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An RNA chaperone, AtCSP2, negatively regulates salt stress tolerance

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Abbreviations: CSD, cold shock domain; CSP, cold shock protein.

Cold shock domain (CSD) proteins are RNA chaperones that destabilize RNA secondary structures. Arabidopsis Cold Shock Domain Protein 2 (AtCSP2), one of the 4 CSD proteins (AtCSP1-AtCSP4) in Arabidopsis, is induced during cold acclimation but negatively regulates freezing tolerance. Here, we analyzed the function of AtCSP2 in salt stress tolerance. A double mutant, with reduced AtCSP2 and no AtCSP4 expression (atcsp2–3 atcsp4–1), displayed higher survival rates after salt stress. In addition, overexpression of AtCSP2 resulted in reduced salt stress tolerance. These data demonstrate that AtCSP2 acts as a negative regulator of salt stress tolerance in Arabidopsis.

The cold shock domain (CSD) contains about 70 amino acid residues including 2 consensus RNA binding motifs (RNP-I and RNP-II).1,2 CSD is a nucleic acid-binding domain that is highly conserved among bacteria, animals, and plants.3,4 There is a large superfamily of CSD proteins in these organisms. The bacterial cold shock protein (CSP) is a small protein composed of only one CSD.4 Escherichia coli contains 9 CSP members (cspA to cpl) and 4 of them (cspA, cspB, cspG, and cpl) are induced in response to cold shock.5 It has been demonstrated that a quadruple mutant of cspA, cspB, cspG, and cpe results in growth defects at low temperature.6 CSPs can bind RNA and melt RNA secondary structures in vitro and in vivo.7,8 Therefore, it has been suggested that bacterial CSPs act as RNA chaperones that destabilize RNA secondary structures formed under low temperatures and thereby promote efficient transcription and translation.

CSD proteins have also been extensively studied in animals. Animal CSD proteins contain various C-terminal auxiliary domains in addition to CSD. Y-box binding protein 1 (YB-1) is the most extensively studied CSD protein in vertebrates. YB-1 consists of 3 domains: the N-terminal alanine/proline rich domain, the CSD, and the C-terminal domain with alternating clusters of positively and negatively charged amino acid residues.9,10 YB-1 is a multifunctional DNA/RNA binding protein that is involved in DNA repair, pre-mRNA splicing, transcription, and translation of several genes whose products are involved in cell division, stress response, immune response, drug resistance, etc.10 It has been demonstrated that disruption of YB-1 results in embryonic or perinatal lethality in mice.11,12 In addition, mouse embryonic fibroblasts (MEFs) from YB-1-deficient embryos show increased sensitivity to oxidative, genotoxic, and oncogene-induced stresses.11 However, YB-1 deficiency causes no global changes in the transcriptome and proteome in freshly isolated MEFs.11 YB-1 may be involved in the selective expression of genes whose proteins are essential for cell proliferation and stress response.13

Another class of widely studied animal CSD proteins is LIN28. LIN28 was first identified as a key regulator of developmental timing in Caenorhabditis elegans.14 LIN28 is an evolutionarily conserved RNA binding protein that contains N-terminal CSD and C-terminal retroviral-type Cys-Cys-His-Cys (CCHC) zinc fingers.14 LIN28 is highly expressed in embryonic stem (ES) cells and decreases during ES cell differentiation.15,16 Recently, LIN28, together with 3 transcriptional factors has been used to reprogram somatic cells into induced pluripotent stem cells.17 At the molecular level, LIN28 suppresses the maturation of let-7 microRNAs which are absent in ES cells, but accumulate during ES cell differentiation.18 Therefore, it was suggested that LIN28 plays an important role in blocking cell differentiation. In addition to microRNA regulation, it has been reported that LIN28 acts as a translational activator or repressor in ES and differentiated cells, respectively.19,20

Plant CSD proteins typically contain a large glycine-rich region interspersed with CCHC zinc fingers as a C-terminal auxiliary domain.22 The first characterized plant CSD protein was wheat cold shock protein 1 (WCSP1) which was identified as a cold-induced gene in winter wheat.23 WCSP1 can melt double-stranded RNA/DNA and can complement a cold sensitive phenotype of an E. coli csp quadruple mutant.24 Therefore, it was...
suggested that WCSP1 shares a function with E. coli CSPs for cold adaptation. Arabidopsis thaliana has 4 genes that encode CSD proteins (AtCSP1 to AtCSP4). Among them, AtCSP3 is the most extensively studied and its function in acquiring abiotic stress tolerance has been demonstrated. Compared with wild type, a knockout mutant of AtCSP3 is more sensitive to freezing, drought, and salt stresses. Conversely, overexpression of AtCSP3 confers enhanced stress tolerance. AtCSP3 regulates the expression of abiotic stress responsive genes, but the functions of these genes are mostly unknown. AtCSP3 interacts with different classes of proteins associated with RNA, including decapping protein 5, poly(A)-binding proteins, and RNA helicases. During abiotic stress, AtCSP3 may regulate gene expression through mRNA stability, splicing, and translation.

AtCSP2 shows the highest level of expression among the AtCSPs. Although AtCSP2 is up-regulated during cold acclimation, a double mutant, with reduced levels of AtCSP2 and no expression of AtCSP4 (the closest paralog of AtCSP2), shows higher freezing tolerance than wild-type after cold acclimation. By contrast, overexpression of AtCSP2 results in decreased freezing tolerance under cold-acclimated condition. Interestingly, both double mutants and AtCSP2-overexpressors show no difference in freezing tolerance under the non-acclimated condition. It is known that C-repeat binding factors (CBFs) confer freezing tolerance through activation of the expression of cold-regulated (COR) genes. AtCSP2 negatively regulates the expression of CBFs and COR genes during cold acclimation. AtCSP2 is a negative regulator of the CBF-dependent pathway for cold acclimation. AtCSP2 is also a negative regulator of seed germination.

AtCSP2-overexpressing lines show retarded germination as compared with the wild type. Overexpression of AtCSP2 results in reduced expression of an ABA catabolic gene (CYP707A2) and gibberellin biosynthesis genes (GA20ox and GA3ox). The ABA levels in AtCSP2-overexpressing seeds are higher than those in the wild type. Therefore, it is proposed that AtCSP2 negatively regulates seed germination by controlling ABA and GA levels.

It has been reported that expression of AtCSP2 is also upregulated by salt treatment. In this paper, we examined whether AtCSP2 is associated with salt stress tolerance. Since AtCSP2 and AtCSP4 redundantly regulate freezing tolerance in Arabidopsis, we utilized the previously-isolated atcsp2–3 atcsp4–1 mutant lines (#1 and #2) for the analysis.

Figure 1. Salt tolerance of the atcsp2–3 atcsp4–1 double mutant. (A) Phenotype of atcsp2–3 atcsp4–1 plants after recovery from salt treatment (185 mM NaCl, 3 days). The photograph was taken 4 d after recovery growth on MS medium without salt. (B) Survival rates were calculated from 3 independent experiments (n = 21). Data represent the means ±SD.

Figure 2. Salt tolerance of AtCSP2-overexpressing lines. (A) Phenotype of AtCSP2-overexpressing lines after recovery from salt treatment (185 mM NaCl, 2.5 days). The photograph was taken 4 d after recovery growth on MS medium without salt. (B) Survival rates were calculated from 3 independent experiments (n = 25). Data represent the means ±SD.
of salt stress tolerance. For the salt tolerance test, 7-day-old wild type (Col-0) and atcsp2–3 atcsp4–1 seedlings were transferred to MS medium supplemented with 185 mM NaCl and grown for 3 d. Survival rates were determined 4 d after transfer back to MS medium. Compared to wild type, atcsp2–3 atcsp4–1 showed significantly increased survival rates after salt stress (Fig. 1A and B). The atcsp2–3 atcsp4–1 plants had survival rates of 73% and 69.8% (#1 and #2, respectively), while the survival rate of wild type plants was 23.8% (Fig. 1B). These data indicate that AtCSP2 has negative effects on salt stress tolerance in Arabidopsis.

To further examine the effect of AtCSP2 on salt stress tolerance, AtCSP2-overexpressing lines (35S::AtCSP2–18, 20, and 26) 32 were used for analysis. Eight-day-old seedlings were transferred to MS medium supplemented with 185 mM NaCl and grown for 2.5 d. The survival rate was calculated 4 d after transfer back to MS medium. All 35S::AtCSP2 lines exhibited substantially lower survival rates than wild-type plants after salt stress (Fig. 2A and B). The survival rate of wild type plants was 86.7%, while 3 35S::AtCSP2 lines showed survival rates of 29.3%, 14.7%, and 50.7% (for lines 18, 20, and 26, respectively) (Fig. 2B). Taken together, these data indicate that, in addition to freezing tolerance, AtCSP2 acts as a negative regulator of salt tolerance in Arabidopsis.

Our data suggest that AtCSP2 is involved in attenuating salt stress signaling. It has been reported that expression of CBF3 and RD29A (a CBF regulon gene) is significantly up-regulated by salt stress. 37,38 In addition, according to data from the public microarray database (BAR; http://www.bar.utoronto.ca/), CBF3 is transiently upregulated in both shoots and roots during a 24-h salt treatment. Following the initiation of salt treatment, the CBF3 transcript reaches maximum levels at hour 3 in shoots (6.5-fold up-regulated) and at hour 6 in roots (33.3-fold upregulated). Furthermore, overexpression of CBF3 confers salt stress tolerance in Arabidopsis. 39 Our previous research showed that AtCSP2 negatively regulates expression of CBF3 and RD29A during cold acclimation. 32 From these data, it is reasonable to speculate that AtCSP2 also attenuates the CBF pathway under salt stress conditions.

Attenuation of stress-signaling pathway by negative regulators is an important process for the proper management of abiotic stress responses. Under cold stress, the expression of CBF genes peaks within several hours and decreases quickly thereafter. 40,41 It has been demonstrated that expression of CBF3 is tightly regulated by negative regulators HOS1 (RING E3 ligase) and MYB15 (R2R3 type MYB transcriptional factor) during cold acclimation. 32,43 Indeed, constitutive overexpression of one of the CBF genes resulted in severe growth retardation. 44,45 Therefore, it is thought that negative regulators are necessary for preventing detrimental effects caused by over-activation of stress responses during the process of plant growth and development.

Our current and previous studies have shown that AtCSP2 negatively regulates abiotic stress tolerance and seed germination by altering expression of genes critical for these responses (Fig. 3). However, the mechanisms by which AtCSP2 regulates gene expression is still unclear. As an RNA chaperone, AtCSP2 may regulate processing and stability of mRNA and/or translation to attenuate signaling during abiotic stress and germination. Consistent with this hypothesis, our preliminary data using yeast 2-hybrid system suggested that AtCSP2 interacts with the nuclear poly(A)-binding proteins PABN2 and PABN3 (data not shown). In order to further characterize the function of AtCSP2, it will be necessary to identify functional complex involving AtCSP2 and target mRNAs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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