Abstract
Rab-GDP dissociation inhibitor protein (rab-GDI) plays an essential role in secretory pathways. To date, the question of multiplicity of GDI isoforms in higher plants remains open and dbEST search, PCR and Southern blot analysis have been used to assess the isoform composition of rab-GDI in Arabidopsis thaliana. A novel rab-GDI isoform, \( \alpha \)-rGDI2, was identified which shares 90% identity in amino acid sequence with the known \( \alpha \)-A. thaliana GDI and significantly differs from it by the structure of its 5' and 3' untranslated regions.

Key words: Rab-GDI, Arabidopsis, secretion, isoforms.

Rab GDP dissociation inhibitors (GDIs) are cytosolic proteins that bind the GDP-bound form of prenylated rab GTPases with high specificity, retrieve them from their fusion targets and deliver them to specific membrane compartments (Schalk et al., 1996). Yeast contains only one GDI gene (Garrett et al., 1994). Several GDI isoforms exist in mammals, which suggests that they may have overlapping functions; however, significance of these differences has yet to be fully understood (see discussion by Benhar et al., 1997; Shisheva and Czech, 1997, and references therein).

Genomes of Volvox and Chlamydomonas contain only one rab-GDI gene (Beyser and Fabry, 1996). Two laboratories have recently determined the cDNA sequence for \( \alpha \)-A. thaliana rab-GDI, termed \( \alpha \)-rGDI1 (EMBL D83531, Ueda et al., 1996; Y07961, Zarsky et al., 1997). The presence of two or three GDI genes in the \( \alpha \)-A. thaliana genome has been reported based on Southern blot analysis (Ueda et al., 1996). However, Zarsky et al. (1997) argue, also on the basis of Southern blot analysis, that the \( \alpha \)-A. thaliana genome contains only one gene for rab-GDI.

This work was undertaken in an attempt to characterize the isoform composition of higher plant rab-GDI. A dbEST BLAST search (http://expasy.hcuge.ch/cgi-bin/BLAST.pl; Altschul et al., 1990) using a maize rab-GDI fragment (GELPQGFARSLAVYGGTYM, identified previously by PCR), as well as conserved sequences from mammalian and yeast GDIs, resulted in several clones from \( \alpha \)-A. thaliana and \( \alpha \)-Oryza sativa (not shown) apparently encoding GDI-like proteins.

Fig. 1. Comparison of the plant rab-GDI amino acid sequences. Designations: At1 and At2, \( \alpha \)-GDI1 and \( \alpha \)-GDI2, respectively; Vc, rab-GDI from Volvox carteri. Multiple alignment was done using the CLUSTAL program (PCGene, IntelliGenetics) using default parameters. Three regions, SCR1A, SCR1B and SCR3B, highly conserved in the members of the rab-GDI family (Schalk et al., 1996) are shown under the alignment. \( \alpha \)-rGDI2 structure confirms several substitutions in SCRs found in plants (double underlined). The structure of the GDI C-terminus is predicted to influence strongly the folding of the N-terminal domain supposed to form the rab-binding region (Schalk et al., 1996). Notably, the C-terminus of \( \alpha \)-GDI2 is truncated by one Glu residue as compared to \( \alpha \)-GDI1, which would diminish its overall negative charge.

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Most of the *A. thaliana* clones showed very high sequence identity to each other in the reliably sequenced regions and appeared to encode *AtGDI1*. Yet, one clone (172L4T7) showed a notable number of amino acid substitutions. Further sequencing of this clone showed that some of them were due to sequencing errors. Nevertheless, the available partial amino acid sequence of 172L4T7 (297 C-terminal amino acids) was clearly distinct from the structure of *AtGDI1*. The lacking N-terminal sequence was obtained by 5'-RACE with a specific antisense primer using *A. thaliana* cDNA as a template and was also found to contain amino acid substitutions as compared to *AtGDI1*. The putative novel isoform (EMBL accession number AJ001397) is termed *AtGDI2* (Fig. 1). Comparison between the untranslated regions of the cDNAs encoding *AtGDI1* isoforms is shown in Fig. 2. The differences in the *AtGDI2* nucleotide sequences determined by us and by T Ueda and coworkers (AB005560) may reflect spontaneous mutations in the *A. thaliana* lines maintained independently in different laboratories; alternatively they may indicate that *AtGDI2* is encoded by two different genes which diverged very recently in evolution.

The probes *AtGDI1* and *AtGDI2* were used for Southern blot analysis. These probes show partial selectivity for the respective GDI isoforms (not shown). The band pattern obtained with the full-length *AtGDI1* cDNA as a probe was consistent with the results of Ueda et al. (1996) suggesting the presence of at least two GDI genes. The partial selectivity of the two probes allows the lower and upper EcoRI fragments (see Fig. 8 in Ueda et al., 1996) in the 5'-7 kb region to be ascribed putatively as *AtGDI1* and *AtGDI2*, respectively (data not shown).

Therefore, a single rab-GDI isoform is not a characteristic feature of plants, but rather may be that of the unicellular organisms (yeast, *Volvox*, *Chlamydomonas*) irrespective of the kingdom they belong to. Functional comparison of the two *A. thaliana* GDI isoforms will be the subject of future work.

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*Arabidopsis thaliana* var. *Columbia* cDNA clones H10B3T7, 172L4T7 and VB18-10992 were obtained from the *Arabidopsis* Biological Resource Centre (ABRC, Ohio, USA). We thank Anne Kearn for excellent technical assistance. D.E.E. is a Royal Society 1983 University Research Fellow.

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**References**


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