The EMBO Journal vol.4 no.11 pp.2831–2838, 1985

Cell-type preference of immunoglobulin κ and λ gene promoters

Didier Picard and Walter Schaffner

Institut für Molekularbiologie II der Universität Zürich, Hönggerberg, CH-8093 Zürich, Switzerland

Communicated by W.Schaffner

Immunoglobulin gene constant regions are known to be associated with strictly tissue-specific enhancer elements. Until recently the promoter of the variable region, which becomes linked to the constant region by somatic rearrangement, could have been viewed as a passive recipient of the enhancer stimulus. Here we show that the promoters of the immunoglobulin κ and λ light chain genes are ~20–30 times more active in lymphoid cells than in non-lymphoid cells. To avoid the problem of differential mRNA stability upon transfection of immunoglobulin genes into non-lymphoid cells we have constructed chimeric genes. All κ mRNA sequences were progressively deleted to fuse the κ gene promoter to a globin gene coding body. A similar chimeric gene was constructed with the promoter of the λ gene. The cell-type preference of the promoter may be exploited during B-lymphocyte differentiation to regulate the immunoglobulin gene promoter independently from the enhancer.

Key words: immunoglobulin gene/promoter/enhancer/tissue-specificity/transfection

Introduction

For the understanding of the developmental regulation of eukaryotic gene expression the discovery of tissue- and cell-type-specific cellular enhancers has been a major contribution (for review, see Picard, 1985). Cellular enhancers were first identified within immunoglobulin (Ig) heavy chain (Banerji *et al.*, 1983; Gillies *et al.*, 1983; Neuberger, 1983; Rabbitts *et al.*, 1983; Hayday *et al.*, 1984) and light chain (Picard and Schaffner, 1984a; Queen and Stafford, 1984) genes and have subsequently been found in several other cellular genes (Gillies *et al.*, 1984; Edlund *et al.*, 1985; Prochownik, 1985; see also, Gluzman, 1985).

The finding of a tissue-specific enhancer associated with the Ig constant region has led to the following simplified view of Ig gene activation (Banerji *et al.*, 1983): first, the enhancer activates the unrearranged constant region which becomes transcribed from pseudopromoters; second, after formation of a functional Ig gene by somatic rearrangement (reviewed in Tonegawa, 1983) the promoter of the variable region juxtaposed to the enhancer is activated to generate genuine Ig mRNA. Recent investigations have shown that post-transcriptional mechanisms are involved in controlling the large increase in the steady-state mRNA level during terminal differentiation to plasma cells at least in the case of Ig heavy chain genes (Mather *et al.*, 1984; Yuan and Tucker, 1984; Gerster, Picard and Schaffner, unpublished results).

The promoter, however, is not simply a passive partner of the enhancer, but rather contributes itself to the tissue-specific or stage-specific regulation of Ig gene transcription. This has recently been shown by uncoupling an Ig heavy chain gene promoter from the rest of the gene (Mason *et al.*, 1985; Grosschedl and Baltimore, 1985).

Here we present direct evidence for a 20- to 30-fold tissue preference of both the Ig κ and the Ig λ light chain gene promoters. By linking the κ gene promoter devoid of any κ mRNA sequences to a different coding body we could circumvent the problem of differential transcript stability which has hampered the interpretation of previously published work (Falkner *et al.*, 1984; Foster *et al.*, 1985). The tissue-specificity of Ig promoters could allow for an independent regulation of the Ig promoter during B-lymphocyte differentiation.

Results

Most genes appear to require a transcriptional enhancer for efficient transcription in transfection experiments. Since immunoglobulin genes contain a strongly tissue-specific enhancer (Banerji *et al.*, 1983; Gillies *et al.*, 1983; Hayday *et al.*, 1984; Picard and Schaffner, 1984a) we decided to link the Ig κ light chain gene of the mouse to an SV40 enhancer to be able to study its expression in non-lymphoid cells as well.

The first series of experiments was carried out with a somatical-

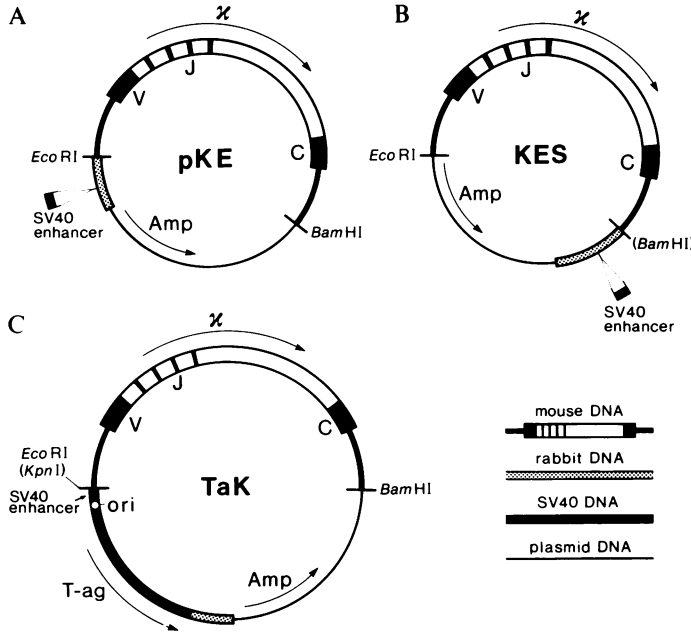

Fig. 1. Structure of immunoglobulin κ gene recombinants. **(A)** Map of pKE, a clone with the SV40 enhancer 1.5 kb upstream of the κ gene. Clone pK (see Materials and methods) lacks the SV40 enhancer. **(B)** Map of KES, a clone with the SV40 enhancer 6 kb downstream of the κ gene promoter. **(C)** Map of TaK, a clone containing the entire SV40 early region including the SV40 enhancer in a head-to-head arrangement with the κ gene. V, J and C, variable, joining, and constant regions of the κ gene, respectively; Amp, β-lactamase gene; ori, SV40 origin of replication; T-ag, SV40 T-antigen.

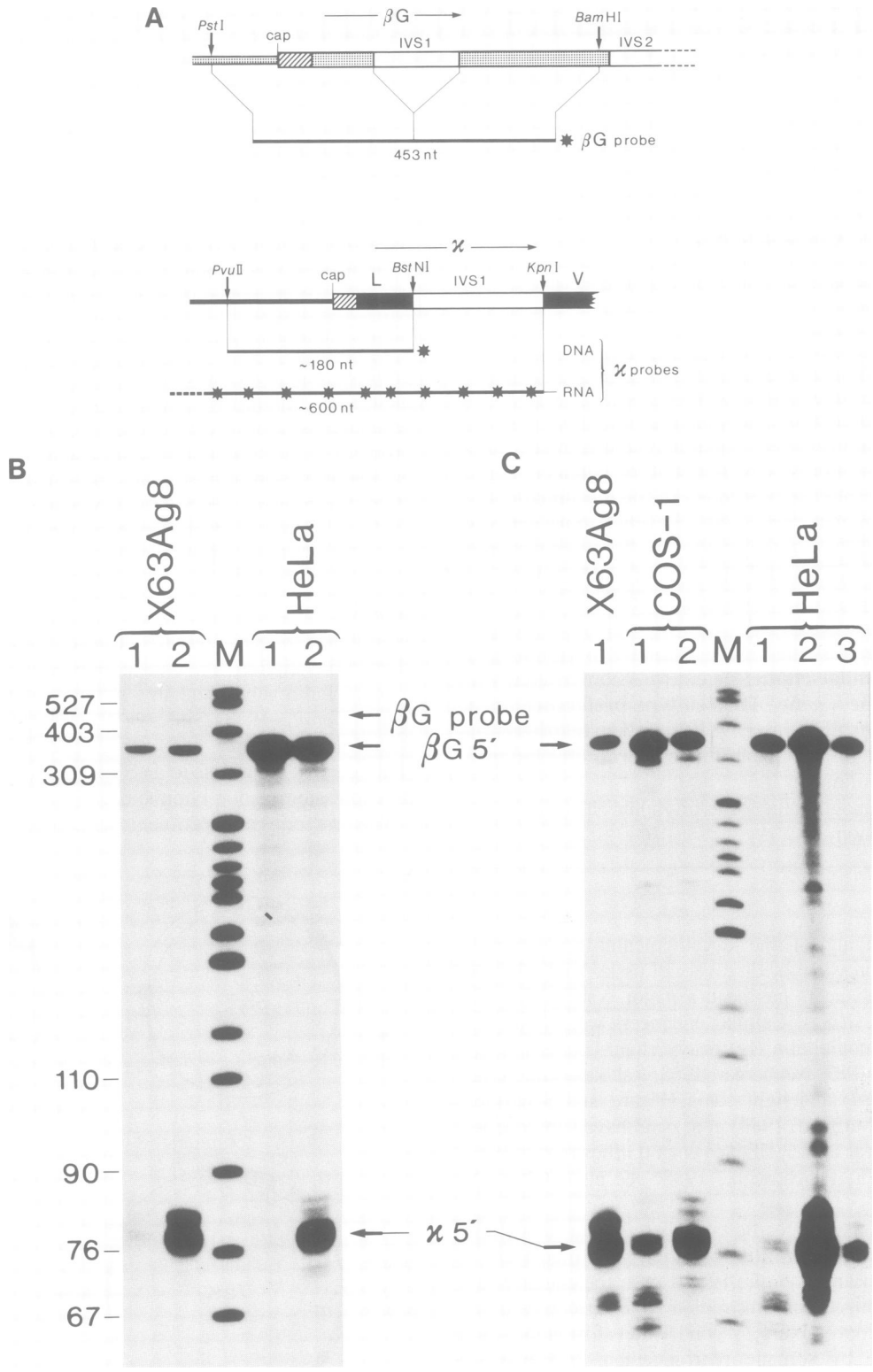

Fig. 2. The immunoglobulin κ gene is correctly transcribed in non-lymphoid cells. **(A)** Schematic representation of the probes used in the RNA analysis. Single-stranded DNA probes were end-labelled as indicated by the terminal asterisks. The β-globin gene probe shown at the top is derived from a clone lacking the first intervening sequence (IVS1) and has been described earlier (de Villiers and Schaffner, 1983). The κ gene DNA probe shown at the bottom is the same as the one used by Queen and Baltimore (1983). The homogeneously labelled complementary-strand RNA probe for the κ gene (only used for the experiment in Figure 4B) extends to the *Acc*I site ~400 bp upstream of the cap site. The hatched boxes represent untranslated leader regions. IVS, intervening sequence; L, leader peptide; V, variable region; nt, nucleotides. **(B)** S1 nuclease mapping of RNA from cells transfected with clone pK **(lanes 1)** or pKE **(lanes 2)** mixed with a β-globin gene-SV40 enhancer recombinant at a 3:1 mass ratio. **Lane M**, end-labelled marker DNA fragments (sizes in nucleotides) of pBR322 digested with *Hpa*II. Myeloma X63Ag8 cells and HeLa cells were transfected by the DEAE-dextran technique and the calcium phosphate co-precipitation technique, respectively. **(C)** S1 nuclease mapping of RNA from cells transfected with clone KES **(lanes 1)**, TaK **(lanes 2)** or pKE **(lane 3)** mixed with the same internal standard as in **(B)**. **Lane M**, marker DNA fragments. Myeloma X63Ag8 cells were transfected by the DEAE-dextran technique; HeLa and COS-1 cells by the calcium phosphate co-precipitation technique.

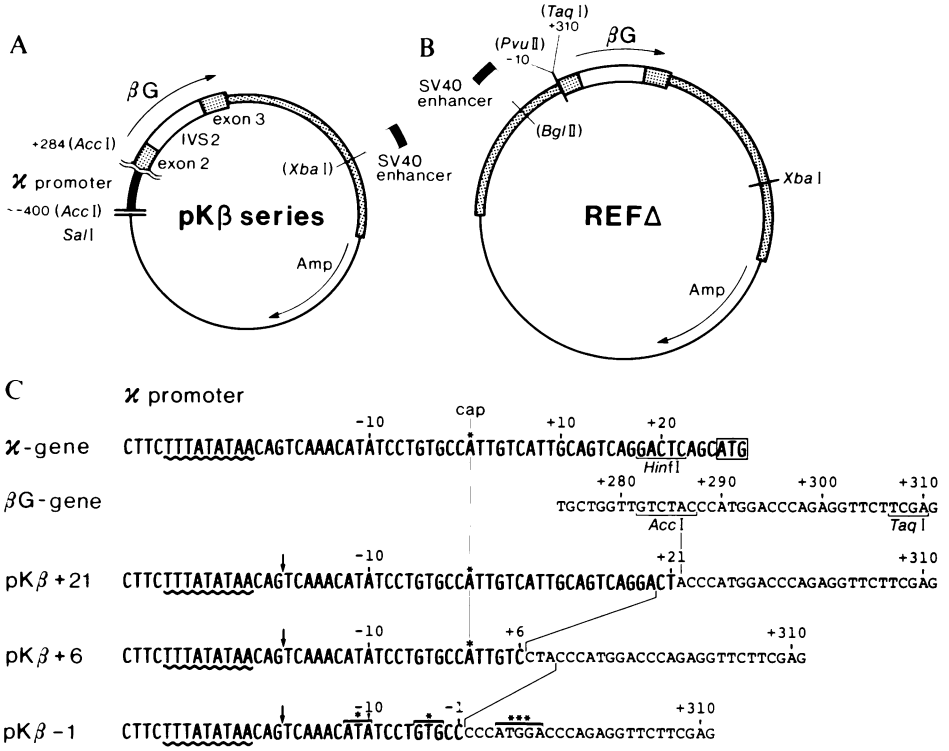

Fig. 3. Chimeric genes with the κ gene promoter. (**A**) Map showing the overall structure of the clones of the pKβ series (pKβ+21, pKβ+6 and pKβ−1). The κ promoter (thick black bar) is fused to the rabbit β-globin gene (stippled parts) within exon 2. The junction is indicated by two parallel wavy lines. (**B**) Map of REFΔ, the internal reference gene clone. The rabbit β-globin gene was modified by deleting the sequences from position −10 to about +310 with respect to the cap site. (**C**) Detailed sequence of the junctions in clones pKβ+21, pKβ+6, and pKβ−1. In the top line TATA box (wavy line), cap site (asterisk) and initiation codon (boxed) of the wild-type κ gene are indicated. In the second line the sequence of the β-globin gene around the junction region is shown (numbering relative to the β-globin gene cap site). The small vertical arrow above the sequence indicates a cryptic 3′ splice site. In the pKβ−1 sequence brackets with asterisks denote transcription initiation regions, the most predominant one being marked with three asterisks.

ly rearranged κ light chain gene on a 6.5-kb *Eco*RI-*Bam*HI DNA fragment (recombinant pK; see legend to Figure 1A). This includes ~1 kb of flanking sequences both upstream and downstream. We inserted the SV40 enhancer as a 200-bp fragment either 1.5 kb upstream (recombinant pKE; Figure 1A) or 6 kb downstream (recombinant KES; Figure 1B) of the cap site. In addition, we linked the entire SV40 early region including the enhancer to the κ gene in a head-to-head arrangement such that the SV40 enhancer is ~1 kb upstream of the κ gene cap site (recombinant TaK; Figure 1C).

Correct transcription of the κ gene in non-lymphoid cells

To compare the expression of the κ gene either with only its own enhancer (recombinant pK) or linked to the SV40 enhancer (pKE) we introduced these recombinants into X63Ag8 mouse myeloma cells and human HeLa cells by the DEAE-dextran transfection technique and the calcium-phosphate co-precipitation technique, respectively. As an internal standard a rabbit β-globin gene linked to the SV40 enhancer was mixed in. Cytoplasmic RNA was isolated ~42 h after transfection and analysed by S1 nuclease mapping using end-labelled single-stranded DNA probes (Figure 2A). Figure 2B shows that the expression of the κ gene is strongly stimulated by the SV40 enhancer (lanes 2) both in myeloma cells and in HeLa cells. In fact, in HeLa cells, transcripts with the correct κ mRNA 5′ end as determined by Queen and Baltimore (1983) are only observed under the influence of the SV40 enhancer (recombinant pKE; compare lanes 1 and 2 in Figure 2B). In myeloma cells the expression of the κ gene is also low unless it is linked to the SV40 enhancer (compare lanes 1 and 2 in Figure 2B) or analysed on a replicating template (see Queen and Baltimore, 1983). Thus, in the presence of the SV40 enhancer, the contribution of the κ gene enhancer is negligible. This was anticipated from our earlier finding that the κ gene enhancer has only 5% of the activity of the Ig heavy chain gene enhancer in transfection experiments (Picard and Schaffner, 1984a).

To substantiate further the correct expression obtained with the recombinant pKE in HeLa cells, we included the plasmids KES and TaK (Figure 1) and transfected monkey kidney COS-1 and human HeLa cells in parallel. Clearly, all of the recombinants give transcripts with the correct κ mRNA 5′ ends both in COS-1 and HeLa cells (Figure 2C). Hence the SV40 enhancer stimulates transcription of the κ gene irrespective of its position either 1.5 kb upstream (recombinant pKE; lane 3) or 6 kb downstream (recombinant KES; lanes 1) with respect to the cap site although in the latter case the expression is slightly weaker and several shorter bands appear. A considerable amount of those incorrect ends is also seen with the recombinant TaK (Figure 2C, lanes 2) which can replicate in COS-1 and HeLa cells. However, TaK yields predominantly a high level of κ transcripts with the authentic 5′ end. It is noteworthy that the same incorrect ends are also seen upon transfection into myeloma cells, albeit relatively less intensely (X63Ag8 lane 1 in Figure 2C). The presence of incorrect ends is most likely due to transcription from pseudopromoter(s) and subsequent splicing into the untranslated leader region of the κ mRNA. In the absence of an enhancer, transcripts from such pseudopromoters may reach a considerable level through template amplification, as has been observed by Queen and Baltimore (1983) who introduced an enhancer-less κ gene on a replicating template into COS-7 cells. In fact, transcription

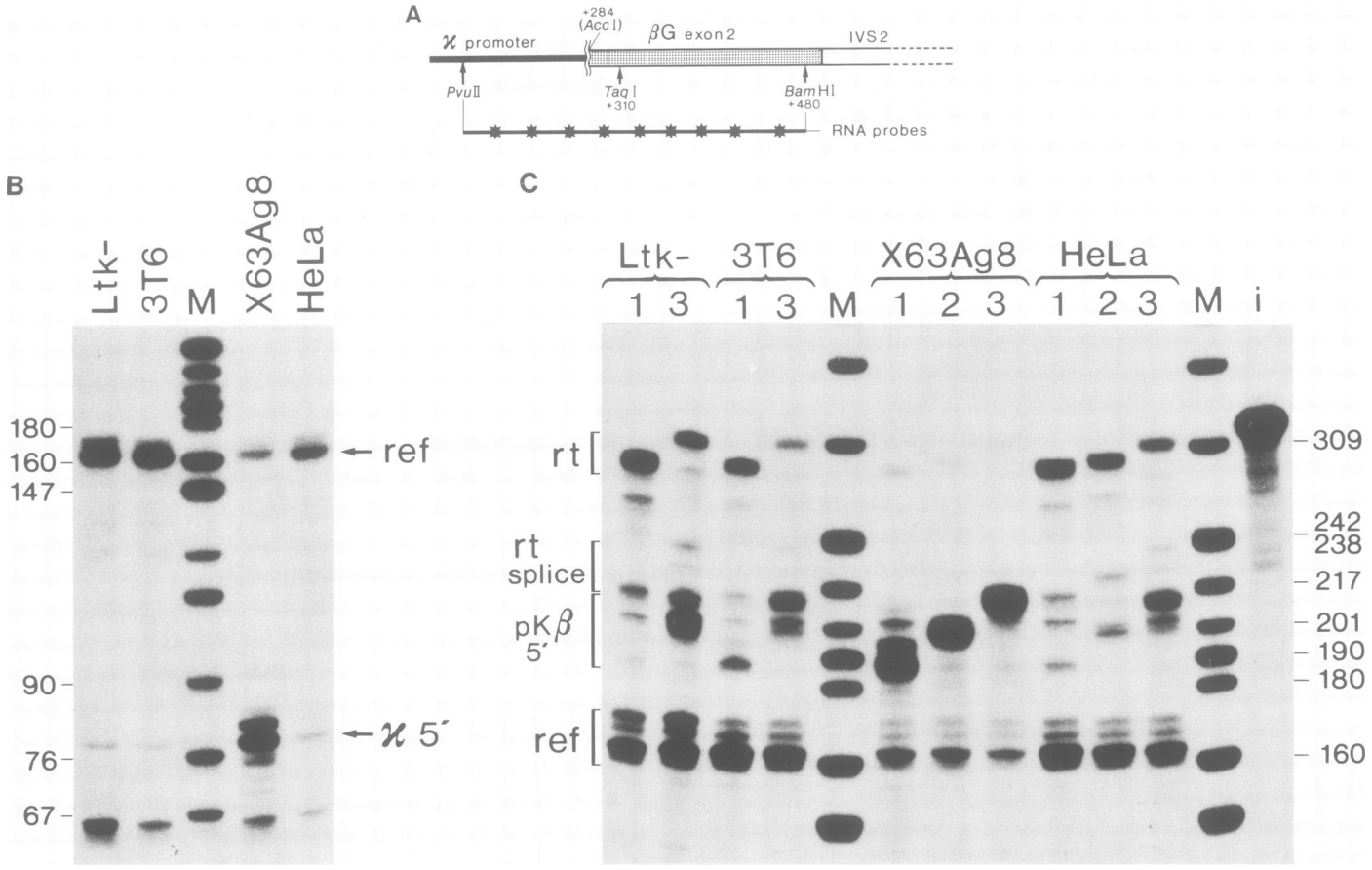

Fig. 4. The Ig κ gene promoter displays tissue preference. **(A)** Schematic representation of the homogeneously labelled RNA probes used to map transcripts from the chimeric genes of the pKβ series. The complementary strand RNA probes were synthesized by the SP6 RNA polymerase system (Melton *et al.*, 1984). The thin line extending the RNA probes at the *Bam*HI end indicates 33 additional nucleotides from the polylinker/SP6 promoter sequence. The two parallel wavy lines indicate that the junction sequences differ amongst the clones of the pKβ series (see Figure 3C). Therefore transcripts from each of the clones of the pKβ series were mapped with their corresponding RNA probe. The homology of the probe to the reference gene clone REFΔ only extends to the *Taq*I site. **(B)** RNA mapping of RNA from cells transfected with a mixture (4:1 mass ratio) of clones KES and REFΔ. All cell lines were transfected by the DEAE-dextran transfection technique as discussed in the text. The RNA probe for the κ gene is shown in Figure 2A. The RNA probe complementary to clone pKβ−1 was used to map the reference transcripts from REFΔ. **Lane M**, end-labelled marker DNA fragments (sizes in nucleotides) of pBR322 digested with *Hpa*II; ref, band corresponding to transcripts from the reference gene. **(C)** RNA mapping analysis of RNA from cells transfected with the pKβ series. Each of the pKβ clones was mixed with REFΔ at a 4:1 mass ratio and transfected by the DEAE-dextran technique as discussed in the text. RNA from cells transfected with pKβ−1/REFΔ **(lanes 1)**, pKβ+6/REFΔ **(lanes 2)** and pKβ+21/REFΔ **(lanes 3)** was mapped with the RNA probe complementary to pKβ−1, to pKβ+6, and to pKβ+21, respectively. **Lanes M**, end-labelled marker DNA fragments; **lane i**, input RNA probe complementary to pKβ−1. Bands corresponding to unspliced (rt) and spliced (rt splice) readthrough transcripts, correct 5′ ends of transcripts from the pKβ series (pKβ 5′), and reference gene transcripts from REFΔ (ref) are indicated.

from pseudopromoters has been encountered in gene transfer experiments with many different genes. Such transcripts are often initiated far upstream of the gene of interest and are then detected as additional protected fragments due to splicing into (cryptic) 3′ splice sites (see, for example, Grosveld *et al.*, 1982; Picard and Schaffner, 1983; Gillies and Tonegawa, 1983).

Despite the surprisingly efficient expression of the κ gene in non-lymphoid cells, a comparison (see Figure 2C) of the κ gene signal to the internal standard (β-globin gene) suggests a preferential expression of the κ gene in myeloma cells. However, to investigate the tissue-specificity of κ gene expression we decided to transfect all the different cell lines by the same technique. This was not the case in the experiments shown in Figure 2 because the different cell lines were transfected by the respective optimal transfection technique.

The κ gene promoter shows tissue preference

To demonstrate a possible contribution of the promoter to the tissue-specific expression of the κ gene it is an important prerequisite that the κ gene promoter is linked to a different coding region to rule out differential stability of the κ mRNA in lymphoid cells as compared with non-lymphoid cells. Figure 3A outlines the basic features of the recombinants of the pKβ series in which the κ promoter region up to position −400 from the cap site was fused to the rabbit β-globin gene at the *Acc*I site within the second exon. The SV40 enhancer was inserted downstream of this chimeric gene. The precise junction sequences of the pKβ recombinants are shown in Figure 3C. pKβ+21 was constructed by exploiting restriction sites. It still retains 21 nucleotides from the untranslated leader of the κ mRNA. This leader portion was progressively deleted from the 3′ side by *Bal*31 nuclease digestion to yield clones pKβ+6 and pKβ−1. In pKβ−1 all of the κ mRNA leader has been deleted including the A at position +1 which is thought to serve as cap site for wild-type κ mRNA (Queen and Baltimore, 1983). We also modified the β-globin gene included as internal control to be able to distinguish the reference transcripts from transcripts from pKβ recombinants using the very same probe in an RNA mapping

analysis. In this reference plasmid, designated REFΔ, a deletion extending from position −10 to +310 with respect to the cap site is introduced into the β-globin gene (Figure 3B). The expression of this modified globin gene is not impaired as compared with that of a wild-type β-globin gene (data not shown) although the start sites appear to be scattered (see Figure 4C).

The recombinants pKβ−1, pKβ+6 and pKβ+21 as well as KES (entire κ gene; see Figure 1B) were transfected in parallel into meyloma X63Ag8 cells, human HeLa and mouse fibroblast 3T6 and Ltk$^-$ cells. To be able to compare the results from such diverse cell lines the DEAE-dextran transfection technique was applied as follows: the transfection cocktail including the internal standard REFΔ was made up as a large pool and then split to the plates with the different cell lines. Furthermore all cell lines were grown in the same medium. 40 h after transfection the cytoplasmic RNA was analysed by hybridization to homogeneously labelled RNA probes synthesized with the SP6 RNA polymerase system (Figures 2A and 4A). Transcripts from pKβ−1, pKβ+6 and pKβ+21 had to be mapped with their corresponding RNA probe due to the different junction points. The reference transcripts from REFΔ are expected to protect a fragment of slightly more than 160 nucleotides with any of the RNA probes (Figure 4A).

The autoradiograph of a representative RNA mapping experiment is shown in Figure 4B and C. The result obtained with the recombinant KES (Figure 4B), which contains the entire κ gene with an SV40 enhancer downstream of it (Figure 1B), confirms the tissue-specific expression of the κ gene already suggested by the experiments discussed above. Densitometric scanning of the autoradiograph reveals a 30-fold tissue preference. The best evidence for a tissue preference of the κ promoter is provided by the experiments with the chimeric constructs of the pKβ series (Figure 4C). Whereas the three non-lymphoid cell lines give essentially the same result, all pKβ clones yield considerably more transcripts with the expected 5′ ends in myeloma X63Ag8 cells.

Specifically, pKβ+21 gives ~17 times more transcripts with the correct cap site (in addition to the same incorrect ends seen with the entire κ gene as discussed above) in myeloma cells than in HeLa cells, and with pKβ+6 the difference is ~25-fold. Chimeric transcripts from both pKβ+21 and pKβ+6 seem to initiate at the correct κ mRNA cap site (see Figure 3C). Recombinant pKβ−1, which retains no κ mRNA sequence, shows a slightly more complex pattern. Since the κ gene cap site is deleted several other purines seem to be chosen for initiation as indicated by the size of the protected bands of ~187, 196, and 203 nucleotides (Figure 4C, lanes 1). In myeloma cells transcripts initiate predominantly at the sequence CATGGA within the globin gene portion and, to a lesser extent, ~5 and 10 bp upstream of the wild-type cap site (indicated by asterisks in Figure 3C). In HeLa and mouse fibroblast cells the same initiation sites are used as well although the relative usage of the three sites varies in the three different non-lymphoid cell lines. If these three bands are added up for quantitation, pKβ-1 is found to be expressed ~25 times more efficiently in myeloma X63Ag8 cells. The same result was obtained in comparison with the expression in another B-cell line, the hybridoma Sp6BU6BU (data not shown).

In addition to specifically initiated transcripts, all three chimeric recombinants give rise to relatively strong readthrough transcription in both the myeloma cell and the non-lymphoid cell lines originating further upstream than the *Pvu*II site at −105 which demarcates the end of the probe. Since RNA probes synthesized by SP6 polymerase contain additional prokaryotic sequences (Melton *et al.*, 1984), protection of the probe by readthrough

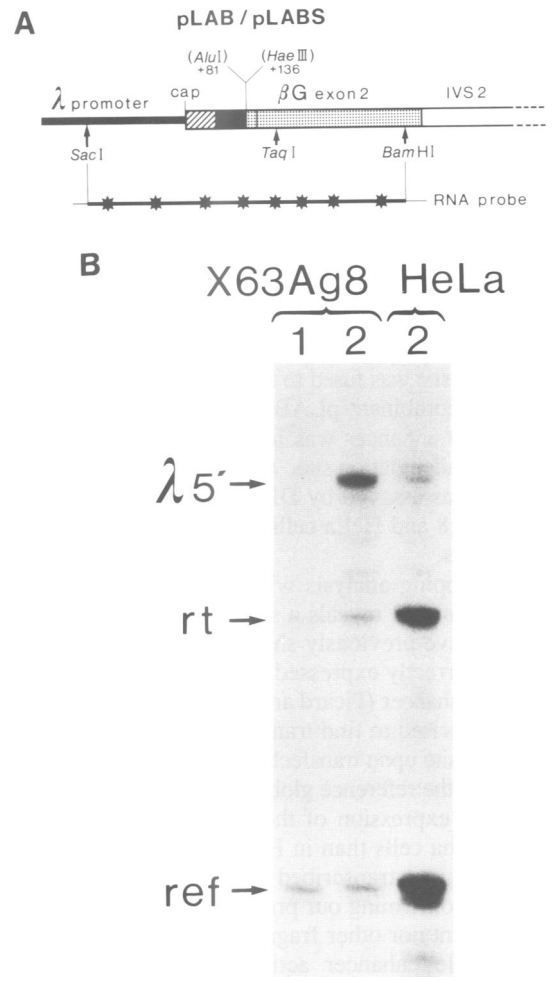

Fig. 5. Similar tissue preference of the Igλ gene promoter. **(A)** Schematic representation of the chimeric gene in the clones pLAB and pLABS. The junction region is shown with the Igλ gene promoter (thick black bar) and λ mRNA leader region, and the rabbit β-globin gene coding body (stippled). The chimeric gene lacks the first intervening sequence of the globin gene. Clone pLABS contains an SV40 enhancer downstream of the chimeric gene (see Materials and methods for further details). The complementary-strand RNA probe used to map transcripts from pLAB(S) is indicated at the bottom. The homology of the probe to the reference gene clone REFΔ extends to the *Taq*I site. IVS2, intervening sequence 2. **(B)** RNA mapping analysis of RNA from HeLa and myeloma X63Ag8 cells transfected with pLAB **(lane 1)** or pLABS **(lanes 2)**. Each of the clones was mixed with REFΔ at 5:1 mass ratio. Transfections were done by the DEAE-dextran technique as discussed in the text. Bands corresponding to the correct 5′ end of transcripts from clone pLABS (λ5′), transcripts from the reference gene clone REFΔ (ref), and presumptive spliced readthrough transcripts (rt) are pointed out.

transcripts is readily distinguishable from residual undigested probe. The bands of ~210, 219, and 232 nucleotides observed with pKβ−1, pKβ+6, and pKβ+21, respectively, can be interpreted as readthrough transcripts which are subsequently spliced into a cryptic 3′ splice site (sequence $(Py)_7N_7AG$; indicated by arrow in Figure 3C) at position −18 with respect to the κ mRNA cap site.

Two conclusions can be drawn from this experiment. First, our chimeric constructs provide direct evidence for a 20- to 30-fold tissue preference of the κ gene promoter. Second, the finding that the extent of tissue specificity observed with the entire κ gene and the chimeric constructs is essentially identical indirectly suggests that the κ mRNA is as stable in HeLa, 3T6

or Ltk$^-$ cells as it is in myeloma cells. One could argue that we have underestimated the degree of tissue preference of the κ promoter because our internal standard, the β-globin reference gene, is also preferentially active in a cell of hematopoietic origin such as a myeloma cell. However, we have obtained essentially identical results with the thymidine kinase gene from herpes simplex virus I as internal standard (data not shown).

Similar tissue preference of the λ gene promoter

To determine whether the promoter of the murine Ig λ light chain gene also displays a tissue preference, we analysed the expression of chimeric construction. The λ gene promoter region including sequences 1400 bp upstream and 81 bp downstream from the λ mRNA cap site was fused to the rabbit β-globin gene coding body to yield recombinant pLAB (Figure 5A). In recombinant pLABS an SV40 enhancer was inserted downstream of the β-globin gene polyadenylation site. The transient expression of this chimeric gene was assayed by DEAE-dextran transfection into myeloma X63Ag8 and HeLa cells as described above for the κ gene experiments.

The RNA mapping analysis with a homogeneously labelled RNA probe (Figure 5) reveals a situation similar to the κ gene promoter. We have previously shown that the immunoglobulin λ gene can be correctly expressed in HeLa cells when it is linked to the SV40 enhancer (Picard and Schaffner, 1983). Therefore we were not surprised to find transcripts initiated at the authentic λ mRNA cap site upon transfection of pLABS into HeLa cells. However, taking the reference globin gene of recombinant REFΔ as standard, the expression of the chimeric gene is ~30-fold higher in myeloma cells than in HeLa cells. The enhancer-less plasmid pLAB is not transcribed to a detectable level even in myeloma cells, confirming our previous finding that neither the promoter fragment nor other fragments from the mouse λ gene contain detectable enhancer activity (Picard and Schaffner, 1984a). Readthrough transcripts most likely account for the band between those diagnostic for transcripts from the chimeric gene and the reference gene. This band maps exactly to the junction between λ mRNA leader and globin gene exon. The sequence immediately preceding the junction reads $(Py)_{11}N_7AG$ and is probably used as a cryptic 3′ splice site for readthrough transcripts initiated far upstream. This is reminiscent of similar transcripts found with the pKβ series (see above).

Discussion

Tissue preference of κ and λ gene promoters

By transfection experiments we present direct evidence that the promoters of both the immunoglobulin κ and the λ light chain genes display a tissue-preference being 20- to 30-times more active in myeloma cells than in non-lymphoid cell lines. To show that the higher expression in myeloma cells is due to a tissue-specific regulation of the promoter rather than differential stability of the Ig mRNA, we have uncoupled the κ and the λ promoters from the rest of the genes and fused them to a β-globin gene coding body. The experiments with one of our κ promoter-globin gene chimeras (pKβ−1; see Figure 3) is particularly conclusive since it contains no κ mRNA sequence.

The κ promoter is contained within ~100 bp upstream of the cap site (Falkner and Zachau, 1984; Bergmann *et al.*, 1984) including a highly conserved octanucleotide which is, however, also found in the promoter of genes active in non-lymphoid cells (Falkner and Zachau, 1984; Parslow *et al.*, 1984). It will be interesting to determine which sequence elements confer the tissue-specificity and how these elements are intermingled with constitutive regulatory elements.

Although we observe a clear tissue-specific regulation of both Ig light chain gene promoters, it should be emphasized that this is not a strict tissue-specificity. This is particularly evident considering our experiments carried out with the entire κ gene transcription unit. When linked to the SV40 enhancer to ensure efficient transcription in different cell-types, correctly initiated transcripts are readily detectable upon transfection into HeLa cells as well as two different mouse fibroblast cell lines, though at a lower level than in myeloma cells. Remarkably, the SV40 enhancer can exert its effect in non-lymphoid cells even over a large distance such as 6 kb from downstream of the κ gene promoter. We have previously shown that the λ gene is only well expressed in HeLa cells when the SV40 enhancer is 150 bp upstream of the cap site (Picard and Schaffner, 1983). However, in certain constructions the SV40 enhancer can also be further away from the λ gene promoter (our unpublished results; see also pLABS in Figure 5).

Comparison with other tissue-specific promoters

A moderate tissue-specificity comparable with that of the Ig light chain gene promoters has recently been described for an Ig heavy chain gene promoter (Grosschedl and Baltimore, 1985) and for the rat insulin gene promoter (Edlund *et al.*, 1985). In the latter case the upstream region can be functionally dissected into a highly tissue-specific enhancer element and a promoter component which has only a 10-fold preference for the insulin-expressing islet cells when linked to a constitutive enhancer.

A seemingly strict tissue-specificity of the promoter has been found with chimeric constructs for another immunoglobulin heavy chain gene (Mason *et al.*, 1985). It has also been suggested for two different κ gene clones (Falkner *et al.*, 1984; Foster *et al.*, 1985), although based on experiments in which differential transcript stability could not be formally ruled out. Nevertheless it is surprising that Foster *et al.* (1985) could hardly detect any specific transcripts in fibroblast cells upon transfection of recombinants with the very same κ gene clone used in our analysis.

Whereas there is perfect agreement on the phenomenon of cell-type-specific regulation of Ig gene promoters, the various groups including our own have proposed somewhat different degrees of tissue-specificity. These differences are most likely due to the fact that different cell lines, test constructions and constitutive viral enhancers therein have been used.

Why are immunoglobulin gene promoters tissue-specific?

The regulatory elements of both the insulin and the κ genes comprise not only a tissue-specific enhancer, but also a promoter with tissue preference. In the insulin gene both elements are tightly associated, whereas the immunoglobulin gene enhancer is uncoupled from the promoter and positioned far downstream within an intron (see model in Figure 6).

Linked to a tissue-specific enhancer element, an enhancer-dependent promoter may not have to be very tissue-specific to ensure proper developmental regulation. For the insulin gene two controlling elements may nonetheless be safer than just one. It is less clear why the promoter of an immunoglobulin gene should be tissue-specific at all. Note that the enhancer, which seems to be exclusively activated in the lymphocyte lineage, is associated with the constant region and becomes linked to the variable region promoter by somatic rearrangement only late in B-lymphocyte differentiation. On the other hand, it has been found that Ig heavy chain gene variable regions are transiently expressed just before somatic rearrangement (Yancopoulos and Alt, 1985), and that unrearranged λ variable (Picard and Schaffner, 1984b) and

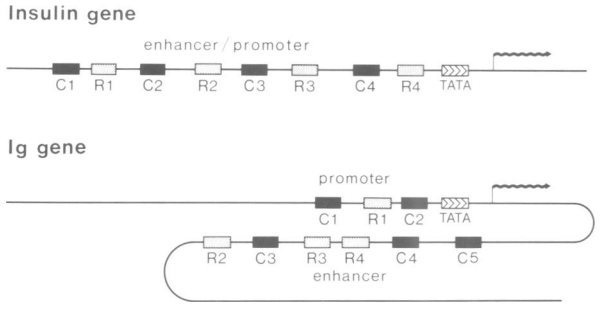

Fig. 6. A model for tissue-specific transcriptional regulation: comparison of immunoglobulin and insulin genes. Whereas insulin enhancer and promoter are contiguous (Edlund *et al.*, 1985), the immunoglobulin enhancer is located downstream of the cap site within the gene (Banerji *et al.*, 1983; Gillies *et al.*, 1983; Neuberger, 1983; Rabbitts *et al.*, 1983; Hayday *et al.*, 1984; Picard and Schaffner, 1984a; Queen and Stafford, 1984). Involvement of further elements is not excluded. Tissue-specific enhancer and promoter are both viewed as being composed of one or several intermingled constitutive (C) and regulatory (R) DNA sequence motifs (arbitrary number and order in the figure) which determine the level and the tissue-specificity of transcription by the cooperative interaction with protein factors (see also review of Serfling *et al.*, 1985; Gluzman, 1985). In either gene the sequence elements just upstream of the TATA box cannot induce high levels of transcription by themselves but will do so in conjunction with additional elements from the enhancer.

possibly some κ variable regions (D.Picard, unpublished results) are transcribed in myeloma cells, albeit at a very low level. Considering these latter observations it is conceivable that the cell-type specificity of Ig gene promoters is exploited at some stage of B-cell differentiation to regulate the promoter independently from the enhancer.

Materials and methods

Cell growth and transfection

All cell lines were grown in DMEM. For human HeLa, monkey kidney COS-1 (Gluzman, 1981), mouse fibroblast 3T6 (a gift from W.Topp, Cold Spring Harbor) and Ltk$^-$ (a gift from C.Weissmann, Zürich) cells the medium was supplemented with 2.5% fetal calf serum and 2.5% calf serum. For mouse myeloma X63Ag8 cells (Köhler and Milstein, 1975) the medium contained 10% fetal calf serum and 50 μM β-mercaptoethanol. The latter medium was used when all cell lines were grown in the same medium.

The calcium phosphate transfection protocol was that of Graham and van der Eb (1973) and of Wigler *et al.* (1978) with the modifications described in Weber *et al.* (1984). Transfection by DEAE-dextran was as described (Banerji *et al.*, 1983) except that after removal of the transfection cocktail HeLa and 3T6 cells were incubated for 4 min with 25% (v/v) dimethylsulfoxide (DMSO) in TBS buffer (25 mM Tris-HCl, 137 mM NaCl, 5 mM KCl, 0.7 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.6 mM Na_2HPO_4, pH 7.4; Kimura and Dulbecco, 1972), and X63Ag8 and Ltk$^-$ cells for 5 min with 17.5% (v/v) DMSO in TBS. The cells were then washed twice with TBS and incubated with fresh medium.

DNAs

All clones were constructed according to standard recombinant DNA techniques (Maniatis *et al.*, 1982) based on the following genes: the κ light chain gene from the mouse myeloma MOPC-41 (Seidman and Leder, 1978; Seidman *et al.*, 1979), the λI light chain gene from the mouse myeloma HOPC2020 (Brack and Tonegawa, 1977; Bernard *et al.*, 1978), the rabbit hemoglobin β1 gene (Maniatis *et al.*, 1978; van Ooyen *et al.*, 1979), and the SV40 early region (Tooze, 1981).

pK. The κ gene was cloned as a 6.5-kb *Eco*RI-*Bam*HI DNA fragment into the *Eco*RI-*Bam*HI restricted clone pβG (Banerji *et al.*, 1983). It thus retains ~1.2 kb of 3′-flanking sequences from the rabbit β-globin gene.

pKE. Using synthetic linkers the SV40 enhancer was cloned into pK at the *Xba*I site within the rabbit DNA. The SV40 enhancer consisted of a 196-bp fragment extending from the *Kpn*I site of SV40.

KES. The κ gene as a 6.5-kb *Eco*RI-*Bam*HI fragment was first inserted into pBR327 (Soberon *et al.*, 1980) to give clone p7K. the *Bgl*II fragment from clone pβGXsv512 (Banerji *et al.*, 1983), which contains the SV40 enhancer fragment embedded in 1.2 kb of 3′-flanking sequences from the rabbit β-globin gene, was then inserted into the *Bam*HI site of p7K.

TaK. The entire SV40 early region was inserted into pK as an *Eco*RI-*Bam*HI fragment (*Eco*RI linker at *Kpn*I site of SV40) fusing the SV40 *Bam*HI site to the *Bgl*II site within the rabbit DNA of pK.

REFΔ. To introduce an internal deletion into the rabbit β-globin gene ('clone 3' in de Villiers *et al.*, 1983) the *Pvu*II site at position −10 with respect to the cap site was fused to the *Taq*I site at +310. The protruding end of the latter was trimmed with S1 nuclease. The SV40 enhancer is at position −425.

pKβ series. The κ gene promoter region was introduced into clone pβG (Banerji *et al.*, 1983) as indicated in Figure 3. To construct pKβ+21 the *Hinf*I site within the leader of the κ mRNA was partially filled in with dATP to match the *Acc*I site at position +284 of the globin gene. The sequence of the *Bal*31 nuclease deletion mutants pKβ+6 and pKβ−1 was verified by chemical sequencing (Maxam and Gilbert, 1980) from the *Taq*I site at position +310 in the globin gene. The 196-bp SV40 enhancer fragment was inserted at the *Xba*I site downstream of the globin gene.

pLAB/pLABS. The upstream region of the Igλ gene was fused to the rabbit β-globin gene in clone pβGX and pβGXsv512 (Banerji *et al.*, 1983) to give recombinants pLAB and pLABS, respectively. pLABS thus contains the 196-bp SV40 enhancer fragment downstream of the globin gene. The λ gene sequence extends from the *Eco*RI site 1.4 kb upstream to the *Alu*I site 81 bp downstream of the λ gene cap site. The *Alu*I site was fused to the *Hae*III site within the first exon of the globin gene (position +136 with respect to the globin gene cap site). pLAB and pLABS lack the first intron of the globin gene. The bacterial plasmid is pBR327 (Soberon *et al.*, 1980) with its β-lactamase gene in opposite orientation to the eukaryotic fusion gene.

RNA analysis

Total cytoplasmic RNA was isolated 40−42 h after transfection as described by de Villiers and Schaffner (1983). Residual input plasmid DNA was removed from RNA preparations by RNase-free DNase (Picard and Schaffner, 1983). S1 nuclease mapping was performed with the single-stranded DNA probes shown in Figure 2A as described (Weaver and Weissmann, 1979; de Villiers and Schaffner, 1983). RNA mapping experiments with the complementary-strand RNA probes (SP6 RNA polymerase system) shown in Figures 2A, 4A and 5A were essentially done as described by Melton *et al.* (1984) except that only 8 μg/ml RNase A and 60 u/ml RNase T1 were used. Autoradiographs were quantitated by densitometric scanning and scintillation counting of excised bands.

Acknowledgements

We are particularly indebted to Fritz Ochsenbein for preparing the excellent graphic figures. Furthermore, we thank Greg Gilmartin for critical reading of the manuscript. This work was supported by a Swiss National Research Foundation grant to W.S. and by the Kanton of Zürich.

References

Banerji,J., Olson,L. and Schaffner,W. (1983) *Cell*, **33**, 729-740.
Bergman,Y., Rice,D., Grosschedl,R. and Baltimore,D. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 7041-7045.
Bernard,O., Hozumi,N. and Tonegawa,S. (1978) *Cell*, **15**, 1133-1144.
Brack,C. and Tonegawa,S. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5652-5656.
de Villiers,J., Olson,L., Banerji,J. and Schaffner,W. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **47**, 911-919.
de Villiers,J. and Schaffner,W. (1983) in Flavell,R.A. (ed.), *Techniques in Nucleic Acid Biochemistry*, Vol. **B5**, Elsevier, Ireland, pp. 1-20.
Edlund,T., Walker,M.D., Barr,P.J. and Rutter,W.J. (1985) *Science*, in press.
Falkner,F.G. and Zachau,H.G. (1984) *Nature*, **310**, 71-74.
Falkner,F.G., Neumann,E. and Zachau,H.G. (1984) *Hoppe-Seyler's Z. Physiol. Chem.*, **365**, 1331-1343.
Foster,J., Stafford,J. and Queen,C. (1985) *Nature*, **315**, 423-425.
Gillies,S.D. and Tonegawa,S. (1983) *Nucleic Acids Res.*, **11**, 7981-7997.
Gillies,S.D., Morrison,S.L., Oi,V.T. and Tonegawa,S. (1983) *Cell*, **33**, 717-728.
Gillies,S.D., Folsom,V. and Tonegawa,S. (1984) *Nature*, **310**, 594-597.
Gluzman,Y. (1981) *Cell*, **23**, 175-182.
Gluzman,Y. (ed.) (1985) *Genetic Elements Regulating Transcription in Eukaryotes*, published by Cold Spring Harbor Laboratory Press, NY, in press.
Graham,F.L. and van der Eb,A.J. (1973) *Virology*, **52**, 456-467.
Grosschedl,R. and Baltimore,D. (1985) *Cell*, **41**, 885-897.
Grosveld,G.C., de Boer,E., Shewmaker,C.K. and Flavell,R.A. (1982) *Nature*, **295**, 120-126.
Hayday,A.C., Gillies,S.D., Saito,H., Wood,C., Wiman,K., Hayward,W.S. and Tonegawa,S. (1984) *Nature*, **307**, 334-340.

Kimura,G. and Dulbecco,R. (1972) *Virology*, **49**, 394-403.
Köhler,G. and Milstein,C. (1975) *Nature*, **256**, 495-497.
Maniatis,T., Hardison,R.C., Lacy,E., Lauer,J., O'Connell,C., Quon,D., Sim,G.K. and Efstratiadis,A. (1978) *Cell*, **15**, 687-701.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
Mason,J.O., Williams,G.T. and Neuberger,M.S. (1985) *Cell*, **41**, 479-487.
Mather,E.L., Nelson,K.J., Haimovich,J. and Perry,R.P. (1984) *Cell*, **36**, 329-338.
Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499-560.
Melton,D.A., Krieg,P.A., Rebagliati,M.R., Maniatis,T., Zinn,K. and Green,M.R. (1984) *Nucleic Acids Res.*, **12**, 7035-7056.
Neuberger,M.S. (1983) *EMBO J.*, **2**, 1373-1378.
Parslow,T.G., Blair,D.L., Murphy,W.J. and Granner,D.K. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2650-2654.
Picard,D. (1985) in Maclean,N. (ed.), *Oxford Surveys on Eukaryotic Genes*, Vol. **2**, Oxford University Press, pp. 24-47.
Picard,D. and Schaffner,W. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 417-421.
Picard,D. and Schaffner,W. (1984a) *Nature*, **307**, 80-82.
Picard,D. and Schaffner,W. (1984b) *EMBO J.*, **3**, 3031-3035.
Prochownik,E.V. (1985) *Nature*, **316**, 845-848.
Queen,C. and Baltimore,D. (1983) *Cell*, **33**, 741-748.
Queen,C. and Stafford,J. (1984) *Mol. Cell. Biol.*, **4**, 1042-1049.
Rabbitts,T.H., Forster,A., Baer,R. and Hamlyn,P.H. (1983) *Nature*, **306**, 806-809.
Seidman,J.G. and Leder,P. (1978) *Nature*, **276**, 790-795.
Seidman,J.G., Max,E.E. and Leder,P. (1979) *Nature*, **280**, 370-375.
Serfling,E., Jasin,M. and Schaffner,W. (1985) *Trends Genet.*, **1**, 224-230.
Soberon,X., Covarrubias,L. and Bolivar,F. (1980) *Gene*, **9**, 287-305.
Tonegawa,S. (1983) *Nature*, **302**, 575-581.
Tooze,J. (ed.) (1981) *DNA Tumor Viruses*, published by Cold Spring Harbor Laboratory Press, NY.
van Ooyen,A., van den Berg,J., Mantei,N. and Weissmann,C. (1979) *Science (Wash.)*, **206**, 337-344.
Weaver,R.F. and Weissmann,C. (1979) *Nucleic Acids Res.*, **7**, 1175-1193.
Weber,F., de Villiers,J. and Schaffner,W. (1984) *Cell*, **36**, 983-992.
Wigler,M., Pellicer,A., Silverstein,S. and Axel,R. (1978) *Cell*, **14**, 725-731.
Yancopoulos,G.D. and Alt,F.W. (1985) *Cell*, **40**, 271-281.
Yuan,D. and Tucker,P.W. (1984) *J. Exp. Med.*, **160**, 564-583.

Received on 26 July 1985