A tissue-specific transcription enhancer element in the human immunoglobulin λ light chain locus

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An 8.0-kb *Eco*RI fragment of the human immunoglobulin (Ig) λ light chain locus carrying the Ke^-Oz^- and Ke^-Oz^+ constant region genes and flanking sequences was studied for the presence of a transcriptional enhancer. Two types of assays were used. In the first, we measured the ability of recombinant plasmids carrying the 8.0-kb Ig fragment covalently linked to the aminoglycoside phosphotransferase (*aph*) gene to transform mouse myeloma or mouse fibroblast cells to geneticin resistance. In the second, we used RNA spot and Northern blot hybridization analyses to determine the relative levels of *aph* specific RNA transiently expressed after DNA transfection. In fibroblast cells, the transformation frequencies were independent of the presence of the Ig fragment in the vector and there was no difference in the level of transient expression of the *aph* gene. In myeloma cells, the Ig fragment enhanced at least 10-fold both the number of transformants and the level of *aph* gene expression over that obtained with vector alone. These results indicate the presence of a tissue-specific transcriptional enhancer in the 8.0-kb *Eco*RI fragment of the human Ig C_λ locus.

Enhancer *Immunoglobulin* *Gene transfer*

1. INTRODUCTION

Transcriptional enhancers are positive regulatory elements which have the following common properties: They are short *cis*-acting DNA sequences which affect transcription of nearby genes. Their action is independent of whether they are located upstream or downstream of the gene concerned and does not depend on the enhancer orientation. They are effective over long distances and can display cell type and species specificity. Comparison of several enhancer sequences suggests they have a consensus core sequence, $GXTGTGG^{TTT}_{AAA}$ [1].

Enhancers were first discovered as components of some viral genomes, viz., SV40 [2,3], polyoma virus [4,5], bovine papillomavirus (BPV-1) [6], retroviruses [7,8] and Herpes simplex virus (HSV-1) [9]. Studies in several laboratories have demonstrated the presence of cellular enhancers in the mouse Ig heavy chain locus [10–12] and in the mouse Ig K light chain locus [13]. Similarly, a human tissue-specific enhancer element has been described in the human Ig heavy chain locus in a non-Hodgkin's lymphoma cell line where this enhancer is translocated next to, and activates, the cellular myc oncogene [14].

Since enhancers have been found in the constant regions of both Ig heavy and Ig C_k loci we decided to examine whether a similar transcriptional regulatory element is present in the Ig C_λ locus. Results are presented in this report.

2. MATERIALS AND METHODS

2.1. *Cells and plasmids*

Mouse $LATK^-$ fibroblast, mouse AG653 and rat Y3AG-1-2-3 myeloma cells were grown in monolayers in Ham's SF12 medium (Flow Laboratories) containing 15% Hyclone serum

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(Sterile Systems). The bacterial strain *Escherichia coli* Hb101 carrying the plasmid pNϵ1 was a gift from P. Montague. The lambda recombinant phage λ22-λ5 which contains an 8.0-kb *Eco*RI fragment carrying the Ke^-Oz^- and Ke^-Oz^+ genes of human Ig C_λ locus was isolated from a chromosome 22 library [15] and was obtained from T. Rabbits [16]. The insert was subsequently cloned in the single *Eco*RI site of plasmid pUC8 to obtain plasmid pUL1. Plasmids pAM91 [17] and pHR28 (A. Sproul and G. Birnie, unpublished) carried mouse actin and human ribosomal DNA sequences, respectively.

2.2. *Plasmid constructions (fig.1)*

The 8.0-kb fragment of human genomic Ig Cλ DNA was isolated after digestion of recombinant pUL1 with *Eco*RI and separation from the bacterial plasmid DNA by agarose gel electrophoresis. The pNϵ1 vector DNA was restricted with *Eco*RI, treated with alkaline phosphatase [22] and mixed with the 8.0-kb human DNA fragment in equimolar proportions. The two DNAs were ligated with T4 DNA ligase and used to transform *E. coli* Hb101 [23]. Ampicillin-resistant clones were obtained and screened by the hybridization technique in [24] using the human insert DNA as a probe. The orientation of the insert Ig C_λ DNA was determined by restriction enzyme digests. Recombinant plasmids pNϵIG1 and pNϵIG2 contained the human DNA in the 5′-3′ or 3′-5′ orientation, respectively, relative to the *aph* gene.

2.3. *DNA-mediated gene transfer*

Transfections of $LATK^-$ and AG653 mouse cells were carried out using the calcium phosphate

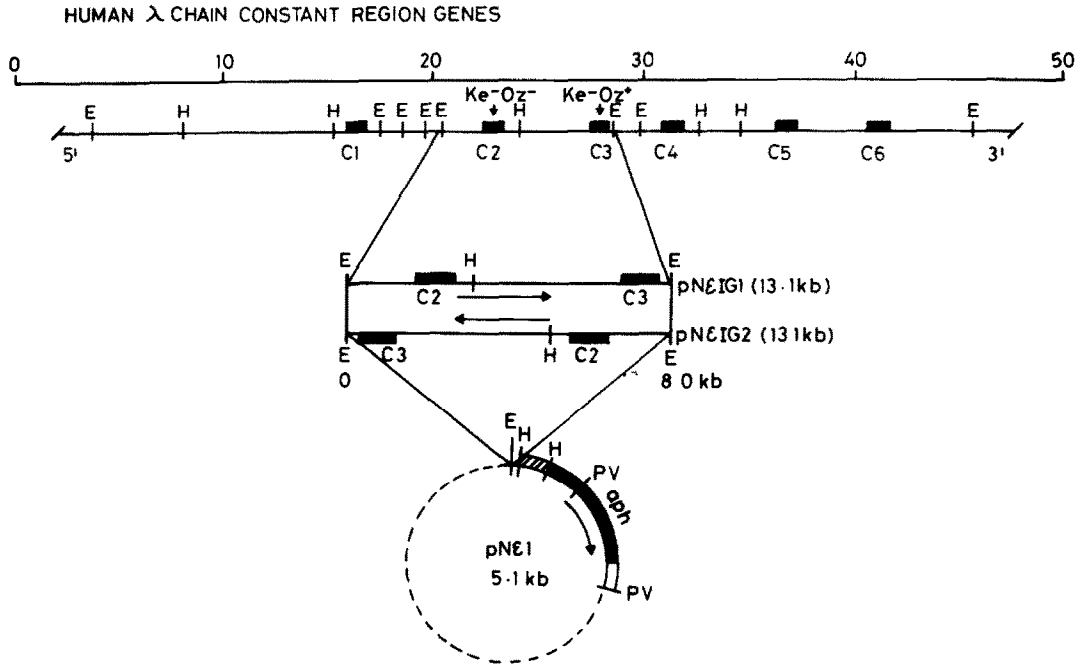

Fig.1. Physical map of the human Ig C_λ locus and structures of *aph* recombinants. The arrangement of a cluster of 6 Cλ genes (filled boxes) along chromosomal DNA is shown at the top [18]. Plasmid pNϵ1 contains the bacterial Tn5 encoded *aph* gene derived from plasmid, pAG60 [19]. The *aph* gene is under the 5′ transcriptional control of the human epsilon globin gene promoter [20] and the 3′-polyadenylation signal of HSV-1 *tk* gene. Plasmid pNϵ1 was constructed by P. Montague. The epsilon globin promoter of 197-bp *Bam*H1-*Pvu*II fragment was converted to a *Hin*dIII fragment using molecular linkers and was derived from plasmid pTKϵ1 [21]. The arrows indicate the direction of transcription; the broken line indicates plasmid pBR322 sequences; the filled box the *aph* structural sequences; the hatched box the human epsilon globin promoter; the open box the polyadenylation signal sequences from the HSV-1 *tk* gene. Plasmids pNϵIG1 and pNϵIG2 were obtained by inserting the 8.0-kb *Eco*RI fragment of Ig C_λ locus into the single *Eco*RI site of pNϵ1. The plasmid maps are not drawn to scale. E, *Eco*RI; H, *Hin*dIII; Pv, *Pvu*II.

technique [25] with the following modifications: 0.5 ml of DNA-calcium phosphate co-precipitate was added to 5 ml medium containing 1×10^6 recipient cells growing exponentially in a 25 cm^2 flask. For the myeloma cells, specially treated flasks (primaria, Falcon) were used to obtain attachment of more than 90% of the cells. After 24 h the medium was replaced with fresh nonselective medium (SF12 containing 15% Hyclone serum) for an additional 24 h. For short-term or transient expression analysis, the cells were harvested by trypsinization. For the long term transformation assay, the medium was changed to selective medium containing geneticin (Gibco) at 200 μg/ml for LATK$^-$ and 800 μg/ml for AG653 cells. For the liquid assay the medium was changed every 3 days for up to 2 weeks before colonies were counted using an inverted microscope. The semisolid medium assay which used 0.9% methocel has been described [26].

2.4. *Isolation and analyses of RNA*

Total RNA extraction 48 h after transfection [21], RNA spot hybridization analysis [27] and Northern blot hybridization analysis [28] have been described in detail elsewhere. ^{32}P-labelled DNA probes with specific activities of $1-2 \times 10^8$ cmp/μg were made by nick-translation [29]. Filter hybridization was carried out in 5 × SSC, 50% formamide for 24 h at 42°C with 10 ng/ml probe as in [30].

3. RESULTS

3.1. *Long-term transformation assays*

First we established conditions for transforming LATK$^-$ mouse fibroblast and AG653 mouse myeloma cells with recombinant plasmids carrying the bacterial Tn5 encoded aminoglycoside phosphotransferase (*aph*) gene as dominant selectable marker. In particular we used the pNϵ1 recombinant carrying the *aph* gene under the 5′-transcriptional control sequences of the human epsilon globin gene promoter and the 3′-polyadenylation signal sequences of the HSV-1 *tk* gene. We have shown previously that similar hybrid gene constructs involving the human epsilon globin promoter fused to the structural sequences of the *tk* gene could functionally replace the *tk* promoter [6,21,31].

The strategy was then to investigate the

Table 1

Transformation of fibroblast LATK$^-$ and myeloma AG653 mouse cells with *aph* recombinants

Donor DNA[a]	Orientation of Ig C_λ genes[b]	Recipient cells	Transformation ratios[c]	
			Liquid assay	Semisolid assay
pNϵ1	–	LATK$^-$	1.0 ± 0.5	1.0 ± 0.5
pNϵIG1	same	LATK$^-$	1.1 ± 0.4	1.0 ± 0.5
pNϵIG2	opposite	LATK$^-$	1.2 ± 0.6	0.8 ± 0.4
pNϵ1	–	AG653	1.0 ± 0.7	1.0 ± 0.6
pNϵIG1	same	AG653	9.8 ± 6.2	12 ± 4.6
pNϵIG2	opposite	AG653	12 ± 8.0	13 ± 4.0

[a] Donor DNAs (0.01–10 μg) were mixed with carrier salmon sperm DNA at a final concentration of 20 μg/ml and transformation was carried out using the calcium phosphate technique as described in section 2

[b] With respect to the *aph* gene

[c] Relative to pNϵ1 transformation frequencies for each cell type

Transformation frequencies for LATK$^-$ cells were in the range of 150–250 and for AG653 0.5–2 colonies/μg pNϵ1 DNA. The average and standard deviations of transformation frequencies expressed as colonies/μg DNA were derived from the counts of 8 flasks per donor DNA from two independent experiments (liquid assay) or 4 plates per donor DNA from one experiment (semisolid assay)

whereas pNεIG2 carried the Ig C_λ genes in the opposite orientation (fig.1).

The results of the transformation of LATK$^-$ mouse fibroblast and AG653 mouse myeloma cells are shown in table 1. The transformation frequencies of LATK$^-$ cells with vector alone (pNε1) or with recombinants pNεIG1 and pNεIG2 were the same. However, transformation frequencies were approx. 10-times higher for the myeloma cells with recombinants pNεIG1 and pNεIG2 than with pNε1. Clearly the presence of the human Ig DNA resulted in cell specific differences in transformation frequencies. Nevertheless it should be noted that using the same pNε1 recombinant, i.e., vector alone, the actual transformation frequencies of myeloma cells were at least 50-times lower than fibroblast cells. This probably reflects the lower capacity of myeloma cells to acquire exogenous DNA in a stable manner and express it. On the other hand LATK$^-$ cells are known to be among the most competent mammalian cells for transformation [32].

In a preliminary long-term transformation assay of rat myeloma Y3AG-1-2-3 cells with the plasmids pNε1, pNεIG1 and pNεIG2 a similar response to that of mouse AG653 cells was obtained. That is, approx. 10-times higher transformation frequencies were obtained with the pNεIG1 and pNεIG2 than with pNε1.

3.2. *Short-term transfection assays (transient expression)*

To discover whether the differences in transformation frequency of the donor DNAs in myeloma cells were reflected in short-term transfection assays, expression of the *aph* gene was measured 48 h after treatment with the DNA calcium phosphate co-precipitates. The relative levels of

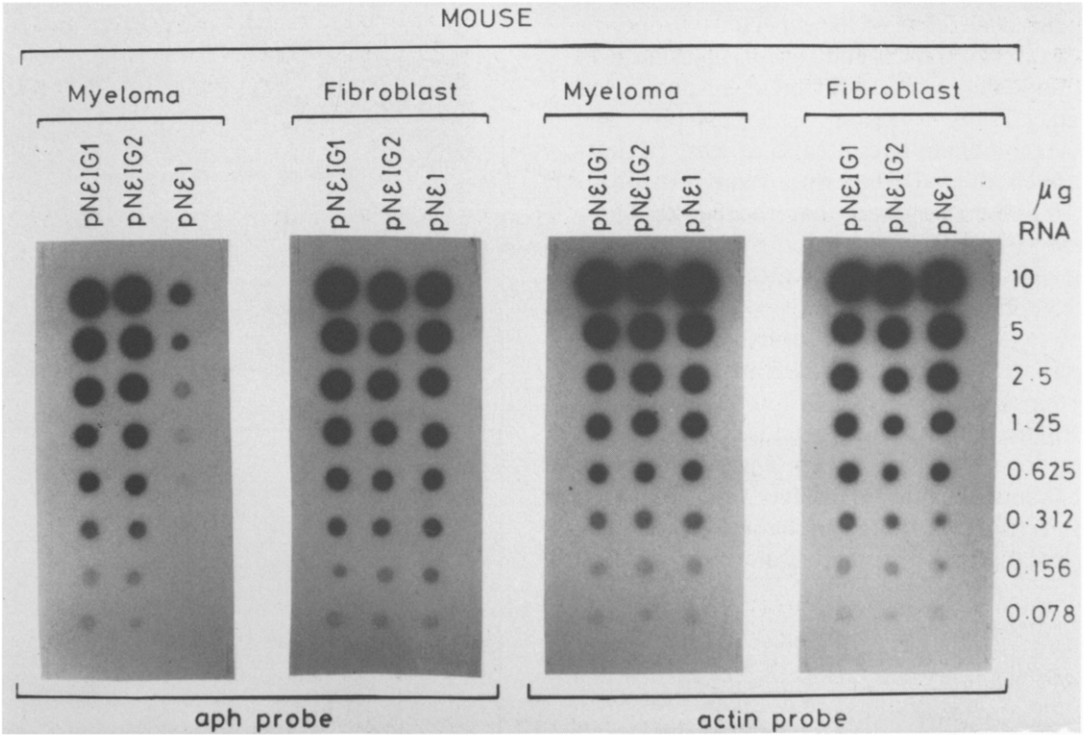

Fig.2. Autoradiographs of spot hybridization assays of *aph* and actin transcripts at 48 h post-transfection of AG653 myeloma and LATK$^-$ fibroblast mouse cells with recombinants pNε1, pNεIG1 and pNεIG2. Two-fold serial dilutions of total cell RNA were spotted on to nitrocellulose [27]. ^{32}P-labeled *aph* (the *Pvu*II fragment carrying the structural gene, see fig.1) and actin (plasmid pAM91) DNAs were used as probes.

aph RNA transcripts were determined using an RNA spot hybridization assay [27] and the results are shown in fig.2.

In LATK$^-$ cells the levels of *aph* RNA transcripts were the same for all the 3 plasmids tested. However, AG653 myeloma cells transfected with recombinants pNϵIG1 or pNϵIG2 had 10–20-times higher levels of *aph* RNA transcripts than cells transfected with pNϵ1. To check that equivalent amounts of RNA from each sample were spotted onto nitrocellulose, the filters were hybridized with pAM91 (mouse actin) or pHR28 (human ribosomal) DNA probes. With both actin (fig.2) and ribosomal (not shown) probes it was found that there was no substantial difference in the level of hybridization of dots and therefore the amount of actin or ribosomal RNA present in these samples was the same.

Northern blot hybridization analysis of RNAs from similar short term transfection experiments is shown in fig.3. Probing blots using *aph* DNA showed that in LATK$^-$ fibroblast cells approximately the same levels of the expected *aph* specific 1.2-kb transcript were found for all 3 plasmids. In AG653 myeloma cells a different response was observed. Transformation with pNϵIG1 and pNϵIG2 recombinants resulted in at least 10-times higher levels of *aph* transcripts than with pNϵ1. These findings also show that the 8.0-kb Ig C_λ fragment affects gene expression by regulating the amount of full-length *aph* mRNA present in transfected cells (fig.3).

Taken together, the RNA spot hybridization and the Northern blot hybridization analyses suggest that a major effect of the 8.0-kb human Ig C_λ DNA fragment on the *aph* gene is at the level of transcription. This is consistent with the presence of an enhancer element. However, alternative explanations, e.g., alterations in the stability of RNA transcripts, cannot be excluded at present.

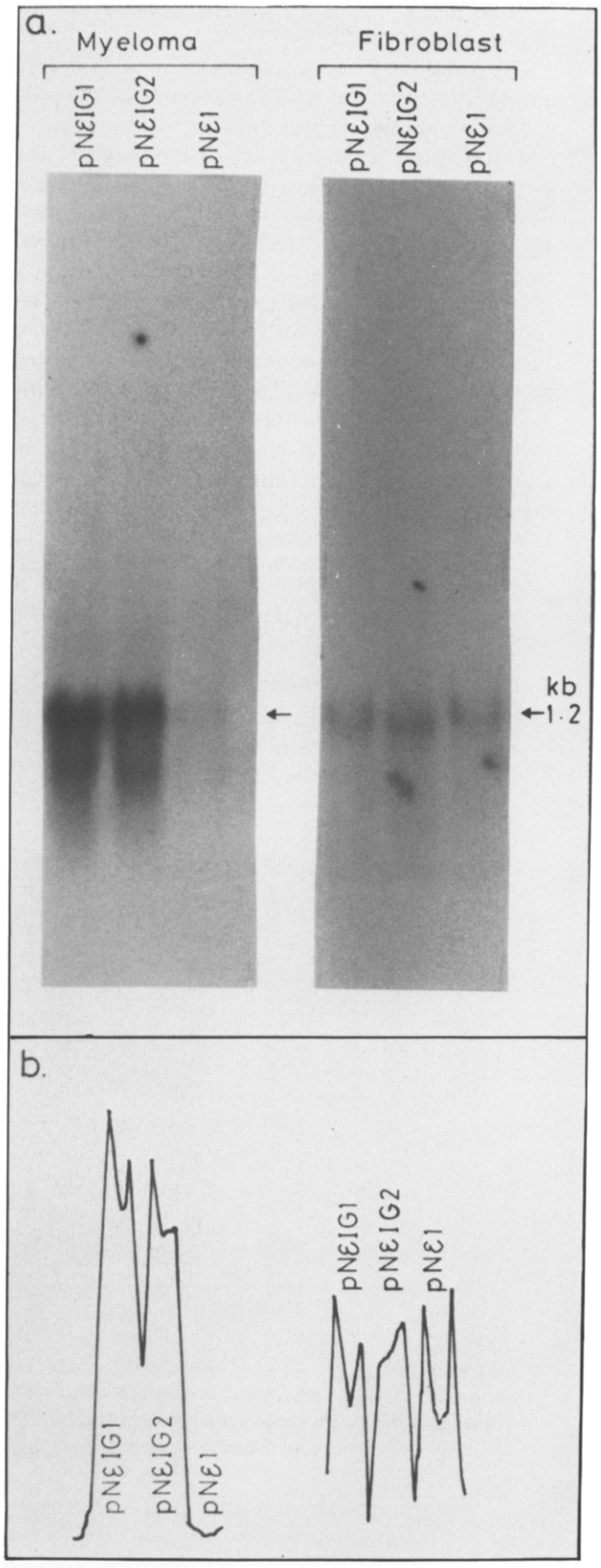

Fig.3. *aph* RNA in transfected cells. 20 μg total cell RNA from AG653 myeloma and LATK$^-$ fibroblast mouse cells transfected with the DNAs indicated were fractionated on a 1% formaldehyde–agarose gel, blotted onto nitrocellulose and probed with ^{32}P-labelled *Pvu*II DNA fragment carrying the *aph* gene (see fig.1). (a) The autoradiographs. (b) Scanning across the 1.2-kb band.

4. DISCUSSION

Here, we have tested for the presence of an enhancer in the unrearranged human Ig C_λ locus. Recombinant plasmids carrying an 8.0-kb Ig C_λ DNA fragment inserted in either orientation upstream of the *aph* gene were used to transfect mouse LATK$^-$ fibroblast and mouse AG653 myeloma cells. Two types of assays were used. In long-term transformation assays, the transformation frequencies of recipient LATK$^-$ and AG653 cells to geneticin resistance were measured. In the short-term transfection assay, the levels of *aph* transcripts present in the recipient cells 48 h post-transfection were analyzed by RNA spot and Northern blot hybridization. Both assays suggested the presence of a tissue-specific transcriptional enhancer sequence encoded in the 8.0-kb *Eco*RI fragment of the human Ig C_λ locus. Our results are also consistent with previous findings with viral transcriptional enhancers that there is a good correlation between the transient levels of expression of the marker *aph* gene and subsequent transformation frequencies [21,32].

It has been suggested for the Ig heavy chain locus that the tissue-specific enhancer element might have a role in cell differentiation [10]. This tissue specificity could be the result of a factor or factors present only in lymphoid (myeloma) cells which regulate human Ig expression during B cell ontogeny. The quantitative differences in the level of Ig gene expression at different stages of B cell development suggest that the enhancer function may be stage specific. Similar suggestions can be made for enhancers in the Ig C_k and C_λ loci. The mechanism of action of tissue- or species-specific enhancers is not known although some plausible models could involve DNA–protein interactions or effects on DNA conformation acting in *cis* [31,32].

It has recently been shown that in certain Burkitt's lymphoma cell lines translocation of the cellular *myc* oncogene close to the heavy chain enhancer results in elevated *myc* gene expression [14]. It is tempting to speculate that such an enhancer element in the Ig C_λ locus might play a similar role in the transcriptional activation of the *c-myc* oncogene in the less common Burkitt's lymphomas where an 8:22 chromosomal translocation places the Ig C_λ next to the *myc* oncogene. Such a chromosomal translocation has recently been described which also displays elevated expression of the *c-myc* oncogene [33]. Experiments are in progress to localize precisely and to sequence the Ig C_λ transcriptional regulatory element.

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