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Molecular and Biochemical Parasitology 69 (1995) 131–134

MOLECULAR AND BIOCHEMICAL PARASITOLOGY

A homologue of the nuclear GTPase Ran/TC4 from *Trypanosoma brucei*

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Received 28 October 1994; accepted 17 November 1994

Keywords: GTPase; *Trypanosoma brucei*; G protein; Molecular cloning

Small GTPases related to the protooncogene product ras have been implicated as being involved in a very large number of cellular processes. The ras superfamily includes the rac and rho proteins, involved in oxidative burst and cytoskeletal organisation respectively and the rab proteins, important in vesicle trafficking [1]. The rab family is most closely related to a further subset, the ran/TC4 GTPases, which are important both for nuclear import and control of the cell cycle [2–4]. All of the small GTPases have a molecular mass of 20–25 kDa, most are isoprenylated at the C-terminus by a farnesyl or geranylgeranyl moiety, a notable exception being ran. In all of the superfamily members the residues which make up the GTP-binding site are highly conserved in four blocks, and this accounts for a large part of the homology of the N-terminal portion. A fifth homology block (number 2 in Fig. 1) is the effector domain, which, in the case of ras and several other superfamily members, interacts with the GTPase activating protein. The C-terminal third is hypervariable and has limited homology between different GTPases. The GTPases are believed to function by switching between two states, a GTP-bound form and a GDP-bound form. Conversion from one to the other is achieved by GTP-hydrolysis or guanine-nucleotide exchange. As part of our studies on trafficking in trypanosomes we have cloned and analysed a member of the G protein superfamily homologous to ran.

A polymerase chain reaction (PCR) was performed using total *Trypanosoma brucei brucei* procyclic cDNA as template, using a miniexon forward primer (GGCCAGGATCCCGCTATTATTAGAA-CAGTTTCTGTACT) and a degenerate reverse primer (GGCCGAATTCYTCYTGNCCNGCNGTR-TCCCA (512-fold degeneracy)) designed to the highly conserved WDTAGQE box of the GTP binding site. The resulting PCR fragments were cloned into pBluescript, and sequenced by the dideoxy pro-

Abbreviations: PCR; Polymerase chain reaction

Note: Nucleotide sequence data reported in this paper are available in the EMBL, GenBank™ and DDJB data bases under the accession number U17085 and U17086 .

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SSDI 0166-6851(94)00209-6

cedure. Several distinct products were obtained from this reaction, and one of them, designated rtb2 (for *r*as homologue in *T. b*rucei)was studied further.

The rtb2 pBluescript insert was used to probe a λZAP trypanosome cDNA library (kindly provided by J. Mansfield and derived from bloodstream form RNA from *T. brucei rhodesiense*). A positive clone was identified and found to contain a near full length copy of the cDNA corresponding to the rtb2 PCR clone. The entire insert was sequenced using dye-terminator chemistry on an Applied Biosystems 373 DNA sequencer and the resulting data were analysed by a FASTA search [5]. Rtb2 was found to have the highest homology to ran/TC4 from a number of organisms and scored significantly greater for this class of small G-proteins than for any other G-protein class, including the closely related rabs (data not shown).

The deduced rtb2 protein sequence, aligned with ran/TC4 homologues from several organisms is shown in Fig. 1. The five homology blocks (1 and 3–5 of the GTP-binding site, and 2 the effector domain) are well conserved and there are no clear differences between rtb2 and the other ran sequences that set it apart. Homology at the C-terminal region is characteristically poor (approximately residue 160 onwards). Rtb2 terminates with a glutamate followed by two aspartate residues, and although none of the other ran sequences terminate in EDD, two of the other four proteins contain an EDD sequence in the terminal pentapeptide and all have highly acidic C-terminal sequences. This conservation may indi-

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                            1                        2
Rtb2      MQASST-ADC-VATFKLVLVGDGGTGKTTFVKRHLTGEFEKRYVATVGVD 50
Spi1      .-.Q--.PQ-N.PT...........................K.I.....E
PfRan     .DSQ--EYI-.-PQY..I......V................K.IP.L...
TC4       .A.Q-GEPQ-..-Q...........................K....L..E
Dd.TC4    .-.---E-K-.-EQI.........V......Q........P..IP.L..S
                         3
Rtb2      VHPLTFHTNRGKICFNCWDTAGQEKFGGLRDGYYIEGQCAIIMFDVTSRN 100
Spi1      ....H....F......V..................Q...G.........I
PfRan     ....K.Q..F...Q..V..................KSD........S..I
TC4       ....V......P.K..V..................QA............V
Dd.TC4    ....I.Y..F...H..V..................Q.N...........I
                                    4
Rtb2      TYKNVPNWHRDITGVCDNIPIVLVGNKVDCAERQVKAKMITFHQKG-LQY 150
Spi1      ......H.W..LVR..E......C.....VK..K....A....R.KN...
PfRan     ......N.Y....RV.ET..M........VKD....SRQ.Q..R.RN...
TC4       ...........LVR..E......C.....IKD.K....S.V..R.KN...
Dd.TC4    S........S.L.R..E......C.....VKD.K..PSQ.V..RRYN.S.
              5
Rtb2      YDISAKSNYKPSEKTVPV-ARKELANDPNLTLVKAPMLD-PNVQPLTAEQ 200
Spi1      .........-NF..PFLWL...-.VG....EF.AS.A.AP.E..VDQQ--
PfRan     .....R...-NF..PFLWL..R-.S.Q...VF.GEHAKA-.EF.IDLN--
TC4       .........-NF..PFLWL...-.IG....EF.AM.ALAP.E.VMDP.--
Dd.TC4    ..V......-NF..PF-.LTSK-.LGNKAV...QQ.T.KL.ETVLDSN--

Rtb2      LQALQE-E-ARAVENVVLPMGEDD
Spi1      .L.QYQQ.MNE.-AAMP..-D...ADL
PfRan     IVREA.K.LEQ.-AA.AI--D.E.IEN
TC4       .A.QY.HDLEV.-QTTA..-D...-DL
Dd.TC4    .MSLY.K.V.D.-AALP..--..NDDL
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Fig. 1. Rtb2 encodes the *T. brucei* homologue of ran. Comparison of the deduced amino acid sequence of rtb2 with the deduced amino acid sequences of a number of the TC4/ran subfamily. Spi1 from *Schizosaccharomyces pombe* [8], PfRan from *Plasmodium falciparum* [9], TC4 from *Homo sapiens* [10] and Dd.TC4 from *Dictyostellium discoideum* [11]. Dots indicate residues identical to rtb2, dashes gaps introduced to maximise the alignment. The five regions involved in GTP/GDP binding are indicated in bold. The amino acid sequence was deduced from the λZAP clone except for the first six amino acids which were deduced from the initial PCR-generated cDNA. The sequences of these two clones were identical in the region of overlap (approx. 200 nucleotides). No full-length cDNA could be found in the λZAP library.

cate a role for the C-terminal region in the function of ran.

The rtb2 PCR clone was used as a probe in both Southern analysis of *T. b. brucei* genomic DNA and in northern analysis of poly(A)$^-$ selected RNA from both procyclic and bloodstream forms. Southern analysis demonstrates that the rtb2 gene is probably single copy based on single and double restriction analysis of genomic DNA (Fig. 2A and data not shown). Northern blot analysis demonstrates that the rtb2 message is constitutively expressed, with a size of about 3 kb. As the 5′ UTR of the rtb2 PCR clone was approx. 200 bp, and the open reading frame encoding rtb2p is approx. 800 bp, it is possible that the 3′ UTR of the message is quite large, but the functional significance of this cannot be assessed from our current data.

Ran is one of the soluble factors required for the import of nuclear localisation signal (NLS)-containing peptides into the nucleus [4,6]. In *S. pombe* there is only one ran homologue (Spi1), but in *S. cerevisiae* there are two closely related forms, GSP-1 and GSP-2. ran proteins are abundant, and not lipid modified [3]. The sequence of rtb2 is consistent with this, as the C-terminal sequence is not homologous with a CAAX-box type isoprenylation signal. Ran is typically found in a complex with a 45 kDa chromatin-binding protein, RCC1, in the nucleus [7].

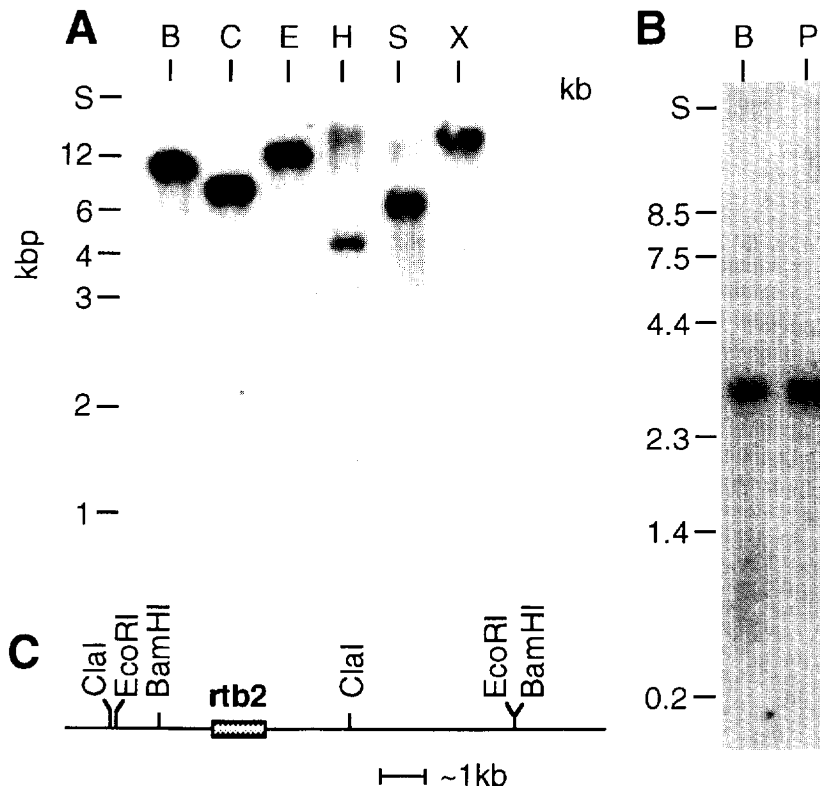

Fig. 2. Genomic organisation and mRNA expression of rtb2. (A) Southern analysis of trypanosome genomic DNA with rtb2 probe. Lanes 1–6, 5 μg DNA digested with the following restriction enzymes: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; S, *Sac*II and X, *Xho*I. (B) Northern analysis of 0.5 μg poly(A)$^+$ RNA from bloodstream (lane B) and procyclic (lane P) form trypanosome. (C) Partial restriction map of the region around the rtb2 locus based on single and double restriction analysis of genomic DNA. Methods: Agarose gel electrophoresis and transfer to nylon membranes of DNA fragments and poly(A)-selected RNA was performed using standard methods. Specific sequences were detected by hybridizing with random-primed rtb2 α-^{32}P-labeled probe overnight and washing with 0.1 × SSC, 0.1% SDS, 65°C for Southern blots, and with 0.5 × SSC, 65°C for northern blots. Migration positions of molecular mass markers are indicated at the left of the relevant panels. S designates the slot position.

RCC1, first identified in HeLa cells, appears to act to maintain ran in the GTP-bound state, and in this role ran has been implicated in the control of cell cycle and the mating response in yeast. Exactly how ran functions in either of its roles is not known at present. Its precise function in *T. brucei* likewise is unknown, but the identification of a homologue is suggestive that control of cell cycle and possibly differentiation may be under the control of this G-protein as in other eukaryotes.

Acknowledgements

We thank Dr John Mansfield (University of Wisconsin) for the λZAP cDNA library, Lyle Uyetake for the poly(A)$^+$ RNA, and Drs Keith Wilson and Ian Manger for invaluable advice.

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