Isolation and characterization of a cDNA encoding a Translin-like protein, TRAX

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Abstract Translin is a DNA binding protein which specifically binds to consensus sequences at breakpoint junctions of chromosomal translocations in many cases of lymphoid malignancies. To investigate its functional significance at such recombination hotspots, we examined whether Translin interacts with other proteins using a yeast two-hybrid system and identified an associated 33 kd protein partner, TRAX, with extensive amino acid homology. The TRAX protein was established to contain bipartite nuclear targeting sequences in its N-terminal region, suggesting a possible role in the selective nuclear transport of Translin protein lacking any nuclear targeting motifs.

Key words: Chromosomal translocation; DNA binding protein; Nuclear transport

1. Introduction

Aberrant chromosomal rearrangements in human lymphoid neoplasms have been found to occur frequently at chromosomal loci where T-cell receptor (TCR) or immunoglobulin (Ig) genes are located, and appear to play a significant role in tumor etiology by dysregulating expression of cellular proto-oncogenes [1]. It has been suggested that the V-D-J recombinase is involved in such interchromosomal rearrangements by recognizing heptamer-nonamer signal sequences [2–4]. In previous studies, however, we found consensus sequences not resembling the signal sequences for Ig/TCR recombinase at breakpoint junctions of chromosomal translocations in many cases of lymphoid neoplasms [5–7]. Subsequently, a novel protein, Translin, exhibiting general binding activity to the above consensus sequences was identified and its cDNA was cloned [8]. Biochemical and electron microscopic studies revealed that Translin polypeptides are held together by disulfide bonds [8] and the subunit of native Translin is a ring-shaped structure which is responsible for its binding to target sequences situated only at single-strand DNA ends (manuscript in preparation). In the present investigation, to provide further insight into Translin function, we used a yeast two-hybrid system [9] to examine whether it might be a member of a multicomponent complex and cloned a cDNA encoding TRAX (i.e. Translin-associated factor X) showing extensive homology with Translin.

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2. Materials and methods

2.1. Yeast two-hybrid cloning

DNA encoding the Translin domain was cloned into the yeast GAL4 DNA-binding domain vector, pGBT9 (Clontech). The resulting plasmid, GAL4bd-Tra (ORF), was used as bait to screen a human spleen cDNA library in two-hybrid interaction analysis which was performed following the Matchmaker Two-Hybrid System Protocol (Clontech). Positive yeast clones were screened by activation of *his* and *lacZ* reporter genes. After transformation of yeast DNA into *E. coli*, pGAD10 plasmids (Clontech) containing cDNA clones were identified by restriction mapping and further characterized by DNA sequencing.

2.2. DNA sequencing

The cDNA inserts of the isolated clones were subcloned into pBluescript SK (Stratagene) and DNA sequencing was performed by the dideoxy chain termination method, using a Taq dye primer cycle sequencing kit and an ABI 373A DNA sequencer (Applied Biosystem). The final sequence was confirmed from analyses of both strands.

2.3. In vitro interaction assays

GST fusion protein containing the Translin domain was expressed using pGEX vectors (Pharmacia) and tested for interaction with ^{35}S-labeled TRAX fusion protein (41 kDa) and β-galactosidase fusion protein (118 kDa) prepared using an in vitro transcription and translation system with pCITE-4a(+) (Amersham). For each in vitro binding assay, aliquots of glutathione-Sepharose beads (Pharmacia) carrying the same amounts of GST or GST/Translin fusion proteins were incubated with ^{35}S-labeled TRAX and β-galactosidase fusion protein in low-stringency NP-40 buffer (20 mM HEPES [pH 7.6], 100 mM NaCl, 0.1% NP-40, 1 mM EDTA) at 4°C for 1 h. After washing several times with the same buffer, proteins on the beads were subjected to 10% SDS-polyacrylamide gel electrophoresis and the amounts of radioactivity were measured with a Fujix BAS 2000 Bio-imaging Analyzer.

2.4. Amplification of cDNA ends

5′-Amplification of cDNA ends was carried out using a 5′-RACE system (Gibco-BRL). First-strand cDNA was synthesized from poly(A)+ RNA of NALL-1 cells (non-T, non-B-ALL) using Moloney murine leukemia virus reverse transcriptase (M-MLVRT) and an F2-specific primer, 5′-GAGACAGCTTCCACATATTC-3′ (primer F2-R1, complementary to nt 536–555). An anchor sequence was then added to the 3′ end of the cDNA using terminal deoxynucleotidyl transferase (TdT). A nested PCR amplification was performed using a primer complementary to the anchor and specific primers, 5′-CAACAGTTATATCCCGACTAAG-3′ (primer F2-R2, complementary to nt 335–356) and 5′-TGAGACGAGCGGAGACTGAA-3′ (primer F2-R3, complementary to nt 124–143). The resulting PCR products (200 bp) were cloned into pBluescript II and four clones were selected for further sequencing.

3. Results and discussion

3.1. Molecular cloning of TRAX

We examined whether Translin might be a member of a multicomponent complex using a yeast two-hybrid system. DNA encoding the full-length Translin was cloned into the yeast GAL4 DNA-binding domain vector and used as bait to

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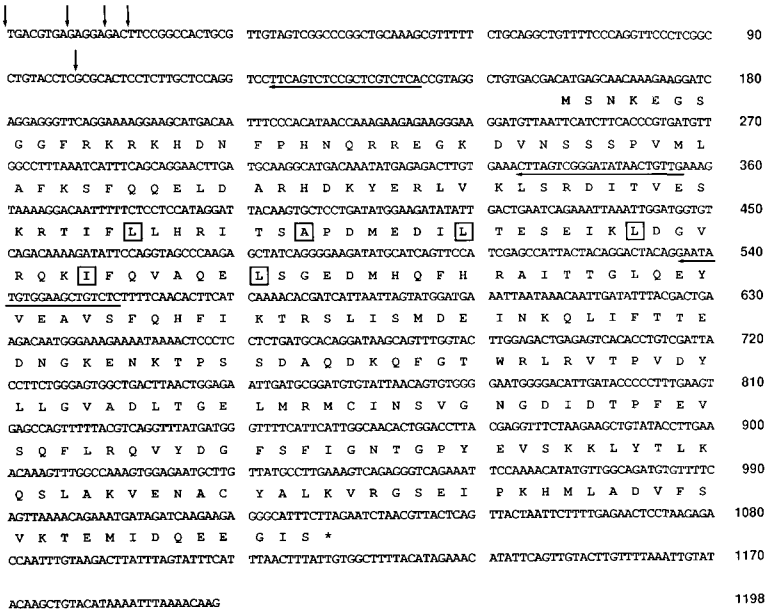

Fig. 1. The nucleotide sequence of the TRAX cDNA and its deduced amino acid sequence. The isolated clone (F2; nt 100–1198) and its 5′-RACE product (nt 1–143, 9–143, 14–143, 17–143) were combined to construct the composite cDNA. The nucleotide sequence and the deduced 290 amino acids of the encoded protein are shown. The stop codon is indicated by an asterisk. The periodic repetition of hydrophobic amino acids is shown in the open box. The horizontal arrows indicate the sequences targeted by the specific primers (F2-R1, F2-R2, F2-R3) used for the 5′-RACE system. The 5′ ends of the F2 clone and the four 5′-RACE products are indicated by the vertical arrows.

screen a human spleen cDNA library in two-hybrid interaction analyses. From screening 6.2×10^6 individual colonies for activation of the *his* reporter gene, 31 clones were obtained. Of these 31 clones, six were selected by activation of *lacZ* reporter genes, but five turned out to be non-specific. We finally selected the one clone F2 whose product gave specific activation with the Translin bait. Nucleotide sequence analysis of this clone revealed an open reading frame (ORF) encoding a protein of 290 amino acids with a predicted molecular weight of 33 kDa (GenBank accession number X95073) (Fig. 1). Since there were no stop codons upstream of the predicted start methionine, we used RACE PCR to extend the insert cDNA in the 5′ direction. Analysis of four resultant clones resulted in the identification of almost identical additional 83, 86, 91 and 99 nt sequences preceding the 5′ end of the original clone. We assumed that the ATG codon at positions 161–163 of the most extended cDNA was the translational initiation codon and named the encoded protein TRAX (i.e. Translin-associated factor X). The TRAX amino acid sequence contains six potential protein kinase C phosphorylation sites (amino acids 2–4, 67–69, 177–179, 240–242, 245–247, 277–279), eight CK2 phosphorylation sites (amino acids

Table 1
Interactions of TRAX and Translin fusion proteins determined by activation of the *lacZ* reporter gene

DNA-binding hybrid	Activation hybrid	Colony color (*lacZ* activity)
A: GAL4bd	GAL4ad-TRAX	White
B: GAL4bd-Tra (ORF)	GAL4ad-TRAX	Blue
C: GAL4bd-lamin	GAL4ad-TRAX	White
D: GAL4bd-p53	GAL4ad-TRAX	White
GAL4bd-Tra (ORF)	GAL4ad	White

Yeast strains HF7c and SFY526 were cotransformed with the GAL4 DNA-binding domain vector pGBT9 encoding the full-length Translin, GAL4bd-Tra (ORF), human lamin C (amino acids 66–230), GAL4bd-lamin, and murine p53 (amino acids 72–390), GAL4bd-p53, and the GAL4 transcription activation domain vector pGAD10 encoding the full-length TRAX, GAL4ad-TRAX. Each transformation mixture was plated on plates lacking tryptophan and leucine, incubated at 30°C for 3 days, and then transferred to nitrocellulose filters for determination of β-galactosidase activity.

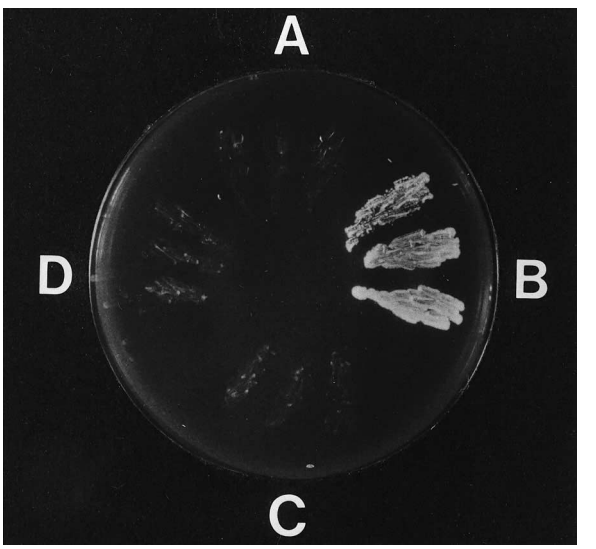

Fig. 2. Interaction of Translin and TRAX fusion proteins determined by *his* reporter gene activation. The yeast strain HF7c was cotransformed with the GAL4 DNA-binding domain vector pGBT9 encoding the domains of various proteins. A: GAL4bd, B: GAL4bd-Tra (ORF), C: GAL4bd-lamin, D: GAL4bd-p53, and the GAL4 transcription activation domain vector, pGAD10, encoding the full-length TRAX, GAL4ad-TRAX. Transformants were streaked onto plates lacking tryptophan and leucine, and incubated at 30°C for 3 days. Three individual colonies appearing on the plates were then streaked onto a plate lacking tryptophan, leucine and histidine, and incubated at 30°C for 2 days.

2–5, 79–82, 88–91, 109–112, 144–147, 155–158, 183–186, 213–216) and one *N*-glycosylation site (amino acids 30–33).

3.2. *TRAX specifically interacts with Translin*

To confirm that TRAX interacts specifically with Translin, DNA encoding the full-length TRAX was cloned into the yeast GAL4 transcription activation domain vector and the resulting plasmid, GAL4ad-TRAX was cotransformed with a GAL4 binding domain vector encoding the domains of various proteins including Translin. Interactions were determined in terms of the ability to activate a *lacZ* reporter construct (Table 1), as well as the *his* reporter construct, and to confer growth in the absence of histidine (Fig. 2). The GAL4ad-TRAX fusion protein interacted with Translin, GAL4bd-Tra (ORF), but not with human lamin C, GAL4bd-lamin and murine p53, GAL4bd-p53.

To examine further the specificity of the interaction between TRAX and Translin observed in the two-hybrid system, the GST-Translin fusion protein, GST-Tra (ORF), was tested for interaction with ^{35}S-labeled TRAX and β-galactosidase prepared by in vitro transcription and translation. As observed in the two-hybrid system, the GST-Tra (ORF) specifically associated with TRAX, but not β-galactosidase (Fig. 3).

3.3. *Comparison of the deduced amino acid sequence of TRAX with that of Translin*

GenBank data base searches using FASTA programs revealed a 28% identity between TRAX and Translin (Fig. 4). While homology was found to exist throughout the two molecules, the C-terminal regions (amino acids 180–264 of TRAX) proved to be the most conserved (38% identity). TRAX contains a previously described bipartite nuclear targeting motif [10] in its N-terminal region, comprising two basic amino acids, a spacer region of any 10 amino acids and a basic cluster in which at least three out of the next five amino acids are basic (**RK**RKhdnfphnq**RR**eg**K**, amino acids 11–27).

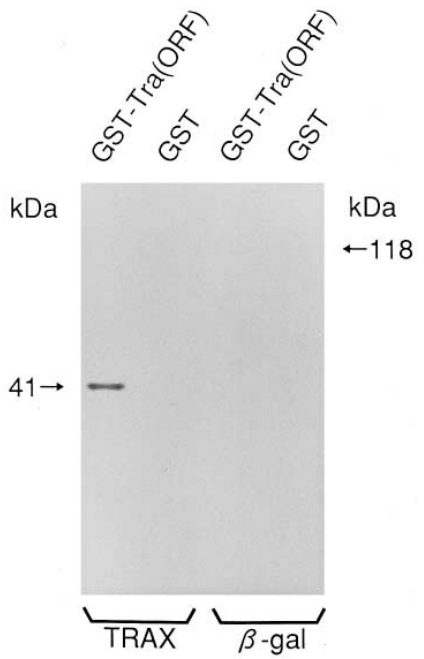

Fig. 3. In vitro interaction of [^{35}S]TRAX with Translin. ^{35}S-Labeled TRAX or β-galactosidase fusion proteins were incubated with GST and GST/Translin fusion protein, GST-Tra (ORF) and processed as described in Section 2.

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TRAX     MSNKEGSGGF RKRKHDNFPH NQRREGKDVN SSSPVMLAFK SFQQELDARH DKYERLVKLS  60
Translin ---------- ---------- ---------- -MS-VSEIFV ELQGFLAAEQ DIREEIRKVV  28

TRAX     RDITVESK-R TIF-LLHRIT S-APDMEDIL TESEIKLD-- -G-VRQKIFQ VAQELSGEDM 113
Translin QSL--EQTAR EILTLLQGVH QGAG-FQDIP KR-CLKAREH FGTVKTHLTS LKTKFPAEQY  84

TRAX     HQFHRAITTG LQEVVE-AVS FQHFIKTRSL ISMDEI-NKQ L-IFTT-EDN GKENKTPSSD 169
Translin YRFHEHWRFV LQRLVFLAA- FVVYLETETL VT-REAVTEI LGIEPDRE-- ---------- 130

TRAX     AQDKQFGTWR LKVTPVDYLL GVADLTGELM RMCINSVGNG DIDTPFEVSQ FLRQVYD-GF 228
Translin ---K--GFH- LDV--EDYLS GVLILASELS RLSVNSVTAG DYSRELHIST FINEL-DSGF 181

TRAX     SFIGNTGPYE VS-KKLY-TL KQSLAKVENA CYALKVRGSE IPKH-MLADV FSVKTEMIDQ 285
Translin RLL-NLKND- -SLFKRYDGL KYDVKKVEEV VYDLSIRG-- FNKETAAACV EK-------- 228

TRAX     EEGIS                                                          290
Translin -----                                                          228
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Fig. 4. Alignment of the human TRAX and Translin proteins. The alignment was maximized by introducing insertions marked by dashes. Residues conserved in the two sequences are boxed. The bipartite nuclear targeting motif in the N-terminal region of TRAX is underlined.

Our previous studies indicated the native form of Translin to be present in the cytoplasm of cell lines of various lineages, while nuclear localization was limited to lymphoid lineage cells with rearranged Ig and TCR loci. These observations raise the intriguing possibility that nuclear transport of Translin is regulated in a physiologically significant way. Translin, however, does not contain any nuclear targeting sequences. Therefore, it is conceivable that other factors such as TRAX with nuclear targeting motifs are involved in the active nuclear transport of Translin. The TRAX has a heptad repeat of hydrophobic amino acids, leucine, alanine, leucine, leucine, isoleucine and leucine (amino acids 73, 80, 87, 94, 101 and 108 respectively), which is consistent with the hypothetical structure referred to as the 'leucine zipper'. The question now has to be addressed whether TRAX homodimerizes or heterodimerizes with Translin by this putative leucine zipper motif, for regulating cellular functions, reminiscent of observations with Bcl-2-Bax [11] or Myc-Max interactions [12–15].

Northern blot analysis of TRAX gene expression revealed a single transcript of approximately 2.7 kb and the distribution of TRAX mRNA in various tissues was like that of Translin (data not shown). All of these results, including the extensive amino acid homology and the similar transcription pattern of mRNA, together with the evidence of in vivo and in vitro interaction of the two proteins, suggest that TRAX is a member of the Translin protein family.

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