Fig. 4. The spatially resolved average pair interaction energy $<u_{ij}(r, \Omega)>$ for SPC/E water at 25°C. The isosurfaces represent $<u_{ij}(r, \Omega)> = -10$ kJ/mol, and hence, any molecules found within the regions enclosed by these surfaces interact with the central molecule on average with an energy less than −10 kJ/mol. Only one hemisphere of the full distribution is shown where the central molecule has been included to define the local frame. The surfaces have been colored according to their separation as in Fig. 2.

pling of certain rotational and translational motions of the central molecule (*12*) (this "rocking" motion of water molecules also accounts for the single feature due to the two H-bond–donating neighbors). This bridge between the H-bond–donating neighbors and the nontetrahedral coordination becomes evident in the oxygen density map at a higher threshold [$g_{OO}(r, \Omega) \approx 1.25$] for TIP4P than for either PPC or SPC/E water [$g_{OO}(r, \Omega) \approx 1.15$], indicating stronger couplings in the former liquid.

Further insights into the local structure in these liquids are provided in Fig. 3, which shows an overlay of the hydrogen and oxygen density maps for SPC/E water. The hydrogens of the H-bond–donating neighbors are clearly resolved into two cupped features "sandwiching" the oxygen density below the central molecule, whereas the two hydrogens of each of the H-bond–accepting neighbors appear as single featureless caps somewhat beyond their respective oxygen features above the central molecule. Figure 3 exhibits one additional hydrogen feature due to the neighbors in nontetrahedral coordination. This local maximum in the hydrogen density appears at separations of 4.0 to 4.4 Å, corresponding to the rather shallow second minimum in $g_{OH}(r)$. Behavior similar to that observed in Fig. 3 can be found for the TIP4P and PPC systems.

To demonstrate further the utility of spatial maps (as opposed to simple 1D radial analysis) we have also spatially resolved the average pair interaction energy for SPC/E water. This function is presented in Fig. 4 where isosurfaces corresponding to −10 kJ/mol, an accepted limit for H-bond energies in water (*14*), are shown. Thus, from an energetic point of view, molecules found within the boundaries of the features in Fig. 4 will on average be "H-bonded" to the central molecule. Comparison of the features due to "H-bonded" neighbors in Figs. 2 (density maps) and 4 (potential map) reveals that, although they are qualitatively similar, a geometric criterion based on the oxygen-oxygen SDF will be more selective. From Fig. 4 we observed that the average pair interaction for nontetrahedral (interstitial) neighbors is greater than −10 kJ/mol, although a significant fraction of the molecules making up this coordination would be labeled as H-bonded from an energetic viewpoint (*11*).

Soper (*16*) has argued that the SDF for water can be determined from experimental data. His approach (*16, 17*) uses a maximum entropy constraint to estimate a full molecular pair distribution function that is consistent with the atom-atom RDFs obtained from neutron scattering (*18*). Soper's results [see figure 7 of (*16*)] strongly support our model calculations.

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Dependence of Enhancer-Mediated Transcription of the Immunoglobulin μ Gene on Nuclear Matrix Attachment Regions

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Transcription of the immunoglobulin μ heavy chain locus is regulated by an intronic enhancer that is flanked on both sides by nuclear matrix attachment regions (MARs). These MARs have now been shown to be essential for transcription of a rearranged μ gene in transgenic B lymphocytes, but they were not required in stably transfected tissue culture cells. Normal rates of transcriptional initiation at a variable region promoter and the formation of an extended deoxyribonuclease I (DNase I)–sensitive chromatin domain were dependent on MARs, although DNase I hypersensitivity at the enhancer was detected in the absence of MARs. Thus, transcriptional activation of the μ gene during normal lymphoid development requires a synergistic collaboration between the enhancer and flanking MARs.

The murine immunoglobulin heavy chain (Ig_H) locus has been studied as a model of tissue-specific gene regulation. Transcriptional control elements reside within the promoters associated with the variable region (V_H) gene segments, the intronic μ enhancer, and the enhancers located at the 3′ end of the locus (*1*). Although these elements contribute to B cell–specific gene expression in transfection assays, only the intragenic enhancer region has been shown to direct efficiently lymphoid-specific transcription of a rearranged μ gene and heterologous reporter genes in transgenic mice (*2*). Delineation of this enhancer in transfection assays indicated that a 220–base pair

(bp) core element, E_μ, which comprises multiple and functionally redundant binding sites for lymphoid-specific and ubiquitous transcription factors, is necessary and sufficient to govern transcriptional stimulation (*1*).

The μ enhancer core is flanked on both sides by AT-rich sequences that mediate an association with the nuclear matrix or scaffold (*3*). Although such matrix attachment regions (hereafter referred to as MARs) are operationally defined by their capacity to mediate matrix association, they appear to participate in diverse processes. One class of MAR, exemplified by the chicken lysozyme A elements, may contribute to gene control by forming structural or topological boundaries between distinct domains in eukaryotic chromosomes and thereby reduce position effects within the integrated chromosomal, but not episomal, state (*4*). This type of MAR has properties reminiscent of chromosomal domain boundary elements such as the specialized chromatin structures that flank the *Drosophila* heat shock locus and the insulator elements at the 5′ end of the chicken β-globin locus (*5*). These elements protect genes from chromosomal position effects when placed outside the enhancer-promoter-gene locus, and they interfere with expression when placed between promoter and enhancer sequences. A potentially different type of MAR has been identified in the Ig heavy and light chain loci. These MARs are located adjacent to the intragenic enhancers, between the promoter and enhancer (*3*), and have not been shown to contribute to transcriptional control in either transient or stable transfection assays in B lymphoid cell lines (*6*, *7*). The MARs that flank the μ enhancer core, however, have been implicated in negative regulation (*8*, *9*) and contain binding sites for two nuclear factors, NFμNR (*9*) and SATB-1 (*10*), that may participate in the repression of enhancer activity in non–B lymphoid cells.

To examine the contribution of MARs to Ig_H transcriptional control, we generated transgenic mouse lines with rearranged genomic Ig μ genes that contained either the 220-bp core enhancer without flanking MARs (ΔMAR) or the MARs without the 220-bp core enhancer (μΔ4) (Fig. 1A). Transgene expression in these mice was analyzed in defined populations of pre-B cells derived by immortalization with the Abelson–murine leukemia virus (A-MuLV) (*11*). In the three transgenic lines harboring the rearranged wild-type μ gene (μwt), transgene expression was detected at a high and relatively constant level irrespective of chromosomal location (Fig. 1B). In contrast, the number of specific μ transcripts was markedly reduced in transgenic lines containing the ΔMAR or μΔ4 genes. Quantitation of transgene expression in these pre-B cell lines indicated that removal of the MARs decreased the abundance of transgene-specific mRNA by a factor of 35 to >1000. Moreover, the variability in the level of expression among the different ΔMAR lines was markedly increased relative to the small fluctuations among different μwt lines shown here and previously (*11*). Thus, in the absence of the MARs,

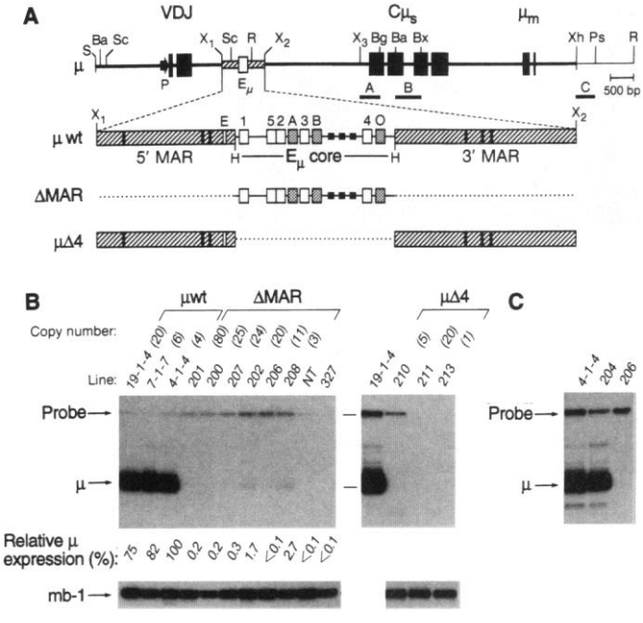

Fig. 1. Structure of μ transgene constructs and analysis of transgene expression in pre-B cells. (**A**) The rearranged genomic μ gene is shown as a bold line at the top. This gene has been previously described (*11*). Briefly, the V_H variable region promoter is represented by the arrow labeled P, the core enhancer is shown as an open box (E_μ), and the MARs are shown as hatched bars. The rearranged V_HDJ_H variable region and the Cμ constant region exons are indicated by the filled boxes, with μ_s and μ_m representing exons encoding the secreted and membrane-bound forms of the μ protein. The thin line adjacent to the Xho I site extends the end of the transgene into the genomic map of the endogenous μ locus from which the transgene was derived. Relevant restriction sites are S, Sal I; Ba, Bam HI; Sc, Sca I; X_1 to X_3, Xba I sites 1 to 3; R, Eco RI; Bg, Bgl II; Bx, Bst XI; Xh, Xho I; Ps, Pst I; and H, Hinf I. Fragments used to make hybridization probes are shown as small bars below the top line and labeled A, B, and C. The μwt, ΔMAR, and μΔ4 transgenes are identical outside of the X_1-X_2 fragment, which contains E_μ and MARs in μwt, only E_μ in ΔMAR, and only the MARs in μΔ4 (*33*). The E_μ core, bracketed by Hinf I sites, contains non–B cell–restricted factor binding sites, termed μE boxes (open boxes, numbered 1 to 5) and the μA, μB, and octamer (O) binding sites for putative B cell–specific regulators (stippled boxes) (*1*). Black bars within the hatched MARs represent binding sites for the NFμNR (*9*) and SATB-1 (*10*) factors; the open bar denotes the E (Ig/EBP-1) site (*1*). Dots indicate sequences that have been removed. (**B**) S1 nuclease protection assay of cytoplasmic RNA from transgenic pre-B cells. For the detection of correctly initiated μ transgene mRNA, 3 μg of cytoplasmic RNA from transgenic pre-B cells were annealed with a transgene-specific, single-stranded, antisense DNA probe, digested with S1 nuclease, and processed as described (*11*). Transcripts initiating at the V_H promoter migrate at position labeled μ. Undigested, full-length probe migrates at position labeled Probe. The abundance of mb-1 transcripts, which are expressed in a B cell–specific fashion (*34*), served as an internal RNA control (*35*). The level of specific transgene expression, relative to μwt line 4-1-4, was determined by analysis of this and other S1 nuclease protection experiments (*36*). NT is a nontransgenic sample, and 327 is a previously described transgene in which the Sc-R enhancer fragment has been deleted (*11*). (**C**) S1 nuclease protection analysis of RNA from transgenic pre-B cells of ΔMAR lines 204 and 206 and μwt line 4-1-4.

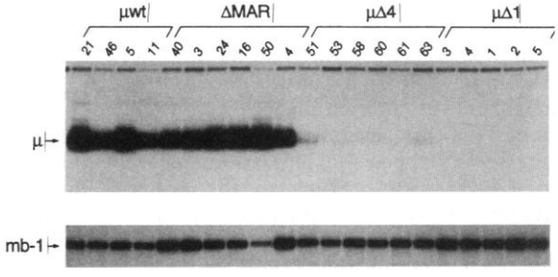

Fig. 2. Expression of wild-type and mutant μ genes in stably transfected M12 B cells. Cytoplasmic RNA (10 μg) from numerically designated M12 cell clones (*37*), shown by DNA blot analysis to harbor the indicated μ genes, was analyzed for the presence of specific μ transcripts by S1 nuclease protection as described in the legend to Fig. 1B. The abundance of mb-1 transcripts served as an internal RNA control.

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the μ transgene is impaired in its ability both to undergo transcriptional activation and to overcome the variability associated with chromosomal position effects, despite the presence of the intronic enhancer. S1 nuclease protection analysis yielded identical results with transgenic spleen RNA and the RNA isolated from transgenic pre-B cells, with the exception of ΔMAR line 204, which was transcriptionally active in the pre-B cell lines but not in spleen (Fig. 1C) (*12*). We have observed this upregulation in independently derived pre-B cells from line 204, suggesting that the ΔMAR gene may be integrated at a chromosomal locus that becomes transcriptionally permissive as a consequence of A-MuLV transformation.

The requirement of the MARs for μ gene expression in transgenic mice was not anticipated from the results of previous tissue culture transfection experiments, which indicated that the enhancer was necessary and sufficient for a high level of transcription (*7*). To address this discrepancy, we stably transfected the same μ gene constructs used for germ-line transformations into a B lymphoid cell line (Fig. 2). As a control, we included a deletion mutant, μΔ1, that lacks both the MARs and the intronic enhancer and which has been previously shown to be transcriptionally inactive in both transfection and germline transformation assays (*13*). Consistent with previous transfection data, both the μwt and ΔMAR genes generated comparable numbers of correctly initiated μ gene transcripts. Expression of the μΔ4 and μΔ1 genes, both lacking the core enhancer, was very low or not detectable. In these experiments, the selectable marker construct, SV2neo, did not contain a MAR (*14*). Furthermore, the SV40 enhancer-promoter region of this construct did not compensate for the deleted μ enhancer in cells containing either the μΔ4 or μΔ1 genes and is, therefore, not likely to account for the expression of the transfected ΔMAR genes.

To determine whether the MARs contribute to μ transgene control at the transcriptional or posttranscriptional level, we performed nuclear run-off assays. Nuclei were isolated from transgenic pre-B cells and incubated under conditions in which nascent RNAs were elongated in the presence of [α-^{32}P]UTP (uridine 5′-triphosphate) (*15*). The labeled transcripts were then hybridized to various denatured transgene-specific and control plasmids (Fig. 3). Transcription across the transgenic V_HDJ_H exon was detected only in cells from the μwt mouse, not in cells from nontransgenic or ΔMAR mice. Therefore, even in the presence of the enhancer, transcriptional stimulation at the V_H promoter is significantly reduced and likely accounts for the reduction in steady-state mRNA abundance observed in transgenic ΔMAR mice. Thus, regulation of μ gene expression by MARs can be detected in germline transformations but not in transfection assays.

In addition to transcriptional stimulation, the function of enhancers has been correlated with localized alterations in chromatin structure that can be detected as nuclease-hypersensitive sites. To examine enhancer function at this level, we analyzed the ΔMAR transgenes for the presence of deoxyribonuclease I (DNase I)–hypersensitive sites. Nuclei from transgenic ΔMAR pre-B cell lines in which the V_H promoter was active (204) or inactive (207 and 201) were treated with various amounts of DNase I (*16*) and analyzed by DNA blot hybridization. A prominent region of DNase I hypersensitivity was detected at the core enhancer in all ΔMAR lines examined (Fig. 4A), whereas hypersensitivity at the V_H promoter was observed only in the transcriptionally active line 204 (*12*). These data indicate that the enhancer core is sufficient to establish localized regions of accessibility in chromatin, even though, in the absence of the MARs, it is defective in its ability to stimulate transcription at the variable region (V_H) promoter. As anticipated from previous studies, no DNase I hypersensitivity at the ΔMAR enhancer core was detected in liver (*12*), in which the enhancer is inactive, suggesting that the enhancer is occupied by factors selectively active in pre-B cells (*17*). Moreover, the ΔMAR transgene was not expressed in liver (*12*), suggesting that the MARs participate in gene control by increasing expression in lymphoid cells rather than by decreasing expression in nonlymphoid cells.

To examine whether the localized alter-

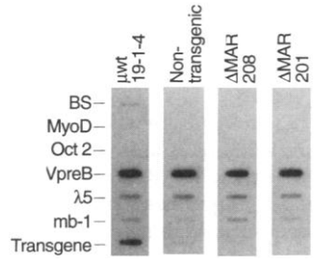

Fig. 3. Rate of transcription of μ transgenes in pre-B cells. Labeled nascent RNAs were hybridized to immobilized plasmid-borne complementary DNAs (cDNAs) as described (*15*). The cDNAs corresponded to pre-B cell–specific VpreB and λ5 genes, the B cell–specific mb-1 gene (*34*), and control sequences including the MyoD gene (*38*) and the Bluescript plasmid vector (Stratagene) into which the cDNAs were inserted (BS). The transgene-specific target sequence was the V_HD region extending from an Nde I site at −1 to a Kpn I site at +490.

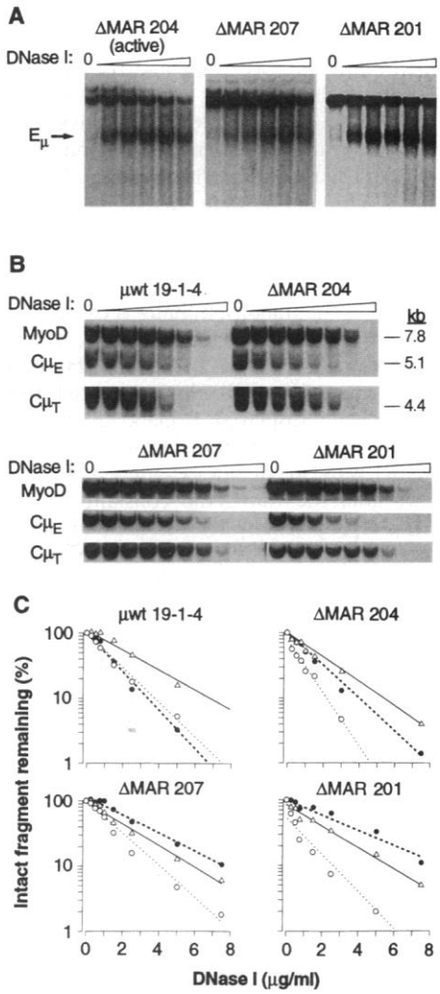

Fig. 4. DNase I hypersensitivity and general DNase I sensitivity of Ig transgenes. (**A**) For analysis of DNase I hypersensitivity, DNase I–treated genomic DNA was cleaved with Sca I and Bgl II, fractionated in a 1% TAE agarose gel, denatured and transferred to filters as described (*39*), and hybridized with probe A (Fig. 1A). The position of the intronic enhancer is indicated by the arrow. (**B**) For analysis of general DNase I sensitivity, 20 μg of genomic DNA, isolated from DNase I–treated nuclei, was digested with Bam HI and Eco RI and hybridized with a probe for the MyoD gene, or with probes C and B for the endogenous Cμ$_E$ and the transgenic Cμ$_T$ loci, respectively (Fig. 1A). Probes were sequentially hybridized to the same DNA blot [probes were removed from the filters by incubation in prehybridization solution at 65°C for ≥3 hours (*39*)] except for the filter containing the μwt 19-1-4 and ΔMAR 204 samples, for which the MyoD and Cμ$_E$ probes were used together. Molecular sizes are indicated in kilobases. (**C**) MyoD (△), Cμ$_E$ (○), and Cμ$_T$ (●) hybridization signals were quantitated with a PhosphorImager (Molecular Dynamics). Values on the *y* axis reflect the amount of intact restriction fragment remaining at each concentration of DNase I, expressed as a percentage of the signal obtained in the absence of DNase I. For some instances in which the last lane of one DNase I titration was next to the first lane of another DNase I titration, the values were not reliable and were therefore not included in the graphs.

ation in chromatin associated with the 220-bp E_μ fragment correlated with a more extensive reorganization of the flanking chromatin structure, we compared the rates of DNase I digestion of various transgenes with those of transcriptionally active and inactive endogenous genes. Digestion rates of both DNase I–resistant and DNase I–sensitive chromatin were determined with probes specific for the transcriptionally inactive MyoD gene, which resides within a 7.8-kb Bam HI–Eco RI genomic fragment, as well as the transcriptionally active endogenous μ constant region $C\mu_E$ (*18*), which is contained within a 5.1-kb Bam HI–Eco RI fragment (Fig. 4B). As anticipated, the $C\mu_E$ region displayed a markedly higher sensitivity to digestion by DNase I than the MyoD gene in all lines examined. In the μwt line, the transgenic constant region $C\mu_T$, represented by a 4.4-kb genomic fragment, displayed a DNase I sensitivity indistinguishable from that of the endogenous $C\mu_E$ region. In contrast, the transcriptionally inactive ΔMAR transgenes were resistant to DNase I. In the ΔMAR line 204, in which expression levels are identical to those in μwt pre-B cells, we observed an intermediate level of DNase I sensitivity, possibly due to the DNase I sensitivity of only a few copies of the tandemly integrated transgenes. The differences in general DNase I sensitivity of the transgenes were quantitated by plotting the extent of chromatin digestion as a function of DNase I concentration (Fig. 4C).

As an additional assay for enhancer function, we examined whether the ΔMAR transgenes were able to direct the synthesis of noncoding, intron-containing "Iμ" transcripts that initiate within a broad region near the 3′ boundary of the core enhancer (*19*). The synthesis of these enhancer-proximal transcripts can be detected prior to any Ig_H gene segment rearrangements and has been proposed to reflect the function of the intronic enhancer and to mediate accessibility at the μ heavy chain locus (*20*). We used an antisense S1 probe that permitted the simultaneous detection of Iμ transcription from both the endogenous and ΔMAR loci (Fig. 5). Iμ transcripts were detected at various levels in six of seven pre-B cell lines carrying the ΔMAR transgene (Fig. 5). The abundance of transgenic IμΔMAR transcripts was similar to or exceeded that of the endogenous Iμ transcripts, providing evidence that the enhancer core is functional in its ability to stimulate transcription at a proximal promoter. The low levels of endogenous Iμ transcripts observed in lines 200, 201, and 202 may reflect differences in the expression or rearrangement status of the endogenous heavy chain locus. Together, these data suggest that the MARs participate in enhancer-dependent activation of the V_H promoter by facilitating communication between the enhancer and promoter or by propagating an enhancer-induced local alteration in chromatin structure. The latter view is supported by recent experiments in which we have shown that a 95-bp enhancer core fragment, which lacked both the enhancer-proximal Iμ promoter and the flanking MARs, conferred accessibility on a closely linked prokaryotic T7 promoter but not on a T7 promoter at a distance (*12*, *21*).

The requirement for the flanking Ig_H MARs in germline transformation assays suggests that these MARs may be functionally similar to sequences that flank the intronic adenosine deaminase enhancer and are also required for expression in germline, but not transfection, assays (*22*). The dependence on these flanking sequences could be related to differences in the methylation status of the DNA. DNA that is integrated at the one-cell stage of mouse embryogenesis is methylated before the gene is expressed at subsequent developmental stages (*23*). Transfection of in vitro–methylated immunoglobulin κ light chain gene constructs into cultured B lymphoid cells has indicated that expression and demethylation of the κ gene is dependent on both an intronic enhancer and a flanking MAR (*24*). Although studies in transgenic mice did not reveal a role for this MAR in transcriptional activation (*25*), recent studies have indicated that both elements are required for somatic hypermutation (*26*). A functional role for the μ 5′ flanking MAR in its native genomic context was also suggested by experiments in which the *neo*^{r} gene was inserted into the 5′ MAR by homologous recombination (*27*).

The mechanism by which the μ intronic MARs synergize with the core enhancer to confer function on a distal promoter is still obscure. Recent studies have suggested that high mobility group–I(Y)–like proteins, which can preferentially bind to the AT-rich MARs, mediate a partial displacement of histone H1, resulting in the formation of an "open" and DNase I–sensitive chromatin domain (*28*). In the absence of the MARs, the destabilization of template-associated histone H1 would be abrogated, preventing transcriptional activation. In one scheme, the binding of specific proteins to MAR sequences or the potential of these AT-rich sequences to become stably unwound (*29*) could directly lead to the formation of a transcriptionally permissive chromatin structure. Alternatively, the MARs could act indirectly by mediating the formation of a nucleoprotein complex at the enhancer that is functionally distinct from that formed in the absence of MARs. According to this view, the nucleoprotein complex formed in the presence of MARs may include factors analogous to the genetically defined SNF/SWI gene products in yeast, which appear to be required for transcriptional activation in nuclear chromatin (*30*). Related to these chromatin models, the MARs could participate in the formation of a topologically constrained domain in chromosomes. Consistent with this proposal, transcriptional initiation at the V_H promoter, but not the adenovirus late promoter, has been shown to be enhanced by negative supercoiling (*31*).

Whatever the mechanism of MAR function in the Ig_H locus, these sequence elements are crucial for the activation of a distal promoter independent of the chromosomal context. The combination of MARs and the μ enhancer core may constitute a regulatory unit functionally analogous to the locus control region in the human β-globin gene cluster that regulates gene expression over large chromosomal distances (*32*).

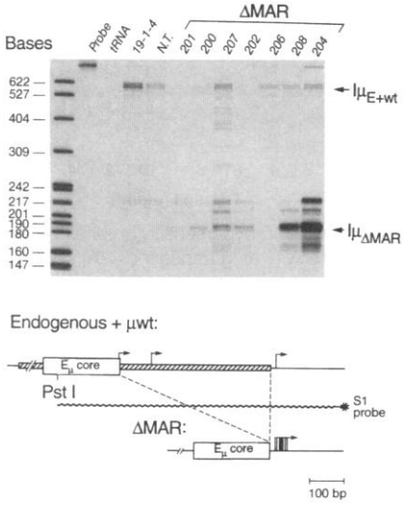

Fig. 5. S1 nuclease protection analysis of enhancer-proximal transcripts. RNA samples (15 μg) described in the legend to Fig. 1B or yeast tRNA was annealed to an 840-nucleotide, end-labeled, single-stranded, antisense DNA probe as described (*35*). Molecular sizes are indicated in bases. A schematic outline of the experiment is shown below. The open box and hatched bars represent the enhancer core and MARs, respectively. Sequences removed during the construction of ΔMAR are indicated by dashed lines, and the single-stranded DNA probe is shown as a wavy line. Arrowheads within the hatched bar denote transcription start sites in both the endogenous μ locus (E) and the μwt transgene (wt). Iμ transcripts specific to the ΔMAR transgene are indicated as $I\mu_{\Delta MAR}$ and are represented by a bold vertical arrow in the schematic diagram. Other, less prominent transcripts are also present.

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16. Nuclei were prepared by dounce homogenization of transgenic pre-B cells in nuclei isolation buffer [0.32 M sucrose, 3 mM $CaCl_2$, 2 mM magnesium acetate, 0.1 mM EDTA, 10 mM tris-HCl (pH 7.4), 1 mM dithiothreitol] containing 0.15% NP-40, incubated with DNase I, and processed (*39*).
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33. The functionally rearranged genomic μ gene, μwt, has been described previously (*11*). For construction of ΔMAR, the 220-bp Hinf I fragment was modified by the addition of Xba I linkers and was inserted into Xba I–digested p1-27 (*35*) to create ΔMARμ-1. This substitution results in the removal of 344 bp and 426 bp from the 5′ end (X_1-H) and 3′ end (H-X_2) of the 1-kb, Eμ-containing X_{1-2} fragment, respectively. ΔMARμ-1 DNA was digested with Nde I (at −1) and Hpa I (at +2586), and the internal fragment was inserted into Nde I– and Hpa I–digested μwt to produce ΔMAR. For construction of μΔ4, the Hinf I sites flanking the 5′ and 3′ ends of the 220-bp enhancer core were converted to Not I and Eag I sites, respectively, to produce pμNE. After digestion of pμNE with Eag I, self-ligation of the large fragment yields μΔ4. For microinjection, plasmids were digested with Sal I and Xho I, and inserts were either excised from low melt agarose as described (*11*) or electroeluted in an Elutrap chamber (S & S), concentrated by butanol extraction, extracted with phenol, precipitated, and resuspended in 10 mM tris-HCl (pH 7.4) and 0.1 mM EDTA.
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35. For the detection of enhancer-proximal, Iμ transcripts, a single-stranded DNA probe 5′ end-labeled with ^{32}P was prepared by extending an oligonucleotide primer (BF-16, 5′-CAGAAGCCACAACCATACATTCCCA) that is complementary to a sequence 220 bp 3′ to X_2 with a Pst I–digested p1-27 template (containing a μwt fragment that extends from −18 to +2603): A linear polymerase chain reaction was performed by mixing 2.5 μl of heat-inactivated kinase reaction mixture, which contained 40 ng of the BF-16 oligonucleotide, with 2 μg of digested p1-27, 100 μM each of nucleoside triphosphates (dATP, dTTP, dCTP, and dGTP), 5 units of Taq DNA polymerase (Promega), and 2 μl of 10× Taq buffer (supplied by manufacturer; containing 15 mM $MgCl_2$) in a final volume of 20 μl, and subjecting the mixture to 20 rounds of thermal cycling for 1 min each at 94°C, 55°C, and 72°C. Samples were applied to a 5% polyacrylamide gel containing 50% (w/v) urea. The appropriate gel slice was excised and the probe was purified by electroelution (Elutrap). mb-1 transcripts were detected with a single-stranded antisense DNA probe, which was synthesized in a linear polymerase chain reaction from Asp718-digested $pMB1_{T3}$ (which contains the mb-1 cDNA beginning with the AUG codon at +27) and a ^{32}P-labeled oligonucleotide primer, mb1-1 (5′-CACCGTCAGGGATGGTGGACC, extending from +127 to +147) [A. Travis, J. Hagman, R. Grosschedl, *Mol. Cell. Biol.* **11**, 5756 (1991)]. S1 reactions were performed as described (*11*).
36. Results shown in Fig. 1B, together with additional lanes containing 30 μg of the same RNA samples processed in parallel, were analyzed with a PhosphorImager (Molecular Dynamics). Relative levels of expression were determined by taking raw values corresponding to the amount of correctly initiated, transgene-specific mRNA, subtracting background, and dividing by the total level of expression in μwt line 4-1-4.
37. M12 B cells (2×10^7) [K. J. Kim *et al.*, *J. Immunol.* **122**, 549 (1979)] were subjected to electroporation (Bio-Rad Gene Pulser; 0.25 kV, 960 μF in 0.4-cm cuvettes) at room temperature in phosphate-buffered saline containing both 20 μg of plasmid DNA linearized at a unique Xho I site at the 3′ end of the gene and 1 μg of Eco RI–linearized SV2neo. After 24 hours, cells were diluted to a density of 10^5/ml with complete RPMI medium containing Geneticin (2 mg/ml) (Gibco), and 1-ml aliquots were seeded into 24-well plates. Clones were picked 10 to 14 days later and maintained in nonselective medium.
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Treatment of Murine Lupus with CTLA4Ig

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The interaction of B7-related molecules on antigen-presenting cells with CD28 or CTLA-4 antigens on T cells provides a second signal for T cell activation. Selective inhibition of the B7-CD28 or B7–CTLA-4 interactions produces antigen-specific T cell unresponsiveness in vitro and suppresses immune function in vivo. To determine whether selective inhibition of the B7-CD28 or B7–CTLA-4 interactions could suppress spontaneous autoimmune disease, a B7-binding protein was generated by genetic fusion of the extracellular domain of murine CTLA-4 to the Fc portion of a mouse immunoglobulin G2a monoclonal antibody (muCTLA4Ig). In lupus-prone NZB/NZW filial generation (F_1) mice, treatment with muCTLA4Ig blocked autoantibody production and prolonged life, even when treatment was delayed until the most advanced stage of clinical illness. These findings suggest a possible role for human CTLA4Ig in the treatment of autoimmune diseases in humans.

Systemic lupus erythematosus (SLE) is a life-threatening autoimmune disease that is characterized by the production of diverse autoantibodies (*1*). Some of these autoantibodies cause damage directly as a consequence of their specificity (for example, autoimmune hemolytic anemia is caused by antibodies to red blood cells), whereas other autoantibodies cause damage indirectly as a consequence of the formation and deposition of immune complexes (for example, immune complex glomerulonephritis is caused by antibodies to nuclear antigens). Other studies have shown that, both in humans with SLE and in murine models for SLE, the production of pathologic autoantibodies by B cells is dependent on stimulatory influences from $CD4^+$ T cells (*1*, *2*).

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