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Plant Gene Register

Cloning and Sequencing of the cDNA for *cas17*, a Cold Acclimation-Specific Gene of Alfalfa[1]

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We have previously reported on two cold acclimation-specific (*cas*) genes from alfalfa, *cas18*, coding for a dehydrin-related protein (Wolfraim et al., 1993), and *cas15*, coding for a putative nucleus-targeted protein (Monroy et al., 1993).

We report here the cloning and sequencing of a new cDNA clone, pAcs784A, corresponding to a gene, *cas17* (*cas* gene coding for a 17-kD protein), that is induced by cold. Two-week-old seedlings of a freezing-tolerant cultivar of alfalfa (*Medicago sativa* L. ssp. *falcata* cv Anik) were cold acclimated at 5°C for 1 week. A λ Uni-Zap cDNA library was constructed using poly(A)$^+$ RNA isolated from these cold-acclimated seedlings. The library was screened by in situ plaque hybridization using a partial-length cDNA clone (pSM784) that has been previously shown to correspond to a cold-inducible gene family (Mohapatra et al., 1989). The cDNA clone described here, pAcs784A, represents the gene *cas17*, which shows extensive homology with *cas18* (Wolfraim et al., 1993) and belongs to the same gene family.

The characteristic features of the cDNA clone pAcs784A are described in Table I. It is 809 bp long, contains an open reading frame of 480 bp, and includes a putative polyadenylation signal. It can encode a polypeptide (CAS17) of 160 amino acid residues with a predicted molecular mass of 16.7 kD. CAS17 is rich in Gly and Thr, which together constitute 42 mol % of the protein. Of the 160 amino acid residues of the CAS17 sequence, 102 (64%) match those of CAS18 reported earlier (Wolfraim et al., 1993). CAS17 also shows high sequence homology to members of the Group 2 LEA/dehydrin/RAB family of proteins. Two similar motifs common to CAS18 and members of the Group 2 LEA/dehydrin/RAB proteins also occur in CAS17: DKIKEKIPG and GEKKGIMEKIKEKIPG.

As was the case with CAS18 (Wolfraim et al., 1993), there are regions of extensive $(Gly\text{-}Thr)_n$ repeats, where n is greater than 2. One such repeat consists of 10 consecutive Gly-Thr pairs. Such $(Gly\text{-}Thr)_n$ repeats are characteristic of clock proteins of other organisms (Li-Weber et al., 1987). Circadian regulation of plant chilling resistance has been demonstrated (Rikin, 1991), and low temperature has been shown to interrupt circadian regulation of transcriptional activity in chilling-sensitive plants (Martino-Catt and Ort, 1992). It is possible that in chilling- and freezing-resistant plants, new isoforms of "clock" proteins are synthesized to reestablish the circadian rhythm of transcriptional activity. CAS17 also shows considerable homology to the Group 2 LEA/dehydrin/RAB proteins. The latter accumulate in response to drought stress and are believed to protect the plant by solvating cellular structures, in part through the action of the hydroxyl groups of such hydroxylated amino acids as Thr (Baker et al., 1988). We speculate that the hydroxyl groups of Thr, abundant in CAS17, may serve a similar function during freezing-induced dehydration.

Table I. *Characterization of cDNA clone pAcs784A from alfalfa*

Organism:	*Medicago sativa* L. ssp. *falcata* cv Anik.
Gene, Gene Product:	cDNA encoding a Gly- and Thr-rich protein.
Location on Chromosome:	Multigene family of unknown location.
Function:	Encodes a putative desiccation protectant.
Expression:	Reversible induction in response to low temperature.
Techniques:	A cDNA library was constructed in λ Uni-ZapII XR (Stratagene) with poly(A)$^+$ RNA from seedlings cold acclimated for 7 d. The library was screened with a radiolabeled insert from clone pSM784 (Mohapatra et al., 1989). Other techniques included dideoxy sequencing (of both strands) on double-stranded DNA using synthetic oligonucleotide primers, and computer analysis of sequence data.
Features of cDNA Structure:	Contains an open reading frame of 480 bp.
G + C Content:	37.6%
Structural Features of the Protein:	Consists of 160 amino acid residues; predicted molecular mass 16,673 D. Rich in Gly (25.0 mol %) and Thr (16.9 mol %). Contains extensive $(Gly\text{-}Thr)_n$ repeats. Highly homologous to previously reported CAS18 and to the drought-induced proteins of the Group 2 LEA/dehydrin/RAB family.
Antibodies:	Not available.

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