Rapid Communication

Cortical actin filaments in guard cells respond differently to abscisic acid in wild-type and abi1-1 mutant Arabidopsis

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Abstract. Cortical actin filaments in guard cells of Commelina communis L. show signal-specific organization during stomatal movements [S.-O. Eun and Y. Lee (1997) Plant Physiol 115: 1491–1498; S.-O. Eun and Y. Lee (2000) Planta 210: 1014–1017]. To study the roles of actin in signal transduction, it is advantageous to use Arabidopsis thaliana (L.) Heynh., an excellent model plant with numerous well-characterized mutants. Using an immunolocalization technique, we found that actin deployments in guard cells of A. thaliana were basically identical to those in C. communis: actin proteins were assembled into radial filaments under illumination, and were disassembled by ABA. In addition, we examined actin organization in an ABA-insensitive mutant (abi1-1) to test the involvement of protein phosphatase 2C (PP2C) in the control of actin structure. A clear difference was observed after ABA treatment, namely, neither stomatal closing nor depolymerization of actin filaments was observed in guard cells of the mutant. Our results indicate that PP2C participates in ABA-induced actin changes in guard cells.

Key words: Abscisic acid – Actin – Arabidopsis (abi1-1 mutant) – Guard cells – Mutant (abi1-1)

Abscisic acid (ABA), a potent stimulus for stomatal closing, induces a drastic change in the actin organization of C. communis guard cells (Eun and Lee 1997). Implication of actin in ABA signaling is supported by a study using a stabilizer for actin filaments in which an application of phalloidin interfered with ABA-induced stomatal closing (Kim et al. 1995). Furthermore, an electrophysiological study showed a functional association between actin and K+–channel activities (Hwang et al. 1997).

Stomatal regulation by a guard-cell-specific, ABA-activated kinase (Li et al. 2000) and suppression of stomatal responses to ABA by inhibitors and mutations of protein phosphatases (Leung et al. 1994; Meyer et al. 1994; Schmidt 1995), provide evidence that protein phosphorylation and dephosphorylation play important roles in ABA signaling in guard cells. Protein (de)phosphorylation is also a major mechanism by which actin polymerization is modulated in animal cells. Recent literature implicates protein kinases and phosphatases in the control of the actin cytoskeleton in plant cells as well (Menzel et al. 1995; Ressad et al. 1998; Smertenko et al. 1998; Yokota et al. 2000).

Actin filaments in mature guard cells have been visualized in many species including A. thaliana (Kost et al. 1998; reviewed in Hwang et al. 2000). However, with the exception of C. communis, stimulus-dependent actin reorganization in guard cell signaling has not been investigated in detail. In the present work, we aimed to investigate actin deployments in guard cells of A. thaliana and to test whether a protein phosphatase type 2C (PP2C) is a possible actin modulator in plant cells, by comparing ABA-dependent actin reorganization in guard cells of the wild type and those of abi1-1, a mutant which is the result of a single base substitution in the gene encoding PP2C (Leung et al. 1994; Meyer et al. 1994).

Plant material

Wild type seeds of Arabidopsis thaliana (L.) Heynh (ecotype Landsberg erecta) were germinated under continuous light at 22 °C on a solid medium containing Hoagland solution (Sigma, St. Louis, Mo., USA) and 0.7% agar. In order to confirm the ABA-insensitivity of the abi1-1 line (Koornneef et al. 1984), the mutant line was germinated in the presence of 1–10 μM ABA. Abscisic acid as low as 1 μM in the germination medium was enough to select abi1-1 seedlings, the guard cells of which were totally insensitive to ABA when tested on mature leaves. Germinated seedlings were transferred to soil and grown in a growth chamber with cycles of 8 h light/16 h dark and of 22/18 °C, respectively, to promote leaf

Abbreviations: ABA = abscisic acid; PP2C = protein phosphatase type 2C

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development. Cool-white fluorescent lamps provided light at a photon flux density (PFD) of 100–150 μmol m⁻² s⁻¹.

Assay of stomatal aperture

Stomata were opened by floating rosette leaves of 5- to 6-week-old plants on a medium containing 50 mM KCl, 10 mM K⁺-Mes buffer (pH 6.1) under white light of PFD 150 μmol m⁻² s⁻¹ for 2–3 h. Abscisic acid was added to the medium to 10 μM, and leaves were treated for 50 min. Epidermal tissue was peeled from the lower surface of leaves at the end of each treatment. Stomatal apertures of mature guard cells (20–22 μm long) were measured using an eyepiece micrometer. The average and the standard errors of combined measurements from separate experiments are shown. Chemicals were purchased from Sigma (St. Louis, Mo., USA) unless otherwise specified.

Immunolocalization of actin filaments in guard cells

For visualization of actin filaments in A. thaliana guard cells, we modified the technique developed for labeling the actin cytoskeleton in C. communis guard cells (Eun and Lee 1997). In brief, epidermal peels were fixed for 1 h in 50 mM Pipes buffer (pH 6.8) containing 200 μM m-maleimidobenzoyl N-hydroxysuccinimide ester, 1% (v/v) dimethyl sulfoxide, 0.05% (v/v) Triton X-100, 5 mM EGTA, 5 mM MgCl₂, and 0.5 mM phenylmethylsulfonyl acid. After the samples had been washed with 50 mM Pipes buffer (pH 6.8) containing 5 mM EGTA and 5 mM MgCl₂, they were frozen in liquid nitrogen and fractured. The epidermal fragments were incubated with actin antibody (Amersham), with biotinylated goat anti-mouse IgM (Amersham) and with fluorescein isothiocyanate-conjugated streptavidin. Samples were observed under a fluorescence microscope (Nikon, Optiphot-2). Images were recorded on T-Max 400 film (Kodak, NY, USA) using a Microflex UFX-DX photographic attachment (Nikon, Microflex UFX-DX).

Organization of actin filaments in guard cells of the wild type

Well-established genetic information and methods of molecular manipulation, together with numerous mutants, make Arabidopsis an excellent model plant to study signal transduction of plant cells. For example, the mutants abil-1 and abil-2 were useful for demonstrating PP2C involvement in ABA-induced activation of anion channels (Allen et al. 1999).

Our immunofluorescence micrographs show that the overall arrangements of actin in guard cells of wild-type A. thaliana were consistent with those seen in C. communis (Eun and Lee 1997): actin was assembled into radial filaments in the cortex of guard cells when stomata were open under illumination (Fig. 1A), and they were de-polymerized when stomata were closed after exposure to ABA (Fig. 1B). In some of those ABA-treated guard cells, long filaments of a random distribution were observed in the subcortical region (Fig. 1C).

Organization of actin filaments in guard cells of abil-1

Guard cells of abil-1 plants that were selected for their ABA insensitivity during germination were totally insensitive to ABA (Fig. 2). When stomata were open under white light, actin filaments in guard cells of abil-1 plants were organized similarly to those of the wild type (Fig. 1D). However, a clear difference was noticed after ABA treatment: actin arrays in the mutant seemed to be persistent (Fig. 1E,1F) unlike those of the wild type, which lost their filamentous architecture in the presence of ABA. As in other types of cells, the density and the degree of actin polymerization in guard cells of a single treatment group vary. To ensure that the observed actin patterns are representative ones, we classified guard cells into three groups according to their actin patterns in the cortex: (a) cells with long radial filaments, (b) cells with short fragments in the radial arrangement, and (c) cells with a mainly spotty pattern. Table 1 indicates that the long radial actin filaments of illuminated guard cells are disassembled by ABA in the wild type, but not in abil-1.

![Fig. 1. Actin organization in A. thaliana guard cells.](image-url) Rosette leaves of the wild type (A–C) and abil-1 mutant (D–F) were floated on 50 mM KCl/10 mM K⁺-Mes buffer (pH 6.1) under white light. The leaves were further illuminated for 50 min in the absence (A, D) or the presence (B, C, E, F) of 10 μM ABA, and then epidermal tissues were peeled, fixed and labeled with actin antibodies. Each panel shows a pair of labeled guard cells except in B, where only the guard cell on the left is labeled. The diffuse staining in the guard cell on the right is from auto-fluorescence that is commonly observed among unlabeled guard cells. The spotty fluorescence in the cell on the left in B is in contrast to the long filamentous pattern in guard cells of A, D, E and F. In F, the guard cells (white) and the stomatal opening (black) are outlined. The fluorescent images do not show the actual size of stomata, since it is altered during fixation (Eun and Lee 1997), and differs depending on the focal plane of observation. The cells are focused near the periclinal surface to show cortical actin filaments except in C, where the focus was at a subcortical region near the nucleus. Bar = 10 μm.