# ORDERED ACTIVATION OF THE Igλ 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 Igλ) 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 Igλ locus is the last to be activated (8).

B cells expressing λ L chains have frequently rendered one or both Jκ alleles nonfunctional 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 (κde) was also found at the human Igκ 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′-situated 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 *Vλ* genes while growing in culture. In these lines RS recombination occurred either at the same time as or before Vλ rearrangements, suggesting that RS recombination is functionally correlated with the activation of the Igλ 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). B1P8-7, 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 $^{32}$P-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 Vλ1-specific probe, we used a 0.85-kb, Xba fragment (16) from the Vλ1.

*Genomic Cloning.* Genomic libraries were cloned as Hind III fragments into λ Charon 28 (B allele of B1P8-7b) and as Eco RI fragments into λ Charon NM 1149 (The A allele of B1P8-7b

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[1] *Abbreviation used in this paper:* RS, recombining sequence.

and the B alleles of B1P8-7-2 and B1P8-7-3). 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 Igλ locus was activated during the culture of two of these transfectants, B1P8-7 and B3P8-17. The original B1P8-7 culture (B1P8-7a) carried two VκJκ rearrangements and produced μ as well as κ L chain (data not shown). During a culture period of several weeks, B1P8-7 (then named B1P8-7b) performed further rearrangements on both Jκ alleles and became $\kappa^-$. The two Jκ alleles of B1P8-7b 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 B1P8-7b 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 B1P8-7b 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 B1P8-7b are nonproductive explaining the absence of κ L chain in this cell line.

On the A allele of B1P8-7b, 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 B1P8-7b, the RS element is retained in the germline configuration. The B1P8-7b 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 B1P8-7b subclones. The Vκ10Jκ5 rearrangement of the B allele of B1P8-7b, however, is deleted in three of the B1P8-7b subclones (Fig. 1 *a*, *lanes 1, 2,* and *3*) and the same subclones also show the replacement of the RS germline fragment (Fig. 1, $RS^0$) 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 B1P8-7b subclones a Vκ to RS joint resulted in the deletion of all Jκ hybridizing sequences on the B allele of B1P8-7b. The VκRS rearrangements of two subclones of B1P8-7b were cloned and sequenced. They are either a Vκ23 to RS (B1P8-7b-2) or a Vκ10b to RS (B1P8-7b-3) rearrangement (Fig. 2). The 4.5-kb Vκ to RS rearrangement seems to be a frequent and ongoing event in the culture of B1P8-7b 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 B1P8-7b and its subclones with a Vλ-specific probe (16, 18, 19). A Vλ2 to Jλ2 rearrangement of 6.4-kb was found in several B1P8-7b 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

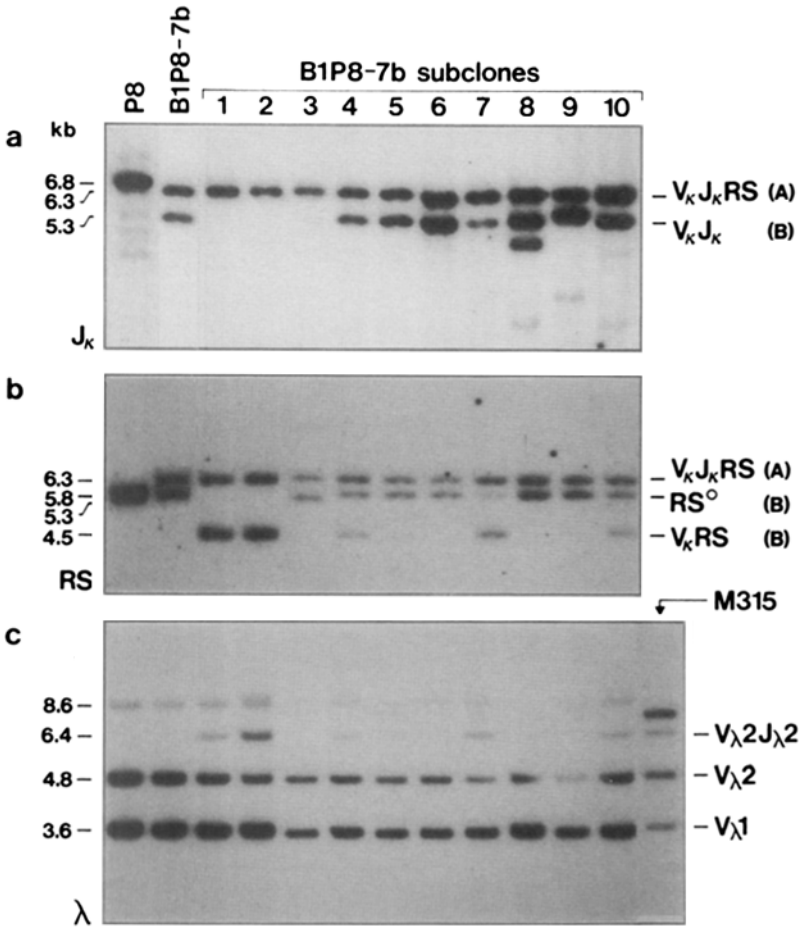
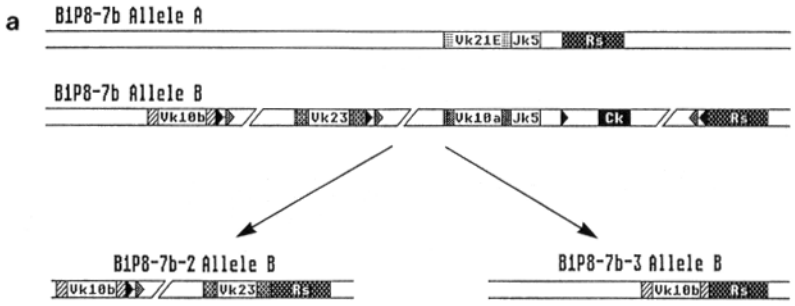



FIGURE 1. Southern blot analysis of Jκ (*a*), RS (*b*), and Vλ (*c*) rearrangements in the cell line P8, the c-*myc* transfectants B1P8-7b, and 10 of the B1P8-7b subclones. The sizes of restriction fragments carrying unrearranged (see lane *P8*) or rearranged elements are given on the left in kb. The type of rearrangement on the two Jκ alleles (*A* and *B*) is given on the right of the panel. Fig. 3 *c*, lane *M315*, shows a parallel analysis of the myeloma line MOPC315 carrying a $V\lambda_1J\lambda_1$ and a $V\lambda_2J\lambda_2$ rearrangement. The DNA of the different cell lines was digested either with Eco RI and Bam HI (*a* and *b*) or with Eco RI alone (*c*).

rearrangement. This strong correlation between RS and Vλ rearrangements was also seen in the analysis of secondary B1P8-7b subclones (Fig. 3). Six of nine subclones of B1P8-7b-10 carried the 4.5-kb VκRS and the Vλ2Jλ2 rearrangement, while in the three remaining subclones, both rearrangements were absent (see Fig. 3).

In other derivatives of B1P8-7b, Vλ rearrangements did not necessarily happen simultaneously with RS rearrangements. For example, two RS rearrangements had occurred in B1P8-7b-3 but only one of its subclones performed a Vλ1 to Jλ1 rearrangement (Fig. 3). Thus, RS rearrangements can occur independently of rearrangements at the Igλ locus. Conversely, however, Vλ rearrangements were found in only




b

B1P8-7b Allele A

| | |
|---|---|
| Vκ21E | TGT CAG CAC AGT AGG GAG CTT CCT |
| Vκ21EJκ5− | TGT CAG CAC AGT AGG GAG CTT CCC ACG TTC GGT GCT |
| Jκ5 | CACTGTGG CTC ACG TTC GGT GCT |

B1P8-7b Allele B

| | |
|---|---|
| Vκ10a | TGC CAA CAG GGT AAT ACG CTT C CT |
| Vκ10aJκ5− | TGC CAA CAG GGT AAT ACG CTT (C) CTC ACG TTC GGT GCT |
| Jκ5 | CACTGTGG CTC ACG TTC GGT GCT |

B1P8-7-2 Allele B

| | |
|---|---|
| Vκ23 | TGT CAA CAG AGT AAC AGC TGG CCT |
| Vκ23RS | TGT CAA CAG AGT AAC AGC TGG CCC CTA GTG GCA GCC |
| RS | CACTGTGAGG ACC CTA GTG GCA GCC |

B1P8-7-3 Allele B

| | |
|---|---|
| Vκ10b | TGT CAG CAG TAT AGT AAG CTT CCT C |
| Vκ10bRS | TGT CAG CAG TAT AGT AAG CTT CCT CGA CCC TAG TGG |
| RS | CACTGTGA GGA CCC TAG TGG |

FIGURE 2. Sequence analysis of the Jκ alleles (*A* and *B*) of B1P8-7b and of two of its subclones, B1P8-7b-2 and B1P8-7b-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 B1P8-7b 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 B1P8-7b. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00804.

those cells of the B1P8-7 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 B1P8-7b-6), both Vλ and RS rearrangements were absent (Fig. 3).

In the B1P8-7 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

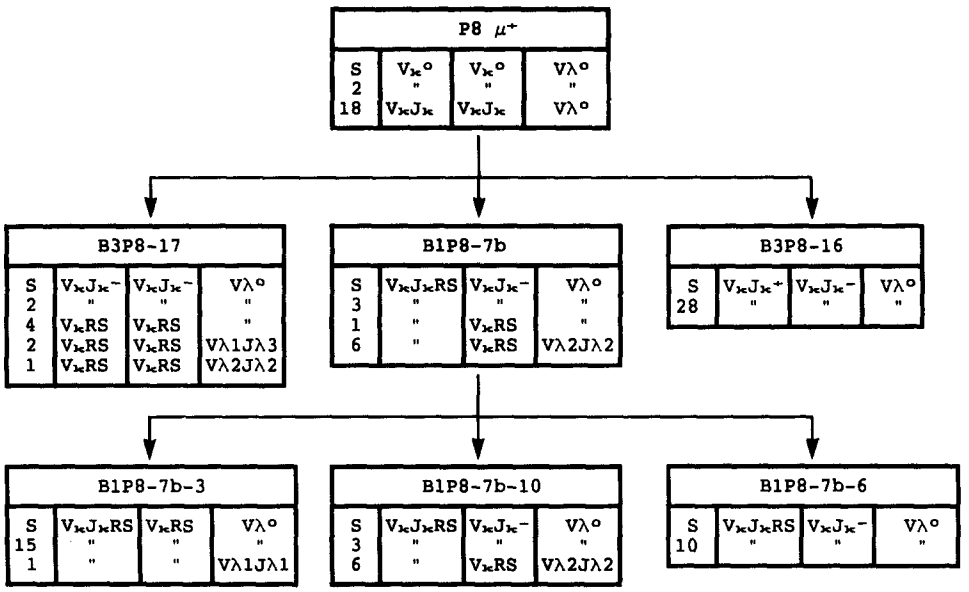



FIGURE 3. Analysis of Vκ, RS, and Vλ rearrangements in subclones of c-*myc*-transfected Abelson lines. The genotypes of the two Jκ alleles and one Vλ allele are shown. (*s*, number of subclones with a given genotype; ", identical genotype to parents). Data for P8 are taken from reference 7.

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 $\kappa^-$ 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).

## Summary

Derivatives of the μ-producing Abelson line P8 have been analyzed for L chain gene rearrangements. Two of seven clones studied assembled their *Vλ* genes while growing in culture. *Vλ* 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 $\kappa^-$ pre-B cells; and (*b*) the deletion or inactivation of sequences lying between Jκ and RS is a requirement for the activation of the Igλ locus.

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