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TRANSFER AND EXPRESSION OF IMMUNOGLOBULIN GENES

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INTRODUCTION

Over the last two decades a great deal of information has accumulated on the structure and function of the immunoglobulin molecules. Structural studies of the myeloma proteins defined the variable and constant regions. Analysis of the variable regions showed the existence of hypervariable regions (1) and began to give insight into the nature of the interaction between the antibody molecule and its specific antigen. The existence of both variable and constant regions on the same molecule gave rise to the concept "two genes—one polypeptide chain" (2), which challenged then-accepted genetic ideas.

With the advent of modern molecular biologic techniques it became clear that the immunoglobulin (Ig) molecule, both heavy and light chain, was encoded by multiple DNA segments. In order to generate a gene encoding a functional Ig molecule, somatic rearrangements of distinct DNA segments must take place. For a complete light-chain gene a V region must be brought next to a J segment to create an active transcription unit. For a heavy-chain gene, V, D, and J segments must be assembled next to a constant-region gene; the initial constant-region gene utilized is the μ gene. In addition, during immune response maturation heavy-chain class switching can occur, whereupon VDJ is recom-

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bined next to a different constant-region gene to change the class but not the specificity of the molecule being synthesized.

In addition to somatic rearrangement, the expression of immunoglobulin (Ig) genes must be regulated during B-lymphocyte differentiation. Transcription of unrearranged V_κ gene segments is not detected prior to rearrangement (3). Rearrangement is necessary but not sufficient for expression (4–7). When B lymphocytes develop into antibody-secreting plasma cells the rate of transcription of the rearranged immunoglobulin genes increases greatly so that in some plasma cells immunoglobulin transcripts may constitute 10% of the mRNA population (8). DNA sequence studies of genes give little insight into the mechanisms of their control. However, if one can modify genes in vitro and transfect them into cells of the appropriate phenotype, it is possible to begin to define the regions of the Ig genes that are important for the regulation of their expression and to identify the basis for differential immunoglobulin gene expression at different stages of lymphocyte differentiation.

The study of the structure and function of the immunoglobulin molecule has been of great interest because of the ability of the immunoglobulin molecule to react with a diverse family of ligands, because different immunoglobulin molecules contain different effector functions, and because of the biologic importance of antibody molecules. The use of myelomas, and more recently hybridoma proteins, has permitted the study of homogeneous populations of antibodies. However, in these cases one is limited to the study of a protein the animal happens to produce. DNA-mediated transfection and immunoglobulin gene expression provide an important new tool for the study of immunoglobulin molecules. By using this technique it will be possible to study the function of novel chain combinations and novel chain structures created in vitro and then expressed following gene transfection. Additionally, in vitro site-specific mutagenesis techniques can be used to construct specific mutations in immunoglobulin genes that can be expressed after transfection. Because sufficient quantities of immunoglobulin are produced in the transformants, quantities of protein necessary for detailed analyses can be obtained.

VECTORS, SELECTIVE TECHNIQUES, AND TRANSIENT VS STABLE EXPRESSION

When transfecting cells two basic approaches can be used. Transient expression can be assayed, or stable transformants can be selected.

To study transient expression the foreign DNA is introduced into the recipient cells; then, following an appropriate interval (usually 48–72 hr), the cell population is harvested and expression is assayed. This method allows an answer to be obtained rapidly; and, since no selection of the recipient population is required, no selectable marker is necessary in the transfecting vector.

In most cases, expression in a transient assay is monitored by S1 analysis of the mRNA produced (9). This provides the most sensitive assay available, and sensitivity is often required for transient assays where little material is available. To increase the sensitivity of the transient assay, vectors are used that replicate in the recipient cells and so increase the gene copy number. For COS cells (10), a monkey kidney cell line that contains an integrated and expressed copy of SV40 T antigen, vectors containing an SV40 *ori* are suitable. These vectors, however, do not replicate in mouse cells. To overcome this shortcoming, vectors containing a polyoma early region can be used in mouse cells (11–15). These replicate to high copy number (50,000–400,000 copies per cell) and so provide gene amplification in the transient assay. Using these vectors, sufficient expression is obtained to permit visualization of Ig proteins on SDS-polyacrylamide gels following radiolabeling and immunoprecipitation (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished).

As an alternative to transient expression assays, expression by stable transformants can be studied. The disadvantage of these experiments is that they are much more time consuming than those for transient expression. Two to four weeks are required to accumulate enough cells for analysis. Such experiments have the advantage of providing a cloned population of cells for long-term analysis. In addition, sufficient expression from a single or low copy number of transfected genes can be obtained.

In transient expression 30–80% of the treated cells will express the transfected genes. However only 10^{-3} to 10^{-6} cells will go on to become stably transformed. Therefore, selective techniques are required that permit the isolation of the rare stably transformed cells from among the many nontransformed cells.

The initial selective technique developed used the expression of the thymidine kinase gene from *Herpes simplex* as a selectable marker (16, 17). Cells that were deficient in endogenous thymidine kinase were transfected with vectors containing the viral enzyme. Only recipient cells that expressed viral thymidine kinase could survive selection in HAT medium (hypoxanthine/aminopterin/thymidine) (18, 19). This proved to be an effective selective technique, but it could be applied only to recipient cell populations deficient in endogenous thymidine kinase. Since it is not a trivial matter to put a drug marker into a cell type, one was limited in the number of potential recipients using these vectors.

To circumvent this problem vectors with dominant selectable markers have been developed. Dominant-acting genetic markers produce a selectable change in the phenotype of normal cells. When a dominant selectable marker is used, the recipient cell population need not be drug marked. Therefore, with a dominant marker any cell can be used as a recipient.

One dominant selection utilizes expression of the bacterial xanthine-guanine

phosphoribosyltransferase gene (*gpt*) (14, 15). The enzyme encoded by this gene, 5-phospho-α-D-ribose-1 diphosphate:xanthine phosphoribosyltransferase (XGPRT), differs from the analogous mammalian enzyme, hypoxanthine-guanine phosphoribosyltransferase (HGPT) in that it can efficiently use xanthine as a substrate in nucleotide synthesis while the mammalian enzyme cannot; mammalian cells do not convert xanthine to xanthylic acid or to guanylic acid at a significant rate. Therefore, in the presence of mycophenolic acid (which inhibits the conversion of IMP to XMP) and xanthine, only cells that express bacterial XGPT survive (Figure 1). Growth in mycophenolic acid + xanthine provides a dominant selection system for unmarked cell lines. The bacterial XGPRT can also be used to complement the enzymatic deficiency in HGPT-deficient cell lines; expression of the XGPRT permits HPGT-deficient cells to survive HAT selection.

A second selectable system relies on acquisition of resistance to the aminoglycoside antibiotic G418 (20). The structure of G418 resembles gentamycin, neomycin, and kanamycin; but, unlike these related compounds, G418 interferes with the function of 80S ribosomes and blocks protein synthesis in eukaryotic cells. Bacterial phosphotransferases inactivate this class of antibiotic (21). Therefore when the phosphotransferase from Tn5 (designated *neo*) is included in a mammalian transcription unit, it confers resistance to G418 when introduced into eukaryotic cells (22).

Any number of different vectors are available for gene expression. The most commonly used vectors to date in lymphoid cells have been the pSV2 vectors (14, 15, 22). The prototype vector with its essential features is shown in Figure 2. These vectors contain the pBR322 *ori* and ß-lactamase gene. Thus they can be propagated and selected as plasmids in *E. coli*. This facilitates in vitro

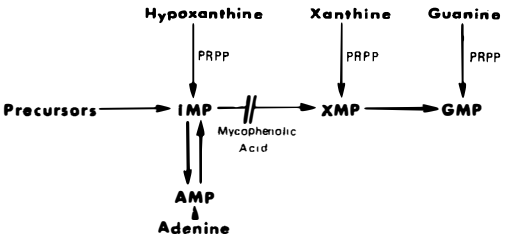

Figure 1 Pathway of purine nucleotide synthesis. Salvage of free purines occurs by condensation with phosphoribosyl pyrophosphate (PRPP). Adenine phosphoribosyl transferase (APRT) accounts for the formation of AMP from adenine and hypoxanthine phosphoribosyltransferase (HPRT) converts hypoxamine to IMP (inosinic acid). No mammalian enzyme is known that can convert xanthine to XMP (xanthylic acid). Mycophenolic acid, an inhibitor of IMP dihydrogenase (19a) prevents the formation of XMP and thereore of GMP. Since normal mammalian cells do not convert xanthine to XMP, they cannot grow in medium containing mycophenolic acid supplemented with xanthine. However, cells that express the bacterial XGPRT gene can grow under these conditions.

manipulation of these vectors and easily enables one to obtain the quantities of DNA required for transfection. In addition these vectors contain a mammalian transcription unit with a protein coding sequence joined at its 5′ end to a segment containing the SV40 early region promoter and at its 3′ end to an SV40 segment containing an intervening sequence and polyadenylation signal. This transcription unit is used to direct the transcription of the selectable marker. The presence of the SV40 early promoter permits the expression of the bacterial gene in mammalian cells. Restriction endonuclease sites (Pstl, BamHl, and EcoRl) are available in regions of extraneous DNA into which can be inserted the additional genes of interest. These vectors need not be propagated as viruses; therefore, there is no theoretical limit on the size of the genes that can be inserted, and genes larger than 25 kb have been used with ease (23).

Many modifications of the different vector systems exist, depending on the assay to be used. As mentioned earlier, the most significant modification entails including the polyoma early region in the vector so that it can replicate and be amplified in mouse cells.

METHODS OF TRANSFECTING CELLS

Three basic techniques have been used to introduce DNA into recipient lymphoid cells: $CaPO_4$ precipitation, treatment with DEAE-dextran, and protoplast fusion.

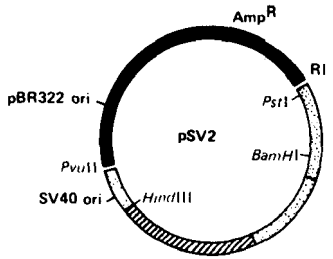

Figure 2 Structure of the pSV2 vectors. pBR322 DNA is represented by a solid black line and contains the plasmid origin of replication and ß-lactamase gene. The hatched segments represent a gene that provides a selectable marker in eukaryotic cells. The two commonly used genes are *gpt* and *neo*. The stippled segments are derived from simian virus 40 (SV40). The Pvu II to Hind III segment contains the SV40 origin of DNA replication (*ori*) and early promoter. The SV40 sequences immediately downstream of the selectable marker contain a splice and polyadenylation site. The early promoter, splice, and polyadenylation sites constitute a transcription unit that permits the expression of a bacterial gene in a eukaryotic cell. The EcoRI, Pst I and BamHI sites are located in SV40 sequences that are not necessary for vector function and thus provide convenient sites into which other genes can be inserted. [Diagram adapted from (14)]

$CaPO_4$ precipitation has been used to introduce DNA into lymphoid cells for stable transfection experiments (24, 25) or to study transient expression (26–28). The basic method used is that of Graham & van der Eb (29) with the modification of Chu & Sharp (30) for suspension cells. Using $CaPO_4$ sufficient expression is achieved for either enzyme analysis assay (28) or S1 analysis of the recipients (26, 27). Stable transfectants can be recovered at a frequency of approximately 10^{-5} to 5×10^{-6} from myeloma cells treated with $CaPO_4$ precipitated DNA (25).

Treatment with DEAE-dextran has also been used to introduce DNA into lymphoid cells (11–13, 27, 31) and in many cases is less toxic to the cells than $CaPO_4$. Using this method 60–70% of treated myeloma cells were found to express antigens encoded by the transfecting DNA 48 hr after treatment (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished), and sufficient mRNA was produced to permit S1 analysis of the recipient cells (11, 12, 27).

The most efficient way of introducing DNA into lymphoid cells may be by protoplast fusion. In this method, *E. coli* bearing the plasmids of interest are treated with lysozyme to remove the bacterial cell wall (32). The resulting spheroplasts are then fused to the myeloma cells using methods similar to those used to create somatic cell hybrids. This method has been used to study both transient expression (13) and stably transformed cells (23, 25, 33–35). Using this method, transfection frequencies ranging from 10^{-4} (23, 33) to as high as 10^{-3} (34) have been observed.

Gene transfection into a wide variety of lymphomas, hybridomas, and myelomas has now been reported. These include the mouse myelomas J558 (25, 34, 35), MPC-11 (12), and X63-Ag8 (31, 27); the rat myeloma Y3 (25); hybridomas 27–44 (25), SP/20Ag14, and SP6 and their mutants (23, 33); and the lymphomas BW5147 (13,25), 18–81 (24), and 70Z (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished). Under the proper conditions and with the appropriate vector, all of these cell lines appear to be potential recipients. Including Ig sequences in the vector often increases the ability to transfect lymphoid cells. When a polyoma vector is used, transient expression of T antigen in 70Z and 18–81 is observed only when the heavy-chain gene is contained within the vector (R. J. Deans, K. A. Denis, A. Taylor, R. Wall, unpublished). Including light chain in the pSV2*gpt* vector increased the transfection frequency of the J558 myeloma 10- to 100-fold (25). However, comparable transfection frequencies were observed whether or not the kappa light chain was included in the pSV2-*neo* vector (33). The exact mechanisms by which the Ig sequences increase the transfection frequency have not yet been defined. However, evidence is accumulating that Ig genes contain lymphoid-specific enhancer regions (see below for discussion) and that these facilitate the expression of the selectable markers.

EXPRESSION OF IMMUNOGLOBULIN MOLECULES

When the MOPC-41 kappa light chain was inserted into the pSV2-*gpt* vector between the EcoR1 and BamHl sites, stable transfectants of the A-MuLV transformed cell line 81–A2 were recovered that synthesized detectable quantities of kappa light chain. The kappa light chain was oriented in the pSV2-*gpt* vector so that its direction of transcription was opposite to that of the *gpt* gene from the SV40 promoter (24). The kappa specific mRNA was found to be of three sizes in the transfectants: 1.2 kb, 1.9 kb, and 2.6 kb. The 1.2 kb transcript represented the correctly processed mRNA while the 1.9 and 2.6 kb transcripts were incorrectly processed and still contained the kappa intervening sequence. The transfected kappa chain was able to assemble with the resident γ_{2b} heavy chain so that H_2L_2 molecules were observed in the cytoplasm. LPS stimulation increased the level of synthesis of the transfected kappa chain synthesis about 5-fold, just as it does in the parental Abelson line, so that the final level of kappa synthesis was about 1/15 of that observed in the MPC-11 myeloma. Thus the gene sequences responsive to LPS stimulation are contained within the transfected gene fragment. However, for the transfected light chain, LPS stimulation effects preferential increase in the quantity of the aberrant 1.9-kb transcript.

The S107A kappa light-chain gene was also capable of transfection and expression in stable transformants when included in the pSV2-*gpt* vector (25). The light chain was oriented in the vector such that its direction of transcription was opposite to that of the *gpt* gene from the SV40 promoter; however, in subsequent experiments the orientation of the light-chain gene was reversed and the same level of kappa chain expression was observed (S. L. Morrison, V. T. Oi, unpublished observation). Thus the transcription from the SV40 promoter appears to neither facilitate nor interfere with kappa chain expression. Two-dimensional gel analysis of the kappa chain produced by the cells transfected with the S107A light chain showed it to be indistinguishable from that produced by the S107 myeloma. In addition, when the S107A kappa light chain was produced in the absence of heavy chain, it was not secreted, just as it is not secreted in the parental myeloma in the absence of heavy-chain synthesis. However, in the presence of heavy chain it can assemble and be secreted as part of an H_2L_2 molecule.

The light chain from a TNP-specific hybridoma was capable of expression when ligated into the pSV2-*neo* vector and transfected into a recipient hybridoma cell line (33). Expression occurred whether the light chain was in the same or opposite orientation from the SV40 promoter. As noted by previous investigators, variability of expression occurs among different independent transformants; however, in this case expression of light chain seemed consis-

tently better when the light chain was oriented such that its transcription was in the opposite direction from transcription from the SV40 promoter. The recipient cell line originally synthesized antibodies specific for TNP but had lost the ability to synthesize the TNP-specific light chain. Transfection with the TNP-specific light chain restored the ability of the Ig to bind antigen and the secretion of TNP-specific IgM. The hemagglutination titer of anti-TNP antibody in some transformants was comparable to that of the parental anti-TNP hybridoma.

Transfection of the S107A light chain into a heavy-chain producing cell line isolated from an anti-PC hybridoma was also able to restore the ability of this cell line to bind antigen (C. Desmeyard, S. L. Morrison, M. D. Scharff, unpublished observation). In these transfectants there was also an increase in cytoplasmic heavy chain as judged by immunofluorescence; presumably this occurred because the light chain interacted with the cytoplasmic heavy chain to protect it from degradation.

It is also possible to get efficient expression of heavy chain following gene transfection. When the γ_{2b} heavy chain from the MOPC-141 myeloma was included in the pSV2-*gpt* vector and used to transfect the J558L cell line, stable transfectants synthesizing approximately 20% of the amount of heavy chains synthesized by the parental myeloma were isolated (34). The heavy mRNA was 1.7 kb in length and of a discrete size; the 1.7 kb size is that expected for a secreted γ_{2b} protein. The γ_{2b} gene included in the expression vector did not include the membrane-specific exon and so could not code for mIg. Transcripts 1.7 kb in size were also observed when this γ_{2b} gene was used to transfect mouse L cells. In the J558L myeloma, which synthesizes lambda light chain, assembly between the γ_{2b} and the lambda light chain occurred such that an anti-γ_{2b} antiserum immunoprecipitated both chains.

By transferring a heavy-chain gene from an anti-TNP-specific myeloma into a light chain–producing variant of that myeloma, it was possible to express a pentameric IgM molecule with anti-TNP activity (23). Cotransfer of specific light chain and heavy chain into a nonproducing myeloma also resulted in the production of pentameric, antigen-binding antibody. Analysis of a transformant obtained using the heavy chain into a light chain–producing myeloma showed that it synthesized about 25% as much protein as the parental myeloma. A transformant obtained after cotransfer of heavy plus light chain synthesized about 10% of the normal amount of IgM. The heavy-chain gene used in these experiments contained the membrane exon, and two mRNA species—a 2.7-kb species presumably with the membrane exon, and a 2.4-kb species coding for the secreted protein—were synthesized in the transformants. However, no membrane-specific protein could be detected either by immunofluorescence or by biosynthetically labeling with radioactive amino acids and immunopre-

cipitation. These results suggest either that the 2.7-kb mRNA is an aberrant transcript, that it is not translated, or that its product is rapidly degraded.

Using a transient expression assay, R. J. Deans and co-workers observed the production of both secreted and membrane alpha chain in NS-1, a light chain–producing myeloma. The rearranged alpha-chain gene from the myeloma M603 was inserted into a polyoma vector. The alpha-chain gene was oriented such that it was expressed either using its own promoter or using the late polyoma transcription signals. No difference in expression was observed with these two different promoters. Immunofluorescence revealed both cytoplasmic and surface IgA, and RNA dot blots showed the presence of both membrane and secretory forms of the mRNA. NS-1 clones transfected with alpha gene synthesized 3–8% of their protein as Ig while BW5147, a thymoma, and 3T3 cells synthesized only 0.3% and 0.8%, respectively, of their protein as Ig. NS-1 transfectants secreted approximately 25% as much IgA in a 72-hr period as did a myeloma. It is however difficult to relate these figures to normal alpha synthesis since the myeloma contains only one active chromosomal gene while the polyoma transfectants contain 50,000–400,000 extrachromosomal copies of the gene per cell.

With the demonstration that it is possible to produce both heavy and light chains efficiently by gene transfection, it is now possible to begin to produce novel Ig molecules using this technique. To this end the variable region from the heavy chain of an anti-*p*-azophenylarsonate-specific hybridoma has been fused to the kappa constant-region gene (J. Sharon, M. L. Gefter, T. Manser, S. L. Morrison, V. T. Oi, M. Ptashne, unpublished). The chimeric gene is expressed as a kappa-sized (approximately 1.2 kb) mRNA that hybridized both to a V_H-specific and to a C_κ-specific probe. The mRNA is translated into a chimeric protein of approximately 25,000 daltons that reacts with both anti-kappa- and anti-$Id_{(heavy)}$-specific antisera and is secreted. This demonstrates that the splice donor from the V_H can be successfully spliced to the C_κ acceptor and generate a mRNA still in reading frame. The chimeric light chain is synthesized in only about 10% of the quantity of the endogenous lambda chain produced by the recipient myeloma, but it still constitutes about 1% of the cell protein. In vitro reassociation experiments show that the V_HC_κ chimeric protein can associate with the light chain from a myeloma of the same specificity to generate a heterodimer that reacts with antigen.

In similar experiments the V_H from the S107 heavy chain has been fused to C_κ (V. T. Oi and S. L. Morrison, unpublished). This chimeric gene has been co-transfected with the S107A light-chain gene into the hamster lymphoma GD-36. Once again the chimeric gene is expressed as a protein and the V_HC_κ and light-chain proteins are found in the secretions of the lymphoma.

Gene transfection can also be used to study regulation of DNA rearrange-

ment and other events that occur during lymphocyte differentiation. K. Blackwell & F. W. Alt (unpublished) constructed a plasmid with a murine heavy-chain D segment (DQ52) separated from a part of the J_H cluster containing JH_3 and JH_4 by a *Herpes* thymidine kinase gene. This plasmid was co-transfected with pSV2-*neo* into a *tk*$^-$ derivative of the Abelson line 38B9, which rearranges its own heavy-chain genes in culture. Stable transformants were then selected for loss of expression of the *tk* gene. Surviving cells were found to have completed rearrangements of either JH_3 or JH_4. One rearrangement, a D-J_3 joint, has been cloned and sequenced. The D and JH_3 segments were found to have recombined in the appropriate regions. These experiments show that the D–J joining is not a function of the chromosomal location of the Ig genes.

TISSUE SPECIFICITY OF IMMUNOGLOBULIN EXPRESSION

Igs are the products of cells of the B lymphoid series and are not observed to be synthesized in other cell types. At least part of this preferential synthesis is a result of Ig genes' being rearranged only in B lymphocytes. Now, using gene transfection, it is becoming apparent that regulation in addition to rearrangement is responsible for specificity of expression.

The first experiment to demonstrate clearly that nonlymphoid cells do not efficiently synthesize Ig proteins from the Ig promoter was that of Falkner\& Zachau (36). When monkey cells (CV1) were transfected with plasmids containing light-chain genes, light-chain-specific mRNA was seen only if the kappa-chain gene was placed near (within 31 bp) the SV40 early promoter. When 0.8 or 3.0 kb of mouse DNA were interspersed, such that the Ig promoter region should be intact and capable of functioning, transcripts containing kappa-specific sequences were heterogeneously large. Kappa-specific translation products could be observed in CV1 cells containing kappa mRNA. When mouse L cells were used as a recipient, no kappa-specific mRNA or protein could be detected.

Similar results have been obtained with the lambda light-chain gene (26). In order to achieve efficient lambda-chain expression in HeLa cells the SV40 enhancer element has to be included 150 base pairs upstream of the cap site of the lambda chain. In this case the lambda transcripts had the same '5-end as authentic mRNA. If the lambda-chain promoter was separated from the SV40 enhancer sequences by more than 1 kb pair of spacer DNA, then lambda-chain transcripts were not correctly initiated in HeLa, CV1, or 3T6 cells.

The tissue specificity of expression is not limited to cells of the nonlymphoid series. In one case a myeloma recipient from a different species (mouse gene

into rat myeloma) was unable to express the transfected Ig gene efficiently (25). In addition, thymoma (BW5147) cells do not efficiently synthesize transfected Ig genes. In a stable transfection assay, L chain synthesis was not detected in BW5147 (25). However, on further examination thymoma transfectants were found to synthesize kappa light chains, but the quantity was less than 5% of that synthesized by a myeloma recipient (S. L. Brown, S. L. Morrison, unpublished).

To compare transcripts initiated from the Ig promoter with those initiated from a different promoter in the same cell lines, Stafford & Queen (12) constructed a vector containing pSV2-*neo*, the MOPC-41 kappa chain, and the polyoma T antigen and *ori* to permit replication in mouse cells. Since light chain and *neo* are on the same vector they serve as internal standards for each other. Mouse 3T3, L, and MPC-11 myeloma cells were transfected with these vectors and RNA prepared and quantitated 48 hr after transfection. All three recipients synthesized the same quantity of *neo*-specific mRNA. Kappa-specific transcripts were seen only in MPC-11. These results suggest that non-lymphoid cells fail to transcribe the transfected immunoglobulin gene.

Rearranged kappa-chain genes introduced into the mouse genome by microinjection of nuclei also show tissue specificity of expression (37). Spleens of transgenic mice were found to have large quantities of mRNA originating from the injected rearranged gene. Up to 50% of the total kappa mRNA in spleens from positive mice were found to bear the V region of the injected gene while less than 4% of the total kappa mRNA from normal littermates was positive. Spleens of transgenic mice also contained 1.5–2 times as much mRNA as normal controls, so the level of expression of the injected gene is relatively high. Examination of livers from injected mice showed no evidence of kappa expression. Therefore, even though hepatocytes contain the rearranged kappa gene, they cannot express it.

EXPRESSION OF NON-Ig IMMUNE RELATED MOLECULES

With the ability to transfect and express genes in lymphoid cells, one can also study the expression and function of non-Ig, immune related molecules. To this end $A\beta^k$ in the pSV2-*gpt* vector was introduced into an Ia^+ B lymphoma (38). Of the transfectants analyzed, one had several copies of the gene while the other had only one. The level of $A\beta^k$ expression in the transfectants was similar to that of $I\text{-}A^k$ in B-cell lines; the transfectant with several gene copies appeared to make slightly more than the transfectant with a single copy. The transfected genes are expressed so that they can be recognized by the appropriate monoclonal antibodies and can stimulate a MLR response. The trans-

fected gene functions in the antigen-dependent activation of an I-A^k restricted T-cell clone. This methodology clearly can be used for the further detailed analysis of the structure and function of I-A molecules.

CONTROL REGIONS IN Ig GENES

Heavy Chains

Although much has recently been learned about the structure of the Ig genes, little is known about their regulation and control. Analysis of transfected genes provides one way to investigate Ig regulation. One can begin to find answers to such questions as why Ig V regions are transcribed only following rearrangement, how Ig synthesis is amplified as a B lymphocyte matures to a plasma cell, and how the cell makes a choice of membrane vs secreted forms of the mRNA and protein.

Regulation of heavy-chain synthesis has been studied in myeloma cells transfected with heavy-chain genes or gene fragments. J558 cells transfected with a γ_{2b} heavy-chain gene effectively synthesized that protein (34). However if the 1-kb Xba I fragment with the internal EcoR1 was deleted from the IVS heavy chain, synthesis decreased to 5% of that seen using an intact heavy-chain gene (see Figure 3). Most of the intervening sequence can be deleted with no influence on heavy-chain synthesis as long as sequences within the 1-kb Xba I fragment are present. These necessary sequences can be inserted in either orientation at the Xba I site 5′ of the heavy-chain gene or within the IVS. When Δ1 was assayed it was found to be active in expression; Δ2 was found to be inactive in Ig expression (Figure 3). Fine structure analysis has

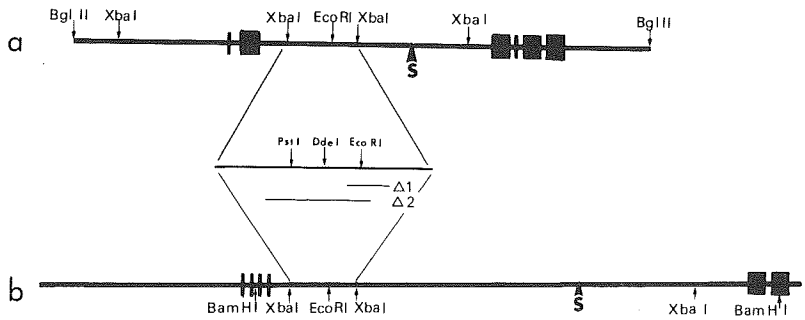

Figure 3 Schematic representation of the heavy-chain gene fragments used for assay of enhancer activity. *a:* The functionally rearranged γ_{2b} gene from the MOPC-141 myeloma (34). Solid boxes represent exons. The 1-kb Xba I fragment with enhancer activity is shown on a larger scale. Sequences deleted from the Xba I fragment are indicated by a solid line. *b:* The μ heavy-chain locus. Banerji et al (31) assayed gene fragments from an unrearranged μ gene. Mercola et al (28) assayed fragments from a rearranged μ gene. However, the fragments were from 3′ of the VDJ joining and so were not affected by gene rearrangement.

shown that most active sequences are contained in a segment lying between the Pst I and Dde I sites within the Xba I fragment. These sequences are effective not only in enhancing transcription of the Ig promoter, but also permit transcription of an enhancerless SV40 promoter. The effect of the sequence appears to be lymphoid-cell specific. When heavy-chain expression is studied in L cells the presence or absence of the 1-kb Xba fragment does not affect the level of heavy-chain synthesis. In addition, this sequence does not permit the expression of an enhancerless SV40 promoter in L cells.

A slightly different approach was used by Banerji (31) to identify enhancing sequences within the heavy-chain IVS. In this case the three Xba I fragments from an unrearranged μ chain gene were tested for their ability to increase the synthesis of T antigen in a vector lacking the SV40 enhancer sequence. The 1-kb Xba I fragment was found to enhance T-antigen expression in myeloma but not HeLa cells. It functioned only in *cis*. This same gene fragment could enhance the expression of β-globin gene transcripts in myeloma cells when located 500 bp upstream or 2500 bp downstream of the β-globin promoter. The Ig enhancer does not function in 3T6, mink lung cells, or human HeLa cells but does function in all myeloma lines tested. Fine structure mapping indicates that sequences important for enhancing are distributed over most of the EcoRI to Pst I fragment of the 1-kb Xba I piece.

Transcriptional enhancer elements have also been detected in the heavy-chain IVS when assayed in a nonlymphoid system (28). In this case an enhancerless SV40 promoter was used to direct transcription of the bacterial chloramphenicol acetyltransferase (CAT) gene. The enhancer activity of various gene fragments was quantified by measuring CAT activity in transfected COS cells. COS cells are an SV40-transformed monkey cell line that constitutively express T antigen (10). The 7.6-kb J_H-C_μ BamHI fragment containing the heavy-chain intervening sequence was capable of enhancing CAT transcription when cloned 3′ to the gene in the "antisense" orientation, but not in the opposite orientation. The Ig sequence had about 60% of the activity of the SV40 enhancer and did not function by increasing gene copy number. When the IVS was divided at the EcoR1 site, both sequences 5′ and 3′ of the EcoR1 site had enhancer activity, the 3′ piece having greater activity. Using this same assay system it is not possible to demonstrate enhancer activity of the J_H-C_μ sequence in CV1 cells, the nontransformed parent line to COS cells. In addition it is unclear how this enhancement in a nonlymphoid system relates to the lymphoid-specific enhancement described above.

Light Chain

Experiments have also identified sequences within the kappa-chain IVS that are necessary for efficient expression of kappa light chain. Queen & Baltimore (11) studied regulation of kappa expression in a transient expression assay. A

vector was constructed with the SV40 origin (minus enhancer) to permit replication in COS cells, and the polyoma early region to permit replication in mouse cells. The kappa-chain gene was inserted so that a SV40 splice and poly A site were 3′ to the gene, so that truncated kappa transcripts could be correctly polyadenylated and processed. Two kappa genes were studied: an intact MOPC41 gene, and an altered MOPC41 gene terminating at the Hind III site within the major intervening sequence (see Figure 4). When these vectors were used to transfect COS cells, both vectors gave rise to the same transcripts in approximately equal quantities. The transcripts originated about 20 bp before the AUG codon used to initiate translation. When the mouse myeloma MPC-11 was used for transfection, the vector with the intact kappa-chain gene gave rise to transcripts originating 20–30 bp before the AUG. When the truncated kappa chain was used, no transcripts originating at the correct 5′ end were observed. Sequences within the kappa-chain gene 3′ of the Hind III site therefore appear necessary for its expression in lymphoid cells.

Picard & Schaffner (unpublished observations) searched for enhancing sequences within the kappa IVS by seeking to identify gene fragments that would enhance expression from a β-globin promoter in a mouse myeloma. When the Hind III-BamHI piece that includes the constant region from kappa chain was included in the vector, correctly initiated β-globin transcripts were seen. Recombinants with λ gene fragments yielded correct β-globin transcripts in neither a kappa nor a lambda myeloma cell line. Fine structure analysis indicated that the entire kappa-chain enhancing activity was contained on a 473 bp Alu I fragment which included a DNase-hypersensitive site (39). The kappa-chain enhancer appears lymphocyte specific because when it was included in the vector, correct β-globin transcripts were not observed in 3T6 cells. In the assay system used, the κ gene enhancer was only about 5% as active as the heavy-chain enhancer, and LPS treatment did not potentiate its activity.

The experiments of Sharon et al (unpublished) demonstrate that the light-chain enhancer can function to enhance transcription from the heavy-chain

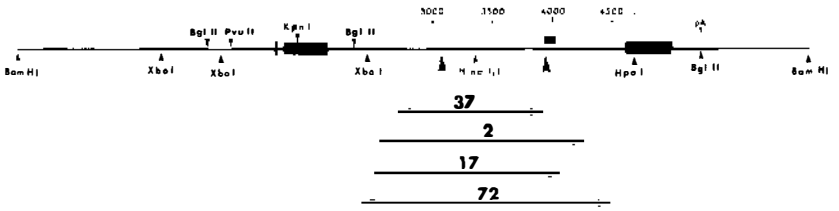

Figure 4 Partial restriction map of the rearranged kappa-chain gene from S107A. The extent of a series of Bal 31 deletions is indicated by solid lines under the gene. Hypersensitive sites in the IVS are indicated by arrowheads (39,40), while the highly conserved sequence is identified by a solid line above the gene (41).

promoter. In the construction lacking the Xba I fragment but containing the Hind-Bam piece from light chain, the V_H was effectively expressed. Adding the Xba I piece into the vector did not increase the level of transcription. Thus in this system the kappa-chain enhancer was fully active and its activity could not be further increased by the heavy-chain enhancer.

To investigate the extent of the regulatory sequences within the kappa IVS, we constructed a series of Bal31 deletions centering around the Hind III site (S. L. Morrison, V. T. Oi, unpublished). Attention was focused on identifying a region within the IVS necessary for kappa-chain synthesis. The Bal31 deletions were assayed both in a hamster lymphoma (GD36) and mouse myeloma (J558L). In both cell lines deletion 37 was found to be functional in directing kappa light chain synthesis while Δ2, Δ17, and Δ72 were decreased in the level of light-chain synthesis. The deletions were reassorted about the Hind III site and either the 5′ or 3′ side of the deletion assayed for its influence on L-chain expression. When assayed in GD36 the 3′ side of Δ2, Δ17, and Δ72 did not direct light-chain synthesis while the 3′ side of 37 and all 5′ deletions were effective in directing L-chain synthesis. A sequence lying 3′ of the terminus of Δ37 and 5′ of the terminus of Δ72 was thus implicated as being important for light-chain synthesis. The region implicated contains the DNase-hypersensitive site seen in the kappa chain of the lymphoma 7OZ (39), one of the DNase-hypersensitive sites seen in a mouse myeloma (40), and the region of sequence that is highly conserved between mouse, rabbit, and human (41). This region therefore appears to contain enhancer-like sequences necessary for expression in a lymphoma.

When the deletions were assayed in a mouse myeloma a different result was obtained. In the myeloma Δ2, Δ17, and Δ72 were deficient in their synthesis of Ig. However, when the 3′ and 5′ sides of the deletions were assayed, both were found to be active in directing light-chain synthesis. Therefore, in the myeloma cell lines two regions, one 5′ to the Hind III site, the second 3′ to the Hind III were shown to have enhancing activity. If both regions are deleted the kappa gene cannot be expressed following transfection. However, presence of either the 5′ or the 3′ sequence is adequate to permit kappa production. In fact, in contrast to studies of lymphoma, DNase sensitivity studies by Chung et al (40) have identified two DNase-sensitive regions in the IVS of kappa genes in myeloma cell lines, one 5′ of the Hind III site and the second 3′ of the Hind III site. It must now be shown that it is this second region that enhances the expression of kappa light chains in transfected J558 cells.

CONCLUSIONS AND PROSPECTS

Although gene transfection has only been used for a short time to study Ig synthesis, it has already proven itself a valuable tool.

Transfection of Ig genes has been used to identify the first cellular enhancer elements and to localize them to the intronic sequences of Ig H- and L-chain genes. Furthermore, these enhancer-like sequences have been demonstrated to function only in B-cell lines; these results have led to the concept that cellular enhancer elements may provide the basis for tissue-specific or promoter-specific regulation of genes. A corollary to this is that genes coordinately expressed during differentiation may be controlled by recognition of shared sets of enhancer sequences. Experiments in the future must be designed to identify the molecular basis of enhancer function.

In addition to furthering our knowledge of Ig gene controlling elements, gene transfection also makes possible advances in our understanding of Ig structure and function. Transfected genes are expressed in sufficient quantity to permit the isolation of protein for detailed studies. Assembly between either a resident Ig chain and a transfected Ig chain or between two simultaneously transfected genes occurs, and the assembled molecules are inserted into membranes and/or secreted. Thus it is possible to generate cell lines making novel combinations of heavy and light chain. In addition, chimeric genes encoding molecules with reassorted variable and constant regions can be made. These Igs will provide valuable new reagents for immunochemical analyses. Using this methodology, we are on the threshold of a new understanding of antibody structure and function, and of the cell biology of Ig biosynthesis.

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