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Identification and characterization of a lower plant Ypt/Rab guanosine dissociation inhibitor (GDI)

Kurt Beyser, Stefan Fabry*

Universität Regensburg, Institut für Biochemie, Genetik und Mikrobiologie, Lehrstuhl für Genetik, Universitätsstraße 31, D-93040 Regensburg, Germany

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Abstract The cDNA encoding a Ypt/Rab guanosine dissociation inhibitor (Ypt-GDI) was isolated from the multicellular green alga *Volvox carteri*, representing the first complete plant *gdi* gene described. The *gdiV1* gene occurs as a single copy in the algal genome, indicating that its product regulates all YptV proteins from *Volvox*. The derived GDI protein (GDIV1p) shows high similarity to animal and fungal GDIs. A specific antibody developed against GDIV1p detected the protein throughout the whole *Volvox* life-cycle. GDIV1p was localized in the cytoplasm and in the algal flagellum. This is in line with earlier findings of a dual localization of Ypt proteins both in the cell body and in the motility organelle, and indicates a novel role of the GDI/Ypt system, possibly in intraflagellar transport.

Key words: Ypt/Rab protein; Green alga; Transport; *Volvox*; *Chlamydomonas*; Flagellum

1. Introduction

The characteristic cyclic activities of small GTPases, GTP hydrolysis and GDP-GTP exchange, are both regulated by a number of specific accessory proteins [1]. One crucial factor is guanosine dissociation inhibitor (GDI), which locks the small G protein in the inactive, GDP-bound state, and, more importantly, also functions as a chaperon releasing GDP-bound small G proteins from their target membrane and storing them in the cytoplasm as an inactive GDI-GTPase complex, until another round of GTP binding and hydrolysis is initiated [2]. The different small G protein subfamilies are regulated by class-specific GDIs. So far, specific GDIs for the Ypt/Rab, Rho, and Rac subfamilies have been described [1]. Within a subfamily, a single GDI can interact with many G protein members [2]. Ypt/Rab-GDI is the best characterized molecule with respect to function [3] and structure [4], and is known in animals and yeast so far. Mammalian cells often contain more than one Ypt/Rab-GDI homologue [5]. In contrast, no GDI has been published from a plant up to now. Here, we report the cloning of a cDNA (*gdiV1*) encoding the Ypt-GDI from the simple multicellular green alga *Volvox carteri*, and describe characteristic features of the gene product, GDIV1p.

Our laboratory has chosen *V. carteri* as a model organism to understand Ypt/Rab function in plants. This alga contains at least six Ypt/Rab proteins (YptV1p–YptV6p [6–8]). For all of them, a homologue in the closely related unicellular species *Chlamydomonas reinhardtii* was also recently detected [8]. Subcellular immunolocalization studies on the algal Ypt proteins demonstrated that they are specifically associated with membranes of organelles of the exocytotic and endocytotic pathways [9] or with the plasma membrane [10], which is in accordance with established functions of Ypt/Rab proteins in intracellular vesicle transport [11]. Surprisingly, however, with the exception of YptV2p, all algal Ypt proteins could also be localized within the membrane-matrix fraction of algal flagella ([9], and unpublished data). This organelle consists of more than 200 different polypeptides which are assembled into a highly organized, dynamic structure [12]. Rapid and continuous intraflagellar transport (IFT) can be observed in *C. reinhardtii* [13,14], but no carrier vesicles typical for Ypt/Rab-dependent membrane transport have been described in flagella to date [15]. Thus, the flagellar location of small G proteins raises novel questions, focussing on a possible function in IFT [9]. In this study, we show that a central Ypt regulator, Ypt-GDI, is present in the algae, and can be localized both in the cytoplasm and in flagella of *V. carteri* and *C. reinhardtii*. This indicates that, like in the cell body, a full GDI-assisted GDP-GTP cycling of Ypts occurs in the eukaryotic motility organelle.

2. Material and methods

2.1. Strains, plasmids, and DNA preparations

Female strains HK10 and EVE of the green alga *V. carteri* f. *nagariensis* [16] were grown under a 16/8 h light/dark regime and synchronized as described [17,18]. *C. reinhardtii* strain *cw15* was cultivated according to Harris [19]. *E. coli* K-12 DH10B (Bethesda Research Laboratories) was used for plasmid cloning; *E. coli* K-12 BL21(DE3) [20] served as a host for heterologous protein expression. The plasmids for cloning the PCR-amplified *ypt* cDNA was pUC18 [21], the expression vector used for production of a recombinant, *His*-tagged GDI protein (rGDIV1p) was pET19b (Invitrogen).

Genomic DNA from *V. carteri* was isolated as described [22]. *C. reinhardtii* DNA was prepared as follows. A 10 ml culture (5×10^6 cells/ml) was harvested (5000 rpm, 5 min), and incubated in 0.5 ml 10 mM Tris-HCl pH 7.5, 20 mM EDTA, 1% (w/v) SDS at 37°C for 1 h. 0.13 vol. 2 M KCl was added, and the mixture held on ice (15 min). After centrifugation (10000 rpm, 10 min), the supernatant was extracted twice with phenol (water-saturated, not buffered):chloroform (1:1) and once with chloroform. 1/10 vol. 3 M NaAc (not buffered) and 1 vol. isopropanol were added to precipitate the DNA (−20°C, 30 min; 10000 rpm, 15 min). The pellet was washed (70% EtOH) and resuspended in 50 µl TE containing 200 ng/ml RNase A. For Southern blot analysis, 2 µg genomic DNA was used per restriction digest. Plasmid DNA was purified according to a modified small-scale method [23].

2.2. Cloning of the gdi cDNA

cDNA was generated by reverse transcription of 6 µg total *V. carteri* RNA using an oligo-dT(33) primer [10]. PCR was performed using two primers derived from highly conserved protein segments present in animal and yeast GDIs (upstream: 5′-TAGCGG(C/I)AA(A/G)AA(A/G)GT(C/I)(C/T)T-(C/I)CA-3′; downstream: 5′-GGCTG(C/I)GG(C/I)A(A/G)(C/T)TC(C/I)CC(C/I)A(A/G)(C/I)CC(A/G)TA-3′; templates: 1:100 diluted first strand cDNA template, or 10^6 phages from a *V. carteri* λZAPII-Express cDNA library (B. Cresnar,

*Corresponding author. Fax: (49) (941) 943-3163.
E-mail: stefan.fabry@biologie.uni-regensburg.de

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unpublished); cycling conditions: 94°C, 1 min; 52°C, 1 min; 72°C, 1 min 15 s; UNO thermal cycler, Biometra). PCR fragments were cloned into the *Xba*I/*Xma*I site of pUC18 by the DISEC-TRISEC PCR cloning method [24], sequenced, and used as a probe to screen 2×10^5 plaques of the cDNA library. Positive clones were subjected to in vivo excision [25], and the inserts of the resulting recombinant plasmids (pBK-CMV; Stratagene) sequenced on both strands.

To construct an in-frame *His*-tag-*gdi* fusion for expressing and purifying rGDIV1p, the sequence context around the putative start codon was changed to obtain a *Nde*I restriction site by PCR mutagenesis. A 1350 bp *Nde*I-*Bam*HI cDNA fragment was cloned, verified by sequencing, and inserted into *Nde*I/*Bam*HI-linearized pET19b, resulting in plasmid pET::*His-gdiV1*.

2.3. Expression and purification of recombinant GDIV1p in E. coli

pET::*His-gdiV1*-transformed *E. coli* BL21(DE3) were grown at 37°C to $OD_{600}=1.0$, induced with 1 mM IPTG, and harvested 30 min post induction. Cells were lysed (French Press), lysates cleared by centrifugation (39 000×*g*, 4°C, 20 min), and the supernatant was applied to a Zn^{2+}-loaded fast-flow chelating Sepharose column (2×1 cm; Pharmacia). After washing with 40 ml buffer A (1 M NaCl; 50 mM Na-phosphate pH 8.0; 5 mM imidazole; 0.05% Tween 20; 10 mM β-mercaptoethanol), rGDIV1p protein was eluted by a linear imidazole gradient (5–400 mM in buffer A, flow rate 0.5 ml/min; GRADI-FRAC separation system, Pharmacia). Following a check of rGDIV1p concentration and purity in relevant fractions by SDS-PAGE [26], fractions were pooled, dialyzed (25 mM Na-phosphate, 300 mM NaCl), and stored at −20°C.

2.4. Generation of a specific polyclonal anti-GDIV1p serum

For the primary immunization of a rabbit, 100 μg rGDIV1p was suspended in Freund's complete adjuvant and injected subcutaneously. Three booster injections (100 μg rGDIV1p in Freund's incomplete adjuvant) were then applied at 4-week intervals. Serum was collected 10 days after each booster injection, and tested. The final serum was affinity-purified by adsorption to an affinity column (1 g CNBr-coupled rGDIV1p Sepharose), elution by 100 mM glycine pH 2.5, and neutralization by 1/7 vol. 1 M Tris-HCl pH 8.8.

2.5. Isolation of algal crude proteins, membrane fractions, and flagella

Denatured total proteins of *V. carteri* were extracted by collecting about 1000–10 000 spheroids (40 μm nytex filter) and boiling them for 5 min in the presence of SDS (2 ml Laemmli buffer [26] lacking bromophenol blue and β-mercaptoethanol). The protein content was measured according to Lowry [27] using the DC Protein Assay of Bio-Rad. Crude extracts were stored at −20°C until use for SDS-PAGE. Particulate fractions were collected by serial centrifugation (3000×*g*, 10 min; 10 000×*g*, 20 min; 40 000×*g*, 1 h; and 100 000×*g*, 2 h) after sonication (4×15 s; 80 W) of about 10 000 *Volvox* spheroids in presence of sorbitol buffer (50 mM HEPES, 330 mM sorbitol, 2 mM EDTA, 100 μM PMSF). The 100 000×*g* supernatant, and all particulate fractions resuspended in a respective volume of sample buffer, were boiled in the presence of SDS (5 min), and stored at −20°C until use for SDS-PAGE and Western blot analysis.

Flagella from *V. carteri* and *C. reinhardtii* were prepared, extracted and fractionated essentially as described earlier [9].

2.6. Indirect immunofluorescence

Growth of *V. carteri* spheroids, preparation of the gonidial cells, mounting, fixation and permeabilization were performed as described [10]. Cells were then washed 3× in PBS, incubated with affinity-purified primary antibody (diluted 1:30 in PBS) at 12°C for 14 h, washed in PBS (3×), and exposed to the secondary antibody (goat anti-rabbit, coupled to fluorescein isothiocyanate (Sigma); dilution 1:1000 in PBS; 37°C; 1 h). Controls were treated similarly, but without primary antibody, or with a primary antibody that was preadsorbed to rGDIV1p. All samples were washed with PBS (10 min) before applying anti-fading solution (Citifluor AF1; Chemical Laboratory, University of Kent), and viewed in a Zeiss epifluorescence microscope (Axioskop 20).

3. Results

3.1. Isolation of a gdi cDNA from the green alga V. carteri

Using a primer pair designed according to two of the best conserved sequence regions in animal and yeast GDI (compare Fig. 2), we amplified a unique 600 bp fragment directly from 10^6 phages of a *V. carteri* λZAPII-Express cDNA library, or from reverse-transcribed total *V. carteri* RNA. The sequences of both PCR fragments were identical. Com-

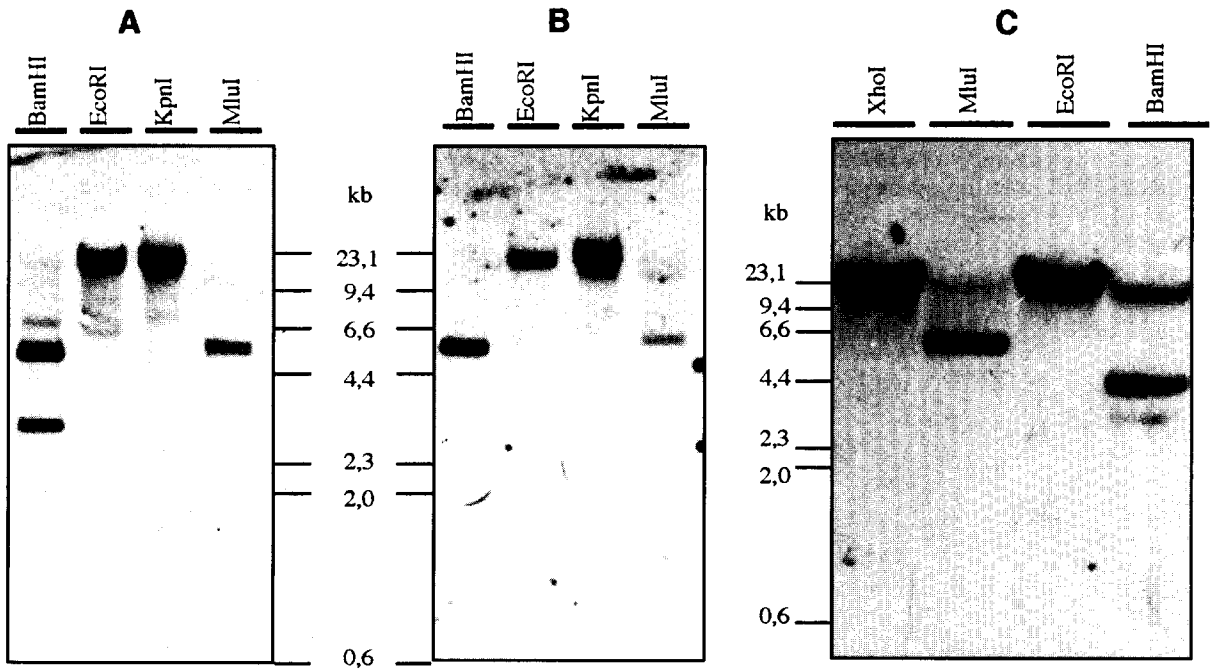

Fig. 1. Southern hybridization of genomic *V. carteri* (A, B) and *C. reinhardtii* DNA (C) probed with *gdiV1* cDNA (A, C: with entire coding sequence; B: with a 760 bp *Bam*HI-*Mlu*I subfragment). Numbers mark the positions of the *Hin*dIII fragments of λDNA used as size marker. Enzymes for digestions are given at the top of each blot. Results were identical irrespective of high-stringency (60°C) or low-stringency (42°C) hybridizations.

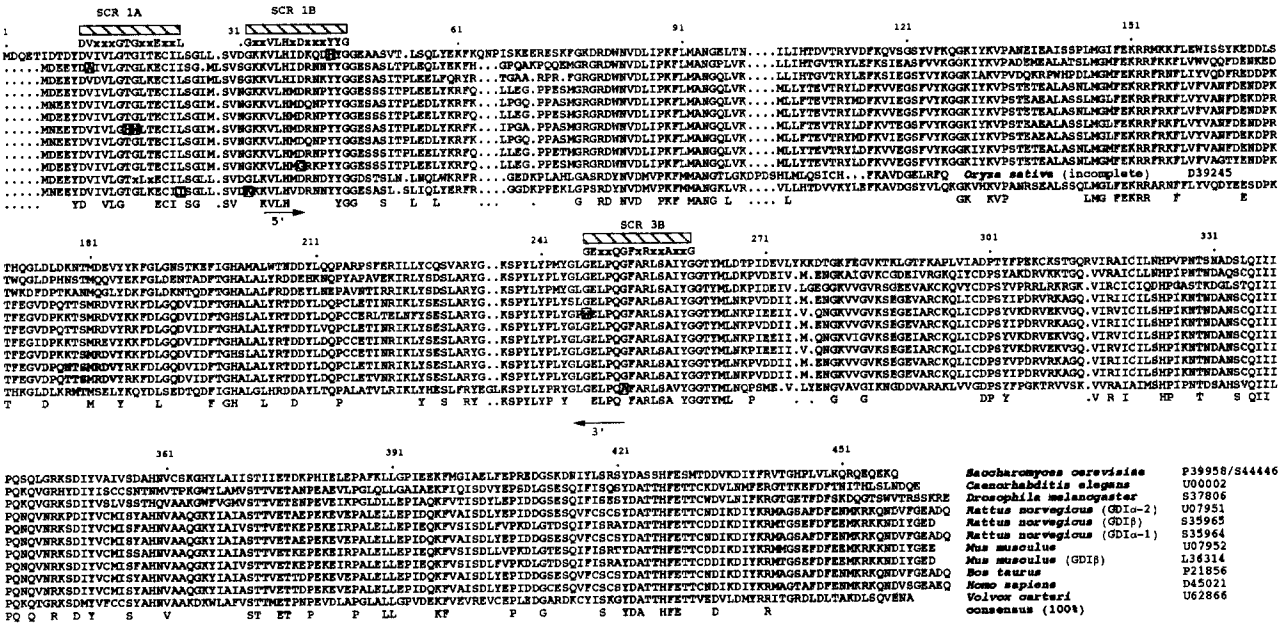

Fig. 2. Comparison of the deduced GDIV1p amino acid sequence with related members of the Ypt/Rab-GDI family. Sequence sources (species and GenBank accession numbers [release June 8, 1996]) are given after each sequence. The incomplete *Oryza sativa* GDI fragment is from an EST. The alignment is based on a multiple sequence comparison performed with the PILEUP program of the GCG software package (version 7). Residues conserved in all sequences are given at the bottom (consensus 100%). Three structurally and functionally defined SCRs [4] are indicated by bars, and their consensus sequences shown above the alignment; residues in individual GDIs deviating from the SCRs are boxed. Positions from which PCR primers (this work) were derived are marked by arrows.

parison with animal *gdi* genes and the derived proteins (see also below) showed that they contained a partial, *gdi*-like cDNA sequence. Conventional library screening with the PCR fragment as a probe revealed three independent clones, one of which contained a 2530 bp fragment representing a full-length *gdi* cDNA. This sequence consisted of 76 bp 5′ UTR, 1329 bp ORF encoding a 443 amino acid polypeptide, and 1111 bp 3′ UTR terminating with the polyadenylation signal TGTAT similar to the algal consensus (TGTAA), a 14 bp spacer, and the poly(A) tail. The bona fide start codon was defined on the basis of (i) great similarity of the encoded N-terminal sequences with animal homologues, and (ii) an in-frame stop codon present 57 bp upstream. The codon usage for all amino acids (except cysteine) shows strong preference for G or C in wobble positions indicative of a *Volvox* high-expression gene [28]. The gene was termed *gdiV1*. The complete sequence is deposited in GenBank under accession number U62866.

3.2. *Volvox and Chlamydomonas contain a single gdi gene*

The *gdi* gene copy number was determined by Southern blot analysis of genomic alga DNA (Fig. 1). In the initial screen, when the complete *gdiV1* coding region was used to probe the *V. carteri* genome, two hybridizing bands were obtained with *Bam*HI and *Mlu*I, but only single ones with *Eco*RI and *Kpn*I (Fig. 1A). Use of a 760 bp *gdiV1 Bam*HI-

Table 1
Percent identity between Ypt/Rab-GDI proteins[a]

	Volvox	*Oryza*	*Homo*	*Bos*	*Mus* β	*Mus* x[b]	*Rat.* α-1	*Rat.* β	*Rat.* α-2	*Droso.*	*Caeno.*	*Sacch.*
Volvox		69.2	61.3	63.1	61.3	61.3	63.1	61.3	63.1	64.5	68.2	58.0
Oryza	n.d.		55.6	57.4	55.5	55.6	57.4	55.6	57.4	54.7	56.6	49.6
Homo	55.3	n.d.		97.3	86.6	86.7	98.2	87.5	98.2	75.7	70.2	61.3
Bos	55.6	n.d.	97.1		88.4	87.5	99.1	88.4	99.1	77.5	72.1	62.2
Mus β	54.9	n.d	84.9	86.5		93.8	89.2	100	89.3	75.7	72.1	61.3
Mus x	54.6	n.d.	85.2	86.7	95.5		88.4	93.8	88.4	75.7	72.1	59.5
Rat. α-1	55.5	n.d.	97.1	98.7	86.5	86.7		89.3	100	77.5	72.1	62.2
Rat. β	53.3	n.d.	83.1	84.5	98.0	93.5	84.5		89.3	75.7	72.1	61.3
Rat. α-2	55.1	n.d.	96.7	98.4	86.3	86.5	99.3	84.3		77.5	65.1	62.2
Droso.	56.4	n.d.	64.5	66.6	64.6	64.9	66.4	62.8	65.9		80.9	62.5
Caeno.	55.6	n.d.	64.1	65.6	63.9	64.8	65.6	63.0	65.4	67.6		64.9
Sacch.	53.3	n.d.	54.4	54.9	54.9	54.4	54.9	53.8	54.4	52.6	55.7	

Upper triangle: sequence comparison using residues 1–126 including an *Oryza sativa* EST fragment; lower triangle: total sequence comparison excluding *Oryza*.
For full species designation see Fig. 2.
[a]calculated by the GAP program of GCG with gap penalty of 3.0 and gap length penalty of 0.1.
[b]x = unspecified GDI variant.
n.d. = not determined (lack of data).

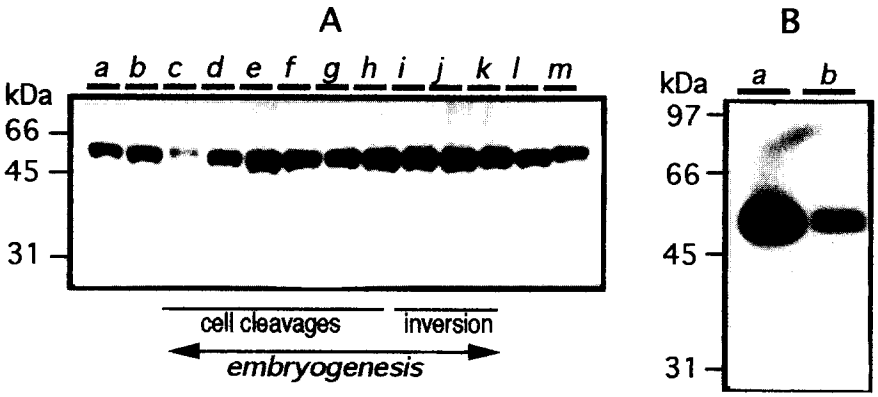

Fig. 3. Immunochemical detection of GDIV1p in algal protein extracts. Anti-GDIV1p was affinity-purified, 1:200 diluted, and detected by ECL (Amersham). A: GDIV1p in total protein extracts of synchronized *V. carteri* cells during the 48 h vegetative life-cycle. Equal protein amounts (20 μg) were loaded, and the following stages examined: (a) hatching of juveniles; (b) growth and cytodifferentiation; (c) onset of embryogenesis; (d–h) 4-cell, 8-cell, 16-cell, 64-cell and 1000-cell stages of embryos, respectively, (i–k) start, midphase, and end of embryo inversion, (l) embryo expansion; (m) start of hatching. For more details of the life-cycle see [10]. B: GDIV1p in total protein (30 μg) from *V. carteri* (a) and *C. reinhardtii* (b).

*Mlu*I cDNA subfragment for hybridization, however, specifically eliminated one band in the *Bam*HI and *Mlu*I digests (Fig. 1B), demonstrating that the second bands were caused solely by internal (in case of *Bam*HI obviously intron-located) cutting sites in the *gdiV1* gene, and not by a second *gdi* gene copy. In addition, in three out of four digests of genomic DNA of the closely related unicellular alga *C. reinhardtii* (Fig. 1C), single bands were detected with the *gdiV1* probe, indicating that *Chlamydomonas* also contains a homologous, single *gdiC* gene.

3.3. The derived GDIV1p protein is highly similar to animal and yeast counterparts

Amino acid alignment of the derived GDIV1p protein with known eukaryotic Ypt/Rab-GDI sequences (Fig. 2) was performed. GDIV1p showed nearly similar identity values to GDI of yeast, fruit fly, nematode, and mammals (53–56%) (Table 1). Identities within the first 126 amino acids of GDI (which allowed inclusion of a partial N-terminal sequence of a higher plant GDI from *Oryza sativa*) were between 58% (yeast) and 69% (rice) (Table 1). Curiously, GDIV1p$^{1-126}$ and the respective GDI fragment from *Caenorhabditis elegans* shared 68% identical residues, but the *Oryza* fragment only 57% with the nematode. Thus, whether or not a real closer relationship of GDIs from higher and lower plants, compared to animal and fungal counterparts, is present must remain open until a full-length plant GDI sequence is reported.

3.4. Analysis of algal GDI protein abundance in Volvox and Chlamydomonas

The specificity of a polyclonal anti-GDIV1p serum was confirmed by 1D and 2D gel electrophoresis and Western blot (not shown). In SDS gel-separated total crude protein extracts of synchronized *V. carteri* cells, a single prominent band of 50 kDa correlating well with the calculated molecular weight of 49.5 kDa for GDIV1p could be detected throughout all stages of the life-cycle examined (Fig. 3A). A relative increase in protein amount after onset of embryogenesis was observed, indicating upregulation of GDI expression during cell cleavages.

In *C. reinhardtii* total crude protein extracts, anti-*Volvox* GDI cross-reacted specifically with a 50 kDa protein (Fig. 3B), which is consistent with the Southern blot results (Fig. 1C) indicating the presence of a highly homologous GDI protein in *Chlamydomonas*.

Ypt/Rab-GDI is a predominantly cytosolic protein [11]. We next examined the intracellular localization of GDIV1p in *V. carteri*. As demonstrated in Fig. 4, after differential fractionation of *V. carteri* total cell extracts, the majority of the immunoreacting GDIV1p protein remained in the soluble fraction, consistent with a cytosolic location. Smaller GDIV1p amounts were also observed in particulate fractions, with the greatest portion present in the $10\,000\times g$ fraction. In contrast, most YptV proteins examined in parallel were detected exclusively in the particulate (mainly $10\,000\times g$) fractions, confirming their predominant membrane association (Fig. 4). Only YptV3p was also detected in a large amount in the final supernatant, supporting earlier observations (H. Huber, unpublished) that YptV3p has an exceptional intracellular distribution.

The cytosolic GDIV1p localization was confirmed by indirect immunofluorescence. For the sake of maximum resolution, the large reproductive *V. carteri* cells (gonidia) are shown. Anti-GDIV1p (Fig. 6A), but not the preimmune serum (Fig. 6B), stained a broad perinuclear region, and some radial extensions representing the cytoplasm in *V. carteri* gonidia.

3.5. GDI is also localized in the membrane-matrix fraction of algal flagella

Interestingly, as demonstrated previously for several Ypt proteins [9], extracts of isolated flagella from both *V. carteri* and *C. reinhardtii* also contained GDI. This is first shown by Western blot analysis (Fig. 5A). When flagella were further subfractionated, GDI was detected only in the membrane-matrix fraction, but not in the flagellar axoneme (Fig. 5B). Isolated *V. carteri* flagella subjected to immunofluorescence also revealed specific staining compared to the control (preadsorbed serum), supporting the Western blot results (Fig. 6C,D). These data correlate well with the flagellar Ypt distribution, and suggest that the flagellar location of small

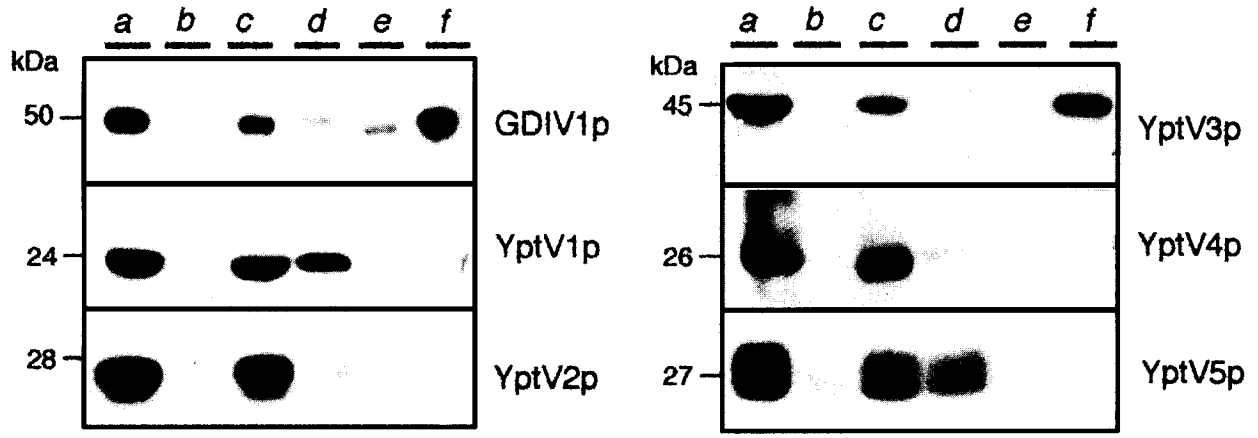

Fig. 4. GDIV1p and YptV proteins in fractionated crude *V. carteri* cell extracts. Anti-GDIV1p (this work), and anti-YptVps [8–10] were affinity-purified, 1:200 diluted, and detected by ECL. (a) Control: crude total protein extract (15 μg); (b–e) 3000×*g*, 10000×*g*, 40000×*g*, 100000×*g* particulate fractions, respectively; (f) 100000×*g* supernatant (in fractions and supernatant, equal volumes corresponding to 7.5 μg total protein were loaded).

GTPases together with a specific regulator is of functional importance.

4. Discussion

The elucidation of the mechanisms of Ypt/Rab small G protein function in animals and yeast has made considerable progress within the last years [11,29]. According to the SNARE hypothesis of intracellular transport, they fulfill specific control functions at different transport steps during the docking of membrane vesicles via SNAP receptors (SNAREs) to their target membrane [30,31]. However, this picture may describe neither the complete spectrum of cellular Ypt/Rab action, nor all ways of intracellular membrane transport [32–34].

GDI is one of the central molecules involved in Ypt/Rab regulation. It governs both the intracellular Ypt/Rab distribution and their GTP-GDP cycle [11]. Many – if not all – small GTPases of the Ypt/Rab family within an organism can be regulated by the same Ypt/Rab-GDI. First insights into the specificity of GDI-Rab interaction have been reported [4,35], but detailed mechanisms remain to be determined.

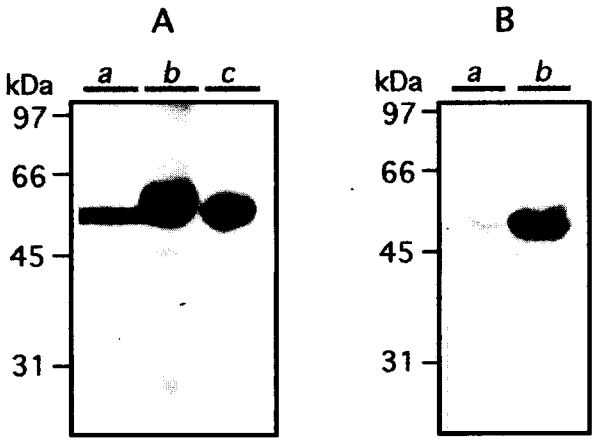

Fig. 5. A: GDIV1p in protein extracts of isolated flagella from *V. carteri* (a, 15 μg) and *C. reinhardtii* (c, 30 μg), and in a total *C. reinhardtii* protein extract (b, 30 μg). B: GDIV1p in flagella subfractions; (a) axonemal fraction; (b) membrane-matrix fraction. Equivalent portions of each fraction, corresponding to 20 μg total flagellar proteins, were separated, immuno-stained, and ECL-developed.

Rab-gdi genes have been cloned from animal and yeast cells so far. In plants, to our knowledge, no GDI homologue is published yet. Here, we describe a complete *gdi* gene, and characterize the gene product, from the green alga *V. carteri*. Southern and Western analyses indicate the presence of a homologous GDI in the related unicellular green alga *C. reinhardtii*, consistent with the very similar Ypt equipment of these two species [8]. Experiments to isolate the *C. reinhardtii gdi* gene are in progress. The lower plant *gdi* genes are single copies in the algal genomes, which is in obvious contrast to at least higher animals, where several different variants of Rab-GDI have been found in one cell. Different functional specificities between these variants are not known, however [5].

Comparison of the different Rab/Ypt-GDI primary structures (Fig. 2) showed considerable similarity (>50% total amino acid identity), indicating high conservation of this protein throughout the eukaryotic kingdom. Algal GDIV1p is nearly equally similar to mammalian, insect, worm, and fungal Rab-GDI, but a rice EST-derived sequence shares greater similarity with GDIV1p than with the remaining sequences (Table 1). Interestingly, GDIV1p shows unique deviations within three 'sequence-conserved regions' (SCRs), which have recently been defined as the best conserved structural and functional Rab/Ypt-GDI cores [4]. Two of the deviations are conservative (Leu-Ile in SCR1A, Gly-Ala in SCR3B), but one introduces an additional positive charge (Gly-Lys in SCR1B; Fig. 2, boxes). Whether these differences have specific functional meanings remains to be seen.

An examination of GDIV1p abundance throughout the *V. carteri* life-cycle demonstrated protein presence in all stages, as expected for a protein with central cell functions. But unlike most YptVps with a constant expression level [9], GDIV1p amounts increased during embryogenesis. A higher demand for GDI in transport processes during the rapid generation of ca. 2000 daughter cells from each gonidium might explain this upregulation.

GDIV1p was predominantly found in the cytoplasm (100000×*g* supernatant; perinuclear immunofluorescence) of

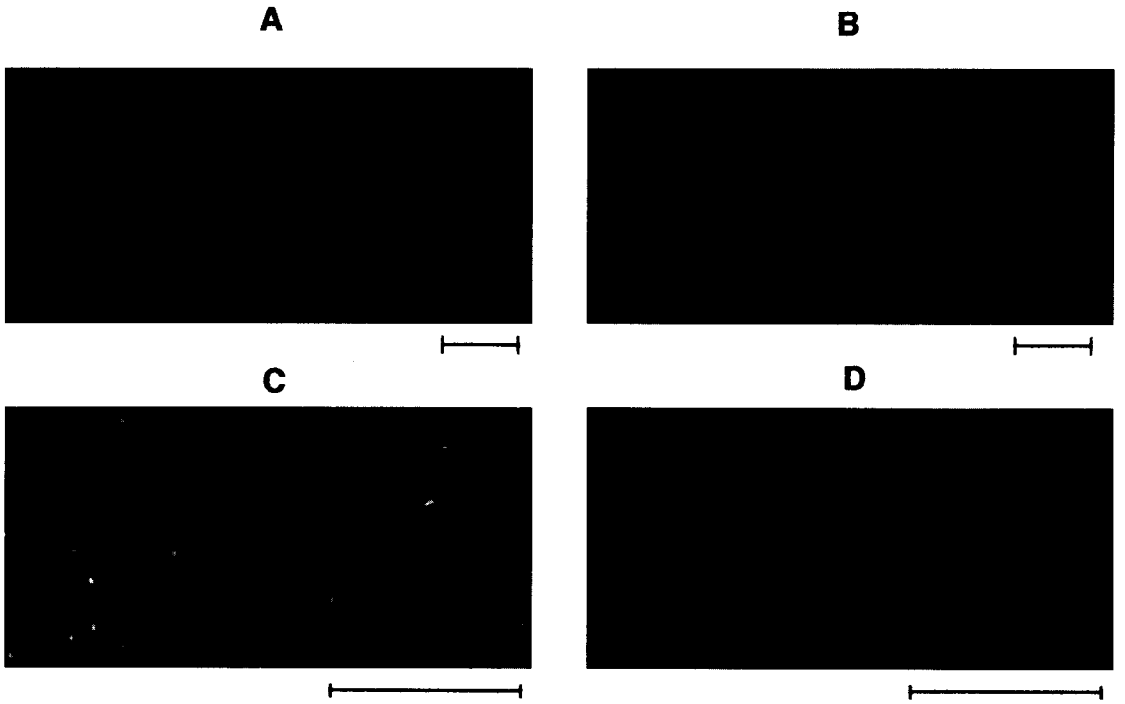

Fig. 6. Immunofluorescence labelling of *V. carteri* gonidia (A,B) or isolated flagella (C,D). A: Immunofluorescence with affinity-purified anti-GDIV1p (1:30) showing perinuclear cytoplasm staining. B: Control staining of gonidia with preimmune serum (1:100). C: Immunofluorescence of isolated flagella probed with anti-GDIV1p (1:30). D: Control staining of the flagella preparation with anti-GDIV1p (1:30) which was preadsorbed to purified rGDIV1p. Bars = 20 μm.

V. carteri cells as expected for a cytosolic protein. Smaller amounts detectable in the particulate fractions (Fig. 4) possibly represent subpools involved in membrane interaction. Interestingly, the main membrane-associated GDI subpool was found in the same fraction ($10000 \times g$) where the bulk of YptV proteins could be detected, suggesting that this GDIV1p subset represents YptVp-interacting complexes at membranes. In contrast, the bulk of the cytosolic GDIV1p in *Volvox* seems not YptVp-bound (lack of colocalizing YptVps – except YptV3p – in the supernatant), indicating that these protein complexes either comprise a small fraction which is beyond the detection level, or that the cytosolic GDIV1p-bound YptVps are lost during preparation.

In plants, the analysis of Ypt/Rab GTPases is generally still in a less advanced state (cf. [36–38]). Nevertheless, the current progress encourages us to predict that, besides confirmation of established functions, fascinating novel aspects can evolve from the study of green organisms' Ypt/Rabs [39–41]. In accordance with this, we recently reported that Ypt proteins can be found not only in the cell body, but also in the flagella of the green algae *V. carteri* and *C. reinhardtii* [9], which is in line with a previous report already noting GTPase activities in *Paramecium* cilia [42]. Here, we demonstrate a flagellar localization of algal Ypt/Rab-GDI in addition to its presence within the cell body.

Multifunctionality is a recently growing property of many small G proteins [43–46]. Thus, besides function in intracellular transport, Ypt/Rabs may also play a role in the recently detected intraflagellar transport processes, possibly catalyzing specific protein-protein interactions. Our demonstration of a cytoplasmic Ypt/Rab-GDI present in flagella of *Volvox* and *Chlamydomonas* suggests that (i) regulated GDP-GTP cycles and (ii) membrane recruitment of Ypts, both of which are prerequisites for their proper action, occur in the motility organelle, opening interesting possibilities for further studies on intraflagellar transport.

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