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33

Immunoglobulin Gene Rearrangement in Immature B Cells

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Summary. Two types of immature B cells, namely fetal liver hybridomas and the leukemic cell line 70Z/3, both of which have cytoplasmic μ chains but no light chains, were examined for DNA rearrangements of their light chain and heavy chain immunoglobulin genes. In the fetal liver hybridomas, which were constructed from fetal liver cells and a tumor cell, no light chain gene rearrangement was observed, whereas in the 70Z/3 cell line a κ light chain rearrangement probably occurred. The results suggest that, although the lack of light chain synthesis can be due to a lack of gene rearrangement, there may also be transcriptional regulation, which may also be important for the expression of light chain immunoglobulins in immature B cells.

The expression of a λI light chain from mouse immunoglobulin has been linked to a DNA rearrangement; this rearranged fragment joins the major part of the gene for the variable (V) region to a short DNA segment called J (*1*). In the κ light chain system an analogous event takes place, namely one of a few hundred Vκ DNA segments joins to one of five Jκ DNA segments to create a complete V gene (*2–4*). This DNA rearrangement has been demonstrated in λ- and κ-producing myelomas.

Evidence has been presented from studies on cloned immunoglobulin heavy chain genes that the rearrangement of DNA occurs prior to the expression of a heavy chain (*5–7*). Furthermore, DNA sequencing studies suggest that a complete, heavy chain V gene is composed of at least three DNA segments, an embryonic V segment, a D segment, which encodes the HV3 region (third hypervariable region), and a J segment (*5–7*). Thus, for the light chain as well as the heavy chain immunoglobulins of the mouse, expression appears to be dependent on somatic rearrangement of DNA sequences.

The cells that have been used extensively for this type of analysis are from plasmacytoma tumors. Essentially, these cells represent the terminally differentiated state of an immunoglobulin-producing cell. The DNA used for comparison studies is often isolated from 10- to 12-day-old mouse embryos or sperm. The stages of cell differentiation that encompass the period between these two extremes have not been easily accessible, although the use of Abelson virus (*8*) or hybridoma technology (*9, 10*) may solve this problem.

Sensitive immunofluorescent techniques have been used to identify a population of immature lymphocytes in the livers of 12- to 15-day-old fetal mice. These cells, which contain immunoglobulin in the cytoplasm but not on the surface (*11*), have been referred to as pre-B cells because of accumulating evidence that they are the progenitors of B lymphocytes (*12*). Recent evidence suggests that these cells pass through a stage in which μ chains are synthesized in the absence of detectable light chains (*9, 10*). We now describe two types of pre-B cells that we have characterized with respect to the possible DNA rearrangements involving immunoglobulin genes. One type of pre-B cell is found in hybridomas prepared from fetal liver by Burrows *et al.* (*10*). The hybridomas were prepared from BALB/c (17-3-5) or C57 Black (4-9-12-7) with a nonimmunoglobulin-producing plasmacytoma Ag8653. The other type is from a cell line of murine leukemia referred to as 70Z/3, which was established in vitro by Paige *et al.* (*13*). Both of these types of pre-B cells have intracellular μ chains but no light chains.

The presence of μ chains in the cytoplasm of the cells suggests that there may have been a DNA rearrangement involving the heavy chain J segment (*5–7*). We and others have cloned the genomic DNA fragment containing the Cμ gene and have shown by heteroduplex analysis and by DNA sequencing that there are four J segments located between 7.6 and 8.7 kilobase pairs (kbp) to the 5′ side, relative to the direction of transcription, of the Cμ gene (Fig. 1a) (*5–7*). The four J-coding DNA segments are themselves located on a 6.4-kbp Eco RI fragment. A 0.8-kbp DNA fragment obtained by digestion with Eco RI and Xba I (see Fig. 1a) was isolated from the 3′ end of the 6.4-kbp Eco RI fragment and used as a probe to detect possible rearrangements in the 6.4-kbp Eco RI fragment in the fetal liver hybridoma cells which make μ chain only, and in the 70Z/3 cell line. The probe hybridizes only to the 6.4-kbp Eco RI band in the DNA of both the Eco RI–digested BALB/c mouse embryo and the Eco RI–digested C57BL/6 mouse kidney (Fig. 1b). The DNA isolated from the parent myeloma Ag8653 and digested with Eco RI also has one positive band at 6.4 kbp. This myeloma has lost the capacity to synthesize both heavy chains and light chains. In contrast, the Southern blots (*14*) for the two hybridomas were quite different. In Eco RI–digested DNA from hybridoma 4-9-12-7, which is derived from C57BL/6, there are three bands that hybridize with the probe. One band is at 6.4 kbp and corresponds to the embryo-type band. This band could arise from either the fetal liver cell or the myeloma parent. Two other hybridizable bands at 5.0 and 2.7 kbp are present only in the DNA of this hybridoma and are, therefore, likely to have arisen from the fetal liver cell. Presumably one or both of these fragments were generated as a consequence of rearrangement that led to the creation of a complete V gene active in the hybridoma. The two rearranged fragments may originate from two different copies of chromosome 12, since it is not uncommon to find more than one nonembryo-type fragment in myeloma cells (*15*). Alternatively, the two fragments could be explained by assuming that the fusion process actually occurred between three cells—the myeloma cell and two cytoplasmic, μ chain fetal liver cells. The

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pattern of hybridizable bands in hybridoma 17-3-5, which is derived from BALB/c, is also shown in Fig. 1b. One band appears to be identical to the embryonic 6.4-kbp band, while another band appears at 5.7 kbp. We have some evidence that the 70Z/3 cell line also contains a rearranged heavy chain J segment (*16*).

On the basis of previous results (*5–7*), the most likely explanation for the DNA rearrangement seen within the 6.4-kbp Eco RI fragment containing the heavy chain J's is that embryonic V, D, and J DNA segments have been joined to create a complete V gene. The results presented here do not in themselves describe a V gene at the DNA level, which can only be done by cloning and DNA sequencing, but strongly support the above conclusion. Thus, these results, as well as the light chain data presented below, are consistent with the idea that expression is linked to the rearrangement of the DNA.

The light chain immunoglobulins in the mouse are either λ or κ type. The κ light chains are encoded in a few hundred germ-line V DNA segments, five J DNA segments, and probably one C DNA segment. To look for possible DNA rearrangements of the κ genes we prepared a chimeric plasmid, containing the Cκ DNA segment on a Hind III fragment inserted into the plasmid vector pBR322 (*15*). The plasmid was then used as a probe in Southern blot analysis of DNA's from Bam HI–digested BALB/c mouse embryo, myeloma Ag8653, and hybridomas 17-3-5 and 4-9-12-7 or from Eco RI–digested 70Z/3 and BALB/c mouse embryo. The probe has homology to two Bam HI fragments of embryonic origin, a 1.8-kbp fragment and a 13-kbp fragment, and in the Eco RI digests to one band of 15 kbp (Fig. 2a). In the Bam digests, the five J-coding DNA segments are located on the 13-kbp fragment, and any DNA rearrangement involving the J sequences would very likely alter the size of that fragment. A similar situation would occur for the 15-kbp Eco RI fragment.

Southern blot analysis of the DNA from Ag8653 revealed three hybridizable bands. The band at 1.8 kbp corresponds to the embryo band at the same position, but the bands at 6.5 and 7.6 kbp are unique to Ag8653. Cloning of these two rearranged bands from P3 DNA, from which Ag8653 was originally derived, revealed that one of the bands corresponds to a legitimate V-J joining, whereas the other corresponds to a rearrangement that occurred in the 5′ flanking region of Cκ, but that did not involve a Jκ or Vκ DNA segment (*17, 18*). Each of the

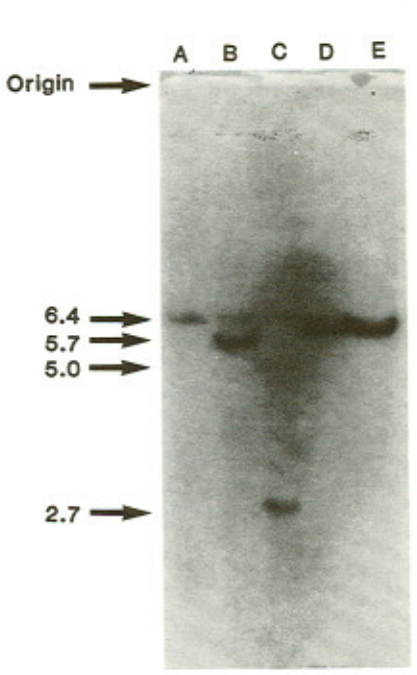
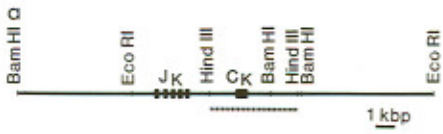
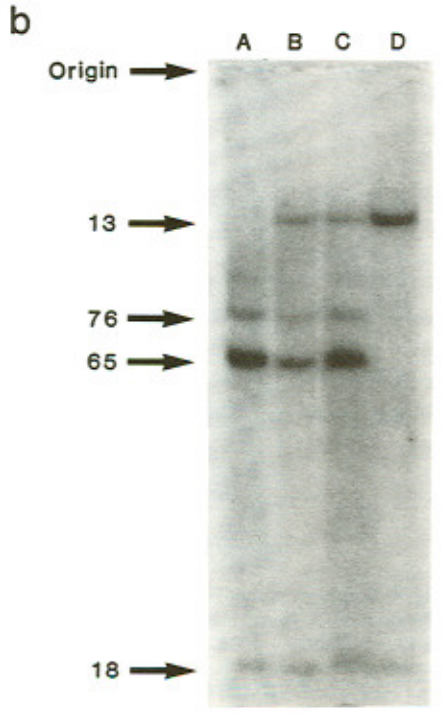
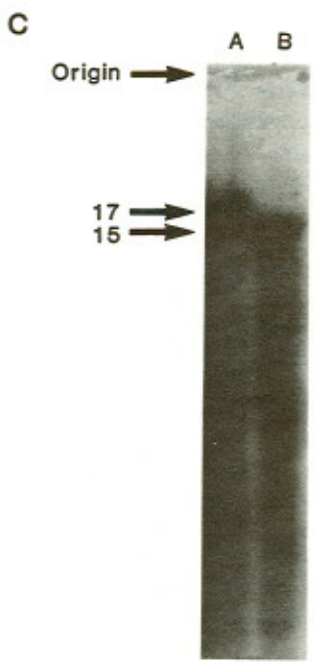


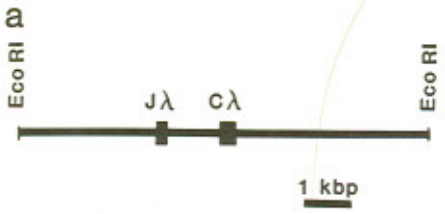
Fig. 1. (a) Physical map of a clone containing the Cμ DNA segment in mouse embryo DNA. The clone was isolated as described in Maki *et al.* (*5*), and the position of the four J DNA segments as in Sakano *et al.* (*7*). Probe for Southern blot hybridization was a 0.8-kbp Xba I–Eco RI fragment indicated by the broken line. (b) Southern hybridization of Eco RI–digested DNA from (band A) Ag8653, (band B) 17-3-5, (band C) 4-9-12-7, (band D) BALB/c mouse embryo, and (band E) C57BL6 mouse kidney, with the 0.8-kbp probe described above. The digested DNA (10 μg) was electrophoretically separated on a 0.8 percent agarose gel at 1 mA/cm for about 24 hours. The DNA was then transferred to a nitrocellulose filter (Schleicher & Schuell, BA85) essentially as described (*14*). Filters were hybridized in the presence of a nick-translated probe 1×10^7 count/min with the procedure of Wahl *et al.* (*22*). Molecular sizes of the bands are indicated in kilobase pairs.

Fig. 2. (a) Physical map of a cloned insert containing the Cκ DNA segment isolated from mouse embryo DNA. The clone was isolated as described in (*17*). The position of the five Jκ DNA segments was as described by Sakano *et al.* (*3*) and Max *et al.* (*4*). The probe used for Southern blot hybridization was a 4-kbp Hind III fragment that had been inserted into the plasmid pBR322 (*15*). (b) Southern blot hybridization of Bam HI–digested DNA from (band A) Ag8653, (band B) 17-3-5, (band C) 4-9-12-7, and (band D) BALB/c mouse embryo, with the plasmid containing the Hind III fragment discussed in (a). Procedures were essentially those described in Fig. 1. Molecular sizes of the bands are indicated in kilobase pairs. (c) Southern blot hybridization of Eco RI–digested DNA from (band A) 70Z/3 and (band B) BALB/c mouse embryo, with the plasmid discussed in (a). Molecular sizes of the bands are indicated in kilobase pairs.









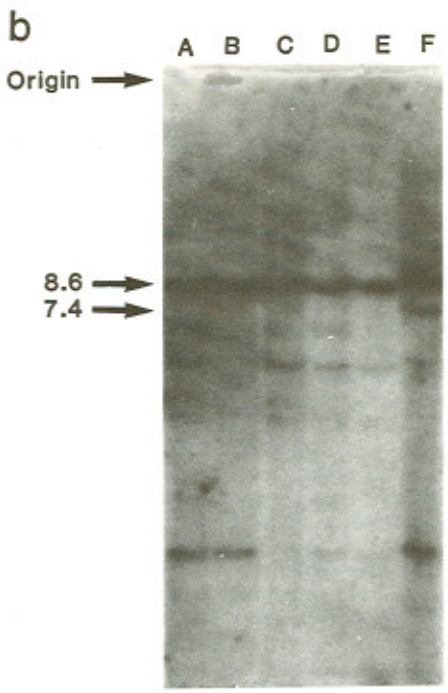


Fig. 3. (a) Physical map of a cloned insert containing the CλI DNA segment isolated from mouse embryo (*1*). (b) Southern blot hybridization of Eco RI–digested DNA from (band A) Ag8653, (band B) 17-3-5, (band C) 4-9-12-7, (band D) BALB/c mouse embryo, (band E) C57BL6 mouse kidney, and (band F) H2020, with the 0.6-kbp Hha I–Hae III fragment described in the text. Procedures were essentially those described in Fig. 1. Molecular sizes of the bands are indicated in kilobase pairs.

DNA's isolated from the two fetal liver hybridomas has four hybridizable bands (Fig. 2b). Two of the bands, at 6.5 and 7.6 kbp, can be considered to have arisen from the DNA of Ag8653. The other two bands correspond to positions of 1.8 and 13 kbp which are precisely the size of the Bam HI fragments in mouse embryo DNA. To exclude the possibility that a rearranged band from one or both of the hybridoma DNA's happens to coincide with one of the nonembryonic bands of Ag8653 origin, we performed similar gel blot analyses with Eco RI–digested DNA as well as with Eco RI plus Bam HI double-digested DNA (data not shown). None of the Southern blots indicated additional rearranged bands in the hybridoma DNA's that could not be accounted for by the DNA of Ag8653 or of mouse embryo. On the basis of these observations we conclude that no V-J type rearrangement has occurred for the κ light chain genes in the fetal liver hybridomas.

In contrast to the fetal liver hybridomas, results with the 70Z/3 line suggested that there had been a rearrangement of the DNA near the Cκ DNA segment. The 70Z/3 line contains one band at 15 kbp which appears to be identical to that in mouse embryo DNA (Fig. 2c). In addition, a band at 17 kbp is present in 70Z/3, but not in mouse embryo DNA. From this result we conclude that it is likely that a rearrangement involving the Jκ DNA segments has taken place in 70Z/3. This conclusion is supported by the observation that 70Z/3 can be induced to synthesize κ light chains in the presence of lipopolysaccharide (*13*).

Before one can conclude that no light chain gene rearrangement has taken place in the fetal liver hybridomas, the other light chain system in the mouse, namely the λI and λII genes, must be investigated. Since, for each of λI and λII chains, there apparently exists only one germ-line V DNA segment and one C DNA segment, as well as one J DNA segment, V-J joining in either of the two genes should generate a fragment of a size characteristic of that gene. It had been previously shown that the Eco RI fragment containing the CλI DNA segment is 8.6 kbp and that there is a J DNA segment of 1.2 kbp on the 5′ side of the C DNA segment (Fig. 3a), while the rearranged VλI + CλI Eco RI fragment is 7.4 kbp (*1*). A 0.6-kbp Hha I–Hae III fragment containing the CλI DNA segment was prepared from complementary DNA (cDNA) made from the λI-producing myeloma H2020 and used for Southern blot analysis of Eco RI–digested DNA from mouse embryo, Ag8653, 17-3-5, 4-9-12-7, and H2020, a λI producer. The results shown in Fig. 3b demonstrate the presence of the 8.6-kbp embryo-type fragment in the two hybridomas, but there is complete absence of a band at 7.4 kbp, which can be seen in the λI producer H2020. Several cross-hybridizable bands observed with the CλI probe may represent CλII or CλIII but were not characterized further.

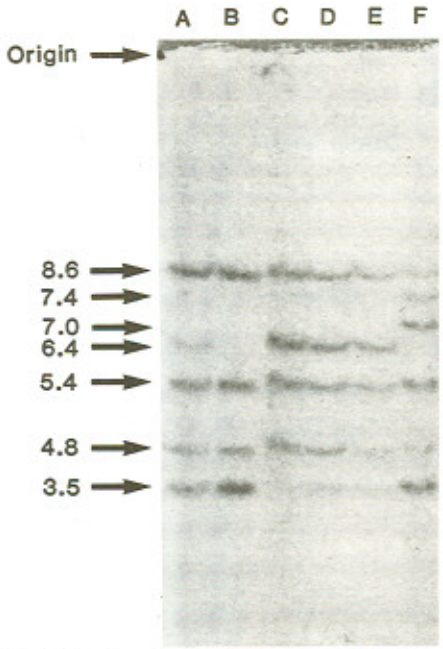


Fig. 4. Southern blot hybridization of Eco-RI–digested DNA from (band A) Ag8653, (band B) 17-3-5, (band C) 4-9-12-7, (band D) BALB/c mouse embryo, (band E) C57BL6 mouse kidney, and (band F) MOPC315, with a VλII + CλII cDNA (*19*). Procedures were essentially those described in Fig. 1. Molecular sizes of the bands are indicated in kilobase pairs.

Light chain genes of λII were examined with the use of complementary DNA prepared from the λII-producing myeloma MOPC315 and inserted into the plasmid vector pBR322 (*19*). On the basis of previous results obtained by gel blot analysis as well as by cloning and DNA sequence analysis, we could identify the 4.8-kbp Eco RI band in mouse embryo as being the VλII DNA segment, while the 3.5-kbp band represents the VλI DNA segment resulting from hybridization between the two V DNA segments (*1*). There are three additional bands in mouse embryo at 8.6, 6.4, and 5.4 kbp that we have not unambiguously identified (Fig. 4). It is likely, on the basis of recent amino acid sequencing studies, which indicate a high degree of homology between the λII and λIII genes (*20*), that some of these additional bands may represent the VλIII DNA segment and the CλIII DNA segment. The pattern from MOPC315 DNA, which is a λII producer, reveals an additional band at 7 kbp which is probably the rearranged VλII + CλII gene. Comparison of the Southern blots of the DNA's for the two hybridomas as well as for Ag8653 with the pattern observed for mouse embryo and MOPC315 DNA's shows clearly that the hybridomas contain only those bands seen in embryo DNA and, in particular, do not contain the rearranged band found in MOPC315. The lack of the 6-kbp band in the hybridoma 17-3-5 is somewhat curious, since this band is present in DNA from both mouse embryo and Ag8653. Possibly the chromosome carrying this gene was lost during

fusion or subsequent propagation of cells. The results of the λII analysis strongly suggest that no DNA rearrangement that could be considered as a rearrangement to activate the synthesis of λII light chains has occurred in the two fetal liver hybridomas.

We have shown by Southern blot analysis that there has been a DNA rearrangement on the 5′ side of the Cμ gene in both of the fetal liver hybridomas as well as in the 70Z/3 line. This rearrangement most likely involves the heavy chain J segments. As a result of previous observations it seems reasonable to infer that this rearrangement is linked to the expression of the μ chain in these cells (*5–7*). The fetal liver hybridoma cells appear not to have any light chain gene rearrangement. Since no light chains have been identified in these cells, we would conclude that these cells cannot synthesize light chains because they lack a functional rearrangement for the light chain V and C DNA segments. If cell fusion and subsequent growth of the fused cells do not alter the organization of immunoglobulin genes, the above conclusion can be extended to the cytoplasmic μ chain pre-B cells that are present in the fetal livers.

Although rearrangement of the light chain gene apparently is required for expression, in itself it may not be the only requirement, as suggested by experiments with the 70Z/3 line in which light chain gene rearrangement is present, yet no light chains are synthesized unless lipopolysaccharide is present. The result suggests that in the cells with μ chains only there may be at least two levels of control for the synthesis of light chains. The first level is the rearrangement of a V DNA segment and a J DNA segment to create a complete gene, without which a complete light chain is not synthesized. The second level of control, which can only become operative after DNA rearrangement has taken place, is a transcriptional regulation like that in the 70Z/3 line. If the second form of control is present to a significant degree in fetal liver cells, then one might predict the finding of some fetal liver hybridomas that have cytoplasmic μ chains and no light chains, but still have rearranged genes for light chains.

Why do immunoglobulin μ cells exist? Although the accumulated evidence is still somewhat limited for an adequate explanation of the asynchronous onset of immunoglobulin synthesis, three broad categories may be considered. (i) This finding simply represents an ontogenic event in the differentiation of B lymphocytes. (ii) The onset of μ synthesis serves as a proliferative, antigen-independent stimulus that allows for the expansion of clones of cells expressing a particular heavy chain variable region (*8*). (iii) These free μ chains are capable of interacting with the external environment via their V regions at a time prior to their ability to synthesize complete antibody molecules, thus moderating possible deteriorative reactions while allowing for tolerance induction and network formation. Evidence to support the third possibility has been found by Levitt and Cooper (*9*) who recently reported that pre-B cells are capable of secreting μ chains before the synthesis of light chains. Furthermore, the 70Z/3 pre-B cell line can be induced to express the μ chain on the cell surface in the absence of light chains (*21*). The analysis of additional hybridomas as well as other cell types with similar characteristics or origins (or both) should be helpful in determining which of these possibilities is correct.

References and Notes

1. C. Brack and S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5652 (1977); C. Brack, M. Hirama, R. Lenhard-Schuller, S. Tonegawa, *Cell* **15**, 1 (1978).
2. N. Hozumi and S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3628 (1976); T. H. Rabbits, *Nature (London)* **275**, 291 (1978); J. G. Seidman, E. E. Max, P. Leder, *ibid.* **280**, 370 (1979).
3. H. Sakano, K. Hüppi, G. Heinrich, S. Tonegawa, *Nature (London)* **280**, 288 (1979).
4. E. E. Max, J. G. Seidman, P. Leder, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3450 (1979).
5. R. Maki, A. Traunecker, H. Sakano, W. Roeder, S. Tonegawa, *ibid.* **77**, 2138 (1980).
6. M. M. Davis, K. Calame, P. W. Early, D. L. Livant, R. Joho, I. L. Weissman, L. Hood, *Nature (London)* **283**, 733 (1980); P. Early, H. Huang, M. Davis, K. Calame, L. Hood, *Cell* **19**, 981 (1980).
7. H. Sakano, R. Maki, Y. Kurosawa, W. Roeder, S. Tonegawa, *Nature (London)* **286**, 676 (1980).
8. D. Baltimore, N. Rosenberg, O. N. White, *Immunol. Rev.* **48**, 3 (1979); E. J. Siden, D. Baltimore, D. Clark, N. B. Rosenberg, *Cell* **16**, 389 (1979).
9. D. Levitt and M. D. Cooper, *Cell* **19**, 617 (1980).
10. P. Burrows, M. Lejeune, J. F. Kearney, *Nature (London)* **280**, 838 (1979).
11. D. G. Osmond and G. J. V. Nossal, *Cell. Immunol.* **13**, 132 (1974); J. J. T. Owen, D. E. Wright, S. Habu, M. C. Raff, M. D. Cooper, *J. Immunol.* **118**, 2067 (1977); M. C. Raff, M. Megson, J. J. T. Owen, M. D. Cooper, *Nature (London)* **259**, 224 (1976).
12. M. D. Cooper, J. F. Kearney, P. M. Lydyard, C. E. Grossi, A. R. Lawton, *Cold Spring Harbor Symp. Quant. Biol.* **41**, 139 (1976); F. Melchers, J. Andersson, R. A. Phillips, *ibid.*, p. 147; P. D. Burrows, J. F. Kearney, A. R. Lawton, M. D. Cooper, *J. Immunol.* **120**, 1526 (1978).
13. C. J. Paige, P. W. Kincade, P. Ralph, *J. Immunol.* **121**, 641 (1978); R. P. Perry and D. E. Kelley, *Cell* **18**, 1133 (1979).
14. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
15. Plasmid prepared containing the Hind III fragment mentioned was prepared and supplied by P. Kennedy.
16. R. Maki and S. Tonegawa, unpublished results.
17. R. Lenhard-Schuller, B. Hohn, C. Brack, M. Hirama, S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4709 (1978).
18. R. Wilson, J. Miller, U. Storb, *Biochemistry* **18**, 5013 (1979); P. Kennedy and S. Tonegawa, unpublished results.
19. The complementary DNA from MOPC315 containing VλII + CλII was a gift from R. Schwartz and M. Gefter.
20. H. Eisen, unpublished results.
21. C. J. Paige, P. W. Kincade, P. Ralph, in preparation.
22. G. M. Wahl, M. Stern, G. R. Stark, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3683 (1979).

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