

REVIEW ARTICLE

HSP90 and co-chaperones: a multitaskers' view on plant hormone biology

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In order to survive under ever-changing conditions plants must be able to adaptively respond to their environment. Plant hormones and the signaling cross-talk among them play a key role in integrating external and internal cues, enabling the plants to acclimate accordingly. HSP90 and several of its co-chaperones are known as pleiotropic factors involved in the signaling pathways of multiple stress responses, including temperature, drought, and pathogen infection. Recently, hormone receptor components for auxin and jasmonic acid, respectively, have been identified as clients of the HSP90 chaperone system, suggesting a direct HSP90-dependent link to hormone signaling. In this review, we give an overview of the multiple roles of HSP90 and its co-chaperones in plant hormone biology and discuss the largely unexplored targets for signal integration that the activity of these apparent multitaskers may suggest.

Keywords: abiotic stress; auxin; biotic stress; chaperone; co-chaperone; HSP90; p23; plant hormone

HSP90 is an evolutionarily highly conserved pleiotropic molecular chaperone, which is essential for survival in eukaryotes under physiological and stressed conditions. A striking amount of 1–2% of the total cellular protein is represented by HSP90 isoforms, which can increase to 4–6% upon exposure to different kinds of stress [1–3]. HSP90 is a key component for the maintenance of cellular homeostasis, and in concert with HSP90 associated co-chaperones constitutes the HSP90 chaperone machinery [4]. The HSP90 chaperone machinery controls multiple cellular processes by regulating maturation, stability, activity, and turnover of its substrates, so called client proteins (for closer information on the HSP90 chaperone cycle, see Box 1).

HSP90 clients may have diverse and unrelated molecular and physiological functions, but often are part of constitutive signaling cascades or the response to stress [5–7]. In plants, HSP90 has been directly and indirectly implied in a broad spectrum of physiological processes ranging from plant growth and development to abiotic and biotic stress responses [8–12], hormone signaling [13–16], anterograde transport of proteins to the chloroplast [17,18], and circadian clock regulation [19] (Fig. 1).

Surprisingly, HSP90-clients do not share any obvious homologies in terms of sequence or structure and the determining factors of the HSP90-client status are still largely obscure [4]. It is, however, clear that the

Abbreviations

ABA, abscisic acid; ABC, ATP-binding cassette; AUX/IAA, auxin/indole acetic acid; BAK1, BRI1-associated receptor kinase1; BES1, BRI1-EMS suppressor 1; BIN2, brassinosteroid insensitive2; BR, brassinosteroids; BRI1, brassinosteroid insensitive1; BZR1, brassinazole resistant1; CFTR, cystic fibrosis transmembrane conductance regulator; COI1, coronatine insensitive 1; FKBP, FK506-binding protein; GA, gibberellic acid; Hsf2A, heat shock factor 2A; HSP, heat shock protein; MAX2, more axillary growth 2; PAMP, pathogen-associated molecular patterns; PAS1, pasticcino1; PAT, polar auxin transport; PP5, protein phosphatase 5; RAR1, required for MLA12 resistance 1; SCF, SKP1-CUL1-F-box; SGT1, suppressor of G2 allele SKP1; TIR1/AFB, transport inhibitor response1/auxin signaling F-box; TOC, translocon of the outer membrane of chloroplasts; TWD1, twisted dwarf 1; VLCFA, very-long fatty acid; ZTL, zeittlupe.

Box 1: HSP90 co-chaperones and the HSP90 chaperone cycle

The processing of a client protein by the HSP90 chaperone machinery involves large-scale conformational changes of HSP90, which are fueled by ATP-binding and hydrolysis cycles of its N-terminal ATPase domain. Misfolded or newly synthesized proteins that require the HSP90 machinery to adopt a functional conformation are first recognized by the generalist protein folding chaperones heat shock protein 40 (HSP40), also called DNAJ proteins, and heat shock protein 70 (HSP70), which unlike HSP90 bind to unfolded substrates. Subsequently, client protein transfer is facilitated by HSP70-HSP90 organizing protein (HOP), which can simultaneously interact with HSP70 and HSP90. This interaction involves two separate tetratricopeptide-repeat (TPR) domains of HOP which bind HSP70 and HSP90 *via* their C-terminal conserved EEVD and MEEVD amino acid motifs, reviewed in [4].

In a next step, HOP and HSP70 can be displaced from the HSP90-client complex by another TPR containing protein, such as multi-domain immunophilins or protein phosphatase 5 (PP5). Triggered by the dissociation of HOP, nucleotide binding of the N-terminal ATPase is promoted [4]. In this conformation, a further co-chaperone, the small protein p23, binds to the N-terminal regions of HSP90 which decreases HSP90 ATPase activity thereby stabilizing the complex [133] (Fig. B1). Upon ATP hydrolysis, the client protein is released and the co-chaperone complex may disassemble. In addition, the HSP90 machinery may appropriate interactions with different regulatory, client-specific or species-specific co-factors, which may define an intricate network of alternative chaperone cycles.

The unique ATP binding and hydrolysis mechanism of HSP90 [4,134] constitutes the basis for the extraordinary specificity of pharmacological HSP90 ATPase inhibitors, such as Geldanamycin (GDA, see Fig. B1), Radicicol and derivatives thereof, which competitively bind to the ATP binding pocket with high affinities and thereby may effectively trap HSP90 in a conformation that prohibits dynamic client interactions [134,135]. In many cases, this may lead to recruitment of clients to their degradation pathway [136]. Furthermore, some compounds, such as [−]-epigallocatechin gallate ([−]-EGCG), may also inhibit HSP90 activity by binding to its C-terminus, potentially interfering with client or co-chaperone interactions [137,138].

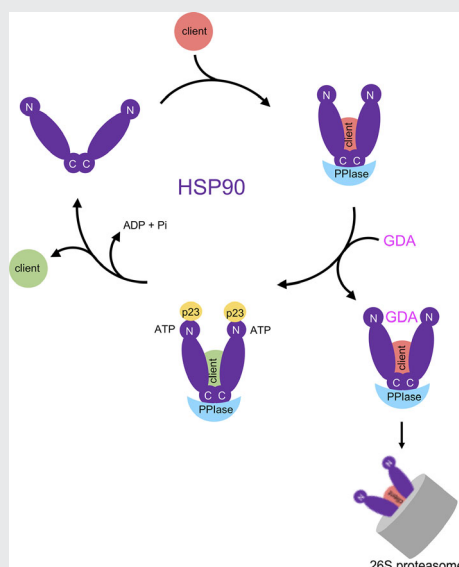


Fig. B1. The chaperone cycle of HSP90 and Effects of HSP90 inhibition. Binding of an unfolded or denatured client protein (red) to HSP90 is mediated by the early foldosome components HSP40/HSP70 and HOP (not shown). Upon ATP-binding, HSP90 adopts to a ‘closed’ conformation. In this stage, HSP90 is in association with the co-chaperone p23 and a TPR-containing protein (exemplified by PPIase). Upon ATP hydrolysis, co-chaperones dissociate and the natively folded client protein (green) is released. HSP90 ATPase inhibitors, such as geldanamycin (GDA), competitively bind the N-terminal ATP-binding pocket of HSP90, leading to arrest of the chaperone cycle and targeting of the client-HSP90 complex for proteasomal degradation.

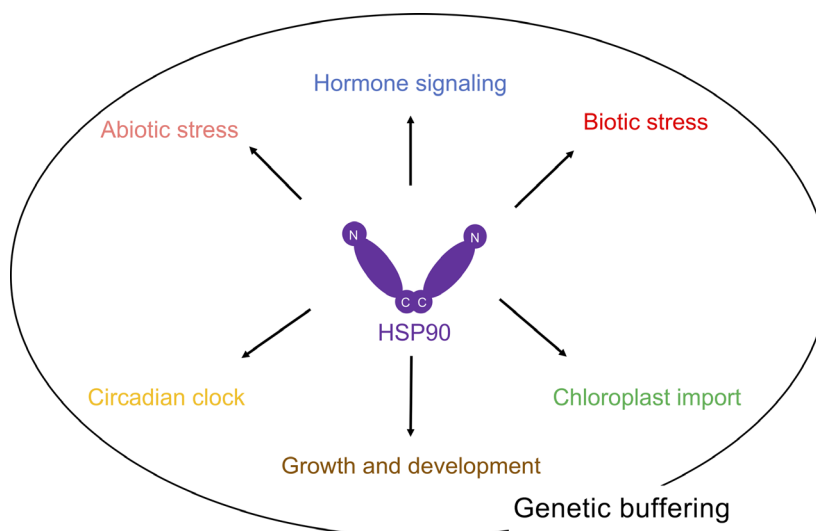


Fig. 1. HSP90s are involved in various physiological and molecular signaling pathways in plants. In plants, HSP90 has been directly and indirectly implied in a broad spectrum of physiological processes ranging from plant growth and development to abiotic and biotic stress responses, hormone signaling, anterograde transport of proteins to the chloroplast and circadian clock regulation. These processes condense to a role of HSP90 in a phenomenon, called genetic buffering conferring robustness to a process by buffering the mutant perturbation of these systems. Please note that in plants this phenomenon has strictly been demonstrated only for the *TIR1* gene for that HSP90 buffers a deleterious point mutation during auxin responses [15].

HSP90 co-chaperone system binds to proteins in their native or near-native conformations and has the ability to stabilize intrinsically labile proteins under normal conditions and likewise prevents destabilization of functional proteins under denaturing conditions [4]. Conversely, the HSP90 chaperone system may rectify effects of cryptic deleterious mutations that may lead to protein folding defects by providing a scaffold for the adoption of functional conformations. Ultimately, these properties condense to the role of HSP90 in a phenomenon, called genetic buffering [15,20,21]. In this process, HSP90 confers robustness to metabolic function and developmental processes by masking genetic polymorphisms or effects of adverse environment, effectively buffering the perturbation of these systems at the phenotype level. A series of compelling studies have demonstrated, that reduction in HSP90 activity either through mutation, RNA-interference or pharmacological inhibition led to the accumulation of variable line-specific phenotypic traits in accessions of *Arabidopsis* and *Drosophila* [20–23]. Under optimal growth conditions variable phenotypes occurred at relatively lower penetrance than upon application of mild stress. As under stressed conditions, the requirement for HSP90 increases and HSP90 may be sequestered to multiple compromised systems, this may expose pre-existing HSP90-buffered cryptic polymorphisms at the phenotypic level. If selected for, these traits may be

heritable to the next generation, providing a molecular explanation for the long-known process of apparent assimilation of environment-induced acquired characteristics in plants and animals [24–27]. It is therefore believed that HSP90 may constitute a motor for adaptive evolutionary processes in response to changed environmental conditions.

HSP90 isoforms in the model plant *Arabidopsis*

The model plant *Arabidopsis* encodes for seven HSP90 isoforms, of which four (HSP90.1–4) are cytoplasmic. HSP90.5 is localized to the chloroplast [28], HSP90.6 to the mitochondria [1] and HSP90.7, also called Shepherd (SHD) to the ER lumen [29]. In crop plants, the HSP90 family seems to be expanded: 9, 12, and 15 putative isoforms were identified in soybean, rice, and maize, respectively [30].

Of the cytoplasmic isoforms of *Arabidopsis*, HSP90 isoforms 2 through 4 are highly similar, with amino acid sequence identities between 96% and 98%, whereas HSP90.1 is about 88% identical to the other cytoplasmic isoforms [1]. All four genes locate to the same genomic region of chromosome 5, where the genes for the isoforms 2, 3, and 4 directly flank each other in a region only 1.5 kB downstream of the HSP90.1 open reading frame [1]. The presence of this

HSP90 gene cluster and their high degree of homology may suggest a common origin through recent gene duplication [1].

The high degree of sequence homology between cytoplasmic HSP90 isoforms in plants may suggest functional redundancy among HSP90 cytoplasmic isoforms explaining the low penetrance phenotypes that can be observed in the single loss-of-function mutants [21,31]. Indeed, a RNAi construct likely targeting a highly conserved region in all four isoforms was not transformable in *Arabidopsis*, leading authors to conclude that highly efficient HSP90 knock-down might yield in lethality [22]. Further support for this is provided by a study in which the generation of cytoplasmic HSP90 double mutants was attempted. While plants homozygous for the *hsp90.1* and heterozygous for the *hsp90.2* loss-of-function mutation could be identified, double mutants were not obtained by self-propagation [11]. These plants showed severe stunted phenotypes and segregated lethal progeny, indicating, that a threshold amount of functional cytoplasmic HSP90 is necessary for viability [11].

While functional differences of cytoplasmic and organellar HSP90 are described in plants and other organisms [28,29,32,33], much less is known about sub-functionalization of individual cytoplasmic isoforms. In metazoan systems, two groups of cytoplasmic HSP90, HSP90 α (inducible) and HSP90 β (constitutive), may have redundant as well as specific functions in different processes [34,35]. In *Arabidopsis* only isoform HSP90.1 is a *bona fide* heat shock inducible protein, whereas the other cytoplasmic isoforms are constitutively expressed at high levels [8,12]. *Arabidopsis* HSP90.1 and HSP90.3 have furthermore been shown to be differentially expressed during different developmental processes [36]. In contrast, detailed expression studies of the 21 *Nicotiana tabacum* HSP90 isoforms revealed, that HSP90 isoforms phylogenetically clustering with *Arabidopsis* HSP90.1 are highly stress inducible [37]. In other respects, most studies have not included all four cytoplasmic isoforms in their experimental setups limiting our current understanding on their functional divergence.

HSP90 co-chaperones in *Arabidopsis*

In analogy to animals and yeast, regulatory and/or client-specific co-chaperones may interact with the HSP90 co-chaperone machinery during client processing in plants (Box 1), which may influence HSP90 ATPase activity, direct the client protein selection or recruit additional co-factors to form multi-protein complexes. HSP90 co-chaperones, like HSP90 are

often multifunctional proteins, which besides their participation in the HSP90-co chaperone system may conceivably also have HSP90 independent activities.

The *Arabidopsis* genome encodes for a large complement of HSP40/DNAJ proteins and HSP70 [38,39]. HOP isoforms are represented by three genes [40]. p23 proteins are encoded by two genes in *Arabidopsis* [41], while AT3G12050 represents the *Arabidopsis* homolog to Activator of HSP90 ATPase (Aha1). Aha1 is a HSP90 co-chaperone that binds to the N-terminal HSP90 domain and inversely to p23 positively regulates HSP90 ATPase activity [4,42] but has so far not been characterized in plants.

TPR-containing immunophilins are represented by five genes: four members belong to the FK506-binding protein (FKBP) family, which are known to be involved in stress responses and hormone related processes, while the cyclophilin40 (CYP40) homolog, Squint (SQN), is involved in vegetative shoot development and Clavata signaling [43,44]. Squint interacts with HSP90 to mediate small RNA duplex loading of the Argonaute1 (AGO1) dependent RNA-induced silencing complex (RISC), which is essential for post-transcriptional gene silencing (PTGS) in microRNA regulated developmental pathways [45,46].

In *Arabidopsis*, a single copy gene encodes for the TPR-domain containing protein phosphatase 5 (PP5), which reportedly plays a role in heat stress resistance [47]. Yeast PP5 phosphatase activity is activated upon HSP90-binding, which influences client protein maturation by dephosphorylating HSP90 and cell division cycle 37 (CDC37), reviewed in ref. [4]. CDC37 is a HSP90 co-chaperone, which is specific to – and essential for the stabilization of HSP90-dependent kinases in yeast and animals [4]. Astonishingly, this important co-chaperone is not conserved in plants, indicating that kinase client proteins may be stabilized through a different mechanism.

Carboxyl terminus of Hsc70-interacting protein (CHIP; 1 gene in *Arabidopsis*) is a TPR-containing U-box ubiquitin ligase which acts as a co-chaperon in the HSP70/HSP90 pathway, thus acting in the quality control of protein folding and the targeting of client proteins for proteasomal degradation [48,49].

Several more TPR-domain containing proteins were predicted *in silico* to interact with HSP70/HSP90 based on the properties of their TPR-domain. Intriguingly, several homologs of known mammalian and yeast HSP90 co-chaperones were found in *Arabidopsis* [50]. TPR5 (AT1G56440) was reported to play an important role in cell division regulation [51] and is the *Arabidopsis* homolog of the yeast co-chaperone Tah1. AT5G03160 may correspond to human Trp2, whereas

Arabidopsis TPR2 (AT1G04130) may correspond to human TTC4 and yeast Cns1, all known co-chaperones of HSP90 [4].

Suppressor of G2 allele SKP1 (SGT1) is a further HSP90 co-chaperone harboring p23-like and TPR domains, which is required for innate immunity in plants and animals and was shown to be involved in hormone signaling in plants [14,16,52–55]. SGT1 is represented by two genes in *Arabidopsis* (SGT1a and SGT1b), which have redundant roles in development as indicated by the embryo lethality of the double mutant [56]. However, only SGT1b is required in innate immunity and plant hormone signaling [14,56]. Required for MLA12 resistance 1 (RAR1; 1 gene in *Arabidopsis*) is a Cysteine- and histidine-rich (CHORD)-domain containing protein which together with HSP90 and SGT1 participates in immune receptor complexes and is essential for disease resistance [53–55,57]. TOC64 is a plant specific co-chaperone, which was shown to interact with HSP90 in pea and may be involved in the HSP90-dependent delivery of chloroplast proteins to the TOC (Translocon of the outer membrane of chloroplasts)-complex [17,58].

Further co-chaperone families of the HSP90 machinery are known from other organisms, for which no orthologs are found in plants, reviewed in ref. [4]. It may, however, be conceivable, that roles of the HSP90 machinery pertaining to these co-chaperones may rely on the independent evolution of functional analogs in plants.

Roles of HSP90 and its co-chaperones in stress

Abiotic stress

While the role of the HSP40/HSP70 chaperone machinery in abiotic stress response is well documented [59], HSP90s may have a direct protective capacity to abiotic stress as well as a role in its signal transduction [60–63]. Surprisingly, however, over-expression of endogenous organellar as well as cytoplasmic HSP90 isoforms in *Arabidopsis* has been reported to increase susceptibility to salt, drought, and oxidative stresses [61,64], while expression of homologous *Glycine max* HSP90s in *Arabidopsis* resulted in partial protection against heat, salt, and oxidative stress [63]. Moreover, pharmacological inhibition of HSP90 by GDA or radicicol treatment (Box 1) triggers heat-inducible gene expression as well as a heat acclimation response [60]. Acute heat shock appears to temporarily reduce the cytoplasmic HSP90 activity, as indicated by the diminished activity of the mammalian

glucocorticoid receptor (GR), a well-known HSP90-dependent protein when exogenously expressed as a reporter in *Arabidopsis* [60]. A possible scenario which may accommodate all of the above observations, may be given by HSP90 executing an active suppressing role on stress responses by binding to and inhibiting the activity of constitutively expressed stress-related transcription factors such as heat shock factor A1d, HsfA1d, under control conditions. Under acute stress, HSP90 may be temporarily depleted as it is recruited to stress-labile proteins thereby releasing inhibition on stress response induction [9,60].

Furthermore, HSP90 and its associated TPR-containing FKBP co-chaperones, ROF1 and ROF2, have been shown to participate in the acquisition of long-term thermotolerance [65–67]. Strikingly, *rof1* mutants are deficient in acclimation to elevated temperature and are very susceptible to heat shock, whereas *rof2* mutants show higher steady-state levels of heat shock related protein expression and increased tolerance toward heat stress [65–67]. This dichotomy was proposed to involve antagonistic roles of ROF1 and ROF2 in an auto-inhibitory feedback loop regulating the heat stress response: under physiological conditions, ROF1 is bound to HSP90.1 in the cytoplasm, whereas upon heat shock the transcription factor heat shock factor 2A (Hsf2A) is induced and forms a cytoplasmic complex with ROF1 and HSP90. Subsequently, this ternary complex is shuttled to the nucleus where it induces the expression of heat acclimation genes including ROF2. After a lag period of recovery, ROF2 interacts with the HSP90-ROF1-Hsf2A complex abrogating its transcriptional activity [66]. Likewise, ROF1 has been identified as a component of osmotic stress tolerance [68] and ROF2 as a regulator of intracellular pH homeostasis [69]. The wheat homolog of ROF2, FKBP73, in conjunction with HSP90 and HOP has been found to interact with freshly synthesized *Arabidopsis* chloroplast pre-proteins in wheat germ lysate-based assays [18] implying a potential contribution to the HSP90-dependent aspects of chloroplast protein precursor stability regulation and delivery to the TOC-complex [17,58]. Three *Arabidopsis* HOP isoforms have been shown to interact with HSP90.1 and play a major role in long-term acquired thermotolerance and protein quality control during heat shock as evidenced by the over-accumulation of insoluble ubiquitinated proteins in the triple mutant [40]. A similar accumulation of ubiquitinated insoluble proteins was observed in mutants of CHIP during heat stress, while in conjunction with HSP70 CHIP has been previously implied in the degradation of misfolded chloroplast precursor proteins [70,71]. These chaperons may be

involved in the integration of temperature signals and responses to regulate plastidial protein delivery, which is known to be highly affected by changing temperatures [72].

Biotic stress

In plants, cytoplasmic HSP90s are known to play an important role in biotic stress responses. In conjunction with the co-chaperones, SGT1 and RAR1, and to a lesser extent PP5, the HSP90-machinery stabilizes resistance (R) proteins of the intracellular nucleotide-binding site (NLS) and LRR domain containing receptor (NLR) family, which are responsible for viral and bacterial pathogen effector recognition and the triggering of defense responses [10,11,31,53,54,73,74]. Furthermore, ROF2 was suggested to be involved in the defense against bacterial pathogen *Pseudomonas syringae* against by an as of yet unknown mechanism [75]. Research in rice provided evidence that the homolog of *Arabidopsis* chitin-elicitor receptor kinase 1 (CERK1), a subunit of the chitin co-receptor complex which is involved in pathogen-associated molecular patterns (PAMP)-triggered immunity, is dependent on HSP90 and the co-chaperone HOP for its delivery to the PM, which thereby participate in anti-fungal immunity [76]. Moreover, research in tobacco and *Arabidopsis* implied HSP90 and SGT1 in resistance against herbivorous insects [22,77].

Structural insights of immunity-related HSP90/co-chaperone-related complexes [55] and roles of HSP40/HSP70 proteins and HSP90 in pathogen defense [78] are excellently reviewed elsewhere.

Roles of HSP90 and its co-chaperones in hormone biology

Auxins

Auxins are a group of growth modulating plant hormones coordinating copious developmental and stimulus-responsive physiological processes in virtually all aspects and stages of the plant life cycle [79–81]. Importantly, auxins are polarly distributed across plant tissues and organs and share similar morphogen-like activities in plant development [82,83]. Auxin is perceived by a nuclear co-receptor complex consisting of a transport inhibitor response1/auxin signaling F-box (TIR1/AFB) family F-box protein partnered with an auxin/indole acetic acid (AUX/IAA) family transcriptional repressor, which are subunits of a SKP1-CUL1-F-box (SCF)-type ubiquitin-protein ligase E3 complex, termed SCF^{TIR1/AFB} [84–86].

The plant hormone auxin is known to be involved in the temperature-induced adaptation of plant growth and development. It has been shown, that the transcription factor phytochrome interacting factor 4 (PIF4) mediates temperature-dependent regulation of auxin biosynthetic genes thereby integrating temperature-sensing and the auxin developmental program [87,88]. Interestingly, HSF transcription factors as well as proteins transcriptionally regulated upon heat shock have so far not been implied in this regiment. Recent evidence has demonstrated a second branch of integration of temperature and auxin, which is dependent on auxin signaling by TIR1/AFB and HSP90, as well as the co-chaperone SGT1b [16]. While a defect in hypocotyl elongation in the *tir1-1* mutant at elevated temperatures (28 °C) has been noted early on [89], HSP90 and cognate co-chaperones were recently shown to stabilize TIR1 and AFB2 upon increasing temperature [16]. Pharmacological HSP90 inhibition furthermore resulted in their proteasomal degradation and interfered with the auxin signaling output [16]. HSP90-mediated auxin receptor stabilization may therefore be involved in the promotion of various temperature-dependent auxin related phenotypes such as increased root and hypocotyl elongation [16]. Together with the finding that HSP90 can buffer auxin responsive phenotypes in plants harboring a cryptic deleterious point mutation in the TIR1 gene, this is strong evidence to support an HSP90 client status of these auxin receptors [15]. A mutant allele of SGT1b, enhancer of *tir1* auxin resistance 3 (*eta3*), has previously been identified in a genetic screen as an enhancer of the *tir1-1* mutant, suggesting its involvement in auxin signaling [90], while knock down of both *SGT1a* or *SGT1b* by artificial microRNAs (amiRNAs) resulted in lower TIR1 accumulation in a protoplast system [14]. Co-IP experiments *in vivo* and *in vitro* revealed, that TIR1, SGT1, and HSP90 directly interact and form a complex which may also involve the co-chaperone RAR1. Interestingly, the mutant SGT1b protein encoded by the *eta3* mutation showed reduced binding to TIR1 in *in vitro* pull-down experiments, suggesting a direct role of SGT1b in the stabilization of TIR1 [16] (Fig. 2).

Interestingly, HSP90 inhibitors interfere with gravitropic root bending, a physiological process that relies primarily not on auxin signaling but on the polar transport of this hormone [16]. Polar auxin transport (PAT) is mediated by three major protein families: secondary active efflux carriers of the PIN-FORMED family, cellular auxin uptake permeases of the AUX1/LAX family and primary active exporters of the subfamily B of ATP-binding cassette (ABC) transporters (ABCBs) [79]. As of yet, no direct link between HSP90

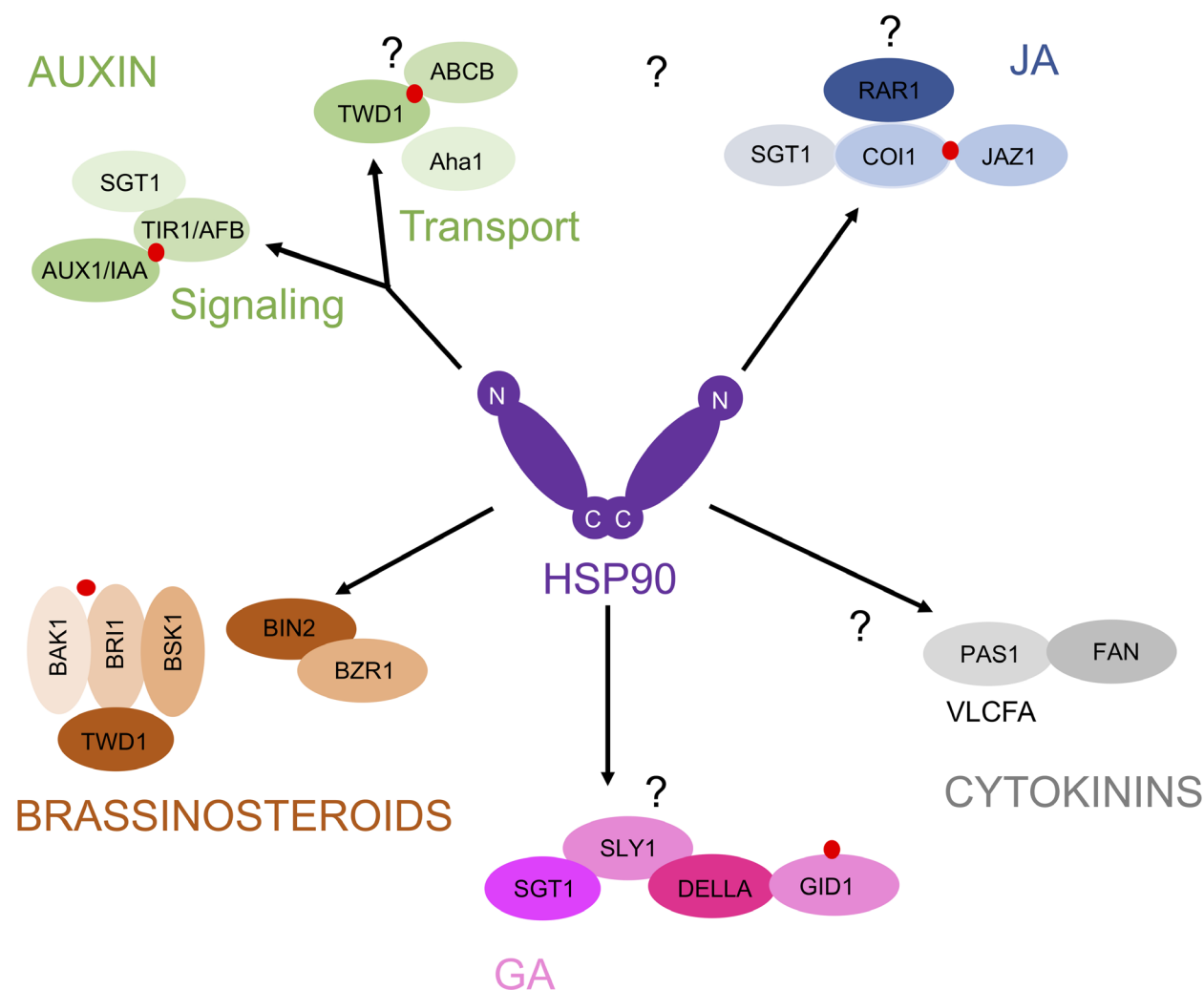


Fig. 2. Roles of HSP90 and its co-chaperones in hormone biology. Overview on the described HSP90 clients (arrow connection with HSP90) and their direct involvement in the hormone (red dots, respectively) signaling pathways of auxin (IAA), jasmonic acid (JA), cytokinins, GA and brassinosteroids. The HSP90-SGT1 co-chaperone module is found in SCF-type E3-ubiquitin ligase hormone receptors for auxin, JA and possibly GA. Note that HSP90 beside an effect on auxin signaling might also control ABCB-mediated auxin transport *via* interaction with the FKBP42, TWD1, although the impact of auxin on TWD1-ABCB interaction is currently not understood [91]. Also, that TWD1 is a component of auxin transport and brassinosteroid signaling pathways; question marks indicate likely interactions that await a final proof.

and PAT has been drawn, however, several co-chaperones of HSP90 have been found to be involved in the regulation of PAT.

Mutants of the two isoforms of the *Arabidopsis* homolog to mammalian p23 exhibit altered auxin distribution patterns in the root tip and delocalization as well as transcriptional repression of PIN proteins. This may explain a short root phenotype, shorter meristem length and reduced cell number in the division zone and implies p23 isoforms as regulators of PAT [41]. Moreover, the *Arabidopsis* TPR-immunophilin FKBP42, twisted dwarf 1 (TWD1), was shown to interact with HSP90 both *in vitro* and *in vivo* [92,93].

The *twd1* mutant shows severe defects in auxin transport (down to 14% of the wild type, depending on the method [93–95]). TWD1 is a membrane-associated protein localizing to the ER, PM, and vacuolar membranes in most tissues of *Arabidopsis* [93,95,96]. TWD1 was suggested to control the maturation of auxin transporting ABCB-transporters at the ER surface and their ER-to-PM progression [93–96]. In particular, the auxin transporting ABCB isoforms ABCB1, ABCB19, and ABCB4 are direct interaction partners of TWD1 and depend on TWD1-mediated chaperone activity for their efficient function [93–96] (Fig. 2). Whether p23 and TWD1 may be involved in

the same module of PAT regulation is unknown. However, PIN proteins, while affected in the p23 mutants, do not show altered localization in the *twl1* mutant TWD1 [94]. Intriguingly, the plant HSP90 co-chaperone orthologue of human Aha1 (At3g12050), which has not been characterized in plants but is known to regulate HSP90 ATPase activity in an opposite manner to p23 [97], was found in co-IP eluates of TWD1 [92].

While the precise mechanism of ABCB regulation by TWD1 is still not resolved, it was speculated to have some degree of analogy to the regulation of human ABCC7, also known as cystic fibrosis transmembrane conductance regulator (CFTR), an ATP-gated chlorine ion channel, mutation of which results in the protein-folding related disease cystic fibrosis [98]. Intriguingly, CFTR is a HSP90 client [32,99] and its regulation involves Aha1 [100] and the multifunctional immunophilin FKBP38, which has a homologous domain structure to TWD1 [101,102].

Brassinosteroids

Brassinosteroids (BR) are steroidal plant hormones regulating various developmental processes and both biotic and abiotic stress responses (reviewed in ref. [103]). BR and auxin signaling outputs are known to have an extensive transcriptional overlap and to be responsive to external stimuli in dependence of PIF transcription factors [104]. The BR co-receptor complex consists of the leucine-rich repeat receptor-like kinase, brassinosteroid insensitive1 (BRI1) and the leucine-rich repeat co-receptor kinase, BRI1-associated receptor kinase1 (BAK1) [103]. The latter, as well as the related somatic embryogenesis receptor kinases (SERKs), are also well known as shared co-receptor kinases of leucine-rich repeat receptor-like kinases in PAMP and damage-associated molecular pattern (DAMP) signaling [105]. BR signaling transduction involves the downstream inactivation of the GSK3-like kinase, brassinosteroid insensitive2 (BIN2), which releases the transcription factors, brassinazole resistant1 (BZR1) and its functionally related paralog, BRI1-EMS suppressor 1 (BES1), from inhibitory phosphorylation allowing for the induction of BR inducible genes, reviewed in ref. [103].

HSP90 has been implied in the BR signaling pathway based on direct interaction with BIN2, BZR1, BES1 as well as due to the differential expression of BR-responsive genes upon pharmacological HSP90 inhibition [106,13] (Fig. 2). More specifically, HSP90.3 was shown to be necessary for maintenance of the dephosphorylated state of BES1 [106], while HSP90.1 and HSP90.3 may act in nucleocytoplasmic export of

BIN2, thereby depleting BIN2 in the nucleus upon BR signaling, which is likely to alleviate BIN2 inhibitory activity on BZR1 and BES1 [13].

An apparent role of TWD1 in the response to BR was noticed shortly after its involvement in auxin biology was proposed [107]. The *twl1* mutant failed to differentially express BR-responsive genes upon exogenous application of 24-epibrassinolide and root growth showed a much-decreased sensitivity toward BR even at very high concentrations [107]. Furthermore, introgression of *twl1* into BR biosynthesis mutants produced phenotypes that were overall reminiscent of *BRI1* loss-of-function [107]. Recent efforts have shed light on the mechanistic relationship between TWD1 and BR, demonstrating a role of TWD1 in BR signaling. Two independent studies reported that TWD1 interacts directly with the kinase domain of BRI1 and BAK1 in a BR-independent manner [108,109]. However, TWD1 appears not to regulate BR co-receptor maturation at the ER, as suggested by their unchanged PM localization and abundance in *twl1* mutants [108,109]. Instead, TWD1 was found to be required for efficient auto-phosphorylation of both co-receptors upon BR perception and therefore their signal transduction activity [109] (Fig. 2). Moreover, BZR1 was found to accumulate in its inactive hyperphosphorylated form in the *twl1* mutant [108]. It was therefore proposed that TWD1 might act as a positive regulator of BR-signal transduction upstream of BIN2 and that TWD1 may exert a scaffolding function for the cytoplasmic portion of the BR co-receptor complex at the PM, maintaining its signaling competent conformational state.

Interestingly, TWD1 is a component of both auxin transport and brassinosteroid signaling pathways in that TWD1 integrates several functional networks in the cell by protein-protein interaction [110], of which one might be shared with HSP90 and/or p23/Aha1. How these findings relate to the observation that the *abcb1/abcb19* double mutant largely phenocopies the *twl1* mutant [93] and whether this includes BR-related phenotypes is currently unresolved. Furthermore, it is important to note that the MAMP-signaling branch of BAK1 in conjunction with the bacterial flagellin receptor flagellin insensitive 2 (FLS2) is not dependent on TWD1 [108]. Interestingly, the *twl1* mutant reacted to treatment with the minimal flagellin peptide epitope flg22 rather more strongly than wild type [108]. This may be in line with the known antagonism between BR and flg22 signaling or related to a negative role of TWD1 chaperone activity to regulate BAK1 availability for immune complex formation [103,108]. Interestingly, SGT1b was found to be dispensable for BR

signaling and the interference with flg22 induced gene regulation, while auxin, gibberellic acid (GA), and jasmonate treatment blocked flg22 signaling in a SGT1b-dependent manner [14]. Whether hormone effects on flg22 signaling in the *twi1* mutant are compromised is not known.

Cytokinin

Pasticcino1 (PAS1) is a TPR-containing FKBP-type immunophilin with analogous domain organization to the *Arabidopsis* ROF proteins [111]. To date, the interaction of HSP90 with PAS1 has not been shown, but considered likely [111]. The *pas1* mutant was isolated in a screen for cytokinin hyper-responsive mutants and shows a dwarf phenotype, ectopic cell divisions and formation of callus-like growths upon exogenous cytokinin application, implying a role of PAS1 in the differential regulation of cell division and differentiation by suppression of cytokinin signals [112–114]. While some primary cytokinin response markers are enhanced in *pas1*, auxin-induced transcription of two Aux/IAA genes is dramatically abated [112].

Originally thought to be a nuclear protein, the C-terminal domain of PAS1 was more recently shown to mediate its native subcellular localization to cytoplasmic structures, where it may be ER-associated [115,116]. PAS1 has been reported to act as a scaffold of ER-resident very-long fatty acid (VLCFA) biosynthesis complexes and to mediate the nuclear shuttling of FKBP-associated NAC (FAN) transcription factors in dividing cells, as well as upon auxin signaling [116,117] (Fig. 2). Interestingly, it was shown, that both PIN1 and AUX1 were mildly affected in PM targeting by *PAS1* loss-of-function, while the *pas1* mutant additionally shows substantial changes in sphingolipid and sterol composition due to the destabilization of VLCFA biosynthesis [117]; VLCFAs are a known factor in PIN trafficking [118] and ABCB auxin transporter stabilization [119].

Jasmonic acid

Analogously to auxin perception by the SCF^{TIR1/AFB} complex, the jasmonate (JA) receptor is a SCF E3 ubiquitin ligase complex incorporating the F-box protein coronatine insensitive 1 (COI1) and jasmonate-ZIM domain proteins (JAZs). These are transcriptional repressors, which are targeted for proteasomal degradation upon JA signaling, thus activating the JA-inducible transcriptional response (reviewed in ref. [120]. JA is involved in multiple aspects of the activation of the plants defense response towards fungal and

herbivorous attack, whereas in contrast, it suppresses MAMP-triggered immunity [120]. Furthermore, JA is a major factor in the systemic wounding response [121] and has key roles in plant growth and development [120]. A SGT1b defective allele was isolated in a screen for mutants insensitive to JA-mediated suppression of flg22-elicited gene expression [14] and has been suggested to be involved in JA-signaling previously [122]. COI1 levels were dramatically reduced by *sgt1b* knock-out or by *SGT1a/SGT1b* knock-down [14]. Physical interaction of COI1 with SGT1b and HSP90 in planta subsequently suggested that COI1 is a HSP90 client protein [14] (Fig. 2). The COI1 chaperone complex was furthermore shown to also involve HSP70 and RAR1 [14]. In RNAi lines targeting cytoplasmic HSP90, JA-responsive genes were differentially regulated upon treatment with the JA analog coronatine, which could be reproduced in seedlings treated with GDA or the HSP70 inhibitor, Ver155008, demonstrating an involvement of SGT1b, HSP70, and HSP90 in the response to JA [14].

Evidence for other hormones

Circumstantial evidence suggests that the involvement of HSP90 and associated co-chaperones in hormone signaling may be broad. The same study reporting the client status of COI1 and stabilization of TIR1 by HSP90 and SGT1b also observed that suppression of flg22-elicited gene expression upon flg22 treatment was absent in the presence of GA in the *sgt1* mutant [14]. GA, similarly to auxin and JA, is perceived by a SCF E3 ubiquitin ligase complex incorporating the F-box protein, sleepy1 (SLY1), and involving the DELLA transcriptional repressors [123]. Based on this finding, it was suggested [14] that the involvement of HSP90 and associated co-chaperones may extend to a broader range of SCF complexes involved in hormone signaling, including GA. HSP90 has furthermore been shown to be involved in the stabilization of the F-box protein, zeitlupe (ZTL), which is part of a SCF-complex involved in circadian clock regulation [19]. The plant hormone, strigolactone, is perceived by a SCF complex incorporating the F-box protein more axillary growth 2 (MAX2) [124,125]. As MAX2 is phylogenetically closer to TIR1 and COI1 than to ZTL [126], it would be conceivable that the HSP90/SGT1b client status may be conserved. MAX2 is furthermore an interaction partner of the transcription factors, BES1 and BZR1, which are HSP90 clients in the BR-signaling pathway and are degraded in presence of strigolactone in a MAX2-dependent fashion [127].

HSP90 has furthermore been proposed to have a role in the signal transduction of abscisic acid (ABA) based on evidence that genetic and pharmacological interference with HSP90 resulted in perturbation of ABA-induced stomatal closure [128]. Additionally, ABA-related transcriptional effects were noted in *hsp90* mutants as well as in *HSP90* RNAi lines [22]. At moment, it is unclear whether these are indirect effects or may represent an involvement of HSP90 via a client protein in ABA-signal transduction. In the latter case, a client protein would likely be downstream of ABA perception as radical treatment did not compromise the ABA-mediated activation of components of the early ABA signal transduction pathway [22].

The *Arabidopsis* TPR-domain protein TPR1 was predicted to have the potential to interact with HSP90 *in silico* and shown to do so *in vitro* [50]. AtTPR1 and its tomato homolog, SITPR1, have been shown to interact with the ethylene receptors, ERS1 and NR, ETR1, respectively [129,130]. Both in tomato and *Arabidopsis*, transgenic over-expressor lines of *TPR1* are stunted and less fertile. Furthermore, they reveal an enhanced sensitivity to ethylene and display a constitutive ethylene response in etiolated seedlings, which was irresponsive to ethylene biosynthesis inhibitors [129,130]. It was furthermore suggested that TPR1 may act independently or downstream of ethylene perception as overexpression of *TPR1* did not affect ethylene insensitivity of the ETR1 mutant allele, *etr1-1* [130].

Conclusions and perspectives

In this review, we have summarized our current understanding of the involvement of HSP90 and some of its co-chaperones in multiple plant signaling and regulation networks. Several HSP90 clients have been found in plants and similarly, multiple plant orthologues of mammalian and yeast HSP90 co-chaperones have been identified as regulators of various aspects in plant physiology, but a synthetic understanding of the HSP90 system in plants is so far lacking.

Our analysis uncovered that in plants HSP90 are indeed multitaskers and involved in a broad spectrum of physiological processes (Fig. 1). Obviously, many (if not all) of these signaling pathways are interconnected. The pleiotropic nature of the HSP90 chaperone system may suggest a role for HSP90 and its cognate co-chaperones in integrating adaptive responses, thereby enabling the plant to express a balanced reaction to environmental change. New and exciting future research avenues may emerge from the identification of novel client proteins and addressing links in the

network of their biological activity given by a shared chaperone system. Importantly, insights into the diverse functions of HSP90 and its co-chaperones and how the apparent multitasking functions of these proteins may relate to an integration of adaptive responses may arise from the detailed study of the HSP90 interactome under different (stress) conditions, upon hormone treatments and/or in the presence/absence of HSP90 co-chaperones. Due to the high degree of conservation of the HSP90 co-chaperone system across all domains of life, exciting avenues of research may also arise from the careful study of such approaches, which have already been carried out in non-plant systems [131,132].

The proposed integrative nature of HSP90s may also be underlined by a deeper investigation of the putative involvements of HSP90s and their associated co-chaperones in hormone signaling. HSP90 and/or its co-chaperones have so far been linked (with the exception of salicylic acid) to all major classes of plant hormones, notwithstanding that in some cases evidence is still circumstantial and in need of a deeper investigation (Fig. 2). Importantly, our analysis reinforces the notion that in these complexes the F-box protein may be the primary target of the HSP90 co-chaperone machinery. This is underlined by the fact that SGT1b binding to COI1 is independent of *Arabidopsis* S-phase kinase-associated protein 1 (ASK1), a core component of the SCF E3 ligase complex, that is shared in different SCF hormone receptor complexes [14]. The finding that HSP90 and associated co-chaperones are involved in the stabilization of receptor complexes involved in hormone signaling, may open the possibility, that HSP90 may act as a universal stabilizer of SCF-type E3-ubiquitin ligase hormone receptor complexes. Remarkably, the SGT1(-RAR1) co-chaperone module has so far always been found in association with SCF-type hormone receptor complexes as well as participating in the stabilization of immune receptors, while apparently RAR1 is crucial for disease resistance but dispensable for the response to auxin [14,16,55].

Furthermore, the dichotomy of FKBP42/TWD1 and SGT1b in terms of their apparent involvement in multiple pathways (Fig. 2) may be a worthwhile example to study the impact of co-chaperones on the sub-functionalization of HSP90 co-chaperone complexes. The puzzling spectrum of instances in which the HSP90 co-chaperone system appears to play a crucial regulatory role make it challenging to disentangle described effects and to assign specific phenotypes and physiological reactions unambiguously and directly to a singular cause.

The above further suggests that the HSP90 system may have contributed to plant adaptation and developmental diversity by supporting a functional radiation of protein families *via* the stabilization of spontaneous mutations in client proteins found in hormone signaling modules, representing the central units for growth and development regulation [1]. In fact, some putative HSP90 client protein families (i.e., F-box, R-proteins, ABC transporters) are expanded in plants [2]. It may therefore be hypothesized, that HSP90 may have contributed to protein neo-functionalization, which may have been a driving force for the adaptation to the challenges of a sessile life style of plants. An investigation of this new role for HSP90s has highest priority and could be achieved by comparing genomic annotations of HSP90 client proteins (and co-chaperones) and their interactomes in different species and the protein family-wide analysis of the HSP90 client status.

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