

Molecular cloning and sequencing of the cDNA encoding plant nuclear matrix endonuclease*

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We have found recently that the level of the 32 kDa endonuclease in the White bush seedlings (*Cucurbita pepo* var. *patissonina*) increases when cell growth is phytohormone stimulated [1]. The enzyme in such cells is associated with the nuclear matrix *via* the 65 kDa polypeptide; it is able to cleave only a single strand in double stranded DNA, the reaction being dependent on DNA structure. Antibodies against the 32 kDa endonuclease were found to inhibit DNA synthesis *in vitro* and recently it was evidenced that the inhibition occurs at the initiation step [2]. This suggests that the 32 kDa endonuclease is involved in the cell cycle regulation and plays some role in cell ageing and death [3]. To investigate in more detail the physiological significance of the 32 kDa protein we have isolated and sequenced the cDNA clone encoding this endonuclease.

During the course of immunoscreening of our lambda-ZAP II expression library derived from *Cucurbita* mRNA using an antibody against the 32 kDa endonuclease, cDNA clone A215 was isolated. For further investigation, the clone was expressed under β -galactosidase promoter control in *E. coli* DH5 α cells, in the presence of 5 mM isopropyl- β -D-thiogalactopyranoside, and the product, when analysed on DNA/polyacrylamide gel [4] showed nuclease activity. This positive clone was subcloned into pBlue-script II SK and both DNA strands were sequenced (Fig. 1). The cDNA sequence has been

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1  GGCAGCGAAGTACGCGACAGCGCTTGGTCTCTCCGCGAATGATGCGCCCT
58  CTGAAAGCAGATAGATCTAAAGCGTCTCCCTCTGCTGCGA   ATG GCG GGT GCG
                                         M A C R 4
118  TCT TCC GCT GCG GAG GAG TTT GAT TAC CTT GCT AAG CTT GCG GAG
S S P R R E E Y V Y L A K L A K 19
156  CAG GCG GAG GCG TAC GAG GAG ATG GAT TTT ATG GAA AAG GCG
Q A K R Y F E E N V E P H E E V 34
201  TCC GCG GCG GTA GAG AAG GAG GAG CTT ACG GCG GAG GAG GCG AAG
S A A V D M E E L T V E E R H 49
246  CTT CTC TCT GAT GCT TAC AAG AAT GTT AAT GGA GCG GGT AGA GCT
L L S V A V E M Y I G A R R A 61
301  TCC TCG AAG AAT AAT TCC TCC AAT GAG GAG AAG GAG GAG ACG GGA
S W W T I T S I E Q E E E S R 73
336  GCG AAG GAT GAT CAT GTC TCG ATC ATC GGA GAT TAC AGA GCG AAG
G W D D H V S I I R D Y R S K 91
391  AAT GAG AAT GAG GCG TCT AAG ATC TCG GGA TTC GAG ATC CTT AAT
I E T E L S R I C G F R I L W 109
434  CTT CTT GAC TCG GCG CTT AAT GCG TCC GCT GGT TCC GGA GAG TCC
L L D S R L I P S A A S G D S 124
481  AAG GTC TTT TAT CTC AAA ATG AAG GCG GAT TAT CAT AGA TAC CAG
K V F Y L E M E G D V H R Y L 139
526  GCT GAA TCC AAG ACC GGA GCG GAA GGA AAG GAA GCT GCG GAA AGT
A C P E T D A E R E A R E S 154
571  ACG CTC AAT GCT TAT AAA TCT GCT GAG GAT AAT GCA AAT GCT GAA
Y L T A V E S A Q D I A M A K 169
616  CTT CTT GCT ACT CAC GCG ATA GSA TTT GCG CAG GGT TTC AAG TTC
L P F T W P I R L G L A L H F 184
661  TCA GTC TTC TAC TAT GAG AAT CAG AAT TCC GCT GAT GCT GCT TCC
S V F Y Y E I L M S P D R A C 199
706  ACG GCT GCT AAA GAG GCT TTC GAG GAG GCA AAT GCT GAA TTC GAT
S L A E Q A F D E A I A E L D 214
751  AAT CAG GCG GAA GAA TCA TAC AAA GAT ACG AAT TTC ATC ATG GAA
Y L C K E S Y R D S T L I N D 229
796  CTT CTT GCT GAC AAG CTC ACC CTA TGG ACT TGG GAG ATC AGA GGT
L L R R M L T L W T S D I R G 244
841  AAT AGC GAG GAG ATC AAA GAA GGA GCT TCC AAA CAG GAT TCT AGT
S R D D I K E A A P E R D C T 259
886  AAT AGT AAT GAG GAA ATC GAT TTC CAG GCG GAG GAT TGA APT 101
H S S E Q I D L H V D D S T S 274
931  GCT TAACTGCTTATGCTGAGGAGTGGCTGCTTGGAGCTTGGCTTGGCTTGGCTTGG
P
989  TATGCGAGCTATCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGG
1046  CTGCTGCTGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGG
1103  GAGTCTCTGGAATGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGGCTTGG
1160  AAAAAAANAAGCTCA

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Fig. 1. The sequence of cDNA encoding the 32 kDa endonuclease.

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deposited in the EMBL/GenBank under accession number X76086.

A search through the EMBL database revealed that the A215 clone was highly homologous (65%) to the 14-3-3 protein from human and bovine brain [5]. The 14-3-3 protein functions as a protein kinase dependent activator of the neurotransmitter synthesis pathway and as an inhibitor of protein kinase C. We report for the first time the presence in *Cucurbita* cells of an endonuclease homologous to the 14-3-3 protein from human and bovine brain.

Using an endonuclease probe consisting of the 1.2 kb *Xba*I-*Xho*I fragment from the coding region we found that under stringent wash condition a single message could be identified at 1.6 kb (Fig. 2). Figure 2 shows that endonuclease mRNA was predominantly synthesized in cotyledons (lane 5) and seedlings (lane 6). The roots (lanes 1, 2) and stems (lanes 3, 4) showed only trace amounts of 1.6 kb mRNA.

The presence of endonuclease mRNA was detected in potato plants as well. Amongst several organs and tissues analysed (leaf, node, internode, root, tuber) young potato tuber (lane 7) and mother tuber (lane 8) showed the highest nuclease mRNA content.

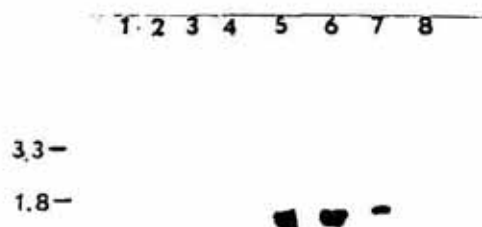


Fig. 2. Northern blot analysis of *Cucurbita pepo* RNA.

Total RNA (50 µg) isolated from roots (1, 2), stems (3, 4), seedlings (5) or cotyledons (6) was fractionated by agarose gel electrophoresis under denaturing conditions. Hybridizations were performed using ³²P-labelled A215 cDNA. Positions of RNA standards (kb) are indicated on the left side. Lanes 7 and 8 represent RNA isolated from potato Desiree young and mother tubers, respectively.

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