The EMBO Journal Vol.2 No.8 pp.1373 – 1378, 1983

Expression and regulation of immunoglobulin heavy chain gene transfected into lymphoid cells

Michael S. Neuberger

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Communicated by César Milstein
Received on 5 May 1983

A plasmid including a mouse immunoglobulin μ gene was transfected into the IgG-secreting human lymphoid line HMy2 and mouse B- and pre-B-cell lines WEHI 231 and 18-81; stably transfected cells were selected. Transfected HMy2 cells synthesized mouse immunoglobulin μ chains as a major secreted protein but the WEHI 231 and 18-81 transfectants transcribed the introduced μ gene at lower levels. In HMy2 transfectants, most of the transcription of the introduced heavy chain gene initiated 40 and 62 bp upstream of the beginning of the V_H exon translation start, although a small proportion of transcripts initiating further upstream was detected. WEHI 231 and 18-81 transfectants gave a much higher proportion of upstream initiation. Transient expression of the V_H exon was monitored following transfection of mouse myeloma with the V_H gene DNA in various plasmid constructs. V_H transcription was only observed if the plasmids contained a segment derived from the large V_H-C_H intron of the immunoglobulin heavy chain locus. This segment, located between J_H and switch regions, functioned both downstream of the V_H exon and upstream in either orientation. The existence of a transcription enhancer element in this region is therefore proposed.

Key words: DNA transfection/immunoglobulin gene expression/transcription enhancer/transcription initiation

Introduction

Little is known about the control of immunoglobulin gene expression. In particular, why is it that unrearranged V genes are very poorly transcribed (Mather and Perry, 1981), whereas following integration with a J_H segment, high level transcription is observed? An attractive model to account for this observation is that DNA rearrangement brings the V_H gene into proximity with some cellular transcription enhancer sequence. It is nevertheless necessary to explain how, on differentiation of a B-cell into a plasma cell, a large increase in the rate of V_H gene transcription results, whereas no concomitant DNA alteration has been observed. In order to study these problems, various plasmids were constructed containing a V_H gene and were introduced into different lymphoid cell lines.

The introduction of immunoglobulin gene DNA into cells should not only allow a study of the regulation of expression of these genes but also open up the possibility of making new antibodies or antibody-based proteins by *in vitro* mutagenesis and DNA transfection.

Results

Plasmid construction

Plasmid pSV-Vμ1 (Figure 1) was used for the stable transfection of lymphoid cells. This plasmid is based on the vector pSV2gpt (Mulligan and Berg, 1981) which contains the *Escherichia coli gpt* gene (encoding xanthine-guanine phosphoribosyl transferase activity) with *gpt* transcription being driven by SV40 signals. Plasmid pSV-Vμ1 was made by inserting a complete V_H-Cμ gene between the *Eco*RI and *Bam*HI sites of pSV2gpt. The V_H segment of plasmid pSV-Vμ1 was put together in two parts using a cDNA clone and a genomic clone isolated from the hybridoma cell line B1-8.δ1; this cell line secretes an IgD, λ_1 antibody with specificity for 4-hydroxyl-3-nitrophenacetyl (NP) (Neuberger and Rajewsky, 1981). The cDNA clone, $pV_{B1\text{-}8}$, includes the V_H region of the B1-8.δ1 heavy chain; the sequence of this cDNA clone indicates an assignment of the beginning of the $C_H\delta$1 exon which differs from the one proposed by Tucker *et al.* (1980) (see Figure 2). The genomic clone, λV_{47}, contains an unrearranged V_H gene homologous to $pV_{B1\text{-}8}$. The V_H segment of pSV-Vμ1 was constructed using λV47 to provide the 5′-flanking sequence and coding sequence up to the *Pst*I site (nucleotide position 490 in Figure 2), whilst cDNA clone $pV_{B1\text{-}8}$ provided the rest of the V_H region from the *Pst*I site through V_H and the D segment to the *Cvn*I site located at the 3′ end of J_H2. This *in vitro* rearranged V_H gene was then joined to a Cμ gene derived from sperm DNA as described in Figure 1. Thus, the sequence of pSV-Vμ1 in the region encoding the secreted portion of the heavy chain variable region is identical to that of the corresponding region of the expressed V_H gene of B1-8.δ1; the pSV-Vμ1 V_H segment is therefore designated V_{NP}.

Stable transfection of lymphoid cells

Fusion with bacterial protoplasts was used to deliver pSV-Vμ1 DNA into cells of the human lymphoid line HMy2. Transformants were selected as described in Materials and methods and, after 3 weeks, HAT-resistant clones had appeared in 12 out of 24 wells. Immunofluorecence analysis revealed that, out of nine wells tested, six wells contained cells of which between 60% and 100% stained brightly in the cytoplasm with a monoclonal anti-mouse μ antibody. Cells from several of these wells positive for mouse μ were cloned and one clone, HMy2-V3, chosen for further study.

Cell line HMy2-V3 secretes mouse immunoglobulin μ chains, in association with the human light chain, as a major secreted protein (Figure 3). This cell line has been maintained in culture for several months and has remained positive for mouse μ production. Analysis of surface-stained HMy2-V3 cells in the fluorescence-activated cell sorter (data not shown) demonstrated that, whilst staining brightly with the monoclonal anti-HLA antibody W6/32, they stained very weakly if at all with anti-mouse μ. Thus HMy2-V3 makes mouse μ chains as a major part of the secreted protein whilst making little if any as membrane IgM.

Transfection of pSV-Vμ1 DNA into the mouse B-cell lymphoma WEHI 231 and the Abelson murine leukaemia virus transformed pre-B-cell line 18-81 was achieved both by protoplast fusion and DEAE-dextran facilitated entry. WEHI 231 gave cell growth in the selective medium in six out of 48 wells following protoplast fusion and four out of 24 wells were positive following incubation with DNA and DEAE-dextran.

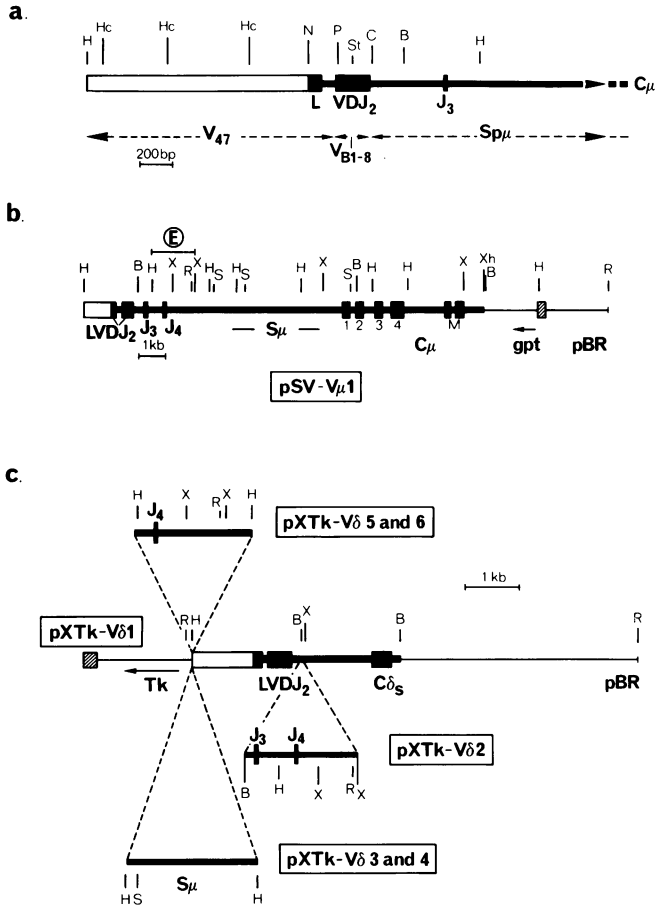

Fig. 1. Structure of plasmids. **(a)** The structure of the V_H gene, V_{NP}, of plasmid pSV-Vμ1. The open box indicates sequences upstream of the initiator codon, filled boxes V_{NP} exons and thick horizontal lines intron sequence of the immunoglobulin heavy chain locus. The segment from the *Hind*III site (H) to the *Pst*I site (P) is derived from λV47, the segment from the *Pst*I site to the *Cvn*I site (C) is from $pV_{B1\text{-}8}$ and all the C_H locus DNA to the right of the *Cvn*I site is from the sperm μ clone ChSpμ27. **(b)** The structure of plasmid pSV-Vμ1 is illustrated, arbitrarily linearized between the *Eco*RI and *Hind*III sites bordering V_{NP}; the thin horiziontal line indicates pSV2gpt vector sequence. The hatched box represents the SV40 *Hind*III-*Pvu*II fragment of pSV2gpt which includes the replication origin, early and late promoters and the 72-bp (SV40 enhancer) repeats. In comparison with the mouse genomic μ gene, pSV-Vμ1 contains a deletion of repetitive μ switch region sequence (Sμ) inherited from ChSpμ27. The region within which the immunoglobulin heavy chain enhancer element is assigned is depicted by the symbol (E). **(c)** pXTk10-based vectors are illustrated, linearized at an *Eco*RI site. The smallest plasmid is pXTk-Vδ1 and pXTk-Vδ2, 3, 4, 5 and 6 contain C_H locus DNA inserted into pXTk-Vδ1 as indicated. The hatched box designates the SV40 origin '*Hind*III'-*Eco*RII fragment which includes the replication origin and early promoter but neither the late promoter nor an intact copy of the 72-bp repeat; the '*Hind*III' site at the edge of this SV40 box has been destroyed (Pelham, 1982). The *Bam*HI site (B) between J_H2 and $C\delta_s$ is the border of J_H region and Cδ region DNA in pXTk-Vδ1, 3, 4, 5 and 6 whereas the *Xba*I site (X) at the right end of the J_H3-J_H4 insert constitutes this order in pXTk-Vδ2. The inserts in pXTk-Vδ3 and 5 are in the orientation shown, whereas pTk-Vδ4 and 6 include these inserts in the opposite orientation. B: *Bam*HI, C: *Cvn*I; H: *Hind*III; Hc: *Hinc*II; N: *Nco*I; P: *Pst*I; R: *Eco*RI; S: *Sac*I; St: *Stu*I; X: *Xba*I and Xh: *Xho*I.

The corresponding figures for 18-81 was growth in four out of 48 wells following protoplast fusion compared with five out of 24 wells after DEAE-dextran treatment. Thus the two procedures are of comparable efficacy in the stable transfection of WEHI 231 and 18-81 cells. pSV-Vμ1 transfected cells WEHI-V4, WEHI-V5 and 18-81-V21 were chosen for further analysis.

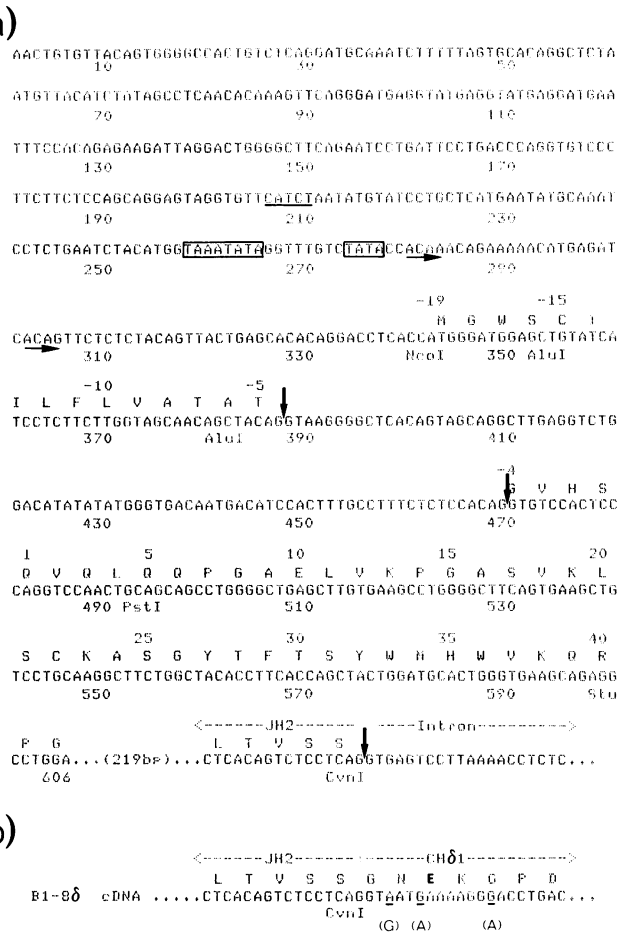

Fig. 2 Sequences of V_{NP} and B1-8.δ1 cDNA clone $pV_{B1\text{-}8}$. **(a)** The sequence of V_{NP} is presented with vertical arrows indicating splice junctions; TATA box-like sequences (boxed), RNA cap sites (horizontal arrows), and a possible upstream element (underlined) are also illustrated. The first three bases of the sequence, AAC, are part of a *Hinc*II site, which is referred to as the *Hinc*II site at position 1. Sequences were determined by the chain terminator method (Sanger *et al.*, 1977). **(b)** Sequence around the J_H2-$C_H\delta1$ junction in B1-8.δ1 cDNA clone, $pV_{B1\text{-}8}$. The sequence through the V, D and J_H2 is the same as that determined for a cDNA clone from the B1-8μ cell line (Bothwell *et al.*, 1981). The sequence of $C_H\delta1$ in the region presented here and for a further 190 bp is the same as that presented by Tucker *et al.* (1980) for BALB/c genomic $C_H\delta1$ except: (i) differences at the positions underlined, which may be due to allotypic differences between the δ genes of C57BL/6 and BALB/c mice [the nucleotides at these positions in the Tucker *et al.* (1980) sequence are given in brackets]; (ii) a different 5′ border of $C_H\delta1$ to that proposed by Tucker *et al.* (1980). The sequence proposed here is in complete agreement with the amino acid sequence of B1-8.δ1 δ chains (Dildrop *et al.*, 1982). This reassignment of the $C_H\delta1$ splice acceptor junction gives a better fit to the canonical splice acceptor sequence and deletes seven amino acids from the Tucker *et al.* (1980) prediction of the $C_H\delta1$ amino acid sequence.

Transcription of the transfected gene

Owing to the fact that untransfected WEHI 231 and 18-81 both express a rearranged μ gene, expression of the pSV-Vμ1 heavy chain gene in the transfected cells was assayed by hybridization with a V_{NP} probe. Nuclease S1 mapping was performed after hybridization of total cytoplasmic RNA from the lymphoid cells with an internally-labelled, single-stranded V_{NP} DNA probe extending from the *Bam*HI site located 197 bp beyond the end of J_H2 in pSV-Vμ1 DNA back to the *Pst*I site located at amino acid position 4 of V_{NP}. Correctly spliced V_{NP}-μ transcripts should protect a 350-base stretch of this 570-base radioactive probe. HMy2-V3, WEHI-

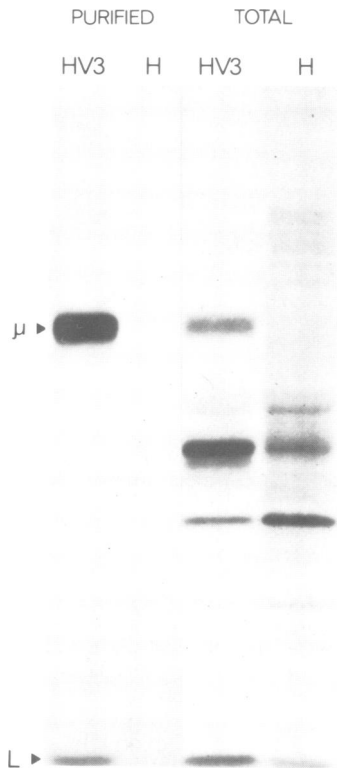

Fig. 3. Immunoglobulin secretion by HMy2-V3. Culture supernatants from HMy2 (H) and HMy2-V3 (HV3) cells incubated (3 h) in medium containing [^{35}S]methionine were reduced and analysed on a 9% SDS/polyacrylamide gel before and after purification on SM1/45 (anti-mouse μ)-Sepharose. The positions of mouse μ and human light chain (L) are indicated.

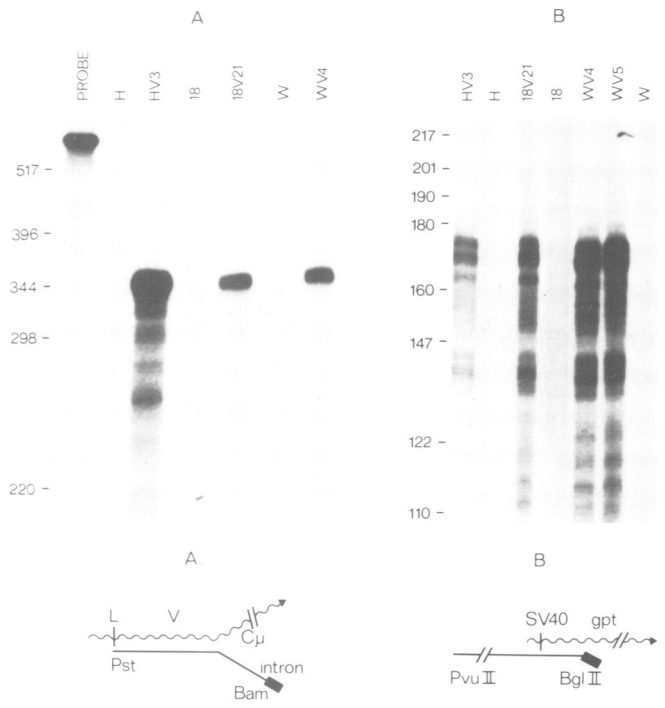

Fig. 4. Determination of V_{NP} and *gpt* transcripts by S1 mapping. (**A**) RNA samples were hybridized with an internally labelled 570-nucleotide probe synthesized from an M13mp9 clone containing a V_{NP} fragment extending from the *Bam*HI site between J_H2 and J_H3 to the *Pst*I site in V_{NP}. A schematic representation of the S1 mapping is given with a wavy line denoting RNA, full line denoting probe and a box depicting M13 sequencing primer and polylinker sequence. 2 μg of HMy2-V3 RNA was used whereas 5 μg of RNA was used from the other cell lines. H, HMy2; W, WEHI 231; and 18, 18-81. (**B**) S1 mapping of *gpt* transcripts with an internally labelled probe made from an M13mp11 clone whose insert extends from the *Bgl*II site in *gpt* to the *Pvu*II site flanking the *gpt*-distal side of the SV40 DNA upstream of *gpt* in pSV2gpt. 5 μg of DNA from each cell line was used. WEHI-V5 (WV5) is a PSV-Vμl transfectant of WEHI 231 that does not synthesize detectable V_{NP} transcripts.

V4 and 18-81-V21 all synthesize correctly spliced RNA hybridizing with the V_{NP} probe, whereas the untransfected parental cell lines do not (Figure 4A). The level of V_{NP}-μ RNA is similar in 18-81-V21 and WEHI V4, but considerably higher (~20-fold) in HMy2-V3.

The level of *gpt* transcription was also monitored by S1 mapping using a probe extending from the *Bgl*II site in *gpt* to a *Pvu*II site located at the end of the late promoter side of the SV40 DNA upstream of the *gpt* gene. Protected fragments in the expected size range of 170 to 180 bp were observed (Figure 4B). Cells from several wells of transfected HMy2, WEHI 231 and 18-81, which grew in selective medium and where were negative for V_{NP} expression, all proved positive for *gpt* transcription. This demonstrates that the failure of these cells to give detectable V_{NP} transcripts is not due to failure in taking up pSV-Vμ1 DNA.

The level of *gpt* transcription was lower in HMy2-V3 than in the transfected WEHI 231 and 18-81 clones. This suggests that the higher level of V_{NP} transcripts detected in HMy2-V3 is not due to higher pSV-Vμ1 DNA copy number, but, rather to a higher rate of transcription. This conforms well to the pattern of immunoglobulin expression in untransfected cells, namely that, whilst pre-B cells and B-cells make similar levels of μ homologous RNA, IgM-secreting plasmatocytomas make ~30–100 times more μ RNA.

Sites of transcription initiation

To locate the start-point of V_{NP} transcription, reverse transcriptase extension assays were performed on a radioactive, 152-base single-stranded V_{NP} restriction fragment primer using HMy2-V3 RNA as the template (Figure 5A). With HMy-2-V3 RNA, but not with RNA from HMy2, elongation of the primer is observed with two major stop-points, yielding fragments of 281 and 259 bp although a trace amount of longer cDNA was detected in the autoradiograph. These fragments correspond to transcription initiation at positions 280 and 302 in the sequence shown in Figure 2, which are 62 bp and 40 bp upstream of the initiator ATG codon. This suggests RNA cap sites of sequence CCACAAA and TCACAGTT (positions 278 and 300), TATA boxes TAAATATA and TATA (positions 258 and 274) and an upstream element CATCT (position 206) which conforms well to the consensus sequence CAATCT which is often found 50–60 bp upstream of the TATA box in eukaryotic promoters (Benoist *et al.*, 1980).

Nuclease S1 mapping experiments were performed to confirm the position of V_{NP} transcript initiation using a DNA probe extending from the *Alu*I site (nucleotide position 315) back to the *Hinc*II site (nucleotide position 1). HMy2-V3 RNA gave protected fragments of 125 and 145 bp as well as a 'ladder' of fragments smaller than 65 bp (Figure 5B). These results suggest transcription initiating or splicing at around positions 207 and 227 in the sequence presented in Figure 2. [The sizes of the S1 protected fragments obtained following hybridization of HMy2-V3 RNA with a probe extending from the *Pst*I site (nucleotide position 490) back to the *Hinc*II site (at position 1) supported this interpretation (data not

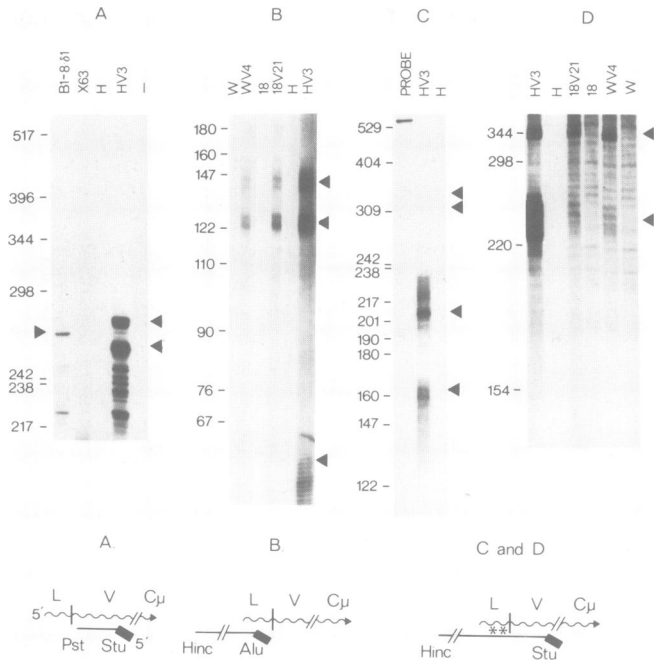

Fig. 5. Determination of V_{NP} transcription initiation point. **(A)** Primer extension. An internally labelled, 152-base primer was synthesized using an M13mp11 clone whose insert extended from the *Stu*I site (amino acid 40) to the *Pst*I site (amino acid 4) in V_{NP}. Reverse transcriptase-catalysed extension of this primer was carried out using total cytoplasmic RNA of B1-8.δ1, X63Ag8 (the fusion partner of hybridoma B1-8.δ1), HMy2 and HMy2-V3 as template and (−) control with no RNA to detect bands caused by self-priming. **(B)** S1 nuclease assays after hybridizing RNA with a 395-base probe made from an M13mp10 clone whose insert extends from the *Alu*I site in V_{NP} (position 352 in the sequence in Figure 2) to the *Hinc*II site (at position 1). **(C)** S1 nuclease assays after hybridizing RNA with a 580-base probe made from an M13mp10 clone whose insert extends from the *Stu*I (amino acid position 40 of the V) to an *Nco*I site (amino acid −19) on DNA of $pV_{B1\text{-}8}$ and continues further from the *Nco*I site on V_{47} DNA to the *Hinc*II site (nucleotide position 1 in Figure 2). **(D)** Exonuclease VII mapping using the same probe as in **(C)**. A schematic representation of these mappings is presented, the same symbols being used as in Figure 4 except that an asterisk indicates a single base mismatch between DNA probe and RNA. Arrows are used in **(A)** to indicate reverse transcriptase major stop-points and in **(B)**, **(C)** and **(D)** to indicate nuclease protected fragments discussed in the text.

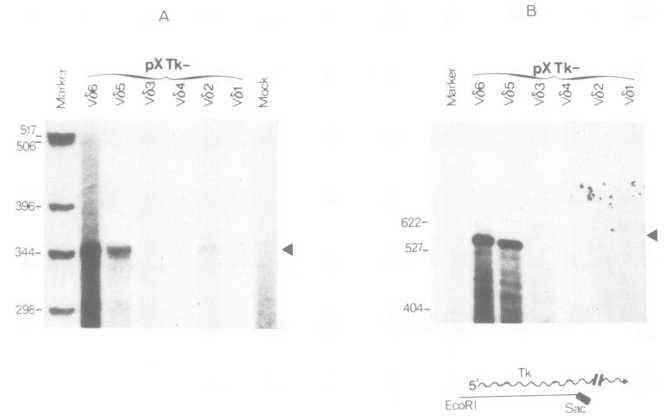

Fig. 6. S1 analysis of V_{NP} and *Tk* transcripts in transiently infected MOPC 315.26 cells. **(A)** Spliced V_{NP} transcripts (arrowed) were measured by S1 mapping using the same 570-base probe as in Figure 4A. RNA samples were from mock-transfected (no DNA) cells and cells transfected with pXTk-Vδ plasmids. This experiment has been repeated three times and in all cases signals are seen with pXTk-Vδ2, 5 and 6 but not, even on long exposures, with pXTk-Vδ1, 3 and 4. **(B)** The same RNA samples as in **(A)** were used for S1 mapping with a 714-base probe made from an M13mp10 clone whose insert extended from the *Sac*I site in *Tk* back to the *Eco*RI site 80 bases upstream of the *Tk* transcription initiation point. The arrow indicates correctly initiated *Tk* transcripts.

shown)]. These data are clearly at variance with the results of the primer extension; a likely explanation for the discrepancy is that although there is a small amount of upstream initiation, most of the V_{NP} transcription in HMy2-V3 starts 62 and 40 bp 5′ of the initiator ATG codon but these transcripts contains too short a stretch of homology with the DNA probe to be detected under the S1 mapping conditions used. To construct a probe containing a greater length of homology with the RNA, an M13 clone was made whose insert started at the *Stu*I site in the V (position 598) and extended back through B1-8.δ1 cDNA clone pV_{B1-8} (thus lacking the leader-V intron) to the *Nco*I site (position 340) and was further extended with V_{47} DNA to the *Hinc*II site at position 1. Due to single base differences between pV_{B1-8} and V_{47} in the leader sequence (positions 362 and 373), there will be two single base mismatches between the DNA probe made from this M13 clone and the V_{NP} transcripts. S1 mapping using this probe (Figure 5C) results in protection of major species in the ranges 215−240 bp (attributable to initiation at positions 280 and 302), 150−165 bp (which can be assigned to cleavage in the mismatch region) as well as minor species of 330 and 310 bp which could be due to the same upstream-initiated transcripts observed in Figure 5B. Use of exonuclease VII, which is a 5′→3′ and 3′→5′ exonuclease but has no endonucleolytic activity, confirmed the assignment of these bands (Figure 5D). The same pattern was obtained as with S1 mapping except for the lack of the 150−165 bp fragments (which were attributed to S1 cleavage in the mismatch region) and the upward shift of the other bands by ~15 nucleotides, consistent with exonuclease VII not digesting right in to the borders of the RNA-DNA hybrids (Chase and Richardson, 1974).

The data in Figure 5 also show that whilst in WEHI-V4 and 18-81-V21 the level of transcription initiating at positions 280 and 302 is low, the level of 'upstream-initiated' transcription in these cells is of a similar order to that in HMy2-V3. The nature of this transcription is discussed below.

Transient expression

Immunofluorescence analysis of MOPC 315.26 mouse myeloma cells 2 days after they had been transfected with pSV-Vμ1 DNA by use of DEAE-dextran revealed that 0.8% of the cells stained brightly in the cytoplasm with monoclonal anti-μ antibody. It was therefore clear that transient expression assays could be used to test for regions of DNA necessary for immunoglobulin gene expression in myeloma cells. A plasmid, pXTk-Vδ1, was constructed in which V_{NP} is linked to the $C\delta_s$ exon (Figure 1); $C\delta_s$ is the 3′ exon of the gene for secreted mouse immunoglobulin δ chains and encodes the carboxy-terminal 21 amino acids and 3′-untranslated region. pXTk-Vδ1 contains no intact copy of the SV40 72-bp transcription enhancer element. Plasmid DNA of pXTk-Vδ1 and its derivatives (Figure 1) was transfected into MOPC 315.26 cells and V_{NP} transcripts assayed in cytoplasmic RNA prepared from these cells 48 h after transfection. No transcripts could be observed using pXTk-Vδ1, but a clear signal due to correctly spliced V_{NP} transcripts was obtained with pXTk-Vδ2 (Figure 6A). pXTk-Vδ2 differs from pXTk-Vδ1 in containing, downstream of V_{NP}, a 2-kb restriction fragment derived from the large intron of the C_H locus. V_{NP} transcripts were also observed following transfection with pXTk-Vδ5 and pXTk-Vδ6; these

plasmids contain a *Hind*III fragment which overlaps the portion of the large C_H intron present in pXTk-Vδ2, but placed upstream (instead of downstream) of V_{NP} in either orientation. No V_{NP} transcripts were detected following infection with pXTk-Vδ3 and pXTk-Vδ4, which contain Sμ switch-region sequences (S_μ) upstream of the V_{NP}.

Assays for thymidine kinase (*Tk*) gene expression showed that *Tk* transcripts could be clearly detected in cells transfected with pXTk-Vδ5 or pXTk-Vδ6. However, transfections with pXTk-Vδ2 gave very low levels of *Tk* transcripts and none could be detected with pXTk-Vδ1, 3 or 4 (Figure 6B). These results define a transcription enhancer element located within a 1600-bp region between the J_H segments and the switch region of the immunoglobulin heavy chain locus as discussed below.

Discussion

Owing to the diversity of V regions expressed by lymphoid cells, there is no panel of cell lines expressing the same immunoglobulin heavy chain gene and representing different stages of lymphoid ontogeny. The work presented confirms that transfection of an immunoglobulin heavy chain gene into different cell lines can yield valuable information on the control of heavy chain gene expression. It is shown here that, by use of DEAE-dextran to facilitate DNA uptake by myeloma cells, transient expression of transfected immunoglobulin gene DNA is achieved in 0.8% of the cell population. This contrasts with a stable transfection frequency of $\sim 10^{-6}$, indicating that the low frequency with which stably transfected clones are isolated is not solely due to inefficient DNA uptake.

Expression of a heavy chain gene in which all sequences 5′ and 3′ of the V_H region are derived from germline DNA has been obtained thus demonstrating that no DNA rearrangements apart from V_H-D-J_H joining are required for immunoglobulin μ heavy chain gene expression.

Major transcription initiation

Transfectants of the IgG-secreting human lymphoid line HMy2 transcribed the introduced mouse V_H gene at high levels, secreting mouse μ chains. Both primer extension and nuclease protection data demonstrated that the major portion (>90%) of V_{NP} transcription in HMy2-V3 starts at positions 62 bp and 40 bp upstream of the initiator ATG codon. Sequences characteristic of eukaryotic promoters are found upstream of these initiation points (Figure 2) and transcription of the B1-8.δ1 V_H gene (which is homologous to V_{NP}) is here shown to initiate in a similar position (Figure 4A). In mouse myeloma, V_H gene transcription has been shown to start 63 bp up from the initiator ATG in HPCM2 (Clarke *et al.*, 1982) and 30 bp from the ATG in MC101 (Kataoka *et al.*, 1982).

Upstream initiation

In the pre-B and B-cell transfectants, as well as in HMy2-V3, V_{NP} transcription starting upstream of the HMy2-V3 major initiation points was observed. The absolute level of this upstream initiation was roughly similar in all three cell types and thus, whilst constituting a minor part of the HMy2-V3 V_{NP} transcripts, upstream initiation accounted for a major proportion in WEHI-V4 and 18-81-V21. The start-point of this upstream initiation cannot at present be assigned. Examination of the sequence presented in Figure 2 shows the presence of a TATA box-like sequence, TAATAT at position 210, which could promote transcription initiation at position 227, although this would mean an unusually short distance between TATA box and RNA cap site. Alternatively, there are several sequences in this region resembling splice acceptor junctions.

It is interesting in this context to note that, on transfection of the V_{NP} gene in various plasmid constructs into fibroblast cells, enhancer-independent transcription is observed which is similar to the upstream initiation in the lymphoid cell transfectants as judged by nuclease mapping (unpublished results). If the upstream-initiated transcripts splice in to positions 207 and 227, then it is clear that these transcripts could originate either somewhere in the plasmid vector or in a 1100-bp region of V_{NP} 5′-flanking sequence included in the plasmid constructs. Thus, either the upstream initiation is a transfection artefact or these observations raise the intriguing possibility that, in pre-B-cell and B-cell, much of the V_H gene transcription originates at a position upstream of the major initiation observed in myeloma.

Transcription enhancer element

The data presented here strongly suggest the existence of a transcription enhancer element located between the J_H region and the μ switch region (S_μ) as depicted in Figure 1. In particular, it has been demonstrated that this region is required for the expression of V_{NP} transcripts in transiently infected mouse myeloma cells and can function at some distance from the V_{NP} promoter, downstream or upstream and in either orientation. It was also observed that if the pXTk-Vδ plasmids used for transfection included the 'immunoglobulin locus enhancer element' in either orientation upstream of the *Tk* gene, then *Tk* transcripts were observed. However, *Tk* transcripts were not detected following transfection with plasmids not containing the putative enhancer element. In pXTk-Vδ2, the 'immunoglobulin locus enhancer element' is separated from the *Tk* promoter by the V_{NP} promoter on the one side and the SV40 early promoter on the other. The weak enhancement of *Tk* transcription observed with pXTk-Vδ2 is thus entirely consistent with the observations of de Villiers *et al.* (1982) who showed that, using the SV40 enhancer, transcription initiated from the enhancer-distal of two tandemly arranged promoters is very weak unless the enhancer-proximal promoter is mutated.

Thus, the postulated immunoglobulin heavy chain locus transcription enhancer element shows the characteristic properties demonstrated for viral enhancer elements (de Villiers *et al.*, 1981; Banerji *et al.*, 1981; Moreau *et al.*, 1981) and suggests that cellular homologues of viral enhancers are indeed implicated in the control of eukaryotic cellular gene expression. The existence of the C_H locus enhancer element would not only explain the high level of expression in myeloma cells of rearranged as opposed to unrearranged V_H genes, but modulation of its activity could also be responsible for differences in the levels of rearranged V_H gene transcription that are observed during B-cell development. The location of the enhancer element is identified in this work as being between a *Hind*III and an *Xba*I site, as depicted in Figure 1; it is, however, clearly likely that the enhancer lies 3′ to J_H4. The enhancer element would therefore be maintained in V_H rearrangements to any of the four J_H segments and would retain its position with respect to the V_H exon following heavy chain class-switching. Deletions extending into the large intron of the C_H locus but which do not abolish immunoglobulin heavy chain expression have been observed (Dunnick *et al.*, 1980;

Köhler *et al.*, 1982); however, none of these deletions removes the region to which the enhancer is assigned.

Materials and methods

DNA and reagents

A cDNA clone, $pV_{B1\text{-}8}$, including the V_H region of hybridoma B1-8.δ1 (Neuberger and Rajewsky, 1981) was constructed by standard procedures and used to isolate a phage λ clone containing an unrearranged V_H gene, λV_{47}, from a genomic library of B1-8.δ1 (unpublished work).

Plasmid pSV-μ was constructed by inserting an *Xho*I- *Bam*HI adapter in the *Bam*HI site of pSV2gpt (Mulligan and Berg, 1981) and then introducing the *Eco*RI-*Xho*I Cμ fragment of ChSpμ27 (Calame *et al.*, 1980) between the *Xho*I and *Eco*RI sites of this plasmid. A rearranged V_H gene, V_{NP}, was assembled using plasmid πVX (gift of B. Seed) as described in Figure 1 and an *Eco*RI fragment of this πXV-based construct introduced into pSV-μ to yield pSV-Vμ1. Plasmid pXTk-Vδ1 is based on vector pXTk10 (Pelham, 1982) and contains the *Hind*III-*Bam*HI fragment of pSV-Vμ1, which includes the rearranged V_H gene, linked to a *Bam*HI-*Bam*HI fragment of phage Ch257.3 (Liu *et al.*, 1980) which contains an exon, $C\delta_s$, encoding the carboxy-terminal 21 amino acids and 3′-untranslated region of the secreted mouse δ chain.

Monoclonal anti-mouse μ antibody SM1/45 was provided by Y. Argon.

Cell lines and transfection

HMy2 (Edwards *et al.*, 1982) and MOPC315.26 (an α-heavy chain-loss variant of mouse meyloma MOPC315; Winberry *et al.*, 1980) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS) and WEHI 231 and 18-81 were grown in the same medium supplemented to 50 μm with 2-mercaptoethanol.

Fusion between HMy2 (2×10^7 washed cells in DMEM, 10% sucrose, 7 mM $MgCl_2$) and bacterial protoplasts [prepared as described by Schaffner (1980) from 50 ml of culture incubated at 37°C for 16 h with 50 μg/ml chloramphenicol at a cell density of 5×10^8/ml] was achieved by pelleting the protoplasts (4000 *g*, 20 min) which had been diluted with 3 volumes of DMEM, 7% sucrose, 10 mM $MgCl_2$ and then centrifuging the HMy2 cells (500 *g*, 10 min) on to the protoplast pellet. Fusion was performed using PEG 1500 and both fusion and selection in HAT medium after placing the cells in two 24-well plates were as described by Galfré and Milstein (1981) except that the selective medium contained gentamycin.

For DEAE-dextran facilitated DNA uptake, 2×10^8 washed WEHI 231 or 18-81 cells were incubated for 20 min in 2.5 ml DMEM containing 40 μg DNA and 200 μg/ml DEAE-dextran (mol. wt. 500 000). After washing, cells were plated in two 24 well plates and selective medium [as described by Mulligan and Berg (1981), except that 8 μg/ml mycophenolic acid (gift from Eli Lilly) was used] applied after 2 days. For transient transfection assays, MOPC315.26 (4×10^6 cells) were incubated for 30 min with 8 μg DNA, 250 μg/ml DEAE-dextran in 1.5 ml DMEM and, after washing, incubated for 2 days in DMEM, 10% FCS.

Analysis of transcripts

RNA was prepared by phenol extraction in the presence of 1% SDS and ethanol precipitation of Nonidet P-40 cell lysates from which nuclei had been pelleted. Single-stranded, internally labelled probes for S1 mapping were prepared using DNA polymerase I Klenow fragment to catalyse extension of the M13 universal sequencing primer on a template of single-stranded DNA of an appropriate M13 clone (~0.5 μg) using [α^{32}P]dATP. After cutting with an appropriate restriction endonuclease, the single-stranded DNA probe was purified from a polyacrylamide-urea sequencing gel. Probes were hybridized with RNA (5 μg) for 6–16 h at 42°C in 50% formamide, 0.5 M NaCl, 40 mM PIPES (pH 6.4), 1 mM EDTA, prior to digestion with nuclease S1 (BRL; 100 units, 30 min 37°C) or exonuclease VII (BRL; 3 units, 6 h, 37°C) (Berk and Sharp, 1978). The same hybridization procedure, except for a shorter time (2 h) was used prior to ethanol precipitation and primer extension by reverse transcriptase. The extension was performed by redissolving the RNA/DNA in reverse transcriptase buffer (Wickens *et al.*, 1978) containing 0.25 mM dNTPs and incubating with reverse transcriptase (4 units) for 1 h at 42°C. The products of S1 mapping and primer extension were analysed on sequencing gels using *Hin*fI and *Hpa*II digests of pBR322 as well as chain terminator sequencing tracks as markers.

Acknowledgements

I thank P. Berg, F. Blattner, L. Hood and H. Pelham for gifts of phage and plasmid clones, G. Grosveld for *Cvn*I endonuclease, Y. Argon for anti-μ antibody and Eli Lilly for mycophenolic acid. I also thank César Milstein for helpful suggestions and for criticising the manuscript.

References

Banerji,J., Rusconi,S. and Schaffner,W. (1981) *Cell*, **27**, 299-308.
Benoist,C., O'Hare,K., Breathnach,R. and Chambon,P. (1980) *Nucleic Acids Res.*, **8**, 127-142.
Berk,A.J. and Sharp,P.A. (1978) *Cell*, **14**, 695-711.
Bothwell,A.L.M. Paskind,M., Reth,M., Imanishi-Kari,T., Rajewsky,K. and Baltimore,D. (1981) *Cell*, **24**, 625-637.
Calame,K., Rogers,J., Early,P., Davis,M., Livant,D., Wall,R. and Hood,L. (1980) *Nature*, **284**, 452-455.
Chase,J.W. and Richardson,C.C. (1974) *J. Biol. Chem.*, **249**, 4553-4561.
Clarke,C., Berenson,J., Goverman,J, Boyer,P.D., Crews,S., Siu,G. and Calame,K. (1982) *Nucleic Acids Res.*, **10**, 7731-7749.
de Villiers,J., Olson,L., Tyndall,C. and Schaffner,W. (1981) *Nucleic Acids Res.*, **10**, 7965-7976.
de Villiers,J., Olson,L., Banerji,J. and Schaffner,W. (1982) *Cold Spring Harbor Symp. Quant. Biol.*, **57**, in press.
Dildrop,R., Brüggemann,M., Radbruch,A., Rajewsky,K. and Beyreuther,K. (1982) *EMBO J.*, **1**, 635-640.
Dunnick,W., Rabbitts,T.H. and Milstein,C. (1980) *Nature*, **286**, 669-675.
Edwards,P.A.W., Smith,C.M., Neville,A.M. and O'Hare,M.J. (1982) *Eur. J.Immunol.*, **12**, 641-648.
Galfré,G. and Milstein,C. (1981) *Methods Enzymol.*, **73**, 1-46.
Kataoka,T., Nikaido,T., Miyata,T., Moriwaki,K. and Honjo,T. (1982) *J. Biol. Chem.*, **257**, 277-285.
Köhler,G., Potash,M.J., Lehrach,H. and Shulman,M.J. (1982) *EMBO J.*, **1**, 555-563.
Liu,C.-P., Tucker,P.W., Mushinski,J.F. and Blattner,F.R. (1980) *Science (Wash.)*, **209**, 1348-1353.
Mather,E.L. and Perry,R. (1981) *Nucleic Acids Res.*, **9**, 6855-6867.
Moreau,P., Hen,R., Wasylyk,R., Everett,R., Glaub,M.P. and Chambon,P. (1981) *Nucleic Acids Res.*, **9**, 6047-6068.
Mulligan,R.C. and Berg,P. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 2072-2076.
Neuberger,M.S. and Rajewsky,K. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 1138-1142.
Pelham,H.R.B. (1982) *Cell*, **30**, 517-528.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.
Schaffner,W. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 2163-2167.
Tucker,P.W., Liu,C.-P., Mushinski,J.F. and Blattner,F.R. (1980) *Science (Wash.)*, **209**, 1353-1360.
Wickens,M.P., Buell,G.N. and Schimke,R.T. (1978) *J. Biol. Chem.*, **253**, 2483-2495.
Winberry,L., Marks,A. and Baumal,R. (1980) *J. Immunol.*, **124**, 1174-1182.