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Intervening sequence of DNA identified in the structural portion of a mouse β-globin gene

(cloning/R-loop mapping/restriction endonuclease mapping/DNA sequencing)

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ABSTRACT **The unusual electron microscopic appearance of a hybrid formed between 9S mouse β-globin mRNA and its corresponding cloned gene segment is caused by at least one, and possibly two, intervening sequences of DNA that interrupt the mouse β-globin gene. Such an interpretation is consistent with a paradoxical restriction site pattern previously noted in this gene and with the nucleotide sequence of that portion of the gene that spans both structural and intervening sequences. The large intervening sequence, approximately 550 base pairs in length, occurs in the structural globin sequence and immediately follows the β-globin codon corresponding to amino acid 104. A smaller, putative intervening sequence is located close to the 5′ end of the β-globin-coding sequence but may reside beyond its initiation codon. The β-globin gene thus appears to be encoded in two, and possibly three, discontinuous segments.**

We have recently cloned a segment of mouse DNA containing a β-globin gene and its surrounding nucleotide sequences (1). When hybridized to 9S globin mRNA and visualized in the electron microscope, this segment of DNA has an unusual appearance which, together with a paradoxical restriction site pattern, can best be explained by assuming that a large interruption occurs within the globin coding sequence (1, 2). Because the notion that structural genetic information is encoded in a continuous linear fashion seemed most straightforward and reasonable, this interpretation required circumspection. This is so despite the important discovery that some—but not all—reiterated 28S ribosomal genes of *Drosophila melanogaster* contain internal sequences not present in mature 28S ribosomal RNA (3–6) and the more recent and equally important discovery that the 5′ or "leader" segments of adenovirus and simian virus 40 (SV40) mRNAs are encoded at sites remote from the structural viral genes (7–12).

In this paper we present electron microscopic and restriction endonuclease analyses that indicate that the mouse β-globin gene is interrupted within its amino acid coding sequence by an ~550-base-pair segment of DNA whose sequence does not appear in mature 9S globin mRNA. To reach this conclusion, we have determined the nucleotide sequence of a portion of the globin gene and the adjacent intervening sequence.* In addition, using the electron microscope, we have observed a second, smaller, looped-out structure close to the 5′ end of the globin coding sequence, suggesting the presence of a second intervening sequence and the possibility that the β-globin gene is encoded in *three* discontinuous segments of DNA. The fact that structural genes may be encoded in discontinuous segments of DNA provides a new perspective from which to consider such questions as gene regulation and mRNA biosynthesis as well as processes that might be involved in generating diversity in multigene families such as the immune and histocompatibility systems.

MATERIALS AND METHODS

Preparation of DNA. The plasmid pMB9·βG2, which contains a segment of mouse DNA originally cloned from the plasmacytoma MOPC-149, was constructed *in vitro* from recombinant phage λgt*WES*·βG2 DNA (1) and pMB9 DNA. *Eco*RI fragments of λgt*WES*·βG2 DNA and pMB9 were ligated in the presence of 50 μM ATP and T4 DNA ligase at 14.5° for 36 hr and used to transform the Ek2 *Escherichia coli* strain, χ1776. Transformed colonies were selected on LB agar plates in the presence of diaminopimelic acid (100 μg/ml), thymidine (40 μg/ml), and tetracycline (12.5 μg/ml). Those containing the 7.0-kilobase (kb) mouse genomic DNA fragment were identified by the Grunstein and Hogness procedure (13) with ^{32}P-labeled mouse globulin cDNA as a hybridization probe.

Colonies of χ1776 [pMB9·βG2] were grown in brain heart infusion medium supplemented with diaminopimelic acid and thymidine to an OD_{590} of 1.2, and the plasmid content was amplified by growth for 18 hr in the presence of chloramphenicol at 15 μg/ml. The cells were lysed by the procedure of Meagher *et al.* (14), without treatment with RNase A. Ribosomal RNA was removed by extraction of the nucleic acid ethanol precipitate in 1 M NaCl/0.05 M Tris·HCl, pH 8.0/10 mM EDTA, followed by chromatography on a Sepharose 2B column in the same buffer.

The 7.0-kb mouse genomic DNA *Eco*RI fragment was purified from λgt*WES*·βG2 or pMB9·βG2 by using the electroelution agarose gel electrophoresis apparatus of Polsky *et al.* (15). The DNA was recovered from electrophoresis buffer by ethanol precipitation.

Restriction Endonuclease Mapping. Restriction endonuclease mapping of pMB9·βG2 DNA and pMB9 DNA was performed by using 0.5–1.0 μg of DNA under standard conditions for *Bam*HI (16), *Hin*dIII (17), *Hin*cII (18), and *Pst* I (19). The fragments were analyzed on 1–2% discontinuous agarose gels (20) and bands were visualized by ethidium bromide staining. The DNA bands were transferred to Millipore filters by Southern's technique (21) and hybridized to ^{32}P-labeled mouse globin cDNA as described (1).

Electron Microscopy. The formation of R loops (4, 22) between the 7.0-kb βG2 DNA fragment and mouse globin mRNA was performed with 50 μg of DNA and 50 μg of RNA per ml in 70% formamide/0.1 M *N*-[tris(hydroxymethyl)methyl]-

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Abbreviations: SV40, simian virus 40; kb, kilobase.

* We refer to this type of sequence as an "intervening sequence" to distinguish it from insertion sequences which have specific biological and structural properties not yet demonstrated for intervening sequences.

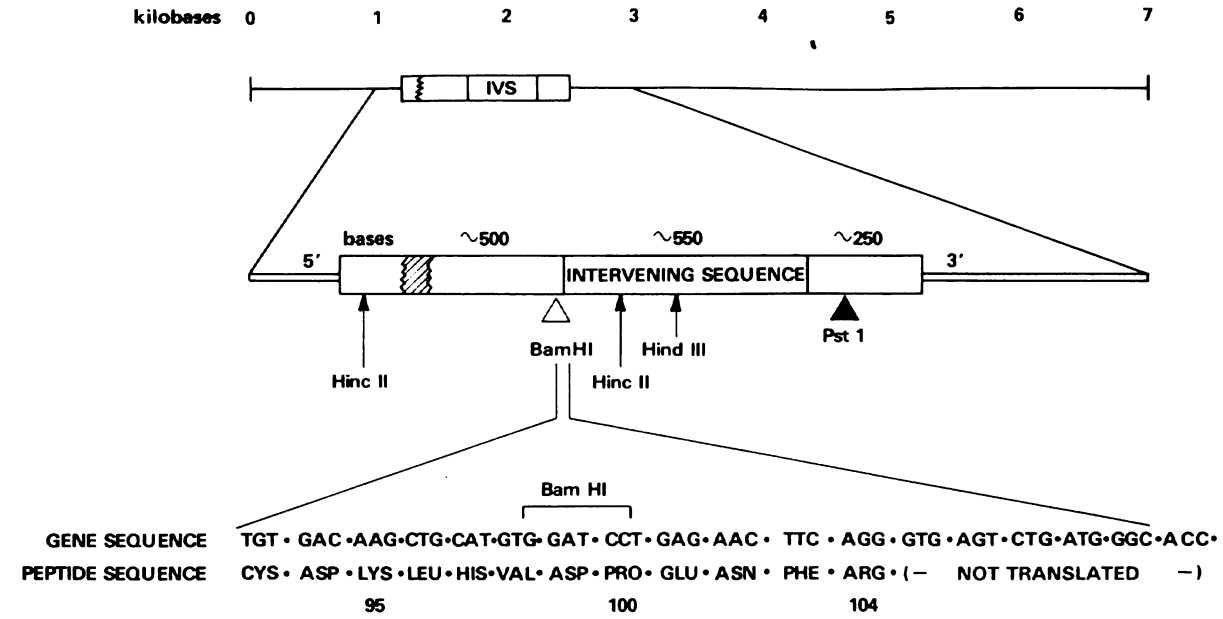

FIG. 1. Restriction endonuclease map and nucleotide sequence of βG2 DNA. The top line represents the position of the β-globin gene within the cloned 7.0-kb mouse DNA fragment, drawn with the sequence corresponding to the 5′ end of globin mRNA at the left. The entire gene is indicated by the boxes, with the 550-base-pair intervening sequence labeled "IVS." The approximate location of the smaller 5′ interruption is drawn as a hatched box. The middle line represents an enlargement of the hybridizing sequences in βG2. The restriction sites are as indicated. The third line is the nucleotide sequence of the anti-coding strand in the region immediately adjacent to the *Bam*HI site of βG2. The amino acid assignments for this region are drawn below. Maps were constructed from gel analyses followed by *in situ* hybridization to globin [^{32}P]-cDNA.

glycine (tricine)·NaOH (pH 8.0)/0.5 M NaCl/10 mM EDTA at 52° for 16 hr. The sample was diluted with 20–50 volumes of the same solution and spread onto a hypophase of H_2O. The DNA was picked onto parlodion-coated grids, stained with uranyl acetate, and shadowed with platinum/palladium and carbon. The grids were viewed with a Philips 300 electron microscope.

SV40 DNA containing poly(T) "tails" was prepared by treating supercoiled DNA with DNase (0.5 ng/ml) in 50 mM Tris·HCl, pH 8.0/5 mM EDTA/30 mM $MgCl_2$ at 37° for 10 min to introduce single-stranded nicks (23). After phenol extraction and ethanol precipitation, the form II SV40 DNA was incubated at 0.5 mg/ml in 0.1 M KH_2PO_4/50 mM cacodylic acid, pH 7.0/1 mM 2-mercaptoethanol/1 mM $CoCl_2$/3 mM dTTP/ and terminal deoxynucleotidyl transferase (500 units/ml) for 2 hr at 37° (24). R loops between βG2 DNA and globin mRNA were formed as before except that the DNA concentration was 10 μg/ml and the RNA concentration was 3.5 μg/ml. After 16 hr, SV40 DNA–poly(T) was added to a final concentration of 5 μg/ml and the mixture was incubated at 52° for several hours before being spread as described above.

DNA Sequence Analysis. pMB9-βG2 DNA (100 μg) was cleaved with *Bam*HI, treated with bacterial alkaline phosphatase (25), and labeled at the 5′ ends with polynucleotide kinase (New England Biolabs) in the presence of [γ-^{32}P]ATP (25). The DNA was cleaved with *Hin*dIII, and the 250- and 800-base-pair *Bam*/*Hin*dIII fragments were isolated on 2% agarose gels. The fragments were chemically modified and analyzed as described by Maxam and Gilbert (25).

RESULTS

Paradoxical Restriction Sites in βG2. Our earliest restriction endonuclease analyses of the cloned mouse β-globin gene indicated that two enzymes, *Hin*dIII and *Sst* I, both of which *failed* to cleave a cloned reverse transcript of β-globin mRNA (26), *readily cleaved* the structural sequence cloned from genomic DNA (1). In order to simplify further restriction analyses and eventual nucleotide sequence determination, the cloned mouse *Eco*RI fragment, βG2, was transferred from the cloning vector, λgt*WES*·βG2, to the *Eco*R1 site of the smaller plasmid, pMB9. In this vector several additional restriction sites were located (relative positions are indicated in Fig. 1). Two of these, the *Pst* I site at 2.32 kb and the *Hin*cII site at 1.26 kb, cleaved within the region that hybridized to globin mRNA, yet they were approximately 1.1 kb apart, *or almost twice the length of globin mRNA*. The *Bam*HI site, corresponding to amino acids in positions 98–100 in mouse β-globin, was 620 base pairs away from a *Pst* I site which should correspond to one of two adjacent possible sites at positions 124–127 and should, therefore, have been located only 75 base pairs away. Although several trivial models could have explained these unexpected results, the possibility that the globin gene was interrupted by an intervening sequence of DNA seemed most likely in view of electron microscopic analyses that were being carried out simultaneously (2).

Electron Microscopic Appearance of β-Globin Gene: β-Globin mRNA Hybrids. The powerful R-loop technique (4, 22), by which a DNA duplex is partially denatured to allow a complementary RNA strand (in this case, globin mRNA) to hybridize and displace a single-stranded region of DNA, permitted us to visualize the mouse globin gene directly (Fig. 2). Surprisingly, instead of a single continuous R loop within the isolated 7-kb DNA fragment, *two* R loop structures were visualized, virtually adjacent to one another but separated by a looped-out, double-stranded region for which there was no displaced single-stranded molecule. The interpretation of such a structure, as illustrated in the line drawing (Fig. 2 *inset*), requires that single-stranded globin mRNA hybridize to two *discontinuous* regions of the genomic fragment. Support for this conclusion was drawn from single-stranded DNA·RNA hybrids in which the double-stranded region is interrupted by a looped-out segment of single-stranded DNA (data not shown).

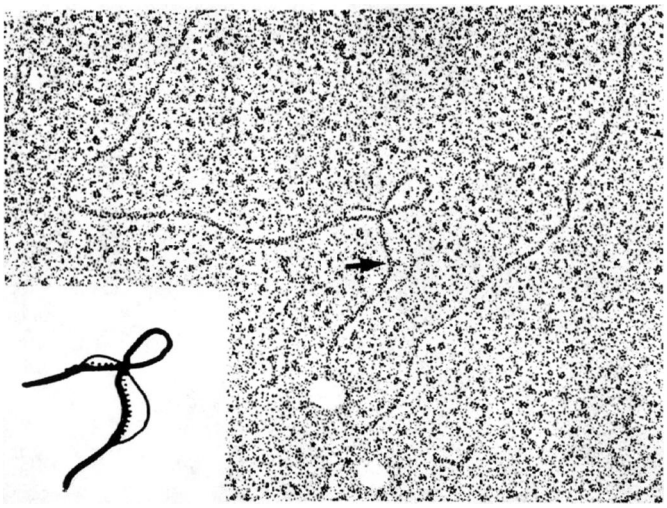

FIG. 2. Electron micrograph of an R loop between gtWES·βG2 and globin mRNA. λgt*WES*·βG2 DNA and globin mRNA were incubated to form R loops. (*Inset*) Drawing of the structure, with the heavy lines representing double-stranded DNA, the narrow lines representing single-stranded DNA, and the dotted line-heavy lines indicating the DNA-mRNA hybrid. (×78,400.)

Mapping the Discontinuous Globin Gene Segments. A series of R loops formed between the βG2 7.0-kb fragment and globin mRNA were measured (Fig. 3). The two loops were unequal in size. The mean (±SD) length of the loops, with the 7.0 kb fragment as a standard, was 143 ± 47 base pair for the smaller, right-hand loop and 278 ± 80 for the left-hand loop. These measurements are underestimates, probably as the result of strand migration at the ends of the loops. [White and Hogness (4) have also noted that R-loop measurements underestimate the size of ribosomal RNA coding sequences.] The intervening nonhybridizing DNA was estimated by this procedure to be 588 ± 88 base pairs long.

The orientation of β-globin mRNA within the two loops was determined by using an electron microscopic procedure devised by Bender and Davidson (24). R loops were hybridized to SV40 DNA to which poly(T) oligomers or "tails" had been attached at single-strand nicks. The poly(T) tails identify the 3′ poly(A) ends of the globin mRNA; a typical molecule is shown in Fig. 4. In each of the 10 molecules observed, SV40 DNA–poly(T) hybridized at the right-hand end of the globin sequence, yielding the orientation shown in Fig. 1. Therefore, the 5′ end of the globin mRNA structural sequence is preceded by approximately 1300 base pairs of DNA in this fragment.

The position of the intervening sequence relative to the restriction endonuclease sites shown in Fig. 1 was confirmed by forming R loops between globin mRNA and *Bam*HI, *Hin*dIII, or *Pst* I fragments of pMB9·βG2 DNA. When isolated *Bam*HI fragments were examined for the presence of R-loop structures, only the 3′ smaller loop was observed, and it was held together by approximately 625 base pair of nonhybridizing DNA (Table 1). This result implies that the *Bam*HI site at 1.7 kb (Fig. 1) is very close to, or within, the 5′ R loop. Cleavage with *Hin*dIII followed by R-loop formation yielded two separate R-loops. The 3′ loop was adjacent to 380 base pair of DNA and the 5′ loop, to 235 base pair of DNA. These measurements clearly place the *Hin*dIII site within the intervening sequence.

The *Pst* I site at 2.32 kb was mapped by this method as being immediately adjacent to or within the 3′ loop; this R loop was not observed in a *Pst* I digest. Instead, the 5′ loop was adjacent to 550 base pairs of nonhybridizing DNA (Table 1). In order to determine whether the *Pst* I site occurred within the 3′ loop,

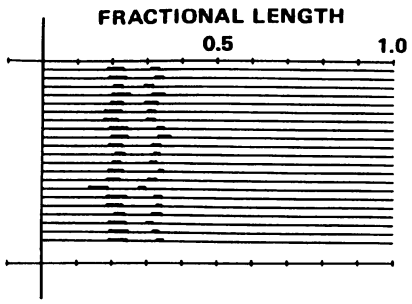

FIG. 3. Distribution of R loops in βG2 DNA. R loops between the 7.0-kb βG2 DNA and globin mRNA were measured by using a Numonics digitizer, and the size and position of the two loops were plotted on a scale of 0 to 1, with one representing the full-length 7.0-kb fragment. The R loops are indicated by the double lines.

pMB9·βG2 DNA was cleaved with a combination of *Pst* I and *Hin*dIII. The 0.37-kb fragment generated between 1.95 and 2.32 kb hybridized to globin cDNA, indicating that the *Pst* I site must cleave within the coding sequence itself (data not shown).

Base Sequence Determination of the Region Spanning the Globin Gene and the Large Intervening Sequence. Our interpretation of the R-loop and restriction site analyses could be confirmed directly by determining the nucleotide sequence of a region of the gene spanning the coding and intervening sequences. A single *Bam*HI site would be expected to correspond to mouse β-globin amino acids 98–100 [such a site has been found in a cloned rabbit β-globin mRNA sequence (27)]. This *Bam*HI site should be very close to the sequence border. By starting at this site and using the Maxam and Gilbert procedure (25), a sequence of approximately 20 base pairs was determined in both the coding and intervening sequences directions (Fig. 5, and summarized in Fig. 1). The DNA coding sequence determined agrees exactly with the known amino acid sequence from positions 93 to 104 of mouse β-globin major and minor chains. However, at positions 105–110, which in β-globin correspond to the sequence Leu-Leu-Gly-Asn-Met-Ile, a nonglobin sequence, Val-Ser-Leu-Met-Gly-Thr, is encountered. No simple substitution, addition, or deletion within these 18 nucleotides can generate a correct β-globin amino acid sequence. This result establishes the existence of the intervening sequence precisely following the codon for amino acid 104. The remaining 40 amino acids must, therefore, be encoded 550 bases away.

A Second Intervening Sequence? During the measurement of the 5′ R loops generated in *Hin*dIII and *Pst* I digests, it was noted that the single-stranded DNA side of the loop was approximately 25% longer than the double-stranded DNA·RNA side. This discrepancy was greater than that expected for the

Table 1. Length of R loops formed between globin mRNA and restriction fragments of pM9·βG2 DNA

Restriction enzyme	5′ loop	Intervening sequence		3′ loop
*Eco*RI	278 ± 8	588 ± 88		143 ± 47
*Bam*HI	Not seen	624 ± 70		247 ± 56
*Hin*dIII	234 ± 55	236 ± 20	619	—
	—	383 ± 33		197 ± 33
Pst I	322 ± 95	550 ± 75		Not seen

R loops were formed between globin mRNA and restriction fragments of pMB9·βG2 DNA as indicated. The lengths (base pairs) of the R loops and the intervening sequences were determined on 25–50 molecules with SV40 DNA as an internal standard. Results are expressed as mean ± SD.

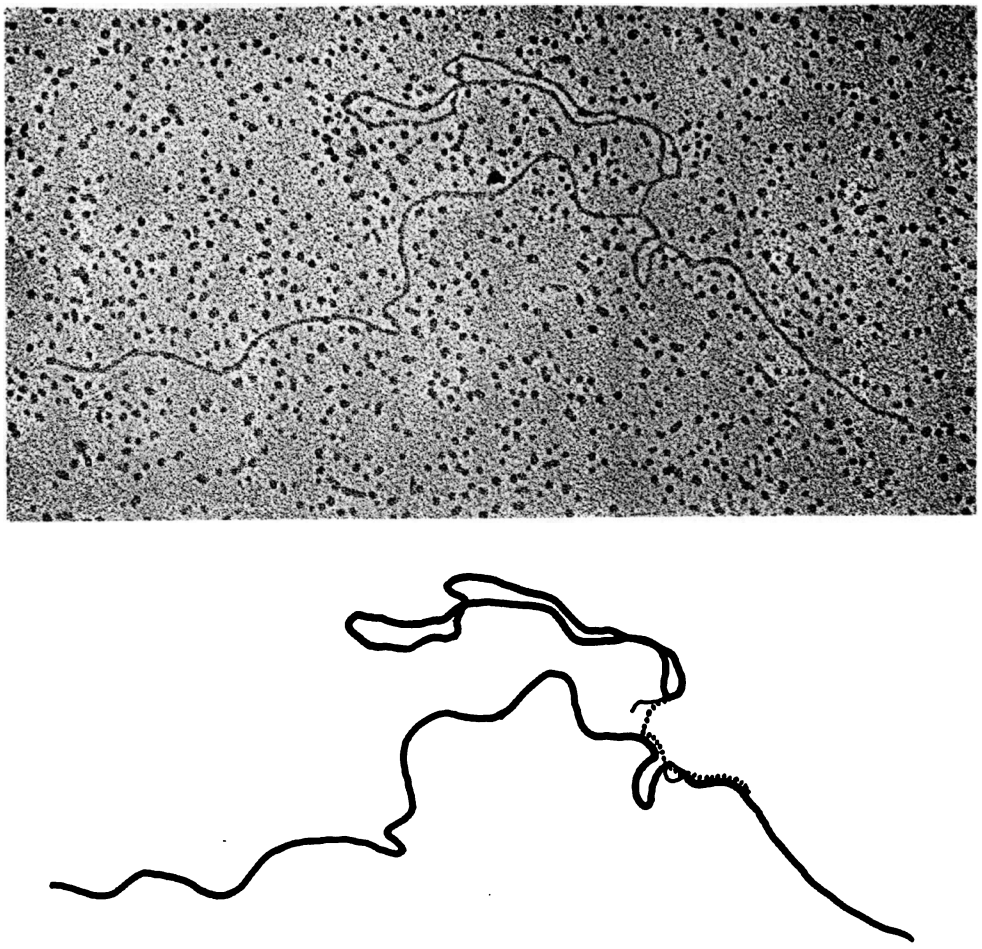

FIG. 4. Orientation of globin mRNA within the R-loop structure. R loops between βG2 DNA (the linear molecule) and globin mRNA were incubated in the presence of SV40 DNA containing poly(T) tails (the circular molecule). The drawing illustrates the molecule, with heavy lines representing double-stranded DNA, narrow lines representing single-stranded DNA, and the dotted line representing globin mRNA. (×71,200.)

difference in measurements between single- and double-stranded DNA. At the same time, a small knot or loop (shown by the arrow in Fig. 2) was observed in each of more than 40 molecules examined. This second, smaller structure occurs very close to what must be the initial globin codons in βG2 DNA and might represent an additional intervening sequence.

DISCUSSION

Intervening Sequences: A General Feature of Globin (and Other) Genes? The possibility that the intervening sequence we observe is a consequence of the cloning procedure appears unlikely because we found characteristic R-loop structures and restriction sites in each of the three independently isolated nonsibling clones of βG2 that we obtained. The further possibility that this structure arises during passage of plasmacytoma cells (from which the DNA was originally obtained) is also unlikely in view of the fact that we have cloned a second, nonallelic β-globin gene and found that it displays the same R-loop structure observed in βG2 (unpublished data). Further evidence suggesting the generality of this structure comes from simultaneous work by Jeffreys and Flavell (28) who, using *in situ* hybridization and restriction site analyses, demonstrated the presence of an intervening sequence in the β-globin gene from several normal rabbit tissues including erythropoietic spleen, a tissue that is actively synthesizing globin. This latter observation suggests that such an intervening sequence does not inactivate an expressed gene. Convincing genetic and structural evidence on this point was provided by Goodman *et al.* (29) who demonstrated that actively utilized yeast suppressor tRNA genes contain a 14-base-pair intervening sequence immediately adjacent to the tRNA anticodon. This sequence is not present in mature yeast tRNAs.

Production of Small mRNAs from Large Genes. There are several possible mechanisms that might prevent intervening sequences from being represented in mature mRNA. In view of the fact that a 15S precursor of 9S β-globin mRNA has been demonstrated by a number of laboratories (30–34), it is simplest to propose that this sequence is transcribed, and eliminated by some *bighting-ligating reaction* that, in the case of β-globin mRNA, would decrease the 1200–1500 base precursor to the 600 base cytoplasmic form. Evidence for the processing of "leader" sequences by elimination from larger sequences of viral mRNA transcripts is beginning to accumulate (ref. 35; G. Kitchingman and H. Westphal, personal communication).

Possible Relevance to Other Genetic Systems and Certain Inherited Disorders. Although the advantage to the cell of introducing interruptions in gene segments is not obvious, it is possible that the joining of segmented genes may be a means of generating additional genetic information or diversity, a mechanism proposed long ago for immunoglobulin genes (36). Indeed, immunoglobin constant and variable region genes appear separate in embryonic tissue (37, 38). The contiguous joining of one or another variable gene with a constant region

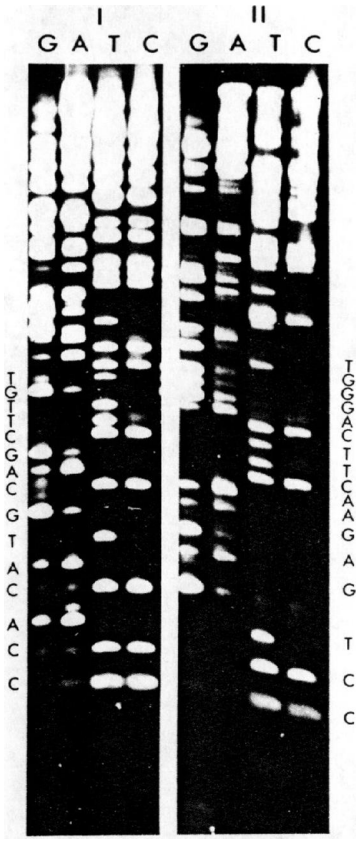

FIG. 5. Nucleotide sequence adjacent to *Bam*HI site. pMB9-βG2 was cleaved with *Bam*HI, and the 5′ ends were dephosphorylated and labeled with [γ-^{32}P]ATP and cleaved with *Hin*dIII. Fragment I (*Left*), containing sequences from the *Bam*HI site to the *Hin*dIII site in the intervening sequence, and fragment II (*Right*), from the *Bam*HI site to a *Hin*dIII outside the 5′ coding region at 0.9 kb, were isolated, treated according to Maxam and Gilbert (25), and run on 20% polyacrylamide gels. The autoradiographs of the gels are shown.

gene may not occur at the level of DNA but rather during or after transcription. We recently cloned two variable region genes from committed immunoglobulin-producing cells and found that they are clearly *not contiguous* with constant region sequences (unpublished data). A similar mechanism might apply to the analogous histocompatibility antigens and be involved in generating their enormous diversity.

Because joining now becomes a requirement of a discontinuous gene sequence, it is reasonable to expect that certain mutations will interrupt this process. For example, thalassemias of the type associated with decreased production of normal globin chains might be altered in a "silent" intervening sequence rather than in regions conventionally considered. It has already been suggested that Lepore-like crossovers which produce decreased amounts of defective globin chain might be effected in this way (39). In any event, it is clear that these intervening sequences pose new and unexpected question in regard to genetic organization and the assembly of genetic information.

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1. Tilghman, S. M., Tiemeier, D. C., Polsky, F., Edgell, M. H., Seidman, J. G., Leder, A., Enquist, L. W., Norman, B. & Leder, P. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4406–4410.
2. Leder, P., Tilghman, S. M., Tiemeier, D. C., Polsky, F. I., Seidman, J. G., Edgell, M. H., Enquist, L W., Leder, A. & Norman, B. (1977) *Cold Spring Harbor Symp. Quant. Biol.*, in press.
3. Glover, D. M. & Hogness, D. S. (1977) *Cell* **10**, 167–176.
4. White, R. L. & Hogness, D. S. (1977) *Cell* **10**, 177–192.
5. Wellauer, P. K. & Dawid, I. B. (1977) *Cell* **10**, 193–212.
6. Pelligrini, M., Manning, J. & Davidson, N. (1977) *Cell* **10**, 213–224.
7. Berget, S. M., Moore, C. & Sharp, P. A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3171–3175.
8. Chow, L. T., Gelinas, R. E., Broker, T. R. & Roberts, R. J. (1977) *Cell* **12**, 1–8.
9. Klessig, D. F. (1977) *Cell* **12**, 9–21.
10. Kitchingman, G. R., Lai, S.-P. & Westphal, H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4392–4395.
11. Celma, M. L., Dhar, R., Pan, J. & Weissmann, S. M. (1977) *Nucleic Acids Res.* **4**, 2549–2559.
12. Aloni, Y., Dhar, R., Laub, O., Horowitz, M. & Khoury, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3686–3690.
13. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
14. Meagher, R. B., Tait, R. C., Betlach, M. & Boyer, H. B. (1977) *Cell* **10**, 521–536.
15. Polsky, F., Edgell, M. H., Seidman, J. G. & Leder, P. (1978) *Anal. Biochem.*, in press.
16. Wilson, G. A. & Young, F. E. (1975) *J. Mol. Biol.* **97**, 123–125.
17. Murray, K. & Murray, N. E. (1975) *J. Mol. Biol.* **98**, 551–564.
18. Landy, A., Ruedisueli, E., Robinson, L., Foeller, C. & Ross, W. (1974) *Biochemistry* **13**, 42134–2142.
19. Smith, D. I., Blattner, F. R. & Davies, J. (1976) *Nucleic Acid Res.* **3**, 343–353.
20. Helling, R. B., Goodman, H. M. & Boyer, H. W. (1974) *J. Virol.* **14**, 1235–1244.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
22. Thomas, M., White, R. L. & Davis, R. W. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2294–2298.
23. Sambrook, J. & Shatkin, A. J. (1969) *J. Virol.* **4**, 719–726.
24. Bender, W. & Davidson, N. (1976) *Cell* **7**, 595–607.
25. Maxam, A. M. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560–564.
26. Rougeon, F. & Mach, B. (1977) *Gene* **1**, 229–239.
27. Efstratiadis, A., Kafatos, F. C. & Maniatis, T. (1977) *Cell* **10**, 571–585.
28. Jeffreys, A. J. & Flavell, R. A. (1977) *Cell*, in press.
29. Goodman, H. M., Olson, M. V. & Hall, B. D. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5453–5457.
30. Ross, J. (1976) *J. Mol. Biol.* **106**, 403–420.
31. Curtis, P. J. & Weissmann, C. (1976) *J. Mol. Biol.* **106**, 1061–1075.
32. Kwan, S.-P., Wood, T. G. & Lingrell, J. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 178–182.
33. Bastos, R. N. & Aviv, H. (1977) *Cell* **11**, 641–650.
34. Curtis, P. J., Mantei, N., Van den Berg, J. & Weissmann, C. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3184–3188.
35. Berget, S. N., Berk, A. J., Harrison, T. & Sharp, P. A. (1977) *Cold Spring Harbor Symp. Quant. Biol.*, in press.
36. Dreyer, W. J. & Bennett, J. C. (1965) *Proc. Natl. Acad. Sci. USA* **54**, 864–869.
37. Hozumi, N. & Tonegawa, S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3628–3632.
38. Tonegawa, S., Brack, C., Hozumi, N. & Schuller, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3518–3522.
39. Ramirez, F., Mears, G., Nudel, U., Bank, A., Luzzatto, L., Gambino, R., Cimino, R. & Quattrin, N. (1977) *Nature*, in press.