

Nucleotide sequence of the promoter region from kiwifruit actinidin genes

Jeannette Keeling, Peter Maxwell and Richard C. Gardner*

Centre for Gene Technology, Department of Cellular and Molecular Biology, University of Auckland, Private Bag, Auckland, New Zealand (*author for correspondence)

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We are interested in the tissue and developmental specificity of actinidin, a cysteine protease that is highly expressed in fruit of *Actinidia deliciosa* (kiwifruit). The protein is expressed from a multi-gene family [2]. Initial screening of a genomic library has revealed a large and diverse family of actinidin-related genes; the first four of these characterised are probably all pseudogenes (P. Maxwell, unpublished data). We wish to isolate the promoter region from the actinidin gene(s) that are the most highly expressed [1, 2].

Inverse polymerase chain reaction (IPCR) provides a method of cloning segments of chromosome adjacent to areas for which sequence data are available. The protocol uses two outward-facing primers to amplify sections of DNA after circularisation of restriction fragments by ligation [3]. We describe here the results of an experiment using high-stringency IPCR to isolate promoter regions from actinidin genes.

In trial reactions to optimise the stringency of PCR, 65 °C was the highest annealing temperature that gave good amplification of an actinidin cDNA clone using oligonucleotides oAc1 and oAc6 (see Fig. 1 for location on the actinidin gene). Amplification of chromosomal DNA at this temperature produced a single band rather than the smear obtained when 50 °C annealing was employed (data not shown). For the IPCR, two oligonucleotides, oAc1 and oAc12 (see Fig. 1), were used as primers on 1 µg of chromo-

somal DNA from the 'Hayward' cultivar of kiwifruit that had been cut with a variety of restriction enzymes and circularised by ligation at 1 µg/ml. The primers gave rise to a 650 bp fragment when *Msp*I and *Taq*I were used together. A product was also obtained with *Sau* 3A digestion, but its size suggested that it contained only a small region upstream of the gene. No products were obtained with *Msp*I alone or with four other enzymes used.

The 650 bp fragment was cloned into *Sma*-cut Bluescribe; six isolates were sequenced completely, and two partially. Two of the clones had identical sequences; one of these (pKIWI37) is shown in Fig. 2. Several features of the sequence deserve comment. First, the oligonucleotide sequences and the *Msp*/*Taq* junction are present at the expected locations. Second, the sequence of the 93 nucleotide transcribed region (excluding oligos) corresponds precisely with the nucleotide

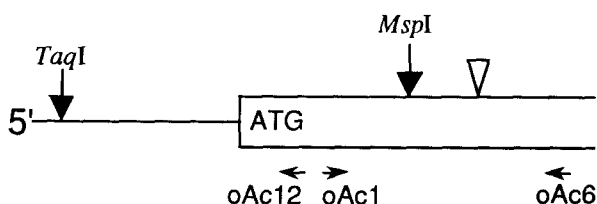


Fig. 1. Location of primers and relevant restriction sites on the actinidin gene. The open triangle indicates the presence of two introns between the *Msp*I site and oligo oAc6 (P. Maxwell, unpublished data).

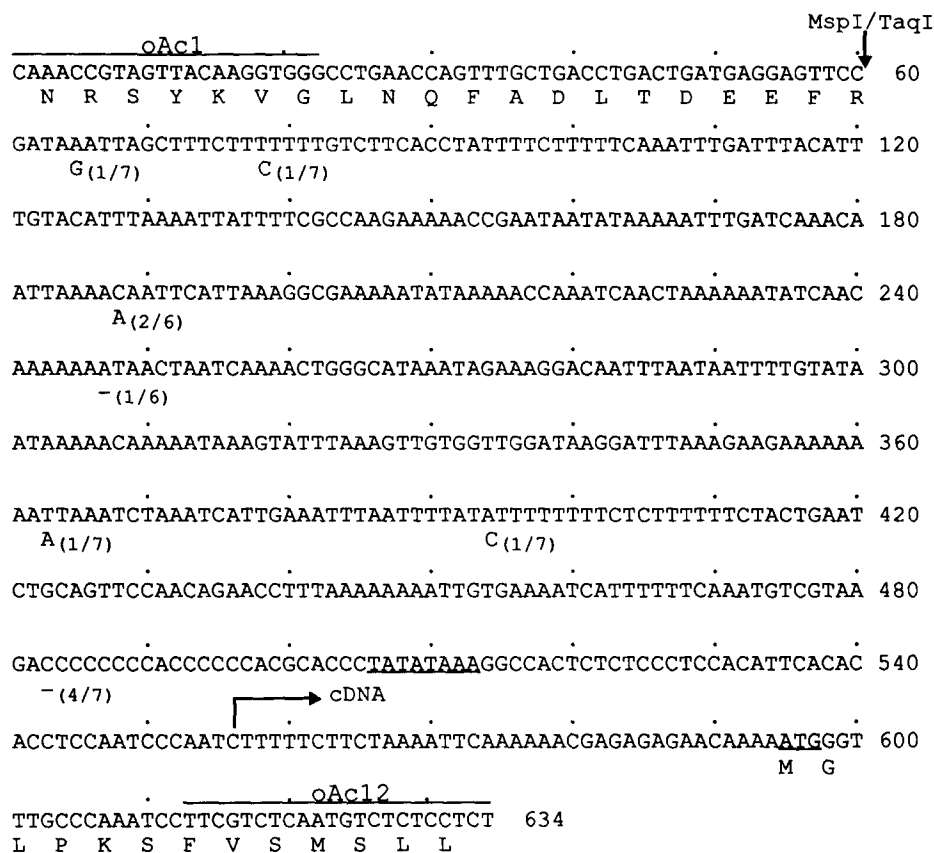


Fig. 2. Nucleotide sequence of pKIWI37. The locations of the oligos, the *MspI*/*TaqI* junction site, and the 5' end of the cDNA sequence are shown above the sequence. The probable initiation codon and a possible TATA box are underlined. Variations in the sequence of the other clones are indicated below the sequence, with the fraction of clones containing the particular variant shown in brackets. Nucleotides 1–60 in the pKIWI37 sequence correspond to nucleotides 276–335 (amino acids 81–100) in the cDNA sequence [1], while nucleotides 558–633 correspond to nucleotides 1–76 (amino acids 1–13).

sequence of two full-length cDNA clones [1]. Third, the sequence contains 497 bp of new sequence upstream of the 5' end of the longest cDNA clone. Fourth, variants of the sequence were found. Some variants may represent errors generated during the 50 cycles of PCR, while others may have been derived from different genomic copies of actinidin. In particular, the presence of a C at nucleotide 483 in three clones that is absent in another four, suggests that PCR products were derived from at least two different genes. Finally, the sequences are all highly AT-rich in the upstream regions and contain a possible TATA box 50 bp upstream of the end of the cDNA sequence. Work is currently in progress to determine whether these putative promoters are functional.

Acknowledgements

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