Trypanosoma brucei: Molecular Cloning of Homologues of Small GTP-Binding Proteins Involved in Vesicle Trafficking

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FIELD, M. C., AND BOOTHROYD, J. C. 1995. Trypanosoma brucei: Molecular cloning of homologues of small GTP-binding proteins involved in vesicle trafficking. Experimental Parasitology **81,** 313–320. Members of the rab subfamily of GTPases have been implicated as important components in vesicle trafficking in the eukaryotes. Individual rab proteins have a remarkable degree of specific subcellular localization. As a first approach to the study of these proteins in the protozoan parasite Trypanosoma brucei we have undertaken a survey of the rab subfamily using a strategy based on degenerate polymerase chain reaction utilizing the miniexon and the highly conserved WDTAGQE box which comprises part of the GTP-binding site. A number of T. brucei partial cDNAs were obtained from procyclic stage cDNA, and seven of these clones (designated rtb1, 3–7, 9) were clearly members of the rab family. Northern analysis of poly(A)-selected RNA indicates that rtb1, 3, 4, and 7 are constitutively expressed at low levels in both life-stages of T. brucei. By Southern analysis of trypanosome genomic DNA and specific PCR from an isolated genomic clone, we show that two of the genes, RTB1 and RTB7, are adjacent or at least closely linked in the T. brucei genome, while the other five are dispersed. These data provide important molecular reagents for dissecting the unusual secretory pathway in this organism. © 1995 Academic Press, Inc.

INDEX DESCRIPTORS AND ABBREVIATIONS: G protein; kinetoplastida; molecular cloning; rab protein; secretion; trypanosoma; vesicle trafficking; BiP, heavy chain binding protein; BSF, bloodstream form; DNA, deoxyribonucleic acid; GPI, glycosylphosphatidylinositol; RNA, ribonucleic acid; PCR, polymerase chain reaction; PGK, phosphoglycerate kinase; VSG, variant surface glycoprotein. The IUB degenerate nucleotide alphabet is used where appropriate.

INTRODUCTION

The processes of secretion and the uptake of nutrients and membrane components in the African trypanosome Trypanosoma brucei remain almost completely unexplored at the molecular level. The parasite recycles cell surface proteins (Coppens et al. 1987), secretes material into the medium (Olsson et al. 1993), endocytoses membrane components (Frevert and Reinwald 1988), and, in the BSF takes up at least two nutrients, low density lipoprotein and transferrin, by receptor-mediated endocytosis (Frevert and Reinwald 1988). Several studies indicate

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that T. brucei is capable of an array of post-translational processing events, including N-glycosylation (Zamze 1991) and GPI-anchor addition (Cross 1990). This latter modification is of particular relevance as the GPI-anchor is the mechanism of membrane attachment for a number of high abundance kinetoplastida cell surface antigens, including the variant surface glycoproteins (VSGs) and the procyclins of T. brucei (reviewed in McConville and Ferguson 1993, Englund 1993). Morphologically the T. brucei endoplasmic reticulum and Golgi structures are essentially the same as those encountered in other eukaryotes (Duszenko et al. 1988; Bangs et al. 1993). However, in the kinetoplastida cell surface membrane traffic is highly unusual; all exocytosis and endocytosis take place through a unique organelle, the flagellar pocket, a specialized cup-shaped invagination of the

plasma membrane located at the base of the flagellum which accounts for ~2% of the parasite cell surface (Webster and Russell 1993). Hence a detailed understanding of vesicle trafficking events in *T. brucei* may reveal important new mechanisms for how secretion can be directed to a single highly specialized area of the cell surface, and how sorting can occur within such a restricted region.

Recent data have indicated that several aspects of the basic machinery of secretion among the eukaryotes are conserved: one particularly striking observation is that a number of GTP-binding proteins, or "rabs," closely homologous to the *ras* protooncogene, are associated with specific steps in exocytosis, endocytosis, and recycling (Pfeffer 1992). While the precise role that each rab plays has yet to be fully defined, these proteins are clearly intimately associated with control of the process of vesicle budding and targeting. The apparent specificity with which rab proteins are localized to specific compartments makes them ideally suited as markers. Apart from BiP (Bangs *et al.* 1993) there are at present no defined secretory pathway markers in *T. brucei*, nor have any of the components involved in vesicle trafficking been identified. Rab proteins are obvious candidates as markers for subcellular compartments in this organism as well as constituting important factors in the exocytic/endocytic pathway. Information from studies of other systems would also allow specific manipulation of the *T. brucei* exocytic and endocytic pathways in a rational manner.

In this paper, we report the identification, using a degenerate PCR approach, of a number of *T. brucei* rab partial cDNAs, which we have designated rtbs for rabs from *T. brucei*. We demonstrate that several of these genes are apparently expressed, and that at least two of the RTB genes are closely linked in the *T. brucei* genome, raising the possibility that genes for small G-proteins in this organism may be clustered in functional arrays. The identification and characterization of a cDNA for a related G-protein, RAN/TC4, also isolated by this strategy, has been reported previously (Field *et al.* 1995).

MATERIALS AND METHODS

Genomic DNA was obtained from cultured procyclic form *T. brucei brucei* 427 strain, using the method of Medina-Acosta and Cross (1993). *T. b. brucei* total RNA and poly(A)-selected RNA, isolated from strain 427, were the gift of L. Uyetake, Stanford University. Plasmid isolations were performed using either the "Wizard" miniprep procedure (Promega Inc.) or with Qiagen anion exchange columns (Qiagen Inc.), as described by the manufacturers. A *T. b. brucei* genomic Sau3A partial digest library, in λFIX was the gift of Dr. G. Roberts (UCSF, San Francisco, CA). All bacteriophage procedures were performed using standard protocols (Ausubel *et al.* 1994). Isolation of λ DNA was performed with a kit from Qiagen Inc. Oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer and used without further purification. All restriction endonucleases were from New England Biolabs Inc. T4 DNA ligase was from Boehringer-Mannheim. AmpliTaq thermostable DNA polymerase was from Perkin–Elmer Inc. Modified reverse transcriptase (Superscript) was from GibcoBRL. The pCRScript blunt-end cloning kit was from Stratagene Inc. (La Jolla, CA). dNTPs and buffers were from Perkin–Elmer Inc., and pBluescript was from Stratagene.

Agarose gel electrophoresis of DNA fragments was performed in TBE buffer. DNA was recovered from gel slices with GeneClean (Bio101 Inc.). For genomic blots ~1 μg of digested DNA was transferred to nylon membranes under alkaline conditions following depurination. For Northern blots, ~1 μg of poly(A)-selected RNA was fractionated by agarose gel electrophoresis in the presence of formamide and transferred to nylon membranes using standard protocols (Ausubel *et al.* 1994). Membranes were UV crosslinked and then blocked in 5× Denhardt's for at least 1 hr before probing (Maniatis *et al.* 1982). Nucleic acids were detected by hybridizing with random-primed (Boehringer-Mannheim) α-^{32}P-labeled dCTP probes. The Southern blots were washed at high stringency (0.1× SSC, 0.1% SDS, 65°C), and at lower stringency (0.5× SSC, 65°C) for Northerns. After washing, membranes were exposed to X-OMAT-AR (Kodak) X-ray film at −80°C.

For degenerate PCR the following primers were used: Miniexon: GGCCAGGATCCCGCTATTATTAGAA-CAGTTTCTGTACT, and WD box: GGCCGAATTCYT-CYTGNCCNGCNGTRTCCCA (512-fold degeneracy). Miniexon primer contains a *Bam*HI site and WD box an *Eco*RI site for cloning. PCR was performed under standard conditions with 50 p*M* each primer and a MgCl$_2$ concentration of 2.5 m*M* (Ausubel *et al.* 1994) in a Perkin–Elmer 480 thermocycler with the following program: 94°C for 1 min, 48°C for 1 min, 72°C for 1 min for 5 cycles, and 94°C for 1 min, 55°C for 1 min, 72°C for 1 min for 35 cycles followed by 15 min at 72°C. PCR products, following digestion with *Bam*HI and *Eco*RI, were ligated into pBluescript. PCR from λFIX clones for identification of RTB1 and RTB7-containing clones used the WD box and RTB1FUS CGTGGATCCATGATCACAG-

CAGCTTCC and RTB7PCR CATGAAAGAGGAAC-CCGC. PCR products from genomic clone R111 were ligated into pCRScript and sequenced. Sequence data were obtained by dideoxy sequencing as described by the manufacturer (Amersham International, Inc.). Sequence data were used to interrogate GenBank/EMBL using Fasta. Alignments were performed using the Clustal algorithm from Sequence Navigator V1.0 (Perkin-Elmer, Inc., Foster City, CA). Phylogenetic reconstructions were done using PAUP V3.0 (Swafford 1993). Bacteriophage libraries were plated out on *Escherichia coli* XL-1Blue MRA P2 lawns and screened using random primer-labeled fragments from the rtb1 cDNA PCR product. The filters were washed at high stringency (0.1× SSC, 0.1% SDS, 65°C) before exposure to X-ray film. Secondary plaques were rescreened and the tertiary plaques analyzed by PCR. Clones scoring positive for both rtb1 and rtb7 were further analyzed by Southern blotting and sequencing of the PCR products.

RESULTS

In order to obtain partial clones of rab cDNAs an adaptation of the strategy described by Zerial *et al.* (1992) was used to PCR amplify portions of rab cDNAs from total procyclic cDNA. A PCR reaction, using a sense primer to the 39 nucleotide miniexon and an antisense degenerate primer to the highly conserved WDTAGQE sequence present in the second homology region of all rab proteins so far identified (see Materials and Methods for description of primers) was performed. When the products of the PCR reaction were visualized by ethidium staining following agarose gel electrophoresis it was apparent that a number of discrete products had been obtained, ranging in size from ~200 to ~400 bp (data not shown). A single prominent band at ~200 bp was isolated and cloned. Se-

quence analysis of 20 independent clones demonstrated that they all contained the same insert, and the clone was designated rtb1 (see Fig. 1). When the remaining products (>200–400 bp) were cloned and sequenced eight further distinct sequences were obtained. One of these, rtb9, was almost identical to rtb4, except for five nucleotide differences that rendered the reading frame of rtb9 closed (data not shown). The hypothetical translations of the remaining sequences are shown aligned in Fig. 1 (the DNA sequences of these clones have been deposited in GenBank; see Materials and Methods). The preponderance of the rtb1 sequence within the PCR products may be at least partially due to this fragment being the smallest product and therefore being preferentially amplified, as the rtb3 message is apparently more abundant (Fig. 2). The small size of the RTB1 cDNA fragment is because there are only 12 nucleotides in the 5' untranslated region between the miniexon and the initiation ATG (Fig. 4).

Fasta searches confirmed that each of the rtb clones, except rtb2, were indeed highly homologous to the rab subfamily of small G-proteins. The fasta scores were always significantly higher against rab sequences than for other small G-proteins. Additional investigation of rtb2 determined that this clone was derived from an mRNA for the *T. brucei* homologue of the nuclear G-protein RAN/TC4 (Field *et al.* 1995).

From the distribution of the frequency of the clones isolated by this procedure (Table 1) it is proposed that only a subset of the total *T. brucei*

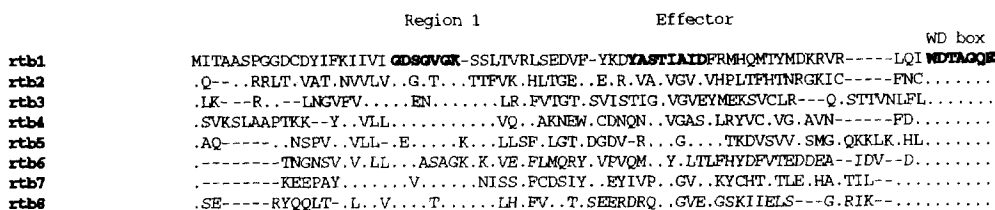|	Region 1	Effector	
			WD box
rtb1	MITAASPGGDCDYIFKIIVI GDSGVGK-SSLTVRLSEDVP-YKDYASTIAIDFRMHQMTYMDKRVR-----LQI WDTAGQE		
rtb2	.Q--..RRLT.VAT.NVVLV..G.T...TTFVK.HLTGE..E.R.VA.VGV.VHPLTFHTNRGKIC-----FNC.......		
rtb3	.LK---R..--LNGVFV.....EN.......LR.FVTGT.SVISTIG.VGVEYMEKSVCLR---Q.STTVNLFL.......		
rtb4	.SVKSLAAPTKK--Y..VLL...........VQ..AKNEW.CDNQN..VGAS.LRYVC.VG.AVN-----PD........		
rtb5	.AQ-----..NSPV..VLL-.E.....K...LLSF.LGT.DGDV-R...G....TKDVSVV.SMG.QKKLK.HL.......		
rtb6	.--------TNGNSV.V.LL...ASAGK.K.VE.FLMQRY.VFVQM..Y.LTLFHYDFVTEDDEA--IDV--D........		
rtb7	.--------KEEPAY.......V......NISS.FCDSIY..EYIVP..GV..KYCHT.TLE.HA.TIL--..........		
rtb8	.SE-----RYQQLT-.L..V....T......LH.FV..T.SEERDRQ..GVE.GSKIIELS---G.RIK--..........		

FIG. 1. Alignment of N-terminal sequences of rtb clones. The inferred amino acid sequences of the partial cDNA clones encoding the N-terminal portions of *T. brucei* rab proteins are shown. Identity with rtb1p is designated by a dot, and gaps introduced into the alignment are indicated by a dash. The single letter amino acid code is used. The residues comprising region 1 of the GTP-binding site, the effector domain, and the sequence against which the degenerate oligonucleotide WD box was designed are shown in bold. The natural sequence in the region of the WD box primer cannot be deduced from this analysis.

rab family has been cloned as one in three of the sequence classes is represented by only a single clone in the pool analyzed. Therefore the total RTB family could reasonably be expected to contain at least 10 to 15 members. This is consistent with data indicating that ~30 rabs may be present in mammalian cells (Chavrier *et al.* 1992), while there are probably less than 10 rabs in yeast. All the PCR products analyzed had the complete miniexon sequence reconstructed at the 5' end, indicating that they were derived from authentic mRNAs (the miniexon primer lacked the 3'-most six nucleotides of the miniexon sequences). The variance in the size of the inserts (200–400 bp) was attributable to the length of the 5' untranslated region, ranging from 12 bp in rtb1 to ~200 bp for rtb2. The significance of this is not known. Phylogenetic reconstruction using PAUP did not reveal any clustering of the rtb sequences among themselves (data not shown; Swafford 1993).

A Northern blot analysis of rtb1, 3, 4, and 7 was performed to determine approximate expression levels and molecular weights of mes-
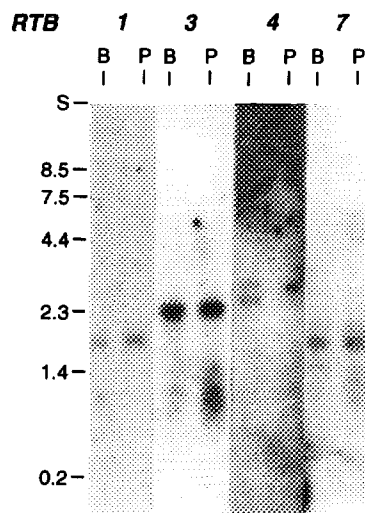
TABLE 1

Frequency of Recovery of rtb Sequences by Degenerate PCR

Sequence	Incidence (%)
Rtb1	46
Rtb2	16
Rtb3	14
Rtb4	4
Rtb5	8
Rtb6	2
Rtb7	6
Rtb8	2
Rtb9	2

Note. Clones generated by a degenerate PCR reaction using *T. brucei* procyclic RNA as template with WD box and miniexon primers (see Materials and Methods) were assigned to sequence class by dideoxy sequence analysis. A total of 50 separate clones were analyzed. Note that the dominant product of 200 bp (rtb1) was partially removed from the mixture used in this analysis, although it is still the major product. The occurrence of a number of low incidence clones is indicative that the entire pool has not been defined by this screen.

FIG. 2. Selected rtb clones are constitutively expressed. Northern blot analysis of rtb1, 3, 4, and 7. All probes showed specific hybridization to a single RNA species. S, slot; migration positions of coelectrophoresed molecular weight markers are shown to the left in kilobases. Lanes: P, procyclic; B, bloodstream form. Probes are indicated at the top.

sages, and to compare expression in procyclic and bloodstream form (BSF) (Fig. 2). Signal intensities were generally low compared to other trypanosome mRNAs (data not shown) consistent with observations in other cell types that both the rab messages and proteins are present in low abundance. In fact, the rtb2 (RAN/TC4) message was apparently the most abundant, consistent with a greater abundance of the RAN protein compared to rabs (Field *et al.* 1995). The message sizes for all the rtbs analyzed were in the range of 1.5–3.0 kb (Fig. 2). Only rtb4 demonstrated evidence for developmental regulation, being somewhat less abundant in the procyclic form. However, as rtb4 was initially identified by PCR using procyclic cDNA as template, expression clearly cannot be fully suppressed in this form. The minor bands observed with some probes may represent rare alternatively spliced forms of the mRNA and/or cross hybridization to related mRNAs (but see below).

In view of both the polycistronic nature of transcription and a number of examples of linked genes in *T. brucei* (Chung and Swindle

1990; Tschudi and Ullu 1988; Gibson *et al.* 1988) we next chose to examine the genomic arrangement of the rtb genes. Southern blot analysis using genomic DNA digested with a selection of six restriction enzymes for each of the rtb clones was performed at high stringency. These single digest data demonstrated similar patterns for rtb1 and rtb7 while the other clones gave unique patterns (Fig. 3A). Southern blot

analysis following double restriction digestion confirmed the similarity of the hybridization patterns for rtb1 and rtb7 (Fig. 3B). The smallest common fragment that was detected was ~5 kb, suggesting that rtb1 and rtb7 may be linked. These data indicate that while the rtb genes are not clustered as a large single array, the apparent linkage of rtb1 and rtb7 could suggest the presence of more limited linkage groups. They
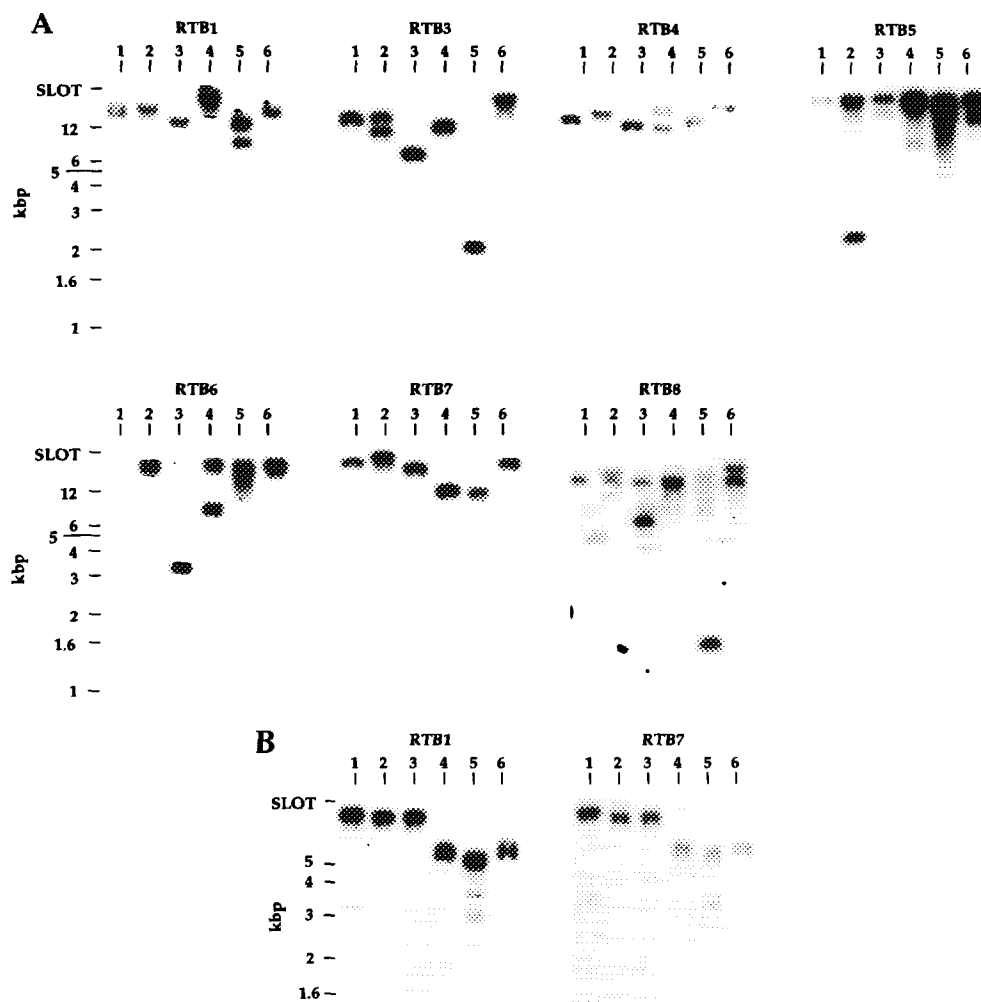

FIG. 3. Southern analysis of rtb1 and 7 indicates that they are linked in the *T. brucei* genome. (A) Single restriction digest genomic Southern analysis of rtb genes. Note that digestion of the DNA in lane 4 of the RTB1 panel was incomplete, resulting in anomalous hybridization position. Enzymes used were: lanes 1, *Bam*HI; lanes 2, *Cla*I; lanes 3, *Eco*RI; lanes 4, *Hin*dIII; lanes 5, *Sac*II; lanes 6, *Xho*I. (B) Double restriction digest genomic Southern analysis of rtb1 and rtb7. Enzymes used were: lanes 1, *Bam*HI + *Cla*I; lanes 2, *Bam*HI + *Eco*RI; lanes 3, *Eco*RI + *Cla*I; lanes 4, *Bam*HI + *Sac*II; lanes 5, *Cla*I + *Sac*II; lanes 6, *Eco*RI + *Sac*II. Migration positions of coelectrophoresed molecular weight markers are shown at left in kbp.

also suggest that the minor bands seen in the Northern blot analyses do not represent cross-hybridization to related mRNAs unless the genes involved are adjacent (or nearly so) in the genome.

In a small number of the digests two bands of approximately equal intensity were observed that hybridized to the probe, e.g., lane 5 in RTB1, lane 2 in RTB3. This was not due to the presence of a restriction site within the probe region. Two potential explanations include allelic differences in restriction sites or hybridization to highly related nearly identical genes which are not resolved with most of the enzymes used in this study. From the data here it is not possible to discriminate between these alternatives.

In order to rigorously confirm the assignment of linkage between rtb1 and rtb7 and to investigate the genomic region around these genes in more detail a λFIX genomic *T. brucei* library was screened using the rtb1 partial cDNA as a probe. Several dozen positive clones of ~50,000 plaques were identified and 6 were purified to homogeneity. Analysis of these genomic clones by PCR using primers specific for rtb1 and rtb7 using the phage DNA as template (see Fig. 4 and Materials and Methods for details) revealed that of the 6 clones 4 produced specific products for both rtb1 and rtb7 of the expected size. Two of these double positive clones, R111 and R131, were selected for further analysis. The PCR products for rtb1 and rtb7 generated from both R111 and R131 were independently cloned into pCRScript and sequenced. The products from clone R111 were identical to the respective rtb cDNA clone (Fig. 4) except for a base inversion at nucleotides 135 and 136 in the rtb7 sequence. The products from R131 were unrelated to G-protein gene sequences or to anything in the GenBank/EMBL databases, and therefore probably arose by fortuitous primer annealing. This is also consistent with the observation that the restriction maps of R111 and R131 are not related (data not shown). Therefore these data indicate that the genes encoding rtb1 and rtb7 are indeed contained within a contiguous DNA segment of the *T. brucei* genome.

DISCUSSION

The molecular basis for secretion in *T. brucei* has yet to be investigated in great detail. It is clear that there is a high degree of similarity in the processing of polypeptides by the trypanosome exocytic machinery and that seen in the higher eukaryotes (e.g., Bangs *et al.* 1986; Duszenko *et al.* 1988), yet there is also considerable evidence that some aspects of the secretory path are novel: the apparent restriction of all trafficking to the flagellar pocket, ~2% of the cell surface area, represents a remarkably high level of membrane specialization (Webster and Russell 1993). With the exception of the homologue of the heavy chain binding protein, BiP, none of the components of the *T. brucei* secretory system have been cloned (Bangs *et al.* 1993). We have used a strategy to analyze the rab family which is of particular interest because numerous studies have implicated these proteins as important components of the secretory system (reviewed in Ferro-Novick and Novick 1993, Nuoffer and Balch 1994). Their specific association with a restricted component of the exo/endocytic system makes these proteins attractive as markers for further dissection of the process of exocytosis in *T. brucei*.

In this paper we report the partial cDNA sequences of seven small G-proteins, all clearly members of the *T. brucei* rab subfamily. Based on the frequency of the various PCR products in the initial screen we propose that the number of proteins comprising the rab family in *T. brucei* is probably similar to that found in the higher eukaryotes, i.e., of the order of 20 members. We have designated these clones by the acronym rtb for rabs from *T. brucei*.

Southern blot analysis (this paper and Field *et al.* 1995) demonstrated that six of the rtb genes were not closely adjacent to each other in the *T. brucei* genome: at high stringency, only single bands were generally seen with each of these rtb probes, suggesting that very close homologues to the sequences obtained by PCR were not present elsewhere in the genome. This is consistent with the sequence data for the PCR products, as the sequences are clearly derived from

RTB1FUS

RTB1cDNA CGC TAT TAT TAG AAC AGT TTC TGT ACT ATA TTG AGT CCA CTT CGC ATG ATC ACA GCA GCT
TRANS   M   I   T   A   A

genomic   ... ...
RTB1cDNA TCC CCT GGT GGG GAC TGC GAC TAC ATA TTC AAG ATT ATT GTC ATT GGC GAC AGT GGC GTT
TRANS   S   P   G   G   D   C   D   Y   I   F   K   I   I   V   I   G   D   S   G   V

genomic   ... ...
RTB1cDNA GGG AAG TCT TCC CTT ACG GTG CGG CTC TCT GAG GAT GTT TTT TAC AAG GAT TAC GCA TCC
TRANS   G   K   S   S   L   T   V   R   L   S   E   D   V   P   Y   K   D   Y   A   S

RTB1cDNA ACC ATC GCA ATT GAT TTT CGA ATG CAC CAA ATG ACG TAC ATG GAT AAG CGT GTC CGC TTG
TRANS   T   I   A   I   D   F   R   M   H   Q   M   T   Y   M   D   K   R   V   R   L

WD box

RTB1cDNA CAA ATA TGG GAC ACC GCC GGA CAA GAG
TRANS   Q   I   W   D   T   A   G   Q   E

RTB7PCR

RTB7cDNA GGG AAG GAA ATA TAG GGT TAG GTG AAG TTT CCT TAC ATG AAA GAG GAA CCC GCT TAC AAG
TRANS   M   K   E   E   P   A   Y   K

RTB7cDNA ATT ATA GTA ATT GGT GAT GTG GGT GTA GGG AAG AGC AAC ATC TCA TCG CGG TTC TGT GAC
TRANS   I   I· V   I   G   D   V   G   V   G   K   S   N   I   S   S   R   F   C   D

genomic   .. ..t g.. ... ...
RTB7cDNA AGC ATT TAT TAC GAG TAC ATT GTT CCA ACG ATT GGC GTG GAC TTC AAG TAT TGC CAT ACC
TRANS   S   I   Y   Y   E   Y   I   V   P   T   I   G   V   D   F   K   Y   C   H   T

genomic   ... ...   **WD box**
RTB7cDNA ACA ACT TTA GAA AAG CAC GCG CGC ACC ATC TTG CTT CAG ATT TGG GAT ACC GCC GGC GAC
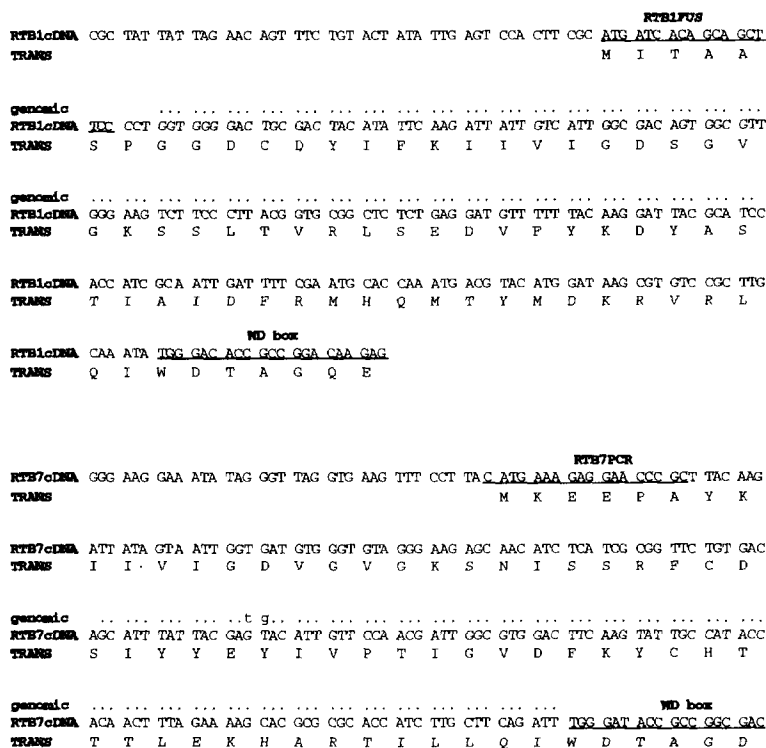TRANS   T   T   L   E   K   H   A   R   T   I   L   L   Q   I   W   D   T   A   G   D

Fig. 4. PCR analysis of genomic clone R111 confirms linkage of Rtb1 and Rtb7. Uppercase nucleotides designate the sequence determined for the partial cDNA PCR clones, with the translated open reading frame shown below. Lowercase nucleotides designate the genomic DNA-derived sequences as obtained by PCR from clone R111 where different from the cDNA. Identities are indicated by a dot. Positions of the complementary regions of the primers are designated by underlining of the cDNA sequence (additional nucleotides added to the 5' end of each primer to provide a cloning site are not shown for clarity). PCR reactions were performed using either RTB1FUS and WD box for RTB1 or RTB7PCR and WD box for RTB7 specific primer sets. The products were directly cloned into pCRScript and sequenced. The natural sequence in the region of the WD box primer cannot be deduced from this analysis.

related but distinct messages. RTB 1 and 7 are physically linked based on Southern blot data and the isolation of a genomic clone containing both genes, but sequence data encompassing the N-terminal third of the ORFs do not suggest that these genes are more similar to one another than to the other members of the rtb family, suggesting that they are not the result of a simple recent tandem duplication. Full-length sequence analysis is required to resolve this issue and is in progress. In the case of VSG, genes encoding the VSG basic copies are grouped together as an array, but those comprising the most closely related group, i.e., a family, are often dispersed among several clusters, e.g., the 117 VSG fam-

ily (Beals and Boothroyd 1992). Although the rtb gene family is certainly much smaller than the VSG gene family, from the results presented here it is a distinct possibility that the genomic organization has similarities to the VSG gene repertoire. As we estimate that there may be several more rtb genes that have not been detected in this study, it remains a possibility that the apparently unlinked rtb genes are close to other G-protein genes.

The clones described in this report represent key reagents for beginning to dissect the secretory pathway in trypanosomes. In addition, we have evidence for the linkage of at least two of the rab genes within the trypanosome genome,

which raises the possibility that some genes encoding small G-proteins are preferentially clustered into arrays. Further analysis of the genomic context of these genes may provide yet more members of the rtb family, while the study of subcellular localization of the proteins encoded by the rtb genes, together with functional disruption experiments, will be a new approach into dissection of mechanisms of vesicle trafficking in *T. brucei*.

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