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An *Arabidopsis thaliana* Thionin Gene Is Inducible via a Signal Transduction Pathway Different from That for Pathogenesis-Related Proteins[1]

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Two cDNAs encoding thionin preproproteins have been isolated from *Arabidopsis thaliana*. The corresponding genes have been designated *Thi2.1* and *Thi2.2*. Southern blot analysis suggests that *A. thaliana* most probably contains single genes for both thionins. *Thi2.2* transcripts have a low basal level in seedlings and show circadian variation. *Thi2.2* transcripts were also detected in rosette leaves. No potent elicitors have been found for the *Thi2.2* gene. Transcripts of the *Thi2.1* gene are not detectable in seedlings but are present in rosette leaves and at a very high level in flowers and in siliques. The expression of the *Thi2.1* gene is highly inducible in seedlings by pathogens, silver nitrate, and methyl jasmonate, but not by salicylate, indicating that the gene is induced by a signal transduction pathway that is at least partly different from that for the pathogenesis-related proteins.

Plants are constantly threatened by a variety of pathogens. As they have evolved, they have developed several different resistance mechanisms. Among these are physical barriers (Ride, 1985) and the production of antimicrobial substances. Well known among the postinfection products are the low-molecular weight phytoalexins (Dixon, 1986) and the PR proteins (Linthorst, 1991). The latter include hydrolytic enzymes, chitinases and glucanases, as well as antimicrobial proteins of the thaumatin group. Another group of defense-related proteins are the thionins (Garcia-Olmedo et al., 1989; Bohlmann and Apel, 1991; Bohlmann, 1994). They are Cys rich, have a molecular weight of about 5000, are synthesized as preproproteins, and are usually basic and toxic for several biological systems (Vernon, 1992). The toxicity against phytopathogenic bacteria led Fernandez de Caleya et al. (1972) to propose a role for thionins in plant defense.

Experimental evidence for leaf-specific thionins of barley supports this view. Cultivated barley (*Hordeum vulgare* L.) and wild barley species contain a large multigene family for leaf thionins (Bohlmann and Apel, 1987; Gausing, 1987; Bohlmann et al., 1988; Bunge et al., 1992). The mature leaf thionins are found in the vacuole (Reimann-Philipp et al., 1989b) and in the cell wall (Bohlmann et al., 1988). The outer cell walls of epidermal cells have especially high concentrations of thionins (Reimann-Philipp et al., 1989a). Thionins that were isolated from leaves or the endosperm of barley were both toxic to plant pathogenic fungi and bacteria in vitro (Bohlmann et al., 1988; Florack et al., 1993; Molina et al., 1993; Terras et al., 1993). The accumulation of barley leaf thionins is inducible by phytopathogenic fungi. Infection by *Septoria nodorum* results in strong expression of leaf thionins in barley coleoptiles (Titarenko et al., 1993). Mildew infection of barley leaves leads to a transient rise in the transcript level, with a maximum after 24 h (Bohlmann et al., 1988; Boyd et al., 1994) in compatible and incompatible interactions. The accumulation of thionins after mildew infection has also been investigated by immunogold labeling. It was found that, in a compatible interaction, thionins were not detectable in the cell wall around the infection peg and in the papillae. In contrast, in an incompatible interaction, thionins were found in the papillae and in the cell wall surrounding the infection peg (Ebrahim-Nesbat et al., 1989, 1993). The accumulation of barley leaf thionins can also be induced by heavy metals (Fischer et al., 1989), jasmonic acid (Andresen et al., 1992), INA (Wasternack et al., 1994), and salicylate (Kogel et al., 1995).

Jasmonic acid or methyl jasmonate (Gundlach et al., 1992) and salicylic acid (Dempsey and Klessig, 1994) are believed to play a role in the defense reaction of plants against phytopathogens. INA has been shown to induce systemic resistance in several plant species, including *Arabidopsis thaliana* (Métraux et al., 1991; Uknes et al., 1992). Further support for a role of thionins as defense proteins comes from the observation that expression of an endosperm-specific hordothionin in transgenic tobacco leads to an enhanced resistance against a phytopathogenic bacterium (Carmona et al., 1993).

Taken together, research on thionins of barley has provided several lines of experimental evidence for a role of thionins in the resistance mechanism of higher plants, but, as for other putative defense proteins, definite proof is still missing. One way of further testing the proposed function of thionins would include the use of mutants or transgenic plants that alter the expression level of thionins, and to analyze the resistance of these plants against pathogens.

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Abbreviations: INA, 2,6-dichloroisonicotinic acid; MS, Murashige and Skoog; PR, pathogenesis-related; RFLP, restriction fragment length polymorphism.

Barley, however, is not routinely transformable. Another problem is the large number of closely related leaf thionin genes in barley (Bohlmann et al., 1988), which makes it difficult to study the expression of single genes (Bohl and Apel, 1993; Ebrahim-Nesbat et al., 1993). Therefore, we have looked for thionin genes in other plants that contain a smaller number of thionin genes and can easily be transformed. Using PCR, thionin genes were detected in *A. thaliana*. As a first step of a detailed analysis of thionins as putative defense factors, we report here the isolation and characterization of two cDNAs that encode typical thionins. Northern blot studies have revealed that one gene is highly inducible by plant pathogens as well as by silver nitrate and methyl jasmonate via a signal transduction pathway, which is at least partly different from that for PR proteins.

MATERIALS AND METHODS

Isolation of Genomic PCR Fragments

Two primers (TH.25: 5'-AAT CAA GTC GAA GCA AA/CG AG/CT TGT/C TG-3'; TH.26: 5'-CAG AAA C/TT/CA GAA/G CAT/G GCG TTG T/GTG/A CA-3') were derived from the sequence of the crambin cDNA (Schrader-Fischer and Apel, 1994) and used for PCR with genomic DNA from the *Arabidopsis thaliana* ecotype Zürich. PCR was done in a total volume of 50 μL containing 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.25 mM dNTP, 10 pM of each primer, 1 unit of *Taq* polymerase, and 1 μg of DNA for 30 cycles at 95°C for 30 s, 48°C for 30 s, and 72°C for 2 min. After phenol extraction and ethanol precipitation, the fragments were separated on a 1.5% Seaplaque GTG (FMC Bioproducts, Rockland, ME) agarose gel. Bands with a length of about 450 and 500 bp were cut out and cloned into the *Sma*I site of pUC18.

Screening of cDNA Libraries

cDNA libraries of the ecotypes Columbia and Landsberg erecta were provided by Dr. Dorothee Staiger and Martin Leube (ETH, Zürich, Switzerland), respectively, and were screened as described by Sambrook et al. (1989) using the two radiolabeled PCR probes.

Sequencing

All clones were sequenced by the dideoxynucleotide chain termination method (Sanger et al., 1977) in both orientations. Sequences were analyzed with the University of Wisconsin Genetics Computer Group programs (Devereux et al., 1984). Isoelectric points were determined with the Genetics Computer Group "Isoelectric" program. Cys residues were excluded in the calculation because it has been shown that the Cys residues of the thionins are involved in disulfide bridges, and the same is probably true for the Cys residues of the acidic domain.

Growth and Treatment of Plants

The following *A. thaliana* ecotypes were used: Col-2, Ler, Ws-0, Zürich. Plants were grown in soil in a greenhouse for seed production, isolation of genomic DNA, or isolation of RNA from different organs. For treatment with chemicals or pathogens, seeds were sterilized, sown on MS plates (Murashige and Skoog, 1962) containing vitamins (2 mg/L Gly, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine-HCl, 0.1 mg/L thiamine-HCl), 2% Suc, and 0.8% agar, stored at 4°C for 2 d, and grown in a growth chamber (16 h light, 20°C, 8 h dark, 18°C) for 14 d. Plants were vacuum infiltrated (Young, 1974) with the test solutions in a desiccator 3 h after the start of the light period, dried for 3 h in a laminar flow-clean bench (Gelaire, Gruppo Flow, Opera, Italy), and put back into the growth chamber for various times.

Methyl jasmonate (Serva, Paramus, NJ) was used as a 100 μM solution in 0.02% (v/v) acetone. Sodium salicylate was used as a 1 mM solution and ethephon was used at a concentration of 0.01% (w/v).

Fusarium oxysporum f.sp. *matthiolae* (strain 247.61, Centraalbureau voor Schimmelcultures, Baarn-Delft, The Netherlands) was grown on potato dextrose agar at room temperature for 2 to 3 weeks. Spores were taken up in sterile water, filtered through Miracloth (Calbiochem), and counted with a Fuchs/Rosenthal (Roth, Karlsruhe, Germany) chamber. Spore suspensions were diluted to 10^6 and 10^7 spores mL^{-1}. Seedlings were grown as before and sprayed with a spore suspension (4 mL/9-cm Petri dish). Petri dishes were closed and incubated again in the growth chamber (the first 24 h in the dark) until harvest of the infected plants.

Northern Blots

Plants were treated as described above. Plants grown on MS medium in Petri dishes were harvested by pouring liquid nitrogen onto the plates. Plant material was ground in liquid nitrogen and RNA was prepared as described by Melzer et al. (1990).

Total RNA (20 or 30 μg) was separated on denaturing 1.0% agarose gels (Ausubel et al., 1994). Ethidium bromide was included to verify equal loading of RNA. After transfer to GeneScreen membranes (NEN), filters were hybridized with 10^6 cpm mL^{-1} oligolabeled ^{32}P probes (Feinberg and Vogelstein, 1983) in HYBSOL (Yang et al., 1993).

The following probes were used: *Thi2.1* (AthTH1) and *Thi2.2* (AthTH2) cDNAs, and *A. thaliana* PR1 and PR5 probes, which were amplified with specific primers according to the sequences published by Uknes et al. (1992). PR1 and PR5 probes were used together. Filters were washed for 15 min at 60°C with 2× SSC and then with 0.5× SSC. Filters were exposed to X-Omat-AR (Kodak) films at −80°C for 1 to 4 d. Probes were stripped off the membrane by boiling in 0.1% SDS according to the manufacturer's instructions.

Southern Blots

Genomic DNA from 6-week-old plants was isolated according to the method of Murray and Thompson (1980) and purified on a cesium chloride gradient. Three micrograms of DNA were digested with restriction enzymes (Boehringer Mannheim) according to the manufacturer's

instructions and separated on a 0.8% agarose gel. Afterward, the DNA was transferred to Pall Biodyne A membranes (Pall, Muttenz, Switzerland), and blots were hybridized (Sambrook et al., 1989) with ^{32}P-labeled *Thi2.1* and *Thi2.2* cDNA probes. Filters were washed twice with 2× SSC, 0.1% SDS at 45°C, once with 0.1× SSC, 0.1% SDS at 65°C, and exposed for 4 d. Membranes were stripped as described above.

RESULTS

Isolation of Two Thionin cDNAs from *A. thaliana*

We used primers derived from the sequence of the crambin cDNA (Schrader-Fischer and Apel, 1994) for a PCR with genomic *A. thaliana* DNA (crambin is a seed-specific thionin from the crucifer *Crambe abyssinica*). After separation on agarose gels, two bands were identified that corresponded in size to fragments expected from thionin genes (Fig. 1). DNA from these bands was cloned into pUC18. Two different genomic fragments were obtained that were shown to be homologous to thionin genes by sequencing (data not shown). These two fragments were used as hybridization probes to screen cDNA libraries (see "Materials and Methods"). Several cDNAs were obtained and sequenced. They all corresponded to the two different PCR fragments.

The sequences of the two longest cDNAs (AthTH1 and AthTH2) are shown in Figure 2. Both encode typical thionin preproproteins, which is evident from a comparison of the deduced protein sequences with those of other thionin preproproteins (Fig. 3). Both *A. thaliana* thionins have six Cys residues, are basic (IEP *Thi2.1* thionin = 12.36; IEP *Thi2.2* thionin = 10.63), and have a Tyr residue at position 13 (see Fig. 3), as have all other toxic thionins (Bohlmann, 1994). At the C terminus both clones encode typical acidic domains (IEP *Thi2.1* acidic domain = 4.59; IEP *Thi2.2* acidic domain = 4.28) with six conserved Cys residues, whereas typical signal sequences are found at the N terminus. A

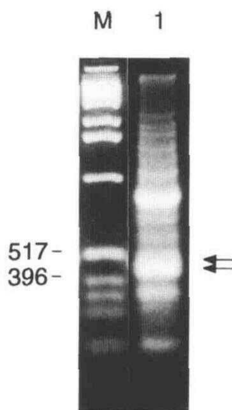

Figure 1. Separation of PCR products amplified with thionin-specific primers on a 1.5% Seaplaque GTG agarose gel. Arrows indicate the bands that were isolated for cloning. Values for size markers are given in bp.

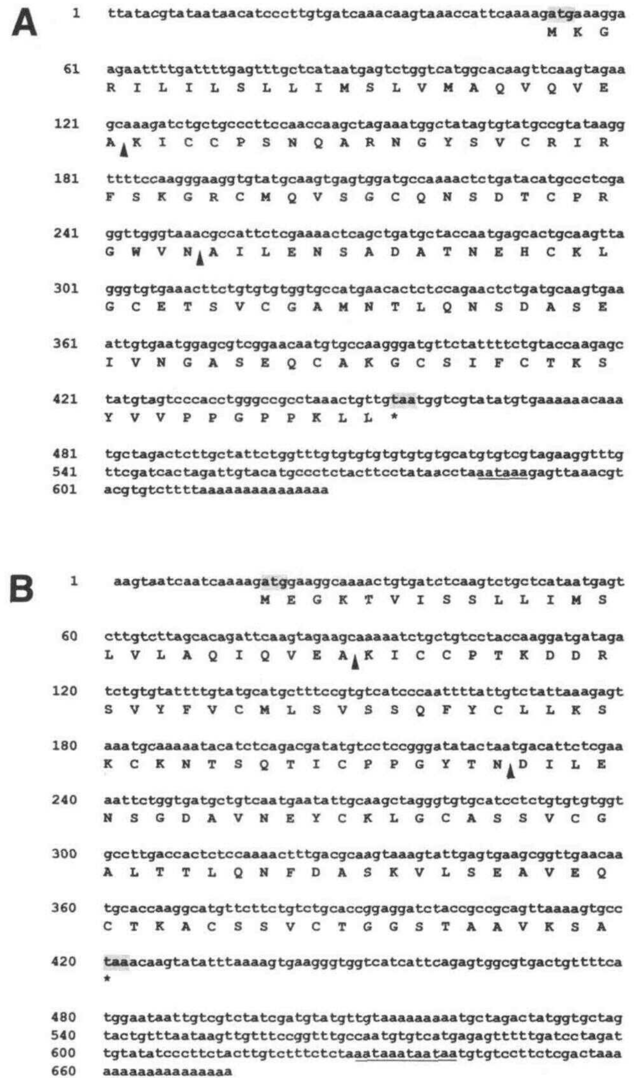

Figure 2. Sequences of the cDNA AthTH1 (A) from the ecotype Columbia and the cDNA AthTH2 (B) from the ecotype Ler. The protein sequence of the encoded preproprotein is given in the one-letter code. Start and stop codons are shaded. Arrowheads indicate the borders of the mature thionin, deduced from a comparison with other thionins. The putative polyadenylation site is underlined.

comparison of the two cDNAs shows 71% homology for the whole coding region. At the protein level there is only 53% identity of amino acids. If the three domains of the preproprotein are viewed separately, the identity values at the protein level are 71 (signal sequence), 42 (thionin), and 57% (acidic domain). Since both thionins have six Cys residues in homologous positions to crambin, the genes are designated *Thi2.1* and *Thi2.2* (Bohlmann et al., 1994).

Genomic DNA from three different ecotypes was digested with different restriction enzymes and separated on an agarose gel (Fig. 4). The blot was first hybridized with a *Thi2.1*-specific probe and, subsequently, after removing the *Thi2.1*-specific probe, rehybridized with a *Thi2.2*-specific probe. After high-stringency washes, probe *Thi2.2* detected

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Position           -20        -10         1         10         20         30         40
                                          ▼
HTH1         MG----LKGV MVCLLILGLV LEQVQVEGKS CCRSTLGRNC YNLCRVRGAQ K-LCAGVCRC KLTSSGKCPT
Hv-DB4       MAPSKSIKSV VICVLILGLV LEQVQVEGKS CCKATLARNC YNTCHFAGGS RPVCAGACRC KIISGPKCPS
Vis A3       MEVVRGSSLV LLVLLLGALL VSN--VESKS CCPNTTGRNI YNACRLTGAP RPTCAKLSGC KIISGSTCPS
CRAMBIN      MEG----KTV ILSVLIMSLV MSYNQVEATT CCPSIVARSN FNVCRLPGTS EAICATYTGC IIIPGATCPG
Ath-Thi2.1   MKG----RIL ILSLLIMSLV MAQVQVEAKI CCPSNQARNG YSVCRIRFSK GR-CMQVSGC QNS--DTCPR
Ath-Thi2.2   MEG----KTV ISSLLIMSLV LAQIQVEAKI CCPTKDDRSV YFVCMLSVSS QFYCLLKSKC KNTSQTICPP

Position           50         60         70         80         90        100        110
                ▼
HTH1         GFPKLALVSN SDEPDTVKYC NLGCRASNCD YM--VNAAAD DEEMKLYLEN CGDACVNFCN GDAGLTSLTA ---
Hv-DB4       DYPKLNLLPE SGEPDVTQYC TIGCRNSVCD NM---DNVFR GQEMKFDMGL CSNACARFCN DGAVIQSVEA ---
Vis A3       DYPKF----- --------YC TMGCESSQCA -----NSNGD AEAVR----- CKTACSDLCN QDVD----DA ---
CRAMBIN      DYANNILKNS AQGNAVNEYC KWGCASSVCG ALTNLQNSDA REIVNGAVRQ CTNACSDFCT NG---SAKAV ETA
Ath-Thi2.1   GWVNAILENS A--DATNEHC KLGCETSVCG AMNTLQNSDA SEIVNGASEQ CAKGCSIFCT KSYVVPPGPP KLL
Ath-Thi2.2   GYTNDILENS --GDAVNEYC KLGCASSVCG ALTTLQNEDA SKVLSEAVEQ CTKACSSVCT GG---STAAV KSA
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Figure 3. A comparison of the preproprotein sequences deduced from the two *A. thaliana* cDNA clones with the sequences from other thionin precursor proteins. Cys residues are shaded. The amino acid at position 13 is given in white. Arrowheads indicate the borders of the mature thionin. Numbering starts with the thionin domain. The alignment was produced with the Genetics Computer Group program "Clustal." The sequences are from HTH1 (Rodriguez-Palenzuela et al., 1988), *Hv*-DB4 (Bohlmann and Apel, 1987), *Vis* A3 (Schrader and Apel, 1991), and crambin (Schrader-Fischer and Apel, 1994). *Ath-Thi2.1* and *Ath-Thi2.2* sequences are from this work.

two bands for *Ssp*I and only one band in the case of the other enzymes, and probe *Thi2.1* detected one band with every enzyme, indicating that there is probably only one gene for each thionin. At very low stringency a few other bands were detected, indicating that *A. thaliana* might contain one or two more thionin genes (data not shown). It is interesting that the *Thi2.2* probe detected RFLPs with every restriction enzyme used, whereas the *Thi2.1* probe detected no RFLP (Fig. 4).

Developmental Expression of the Thionin Genes

The developmental expression of both thionin genes was investigated with northern blots. Total RNA was isolated from different organs and developmental stages (Fig. 5) and analyzed on northern blots. Hybridization with a *Thi2.1*-specific probe revealed a very high transcript level in siliques and in flowers as well as a signal in rosette leaves, but no signal was detected in seedlings, cauline leaves, stems, and roots. Hybridization with a *Thi2.2*-specific probe gave signals in seedlings and rosette leaves, but only a barely detectable signal in siliques and no signals in cauline leaves, stems, flowers, and roots (Fig. 5).

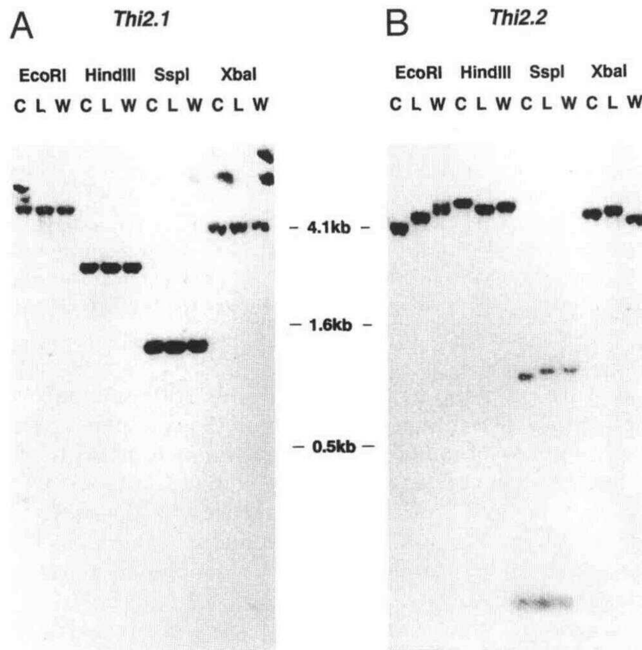

Figure 4. Genomic blot probed with *Thi2.1*- (A) and *Thi2.2*-specific (B) probes. The restriction enzymes are indicated above the lines. For each enzyme the following ecotypes were used from left to right: Col-2 (C), Ler (L), Ws-0 (W).

Induction by Phytopathogens

Barley leaf thionins have been shown to be inducible by phytopathogenic fungi. Preliminary experiments with *A. thaliana* indicated that *Thi2.1* can also be induced by phytopathogenic fungi and bacteria (data not shown). Therefore, we conducted an experiment with *F. oxysporum* f.sp. *matthiolae* under controlled conditions (Fig. 6). This isolate of *F. oxysporum* gives a compatible interaction with seedlings of the ecotype Col-2. Seedlings were sprayed with two different spore densities and mRNA was isolated at 1,

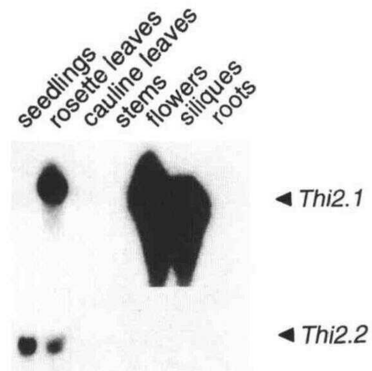

Figure 5. Northern blots hybridized with *Thi2.1*- and *Thi2.2*-specific probes. Thirty micrograms of total RNA isolated from different parts of the ecotype Col-2 were used. Exposure was for 1 (*Thi2.1*) or 4 d (*Thi2.2*). Seedlings were grown on MS medium. All other plant organs were isolated from plants that were grown in soil in the greenhouse.

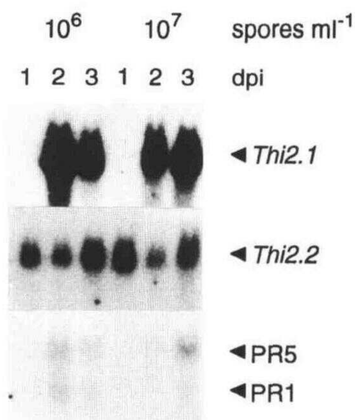

Figure 6. Induction of *Thi2.1* by *F. oxysporum* f.sp. *matthiolae*. Total RNA was isolated from seedlings infected as described in "Materials and Methods." Northern blots (20 μg of total RNA) were hybridized with probes for *Thi2.1*, *Thi2.2*, PR1, and PR5. dpi, Days postinfection.

2, and 3 d postinfection. The northern blot shows that the *Thi2.1* gene is strongly induced at 2 and 3 d postinfection, whereas the transcript level for *Thi2.2* remains at the level of untreated controls. Compared to *Thi2.1*, the induction of PR1 and PR5 is much lower (Fig. 6).

Induction by Chemicals

Previously, various chemicals and other abiotic elicitors have been shown to induce barley thionins, PR proteins, or phytoalexins. We have tested several of these elicitors in *A. thaliana* for their ability to induce thionin gene expression in comparison with their effects on PR1 and PR5 genes. Seeds were germinated on MS plates and grown for 14 d under a day/night cycle. The various test solutions were infiltrated as described in "Materials and Methods." RNA was analyzed on northern blots (Fig. 7). *Thi2.1* transcripts were not detectable in control seedlings, but the gene was highly inducible by silver nitrate and methyl jasmonate. The induction by silver nitrate gave a transcript maximum at 6 h after treatment, as shown in several independent experiments, whereas later the level declined again. Methyl jasmonate did not show a clear maximum, but the transcript level remained high for the total period tested (24 h). PR1 and PR5, however, were not induced by silver nitrate or methyl jasmonate. No *Thi2.1* signal was detectable after treatment with salicylate or ethephon for 48 h. On the other hand, salicylate induced PR1 gene expression, and ethephon induced both PR1 and PR5 gene expression with a weak signal at 24 h and a strong signal at 48 h. No induction of the *Thi2.1* gene was observed (data not shown) after treatment with lead nitrate, vanadate, and hormones (ABA, indole acetic acid, zeatin, and GA_3, each at 100 μM).

The expression of the *Thi2.2* gene is regulated differently (Fig. 7). A control experiment with untreated seedlings and seedlings treated with water shows only that the transcript level for *Thi2.2* displays circadian variation and that the stress of the infiltration procedure does not influence the transcript level. No potent elicitors have been found that induce the expression of the *Thi2.2* gene. Salicylate, ethephon, methyl jasmonate, and silver nitrate were all ineffective. Furthermore, lead nitrate, vanadate, and the various hormones mentioned above also did not affect the transcript level of the *Thi2.2* gene (data not shown).

DISCUSSION

Isolation of Two *A. thaliana* Thionin cDNAs

We have isolated and characterized two different and complete thionin cDNAs from *A. thaliana*. The identity of these cDNAs could be verified by a comparison of the deduced protein sequences, encoded by the two cDNAs, with the sequences of other thionin preproproteins (Fig. 3). The *A. thaliana* cDNAs also encode preproproteins containing a signal sequence, the thionin domain, and an acidic domain. Furthermore, all Cys residues are conserved. The basic nature of the two deduced thionins and the Tyr residue at position 13 indicate that both proteins are probably toxic (Bohlmann, 1994).

A comparison of the two *A. thaliana* cDNAs shows 71% identity at the nucleotide level of the coding region and only 53% identity at the protein level. If the different parts of the preproprotein are viewed separately, the differences are higher between the thionin parts than between the acidic domains or between the signal sequences. This phenomenon has been observed before with other thionin precursors (Bunge et al., 1992; Castagnaro et al., 1992) and might be due to a high selection pressure exerted on the thionins by, for instance, genetically variable pathogens such as mildew (Bohlmann, 1994).

Genomic Southern blots (Fig. 4) revealed that both *A. thaliana* thionins are most likely encoded by single genes. Except for *SspI*, in the case of the *Thi2.2* gene, every enzyme that was used detected only a single band. Furthermore, multiple cDNA clones were sequenced and all had the same sequence. Genomic clones have been isolated that correspond to the two cDNAs (A. Vignutelli, unpublished results). A closer look at the genomic blots revealed that the *Thi2.2* probe detected RFLPs for every enzyme and every ecotype that was used, whereas the *Thi2.1* probe detected no RFLP. This is rather surprising, given the scarcity with which RFLPs are usually detected. It indicates that the *Thi2.2* gene might be located in a region of the *A. thaliana* genome with a high evolution rate. Another explanation could be that the *Thi2.2* gene itself is evolving rapidly, perhaps in the promoter region or in the 3′ noncoding region, producing abundant RFLPs.

Low-stringency washes of the Southern blots revealed that *A. thaliana* might contain one or two more thionin genes in addition to the two for which we have isolated cDNAs. Nevertheless, compared to barley (Bohlmann et al., 1988), the total number of thionin genes in *A. thaliana* is much lower and the differences at the nucleotide level are much higher. This makes it possible to study the expression of single genes by northern blot analysis, as has been shown in this work, and allows screening for thionin mutants.

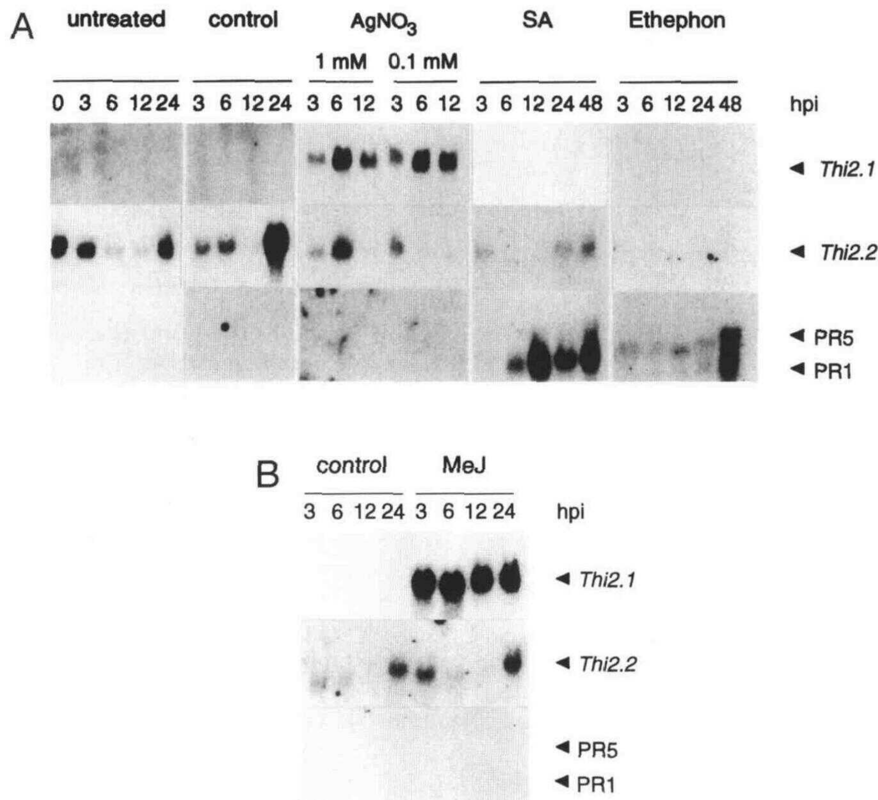

Figure 7. Induction by chemicals. Seedlings were grown on MS plates and infiltrated with test solutions as described in "Materials and Methods." Note that all treatments started at the same daytime. Northern blots were prepared with 30 μg of total RNA and hybridized with probes for *Thi2.1*, *Thi2.2*, PR5, and PR1. A, Untreated seedlings (not infiltrated); control (infiltrated with water); $AgNO_3$, infiltrated with silver nitrate; SA, infiltrated with sodium salicylate (1 mM). B, Control (infiltrated with 0.02% acetone); MeJ, infiltrated with 0.1 mM methyl jasmonate in 0.02% acetone. hpi, Hours postinduction.

Regulation of *Thi2.1* and *Thi2.2*

The *Thi2.2* gene has a low basal transcript level in seedlings and rosette leaves. In seedlings, its transcript level might be under circadian control (Fig. 7A). Whether the *Thi2.1* gene might be regulated similarly is not known because the transcript level in seedlings is below the detection limit (Fig. 7A). A very high *Thi2.1* transcript level was found in flowers and in siliques (Fig. 5). A similar phenomenon has been described for tobacco PR proteins, some of which are developmentally expressed in flowers (e.g. Lotan et al., 1989; Coté et al., 1991). An expression of defense proteins in flowers and fruits would particularly protect the reproductive organs of the plant. Seeds are similarly protected by a variety of antimicrobial proteins.

The *Thi2.1* gene was inducible in seedlings by phytopathogens, whereas *Thi2.2* was not. The response to *F. oxysporum* f.sp. *matthiolae* has been tested with two different spore densities, both of which led to an increase of the *Thi2.1* mRNA level (Fig. 6). Compared to PR1 and PR5 (Fig. 6), the induction of *Thi2.1* was more pronounced. It remains to be seen if the response in incompatible interactions is even faster and stronger, which has been demonstrated for many plant-fungus interactions. Preliminary results (P. Epple, unpublished data) indicate that this gene is also inducible by other phytopathogenic fungi and bacteria.

The response to chemical elicitors is different for both thionin genes. No elicitors have been found for the *Thi2.2* gene so far. The *Thi2.1* gene, on the other hand, is strongly inducible by both methyl jasmonate and silver nitrate, but PR1 and PR5 genes are not. Silver nitrate is well known as an elicitor of phytoalexins (e.g. Tsuji et al., 1992), and methyl jasmonate or jasmonic acid are strong elicitors of barley leaf thionins (Andresen et al., 1992) and other defense-related proteins (e.g. Gundlach et al., 1992). Typical PR protein elicitors such as ethephon and salicylate were not effective in inducing the expression of the two thionin genes (Fig. 6). Ethylene, produced by ripe apples, was also not effective (data not shown). It has been shown recently that ethylene induces the accumulation of PR proteins in tobacco (Raz and Fluhr, 1993).

In *A. thaliana*, however, only a hevein-like protein, and not PR1, PR2, or PR5, is induced by ethylene (Lawton et al., 1994). On the other hand, ethephon is an inducer of PR proteins in *A. thaliana* due to the liberation of acids (Lawton et al., 1994). Salicylic acid is involved in systemic acquired resistance (Ryals et al., 1994) and, as such, is an inducer of PR proteins in *A. thaliana* (Uknes et al., 1992) and other

plants. Since neither ethylene or ethephon nor salicylate induced the accumulation of *Thi2.1* mRNA, the signal transduction pathway for this gene must be different at least in part from that for PR proteins. Therefore, thionins do not seem to play a role in systemic acquired resistance, and their induction might therefore be local. This will be studied further with promoter-*uidA* fusions.

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