HSP90 regulates temperature-dependent seedling growth in *Arabidopsis* by stabilizing the auxin co-receptor F-box protein TIR1

Renhou Wang1, Yi Zhang1, Martin Kieffer2, Hong Yu1, Stefan Kepinski2 & Mark Estelle1

Recent studies have revealed that a mild increase in environmental temperature stimulates the growth of *Arabidopsis* seedlings by promoting biosynthesis of the plant hormone auxin. However, little is known about the role of other factors in this process. In this report, we show that increased temperature promotes rapid accumulation of the TIR1 auxin co-receptor, an effect that is dependent on the molecular chaperone HSP90. In addition, we show that HSP90 and the co-chaperone SGT1 each interact with TIR1, confirming that TIR1 is an HSP90 client. Inhibition of HSP90 activity results in degradation of TIR1 and interestingly, defects in a range of auxin-mediated growth processes at lower as well as higher temperatures. Our results indicate that HSP90 and SGT1 integrate temperature and auxin signalling in order to regulate plant growth in a changing environment.
Temperature is a key environmental factor with diverse effects on plant growth and development. Extremely high temperature can severely impact growth, and plants have evolved complex mechanisms to adapt to heat stress. Extensive studies have revealed that various heat stress response factors (HSFs) and heat-shock proteins (HSPs) play central roles in heat sensing and signalling. Apart from the classical heat-shock response, a mild change in ambient temperature (between 16 and 29°C) can dramatically alter plant growth and development, and significantly affect crop yield. So far, HSFs and HSPs have not been shown to play a role in these responses. In contrast, recent studies indicate that the transcription factor phytochrome-interacting factor 4 (PIF4) and the versatile plant hormone auxin play pivotal roles in ambient temperature-regulated hypocotyl growth in *Arabidopsis*. At higher temperature, PIF4 acts to promote expression of several auxin biosynthetic genes resulting in increased auxin levels and hypocotyl growth. Ambient temperature signalling also appears to involve changes in chromatin structure mediated by histone H2A.Z. However, the precise mechanism of thermosensing remains unclear. Auxin is perceived by the TRANSPORT INHIBITOR RESPONSE1/AUXIN RESPONSE F-box (TIR1/AFB) auxin co-receptors. These proteins are subunits of ubiquitin protein ligases called SCF-TIR1/AFB together with CULLIN1, *Arabidopsis* SKP1-related and RING BOX1. Auxin acts by directly binding to the TIR1/AFB proteins, and promoting the degradation of transcriptional repressors called Aux/IAA proteins. The regulation of Aux/IAA degradation has been extensively studied. In contrast, there is little information on the regulation of TIR1/AFB stability. We have recently shown that TIR1 is an unstable protein in seedlings raising the possibility that changes in TIR1 stability may influence auxin signalling. For example, we show that stability of the TIR1/AFB proteins is affected by their ability to assemble into an SCF complex.

Assembly and regulation of SCF complexes such as SCF-TIR1 is a highly dynamic process that involves a number of proteins and protein complexes including the ubiquitin-like protein Nedd8 (RELATED TO UBQUITIN in *Arabidopsis*), the COP9 signalosome (CSN) complex, and the cullin-associated Nedd8-disassociated (CAND1) protein. These proteins act to regulate activity of the SCF and enable the exchange of substrate adapters as required during changing cellular conditions. As the *Arabidopsis* genome encodes hundreds of FBPs, the challenge of identifying the possibility that changes in TIR1 stability may influence auxin signalling is particularly acute. Indeed, the RUB1, CSN and CAND1 have all been implicated in SCF-TIR1/AFB function.

In addition to these factors, genetic studies show that a protein called SUPPRESSOR OF G2 ALLELE SKP1 (SGT1) is required for auxin response. There are two SGT1 genes in *Arabidopsis* called SGT1a and SGT1b. A mutant allele of SGT1b, called enhancer of tir1 auxin resistance 3 (eta3), was isolated as an enhancer of the tir1-1 mutant, implicating SGT1 in auxin response. SGT1 is a co-chaperone of HEAT SHOCK FACTOR 90 (HSP90) and has been implicated in a variety of processes in eukaryotes. The protein was first identified in budding yeast and is required for assembly of kinetochores and activity of the E3 ligase SCF-Cdc4p. SGT1 and HSP90 play critical roles in regulating abiotic and biotic stress responses. They are required for normal function of nucleotide-binding domain leucine-rich repeat (NLR) immune sensor proteins in both plants and animals. However, their role in ambient temperature sensing and signalling has not been investigated.

In this study, we show that the HSP90-SGT1 chaperone system is required for the plants’ response to an increase in ambient temperature. TIR1 is rapidly stabilized at 29°C, a change that is HSP90 dependent and associated with increased seedling growth. Further, we show that both HSP90 and SGT1 are in a complex with TIR1 in plants. In addition, the mutant SGT1b protein encoded by the *eta3* gene displays reduced binding to TIR1, suggesting that auxin resistance in *eta3* is directly related to TIR1 levels or function. Our data suggest that the HSP90-TIR1 module integrates environmental temperature and auxin signalling to regulate plant development and reveals a link between the molecular networks regulating plant growth response to ambient temperature and the HSPs-HSFs module that plays a central role in heat sensing and signalling.

**Results**

Temperature-dependent growth requires HSP90. HSP90 has been implicated in a wide range of signalling pathways in both plants and animals. In animals, HSP90 has been shown to play a fundamental role in heat-shock responses and numerous processes in non-stress conditions. To determine if HSP90 has an important role in high ambient temperature promoted plant growth, we grew *Arabidopsis* seedlings at 22 and 29°C and treated them with the HSP90 inhibitor geldanamycin (GDA). GDA is a highly specific inhibitor of HSP90 activity without known off-target activity. In this experiment, 5-day-old seedlings were transferred from 22 to 29°C and hypocotyl length was measured after an additional 4 days in the absence and presence of GDA. As expected, hypocotyl length was stimulated by growth at the higher temperature (Fig. 1a). However, in the presence of GDA this effect was eliminated.

In our previous work, we reported that increased ambient temperature also altered lateral root formation, although we did not provide details. We therefore examined the roots of seedlings shifted to 29°C as described above. The results in Fig. 1b show that this treatment enhanced lateral root formation. Surprisingly, we found that elevated temperature also significantly promoted primary root growth (Fig. 1c). To confirm that these responses are related to auxin, we used the *tir1-1 afb2-3* double mutant, known be auxin resistant and display reduced auxin-induced lateral root formation. We found that the double mutant is also affected with respect to temperature-induced growth responses indicating that the auxin receptors are required for these responses (Supplementary Fig. 1a,b). As for the hypocotyl response, we found that GDA inhibited temperature-induced changes in root growth (Fig. 1b,c). These results indicate that HSP90 is required for the effects of elevated temperature on both root and hypocotyl growth. We also tested the effects of two other structurally unrelated HSP90 inhibitors on the temperature response. Radicicol inhibits HSP90 by binding to the ATP-binding site, whereas (-)-epigallocatechin gallate binds to the C-terminus and inhibits dimerization of the protein. Both inhibitors affected seedling growth in the same way as GDA (Supplementary Fig. 2a–f).

According to the model established from studies of hypocotyl growth, elevated temperature stimulates accumulation of auxin, which subsequently activates auxin responsive genes. To determine if HSP90 is required for the effect of temperature on auxin-responsive genes, we tested the effect of GDA on expression of the auxin reporter *DR5:GUS* at 22 and 29°C. GUS levels were strongly elevated at 29°C in both the root and shoot but as for the growth responses, this effect was strongly inhibited by GDA (Fig. 1d). We also examined three auxin responsive genes, *GH3.17*, *IAA19* and *IAA5*, and found that in each case, GDA prevented the characteristic positive effect of 29°C on gene expression (Supplementary Fig. 3). Thus, our data suggest that HSP90 plays an important role in high temperature-regulated...
plant growth by affecting auxin-related processes. However, GDA did not affect the expression of YUCCA8, an auxin biosynthetic gene that is activated by high temperature (Supplementary Fig. 3)\(^3\). This result implies that HSP90 does not regulate the temperature growth response by modulating auxin synthesis, but rather through other processes such as auxin signalling.

The chaperone HSP90 is required for auxin response. The results described above suggest that HSP90 affects temperature-promoted growth by regulating auxin response. This is consistent with earlier studies, which showed that the HSP90 co-chaperone SGT1 is required for auxin response in Arabidopsis\(^3\). To determine if HSP90 is required for auxin signalling apart from the temperature response, we examined the effect of GDA on a variety of auxin growth responses. First, we examined the effect of GDA on auxin-induced hypocotyl elongation. Treatment of light-grown seedlings with 2 \(\mu\)M IAA had a pronounced effect on hypocotyl elongation as previously reported (Supplementary Fig. 3a)\(^3\). However, this effect was completely eliminated in the presence of 5 \(\mu\)M GDA. Similarly, the effect of IAA on lateral root formation and primary root growth was strongly suppressed by GDA (Supplementary Fig. 3b–d). Finally, root gravitropism, an auxin-mediated process, was inhibited by GDA (Supplementary Fig. 3a). In addition, we found that radicicol also inhibited IAA-induced hypocotyl elongation and lateral root formation (Supplementary Fig. 4a–c). These results indicate that HSP90 is broadly required for auxin-dependent growth responses.

Auxin is known to act by regulating gene expression\(^4\). To determine if HSP90 is required for auxin-regulated transcription, we examined the effect of GDA on the auxin reporter DR5:GUS. As expected treatment of DR5:GUS seedlings with auxin resulted in a strong increase in GUS levels (Fig. 2e). Treatment with GDA substantially inhibited this response. We also examined the response of four auxin-regulated genes and obtained similar results. In the presence of GDA, the effect of IAA on gene expression was strongly inhibited. In the case of IAA14, the inhibition was almost complete (Supplementary Fig. 4d).

HSP90 and high temperature stabilize the auxin co-receptors. Experiments in human cells have shown that HSP90 interacts with a large number of E3 ligases\(^3\) and in plants, the F-box protein ZEITLUPE (ZTL) is a client of HSP90 (ref. 32). Loss of HSP90 activity resulted in decreased stability of ZTL and a defect in circadian rhythm. As auxin is perceived by the TIR1/AFB auxin co-receptors, we wondered if these proteins might also be HSP90 clients. To test this possibility, we utilized pTIR1:gTIR1-VENUS and pAFB2:gAFB2-VENUS reporter lines. These lines were generated by amplifying the respective gene sequences, including introns and 4 kb of 5’ sequence, and placing YFP at the C-terminus of the gene. The constructs were introduced into tir1-1 and afd2-3 plants, respectively. Both tir1-1 and afd2-2 display modest resistance to IAA\(^3\) (Supplementary Fig. 5). Auxin response was restored by the transgenes (Supplementary Fig. 5). Confocal imaging of the root tips of pTIR1:gTIR1-VENUS and pAFB2:gAFB2-VENUS plants revealed that both proteins accumulate in most cell types in this region of the root and are primarily localized to the nucleus (Supplementary Fig. 5). When seedlings are treated with GDA for 24 h, there is a dramatic loss of TIR1-VENUS and AFB2-VENUS in all cell types even though this treatment does not result in a change in TIR1 or AFB2 transcript level (Supplementary Fig. 6). A similar effect was observed using a pTIR1:cTIR1-GUS line (Supplementary Fig. 6). Because HSP90 has many client proteins, it is possible that GUS or VENUS is destabilized by GDA. To exclude this possibility, we treated the pTIR1:GUS and pUBQ10:CFP-VENUS lines with GDA. The results shown in Supplementary Fig. 7a,b indicate that levels of the two reporter proteins are not affected by GDA.
We next asked if TIR1 degradation upon GDA treatment required the proteasome. *pTIR1:gTIR1-VENUS* seedlings were treated with GDA ± the proteasome inhibitor bortezomib for 4 h before imaging. The results in Fig. 3j show that TIR1-VENUS levels were restored by bortezomib indicating that inhibition of HSP90 results in degradation of TIR1 by the proteasome.

Our data suggest that HSP90 regulates the temperature growth response by enhancing auxin signalling rather than auxin biosynthesis (Fig. 1 and Supplementary Fig. 4). One possible mechanism is that high temperature promotes the accumulation of the TIR1/AFBs and that HSP90 is required for this process. To test this hypothesis, we first examined TIR1, AFB2 and AFB3 RNA levels in temperature-shifted seedlings by quantitative reverse transcription–PCR (qRT–PCR). The levels of these three transcripts were largely unchanged by the treatment (Supplementary Fig. 6b). We next examined TIR1–VENUS in the *pTIR1:gTIR1-VENUS* line after temperature shift. In this case, we observed a substantial increase in TIR1-VENUS levels in seedlings shifted to 29°C (Fig. 4a). In the presence of GDA, this response was completely inhibited indicating that it requires HSP90. Similar results were obtained using the radiciol and ECGC (Supplementary Fig. 6a). As for seedlings at 22°C, bortezomib restored TIR1-VENUS levels in the presence of GDA indicating that TIR1 is degraded by the proteasome at 29°C as well (Fig. 3j). TIR1-VENUS levels were also strongly induced by 29°C in the hypocotyl consistent with the growth response observed in this tissue at higher temperature (Fig. 4b). A similar result was observed in the TIR1:cTIR1-GUS plants, further demonstrating that the effect of high temperature and GDA is not on the green fluorescent protein (GFP) protein itself (Supplementary Fig. 6c).

We next performed a time-course experiment to determine how quickly TIR1 levels changed. Remarkably, we found that TIR1 levels increase within 1 h and substantially increase within 2 h (Fig. 4c). Treatment with GDA strongly inhibited this response.

As elevated temperature does not affect transcription of the auxin co-receptor genes, it is possible that changes in stability are responsible for elevated TIR1 levels. To investigate this, we performed a cycloheximide experiment. The results in Fig. 4e,f show that TIR1–VENUS is substantially more stable at 29°C than at 22°C. The levels of TIR1 are essentially unchanged over 4 h at 29°C, whereas they drop rapidly at 22°C. In addition, GDA treatment reduced stability of TIR1–VENUS at 29°C, indicating the increase in stability at elevated temperature is HSP90 dependent.

One possible explanation for the increased TIR1–VENUS stability at higher temperature is an increase in HSP90 levels. We next asked if TIR1 degradation upon GDA treatment required the proteasome. *pTIR1:gTIR1-VENUS* seedlings were treated with GDA ± the proteasome inhibitor bortezomib for 4 h before imaging. The results in Fig. 3j show that TIR1-VENUS levels were restored by bortezomib indicating that inhibition of HSP90 results in degradation of TIR1 by the proteasome.

As HSP90 is required for lateral root development, we also imaged lateral root primordia in the reporter lines. We have previously shown that TIR1 and AFB2 are required for lateral root formation. As expected, the TIR1–VENUS and AFB2–VENUS proteins were observed in cells of stage II primordia (Fig. 3c,g). In contrast, very little VENUS signal is observed after GDA treatment (Fig. 3d,h). These results indicate that HSP90 is required for stability of TIR1 and AFB2 and further suggest that the effects of reduced HSP90 activity on auxin response are due to reduced levels of these auxin co-receptors.

TIR1 co-immunoprecipitates HSP90 and SGT1. As HSP90 is required for TIR1 stability, it is possible that HSP90 interacts, directly or indirectly, with the auxin co-receptor. To test this possibility, we performed an immunoprecipitation experiment using *pDEX:TIR1-Myc* plants after dexamethasone treatment and tested for co-IP of HSP90. The *pDEX:TIR1-Myc* line was described previously. The results in Fig. 5a show that HSP90 co-IPs with TIR1-Myc, indicating that HSP90 is present in a complex that includes TIR1. A similar experiment was done with the *pTIR1:gTIR1-VENUS* line and again HSP90 was recovered together with TIR1–VENUS (Fig. 5b).
The HSP90 co-chaperone SGT1 has been implicated in auxin signalling. An Arabidopsis mutation in the SGT1b gene, called eta3, confers auxin resistance and reduced lateral root formation, defects that are similar to those observed in GDA-treated seedlings. To determine if SGT1 also interacts with TIR1, we tested for co-IP with TIR1-Myc and TIR1-VENUS using an SGT1 antibody generated with a SGT1 peptide. The antibody recognizes both SGT1a and SGT1b in plant extracts (Supplementary Fig. 4).

As seen in Fig. 5a, b, SGT1b is recovered with both TIR1-Myc and TIR1-VENUS.

To determine if HSP90 and/or SGT1b interact directly with TIR1, we performed a series of pull-down experiments. Glutathione S-transferase (GST)-tagged SGT1b and HSP90 synthesized in Escherichia coli were incubated with TIR1-myc synthesized in a TNT (Promega TNT Quick Coupled Transcription/Translation system) extract. The results in Fig. 5c, d show that TIR1-Myc is recovered in both the GST-SGT1b and GST-HSP90 pull-down indicating that both proteins interact directly with TIR1 in this assay. In previous studies, a region near the C-terminus of SGT1 called the SGT1-Specific (SGS) domain was shown to interact with the LRR domain of NLR proteins. There is also evidence from other systems that this domain interacts with LRRs. In addition, the eta3 mutation results in a truncated protein lacking the C-terminal 36 amino acids of the SGS domain.

To determine if the SGS domain of SGT1b is required for interaction with TIR1, we repeated the pull-down experiment with mutant forms of SGT1b lacking the entire SGS domain or the last 36 amino acids as in eta3. We found that both mutant forms are deficient in TIR1-myc binding, indicating that the SGS domain is responsible for the interaction with TIR1.

To further explore the interaction between these proteins, we used the bimolecular fluorescence complementation (BiFC) system to test for interactions between TIR1, SGT1b and HSP90 in plant cells. cYFP and nYFP protein fusions were agro-infiltrated into tobacco leaf epidermal cells. Figure 5e is the negative control. The results clearly show that SGT1b interacted with HSP90 as expected (Fig. 5i, j) and that the interaction occurred in both the cytoplasm and the nucleus. In addition, we found that TIR1 interacted with SGT1b in the nucleus (Fig. 5g, h). However, we did not detect an interaction between TIR1 and HSP90 with this approach (Fig. 5f).
results are consistent with a recent report showing that TIR1 interacts with SGT1b, HSP90 and HSP70 in Arabidopsis protoplasts.

As there are 5 HSP90 genes encoding cytoplasmic forms of the protein, a genetic analysis of HSP90 function is challenging. We attempted to knock down HSP90 levels using an artificial microRNA but the resulting lines were very unstable. As an alternative, we crossed the pTIR1:gTIR1-VENUS transgene into the eto3 mutant and determined TIR1-VENUS levels by protein blot. The results in Fig. 5k show that TIR1-VENUS levels are reduced in eto3 plants compared with the wild type at 22°C. TIR1-VENUS levels increase at 29°C in wild-type plants as anticipated. However, in the eto3 background, this increase did not occur. These results provide genetic confirmation that the SGT1-HSP90 complex is required for TIR1 stability. In addition, they provide a clear molecular explanation for the auxin-resistant phenotype of the eto3 mutant.

**Discussion**

The TIR1/AFB auxin co-receptors promote degradation of the Aux/IAA transcriptional repressors in response to auxin. Although the regulation of Aux/IAA degradation has been extensively studied, we know little about the control of TIR1 stability or the role that changes in TIR1 level may have in auxin signalling. In a recent study, we demonstrated that TIR1 is an unstable protein. Here we show that the molecular chaperone...
HSP90 acts to stabilize TIR1 and the related AFB2 protein. We demonstrate that TIR1 is in a complex with both HSP90 and the co-chaperone SGT1. Earlier genetic studies demonstrated that a mutation in SGT1b (eta3) resulted in both auxin and jasmonic acid (JA) resistance, but the basis for these defects was not clear. We find that SGT1 binds directly to TIR1 in vitro through its SGS domain. This is consistent with reports that the SGS domain binds LRR domains in other client proteins including plant NLRs. As the eta3 mutation results in the loss of 36 amino acids at the C-terminus of SGT1b, including much of the SGS domain, our results provides a molecular explanation for the eta3 auxin phenotype. Further, we find that the consequence of loss of SGT1b function in eta3 is a reduction in TIR1 levels, consistent with a role for the SGT1-HSP90 co-chaperone–chaperone pair in TIR1 stability. A recent report demonstrated that HSP90 and SGT1b interact with the JA receptor COI1, an F-box protein closely related to TIR1.

A mutation in the SGT1b gene results in a reduction in COI1 levels and JA response. These workers also showed that SGT1b and HSP90 form a complex with TIR1 in protoplasts. Interestingly, HSP90 has also been reported to function in brassinosteroid (BR) signalling. In this case, HSP90 interacts with the BIN2 protein kinase and promotes nuclear localization. As BR is also involved in hypocotyl elongation and displays interactions with auxin, as well as other hormones, it is possible that changes in BR signalling may also contribute to the effects of GDA on auxin-induced hypocotyl growth. However, because the role of BR in the temperature response is less than that of auxin, and because we have shown that a number of other auxin response are affected by GDA, we think that a reduction in TIR1 levels is likely to be the primary cause of reduced hypocotyl elongation. Further studies are required to clarify this issue. In any case, these results highlight the importance of the HSP90 system in hormone signalling in plants, as in animals.
The growth response of *Arabidopsis* hypocotyls to a mild increase in ambient temperature is well documented. According to the current model, the hypocotyl response is caused by the PIF4-regulated accumulation of auxin.3,9 Our data indicate that this represents only part of the response and that HSP90-mediated increase in TIR1 levels is also required. We also show that elevation of ambient temperature from 22 to 29 °C has a broad effect on seedling growth with increased lateral root formation and primary root elongation. These changes are associated with a rapid increase in both TIR1 and HSP90 levels. HSP90 is required for maintaining TIR1 levels probably by protecting it from degradation through a proteasome-mediated pathway. The detailed mechanism of TIR1 stabilization at elevated temperature is unclear. It is likely that HSP90 helps to maintain TIR1 in its native folded state. As the temperature rises, increased HSP90 further stabilizes TIR1 to promote growth. In this way, the HSP90-SGT1 chaperone system acts to integrate temperature and auxin signalling during seedling development.

The mechanism of HSP90 action is poorly understood but appears to be complex. The protein has a very flexible and dynamic structure and cooperates with many co-chaperones with diverse activities. Some co-chaperones act to regulate the ATPase activity of HSP90, but this is not the case with SGT1. Extensive studies of plant NLR proteins led to a model in which SGT1 binds to both the NLR protein, through the SGS domain, and the HSP90 dimer, via a second SGT1 domain called the CS (Chord and SGT1). In the case of NLRS, the RAR1 protein also binds to both HSP90 and SGT1, possibly acting to stabilize a functional complex. The protein has a very flexible and dynamic structure and cooperates with many co-chaperones with diverse activities. Some co-chaperones act to regulate the ATPase activity of HSP90, but this is not the case with SGT1. Extensive studies of plant NLR proteins led to a model in which SGT1 binds to both the NLR protein, through the SGS domain, and the HSP90 dimer, via a second SGT1 domain called the CS (Chord and SGT1). In the case of NLRS, the RAR1 protein also binds to both HSP90 and SGT1, possibly acting to stabilize a functional complex. However, RAR1 is not required for auxin response suggesting that SGT1 is sufficient to promote the interaction between TIR1 and HSP90. Alternatively, an unknown protein may serve the function of RAR1. Finally, we note that HSP90 interacts directly with TIR1 in vitro. Although we were not able to demonstrate this interaction in vivo, it is possible that the inherent affinity of HSP90 for TIR1 overcomes the requirement for RAR1. In general, HSP90 is thought to act late in the protein folding pathway, including on completely folded proteins.49,50 Because most of the 18 LRRs in TIR1 contribute to the auxin-binding pocket, HSP90, in concert with SGT1 may have an important role in maintaining the properly folded protein. Although our current model is that SGT1 acts in concert with HSP90 to regulate TIR1 folding and/or stability, it is also possible that SGT1 has a function that is independent of HSP90.

The responses of plants to a mild increase in ambient temperature and a heat shock are very different5. The former leads to increased plant growth, whereas the latter inhibits plant growth. Although HSFs and HSPs have been extensively studied in the context of a heat stress response, the function of these proteins in the response to mild temperature changes have not been reported. However, accumulating reports show that mutations or overexpression of HSFs or HSPs can strongly alter plant growth at normal temperatures, suggesting that these proteins may be important for plant growth in the absence of stress.51–56 Our data suggest that in addition to their roles in heat stress response, the HSP and HSF pathways are also required for regulating plant growth in response to a mild increase in ambient temperature. Therefore, these different temperature responses may share thermosensors and early signalling components. In natural conditions, the environmental temperature rises gradually rather than abruptly. It thus makes sense that plants have at least partially overlapping pathways to sense and respond to growth-promoting temperatures and stressful heat shock. A mild increase in ambient temperature may promote plant growth and at the same time prime the plant for imminent heat stress. For example, a warm night or dawn may be indicative of upcoming heat at noon. Indeed, it has been reported that a gradual rise in temperature is more effective than an abrupt temperature increase in inducing acquired thermotolerance.5,57

**Methods**

**Plant materials and growth conditions.** *Arabidopsis thaliana* ecotype Col-0 was used in all experiments. The pC5890:CFP-VENUS line was provided by Julian Schroeder.38 The pTIR1::GFP-TIR1-VENUS and pAFB2:GFP-TIR2-VENUS constructs were made by fusing in frame a genomic fragment consisting of a 4-kb promoter, the CDS of each gene and a VENUS-coding region in frame, followed by a 2-kb 3’ end region of each gene. Traditional cloning strategies including PCR, endonuclease digestion and ligation were adopted to introduce the fragments into a binary vector pC5890 (ref. 19). More details about the primers used for gene cloning are shown in Supplementary Table 1. The resulting pTIR1::GFP-TIR1-VENUS and pAFB2-GFP-VENUS were used to transform tir1-1 and afb2-3, respectively.41,60

Sterilized seeds were sown on ⅔ MS media, and stratified at 4 °C for 3 days. All the hypocotyl and root assays were performed in short days (8 h light:16 h dark) at a fluence rate of 60 μmol m⁻² s⁻¹. Except where described otherwise, seedlings were grown for 4 days before treatments.

**RNA extraction and qRT-PCR.** Total RNA was extracted using RNAasey Plant Mini Kits (Qiagen), and an on-column DNase digestion step was included during RNA extraction as recommended. cDNA was synthesized using SuperScript III (Life Technologies) and analysed by quantitative PCR on a CF960 Real-Time System (Bio-Rad). RNA levels were quantified against transcripts of the GACP2 gene (At1G13440). The oligos used for PCR are listed in Supplementary Table 1.

**GUS staining.** For GUS staining seedlings were stained in 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1% X-glc, 0.5 mM K₃Fe(CN)₆, 1 mM K₃Fe(CN)₄, and 0.05% Triton X-100 for 3 h followed by incubation in 70% (vol/vol) and then absolute ethanol for 1 h and overnight, respectively.

**Confocal microscopy.** Seedlings were imaged on a laser scanning confocal microscope (Zeiss MLS 710). To visualize root anatomy, they were stained in 4-μm propidium iodide (10 μg/ml, rinsed with water, and imaged). Image J was used to quantify the intensity of yellow fluorescent protein (YFP) fluorescence signal in individual nuclei. Mean intensity was computed from all the nuclei in an image. Each image comprised 430 μm of the root tip measured from root cap, equivalent to one field of view with a ×20 objective lens. At least four images were used for each time point.

**Immunoprecipitation and in vitro pull-down experiments.** For the immunoprecipitation assays, total proteins were extracted from 7-day-old pGFP-TIR1-Myc seedlings that were treated with or without 30 μM dexamethasone (DEX) for 24 h. Two milligrams of total proteins from each treatment were used for the immunoprecipitation. Four micrograms of anti-Myc monoclonal antibody (Covance, MMS-190R) was crosslinked by BS3 (Thermo Scientific Pierce, P121580) to Protein G Dynabeads (Life Technologies, 10004D) for 4 h at 4 °C. The complexes were washed five times and then applied to SDS-PAGE for protein blot detection. Anti-Myc (Roche, 1181415001 at 1:5,000 dilution), anti-HSP90 (Enzo Life Sciences AD1-SPA-835-F; 1:2,000) and anti-SGT1 (Customized anti sera. ProSci Incorporated, 1:5,000) were used in this study (Supplementary Fig. 8). The sequence of the antigen peptide used to raise the antibody was C-DWDKLEÅEVKQKKEKDE. The TIR1-Myc co-IP experiment was repeated three times with similar results. For the co-IP experiments with TIR1-VENUS samples, the same experimental procedures were performed except 4 μg of anti-GFP antibody (Invitrogen, A11122) was used. The co-IP products and input samples were detected by anti-GFP (Invitrogen, A11122, at 1:2,500 dilution), anti-HSP90 (Enzo Life Sciences AD1-SPA-835-F; 1:2,000) and anti-SGT1 (Customized anti sera. ProSci Incorporated, 1:5,000).

To express recombinant proteins for in vitro pull-down assays, CDSSs of *Arabidopsis* HSP90.2, IAA7, SGT1b, SGT1B seedlings that were treated with or without 30 μM dexamethasone (DEX) for 24 h. Two milligrams of total proteins from each treatment were used for the immunoprecipitation. Four micrograms of anti-Myc monoclonal antibody (Covance, MMS-190R) was crosslinked by BS3 (Thermo Scientific Pierce, P121580) to Protein G Dynabeads (Life Technologies, 10004D) for 4 h at 4 °C. The complexes were washed five times and then applied to SDS-PAGE for protein blot detection. Anti-Myc (Roche, 1181415001 at 1:5,000 dilution), anti-HSP90 (Enzo Life Sciences AD1-SPA-835-F; 1:2,000) and anti-SGT1 (Customized anti sera. ProSci Incorporated, 1:5,000).

**Bimolecular florescence complementation.** For the BiFC assay, sequences encoding TIR1, HSP90.2 and SGT1b were cloned into pENTR/D-Tops (Life Technologies, K240020SP) cloning vector and then into pDEST17 (Life Technologies) using Gateway cloning (Life Technologies, 11803-012). Primers used for the cloning are listed in Supplementary Table 1. GST-tagged proteins were purified from *E. coli* following a protocol described previously51. TIR1-Myc protein was synthesized in vitro using a TNT transcription and translation system. Equal amount of GST-tagged proteins were mixed with TIR1-Myc and incubated at 4 °C for 4 h followed by washing and protein blots to detect the recovered TIR1-Myc levels. Anti-Myc (Roche, 1181415001 at 1:5,000 dilution and anti-GST (Sigma-Aldrich, G7781-2ML) at 1:5,000 dilution were used for the western blots. Original blot images are shown in Supplementary Fig. 9.
Table 1. The nYFP (pDEST–VYNE(R)GW) and cYFP (pDEST–VYCE(R)GW) vectors are generous gifts from Dr Rainer Waadt and were described previously41. The resulting binary vectors carrying DNA sequences encoding the proteins fused to nYFP and cYFP were transfected into Agrobacteria strain GV3101 (pMP90). Equal amount of Agrobacteria carrying vectors for expressing nYFP- and cYFP-fusion proteins were mixed and Nicotiana benthamiana leaves were infiltrated with the Agrobacterium solution following a protocol as described previously82. Briefly, Agrobacteria carrying each construct were sedimented by centrifugation and resuspended in a solution of 10 mM MgCl2 and 150 μg ml−1 acetosyringone to give a final OD600 of 0.8. The cells were then incubated at room temperature for 3 h before being infiltrated into the abaxial surface of ~4-week-old N. benthamiana leaves.

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**Acknowledgements**

This work was supported by NIH Grant R01GM43644 (to M.E.), the Gordon and Betty Moore Foundation (M.E.), the Howard Hughes Medical Institute (M.E.) and by BBSRC grant BB/L010623/1 (S.K.).

**Author contributions**

R.W., Y.Z., S.K. and M.E. designed the experiments. R.W., Y.Z., H.Y. and M.K. performed the experiments. R.W., Y.Z., H.Y. and M.E. analysed the data. R.W., Y.Z. and M.E. wrote the manuscript.

**Additional information**

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Wang, R. et al. HSP90 regulates temperature-dependent seedling growth in Arabidopsis by stabilizing the auxin co-receptor F-box protein TIR1. *Nat. Commun.* **7**:10269 doi: 10.1038/ncomms10269 (2016).

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Corrigendum: HSP90 regulates temperature-dependent seedling growth in *Arabidopsis* by stabilizing the auxin co-receptor F-box protein TIR1

Renhou Wang, Yi Zhang, Martin Kieffer, Hong Yu, Stefan Kepinski & Mark Estelle

*Nature Communications* 7:10269 doi: 10.1038/ncomms10269 (2016); Published 5 Jan 2016; Updated 11 May 2016

This Article contains errors in the labelling of the x axis in Supplementary Figure 3 (lower panel). The x axis in this panel should have been labelled '22 °C Mock; 22 °C GDA; 29 °C Mock; 29 °C GDA'. The correct version of this figure appears below.