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Short sequence-paper

Characterization of a cDNA encoding a rice mitochondrial voltage-dependent anion channel and its gene expression studied upon plant development and osmotic stress[1]

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Abstract

The voltage-dependent anion channel (VDAC) of mitochondria forms a large pore in the outer envelope membrane. Here, the full *Oryza sativa* OSVDAC1 cDNA was sequenced and is shown to belong to a small multigene family in the rice genome. This cDNA is 1093 bp long and codes for a protein of 274 amino acids. Expression studies of the *osvdac1* gene show a regulation of its level in function of the plantlets maturation and organs. In contrast with several bacterial porins, osmotic stress does not have any effect on the plant *osvdac1* gene expression.

Keywords: Rice; Stress; Voltage-dependent anion channel; Development

The outer membrane of mitochondria is permeable to molecules with a molecular weight up to 6 kDa due to the presence of voltage-dependent anion channel (VDACs) also called mitochondrial porins by analogy with the proteins forming pores in the outer membrane of Gram-negative bacteria [1]. Genes encoding VDAC proteins have been cloned from mammalian, fungal and plant species [2–4]. Multiple *vdac* genes have been found in human and plants indicating that the VDAC protein belongs to a multigene family [2,5,6]. All VDACs described to date have similar channel activity. However, there is little amino acids sequence conservation (less than 50%) between VDACs of plants, animals and fungi. It has been shown that permeability of the VDAC to ADP is regulated by NADH and NADPH and that the VDAC controls the release of cytochrome *c* into the cytoplasm [7,8]. This suggests that VDACs play a key role in the mitochondrial respiration and in the release of death-promoting factors in the cytosol. In addition, regulation of the *vdac* gene expression could also control the mitochondrial functions. In plants, the mitochondrial respiration is strongly affected by environmental stress conditions [9]. Very few data dealing with the regulation of genes encoding mitochondrial membrane proteins are available and the regulation of the plant *vdac* in response to environmental factors has not been investigated so far.

Here, we report on the molecular identification of a *vdac* cDNA from rice. The secondary structure and

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[1] OSVDAC1 accession number: Y18104.

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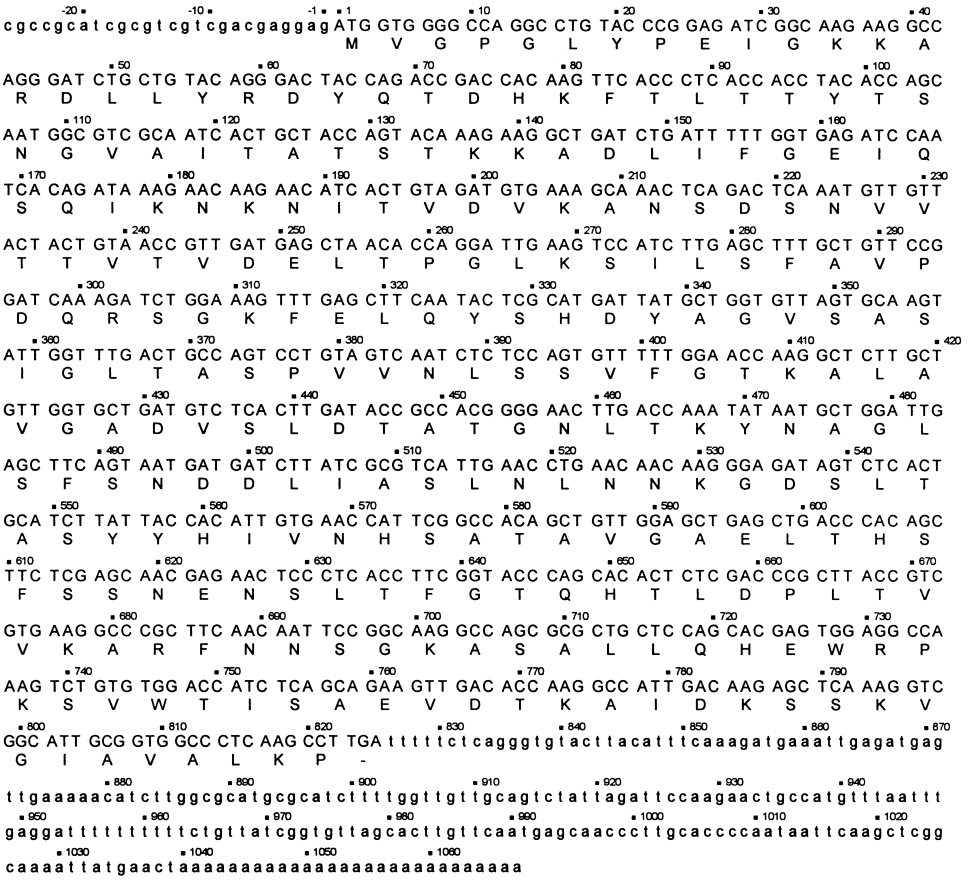

Fig. 1. Nucleotide sequence of the rice OSVDAC1 cDNA and deduced primary structure of the protein.

several amino acids sequence motifs were predicted from the primary structure of the VDAC protein. The expression and the regulation of the *vdac* gene has been investigated with respect to the plant development and in response to osmotic stresses.

The EST database of GenBank was screened for homologous known plant porins. Several ESTs corresponding to different rice VDACs were identified from NIAR/STAFF. One of these (RICC3065A) was selected for further analysis and named OSVDAC1. The full sequence of this clone was determined using the dideoxynucleotide chain-termination method with automatic laser fluorescence (Pharmacia). The cDNA insert has a size of 1093 bp and contains a minimum open reading frame of 822 nucleotides encoding a putative protein of 274 amino acids having a predicted molecular weight of 29.2 kDa (Fig. 1). The deduced amino acid sequence of the OSVDAC1 was compared with the published plant porin sequences. The highest similarity (90%) and identity (84%) were found with the wheat TAVDAC1 [6]. As shown in Fig. 2, comparisons between porin sequences of plants indicates similarities ranging from 77 to 80% and identities ranging from 64 to 69%.

Computer analysis of the OSVDAC1 amino acid sequence [10] exhibits a motif conserved in all eukaryotic porins (in His-217; [Y/H]-x(2)-D-[S/P/A]-x-[S/T/A]-x(3)-[T/A/G]-[K/R]-[L/I/V/M/F]-[D/N/S/T/A]-[D/N/S]-x(4)-[G/S/T/A/N]-[L/I/V/M/A]-x-[L/I/V/M/Y]) and a leucine zipper motif (in Leu-147; L-x(6)-L-x(6)-L-x(6)-L). Several putative sites of phosphorylation have been detected: protein kinase C ([S/T]-x-[R/K]), Ser-44, Thr-45, Ser-102, Ser-232, Ser-263; caseine kinase II ([S/T]-x(2)-[D/E]), Thr-81, Ser-164, Ser-205; tyrosine kinase ([R/K]-x(2,3)-[D/E]-x(2,3)-Y), Lys-12. Moreover, five putative glycosylation sites (N-x-[S/T]) were found, respectively, at Asn-63, Asn-129, Asn-153, Asn-190, Asn-230. They could provide regulatory sites for the gating of the channel. The hydropathy analysis of the OSVDAC1 was in-

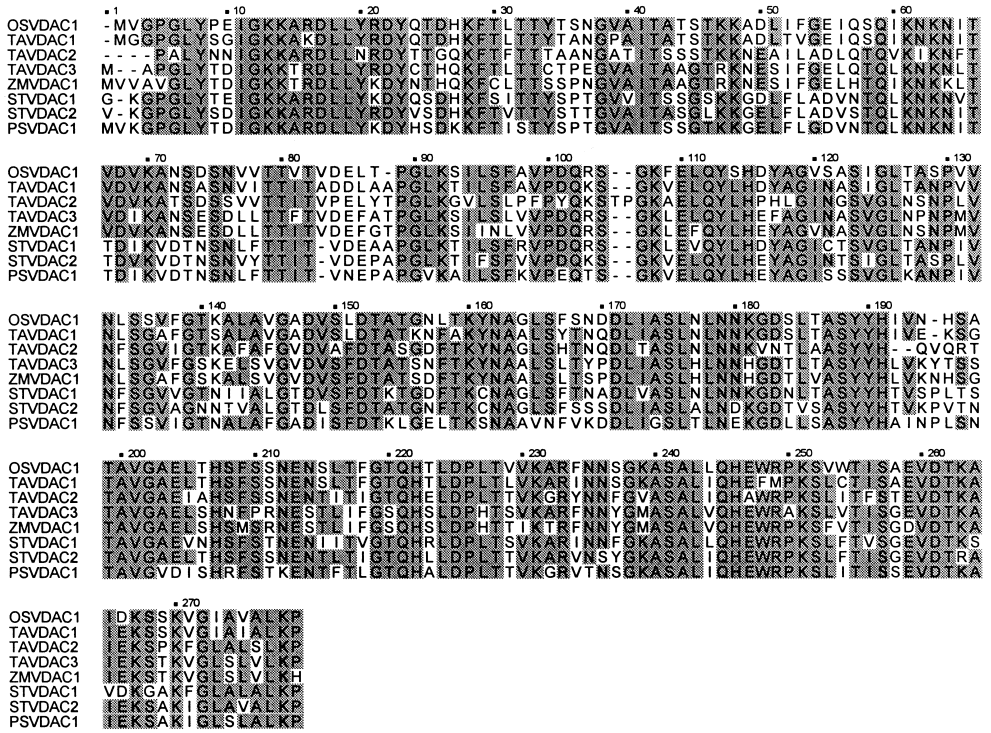

Fig. 2. Comparison of the deduced rice OSVDAC1 amino acid sequence with wheat TAVDAC1, TAVDAC2, TAVDAC3 [6], maize ZMVDAC1 [4], potato STVDAC1, STVDAC2 [5] and pea PSVDAC1 [4]. Shaded boxes indicate conserved amino acid residues. The sequences were assembled and analyzed using the GeneCompar program (Vauterin, Applied Maths, Kortrijk Belgium).

vestigated using the Vogel and Jähnig algorithm [11] to get information about the secondary structure of the protein (Fig. 3). The results suggest that the OSVDAC1 protein would have 9–11 putative transmembrane β-sheets. Plotting the amino-terminal region (from Pro-4 to Asp-21) along a helical wheel indicates that it could form an amphipathic α-helix. These predictions for the secondary structure are similar to those of other members of the VDAC family [3,6,12]. They could be essential for both structure and function of the channel. β-Sheets are assumed to form a cylindrical barrel through the outer mitochondrial membrane and the α-helix is thought to be a targeting signal for insertion of the protein into the mitochondrial outer membrane [12].

To check for the presence of a gene family encoding porins in the rice genome, the full OSVDAC1 cDNA was used as ^{32}P-labeled probe against the rice genome DNA digested with various restriction enzymes (Fig. 4). Strongly and weakly hybridized bands suggested the presence of genomic DNA fragments with, respectively, high and low homology to the OSVDAC1 cDNA. These results indicate that the OSVDAC1 gene belongs to a small multigene family in the rice genome which is consistent with the presence of different porin ESTs of rice in the database. Several VDAC isoforms have also been determined in human [2,13] and in plant cells from potato and wheat [5,6]. However, the physiological significance of mitochondrial porin isoforms in plants is not yet established. It has been suggested that the binding of hexokinase to the porin may allow a preferential access to mitochondrial ATP [14,15]. Studies of the human porin isoforms have shown that only one isoform is able to bind to hexokinase [2]. Moreover, in plants, the three wheat VDAC isoforms have specific electrophysiological properties when expressed in a *vdac*-minus yeast [16]. Thus, one can expect that the different VDAC isoforms of the rice genome have a specific function in the plant cells.

Northern blot analyses were performed to study the expression patterns of the *osvdac1* in response to plant growth and osmotic stress. Our results show that the *osvdac1* is expressed in all the plantlets

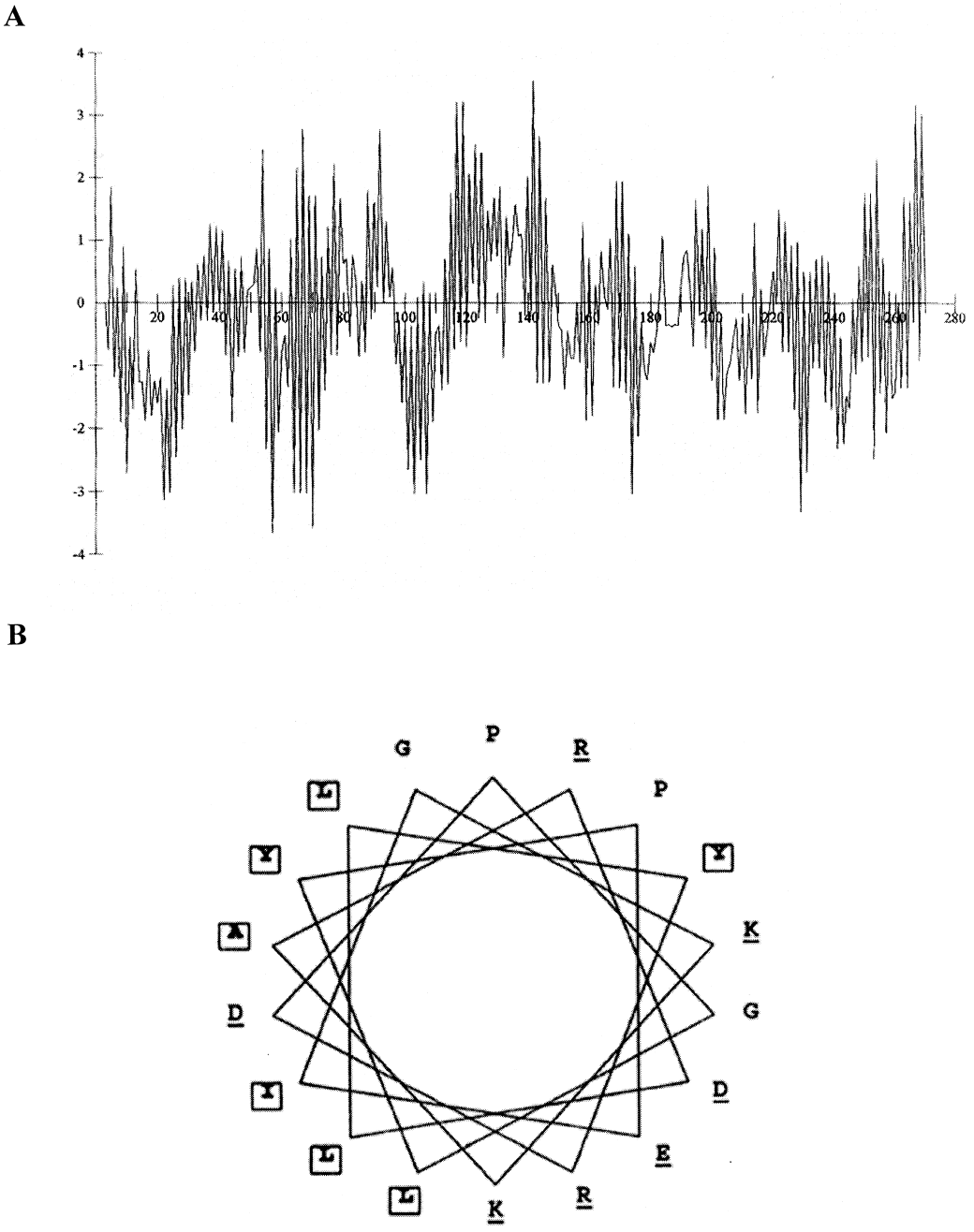

Fig. 3. Hydropathy analysis. (A) Evaluation of the potential for each stretch of amino-acids to form transmembrane β-sheets in the OSVDAC1 porin. (B) Helical prediction of the OSVDAC1 N-terminal region. Bold amino acids are hydrophobic and underlined amino acids are hydrophilic.

extracts. However, its transcript level is the highest during the first days following the germination and decreases in the later growth stages (Fig. 5A). In addition, the *osvdac1* expression level is higher in the shoots than in the roots (Fig. 5B). These different levels of *osvdac1* gene expression suggest a regulation of the amount of OSVDAC1 transcripts during the plant growth and a differential expression of the *osvdac1* gene in function of the different organs. A similar gene expression pattern influenced by plantlets maturation was observed for the three wheat *vdacs* isoforms. However, no shoot *vdac* enhanced expres-

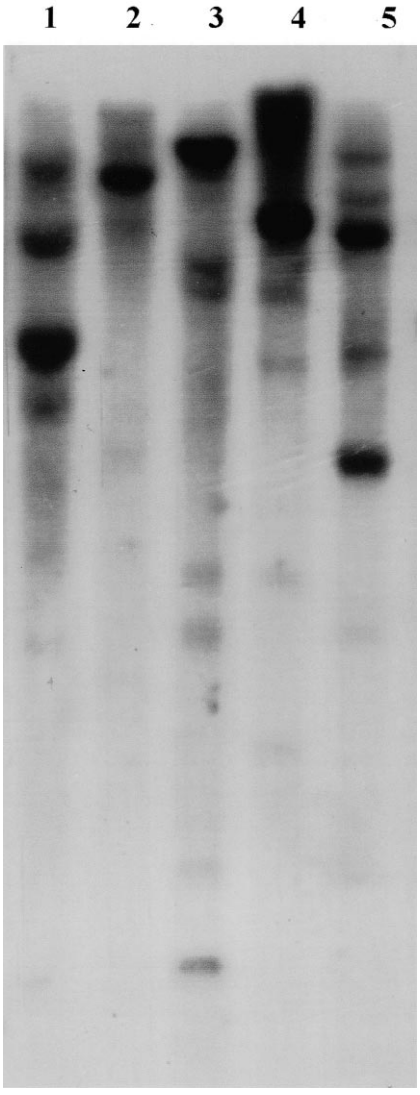

Fig. 4. Southern blot of the *osvdac1* gene. *O. sativa* DNA was digested with different restriction enzymes (lane 1, *Hin*dIII; lane 2, *Bam*HI; lane 3, *Sac*I; lane 4, *Xho*I; lane 5, *Eco*RI) and fractionated on 0.8% agarose gel before the transfer by gravity blotting onto positively charged nylon membrane. Blot was hybridized with the full-length ^{32}P-labeled OSVDAC1 cDNA at 62°C and washed in 2×SSC (0.3 M NaCl, 0.03 M $C_6H_5Na_3O_7{\cdot}2H_2O$) first at room temperature for 20 min and then at 42°C for 40 min.

sion was found in wheat [6]. The high *osvdac1* gene expression level observed in the very young plantlets could be explained by the high-energy demand for growth during the first days following the seed germination. This result is also consistent with the increase of mitochondrial biogenesis observed during the seed germination [17].

Osmotic regulation of the bacterial outer membrane porin expression is well-documented [18–20]. These studies have demonstrated that the *E. coli OmpC* porin gene is preferentially expressed in an environment of high osmolarity and *E. coli OmpF* porin gene in a medium of low osmolarity. Therefore, the effect of water stress on the *osvdac1* gene expressions was investigated on the 7-day-old plantlets. The expression of an osmotic stress induced gene, *sal*T [21], was also studied as control experiment to show that plants did effectively respond to the applied stress. As shown in Fig. 6, the *osvdac1* gene is well expressed in the 7-day-old plantlets grown on MS medium [22] and *sal*T gene is not detectable in the same conditions. In contrast, the expression of *osvdac1* gene remains very stable and that of *sal*T gene is strongly induced when the plantlets were transferred 24 h on the same medium supplemented with either 200 mM NaCl or an iso-osmotic solution of mannitol. These results indicate that, in contrast to genes encoding bacterial porins,

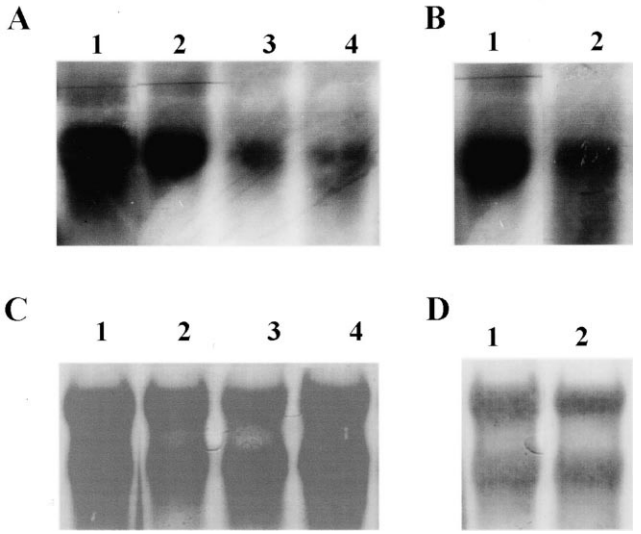

Fig. 5. (A) Total RNA extracted from rice shoots material of 1 (lane 1), 3 (lane 2), 8 (lane 3), and 11 (lane 4) days old. (B) Total RNA extracted from rice shoots (lane 1) and roots (lane 2). Samples were fractionated on 1% agarose gels that contained formaldehyde before the transfer by gravity blotting onto positively charged nylon membrane. Blot was hybridized with the full-length ^{32}P-labeled OSVDAC1 cDNA at 62°C and washed in 2×SSC (0.3 M NaCl, 0.03 M $C_6H_5Na_3O_7{\cdot}2H_2O$) first at room temperature for 20 min and then at 42°C for 40 min. (C,D) The corresponding membranes were stained with Methylene blue to verify the equal amount of transferred RNA.

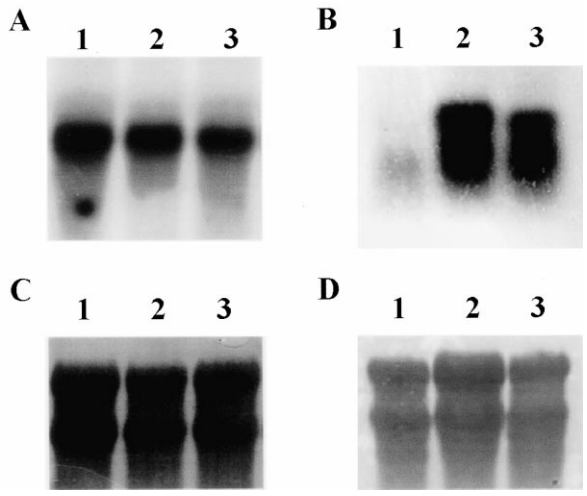

Fig. 6. Total RNA extracted from 7-day-old rice plantlets incubated 1 day in MS medium (lane 1) and in MS medium supplemented with, respectively, 200 mM NaCl (lane 2), iso-osmotic solution of mannitol (lane 3). Samples were fractionated on 1% agarose gels that contained formaldehyde before the transfer by gravity blotting onto positively charged nylon membrane. Blots were hybridized with the full-length ^{32}P-labeled OSVDAC1 cDNA (A) or SalT cDNA (B) at 62°C and washed in 2×SSC (0.3 M NaCl, 0.03 M $C_6H_5Na_3O_7{\cdot}2H_2O$) first at room temperature for 20 min and then at 65°C for 40 min. The corresponding membranes were stained with Methylene blue to verify the equal amount of transferred RNA (C,D).

the rice *osvdac1* expression is not osmotic stress responsive.

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