Germ Line Transcription of the Immunoglobulin Heavy Chain Locus Directs Production of μ Chain without VDJ

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Abstract

Immunoglobulin VDJ recombination is associated with transcriptional activation of the Ig variable region elements. We have previously described a novel Ig μ chain protein and mRNA produced by pre-B cell hybrids from normal and X-linked agammaglobulinemic bone marrow. We have now characterized the mRNA encoding this protein and find that it is composed of a 5′ leader sequence spliced to Cμ (LS-Cμ), lacking the variable (V), diversity (D), and joining (J) gene sequences. The leader sequence is encoded by a novel exon 16 kb upstream of the JH locus. Transcription of the germ line heavy chain locus from this LS exon results in transcriptional activation of the JH locus, apparently the initial step in commitment to B lymphoid development. Polymerase chain reaction amplification of normal bone marrow shows that these germ line LS-Cμ transcripts are a product of bone marrow pre-B cells. Production of LS-Cμ commences a sequential process of transcriptional activation, with concordant translation of Ig rearrangement intermediates, in the process of creating a productive VDJ rearrangement. (J. Clin. Invest. 1992. 89:2046–2052.) Key words: immunoglobulin • recombination • B lymphocytes • leader sequence • pre-B cells

Introduction

The genes encoding the variable regions of immunoglobulin are assembled in developing lymphocytes by selection and recombination of diverse gene elements. The Ig heavy chain V region is formed by recombination of variable (VH), diversity (D), and joining (J) gene elements (1, 2) in an ordered rearrangement process (3). First a D gene element is recombined to a JH segment, followed by recombination of a VH to the formed DJH structure. The sequential steps of recombination are catalyzed by a single recombinase (4). The steps of recombination are associated with transcriptional activation of the recombinating gene elements, suggesting that the recombinase is directed in its sequential steps by control of access (5, 6). In VH to DJH recombination, the DJH intermediate is transcribed from promoters upstream of several of the D gene elements (7, 8), and unrearranged VH elements are transcribed from the promoters required for mature VDJμCμ transcription. Transcription of the unrearranged JH locus has been reported (6), but the transcripts and promoter that directs this transcription have not been characterized.

The human antibody deficiency disease X-linked agammaglobulinemia (XLA) results from failure of B lymphocyte development (9). Patients with the major phenotype of XLA have normal numbers of pre-B cells in bone marrow, but only limited numbers of mature B lymphocytes in peripheral circulation (10). We have previously proposed that the arrest in B lymphoid development in XLA results from failure of Ig V(D)J recombination (11). This proposal was based on the identification of truncated μ chains produced as mRNA and translated polypeptides in pre-B cell hybrids derived from the bone marrow of patients with XLA. This novel transcript was identified in some pre-B cell hybrids from normal fetal liver, but was the only product identified in hybrids from three patients with XLA.

We have now characterized these truncated μ chains, and report that they are novel transcripts of the unrearranged Ig heavy (H) chain locus, representing transcriptional activation of the JH locus. The transcripts are composed of a 5′ leader sequence spliced to the μ constant region (LS-Cμ), lacking the V, D, or J sequence characteristic of the previously described DTμ (DJH rearrangement) or mature μ (VDJμCμ). LS-Cμ is transcribed from an exon 16 kb upstream of the JH locus in the unrearranged H chain locus. Transcription from this exon entails transcriptional activation of the unrearranged JH elements. By polymerase chain reaction (PCR) amplification, we have identified LS-Cμ transcripts in normal bone marrow, further indicating that it represents a normal product of VDJ recombination.

Methods

The cell hybrids are as described previously (11, 12). Normal human bone marrow was obtained by iliac crest biopsy from a normal volunteer, with aspiration into preservative-free heparin as anticoagulant. Written informed consent was obtained following procedures reviewed and approved by the Committee on Clinical Investigation, Children's Hospital, Boston. Mononuclear cells were separated by buoyant density centrifugation on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ), and adherent cells were removed by adherence to plastic petri dishes overnight. For nucleic acid preparation, cells were pelleted by centrifugation, the medium aspirated, and stored at −80° until used to make RNA or DNA.

1. Abbreviations used in this paper: D, diversity; H, heavy; J, joining; LS, leader sequence; LS-Cμ, 5′ LS spliced to the μ constant region; PCR, polymerase chain reaction; VH, variable; XLA, X-linked agammaglobulinemia.

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RNA was isolated by guanidine-HCl lysis as described by Chirgwin et al. (13), followed by differential ethanol precipitation to remove DNA. PolyA-containing sequences were enriched by chromatography in oligo-dT cellulose. cDNA was synthesized with cloned Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD) using oligo-dT or random hexamers (Promega Corp., Madison, WI) for priming. A cDNA library was constructed from oligo-dT–primed cDNA by annealing dC-tailed cDNA into dG-tailed pBR322. The insert from the Cμ plasmid pμHM2 and a 61-bp EcoRI-HinfI fragment at the 5' end of Cμ (nt 47–108, see [9]) were used to screen the library. The inserts of pμHM2 and μb9 were later subcloned into pUC18. LS-Cμ sequence was determined by both Maxam and Gilbert's (14) and diodeoxy methods (15), and the sequence was determined for both strands. Genomic DNA was extracted, digested with restriction endonucleases, and Southern blotted to nitrocellulose (Schleicher & Schuell, Inc., Keene, NH) as described by Maniatis et al. (16). 5' terminal cDNA probes from μb9 and 73 2-33 were EcoRI fragments of cDNA clones isolated by electrophoresis through a 5% acrylamide gel (14). EcoRI sites are present at the 5' terminus of these cDNAs (added by adapters for cloning) and at nucleotide 47 of the Cμ sequence.

Polymerase chain reaction amplifications were performed in a thermal cycler (Perkin-Elmer Cetus Instruments, Norwalk, CT) with buffers, dNTPs, and Taq1 polymerase from Perkin-Elmer Cetus. PCR for Cμ was synthesized with random primers, and incorporation of [32P]dCTP in an aliquot used to quantitate the cDNA. Primers were synthesized by Dr. A. Whitehead (Harvard Medical School, Boston MA), using a synthesizer (Applied Biosystems, Inc., Foster City, CA). The primers used were two nested primers for Cμ:

CMI 5' TGATGGAGTCGGGAAGGAAGTCCTGT 93–127 of Cμ
CMI 5' TGCTGACCTTTCGACACCGTGGTCGTGTGCGGT 247–278 of Cμ.

These are reverse complement sequences of the coding sequence of Cμ.

LSP 5' TTCCATTGCTTTGATCAGCTCAGACTGAACACACAGAGCTGT –84 to –48 of μb9.

Three primers from Vμ gene 5' flanking leader sequence 1st exons were:

61RILS 5' TCACCAAGAGCCTCCAGACAAT –158 to –138 of 6-1R1 (Vμ6)

19IIIS 5' TACCTTTCTGAGGTCTCTGGACC –173 to –153 of 1-9II (Vμ4)

212LS 5' CCTCTTCTACAGAAGCTCT –181 to –163 of 21-2 (Vμ1).

These primers were derived from sequences published by Berman et al. (17). A map of the Ih chain locus, and the positions of these primers is given in Fig. 1. The positions listed are nucleotides upstream from the 5' end of the FR1 sequence, which places them in comparable positions to LSP with respect to the leader sequence first exon of these Vμ gene elements. cDNA was first amplified with CMI as 3' primer. If no product was detected in an agarose gel, 1 μl of a 1:20 dilution of the primary PCR was reamplified with either CMIII or CMI nested primer.

The conditions for PCR amplification were 94° (denaturation) for 1 min, 70° (annealing) for 1 min, 72° (reaction) for 3 min, for 30 cycles. The annealing temperature was reduced to 65° for the LSP/CMI reaction with normal bone marrow. Precautions were taken to prevent cross-contamination.

Primers to amplify a fragment entirely were used as positive control for amplification. These primers:

CM4R
5' CAACCTTGAGTGCCTGTCAGAGATCTC –700 +700 reverse complement of Cμ coding strand and CM5F
5' AAGACGTGCCTCTTCCAGTGATTG –299 +323 of Cμ coding strand

yielded a 433-bp amplification product. We used these primers as control in the following way: All reagents for amplification of an experimental cDNA except primers were mixed in a single tube. These reagents included the test cDNA and Taq1 polymerase. 98-μl aliquots were placed in individual tubes and the relevant primers (e.g., 61RILS and CM4, or CM4R and CM5F) were added, followed immediately by thermal cycling for amplification. The CM4R/CM5F reaction then served as a test for addition of all necessary reagents for the amplification reaction and provided a control for comparison of amplification between experiments run at different times.

Amplification products were identified by Southern blotting of samples in agarose gels. The DNA was transferred either to nitrocellulose as previously described (16) or to Sure Blot Hybridization Membrane (Oncor Inc., Gaithersburg, MD) following the manufacturer's instructions. Blots were probed with the insert Cμ fragment from the pμHM2 plasmid.

Figure 1. Map of primers used for PCR examination of Ig transcripts. (A) The germ line Ig heavy chain locus, not shown to scale, is shown. Vμ gene elements are carried in the genome over a large region starting ~90 kb upstream of the Cμ coding sequence. The single element Vμ6 gene, named 6-1R1 by Berman et al. (17) is the most 3' of the Vμ gene elements. The locations of the Vμ4 (1-9II) and Vμ1 (21-2) gene elements, and the Vμ6 gene elements that have near identity to the μb9 (LSP primer) leader sequence are not known. The LS exon is not shown. (B) The structure of Vμ gene elements is shown. A leader sequence first exon is separated from the remainder of the leader sequence (second exon) and Vμ coding sequence by an intron of ~100 bp. The primers 61RILS, 19IIIS, and 212LS were taken from sequences of the first leader sequence exons of the 6-1R1, 1-9II, and 21-2 Vμ gene elements. These primers are analogous in position to the LSP primer on the 56P1 Vμ gene element. (C) The positions of the leader sequence and Cμ primers on LS-Cμ germ line and VμDJμCμ mature transcripts are shown. The LSP primer yielded PCR amplification products with both germ line and mature transcripts (see Fig. 2 and Results). The 61RILS, 19IIIS, and 212LS primers yielded PCR amplification product only with mature transcripts (see Results).
Phage clones spanning the Jμ to Cμ intron were CH 4-22 (a twin to CH 4-38 [18]) and CH 28-6, provided by P. Leder (Harvard Medical School), as described by Ravetch et al. (18) and clone C7p6.2 was as described by Rabbits et al. (19). Cosmid C17p1, c17p3, and Cos6b, provided by H. Schroeder (University of Alabama at Birmingham) and D. Cox (Hospital for Sick Children, Toronto), were as described by Schroeder et al. (20).

**Results**

cDNA sequence encoding the truncated μ chain. Four pre-B cell hybrids were previously described (11). LSM-T, LSM-A, and LSM-SB21 resulted from fusion of mononuclear cells from bone marrow of three different patients with X-linked agammaglobulinemia. LSH6 was a clone derived from fusion with normal fetal liver pre-B cells. This clone was selected for study because it produced a truncated μ chain that comigrated with the μ chain produced by the XLA hybrids. The LSM 2.7 human myeloma used as fusion partner made no detectable Ig as protein or RNA, and had deleted the Cμ gene (12, and unpublished results). The four hybrids (LSM-T, LSM-A, LSM-SB21, and LSH6) were previously characterized to produce truncated μ heavy chain without variable region as both mRNA and polypeptide. μ chain proteins were reduced in size to 54 and 57 kD, compared with 66 and 69 kD for normal μ chain (secretory and membrane forms). mRNA-encoding μ chain was reduced in size, due to absence of 300 nt of 5′ terminal sequence encoding the V region.

To determine the 5′ terminus of the truncated μ chains, a cDNA library was constructed by oligo-dT priming of mRNA from the LSH6 hybrid. Two clones encoding μ chain were isolated. Restriction mapping of the clones confirmed that there were no deletions of the Cμ sequence. The sequence of the clones was determined from the EcoRI site at nucleotide 47 of Cμ. The sequence lacks Vμ D, or Jμ gene sequence of mature μ chain or previously characterized immature Δμ. In place of this sequence, one clone (μb9) has an 88-nt sequence that has near identity to the first LS exon of two human Vμ gene elements (Fig. 2) (21). The second clone has an identical sequence but extends only 63 nt upstream of Cμ. The putative LS has an initiation codon (ATG) 61 nt upstream of the 5′ terminus of Cμ and an open reading frame downstream. The ATG is in phase for translation of Cμ. Compared with the product of primer-extension experiments, the longer clone is missing 65 nt of the full message, presumably 5′ untranslated sequence.

Because of the size of the cDNA sequence, its open reading frame, and the absence of other detectable μ chain message, it seems likely that this truncated message encodes the 54/57 kD protein identified in the pre-B cell hybrids. Since leader sequences are removed from the nascent polypeptide with passage into the lumen of the endoplasmic reticulum, this truncated polypeptide is Cμ, and the transcript is LS-Cμ. This novel μ chain is the product of the pre-B parents of these hybrid cells. The myeloma parent of these hybrids could not have contributed the novel μ chains because it lacks the Cμ gene by Southern blotting and produces no detectable μ chain RNA, either by Northern blotting or by PCR amplification (unpublished results).

**LS-Cμ is a product of normal pre-B cells.** Immune fluorescence experiments previously suggested that some pre-B cells from normal bone marrow produced μ chain without V region antigen (11). To determine whether LS-Cμ is a product of (these) normal pre-B cells, we examined normal bone marrow directly by PCR amplification. cDNA prepared from total RNA of normal human bone marrow was amplified with primers derived from Cμ and LS. A DNA fragment of 360 bp, the size predicted for the 5′ terminus of LS-Cμ with these primers, was identified (Fig. 3). This product was isolated from a gel, reamplified, and cloned into pUC18. The sequence of this molecule has near identity to the LS cDNA clone from the LSH6 pre-B cell hybrid (Fig. 3 B). The three nucleotides that differ are conservative for known leader sequence functions. The differences might result from an artifact of PCR amplification. However, isolation of seven independently derived PCR clones with this sequence from this sample of normal bone marrow leads us to suspect that this sequence represents polymorphism in the LS. Truncated μ chains composed of LS-Cμ are likely candidates for the product of normal bone marrow pre-B cells previously identified to produce μ chain without V region antigen.

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**Figure 2.** Sequence of cDNA clone μb9 from the pre-B hybrid LSH6. An ATG initiation codon is located at position −43, identical in location to the initiation codon for the known leader sequence first exon of the Vμ gene element 56P1 cloned as cDNA from fetal liver (21). 56P1 is spliced to the second exon of the Vμ gene element, containing the remainder of the leader sequence and the coding sequence of the variable region. μb9 is spliced to the 5′ terminus of Cμ, providing the initial A nucleotide to the 5′ terminal GA, necessary for in frame translation of Cμ. There are two single base differences between μb9 and 56P1. The single nucleotide change in the coding region of this proposed leader sequence results in conservative substitution of Thr for Ser. The μb9 sequence has been submitted to GenBank under accession number M81847.
LS-Cμ is transcribed before recombination. Southern blotting of the JH locus from the pre-B cell hybrids suggests that LS-Cμ results from transcriptional activation of the Ig heavy chain locus before recombination. HindIII/BamHI double digestion was used to separate the JH locus from Cμ. Genomic DNA from the four pre-B cell hybrids contained a 7-kb band that comigrates with the embryonic (unrearranged) JH locus band from an unrearranged T cell line (Fig. 4). This band is present against a background of multiple rearranged JH bands that are contributed by the myeloma parent of the hybrids, and is the only band unique to the hybrid cells that are shared in all four hybrids. All of the other JH bands in the pre-B cell hybrids are also present in the myeloma parental genomic DNA. We suggest that LS-Cμ is transcribed from the unrearranged H chain locus.

Does LS-Cμ result from transcriptional activation of VH gene elements? Cμ with a leader sequence nearly identical to the leader sequence first exon of some VH gene elements might result from transcriptional activation of the entire H chain locus, including the VH gene elements, or from transcription from a specific LS exon upstream of the Cμ locus. For LS-Cμ to result from transcriptional activation of VH genes, long transcripts spanning more than 100 kb from the unrearranged VH gene elements to Cμ would utilize the splice sequence at the 3' end of some or all VH gene element first exons, yielding truncated μ chains with leader sequence first exons of diverse origin. We tested for truncated μ chains with alternate VH leader sequences by PCR amplification of LSH6 pre-B cell hybrid cDNA with primers specific for leader sequence first exon of several VH gene elements. Primers used were specific for VH6.
Figure 4. J_H gene loci in pre-B cell hybrids. Genomic DNA from the pre-B cell hybrids (10 μg) was digested with BamHI and HindIII, Southern blotted, and probed with the 2-kb Sau3A fragment spanning the J_H locus from J_H2 to J_H6 (18). LSM 2.7 parent human myeloma (P) and CEM T cell line with J_H genes in embryonic (unarranged) configuration (E) are shown as controls. A band unique to the four pre-B cell hybrids comigrates with the 7-kb band with unarranged J_H locus from CEM. Molecular weight markers are λ DNA digested with HindIII.

(single element family that is most proximal to Cµ) and 1-9II (V_H4 gene family) and 21-2 (V_H1 gene family). Amplification product was identified only with the LSP primer derived from the μb9 sequence (data not shown), indicating that LS-Cµ results from transcription from a specific LS exon.

Mapping of the LS exon. The absence of transcriptional activation of V_H gene elements suggests that LS-Cµ results from transcription of a specific LS exon upstream of Cµ. Direct cloning of the LS exon has been hindered by the near identity of μb9 with the leader sequence first exons of some V_H gene elements, and the apparent polymorphism of the LS between different individuals. As an alternate approach, we have assumed that the LS exon lies between the V_H6 gene element, the most 3' V_H gene element, and Cµ, and restriction mapped a collection of plasmid, phage, and cosmids spanning this region.

Nelson et al. (22) identified transcriptional activation of the Cµ locus arising from the J_H to Cµ intron. Toned sterile μ transcripts, they have not been found to be processed into mRNA or to yield a sequence that can be translated. To rule out LS-Cµ transcription arising from this intron, we examined phage clones that span this region. A battery of LS probes, both oligonucleotides and cDNA, did not hybridize with Southern blots of these clones (the phages used are shown in Fig. 5, the negative blot is not shown). The plasmid clone Ccp9.2 from T. Rabbitts (Medical Research Council Laboratory for Molecular Biology, Cambridge, England) was examined also, but is not shown (19). To allow for polymorphism, these hybridizations were carried out at reduced stringency (42° and 6× standard saline citrate [SSC]). This suggests that LS transcription must arise from an exon upstream of the J_H locus.

Southern blotting of cosmids clones spanning the region from the most 3' V_H gene element, V_H6, to the J_H locus indicates that the LS exon lies 16–18 kb upstream of J_H1. Examination of the cosmids clone cos6 with 5' terminal fragments from the μb9 and 73 2-33 cDNAs identified a 9-kb HindIII fragment that is 14–23 kb upstream of J_H1 (Fig. 5). The μb9 cDNA probe was removed at temperatures above 48° and salt below 2X SSC, while the 73 2-33 probe was resistant to melting to 55° and 0.1X SSC, further evidence for polymorphism in the LS. Digestion of cos6 with additional enzymes refined this map, locating the LS exon to a 2.3-kb SacI fragment that lies 14–16 kb upstream of J_H1. No additional fragments that hybridize with LS probes were found on the other cosmids clones extending to the V_H6 gene element (Fig. 5). This location for the LS exon is exactly analogous to the κ promoter upstream of the Jx locus (23), and suggests that the LS exon serves as a transcriptional promoter for the J_H locus.

Transcription from the LS exon yields a 26–28-kb primary transcript with splice donor sites at the 3' end of the LS and at the 3' ends of each J_H element, with a single splice acceptor site at the 5' end of Cµ. LS-Cµ mRNA was only identified from

Figure 5. Mapping of the LS exon. The phage and cosmids clones used to map the LS exon are shown below the map of the region from V_H6 to Cµ on chromosome 14. The phage clones CH 28-6 and CH 4-22 (twin to CH4-38), as described by Ravetch et al. (18), showed no hybridization with LS probes, either oligonucleotides, or 5' EcoRI restriction fragments from the cDNA clones μb9 and 73 2-33, when stringency was reduced to 42° at 2X SSC. The cosmids, as described by Schroeder et al. (20) showed hybridization of both oligonucleotides and cDNA restriction fragments with the 9.0-kb HindIII band shown on the map. The oligonucleotides melted from this band above 42° at 2X SSC. The cDNA restriction fragment from μb9 was stable to 48° at 2X SSC while the cDNA fragment from 73 2-33 was stable to 0.1X SSC at 55°. SacI digestion refines this map, identifying a 2.3-kb fragment at the 3' end of the 9.0-kb HindIII fragment. Upstream in this map is to the left, with the V_H6 gene element 77 kb 5' to the J_H gene elements. The scale above the top line is in kb. The map locations of the gene elements shown are derived from Schroeder et al. (20).
splicing of the LS splice donor site to the Cμ splice acceptor site. This product is translated into polypeptide. Alternate splicing from the Jμ element splice donor sites would yield 16–17-kb RNAs that likely could not be translated. These alternate products would not have been identified by PCR amplification.

Discussion

We have described a novel Ig H chain transcript that arises from an exon upstream of the JH locus before recombination. LS-Cμ production may represent the initial step in recombinational access for the unarranged H chain locus, directing transcriptional control of the JH elements before DJH recombination. The recombinase has been proposed to be directed in its sequential steps of catalysis by control of access (24). This proposal is based on identification of transcription of the DJH intermediate and unarranged Vμ gene elements in Abelson virus–transformed murine pre-B cells (5). Transcriptional activation of the light chain Jα locus has been described, and Vμ to Jα recombination is associated with transcriptional activation from the kα exon (6, 25). The LS exon serves as an analogous promoter for the H chain, directing transcriptional control of the unarranged JH locus. LS-Cμ transcripts are processed into mRNA and translated into polypeptide.

The first three steps in Ig recombination are associated with transcriptional activation. The unarranged JH locus is transcribed from the LS exon before D to JH recombination (6). Unarranged Vμ gene elements and the DJH intermediate step of recombination are transcribed during Vμ to DJH recombination (5, 6). The murine light chain locus has a leader sequence exon, kα, analogous to the human H chain LS exon (23, 25). kα has near identity to the first leader sequence exon of Vμ gene elements, and lies 3–4 kb upstream of the Jα locus. Transcripts from the kα exon are processed into truncated k chain mRNA. In addition to the k chain locus, T cell antigen receptor gene elements likely are transcribed. Dβ elements direct transcription and likely translation of the DJβH intermediate (26). Transcripts of Tα and Tβ have been described in human B cell precursor cell lines (27), and appear to be transcribed in T cells as well (28). Transcriptional activation of gene segments during recombination of Ig and T cell antigen receptors appears to be a rule.

mRNA transcripts of the same size as LS-Cμ have been identified previously in murine cell lines, but not further characterized (29, 30). Kemp et al. (29) found a 1.9-kb Cμ transcript as the only Ig present in T cell and myeloid cell lines with unarranged Ig loci, including the T cell line EL4. Schlissel et al. (6) described a μ0 PCR amplification product that resulted from transcription of the unarranged JH elements in EL4. The PCR based assay used by Schlissel et al. (6) would not identify mRNA derived from an LS exon that was processed as LS-Cμ. However, alternative processing of LS-Cμ primary transcripts, using the splice donor sites at the 3' ends of each JH locus, would yield RNAs that amplify as μ0. We suggest that there is a murine analogue of LS-Cμ and the LS exon, leaving open the question of how alternative processing of these transcripts is regulated.

A mechanical interpretation of the access model entails sequential activation, first of the JH locus, then of D gene elements for D to JH recombination, then of Vμ gene elements for Vμ to DJH recombination. The linear arrangement of the gene elements does not fit with a strict interpretation of this model.

One human D gene element is located within the JH gene locus (18), and several D gene elements are upstream of the most 3' VH gene element (31). An alternate explanation of the observed transcriptional activation is simply that the entire locus opens up in preparation for recombination, transcription occurs coincidentally, and the sequential steps of recombination are regulated by a secondary mechanism. This view is consistent with a report of differential regulation for D to JH versus Vμ to DJH recombination in murine transfection vectors (32). In this view, conservation of transcriptional promoters upstream of the JH and Jk loci, and of the D gene elements is not strictly required for recombination. Instead, they could then be viewed either as evolutionary relics, or could be of import for translation of the intermediate structures into protein.

The significance of translation of these immature products into protein is not clear. LS-Cμ transcripts are clearly translated into truncated μ chains by the pre-B cell hybrids previously described and likely represent the 3–5% of pre-B cells from normal bone marrow previously identified to be Cμ + Vμ by immune fluorescence (9). Cell-free translation of LS-Cμ from the pre-B cell hybrids showed that some of the mRNA includes the 3' μ exon necessary for insertion in the cell membrane, raising the possibility of these molecules posing as membrane Ig. Dμ intermediates are translated into protein in both murine and human cells (7, 8, 33). Again, some of these molecules include the μ exon, and are known to be expressed as membrane Ig. The truncated μ chains, both LS-Cμ and Dμ, may combine with the light chain surrogates lambdα5 and Vpre-B to form a pseudo-immunoglobulin molecule without variable region (34, 35). Polypeptides with sequence predicted by the kα nucleic acid sequence have been reported (36, 37). The Dβ transcripts of T cell antigen receptor have an upstream ATG initiation codon with following open reading frame with the properties of a leader sequence, suggesting that this intermediate may be translated as well (26). The conservation of transcription and translation of the several steps of recombination in both murine and human cells is the strongest argument for a regulatory role of these intermediate products.

We have previously proposed that the failure of B cell development in patients with XLA results from a failure of H chain VDJ recombination (11). VDJ recombination is the primary event in pre-B cells. LS-Cμ was the only Ig product found in uncloned pre-B cell hybrids from three XLA patients. In contrast, only 3 of 15 pre-B cell hybrids derived from normal fetal liver produced LS-Cμ (unpublished results). Determination of whether LS-Cμ is the predominant product of XLA bone marrow pre-B cells, by direct examination of the products of XLA bone marrow pre-B cells, will be reported in a later publication.

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