

# cDNA cloning and characterization of tobacco ABC transporter: *NtPDR1* is a novel elicitor-responsive gene<sup>1</sup>

Michiko Sasabe, Kazuhiro Toyoda, Tomonori Shiraishi, Yoshishige Inagaki, Yuki Ichinose\*

Laboratory of Plant Pathology and Genetic Engineering, Faculty of Agriculture, Okayama University, Tsushima-naka 1-1-1, Okayama 700-8530, Japan

Received 3 January 2002; revised 25 March 2002; accepted 28 March 2002

First published online 16 April 2002

Edited by Marc Van Montagu

**Abstract** We isolated an INF1 elicitin-inducible cDNA encoding a pleiotropic drug resistance (PDR)-type ATP-binding cassette (ABC) transporter homolog (*NtPDR1*) in suspension-cultured tobacco Bright Yellow-2 (BY-2) cells by application of differential display PCR. The *NtPDR1* (*Nicotiana tabacum* PDR protein 1) gene also encodes a 162 kDa protein that includes two putative hydrophilic domains containing the ABC signature motif and two putative hydrophobic domains. Expression of the *NtPDR1* gene was rapidly and strongly activated by treatment of BY-2 cells with INF1 elicitor. Further, treatment of BY-2 cells with flagellin, a bacterial proteinaceous hypersensitive reaction elicitor, or yeast extract, a general elicitor, also induced *NtPDR1* gene expression. These results indicate that *NtPDR1* may be involved in the general defense response in tobacco. This is the first report that microbial elicitors induce the expression of a plant ABC transporter gene. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

**Key words:** ATP-binding cassette transporter; Tobacco Bright Yellow-2; Elicitor; Defense response

## 1. Introduction

Plants have highly sensitive systems to protect themselves from different microbial pathogens. In past years, there have been various investigations of defense systems such as the hypersensitive reaction (HR), including cell death, oxidative burst and defense gene expressions, or systemic acquired resistance. The complicated networks for the induction of defense responses in plants include the recognition, amplification and transduction of signals induced by pathogen attack. After

recognition of the signal molecules derived from infection with pathogens by a putative receptor molecule, second messengers transduce the information to the nucleus, activating a number of defense-related genes. Many attempts in several model systems have demonstrated that de novo protein synthesis and gene expression are required for induction of effective defense responses and acquisition of resistance [1]. Thus, a variety of genes are found to be induced specifically during defense responses. These genes potentially encode transcriptional regulators, enzymes related to secondary metabolic pathways leading to synthesis of phytoalexins and pathogenesis-related (PR) proteins [2]. However, many genes related to the defense responses are still uncharacterized [3–5].

The ATP-binding cassette (ABC) transporter superfamily comprises ATP-driven efflux pumps in the membrane that export a wide spectrum of cytotoxic compounds [6,7]. This superfamily is very large and exists in diverse species, including bacteria, yeasts, mammals and plants. In mammals, many ABC transporter proteins have already been identified and are considered to be of clinical importance. Over 20 plant ABC transporter genes have been isolated so far, and many other ABC transporter gene homologs of *Arabidopsis* are registered in the genome databases and classified by their sequence homology [8]. One group of them was localized on the vacuolar membrane and its reported function is to transport glutathione *S*-conjugates (GS conjugates) and/or chlorophyll catabolites [9–12]. However, the role of most plant ABC transporters has not been clarified. Recently, it has also been reported that some ABC transporters of fungal pathogens are important in pathogenesis in order to protect the fungus itself against plant defense mechanisms [13,14]. On the other hand, in *Nicotiana plumbaginifolia*, one of the ABC transporters, NpABC1, is suggested to be involved in the secretion of a secondary antifungal terpenoid such as sclareolide, a close analog of sclareole, that plays a role in plant defense [15]. Nevertheless, the involvement of the plant ABC transporter in the interaction with pathogens is not known at all.

INF1 elicitor, an elicitor of the late-blight pathogen *Phytophthora infestans*, induces typical HR on tobacco [16]. Our previous report suggested that a set of genes are involved in the INF1 elicitor-induced defense responses in Bright Yellow-2 (BY-2) cells [17]. To further understand the molecular mechanisms of plant disease resistance, we performed differential display in a model system with INF1 elicitor and tobacco BY-2 cells, and cloned an ABC transporter homolog as one of the INF1 elicitor-induced genes. Here we report that the ABC transporter gene or the gene product might be involved in the general defense response in plants.

\*Corresponding author. Fax: (81)-86-251 8308.

E-mail address: yuki@cc.okayama-u.ac.jp (Y. Ichinose).

<sup>1</sup> The nucleotide sequence reported in this paper has been submitted to the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession number AB075550.

**Abbreviations:** ABA, abscisic acid; ABC, ATP-binding cassette; HR, hypersensitive reaction; LB, Luria–Bertani; MDR, multidrug resistance; MeJA, methyl jasmonic acid; MRP, MDR-associated protein; NBF, nucleotide-binding fold; *NtPDR1*, *Nicotiana tabacum* PDR protein 1; PAL, phenylalanine ammonia-lyase; PDR, pleiotropic drug resistance; PR, pathogenesis-related; RACE, rapid amplification of the cDNA end; SA, salicylic acid; TMD, transmembrane domain

## 2. Materials and methods

### 2.1. Plant cells

Suspension-cultured cells of tobacco BY-2 in MS medium supplemented with sucrose (30 mg/ml), thiamine (1 µg/ml), myo-inositol (100 µg/ml),  $\text{KH}_2\text{PO}_4$  (200 µg/ml) and 2,4-dichlorophenoxyacetic acid (0.2 µg/ml) were freshly reinoculated every week and agitated at 130 rpm at 27°C [18]. The treatment with elicitor (10 µg/ml as a bovine serum albumin (BSA) equivalent), flagellin (10 µg/ml as a BSA equivalent), yeast extract (5 mg/ml) or various chemicals was performed 3 days after reinoculation, and the cells were collected at intervals by centrifugation.

### 2.2. Preparation of elicitor

INF1 elicitor (*inf1* gene product of *P. infestans*) was prepared as a recombinant protein using the method previously described [17].

Preparation of flagellin<sup>Pst</sup>, flagellin protein of *Pseudomonas syringae* pv. *tomato*, followed the method of Taguchi et al. [19]. Briefly, bacteria were incubated for 24 h at 27°C in Luria–Bertani (LB) medium containing 10 mM  $\text{MgCl}_2$  and then for 24 h at 23°C in minimal medium (50 mM potassium phosphate, 7.6 mM  $(\text{NH}_4)_2\text{SO}_4$ , 1.7 mM  $\text{MgCl}_2$  and 1.7 mM NaCl, pH 5.7) supplemented with 10 mM each of mannitol and fructose. The cultured medium was centrifuged at 7000×g for 10 min. After separation of flagella from bacteria by vortexing in phosphate buffer (50 mM sodium phosphate, pH 7.0) and centrifugation at 10000×g for 30 min, the supernatant was further centrifuged at 100000×g for 30 min. The resultant pellet of crude flagella was suspended in 0.1 M glycine–HCl buffer (pH 2.0) for dissociation. After recentrifugation at 100000×g for 30 min, the supernatant was collected as the flagellin<sup>Pst</sup> preparation, and used after the pH was adjusted to 7.0 with NaOH.

Yeast extract (Difco, USA) was dissolved in water and filtered through a 0.45 µm pore filter disk (Kurabo, Japan), and this was used as the general elicitor. The final concentration of yeast extract was 5 mg/ml; yeast extract at this concentration did not induce cell death after treatment of BY-2 cells for 24 h.

### 2.3. Differential display PCR

Differential display was carried out according to the method of Yoshida et al. [20]. mRNA (0.5 µg) purified by PolyAtract<sup>®</sup> mRNA Isolation System IV (Promega, USA) was used as the template to generate cDNA in 25 µl of reaction mixture containing 10 mM dNTP, 160 µM random primer, 100 U RNase inhibitor and 20 U RAV-2 reverse transcriptase (Takara, Japan). Reactions were diluted (1:3), and 1 µl was used as a template for differential display PCR. DNA was amplified in 50 µl of the PCR reaction mixture, containing cDNA, 10 mM dNTP, 50 µM arbitrary 12-mer primer (DNA Oligomer (12) set; Wako Pure Chemical Industries, Japan) and 5 U AmpliTaq<sup>®</sup> DNA polymerase (Roche, USA) on a Takara PCR Thermal Cycler TP240 (Takara) under the following conditions: one cycle of 5 min at 94°C, 40 cycles of 30 s at 94°C, 1 min at 40°C, 2 min at 72°C. Amplified DNA was separated on 1.5% agarose gel and stained with ethidium bromide. The nucleotide sequence of the G27 primer, one of arbitrary primers, was 5'-GGCGTGGAAGGA-3'.

### 2.4. Isolation of *Nicotiana tabacum* pleiotropic drug resistance protein 1 (*NtPDR1*) cDNA and determination of nucleotide sequence

To determine the *NtPDR1* cDNA sequences, a cDNA library was constructed from BY-2 cells treated with INF1 elicitor for 6 h using a ZAP-cDNA<sup>®</sup> synthesis kit and a Gigapack<sup>®</sup> III Gold cloning kit (Stratagene, USA) following the manufacturer's instructions. Screening of the cDNA library was carried out at 42°C overnight in 5×SSC (150 mM NaCl, 15 mM  $\text{Na}_3\text{-citrate}$ , pH 7.0), 50% formamide, 0.02% SDS, 2% blocking reagent (Boehringer Mannheim, Germany) and 0.1% lauroylsarcosine. The DNA probe was made using a PCR digoxigenin (DIG) probe synthesis kit (Boehringer Mannheim). To obtain the complete 5'-sequence of the *NtPDR1* gene, 5'-rapid amplification of the cDNA end (RACE) PCR was performed with a Marathon<sup>™</sup> cDNA amplification kit (Clontech, USA) following the manufacturer's instructions. The gene-specific primer for 5'-RACE PCR was designed from the cDNA sequence obtained from the cDNA library. DNA sequence analysis of these clones was performed with an ABI PRISM<sup>®</sup> 310 genetic analyzer (Perkin Elmer Applied Biosystems Japan, Japan).

### 2.5. Northern blot analysis

15 µg of total RNA was separated on a 1% agarose gel containing formaldehyde and blotted onto a Hybond<sup>®</sup>-N<sup>+</sup> nylon membrane (Amersham Pharmacia Biotech, UK). Equalized loading of RNA was visualized by ethidium bromide staining of rRNAs. The RNA probes were made using an RNA transcription kit (Stratagene) and labeled with DIG-UTP by DIG RNA Labeling Mix (Boehringer Mannheim). Hybridization was performed at 68°C overnight in 5×SSC, 50% formamide, 0.02% SDS, 2% blocking reagent (Boehringer Mannheim) and 0.1% lauroylsarcosine. The final washing step was performed at 68°C in 0.1×SSC and 0.1% SDS. Hybridized mRNAs were detected with anti-DIG antibody conjugated with alkaline phosphatase (Boehringer Mannheim) and its chemiluminescent substrate, CDP-Star<sup>®</sup> (Boehringer Mannheim).

### 2.6. Application of chemicals

To analyze the expression pattern of *NtPDR1*, we used K252a (0.2 µM) as a protein kinase inhibitor, cantharidin (0.2 µM) as a protein phosphatase inhibitor, cycloheximide (15 µM) as an inhibitor of de novo protein synthesis and EDTA (3 mM), EGTA (5 mM) as chelators or  $\text{LaCl}_3$  (0.5 mM) as a  $\text{Ca}^{2+}$  channel blocker. To study the effect of phytohormones, abscisic acid (ABA; 1, 5, 20 µM), methyl jasmonic acid (MeJA; 1, 5, 20 µM) and salicylic acid (SA; 20, 50, 500 µM) were applied to BY-2 cells. The concentration of all tested chemicals is the maximum within the range of non-toxicity when they are used alone for 24 h on BY-2 cells. All chemicals were produced by Sigma (Sigma-Aldrich, USA).

## 3. Results and discussion

### 3.1. Isolation of *NtPDR1* gene by differential display

To isolate differentially expressed genes during the defense response, we compared mRNAs of BY-2 cells treated with INF1 elicitor and LB medium (control) for 1 or 3 h by the

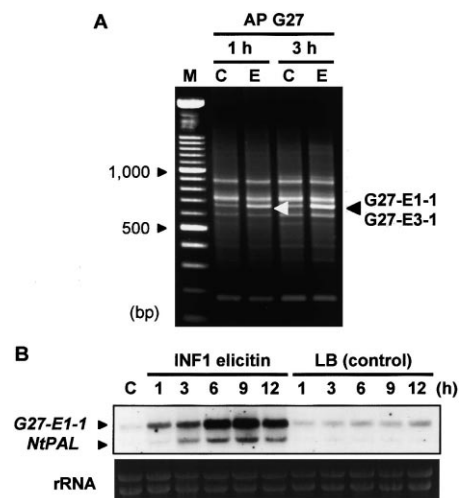


Fig. 1. Isolation of INF1 elicitor-responsive gene by differential display. A: Differential display PCR was carried out using BY-2 mRNA and G27 primer. Each mRNA was prepared from BY-2 cells at 1 or 3 h after treatment of control (equal volume LB medium to INF1 elicitor (v/v), represented as C) or INF1 elicitor (10 µg/ml, represented as E). G27 primer was used as an arbitrary primer (represented as AP G27). Arrowheads indicate the DNA fragments, G27-E1-1 and G27-E3-1, whose intensities were enhanced after elicitor treatment. Lane M denotes the molecular size marker of a 100 bp ladder. B: The expression of *NtPAL* and *G27-E1-1* induced by INF1 elicitor in BY-2 cells. Total RNAs were collected from BY-2 cells treated with INF1 elicitor (10 µg/ml) or LB medium (control), and analyzed by Northern blot using a *G27-E1-1* fragment and the *NtPAL* gene [17] as probes. Each signal is indicated by an arrowhead. Control RNA (denoted as C) was extracted from non-treated BY-2 cells.

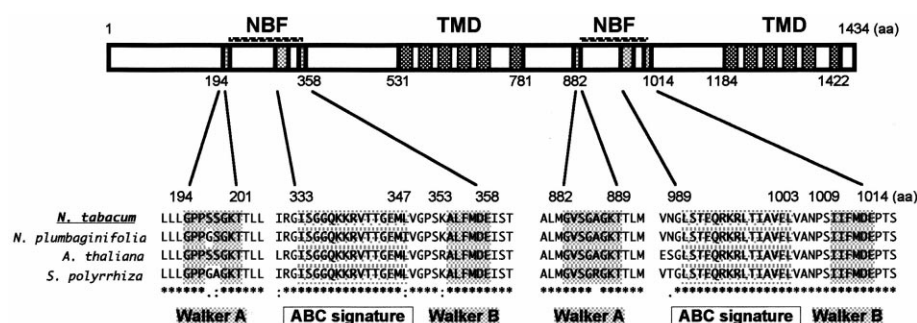


Fig. 2. Structural analysis of *NtPDR1* gene. The structure of *NtPDR1* was predicted by scanning the deduced amino acid sequence with the InterPro database [32] and PSORT program [25], and is presented as a schematic model. NBF and TMD indicate the predicted location of NBFs and TMDs, respectively. Black shading in the TMD shows transmembrane-spanning  $\alpha$ -helices. Multiple alignment of the part of NBF is presented under the schematic model. Nucleotide-binding sites (Walker A, B) and ABC signature motifs of *NtPDR1* were aligned to *N. plumbaginifolia* NpABC1 (accession no. AJ404328-1) [15], an *A. thaliana* ABC homolog (AC013453-3), *S. polyrrhiza* TUR2 (Z70524-1) [23] using the CLUSTAL W program [33]. The numbers indicate the position of the amino acid in *NtPDR1*. Below the amino acid sequences, identical, similar and the same hydrophobic residues in all four sequences are marked by asterisks, colons and points, respectively. The Walker A, B motifs and ABC signature motifs are shown with shading in the sequence.

random-amplified polymorphic DNA/reverse transcription-PCR differential display method [20]. Among the 32 primers tested, we obtained 19 elicitor-responsive fragments. Two of them, G27-E1-1 and G27-E3-1 fragments, were independently amplified with G27 primer from mRNAs in BY-2 cells treated with elicitor for 1 h and 3 h, respectively. Their nucleotide sequences were identical, suggesting that they were transcribed from the same gene (Fig. 1A). To investigate the expression of the corresponding gene we performed Northern blot analysis of INF1 elicitor-treated BY-2 cells using the G27-E1-1 fragment as a probe. This gene was rapidly and strongly induced by treatment with INF1 elicitor in BY-2 cells (Fig. 1B). The expression level of this gene was much stronger than that of the *N. tabacum* phenylalanine ammonia-lyase (*NtPAL*) gene, which is known as a general defense gene (Fig. 1B). Thus, the expression of both genes reached their maximum level 9 h post-treatment and showed a similar pattern.

### 3.2. Structural analysis of *NtPDR1* gene

For further analysis, cDNA clones corresponding to G27-E1,3-1 fragments were isolated from a cDNA library prepared from elicitor-treated cells. About 3.3 kb and 1.47 kb cDNAs were obtained from the cDNA library and 5'-RACE PCR, respectively. Both cDNAs were connected at the overlapping region and produced a 4673 bp cDNA. This gene potentially

encoded a 162 kDa protein with 1434 amino acids, and showed a high homology to the ABC transporter family. The ABC transporter superfamily has several subfamilies distinguished on the basis of the topology of transmembrane domain (TMD) and the cytosolic loop corresponding to the nucleotide-binding fold (NBF), which is conserved in a family of ATP-binding proteins [21,22]. In the main two subfamilies, the MDR (multidrug resistance) subfamily has the [TMD-NBF]<sub>2</sub> configuration, and the PDR subfamily conserves reverse topology in comparison with the MDR subfamily. Our isolated gene showed a homology especially to PDR-type ABC transporters of various species including plants; therefore, we designated this clone *NtPDR1* (*N. tabacum* PDR protein 1). The deduced amino acid sequence of *NtPDR1* is homologous, with 84.1%, 71.7% and 70.3% identical residues, to *NpABC1* of *N. plumbaginifolia* [15], the *Arabidopsis* ABC transporter homolog and *TUR2* of *Spirodela polyrrhiza* [23], respectively. They all belong to the PDR subfamily and have the [NBF-TMD]<sub>2</sub> topology (Fig. 2). The predicted *NtPDR1* protein consists of two repeated halves, each comprising one cytoplasmic hydrophilic region containing the NBF followed by one hydrophobic region, integrated in the membrane through six transmembrane-spanning  $\alpha$ -helices (Fig. 2). Each ABC transporter domain in hydrophilic regions

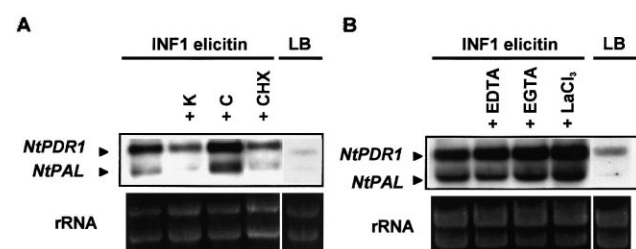


Fig. 3. Regulation of *NtPDR1* and *NtPAL* expression. A: Effects of inhibitors of protein kinase (0.2  $\mu$ M K252a; +K), protein phosphatase (0.2  $\mu$ M cantharidin; +C) and de novo protein synthesis (15  $\mu$ M cycloheximide; +CHX) on the expression of *NtPDR1* and *NtPAL* genes induced with INF1 elicitor (10  $\mu$ g/ml) in BY-2 cells. B: Effects of chelators of cations (3 mM EDTA, 5 mM EGTA) and a  $\text{Ca}^{2+}$  channel blocker (0.5 mM  $\text{LaCl}_3$ ) on the expression of *NtPDR1* and *NtPAL* genes induced with INF1 elicitor (10  $\mu$ g/ml) in BY-2 cells 3 h after treatment. All chemicals were added 30 min prior to treatment of BY-2 cells with elicitor or LB medium (control).

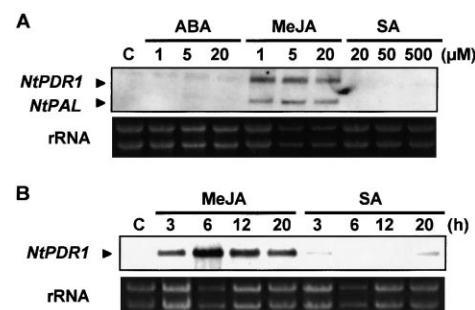


Fig. 4. Effects of phytohormones on the expression of *NtPDR1*. A: Effects of ABA (1, 5, 20  $\mu$ M), MeJA (1, 5, 20  $\mu$ M) and SA (20, 50, 500  $\mu$ M) on the expression of *NtPDR1* and *NtPAL* genes in BY-2 cells. Control RNA (C) was extracted from non-treated BY-2 cells. All phytohormones were treated for 3 h. B: Time course analysis of the expression of the *NtPDR1* gene in BY-2 cells treated with MeJA (20  $\mu$ M) or SA (500  $\mu$ M). Control RNA (C) was extracted from 0-time non-treated BY-2 cells.



contains ABC transporter nucleotide-binding motifs, Walker A (P-loop) and Walker B [24,21], and the ABC signature motif [21] between Walker A and B, which are highly conserved among known ABC transporters, especially in plants (Fig. 2).

In the EST database of *Arabidopsis thaliana*, over 100 ABC transporter homologs are registered, but plant ABC transporters are not as clearly classified by the functions as those of animals or yeasts are. When we constructed a phylogenetic tree with the deduced amino acid sequences of *NtPDR1* and some plant ABC transporters, it showed that *NtPDR1*, *NpABC1*, *TUR2* and one of the *Arabidopsis* ABC transporter homologs could form one subclass (data not shown) in the PDR subfamily. The MRP (MDR-associated protein) subfamily of *Arabidopsis* ABC transporters, which have modified [TMD-NBF]<sub>2</sub> topology, are relatively well characterized in plants. AtMRP1–3 have been reported to transport GS conjugates or chlorophyll catabolites to vacuoles for cell detoxification [9–12]. According to a prediction of localization of *NtPDR1* protein by the PSORT program [25], *NtPDR1* is likely to exist on the plasma membrane like *NpABC1* [15]. [NBF-TMD]<sub>2</sub>-type ABC transporters localized on the plasma membrane might have a specific conserved function different from that of [TMD-NBF]<sub>2</sub>-type ABC transporters, including the MRP subfamily.

### 3.3. Regulation of *NtPDR1* gene expression

To examine how the expression of *NtPDR1* is regulated, the effects of several chemicals that affect various signaling pathways of elicitor-induced defense responses in BY-2 cells [17] were investigated by Northern blot analysis. Fig. 3 shows the results of *NtPDR1* (upper band) and *NtPAL* (lower band) mRNA accumulation on BY-2 cells 3 h after treatment with elicitor in the presence of each inhibitor. K252a, a protein kinase inhibitor, remarkably inhibited the expression of elicitor-induced *NtPDR1* and *NtPAL* (Fig. 3A). In contrast, cantharidin, a protein phosphatase inhibitor, accelerated the expression of these genes (Fig. 3A). These results indicate that the expression of *NtPDR1* requires a protein phosphorylation

event, as *NtPAL* does. In addition, expression of *NtPDR1* was suppressed by cycloheximide, indicating that de novo protein synthesis is also required for elicitor-induced expression of *NtPDR1* (Fig. 3A). On the other hand, we tested the effect of chelators of cations and a Ca<sup>2+</sup> channel blocker to investigate whether these chemicals affect *NtPDR1* mRNA accumulation. However, these chemicals did not have significant effects on the expression of *NtPDR1* and *NtPAL*. Interestingly, the regulation pattern of *NtPDR1* expression exactly followed that of *NtPAL*, a typical defense gene (Fig. 3A,B). The expression of defense-related genes in plants often shows the same activation pattern both temporally and spatially. These results suggest that the expression of *NtPDR1* and *NtPAL* might be regulated under the same signal transduction pathway.

### 3.4. Effect of phytohormones on expression of *NtPDR1*

Smart and Fleming have reported that *TUR2*, an ABC transporter homolog of *S. polyrrhiza*, an aquatic plant, was induced by ABA [23]. Because *NtPDR1* has high homology to *TUR2*, we tested the effect of ABA, in addition to MeJA and SA, representative plant hormonal signal molecules during defense responses, on the expression of *NtPDR1* and *NtPAL* in BY-2 cells. As shown in Fig. 4A, no concentration of ABA (1, 5, 20  $\mu$ M) or SA (20, 50, 500  $\mu$ M) induced significant activation of either the *NtPDR1* gene or the *NtPAL* gene. However, *NtPDR1* and *NtPAL* genes were activated by MeJA, even at very low concentrations (1, 5, 20  $\mu$ M). Moreover, when we successively analyzed the expression of *NtPDR1* by MeJA (20  $\mu$ M) or SA (500  $\mu$ M), it was clearly responsive to MeJA but not to SA (Fig. 4B). SA and JA are important compounds related to the signal transduction pathways triggered by pathogens or wounding, and are known to induce the expression of specific genes such as acidic or basic PR protein genes, respectively [26]. Our results showed that MeJA but not SA specifically induced expression of the *NtPDR1* gene, which suggests that the *NtPDR1* gene is classified as a JA-responsive defense-related gene like the basic PR protein genes, whereas the expression of *NtPDR1* gene was not induced by ABA. The function of ABA is the promotion of abscission of fruits or leaves or adaptation to environmental stresses, such as cold or osmotic stress. Although some functions of ABA are similar to JA, ABA and JA differed in their ability to induce expression of the *NtPDR1* gene. Defense responses against insects or pathogens [27] or the increase of secondary metabolites accumulated by some elicitors [28,29] are phenomena known to be affected by JA or its derivatives but not ABA. Thus, the responsiveness of the *NtPDR1* gene to JA suggests that the function of *NtPDR1* protein is linked with plant defense responses.

### 3.5. Expression of *NtPDR1* gene by treatment with various elicitors

The induced expression of the *NtPDR1* gene by other elicitor molecules was examined. Accumulation of the *NtPDR1* transcript was detected in BY-2 cells after treatment with the bacterial HR elicitor flagellin<sup>Psto</sup>, which was prepared from *P. syringae* pv. *tomato*, a non-pathogen to tobacco. The expression of *NtPDR1* was strongly increased within 3 h and reached its maximum level 6 h after the treatment with flagellin (Fig. 5, upper panel). Subsequently, the accumulation of *NtPDR1* mRNA diminished to the control level by 12 h after

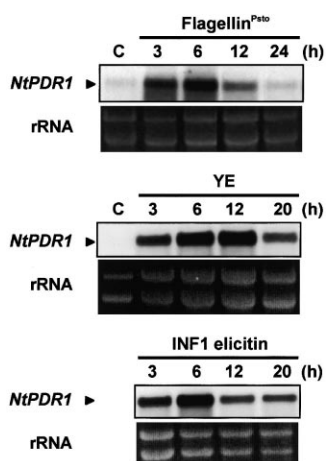


Fig. 5. Effects of different elicitors on expression of *NtPDR1* gene in BY-2 cells. RNAs were successively prepared from BY-2 cells treated with flagellin<sup>Psto</sup> (10  $\mu$ g/ml), yeast extract (YE; 5 mg/ml) and INF1 elicitor (10  $\mu$ g/ml). Control RNAs in treatment of flagellin<sup>Psto</sup> (denoted as C in upper panel) and yeast extract (C in middle panel, this is also the control for elicitor treatment) were extracted from 0-time non-treated BY-2 cells.

treatment. Thus, the expression profile was very similar to that after treatment of INF1 elicitor (Fig. 5, lower panel). Yeast extract, which is known as a general elicitor that does not induce cell death, also activated the expression of *NtPDR1* within 3 h after treatment, and the maximum level of the expression of *NtPDR1* continued 6–12 h after treatment. These findings indicate that the expression of *NtPDR1* is induced by diverse elicitor molecules derived from fungi, bacteria or yeast. This suggests that *NtPDR1* is a general defense gene like the *PAL* or *PR* genes and might be involved in a defense response in tobacco. Involvement of the ABC transporter in plant defense responses has been speculated from recent reports of the isolation of an ABC transporter homolog as a candidate for a defense-related gene from systematic analysis in different model systems [3,5]. Here we have first demonstrated that PDR-type plant ABC transporter gene shows the specific expression by various stimuli which promote the defense response.

The substrates of *NtPDR1* are unknown at present, and, therefore, the role of *NtPDR1* protein in defense response is still unclear. *NtPDR1* might contribute to cell detoxification by the transportation of intracellularly accumulated phenolic compounds during the defense response, or the secretion of anti-pathogenic compounds such as phytoalexins to attack extracellular pathogens. It has been reported that the accumulation of *NpABC1* protein was induced by antifungal terpenoids, and *NpABC1* is thought to excrete them from the cells [15]. The high similarity of deduced amino acid sequences between *NtPDR1* and *NpABC1* might show that *NtPDR1* also has the function of the secretion of some antifungal compounds as *NpABC1*. Recently it was demonstrated that *Pti3*, which was isolated as the factor interacting with *Pto*, a resistance gene product of tomato to *P. syringae*, by a two-hybrid system, encodes an ABC transporter homolog [30]. This interaction between *Pto* and *Pti3* (ABC transporter) was reduced when *Pto* was mutated at the site required for the induction of HR; therefore, expression of the ABC transporter seems to be involved in the induction of HR [30]. In the nematode, it has been reported that the expression of an ABC transporter homolog, the *ced7* gene, was stimulated in both dying cells and surrounding engulfing cells during programmed cell death in embryogenesis [31]. These reports indicate the possible involvement of the ABC transporter in the regulation of programmed cell death in multicellular organisms and raise the possibility that *NtPDR1* also controls cell death or cell survival during the HR induced by elicitor.

Further functional analysis is required to understand the role of *NtPDR1* and its correlation to plant defense mechanisms.

**Acknowledgements:** We thank Prof. Sophien Kamoun, Ohio State University, for the gift of the *inf1* gene and Dr. Fumiko Taguchi in our laboratory for the preparation of flagellin. This work was supported by a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists, and also by Grants-in-Aid for Scientific Research on Priority Areas (No. 12052215) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## References

- [1] Dixon, R.A. and Lamb, C.J. (1990) *Annu. Rev. Plant Pathol. Plant Mol. Biol.* 41, 339–367.
- [2] Rushton, P.J. and Somssich, I.E. (1999) in: *Transcriptional Regulation of Plant Genes Responsive to Pathogens and Elicitors* (Stacey, G. and Keen, N.T., Eds.), Plant-Microbe Interact. Vol. 4, pp. 251–274, APS Press, St. Paul, MN.
- [3] Ichinose, Y., Tiemann, K., Schwenger-Erger, C., Toyoda, K., Hein, F., Hanselle, T., Cornels, H. and Barz, W. (2000) *Z. Naturforsch. [C]* 55, 44–54.
- [4] Etienne, P., Petitot, A.S., Houot, V., Blein, J.P. and Suty, L. (2000) *FEBS Lett.* 466, 213–218.
- [5] Xiong, L., Lee, M.W., Qi, M. and Yang, Y. (2001) *Mol. Plant-Microbe Interact.* 14, 685–692.
- [6] Higgins, C.F. (1992) *Annu. Rev. Cell Biol.* 8, 67–113.
- [7] Senior, A.E., al-Shawi, M.K. and Urbatsch, I.L. (1995) *FEBS Lett.* 377, 285–289.
- [8] Sanchez-Fernandez, R., Davies, T.G.E., Coleman, J.O.D. and Rea, P.A. (2001) *J. Biol. Chem.* 276, 30231–30244.
- [9] Lu, Y.P., Li, Z.S. and Rea, P.A. (1997) *Proc. Natl. Acad. Sci. USA* 94, 8243–8248.
- [10] Lu, Y.P., Li, Z.S., Drozdowicz, Y.M., Hortensteiner, S., Martinoia, E. and Rea, P.A. (1998) *Plant Cell* 10, 267–282.
- [11] Tommasini, R., Vogt, E., Schmid, J., Fromentau, M., Amrhein, N. and Martinoia, E. (1997) *FEBS Lett.* 411, 206–210.
- [12] Tommasini, R., Vogt, E., Fromentau, M., Hortensteiner, S., Matile, P., Amrhein, N. and Martinoia, E. (1998) *Plant J.* 13, 773–780.
- [13] Urban, M., Bhargava, T. and Hamer, J.E. (1999) *EMBO J.* 18, 512–521.
- [14] Schoonbeek, H., Del Sorbo, G. and De Waard, M.A. (2001) *Mol. Plant-Microbe Interact.* 14, 562–571.
- [15] Jasinski, M., Stukkens, Y., Degand, H., Purnelle, B., Marchand-Brynaert, J. and Boutry, M. (2001) *Plant Cell* 13, 1095–1107.
- [16] Kamoun, S., van West, P., de Jong, A.J., de Groot, K.E., Vleeshouwers, V.G.A.A. and Govers, F. (1997) *Mol. Plant-Microbe Interact.* 10, 13–20.
- [17] Sasabe, M., Takeuchi, K., Kamoun, S., Ichinose, Y., Govers, F., Toyoda, K., Shiraishi, T. and Yamada, T. (2000) *Eur. J. Biochem.* 267, 5005–5013.
- [18] Nagata, T., Okuda, K. and Takeda, I. (1982) *Plant Cell Rep.* 1, 250–252.
- [19] Taguchi, F., Shimizu, R., Nakajima, R., Toyoda, K., Shiraishi, T. and Ichinose, Y. (2002) submitted.
- [20] Yoshida, K.T., Naito, S. and Takeda, G. (1994) *Plant Cell Physiol.* 35, 1003–1009.
- [21] Higgins, C.F., Hyde, S.C., Mimmack, M.M., Gileadi, U., Gill, D.R. and Gallagher, M.P. (1990) *J. Bioenerg. Biomembr.* 22, 571–592.
- [22] Hung, L.W., Wang, I.X., Nikaido, K., Liu, P.Q., Ames, G.F. and Kim, S.H. (1998) *Nature* 396, 703–707.
- [23] Smart, C.C. and Fleming, A.J. (1996) *J. Biol. Chem.* 271, 19351–19357.
- [24] Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) *EMBO J.* 1, 945–951.
- [25] Nakai, K. and Kanehisa, M. (1991) *Proteins* 11, 95–110.
- [26] Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N. and Ohashi, Y. (1998) *Plant Cell Physiol.* 39, 500–507.
- [27] Creelman, R.A. and Mullet, J.E. (1997) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 355–381.
- [28] Gundlach, H., Muller, M.J., Kutchan, T.M. and Zenk, M.H. (1992) *Proc. Natl. Acad. Sci. USA* 89, 2389–2393.
- [29] Mueller, M.J., Brodschelm, W., Spannagl, E. and Zenk, M.H. (1993) *Proc. Natl. Acad. Sci. USA* 90, 7490–7494.
- [30] Sessa, G., D’Ascenzo, M. and Martin, G.B. (2000) *EMBO J.* 19, 2257–2269.
- [31] Wu, Y.C. and Horvitz, H.R. (1998) *Cell* 93, 951–960.
- [32] Apweiler, R., Attwood, T.K., Bairoch, A., Bateman, A., Birney, E., Biswas, M., Bucher, P., Cerutti, L., Corpet, F., Croning, M.D.R., Durbin, R., Falquet, L., Fleischmann, W., Gouzy, J., Hermjakob, H., Hulo, N., Jonassen, I., Kahn, D., Kanapin, A., Karavidopoulou, Y., Lopez, R., Marx, B., Mulder, N.J., Oinn, T.M., Pagni, M., Servant, F., Sigrist, C.J.A. and Zdobnov, E.M. (2001) *Nucleic Acids Res.* 29, 37–40.
- [33] Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) *Nucleic Acids Res.* 22, 4673–4680.