GENE NOTE

A novel Arabidopsis thaliana dynamin-like protein containing the pleckstrin homology domain

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Abstract

A full-length cDNA encoding a novel type of plant dynamin-like protein, ADL3, was isolated from Arabidopsis thaliana. ADL3 is a high molecular weight GTPase whose GTP-binding domain shows a low homology to those of other plant dynamin-like proteins. ADL3 contains the pleckstrin homology domain as is in mammalian dynamins, although other plant dynamin-like proteins reported lack this domain. The ADL3 gene was expressed weakly in various tissues, except for siliques with high level expression, which is distinct from the case for other plant dynamin-like protein genes. Taken together, it is predicted that the mode of activation of ADL3 is different from those of other plant homologues.

Key words: Arabidopsis thaliana, dynamin-like protein, pleckstrin homology (PH) domain.

Dynamins, the high molecular weight GTPases, play a key role in scission event common in various type of endocytosis at plasma membrane (van der Bliek, 1999). Dynamin usually consist of N-terminal GTPase domain, pleckstrin homology (PH) domain and C-terminal proline/arginine-rich domain (PRD) with a conserved domain of unknown function and coiled-coil domain. The PH domain and PRD are well known to have the capacity to exert regulatory effects on the GTPase activity (Lin et al., 1997; Herskovits et al., 1993). Dynamin-like proteins have been identified in higher plants. It is notable that all plant dynamin-like proteins reported lack the PH domain and PRD. In yeast, a dynamin-like protein, Vps1p, is involved in protein transport from Golgi to an endosomal compartment (Wilsbach and Payne, 1993), while another dynamin-like protein, Mgm1p, plays a role in mitochondria maintenance (Jones and Fangman, 1992). Interestingly, these yeast proteins also lack the PH domain and PRD. It is therefore probable that plant dynamin-like proteins also function in intracellular events other than in endocytosis. Indeed, soybean PDL (plant dynamin-like) is required for the formation of cell plates from Golgi (Gu and Verma, 1996) and Arabidopsis ADL1 (Arabidopsis dynamin-like 1) functions in biogenesis of thylakoid membranes in chloroplasts (Park et al., 1998). However, the existence of the PH domain in plants was currently confirmed by the molecular cloning of Arabidopsis cDNAs for phosphatidylinositol 4-kinase (PI 4-K) (Stevenson et al., 1998) and pleckstrin homologue (Mikami et al., 1999), which led us to search a plant dynamin-like protein containing the PH domain.

Searching of the plant expression sequence tag (EST) databases against human pleckstrin resulted in the discovery of an Arabidopsis EST clone, 208A13T7 (GenBank accession number N37665), encoding a part of the PH domain. A DNA fragment corresponding to the EST sequence was amplified by polymerase chain reaction (PCR) and used as a probe for screening of an Arabidopsis cDNA library. The largest cDNA insert, 3172 bp, was sequenced, yielding a largest cDNA insert, 3172 bp, was sequenced, yielding a

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of protein localization sites (http://www.nibb.ac.jp:8000) indicate that the N-terminal extension of ADL3 is not typical signal sequence and that ADL3 has a potential to translocate to chloroplast stroma, mitochondrial matrix space, microbody, and nucleus. It is, however, possible that ADL3 associates with membranes via the interaction between its PH domain and phosphoinositides of membranes. Moreover, the ADL3 mRNA was expressed weakly in various tissues, except pleckstrin homology domain that binds phosphatidylinositol 4-monophosphate.

**Fig. 1.** Structural characteristics of ADL3. (A) Schematic representation of *Arabidopsis* dyanin-like proteins and human dyanin-1. For the cloning of the *ADL3* cDNA, a DNA fragment corresponding to the EST, 203A13T7, was amplified from an *Arabidopsis* cDNA library by PCR at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min for 25 cycles with two synthetic oligonucleotide primers 5′-GAGAAGATCCGAGGAG-3′ (forward) and 5′-TTTACGTCCTGAAGATCTG-3′ (reverse) and used as a probe for screening of the same *Arabidopsis* cDNA library. Among positive clones, the largest cDNA was used for further analysis. (B) Alignment of the PH domain of ADL3 and human dyanin-1. Seven β-sheet and an α-helix in the PH domain are underlined and double-underlined, respectively. (C) Multiple amino acid sequence alignment of human dyanin-1 and *Arabidopsis* dyanin-like proteins. Residues indicated in white letters on a black background are identical in 3 to 4 sequences and those possibly involved in GTP-binding are indicated by asterisks. Accession numbers: ADL1, L38614; ADL2, AF012833; human dyanin 1, Q05193. For all panels, numbers refer to amino acid positions relative to the first residue of each protein.

**Fig. 2.** Tissue distribution of the *ADL3* mRNA. Total RNA (40 μg) was separated on 5% formaldehyde/1% agarose gel, blotted onto Hybond-N membrane (Amersham), and hybridized to the 32P-labelled full-length *ADL3* cDNA at 42 °C. The final wash was in 0.1 x SSC/0.1% (w/v) SDS at 65 °C. Total RNA blotted onto a nitrocellulose filter was stained with methylene blue to compare the amounts of RNA in each lane (bottom). Signals were visualized with a Fuji BAS-2000 biomage analyser. F, flowers; Sq, siliques; St, stems; L, leaves; R, roots.

findings, it is highly possible that the mode of activation of ADL3 is different from those of other plant dyanin-like proteins and that ADL3 presumably perform a role different from other related proteins. Therefore, functional characterization of ADL3 must provide new insight into the biological significance of dyanin-like proteins in higher plants.

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