Altered expression of cytosolic/nuclear HSC70-1 molecular chaperone affects development and abiotic stress tolerance in Arabidopsis thaliana

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Abstract

Molecular chaperones of the heat shock cognate 70 kDa (HSC70) family are highly conserved in all living organisms and assist nascent protein folding in normal physiological conditions as well as in biotic and abiotic stress conditions. In the absence of specific inhibitors or viable knockout mutants, cytosolic/nuclear HSC70-1 over-expression (OE) and mutants in the HSC70 co-chaperone SGT1 (suppressor of G2/M allele of skp1) were used as genetic tools to identify HSC70/SGT1 functions in Arabidopsis development and abiotic stress responses. HSC70-1 OE caused a reduction in root and shoot meristem activities, thus explaining the dwarfism of those plants. In addition, HSC70-1 OE did not impair auxin-dependent phenotypes, suggesting that SGT1 functions previously identified in auxin signalling are HSC70 independent. While responses to abiotic stimuli such as UV-C exposure, phosphate starvation, or seedling de-etiolation were not perturbed by HSC70-1 OE, it specifically conferred γ-ray hypersensitivity and tolerance to salt, cadmium (Cd), and arsenic (As). Cd and As perception was not perturbed, but plants overexpressing HSC70-1 accumulated less Cd, thus providing a possible molecular explanation for their tolerance phenotype. In summary, genetic evidence is provided for HSC70-1 involvement in a limited set of physiological processes, illustrating the essential and yet specific functions of this chaperone in development and abiotic stress responses in Arabidopsis.

Key words: Arsenic, auxin, cadmium, co-chaperone, DnaK, γ-ray, heat shock, HSC70, HSP70, meristem, SGT1, UV-C.

Introduction

Among all molecular chaperones, the 70 kDa heat shock proteins constitute a large and highly conserved family of chaperones in both prokaryotes (DnaK) and eukaryotes (HSP70). HSP70 chaperones are involved in many aspects of protein folding: folding of newly synthesized proteins, prevention of aggregation and refolding of stress-denatured proteins, disaggregation of protein aggregates, control of activity and stability of regulatory proteins, protein translocation through membranes, and degradation of misfolded proteins (Young et al., 2003). The HSP70 chaperones control cellular protein homeostasis through ATP-dependent cycles and essentially bind non-native polypeptides and folding intermediates that are either newly synthesized or stress induced (Erbse et al., 2004; Bukau et al., 2006). In eukaryotes, HSP70s are essential for cellular viability and are present in almost every subcellular compartment: cytosol, nucleus, endoplasmic reticulum, mitochondria, and chloroplasts (Mayer and Bukau, 2005). Despite their physiological and medical importance, their functional analysis remains very limited by (i) gene redundancy which limits genetic analysis; (ii) their essential functions which do not permit the characterization of viable null mutants; and
particularly in plants. HSP70 substrates and their functions in eukaryotes, and Chiosis, 2006). For these reasons, little is known about (iii) the absence of specific and efficient inhibitors (Brodsky and Chiosis, 2006). The Arabidopsis genome encodes 14 HSP70s of the DnaK subfamily, five of which (HSC70-1 to HSC70-5; for heat shock cognate) are predicted to be cytosolic/nuclear (Lin et al., 2001). While the loss of single cytosolic/nuclear HSC70 genes is tolerated (Noël et al., 2007), HSC70 genes were shown to be globally essential in Arabidopsis as observed in other eukaryotes (Sung and Guy, 2003; Noël et al., 2007). In addition, single HSC70 mutants were never identified in forward genetic approaches nor did those display any mutant phenotypes in forward genetic screens, probably due to a high level of functional redundancy. An important tool to assign those functions to cytosolic/nuclear HSC70 was thus the physiological characterization of transgenic plants overexpressing HSC70-1 as a genetic tool to deregulate chaperone function (Sung and Guy, 2003). HSC70-1 overexpression (OE) caused a general OE of other cytosolic/nuclear HSC70 genes and caused an enhanced heat shock tolerance, dwarfism, and a modified root architecture (Sung and Guy, 2003). In addition, cytosolic/nuclear HSC70s are also important for controlling resistance to bacterial and oomycete pathogens (Noël et al., 2007). However, substrates for Arabidopsis HSC70 chaperones involved in those responses remain to be identified.

Chaperone activities are modulated and/or coordinated by a complex network of co-chaperones that define balance of protein assembly and degradation by the ubiquitin–proteasome machinery (Erbse et al., 2004; Bukau et al., 2006). The co-chaperone archetype is DnaJ/HSP40 which enhances the ATPase activity of DnaK/HSP70. Other co-chaperones such as Hop/Sti1 (for Hsp70/Hsp90-organizing protein) can bind to both HSP90 and HSP70 and act as a scaffold to bridge HSP70 and HSP90 activities (Hernandez et al., 2002). For example, in mammals, nascent steroid receptors are recruited to HSP70 by HSP40 and later transferred by the Hop co-chaperone to HSP90 to complete their maturation. SGT1 (for suppressor of G2/M allele of skp1) is a conserved and essential co-chaperone that binds to HSP90 (Bansal et al., 2004) and HSP70 (Noël et al., 2007; Spiechowicz et al., 2007). SGT1 has been implicated in cell cycle progression and adenyl cyclase activity in yeast (Kitagawa et al., 1999; Dubacq et al., 2002). SCF E3 ubiquitin ligase-dependent signalling (Kitagawa et al., 1999; Gray et al., 2003), immunity, and heat shock tolerance in plants (Austin et al., 2002; Noël et al., 2007). Because most of those physiological responses depend on leucine-rich repeat (LRR) proteins, SGT1 proteins have been proposed to be essentially involved in the folding of LRR proteins (Stuttmann et al., 2008). Despite recent in vitro studies which suggest a potential co-chaperone activity for SGT1 alone (Zabka et al., 2008), it is rather considered as an HSP70/90 scaffold protein as it has so far failed to affect HSP90 ATPase activity in vitro (Catlett and Kaplan, 2006). Two genes, SGT1a and SGT1b, encode SGT1 proteins in Arabidopsis which both interact in planta with cytosolic/nuclear HSC70 (Noël et al., 2007). While double mutants are lethal at the embryonic stage, single mutants are fully viable (Austin et al., 2002; Azevedo et al., 2006). SGT1a and SGT1b both function in plant immunity against host pathogens (Austin et al., 2002; Azevedo et al., 2006), heat shock tolerance (Noël et al., 2007), and responses to the plant hormones auxin and jasmonic acid (Gray et al., 2003). Because auxin modulates cell expansion, and initiation and emergence of lateral roots, this hormone is a key regulator of root development and architecture. In conclusion, tolerance to heat shock, immunity, and root architecture represent an important functional overlap between SGT1 and cytosolic/nuclear HSC70 roles in Arabidopsis, suggesting that the SGT1/HSC70 complex has important functions in plant physiology.

Taking advantage of plants overexpressing HSC70-1 as well as single sgt1 mutants, multiple tests were conducted to identify new physiological responses and stress adaptations genetically dependent on HSC70 and/or SGT1 in Arabidopsis.

Materials and methods

Plant material and general growth conditions

All Arabidopsis thaliana genotypes used in this study are from the Col-0 ecotype. The following mutants were used: sgt1a (Gray et al., 2003), cad1-3 (Howden et al., 1995a), axr1-3 (Lincoln et al., 1990), GT92 (Davies et al., 1994), hsc70-1;1 (SALK_135531; Noël et al., 2007), hsc70-2;1 (SALK_085076; Noël et al., 2007), and hsc70-3;1 (GK_758E01; Noël et al., 2007). Two independent homozygous Col-0 plants over-expressing HSC70-1 (Sung and Guy, 2003) were used: lines 8-7 (single insertion) and 8-9 (multiple insertions). A T-DNA insertion mutant (GK_266H09) situated in the third exon of SGT1a was obtained from the GABI-KAT collection and renamed sgt1a-2 (Supplementary Fig. S1A available at JXB online). The insertion creates an early stop codon at the end of the region coding for the TPR domain and, accordingly, no SGT1a protein can be detected by an antibody directed against the SGT1a C-terminus (SGS antibody; Supplementary Fig. S1B). Plants were grown in soil in a walk-in chamber under short day conditions (8 h light/16 h darkness) at 21 °C/18 °C (light/dark) and a light intensity of 200 μmol photons m−2 s−1. Unless stated otherwise, plants were grown in sterile conditions on vertically oriented MS/10 medium containing 0.5% sucrose in a white light growth chamber under a 16 h photoperiod at 24 °C/21 °C (light/dark).

Plant growth conditions during heavy metal and metalloid treatments

Sterile seeds were plated on half-strength Hoagland medium (H/2; pH 5.2) and incubated for 1 d at 4 °C. Plants were grown in vertical orientation with an 8 h light/16 h darkness photoperiod at 21 °C/18 °C, respectively, and a light intensity of 100 μmol photons m−2 s−1. Six-day-old seedlings were transferred to H/2 medium supplemented or not with CdCl₂ or NaAsO₂. Root length was measured 13 d later.
Samples for biochemical analyses were harvested 21 d after transfer. All experiments were performed at least three times.

**Measurements of root architecture and hypocotyl length**

In order to determine root and hypocotyl length, photographs were taken from at least 10 plants per condition and their roots measured using the ImageJ software (http://rsb.info.nih.gov/ij/). The number of lateral roots was scored under the binocular at 7, 14, and 21 d post-germination. Hypocotyl length was determined 7 d post-germination as described (Gray et al., 2003). All experiments were performed at least three times.

**Root growth inhibition assays**

For root growth inhibition assays, 5-d-old seedlings were transferred to MS/10 medium containing different concentrations of synthetic auxin 2,4-D (Sigma), NaCl, and mannitol. Root elongation was measured 3 d after transfer of at least 10 plants per condition. All experiments were performed at least three times.

**Size measurements of root cells and meristems**

To measure cell length, roots were briefly stained with ruthenium red and observed with a bright field microscope (Leica DMRXA, ×20 objective). At least 10 cells were measured on six different roots using a micrometric lens. Cell sizes were determined in the differentiated zone. To estimate the size of the proximal meristem, the number of non-differentiated cells in the cortex was measured as described (Casamitjana-Martinez et al., 2003). Propidium iodide-stained roots were observed by confocal laser scanning microscopy using a Leica SP2 AOBS inverted confocal microscope (Leica Microsystems, Germany) equipped with an argon ion laser. Propidium iodide was excited at 514 nm and imaged using a custom 610–720 nm band pass emission filter. All experiments were performed at least three times.

**Measurements of leaf epidermal cell density**

In order to estimate epidermal cell density, epidermal peels were prepared as described (Forestier et al., 1998) from fully expanded leaves of plants grown in soil for 4 weeks under short day conditions. Peels were briefly stained in ruthenium red and imaged using a bright field microscope (Leica DMRXA, ×40 objective). The number of epidermal cells was counted (guards cells excluded) on at least three different leaves from three different plants. The experiment was performed three times.

**Western blot analyses**

Proteins were separated by SDS-PAGE on 12% gels and transferred onto nitrocellulose membranes. The following antibodies were used: monoclonal mouse anti-spinach HSP70 (Stressgen SPA-817, Victoria, Canada); rabbit anti-SGS antibody (S Betsuyaku, A Takahashi, K Shirasu, and J Parker, unpublished); and goat anti-rabbit IgG–alkaline phosphatase (AP) conjugate (Sigma A3687, St Louis, MO, USA). AP activity was detected with p-nitroblue tetrazolium.

**Pigment analyses**

Anthocyanin and chlorophyll/carotenoid contents were measured on leaf discs as described (Ticconi et al., 2001; Tzvetkova-Chevvolleau et al., 2007). All experiments were performed at least three times.

**Quantitative RT-PCR**

Plants grown in hydroponic conditions were supplied with 5 μM or 50 μM CdSO₄, and roots were sampled at 0 h and 6 h as described (Herbette et al., 2006). RNA extraction, cDNA synthesis, quantitative reverse transcription-PCR (RT-PCR), and data processing were performed as described (Herbette et al., 2006). Three independent RT-PCR experiments were performed on three independent biological samples.

**UV-C and γ-ray treatments**

To test for UV-C tolerance, 4-d-old seedlings grown horizontally on MS/10 were given a single dose of UV-C (254 nm; 0–50 kJ/m²) using a Stratalinker 2400 (Stratagene, La Jolla, CA, USA). Lids were removed during exposure. Plants were observed 6 d and 13 d post-exposure. To assay for γ-ray sensitivity, 4-d-old seedlings grown horizontally on MS/10 were given a single dose of γ-rays with a 60Co source (45 Gy min⁻¹) and photographed 12 d post-irradiation.

**Glutathione (GSH) and phytochelatin (PC) dosage**

Quantification of GSH and PC was performed fluorometrically by HPLC after monobromobimane derivatization as described (Picault et al., 2006).

**Quantitative determination of metal content**

Plant shoots were mineralized and analysed using an inductively coupled plasma-optical emission spectroscopy apparatus (IPC-OES, Varian Vista MPX) as described (Picault et al., 2006).

**Results**

**Dwarfism of plants overexpressing HSC70-1 is explained by a reduction in cell numbers, not size**

The cytosolic/nuclear HSC70 and SGT1 proteins assemble into complexes which mount post-invasive plant immunity and heat stress tolerance (Noël et al., 2007). It was thus decided to analyse two independent representative lines overexpressing HSC70-1 by 4- and 7-fold (8-9 and 8-7, respectively; Sung and Guy, 2003) and two sgt1 mutants. The sgt1a-2 line was isolated in the GABI-KAT collection (GK_266H09) and only produces a truncated fragment
corresponding to the N-terminal TPR domain of SGT1a (Supplementary Fig. S1 at JXB online). Accordingly, no full-length SGT1a protein is detected with the SGS antibody directed against the C-terminal part of SGT1a. The sgt1beta3 mutant produces a non-functional SGT1b protein that lacks its last 36 amino acids and is unable to interact with HSC70s (Gray et al., 2003; Noël et al., 2007). A detailed phenotypic characterization of these lines relative to their wild-type Col-0 was conducted.

While the biomass of the aerial parts of wild-type and sgt1 mutant plants was not significantly affected, HSC70-1 OE reduced the biomass by 25–70% in a HSC70-1 dose-dependent manner (Fig. 1A, B). This difference in biomass is not due solely to a reduction in total leaf surface (Sung and Guy, 2003), but also to a 20–30% reduction of the leaf number compared with wild-type or sgt1 mutants (Fig. 1C). To explain these observations, leaf epidermal cell density was determined in all lines (Fig. 1D). This parameter was not changed in lines 8-7 and 8-9 relative to Col-0, suggesting that HSC70-1 OE affects total cell number per leaf rather than cell size and that the activity of the apical meristem might be affected by HSC70-1 OE.

This hypothesis was tested in the root tissues whose cellular organization is better defined. The primary root of plants grown in vitro was also 50% shorter in lines 8-7 and 8-9 than in the wild-type and sgt1 mutant roots (Fig. 2A). Single cell length measurements above the differentiation zone of the root showed that all lines had a very similar cell length (Fig. 2B) that could not account for the difference in primary root length. Indeed, lines 8-7 and 8-9 contain 30–40% fewer cells than wild-type plants (Fig. 2C), suggesting that meristematic activity might be affected. In addition, the daily rate of primary root elongation in HSC70-1 OE lines was reduced by a factor of 3 compared with the wild type.

Fig. 1. General growth phenotypes of sgt1 mutants and HSC70-1 overexpression lines grown in soil for 4 weeks under short day conditions. (A) Picture of one representative plant per genotype. Scale bar = 1 cm. (B) Average rosette fresh weight (FW). (C) Number of leaves per rosette. (D) Epidermal cell density. Error bars indicate the standard deviation. At least three independent experiments gave similar results. *, statistically significant differences for values compared with the wild type as determined by Student’s t-test (P < 0.01).
Fig. 2. Cellular basis for dwarfism of plants overexpressing HSC70-1. (A) Average primary root length of 1-week-old plantlets grown in vitro \((n=6)\). (B) Average length of cells above the differentiation zone \((n=60)\). (C) The number of cells per root was extrapolated from measurements performed in (A) and (B). (D–F) One-week-old seedlings were observed by confocal laser scanning microscopy to determine the number of cortex cells in the proximal meristem (PM). The arrow indicates the transition zone (TZ) before cells enter the elongation–differentiation zone (EDZ). Scale bar \(= 80 \mu m\). (D) Values are the average of at least 10 meristems. At least three independent experiments gave similar results. Error bars indicate the standard deviation. *, statistically significant differences for values compared with the wild type as determined by Student’s \(t\)-test \((P < 0.01)\).
and sg1 mutants (Supplementary Fig. S2A at JXB online). The number of cortex cells in the proximal meristem was therefore measured as described (Casamitjana-Martinez et al., 2003, Fig. 2D–F). HSC70-1 OE caused a dose-dependent reduction of proximal meristem size, suggesting a reduced meristematic activity. In conclusion, these results indicate that the general dwarfism induced by HSC70-1 OE is predominantly due to a defect in maintenance or activity of the root and apical meristems, a function which had not so far been attributed to any cytosolic/nuclear HSC70 molecular chaperones in plants.

The modified root architecture caused by HSC70-1 OE is not caused by a general alteration of auxin signalling

Plants overexpressing HSC70-1 were shown to have shorter and more branched roots than the wild type (Sung and Guy, 2003), this latter process being strongly dependent on the plant hormone auxin. Parameters of the root architecture of the various lines were first measured in greater detail simultaneously (Supplementary Fig. S2 at JXB online). As shown in Fig. 3A, the lateral root density of lines 8-7 and 8-9 was increased by 40% compared with Col-0 and the sg1a-2 mutant while it was significantly reduced in the sg1b eta3 mutant as reported (Gray et al., 2003). Interestingly, the absolute number of lateral roots per plant was not significantly modified (Fig. 3B, white bars). Thus, the increased density of lateral roots is caused by a shorter primary root and slower primary root growth (Fig. 3B, Supplementary Fig. S2A) rather than by an enhanced lateral root initiation/emergence. Since auxin is a key hormone for lateral root induction and emergence, two auxin-dependent responses were tested, i.e. hypocotyl elongation and root growth inhibition. In both instances, responses of plants overexpressing HSC70-1 were similar to those of the wild type (Fig. 3C, D). Auxin signalling mutants sg1b eta3 and axr1-3 had shorter hypocotyls and reduced root growth inhibition as expected. Therefore, the modified root architecture of HSC70-1 OE lines could not be attributed to any altered auxin-dependent responses but was more likely to be due to reduced primary root growth.

HSC70-1 OE confers salt-resistant root growth

Besides the essential roles of HSC70/SGT1 in plant development, the HSC70-1/SGT1 complex also controls

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**Fig. 3.** Root architecture and auxin-dependent responses in lines overexpressing HSC70-1. (A) The number of emerged lateral roots relative to primary root lengths between 13 d and 21 d post-germination was measured as a quantitative parameter to evaluate modifications in root architecture. n > 12. (B) The numbers of lateral roots per primary root (white bars) and primary root length (black bars) 17 d after germination are shown. The same samples as in (A) and in Supplementary Fig. S2B at JXB online. (C) Inhibition of root elongation by increasing concentrations of the synthetic auxin 2,4-D. Data points are averages from at least 10 seedlings. At least three independent experiments gave similar results. Standard deviations were < 10%. (D) Hypocotyl length of 1-week-old seedlings. Error bars indicate the standard deviation. One experiment from three is shown. *, statistically significant differences for values compared with the wild type as determined by Student’s t-test (P < 0.01).
HSC70-1 OE does not confer general osmotic protection but a more specific salt tolerance. These observations prompted determination of the mineral composition of this set of lines after mineralization and ICP-OES analysis. Among all ions measured (Co, Al, P, Zn, Sr, Mo, Ca, Cu, Fe, K, Mg, and Na), only a 3- to 4-fold increase in Na concentration in the leaves of the plants overexpressing HSC70-1 relative to Col-0 and sgt1 mutants (Fig. 4B, data not shown) was observed when grown in soil. No significant changes in mineral composition could be detected in HSC70-1 OE lines cultivated on H/2 (0.02 M Na) and on MS/10 (4 M Na) compared with Col-0 (data not shown). These results indicate that HSC70-1 OE confers some Na hyperaccumulation and tolerance in Arabidopsis.

HSC70-1 OE confers hypersensitivity to γ-radiation but not UV-C

Tolerance to UV-C and γ-rays, two types of radiation causing a wide variety of DNA breaks and damage (Sutherland et al., 2000), was tested on 4-d-old plants. All lines showed similar sensitivity to a wide range of UV energies (0–50 kJ/m²; data not shown). In contrast to sgt1 mutants and Col-0, lines 8-7 and 8-9 showed a HSC70-1 dose-dependent hypersensitivity to γ-radiation visible at 100 Gy and 125 Gy (Fig. 5A). Indeed, while other genotypes could recover some meristematic activity and produce new leaves after 12 d, lines 8-7 and 8-9 produced no or few new leaves once irradiated. Line GT92 (Davies et al., 1994) was used as a mutant hypersensitive to γ-ray exposure. In Fig. 5B, two Col-0 seeds were sown on the same plate with 8-9 seeds. At 10 d after irradiation at 100 Gy, a clear growth differential is observed. Therefore, HSC70-1 OE specifically compromises tolerance to γ-irradiation, but not to UV-C exposure.

HSC70-1 OE confers tolerance to Cd(II) and As(III)

Publicly available microarray experiments suggest that mRNA accumulation of several HSC70 genes is modified in root and shoot tissues upon exposure to Cd(II) in hydroponic cultures (Herbette et al., 2006). A detailed validation of this data set was therefore conducted by quantitative RT-PCR experiments (Fig. 6A). It could be shown that accumulation of HSC70-2, HSC70-4, and HSC70-5 mRNA is rapidly (as soon as 2 h; data not shown) and strongly [as much as 19 times for HSC70-4 after 6 h treatment with 50 µM Cd(II)] increased in root tissue exposed to Cd(II). HSC70-3 mRNA accumulated to a lesser extent. HSC70-1 and SGT1b levels remained globally unchanged. SGT1a expression was also increased 5- to 9-fold by Cd(II). Based on this experiment, the potential contribution of those genes to tolerance to the heavy metal cadmium and the metalloid arsenate was tested.

To assay for tolerance, 1-week-old seedlings were transferred onto medium containing 0–50 µM Cd(II) and 0–10 µM As(III), and primary root growth was imaged 6 d after transfer. Root growth from the sgt1beta3, hsc70-1;1, hsc70-2;1, and hsc70-3;1 mutants was indistinguishable from that of the wild type Col-0 (Fig. 6B, data not shown).
Root growth of the phytochelatin synthase 1 mutant cad1-3 was, as expected, severely impaired on both Cd(II)- and As(III)-containing media. Interestingly, primary root growth of lines 8-7 and 8-9 was only weakly affected by both 50 μM Cd(II) and 10 μM As(III), conditions under which 75% inhibition of Col-0 root growth was observed. Furthermore, 10 μM As(III) did not induce lateral root growth and root architecture remodelling as observed in the wild type Col-0 (Fig. 6C). Fresh weight measurements of the aerial parts 21 d post-transfer were poorly informative since both Cd(II) and As(III) had only a limited impact on fresh weight under the conditions used here (data not shown). GSH and PCs, small polypeptides synthesized non-transcriptionally from GSH, both act as chelators of heavy metals. PCs and GSH play critical roles in their detoxification, as illustrated by identification of Cd-sensitive mutants cad1-3 (PC-deficient, Fig. 7C, Howden et al., 1995a) and cad2 (GSH-deficient, Howden et al., 1995b). GSH and PC contents were measured (Fig. 7). GSH levels in lines 8-7 and 8-9 were similar to those of the wild-type control (Fig. 7A). PC synthesis was also induced normally by Cd(II) and to a lesser extent by As(III)). It is noteworthy that no constitutive PC synthesis was observed in the absence of Cd(II) or As(III), indicating that heavy metal perception by the plant and downstream signalling leading to PC synthesis is not compromised by HSC70-1 OE. To search for a molecular basis for Cd(II) and As(III) tolerance in lines 8-7 and 8-9, the Cd and As contents in the aerial parts of seedlings were measured using ICP-OES analysis (Fig. 7C). No Cd or As could be detected in control conditions. While As levels did not vary in the lines tested, lines overexpressing HSC70-1 contained 20–25% less Cd than Col-0 and the sgt1beta3 mutant when grown on 50 μM Cd(II). Therefore, reduced uptake or enhanced efflux of Cd(II) but not As(III) might serve as one possible molecular explanation for Cd tolerance conferred by HSC70-1 OE.
Discussion

In this study, the genetic evidence for HSC70-1 implication in meristem activity and several physiological responses such as tolerance to heavy metals, salt, and γ-rays in *Arabidopsis* is provided.

**HSC70-1 OE to investigate HSC70 functions in plants**

Despite the essential functions carried out by HSC70 in eukaryotes, little is known about their specific contributions to signalling pathways and their molecular targets. The genetic analysis of this small gene family is limited by functional redundancy and lethality of multiple mutants. For instance, the *hsc70-2/hsc70-4* double mutant is seedling lethal while single mutants did not display any mutant phenotypes in plant immunity or tolerance to heat stress and heavy metal (Noël *et al.*, 2007; data not shown). Furthermore, in contrast to HSP90 activity which can be specifically inhibited *in vivo* using pharmacological agents, a specific and potent HSC70 inhibitor is still to be identified. For these reasons, an extensive characterization of plants overexpressing HSC70-1 initially described by Sung and Guy (2003) was performed.

**Physiological importance of the HSC70/SGT1 chaperone complex**

Similarly to HSC70, *SGT1a* and *SGT1b* constitute a small gene family with redundant and globally essential functions grown as in (A). Scale bar = 8 mm. Shoot fresh weight (FW) was determined 21 d post-transfer to Cd(II)- and As(III)-containing media. Error bars indicate the standard deviation. One representative experiment from three is shown.
Physiological responses dependent on HSC70-1 such as tolerance to γ-radiation, Na, As, or Cd. SGT1a expression, which to date was known to be up-regulated only by heat shock and interaction with an incompatible pathogen (Noël et al., 2007), is also induced by Cd, and this could suggest the implication of SGT1 in Cd tolerance. Reciprocally, HSC70-1 has been implicated in maintenance/activity of the root and apical meristem, reminiscent of SGT1 functions in cell cycle progression through the G1/S and G2/M checkpoints in yeast (Kitagawa et al., 1999). Finally, despite the clear implication of SGT1 in auxin signalling (Gray et al., 2003), HSC70-1 OE did not affect any of the tested responses to this hormone. The observed modification of root architecture caused by HSC70-1 OE is more likely to be due to a reduced primary root growth than a defect in auxin-dependent lateral root initiation or emergence. In summary, with the limits of the genetic tools available to dissect SGT1/HSC70 functions, a clear conclusion could not be drawn on contributions of the HSC70/SGT1 complex to those physiological responses. This could indicate that SGT1 is simply not involved or that SGT1 levels in single sgt1 mutants stay above the threshold needed to accomplish these functions. This latter hypothesis is reminiscent of SGT1 functions in plant immunity and auxin signalling where SGT1a is not genetically required but can still compensate for the loss of the SGT1b protein when overexpressed (Azevedo et al., 2006).

Heavy metal and metalloid tolerance conferred by HSC70-1 OE

On the one hand, induction of the expression of genes for chaperones (including HSP70) has long been used as a sensor for heavy metals but it is not very specific since many abiotic and biotic stresses will also induce their expression. For instance, cadmium and arsenite increased expression of small HSP in rice (Guan et al., 2004) and 81 kDa HSP in Arabidopsis (Takahashi et al., 1992). In the present study, it was shown that expression of HSC70-2, HSC70-4, and HSC70-5 is strongly up-regulated in the presence of cadmium. In particular, HSC70-5 expression, which is very low in normal physiological conditions (Sung et al., 2001), was strongly induced by Cd, while pathogen challenge had little effect on its expression (Noël et al., 2007). As such, it might constitute a more specific marker for exposure to Cd than other cytosolic/nuclear HSC70 genes. On the other hand, little evidence for the contribution of cytosolic/nuclear HSC70 to heavy metal tolerance is yet available. For instance, HSP70 OE was shown to confer antimony tolerance to the protozoan parasite Leishmania (Brochu et al., 2004), and heat shock was shown to protect tomato cell cultures from Cd-induced membrane damage (Neumann et al., 1994). Interestingly, the tolerance conferred by HSC70-1 OE in Arabidopsis seems somewhat limited to Cd and As since preliminary experiments indicate that tolerance to antimony (SbIII) and zinc was not affected by HSC70 OE (A-C Cazalé and LD Noël, unpublished data). A protective effect of DnaK/Hsp70 OE has not been
reported in other organisms, but the loss of HscC, one of the three DnaK homologues from Escherichia coli, specifically conferred sensitivity to Cd, possibly due to a defect in the Cd export machinery or detoxication (Kluck et al., 2002). In Arabidopsis, the reduction of Cd accumulation by HSC70-1 OE in the aerial parts may be caused by a defect in the Cd import system or an increased Cd efflux and thus provide a possible explanation for the Cd tolerance of HSC70-1-overexpressing lines. While wild-type plant growth is strongly hampered on weakly contaminated soils and their heavy metal content elevated, HSC70-1 OE might be relevant to produce tolerant plants with lower internal heavy metal content.

Supplementary data

Fig. S1. Molecular characterization of the sgt1a-2 mutant.

Fig. S2. Root architecture parameters of HSC70-1 overexpression lines.

Fig. S3. Inhibition of root growth by increasing concentrations of mannitol.

Fig. S4. Anthocyanin accumulation in sgt1 mutants and HSC70-1 overexpression lines.

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