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# Sequence relationships between putative T-cell receptor polypeptides and immunoglobulins

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*Comparison of the sequence of a cloned T cell-specific cDNA with those of cross-reacting cloned cDNAs isolated from a thymocyte library indicates the presence of variable, constant and joining regions remarkably similar in size and sequence to those encoding immunoglobulin proteins. Together with the evidence for somatic gene rearrangements reported in the accompanying paper, this strongly suggests that the TM86 cDNA clone encodes one chain of the T-cell receptor for antigen.*

IN THE preceding paper[1] we reported the isolation of a cDNA clone, TM86, which represents a species of mRNA that is expressed in T cells but not in B cells, encodes a membrane-associated protein, and is rearranged in T cells. We report here our further analysis by nucleotide sequencing of clone TM86, and also of several cross-reacting clones isolated from a thymocyte cDNA library. Our data reveal a remarkable resemblance between the amino acid sequences of the proteins encoded by the mRNAs represented by the TM86 and related cDNA clones and the immunoglobulin proteins of B cells.

Immunoglobulin proteins have a characteristic primary and secondary structure made up of a series of intrachain disulphide-bonded domains (as reviewed in ref. 2). Both heavy and light immunoglobulin chains begin with a leader peptide of 17–29 residues, followed by a variable region of 94–97 residues then a joining region of 13–17 residues (in heavy chains the variable and joining regions are separated by short 'diversity' elements of 1–14 residues). These elements are encoded separately in the germ-line genome by gene segments that are brought together during B-cell ontogeny[3–6] to generate an enormous range of different antibody genes. The domains of the constant regions of immunoglobulins (of variable number depending on polypeptide chain type and class) are encoded by separate exons[7], but they do not appear to rearrange during development. The sequences of our T cell-specific cDNA clones has revealed a similar arrangement of constant and variable regions, strongly suggesting that the mRNAs that they represent encode polypeptide components of the T-cell antigen receptor.

## Characterization of cDNA clones

In view of the evidence presented in the preceding paper that the region of the genome represented by cDNA clone TM86 is rearranged in T cells, we sought to isolate cDNAs representative of independent rearrangement events by screening a thymocyte cDNA library (provided by C. Benoist) with TM86 using standard conditions[8]. Positive clones were subcloned into the *Eco*RI site of plasmid pUC9 (ref. 9). Partial restriction maps and the sequencing strategies[10] are shown in Fig. 1 for TM86 and the three thymus-derived clones 86T1, 86T3 and 86T5. The 3′ termini of the 86T series of clones are all identical, as each clone has an internal *Eco*RI site in that region, and the variation in position of the 5′ termini is presumably due to random chain termination during library construction.

As the largest mRNA detected on Northern blots with TM86 has a size of ~1,300 bases[1], by subtracting a poly(A) tail of

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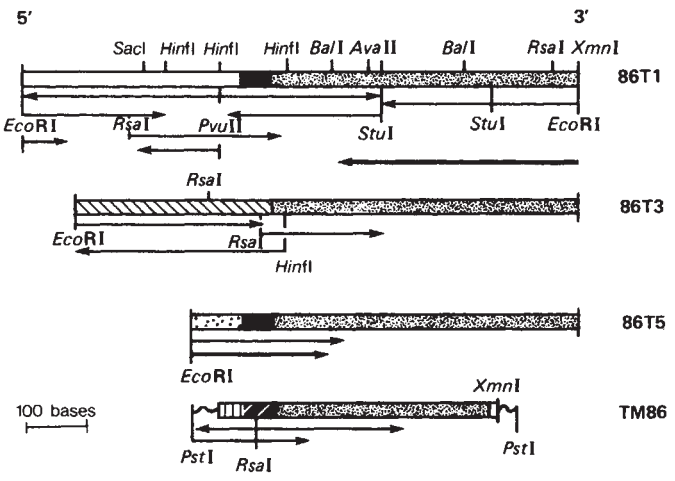


**Fig. 1** cDNA clone sequencing strategy and regions of similarity. The TM86 cDNA clone was obtained as described in the accompanying article[1], and the insert excised by *Pst*I from the plasmid vector. The insert was $^{32}$P-labelled by nick-translation[8] and used to screen a λgt-10 cDNA library constructed from total thymocyte poly(A)$^+$ RNA from young, BALB/c mice (T. Huynh and R. W. Davis, unpublished protocol). Shaded areas indicate major similarities between the different cDNA clones. The 5′ → 3′ orientation was deduced from the large open reading frame of 86T1. Restriction sites indicated for 86T1 are shared within regions having the same shading on the cDNA clones. Sequencing was by the procedure of Maxam and Gilbert[10], with thick arrows representing 3′-end labelling and thin arrows 5′-end labelling.

150–250 bases we would expect a cDNA insert size of around 1,100 bases. Thus, the 938-base insert of clone 86T1 should contain most of the sequence of the mRNA it represents. The insert of 86T1 was completely sequenced and compared with partial sequences of the other clones (see Fig. 1). As implied by Fig. 1, the 3′ end of TM86 appears to be different from those of the 86T series, which may have a bearing on thymic maturation and will be the subject of a separate report.

The nucleotide sequences and predicted amino acid sequences of the four different cDNA clones are given in Fig. 2. It can be seen that an open reading frame exists throughout the entire 86T1 insert, beginning with a methionine codon 22 nucleotides from the 5′ end and extending for 306 amino acid residues through the 3′ *Eco*RI site. The positions of cysteine residues and five potential carbohydrate attachment sites are indicated. Several interesting points are evident from these sequences: (1) Although all of the clones have identical 3′ end sequences (except where noted above for TM86) the 5′ end sequences are all very different. We have recently sequenced the entire 'constant' region of 86T3 (D. Becker and M.M.D., unpublished results) and it differs from the sequence of 86T1 by only a single nucleotide. This arrangement of constant and variable regions is similar to that observed with immunoglobulin cDNA clones. (2) Immediately following the methionine initiation codon there is a stretch of hydrophobic residues corresponding to the expected leader peptide (the sequence Leu-Leu-Leu is common in immunoglobulin κ light-chain leader peptides). (3) 86T1 and 86T5 share a 16-residue sequence between the constant and variable regions that is not found in the sequences of 86T3 and TM86, suggesting the existence of an independently assorting *J*-like region. (4) The positions of cysteine, and other, residues is similar to that of immunoglobulin and related molecules. (5) Clone 86T3 appears to be derived from a non-functional mRNA as it has stop codons in every reading frame.

## Similarity to immunoglobulins

To identify possible evolutionary relationships between the sequence of cDNA clone 86T1 and other known sequences, we searched the Dayhoff protein sequence data bank using the rapid comparison programs of Wilbur and Lipman[11]. From the Dayhoff bank of ~2,300 sequences, 25 were found to have a similarity to the 86T1 sequence greater than, or equal to, five standard deviations from the mean. Of these 25 sequences, 24 were immunoglobulin sequences and one was of a class II human major histocompatibility molecule (HLA-DC1α). Furthermore, it was found that the apparent variable region of 86T1 matched with variable regions of immunoglobulins and the constant region with immunoglobulin constant regions (and in one case with the second domain of HLA-DC1α, which has previously been shown to have a sequence related to those of immunoglobulins[12]).

Figure 3 gives examples of sequences from the Dayhoff bank which match with parts of the 86T1 sequence. The closest match was found with a sequence just N-terminal of the third hypervariable region of the heavy chain of the monoclonal antibody 93G7 (ref. 13). This is the most highly conserved sequence of immunoglobulin variable regions, and surrounds the second cysteine at residue 92 which forms part of the characteristic intrachain disulphide bond of the variable regions domain[2].

Figure 4 shows a more detailed comparison between the 86T1 sequence and those of murine immunoglobulin variable and constant regions, and demonstrates the clear immunoglobulin-like character of most of the 86T1 sequence. The similarity of the variable region of 86T1 to three different immunoglobulin variable region sequences[13–15] is shown in Fig. 4*a*. The degree of similarity between 86T1 and the immunoglobulin variable regions is comparable to that between the variable regions of immunoglobulin heavy and light chains. Of the 18 invariant residues of murine κ variable regions and the 10 invariant residues of the heavy-chain variable regions, 13 and 6, respectively, are found in the 86T1 sequence. The outermost two cysteines of the 86T1 variable region are separated by a similar number of residues (68) to those separating the cysteines of immunoglobulin variable regions that form the intrachain disulphide bonds[2] (65 amino acids for light chains and 70 amino acids for heavy chains). The alignment in Fig. 4 also predicts that the putative leader peptide of 86T1 will be cleaved just before the asparagine (N) residue at position 20 (Fig. 2).

This similarity to immunoglobulins is also shown by the constant region of 86T1, particularly around the cysteine residue at position 164 (Fig. 4*b*). It is interesting to note that the region N-terminal of this cysteine is more similar to light chains and the C-terminal region is more similar to heavy chains. The similarity of the 86T1 sequence around the last cysteine residue (position 260) to both λ and κ light chains may be important as in light chains this is the cysteine that forms an interchain disulphide bond with the heavy chain. The similarity in sequence between 86T1 and immunoglobulins is so pronounced that it might at least in part explain the cross-reactivity of B-cell anti-idiotypic antisera with T cells[16–18]. In contrast to this amino acid sequence similarity, the nucleotide sequence of 86T1 has diverged sufficiently from those of immunoglobulin genes to explain the negative results of many investigators who have used immunoglobulin gene probes to search for the genes encoding the T-cell receptor[19–22].

## Joining region-like elements

As indicated in Fig. 2, 16 amino acids are shared between 86T1 and 86T5 but not with the other two clones that we have sequenced, 86T3 and TM86. As this falls precisely into the region occupied by joining (*J*) region elements in immunoglobulins (between the variable and constant regions) we compared this region in 86T1 with the equivalent regions of 86T3 and TM86, as well as with the consensus sequences from each of the three different immunoglobulin *J* region elements[2] (Fig. 5). As shown in Fig. 5, the putative *J* regions of 86T1 and TM86 both have very similar sequences to those of all the immunoglobulin *J* regions, with no need for gaps to be introduced. As with the variable region similarities, the T-cell *J*

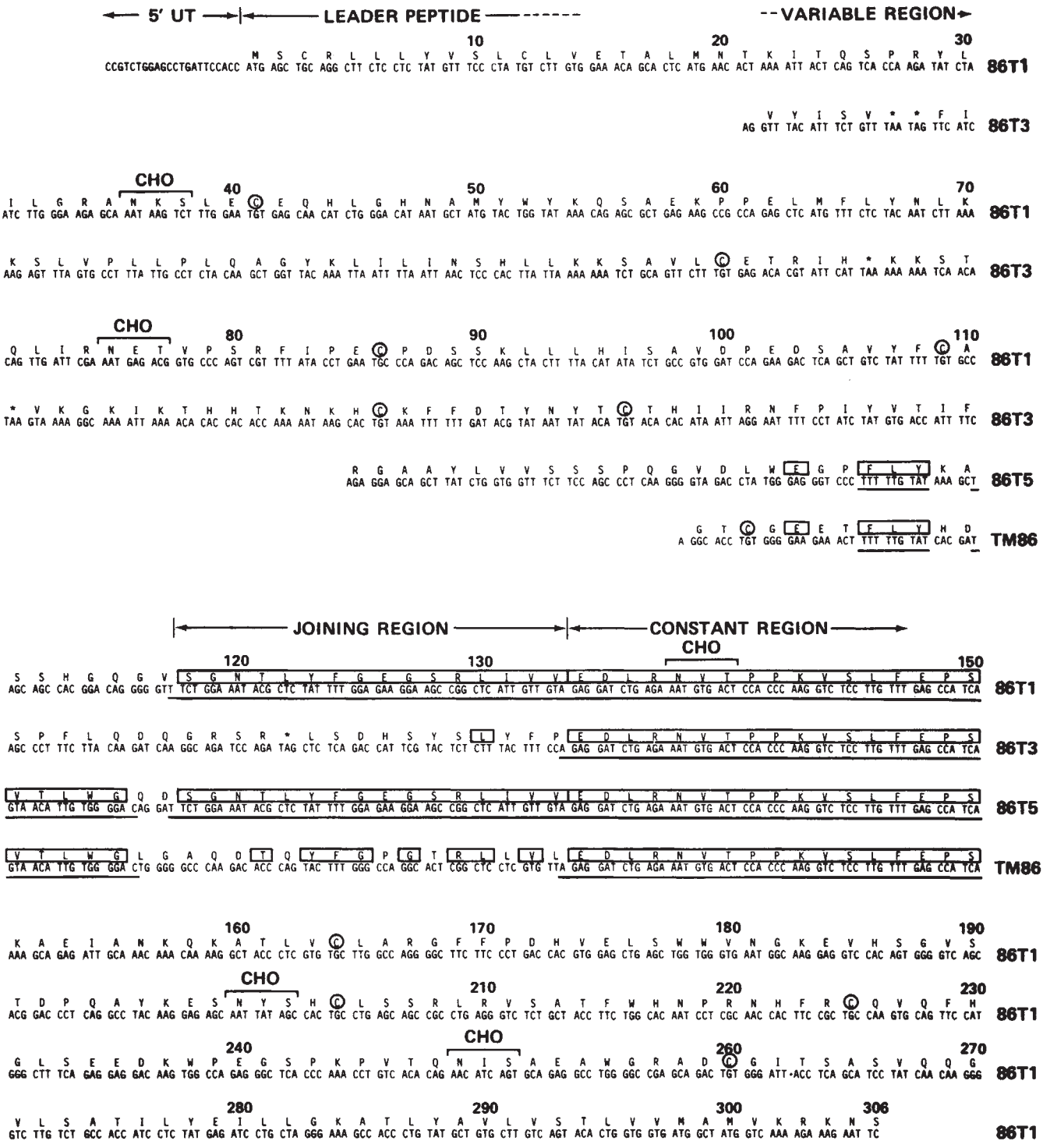


**Fig. 2** Sequence of TM86 and related cDNA clones. This figure shows the complete nucleotide sequence of 86T1 and partial sequences of the other cDNA clones, indicating the 5′ untranslated region (UT), the putative leader polypeptide, and the variable, joining and constant regions. Amino acid similarities are boxed, and nucleotide sequence similarities are underlined. The numbering follows the predicted amino acid sequence of 86T1. Cysteine residues are circled and possible carbohydrate (CHO) attachment sites are indicated (following the formula: N–X–S or N–X–T)[31].

regions show an approximately equal degree of similarity to each of the different immunoglobulin *J* region types, similar to the relationship between the heavy-chain and light-chain *J* regions, but not as pronounced as that between light-chain *J* regions. In terms of size (16 amino acids) they are more closely related to heavy-chain *J* regions (17 amino acids) than light-chain *J* regions (13 amino acids) but since the putative receptor chains are approximately equal in size[23–25], the same constraints may not apply.

In addition to the *J* elements, the adjacent 5′ regions of 86T5 and TM86 between residues 103 and 115 (Fig. 2) are very similar. In particular, the 17-nucleotide and 9-nucleotide identities between these two cDNA clones suggest the existence of other possible 'mini-gene'[26] elements possibly analogous to the

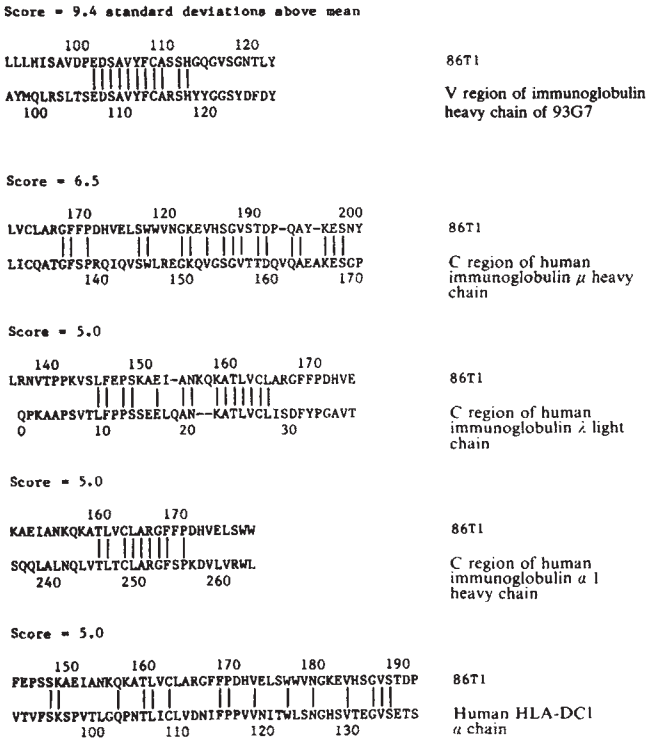


**Fig. 3** Search of the Dayhoff sequence data bank for sequences closely related to that of 86T1 using the program of Wilbur and Lippman[11]. Representative similarities are shown as they appear from the search with the matches indicated (the computer program focuses on short stretches of homology and thus all of the possible matches are not shown). Gaps are represented as dashes (–).

*D* region of heavy-chain immunoglobulins[6,27,28]. An alternative explanation would be that these similarities represent highly conserved sequences of related variable-region genes.

## Structural predictions

A number of groups have devised formulae to predict from amino acid sequences which portions of a molecule are embedded in membranes and which are exposed. These predictions are based on weighting formulae derived partly from measurements of solubility in organic and aqueous solvents and partly from empirical considerations. One such formula is that of Kyte and Doolittle[29] which is particularly suited for cell-surface molecules as most of the now considerable literature on the structure of such molecules has been taken into account. This 'hydropathicity' plot is shown in Fig. 6 and indicates a number of important features: first, that the 86T1 protein has the alternating hydrophobic–hydrophilic stretches characteristic of globular proteins[29]; second, that the predicted leader polypeptide occurs in a very adequate hydrophobic environment; and most importantly, it predicts a possible transmembrane region spanning the end of the 86T1 sequence, followed by a string of positive charges (Lys-Arg-Lys) which are characteristic of the cytoplasmic portion of a number of lymphocyte cell-surface markers[2]. The major difficulty with this prediction is the lysine residue (K) at position 284, in the middle of the putative transmembrane region, which is not characteristic, but may be masked by a salt bridge to another protein (possibly the other chain of the heterodimer).

## Discussion

For the following reasons the data presented in this and the accompanying paper[1] indicate the considerable likelihood that the mRNAs related to the TM86 cDNA clone encode one chain of the T-cell antigen receptor.

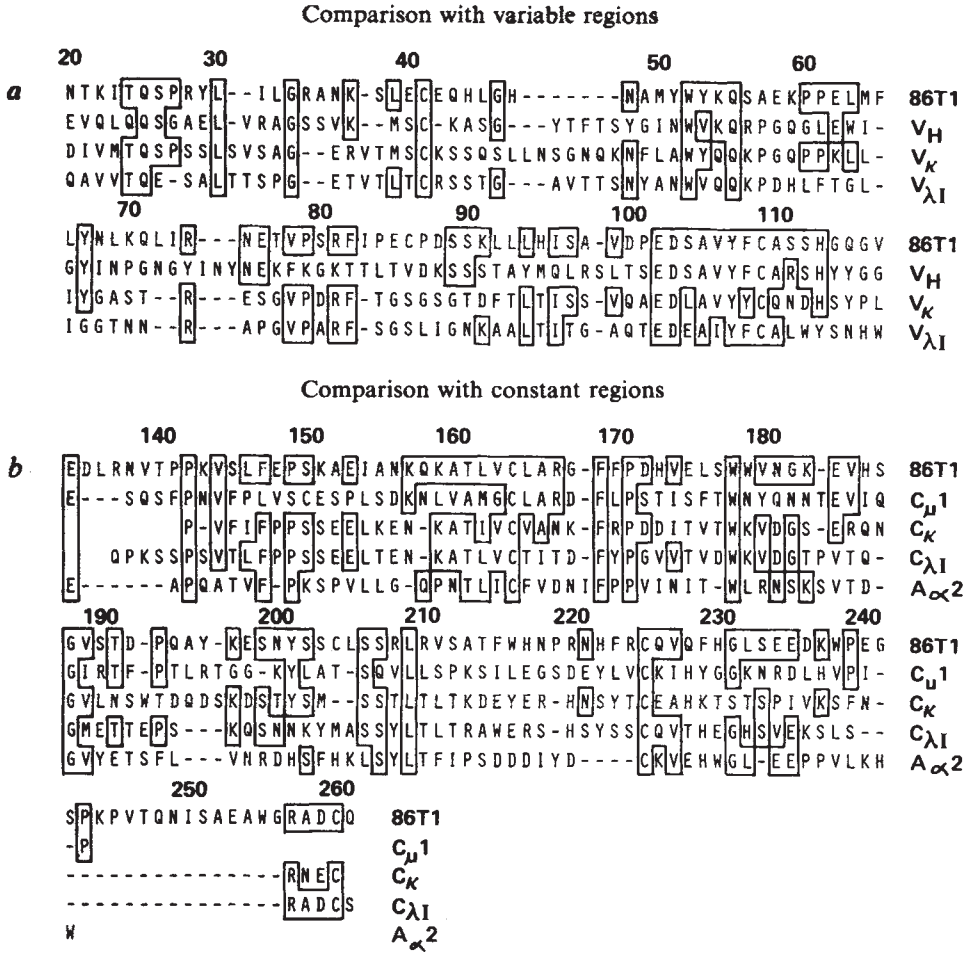


**Fig. 4** Similarities of the 86T1 amino acid sequence to variable (*a*) and constant (*b*) regions of murine immunoglobulins and to the second domain of the murine Ia–$A^d_\alpha$ (ref. 32) (the murine equivalent of the human HLA chain $DC_\alpha$). Similarities are boxed with gaps represented as dashes (–). *a* Shows a comparison of the variable-region immunoglobulin sequences with the 5′ region of 86T1; *b* shows a comparison of the 3′ region of 86T1 with immunoglobulin constant-region sequences and a region of the sequences of $A^d_\alpha$. The 9367′CL sequence also includes part of the *D* (diversity) region. The $V_\kappa$ is from MOPC 603[14] and the $V_\lambda$ sequence from MOPC 104e[15].

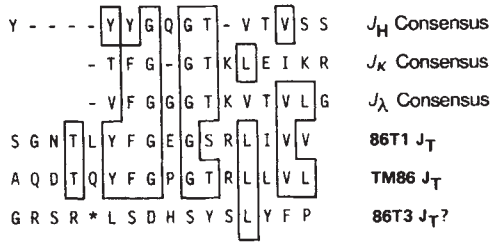


**Fig. 5** Joining region sequence similarities between immunoglobulin consensus *J* sequences[2] and the apparent T-cell *J* regions. Similarities are boxed and dashes (–) indicate a non-conserved residue between the *J* region elements of a particular immunoglobulin.

The first reason is the consistent rearrangements seen in Southern blot analyses of T-cell hybridomas and lymphomas, and the fact that these appear to vary in T cells of different specificities[1]. Second, the analysis presented here shows the very close similarity of the amino acid sequences predicted by our cDNA sequences to immunoglobulin variable, constant and joining regions (Figs 3–5). This similarity is much closer than that of the postulated 'super-family' of immunoglobulin-like molecules which includes a wide variety of very distantly related cell-surface glycoproteins[30], as judged by the outcome of the computer search (Fig. 3) and the overall similarities presented in Figs 4 and 5. Third, the finding of sequence differences and similarities between the set of cDNA clones illustrated in Fig. 2 and that these can be divided into 5′ variable, 3′ constant and intermediate joining regions of approximately the same size as those of immunoglobulins.

Given these considerations, we conclude that the locus described here represents a type of immunoglobulin gene specifically rearranged and expressed in at least some subsets of T lymphocytes, and that it must almost certainly play a part in antigen recognition by T cells. Supporting this later contention is recent work which indicates that antisera raised against synthetic peptide fragments of 86T1 can significantly inhibit the antigen-dependent release of interleukin 2 by T helper hybridomas (J. Rothbard, S.M.H., R.H. Schwartz and M.M.D., manuscript in preparation.

On the basis of the data that we have presented we suggest that the structure of the 86T1 gene is that shown in Fig. 7, with a 19-amino acid leader polypeptide, a 98-amino acid variable region, a 16-amino acid *J* region and a single globular constant-region domain followed by transmembrane and cytoplasmic portions. By analogy to immunoglobulin, the two outermost cysteines in each globular domain would be linked (as shown in the figure) and the last cysteine at position 260 would be bound to the other chain of the receptor heterodimer. It is not clear why there is an extra cysteine within each domain, but it should be noted that rabbit light-chain immunoglobulins also have an additional extra cysteine within each domain (and at the same position in the constant region); this may confer additional stability on the native molecule, perhaps through inter-chain bonding. The protein predicted from the 86T1 nucleotide sequence has a molecular weight of 34,000 (34K), and the processed molecule would be 32K, plus whatever additional coding sequence remains in the full-sized mRNA. Given the many glycosylation sites this is thus far compatible in size with either of the two 40–48K putative T-cell receptor polypeptide chains seen in the mouse[24,25].

Another interesting finding is the relative lack of immunoglobulin variable region homology found in the 86T5 and TM86 fragments shown in Fig. 2. 86T5, in particular, has no cysteine

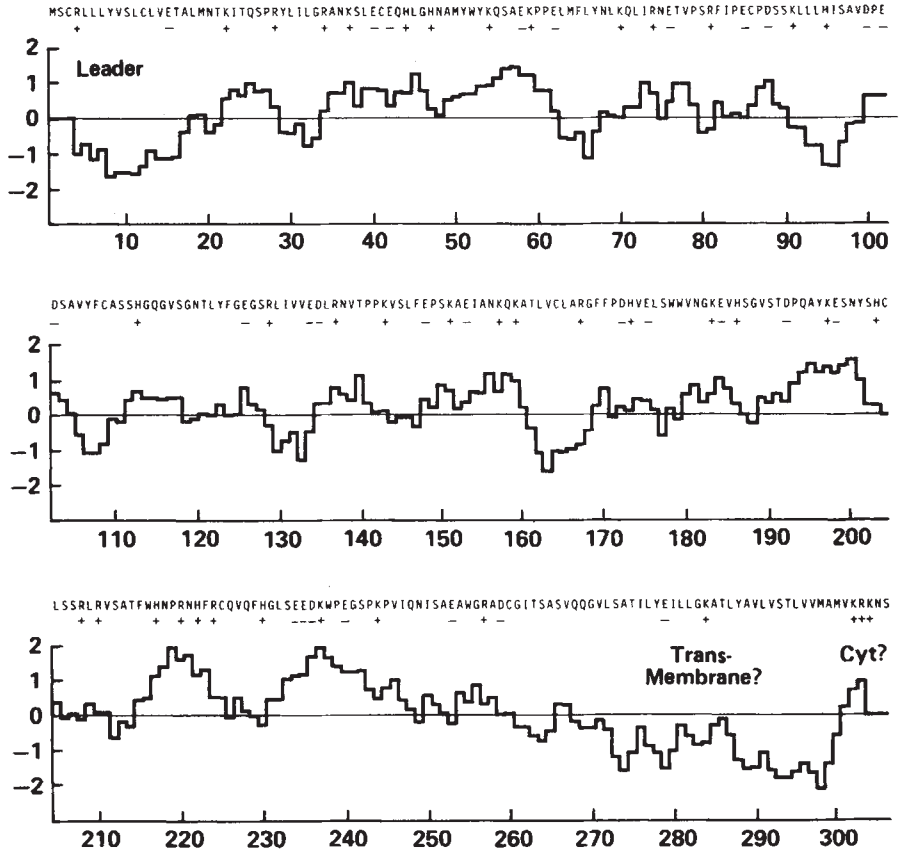


**Fig. 6** Kyte–Doolittle 'hydrophathicity' plot. The weighting system of Kyte and Doolittle[29] was used to predict membrane-embedded and hydrophilic stretches in the sequence of 86T1: negative values indicate the former and positive values the latter (see text). The numbers 1 and 2 in the figure represent the numbers 10 and 20 used in ref. 29.

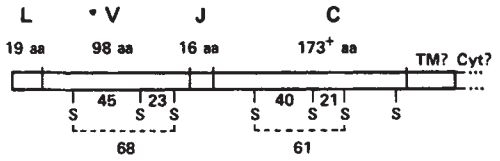


**Fig. 7** Suggested structure of the 86T1 gene with leader (L), variable (V), joining (J) and constant (C) regions indicated together with the length in amino acids (aa). Also illustrated are the positions of sulphydryl side chains of the cysteine residues (S) and possible intrachain bonding, based on the immunoglobulin analogy. The transmembrane (TM) and cytoplasmic (Cyt) portions of the molecules, based on the analysis in Fig. 6, are also shown.

residue even remotely near the one at position 109 of 86T1, which is so highly conserved in all immmunoglobulin variable regions. TM86 has a cysteine nearby at position 101, but appears to lack some of the surrounding conserved sequences. This may be the result of either a large number of non-functional transcripts (particularly in the thymus) or a wide variation in the structural requirements for a binding site.

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*Note added in proof*: Recent sequence analysis (Y. Chien, J.K. and M.M.D., work in progress) of a cDNA clone which extends further at the 3′ end than 86T1 indicates the presence of a stop codon immediately after the equivalent of residue 306 in Fig. 2. Therefore, the predicted amino acid sequence of 86T1 is most likely to be of the complete molecule.

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## LETTERS TO NATURE

# Pioneer 10 search for gravitational waves—no evidence for coherent radiation from Geminga

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**It has been reported[1] that an observed 160-min solar oscillation may be caused by gravitational radiation from the intense $\gamma$-ray source Geminga, a source whose period according to the report is exactly one cycle per year different from the solar oscillation period. This remarkable coincidence has motivated us to look for a sinusoidal component having a period near 160 min in Doppler data from the 1981 gravitational wave search with Pioneer 10. We find no such component in our most quiet data. Thus, the amplitude of a possible sinusoidal variation is no more than $3.3\times10^{-14}$ in fractional Doppler frequency, which for the direction of Geminga translates into an upper bound of $2\times10^{-14}$ for the polarization component of gravitational spatial strain potentially observable. This places rather stringent limits on the amount of gravitational radiation available to excite the 160-min normal mode of the Sun.**

We have examined Doppler data taken by the NASA/JPL Deep Space Network with Pioneer 10 over a 3-week period from 14 November to 8 December 1981 during solar opposition; at such times low-frequency phase fluctuation caused by scattering by free electrons in the interplanetary plasma is at a minimum. The data represent part of the record from gravitational wave searches with both Pioneer 10 and Pioneer 11 during their extended Heliospheric Mission. Data are also available from the 1982 opposition of Pioneer 10, as well as from two oppositions of Pioneer 11 for that matter, but these data have not been reduced to a point where they are useful for a search for the specific gravitational wave signal of interest here. The standard deviation of the Doppler noise from the best 1981 Pioneer 10 data is $\sim2\times10^{-13}$ in fractional frequency ($\Delta\nu/\nu$) over a bandwidth of $4\times10^{-5}$ to $\sim2\times10^{-3}$ Hz. The low-frequency cutoff is set by the round-trip light-time (or 'storage' time) of the microwave link while the system noise limits the high-frequency region[2–4].

The long light time $l$ to Pioneer 10, now beyond the orbit of Neptune, is a definite advantage in searching for long-period coherent gravitational waves. During the 1981 opposition $l\sim$ 12,490 s and the ratio $4\pi l/P\sim16.4$ rad, where $P$ is the period of the hypothesized gravitational wave. This ratio enters in a fairly complicated way with the cosine of the separation angle on the sky between Pioneer 10 and Geminga, $\mu=\cos\theta$, in determining the relation between the strain amplitude $h$ of the gravitational wave and the amplitude of the fractional Doppler response $\Delta\nu/\nu$ at the same frequency. The right ascension and declination of Pioneer 10 on 28 November 1981 was 4 h 4.05 min and 24.097° respectively, while Geminga has a position of 6 h 31 min and 17°48′, both positions given with respect to the mean equator and equinox of 1950.0. This yields $\mu=0.82$. From the 'three-pulse' response of the Doppler system[5], we conclude by summing the three phasors of the coherent Geminga signal that the observable polarization component of strain amplitude of a gravitational wave at Earth is amplified by a factor of 1.63 in the Doppler data.

Two approaches were used to search for a periodic signal from Geminga in the reduced Doppler record. In the first approach, three selected long passes of Doppler data of ~12 h each were transformed into the frequency domain by a fast Fourier transform operating on 512 data, sampled at 100-s intervals. The three spectra were averaged with equal weight given to each; the resulting spectrum is shown in Fig. 1. No