

DEMONSTRATION OF  $\delta$ REC-PSEUDO  $J\alpha$   
REARRANGEMENT WITH DELETION OF THE  
 $\delta$  LOCUS IN A HUMAN STEM-CELL LEUKEMIA

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The TCR- $\delta$  locus is located on chromosome 14, band q11.2, interspersed within the TCR- $\alpha$  locus (1-5). These two loci, while physically linked on one chromosome, have a precise pattern of differential expression during thymic development (3), implying an efficient regulatory mechanism that distinguishes them (6). It has recently been proposed (7) that this control might be exerted by a rearrangement that serves to delete the delta locus. A rearrangement between a  $\delta$  recombining element ( $\delta$ Rec) lying 5' of the diversity (D) $\delta$  and joining (J) $\delta$  elements, and a pseudo  $J\alpha$  gene (lying 3' of the  $\delta$  locus) was inferred that would delete the  $\delta$  locus and thus preclude TCR- $\delta$  usage. This inference was based on the cloning and sequencing of thymic extrachromosomal circular DNA excision products. This deletional event would preclude utilization of the D $\delta$  elements in TCR- $\alpha$  gene rearrangement. This is supported by numerous experiments involving the cloning of rearranged TCR- $\alpha$  genes.

We have sequenced a direct site-specific rearrangement between the  $\delta$ Rec and pseudo  $J\alpha$  genes in the human leukemic stem-cell line DU.528 (8, 9). This rearrangement resulted in the deletion of the  $\delta$  locus and supports the previous predictions regarding such rearrangements.

Materials and Methods

*Cell Line.* The cell line DU.528 was derived from a primary leukemia with clinical and immunophenotypic features of an early T cell precursor acute lymphoblastic leukemia. The cells are CD7<sup>+</sup>, CD3<sup>-</sup>, CD4<sup>-</sup>, CD8<sup>-</sup> and display stem-cell characteristics with the ability to differentiate into multiple lineages (8, 9).

*Southern Blot Analysis.* DNA was extracted from the cell line DU.528 and Southern blot analysis was performed as previously described (10, 11). The  $J\alpha$ 75 probe was a gift of M. Minden (reference 12; "J $\alpha$ G"). The configuration of the J $\delta$ 1 and other  $\delta$  probes have been described previously (7) and were recloned in our laboratory.

*Genomic Library Preparation and Analysis.* A genomic library was constructed in EMBL-3 (Promega-Biotec, Madison, WI) with partial *Mbo* 1-digested DNA from DU.528. Subclones were prepared in pGEM7Zf (Promega-Biotec) and phage M13 vectors. The dideoxy chain-termination method (13) was used for DNA sequencing.

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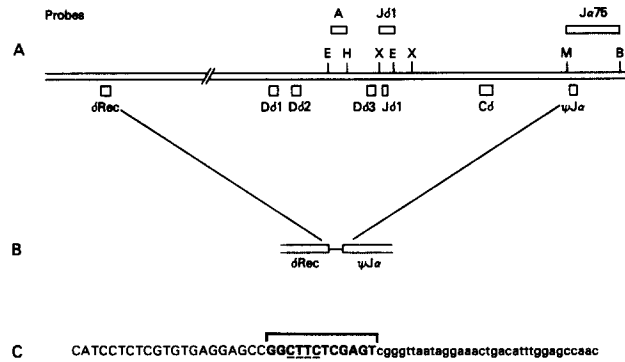


FIGURE 1. (A) Germline configuration of the  $\delta$  locus with flanking  $\delta$ Rec and pseudo J $\alpha$  ( $\psi$ J $\alpha$ ) genes. Probes were Eco RI(E)-Hind III (H) fragment from between D $\delta$ 2 and D $\delta$ 3 (probe A); Xba (X)-E J $\delta$ 1 probe and Mbo I(M) Bam HI(B) J $\alpha$ 75 probe. Not all sites are shown. (B) The  $\delta$ Rec/ $\psi$ J $\alpha$  rearrangement is shown schematically with deletion of intervening DNA. (C) Sequence of  $\delta$ Rec (upper case letters) and pseudo J $\alpha$  (lower case) with intervening nucleotides (overlined). The four nucleotides possibly from D $\delta$ 2 are indicated (broken line).

## Results

**A Rearrangement Detected 75 kb 5' to Constant  $\alpha$ .** A J $\alpha$  probe to the region 75 kb 5' to the constant (C) $\alpha$  gene detected a rearrangement in the stem-cell line DU.528. This rearrangement was cloned and sequenced. The 5' end of the sequence was identical to that previously described for the  $\delta$ Rec element (uppercase letters, Fig. 1 C) and the 3' end (lowercase) was identical to a pseudo J $\alpha$  gene (7). Between these two elements were 12 nucleotides (overlined, Fig. 1) not identified as belonging to either and consistent with "N-region" addition by the enzyme terminal deoxynucleotidyl transferase (TDT). However, the 4-bp motif CTTC found within the D $\delta$ 2 (formerly D $\delta$ 1) (7, 14) segment is also present in the 12-base stretch. Alternatively, therefore, it is possible that the 12-bp addition was formed by N-region addition of two G nucleotides between  $\delta$ Rec and D $\delta$ 2 and N-region addition of six nucleotides TCGAGT between D $\delta$ 2 and pseudo J $\alpha$ . Consistent with this latter possibility, N-region addition by TDT tends to be G rich (15).

**The  $\delta$ Rec-Pseudo J $\alpha$  Rearrangement Deletes the  $\delta$  Locus.** A probe from between D $\delta$ 2 and D $\delta$ 3 revealed this region was deleted from both chromosomes 14 in the cell line

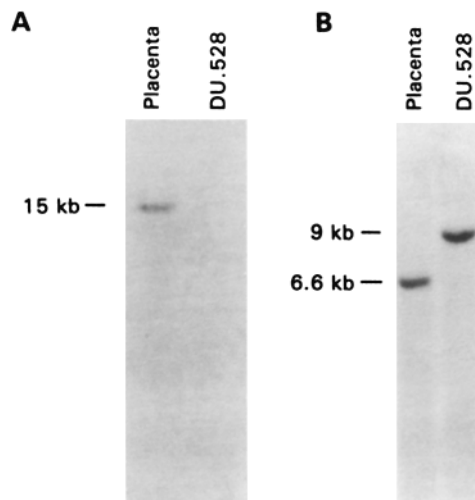


FIGURE 2. Southern blot analysis of germline DNA and DU.528 DNA. (A) Deletion of DNA between D $\delta$ 2 and D $\delta$ 3 from both chromosomes 14 in DU.528 detected by Probe A (Fig. 1). Restriction enzyme, Bam HI. (B) Single rearrangement in DU.528 DNA probed with J $\delta$ 1 probe (Fig. 1). Note absence of germline band in DU.528, consistent with deletion of the J $\delta$ 1 due to  $\delta$ Rec-pseudo J $\alpha$  rearrangement. Restriction enzyme, Hind III.

(Fig. 2 *A*). A  $J\delta 1$  probe showed a single rearranged  $J\delta 1$  band with absence of the germline configuration (Fig. 2 *B*). Consistent with this observation, only a single class of  $J\delta 1$  rearranged clones was identified from a genomic library screened with the  $J\delta 1$  probe. This rearrangement corresponded to a translocation between chromosome 1p33 and 14q11 that is the subject of a separate report. Taken together, these data show that the rearrangement between  $\delta$ Rec and pseudo  $J\alpha$  deleted the  $D\delta/J\delta$  region on that chromosome, while events involving the translocation deleted the  $D\delta$  elements on the other chromosome (16).

### Discussion

We have cloned and sequenced a direct rearrangement between the  $\delta$ Rec element and a pseudo  $J\alpha$  gene. There were 12 intervening nucleotides consistent with either "N-region" addition or a  $\delta$ Rec- $D\delta 2$ -pseudo  $J\alpha$  rearrangement. This rearrangement deleted the  $\delta$  locus.

A model of T cell ontogeny has recently been proposed in which either productive TCR- $\delta$  rearrangement or deletion of the TCR- $\delta$  locus would separate the  $\gamma/\delta$  and  $\alpha/\beta$  classes of T cells (7). The prediction regarding deletion of the delta locus in T cells undergoing TCR- $\alpha$  rearrangements was based on sequence analysis of extrachromosomal, circular-DNA from human thymus. This report confirms the existence of such rearrangements by analysis of a direct recombination that deletes the  $\delta$  locus in a human leukemic stem-cell line. This cell is  $CD7^+$  but  $CD3^-$ ,  $CD4^-$ ,  $CD8^-$  and is able to differentiate into multiple lineages. Given the phenotype of this cell, it is likely that the  $\delta$ Rec and pseudo  $J\alpha$  rearrangement is an event that occurs early in T cell development and before rearrangement of the TCR- $\alpha$  locus.

The presence of intervening nucleotides was not predicted and could be attributed to either "N-region" sequence alone or a  $\delta$ Rec- $D\delta 2$ -pseudo  $J\alpha$  rearrangement with addition of "N-region" sequences. While the extra bases could be added by non-TDT mechanisms, the presence of the intervening nucleotides might suggest that the rearrangement occurred at an early time in lymphoid ontogeny when the enzyme or enzymes involved in "N-region" addition (e.g., TDT) are active.

The demonstration of a direct rearrangement between  $\delta$ Rec and pseudo  $J\alpha$  genes provides additional support for the view that this rearrangement defines an intermediate event in the process of differentiation towards the  $\alpha/\beta$  lineage.

### Summary

It has been hypothesized that a rearrangement between the  $\delta$  recombining element ( $\delta$ Rec) and a pseudo  $J\alpha$  gene serves to delete the TCR- $\delta$  locus before rearrangement of the TCR- $\alpha$  genes. We have now sequenced a direct, site-specific rearrangement between the  $\delta$ Rec element and a pseudo  $J\alpha$  gene in a human leukemic stem-cell line. Putative "N-sequence" addition was noted at the site of recombination, suggesting that this event occurred at a time when the enzyme(s) involved in N-region addition were active in this cell. This provides support for the view that deletion of the TCR- $\delta$  locus is required before rearrangement of the TCR- $\alpha$  chain genes.

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