Immunoglobulin \( \kappa \) Gene Expression after Stable Integration

I. ROLE OF THE INTRONIC MAR AND ENHANCER IN PLASMACYTOMA CELLS*

(Received for publication, July 5, 1989)

Veronica C. Blasquez, Ming Xu, Steven C. Moses, and William T. Garrard‡
From the Department of Biochemistry, the University of Texas Southwestern Medical Center, Dallas, Texas 75235

Rearranged MOPC41 immunoglobulin \( \kappa \) gene constructs have been stably introduced into cultured S194 mouse plasmacytoma cells to investigate the effects of deleting the intronic enhancer and/or matrix association region (MAR) on gene expression. Intact single-copy \( \kappa \) genes containing 1.5 kilobase pairs of upstream and 8.5 kilobase pairs of downstream flanking sequences exhibited sensitivity to chromosome position effects and were expressed at a mean level of 27% relative to the endogenous \( \kappa \) gene expression or only 6% with respect to the MOPC41 \( \kappa \) mRNA levels in the tumor. Deletion of the intronic MAR led to a 4-fold decrease in expression, while deletion of both the MAR and enhancer led to an 11-fold decline. These effects were dampened by preselecting for integration into a transcriptionally poised chromatin location as demonstrated by linkage to a selectable marker which lacked both a MAR and an enhancer. Significant, we found that sequences downstream of the poly(A) addition site compensated 150-fold for deletion of the intronic enhancer.

The immunoglobulin genes provide a powerful biological system for the study of the regulation of tissue-specific gene expression. During B lymphocyte differentiation in the mouse, one of several hundred variable regions (V,\(^1\)) of the \( \kappa \)-immunoglobulin light chain genes is selected for a recombination event with one of four functional joining regions (J,\(^1\)) immediately 5' to a single constant region (C,\(^1\)) (for review, see Refs. 1 and 2). This process of somatic recombination leads to transcriptional activation because it brings together two cis-acting DNA sequences that normally are far apart in the genome: a promoter element (P,\(^1\)) located immediately 5' of the V, exon (5'-6) and an enhancer element (E,\(^1\)) positioned in the intron just upstream of the (C,\(^1\)) exon (5, 7-9). More recently, these cis-acting sequences have been functionally dissected (10-15), some of the corresponding trans-acting proteins have been identified (16-21) and purified (22, 23), and several cDNAs encoding these proteins have been cloned (24-28). Interestingly, part of the regulation is negative, being mediated by an inhibitor IxB, that complexes with NF-\( \kappa \)B, a positive protein activator which binds to the intronic enhancer (29, 30).

In addition to the promoter and enhancer elements, a matrix association region (MAR or M,\(^1\)) resides adjacent to the enhancer within the major intron in the mouse, rabbit, and human \( \kappa \) genes (31-34). Since the MAR interacts with the nuclear matrix or scaffold, it appears to play a fundamental role in chromosomal loop organization of the \( \kappa \) gene locus (31). This sequence contains sites that interact with topoisomerase II (31, 32) and, therefore, conceivably could participate as a swivel to regulate torsional stress during transcription (35, 36). Interestingly, rabbit \( \kappa i\)-immunoglobulin genes that have spontaneously deleted a 160-bp segment of the MAR express 4-fold lower than their undeleted counterparts (37), and expression of the rabbit \( \kappa i\) gene, in which the entire MAR and other intronic sequences have been deleted (32, 33), is very low and often undetectable (33).

Here we address the biological significance of the intronic MAR and enhancer on transcription of the mouse \( \kappa \) gene in plasmacytoma cells. Since previous studies employing only transient expression to evaluate the importance of these sequences have failed to detect a function for the MAR (5, 9), we decided to employ stable integration experiments, in which bona fide chromatin structures are assembled and stably propagated to daughter cells. We have also evaluated the effect on gene expression of integration into different chromosomal positions and of linkage to a transcribed selectable marker. Our results demonstrate that all these parameters affect gene expression and that the MAR and the enhancer each contribute a significant quantitative effect on expression. Furthermore, we have found that sequences downstream of the poly(A) site compensate 150-fold for deletion of the intronic enhancer.

**EXPERIMENTAL PROCEDURES**

Recombinant DNA Plasmids and Hybridization Probes—An EcoRI-BamHI 7.3-kb fragment containing the functional \( \kappa \) gene of mouse myeloma MOPC41 and a 6.3-kb derivative lacking both the MAR and enhancer were obtained from David Baltimore’s Laboratory (Massachusetts Institute of Technology) (5). These fragments were subcloned into the EcoRI-BamHI site of pBR322. The MAR was deleted from the 7.3-kb construct by cleaving at the HindIII site and digesting with Bal31. Synthetic HindIII linkers were ligated onto the intron just upstream of the (C,) exon (5, 7-9). More recently, these cis-acting sequences have been functionally dissected (10-15), some of the corresponding trans-acting proteins have been identified (16-21) and purified (22, 23), and several cDNAs encoding these proteins have been cloned (24-28). Interestingly, part of the regulation is negative, being mediated by an inhibitor IxB, that complexes with NF-\( \kappa \)B, a positive protein activator which binds to the intronic enhancer (29, 30).

In addition to the promoter and enhancer elements, a matrix association region (MAR or M,\(^1\)) resides adjacent to the enhancer within the major intron in the mouse, rabbit, and human \( \kappa \) genes (31-34). Since the MAR interacts with the nuclear matrix or scaffold, it appears to play a fundamental role in chromosomal loop organization of the \( \kappa \) gene locus (31). This sequence contains sites that interact with topoisomerase II (31, 32) and, therefore, conceivably could participate as a swivel to regulate torsional stress during transcription (35, 36). Interestingly, rabbit \( \kappa i\)-immunoglobulin genes that have spontaneously deleted a 160-bp segment of the MAR express 4-fold lower than their undeleted counterparts (37), and expression of the rabbit \( \kappa i\) gene, in which the entire MAR and other intronic sequences have been deleted (32, 33), is very low and often undetectable (33).

Here we address the biological significance of the intronic MAR and enhancer on transcription of the mouse \( \kappa \) gene in plasmacytoma cells. Since previous studies employing only transient expression to evaluate the importance of these sequences have failed to detect a function for the MAR (5, 9), we decided to employ stable integration experiments, in which bona fide chromatin structures are assembled and stably propagated to daughter cells. We have also evaluated the effect on gene expression of integration into different chromosomal positions and of linkage to a transcribed selectable marker. Our results demonstrate that all these parameters affect gene expression and that the MAR and the enhancer each contribute a significant quantitative effect on expression. Furthermore, we have found that sequences downstream of the poly(A) site compensate 150-fold for deletion of the intronic enhancer.

**EXPERIMENTAL PROCEDURES**

Recombinant DNA Plasmids and Hybridization Probes—An EcoRI-BamHI 7.3-kb fragment containing the functional \( \kappa \) gene of mouse myeloma MOPC41 and a 6.3-kb derivative lacking both the MAR and enhancer were obtained from David Baltimore’s Laboratory (Massachusetts Institute of Technology) (5). These fragments were subcloned into the EcoRI-BamHI site of pBR322. The MAR was deleted from the 7.3-kb construct by cleaving at the HindIII site and digesting with Bal31. Synthetic HindIII linkers were ligated onto the intron just upstream of the (C,) exon (5, 7-9). More recently, these cis-acting sequences have been functionally dissected (10-15), some of the corresponding trans-acting proteins have been identified (16-21) and purified (22, 23), and several cDNAs encoding these proteins have been cloned (24-28). Interestingly, part of the regulation is negative, being mediated by an inhibitor IxB, that complexes with NF-\( \kappa \)B, a positive protein activator which binds to the intronic enhancer (29, 30).

In addition to the promoter and enhancer elements, a matrix association region (MAR or M,\(^1\)) resides adjacent to the enhancer within the major intron in the mouse, rabbit, and human \( \kappa \) genes (31-34). Since the MAR interacts with the nuclear matrix or scaffold, it appears to play a fundamental role in chromosomal loop organization of the \( \kappa \) gene locus (31). This sequence contains sites that interact with topoisomerase II (31, 32) and, therefore, conceivably could participate as a swivel to regulate torsional stress during transcription (35, 36). Interestingly, rabbit \( \kappa i\)-immunoglobulin genes that have spontaneously deleted a 160-bp segment of the MAR express 4-fold lower than their undeleted counterparts (37), and expression of the rabbit \( \kappa i\) gene, in which the entire MAR and other intronic sequences have been deleted (32, 33), is very low and often undetectable (33).

Here we address the biological significance of the intronic MAR and enhancer on transcription of the mouse \( \kappa \) gene in plasmacytoma cells. Since previous studies employing only transient expression to evaluate the importance of these sequences have failed to detect a function for the MAR (5, 9), we decided to employ stable integration experiments, in which bona fide chromatin structures are assembled and stably propagated to daughter cells. We have also evaluated the effect on gene expression of integration into different chromosomal positions and of linkage to a transcribed selectable marker. Our results demonstrate that all these parameters affect gene expression and that the MAR and the enhancer each contribute a significant quantitative effect on expression. Furthermore, we have found that sequences downstream of the poly(A) site compensate 150-fold for deletion of the intronic enhancer.
pAT153. A 449-bp Delc-Acll fragment of this insert was gel-purified and used as the V,41 probe. The J, probe is a HindIII-BglII fragment which contains the entire germ line J, region subcloned into pSP64 (probe A in Ref. 41). The C, probe is a 1.7-kb HindIII-BglII fragment obtained from the plasmid pG19/45 (31). The probe used to detect mouse anti-human IgG antibodies containing part of the cDNA sequence encoding human β-actin (42).

The selectable marker pBneo which was used in unlinked transfections contained the neo' gene inserted into the vector pXXH (43, 44). For the linked transfections, neo' constructs were ligated to an enhancerless selectable marker. This construct was created using the plasmid pAC1, obtained from Richard Myers (University of California, San Francisco), containing neo' linked to 680 bp of the herpes simplex virus thymidine kinase (HSV-tk) promoter in a pSP6 vector. We inserted a Safl fragment that contains the SV40 small t intron and poly(A) site (obtained from pBneo) into the Safl site located 3' of the neo' gene in pAC1. The resulting construct is termed pE'Tk-neo (see Fig. 1). The long form of the X gene constructs (15-16-kb EcoRI fragments) were inserted at the EcoRI site of pE'Tk-neo to generate plasmids for the linked transfection experiments. All plasmids used for transfection were prepared by the alkaline lysis method and purified by CsCl equilibrium centrifugation (45).

Cell Culture and Transfection—S194 plasmacytoma cells, obtained from American Type Culture Collection, were grown in Dulbecco's modified Eagle's medium containing 13% horse serum, 2.0 mM glutamine, and 50 mg/ml of gentamicin. Cells were grown in suspension for 3 h. The lysate was extracted successively with phenol:chloroform (1:1) and with chloroform:isoamyl alcohol (24:1). Resulting samples were ethanol-precipitated directly and resuspended in TE buffer, adjusted to 0.3 M sodium acetate, and subjected to multiple cycles of washing with 70% ethanol and precipitation with 3.0 M LiCl, repelleted, and resuspended in 0.5 ml of RNA solubilization buffer (10 mM Tris, pH 7.5, 1 mM EDTA, 0.5% SDS) at -20°C by adding 0.05 volume of 5.0 M ammonium acetate and 2.5 μl of ethanol. RNA pellets were washed in 100 mM sodium acetate buffer, 50 mM sodium phosphate, pH 7.0, 0.1% SDS and resuspended in water for 5 min, then with 4 changes of 0.1 × SSC, 10 mM sodium phosphate, pH 7.0, 0.5% SDS at 65°C for a total of 2 h. After washing, air-dried filters were exposed to preflashed x-ray films (49).

Isolation of DNA and Northern Analysis—DNA was isolated from plasmacytoma cells by the LiCl-guanidine monothiocyanate procedure (51). DNA samples from MOPC41 plasmacytoma cells were also used in Southern analysis of genomic DNA. Genomic DNA was isolated by 2-3 min vortexing at maximum speed setting in 1.0 ml of lysis buffer (5.0 M guanidine monothiocyanate, 10 mM EDTA, 50 mM Tris-HCl, pH 7.5, and 8% (v/v) β-mercaptoethanol) (added just before centrifugation of bone marrow). DNA was precipitated at 4°C overnight by the addition of 7 ml of 4.0 M LiCl. Mixtures were transferred to a baked 15-ml Corex tube, and the RNA was pelleted by centrifugation at 9500 g for 20 min in a swinging bucket rotor at 4°C. RNA pellets were washed in 1 ml of lysis buffer containing 50 mM sodium phosphate, pH 7.0, 1.0 mM EDTA, 0.5% SDS, and resuspended in 0.5 ml of RNA solubilization buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% SDS). Mixtures were vortexed, heated at 50°C for 1 min to facilitate dissolution, and then extracted with phenol/chloroform (1:1). RNA was precipitated at -20°C by adding 0.05 volume of 5.0 M ammonium acetate and 2.5 μl of ethanol. RNA pellets were suspended in 10 μl of diethylpyrocarbonate-treated water and quantitated at 260 nm. RNA was resolved by electrophoresis through a 1.4% formaldehyde-agarose gel as described (45), except that the running buffer consisted of 20 mM sodium phosphate, pH 7.1. After electrophoresis, gels were washed for 6 × 10 min with 65°C water, stained with ethidium bromide (0.5 μg/ml) for 10 min, destained, and photographed. Autoradiograms of the RNA was blotted onto nitrocellulose filters, and hybridized with probes in buffer containing 50% formamide, 5 × SSC, 50 mM sodium phosphate, pH 7.0, 10 × Denhardt's solution, 0.1% SDS, 0.1% sodium pyrophosphate, and 250 μg/ml carrier DNA at 42°C for 16-20 h. After hybridization, filters were washed with V,41, were washed for 10 min in 2 × SSC, 0.5% SDS, 0.1% sodium pyrophosphate at room temperature, then four times for 15 min in 0.1 × SSC, 0.5% SDS, 0.1% sodium pyrophosphate at 65°C, and finally with 0.1 × SSC twice at 65°C. Filters probed with human actin were washed in 2 × SSC, 1% SDS, 0.1% sodium pyrophosphate twice for 10 min at room temperature, then twice for 45 min at 65°C. Blots were exposed for 30 min to 2 weeks to preflashed x-ray films at -70°C (49) and quantitation of hybridization signals was performed by densitometric scanning using a Hoefer densitometer linked to a Bio-Rad integrator. For quantitation of the V,41 mRNA, since linear intensities of the various samples could not always be obtained from a single film, several films were scanned and related to one another by a common sample that exhibited linear responses in both films. The V,41 signals were corrected for RNA loading by dividing by the actin signal and related to MOPC41 expression. Since equal amounts of MOPC41 tumor RNA were hybridized, and both the human actin probe as well as the SI94 cell RNA (see Fig. 3, A and B), quantitation of mRNA signals as percent MOPC41 was achieved simply as per μg of RNA loaded and also by way of including a common positive clone standard that had been carefully quantitated with respect to an equivalent mass of MOPC41 RNA.

RESULTS

General Experimental Approach—We decided to utilize the technique of stable integration to investigate the effects of deleting potentially important sequences on immunoglobulin κ gene expression in plasmacytoma cells. This approach allows the introduction gene to be established, maintained, and propagated in bona fide chromatin structures enabling evaluation of the function of a sequence in a nearly normal in vivo environment. We selected electroporation as the method to introduce DNA molecules into cells since previous studies demonstrated that the technique is very efficient and provides low copy number integrants which are desirable for ease in data interpretation (20, 46, 53). Mouse SI94 plasmacytoma cells were chosen as recipient cells since they have not been employed successfully earlier by Atchison and Perry (20) for gene expression studies. Finally, the MOPC41 κ gene was used since its sensitivity to mutations within the intron enhancer has been previously studied extensively by transient expression assays (5, 7, 9). Fig. 1A shows the three constructs that
were employed, termed MAR'-ENH', MAR'-ENH', and MAR'-ENH', which contain or lack deletions within the intron spanning the MAR sequence (0.5 kb) or both the MAR and enhancer sequences (1 kb). We also employed either a short form (SF) or a long form (LF) of these constructs; the long form contains about 8.5 kb of additional 3' sequence, including the natural transcription termination region (41). We introduced vector-free SF and LF constructs into S194 cells by unlinked co-transformation, thereby avoiding the potential influences of adjacent prokaryotic sequences or transcribed genes. In addition, we directly assessed the effects of linkage to a transcribed gene for the LF constructs by employing the newly designed enhancerless selectable marker, termed pE' tk-neo, shown in Fig. 1B; this DNA construct also lacks a MAR. The k gene constructs were inserted in the divergent transcriptional orientation with respect to neo'. The organization of different sequences is designated in the key.

Identification of Transformants by Genomic Southern Analysis—For ease in data interpretation we decided to quantitate expression only of those MOPC41 k gene constructs which had undergone single-copy integrations. We therefore performed genomic Southern analysis on DNA isolated from the G418 resistant clones, first to identify cell lines which had taken up MOPC41 k genes, and second to exclude from these clones those which had integrated either tandem copies or aberrant forms of the introduced DNA. The endogenous S194 transcripts do not yield a detectable hybridization signal in response to this MAR', respectively. In the case of the LF construct, the multiple copies of independently integrated SF and LF constructs, respectively. In the case of the LF construct, the clones obtained from three independent transfection experiments. A Vx probe was used to detect transcripts from the introduced k genes. The endogenous S194 transcripts do not yield a detectable hybridization signal in response to this.
The presence of the intronic MAR contributes to the maximal level of expression of the mouse κ gene although its presence is not absolutely essential for expression.

ThePresence of an Adjacent Transcribed Gene Eliminates the Effect of Deleting the MAR—Previous studies employing transient expression had failed to detect a significant effect of deleting the MAR sequence on transcription (5, 9). Since this work involved the presence of another transcriptional unit on the test constructs, we decided to investigate the effect of a linked active gene on the expression of flanking κ genes. For this purpose we inserted the κ gene constructs into a vector containing an enhancerless selectable marker (Fig. 1B). Linear forms of this construct were then stably integrated into S194 cells, and clones containing single copies of the introduced genes were subjected to Northern analysis. As shown in Fig. 4, the mean level of κ gene expression was reduced only 1.6-fold by deleting the MAR in these linked experiments, a difference that was found not to be statistically significant. In fact, if the highest expressor in each group is eliminated from the analysis, the mean expression levels between these two groups become equal. We conclude that linkage to a transcribed gene minimizes the effect of the MAR deletion.

Downstream Sequences Compensate for Deleting the Intronic Enhancer—We obtained an interesting and somewhat unexpected result when we evaluated the effect of deleting both the MAR and the enhancer in SF and LF constructs that had been transfected by unlinked cotransformation. As expected, the expression of the MAR'ENH' SF construct was almost completely abolished (Fig. 5), exhibiting a mean level of only 0.004% with respect to MOPC41, about a 1000-fold reduction per gene copy compared to the MAR'ENH' SF and LF constructs, but a significant effect on expression of deleting the MAR (p < 0.05). Therefore, these results indicate that the intronic MAR contributes to the maximal level of expression of the mouse κ gene although its presence is not absolutely essential for expression.

The presence of another transcriptional unit on the test constructs, we decided to investigate the effect of the MAR deletion. The mean levels of κ gene expression for the SF and LF, respectively (Fig. 3). Analysis of the statistical significance of these results using a parametric test (Welch's approximation to the Student's t test) and/or a nonparametric test (Mann-Whitney U test) revealed no significant difference between the expression levels of the MAR'ENH' SF and LF constructs, but a significant effect on expression of deleting the MAR (p < 0.05). Therefore, these results indicate that the intronic MAR contributes to the maximal level of expression of the mouse κ gene although its presence is not absolutely essential for expression.

The Presence of an Adjacent Transcribed Gene Eliminates the Effect of Deleting the MAR—Previous studies employing transient expression had failed to detect a significant effect of deleting the MAR sequence on transcription (5, 9). Since this work involved the presence of another transcriptional unit on the test constructs, we decided to investigate the effect of a linked active gene on the expression of flanking κ genes. For this purpose we inserted the κ gene constructs into a vector containing an enhancerless selectable marker (Fig. 1B). Linear forms of this construct were then stably integrated into S194 cells, and clones containing single copies of the introduced genes were subjected to Northern analysis. As shown in Fig. 4, the mean level of κ gene expression was reduced only 1.6-fold by deleting the MAR in these linked experiments, a difference that was found not to be statistically significant. In fact, if the highest expressor in each group is eliminated from the analysis, the mean expression levels between these two groups become equal. We conclude that linkage to a transcribed gene minimizes the effect of the MAR deletion.

Downstream Sequences Compensate for Deleting the Intronic Enhancer—We obtained an interesting and somewhat unexpected result when we evaluated the effect of deleting both the MAR and the enhancer in SF and LF constructs that had been transfected by unlinked cotransformation. As expected, the expression of the MAR'ENH' SF construct was almost completely abolished (Fig. 5), exhibiting a mean level of only 0.004% with respect to MOPC41, about a 1000-fold reduction per gene copy compared to the MAR'ENH' constructs (Fig. 3, C and D). In striking contrast, the same deletion in the LF construct resulted in a mean expression value of 0.56% with respect to MOPC41 RNA standard, only 11-fold lower than the mean value of 6.2% exhibited by the corresponding intact LF construct MAR'ENH' (Fig. 3D). Thus, the 8.5 kb of downstream sequences yield approximately a 150-fold compensation for deletion of the MAR and en-

**Fig. 3. Deletion of the intronic MAR reduces κ gene expression.** Total RNA was isolated from independent clones containing SF MAR'ENH' or MAR'ENH' constructs (A) or the corresponding LF constructs (B). RNA samples from S194 and a 2-fold dilution from MOPC41 tumor are included as controls. Duplicate Northern blots were hybridized with the Vκ and actin probes. Indicated below the blots are the number of intact gene copies of the constructs which independently integrated at different chromosomal positions in each clone. Densitometric scans of A and B quantitated as shown in C and D. The dashed lines and numbers represent the means for each group.

**Fig. 2. Genomic Southern analysis of G418-resistant S194 clones.** A, identification of S194 cell clones cotransfected with the MAR'ENH' LF construct. Genomic DNA, isolated from 21 G418-resistant clones, was digested with BgIII, resolved on a 0.9% agarose gel, and transferred to nitrocellulose. The resulting filters were hybridized with a Jκ probe. Lanes marked Std contained dilutions of the recombinant plasmid bearing MAR'ENH' LF cut with EcoRI-BgIII. Lanes depicted a-f correspond to positive clones. Shown on the left are the migration positions of size standards corresponding to a HindIII digest of bacteriophage λ DNA and a HaeIII-digest of φX174 DNA. B, genomic DNA was isolated from clones a-f, digested with the indicated enzymes, and subjected to Southern analysis as indicated. κ3, endogenous κ genes; κf, transfected κ genes; arrows indicate the band positions characteristic for tandem integrations. Lane symbols are as in A.
the effect of deleting the MAR. Total RNA was isolated from independent clones obtained from linked transfection experiments of MAR+ENH+ LF and MAR+ENH+ LF constructs. Data were quantitated as described in Fig. 3.

hancer. Since there was no significant difference in expression between the SF and LF forms of the MAR+ENH+ constructs (Fig. 3, C and D), we conclude that the downstream sequences primarily compensate for the enhancer deletion and not for deletion of the MAR. Since three out of four MAR+ENH+ SF clones shown in Fig. 5 actually gave rise to correct-sized transcripts that could be visualized after autoradiographic exposure for 2 weeks (data not shown), the inability of these clones to express must indeed be due to inefficient expression as opposed to any aberration occurring in the integrated genes, a point also confirmed by Southern blot analysis (data not shown). Further analysis, however, has revealed that in two out of eight clones isolated from MAR+ENH+ SF transfections, expression was 40–80-fold better than the mean value reported above. Due to this low frequency of occurrence, we suggest that these abnormally high expressors arise from integration of the transduced gene next to endogenous enhancers.

When the MAR+ENH+ LF construct was tested by linkage to the enhancerless selectable marker, the expression level increased further about 2-fold (from 0.56% for unlinked to 1.2% for linked per gene copy), leading only to an overall 6-fold reduction in the mean level of expression relative to the intact gene (data not shown). Thus, just as was the case for the effect of the MAR deletion on κ gene expression, linkage to a transcribed gene also dampened the additional effects of deleting the enhancer.

**DISCUSSION**

**MARs Appear to Serve a Positive Role in Transcription**—The positioning of MARs next to enhancers has been evolutionarily conserved in the immunoglobulin genes (31, 32, 54) suggesting that these attachment site sequences may play a role in transcription. We tested this hypothesis directly by determining the effect of deleting the element from a functionally rearranged immunoglobulin κ gene after stable integration into the genome of plasmacytoma cells. Our results demonstrate that deletion of the MAR reduced the mean steady-state level of κ mRNA about 3–4-fold. Interestingly, a natural polymorphism in the κ gene of different strains of rabbits exhibits a quantitative effect very similar to our results; an allele that has spontaneously deleted a 160-bp segment of the MAR is expressed 4-fold lower than its undeleted counterpart in heterozygotes (37).

The quantitative reduction in κ mRNA levels upon deleting the MAR is probably not a trivial effect of altering the distance between the promoter and the intrinsic enhancer. In the intact MOPC41 κ gene these sequences are 3.8 kb from one another, and the MAR deletion brings them closer by only 550 bp. Furthermore, different J regions are normally utilized for κ gene recombinational events, leading to natural variation in promoter and enhancer distances of more than 1 kb.

The quantitative reduction in κ mRNA levels upon deleting the MAR is also probably not a trivial consequence of altering primary transcript stability or splicing efficiency, since coupling the same construct to a transcribed selectable marker essentially eliminated the effect of the mutation. In addition, earlier transient expression assays employing vectors containing linked active genes failed to detect any effect of deleting the MAR on steady-state mRNA levels (5, 9). These observations suggest that the role of the MAR may be to set up a favorable chromatin environment for transcription. If one preselects for a transcriptionally poised nuclear location, then the MAR appears to be no longer necessary for optimal transcription. We favor this interpretation since the selectable marker employed in these linked transformation experiments lacks both a MAR and an enhancer.

Other studies also implicate MARs as playing a positive role in transcription. The Drosophila fushi tarazu gene appears to be more sensitive to chromosomal position effects when lacking terminal MARs (discussed in Ref. 56). A β-globin minilocus can be created that exhibits expression that is roughly proportional to gene copy number and independent of chromosomal integration site (57); this gene construct appears to have MARs at each end (58). In these cases, however, the MARs studied were intergenic, unlike the intrinsic MAR of the κ gene. Furthermore, since the only significant MAR associated with the κ gene constructs employed here is intronic (31), perhaps the high sensitivity of the intrinsic κ gene constructs to chromosomal position effects is related to a lack of MARs at either end. Such sequences could serve as a boundary to buffer adverse effects from adjacent chromatin structures (55). We are currently testing this hypothesis.

How does the intrinsic MAR function to increase κ gene expression? The sequence could serve to target integration events to favorable chromosomal locations, although we have not detected any effect on the transformation frequency upon deleting the sequence (data not shown). On the other hand, the MAR could target the chromatin to a specific subnuclear location enriched in the transcription machinery. The MAR also could serve as a substrate for topoisomerase II (31, 32) to relax torsional stress introduced by transcription (35, 36).
Immunoglobulin κ Gene Expression in Plasmacytoma Cells

Clearly more work will be necessary to distinguish among these and other alternatives.

Downstream Sequences Compensate for Deletion of the Enhancer but not the MAR—Whereas overall expression was reduced 3-4-fold upon deletion of the MAR in the short and long forms of the MOPC41 κ gene constructs, additional deletion of the enhancer completely inactivated the short form, a more dramatic result than has been previously observed for the same gene (5, 9). It should be noted, however, that these previous studies used transient expression assays as opposed to stable integration, and other active transcriptional units were linked to the test constructs in addition to the presence of the polyoma enhancer and origin of DNA replication (5, 9). These differences could serve to dampen the effects of mutations on gene expression. Such has been demonstrated to be the case when the polyoma origin is present (9) or when another actively transcribed gene is linked to the test gene (Fig. 4).

Deletion of the MAR and enhancer, which led to inactivation in the short form construct, was compensated about 150-fold by the downstream sequences present on the long form. The MAR-ENH-LF construct expressed at a mean level only 11-fold lower than that of the intact gene. If the effect of the MAR deletion, then deletion of the enhancer, but not the MAR, would be predicted to cause only a 3-fold reduction in the mean level of expression of the long form.

The nature of the cis-acting elements that compensate for deletion of the intronic enhancer remain to be elucidated. Previous studies that led to the identification of the intronic enhancer employed transient expression and the gene constructs studied lacked the downstream region included here (5, 7, 9). Contained within the 8.5 kb of the downstream region is the natural transcription termination region, which resides about 2 kb 3’ of the poly(A) addition site (41). In addition, the downstream region contains about 5 kb of repetitive sequences, including the dispersed elements R, B1, and B2, as well as simple sequence tandem repeats (59–62).

Interestingly, this downstream region has been implicated to be necessary for expression in transgenic mice (63), and in the accompanying paper (51) we demonstrate that the long form of the MOPC41 gene is expressed tissue-specifically in transgenic mice in spite of the lack of the intronic enhancer and MAR. Furthermore, after submission of this work, Meyer and Neuberger (64) reported the identification of a B-cell-specific enhancer in this downstream region.

The function of immunoglobulin gene enhancers appears to be connected to the stage of B cell development. In pre-B cells, transcriptional activation of the κ-immunoglobulin gene requires the continual presence of NF-κB, one of the factors that associates with the intronic enhancer (20, 65). However, in the plasmacytoma S107, NF-κB appears not to be required for active endogenous κ gene transcription (20, 66), consistent with the results reported here that other elements can substitute for the intronic enhancer. In addition, previously activated κ gene transcription is not enhanced by NF-κB in a particular hybridoma cell line (66), and in another system transcription can be induced by γ-interferon without activation of NF-κB (67). Similarly, the presence of the heavy chain enhancer is apparently necessary for continuous active transcription of linked genes in pre-B cells (68), but spontaneous deletion of the heavy chain enhancer can occur without transcriptional inactivation in plasmacytoma cells, again suggesting compensation by other elements (69–73).

Acknowledgments—The technical assistance of L. Garrett, Y. Mehrotra, and D. Shade is gratefully acknowledged. We thank D. Baltimore, P. Bird, P. Leder, R. Myers, and J. Sambrook for kindly providing recombinant DNA clones. We greatly appreciate the stimulating discussions with Dr. R. G. Mage.

REFERENCES

40. Xu, M. (1989) Analysis of Transcription of the Mouse Immunoglobulin κ Gene. Ph.D. dissertation. The University of Texas Southwestern Medical Center, Dallas, TX
Immunoglobulin Gene Expression in Plasmacytoma Cells