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Isolation and characterization of a cDNA clone corresponding to the mouse *t*-complex gene *Tcp-1x*

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Summary

The mouse *t* complex on chromosome 17 is known to harbour many genes which have an important role in spermatogenesis. One of these, *Tcp-1* has been cloned and shown to code for a protein probably essential for acrosome formation. During the isolation of a cDNA for *Tcp-1* two other homologous sequences were recognized and described as *Tcp-1x* and *Tcp-1y*. In this paper we describe the isolation of a cDNA which has been shown by *in situ* hybridization to correspond to the *Tcp-1x* gene. Sequence analysis has confirmed that a 140 bp region of homology between *Tcp-1* and *Tcp-1x* lies in the 3′ portion of both genes. Northern blotting has revealed that the *Tcp-1x* gene is expressed abundantly in liver where two transcripts are detectable and hybrid selection shows that the gene codes for a 37 kDa protein. A search of the DNA databases has failed to find any significant homology between *Tcp-1x* and any other sequences apart from *Tcp-1* .

1. Introduction

The mouse *t* complex on chromosome 17 is known to harbour genes which influence male fertility and embryonic development (Bennett, 1975; Silver, 1985). A number of genes have been cloned from the region, especially by searching for genes expressed uniquely or predominantly in male germ cells (Rappold *et al.* 1987; Schimenti *et al.* 1988). The first such gene to be cloned and identified was *Tcp-1* (Willison *et al.* 1986). *Tcp-1* was first described by Silver *et al.* (1979) as an abundant protein in germ cell extracts which was polymorphic between wild type mice and mice harbouring a complete *t* haplotype. The gene *Tcp-1* is now known to code for a protein which is associated with the cytoplasmic aspect of the *trans*-Golgi network and is probably essential for acrosome formation in developing spermatids (Willison *et al.* 1989).

During the isolation of *Tcp-1* two other cross-hybridizing sequences were also identified (Willison *et al.* 1986). One of these, called *Tcp-1x* was shown to map very close to *Tcp-1* in the *t* complex. A second called *Tcp-1y* was not linked to *Tcp-1* and its precise chromosomal location is still unknown. In this paper we describe the isolation and characterisation of a cDNA clone corresponding to the *Tcp-1x* gene and discuss its relationship to *Tcp-1*.

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2. Materials and methods

The 8·5-day mouse embryo library was a kind gift from Dr B. Hogan and was cloned into the vector λgt10. All manipulations involving DNA and RNA used standard technology. RNA was prepared using the method of Auffray & Rougeon (1980). Hybrid selection was performed as described previously (Willison *et al.* 1986). *In situ* hybridization was carried out as described in Lyon *et al.* (1986), 1988) on mitotic cells heterozygous for the T(1:17)190H reciprocal translocation. DNA sequencing was carried out using the sequenase enzyme and oligonucleotide primers made in-house.

3. Results

The 3′ portion of a full-length cDNA clone for *Tcp-1* was used to screen an 8·5-day mouse embryo cDNA library. Ten positively hybridizing plaques were picked and rescreened. On subcloning the inserts from the recombinant phage into the plasmid pUC9 and analysing their structure using restriction enzymes two

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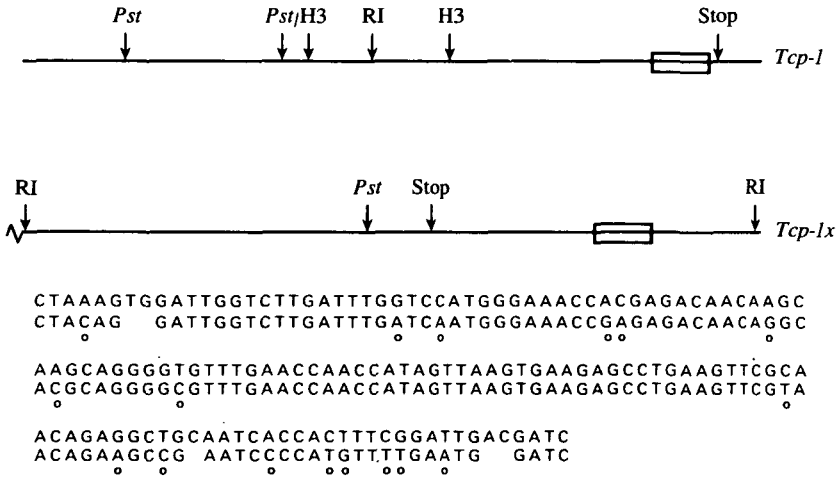

Fig. 1. Top: Restriction map of the *Tcp-1* and *Tcp-1x* cDNA clones. Restriction sites for the enzymes *Eco*R I, *Hin*d III and *Pst* I are indicated. The region of homology is shown as a box. Codons closing the open reading frame are indicated. *Eco*R I sites on *Tcp-1x* are cloning sites. Bottom: DNA sequence of the region of homology between *Tcp-1* and *Tcp-1x*. The sequence is drawn to allow a direct comparison with the published *Tcp-1* sequence. Altered nucleotides are indicated (o) and spaces have been left to achieve the best fit. *Eco*R I sites on *Tcp-1x* are cloning sites.

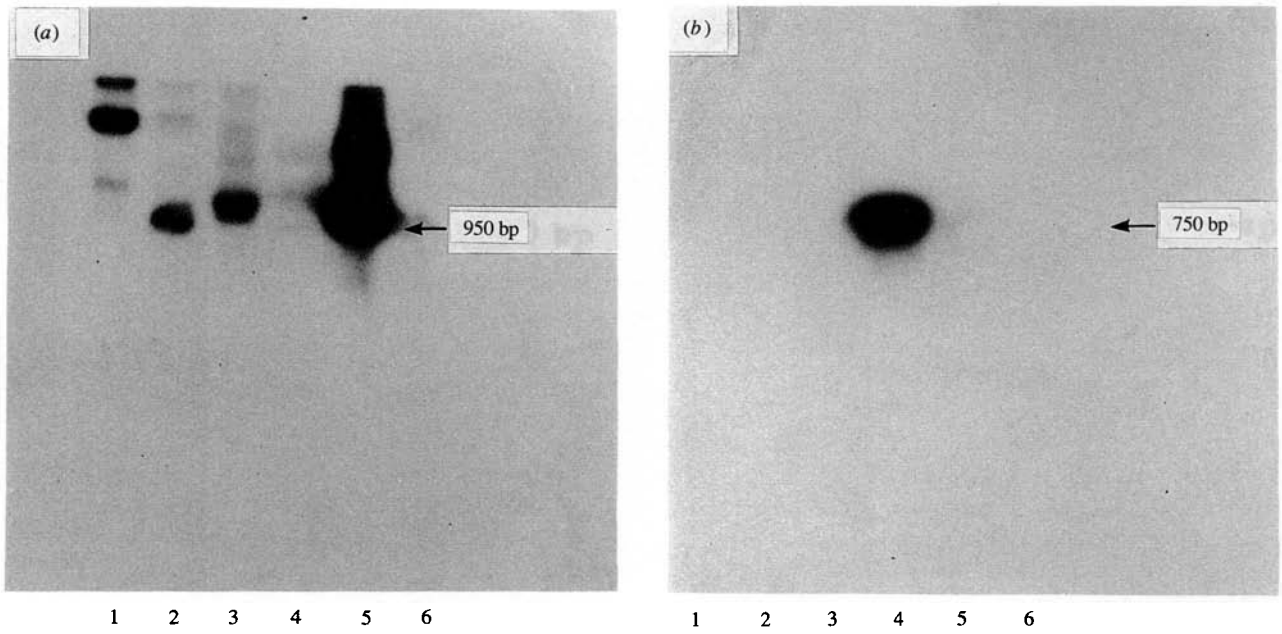

Fig. 2. *Tcp-1* and *Tcp-1x* cDNA clones were digested with the enzymes as indicated and the DNA fragments blotted and probed with either (*a*) the insert from clone 2D3′ or (*b*) the insert from clone 2D5′. 1, *Tcp-1* digested with *Eco*R I. 2, *Tcp-1* digested with *Hin*d III. 3, *Tcp-1* digested with *Pst* I. 4, 2D5′ digested with *Eco*R I and *Pst* I. 5, 2D3′ digested with *Eco*R I and *Pst* I. 6, pUC digested with *Eco*R I.

types of recombinant were identified (Fig. 1). The first was identical to *Tcp-1* but the second had a different pattern of restriction sites and in particular lacked any internal *Hin*d III sites of which there are two in *Tcp-1*. This new class of cDNA clones were presumed to be copies of mRNA transcribed from either the *Tcp-1x* or *Tcp-1y* genes.

One member of this new class of recombinants was selected for further study. This clone, called 2D, had an insert of about 1·7 kb and the region of this new clone homologous to *Tcp-1* was identified by Southern blotting. Using the enzymes *Eco*R I and *Pst* I the cDNA insert of clone 2D could be excised and cut into two fragments of approximately 950 and 750 bp. These two fragments have been subcloned into pUC9 and are referred to as clones 2D3′ and 2D5′ respectively. On probing a Southern blot of a *Tcp-1* cDNA clone digested with *Eco*R I, *Hin*d III or *Pst* I with the insert from the 2D3′ clone, bands were detected at 4·0, 0·8 and 1·0 kb respectively (Fig. 2). These bands are consistent with the previously reported restriction map of *Tcp-1*. In contrast, the same blot probed with the insert from the 2D5′ clone gave no hybridization to the *Tcp-1* sequences (Fig. 2). This result confirms that the region of homology between *Tcp-1* and clone 2D is in the 3′ portion of

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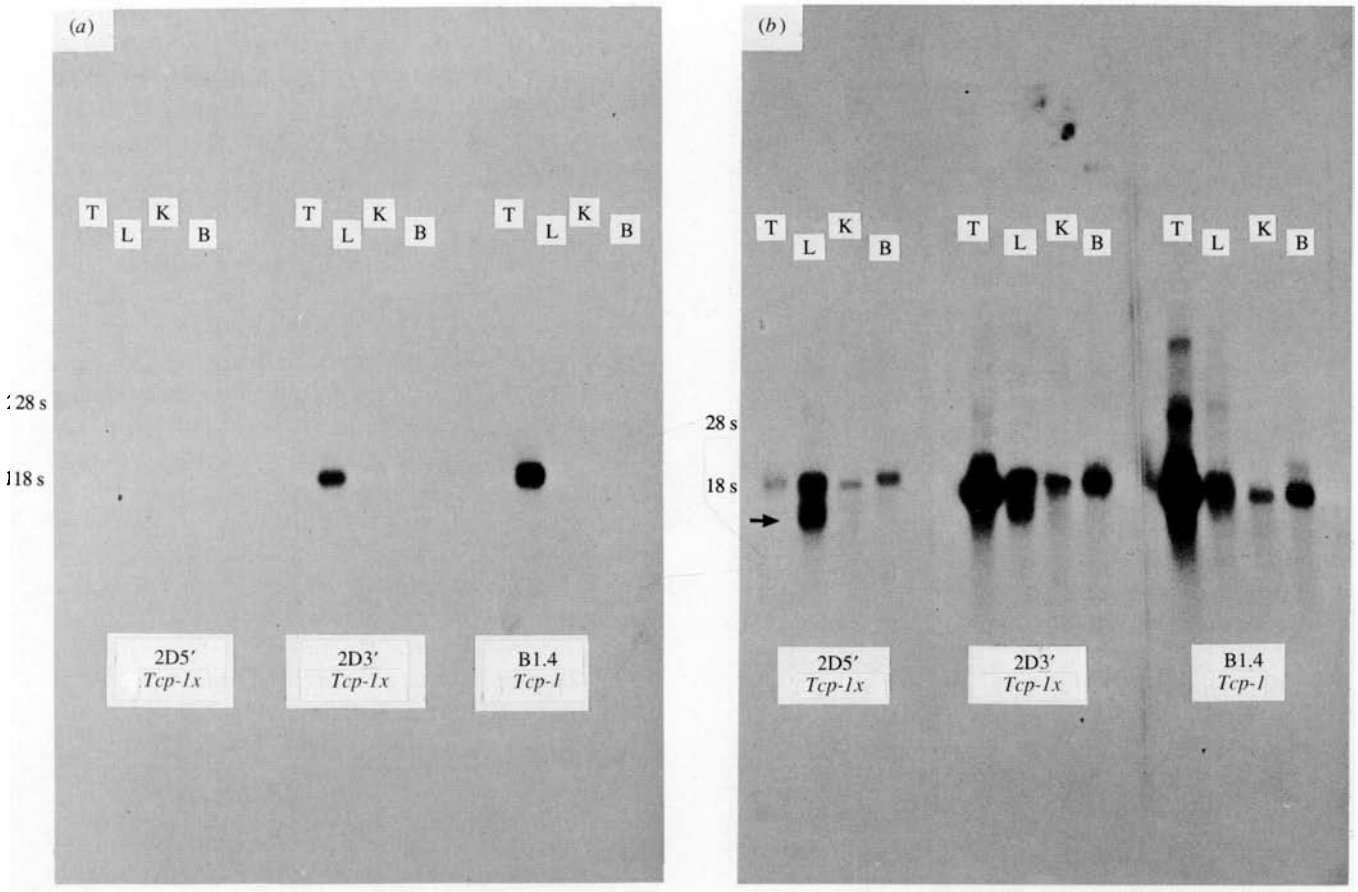

Fig. 3. Northern blot of RNA isolated from Testis (T), Liver (L), Kidney (K) and Brain (B) and hybridized with probes as indicated. B1.4 is the 3′ portion of the *Tcp-1* clone. (*a*) A 6-h exposure; (*b*) a 24-h exposure. All probes were of approximately the same specific activity. The arrow indicates the position of the liver specific transcript.

both cDNAs. It also indicates that the 2D5′ clone can be used to study expression of clone 2D in the absence of any cross hybridization with *Tcp-1*.

When labelled probes made from *Tcp-1* are hybridized to Northern blots of RNA prepared from a range of tissues a strong signal is detected with testis RNA after exposure for 5–6 h (Fig. 3); only after a much longer exposure (24 h) is any signal detected in other tissues. When a probe was made from the 750 bp insert of clone 2D5′ and hybridized to a Northern blot no signal was detectable with any tissue over a 6 h exposure (Fig. 3). On longer, overnight, exposure strong hybridization could be detected in the lane containing liver RNA with two transcripts visible (Fig. 3). The upper of these two transcripts was about 2 kb in size and was also detectable at much lower levels in the other tissues analysed, but the lower one of 1·8 kb was only present in liver. This lower transcript could also be detected in liver after overnight exposure using the 3′ portion of the 2D probe, as would be predicted, but was not detectable using the *Tcp-1* probe due to the limited homology between this probe and the transcript (Fig. 3).

Hybrid selection was performed with the 2D5′ clone to identify the protein that the gene coded for. Using total RNA isolated from the liver of a C57BL/6J mouse a protein of approximately 37 kDa was identified in the *in vitro* translation products (Fig. 4). A protein of the same size was obtained using liver RNA from a *t* mutant (t^{w2}/t^{w1}) (data not shown). It was not possible to select any specific product from testis RNA with this clone.

To determine the identity of clone 2D the 5′ portion of the clone which showed no homology to *Tcp-1* was used in *in situ* hybridization to mitotic cells of the genotype T190/++. The translocation splits chromosome 17 at the B band so that the proximal bands (17A-B) are carried on a short marker and the distal bands on a long marker chromosome (Lyon *et al.* 1986). On both the short marker and on the normal chromosome 17 also present, the centre of the grain distribution was found to be at the junction of bands 17A2/A3 (Fig. 5). Within the limits of resolution of the technique this places clone 2D in the same position as *Tcp-1* and confirms its identity as *Tcp-1x*.

Figure 6 shows the DNA sequence of clone 2D. The sequence, bounded by *Eco*R I restriction cloning sites, is 1687 nucleotides in length and has an open reading frame coding for a protein of 30 594 Da. In comparison with the products of hybrid selection this suggests that clone 2D lacks about 150 nucleotides of coding sequence at the 5′ end. Comparison with sequences in

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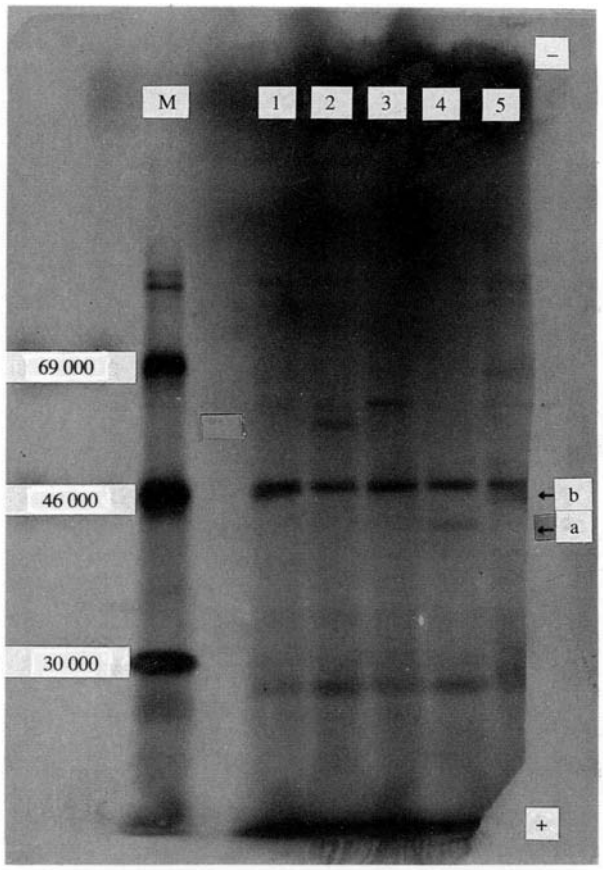

Fig. 4. Hybrid selection/translation products. Lane 1, zero RNA control. Lanes 2, 3, products selected by two cDNA clones only expressed in testis, from testis RNA. Lane 4, products selected by clone 2D5′ using liver RNA. Lane 5, products selected by a β-actin cDNA clone. a, indicates the position of the approximately 37000 Da protein selected by clone 2D5′ and b is the position of β-actin. M is a lane of markers; bovine serum albumin (69000 Da); ovalbumin (46000 Da); carbonic anhydrase (30000 Da). Translation products were separated on 10% polyacrylamide gels and fluorographed.

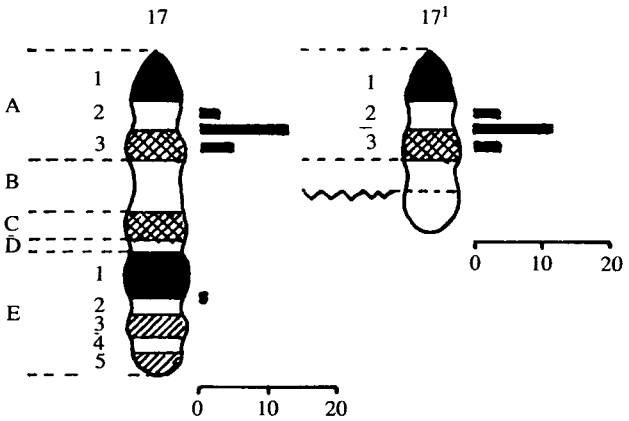

Fig. 5. Grain distribution on chromosome 17 and on the 17^1 translocation after hybridization of the insert of clone 2D5′ to G-banded metaphase chromosomes. 18·7% of the grains were found on the 17 chromosome at segment A2–A3 and 20·5% on the same segment of the 17^1 chromosome. 200 mitotic cells were examined and 112 grains were scored.

the EMBL and GENBANK databases reveals no significant homology except to *Tcp-1* (Willison *et al.* 1986). Analysis of the open reading frame shows that there is a potential *N*-glycosylation site at amino acid 143, and potential protein kinase C phosphorylation sites at amino acids 10, 101, 102 and 126. Analysis of the amino-acid composition shows that clone 2D protein has a high percentage of hydrophobic amino acids including a stretch of 17, ending 3 amino acids from the c terminus, which may indicate that the protein is membrane bound. This will have to await further verification. As expected from the Southern blotting data the region of homology between the *Tcp-1* and *Tcp-1x* clones is in the 3′ portion of the cDNA. A region of 140 nucleotides corresponding to the terminal 140 nucleotides of coding sequence of *Tcp-1* show 85% homology to 3′ non-coding sequences in *Tcp-1x* (Fig. 1). Sequence obtained from two separate isolates of the *Tcp-1x* clone reveal that this region of sequence is inverted in relation to the *Tcp-1* sequence, but the significance of this is unclear. The 3′ terminus of the *Tcp-1x* clone has a poly(A) tail and a polyadenylation site 20 nucleotides upstream.

4. Discussion

The work described here reports the identification and characterization of a cDNA clone corresponding to the *Tcp-1x* gene previously described as being in close linkage with *Tcp-1* on mouse chromosome 17 (Willison *et al.* 1986). Although the gene was isolated from a mouse embyro library there is no evidence that *Tcp-1x* has any specific role in mouse development and Northern blotting data has failed to reveal any significant variation in expression during embryogenesis between days 11 and 21 (data not shown). *Tcp-1x* is expressed predominantly in the liver where it hybridizes to two transcripts, one of which is only found in the liver, and is barely detectable in the testis. This is in contrst to *Tcp-1* which is abundantly expressed in the testis and only faintly detectable in the liver. This pattern of reciprocal expression is interesting in view of their close linkage; analysis of genomic clones suggest that they are within 10 kb of each other (Willison *et al.* 1986). It should be noted, however, that the sequence identity between *Tcp-1* and *Tcp-1x* will lead to cross hybridization on Northern blots if probes containing the homologous 3′ sequences are used. The sequence data suggest very strongly that the homology between the two clones probably has no functional significance. Firstly the region of homology is inverted in the *Tcp-1x* clone in relation to *Tcp-1* and secondly the region of homology is within the 3′ untranslated region of the former but is translated in the latter. This would suggest that the homology has arisen by some rearrangement of DNA in this region, the sort of event which is known to have happened many times within the *t* complex (Herrmann *et al.* 1986).

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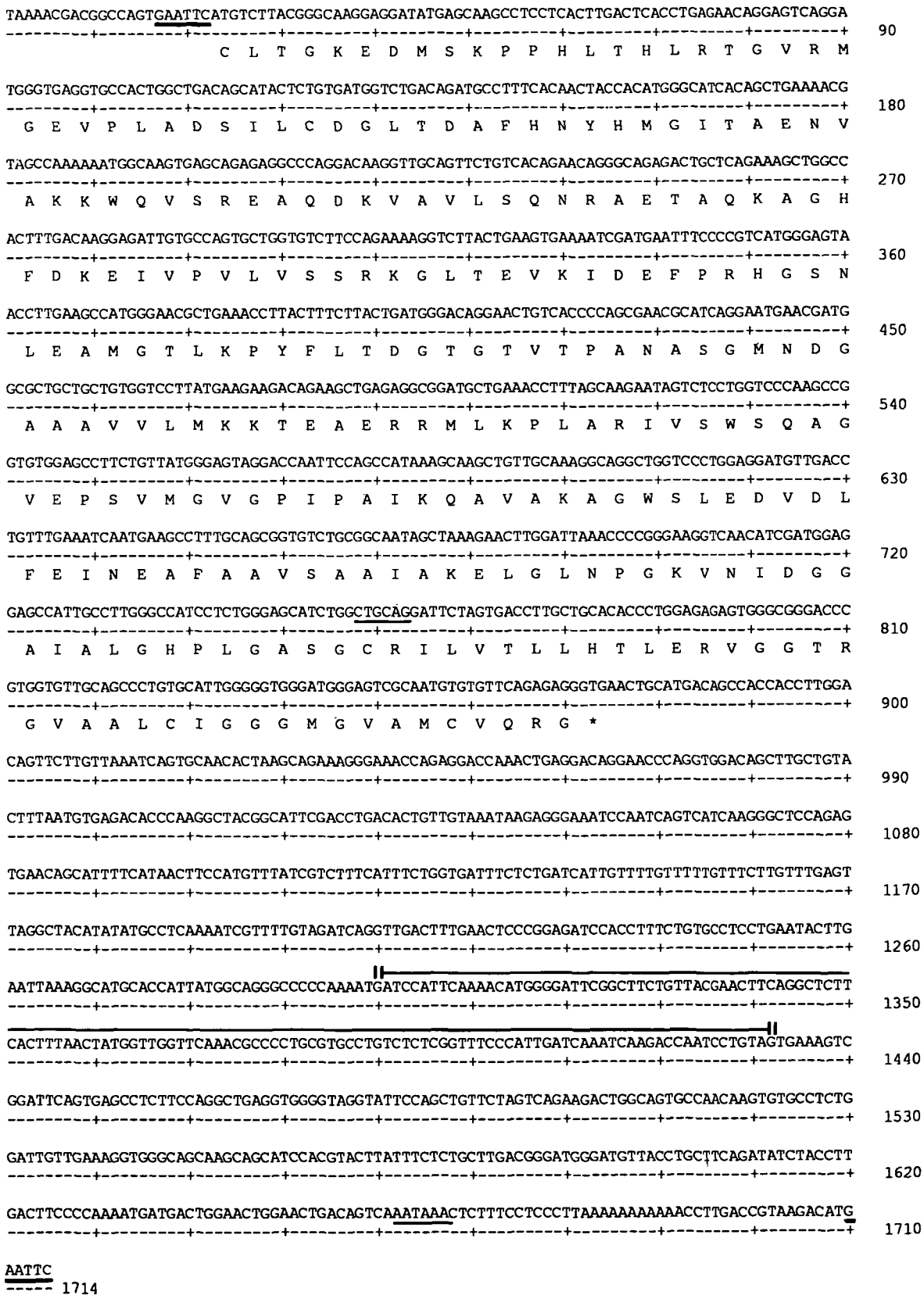

Fig. 6. Partial cDNA sequence of clone 2D. The *Eco*R I sites and *Pst* I site and the poly(A) addition signal are underlined. The region of homology between *Tcp-1* and *Tcp-1x* is indicated (⊢⊣).

Tcp-1 was mapped by *in situ* hybridisation to band 17A3 on unbanded chromosomes by measuring silver grain positions relative to the distal and proximal ends of the T190H short marker and also by lining grain bearing short markers alongside a G-banded example (Lyon *et al.* 1986). However, some reservations were expressed regarding the accuracy of the placement because of the large grain sizes relative to the bands

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and a position at the proximal margin of band 17B could not be excluded. For the same reason, the position of *Tcp-1* could equally be located at the proximal margin of 17A3, the location close to that determined for *Tcp-1x* using banded examples of both the small marker and the normal chromosome 17. In addition, the smaller grains enabled a more accurate placement to be made for *Tcp-1x* in relation to the bands. We therefore conclude that in view of the different methods used, the apparent difference in location of *Tcp-1* and *Tcp-1x* is insufficient to be meaningful.

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