Structure and expression of murine germ-line immunoglobulin ϵ heavy chain transcripts induced by interleukin 4

(isotype switching/ C_{ϵ} gene/germ-line transcription)

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ABSTRACT The murine lymphokine, interleukin 4 (IL-4) is able to specifically promote isotype switching to IgG1 and IgE in cultures of mitogen-stimulated B cells. Emerging evidence suggests that germ-line immunoglobulin heavy chain gene transcription may direct switching by modulating switch-region accessibility to a recombinase. In this study, cloned cDNA copies of the germ-line ϵ heavy chain transcript have been used to determine the genomic organization of this transcription unit. The 5' end of these transcripts are derived from an exon, denoted I_{ϵ} , located 2 kilobases 5' of the C_{ϵ} switch region [$C_{\epsilon} = \epsilon$ heavy chain constant (C) region gene]. Nucleotide sequence analysis reveals that this RNA does not encode a protein, as the I_{ϵ} exon contains termination codons in all reading frames. Germ-line ϵ chain transcripts can be detected in cultures of normal splenic B cells treated with IL-4 within 24 hr, and this expression correlates with subsequent switching to C_{ϵ} . Consistent with the IL-4 inducibility of this RNA is the identification of a motif upstream from the site of transcription initiation that closely resembles a transcription element implicated in the IL-4 regulation of the gene encoding the murine class II histocompatibility antigen, $A\alpha^k$. These data lend support to the accessibility model of isotype switching and implicate IL-4 in the transcriptional activation of the C_{ϵ} locus.

The immunoglobulin heavy (H) chain constant region (C_H) determines the effector function of an antibody. For example, IgM is produced early in an immune response and is an effective opsinizing antibody, whereas the symptoms of atopic allergy arise from histamine release triggered by IgE binding to mast cells. In the mouse there are eight classes of immunoglobulin, each encoded by a distinct immunoglobulin C_H gene (designated *Igh-C*). The order of these genes on chromosome 12, which together span ≈ 180 kilobases (kb) are: 5' – μ , δ , $\gamma 3$, $\gamma 1$, $\gamma 2b$, $\gamma 2a$, ϵ , and α – 3' (1).^{*} During B-cell development, immunoglobulin H-chain gene segments are assembled by two types of DNA rearrangements (2). The first involves the fusion of H-chain variable (V_H), diversity (D_H), and joining (J_H) elements into a contiguous sequence upstream of the C_{μ} gene. A second, distinct rearrangement, termed “switch recombination” juxtaposes the assembled $V_H D_H J_H$ genes upstream of another C_H gene, with the resultant deletion of the intervening DNA (for review, see ref. 3). This recombination occurs between regions of repetitive DNA sequence [switch (S) regions] that are located 5' of each C_H gene.

The synthesis of specific immunoglobulin isotypes in an immune response is regulated by the nature of the antigen and the helper T cells (4). T-cell independent antigens and polyclonal mitogens such as bacterial lipopolysaccharide (LPS) promote a modest degree of isotype switching to $C_{\gamma 2b}$ and $C_{\gamma 3}$

(5). T cell-dependent regulation of immunoglobulin isotypes appears to be mediated by certain T cell-derived lymphokines (for reviews, see refs. 6 and 7). Splenic B-cell cultures treated with LPS and interleukin 4 (IL-4) preferentially switch to IgG1 (8) and IgE (9) and down-regulate the synthesis of IgG2b and IgG3 (10). In contrast, γ interferon, while appearing to promote switching to IgG2a (10, 11), inhibits the synthesis of IgG1, IgG2b, IgG3, and IgE (10).

Two models have been proposed to account for regulated isotype switching. The first implies that isotype-specific recombination enzymes mediate switching through the recognition of repeated motifs that are unique to a particular S region (12). In the second model, switching is performed by a common recombinase, with the specificity of this rearrangement determined by S region accessibility (13, 14). Experimental evidence favors the latter model. The “accessibility model” is based upon the observation that, in certain B-cell lines, the expression of specific germ-line C_H transcripts precedes switching to the corresponding C_H genes (13, 14). Germ-line $C_{\gamma 2b}$ and $C_{\gamma 1}$ transcripts were subsequently shown to be induced in splenic B-cell cultures by LPS (15) and IL-4 (16–18), respectively, prior to switching to these isotypes. A mechanism favored by the association of germ-line transcription with preferential switching is that lymphokines and mitogens could direct switching by transcriptional regulation of recombination-enhancing elements located near C_H genes. Germ-line transcription promoted by these elements could alter S -region chromatin structure, increasing accessibility of these sequences to a recombinase.

Here, the topography of the germ-line ϵ transcription unit deduced from the sequence of cDNA copies of germ-line ϵ RNA is described,[†] and the expression of these transcripts is shown to be inducible in normal B cells by IL-4.

MATERIALS AND METHODS

Cell Culture. The murine B lymphoma I29 was maintained as described (13). Splenic cells were cultured at a density of 10^6 cells per ml in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 50 μ M 2-mercaptoethanol, 25 μ g of LPS (GIBCO) per ml, 10^3 units of recombinant murine IL-4 per ml, or a combination of LPS and IL-4 at these concentrations. Murine IL-4 was prepared from the supernatant of COS cells transiently transfected with the simian virus 40–IL-4 expression vector pcDSR α 205 as described (19).

Abbreviations: IL-4, interleukin 4; LPS, lipopolysaccharide; H, heavy; C, constant; V, variable; J, joining; D, diversity; S, switch. ^{*}These designations will be used for *Igh-C* mouse genes that have been assigned respectively the following names (C_H product in parentheses): *Igh-6* (IgM), *Igh-5* (IgD), *Igh-8* (IgG3), *Igh-4* (IgG1), *Igh-1* (IgG2a), *Igh-3* (IgG2b), *Igh-7* (IgE), and *Igh-2* (IgA).

[†]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M31133).

RNA Hybridization. Poly(A)⁺ RNA isolated from I29 or splenic B cells was fractionated on 1% formaldehyde agarose gels (20) and transferred onto nitrocellulose membranes. Filters were prehybridized in 40% formamide/5× SSC (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7)/0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% albumin/100 μg of denatured salmon sperm DNA per ml and then hybridized for 16–18 hr at 37°C with probes at 2–4 × 10⁶ cpm/ml. Filters were washed in 6× SSC/0.1% sodium dodecyl sulfate (SDS) at 50°C and exposed for autoradiography. All probes were labeled by random primer extension (21) to specific activities of 5 × 10⁸–10⁹ cpm/μg.

cDNA Cloning. Poly(A)⁺ RNA isolated from the I29 was used to synthesize cDNA, which was cloned into the bacteriophage vector λgt10 (22). Approximately 5 × 10⁵ recombinant phage were screened by plaque hybridization (23) using as a probe a 1.2-kb *Pst* I restriction fragment located 2 kb 5' to the murine *C_ε* S region (1).

Nucleotide Sequencing. Two independent cDNA clones, ε1 and ε2, and the 1.2-kb *Pst* I fragment located 2 kb 5' to the *C_ε* S region (see Fig. 2B) were subcloned into the M13 phage vectors mp18 and mp19 (24) and sequenced by the dideoxynucleotide chain-termination method (25) according to the strategies outlined in Fig. 2.

S1 Nuclease Mapping. A 560-base-pair (bp) *Sph* I/*Bal* I fragment spanning the 5' end of the *I_ε* exon was end-labeled with [γ-³²P]ATP (3000 Ci/mmol; Bresatech, Adelaide, Australia; 1 Ci = 37 GBq) at the *Bal* I site by using polynucleotide kinase to a specific activity of 10⁷ cpm/μg and was purified from low-melting agarose. Total RNA isolated from I29 or L cells (50 μg) was hybridized to an excess of the denatured DNA probe at 50°C as described (26). Samples were digested with 200 units of nuclease S1 (Boehringer) per ml at 37°C for 45 min and fractionated on a 5% acrylamide/8 M urea gel.

RESULTS

Cloning of Germ-Line ε Transcripts. The murine B-cell lymphoma I29 is able to spontaneously switch from IgM to C_{γ2a}, C_ε, or C_α (13). Switching to these isotypes is preceded by germ-line transcription of the corresponding C_H genes (13), which led to the proposal that directed switching may result from transcription-induced accessibility of switch regions to a recombination enzyme(s) (13, 14). To understand how IL-4 promotes switching to C_{γ1} and C_ε and what role germ-line transcription plays, the structure of the germ-line ε transcripts from I29 was characterized.

The germ-line ε RNA in I29 is ≈1.7 kb, slightly smaller than the secreted form of a V_H-containing ε mRNA (27). As the V_HD_HJ_H gene expressed from the functional allele of I29 does not hybridize to the germ-line ε transcript (unpublished results), and as the C_ε region, 3' untranslated region, and poly(A) tail together would only constitute 1.45–1.5 kb of this RNA, it was likely by analogy with the germ-line γ2b transcripts (28) that the 5' end of the germ-line ε RNA would map upstream of the S_ε region. Therefore, cloned genomic sequences located 5' of S_ε, which together span 3.5 kb, were used as hybridization probes to try to locate sequences that contribute the additional 250 bp to the germ-line transcript. These data are summarized in Fig. 1. A 1.2-kb *Pst* I fragment (Fig. 2B, probe c) located 2 kb upstream of the S_ε region hybridized to a 1.7-kb RNA expressed in surface IgM-positive I29 cells (lane c) that was the same size as the germ-line transcript detected with a C_ε probe (lane a). Genomic sequences immediately 5' (lane b) and 3' (lane d) of this *Pst* I fragment failed to hybridize to the germ-line ε RNA, indicating that this region appeared to encompass an exon (designated I_ε) unique to the germ-line ε transcript.

To understand in detail how the structure of the germ-line ε RNA is related to the topography of the C_ε locus, a cDNA

FIG. 1. Detection of an exon unique to the germ-line ε transcript by Northern blot analysis. Five microgram samples of I29 poly(A)⁺ RNA were fractionated on a 1% formaldehyde/agarose gel and transferred to nitrocellulose membrane filters. Individual lanes were hybridized with DNA probes located within those regions of the C_ε locus shown in Fig. 2B. Lanes: a, probe a, a 1-kb *Hind*III/*Xba* I fragment spanning part of C_ε exon 3 and all of exon 4; b, probe b, a 1.05-kb *Bam*HI/*Pst* I fragment 3 kb 5' of the S_ε region; c, probe c, a 1.2-kb *Pst* I fragment located 2 kb upstream of S_ε; d, probe d, a 1-kb *Pst* I/*Stu* I fragment immediately 5' of S_ε. Lane a was subjected to autoradiography for 24 hr, whereas lanes b, c, and d were exposed for 4 days. The size estimate of 1.7 kb for the germ-line ε RNA in I29 was based on the mobility of RNA size markers (BRL). Bands larger than 1.7 kb are precursor transcripts (unpublished results).

library generated from I29 poly(A)⁺ RNA was screened for cloned copies of the germ-line ε transcript by using the 1.2-kb *Pst* I fragment as a probe. Two positively hybridizing clones, designated λε1 and λε2 with cDNA inserts of 1.50 and 1.55 kb, respectively, were chosen for further analysis. The strategies used to determine the nucleotide sequence of the ε2 cDNA and the genomic region encompassing the I_ε exon are shown in Fig. 2 A and B, respectively. The nucleotide sequence of the 1.2-kb *Pst* I fragment containing the I_ε exon is shown in Fig. 3.

The ε2 cDNA sequence of 1536 nucleotides reveals that the germ-line ε RNA, with the exception of the 5' 96 nucleotides, shares complete identity to the C_ε gene across C region domains C_{H1} to C_{H4} and utilizes the secreted domain and normal transcription termination signals. Moreover, both ε1 and ε2 have poly(A) tails, confirming that the 3' end of the cDNAs are complete. The 5' 96 nucleotides of the ε2 cDNA correspond to nucleotides 773–869 in the *Pst* I genomic fragment encompassing the I_ε exon (Fig. 3), proving that the 5' end of the germ-line ε RNA does indeed map to this region of the H-chain locus. In Fig. 4, the sequence from the 5' end of the ε2 cDNA is compared with that of the C_{H1} exon of C_ε and its 5' flanking DNA and with the 3' region of the I_ε exon. There is a consensus splice donor signal (nucleotides 868–872; Fig. 3) located at the site where the sequence of I_ε diverges from that of ε2, and the normal splice signal 5' of the C_ε C_{H1} exon is used as the splice acceptor. Thus, the germ-line ε RNA is generated from a primary transcript by a splice that removes the intervening sequence between I_ε and C_ε, including S_ε.

To identify the site(s) of transcription initiation, the germ-line ε RNA was subjected to S1 nuclease mapping. By using the strategy outlined in Fig. 5B, a 560-bp *Sph* I/*Bal* I fragment that should encompass the 5' end of the I_ε exon was used to determine the site at which the germ-line ε transcripts initiate. I29 RNA (Fig. 5A, lane D) protected a dominant band of ≈215 nucleotides, indicating that the I_ε exon must be between 240 and 245 nucleotides in length. Longer exposures of S1 nuclease gels (not shown) revealed two sets of protected fragments 50–60 and 68–85 bp in length. These may represent minor sites of transcription initiation. A low abundance of the germ-line ε RNA in I29 prevented a precise assignment of the transcript initiation site by primer-extension analysis, so it is not possible to completely rule out that the 5' end of this transcript is derived from a small exon upstream of I_ε. This

FIG. 2. Schematic diagrams of the germ-line ϵ cDNA clone and the C_ϵ locus. (A) Sequence strategy of 1.55-kb germ-line $\epsilon 2$ cDNA: arrows indicate the direction and distance sequenced of each restriction fragment. All sequencing utilized restriction sites within the cDNA with the exception of a region corresponding to domain 4, which was sequenced by using a 17-nucleotide primer complementary to nucleotides 1984–2000 of the sequence of murine C_ϵ (33). This is indicated by the arrow with the bar. Bracketed *EcoRI* sites at the ends of the cDNA correspond to restriction sites added during the cloning process. The stippled region corresponds to the coding region ($CH1$ – $CH4$) of the C_ϵ gene, the filled area corresponds to the 3' untranslated region, and the open area corresponds to the 5' germ-line exon I_ϵ . The wavy line spanning the junction of C_ϵ domain $CH1$ and the I_ϵ exon corresponds to the sequence shown in Fig. 4.2. (B) Organization of the germ-line C_ϵ locus. The restriction map of the germ-line C_ϵ locus is based upon the data of Shimizu *et al.* (1). B, *Bam*HI; Bg, *Bgl*II; Pst, *Pst*I; Sp, *Sph*I; X, *Xba*I; St, *Stu*I; Hc, *Hinc*II. The location of the hybridization probes a, b, c, and d, described in Fig. 1, are displayed as bars. The wavy line 1 spanning the 3' end of the I_ϵ exon corresponds to the sequence shown in Fig. 4, line 1, and the wavy line 3 at the 5' end of the C_ϵ $CH1$ exon corresponds to the sequence shown in Fig. 4, line 3. The sequencing strategy of the 1.2-kb *Pst* I fragment encompassing I_ϵ is displayed in the expanded region. The jagged line at the 5' end of I_ϵ signifies that the precise cap site of the germ-line C_ϵ RNA has yet to be determined.

however is unlikely as the 5' boundary of the I_ϵ exon mapped by S1 nuclease does not contain a sequence homologous to a RNA splice acceptor signal. Therefore, the size of the major germ-line ϵ transcript determined by cDNA cloning and S1 nuclease mapping is ≈ 1.65 kb, which is in good agreement with Northern blot estimates. As analysis of the I_ϵ sequence (Fig. 3) reveals that there are stop codons in all three reading frames. Although one ATG codon is in the same reading frame as C_ϵ , the open reading frame rapidly terminates within the I_ϵ exon. Therefore, the germ-line ϵ transcript is a non-coding or sterile RNA.

A Motif Highly Homologous to an IL-4-Responsive Transcription Element Is Located Upstream of the Germ-Line ϵ Transcription Initiation Site. The sequence of the region immediately upstream of the initiation site for the germ-line ϵ RNA is shown in Fig. 3. The promoter for this transcript appears to belong to that class of RNA polymerase II promoters that do not contain motifs related to a TAATA or

CAAT consensus sequence (29). It also lacks the G+C-rich binding sites of the transcription factor Sp1, which are often associated with the promoter of housekeeping genes (30). Instead, the sequence immediately upstream of the transcript initiation site (box II, nucleotides 608–623) is related to a 17-bp element that has been shown to be sufficient for terminal deoxynucleotidyltransferase gene expression (31). It remains to be determined if this sequence functions as the promoter for the germ-line transcription unit. Approximately 150 nucleotides upstream from the transcriptional start site (box I, nucleotides 486–499) is a 14-bp motif highly homologous to two elements upstream of the gene encoding the murine class II histocompatibility antigen $A\alpha^k$ that specifically bind a nuclear protein induced in B cells by IL-4 (32). The sequence upstream of I_ϵ , 5'-ATGGTGACTGCACG-3', is identical to the consensus sequence for the $A\alpha^k$ elements (5'-ATGNTGCNTNNAAG-3') at 12 of 14 nucleotides, with no insertions or deletions. No other sequences homologous

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Pst I
CTGCAGCTGAGACAGACACTACTAGTACCCCATGAAAGCTGCTGAGCCAAAGCCAGCCCTCACACCATCTTTACCCTCATCCCTCCCTCAGTGCAGAC 100
ATAGACCACAGGCCTGGAAGAGACGTTAGCTGTTTCTACACAGCTCCGTGAAACCCAGTCACAACCCAGATGTGCTCTGTCTCTTGGACTCCTTGCCAG 200
AGTAGCAGGTAGAGGACCTCAAGCTGAAAGATAATCACTTGTGAGTGGGCACGAGGGAAGGCCACTGTCCCTCGCATGCCAGCTCCAAAGCTGATACAGG 300
AACTAGGGTGCCTCTATCAGAGGCCCTGCAATGTCATATCTGGCCACAGGCTGTTCTCTTTGTGACCATTAATAACTTACAAAGTGACAGCCACACT 400
CCCCGTAAGGCTGCCAAAGGAACAGAAAAAGCAATGGCGAGGGTGTAGTCTGCCTCAGGGCAGTGACACTCCAAGGGGCAGGCATGGTGACTGCACG 500
ACACACATGCAAGGCTTTAATACGAGAGCTATGCAAGGAGACCTGGGATCAGACGATGGAGAATAGAGAGCCTTGACAGAGTGTGAGGTTGTCTCTCT 600
AGAAAGAGGCCTCACCTGAGAGCCACTGTGCCTTAGTCAACTTCCAAGAACAGAAATCAAAGGGAATTCCTCAAGGCTGTAAAGCCGGGGGTTCCCAAC 700
CCACTTTTAGCTGAGGGCACTGAGGCAGAGCGGCCCTTAGGTACTACCATCTGGGCATGAATTAATGGTTACTAGAGATTACAAACGCTGGGAGCCTGC 800
ACAGGGGGCAGAAAGATGGCTTCGAAATGAAGACAGTCTGGCCAGCCACTCACTTATCAGAGGACCTCAGGTATTACAACCCATGGGACCCCTGAGCAAAAGG 900
GTTTGCTTAAGGAGAAGGGACAAACAGGTTACAGGGTCTGGGTGGGGAAGGGGACACCTGGGCTGCCTTCTAATGTGGACAGTCTCTTGACCACCGAAT 1000
GTCCTTCAGCTATCACTTCCCTGCACTAAGGCACACAGGTATTAGAAATCTGATAGCTATTATCATGAAGACGGGGGACTGTGGATCTCAACAGAGAGGG 1100
CTGAACCAAGATAAACTGAATATGTTGTGAGAACTCAAAACTGCAG
Pst I
1148

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FIG. 3. Nucleotide sequence of the I_ϵ exon and flanking regions. The I_ϵ exon is displayed as the slanted nucleotides, and the 5' and 3' flanking regions are shown as upright nucleotides. The 3' splice donor signal is underscored by dots. The approximate location of the germ-line ϵ transcript cap site mapped by S1 nuclease is shown, although the precise nucleotide(s) remains to be determined. Termination codons in the I_ϵ exon are underlined, and the asterisk denotes the 5' end of the $\epsilon 2$ cDNA clone. Boxed region I is the motif homologous to the transcription element for the $A\alpha^k$ gene that binds an IL-4-inducible nuclear protein, and boxed region II represents the putative promoter element.

FIG. 4. Nucleotide sequence of the germ-line ϵ RNA. The sequence from the 5' end of the ϵ 2 cDNA (line 2) is compared with that of the 3' junction of the I_ϵ exon (line 1) and the 5' junction of the C_ϵ CH1 exon (line 3). The regions the sequences correspond to are shown in Fig. 2. The I_ϵ exon and 3' flanking sequence are represented by capital letters, while lower case letters denote the C_ϵ CH1 exon and 5' flanking sequence. The consensus splice donor and acceptor signals (39) are underscored by the dots.

to known recognition elements for DNA binding proteins have been identified within the 600-nucleotide region 5' of the I_ϵ exon. The presence of this motif may indicate that IL-4 promotes switching to C_ϵ as a direct result of an IL-4-inducible nuclear protein binding to this site and inducing germ-line transcription.

IL-4 Induces Germ-Line ϵ Transcription in Normal B Cells. To determine if IL-4 could induce germ-line ϵ transcription in normal B cells, poly(A)⁺ RNA isolated from splenic B-cell cultures treated with IL-4 or a combination of LPS and IL-4 for periods of 1–3 days was analyzed by RNA blotting. These results are summarized in Fig. 6. Either IL-4 alone (lanes 4 and 5) or in combination with LPS (lanes 6–8) could induce the expression of the 1.7-kb germ-line ϵ transcript. Expression was detected as early as 24 hr, and in the LPS/IL-4-treated cultures, it appeared to reach steady-state levels by 48 hr. Cultures treated with IL-4 alone could not be monitored for periods later than 48 hr, as cell death became too great after this time. The expression of germ-line ϵ transcripts in B cells stimulated with LPS and IL-4 precedes switching to IgE, which is first detected at day 3 in these cultures (unpublished results). Although LPS treatment alone was unable to induce germ-line ϵ expression in splenic

B cells (lanes 1–3), transcript levels in LPS/IL-4-treated cultures after 48 hr were $\approx$ 2-fold higher than that seen in cultures incubated in IL-4 alone for the same period of time. While this difference can be most easily explained by LPS-mediated B-cell proliferation in the LPS/IL-4-treated culture, it remains a possibility that LPS enhances the IL-4-mediated induction of these transcripts.

DISCUSSION

An increasing body of data favors a model in which germ-line transcription of C_H genes induced by mitogens and lymphokines may promote isotype switching by altering the chromatin structure of a switch region, thereby increasing the accessibility of these sequences to a recombinase. As a first step in determining the role of germ-line ϵ transcription in switching, cDNA copies of the germ-line ϵ RNA were cloned and the transcription unit was characterized.

Germ-line ϵ transcription appears to initiate 2 kb upstream of S_ϵ , proceeds through S_ϵ , and terminates downstream of C_ϵ . The intervening sequence separating the noncoding I_ϵ exon and C_ϵ is spliced from the primary transcript to generate the 1.7-kb germ-line RNA. Therefore, the structure of this transcript makes it highly unlikely that the germ-line ϵ RNA in I29, a cell line that expresses several germ-line RNAs, arose from a single, long transcript that was differentially processed in a manner analogous to that used in the coexpression of IgM and IgD (33). Although the germ-line ϵ , γ_{2b} (28), μ (34), and α (unpublished results) transcription units are very similar in overall organization, the germ-line I_ϵ , $I_{\gamma_{2b}}$, I_μ , and I_α exons share no significant homology. Therefore, there is no evidence supporting the derivation of the germ-line exons from a common ancestral sequence.

While the control of DNA accessibility has been used to regulate DNA recombination in a number of genetic systems (35), it is possible that germ-line transcription may only be a manifestation of other events responsible for targeting S

FIG. 5. S1 nuclease mapping of the germ-line ϵ transcription initiation site. Total RNA from either L cells or I29 cells was annealed to a 560-bp Bal I/ Sph I restriction fragment labeled with polynucleotide kinase to a specific activity of 10^7 cpm/ μ g and treated with S1 nuclease, and the products were fractionated on a 5% polyacrylamide/urea gel. The size of the major protected fragment (215 nucleotides) was estimated from the relative mobility of radio-labeled Hae III-digested ϕ X174 DNA. Lanes: A, Hae III-cleaved ϕ X174 DNA; B, labeled Bal I/ Sph I fragment with S1 nuclease omitted; C, L-cell RNA control; D, I29 cell RNA. The band of $\approx$ 300 nucleotides in lane D is artifactual, as it is sometimes seen in L-cell or yeast tRNA-only controls. (B) Diagrammatic summary of S1 nuclease protection data. The open box corresponds to the I_ϵ exon, SD is the splice donor signal, and the asterisk indicates the labeled Bal I restriction site.

FIG. 6. Induction of germ-line ϵ RNA in splenic B cells stimulated with IL-4. Samples (5 μ g) of poly(A)⁺ RNA isolated from mitogen- and/or lymphokine-treated splenic B cells was fractionated on a 1% formaldehyde/agarose gel, transferred to nitrocellulose membrane, and hybridized with the 1.2-kb Pst I fragment that encompasses I_ϵ . The filter was subjected to autoradiography for 4 days. Lanes: 1–3, LPS-stimulated splenic cells harvested after 24, 48, and 72 hr, respectively; 4 and 5, IL-4-stimulated cells harvested after 24 and 48 hr; 6–8, LPS/IL-4-stimulated cells harvested after 24, 48, and 72 hr.

regions for recombination. However, the identification of a motif 5' to the initiation site of germ-line ϵ transcription that is highly homologous to an IL-4-responsive transcription element, provides strong support for the idea that IL-4 does indeed promote switching to C_ϵ by direct transcriptional activation of the locus. If this element is important in the regulation of germ-line ϵ transcription by IL-4, then it is likely that a similar motif will be present in the region 5' to $S_{\gamma 1}$, where germ-line $\gamma 1$ transcription is thought to initiate (17, 18). The induction of germ-line ϵ transcripts in normal B cells by IL-4 alone, may indicate that, like the commitment to IgG1 switching (36), IL-4 can initiate the switch to ϵ independently of mitogen.

Even if germ-line C_H transcripts play a direct role in switching it is unclear what this could be. If transcription merely serves to make S regions accessible to the recombinase, then these transcripts are a nonfunctional by-product of this process. Alternatively, although germ-line ϵ and $\gamma 2b$ transcripts do not encode proteins, these RNAs may still play a functional role in the switching process. For example, RNA has been shown to be part of the catalytic ribonucleoprotein telomerase in *Tetrahymena* (37).

Several lines of evidence suggest that the switch recombination enzyme(s) is regulated independently of the signals that selectively target an S region for recombination. The pre-B-cell lines 18-81 and 38B9 are both able to correctly rearrange introduced copies of S region substrates at a similar frequency, indicating that both cell lines contain the recombinase activity (38). However, only in line 18-81 does the endogenous $C_{\gamma 2b}$ gene undergo switching, and this correlates with the expression of germ-line $\gamma 2b$ transcripts. Furthermore, treatment of B cells with IL-4 alone induces the expression of germ-line $\gamma 1$ (18) and ϵ transcripts but does not result in the production of these isotypes in the absence of a mitogenic signal (36). Taken together, these findings are consistent with switching requiring at least two events; mitogenic stimulation of resting B cells by agents such as LPS may be linked to the expression of the recombinase, whereas other events, such as germ-line transcription, target recombination to a particular S region.

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