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SHORT REPORT

Structure, expression and chromosomal mapping of TKT from man and mouse: a new subclass of receptor tyrosine kinases with a factor VIII-like domain

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Using a polymerase chain reaction-mediated approach we have characterized cDNAs from human and mouse origin representing a novel type of receptor protein tyrosine kinase (RTK). The deduced amino acid sequence (855 amino acids) of the longest open reading frame has a unique extracellular region encompassing a factor VIII-like domain, not previously described for RTKs. The most closely related RTKs are members of the neurotrophin receptors (TRK), which showed 47–49% homology with the kinase domain of the new RTK. Therefore, the new gene has been called *TKT* (*T*yrosine-*K*inase related to *T*RK). TKT orthologs from man and mouse were 98% similar. In both species a major transcript of 10 kb was found to be expressed at high levels in heart and lung. Low levels of this mRNA-species were detected in human brain, placenta, liver, skeletal muscle, kidney and in mouse brain and testis. Analysing human/mouse somatic cell hybrids we demonstrated that *TKT* segregates with human chromosome 1.

Receptor tyrosine kinases (RTKs) play a key role in the communication of cells with their microenvironment. These molecules are involved in the regulation of cell growth, differentiation and metabolism. In several cases the biochemical mechanism by which RTKs transduce signals across the membrane has been shown to be ligand induced receptor oligomerization and subsequent intracellular autophosphorylation. This autophosphorylation leads to phosphorylation of cytosolic targets as well as association with other molecules, which are involved in pleiotropic effects of signal transduction. RTKs have a tripartite structure with extracellular, transmembrane and cytoplasmic regions. The intracellular portion of RTKs harbours the protein tyrosine kinase (PTK) – domain of the molecule. There appear to be at least six subclasses of RTKs: EGF-receptor (Ullrich *et al.*, 1984); insulin-receptor (Ebina *et al.*, 1985; Ullrich *et al.*, 1985), PDGF-receptor (Yarden *et al.*, 1986; Claesson-Welsh *et al.*, 1989), FGF-receptor (Lee *et al.*, 1989; Holtrich *et al.*, 1991), EPH/ELK (Hirai *et al.*, 1987; Böhme *et al.*, 1993) and TRK (Martin-Zanca *et al.*, 1989).

RTK-genes were characterized by applying the polymerase chain reaction (PCR) in combination with degenerate oligonucleotide primers based upon conserved motifs of the kinase domain of PTKs (Wilks, 1989; Holtrich *et al.*, 1991). In a more direct approach we identified a new member of the EPH/elk-family of RTKs: we utilized oligonucleotide primers specifically designed to a highly conserved N-terminal motif (CKETFNL) of EPH/elk-RTKs and a motif of the kinase region (SDVWS) in RNA-PCRs. 5′ and 3′ elongation of the primary PCR-product allowed to isolate a new gene *HEK2* as a new member of this family (Böhme *et al.*, 1993).

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PCR-mediated isolation of a novel RTK-gene

To identify additional members of the EPH/elk-family we utilized a different combination of primers designed according to the above mentioned motifs for PCR with cDNA templates from human embryonic RNA. This amplification gave rise to a fragment of 800 bp which differed from the anticipated PCR-product of 2 kb derived from members of the EPH/elk-family. Nested primers for the PTK-specific motifs HRDLA and SDVWS were used to verify the identity of this PCR-product and gave rise to the expected 200 bp-product. The original 800 bp-product, designated K1, was sequenced and subsequently used as a probe to screen cDNA libraries from human heart and thymus (2×10^6 recombinant clones each). Several overlapping clones spanning 2.3 kb were isolated. Anchored and ligation-mediated PCR was performed to extend the sequence in 3′ and 5′ direction (Böhme *et al.*, 1993).

TKT represents a new subclass of RTKs

Figure 1 shows the composite nucleotide sequence of 3.1 kb of the K1 cDNA. An open reading frame begins with an ATG codon at nucleotide 354 and ends at an in-frame stop codon at position 2919. Several features of the sequence indicate that the ATG codon at position 354 is used for the initiation of translation: it is surrounded by a sequence that is in agreement with Kozak's rule (Kozak, 1984) and the following DNA sequence predicts a hydrophobic signal peptide. Furthermore there are termination codons upstream of the ATG codon in all three reading frames.

The deduced polypeptide contains a second hydrophobic stretch of amino acids (residues 400–421), which represents a transmembrane domain followed by a basic stop transfer motif. This suggests that the

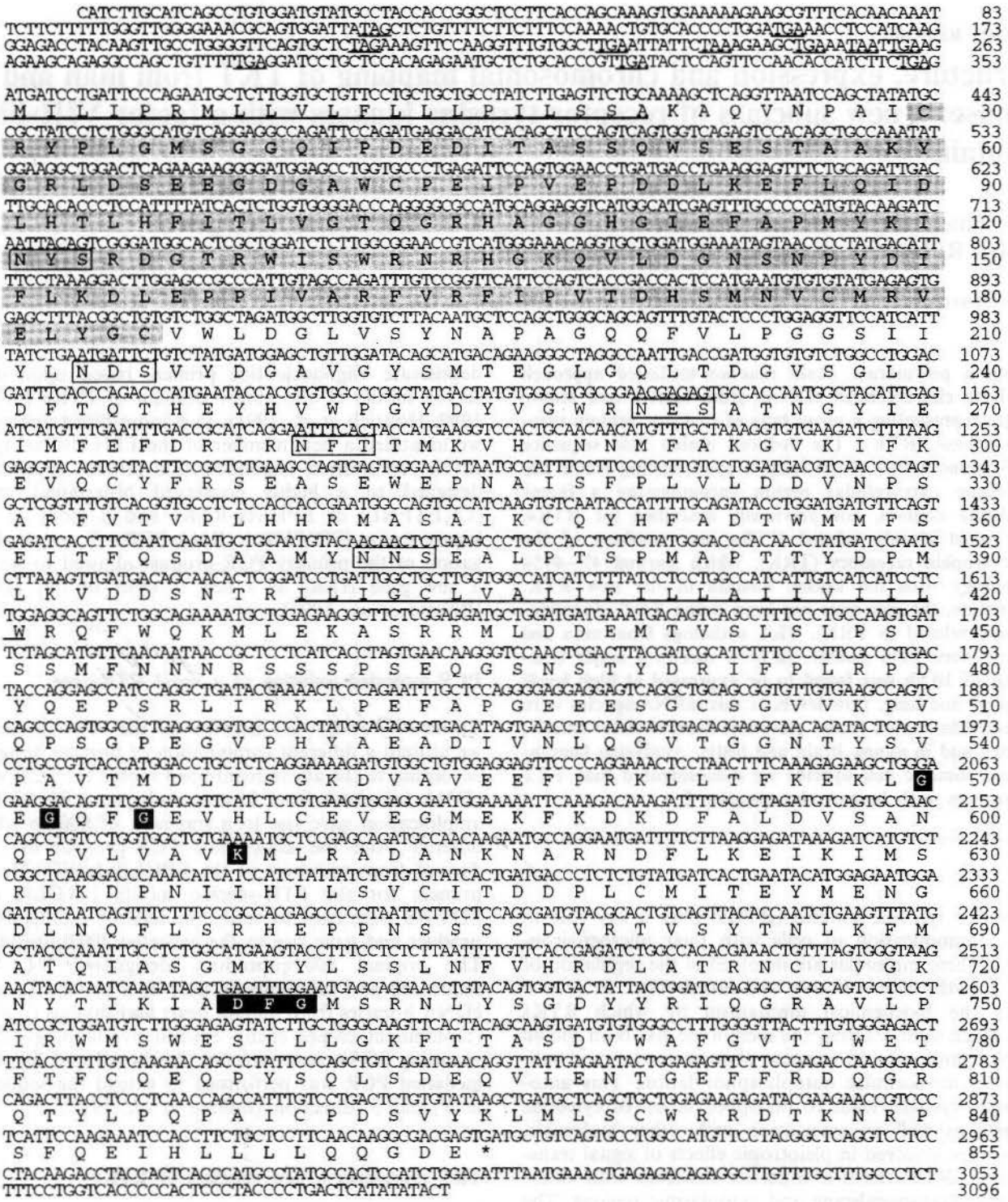

Figure 1 Nucleotide and deduced amino acid sequence of TKT. The deduced amino acid sequence in one letter code is given below the nucleotide sequence. The putative signal peptide and the transmembrane domain are underlined. Potential sites for N-glycosylation in the extracellular region are boxed. The invariant motifs of the kinase domain (Hanks *et al.*, 1988) are given in inverted letters: the consensus sequence GxGxxG of nucleotide binding proteins and PTKs, the conserved lysine residue involved in the phosphotransfer reaction and the invariant residues (DFG) implicated in ATP-binding. The factor VIII-like sequence in the extracellular part is shaded

putative K1 protein is an integral membrane protein (von Heijne, 1986; Singer, 1990). The extracellular region contains 399 amino acids with five potential N-linked glycosylation sites. The cytoplasmic portion consists of 434 amino acids and encompasses a juxtamembrane domain of 139 amino acids and a kinase domain that contains all characteristic features of PTKs (Figure 1) (Hanks *et al.*, 1988). A putative autophosphorylation site is found at position 740. A kinase insert as well as a C-terminal tail are missing. In other RTKs these regions were shown to contain phosphotyrosine residues which interact with SH2-domains. The 3′ untranslated sequence encompasses 178 nucleotides. A potential polyadenylation signal is missing.

Comparison of the K1 amino acid sequence with known sequences revealed that K1 is a member of the RTK family, but does not belong to one of the known subclasses. The most closely related RTKs are members of the neurotrophin receptors (TRK, Martin-Zanca *et al.*, 1989), which showed 47–49% homology with K1 in the kinase domain (Figure 2). Thus we named the K1 gene *TKT* (pronounced ticket): *T*yrosine-*K*inase related to *T*RK. Comparing the kinase domains of TKT with those of the trk-family and various types of insulin receptors instead of a consecutive alignment TKT exhibits three insertions which are between two and 11 amino acids in length (Figure 2).

TKT contains a factor VIII-like domain

The extracellular regions of RTKs contain certain features which distinguish individual families of RTKs: To date, cysteine-rich regions, immunoglobulin-like domains and repeats of the EGF-like type and the fibronectin-type have been found to be components of the extracellular portion of RTKs (Hirai *et al.*, 1987; Yarden & Ullrich, 1988; Lindberg & Hunter, 1990; Ziegler *et al.*, 1993). These motifs could not be detected in the deduced amino acid sequence of TKT. Interestingly, a computer-aided homology search revealed similarities with domains of other proteins. Figure 3 shows a domain (amino acids 30–185) which begins eight residues after the presumptive cleavage site of the signal peptide and is homologous to both C-units at the carboxyterminus of factor VIII, a component of blood coagulation (Gitschier *et al.*, 1984; Vehar *et al.*, 1984). These two C-units within factor VIII have 37% homology with each other. Homology of TKT to the C1-unit and the C2-unit of factor VIII within a stretch of 156 amino acids was determined to be 35% and 30% respectively. Homologies to other proteins, which contain factor VIII-like sequences were also observed: (a) The 156 amino acid region of TKT is 27 and 33% homologous to the C1- and C2-unit, respectively, of a surface protein of mouse mammary epithelial cells (MFG-E8: milk fat globule membrane protein, Stubbs

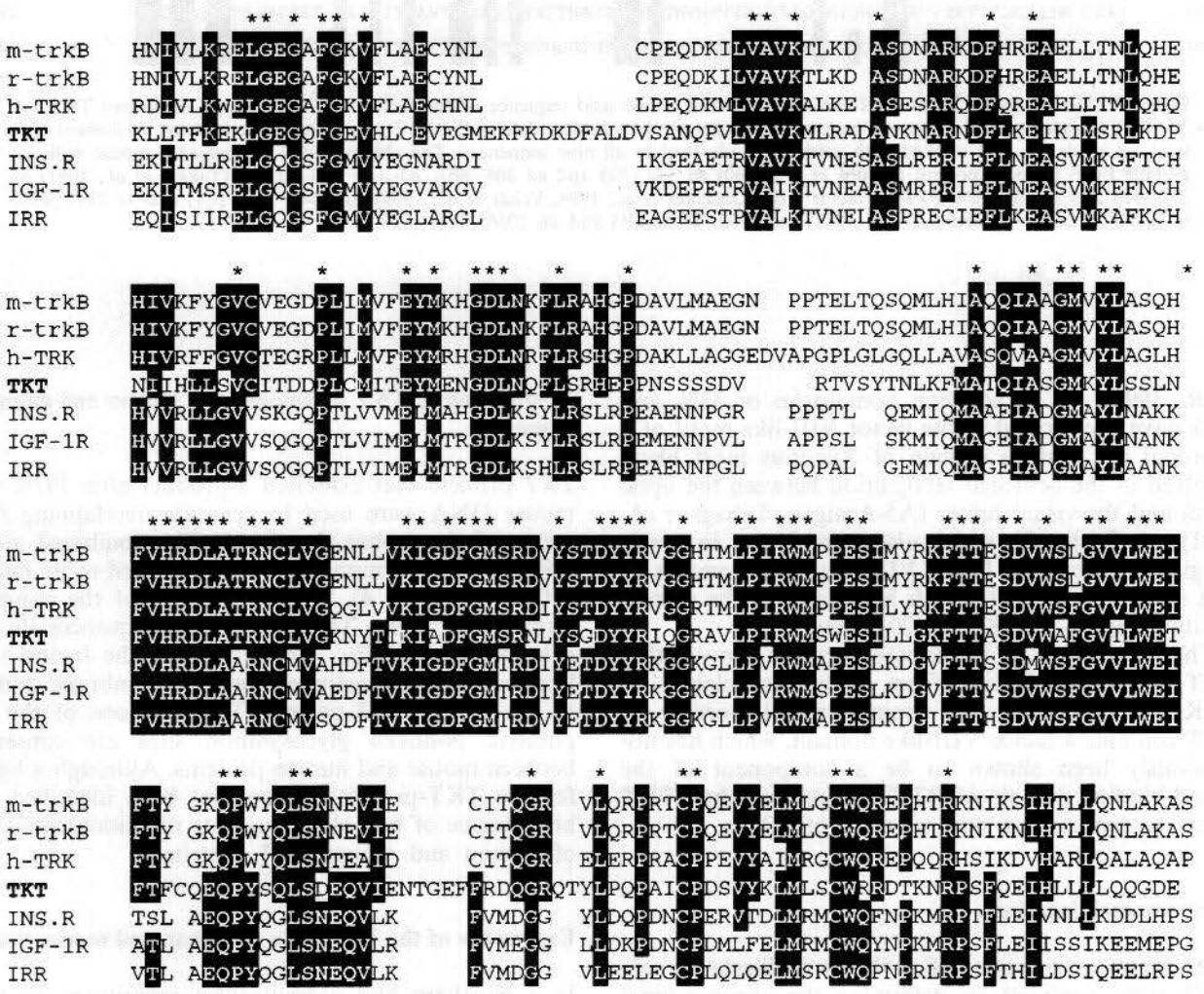

Figure 2 Comparison of the TKT kinase domain with several others RTKs. Amino acid sequences of the kinase domains (Hanks *et al.*, 1988) of human TKT, human TRK (h-TRK; Martin-Zanca *et al.*, 1989), mouse trkB (m-trkB; Klein *et al.*, 1989), rat trkB (r-trkB; Middlemas *et al.*, 1991), the insulin receptor (INS.R; Ebina *et al.*, 1985; Ullrich *et al.*, 1985), the insulin like growth factor-1 receptor (IGF-1R; Ullrich *et al.*, 1986) and the insulin receptor-related receptor (IRR; Shier & Watt, 1989) were aligned using the Tree program of the HUSAR software package (DKFZ, Heidelberg) based on the progressive alignment method of Feng and Doolittle (1987). If all members of at least two of the three subgroups (i.e. TRK-, insulin receptor- and TKT-subgroup) share identical residues, amino acids are given in inverted letters

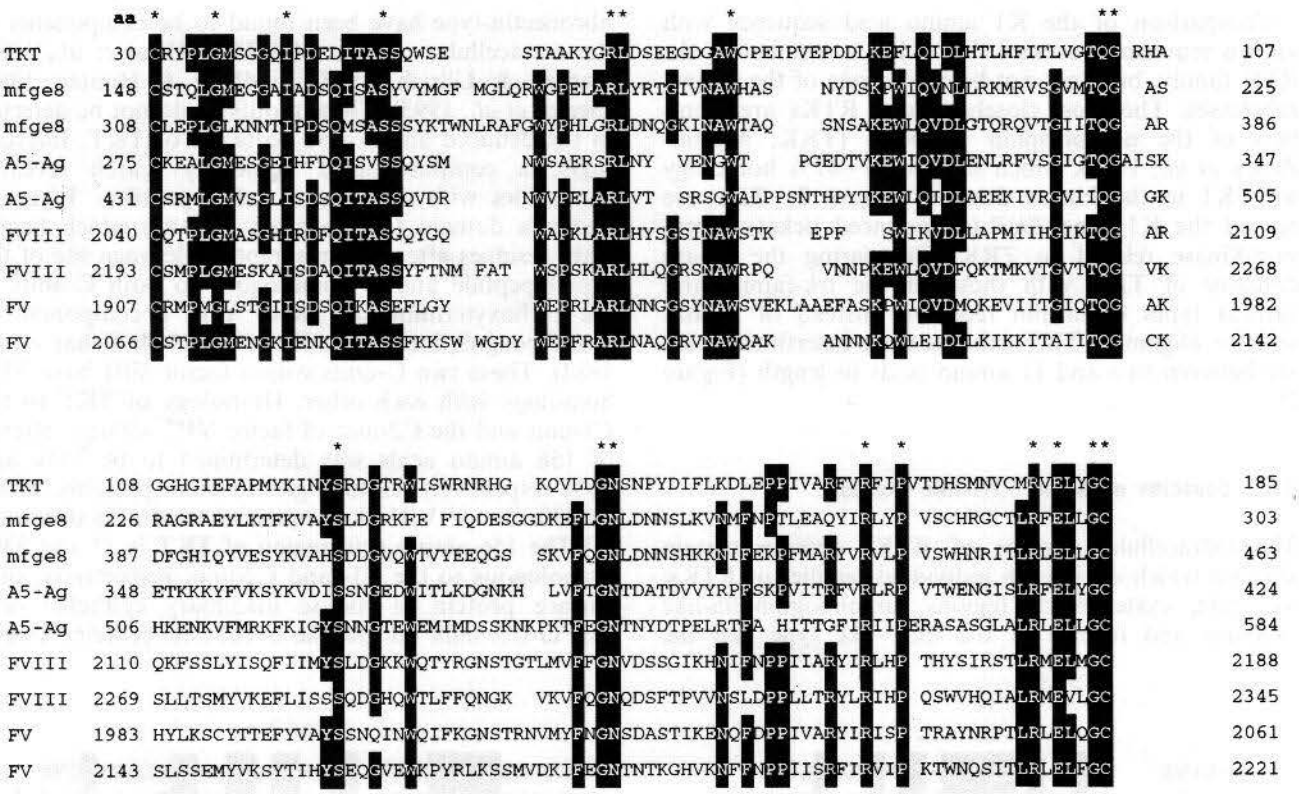

Figure 3 Homology of factor VIII-like domains. The amino acid sequence of the factor VIII-like domain of human TKT (aa 30–185) was aligned with homologous regions of other proteins as described in Figure 2. Amino acids are inverted if identical in at least six sequences and marked with asterisks if identical in all nine sequences. The abbreviations are: *mfge8* – mouse milk fat globule EGF factor 8 protein (Stubbs *et al.*, 1990) aa 148–303 and aa 308–463; *A5-Ag* – A5-antigen (Takagi *et al.*, 1991) aa 275–424 and aa 431–584; *FVIII* – factor VIII (Gitschier *et al.*, 1984; Vehar *et al.*, 1984), aa 2040–2188 (C1) and aa 2193–2345 (C2); *FV* – factor V (Jenny *et al.*, 1987), aa 1907–2061 (C1) and aa 2066–2221 (C2)

et al., 1990); (b) In addition homologies of 33% and 32% have been found to the factor VIII-like motif of a neuronal cell surface protein of Xenopus most likely involved in the neuronal recognition between the optic fibres and the visual center (A5-Antigen, Takagi *et al.*, 1991); (c) Factor V, which also participates in blood coagulation, contains factor VIII-related C-domains as well (Jenny *et al.*, 1987) with homology to the corresponding region of TKT of 29% and 33%.

The comparison of the kinase domains showed that TKT is clearly distinct from known subclasses of RTKs. Furthermore, the aminoterminal portion of TKT contains a factor VIII-like domain, which has not previously been shown to be a component of the ligand-binding domain of RTKs. Taken together, TKT seems to represent a new subclass of RTKs.

Chromosomal location

Human/mouse somatic cell hybrids (Willecke *et al.*, 1990) were analysed to determine the chromosomal localization of *TKT*. In PCR with primers which amplify human but not mouse genomic DNA, we demonstrated that *TKT* segregates with the human chromosome 1 and is located in the region 1q12-qter, which is the same as for *TRK* (1q23-1q24, Morris *et al.*, 1991).

Comparison of TKT orthologs from human and mouse tissues

TKT primers that exhibited a product after PCR with mouse DNA were used to generate overlapping fragments of the mouse *tkt* cDNA. The combined amino acid sequence of mouse TKT was aligned to its human ortholog (Figure 4). The comparison of the complete human and mouse TKT-amino acid sequences showed a homology of 98%. We also found the factor VIII-like sequence upstream of the transmembrane domain of the mouse TKT-protein. The locations of the five putative N-linked glycosylation sites are conserved between mouse and human proteins. Although a ligand for the TKT-protein has not yet been identified, the high degree of homology suggests functional similarity of human and mouse TKT-proteins.

Expression of the TKT gene in human and mouse tissues

In a Northern blot hybridization experiment we used poly(A)$^+$ RNA from human adult tissues to determine the pattern of *TKT*-expression (Figure 5a). A 229 bp-*TKT*-fragment (probe I) representing a portion of the putative aminoterminal region (nt pos. 667–895) was used as probe. In a Southern blot analysis this probe detected EcoRI-fragments of 7 and 3 kb as well as

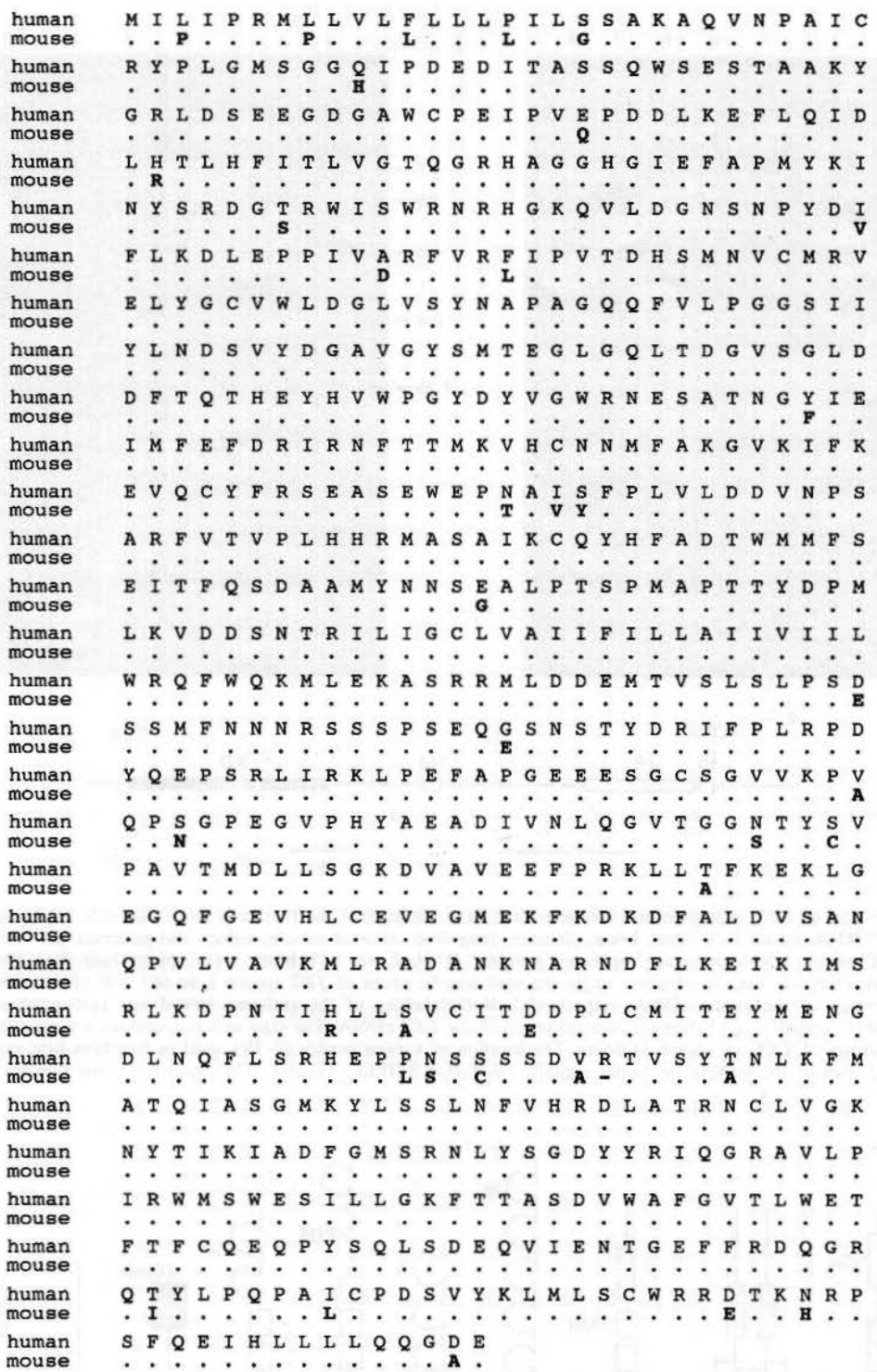

Figure 4 Comparison of the deduced amino acid sequences of human and mouse TKT. The nucleotide sequence of mouse *tkt* cDNA was determined using RNA-PCR with different primers derived from the human cDNA sequence. The deduced amino acid sequences of human (1–855) and mouse (1–854) TKT were compared and one gap has been introduced for optimal alignment. Residues identical to the human sequence are replaced by dots

HindIII- and PstI-fragments of 5 kb each, which indicated that the specifity of the probe and the stringency conditions were sufficient for the discrimination between *TKT* and related genes. The Northern blot was standardized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe.

Using probes I, II, III and IV in separate hybridization experiments a major 10 kb-transcript was found at high levels in heart and lung, with lower levels in brain, placenta, liver, skeletal muscle, pancreas and kidney (Figure 5a and c). With the same set of probes a second signal was detected at lower intensity at 4.5 kb in the above mentioned tissues except in brain. Various additional weak bands were observed at 8.0, 3.6, 2.4 and 1.7 kb.

In a second Northern blot hybridization experiment we used poly(A)$^+$ RNA from mouse tissues and probe I (Figure 5c) derived from mouse cDNA. As shown in

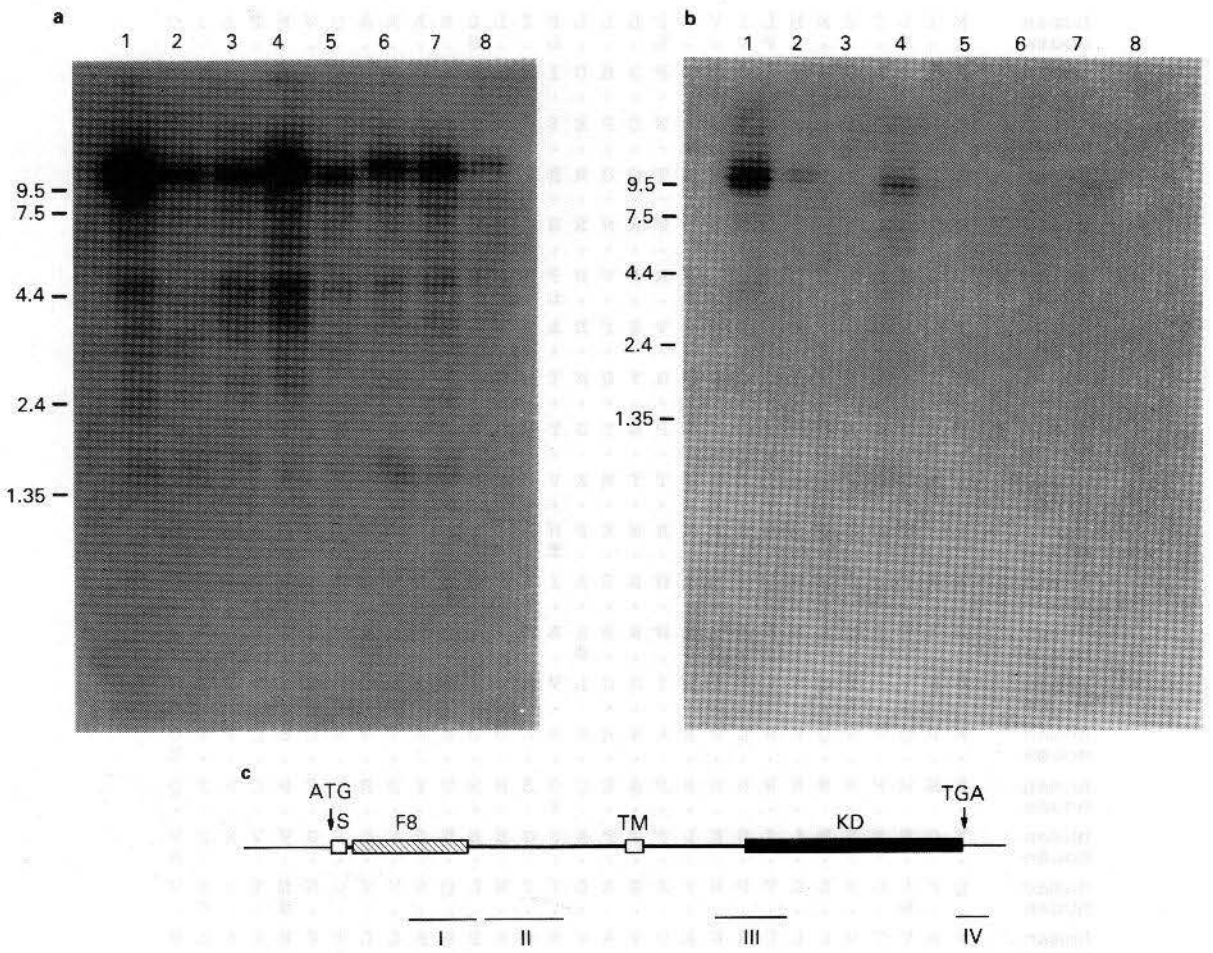

Figure 5 Expression of *TKT* in human and mouse tissues. (**a**) Each lane of the Northern blot (Clontech, USA) contained 2 μg human poly(A)$^+$ RNA. Lanes 1–8: heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. (**b**) Each lane of the Northern blot (Clontech, USA) contained 2 μg mouse poly(A)$^+$ RNA. Lanes 1–8: heart, brain, spleen, lung, liver, skeletal muscle, kidney and testis. PCR was used to obtain a single stranded specific probe of *TKT* (probe I, nt 667–895 of the human sequence and the same region of the mouse cDNA, respectively). Radiolabelling of the antisense strand was performed using 250 μCi [α-^{32}P]dCTP (6000 Ci mmol^{-1}). (**c**) Schematic representation of the *TKT* cDNA. The start and stop-codons are indicated by arrows, characteristic features of *TKT* are shown as boxes. The location of various probes (I–IV), used in Northern blot experiments are given as vertical lines at the bottom (S: signal peptide; F8: factor VIII-like domain; TM: transmembrane domain; KD: kinase domain)

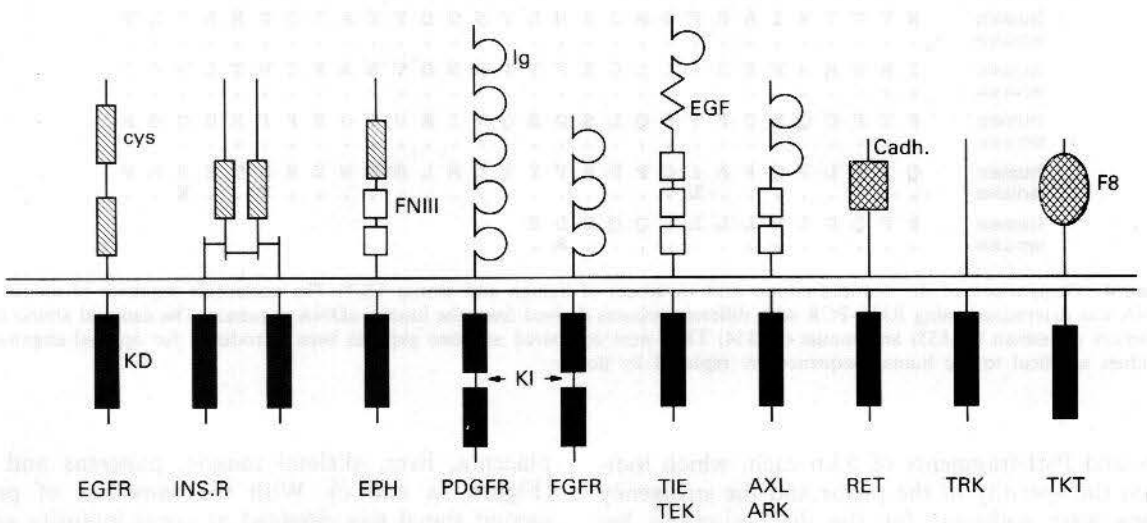

Figure 6 Structural motifs of receptor tyrosine kinases. Various subtypes of RTKs are shown schematically with their intracellular region (KD = kinase domain, KI = kinase insert) as well as their structural motifs in the extracellular regions: cysteine-rich regions (cys), immunoglobulin-like domains (Ig), EGF-like repeats (EGF) and fibronectin-type-III like repeats (FNIII) as well as the cadherin-related domain (Cadh.) of RET and the factor VIII-like domain (F8) of TKT. EGFR (Ullrich *et al.*, 1984), INS.R (Ebina *et al.*, 1985; Ullrich *et al.*, 1985), EPH (Hirai *et al.*, 1987); PDGFR (Yarden *et al.*, 1986; Claesson-Welsh *et al.*, 1989), FGFR (Lee *et al.*, 1989), TIE (Partanen *et al.*, 1982), TEK (Dumont *et al.*, 1993; Ziegler *et al.*, 1983), AXL (O'Bryan *et al.*, 1991), ARK (Rescigno *et al.*, 1991), RET (Takahashi & Cooper, 1987; Iwamoto *et al.*, 1993), TRK (Martin-Zanca *et al.*, 1989)

Figure 5b mouse *tkt* shows high expression of a 10 kb transcript in heart and lung. Low levels of transcripts were detected in mouse brain and testis. Additional smaller transcripts were detected at lower frequency of expression.

These observations indicate that different *TKT* mRNA species are derived from one gene and may be generated by alternative splicing or by selective use of different polyadenylation sites.

Through molecular cloning and sequencing of a 3096 nt cDNA, we have determined the primary structure of TKT. The 885 residue polypeptide corresponds to a classical RTK with tripartite structure. While all RTKs share a common cytoplasmic kinase domain, the extracellular ligand binding portion of the molecule is composed of various structural motifs: Ig-like, EGF-like, FNIII-like and cysteine-rich domains (Figure 6). TKT enriches this spectrum by a factor VIII-like sequence. The human factor VIII is a trace plasma glycoprotein, which plays a key role in normal blood coagulation (Gitschier *et al.*, 1984; Vehar *et al.*, 1984). In addition to the TKT-receptor the mouse mammary epithelial cell surface protein (MFG-E8) also shows considerable homology to factor VIII. This mouse protein is involved in lactogenesis. In this process the apical surface of mammary epithelial cells becomes highly specialized and participates in the triglyceride secretion into milk. The triglyceride droplet is enclosed in the milk fat globule membrane (MFGM), which contains a high percentage of the factor VIII-related protein (Stubbs *et al.*, 1990). The A5-antigen which has homologies to factor VIII as well, is a neuronal cell surface protein of Xenopus and seems to be involved in neuronal recognition processes between optic nerve fibers and the visual center (Takagi *et al.*, 1991). Furthermore blood vessels can be highlighted by staining endothelial cells for factor VIII, which is localized on their surface. Taken together factor VIII and related molecules are found on or within the membrane and seem to play a role for cell surface recognition and adhesion. The TKT-receptor which has a factor VIII-related domain in its extracellular region may combine these functions with transmembrane signal transduction.

Note added in proof

TKT-accession number: X74764.

During the review process of this report a publication by Johnson *et al.* (*Proc. Natl. Acad. Sci. USA*, **90,** 5677–5681) appeared describing a RTK with a structure similar to TKT which they named DDR. This protein shares 73% homology with TKT and shows a factor VIII-like domain as well. Thus, the new RTK-subclass contains at least two members.

Acknowledgements

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References

Böhme, B., Holtrich, U., Wolf, G., Luzius, H., Grzeschik, K.-H., Strebhardt, K. & Rübsamen-Waigmann, H. (1993). *Oncogene*, in press.

Claesson-Welsh, L., Eriksson, A., Westermark, B. & Heldin, C.-H. (1989). *Proc. Natl. Acad. Sci. USA*, **86,** 4917–4921.

Dumont, D.J., Gradwohl, G.J., Fong, G.-H., Auerbach, R. & Breitman, M.L. (1993). *Oncogene*, **8,** 1293–1301.

Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Graf, L., Clauser, E., Ou, J.-H., Masiarz, F., Kan, Y.W., Godfine, I.D., Roth, R.A. & Rutter, W.J. (1985). *Cell*, **40,** 747–758.

Feng, D.-F. & Doolittle, R.F. (1987). *J. Mol. Evol.*, **25,** 351–360.

Gitschier, J., Wood, W.I., Goralka, T.M., Wion, K.L., Chen, E.Y., Eaton, D.H., Vehar, G.A., Capon, D.J. & Lawn, R.M. (1984). *Nature*, **312,** 326–330.

Hanks, S.K., Quinn, A.M. & Hunter, T. (1988). *Science*, **241,** 42–52.

Hirai, H., Maru, Y., Hagiwara, K., Nishida, J. & Takaku, F. (1987). *Science*, **238,** 1717–1720.

Holtrich, U., Bräuninger, A., Strebhardt, K. & Rübsamen-Waigmann, H. (1991). *Proc. Natl. Acad. Sci. USA*, **88,** 10411–10415.

Iwamoto, T., Taniguchi, M., Asai, N., Ohkusu, K., Nakashima, I. & Takahashi, M. (1993). *Oncogene*, **8,** 1087–1091.

Jenny, R.J., Pittman, D.D., Toole, J.J., Kriz, R.W., Aldape, R.A., Hewick, R.M., Kaufman, R.J. & Mann, K.G. (1987). *Proc. Natl. Acad. Sci. USA*, **84,** 4846–4850.

Klein, R., Parada, L.F., Coulier, F. & Barbacid, M. (1989). *EMBO J.*, **8,** 3701–3709.

Kozak, M. (1984). *Nucleic Acids Res.*, **12,** 857–872.

Lee, P.L., Johnson, D.E., Cousens, L.S., Fried, V.A. & Williams, L.T. (1989). *Science*, **245,** 57–60.

Lindberg, R.A. & Hunter, T. (1990). *Mol. Cell. Biol.*, **10,** 6316–6324.

Martin-Zanca, D., Oskam, R., Mitra, G., Copeland, T. & Barbacid, M. (1989). *Mol. Cell. Biol.*, **9,** 24–33.

Middlemas, D.S., Lindberg, R.A. & Hunter, T. (1991). *Mol. Cell. Biol.*, **11,** 143–153.

Morris, C.M., Hao, Q.L., Heisterkamp, N., Fitzgerald, P.H. & Groffen, J. (1991). *Oncogene*, **6,** 1093–1095.

O'Bryan, J.P., Frye, R.A., Gogswell, P.C., Neubauer, A., Kitch, B., Prokop, C., Espinosa III, R., Le Beau, M.M., Earp, H.S. & Liu, E.T. (1991). *Mol. Cell. Biol.*, **11,** 5016–5031.

Partanen, J., Armstrong, E., Mäkelä, T.P., Korhonen, J., Sandberg, M., Renkonen, R., Knuutila, S., Huebner, K. & Alitalo, K. (1992). *Mol. Cell. Biol.*, **12,** 1698–1707.

Rescigno, J., Mansukhani, A. & Basilico, C. (1991). *Oncogene*, **6,** 1909–1913.

Shier, P. & Watt, V.M. (1989). *J. Biol. Chem.*, **264,** 14605–14608.

Singer, S.J. (1990). *Ann. Rev. Cell. Biol.*, **6,** 247–296.

Stubbs, J.D., Lekutis, C., Singer, K.L., Bui, A., Yuzuki, D., Srinivasan, U. & Parry, G. (1990). *Proc. Natl. Acad. Sci. USA*, **87,** 8417–8421.

Takagi, S., Hirata, T., Agata, K., Mochii, M., Eguchi, G. & Fujisawa, H. (1991). *Cell*, **7,** 295–307.

Takahashi, M. & Cooper, G.M. (1987). *Mol. Cell. Biol.*, **7,** 1378–1385.

Ullrich, A., Coussens, L., Hayflick, J.S., Dull, T.J., Gray, A., Tam, A.W., Lee, J., Yarden, Y., Libermann, T.A., Schlessinger, J., Downward, J., Mayes, E.L.V., Whittle, N., Waterfield, M.D. & Seeburg, P.H. (1984). *Nature*, **309,** 418–425.

Ullrich, A., Bell, J.R., Chen, E.Y., Herrera, R., Petruzelli, L.M., Dull, T.J., Gray, A., Coussens, L., Liao, Y.-C., Tsubokawa, M., Mason, A., Seeburg, P.H., Grunfeld, C., Rosen, O.M. & Ramachandran, J. (1985). *Nature*, **313,** 756–761.

Ullrich, A., Gray, A., Tam, A.W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Bon, T.L., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, J. & Fujita-Yamaguchi, Y. (1986). *EMBO J.*, **5,** 2503–2512.

Vehar, G.A., Keyt, B., Eaton, D., Rodriguez, H., O'Brien, D.P., Rotblat, F., Oppermann, H., Keck, R., Wood, W.I., Harkins, R.N., Tuddenham, E.G.D., Lawn, R.M. & Capon, D.J. (1984). *Nature*, **312,** 337–342.

von Heijne, M. (1986). *Nucleic Acids Res.*, **14,** 4683–4690.

Wilks, A.F. (1989). *Proc. Natl. Acad. Sci. USA*, **86,** 1603–1607.

Willecke, K., Jungblut, S., Dahl, E., Hennemann, R., Heynkes, R. & Grzeschik, K.-H. (1990). *Eur. J. Cell Biol.*, **53,** 275–280.

Yarden, Y., Escobedo, J.A., Kuang, W.-J., Yang-Feng, T.L., Daniel, T.O., Tremble, P.M., Chen, E.Y., Ando, M.E., Harkins, R.N., Francke, U., Fried, V.A., Ullrich, A. & Williams, L.T. (1986). *Nature*, **323,** 226–232.

Yarden, Y. & Ullrich, A. (1988). *Ann. Rev. Biochem.*, **57,** 443–478.

Ziegler, S.F., Bird, T.A., Schneringer, J.A., Schooly, K.A. & Baum, P.R. (1993). *Oncogene*, **8,** 663–670.