

Molecular cloning and sequencing of the cDNA encoding plant 22 kDa nuclease*

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Nucleases are the crucial enzymes in the metabolism of nucleic acids. They also take part in the programmed cell death (apoptosis). It has been suggested that at least two nucleases of molecular mass of 18 kDa and 30 kDa are involved in the apoptotic cascade. The reported correlation between occurrence of the 18 kDa nuclease and DNA degradation made this protein a candidate for the apoptotic nuclease [1, 2]. All data accumulated so far concern human and animal enzymes, nothing being known about their plant counterparts.

We have found recently, in *Cucurbita pepo* seeds a nuclease of low molecular mass 22 kDa. The purified enzyme gives on polyacrylamide gel a single band indicative of its homogeneity.

The enzyme exhibited 1.5 fold higher activity towards denatured DNA as compared with that towards native DNA, and was inactive towards RNA. It digested DNA to 3'-P-nucleotides, and preferentially cleaved the A-T inter-nucleotide bonds.

The enzyme was activated by divalent cations like Mg^{2+} (3.0 mM), Ca^{2+} (5.0 mM), and Mn^{2+} (0.5 mM), while EDTA (50 mM) caused complete loss of enzyme activity.

We have isolated polyclonal antibodies against the 22 kDa nuclease. In the course of screening our lambda-ZAP II expression library, derived from *Cucurbita* mRNA with antibodies against the 22 kDa endonuclease, a cDNA clone (900 bp), r4, was isolated. For further studies the clone was expressed in *E. coli*

DH5 α cells under β -galactosidase promoter control in the presence of 5 mM isopropyl- β -D-thiogalactopyranoside (IPTG). The product was analysed on DNA-acrylamide gel [3] and showed nucleolytic activity (Fig. 1). The two bands (lane 1) resulted from initiation of protein synthesis at the β -galactosidase ATG site (fusion protein, upper band) and at the same sequence inside the cDNA open reading frame.



Fig. 1. Electrophoresis of *E. coli* cell extract on DNA-polyacrylamide gel. The bands appeared as a result of DNA degradation.

Lane 1, nonstimulated cells; lane 2, cells stimulated with 5 mM IPTG.

This positive clone was subcloned into pBlue-script II SK vector and both strands were sequenced. The cDNA insert proved to contain two restriction sites for *Asp*718 and *Eco*R1.

A search of the EMBL database showed that the r4 clone was highly homologous to the biotin binding proteins.

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Using an endonuclease probe consisting of 700 bp *EcoRI-XhoI* fragment from the coding region, we found that under stringent wash condition the single message was identified at 1.2 kb.

Treating the genomic DNA with the restriction enzymes *XbaI-KpnI-XhoI* we found in Southern blotting the shortest DNA fragment of about 1.4 kb specifically recognized by the nuclease cDNA. This fragment represents probably the entire gene for the 22 kDa nuclease (Fig. 2).

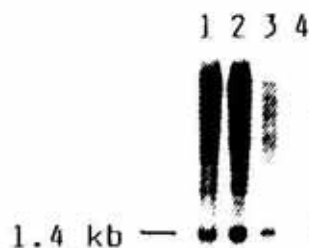


Fig. 2. Southern blot analysis of genomic DNA (10 µg) isolated from leaves (lane 1), cotyledons (lane 2), stems (lane 3) and roots (lane 4) of *Cucurbita pepo*. DNA was treated with *XbaI-KpnI* restriction enzymes. The blot was probed with the *EcoRI-XhoI* nuclease cDNA fragment labelled (multiprime reaction) with [³²P]-dCTP.

To our best knowledge, this is the first report about cloning and sequencing of a low molecular mass nuclease from plant cell.

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