Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 4045–4049, June 1985
Biochemistry

Alternative splicing patterns in an aberrantly rearranged immunoglobulin κ-light-chain gene

(plasmacytoma/fragment gene/cDNA clone/intervening sequences/RNA splice sites)

SANTOSH K. SIKDER*†, ELVIN A. KABAT*†‡§, AND SHERIE L. MORRISON*†

Departments of *Microbiology, ‡Human Genetics and Development, and §Neurology, and †The Cancer Center/Institute for Cancer Research, College of Physicians and Surgeons, Columbia University, New York, NY 10032

Contributed by Elvin A. Kabat, February 25, 1985

ABSTRACT **Using a 15-nucleotide primer specific for the immunoglobulin κ-chain gene, we synthesized cDNA from the mRNA of an anti-α(1→6)dextran hybridoma. The hybridoma had been produced using MPC-11 as the parental myeloma. Hybridization and sequence analysis of one clone showed that it was derived from a 1.2-kilobase (kb) κ-chain mRNA that lacked a joining minigene segment (*J*). The mRNA had the leader region correctly spliced to the variable region (*V*) but, in the absence of a *J*, V_κ was flanked by 62 nucleotides (3202–3263) from the intervening sequence (between *J5* and the κ-chain constant region gene C_κ) before being spliced to C_κ. This mRNA originated from the κ-chain-fragment gene of MPC-11 but differed from the previously described 0.8-kb κ-chain-fragment mRNA [Choi, E., Kuehl, W. M. & Wall, R. (1980) *Nature (London)* 286, 776–779; Seidman, J. G. & Leder, P. (1980) *Nature (London)* 286, 779–783] in which the leader sequence is spliced directly to C_κ. This 1.2-kb mRNA was present as a polyadenylylated species in total cellular RNA but could not be detected in cytoplasmic RNA. Thus, it either failed to be transported out of the nucleus or was rapidly degraded in the cytoplasm. These studies show that transcripts of the κ-chain-fragment gene are processed by two distinct splicing pathways to yield either a 0.8-kb mRNA with the leader region spliced directly to C_κ or a 1.2-kb mRNA with leader, *V*, 62 nucleotides of the intervening sequence, and C_κ.**

An immunoglobulin κ-chain structural gene is composed of four discrete germ-line genetic segments: a leader segment (*L*) encoding the NH_2-terminal (5′) end of the leader sequence, a variable segment (*V*) encoding four residues at the COOH-terminal end of the leader plus amino acid residues 1–95 of the mature κ light chain, a joining segment (*J*) encoding residues 96–107, and a constant region segment (C_κ) encoding residues 108–214 (1–3). Prior to expression, the *L* and *V* sequences are at an as yet undefined distance from the *J* and *C*. When κ-chain expression is initiated, one of the several hundred *V* genes recombines next to one of the four functional *J* genes (1–3). The active gene is in three separate coding segments: *L*, a *V-J* recombined gene, and a *C* gene. The introns, separating *L* from the *V-J* and the *V-J* from C_κ, are included in the primary transcript; they are removed by splicing so that in the mature cytoplasmic mRNA *L*, *V*, *J*, and *C* are contiguous (1–6).

There are two rearranged κ-chain genes in the mouse myeloma MPC-11 (7). One, with a normal *V-J* rearrangement, encodes the wild-type light chain found in the cell line. The second represents an aberrant rearrangement in which *V* has been joined to nucleotide 3202 of the intervening sequence (IVS) (8), eliminating all of the *J* with their donor splice sites. Presumably this aberrant rearrangement resulted from homologous recombination between the -C-A-C-A-G-T-G-A-T-A- sequence flanking the *V* and -C-A-C-A-G-T-G-A-T-A- within the IVS. An aberrant recombination event has been found to occur in several mouse myelomas at this position (8–10). Studies by others have shown that this aberrantly rearranged gene is transcribed; in the absence of the normal donor splice site associated with *J*, the *L* is spliced directly to C_κ (8, 9). This mRNA is translated into a κ C-fragment protein (7, 11, 12).

In the present studies, we demonstrate a second splicing pattern for the aberrantly rearranged κ-chain gene. By cloning and sequencing cDNA produced from the mRNA of a hybridoma made using MPC-11 as a parent, and by analyzing a variant of MPC-11 which transcribes only the aberrantly rearranged κ-chain gene, we show that an mRNA the size of that of a normal light chain transcribed from the aberrantly rearranged gene is also present. In this mRNA, *L* is correctly spliced to *V*; however, in the absence of a *J* sequence and splice site, nucleotide 3263 of the IVS is spliced to the normal acceptor in C_κ. This spliced mRNA is polyadenylylated. However, it cannot be detected as a mature cytoplasmic mRNA.

MATERIALS AND METHODS

Cell Lines and Tumors. Line 45.21.1 is an IgA(κ)-secreting hybridoma of C57BL/6JA mouse origin producing antibody specific for an α(1→6)-linked dextran (13). In addition to the specific light chain (spleen-chain derived), a nonspecific κ light chain and a fragment light chain are both produced by the myeloma fusion partner 45.6.TG.1.7.5 (derived from the parental plasmacytoma MPC-11) (14). NP.2 is a "nonproducing" variant cell line, isolated from MPC-11 cells by nitrosoguanidine mutagenesis, which produces only the fragment light chain (7). The cell lines were grown and maintained in Iscove's modified Dulbecco's medium (GIBCO) containing 10% fetal calf serum.

Enzymes and Reagents. Restriction endonucleases, DNA polymerase I and Klenow fragment, and T4 polynucleotide kinase were purchased from New England Biolabs and Bethesda Research Laboratories and were used according to the suppliers' recommendations. Terminal deoxynucleotidyltransferase was from P-L Biochemicals, calf intestinal alkaline phosphatase, and nuclease S1 were from Boehringer Mannheim. Avian myeloblastosis virus reverse transcriptase was from J. Beard (Life Sciences, St. Petersburg, FL). [α-^{32}P]dCTP (3000 Ci/mmol; 1 Ci = 37 GBq) was from Amersham and [γ-^{32}P]ATP (7000 Ci/mmol) from ICN.

General Methods. Total cellular RNA was isolated from cells by the guanidinium thiocyanate method (15), and cytoplasmic RNA was isolated by treating the cells with 0.5%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: L, leader segment; V, variable region; J, joining segment; IVS, intervening sequence; C, constant region; kb, kilobase(s); bp, base pair(s).

Nonidet P-40 and removing the nuclei by centrifugation as described (16). Poly(A)$^+$RNA was enriched by chromatography through an oligo(dT)-cellulose column (17).

Southern blot analysis was done as described (18), after DNA cleaved with restriction endonucleases was electrophoresed in a 1% agarose gel. Probes (specific activity 2–4 × 10^8 cpm/μg) were made by nick-translation (19) of specific DNA fragments or plasmids, using [α-^{32}P]dCTP and DNA polymerase I.

For RNA analysis, both total (10–15 μg) and poly(A)$^+$RNA (100–400 ng) were fractionated by electrophoresis in 0.8% agarose gel in 2.2 M formaldehyde/20 mM 3-(*N*-morpholino)propanesulfonic acid (Mops), pH 7.0/5 mM sodium acetate/1 mM EDTA (20). RNA was blotted onto nitrocellulose essentially as described by Thomas (21). Plasmid DNA was isolated by the alkaline quick-lysate method (22). Nuclease S1 analysis was done as described (23).

DNA Fragments Used as Probes. A mouse embryo κ-chain-specific clone with a 16-kilobase (kb) *Eco*RI fragment in pBR322 (24) was obtained from J. Seidman; 5.5 kb of it, containing J_κ and C_κ including the IVS, is shown in Fig. 1. The following fragments were isolated from this clone and used as probes: κJ-IVS, 2.7-kb *Hin*dIII fragment; κJ1-5, 1.7-kb *Hin*dIII–*Bgl* II fragment; κIVS, 1-kb *Bgl* II–*Hin*dIII fragment. In addition, a fragment was also used which corresponded only to the C of the κ light chain (Cκ).

Preparation of cDNA. A 15-nucleotide primer 3′ A-C-G-T-G-G-T-T-G-A-C-A-T-A-G 5′ from the 5′ end of C_κ (i.e., nucleotides 4626–4640; *C* starts at 4616) was synthesized with an Applied Biosystems 380A DNA Synthesizer (Foster City, CA), through the kindness of A. Efstratiadis and A. Murphy.

Using this primer, double-stranded cDNA was made (by a procedure supplied by Chien, Y., Crabtree, G., Lis, J. & Kemp, D.) by reverse transcription of 100 μg of total RNA (about 2 μg of poly(A)$^+$ RNA). RNA was denatured by heating at 105°C for 2 min in a saturated NaCl bath, and second-strand synthesis was with DNA polymerase I (Klenow fragment). The double-stranded cDNA was treated with nuclease S1 and size-fractionated on a Sepharose 4B column. The excluded fractions from 400–500 base pairs (bp) were combined, tailed with oligo(dC), and annealed to *Pst* I-cut, oligo(dG)-tailed pBR322 DNA. Transformation of *Escherichia coli* RR1 was as described by Kushner (25). Transformants were selected for their resistance to tetracycline, and recombinants were identified by their loss of ampicillin resistance. The plasmid DNA to be sequenced was purified from 1–2 liters of culture.

Sequence Analysis of cDNA. DNA fragments were excised from pBR322 and subcloned by insertion into the *Pst* I site of plasmid pUC8 (26). Both strands were sequenced by the chemical-modification procedures of Maxam and Gilbert (27). One portion (≈10 μg) of the recombinant plasmid, containing the fragment within the polylinker, was cut with *Hin*dIII and treated with phosphatase, and 5′ ends were labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. The linear labeled plasmid then was cut at the other end of the polylinker with *Eco*RI to give the fragment of interest labeled at one end; this was isolated by electrophoresis in a 5% polyacrylamide gel and used for sequencing in one direction. The other strand was sequenced in a similar way, except it was first cut with *Eco*RI, labeled, and then cut with *Hin*dIII. Since the κ-chain cDNA had an internal *Pst* I site, two fragments were cloned from pBR322 into pUC8.

RESULTS

Identification of a cDNA Clone Produced from the mRNA Transcribed from the Aberrantly Rearranged κ Gene. cDNA was made from total cellular RNA (isolated from anti-dextran hybridoma (45.21.1) with a 15-nucleotide primer corresponding to the 5′ end of C_κ. The clones were initially probed with κJ-IVS (2.7 kb), containing five *J*s and a flanking sequence from the intron between *J5* and *C* (Fig. 1). One colony that was positive by hybridization contained a cDNA insert of 470 bp, which on *Pst* I digestion gave two bands of 320 bp and 150 bp. Southern blot analysis showed that the 320-bp fragment did not hybridize with the κJ1-5 (1.7 kb) probe but did hybridize strongly with the κIVS probe (Fig. 1) (data not shown). The 150-bp fragment did not hybridize with either probe. Thus, the cloned cDNA did not contain a *J* sequence but did contain a portion of the IVS 3′ of *J5*.

DNA Sequence of the cDNA Clone. Fig. 2 gives the nucleotide sequence of the cDNA clone. The *L* and *V* sequences and the stretch of IVS correspond exactly to the sequence reported by Seidman and Leder (8) for the aberrantly rearranged κ-chain gene of MPC-11; we have identified the few nucleotides that they did not determine; and we find nucleotide 108 to be adenylate, not guanylate as they reported. In addition, our 5′ untranslated region differs from the sequence they propose for the region 5′ of the gene, but their sequence contains many undetermined residues. Most significantly, our cDNA is quite different from the structure they proposed for the mature mRNA transcribed from this gene. In the absence of a *J*, they proposed that *L* is spliced directly to C_κ and that splicing between *L* and *V* does not occur. Our cDNA clone was made from an mRNA in which *L* was spliced to *V*; in the absence of a *J*, C_κ is spliced to nucleotide 3263 within the IVS. The 62 IVS nucleotides contain an in-frame stop codon.

Analysis of RNA. Our cDNA was made by using total cellular RNA and would, therefore, contain both cytoplasmic and nuclear RNA. To determine whether this aberrant spliced transcript was present in the cytoplasm and whether it was synthesized by NP.2 cells, which transcribe only the aberrantly rearranged gene, we fractionated total RNA from the anti-dextran hybridoma 45.21.1 and from NP.2 and cytoplasmic RNA from NP.2 in agarose gels and transferred the fractionated RNA to nitrocellulose filters. When the fractionated RNA was hybridized to a C_κ-specific probe, bands of 0.8 and 1.2 kb were seen in 45.21.1 and NP.2 total RNA (Fig. 3 *A* and *B*, lanes 1); however, cytoplasmic RNA from NP.2 yielded only a 0.8-kb hydridizing band (Fig. 3*C*, lane 1). When the *Bgl* II-*Hin*dIII IVS fragment (κIVS) was

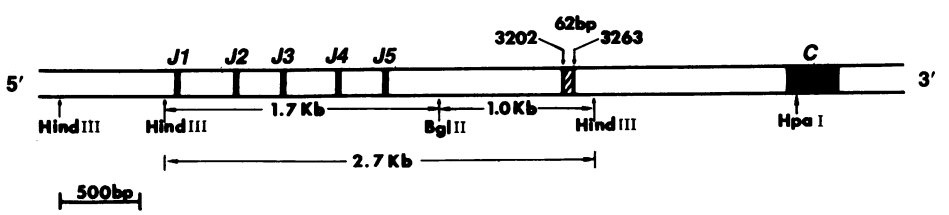

FIG. 1. A 5.5-kb DNA segment of a mouse embryo κ-chain-specific clone with a 16-kb *Eco*RI fragment in pBR322. This segment contains J_κ and C_κ including the IVS. Probes used were the 2.7-kb *Hin*dIII fragment (κJ-IVS), the 1.7-kb *Hin*dIII–*Bgl* II fragment (κJ1-5), and the 1.0-kb *Bgl* II–*Hin*dIII fragment (κIVS). The hatched region shows nucleotides 3202–3263 (62 bp) within the IVS (see ref. 24).

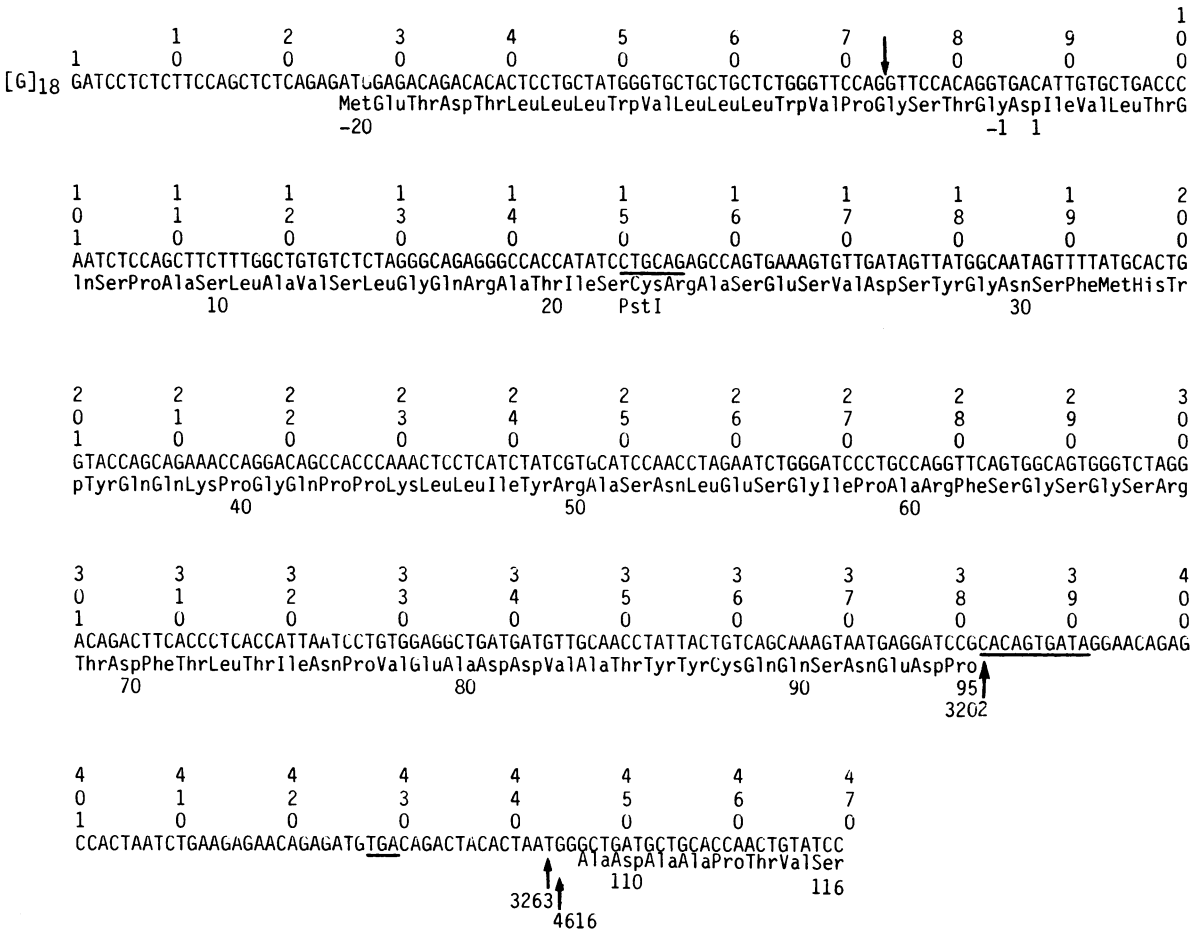

FIG. 2. Nucleotide sequence of the cDNA insert (clone 8, 470 bp). The arrow (↓) indicates the *L→V* splicing site. The *Pst* I site, the proposed site of recombination (-C-A-C-A-G-T-G-A-T-A-), and an in-frame stop codon within the IVS are underlined. IVS nucleotides 3202–3263 are indicated (germ-line numbering from ref. 24). These 62 nucleotides agree with the published sequence 3′ of the aberrantly rearranged V_κ in MPC-11 (8). The aberrantly rearranged κ-chain gene has been shown to have the same leader and variable regions as the productively rearranged light chain gene of MOPC-321 (28).

used for hybridization, 45.21.1 and NP.2 total RNA showed a band at 1.2 kb as well as some larger transcripts (Fig. 3 *A* and *B*, lanes 2). Cytoplasmic RNA from NP.2 showed no hybridization with this probe (Fig. 3*C*, lane 2). When the

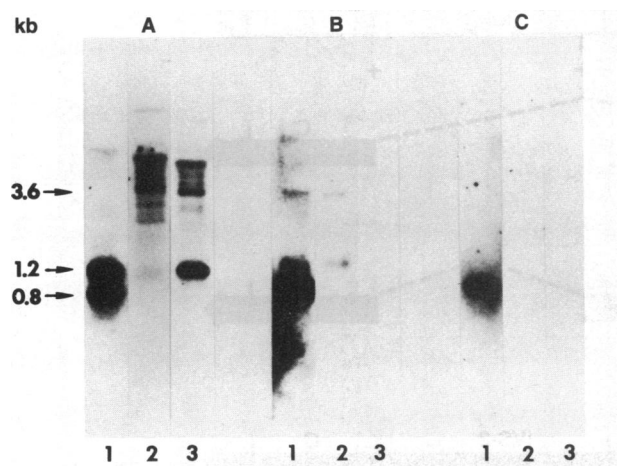

FIG. 3. Analysis of total cellular RNA from 45.21.1 cells (*A*), total cellular RNA from NP.2 cells (*B*), and cytoplasmic RNA from NP.2 cells (*C*). Lanes 1–3 in each panel show the hybridization patterns with probes C_κ, κIVS, and κJ1-5, respectively. Probe C_κ corresponds to a *C* fragment of κ light chain. For details of probe κIVS and κJ1-5, see Fig. 1.

κJ1-5 probe was used, 45.21.1 RNA showed a strongly hybridizing band of 1.2 kb, with additional bands of about 3.5, 5.6, and 6.5 kb (Fig. 3*A*, lane 3); no bands were seen in either NP.2 total or cytoplasmic RNA (Fig. 3 *B* and *C*, lanes 3). Taken together, these data show that 45.21.1 total RNA contains a 1.2-kb mRNA that hybridizes to a fragment from the IVS; this RNA corresponds to our cDNA. Total RNA from NP.2 also contains the 1.2-kb species. Cytoplasmic RNA from NP.2 contains only the 0.8-kb, light chain fragment-related species previously described. Since the 1.2-kb RNA species was not present in the cytoplasm of NP.2 cells but was detected only in total RNA, it must be found only in the nucleus. It is not clear why Choi *et al.* (9) failed to detect this species when they analyzed nuclear RNA from NP.2, except that their method of RNA isolation differed from ours.

To explore the question of whether this RNA transcript was polyadenylylated, poly(A)$^+$RNA was separated on an oligo(dT)-cellulose column and the poly(A)$^+$ and poly(A)$^-$ fractions were analyzed, along with total RNA from NP.2 and 45.21.1 cells in parallel lanes (Fig. 4, lanes 1–4), by hybridization with our cDNA clone (clone 8). The poly(A)$^+$ fraction (lane 3) contained two species of RNA, of 1.2 and 0.8 kb, in addition to a 3.6-kb band; the poly(A)$^-$ fraction (lane 4) did not show any of these bands. The cytoplasmic RNA from NP.2 gave only a 0.8-kb band (lane 5). When the 150-bp *Pst* I fragment from clone 8, containing 5′ untranslated region, *L*, and sequence encoding amino acids 1–23 (Fig. 2) was used as the probe, poly(A)$^+$ RNA from NP.2 showed both the 1.2- and 0.8-kb bands (data not shown). Nuclease S1 analysis was

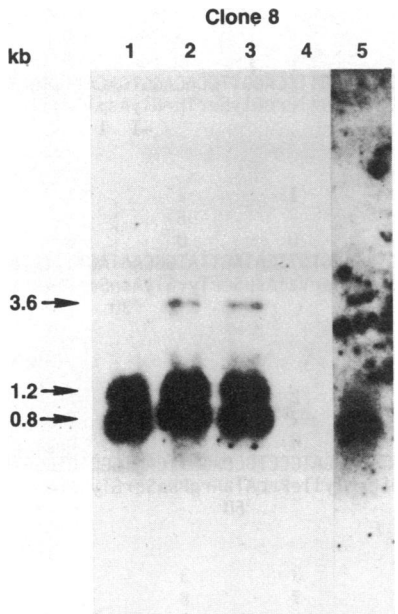

FIG. 4. Analysis of RNA with cDNA (clone 8) as a probe. Lanes: 1, total cellular RNA from 45.21.1 cells; 2, total RNA from NP.2 cells; 3, poly(A)$^+$ RNA fraction from NP.2 total RNA; 4, poly(A)$^-$ RNA fraction from NP.2 total RNA; and 5, cytoplasmic RNA from NP.2 cells.

also done with DNA from a genomic clone of C_κ cut at the *Hpa* I site in C_κ (Fig. 1) and labeled with T4 DNA polymerase. Total RNA from 45.21.1 and NP.2, as well as NP.2 cytoplasmic RNA, protected a fragment of the same size, indicating that all κ mRNA in the cells has the same 3′ terminus (data not shown). Taken together, these data established that in the MPC-11 derived cell line NP.2 (as well as in hybridomas made using MPC-11 as a parent), the aberrantly rearranged κ-chain gene gives rise both to a 0.8-kb RNA produced by $L \rightarrow C$ splicing and to a 1.2-kb transcript in which L is spliced to V and C_κ is spliced to residue 3263 of the IVS. However, this 1.2-kb transcript was not seen in the cytoplasmic mRNA but could be detected only when total cellular RNA was analyzed.

DISCUSSION

This study of the aberrantly rearranged κ-chain gene of the MPC-11 myeloma cell line derives from an observation made while analyzing cDNA synthesized from an antidextran hybridoma (13) obtained using a myeloma line derived from MPC-11. During screening of the cDNA with the κJ-IVS (2.7-kb) probe, a clone was identified that had an insert of 470 bp. This clone, clone 8, did not hybridize with the κJ1-5 probe but hybridized strongly with the κIVS probe, a 1.0-kb *Bgl* II–*Hin*dIII fragment from the intron between *J5* and *C* (Fig. 1).

Previous studies have shown that two independent κ-chain genes are rearranged in mouse myeloma MPC-11 (8, 9). One encodes the normal light chain. The other is an aberrant rearrangement in which *V* has been joined to nucleotide 3202 of the IVS, deleting all of the *J*s but leaving the rest of the IVS and C_κ intact.

Nucleotide sequencing of the cDNA insert in clone 8 (Fig. 2) shows it to have the *L* and *V* sequences of the aberrantly rearranged κ-chain gene of MPC-11 but with *L* and *V* spliced as in a normal light chain. However, because *V* lacks a *J* with its splice site, C_κ is spliced to nucleotide 3263 of the IVS. Thus, in this mRNA, *V* is separated from *C* by 62 nucleotides of the IVS (nucleotides 3202–3263).

In the initial description of the aberrantly rearranged κ-chain gene, it was suggested that transcripts from that gene might be processed in two different ways (Fig. 5, modes *a* and *c*). In these initial studies, only mRNA that had been spliced using pathway *a* was identified. This mRNA encodes the C_κ-fragment protein which is observed in MPC-11 cells (7, 11, 12). Our findings show an alternative splicing pattern (Fig. 5, mode b) which generates an mRNA corresponding to our cDNA. In this case, *L* is spliced to *V* through the normal consensus sequence 5′··· G/**G-T**···intron···**A-G**/G···3′ (slashes indicate the endpoints of the intron). The joining of the IVS (nucleotide 3263) to C_κ (nucleotide 4616) uses the sequence 5′···T/**G-T**···intron···**A-G**/G···3′. Several examples of this splice site have been reported (29–32).

The question arises as to why a transcript corresponding to our cDNA was not found in the cytoplasm. It is possible that it represents an intermediate in splicing and that *V*–IVS will be removed in a second step. However, this seems unlikely

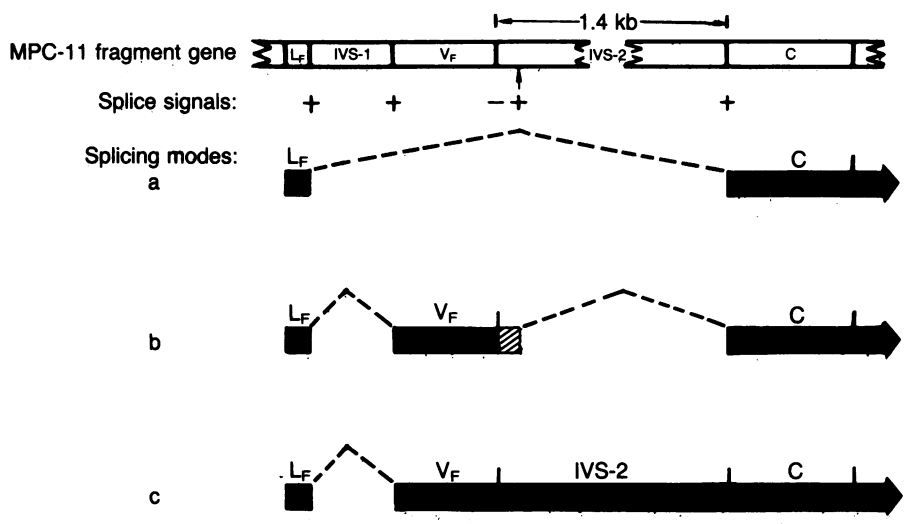

FIG. 5. Three possible splicing modes for RNA transcripts of the MPC-11 κ-chain-fragment gene. Mode a generates a 0.8-kb mRNA; mode b, a 1.2-kb mRNA corresponding to our cDNA; and mode c, a theoretically larger mRNA containing the intact 1.4-kb IVS. Sequences that have been shown to be used to generate spliced transcripts are indicated by +; the splice junction that is missing because of the aberrant recombination is indicated by −. An arrow shows nucleotide position 3263 within the IVS-2. The hatched region (mode b) corresponds to 62 IVS nucleotides (3202–3263) (see legends to Figs. 1 and 2).

since the splice signals would be 5'···G/G-T···A-T/G···3' and there are no examples of A-T/G as a splice acceptor (29).

Polyadenylylation and splicing are reported to be two of the processing steps important for the formation of mature eukaryotic mRNAs, which are transported to the cytoplasm (33, 34). The mRNA product corresponding to our cDNA (splicing mode b, Fig. 5) was found to accumulate in the nucleus and could not be identified in the cytoplasm, although it is polyadenylylated and spliced. However, the mRNA contains a small fragment of IVS between *V* and C as a consequence of the unusual splicing pattern. It is possible that these sequences from the IVS prevent transport to the cytoplasm. Alternatively, the mRNA might be transported to the cytoplasm but rapidly degraded because of the IVS. Further experiments are required to distinguish between these possibilities.

E.A.K. thanks Mark Davis for teaching him cDNA cloning in his laboratory at the National Institutes of Health and David Givol for teaching him sequencing at the Weizmann Institute. We wish to thank Saul Silverstein and Fred Alt for helpful comments on the manuscript and Ron DePinho for assistance with sequencing. This work was supported by the National Science Foundation (Grant PCM-81-02321 to E.A.K.), the National Institutes of Health (Grant 1R01 AI19042 to E.A.K., Grants CA16858 and CA22736 to S.L.M., and Cancer Support Grant CA13696 to Columbia University), and the American Cancer Society (Grant IMS-360 to S.L.M.). S.L.M. is the recipient of Research Career Development Award AI00408 from the National Institutes of Health.

1. Max, E. E., Seidman, J. G. & Leder, P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3450–3454.
2. Sakano, H., Huppi, K., Heinrich, G. & Tonegawa, S. (1979) *Nature (London)* **280,** 288–294.
3. Adams, J. M. (1980) *Immunol. Today* **1,** 10–17.
4. Seidman, J. G. & Leder, P. (1978) *Nature (London)* **276,** 790–795.
5. Brack, G., Hirama, M., Lenhard-Schuller, R. & Tonegawa, S. (1978) *Cell* **15,** 1–14.
6. Seidman, J. G., Max, E. E. & Leder, P. (1979) *Nature (London)* **280,** 370–375.
7. Kuehl, W. M. & Scharff, M. D. (1974) *J. Mol. Biol.* **89,** 409–421.
8. Seidman, J. G. & Leder, P. (1980) *Nature (London)* **286,** 779–783.
9. Choi, E., Kuehl, W. M. & Wall, R. (1980) *Nature (London)* **286,** 776–779.
10. Perry, R. P., Kelley, D. E., Coleclough, C., Seidman, J. G., Leder, P., Tonegawa, S., Matthyssens, G. & Weigert, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1937–1941.
11. Rose, S. M., Kuehl, W. M. & Smith, G. P. (1977) *Cell* **12,** 453–462.
12. Burstein, Y. & Schechter, I. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 716–720.
13. Sharon, J., Kabat, E. A. & Morrison, S. L. (1981) *Mol. Immunol.* **18,** 831–846.
14. Laskov, R. & Scharff, M. D. (1970) *J. Exp. Med.* **131,** 515–541.
15. Chirgwin, J. M., Przybyla, A. E., McDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
16. Matsuuchi, L. & Morrison, S. L. (1982) *Mol. Cell. Biol.* **2,** 1134–1144.
17. Aviv, M. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
18. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
19. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
20. Goldberg, D. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5794–5798.
21. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
22. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7,** 1513–1523.
23. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–732.
24. Max, E. E., Maizel, J. V., Jr., & Leder, P. (1981) *J. Biol. Chem.* **256,** 5116–5120.
25. Kushner, S. R. (1978) in *Genetic Engineering*, eds. Boyer, H. B. & Nicosia, S. (Elsevier/North-Holland, Amsterdam), p. 17.
26. Vieira, J. & Messing, J. (1982) *Gene* **19,** 259–268.
27. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
28. Zeelon, E. P., Bothwell, A. L. M., Kantor, F. & Schechter, I. (1981) *Nucleic Acids Res.* **15,** 3809–3820.
29. Mount, S. M. (1982) *Nucleic Acids Res.* **10,** 459–472.
30. Sun, S., Slightom, J. & Hall, T. C. (1981) *Nature (London)* **289,** 37–41.
31. Yamada, Y., Avvedimento, V. E., Mudryi, M., Ohkubo, H., Vogeli, G., Meher, I., Pastan, I. & de Crombrugghe, B. (1980) *Cell* **22,** 887–892.
32. Perricaudet, M., Le Moullec, J.-M., Tiollas, P. & Petterson, U. (1980) *Nature (London)* **288,** 174–176.
33. Nevins, J. R. (1983) *Annu. Rev. Biochem.* **52,** 441–466.
34. Wickens, M. & Stephenson, P. (1984) *Science* **226,** 1045–1051.