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Short communication

Isolation of a mRNA encoding a nucleoside diphosphate kinase from tomato that is up-regulated by wounding

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Abstract

A cDNA clone (TAB2) encoding a nucleoside diphosphate (NDP) kinase has been isolated from a tomato (*Lycopersicon esculentum* Mill. cv. Ailsa Craig) cDNA library. The clone is 590 bp long and exhibits a high degree of sequence identity with spinach NDP kinases I and II, *Pisum sativum* NDP kinase I, *Arabidopsis thaliana* NDP kinase, *Drosophila melanogaster* NDP kinase, *Dictyostelium discoideum* NDP kinase and human Nm 23-H1 and Nm23-H2. Northern analysis has revealed that the mRNA encoded by TAB2 is up-regulated in both leaf and stem tissue in response to wounding. The increase is apparent within 1 h of wounding and is not further elevated by application of ethylene. Southern blot analysis indicates that TAB2 is a member of a small gene family.

Nucleoside diphosphate (NDP) kinase catalyses the transfer of the γ-phosphate of 5′-triphosphate nucleotide to 5′-diphosphate nucleotides involving a high-energy phosphohistidine intermediate [10]. Recently, research has been given new impetus by the discovery that NDP kinase has significant homology to the Nm 23 and awd proteins [17] which have been linked with mammalian tumour metastasis and the development of *Drosophila melanogaster* respectively. It has been demonstrated that NDP kinase converts GDP-bound ADP ribosylation factor (ARF) to GTP-bound ARF in the absence of nucleotide exchange [12] and that NDP kinase is stimulated by cell surface cAMP receptors [4]. Therefore, NDP kinase may mediate hormone action by manufacturing GTP for GTP-binding protein activation.

Over the last year, reports have been made of the identification of cDNAs encoding NDP kinases in higher plants, and in spinach two forms of the enzyme have been characterised at the protein and nucleotide levels [9, 19]. It has also recently been postulated that the nucleotide regulation of a plasma membrane NADH oxidase in

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X75324.

soybean may be mediated by a NDP kinase [8]. In this paper we describe the isolation of a cDNA clone that encodes a putative NDPK I from tomato that has close sequence identity with both the major plant and animal forms of the enzyme. Of major significance is the observation that the expression of the gene encoding this NDPK I is up-regulated rapidly in response to wounding in leaf and internode tissue.

The growth of tomato plants, preparation of explants and determination of abscission has been described previously [13, 16]. Total RNA was extracted from leaf abscission zone tissue as described [3] and 5 μg of poly(A)$^+$ RNA was used to construct a cDNA library in the λ Zap vector (Stratagene, USA). The library was differentially screened against RNA extracted from non-abscission zone tissue [11] and several cDNAs of interest isolated.

Northern analysis was performed with each clone to determine the precise pattern of transcript accumulation over the time course of abscission. Equal amounts of RNA from 0, 12, 24, 36 and 48 h ethylene-exposed abscission zone and non-zone tissue were used and probed as described [3]. The mRNA coding for one of the clones, TAB2, accumulated in both zone and non-zone tissue within 12 h exposure to ethylene and this elevated level was maintained (not shown). To determine whether the accumulation of this transcript was related to the presence of ethylene or was promoted by the wounding associated with explant generation, RNA was extracted from non-zone (stem) tissue exposed to ethylene for 12 and 24 h of incubated in the absence of the gas, and subjected to northern analysis. It is evident from Fig. 1 that the accumulation of TAB2 mRNA in explant tissue occurred irrespective of exposure to ethylene within 12 h of excision from the plant. To investigate whether an increase in the expression of TAB2 mRNA was a general feature of wounded tissue, total RNA from leaves 0, 1, 2, 6 and 12 h after wounding was isolated using the method described [15] and a northern analysis undertaken as before. At 1 h after wounding an elevation in the level of TAB2 transcript could be detected and this signal

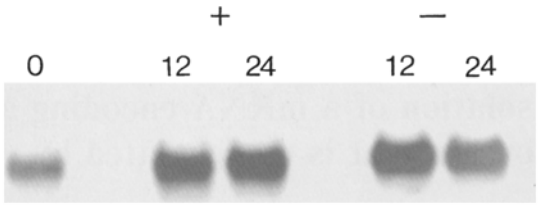

Fig. 1. Northern blot analysis of mRNA isolated from stem tissue incubated in the presence (+) or absence (−) of 10 μl/l of ethylene for 12 and 24 h. Total RNA (10 μg per lane) was probed with labelled TAB2 cDNA and exposed to X-ray film for 24 h at −70 °C with intensifying screens.

reached a plateau by 6 h (Fig. 2). Both strands of the TAB2 cDNA insert were sequenced and a database search revealed the clone to have a high degree of sequence identity at the amino acid level (Fig. 3) with spinach NDPK I and II (88% and 64% respectively), *P. sativum* NDPK I (86%), *A. thaliana* NDPK (80%), the *Drosophila melanogaster* awd gene (70%), *Dictyostelium discoideum* NDPK (66%) and human hNm 23-H1 (66%). TAB2 has 144 amino acid residues but does not appear to contain a full open reading frame (ORF). Based on the ORFs for other plant NDP kinases it would appear that TAB2 is 3–5 amino acids short of encoding the entire open reading frame. Subsequent rescreening of the cDNA library failed to isolated a full-length clone.

The TAB2 sequence possesses several of the major functional groups associated within NDP kinases. The histidine residue corresponding to His^{111} and consensus sequence Gly- X- Gly- X- X- Gly, present as Gly^8- Val- Gln- Arg- Gly- Leu- Val- Gly^{15}, have both been proposed to be ATP-binding sites [4, 5]. The highly conserved motif Asp^{100}- Phe^{101}, which seems to be important for

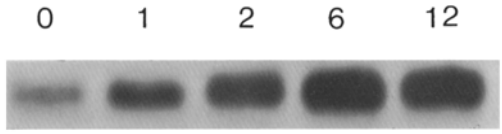

Fig. 2. Northern analysis of mRNA isolated from leaf tissue wounded for 0, 1, 2, 6 or 12 h. Total RNA (10 μg per lane) was probed with labelled TAB2 cDNA and exposed to X-ray film for 48 h at −70 °C with intensifying screens.

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Tom NDPK 1      FIMIKPDGVQRGLVGEIISRFEKKGFSLKGLKLITVDRAF
Spinach NDPK 1  ..............................A..FVN...P.
P.Sativum       ..............................Y.....FVN....
Arabidopsis     .............I..V.C.......T.....S.E.S.
Drosophila      ..............K..E...Q...K.VA..FTWASKEL
Spinach NDPKII  Y.....................K.I...MYPCPKEL
D. discoideum   .LAV.....A.........A.Y.....V.V...QLVPTKDL
hNm23- H1       ..A..............K...Q...R.V...FMQASEDL

Tom NDPK 1      AEKHYADLSAKPFFNGLVEYIVSGPVVAMVWEGKGVVATG
Spinach NDPK 1  ..............................................
P.Sativum       ..............S...D..I.......I..N....T..
Arabidopsis     .....E...S.S..S...D..........I.......L..
Drosophila      L.........R...P...N.MN.....P.....LN..K..
Spinach NDPKII  ..E..K..K..S..Q..ID..T.....C.A...V....SS
D. discoideum   ..S...EHKER...G...SF.T.........F.......SA
hNm23- H1       LKE..V..KDR...A...K.MH...........LN..K..

Tom NDPK 1      RKIIGATNPLESAAGTIRGDFAIDIGRNVIHGSDAVESAR
Spinach NDPK 1  ...L......A.EP.......................D..T
P.Sativum       .........AQ.EP.........................N
Arabidopsis     .........AA.EP.......C.QV...I.....S.....
Drosophila      .QML.....AD.LP.......C.QV...I.........E
Spinach NDPKII  ..L....D..QAEP......L.VQT....V.....SPDNGK
D. discoideum   .LM..V....A..P.S.....GV.....I.....S....N
hNm23- H1       .VML.E...AD.KP.......C.QV...I.....S....E

Tom NDPK 1      KEIALWF-PEGIAEWQSSLHSCIYE
Spinach NDPK 1  .......-.D.VVH.......W...
P.Sativum       .......-..A...E.....W...
Arabidopsis     .......-.D.PVN....V.PWV..
Drosophila      .......NEKEL.T.TPAAKDW...
Spinach NDPKII  R..G...KEGE.CQ.TPAQAPWLR.
D. discoideum   R......K..ELLTEVKPNPL-L..
hNm23- H1       ...G...H..ELVDYT.CAQNW...
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Fig. 3. Comparison of the amino acid sequence of TAB2 with those of other NDP kinases. Matching amino acids are indicated by a dot.

catalytic sites [2], is also present. In tomato, the region common to protein kinases (Ala- X- Lys) in which the lysine residue is essential for catalytic activity [6] has a minor difference, becoming Gly^{38}- Leu- Lys^{40}. This is the same as that found in spinach NDPK II but, interestingly, is not found in sequences that show greater overall homology to pTAB2. A hydropathy profile [7] shows no evidence for a signal peptide sequence at the amino acid terminal. In addition, extraction of genomic DNA as previously described [3] and probing of a Southern blot [14] using radiolabelled TAB2 shows that a small gene family is probably responsible for its expression (Fig. 4).

As yet, no information is available as to the role of NDP kinases in plant growth and development [18]. Our results demonstrate that the expression of TAB2 is stimulated rapidly in tomato leaf and stem tissue after mechanical wounding. Furthermore, there is no evidence that the up-regulation of TAB2 mRNA on wounding is influenced by ethylene. In the light of recent research linking NDP kinases with GTP-binding proteins [1, 8, 12], it seems possible that the mRNA coding for TAB2 may play a role in the perception and/or transduction of a wound-related signal. Further work will be necessary to determine whether its expression may be linked to the expression of

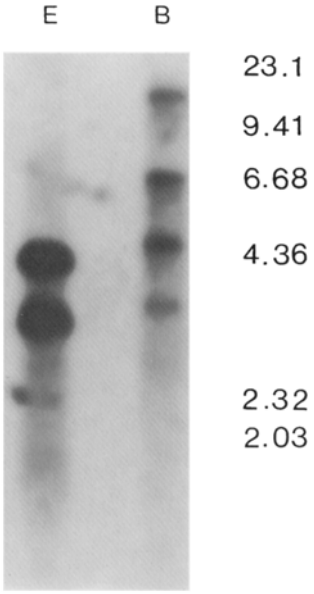

Fig. 4. Genomic Southern blot of tomato DNA. DNA was digested with *Eco* RI (E) and *Bam* HI (B) restriction enzymes and probed with labelled TAB2 cDNA. The positions of *Hin*d III-digested Lambda DNA size markers are shown. The blot was exposed to X-ray film for 3 days at −70 °C with intensifying screens.

genes regulating ethylene biosynthesis and whether its up-regulation in plants is of a local or systemic nature.

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