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Transposon tagging of tobacco mosaic virus resistance gene *N*: Its possible role in the TMV–*N*-mediated signal transduction pathway

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ABSTRACT **Plants can recognize and resist invading pathogens by signaling the induction of rapid defense responses. Often these responses are mediated by single dominant resistance genes (*R* genes). The products of *R* genes have been postulated to recognize the pathogen and trigger rapid host defense responses. Here we describe isolation of the classical resistance gene *N* of tobacco that mediates resistance to the well-characterized pathogen tobacco mosaic virus (TMV). The *N* gene was isolated by transposon tagging using the maize Activator (*Ac*) transposon. We confirmed isolation of the *N* gene by complementation of the TMV-sensitive phenotype with a genomic DNA fragment. Sequence analysis of the *N* gene shows that it encodes a protein with an amino-terminal domain similar to that of the cytoplasmic domains of the *Drosophila* Toll protein and the interleukin 1 receptor in mammals, a putative nucleotide-binding site and 14 imperfect leucine-rich repeats. The presence of these functional domains in the predicted *N* gene product is consistent with the hypothesis that the *N* resistance gene functions in a signal transduction pathway. Similarities of N to Toll and the interleukin 1 receptor suggest a similar signaling mechanism leading to rapid gene induction and TMV resistance.**

The hypersensitive response (HR), characterized by rapid, localized cell death and tissue necrosis at the site of pathogen ingress, is an active, primary, defense response that occurs in a wide variety of plants. The HR is associated with the rapid induction of a number of biochemical, physiological, and molecular processes that prevent pathogen growth and spread. Some of the responses of cells undergoing HR include the oxidative burst leading to production of reactive oxygen intermediates (ROIs), alteration of membrane potentials, increases in lipoxygenase activity, production of antimicrobial compounds, such as phytoalexins, lignin deposition, and the expression of defense-related genes (1–3). The molecular and biochemical processes that trigger HR in plants are largely unknown. However, the induction of HR generally occurs following an interaction between the product of a resistance (*R*) gene and a corresponding pathogen avirulence (*Avr*) gene product (4). This "corresponding genes" hypothesis was first described by Flor (5) in studies of the interaction of flax with the rust fungus, *Melampsora lini*. Since then, this simple genetic relationship has been described for numerous plant–pathogen interactions. Plant *R* genes that mediate HR are often single, dominant loci whose products may function as specific receptors which interact, either directly or indirectly, with elicitors produced by *Avr* genes carried by pathogens (6). This receptor–elicitor interaction has been hypothesized to initiate signal-transduction pathways leading to expression of disease-resistance responses (4, 7).

Several avirulence genes from different pathogens corresponding to specific *R* genes have been cloned (8). In some cases, the role of the avirulence gene product as an elicitor has been established (9–12). However, the lack of knowledge about the nature of *R* genes or their protein products has limited the study of their role in pathogen recognition, signal transduction, and activation of HR. To date, two disease-resistance genes have been cloned: the *HM1* gene of maize and the *Pto* gene of tomato. The *HM1* gene, which confers resistance to the fungal pathogen *Cochliobolus carbonum* Nelson race 1, was cloned by transposon tagging (13). *HM1* encodes an NADPH- and NADH-dependent *Helminthosporium carbonum* (HC) toxin reductase responsible for detoxification of the cyclic tetrapeptide HC toxin produced by *C. carbonum* race 1 (14). The *Pto* gene, which confers resistance to *Pseudomonas syringae* pv. tomato carrying the *avrPto* gene, was isolated by positional cloning (15). The amino acid sequence of Pto does not suggest that it acts as a receptor, but it shows significant similarity to serine/threonine kinases. The Pto kinase may interact directly or indirectly with the *avrPto*-encoded elicitor molecule, then phosphorylate a subsequent modulator of the resistance responses, thereby participating in a signal-transduction cascade. Molecular characterization of cloned *R* genes and isolation of new *R* genes which confer resistance to viral, fungal, or bacterial pathogens is necessary to understand their role in mediating resistance.

Genetic approaches to obtain loss-of-function mutants with altered resistance responses offer powerful means to isolate *R* genes and gain insight into their functions. The interaction between the resistance gene *N* of tobacco and tobacco mosaic virus (TMV) has long served as a classical model for the study of plant-resistance responses to pathogens and provides an excellent system for this approach. The dominant *N* gene was introgressed into tobacco cultivars by crossing the TMV-resistant (TMV^R) species *Nicotiana glutinosa* to TMV-sensitive (TMV^S) *Nicotiana tabacum*, followed by repeated backcrosses to a recurrent TMV^S tobacco parent (16). Plants bearing the

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Abbreviations: TMV, tobacco mosaic virus; HR, hypersensitive response; IL-1R, interleukin 1 receptor; NBS, nucleotide-binding site; LRR, leucine-rich repeat; RFLP, restriction fragment length polymorphism; ROI, reactive oxygen intermediate; N^{tr}, truncated form of N protein.

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N gene (*NN* tobacco) mount an HR to all strains of TMV except the ToMV-ob strain, thereby preventing the systemic spread of the virus beyond the initial infection site. In contrast, TMV^S tobacco cultivars (*nn* tobacco) allow TMV to spread systemically and develop mosaic symptoms characterized by intermittent areas of dark green tissue mixed with light green leaf tissue.

TMV, the type member of the tobamovirus group, is mechanically transmitted and one of the most extensively characterized pathogens of plants. The genome of TMV consists of a single positive-sense RNA of 6395 nucleotides and encodes at least four proteins (17). Two proteins, 126 kDa and 183 kDa, are synthesized from genomic RNA and are involved in viral replication. The 30-kDa, cell-to-cell movement protein and 17.5-kDa viral coat protein are translated from subgenomic RNAs. The precise viral component that elicits *N*-mediated HR has not been strictly defined. However, one study suggests that the 126-kDa replicase protein is required for HR induction in *NN* tobacco (18).

The precise events required for the TMV–N-induced HR and the subsequent defense pathway have not been established. It has been hypothesized that *N* gene product may function as a receptor capable of recognizing a component of TMV, thereby initiating subsequent defense responses. To begin elucidation of the molecular and biochemical basis of *N*-mediated TMV resistance, we have isolated the *N* gene from tobacco by transposon tagging using the maize transposon *Ac*. A positive mutant-selection scheme was developed to isolate loss-of-function mutants unable to respond to TMV with HR (TMV^S mutants). One TMV^S mutant carried an unstable mutation that correlated with the presence of an *Ac* transposon. Genomic sequences flanking the *Ac* transposon were used as probes to isolate genomic clones containing *N* sequences. A genomic DNA fragment containing the *N* gene conferred TMV resistance to TMV^S tobacco. The amino acid sequence derived from both genomic and cDNA clones suggests that the *N* gene encodes a protein with an amino-terminal domain similar to the cytoplasmic domains of the *Drosophila* Toll protein (19) and the mammalian interleukin 1 receptor (IL-1R) (20). It also contains a nucleotide-binding site (NBS) and a leucine-rich repeat (LRR) region. The presence of these three domains suggests that the N protein represents a class of disease-resistance proteins active in signal-transduction pathways. The potential function of N in the TMV-dependent HR will be discussed with respect to features central to Toll and IL-1R signaling pathways.

RESULTS AND DISCUSSION

Isolation of an Unstable TMV-Susceptible Mutant. We utilized the Activator (*Ac*) transposon of maize (21) for insertional mutagenesis and isolation of the *N* gene. In tobacco, critical functional aspects of *Ac* transposition in maize are retained (22). These include conservation of *Ac* structural integrity following transposition, generation of an 8-bp duplication at the site of insertion, generation of footprints upon excision, and continued transposition over several generations (23). The *Ac* transposon was introduced into the TMV^R tobacco cultivar Samsun NN, which is homozygous for the *N* gene.

The isolation of *Ac*-induced mutations of *N* required generation and screening of large populations of F_1 seedlings heterozygous for *N* (*Nn*) to identify potential loss-of-function mutations. These mutants would be identified as plants unable to mount a TMV-dependent HR. Therefore, we developed and utilized a positive mutant-selection scheme that exploited the reversible temperature sensitivity of the *N*-mediated HR to TMV. At temperatures below 28°C, plants carrying the *N* gene can mount an HR in response to TMV infection. However, it has been shown that some component of the *N*-mediated HR is temperature sensitive, and it is reversible. Thus, at temperatures above 28°C, plants carrying the *N* gene do not generate HR in response to TMV infection but allow TMV to spread systemically. When these systemically infected plants are shifted to a permissive temperature (21°C), they develop lethal systemic HR. However, when plants infected with TMV are maintained at elevated temperatures (above 28°C) and then returned to a permissive temperature (21°C), they will develop lethal systemic necrosis (systemic HR). Those plants bearing an inactive, mutant allele of *N* were anticipated to survive the temperature shift.

We have developed a positive mutant selection scheme to isolate TMV^S mutants (Fig. 1). Eight-week-old *nn* and *Nn* seedlings were subjected to one of the following treatments: control inoculation with buffer (50 mM sodium phosphate, pH 7.0) and Celite (Fisher Scientific) abrasive (Fig. 1, panel 1), inoculation with Celite and TMV (U1 strain) at 21°C (Fig. 1, panel 2), and inoculation with Celite and TMV at 30°C

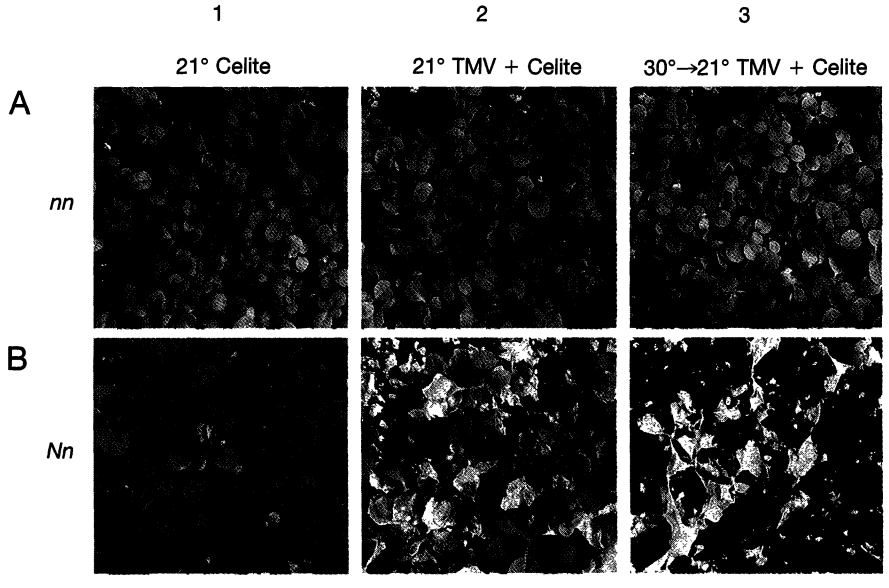

FIG. 1. A positive-selection scheme for TMV^S mutants. Eight-week-old *nn* (*A*) or *Nn* (*B*) seedlings were inoculated with buffer and Celite abrasive at 21°C (panel 1), TMV and Celite suspension at 21°C (panel 2), or TMV and Celite suspension at 30°C, followed by a temperature shift to 21°C 3 days postinoculation (panel 3). Seedlings were scored for TMV-induced HR on the fifth day postinoculation.

followed by a temperature shift to 21°C 3 days postinoculation (Fig. 1, panel 3). Seedlings were scored for survival 5 days postinoculation. The *nn* plants (Fig. 1*A*) were unaffected by the three treatments. The *Nn* seedlings were unaffected by the control treatment (Fig. 1*B*, panel 1). The *Nn* seedlings did develop localized HR in response to TMV infection at 21°C (Fig. 1*B*, panel 2). However, when the *Nn* seedlings were first inoculated at 30°C then transferred to 21°C, nearly all of them succumbed to lethal systemic HR (Fig. 1*B*, panel 3). The few plants that survived this treatment were termed TMV^S mutants.

We used this positive-selection scheme to isolate TMV^S mutants potentially bearing *Ac*-induced mutations of *N*. *Nn* seedlings (64,000) from crosses between three *NN*::*Ac* parents and SR1 nn and a control population of 29,000 *Nn* seedlings from one cross between Samsun NN and SR1 nn were produced. Eight-week-old *Nn* seedlings were inoculated with TMV at 30°C and maintained at that temperature for 3 days to allow TMV to establish a systemic infection. At 3 days postinoculation, the seedlings were transferred to 21°C to induce *N*-dependent, lethal systemic HR. The surviving seedlings were scored 2 days after the temperature shift, when nearly all other seedlings had succumbed to the temperature-induced, lethal systemic HR. Three rounds of TMV inoculation and temperature shifting were applied to seedlings to minimize the isolation of escapers.

We isolated a total of 47 TMV^S mutants from *Nn* populations; 11 mutants were isolated from the F_1 population derived from the control cross between Samsun NN and SR1 nn, while 36 mutants were isolated from three F_1 populations derived from crosses between *NN*::*Ac* parents and SR1 nn. The frequency of loss of resistance to TMV among Samsun NN × SR1 nn and *NN*::*Ac* × SR1 nn progeny was similar and was 3.8×10^{-4} and 5.6×10^{-4}, respectively. The ability to obtain TMV^S mutants at similar frequencies from *Nn* populations with or without *Ac* indicated that the *N* gene has a high spontaneous mutation rate. Fifteen homozygous mutant lines containing *Ac* were used for further molecular and genetic analyses.

Mutant lines homozygous for their loss-of-function mutations were selfed, and the progeny were examined for stability of the TMV^S mutation. We determined that one mutant line (D11-1) had an unstable TMV^S mutation. Out of 145 selfed progeny examined, 20 were TMV^R, 68 were TMV^S and 57 displayed sectors of TMV^R tissue in a TMV^S background ($TMV^{R/S}$). The selfed progeny of the other 14 lines displayed only the TMV^S phenotype, indicating that they possessed nonreverting mutations of *N*. Mutations caused by *Ac* transposons can revert and lead to expression of an unstable phenotype. Therefore, we postulated that the D11-1 mutant line bore an *Ac* insertion in the *N* gene.

Insertional Mutagenesis of *N* by *Ac10*. If *Ac* had inactivated the *N* gene, the $TMV^{R/S}$ phenotype (sectored phenotype) and the *Ac* transposon causing the mutation were expected to cosegregate with an *N*-linked restriction fragment length polymorphism (RFLP) marker. We have isolated such a marker, Nt-1, that identifies an *N*-linked RFLP, Nt-1G. The Nt-1G RFLP is linked to *N* by less than 0.25 cM. To assess the linkage of the $TMV^{R/S}$ phenotype with the Nt-1G RFLP, a population of 80 progeny plants from the cross of C2-2, the TMV^S mutant (N*n) progenitor of the D11-1 mutant line, with SR1 nn were inoculated with TMV and scored for their phenotypes. Twenty-six of these progeny plants displayed the $TMV^{R/S}$ phenotype and 54 were TMV^S. Southern blot hybridization analysis with the Nt-1 marker showed that 39 of 80 progeny plants were heterozygous for the Nt-1G RFLP, as expected. The 26 $TMV^{R/S}$ plants possessed the Nt-1G RFLP, demonstrating that the unstable mutation was linked to *N*. Furthermore, two *Ac*-hybridizing bands (10.2 and 8.0 kb) termed *Ac10* and *Ac8*, respectively, were identified only in plants that bore the Nt-1G RFLP. These results indicated that *Ac10* and *Ac8* were candidates for the insertional inactivation of *N*.

To determine which of these *Ac* transposons was inserted at the *N* locus, we analyzed progeny of a germinal revertant crossed with SR1 nn. Ninety-five progeny plants from this cross (termed the E501 population) were inoculated with TMV and scored for their phenotypes. The DNA from 54 TMV^R plants were subjected to Southern blot hybridization analysis with the 5′ *Ac* probe. *Ac10* was not present in any of the 54 plants, whereas *Ac8* was observed in 52 plants. The absence of *Ac10* and the presence of *Ac8* in the E501 population established *Ac10* as the transposon tagging the unstable mutation of *N*.

Genomic DNA flanking the *Ac10* insertion site was isolated and used to confirm that genomic DNA fragments were disrupted by the *Ac10* insertion in TMV^S. All 54 TMV^R E501 plants contained the wild-type genomic fragment, indicating that genomic sequences were restored to wild type in the revertant parent. Interestingly, a number of E501 plants were TMV^S and possessed the wild-type genomic fragment, indicating that excision of *Ac10* does not always lead to phenotypic reversion to TMV resistance. These plants were thought to have arisen due to imprecise excision of *Ac10*. *Ac* creates a direct 8-bp target-site duplication upon insertion and upon excision often leaves behind portions of the duplication, known as "footprints." These footprints could result in insertions, deletions, or substitutions that could render a gene product nonfunctional if located in an open reading frame (22, 24). Sequence analysis of genomic DNA flanking *Ac10* showed that it was flanked by a direct 8-bp duplication (Fig. 2).

Sequence analysis of cDNA clones showed that *Ac10* was inserted in a large open reading frame. Therefore, imprecise excision could result in the production of a nonfunctional polypeptide. Sequence analysis of seven independent TMV^S plants lacking *Ac10* showed that each contained frame-shift mutations that would result in truncation of the predicted polypeptides either four or nine amino acids from the *Ac10* excision site (Fig. 2). Sequence analysis of the excision sites of seven independent germinal revertants showed no changes in

			Phenotype
Wild type			
	-AT TTG CCG-		TMV^R
***Ac10* insertion**			
	-AT TTG CCG//*Ac10*//AT TTG CCG-		TMV^S
***Ac10* excision**			
***N** footprints*			
F501-48	-AT TTG CCC TTT GCC GTC	-9 aa- ◊	TMV^S
F501-64	-AT TTG CCT GCC GTC	-9 aa- ◊	TMV^S
E501-2	-AT TTG CTT TGC CGT	-4 aa- ◊	TMV^S
E501-3	-AT TTG CCA TTT TGC CGT	-4 aa- ◊	TMV^S
E501-9	-AT TTG CCC CGT	-4 aa- ◊	TMV^S
E501-16	-AT TTG CCC TTT GCC GTC	-9 aa- ◊	TMV^S
E501-28	-AT TTG CCC TTT GCC GTC	-9 aa- ◊	TMV^S
***N* revertants**			
D112-15	-CAT TTG CCG TCT-		TMV^R
F501-34	-CAT TTG CCG TCT-		TMV^R
F501-45	-CAT TTG CCG TCT-		TMV^R
F501-65	-CAT TTG CCG TCT-		TMV^R
F501-66	-CAT TTG CCG TCT-		TMV^R
F501-67	-CAT TTG CCG TCT-		TMV^R
F501-68	-CAT TTG CCG TCT-		TMV^R
F501-69	-CAT TTG CCG TCT-		TMV^R

FIG. 2. Sequence analysis of the target site of *Ac10* insertion and excision sites. Upon insertion of *Ac10*, 8 bp of the wild-type *N* sequence (ATTTGCCG) were duplicated. Triplets of bases indicate codons in the cDNA sequence. Sequences remaining in sensitive plants following excision of *Ac10* are underlined. Asterisk indicates the occurrence of premature stop codon. E501 are offspring of the germinal revertant, D112-15, crossed with SR1 tobacco, and F501s are selfed offspring of a TMV^S mutant. The phenotype of the plants bearing *Ac10* insertions or excisions is indicated.

sequence compared with wild type (Fig. 2). These data provided further evidence that *Ac10* was inserted in the *N* gene.

Resistance to TMV Is Conferred by a Genomic DNA Fragment. To confirm the isolation of the *N* gene and to determine if this single gene was sufficient to confer resistance to TMV in tobacco, we transformed the full-length *N* gene into the TMV^S cultivar SR1 nn. The full-length gene was isolated from an *N. glutinosa* λ DNA library. A DNA fragment was determined to contain the full-length *N* gene by comparative restriction enzyme mapping, Southern blot hybridization, and sequence analysis of genomic and cDNA clones. This fragment was subcloned into the transferred DNA (T-DNA) vector pOCA28 to generate the pTG38 construct, which was transformed into SR1 nn tobacco by *Agrobacterium*-mediated transformation. T_0 plants were regenerated and inoculated with TMV (U1 strain) 1 week after transfer to soil. Eleven of twelve pTG38 transformants responded to TMV with HR. The development of HR lesions was indistinguishable from those formed by Samsun NN control plants that were regenerated simultaneously. This result confirmed that the *N* gene was isolated and was sufficient to confer resistance to TMV in tobacco.

Structure of the *N* Gene. The sequence analysis of cDNA and genomic DNA clones indicates that the *N* gene may encode full-length (N) and truncated (N^{tr}) forms of proteins (Fig. 3). The *N* gene contains five exons that are spliced together to form a single open reading frame of 3432 nt with the potential to encode a protein of 1144 amino acids and a deduced molecular mass of 131.4 kDa (N; Fig. 3). The N^{tr} form results from alternative splicing of a 70-bp exon to form a 1956-nt open reading frame, which encodes a truncated polypeptide of 652 amino acids and a deduced molecular mass of 75.3 kDa (N^{tr}; Fig. 3). N^{tr} is identical to the amino terminus of N except for the 36 additional amino acids at the carboxyl terminus. Sequence analysis of N^{tr} does not suggest a possible function for these extra 36 amino acids. The functional significance of N^{tr} in *N*-mediated resistance is not known; however, on the basis of knowledge of the roles of truncated forms of other proteins, we hypothesize that N^{tr} may act as a dominant negative regulator of the function of the full-length N protein. Alternatively, N^{tr} may be required for signal transduction or in an interaction with the TMV elicitor (ligand). In many cell-surface receptors (cytokine receptors and growth-factor receptors), truncated and soluble forms of receptors have been reported (25, 26). These naturally occurring truncated forms of receptors compete with full-length forms for ligand binding or in receptor–receptor interaction (27, 28). In mouse pro-B cells, the truncated form of erythropoietin receptor (EPOR-T) acts as a dominant negative regulator of cell growth and prevention of apoptosis (26, 29). In contrast, the truncated form of the Toll receptor enhances the full-length Toll-receptor function (30). It will be interesting to determine the temporal and spatial regulation of N and N^{tr}, and their functional role if any in *N*-mediated signal-transduction pathway.

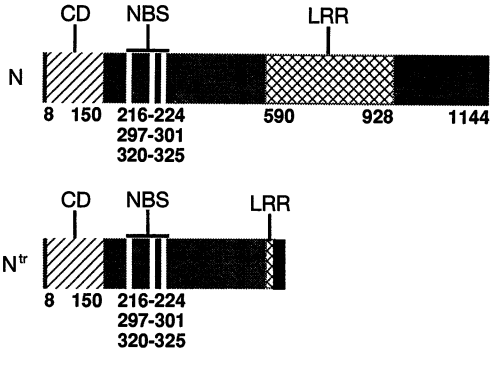

FIG. 3. Schematic diagram of the N and N^{tr} proteins. An analysis of N protein amino acid sequence identified three domains of possible functional significance. These domains are indicated and shown to scale within the full-length N protein. CD, putative cytoplasmic domain of N with sequence similarity to the cytoplasmic domains of Toll, IL-1R, and MyD88; NBS, putative nucleotide-binding site comprising three motifs from amino acid 216 to 325; LRR, leucine-rich repeat region consisting of 14 imperfect tandem leucine-rich repeats. Alternative splicing yields a truncated protein, N^{tr}. N^{tr} is identical to the amino terminus of N except for the carboxyl-terminal 36 amino acids, indicated by the black box next to the LRR on the right.

The predicted N protein does not contain a signal sequence or a potential transmembrane hydrophobic region, suggesting that it is a cytoplasmic protein. There are eight putative N-linked glycosylation sites (N-X-S/T) in N protein. Comparison of the amino acid sequence of the N protein with the GenBank data base (release 82.0) by using the BLAST program (31) revealed three domains that may be of functional significance. In the amino-terminal region, the N protein contains a nucleotide-binding site (NBS). Usually, three separate polypeptide motifs are required to form an NBS: P-loop (kinase 1), kinase 2 and kinase 3a (32). The first motif of the NBS, the P-loop, functions in binding the phosphates of ATP/GTP and is found in N between amino acids 216 and 224 (GMGGVGKTT, which matches the consensus sequence A/GXXXXGKS/T). A second motif, kinase 2, is defined by four consecutive hydrophobic amino acids followed by an invariant aspartate (D) and is found between amino acids 297 and 301 (LIVLD). This aspartate residue catalyzes the reversible transfer of divalent cations (Mg^{2+} or Ca^{2+}) in phosphate transfer reactions. The third motif, kinase 3a, found between amino acids 320 and 325 (FGNGSR) is involved in purine or ribose binding and commonly contains a tyrosine (Y) or arginine (R). The NBS is found in many families of proteins, such as adenylyl kinases, the ras family of proteins, ATPases, and elongation factors (33). The GTP-bound state of $p21^{ras}$ is necessary for signaling effector proteins involved in cell growth and differentiation (34). The presence of an NBS close to the amino terminus of the predicted N protein similar to that observed in ras and adenylate kinase suggests that the binding of ATP or GTP is required for N protein function.

The amino acid sequence of the N protein from 590 to 928 contains 14 imperfect LRRs. Each repeat is composed of approximately 26 amino acids with a leucine-rich motif, PXX-*a*XXLXXLXXLXLXXXXXLXXL (where *a* is an aliphatic amino acid and X is any amino acid). LRRs have been identified in a variety of proteins from a wide range of species, including yeast adenylyl cyclase (35), *Drosophila* Toll (19), human platelet membrane glycoprotein Ibα chain (36), *Drosophila* Chaoptin (37), and *Arabidopsis* receptor-like transmembrane kinases (38–40). Several possible functions have been suggested for LRRs, which may be extracellular or cytoplasmic. They could play a role in protein–protein interactions (36, 41, 42) or cell adhesion, or mediate interactions between the protein and cellular membranes (35, 43).

The amino-terminal amino acids of N (from 8 to 150) are similar to the cytoplasmic domains of the *Drosophila* Toll protein (55% similar; ref. 19), the human IL-1R (49% similar; ref. 20), and an IL-6-induced, primary-response protein in mouse myeloid cells, MyD88 (44% similar; ref. 44).

Proposed Role for N in Signal Transduction. Increasingly, evidence suggests that plant cellular-defense responses may be analogous to the "natural" or innate immunity of vertebrates and insects (Fig. 4). In vertebrates and insects, innate immunity is characterized by rapid induction of gene expression following microbial infection. A characteristic feature of disease resistance in plants is the rapid induction of HR. In mammalian and *Drosophila* innate immune responses, perception of signals produced by invading bacterial pathogens results in translocation of Rel-related transcription factors from the cytoplasm to the nucleus. These transcription factors, NF-κB in mammals (45) and Dif in *Drosophila* (46), induce defense genes upon binding to κB-like motifs in the promoters of these

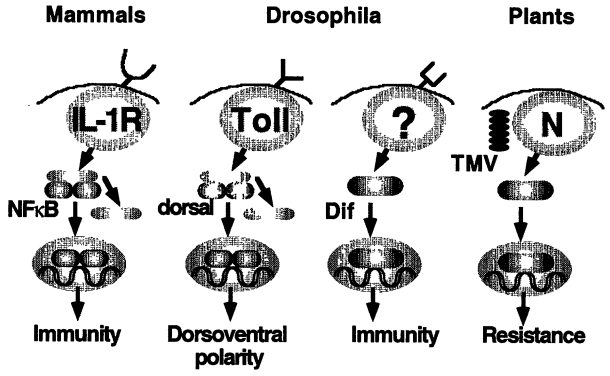

FIG. 4. Comparison of immune and developmental pathways of different organisms adopted from Hultmark (52).

genes (47, 48). In the mammalian immune system, binding of IL-1 to the IL-1R induces translocation of NF-κB to the nucleus, leading to gene expression and induction of acute-phase responses. The receptor that activates Dif in *Drosophila* upon microbial infection has not been identified. The cytoplasmic domain of the IL-1R, which is involved in transducing the signal required for translocation of NF-κB, has sequence and functional similarity with the cytoplasmic domain of another *Drosophila* protein, Toll (19). In *Drosophila* development, the perception of an unknown extracellular signal by Toll results in translocation of Dorsal, a homologue of NF-κB. Translocation of Dorsal to the nucleus induces the expression of zygotic genes involved in dorsoventral polarity (49).

Mutations in conserved amino acids within the cytoplasmic domains of Toll and the IL-1R indicate that this domain is required for nuclear factors NF-κB and Dorsal signal transductions (30, 50, 51). The presence of a similar domain in the amino terminus of the N protein suggests that it may trigger an intracellular signal-transduction cascade related to the Toll and IL-1R pathways (Figs. 4 and 5). Analogous to the Toll and IL-1R systems, the N protein may function as a receptor that interacts directly with the TMV gene product which elicits the *N*-mediated HR. The introduction of the *N* gene alone was sufficient to confer the TMV^R phenotype to SR1 nn, which suggests that the *N* gene product may be the determinant for TMV perception and induction of HR.

The elicitor of the *N*-mediated HR has not been strictly defined. However, the 126-kDa TMV replicase gene product may be involved (18). In *N*-mediated HR, TMV replication and gene expression are required because neither the virion nor

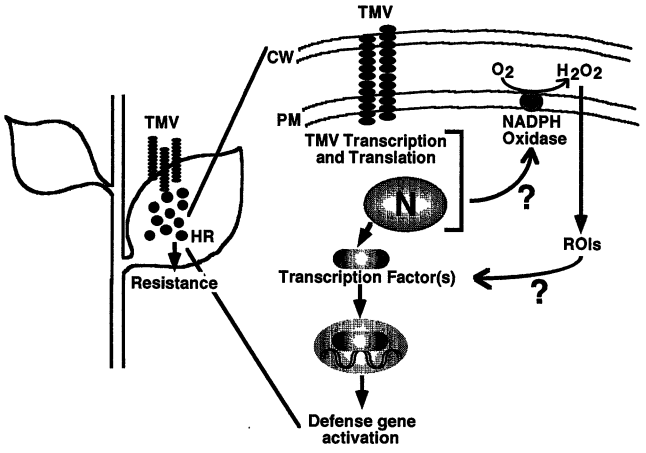

FIG. 5. Model showing possible role of the *N* gene product in signal perception and transduction upon TMV infection leading to HR and defense-gene activation. CW, cell wall; PM, plasma membrane; ROIs, reactive oxygen intermediates.

coat protein alone can induce HR. TMV particles gain access to the cytoplasm of plant cells by mechanical damage. In the cytoplasm, TMV particles are uncoated by cytoplasmic ribosomes, and the genome is translated (53). The first protein produced is the replicase, which in turn leads to multiplication of the viral genome within the cytoplasm. Since the life cycle of TMV is carried out in the cytoplasm of plant cells, it would seem appropriate that *N* would encode a cytoplasmic receptor which could interact with the intracellular TMV-encoded elicitor. Based on the similarity of N with the cytoplasmic domains of Toll and IL-1R, it is tempting to speculate that N might activate a Rel-related transcription factor that induces the expression of genes responsible for the *N*-mediated HR.

The events immediately following TMV infection and preceding HR are not well defined. However, it has been widely observed that HR is preceded by a rapid outburst of ROIs (O_2, H_2O_2, and OH^-). A plasma membrane multisubunit NADPH oxidase complex, similar to mammalian phagocytes, might be involved in the release of ROIs in plants (3). In *NN* tobacco, the outburst of ROIs has been reported to occur as early as 10 min following TMV infection and was found to be NADPH- and Ca^{2+}-dependent (54). ROIs in prokaryotes and eukaryotes have been implicated in the induction of gene expression mediated by redox-regulated transcription factors (55). In the mammalian innate immune response, ROIs have been shown to induce acute-phase response genes by activating the transcription factors, NF-κB (56) and AP-1 (57, 58). The *N*-mediated, rapid induction of intracellular ROIs indicates that a redox-regulated transcription factor might be involved in the induction of defense responses to TMV.

In summary, we have cloned the plant disease-resistance gene *N* that confers resistance to the viral pathogen TMV. The amino acid sequence of the encoded N protein contains domains which suggest a role for N in signal transduction leading to HR. The identification of these sequences within N will facilitate systematic dissection of the biochemical and molecular basis of the disease-resistance response to TMV in tobacco.

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