ORDERED ACTIVATION OF THE Igα LOCUS IN
ABELSON B CELL LINES

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During B cell development, V genes are assembled at the three different Ig gene loci (IgH, Igκ, and Igα) in a controlled and ordered fashion (1, 2). V gene rearrangements start at the IgH locus (3, 4) and continue at the Igκ locus in μ-producing cells (5-7), while the Igα locus is the last to be activated (8).

B cells expressing λ L chains have frequently rendered one or both Jκ alleles non-functional by the rearrangement of a recombining sequence (RS) 1 element (9-11). The RS element lies 3' of the mouse Cκ exon and is bordered at its 5' side by a 23-bp heptamer nonamer joining signal (9, 10). A homologous element called the κ-deleting element (kde) was also found at the human Igk locus (11) and has recently been mapped 24 kb downstream of the Cκ exon (12). Rearrangements of these elements involve either an isolated heptamer in the Jκ-Cκ intron or a 5'-situating Vκ gene segment and thus result in the deletion of part or all of the Jκ locus (10, 13). Due to the lack of suitable cellular models, the order and function of RS rearrangement in developing B cells is still unclear.

We have extensively subcloned and analyzed c-myc transfectants of P8, a μ-producing derivative of the Abelson line 300-19 (6, 14, 15). Two of seven c-myc-transfected Abelson lines assemble Vα genes while growing in culture. In these lines RS recombination occurred either at the same time as or before Vα rearrangements, suggesting that RS recombination is functionally correlated with the activation of the Igα locus.

Materials and Methods

Cell Lines. P8 is a derivative of the Abelson line 300-19. It carries a VDJ and a VDJ rearrangement at the IgH loci and produces intracellular μ chains. While growing in culture, P8 rearranges its κ loci (6). BIP8-7, B3P8-16, and B3P8-17 are c-myc-transfected derivatives of P8 in which the expression of the transfected c-myc gene has been proven via Northern blot analysis (14). Subclones of these lines were isolated by limiting dilution.

Southern Blot Analysis. Approximately 15 μg of genomic DNA was digested by appropriate restriction enzymes, subjected to electrophoresis through 1% agarose, blotted onto nitrocellulose, and assayed for hybridization to 32P-labeled probes. The Jκ-specific probe (5) was the 2.7-kb Hind III fragment carrying the Jκ segments; the RS-specific probe (9) was the 0.8-kb Sau 3A fragment from the RS region; and as a Vκ1-specific probe, we used a 0.85-kb, Xba fragment (16) from the Vκ1.

Genomic Cloning. Genomic libraries were cloned as Hind III fragments into λ Charon 28 (B allele of BIP8-7b) and as Eco RI fragments into λ Charon NM 1149 (The A allele of BIP8-7b

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1 Abbreviation used in this paper: RS, recombining sequence.
and the B alleles of BIP8-7-2 and BIP8-7-3). Libraries were screened either with a Jκ-specific or an RS-specific probe (for probes, see above).

DNA Sequencing DNA fragments were cloned into the plasmids pUC 18 and 19, respectively, and sequenced according to the dideoxy chain termination method (17).

Results and Discussion

Seven c-myc transfectants of P8 were subcloned and analyzed for Vκ, Vλ, and RS rearrangements. The Igκ locus was activated during the culture of two of these transfectants, BIP8-7 and BIP8-17. The original BIP8-7 culture (BIP8-7a) carried two VκJκ rearrangements and produced μ as well as κ L chain (data not shown). During a culture period of several weeks, BIP8-7 (then named BIP8-7b) performed further rearrangements on both Jκ alleles and became κ-. The two Jκ alleles of BIP8-7b are detected on a Southern blot as Bam HI-Eco RI fragments of 6.3 kb (allele A) and 5.3 kb (allele B) (see Fig. 1 a). Both Jκ alleles of BIP8-7b were cloned and sequenced. They each carry a Vκ to Jκ5 rearrangement using a Vκ element of either the Vκ21E (allele A) or the Vκ10 subgroup (allele B). The Vκ21E to Jκ5 joint of the BIP8-7b placed the Vκ and Jκ coding sequence in the same reading frame while the Vκ10 and Jκ5 segments were joined out of phase (Fig. 2 b, circled nucleotide). However, the correctly rearranged Vκ21E segment is not functional because it carries a 1-bp deletion in the leader sequence resulting in a frame shift (data not shown). Thus, both VκJκ rearrangements of BIP8-7b are nonproductive explaining the absence of κ L chain in this cell line.

On the A allele of BIP8-7b, the RS element is rearranged into the Jκ-Cκ intron. This VκJκRS rearrangement lies on the 6.3-kb Bam HI-Eco RI fragment that is detected on the Southern blot by the Jκ and the RS probe (Fig. 1, a and b). On the B allele of BIP8-7b, the RS element is retained in the germline configuration. The BIP8-7b culture was subcloned and 10 of its subclones were analyzed for further Jκ, RS, and Vλ rearrangements. The 6.3-kb VκJκRS rearrangement remains unchanged in all BIP8-7b subclones. The Vκ10Jκ5 rearrangement of the B allele of BIP8-7b, however, is deleted in three of the BIP8-7b subclones (Fig. 1 a, lanes 1, 2, and 3) and the same subclones also show the replacement of the RS germline fragment (Fig. 1, RS') by rearranged RS fragments of either 4.5 kb (Fig. 1 b, lanes 1 and 2) or 5.3 kb (Fig. 1 b, lane 3). Apparently, in all three BIP8-7b subclones a Vκ to RS joint resulted in the deletion of all Jκ hybridizing sequences on the B allele of BIP8-7b. The VκRS rearrangements of two subclones of BIP8-7b were cloned and sequenced. They are either a Vκ23 to RS (BIP8-7b-2) or a Vκ10b to RS (BIP8-7b-3) rearrangement (Fig. 2). The 4.5-kb Vκ to RS rearrangement seems to be a frequent and ongoing event in the culture of BIP8-7b subclones, because it was also detected submolarly in the subclones 4, 5, 7, and 10 (Fig. 1 b).

We next analyzed the Igλ locus of BIP8-7b and its subclones with a Vλ-specific probe (16, 18, 19). A Vλ2 to Jλ2 rearrangement of 6.4-kb was found in several BIP8-7b subclones (Fig. 1 c, lanes 1, 2, 4, 5, 7, and 10). In most cases this Vλ2 to Jλ2 rearrangement was only submolarly present, suggesting that it was happening in part of the analyzed culture. Interestingly, however, the Vλ2 to Jλ2 rearrangement occurred only in those subclones that had undergone or were undergoing Vκ to RS rearrangements (Fig. 1, compare b and c). Furthermore, for each subclone the intensity of the signal for the Vλ2Jλ2 rearrangement was similar to that of the VκRS
rearrangement. This strong correlation between RS and V\(\lambda\) rearrangements was also seen in the analysis of secondary BIP8-7b subclones (Fig. 3). Six of nine subclones of BIP8-7b-10 carried the 4.5-kb V\(\kappa\)RS and the V\(\lambda2\)J\(\kappa2\) rearrangement, while in the three remaining subclones, both rearrangements were absent (see Fig. 3).

In other derivatives of BIP8-7b, V\(\lambda\) rearrangements did not necessarily happen simultaneously with RS rearrangements. For example, two RS rearrangements had occurred in BIP8-7b-3 but only one of its subclones performed a V\(\lambda1\) to J\(\lambda1\) rearrangement (Fig. 3). Thus, RS rearrangements can occur independently of rearrangements at the Ig\(\lambda\) locus. Conversely, however, V\(\lambda\) rearrangements were found in only
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Figure 2. Sequence analysis of the Jκ alleles (A and B) of BIP8-7b and of two of its subclones, BIP8-7b-2 and BIP8-7b-3. (a) Organization of Vκ, Jκ, and RS elements at each allele. Available heptamer-nonamer joining signals are drawn as filled or shadowed triangles, respectively. The subgroup of the rearranged Vκ segment was determined by a comparison of its sequence with published sequences (24). The order of unrearranged Vκ segments on the B allele of BIP8-7b is tentative. (b) Characterization of the cloned VκJκ5 and VκRS rearrangements. Sequences at the joint are compared with either the Jκ5 or RS germline (10, 25) sequence as well as to published Vκ sequences of the indicated subgroups (24). 5' heptamer sequences of the joining signal are boxed. The recombination points are indicated by arrows. The circled nucleotides indicate the out of phase joint between the Vκ10a and Jκ5 coding sequence on the B allele of BIP8-7b. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00804.

those cells of the BIP8-7 lineage that had destroyed both Jκ alleles via an RS rearrangement. This correlation was also found in B3P8-17, an independent derivative of P8. Three subclones of B3P8-17 carried a Vλ1Jλ3 or a Vλ2Jλ2 rearrangement, as well as VκRS complexes, on both Jκ alleles (see Fig. 3). In other cell lines (B3P8-16 and BIP8-7b-6), both Vλ and RS rearrangements were absent (Fig. 3).

In the BIP8-7 cell lineage, Jκ, RS, and Vλ rearrangements occurred in an ordered fashion. After multiple Vκ to Jκ joints, an RS rearrangement occurred first on one Jκ allele. The rearrangement of the RS element on the second Jκ allele was often followed by the activation of the Igλ locus. This order of rearrangement events is in accordance with the analysis of most mouse and human λ-producing myeloma
FIGURE 3. Analysis of Vx, RS, and Vl rearrangements in subclones of c-myc-transfected Abelson lines. The genotypes of the two Jx alleles and one Vl allele are shown. (s, number of subclones with a given genotype; *, identical genotype to parents). Data for P8 are taken from reference 7.

lines (9, 11) although exceptions to the rule have been found (20, 21). A correlation between λ and RS rearrangements has also been described in the Abelson line ABC-1 (22).

RS rearrangements may be initiated only in κ- pre-B cells since both VκJκ complexes of B1P8-7b were unproductive. Unproductive VκJκ rearrangements were recently also found on each VκJκRS allele of a human λ-producing B cell (13).

How can the correlation found between RS and Vλ rearrangements be explained? Rearrangements of RS and Vλ segments may be controlled by the same factors that would in general first activate RS rearrangements, because during B cell development the Igκ locus is “opened” earlier than the Igλ locus (23). A functional role of RS rearrangements was proposed in two alternative models, suggesting that either an activation signal would be generated by the product of an RS rearrangement or a repressing signal would be removed by the deletion of inhibitory sequences or genes lying between the Jκ and RS elements (22). The existence of a regulatory active VκRS protein seems unlikely because Vκ and RS sequences are often joined in different reading frames (9), all of which are terminated soon after the VκRS junction (see also VκRS sequences of B1P8-7b-2 and B1P8-7b-3 in Fig. 2 b). The fact that in the cell lineage, λ rearrangements were only found in cells with two RS rearrangements suggests that the deletion of sequences lying between Jκ and RS is a requirement for the activation of the Igλ locus. In some B cells these sequences may be inactivated by different means, explaining why in a few λ-producing B cells only one RS rearrangement is found (9).
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Summary

Derivatives of the μ-producing Abelson line P8 have been analyzed for L chain gene rearrangements. Two of seven clones studied assembled their Vκ genes while growing in culture. Vκ gene rearrangements occurred only in those Abelson subclones that either were rearranging or had rearranged their recombining sequence (RS) element on both Igκ alleles. Our data suggest that (a) RS rearrangements are preferentially initiated in κ+ pre-B cells; and (b) the deletion or inactivation of sequences lying between Jκ and RS is a requirement for the activation of the Igκ locus.

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