degradation system.[7,8] We examined whether HSP90 interacted with TIR1 and found that FLAG-TIR1 and HSP90 formed a complex in an Arabidopsis leaf extract. In addition, the interaction of HSP90.2 and TIR1 was confirmed by a BiFC analysis. We also examined the effect of HSP90 inhibitors on the localization of TIR1-GFP. TIR1-GFP is accumulated in the nuclei under normal conditions; after RAD or GDA treatment most TIR1-GFP remained in the nucleus, but some TIR1-GFP was observed in the cytosol. This suggests that HSP90 aided nuclear localization of TIR1.

In the nucleus, HSP90 and TIR1 form a complex that appears to function as a chaperone for TIR1. Together with the report of Wang et al.,[12] our study[9] suggests that HSP90 works with the co-chaperone SGT1b as a chaperone of the SCF^{TIR1} complex and regulates its auxin receptor function in the nucleus. It is possible that under severe stress conditions when chaperone activity of HSP90 is low, TIR1 can no longer function as an auxin receptor; its protein might be misfolded and a functional SCF^{TIR1} complex is not formed (Fig. 1).

HSP90 can mask genetic or epigenetic mutations in eukaryotes to create cryptic phenotypes in a phenomenon known as buffering. The cryptic phenotypes can be made visible by perturbing HSP90 activity, such as by increasing the temperature or adding a low concentration of HSP90 inhibitor.[13-18] In the *tir1-1* mutant, the TIR1 protein has an amino acid substitution (G147D); in the T-DNA insertion mutant, *tir1-10*, TIR1 is absent. The *tir1-1* mutation causes a cryptic auxin response defect.[9] We found that the induction of *IAA1* and *IAA5* genes in the *tir1-1* mutant was almost the same after auxin treatment as the wild type, while expression of the genes was strongly reduced in the *tir1-10* mutant.[9] Interestingly, the expression level of auxin-inducible genes were decreased with low concentration of HSP90 inhibitors in *tir1-1* suggesting that HSP90 cover the cryptic mutation of TIR1-1 function.[9] These findings suggest that HSP90 can buffer the effects of a point mutation in its target protein TIR1. We hypothesize that HSP90 may assist the mutated TIR1^{G147D} protein to recover receptor function in

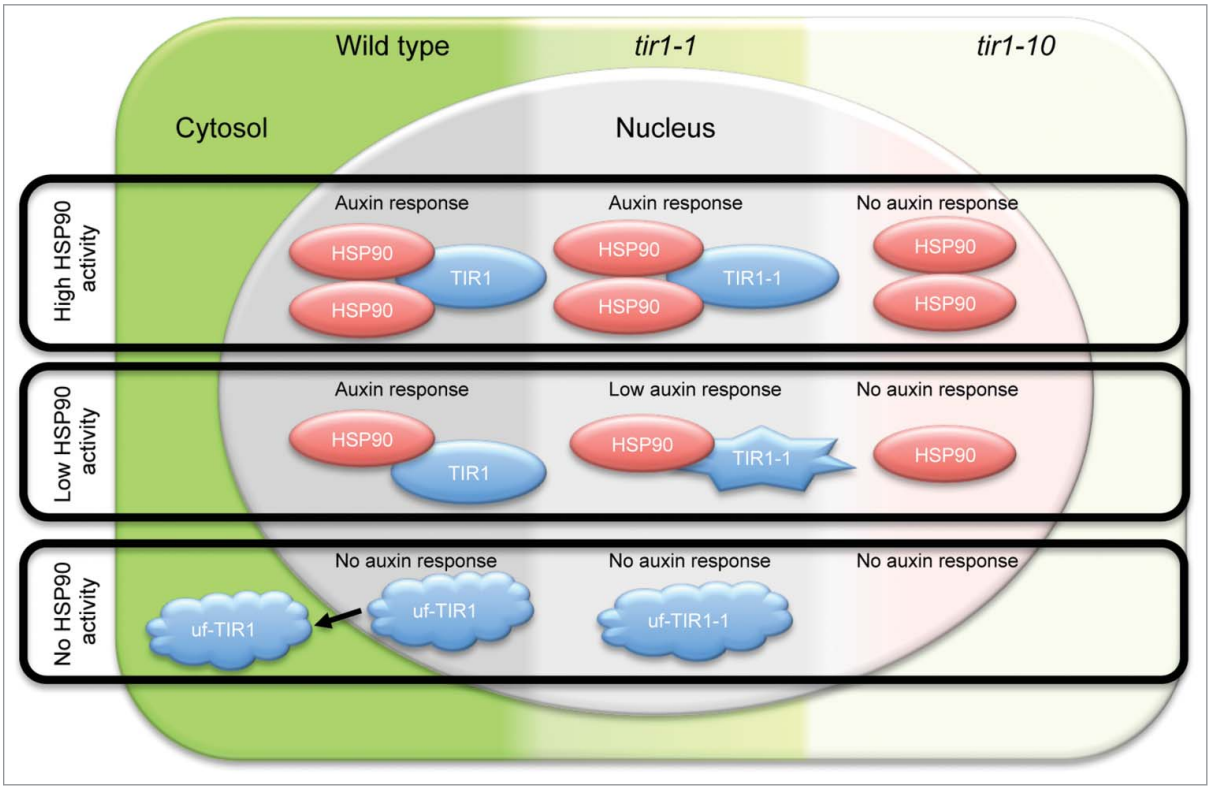

Figure 1. Schematic model for the function of Arabidopsis HSP90 as a chaperone for TIR1 in auxin signaling and for its buffering function in wild type (WT) and *tir1* mutants. TIR1 needs the chaperone activity of HSP90 to function as a receptor for auxin and to promote an auxin response. HSP90 positively regulates the auxin response by stabilizing TIR1 function. Under conditions when no HSP90 activity occurs, the TIR1 is mostly non-functional and some unfolded TIR1 (uf-TIR1) proteins are exported to the cytosol, causing a severe attenuation in the auxin response. TIR1-1 protein with a point mutation may require much more HSP90 chaperone activity compared to wild type TIR1. Therefore, in low HSP90 activity conditions, mutated TIR1-1 cannot keep its function; as a result, the *tir1-1* mutant displays its partially defective auxin response phenotype. In the *tir1-10* mutant, the auxin response is severely impaired because the TIR1 protein is absent.

the *tir1-1* mutant; $TIR1^{G147D}$ may require more HSP90 chaperone activity compared to wild-type TIR1, and the *tir1-1* mutant is therefore hypersensitive to inhibition of HSP90 (Fig. 1). In the presence of a low concentration of HSP90 inhibitor, as in low HSP90 activity conditions, HSP90 could not buffer the mutation of TIR1 and the phenotype of the *tir1-1* mutant was exposed (Fig. 1). The auxin response phenotype was not cryptic in the *tir1-10* mutant because it is a null allele of TIR1 (Fig. 1). Our findings support the concept that HSP90 can produce cryptic phenotypes following mutation in a target protein such as auxin receptor TIR1[9].

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

ORCID

Ikuko Hara-Nishimura http://orcid.org/0000-0001-8814-1593
Kenji Yamada http://orcid.org/0000-0003-4872-3729

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