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Rearrangements to the JP1, JP and JP2 segments in the human T-cell rearranging gamma gene (TRGγ) locus

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In the human T-cell rearranging gamma (TRGγ) locus, five joining (J) segments have been identified: J1, J2 and three additional segments JP, JP1 and JP2. We report the sequence of the germline JP1 segment and compare it with the other human and mouse Jγ segments. We also demonstrate that rearrangements to the three additional Jγ segments can be identified by hybridization of the *Kpn*I digests to the Jγ1 probe pH60. Since rearrangements to J1 or J2 can be assigned, using the same pH60 probe, to one of the nine variable (V) γ genes known to rearrange [(1987) EMBO J. 6, 1945–1950], our results show that a unique probe can detect all the TRGγ rearrangements and be particularly useful for assessing the preferential usage of Vγ and Jγ segments in the TRG γ-expressing cells.

T-cell; Lymphocyte; Rearrangement; γ-Chain; Leukaemia; Lymphoma

1. INTRODUCTION

The human T-cell rearranging γ gene (TRGγ) has been identified [1,2] by homology with a mouse gene that has been shown to undergo rearrangement specifically in T-cells [3,4]. Human TRGγ genes have been mapped to chromosome 7 [5] at band 7p15 [2]. The TRGγ locus like other studied rearranging genes has variable (V) region genes, joining (J) and constant (C) region segments which join during the early stage of T-cell differentiation [6]. Two human constant-region genes have been identified [1,7–9] which are linked to each other at 16 kilobases [1,9]. 14 variable γ genes belonging to four subgroups [7,10,11] are located upstream of the two Cγ genes (fig.1A). Nine Vγ genes belong to subgroup I [10,11] whereas subgroups II, III and IV each consist of a single gene, respectively designated V9, V10 and V11 [7,10,11]. Two joining gene segments, Jγ1 and Jγ2 were first identified upstream of Cγ1 and Cγ2 [7], as well as a Jγ segment designated JP [10] upstream of Jγ1. More recently, two additional Jγ gene segments, JP1 and JP2, have been located in the Cγ1 and Cγ2 loci, respectively [12,13] (fig.1). It is of interest to know the germline sequence of these segments to evaluate the diversity of the V-J junction. So far, germline sequences of four Jγ segments have been published [7,10,13]. We now report the sequence of the germline JP1 and compare it with the other human and mouse Jγ segments. We previously showed that rearrangements to J1 or J2 could be assigned, using the Jγ1 probe pH60 [7] to one of the nine Vγ genes known to rearrange [11]. We now demonstrate that rearrangements to the additional Jγ segments, JP1, JP and JP2 can be identified by hybridization of the *Kpn*I digests to the Jγ1 probe pH60. A unique probe can therefore detect all the TRGγ rearrangements.

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2. MATERIALS AND METHODS

Southern filter hybridization [14] was carried out with 10 μg genomic DNA using nick-translated probes [15]. Conditions for hybridization, washing and monitoring have been described [10]. Nucleotide sequence analysis was carried out by the dideoxy chain-termination procedure [16] in M13 vectors [17].

2.1. *Probes*

The Jγ probe, pH60, containing the 700 base pair (bp) *Hin*dIII-*Eco*RI from M13H60 [1] subcloned in pUC9, includes the Jγ1 segment [7], this Jγ1 probe cross-hybridizing with Jγ2 but not with the additional Jγ segments. The VγI probe, 1.1 kilobase (kb) *Sac*I Vγ3 fragment from λSH4 [10], detects the nine Vγ genes belonging to subgroup I [11]. The VγII probe, a 400 bp *Pst*I-*Acc*I isolated from K20PR [7], contains V9 and detects the single gene belonging to subgroup II [10]. The VγIII probe, a 700 bp *Pst*I-*Eco*RI Vγ10 fragment from λR12, contains the 5'-region of Vγ10 and detects the single gene belonging to subgroup III [11].

3. RESULTS AND DISCUSSION

3.1. *Sequence of the germline JP1 segment*

Restriction maps from two λ phage clones isolated from a MOLT4 library showed by comparison with published maps of the human TRGγ locus [9–11] that two rearrangements occurred, V2-JP1 on one chromosome and V2-JP2 on the other (Rabbitts, T.H. et al. and [18]). Restriction maps showed that JP1 and JP2 are in the proximity of a *Hin*dIII site which is absent from the MOLT4 clones, as a consequence of the rearrangement (fig.1B). In unrearranged clones these additional J segments are localized in a 2.4 kb and a 1.1 kb *Eco*RI fragment located respectively upstream of those containing JP and J2 [9]. A 2.4 kb *Eco*RI fragment containing the JP1 segment was obtained from λRγ, a clone isolated from a phage library of the Burkitt's lymphoma cell line Raji [1]. *Hin*dIII-*Eco*RI subclones were sequenced from the *Hin*dIII site. The JP1 germline sequence which encodes 19 amino acid residues appears in fig.2. The conserved heptamer-nonamer sequences, proposed to be involved in the V-J joining, are separated by a 12-base spacer at the 5'-end of the J segment, whereas a conserved splice site is found at its 3'-end. Therefore, JP1 appears to be a functional gene segment.

3.2. *Comparison of the human and mouse Jγ segments and duplication in the TRGγ locus*

In fig.2, JP1 is compared to the other human and mouse Jγ gene segments. Both JP1 and JP2 are 19 amino acids long, as are the mouse Jγ gene segments. Their protein sequences share a homology of 57 and 52% respectively with those of the murine J1/J4 segments [4]. This homology between species is similar to and even higher than that existing between the mouse J1 and J2 [19,20] gene segments (which are 52% homologous).

As noted in the restriction maps and underlined by the repetition of characteristic *Kpn*I or *Xho*I sites (fig.1B), the human Cγ locus underwent recent duplication. A homology of up to 98% has been found previously from nucleotide sequence comparison of the 2.1 kb *Hin*dIII fragments which contain Jγ1 and Jγ2 (fig.1B), respectively ([7] and Rabbitts, T.H. and Lefranc, M.-P., unpublished). Comparison of the restriction sites in regions encompassing the JP1 and JP2 segments in fig.1B reveals that these two regions also result from a recent duplication, although the homology is lower. Indeed, the JP1 and JP2 segments have a homology of 63% for the amino acid sequence and 80% for the nucleotide sequence compared to the 100% homology observed between the J1 and J2 segments (one allelic form of J1 has one silent nucleotide substitution [7] whereas the other [10] is perfectly homologous to J2). Strikingly, the region encompassing the JP segment has no equivalent in the Cγ2 locus [10] and this is of interest, since most of the T-cells expressing γ-chain seem to use to JP segment [22].

3.3. Kpn*I digests and rearrangement assignments to JP1, JP, and JP2*

When a Vγ gene is rearranged to J1 or J2, it is possible to identify this gene by *Eco*RI, *Hin*dIII and *Bam*HI digestion and hybridization to the Jγ1 probe pH60 [11]. We now report that using the same probe, rearrangements to the additional Jγ segments JP1, JP and JP2 can also be identified when DNAs are digested with the *Kpn*I restriction enzyme.

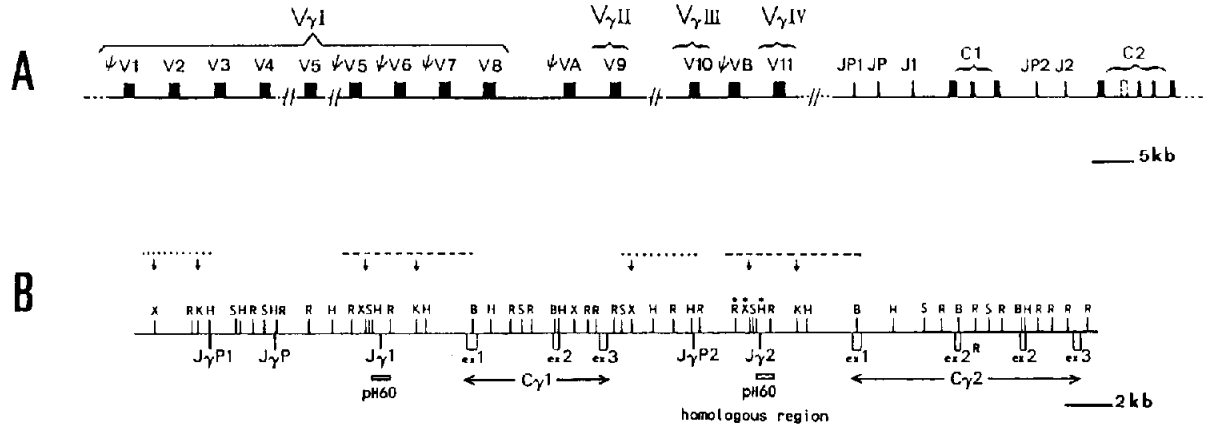

Fig.1. Organization of the human TRGγ locus. (A) Schematic representation of the human TRGγ locus [12]. For detailed maps, see [9–11] and panel B. (B) Restriction map of the human TRGγ constant region locus ([9] and this paper). Restriction sites: B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; S, *Sac*I; X, *Xho*I. Asterisks indicate polymorphic restriction sites [9]. Duplicated regions are shown by dashed lines.

The VγI and VγIII subgroup genes have an internal *Kpn*I site [10,11]. According to the J segment to which the VγI or VγIII gene is rearranged, *Kpn*I restriction fragments of different sizes can be detected: 1.8 kb (J1 or J2), 8.5 kb (JP1), 4.7 kb (JP2) (fig.3A). This is also shown in fig.4 where the 8.5 kb and 4.7 kb *Kpn*I bands detected in MOLT4 DNA (lane 1) and the 1.8 kb band observed in JM (lane 2) correspond respectively to the V2JP1, V2JP2 (MOLT4) and V8-J2 (JM) rearrangements [11,18]. Only one example of a Vγ gene belonging to the VγI or VγIII subgroup and involving JP has been described so far, a V2-JP out-of-frame rearrangement in the F8 cell line [10] which shows a 5.9 kb *Knp*I band (fig.4, lane 3).

Two genes V9 and V11 (respectively, single members of the VγII and VγIV subgroups) have no internal *Kpn*I site [7,11] and display characteristic *Kpn*I bands when rearranged to J1 or J2: 7.5 kb for V9 and 6 kb for V11 (fig.3B). As

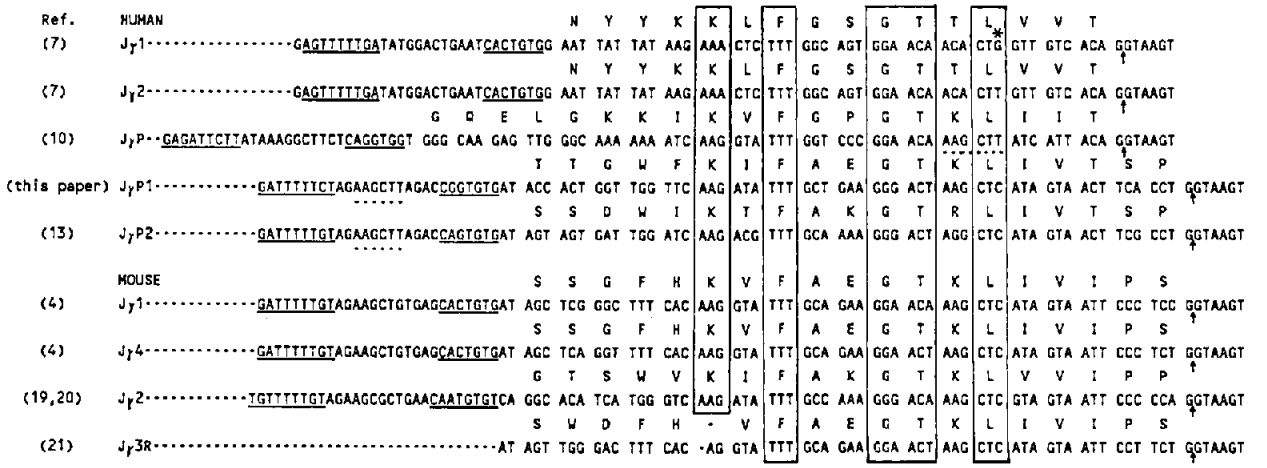

Fig.2. Sequence of the germline JP1 segment and comparison with the known human and mouse Jγ gene segments. Heptamer and nonamer sequences are underlined. Dashed lines indicate *Hin*dIII sites. The splicing sites are denoted by arrows. An asterisk indicates the nucleotide which can be either G or T (silent substitution) in the Jγ1 sequence [7,10].

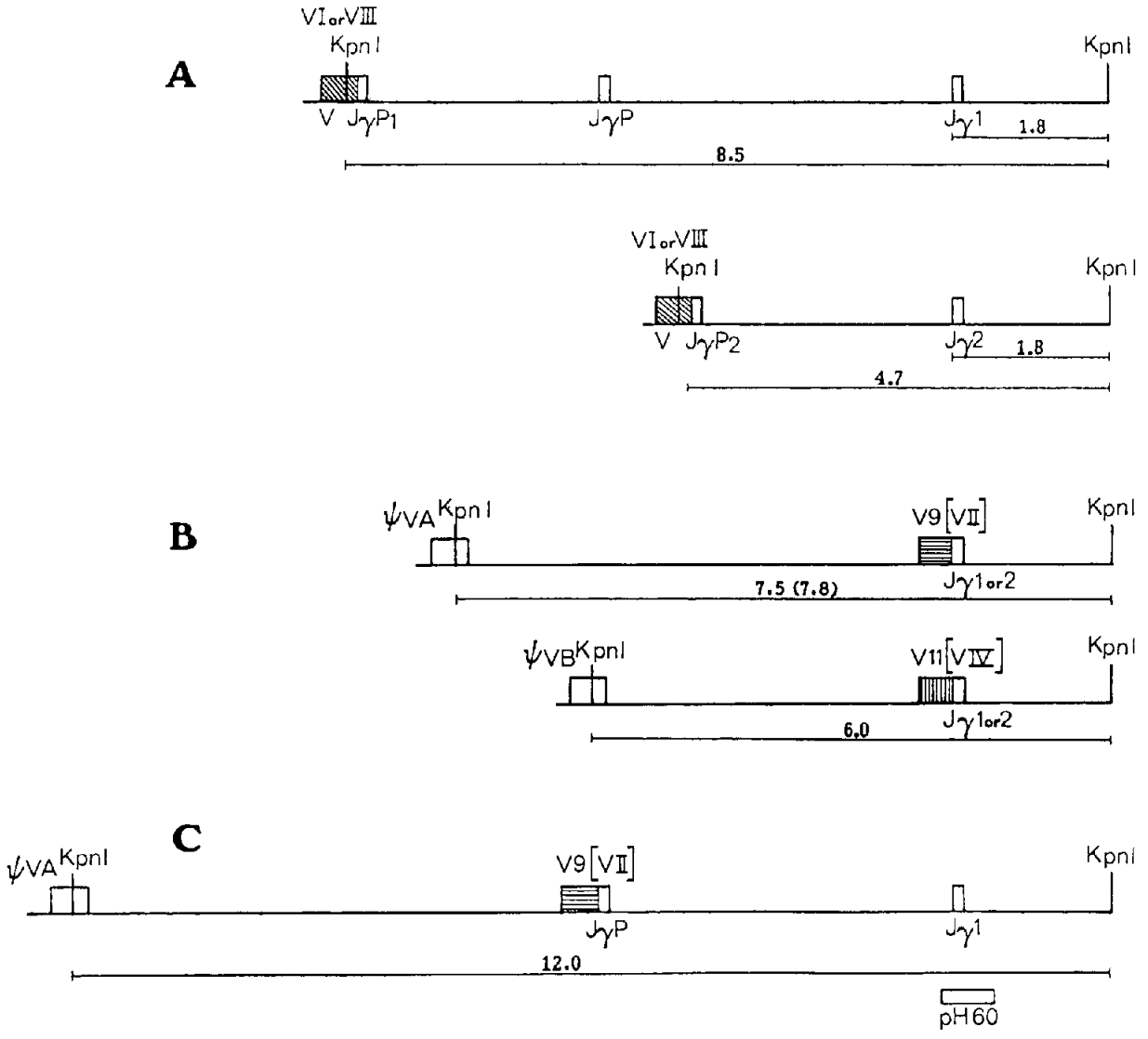

Fig.3. *Kpn*I restriction fragment sizes when Vγ genes are rearranged to the different Jγ gene segments. (A) Rearrangements of the VγI or VγIII subgroup genes to the JγI, Jγ2, JP1 or JP2 segments. The VγI subgroup genes which rearrange are V2, V3, V4, V5, ψV7 and V8 [10,11]. V10 is the single member of the VγIII subgroup [11]. (B) Rearrangement of the V9 and V11 genes (respectively, single members of the VγII and VγIV subgroups) to the Jγ1 or Jγ2 gene segments [7,10,11]. (C) V9-JP rearrangement observed in TRGγ$^+$ cells [22]. A *Hin*dIII restriction fragment length polymorphism (RFLP) due to a 300 bp insertion/deletion exists for one of the fragments located between ψVA and Vγ9 [11], which explains the *Kpn*I RFLP (7.5 or 7.8 kb *Kpn*I bands) mentioned in panel B. The *Kpn*I restriction fragment sizes have been deduced from cloned DNA fragments [9–11].

an example, the 6 kb *Kpn*I band detected in JM (fig.4, lane 2) corresponds to a V11-J1 rearrangement ([11] and unpublished).

These *Kpn*I bands are detected, in addition to the 16 kb (J2) and 9 kb (J1) germline bands when thymus or peripheral T-lymphocytes are digested with *Kpn*I and hybridized to pH60 (fig.4, lane 4) (our data and [23]). All these bands have been identified in T-cell clones displaying one or the other of these rearrangements. Interestingly, CD3$^+$

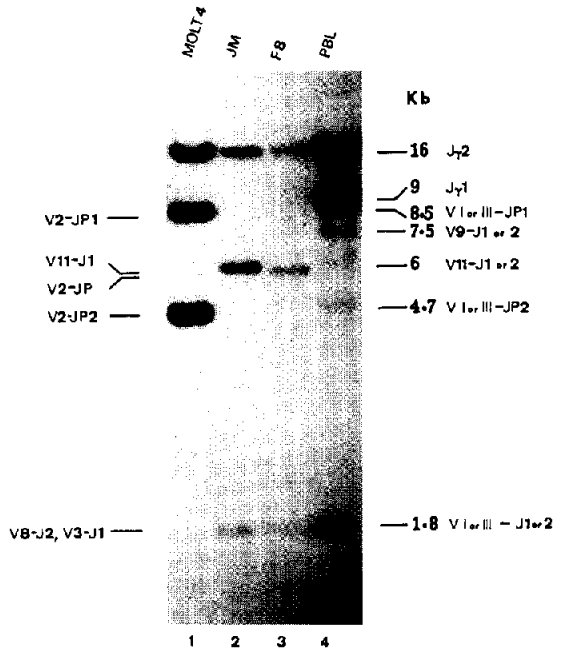

Fig.4. Southern hybridization of pH60 to *Kpn*I-digested DNA from T-cells (lanes 1–3) and peripheral blood leukocytes (PBL) (lane 4). Lanes: 1, MOLT4; 2, JM; 3, F8; 4, PBL of a normal donor. On the left are indicated rearrangements observed in MOLT4: V2JP1 and V2JP2 (see text); JM: V11JP1, V8J2 ([11] and unpublished); F8: V2JP, V3J1 [10]. The sizes of the rearrangements observed in PBL and their assignment are given on the right.

TCR$\alpha\beta^-$ clones expressing γ and recognized by the monoclonal antibody anti-Ti γ A (TRGγ^+ cells) [24] display a productive V9-JP rearrangement identified by a characteristic 12 kb *Kpn*I band (fig.3C) [22]. Altogether these results show that *Kpn*I digests hybridized to pH60 allow the identification of rearrangements occurring to the additional Jγ segments (JP1, JP or JP2). Moreover, the precise assignment of the Vγ genes involved in these rearrangements can be made using specific Vγ probes (see section 2). Table 1 shows the *Eco*RI restriction fragments allowing the identification of the VγI genes rearranged to JP1 or JP2. A V9-JP rearrangement can be confirmed by hybridization of an *Eco*RI digest to the VγII probe (a 2 kb *Eco*RI rearranged band instead of the 5.2 kb germline one) [11,22] and a V10-JP1 rearrangement can be confirmed by hybridization of a *Hin*dIII digest to the VγIII probe (a 4.2 kb *Hin*dIII rearranged band instead of the 3.5 kb germline band) [11].

Table 1

Assignment of the VγI genes joined to JγP1 or JγP2 (*Eco*RI digests, hybridized to the VγI probe)

	JP1	JP2
V2	2.1 (18.5)	0.5 (14.5)
V3	6.6	5.0
V4	2.1 (28)	0.5 (24)
V5	3.4	1.8
ψV7	4.3	2.7
V8	5.4	3.8

Sizes of the *Eco*RI rearranged bands are in kilobases (kb). Sizes of the *Bam*HI bands for the V2 and V4 rearrangements are given in parentheses

4. CONCLUSION

We reported the sequence of the human germline JP1 segment and compared it with the other human and mouse Jγ gene segments. This segment, as well as the other additional Jγ segments, JP and JP2, have been shown to be used in T-cell clones. The function of the TRGγ-expressing cells remains unclear. It is therefore of interest to characterize fully the V-J rearrangements undergone by these cells. We previously showed that rearrangements to J1 or J2 could be assigned to one of the nine Vγ genes known to rearrange [11]. Here, we demonstrated that *Kpn*I digests hybridized to the Jγ1 probe, pH60, allow a characterization of the rearrangements occurring to the JP1, JP or JP2 segments. Together these results show that a unique probe, pH60, can detect all rearrangements in the TRGγ locus whatever the Jγ segment involved in the rearrangements. T-cells displaying only one (or none) TRGγ rearrangement with *Eco*RI, *Hin*dIII and *Bam*HI [11] should be digested with *Kpn*I in order to detect rearrangements to the additional Jγ segments. In particular, the V9-JP rearrangement frequently observed in TRGγ^+ cells [22] is detected with the *Kpn*I digest (the *Bam*HI rearranged band has virtually the same size as the germline band and the *Eco*RI and *Hin*dIII bands are in germline configuration). Moreover, it is also possible to identify (or confirm) the V gene rearranged to an addi-

tional Jγ segment by using specific VγI, VγII or VγIII probes. Such precise determination of the Vγ-Jγ rearrangements should help in elucidating the function of the γ protein by assessing the preferential usage of given V and J segments in the TRGγ-expressing cells.

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