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Update section

Short communication

Nucleotide sequence of an osmotin-like cDNA induced in tomato during viroid infection

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Abstract

A cDNA library from tomato planta macho viroid (TPMV)-infected tomato was constructed. The library was screened at low stringency with a tobacco PR-R cDNA probe. An 832 bp cDNA from a mRNA present only in infected tissue was isolated. Nucleotide sequence showed high homology with the osmotin from both tobacco and tomato (NP24). This cDNA probably corresponds to the AP24 and P23 proteins previously described in tomato and induced upon fungal and viroid infection.

The pathogenesis-related (PR) proteins are a group of structurally diverse proteins, apparently ubiquitous in plants, induced by a variety of agents, ranging from ethylene to pathogens [3]. Although it is known that these proteins are involved in systemically acquired resistance, and in general to the stress response, it is not clear what is their precise role in these phenomena [9].

The viroids are plant pathogens consisting of single-stranded, circular, unencapsidated RNA, and do not code for any protein. It has been reported that viroids are able to induce the synthesis of PR proteins in several plant hosts. Indeed, a tomato protein, P23, induced by citrus exocortis viroid (CEV) and highly homologous to tomato osmotin (NP24), has been isolated recently [2, 6]. The latter is induced as a response to high salt concentrations, but its proposed role in adaptation to osmotic stress has not been confirmed [2]. These proteins belong to the PR-5 family of the PR proteins. This group also includes the sweet-tasting protein thaumatin and a bifunctional α-amylase/proteinase inhibitor from maize [3, 7].

In this work we describe the cloning and sequencing of a cDNA from tomato infected with

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X66416 (*L. esculentum* mRNA tpm1).

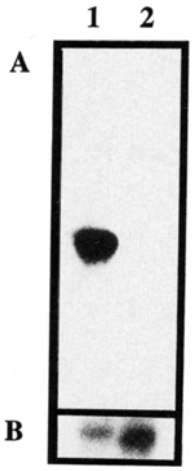

Fig. 1. A. Northern analysis of total RNA from TPMV-infected (lane 1) and healthy (lane 2) tomato leaf tissue. The RNA was transferred to a nylon-based membrane and hybridized using ^{32}P-labelled TPM1 cDNA as probe. B. Control as in A, but using *A. thaliana* tubulin as probe.

tomato planta macho viroid (TPMV). Poly(A)$^+$ RNA was isolated from leaves of TPMV-infected tomato (cv. Rio Grande), and its complementary DNA synthesized and cloned in λgt-10. The resulting library was screened with tobacco PR-R (PR-5) cDNA as probe (a kind gift from J. Ryals, CIBA-Geigy). We selected the largest cDNA (TPM1), which produced a strong positive hybridization signal, for further analysis and sequencing.

Northern analysis showed that TPM1 expression is induced to high levels as a result of viroid infection. These results are shown in Fig. 1A. Total RNA from healthy and TPMV-infected tomato leaves (28 days after inoculation) was blotted onto a nylon membrane. The membrane was hybridized at high stringency conditions using the TPM1 clone as probe. No hybridization signal is visible in the lane corresponding to RNA from healthy tissue (lane 2), but a strong one is evident in that of infected tissue (lane 1). The signal corresponds to a messenger RNA ca. 1000 bases long. This result suggests that the gene encoding the TPM1 protein is not expressed in normal (uninoculated) conditions, and is strongly induced as a response to viroid infection. As a control, we hybridized identical RNA samples against a β-tubulin genomic probe from *Arabidopsis thaliana* [4]. As a contrast to TPM1, β-tubulin RNA expression was usually similar or even a little higher in healthy tissue than in TPMV-infected tomato tissue (Fig. 1B).

The sequence of the cDNA is shown in Fig. 2, which also features the sequence of the predicted protein encoded by the most probable open reading frame (ORF). The cDNA is 832 bp long and the ORF is 714 bp long. This sequence has two possible polyadenylation signals (underlined). The absence of methionine at its 5′ indicates that this clone is lacking part of the signal sequence typically present in PR5 proteins. Computer analysis suggests that the first 13 amino acids are likely to be part of a signal peptide. The position between Ala^{13} and Ala^{14} (see Fig. 2) is a probable signal peptide processing site that conforms to the (−3, −1) rule [8]. The analysis also reveals a high homology (nearly 90%) to tomato osmotin (NP24) cDNA. In addition, the predicted mature N-terminus of TPM1 (starting from Ala^{14}) is 100% homologous to the reported N-terminal sequences of viroid-induced protein P23 and to the fungus-induced protein with antifungal properties AP-24, suggesting that all these proteins might be equivalent [6, 10]. Figure 3 shows the homology of the N-terminus with NP24, AP24, P23 as well as to other members of the PR-5 family, such as thaumatin, PR-R and zeamatin, a protein from maize with a suggested activity to permeabilize membranes [5, 7]. It remains to be demonstrated if more members of the PR5 group also display that activity and its importance in their suggested biological properties (e.g., antifungal activity or osmotic stress tolerance) [7].

The predicted protein is moderately hydrophobic, with a GRAVY of −3.96. According to the calculated amino acid sequence of TPM1, its predicted secondary structure should be predominantly β with a high content of β turns and a very small content of α helices. Since all the PR-5 proteins whose full sequence is known have all 16 of their cysteine residues in conserved positions, we could expect a tertiary structure similar to the one reported for thaumatin [1].

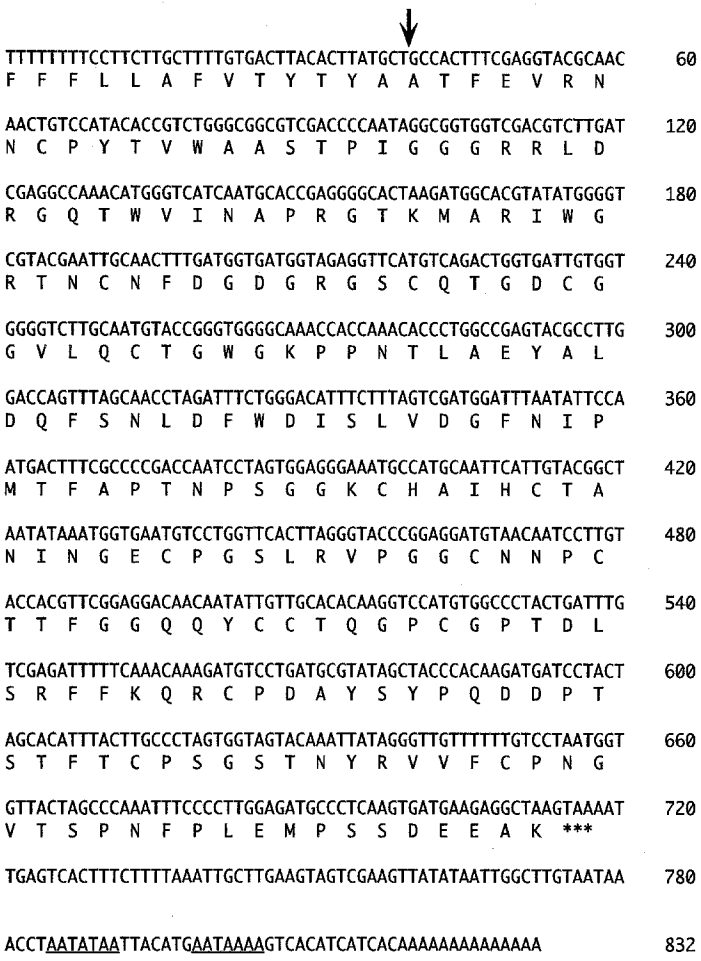

Fig. 2. Nucleotide sequence and translation of TPM1 cDNA. The arrow indicates the predicted processing site of the signal peptide. The sequences underlined are possible polyadenylation signal.

These results support notion that the induction of PR5 proteins is a general response of plants to stress situations that may well include agents as diverse as high salt concentration, fungi and viroids.

	10	20		
TPM1	ATFEVRNNCP	YTVWAASTPI	-----GGGRR	LD
NP24	ATIEVRNNCP	YTVWAASTPI	-----GGGRR	LD
AP24	ATFEVRNNCP	YTVWAASTPI	-----GGGRR	LD
P23	ATFEVRNNCP	YTVWAASVPV	-----GGGRQ	LN
PR-R	ATFDIVNQCT	YTVWAAASPG	------GGRQ	LN
THAUMATIN	ATFEIVNRCS	YTVWAAASKG	DAALDAGGRQ	LN
ZEAMATIN	AVFTVVNQCP	FTVWAASVPV	-----GGGRQ	LN
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Fig. 3. Comparison of the N-terminus of the predicted mature TPM1 protein with osmotin (NP24) and other members of the PR-5 family. The asterisks indicate the conserved amino acids in the first 20 residues (before any gaps are introduced). Sources for N-terminal sequences: NP24 [2], AP24 [10], P23 [6], PR-R [7], thaumatin [7], zeamatin [7].

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