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Update section

Sequence

Nucleotide sequence of a rice *rab16* homologue gene

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We report here the nucleotide sequence of a rice (*Oryza sativa* cv. Akitakomachi) cDNA, a homologue of *rab16A–D* [5, 10]. This cDNA clone, designated *rab25*, was obtained from a rice cDNA library [1] prepared from abscisic acid (ABA)-treated suspension calli, by a simple subtractive method (K. Aguan *et al.*, in preparation). The rice embryonic callus, originating from the scutellum, was cultured as described previously [4]. To eliminate any effect of light, the cultured cells were placed under continuous dim (200 lux) light. *rab25* is 920 bp long and contains an open-reading frame of 687 bp, which is translated into 228 amino acids corresponding to a protein of M_r 24722 (Fig. 1). At the nucleotide sequence level, *rab25* shows 63.5%, 60.2%, 59.7% and 51.0% homology with barley dehydrin 9 [2], rice *rab16* [5, 10], maize *rab17* [8] and barley ABA-induced gene (pHVA1-1) [3], respectively. Transcription of *rab25* in rice calli was readily induced by either ABA (10 μM) treatment or high osmotic (0.5 M mannitol) stress (Fig. 2, lanes 3–6), as already reported by others [5, 10], but was rather repressed at low temperature even after 24 h of incubation compared with the control (25 °C) (Fig. 2, lanes 1 and 2). Time-course analysis showed that transcription of *rab25* was not induced at an earlier stage of low-temperature treatment (data not shown). This suggested that the endogenous ABA content of rice calli was not increased at a level high enough to induce the expression of the *rab* gene under the present conditions. To reach such a conclusion, however, a direct measurement of endogenous ABA content in rice calli after low-temperature treatment is required. The size of the *rab25* transcript was estimated to be about 1.1 kb using 25S rRNA (3.4 kb) and 17S rRNA (1.8 kb) as markers. The transcriptional start site of the gene was determined by a primer extension using the gene-specific 27-mer (nucleotide positions 87 to 61 in Fig. 1.) *rab25* lacks 26 nucleotides at the 5′ end (data not shown). Thus the 5′ non-coding region of this gene is 86 bp long, and it is in good agreement with those (80 to 141 bp) of *rab16A–D* [10]. Rab25 protein product showed high amino acid sequence homology with Rab16A–D, Rab 17 and Dehydrin 9, and the proteins also shared three characteristics: two repeated 14-mer sequences (KKSIKEKIKEKLPG), a series of 7 to 9 serine residues, and a histidine residue at the carboxyl terminus. The protein product of the ABA-induced gene (pHVA1-1) in barley [3] did not show significant homology with these three Rab

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X57327.

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GGCTCAAAGAGGGTAACCGAGTTAGAAACTGAGGAGGAAGCAACTCGACGACACCAACCA   60

ATGGCGGAGCACGCCACGGGAGTGTACGGGCACCCGTACCCGCGCGTCGACCAGTACGGG  120
Met Ala Glu His Ala Thr Gly Val Tyr Gly His Pro Tyr Pro Arg Val Asp Gln Tyr Gly   20

AACCCTGTGCCGCCGGTCGACCAGTACGGCAACCCCGTCCCGGACGAGCCGGCGCCGCGC  180
Asn Pro Val Pro Pro Val Asp Gln Tyr Gly Asn Pro Val Pro Asp Glu Pro Ala Pro Arg   40

GACACGGCCGCGGGGTACGTGGCGCCGCCGGACCCCGCGGTGTCGACTGGCGACTACGGC  240
Asp Thr Ala Ala Gly Tyr Val Ala Pro Pro Asp Pro Ala Val Ser Thr Gly Asp Tyr Gly   60

CTCGCCGGCGCGGAGGCGCCGGCACCCGCACGAGAGCGCGGTGATGAGCGGCGCAGCCGG  300
Leu Ala Gly Ala Glu Ala Pro Ala Pro Ala Arg Glu Arg Gly Asp Glu Arg Arg Ser Arg   80

CGCTGCCGTCGCACCAGGAGGCGAGGCGTACACGCGCGACGGCGGCGGCGTAGTTCCCCC  360
Arg Cys Arg Arg Thr Arg Arg Arg Gly Val His Ala Arg Arg Arg Arg Arg Ser Ser Pro  100

GGCCGGCGAGAAGACGTTCGCCTACGAGGGCACGGTCAGCGCCGCCGGCTCACGGGCGCC  420
Gly Arg Arg Glu Asp Val Arg Leu Arg Gly His Gly Gln Arg Arg Arg Leu Thr Gly Ala  120

TCCGGGCAGCTCCAGCCCACCACCAGGGAGGAGGGGCACACGACGCTCGGCGAGACGTTG  480
Ser Gly Gln Leu Gln Pro Thr Thr Arg Glu Glu Gly His Thr Thr Leu Gly Glu Thr Leu  140

CGCCGCTCCGGCAAATCCAGCTCCAGCTCCAGCTCGTCGTCGGAGGATGACGGGCAAGGT  540
Arg Arg Ser Gly Lys Ser Ser Ser Ser Ser Ser Ser Ser Glu Asp Asp Gly Gln Gly  160

GGGCGGAGGAAGAAGAAGAGCATCAAGGAGAAGATAAAGGAGAAGCTTCCCGGCAGCCAC  600
Gly Arg Arg Lys Lys Lys Ser Ile Lys Glu Lys Ile Lys Glu Lys Leu Pro Gly Ser His  180

AAGCAGGAGGAGCAGAAGCAAGCTGGCCACACGGCTCCGGCGGCCGGGACGGGGACGGGG  660
Lys Gln Glu Glu Gln Lys Gln Ala Gly His Thr Ala Pro Ala Ala Gly Thr Gly Thr Gly  200

ACGGGGACGCATGCAGCGGGGAAGCACGAGAAGAAGGGCATCGTGGAGAAGATCAAGGAG  720
Thr Gly Thr His Ala Ala Gly Lys His Glu Lys Lys Gly Ile Val Glu Lys Ile Lys Glu  220

AAGCTCCCCGGCCACGGCCACCACTGAGCGAGAGCTCGCGCGCACGCACTTTCATCGGTT  780
Lys Leu Pro Gly His Gly His His *                                              228

GACGTTCGTGCAACTGTCCATGCATGTATGTATAATACCAGTCGTGTTTCAGTTCGTTAA  840

TTTTACGGTCGATGTGTGGTCTCGGTAAAACTAGGTGGTGTACAGTGCGTCTATGCATGT  900

ACGGTGTATCCAATAATCCA                                           920
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Fig. 1. Nucleotide sequence of *rab25*. The DNA and deduced amino acid sequences are numbered from the first nucleotide in the clone and from the first methionine, respectively. The asterisk indicates the termination codon. DNA sequencing of both strands was done according to the method of Sanger *et al.* [7].

proteins, although its C-terminus was still histidine.

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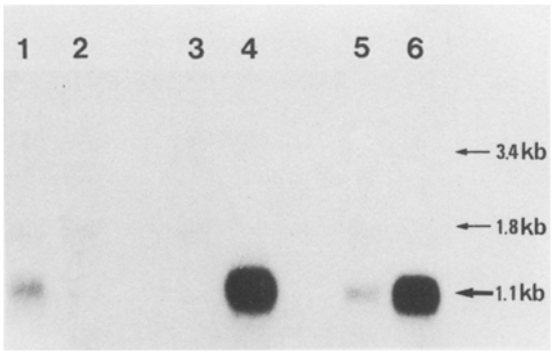

Fig. 2. Expression patterns of the *rab25* transcript in rice suspension cells. Five micrograms of poly(A)$^+$ RNA in each lane, prepared as described previously [6, 9], was fractionated on a 1.0% agarose, 6.6% formaldehyde gel and blotted onto a Hybond-N (Amersham) membrane filter. Hybridization with ^{32}P-labeled *rab25* insert DNA, and washing conditions have been described previously [6]. RNAs were prepared from (lane 1) calli grown at 25 °C for 24 h; (lane 2) calli grown at 5 °C for 24 h; (lane 3) 25 °C for 3 h; (lane 4) same as lane 3, except for addition of 10 μM abscisic acid; (lane 5) 25 °C for 3 h; (lane 6) same as lane 5, except for addition of 0.5 M mannitol. The rice suspension cells were exposed to low temperature (5 °C) as follows: at zero time, the temperature (25 °C) is down-shifted at 2 °C/h, until 9 h later the temperature reached 5 °C, the calli were then incubated for another 15 h at 5 °C.

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