

Sequences of $C\mu$ and the V_H1 Family in LG7, a Clonable Strain of *Xenopus*, Homozygous for the Immunoglobulin Loci

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Twenty-eight heavy-chain variable (V_H1) region genes and the immunoglobulin (IgM) heavy-chain constant region of an isogenic *Xenopus* hybrid, *X. laevis/X. gilli*, LG7, have been sequenced. The LG7 clone represents the first *Xenopus* hybrid that is homozygous for the IgH locus. The V_H1 family was specifically investigated because V_H1 genes are used by the antibodies produced during the *Xenopus* antidinitrophenol (DNP) response. These V_H1 germ-line sequences establish a so-called "dictionary" that is available for studying somatic hypermutational mechanisms in immunized frogs.

KEYWORDS: Isogenic *Xenopus*, immunoglobulin, genes.

INTRODUCTION

The gynogenetic development of diploid eggs produced by *X. laevis/X. gilli* hybrids (LG) leads to the production of clones of heterozygous isogenic females (Kobel and Du Pasquier, 1975). The development of the different hybrids and the ease with which large numbers of them can be produced has been a major advantage in the studies of various aspects of the *Xenopus* immune system, particularly antibody diversity (Wabl and Du Pasquier, 1976; Du Pasquier and Wabl, 1978). As useful as they are, these clonable animals are still heterozygous and therefore genetically complex. It would considerably simplify immunogenetic studies if some LG hybrid clones could be made homozygous at the major histocompatibility complex (MHC) or immunoglobulin (Ig) loci. We report here such a clone, LG7, that is homozygous at the Ig heavy-chain (H) locus. In addition, we report most of its V_H1 germ-line sequences as well as the sequence of its IgM (μ) heavy-chain gene. This V_H family is involved in the antidinitrophenol (DNP) response, one of the best studied *Xenopus* antibody responses (Brandt et al., 1980; Schwager et

al., 1988). Thus, these germ-line sequences establish a "dictionary" necessary for future studies on somatic generation of the antibody repertoire in this species.

RESULTS

LG7 Is Homozygous for the Ig Loci

LG7 frogs first attracted our interest because they yielded fewer restriction fragments than other LG hybrids (Schwager et al., 1988). Whether this was due to homozygosity or to a Ig H chromosome loss was not known. To determine the cause of LG7's unique restriction fragment length polymorphism (RFLP) pattern, we decided to first use a variety of techniques. The previous RFLP analyses were confirmed by testing different digests of LG genomic DNAs with probes from all 11 V_H families, light-chain probes for $V\rho$ and $C\rho$ (kindly provided by our colleague J. Schwager; Schwager et al., 1991) and $C\mu$ probes. The individual patterns could be grouped into four RFLP classes, identical for each probe (LG3,15; LG17,5,46; LG14,6; and LG7). LG7 consistently gave half as many restriction fragments as the other LG7 hybrids and Fig. 1 shows examples of Southern blot hybridized with

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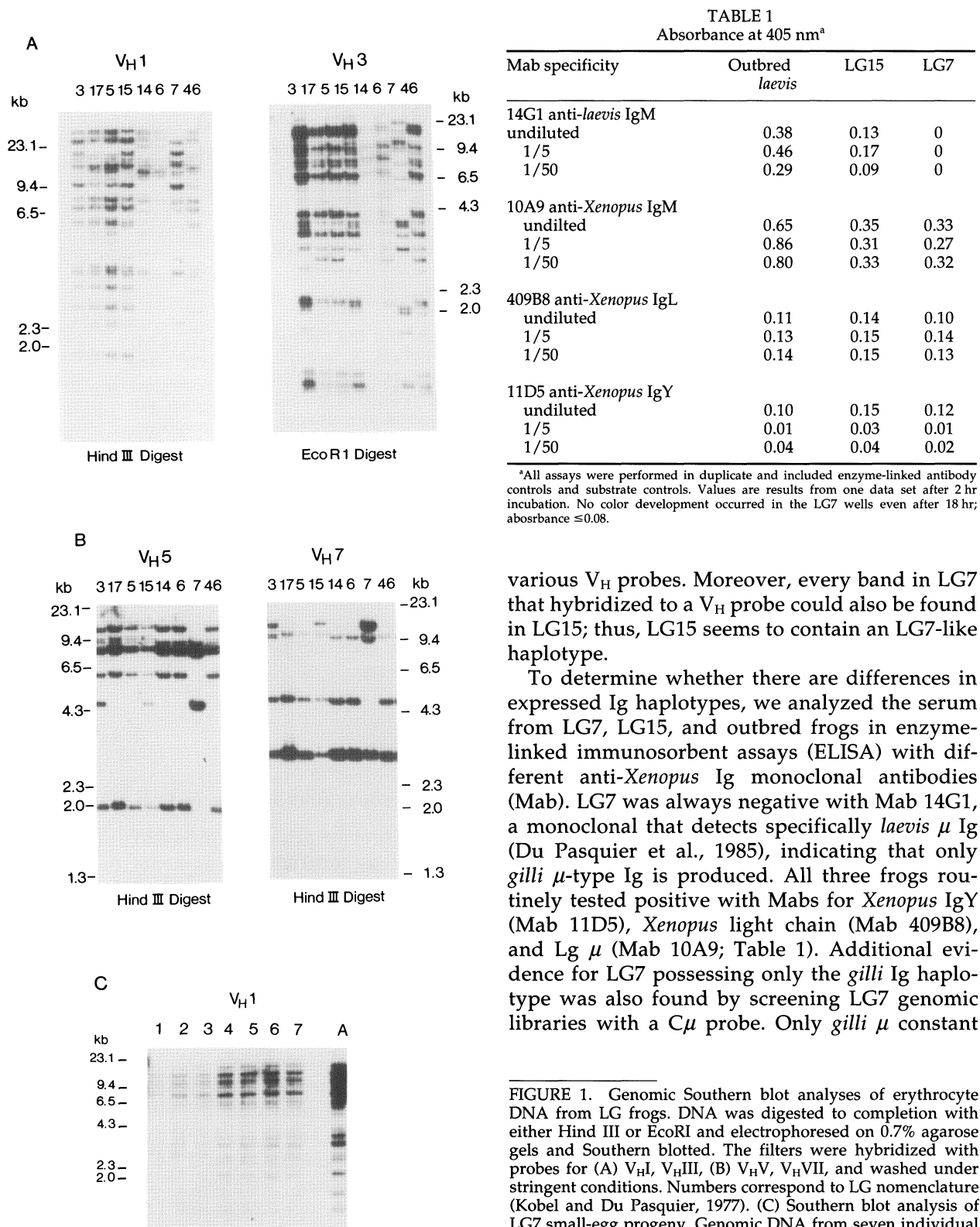


TABLE 1
Absorbance at 405 nm^a

Mab specificity	Outbred <i>laevis</i>	LG15	LG7
14G1 anti- <i>laevis</i> IgM			
undiluted	0.38	0.13	0
1/5	0.46	0.17	0
1/50	0.29	0.09	0
10A9 anti- <i>Xenopus</i> IgM			
undiluted	0.65	0.35	0.33
1/5	0.86	0.31	0.27
1/50	0.80	0.33	0.32
409B8 anti- <i>Xenopus</i> IgL			
undiluted	0.11	0.14	0.10
1/5	0.13	0.15	0.14
1/50	0.14	0.15	0.13
11D5 anti- <i>Xenopus</i> IgY			
undiluted	0.10	0.15	0.12
1/5	0.01	0.03	0.01
1/50	0.04	0.04	0.02

^aAll assays were performed in duplicate and included enzyme-linked antibody controls and substrate controls. Values are results from one data set after 2 hr incubation. No color development occurred in the LG7 wells even after 18 hr; absorbance ≤ 0.08 .

various V_H probes. Moreover, every band in LG7 that hybridized to a V_H probe could also be found in LG15; thus, LG15 seems to contain an LG7-like haplotype.

To determine whether there are differences in expressed Ig haplotypes, we analyzed the serum from LG7, LG15, and outbred frogs in enzyme-linked immunosorbent assays (ELISA) with different anti-*Xenopus* Ig monoclonal antibodies (Mab). LG7 was always negative with Mab 14G1, a monoclonal that detects specifically *laevis* μ Ig (Du Pasquier et al., 1985), indicating that only *gilli* μ -type Ig is produced. All three frogs routinely tested positive with Mabs for *Xenopus* IgY (Mab 11D5), *Xenopus* light chain (Mab 409B8), and Lg μ (Mab 10A9; Table 1). Additional evidence for LG7 possessing only the *gilli* Ig haplotype was also found by screening LG7 genomic libraries with a C μ probe. Only *gilli* μ constant

FIGURE 1. Genomic Southern blot analyses of erythrocyte DNA from LG frogs. DNA was digested to completion with either Hind III or EcoRI and electrophoresed on 0.7% agarose gels and Southern blotted. The filters were hybridized with probes for (A) V_HI, V_HIII, (B) V_HV, V_HVII, and washed under stringent conditions. Numbers correspond to LG nomenclature (Kobel and Du Pasquier, 1977). (C) Southern blot analysis of LG7 small-egg progeny. Genomic DNA from seven individual LG7 small-egg tadpoles (numbered 1-7) and an adult LG7 (labeled A) were digested with EcoRI and hybridized with a V_H probe. Markers (in kb) are indicated in the margins.

regions could be sequenced from two different LG7 genomic libraries. Even though *laevis* and *gilli* C_μ cross-hybridize and share long stretches of sequence identity, the introns are of different sizes, with characteristic repeats and thus are easy to identify (Du Pasquier, unpublished).

Although the foregoing data did not exclude loss of a chromosome or deletion of one of the Ig loci, additional experiments do so. Chromosome spreads of phytohemagglutinin-stimulated LG7 spleen cells contained 36 chromosomes, the number expected of LG hybrids. Moreover, when Southern blots were titrated with C_μ and C_ρ probes, the signal intensity of LG7 genomic DNA was equal to that of LG15 and LG14, that is, LG7 contains the same number of copies of C_μ and C_ρ as LG15 and LG14 (data not shown).

LG hybrids produce two types of eggs, small eggs that can be either normal haploids or aneuploids, and large genetically identical diploid eggs that are used to propagate the species. If LG7 was heterozygous, segregation of IgH (or any other gene family) polymorphism should be detected in progeny. However, the segregation cannot be followed in the large (2n) eggs because they are always identical. Instead, the small eggs (1n) that contain a segregated set of chromosomes must be analyzed. We compared in Southern blots digests of genomic haploid LG7

embryo DNA with adult LG7 DNA. The RFLP pattern of every haploid embryo tested was identical with the adult parent LG7 pattern when tested with different V_H probes and with a C_μ probe (Fig. 1C). Thus, LG7 is homozygous at the IgH locus. This and the fact that the LG7 pattern of Ig H-chain genes is identical to half of the pattern of LG15 Ig genes suggests that LG7 is homozygous for at least the *gilli* Ig H-chain gene set present in LG15.

LG7 Is Derived from the Gynogenetic Development of an LG15 Small Egg

Small eggs rarely develop beyond hatching unless the second polar body is not extruded so that diploidy is restored and a viable embryo is created. This is a rare occurrence. When small egg embryos do survive, their genetic loci will still be heterozygous if there is a crossover between a locus and the centromere. Thus, heterozygosity is a function of gene position; heterozygosity increases with increasing distance from the centromere (Nace et al., 1970; Kobel and Du Pasquier, 1977).

We believe that the LG7 hybrid developed from an accidentally selected viable diploid LG15 small egg (see Fig. 2). Because all LG hybrids are tested by skin graft as a routine control, this

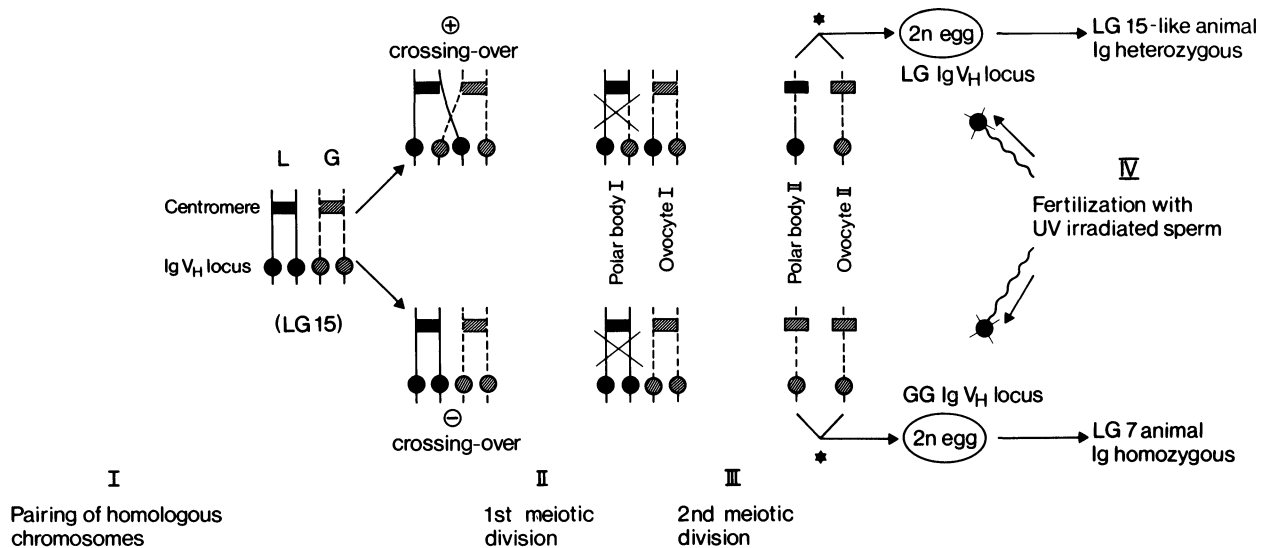


FIGURE 2. Scheme depicting LG7 origins. Pairing of *laevis* (solid lines) and *gilli* (hatched lines) Ig H-chain containing chromosomes in meiosis. Upper pathway generates an LG15-like animal; the lower pathway generates the homozygous LG7 animal. The asterisks (*) represent the point where the prevention of the second polar body extrusion occurs and a viable diploid egg is produced.

animal was easily isolated. Later, it was found to produce both big and small eggs, thus making it clonable.

Genomic V_H1 Gene Sequences

Three genomic libraries representing altogether 1.7×10⁶ recombinants phage (three to four genome equivalents) were made from LG7 red cell genomic DNA and screened unamplified with a *Xenopus* family V_H1 cDNA probe at high stringency. A total of twenty eight different V_H1 elements were isolated and sequenced, and the alignments of their encoded amino acids are shown in Fig. 3. All exhibit a typical V_H gene structure (Kabat et al., 1991) and all contain specific features characteristic of *Xenopus* V_H1 genes. The complementarity-determining regions (CDRs) show limited variability and many different genes contain identical CDR1s and very similar if not identical CDR2s. The CDR2s exhibit the most sequence divergence which is restricted to their amino-terminal (NH₂-) portions (Schwager et al., 1988, 1989). Six sets of the genomic V_H1s contain identical CDR1s and three sets contain identical CDR2s (Table 2). The CDR1s are fifteen nucleotides in length and can differ by as much

as 60%. Only the nucleotides at positions 100–103, encoding MET 34, never vary. Genomic g44A was the only gene that contained a CDR1 identical to one published previously from a *Xenopus laevis* genome (p LL1.4; Schwager et al., 1989). The largest sequence difference found in the V_H1 CDR1s is a change of nine nucleotides (g46C vs. g345A). LG7 V_H1 CDR2s vary in length from 48–54 nucleotides (16–18 amino acids) and can differ by as much as 43% (g2A vs. g345A). Overall nucleotide sequence identities range from 82–96%, and as expected, the framework (FR) regions are the most highly conserved. The V_H1 nucleotide sequences beginning with the initiation codon (ATG) of the split leader and

TABLE 2
V_H1 Genomics That Share CDRs

CDR1s	CDR2s
g7C, g15	g7B, g7C
g4A, g10A	g342A, g342B
g35, g2A, g13	g352, g2B
g343, g10B	
g342A, g342B	
g22, g349B	

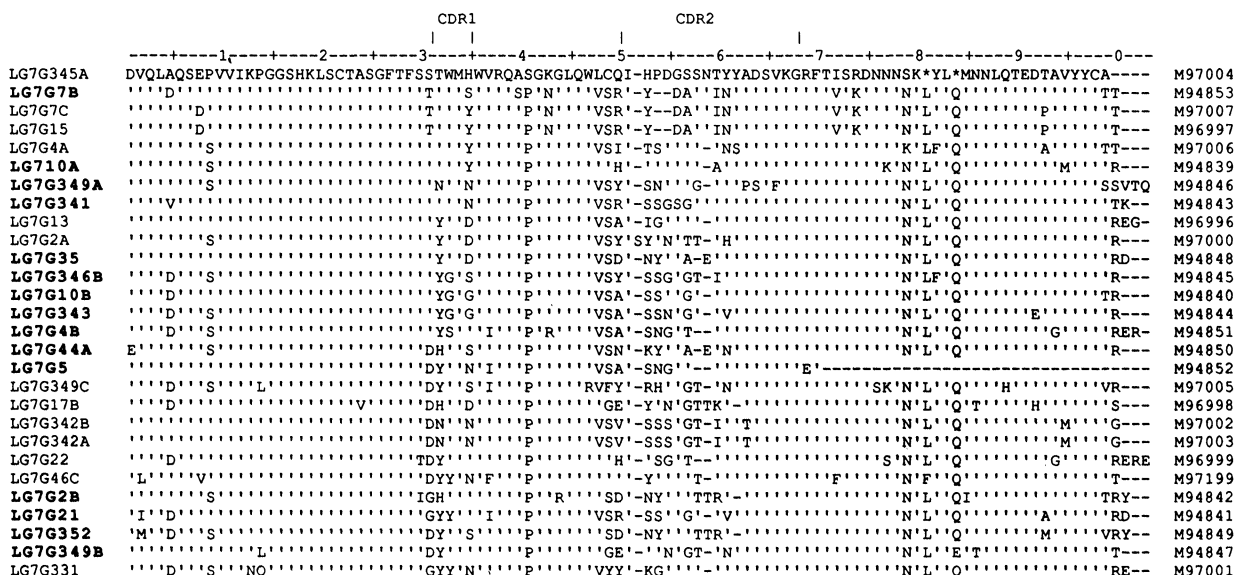


FIGURE 3. Alignments of encoded amino acids of V_H1 genomic genes (in one letter code) beginning with residue 1. The V_H1 genes in bold type were found expressed in a cDNA library made from immunized LG7 frogs. Sequence identities are indicated by (') and gaps (-) are introduced to maximize homology. The asterisks (*) indicate stop codons. The CDR boundaries are marked and are according to Kabat et al. (1991). Genes g345A and g342A are pseudogenes and gene g5 is truncated due to only being partially sequenced. Master sequence was chosen for length. GenBank accession numbers are at the right margin.

continuing to the conserved nonamer of the recombination signal sequence (RSS) are aligned in Fig. 4. The conserved octamer ATGCAAAT and sequences analogous to a TATA box that are found 5' of all V_H genes (Parslow et al., 1984) and act as the Ig H-chain promoter (Wirth et al., 1987) were identified in twenty five of the V_H1 genes. Three of the genes, g349A, g349C, and g343, were cloned using a restriction site just 3' of their leader sequences and so their promoters were not identified. Genes g349A and g343 are both viable V_H genes because they are found expressed in an LG7 cDNA library (Wilson et al., 1992). Very little sequencing was performed 5' of the leader sequences and consequently no second possible promoter regions, as have been previously identified in V_H1 genes (Schwager et al., 1989), were found. The LG7 V_H1 leader regions range from sixteen to nineteen amino acids in length and several genes (e.g., g7B, g7C, g2A, g35) contain almost identical leader sequences. Gene g44A has the longest leader intervening intron of 108 nucleotides (Fig. 4A). The V_H1 genes varied in overall length from 98–101 amino acids. The heptamers of the RSS are identical in all but one of the V_H1 genes. Two nucleotide substitutions in g22 change its heptamer to CACACTA. The nonamer sequences are much more variable and only six genes contain the concensus ^G/_ACAAAAACA previously identified as the V_H1 nonamer (Schwager et al., 1989). The RSS 23-bp spacers vary by a wide range. Six genes contain identical 23-bp spacers, whereas others differ by more than 50% (g345A vs. g352).

Many of the genomic V_H1s were isolated more than once from both the same library and from the different libraries. Eight of the recombinant phage contained more than one V_H1 gene (e.g., g349A, B, C) and at least four recombinant phage (from seven chosen at random) tested positive for members of additional V_H families by hybridization with family-specific oligonucleotide probes (Haire et al., 1990). Three of the recombinant phage contained V_H II hybridizing fragments and one different recombinant phage contained a V_H VIII hybridizing fragment (data not shown). These results in part agree with recent reports that show evidence for interpersions of *Xenopus* V_H families (Schwager et al., 1989; Haire et al., 1991).

Two of the twenty eight genomic genes are clearly pseudogenes. Gene g345A contains two

stop codons in its FR3 region, beginning at nucleotide positions 238 and 247, and gene g242A contains a stop codon in its leader sequence. Genes g342A and 342B are located on the same recombinant phage and differ by four bases. Both genes contain an extra different base in their leader intron sequences and, more important, g342A contains two extra bases in its leader, one of which is responsible for generating the stop codon (TAA) directly after the initiation codon ATG. The close sequence similarities and their close association in the DNA suggest that these two genes are the result of a recent gene-duplication event. Genes g7B and g7C probably also represent another example of recent gene duplication; they are found on the same recombinant phage and differ by thirteen bases in their coding regions. Genes g15 and g7C only differ by one base in their CDR2 regions, and are identical everywhere else from 100 bp 5' of their octamer to 80 bp 3' of their RSS sequences (data not shown). Recombinant phage 15 and 7 are different by RFLP analysis, which suggests that these two sequences represent two genes. However, this evidence, even considered with the low (practically nonexistent) frequency of sequencing error (see Materials and Methods) of our reactions is inconclusive. Both genes g7B and g7C were repeatedly isolated from all libraries, whereas g15 was found only once. Also the EcoR1 fragment containing g15 is approximately equal in size to the EcoR1 fragment containing g7C, and so the difference in the two may well be the result of a sequencing artifact.

IgM Heavy-Chain Region

The four exons (C_μ1 through C_μ4) encoding the *gilli* C_μ chain of LG7 are shown in Fig. 5. The coding region encompasses 4163 bp from the first amino acid of c_μ1 to the polyadenylation signal. The four exons and their splice sites were identified by comparison with the published *laevis* μ heavy-chain cDNA sequence (Schwager et al., 1988) and with LG7 μ cDNA sequences (Wilson et al., 1992). The splice sites obey the traditional rule encountered so far for all Ig genes, the splice junction occurs between the first and second base of the joining amino acid (Brüggerman, 1987). In two places, the exon boundaries of the *gilli* μ gene differ from the boundaries predicted from the *laevis* μ cDNA for the C_μ2 exon. The LG7 C_μ2

B

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-----1-----2-----3-----4-----5-----6-----7-----8-----9-----0-----1-----2
LG7G345A GATGTGCAACTTGCCAGTCAGAGCCAGTGGTGATAAAGCCGGGAGGTCTCACAACTGTCTCGCACAGCCTCTGGGTTACACATTCAGTAGGCATGGATGCACCTGGGTTAGACAGGCT
LG7G7B .....AT.....C.....C.....AG.....T.....
LG7G7C .....T.....C.....A.....C.....T.....
LG7G15 .....T.....C.....A.....C.....T.....
LG7G4A .....A.....T.....T.....C.....T.....A.....
LG710A .....T.....T.....C.....T.....
LG7G349A .....A.....T.....T.....AC.....A.....
LG7G341 .....A.....T.....C.....A.....
LG7G13 .....A.....T.....TAC.....G.....
LG7G2A .....A.....T.....TAC.....G.....
LG7G35 .....A.....T.....TAC.....G.....
LG7G346B .....A.....A.....T.....TATG.....AG.....
LG7G10B .....A.....A.....T.....TATG.....AG.....
LG7G343 .....A.....A.....T.....TATG.....GG.....
LG7G4B .....A.....A.....T.....TTAC.....CT.....A.....
LG7G44A .....A.....T.....C.....GATCAC.....AG.....
LG7G5 .....A.....T.....GA.....TAC.....A.....A.....
LG7G349C .....A.....A.....T.....AG.....TAC.....AG.....A.....
LG7G17B .....A.....AT.....T.....GATCAC.....G.....A.....
LG7G342B .....A.....T.....TA.....GAT.....AC.....A.....
LG7G22 .....A.....A.....C.....GATTAC.....
LG7G46C .....T.....T.....T.....T.....GATTAT.....AT.....A.....T.....
LG7G2B .....A.....A.....T.....T.....G.....TCAC.....
LG7G21 .....CA.....A.....T.....G.....TTAT.....AT.....A.....
LG7G352 .....CA.....A.....T.....T.....GATTAC.....AG.....
LG7G349B .....A.....T.....T.....GATTAC.....
LG7G331 .....A.....A.....T.....C.....A.....G.....TTAT.....AT.....A.....

-----1-----2-----3-----4-----5-----6-----7-----8-----9-----0-----1-----2
LG7G345A TCTGGGAAAGGATTACAGTGGTTATGTCAAATTCACCCGTATGGGAGTAGTAACACATACTATGCTGATTCAGTCAAAGGAAGATTCACCATCTCCAGAGACAAATAAACAGCAAGTAA
LG7G7B .....T.....G.....G.....C.....GT.....AT.....TGA.....CA.....T.....TA.....A.....T.....G.....A.....A.....A.....T.....
LG7G7C .....C.....T.....G.....G.....C.....GT.....AT.....TGA.....CA.....T.....TA.....A.....T.....G.....A.....A.....A.....T.....
LG7G15 .....C.....T.....T.....G.....G.....C.....GT.....AT.....TGA.....CA.....T.....TA.....A.....T.....G.....A.....A.....A.....T.....
LG7G4A .....C.....T.....G.....G.....C.....ATT.....CAC.....AGC.....A.....C.....G.....T.....G.....A.....A.....A.....AG.....T.....
LG710A .....C.....T.....C.....A.....T.....G.....G.....T.....T.....G.....A.....A.....A.....G.....A.....T.....
LG7G349A .....C.....T.....A.....G.....G.....C.....T.....T.....AGTAA.....A.....G.....CC.....AGC.....T.....T.....A.....A.....T.....
LG7G341 .....C.....T.....G.....G.....C.....GT.....AG.....AGC.....G.....A.....TG.....G.....T.....T.....A.....A.....T.....
LG7G13 .....C.....T.....G.....G.....C.....GCT.....ATAGG.....CA.....C.....T.....T.....A.....A.....T.....
LG7G2A .....C.....T.....G.....C.....T.....T.....AGTTACCCAAATG.....A.....C.....CA.....C.....T.....C.....T.....T.....A.....A.....T.....
LG7G35 .....C.....T.....G.....C.....G.....T.....A.....TAC.....C.....GC.....G.....T.....T.....A.....A.....T.....
LG7G346B .....C.....T.....G.....G.....C.....T.....T.....AG.....AGC.....G.....G.....C.....T.....T.....T.....A.....A.....T.....
LG7G10B .....C.....T.....G.....G.....C.....GCT.....AG.....AGC.....G.....G.....T.....T.....T.....A.....A.....T.....
LG7G343 .....C.....T.....G.....G.....C.....GCT.....AG.....AGCA.....G.....G.....GT.....T.....T.....A.....A.....T.....
LG7G4B .....C.....T.....G.....G.....C.....GCG.....AG.....AA.....GA.....C.....C.....T.....T.....T.....A.....A.....T.....
LG7G44A .....C.....T.....G.....C.....A.....T.....A.....ATA.....C.....GC.....G.....G.....A.....T.....T.....A.....A.....T.....
LG7G5 .....C.....T.....G.....G.....C.....GCG.....AG.....AA.....G.....T.....GA.....T.....T.....A.....A.....T.....
LG7G349C .....C.....T.....G.....G.....T.....T.....AAGG.....A.....AG.....C.....A.....T.....T.....TC.....A.....A.....T.....
LG7G17B .....C.....T.....G.....G.....T.....T.....A.....G.....C.....A.....G.....T.....T.....T.....A.....A.....T.....
LG7G342B .....C.....T.....G.....G.....C.....GTT.....AG.....AG.....AGC.....G.....C.....T.....A.....G.....T.....T.....A.....A.....T.....
LG7G342A .....C.....T.....G.....G.....C.....GTT.....AG.....AG.....AGC.....G.....C.....T.....A.....G.....T.....T.....A.....A.....T.....
LG7G22 .....C.....T.....G.....C.....T.....T.....GA.....C.....C.....T.....T.....T.....G.....A.....T.....
LG7G46C .....C.....T.....G.....T.....T.....T.....A.....C.....T.....T.....T.....T.....T.....T.....TC.....
LG7G2B .....C.....T.....C.....G.....T.....A.....TA.....CA.....CG.....T.....G.....T.....T.....A.....A.....T.....
LG7G21 .....C.....T.....G.....G.....C.....GT.....AAG.....AGC.....C.....G.....GT.....T.....T.....T.....A.....A.....T.....
LG7G352 .....C.....T.....C.....G.....T.....A.....TA.....CA.....CG.....T.....T.....T.....T.....A.....A.....T.....
LG7G349B .....C.....T.....G.....G.....T.....A.....A.....G.....C.....A.....T.....T.....T.....A.....A.....T.....
LG7G331 .....C.....T.....G.....G.....A.....T.....T.....A.....AGG.....T.....T.....T.....A.....A.....T.....

-----1-----2-----3-----4-----5-----6-----7-----8-----9-----0-----1-----2
LG7G345A TATCTGTAAATGAACAATCTACAAACTGAAGACACTGCCGTGTTATTACTGTGCTGG-----CACAGTGAGAAAGGATCCCAGAGCAGTCGACGAAAACA
LG7G7B .....C.....A.....T.....C.....T.....ACAGA-----TAG.....T.....A.....AC.....
LG7G7C .....C.....T.....C.....T.....ACAGA-----TAG.....T.....A.....AC.....
LG7G15 .....C.....T.....C.....T.....ACAGA-----TAG.....T.....A.....AC.....
LG7G4A .....T.....C.....T.....G.....T.....ACAG-----GC.....G.....G.....ATT.....T.....G.....CCT.....TAAGGT.....GT.....
LG710A .....C.....T.....C.....A.....T.....ACAG-----TAG.....T.....A.....AC.....
LG7G349A .....C.....T.....T.....CTGTGACACAGTGA.....C.....C.....C.....TG.....A.....A.....TGA.....C.....
LG7G341 .....C.....T.....C.....T.....ACGAAG-----C.....TA.....A.....A.....T.....
LG7G13 .....C.....T.....A.....AGAGGGA-----C.....A.....A.....A.....T.....T.....
LG7G2A .....C.....T.....C.....T.....ACA-----T.....A.....A.....A.....C.....AC.....
LG7G35 .....C.....T.....C.....T.....AGACA-----C.....A.....A.....A.....TG.....A.....
LG7G346B .....T.....C.....T.....G.....A.....A.....AGA-----C.....A.....A.....A.....AGTGAAC.....A.....
LG7G10B .....C.....T.....C.....T.....A.....A.....AGA-----C.....A.....A.....A.....A.....
LG7G343 .....C.....T.....T.....A.....A.....T.....A.....AGA-----C.....A.....TAG.....T.....A.....T.....
LG7G4B .....CC.....C.....T.....G.....T.....A.....AGAGAGA-----C.....AC.....T.....T.....TAA.....T.....
LG7G44A .....C.....T.....C.....T.....A.....A.....ACA-----C.....A.....A.....A.....A.....
LG7G5 .....-----
LG7G349C .....C.....T.....C.....T.....A.....AGACA-----C.....C.....ATGACA.....AA.....C.....C.....ATTGCTCTT
LG7G17B .....C.....T.....C.....T.....CA-----C.....G.....A.....GAATGAG.....TTC.....C.....GA.....T.....CT
LG7G342B .....C.....T.....C.....T.....A.....A.....AGA-----C.....A.....A.....A.....T.....
LG7G342A .....C.....T.....C.....T.....A.....A.....AGA-----C.....A.....A.....A.....T.....
LG7G22 .....C.....T.....C.....T.....G.....T.....A.....AGAGAGAGAG-----C.....A.....C.....A.....A.....TG.....
LG7G46C .....C.....T.....C.....T.....ACATG-----C.....TAGC.....T.....GTGCAGC.....AC
LG7G2B .....C.....T.....C.....T.....A.....A.....ATACA-----C.....A.....A.....A.....TCA.....AT.....
LG7G21 .....C.....T.....C.....T.....G.....A.....AGATG-----C.....TAG.....T.....A.....T.....
LG7G352 .....C.....T.....T.....G.....T.....T.....C.....T.....A.....ATATA-----A.....AAGCAATG.....CAA.....AACTCCA.....TTCG.....T
LG7G349B .....CG.....C.....T.....A.....A.....ACAGA-----C.....TAG.....A.....A.....A.....T.....
LG7G331 .....C.....C.....T.....G.....A.....A.....AGAGA-----C.....A.....A.....A.....AC.....G

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FIGURE 4

1 GTAATAAGAAATGTCAAAACCCACAGAGAGCCTGTGAAGATTGTTCTTTGCACTAACATCAAAACTAACCTTAACCTCTCCACAAACCTGGTTCAGTGAAATTC 107

108 TACATTTTCATCTAACACCTTGTAATCATTACCATCTCTAATCCTAAACAATACTCATTCTCCCTTCTCTTTTGGCTTGATTCTCAG Ala Thr Ser 210
CT ACT TCA

211 Lys Ser 10 15 20 25 30
Asn Pro Pro Ser Leu Phe Pro Leu Ile Ser Cys Gly Glu Ser Met Asp Pro Val Thr Ile Gly Cys Leu Ala Lys Asp Phe
AAC CCC CCA TCC CTT TTT CCA CTC ATT TCT TGT GGG GAG TCT ATG GAC CCA GTC ACC ATT GGT TGT TTG GCC AAA GAT TTC 291

292 Leu Pro Glu Thr Ile Ser Phe Ser Trp Gly Asp Lys Asn Asn Ala Ser Tyr Ser Thr Gly Leu Lys Ser Tyr Lys Pro Val
CTC CCT GAA ACT ATT AGC TTC AGC TGG GGA GAT AAG AAC AAT GCC AGT TAC TCC ACT GGC CTC AAG AGC TAT AAA CCT GTG 372

373 Met Gln Ser Ser Gly Thr Tyr 65 70 75 80
ATG CAA TCT TCC GGC ACC TAC TCA GCC AGC TCC CAA GTC AAC GTT GCC TCT GCA GTC TGG GAC AAA AGT GAA CCA TTT TAC 453

454 85 90 Asp Thr Ile 95 Leu 100 Asp Pro
Cys Asn Ala Lys His Leu Glu Ile Thr Lys Ser Val Glu Val Lys Lys Gly Thr V
TGC AAT GCC AAA CAC CTG GAG ATC ACT AAG AGT GTG GAG GTC AAG AAA GGT ACA G GTAACCTATTTTGAACCTTAAATTCACATAGA 541

542 AATATATTACTCTCTCTATATTTTATGGCCATGAAACATATTTACTTATTGATAAACAGCTCCAAGAATCAACACCCTGATTCAATACAAGTAAGATTATTACT 648

649 CAGTGATTCTGTCGGAACCACTTTATATTGAGCACAATAATAAATAAATTTCTTATAGAAGTAATAAAAAATAATTTCTTATATACCTATAATATATATATTTT 755

756 TTTAATAGAGATGCTGCAAGTGAATGCATAACTGCATACATATCAAGCAGCCATGCATGAAATATGTGTATCAATAATGTCTCTTTTAAAGGGGTGATATGGC 862

863 AATCCTAATGAGAATACTTTGGTATGCATCTAATACAATAGGCTGAACGCACAACCCCTACCAAACGATCATATTTCTCTAGGTATTAGTAAACAATAAAT 969

970 GAAAACCTCAGGTGATTAATAATGCTAATAAATAATGTGTGTGTGTAGTGTCTAGAAAGTAAATGTTAATTTCCACATAAGGCTGTGTCAACAATTA 1076

1077 GGTATTAGCAGCAACTTTCAACTGTCTGCAAAACAGGATGGATGTTCTTGAATCTTGGGTTTTTCTGTTTTTTCATTAATTTGGAGAACCATGACTTTAGA 1183

1184 TACTAAAATATGTGAACATAAGAAAAAACTGGGTATGTCATCAGAATGGTATCTCAAATTACAGGGTAGTGTCTAGTTATTACATAGAAAAAGCAAAACCATAGC 1290

1291 AAGAGAAGAAATGCTTGTTCAAATAGGACTCTATGGGAAATGACAAAAGGTAATTTAAAGGTGAACACTCCCTTTAATCTTTCCAATTGGCATAATAATCTACTTG 1397

1398 CTTTAAAGAAAAACAAGGAACCTTTTTGGAGCCAGTTTCAAGTAGCAGCTACTTTCCCTGTGTGACATAAGCCTGACTGTGAAATTTATCTTTTGCATTCATAAA 1504

1505 TTTAAACTAATAAACTAATAGAAGTTACATGTAGTACACAACAGAATGTGAACACTAGAAATGATATTTCCCTTCCAATATTTTGAATAATCTACTGGTTATAT 1611

1612 ACAAATGTGACAAAAAACTGAACCTCTTTTATATCGGTATTTCAGTATACACTGAGGACAGATTTATTAATGTTGGTGTAAAAATGGTGAATAATACACCACAC 1718

1719 GTCTCCAGCATTGCCGTATAAGAATGGTGTATTTTTTTTACGCACTTTTCTTTGACCAACTACTGGCAGAACTTACTTATAAAGGTCTGAATCGATGTGAAATAG 1825

1826 CATTACATGGCATAAAAAAACAACCTTGATAAATTCGCCATTTTACATGGCTTTCTACACCGTTTTTTTTGCCAAAATTCGTTTTTACACAGATAACTATGC 1932

1933 CCCTGAATGTACATATTGCATACAAAAATACTGAAGAATGATACATAAAATGAATAGGCCCTGCCAAAAGAGTTTACGGAGTGACCAGCTGTAGAGTTGACA 2039

2040 TAAATTTATTTACCCTTTTTTTGTTTATTTGTATCAACACTCACTTTTCTTAAATTAAGGAGTAACACATCATTAGCTTGAAGTATACCCGAAATGCGTCTCTGT 2146

2147 Lys Pro 110 115
al Asn Lys Val Glu Lys Pro Val Val Ser Ile His Pro Pro
TG AAT AAA GTT GAA AAA CCA GTT GTG TCT ATT CAC CCT CCA 2236

2237 Ser Lys Asp Ala Leu Ala Leu Asn Glu Ser Leu Phe Ile Val Cys Leu Ala Thr Asn Phe Asn Pro Lys Asn Ile Val Ile
TCC AAG GAT GCT CTT GCC TTG AAT GAA AGC CTC TTT ATC GTA TGT CTT GCA ACG AAT TTT AAT CCC AAA AAT ATA GTA ATT 2317

2318 145 150 Thr 155 160 165 Arg 170
Lys Trp Leu Lys Asn Gly Asn Gln Thr Lys Glu Gly Val Arg Val Glu Glu Pro Val Glu Asp Lys Lys Gly Gly Tyr Glu
AAA TGG CTA AAG AAT GGG AAC CAG ACA AAA GAA GGT GTG AGA GTT GAA GAA CCT GTT GAA GAC AAA AAG GGA GGA TAT GAG 2398

2399 Thr Thr Ser Tyr Leu Ser Ile Thr Arg Lys Glu Trp Asp Leu Asp Thr Leu Tyr Ser Cys Val Val Glu His Ala Gly Ser
ACA ACA TCC TAT CTC TCC ATA ACT AGA AAG GAA TGG GAT TTG GAT ACT TTG TAC TCA TGT GTA GTT GAA CAT GCA GGA TCG 2479

2480 Ala 200 205 210
Gly Ser Leu Gln Glu Lys Asn Met Ser Lys Ser Leu Met Cys A
GGT TCC TTA CAA GAG AAG AAT ATG AGC AAA TCA CTA ATG TGT G GTAAGTCTTGTGTGATGTGATGCTTATTTGTTCTACAGATAAGGATCAGTT 2571

2572 TTAATTAAGAAATTAATTTATGTATAAGTGTAAATACTGTGGGTCATATTTGCTTCAATGCATGTTGGTACATATATAGTTACATATAGTCTTTATCTGATTATGA 2678

2679 CCAATAAAAGCCAAAATATACCAATTTTGAAGGTATTTTTGGCTGTAACAGTGTGAGAACCCGCACTCACCTCTCCTTAGTACTAATAGCCAGGTATATATAT 2785

2786 ATGAGAGAGATGTCAGGGTATTTTTTGATGTCATAGTGTATGCACACTATTTAAAGGGTTGGTCCCTGTGAATTCAGGTGAGTGAACAGCATCT 2892

2893 CGTTTTCTATTTAAACTGCTTATGGGGCTAAACATGAAGTGTATGATCGGTTATTTGGTTGAACAAAGCCAAGTAGACTGGCATGTTTGCACAATGTAAGGGTGGC 2999

3000 TTAATAAATGTTTGTATTGAGGGAATTTAACATAAATAATGTATAAATTTGAGTATGTTATCTCACTATTTTTTTTCCCTTTTTTTTCCCTTTTTTTTCCCTTT 3106

FIGURE 5

Mabs (like 14G1, see the foregoing) can be produced.

Only 650 bp beyond C_{μ4} were sequenced and no transmembrane exons were found in that stretch. The segments encoding the *Xenopus* transmembrane region have been published previously (Du Pasquier and Schwager, 1989) and a complete map of the *Xenopus* IgH locus will be

published elsewhere (Du Pasquier, in preparation). The *gilli* μ locus is also characterized by a long intron between C_{μ1} and C_{μ2} (1618 bp). This intron contains sequences similar to some enhancer motifs described for mammalian Igs (Staudt and Lenardo, 1991). An octamer and sequences analogous to $\mu e4$ and $\mu e5$ are identified in Fig. 5.

3107	TTCCTCAG	sp	Thr	His	Ile	Thr	Pro	Thr	Ser	Ile	Gln	Val	Ile	Thr	Ile	Pro	Pro	Ser	Leu	Glu	Ser	Ile	Phe	Glu	Lys	3185			
		AC	ACC	CAT	ATA	ACA	CCC	ACT	AGT	ATC	CAA	GTA	ATC	ACT	ATT	CCA	CCA	TCA	TTA	GAG	AGC	ATT	TTT	GAG	AAA				
3186	AAA	TCT	GCC	ACA	CTT	ACC	TGC	CTG	GTC	AGT	AAT	ATG	GCT	AAC	TCT	GAG	GAT	TTG	AGA	TCA	ATA	TCC	TGG	TTT	AAA	AAA	TCT	3266	
3267	GGT	ACT	CAA	GAG	ATA	CCA	TTG	AAA	ACA	GAA	CTG	GGA	GAT	GCA	ATC	TAT	AAC	GAT	AAC	CGC	ACC	TAT	TCT	GTA	AAA	GGA	ACC	3347	
3348	ACC	ACT	GTC	TGC	GCT	GAT	GAA	TGG	AAT	AAC	GAC	AAG	TTT	GTC	TGC	AAA	GTG	GAA	CAC	ACA	GAG	CTG	GCT	TCA	GTG	AAG	GAG	3428	
3429	GTC	TTT	CTC	TTT	AAA	GAA	AAA	G	GTA	AGGCCATCTACCTGTACTGCACAGTACTAATCAGACAGAACAAC	TATAATAAAATAGCCATTATACTTGATACC																3527		
3528	AATATAGCACACATTTTTCATGTGCAGAGGTTGTTTATTCATCCACTGATCTGATAGCTATTATTAAATATAACAATTAATAAAATTTCTATTAGGGTCTGTTTAAAGCAC																											3634	
3635	CAAAGACCAACATCTGTAGAAAGTATAATTTTCTACAATAGAGTAGATCATCAACCTGTAATAAAATTTGTGACATCCATTGCGAGTTGTATGTTAAGGGTGTMTTT																											3741	
3742	ACTGATATCTGTAGAGGAAGCATTGTGTGAGTAATCCCTTCAAAAACCTTTGAGATTGTGAACTCCTAATTTTCTATTMTTAAAACAG																											3842	
3843	CCA	TCT	GTT	TAT	GTT	TTC	CCA	CCA	CCT	CTT	GAG	GAA	TTG	TCT	AAG	AGA	GAA	ACT	GCC	ACC	TTG	ACA	TGC	TTG	GTT	AAA	GGG	3923	
3924	TTC	AGC	CCC	TCT	GAA	ATA	TTT	GTA	AAA	TGG	CTT	CAC	AAC	AAT	GAG	GCG	GTT	CCA	AAA	CAA	AAT	TAC	ATA	AAT	ACC	AGC	ATC	4004	
4005	AAT	ASP	Glu	Leu	Tyr	Pro	Lys	Gly	Gln	Lys	Ser	Gly	Lys	Phe	Phe	Leu	Tyr	Ser	Leu	His	Thr	Ile	Asp	Phe	Lys	Asp	Trp	4085	
4086	GAT	GCT	GGT	GAT	AGT	TTT	TCC	TGT	GTG	GTT	GCC	CAT	GAG	TCA	TTA	CCA	CTT	CAG	CTG	ACC	CAA	AGG	AGC	ATT	GAC	AAG	TCT	4166	
4167	TCT	GGT	AAA	CCT	ACT	AAC	GTG	AAT	GTG	TCC	CTC	GTC	TTG	TCT	GAT	ACC	TGT	TAGTGATCATCTCCAAAGACCC	TATAACTCCATCTCATG									4256	
4257	TTCTTCCCTATATGGTGAATAGGACAAGGCGGAGGAGAAGTGTGACTTTATGTTTGTCTGTCTGTCTTTGTCATATTTTTGTCGTGCAAAATTTAAAGTAGAATA																												4363
4364	AAAATCAATTTATACAATGTTTGTGTTGTATGCATGCAATTTGGGAAAGAAGTGGTGAATTTGGAAAAATGAAATACTTCACCAGCTGGTTTGTAGCCCTGCATTAGGGAT																												4470
4471	TTGAATCCAGATACACCTACACCTTTTCATAAAAACCCACAGCCATTAGCTTCAGTAGGAATCCAAAAGCTCAGAGCTGCATAGGGTAAGAACCTTAACAAAGTCCAC																												4577
4578	ATTACTACTTGATTAGTTTCATTATATAAAGAAATTACATATGACTAGGAAAGGGAATGCTTACTAGCCAAGATGTCCAAAATAAACATCATTCAGTTAATTTGAAAGT																												4684
4685	GTTTCATGTGCTTTCTATGTTATAGGCAATGTGTGTTCAACTTAGAACATATGAGACAAAACAACTGTTATGGGCATATTTTTCAAAAGTGTGAAATTAATCCCACT																												4791
4792	TTCAATCTTTTCTACTGTTTTTGTGTACTACAAG																											4830	

FIGURE 5. Nucleotide and inferred amino-acid sequences of the LG7 IgM heavy-chain gene. The inferred amino acids are indicated above the nucleotides. Only the amino acids of *laevis* μ (from the cDNA sequence; Schwager et al., 1988) that differ from *gilli* μ are shown. These residues are shown above the LG7 *gilli*-encoded amino acids. Sequence motifs in the CH1 to CH2 intron that are similar to Ig-enhancer sequences are underlined. These include a SITE E, E-boxes ($\mu e5$, $\mu e3$, $\mu e4$), and a μB motif. A sequence identical to the KBF-A motif in the kappa-chain enhancer is underlined and overlined. The octamer sequence is double underlined. Ig-enhancer sequences are from Staudt and Lenardo (1991). Polyadenylation sites are in bold. GenBank accession number of *gilli* μ is M97008.

DISCUSSION

In this study, we examined a clone of isogenic *Xenopus*, the *X. laevis*/*X. gilli* hybrid LG7, which probably developed accidentally from a LG15 small egg (Kobel and Du Pasquier, 1986) with a recombined genome. Because this clone is homozygous at the heavy-chain locus, its genetic simplicity makes it the best available strain for comparing germ line with cDNA Ig sequences. These comparisons are necessary to detect the presence of somatic mutations, an issue that has remained unanswered in the lower vertebrates for the past 18 years, ever since the major differences in antibody repertoires were first reported between amphibians and mammals.

In addition to establishing a dictionary of V_{H1} sequences from LG7, we have sequenced the μ -chain gene. This will be useful for future studies with conventional heterozygous LG hybrids in order to identify origins of μ cDNAs.

All of the twenty eight V_{H1} genes sequenced exhibited the typical vertebrate V_H -gene structure (Kabat et al., 1991) and sequence identities ranged from 82–96%, thus easily fulfilling the criteria of V_H -gene family membership (Brodeur and Riblet, 1984). In addition, all of the V_{H1} s contain specific features and conserved nucleotides previously described for *Xenopus* V_{H1} genes (Schwager et al., 1989). Many shared CDR1s and CDR2s are similar even though by overall comparison they show the most sequence divergence. Most of this variability is in the first eight to ten amino acids of the CDR2s. The LG7 V_{H1} sequences further reinforce, by allowing for greater comparisons, the hypothesis that the low heterogeneity of the *Xenopus* V_H pool (at least for this family) may be the result of recent expansion events (Schwager et al., 1989; Du Pasquier and Schwager, 1991).

We are aware that the screening process may have missed genes and that calculating V_H family size by Southern blotting can be inaccurate because restriction fragments may contain more than one gene or may comigrate in the gel. The size of the LG7 V_{H1} family was originally estimated to contain at a maximum thirty genes and Southern blot analyses with frequent six or even four base cutters are consistent with this estimate (data not shown). Because studies in outbred *laevis* and other LG hybrids place the V_{H1} family size at approximately sixty in heterozygous indi-

viduals (Schwager et al., 1989; Haire et al., 1990), we believe that very few LG7 genes are missing. Indeed, when the twenty eight germ-line genes sequenced in this study were used to analyze LG7 somatic mutants (Wilson et al., 1992), only four out of fifty five cDNAs could not be unambiguously assigned to one of these germ-line V_{HS} .

The LG7 *gilli* $C\mu$ segment conforms to the organizational pattern seen in all other vertebrate $C\mu$ loci. There are four exons that encode the four protein domains of the secreted μ heavy chain and a detailed analysis of the inferred amino acid identities between *Xenopus* μ chain and those of other vertebrates can be found in Schwager et al. (1988). One unusual feature of the *gilli* μ gene is the presence of enhancer motifs in the $C\mu 1$ to $C\mu 2$ intron. Whether these sequences have any enhancer function remains to be investigated. Enhancer like sequences are also found 5' of the putative switch region of the *Xenopus laevis* IgM gene (Du Pasquier, unpublished), a location analogous to the site of the mammalian IgH-chain enhancer. The $C\mu 1$, $C\mu 2$ intron length, however, is not unusual. Introns of similar lengths are found between $C\mu 1$ and $C\mu 2$ of the channel catfish, *Ictalurus punctatus*, and between $C\mu 2$ and $C\mu 3$ of the horned shark, *Heterodontus francisci* (Wilson et al., 1990; Kokubu et al., 1988).

The LG7 hybrids are the first *Xenopus* clones to be homozygous at their IgH locus and preliminary evidence indicates that they may well be homozygous for the ρ light-chain locus. Except for the MHC locus, which is heterozygous (Bernard et al., 1979), homozygosity at other LG7 genetic loci has yet to be investigated. In the future, it may well prove to be worthwhile to create other clonable homozygous LG hybrids by using pressure or cold-temperature shock to increase the frequency with which the second meiotic division does not occur. This technique was previously exploited to study histocompatibility antigens (Kobel and Du Pasquier, 1977).

MATERIALS AND METHODS

Genomic DNA Preparation and Library Construction

High-molecular-weight DNA was prepared from *Xenopus* LG erythrocytes lysed in TES buffer (10 mM Tris, pH 8.0, 10 mM EDTA, 400 mM

NaCl, 0.2% SDS), containing 100 μg/ml proteinase K. LG7 small-egg progeny DNA was prepared in an identical manner except that tissues from 3-day-old tadpoles were homogenized in TES buffer. DNA was extracted as previously described (Kiefer, 1990).

LG7 genomic libraries were made from DNA partially digested with Sau3A or Mbo1 and size fractionated by sucrose gradient centrifugation (Davis et al., 1986). Fragments of approximately 20 kb were packaged into EMBL3 vectors (Stratagene) following manufacturers' recommended protocol. The libraries contained approximately 3.6×10^5 (Sau3A) and 5.6×10^5 (Mbo1) recombinants. All libraries were screened unamplified with V_{H1} or C_μ probes, as previously described (Wilson et al., 1986). Identical V_{H1} genes were isolated from all three libraries. The C_μ genes were isolated and sequenced from the library Sau3A 3.

Southern Blot Analysis

Genomic DNA (5–10 μg) was digested to completion with restriction enzymes, electrophoresed on 0.7% agarose gels, transferred to nitrocellulose or nytran filters (Schleicher and Schuell) and hybridized (Schwager et al., 1991). All probes were labeled by the method of Feinberg and Vogelstein (1983). After overnight hybridization, the Southern blots were washed at high stringency (65°C, 0.1×SSC, 0.1% SDS). X-ray film exposure was at –80°C. The DNA probes for V_{H1}, V_ρ, C_ρ were kindly provided by our colleague, J. Schwager.

Subcloning and Sequencing

Fragments containing V_{H1} genes were subcloned into Bluescript plasmid vectors (Stratagene) and sequenced on both strands by primer extension using the dideoxynucleotide triphosphate chain termination reaction method (Sanger et al., 1977). Synthetic oligonucleotides were prepared by H. R. Kiefer's laboratory (Basel Institute for Immunology). The error frequency of the modified T7 polymerase (USB) used in our sequencing reactions must be very low because two independent genomic C_μ clones were sequenced (4830 bp each) with no base differences being found.

Computer Analysis

DNA sequences were aligned in pairs with a program based on the algorithm of Needleman and Wuntsch (1970). Each sequence was aligned to a "master" and their pairwise alignments were used as input for a multiple alignment program based on a heuristic algorithm. All computer programs were written by Charles Steinberg (Basel Institute for Immunology).

Enzyme-Linked Immunoabsorbent Assay and Chromosome Analysis

Serum was obtained from LG7, LG15, and outbred *laevis* frogs (two each) and diluted 1/10 in amphibian PBS (standard PBS diluted 1.3 fold). Microtitre plate wells were coated with 50 μl of diluted frog serum overnight at 4°C. After washing the plates, assays were performed using the β-Galactosidase Hybridoma Screening Kit (BRL) following the manufacturer's recommended procedure. The primary antibodies were Mabs 14G1, 10A9, 409B8, and 11D5 (Du Pasquier et al., 1985).

Chromosome spreads were prepared using a procedure previously described (Du Pasquier et al., 1985).

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