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BRIEF COMMUNICATION

Effect of low temperature on *profilins* and *ADFs* transcription and actin cytoskeleton reorganization in *Arabidopsis*

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Abstract

In the present study, we found that the expression patterns of the vegetative *profilins* and actin depolymerizing factors (*ADF*s) were specifically altered under low temperature (17 ºC) stress using a real-time PCR. Our results show that also reorganization of the actin cytoskeleton was triggered by a low temperature. Facilitation of microfilament (MF) assembly by phalloidin treatment resulted in an enhanced low temperature stress tolerance, whereas blocking MF assembly with latrunculin B resulted in enhanced low temperature stress sensitivity. Our results show that the specific members of the vegetative profilins and ADFs might participate in regulating the response of plants to a low temperature stress, and the actin cytoskeleton is vital for the tolerance of *Arabidopsis* seedlings to low temperature stress.

Additional key words: latrunculin B, microfilaments, phalloidin, stress tolerance.

Recently, the changes in plant actin cytoskeleton have been implicated in response to numerous environmental stimuli, *e.g.*, gravity, mechanical stress, salinity, and low temperature (Wasteneys and Yang 2004, Huang *et al.* 2007). In tobacco BY-2 cells, a low temperature stimulates the depolymerization of microfilaments (MFs) (Pokorna *et al.* 2004). In winter oilseed rape suspension cells, the freezing-induced depolymerization of MFs was sensitive to the cell growth phase (Egierszdorff and Kacperska 2001). Rearrangement of MFs could activate the expressions of cold-inducible genes, such as *cas30* and *BN115*, during a cold stress (Orvar *et al.* 2000, Sangwan *et al.* 2001).

The function of MFs depends on the balance between G-actin and F-actin rates, and many actin-binding proteins (ABPs) can regulate these rates. Profilin (PRF) and the actin depolymerizing factor (ADF) in vertebrates and yeast are two of the most highly and widely expressed types of ABPs. Increasing evidence has suggested that also in plants, PRFs and ADFs play prominent roles in modulating the dynamics of MFs and responding to a wide variety of environmental stresses (Aderem 1992, Machesky and Pollard 1993, Bamburg 1999). An ADF can be activated during a cold acclimation and increases freezing tolerance in wheat (Ouellet *et al.* 2001). In the present study, the differential expression patterns of plant vegetative *PRFs* and *ADFs* genes suggest their roles in response to low temperature stress. In addition, our results also suggest that the actin cytoskeleton plays a crucial role in responses to low temperature stress in *Arabidopsis*.

To obtain a further insight into the roles of vegetative PRFs and ADFs under low temperature stress, 2-week-old *Arabidopsis thaliana* L. seedlings exposed to a low temperature stress (17 °C) were sampled at designated time points (0, 1, 3, 6, 12, and 24 h) for analysis of gene expression by quantitative real-time PCR. Control *A. thaliana* seedlings were kept at a temperature of 22 ºC. The real-time PCR analysis was performed according to the method described by De Silva *et al.* (2011). Primers are shown in Table 1.

For visualization of the dynamic MF networks in

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Abbreviations: ABPs - actin-binding proteins; ADFs - actin depolymerizing factors; GFP - green fluorescent protein; Lat B - latrunculin B; MF - microfilament; PRF - profilin.
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Table 1. List of primers used for the real-time PCR experiments conducted in this study.

Genes	Forward primer sequences (5’-3’)	Reverse primer sequences (5’-3’)
Profilin 1	GAGCTGTGATCCGAGGGAAG	TCATCGTAGAAGCCAAAGACCA
Profilin 2	GGTTGTCCAAGTCTACAAACCAAA	GCCACGACATCTTTCTTCTTCTC
Profilin 3	ATGTTGCAGGCAACCGCCTC	TGGGGCAAGTGTTCCAGGTGT
ADF 1	GGGAACTAGATGGGATTCAAGTAGAG	GGCTCCTGAAAACATCGAGATC
ADF2	GCCAAAGTGAGAGACAAGATGATTT	GAATTCCATCTAGTTCTCTCTTGAACCT
ADF3	TCGGTTGAATCAAACTTTTTCGT	GGTACCGTCACAGCAAACTTTAGG
ADF4	TGTTTTCTATTCTCTTACAGTCTCTTGTGA	CAGAGAACAGACCAGACAGATAGAATG
ADF5	CGTTTGTGTTTTGATTGTGTTGTTAA	CCGTTACTCGTAGGACAAATTCG
ADF6	AGCTACTGATCCTACTGAGGTTGATCT	GCAATCTTGCTTGCTCTCAGTTC
ADF9	CTCAAAATATAACGAAAGAACAAGAAGACA	CACTCGTCGCCGTCTTCAA
UBQ-10	AACTTTGGTGGTTTGTGTTTTGG	TCGACTTGTCATTAGAAAGAAAGAGATAA

hypocotyl cells, we used an *Arabidopsis* line FABD2 expressing a fusion construct of the green fluorescent protein (GFP) with actin-binding domain 2 (ABD2) of the plant actin-binding protein fimbrin (Voigt *et al.* 2005). The seedlings were observed with respect to a low temperature stress-induced dynamics of MF reorganization. Fluorescence images were captured with a confocal laser scanning microscope (*Olympus DP72*, Tokyo, Japan) equipped with a 40× objective. The GFP fluorescence images were collected using a 488 nm excitation laser line and a 505 - 530 nm band pass emission filter. To measure the amount of F-actin in hypocotyl cells, the fluorescent images were captured under the same conditions. The images were analyzed using *Image J*, and the amounts of F-actin were calculated by measuring the number of pixels per square millimeter of individual cells.

Four-day-old seedlings were pre-treated with latrunculin B (Lat B) at 22 °C for 60 min (Sangwan *et al.* 2001) or phalloidin for 12 h (Wang *et al.* 2009), then the seedlings were subjected to the low temperature stress as described above. Five days after the treatments, we measured the root lengths.

In higher plants, profilin 1 (PRF1), profilin 2 (PRF2), and profilin 3 (PRF3) are present in all vegetative tissues (Kandasamy *et al.* 2002). Under the low temperature stress, the transcriptions of *PRF1* and *PRF2* genes were increased. The *PRF2* exhibited the highest expression among the three genes, and it was highest after 6 h at the low temperature. The transcription of *PRF1* increased mildly (Fig. 1*A*). It is possible that *PRF2* played an important role in the response to the low temperature stress and that the functions of PRF1 and PRF2 are partially redundant.

The *Arabidopsis ADF* gene family is phylogenetically grouped into four ancient subclasses: subclass I *ADFs* includes *ADF1*, *2*, *3*, and *4*; subclass II includes IIa (*ADF7* and *10*) and IIb (*ADF8* and *11*); subclass III includes *ADF5* and *9*; *ADF6* is the only subclass IV member. The members of subclasses I, III, IV are expressed in vegetative tissues (Ruzicka *et al.* 2007). Here, we examined the transcription of *ADF* subclasses I, III, and IV under the low temperature stress. The expression patterns of the *ADF*s in subclasses I and III

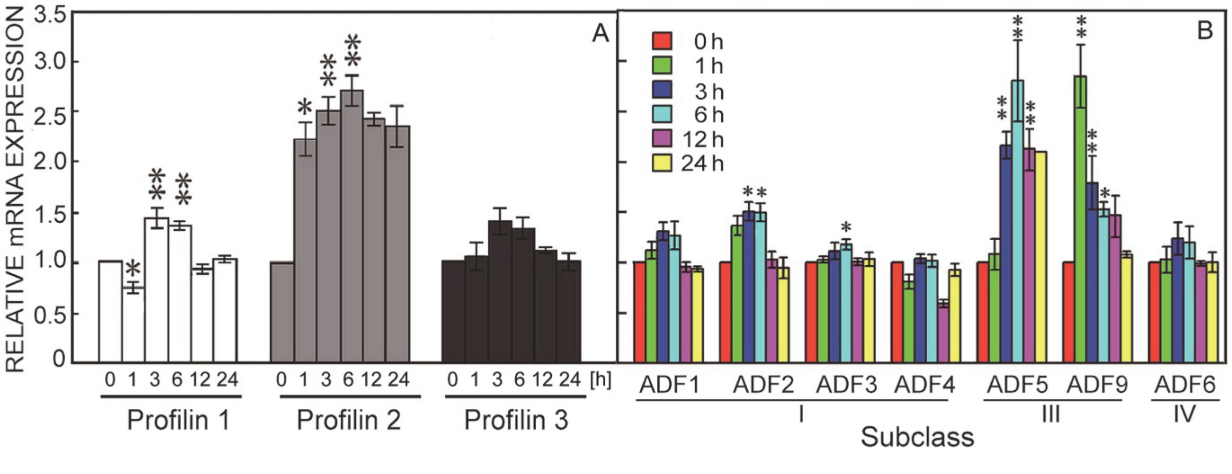

Fig. 1. Expression profiles of genes coding *Arabidopsis* profilins (*A*) and ADFs (*B*) under a low temperature stress of different duration. Expression was analyzed by real-time PCR with *UBQ-10* as internal control. The *vertical bars* indicate means ± SE of three biological replicates. Statistical significance was determined (* - $P \leq 0.05$, ** - $P \leq 0.01$) by Student’s *t*-tests.

were induced significantly except *ADF1* and *ADF4*. The transcriptions of *ADF2* and *ADF3* reached a maximum after a 6-h stress, and the transcription of *ADF9* reached a maximum already after 1 h. This result might indicate that *ADF9* responded quickly to the low temperature, whereas *ADF5* increased continuously. The *ADF4* and *ADF6* were induced marginally (Fig. 1*B*). Overall, the transcription of the *ADFs* in subclasses I, III, and IV were induced by the low temperature stress, and the subclass III *ADFs* responded most strongly. These results imply that the subclass III *ADFs* might be the most important response genes under the low temperature stress.

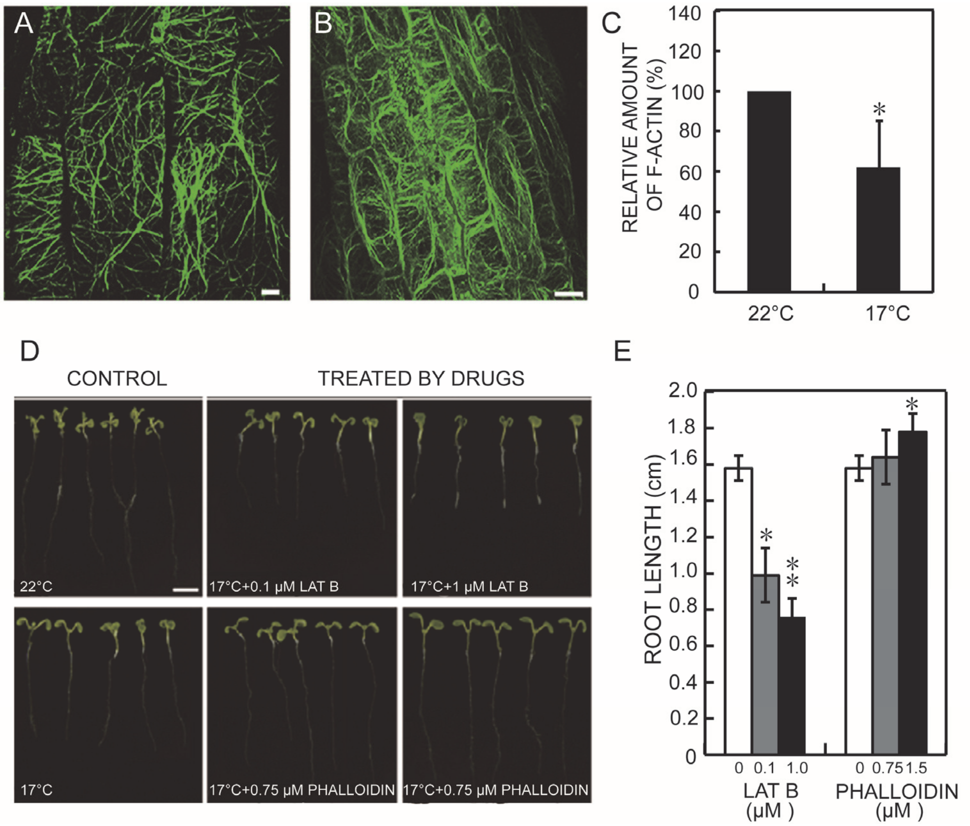

Fig. 2. The effect of low temperature stress on microfilament (MF) organization in *Arabidopsis* hypocotyl cells. Fluorescence images of MFs in the hypocotyl cells under a control temperature of 22 ºC (*A*) and a low temperature of 17 °C for 24 h (*B*); the *bar* is 10 µm. *C* - Measurements of the relative amount of F-actin in seedlings grown at 22 and 17 °C for 24 h. *D* - Seedlings grown at the control temperature for 4 d, then transferred to 17 °C for 5 d, and subsequently grown at 17 °C with MF-targeting drugs at varying concentrations: 0.1 µM latrunculin (Lat) B, 1 µM Lat B, 0.75 µM phalloidin, and 1.5 µM phalloidin. *E* - The root length of seedlings subjected to 17 °C and Lat B or phalloidin. Means ± SE, *n* = 20. Statistical significance was determined (* - $P \leq 0.05$, ** - $P \leq 0.01$) by Student's *t*-tests.

The results indicate that the low temperature stress impacted expression profiles of *profilins* and *ADFs*. Then, to investigate whether they respond to a low temperature by influencing the organization of the actin cytoskeleton, we examined the MFs in *Arabidopsis* hypocotyl cells. Normally, the actin cytoskeleton in hypocotyl cells is arranged in a fine structure with longitudinal cables and transverse perinuclear arrays (Fig. 2*A*). After the treatment at 17 °C for 24 h, the longitudinal cables disappeared, and the transverse MFs were neatly arranged but not around the nucleus (Fig. 2*B*). The relative amount of F-actin decreased (Fig. 2*C*). These results suggest that the observed MF cytoskeletal reorganization was closely related to low temperature tolerance. To test this hypothesis, we used Lat B and phalloidin to modulate the MF organization and examined the effects of the drugs on *Arabidopsis* seedlings under the low temperature stress. Four-day-old seedlings were transferred to a Murashige and Skoog medium supplemented with Lat B or phalloidin and then subjected to the temperature of 17 °C for 5 d (Fig. 2*D*). The root length of the seedlings decreased as the Lat B concentration increased, and it increased when phalloidin was present (Fig. 2*E*), with the latter phenomenon occurring in a concentration-dependent manner. These results suggest that the treatment with Lat B, a destabilizer, reduced the ability of the *Arabidopsis* seedlings to withstand the low temperature stress, whereas their ability to endure this stress was increased when phalloidin was added as stabilizer. Therefore, we conclude that the rearrangement of the MFs could be viewed as active response to the low temperature stress.

In conclusion, the results show that the low temperature stress caused reorganization of the actin cytoskeleton; depolymerization of the MFs induced a plant sensitivity to the low temperature stress, and polymerization of the MFs improved the ability of the plants to withstand the low temperature stress. We should clarify the potential mechanism underlying the response of profilins and ADFs to low temperature stress in future studies.

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