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Biochimica et Biophysica Acta 1306 (1996) 9–13

BBA Biochimica et Biophysica Acta

Short sequence-paper

Isolation of a cDNA clone, TRX, encoding a human T-cell lymphotrophic virus type-I Tax_1 binding protein

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Received 3 January 1996; accepted 9 January 1996

Abstract

Tax_1 is essential for human T-cell lymphotropic virus type I (HTLV-I) virus replication and transformation. We have identified and characterized a Tax_1 binding protein, TRX, by cDNA screening of a Jurkat T-cell cDNA library. TRX mRNA is ubiquitously expressed in human tissues tested and cell lines analyzed.

Keywords: HTLV-1; Tax_1; Tax_1 binding protein

The T-cell lymphotropic virus type-I, HTLV-I, is associated with an aggressive malignancy of mature $CD4^+$ T-cells, adult T-cell leukemia (ATL), and tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) [1,2]. HTLV-I encodes a 40-kDa protein, Tax_1, which plays a key role in viral replication, transformation and gene regulation. Tax_1 is a potent transactivator of viral and cellular promoters [3–9]. Tax_1 mediates its transcriptional activity through association with cellular transcription factors [10–13]. Several Tax_1 binding proteins have been characterized through experiments designed to identify cis-acting elements and DNA-binding proteins, through which Tax_1 regulates transcription [14–16]. Tax_1 binding proteins, which play an important role in cellular proliferation and transformation, may have been missed by this approach. To circumvent this problem, we have used purified, biologically active, recombinant Tax_1 to screen a T-lymphocyte λgt11 cDNA expression library. We now report the characterization of a cDNA clone that encodes a protein, TRX (*T*ax *R*eactive protein *X*), which physically interacts with Tax_1. The TRX cDNA encodes a 221 amino acid open reading frame, producing a 27 kDa protein. TRX mRNA is ubiquitously expressed in normal human tissues.

TRX was originally identified following the screening of a human Jurkat T-cell cDNA library in a λgt11 vector. A total of 5×10^5 phage plaques were transferred to nitrocellulose filters (Schleicher and Schuell, BA38), incubated with bacterially expressed purified Tax_1 protein, washed, and further incubated with a cocktail of four monoclonal antibodies against Tax_1 (TAbs) (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH). The filters were then probed with sheep anti-mouse monoclonal antibodies conjugated to alkaline phosphatase (SAM-AP). The location of positive plaques was visualized using chromophore precipitation with NTB and BCIP [17] (Fig. 1A, left). These positive plaques were picked, replated, and subjected to three additional rounds of screening and purification until a homogeneous clone of the Tax_1 binding protein was identified (Fig. 1A, right). To demonstrate the specificity of the interaction between Tax_1 and the positive clone, additional control experiments were performed. The filters were cut into quadrants and each was subjected to a different assay (Fig. 1B, left). Quadrant 1: Tax_1, followed by TAbs and SAM-AP. Quadrant 2: Mock HB101 extract followed by TAbs and SAM-AP.

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PII S0167-4781(96)00012-7

Quadrant 3: TAbs followed by SAM-AP. Quadrant 4: SAM-AP antibody alone. A negative control wild-type λgt11 phage stock was used in parallel (Fig. 1B, right).

To further test the interaction of our clone with Tax_1, the purified phages were grown to near confluence and a plate lysate was prepared [17]. The proteins were concentrated using TCA precipitation, separated by SDS-PAGE electropheresis and transferred to a nitrocellulose filter. Following a denaturation/renaturation step, the blot was incubated with purified Tax_1 or HB101 extract, washed and incubated with TAbs. Tax_1 was shown to specifically interact with the 132 kDa β-galactosidase/TRX fusion protein (Fig. 2, lane 1). Tax_1 did not interact with any proteins in control λgt11 phage plate lysate (Fig. 2, lane 2).

Next, the insert of a single positive plaque was cloned into a pCRII vector (Invitrogen). An in vitro translation reaction using this plasmid resulted in the synthesis of the 23 kDa TRX protein (Fig. 3, lane 1). Tax_1 protein, purified from baculovirus or *Escherichia coli* (*E. coli*), was used to coimmunoprecipitate the ^{35}S-labelled TRX protein from solution (Fig. 3). TRX was precipitated only in the presence of Tax_1 or anti-Tax antibody TAb 172 (Fig. 3, lanes 3 and 5). Control IgG_{2a} sera did not precipitate TRX (Fig. 3, lanes 2 and 4).

Following isolation and sequencing of the full-length cDNA clone, computer analysis of the sequence revealed a 221 amino acid open reading frame within the insert (Fig. 4A). The 340 bases downstream of the TGA stop codon contain Alu repetitive elements (data not shown). Fig. 4B

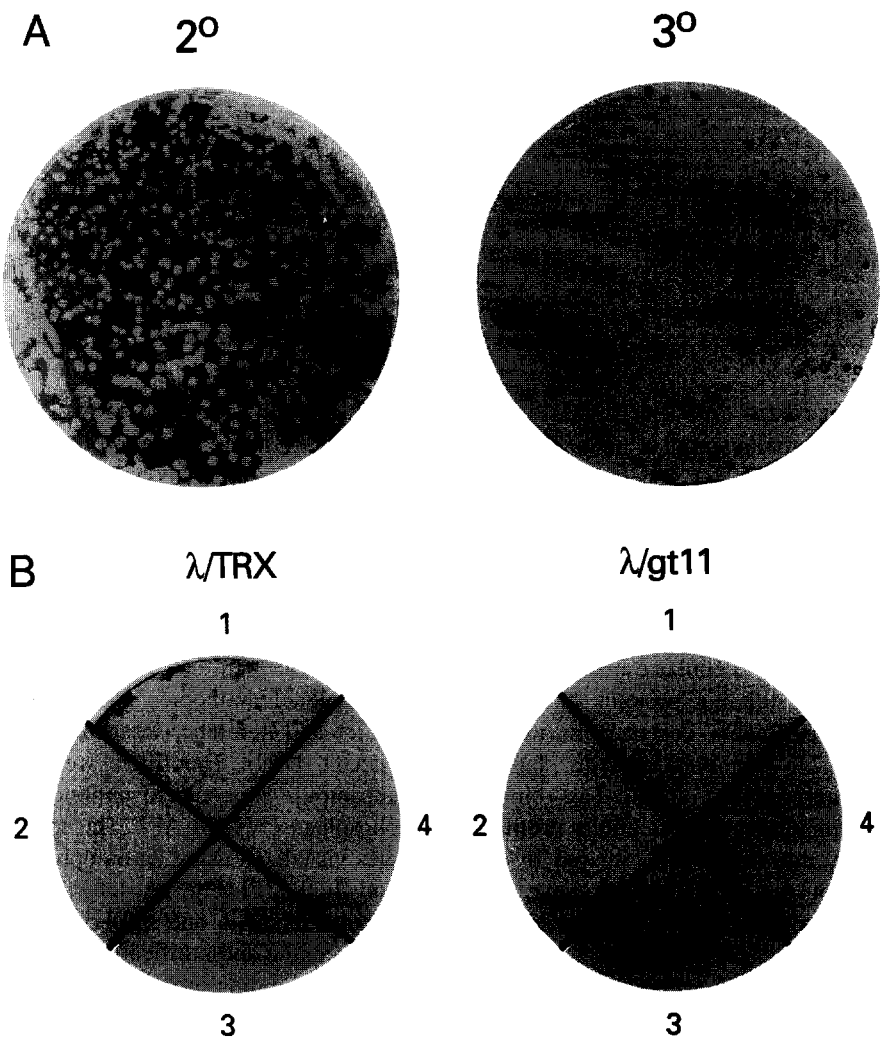

Fig. 1. (A) Secondary and tertiary screens of the Jurkat T-cell library using purified Tax_1 (5 μg/ml). 10^3 plaques/plate were transferred to each filter. Following an incubation with Tax_1, the filters were washed twice and probed with 1:7500 SAM-AP. The filters were developed using 3.3 μl/ml and 4.4 μl/ml of BCIP and NTB respectively [17]. Positive plaques are identified by the dark chromophore staining of the periphery of the plaque. (B) Quaternary screens of TRX and control λgt11 phage. Each quadrant was subjected to a different control assay. Q1: Tax_1 + TAbs + SAM-AP. Q2: HB101 (mock Tax_1 extract) + TAbs + SAM-AP. Q3: TAbs + SAM-AP. Q4: SAM-AP.

represents a computer-generated hydrophilicity plot of the TRX protein. TRX is predominately hydrophobic in its N-terminus and hydrophilic in its C-terminus.

The expression pattern of TRX was analyzed using a multiple tissue Northern blot (Clonetech, Palo Alto, CA). An anti-sense oligonucleotide corresponding to the first fifty bases of the TRX sequence was used as a hybridization probe to analyze a multiple tissue Northern blot as described [18]. A predominant 3.5 kb band was observed in all the tissues. Minor RNA species of 9.5 and 2.3 kb

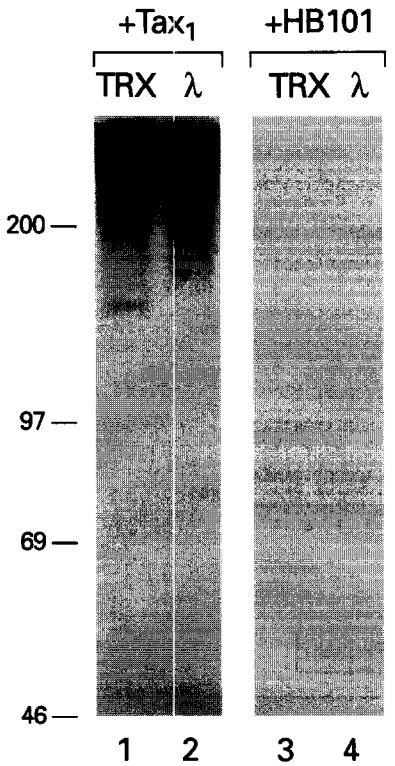

Fig. 2. Far Western blot. Phage proteins from a TRX plate lysate were TCA concentrated, electrophoresed through a 7% acrylamide gel and subsequently transferred to a nitrocellulose filter overnight. Following denaturation for 30 min in 6 M guanidinium-HCl/X-50 (50 mM Hepes, pH 7.9, 20% glycerol, 50 mM KCl, 1mM EDTA, 1 mM DTT, 0.5 mM PMSF), renaturation for 30 min in 3 M guanidinium-HCl/X-50 and 30 min in X-50/5% non-fat dry milk Western blot buffer [17], the filters were incubated in 5 μg/ml of purified Tax_1 or HB101 mock extract, washed twice, and probed with 1:1000 TAbs. The immune complex representing the interaction of Tax_1 with the β-galactosidase/TRX fusion protein was visualized using ^{125}I Protein A. Lane 1: TRX phage lysate + Tax_1. Lane 2: λgt11 phage lysate + Tax_1. Lane 3: TRX phage lysate + HB101. Lane 4: λgt11 phage lysate + HB101.

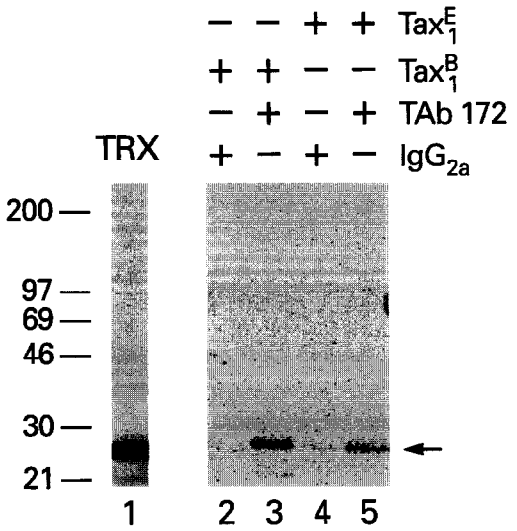

Fig. 3. Coimmunoprecipitation of TRX with Tax_1. Five microliters of ^{35}S-labelled TRX was diluted in 200 μl of X-50/0.2% NP-40 and incubated with 2 μg of purified Tax_1 for 3 h. Immunoprecipitations were performed using an anti-Tax_1 antibody, TAb 172 (lanes 3 and 5), or a control antibody (lanes 2 and 4). Input TRX is found in lane 1.

were also detected, possibly indicative of unspliced and multiple spliced forms of the TRX mRNA, respectively (Fig. 5A). The same blot was re-probed for actin (Fig. 5B) [19]. Interestingly, Western blot analysis revealed that stable TRX protein expression is limited to lymphocytes and lymphocytic-derived cells lines (data not shown).

We have isolated and characterized a novel Tax_1 binding protein, TRX, whose mRNA is ubiquitously expressed while the protein expression is restricted to lymphocytes. The specificity of the Tax_1/TRX interaction was demonstrated by three different assays, including cDNA screening, far Western blotting and coimmunoprecipitation. A search against GenBank failed to identify any proteins with complete homology to TRX. Recent evidence suggests that TRX forms a complex with the cdc2/cyclin B kinase complex (data not shown). Experiments are in progress to establish the functional relevence of this interaction.

The authors would like to thank Drs. Gilberto Fragoso and William Whalen for their help with the computer programs and Dr. Ravi Dhar for many helpful discussions. The sequence of TRX was submitted to GenBank under the following accession number: U25801.

A

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ATGCCCGGCAGTTCATCATCTCCTGGATCCTGGTTCTGGAGTCGGTGCCAGA
-----+---------+---------+---------+---------+---------+------+
TACGGGCCGTCAAGTAGTAGAGGACCTAGGACCAAGACCTCAGCCACGGTCT
M  P  G  S  S  S  S  P  G  S  W  F  W  S  R  C  Q  T

CATTAACCTGCTGGATTACTGCCGGAGAGTCCTGGATGGACTCTTCCAGAGTCCTGGGTG
241 ---------+---------+---------+---------+---------+--------+300
GTAATTGGACGACCTAATGACGGCCTCTCAGGACCTACCTGAGAAGGTCTCAGGACCCAC
  L  T  C  W  I  T  A  G  E  S  W  M  D  S  S  R  V  L  G  D

ACAATGGCAAAGAGATTCGCAAAATGTGTGAGGTTGTTCTTGGAGAATTCTTAAAAGAAA
301 ---------+---------+---------+---------+---------+--------+360
TGTTACCGTTTCTCTAAGCGTTTTACACACTCCAACAAGAACCTCTTAAGAATTTTCTTT
  N  G  K  E  I  R  K  M  C  E  V  V  L  G  E  F  L  K  E  I

TTAAGAAGAACCCCTCCAGTGTGAAGTTTCCTGAGATGGCCAACATCCCGGTGATCCACT
361---------+---------+---------+---------+---------+--------+420
AATTCTTCTTGGGGAGGTCACACTTCAAAGGACTCTACCGGTTGTAGGGCCACTAGGTGA
  K  K  N  P  S  S  V  K  F  P  E  M  A  N  I  P  V  I  H  C

GCCAGACAACAGATGACCTCATCCAGCTGACAGCCATGTGCTGGATGCGGGAGTTCATCC
421---------+---------+---------+---------+---------+--------+480
CGGTCTGTTGTCTACTGGAGTAGGTCGACTGTCGGTACACGACCTACGCCCTCAAGTAGG
  Q  T  T  D  D  L  I  Q  L  T  A  M  C  W  M  R  E  F  I  Q

AGCTGGCGGGCCGCGTCATGCTGCCTTACTCCTCCGGGATCCTGACTGCTGTCTTGCCCT
481---------+---------+---------+---------+---------+--------+540
TCGACCGCCCGGCGCAGTACGACGGAATGAGGAGGCCCTAGGACTGACGACAGAACGGGA
  L  A  G  R  V  M  L  P  Y  S  S  G  I  L  T  A  V  L  P  C

GCTTGGCCTACGATGACCGCAAGAAAAGCATCAAAGAAGTGGCCAACGTGTGCAACCAGA
541---------+---------+---------+---------+---------+--------+600
CGAACCGGATGCTACTGGCGTTCTTTTCGTAGTTTCTTCACCGGTTGCACACGTTGGTCT
  L  A  Y  D  D  R  K  K  S  I  K  E  V  A  N  V  C  N  Q  S

GCCTGATGAAGCTGGTCACCCCCGAGGACGACGAGCTGGATGAGCTGAGACCTGGGCAGA
601---------+---------+---------+---------+---------+--------+660
CGGACTACTTCGACCAGTGGGGGCTCCTGCTGCTCGACCTACTCGACTCTGGACCCGTCT
  L  M  K  L  V  T  P  E  D  D  E  L  D  E  L  R  P  G  Q  R

GGCAGGCAGAGCCCACCCCTGACGATGCCCTGCCAAAGCAGGAGGGCACAGCCAGTGGGT
661---------+---------+---------+---------+---------+--------+720
CCGTCCGTCTCGGGTGGGGACTGCTACGGGACGGTTTCGTCCTCCCGTGTCGGTCACCCA
  Q  A  E  P  T  P  D  D  A  L  P  K  Q  E  G  T  A  S  G  C

GCACTGAGGCGCACGACTTCCGTGACATCCCTCCAATGATGCGCAACTCCCTACTCGTGA
721---------+---------+---------+---------+---------+--------+780
CGTGACTCCGCGTGCTGAAGGCACTGTAGGGAGGTTACTACGCGTTGAGGGATGAGCACT
  T  E  A  H  D  F  R  D  I  P  P  M  M  R  N  S  L  L  V  R

GAAAACGTGAGACCAACCCAAACGGGGAGACACTCTGCAGAGTGACCAGCGCTTCAAAAG
781---------+---------+---------+---------+---------+--------+840
CTTTTGCACTCTGGTTGGGTTTGCCCCTCTGTGAGACGTCTCACTGGTCGCGAAGTTTTC
  K  R  E  T  N  P  N  G  E  T  L  C  R  V  T  S  A  S  K  V

TGTCAAGTTTATGA
841---------+-
ACAGTTCAAATACT
  S  S  L  *
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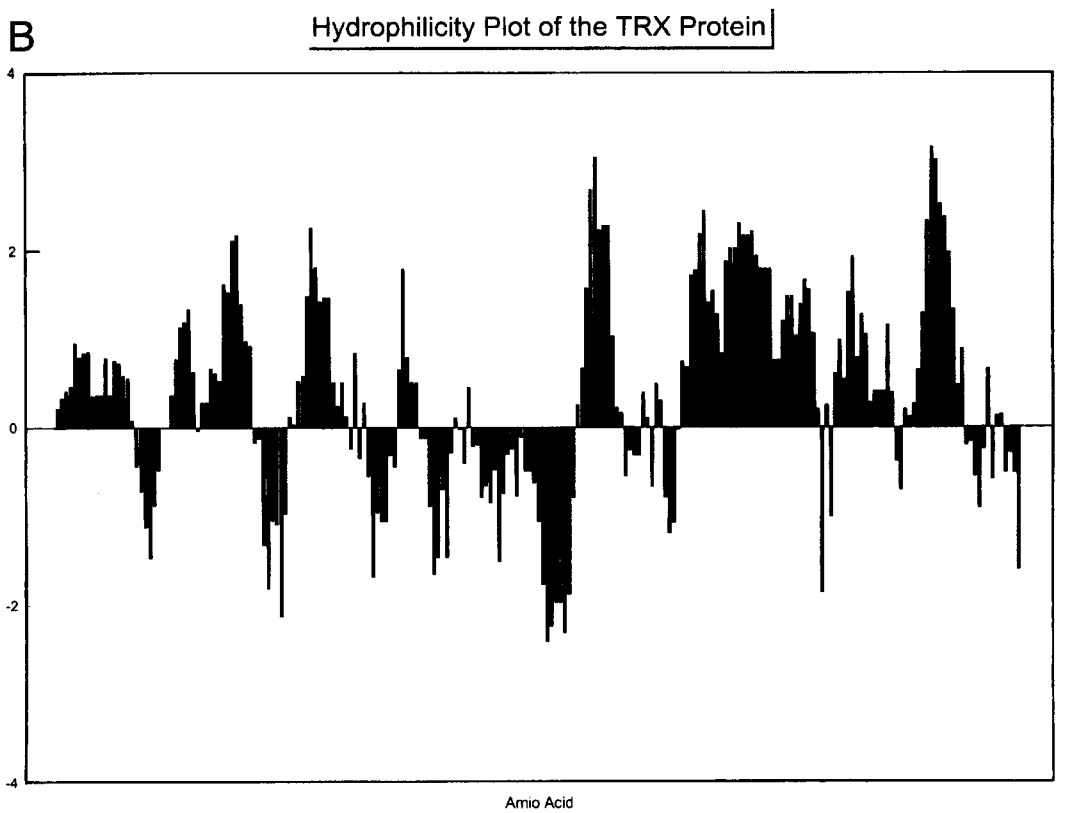
B



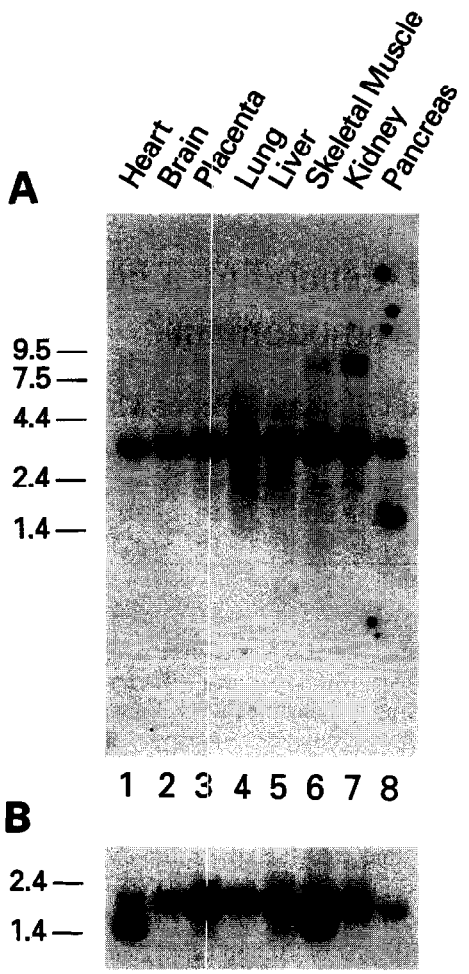


Fig. 5. (A) The mRNA expression pattern of TRX in a variety of human tissues. A 50-base oligonucleotide corresponding to the 5′ end of TRX was used as a probe in this northern blot. A predominant 3.5 kb mRNA is observed in all the tissues, while a smaller 2.3 kb species is seen in samples taken from human lung, liver, skeletal muscle and kidney. The latter two samples also contain a 9.5 kb mRNA. (B) The above filter was stripped and re-probed for actin.

References

[1] Bangham, C.R.M., Daenke, S., Phillips, R.E., Cruickshank, J.K. and Bell, J.I. (1988) EMBO J. 7, 4179–4184.
[2] Bhagavati, S., Ehrlich, G., Kula, R.W., Kwok, S., Sninsky, J., Udani, V. and Poiesz, B.J. (1988) N. Engl. J. Med. 318, 1141–1147.
[3] Brady, J., Jeang, K.T., Duvall, J. and Khoury, G. (1987) J. Virol. 61, 2175–2181.
[4] Felber, B.K., Paskalis, H., Kleinman-Ewing, C., Wong-Staal, F. and Pavlakis, G.N. (1985) Science 229, 675–679.
[5] Jeang, K.-T., Brady, J., Radonovich, M., Duvall, J. and Khoury, G. (1988) in Mechanisms of control of gene expression (Cullen, B., Gage, L.P., Siddiqui, M.A.Q., Skalka, A.M. and Weissbach, H., eds.), pp. 181–189, Alan R. Liss, New York.
[6] Jeang, K.-T., Widen, S.G., Semmes IV, O.J. and Wilson, S.H. (1990) Science 247, 1082–1084.
[7] Alexandre, C., Charnay, P. and Verrier, B. (1991) Oncogene 6, 1851–1857.
[8] Alexandre, C. and Verrier, B. (1991) Oncogene 6, 543–551.
[9] Fjuii, M., Sassone-Corsi, P. and Verma, I.M. (1988) Proc. Natl. Acad. Sci. USA 85, 8526–8530.
[10] Beimling, P. and Moelling, K. (1992) Oncogene 7, 257–262.
[11] Marriott, S.J., Lindholm, P.F., Reid, R.L. and Brady, J.N. (1991) New Biologist 3, 678–686.
[12] Fujii, M., Tsuchiya, H., Chuhjo, T., Akizawa, T. and Seiki, M. (1992) Genes Dev. 6, 2066–2076.
[13] Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M. and Roeder, R. (1993) Nature 362, 179–181.
[14] Hirai, H., Fujisawa, T., Suzuki, K., Ueda, M., Muramatsu, A., Tsuboi, A., Arai, N. and Yoshida, M. (1992) Oncogene 7, 1737–1742.
[15] Suzuki, T., Fujisawa, J.-I. and Yoshida, M. (1993) Proc. Natl. Acad. Sci. USA 90, 610–614.
[16] Zhao, L.-J. and Giam, C.-Z. (1991) Proc. Natl. Acad. Sci. USA 88, 11445–11449.
[17] Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual (Ford, N., Nolan, C., Ferguson, M. eds.), pp. 18.60–18.72. Cold Spring Harbor Press, New York.
[18] Dittmer, J., Gitlin, S.D., Reid, R.L. and Brady, J.N. (1993) J. Virol. 67, 6087–6095.
[19] Tendler, C.L., Greenberg, S.J., Blattner, W.A., Maans, A., Murphy, E., Fleisher, T., Hanchard, B., Morgan, O., Burton, J.D., Nelson, D.L. and Waldmann, T.A. (1990) Proc. Natl. Acad. Sci. USA 87, 5218–5222.

Fig. 4. (A) The DNA and amino acid sequence of the TRX open reading frame (Program Manual for the Wisconsin Package, Version 8.0, Genetics Computer Group, Madison, Wisconsin). (B) Computer generated hydrophilicity plot of TRX.