ations occur in human tumor-derived cell lines and solid tumors that display a high frequency of LOH on chromosome 11q23. Fifty-five percent (6 out of 11) of the tumors with *PPP2R1B* alterations have full or partial deletions of one allele, combined with deletions or missense alterations in conserved regions of the other allele, leaving the cells functionally null for *PPP2R1B*. The genes encoding the many isoforms of the PP2A-B subunit make up a large and diverse multigene family. At least five isoforms of the PP2A-B subunit have been discovered and map to other regions of the human genome that display frequent LOH in cancer (*30*). This suggests that the search for mutations in other components of the PP2A family and the characterization of the roles PP2A plays in tumor development may open new avenues for diagnosis and therapy of cancer.

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18. Gel-purified PCR products were cloned with the TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA). DNA from a single transformed colony was isolated and sequenced by means of automated DNA sequencing.
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31. Cells were lysed, proteins were separated by electrophoresis, and gels were transferred to Millipore Immobilon-P membrane. Immunodetection was carried out with goat anti-human PP2A-Aβ immunoglobulin and secondary horseradish peroxidase–conjugated donkey anti-goat immunoglobulin (Santa Cruz Biotechnology). Immunodetection was carried out with enhanced chemiluminescence (Amersham Life Sciences). Goat anti-human actin immunoglobulin was added as a control.
32. Cell lysates were immunoprecipitated with rabbit anti-human PP2A-C immunoglobulin (Promega) and protein G–agarose (Boehringer Mannheim). The precipitate was collected by centrifugation, and the pellet was washed with cold phosphate-buffered saline. The precipitate was then analyzed on an 8% SDS-polyacrylamide gel. Protein immunoblot detection was carried out with purified goat anti-human immunoglobulin against PP2A-Aβ and PP2A-C and secondary horseradish peroxidase–conjugated donkey anti-goat immunoglobulin (Santa Cruz Biotechnology).

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Role of Farnesyltransferase in ABA Regulation of Guard Cell Anion Channels and Plant Water Loss

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Desiccation of plants during drought can be detrimental to agricultural production. The phytohormone abscisic acid (ABA) reduces water loss by triggering stomatal pore closure in leaves, a process requiring ion-channel modulation by cytoplasmic proteins. Deletion of the *Arabidopsis* farnesyltransferase gene *ERA1* or application of farnesyltransferase inhibitors resulted in ABA hypersensitivity of guard cell anion-channel activation and of stomatal closing. *ERA1* was expressed in guard cells. Double-mutant analyses of *era1* with the ABA-insensitive mutants *abi1* and *abi2* showed that *era1* suppresses the ABA-insensitive phenotypes. Moreover, *era1* plants exhibited a reduction in transpirational water loss during drought treatment.

Protein farnesylation, a posttranslational modification process, mediates the COOH-terminal lipidation of specific cellular signaling proteins, including Ras, guanosine triphosphatases (GTPases), trimeric GTP-binding protein, nuclear lamin B, and yeast mating pheromone **a**-factor (*1*). In each of these cases, farnesylation increases membrane association and cellular activity of these proteins. Thus, farnesylation plays an essential role in signal transduction cascades of yeast and mammalian cells (*1*).

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In plant cells, farnesyltransferase (FTase) activities have been identified, and changes in FTase activity during cell growth and division have been demonstrated (*2*, *3*). In *Arabidopsis*, recessive mutations in the *ERA1* gene, which encodes the FTase β subunit, were identified and have been shown to prolong seed dormancy due to an enhanced response to ABA (*4*). This suggests that farnesylation may be essential for negative regulation of ABA signaling in seeds.

Plants lose over 90% of water by transpiration through stomatal pores formed by pairs of guard cells on the leaf surface. The hormone ABA is synthesized in response to drought stress and triggers a signaling cascade in guard cells that results in stomatal closing (*5*, *6*). Studies have indicated that activation of anion channels in the plasma membrane of guard cells is required during ABA-induced stomatal closing (*6–8*). Coupling of intracellular signaling proteins to membrane ion channels is essential

for this ABA-mediated response (*6*, *8*). To investigate whether cytoplasmic regulators are linked to ABA regulation of ion channels by farnesylation, we analyzed the effects of two competitive FTase inhibitors, α-hydroxyfarnesylphosphonic acid (HFPA) (*9*) and manumycin (*10*) on ion channels and stomatal movements. Whole cell patch-clamp current recordings (*11*) showed that, in the presence of ABA, exposure of *Arabidopsis* guard cells to HFPA significantly increased ABA activation of anion currents (Fig. 1, A and B) ($P < 0.004$). In the absence of ABA, HFPA did not enhance anion currents (Fig. 1B) ($P > 0.5$). Stomatal aperture measurements (*12*) showed that HFPA treatment also increased the ABA sensitivity of stomatal closing (Fig. 1C) ($P < 0.001$ at 5 μM ABA). ABA-hypersensitive stomatal closure was also detected by use of a different FTase inhibitor, manumycin ($n = 3$ experiments, 480 stomata).

Previous studies have shown that a pea FTase β subunit is expressed in meristematic tissues (*13*), and mRNA of the *Arabidopsis* *ERA1* FTase accumulates in flower buds (*4*). To determine whether *ERA1* is also expressed in guard cells, we analyzed transgenic plants expressing *ERA1* promoter–GUS constructs in mature leaves (*14*). In intact leaves, *Arabidopsis* guard cells showed GUS activities, indicating that the *ERA1* gene is expressed in guard cells (Fig. 1D), in addition to expression in other vegetative tissues.

A fast-neutron mutant allele, *era1-2*, in which the entire *ERA1* gene is deleted, was used to determine whether *ERA1* directly affects guard cell ABA signaling. Stomatal aperture measurements showed that the *era1-2* mutation caused ABA hypersensitivity of stomatal closing (Fig. 2A) ($P < 0.001$ at 10 μM ABA). In the absence of exogenous ABA, stomatal apertures in *era1-2* were slightly smaller than those in wild-type (WT) control plants under the imposed conditions (Fig. 2A). When the KCl concentration in solutions and light intensity were increased during stomatal opening, stomata in *era1-2* opened as wide as those in WT plants but continued to show ABA hypersensitivity of stomatal closing (*15*), indicating that smaller stomatal apertures in *era1-2* might be due to hypersensitivity to endogenous ABA.

We examined whether the *era1* mutation affects ABA regulation of guard cell anion channels (*11*). In the absence of ABA, *era1-2* did not cause constitutive enhancement of anion currents under the imposed conditions (Fig. 2, B and C). In the presence of 10 μM ABA, *era1-2* mutation consistently caused increased activation of anion currents compared to WT [Fig. 2, B ($n = 30$) and C ($n = 49$)] (*16*). Current voltage analyses showed that ABA-activated steady-state anion currents were substantially larger in *era1-2* than in WT guard cells (Fig. 2D) ($P < 0.003$ at −145 mV). Interestingly, transient depolarization-activated outward-rectifying K^+ currents in the plasma membrane of guard cells were enhanced by the *era1* mutation in the absence of ABA. For example, peak currents at +100 mV were 254 ± 24 pA ($n = 18$) in WT and 411 ± 36 pA ($n = 15$) in *era1-2* guard cells (*11*).

The above data show that deletion of the *ERA1* FTase gene causes ABA hypersensitivity of anion-channel activation and of stomatal closing. The findings that FTase inhibitors mimic the *ERA1* deletion mutation in WT plants (Fig. 1) suggest that ABA hypersensitivity in *era1-2* is not due to a long-term effect of FTase deletion during guard cell maturation. Rather, these data suggest that FTases modulate a negative regulation pathway of guard cell ABA signaling.

The ABA-insensitive mutant loci *abi1* and *abi2* (*17*) encode type 2C protein phosphatases (PP2C) (*18*–*20*). Recent studies have led to models in which these PP2Cs may function as negative regulators in ABA signaling (*8*, *21*). ABI1 and ABI2 do not have farnesylation consensus sequences. To test whether the *era1* and *abi* mutations interact genetically, we generated homozygous double mutants of *era1/abi1* and *era1/abi2* (*22*). As previously reported, activation of anion currents by ABA is impaired in the *abi1* and *abi2* mutants (Fig. 3, A and B), consistent with impairment in ABA-induced stomatal closing (*8*, *17*, *23*). ABA (10 μM) was sufficient to activate anion channel currents in both the *era1/abi1* (Fig. 3A) and *era1/abi2*

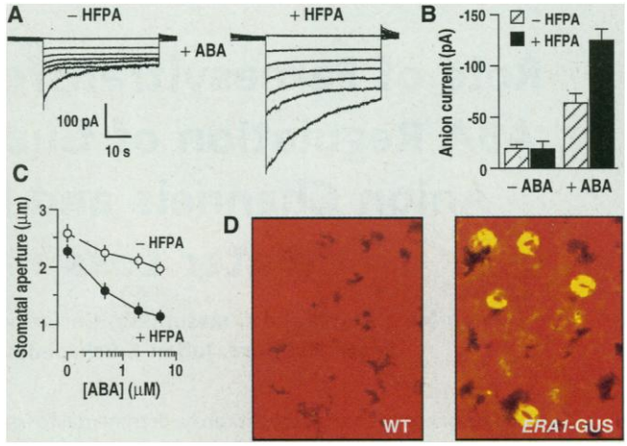

Fig. 1. Protein farnesyltransferase inhibitors affect ABA signaling in *Arabidopsis* guard cells. **(A)** The FTase inhibitor HFPA causes increased activation of ABA-regulated anion currents in *Arabidopsis* guard cells. Guard cells were treated with 10 μM ABA in the absence (–HFPA) or presence (+HFPA) of 2 μM HFPA. Whole cell patch-clamp currents were recorded from a holding potential of +30 mV with membrane voltage steps ranging from −145 mV to +35 mV in +30-mV increments (*8*). **(B)** Average magnitudes of steady-state anion currents recorded at −145 mV in the absence and presence of 2 μM HFPA with or without 10 μM ABA. Experiments were performed as in (A). Currents at the end of −145-mV voltage pulses were averaged ($n = 10$ to 12 guard cells for each condition) (*11*). **(C)** HFPA causes increased ABA sensitivity of stomatal closing. Intact leaves were floated in solution with or without 2 μM HFPA under light for 2 hours to induce stomatal opening. Then ABA at indicated concentrations was added to the bath solution to assay stomatal closing (*12*). Data from four separate experiments ($n = 80$ stomata per data point) are shown. **(D)** ERA1 FTase expression patterns in intact leaves of WT *Arabidopsis* and *ERA1*-promoter–GUS transgenic plants. Transgenic plants contained an *ERA1*-promoter GUS fusion (*ERA1*-GUS) and were exposed to blue light. Gene expression resulted in cleavage of the Imagene Green dye to give yellow fluorescence on a red chlorophyll autofluorescent background in intact leaves. Wild-type plants or empty vector–transformed plants were used as controls.

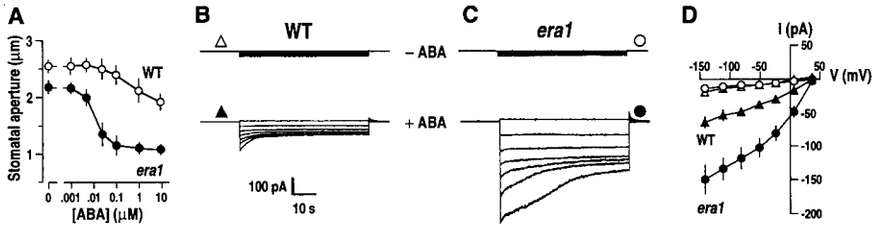

Fig. 2. The FTase deletion mutant *era1-2* causes ABA hypersensitivity of anion-channel activation and of stomatal closing. **(A)** Comparison of ABA-induced stomatal closing in wild-type (WT) and the *era1-2* mutant. Data from three representative ($n = 60$ stomata per data point) experiments out of nine are shown. **(B and C)** Whole-cell currents recorded in the absence (–ABA) or presence of 10 μM ABA (+ABA) in WT (B) and in *era1-2* mutant (C) guard cells. ABA (10 μM) was added to pipette and bath solutions (*8*). Voltage protocols in (B) and (C) were the same as in Fig. 1A. **(D)** Steady-state current-voltage relationships show increased ABA activation of anion currents in *era1-2* guard cells compared with those in WT guard cells. Recordings were performed as in (B) and (C) ($n = 14$ to 27 cells averaged per curve). Symbols are as in (B) and (C).

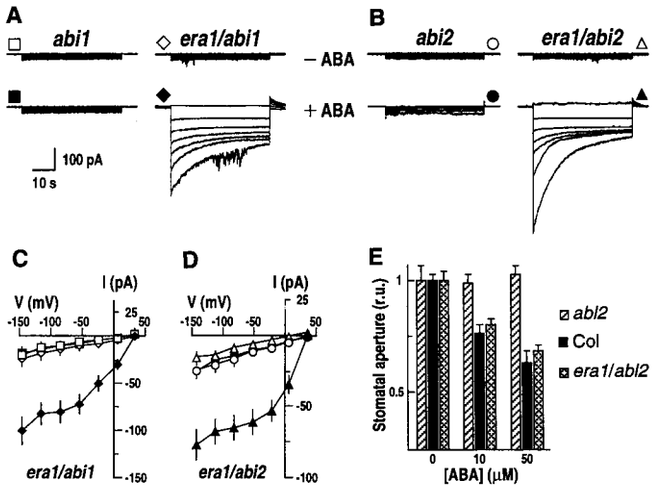

Fig. 3. ERA1 FTase deletion suppresses the ABA-insensitive (semi-) dominant mutations in *era1/abi1* and *era1/abi2* homozygous double mutants. (**A**) ABA activation of anion-channel currents was analyzed in the absence (–ABA) or presence of 10 μM ABA (+ABA) in *era1/abi1* double-mutant guard cells (right) and compared to *abi1* mutant guard cells (left). (**B**) ABA activation of anion-channel currents was analyzed in the absence or presence of 10 μM ABA in *era1/abi2* double-mutant guard cells and compared to *abi2* mutant guard cells. Time and current scale bars refer to both (A) and (B). (**C** and **D**) Steady-state current-voltage relationships as recorded in (A) and (B), respectively. Symbols in (C) and (D) correspond to those in (A) and (B), respectively (n = 5 cells for *abi1* and *abi2*; n = 10 to 16 cells for *era1/abi1* and *era1/abi2* per condition). (**E**) ERA1 FTase mutation partially suppresses the ABA insensitivity of stomatal closing in the *abi2* mutant. ABA-induced stomatal closures in *abi2* and *era1/abi2* double mutant were compared. Stomatal apertures of *abi2*, Colombia WT, and *era1/abi2* were normalized with respect to the apertures in the absence of ABA, respectively, for comparison; r.u., relative unit. Data from three separate experiments are shown (n = 60 stomata per bar).

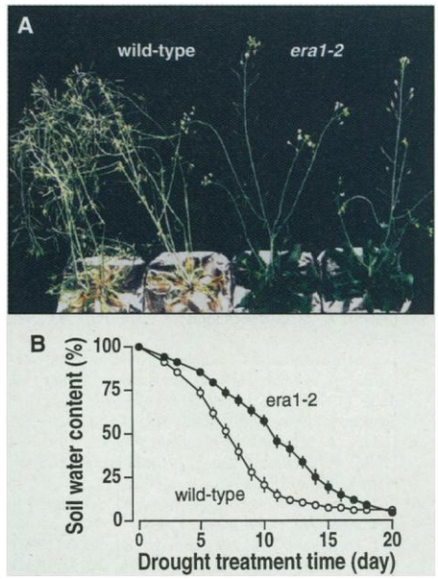

Fig. 4. Reduced wilting of *era1-2* plants during drought stress. (**A**) Both WT and *era1-2* plants were grown under normal watering conditions for ~21 days and then subjected to drought stress by completely terminating irrigation. Pots were covered to minimize soil evaporation. Photo shows four representative plants out of 32 after 12 days of drought stress (*26*). (**B**) Changes in soil water content during drought stress treatment of WT and *era1-2* plants.

double mutants (Fig. 3B). Steady-state current-voltage relations showed that ABA activation of anion currents was restored in these two double mutants (Fig. 3, C and D). Furthermore, ABA-induced stomatal closing was restored in the *era1/abi1* double mutant (*24*) and in the *era1/abi2* double mutant (Fig. 3E). Stomatal responses of the *era1/abi2* double mutants were similar to those of WT plants, but did not fully show the *era1* phenotype. Stomata of *era1/abi1* showed less ABA sensitivity than *era1/abi2*, but suppression of the *abi1* phenotype was clear (*24*). The ABA insensitivities of *abi1* and *abi2* in seed germination (*17*) were also suppressed in these double mutants with a sensitivity sequence of *era1* > *era1/abi1* ≈ *era1/abi2* > Ler WT > Col WT > *abi1* ≈ *abi2*, where Col WT and Ler WT are Colombia WT and Lansberg *erecta* WT, respectively (*25*).

Because deletion of the *ERA1* FTase potentiates ABA-induced anion currents and stomatal closing in epidermal strips and partially suppresses the *abi1* and *abi2* mutations, we investigated whether whole plant transpiration is reduced during drought. Both WT plants and *era1-2* plants were grown and watered for ≈21 days, and then subjected to drought stress by terminating irrigation (*26*). Wild-type and *era1-2* plants showing similar developmental stages and similar number of leaves were specifically selected for drought treatments, and evaporation from soil was minimized by covering the soil in pots. After 12 days of drought treatment, WT plants showed severe wiltiness and chlorosis of rosette leaves. In contrast, *era1-2* plants were turgid and leaves remained green (Fig. 4A). Soil water content in pots of *era1-2* plants decreased more slowly during drought stress than those of WT plants (Fig. 4B), consistent with plant phenotypes. The *era1-2* plants also showed slowed growth, which may be partially due to increased stomatal closing and reduced carbon fixation, or to *ERA1* expression in several vegetative tissues (*4*, *27*), or both. When pots were not covered, the reduced wiltiness of *era1-2* plants was visible although less pronounced. Transpiration rates of WT leaves were 2.8 ± 0.3–fold larger than those of *era1-2* plants after 10 days of drought (*26*). Stomatal apertures of both WT and *era1-2* decreased during drought (*24*). However, stomatal apertures of *era1-2* decreased faster and were smaller than those of WT during drought (for example, 1.08 ± 0.05 versus 1.24 ± 0.03 μm after 4 to 5 days of drought in noncovered pots; n = 75, P < 0.02). These results show that *ERA1* deletion decreases the transpiration rate of leaves and consequently slows desiccation during drought.

Protein farnesylation plays important and diverse roles in cellular processes and signal transduction cascades, which control cell growth, division, morphology, and visual signaling in eukaryotic cells (*1*–*3*). Competitive FTase inhibitors, as used here (Fig. 1), reduce Ras-mediated tumor growth (*10*, *28*). However, viable null mutants in FTase genes have not yet been found in other multicellular eukaryotes (*1*), and ion-channel modulation by FTases has not yet been reported. In plants, roles for protein farnesylation have been demonstrated in cell cycle regulation (*2*, *3*, *13*) and in seed germination (*4*). The only plant protein of known function shown to be farnesylated in vivo thus far is ANJ1, which is a homolog of the bacterial molecular chaperone DnaJ (*29*).

Although *era1* affects other signal transduction processes (*27*), we demonstrate in guard cells a function for protein farnesylation in regulation of ion channels, stomatal movements, and transpirational water loss by modulation of the ABA signaling cascade. Partial suppression of the ABA-insensitive phenotypes of the *abi1* and *abi2* mutants by *ERA1* deletion suggests that the target of the *ERA1* FTase may function downstream or parallel to these ABI protein phosphatases. We propose that the ERA1 FTase plays a major role in linking undetermined soluble negative regulatory proteins to plasma membrane ion-channel regulation in guard cells. Modulation of ERA1 or its targets, specifically in guard cells or other cell types, will allow further analysis of ERA1 effects on gas exchange, growth, and development. In conclusion, using several approaches, we provide evidence for a mechanism causing ABA hypersensitivity in guard cell signaling.

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11. *Arabidopsis thaliana* [Columbia ecotype, *era1-2* (*4*), *era1/abi1*, and *era1/abi2* (*22*)] plants were grown in a controlled environment growth chamber with a 16:8 hour light:dark cycle. Guard cell protoplasts were isolated by enzymatic digestion of leaf epidermal strips (*8*). Whole cell patch-clamp experiments were performed, and data were analyzed as described (*8*). The solutions used in patch-clamp experiments contained 150 mM CsCl, 2 mM $MgCl_2$, 6.7 mM EGTA, 3.35 mM $CaCl_2$, 5 mM tris-GTP, 5 mM Mg-ATP, and 10 mM Hepes-tris (pH 7.1) in the pipette and 30 mM CsCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM MES-tris (pH 5.6) in the bath. For transient K^+ current ("IAP") recordings, standard solutions were used [Z.-M. Pei, V. M. Baizabal-Aguirre, G. J. Allen, J. I. Schroeder, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6548 (1998)]. Abscisic acid ([±]-*cis*,*trans*-ABA; Sigma) was freshly added to the bath and pipette solutions. Osmolalities of solutions were adjusted to 485 mmol/kg for bath and 500 mmol/kg for pipette by addition of D-sorbitol. In ≈30% of guard cells, anion currents did not respond to ABA or HFPA. For unbiased data analysis, nonresponding cells were included in all reported data averages (omission of nonresponding cells would not change conclusions). Statistical analyses were performed with EXCEL (Microsoft). Data are presented as the mean ± SEM.
12. Stomatal aperture measurements were conducted as described (*8*). Detached rosette leaves were floated in solutions containing 20 mM KCl, 1 mM $CaCl_2$, and 5 mM MES-KOH (pH 6.15) and exposed to light at a fluency rate of 300 μmol m^{-2} s^{-1}. Subsequently, the indicated concentrations of ABA, or 2 μM HFPA or 5 μM manumycin, or ABA and 2 μM HFPA or 5 μM manumycin were added to the solutions to assay for stomatal closing. After treatments for 2 hours, stomatal apertures were observed with a digital video camera attached to an inverted microscope. Stomatal density was not affected in *era1-2*.
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16. In addition, ABA activation of anion-channel currents was also analyzed at 1 and 50 μM ABA (n = 23 and 28 cells for WT and *era1-2*, respectively). Activation of anion currents was also potentiated in *era1-2* compared with WT at 1 μM ABA, whereas at 50 μM ABA both WT and *era1-2* responses were similar.
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22. Double mutants of *era1/abi1* and *era1/abi2* were generated by crossing *era1-2* into *abi1-1* and *abi2-1* respectively. F_2 seeds were screened for ABA insensitivity to select for *abi1* or *abi2*. In the next generation seeds were screened for ABA supersensitivity (*era1/era1*). Supersensitive seeds were advanced to the next generation. Homozygous double mutants were identified by PCR amplification with primers of 5′-GATATCTCCGCCGGAGAT-3′ and 5′-CCATTCCACTGAATCACTTT-3′ for *abi1-1*, and 5′-CATCATCTGCTATGGCAGG-3′ and 5′-CCGGAGCATGAGCCACAG-3′ for *abi2-1*, as described (*19*). The *era1-2* deletion was verified by Southern (DNA) blot with *ERA1* cDNA as a probe. The *era1/abi1* mutant was further verified by back crosses to both parents to confirm genetically that it was a double mutant.
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24. C. M. Kwak, data not shown (n = 960 stomata for *era1/abi1 experiments*; n = 220 stomata for drought experiments).
25. C. M. Kwak, data not shown. ABA inhibition of seed germination was analyzed as described (*17*). Germination of seeds was defined as positive when a radical tip had fully penetrated the seed coat (n = 50 per condition). Each experiment (n = 25) included conditions comparing the indicated lines at multiple ABA concentrations.
26. Transpiration rate and soil moisture were measured as described [N. Vartanian, L. Marcotte, J. Giraudat, *Plant Physiol.* **104**, 761 (1994)]. For drought experiments, seeds of both WT (Col) and *era1-2* were germinated in individual pots each containing the same amount of prewetted soil. Plants were grown under constant light (200 μmol m^{-2} s^{-1}) and watered by irrigation until just before the plants bolted (≈3 weeks). Because *era1* affects growth, WT and *era1-2* plants (n = 16 each) were selected that were at the same developmental stages and had similar numbers of leaves. At this point pots were removed from water and allowed to dry over time. Evaporation from soil was reduced by covering the soil surface with tinfoil so that water loss occurring primarily through plant transpiration could be quantified. Watered control plants were also analyzed. Pots were weighed every day at the same time. Pots containing no plants were subjected to the same treatments to determine the background rate of water loss.
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Cell Surface Trafficking of Fas: A Rapid Mechanism of p53-Mediated Apoptosis

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p53 acts as a tumor suppressor by inducing both growth arrest and apoptosis. p53-induced apoptosis can occur without new RNA synthesis through an unknown mechanism. In human vascular smooth muscle cells, p53 activation transiently increased surface Fas (CD95) expression by transport from the Golgi complex. Golgi disruption blocked both p53-induced surface Fas expression and apoptosis. p53 also induced Fas-FADD binding and transiently sensitized cells to Fas-induced apoptosis. In contrast, *lpr* and *gld* fibroblasts were resistant to p53-induced apoptosis. Thus, p53 can mediate apoptosis through Fas transport from cytoplasmic stores.

p53 is the most commonly mutated gene in human cancer (*1*). p53 is a sequence-specific transcription factor, whose transcriptional targets induce growth arrest and apoptosis (*2*). Although its tumor suppressor function requires both activities, some human tumor–derived p53 mutants transactivate p53-responsive promoters and induce growth arrest, implying that apoptosis is the more potent mechanism (*3*). Depending on cell type, p53-induced apoptosis either requires transcriptional activation (*4*) or occurs without new RNA and protein synthesis (*5*). The occurrence of mutants that transactivate p53 targets but are defective for apoptosis (*6*), or vice versa (*7*), suggests that p53 induces apoptosis through transactivation-dependent and -independent mechanisms, implying a structural