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A Lymphocyte-Specific Cellular Enhancer Is Located Downstream of the Joining Region in Immunoglobulin Heavy Chain Genes

Julian Banerji, Laura Olson, and Walter Schaffner
Institut für Molekularbiologie II
der Universität Zürich, Hönggerberg
CH-8093 Zürich, Switzerland

Summary

Transcriptional enhancers, originally discovered in viral genomes, are short, *cis*-acting, regulatory sequences that strongly stimulate transcription from promoters of nearby genes. We demonstrate the existence of an enhancer within a mouse immunoglobulin heavy chain gene. A DNA fragment located between the joining region and the switch recombination region in the intron upstream of the immunoglobulin μ constant region has been linked, in both orientations, to genes coding for rabbit β-globin or SV40 T antigen. This element enhances the number of correct β-globin gene transcripts by at least two orders of magnitude and also stimulates production of T antigen. It acts from several hundred to several thousand base pairs up or downstream of a promoter without amplifying template copy number. Of the various cell lines tested, the immunoglobulin gene enhancer functions only in lymphocyte-derived (myeloma) cells. We propose that this tissue-specific enhancer contributes to the activation of somatically rearranged immunoglobulin variable region genes and possibly to abnormal expression of other genes (e.g. *c-myc*) that become translocated to its domain of influence.

Introduction

The enormous repertoire of genes coding for immunoglobulin (Ig) molecules is achieved, in part, by rearranging pieces of DNA that are noncontiguous in chromosomes of germ-line cells (Hozumi and Tonegawa, 1976; reviewed by Tonegawa, 1983). To create a functional Ig heavy chain gene, one of a large number of variable (V) region DNA segments (Cory and Adams, 1980; Rabbitts, Matthyssens, and Hamlyn, 1980) is joined to one or more diversification (D) elements (Schilling et al., 1980; Sakano et al., 1981), which is joined to one of four joining (J) regions (Early et al., 1980; Sakano et al., 1980) upstream of the constant region exons (Gough et al., 1980; Rogers et al., 1980) of the complete gene.

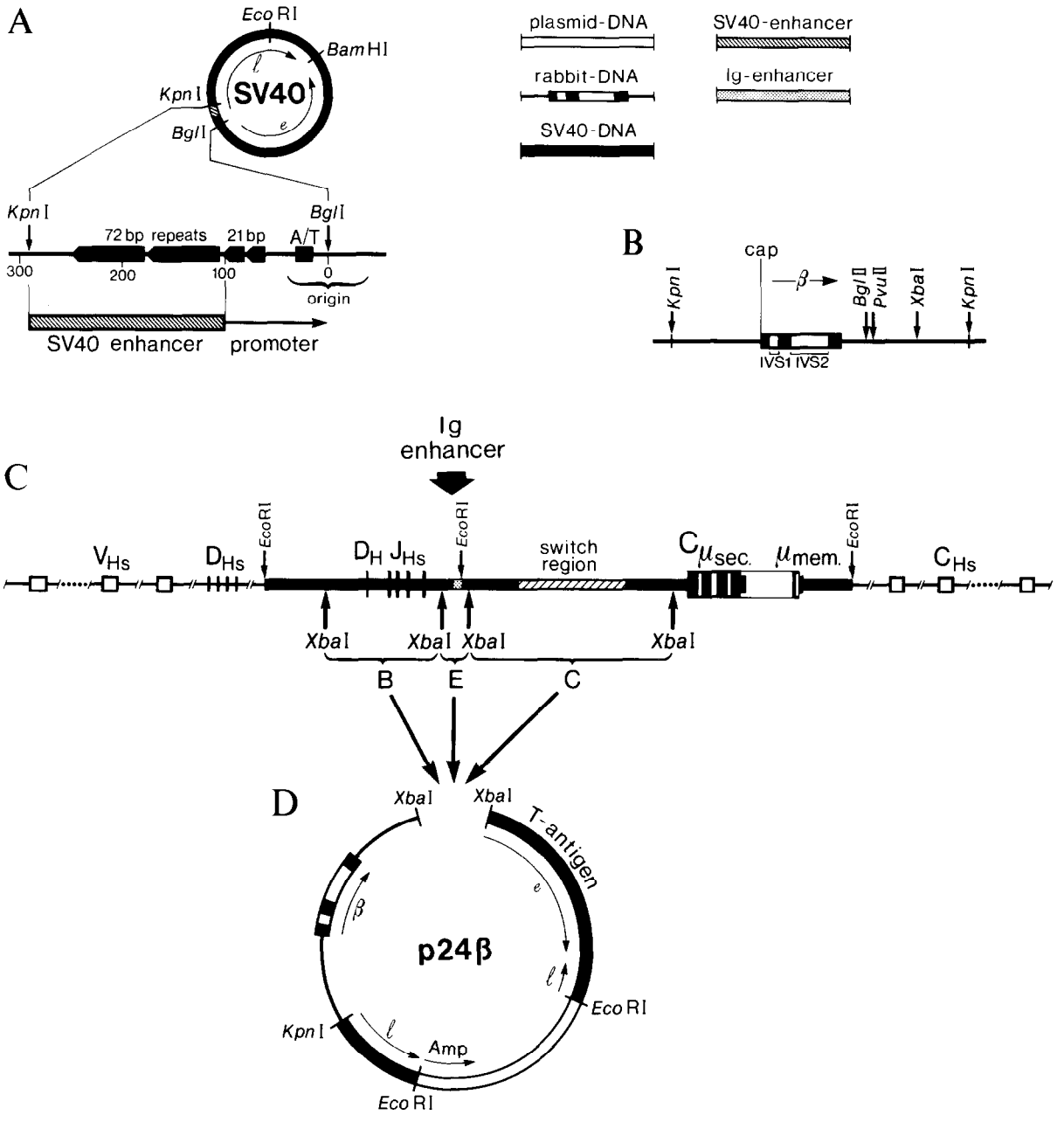
In myeloma cells the number of transcripts derived from a functionally rearranged kappa light chain V region gene is about four orders of magnitude higher than those derived from unrearranged V_K sequences (Mather and Perry, 1981). In the similarly organized Ig heavy chain genes, the DNA upstream from each variable region remains essentially unchanged by the rearrangements that create a transcriptionally active Ig heavy chain gene (Davis et al., 1980; Sakano et al., 1980; Clarke et al., 1982). Therefore, transcription of the functionally rearranged gene might be a consequence of signals encoded in the newly juxtaposed downstream sequences.

A DNA sequence that has been directly shown to influence transcription in such a fashion is the enhancer element in the genome of simian virus 40 (SV40). In a manner independent of distance and orientation, this DNA element can serve, in cis, to enhance the transcription of a nearby β-globin gene by more than two orders of magnitude even when it is located several thousand base pairs downstream from the promoter (Banerji, Rusconi and Schaffner, 1981; see also Moreau et al., 1981). Enhancers have also been identified in the genomes of polyoma virus (de Villiers and Schaffner, 1981), bovine papilloma virus (BPV) (Lusky, Berg, and Botchan, 1982), and Moloney sarcoma virus (MSV) (Laimins et al., 1982). Viral enhancers function in a wide variety of cell types but perform optimally in their natural host cells (de Villiers and Schaffner, 1981; Laimins et al., 1982; de Villiers et al., 1982b). They have proved to be useful for recombinant DNA studies of gene expression (for a review see Banerji and Schaffner, 1983; de Villiers and Schaffner, 1983). In particular, the SV40 enhancer has allowed efficient expression of an Ig lambda gene in HeLa cells, where it is not otherwise expressed (Picard and Schaffner, 1983). However, a nonviral enhancer with the above described properties has not yet been described in association with a cellular transcription unit, though it remains to be seen whether the "upstream sequences" identified in certain cellular promoters (e.g., see Grosschedl and Birnstiel, 1980) are functionally related to enhancers.

We tested the hypothesis that an enhancer element is encoded in the DNA of the mouse immunoglobulin constant region locus. Using a transient expression assay, we found such a DNA element in the intron between the J_H and the μ constant region exons. This enhancer element behaves similarly to the SV40 viral enhancer except for a striking tissue specificity—it functions in B-lymphocyte-derived (myeloma) cells, but not in other cell types we have tested.

Results

We began our studies with cloned DNA consisting of two Eco RI fragments encoding the J_H cluster and the μ constant region of the unrearranged (germ-line) mouse immunoglobulin heavy chain gene (clone M 23; J. Banerji, Master's thesis, State University of New York at Stony Brook, 1980; Marcu et al., 1980; a gift from K. Marcu). This DNA was digested with the restriction endonuclease Xba I, which released three fragments of interest encompassing almost the entire intron between the J and constant region exons. These three fragments, labeled B, E, and C (Figure 1C), were subcloned in either orientation into the plasmid p24β (Figure 1D). This plasmid contained both a rabbit β-globin gene (Figure) and SV40 sequences coding for T antigen, including the early promoter but


A
Eco RI
Bam HI
Kpn I
SV40
Bgl I
Kpn I
72 bp repeats
21 bp
A/T
Bgl I
300
200
100
0
origin
SV40 enhancer
promoter
plasmid-DNA
SV40-enhancer
rabbit-DNA
Ig-enhancer
SV40-DNA
B
cap
Kpn I
Bgl II
Pvu II
Xba I
Kpn I
IVS1 IVS2
C
Ig enhancer
EcoRI
EcoRI
EcoRI
VHs
DHs
DH JHs
switch region
Cμsec.
μmem.
CHs
Xba I
Xba I
Xba I
Xba I
B
E
C
D
Xba I
Xba I
T-antigen
p24β
Eco RI
Kpn I
Amp
Eco RI
E
enhancer
Xba I
Kpn I
pβG
Amp
Kpn I
F
Kpn I
Xba I
Bgl I
1
2
3
4
X
P
E
X
(K)
E
P

excluding the SV40 enhancer (Figure 1A). The six recombinants were named p24βB+, p24βB−, p24βE+, p24βE−, p24βC+, and p24βC−. As a negative control we used the plasmid p24β (the parental recombinant without an insert), and as a positive control we used pDept (containing the early region of SV40, including the viral enhancer shown in Figure 1A). These eight recombinants were transfected into HeLa cells which, after 2 days, were fixed and stained by indirect immunofluorescence for T antigen. No fluorescent cells were found (i.e., no enhancer activity was detected) when any of the three Ig segments were tested in HeLa cells (Figure 2).

The situation was different when we transfected the recombinants into a cultured mouse myeloma cell line, X63-Ag8, derived from B-lymphocytes in which immunoglobulin genes are normally heavily transcribed. Using a modified DEAE-dextran transfection protocol and the transient assay described above, a bright nuclear T-antigen fluorescence was seen in a fraction of the cells transfected with the recombinants p24βE+ or p24βE− (containing the Ig Xba I E fragment), or with pDept (the positive control containing the SV40 enhancer), but not with any of the other recombinants (Figure 2 and data not shown).

The Ig Enhancer Acts Only in *Cis*

To determine whether the enhanced expression of T antigen observed with the immunoglobulin Xba I E fragment was a *cis*- or a *trans*-acting phenomenon, we cotransfected two DNA clones into X63-Ag8 cells: one, pTAR7, contained the Xba I E fragment devoid of SV40 sequences (see Experimental Procedures for a complete description of this and other clones); the other, pET3, contained the SV40 early region devoid of an enhancer. No T-antigen-positive cells were detected in the transient expression assay, indicating that the immunoglobulin Xba I E fragment cannot act in *trans* but only in *cis* (Table 1).

Long-Range Effect

To determine whether this fragment could also enhance the correct expression of another gene, such as the rabbit β-globin gene, seven of the eight recombinants used above were again transfected into X63-Ag8 cells. However, as a positive control we used pβGXsv512, consisting of a cloned β-globin gene pβG (see Figure 1E) with a small fragment of SV40 containing the enhancer inserted 1.5 kb behind the gene. Thirty-six hours after transfection the cells were harvested, their RNA was extracted, and the number of correctly initiated β-globin transcripts was measured by the S1 nuclease hybridization assay (Figure 3A).

The results (Figure 3B) show that in myeloma cells the number of correctly initiated β-globin gene transcripts is greatly increased in clones containing the Ig Xba I E fragment in either orientation but not in clones containing the B or C fragments. Thus this Ig gene segment is similar to the SV40 enhancer in that it can serve, in a transient expression assay, to enhance transcription of a gene when it is placed in either orientation, up to 500 bp upstream, or 2500 bp downstream, from the promoter of a nearby gene.

Enhancement Does Not Require Template Amplification

The clones p24βE+ and p24βE− contain more viral sequences than pβGXsv512, the positive control. To determine whether part of the Ig enhancer effect was due to template amplification via the SV40 origin mediated by enhanced levels of T antigen, appropriate recombinants were restricted with the endonucleases Kpn I and Bgl I. A fragment of DNA containing the β-globin gene, linked in some cases to an SV40 or an Ig enhancer but devoid of a functional SV40 replication origin and the T-antigen structural gene, was purified by gel electrophoresis (see Figure 1F). These linear DNAs were transfected into X63-Ag8 cells. S1 mapping of the resultant transcripts again showed that the Ig enhancer is more effective in myeloma cells than the SV40 enhancer, and that the observed enhancement of at least two orders of magnitude was not due to T-antigen-mediated replication of the transfected templates (Figure 3C).

We also conducted a transient expression assay in the presence of hydroxyurea, which inhibits DNA replication (Manteuil and Girard, 1974). We used three clones containing the T-antigen coding region and the SV40 early promoter, pET3, pDept, and p24βE+. Clone pET3 contained no enhancer; pDept included an SV40 viral enhancer; and p24βE+ included an Ig enhancer. Each recombinant was transfected onto two plates of X63-Ag8 cells. In addition, pDept was transfected onto two plates of HeLa cells. One plate of each pair was treated normally; the

Figure 1. Schematic Representation of Recombinant DNAs Used

(A) Map of the 5.2 kb SV40 genome (Tooze, 1981) with the Kpn I to Bgl I region expanded to show SV40 enhancer (fine hatching) and SV40 promoter.

(B) Map of the rabbit hemoglobin β1 gene within a 4.7 kb Kpn I fragment showing relevant restriction sites.

(C) Schematic diagram of the immunoglobulin heavy chain gene family in its germ-line configuration. Thin lines: regions not drawn to scale. Thick lines: two Eco RI fragments containing the μ constant region (Marcu et al., 1980). Coarse hatching in switch recombination region indicates sequences that are deleted in this phage clone. Stippled area under large arrow shows location of the Ig enhancer. Arrows pointing upwards mark the restriction sites used to generate Ig Xba I fragments B, E, and C.

(D) Map of p24β, a clone containing a unique Xba I site into which DNA fragments were cloned and assayed for their ability to enhance expression of β-globin or T antigen (the synthetic Xba I linker in SV40 is located 99 bp from the Bgl I site between the 72 bp and 21 bp tandem repeats; a gift from Y. Gluzman; see also Figure 1A). Clones thus generated were designated p24βB+ and p24βB− (Xba I B fragment in either orientation), p24βE+ and p24βE− (Xba E enhancer fragment in either orientation).

(E) Map of pβG, a clone containing only the 4.7 kb Kpn I fragment with the rabbit β-globin gene. This clone was used to test enhancer DNA fragments as above—e.g., clone pβGxba32 contained the SV40 enhancer.

(F) Schematic diagram of Kpn I–Bgl I fragments used for the experiment shown in Figure 3C. (1) derived from p24β (without enhancer); (2) and (4) derived from p24βE+ and p24βE−, respectively (with Ig enhancer); (3) derived from pβGxba32 (with SV40 enhancer). (K) refers to a destroyed Kpn I site.

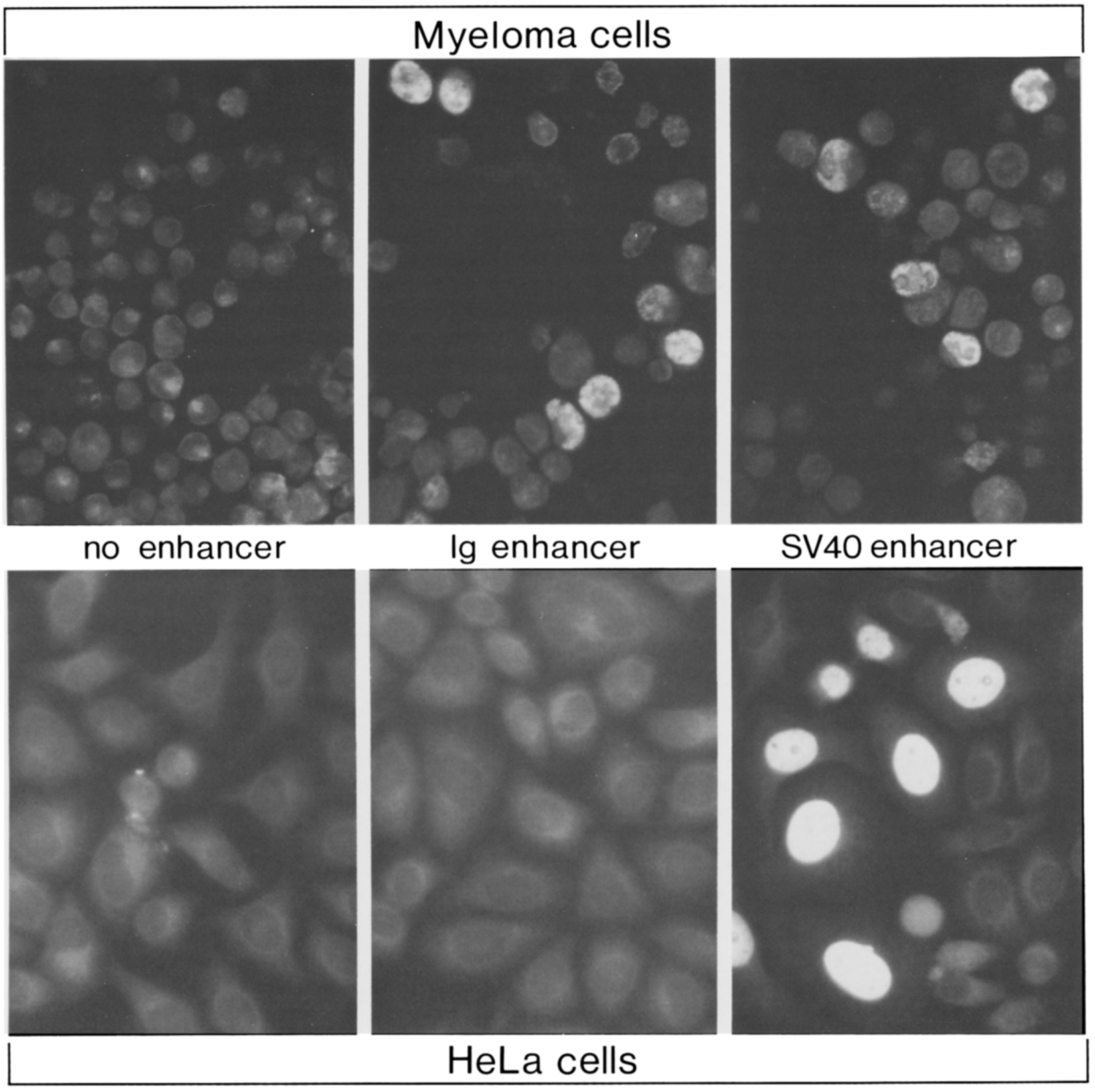


Figure 2. Myeloma-Cell-Specific Activity of the Ig Enhancer

SV40 T-antigen clones were transfected in parallel into both myeloma X63-Ag8 cells and HeLa cells. Thirty-six hours later, cells were fixed and stained for nuclear T antigen by indirect immunofluorescence (see Experimental Procedures). Left: negative control, clone p24β with a deletion of the entire 72 bp repeat enhancer region (for details see Figure 1 and Experimental Procedures). Center: clone p24βE+ with the Ig enhancer replacing the SV40 enhancer. Nuclear T-antigen fluorescence is visible in transfected myeloma cells but not in HeLa cells (see also Table 1). Right: positive control, clone pDept, which contains the SV40 enhancer in its wild-type configuration.

other was treated with hydroxyurea (see Experimental Procedures). When the levels of T-antigen expression directed by an SV40 and an Ig enhancer were compared, the percentages of T-antigen-positive cells were about the same, either with hydroxyurea treatment or without (see Table 1). The percentage of T-antigen-positive cells was always smaller under conditions of hydroxyurea treatment. However, this appears to be a general phenomenon since it is also seen in hydroxyurea-treated HeLa cells transfected with pDept (containing the SV40 enhancer). Essentially the same results were obtained when DNA replication was inhibited by cytosine arabinoside (araC) (data not shown). We conclude that the enhancing ability of the Ig Xba I E fragment parallels that of the SV40 enhancer, which does not act via template amplification (Banerji et al., 1981).

Cell-Type Specificity

We wanted to know if the Ig enhancer would function in mouse cells other than the X63-Ag8 cell line derived from

Table 1. Enhancer-Directed T-Antigen Synthesis in Transfected Cells

T Antigen Clone (Enhancer)	Cells (Type)	Total Cells[a] (per 8 × 8 mm)	Positive for T Antigen	Duplicate Plate Treated with Hydroxyurea: Total Cells (per 8 × 8 mm)	Duplicate Plate Treated with Hydroxyurea: Positive for T Antigen
pET3 (no enhancer)	X63-Ag8 (myeloma)	50,000	0	14,000	0
p24βE+ (Ig enhancer)	X63 Ag8	54,000	2130 (4%)	11,500	158 (1.4%)
pDept (SV40 enhancer)	X63-Ag8	51,000	2860 (6%)[b]	13,000	187 (1.4%)
pDept	HeLa (human carcinoma)	49,000	8400 (17%)	21,000	1160 (6%)
pET3 (no enhancer) and pTAR7 (Ig enhancer but no T antigen gene)	X63-Ag8	46,000	0		

All transfections were done without chloroquine (see Experimental Procedures).

[a] The total number of cells in the 8 × 8 mm area was extrapolated from counting three areas of 0.145 mm^2 each (400-fold magnification); that is, a total of 150–300 cells was counted. The number of T-antigen-positive cells was counted either in the entire 8 × 8 mm area or in a defined section thereof such that at least 100 positive cells were scored.

[b] pDept, with the wild-type arrangement of the SV40 enhancer-promoter, gives higher values of T-antigen-positive cells than p24βE+ containing the Ig enhancer and the truncated T-antigen gene. However, when both enhancers are explanted and placed 2500 bp downstream of the β-globin gene, the Ig enhancer is stronger than the SV40 enhancer.

mouse lymphocytes. In other words, is its inability to function in human HeLa cells a species restriction, or would the enhancer also be nonfunctional in mouse 3T6 cells, implying that it is cell-type specific? Transfections into several different cell lines are compared in Table 2. The Ig enhancer does not function detectably in the cell line 3T6, derived from mouse fibroblast, nor does it function in mink lung cells or human HeLa cells (however, we cannot exclude a weak but significant activity below the threshold of the immunofluorescence assay). In contrast, the Ig enhancer functions well in all mouse myeloma cells tested: X63-Ag8; and two related cell lines, F0 and Sp6Bu6Bu.

Delimitation of Important Sequences

To narrow the region responsible for the enhancing effect, we constructed the deletion mutants shown in Figure 4. The Xba I E fragment can be cleaved once by each of the endonucleases Pst I or Eco RI. We tested both the large and the small Pst I to Xba I fragments in clones named pPTA-L and pPTA-S, and both the large and the small Eco RI to Xba I fragments in clones named pET1 and pET3. After assaying these clones for enhanced T-antigen expression (see Figure 4 for results), we concluded that most, if not all, of the enhancing activity is in the central Eco RI to Pst I fragment. This fragment was sequenced in its entirety from both ends (Figure 5A).

A 171 bp Alu I restriction fragment (Figure 4) from the central portion of the Eco RI–Pst I region was subcloned into a Pvu II site behind the β-globin gene (Figure 1B) in the clone pβGX (Figure 1E). This clone, called pβGXalu, was transfected into X63-Ag8 cells along with five other clones, and the β-globin gene transcripts were analyzed as before. The other clones were all derivatives of the parental clone pβG. The first, pβG, contained no insert; the second, pβGXsv512, contained the SV40 enhancer; the third, pβGXpy, the polyoma enhancer (de Villiers et al., 1982b; a gift from J. de Villiers); and the last two, pTAR1 and pTAR7, contained the Xba I E fragment cloned in either orientation. The SV40 and polyoma virus enhancers and the Ig enhancer Alu I subfragment were about equally efficient, whereas the Ig Xba I E fragment was two to three times more efficient than the viral enhancers or the Alu I subfragment (data not shown).

To delineate further the sequences responsible for the enhancing effect, exonuclease Bal 31 was used to generate a series of deletion mutants. The large Eco RI to Xba I fragment was subjected to resection from the Eco RI site, and synthetic Xho I linkers were used to mark the mutant breakpoints. The fragments were then inserted into a plasmid vector pBAG, containing SV40 T-antigen coding sequences including the early promoter, but excluding the SV40 enhancer. These recombinants were then transfected into X63-Ag8 cells, which were assayed for enhanced T-antigen expression. A fragment retaining considerable enhancer activity, pBAG-A, was then subjected to a Bal 31 resection from the other (Xba I) end, and synthetic Xba I linkers were used to mark these deletion endpoints. The new deletion fragments were replaced into the vector pCAB and assayed for T-antigen expression. Figure 4 depicts the entire collection of DNA fragments that were generated and tested for enhancer activity, along with an indication of their relative enhancing abilities. The endpoint of each deletion mutant was determined by sequencing.

The results of the Bal 31 resection indicate that sequences important for the enhancing effect are distributed over most of the 300 bp Eco RI to Pst I fragment. Similar results have been obtained by testing deletion mutants of the SV40 and polyoma enhancers (Benoist and Chambon, 1981; S. Lupton and R. I. Kamen, personal communication), suggesting that an enhancer does not consist of one short sequence that is necessary and sufficient for full activity. Rather, a number of short but significant homologies have been found by computer-aided comparison of this sequence with those of viral enhancers from SV40, BKV, polyoma virus, adenovirus (E1a), MSV, and BPV (see Gluzman and Shenk, 1983). However, when any arbitrary

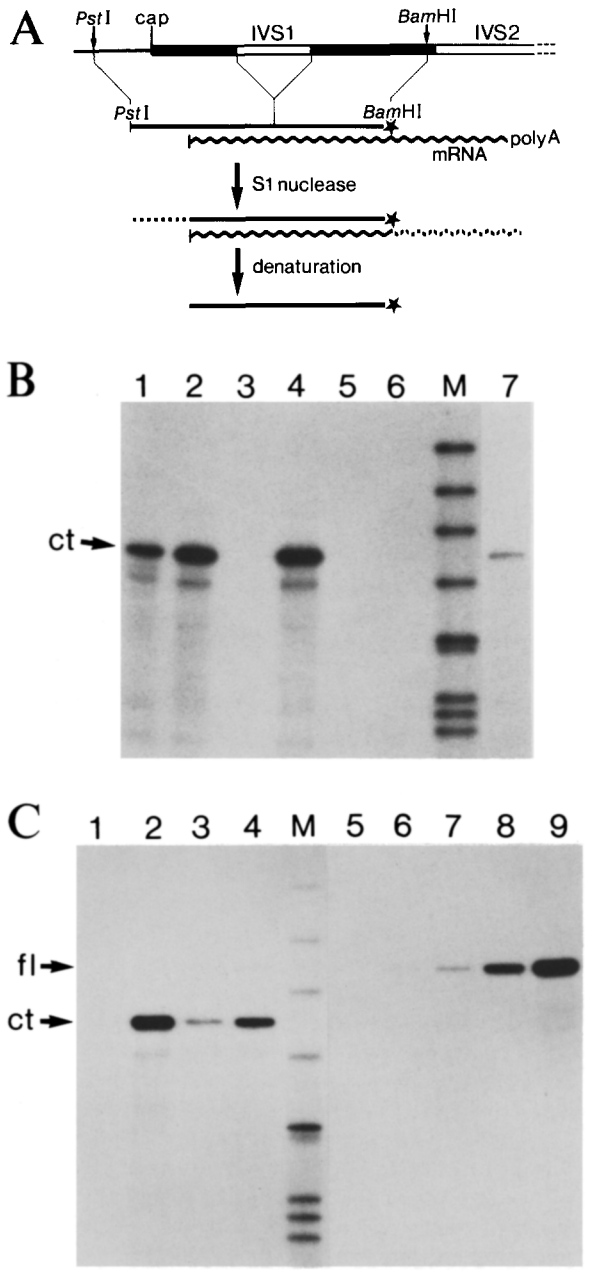


Figure 3. β-Globin Gene Transcription Directed by the Ig Enhancer

(A) S1 nuclease mapping scheme (Weaver and Weissmann, 1979). A β-globin gene clone lacking the first intervening sequence (IVS1; see also Figure 1B; Weber et al., 1981; a gift from H. Weber) was used as a radioactive probe (for further details see Rusconi and Schaffner, 1981). DNA end-labeled at the Bam HI site was hybridized to unlabeled RNA from 10^6 transfected X63-Ag8 myeloma cells, treated with S1 nuclease, denatured, fractionated by gel electrophoresis, and autoradiographed.

(B) Gel autoradiography. Hybridization to RNA from cells transfected with recombinant pβGXsv512, which contains the SV40 enhancer downstream of the β-globin gene (lane 1; positive control; Figure 1E); with the recombinant p24βE+ containing the Ig Xba I E enhancer fragment in the same orientation with respect to the β-globin gene promoter as it is to the Ig gene promoter in vivo (lane 2); with the parental globin recombinant p24β not containing an enhancer (lane 3, negative control); with the recombinant p24βE− containing the Ig enhancer fragment in the inverted orientation (lane 4); with recombinants p24βB+ and p24βB−, a 1:1 mixture with both orientations of the Xba I B fragment (lane 5); with p24βC+ and p24βC−, a 1:1 mixture with both orientations of the Xba I C fragment (lane 6; see also Figure 1D). (Lane 7) Control hybridization to rabbit reticulocyte RNA. (Lane M) End-labeled marker DNA fragments (pBR322 digested with Hpa II). ct: fragment with correct terminus, mapping 354 nucleotides upstream from the Bam HI site.

(C) Gel autoradiography after transfection of purified linear template DNA devoid of the SV40 replication origin and T-antigen coding sequences (see Figure 1F). (Lane 1) Hybridization to RNA from cells transfected with the large Kpn I–Bgl I fragment of p24β (not containing an enhancer). (Lane 2) Kpn I–Bgl I fragment of p24βE + (enhancer). (Lane 3) Kpn I–Bgl I fragment of pβGxba32 (SV40 enhancer; see also Figure 1E). (Lane 4) Kpn I–Bgl I fragment of p24βE − (inverted Ig enhancer). (Lanes 5–9) A dilution series with the full-length probe. The dilution factor between neighboring lanes is 3.16—i.e., lanes 7 and 9 are one order of magnitude apart. (Lane M) Marker DNA fragments. fl: full-length probe (453 nucleotides). ct: correct terminus fragment (354 nucleotides).

Table 2. Cell-Type Specificity of the Ig Enhancer

DNA Clone[a] (Amount)	Cells (Species)	Total Cells (per 8 × 8 mm)	Positive for T Antigen
p24βE+ (0.25 μg)	X63-Ag8 (mouse)	31,000	1,320 (4%)
pDept (0.25 μg)	X63-Ag8	41,000	2,920 (7%)
pDept (0.025 μg)	X63-Ag8	32,000	502 (1.6%)
pDept (0.0025 μg)	X63-Ag8	38,000	59 (0.2%)
p24βE+ (0.25 μg)	3T6 (mouse)	27,000	0
pDept (0.25 μg)	3T6	35,000	3,700 (11%)
pDept (0.025 μg)	3T6	30,000	790 (3%)
pDept (0.0025 μg)	3T6	34,000	62 (0.2%)
p24βE+ (0.25 μg)	Lung (mink)	30,000	0
pDept (0.25 μg)	Lung	32,000	2,820 (9%)
pDept (0.025 μg)	Lung	33,000	380 (1.2%)
pDept (0.0025 μg)	Lung	36,000	12 (0.03%)
p24βE+ (0.25 μg)	HeLa (human)	47,000	0
pDept (0.25 μg)	HeLa	43,000	13,000 (30%)
pDept (0.025 μg)	HeLa	45,000	2,240 (5%)
pDept (0.0025 μg)	HeLa	40,000	240 (0.6%)

[a] All transfections were done using 35 mm plates where 0.25 μg DNA are normally used (see Experimental Procedures).

criterion is used to compare these enhancers, no most compelling "core" sequence can be extracted. The polyoma enhancer shares many homologies with the Ig enhancer. However, except for homology blocks K, N, and C, these homologies cannot be assembled into a distinct spatial framework (Figure 5B). Some sequences seem to be absolutely required for enhancer activity: T-antigen expression is no longer detectable in mutants deleted extensively from either end. This central region contains a duplication of the sequence 5′-GTGGTTT-3′, which also appears on the opposite strand. This sequence, or its derivatives, appears in other enhancers (see Weiher et al., 1983) and is present 150 bp upstream, and on the other strand 150 bp downstream, of the DNAase I hypersensitivity site (Chung et al., 1983) of the Ig kappa light chain intron (Max et al., 1981).

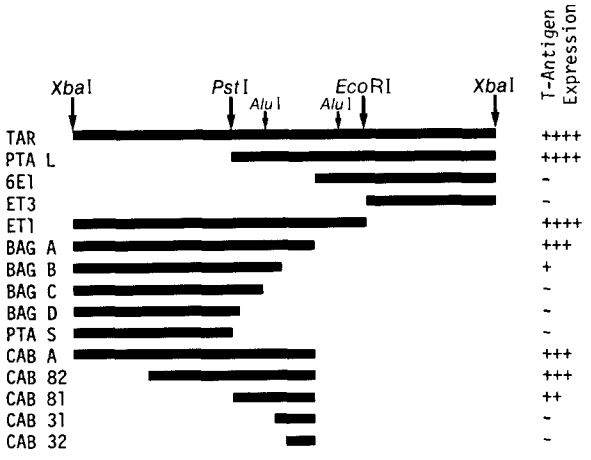


Figure 4. Localization of the Ig Enhancer Activity

Top line: the Xba I E fragment. Black bars indicate DNA still present in the deletion mutants. Immunofluorescence quantitation of the fraction of T-antigen-positive cells was done as indicated in Table 1. – no positive cells, + less than 5%, ++ between 5% and 30%, +++ between 30% and 75%, ++++ more than 75% of the fraction of T-antigen-positive cells seen with the standard recombinant p24βE+. Endpoints of the Bal 31 resection series are as follows: p6E1 extends up to and includes base pair 198; pBAG-A, base pair 198; pBAG-B, base pair 120; pBAG-C, base pair 77; pBAG-D, base pair 21; pCAB-A, base pair 198, pCAB-81, base pair 24; pCAB-31, base pair 138; pCAB32, base pair 164 (see Figure 5 for numeration).

Discussion

We have shown that the mouse genome contains a sequence that, in *cis*, enhances the expression of a nearby gene. Simple deletion of this region from the Ig transcription unit and measurement of a reduction in the number of Ig mRNAs would not allow one to distinguish whether this region acts in *cis* or in *trans* to enhance transcription or to ensure the stability (or processing) of the primary transcript. Since DNA segments that act in *trans* to stimulate transcription have recently been identified in the genomes of pseudorabies virus and adenovirus (see Gluzman and Shenk, 1983), we have explicitly demonstrated here that the Ig enhancer does not act in *trans*. We also show that it is not required in *cis* for RNA processing by explanting the enhancer outside of a transcription unit and showing that the transcripts are correctly initiated. We considered it necessary to S1 map the transcripts since it has been shown (Picard and Schaffner, 1983) that abundant production of Ig transcripts that appear properly sized on Northern blots can result from aberrant splicing of longer transcripts that initiate at sites farther upstream on the same molecule (e.g., at SV40 control signals). Such aberrant transcripts have been found to direct the synthesis of Ig protein. (M. Neuberger, personal communication).

Placement of the enhancer element in either orientation from several hundred to several thousand base pairs up or downstream from a promoter can increase the number of correctly initiated transcripts of the β-globin gene by at least two orders of magnitude. It has also been shown that this Ig μ Xba I E fragment enhances correct transcription from the promoter of an immunoglobulin lambda light chain gene (D. Picard and W. Schaffner, unpublished results). The Ig enhancer element functions in a manner similar to the SV40 viral enhancer in that it does not act by raising the copy number of the transfected template. It differs from the SV40 enhancer in that it functions only in myeloma cells, derived from B-lymphocytes, but not in cells that stem from mouse fibroblasts or from human or mink epithelial cells. Though studies comparing the efficiencies of mouse viral enhancers in mouse and primate cells have noted a species preference for these enhancers (de Villiers et al., 1982b; Laimins et al., 1982), the Ig enhancer is the first example of an enhancer that functions in mouse cells of one specific lineage, but not another.

We propose that this cell-type-specific enhancer element serves to enhance correct transcription from the promoter of the most proximal upstream immunoglobulin variable region which becomes joined to the Ig constant region by the somatic recombination events that create a complete Ig heavy chain gene. Furthermore, because of its location in front of the switch recombination region, this DNA element would continue to be associated with, and would promote transcription from the original variable half of that gene throughout subsequent class switching events. Thus, whether the class switch involves actual DNA rearrangements (Kataoka et al., 1980; Davis et al., 1980; Sakano et al., 1980) or novel splicing of longer transcripts (Maki et al., 1981; Yaoita et al., 1982), normal B-cells probably use the enhancer described here to express Ig heavy chains of all classes (Figure 6). In addition, the most proximal upstream V region promoter probably acts as a sink or "road block" for information traveling along the DNA from the enhancer (see below), preventing enhanced transcription from more distal V region promoters.

The model that this region of the genome contains sequences that are important for the expression of Ig genes is compatible with observations of Alt et al. (1982a): Continued growth of transformed lymphoid cells in culture leads to deletions in the tandemly repeated switch sequences, similar to those which have been previously described (Marcu et al., 1980). One of these deletions extends into the Xba I E fragment; and the loss of the ability to express Ig heavy chains has been correlated, in one instance, with the loss of sequences that we have now shown to contain the Ig enhancer.

There are reports that the unrearranged μ constant region is transcribed to some extent (Kemp et al., 1980; Alt et al., 1982b). The 5′ ends of these "sterile" μ transcripts have been mapped to a position close to the location of the Ig enhancer described here (Nelson et al., 1983). We previously found that information is transmitted in both directions from an enhancer to proximal promoters (a property that seems intrinsic to all enhancers), even from a position 3′ to the promoter (Banerji et al., 1981). We also noted that functional promoters and plasmid sequences attenuate the propagation of this information along the DNA, though long stretches of DNA from be-

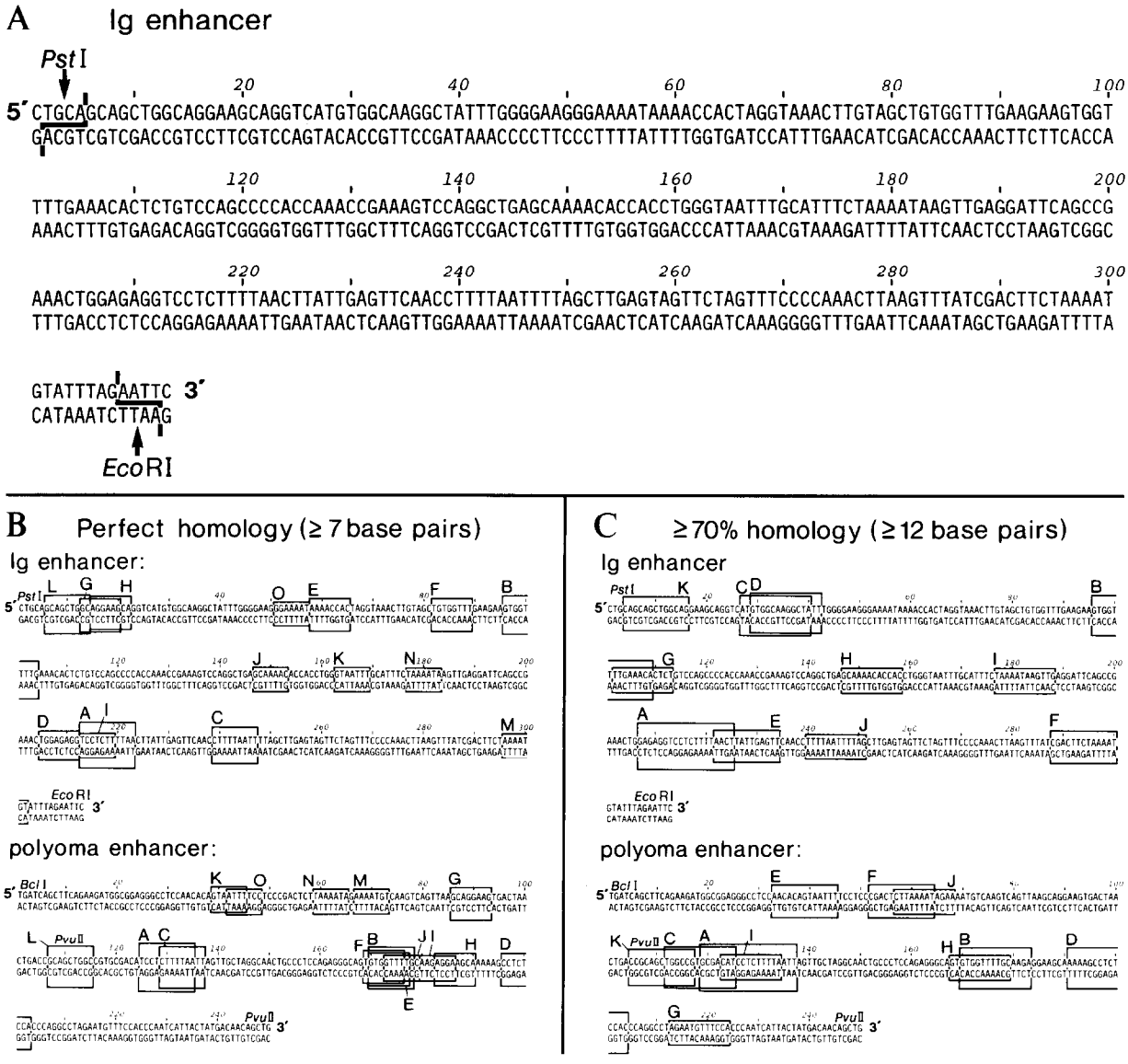


Figure 5. Nucleotide SEquence of the Ig Enhancer and a Comparison with the Polyoma Virus Enhancer

(A) Nucleotide sequence of the 313 bp Pst I to Eco RI Ig enhancer segment from the unrearranged Ig μ constant region. The sequence of part of this region, from a rearranged Ig gene in the IgM expressing plasmcytoma HPC76, has been presented before (Gough and Bernard, 1981). Among several differences between our sequence and theirs is a 6 bp deletion (our coordinates 166–171) of unknown physiological relevance.

(B and C) Homologies between the Ig and polyoma enhancers according to different criteria. Homologous sequences are boxed; any pair of short homologies is labeled identically (not all homologies are on the same DNA strand). The polyoma sequence is from Tyndall et al. (1981).

tween the mouse major and minor β-globin genes have no attenuating effect in these experiments (Banerji and Schaffner in de Villiers et al., 1982a). Enhancer-activated pseudopromoters have indeed been found in plasmid DNA (Wasylyk et al., 1983; see also Moreau et al., 1981). It seems likely therefore that the sterile μ transcripts are due to transcription from pseudopromoters located near the Ig enhancer (see also Figure 6). Sterile kappa transcripts that begin at a pseudopromoter upstream of the J segments of the unrearranged kappa light chain constant region (Van Ness et al., 1981) may arise by a similar mechanism.

The chromatin of actively expressed Ig kappa genes contains a general DNAase I sensitivity (Storb et al., 1981), and specific DNAase I hypersensitivity sites exist in the intron between the J region and constant region of Ig genes (Parslow and Granner, 1982; Chung et al., 1983). Such DNAase I hypersensitivity sites are also present in polyoma or SV40 chromatin extracted from infected cells (Herbomel et al., 1981; Cremisi, 1981). These sites coincide with the locations of the papovavirus enhancers. In other genes (putative) enhancers might also be situated near such hypersensitivity sites (Muskavitch and Hogness, 1982).

If enhancers activate genes within their domain of influ-

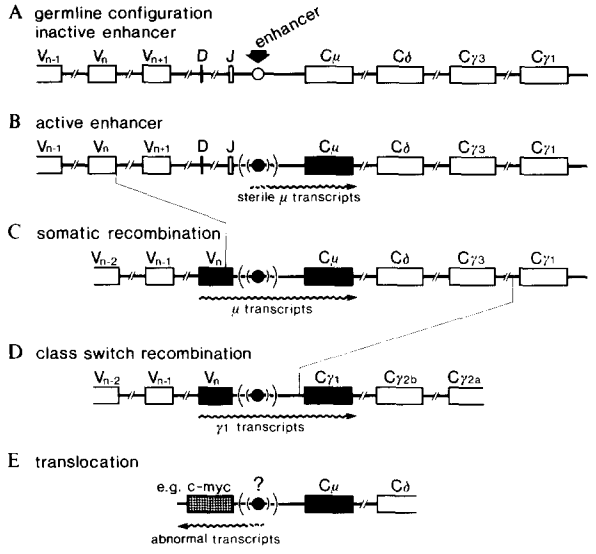


Figure 6. Model of the Ig Enhancer Effect on Gene Transcription

(A) Germ-line configuration of the Ig heavy chain gene family. This region is not transcribed when the Ig enhancer is inactive.

(B) Cells in which the enhancer is active give rise to sterile μ transcripts that start at pseudopromoters in the unrearranged locus.

(C) Creation of a functional Ig gene by somatic recombination (VDJ joining) gives rise to correct Ig μ heavy chain gene mRNAs. Alternate processing of longer transcripts can give rise to mRNAs coding for Ig heavy chains of other classes.

(D) Class switching by allelic deletion gives rise to mRNAs coding for Ig heavy chains of invariant idiotype but different isotype.

(E) Aberrant translocation of the Ig enhancer to the vicinity of other, nonimmunoglobulin genes gives rise to transcripts beginning at nearby promoters and pseudopromoters. (The last line of this figure describes a hypothetical arrangement. In fact, it has been shown, in the case of a mouse plasmacytoma, that the 5′ end of *c-myc* and the 5′ end of a functionally rearranged Ig gene (including the Ig enhancer) have been juxtaposed by a reciprocal crossover occurring within both genes (Cory et al., 1983; see also Stanton et al., 1983; Shen-Ong et al., 1982). Taub et al., 1982, have described lymphomas in which the human Ig μ constant region is juxtaposed to a *c-myc* gene; however, the location of the putative human Ig enhancer is unknown.

ence, it follows that when they are translocated they may also cause alterations in the transcription pattern of the newly juxtaposed DNA. Oncogenically transformed animal cells have novel DNA sequences inserted near real (or presumed) cellular oncogenes in chicken bursal lymphomas (Hayward et al., 1981; Neel et al., 1981; Payne et al., 1982), in mouse mammary tumors, thymic lymphomas, and plasmacytomas (Nusse and Varmus, 1982; Tsichlis et al., 1983; Shen-Ong et al., 1982; Cory et al., 1983; Stanton et al., 1983), and in human Burkitt's lymphomas (Taub et al., 1982; see also Figure 6). Transcriptional enhancers may be responsible for altering gene expression in many of the above examples, and we consider it likely that enhancers are integrally involved in the normal (and abnormal) development of eucaryotic cells.

Experimental Procedures

Construction of Recombinant DNAs

All clones were constructed according to standard recombinant DNA techniques (Maniatis et al., 1982). Clones not described in detail in the text were made as follows. For pDept, the small Eco RI to Bam HI fragment of pML (Lusky and Botchan, 1981) was replaced by the large Kpn I to Bam HI fragment of SV40 (see Figure 1A) using Eco RI linkers at the Kpn I site. For pET1, the Eco RI fragment of p24βE+ containing part of the Ig Xba I E fragment and all of the SV40 early region was cloned into pUC8 (Viera and Messing, 1982) with the enhancer being proximal to the polylinker. pET3 is a derivative of p24βE−, analogous to pET1. pTA-S is a derivative of p24βE+, lacking the Pst I fragment which encompasses part of the Ig Xba I E fragment and part of the β-globin gene. pTA-L is a derivative of p24βE−, analogous to pTA-S. pβG is the 4.7 kb Kpn I fragment containing the rabbit hemoglobin β1 gene (a gift from T. Maniatis) cloned into the Kpn I site of pJC-1 (a gift from J. Jenkins; see Banerji et al., 1981; see Figure 1E). pβG− is a derivative of pβG containing the Kpn I insert in the opposite orientation. pβGX is a derivative of pβG containing an Xho linker at the Bgl II site (shown in Figure 1B) behind the β-globin gene. pBAG is a derivative of p24β with its β-globin gene replaced by that from pβGX. This clone was used as a recipient vector for the Xba I to Xho I fragments of the Bal 31 resection. pCAB is a derivative of pET1 with the Bgl II to Xba I fragment of pBAG-A replacing the enhancer-containing Xba I to Bam HI (polylinker) fragment of pET1. It was used as a recipient vector for Xba I to Xho I fragments of the second Bal 31 resection. pTAR1 is the Ig Xba I E fragment cloned into the Xba I site of pβG−. The orientation of the insert with respect to the β-globin gene is the same as that of p24βE+ (Figure 1F, second line). pTAR7 is identical with pTAR1 except for the inversion of the Xba I insert. pβGXalu is the Alu fragment depicted in Figure 4 inserted into the Pvu II site (shown in Figure 1B) behind the globin gene of pβGX. pβGXpy is the 246 bp Bcl I to Pvu II fragment containing the polyoma enhancer (de Villiers and Schaffner, 1981; see also Figure 5) cloned with Xho I linkers into pβGX. pβGXsv512 is a 201 bp fragment extending from the Kpn I site of SV40 and containing the SV40 enhancer, cloned with Xho I linkers into pβGX. pβGxba32 is a 366 bp Kpn I to Hind III fragment containing the SV40 enhancer cloned with synthetic linkers into the Xba I site of pβG (see Figure 1E). p6E1 is a derivative of p24βE+ digested with Bgl II, then digested with Bal 31, and finally reclosed in the presence of Xho I linkers.

Cell Growth and Transfection with DEAE-Dextran

Cell Growth

Myeloma cells X63-Ag8 (Köhler and Milstein, 1975; a gift from S. Y. Chung), F0 (Fazekas de St. Growth and Scheidegger, 1980; a gift from H. Hengartner), and Sp6Bu6Bu (a gift from G. Köhler) were grown in Dulbecco's modified Eagle's medium with 4.5 g glucose/liter (Gibco), containing 2.5% calf serum and 12.5% fetal calf serum, and buffered with 3.7 g sodium bicarbonate/liter. Nouse 3T6 (a gift from G. Magnusson), mink lung (a gift from H. Diggelmann), and HeLa cells (a gift from U. Pettersson) were grown in the same medium except that the fetal calf serum concentration was 2.5%. Cells were seeded the day before transfection. At the time of transfection, they were less than 20% confluent (for HeLa and mink cells, 50% confluent). Myeloma cells were by then moderately adhered to the petri dish such that they were not washed off the plate upon careful changes of the medium.

DNA

For the transfection of one large (90 mm diameter) petri ddish, 1 μg of the appropriate DNA clone was diluted with Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.8]) to 50 μl. Tris-buffered saline, 250 μl (TBS; 25 mM Tris-HCl, 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM Na_2HP04 [pH 7.4], modified from Kimura and Dulbecco, 1982), was then added, and the sample was mixed well at room temperature. A solution of DEAE-dextran, 300 μl (Pharmacia; M_r 2 × 10^6; aliquots of 1 mg/ml in TBS kept frozen), was added, and the sample was mixed well at room temperature.

Transfection

The medium from the plate was carefully replaced by 10 ml TBS (90 mm plate). TBS was aspirated, and another ml TBS was added and aspirated. The DNA sample (0.6 ml, 1 μg DNA in TBS with 0.5 mg/ml DEAE-dextran) was added to the center of the plate. The plate was kept at room temperature for 30 min with occasional tilting. The DNA was then aspirated and carefully replaced by 10 ml TBS. TBS was aspirated and replaced by 10 ml tissue culture medium containing 0.1 mM chloroquine diphosphate (Luthman and Magnusson, 1983; Sigma; mN stock solution in TBS, aliquots kept frozen in the dark; chloroquine treatment increased the number of T-

antigen-positive cells 2 to 5 fold under our assay conditions). After 5 hr of incubation at 37°C, the chloroquine medium was replaced by standard tissue culture medium and incubation at 37°C was continued for another 30–35 hr. During the incubation time, cells were not allowed to become overcrowded but were split into two plates. RNA analysis was performed as described (Weaver and Weissmann, 1979; de Villiers and Schaffner, 1983). For immunofluorescence analysis, transfections were scaled down to 35 mm plates, and only 0.25 μg DNA was used.

Fixation of Cells for the Immunofluorescence Assay

The usual fixation procedure, in which the fixer is added to the wet cell monolayer after washing once with TBS (Banerji et al., 1981) gave best results with fibroblast and epithelial cells. However, for myeloma cells the procedure had to be modified as follows. Medium was replaced by TBS, which was also aspirated. The plate, without a lid, was put in an upright position and allowed to dry completely in the airstream of the laminar flow hood. Fixer (70% v/v methanol, 30% acetone, kept at −20°C) was added, and the plate was incubated at −20°C for 15 min. The fixer was aspirated, and the plate was completely dried and processed for immunofluorescence as described in Banerji et al. (1981).

Inhibition of DNA Replication

After DNA transfection with DEAE-dextran, chloroquine treatment was omitted because cells are killed by treatment with both chloroquine and hydroxyurea. Instead, cells were incubated in medium containing 10 mM hydroxyurea (Sigma; stock aliquots of 1 M in H_2O kept frozen) for 35 hr and then fixed for immunofluorescence. For some experiments DNA replication was also inhibited with cytosine arabinoside (Sigma) at a final concentration of 0.1 mM, with the other conditions as described above. Mitotic cells are no longer visible in the cell population upon treatment with either of the two inhibitors.

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Note Added in Proof

After completion of our experiments we learned that S. Gillies et al. (this issue) and M. Neuberger (EMBO J., in press) have reached a similar conclusion about the presence of a myeloma-specific enhancer element in the intron of the Ig heavy chain constant region.