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Promoter and enhancer elements in the rearranged α chain gene of the human T cell receptor

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We cloned and compared the sequence of a rearranged human T cell receptor (TCR) VαJα gene and its germline counterparts. The only difference in the coding region sequence was confined to the joining region where three nucleotides, TTG, unaccountable by either Vα or Jα sequence, were present. By nuclease S1 mapping we identified the mRNA start of the α chain 70 nucleotides upstream from the initiator ATG. A 600 bp fragment containing the sequences upstream to the ATG drives the expression of the bacterial chloramphenicol acetyltransferase (CAT) gene. This promoter activity is T cell specific since it can be demonstrated in human T cells but not in B cells or HeLa cells. A 1.1 kb *Bam*HI–*Hin*dIII fragment located 5′ to the first exon of the Cα gene was found to enhance transcription from either the heterologous SV40 promoter or the homologous TCR α chain promoter. This enhancement activity was independent of the location of the fragment with respect to CAT and was specific to lymphoid cells (either T or B cells) but cannot be demonstrated in HeLa cells.

Key words: VJ rearrangement/mRNA start/CAT assay/tissue specificity

Introduction

The T cell receptor (TCR), like immunoglobulins, is encoded by gene segments which are rearranged during ontogeny to generate the transcription unit of the receptor polypeptide chains (Hedrick *et al.*, 1984; Siu *et al.*, 1984). The Tα chain locus consists of ~50–100 Vα gene segments separated by an unknown distance from at least 20 joining (Jα) gene segments which are dispersed over >60 kb of DNA upstream of a single Cα gene segment (Mak and Yanagi, 1984; Davis, 1985; Yoshikai *et al.*, 1985; Winoto *et al.*, 1985). Diversification of Tα may result from germline diversity of many genes, combinatorial joining allowing random association of V and J genes and junctional diversity due to imprecise joining and addition of extra nucleotides to the V–J junction. It is not clear yet whether somatic hypermutations of the V genes play a role in generating Tα diversity (Fink *et al.*, 1986). To analyse this point it is desirable to compare the sequence of the germline and rearranged Vα genes.

The study of transcription regulatory elements in the TCR genes is less advanced. In view of the key position of T cells in the immune system it seems extremely important to analyse these regulatory elements, their tissue specificity and the factors which control their activity. In functionally rearranged immunoglobulin genes the enhancer element located between the J and the C region is brought into functional proximity with the V gene promoter after VDJ joining, and enhances transcription from the promoter located 5′ to the V gene (Banergi *et al.*, 1983; Gillis *et al.*, 1983; Neuberger, 1983). A similar gene rearrangement exists also in the Tα gene and it is likely, therefore, that an enhancer element will also be located between the Jα and Cα genes. However, the distribution of Jα segment over a very long stretch of DNA hampered the analysis of such an element. On the basis of limited homology between IgH and Tα we speculated that the region upstream to Cα may be a candidate for an enhancer of the TCR α gene.

To study the regulatory elements of the Tα gene we cloned the Cα gene, the rearranged VαJα gene as well as its germline Vα and Jα counterparts from the human T cell leukemia line HD-Mar (Ben-Bassat *et al.*, 1980). The Vα gene joined a Jα gene 3 kb from Cα; it is expressed in the mRNA of HD-Mar and therefore allows the analysis of the mRNA start by nuclease S1 protection experiments. A *Bgl*II–*Bam*HI fragment which includes 600 bp 5′ to the ATG of the Vα gene contains a promoter activity that can drive the expression of the bacterial chloramphenicol acetyltransferase (CAT) gene in T cells. This activity is greatly enhanced by the 1.1 kb *Bam*HI–*Hin*dIII fragment located 5′ to the first exon of the Cα gene. The location of this enhancer implies a long range effect of this element *in vivo*.

Results

Structure of the germline and rearranged VαJα of TCR from HD-Mar

DNA from the HD-Mar cells (Ben-Bassat *et al.*, 1980) was analysed for the rearrangement of the Tα gene by digestion with various restriction enzymes and hybridization of their Southern blots to the radioactively labeled Jα fragment. Digestion by *Bam*HI or *Hin*dIII revealed one germline band of 3.7 kb which is present in both placenta and HD-Mar DNA and rearranged bands of 3.5 kb and 4.0 kb in the *Bam*HI and *Hin*dIII digest respectively, which were detected only in HD-Mar DNA. The rearranged *Hin*dIII fragment of Jα (p24.1) as well as the germline Jα fragment (p3.3) were cloned into pBR322. Mapping of the two clones indicates that they share an identical restriction enzyme pattern downstream from the *Pst*I site (Figure 1) but differ in the restriction enzyme pattern upstream to the *Pst*I site. Figure 1 also shows the identify of p3.3 with the *Hin*dIII fragment of λC3 which was isolated from a human gene library (Maniatis *et al.*, 1978) by the Cα probe and contains both Cα and Jα sequences. The 1.7 kb *Hin*dIII–*Bam*HI fragment of p24.1 (Figure 1) was used as a probe to isolate genomic clones from a human gene library. One of the strongly hybridizing clones was further analysed and found to contain an insert of 15 kb which included a 3.6 kb *Eco*RI fragment (pV.1) that hybridized to the *Eco*RI–*Bam*HI fragment of p24.1. Restriction enzyme mapping of pV.1 revealed a pattern identical to that of p24.1 upstream to the *Bam*HI site, but a different pattern downstream to the *Bam*HI site (Figure 1). Hence it is likely that p24.1 is a joined product

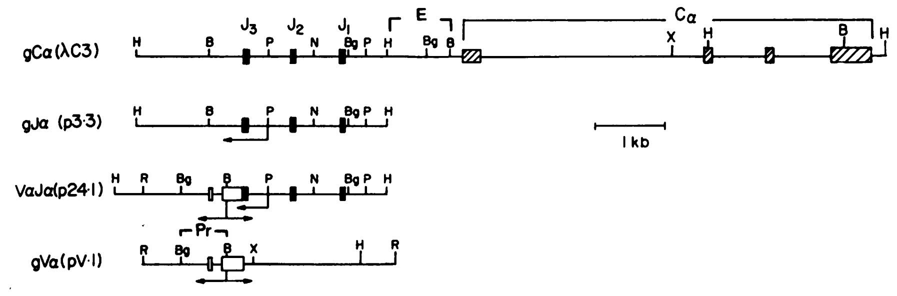

Fig. 1. Physical map of the isolated clones used in the analysis of VαJα rearrangement. gCα(λC3) is a portion of a recombinant phage isolated from human genomic library (Maniatis *et al.*, 1978) using the human cDNA clone pHαT1 (a gift of Dr C.Croce) as a probe. The position of Jα and Cα regions was determined by hybridization to pHαT1 and Jα probes and comparison with the previously published map (Yoshikai *et al.*, 1985). Plasmids p3.3 and p24.1 were cloned in pBR322 from the *Hin*dIII digested DNA of HD-Mar (see Materials and methods). pV.1 is an *Eco*RI fragment from a recombinant phage isolated from human gene library using the *Eco*RI–*Bam*HI fragment of p24.1 as a probe. Open boxes represent exons of Vα, filled boxes represent Jα and hatched boxes refer to Cα exons. The numbering of Jα begins with Jα1, being the nearest to Cα according to the data of Yoshikai *et al.* (1985). B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; N, *Nco*I; P, *Pst*I; R, *Eco*RI; X, *Xba*I. Arrows indicate the sequencing strategy. E and Pr indicate the fragments used to test enhancer and promoter respectively.

```
                                                                       <-------- L
                                M   Y   I   V   C   N   Y   C   V   F   L   G   L   W
VαJα   GGCATCTCTG TAGAAACATA ATG TAT ATT GTT TGC AAT TAT TGT GTA TTC CTA GGC CTG TGG G/GTAGAAA TATGAGTGGT
gVα    ---------- ---------- --- --- --- --- --- --- --- --- --- --- --- --- --- --- - ------- C---------

VαJα   TTTTTCTCTA ACAAAGGGAC GACTGGGGAG AGTGGGGGGC CCATGAAAAG ATGATTTAAA TTCTGGGTAC GAAGTGTCCC CTACCCAATC
gVα    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                                                         1                                   10
                                              G   V   W   S   Q   Q   K   E   V   E   Q   D   P   G   P
VαJα   TGTCTTCTCT GTTCTGACTG CTGTCTTTTT TCACAG/GG GTT TGG AGC CAA CAG AAG GAG GTG GAG CAG GAT CCT GGA CCA
gVα    ---------- ---------- ---------- ------ -- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                   20                                      30
        L   S   V   P   E   G   A   I   V   S   L   N   C   T   Y   S   N   S   A   F   Q   Y   F   M   W
VαJα   CTC AGT GTT CCA GAG GGA GCC ATT GTT TCT CTC AAC TGC ACT TAC AGC AAC AGT GCT TTT CAA TAC TTC ATG TGG
gVα    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                   40                                      50                                      60
        Y   R   Q   Y   S   R   K   G   P   E   L   L   M   Y   T   Y   S   S   G   N   K   E   D   G   R
VαJα   TAC AGA CAG TAT TCC AGA AAA GGC CCT GAG TTG CTG ATG TAC ACA TAC TCC AGT GGT AAC AAA GAA GAT GGA AGG
gVα    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                   70                                      80
        F   T   A   Q   V   D   K   S   S   K   Y   I   S   L   F   I   R   D   S   Q   P   S   D   S   A
VαJα   TTT ACA GCA CAG GTC GAT AAA TCC AGC AAG TAT ATC TCC TTG TTC ATC AGA GAC TCA CAG CCC AGT GAT TCA GCC
gVα    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
gJα                                                                                        TCAAGAAGGT TATCT

                   90 <------- V   N   J  -------->            100                                     110
        T   Y   L   C   A   M   S   C   S   S   A   S   K   I   I   F   G   S   G   T   R   L   S   I   R
VαJα   ACC TAC CTC TGT GCA ATG AGT TGC AGC AGT GCT TCC AAG ATA ATC TTT GGA TCA GGG ACC AGA CTC AGC ATC CGG
gVα    --- --- --- --- --- --- --C GCACAGTGC TCCCCAGACA CTTGCTTGCA GTCTGTACCC AAACCCAGGA
gJα    CAAA GACCTTACCC ACAGTGG GGG TA- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

        P
VαJα   CCA A/GTAAGTAGA ATGAAGCAGG AGAGCAAGGG
gJα    --- - --------- ---------- ----------
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Fig. 2. Comparison of the DNA sequence of germline gVα and gJα with that of the rearranged VαJα. The three sequences are aligned and the predicted amino acids are indicated. The sequence of VαJα is from p24.1, of gVα from pV.1 and of gJα from p3.3 (Figure 1). Recombination signals of gJα and gVα are boxed. A dash (–) indicates nucleotide identity to the VαJα sequence. The Jα sequence is identical to the published sequence (nucleotides 468–596 in Figure 4; Yoshikai *et al.*, 1985) and is referred to here as Jα3 (see Figure 1). L, leader; V, Vα; J, Jα.

of p3.3 and pV.1 (Figure 1) and presumably contains the rearranged VαJα.

To characterize the rearranged Jα clone (p24.1), we sequenced the region around the joining point and compared it with the sequence of the germline Jα clone (p3.3) as well as with that of pV.1 clone (see Figure 1 for sequence strategy). The data (Figure 2) indicate that the DNA sequence that joins Jα has the characteristic feature of Vα of the T cell antigen receptor. It contains 14 codons for a hydrophobic leader, followed by an intron of 143 bp and Vα exon of 97 codons. The sequence of the J region, and downstream from it, is identical to the published Jα (Yoshikai *et al.*, 1985) which is the third one from Cλ (Figure 1).

RNA transcript analysis

The *Bam*HI–*Pst*I fragment of p24.1 (Figure 1) was used as a probe to anlayse a Northern blot of HD-Mar. The results (Figure 3a) show that this probe hybridized to a 1.6 kb mRNA in HD-

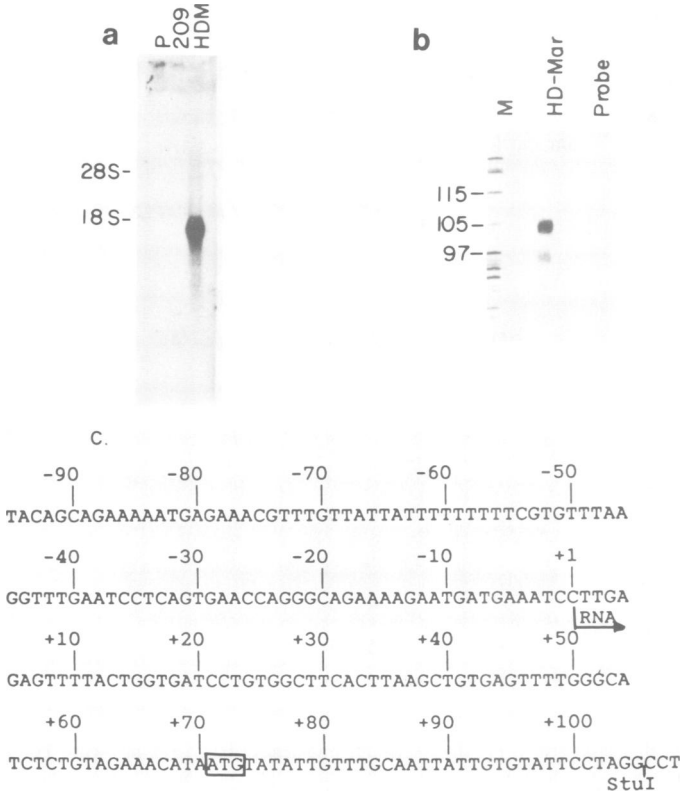

Fig. 3. RNA transcript of Tα in the human T cell line HD-Mar. (a) Northern blot analysis of poly(A)$^+$ mRNA from HD-Mar, from the human B cell line 209 and from human placenta (P). The *Bam*HI−*Pst*I fragment from p24.1 was used as a probe to detect the Tα gene transcript. (b) Determination of the mRNA start site of the α chain mRNA using the nuclease S1 protection analysis. The probe was the 650 nucleotides, *Bgl*II−*Stu*I fragment. The reactions were analysed on 6% acrylamide−urea gels alongside the sequence of the M13 clone containing the *Bgl*II−*Stu*I insert. The size of the protected fragment, 105 nucleotides, was determined from this sequence. M, the A lane of the sequencing reaction. HD-Mar, RNA and probe; Probe, no RNA. (c) Nucleotide sequence of the region upstream to the Vα relative to the transcription initiation site. The leader ATG is boxed and the *Stu*I site 35 bp downstream to the ATG is marked.

Mar but not in the B cell line 209 or placenta DNA. This size of mRNA corresponds to that of the expressed Tα mRNA and indicates that p24.1 contains a productively rearranged Vα which is transcribed into an α chain mRNA.

To locate the mRNA start site we used nuclease S1 protection analysis. The probe for this experiment was the 650 bp *Bgl*II−*Stu*I fragment, spanning the region upstream from nucleotide 35 (*Stu*I site Figure 3c) of the leader, that was subcloned in M13. The uniformly labeled fragment was hybridized to RNA derived from HD-Mar and digested with nuclease S1 as described (Weaver and Weissman, 1979). Figure 3b shows a minor and a major protected fragment in HD-Mar RNA which are 95 and 105 nucleotides long respectively. These protected fragments contained 35 p of the leader sequence (from the ATG to *Stu*I, Figure 3c) and 70 bp (for the major fragment) upstream to the ATG, indicating that the major mRNA start site is 70 bp upstream to the ATG. Our assignment of the major mRNA start site is based on the assumption that there are no additional introns/exons upstream to this point. The absence of consensus splice site in the sequence at this point (Figure 3) supports this assumption. The DNA sequence upstream to the mRNA start site does not contain a consensus TATA box or CAAT box.

Functional analysis of the TCR α chain enhancer and promoter elements

Since the TCR genes share structural homology with the immunoglobulin genes, we reasoned that elements controlling transcription may be located in similar positions. First, the promoter, immediately upstream of the mRNA start sites, was cloned to direct expression of CAT in pαTVPCAT. This was accomplished by ligating the 0.7 kb *Bgl*II−*Bam*HI fragment from p24.1 (Pr in Figure 1) to the *Bgl*II site of pCAT3M (Laimins *et al.*, 1984) to generate pαTVPCAT (Figure 4a). Second, we searched for a potential enhancer element which might be analogous to the immunoglobulin heavy chain enhancer located upstream of the first exon of Cμ (Banergi *et al.*, 1983; Gillies *et al.*, 1983). Analysis of the published sequence upstream to the first exon of the Cα gene of the TCR revealed a duplicated decamer separated by 35 bp. The sequence of this decamer, TTCTGTGGCA (nucleotides 2947−2957 and 2992−3002, Yoshikai *et al.*, 1985), shares 9 bp with the sequence of the enhancer core of immunoglobulin heavy chain TCATGTGGCA (Church *et al.*, 1985; Schlokat *et al.*, 1986). This sequence was introduced as a *Bam*HI−*Hin*dIII fragment from Cλ3 (E in Figure 1) both upstream and downstream of the Vα promoter in pαTVPCAT resulting in pαTVPCAT·E1 and E2 respectively. In addition, this putative enhancer was inserted upstream and downstream of the SV40 promoter driven CAT gene in pA10CAT as pA10CAT·E1 and E2 respectively (Figure 4a). The CAT expression of these various promoter−enhancer constructs was tested in three human cell systems: the non-lymphoid HeLa cell line, the T cell HD-Mar and the B cell GM 4672 (Figure 4b). pαTVPCAT which contains the Vα promoter without enhancer element was only able to direct CAT expression in the T cell. The 1.1 kb *Hin*dIII−*Bam*HI fragment enhances this Vα promoter activity in T cells and also facilitates CAT expression in B cells but not in HeLa cells. These results indicate that the Vα promoter is T cell specific, whereas the Tα enhancer is lymphoid cell (T and B) specific. The enhancement effect was independent of its location 5′ or 3′ to the CAT gene. In this particular experiment >90% of chloramphenicol acetylation was observed. In another experiment, where different time points were taken from the acetylation reaction, we found that in the linear portion of the reaction the enhancement was ~14-fold in HD-Mar (Figure 5). The Tα enhancer can drive the CAT expression also from the heterologous SV40 promoter. The constructs pA10CAT·E1 and E2 showed enhanced CAT activity as compared with pA10CAT in either T or B cells but not in HeLa cells. When the location of the enhancer was 5′ to the CAT gene in these constructs, the enhancement was 2-fold (in B cells) and 3-fold (in T cells) more effective than if it were located 3′ to the CAT gene. The enhancement compared with pA10CAT was 8- and 25-fold in T cells and B cells respectively (Figure 4b). These results indicate that the sequence immediately upstream to the Cα of the TCR contains an enhancer element which can increase expression from either homologous or heterologous promoter.

Discussion

We have cloned and analysed a productively rearranged VαJα gene of the TCR from a T cell line HD-Mar, as well as the relevant germline Vα gene and Jα region. The sequence of the HD-Mar Vα segment is quite different from most published human Vα sequences and suggests that this Vα belongs to a small family of Vα genes. This is also supported by Southern blot hybridization with the Vα probe (*Bam*HI−*Xba*I fragment from

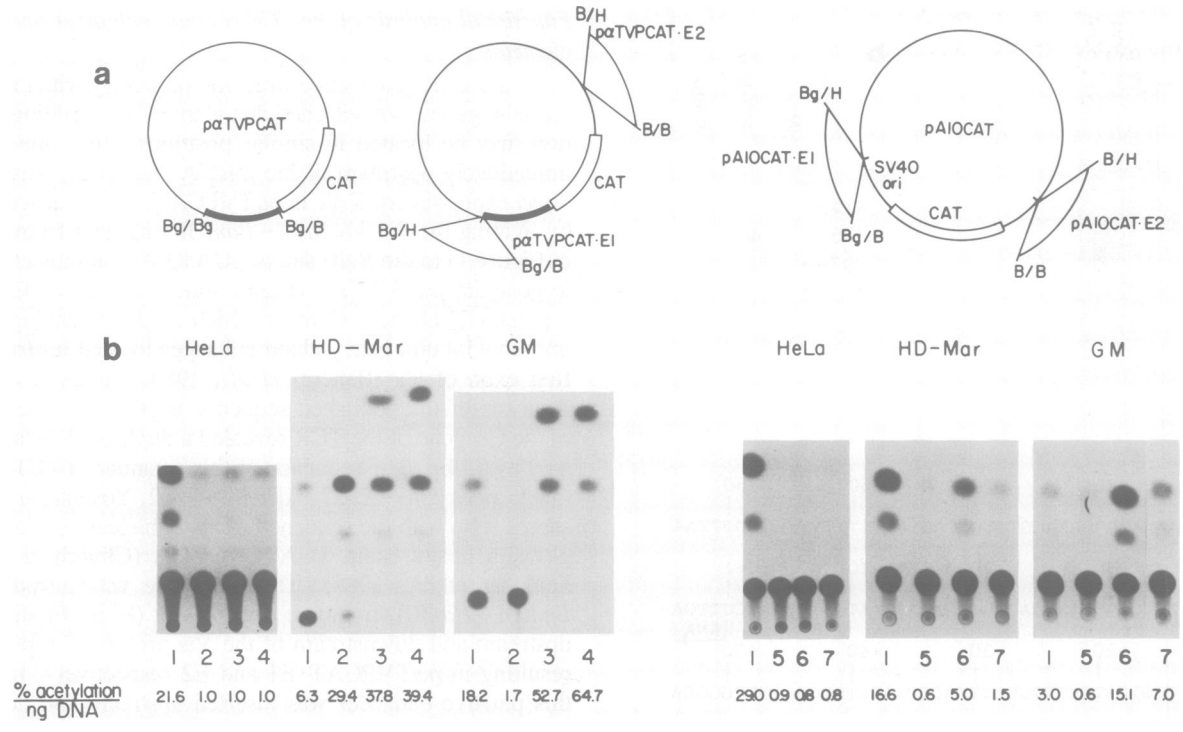

Fig. 4. CAT assay of the promoter and enhancer elements of the TCR. (**a**) Left hand side construct, the *Bgl*II−*Bam*HI fragment of p24.1 (Pr in Figure 1) was ligated to the *Bgl*II site of pCAT3M to generate pαTVPCAT where the Vα promoter is linked to the CAT gene. Middle construct, the *Hin*dIII−*Bam*HI fragment of λC3 (E in Figure 1) was ligated to either *Bgl*II or *Bam*HI sites of pαTVPCAT, after fusing the ends, to generate pαTVPCAT·E1 and pαTVPCAT·E2 respectively. Right hand construct, the *Hin*dIII−*Bam*HI fragment of λC3 was ligated to pA10CAT, which contains the SV40 promoter but no enhancer, similar to that described for pαTVPCAT. (**b**) CAT assays of extracts from transfected cells. (**1**) pSV2CAT; (**2**) pαTVPCAT; (**3**) pαTVPCAT·E1; (**4**) pαTVPCAT·E2; (**5**) pA10CAT; (**6**) pA10CAT·E1; (**7**) pA10CAT·E2. The numbers under the autoradiograms of the CAT assay represent CAT activity calculated from per cent acetylation divided by ng of transfected plasmids (see Materials and methods). In some of these experiments the conversion to acetyl chloramphenicol was >90% and outside the linear range of the enzyme activity. For additional data see text.

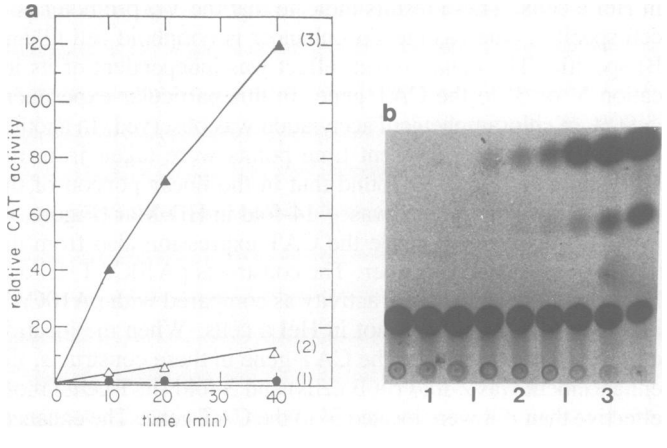

Fig. 5. Time course of CAT synthesis in HD-Mar. Replica cultures (10^7 cells) of HD-Mar were transfected with various CAT-containing plasmids by the protoplast fusion method. CAT activity was assayed on 1/5 of the cell lysates at different time points. The relative CAT activity (**a**) was calculated from the radioactivity data (**b**) and the plasmid content (see Materials and methods). **Lane 1**, pCAT3M; **lane 2**, pαTVPCAT; **lane 3**, pαTVPCAT·E1. The constructs are described in Figure 4.

pV.1) of *Eco*RI digested human genomic DNA which revealed one predominant band of 3.6 kb and three weakly cross-hybridizing bands. The most closely related Vα regions are the human Vα HAP-26 (Yoshikai *et al.*, 1986) and the mouse Vα5H (Winoto *et al.*, 1985) which share homology of 73 and 62% at the amino acid level respectively with the cloned Vα from HD-Mar. Comparison of the sequences clearly shows that pV.1 is the germline counterpart of the Vα region of p24.1. They are identical not only in the coding region but also in the intron and in the sequence upstream to the ATG. The data show no evidence of extensive somatic mutation. There is only one difference located in the intron sequence (T/C) between the germline and the rearranged Vα gene. This can be due to either polymorphism or somatic mutation. The major difference between the germline Vα and Jα and the rearranged VαJα is at the joining site. The sequence (Figure 2) shows that the joining occurred six nucleotides after the recombination signal of the Jα and two nucleotides before the recombination signal of the Vα with the addition of three unrelated nucleotides at the junction (TTG) which represent N-region (Alt and Baltimore, 1982) and resulted in a new amino acid (Cys) at the VαJα junction. The joining at alternate sites and addition of nucleotides at the joining site may be the major diversification mechanism in the α chain of the TCR.

Nuclease S1 protection analysis of the TCR α chain transcript in HD-Mar revealed a transcription start site which is 70 nucleotides upstream to the ATG. The region upstream to Vα is a functional promoter since it can drive the expression of CAT in the pαTVPCAT construct (Figure 4). This promoter activity is specific for T cells and was demonstrated in the T cell line HD-Mar, but not in B cells or HeLa cells.

In this study we have also demonstrated an enhancer element in the *Hin*dIII−*Bam*HI fragment which is located between Cα and the first Jα (Figure 1). Figure 4 shows that this fragment enhances CAT expression from either SV40 promoter (pA10CAT·E1 and pA10CAT·E2), or Vα promoter (pαTVPCAT·E1 and pαTVPCAT·E2). The enhancement is independent of the location of this fragment 5′ or 3′ to the CAT gene. It is of

interest that the enhancer operated in both T cells and B cells, but not in HeLa cells. In B cells the Vα promoter does not drive the expression of CAT, but when linked to the enhancer the expression of CAT is similar to that found in T cells. Such promoter−enhancer synergism was also described for immunoglobulins (Gracia *et al.*, 1986). This implies that the T cell enhancer is lymphoid cell (B and T) specific. There are also reports that the Ig enhancers operate in some T cells (e.g. Molt 4, Scholer and Gruss, 1985). A possible explanation for this may be the homology between the repeated decamer (TTCTGTGGCA) of the *Hin*dIII−*Bam*HI fragment and the enhancer core of Ig heavy chain (TCATGTGGCA) which suggest that some transcription factors in both cell types recognize a very similar sequence. Studies on Ig enhancer indicated multiple nuclear factors which interact with various sequence elements in the enhancer, some of them specific to B cells and some not (Weinberger *et al.*, 1986; Sen and Baltimore, 1986). Further analysis of the Tα enhancer is required in order to dissect it to specific and general elements. It is of interest that the sequence upstream to the Cβ2 gene of the mouse TCR contains an octamer repeat, CTGTGGCA, separated by 150 bp. This octamer is identical to eight nucleotides of the decamer in Tα sequence and we speculate therefore that the enhancer activity of the β chain of the TCR may be located upstream to Cβ2.

The homology between TCR and Ig genes in their structure, organization and mechanism of rearrangement is well established. Our results indicate that this homology is preserved also for the transcription regulatory elements. The Vα promoter is close to the initiator ATG and the 5′ non-translated region is short, as is the case for Ig. The enhancer described here is located immediately upstream to Cα in the intron between Jα and Cα, and this is also similar to the enhancer location in Ig.

Another important point raised by the location of the enhancer is its possible long range effect on the Vα promoter. Because of the distribution of Jα segments over a long distance (Yoshikai *et al.*, 1985) it is likely that, unless other enhancer elements are dispersed within the Tα locus, the Tα enhancer described here will exert its effect on promoters of Vα which joins Jα at least 60 kb away from the enhancer. By analogy this long range effect may be true also for the Ig enhancers. Indeed, Wang and Calame (1985) showed that the H chain enhancer can activate transcription of a V_H promoter located 17.5 kb away, and Atchison and Perry (1986) demonstrated that the effect of the κ chain enhancer on its promoter is the same whether the distance between them is 1.7 or 7.7 kb. It is likely therefore, that such a long distance effect of the immune system gene enhancers will also influence the transcription of other genes, e.g. oncogenes, which are translocated to the Ig or TCR locus. For example, the *bcl2* in follicular lymphoma is translocated to the Ig heavy chain J region but the *bcl*-2 gene promoter which is probably deregulated by the Ig enhancer is >60 kb upstream to it (Tsujimoto *et al.*, 1987).

Materials and methods

Cells and reagents

The human T cell line HD-Mar, kindly provided by Dr H.Ben-Bassat (Ben-Bassat *et al.*, 1980), the human B cell lines 209 (a gift of Dr G.Lenoir, Lyon, France) and GM 4672 (Satoh *et al.*, 1983) were grown in RPMI 1640 supplemented with 15% fetal calf serum (FCS). HeLa cells were grown in Dulbecco's modified Eagle's medium containing 10% FCS. Human Jα probe and Cα cDNA (pHαT1) were a gift of Dr C.Croce. The CAT-containing vectors pSV2CAT (containing SV40 promoter and enhancer, Gorman *et al.*, 1982), pA10CAT (containing SV40 promoter without enhancer, Gorman *et al.*, 1972) and pCAT3M (containing neither SV40 enhancer nor promoter, Laimins *et al.*, 1984) were a gift of Dr P.Gruss.

DNA and RNA methods

DNA from HD-Mar cells was digested with *Hin*dIII and fractionated by electrophoresis through 0.7% agarose gels. The gel band containing the two fragments hybridizing to the Jα probe was sliced, electroeluted onto DEAE filter NA45 (Schleicher and Shuell) and the DNA was recovered by elution with 1 M NaCl and 0.05 M arginine, at 67°C, for 2 h followed by ethanol precipitation. The DNA was ligated to the *Hin*dIII-cut, dephosphorylated pBR322 (New England Biolabs) and was used to transform *Escherichia coli* HB101. Positive colonies (one positive clone per 16 000 colonies) were identified by the TCR Jα probe.

DNA sequence was determined by subcloning the desired fragments (Figure 1) into M13 and sequencing by the chain termination method (Sanger *et al.*, 1977). In some cases the sequence was determined by the chemical degradation method (Maxam and Gilbert, 1980).

Cytoplasmic RNA from cells was prepared by standard methods, and poly(A)-containing RNA was selected as described (Aviv and Leder, 1972). Northern blots of mRNA were performed according to Thomas (1980).

Nuclease S1 protection analysis

The 650 bp *Bgl*II−*Stu*I fragment from p24.1 containing the sequence upstream to nucleotide 35 of the leader (Figure 2) was subcloned into the *Hin*cII site of M13mp19. This clone in its single strand form was used to generate the probe for S1 mapping by extension of the M13 primer, digestion with *Bam*HI and *Hin*dIII and isolation on a 5% polyacrylamide−urea gel. For nuclease S1 protection analysis 10 μg of cystoplasmic RNA from either HD-Mar cells or placenta were hybridized with 50 000 d.p.m. of the probe in 20 μl of 80% formamide, 40 mm Pipes pH 6.4, 0.4 M NaCl and 1 mM EDTA. The hybridization reaction was incubated at 72°C for 15 min and at 42°C for 16 h. The reactions were processed as described (Weaver and Weissman, 1979) and analysis on 6% polyacrylamide−urea gels.

DNA transfections

Transfection of plasmid DNA into HeLa cells was by the calcium phosphate method, essentially as described (Graham and Van der Eb, 1973). Transfection of plasmids into B (GM 4672) and T (HD-Mar) cells as well as HeLa cells was performed by a modification of the protoplast fusion technique (Sandri-Goldin *et al.*, 1981). Bacteria containing the appropriate plasmid were grown at 37°C in the presence of chloramphenicol (170 μg/ml). Twenty-five milliliters of an overnight culture were precipitated and resuspended in 1.25 ml of 0.05 M Tris−HCl containing 20% sucrose, and 0.25 ml of freshly prepared lysozyme (5 mg/ml) was added. The bacteria were incubated for 6 min on ice followed by the addition of 0.5 ml of 0.25 M EDTA (pH 8) and incubation for an additional 5 min on ice. After addition of 0.5 ml of 0.05 M Tris−HCl (pH 8) the bacteria were transferred into 37°C for 10 min. The protoplasts were diluted with 10 ml of RPMI 1640 medium containing 10% sucrose and 10 mM $MgCl_2$ and incubated for 10 min at room temperature. For DNA transfection, 10^7 cells were spun down and resuspended in 1 ml of RPMI 1640 medium at room temperature followed by the addition of 3 ml of the protoplast preparation. The cells were precipitated at 1500 r.p.m. and resuspended in 1 ml of warm (37°C) 45% polyethylene glycol (mol. wt 1450, Sigma) in RPMI for 1 min, followed by the addition of 9 ml of medium. The cells were spun down, and the pellet was resuspended in 20 ml of RPMI 1640 containing 15% FCS and kanamycin (100 μg/ml) and grown in 250-ml flasks for 48 h at 37°C.

CAT assay

Approximately 40 h after transfection, the cells were divided so that one half of the cells were used for quantitation of the plasmid content and the remaining cells were lysed and sonicated for CAT assay as described (Gorman *et al.*, 1982). The percentage of the acetylated forms of [^{14}C]chloramphenicol (New England Nuclear Corp.) was determined by cutting out the radioactive spots and counting in a liquid scintillation counter. The results of the CAT activity were normalized to the amount of plasmid DNA in the transfected cells. To quantitate intracellular plasmid DNA in the transfected cells, the cells were washed with phosphate-buffered saline and plasmid DNA was isolated as described by Hirt (1967). The DNA was digested with *Eco*RI and electrophoresed on 0.8% agarose gels in parallel with known quantities of the plasmid. Southern blots of these gels were hybridized to the 1.7 kb *Eco*RI fragment of the CAT gene and the resultant radiograms were quantitated by densitometry.

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