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Tissue Specificity of the Initiation of Immunoglobulin κ Gene Transcription

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Summary: The transient transcription of a rearranged mouse immunoglobulin κ gene was studied in a monkey fibroblast cell line. The gene was inserted into an SV40 expression vector and the calcium phosphate coprecipitation method was used for transfection. The transcripts were correctly spliced; transcription, however, was initiated within the vector and not at the correct site 23–26 bp upstream of the gene, irrespective of the length of the upstream sequences (90, 160, 370, and 870 bp) in the plasmid constructs. In contrast, accurately initiated transcripts were observed when a plasmid containing the κ gene with 870 bp of its upstream sequence was introduced into a lymphoid cell line; the plasmid was constructed from the pSV2-gpt vector and the electric impulse method was used for gene transfer in most experiments.

Tissue-specific expression of κ light chain genes in lymphoid cells is known to depend on the presence of an enhancer element in the J-C intron. The results reported in this paper suggest that the sequence elements pd and dc which are located upstream of the leader gene segment also act in a tissue-specific manner and that it is the initiation of transcription which is a tissue-specific event.

Gewebsspezifität der Transkritionsinitiation eines Immunglobulin-Gens vom κ-Typ

Zusammenfassung: In einer Zellinie von Affen-Fibroblasten wurde die transiente Transkription eines umgelagerten Maus-Immunglobulin-κ-Gens untersucht. Das in einen SV40-Expressionsvektor eingebaute Gen konnte mit Hilfe der Calciumphosphat-Copräzipitationsmethode in die Zellen eingeführt werden. Die Transkripte wurden korrekt gespleißt, jedoch war die Transkription innerhalb des Vektors initiiert worden und nicht an der richtigen Initiationsstelle, 23–26 bp stromaufwärts vom Gen. Die Initiationsstelle war nicht abhängig von der Länge der 5′-seitig gelegenen Sequenz (90, 160, 370 oder 870 bp). Im Gegensatz dazu wurden korrekt initiierte Transkripte beobachtet, wenn ein Plasmid mit einem κ-Gen, einschließlich der 870 bp langen, strom-

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Abbreviations:
Hepes: 4-(2-Hydroxyethyl)-1-piperazinethanesulfonic acid;
PBS: phosphate-buffered saline;
NP40: ethylphenylpolyethyleneglycol (detergent).

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aufwärts gelegenen Sequenz, in eine lymphoide Zellinie eingeführt wurde; das Plasmid wurde mit Hilfe des pSV2-gpt-Vektors konstruiert. Für den Gen-Transfer wurde in den meisten Experimenten die elektrische Impulsmethode verwendet.

Die gewebsspezifische Expression von κ-Kettengenen in lymphoiden Zellen ist bekanntlich von der Gegenwart eines „enhancer"-Elements im J-C-Intron abhängig. Die in dieser Arbeit mitgeteilten Ergebnisse lassen vermuten, daß auch die stromaufwärts vom „leader"-Gensegment lokalisierten Sequenz-Elemente pd und dc gewebsspezifisch wirksam werden, und daß dabei die Initiation der Transkription der gewebsspezifische Schritt ist.

Key-words: Coordinate gene expression, electric field-mediated gene transfer, immunoglobulin genes, tissue-specific initiation.

The transfer of cloned genes into eukaryotic cells has become a powerful tool in the study of gene expression. One of the most widely applied techniques for gene transfer is the delivery of DNA as a calcium phosphate coprecipitate into fibroblasts[1]. Studies with immunoglobulin κ genes in the fibroblast system have shown that the κ gene is not transcribed correctly and that viral enhancer sequences, which increase e.g. the transcription of β globin genes by orders of magnitude[2] cannot activate κ gene transcription[3,4]. For an immunoglobulin λ gene under certain conditions (i.e. by insertion of an SV40 72 bp repeat close to the capping site) correctly initiated transcripts were obtained in fibroblasts[5].

Efficient expression of immunoglobulin κ genes was observed when they were introduced into cells of the B-lymphoid lineage where these genes are normally expressed[6–8]. By directly comparing κ gene expression in non-lymphoid and lymphoid cells using a transient transcription assay it was found that this gene is correctly transcribed only in the latter cell type[9]. This seems to be due to an enhancer element in the large intron which is active in lymphoid cells and inactive in fibroblasts[10,11]. We have shown recently that the −90 to −160 bp region upstream of the initiator codon is also essential for correct κ gene transcription in lymphoid cells[12] but the tissue specificity of the region was not demonstrated. The region contains the highly conserved decanucleotide sequence TNATTTGCAT (dc) and the moderately conserved pentekaideca nucleotide sequence TGCA$^{G}_{C}$CTGTGNCCAG (pd). Thus the κ gene promoter consists of the pd and dc elements and more general promoter elements like the TATA box and cap site. By comparing the 5′ flanking regions of several immunoglobulin light chain genes the sequence ATTTGCAT, which is part of the dc sequence, has also been identified by Parslow et al.[13].

To find an answer to the unsolved question whether the cell type specificity observed in immunoglobulin κ gene transcription resides only in the intron enhancer region or also in the κ promoter region, we now examined the role of the upstream region of a mouse κ gene in a fibroblast expression system and compared the results with those obtained in a lymphoid cell system.

Results

Construction of recombinant plasmids

Transient expression studies in fibroblasts with the mouse immunoglobulin κ gene T1 had shown that an activation of this gene by a viral enhancer over long distances was not possible[3]. We have now constructed the plasmids shown in Fig. 1A for two reasons. We wanted to know firstly whether the viral enhancer activates the gene when it is located nearby or at an intermediate distance, and secondly, whether the κ promoter region plays a role in the fibroblast expression system. The plasmids contain sequences of pBR322, the κ gene T1[14] and an SV40 vector fragment which consists of the gene encoding the large T antigen, the origin of replication, and the early and late promoters including the enhancer sequences. The κ gene region was truncated in its 5′ end (at the *Taq*I, *Hae*III, and

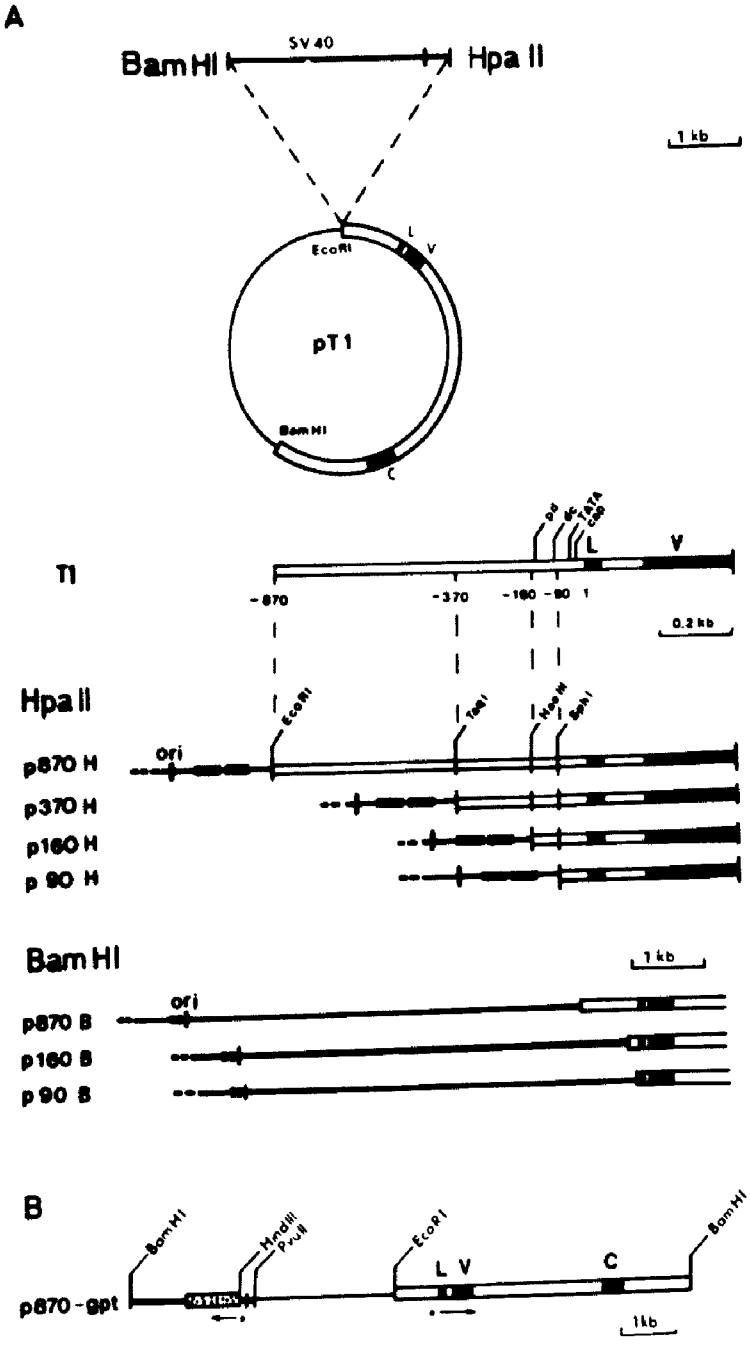

Fig. 1. Recombinant plasmids used for transfection experiments.

A. pBR322 sequences are shown as thin lines, SV40 sequences as bold lines, the two black segments represent the 72 bp repeats (enhancer) with the origin of replication (ori). The mouse DNA including the κ gene T1 with its leader (L), variable (V), and constant (C) region segment is shown as a double line, the exons are the black segments. The numbers of the plasmids refer to the length of the 5′ flank upstream of the coding region of the κ gene. In a scheme the construction and structure of the *Hpa*-series (H) and *Bam*-series (B) of plasmids are shown. In the – 90 to – 160 bp region the two conserved sequence elements pd and dc (see text) are indicated which are absent in the p90H and p90B constructs.

B. The plasmid p870-gpt used for the myeloma cell transfection experiments is shown in linearized form. The stippled segment is the *E. coli* gpt gene; the *Hin*dIII-*Pvu*II fragment contains the SV40 origin of replication.

*Sph*I sites) and ligated with the SV40 vector. Two series of plasmids were obtained. The *Hpa*-series plasmids p90H, p160H, p370H, and p870H contain the κ gene with 90, 160, 370, and 870 bp, respectively, of its upstream region directly followed by the SV40 72 bp repeats; in the *Bam*-series plasmids p90B, p160B, and p870B the viral enhancer is separated from the κ coding region by 2.8 kb or more. In the case of the p90H and p90B constructs the region containing the pd and dc elements[12] is deleted.

For the lymphoid cell transfection experiments the plasmid p870-gpt (Fig. 1B)[12] was used. In this construct the κ gene T1 with 870 bp of its upstream region is inserted into the vector pSV2-gpt[15]. This vector contains the *E. coli* gpt gene which, on transfer into eukaryotic cells, can be used as a dominant selection marker.

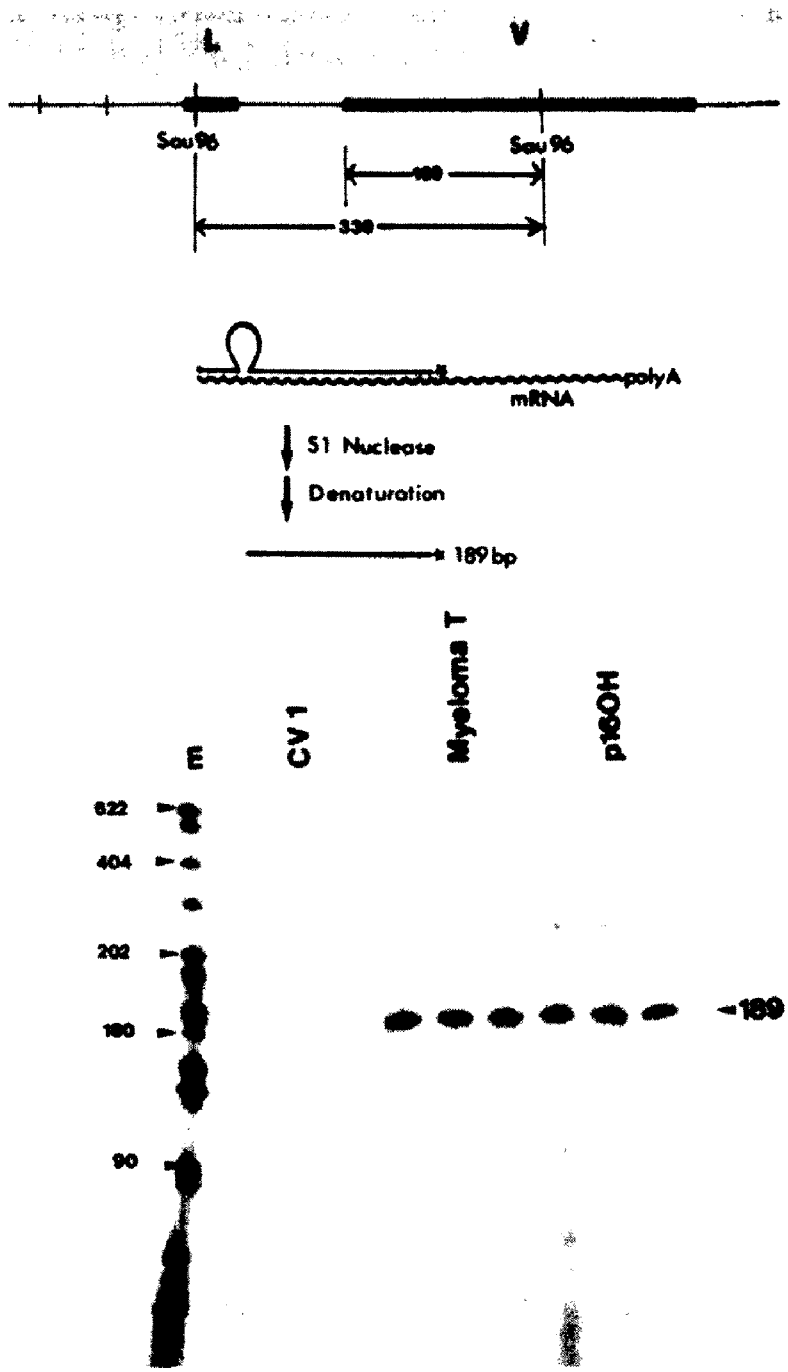

Fig. 2. κ transcripts obtained from transfected monkey fibroblasts are correctly spliced.

In the upper part of the figure the S1-mapping strategy is shown. The ^{32}P-end-labelled 339 bp DNA probe was hybridized to total cellular RNA, digested with S1 nuclease, denatured, and separated on a 6% polyacrylamide-urea gel (lower part of the figure). Parallel experiments were carried out with RNA from fibroblast cells (CV1), transfected fibroblast cells (p160H), and the B-cell tumor called myeloma T[14,38] from which the κ gene T1 was derived. Three different S1 nuclease concentrations were used (1000, 2000, and 3000 U/ml). The weak bands in the p160H tracks (larger and smaller than 189 nucleotides) are resistant to S1 nuclease digest. As a marker (m) an end-labelled *Hpa*II digest of pBR322 is shown.

Efficient transcription and splicing of κ mRNA in monkey fibroblast but no correct initiation

To learn more about transcription of the κ gene in a heterologous expression system the plasmids described in Fig. 1A were transfected into monkey kidney cells (CV1-cells), following a transient expression protocol[16]. S1 protection experiments[17] with total cellular RNA were performed according to the strategy shown in Fig. 2. The probe was a 339 bp Sau96 fragment which included the small intron. If the transcript is correctly spliced, the same 189 residue protected fragment should be present as it was formed from the transcripts of myeloma T cells. From all *Hpa*- and *Bam*-series plasmids this fragment was obtained (at similar levels) as it is shown in Fig. 2 with p160H as an example; it was lacking, however, in non-transfected CV1 cells. This

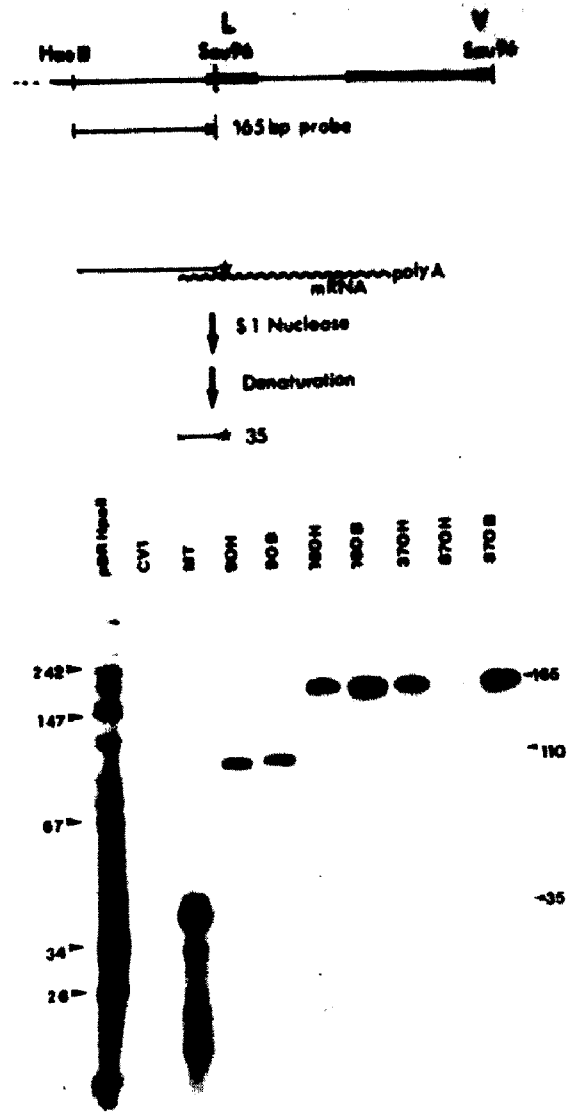

Fig. 3. No correct initiation of κ gene transcription in the fibroblast-SV40 vector system.

In the upper part the strategy for the determination of the 5′ ends of the κ transcripts is shown. The end-labelled probe (10 ng, 2×10^7 cpm) was hybridized with total cellular RNA (10 μg), digested with S1 nuclease (1000 U/ml; 40 min at 30 °C) and sized on a denaturing 12% polyacrylamide-urea gel (lower part). The 35 residue band in the myeloma T control (MT) indicates correct 5′ ends and is seen neither in the negative control (CV1) nor in the RNA from the CV1 cells transfected with the *Hpa*- and *Bam*-series plasmids. The origin of the 110 and 165 residue bands is explained in the text. Additional faint bands probably result from incomplete S1 digestion.

shows that in the monkey fibroblast in-vivo expression system easily detectable amounts of κ RNA were formed which were correctly spliced in the first intron. κ-specific transcripts were not detected when plasmids lacking the SV40 enhancer (e.g. pT1; Materials and Methods) were used for transfection (data not shown).

In order to assay in the transfected cells for correctly initiated κ mRNA, S1 mapping was performed[17,18], using as a probe the 165 bp *Hae*III-Sau96 fragment (Fig. 3), ^{32}P-labelled at its 5′ ends by polynucleotide kinase. Only the experiment with myeloma T RNA resulted in the predicted 34–36 residue band, indicating an origin of transcription 23–26 bp upstream of the coding sequence[19]. With the RNA preparation from the other transfectants no such band was observed. In the experiments with the RNAs extracted from cells transfected with the p90H and p90B plasmids, which lack the segment including the dc and pd elements, a 110-residue band was obtained. This indicates protection of the probe up to the *Sph*I site at position −90 (Fig. 1) where the vector sequences begin and does not reflect a new cap site. The band shows that transcription starts upstream of the *Sph*I site at vector-provided promoters which suggests that sequences necessary for correct initiation of κ gene transcription in the myeloma cell

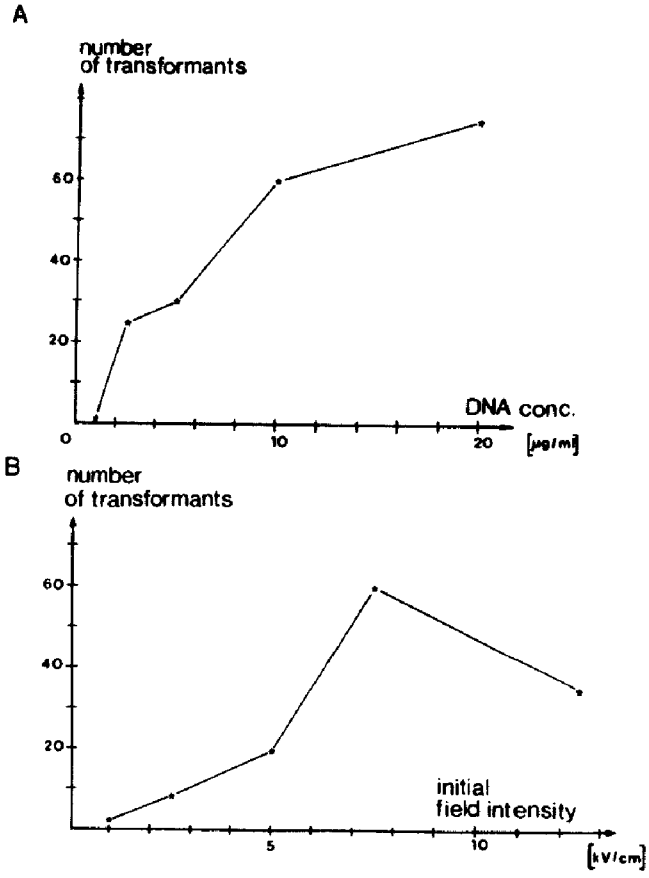

Fig. 4. Dependence of transformation frequencies on (A) DNA concentration and (B) initial field strength.
A. Increasing amounts of supercoiled p870-gpt plasmid DNA were added to X63Ag8.653 cell samples (1.3×10^7 cells/ml; sample volume 0.31 ml). Three pulses of 5 kV cm^{-1} at a pulse duration of 4.6 µs were applied to each sample at the various DNA concentrations at 20 °C (sample resistance 220 Ω; discharge capacitor 21 nF). Cell clones were counted 17 days after the beginning of selection. The number of transformants refers to the number of transfected cells in the sample. B. Cell samples (1.3×10^7 cells/ml; sample volume 0.31 ml) were subjected to electric impulses of increasing field strength in the presence of constant amounts of supercoiled p870-gpt plasmid DNA (10 µg/ml). At higher strength of the electric field (12.5 kV cm^{-1}) spontaneous lysis of about 20% of the cells occurs immediately after the shock. Cells were further treated as described in Materials and Methods.

system[12] do not influence transcription in the fibroblast cell system. The other transfectants (cells transfected with p160H, p160B, p370H, and p870B) also showed no correct initiation. The strong 165 residue band (Fig. 3) indicates protection of the full length probe. Since a double-stranded end-labelled DNA probe was used, this band could also result from renaturation, but under the S1 digest conditions used this band was normally very weak (Fig. 3, MT and p90H, p90B tracks); its appearance therefore reflects full length protection of the probe, indicating that transcription was initiated upstream of the normal κ cap site in vector-derived sequences. This view is supported by Northern blot analyses where only incorrectly large-sized molecules were detected (data not shown). In summary, the experiments in the fibroblasts SV40 vector system show that correct initiation of κ gene transcription does not occur and can also not be activated by reducing the distance between the κ gene and the viral enhancer sequences.

Electric impulse method for DNA transfer into lymphoid cells

Our first transfection experiments with the lymphoid cell line X63Ag8.653[20] using the calcium phosphate coprecipitation technique[1] and the plasmid p870-gpt (Fig. 1B) were not successful because, as we know now, this cell line does not tolerate long exposure to high concentrations of calcium ions and of mycophenolic acid. The electric impulse method of gene transfer, on the other hand, led to large numbers of transformants when, additionally, the concentration of mycophenolic acid in the selective medium was reduced drastically. The electroporation method involves subjecting cell suspensions in the presence of dissolved plasmid DNA to short electric impulses[21]; it appears to work by electrically induced pore formation in the cell membranes[22]. The number of transformants increases with increasing concentration of dissolved plasmid DNA. The optimum range of the initial field intensity of the pulses was 7–9 kV cm^{-1} (Fig. 4). The transformation frequency also depends strongly on the lymphoid cell line used. The cell line X63Ag8.653 yields a transformation frequency of about 5×10^{-5} cells per µg of DNA; the frequency is higher by a factor of ten for the cell line NS1. The transformation frequency increased when linearized plasmid DNA was used, but in this case most transformants had taken up κ genes which were no longer intact[23]. After the electric field method had been

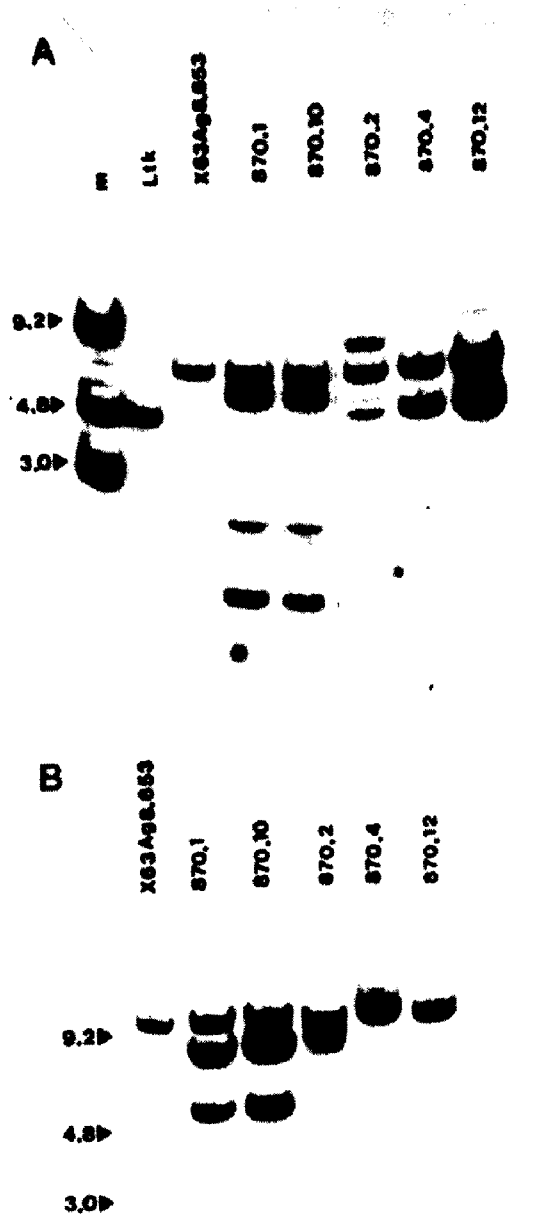

Fig. 5. After transfection the lymphoid cells contain only few copies of the κ gene.
A. *Hin*dIII digests of DNAs (15 μg) from mouse Ltk$^-$ cells (Ltk), X63Ag8.653 lymphoid cells and from transformed cell clones were separated on a 1.0% agarose gel, transferred onto nitrocellulose filters and hybridized with a C_κ probe. This probe consists of the *Hin*dIII-*Bam*HI mouse C_κ fragment cloned in pBR322[14]. Clones 870.1 and 870.10 show nearly identical banding patterns except for one additional weakly hybridizing fragment in clone 870.10. The hybridization marker (m) consists of known C_κ gene-containing fragments.
B. Southern blot hybridization analysis of *Eco*RI digests with the same DNAs as in A. The blots were exposed for two days with intensifier screens.

established we found that X63Ag8.653 cells could also be transformed by the calcium phosphate coprecipitation technique, when the exposure time of the cells in the medium containing high concentrations of calcium ions was reduced and the proper selection conditions were used (Materials and Methods).

The gpt-positive stable transformants were selected with mycophenolic acid[15]. Several lymphoid cell lines (e.g. X63Ag8.653; NS1; hybridomas derived from those two lines) are very sensitive to inital high doses of mycophenolic acid (> 1 μg/ml); lysis of all transfected cells occurs within 5 days. Stable transformants were obtained, how-

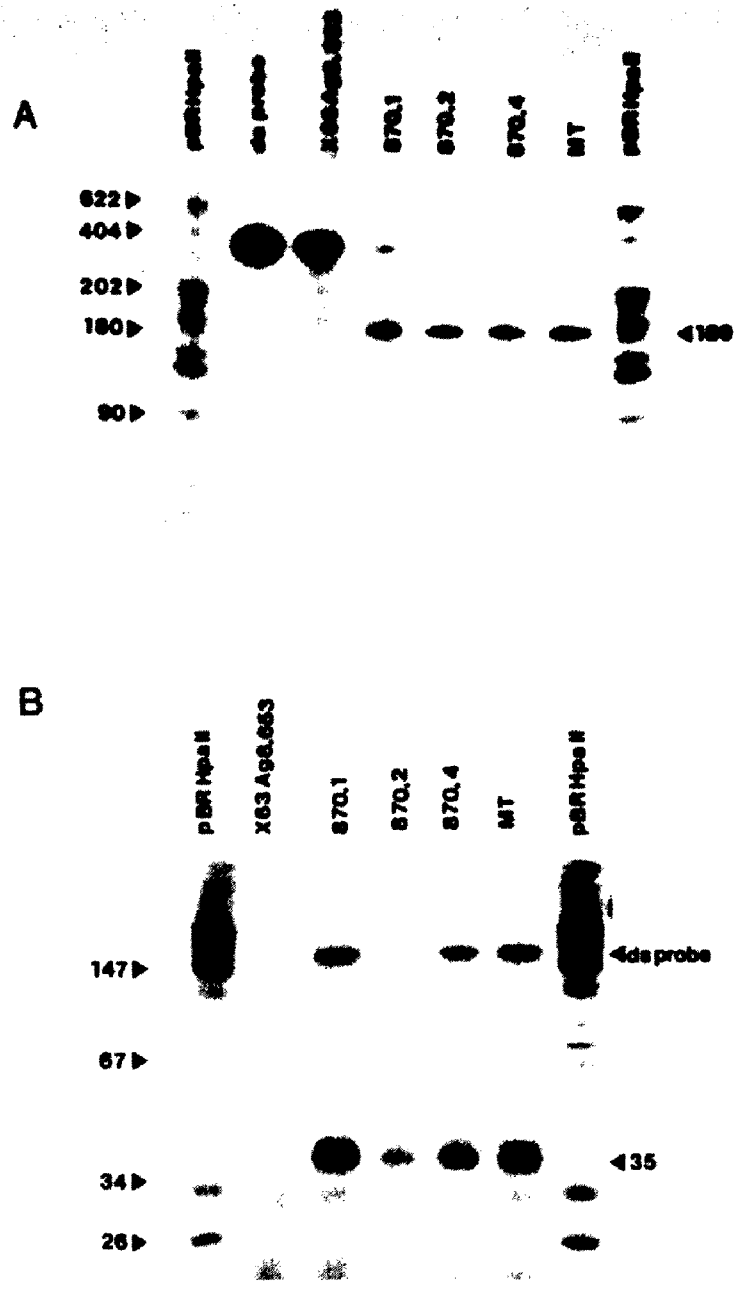

Fig. 6. In transformed lymphoid cell clones normal splicing and accurate initiation of transcription occurs.

A. The intron-exon border of the first intron was mapped as in Fig. 2. The marker is a ^{32}P-end-labelled *Hpa*II digest of pBR-322. The negative control was done with RNA extracted from the X63Ag8.653 cell line and the positive control was performed with RNA extracted from myeloma T (MT). B. The 5′ ends were mapped as described in Fig. 3. The 35 nucleotide band found for myeloma T (MT) and the transformed cell lines (870.1, 870.2, and 870.4) shows correct initiation of transcription. As the negative control RNA from non-transfected X63Ag-8.653 cells was used. Faint bands in the lower part of the autoradiograph are due to incomplete S1 digestion.

ever, when an initial low dose (0.25 μg/ml) was increased stepwise (Materials and Methods).

Screening for κ protein production was carried out by an enzyme-linked immunoassay[3]. 5–7% of the clones resistant to mycophenolic acid were also positive for κ determinants. The transformed cell lines 870.1, 870.2, 870.4, 870.5, 870.10, and 870.12 resulted from this screening step.

Electric field-mediated gene transfer leads to the integration of only a few κ gene copies into the host genome

The number of the transferred κ sequences in the myeloma cell clones was estimated by Southern blot hybridization with a C_κ probe (Fig. 5). Our X63Ag8.653 line does not contain a germline C_κ gene as it was observed for instance in Ltk$^-$ cell DNA (Fig. 5). We find one rearranged κ gene

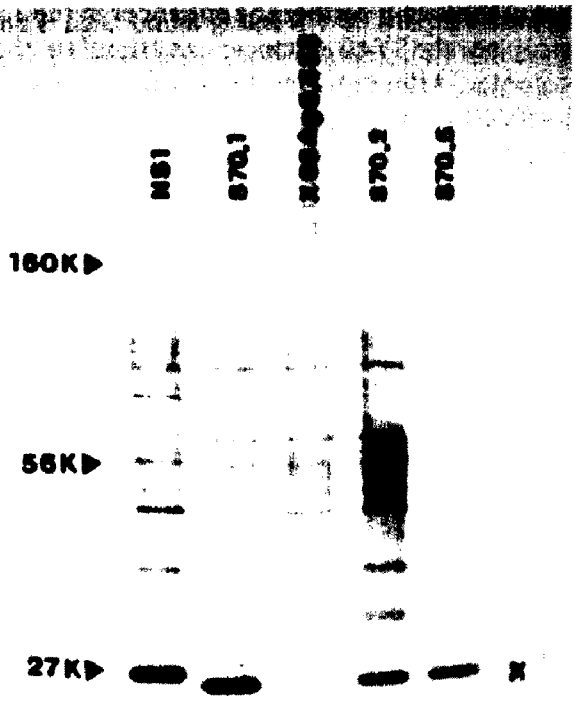

Fig. 7. High levels of κ protein expression in lymphoid cell transformants.

To compare intracellular amounts of κ chains, cells were analysed as described in Materials and Methods. Labelled, affinity-purified κ chains from 2×10^6 cells were applied to each track. The faint bands in the upper part of the fluorograph are due to non-specific binding of labelled proteins to protein A Sepharose. 27 K is the molecular mass (in kDa) of the κ protein.

allele from which, however, no transcript is produced[12]. The original X63Ag8.653 cell line has two κ gene alleles and produces high levels of κ mRNA but no κ protein[24,25]. Our X63Ag8.653 line is a subline derived by cloning which has lost the transcriptionally active κ allele.

The transformed myeloma cell clones (870.1, 870.2, 870.4, 870.10, and 870.12) show, in addition to the κ gene provided by the host cell line, low copy numbers of the transfected sequences. The clones have taken up only one to three copies as compared to the endogenous κ gene as an internal standard. Southern blot hybridization with digests of restriction nucleases cleaving only once in the p870-gpt sequence (*Eco*RI digest, Fig. 5B; *Bam*HI digest not shown) confirm that the plasmids are integrated into the host cell genome.

In the fifteen cell lines which had been transformed by the electric impulse method only few plasmid copies were integrated. In contrast, using the calcium phosphate coprecipitation technique with the same plasmid (p870-gpt) and the same selection procedure we found in two out of five clones high numbers of plasmid copies. Since they all reside in one or two restriction fragments, they may have arisen by gene amplification events[23].

Southern blot analysis of *Hin*dIII-digested DNA from the cell lines 870.4 and 870.12 showed that in addition to the endogenous κ gene a second band occurs, representing about one copy of the transferred plasmid (Fig. 5A). From the same DNAs digested with *Eco*RI (Fig. 5B) and *Bam*HI (data not shown) only one C_κ-containing fragment is released. (In the *Bam*HI digest an additional minor band probably representing a weakly hybridizing flank was found.) The conclusion from this finding is that either the integration of the transferred plasmid has occurred near the host cell κ gene locus, or that the *Eco*RI or the *Bam*HI fragments containing the transferred κ gene accidentally have the same size as the endogenous band.

DNA from the cell lines 870.1 and 870.10 yielded nearly identical cleavage patterns with several restriction nucleases; only slight differences (faint additional bands, probably reflecting hybridizing flanks) were observed. Since they produce very different levels of RNA[23] they are probably individual clones, although they were isolated from the same culture plate.

In the transformed lymphoid cells correct transcripts and normal κ proteins are made

S1 protection experiments indicated that the transcripts obtained from the transformed lymphoid cell lines were correctly spliced in the small intervening sequence (Fig. 6A). These transcripts, in contrast to those in monkey fibro-

blasts, are correctly initiated as shown by S1 mapping (Fig. 6B).

The production of κ protein chains was studied by in vivo labelling experiments with [^{35}S]-methionine. A fluorograph of the electrophoretically separated proteins is shown in Fig. 7. The host cell line X63Ag8.653 did not produce κ protein chains. The myeloma cell line NS1[26] was taken as a positive control. This cell line does not secrete κ chains, but accumulates moderate levels of κ proteins intracellularly. In comparison to this cell line the transformants produced similar amounts. Since they integrated only one to three κ gene copies the efficiency of transcription seems to be similar to that observed with the κ gene of NS1 cells.

Discussion

Gene transfer systems with lymphoid cells have been developed by several groups using different techniques. Initial difficulties to transfect the X63Ag8.653 cell line by the common gene transfer techniques prompted us to investigate the conditions at which cloned genes could be introduced into this cell line by the electric impulse method. A high yield of transformants was obtained when dense cell suspensions in the presence of high concentrations of plasmid DNA were subjected to electric impulses. At moderate initial field strengths nearly no spontaneous lysis of cells occurred, so that cell lines which poorly tolerate other procedures (e.g. exposure to polyethylene glycol or to a medium containing high concentrations of calcium ions) could be transfected efficiently. Since only few gene copies are taken up by the cells it is possible to study gene expression at a physiologically low gene dosage. In comparison to the calcium phosphate coprecipitation technique[1,16], or the protoplast fusion technique[27], the electric impulse method is very simple and rapid, but it requires a special electric field-generating device (for details see Neumann et al.[21]).

In *non-lymphoid cells* transient expression experiments with κ gene- and SV40-containing plasmids result in the production of easily detectable levels of κ-specific transcripts. These transcripts initiate, however, at promoters of the vector which means that in these cells neither the enhancer in the J_κ-C_κ intron nor the SV40 enhancer can stimulate the κ promoter. After reducing the distance between the SV40 72 bp repeats and the κ promoter no induction of authentic transcripts could be observed either. This may be due to the fact that our plasmid constructs contain an intact SV40 early region and express T antigen after transfection. Therefore dominating SV40 promoters and viral control mechanisms may interfere with an SV40 enhancer action on the κ promoter (e.g. ref.[2,28]). Only low levels of incorrectly initiated κ transcripts were obtained in mouse L cells stably transformed with κ gene-containing plasmids which either contain viral enhancer sequences[4] or lack them.[23] Our conclusion is, therefore, that the immunoglobulin κ gene promoter is inactive in non-lymphoid cells regardless of the transfection assay and the vector system used.

In *lymphoid cells* accurate κ gene transcription was obtained in transient expression systems[9] and in permanently transformed cells[6–8,12]. The high level of expression which we observed was due to only few gene copies introduced into the host cell genome. The transferred κ genes apparently are transcribed at a similar efficiency as the endogenous κ gene in lymphoid cells. The high level of expression is due to a tissue-specific enhancer element in the large intron[11,29], while correct initiation of κ gene transcription is dependent on the presence of certain sequences upstream of the genes[12]. In fibroblasts these sequences do not direct correct κ gene transcription. Thus cell type specificity of immunoglobulin κ gene transcription is characteristic not only of the enhancer in the J_κ-C_κ intron but also of the region upstream of the κ gene. This region contains conserved sequence elements[12,13] which we call dc and pd (Fig. 8). dc- and cd-related sequences are located also in regulatory regions upstream of several prokaryotic and eukaryotic genes, e.g. the catabolite-sensitive genes of *E. coli;* for review see refs.[23,30], and some *Drosophila* heat shock genes[31]. Since not only dc but also pd and enhancer-related sequences occur elsewhere in the genomes of various organisms including mouse (e.g. ref.[12]) it may be the specific location of the three elements around the κ chain gene which is responsible for the cell type specificity of expression.

In the κ light chain genes the dc and pd elements appear to serve a promoter function by directing the correct initiation of transcription. In addition, they resemble enhancer elements in their sequences[12] and in the tissue specificity of their function. In the histone gene system of several organisms a dc element may also possess another property of enhancer elements, i.e. bidirectional action: it is located between H2A and H2B genes which are arranged in opposite polarity in the genomes of several organisms[32,33].

The *regulation* of immunoglobulin gene expression is a complex process; for review see refs.[34,35]. κ gene transcription itself depends on enhancer and promoter sequences including the pd and dc elements. The dc element together with its inverted and complementary form (cd = 5′-ATGCAAATNA-3′) found upstream of immunoglobulin heavy chain genes, may also be involved in the *coordinate expression* of heavy- and light-chain genes[12,13]. dc and cd elements might be recognition sequences for transacting factors such as DNA binding proteins.

It is tempting to speculate that dc and cd elements are involved in the coordinate expression not only of immunoglobulin heavy and light chain genes, but also of other gene pairs or gene clusters. In addition to the histone H2A-H2B genes (see above) two *Drosophila* genes should be mentioned: the 74F gene[36] and the glue protein gene Sgs4[37], which carry on their 5′ sides a dc element and a cd-related element, respectively. Thus dc and cd sequences may be a novel class of regulatory elements contributing to the molecular basis of coordinate expression in certain classes of eukaryotic genes.

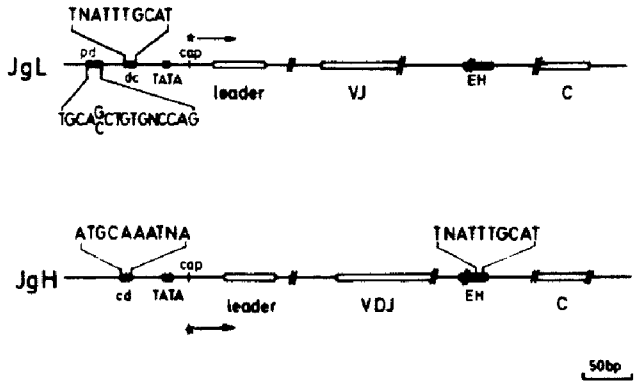

Fig. 8. Schematic presentation of rearranged immunoglobulin light and heavy chain genes (IgL, IgH) with sequence elements, probably involved in the coordination of their transcription.

The immunoglobulin gene segments are shown as open bars. EH is the enhancer region. The asterisk marks the initiation and the arrow the direction of transcription. The pd and dc elements were defined by comparing the 5′ flanks of 12 mouse and human light chain genes (one representative each of the different groups or subgroups). The result is: 5′-NNC$_7$($T_{10}G_{10}C_{11}A_8\overset{G}{C}_5{}_7$-$C_7T_9G_8T_6G_6A_6C_7C_7A_7G_6$)NNN-distance 17 bp in 6 sequences; others vary from 1 to 47 bp – ($T_9G_8A_9T_{12}$-$T_{12}T_{12}G_{12}C_{12}A_{12}T_{12}$)$A_7T_7$NN- distance 80–90 bp to start codon ATG-3′. The subscripts indicate the frequencies of the most abundant nucleotides for every position. The pd and dc elements are enclosed in brackets. N designates positions with less than 50% conservation. The defined sequences are confirmed when all 22 known sequences of 5′ flanks of light chain genes are compared, although the drop-off at the edges of the elements is less pronounced. Pseudogenes are not included. The degree of conservation for the positions was calculated by a computer program (program DNBCGP, developed by P.S. Neumaier). For details, also on the cd element, see Falkner[23].

Materials and Methods

Construction of recombinant plasmids

To construct the plasmids p90H, p160H, p370H p870H and their orientational isomers p90B, p160B and p870B, the 5′-flanking regions of the κ gene T1 were shortened at its *Taq*I, *Hae*III and *Sph*I sites which were converted into *Eco*RI sites[12]. The *Eco*RI-*Bam*HI fragments of the κ gene region were inserted into pBR-322, resulting in the intermediate plasmids p370, p160, and p90 which are analogues to pT1. pT1 contains the full length *Eco*RI-*Bam*HI κ fragments with 870 bp of its upstream region, inserted into pB322[3]. The intermediate plasmids were linearized with *Eco*RI, treated with alkaline phosphatase, and ligated with the SV40 vector fragment. To obtain this fragment a *Hpa*II-*Bam*HI digest of SV40 DNA was incubated with the Klenow fragment of DNA polymerase to fill in the ends and *Eco*RI linkers were added. The linkers were cut with *Eco*RI and the SV40 fragment was purified by agarose gel electrophoresis. The structures of the resulting *Hpa*-series plasmids p90H, p160H, p370H, and p870H and of the *Bam*-series plasmids p90B, p160B, and p870B were confirmed by restriction enzyme analyses.

Cell culture

African green monkey kidney cells (cell line CV1) were cultured in Dulbecco's modified Eagle's medium, containing 10% newborn calf serum and 150 μg/ml glutamine. Mouse lymphoid cell lines (X63Ag8.653, NS1 kindly provided by *H. Saumweber*, Tübingen) were cultured in RPMI 1640 medium supplemented with 10% inactivated fetal calf serum and 150 μg/ml glutamine.

Transfection of lymphoid cells

In a standard electric impulse experiment, 6×10^5 lymphoid cells (log-phase) were suspended in 0.31 ml Hepes-buffered saline (25mM Hepes, 150mM NaCl, 5mM KCl, 1mM Na_2HPO_4, pH 7.2) containing 50 μg/ml dissolved supercoiled plasmid DNA. After 10 min (DNA adsorption onto the cell surface) the suspension was subjected to three successive electric impulses of an initial field strength of 6–7 kV/cm. Since the resistance of the suspension is $R = 220\ \Omega$ and the discharge capacity is $C = 21$ nF, the effective pulse duration is $RC = 4.6$ μs. 10 min after the pulsing (penetration of DNA into the cell) the cells were resuspended in 10 ml of fresh medium. For further technical details see Neumann et al.[21].

The transfection of X63Ag8.653 cells by the calcium phosphate coprecipitation technique with p870-gpt supercoiled plasmid DNA without carrier DNA was performed as described by Chu and Sharp.[16] $1-2 \times 10^7$ cells were suspended in 0.5 ml of a DNA precipitate and incubated for 15 min at room temperature. The suspension was diluted tenfold with fresh medium and incubated for 1–2 h. The medium was then sucked off and replaced by a fresh one. After three days selection was carried out as described below.

Selection

Transfected cells (X63Ag8.653, NS1) were grown for three days without selective pressure in RPMI 1640 medium containing penicillin and streptomycin. Then a selective medium containing 250 μg/ml xanthine, 15 μg/ml hypoxanthine, 150 μg/ml glutamine, antibiotics, and 0.25 μg/ml mycophenolic acid (Lilly Research Labs, Indianapolis) was used for six days. Cells were grown for six days with 0.25 and for another six days with 0.5 μg/ml mycophenolic acid. Further cultivation was done with 2 μg/ml mycophenolic acid. Cell clones were visible within 12 (NS1) to 18 (X63Ag8.653) days. Subcloning was done by micromanipulation after a short trypsin digest of the adherently growing clones.

Analysis of in vivo labelled proteins

1×10^7 myeloma cells were incubated in 1 ml of methionine-free medium (Gibco) without serum but supplemented with 150 μg/ml glutamine. After 30 min the medium was changed and 50 μCi/ml [^{35}S]methionine was added. The cells were incubated for 3 h and resuspended every 15 min. They were then washed once in phosphate buffered saline (PBS). The cell pellet was resuspended in 200 μl of PBS/0.1% NP40 and lysed by four freeze-thaw cycles. Adsorption of the labelled κ chains to protein A-Sepharose, polyacrylamide gel electrophoresis and fluorography were done as described by Falkner and Zachau[3].

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Literature

1 Graham, F.L. & van der Eb, A.J. (1973) *Virology* **52**, 456–467.
2 Banerji, J., Rusconi, S. & Schaffner, W. (1981) *Cell* **27**, 299–308.
3 Falkner, F.G. & Zachau, H.G. (1982) *Nature (London)* **298**, 286–288.
4 Gillies, S.D. & Tonegawa, S. (1983) *Nucleic Acids Res.* **11**, 7981–7997.
5 Picard, D. & Schaffner, W. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 417–421.
6 Rice, D. & Baltimore, D. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7862–7865.
7 Ochi, A., Hawley, R.G., Shulman, M.J. & Hozumi, N. (1983) *Nature (London)* **302**, 340–342.
8 Oi, V., Morrison, S., Herzenberg, L.A. & Berg, P. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 825–829.
9 Stafford, J. & Queen, C. (1983) *Nature (London)* **306**, 77–79.
10 Queen, C. & Baltimore, D. (1983) *Cell* **33**, 741–748.
11 Picard, D. & Schaffner, W. (1984) *Nature (London)* **307**, 80–82.
12 Falkner, F.G. & Zachau, H.G. (1984) *Nature (London)* **310**, 71–74.
13 Parslow, T.G., Blair, D.L., Murphy, W.J. & Granner, D.K. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2650–2654.
14 Steinmetz, M. & Zachau, H.G. (1980) *Nucleic Acids Res.* **8**, 1693–1707.
15 Mulligan, R.C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2072–2076.
16 Chu, G. & Sharp, P.A. (1981) *Gene* **13**, 197–202.
17 Berk, A.J. & Sharp, P.A. (1977) *Cell* **12**, 721–732.
18 Weaver, R.F. & Weissmann, C. (1979) *Nucleic Acids Res.* **7**, 1175–1193.
19 Bodary, S. & Mach, B. (1982) *EMBO J.* **1**, 719–724.

20 Kearney, J.F., Radbruch, A., Liesegang, B. & Rajewsky, K. (1979) *J. Immunol.* **123**, 1548–1550.
21 Neumann, E., Schaefer-Ridder, M., Wang, Y. & Hofschneider, P.H. (1982) *EMBO J.* **1**, 841–845.
22 Sugar, I.P. & Neumann, E. (1984) *Biophys. Chem.* **19**, 211–225.
23 Falkner, F.G., Thesis Universität München 1984.
24 Perry, R.P., Kelley, D.E., Coleclough, C. & Kearney, J.F. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 247–251.
25 Mather, E.L. & Perry, R.P. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4689–4693.
26 Köhler, G., Howe, S.C. & Milstein, C. (1976) *Eur. J. Immunol.* **6**, 292–295.
27 Schaffner, W. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2163–2167.
28 Wasylyk, B., Wasylyk, C., Augereau, P. & Chambon, P. (1983) *Cell* **32**, 503–514.
29 Queen, C. & Stafford, J. (1984) *Mol. Cell. Biol.* **4**, 1042–1049.
30 de Crombrugghe, B., Busby, S. & Buc, H. (1984) in *Biological Regulation and Development* (Goldberger, R.F. & Yamamoto, K.R., eds.) Vol. 3B, pp. 129–167, Plenum Press, New York.
31 Wu, C. (1984) *Nature (London)* **309**, 229–234.
32 Harvey, R.P., Robins, A.J. & Wells, J.R.E. (1982) *Nucleic Acids Res.* **10**, 7851–7863.
33 Hentschel, C.C. & Birnstiel, M.L. (1981) *Cell* **25**, 301–313.
34 Wall, R. & Kuehl, M. (1983) *Annu. Rev. Immunol.* **1**, 393–422.
35 Morrison, S. & Oi, V. (1984) *Annu. Rev. Immunol.* **2**, 239–256.
36 Möritz, Th., Edström, J.E. & Pongs, D. (1984) *EMBO J.* **3**, 289–295.
37 McGinnis, W., Shermoen, A.W., Heemskerk, J. & Beckendorf, S.K. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1063–1067.
38 Altenburger, W., Steinmetz, M. & Zachau, H.G. (1980) *Nature (London)* **287**, 603–607.