Contribution of Promoter to Tissue-Specific Expression of the Mouse Immunoglobulin Kappa Gene

Abstract. *The immunoglobulin kappa (κ) gene promoter was activated by a "neutral" enhancer derived from Harvey murine sarcoma virus (HaMuSV) in immunoglobulin-producing myeloma cells, regardless of the enhancer's orientation or position in the vector. In one fibroblast line (3T3) the immunoglobulin κ gene promoter was completely inactive when linked to the HaMuSV enhancer, whereas in mouse L cells, promoter activity was observed only with the HaMuSV enhancer in tandem with the immunoglobulin κ gene promoter. The differential behavior of the gene promoter, when activated by a neutral enhancer in these three murine cell lines, suggests that promoter sequences contribute to the tissue-specific expression of this gene.*

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Most developmentally regulated genes exhibit tissue-specific expression. Such specificity is thought to be conferred by interaction of DNA sequences—within or near the genes—with regulatory molecules in specialized cells. Efforts have been made to identify the DNA sequences involved in tissue-specific expression by construction of test vectors containing these functional elements linked to a marker gene. The test vectors are introduced into cells that do or do not express the gene of interest. The newly introduced, or exogenous, DNA sequences are thought to interact with the same regulatory molecules as endogenous genes within such cells and to exhibit the same pattern of function as the endogenous genes.

Regulatory sequences that contribute to tissue-specific expression of particular genes have been identified on the 5′ side of the promoter, within the promoter, or on the 3′ side of the promoter and within the body of the gene (*1*). Certain of these elements exhibit the same properties as enhancers first identified in viral genomes—namely, they increase promoter function when placed in either orientation and at variable distances from the gene (*2*). Transfection of rearranged immunoglobulin heavy and light chain genes into both lymphoid and nonlymphoid cells has led to the identification of an enhancer in the intron between the joining (J) and constant (C) coding region (J-C intron) (*3–8*). However, unlike the viral enhancers, immunoglobulin (Ig) gene enhancers can activate either homologous or heterologous promoters but only in lymphoid cells. This indicates that the enhancer contributes to tissue-specific gene expression. Recently, conserved sequences upstream from the promoter TATA box of the Ig κ gene have been shown to be essential for efficient and correct initiation of gene transcription in lymphoid cells (*9–11*). We designed experiments to determine whether the promoter or flanking sequences immediately 5′ of the Ig κ gene also contribute to the tissue-specific expression of this gene.

The cell-type specificity of the Ig κ gene promoter was characterized with the use of the coding sequences of two test genes: chloramphenicol acetyl transferase (CAT) for transient expression assays, and the gene that confers neomycin (Neo) resistance for stable transformation assays (Fig. 1). The selection of cells as recipients for the test vectors was based on whether they did or did not express the differentiated phenotype of antibody production. J558 myeloma cells (obtained from the American Type Culture Collection) produce IgA ($\alpha_1\lambda_1$), whereas L cells and 3T3 cells are fibroblast lines in which endogenous or transferred Ig genes are not expressed (*5–7*).

The design of our experiments required the use of an enhancer that was active in the test cell lines. Viral enhancers often exhibit species but not cell-type specificity. To verify that the Harvey murine sarcoma virus (HaMuSV) enhancer functions in 3T3, L, and myeloma cells, we linked it to the SV40 early promoter in a test vector. This promoter does not exhibit tissue specificity. We construct vectors containing the HaMuSV enhancer in either orientation 5′ to the SV40 early promoter by using the vector $pAT_{10}Cat2$; this vector lacks the SV40 enhancer element (*12*). The HaMuSV enhancer was inserted into the Bgl II site 5′ to the promoter. The $pAT_{10}CAT$ vector generated no CAT enzyme in these cells in the absence of an enhancer, but the HaMuSV enhancer in either orientation resulted in CAT activity (data not shown).

The HaMuSV enhancer activated the Ig κ promoter in myeloma cells regardless of the enhancer's orientation or position within the vector (Fig. 2, C and D), although the level of CAT activity observed with the various vectors ranged from 20 to 105 percent of that seen with pRSVCat, a standard vector in which CAT gene expression depends on the strong enhancer and promoter from the Rous sarcoma virus genome. Acetylation of chloramphenicol was linear for at least 1 hour in extracts of myeloma cells so that the 1-hour values were used to compare the relative activities. In contrast, no CAT activity was generated in 3T3 cells transfected with vectors containing the Ig κ gene promoter and the HaMuSV enhancer. Similarly, the Ig κ gene promoter with 625 base pairs (bp) of 5′ flanking sequence was inactive in L cells when introduced in vectors containing the HaMuSV enhancer (Fig. 2, A and B). Activity of the Ig κ gene promoter was observed only with the shorter promoter having 225 bp of 5′ flanking sequence (P1) and then only with the HaMuSV enhancer in the tandem (E_1) orientation (Fig. 1A). The basic pattern of CAT gene expression in test vectors was reproducible with two different plasmid DNA preparations and a minimum of three DNA transfection studies for each vector.

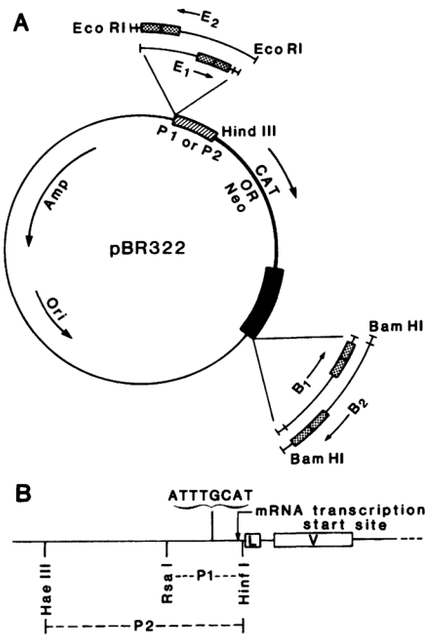
The ability of the HaMuSV enhancer to activate the Ig κ gene promoter was

Table 1. Transformation frequency of L and J558 cells with Neo test vector with 1 μg of DNA per 10^6 cells and 10 μg of DNA per 3×10^6 cells, respectively. L cells transfected with vectors containing the Neo gene were plated at 10^6, 5×10^5, and 10^5 cells per 100-mm dish. Regular culture medium was replaced with that containing G418 (Gibco) at a concentration of 400 μg/ml (*13*) 24 to 48 hours after transfection. Colonies were counted 2 weeks after the initiation of selection by fixation in 10 percent formaldehyde and staining with methylene blue. Twenty-four to 48 hours after transfection with Neo factors, the J558 myeloma cells were replaced in medium containing G418 (400 μg/ml) into three 24-well microtiter plates. Transformation frequency of J558 cells was determined by calculating the proportion of wells that contained growing cells at 2 to 3 weeks.

Vector	Transformation frequency ($\times 10^5$)	
	L	J558
P1Neo	0.1	0.01
E_1P1Neo	126	1.0
E_2P1Neo	11	0.8
E_1P2Neo	10.8	0.7
E_2P2Neo	11	1.0
pSV2Neo	116	1.0

also studied in a stable transformation assay with vectors containing the Neo gene (Fig. 1). Comparison was made to pSV2Neo (*13*) to allow for normalization of differences in transfection frequencies. Vectors containing the HaMuSV enhancer in either orientation 5′ to the promoter gave comparable transformation frequencies in J558 myeloma cells (Table 1). The transformation frequency was comparable to that observed with the control vector pSV2Neo. In contrast, in L cells a transformation frequency comparable to that of the control vector was observed only with the truncated Ig κ gene promoter (P1) and then only with the enhancer in tandem to the promoter (E_1P1Neo in Fig. 1). The transformation frequency achieved with vectors having the HaMuSV enhancer in the other orientation or associated with the longer Ig κ promoter (P2) was one-tenth that with pSV2Neo (Table 1).

Failure of the HaMuSV enhancer to activate the Ig κ gene promoter in nonlymphoid cells suggests that the promoter and 5′ flanking region may contain regulatory sequences that contribute to the tissue-specific expression of Ig κ genes, perhaps by binding to lymphoid



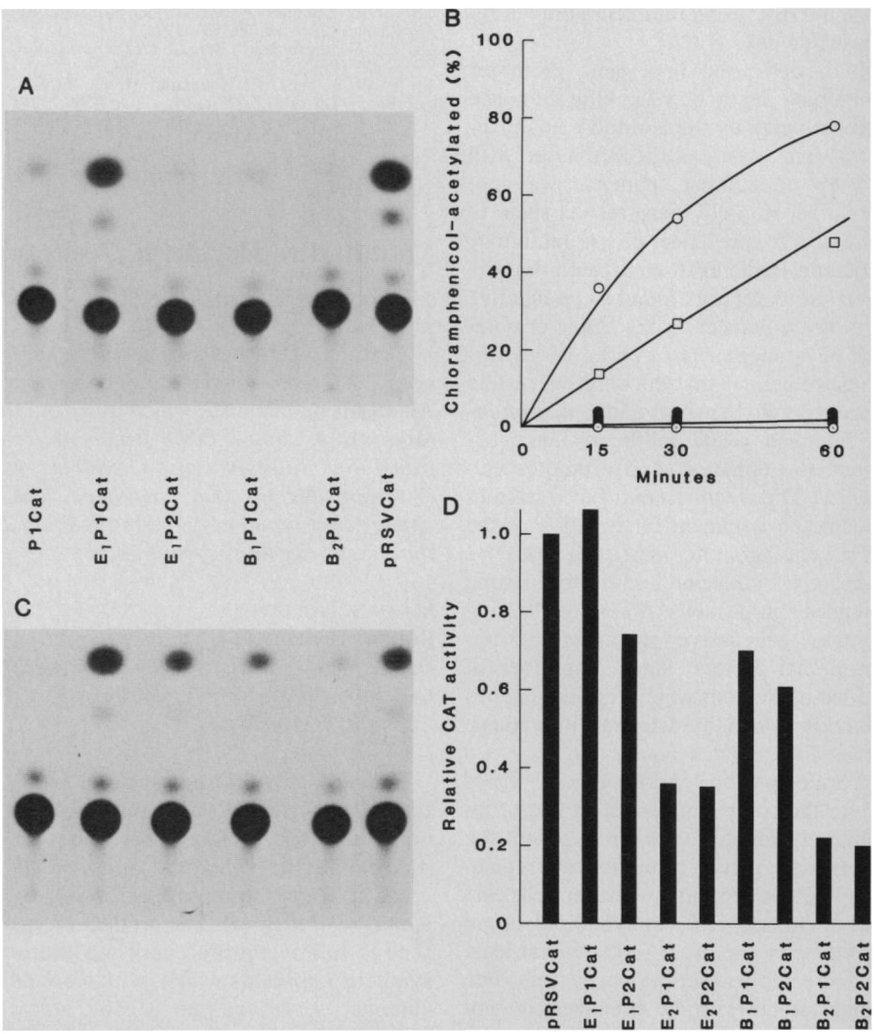


Fig. 1 (left). (A) General map of the test vector, showing the 75-bp repeat of HaMuSV (dotted)—the direction of the arrow indicates the orientation of the enhancer as defined by its position relative to its native promoter; the Ig κ gene promoter (P1, 250 bp and P2, 650 bp) (hatched); the SV40t splice and polyadentylation sequences (solid); the BR322 vector; and the test gene. (B). Map of the 5′ end of the Ig κ gene indicating the promoter fragments used in construction of the test vectors. L designates the coding sequence for the leader region and V the coding sequence for the variable region. The conserved octanucleotide sequence (*9*) is shown within brackets. The CAT and Neo genes were isolated from the plasmids pSV2Cat (*16*) and pSV2 Neo (*13*) respectively, as Hind III–Bam HI fragments. The Ig κ V-region gene promoter was purified from a plasmid containing the V-region gene with 5′-flanking sequences originally isolated from DNA of MOPC 173B myeloma cells (*17*) (provided by E. Max, National Institutes of Health). DNA fragments of 250 bp (P1) or 650 bp (P2) were isolated as shown; standard procedures were used to convert the 5′ and 3′ ends of these fragments into Eco RI and Hind III ends, respectively, with synthetic linkers (New England Biolabs). A 500-bp DNA fragment containing the enhancer of the long terminal repeat of HaMuSV was isolated from plasmid pM13 (*18*) (provided by G. Hager, National Institutes of Health). This fragment contains 250 bp of the U3 region, including the 75-bp direct repeats, and excludes promoter sequences between −160 and the start site for transcription. The HaMuSV enhancer was studied in both orientations either 5′ to the promoter in the Eco RI site (E_1 and E_2) or 3′ to the test gene in the Bam HI site (B_1 and B_2). The plasmids containing the HaMuSV enhancer indicate the position of the enhancer (E_1, E_2, B_1, or B_2), the nature of the promoter fragment (P1 or P2), and the identity of the test gene (CAT or Neo). Fig. 2 (right). (A and B) The function of the Ig κ promoter is observed only when the HaMuSV enhancer is in tandem with the promoter L cells. (C and D) the HaMuSV enhancer activates the Ig κ promoter when placed 5′ or 3′ to the promoter in both orientations in J558 myeloma cells. Designation of the vectors is as described in Fig. 1. The CAT enzyme activity obtained on transfection of the designated test vectors was normalized to that of pRSVCat (*19*) in each cell line. L cells were transfected by the DNA-$CaPO_4$ coprecipitation method (*20*) with either 10 μg of CAT gene test vectors per 10^6 cells without carrier DNA. Six hours later the cells were treated with 15 percent glycerol (*21*) for 2 minutes at room temperature and then maintained in regular growth medium. The J558 myeloma cells were transfected with 10 μg of vector DNA by a modified DEAE-dextran method (*22*). Cells transfected with vectors containing the CAT gene were harvested after 44 to 48 hours, and the assay was carried out on cell extracts by a modification of the method of Gorman *et al.* (*16*). The pRSVCat vector was used as a control in the transient expression assays to allow for normalization of differences in transfection frequencies of these cell lines. For the data displayed in (A) and (C), 5 μg or 50 μg of extract from L cells or J558 myeloma cells, respectively, were assayed for 1 hour at 37°C. The data displayed in (B) were obtained by analyzing 15 μg of extract from transfected L cells for 15, 30, or 60 minutes: (○) pRSVCAT; (□) E_1P1Cat; (●) E_1P2Cat; (■) B_1P1Cat; and (⊙) B_1P2Cat. (D) CAT activity measured in extracts of myeloma cells after transfection with the designated vectors; activity is normalized to that obtained with pRSVCat.

cell–specific regulatory factors. Indeed, a highly conserved octanucleotide sequence has been identified approximately 70 bp upstream from the transcriptional initiation start site of all sequenced Ig κ genes (*9, 11*). The region containing the octanucleotide sequence is essential for correct initiation of transcription from the Ig κ promoter function in lymphoid cells (*10*). Results analogous to our data have been reported, and RNA mapping studies verified that correct initiation from the Ig κ promoter occurs only in Ig-producing cells (*14*).

In L cells, the Ig κ gene promoter containing 225 bp of 5′ flanking sequence was activated by the HaMuSV enhancer in tandem, whereas the promoter with 625 bp of flanking sequence was not activated. Initially these results seem to suggest the presence of an inhibitory sequence in the upstream region, but the short promoter was found to be inactive with the enhancer in the other orientation or at another position in the vector. Thus we believe that the different results obtained with the short and long promoter fragment relate to the distance between the enhancer and promoter elements. The requirement for a tissue-specific environment for function of the Ig κ gene promoter can apparently be overcome by placing it close to a strong enhancer in L cells. These cells may contain permissive nonspecific transcriptional factors, since a number of cloned, developmentally regulated genes function when transferred into these cells.

Tissue-specific expression of genes under the control of *cis*-acting transcriptional elements is thought to reflect the interaction of such elements with regulatory factors present in certain cells and not in others. Cells of lymphoid origin would seem therefore to contain at least two specific transcriptional factors, one that interacts with the enhancer element (*15*), and a second that interacts with sequences within the promoter. Both the Ig enhancer and promoter can be shown to function independently in lymphoid cells with a nontissue-specific promoter or enhancer, respectively, but neither the Ig promoter or enhancer will function consistently in nonlymphoid cells.

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Mutation to Herbicide Resistance Maps Within the *psbA* Gene of *Anacystis nidulans* R2

Abstract. *A* psbA *gene encoding the target of photosystem II herbicide inhibition, the 32,000-dalton thylakoid membrane protein, has been cloned from a mutant of* Anacystis nidulans *R2, which is resistant to 3-(3,4-dichlorophenyl)-1,1-dimethylurea-(diuron). A cloned DNA fragment from within the coding region of this gene transforms wild-type cells to herbicide resistance, proving that mutation within* psbA *is responsible for that phenotype. The mutation consists of a single nucleotide change that replaces serine at position 264 of the wild-type protein with alanine in that of the diuron-resistant mutant.*

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Mutation of a chloroplast gene, *psbA*, has been correlated with resistance to urea- and triazine-class herbicides in *Amaranthus hybridus* and *Solanum nigrum* (*1, 2*) and the green alga *Chlamydomonas reinhardii* (*3*). The product of this gene is an integral protein of the photosynthetic apparatus which, with a bound quinone, serves as the second stable electron acceptor of photosystem II (PS II) (*4*), termed Q_B (*2*). The Q_B protein is the site of herbicide binding (*5*). We have isolated a *psbA* gene from a diuron-resistant mutant of the cyanobacterium *Anacystis nidulans* R2 (*6*) and report that a cloned internal fragment of the mutant gene can transform wild-type *A. nidulans* cells to diuron resistance. Like the mutant from which the diuron-resistance gene was obtained (*6*), cells transformed with the internal fragment are also resistant to the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamine-*s*-triazine) and the quinone analog 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO), indicating that the same mutation in the *psbA* gene affects interaction with each of these inhibitors of photosynthetic electron transport.

When chromosomal DNA from *A. nidulans* is digested with the restriction enzyme Eco RI and probed with cloned *psbA* genes from spinach chloroplast DNA (*7*) or from *Anabeana* 7120 (*8*), three bands of approximately 11, 9, and 7 kilobase pairs (kb) are detected with equal intensity of hybridization (data not shown). At least two of these bands (11 and 9 kb) carry complete structural genes; the extent of *psbA* coding sequence on the 7-kb fragment has not yet been determined. The 11-kb Eco RI fragment was isolated from a bacteriophage λ gt7-ara6 (*9*) library of DNA fragments (*10*) from the *A. nidulans* mutant R2D2-X1 (*6*). This cloned DNA was able to transform wild-type *A. nidulans* cells to diuron resistance, presumably by homologous recombination (*11*). No diuron-resistant transformants were obtained when recombinant λ DNA carrying the 9-kb fragment from resistant-cell DNA was incubated with wild-type cells. Analysis of fragments of the diuron resistance-conferring λ clone by hybridization with the spinach *psbA* probe, and assays of the ability to transform cells to diuron resistance indicated tight linkage of the herbicide-resistance locus and *psbA* gene homology.