The importance of the 3′-enhancer region in immunoglobulin κ gene expression

Kerstin B.Meyer, Melanie J.Sharpe, M.Azim Surani[1] and Michael S.Neuberger*
MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH and [1]Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, UK

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ABSTRACT

The first enhancers to be identified in the immunoglobulin gene loci are located in the J-C intron. However, deletion of the immunoglobulin κ intron-enhancer has little effect on the transcription of κ transgenes. Here we ask whether the second κ enhancer which we recently identified at the 3′-end of the locus plays a role in κ gene expression. We show that its omission leads to 20 – 40 fold lower expression of κ transgenes and to poor allelic exclusion. Transfection experiments show that activity of the 3′-enhancer, like that of the κ-intron enhancer, can be induced in a pre-B cell line by incubation with bacterial lipopolysaccharide. Whereas induction of the κ-intron enhancer is due to induction of NF-κB activity, deletion mapping of the 3′-enhancer localises its activity to a 50 nucleotide region that lacks an NF-κB site; indeed the 3′-enhancer allows κ expression in a cell line which lacks NF-κB. Thus, both the 3′- and intron-enhancers can be induced at the same stage of differentiation but by distinct pathways. Furthermore, unlike the intron-enhancer, the 3′-enhancer plays a critical role in the transcription of rearranged immunoglobulin κ genes.

INTRODUCTION

The regulation of transcription in mammalian cells involves multiple elements that act together to ensure high levels of gene expression. As regards the immunoglobulin [Ig] genes, the first enhancer elements were identified in the major introns of both the heavy [IgH] (1–3) and κ light chain (4,5) loci. These enhancers are sufficient to direct lymphoid-specific gene expression when linked to heterologous genes (6,7). All three immunoglobulin loci also contain lymphoid-specific promoters upstream of their V-region genes (8–10).

Several lines of evidence indicate that a model in which Ig transcription is solely regulated by a V gene promoter coupled to an intron-enhancer must be an oversimplification. In the case of the κ locus, transgenes that just contained all the previously identified κ transcription elements were nevertheless not expressed at high level unless present in high copy number (11,12, 41). Conversely, the endogenous κ gene in the S107 plasmacytoma is well expressed despite the fact that the intron-enhancer is not active in this line (13,14). As regards the IgH locus, several cell lines actively transcribe their IgH genes although the intron-enhancer has been deleted (15–18). These observations led us to look for additional lymphoid-specific Ig enhancers and we identified such an enhancer at the 3′-end of the κ locus, sandwiched between the C_κ exon and the RS sequence (19). An enhancer has also recently been identified at the 3′-end of the IgH locus (20). However, a role for these additional enhancers in Ig transcription has not been established.

Here we address the question of whether the 3′ enhancer does indeed play a role in κ light chain expression. We show that inclusion of DNA spanning this region is necessary in order to achieve high level κ expression in transgenic mice. Both enhancers can be activated by lipopolysaccharide treatment of transfectants of pre-B cell lines. However, unlike the κ-intron enhancer, the activity of the 3′-enhancer is not dependent on the NF-κB transcription factor and the 3′-enhancer therefore allows κ expression in a cell-line that lacks NF-κB. Thus, the two enhancers must be activatable by different pathways.

MATERIALS AND METHODS

DNA and plasmids

The short κ construct used for creating the Sκ transgenic mice has been previously described (12). For the creation of the long construct, a rat C_κ fragment of the LOU allele (21) was modified by site directed mutagenesis (22) to create *Hpa*I and *Bgl*II sites at positions corresponding to those found in mouse C_κ (23). The 470 nt *Hpa*I–*Bgl*II rat C_κ fragment was then excised and this fragment ligated in place of the equivalent mouse C_κ fragment in a mouse genomic *Xba*I–*Bam*HI subclone in pUC18. This composite C_κ was combined with the rearranged mouse V_κOx 1 (1.4 kb *Eco*RI -*Xba*I) to form a 5.2 kb *Eco*RI-*Bam*HI fragment in a pUC vector with a *Sma*I site flanking the *Bam*HI. An 8 kb *Sac*I-*Eco*RI fragment containing mouse genomic DNA downstream of C_κ (including the 3′-enhancer) was subcloned into pUC18, excised as a *Sma*I-*Eco*RI fragment, and ligated together with the 5.2 kb V_κOx-1/ composite-C_κ *Eco*RI-*Sma*I fragment in the *Eco*RI site of the vector pSV2neo.

Derivatives of the long construct that differed in the C_κ

* To whom correspondence should be addressed

3'-region were created as described in the legend to Figures 4 and 7. For deletion analysis of the 3'-enhancer, deletions within fragment **f** were generated using a Bluescript KS+ vector; single-stranded DNA was prepared by superinfection with helper virus and site directed mutagenesis carried out as described elsewhere (22). Plasmids pβ128 and πSVHPα2 (31) were gifts from K. Weston and R. Treisman. Plasmid pk3'βG contains the *Xba*I-*Sac*I 3'-enhancer fragment introduced upstream of an enhancerless β-globin gene in a pUC12 derivative that contains a *neo* transcription unit (gift of P. Berg) under control of the HSV *tk* promoter.

Transgenic mice and hybridomas

Derivation of transgenic mice lines carrying the Sκ construct has been described (12,24). Previously these have been referred to as κOx lines but in this work κOx^2 is designated Sκ^2, κOx^4 is Sκ^4 *etc*. The Lk mice were generated by microinjection of a vector-free *Eco*RI 13.5 kb fragment into the pronucleus of (C57BL/6×CBA) female×(C57BL/6×CBA) male eggs as previously described (7). Positive offspring were identified by Southern blot analysis of tail DNA or by serum ELISA and bred with BALB/c mice. Hybridomas were established from adult mice by fusion of spleen cells with the myeloma NSO (25).

Serological assays and immunofluorescence

Transgenic κ chains were detected by an ELISA using the mouse anti-rat κ monoclonal antibody MARK-1 as previously described (12). Cytoplasmic immunofluorescence of permeabilised spleen cells attached to slides (12) was performed using FITC-conjugated OX-20 (Serotec) to detect mouse κ; to detect transgenic κ, we used biotinylated sheep anti-rat antiserum in the presence of 1% normal mouse serum chain followed by streptavidin conjugated to TEXAS-red (Amersham).

DNA transfection and ribonuclease protection assay

MPC11 BU4 was obtained from B. Wasylyk; S107 (originally from the Salk Cell Bank), NSO (26) and 70Z/3 (27) from our laboratory culture collection and maintained in Dulbecco's modified Eagle's medium containing 10% foetal calf serum. Stable transfection was achieved by electroporation (28); the S107, NSO and 70Z/3 transformants were selected 24 h after transfection by resistance to G418 (Gibco) at an initial concentration of 0.5 mg/ml and this selective concentration increased to 1mg/ml (S107) or 2mg/ml (NS0, 70Z/3) 6 days after transfection. MPC11 was transiently transfected by calcium phosphate coprecipitation (29) and total cytoplamic RNA was prepared by NP40 lysis and subsequent phenol extraction of the cytosolic fraction 38–42 h later. Ribonuclease protection assays (30) were carried out using β-globin, hsp cognate or C_κ probes as previously described (8,31).

RESULTS

High level expression in transgenic mice of κ genes that include the 3'-enhancer

Transgenic mouse lines were established in order to ascertain whether sequences located downstream of Cκ affected κ gene expression. The transgenes were composed of a mouse V_κ gene linked to a rat Cκ. The presence of the rat constant region meant that the transgene could be distinguished from the endogenous κ at the protein level using anti-rat κ antibodies and at the nucleic acid level by a ribonuclease protection assay. The V-region was characteristic of antibodies directed against the hapten phenyl-oxazolone. Two transgenes were used: a long one (Lκ) which includes both the intron- and 3'-enhancers and a short one (Sκ) which contains only the intron-enhancer (Figure 1). Four lines of transgenic mice (designated Sκ^2, Sκ^4, Lκ^3 and Lκ^6) which harboured between 2 and 5 copies of the transgene were used for most of the analyses, although a high-copy (Sκ^5) line which carried 30–50 copies of the short κ construct was also included for comparison.

Transgene expression was analysed by immunofluorescence of splenic B cells from adult mice. In Sκ^4 animals carrying the short κ construct, transgene expression was only clearly detectable in some 5–7% of splenic B cells; nearly all the B cells expressed an endogenous mouse light chain (Figure 2). A similar pattern of expression was found in the Sκ^2 mice (not

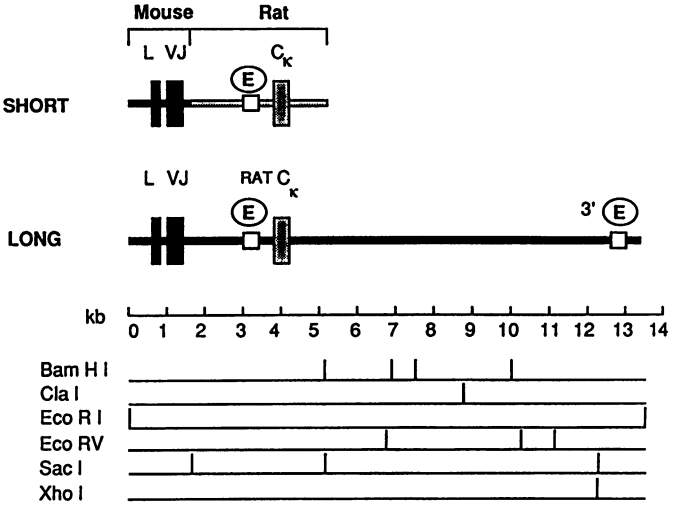

Fig. 1. The κ gene constructs. Sequences of mouse origin are shown thickly stippled, rat sequences are thinly stippled and enhancers are in open boxes.

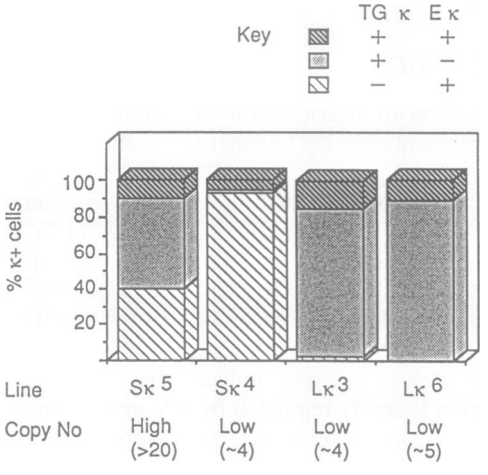

Fig. 2. Immunofluorescence analysis of κ gene expression in the transgenic mice. The histograms depict the percentage of κ$^+$ cells in the spleens of the adult transgenic mice that stained for either: transgenic rat κ and endogenous mouse κ (TGκ$^+$, Eκ$^+$); transgenic κ only (TGκ$^+$, Eκ$^-$) or endogenous κ only (TGκ$^-$, Eκ$^+$).

shown). The low level of expression can to some extent be compensated for by a large increase in transgene copy number since in the $S\varkappa^5$ mice (which harbour a high transgene copy number) a greater proportion of the B cells stained for the transgene (Figure 2).

The results obtained with the long $\varkappa$ construct contrast sharply with those obtained with the short one. In the $L\varkappa^3$ line, 80–85% of the adult splenic B-cell population expressed only the transgene, with the remaining 10–15% of the B cells coexpressing an endogenous light chain (Figure 2). Transgene expression is even more dominant in the $L\varkappa^6$ line with about 90% of the splenic B cells expressing only rat $C_\varkappa$. Thus expression levels from the long $\varkappa$ construct are sufficient to effect allelic exclusion.

The difference between the transgenes is also evident from the titres of serum antibody. ELISA assays reveal that whereas the titre of antibody containing transgenic $\varkappa$ chains in the sera of adult $S\varkappa^2$ and $S\varkappa^4$ mice is about 100 mg/ml, the corresponding titre in the $L\varkappa$ mice is some 10–40 fold higher. However, the major contribution of cellular selection in determining the overall *in vivo* pattern of transgene expression (12) makes such comparisons of serum titres very crude. We therefore compared transgene expression in hybridomas generated by fusing spleen cells from the transgenic mice with the NS0 plasmacytoma. As regards both the $L\varkappa^3$ and $L\varkappa^6$ mice, the pattern of light chain expression in the hybrids paralleled what was observed in the immunofluorescence analysis of the splenic B cells. In contrast, in the $S\varkappa^4$ mice we found that 60% of the hybrids that made an endogenous $\varkappa$ chain now co-expressed a transgene light chain; the same held true for the $S\varkappa^2$ mice. These results with the hybridomas obtained from the $S\varkappa$ mice differ from what was observed with the primary B cells. This presumably reflects the fact that transgene expression in many primary cells is below our level of detection; this low level is increased on fusion. Titrations of transgene expression in supernatants from representative clones of the $S\varkappa$ and $L\varkappa$ mice are shown in Figure 3; the transgene expression in the hybridomas from the low-copy $L\varkappa$ mice is some 20–40 fold higher than from the low-copy $S\varkappa$ mice. Thus, the short $\varkappa$ transgene is only poorly expressed and is relatively ineffective in mediating feedback inhibition of endogenous light chain gene rearrangement. However, results with the long $\varkappa$ construct show that sequences downstream of the constant region have a dramatic effect in increasing $\varkappa$ gene expression in transgenic mice and in allowing effective allelic exclusion of endogenous $\varkappa$ expression.

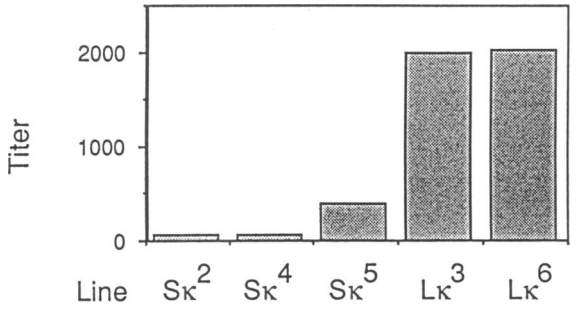

Fig. 3. Titration of the transgenic light chain secreted by hybridomas from the transgenic mice. Supernatants of hybridoma cells grown to saturation in DMEM/10% FCS were assayed for rat $\varkappa$ determinants. Titres are given as the dilution of supernatant required to react with and sequester 50% of the anti-rat $\varkappa$ antibody used in the ELISA. The assays have been carried out several times using multiple clones; in each batch of assays of hybridoma supernatants from the same mouse line, the scatter of individual titres was such that the s.e.m. always amounted to less than 25% of the mean.

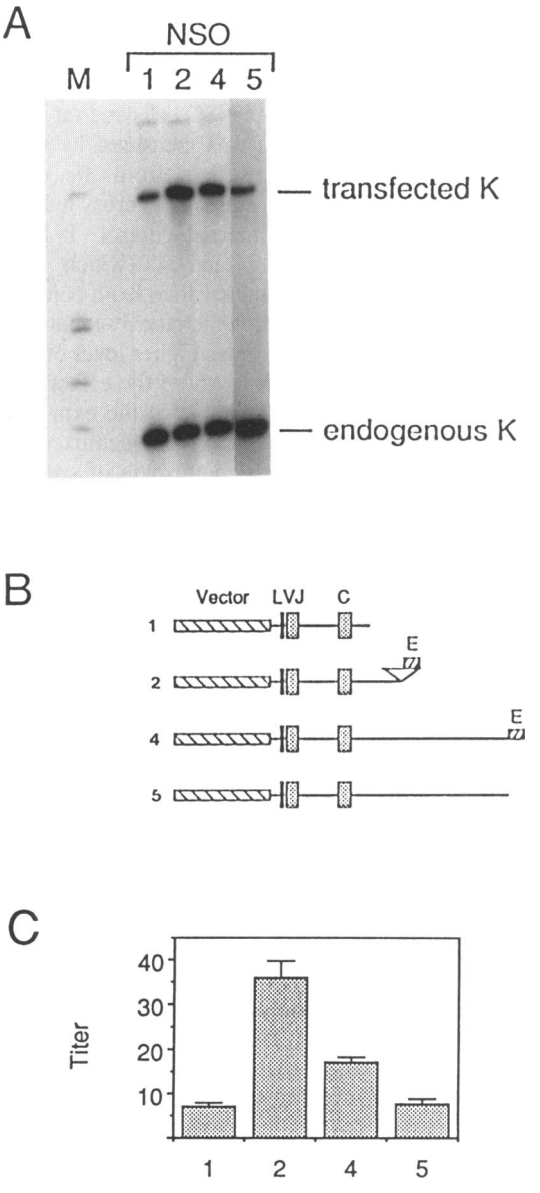

Fig. 4. Expression of $\varkappa$ genes transfected into NS0. **(A)** Ribonuclease protection assay of transcription from the transfected $\varkappa$ gene of NS0 cells stably transfected with the constructs shown in panel B. For each construct, transcripts of the transfected and endogenous $\varkappa$ genes were assayed in samples of RNA that were prepared from pools of 12 wells each of which contained multiple clones. The positions of size markers (a *Hpa*II digest of pBR322) are shown in the lane marked 'M'. **(B)** The plasmids used. All are based on the long construct depicted in Fig. 1. Plasmid 1 contains the *Eco*RI-*Bam*HI $V_\varkappa$-$C_\varkappa$ segment of the long construct cloned between the *Eco*RI and *Bam*HI sites of pSV2neo; the *Bam*HI site is located 1.2 kb downstream of the $C_\varkappa$ polyadenylation site; plasmid 2 was created by digesting plasmid 4 with *Eco*RV and self-ligation; plasmid 4 is the long construct depicted in Fig. 1 cloned into the *Eco*RI site of pSV2neo; plasmid 5 carries the *Eco*RI-*Xho*I $V_\varkappa$-$C_\varkappa$ segment of the long construct cloned between the *Eco*RI and *Xho*I sites of a pSV2neo-derivative that has *Xho*I linkers in the *Bam*HI site. **(C)** Expression of the transgenic $\varkappa$ chain in supernatants of individual wells of NS0 transfectants depicted as a histogram. Cells were grown to saturation in DMEM/10% FCS. Titres are given as the dilution of the culture supernatant that is required to react with and sequester 50% of the anti-rat $\varkappa$ antibody used in the ELISA and are given as the mean from more than 10 individual clones. The standard error of the mean is indicated by error bars.

The 3'-enhancer increases κ expression in a stably transfected plasmacytoma

Presumably it is the 3'-enhancer that is responsible for the increased transcriptional activity of the long as opposed to the short κ construct. In order to test this directly, we resorted to stable transfection assays in the NS0 plasmacytoma. A series of constructs with and without the 3'- enhancer were generated that were based on the long κ construct. The constructs 1, 2, 4 and 5 (Figure 4) were transfected into NSO cells and rat κ expression in pools of stable transfectants was measured by ribonuclease protection assays. Sterile transcripts from the endogenous, aberrantly rearranged mouse κ gene of NS0 cross-hybridized with the probe and served as an internal control. It is clear that expression from constructs 2 and 4 (which include the 3'-enhancer) was significantly higher than from constructs 1 and 5 (which do not). Construct 2, in which the 3'-enhancer has been brought closer to the C_κ exon, gives higher level of κ transgene expression than does construct 4 where the effect is relatively modest (see also ref. 32). To ensure that the expression levels seen in ribonuclease protection assays from the mixed populations are not biased due to differential outgrowth of specific clones, we analysed serum titres of rat κ chains in individual wells which represent distinct integration events. The results (Figure 4C) reflect those seen in the ribonuclease protection assays with the pools. Thus, the 3'-enhancer does makes a difference to κ gene expression in stably transfected plasmacytoma. The effect is considerably less than that observed with the transgenic mice. This is scarcely surprising as the κ transcription unit in the transfected NS0 is linked to the active *neo* gene with its adjacent SV40 enhancer; this could compensate to a large extent for the lack of the downstream enhancer in constructs 1 and 5.

The 3'-enhancer is inducible in the 70Z/3 cell line

The expression of the κ locus is induced at the pre-B cell stage. The κ-intron enhancer is inducible in the 70Z/3 cell line which carries a rearranged but transcriptionally silent κ gene. This correlates with the induction of the nuclear factor NF-κB, a component which is essential for the funtion of the κ-intron enhancer (33,34). The observation here, that the presence of the 3'-enhancer on a κ transgene is important in allowing it to mediate allelic exclusion of endogenous gene rearrangement, suggested that the 3'-enhancer might be active at an early stage in B cell development. We therefore analysed the activity of the 3'-enhancer in 70Z/3 cells. Plasmid pk3'βG, which carries the 3'-enhancer linked to a β-globin reporter gene cloned into a vector that contains a *neo* gene under control of the enhancerless herpes simplex virus thymidine kinase promoter, was transfected into 70Z/3 by electroporation. The cells were then plated out in 24 wells and G418-resistant clones appeared in all wells; these were pooled for subsequent analysis of transcriptional activity of the β-globin reporter gene. As internal control, a constitutively expressed heat shock cognate gene was employed. Despite the fact that stable transfection using a selectable marker probably ensures that the exogenous gene is present in a region of active chromatin, the β-globin gene was transcriptionally silent in the population of transfectants. However, after 16 hours of treatment with LPS, β-globin transcripts were clearly detectable (Figure 5, lanes 1 and 2); the same treatment also induced expression of the endogenous κ gene (Figure 5, lanes 3 and4). Analogous results were obtained with transfectants of another pre-B cell line, 300−19P (data not shown). As LPS does not induce expression from an enhancerless β-globin gene in transfected pre-B cells

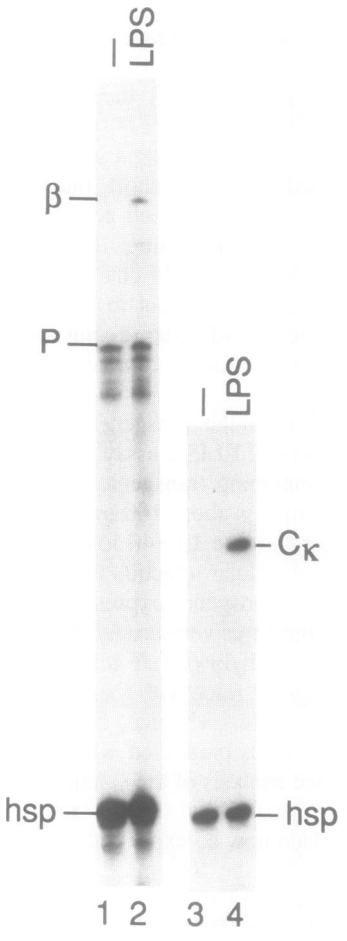

Fig. 5. The 3'-enhancer is inducible in 70Z/3 cells. Ribonuclease protection mapping of RNA from 70Z/3 cells stably transfected with pk3'βG either uninduced (−) or treated for 16 h with LPS. The same RNA samples were mapped with probes for β-globin and for an hsp-cognate [as an internal reference] (lanes 1 and 2) and C_κ and hsp-cognate probes (lanes 3 and 4). Correctly initiated transcripts are indicated; P denotes undigested probe.

(8), it appears that activity of the 3'-enhancer can be induced by LPS in pre-B cells.

Functional dissection of the 3'-enhancer

The activty of the κ3' enhancer thus parallels that of the κ-intron enhancer raising the possibility that the two elements are both controlled by the same nuclear factor. The induction of κ transcription in several pre-B cell lines is known to correlate with the activation of the intron enhancer and the translocation of one of its cognate binding factors (NF-κB) into the nucleus (35). We have previously noted the presence of a sequence related to the NF-κB binding motif in the region of the 3'-enhancer (19); we therefore wished to delimit the active part of the 3'-enhancer more precisely in order to discover whether the activation of the intron- and 3'-enhancers could be induced by a common mechanism.

The 800 base pair *Sac*I-*Xba*I 3'-enhancer fragment was subcloned and linked to a human β-globin gene, which served as reporter gene in transient transfection assays. The constructs carrying the inserts shown in Figure 6A were transfected into the MPC11 plasmacytoma along with a human α-globin reference plasmid; cytoplasmic RNA was prepared 38−42 hrs after transfection. The amount of β- and α-globin RNAs were measured by ribonuclease protection assay (Figure 6C).

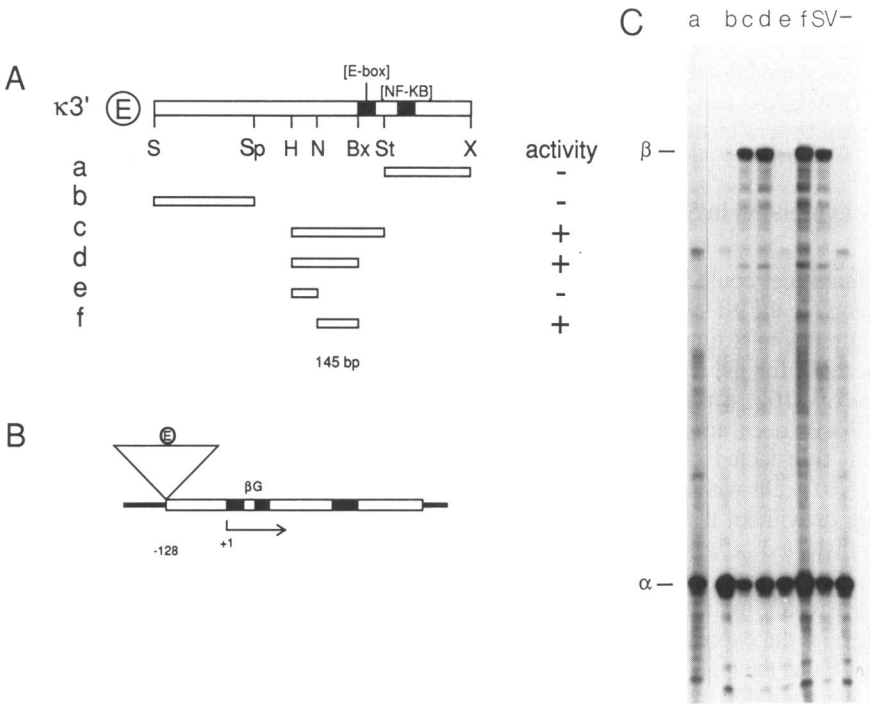

Fig. 6. Delimitation of enhancer activity. (**A**) Map showing the subfragments of the 808bp *Sac*I-*Xba*I k3'-enhancer that were assayed for activity. Restriction sites are abbreviated: S, *Sac*I; Sp, *Ssp*I; N, *Nco*I; Bx, *Bst*XI; H, *Hae*III and St, *Sty*I. For the latter two enzymes, only relevant sites are shown. The solid boxes represent sequence homologies identified previously (Meyer and Neuberger, 1989) to the IgH-intron and the κ-intron enhancers. (**B**) A schematic representation of plasmid pβ128 used for the enhancer assays. (**C**) Ribonuclease protection assay of β- and α-globin RNAs in MPC11 cells transiently transfected with pb128 derivatives carrying enhancer subfragments fragment **a**–**f**, the SV40 enhancer (SV) or no enhancer (–) at position –128 of pβ128.

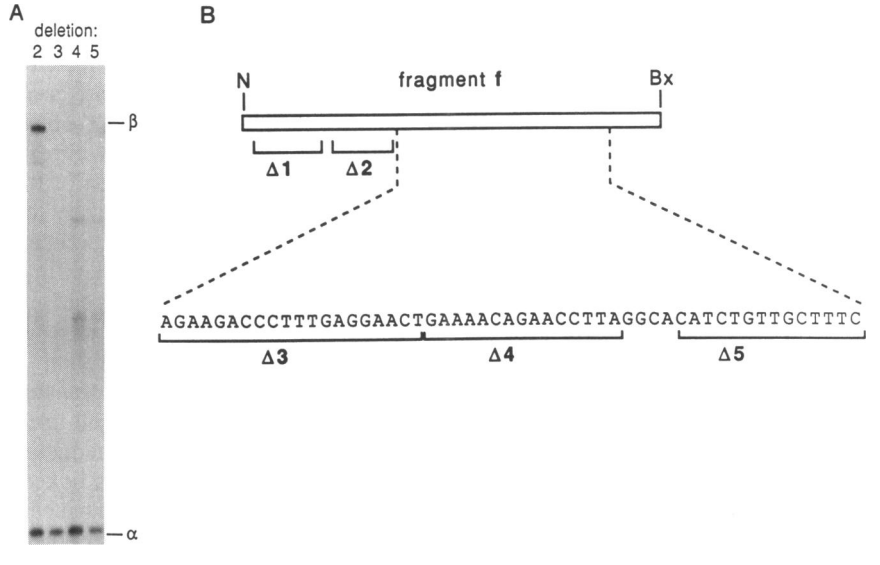

Fig. 7. Functional deletional analysis of enhancer subfragment **f**. (**A**) MPC11 cells were transfected with a Bluescript KS+ vector (Stratagene) carrying the deletion derivatives of enhancer subfragment **f** placed 128 nt upstream of the human β-globin gene. An α-globin reference plasmid was co-transfected and globin transcripts were mapped by ribonuclease protection assays. (**B**) Sequence of the deletions which abolish enhancer activity. Internal deletions were made within enhancer subfragment **f** with the following deletion endpoints using the numbering of ref. (19): Δ1 deletion (nt 389–411, data not shown); Δ2 (nt 416–436); Δ3 (nt 437–456); Δ4 (nt 457–471) and Δ5 (nt 476–489). The deleted DNA was substituted by the sequence CTCGAG.

Comparison of the activities of the various subfragments reveals that full activity was contained with a 145 base pair region (fragment f). This sequence does not overlap the homologies that we had previously noted to the NF-κB binding, the E2/E3 region of the mouse IgH intron-enhancer or the interferon consensus. Thus the activity of the enhancer must be caused by sequence motifs that we had not previously identified on the basis of homology searches. To locate such motifs more precisely, we made a number of approximately 20 base pair deletions within fragment f by site-directed mutagenesis. Enhancer activity is not

impaired by deletions 1 or 2, but deletions 3, 4 and 5 abolish function (Figure 7). Interestingly, Δ3 spans a sequence (TTTGAGGAA) which shows good homology to a region of the mouse IgH enhancer (TTTGGGGAA) that binds a nuclear factor and is implicated in lymphoid-specific transcriptional activity (36–38).

The 3′-enhancer allows κ expression in S107 cells which lack NF-κB

Since the activity of the 3′-enhancer could be located to a region that does not include homology to the NF-κB binding motif, NF-κB is presumably not required for its function. Therefore, the 3′-enhancer might well account for the transcriptional activity of the endogenous κ gene of S107, a plasmacytoma cell line which lacks functional NF-κB, does not activate the κ-intron enhancer and in which many transfected κ genes are silent (13). To test this, we transfected S107 cells with constructs that carried the rearranged κ gene with or without the 3′ enhancer (Figure 8). Expression of the transfected κ gene was analysed by ribonuclease protection assays from pools of stable transfectants. Whereas constructs 2 and 3 which both carry the 3′-enhancer were transcribed at high levels, the expression of construct 1 is scarcely detectable. To ensure that the high levels of transcription seen for construct 2 and 3 were not due to single positive clones having outgrown an otherwise negative population, we assayed individual wells and found that for both these constructs all wells tested were positive for rat C_κ with high expression levels. In sharp contrast, expression levels of construct 1 were below the levels of detection in all wells tested. These results strongly support the proposal that it is the 3′-enhancer which drives expression of the endogenous κ gene of S107 and demonstrates that NF-κB is indeed not required for the activity of this enhancer. Interestingly, the presence of the SV40 enhancer in the vector is not able to compensate for the lack of the 3′-enhancer in construct 1; this is in keeping with the fact that the SV40 enhancer is relatively weak in S107 cells, possibly because its activity in plasmacytoma cells depends considerably upon NF-κB (13).

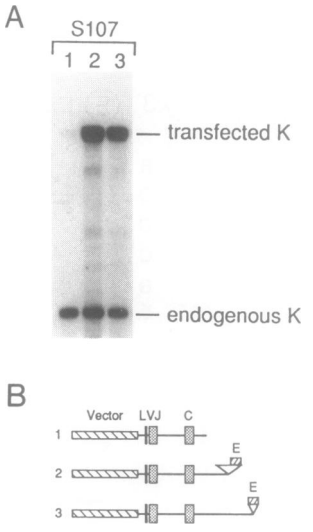

Fig. 8. The 3′-enhancer allows expression of transfected κ genes in the S107 plasmacytoma. (**A**) Ribonuclease protection assay of κ transcription in stable transfectants of plasmacytoma S107. RNA was prepared from pools of 24 wells. (**B**) Plasmids 1 and 2 are described in the legend to Fig. 4; plasmid 3 was derived from the plasmid 4 (described in Figure 4) by internal deletion using *ClaI* and *XhoI*.

DISCUSSION

We were originally moved to look for additional transcription elements in the κ locus because of the poor transcriptional activity of short κ transgenes and because the endogenous κ locus of plasmacytoma S107 is transcribed despite the lack of an active intron-enhancer in this cell line. We identified an additional enhancer which was located downstream of the constant region (19). Here we show that this 3′-enhancer does indeed play a role in κ gene expression, both in transgenic mice and in transfected cell lines. This enhancer is in fact located about 13 kb downstream of the promoter of a functionally rearranged κ gene; however there are already precedents for functionally important transcription elements being located at considerably greater distances from the transcription start site (see, for example ref. 39).

Several groups have established mouse lines transgenic for rearranged κ genes in order to answer questions concerned with the feedback regulation of immunoglobulin gene rearrangement or immunological tolerance. A detailed comparison of the results obtained here with those from other transgenic mice is complicated by the facts that (i) the different transgenes are not matched; (ii) high transgene copy number can compensate for absence of the 3′-enhancer and (iii) integration position could affect transcriptional activity, although we have not seen strong effects of this nature here. Nevertheless, the efficacy of the previously described transgenes in directing κ gene expression is largely consistent with the results we obtain here. Thus, good levels of expression were achieved with long transgenes which included the 3′-enhancer; much poorer expression was obtained with shorter constructs unless present in high copy number (11, 12, 40–43). Also cell lines have, of course, been previously established which carry transfected κ genes. Again, the results of these earlier experiments are easily interpreted in the light of our finding of a role for the 3′-enhancer. Thus, Blasquez et al. (32) found that sequences downstream of C_κ can compensate for the otherwise poor expression of transfected genes which lack the intron-enhancer; this is likely to reflect the existence of the 3′-enhancer. Similiarly, in experiments (13) in which transfected κ genes were found to be silent in the S107 plasmacytoma (in which the intron-enhancer is not functional due to the lack of active NF-κB), the transfected genes did not include the 3′-enhancer. Our demonstration that the 3′-enhancer can potentiate the expression of transfected κ genes which would otherwise be inactive in this cell-line is consistent with the recent demonstration (45) that the 3′-enhancer can activate the expression of a heterologous linked gene in S107 cells.

Could the intron- and 3′-enhancers have different roles? The intron-enhancer is not able to potentiate κ transcription in the S107 plasmacytoma; as we show here, this function can be carried out by the 3′-enhancer. Similarly, functionally rearranged κ genes which lack the intron-enhancer but retain the 3′-enhancer are well expressed in transgenic mice (43). Thus, the intron-enhancer does not appear to be needed to achieve good κ expression levels. In contrast, our results show that the 3′-enhancer is needed for such expression and the intron-enhancer cannot fully compensate for the lack of the 3′-enhancer; short κ constructs are only poorly expressed in transgenic mice. These findings all point to the 3′-enhancer playing a more important role than the intron-enhancer in regulating the level of κ transcription, particularly during the later stages of B cell differentiation. What about the

initial activation of light chain expression? Results with constructs lacking the intron-enhancer (43) or the 3′-enhancer (described here) indicate that either of the two enhancers on their own is sufficient to allow activation of rearranged κ transgenes in transgenic mice. At present, it is not known which of the two enhancers is activated first. Analysis of day 17 foetal liver from mouse lines carrying either the long transgene or a high copy number of the short one does not reveal any clear difference in the time of activation of the two transgenes (data not shown). Furthermore, the fact that lipopolysaccharide stimulation of κ gene expression in the early-B cell line 70Z/3 correlates with activation of the intron-enhancer also suggests that activation of the 3′-enhancer is unlikely to precede that of the intron enhancer. An attractive possibility given the location of the intron-enhancer close to the J_κ segments is that this enhancer plays an important role in regulating V_κ-J_κ joining. In fact, Goodhardt et al. (45) have introduced into mice an unrearranged chimaeric V_κ-J_κ-C_κ transgene that includes the intron- but probably not the 3′-enhancer; V_κ-J_κ rearrangement does indeed occur in the lymphoid tissues of these mice although it is detected in both B and T cells.

Two factors have been specifically implicated in the induction of the intron-enhancer in pre-B cells: NF-κB and κBF-A (33,46). The fact that the 3′-enhancer does not depend on NF-κB is, of course, entirely in keeping with the activity of this enhancer in S107 cells. There is no good homology to the κBF-A motif within the 3′-enhancer; moreover the κBF-A factor is found in pre-B cells but not in plasmacytomas MPC11 and S107 where we have shown this enhancer to be active. Thus, the 3′-enhancer must be induced by a pathway distinct from that used by the intron-enhancer. Interestingly, there is a homology (8/9 match) between the core region of the κ3′-enhancer and a segment (μB) immediately downstream of the μE3 binding site of the mouse IgH-intron enhancer; this segment has been implicated in the binding of a lymphoid-specific factor involved in transcriptional activation (36–38). Clearly, however, further analysis is needed to compare the pathways of induction of the κ-intron and κ3′-enhancers.

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