ing from positions 28 to 1477(*Escherichia coli* 16S rRNA numbering) by using distance matrix and maximum-likelihood methods and the ARB program package [J. Felsenstein, *PHYLIP (phylogeny inference package), version 3.5c* (University of Washington, Seattle, WA, 1993); B. L. Maidak *et al.*, *Nucleic Acids Res.* **24**, 82 (1996); O. Strunk and W. Ludwig, *ARB: A Software Environment for Sequence Data* (Technische Univ., Munich, Germany, 1996)].

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Alterations of the *PPP2R1B* Gene in Human Lung and Colon Cancer

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The *PPP2R1B* gene, which encodes the β isoform of the A subunit of the serine/threonine protein phosphatase 2A (PP2A), was identified as a putative human tumor suppressor gene. Sequencing of the *PPP2R1B* gene, located on human chromosome 11q22-24, revealed somatic alterations in 15% (5 out of 33) of primary lung tumors, 6% (4 out of 70) of lung tumor–derived cell lines, and 15% (2 out of 13) of primary colon tumors. One deletion mutation generated a truncated PP2A-Aβ protein that was unable to bind to the catalytic subunit of the PP2A holoenzyme. The *PP2R1B* gene product may suppress tumor development through its role in cell cycle regulation and cellular growth control.

Carcinomas of the lung and colon account for over 270,000 new cases of cancer each year in the United States (*1*). Many human cancers contain mutations in tumor suppressor genes, which often map to genomic regions that exhibit allelic loss, or loss of heterozygosity (LOH), in tumors. LOH at chromosome 11q22-24 has been associated with lung, colon, breast, cervical, head and neck, and ovarian cancers, as well as melanoma (*2*). Introduction of a normal chromosome 11, or a derivative t(X;11) chromosome containing 11pter-q23, can reverse the tumorigenic potential of lung (*3*), breast (*4*), and cervical carcinoma cells (*5*) and Wilms tumor (*6*) when introduced into nude mice. These studies suggest that one or more tumor suppressor genes are located centromeric to the t(X;11) breakpoint at 11q23.

To identify the tumor suppressor genes on chromosome 11 that are inactivated in lung cancer, we mapped the t(X;11) breakpoint relative to regions of frequent LOH (*7*) and defined a minimum critical region between *D11S1394* and *D11S689* (Fig. 1A). This region of chromosome 11q23 demonstrated high-frequency LOH in a variety of cancers, including lung and colon tumors. Evaluation of LOH in 28 lung cancer and paired normal cell lines revealed allelic loss in 71% of the cancer cell lines. Two polymorphic DNA markers, *D11S1647* and *D11S1987*, showed allelic loss in 42.9 and 46.2% of the cancer cell lines, respectively (*8*), and both markers were lost in 28.6% of the cell lines.

On the basis of these results, the region between *D11S1394* and *D11S689*, and especially that between *D11S1647* and *D11S1987*, was systematically surveyed for candidate tumor suppressor genes. Over 100 candidate genes and expressed sequence tag (EST) markers were identified from a radiation hybrid map of human chromosome 11 (available at http://ftp.well.ox.ac.uk) and from the Human Gene Map (available at http://www.ncbi.nlm.nih.gov/SCIENCE96/ResTools.html). One of the EST sequences (*M65254*) was found to correspond to a subunit of the serine/threonine protein phosphatase 2A (PP2A). PP2A is an important regulatory enzyme that down-regulates the mitogen-activated protein kinase (MAPK) cascade, relays signals for cell proliferation, and has been linked to carcinogenesis (*9*). The PP2A holoenzyme exists in several trimeric forms consisting of a 36-kD core catalytic subunit, PP2A-C; a 65-kD structural/regulatory component, PP2A-A; and a variable regulatory subunit, PP2A-B, which confers distinct properties on the holoenzyme. Each subunit exists as multiple isoforms encoded by different genes, so that there are many forms of the PP2A trimer, differing in expression patterns and specificity. The gene identified at chromosome 11q23, denoted *PPP2R1B* according to standardized nomenclature, encodes the β isoform of the structural/regulatory A subunit PP2A-Aβ. PP2A-Aβ is necessary for interaction of the catalytic PP2A-C and variable PP2A-B subunits and is critical for phosphatase activity (*10*).

The precise physical location of *PPP2R1B* was determined by colocalizing it within P1-derived artificial chromosome (PAC) (*11*) clones that contained sequence-tagged sites (STSs) on chromosome 11q22-23 (Fig. 1A). PAC clone pDJ433L20 contained *PPP2R1B* as well as markers *SHGC9837* and *D11S966E* and was localized to a 15 centiray region between markers *D11S1647* and *D11S1987* on 11q22.2 (*12*). Thus, the *PPP2R1B* gene is located in a region showing high-frequency LOH (Fig. 1A).

The complete sequence of *PPP2R1B* was determined by a combination of EST analysis (*13*), cDNA sequencing (*14*), and 5′ rapid amplification of cDNA ends (RACE) (*15*). The sequence predicts a 601–amino acid protein with extensive homology to the PP2A-Aβ subunits of pig and *Xenopus* and to the human PP2A-Aα isoform (Fig. 1B). Both PP2A-Aα and PP2A-Aβ are composed of 15 internal repeat sequences, each consisting of two amphipathic helices necessary for binding PP2A-B and PP2A-C (*16*).

To determine whether human tumors contain alterations in *PPP2R1B*, we amplified the coding regions by reverse transcriptase–polymerase chain reaction (RT-PCR) (*17*) and carried out direct DNA sequencing of samples from 130 cancer-derived cell lines and 70 primary tumors, including lung, colon, breast, and cervical tumors. Two of the tumors generated altered *PPP2R1B* amplification products. H1450 cells had a 1.8-kb product representing the wild-type *PPP2R1B* and a second 1-kb product (Fig. 2A). Sequencing of the latter revealed an internal in-frame deletion of 867 base pairs (bp), which is predicted to produce a truncated PP2A-Aβ lacking amino acids 230 to 518 (Fig. 2B). The 1.8-kb PCR product contained an $A_{1540} \rightarrow G$ transition, which changes a highly conserved Asp_{504} to Gly. Thus, both alleles of *PPP2R1B* are altered and possibly inactivated, whereas the matched lymphoblastoid cell line from the same patient, BL7, con-

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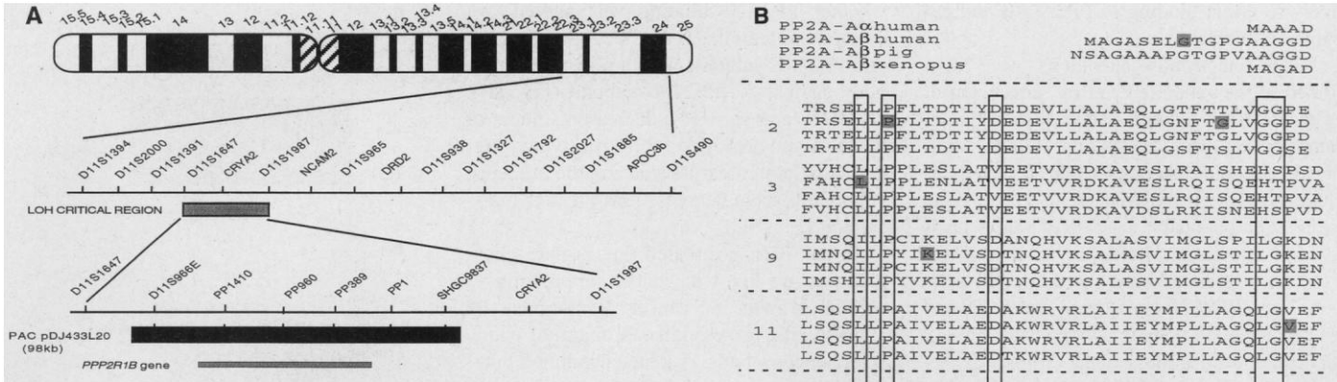

Fig. 1. (**A**) Physical map of chromosome 11q23-24 showing *PPP2R1B* and the LOH critical region. Polymorphic markers from chromosome 11 radiation hybrid maps were used for LOH analysis (*7*). Expansion of the map and high-resolution mapping were done by PAC clone isolation (*11*). Markers PP1410, PP960, PP389, and PP1 were derived from portions of an EST sequence (GenBank accession number M65254). (**B**) The predicted amino acid sequence of PP2A-Aβ, determined from the sequence of *PPP2R1B*. Alignment of human PP2A-Aα with human PP2A-Aβ, pig PP2A-Aβ, and *Xenopus* PP2A-Aβ. Boxed amino acids represent highly conserved residues defining 15 internal repeats (*14*). The sequence is separated according to repeats; numbers at left indicate the repeat number (dashed lines separate discontinuous sequence). Shaded amino acids, indicated in the human PP2A-Aβ sequence, represent mutations found in lung and colon tumors. Sequence alignment was carried out with Multiple Sequence Alignment (Infomax, Bethesda, Maryland) and MEGALIGN (DNAStar, Madison, Wisconsin). Portions of the *PPP2R1B* sequence were determined previously (*14*) (GenBank accession number M65254). The complete sequence of the human *PPP2R1B* gene has been deposited in GenBank (accession number AF087438).

tains no detectable alterations. In H220 cells, one of the *PPP2R1B* alleles had a 143-bp deletion, resulting in a frameshift and a loss of 83 amino acids at the COOH terminus of PP2A-Aβ (Table 1).

DNA sequencing of PCR amplification products revealed 18 alterations in *PPP2R1B* in 11 tumor-derived cell lines and primary tumors (Table 1). The alterations include deletions, frameshifts, and point mutations leading to nonconservative amino acid substitutions (Table 1). Cell line H2009 carries one allele with a G_{298}→A transition (Fig. 2C), which changes a conserved Gly^{90} to Asp, as well as a wild-type *PPP2R1B* allele. The matched lymphoblastoid cell line BL2009 has both G_{298}→A and wild-type coding sequences (*18*), which suggests that the lung cancer patient from which these cells are derived harbors a germline mutation in the *PPP2R1B* gene. Although it is possible that this alteration is a polymorphism rather than a mutation, it was not detected in 200 other normal and malignant cell lines. The alteration Gly^{90}→Asp was found in two separate tumors and not in surrounding normal tissue, which suggests that this site may be a hot spot for mutation. One colon adenocarcinoma, T25, had two alterations in the same *PPP2R1B* allele (Table 1). Many of the alterations affect conserved amino acids within the repeat sequences necessary for PP2A-Aβ binding to the catalytic C subunit (*16*) and thus may destabilize the enzyme complex. In particular, deletions and frameshifts that alter repeats 11 to 15 (Fig. 1B) are

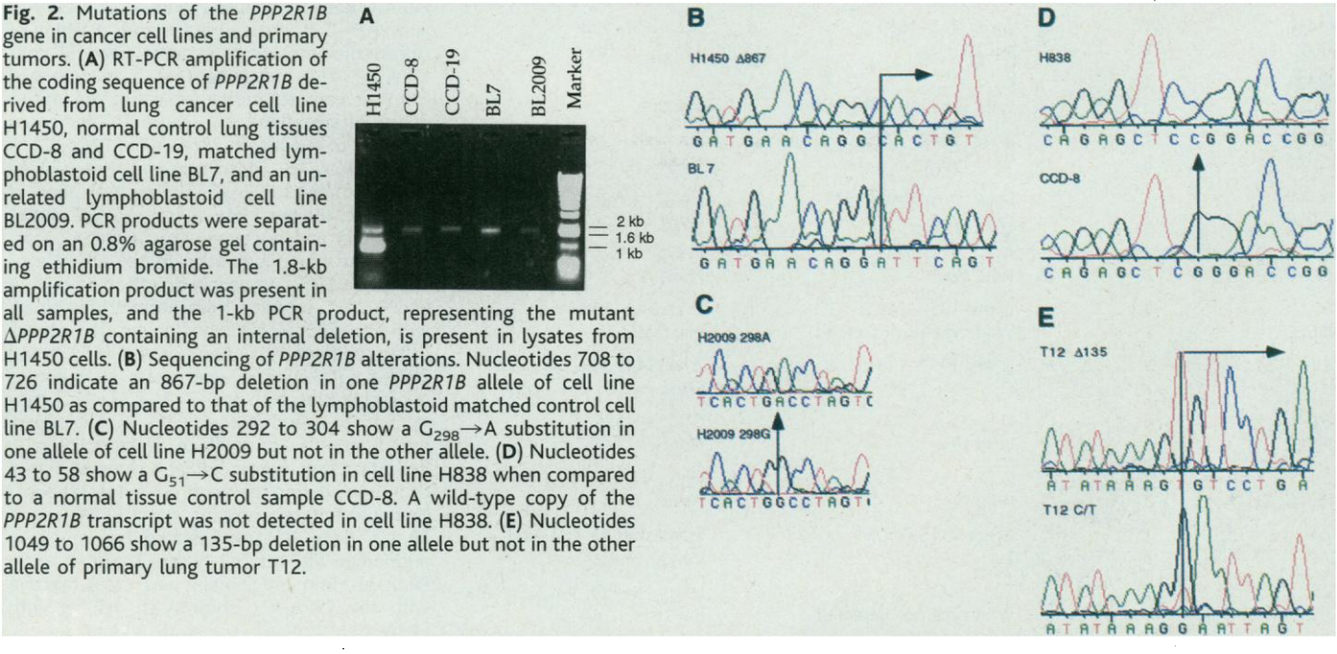

Fig. 2. Mutations of the *PPP2R1B* gene in cancer cell lines and primary tumors. (**A**) RT-PCR amplification of the coding sequence of *PPP2R1B* derived from lung cancer cell line H1450, normal control lung tissues CCD-8 and CCD-19, matched lymphoblastoid cell line BL7, and an unrelated lymphoblastoid cell line BL2009. PCR products were separated on an 0.8% agarose gel containing ethidium bromide. The 1.8-kb amplification product was present in all samples, and the 1-kb PCR product, representing the mutant Δ*PPP2R1B* containing an internal deletion, is present in lysates from H1450 cells. (**B**) Sequencing of *PPP2R1B* alterations. Nucleotides 708 to 726 indicate an 867-bp deletion in one *PPP2R1B* allele of cell line H1450 as compared to that of the lymphoblastoid matched control cell line BL7. (**C**) Nucleotides 292 to 304 show a G_{298}→A substitution in one allele of cell line H2009 but not in the other allele. (**D**) Nucleotides 43 to 58 show a G_{51}→C substitution in cell line H838 when compared to a normal tissue control sample CCD-8. A wild-type copy of the *PPP2R1B* transcript was not detected in cell line H838. (**E**) Nucleotides 1049 to 1066 show a 135-bp deletion in one allele but not in the other allele of primary lung tumor T12.

likely to affect binding of PP2A-Aβ and enzyme activity (*16*).

Single nucleotide alterations were confirmed at the genomic level by sequencing of PCR-amplified genomic sequence from tumor samples T64, T68, H838, and H2009. Additional noncoding alterations were detected, including a G→T transversion in H220 in an exon/intron boundary, which presumably leads to a splicing error resulting in the loss of one exon, as well as the observed frameshift deletion. The *PPP2R1B* genes from tumors T9 and T12 each carry a point mutation inside intron 8 that could also lead to splicing errors responsible for the observed deletion. None of the sequence changes identified in primary tumor genomic DNA were detected in the genomic DNA from adjacent normal tissue (*19*).

To determine if the sequence alterations in *PPP2R1B* might produce biochemical changes leading to altered PP2A activity, we performed immunoblot analysis with anti–PP2A-Aβ immunoglobulin. Lung cancer line H1450 has two *PPP2R1B* alterations, a deletion in one *PPP2R1B* allele, and a missense mutation in the other allele of *PPP2R1B*. Lysates of H1450 were found to contain an immunoreactive truncated PP2A-Aβ protein of 45 kD in addition to the wild-type 65-kD PP2A-A protein (Fig. 3A). This alteration might be expected to compromise a highly conserved region of the protein necessary for PP2A-Aβ to form a functional trimer with PP2A-B and PP2A-C (*20*). To determine whether PP2A-C binding was altered, we coimmunoprecipitated the PP2A-A, PP2A-B, and PP2A-C subunits with a polyclonal antibody against the PP2A-C subunit (Fig. 3B). H1450 lysates showed a decreased amount of PP2A-A and no 45-kD PP2A-Aβ, which suggests that both the truncated and the missense A subunits have decreased affinity for the C subunit (Fig. 3C).

It has been postulated that protein phosphatases are involved in the suppression of cellular growth and cancer development by antagonizing protein kinases, many of which act as oncoproteins. A gene encoding a putative protein tyrosine phosphatase, *PTEN/MMAC1*, was recently shown to be mutated in human brain, breast, and prostate cancer (*21, 22*). PP2A also plays an important role in cell cycle checkpoint control (*23*), inhibits nuclear telomerase activity (*24*), and is a target for chemical tumor promoters (*25*), DNA tumor viruses, oncogenes, and components of cell growth control. Polyomavirus T antigen and SV40 small t antigen bind to PP2A-A, displacing PP2A-B and inhibiting PP2A phosphatase activity, interactions that are thought to mediate viral transformation (*26, 27*). The *HOX11* oncogene interacts with PP2A, inhibiting activity and disrupting a G_2/M cell cycle checkpoint (*28*). Casein kinase 2α inhibits cellular transformation by *ras* and suppresses cell growth by enhancing PP2A activity (*29*).

Our results show that *PPP2R1B* alter-

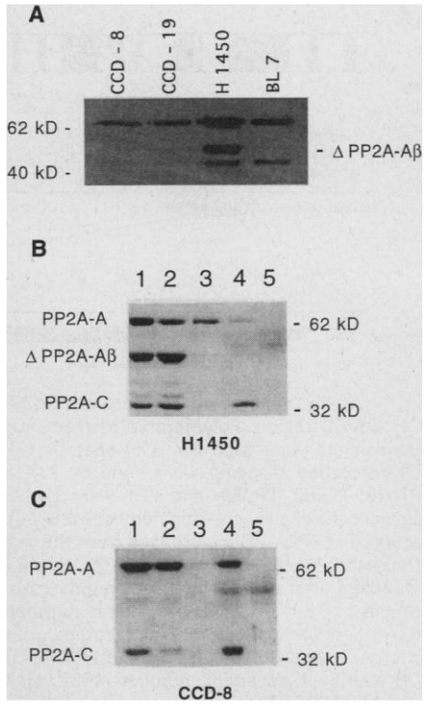

Fig. 3. (**A**) Identification of PP2A-Aβ proteins by immunoblotting. PP2A-Aβ was detected in lysates of normal lung tissue samples CCD-8 and CCD-19, cell line H1450, and BL7 lymphoblastoid control cells, with anti–PP2A-Aβ immunoglobulin (*31*). The 65-kD PP2A-Aβ protein was detected in all cells tested, and H1450 lysates contain an additional 45-kD band representing the mutant subunit. The 40-kD band is an actin control. (**B**) Coimmunoprecipitation of PP2A-A and PP2A-C subunits from H1450 cells with anti–PP2A-C immunoglobulin (*32*). Immunoblots of whole-cell lysates with both anti–PP2A-A and anti–PP2A-C show (lane 1) full-length PP2A-A (65 kD), truncated ΔPP2A-Aβ (45 kD), and PP2A-C (36 kD). The supernatant after immunoprecipitation with anti–PP2A-C (lane 2) contains PP2A-A, Δ PP2A-Aβ, and PP2A-C. The supernatant after washing of the immunoprecipitation pellet (lane 3) contains PP2A-A, indicating that it is dislodged from the complex during the wash. The immunoprecipitate (lane 4) contains decreased amounts of full-length PP2A-A and PP2A-C but lacks ΔPP2A-Aβ, which suggests that ΔPP2A-Aβ cannot bind PP2A-C. The control (lane 5) represents the reactivity of anti–PP2A-C immunoglobulin and horseradish peroxidase–conjugated anti-immunoglobulin in the absence of cell lysates. (**C**) Immunoprecipitation of PP2A-A and PP2A-C subunits from normal lung tissue with anti–PP2A-C immunoglobulin. The whole-cell lysate (lane 1) contains PP2A-A (65 kD) and PP2A-C (36 kD). The supernatant after immunoprecipitation with anti–PP2A-C (lane 2) contains PP2A-A and PP2A-C (lane 2). The supernatant after washing (lane 3) lacks PP2A-A, indicating that normal PP2A-A remains bound to PP2A-C during the wash procedure. The immunoprecipitate contains both PP2A-A and PP2A-C (lane 4). Lane 5 is a control lane with antibodies without cell lysate. The goat anti–PP2A-Aβ immunoglobulin was cross-reactive with the PP2A-Aα isoform as well as with PP2A-Aβ.

Table 1. *PPP2R1B* sequence alterations in lung and colon cancer; nts, nucleotides; aa, amino acid; CAC, colon adenocarcinoma; SCLC, small-cell lung carcinoma; NSCLC, non–small-cell lung carcinoma; Δ, deletion; →, nucleotide or amino acid alteration. "Wild type not detected" indicates the probable deletion of the allele through LOH.

Tumor	Allele	cDNA alteration	Predicted effect
H1450	1	Δ nts 717–1583	Δ aa 230–518
SCLC	2	$A_{1540} \rightarrow G$	$Asp_{504} \rightarrow Gly$
H838	1	$G_{51} \rightarrow C$	$Gly_8 \rightarrow Arg$
NSCLC	2	Wild type not detected	Inactive
H220	1	Δ nts 1584–1726	Frameshift aa 519–601
SCLC	2	Wild type	None
H2009	1	$G_{298} \rightarrow A$ (germline)	$Gly_{90} \rightarrow Asp$
NSCLC	2	Wild type	None
T11	1	$A_{1056} \rightarrow G$	$Lys_{343} \rightarrow Glu$
NSCLC	2	Wild type	None
T9	1	Δ nts 1057–1191	Δ aa 344–388
NSCLC	2	Wild type not detected	Inactive
T12	1	Δ nts 1057–1191	Δ aa 344–388
NSCLC	2	$C_{222} \rightarrow T$	$Pro_{65} \rightarrow Ser$
T64	1	$G_{298} \rightarrow A$	$Gly_{90} \rightarrow Asp$
NSCLC	2	Wild type	None
T68	1	$G_{298} \rightarrow A$	$Gly_{90} \rightarrow Asp$
NSCLC	2	Wild type	None
T24	1	Δ nts 1315–1505	Frameshift aa 422–601
CAC	2	$T_{1663} \rightarrow C$	$Val_{545} \rightarrow Ala$
T25	1	$T_{331} \rightarrow C$, $T_{1372} \rightarrow C$	$Leu_{101} \rightarrow Pro$, $Val_{448} \rightarrow Ala$
CAC	2	Wild type not detected	Inactive

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*). 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

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