Plant Molecular Biology **18**: 623–627, 1992.

Update section

Short communication

Isolation and characterization of a plant cDNA showing homology to animal glutathione peroxidases

M.C. Criqui, E. Jamet, Y. Parmentier, J. Marbach, A. Durr and J. Fleck*
*Institut de Biologie Moléculaire des Plantes du C.N.R.S., 12 rue du Général Zimmer F-67000 Strasbourg, France (*author for correspondence)*

Received 17 July 1991; accepted in revised form 16 October 1991

Key words: btuE, glutathione peroxidase, *Nicotiana sylvestris*, protoplast, stress

Abstract

A cDNA library from freshly isolated protoplasts was differentially screened using cDNAs from mesophyll cells, stressed leaf strips and cell suspension cultures. One of the selected clones, 6P229, turned out to encode a putative polypeptide showing homology to the *btuE* periplasmic protein of *Escherichia coli* and to animal selenium-dependent glutathione peroxidases. A major difference was that the putative selenocysteine in the active site was not encoded by the termination codon TGA. The 6P229 gene was found to be expressed in germinating seeds, in apex and in flowers, as well as in stressed tissues. This pattern of expression would be consistent with a key role in cellular metabolism such as defense against oxidative stresses.

In order to understand how freshly isolated protoplasts of higher plants can re-enter the cell cycle, we are looking for genes expressed at a high level in protoplasts. A cDNA library from 6 h old protoplasts prepared from *Nicotiana sylvestris* leaves was constructed in λgt10. It was differentially screened using cDNAs from leaves, leaf strips maintained in the medium used for protoplast preparation but devoid of enzymes, and actively dividing cell suspension cultures. About 10% of the clones gave no signal or a faint signal after this triple screening and corresponded to genes highly expressed in protoplasts. Clone 6P229 was one of those.

The insert of 6P229 was subcloned in Bluescript vector (Stratagene) and sequenced by the dideoxy chain termination method [14] (Fig. 1A). Additional clones were isolated after a second screening of the library: all had the same nucleotide sequence. Our longest cDNA clone was 810 nucleotides in length and was terminated by a poly(A) sequence. Direct cDNA sequencing on

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X60219.

```
  1  AAAAATCTCAATTTCCTTAGGCAATTTTCTTCCATTCTAAAGCCAACCCATTTCAATTCTGTATCTATTCAGCCGATCCAAACAGTTCTT
 91  TCCAATTCTATTGTTTCAGCTAAGAGATTTGAGTTGTTTTGTTTAAGATCAGATTATAGTACCATGGCCAGTCAATCCAGCAAGCCTCAA
  1                                                                  M  A  S  Q  S  S  K  P  Q
181  TCTATTTATGACTTCACTGTCAAGGATGCTAAGGGTAATGATGTTGATCTCAGCATTTACAAGGGAAAGGTCCTTATTATTGTCAATGTT
 10  S  I  Y  D  F  T  V  K  D  A  K  G  N  D  V  D  L  S  I  Y  K  G  K  V  L  I  I  V  N  V
271  GCATCACAGTGTGGTCTGACAAATTCGAACTATACTGACTTGACCGAGATATACAAGAAGTACAAGGATCAAGGTTTGGAGATTCTTGCA
 40  A  S  Q  C  G  L  T  N  S  N  Y  T  D  L  T  E  I  Y  K  K  Y  K  D  Q  G  L  E  I  L  A
361  TTCCCTTGCAACCAGTTCGGTGGGCAGGAGCCTGGAAGCATTGAAGAGATCCAGAACATGGTTTGCACTCGCTTCAAGGCCGAGTACCCA
 70  F  P  C  N  Q  F  G  G  Q  E  P  G  S  I  E  E  I  Q  N  M  V  C  T  R  F  K  A  E  Y  P
451  ATATTCGATAAGGTTGATGTGAATGGTGATAATGCTGCTCCACTGTATAAGTTCTTGAAATCAAGCAAAGGTGGGTTCTTTGGAGATAGT
100  I  F  D  K  V  D  V  N  G  D  N  A  A  P  L  Y  K  F  L  K  S  S  K  G  G  F  F  G  D  S
541  ATCAAGTGGAACTTCTCCAAATTCCTTGTTGACAAAGAAGGAAACGTCGTCGATCGCTACTCTCCAACCACTACTCCAGCTAGCATGGAG
130  I  K  W  N  F  S  K  F  L  V  D  K  E  G  N  V  V  D  R  Y  S  P  T  T  T  P  A  S  M  E
631  AAGGATATCAAGAAACTTTTGGGTGTTGCTTAAGCTCTGGATTGATCTGCATCTGCATTCCTCTACTAGAAATAATGAGAGACTACTATA
160  K  D  I  K  K  L  L  G  V  A  *
721  GTGAATAAGTTCTGTGTTGATGTAATTTGCTTCTTGTTTTGCCACTACTTTGTTCAGTTGCTTGTATAATTTCAATATTGAATACATTTC
811  CAATGA  816
```

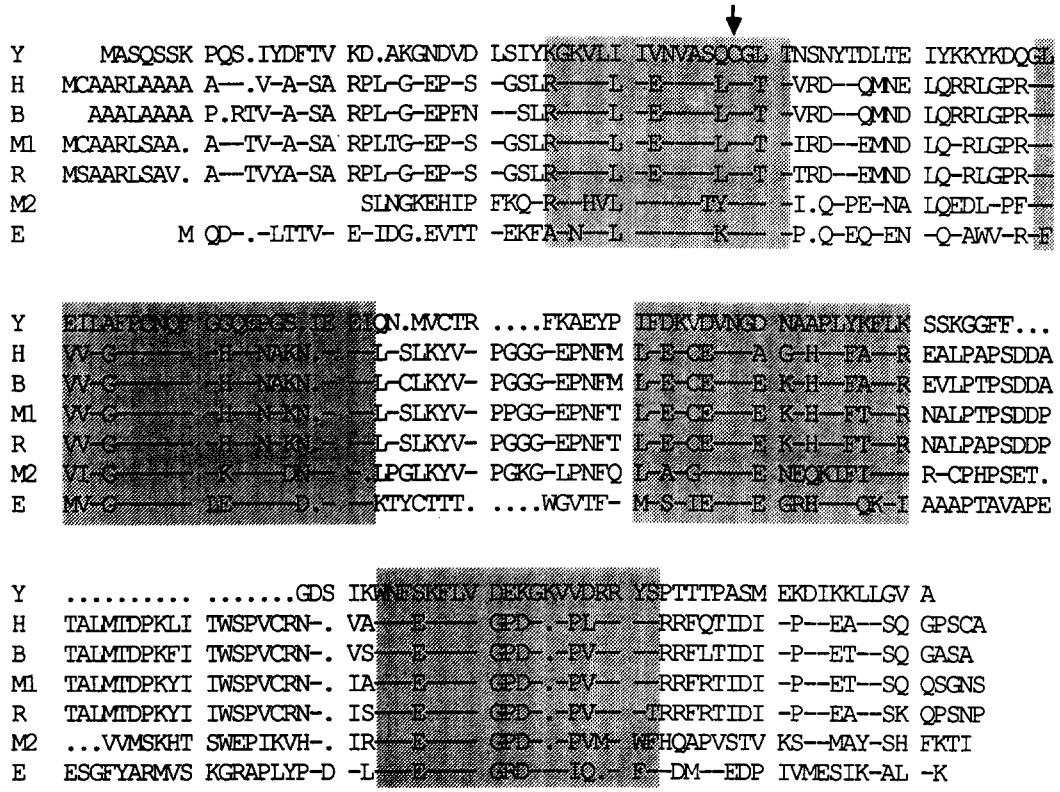

Fig. 1. A (top). The nucleotide and derived amino acid sequences of clone 6P229. Potential polyadenylation sites are underlined. B (bottom). Homologies to animal glutathione peroxidases and to the *E. coli btuE* periplasmic protein. Dots correspond to gaps introduced to maximize homologies. Identical amino acids are indicated by bars. Four conserved domains are boxed. SeC position is indicated by an arrow. Y stands for 6P229, H for human [11], B for bovine [7], M1 for mouse [3], R for rat [16], M2 for mouse [6], E for *E. coli* [5].

total RNAs was performed [10] and showed a strong arrest in elongation close to the end of this clone. The size of the corresponding mRNA was determined from northern analysis and was found to be about 1 kb. These results indicate that the longest cDNA recovered was probably nearly full length. The longest open reading frame gave rise to a polypeptide of 224 amino acids but showed no initiation codon close to the 5′ end of the clone. The Met codon at position 52 is likely to be the initiation codon both because our cDNA clone is nearly full-length (see above) and because of the results from sequence comparisons to known genes (see below). The encoded protein would then be 169 amino acids in length and have a calculated molecular mass of 18755 Da.

Computer search in Swissprot and Genbank databanks revealed homologies to several animal selenium-dependent glutathione peroxidases (bovine [7], mouse [3], human [11], rat [16], rabbit [1]), to a clone related to glutathione peroxidase isolated from a murine epididymal cDNA library [6], and to the *E. coli btuE* periplasmic protein which function is unknown [5, 13]. It should be noted that the murine cDNA has not yet been shown to encode a selenium-dependent glutathione peroxidase (J.P. Dufaure, personal communication). The average degree of homology of the 6P229 polypeptide to all these proteins is about 33% over the complete amino acid sequence, while the average degree of similarity is about 47%. However, the four domains which are boxed in Fig. 1B show a much higher degree of conservation in all polypeptides, varying between 65% homology and similarity in the fourth domain, and 70% homology and 82% similarity in the first domain.

Clone 6P229, the mouse cDNA related to glutathione peroxidase and *btuE* have either a TGT or a TGC codon for the active site cysteine (indicated by an arrow in Fig. 1B). However, all animal glutathione peroxidases use the termination codon TGA for the selenocysteine (SeC) [3]. This might indicate that 6P229 might not be a selenium-dependent glutathione peroxidase. As mentioned above, it might also be true for the mouse cDNA. Moreover, there is no glutathione peroxidase activity in *E. coli* and the function of the *btuE* gene is not known at the moment [13]. Interestingly, some amino acids are conserved between 6P229 and the mouse cDNA related to glutathione peroxidase and/or the *E. coli btuE* polypeptide. However, both selenium-dependent and -independent glutathione peroxidase activities have been described in algae [12] and in higher plants [4]. But no partial protein sequence of these activities is available.

Southern analysis indicated that the 6P229 gene was present in a small number of copies in the genome of *N. sylvestris* (Fig. 2). Indeed after restriction with *Eco* RI, *Eco* RV, *Hin*d II and *Hin*d III, only two to three bands appeared. It should be noted that there is one *Eco* RI site and one *Hin*d II site in the 6P229 cDNA. It suggested that there were at most two copies of 6P229 per haploid genome.

The expression of 6P229 was analysed in protoplasts and protoplast-derived cultures, in various tissues as well as after different stresses (Fig. 3). It was shown to be expressed very early

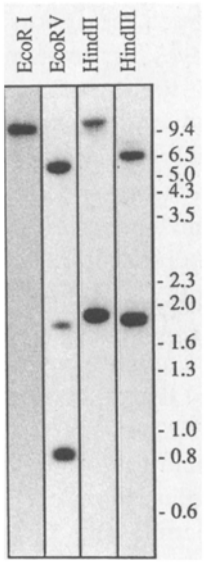

Fig. 2. Genomic Southern blots of *Nicotiana sylvestris*. DNA (5 μg per lane) was digested with *Eco* RI, *Eco* RV, *Hin*d II and *Hin*d III. The nylon membrane (Amersham) was hybridized to radiolabelled 6P229. The position of size markers (kb) is indicated. All procedure was performed according to Jamet *et al.* [8].

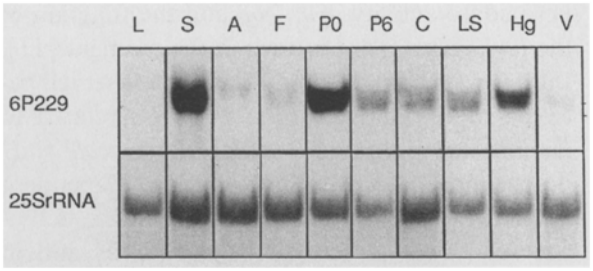

Fig. 3. Expression of 6P229 in different tissues, in mesophyll protoplasts and after various stresses. L, leaf; S, germinating seeds; A, apex; F, flowers; P0, 0-h-old protoplasts; P6, 6-h-old protoplasts; C, 7-day-old cell suspension cultures; LS, 24-h-old leaf strips; Hg, leaves harvested 6 h after $HgCl_2$ treatment; V, leaves harvested 72 h after GTAMV infection. 5 μg total RNAs per sample were electrophoresed in an agarose-formaldehyde gel and subsequently transferred to a nylon membrane (Amersham). Hybridization was performed using radiolabelled 6P229 as probe. Hybridization of the same membrane to a probe specific for 25SrRNA is given as a control to check the integrity of RNAs. All procedures were according to Jamet *et al.* [9].

after protoplast isolation following an overnight digestion (see [9] for protoplast preparation technique). The level of expression decreased and the expression was maintained in protoplast-derived cultures at a low level. It was not found to be expressed in leaves, nor in stems and roots (data not shown), but was expressed at a high level in germinating seeds and at a lower level in apex and in flowers. Different stresses have also been tested: the immersion of leaf strips in the protoplast isolation medium for 24 h to mimic the stress undergone by protoplasts during isolation, the vaporisation of a 0.1% $HgCl_2$ solution on leaves, the inoculation of leaves with local lesion forming GTAMV (green tomato atypical mosaic virus) [15]. All caused the expression of 6P229.

Sequence comparisons indicated that the 6P229 cDNA had four domains of high homologies to animal glutathione peroxidases and to the *E. coli btuE* periplasmic protein of unknown function. Its induction in specific tissues, in protoplasts and in response to various stresses was consistent with a key role in defense against oxidative stresses as described for the superoxide dismutase gene [2]. 6P229 is thus a good candidate to encode a plant glutathione peroxidase.

Acknowledgements

We wish to thank Dr Logemann for the gift of the 25S rRNA probe and Prof Dufaure for critical reading of the manuscript. This work was supported by the C.N.R.S. and by a grant from Ministère de la Recherche et de la Technologie to M.-C.C.

References

1. Akasaka M, Mizoguchi J, Yoshimura S, Watanebe K: Nucleotide sequence of a cDNA for rabbit glutathione peroxidase. Nucleic Acids Res 17: 2136 (1989).
2. Bowler C, Alliotte T, De Loose M, Van Montagu M, Inzé D: The induction of manganese superoxide dismutase in response to stress in *Nicotiana plumbaginifolia*. EMBO J 8: 31–38 (1989).
3. Chambers I, Frampton J, Goldfarb P, Affara N, McBain W, Harrison PR: The structure of the mouse glutathione peroxidase gene: the selenocysteine in the active site is encoded by the 'termination' codon, TGA. EMBO J 5:1221–1227 (1986).
4. Drotar A, Phelps P, Fall R: Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci 42: 35–40 (1985).
5. Friedrich MJ, De Veaux LC, Kadner RJ: Nucleotide sequence of the *btuCED* genes involved in vitamin B_{12} transport in *Escherichia coli* and homology with components of periplasmic-binding protein-dependent transport systems. J Bact 167: 928–934 (1986).
6. Ghyselinck NB, Dufaure JP: A mouse cDNA sequence for epididymal androgen-regulated proteins related to glutathione peroxidase. Nucl Acids Res 18: 7144 (1990).
7. Gunzler WA, Steffens GJ, Grossman A, Kim SMA, Otting F, Wendel A, Flohe L: The amino-acid sequence of bovine glutathione peroxidase. Hoppe-Seyler's Z Physiol Chem 365: 195–212 (1984).
8. Jamet E, Durr A, Fleck J: Absence of truncated genes in the amphidiploid *Nicotiana tabacum*. Gene 59: 213–221 (1987).
9. Jamet E, Parmentier Y, Durr A, Criqui M-C, Fleck J: Is ubiquitin involved in the dedifferentiation of higher plant cells? Cell Diff Dev 29: 37–46 (1990).
10. Lamattina L, Weil JH, Grienenberger JM: RNA editing at a splicing site of NADH dehydrogenase subunit IV gene transcript wheat mitochondria. FEBS Let 258: 79–83 (1989).
11. Mullenbach GT, Tabrizi A, Irvine BD, Bell GI, Hallewell RA: Sequence of a cDNA coding for human glutathione peroxidase confirms TGA encodes active site selenocysteine. Nucl Acids Res 15: 5484 (1987).
12. Overbaugh JM, Fall R: Characterization of a selenium-

independent glutathione peroxidase from *Euglena gracilis*. Plant Physiol 77: 437–442 (1985).
13. Rioux C, Kadner RJ: Vitamin B_{12} transport in *Escherichia coli* K12 does not require the *btuE* gene of the *btuCED* operon. Mol Gen Genet 217: 301–308 (1989).
14. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467 (1977).
15. Van Regenmortel MHV: Tobamoviruses. In: Kurstak E (ed) Handbook of Plant Virus Infections and Comparative Diagnosis. Elsevier/North Holland Medical Press, Amsterdam (1981).
16. Yoshimura S, Takekoshi S, Watanebe K, Fujii-Kuriyama Y: Determination of nucleotide sequence of cDNA coding rat glutathione peroxidase. Nucl Acids Res 17: 2136 (1989).