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Genetics

Deletion of a B-cell-specific enhancer affects transfected, but not endogenous, immunoglobulin heavy-chain gene expression

(mouse myeloma cells/transcriptional regulation/gene transfer)

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ABSTRACT **A transcriptional enhancer has recently been identified between the variable and constant region coding segments of an assembled immunoglobulin heavy-chain gene. This enhancer is required for efficient expression of such genes when transfected into myeloma cells. A class switch rearrangement in the mouse myeloma cell line 9.9.2.1 has resulted in deletion of this enhancer, and yet this cell line continues to produce heavy chains at a high level. Cell line 9.9.2.1 is a γ2a-producing class-switch variant derived from the γ2b-producing myeloma cell line, MPC11. We demonstrate that despite the high level of heavy-chain production in 9.9.2.1, the cloned 9.9.2.1 heavy-chain gene is not expressed efficiently when transfected into myeloma cells. Efficient expression after transfection can be obtained only if the deleted enhancer is reinserted into the gene. The implication of this finding for the role of this enhancer in establishing and/or maintaining immunoglobulin heavy-chain gene expression is discussed.**

Enhancers are short DNA sequences that can regulate gene expression by increasing the transcription of nearby genes (reviewed in ref. 1). Enhancers appear to function in a manner that is relatively independent of their position and orientation with respect to the activated gene. An enhancer has been identified within the intervening sequence separating variable- and constant-region coding segments (the major intron) of rearranged immunoglobulin heavy-chain genes (2–4). This enhancer was found to be essential for the efficient expression of heavy-chain genes when transfected into B-lymphocyte-derived cells, but it had no effect on expression of these genes when transfected into nonlymphoid cells (2, 3). However, transfection experiments do not establish whether or not this enhancer is also essential when the B-cell heavy-chain gene is present in its natural chromosomal location.

The 9.9.2.1 myeloma cell line is an $IgG_{\gamma 2a}$-producing class-switch variant that was isolated from the $IgG_{\gamma 2b}$-producing, MPC11 cell line via an "intermediate," 9.7.1 (5). The class switch from γ2b to γ2a heavy-chain production in 9.9.2.1 cells is the result of a gene rearrangement whose 5′ breakpoint maps upstream of the recently described heavy-chain enhancer and whose 3′ breakpoint lies 6.5 kilobases (kb) upstream of γ2a constant-region coding sequences ($C_{\gamma 2a}$) (Fig. 1) (6). The result is deletion of the enhancer and $C_{\gamma 2b}$ so that MPC11 heavy-chain variable-region coding sequences (V_{MPC11}) are now transcribed in association with $C_{\gamma 2a}$. We find that, despite deletion of the enhancer, 9.9.2.1 cells continue to produce high levels of immunoglobulin. Since the 3′ breakpoint of the class-switch rearrangement in 9.9.2.1 is 6.5 kb upstream of $C_{\gamma 2a}$, we postulated (6) that these $C_{\gamma 2a}$ flanking sequences, now constituting part of the intron separating V_{MPC11} and $C_{\gamma 2a}$, might include a region with

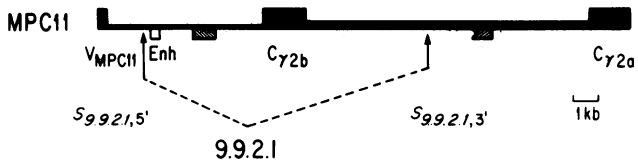

FIG. 1. Scheme of the class-switch rearrangement that has occurred in the MPC11 heavy-chain gene to give rise to the 9.9.2.1 class-switch variant cell line. Arrows point to the 5′ and 3′ breakpoints of the class-switch rearrangement. Black boxes represent variable- and constant-region coding sequences. Cross-hatched boxes represent regions consisting of tandemly repeated sequences. Region containing the heavy-chain enhancer is indicated as an open box (Enh).

enhancer function. To test this, we cloned the 9.9.2.1 heavy-chain gene into a simian virus 40 expression vector, pSV2gpt (7, 8), with and without addition of a DNA fragment containing the previously described heavy-chain gene enhancer. These plasmids were used to transfect the J558L myeloma cell line. We also transfected J558L cells with a heavy-chain gene similar to the one expressed by MPC11, both before and after removal of the enhancer. Our results indicate that the cloned 9.9.2.1 heavy-chain gene does not contain sequences with enhancer function and that for efficient expression of this gene in transfection assays, it must be modified by addition of the previously identified enhancer region.

MATERIALS AND METHODS

Cell Lines. 45.6.2.4 is a γ2b/κ-producing myeloma cell line derived from the BALB/c mouse tumor, MPC11 (9). We refer to this cell line throughout the text as MPC11. Cell line 9.7.1 is a variant cell line isolated after mutagen treatment of 45.6.2.4 cells (10). It produces an abnormally large heavy chain encoded by a gene that contains both $C_{\gamma 2b}$ and $C_{\gamma 2a}$ sequences (6, 11). Cell line 9.9.2.1 is a γ2a/κ-producing class-switch variant that arose spontaneously from 9.7.1 (5). M319.2 is a γ2a/κ-producing switch variant that was isolated directly from mutagenized MPC11 cells (10). J558L produces λ light chains but no immunoglobulin heavy chains, and it was isolated as a spontaneous variant of the α/λ-producing J558 myeloma cell line (12). All of the cell lines were maintained in Dulbecco's modified Eagle's medium containing 15% horse serum, 100 units of penicillin per ml, and 100 μg of streptomycin per ml.

Plasmid Construction. *Eco*RI restriction fragments containing the γ2a and γ2b constant region coding sequences ($C_{\gamma 2a}$ and $C_{\gamma 2b}$) were subcloned from the recombinant λ phages $ChM_{\gamma 2a}$-9 (13) and RBL216 (14), respectively, into the *Escherichia coli* plasmid pBR325. *Eco*RI/*Bgl* II fragments (4.3 kb) were isolated from these subclones and ligated to

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Abbreviation: kb, kilobase(s).

*Eco*RI/*Bam*HI digested, calf intestinal phosphatase-treated pSV2gpt vectors (7, 8). The resultant plasmids were designated pγ2a and pγ2b. The *Eco*RI/*Bgl* II fragments contain $C_{\gamma 2a}$ (or $C_{\gamma 2b}$) and flanking sequences (see Fig. 3), including the polyadenylylation site for the secreted form of the heavy chains; they do not contain the membrane exons of either constant region gene.

*Eco*RI restriction fragments containing the promoter region (G. Gilmore, personal communication) and transcriptional start site, the leader and variable region coding sequences (V_{MPC11}), and most of the major introns of the 9.7.1 (6.0 kb) and the 9.9.2.1 (7.9 kb) heavy-chain genes were subcloned from the recombinant phages Ch9.7.1 (6) and Ch9.9.2.1 (6), respectively, into the *Eco*RI-digested, calf intestinal phosphatase-treated plasmids pγ2a and pγ2b. The resultant plasmids containing both V_{MPC11} and $C_{\gamma 2a}$ (or $C_{\gamma 2b}$) in the same transcriptional orientation were designated p97γ2a or p97γ2b (which contain the previously described enhancer) and p99γ2a or p99γ2b (which are missing the enhancer).

The 1.0-kb *Xba* I fragments containing the enhancer were removed from p97γ2a and p97γ2b by partially digesting plasmids with *Xba* I, isolating the appropriately sized linear fragments from a low-melting-point agarose gel, and then recircularizing the isolated fragments. The resultant plasmids were designated p97ΔEnhγ2a and p97ΔEnhγ2b. The same 1.0-kb *Xba* I fragments were inserted into *Xba* I sites in the plasmids p99γ2a and p99γ2b (see Fig. 3) to form the plasmids designated p99Enhγ2a and p99Enhγ2b.

Transfections. J558L cells were transfected by protoplast fusion (15) essentially as described by Oi *et al.* (12) except that polyethylene glycol (50%) was dissolved in a calcium-deficient medium (150 mM NaCl/5 mM KCl/8 mM sodium phosphate buffer, pH 7.2/0.2% D-glucose). Then, 10^5 or 10^4 cells were plated in 24- or 96-well plates, respectively, and colonies of transformants were visible in 7–14 days. The frequency of transformation ranged from 0.2 to 1.8 transformants per 10^5 cells. In most transfection experiments, transformants arose in <37% of the culture wells, indicating that each transformant line was most likely derived from a single cell.

ELISA. Secreted immunoglobulins were detected by ELISA. Immunlon 2 plates (Dynatech) were coated overnight at 4°C with 50 μl of affinity-purified goat anti-mouse IgG (0.1 mg/ml), Fc fragment-specific (Cappel Worthington); 50 μl of serial dilutions from 48-hr culture supernatants of 1×10^6 cells was incubated with the coated wells for 1 hr at room temperature. Specific immunoglobulin was detected with alkaline-phosphatase-conjugated goat anti-mouse immunoglobulins (Cappel Worthington), using *p*-nitrophenol phosphate as the enzyme substrate. The assay was quantitated in an ELISA plate reader (Bio-Tek, Burlington, VT).

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Cells (5×10^6) were cultured in the presence of 100 μCi of [^{35}S]methionine for 3 hr at 37°C (1 Ci = 37 GBq). Cells were lysed with 0.5% Nonidet P-40 and precipitated with heat-killed *Staphylococcus aureus*, Cowan Strain I. The A protein from *S. aureus* binds mouse IgG2a and IgG2b. Immunoglobulin was eluted from the *S. aureus* by the addition of gel sample buffer containing 2.3% $NaDodSO_4$. The eluted protein was reduced with 2-mercaptoethanol and applied to 10% $NaDodSO_4$/polyacrylamide gels (16). For the experiment shown in Fig. 2, cells were not incubated with [^{35}S]methionine prior to lysis and *S. aureus*-precipitated immunoglobulins were visualized by Coomassie blue staining.

Southern Analysis. Agarose gel electrophoresis, transfer to nitrocellulose, and DNA hybridizations were performed as described (11). Restriction enzyme-digested DNA (9 μg) was run in each lane, the amount of DNA having been quantitated by a diaminobenzoic acid assay (17).

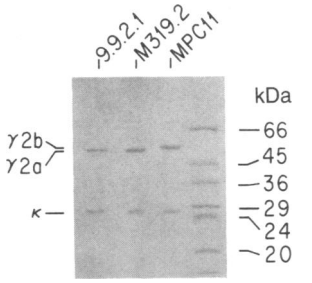

FIG. 2. Coomassie blue-stained 10% $NaDodSO_4$/polyacrylamide gel comparing precipitated immunoglobulin from the MPC11, M319.2, and 9.9.2.1 cell lines. Each gel lane contains immunoglobulin from 3×10^6 cells.

RNA Blot Analysis. RNA blots were performed essentially as described by Maniatis *et al.* (18). Twenty micrograms of total cytoplasmic RNA (estimated from the OD_{260} of the RNA solutions) was run in each lane.

RESULTS

To assess the effect of the heavy-chain gene enhancer deletion on the amount of heavy chain produced in 9.9.2.1, heavy-chain levels in this cell line were compared with those of related cell lines. Precipitated immunoglobulins from equal numbers of MPC11, M319.2, and 9.9.2.1 cells were compared after $NaDodSO_4$/polyacrylamide gel electrophoresis and staining with Coomassie blue. M319.2 is, like 9.9.2.1, an $IgG_{\gamma 2a}$-producing class-switch variant derived from MPC11 (10). However, the γ2a heavy-chain gene that is formed after M319.2's class-switch rearrangement still contains the heavy-chain gene enhancer (6). As shown in Fig. 2, the amount of heavy chain produced by 9.9.2.1 cells is equal to the amounts produced by the enhancer-containing MPC11 and M319.2 cell lines.

The possibility that a second enhancer had replaced the deleted one in the 9.9.2.1 heavy-chain gene was tested by quantitating the expression of the cloned 9.9.2.1 heavy-chain gene after transfection into another myeloma cell line. For these studies, a heavy-chain gene containing the previously described enhancer and the 9.9.2.1 heavy-chain gene, both with and without the addition of that enhancer, were cloned into the pSV2gpt expression vector (7, 8). The resulting plasmids were transfected into myeloma cells and stable transformants for bacterial xanthine/guanine phosphoribosyltransferase (Eco gpt) expression were isolated by selection in medium containing mycophenolic acid and xanthine (7, 8). Restriction maps of the plasmid constructions used in these transfection studies are shown in Fig. 3. Plasmid p99γ2a is a reconstruction of the entire expressed heavy-chain gene in 9.9.2.1 (6). Plasmid p99Enhγ2a differs from p99γ2a only by the insertion of a 1.0-kb *Xba* I fragment containing the previously identified enhancer.

Plasmid p97γ2a includes an *Eco*RI fragment containing the variable region and major intron (including the known enhancer) of the expressed heavy-chain gene from another MPC11 class-switch variant, 9.7.1. The one detectable difference between MPC11 and 9.7.1 in the DNA included within this fragment is the loss of an *Eco*RI recognition site in 9.7.1 (the usual position of this site in the MPC11 gene is designated by an asterisk in Fig. 3) (6). Since the loss of this site simplified our constructions, we used 9.7.1-derived sequences in these cloning experiments instead of those derived from MPC11. Plasmid p97ΔEnhγ2a is the same as p97γ2a except that it is missing the 1.0-kb *Xba* I fragment that contains the enhancer.

Also constructed were plasmids identical to the ones depicted in Fig. 3 except that the *Eco*RI/*Bgl* II fragments that contain the γ2a constant region were replaced with *Eco*RI/*Bgl* II fragments containing the γ2b constant region. Comparisons of transformants obtained by transfection with γ2a-containing plasmids versus γ2b-containing plasmids al-

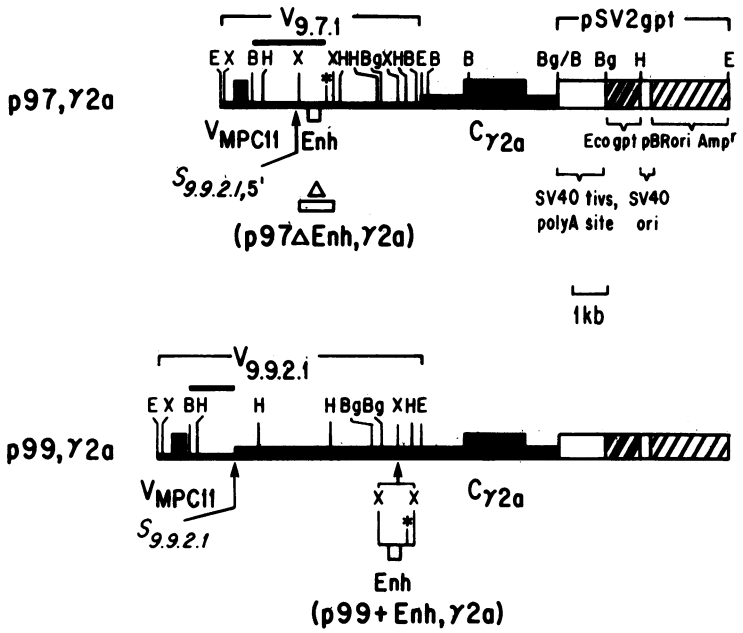

FIG. 3. Restriction maps of plasmid constructions used in transfection experiments. Eco gpt coding sequences and simian virus 40 (SV40)-derived sequences present in the pSV2gpt vector are indicated. Black boxes represent the variable- and constant-region coding sequences of the heavy chain. The 5' class-switch rearrangement breakpoint of 9.9.2.1 ($S_{9.9.2.1}$) is indicated on the p97,γ2a and p99,γ2a restriction maps. The $V_{9.7.1}$ and $V_{9.9.2.1}$ *Eco*RI fragments differ 3' of this switch site. The heavy-chain enhancer is represented by an open box (Enh). The deletion and insertion of the 1.0-kb *Xba* I fragment used to generate p97,ΔEnhγ2a and p99,Enhγ2a, respectively, are indicated. E, *Eco*RI; X, *Xba* I; B, *Bam*HI; H, *Hin*dIII; Bg, *Bgl* II; * corresponds to the location of an *Eco*RI site in MPC11 that is missing in 9.7.1 (see text). Black bar above each restriction map indicates the sequences recognized by the pJ11 probe.

lowed us to control for possible class-specific effects (e.g., transcriptional control sequences present in or near one constant region gene but not the other). This control is important because 9.9.2.1 and MPC11 cells produce immunoglobulins of different classes.

The resultant recombinant plasmids were transfected into J558L cells by protoplast fusion (12, 15). J558L is a myeloma cell line that no longer synthesizes a heavy chain but that continues to produce a λ light chain (12). A heavy chain produced through the expression of an exogenous gene introduced into J558L by transfection can associate with this λ light chain so that complete immunoglobulins (two heavy chains, two light chains) are secreted from the cell. We measured the amount of immunoglobulin secretion ($IgG_{\gamma 2a}$ or $IgG_{\gamma 2b}$) occurring in stable transformants by performing ELISAs on 48-hr supernatants collected from cultures initiated with equal numbers of cells. Nine of 13 transformants obtained by transfection with p97γ2a had accumulated relatively high levels of antibody in the 48-hr culture supernatants (Fig. 4*A*). The four exceptions were shown by genomic DNA analysis to have partially deleted and/or rearranged copies of the transfected plasmids integrated in their genomes (data not shown). In contrast, 6 of 6 transformants obtained by transfection with p97ΔEnhγ2a had accumulated less antibody by a factor of ≈40 in the 48-hr culture supernatants, and 13 out of 13 transformants obtained by transfection with p99γ2a secreted little or no antibody. These results suggest that there is no sequence present in the cloned 9.9.2.1 heavy-chain gene that can act as an enhancer for transfected gene expression. However, 8 of 12 transformants obtained by transfection with p99Enhγ2a secreted high levels of antibody. This last result demonstrates that the cloned 9.9.2.1 heavy-chain gene is capable of being expressed at high levels after transfection if provided with the necessary enhancer. Similar results were obtained with transformants that had received the Cγ2b-containing plasmids (Fig. 4*B*).

It should be noted that there are 1.5 kb of 5' flanking sequences to $C_{\gamma 2b}$ in the major intron of p97γ2b that were not present in the corresponding intron of plasmid constructions used in earlier studies describing the enhancer (2). Inefficient heavy-chain gene expression in transformants containing p97ΔEnhγ2b indicate that these additional sequences have no enhancer function.

Since in the ELISAs used, intact heavy chains would not be distinguished from aberrant heavy chains still reactive with the antibodies used in the assay, we extended our analysis of the immunoglobulins produced by the different classes of transformants. Biosynthetically labeled ([^{35}S]-methionine) immunoglobulin was precipitated from transformants and analyzed on $NaDodSO_4$/polyacrylamide gels under reducing conditions. As shown in Fig. 5, the heavy chains produced in the transformants were of the same molecular weight as those produced in 9.9.2.1 cells. Only the transformants that had received plasmids containing the

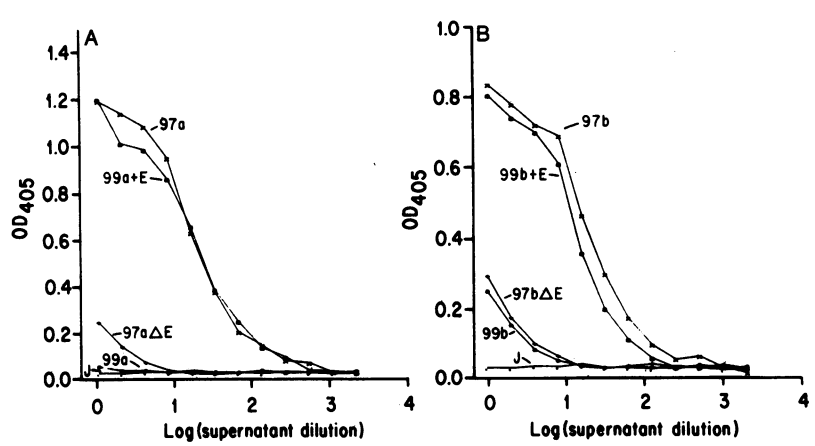

FIG. 4. Titration curves of immunoglobulin accumulated in culture supernatants as measured by ELISA. Supernatants were collected from 1×10^6 cells after 48 hr of culture. In the ELISA, alkaline phosphatase-conjugated goat antibodies to mouse immunoglobulins were used as a second-step reagent. The reaction of this reagent with *p*-nitrophenol phosphate yields a colored product with an absorbance peak at 405 nm. Each curve is an average of three independent transformants that were transfected with the same plasmid construction. (*A*) 97a, transformants obtained after transfection with p97γ2a; 99a+E, p99Enhγ2a; 97aΔE, p97ΔEnhγ2a; 99a, p99γ2a; J, J558L. (*B*) 97b, p97γ2b; 99b+E, p99Enhγ2b; 97bΔE, p97ΔEnhγ2b; 99b, p99γ2b; J, J558L.

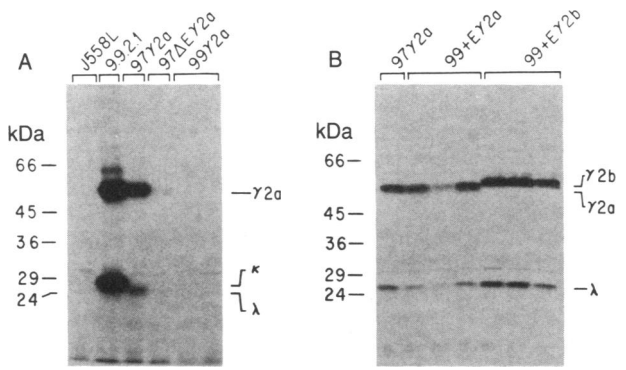

FIG. 5. Autoradiograms of 10% NaDodSO$_4$/polyacrylamide gels showing biosynthetically labeled ([^{35}S]methionine) immunoglobulin. Each lane was loaded with immunoglobulin isolated from an independently transformed cell line. The J558L λ light chain is only precipitated by *S. aureus* when it is associated with a heavy chain. Transformant lines (left to right): (*A*) 97γ2a-3; 97ΔEγ2a-4; 99γ2a-2, -1 (*B*) 97γ2a-3; 99+Eγ2a-2, -6, -15; 99+Eγ2b-1, -2, -3.

known enhancer made high levels of heavy chain. There was again no indication that the cloned 9.9.2.1 heavy-chain gene was being expressed in the transformants.

To verify that all classes of transformants had received intact copies of the transfected genes, and to determine the copy number of the integrated plasmids, genomic DNA was isolated from the transformants and analyzed. DNA was digested with *Eco*RI, size-fractionated on agarose gels, and blotted to nitrocellulose. The blots were then probed with pJ11, which hybridizes to sequences between the *Bam*HI site and the missing *Eco*RI site in the major intron of the 9.7.1 heavy-chain gene and to sequences between the *Bam*HI site and the switch site in the 9.9.2.1 heavy-chain gene (indicated by the solid bars in Fig. 3). The pJ11 probe also recognizes sequences endogenous to the recipient J558L cells because two restriction fragments are detected with this probe in size-fractionated *Eco*RI-digested J558L DNA (Fig. 6*A*). No additional bands are seen in DNA from transformants obtained by transfection with the pSV2gpt vector alone. Additional bands of the predicted sizes (6.0 kb for p97γ2a, 5.0 kb for p97ΔEnhγ2a, and 7.9 kb for p99γ2a) are seen, however, in *Eco*RI-digested DNA from transformants obtained by transfection with plasmids containing the cloned heavy-chain genes (Fig. 6). When the same blot is erased and probed with pSV2gpt sequences, additional bands, again of the predicted sizes (5.4 kb for pSV2gpt and 9.4 kb for the others) are detected (data not shown). Predicted patterns are also seen in blots of *Hin*dIII-digested DNA from transformants obtained by transfection with p99γ2a and probed with the entire transfected plasmid (data not shown). The results indicate that most transformants (in all the transformant classes) carried intact integrated copies of the transfected genes.

It is also evident from DNA hybridization patterns that all transformants that had received p97γ2a had only a few integrated copies of the transfected gene. In contrast, while some transformants that had received p97ΔEnhγ2a or p99γ2a also had only a few integrated copies of the transfected genes, others carried these genes in much higher copy numbers (Fig. 6). The amount of DNA loaded in each gel lane was normalized by diaminobenzoic acid assays (17) and by comparing the intensity of the hybridization signal from J558L-derived pJ11 hybridizing fragments. The level of heavy-chain production in transformants with only a few integrated copies of enhancer-containing genes was ≈40-fold higher than that of transformants that had perhaps 20 times the number of integrated enhancer-deficient gene copies. Interestingly, no differences in heavy-chain production were seen between transformants with very high copy numbers of enhancer-deficient genes and those with only a few copy numbers of the same genes.

To determine whether the differences in antibody levels among the transformants corresponded to equivalent differences in immunoglobulin mRNA levels, total cytoplasmic RNA was isolated from representative transformants, size-fractionated on denaturing agarose gels, blotted to nitrocellulose, and hybridized with a γ2a cDNA probe (Fig. 7). High levels of specific message were seen only in transformants obtained after transfection with enhancer-containing plasmids. The blots were erased and probed with a λ light-chain cDNA to verify that each lane had been loaded with approximately equal amounts of intact RNA (data not shown). These results suggest that the differences seen in heavy-chain production between the various classes of transformants reflect differences in steady-state mRNA levels. No heavy-chain mRNA was detected in transformants obtained by transfection with p99γ2a, again indicating the lack of an enhancer in the cloned 9.9.2.1 heavy-chain gene.

DISCUSSION

In earlier studies, we showed that in the 9.9.2.1 myeloma line, and possibly in another class-switch variant cell line, the heavy-chain gene enhancer has been deleted as a result of class-switch rearrangement (6). We now demonstrate that in 9.9.2.1, despite the deletion of the heavy-chain gene enhancer from the major intron of the expressed γ2a gene, a high level

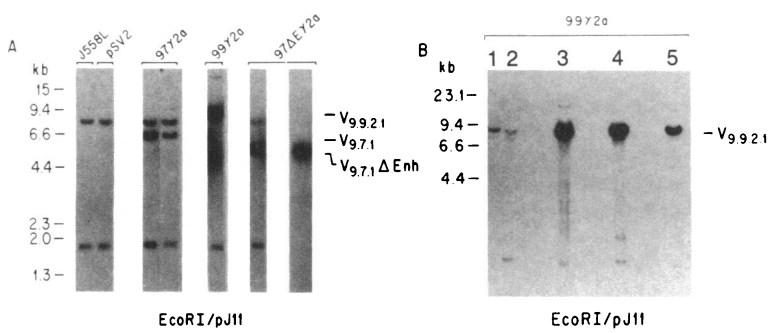

FIG. 6. Southern analysis of genomic DNA isolated from transformed cell lines. (*A*) DNA was digested with *Eco*RI, size-fractionated on an agarose gel, blotted to nitrocellulose, and then hybridized with pJ11. Transformant lines (left to right): 97γ2a-12, -3; 99γ2a-5; 97ΔEγ2a-4, -7. Exposure time for the 97ΔEγ2a-7 lane was 1/24th that of the other lanes. The genome of this transformant contains many copies of the transforming plasmid (see text). (*B*) p99γ2a transformants containing a few (lanes 1 and 2) or many (lanes 3, 4, and 5) copies of the transforming plasmid. DNA was treated as in *A*. Transformant lines (left to right): 99γ2a-5, -6, -2, -11, -15. Extra hybridization bands found in some transformants are most likely "junctional" fragments, which are formed at the site of plasmid integration and contain both plasmid and genomic sequences.

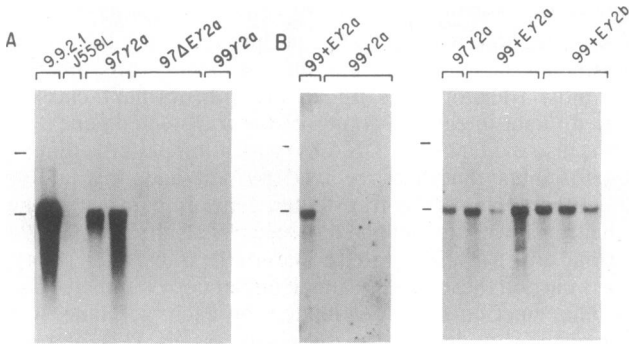

FIG. 7. RNA blot analysis of total cytoplasmic RNA isolated from transformed cell lines. Twenty micrograms of RNA was loaded in each lane, size-fractionated on agarose gels, blotted to nitrocellulose, and hybridized with a $C_{\gamma 2a}$ cDNA. Transformant lines (left to right): (*A*) 97γ2a-3, -9; 97ΔEγ2a-4, -6; 99γ2a-2. (*B*) 99+Eγ2a-2; 99γ2a-2, -5, -7; 97γ2a-3; 99+Eγ2a-2, -6, -15; 99+Eγ2b-1, -2, -3. Migration distances of 28S and 18S ribosomal RNAs are indicated to the left of each autoradiogram.

of heavy-chain gene expression is maintained. Recently, efficient heavy-chain gene expression after enhancer deletion has also been described in an Abelson viral-transformed pre-B-cell line (19) and in a hybridoma IgD-producing class-switch variant line (20). In both of these reports, enhancer deletion was implicated by restriction mapping of total genomic DNA. Since in these cases, and in the case of 9.9.2.1, it was clearly possible that a second enhancer had replaced the deleted enhancer, we initiated studies of the expressed heavy-chain gene cloned from 9.9.2.1.

Our studies demonstrate that despite the high levels of heavy-chain production in 9.9.2.1, the cloned 9.9.2.1 heavy-chain gene cannot be expressed efficiently after transfection into J558L cells unless the deleted enhancer is reinserted into the gene. These results imply that there is no "substitute" enhancer that has taken the place of the deleted enhancer in the 9.9.2.1 heavy-chain gene. We cannot rule out the possibility that a second enhancer is present farther upstream (5′ with respect to transcription) of the expressed variable-region gene or farther downstream (3′) of the expressed γ2a constant-region gene. However, our p99γ2a construction includes 11.6 kb 3′ of the heavy-chain gene promoter region. With respect to 5′ flanking sequences, observed differences in the transcriptional activity of rearranged and unrearranged (germline) variable-region genes have been interpreted as an indication that elements conferring high transcriptional activity upon rearranged immunoglobulin genes do not lie 5′ of the variable-region coding segments (21). Recently, Mercola *et al.* (22) failed to find transcriptional enhancers in sequences up to ≈5.7 kb 5′ of a germline heavy-chain variable-region gene.

It is conceivable that a second type of enhancer element may be present in the 9.9.2.1 heavy-chain gene that can be recognized in 9.9.2.1 cells but not in J558L cells. This apparent cell specificity might simply reflect the loss, in J558L, of a function common to most myelomas. This hypothesis is currently being tested by transfecting the cloned 9.9.2.1 heavy-chain gene into a nonproducing (heavy-chain gene deletion) variant of the 9.9.2.1 cell line.

The results reported here could be taken to imply that while the heavy-chain gene enhancer is essential in transfection experiments, it may not be essential when the heavy-chain gene is present in its natural position on mouse chromosome 12. This could mean that the enhancer does not regulate normal endogenous heavy-chain gene expression. Alternatively, the enhancer may have a role in normal heavy-chain gene expression, but other long-range *cis*-acting elements present on chromosome 12 may be capable of compensating for its absence.

A model consistent with the results reported here is that the enhancer may be needed only initially to establish high-level transcription of the heavy-chain gene. Once the transcriptional rate of the gene is established, it may remain unchanged throughout subsequent cell divisions regardless of the presence or absence of the enhancer. According to this model, the transfected gene, upon entry into the cell, would require the action of the enhancer to establish its high-level expression. Presumably, after this initial activation, the enhancer would also cease to be necessary to the transfected gene. Deletion of the enhancer from the heavy-chain gene of a stable transformant would be predicted to have little or no effect on heavy-chain production. If the tissue specificity of the heavy-chain enhancer is due to the tissue-specific expression of a *trans*-acting factor (22, 23), this factor must continue to be produced in myeloma cells, because in cell lines such as J558L, high-level expression of transfected heavy-chain genes remains enhancer dependent. The continued production of this factor, despite the postulated loss of a requirement by the endogenous heavy-chain gene for the enhancer, might be explained if the factor had additional regulatory roles in these cells.

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