Role of the Intronic Elements in the Endogenous Immunoglobulin Heavy Chain Locus

EITHER THE MATRIX ATTACHMENT REGIONS OR THE CORE ENHANCER IS SUFFICIENT TO MAINTAIN EXPRESSION

High level expression in mice of transgenes derived from the immunoglobulin heavy chain (IgH) locus requires both the core enhancer ($E_{\mu}$) and the matrix attachment regions (MARs) that flank $E_{\mu}$. The need for both elements implies that they each perform a different function in transcription. While it is generally assumed that expression of the endogenous IgH locus has similar requirements, it has been difficult to assess the role of these elements in expression of the endogenous heavy chain gene, because B cell development and IgH expression are strongly interdependent and also because the locus contains other redundant activating elements. We have previously described a gene-targeting approach in hybridoma cells that overcomes the redundancy problem to yield a stable cell line in which expression of the IgH locus depends strongly on elements in the MAR-$E_{\mu}$-MAR segment. Using this system, we have found that expression of the endogenous $\mu$ gene persists at substantially (~50%) normal levels in recombinants which retain either the MARs or $E_{\mu}$. That is, despite the dissimilar biochemical activities of these two elements, either one is sufficient to maintain high level expression of the endogenous locus. These findings suggest new models for how the enhancer and MARs might collaborate in the initiation or maintenance of transcription.

Expression activating elements in the immunoglobulin heavy chain (IgH) locus (Fig. 1) have been identified and characterized in diverse ways. An enhancer lying in the VDJ-$E_{\mu}$ intron was originally detected by its capacity to stimulate transcription from linked reporter genes that were transfected into myeloma cell lines (1, 2). Experiments using transgenic cell lines from which this enhancer could be deleted after transcription had been initiated indicated that the enhancer was required to maintain high level expression (3, 4). The enhancer-bearing segment can be divided into two subregions on the basis of sequence motifs and protein-binding characteristics. Thus, multiple transcription factors that bind to characteristic motifs define a central core ($E_{\mu}$) region; this region is flanked by matrix attachment regions (MARs) identified by their affinity for the nuclear matrix (reviewed in Refs. 5 and 6). Both elements, $E_{\mu}$ and the MARs, are required for high level expression of IgH-derived transgenes in mice, albeit not in stably transfected cell lines (7). The switch (S) regions were first recognized as repetitive DNA segments that lie 5′ of the exons encoding heavy chain constant regions and which are the sites of most of the breakage and rejoining events underlying the heavy chain isotype switch. These switch regions also harbor elements that stimulate expression of IgH-derived reporter genes in transgenic mice, but again this activity has not been detected in assays of stably transfected cell lines (8, 9). That all three types of elements are required for high level expression in transgenic mice indicates that each element performs a different and necessary function in transcription. Evidence of the $E_{\mu}$-MAR collaboration can also be seen in assays of chromatin accessibility (10) and DNA demethylation (11).

It is generally assumed that the expression-activating elements defined in transgene expression assays function similarly in the transcription of genes in their endogenous chromosomal loci. Thus, while the intronic MARs and $E_{\mu}$ core enhancer are not needed to maintain expression of the endogenous IgH locus in some B-cell lines (12–15), this difference is generally ascribed to the presence of other, functionally redundant, activating elements present in the IgH locus but absent from the transgenes, viz., the MARs that are located 5′ of the Cy exons (16) and enhancers that are located 3′ of the Ca exons (reviewed in Ref. 17). In fact, the capacity of the intronic elements to contribute to IgH expression can be observed under conditions that ablate this redundancy. Thus, targeted replacement of one of these 3′ $E_{\alpha}$ enhancers by the neo gene extinguishes IgH expression specifically in those myeloma cells that lack the intronic activating elements (18).

We have previously described a related method of ablating redundancy in which introduction of the gpt cassette 3′ of the endogenous $\mu$ heavy chain gene of hybridoma cells apparently insulates the transcription unit from other activating elements and renders $\mu$ expression dependent on the intronic activating elements (6). That is, in this configuration, deletion of the 5′-MAR-$E_{\mu}$-3′-MAR segment depresses $\mu$ expression to ~2% the normal level (6). Although the mechanism by which the gpt cassette renders the $\mu$ gene expression dependent on the intronic elements is unknown, this approach offers the possibility of examining how these elements function in a reproducible and nearly normal context. Thus, using this system we showed that recombinants that retain a substantial segment of the MARs maintain $\mu$ expression at a high (~60%) normal level. In the context of models in which $\mu$ expression requires both MAR and $E_{\mu}$ functions, this result implies that other enhancers in
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FIG. 1. Structure of IgH loci and targeting vectors. A, structure of the endogenous IgH locus of Sp6 hybridoma cells. This diagram shows position of relevant restriction sites and elements of interest: MARs, Eμ enhancer, and Sμ switch region. B, generation of targeted recombinants. As illustrated here, the targeted recombinants were produced by recombination between the transfected vector and the endogenous IgH locus of the mutant hybridoma igm692, which lacks the Cμ1 and Cμ2 exons and a segment of the switch region (22, 32). The construction of the targeting vectors is described under "Materials and Methods." The selection of targeted recombinants in MHX medium, screening for normal IgM production and DNA junctions, and composition of the probes and primers are described under "Materials and Methods." C, structure of recombinant IgH loci. The recombinants bear the indicated parts of the Nael-SnaI (b) segment of the JH-Cμ switch region. The indicated Nael site and define segment B represented in the targeting vector (see B, above). As indicated, the Eμ and MAR segments were designed to overlap by 13–17 nucleotides in an effort to ensure that no enhancer-specific site was interrupted. These short segments are expected to contribute no significant MAR activity (23, 33).

intrinsic elements from the active IgH locus of a mouse hybridoma cell line, Sp6, which produces IgM(κ) specific for the hapten, triaminophenyl (6, 24). This system makes use of a mutant hybridoma, igm692, which encodes a truncated immunoglobulin μ heavy chain lacking the Cμ1 and Cμ2 domains (Fig 1). The targeting vector bears the bacterially derived gpt gene for guanine-xanthine phosphoribosyltransferase, which allows cells to convert exogenous xanthine to XMP and to grow

RESULTS

Construction of Targeted Recombinants—We have previously described a gene-targeting system to remove various

MER, Eμ’ MAR’, and Eμ’ MAR’ recombinants, respectively. The recombinant IgH locus can act on the μ gene, but that other MARs in the locus cannot. We have now tested this interpretation by examining the properties of the reciprocal recombinant that retained Eμ but lacked the MARs. As reported here, we have found unexpectedly that this recombinant also continued to express the μ gene at a high (~50%) normal level, a result that implies that other MARs in the recombinant IgH locus, but not other enhancers, can act on the μ gene. These observations then pose the paradox that high level μ expression in the recombinant hybridoma cells requires a MAR or an enhancer, even though the recombinant IgH locus can supply both of these functions. We present two models that might account for this puzzling combination of observations.

MATERIALS AND METHODS

Construction of Targeting Vectors—We have previously described the Eμ’ MAR’ recombinants (6). Fig. 1 illustrates the construction of the three targeting vectors, p0, pEμ, and pMM, used to generate the Eμ MAR, Eμ’ MAR’, and Eμ’ MAR’ recombinants, respectively. The targeting vectors are composed from seven DNA segments (6). Thus, the NcoI-NaeI segment A included the VDJ3 nucleotides of GenBankTM/EMBL accession number X56936. Segment B was specific for each recombinant, such that p0 lacked the entire 5’-MAR-Eμ’-MAR segment, while pEμ and pMM included the Eμ and 5’-MAR’-MAR’ segments, respectively, as illustrated in Fig. 1C. Segments D (bounded by restriction sites SnaI-SphI) and F (bounded by restriction sites BglII-NdeI) are included in entries MUSICD07-09. Segment C was derived from the multilonging site of two vectors and has the sequence: GC-GGCCGCTCGAGTGACCATTGGAAGCTCGTGCTACCCATAC, where the lowercase letters denote the sequence beginning in the 3’-half of the site denoted SnaI (b) (Fig. 1A). Segment E (bounded by restriction sites SphI-BamHI) was derived from pSV2gpt (19). Segment G corresponds to pGEM5 (Promega Biotech). The notation B/B indicates that segments E and F were joined by ligating the BamHI-generated end of E with the BglII-generated end of F. Segments A and C–G were obtained from the transfer vector “D” (6) by digesting with NotI and either Nael or its isoschizomer NgoM1 and purified by gel electrophoresis. The insert segments denoted B were constructed by PCR using the primers defined below in which restriction sites were fused to sequences that would pair with specific regions of the intronic DNA. Thus, the Eμ insert was generated from plasmid pSp6 (20) by PCR using primers 5 and 6. Primers 7 and 8 were used to amplify the MARs-containing insert from plasmid pμΔ4, kindly provided by R. Grosschedl (7). The NotI and Nael/NgoM1 sites in the primers are italicized.

Cell Culture and Transfection—Previous publications have described the wild-type Sp6 (21) and mutant igm692 (22) and X10 (23) cell lines and the methods for cell culture and transfection by electroporation (24). As illustrated in Fig. 1, targeted recombinants were identified by screening either for the correct 5’ junction using primers 1 and 2 (see below) or by testing culture fluid for the presence of normal IgM using a Cμ1-specific ELISA. Transfectants satisfying these criteria were further tested for both correct junctions using primers 3 and 4 (see below). To ensure that the recombinants had not acquired additional vector DNA, genomic DNA was digested with BamHI and analyzed by Southern blot probing with the Cμ1-specific ELISA. Transfectants satisfying these criteria were further tested for both correct junctions using primers 3 and 4 (see below). To ensure that the recombinants had not acquired additional vector DNA, genomic DNA was digested with BamHI and analyzed by Southern blot probing with the Cμ1–2 (XbaI-BamHI) and Cμ3–4 (HindIII-HindIII) fragments. In most cases only the predicted bands were seen, and only recombinants with the correct structure were analyzed for μ mRNA production.

Analysis of DNA Structure and mRNA Production—DNA structure was analyzed by Southern blot and other methods, as described (24). The primers used for PCR amplifications were as follows. Primer 1, 5’-ACATGGAGTGCTCAGAGCTGACGACA-3’ (5’ of VH homology region); primer 2, 5’-GGTGACGGACTCATCTCCATATGGT-3’ (in multicloning site); primer 3, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’ (in multi-

clamation site); primer 4, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’ (in gpt gene); primer 5, 5’-GGTGACGGACTCATCTCCATATGGT-3’ (5’ of VH homology region); primer 6, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’ (in gpt gene); primer 7, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 8, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 9, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 10, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 11, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 12, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 13, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 14, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 15, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 16, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 17, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 18, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’; primer 19, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’.

RNA was analyzed by Northern blot, as described (24).

NaeI I and either

BglII HI and analyzed by Southern blot and other methods, as described (24). The primers used for PCR amplifications were as follows. Primer 1, 5’-ACATGGAGTGCTCAGAGCTGACGACA-3’ (5’ of VH homology region); primer 2, 5’-GGTGACGGACTCATCTCCATATGGT-3’ (in multicloning site); primer 3, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’ (in gpt gene); primer 4, 5’-GATCAAGAGGCTCTCAACGGTCATGTGGT-3’ (5’ of VH homology region); primer 5, 5’-GGTGACGGACTCATCTCCATATGGT-3’ (5’ of VH homology region); primer 6, 5’-GGTGACGGACTCATCTCCATATGGT-3’ (5’ of VH homology region); primer 7, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 8, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 9, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 10, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 11, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 12, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 13, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 14, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 15, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 16, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 17, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 18, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 19, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 20, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 21, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 22, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 23, 5’-GGTGACGGACTCATCTCCATATGGT-3’; primer 24, 5’-GGTGACGGACTCATCTCCATATGGT-3’.
in medium containing xanthine and mycophenolic acid (MHX medium) (19). Homologous recombination between the targeting vector and the endogenous μ gene can restore the missing exons and render the recombinants gpt<sup>+</sup>, so that they can then be selected in MHX medium. This feature makes it possible to use an ELISA specific for the C<sub>μ1</sub> domain to detect targeted recombinants, if the recombinants express the μ gene at >0.1% of the normal level. We have shown that recombinants bearing an intact 5′-MAR-E<sub>μ</sub>-3′-MAR segment express the μ gene at the same level as the parental hybridoma (24).

As noted above, Forrester et al. (7) reported that high level μ expression in transgenic mice requires both E<sub>μ</sub> and MARs. Consistent with their findings, our analysis of nested deletions that removed various segments of the JH-C<sub>μ</sub> intron from the endogenous locus indicated that μ expression depends strongly on some component in the MAR-E<sub>μ</sub>-MAR segment and that the level of μ expression was closely related to the extent of remaining MAR DNA (6). Because of the importance of relating analyses of transgene and endogenous gene expression, we have followed their definition of these elements very closely. Accordingly we have constructed the pE<sub>μ</sub>, pMM, and p0 targeting vectors to generate the E<sub>μ</sub>-MAR<sup>+</sup>, E<sub>μ</sub> MAR<sup>−</sup>, and E<sub>μ</sub>′ MAR recombinants, respectively, such that these recombinants bear very nearly the same DNA segments that were used in the transgene expression study. Thus, the E<sub>μ</sub>-MAR<sup>+</sup>, E<sub>μ</sub>′MAR<sup>−</sup>, and E<sub>μ</sub> MAR recombinants bear the complete 5′-MAR-E<sub>μ</sub>-3′-MAR segment, the E<sub>μ</sub> segment, and the 5′-MAR-3′-MAR segment, respectively (Fig. 1).

To generate the recombinants we transfected linearized vector DNA into the igm692 recipient cells and plated these cells at limiting dilution in MHX medium (Table I). The initial MHX<sup>+</sup> recombinants which we generated with the p0 and pE<sub>μ</sub> vectors were identified by PCR amplification of the 5′-junction fragment (Fig. 1). These recombinants produced detectable levels (>0.1%) of normal IgM, as measured using a C<sub>μ</sub>-specific ELISA, suggesting that these two screening methods might be used interchangeably. For this reason, most subsequent recombinants were identified by first testing culture fluid with the C<sub>μ</sub>-specific ELISA and then testing positive colonies for proper junctions by PCR. The recombinants were then subcloned at 0.1 cell/well. To assure that only a single copy of the vector had inserted into the recombinant loci, we analyzed the μ-gpt DNA by Southern blots of genomic DNA (results not shown). Multiple independent recombinants that yielded only the predicted bands were then studied further.

In principle, the structure of the intron in the targeted IgH locus could affect expression of the linked gpt gene. However, an effect that prevented growth in MHX medium would have been evident as a marked decrease in the frequency of targeted MHX-resistant transfectants. As shown in Table I, we recovered all recombinants at frequencies ranging from 2 × 10<sup>−4</sup> to 5 × 10<sup>−6</sup>. We interpreted this relatively consistent recovery frequency to mean that the selected phenotype was typical of the recombinant structure, i.e. the selected phenotype did not require a (rare) mutation in addition to recombination.

**Effects of Deletions on μ mRNA Production**—To assess the importance of individual intronic elements in μ expression, we measured the level of μ mRNA in recombinants of each structure, as well as the level of actin mRNA, which is expected not to be affected by the intronic deletions. As a negative control for specificity we included RNA from the Sp6-derived mutant X10, which has a deletion of the entire μ gene (denoted Δμ1) (23).

Representative results for particular recombinants are shown in Fig. 2. For each lane the indicated ratio of μ to actin mRNA levels was calculated and then expressed as the percent of the value obtained for one of the E<sub>μ</sub>-MAR<sup>+</sup> recombinants, E<sub>μ</sub>-MAR<sup>−</sup>-R1; this percentage is listed below each lane of the blot.

We have examined at least four independent recombinants of each deletion type in this same way. Also RNA was prepared and analyzed multiple times from most individual recombinants (blots not shown). From these data we calculated the level of μ production compared with the E<sub>μ</sub>-MAR<sup>+</sup> recombinant, and Table II lists the mean (± S.E.) obtained from these measurements of multiple independent recombinants. As reported previously (6, 24), the E<sub>μ′</sub>MAR<sup>−</sup> recombinants produced approximately the same level of μ mRNA as the Sp6 parental hybridoma, while the E<sub>μ</sub>-MAR<sup>−</sup> recombinants produced only ~6% of the normal level of μ mRNA. The E<sub>μ</sub>-MAR recombinant, which bears both 5′- and 3′-MARs produced ~60% of the normal level of μ mRNA, thus comparable to what we found previously for a recombinant bearing only the 3′-MAR (6). Interestingly, the E<sub>μ</sub>-MAR recombinant produced a similarly high level of μ mRNA (~50% normal). Our previous analysis of the E<sub>μ′</sub>MAR<sup>−</sup> recombinants by nuclear run-on assays showed that their decreased μ mRNA content reflects decreased transcription (6). The relatively high μ mRNA content of the E<sub>μ′</sub>MAR<sup>−</sup> and E<sub>μ</sub>-MAR<sup>−</sup> recombinants therefore indicates that these elements were each sufficient to maintain transcription of the μ gene in the context of the recombinant IgH locus.

**DISCUSSION**

As summarized in the Introduction, high level expression of IgH-derived transgenes in mice requires both E<sub>μ</sub> and MARs, indicating that these elements each provide different and nec-

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**Table I**

**Generation of targeted recombinants**

| Vector DNA | MHX<sup>+</sup>/surviving input cell | 5′-PCR<sup>+</sup>/MHX<sup>+</sup> | C<sub>μ1</sub>+/MHX<sup>+</sup> | Targeted/5′-PCR<sup>+</sup> or C<sub>μ1</sub>+/Targeted/surviving input cell |
|------------|----------------------------------|---------------------------------|----------------------------|--------------------------------------------------|
| pMM        | 30                               | 0.8 × 10<sup>6</sup>            | 5 /12                      | 4 /5                                              | <1 × 10<sup>−7</sup> |
| E<sub>μ</sub> | 40                               | 1.23 × 10<sup>7</sup>           | 9 /35                      | 0 /8                                              | 1 × 10<sup>−6</sup> |
| 80         | 1.351 /10<sup>6</sup>            | 2309                           | 12 /2                      | 2 × 10<sup>−7</sup>                              |
| 250        | 2.8803 × 10<sup>7</sup>          |                                | 21/2880                    | 4 × 10<sup>−7</sup>                              |
| p0         | 50                               | 2.44 × 12 × 10<sup>7</sup>      | 7 /20                      | 6 /7                                              | 6 × 10<sup>−6</sup> |
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**TABLE II**

| Recombinant | Relative μ mRNA | Mean ± S.E. |
|-------------|-----------------|-------------|
| Eμ^−MARC | 90 ± 11 |
| R1 | 100 |
| R4 | 79 ± 25 |
| Eμ^−MARC^ | 64 ± 8.5 |
| R2 | 74 ± 17 |
| R3 | 70 ± 1 |
| R9 | 61 ± 6 |
| R11 | 52 ± 10 |
| Eμ^−MARC | 46 ± 8.9 |
| R5 | 42 |
| R7 | 54 ± 4 |
| R17 | 34 ± 0 |
| R18 | 41 |
| R66 | 58 ± 1 |
| Eμ^−MARC | 6.8 ± 4.9 |
| R3 | 10 |
| R6 | 4.2 ± 1.2 |
| R10 | 4.3 ± 2.2 |
| R14 | 3.6 ± 3.1 |
| R44 | 16.4 ± 6.6 |
| R65 | 2.2 ± 1 |
| Sp6 parental hybridoma | 85 ± 1 |

Recombinants of the indicated structure were generated as described in the text and Table I. Each line represents an independently generated recombinant. One or more RNA preparations were made from each recombinant and analyzed by Northern blot, as illustrated in Fig. 2. The ratio of μ to actin mRNA was measured for each preparation of RNA, and the value obtained for the μ-deleted cell line (Δμ = X10) was subtracted. This difference was then normalized to the value obtained for the Eμ^−MARC recombinant included in each analysis. The values for each independent RNA preparation from each recombinant were then averaged, as presented here with the standard error in the second column. For each set of independent recombinants of the same structure, e.g., the six recombinants with the Eμ^−MARC^ structure, the average values were then combined to obtain the average and standard error shown in the last column.

Figure 2. μ RNA production by recombinant hybridomas. Cytoplasmic RNA was isolated from the indicated cell lines and analyzed by Northern blotting with probes corresponding to the Cμ3–4 exons and actin. The μ deleted cell line, X10, is included as a negative control. The intensity of the μ- and actin-specific bands were quantified by PhosphorImager analysis. The ratio of μ to actin activity was calculated for each cell line. To compensate for the low background of radioactivity due to nonspecific binding, the value for a comparable region of the lane for the μ-deleted cell line (Δμ = X10) was subtracted from the value obtained for the μ band of each recombinant. For the sake of comparing independent blots the net μ/actin value for each recombinant was normalized to the value obtained for the Eμ^−MARC^ recombinant.

The values corresponding to this particular blot are listed below each lane. This same analysis was applied to other independent recombinants as well as the parental Sp6 hybridoma (Sp6/HL subclone), and the compilation of these measurements is presented in Table II.

The observation that these elements are not needed to maintain endogenous IgH expression in B cell lines has suggested either that the locus contains other, functionally redundant elements, or that continued expression of the heavy chain gene in these cell lines does not require enhancers or MARs of any type (12–15). The finding that the 5′-MAR-Eμ3-3′MAR segment is in fact required for continued expression of a transgene in a pre-B cell line then argues that the normal IgH locus has activating elements that are redundant in B cell lines (3, 4). It might, however, be inappropriate to meld these experiments, as transgenes in mice and the endogenous IgH of cell lines have been subjected to ontogenetic effects that might not apply to transgenes introduced into cell lines.

To investigate the role of the intronic activating elements under more uniform conditions, we have exploited our finding that insertion of the gpt cassette into the IgH locus renders expression of the endogenous μ gene dependent on at least one intronic element in the 5′-MAR-Eμ3-3′MAR segment (6). These conditions have allowed us to analyze the role of the MARs and Eμ core enhancer in μ expression, using closely related targeted recombinants that present the μ gene in a constant chromosomal and cellular context. In contrast to the case of μ expression from transgenes in mice where both Eμ and MARs are required, our analysis of μ expression in the recombinant IgH locus of hybridoma cells indicates that high level μ expression required either Eμ or the MARs. Thus, for both the Eμ^−MARC^ and Eμ^−MAR^ targeted recombinant cell lines, μ expression was much higher than the case of the Eμ^−MAR^ locus, and each of the single element loci was roughly comparable with the complete Eμ^−MAR^ locus. These observations lead to an interesting paradox, as follows. On the one hand, the large difference in μ expression between the Eμ^−MARC^ and Eμ^−MAR^ recombinants indicates that μ expression required the Eμ function, i.e., that Eμ function could not be supplied to the Eμ^−MAR^ locus by elements elsewhere in the locus. Similarly, the large difference between the Eμ^−MARC^ and Eμ^−MAR^ recombinants indicates that μ expression required the MAR function and that this function could not be supplied by the locus. Thus, these results indicate that μ expression required both the MAR and Eμ functions, because the locus could supply neither function. On the other hand, the relatively small (~2-fold) difference between the Eμ^−MARC^, Eμ^−MAR^, and Eμ^−MAR^ recombinants recombinants leads to the contrary conclusion, namely that μ expression required neither MAR nor Eμ function, because the locus could supply both functions.

We propose two models to account for this apparent paradox. One possibility is that maintenance of μ expression does not in fact require both Eμ and MAR functions, as it should be noted that the experiments that tested for continued transgene expression in pre-B cell lines examined the effects of deleting a segment which bore both elements and did not test deletions of each element separately (3, 4). That is, the role of Eμ and MARs in initiating expression might be different from their role in maintaining expression. For example, initiation might require demethylation of some special site in the IgH locus, and both Eμ and a MAR might be required to render that site a substrate for demethylation. Conversely, maintenance might be abrogated by methylation, and either Eμ or a MAR might be sufficient to prevent specific methylation.

A second possibility, suggested by the seemingly complementary actions of Eμ and MARs, is that μ expression always requires both an enhancer and MARs, but that one element in or near the transcription unit can collaborate with another (complementary) element elsewhere in the locus. Thus, either Eμ or a MAR would be sufficient to maintain expression of the μ gene in the endogenous locus, because by hypothesis Eμ can...
collaborate with a MAR, or, alternatively, a MAR in the JH-Cμ intron can collaborate with an enhancer. The IgH locus is known to include other enhancers 3' of Ca; as well, other MARs lie between C5 and Cγ3 genes and 5' of the V region promoter (16, 26). To explain why these other enhancers and MARs do not collaborate to activate transcription in the Eμ·MAR·recombinants, we suppose that the functional unit must be properly situated, as noted above. Experiments using a MAR derived from the interferon β gene have also led to the conclusion that the capacity of a MAR to stimulate transcription depends strongly on its position relative to the promoter (27), although the “distance,” 1.5 kilobases between the μ promoter and the intronic MARs, is in the range for which the interferon β MAR was inhibitory.

MARs are found both outside and within transcription units, and those MARs within transcription units are often co-localized with enhancers (28). It has been proposed that this association exists because the functioning of these elements requires their collaboration (29). Thus, MARs might facilitate promoter-enhancer contacts or sequester the enhancer in the vicinity of transcription factors (30). Our results indicate that Eμ and the MARs can function, although there are no (recognizable) complementary elements in their immediate vicinity.

Initiation and maintenance of expression might each require both an enhancer and MARs but differ in their dependence on linkage, e.g. initiation might require close linkage between the enhancer and the MARs, while more separated elements might suffice to maintain expression. That is, the two models proposed above are not mutually exclusive.

To judge from the well studied IgH and β-globin loci, transcriptional activating elements might often be redundant in normal chromosomal genes. As reviewed in the Introduction, the finding that IgH expression was maintained at a high level in B-cell lines that had lost the enhancer needed for transgene expression was an early suggestion that the locus contained other, normally redundant activating elements. Similarly, in the case of the β-globin locus, several DNase hypersensitive sites that are required for high level transgene expression can be deleted from the endogenous locus without greatly altering β-globin gene expression (31). The redundant elements, which permit transcription in the mutant globin and IgH loci, have not been identified. It might be expected that functionally redundant elements would resemble each other, and, indeed, the aforementioned 3' enhancers in the IgH locus are good candidates for the elements that maintain expression in the enhancerless mutant B-cell lines. However, our finding that the MARs and Eμ, two elements that differ substantially in their biochemical properties and activities, are functionally redundant in their capacity to maintain IgH expression underscores the general possibility that redundant elements might appear dissimilar.

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