Structure and expression of \textit{kin2}, one of two cold- and ABA-induced genes of \textit{Arabidopsis thaliana}

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Abstract

We report the isolation of the second member, \textit{kin2}, of a family of two cold-inducible genes of \textit{Arabidopsis thaliana}. The proteins corresponding to the two genes have similarities to the small antifreeze proteins from Winter flounder. \textit{Kin1} and \textit{kin2} are organized in a close tandem array in the genome of \textit{A. thaliana}. Both have three exons separated by introns with approximately the same length and location. The coding regions are highly conserved while the introns and especially the 3' flanking sequences of the mRNAs have diverged. The \textit{kin1} and \textit{kin2} genes are coordinately regulated in the cold. Unlike \textit{kin1}, the \textit{kin2} mRNA has a detectable basal level, and accumulates to a higher level during acclimation. Both mRNAs are induced by 10 $\mu$M ABA but only \textit{kin2} responds strongly to drought and salinity stresses.

We previously described the isolation of the genomic clone containing the cold inducible gene \textit{kin1} from \textit{Arabidopsis thaliana} L. Heynh, strain Colombia [9]. The corresponding cDNA was isolated using the genomic clone as a probe. Of the 21 cDNA clones hybridizing to this probe fourteen were homologous to \textit{kin1} [9] and seven corresponded to another gene, designated \textit{kin2}. Analysis of the sequence of the \textit{kin1} genomic clone suggested that its 3' end contained the beginning of the first exon of \textit{kin2}. In order to find the rest of the \textit{kin2} gene, another genomic library was constructed in lambda EMBL3 (Promega) and screened with the 1.8 kb Eco RI fragment containing the \textit{kin1} gene [9] as a probe. Two recombinant phage were shown to contain sequences corresponding to the \textit{kin2} cDNA by restriction enzyme mapping and hybridization analysis. The longer of the two new clones, extending over both the 5' and 3' ends of the original genomic clone was chosen for further studies. Based on restriction and hybridization analyses of subclones of

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The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X62281.
this genomic clone we concluded that a 2.7 kb Hind III fragment extending 885 bp over the 3' end of the original clone contained the missing 3' region of the kin2 gene.

The nucleotide sequences of the kin2 cDNA and the corresponding genomic clone were determined and compared to the kin1 sequences (Fig. 1a). Like in the kin1 gene, the open reading frame of kin2 is composed of three exons separated by introns. The length of the coding regions and the intron positions are identical to those of kin1. The sequence of the coding region is 95% homologous to that of kin1, while the homologies between the introns are 80% and 65% respectively. In addition, the 3' non-coding regions have diverged significantly. The first intron of the kin2 gene is 12 bp shorter than that of the kin1 gene while the second kin2 intron is 7 bp longer than the corresponding kin1 intron. The exon-intron border sequences are typical of those found in other plant genes [2].

The transcription start sites were identified by primer extension [9]. In both genes, the transcription initiates 60 bp upstream of the translation initiation site (data not shown) and is preceded by putative TATA boxes. Unlike in the kin1 gene, the transcription initiation site of kin2 does not completely match the published consensus sequences for plants (CTCATCA) [7]. The 3' untranslated region of the kin2 gene is longer than that of the kin1 gene. Like the cDNA clones corresponding to kin1, which are very heterogenous at their 3' ends, the kin2 cDNAs also have two polyadenylation sites. Six of the kin2 cDNA clones are polyadenylated at position 785 and one at position 800 (Fig. 1a). This is in accordance with the observation that many plant genes are polyadenylated at multiple sites [4]. The putative polyadenylation signal has one mismatch compared to the eukaryotic consensus AATAAA. Still, it fits within the location, 29 ± 7 bp upstream from the polyadenylation site, suggested for plant genes [8].

The alignment of the deduced amino acid sequences of Kin1 and Kin2 is shown in Fig. 1b. Both sequences are 66 amino acids long, corresponding to polypeptides of 6.5 kDa which is in agreement with the results of in vitro translation of kin1-selected mRNA [9]. They bear 91% homology. The polypeptides are rich in alanine, glycine and lysine. They both contain fifteen alanine and nine lysine residues while Kin1 has nine and Kin2 seven glycine residues. Five of the six altered

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**Fig. 1a. Alignment of the nucleotide sequences of kin1 [9] and kin2.** Identical nucleotides are indicated by dots. The transcriptional start sites are indicated by an arrow and the putative TATA box and polyadenylation signals are marked with bold lines. The polyadenylation sites found in different kin2 cDNA clones are marked with horizontal arrows. Kin1 has seven polyadenylation sites [9]. The coding regions are boxed. The sequences corresponding to the gene-specific probes are underlined.

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**Fig. 1b. Homology between the deduced amino acid sequences of the Kin1 [9] and Kin2 polypeptides.** Identical amino acids are marked with dots.