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SHORT COMMUNICATION

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Genomic structure and chromosomal localization of the gene encoding TRAX, a Translin-associated factor X

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Abstract The *TRAX* gene encodes a Translin-associated 33-kDa protein partner, TRAX. The TRAX protein has extensive amino acid homology with Translin, and contains bipartite nuclear targeting sequences, suggesting a possible role in the selective nuclear transport of Translin lacking any nuclear targeting motifs. In the present study, genomic clones of the human *TRAX* gene were isolated to determine the complete genomic organization. The genomic structure of the human *TRAX* gene was similar to that of the human *Translin* gene, consisting of six exons and five introns, encompassing approximately 27 kb in genomic DNA. Northern blot analysis revealed a predominant transcript of approximately 2.7 kb, and its distribution in various tissues was like that of *Translin*. Chromosomal mapping by fluorescence in situ hybridization (FISH) analysis allowed localization of the *TRAX* gene to human chromosome 1q41.

Key words *TRAX* · Chromosome 1q41 · Nuclear targeting motif · *Translin* · Leucine zipper · Heteropolymer

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Introduction

We have previously identified the Translin-associated protein X, TRAX (Aoki et al. 1997a), a novel protein of 290 amino acids with a predicted molecular mass of 33 kDa, using a yeast two-hybrid system. Translin itself was originally identified as a DNA binding protein that specifically recognized the consensus sequences found at the breakpoints of chromosomal translocations in many cases of lymphoid neoplasms (Kasai et al. 1992; 1994; Aoki et al. 1994; 1995). Electron microscopic and crystallographic investigations have revealed that Translin is a ring-shaped structure with an assembly of eight subunits, and this multimeric protein binds to target sequences situated only at single-strand DNA ends (Kasai et al. 1997). In order to provide further insight into Translin function, we used a yeast two-hybrid system to examine whether it might be a member of a multicomponent complex, and cloned a cDNA encoding *TRAX* (Aoki et al. 1997a). Comparison of the amino acid sequence of TRAX with that of Translin showed 28% identity throughout the two molecules, with 38% identity at the C-terminal regions, suggesting that TRAX is a member of the Translin family. The TRAX protein contains bipartite nuclear targeting sequences in its N-terminal region (Aoki et al. 1997a), suggesting a possible role in the selective nuclear transport of Translin protein lacking any nuclear targeting motifs. It has been reported that TRAX and Translin associate in vivo as components of the NS1 strand-specific DNA binding complex enriched in brain (Taira et al. 1998). Because Translin was found as a homopolymer in lymphoid cells, these results raise the possibility that Translin forms heteropolymers with TRAX in the central nervous system and regulates cellular functions, reminiscent of observations with Bcl-2-Bax (Oltvai et al. 1993) or Myc-Max interactions (Blackwood and Eisenman 1991). Here we report the expression profile, genomic organization, and chromosomal localization of the human *TRAX* gene.

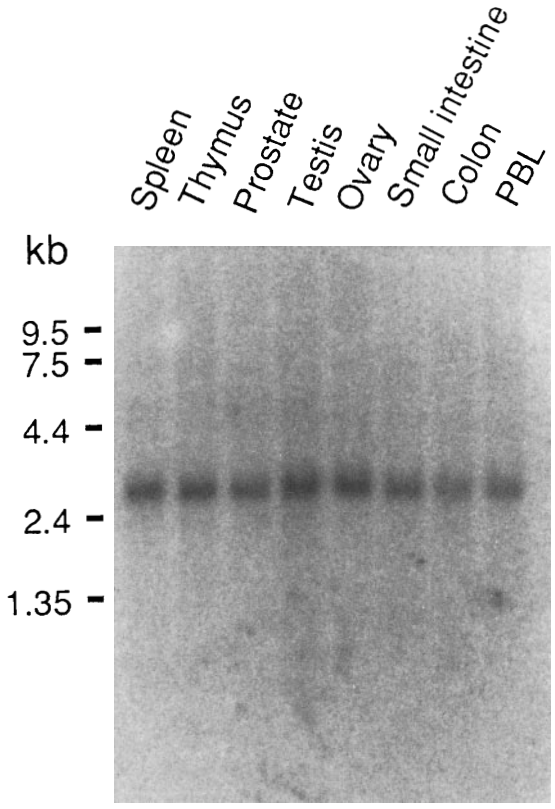

Fig. 1. Northern blot analysis of *TRAX* mRNA in various human tissues. Northern blot analysis of human multiple tissue blots (Clontech, Palo Alto, CA, USA) was performed according to the manufacturer's instructions, using a 0.9-kb TRAX open reading frame (ORF) as the probe. Individual lanes contain 1 μg of poly(A)$^+$ mRNA from the indicated tissues. *PBL*, Peripheral blood lymphocytes

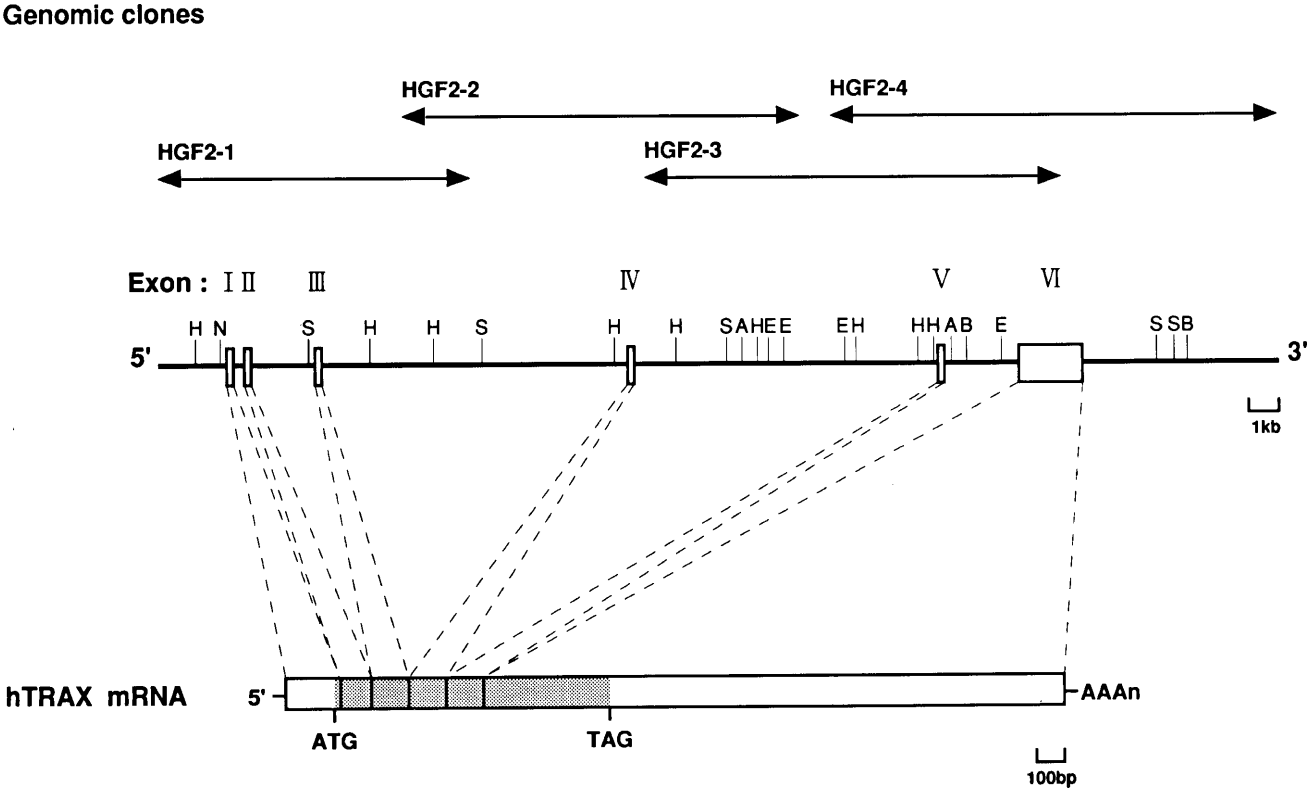

Fig. 2. Genomic structure of the human *TRAX* gene. Schematic representation of intron/exon distribution for the human *TRAX* gene. The exons, *numbered I-VI*, and the introns are represented by *open boxes* and *lines*, respectively. The translated region of mRNA is designated by *filled boxes*. Restriction sites for *Asp*718 (*A*), *Bam*HI (*B*), *Eco*RI (*E*) *Hind*III (*H*), *Not*I (*N*), and *Sst*I (*S*) are shown. The positions of the translation initiation codon (*ATG*) and the termination codon (*TAG*) are also indicated. *HGF2-1*, *HGF2-2*, *HGF2-3*, and *HGF2-4* are form different overlapping clones

Methods and results

Expression profile of human *TRAX* gene

To determine the size and expression pattern of *TRAX* mRNA, multi-tissue Northern blots were probed with a 0.9-kb *TRAX* open reading frame (ORF) encoding a protein of 290 amino acids with a predicted molecular weight of 33 kDa. The autoradiograms of the blots are shown in Fig. 1. A major transcript, of approximately 2.7 kb, was detected in spleen, thymus, prostate, small intestine, colon, and peripheral blood lymphocytes (PBL), and at slightly higher levels in testis and ovary. Thus, the expression profiles in the various tissues suggest that the human *TRAX* gene, like Translin, is expressed ubiquitously in all adult tissues.

Genomic structure of human *TRAX* gene

Screening of a human genomic library with ^{32}P-labeled 1.2-kb *TRAX* cDNA resulted in the isolation of four different overlapping clones, called HGF2-1, HGF2-2, HGF2-3, and HGF2-4, with estimated sizes of 10, 13, 13.2, and 14.3 kb, respectively. They were analyzed by restriction mapping. Comparisons of the *TRAX* genomic and cDNA sequences (GenBank accession number, X95073) revealed that the genomic clones contained all of the sequences characterized in the cDNAs, with five introns, ranging in size from 0.4 to 10.2 kb (Fig. 2). It was also shown that the entire human *TRAX* gene was composed of six exons, encompassing approximately 27 kb in genomic DNA. The sizes of exons 1–6 were more than 175, 105, 115, 131, 128, 1994 bp. All junction sequences proved to be in agreement with the consensus splice signals at intron/exon junctions, i.e., all splice donors were GT and acceptors were AG (Breathnach and Chambon 1981). Thus, the genomic structure of the *TRAX* gene is similar to that of the *Translin* gene, which consists of six exons of size more than 147, 94, 97, 116, 80, 2158 bp (Aoki et al. 1997b).

GeneBank data bank searches using FASTA programs revealed a 28% identity between TRAX and Translin. While homology was found to exist throughout the two molecules, their most conserved C-terminal regions (38% identity) were localized to exon 6 (Aoki et al. 1997a). The TRAX protein has a heptad repeat of hydrophobic amino acids — leucine, alanine, leucine, leucine, isoleucine, and leucine. This putative leucine zipper motif of TRAX for multimer formation was shown to be encoded by exons 3 and 4, while that of Translin was encoded by exon 6. The translation, initiation, and termination sites were localized to exons 1 and 6 in both TRAX and Translin. Like the promoter region of Translin, several promoter-like domains were found in the upstream region of the human *TRAX* gene, and this region was highly GC-rich (data not shown).

Chromosomal location of the human *TRAX* gene

Southern blot analysis revealed that the *TRAX* gene was present as a single copy in the human genome (data not shown). The chromosomal location of the *TRAX* gene was refined by fluorescence in situ hybridization (FISH) on metaphase chromosome spreads, as described previously (Inazawa et al. 1993). Hybridization of the biotinylated probe, HGF2-4, and detection via fluorescein isothiocyanate (FITC) resulted in the observation of specific signals on the long arm of chromosome 1 at band q41 (Fig. 3).

Discussion

We have isolated genomic clones of the human *TRAX* gene and determined the complete genomic structure. Chromosomal mapping by FISH analysis allowed localization of the *TRAX* gene to human chromosome 1q41. Comparison of the human *TRAX* and *Translin* genes revealed that they

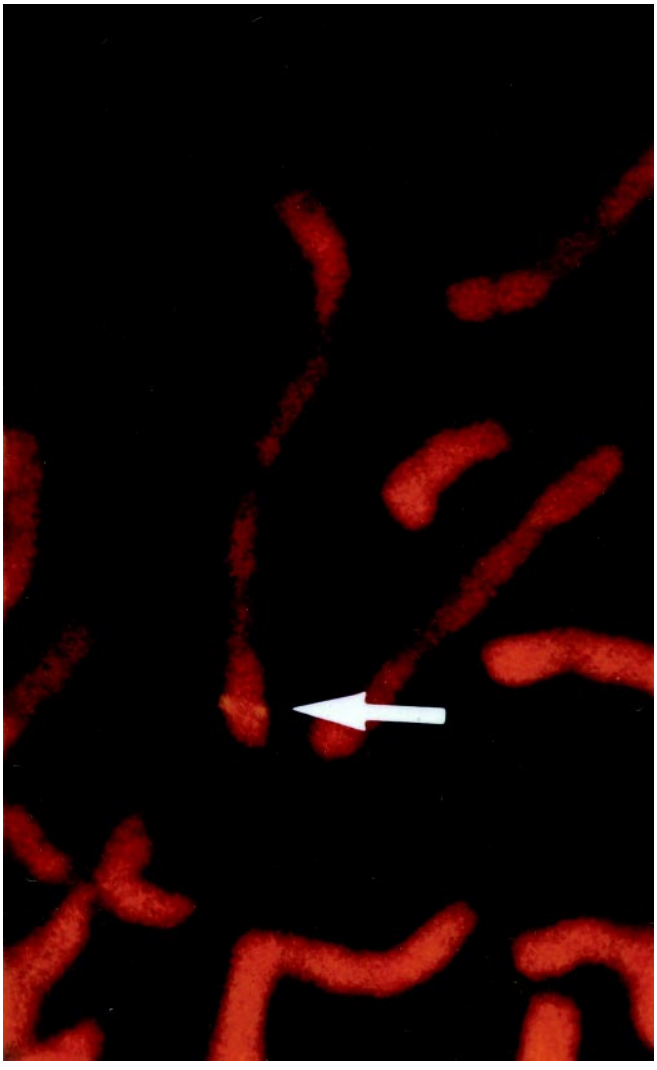

Fig. 3. Chromosomal localization of the human *TRAX* gene. Fluorescence in-situ hybridization was performed with the biotin-labeled probe, HGF2-4, hybridized to human metaphase chromosomes. A partial R-banded prometaphase shows spots (*arrow*) on both chromatids of chromosome 1q41

share not only a high homology in the amino acid sequences of their encoded proteins but also a high degree of structural similarity at the genomic level, each of them consisting of six exons and five introns, encompassing approximately 27 kb in genomic DNA. The expression profiles in various tissues also suggest that TRAX is a member of the Translin family. The TRAX protein has a putative leucine zipper motif, which is thought to heterodimerize with Translin, and contains a bipartite nuclear targeting motif in its N-terminal region. Therefore, it is conceivable that TRAX regulates the active nuclear transport of Translin in a physiologically significant way. It is of particular interest and importance to address the question of what determines the interaction of TRAX and Translin proteins in various tissues. Further studies are now needed to provide clues to understanding the functional implications of TRAX-Translin interactions for human diseases.

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