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Immunology

Regulated expression of an immunoglobulin κ gene introduced into a mouse lymphoid cell line

(Abelson murine leukemia virus/DNA transfection/*gpt* selection/lipopolysaccharide induction)

DOUGLAS RICE AND DAVID BALTIMORE

The Whitehead Institute for Biomedical Research, the Center for Cancer Research, and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Contributed by David Baltimore, September 16, 1982

ABSTRACT **We have introduced a functionally rearranged murine κ light chain immunoglobulin (Ig) gene into an Abelson murine leukemia virus-transformed lymphoid cell line. Plasmid pSV2gpt-κ41, containing the κ light chain gene from the myeloma MOPC41 and the selectable marker gene *gpt*, was introduced into 81A-2 cells by the calcium phosphate coprecipitation technique. Cells expressing the *gpt* gene were selected by growth in medium containing mycophenolic acid. One transfected cell line, κ-2, was shown to make κ mRNA and polypeptide chains and to assemble the κ chain product with γ2b heavy chains to form an apparently complete IgG2b. When bacterial lipopolysaccharide was added to the growth medium, levels of κ mRNA and polypeptide increased, showing regulated expression of the introduced κ gene.**

B cell differentiation proceeds from the "pre-B" lymphocyte, which synthesizes μ immunoglobulin (Ig) heavy chains but no light chains, to the mature B lymphocyte, which synthesizes both heavy and light chains and expresses surface Ig, and finally to the Ig-secreting plasma cell (1–5). The availability of transformed cell analogs has allowed biochemical characterization of these stages of cellular differentiation (6–11). Recently, such studies have contributed greatly to our understanding of the structure of Ig gene segments and the joining of these segments to produce a functionally rearranged Ig gene (12–17).

Although much is now known about Ig gene structure, relatively little is known about the molecular mechanisms that control Ig gene expression. One approach to study such controls is to introduce Ig genes into various cell lines, including both lymphoid cells at various stages of differentiation and nonlymphoid cells. One might then be able to identify control mechanisms unique to lymphoid cells that allow the cells to express, assemble, and secrete Igs. To begin such studies, we have attempted to introduce a functionally rearranged murine κ light chain gene into an Abelson murine leukemia virus (A-MuLV)-transformed lymphoid cell line.

Previous studies have shown that A-MuLV infection of bone marrow or fetal liver cells transforms cells of the B-lymphoid lineage, usually "pre-B" cells (18, 19). Derivatives of one A-MuLV transformant, 18-8, have been shown to switch from μ to γ2b heavy chain synthesis while in culture (20–22). One such derivative, 81A-2, synthesizes γ2b protein, but has lost its κ constant region light chain gene segments (unpublished data). Here we report that, after the introduction of a functionally rearranged κ gene into 81A-2 cells, the κ gene is expressed in a regulated manner.

MATERIALS AND METHODS

Cells. The A-MuLV-transformed cell line 81A-2, a derivative of the line 18-8, synthesizes γ2b heavy chain protein, but no light chain, and has lost its κ constant region genes (refs. 18 and 22; unpublished data). Cells were grown and analyzed for Ig protein synthesis by metabolic labeling and immunoprecipitation as described (18). Nonreduced samples were prepared for electrophoresis as described by Margulies *et al.* (23).

DNA Procedures. The phage λCh4A-41KC21, containing the rearranged genomic κ light chain gene from the myeloma MOPC41, was obtained from P. Leder (12). The 7-kilobase-pair (kbp) *Eco*RI/*Bam*HI fragment containing the κ gene was inserted into *Eco*RI- and *Bam*HI-cleaved plasmid pSV2gpt, obtained from R. Mulligan (24). The resulting plasmid, shown in Fig. 1, is called pSV2gpt-κ41. Ten micrograms of DNA from this plasmid was transfected into 5×10^7 81A-2 cells by a modification of the calcium phosphate technique of Graham and Van der Eb (25). Cells were washed in phosphate-buffered saline (0.14 M NaCl/2.5 mM KCl/16 mM Na_2HPO_4/1.4 mM KH_2PO_4), resuspended in 1 ml of transfection cocktail [made by adding DNA to 1 ml of Hepes-buffered saline, then adding 62.5 μl of 2 M $CaCl_2$ (26)] and incubated 15 min at room temperature. Then 10 ml of medium was added and the cells were incubated at 37°C for 4 hr. Cells were then washed in phosphate-buffered saline, incubated at 37°C for 2 min in 2 ml of Hepes-buffered saline with glycerol (26), and washed again in phosphate-buffered saline. Cells were then resuspended in 10 ml of nonselective medium, grown for 3 days, and then transferred to selective medium [RPMI 1640 medium supplemented with mycophenolic acid at 2 μg/ml, xanthine at 250 μg/ml, hypoxanthine at 15 μg/ml, and glutamine at 150 μg/ml (27)]. Transfected and mock-transfected 81A-2 cells were passaged in selective medium for approximately 3 weeks, until the mock-transfected cells had died. The transfected cells were then cloned by limiting dilution in nonselective medium.

RNA. Total cellular poly(A)-containing RNA was isolated by the guanidine·HCl procedure (28), fractionated according to size by electrophoresis in formaldehyde gels (29), transferred to nitrocellulose, and hybridized with ^{32}P-labeled DNA probes as described (30).

RESULTS

To examine expression of a κ gene from transfected plasmid DNA, the plasmid pSV2gpt-κ41 was constructed to contain the rearranged chromosomal κ light chain gene from the myeloma MOPC41 (12) and the selectable marker gene *gpt*, the gene from *Escherichia coli* that codes for the enzyme xanthine-guanine phosphoribosyltransferase [GPT; EC 2.4.2.22 (27)] (Fig. 1). In mammalian cells grown in media containing inhibitors of purine synthesis (here, mycophenolic acid), expression of the *gpt* gene allows selective cell growth using xanthine as the pre-

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Abbreviations: A-MuLV, Abelson murine leukemia virus; GPT, xanthine-guanine phosphoribosyltransferase; kb, kilobase(s); kbp, kilobase pair(s); LPS, bacterial lipopolysaccharide; SV40, simian virus 40.

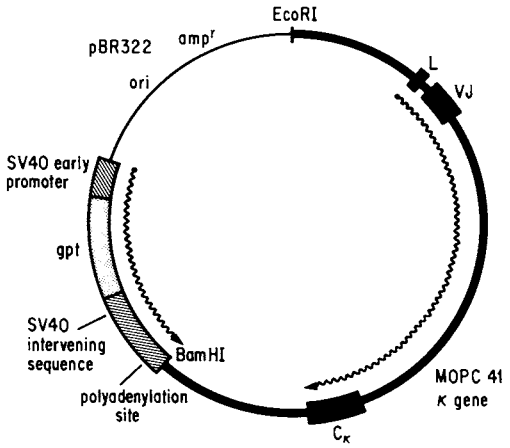

FIG. 1. Structure of the plasmid pSV2gpt-κ41 (11.4 kbp). Mouse DNA sequences containing the rearranged κ light chain gene from the myeloma MOPC41 are represented by the heavy dark line. The leader (L), variable plus joining region (VJ), and constant region (C_κ) of the κ gene are indicated. Simian virus 40 (SV40) sequences, represented by hatched regions, include DNA segments containing the early promoter, the small tumor antigen intervening sequence, and sequences for termination and polyadenylylation of SV40 early transcripts. The *gpt* gene from *Escherichia coli* is shown as a stippled region. *ori*, Origin of replication; *amp*^{r}, ampicillin resistance. Transcription units are indicated by wavy lines.

cursor for synthesis of guanine nucleotides (27). In pSV2gpt, the parental plasmid used for this construction, the *gpt* gene is transcribed from the SV40 early promoter and is followed by a region of SV40 DNA containing the small tumor antigen intervening sequence and signal sequences for transcript termination and polyadenylylation (24). To reduce the possibility of transcription of the κ light chain gene from promoters other than its own, plasmid pSV2gpt-κ41 was designed so that transcription from the SV40 promoter is in opposite orientation from that required for κ gene expression.

The 81A-2 cell line used as recipient of the transfected DNA is an A-MuLV-transformed murine lymphoid cell that synthesizes γ2b heavy chain but no light chain [no C_κ alleles can be detected by hybridization (ref. 22 and unpublished data)]. Plasmid pSV2gpt-κ41 DNA was introduced into 81A-2 cells by the calcium phosphate coprecipitation technique (25). Cells expressing the *gpt* gene were selected by growth in medium containing mycophenolic acid and then cloned by limiting dilution. When DNA from three selected cell lines was prepared and analyzed by hybridization with a κ probe, all three lines were found to have acquired one or a small number of the introduced κ genes. From the pattern of the hybridizing bands, at least two of three lines were judged to be independent transfectants. Eight cell lines were assayed for GPT enzyme activity by the *in situ* gel assay of Mulligan and Berg (24); all eight were positive (data not shown).

When the eight *gpt*$^+$ cell lines were assayed for production of κ protein by metabolic labeling with [^{35}S]methionine and immunoprecipitation with anti-κ antiserum, all eight were found to synthesize a polypeptide which (*i*) was precipitable with anti-κ antiserum (Fig. 2, lane d for clone κ-2 and data not shown for the others); (*ii*) comigrated with the κ chain produced by the myeloma MPC11 (apparent M_r 23,000) (Fig. 2, lane a); and (*iii*) was not evident in the nontransfected 81A-2 parent cell line (Fig. 2, lane b). In the original autoradiogram, the background bands in the M_r 23,000 region are much fainter than reproduced here. Because the 81A-2 cells lack C_κ alleles, none of the background bands are κ light chain. Precipitation of the M_r 23,000

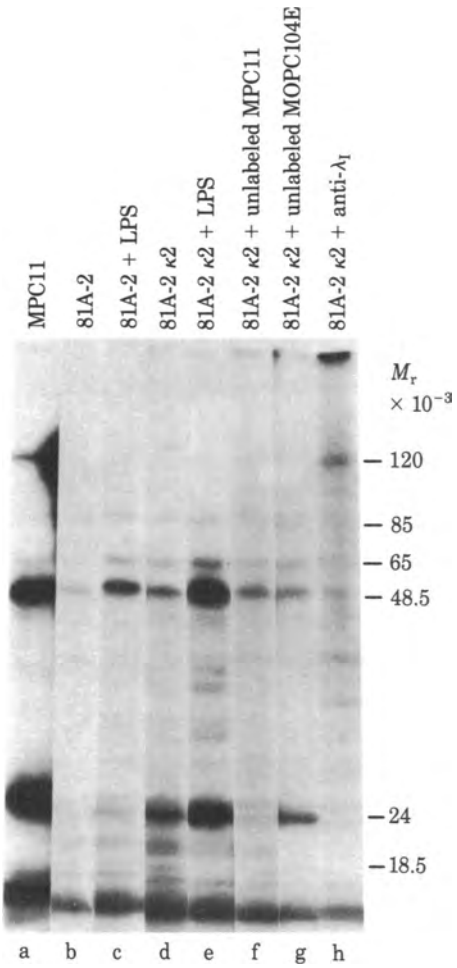

FIG. 2. Polyacrylamide gel electrophoresis of Ig polypeptides synthesized by 81A-2 cells and the transfectant κ-2. Cytoplasmic extracts were prepared from cells labeled with [^{35}S]methionine, immunoprecipitated, and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Lane a, myeloma MPC11 extract immunoprecipitated with anti-κ antiserum; lanes b and c, extract from parent A-MuLV-transformant 81A-2 grown in the absence (lane b) and presence (lane c) of *Salmonella typhimurium* lipopolysaccharide (LPS) and immunoprecipitated with anti-κ antiserum; lanes d and e, extract from the transfectant κ-2 grown in the absence (lane d) and presence (lane e) of LPS and immunoprecipitated with anti-κ antiserum; lanes f and g, extract from the transfectant κ-2 mixed with unlabeled MPC11 extract (lane f) or unlabeled MOPC104E extract (lane g) and immunoprecipitated with anti-κ antiserum; lane h, extract from the transfectant κ-2 immunoprecipitated with anti-λ_I antiserum. Sizes of molecular weight marker proteins are indicated.

polypeptide by anti-κ serum was blocked by competition with an unlabeled MPC11 protein extract (containing authentic κ light chains) but not by an unlabeled MOPC104E protein extract (containing λ_I light chains) (Fig. 2, lanes f and g). Also, the apparent κ chain was not precipitable by anti-λ_I antiserum (Fig. 2, lane h). Therefore, the M_r 23,000 polypeptide appears to be the protein product of the κ light chain gene transfected into the 81A-2 cells.

To examine the RNA produced from the transfected κ gene, poly(A)-containing mRNA was prepared from the parent 81A-2 cell line and the transfectant κ-2. The RNA was size-fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled plasmid DNA probe containing the κ constant region (C_κ), the κ joining (J_κ) segments, and the sequence that intervenes between them. No hybridization was detected to the RNA prepared from the par-

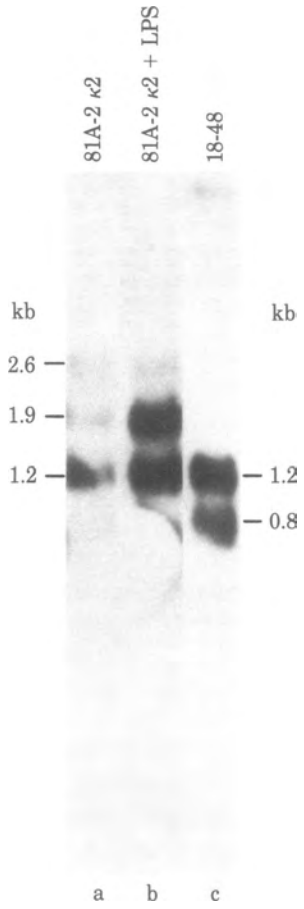

FIG. 3. Analysis of κ RNA transcripts in the transfectant κ-2. Poly(A)-containing RNA was isolated from cells, size-fractionated by agarose gel electrophoresis, transferred to nitrocellulose paper, and hybridized with ^{32}P-labeled DNA from a plasmid containing the sequence from J_κ through C_κ. Lanes a and b, RNA from the transfectant κ-2 grown in the absence (lane a) or presence (lane b) of LPS. Lane c, RNA from the κ-producing A-MuLV-transformed cell line 18-48.

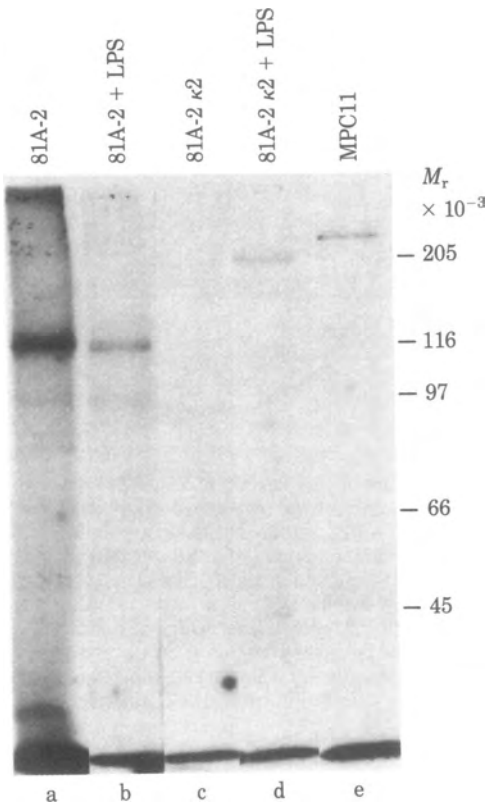

FIG. 4. Polyacrylamide gel electrophoresis of nonreduced Ig synthesized by the κ-2 transfectant. Cytoplasmic extracts were prepared as for Fig. 2. Lanes a and b, extracts from the parent 81A-2 cells grown without (lane a) and with (lane b) LPS and immunoprecipitated with anti-κ antiserum; lanes c and d, extract from κ-2 transfectants grown without (lane c) and with (lane d) LPS and immunoprecipitated with anti-κ antiserum; lane e, myeloma MPC11 extract immunoprecipitated with anti-κ antiserum.

ent line 81A-2 (not shown), but RNA from the κ-2 line contained hybridizing species of approximately 1.2, 1.9, and 2.6 kilobases (kb) (Fig. 3, lane a). The smaller RNA comigrated with authentic κ mRNA from the A-MuLV-transformed cell line 18-48 (Fig. 3, lane c).[The smaller 0.8-kb RNA in 18-48 is an aberrantly small κ transcript (5).] In other experiments, both the 1.9-kb and the 2.6-kb RNAs were found to hybridize strongly to a probe specific for the intervening sequence between J_κ and C_κ and hybridize weakly to a pBR322 DNA probe. Hence, these higher molecular weight species are some type of aberrant RNA. The 1.2-kb species, however, appears to be an authentic κ mRNA transcript in that it hybridizes only to the probe containing κ coding sequences and not to the intervening sequence probe or pBR322.

Because many A-MuLV-transformed lymphoid cell lines increase Ig production when LPS is added to the medium (18, 31), we investigated the effects of LPS on κ chain synthesis in the transfected line. The parent line 81A-2 increases synthesis of γ2b heavy chain protein and mRNA upon induction by LPS (22) (Fig. 2, lane c). When LPS was added to the κ-2 cells, κ light chain synthesis increased approximately 5-fold, to a level approximately 1/15th that of the myeloma MPC11 (Fig. 2, lane e). To determine if the LPS-induced increase in κ chain synthesis was due to an increased content of specific mRNA, the mRNA fraction was prepared from LPS-treated 81A-2 parent cells and transfected κ-2 cells. Again, no κ mRNA species was detected in the parental cells, but the κ-2 cells contained increased levels of the 1.2- and 1.9-kb species (Fig. 3, lane b). Interestingly, the level of the 1.9-kb RNA species increased even more than that of the presumably authentic 1.2-kb RNA species.

Because the transfected cells were producing both γ2b heavy chains and κ light chains, it was possible that the cells could assemble the heavy and light chains into IgG. To examine this question, samples of [^{35}S]methionine-labeled protein extracts were immunoprecipitated with anti-κ antiserum and the nonreduced samples were subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis. The parental 81A-2 cells produced a protein of approximately the correct size for γ2b heavy chain dimers (Fig. 4, lanes a and b; the darker appearance of lane a is due to more labeled extract present). The κ-2 cells produced a protein that migrated slightly faster than the IgG2b produced by the myeloma MPC11 (Fig. 4, lanes d and e) but slower than the bulk of the rabbit IgG antiserum visualized by staining (not shown). In other experiments (not shown) no free κ chain was found in the κ-2 cells, although a significant amount was present in MPC11 cells. Essentially all of the κ chain produced in the κ-2 cells appears to be assembled into IgG2b.

DISCUSSION

The major result of these studies is the demonstration that a functional κ gene can be introduced into a lymphoid cell line in which it will be continuously expressed. This opens the possibility of examining control and rearrangement mechanisms in

lymphoid cells by using inserted genetic elements.

The κ gene introduced into 81A-2 cells apparently functions normally in spite of being in a very unusual context. The gene was in an SV40/pBR322 vector that then integrated into a presumably random site in the cell genome, a site unlikely to be related to the normal location of the κ gene in chromosome 6. In spite of its unusual context, the introduced gene was expressed at about the same level as the resident γ2b heavy chain gene. The κ gene was apparently using its own promoter because in the construction no promoter was provided that faced in the correct direction. It is possible that the SV40 DNA sequences present might have provided some enhancing function for κ expression (32).

The introduced κ gene not only was expressed at a basal level but also was inducible by LPS. The mechanism and function of this induction system are far from clear, but the ability of the introduced κ gene to respond indicates that sufficient κ-related DNA sequences to provide for LPS inducibility were included in the construct. The construct contained, in addition to the V_κ, J_κ, and C_κ coding segments, the intervening sequence between the coding regions and about 1–1.5 kb of DNA both 5′ of $V_\kappa J_\kappa$ and 3′ of C_κ. Any of this extra DNA could be involved in promoter and control functions, but the results make it unlikely that any sequences important for κ expression exist more than 1.5 kb to either side of the coding region.

LPS control of heavy chain expression in 81A-2 cells is allele specific and correlates with a deletion in the intervening sequence between $V_H DJ_H$ and C_μ (22, 33). The productively rearranged heavy chain allele is inducible by LPS and contains this deletion, whereas the other allele, containing a nonproductive rearrangement, lacks the deletion and is not inducible by LPS. Therefore, LPS inducibility of heavy chain seems to be determined at the DNA level. Whether the introduced κ gene is responding directly to LPS or to the product of the heavy chain allele is an open question. The possibility that transcription of the light chain gene is controlled by a product of the heavy chain locus is an interesting possibility and needs further investigation.

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