RESEARCH ARTICLE

Immunoglobulin Heavy-Chain Enhancer Requires One or More Tissue-Specific Factors

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Abstract. *Enhancer sequences are regulatory regions that greatly increase transcription of certain eukaryotic genes. An immunoglobulin heavy-chain variable gene segment is moved from a region lacking enhancer activity to a position adjacent to the known heavy-chain enhancer early in B-cell maturation. In lymphoid cells, the heavy-chain and SV40 enhancers bind a common factor essential for enhancer function. In contrast, fibroblast cells contain a functionally distinct factor that is used by the SV40 but not by the heavy-chain enhancer. The existence of different factors in these cells may explain the previously described lymphoid cell specificity of the heavy-chain enhancer.*

The control of eukaryotic gene expression involves several classes of regulatory elements that act in *cis* to modulate transcriptional activity (*1*). An interesting class of positive regulatory elements, termed enhancers, elevate transcription from eukaryotic promoters (*2*). Enhancers were initially described as a necessary component of some viral promoters, notably those of SV40, polyoma, and retroviruses (*3*), and were subsequently found to enhance transcription of many heterologous viral and cellular promoters (*2, 4*). Cellular enhancers have been described in the immunoglobulin heavy (H)- and κ-chain genes, where they have been postulated to confer transcriptional competence on rearranged V genes during B-cell development (*5, 6*).

Variable (V) and constant (C) gene segments, which undergo rearrangement to form an active immunoglobulin gene, are widely separated on the same chromosome in unrearranged (germline) DNA (*7*). During B-cell maturation, transcription from a V_H or V_κ promoter occurs after the V gene is joined with D (diversity) or J (joining region) gene segments and is juxtaposed with an enhancer located upstream of C_μ or C_κ. In contrast, unjoined V genes are not transcribed at detectable levels even in terminally differentiated plasma cells (*8*). This observation has prompted the suggestion that joining brings the enhancer sufficiently close to a V-gene promoter to allow its activation.

However, there is evidence that activation of immunoglobulin gene transcription has requirements in addition to V-gene rearrangement. (i) Somatic cell hybrids between immunoglobulin-secreting lymphoid cells and nonlymphoid cells usually lose the capacity for antibody expression even though the chromosome containing the rearranged gene is retained (*9*); (ii) a joined κ gene introduced into transgenic mice is expressed only in lymphoid cells (*10*); and (iii) joined immunoglobulin genes are efficiently transcribed following transfection into lymphoid, but not fibroblast, cell lines (*11*). All or part of this tissue specificity may involve the enhancers since the heavy- and κ-chain enhancers function preferentially in plasmacytoma rather than in fibroblast cell lines (*5*). There is now evidence for soluble factors that bind to the enhancer for a ribosomal gene transcribed by polymerase I (*12*) and to the SV40 and murine sarcoma virus (MSV) viral enhancers (*13*). A central goal of our work was to determine whether cellular factors were required for activity of the immunoglobulin heavy-chain enhancer and, if they were, to ask if they were preferentially present in lymphoid cells. Such putative enhancer factors might comprise part of a stable transcription complex (*14*), cause alterations in chromatin structure, or position the gene at a site on the nuclear matrix appropriate for transcription.

By means of a sensitive, reliable procedure for the functional assay of enhancer activity in plasmacytomas and nonlymphoid cells we have shown that VDJ joining brings a V_H gene from a region lacking enhancers into functional proximity with the single enhancer in the region between J_H and C_μ. These data support the model that joined V-gene promoters are activated by nearby enhancers. We performed in vivo competition experiments with a modification of this procedure to demonstrate that molecules present in lymphoid, but not fibroblast, cell lines bind to the heavy-chain enhancer and are required for its activity. There is a requirement for the plasmacytoma-specific molecule (or molecules) by the immunoglobulin enhancer and by the SV40 enhancer in lymphoid cells.

An enhancer is 3′ of J_H, but not 3′ of a germline V gene. Several segments from the intervening sequence between J_H and C_μ and from the region 3′ to an unrearranged V_H gene segment had enhancer activity when assayed in the SV40-transformed monkey kidney cell line, COS (*6*). These results suggested that there might be multiple enhancers within the immunoglobulin heavy-chain locus. In order to assay these sequences for enhancing activity in a lymphoid cell environment, a modified protocol was developed for transient transfection and subsequent analysis of murine plasmacytoma P3X63-Ag8 cells with vectors containing the chloramphenicol acetyltransferase (CAT) gene and cloned regions from heavy-chain genes (legend to Fig. 1).

Portions of the joined V1-C_μ heavy-chain gene (Fig. 1A) from the hybridoma HPCM2, which produces antibodies to phosphorylcholine (*15*), were subcloned into the vector pA10CAT-2 (*16*). The location and sequence of the V1 gene promoter have been determined (*17*). The vector pA10CAT-2 directs transcription of the CAT gene with the SV40 early promoter but lacks a functional enhancer. Since CAT activity is normally absent in mammalian cells, the amount of CAT enzyme produced upon transfection with these constructs is a sensitive measure of the enhancing potential of the cloned sequence. The re-

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gion to be assayed spanned a distance 2 kilobases (kb) upstream of the V1 promoter and extended 10 kb downstream to the second C_μ exon. Only those fragments containing the 1.0-kb Xba I fragment are competent as enhancers (Fig. 1B), confirming previous reports of an enhancer in this region (*5, 6*). The presence of additional sequences active in COS cells may be due to replication of the vector or interaction of the constitutively expressed SV40 T antigen with the promoter region. Thus, only one region of enhancer activity functional in lymphoid cells occurs near a rearranged μ gene. In our assay, the Xba I 1.0-kb enhancer region exhibits between 60 and 200 percent of the activity of the SV40 enhancer, which contains two tandem copies of the 72–base pair (bp) repeat. Like its viral counterparts, the heavy-chain element can enhance transcription in either orientation and from a position either 5′ or 3′ to the coding region (*18*).

Similar results were also obtained with the analogous region from the human μ gene. The intervening sequence from a human μ genomic clone was subcloned in both orientations 3′ to the CAT gene in pA10CAT-2, which produced a vector analogous to construct p4 derived from murine sequences (Fig. 1A). The orientation of fragments ≥ approximately 3 kb in pA10CAT-2 affects the level of enhancement, probably because enhancer-containing regions are located at significantly different distances from the promoter (*19*). Accordingly, we present data from constructs with inserts in the same position and in the sense orientation relative to the CAT gene transcription unit. Following transfection of these constructs into plasmacytoma P3X63-Ag8 cells, CAT assays indicated comparable activity in the μ-intron regions of both species (compare constructs p4 and p9 in Fig. 1B). Constructs containing inserts in the antisense orientation gave 20 percent (human) or 70 percent (mouse) less activity (*18*). These results confirm earlier reports identifying an enhancer in the human intron between J_H and the μ switch region (S_μ) (*20*).

The hypothesis that transcriptional competence is conferred upon the V_H gene promoter by its association with the J_H-C_μ enhancer following rearrangement predicts that no enhancer occurs within a functional distance 3′ of the promoter in its germline configuration. To test this prediction, a region of 17 kb surrounding the germline V1 gene segment was examined for the presence of enhancers functional in P3X63-Ag8 cells (Fig. 1C). As before, large fragments were cloned and tested in both orientations to control for the possibility that an enhancer would be overlooked if situated too far from the SV40 early promoter in the plasmid vector. No enhancing activity was detected in any of the subclones examined (Fig. 1D). These data indicate that, at least for the V1 gene segment, the promoter is moved from a region devoid of enhancer influence to a position adjacent to a strong enhancer as a consequence of VDJ joining.

Heavy-chain enhancer is not utilized in all cells. V-gene transcription could be a direct consequence of rearrangement with an enhancer or could require the contribution of the B-cell environment. Efficient transcription of rearranged light- and heavy-chain genes is obtained when they are transfected into plasmacytomas (*5, 11, 21*), but undetectable or very low transcription is seen upon transfection of rearranged λ, κ, and γ2b genes into fibroblasts (*11, 22, 23*). We obtained similar results when a rearranged μ heavy-chain gene from HPCM2 was transfected into mouse L cells. Although transformants contained one to ten copies of the rearranged gene, they contained less than 0.5 percent of the amount of μ transcripts present in a plasmacytoma control as shown by S1 nuclease-digestion analyses of RNA (*18*). These results suggest that, in addition to the requirement for proximity to an enhancer, transcription from V_H promoters requires factors not present in the fibroblasts.

A similar conclusion can be drawn from experiments assaying heavy-chain enhancer function in nonlymphoid cells. The murine heavy-chain enhancer functions preferentially in mouse lymphoid

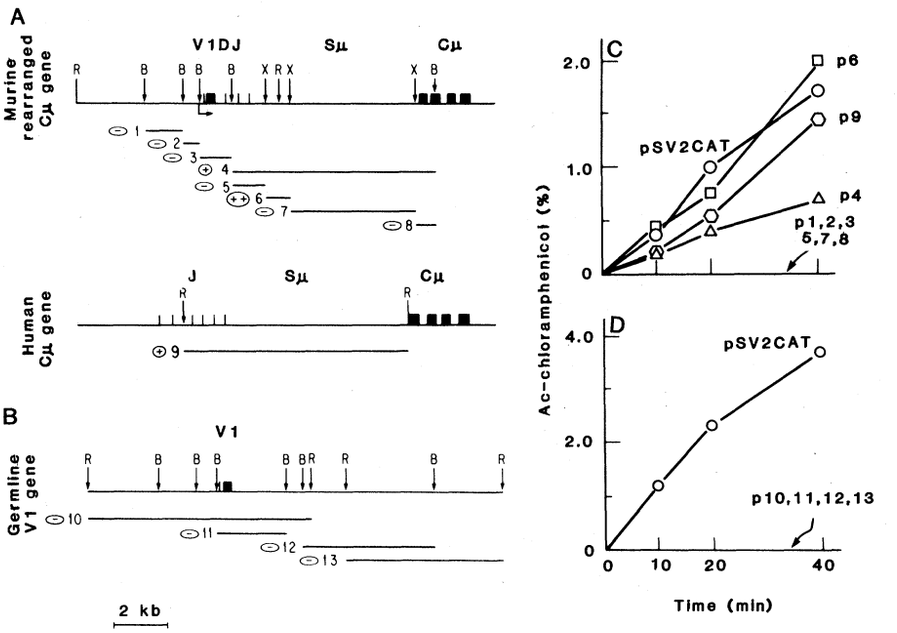

Fig. 1. Enhancer activity in the rearranged and germline heavy-chain genes. Regions of genomic clones subcloned into pA10CAT-2 were assayed for enhancing activity in plasmacytoma P3X63-Ag8 (γ1,κ). Exponentially growing plasmacytoma cells (6×10^6) were transfected with equimolar amounts of the various supercoiled plasmid constructs (corresponding to 80 μg of a 6.6-kb plasmid) by the calcium phosphate coprecipitation technique essentially as described (*29*). Briefly, a precipitate was formed by the dropwise addition of 1 ml of a solution of DNA and $CaCl_2$ (0.25*M*) to 1 ml of 2× HBS (2× HBS is 50 m*M* Hepes, 280 m*M* NaCl, 1.5 m*M* Na_2HPO_4, *p*H 7.05 ± 0.05). Cells, washed in serum-free medium, were gently resuspended in the DNA precipitate mixture and incubated for 20 minutes at 37°C; 8 ml of Dulbecco's modified Eagle medium supplemented with fetal calf serum (10 percent) was then added. Cells were fed 1 day later. Approximately 44 hours after transfection, the cells were harvested and a soluble extract was prepared and assayed for CAT activity as described (*16*) but with the following modifications. Extracts were heated for 7 minutes at 60°C to inactivate an endogenous deacetylase activity. After brief centrifugation at 4°C, 70 μl of the extract supernatants were assayed for CAT activity. Activity was corrected for the number of cells harvested by normalizing to 10^7 cells. These experiments have been repeated a minimum of three times with DNA from different plasmid preparations. (A) Regions of the murine and human μ genes assayed for enhancer activity. Lines under the maps of the genomic clones indicate the regions (1 to 9) subcloned in pA10CAT-2 in both orientations 3′ to the CAT gene. The symbols −, +, and ++ indicate levels of enhancer activity as measured in (B). (B) Results of the CAT enzyme assays for the constructs (p1 to p9) derived from regions shown in (A). Data are given for constructs containing inserts in the sense orientation relative to the CAT gene. (C) Regions of the germline V1 gene segment assayed for enhancer activity. (D) Results of the CAT enzyme assays for the constructs shown in (C). The ordinates in (B) and (D) are the percent of [^{14}C]chloramphenicol converted to the acetylated form.

relative to murine Ltk$^-$ (*5*), human HeLa (*5*), and simian CV1 (*6*) cell lines. Table 1 extends these results to include additional fibroblast, murine, and human lymphoid lines. The immunoglobulin enhancers were more active in murine plasmacytomas than in the fibroblast lines, although low-level enhancer activity was observed in the murine fibroblast lines. Neither the murine nor the human heavy-chain enhancer was active in the human B-cell lines tested, although the SV40 enhancer functioned at levels similar to those observed in the murine lymphoid lines. The reason for this result is not clear although it is known that the human lines produce significantly less antibody than the murine plasmacytomas. The ability of some cell lines to discriminate between the viral and heavy-chain elements is consistent with the hypothesis that discrete cellular factors might be required for efficient enhancer function.

Heavy-chain enhancer activity depends on factor binding. An in vivo competition experiment (Fig. 2) was designed to test the hypothesis that cellular molecules interact directly with enhancer elements. A constant amount of a test plasmid containing an enhancer and the assayable CAT gene was cotransfected into cells with increasing amounts of competitor enhancer sequence-containing vector that did not have the CAT gene. Plasmid vector lacking both the CAT gene and enhancer sequences was also added in order to introduce an equimolar amount of plasmid DNA into the cells for each transfection. If factors required for enhancer function were present at limiting concentrations in the cell, the competing enhancers would cause decreased CAT activity because the test enhancer would not be fully saturated.

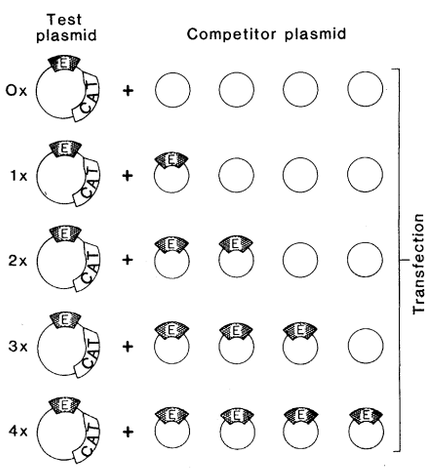

Fig. 2. Schematic outline of the in vivo titration experiments. A constant amount of test plasmid containing the CAT gene was cotransfected with variable amounts of competing enhancer-containing plasmids into P3X63-Ag8 cells. Plasmid DNA devoid of eukaryotic sequences was added so that equimolar amounts of plasmid molecules were introduced into the cells for each transfection. Transfected cells were processed for the CAT assay as described in the legend to Fig. 1.

Nonsaturating levels of CAT activity were attained when test plasmids were transfected under the conditions of the competition experiments, but in the absence of competing enhancers (*18*). Thus, the concentration of putative cellular factors was not initially limiting.

First we examined whether additional copies of the murine heavy-chain enhancer could decrease its own activity. The test construct p6, containing the Xba I 1.0-kb fragment of the HPCM2 heavy-chain gene (Fig. 1A), was transfected into P3X63-Ag8 cells with competing DNA. The enhancer-containing competitor plasmid, pX1.0, contained two copies of the Xba I 1.0-kb fragment. Increasing the amount of pX1.0 in the transfection mixture resulted in a decrease in CAT activity (Fig. 3A). This decrease was observed at all concentrations of pX1.0 tested; at a 12-fold excess of competing enhancer, the level of enhancer-dependent CAT activity declined to 20 to 25 percent of initial levels. To rule out artifacts, a plasmid containing the 1.2-kb Bam HI fragment from the murine heavy-chain gene, a region shown to lack enhancing activity (construct p3 from region 3 of Fig. 1A), was substituted for pX1.0 in the assay. This plasmid did not decrease CAT activity demonstrating that the transfection reaction was not affected by the presence of additional plasmids (*18*). Our interpretation of these results is that the binding of one or more cellular factors present in limiting concentration within the cell is essential for enhancer function.

The corresponding enhancer regions of the human and murine heavy-chain genes share extensive nucleotide sequence homology (*20*). The activity of the human immunoglobulin enhancer is comparable to that of the analogous mouse sequence in a murine cell line (Fig. 1). Thus, the two enhancers may utilize the same factor in plasmacytoma cells. To test this possibility, the 8.3-kb intron region of the human gene inserted into pA10CAT-2 3′ to the CAT gene sequences (construct p9 from region 9 of Fig. 1A) was used as test enhancer. A molar amount of plasmid equivalent to that used with the murine enhancer was transfected under identical conditions. In P3X63-Ag8 cells, a 12-fold excess of competing murine enhancer resulted in a decrease in CAT activity to 9 to 21 percent of that observed in the absence of competing enhancers (Fig. 3B). This result indicates that at least one factor is required in both murine and human cells, suggesting that the mechanism for enhancer recognition and function is conserved across the two species.

Heavy-chain enhancer factor in lymphoid but not fibroblast cells. Activity of the immunoglobulin enhancer is restricted to certain lymphoid cell lines (Table 1). The viral SV40 enhancer, however, has a wider host range, permitting it to function in most fibroblast and lymphoid cell lines. Some quantitative host-cell tropism has been observed for the SV40 enhancer as compared to other viral enhancers and may be the result of the interaction of the enhancer with cellular factors (*13*). To determine whether the SV40 and heavy-chain enhancers are recognized by common or different factors in the lymphoid cell, we used the murine heavy-chain enhancer as the competitor of SV40 enhancer activity. A molar amount of pSV2CAT equivalent to that used for the test plasmids containing the heavy-chain enhancers was cotransfected with competing DNA into P3X63-Ag8 cells. Increasing amounts of the heavy-chain enhancer resulted in a decrease in the CAT signal dependent on the SV40 enhancer (Fig. 4A). The activity at a 12-fold excess of immunoglobulin enhancer was 4 to 18 percent of that seen without competing enhancer. The magnitude of reduction in test SV40 enhancer

Table 1. Heavy chain enhancer activity in various cell lines.

Cell line*	Enhancer†		
	Murine	Human	SV40
	Lymphoid		
Murine			
P3X63-Ag8	+	+	+
S107	+	N.D.	+
Human			
AF10	N.D.	−	+
8226	−	N.D.	+
HH1040	−	N.D.	+
IE 15.1	N.D.	−	+
JL 10.1	N.D.	−	+
	Fibroblast		
Murine			
Ltk$^-$	+/−	+/−	+
ML	+/−	+/−	+
Human			
HeLa	−	−	+
143	−	−	+
Simian			
CV1	−	−	+

*P3X63-Ag8, S107, AF10, and 8226 are myeloma lines; HH1040, IE 15.1, and JL 10.1 are lymphoblastoid lines. †For enhancing activity, + indicates activity equivalent to that of the SV40 enhancer in the particular cell type, +/− indicates levels 10 to 40 percent that of the SV40 enhancer, and − indicates no activity detected; N.D., not determined.

activity was comparable to that seen for the heavy-chain enhancers. The simplest interpretation is that, in the lymphoid cell, both SV40 and heavy-chain enhancer activities depend on a common factor.

We wished to determine whether the factor present in lymphoid cells is the same as that in fibroblast lines where the SV40, but not the immunoglobulin, enhancer is active. Two murine fibroblast lines, Ltk$^-$ and a liver-cell line, ML, were chosen for titration experiments analogous to those in P3X63-Ag8 cells with the SV40 element as the test enhancer and the murine heavy-chain enhancer as competitor. In contrast to the results with the plasmacytoma line, increasing the amount of competing heavy-chain enhancer did not affect SV40 enhancer activity in these cells (Fig. 4A). However, use of another plasmid construction containing the SV40 enhancer as competitor (pSV2NEO) resulted in the complete inhibition of SV40 enhancer-dependent transcription (Fig. 4B). To control for the possibility that sequences present in pSV2NEO other than the SV40 72-bp repeat enhancer might be competing for transcription factors required by pSV2CAT, the vector pA10NEO, which contains the same sequences as pSV2NEO except that all but 21 bp of the tandem 72-bp repeats have been deleted, was used as competitor (Fig. 4B). Increasing amounts of pA10NEO did not affect SV40 enhancer-dependent CAT activity. Furthermore, total inhibition of pSV2CAT activity occurred with pSV2NEO as competitor and pA10NEO as the enhancer-lacking filler plasmid (*18*). These experiments suggest the presence of one or more fibroblast factors required for SV40 enhancer function. However, only in the plasmacytoma cell can the heavy chain enhancer compete for *trans*-acting factors required for SV40 enhancer function. We interpret these results to indicate that different enhancer-binding factors are present in the two types of cell lines tested.

Discussion. Transcription from immunoglobulin V_H promoters is thought to depend on an enhancer sequence (*5, 6*). Our results show that a region of 11 kb surrounding the rearranged μ heavy-chain gene from plasmacytoma HPCM2 contains only one enhancer active in plasmacytoma cells (Fig. 1). Joined V_H promoter regions are brought 2 to 3 kb upstream from the enhancer depending on the particular J_H gene segment used. The region 12 kb downstream from the germline V1 promoter did not contain detectable enhancing activity in lymphoid cells (Fig. 1D). Thus, the V1 promoter is only proximal to an active enhancer after VDJ joining, supporting the hypothesis that enhancers mediate V_H gene promoter activation.

The exact contribution of the enhancer to immunoglobulin expression during B-cell maturation is unclear. Recently, B-cell lines have been reported in which the known heavy-chain enhancer was deleted from the active μ gene yet normal levels of μ chains were made (*24*). This is inconsistent with our results and with the requirement of a transfected γ2b gene for an enhancer (*5*). It is difficult to reconcile these observations, although several explanations are possible. Sequence and functional analyses of the deleted μ genes may yield information regarding the nature of the deleted region, promoter-enhancer interaction, and the role of enhancers during development.

V-gene joining and proximity to an enhancer are not sufficient to activate V1 gene transcription. We and others (*23*)

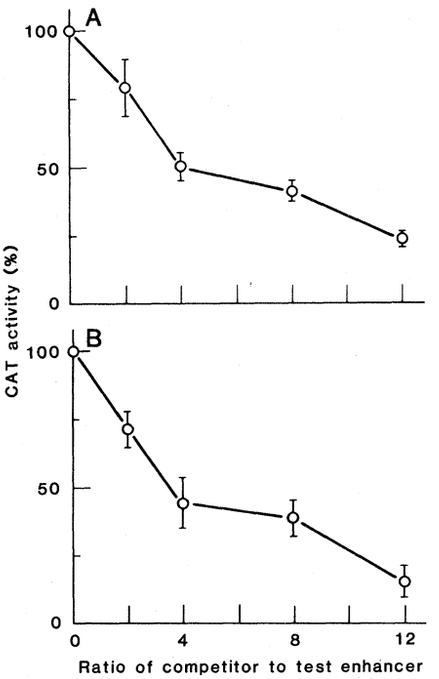

Fig. 3. Competition of the heavy-chain enhancers for a plasmacytoma factor or factors required for enhancer function. Transfected cells were assayed for CAT activity as described in the legend to Fig. 1. The slopes of the enzyme assay curves were used to determine the CAT activity for each transfection. The percentage of activity relative to uncompeted transfection was plotted against the molar ratio of competitor enhancer sequence to test enhancer sequence in the transfection mixture. Error bars represent the variation observed in three separate experiments with P3X63-Ag8 cells and DNA isolated from different plasmid preparations. CAT activity dependent on the murine heavy-chain enhancer (A) and the human enhancer (B) was determined with a pBR322-derived plasmid containing two copies of the Xba I 1.0-kb fragment from the murine μ gene as competitor (pX1.0). Ratios of competitor to test sequence greater than 12 were not achieved because transfection of larger quantities of DNA resulted in cell death. In (A) and (B), 30 μg of construct p6 and 63 μg of construct p9 were used as test plasmids, respectively.

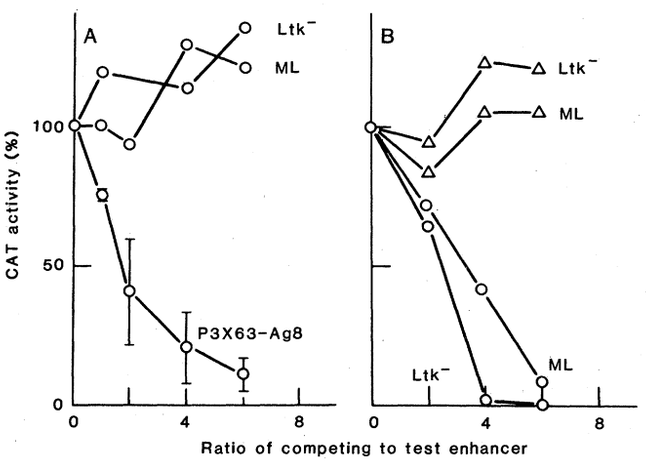

Fig. 4. The heavy-chain enhancer and the SV40 enhancer compete for plasmacytoma, but not fibroblast, factors. The competition experiment is similar to that of Fig. 3 except that a plasmid containing the two 72-bp repeat enhancers of SV40 (pSV2CAT) was used as the test construct. (A) Competition results using the Xba I 1.0-kb fragment from the murine μ gene as competitor enhancer. Since pSV2CAT contains the two 72-bp repeats, the ratio of competing to test enhancer sequences is the molar ratio of enhancer-containing to test plasmid molecules in the transfection mix. (B) Competition results using a plasmid containing the SV40 enhancer region, pSV2NEO (○), or a control plasmid, pA10NEO (△), as competitor. The pSV2NEO and pSV2CAT contain identical SV40 regulatory regions; pA10NEO is similar to pSV2NEO except that the fragment generated by cleavage with Pvu II and Sph I, containing most of the tandem 72-bp enhancer element, has been deleted. Either 23 or 6 μg of pSV2CAT was cotransfected into P3X63-Ag8 cells and the fibroblast lines Ltk$^-$ and ML, respectively, with the competitor DNA. The ML line is a nonvirally transformed murine liver cell line which had been in culture for fewer than 200 population doublings.

have found that transcription of a rearranged heavy-chain gene is not detected following transfection into Ltk$^-$ cells, indicating that a B-cell environment is required for significant expression. The cellular preference of the heavy-chain enhancers (Table 1) suggests that at least one component of the B-cell environment is a factor required for enhancer function.

In vivo competition experiments revealed the presence of a limited concentration of molecules that bind to the heavy-chain enhancer and are required for its activity (Fig. 3). In the plasmacytoma cell, transcription dependent on the SV40 enhancer was also prevented with the heavy-chain enhancer as competitor (Fig. 4A) indicating that at least one common factor is utilized by the heavy-chain and SV40 enhancers. Furthermore, the similar extent of the decrease in the SV40 and heavy-chain enhancer activity (Figs. 3A and 4A) indicates that relatively few, if any, factors exist in the plasmacytoma cell which can substitute for the heavy-chain factor in mediating SV40 enhancer activity. Similar experiments with two murine fibroblast lines demonstrated the existence of a fibroblast enhancer factor functionally distinct from the lymphoid factor (Fig. 4A).

Eukaryotic transcription probably involves multiple factors which interact in complex ways (*14*). Our experiments demonstrate that tissue-specific enhancer recognition factors can be a critical part of this process. Since our assay is a functional one, we identify activities but cannot assign these activities to discrete molecules. We cannot tell if multiple factors are required for enhancer activity or if what we call a factor is a heterogeneous population of factors with overlapping affinities for enhancers. Furthermore, enhancing activity may reflect the presence of a soluble molecule or a component of the nuclear matrix. However, these experiments identify two activities: (i) an activity required for enhancer-dependent transcription present in limiting concentrations, which may or may not reside on the same molecule as (ii) a tissue-specific activity that binds to the enhancer.

On the basis of the differential ability of the heavy-chain enhancer to compete with SV40 enhancer activity in the lymphoid and fibroblast lines, we propose that the plasmacytoma and fibroblast cell lines contain different molecules which bind to enhancers and mediate their function. The SV40 enhancer could have evolved the capacity to bind and utilize the factors from both cell types. Thus, the SV40 element may be the prototype of a class of enhancers which can use the factor or factors present in a wide range of cell types. The heavy-chain enhancer, in contrast, would represent a class of enhancers specific for a particular cell lineage.

Modulation of immunoglobulin enhancer-specific factors could be involved in regulating the levels of immunoglobulin expression during B-cell development. In the murine pre–B-cell line 70Z/3, the κ light-chain gene is rearranged but not transcribed at detectable levels (*25*). Stimulation with lipopolysaccharide induces κ transcription and a deoxyribonuclease I hypersensitive site at the position of the κ enhancer, possibly reflecting the interaction of the enhancer with an induced factor (*25*). It is intriguing to postulate that levels of immunoglobulin factors that bind to the enhancer increase during B-cell ontogeny, contributing to the increased levels of immunoglobulin transcription observed in the terminally differentiated plasma cell. An increase in the cellular concentration of the immunoglobulin enhancer-specific factor or factors could be responsive to the effects of the T-cell growth and differentiation factors thought to be involved in B-cell maturation (*26*).

Recently, viral enhancers having much more stringent tissue specificities than the SV40 enhancer have been described and other cellular enhancers demonstrating tissue specificity have been reported (*27*). There is also increasing evidence for numerous enhancer elements in the genomes of higher organisms (*2*), and cellular factors have been shown to be required for activity of an RNA polymerase I enhancer (*12*) and the SV40 and MSV enhancers (*13*). Based on these data and our competition results, we propose that different types of cells may contain one or more specific enhancer factors which allow particular genes or sets of genes to be expressed in a tissue-specific or developmentally regulated manner (or both). Regulation of enhancer factors is suggested by the hormone dependence of the murine mammary tumor virus (MMTV) enhancer element (*28*).

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