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The chromosomal location of T-cell receptor genes and a T cell rearranging gene: possible correlation with specific translocations in human T cell leukaemia

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We have examined the chromosomal location of human T cell-specific genes which are involved in antigen recognition and of a gene which specifically rearranges in T cells. The genes encoding both the variable and constant region segments of the T cell receptor α chain are found on chromosome 14 while the δ chain gene of the T cell receptor-associated T3 complex is localised to chromosome 11. Further, the two tandemly arranged T cell-specific rearranging genes, designated γ, were mapped to chromosome 7, but apparently not closely linked to the previously mapped T cell receptor β-chain gene. The locations of the three different genes, which undergo rearrangement in T cells, may correlate with the chromosomal breakpoints known to be involved in translocations within abnormal human T cells.

Key words: chromosomal location/T cell antigen receptor/genetic mapping/leukaemia

Introduction

The T cell antigen receptor (TCR) is a cell-surface protein comprising at least five distinct polypeptides; two of these, α and β chains, represents the antigen recognition part (Reinherz *et al.*, 1983), while the T3 complex (δ, ε and γ chains) is associated with α and β chains but, as yet, the role of T3 has not been established (Borst *et al.*, 1983). The genes coding for these various chains (except T3ε and γ) have recently been the subject of study (Chien *et al.*, 1984a; Hedrick *et al.*, 1984; Saito *et al.*, 1984a; Sim *et al.*, 1984; Sims *et al.*, 1984; van den Elsen *et al.*, 1984; Yanagi *et al.*, 1984), and a third type of gene designated γ has been isolated (Saito *et al.*, 1984b), which also undergoes rearrangement in T cells but whose relationship to the TCR is not known [we have designated the homologous human gene TRGγ to avoid confusion with the T3 complex γ gene (Lefranc and Rabbitts, 1985)]. These various T cell genes can undergo specific DNA rearrangement in an analogous way to the immunoglobulin genes (Clark *et al.*, 1984; Chien *et al.*, 1984b; Siu *et al.*, 1984). It seems possible therefore that the TCR genes might be involved in chromosomal translocations in T cell leukaemias, like those present in, for example, the human B cell neoplasia Burkitt's lymphoma (Klein, 1981). As a first step towards examining this possibility, it is necessary to ascertain the chromosomal location of the T cell genes. The human TCR β chain genes are known to occur on chromosome 7 (Collins *et al.*, 1984; Barker *et al.*, 1984; Caccia *et al.*, 1984), and we now report the chromosomal mapping of the TCRα genes, the TRGγ genes and the T3δ gene. The results suggest some interesting possible associations with translocations in human T cell leukaemias.

Results

Characterisation of TCRα and T3δ clones

The cDNA clones used in this study were isolated from the enriched cDNA library (derived from mRNA of the human T cell leukaemic cell line JM) previously described (Sims *et al.*, 1984). A T3δ cDNA clone (pJM3A10) was identified by sequence homology to the previously reported clone (van den Elsen *et al.*, 1984) from a panel of randomly sequenced T cell-specific clones in the JM cDNA library. The pJM3A10 clone sequence was shown to contain 330 bases of T3δ sequence starting at residue 365 of the previous clone and we found no sequence differences (because of this identity these data are not shown). The criteria which previously applied to the identification of the T3δ clone (van den Elsen, 1984) must, of course, also apply to our T3δ clone.

A TCR α chain clone (pJM3E11) was first identified by hybridisation to a mouse α clone TT11 (Chien *et al.*, 1984a) and possesses sequences compatible with previously published α chain peptide sequence data (Fabbi *et al.*, 1984; Hannum *et al.*, 1984), and with a cDNA clone (pGA) prepared from a different human cell line (HPB-MLT) (Sim *et al.*, 1984). The sequence of pJM3E11 is presented in Figure 1, and shows that TCR α chain mRNA is at least 1510 bases long. The clone contains constant region and joining (J) region segments identical with those previously published (Sim *et al.*, 1984). The pJM3E11 clone also encodes untranslated sequences of 128 bases at the 5′ end (including a hydrophobic leader sequence) and 545 bases at the 3′ end including the poly A addition signal sequence (ATTAAA) starting at residue 1500. Although the J segment present in the α-cDNA clones made from JM and HPB-MLT cells is identical, the rest of the variable region is rather different. Sequence homology at the nucleotide level essentially stops after the J segment. The V region base sequences differ widely; since these clones were made from randomly selected T cell lines, the observed divergence in Vα sequence argues that Vα gene diversity does occur in germ line DNA. Examination of the pJM3E11 and pGA sequences shows the presence of an additional sequence (five or six codons in length) in pJM3E11 between the V and J segments (the V regions were aligned using the conserved cysteine residues responsible for the intra-chain disulphide bridges of the V region). A possible explanation for this sequence in the JM cDNA clone is that it derives from a diversity or D segment like those present in TCR β chain genes. (It is of course possible that this sequence comes from a long, germ line V gene).

Chromosomal location of human TCR and T3 genes

As a preliminary analysis of the clones used in this study, we

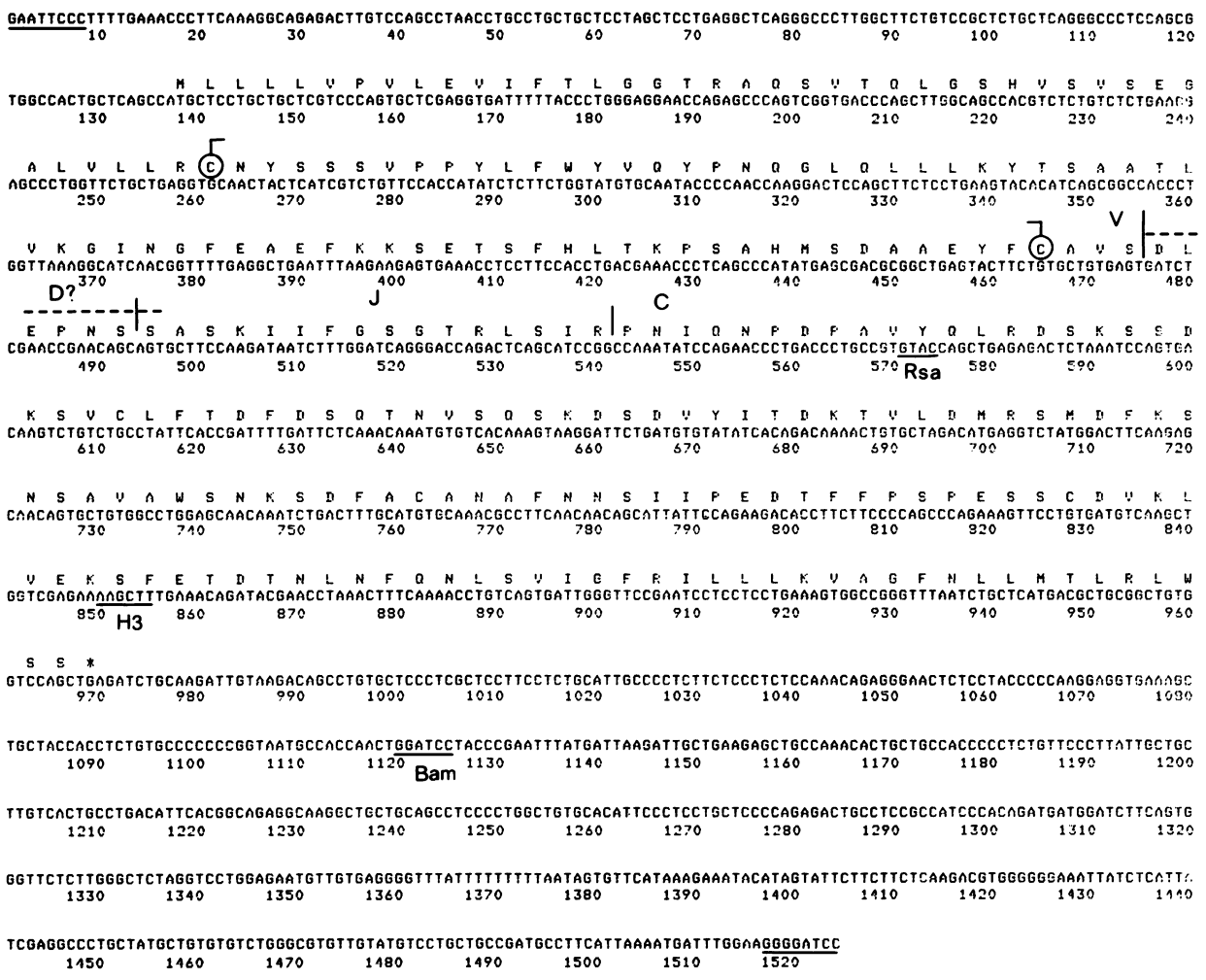

Fig. 1. Nucleotide sequence and derived protein translation of the TCR α chain cDNA clone pJM3E11. The sequence of the inserted cDNA isolated from the JM cDNA library (Sims *et al.*, 1984) was obtained by subcloning restriction enzyme fragments into M13 vectors followed by sequencing using dideoxy chain termination procedures (Sanger *et al.*, 1980). The bases underlined at the 5′ and 3′ end of the sequence occur at the cloning site of the pUC vector (Vieira and Messing, 1982). The *Bam*HI (Bam) and *Hind*III (H3) sites at the 3′ end of the clone represent the internal sites present in the 3′ UT region. The possible D segment is indicated.

investigated their ability to hybridise, in genomic filter hybridisations, with DNA from a mouse-human cell hybrid (FIR5R3) which contains only human chromosomes 14 and 18. Preliminary analysis showed that the α probe hybridised with FIR5R3 DNA, but the T3δ probe did not. The results of a hybridisation experiment using the α cDNA probe with this DNA are shown in Figure 2A. At the stringency of hybridisation used, the α probe does not hybridise effectively with mouse DNA whilst DNA from the human cell line Colon 320 (Alitalo *et al.*, 1983) shows approximately six hybridising bands corresponding to a family of several Vα genes and the single Cα gene. FIR5R3 DNA shares this set of human specific α gene bands (Figure 2A) showing that the α locus resides on either human chromosome 14 or 18. Subsequently we utilised a panel of mouse-human hybrids to demonstrate that the α genes are situated on chromosome 14 (Table I). This panel of somatic cell hybrids included three lines with human chromosome 14, but not 18, and one line with human chromosome 18, but not 14. The results of these hybridisations, summarised in Table I, demonstrate a complete concordance between the human TCR α-specific restriction fragments and chromosome 14 (together with a discordance with chromosome 18). Therefore we conclude that both V and C regions of the α chain genes are situated on human chromosome 14. This supports previous results using a C region probe (Collins *et al.*, 1985).

The T3δ clone did not hybridise with FIR5R3 DNA and thus this gene cannot reside on human chromosomes 14 or 18. To map this locus, therefore, we examined the hybridisation of a different set of mouse-human hybrids with the T3δ probe (Table II). A scrutiny of the various human chromosomes present showed a concordance of T3δ gene hybridisation with chromosome 11; this was confirmed by using DNA from the cell hybrid HORL9D2R1 which only has chromosome 11 plus a fragment of chromosome X (Goodfellow *et al.*, 1982). Figure 2B shows the hybridisation data of the T3δ probe with HORL9D2R1 DNA; this DNA shows the two *Sac*I restriction fragments obtained in the Colon 320 human DNA control whereas no hybridisation was detected with mouse DNA. These data confirm the occurrence of the T3δ gene on chromosome 11. In the future it will be interesting to know whether or not the T3 ε and γ chain genes are also localised on this chromosome.

Chromosomal location of a human T cell rearranging gene, γ

There are at present known to be three genes which undergo rearrangement in T cell DNA, the TCR α and β genes as well

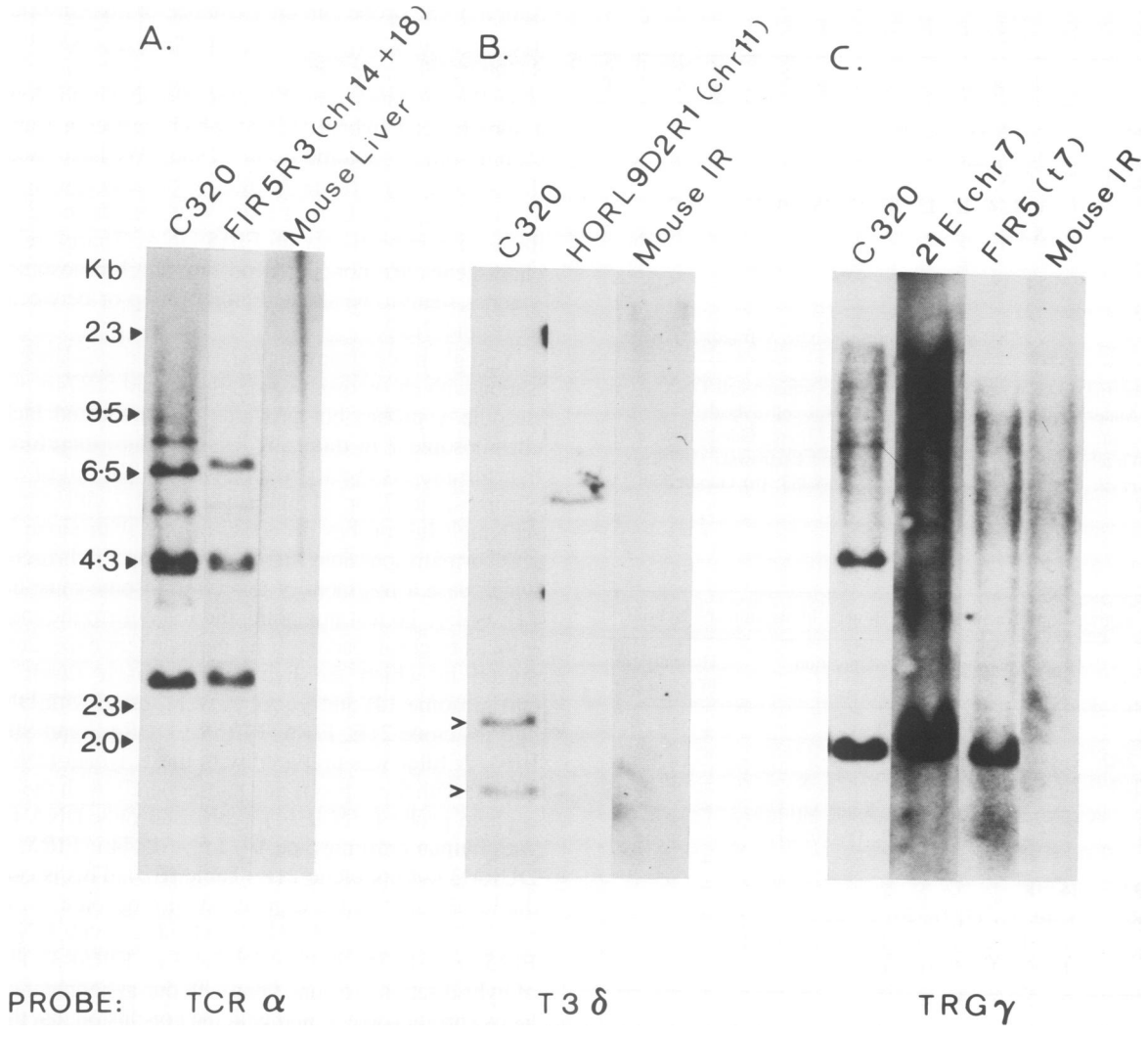

Fig. 2. Filter hybridisation of T cell gene probes and DNA from somatic cell hybrids. Cellulose nitrate filters were prepared, as described in Materials and methods, using the various DNA samples indicated at the top of each panel. DNA was digested to completion with *Hind*III **(panels A** and **C)** or *Sac*I **(panel B)**. C320 represents Colon 320; mouse liver DNA was prepared from BALB/c mice; mouse IR DNA was prepared from mouse L cells. The sizes of restriction fragments were determined by co-electrophoresis of λ phage DNA cut with *Hind*III. **Panel A:** probe pJM3E11 (TCRα). **Panel B:** probe pJM3A10 (T3δ). The two open arrows indicate the two *Sac*I restriction fragments, hybridising with the probe, which correspond to human T3δ gene. **Panel C:** probe: M13H60 (TRGγ).

as a third type of gene, of unknown function, which was first described in mouse (Saito *et al.*, 1984b). The human homologue of this gene has been isolated and the locus has been shown to contain two tandemly duplicated Cγ genes (Lefranc and Rabbitts, 1985). A subclone (M13H60) was isolated from an intervening sequence of a genomic clone. This cloned probe detects *Hind*III fragments of 2.1 and 4.5 kb in human DNA as illustrated by Colon 320 DNA (Figure 2C), but does not cross-hybridise to restriction fragments in mouse DNA under this hybridisation stringency. The γ probe does not hybridise to the FIR5R3 DNA but hybridises strongly with DNA from a hybrid clone 21E which only possesses human chromosome 7 (Croce and Koprowski, 1979). [The 4.5-kb *Hind*III fragment is polymorphic in human DNA (Lefranc and Rabbitts, 1985) and therefore clone 21E is homozygous for the 2.1-kb fragment.] This result, therefore, strongly implies that the γ gene locus is present on human chromosome 7. This conclusion is further supported by the strength of the signal obtained between the γ probe and clone 21E DNA since these cells have multiple copies of this chromosome.

The mapping of the TRGγ to chromosome 7 was confirmed using the panel of cell hybrids listed in Table IIIA. The hybridisation results confirm the localisation of the γ locus to chromosome 7 since there is a concordance between the hybrid-

Table I. Hybridisation of the human TCR α cDNA clone with DNA from mouse-human hybrids

Cell hybrid	Hybridisation of human α genes	% cells with human chromosome	
		14	18
NG2/17Ag68	−	0	90
NG2/15.5Ag14	+	80	40
NG9/16.2Ag75	+	40	0
NG9/16.6Ag80	+	30	60
NG2/15.7Ag71	+	20	0
NG2/19/9.1.21	+	10	0
NG2/15.7Ag7	+	70	20

The TCR α chain probe (pJM3E11) was hybridised with DNA from the various cell lines as described in Materials and methods.
+ indicates the presence of human TCR α genes; − indicates the absence of human TCR α genes.

Table II. Chromosomal assignment of human T3δ gene

Cell hybrid	Human chromosomal composition	Human T3δ gene
(a) 3W4 clone 7	7, 10, 11, 12, 14, 15, 17, 21, X	+
(b) DT1.2R	3, 10, 11, 17, 18, 20, 21, 22	+
(c) HORL9D2R1	11, X	+
(d) SIR74ii	1, 2, 4, 12, 13, 14, 17, 18, 20, 21, X [7]	–
(e) Clone 21E	7	–
(f) FIR5R3	14, 18	–
(g) GPT 17/3	17	–
(h) ThyB 13/3	21, X (undefined chromosome X fragment)	

The T3δ probe (pJM3A10) was used to hybridise somatic cell DNA as described in Materials and methods. References for cell hybrids: (a) Nabholz *et al.* (1983); (b) Swallow *et al.* (1977); (c) Goodfellow *et al.* (1982); (d) Whitehead *et al.* (1982); (e) Croce and Koprowski (1979); (f) Solomon *et al.* (1983); (g) Tunnacliffe *et al.* (1983; (h) Goodfellow *et al.* (1980).

Table IIIA. Segregation of a human TRGγ gene in somatic cell hybrids

Cell hybrid	Human chromosomal composition	Hybridisation of γ genes
(a) C1.21E	7	+
(b) CTP41.2A2	2, [3], 6, 7, 14, [16], X	+
(c) 3W4 Cl. 7	7, 10, 11, 12, 14, 15, 17, 21, X	+
(d) FIR5	7pter – 7q22, 14, 18, Xqter-Xq13	+
(e) SIR74ii	1, 2, 4, 12, 13, 14, 17, 18, 20, 21, X [7]	+
(f) FIR5R3	14, 18	–
(g) DT1.2R	3, 10, 11, 17, 18, 20, 21, 22	–
(h) DUR4.3	3, 5, 10, 11, 12, 13, 14, 15p11-qter, 17, 18, 20, 21, 22, Xq11-qter	–

Table IIIB.

Cell hybrid	Hybridisation probe	Human chromosomal contribution	Presence of human specific gene signal
(a) Cl. 21E	α	7	–
	γ		+
(b) FIR5R3	α	14, 18	+
	γ		–
(c) SIR74ii	α	1, 2, 4, 12, 14, 17, 18, 20, 21, 22, X [7]	+
	γ		+
(d) FIR5	α	7pter-7q22, 14, 18, Xqter-Xq13	+
	γ		+
(e) DUR4.3	α	3, 10, 11, 12, 13, 14, 15p11-qter, 17, 18, 20, 21, 22, Xq11-qter	+
	γ		–

A. Hybridisation of the γ probe M13H60 to various somatic cell hybrids. Cell lines used (see also Table II legend): (b) Jones *et al.* (1976); (h) Solomon *et al.* (1976).

B. Comparative hybridisation properties of γ probe M13H60 and α probe pJM3E11 with somatic DNA from cell hybrids.

isation of the probe and the existence of this chromosome in the hybrids used [except SIR74ii (see below)]. Previously, the TCR β chain gene locus has been placed on a region of the chromosome 7 extending from q22 to the telomere of the long arm using a somatic cell hybrid (FIR5) which carries a translocation of chromosome 7 (Solomon *et al.*, 1983). We have used DNA from this same hybrid to analyse the hybridisation with the M13H60 γ probe (Figure 2C) and found that, unlike the β chain probe, the γ gene hybridises with FIR5 DNA. Therefore, the γ and β chain genes are not linked on human chromosome 7, and the γ genes seem to be situated either on 7p or between the centromere and 7q22.

A single discrepancy appears in the concordance of γ hybridisation with chromosome 7 *viz.* the hybrid SIR74ii, which hybridises the γ probe, but previous data indicated lack of human chromosome 7 in this cell. It is possible, therefore, that the γ hybridisation is due to the presence in SIR74ii of a fragment of chromosome 7 or a minority of cells with chromosome 7. Although Igh probes do not hybridise with clone 21E DNA it was formally possible that γ genes map to chromosome 14 (in which case a fragment of this chromosome must exist in clone 21E cells). To test this point and to confirm the mapping of the γ locus to chromosome 7, an independent experiment was conducted in which the hybridisation of α (itself localised to chromosome 14) and γ probes were tested with DNA from the hybrids clone 21E, FIR5, FIR5R3 DUR4.3 and SIR74ii (Table IIIB). A filter was prepared with these hybrid DNAs, hybridised first with the α probe and subsequently with the γ probe. The α chain probe (pJM3E11) hybridised only DNA from hybrids with human chromosome 14, i.e., SIR74ii, FIR5, FIR5R3 and DUR4.3 but not clone 21E (Table IIIB). This is consistent with the localisation of the α genes to chromosome 14. On the other hand, the γ probe (M13H60) hybridised with DNA from 21E, FIR5 and SIR74ii but not DUR4.3 or FIR5R3. These patterns of hybridisation are consistent with our assignment of the γ chain locus chromosome 7, and with the conclusion that the cell hybrid SIR74ii must contain some human chromosome 7-derived material.

Discussion

We have assigned the human TCR α chain V and C genes to chromosome 14, the T3δ gene to chromosome 11 and the TRGγ to chromosome 7 (7pter to 7q22). The location of the TRGγ to chromosome 7 is of interest because it has recently been shown that the TCR β chain genes also occur in this chromosome (Collins *et al.*, 1984; Barker *et al.*, 1984; Caccia *et al.*, 1984). Similarly, the occurrence of the TCR α chain gene on chromosome 14 is intriguing because the immunoglobulin heavy chain (Igh) locus maps to 14q32.

The mapping of three gene loci capable of undergoing rearrangement in T cells to their respective chromosomes is very interesting because of specific chromosomal translocations known to occur in human T cell leukaemias, and in clonal T cell expansions from patients with ataxia telangiectasia. These abnormalities can involve 7p, 7q and 14q. A frequently occurring abnormality present in T cell chronic lymphocytic leukaemias (T-CLL) is an inversion of the long arm on chromosome 14 (14q11; q32) (Hecht *et al.*, 1984; Zech *et al.*, 1984). The possibility that TCR α chain genes (mapping to chromosome 14) are involved in this inversion, perhaps with an unidentified oncogene, is provocative. It is therefore vital to determine whether the TCR α and/or the Igh genes are rearranged in cells carrying this chromosome 14

inversion. In this context, a preliminary analysis of several samples of T-CLL with inv14 has failed to show rearrangement of the Igh joining region genes (unpublished).

A further interesting correlation concerns the chromosome 7 assignment of TCR β and TRGγ gene loci. It has been shown that human T cell cultures *in vitro* spontaneously generate chromosome abnormalities, involving 14q11 with either 7p13 and 7q3 (Welch and Lee, 1875; Beatty-DeSana *et al.*, 1975; Hecht *et al.*, 1975). Although these were non-malignant cells, the 7q3; 14q11 abnormality has also been observed in adult T cell leukaemia (Fukuhara *et al.*, 1983) and both 7p and 7q; 14q11 translocations have been observed in ataxia telangiectasia patients as a subclinical, but apparently clonal, T cell expansion (Taylor *et al.*, 1981). This is especially interesting in that ataxia telangiectasia patients show a propensity to develop malignant T cell lymphoma. When such a lymphoma develops it can carry the chromosome 14 abnormality (Taylor *et al.*, 1981) indicating that the 14q11 position is crucial to the development of the various T cell malignancies. With the availability of the various T cell probes described here the nature of the genes located near the translocation points is now potentially amenable to study.

Materials and methods

Cell lines

The various cell lines are indicated in the table legends; Colon 320 is a colon carcinoma cell line (Alitalo *et al.*, 1983).

Filter hybridisation

10 μg aliquots of genomic DNA were digested to completion with the relevant restriction enzyme (see legend to Figure 1) and fractionated on 0.8% agarose. After transfer to cellulose nitrate (Southern, 1975) the filters were hybridised as previously described (Sims *et al.*, 1984) using either (A) pJM3E11 TCRα probe, (B) pJM3A10 T3δ probe, or (C) M13H60 TRGγ probe.

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References

Alitalo,K., Schwab,M., Lin,C.C., Varmus,H.E. and Bishop,J.M. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1707-1711.

Barker,P.E., Ruddle,F.H., Royer,H.D., Acuto,D. and Reinherz,E.L. (1984) *Science (Wash.)*, **226**, 348-349.

Beatty-DeSana,J.W., Hoggard,M.J. and Coolege,J.W. (1975) *Nature*, **255**, 242-243.

Borst,J., Alexander,S., Elder,J. and Terhorst,C. (1983) *J. Biol. Chem.*, **258**, 5135-5141.

Caccia,N., Kronenberg,M., Saxe,D., Haars,R., Bruns,G.A.P., Goverman,J., Malissen,M., Willard,H., Yoshikai,Y., Simon,M., Hood,L. and Mak,T.W. (1984) *Cell*, **37**, 1091-1099.

Chien,Y.-H., Becker,D.M., Lindsten,T., Okamura,M., Cohen,D.I. and Davis, M.M. (1984a) *Nature* **312**, 31-35.

Chien,Y.-H., Gascoigne,N.R.J., Kavaler,J., Lee,N.E. and Davis,M.M. (1984b) *Nature*, **309**, 322-326.

Clark,S.P., Yoshikai,Y., Siu,G., Taylor,S., Hood,L. and Mak,T.W. (1984) *Nature*, **311**, 387-389.

Collins,M.K.L., Goodfellow,P.N., Dunne,M.J., Spurr,N.K., Solomon,E. and Owen,M.J. (1984) *EMBO J.*, **3**, 2347-2349.

Croce,C.M. and Koprowski,H. (1979) *J. Exp. Med.*, **140**, 1221-1229.

Fabbi,M., Acuto,O., Smart,J.E. and Reinherz,E.L. (1984) *Nature*, **312**, 269-271.

Fukuhara,S., Hinuma,Y., Gotoh,Y.-I. and Uchino,H. (1983) *Blood*, **61**, 205-207.

Goodfellow,P.N., Banting,G., Levy,R., Povey,S. and McMichael,A. (1980) *Somat. Cell Genet.*, **6**, 777-787.

Goodfellow,P.N., Banting,G., Wiles,M.V., Tunnacliffe,A., Parkar,M., Solomon, E., Dalchau,R. and Fabre,J.W. (1982) *Eur. Immunol.*, **12**, 659-663.

Hannum,C.H., Kappler,J.W., Trowbridge,I.S., Marrack,P. and Freed,J.H. (1984) *Nature*, **312**, 65-67.

Hecht,F., Kaiser-McCaw,B., Peakman,D. and Robinson,A. (1975) *Nature*, **255**, 243-244.

Hecht,F., Morgan,R., Kaiser-McCaw Hecht,B. and Smith,S.D. (1984) *Science (Wash.)*, **266**, 1445-1447.

Hedrick,S.M., Nielsen,E.A., Kavaler,J., Cohen,D.I. and Davis,M.M. (1984) *Nature*, **308**, 153-158.

Jones,E.A., Goodfellow,P.N., Kennett,R.H. and Bodmer,W.F. (1976) *Somat. Cell Genet.*, **2**, 483-486.

Klein,G. (1981) *Nature*, **294**, 313-318.

Lefranc,M.P. and Rabbits,T.H. (1985) *Nature*, in press.

Nabholz,M., Miggiano,V. and Bodmer,W.F. (1969) *Nature*, **223**, 358-363.

Reinherz,E.L., Meuer,S.C. and Schlossman,S.F. (1983) *Immunol. Rev.*, **74**, 83-112.

Saito,H., Kranz,D.M., Takagaki,Y., Hayday,A.C., Eisen,H.N. and Tonegawa,S. (1984a) *Nature*, **312**, 36-40.

Saito,H., Kranz,D.M., Takagaki,Y., Hayday,A.C., Eisen,H.N. and Tonegawa,S. (1984b) *Nature*, **309**, 757-762.

Sanger,F., Coulson,A.R., Barrell,B.G., Smith,A.J.H. and Roe,B.A. (1980) *J. Mol. Biol.*, **143**, 161-178.

Sim,G.K., Yague,J., Nelson,J., Marrack,P., Palmer,E., Augustin,E. and Kappler,J. (1984) *Nature*, **312**, 771-775.

Sims,J.E., Tunnacliffe,A., Smith,W.S. and Rabbitts,T.H. (1984) *Nature*, **312**, 541-545.

Siu,G., Clark,S.P., Yoshikai,Y., Malissen,M., Yanagi,Y., Strauss,E., Mak,T.W. and Hood,L. (1984) *Cel*, **37**, 381-391.

Solomon,E., Bobrow,M., Goodfellow,P.N., Bodmer,W.F., Swallow,D.M., Povey,S. and Noel,B. (1976) *Somat. Cell Genet.*, **2**, 125-140.

Solomon,E., Hiorns,L., Dalgleish,R., Tolstoches,V., Crystal,R. and Sykes,B. (1983) *Cytogenet. Cell Genet.*, **35**, 64-66.

Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.

Swallow,D.M., Solomon,E. and Pajunen,L. (1977) *Cytogenet. Cell Genet.*, **18**, 136-148.

Taylor,A.M.R., Oxford,J.M. and Metcalfe,J.A. (1981) *Int. J. Cancer*, **27**, 311-319.

Tunnacliffe,A., Parkar,M., Povey,S., Bengtsson,B.O., Stanley,K., Solomon,E. and Goodfellow,P. (1983) *EMBO J.*, **2**, 1577-1584.

Welch,J.P. and Lee,C.L.Y. (1975) *Nature*, **255**, 241-242.

Whitehead,A.S., Solomon,E., Chambers,S., Bodmer,W.F., Povey,S. and Fey,G. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 5021-5025.

van den Elsen,P., Shepley,B.-A., Borst,J., Coligan,J.E., Markham,A.F., Orkin,S. and Terhorst,C. (1984) *Nature*, **312**, 413-418.

Vieira,J. and Messing,J. (1982) *Gene*, **19**, 259-268.

Yanagi,Y., Yoshikai,Y., Leggett,K., Clark,S.P., Aleksander,I. and Mak,T.W. (1984) *Nature*, **308**, 145-149.

Zech,L., Gahrton,G., Hammarstrom,L., Juliusson,G., Mellstedt,H., Roberts,K.H. and Smith,C.I.E. (1984) *Nature*, **308**, 858-860.

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