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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* DH5α 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 pBluescript II SK and both DNA strands were sequenced (Fig. 1). The cDNA sequence has been

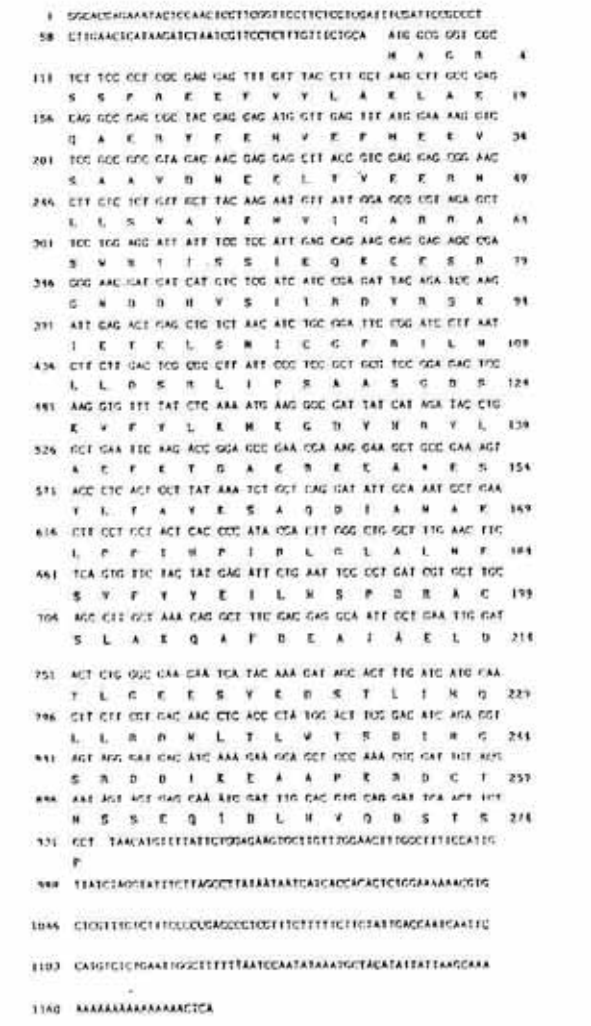

Fig. 1. The sequence of cDNA encoding the 32 kDa endonuclease.

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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 ^{32}P-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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