cerebellar EGL and the dentate gyrus harbor neuroblasts that generally give rise to a single lineage (*22*). Thus, as cells move from a multipotent, less-differentiated state to their terminal phenotype, Atm function after irradiation becomes apparent. Because $Atm^{-/-}$ and $p53^{-/-}$ mice appear neuroanatomically normal (*23*), Atm- and p53-dependent apoptosis is probably distinct from programmed cell death occurring during nervous system development (*24*).

These data establish a role for Atm during radiation-induced apoptosis in select cell populations in the developing CNS. It is possible, at this stage, that neurons require Atm as a component of a survival checkpoint so that developing neural cells that have genomic (or other) damage can be eliminated. Thus, defective Atm may allow genomically compromised neurons to survive, and their accumulated mutations lead to functional deficits later in life. In specific cell populations such as Purkinje or granule cells, this process could lead to selective neurodegeneration as seen in AT (*3*). Further, because disruption of Atm function is protective against irradiation, these findings may have therapeutic implications for attenuating the serious neurological sequelae after craniospinal irradiation for pediatric brain tumors.

REFERENCES AND NOTES

1. K. Savitsky *et al.*, *Science* **268**, 1749 (1995).
2. M. F. Lavin, Y. Shiloh, *Annu. Rev. Immunol.* **15**, 177 (1997).
3. R. P. Sedgwick and E. Boder, in *Handbook of Clinical Neurology*, P. Vinken, G. Bruyn, H. Klawans, Eds. (Elsevier, New York, 1991), vol. 60, pp. 347–423.
4. P. J. McKinnon, *Hum. Genet.* **75**, 197 (1987).
5. G. Rotman and Y. Shiloh, *Bioessays* **19**, 911 (1997).
6. N. Heintz, *Curr. Opin. Neurol.* **9**, 137 (1996).
7. H. A. Soares, J. I. Morgan, P. J. McKinnon, *Neuroscience*, in press; G. Chen and E. Lee, *J. Biol. Chem.* **271**, 33693 (1996).
8. The *neo* gene was used to interrupt exon 57 and replace exon 58 of *Atm* (Genbank number U55702), deleting a region of the phosphoinositol 3-kinase domain as occurs in a number of individuals with AT.
9. C. Barlow *et al.*, *Cell* **86**, 159 (1996); A. Elson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13084 (1996); Y. Xu and D. Baltimore, *Genes Dev.* **10**, 2401 (1996). However, we (M.J.C. and P.J.McK.) do not find abnormalities in the $Atm^{-/-}$ cerebellum using electron microscopy as has been reported by R. O. Kuljis, Y. Xu, M. C. Aguila, and D. Baltimore [*Proc. Natl. Acad. Sci. U.S.A.* **94**, 12688 (1997)].
10. Wild-type ($Atm^{+/+}$) or $Atm^{-/-}$ littermates were used at either 3 or 5 days after birth (P3 or P5; day of birth is P0). Mice were irradiated with 14 gray (Gy) from a Cs irradiator (delivered at a rate of 0.9 Gy/min) and allowed 18, 24, or 48 hours of recovery. Tissues were collected after fixation by transcardial perfusion with 4% paraformaldehyde and subsequently cryoprotected in sucrose and cryosectioned (12-μm coronal sections). Neutral red staining was performed with 1% Neutral red (Aldrich Chemical) in 0.1 M acetic acid (pH 4.8) for 1 min followed by dehydration in ethanol. All data presented compare littermates and are derived from 10 separate litters. All animal experiments were performed according to institutional guidelines and under an Institutional Animal Care and Use Committee (IACUC)–approved protocol.
11. Y. Gavrieli, Y. Sherman, S. A. Ben-Sasson, *J. Cell Biol.* **119**, 493 (1992). ISEL staining was performed on tissue cryosections from P3 animals, 18 hours after irradiation with the FragEL kit (Oncogene Research Products) according to the manufacturer's directions. Sections were counterstained with methyl green (Vector laboratories).
12. P. E. Schauwecker and O. Steward, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4103 (1997).
13. S. W. Lowe, E. M. Schmitt, S. W. Smith, B. A. Osborne, T. Jacks, *Nature* **362**, 847 (1993); A. R. Clarke *et al.*, *ibid.*, p. 849.
14. K. A. Wood and R. J. Youle, *J. Neurosci.* **15**, 5851 (1995); Y. Enokido, T. Araki, K. Tanaka, S. Aizawa, H. Hatanaka, *Eur. J. Neurosci.* **8**, 1812 (1996).
15. M. B. Kastan *et al.*, *Cell* **71**, 587 (1992). A delay or absence of p53 stabilization has been reported in irradiated thymocytes from $Atm^{-/-}$ mice resulting in reduced or normal levels of apoptosis, respectively [C. H. Westphal *et al.*, *Nature Genet.* **16**, 397 (1997); C. Barlow, K. D. Brown, C. X. Deng, D. A. Tagle, A. Wynshaw-Boris, *ibid.* **17**, 453 (1997)].
16. Tissues were harvested 2 hours after 14 Gy of irradiation and homogenized in SDS sample buffer; equal quantities of protein were separated on 10% SDS–polyacrylamide gel electrophoresis gels and transferred to nitrocellulose membranes. Membranes were stained with Ponceau S (Sigma) to confirm equal protein transfer. p53 was detected with antibody to p53 (anti-p53) (Ab-7, Calbiochem) at a dilution of 1/2500 for 1 hour at room temperature. Antibody binding was detected by enhanced chemiluminescence (Amersham). p53 immunohistochemistry was done with anti-p53 (CM5, Vector laboratories) at a dilution of 1/500 by antigen retrieval [C. A. Midgley *et al.*, *J. Cell Sci.* **101**, 183 (1992)]. The Vectastain Elite ABC kit–VIP substrate avidin-biotin immunoperoxidase system (Vector laboratories) was used for visualization of primary antibody binding. Sections were counterstained with methyl green. Data were from five separate litters.
17. $p53^{-/-}$ mice were obtained from Jackson Laboratories (Bar Harbor, Maine) and are described in (*13*). Experiments were performed twice with littermates ($p53^{+/+}$ and $p53^{-/-}$) from two separate litters.
18. R. Baskaran *et al.*, *Nature* **387**, 516 (1997); T. Shafman *et al.*, *ibid.*, p. 520.
19. Z. M. Yuan *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1437 (1997).
20. V. L. Tybulewicz, C. E. Crawford, P. K. Jackson, R. T. Bronson, R. C. Mulligan, *Cell* **65**, 1153 (1991).
21. D. A. Steindler, T. Kadrie, H. Fillmore, L. B. Thomas, *Prog. Brain Res.* **108**, 349 (1996); C. Lois, A. Alvarez-Buylla, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2074 (1993); D. L. Turner and C. L. Cepko, *Nature* **328**, 131 (1987).
22. M. E. Hatten and N. Heintz, *Annu. Rev. Neurosci.* **18**, 385 (1995); W. M. Cowan, B. B. Stanfield, K. Kishi, *Curr. Top. Dev. Biol.* **15**, 103 (1980).
23. Although $p53^{-/-}$ mice are viable, a significant subset of $p53^{-/-}$ embryos develop defects in neural tube closure [J. F. Armstrong, M. H. Kaufman, D. J. Harrison, A. R. Clarke, *Curr. Biol.* **5**, 931 (1995); V. P. Sah *et al.*, *Nature Genet.* **10**, 175 (1995)].
24. R. W. Oppenheim, *Annu. Rev. Neurosci.* **14**, 453 (1991).

25. We thank S. Goff and S. Boast (Columbia University) for providing c-Abl null mice, C. Nagy for help in generating the $Atm^{-/-}$ mice, and S. J. Baker for comments on the manuscript. Supported by the AT Children's Project (P.J.McK.), Cancer Center CORE grant CA-21765, and by the American Lebanese and Syrian Associated Charities (ALSAC) of St. Jude Children's Research Hospital.

27 January 1998; accepted 27 March 1998

COI1: An *Arabidopsis* Gene Required for Jasmonate-Regulated Defense and Fertility

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The *coi1* mutation defines an *Arabidopsis* gene required for response to jasmonates, which regulate defense against insects and pathogens, wound healing, and pollen fertility. The wild-type allele, *COI1*, was mapped to a 90-kilobase genomic fragment and located by complementation of *coi1-1* mutants. The predicted amino acid sequence of the COI1 protein contains 16 leucine-rich repeats and an F-box motif. It has similarity to the F-box proteins *Arabidopsis* TIR1, human Skp2, and yeast Grr1, which appear to function by targeting repressor proteins for removal by ubiquitination.

Jasmonates (JAs), which include jasmonic acid and its cyclopentanone derivatives, are widely distributed throughout the plant kingdom. They are synthesized by the octadecanoic pathway from linolenic acid in undamaged tissues and (apparently) by a different pathway in wounded tissues. JAs affect a variety of processes in plants, including root growth, fruit ripening, senescence, pollen development, tuber formation, tendril coiling, and defense against insect pests and pathogens. They alter gene transcription, RNA processing, and translation (*1*).

Tomato plants respond to injury, such as that caused by chewing insects, by making proteinase inhibitor (pin) proteins that inhibit insect digestive proteases (*2*). JA is required for plant defenses against insect predation (*3*) and acts together with ethylene formed in the wounded tissues to regulate pin gene expression (*4*). In *Arabidopsis*, the functions of JA are defined by the triple mutant *fad3-2 fad7-2 fad8*, which is deficient in linolenic acid,

the precursor to JA (5), and by the *coi1* mutant (6) and other mutants (7) with reduced sensitivity to JA. The *fad3-2 fad7-2 fad8* and *coi1* mutants have deficient wound responses (8, 9) and high mortality from attack by chewing insects (8, 10), and they produce nonviable pollen (5, 6). Treatment of plants with JA corrects these deficiencies in *fad3-2 fad7-2 fad8*, but not in *coi1* (5, 6, 8–10). The *coi1* mutation therefore defines a gene, *COI1*, that functions in the JA signal pathway and is required for pollen development and defense against pests, and also for defense responses to pathogens (11). To investigate its function, we have used a map-based strategy to isolate *COI1*.

We genetically mapped *coi1* in F_2 plants from a cross between the *coi1-1*/*coi1-1* mutant derived from *Arabidopsis* ecotype Columbia (6) and wild-type *COI1*/*COI1* plants of ecotype Landsberg erecta. Tests on 188 plants for genetic linkage between the coi1 phenotype (12) and cleaved amplified polymorphic sequence (CAPS) markers (13) placed *coi1-1* on chromosome 2 flanked by visible markers *as* and *cer8* (Fig. 1A). Further mapping located *COI1* on bacteria artificial chromosome (BAC) D25L8 (Fig. 1B). The *COI1* gene was mapped in D25L8 by screening DNA fragments for transient complementation of *coi1-1* mutants transgenic for the *COI1*-dependent wound- and JA-responsive reporter gene, *PThi2.1-GUS* (Fig. 1, B to E) (14). Fragment 8ks was sufficient to complement *coi1-1* (Fig. 1E); this fragment identified clones 1.8c and 1.9c in an *Arabidopsis* cDNA library (15). The 1.8c cDNA complemented the *coi1-1* mutant in the transient assay (Fig. 1E).

To confirm that 8ks complemented other JA responses in the *coi1-1* mutant, we used *Agrobacterium tumefaciens*–mediated transformation to produce stable transgenic plants (16). We could not achieve direct transformation of *coi1-1* mutants by two different methods that efficiently transformed wild-type plants (16, 17). However, we could successfully transform fertile *COI1*/*coi1-1* plants with 8ks by in planta vacuum-infiltration transformation. About 25% of the untransformed progeny of these plants segregated as *coi1-1* mutants, as expected. However, of 79 seedlings selected from the same seed lot for kanamycin resistance through the neomycin phosphotransferase gene linked to 8ks on the transforming DNA, all exhibited wild-type growth inhibition in re-

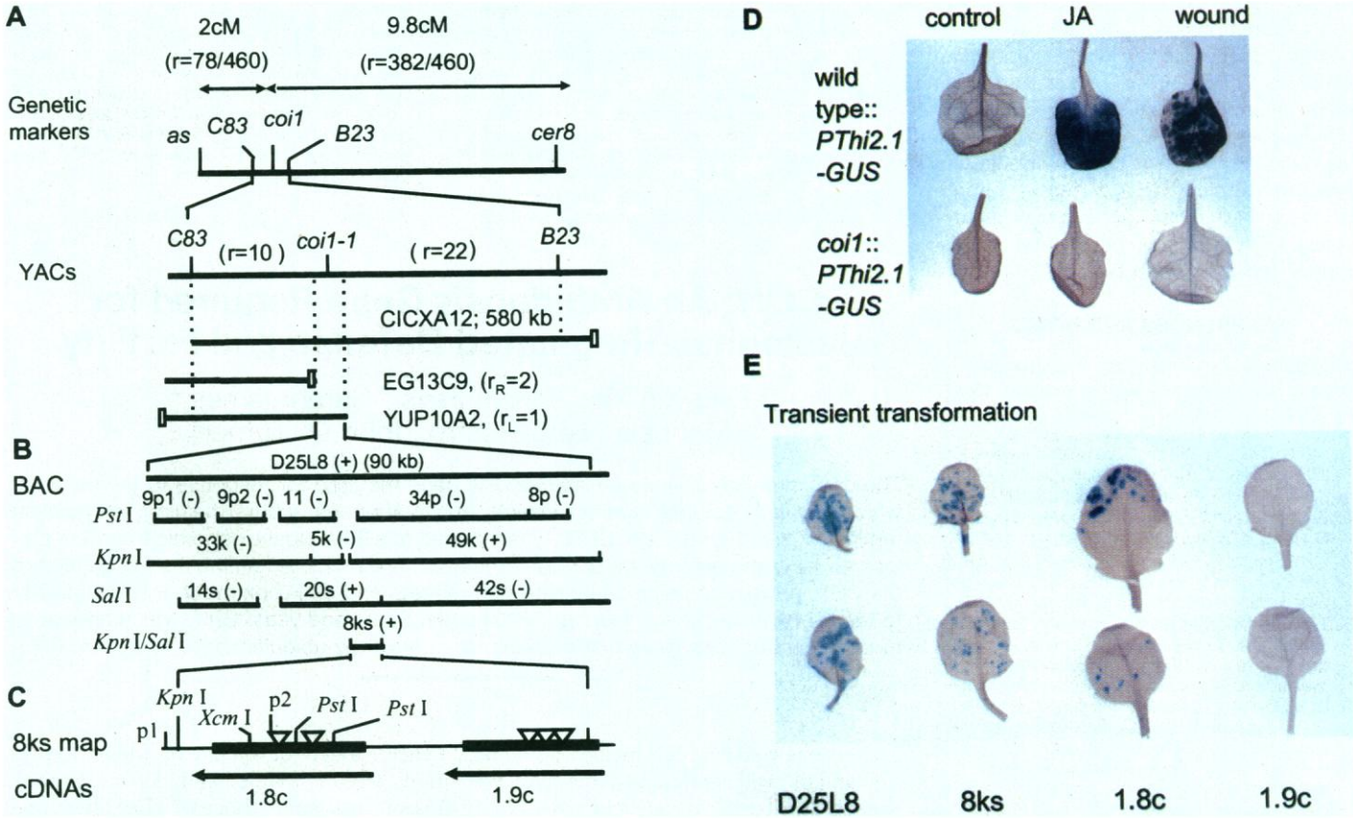

Fig. 1. Genetic and physical mapping of *Arabidopsis* DNA sequences that complement *coi1-1*. (**A**) Genetic mapping of 460 plants with chromosome recombinations between visible markers *as* and *cer-8* placed *coi1-1* between CAPS markers C83 and B23 (24). C83 and B23 were used to screen YAC libraries (25). Numbers of detected chromosome recombinations between the marker and *coi1-1* are denoted by *r*; subscripts L and R indicate markers derived from YAC left and right ends, respectively (open box indicates right end). (**B**) The closest markers flanking *coi1-1*, EG13C9 (right end) and YUP10A2 (left end), hybridized to BAC clone D25L8 (25). Restriction enzyme digestion fragments of D25L8 are named to indicate size (in kilobases) and are marked (+) if they complemented *coi1-1* in the transient transformation assay or (–) if they did not. (**C**) Map of 8ks and adjoining DNA. Thick line shows predicted coding sequences; introns are marked as open triangles; p1 (located outside of 8ks) and p2 (within 8ks) are PCR primers that amplify a 1.5-kb fragment with an internal recognition site for Xcm I (see Fig. 2B). Arrows indicate position and orientation of transcription of expressed sequences 1.8c and 1.9c identified by 8ks in a cDNA library with inserts controlled by the CaMV 35S promoter (15). (**D**) Plants transgenic for *PThi2.1-GUS* (14) were infiltrated with 10 μM methyl jasmonate (JA) or water (control), or wounded by gold particles fired from a particle gun. GUS activity could be detected in wounded and JA-treated wild-type plants, but not in controls or *coi1-1* mutants, indicating that *COI1* is required for JA- and wound-induced expression of the *Thi2.1* gene. (**E**) *coi1-1* plants transgenic for *PThi2.1-GUS* were bombarded (26) with gold particles coated with candidate *COI1* DNA sequences. Histochemical staining revealed sites of transformation as blue spots of GUS activity, indicating complementation of *coi1-1* by DNA from BAC D25L8, fragment 8ks, and cDNA 1.8c. Two leaves are shown for each complementation assay.

sponse to JA and produced fertile pollen. This indicated that the transgene, 8ks, had functionally complemented the *coi1-1* mutation. Similar results indicated that *coi1-1* was complemented by cDNA clone 1.8c but not by cDNA clone 1.9c, nor by the transformation vector. We determined the DNA sequence of the *COI1* gene (*18*) and developed a CAPS marker to identify *coi1-1* mutants that had been complemented by the 8ks transgene.

The DNA sequence of 8ks indicated a single open reading frame that corresponded to cDNA clone 1.8c (Fig. 1C). The corresponding sequence from the *coi1-1* mutant deviated from that of the wild type by a single nucleotide change, G to A, at position +1401 relative to the translation start of 1.8c. This mutation created a polymorphism between DNA digested with Xcm I from wild-type plants and from the *coi1-1* mutant (Fig. 2B). Analysis of the 79 transgenic plants for this polymorphism revealed that 24 were homozygous for the *coi1-1* mutation, one of which is T-36 (Fig. 2); 37 were heterozygous; and 18 were homozygous for the wild type. This agreed with the expected ratio of 1:2:1 (*coi1-1*: heterozygous:wild-type) in the 79 tested plants ($P > 0.3$), and confirms the functional complementation of the *coi1-1* mutant by *COI1* sequences in 8ks.

The *COI1* cDNA sequence predicted a 1779-nucleotide gene product coding for a 592–amino acid protein (Fig. 3). Support for the designation of this open reading frame as *COI1* came from the complementation experiments, described above, and from deviations of the sequences of three *coi1* alleles (*12*) from the wild-type sequence. The DNA sequence of *coi1-1* differed from the wild type by a single nucleotide that converted codon 467 (W) into a translation stop codon. In *coi1-15* a 1-nucleotide deletion at codon 60 (C) caused a frame shift that introduced a translation stop at codon 75. In *coi1-18* a 10-nucleotide deletion, replaced by a ~3-kb insertion, altered the sequence from codon 358 (E) to a translation stop at codon 366. The three mutant alleles were therefore predicted to produce truncated proteins. Northern (RNA) blot analysis revealed that the *COI1* transcript was expressed in similar amounts in untreated, wounded, and JA-treated tissues (*10*).

The deduced amino acid sequence of the COI1 protein contains a degenerate F-box motif (*19*) and 16 imperfect leucine-rich repeats (LRRs) (*20*), consensus xLxxaxxxCxxLxxaxa, where "a" is a hydrophobic amino acid (Fig. 3). Both of these motifs are involved in protein-protein interactions (*20, 21*). Database searches indicated that COI1 was related to three LRR-containing F-box proteins: *Arabidopsis* TIR1 (*22*), to which it has 34% identity, as well as human Skp2 and yeast Grr1 (*19, 21*). TIR1 is required for response to auxin, and *tir1* mutants are fertile plants with reduced sensitivity to root growth inhibition by auxin. However, *coi1* mutants are male sterile and show wild-type sensitivity to growth inhibition by auxin (*10*). Apparently, therefore, COI1 and TIR1 function in separate signal pathways. Both Skp2 and Grr1 regulate cell division, and Grr1 also regulates nutrient uptake (*23*). These and other F-box proteins function as receptors that selectively recruit repressor proteins into a complex required for the ubiquitination of substrates targeted for removal (*21*). We speculate that COI1 may be an F-box protein that recruits regulators of defense response and pollen development for modification by ubiquitination.

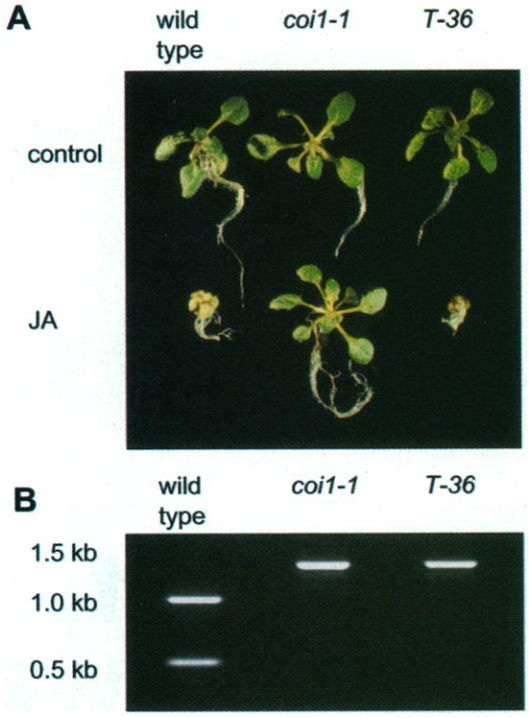

Fig. 2. Stable complementation of the *coi1-1* mutant by 8ks. (**A**) Seedlings of wild type, the *coi1-1* mutant, and line T-36 (transgenic for 8ks) were grown on MS medium (control) or medium containing 50 μM JA (*6*). JA inhibited growth of seedlings of the wild type and T-36, but not the *coi1-1* mutant. (**B**) A 1.5-kb fragment containing part of *COI1* or the *coi1-1* mutant allele could be amplified by the PCR primers p1 (5′-GGTTCTCTTTAGTCTTTAC-3′) and p2 (5′-CAGACAACTATTTCGTTACC-3′) from chromosomal DNA but not from the transgene 8ks (Fig. 1C). Xcm I cleaved the 1.5-kb fragment from wild-type DNA at the recognition site CCA-9N-TGG, but not from the *coi1-1* mutant, in which the Xcm I recognition site was altered by the G1401A mutation to CCA-9N-TGA. The 1.5-kb fragment from T-36 was not cleaved by Xcm I, indicating that this plant was homozygous for the *coi1-1* mutation and was therefore functionally complemented by the transgene 8ks.

F-Box

```
MEDPDIKRCK LSCVATVDDV IEQVMTYITD PKDRDSASLV CRRWFKIDSE TREHVTMALC YTATPDRLSR RFPNLRSLKL   80
                                                        coi1-15  A TLRRLIVLAV DSRT*
                    leucine-rich repeats start
KGKPRAAMFN LIPENWGGYV TPWVTEISNN LRQLKSVHFR RMIVSDLDLD RLAKARADDL ETLKLDKCSG FTTDGLLSIV 160
THCRKIKTLL MEESSFSEKD GKWLHELAQH NTSLEVLNFY MTEFAKISPK DLETIARNCR SLVSVKVGDF EILELVGFFK 240
AAANLEEFCG GSLNEDIGMP EKYMNLVFPR KLCRLGLSYM GPNEMPILFP FAAQIRKLDL LYALLETEDH CTLIQKCPNL 320
EVLETRNVIG DRGLEVLAQY CKQLKRLRIE RGADEQGMED EEGLVSQRGL IALAQGCQEL EYMAVYVSDI TNESLESIGT 400
                                  coi1-18  QK PQISS*
YLKNLCDFRL VLLDREERIT DLPLDNGVRS LLIGCKKLRR FAFYLRQGGL TDLGLSYIGQ YSPNVRWMLL GYVGESDEGL 480
                                                                  coi1-1  *
                leucine-rich repeats finish
MEFSRGCPNL QKLEMRGCCF SERAIAAAVT KLPSLRYLWV QGYRASMTGQ DLMQMARPYW NIELIPSRRV PEVNQQGEIR 560
EMEHPAHILA YYSLAGQRTD CPTTVRVLKE PI*                                                      592
```

Fig. 3. Derived amino acid sequence of *COI1* (*27*). A line over the sequence indicates regions with similarity to previously defined functional domains. The altered sequences of the mutant alleles *coi1-1*, *coi1-15*, and *coi1-18* are indicated. The *COI1* cDNA sequence has been deposited in GenBank (accession number AF036340). The COI1 genomic sequence was simultaneously determined in the *Arabidopsis* genome-sequencing project (accession number AF00210).

REFERENCES AND NOTES

1. G. Sembdner and B. Parthier, *Annu. Rev. Plant Physiol.* **44**, 569 (1993); R. A. Creelman and J. M. Mullet, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**, 355 (1997).
2. C. L. Ryan, *Plant Mol. Biol.* **19**, 123 (1992).
3. H. Pena-Cortes, T. Albrecht, S. Prat, E. W. Weiler, L. Willmitzer, *Planta* **191**, 123 (1993); G. A. Howe, J. Lightner, J. Browse, C. A. Ryan, *Plant Cell* 8, 2067 (1996).
4. P. J. O'Donnell *et al.*, *Science* **274**, 1914 (1996).
5. M. McConn and J. Browse, *Plant Cell* 8, 403 (1996).
6. B. F. Feys, C. E. Benedetti, C. N. Penfold, J. Turner,

ibid. **6**, 751 (1994).
7. P. E. Staswick, W. Su, S. H. Howell, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 6837 (1992); S. Berger, E. Bell, J. E. Mullet, *Plant Physiol.* **111**, 525 (1996).
8. M. McConn, R. A. Creelman, E. Bell, J. Mullet, J. Browse, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5473 (1997).
9. E. Rojo *et al.*, *Plant J.* **13**, 153 (1998).
10. D.-X. Xie, B. F. Feys, S. James, M. Nieto-Rostro, J. G. Turner, unpublished data.
11. I. A. M. A. Penninckx *et al.*, *Plant Cell* **8**, 2309 (1996).
12. *coi1* mutants representing three independent alleles (*coi1-1*, *coi1-15*, and *coi1-18*) were selected as seedlings with green leaves and well-developed roots on MS medium (mineral salts and sucrose, 3% w/v, solidified with agar, 0.6% w/v) supplemented with 50 μM methyl jasmonate (6).
13. A. Konieczny and F. M. Ausubel, *Plant J.* **4**, 403 (1993).
14. A fragment of *Thi2.1* gene [C. E. Eppel, K. Apel, H. Bohlman, *Plant Physiol.* **109**, 1813 (1995)] was used as a probe to identify hybridizing clones in an *Arabidopsis thaliana* ecotype Columbia genomic library. From one clone, a fragment of ~1 kb containing the *Thi2.1* promoter was amplified by the polymerase chain reaction (PCR) with primers that hybridized to vector sequences and to sequences 5′ from the ATG start of *Thi2.1*. This fragment was fused to GUS at the Sma I site of pBI101.3 [R. A. Jefferson, T. A. Kavanagh, M. Bevan, *EMBO J.* **6**, 3901 (1987)] to give *PThi2.1-GUS*. *PThi2.1-GUS* was then introduced into plants by *Agrobacterium tumefaciens*–mediated transformation (*16*). GUS activity was detected histochemically [R. A. Jefferson, *Plant Mol. Biol. Rep.* **5**, 387 (1987)].
15. M. Mindrinos, F. Katagiri, G.-L. Yu, F. M. Ausubel, *Cell* **78**, 1089 (1994).
16. Fragment 8ks (Fig. 1B) and Hind III–Sac I fragments of cDNAs 1.8c and 1.9c (Fig. 1C), containing the CaMV 35S promoter and full-length coding sequence, were each cloned into pBin19 [M. Bevan, *Nucleic Acids Res.* **12**, 8711 (1984)] and then mobilized into *Agrobacterium tumefaciens* pGV3101 by electroporation. Flower buds of wild-type and *COI1/coi1-1* plants were transformed by in planta vacuum infiltration [A. Bent *et al.*, *Science* **265**, 1856 (1994)]. Transgenic plants were selected as seedlings with green leaves and well-developed roots on MS containing kanamycin (50 μg/ml) and confirmed by Southern (DNA) blot analysis.
17. D. Valvekens, M. Van Montagu, M. van Lijsebetten, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5536 (1988).
18. Templates for DNA sequencing were overlapping subclones of fragments 8ks, 1.8c, and 1.9c (Fig. 1C) and fragments amplified from PCR from *coi1* mutants (*12*) using *Pfu* DNA polymerase (Stratagene).
19. C. Bai *et al.*, *Cell* **86**, 263 (1996).
20. B. Kobe and J. Deisenhofer, *Trends Biochem. Sci.* **19**, 415 (1994); S. G. St. C. Buchanan and N. J. Gay, *Prog. Biophys. Mol. Biol.* **65**, 1 (1996).
21. F. N. Li and M. Johnston, *EMBO J.* **16**, 5629 (1997); D. Skowyra, K. L. Craig, M. Tyers, S. J. Elledge, J. W. Harper, *Cell* **91**, 209 (1997).
22. M. Ruegger *et al.*, *Genes Dev.* **12**, 198 (1998).
23. H. Zhang, R. Kobayashi, K. Galaktionov, D. Beach, *Cell* **82**, 915 (1995); J. S. Flick and M. Johnston, *Mol. Cell. Biol.* **11**, 5101 (1991); Y. Barral, S. Jentsch, C. Mann, *Genes Dev.* **9**, 399 (1995).
24. The CAPS marker B23 revealed a DNA polymorphism between ecotypes Columbia and Landsberg erecta when Pst I was used to digest a PCR fragment amplified with the primers 5′-CTGCTTTTTT-GTGAGGTTTTG-3′ and 5′- CTCCACTCGCTAAA-CATCTG-3′.
25. YAC protocols were according to R. Schmidt, G. Cnops, I. Bancroft, C. Dean, *Aust. J. Plant Physiol.* **19**, 341 (1992). YAC libraries were F. Creusot *et al.*, *Plant J.* **8**, 763 (1995); J. Ecker, *Methods* **1**, 186 (1990); E. Grill and C. Somerville, *Mol. Gen. Genet.* **226**, 484 (1991). The IGF BAC library is described at http://194.94.225.1/private_workgroups/pg_101/bac.html.
26. P. Christou and W. F. Swain, *Trends Biotechnol.* **8**, 145 (1990).
27. Abbreviations for the amino acids are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and *, stop codon.
28. We thank J. Tashpulatov and E. Uslu for technical assistance; F. M. Ausubel for the cDNA library; J. Chory for information on C83; R. Schmidt, C. Dean, and D. Bouchez for support in screening YAC libraries and providing YAC clones; M. Estelle and J. Sanchez-Serrano for sharing information before publication; Nottingham Arabidopsis Stock Centre for seed; and the Arabidopsis Biological Resource Centre for restriction fragment length polymorphism clones, DNA libraries, and BAC filters. Supported by grant G01325 from the BBSRC, UK.

11 December 1997; accepted 30 March 1998