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Isolation and Characterization of *ALDH11A5*, a Novel Non-Phosphorylating GAPDH cDNA from *Physcomitrella patens**

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Abstract. *The unique ability to perform targeted gene disruption in mosses has made* Physcomitrella patens *an attractive choice for plant functional genomics. A cDNA* ALDH11A5 *(AY504666) was identified in the plant* P. patens *with significant similarity to the cytosolic NADP*$^+$*-dependent, non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN; EC 1.2.1.9). RNA blot hybridization was used to analyze* ALDH11A5 *mRNA transcript abundance in response to a variety of stresses including high osmoticum (600 mM sorbitol), salinity (300 mM NaCl), and ABA application (50 μM). This is the first report of GAPN from bryophytes, and demonstrates that this unique plant enzyme is not restricted to tracheophytes.*

NADP$^+$-dependent, non-phosphorylating GAPDH (GAPN) catalyzes the irreversible oxidation of glyceraldehyde-3-phosphate to 3-phosphoglycerate (Habenicht et al. 1994). GAPN has no significant sequence homology to the phosphorylating GAPDHs and is a member of the ALDH protein superfamily (Wood & Krayesky 2002). ALDH11 (GAPN) orthologues operate in the cytosol of autotrophic eukaryotes where the enzyme generates NADPH for biosynthetic processes from photosynthetic glyceraldehyde 3-phosphate exported from the chloroplast by the phosphate translocator. ALDH11 is one of the classic glycolytic ''bypass'' reactions unique to plants (Plaxton 1996), and Gao and Loescher (2000) have established that GAPN is the main source of NADPH for mannitol biosynthesis in celery. Although the function of this enzyme in metabolism is not completely understood, it is hypothesized that enhanced GAPN activity allows glycolysis to continue under stress conditions that limit adenylate nucleotide concentrations. To further investigate this hypothesis, we isolated and characterized an ALDH11 orthologue from *Physcomitrella patens*. Model moss systems such as *P. patens*, *Ceratodon purpureus*, and *Tortula ruralis* have been powerful experimental tools for the elucidation of a variety of complex biological processes in plants (Cove 2000; Reski 1999; Schaefer 2002; Wood et al. 2000). The unique ability to perform targeted gene disruption in mosses has made *Physcomitrella* an attractive choice for plan functional genomics.

The complete ORF-containing cDNA sequence ALDH11A5 (AY504666) was derived from the *P. patens* ESTs C_pp004046095 (1,160 bp, R. Reski, unpubl. data), BJ2022081 (590 bp), and BJ72951 (586 bp, Nishiyama et al. 2003). ALDH11A5 is 1,883 bp in length, encoding a 496 amino acid deduced polypeptide (nucleotide 121–1608) with a predicted molecular mass of 53.3 kDa and pI of 6.4 (Fig. 1A) that is 79% identical to ALDH11 orthologues from *Arabidopsis* and celery. The deduced polypeptide contains the ALDH glutamic acid active site signature sequence MELGGNA (residue 264–270, PROSITE PS00687) and the catalytic thiol (residue 291–302, PROSITE PS00070) (Sophos et al. 2001).

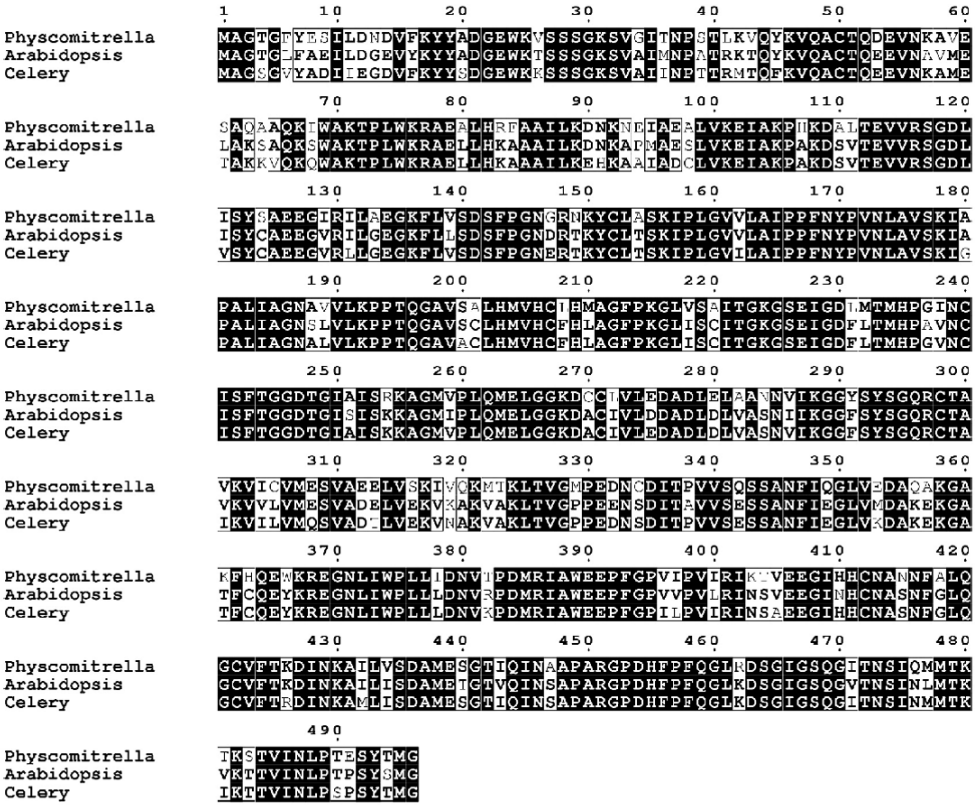
To examine the structural relationship between ALDH11A5 and existing ALDH11 family members, the deduced amino acid sequences were analyzed by the Neighbor-Joining method (Saitou & Nei 1987). The phylogenetic tree assembled from the pairwise alignment of those sequences is depicted in Figure 1B. Previously characterized ALDH11 sequences could be reproducibly grouped into a single angiosperm clade with ALDH11A5 forming a sister clade. Although a novel protein sequence, ALDH11A5 is clearly a member of the ALDH11 protein family.

To study expression levels of ALDH11A5 in response to abiotic stress conditions, *P. patens* plants were subjected to osmotic-stress, salt-stress, and treatment with ABA (a known mediator in stress-responsive signaling pathways). *Physcomitrella*

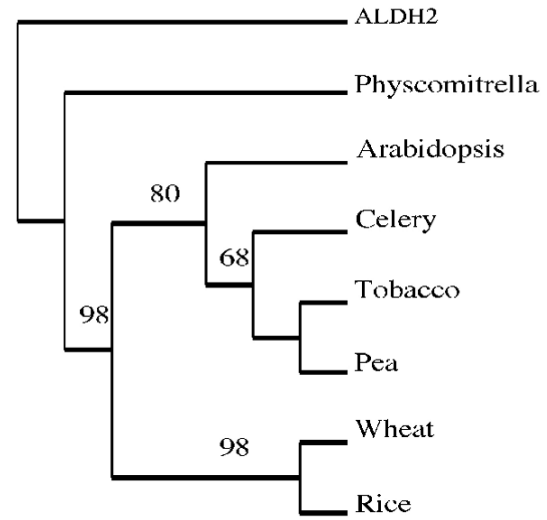
* The nucleotide sequence reported in this paper appears in EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number AY504666.

0007-2745/04/$0.45/0

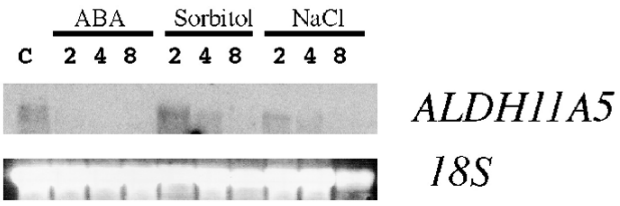
A


Physcomitrella
Arabidopsis
Celery

B


ALDH2
Physcomitrella
Arabidopsis
Celery
Tobacco
Pea
Wheat
Rice
80
68
98
98

C


ABA
Sorbitol
NaCl
C 2 4 8 2 4 8 2 4 8
ALDH11A5
18S

patens plants were grown axenically in liquid Knop medium containing 250 mg l^{-1} KH_2PO_4, 250 mg l^{-1} $MgSO_4 \times 7\ H_2O$, 250 mg l^{-1} KCl, 1,000 mg l^{-1} $Ca(NO_3)_2 \times 4\ H_2O$, 12.5 mg l^{-1} $FeSO_4 \times 7\ H_2O$, pH 5.8, and cultured in Erlenmeyer flasks at 25 ± 1°C under a 16/8 hr light/dark photoperiod with a light intensity of 55 μmol m^{-2} $sec.^{-1}$. (Reski et al. 1994). Plants were subcultured at seven days intervals. For salt and sorbitol treatments, plants were transferred to Knop medium supplemented with 300 mM NaCl or 600 mM sorbitol, respectively. ABA-treatment has been carried out by application of 50 μM (±)-*cis-trans* ABA to the liquid cultures. Total RNA was isolated according to Pawlowski et al. (1994). An RNA gel blot of total RNA isolated from *P. patens* gametophytes was hybridized at 68°C with a ^{32}P-labeled probe (C_pp004046095 PCR amplified from the vector using standard M13–20 and M13 reverse primers) (Fig. 1C). ALDH11A5 steady-state transcript amounts were detectable in untreated plants. Steady-state transcript levels were unchanged after two hours exposure to sorbitol and NaCl in liquid culture, and subsequently declined after prolonged exposure. No transcript was detected after incubation with ABA.

Like all mosses, *P. patens* gametophytes lack stomata. They cannot regulate their intracellular water status by modulating the gas exchange properties of the leaf tissue, and osmotic–stress (such as exposure to salt and sorbitol in cell culture) acts directly upon the protoplasm. ALDH11A5 transcript was stably maintained for two hours in response to 600 mM sorbitol and 300 mM NaCl. Our results suggest that the GAPN glycolytic ''shunt'' may continue to function in response to severe osmotic-stress and salinity-stress. Further molecular and physiological experiments are underway to evaluate the role of ALDH11A5 in plant development and adaptation environmental stresses.

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Figure 1. — A. Alignment of the deduced polypeptide sequence of *Physcomitrella patens* ALDH11A5 (AY504666) with orthologues from *Arabidopsis* (ALDH11A3, NP_180004) and Celery (*Apium graveolens*, AAF08296). Sequences are numbered from the presumed translation initiation methionine (M) and are aligned to give maximal alignment. — B. Phylogram derived from a data set of deduced polypeptides for ALDH11A5 and related deduced polypeptides corresponding to six previously characterized ALDH sequences. The rooted tree (ALDH2, *Nicotiana tabacum*, T02301) was constructed using Clustal-X (Jeanmougin et al. 1998). The data set consisted of the following sequences: *N. tabacum*, P93338; *Pisum sativum*, S43832; *Triticum aestivum*, AAM77679; and *Oryza sativa*, AAM00227. Numbers above the lines represent bootstrap percentages (based on 1,000 replicates). — C. RNA blot analysis of ALDH11A5 using 20 μg of total RNA from *P. patens* gametophytes and probed with a [^{32}P]-labeled cDNA probe. Plants were treated with 50 μM (±)-*cis-trans* ABA, 600 mM sorbitol or 300 mM NaCl for 2, 4, and 8 hr. The 18S rRNA was stained using ethidium bromide.