

## Two regulatory elements for immunoglobulin $\kappa$ light chain gene expression

(transfections/enhancers/promoters)

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**ABSTRACT** By using internal deletions within a rearranged immunoglobulin  $\kappa$  light chain gene, the presence of an intron regulatory sequence (enhancer) has been confirmed. Its presence is required for high-level transcription from a plasmid after transfection into myeloma cells. Transfection efficiency was monitored by the activity of a deleted H4 histone gene included in the plasmid. The intron element could be moved upstream of the gene in both orientations, fulfilling the definition of an enhancer. By using 5' deletions, a second regulatory element was located upstream of the "TATA" box, between positions -69 and -104. These two elements both are required for efficient  $\kappa$  chain gene expression.

The introduction of immunoglobulin genes into lymphocytic cell lines provides a tool for defining important regulatory regions and identifying tissue-specific factors involved in gene expression. The analysis of a variety of eukaryotic genes in this way has revealed the presence of a number of distinct transcriptional elements. The "TATA" box is one that is present in most genes and helps to determine the precise location of the transcriptional start site (reviewed in ref. 1). Several less-defined elements are located upstream of the TATA homology (2-4). Another class of control elements, termed enhancers, are distinguished by their ability to activate transcription units independently of their orientation or precise positioning relative to the promoter element (5-7). We have designed experiments, using a transient transfection assay, which examine regulatory elements required for  $\kappa$  chain gene transcription.

One feature of Ig gene expression is the requirement for chromosomal rearrangements before a functional Ig protein is produced (reviewed in ref. 8). In the case of  $\kappa$  light chain genes, a variable (V) region,  $V_{\kappa}$ , is joined to one of several joining (J) segments,  $J_{\kappa}$ , located upstream of the unique constant (C) region,  $C_{\kappa}$ . The functional rearrangement process takes place only in the B-lymphocyte lineage at a particular stage of differentiation (9). After rearrangement has occurred, B cells mature into plasma cells, which transcribe the rearranged  $\kappa$  chain gene at an increased level compared to the B lymphocyte (10). Regulatory elements recently identified in the J-C intron of  $\kappa$  (11, 12) and heavy (13-15) chain genes offer an explanation of why only the rearranged V gene is transcribed efficiently, whereas unrearranged V genes are transcriptionally inactive.

Previous studies have identified the intron regulatory element of the  $\kappa$  chain gene by 3'-terminal deletions or by moving the intron element near test genes (11, 12, 16). To examine more precisely the regulatory role of the intron, we have made internal deletions within the intron of a rearranged  $\kappa$  chain gene. We have controlled the effects of the deletion by including in the tested DNA constructs a marked H4 histone

gene whose transcription should be independent of deletions elsewhere. We have further examined  $\kappa$  chain gene regulation by making deletions 5' to the gene. These studies have defined two regulatory elements: the intron enhancer and an upstream element that probably includes an octanucleotide sequence highly conserved among V genes (17).

### MATERIALS AND METHODS

**Plasmids.** A 7-kilobase pair (kbp) *EcoRI*-*BamHI* fragment containing the expressed mouse  $\kappa$  chain gene from myeloma MOPC41 (a gift from P. Leder) was bounded by *Sal I* synthetic linkers and ligated with a *Sal I*-*Xho I* vector fragment containing pBR322 "poison-minus" sequences (28), a 0.6-kb modified mouse histone H4 gene, and a 3.6-kb *BamHI* polyoma virus fragment including the origin of replication and the early gene. The resultant plasmid was designated pPy $\kappa$  (Fig. 1A). The modified mouse H4 histone gene was truncated by 50 nucleotides at its 5' end to provide a distinction between its transcripts and those from endogenous mouse H4 genes. The  $\kappa$  light chain gene was inserted in opposite transcriptional orientation to the polyoma segment.

Deletion mutants of the  $\kappa$  chain gene in the  $J_{\kappa}$ - $C_{\kappa}$  intron were constructed as follows: plasmid pBR322 $\kappa$  (in which the  $\kappa$  chain gene was ligated with an *EcoRI*-*BamHI* fragment of pBR322) was cleaved in the  $J_{\kappa}$ - $C_{\kappa}$  intron with either *HindIII* or *Sac I* and subsequently digested with BAL-31 exonuclease. Synthetic *Xho I* linkers were ligated onto the resected ends, and the plasmids were transfected into *Escherichia coli*. Plasmids containing deletions of desired sizes were identified by detailed restriction mapping and are depicted in Fig. 1B. The *EcoRI*-*BamHI*  $\kappa$  chain gene fragments containing the different intron deletions were purified by electrophoretic fractionation, flanked by *Sal I* linkers, and ligated with the *Xho I*-*Sal I* vector fragment described above.

Insertion mutants containing either the  $\kappa$  light chain or the  $\mu$  heavy chain enhancer fragment 5' to  $V_{\kappa}$  were constructed as follows: the *HindIII*-*Mbo I* 819-bp fragment containing the  $\kappa$  chain enhancer, the *Xba I*-*EcoRI* 700-bp fragment containing the  $\mu$  chain enhancer, or the *Pvu II*-*EcoRI* 300-bp fragment that harbors only 30% of the  $\mu$  chain enhancer activity were individually inserted in the unique *Sal I* site of the plasmid containing the H3 $\Delta$ 14 gene, which lacks the  $\kappa$  enhancer sequences and is depicted in Fig. 1B. All fragments were inserted in the correct orientation [ins. $\kappa$ 1, ins. $\mu$ 300 $\kappa$ 1, and ins. $\mu$ 700 $\kappa$ 1(ins. $\mu$ 1)] and in opposite orientation [ins. $\kappa$ 2, ins. $\mu$ 300 $\kappa$ 2, and ins. $\mu$ 700 $\kappa$ 2(ins. $\mu$ 2)] with respect to transcription (see Fig. 3A).

Abbreviations: V, variable; J, joining; C, constant; kbp, kilobase pair; wt, wild-type.

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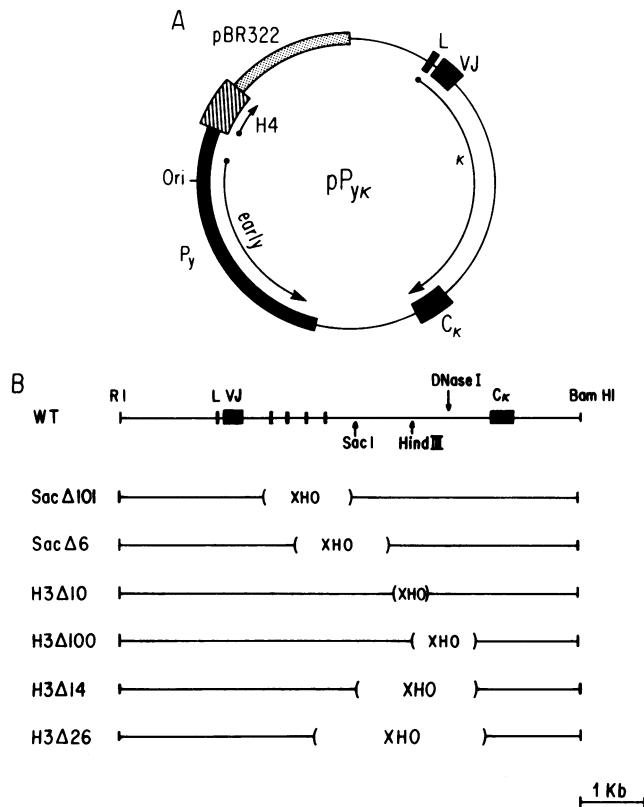


FIG. 1. Schematic diagram of  $\kappa$  chain gene-containing plasmids. (A) Scheme of the pPy $\kappa$  plasmid DNA. The circular map of the plasmid DNA is shown. The plasmid contains a 7-kbp *EcoRI*–*Bam*HI fragment representing the entire genomic rearranged  $\kappa$  light chain gene cloned from MOPC41 myeloma. The leader (L), V–J region, and  $C_{\kappa}$  region of the  $\kappa$  chain gene are indicated by black boxes. pBR322 sequences and mouse histone H4 gene sequences are stippled or hatched, respectively. Polyoma sequences (Py), represented by a thick black line, include the origin of replication and the early region genes. The directions of transcription are shown by arrows. (B) Deletion mutants in the  $J_{\kappa}$ – $C_{\kappa}$  intron. Deletion mutants were constructed as follows. The  $\kappa$  chain gene was cleaved either with *Hind*III or *Sac*I [indicated on the map of the wild-type (wt)  $\kappa$  chain gene] followed by BAL-31 exonuclease digestion. Synthetic *Xho*I linkers were ligated onto the resected ends. The DNA segment deleted in each mutant gene was determined by restriction analysis and is indicated by parenthesis. “DNase I” marks a prominent site of DNase hypersensitivity (18).

To construct the 5' deletion mutants of the  $\kappa$  chain gene (depicted in Fig. 4A), pSV2gpt $\kappa$  plasmids (19) were cleaved at the unique *Eco*RI site about 1.5 kbp upstream of  $V_{\kappa}$ , digested with BAL-31 exonuclease, linked with *Eco*RI synthetic linkers, and recut at the *Xba*I site within the  $J_{\kappa}$ – $C_{\kappa}$  intron. To replace pBR322 sequences 5' to the *Eco*RI site, the *Eco*RI–*Xba*I fragments containing the  $V_{\kappa}$  region were purified after electrophoretic fractionation and recloned in the *Eco*RI–*Xba*I vector fragment of pSV2gpt $\kappa$ . The *Eco*RI–*Bam*HI fragments containing the different 5' deletion mutants of the desired size were then inserted into the unique *Sal*I site of the polyoma-containing vector by using *Sal*I linkers. Plasmids were then selected that contained the  $\kappa$  chain gene inserted in the reverse orientation with respect to transcription of the polyoma early region gene.

**Cell Culture and Transfection.** Transcription of the various  $\kappa$  chain gene constructs was analyzed after transfection into the myeloma cell line MPC11. Cells were maintained in RPMI 1640 medium containing 10% fetal calf serum and 50  $\mu$ M 2-mercaptoethanol. Transient transfections were performed by the DEAE-dextran technique (20).

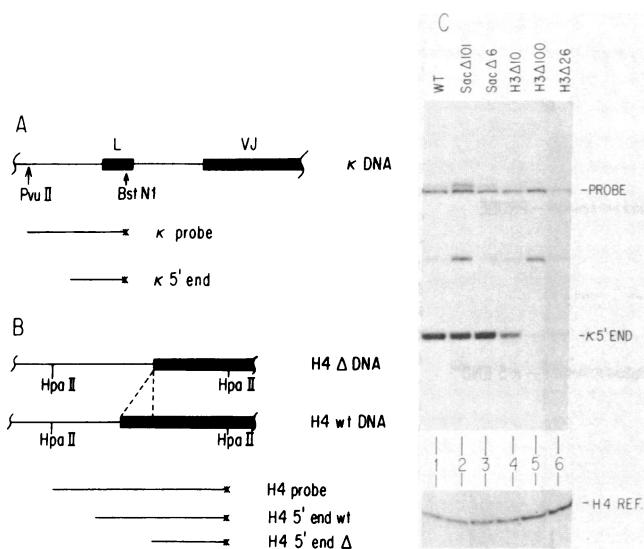
**RNA Preparation and Analysis.** Cytoplasmic RNA was prepared 44 hr after transfection (21). RNA was analyzed by S1 nuclease mapping (22, 23) with a 5'-end-labeled 180-bp *Pvu* II–*Bst*NI double-stranded probe for the  $\kappa$  chain gene (called “ $\kappa$  probe”) and a 265-bp *Hpa* II single-stranded DNA probe for the mouse histone H4 gene (called “H4 probe”). Because the mouse H4 gene inserted in the vector was truncated by 50 bp at its 5' end, the transfected H4 mRNA protects a 99-bp fragment, whereas the endogenous mRNA protects a 175-bp fragment. Hybridizations were carried out at 48°C for 16 hr for both probes. The protected DNA was analyzed by electrophoresis through 8% polyacrylamide/urea gels.

## RESULTS

**Mapping of Regulatory Sequences in the  $\kappa$  Chain Gene.** To identify regulatory sequences in the  $J_{\kappa}$ – $C_{\kappa}$  intron that could influence the rate of  $\kappa$  chain transcription, a series of deletion mutants spanning the entire intron were made in a rearranged, functional  $\kappa$  chain gene by cleavage at unique sites within the intron, followed by BAL-31 exonuclease digestion (Fig. 1B). The wt  $\kappa$  chain gene and the deletion mutant genes were cloned into a vector (Fig. 1A) that contains pBR322 sequences, the early region of polyoma viral DNA including the origin of DNA replication, and a modified mouse H4 histone gene, which had a 50-nucleotide deletion at its 5' end to distinguish its transcripts from those of endogenous mouse H4 RNA. Transcription of the modified mouse H4 histone gene was used to monitor the efficiency of the transfection and the RNA recovery in each experiment. Previous studies have indicated that the presence of polyoma sequences does not perturb regulation but increases the amount of RNA made after transfection of active constructs (ref. 11; unpublished data).

To study the effect of removal of  $J_{\kappa}$ – $C_{\kappa}$  intron sequences on  $\kappa$  chain mRNA accumulation, plasmids containing either wt or deletion mutant genes were transfected into MPC11 cells by using a DEAE-dextran protocol (20). Cytoplasmic RNA was prepared 44 hr after transfection and was assayed by S1 nuclease mapping for transcripts of the  $\kappa$  chain and H4 histone genes using 5'-end-labeled probes (22, 23). For the analysis of  $\kappa$ -specific RNA, the probe was a 180-bp *Pvu* II–*Bst*NI fragment labeled in the leader exon (Fig. 2A). RNA from myeloma cells transfected with the plasmid pPy $\kappa$ , which contains the wt  $\kappa$  chain gene, protected a fragment (Fig. 2C) that comigrated with a fragment protected by mRNA from MOPC41, the myeloma from which the  $\kappa$  chain gene was derived (not shown). This fragment is characteristic of transcripts initiating about 25 bp upstream of the ATG codon (11). (In Fig. 2C, the band between the input probe DNA and the protected fragment is a variable artifact of unknown origin.) For the analysis of the H4 RNA, a 265-nucleotide *Hpa* II fragment was used (Fig. 2B). This probe was labeled within the H4 gene and extended 115 bp upstream of the ATG codon. Protection by H4 mRNA from transfected cells yielded a 99-bp fragment from the transfected modified H4 gene (Fig. 2C Bottom).

Plasmids containing the deletion mutants depicted in Fig. 1B were transfected in parallel with pPy $\kappa$  into MPC11 myeloma cells, and the extracted RNAs were analyzed (Fig. 2C). Plasmids containing deletion mutants Sac $\Delta$ 101 and Sac $\Delta$ 6 produced levels of RNAs similar to that of pPy $\kappa$ , which contained the wt gene. Deletion H3 $\Delta$ 10 produced a slightly reduced amount of  $\kappa$  RNA but a wt level of H4 RNA, suggesting some effect of the deletion on transcription. A marked decrease was seen in the levels of  $\kappa$  mRNA in myeloma cells transfected with pPy $\kappa$ H3 $\Delta$ 100 and pPy $\kappa$ H3 $\Delta$ 26, although no reduction in H4 transcription was evident (Fig. 2, lanes 5 and 6). To quantitate and normalize the levels of  $\kappa$ -specific



**FIG. 2.** S1 nuclease analysis of RNA from transfected MPC-11 myeloma cells. (A) Structure of the 5'-end-labeled  $\kappa$  probe. The leader (L) and V-J segments are indicated by black boxes; intron and 5' noncoding sequences are drawn with a thin line. The structures of the DNA probe, which consists of a 180-bp Pvu II–Bst NI fragment, and of the DNA fragment protected by the 5'-terminal nucleotides of the specific  $\kappa$  chain RNA are shown. The position of the  $^{32}\text{P}$  label is indicated by an asterisk. (B) Structure of the 5'-end-labeled H4 probe. The H4 coding sequences of the wt and deleted ( $\Delta$ ) genes are depicted as black boxes. The structures of the DNA probe, which consists of a 265-bp Hpa II fragment, and the DNA fragments that are protected by the H4-specific transcripts of either the wt or the deleted genes are shown. The position of the  $^{32}\text{P}$  label is indicated by an asterisk. (C) Analysis of  $\kappa$  chain and H4 histone gene transcripts. RNA from myeloma cells transfected with the wt and deletion-mutant  $\kappa$  chain genes (depicted in Fig. 1B) were hybridized to 5'-end-labeled  $\kappa$  and H4 probes and digested with S1 nuclease as described in *Materials and Methods*. The protected fragments were separated by gel electrophoresis and detected by autoradiography. The positions of the full-length DNA probe and of the  $\kappa$  and H4 protected fragments are indicated.

mRNA produced in cells transfected either with wt or with deletion mutant genes, autoradiograms from several independent experiments at several exposures chosen to ensure linearity were scanned by a densitometer for transcript bands specific for H4 histone and  $\kappa$  chain genes. MPC11 cells transfected with deletion mutants H3 $\Delta$ 100, H3 $\Delta$ 14, and H3 $\Delta$ 26 transcribed approximately 1/12th as much  $\kappa$ -specific mRNA as MPC11 cells transfected with the pPy $\kappa$  plasmid containing the wt  $\kappa$  chain gene. These results strongly suggest that DNA sequences deleted in plasmid pPy $\kappa$ H3 $\Delta$ 100 but still present in pPy $\kappa$ Sac $\Delta$ 101 and pPy $\kappa$ Sac $\Delta$ 6 are essential for high levels of  $\kappa$  chain transcription in myeloma cells; deletion H3 $\Delta$ 10 probably comes right to the border of the required sequences. Thus, using internal deletions within the  $J_{\kappa}$ - $C_{\kappa}$  intron, we find a regulatory element in the same region previously defined by long terminal deletions (16) and by its effects on test genes (12).

**Effect of  $\kappa$  and Heavy Chain Sequences on  $\kappa$  Chain mRNA Accumulation.** To determine whether the regulatory sequences located in the  $J_{\kappa}$ - $C_{\kappa}$  intron behave similarly to viral enhancer elements, the following plasmids were constructed. An 819-bp HindIII–Mbo I fragment, containing the regulatory sequences defined by deletion mapping, was inserted in both orientations into a Sal I site located about 1.5 kbp upstream of  $V_{\kappa}$  in the pPy $\kappa$ H3 $\Delta$ 14 plasmid, yielding recombinants pPyins. $\kappa$ 1 and pPyins. $\kappa$ 2 (Fig. 3A). The  $\kappa$  chain was transcribed at very low levels from pPy $\kappa$ H3 $\Delta$ 14, a plasmid

lacking the regulatory sequences (Fig. 3B, lane 1). By contrast, the pPyins. $\kappa$ 1 and pPyins. $\kappa$ 2 plasmids were transcribed at wt levels (Fig. 3, lanes 7 and 8). The HindIII–Mbo I fragment was inserted also in either orientation into a Pvu II site located 128 bp upstream of the ATG codon. These two constructs produced as much  $\kappa$  RNA as did pPy $\kappa$ , pPyins. $\kappa$ 1 and -2 (data not shown). We conclude that the regulatory element located in the  $J_{\kappa}$ - $C_{\kappa}$  intron can enhance transcription when it is placed in either orientation up to 1.5 kbp upstream or 3.2 kbp downstream from the  $V_{\kappa}$  promoter (its normal location), thus behaving similarly to a viral enhancer.

Recently, a lymphocyte-specific enhancer has been identified downstream of the J region in immunoglobulin heavy chain genes (13–15). We have compared the relative strength of the heavy and  $\kappa$  chain enhancer as measured by  $\kappa$  chain gene transcription. For the heavy chain enhancer, two fragments were inserted independently into the Sal I site in plasmids containing the H3 $\Delta$ 14 mutant gene (Fig. 3A). One is a 700-bp Xba I–EcoRI fragment that contains the entire heavy chain enhancer activity, and the second is a 300-bp Pvu II–EcoRI fragment that, when added to a modified heavy chain gene lacking the  $V_{\text{H}}-C_{\mu}$  intron, restores only 30% of the  $\mu$  chain transcription activity (unpublished data). The fragments were inserted in the correct (ins. $\mu$ 700 $\kappa$ 1, ins. $\mu$ 300 $\kappa$ 1) and in the reverse (ins. $\mu$ 700 $\kappa$ 2, ins. $\mu$ 300 $\kappa$ 2) orientation with respect to direction of transcription. The four different  $\mu$  chain enhancer-containing plasmids were transfected into MPC11 cells, and RNA was analyzed for  $\kappa$ - and H4-specific transcripts by S1 mapping. Insertion of the 300-bp heavy chain enhancer fragment restored transcription to wt levels (Fig. 3, lanes 5 and 6), and the 700-bp Xba I–EcoRI heavy chain fragment increased the level of  $\kappa$  chain transcription by 2- to 3-fold over that of the  $\kappa$  enhancer (Fig. 3, lanes 3 and 4). The quantitative values were determined by densitometer scans and were corrected for H4 histone transcription. Thus, we can conclude that the  $\mu$  chain enhancer is 2- to 3-fold more active than the  $\kappa$  chain enhancer when measured on a  $\kappa$  chain gene.

**Mapping of Regulatory Sequences in the Promoter Region of the  $\kappa$  Chain Gene.** To study potential regulatory elements located upstream of  $V_{\kappa}$ , 5' deletion mutants were generated using BAL-31 digestion (Fig. 4A). The wt and the 5' deletion mutant genes were transfected into MPC11 cells, cytoplasmic RNA was isolated, and it was analyzed by S1 nuclease mapping for  $\kappa$ - and H4-specific transcripts. Deletion of sequences upstream of position -104 has no effect on  $\kappa$  chain transcription because deletion mutant 5' $\Delta$ 5 is transcribed at the same efficiency as pPy $\kappa$  (Fig. 4B, lanes 1 and 4). Removal of sequences between positions -69 and -104 upstream of the coding region, which left the TATA sequence intact, abolished transcription completely (lane 2). These results suggest that sequences located upstream of position -104 are not important for  $\kappa$  chain transcription, whereas sequences mapping between positions -69 and -104 are crucial for its transcription.

## DISCUSSION

These studies have contributed to the definition of two DNA sequences required for  $\kappa$  chain gene expression: an enhancer-like element in the  $J_{\kappa}$ - $C_{\kappa}$  intron and an upstream sequence.

The intron sequence has been described (11, 24) and has been shown to enhance transcription of a  $\beta$ -globin gene (12). Another recent study also has examined its activity in  $\kappa$  chain gene constructs (16). The upstream element had not been defined previously, although its presence had been predicted from comparative sequence analysis (17), and it has been found in a parallel study (25).

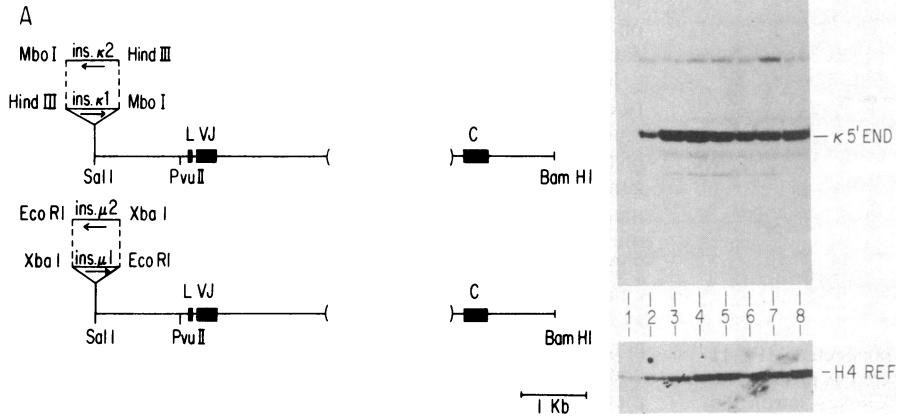


FIG. 3. Enhancement of  $\kappa$  chain gene transcription by either the  $\kappa$  or  $\mu$  chain enhancers. (A) Schematic representation of insertion mutants used. The H3 $\Delta$ 14 gene, which lacks the  $\kappa$  chain enhancer sequence, is shown, and the L, V-J, and C regions are indicated by black boxes. The  $\kappa$  and  $\mu$  chain enhancer fragments described in *Materials and Methods* were inserted in the Sal I site in the correct orientation [ins. $\kappa$ 1, ins.- $\mu$ 300 $\kappa$ 1, and ins. $\mu$ 700 $\kappa$ 1(ins. $\mu$ 1)] and in opposite orientation [ins. $\kappa$ 2, ins. $\mu$ 300 $\kappa$ 2, and ins. $\mu$ 700 $\kappa$ 2(ins. $\mu$ 2)]. The orientation relative to transcription is shown with an arrow. (B) Analysis of the 5' ends of  $\kappa$  chain gene-specific transcripts. RNA from myeloma cells transfected with wt, H3 $\Delta$ 14 deletion mutant (depicted in Fig. 1B), and insertion mutant genes (depicted in Fig. 3A) were hybridized to 5'-end-labeled  $\kappa$  and H4 probes shown in Fig. 2 A and B. After digestion with S1 nuclease, the protected fragments were analyzed by electrophoresis through 8% polyacrylamide/urea gels. The positions of the full-length DNA probe and the protected  $\kappa$  chain and H4 histone DNA fragments are indicated.

**The Enhancer.** The present study shows by internal deletions within the  $J_{\kappa}$ - $C_{\kappa}$  intron that the only intron regulatory element identifiable by these methods is the one seen in previous studies (11, 12). It occurs coincident with a region of sequence conserved between mouse, man, and rabbit  $\kappa$  chain genes and a region of hypersensitivity to deoxyribonuclease digestion (18, 26, 27).

The intron regulatory sequence behaves as an enhancer by conventional criteria: its activity is evident whether it is upstream of the gene or within it and in both orientations. It also stimulates, albeit weakly, transcription of a  $\beta$ -globin gene (12) and a  $\mu$  heavy chain gene (unpublished data). Further studies are required to know whether the "enhancer"

contains a single long regulatory sequence (16) or multiple elements.

The  $\kappa$  chain gene enhancer was about one-third to one-half as potent an activator of  $\kappa$  chain transcription as the heavy chain enhancer that was described recently by several groups (13-15). This contrasts with recently published studies reporting that the  $\kappa$  chain enhancer is only about 5% as active as the heavy chain enhancer when measured on test genes:  $\beta$ -globin gene or enhancerless gene for simian virus 40 tumor antigen (12). Several explanations should be considered for this apparent discrepancy. First, because the  $\kappa$  and heavy chain gene enhancer-promoter regions must somehow allow approximately equal synthesis of the two chains,

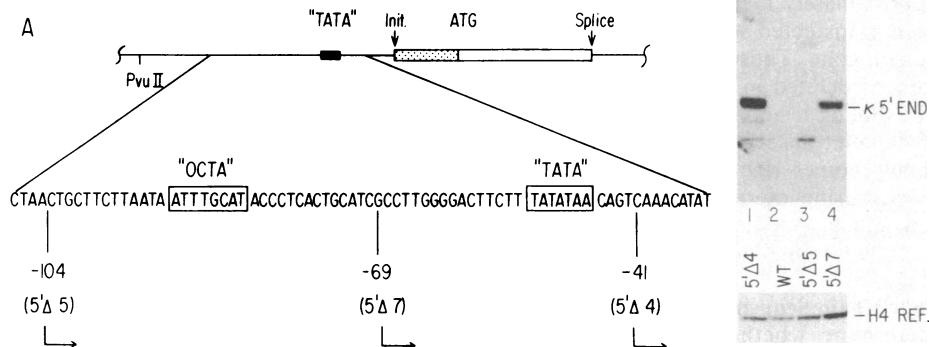


FIG. 4. S1 analysis of RNA from myeloma cells transfected with 5' deletion mutants of the  $\kappa$  chain gene. (A) Schematic representation of  $\kappa$  chain gene 5' deletion mutants. The leader (L), 5' untranslated, and TATA box regions are represented by open, stippled, and black boxes, respectively. The nucleotide sequence between positions -32 and -108 upstream of the coding region is shown. The TATA box and the octanucleotide (OCTA) conserved sequences are boxed. The 5' deletion mutants were generated by using BAL-31 digestion as described. The 5' end points, determined by sequence determination of 5' $\Delta$ 5(-104), 5' $\Delta$ 7(-69), and 5' $\Delta$ 4(-41), are indicated by arrows. (B) Analysis of  $\kappa$  chain and H4 histone gene transcripts. RNA from myeloma cells transfected with wt and 5' $\Delta$ 5, 5' $\Delta$ 7, and 5' $\Delta$ 4 mutant genes were hybridized to 5'-end-labeled  $\kappa$  and H4 probes shown in Fig. 2 A and B. After digestion with S1 nuclease, the protected fragments were analyzed by electrophoresis through 8% polyacrylamide/urea gels. The positions of the full-length DNA probe and the protected  $\kappa$  and H4 fragments are indicated.

there may be a coupling of enhancer and promoter specificity, which may be more prominent for the  $\kappa$  chain gene than for the heavy chain gene. Alternatively, there may be an additional transcriptional regulatory element present in our enhancer-deleted  $\kappa$  chain gene that would elevate the level of  $\kappa$  chain transcription.

**The Upstream Sequence.** Emerging evidence points to the existence of a class of eukaryotic promoters in which the level of transcription is regulated by at least two elements. One, an A/T-rich sequence, is referred to as the TATA box and is located 25 to 30 bp upstream from the transcription initiation site (1). Others are located upstream from the TATA box (2–4). In studying the region 5' to the mouse  $V_{\kappa}$  region, using 5' deletion mutants, we have identified sequences upstream of the TATA box that are essential for  $\kappa$  chain transcription. They are located between position –69 and –104 upstream of the coding region. Deletion of sequences 5' of position –104 had no effect on the efficiency of  $\kappa$  chain transcription, but deletion of sequences between –69 and –104, which left intact the TATA box around position 30 upstream of the initiation site, showed no accumulation of transcripts.

A conserved octanucleotide sequence located upstream of various  $V_{\kappa}$  chain genes was previously identified by sequence examination (17). The inverse sequence was found at the same location 5' to heavy chain  $V$  genes. This octanucleotide sequence has been selectively conserved among diverse  $V$  genes in at least two mammalian species. It is quite likely that our deletions affect transcription as a consequence of the deletion of this octanucleotide, but further work will be required to test this proposition.

A recent study has shown also that upstream deletions abolish mRNA accumulation and presumably affect transcription (25). In that study, two upstream sequences were suggested as important elements: the previously noted octanucleotide (extended by two bases to a decanucleotide) and a further-upstream and less-conserved pentadecanucleotide. Our deletion 5' $\Delta$ 5 removed the pentadecanucleotide but did not appear to reduce transcription, implying that this conserved sequence does not greatly affect transcription, at least in transfected myeloma cells.

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