

germline V_H and different D_H and J_H segments presumably would contribute to the significant antibody repertoire of *Heterodontus*. An absence or reduced degree of combinatorial diversity may account for the remarkable lack of inter-individual variation associated with the short- and long-term-specific immune response of this species^{4,5}. Recently, it has been shown that combinatorial diversity dominates the secondary immune response to 2-phenyl-5-oxazolone, which is accompanied by increases in antigen-binding affinity and differences in fine specificity¹³. In terms of both of these functional parameters, *Heterodontus* lacks a secondary immune response even after long periods of hyperimmunization. In a poikilothermic species whose cell proliferation kinetics may be unsuitable for generating and selecting an extensive repertoire of closely related antigen-binding specificities¹⁴, close linkage and presumably reduced recombination between different clusters of V_H , D_H , J_H and C_H segments may ensure efficient formation of certain antibodies; natural selection could act more efficiently on the different segmental elements contained in a closely linked cluster (without combinatorial joining) than on segments distributed over large chromosomal distances (with combinatorial joining). If combinatorial diversity is not necessarily advantageous, why is the gene segmented and recombined in this manner? Perhaps the rearrangement process has more to do with eventual regulation of the response through idiotype recognition than with the creation of unique combining specificities. The biological consequences of junctional joining on regulatory idiotype expression are known¹⁵ and include examples of D -region changes that alter idiotype but not antigen-binding specificities¹⁶.

The difference in the organization of the elasmobranch V_H and mammalian V_H (and other V) receptor systems is extreme and a hypothetical schematic comparison is given in Fig. 4. The elasmobranch linkage pattern may be representative of a common, ancestral gene family. Although the size of the segmented gene family is unknown, the presence of multiple constant-region segments suggested in these studies raises interesting questions as to their interrelationship and possible functions. A catastrophic loss or successive inactivation of members of this multigene family may have occurred during the vertebrate radiations, as selection favoured a system in which greater somatic variation was introduced. $DQ52$, located ~700 bp 5' to J_H coding regions in human and mouse¹⁷, may be a modern, partial vestige of the elasmobranch-like organization. Alternatively, the gene arrangement may be a unique adaptation of this lower vertebrate. Although the close linkage of the shark V_H , D_H , J_H and C_H resembles the arrangement of the segmental components of the single, functional avian (chicken) λ light-chain gene¹⁸, it remains to be seen whether the arrangement is characteristic of other lower vertebrate V genes. Based on this elasmobranch-mammal comparison, the evolution of the V_H gene family appears to have involved dramatic changes in the chromosomal organization of the individual genetic segments rather than in their numbers or basic mechanisms of recombination.

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A chromosome 14 inversion in a T-cell lymphoma is caused by site-specific recombination between immunoglobulin and T-cell receptor loci

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Specific chromosomal aberrations are associated with specific types of cancer (for review see ref. 1). The distinctiveness of each association has led to the belief that these chromosomal aberrations are clues to oncogenic events or to the state of differentiation in the malignant cell type²⁻⁶. Malignancies of T lymphocytes demonstrate such an association characterized most frequently by structural translocations or inversions of chromosomes 7 and 14 (refs 7-9). Analyses of these chromosomally marked tumours at the molecular level may therefore provide insight into the aetiology of the cancers as well as the mechanisms by which chromosomes break and rejoin. Here we report such an analysis of the tumour cell line SUP-T1 derived from a patient with childhood T-cell lymphoma carrying an inversion of one chromosome 14 between bands q11.2 and q32.3, that is¹⁰, inv(14)(q11.2; q32.2). These are the same chromosomal bands to which the T-cell receptor α -chain^{11,12} (14q11.2) and the immunoglobulin heavy-chain locus¹³ (14q32.3) have been assigned. Our analysis reveals that this morphological inversion of chromosome 14 was mediated by a site-specific recombination event between an immunoglobulin heavy-chain variable region (Ig V_H) and a T-cell receptor (TCR) α -chain joining segment (TCR J_α). S₁ nuclease analysis shows that this hybrid gene is transcribed into poly(A)⁺ RNA.

We began our analysis by studying the organization and structure of the TCR α -chain locus in the SUP-T1 cell line. The germline genomic structure of the TCR α -chain locus comprises a single constant region (C_α) preceded 5' by several joining segments (J_α) dispersed over at least 30 kilobases (kb)¹⁴. The variable regions (V_α) reside at an unknown distance more centromeric to the TCR C_α locus within the same chromosomal band^{15,16}. Using DNA probes containing germline TCR J_α regions spanning 15 kb 5' of TCR C_α , two distinct TCR C_α -linked rearrangements were detected in this cell line. Probe A, from the region 5 kb 5' of TCR C_α , demonstrated a rearrangement seen as a novel 8-kb band on *Hind*III digestion of SUP-T1 genomic DNA (Fig. 1a). Germline 4.3- and 3.6-kb bands were identified with this probe. A second TCR J_α -containing probe, probe B, derived from a region 15 kb farther upstream of TCR C_α , identified a second rearrangement, a novel 2.5-kb band on *Eco*RI-digested genomic DNA (Fig. 1b). With probe B, no germline band was seen in SUP-T1, suggesting that this sequence had been deleted from the allele which contained the more 3' rearrangement. A germline probe taken from a region 2.5 kb farther 5' did not detect any homologous sequences in SUP-T1

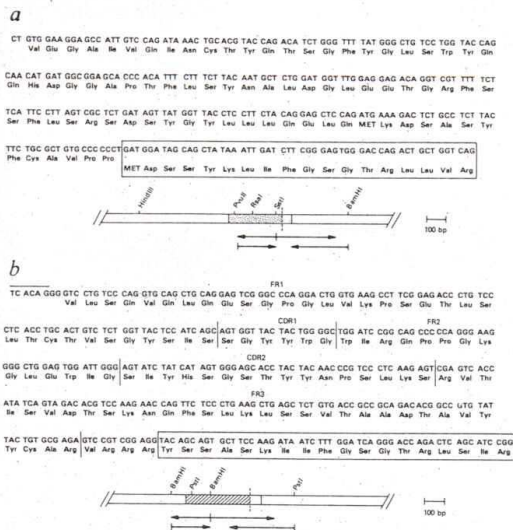


Fig. 2 *a*, Nucleotide sequence and amino-acid translation of the coding sequence of a TCR V_{α} - J_{α} rearranged gene from SUP-T1. The TCR J_{α} sequence is boxed. A possible frameshift occurs at the TCR V_{α} - J_{α} junction. The sequencing strategy is shown underneath. Stippled bar, the TCR V_{α} coding region; open bar represents TCR J_{α} . A vertical dotted line indicates the position of the V - J junction. *b*, Nucleotide sequence and amino-acid translation of the coding sequence of an Ig V_H -TCR J_{α} rearranged gene from SUP-T1. The TCR J_{α} sequence is boxed. Framework (FR) and complementarity-determining (CDR) regions are indicated. The sequencing strategy is shown underneath. ▨, Ig V_H coding region; □, TCR J_{α} . A vertical dotted line indicates the V - J junction. Sequencing was performed by the M13 dideoxy method of Sanger³⁹.

Numerous groups have definitively localized the TCR α -chain gene to 14q11.2 (refs 11, 12) and the immunoglobulin heavy-chain variable and constant regions to 14q32.3 (refs 13, 20 and

The 270-bp Ig V_H probe contained 120 bp of Ig V_H coding region. RNA from SUP-T1 protected a 120-bp fragment, consistent with specific transcription of this Ig V_H (Fig. 4b). The Ig V_H transcript was enriched in the poly(A)⁺ fraction. Additional S₁ nuclease analysis using a probe including the 3' part of Ig V_H, the TCR J_α and intervening sequence, has demonstrated that Ig V_H and TCR J_α are transcribed on the same message. The Ig V_H-TCR J_α junction remains in-frame and, at least theoretically, subsequent translation of this hybrid message into protein would be possible.

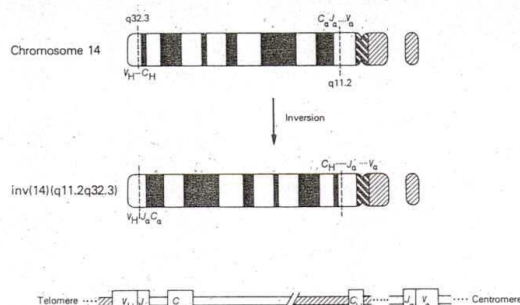


Fig. 3 A model for inversion of chromosome 14 via Ig V_H -TCR J_α site-specific recombination. The chromosomal band localization of the immunoglobulin and T-cell receptor loci and their orientations are shown in the upper idiogram of a normal chromosome 14. Site-specific recombination between an immunoglobulin heavy-chain variable region (V_H) and a T-cell receptor α -chain joining segment (J_α) results in an inversion of chromosome 14 at bands q11.2 and q32.3 (vertical dotted lines), as shown in the lower idiogram. The additional TCR V_α - J_α depicted on the inverted chromosome in the schematic diagram was identified by cloning experiments (data not shown). The orientation of the immunoglobulin and T-cell receptor loci in this schema is based on the cloning experiments presented here (see text). Hatched region, sequences from band q32.3; open region, sequences from band q11.2.

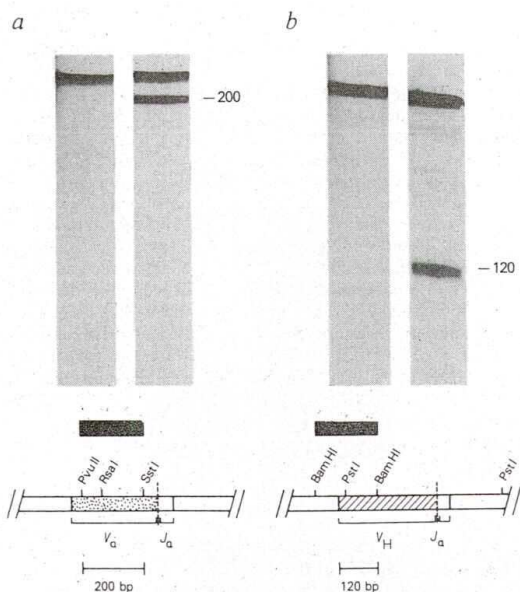


Fig. 4 S_1 nuclease analysis of SUP-T1 RNA. **a**, Transcription of a TCR V_α gene. An S_1 probe (solid bar) was prepared from 200 bp of internal TCR V_α coding sequence and 40 bp of M13 primer. Left-hand lane, control (no RNA); right-hand lane, SUP-T1 poly(A)⁺ RNA. When annealed to poly(A)⁺ RNA, all 200 bases of TCR V_α coding region (stippled region in the schematic diagram) were protected from S_1 nuclease digestion. **b**, Transcription of an immunoglobulin V_H gene. An S_1 probe (solid bar) was prepared from 270 bp of Ig V_H coding and 5' intervening sequence. Left lane, control (no RNA); right lane, SUP-T1 poly(A)⁺ RNA. When annealed to poly(A)⁺ RNA, the 120 bases of the Ig V_H coding sequence were protected from S_1 nuclease. The hatched region in the schematic diagram is the entire Ig V_H coding sequence.

The Ig V_H -TCR J_α recombinant described here clearly demonstrates that one recombination system²³⁻²⁶ can recognize both immunoglobulin²⁷ and T-cell²⁸⁻³¹ substrates and unify them; it also requires the cell to have been in a developmental state where these two distinct and disparate loci were both susceptible to a recombination event. The spacing of the signal sequences 5' of the TCR J_α and 3' of the Ig V_H suggests that the joining event would not require interposition of a D (diversity) segment if previously postulated rules of joining¹⁷ pertained. Current hypotheses suggest that these recombinases may also mediate recombination between immunoglobulin loci and other more divergent genes and signal sequences, possibly resulting in the t(14; 18)^{32,33} or t(11; 14)³⁴ translocations seen in subgroups of B-cell tumours.

The chromosome 14 inversion has been observed only in frankly malignant clones of T cells^{7,8,10}, including four out of five recently described patients with T-cell chronic lymphocytic leukaemia⁸, or in 'normal' T cells from patients with diseases that predispose to the development of leukaemia or lymphoma, such as ataxia telangiectasia³⁵⁻³⁷. Although this karyotypic finding may only reflect the aberrant joining of two distinct loci by a common recombination system at a particular point in lymphocyte development, it is also possible that this chromosomal alteration presages or contributes to malignant transformation of T cells. This might occur by activation of an as yet unidentified oncogene, or perhaps by the formation of a hybrid immunoglobulin-TCR cell-surface receptor providing an inappropriate mitogenic stimulus.

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