The EMBO Journal vol.6 no.7 pp.1967–1974, 1987

The human homologue of the mouse t-complex gene, *TCP1*, is located on chromosome 6 but is not near the HLA region

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Communicated by W.Bodmer

Southern blot analysis indicates that there are four sequences in the human genome related to the mouse t-complex gene Tcp-1. All four genes were cloned and partial sequencing showed that one of them was a functional gene, and the other three were pseudogenes. The human sequences were all ~ 90% related to each other and 82–89% related to the mouse Tcp-1^a sequence. Human *TCP1* cDNA clones from both fibrosarcoma and B cell lines confirmed that there was a single expressed gene. mRNA transcripts of different sizes were accounted for by two different polyadenylation signals. The human *TCP1* gene shared some amino acid substitutions with the mouse t-complex allele (Tcp-1^a) which were not found in Tcp-1^b. The functional human *TCP1* gene was mapped, using a panel of somatic cell hybrids, as well as *in situ* analysis, to the long arm of chromosome 6 at 6q23-qter and thus is not closely linked to the HLA complex on the short arm. For this reason and others it is unlikely that there is a human equivalent of the mouse t-complex.

Key words: human t-complex gene/chromosome 6/mouse gene/comparison

Introduction

Recent molecular and genetic analysis of the mouse t-complex has shown it to be a doubly inverted region of chromosome 17 (Shin *et al.*, 1983; Herrmann *et al.*, 1986) ~30 Mbp long (Herrmann *et al.*, 1987) covering chromosome bands 17A3 and 17B (Lader *et al.*, 1985; Lyon *et al.*, 1986). The distal inversion includes the entire major histocompatibility complex (MHC). All t-chromosomes derive from a single ancestor which propagated successfully due to transmission ratio distortion (TRD), a mechanism whereby heterozygous males (t/+) pass the t-chromosome on to offspring at a higher frequency than the wild-type chromosome 17. TRD requires multiple genetic elements, some of which cause sterility when homozygosed (Lyon, 1984, 1986). It is for this reason that t-chromosomes are suggested to have acted as traps for lethal genes during their evolution — recessive lethals prevent the production of sterile homozygous males competing for females and for food (Lyon, 1986). There have been claims of transmission distortion of MHC markers in man and other species although the degrees of distortion do not approach the 99% observed in mice (Awdeh *et al.*, 1983; Bailey, 1986; Philipsen and Kristensen, 1985).

Recently, a cDNA clone for one of the polymorphic genes in the mouse t-complex, Tcp-1, was identified (Willison *et al.*, 1986). Tcp-1 codes for a protein which is abundantly expressed in testis as well as in other tissues (Silver *et al.*, 1979, 1983; Dudley *et al.*, 1984). In this paper we describe the cloning and characterization of human *TCP1* gene sequences. [Standardized symbols for human genes are used, i.e. upper case italicized letters (Human Gene Mapping 8, 1986).]

Results

The human genome contains four Tcp-1 related sequences

To estimate the number of human sequences homologous to mouse Tcp-1 probes, Southern blots of human DNA, digested with a variety of restriction enzymes, were probed with fragments of cDNA clones (Willison *et al.*, 1986). The fragments were from the extreme 5′ end, the 5′ half or the 3′ half. The results for

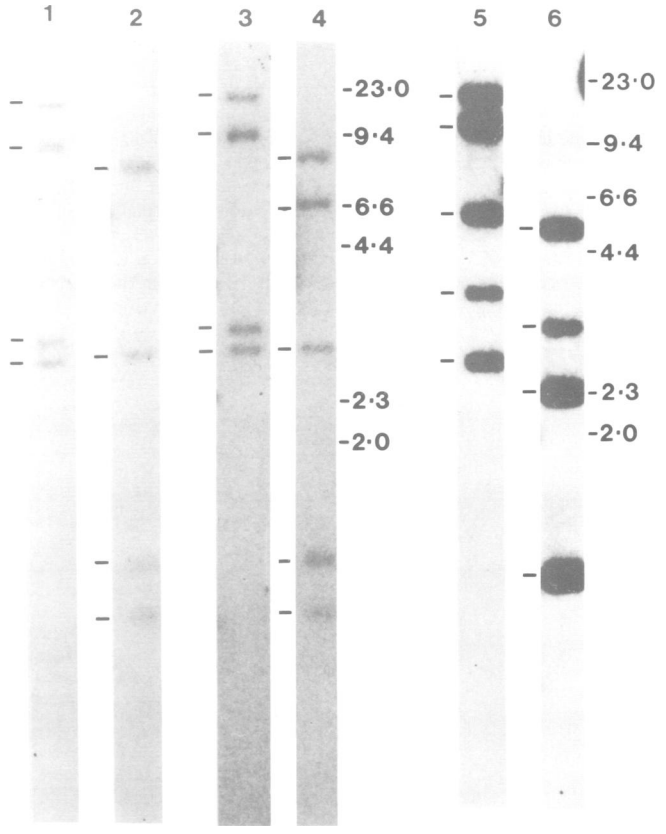

Fig. 1. Southern blots of human DNA probed with mouse Tcp-1 cDNA clone subfragments. Human genomic DNA was digested to completion with *Bam*HI **(lanes 1, 3** and **5)** or *Eco*RI **(lanes 2, 4** and **6)**. The DNA was then electrophoresed, blotted and probed with the extreme 5′ end probe of 133 bp **(lanes 1** and **2)**; a longer 5′ probe p1.2 (position 1–783; **lanes 3** and **4)**, or a human 3′ end cDNA probe pB1.4 hum (covering 580 bases of the 3′ end of the human mRNA). Similar results were obtained with the 3′ end mouse and 3′ end human cDNA probes (Willison *et al.*, 1985). The blots were washed in 0.5 × SSC at 65°C and were exposed to Kodak XAR-5 film at −70°C for 72 h with intensifying screens.

Southern blots of DNA digested with *Bam*HI and *Eco*RI are shown in Figure 1. At least four bands were obtained with all probe/enzyme combinations. When different DNA samples from unrelated individuals were used, similar patterns were obtained, indicating that the *TCP1* sequences are not highly polymorphic and that the multiple bands observed are not simply due to two different alleles at heterozygous loci. These experiments are consistent with the conclusion that there are four highly related *TCP1* sequences in the human genome.

Isolation of human Tcp-1-related genomic clones

Having ascertained that there were four human sequences related to the mouse Tcp-1 gene we cloned them to determine which of them corresponded to the functional gene. A phage λ EMBL 3 genomic library of partially *Mbo*I-digested fragments of a human cell line DNA and a human DNA cosmid library were screened with a human *TCP1* cDNA clone pB1.4 hum which covers 580 bases of the 3′ end of the human mRNA (Willison *et al.*, 1985). A total of 13 genomic clones were obtained. These were analysed by restriction enzyme mapping using the 5′ and 3′ specific mouse Tcp-1 probes. The clones fell into four groups as shown in Table I. Between them the clones accounted for all of the *Bam*HI and *Eco*RI restriction enzyme fragments observed in the genomic Southern blots (Figure 1) confirming that there are four human *TCP1* sequences. Group 1 contained two clones, 6G and 6C, which were positive with both 5′ and 3′ end probes, including the extreme 5′ end probe, indicating that these clones contained complete *TCP1* sequences. Group 2 contained one clone, 6F, which hybridized to the long 5′ end probe, but not to the short, extreme 5′ end probe from the mouse cDNA clone, indicating that the clone does not contain the complete gene. Group 3 contained eight out of the 13 clones. These fell into two sub-groups, except for one cosmid clone, cosI, which carried fragments contained within both sub-groups. Additional mapping, not shown here, indicated that the *TCP1* sequences are spread out over >20 kb on these clones, which explains why they encompass at least two restriction enzyme fragments with all enzymes studied (Table I). Sequence analysis, described below, indicated that the *TCP1* sequence in these clones was interspersed with human repetitive DNA elements. The fourth group contained two more clones, 2A and 6D, which were negative with the extreme 5′ end probe.

Table I. Characterization of human Tcp-1-related genomic clones

Genomic DNA sizes (kb)[a]					Corresponding genomic clones	
*Bam*HI		*Eco*RI		*Pst*I	Group	Clone no.
5′ probe	3′ probe	5′ probe	3′ probe	3′ probe		
2.5	20.0	0.7	1.0	1.9	1	6G,6C
–	12.0	3.0	2.3	4.0/1.9	2	6F
3.0	5.1/3.5	7.5/0.8	5.0/3.0	2.5/0.9	3	1A,1D,4A, 5B,5C,6C, 6E,cosI
–	2.5	8.5	1.0	0.6	4	2A,6D

[a]These figures represent the band sizes found in the genomic clones on the right of the table. Some of the genomic clones contained fragments that did not correspond to these sizes for all three enzyme digests and obviously were fusion fragments with vector sequences (see text and Figure 1). An exception to this is *Pst*I, where there is a known polymorphism (Willison *et al.*, 1985). The 5′ end probe p1.2 covered positions 1–783 and the 3′ end, pB1.4, positions 1025–1810 of the mouse cDNA (Willison *et al.*, 1986). –, These sizes were not recorded because all the clones in these groups contained fusion fragments with the vectors.

Sequence analysis

To determine which of the *TCP1* sequences corresponded to the expressed human *TCP1* gene we sequenced restriction enzyme fragments derived from representative clones from each of the four groups shown in Table I. Sections of these sequences are shown in Figure 2. Three groups of sequences appeared to be pseudogenes. Clone 6C, representative of the first group, contained deletions, one of 13 bp and another of 4 bp. An additional sequence indicated that clone 6C did not contain introns. Sequence

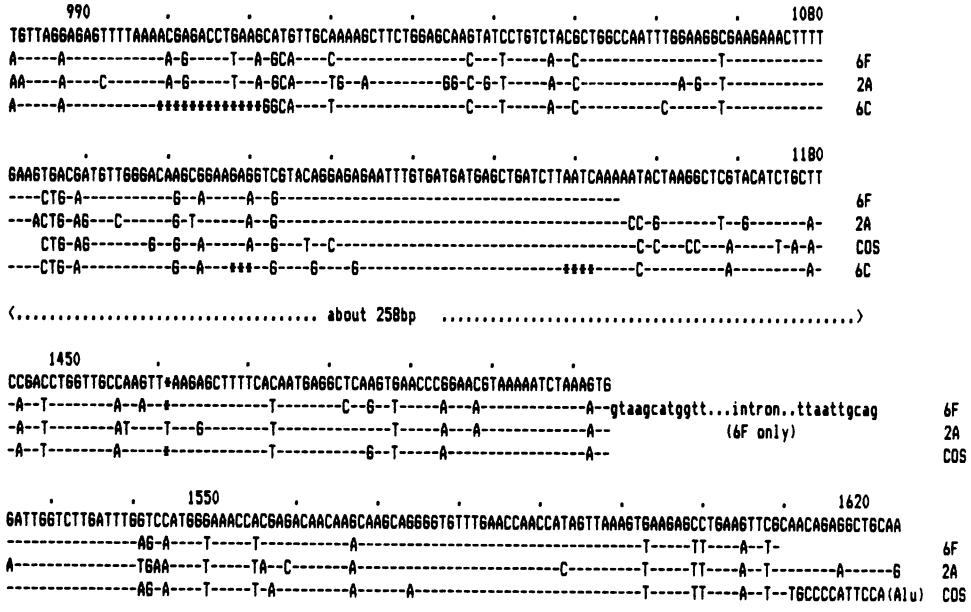

Fig. 2. Compilation of four human *TCP1* sequences. Restriction enzyme fragments from representative clones of the four human *TCP1* sequences described in the text were partially sequenced as described in Materials and methods. Extensive sequence was obtained in some cases, but only areas of considerable overlap and unambiguous sequence are presented. The human sequences are compared with the mouse Tcp-1[b] cDNA sequence, on which the numbering is based (Willison *et al.*, 1986). The only clone which can represent a fully functional gene is 6F, since it contains introns and is colinear with the expressed human and mouse sequences (Figure 3). The other sequences are discussed in the text and in Table II.

of the cosmid clone, cosI, from the third group (Table I), showed that this clone contained several insertions of DNA within the *TCP1* coding region. Sequences homologous to both *Alu* and *Kpn*I families of human repeats were identified, as well as a duplication of 22 bp of the coding region. The sequences in group 4 also lacked introns and contained some small deletions. Only one of the clones, 6F, contained the intron sequences and moreover the intron studied in this clone, shown in Figure 2, corresponds exactly in position to an intron within the mouse Tcp-1 gene (K.Willison, unpublished data). In other respects the 6F sequence was colinear with the expressed mouse sequence, containing a corresponding open reading frame that would encode a protein 98% related to the mouse Tcp-1^a product over the region we have determined. It is concluded that clone 6F contains the expressed human *TCP1* gene and that there is only one functional human *TCP1* gene plus three pseudogenes in the haploid human genome. These pseudogenes are probably of the processed type which are derived by reverse transcription of mRNAs expressed during gametogenesis or embryogenesis (Rogers, 1984). Since the *TCP1* gene is expressed at a high level in germ cells of the testis it is possibly not surprising it gave rise to processed pseudogene copies which were incorporated into the germline.

Table II. Comparison of nucleotide sequence of human and mouse Tcp-1-related genes

	Mouse cDNA (pB1.4)	Clone 6F	Clone 2A	Clone 6C
Clone 6F	89			
Clone 2A	82	92.5		
Clone 6C	88	94	91	
Clone cosI	84	94	93	88

The figures given are of percentage identity, calculated over at least 250 bp, for pairwise combinations of sequences. Part of the data on which these figures are calculated is displayed in Figure 2. The mouse sequence is from Tcp-1^a (Willison *et al.*, 1986).

Relationships of human TCP1 sequences

It was of interest to determine the degree of relatedness of the four human sequences, both to each other, and to the mouse sequence. These data are summarized in Table II. They indicate that, of all the human *TCP1* sequences, the functional human gene is overall the most homologous to the mouse Tcp-1 sequence, which is to be expected. The second conclusion from these data is that the human sequences are all more closely related to each other than they are to the mouse sequence. This may be interpreted to indicate that the three pseudogenes have arisen subsequent to speciation of mouse and man. All three pseudogenes are closely (between 88 and 92.5%) related, which is also consistent with the notion that they arose recently. Mouse appears to have only one Tcp-1 pseudogene, Tcp-1y (Willison *et al.*, 1986).

Human TCP1 cDNA sequences

Although we could only obtain evidence for the expression of one human *TCP1* gene, Northern blot analysis of mRNA from some tissues, for example B cell mRNA, revealed at least two

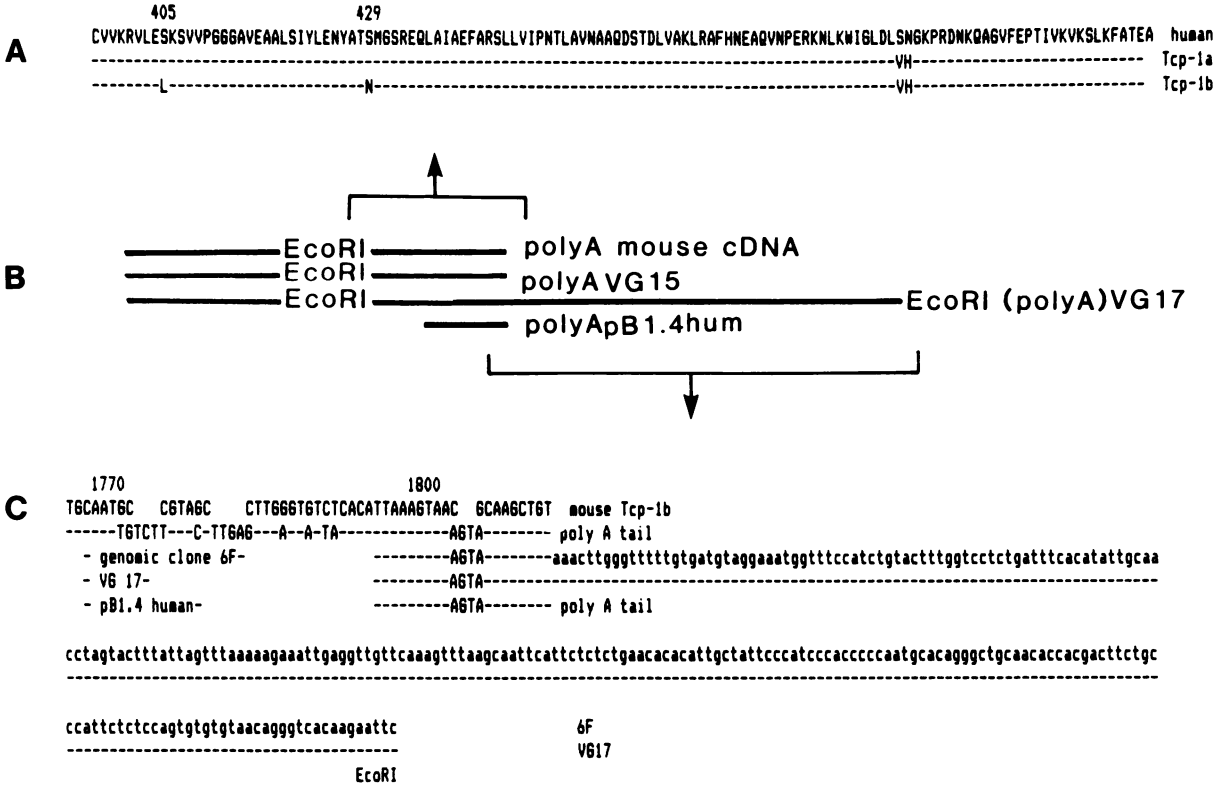

Fig. 3. Comparison of human and mouse Tcp-1 cDNA clones. **(A)** Peptide sequences. The two mouse TCP-1 peptides have been published; TCP1A is taken from t^{w32} and TCP1B from CBA/Ca (Willison *et al.*, 1986). The human sequence is derived from the cDNA clone pB1.4 hum. In TCP1A the leucine → serine replacement at position 405 is the result of a T → C transition which creates a *Taq*I site. This *Taq*I site is characteristic of all t-chromosomes studied so far (Willison *et al.*, 1986). Additional partial sequence from the human genomic clone also indicated that the human sequence was analogous to the Tcp-1^a allele: at aa296, human and Tcp-1^a Cys, Tcp-1^b Tyr; at aa326, human and Tcp-1^a Thr, Tcp-1^b Ser. **(B)** Schematic plan of the cDNA clones showing the extent of each clone. The top line represents the sequence of the mouse cDNA clone Tcp-1^b. VG15 and VG17 are human Tcp-1 cDNA clones from the human lymphoblastoid cell line and pB1.4 hum is from the cell line HT1080. **(C)** Comparison of 3′ end sequences of cDNA clones. Also shown is the cognate sequence from the functional human *TCP1* gene 6F.

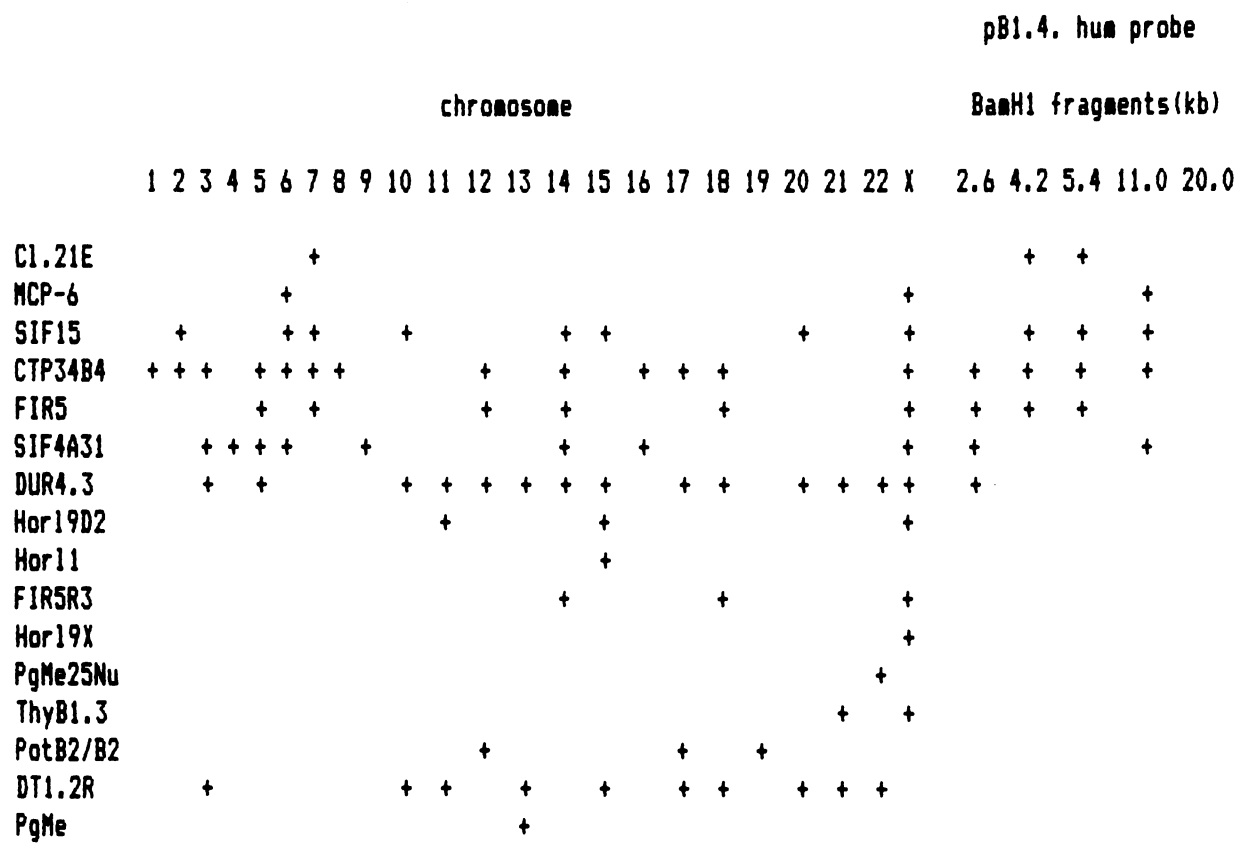

Fig. 4. Mapping of *TCP1* sequences to human chromosomes using a panel of somatic cell hybrids. An example of the data from which this figure is constructed is shown in Figure 5. The hybrid cell lines have been described previously: Cl.21E, Croce and Koprowski (1974); MCP-6, Goodfellow *et al.* (1982); SIF1S, SIF4A31, Edwards *et al.* (1985); CTP34B4, Jones *et al.* (1976); FIR5, FIR5R3, Hobart *et al.* (1981); DUR4.3, Solomon *et al.* (1976); Horl9D2, Horl1, Horl9X, Heisterkamp *et al.* (1982); PgMe25Nu, van Kessel *et al.* (1981); ThyB1.3, Goodfellow *et al.* (1980); PotB2/B2, Andrews *et al.* (1981); DT1.2R, Swallow *et al.* (1977). Hybrid PgMe was a gift from Dr C.Bostock.

different-sized transcripts of ~2.4 kb (weak) and 1.7 kb. To investigate this, and to confirm that clone 6F represented the only expressed gene human *TCP1* gene, clones were isolated from cDNA clone banks made from a human fibrosarcoma cell line HT1080 (Hall and Brown, 1985) and human B lymphoblastoid cell line mRNA (MANN; Heyes *et al.*, 1986) respectively, as described in Materials and methods. The HT1080 cDNA clone was colinear with the mouse cDNA clones described elsewhere (Figure 3; Willison *et al.*, 1986). However, two classes of B cell cDNA clones were apparent when these were restriction mapped (Figure 3). These clones contained an *Eco*RI site within the coding region at the same point as in the mouse Tcp-1 gene and sequence determined from this site indicated that it was equivalent in all of these human clones. The 3′ end sequences fell into two groups, however. Some clones, for example VG15, were polyadenylated at a position analogous to the position in the mouse Tcp-1 cDNA clone, allowing for some minor species differences in the sequences (Figure 3C). However, other clones (two out of 10) continued for a further 239 bp before reaching an *Eco*RI site. Presumably, these transcripts are polyadenylated downstream of this site, at a distance that remains to be determined. We assume that the two different-sized mRNA transcripts observed on Northern blots are due to this differential polyadenylation.

Comparison of human and mouse protein sequences

Having obtained some sequence information from the human gene (6F) and the cDNA clones it was of interest to compare the derived protein sequences with the available mouse sequences. At the protein level the human and mouse sequences were at least 95% related (Figure 3A). Two mouse sequences are available over this region and the human sequence was more analogous to one of them, Tcp-1^a. In other words, the human sequence has some of the features of the mouse sequence characteristic of the t-complex rather than the Tcp-1^b allele which is representative of inbred mouse strains. This was also true of all three pseudogene sequences: over the areas of sequence determined, the peptide sequences specified by the pseudogenes matched Tcp-1^a rather than Tcp-1^b (not shown).

Chromosome mapping

Having characterized the four human *TCP1* sequences we mapped the functional gene. A panel of human/mouse somatic cell hybrid DNAs was screened with the human cDNA clone pB1.4 hum described above (Figure 4). This showed that the functional (6F) gene was on chromosome 6, as the 12.0-kb *Bam*HI band was present on a cell (MCP-6) which contains an X/6 translocation chromosome as its only human material, but was not on other hybrids containing the human X chromosome. Another human *TCP1* gene was also mapped to chromosome 7: hybrid cl.21E, containing only human chromosome 7, gave rise to two positive *Bam*HI bands, of 5.1 and 3.5 kb. As shown in Table I, these bands are characteristic of the genomic clones in group 3 which contained the pseudogene that is dispersed over at least 20 kb.

Only one other gene could be tentatively mapped on this panel: the clones in group 4 (Table I) contained a 2.5-kb *Bam*HI band. This band was not present on hybrids containing chromosomes 6 or 7 and its pattern on the panel of hybrid DNAs was consist-

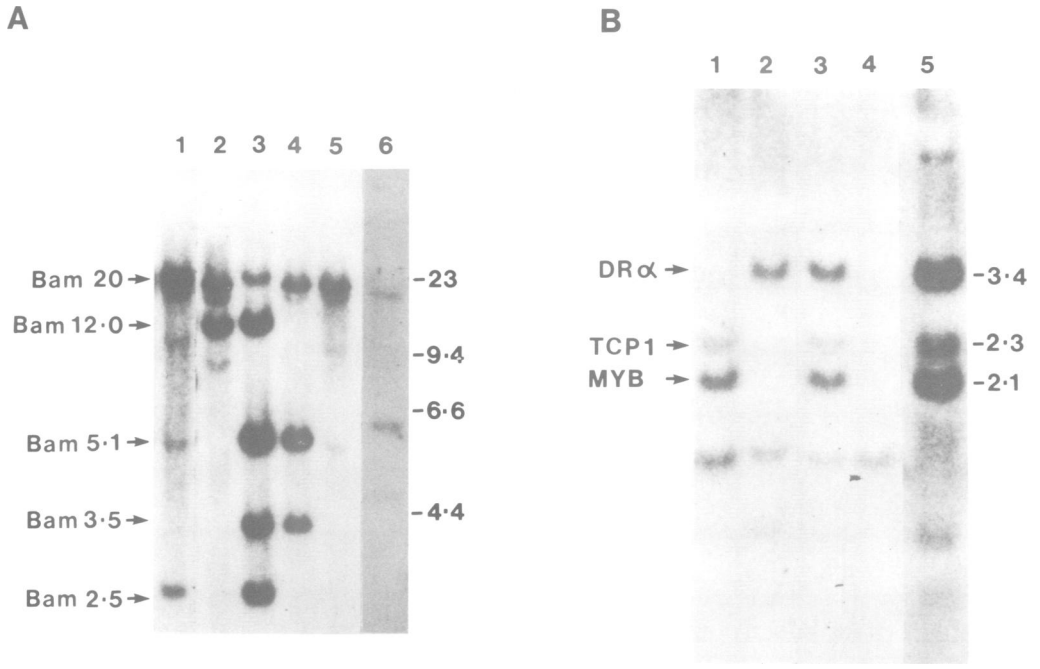

Fig. 5. Southern blots of somatic cell hybrids probed with the human *TCP1* cDNA clone pB1.4 hum. **(A)** *Bam*HI-cut genomic DNA probes with pB1.4 hum. **Lane 1**, hybrid Dur 4.3; **lane 2**, MCP-6; **lane 3**, human DNA; **lane 4**, Cl21E; **lane 5**, mouse PCC4 DNA (see Figures 4 and 6 for a description of the human chromosome contents of the hybrids); **lane 6**, hybrid 56-47 (Nagarajan *et al.*, 1986). **(B)** *Eco*RI-cut genomic DNA from somatic cell hybrids probed simultaneously with the pB1.4 hum human cDNA probe; a DRα cDNA probe (Lee *et al.*, 1982) and a *MYB* oncogene probe (Harper *et al.*, 1983). The bands corresponding to the different probes were determined by probing the filter with each probe individually. **Lane 1**, hybrid EDAG2.9; **lane 2**, hybrid EDAG3R; **lane 3**, hybrid MCP-6; **lane 4**, mouse DNA; **lane 5**, human DNA. EDAG hybrids were a generous gift from Dr Ian Craig, Oxford. Their human chromosome 6 content is shown in Figure 6.

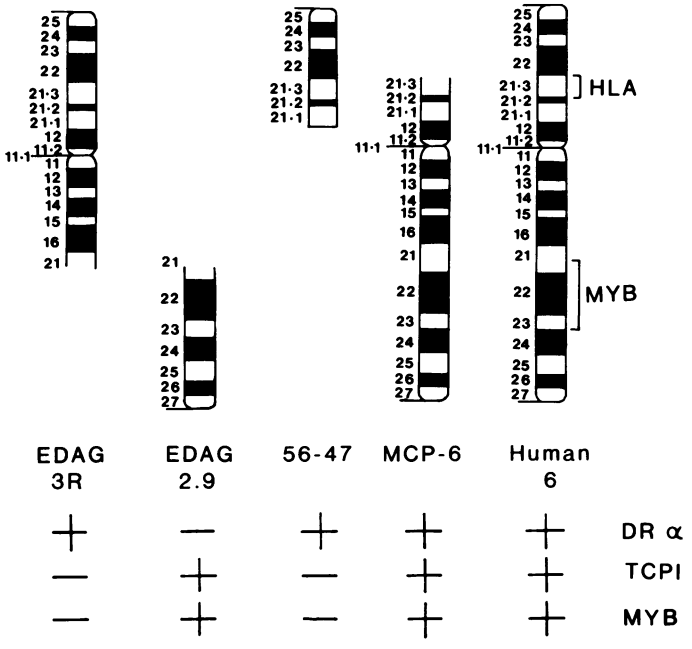

Fig. 6. Outline of chromosome 6 fragments in informative somatic cell hybrids. The hybrids are referenced in Figure 5. The results of probing with the three probes shown in Figure 5 are represented underneath the chromosomes.

ent with chromosome 5. Because we did not have hybrids with small numbers of human chromosomes which included chromosome 5 we could not confirm this assignment and regard it as tentative. The 20-kb *Bam*HI band coincided with a mouse band on these blots and we did not pursue mapping of the gene corresponding to the band (group 1) any further.

Some representative blots from these mapping experiments are shown in Figure 5A and B. In order to localize the 6F gene on chromosome 6 we screened a panel of hybrids containing fragments of this chromosome (Figure 5B). From this analysis the 6F gene was on the long arm of chromosome 6 and not on the short arm, near HLA, as we expected. The fidelity of the hybrid DNA panel was confirmed with known 6p and 6q probes DRα and *MYB* respectively (Figure 5B), and this maps to the functional *TCP1* gene to 6q21-qter (Figure 6).

In situ hybridization techniques were then employed to confirm this localization. As shown in Figure 7, the *TCP1* gene on chromosome 6 is located at 6q23-qter. The chromosome 7 gene is also localized to 7p14-cent. Possible signals were obtained from other chromosomes, including chromosome 5, but there were too few silver grains at these locations to make them statistically significant.

Discussion

In this paper we have shown that the human haploid genome contains one functional *TCP1* gene, highly related to the mouse Tcp-1 sequence, which is located at the distal end of the long arm of human chromosome 6. It is alo concluded that no human *TCP1*-related sequences map near the HLA complex.

Synteny between genes near the mouse H-2 and human HLA complexes is an important consideration in the discussion of possible human t-like genetic effects. A sequence that has been mapped to the short arm of chromosome 6, and was thought to be part of a putative human t-complex was *PGK2* (Szabo *et al.*, 1984) but this locus has recently been identified as a pseudogene (Tani *et al.*, 1985). Thus to the immediate distal side of H-2 there is no synteny since both the loci which map there, *PGK2* and

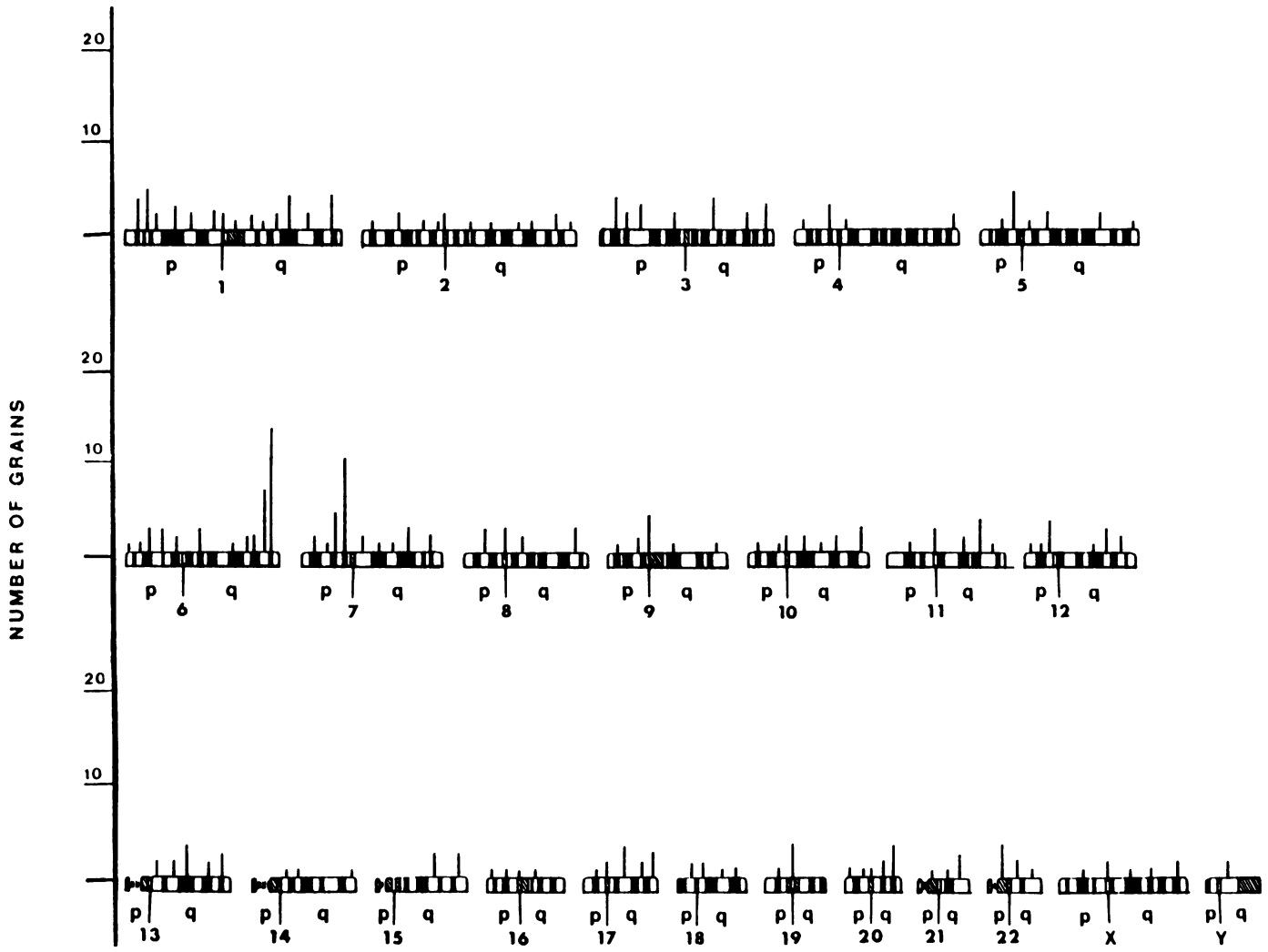

Fig. 7. Diagram showing silver grain distribution over chromosomes of normal lymphocytes following *in situ* hybridization with probe pB1.4 hum. Note the majority of grains localized over chromosome bands 6q2.6 and 7p1.1.

C3, are located on human chromosome 19 and are probably part of a larger syntenic linkage group themselves (Human Gene Mapping 8, 1985).

Two proximal MHC-linked markers that are in analogous locations in mouse and man, *PIM1* and *GLO1* (Weitkamp, 1976; Nagarajan *et al.*, 1986; Ziegler *et al.*, 1985) are not close to any of the breakpoints involved in the inversions that characterize t-chromosomes. However, even this short region of apparent synteny is not simple since *ACRY1* (α crystallin) maps between *GLO1* and H-2K in the mouse (Skow and Donner, 1985) but the corresponding human locus is on chromosome 21 (Quax-Jeuken *et al.*, 1985). *TCP1* may represent an isolated gene like *ACRY1* or may be part of a larger syntenic linkage between 6q and mouse 17. It is relevant to note that *SOD2* maps to 6q although it has not been regionally assigned in the mouse (Szymura *et al.*, 1981). The simple interpretation would be the existence of a syntenic region containing *SOD2* and *TCP1*. This type of syntenic linkage is called syntenic homology: the apparent conservation of linkage groups which cross a centromere (McCusick, 1985) of which there are several examples (Nadeau *et al.*, 1986). It is difficult to determine whether syntenic homology represents a random reassortment of gene order or whether it reflects the outcome of a small number of insertion and/or translocation events. An estimate of the number of syntenic linkage groups between mouse and man is 174 ± 39 (Nadeau and Taylor, 1984) so it is possible that, by chance, *TCP1* is on the same chromosome as the MHC in both species. However, until there is evidence to the contrary it can be assumed that the gene arrangement reflects internal rearrangements of the analogous chromosome in the two species, calling for intrachromosomal inversion or translocation events.

In conclusion, there are as yet no genes on the short arm of human chromosome 6 that could be construed as having genotypic or phenotypic effects of a t-complex nature. The fact that *TCP1* is not near the MHC is particularly significant because it encodes a major polymorphic testis protein that is suggested to account for some part of the t-complex effects on transmission distortion (Silver and Remis, 1987). Another explanation may have to be found for the marginal effects of transmission distortion of human MHC markers that have been recorded so far. It will be interesting to determine whether there are any other t-complex markers near *TCP1* on 6q. It cannot be ruled out that the *TCP1* gene is involved in phenomena similar to those manifested by the mouse t-complex, but involving the long arm of chromosome 6, although we consider this unlikely. Indeed, it seems that the properties of the mouse t-complex are a special case without a direct counter-

part in other species and it is unlikely that a human t-complex could have evolved, given the very different breeding structures of human and mouse populations. The TRD genes may be a feature of the particular inversions that took place once in the generation of the mouse t-complex. These genes may be neomorphs, i.e. they would have been created by the inversions at the junctions between inverted DNA and adjacent DNA or by altering the copy number of sequences at the inversion breakpoints.

How could the evolution of the rodent and primate *TCP1* genes be envisaged? Any model would have to take account of the fact that humans seem to have only one representative of the Tcp-1^a and Tcp-1^b alleles found in the mouse, confirmed by the fact that all three human pseudogenes derive from this sequence. It is suggested that the mouse t-complex has been prone to fixing mutations because these could not be removed easily by recombination, due to the inversions. Thus it is assumed that wild-type chromosome 17 carries the Tcp-1 allele found in other species and the t-complex gene would have diverged somewhat from that sequence. Now that we have shown that portions of the human gene are closer to Tcp-1^a this interpretation may be incorrect. It may well be that genetic isolation between t-complex and 'wild-type' mice led to the independent evolution of two different Tcp-1 alleles but Tcp-1^a has not accumulated mutations due to its isolation. Since t-complex mice may carry the allele equivalent to man, perhaps the mouse t-chromosome could be considered as the 'wild-type' and the chromosome found in most inbred strains a variant form. Any further speculation on the evolutionary history of Tcp-1 alleles would benefit from knowledge of sequences of several mouse strains. Some insight may also come from studies of Tcp-1 alleles in other mammals. Chromosome banding patterns demonstrate that frequent interchromosomal exchanges occurred in the evolution of rodents, compared with primates. In the latter species, chromosome evolution has been very conservative and all chromosomes can be identified (Yunis and Prakash, 1982). In contrast, if rat and mouse chromosomes are compared, they have no autosomes in common, only chromosomal segments (Viegas-Peguignot *et al.*, 1983). The t-complex properties could well have developed only in rodents given this background of chromosome instability.

Materials and methods

Nucleic acid techniques

DNA and RNA isolation as well as Southern and Northern blot analysis were all performed as described elsewhere (Maniatis *et al.*, 1982; Willison *et al.*, 1986).

DNA sequencing

DNA fragments, cut with restriction enzymes, were subcloned from phages and plasmids into M13 mp18 or mp19 vectors and sequenced by the chain termination method of Sanger *et al.* (1977).

Construction and screening of genomic libraries

Partial *Mbo*I-cut MANN cell line DNA was cloned in the EMBL 3 vector (Heyes *et al.*, 1986) and a library of 250 000 recombinant clones was screened as described by Kaiser and Murray (1985). The MANN cosmid library was described in Meunier *et al.* (1986).

cDNA libraries

In order to construct the human B lymphoblastoid cell line cDNA library poly(A)$^+$ mRNA was purified from the cell line LLICRF (Heyes *et al.*, 1986), using guanidinium isothiocyanate, caesium chloride density gradient centrifugation (Chirgwin *et al.*, 1979) and oligo(dT) cellulose chromatography (Collaborative Research Type III). Double-stranded cDNA was prepared using the reverse transcriptase/RNaseH/DNA polymerase I technique (Gubler and Hoffman, 1983), then blunt ended using the Klenow fragment of DNA polymerase and T4 DNA polymerase (Bethesda Research Laboratories). Internal *Eco*RI sites were methylated using *Eco*RI methylase, and *Eco*RI linkers added to the cDNA prior to ligating to *Eco*RI-cut λgt10 arms (Vector Cloning Systems). A cDNA library of 100 000 recombinant clones was propagated on *Escherichia coli* NM 514 cells. The library was screened by filter hybridization at 42°C in 5 × SSC, 50% formamide, 0.1% SDS, 5 × Denhardt's solution, 40 μg/ml salmon sperm DNA with 5 × 10^5 c.p.m./ml ^{32}P-labelled probe prepared by hexamer priming (Feinberg and Vogelstein, 1983). Filters were washed at 50°C in 0.5 × SSC, 0.1% SDS and exposed to Kodak XAR-5 film with intensifying screens for 10 h at −70°C. The cDNA clone pB1.4 hum was obtained by screening a λGT10 cDNA library from a fibrosarcoma cell line, HT1080 (Hall and Brown, 1985) with the mouse Tcp-1 probe, pB1.4 (Willison *et al.*, 1986). This probe pB1.4 hum has been deposited with the ATCC, MD, USA.

Cell lines and somatic cell hybrids

The cell lines have been described previously (Heyes *et al.*, 1986) and the sources of the somatic cell hybrids are listed in the legend to figures pertaining to them.

In situ hybridization

In situ hybridization was performed on chromosomes prepared from PHA-stimulated normal blood lymphocytes, essentially as described by Harper and Saunders (1981). One hundred Q-banded metaphases were photographed prior to hybridization, and the slides were then destained. The pB1.4 hum probe was labelled using oligoprimers and [^{3}H]deoxyribonucleotides to a sp. act. of ~1 × 10^8 c.p.m./μg, and hybridization carried out overnight at a final concentration of either 0.1 μg/ml or 0.02 μg/ml at 37°C. Slides were washed in 2 × SSC at 39°C, dehydrated and dipped in Ilford K-5 emulsion. They were developed after 1−3 weeks and stained with Wrights stain. A total of 290 grains were scored and 25 were located at 6q23-qter, 15 at 7p14-cent.

Acknowledgements

We should like to thank Dr Ian Craig for the EDAG hybrids which were a gift from the DMD group, Genetics Laboratory, University of Oxford. The hybrids were produced as part of work supported by the Muscular Dystrophy Group of Great Britain. We also acknowledge Susan Carson for making the MANN cosmid library. C.Fonasch and A.Ziegler have obtained similar *in situ* hybridization results with the human *TCP-1* probe described here and we thank them for communicating these to us before publication. K.W. and K.D. are supported by the CRC/MRC Joint Committee for the Institute of Cancer Research.

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Received on April 1, 1987