View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Columbia University Academic Commons

Gene, 67 (1988) 41–48
Elsevier

GEN 02428

Molecular cloning of a bovine immunoglobulin lambda chain cDNA

(Recombinant DNA; mammary gland; gene library; nucleotide sequence; pseudogene)

Vladimir N. Ivanov[a], Vladimir A. Karginov[b], Igor V. Morozov[b], Stanislav I. Gorodetsky[a]

[a] N.I. Vavilov Institute of General Genetics, USSR Academy of Sciences, Moscow (U.S.S.R.) and [b] Institute of Bioorganic Chemistry, USSR Academy of Sciences, Siberian Branch, 630090 Novosibirsk (U.S.S.R.) Tel. 35-6391

Received 1 December 1987
Accepted 25 December 1987
Received by publisher 21 March 1988

SUMMARY

A cDNA library of the bovine mammary gland constructed in pBR322 was screened by mRNA hybrid-selected translation and by differential hybridization. Several immunoglobulin (Ig) λ light-chain clones were identified and sequenced. Nucleotide sequence comparison of bovine and human Ig λ chains showed a high degree of homology for constant regions and for J regions. The amino acid (aa) sequence encoded by the constant region of the bovine Ig λ chain cDNA contains 107 aa with differences at 24 aa positions from the human Ig λ chain. Three complementarity-determining regions (CDR1, 2, 3) characteristic of the variable region of bovine Ig λ chain cDNA can be distinguished. The bovine and human sequences display good homology in the framework region 3 (FR3) but only patches of homology throughout the FR2 region. The 5′ end of the bovine Ig λ chain cDNA fragment of clone 1-14E contains five stop codons: two in CDR1, one in FR1 and two in the hydrophobic prepeptide region. These data suggest that the Igλ mRNA of clone 1-14E is transcribed from the V_λ pseudogene.

INTRODUCTION

Mammalian immunoglobulins are important components of colostrum and milk. The concentration of several classes of immunoglobulins in colostrum is higher than in serum. Species that transmit immunoglobulins exclusively in utero (man, rabbit) produce colostrum that is predominantly composed of IgA, while those that transmit immunoglobulins exclusively via the colostrum (cattle, goat, pig), have colostrum predominantly composed of IgG. Bovine colostrum and milk also contain minor concentrations of sIgA and IgM. The immunoglobulins of the lacteal secretions are derived either from blood or from a local compartment of the immune system. The bulk of immunoglobulins is transferred into colostrum and milk transepithelially from the bloodstream. The local immune system of mammary

Correspondence to: Dr. V.N. Ivanov, N.I. Vavilov Institute of General Genetics, USSR Academy of Sciences, 3, Gubkin Street, 117809 Moscow (U.S.S.R.) Tel. 135-1229.

Abbreviations: aa, amino acid(s); bp, base pair(s); C, constant region; CDR, complementarity-determining region; ds, double stranded; FR, framework region; Ig, immunoglobulin; nt, nucleotide(s); SDS, sodium dodecyl sulfate; sIgA, secretory immunoglobulin A; V, variable region.

0378-1119/88/$03.50 © 1988 Elsevier Science Publishers B.V. (Biomedical Division)

glands produces mainly sIgA. Antibodies of this class have a local protective anti-microbial function in mammary gland ducts and in the intestine of newborns (Butler, 1974; 1986).

At present, considerable information is available on organization and expression of immunoglobulin genes of mouse and man (Tonegawa, 1983; Honjo, 1983), although the information on bovine immunoglobulins is rather scarce. The amino acid sequence of bovine immunoglobulins and the nucleotide sequence of their mRNAs are unknown. We used a cDNA library of the bovine mammary gland constructed previously (Gorodetsky et al., 1985) for screening of Igλ light-chain cDNA clones. As a result we isolated clones containing Igλ chain cDNA fragments which were used for sequencing.

MATERIALS AND METHODS

(a) Construction of cDNA library

A cDNA library of the bovine mammary gland was constructed as described earlier (Gorodetsky et al., 1985). The ds cDNA and *Pst*I-digested pBR322 were tailed with about 20 nt of oligo(dC) and oligo(dG), respectively. After annealing, the recombinant plasmids were used to transform *Escherichia coli* JC5183 as described by Kushner (1978).

(b) Isolation and analysis of recombinant DNA

Plasmid DNA isolation, colony hybridization in situ, gel electrophoresis of DNA, nick translation, and blot hybridization were performed according to standard procedures (Maniatis et al., 1982). Hybridization selection-translation followed by 0.1% SDS–12% polyacrylamide gel electrophoresis was performed as described previously (Ivanov et al., 1984).

(c) Nucleotide sequencing

Sequencing was performed by the method of Maxam and Gilbert (1980) after recloning the cDNA fragments into plasmids pUC19 or pGEM1.

RESULTS

(a) Analysis of the cDNA library of the bovine mammary gland

A minilibrary of 350 colonies carrying recombinant plasmids from the cDNA library of the bovine mammary gland were screened with ^{32}P-labelled probes consisting of cloned fragments coding for α_{s1}-, β-, and κ-casein. Twelve, six and four colonies produced hybridization signals with these labelled probes, respectively. The rest of the minilibrary was collected into small groups including three or four clones. Plasmid DNA of these groups of clones was analyzed by mRNA hybrid-selected translation. This assay employed the plasmid DNA to select for specific mRNA by means of hybridization, followed by in vitro translation, immunoprecipitation, and M_r determination of the polypeptide. The immunoglobulin fraction of antiserum to total bovine milk proteins was used for immunoprecipitation. This antiserum contained antibodies to caseins, β-lactoglobulin and IgG. The translation products obtained from our clones are shown in Fig. 1. Lanes 2 and 3 correspond to proteins encoded by total unfractionated mRNA after immunoprecipitation with anti-milk protein antiserum. The mRNA which hybridized with plasmid DNA of one group of clones (1-11G, 1-13U and 1-15F) produced α_{s1}-casein, β-lactoglobulin and a 22-kDa protein p22, whose electrophoretic mobility was in the region of β- and κ-caseins (Fig. 1, lane 4). The next stage consisted of more accurate identification of individual clones. It was shown that the mRNA which hybridized with plasmid DNA of clones 1-15F produced β-lactoglobulin, clone 1-11G, α_{s1}-casein, and clone 1-13U, protein p22 (Fig. 1).

^{32}P-labelled probes containing cloned fragments of β- and κ-casein failed to hybridize with plasmid DNA of clone 1-13U. A preliminary assumption was that the cDNA fragment of clone 1-13U encoded immunoglobulin light chain because of its size of 22 kDa and electrophoretic mobility in the region of β- and κ-caseins. Since we did not have monospecific antisera to individual λ and κ light chains, identification of the fragment was performed by direct nucleotide sequencing. A 300-bp *Pst*I cDNA fragment of clone 1-13U was recloned into the *Pst*I site of plasmid pUC19 polylinker.

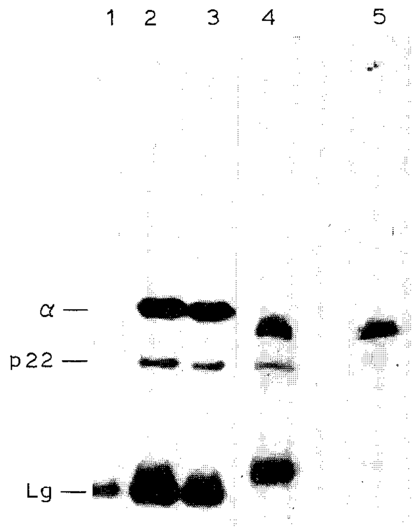

Fig. 1. Hybrid-selected translation. Translation products of total mammary gland mRNA following immunoprecipitation with antibodies to total milk proteins were used as a control (lanes 2 and 3). ^{35}S-labelled translation products of mRNA selected by hybridization were immunoprecipitated and separated by electrophoresis in 0.1% SDS–12% polyacrylamide gel. Total mRNA was hybridized with plasmid DNA of clone 1-15F (lane 1), a group of clones 1-13U, 1-15F and 1-11G (lane 4), and clone 1-11G (lane 5). Shown at the left are the main translation products of the mammary gland mRNA: α_{s1}-casein (α), protein p22 (p22), β-lactoglobulin (Lg).

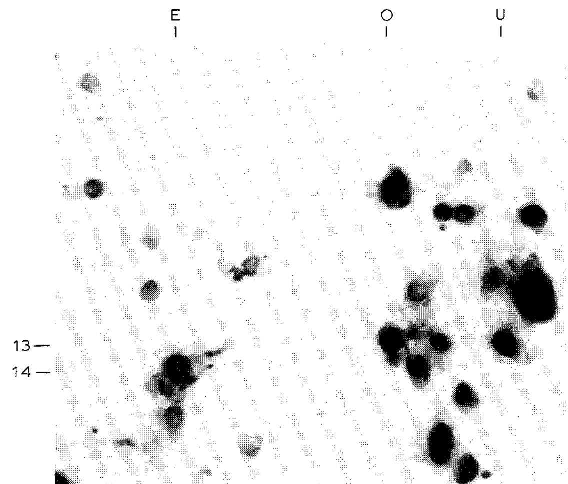

Fig. 2. Screening of the bovine mammary gland cDNA minilibrary. The 350 clones on nitrocellulose were numbered by columns designated A through V and rows designated 1 through 18; only columns E, O, U and rows 13 and 14 are marked on the margins. In situ colony hybridization was carried out with the ^{32}P-labelled probe (10^6 cpm/ml), namely the 300-bp *Pst*I fragment of clone 1-13U, which is the coding fragment of bovine Igλ light-chain cDNA. Hybridization conditions were as described by Maniatis et al. (1982). All colonies of this minilibrary were given the prefix 1, e.g., 1-13U. Clones 1-13O, 1-13U and 1-14E were used for sequencing the bovine Igλ chain cDNA.

Sequencing has shown that this cDNA fragment encoded 67 aa with a high degree of homology to aa 150–217 of the human light chain and a portion of the 3′-nontranslated region. The 300-bp *Pst*I cDNA fragment of clone 1-13U labelled by nick translation was used as a hybridization probe for screening of the minilibrary. About 30 colonies produced positive signals (Fig. 2). The size of cDNA fragments of these clones was usually from 200–400 bp. The largest was an 860-bp insert of clone 1-14E and a 560-bp insert of clone 1-130. The cDNA fragments of these clones were used for restriction map construction and sequencing.

To facilitate the sequencing, the fragments were recloned into the polylinker of plasmids pUC19 or pGEM1.

(b) Sequencing of Ig λ light-chain cDNA

The restriction map of cDNA fragment of clone 1-14E and sequencing strategy are shown in Fig. 3. The exact size of this fragment was 862 bp with 20-bp oligo(dG)-oligo(dC) connectors. Sequence comparison of human Ig λ chain cDNA (Anderson et al., 1985) and a cDNA fragment of clone 1-14E revealed a considerable homology and allowed to identify this cloned fragment as bovine Ig λ chain cDNA (Figs. 4 and 5).

The complete C domain of bovine mature light-chain polypeptide is 107 aa long and is encoded by nt 420 through 740. The constant regions of bovine and human cDNA differ only at 24 aa positions (Fig. 5). There is also a good homology between the J region of human cDNA encoding aa 100–112 and the J region of bovine Ig λ cDNA (Fig. 4). Three complementarity-determining regions (CDR1, 2, 3) characteristic of V-region bovine Ig λ chain can be distinguished. The bovine and human sequences display good homology in the FR3 regions but only patches of homology throughout the FR2 regions. A poor homology is characteristic of the leader peptide regions and FR1 regions, although some aa residues (Ser-10, Gly-15, Gln-24) are conservative. We were surprised that the 5′-coding region of clone 1-14E cDNA contained five stop codons: two in the leader-peptide region, and one each in FR1 and in the

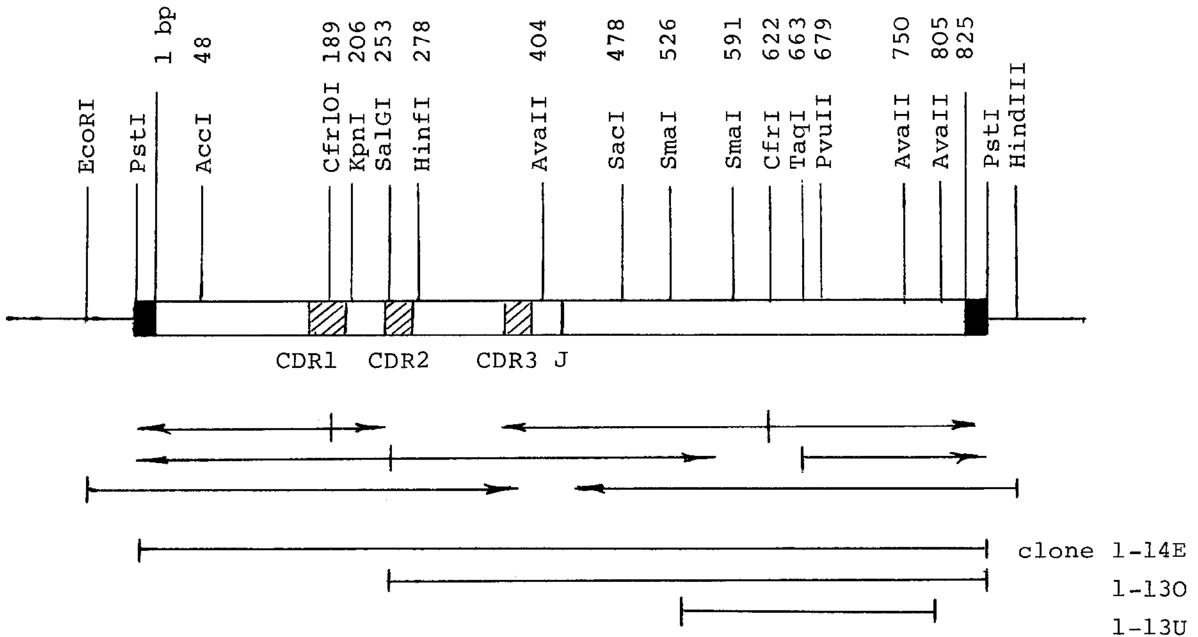

Fig. 3. Restriction map of Ig λ chain cDNA (clone 1-14E) and sequencing strategy. The DNA restriction fragments indicated by arrows were sequenced by the procedure of Maxam and Gilbert (1980). The thin line represents the polylinker of pUC19; blackened boxes are oligo(dG)-oligo(dC) connectors, hatched boxes represent CDR segments, and open boxes are the other regions of Ig λ light-chain cDNA.

CDR1 regions. There was a small deletion in the leader peptide region and no Cys-22 at all. The existence of so many stop codons and other nucleotide sequence changes in the 5′-coding region of the cDNA fragment of clone 1-14E implied that the V region of this clone arose from a pseudogene. We performed additional screening of bovine mammary gland cDNA library containing about 5400 clones to find more Ig λ cDNA clones with 5′ noncoding and 5′-coding regions. A ^{32}P-labelled 270-bp *Pst*I-*Sal*I subfragment of variable region of clone 1-14E was used as a probe. Twenty-four clones produced positive signals with this probe. The size of cDNA fragments was from 200 to 400 bp. Only three clones contained 860-bp cDNA fragments, namely clones 15X, 16F and 23B. cDNA fragments of these clones were recloned into the *Pst*I site of the polylinker of plasmid pGEM1 and used for sequencing. All the fragments had the same nucleotide sequence as the fragment of clone 1-14E with differences only in the connector size. The conclusion was that the non-translated Ig λ mRNA which arose from a pseudogene was widespread in the mRNA population of the bovine mammary gland.

We also sequenced the 570-bp fragment of clone 1-130 encoding Ig λ chain fragment starting at aa 53. Nucleotide sequence comparison of V regions of clones 1-130 and 1-14E is shown in Fig. 6. There are several differences in FR3, a lot of differences in the hypervariable region CDR3 and a few differences in the J region. This suggests that cDNA of clones 1-130 and 1-14E arose from different V_λ genes. Unfortunately, there is no information about the 5′ region of the second V_λ gene. At the same time, C regions of clones 1-14E and 1–130 have the same sequence.

DISCUSSION

The complexity of the Ig λ chain gene locus organization appears to correlate with the incidence of λ chain polypeptides in immunoglobulin molecules. In inbred mice, immunoglobulins with κ chains outnumber those with λ chains by about 20 : 1. The λ chain gene cluster is organized very simply and contains only 2 V_λ, 4 J_λ and 4 C_λ genes (Tonegawa, 1983; Eisen and Reilly, 1985). About 40% of human immunoglobulins contain λ light chain and the rest κ light chain. Human Ig λ chain gene locus organization is more complex. The number of V_λ genes is

```
B   1                        AAAGCATAAAATAAATAAAGGAATAAAGTTTCATTTTTTGTTCC

                 -16
H                G'' 'CA C'' CTT C'C 'C' C'C C'G G'T CAC ''' 'CA '''
B  45    ATG TAT ACT CAT TTA AAA ATT ATC TTA GTA ACA TGA TGC AAG GAT
         Met Tyr Thr His Leu Lys Ile Ile Leu Val Thr xxx Cys Lys Asp
         -17                         -10

H        -3
         ''T
B  90    TGA AAA GTT GCC ATA TTT CCA GTC GCA GTC AAT TCA AAA GTG AGG
         xxx Lys Val Ala Ile Phe Pro Val Ala Val Asn Ser Lys Val Arg
             -1   1                                  10

H
B 135    AGG GGG CAT TCA CTT TGA AAT CCT CTT AGA CAA TAA TAG AAT AAC
         Arg Gly His Ser Leu xxx Asn Pro Leu Arg Gln xxx xxx Asn Asn
                                 20

         29------CDR1-----------------           39
H        ''T 'CC ''C AA' TA' ''' CAG ''' ''T ''G '''
B 180    ATC GGT AGT GCC GGT GTG GCC TGG TAC CAA CAG GTC TCA TCG GGC
         Ile Gly Ser Ala Gly Val Ala Trp Tyr Gln Gln Val Ser Ser Gly
             30                                  40

                             49       ------- CDR2 -------
H                            ''' ''' 'AG GA' 'AC CAA A'' ''T CT' ''' 'TC
B 225    CTC AGA ACC ATC ATC TAT GGT AGT AGT AGT CGA CCC TCG GGG GCT
         Leu Arg Thr Ile Ile Tyr Gly Ser Ser Ser Arg Pro Ser Gly Ala
                                 50

                                 66  69
H        ''T ''T ''G ''' ''T ''' ''C 'GC ''C TC' 'AC T'T ''' TG' ''C
B 270    CCA GAC CGA TTC TCC GGC TCA AAG TCT GGC ACT ACA GGC ACC CTG
         Pro Asp Arg Phe Ser Gly Ser Lys Ser Gly Thr Thr Ala Thr Leu
             60                                  70

                     80                                          91
H        ''' ''' ''' GGA ''G A'' A'' ''G ''' ''' ''T ''C ''C 'A' '''
B 315    ACC ATC ACC TCG CTC CAG GCT GAC GAC GAG GCG GAT TAT TTC TGT
         Thr Ile Thr Ser Leu Gln Ala Asp Asp Glu Ala Asp Tyr Phe Cys
                                 80

            92-----------CDR3-----------  100
H           CAG T'' TTT G'T 'AC 'CC 'A' CAA 'GA ''G ''C ''' G'A '''
B 360    GCA ACT GCT GAC TAC AGT AGG AGT ACT GTT GTT TTT GGC AGC GGG
         Ala Thr Ala Asp Tyr Ser Arg Ser Thr Val Val Phe Gly Ser Gly
             90                                  100

                             110
H        ''' 'AG T'' ''' '''
B 405    ACC AGA CTG ACC GTC
         Thr Arg Leu Thr Val
                         108
```

Fig. 4. Comparison of the nucleotide sequences of the variable regions of the human (H) and bovine (B) Ig λ chain cDNA (clone 1-14E). The human sequence is from Anderson et al. (1985). The prime marks indicate nucleotides common to both sequences. The nucleotides have been numbered for the bovine sequence starting at 1 (left margin). The corresponding amino acids are shown under the nucleotide sequence and are numbered starting at aa 1 (Val), which marks the beginning of the V region. Negative numbers refer to the amino acids of the leader sequence. The human nucleotide sequence is shown only for the region with high homology to the bovine sequence. Numbering of the codons is in accordance with the human Ig λ amino acid sequence (Anderson et al., 1985). The in-phase termination codons are marked xxx.

```
       111                                             120
H      ''A ''' ''' ''' ''' G'T G'C ''' ''' ''' ''T ''' ''' ''A '''

B 420  CTC GGT CAG CCC AAG TCC CCA CCC TCG GTC ACC CTG TTC CCG CCC

       Leu Gly Gln Pro Lys Ser Pro Pro Ser Val Thr Leu Phe Pro Pro
       109                                         120

                           130                                 140
H      ''' T'T ''' ''' ''T C'A 'C' ''' ''' ''' ''A ''' ''' ''' '''

B 465  TCC ACG GAG GAG CTC AAC GGC AAC AAG GCC ACC CTG GTG TGT CTC

       Ser Thr Glu Glu Leu Asn Gly Asn Lys Ala Thr Leu Val Cys Leu
                               130

                                           150
H      ''A ''T ''' ''' ''' ''' ''A GC' ''' ''A ''' 'C' ''' ''' '''

B 510  ATC AGC GAC TTC TAC CCG GGT AGC GTG ACC GTG GTC TGG AAG GCA

       Ile Ser Asp Phe Tyr Pro Gly Ser Val Thr Val Val Trp Lys Ala
           140                                     150

                           160                                 170
H      ''T A'' ''' C'' G'' 'AG GCG GGA ''' ''' ''' ''' ACA C'' '''

B 555  GAC GGC AGC ACC ATC ACC CGC AAC GTG GAG ACC ACC CGG GCC TCC

       Asp Gly Ser Thr Ile Thr Arg Asn Val Glu Thr Thr Arg Ala Ser
                               160

                                           180
H      ''' ''A ''' ''' 'A' ''' ''' ''' ''' ''' ''' ''' ''' ''' '''

B 600  AAA CAG AGC AAC AGC AAG TAC GCC GCC AGC AGC TAC CTG AGC CTG

       Lys Gln Ser Asn Ser Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu
           170                                     180

                           190                                 200
H      ''' CCT GAG C'G ''' ''G ''C C'C AAA ''C ''' ''' ''' C'' '''

B 645  ACG AGC AGC GAC TGG AAA TCG AAA GGC AGT TAC AGC TGC GAG GTC

       Thr Ser Ser Asp Trp Lys Ser Lys Gly Ser Tyr Ser Cys Glu Val
                               190

                                           210
H      ''' ''T ''A ''' ''' ''' ''' GA' ''' ''' ''' GCC ''T A'' ''A

B 690  ACG CAC GAG GGG AGC ACC GTG ACG AAG ACA GTG AAG CCC TCA GAG

       Thr His Glu Gly Ser Thr Val Thr Lys Thr Val Lys Pro Ser Glu
           200                                     210

           217
H      ''' ''A ''' 'TTCTCAT''''-C'''''CA''A''''A'ACTAGA'

B 735  TGT TCT TAG GG------CCCTGGACCCCCACCCTCGGGGGCCCTCTGGCCCACACC

       Cys Ser Stop
           215

B 785  CCCTCCCCCACCTCTCCATGGACCCCTGAGCCCCTACCCAGGTCGCCTCACACAAGGGG

B 844  CCTCTCCCTCCCTCCCTGTTCCTGCTTCTC
```

Fig. 5. Comparison of the nucleotide sequences of the constant region of the human (H) and bovine (B) Ig λ chain cDNA (clone 1-14E). The human sequence is from Anderson et al. (1985). The prime marks indicate nucleotides common to both sequences. Dashes represent the gaps introduced to maximise homologies. The nucleotides have been numbered for bovine sequence starting at 420 (left margin). The corresponding amino acids are shown under the nucleotide sequence and are numbered starting at aa 109 (Leu). The human Ig λ nucleotide sequence codons are shown above the bovine sequence and are numbered according to the human Ig λ amino acid sequence. The last 47 nt of the 3′-noncoding region of bovine Ig λ cDNA are from clone 1-13O.

```
B 130                   ''' ''' ''' ''' ''' ''C ''' ''' ''' ''' ''' ''' ''C
B 14E   AGT AGT AGT CGA CCC TCG GGG GCT CCA GAC CGA TTC TCC GGC TCA
        51                                  60
B 130   ''' ''C ''' 'AC ''' 'C' ''' ''' ''' ''' 'G' ''A ''' ''' '''
B 14E   AAG TCT GGC ACT ACA GGC ACC CTG ACC ATC ACC TCG CTC CAG GCT
                        70                                      80
B 130   ''G ''T ''' ''' ''' ''' ''' ''' 'T' --- 'T' T'T G'T --- '''
B 14E   GAC GAC GAG GCG GAT TAT TTC TGT GCA ACT GCT GAC TAC AGT AGG
                                            90
                                            L---------CDR3-----------
B 130   ''' 'G' AAC ATT 'G' C'' ''C ''' ''' ''' ''' 'C' ''' G'' '''
B 14E   AGT ACT --- --- GTT GTT TTT GGC AGC GGG ACC AGA CTG ACC GTC
        ___97|          98      100
```

Fig. 6. A comparison of the nucleotide sequence of variable regions of bovine Ig λ chain cDNA fragments from clones 1-13O and 1-14E. The prime marks indicate nucleotides common to both sequences; dashes represent the gaps introduced to maximise homologies. The bovine nucleotide sequence codons (clone 1-14E) are numbered in accordance with Fig. 4.

15 to 20 (Anderson et al., 1985). Finally, more than 90% of bovine immunoglobulins contain λ light chains (Butler, 1986). The bovine λ chain locus has probably the most complex organization.

The Ig gene family is rich in pseudogenes. The V_H locus of the human genome contains about 40% pseudogenes and one of its clusters up to 70% pseudogenes (Kodaira et al., 1986). Numerous pseudogenes are characteristic of the chicken λ chain gene family containing 15 V_λ gene (Reynaud et al., 1985; Parvari et al., 1986). Maybe the bovine λ gene family is also rich in pseudogenes. The evidence about pseudogene origin of the mRNA of clone 1-14E is not surprising, but it is very interesting that this messenger is widespread in the total mRNA population of bovine mammary gland. A different hypothesis may be proposed to explain these data, including the loss of the pseudogene's ability to regulate transcription, and a change in stability of the mRNA transcribed from the pseudogene. The one that seemed interesting to us is the reading of pseudogene mRNA in a wrong frame. Indeed, in case of frameshift it is possible to translate polypeptide X containing 28 aa starting from the ATG codon (nt 77–79) to the stop codon (nt 160–162). This polypeptide would be rich in serine and have some degree of homology with the polyserine cluster of bovine β-casein (Fig. 7). One of the main functions of β-

```
                                                    15  16  17  18
                                                    Ser Leu Ser Ser
β-CASEIN                                            AG' CT' TCA ''C
1-14E    77  ATG ATG CAA GGA TTG AAA AGT TGC CAT ATT TCC AGT CGC AGT CAA
             Met Met Gln Glu Leu Lys Ser Cys His Ile Ser Ser Arg Ser Gln
             1   2   3   4   5   6   7   8   9   10  11  12  13  14  15
                                                     __          __

                     19  20  21
                     Ser Glu Glu
β-CASEIN             ''' ''' ''A
1-14E    122 TTC AAA AGT GAG GAG GGG GCA TTC ACT TTC AAA TCC TCT TAG
             Phe Lys Ser Glu Glu Gly Ala Phe Thr Leu Lys Ser Ser Stop
             16  17  18  19  20  21  22  23  24  25  26  27  28
                     __________
```

Fig. 7. Bovine Ig λ chain mRNA (clone 1-14E) read in a wrong frame starting from nt 77. The deduced amino acid sequence of polypeptide X is shown under the nucleotide sequence. The homology region with the polyserine cluster of bovine β-casein (Bayev et al., 1987) is underlined.

casein is to transfer phosphate and calcium by serine-residue phosphorylation. Hypothetic polypeptide X would thus serve as an additional phosphate and calcium transfer agent for the developing organism. If this hypothesis were proved by direct evidence on the existence of this polypeptide in the bovine mammary gland it would be an indication of the molecular substitution of some immunoglobulin pseudogenes as a result of a high mutation hypervariability of these genes.

ACKNOWLEDGEMENTS

We are grateful to N.P. Mertvetsov for helpful discussions.

REFERENCES

Anderson, M.L.M., Brown, L., McKenzie, E., Kellow, J.E. and Joung, B.D.: Cloning and sequence analysis of an Ig λ light chain mRNA in the Burkitt's lymphoma cell line EB4. Nucl. Acids Res. 13 (1985) 2931–2941.

Bayev, A.A., Smirnov, I.K. and Gorodetsky, S.I.: The primary structure of bovine β-casein cDNA. Mol. Biol. (in Russian) 20 (1987) 255–265.

Butler, J.E.: Biochemistry and biology of ruminant immunoglobulins. In Pandey, R. (Ed.), Progress in Veterinary Microbiology and Immunology, Vol. 2. Karger, Basel, 1986, pp. 1–52.

Butler, J.E.: Immunoglobulins of the mammary secretions. In Larson, B.L. and Smith, V.R. (Eds.), Lactation, Vol. 3. Academic Press, New York, 1974, pp. 217–255.

Eisen, H.N. and Reilly, E.B.: Lambda chains and genes in inbred mice. Annu. Rev. Immunol. 3 (1985) 337–365.

Gorodetsky, S.I., Kershulite, D.R., Ivanov, V.N., Kapelinskaya, T.V. and Kaledin, A.S.: Synthesis and cloning of DNA complementary to mRNA from bovine mammary gland. Genetika 21 (1985) 191–200.

Honjo, T.: Immunoglobulin genes. Annu. Rev. Immunol. 1 (1983) 499–528.

Ivanov, V.N., Kershulite, D.R., Bayev, A.A., Akhundova, A.A., Sulimova, G.E., Judinkova, E.S. and Gorodetsky, S.I.: Identification of bacterial clones encoding bovine caseins by direct immunological screening of the cDNA library. Gene 32 (1984) 381–388.

Kadaira, M., Kinashi, T., Umemura, I., Matsuda, F., Noma, T., Ono, Y. and Honjo, T.: Organization and evolution of variable region genes of the human immunoglobulin heavy chain. J. Mol. Biol. 190 (1986) 529–541.

Kushner, S.R.: An improved method for transformation of *Escherichia coli* ColE1-derived plasmids. In Boyer, H.W. and Nicosia, S. (Eds.), Genetic Engineering. Elsevier, Amsterdam, 1978, pp. 17–23.

Maniatis, T., Fritsch, E.F. and Sambrook, J.: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.

Maxam, A.M. and Gilbert, W.: Sequencing end-labelled DNA with base-specific chemical cleavages. Methods Enzymol. 65 (1980) 499–560.

Parvari, R., Zim, E., Lentner, F., Ter-Or, S., Burstein, Y. and Schechter, I.: Analyses of chicken immunoglobulin light chain cDNA clones indicate a few germline V_λ genes and allotypes of C_λ locus. EMBO J. 6 (1987) 97–102.

Reynaud, C.-A., Anquez, V., Dahan, A. and Well, J.-C.: A single rearrangement event generates most of the chicken immunoglobulin light chain diversity. Cell 40 (1985) 283–291.

Tonegawa, S.: Somatic generation of antibody diversity. Nature 302 (1983) 575–581.

Communicated by A.A. Bayev.