Volume 11 Number 22 1983 Nucleic Acids Research

Expression of cloned immunoglobulin genes introduced into mouse L cells

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Received 23 May 1983; Revised and Accepted 18 October 1983

ABSTRACT

Functionally rearranged immunoglobulin heavy-chain (γ_{2b}) and light-chain (λ_1 and κ) genes were introduced into mouse L tk^- cells by co-transformation with the Herpes virus tk gene. Cloned cell lines were selected in HAT medium and tested for the presence of transfected immunoglobulin gene sequences by Southern blotting analysis. It was found that the γ_{2b} gene was accurately transcribed at a low level in transfected mouse L cells and cytoplasmic γ_{2b} heavy-chain protein was detected by immunoprecipitation of cell extracts. Light-chain genes, on the other hand, were not accurately transcribed. Instead, λ_1 or κ RNA species were detected which were approximately 200 to 300 bases longer than the authentic mRNAs. These results suggest that the expression of rearranged heavy-chain and light-chain genes are controlled differently and that these differences can be seen in transfected, non-lymphoid cells.

INTRODUCTION

Immunoglobulin genes require one or more somatic recombination events for the creation of a complete, active transcription unit (1). For light chains, a variable (V) gene segment recombines via a joining (J) segment to the 5'-flanking sequence of the constant (C) region producing a functional VJ-C gene. Heavy-chain gene rearrangement involves multiple steps: the joining of a diversity (D) segment to a J segment prior to VDJ-C joining; and switch-recombination which results in the expression of the same VDJ sequences with different subclasses of heavy-chain constant genes.

While the structure of the DNA sequences for both light-chain and heavy-chain genes has been analyzed in detail, before and after recombination, little is known about how such rearrangements lead to the activation of transcription. Studies with light-chain genes have shown that unrearranged V_κ gene

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segments are not transcribed in lymphoid cells (2), although they have been shown to have active transcriptional promoters when assayed _in vitro_ or in _Xenopus_ oocytes (3). Thus it appears that the joining of sequences from the _C_ region of light-chain (and presumably heavy-chain) genes, activates the otherwise silent _V_ gene promoters.

One method of testing whether rearrangement of immunoglobulin genes alone is sufficient for their expression is to introduce a cloned light-chain or heavy-chain gene from an antibody-secreting myeloma into a non-lymphoid cell type. We have stably transformed mouse L cells with functionally rearranged genes coding for λ_1 or κ light chains, or γ_{2b} heavy chains in order to test whether these genes can be expressed in a non-lymphoid cell. Results indicate that there is a marked difference in the expression of light-chain and heavy-chain genes in these cells.

MATERIALS AND METHODS

Plasmid Constructions. Plasmids ppTL and ppLγ_{2b}VC, containing the Herpes virus thymidine kinase (tk) and rearranged γ2b heavy-chain genes respectively, were constructed as follows. A parental plasmid, ppL, was first constructed by ligating a 2.4 kb BamHI-EcoRI fragment of plasmid pBRd [a derivative of pBR322 not containing the so called "poison" sequences (4)] to a 2.3 kb BamHI-EcoRI fragment of plasmid pMLVTK (5). This latter fragment contains the 3' terminal end of an integrated Moloney leukemia virus including the 3' long terminal repeat (LTR) sequence and 0.7 kb of flanking rat sequences (5).

Plasmids ppTL and ppLγ_{2b}VC were then constructed by inserting the 3.4 kb BamHI (tk gene) fragment or the 9 kb Bgl II (γ_{2b} gene) fragment from phage clone M141-p21 into the BamHI site of plasmid ppL (see Figure 1). Plasmids ppLλ_1VC and ppLκVC were constructed by inserting the 7.4 kb EcoRI fragment from phage clone Ig303 (λ_1) and a 5.6 kb EcoRI fragment from phage clone IgLPC-1 (κ) into the EcoRI site of plasmid ppL (see Figure 6). Plasmid ppγ_{2b}VC (derivative of ppLγ_{2b}VC not containing the LTR) was constructed by first removing the 1.2 kb Kpn I from plasmid ppL (see Figure 1) and then inserting the 9 kb Bgl II fragment

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(γ_{2b} gene) into the BamHI site. The orientation of the γ_{2b} gene was the same as in plasmid ppLγ_{2b}VC.

Cell Culture and Transformation. Mouse Ltk$^-$ aprt$^-$ cells (6), obtained from R. Axel, were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Co-transformation of the γ_{2b} heavy-chain gene and the Herpes virus tk gene was carried out as described (6) using 5 µg of ppLγ_{2b}VC plasmid DNA and 50 ng of ppTL plasmid DNA per ml of calcium phosphate precipitate. Colonies surviving in HAT medium (hypoxanthine, 15 µg/ml; aminopterin, 0.2 µg/ml; and thymidine, 5 µg/ml) were isolated and grown in mass culture for analysis of DNA and RNA.

Analysis of DNA and RNA. High molecular weight cellular DNA was prepared and analyzed by Southern blotting as described (7). Total cellular RNA was prepared by the guanidinium isothiocyanate-CsCl method (8) and enriched for mRNA by oligo (dT)-cellulose chromatography (9). Analysis of RNA by Northern blotting was carried out by electrophoresis on denaturing formaldehyde-agarose gels and transfer to nitrocellulose (10).

The 5' termini of γ_{2b} mRNAs were mapped by primer extension, essentially as described by Ghosh et al. (11). A 55 bp Ava II-Hinf I fragment (primer) was labeled at the 5' end of the anti-message strand by first labeling Hinf I digested DNA with T4 polynucleotide kinase and [γ-^{32}P] ATP (12) followed by restriction with Ava II. Gel-purified primer (20 ng) was mixed with either 0.5 µg of poly A(+) RNA or with 20 µg of total cellular RNA and denatured at 85° in 20 µl of 80% formamide, 0.4 M NaCl, 20 mM Pipes (pH 6.4), 1 mM EDTA for 10 minutes. After hybridization at 52° for 16 hr, DNA-RNA hybrids were dissolved in 300 µl of cold 0.3 M sodium acetate, precipitated with ethanol and suspended in 40 µl of 50 mM Tris, pH 8.3; 60 mM NaCl; 6 mM $MgCl_2$; 10 mM DTT. Reverse tran°scriptase (5U) and unlabeled deoxyribonucleotides (1 mM) were added and the reaction mixtures were incubated at 41° for 1 hr. Ethanol precipitated extension products were then analyzed on 8% sequencing gels (13).

Analysis of Proteins. Biosynthetic labeling of L cells (2×10^6 cells in 60 mm dishes) was carried out in growth medium without methionine and including ^{35}S-methionine at 50 µCi/ml. At the end of the labeling period (2 hr), cells were rinsed twice

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and scraped into PBS, pelleted by centrifugation and suspended in 2 ml of PBS containing 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, and 5 mM EDTA. After 15 minutes on ice, nuclei were removed by centrifugation. Immunoprecipitation was carried out with goat anti-mouse IgG (Fc fragment) antibody (Cappell Laboratories) and Protein A-sepharose beads (14) and proteins were analyzed by electrophoresis (15).

RESULTS

Transfection. The functionally rearranged γ_{2b} heavy-chain gene from myeloma MOPC 141 was originally cloned from a partial EcoRI library into λ phage (16). A large segment of this DNA clone, including the variable (V) region, the 3.5 kb intron between the V and C gene segments, and that portion of the C gene segments coding for the secretary form of the γ_{2b} heavy chain, was subcloned into a plasmid vector containing a retroviral long terminal repeat (LTR) sequence. The purpose of including these viral DNA sequences was to test for a possible enhancing effect on immunoglobulin gene expression by the LTR 72 bp repeat (17). The orientation of the LTR transcriptional promoter is the same as that of the γ_{2b} heavy-chain gene but is located more than 8 kb downstream. Approximately 2.2 kb of γ_{2b} gene 5' flanking sequences and 0.9 kb of 3' flanking sequences are also present in plasmid ppLγ_{2b}VC (Figure 1).

Mouse L tk^- cells were co-transfected with plasmids ppLγ_{2b}VC and ppTL (a plasmid containing the selectable Herpes virus tk gene) using the calcium phosphate precipitation method and tk^+ transformants were isolated and analyzed for the presence of γ_{2b} heavy-chain gene sequences. The hybridization probe used for Southern blotting analysis was the 9 kb Bgl II fragment used to construct plasmid ppLγ_{2b}VC. As expected, this probe detects the homologous unrearranged V_H gene segment, several related (cross-hybridizing) V_H gene sequences and the unrearranged $C\gamma_{2b}$ gene segments present in both the parental L cell and transformed cell lines. Nonetheless, when cellular DNA was digested with EcoRI, transfected γ_{2b} gene sequences (Figure 2, 7.6 and 6.3 kb bands) could be detected above the background of endogenous sequences with a short exposure of the autoradiogram.

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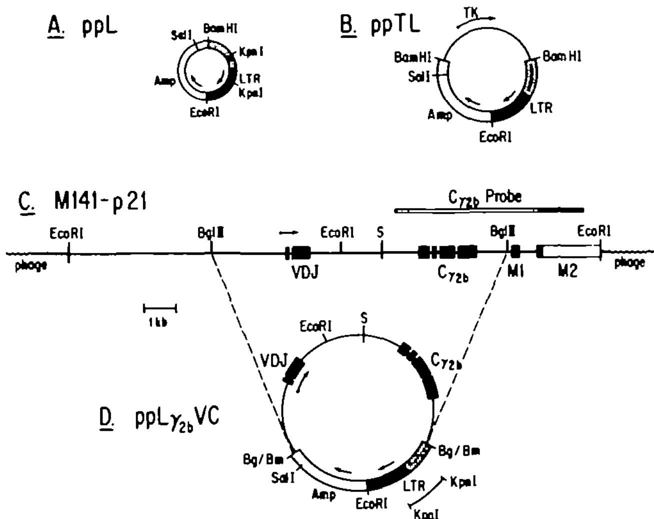

Figure 1
Restriction maps of plasmids used for transfection.
A) Parental plasmid ppL containing sequences from the "poisonless" pBR322 derivative, pBRd (open bars), and plasmid pMLVTK (7). The black bar indicates the retroviral long terminal repeat (LTR) sequence, the hatched bar indicates flanking rat sequences from the integrated provirus, and the stippled bar, the 3' end of Moloney leukemia virus. The direction of transcription from the ampicillin and LTR transcriptional promoters is indicated by arrows.
B) Plasmid ppTL containing the 3.4 kb BamHI (tk gene) fragment from Herpes virus cloned in the BamHI site of plasmid ppL. The direction of transcription of the tk gene is indicated by an arrow.
C) Partial restriction map of phage clone M141-p21, containing the complete γ_{2b} gene. *Only those Bgl II sites used for* subcloning the γ_{2b} gene are shown. Coding regions, identified by R-loop mapping or sequence analysis (30, 31) are shown as filled boxes. The open box indicates the 3' untranslated segment of the M2 exon as determined by Rogers et al. (32). The switch recombination site (S), the direction of γ_{2b} mRNA transcription (arrow), and those sequences used as a probe for RNA blotting ($C\gamma_{2b}$ probe) are also shown.
D) Plasmid ppLγ_{2b}VC containing the 9kb Bgl II fragment from M141-p21 cloned in the BamHI site of plasmid ppL (Bg/Bm indicates the fused restriction sites). The segment indicated by a bar has been deleted in plasmid ppγ_{2b}VC.

Three out of four tk^+ clones were found to contain ppLγ_{2b}VC DNA ranging from one copy per cell (clone G2) to as many as 30 (clone G3). Those clones with multiple copies of the plasmid (G3 and

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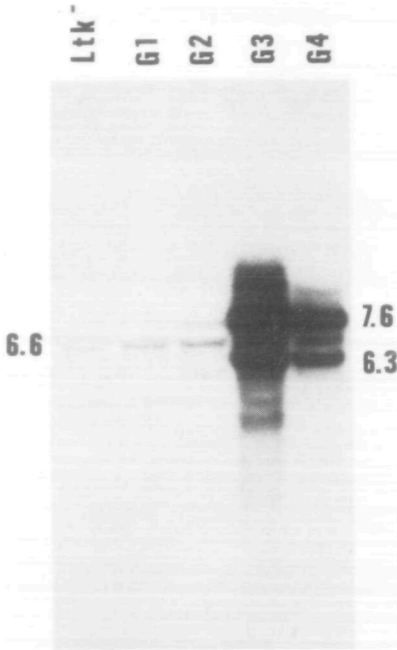

Figure 2
Southern blotting analysis of DNA from transfected L cells. DNA from four tk^+ cell lines, co-transfected with plasmid ppLγ_{2b}-VC and ppTL, was digested with EcoRI, electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose, and hybridized to nick-translated γ_{2b} gene insert (9 kb Bgl II fragment). Numbers on the right indicate the positions of the 7.6 kb and 6.3 kb EcoRI fragments of plasmid ppLγ_{2b}VC. The position of the endogenous Cγ_{2b} gene fragment (6.6 kb) is indicated on the left.

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G4) appear to contain tandem repeat structures, as determined by additional restriction analyses (not shown). This appears to be the result of transfecting with circular plasmid [see for example, Canaani and Berg (18)] and also occurs with plasmid vector DNA (not shown). In the clone containing a single copy of the plasmid (G2), the smaller plasmid fragment migrated faster than the 6.3 kb band and likely represents the junction fragment with cellular DNA or with the co-transfected ppTL plasmid DNA.

Expression of the γ_{2b} heavy-chain gene. Poly A(+) mRNA was prepared from the G2, G3 and G4 cell lines and analyzed by Northern blotting and hybridization with a constant region (Cγ_{2b}) probe. All three cell lines were found to produce mRNA containing γ_{2b} heavy-chain sequences which comigrated with the secretory heavy-chain mRNA from MOPC 141 myeloma cells (Figure 3). The amount of γ_{2b} mRNA in the individual cell lines was roughly proportional to the number of gene copies per cell but the level of expression (i.e. the level of stable mRNA) was at least two orders of magnitude less per gene copy than that in the myeloma. This estimate is based on the fact that clone G2 and

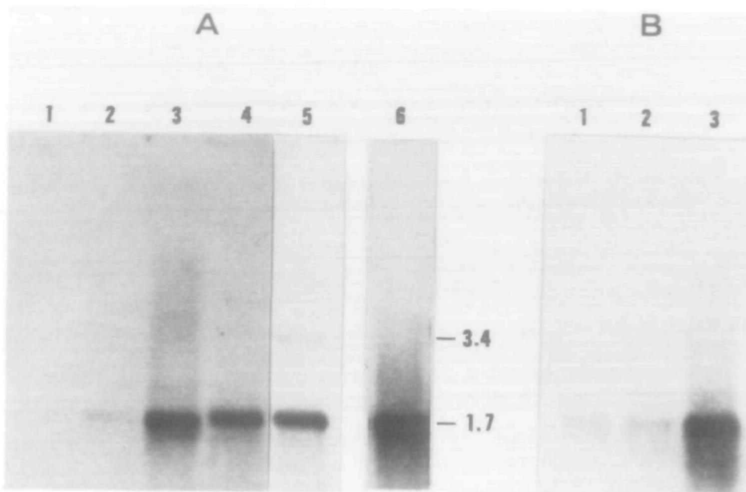

Figure 3
Analysis of γ_{2b} mRNA in transfected L cell lines. Total cell polyA(+) RNA (approximately 0.4 µg per lane) was electrophoresed on a 1% agarose-formaldehyde gel, transferred to nitrocellulose, and hybridized with nick-translated $C\gamma_{2b}$ probe (see Figure 1). (A) RNA from lines Ltk⁻(lane 1), G2 (lane 2), G3 (lane 3), and G4 (lane 4) were run together with total RNA (5 µg) from myeloma MOPC 141 (lane 5). Lane 6 shows a longer exposure of lane 2. The molecular weights of the membrane form (3.4 kb) and secreted form (1.7 kb) of γ_{2b} mRNA are indicated.
B) RNA from lines containing plasmid ppLγ_{2b}VC or ppγ_{2b}VC were compared by Northern gel analysis as in (A). Lane 1, G2 RNA (one copy of ppLγ_{2b}VC per cell); lane 2, G11 RNA (one copy of ppγ_{2b}VC per cell); lane 3, G13 RNA (5 to 10 copies of ppγ_{2b}VC per cell).

MOPC 141 cells contain equivalent amounts of $\gamma 2_b$ mRNA in 0.5 µg of poly A(+) RNA and 5 µg of total RNA, respectively (Figure 3, lanes 4 and 5). Since 5 µg of total RNA contain 50 to 100 ng of poly A(+) RNA, this represents a 5 to 10-fold difference in the amount of γ2b mRNA per cell or a 100-fold difference per gene copy. These results demonstrate that the promoter of a rearranged γ_{2b} heavy-chain gene can be recognized in mouse fibroblasts and that accurate transcription and splicing can occur to produce a stable mRNA.

We next tested whether the expression of the γ_{2b} gene was affected by the enhancer sequence contained in the LTR. L cell clones containing integrated plasmid ppγ_{2b}VC (a derivative lacking most of the LTR sequence--Figure 1) were isolated and

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tested for the expression of γ_{2b} mRNA. As seen in Figure 3B, γ_{2b} mRNA was synthesized in the absence of the LTR enhancer. Furthermore, comparable levels of stable mRNA were found in cell clones containing similar copy numbers of ppLγ_{2b} or ppγ_{2b}VC.

Analysis of the 5' end of γ_{2b} mRNA. In order to more accurately compare the γ_{2b} mRNAs from MOPC 141 and transfected L cells, the 5' termini were analyzed by the primer-extension method. A 55 bp primer from the V region exon was prepared by labeling a 60 bp Hinf I fragment with γ-^{32}P-ATP and polynucleotide kinase and digesting with Ava II. This results in a 55 bp fragment labeled at the 5' terminus of the anti-message strand (see Figure 4). When the 55 bp fragment was hybridized to RNA from the transfected L-cell clone G3 or MOPC 141 myeloma cells and extended with reverse transcriptase, a predominant extension product of 167 bp was detected in both cases (Figure 4, lanes 2 and 3). No such extension product was detected when Ltk$^-$ cell RNA was used (lane 1). The 167 bp extension product most likely corresponds to a cDNA extended from the V gene segment primer, across the splice junction between the leader sequence and V sequence exons, to a mRNA start site approximately 30 bp upstream of the leader exon coding sequence (see Figure 4). No cDNA extension products corresponding to unspliced transcripts (248 nt) were detected. We conclude that the γ_{2b} mRNA produced in L cells is identical to that produced in myeloma MOPC 141 with respect to the length of the 5' terminus and splicing of the first two exons.

These results are in agreement with the mapping data of Kataoka et al. (19) who located the start site of the γ_1 mRNA from myeloma MC101 at 30 bp from the leader exon. This functionally rearranged V_H gene segment shares extensive sequence homology with the V_H gene segment in plasmid ppLγ_{2b}VC, differing only at 10 bp out of 120 bp upstream of the leader exon (19).

Detection of γ_{2b} heavy-chain protein. Transfected L cell clones were permeabilized and stained with fluorosceinated antibody specific for the constant region of mouse IgG. Clones G3 and G4 showed intense cytoplasmic staining in all cells indicating that γ_{2b} heavy chain was synthesized in these cell lines. Clone G2, containing only a single copy of plasmid ppLγ_{2b}VC did not show

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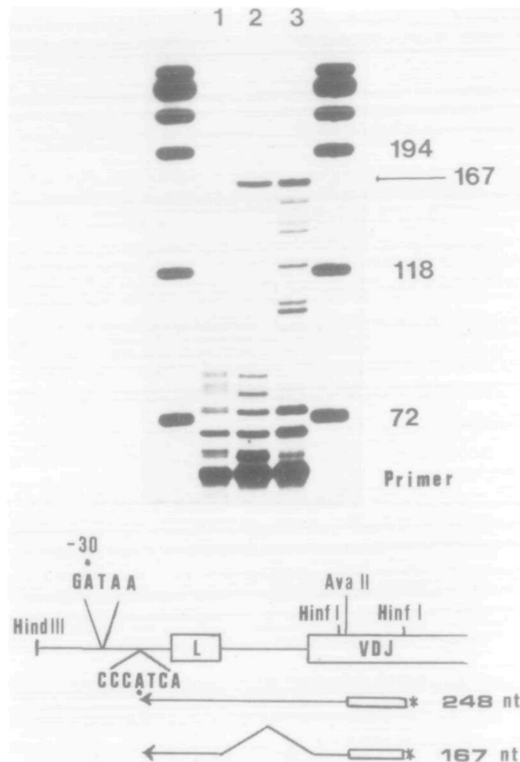

Figure 4
Analysis of the 5'-termini of γ_{2b} mRNA from a transfected L cell line and MOPC 141. Primer-extension reactions (see Methods) contained end-labeled probe hybridized to RNA from Ltk$^-$ cells (lane 1), transfected line G3 (lane 2), or MOPC 141 myeloma cells (lane 3). Extension products were analyzed on an 8% sequencing gel with end-labeled Hae III restriction fragments of ϕX-174 as markers. The major extension product is indicated with an arrow. A schematic representation of the experiment is shown. Open boxes show the leader (L) and VDJ exons of the γ_{2b} gene from MOPC 141. The predicted cDNA products from spliced and unspliced γ_{2b} mRNAs are shown below. The start site of the γ_{2b} mRNA from MOPC 141 was predicted from the data of Kataoka et al. (19) who mapped the start site of the closely related V_H gene from myeloma MC101 to the A residue indicated (see text).

detectable fluorescence (data not shown).

The nature of the protein synthesized in the G3 and G4 clones was examined further by biosynthetic labeling with ^{35}S-methionine, immunoprecipitation with anti-heavy chain antibody and electrophoretic analysis. The major protein

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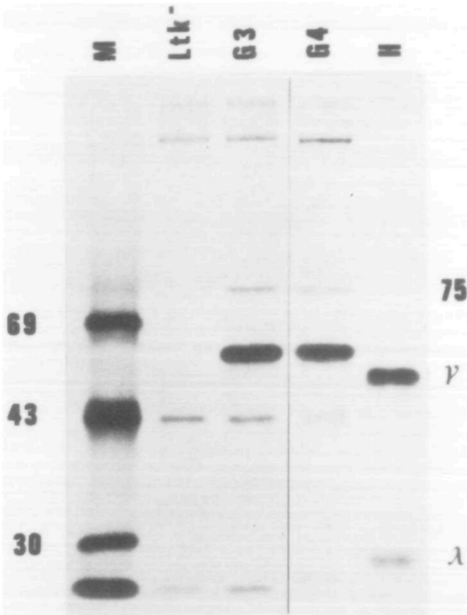

Figure 5
Analysis of immunoprecipitates from ^{35}S-methionine labeled extracts of Ltk⁻ cells and transfected lines G3 and G4. Cell extracts were labeled for 2 hr. as described in Methods, immunoprecipitated with antisera specific for the Fc portion of mouse IgG, and analyzed on an 8.5% polyacrylamide gel under reducing conditions. Molecular weight markers (M) and immuno-precipitated ^{35}S-methionine labeled proteins from a γ_{2b}-producing hybridoma (H) were run as standards. The molecular weight (75 K) of a minor protein detected in lines G3 and G4, and the positions of the γ_{2b} and λ_1 chains synthesized by the hybridoma are indicated on the right.

detected in immunoprecipitates migrated more slowly than marker γ_{2b} heavy chain from a γ_{2b} producing hybridoma (Figure 5), however subsequent studies (20) have shown that a protein with this electrophoretic mobility is synthesized in myeloma cells transfected with the same cloned γ_{2b} gene. No such protein was detected in the parental Ltk⁻ cell line (Figure 5). Thus we conclude that the γ_{2b} heavy-chain is accurately translated in mouse L cells. The difference between the MOPC141 and hybridoma γ_{2b} proteins is not known but may be the result of an additional glycosylation site in the MOPC141 heavy chain.

A second minor protein with a molecular weight of 75K was also detected in the G3 and G4 cell lines and was reproducibly immunoprecipitated with γ_{2b} heavy-chain protein from these cells (Figure 5). Since the γ_{2b} gene introduced into these cells does not contain the M1 and M2 (membrane) exons (see Figure 1), it is unlikely that the 75K protein represents the membrane form of γ_{2b} heavy chain. Instead, this protein may have an affinity for intracellular heavy chains when they are not complexed with light chains. Such a protein has been described in myeloma and hybridoma variants that make only heavy chains (21, 22).

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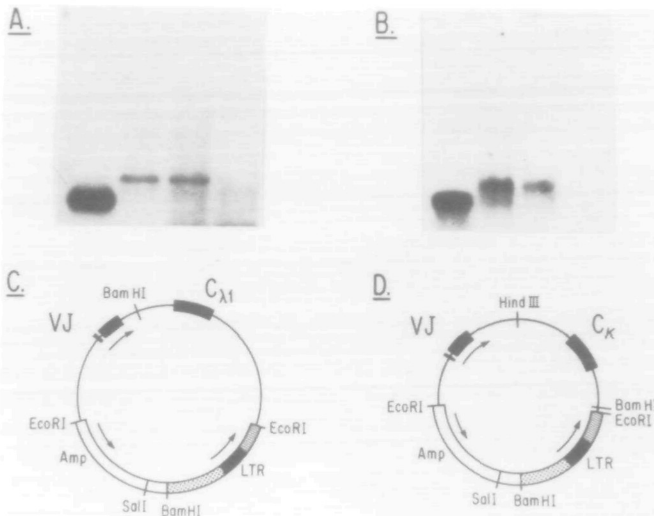

Figure 6
Expression of light-chain RNA in L cell clones transfected with plasmids ppLλ_1VC or ppLκVC. Total cell poly A(+) RNA was analyzed as in Figure 3. (A) Northern blotting analysis of RNA from two lines containing one copy (lane 2) or several copies (> 10, lane 3) of plasmid ppLλ_1VC. Lane 1, λ1 RNA from H2020 myeloma cells; lane 4, RNA from Ltk cells. (B) Northern blotting analysis of RNA from two lines containing one copy (lane 2 or several copies (5 to 10, lane 3) of plasmid ppLκVC. Lane 1, total RNA from myeloma LPC-1; lane, RNA from Ltk cells. (C) Partial restriction map of plasmid ppLλ_1VC. The 7.4 kb EcoRI fragment from phage clone Ig303 [originally cloned from the H2020 myeloma (33)] was subcloned in the EcoRI site of plasmid ppL (Figure 1). (D) Partial restriction map of plasmid ppLκVC. A 5.6 kb EcoRI - BamHI fragment from phage clone IgLPC-1 [cloned from the LPC-1 myeloma (Tonegawa, unpublished)] was converted to an EcoRI-EcoRI fragment by first subcloning in the synthetic polylinker of plasmid pBRX-18 (B. Seed, personal communication). The EcoRI fragment was then subcloned in the EcoRI site of plasmid ppL.

Expression of Light Chain Genes. Functionally rearranged genes for both λ_1 and κ light-chain proteins were subcloned in plasmid ppL and introduced into Ltk$^-$ cells as just described for the γ_{2b} gene. In contrast to the results with transfected heavy-chain genes, neither of the light-chain genes was accurately transcribed in L cells. As seen in Figure 6, λ_1 and κ poly A(+) RNAs of discrete sizes were synthesized at low levels in transfected cells. In both cases, however, the RNA species

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from transfected cells were 200 to 300 bases longer than the authentic λ_1 or κ mRNAs from H2020 (λ_1 producer) or LPC-1 (κ-producer) myeloma cells. The amount of stable λ_1 or κ RNA in the individual clones of L cells was found to be independent of gene copy number, again contrasting with the results with the γ_{2b} gene. The same results were obtained when the light-chain genes were cloned in plasmid ppL at the same site (after converting the BamHI site to an EcoRI site with synthetic linkers) and in the same orientation as the γ_{2b} gene of plasmid ppLγ_{2b}VC (not shown).

The nature of the additional sequences in the λ_1 RNA transcripts was examined by S1 nuclease protection using two single-stranded DNA probes. The first probe spans the λ_1 mRNA splice junction between the leader and VJ exons (splice probe) and the second spans the λ_1 mRNA major start site (5' end probe) utilized in H2020 myeloma cells (Figure 7A). Authentic λ_1 mRNA from H2020 myeloma cells protected fragments of 126 nt (Figure 7B, lane 3) and 80 nt (lane 9) for the splice probe and 5' end probe, respectively. The same fragments were protected by RNA from an X-63Ag8 cell line transfected with the λ_1 gene (lanes 5 and 11).

In contrast, transfected L cell RNA protected the 126 nt fragment corresponding to the VJ splice junction (lane 2) but did not protect any of the 5' end probe (lane 8). These results indicate that the λ_1 RNA produced in transfected L cells is initiated upstream of the normal mRNA start site and is spliced to the acceptor site of the VJ exon. This model is in complete agreement with the results of Picard and Schaffner (23).

The relative amounts of λ_1 mRNA per cell can also be estimated from the data in Figure 7. If we assume that the λ_1 mRNA from H2020 cells is 50% pure, a comparison of band intensities in Figure 7B indicates that the transfected L cell line contains about 2 ng of λ_1 RNA per 5 µg of poly A(+) RNA while the transfected X-63 Ag8 cell line contains about 10 ng of λ_1 per 10 µg of total RNA (or 0.1 to 0.2 µg of poly A(+)RNA). This represents a difference in the level of expression of from 125 to 250-fold between the two cell types.

Since the κ RNA made in transfected L cells (this study) is

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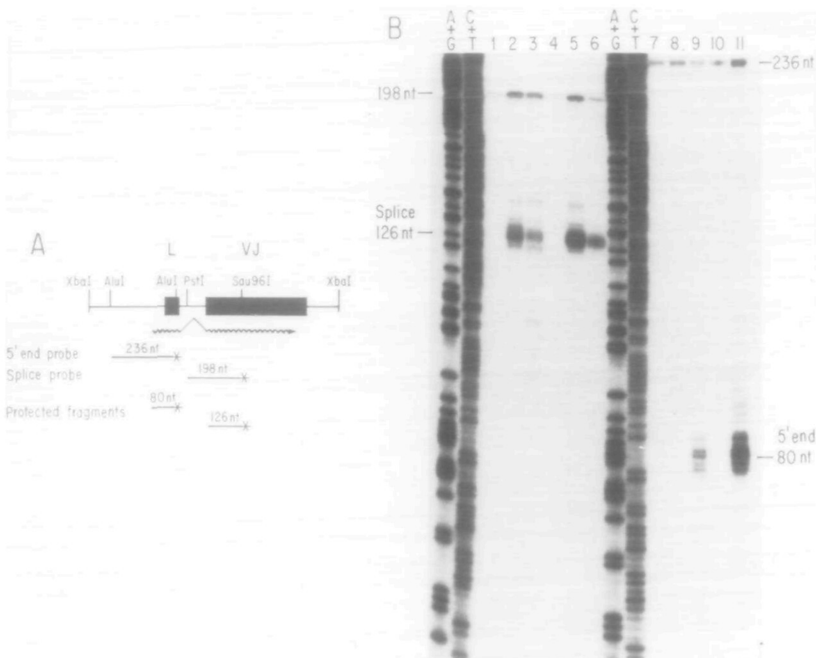

Figure 7
S1 nuclease analysis (34) of λ_1 RNA transcripts in transfected L cells and myeloma cells. (A) A schematic diagram shows the end-labeled, single-stranded probes used to detect the 5' terminus or the splice acceptor site of the VJ exon. The λ_1 mRNA major start site in H2020 myeloma cells was determined by Picard and Schaffner (23). (B) RNA was hybridized to the splice probe (lanes 1-6) or the 5' end probe (lanes 7-11), treated with S1 nuclease and analyzed on an 8% sequencing gel together with A+G and C+T sequencing reactions (size markers). Lanes 1 and 7, 5 µg of poly A(+) RNA from L cells; lanes 2 and 8, 5 µg of polyA(+) RNA from L cells transfected with plasmid ppLλVC; lanes 3 and 9, 2 ng of purified λ_1 mRNA from H2020 myeloma cells; lanes 4 and 10, 10 µg total RNA from X-63Ag8 myeloma cells; lanes 5 and 11, 10 µg total RNA from X-63Ag8 cells transfected with plasmid pSV-λ_1VC; lane 6, same as 5 but 1/5 of the sample was analyzed. Plasmid pSV-λ_1VC was constructed by inserting the 7.4 kb EcoRI fragment (λ_1 gene) into plasmid pSV2gpt (35). Myeloma X-63Ag8 cells were stably transfected as described (20).

also about 300 bases longer than authentic κ mRNA, it is likely that this RNA is also initiated upstream of the normal mRNA start site and spliced to the V_κ exon. This may be a property of light-chain genes when introduced into non-lymphoid cells.

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Apparently the 72 bp repeat sequence contained in the LTR (17) of plasmid ppL is unable to enhance λ_1 or κ mRNA synthesis in the plasmid constructions used in this study. Since this repeat sequence has been shown to be more active as an enhancer in mouse cells than the SV40 repeat sequence (24), we conclude that the lack of an enhancing effect on λ_1, κ and γ_{2b} transcription is due to the distance from the immunoglobulin mRNA start site.

DISCUSSION

We have examined the expression of both light-chain and heavy-chain immunoglobulin genes in a non-lymphoid cell type. Two different types of rearranged light-chain genes, κ and λ_1, were found to behave similarly in stably transformed L cells: the level of expression was independent of the gene copy number and the major poly A(+) RNAs produced were at least 200 bases longer than the authentic κ or λ_1 mRNAs. Preliminary results with the λ_1 RNA, together with the data of Picard and Schaffner (23), suggest that these RNAs are transcribed from upstream promoters and spliced to the _V_ region exons.

In contrast to the results with light-chain genes, the γ_{2b} heavy-chain gene from MOPC 141 was accurately transcribed from its natural promoter in L cells. Furthermore, increased levels of γ_{2b} mRNA were found in cell clones with greater gene copy numbers. Even though the level of γ_{2b} mRNA in transfected L cell clones G3 and G4 was high enough to detect γ_{2b} heavy-chain protein, this level was much lower than that in MOPC 141 myeloma cells. Since the very high levels of immunoglobulin mRNA in plasma cells have been attributed to a combination of messenger stability, rapid processing and a high transcription rate (25), it is not possible to accurately compare the relative rates of γ_{2b} mRNA transcription from the steady-state level of mRNA alone. Nonetheless, we have demonstrated that rearrangement is sufficient for the expression of a heavy chain, but not light-chain immunoglobulin genes.

It has been shown that both light-chain ($\underline{C}_\kappa$) and heavy-chain ($\underline{C}_\mu$) constant region DNA segments are transcriptionally active in lymphoid cells (26, 27) while _V_ DNA segments are not (2). For this reason it has been postulated that the consequence of

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light-chain and heavy-chain gene rearrangement is the conferral of transcriptional competence to the V DNA segment promoters by the activated C DNA segments. Unlike immunocompetent cells, L cells do not transcribe either light-chain or heavy-chain C DNA segments and thus could not confer such an activity on the V DNA segment promoter of a rearranged gene. In fact we and others have recently shown that a tissue specific enhancer element is located in the major intron of this rearranged γ_{2b} gene (20,28,24). This site corresponds to the J_H-C_μ region in germ line DNA and thus explains why the C_μ region is transcriptionally active in lymphoid cells. Since we also showed that this enhancer element is not active in transfected L cells (20), it is difficult to explain why the rearranged γ_{2b} is accurately transcribed (albeit at a low level) in these cells while the light chain genes are not. One possible explanation is that an enhancer-like element, active in non-lymphoid cells, is present in another region of the rearranged heavy-chain gene and that this allows for the low level transcription from the VDJ gene segment promoter.

Although definitive evidence for the presence of a tissue-specific enhancer element associated with the C_κ or $C\lambda$ gene segments has yet to be generated, the data presented in Figure 7 clearly show that the λ_1 gene is expressed at a much higher level in transfected X-63Ag8 cells than in transfected L cells. In addition, the λ_1 mRNA that is produced in X-63Ag8 cells is initiated at the same start site utilized in H2020 myeloma cells. These results strongly suggest that a tissue-specific enhancer sequence is contained within the 7.4 kb EcoRI λ_1 gene fragment and that factors present in lymphoid cells are required for the function of this element. In contrast to the heavy-chain gene, the activation of the λ_1 light-chain gene affects both the site of mRNA initiation and the level at which the gene is expressed.

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