Isolation and characterization of the gene for the murine T cell differentiation antigen and immunoglobulin-related molecule, Lyt-2

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ABSTRACT

We present here the sequence of the 5310 base pair Hind III-cleaved genomic DNA segment that includes the gene for the Lyt-2, a murine differentiation antigen expressed on most immature T lymphocytes as well as the cytotoxic suppressor T cell subset. We also present the complete intron/exon structure of Lyt-2. There are five exons: a fused leader and immunoglobulin variable region like exon, a hinge region exon, a transmembrane exon and two alternatively spliced intracytoplasmic exons (alternative splicing of these exons yields the 38 kDa alpha and 34 kDa alpha′ Lyt-2 polypeptides). The promotor region contains a "TATA" box and sequences homologous to the putative immunoglobulin transcriptional control elements cd/pd. S1 protection analysis reveals that thymocytes, T cells from lymph nodes, and a Lyt-2 transfectant obtained by introduction of total genomic DNA have the same initiation site. In the 3′ region, there is a polyadenylation signal sequence after a 700 bp long 3′ untranslated region.

INTRODUCTION

The murine T lymphocyte differentiation antigen Lyt-2 is a membrane glycoprotein co-expressed with Lyt-3 on immature T cells as well as a distinct subset of T cells: cytotoxic and suppressor cells (1-3). The postulated function of the Lyt-2,3 complex, based on blocking experiments with monoclonal antibodies against Lyt-2, is to serve as an accessory molecule to aid in the binding of cytotoxic T cells (CTL) to non-polymorphic region(s) of the major histocompatibility complex class I molecules expressed on all target cells (4-9). The human homolog Leu-2 has similar functional characteristics and tissue distribution as Lyt-2 (10-12). In thymocytes, the Lyt-2 determinant is found on two polypeptides of 38kDa (alpha) and 34kDa (alpha′), whereas lymph node T cells express only the 38 kDa polypeptide (13-15). This represents a maturation phenotype for Lyt-2 expressing cells. We, and others (16-18) have reported the sequence of two Lyt-2 cDNAs which arise from differential splicing. In addition, we have formally proven that the differential splicing of the primary transcript is responsible for the two differently sized Lyt-2 molecules by utilizing cDNA expression vectors (17). We have also reported that the Lyt-2 molecule is homologous to Leu-2 and belongs to

the immunoglobulin gene super family (16). In this paper, we report the complete sequence, the intron/exon organization and some control elements of the Lyt-2.2 gene.

MATERIALS AND METHODS

Lyt-2 genomic cloning: An Mbo I partial genomic library of B10.A liver DNA, in lamda J1 phage (19), was screened with the insert of pLY2C-1 (16) as a hybridization probe. Plaque hybridization was performed under the conditions described previously (16). Positive clones were picked and DNA was prepared by standard methods. The DNA was cleaved with several restriction enzymes and electrophoresed on a 0.7of L(TK^-) cells with Lyt-2 genomic clones was performed as described (20).

DNA Sequence Analysis: The isolated 5.3-Kbp Hind III fragment digested with Sau3AI, Alu I, and HaeIII was shotgun cloned into mp18 and mp19 phage vector. Single stranded phage DNA was sequenced by the dideoxynucleotide chain termination technique of Sanger, et al. (21) as modified by Messing (22). Forced subcloning of genomic fragments with Aha III, Bam HI, Eco RI, Hind III, Pst I, Xba I, Xho I, was also used to construct the sequence. Analysis of the sequence data was performed using BIONET (NIH Grant 1U41 RRO01681-01) and STADEN (23,24) programs on a VAX 780 computer, and the Beckman MicroGenie sequence analysis program.

RNA Extraction and S1 Nuclease Protection Assay: Total RNA and poly A^+ RNA was obtained as described (25). ^{32}P-labeled single strand probe was synthesized from M13 mp template based on Burke's procedure (26). Annealing of probe fragments with RNAs and S1 nuclease (Pharmacia P-L Biochemicals) digestions were performed as described by Favaloro, et al. (27). The ^{32}P-labeled probes made by this procedure were also used for plaque hybridization.

RESULTS

Isolation of genomic DNA encoding Lyt-2.2

We screened an Mbo I partial genomic library of B10.A liver DNA with the insert of the previously characterized partial Lyt-2 cDNA clone (pLY2C-1) and isolated a lambda j1 phage (L-6CCA) containing a 15-Kbp insert. Hybridization with pLY2C-1 as well as the full length cDNA clone, pLY2C-22 (16), suggested that the Lyt-2 gene is located on a single 5.3-Kb Hind III fragment. This 5.3-kb Hind III fragment was subcloned into the unique Hind III site of pBR322 (p6CCA). In order to establish that the insert of p6CCA contains the entire functional Lyt-2 gene, DNA-mediated gene transfer was performed. About 25% of TK^+

(thymidine kinase) L cells cotransfected with either the L-6CCA or p6CCA DNA were shown to be positive for Lyt-2 by FACS (Fluorescence Activated Cell Sorter) analysis (16). The presence of Lyt-2 molecule was confirmed by immunoprecipitation, showing both 38Kd and 34Kd polypeptides (16). Thus, Lyt-2 alpha and alpha′ chains can be expressed as homo- and heterodimers in the absence of Lyt-3.

Nucleotide sequence and exon/intron structure of the Lyt-2.2 gene

Comparison of the p6CCA sequence with that of full length cDNA (pLY2C-22) (16) reveals the intron/exon boundaries shown in Figure 1a and Table I. The Lyt-2 gene has five exons (Figure 1b): a fused leader and immunoglobulin variable region like exon, a hinge region exon, a transmembrane exon and two intracytoplasmic exons. Thus, each exon corresponds approximately to a functional domain of the molecule; this is similar to other members of the Immunoglobulin superfamily (28), except that Lyt-2 does not have a separate leader exon as do all other sequenced immunoglobulin super family members. We have previously shown that the 38 kDa alpha Lyt-2 peptide is derived from the splicing of all five exons to form an alpha-encoding mRNA and that the
34 kDa alpha′ Lyt-2 peptide is formed by the splicing of the transmembrane donor site directly to the fifth exon, thus deleting exon 4 from the mRNA (17). Loss of this 31 bp exon effects a frameshift and results in the 34 Kd alpha′ Lyt-2 peptide.

Nucleotides at the intron/exon boundaries which conform to consensus splice donor and acceptor sequences (29) are shown in Table I. Only the transmembrane donor, which alternately splices to the fourth or fifth exon, does not have the AG motif on the exon side of the boundary. We note that the mRNA splicing between exons 1 and 2, 2 and 3, 3 and 4, and 3 and 5 occurs between the first and second nucleotides of a codon triplet, as with most immunoglobulin and Class I MHC splice junctions (30). Splicing between exon 4 and 5 joins the second and third nucleotides of a triplet, similar to an IgM membrane-bound exon. (31)

Determination of 5′ end of Lyt-2 message and putative transcriptionalregulatory elements:

A Hinf I-Hinf I fragment (507-559) was used to prime a Hind III- Eco RI (1-745) DNA fragment (which spans the ATG start codon and putative promoter region) cloned into mp19, in M13 primer extension, to produce a ^{32}P-labeled single-strand probe (probe structures in Figure 2a). This probe was hybridized to RNA from thymus, lymph nodes and total genomic Lyt-2 transfectants and then subjected to S1 analysis (Figure 2b). All RNAs protected a 430 bp band (data for lymph nodes not shown). Thymus RNA protected several other bands which, since they were not consistently found in several separate RNA preparations and S1 experiments, are considered to be due to partial RNA degradation. We also identified a 270

A

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AAGCTTGGGAACCCAGAAACCTATCTACAACAAAAACAAAACGGAGACTCCCCA  54
CTCCTCTCCCTTCATGTCCTGTCACTCTCATATTTTTTTTATTAAGATTTAATC  108
TTTATCCTTCTTCTTTCAGCCACTCTTGCAGAAGAACAATTCCTAAGCCTCCCT  162
TCCAAGTGTTTTCTCTTCCCCAAATCTCAGTTTTCAATTCCTCCCACTTCCTCT  216
GCAACGGTCCATTCTCACTCTGAGTTCCCAGCCCCCTAAAAGCTGCTTCACACT  270
CTTTGGTGGGGACTTTGGGTGACATCATATCCTCACATAGCAAATCAGCTCTGT  324
CTCCACCTGGCTAAAGGAGCAGTTTCCCCGACCCTACACGCCTCCCCCACCCCA  378
CCTCCTCCGCCCTGTTCCTGGGCCCCTCCCCTAGAGCCCTAGCTTGACCTAAGC  432

                          METAlaSerProLeuThrArgPheLeuSerL
TCCTTGCTGCTGGAGAGCACACCATGGCCTCACCGTTCACCCGCTTTCTGTCGC  486

euAsnLeuLeuLeuLeuGlyGluSerIleIleLeuGlySerGlyGluAlaLysP
TGAACCTGCTGCTGCTGGGTGAGTCGATTATCCTGGGGAGTGGAGAAGCTAAGC  540

roGlnAlaProGluLeuArgIlePheProLysLysMETAspAlaGluLeuGlyG
CACAGGCACCCGAACTCCGAATCTTTCCAAAGAAAATGGACGCCGAACTTGGTC  594

lnLysValAspLeuValCysGluValLeuGlySerValSerGlnGlyCysSerT
AGAAGGTGGACCTGGTATGTGAACTGTTGGGGTCCGTTTCGCAAGGATGCTCTT  648

rpLeuPheGlnAsnSerSerSerLysLeuProGlnProThrPheValTyrMETA
GGCTCTTCCAGAACTCCAGCTCCAAACTCCCCCAGCCCACCTTCGTTTATATGG  702

laSerSerHisAsnLysIleThrTrpAspGluLysLeuAsnSerSerLysLeuP
CTTCATCCCACAACAAGATAACGTGGGACGAGAAGCTGAATTCGTCGAAACTGT  756

heSerAlaMETArgAspThrAsnAsnLysTyrValLeuThrLeuAsnLysPheS
TTTCTGCCATGAGGGACACCAATAATAAGTACGTTCTCACCCTGAACAAGTTCA  810

erLysGluAsnGluGlyTyrTyrPheCysSerValIleSerAsnSerValMETT
GCAAGGAAAACGAAGGCTACTATTTCTGCTCAGTCATCAGCAACTCGGTGATGT  864

yrPheSerSerValValProValLeuGlnLys
ACTTCAGTTCTGTCGTCCCAGTCCTTCAGAAAGGTTTCGAAGTGCAGGTTCCCG  918

TCTCCTGGCTTTTAGGGGTCACATCTGAACAAGTTTCACCCACATTCCCTCTTC  972
TTAAATCTCTTGGGGCCTCTTGCAGCTCATTTCTATACTGTCCCCATTTTACAG  1026
ACCAAAAAGTTGAGGCTGAGCTCCTGGGTGGACTTGGAAGTGATTTTTAAACGC  1080
CCTGGATCTGGGCTGGACTCTGGATGCACGTGTGATCCTGCCCCTGTTGGCTCC  1134
AGGATAGAATTTTGCTATTGACTCCAGCTTTGGGTTGGTGGAGGCCCGCAGTCCC  1188
TTAGATCTGATCTAGGCACATTGGCCCAAATCACACTTGGCTCCCCGTGTGGCA  1242
GCAGCGGAGTAGCCCAGCACGTATCCAGGGGTTCCTTCTTCCTCTAGTTAAAAC  1296
CTAGGAAACGGCTCCTTCTTGGGTAAAGGCTAAGTGGAAAGGGGCTGGTGCATC  1350

                                              alAsnSerThr
CACAATTCCAGCTTGACCCCGCAGGCACCCTATTGCTTTTGCAGTGAACTCTACT  1404

ThrThrLysProValLeuArgThrProSerProValHisProThrGlyThrSer
ACTACCAAGCCAGTGCTGCGGAACTCCCTCACCTGTGCACCCTACCGGGACATCT  1458

GlnProGlnArgProGluAspCysArgProArgGlySerV
CAGCCCCAGAGACCAGAAGATTGTCGGCCCCGCTGCCTCAGGTTAGTGCTCTCAA  1512

GCGATTGAGATAAGCAGATAGCCTGGGGTGGAAAATCCTGATCTTGGAGGGGAG  1566
ACCTGGACGGGGAGACGTGCTGGGGGCAGGGTTGGGGCCTGTCCAGAGCGTTTC  1620

                              alLysGlyThrGlyLeuAspPheAla
TAAATTCCATTCATTCGGATCTTGTCAGTGAAGGGGACCGGATTGGACTTCGCC  1674

CysAspIleTyrIleTrpAlaProLeuAlaGlyIleCysValAlaLeuLeuLeu
TGTGATATTTACATCTGGGCACCCTTGGCCGGAATCTGCGTGGCCCTTCTGCTC  1728

SerLeuIleIleThrLeuIleCysTyrHisA
TCCTTGATCATCACTCTCATCTGCTACCACAGTAAGTTCTGGGAGTTCCCCCCA  1782

TCCAGCCCCCACCACCCGCCCCACCCCACCCCCGGCCAAGAGACTGCTTTGTGCTC  1836
CAGGAGGAGTCTGCAGCTTTCTGCCTGCTCCAGACAGCCTTCAGGGAGATTACT  1890
TTTAGGTTGGGCAAAAGGGGCAGGGCGTTCCAGCTGACCTATAGGACCAGTGTA  1944
GTGGGTTCTTTCTTGAAGGCAGGAGACAGGTATACCACCCCACTGCTCTTTCCA  1998
CCCTCTGGGAACTTTTCAGAACAGAGCCCTGTTCCAGGATCCCCAAGAGCAGCC  2052
AAATCAGCAGTTAGCACAGCCAGGATAACTTAAGCACACTAAGGAGGGGTTTAC  2106
ATACCGTGAGACCAGCCCCCAAGAAGGCTGAGTCACAACCATGACTGGCTTGAG  2160
ATTTGCCTTAATCCCACCTCACAAACCATTCACTCCTCTGAGTCTATACAGTGA  2214
TTACACCAACCAGGTCAACAGATTCCCACAGAGACTCACATCTCGATTTACAAT  2268
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ACTAGTTAGGTAGCCTAGGATAACCCCAAACTTCTGATCTGATCCTTCTGCCTT  2376
CCTTCATTTTGGGAGTGCTGGGACAAGGCCCATAGCCACCAAAGCAAATTTAAA  2430
ATGTTTTCTTTTATTATGTATTTATGCATTTATTTATTTCGCTTTTTTTTAAAC  2484

GCAGGGTTTCTCTCTCTAGCCCTGGCTCTGCTGGAACTCACCTGTAGACCAGGC  2538
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AGCCTGGCTGACCACAGCCTGCCTGAAATCTTTTATTTTTAATTCCAACTTGAG  2646
GAAAGAGTAGGCAAAGTAGTGTTCACTCCAAGTCTTGAACAGTTCTGAGACTCA  2700
AAGTTCTGGAGCAGAGGAAGCTATAGAAACCGTATCTGGCAGGTATTCATTCTC  2754

                rgSerArgLysArgValCysLysCysProAr
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AACGTCTCTGATCTCTAGGTCTCGTGACGCTGGGCAGCCGTGGTCCCAGTGGTG  2862
AGCTTTGGGAATCTTTCCTAGAGAGAGAGGTCATGCCAGTGAAGTCCTGGTGCC  2916
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TTTCAGGACCTGGAGTGAGCTTGGTAGCTTCTGCAAGACAATTACTTATGCTTA  3132
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 gProLeuValArgGlnGluGlyLysProArgProSerGluLysIleVal *
CAGGCCGCTAGTCAGACAGGAAGGCAAGCCCAGACCTTCAGAGAAAATTGTCTA  3780

AAATGGCACCGCCAGGAAGCTACAACTACTACATGACTTCACAGATCTCTTCTT  3834
GCAAGAGCCAGGCCCTCCTTTTTCAAGTTTCCTGCTGTCTTATGTATTGCCCTC  3888
TGTATTGTTTTAGTAGGGCTGTGATGGGGACAGTTCCTTTTTCTTTATGAATTC  3942
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GGTGGAAACAGCAGGATCTGTAGACCCGGAGAGAACCCAAGCTAGAGGAACCCT  4158
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GTGTCTTTAGTATAGCATGCTTGTGCCTCAAACTGCAAGGAACCAACTGCTATG  4266
AATCTGTGATCTGCTCAGCTCTGGCTGTGAGCATCAGATGTAACAAATCAAAGT  4320
CAGGAGAACTGCCTTTAGCGGTGCACCTGGACATCAGAGCCCCTTGTAAGAACT  4374
GCGAAATTGCTGCCACTCTAGTGGAGCGTACCGTAAATCTGGAAAACACTCCCC  4428
TCTGTCACTCAAGGCTCTAGTAGAATCCAATTAAAGCTATTCAAACCACTGCCG  4482  <- Poly A
CAGACCCTCTCCTGTGTCTGCGTGGAATGGCTCTCTCGACGACGTCGGCAAAGC  4536
GTAGGATTAAACTGACAAACAGATGCAGACAGGAGAGCCGTTGAATCTGAATGT  4590
AATGTCAGGTCGAACCTCGGACTTTTTATAATACAGAAAAAATTCCAGGGGGGA  4644
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CGAAAGGAATTTGCAGGAACTAGATAAATGTTTACAATAGAGATTATCAGCCCT  4752
GCCTAGCATCATCAGCCTACTGCCAGGTAAGAATTTCACACTTTAGTTTAAAAG  4806
CACCAGGGGGTAATTACTCTTGGCAGCCCTCAAGAATAATGTACTGTCACGGAC  4860
CCCTAAATTCCTAGGCCTTGTTAAATCTTATCTTCTCTCCACAATCCCCATTTA  4914
TCAGGAGAGTATCTCAACTTGCTCTTGGCATGGAATGTAGCCTGTACTAAAGAT  4968
TTTTATCTCAGTGAGATTCCCAGGCTCTTTCTGCAGACCAGATGAGCTAATGGC  5022
CTTGTTTATTGTAATGCTTAATTACTTACTGAATAAGTTAAACTCCTTGCTGTT  5076
ATCTGGGATCTTGGAACAATGGGGGGGCTGGGGGGGGGGGCGGGGGGGCTTTAC  5130
CTATCTCAGAATTCAATCTTAAAAGGCACTTACAATAGGCTAATGCTTAATACA  5184
GGCCACATGGATGCATACACTAGACTAACGCGGGAGTGGAAAATTAATTTATGG  5238
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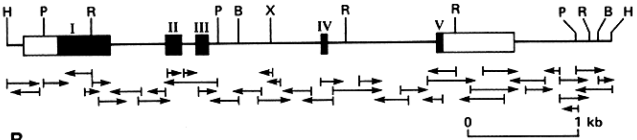


B

Figure 1: Nucleotide sequence and genomic organization of the Lyt-2.2 gene

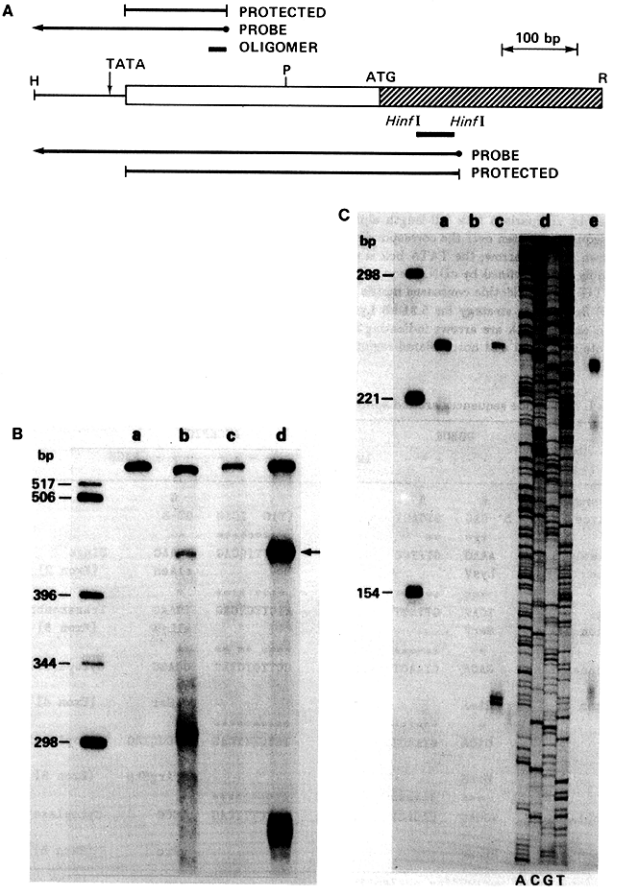
A) The nucleotide sequence of the 5310 base pair genomic Lyt-2.2 is shown. Exonic regions, derived by comparison to a full-length alpha cDNA clone, are noted by the predicted amino acid sequence written over the corresponding nucleic acid sequence. Start site of transcription is shown with an arrow; the TATA box is underlined and cd/pd-related sequences are boxed. The poly A site defined by cDNA sequencing is indicated with an arrow; the AATTAAA and CACTG pentanucleotide consensus motifs are indicated by a broken line above the sequence.

B) Sequencing strategy for 5.31-kb Lyt-2 gene. A schematic of the genomic structure is shown and beneath are arrows indicating the direction of sequencing. Black and open boxes indicate each exon and untranslated regions,respectively. H; Hind III, P; Pst I, R; EcoRI, B; BamH I, X;Xba I

Table I. Nucleotide sequences around splice junctions of the Lyt-2 gene

	DONOR			ACCEPTOR		
EXON	- - - : - - -	INTRON		- - - - : - - -	EXON	
Consensus	A A			G		
splice	5'-CAG	GTGAGT	... ...	(Y)6 XCAG	GT-3'	
	***	** *		*********	*	
Variable-like	AAAG	GTTTGG	... ...	GCTTTTGCAG	TGAAC	Hinge
[Exon 1]	LysV				alAsn	[Exon 2]
	***	** ***		**** ****	*	
Hinge	TCAG	GTTAGT	... ...	ATCTTGTCAG	TGAAG	Transmembrane
[Exon 2]	SerV				alLys	[Exon 3]
	*	******		**** ** **	**	
Transmembrane 1	CACA	GTAAGT	... ...	CCTTGTCTAG	GGAGC	Cytoplasmic
[Exon 3]	HisA				rgSer	[Exon 4]
	*	******		**********	**	
	CACA	GTAAGT		TCTCTTTCAG	GCCGCTAG	Cytoplasmic 1'
	HisS				erArgStp	[Exon 5]
	***	******		**********	*	
Cytoplasmic 1 2	CCCAG	GTGAGT	... ...	TCTCTTTCAG	GCCG	Cytoplasmic
[Exon 4]	ProAr				gPro	[Exon 5]

Y, unspecified pyrimidine nucleotide
N, Unspecified nucleotide
*, Identity to consensus splice sequence


A
PROTECTED
PROBE
OLIGOMER
100 bp
TATA
P
H
ATG
R
HinfI
HinfI
PROBE
PROTECTED
B
a
b
c
d
bp
517
506
396
344
298
C
a
b
c
d
e
bp
298
221
154
A C G T

bp protected band in the total genomic transfectant, possibly due to aberrant trancription initiation. To delineate the initiation site, a synthetic oligomer (CTCAAGGGTCCGGGG) complementary to nucleotides 228 to 242 was used to prime the M13 vector containing the Hind III to Pst I fragment for preparation of single-strand probe. Total RNA from thymocytes and genomic transfectants both protect the oligonucleotide-primed probe to give a 5′ initiation site at nucleotides 123 and 124 in the genomic sequence, thus localizing at least one transcription initiation event to this site (Figure 2c). Position 123/124 is compatible with the 430 bp protected band in the localization experiment described above. Using this initiation site as a landmark, we searched and identified a "TATA" box (TATTAA) 29bp upstream. We found no other "TATA" box sequence or ATG start codon between this initiation site and the methionine start codon defined as the translational initiation point for Lyt-2. Additionally, no sequence corresponding to a presumptive "CAAT" box could be found.

Since it is clear that Lyt-2 is evolutionarily related to immunoglobulin molecules, we searched our genomic sequence for DNA motifs homologous to the defined, though putative, immunoglobulin regulatory elements cd/pd (32,33). A nucleotide stretch bearing homology to the cd consensus sequence and the SV40 enhancer is found beginning at position 308 in Figure 1a with the alignment of homology shown in Table 2a. Downstream of this sequence, starting at position 326 in Figure 1a, there is a DNA motif with homology to pd of immunoglobulins, shown in Table 2b.

Determination of 3′ end of Lyt-2 RNA:

Sequencing of alpha cDNA clone 30-3 localized a poly A addition site to nucleotide 4476

Figure 2: S1 nuclease analysis of transcriptional initiation of Lyt-2.2
A) Probes used for S1 analysis in Figure 2b and 2c. The Hinf I fragment indicated was used to prime the Hind III(H)- Eco RI(R) fragment cloned into mp19 to produce a homogeneously labeled probe. The synthetic oligomer listed in the text was used as primer of the Hind III(H)-Pst I(P) fragment cloned into mp19 to precisely define the initiation site. Open box and shaded box indicate 5′ untranslated region and exon I, respectively.
B) S1 analysis using Hinf I-primed single-strand probe. Probe alone, with no S1 nuclease added to reaction mix (lane a). Probe hybridized with 3μg of poly A^+ thymus RNA (b) 3μg of polyA^+ L cell RNA (c) and 15μg of RNA from L cells transfected with total mouse genomic DNA and selected for amplified Lyt-2.2 expression (d). The fragment size was determined with Hinf I digested pBR322 size marker.
C) Delineation of 5′-most mRNA initiation site using synthetic oligomer. Probe alone, with no S1 nuclease added to reaction mix (lane a) Probe hybridized with 80μg of L cell RNA (b) 15μg of RNA from amplified Lyt-2.2 L cell transfectant(c) and 80μg of total thymus RNA (e). Sequence of Hind III-PstI mp19 probe used in this S1 analysis primed with the same oligomer (d).

Table II. Lyt-2 promoter region has homology to immunoglobulin regulatory sequences and SV40 enhancer.

A)	
cd CONSENSUS	ATGCAAATNA
Lyt-2 (308)	TAGGAAATCA
SV40 enhancer	ATGCAAAGCA
B)	
pd CONSENSUS	TGCAG/CCTGTGNCCAG
LYT-2 (326)	TGCAG CTGGCTAAAG

A) The immunoglobulin cd consensus sequence (31) aligned with a sequence from the Lyt-2 promoter starting at position 308 in the genomic sequence, and core region of the SV40 enhancer.
B) The immunoglobulin pd consensus sequence (31) aligned with a sequence from the Lyt-2 promoter starting at position 326 in the genomic sequence.

in the genomic sequence. We identified a consensus polyadenylation signal AATTAAAA at position 4456 and two consensus pentanucleotide sequences CACTG 5′ (position 4433) and 3′ (4475) to the polyadenylation signal. In order to test for the possibility of other poly A sites 3′ to this site because of the presence of RNA species migrating at 3.0-kb (16), we examined 23 newly isolated, uncharacterized Lyt-2 cDNA clones with two single strand probes derived from M13 vector with the following inserts: Xho I- Pst I (4517-4999) and Stu I - Hind III (4873-5305). No cDNA clones hybridized with these probes. However, 17 out of 23 clones were positive when hybridized with the Sac I - Pst I (4194-4999) probe. Based on these results, we conclude that Lyt-2 has one poly-A site which begins polyadenylation at residue 4476. We found no poly-A sites 3′ to this.

DISCUSSION

We have cloned and analyzed the gene encoding the Lyt-2.2 murine glycoprotein. Combining the results of Southern blot analysis of cloned phage DNAs with full length Lyt-2 cDNA, and transfection experiments, we conclude that the 5.3-Kb Hind III fragment is sufficient to encode the entire Lyt-2 gene. This was confirmed by sequence analysis of this DNA fragment and previously isolated Lyt-2 cDNAs. The Lyt-2 gene has five exons: a fused leader and immunoglobulin variable region-like exon, a hinge region exon, a transmembrane exon and two intracytoplasmic exons. The organization of exons corresponding to functional domains of the protein is similar to other members of the immunoglobulin gene superfamily (28). Lyt-2 is exceptional in that the leader exon is fused to the variable region-like exon, presumably by deletion of the intron since the human and rat homologues both have been shown to have a

separate leader exon (34,35). Sequences at the boundaries of introns and exons predicted by alignment of the alpha and alpha′ cDNAs to the genomic sequence conform to consensus splice sequences for mamalian mRNAs (29). Splicing between the first four exons of Lyt-2 occurs between the first and second nucleotides of the codon, representing what we postulate to be, at least for the variable region-like exon 1, a surviving evolutionary splicing pattern linking Lyt-2 to immunoglobulin genes.

The S1 protection study revealed that the Lyt-2 gene has an unusually long 5′ untranslated region when compared with most other mRNAs studied (36). There is no ATG codon nor "TATA" box-like sequence after the putative initiation site and before the methionine codon of the leader peptide. Preliminary analysis shows that Lyt-2 and its human homologue Leu-2 show significant homology in their 5′ untranslated regions, including a cd-like sequenced (unpublished observation); this may indicate that the structure of the 5′ noncoding region participates in the regulated expression of these genes.

In the promotor region of Lyt-2, there are sequences similar to cd and pd related elements which are considered to be a transcriptional regulatory elements in Ig genes (32,33). The position of these sequences relative to the ATG is similar to that of mouse Vk, 3′Ck, or human V_H (32), except that in Lyt-2, these sequences locate 3′ to the mRNA initiation site because of an unusually long 5′ non coding region in Lyt-2. Considering that Lyt-2 is a member of immunoglobulin superfamily, and linked to the immunoglobulin kappa loci, it will be of interest to know whether these sequences have a similar function, if any, to cd or pd sequences of immunoglobulin genes. On the basis of transfections with Lyt-2 cDNA expression vectors to which we linked the Lyt-2 genomic promoter, we have shown that these constructs allow expression of Lyt-2 (17). However, S1 analysis of these cDNA transfectants and L cells transfected with the isolated 5.3-kb Hind III fragment shows both probes (see Figure 2a) to be completely protected, indicating transcription initiates from upstream of the 5′ Hind III site. Whether this indicates that sequences in the genome upstream of the Hind III site are necessary to correctly initiate transcription, i.e. the start site as defined in total DNA transfectants and thymus, or that vector sequences are influencing transcription initiation remains to be determined.

We and others have shown that two types of mRNA arise by differential RNA splicing of exon 4 (17,18). We have formally proven by transfection with alpha and alpha′ cDNA expression vectors (17) that the two molecular forms of Lyt-2 are due to differential splicing and not post-translational modification (although we have shown that there are slight differences in glycosylation between the two chains). Alternative splicing similar to what we see with

the Lyt-2 gene is known to occur in several other genes, such as proprotachykinin in bovine (37), alpha A-crystallin in mouse (38), r-fibrinogen in rat (39), and myelin basic protein in mouse (40), however the mechanism which produces this alternative splicing is not understood. Since Solnick reported the importance of the secondary structure of the primary transcript as a mechanism for differential splicing (41), we searched for inverted repeats or repetitive sequences in the vicinity of exon 4. There are two long inverted repeats: the first at position 2966-2980 and its inverted homologue at 1796-1783 (ΔG=-25.2 Kcal/mol), and the second at 3594-3612 (ΔG=-22.0 Kcal/mol) with its inverted homologue at 2165-2148. Since a number of other factors probably play crucial roles in the alternative splicing process, we are unable to assign at present the relative importance these inverted repeats have to the differential splicing of Lyt-2.

During the completion of this manuscript, Liaw, et al. (42) published a partial nucleotide sequence of the Lyt-2.2 gene.

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