Cell, Vol. 33, 717–728, July 1983, Copyright © 1983 by MIT

0092-8674/83/070717-12 $02.00/0

A Tissue-specific Transcription Enhancer Element Is Located in the Major Intron of a Rearranged Immunoglobulin Heavy Chain Gene

Stephen D. Gillies, Sherie L. Morrison,* Vernon T. Oi,† and Susumu Tonegawa
Center for Cancer Research
and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
*Department of Microbiology
Columbia College of Physicians and Surgeons
New York, New York 10032
†Department of Genetics and Biochemistry
Stanford University School of Medicine
Stanford, California 94305

Summary

We have studied the DNA sequences required for high level expression of a cloned heavy chain immunoglobulin gene stably introduced into mouse myeloma cells by DNA transfection. We found that DNA sequences derived from the germ line J_H–C_μ region are required for accurate and efficient transcription from a functionally rearranged V_H promoter. Similar to viral transcriptional enhancer elements, these cellular sequences stimulate transcription from either the homologous V_H gene segment promoter or a heterologous SV40 promoter. They are active when placed on the 5′ or 3′ side of the rearranged V_H gene segment and they function when their orientation is reversed. However, unlike viral enhancers, the Ig gene enhancer appears to act in a tissue-specific manner, since it is active in mouse B cells but not in mouse fibroblasts. The nucleotide sequence of the Ig enhancer region contains repeating elements that closely resemble sequence elements found in many viral enhancers. We discuss the possible role of tissue-specific transcription in cell differentiation and malignant transformation.

Introduction

Molecular analyses of immunoglobulin (Ig) genes established that an Ig polypeptide chain is encoded in multiple gene segments scattered along a chromosome of the germ line genome and that these gene segments must be brought together to form a complete Ig gene active in B lymphocytes (For a review see Tonegawa, 1983). This somatic assembly of Ig gene segments is achieved by a series of developmentally-controlled recombination events that occur during the differentiation of B cells.

Two types of recombination occur: V–J or V–D–J joining and switch recombination. The V–J and V–D–J joinings are essential for the formation of the DNA sequence coding for the variable (V) region of the light and heavy chain, respectively, while switch recombination replaces the constant (C) region-coding sequence of the heavy chain of one class (usually μ) with another (γ, ϵ, α, etc.). The exact timing of these recombination events in the course of B cell development has not been determined, but both V–J and V–D–J joining occur before the cell encounters antigens.

One of the most important functions of the V–J and V–D–J joinings is to create complete Ig genes with a diverse set of V region-coding DNA sequences from a limited number of the gene segments carried in the germ line genome (Tonegawa, 1983). Another important function of V–J and V–D–J joining events is in the control of the expression of Ig genes during B cell differentiation. On the basis of the fine structural analysis of Ig genes, it is clear that these joining events are prerequisite for the synthesis of a complete Ig chain: there is no evidence that any unrearranged germ line Ig gene segment can directly participate with its coding capacity in the synthesis of a complete, functional Ig chain (Brack et al., 1978; Bernard et al., 1978; Sakano et al., 1979; Max et al., 1979; Early et al., 1980; Sakano et al., 1980). In fact, it has been shown that in a myeloma cell the RNA transcript of an unrearranged V_κ segment is no more than 0.1 copy per gene, a level at least four orders of magnitude lower than that of the transcript from the rearranged, expressed V_κ segment present in the same cell (Mather and Perry, 1981).

Both the V–J and V–D–J joining events alter the sequence configurations in the 3′ region of the germ line V gene segment, but the 5′ flanking region of the V gene segment, where the transcription promoter and other controlling elements reside, is unaffected by the rearrangement (Bernard et al., 1978 and Clarke et al., 1982). Although a few nucleotide differences have been found between the germ line and somatic sequences in the 5′ flanking region of V gene segments (Sakano et al., 1980), these base substitutions are by-products of the somatic mutation events whose physiological role is to diversify the V-coding sequences (Bernard et al., 1978; Weigert and Riblet, 1976; Selsing and Storb, 1981). These base changes are not systematic and therefore are thought to have no bearing on the control of Ig gene expression (Clarke et al., 1982).

A possible explanation of how the downstream sequence might confer transcriptional competence to the rearranged V gene segment promoter is through transcriptional enhancement. Although the mechanism of this phenomenon is unknown, specific viral DNA sequence elements have been described (Banerji et al., 1981; de Villiers et al., 1981; Levinson et al., 1982) which enhance viral or recombinant cellular gene transcription. Because such enhancer elements can activate transcription from promoters which are located either upstream or downstream, and more than 1 kb away, it is possible that an analogous element might be located near the C gene segment. The observations that the C gene segments, in contrast to the V gene segments, are transcriptionally active in lymphoid cells in the absence of rearrangement support this hypothesis. In this case, promoter-like sequences upstream of the C gene segments are utilized for transcription but the

transcripts are degraded in the nuclei (Kemp et al., 1980; Van Ness et al., 1982).

The recent technical advances for introducing cloned immunoglobulin genes into lymphoid cells (Oi et al., 1983; Rice et al., 1983) have made it possible to study the structure–function relationship between specific DNA sequences and gene expression in these cells. We describe an enhancer element in the major intron of a rearranged γ_{2b} heavy chain gene. This sequence is located between the J_H region and the switch-recombination site utilized in myeloma MOPC 141, i.e., it is derived from sequences upstream of the germ line C_μ gene segment.

Results

High Level Expression of the Heavy Chain Gene Introduced into Myeloma Cells

We previously reported that the functionally rearranged immunoglobulin heavy chain (γ_{2b}) gene from MOPC 141 tumor cells (Sakano et al., 1980) can be accurately expressed at a low level in transfected mouse L cells (Gillies et al., 1983). For the studies presented here, we subcloned the same γ_{2b} gene fragment into plasmid pSV2*gpt* (Mulligan and Berg, 1980), transfected the mouse myeloma line, J558L, and selected for *gpt* gene activity by resistance to mycophenolic acid. This J558L line has lost the ability to express the endogenous immunoglobulin heavy chain gene but continues to synthesize a λ light chain. Furthermore, J558L has been shown to have a relatively high transformation frequency ($>10^{-4}$) when pSV2*gpt* vectors containing light chain genes are used for transfection (Oi et al., 1983).

Using a modified protocol for protoplast fusion (see Experimental Procedures), we found that plasmid pSV-γ_{2b}VC (Figure 1) transforms J558L cells at a frequency of greater than 10^{-3}. This high frequency made it possible to use pools of independently-derived clones of *gpt* transformants to compare the expression of plasmids containing defined deletions with that of the parental plasmid pSV-γ_{2b}VC. The advantage of this method is that the resulting cell lines represent several independent integration events (required for transformation), therefore the level of heavy chain gene expression in a given pool should reflect the average level of the individual clones. Thus the possible effect of the site of integration on the expression of the transfected gene is minimized.

Cell lines obtained by transfection with plasmid pSV-γ_{2b}VC and selection for *gpt* expression (growth in the presence of mycophenolic acid) were found to express high levels of γ_{2b} heavy chain (Figure 2A, lanes 2–5). These levels of expression of the exogenous γ_{2b} genes are estimated to be about 20% of that of the endogenous γ_{2b} gene in MOPC 141. Apparently, this heavy chain can form an immunoglobulin molecule with the λ light chain of myeloma J558L, because the light chain was immunoprecipitated from cell extracts with antiheavy chain antisera and equimolar amounts of heavy and light chain were secreted into the culture medium (Figure 2A, lane 14).

A Deletion of Part of the Major Intron Abolishes the High Level Expression of the Heavy Chain Gene

Deletion mutants of the parental plasmid were constructed to test whether the removal of specific noncoding DNA sequences would affect the expression of the γ_{2b} gene in J558L cells. Because deletions between the VDJ and C_μ exons of an Abelson murine leukemia virus-transformed cell line have been correlated with decreased heavy chain production (Alt et al., 1982), we constructed mutant plasmids with deletions in this region. Two such plasmids, pSV-γ_{2b}3′RΔ1 and pSV-γ_{2b}3′RΔ2 contain overlapping deletions around the unique Eco RI site of the parental plasmid pSV-γ_{2b}VC (Figure 1). These three plasmids were introduced into J558L myeloma cells and the expression of the γ_{2b} heavy chain gene in stably transformed cells was compared.

Cell lines obtained by transfection with plasmid pSV-γ_{2b}3′RΔ1 synthesized high levels (no less than half of the wild type level) of γ_{2b} heavy chain (Figure 2A, lanes 6–9) and secreted immunoglobulin (Figure 2A, lane 15). In contrast, four cell lines obtained by transfection with plasmid pSV-γ_{2b}3′RΔ2 synthesized only low levels (about 5% of the wild type level) of heavy chain (Figure 2A, lanes 10–13). The same results were obtained when subclones of

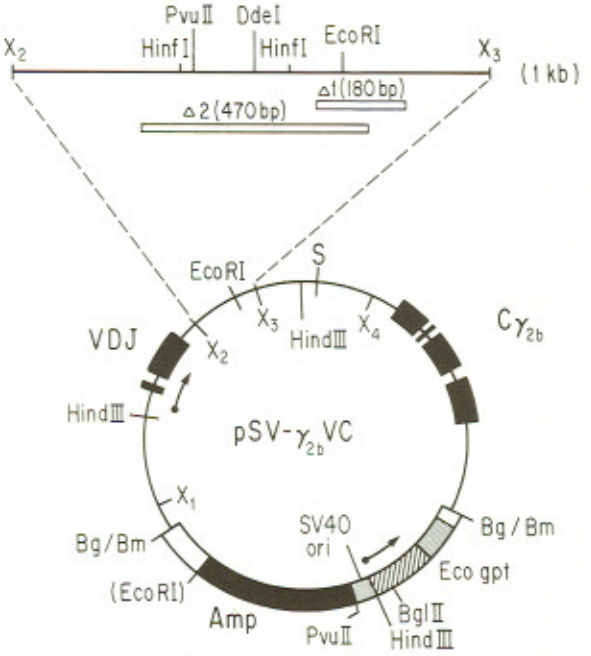

Figure 1. Partial Restriction Map of Plasmid pSV-γ_{2b}VC

A 9 kb Bgl II fragment was inserted into the Bam HI site (indicated by Bg/Bm) of plasmid pSV2*gpt*. The Eco*gpt* gene (wide stripes) is flanked by SV40 sequences (thin stripes) including the origin (ori) of replication and mRNA start site (arrow). The γ_{2b} gene (narrow line) contains VDJ and $C\gamma_{2b}$ exons (solid boxes) and a mRNA start site (arrow) about 30 bp upstream of the VDJ coding sequence (Gillies and Tonegawa, 1983). The switch recombination (S) site is also shown. The DNA segments deleted in plasmids pSV-γ_{2b}3′RΔ1 and pSV-γ_{2b}3′RΔ2 are shown in linear form above the circular map. The sizes of the deletions, as determined by restriction analysis, are indicated. The exact locations of these deletions are shown in Figure 7.

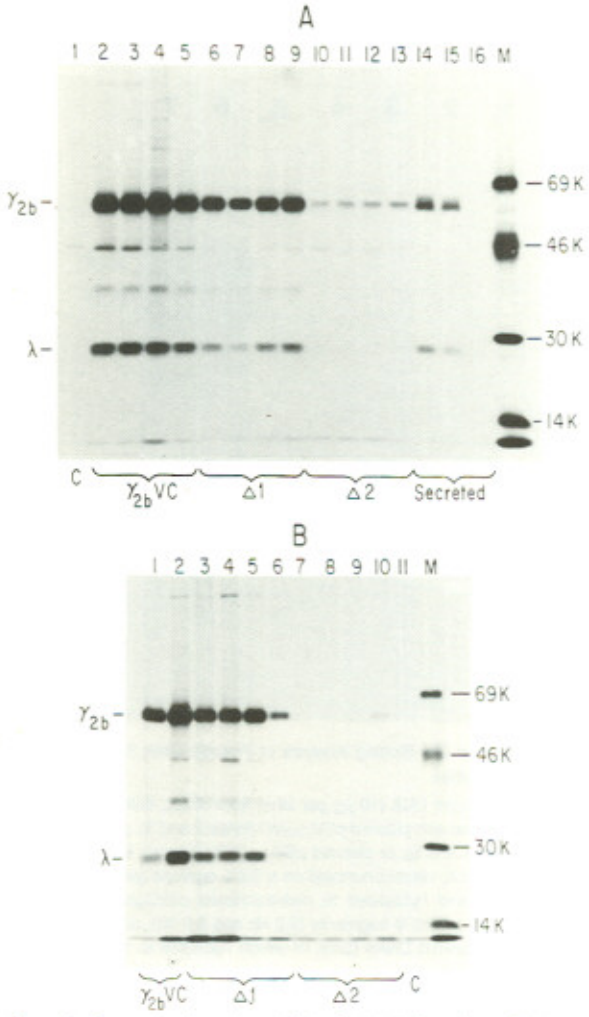

Figure 2. Expression of γ_{2b} Heavy Chain Protein in Transfected Cells

Transfected cell lines were labeled with ^{35}S-methionine and cell extracts were analyzed as described in Experimental Procedures. Immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. (A) Four transfected lines (pools of individual clones) were analyzed for each plasmid tested. Lane 1: control J558L cells; lanes 2–5: cells transfected with plasmid pSV-γ_{2b}VC; lanes 6–9: cells transfected with plasmid pSV-γ_{2b}3′RΔ1; lanes 10–13: cells transfected with plasmid pSV-γ_{2b}3′RΔ2. Secreted proteins from cells transfected with plasmid pSV-γ_{2b}VC (lane 14), plasmid pSV-γ_{2b}3′RΔ1 (lane 15), and plasmid pSV-γ_{2b}3′RΔ2 (lane 16) were immunoprecipitated and analyzed on the same gel. (B) Cell lines subcloned from the transfected cell lines were tested for γ_{2b} heavy chain protein synthesis as in (A). The plasmids used for transfection are indicated below the autoradiogram. Control (C) cell extract is shown in lane 11. The positions of the γ_{2b} heavy chain and λ light chain (synthesized in J558L cells but not immunoprecipitated in the absence of γ_{2b} heavy chain) are indicated.

each pool were tested for γ_{2b} heavy chain expression (Figure 2B), although more variation was observed in the level of expression between individual clones. Nonetheless, these results strongly suggest that DNA sequences deleted in plasmid pSV-γ_{2b}3′RΔ2, but still present in pSV-γ_{2b}3′RΔ1, are essential for the high level expression of heavy chain genes in myeloma cells.

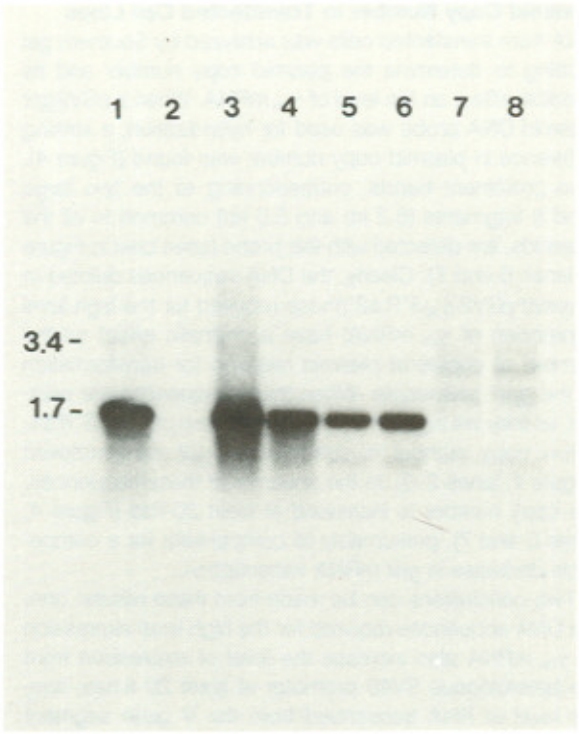

Figure 3. Northern Gel Blotting Analysis of Transfected Cell RNA

Total cell RNA (10 μg per lane) was electrophoresed on a denaturing agarose gel, transferred to nitrocellulase, and hybridized to nick-translated Cγ_{2b} probe. RNA (2.5 μg) from MOPC 141 cells (lane 1) and J558L cells (lane 2) were also analyzed. Two cell lines transfected with plasmid pSV-γ_{2b}VC (lanes 3 and 4), plasmid pSV-γ_{2b}3′RΔ1 (lanes 5 and 6), and plasmid pSV-γ_{2b}3′RΔ2 (lanes 7 and 8) are shown. The positions of the secreted (1.7 kb) and membrane forms (3.4 kb) of γ_{2b} mRNA are indicated.

The Reduced Expression of the γ_{2b} Gene Is at the Level of RNA

The steady-state level of γ_{2b} mRNA in transfected cell lines was analyzed by Northern gel blotting and hybridization with the Cγ_{2b} probe. It should be noted that the γ_{2b} heavy chain gene used in these studies does not contain the exons coding for the membrane form of γ_{2b} (Gillies and Tonegawa, 1983) and thus the only species of mRNA expected in transfected cells is the secreted form (1.7 kb).

As seen in Figure 3, cell lines transfected with plasmid pSV-γ_{2b}VC (lanes 3 and 4) and plasmid pSV-γ_{2b}3′RΔ1 (lanes 5 and 6) contain high levels of the secreted form of γ_{2b} mRNA. The cell lines transfected with plasmid pSV-γ_{2b}3′RΔ2 (Figure 3, lanes 7 and 8) contained much lower levels of γ_{2b} mRNA of the correct size, in agreement with the decreased level of γ_{2b} heavy chain protein (Figure 2). The additional RNA bands seen in lanes 7 and 8 (Figure 3) also contain γ_{2b} sequences but appear to be read-through products of the Ecogpt gene. Data presented below support this explanation, as opposed to the idea that the intron deletion has a deleterious effect on RNA splicing and results in low levels of translatable γ_{2b} mRNA.

Plasmid Copy Number in Transfected Cell Lines

DNA from transfected cells was analyzed by Southern gel blotting to determine the plasmid copy number and its possible effect on the level of γ_{2b} mRNA. When a pSV2*gpt* plasmid DNA probe was used for hybridization, a striking difference in plasmid copy number was found (Figure 4). Two prominent bands, corresponding to the two large Hind III fragments (6.2 kb and 5.0 kb) common to all the plasmids, are detected with this probe (seen best in Figure 4, lanes 6 and 7). Clearly, the DNA sequences deleted in plasmid pSV2-γ_{2b}3′RΔ2 (those required for the high level expression of γ_{2b} mRNA) have a dramatic effect on the number of copies of plasmid required for transformation to the *gpt*$^+$ phenotype. When these sequences are present, as they are in plasmids pSV-γ_{2b}VC and pSV-γ_{2b}3′RΔ1, a low copy number is sufficient for *gpt* transformation (Figure 4, lanes 2–5). In the absence of these sequences, the copy number is increased at least 20-fold (Figure 4, lanes 6 and 7), presumably to compensate for a comparable decrease in *gpt* mRNA transcription.

Two conclusions can be made from these results: one, the DNA sequences required for the high level expression of γ_{2b} mRNA also increase the level of expression from the heterologous SV40 promoter at least 20 times; two, the level of RNA transcribed from the V gene segment promoter is decreased about 400 times per gene copy in the absence of this DNA sequence. This calculation is based on the observed decrease by a factor of 20 in γ_{2b} gene expression as a result of the 3′RΔ2 deletion, and the fact that this decreased level is likely the result of the transcription of at least 20 times as many gene copies.

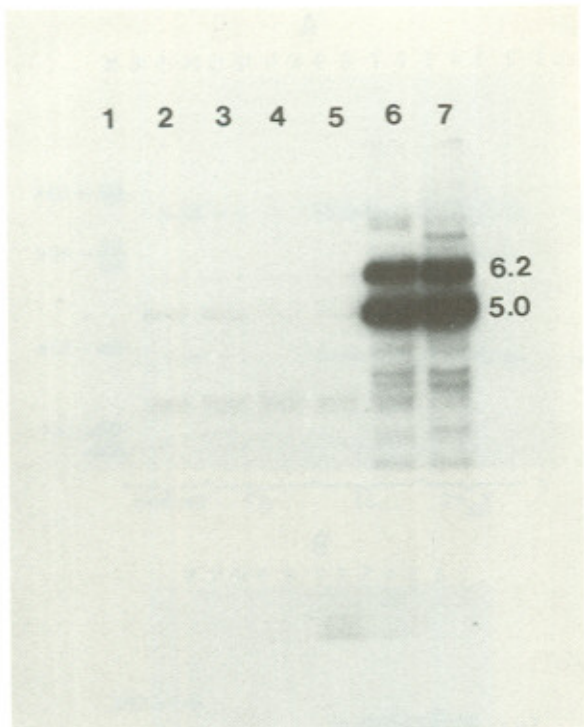

Figure 4. Southern Gel Blotting Analysis of Plasmid DNA Sequences in Transfected Cell Lines

High molecular weight DNA (10 μg per lane) from J558L cells (lane 1), or cell lines transfected with plasmid pSV-γ_{2b}VC (lanes 2 and 3), plasmid pSV-γ_{2b}3′RΔ1 (lanes 4 and 5), or plasmid pSV-γ_{2b}3′RΔ2 (lanes 6 and 7) was digested with Hind III, electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose, and hybridized to nick-translated pSV2*gpt* DNA. The positions of the two Hind III fragments (6.2 kb and 5.0 kb), common to all the transfected plasmid DNAs (both of which hybridize to the pSV2*gpt* probe), are indicated.

DNA Sequences Located in the γ_{2b} Gene Intron Enhance Expression in an Orientation-independent and a Position-independent Manner

The DNA sequences defined as viral enhancer elements have been shown to stimulate the transcription of homologous or heterologous promoters either upstream or downstream, and in either orientation with respect to the direction of transcription (Moreau et al 1981; Wasylyk et al, 1983). In order to test whether the sequences located in the major intron of the γ_{2b} gene (and all other heavy chain genes) behave similarly, we constructed a plasmid with most of the intron sequences deleted. We then inserted a 1 kb Xba I fragment ($X_{2/3}$) containing those intron sequences with potential enhancer activity into either of two sites in either of the two orientations. The first corresponds to the original position of this fragment in the parental plasmid (as part of the VDJ-Cγ_{2b} intron) and the second is approximately 1.4 kb upstream (on the 5′ side of the V gene segment). Four plasmids were obtained which contained the $X_{2/3}$ fragment in the normal or reversed orientation, either upstream or downstream of the mRNA start site (see Figure 5A).

Cell lines obtained by transfection with the plasmids just described were analyzed for the expression of γ_{2b} heavy chain. As seen in Figure 5B, cells transfected with plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$ (with most of the intron deleted) did not synthesize significant levels of γ_{2b} protein (lanes 3 and 4). The insertion of the $X_{2/3}$ fragment into the intron site (the normal position of this fragment) restored the expression of γ_{2b} protein in both the normal (Figure 5B, lanes 5 and 6) or reversed (Figure 5B, lanes 7 and 8) orientations. Similarly, insertion of the same fragment upstream of the V gene segment (on the 5′ side of the transcriptional promoter) in either the normal (Figure 5B, lanes 9 and 10) or the reversed (Figure 5B, lanes 11 and 12) orientation also restored the expression of γ_{2b} protein to normal levels.

These results clearly demonstrate that the intron sequences deleted in the 3′RΔ2 mutant plasmid have a direct effect on transcription in a manner that is analogous to the viral enhancers. They also show that the enhancer function does not require the expression of these sequences in the γ_{2b} gene primary transcript, because movement of the $X_{2/3}$ fragment outside of the transcription unit (i.e., the Xba I, site) had no effect on its ability to function.

Tissue Specificity of the Immunoglobulin Enhancer Element

The rearranged γ_{2b} gene used in these studies is also accurately transcribed in mouse fibroblasts (Ltk$^-$ cells)

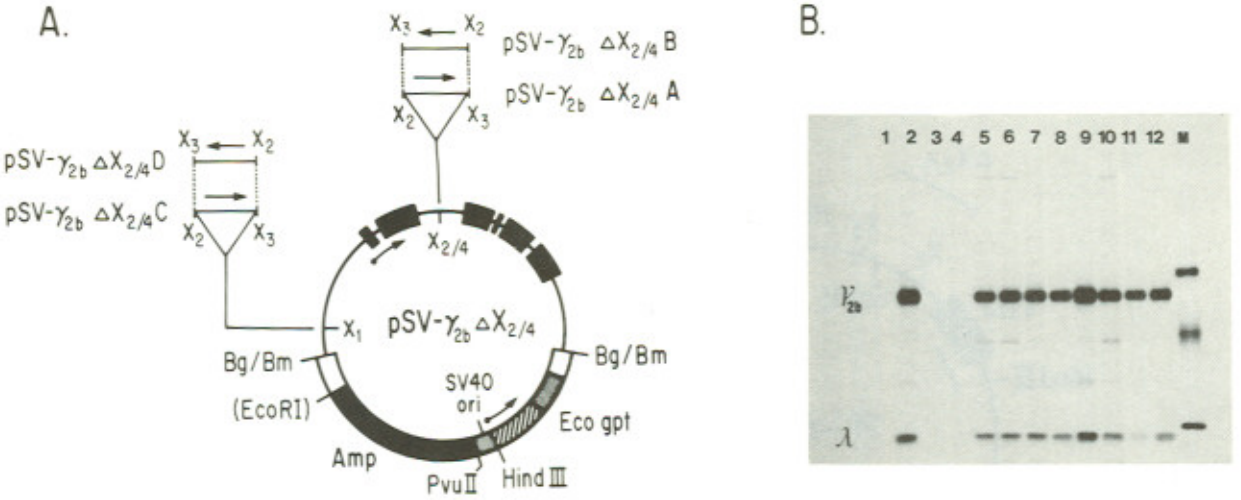

Figure 5. Enhancement of γ_{2b} Gene Expression by a 1 kb Intron Fragment

(A) Restriction map of plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$. This plasmid was constructed from plasmid pSV-γ_{2b}VC (shown in Figure 1) by removing two Xba I fragments from the γ_{2b} gene intron (from the X_2 to the X_4 sites in Figure 1). Derivatives of plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$, labeled A through D, contain inserts of the 1 kb $X_{2/3}$ fragment in the sites indicated and the orientation (relative to transcription) is shown with an arrow. (B) Expression of γ_{2b} heavy chain in cells transfected with the plasmids shown in (A). Analysis was carried out as described in Figure 2. Cell lines tested were J558L (lane 1), and those transfected with plasmid pSV-γ_{2b}VC (lane 2), plasmid pSV2-$\gamma_{2b}\Delta X_{2/4}$ (lanes 3 and 4), plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$A (lanes 5 and 6), plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$B (lanes 7 and 8), plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$C (lanes 9 and 10), and plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$D (lanes 11 and 12).

cotransfected with the herpes virus tk gene and plasmid ppLγ_{2b}VC (Gillies and Tonegawa, 1983). The level of γ_{2b} gene expression in these cells was found to be proportional to the number of transfected genes, but is at least two orders of magnitude less per gene copy than in myeloma cells. Thus it is likely that the enhancer element, described above, does not function in nonlymphoid cells.

In order to compare the levels of γ_{2b} gene expression in fibroblasts, with and without the immunoglobulin enhancer, we first made a deletion mutant, ppL$\gamma_{2b}\Delta X_{2/3}$, lacking these sequences (Figure 6A). We then modified the plasmids so that a high copy number of γ_{2b} genes would be integrated into the transfected L cells and increase the expression of the γ_{2b} gene to an easily detectable level. This was done by inserting a truncated *tk* gene (a 2.3 kb Eco RI fragment containing only limited 5′ upstream sequences) into both the wild type and mutant plasmids. Transformation to the tk^+ phenotype with this fragment requires the transfer of multiple plasmid copies into cells, thus another gene on the same plasmid would also be present at a high copy number in tk^+ transformants (our unpublished results).

Plasmids ppLγ_{2b}-TK and ppL$\gamma_{2b}\Delta X_{2/3}$-TK were introduced into mouse Ltk^- cells and the tk^+ transformants (approximately 50 individual clones) were pooled, grown in mass culture, and tested for the presence of γ_{2b} DNA sequences. As seen in Figure 6B, each transfected cell line contained comparable numbers of tandem, head-to-tail oligomers of either plasmid. Control experiments (not shown) indicate that individually cloned cell lines also contain the same number (about 15 copies per cell) of transfected plasmid DNA. Apparently the copy number is determined by the level of expression of the *tk* gene which, in this case, has been reduced considerably by the deletion of the upstream sequences. To compensate for the low level of expression, multiple copies of the *tk* gene are required for *tk* transformation. This, then, is analogous to the results with pSV2*gpt* vectors described above.

We compared the expression of the normal and mutant γ_{2b} heavy chain genes in these cell lines by Northern gel blotting analysis of total cell RNA. As seen in Figure 6C, the steady-state level of γ_{2b} mRNA is not affected by the deletion of the immunoglobulin enhancer. We concluded that the low level expression of the heavy chain gene in L cells is a result of the fact that this enhancer element is functional only in lymphoid cells.

Additional experiments have been carried out to test the tissue specificity of the immunoglobulin enhancer. We constructed a derivative (pSER) of plasmid pSV2*gpt* lacking most of the SV40 72 bp repeat sequence (see Experimental Procedures). When this plasmid is used to transfect either mouse L cells or J558L myeloma cells, the transformation frequency (relative to that of plasmid pSV2*gpt*) is lowered by more than a factor of 20 (from 2×10^{-3} to 10^{-4} in L cells and from 3×10^{-4} to 10^{-5} in J558L myeloma cells—Table 1). When the 1 kb $X_{2/3}$ fragment containing the immunoglobulin enhancer is inserted into the Eco RI site of plasmid pSER, the transformation frequency is restored to the level of plasmid pSV2*gpt*, but only in myeloma cells. There is no effect on the transformation frequency of plasmid pSER in L cells (Table 1). Thus the enhancing effect on the heterologous SV40 promoter (which controls the Eco*gpt* gene) is also tissue-specific.

Using this same transformation assay we tested smaller restriction fragments for enhancer activity. A 140 bp Pvu II–Dde I fragment (see Figure 1), containing some of the

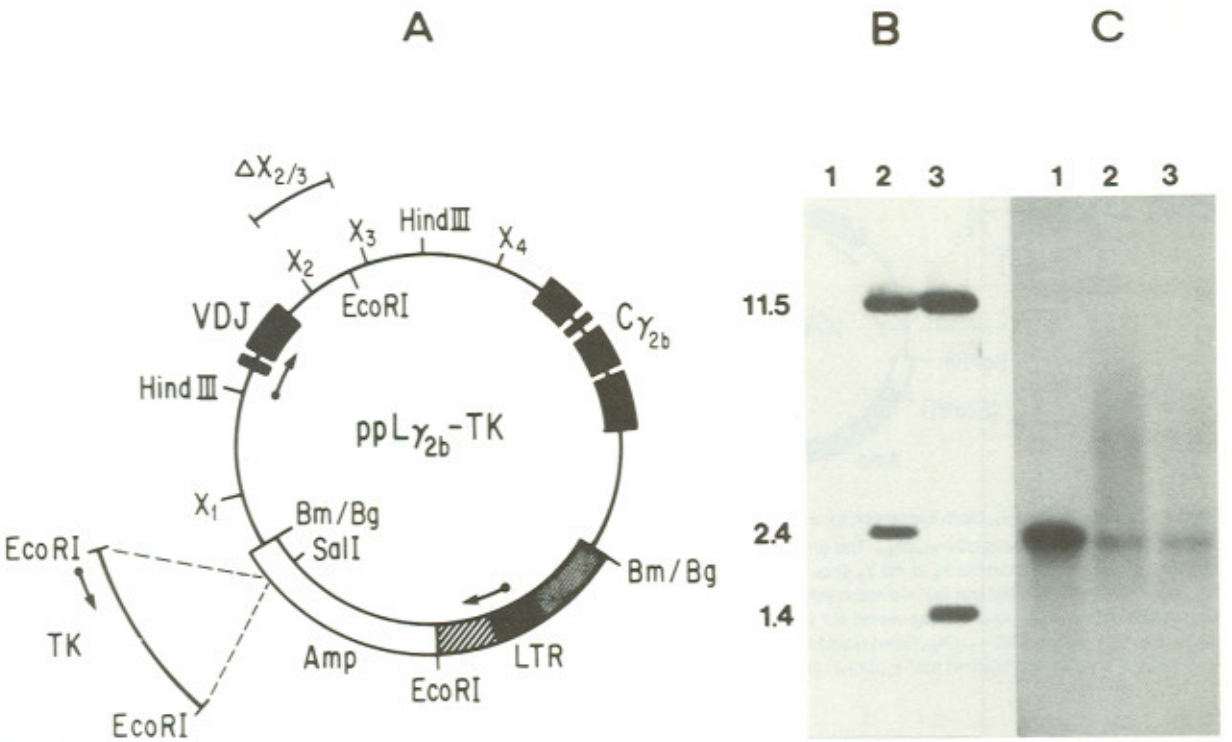

Figure 6. Expression of γ_{2b} mRNA in Mouse L Cells Transfected with Plasmids ppLγ_{2b}-*tk* and ppL$\gamma_{2b}\Delta X_{2/3}$-*tk*

(A) Restriction map of the plasmids used for transfection. Plasmid ppLγ_{2b}-*tk* was constructed by inserting a 2.3 kb fragment of the herpes virus *tk* gene into the unique Sal I site of plasmid ppLγ_{2b}VC (Gillies and Tonegawa, 1983). The direction of transcription of the *tk* gene (arrow) is opposite that of the γ_{2b} gene. The sequences deleted in plasmid ppL$\gamma_{2b}\Delta X_{2/3}$-*tk* are indicated. (B) Southern gel blotting analysis of DNA from L *tk*$^-$ cells (lane 1), and cells transfected with plasmid ppLγ_{2b}-*tk* (lane 2), or plasmid ppL$\gamma_{2b}\Delta X_{2/3}$-*tk* (lane 3). DNA was digested with Hind III and hybridized to nick-translated γ_{2b} DNA (the 9 kb Bgl II fragment used for plasmid construction). (C) Northern gel blotting analysis of total cell RNA from MOPC 141 myeloma cells (lane 1), and cells transfected with plasmid ppLγ_{2b}-*tk* (lane 2), or plasmid ppL$\gamma_{2b}\Delta X_{2/3}$-*tk* (lane 3). Nick-translated Cγ_{2b} probe (Gillies and Tonegawa, 1983) was used for hybridization.

sequences deleted in plasmid pSV-γ_{2b}3′RΔ2, was found to increase the transformation frequency of plasmid pSER by 20-fold in J558L cells but not in mouse L cells (Table 1). Thus we have localized the immunoglobulin enhancer sequence to this portion of the $X_{2/3}$ fragment.

DNA Sequences in the Heavy Chain Gene Intron Resemble Viral Enhancers

Weiher et al. (1983) have suggested that the sequence 5′ GTGG$\frac{AAA}{TTT}$G3′ (where $\frac{A}{T}$ means either A or T appears at that position) represents a crucial core element common to all of the known viral enhancers. Sequence analysis of the $X_{2/3}$ fragment (the 1 kb fragment shown to have enhancer activity—Figure 5) shows that such a sequence is located in the region that is deleted in plasmid pSV-γ_{2b}3′RΔ2 (but not in the 3′RΔ1 mutant) and is present in the 140 bp Pvu II–Dde I fragment. In fact, the sequence 5′GTGGTTT(T)GAA-3′ is present as a closely spaced repeat (Figure 7), oriented in the direction of transcription. The first eight nucleotides of this sequence are also found upstream of the tandem repeat, but oriented in the opposite direction.

Figure 8 shows a comparison of several viral sequences shown to have enhancer activity and the repeat sequences

Table 1. Transformation Frequency of pSV2*gpt* and Derivative Plasmids in J558L Myeloma Cells and L Cells

	Cell Type	
Plasmid	J558L	L Cell
pSV2*gpt*	3×10^{-4}	2×10^{-3}
pSER	8×10^{-6}	1×10^{-4}
pSER-$X_{2/3}$	4×10^{-4}	1×10^{-4}
pSER-$X_{2/3}$(140)	2×10^{-4}	9×10^{-5}

Cells were transfected by protoplast fusion and plated at 10^4 cells per well and 2×10^5 cells per well (J558L) or at 10^4 and 10^5 cells per 100 mm dish (L cells). Selective medium containing mycophenolic acid (6 μg/ml for J558L or 25 μg/ml for L cells) was added at 48 hr and colonies were counted at 10 days (J558L) or at 14 days (L cells). Derivatives of plasmid pSER were constructed by inserting (blunt-end ligating) either the 1 kb $X_{2/3}$ fragment or a 140 bp Pvu II–Dde I [$X_{2/3}$(140)] fragment (see Figure 1) into the Eco RI site.

in the γ_{2b} gene intron. Sequences contained in the Moloney sarcoma virus (MSV) 73 bp repeat sequence appear to be most similar to the immunoglobulin sequence, especially on the 5′ side of the first "core" repeat. The "core" sequence of polyoma virus was most similar to the second "core" repeat, as both contain an additional T residue.

Also shown are two sequences, present in the immu-

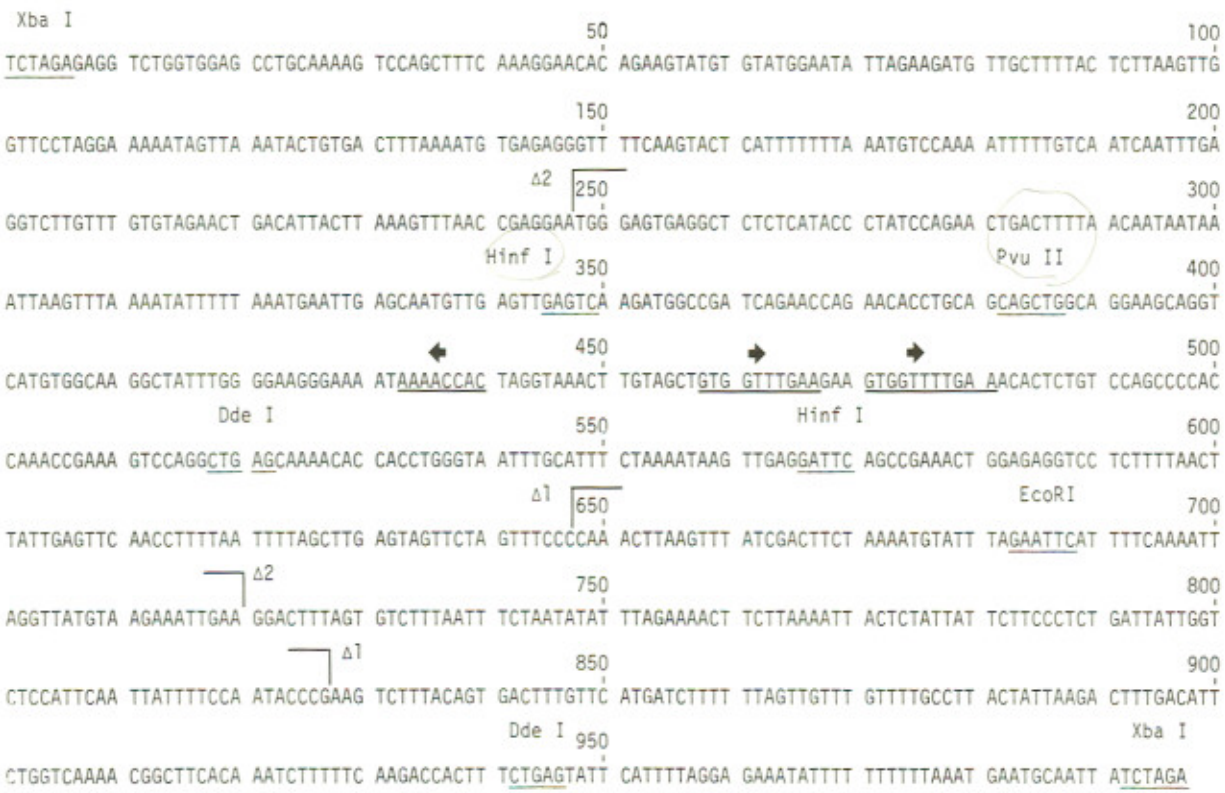

Figure 7. Nucleotide Sequence of the 1 kb $X_{2/3}$ Fragment

DNA sequencing was carried out according to standard procedures (Maxam and Gilbert, 1980). The sequences deleted in plasmids pSV-γ_{2b}3′RΔ1 and pSV-γ_{2b}3′RΔ2 are indicated. The underlined sequences are those similar to the "core" elements common to most viral enhancers (Weiher et al., 1983). Arrows indicate the orientation of the immunoglobulin "core" elements relative to the direction of γ_{2b} mRNA transcription.

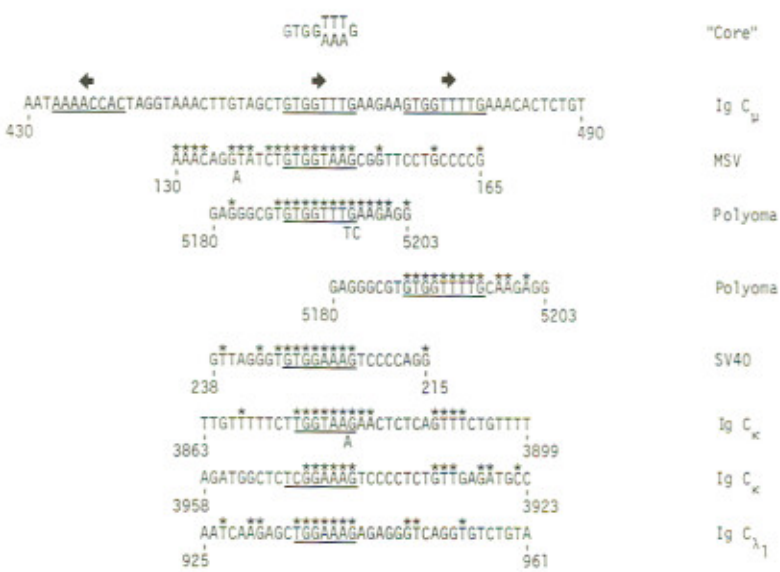

Figure 8. A Comparison of Sequences in the 1 kb $X_{2/3}$ Fragment with Those of Viral Enhancer Elements

Sequences are aligned at the putative "core" (underlined) sequences (Weiher et al., 1983). The residues which match those in the immunoglobulin heavy chain sequence are indicated by an asterisk. These include either an A or T residue in positions 5–7 of the "core" sequence. In some cases a residue has been displaced to maximize the homology. Numberings of the sequences are according to Van Beveren et al., 1981 (MSV); Griffin et al., 1980 (Polyoma); Buchman et al., 1980 (SV40); Max et al., 1981 (IgCκ); Bernard and Tonegawa, unpublished data (Ig $C_{\lambda 1}$).

noglobulin light chain gene intron, that resemble an enhancer element. The existence of such a sequence near the C_κ gene segment is rendered plausible by its proximity to a DNAase I hypersensitive site (Parslow and Granner, 1982). In addition, studies using transfected genes suggest that deletions in this region reduce transcription from the V promoter (V. O. and S. M., unpublished data). A similar core-like sequence is also present near the constant portion of the λ_1 light chain gene, but there is yet no evidence showing that this is part of an enhancer element.

Discussion

Evidence for an Enhancer Element in the Intron of a Heavy Chain Immunoglobulin Gene

Sequences contained in the major intron between the functionally rearranged VDJ and C exons of a heavy chain immunoglobulin gene were shown to be essential for its high level expression in transfected myeloma cells. Although the deletion of these sequences decreases the level of expression in transfected cells 20 times (Figure 2), the actual reduction is probably about 400 times per gene copy. We have tested whether these sequences are analogous to viral enhancer elements. In addition to increasing the level of transcription from homologous promoters, viral enhancers also increase transcription from many heterologous, viral or nonviral promoters. This enhancing activity is independent of the orientation of the enhancer element, relative to the direction of transcription, and is independent of its position as long as the distance between the enhancer and promoter is within several kilobases (Banerji et al., 1981; Moreau et al., 1981; Wasylyk et al., 1983).

These properties also apply to the sequences contained in the heavy chain immunoglobulin gene. Enhancement of the heterologous SV40 promoter occurs when the intact γ_{2b} heavy chain gene is present in plasmid pSV2*gpt* and a low copy number of the recombinant plasmid is sufficient for *gpt* transformation. When the γ_{2b} gene intron sequences are removed, the plasmid copy number increases dramatically to compensate for the decreased expression of the *gpt* gene from the SV40 promoter (Figure 4).

We also found that DNA fragments from the γ_{2b} gene intron can substitute for the SV40 enhancer in plasmid pSV2*gpt* (Table 1). This transformation assay is based on the ability of the DNA fragments, located more than 2 kb away from the SV40 promoter, to enhance the transcription of the *gpt* gene and thereby increase the transformation frequency. Using this method we have shown that most of the enhancing activity can be localized to a 140 bp fragment. The nucleotide sequence in this region contains a repeat sequence which closely resembles the "core" nucleotides found in most viral enhancers (Figure 8).

Finally, we demonstrated that this intron sequence maintains its ability to stimulate transcription of the heavy chain gene when it is moved outside of the γ_{2b} transcription unit (5′ of the mRNA start site) and when its orientation is reversed (Figure 5). These results show that the immunoglobulin intron sequence is an enhancer element and has properties in common with those of viral origin, even though the latter are generally located on the 5′ side of their cognate transcriptional promoters.

The Role of Transcriptional Enhancement in the Regulation of Immunoglobulin Gene Expression

The creation of active immunoglobulin genes through somatic recombination has been studied in detail (reviewed by Tonegawa, 1983), but the mechanism by which this activation is brought about has been a major problem of molecular immunology. The observation that the C_κ and C_μ gene segments are transcriptionally active in lymphoid cells, prior to V–J or V–D–J joining (Kemp et al., 1980; Van Ness et al., 1982), provided the first evidence that sequences downstream of the V gene segment promoter might affect the transcription of the functionally rearranged gene.

The mechanism of this activation can now be explained, at least for the heavy chain gene, by our identification of an enhancer element between the J_H and $C\gamma_{2b}$ gene segments of a functionally rearranged gene. This site corresponds to the J_H–C_μ region of germ-line DNA (see Figure 9). Following VDJ-joining, which occurs before B cells encounter antigens, this enhancer (which would now be part of the major intron of the functionally rearranged μ

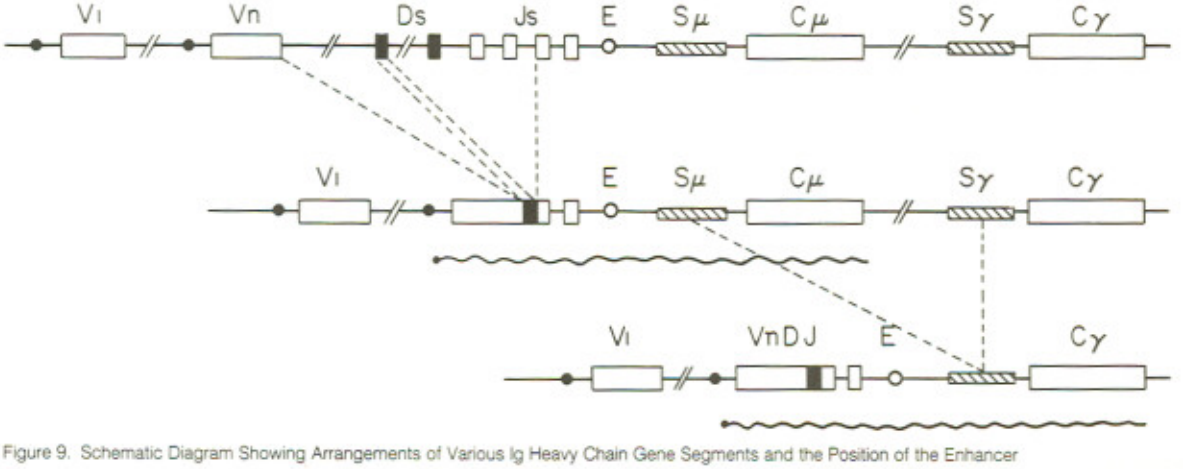

Figure 9. Schematic Diagram Showing Arrangements of Various Ig Heavy Chain Gene Segments and the Position of the Enhancer

Top, middle, and bottom arrangements correspond to germ-line DNA; μ chain-positive, preswitch B cell DNA; and γ chain-positive, postswitch plasma cell DNA, respectively. The enhancer element (O) is located between the J_H segments and C_μ segments in the germ-line DNA and becomes part of the major intron in the active μ chain gene upon V–D–J joining. The same enhancer element is retained in the major intron of the active γ chain gene, which is created by a switch recombination from the μ gene. $S\mu$ and $S\gamma$ refer to the regions in which switch recombinations occur. The wavy lines and the large filled circles (●) represent the primary transcripts and the promoters, respectively. The small filled circles (•) represent the 5′ caps of the RNA molecules.

chain gene) activates the promoter of the rearranged V gene segment. In this way only a single V gene segment (out of several hundred) would be transcriptionally active, and only after functional rearrangement had occurred.

Subsequent to the encounter with antigens and stimulation by T cells, a second type of rearrangement (switch recombination) occurs in heavy chain genes and results in the replacement of the C_μ coding sequence (Figure 9) with those of the other heavy chain classes and subclasses (Maki et al., 1980; Kataoka et al., 1980; Sakano et al., 1980; Davis et al., 1980). In order to function after class switching, the immunoglobulin enhancer would have to be located upstream of the switch region, otherwise it would be deleted along with the C_μ coding sequence. This is in fact the case since its location is more than 1 kb upstream of any known switch sites within the S_μ region. Thus as we have shown for the MOPC 141 γ_{2b} gene, it is likely that the same enhancer is used for the expression of all heavy chain classes following switch recombination, although it is possible that additional regulatory elements may be associated with the individual C region gene segments.

Evidence for Other Cellular Enhancer Elements

The data presented in this report represents the first clear demonstration of an enhancer element being associated with a defined cellular gene. The possibility that enhancers are present in cellular DNA has already been suggested by others. For example, Conrad and Botchan (1982) isolated human DNA sequences which hybridized to the region of SV40 DNA spanning the origin of replication. One of these sequences was found to enhance the efficiency of *tk* transformation in an orientation-independent manner and thus resembles the viral enhancer elements. Furthermore, this DNA sequence cross-hybridized with many sequences in human DNA, suggesting that a family of such elements exists.

Rosenthal and Khoury (personal communication) have likewise isolated a human DNA sequence by virtue of its cross-hybridization with a portion of BK virus DNA. In this case, however, the sequence appears to be unique in the human genome even though it contains repeating 21 bp elements. These repeat elements show some homology to the BK virus enhancer region but appear to be about 8 times less active when tested for enhancing activity in the CAT assay of Gorman et al. (1982).

Thus it is likely that enhancer elements might serve as a general mechanism for gene regulation in eucaryotes. The association of such elements with specific genes is currently being studied in several systems. In addition to our demonstration of an enhancer element in the heavy chain immunoglobulin gene, two of us (V. O. and S. M.) have found that sequences near the C_κ gene segment are essential for the high level expression of κ chains in transfected myeloma cells. In this case, however, the functional similarity of this sequence element to the viral enhancers is less clear.

The use of enhancer elements as regulators of gene expression may not be confined to higher eucaryotic systems. There is evidence (L. Guarente, personal communication) that the yeast iso-1-cytochrome c gene is activated by heme and that sequences upstream of the gene are essential for this effect. Furthermore, the inversion of this activator sequence did not affect the inducibility of expression. This strongly suggests that this region is not simply a component of the transcriptional promoter.

Tissue-specific Enhancer Elements and Their Possible Role in Cell Differentiation

The most interesting property of the immunoglobulin enhancer is its tissue specificity. The MSV and SV40 viral enhancers have been shown to have a certain degree of host cell specificity (Laimins et al, 1982). This effect may also be explained in terms of tissue specificity because the two cell types used for the comparison were derived from different tissues. The immunoglobulin enhancer, on the other hand, functions at a high level in a lymphoid (myeloma) cell type but not at all in another cell type (fibroblast) of the same species. It is likely that this specificity is the result of a factor (or factors), present only in lymphoid cells, which regulates immunoglobulin expression during B cell ontogeny.

At early stages of B cell development, μ heavy chains are expressed at a low level (Levitt and Cooper, 1980). After the B cell encounters antigen and interacts with regulatory T cells, terminally differentiated plasma cells appear and produce very high levels of immunoglobulin (Schibler et al, 1978). The quantitative differences in the level of immunoglobulin gene expression at different stages of B cell development suggest that the enhancer function may be stage-specific. It is also possible that multiple regulatory elements are contained within this enhancer region and that increased levels of expression result from the combined effect of individual enhancers.

Another possible example of a tissue-specific enhancer element has been described in the polyoma virus system (Katinka et al., 1980; Fujimura et al., 1981; Fujimura and Linney, 1982). It was shown that polyoma mutants that acquire the ability to replicate in the otherwise refractory F9 embryonal carcinoma cells contain point mutations and, in some cases, tandem duplications near the region of polyoma DNA which was shown (de Villiers et al., 1981) to have enhancer activity. This result suggests that certain DNA sequences are recognized as an enhancer in cells permissive for polyoma virus but that sequences located at an adjacent site are recognized (after being mutated) in embryonal cells. Thus it appears that polyoma, like immunoglobulin genes, may contain multiple regulatory elements within their enhancer regions.

The mechanism of tissue-specific enhancer is not known and this simply reflects our present ignorance of the mechanism of enhancers in general. Clearly though, the sequence differences and similarities between the various enhancers strongly suggest that specific regulatory proteins recognize these sites. In fact, the glucocorticoid

receptor protein, which binds to the promoter region of mouse mammary tumor virus (MMTV), may be an example of an enhancer binding protein (K. Yamamoto, personal communication). A sequence upstream of the MMTV promoter, essential for hormone responsiveness, was shown to enhance the herpes *tk* gene in an orientation–independent manner and to bind the hormone receptor.

It is tempting to speculate that the presence or absence of such enhancer binding proteins determines whether or not an enhancer functions in a given cell type. Furthermore, a particular enhancer binding protein might recognize many different but related sequences to a greater or lesser extent (or bind with different affinities). In this way it would be possible to activate many individual genes and to express them at different levels. Alternatively, the level of expression of a gene that is controlled by an enhancer may be determined by the distance between this element and the promoter site. It seems likely that some or all of these mechanisms of gene regulation function during the process of cellular differentiation. In this way the expression of multiple genes could be controlled (coordinately expressed) by a relatively small number of regulatory proteins.

Enhancers as Activators of Cellular Oncogenes

One striking case for the role of enhancers in tumorigenesis was provided by studies of avian leukosis virus (ALV) induced chicken B cell lymphomas. In such lymphomas, ALV DNA was found to be integrated adjacent to the *c-myc* gene (Payne et al., 1981), the cellular counterpart of the transforming sequences from the MC29 group of defective retroviruses (Sheiness and Bishop, 1979). Although it was first thought that *c-myc* was activated by a promoter-insertion mechanism (Hayward et al., 1981), it was later shown that ALV insertions could occur in the opposite orientation or downstream of the *c-myc* gene (Payne et al., 1982). Thus the ALV enhancer element was responsible for the increased level of *c-myc* expression and, presumably, for oncogenic transformation.

The role of cellular enhancer elements in the activation of oncogenes has also been suggested by recent findings (reviewed by Klein, 1983). Many murine and human tumors of lymphoid origin have been shown to contain chromosomal translocations in which an oncogene (*c-myc*) has been rearranged to an immunoglobulin C region gene segment. The majority of rearrangements in human Burkitt lymphomas were found to occur at the C_μ region while those in mouse plasmacytomas occur at the C_α region. While the results we have described could account for the activation of *c-myc* in some human C_μ rearrangements (by analogy to the murine C_μ enhancer), the results in the murine system are somewhat unclear. It has not been ruled out, however, that the murine C_α gene segment contains an additional enhancer element.

We are currently investigating the activation of *c-myc* by sequences contained near the *c-myc*-C_μ junction in human DNA. It will be interesting to compare the sequences in this region with those that we showed to contain enhancer activity. Sequences that have been conserved through evolution may also help to identify the critical components of this regulatory element.

Experimental Procedures

Cell Culture and Transfection

The myeloma cell line, J558L, is a heavy chain loss variant of J558 and synthesizes λ light chains (Oi et al., 1983). Cells were grown in Dulbecco's modified Eagle's medium (MEM) containing 10% fetal calf serum. J558L cells were transfected by a modification of the protoplast fusion technique (Sandri-Goldin et al., 1981). Approximately 2×10^6 cells (grown to a density of 4 to 6×10^5 cells/ml) were washed once with serum-free MEM, collected by centrifugation (5 min. at 500 g), and suspended by gentle pipetting in the protoplast suspension (approximately 2×10^9 protoplasts in 4 ml). The cell–protoplast suspension was transferred to a 60 mm dish and centrifuged at 1500 g for 7 min. After gentle aspiration of the supernatant, 1.5 ml of 50% PEG-1500 (in serum-free MEM and prewarmed to 37°C) was added and the dish was spun at 500 g until 90 sec had elapsed from the time of PEG addition. Cells were resuspended by gently pipetting in two 5 ml washes of prewarmed, serum-free MEM which were added to 15 ml of MEM in a 50 ml centrifuge tube. Following centrifugation at 500 g for 5 min, cells were resuspended in growth medium containing kanamycin (100 μg/ml) and plated in 96-well dishes at two densities: 1×10^4 cells per well and 2×10^3 cells per well. After 48 hr selective medium (Oi et al., 1983) was added.

Plasmid Constructions

Plasmid pSV-γ_{2b}VC, containing the γ_{2b} gene from myeloma MOPC 141, was constructed by inserting a 9 kb Bgl II fragment (Gillies and Tonegawa, 1983) from phage clone M141-p21 (Sakano et al., 1980) into the unique Bam HI site of plasmid pSV2*gpt*(RI). This latter plasmid was constructed by mutating the Eco RI site of plasmid pSV2*gpt* (Mulligan and Berg, 1980). The transcription orientation of the γ_{2b} gene is opposite that of the *gpt* gene (Figure 1).

Plasmids pSV-γ_{2b}3′RΔ1 and pSV-γ_{2b}3′RΔ2 were constructed by digesting Eco RI-cut pSV-γ_{2b}VC DNA with exonuclease Bal 31 (1 U/μg of DNA) at 23°C for 2 or 4 min and recircularizing the products with T4 DNA ligase. The extent of the deletions were determined by restriction analysis and DNA sequencing.

Plasmid pSV-$\gamma_{2b}\Delta X_{2/4}$ was constructed by first digesting plasmid pSV-γ_{2b}VC DNA with Bgl II and then partially digesting with Xba I. The 6.5 kb partial digestion product extending from the unique Bgl II site to the Xba I site (X_2) on the 3′ side of the VDJ exon (clockwise on the map in Figure 1) and the 5.1 kb complete digestion product extending from the Bgl II site, counterclockwise to the Xba I site (X_4) on the 5′ side of the $C\gamma_{2b}$ coding region, were gel purified and ligated. The resulting plasmid, pSV-$\gamma_{2b}\Delta X_{2/4}$, was used for the experiment shown in Figure 5. Derivatives of this plasmid (A–D) were constructed by partially digesting with Xba I, treating the DNA with calf intestine alkaline phosphatase, purifying linear full-length DNA, and ligating the products with the 1 kb Xba I fragment extending from the X_2 to X_3 sites ($X_{2/3}$ fragment in Figure 1). The site of insertion and the orientation of the $X_{2/3}$ fragment were determined by restriction analysis.

Plasmid pSER was constructed by digesting plasmid pSV2*gpt* DNA with Sph I and Pvu II and removing the 3′ protruding bases with T4 DNA polymerase (O'Farrell, 1981). The two blunt ends were then ligated to produce a selectable plasmid vector which no longer contains the SV40 enhancer sequence.

Analysis of Transfected Cells

Approximately 10 days after transfection, the cells contained in a single well (from 5 to 10 independent clones) were harvested and grown in mass culture for analysis of protein synthesis and the steady-state level of γ_{2b} mRNA (Gillies and Tonegawa, 1983). Four such pools were analyzed for each plasmid tested as well as subclones obtained by limiting dilution.

Protein synthesis was measured by labeling 5×10^6 cells for 1 hr with ^{35}S-methionine (50 μCi/ml) and analyzing immunoprecipitated cell extracts as described (Gillies and Tonegawa, 1983). Secretion of immunoglobulin

was measured by labeling approximately 2×10^4 cells for 16 hr in 50 μl of normal growth medium containing ^{35}S-methionine (25 μCi/ml). Immunoglobulin was then immunoprecipitated from culture supernatants.

Acknowledgments

We thank Ms. Lena Angman for excellent technical help, Anne Ephrussi for assistance in plasmid constructions, and the Eli Lilly Company for supplying mycophenolic acid. We are indebted to Keith Yamamoto, Leonard Guarente, Nadia Rosenthal, and George Khoury for communicating their unpublished results. S. G. was supported by a postdoctoral fellowship from the Damon Runyon-Walter Winchell Cancer Fund. This work was supported in parts by grants from the National Institutes of Health (AI-17879 and CA-14051).

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

Received May 2, 1983.

References

Alt, F. W., Rosenberg, N., Casanova, R. J., Thomas, E., and Baltimore, D. (1982). Immunoglobulin heavy-chain expression and class switching in a murine leukemia cell line. Nature *296*, 325–331.

Banerji, J., Rusconi, S., and Schaffner, W. (1981). Expression of a β-globin gene is enhanced by remote SV40 DNA sequences. Cell *27*, 299–308.

Bernard, O., Hozumi, N., and Tonegawa, S. (1978). Sequences of mouse immunoglobulin light chain genes before and after somatic changes. Cell *15*, 1133–1144.

Brack, C., Hirama, M., Lenhard-Schuller, R., and Tonegawa, S. (1978). A complete immunoglobulin gene is created by somatic recombination. Cell *15*, 1–14.

Buchman, A. R., Burnett, L., and Berg, P. (1980). The SV40 nucleotide sequence. In DNA Tumor Viruses, J. Tooze, ed. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 799–829.

Clarke, C., Berenson, J., Goverman, J., Crews, S., Siu, G., Boyer, P., and Calame, K. (1982). An immunoglobulin promoter region is unaltered by DNA rearrangement and somatic mutation during B cell development. Nucl. Acids Res. *10*, 7731–7749.

Conrad, S. E., and Botchan, M. R. (1982). Isolation and characterization of human DNA fragments with nucleotide sequence homologies with the simian virus 40 regulatory region. Mol. Cell Biol. *2*, 949–965.

Davis, M., Calame, K., Early, P., Livant, D., Joho, R., Weisman, I., and Hood, L. (1980). An immunoglobulin heavy-chain gene is formed by at least two recombinational events. Nature *283*, 733–738.

de Villiers, J., Olson, L., Banerji, J., and Schaffner, W. (1981). A small segment of polyoma virus DNA enhances the expression of a cloned β-globin gene over a distance of 1400 base pairs. Nucl. Acids Res. *9*, 6251–6264.

Early, P., Huang, H., Davis, M., Calame, K., and Hood, L. (1980). An immunoglobulin heavy chain variable region gene is generated from three segments of DNA: V_H, D and J_H. Cell *19*, 981–992.

Fujimura, F. K., Deininger, P. L., Friedmann, T., and Linney, E. (1981). Mutation near the polyoma DNA replication origin permits productive infection of F9 embryonal carcinoma cells. Cell *23*, 809–814.

Fujimura, F. K., and Linney, E. (1982). Polyoma mutants that productively infect F9 embryonal carcinoma cells do not rescue wild-type polyoma in F9 cells. Proc Nat. Acad. Sci. USA *79*, 1479–1483.

Gillies, S., and Tonegawa, S. (1983). Expression of cloned immunoglobulin genes introduced into mouse L cells. Nucl. Acids Res., in press.

Gorman, C., Moffat, B., and Howard, B. (1982). Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell Biol. *2*, 1044–1051.

Griffin, B. E., Soeda, E., Barrell, B. G., and Staden, R. (1980). Sequence and analysis of polyoma virus DNA. In DNA Tumor Viruses, J. Tooze, ed. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 831–896.

Hayward, W. S., Neel, B. G., and Astrin, S. M. (1981). Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature *290*, 475–480.

Kataoka, T., Kawakami, T., Takahashi, N., and Honjo, T. (1980). Rearrangement of immunoglobulin γ_1-chain gene and mechanism for heavy chain class switch. Proc. Nat. Acad. Sci. USA *77*, 919–923.

Katinka, M., Yaniv, M., Vasseur, M., and Blangy, D. (1980). Expression of polyoma early functions in mouse embryonal carcinoma cells depends on sequence rearrangements in the beginning of the late region. Cell *20*, 393–399.

Kemp, D., Harris, A., Cory, S., and Adams, J. (1980). Expression of the immunoglobulin C gene in mouse T and B lymphoid and myeloid cell lines. Proc. Nat. Acad. Sci. USA *77*, 2876–2880.

Kim, S., Davis, M., Sinn, E., Patten, P., and Hood, L. (1981). Antibody diversity: somatic hypermutation of rearranged V_H genes. Cell *27*, 573–581.

Klein, G. (1983). Specific chromosomal translocations and the genesis of B-cell-derived tumors in mice and men. Cell *32*, 311–315.

Laimins, L. A., Khoury, G., Gorman, C., Howard, B., and Gruss, P. (1982). Host-specific activation of transcription by tandem repeats from simian virus 40 and Moloney murine sarcoma virus. Proc. Nat. Acad. Sci. USA *79*, 6453–6457.

Levinson, B., Khoury, G., Van de Woude, P., and Gruss, P. (1982). Activation of SV40 genome by 72-base pair tandem repeats of Moloney sarcoma virus. Nature *295*, 568–572.

Levitt, D., and Cooper, M. D. (1980). Mouse pre-B cells synthesize and secrete μ heavy chains but not light chains. Cell *19*, 617–625.

Maki, R., Traunecker, A., Sakano, H., Roeder, W., and Tonegawa, S. (1980). Exon shuffling generates an immunoglobulin heavy chain gene. Proc. Nat. Acad. Sci. USA *77*, 2138–2142.

Mather, E., and Perry, R. (1981). Transcriptional regulation of immunoglobulin V genes. Nucl. Acids Res. *9*, 6855–6867.

Max, E. E., Seidman, J. G., and Leder, P. (1979). Sequences of five potential recombination sites encoded close to an immunoglobulin κ constant region gene. Proc. Nat. Acad. Sci. USA *76*, 3450–3454.

Max, E. E., Miazel, J. V., and Leder, P. (1981). The nucleotide sequence of a 5.5 kilobase DNA segment containing the mouse κ immunoglobulin J and C region genes. J. Biol. Chem. *256*, 5116–5120.

Maxam, A. M., and Gilbert, W. (1980). Sequencing end-labeled DNA with base-specific chemical cleavages. Meth. Enzymol. *65*, 499–560.

Moreau, P., Hen, R., Wasylyk, B., Everett, R., Gaub, M. P., and Chambon, P. (1981). The 72 base pair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucl. Acids Res. *9*, 6047–6068.

Mulligan, R. C., and Berg, P. (1980). Expression of a bacterial gene in mammalian cells. Science *209*, 1422–1427.

O'Farrell, P. (1981). Replacement synthesis method of labeling DNA fragments. Bethesda Research Labs Focus *3*(3), 1.

Oi, V., Morrison, S. L., Herzenberg, L. A., and Berg, P. (1983). Immunoglobulin gene expression in transformed lymphoid cells. Proc. Nat. Acad. Sci. USA *80*, 825–829.

Parslow, T. G., and Granner, D. K. (1982). Chromatin changes accompany immunoglobulin κ gene activation: a potential control region within the gene. Nature *299*, 449–451.

Payne, G. S., Courtneidge, S. A., Crittenden, L. B., Fadly, A. M., Bishop, J. M., and Varmus, H. E. (1981). Analysis of avian leukosis virus DNA and RNA in bursal tumors: viral gene expression is not required for maintenance of the tumor state. Cell *23*, 311–322.

Payne, G. S., Bishop, J. M., and Varmus, H. E. (1982). Multiple arrangements of viral DNA and an activated host oncogene in bursal lymphomas. Nature *295*, 209–215.

Rice, D., and Baltimore, D. (1983). Regulated expression of an immunoglobulin κ gene introduced into a mouse lymphoid cell line. Proc. Nat. Acad.

Sci. USA *79*, 7862–7865.

Sakano, H., Huppi, K., Heinrich, G., and Tonegawa, S. (1979). Sequences at the somatic recombination sites of immunoglobulin light-chain genes. Nature *280*, 288–294.

Sakano, H., Maki, R., Kurosawa, Y., Roeder, W., and Tonegawa, S. (1980). Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy-chain genes. Nature *286*, 676–683.

Sandri-Goldin, R. M., Goldin, A. L., Levine, M., and Glorioso, J. C. (1981). High-frequency transfer of cloned herpes simplex virus type 1 sequences to mammalian cells by protoplast fusion. Mol. Cell. Biol. *1*, 743–752.

Schibler, U., Marcu, K. B., and Perry, R. P. (1978). The synthesis and processing of the messenger RNAs specifying heavy and light chain immunoglobulin in MPC-11 cells. Cell *15*, 1495–1509.

Sheiness, D., and Bishop, J. M. (1979). DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. J. Virol. *31*, 514–521.

Selsing, E., and Storb, U. (1981). Mapping of immunoglobulin variable region genes: relationship to the "deletion" model of immunoglobulin gene rearrangement. Nucl. Acids Res. *9*, 5725–5735.

Tonegawa, S. (1983). Somatic generation of antibody diversity. Nature *302*, 575–581.

Van Beveren, C., van Straaten, F., Galleshaw, J. A., and Verma, I. M. (1981). Nucleotide sequence of the genome of a murine sarcoma virus. Cell *27*, 97–108.

Van Ness, B. J., Weigert, M., Coleclough, C., Mather, E. L., Kelley, D. E., and Perry, R. P. (1981). Transcription of the unrearranged mouse C_κ locus: sequence of the initiation region and comparison of activity with a rearranged V_κ–C_κ gene. Cell *27*, 593–602.

Wasylyk, B., Wasylyk, C., Augereau, P., and Chambon, P. (1983). The SV40 72 bp repeat preferentially potentiates transcription starting from proximal natural or substitute promoter elements. Cell *32*, 503–514.

Weigert, M., and Riblet, R. (1976). Genetic control of antibody variable regions. Cold Spring Harbor Symp. Quant. Biol. *41*, 837–846.

Weiher, H., Konig, M., and Gruss, P. (1983). Multiple point mutations affecting the simian virus 40 enhancer. Science *219*, 626–631.