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The HSP90 chaperone complex, an emerging force in plant development and phenotypic plasticity

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The essential cellular functions of the molecular chaperone HSP90 have been intensively investigated in fungal and mammalian model systems. Several recent publications have highlighted the importance of this chaperone complex in plant development and responsiveness to external stimuli. In particular, HSP90 is crucial for R-protein-mediated defense against pathogens. Other facets of HSP90 function in plants include its involvement in phenotypic plasticity, developmental stability, and buffering of genetic variation. Plants have emerged as powerful tools that complement other model systems in attempts to extend our knowledge of the myriad impacts of protein folding and chaperone function.

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Abbreviations

AHA1	Activator of HSP90 ATPase1
Avr	Avirulence
CLV	*CLAVATA*
ER	endoplasmic reticulum
FKBP	FK506-binding protein
HOP	HSP70/HSP90 organizing protein
HR	hypersensitive response
HSP90	heat shock protein90
LRR	leucine-rich repeat
R	Resistance
RAR1	REQUIRED FOR MLA12 RESISTANCE1
RIN4	RPM1-INTERACTING PROTEIN4
RPM1	RESISTANCE TO *Pseudomonas syringae* EXPRESSING AVRRPM1
RPS2	RESISTANCE TO *P. syringae* EXPRESSING AVRRPT2
RPS4	RESISTANCE TO *P. syringae* EXPRESSING AVRRPS4
SGT1	Suppressor of G2 allele of *suppressor of kinetochore protein1*

Introduction

Heat shock protein90 (HSP90) is an abundant, evolutionarily conserved molecular chaperone [1–3]. By definition, molecular chaperones assist other polypeptides in folding and prevent unproductive interactions without becoming part of a final structure [4]. Although essential under physiological conditions, this function is of particular importance under conditions that interfere with protein folding, such as high temperature or other stresses [5]. Hence, HSP90 and many other chaperones are highly stress-inducible. In the widely investigated animal and fungus systems, HSP90 chaperones a diverse set of client (substrate) proteins that are involved in many essential cellular processes (reviewed in [2,3]). Canonical examples of HSP90 clients include mammalian steroid hormone receptors. HSP90 facilitates the maturation of many steroid hormone receptors to a stable conformation in an ATP-dependent manner, enabling them to bind their hormone ligand and to initiate signal transduction. HSP90 functions in complex with various co-chaperones, which modulate its ATPase activity and client protein interactions. For example, the co-chaperone HOP (HSP70/HSP90 organizing protein) is involved in loading the immature client and inhibiting the ATPase activity of HSP90. When HSP90 binds ATP, HOP is exchanged for p23 and prolylisomerases/immunophilins. The substrate is released upon ATP hydrolysis, and can be stimulated by another set of co-chaperones, such as AHA1 (Activator of HSP90 ATPase1). The work of many laboratories indicates that the function of HSP90 and many co-chaperones is conserved among fungi, animals, and plants; however, individual substrates may not be conserved [1–3].

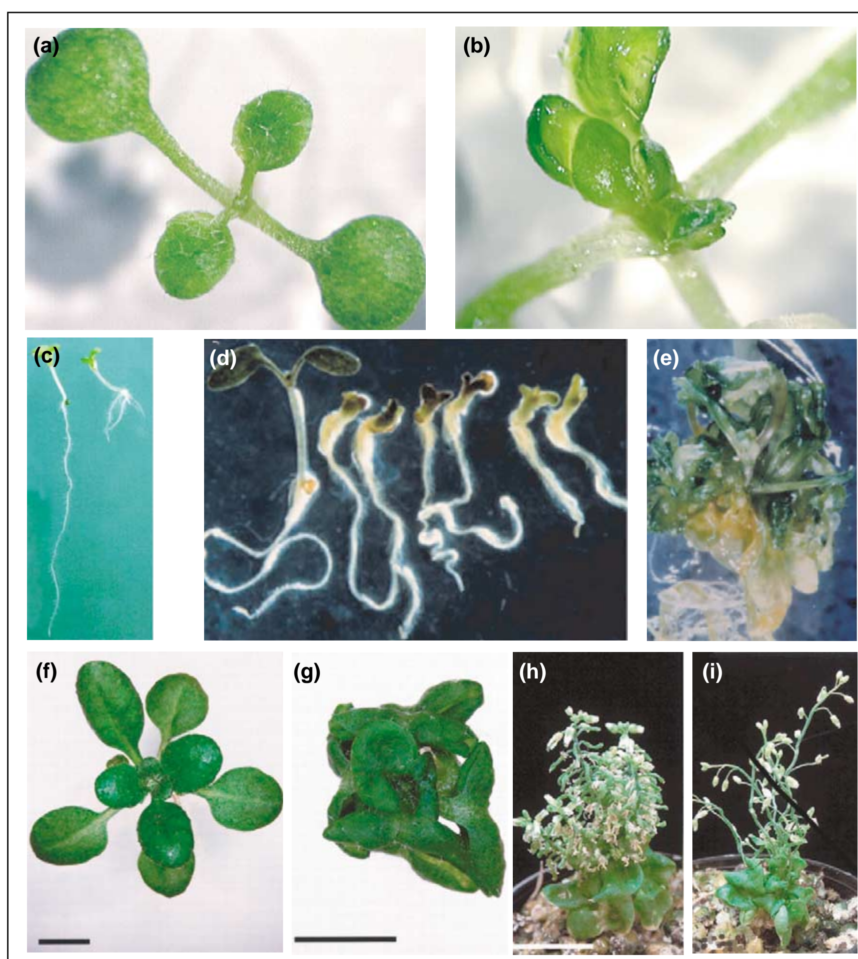
Until recently, only a few laboratories have focused on elucidating the physiological roles of HSP90 chaperone complexes in plants. Recent reports on HSP90's involvement in plant disease resistance and genetic buffering have widened the field and spurred new interest in the research community. Indeed, studying HSP90 in plants has great potential to reveal unknown functional aspects that might be either specific to plants or shared among biological kingdoms. Like the unicellular fungi, sessile plants must sense and respond to changing local environments without the advantage of motility. Like motile animals, higher plants are complex multicellular organisms and are faced with the task of tissue differentiation, a process known to involve HSP90 in animal systems. This combination may enhance the importance of an environmentally responsive chaperone complex such as HSP90, and thereby ease its investigation.

HSP90 in plant development

Seven HSP90 isoforms exist in *Arabidopsis*: four closely related isoforms are cytosolic (HSP90.1–HSP90.4); one is chloroplastic (HSP90.5); one is mitochondrial (HSP90.6); and one is localized in the endoplasmic reticulum (ER) (HSP90.7) [6]. Of the four cytosolic isoforms, one is heat-inducible, whereas the others are constitutively expressed [7]. Lines that have T-DNA insertions in individual cytosolic HSP90s typically appear morphologically wild-type, indicating some degree of isoform redundancy [8•,9••]. Partial inhibition of all *Arabidopsis* HSP90-isoforms with the specific inhibitor geldanamycin results in low-penetrance morphological phenotypes that affect many plant structures (Figure 1). In seedlings, such morphological alterations include epinastic cotyledons, altered rosette symmetry, radially symmetric leaves, and abnormal growth of root hairs [10].

Two mutant organelle-specific HSP90s have been described, both influencing plant development. Mutation of the chloroplast-specific HSP90.5 causes altered response to red light, chlorate resistance, and constitutively delayed chloroplast development in the *cr88* mutant [11•,12,13]. Mutation of the ER-specific HSP90.7 produced floral and shoot meristem phenotypes in the *shepherd* mutant that closely resemble that of the three *clavata* (*clv*) mutants (Figure 1; [14]). The *CLV* genes restrict stem-cell identity and promote cell differentiation as

Figure 1



HSP90 and co-chaperones profoundly affect plant development. **(a,b)** Fourteen-day-old F1 Columbia/Landsberg *erecta* (Ler) seedlings grown on (a) control medium or (b) geldanamycin-containing medium. Emerging rosette leaves are severely phenotypically altered by partial inhibition of Hsp90. **(c)** Morphological differences between seven-day-old wildtype plants (WS, left) and *shepherd* mutants (right). **(d,e)** *pasticcino* mutants have dramatically altered phenotypes throughout development. (d) Seven-day old light-grown seedlings, one wildtype seedling (left), followed by two seedlings each of *pas1*, *pas2* and *pas3*. (e) *pasticcino* mutant after 3 months. **(f–i)** *twisted dwarf1* (*ultracurvata2*) mutants are phenotypically similar to brassinosteroid mutants. Scale bar = 4 mm. (f,g) 23-day-old plant rosettes of (f) Ler wildtype and (g) *ucu-2* plants. **(h,i)** 45-day-old plants with an altered inflorescence phenotype that resembles the phenotypes caused by two different *ucu2* alleles: (h) *ucu2-1* and (i) *ucu2-3*. (a,b) from [10]; (c) from [14]; (d,e) from [50]; (f–i) from [24]. Reproduced with permission from (a–c) Nature Publishing Group, (d,e) the Company of Biologists and (f–i) the American Society of Plant Biologists.

negative regulators of WUSCHEL. CLV3 is a secreted ligand, which binds to a complex of CLV1 and CLV2, which are both transmembrane proteins with extracellular domains [15,16]. As such, CLV1 and CLV2 are probable targets of the ER chaperone machinery. In fact, the overexpression phenotype of CLV3 is suppressed in the *shepherd* mutant [14].

The functional conservation of the cytosolic HSP90 chaperone machinery is best evidenced by the successful maturation of exogenous mammalian steroid hormone receptors in plants [17]. Several plant homologs of co-chaperones, including HOP, p23, and prolylisomerase/immunophilins, have been identified [18,19]. Interference with these plant homologs reveals their diverse roles in plant development, but any role of the cytosolic HSP90s in producing these phenotypes has yet to be investigated.

For example, Berardini *et al.* [20] conducted a mutant screen to identify genes that are involved in developmental transitions of vegetative leaf shape. Mutations in *squint*, the *Arabidopsis* homolog of the mammalian HSP90-interacting prolylisomerase cyclophilin 40, caused an earlier transition from juvenile to adult leaves, as evidenced by leaf shape and trichome distribution.

Mutations in the *Arabidopsis PASTICCINO1* gene, which encodes a protein that is similar to the mammalian prolylisomerase FK506-binding protein (FKBP) 52, cause highly pleiotropic phenotypes including ectopic cell proliferation in cotyledons, extra cell layers in the hypocotyl, and abnormal apical meristems (Figure 1; [21,22]). Both cell elongation and cell proliferation are affected.

TWISTED DWARF1 (AtFKBP42/ULTRACURVATA2) is a FKBP that is known to interact with HSP90 [23]. T-DNA insertion mutants in which this protein is not functional exhibit pleiotropic developmental phenotypes, including reduced height, twisted architecture, and brassinosteroid insensitivity (Figure 1; [24]). It is tempting to speculate that brassinosteroid-mediated signal transduction may require the activity of the HSP90 chaperone complex, similar to steroid-hormone-dependent signaling in other model organisms. The scope of HSP90 involvement in plant development remains to be investigated but will most certainly shed light on the function of the complex HSP90 chaperone machinery.

HSP90 and disease resistance

Plant immunity is mediated by Resistance (R) proteins that allow recognition of specific pathogen molecules (Avirulence [Avr] proteins). Among many downstream effects, the activation of resistance pathways usually induces the hypersensitive response (HR), which involves localized programmed cell death at the point of infection [25,26]. Several recent papers have provided evidence of a role for HSP90 in plant disease resistance. Takahashi *et al.* [8•] examined the effects of HSP90 modulation in *Arabidopsis* on signaling through the resistance pathways containing the *R* genes RPM1 and RPS2. *Pseudomonas syringae* infection causes upregulation of HSP90.1 but not of the other HSP90s. Application of geldanamycin reduced RPS2-mediated bacterial resistance and the HR, yet RPM1-mediated HR was unaffected with a slight decrease in disease resistance. Similarly, a null mutation in *hsp90.1* displayed RPS2- but not RPM1-mediated resistance defects.

In a screen for loss of recognition of avrRpm1, Hubert *et al.* [9••] isolated three mutations in HSP90.2 that are localized to the ATP-binding domain. Similar mutations prevent either ATP binding or ATP hydrolysis in yeast, thus abrogating substrate cycling with HSP90. In contrast to Takahashi *et al.*'s [8•] experiments with *hsp90.1* null mutations, these *hsp90.2* mutations decreased RPM1- but not RPS2-mediated resistance. Null mutations in *hsp90.2* failed to display decreased resistance. These results raise the possibility that the nearly identical cytosolic HSP90s of *Arabidopsis* might have diverged in function. Furthermore, ATP hydrolysis is required for HSP90's disease-related function, suggesting an active chaperoning role.

Three groups [27•–29•] demonstrated a role for HSP90 in disease resistance in *Nicotiana benthamiana*. Using viral-induced gene silencing to decrease chaperone levels, these groups showed that resistance that is mediated by the *R* genes *Pto* against *P. syringae*, *Rx* against potato virus X, and *N* against tobacco mosaic virus were all dependent on HSP90.

In both tobacco [28•] and *Arabidopsis* [8•,9••], several lines of evidence demonstrate an interaction among HSP90, SGT1, and RAR1. The latter two proteins were previously implicated in disease resistance pathways and can interact with each other in the absence of HSP90 [30]. SGT1 is a conserved eukaryotic protein that has functions in cell cycle, kinetochore assembly, and protein degradation [30]. Like established co-chaperones of HSP90, SGT1 contains a tetratricopeptide repeat (TPR) domain, a structural motif that is typically involved in HSP90 binding. Further, the amino-terminal CS domain (cysteine- and histidine-rich [CHORD] and SGT1 domain) of SGT1 shares structural similarity with the HSP90 co-chaperone p23 [30]. *Arabidopsis* SGT1b probably interacts with both the ATPase domain and carboxyl terminus of HSP90 through its CS and TPR domains, respectively. SGT1 is also known to interact with R proteins, presumably with the leucine-rich repeat (LRR) domain [31]. Direct interaction between HSP90 and the LRR domain of *N* has been described in tobacco [28•]. The CHORD-1 of RAR1 appears to interact with the ATPase domain of HSP90 [8•,9••,28•]. As not all HSP90-dependent R proteins appear to require RAR1 [31], other co-factors may perform a similar function.

Drawing an analogy to the mammalian steroid hormone receptor maturation paradigm, one could equate SGT1 with a HOP-like co-chaperone that is involved in substrate loading, and RAR1 is a possible equivalent to p23 or AHA1, which both facilitate the completion of client protein maturation. A closer look at the current data, however, reveals that the situation is far more complex. In the steroid hormone receptor model, the HSP90 complex chaperones a metastable protein, keeping it poised for activation by its hormone ligand [2,3]. By contrast, in the absence of pathogens, R proteins are stabilized and functionally silenced by intramolecular interactions [32]. Several lines of evidence suggest that, upon infection, R proteins acquire a destabilized, degradation-sensitive conformation that exposes their nucleotide-binding site (NBS) domain, which then mediates subsequent signaling [32–34]. Constitutive R protein signaling results in cell death [34–36], which necessitates efficient mechanisms of negative regulation balanced by equally efficient activation upon pathogen recognition. Recent work identified RIN4 as a genetic negative regulator and interacting partner of RPM1 and RPS2 [35,37]. RIN4 becomes modified upon interaction with pathogen virulence proteins and facilitates R protein activation [38]. Do HSP90 and its proposed co-chaperones SGT1 and RAR1 represent additional negative regulators or does the chaperone complex aid R protein signaling?

Several relevant pieces of information allow a model for HSP90 function to be proposed (Figure 2). First, both *rar1* and *hsp90.2.3* mutations result in loss of RPM1-mediated resistance and disappearance of RPM1 protein [9••]. Similarly, silencing of *HSP90* also results in reduced levels of Rx protein, whereas silencing of *SGT1* does not [27•,39]. Further, HR induction and R-protein stability are genetically separable: truncated R proteins that do not have the LRR can induce HR but are unstable; the converse is true for R proteins that lack the amino-terminal domains [36]. Second, RIN4, a negative regulator of RPM1, and HSP90 cannot be co-immunoprecipitated with RPM1 at the same time [9••]. Third, the phenotypes of *rin4* include constitutive RPM1 and RPS2 protein signaling and cell death, and are opposite to those of *rar1* and *hsp90.2-3* [9••,40,41]. In fact, the lethality of *rin4* is delayed by *rar1* [40], suggesting that RAR1 and HSP90 may be functional activators of R-protein signaling.

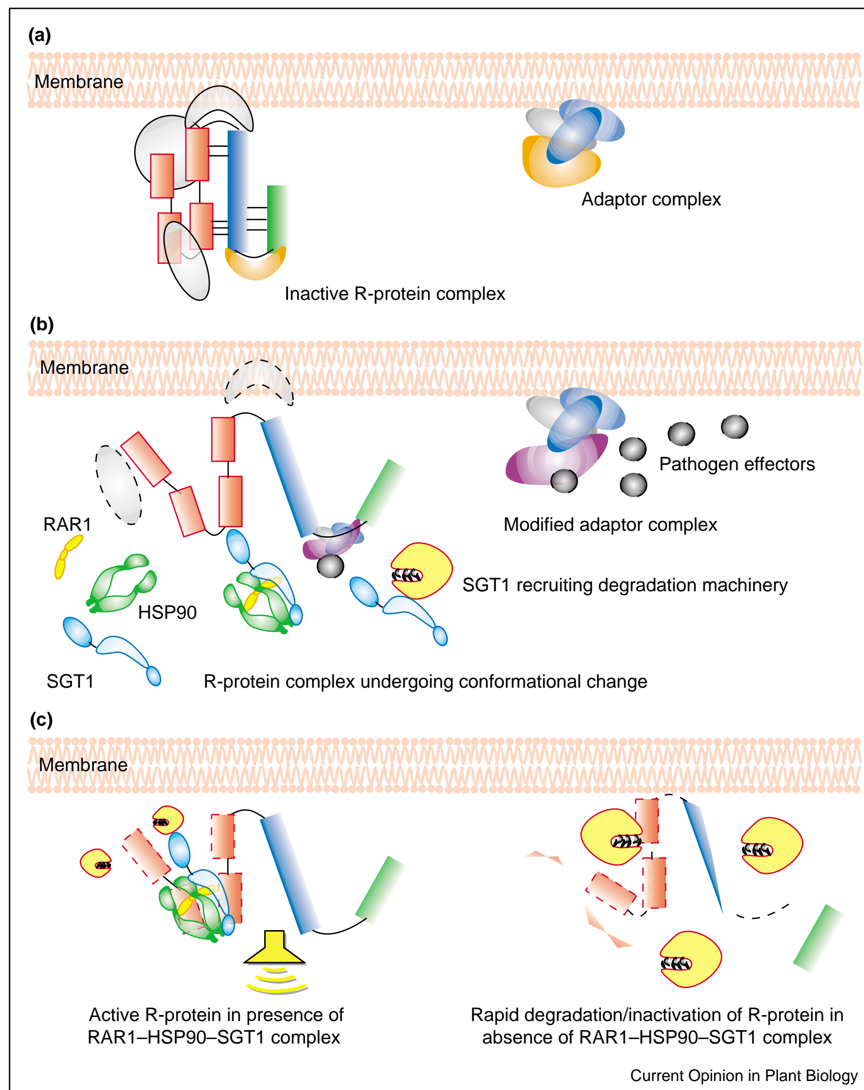
On the basis of these data, we speculate that the modification of RIN4 results in the loss of the inhibitory intramolecular interactions within RPM1 and RPS2. The HSP90–RAR1–SGT1 complex may be targeted to the R protein by SGT1 interaction with the LRR domain. The complex may mediate RIN4 displacement from the R protein and may act to stabilize the R protein in its active conformation. Given SGT1's established function in E3 ubiquitin-ligase recruitment and protein degradation [42], it seems reasonable to hypothesize that SGT1 mediates R protein degradation. When SGT1 is part of the HSP90–RAR1–SGT1 complex, SGT1-mediated R-protein degradation may be slowed for long enough to allow signal transduction to occur. Thus, the system might represent a finely tuned and coordinated balance between the activation of downstream signaling and R-protein inactivation. Although elucidation of the mechanistic details will require many more experimental endeavors, it appears that plants have co-opted a set of evolutionarily conserved proteins to perform a function that has not yet been described in other model systems.

HSP90 as a capacitor of phenotypic variation

The interaction between HSP90 complexes and R proteins might be related to another recently described functional aspect of HSP90. R proteins maintain a high degree of sequence polymorphism [43,44], probably because of the constant fluctuating struggle between plants and pathogens. In particular, the LRR domain, the contact site between the HSP90 complex and the R proteins, is under diversifying selection [45]. HSP90 has been demonstrated to conceal the phenotypic consequences of underlying natural polymorphisms in *Arabidopsis* and *Drosophila* [10,46,47••,48•]. If HSP90 buffering capacity is altered by environmental stress, mutation, or pharmacological interference, such polymorphisms can be phenotypically exposed, prompting the suggestion that HSP90-mediated storage and release may influence evolutionary processes. It is an intriguing idea that the dependence of R protein signaling on HSP90 chaperone activity might allow the toleration of slight structural deviations in R proteins and aid the observed sequence diversification of this protein family [26]. In this context, one should note that overexpression in tobacco of the *Arabidopsis R* gene RPS4 results in activation of HR [36]. This effect is dependent on the endogenous HSP90 and SGT1. These observations suggest that the conserved HSP90 complex has the inherent flexibility to bridge between rapidly diverging, in this case heterologous, R proteins and conserved downstream signaling elements. Such a system might minimize the mutational steps that are necessary for response to a novel virulence factor.

Induced defense responses are a classical example of phenotypic plasticity. Beyond defense, the environmentally responsive HSP90 chaperone complex, via its suggested involvement in multiple signaling cascades, has the potential to be of great importance for sensing the environment and mediating appropriate phenotypic plasticity. Indeed, when *Arabidopsis* seedlings are grown in the dark in the presence of the HSP90-inhibiting drug geldanamycin, the elongation of their hypocotyls is dramatically affected [10]. Similarly, the plasticity of root elongation, the response to gravity, and greening upon light exposure are also HSP90 dependent. Genetic background significantly affected the degree to which these

Figure 2



Model of R-protein activation aided by the HSP90 chaperone complex, which includes RAR1 and SGT1. **(a)** Before infection. Inactive R-proteins are stabilized by intramolecular bonds and negative regulators, including an adaptor (which is RIN4 for RPM1 and RPS2, orange) and other molecules (gray). A second pool of the adaptor may exist that is not bound to the R protein [25]. The coiled-coil (CC)/Toll and Interleukin1 receptor (TIR, green) and nucleotide-binding site (NBS, blue) domains of the R protein are involved in signaling, and the LRR domains (red) mediate interaction with negative and positive regulators of R-protein function. **(b)** Upon infection, pathogen effectors mediate the modification of the adaptor. The modified adaptor (purple) may subsequently trigger a conformational change in the R protein, resulting in the loss of intermolecular bonds and negative regulators (dashed lines). The destabilized R protein is susceptible to degradation by the proteolytic machinery (yellow, red outline), which may be recruited by SGT1 (blue). The Hsp90 chaperone complex, consisting of Hsp90 (green dimer), SGT1 (blue), and RAR1 (yellow), may recognize the destabilized conformation of the R protein. **(c)** Two scenarios are illustrated. On the left, the Hsp90 chaperone complex stabilizes the active conformation of the R protein and slows SGT1-mediated degradation or inactivation for long enough to allow downstream signaling. On the right, the Hsp90 chaperone complex is not functional and rapid degradation or inactivation ensues, leading to disease sensitivity. Schematic modified after [25].

traits were modulated by HSP90. In addition, an increase in trait variance was observed when HSP90 was inhibited, suggesting that the HSP90 chaperone complex stabilizes development in plants [10]. An effect of HSP90 reduction on development has not been observed in the fly *Drosophila melanogaster* [49]. This difference may reflect the enhanced link between the environment and the continuous developmental process of plants. Plants may exploit the environmentally sensitive HSP90 chaperone complex to remodel morphology or to initiate signaling in response to external stimuli.

To date, neither the identity nor frequency of HSP90-buffered polymorphisms in natural populations nor the

molecular underpinnings of HSP90 buffering are known. The plant model *Arabidopsis* offers several advantages for investigating HSP90 influence on phenotypic variation and evolutionary processes: large numbers of clonal seeds, recombinant mapping populations, limited inbreeding depression, and haplotyped natural populations. Employing the knowledge gained in *Arabidopsis* studies, the multiple facets of the HSP90 chaperone complex could then be investigated in natural settings and extended to other plant species, thereby providing a comprehensive view of the role of HSP90 in plant development.

Conclusions

In summary, plants, as sessile but highly differentiated organisms, face constant challenges from a variable environment. Hence, mechanisms that facilitate developmental stability while allowing for programmed environmental responsiveness are likely to be of greater importance in plants than in other model systems. Molecular chaperones, such as the HSP90 chaperone complex, are plausible candidates to accommodate such flexibility. Indeed, HSP90 and proposed co-chaperones are implicated in a host of plasticity responses and developmental processes. Future research must elucidate the mechanistic details and the extent of HSP90 requirement in related processes. In particular, the mostly uncharacterized interplay between environmental signals and HSP90 function might provide a widespread molecular mechanism of phenotypic plasticity.

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