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Bax-induced cell death in tobacco is similar to the hypersensitive response

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ABSTRACT **Bax, a death-promoting member of the Bcl-2 family of proteins, triggered cell death when expressed in plants from a tobacco mosaic virus vector. Analysis of Bax deletion mutants demonstrated a requirement for the BH1 and BH3 domains in promoting rapid cell death, whereas deletion of the carboxyl-terminal transmembrane domain completely abolished the lethality of Bax in plants. The phenotype of cell death induced by Bax closely resembled the hypersensitive response induced by wild-type tobacco mosaic virus in tobacco plants carrying the *N* gene. The cell death-promoting function of Bax in plants correlated with accumulation of the defense-related protein PR1, suggesting Bax activated an endogenous cell-death program in plants. In support of this view, both *N* gene- and Bax-mediated cell death was blocked by okadaic acid, an inhibitor of protein phosphatase activity. The ability of Bax to induce cell death and a defense reaction in plants suggests that some features of animal and plant cell death processes may be shared.**

Programmed cell death (PCD) fulfills the same roles, elimination of unwanted cells during development and sacrifice of diseased cells, in both plants and animals (1–3). However, although some similarities exist between the ultrastructural and physiological hallmarks of PCD in animals and plants (4–6), evidence for common pathways leading to cell death is limited (7, 8).

In plants, the triggering of PCD in response to an invading pathogen results in the formation of a zone of dead cells in the vicinity of the infection site. This killing of host cells, termed the hypersensitive response (HR), is frequently associated with resistance to further pathogen multiplication and spread (9). The HR against tobacco mosaic virus (TMV), in tobacco (*Nicotiana tabacum*) carrying the *N* gene, provides a well studied model of pathogen-induced PCD in plants. The *N* gene, which has been introgressed from *Nicotiana glutinosa* into several other members of the genus *Nicotiana* (subsequently referred to as NN genotype) confers an HR against most strains of TMV. In contrast, *Nicotiana* species lacking the *N* gene (genotype nn) support ongoing accumulation and spread of TMV.

The *N* gene has been cloned and is predicted to encode a cytoplasmic protein with an amino-terminal nucleotide-binding domain and carboxyl-terminal leucine-rich repeats (10). Activation of the *N* gene-mediated HR is temperature sensitive (11) and involves an interaction, either direct or indirect, of the *N* gene product with the virus-encoded 126 kDa elicitor protein (12). Cells undergoing the *N* gene-mediated HR exhibit a sequence of responses that are similar to those described for PCD in animal systems (3), including condensation and vacuolization of the cytoplasm and nuclear DNA fragmentation, culminating in the death of infected cells and the limitation of virus spread (13). The *N* gene-mediated HR in tobacco is highly specific for TMV, and tobacco cultivars lacking the *N* gene are susceptible to TMV infection. Other species in the family Solanaceae are also susceptible to TMV infection; however, transgenic *Nicotiana benthamiana* and tomato engineered to express the *N* gene also exhibit an HR after TMV infection (D. Baulcombe, personal communication; ref. 14), indicating that the downstream effectors of the HR are conserved between tobacco and these related species. Despite the significant progress made in characterizing both the *N* gene product and the TMV elicitor (10, 12), the events leading from pathogen recognition to PCD are poorly understood.

In animal systems, studies of PCD have identified numerous pathways involved in the transduction of death-inducing stimuli to the cellular execution machinery (3, 15). The Bcl-2 family of proteins, which includes key regulators of both cell survival and cell death, is characterized by the possession of at least one of four conserved motifs known as Bcl-2 homology domains (BH1–4) (16). Bcl-2, and related death-antagonist proteins, promote cell survival by blocking the activation of proteases, termed caspases, required for the final execution phase of the cell-death program (16). In contrast, the death agonist members of the Bcl-2 family, of which Bax is a well studied example, appear to be able to disrupt the protective effect of Bcl-2 by the formation of Bcl-2/Bax heterodimers (16, 17).

Although heterodimerization of Bax is an important determinant of cell death, the lethal effect of this protein does not depend entirely on interactions with prosurvival proteins, as Bax can induce cell death irrespective of its ability to dimerize with Bcl-2 (18, 19). Furthermore, Bax is also lethal when expressed in the budding yeast, *Saccharomyces cerevisiae*, even though yeasts express neither Bcl-2-related proteins nor caspases (20). The lethal effect of Bax in yeast is known to depend on BH3, a domain required for homodimerization, thereby implicating Bax homodimerization as a prerequisite for toxicity (21, 22).

A plausible model to explain the caspase independent lethality of Bax in yeast proposes a direct effect of Bax on mitochondria, through its ability to form channels in the outer membrane of the organelle, leading to cytochrome *c* release and organelle dysfunction (23). This model is supported by the requirement for the carboxyl-terminal transmembrane (TM) domain, which targets Bax to mitochondria, in mediating cell death in yeast (20), and also by the finding that Bax stimulates the release of cytochrome *c* from yeast mitochondria (24).

Here we investigate the ability of Bax to induce cell death and trigger a defense-related response in plants when expressed from a viral vector and compare these responses with the HR induced by TMV on tobacco carrying the *N* gene.

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Abbreviations: CP, coat protein; dpi, days postinoculation; GFP, green fluorescent protein; HR, hypersensitive response; OA, okadaic acid; PCD, programmed cell death; TM, transmembrane; TMV, tobacco mosaic virus.

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MATERIALS AND METHODS

Plasmid Constructions. The constructs pTMV.Bax and pTMV.GFP were obtained by insertion of cDNAs, encoding Bax and the green fluorescent protein (GFP) respectively, between the *Pme*I and *Xho*I sites of the TMV vector cDNA in p30B. The modified TMV cDNA in p30B is similar to the previously described TMV vector construct, pTB2 (ref. 25; W. O. Dawson, personal communication). cDNA fragments were prepared by PCR amplification of *gfp* (26) and murine *bax* (17) cDNA templates, by using specific oligonucleotide primers incorporating restriction enzyme recognition sites at the 5′-(*Pme*I) and 3′-termini (*Xho*I) of the amplified sequences. The Bax deletion mutants pTMV.BaxΔBH3 (deleted from amino acids 60 to 75 inclusive), pTMV.BaxΔBH1 (102 to 117), pTMV.BaxΔBH2 (153 to 170), and pTMV.BaxΔTM (166 to 192) were prepared by oligonucleotide directed mutagenesis of pTMV.Bax. The viral genomes encoded by the above plasmids are referred to by deletion of the prefix p from the plasmid name. To construct the fusion between GFP and the Bax TM domain, a PCR fragment encoding the carboxyl-terminal 27 amino acids of Bax was amplified from the *bax* cDNA template and ligated to the 3′-terminus of a *gfp* cDNA, cloned in the vector pT7 blue (Novagen). The cDNA fragment encoding the GFP-TM fusion protein was isolated after digestion with *Eco*RV and *Xho*I and ligated between the *Eco*RV and *Sal*I restriction sites of the potato virus X cDNA vector pTXS.P2C3S (27), to give pTXS.GFP-TM. Virus derived from this plasmid is referred to as PVX.GFP-TM.

***In Vitro* Transcription and Plant Inoculation.** Plasmids harboring the modified TMV cDNAs were linearized with *Kpn*I, and *in vitro* run-off transcripts were synthesized as described previously (28). Transcription reaction products were inoculated directly to 8-wk-old plants by manual abrasion of aluminum oxide dusted leaves. Mock inoculations were performed by similar treatment of leaves with control transcription reactions lacking RNA polymerase. Plants were maintained in controlled environment chambers with a 16-hr photoperiod (400 μE m^{-2} s^{-1}) and were kept at either 32°C or 24°C, as indicated in the text.

Immunoblot Analysis. Protein extraction and Western blot analyses of leaf protein were performed as described previously (28). Total plant protein (10 μg) was electrophoresed on a 15% SDS-polyacrylamide gel. After transfer of protein to nitrocellulose membranes, immunoblotting was performed by using antisera raised in rabbits against either the TMV coat protein (CP) (dilution 1:2,000), Bax [a mixture of antibody raised against amino acids 11–30 of Bax, at 1:1,000 (Santa Cruz Biotechnology) and antibody raised against amino acids 150–165 of Bax, at 1:500 (Alexis Biochemicals, San Diego, CA)], or PR1 (1:5,000; the gift of L. A. J. Mur). Alkaline phosphatase-conjugated anti-rabbit IgG (Sigma) was used as the secondary antibody.

Okadaic Acid Treatment. Okadaic acid (OA) treatment of leaves was performed 24 hr after inoculation of either *Nicotiana edwardsonii* with TMV.GFP or *N. benthamiana* with TMV.Bax. Leaves were left attached to inoculated plants for 24 hr at 32°C. Inoculated leaves were then detached at the petiole and immersed either in a solution of 500 nM OA (Calbiochem), 0.4% ethanol (vol/vol), or in a control solution (0.4% ethanol). After a further 1-hr incubation at 32°C, the detached leaves were transferred to 24°C and maintained under continuous illumination. Leaves were photographed 24 hr after either OA or water treatment (48 hr postinoculation). UV photography was as described previously (27).

Staining of Mitochondria and Confocal Microscopy. Mitochondria were stained by vacuum infiltration of leaves with the fluorescent probe MitoTracker Red (Molecular Probes) at 650 nM in water, as described previously (29). Stained tissue was examined under a Bio-Rad MRC1000 confocal laser scanning microscope using excitation light at either 488 nm, to detect GFP (emission filter 522 nm), or 568 nm, to detect the mitochondrial stains (emission filter 605 nm). Imaging and image processing were as described by Baulcombe *et al.* (27).

RESULTS AND DISCUSSION

Infection of *N. benthamiana* (genotype nn) with a TMV-based vector carrying the murine *bax* cDNA (TMV.Bax, Fig. 1*A*) induced localized tissue collapse and cell death by 2–3 d postinoculation (dpi) (Fig. 1*B*). Areas of collapsed tissue, induced after viral infection, are subsequently referred to as lesions. Both the timing of cell collapse and the phenotype of the lesions were similar to the HR induced by TMV expressing GFP (TMV.GFP, Fig. 1*A*) on *N. edwardsonii* (genotype NN; Fig. 1*B*). Studies in mammalian systems and yeast have identified domains required for the prodeath activity of Bax (20, 21). To determine whether the plant response required the same domains, a series of Bax deletion mutants were expressed from the TMV vector.

Bax mutants lacking the BH3 (TMV.BaxΔBH3), BH1 (TMV.BaxΔBH1), or BH2 (TMV.BaxΔBH2, Fig. 1*A*) domains all induced localized cell death after inoculation to *N. benthamiana* leaves (Fig. 1*B*). At 4 dpi, the average diameter of lesions induced by TMV.Bax [1.26 mm ± 0.20 mm (mean ± SE)] was smaller than lesions induced by the deletion mutants TMV.BaxΔBH1 (2.79 ± 0.31 mm), TMV.BaxΔBH2 (1.87 ± 0.40 mm), and TMV.BaxΔBH3 (2.92 ± 0.47 mm). Lesions induced by TMV.Bax and TMV.BaxΔBH2 were comprised entirely of collapsed cells. In contrast, lesions induced by TMV.BaxΔBH1 and TMV.BaxΔBH3 initially developed a ringspot morphology, with concentric zones of collapsed and viable cells, followed by complete cell collapse at later time points (Fig. 1*B* and data not shown). In contrast to the lethal phenotypes induced by the BH domain deletion mutants, expression of a Bax mutant lacking the carboxyl-terminal TM domain (TMV.BaxΔTM, Fig. 1*A*) gave rise to mild chlorotic symptoms on inoculated leaves (Fig. 1*B*), demonstrating that the TM domain was essential for Bax-induced cell death in plants. However, expression of the TM domain alone, from TMV.TM (Fig. 1*A*), gave a symptomless infection similar in appearance to infections with TMV.GFP on *N. benthamiana* (Fig. 1*B*). Thus the TM domain was necessary, but not sufficient, for the cell death-promoting effect of Bax in plants.

To confirm accumulation of virus and the expression of Bax and the Bax deletion mutants, the level of viral CP and Bax antigen in inoculated leaves was assessed. Protein samples, prepared from *N. benthamiana* leaves inoculated with TMV expressing either Bax or the Bax deletion mutants, were analyzed by Western blotting. At 2 dpi, the highest level of Bax antigen was detected in leaves inoculated with the nonlethal TMV.BaxΔTM, whereas levels of Bax antigen in leaves inoculated with TMV expressing either Bax or the BH domain deletion mutants were low or negligible at this time point (Fig. 1*C*). At later time points, levels of Bax antigen in samples from plants inoculated with either TMV.Bax or TMV.BaxΔBH2 remained low, whereas antigen levels in leaves infected with TMV.BaxΔBH3, TMV.BaxΔBH1, and TMV.BaxΔTM continued to rise (Fig. 1*C*). The low level of Bax antigen detected in TMV.BaxΔBH2-infected leaves most probably reflects the fact that this mutant is deleted across part of the epitope recognized by one of the two antibodies used to detect Bax. CP was detected in all virus-inoculated leaves at 2 dpi, with the lowest levels seen in TMV.Bax-infected samples (Fig. 1*C*). Levels of CP increased with time in all samples except those from TMV.Bax-infected plants, where the level of CP did not increase beyond 3 dpi (Fig. 1*C*). Overall, the lowest levels of both CP and Bax antigen were detected in samples from TMV.Bax-infected leaves (Fig. 1*C*). These results suggested that the lethal response induced by Bax was responsible for

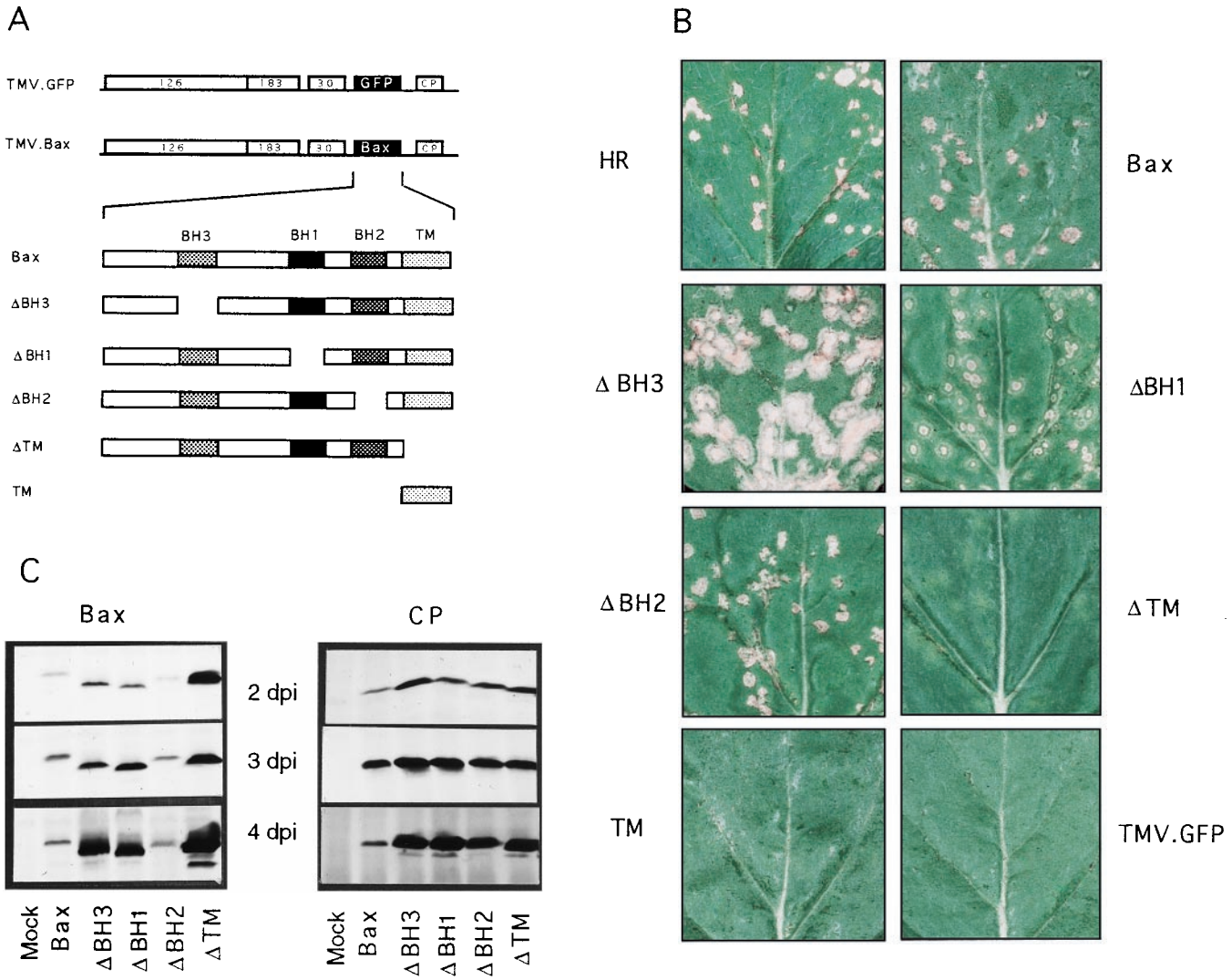

FIG. 1. Phenotypes associated with the expression of Bax and Bax deletion mutants in plants. (*A*) Schematic diagrams of the TMV vector constructs carrying the GFP and Bax cDNAs (not drawn to scale). Open boxes represent viral ORFs with the sizes of the encoded proteins indicated in kDa; CP is the TMV coat protein; filled boxes show the GFP and Bax ORFs. Schematic diagrams of the Bax and Bax deletion mutant ORFs are shown below. (*B*) Comparison of the localized HR induced by TMV.GFP on *N. edwardsonii* with symptoms induced by TMV.Bax, the Bax deletion mutants, and TMV.GFP on *N. benthamiana* at 4 dpi. The Bax deletion mutants used as inoculum are indicated by the deletion of the prefix TMV.Bax from the plasmid name. (*C*) Western blot analysis of protein accumulation in *N. benthamiana* leaves 2, 3, and 4 dpi with TMV.Bax, TMV expressing the Bax deletion mutants and mock inoculated plants. Blots were probed with antiserum raised against either Bax or the TMV CP.

reduced movement and/or replication of the viral vector and that the full-length Bax was a more efficient inducer of cell death than any of the Bax deletion mutants. The differential activity of Bax and the Bax deletion mutants in triggering cell death can be compared with interactions between TMV isolates and tobacco varieties carrying the resistance gene *N′*. TMV isolates that are strong elicitors of the *N′*-mediated HR induce a rapid host response, resulting in small lesions and localization of virus; weak elicitors are slower to induce a host response and give rise to larger spreading lesions and higher levels of virus accumulation (30).

The TM domain is necessary for the lethal effect of Bax in yeast, a property that has been correlated with the role of the TM domain in targeting Bax to mitochondria (20). To determine whether the TM domain of Bax was capable of targeting protein to plant mitochondria, a translational fusion between the GFP and the Bax TM domain (GFP-TM) was prepared and expressed from a potato virus X-based vector, PVX.GFP-TM. Expression of the GFP-TM fusion protein did not induce any obvious cytopathological effects in *N. benthamiana* (data not shown), consistent with the observation that expression of the TM alone was not cytotoxic (Fig. 1*B*). Analysis of tissue infected with PVX.GFP-TM under a confocal laser scanning microscope revealed a punctate distribution of GFP (Fig. 2*A*) that colocalized with the fluorescent signal produced when infected tissue was counterstained with either of the mitochondrial markers MitoTracker Red or hexyl rhodamine B (Fig. 2*B* and data not shown). Thus the presence of the Bax TM domain, as a carboxyl-terminal fusion to the GFP, was capable of targeting GFP to mitochondria. The requirement for the Bax TM domain in triggering cell death in plants, together with the fact that the TM domain alone is sufficient to direct a heterologous protein to mitochondria, raises the possibility that as reported for yeast (20), mitochondrial targeting of Bax is necessary for its prodeath activity in plants.

The similarity between the lesions induced in response to TMV.Bax infection and the *N* gene-mediated HR suggested that a host-defense response was activated in response to Bax. We therefore looked for accumulation of PR1, a protein that is specifically induced during the HR and in response to necrogenic pathogens (31), in tissues infected with TMV expressing Bax and the Bax deletion mutants. In *N. benthami-*

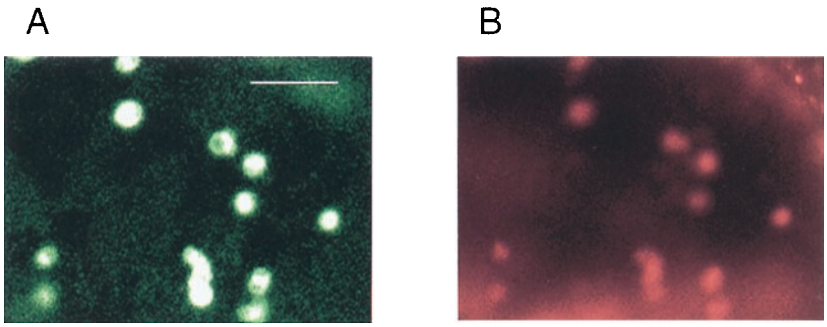

FIG. 2. Mitochondrial localization of the GFP-TM fusion protein. (*A* and *B*) Confocal laser scanning micrographs showing a highly magnified region of a single *N. benthamiana* leaf trichome cell infected with PVX.GFP-TM. The tissue was imaged at 488-nm excitation wavelength to detect GFP (*A*) and 568-nm excitation wavelength to detect the mitochondrial marker MitoTracker Red (*B*). (Bar = 5 μm.)

ana inoculated with either TMV.Bax or TMV.BaxΔBH2, PR1 protein was detected by 2 dpi, reaching maximum levels by 3 dpi in TMV.Bax-infected tissue and by 4 dpi in TMV.BaxΔBH2 infected tissue (Fig. 3*A*). Leaves infected with either TMV.BaxΔBH3 or TMV.BaxΔBH1 accumulated no PR1 protein by 3 dpi; however, by 4 dpi, PR1 was detected in TMV.BaxΔBH1-, but not TMV.BaxΔBH3-infected leaves (Fig. 3*A*). PR1 protein did not accumulate in samples from either TMV.BaxΔTM-infected plants or mock inoculated plants (Fig. 3*A*). Thus, the rapid induction of PR1 accumulation correlated with smaller lesions and reduced virus accumulation (Fig. 1*B* and *C*). The fact that TMV.BaxΔBH3 caused cell collapse and death by 4 dpi (Fig. 1*B*) without inducing PR1 accumulation suggested that cell death *per se* was not directly responsible for activating PR1 synthesis.

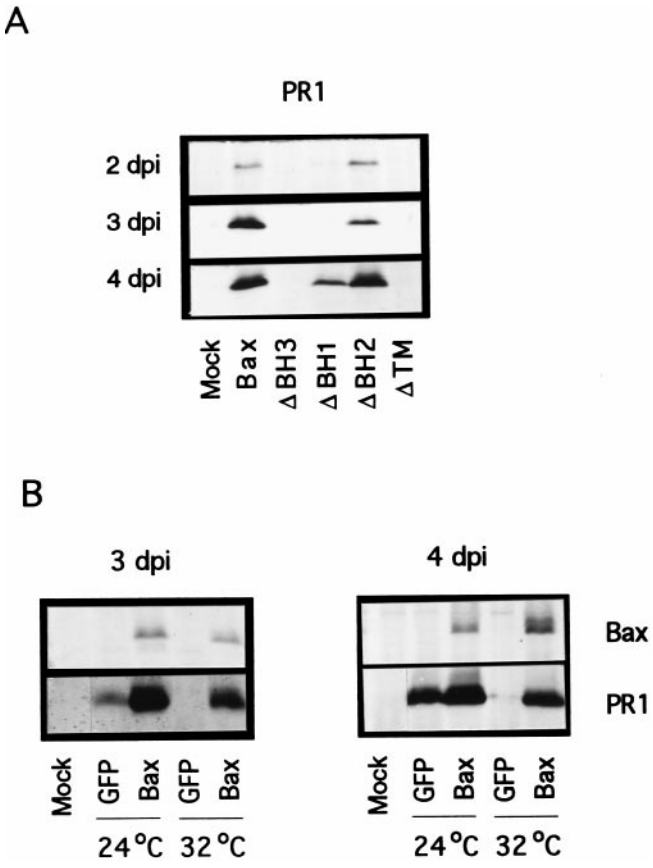

FIG. 3. Analysis of PR1 protein accumulation in virus-infected leaf tissue. (*A*) Accumulation of PR1 protein at 2, 3, and 4 dpi in total protein extracts from inoculated leaves of *N. benthamiana* plants infected with TMV expressing either Bax or the Bax deletion mutants. (*B*) Comparison of PR1 and Bax accumulation in *N. edwardsonii* in response to TMV.GFP and TMV.Bax at 3 and 4 dpi. Plants were incubated at either 24°C (permissive for the *N* gene-mediated HR) or 32°C (nonpermissive for the *N* gene-mediated HR), as indicated.

The analysis of lesion size and the kinetics of PR1 accumulation, induced by the Bax deletion mutants, showed that both BH3 and BH1 were important determinants of the plant response. The BH3 domain is necessary for Bax-induced cytotoxicity in yeast (20, 21), and studies using a yeast two-hybrid system have shown that the minimum requirements for Bax homodimerization are the BH3 domain from one molecule and a region covering both BH3 and BH1 from another (22). In plants, expression of BaxΔBH3 and BaxΔBH1 did induce cell death, but restriction of virus spread and accumulation of PR1 were considerably delayed, suggesting that homodimerization was important for rapid activation of the plant response.

To compare the accumulation of PR1 protein induced by TMV.Bax with its accumulation during the HR in *N* gene tobacco, *N. edwardsonii* plants were inoculated with either TMV.GFP or TMV.Bax. The *N* gene-mediated HR is temperature sensitive, with lesion development and virus resistance occurring only at temperatures below 30°C (11); however, TMV.Bax induced cell death in *N. edwardsonii* at both 24°C and 32°C (data not shown). Western blotting demonstrated accumulation of PR1 protein in TMV.GFP-inoculated plants maintained at 24°C for 3 d after infection, whereas plants kept at 32°C did not accumulate PR1 protein (Fig. 3*B*). Plants inoculated with TMV.Bax accumulated PR1 protein at both 24°C and 32°C, and this response was correlated with the accumulation of Bax protein (Fig. 3*B*). Thus the Bax-induced plant defense response is not temperature sensitive, and similar levels of PR1 protein accumulated in TMV.Bax infected tissue irrespective of whether plants were maintained at 24°C or 32°C (Fig. 3*B*).

OA, an inhibitor of type 1 and type 2A serine/threonine protein phosphatases (32), can block both *N* gene-mediated HR and developmental PCD in plants (33, 34). To determine whether the lethal effect of Bax in plants required phosphatase activity, we treated TMV.Bax-infected leaves with OA. The effect of OA treatment on the development of the TMV.GFP-induced HR in *N. edwardsonii* can be seen in the comparison of infected leaf tissues under white light and ultraviolet light (Fig. 4). Despite the presence of numerous infection sites, seen as green fluorescent spots under UV light, OA completely blocked the development of collapsed cell lesions 24 hr after plants were shifted from 32°C to 24°C (Fig. 4). Treatment of TMV.Bax-infected *N. benthamiana* leaves with OA also blocked the formation of Bax-induced lesions (Fig. 4). However, treatment with OA after the first visible signs of TMV. Bax-induced cell collapse did not prevent the continued development of lesions (data not shown). These results suggest that, like the TMV-mediated HR, the lethal effect of Bax in plants requires reversible-protein phosphorylation in a signal-

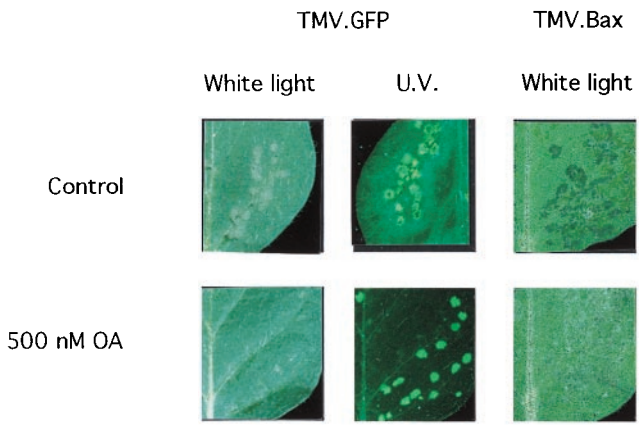

FIG. 4. Effect of OA treatment on the development of the HR and Bax-mediated cell death. *N. edwardsonii* leaves infected with TMV.GFP and *N. benthamiana* leaves infected with TMV.Bax are shown at 2 dpi. After inoculation, plants were maintained at 32°C for 24 hr, then leaves were incubated in either OA or a control solution for 24 hr at 24°C. TMV.GFP-infected leaves are shown under both white light and UV light; TMV.Bax-infected leaves are shown under white light only.

ing pathway that initiates the cell-death program; however, after entering the execution phase of the program, the process becomes irreversible.

From the data presented above, it is clear that, as in other systems, overexpression of Bax induces a lethal effect in plants. Structure-function analysis of Bax-mediated lethality in plants showed greater similarities to results obtained in yeast than to results from studies in mammalian systems (20). Most importantly, the TM domain is essential for the lethal phenotype of Bax in plants and yeast but is nonessential in mammalian cells. This difference is possibly because of the ability of BaxΔTM to associate with mammalian mitochondrial membranes via dimerization to endogenous Bcl-2 family proteins (20). In yeast and plants, endogenous partners for Bax heterodimerization are believed to be absent, thus preventing mitochondrial targeting of BaxΔTM. The observation that the death antagonist protein Bcl-x_L is unable to inhibit the *N* gene-mediated HR to TMV (35) also argues against a role for endogenous Bcl-2-related proteins in the plant HR.

Apparent parallels between the plant HR and animal PCD are intriguing (7, 8), but insufficient information is available on the biochemistry of plant cell execution to allow a direct comparison with animal models of PCD. One similarity between plant and animal cell-death processes is the apparent role of protein phosphatase(s), which is required for developmental and pathogen-triggered PCD in plants (33, 34) and is also implicated in animal PCD where protein phosphatase 2A activity is specifically up-regulated by a cell-death-related protease (36, 37). In plants, the requirement for protein phosphatase activity, both in the Bax-mediated response and the *N* gene HR, raises the possibility that expression of Bax triggered HR-associated events downstream of both the *N* gene-encoded receptor and the temperature-sensitive component(s) of the signal transduction pathway.

A key event in animal PCD is the release of cytochrome *c* from mitochondria into the cytosol, triggering caspase activation and initiating the final degradation phase of the cell-death program (16, 23). The requirement for the Bax TM domain in triggering cell death in plants and the ability of the GFP-TM fusion protein to target mitochondria suggest mitochondrial targeting is also necessary for the plant response. Interestingly, the mammalian protein Apaf1, which activates caspase-9 in the presence of cytosolic cytochrome *c*, contains an amino-terminal nucleotide-binding domain showing homology to the *N* gene product and related plant disease resistance genes (38, 39). Apaf1, when bound to dATP and cytochrome *c*, initiates autoprocessing of procaspase-9 to the active protease; however, activation of caspase-9 is negatively regulated by phosphorylation (40). In addition, both the *N* gene product and Apaf1 possess carboxyl-terminal domains implicated in protein–protein interactions (39). The recent discovery that a caspase-like proteolytic activity is induced in tobacco undergoing the *N* gene-mediated HR (41) also points to the possibility of similar mechanisms controlling the execution of the death program in both plants and animals.

Current evidence linking PCD processes in plants and animals is circumstantial, and not all of the classical hallmarks of programmed cell death in animal systems are observed in plants. For example, oligosomal laddering of nuclear DNA is a common, although not universal, phenotype in animal cells undergoing PCD (42), and oligosomal DNA laddering has also been described in plants undergoing the HR (5, 6). However, in TMV-infected tobacco undergoing the HR, low molecular weight DNA laddering is not observed (43), and no evidence of oligosomal DNA laddering was found in either TMV.Bax-infected leaves or protoplasts (C.L., unpublished data), indicating that not only do plant and animal PCD processes differ, but also not all plant HR responses are identical. However, despite the differences between PCD in plants and animals, the observation that Bax can trigger an active plant defense response indicates that some elements of the PCD effector machinery may be conserved between the plant and animal kingdoms.

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