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Molecular cloning of a bovine immunoglobulin lambda chain cDNA

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

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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 Ig λ 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 exclu-

sively 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

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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.

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 Ig λ light-chain cDNA clones. As a result we isolated clones containing Ig λ 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 ³²P-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).

³²P-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.

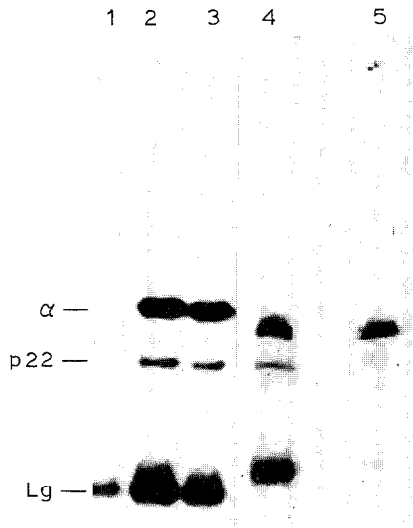


Fig. 1. Hybrid-selected translation. Translation products of total mammary gland mRNA following immunoprecipitation with antibodies to total milk proteins were used as a control (lanes 2 and 3). ^{35}S -labelled translation products of mRNA selected by hybridization were immunoprecipitated and separated 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, β -lactoglobulin (Lg).

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

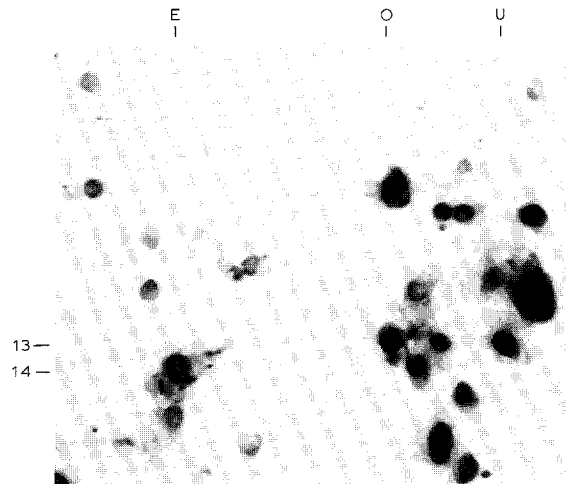


Fig. 2. Screening of the bovine mammary gland cDNA minilibrary. The 350 clones on nitrocellulose were numbered by columns designated A through V and rows designated 1 through 18; only columns E, O, U and rows 13 and 14 are marked on the margins. In situ colony hybridization was carried out with the ^{32}P -labelled probe (10^6 cpm/ml), namely the 300-bp *Pst*I fragment of clone 1-13U, which is the coding fragment of bovine Ig λ 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 Ig λ chain cDNA.

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

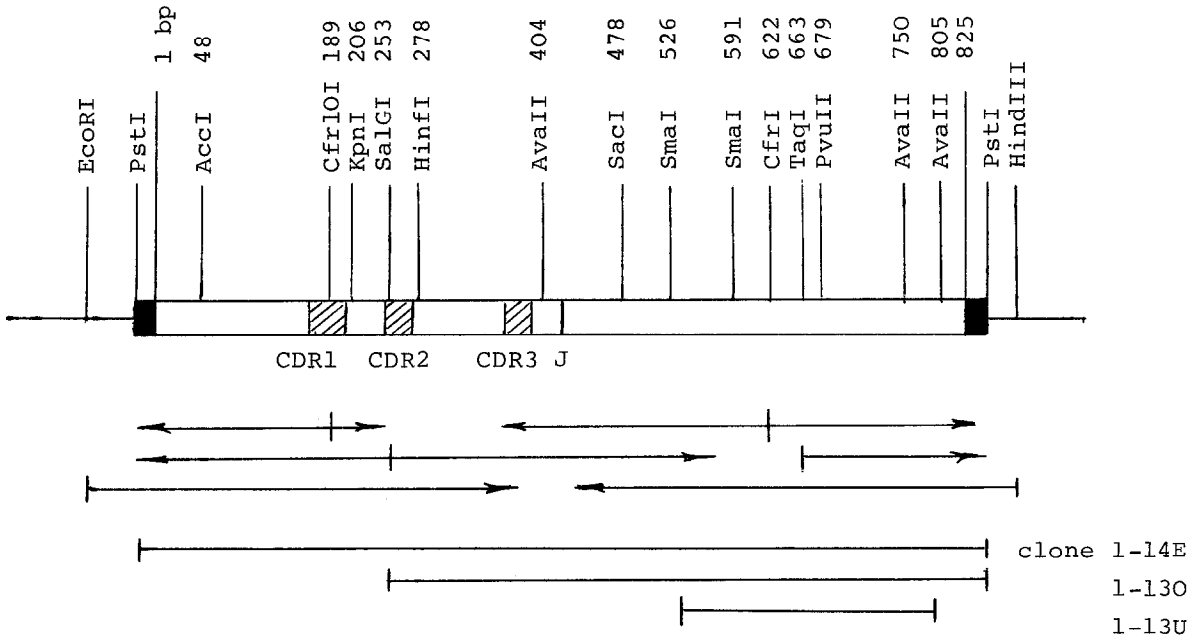


Fig. 3. Restriction map of Ig λ chain cDNA (clone 1-14E) and sequencing strategy. The DNA restriction fragments indicated by arrows were sequenced by the procedure of Maxam and Gilbert (1980). The thin line represents the polylinker of pUC19; blackened boxes are oligo(dG)-oligo(dC) connectors, hatched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

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B 1              AAAGCATAAAATAAATAAAGGAATAAAGTTTCATTTTTTTGTTCC
H              -16
              G' ' 'CA C' ' CTT 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 ' ' C C ' ' C A A ' T A ' ' ' C A G ' ' ' ' ' 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
H
      49          CDR2          69
      ' ' ' ' ' ' '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
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
H      ' ' ' ' ' ' ' 80          91
      ' ' ' ' ' ' 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
H      92-----CDR3----- 100
      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
H      ' ' ' 'AG T' ' ' ' ' ' ' 110
B 405 ACC AGA CTG ACC GTC
      Thr Arg Leu Thr Val
      108

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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.

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H      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

H      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      140

H      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

H      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      170

H      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

H      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      200

H      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

H      217
H      ' ' 'A ' ' ' 'TTCTCAT' ' ' ' -C' ' ' ' ' 'CA' 'A' ' ' 'A'ACTAGA'
B 735 TGT TCT TAG GG-----CCCTGGACCCCCACCCTCGGGGGCCCTCTGGCCCACACC
      Cys Ser Stop
      215

B 785 CCCTCCCCACCTCTCCATGGACCCCTGAGCCCCTACCCAGGTGCCTCACACAAGGGG
B 844 CCTCTCCCTCCCTCCCTGTTCTCTGCTTCTC

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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 1-130.

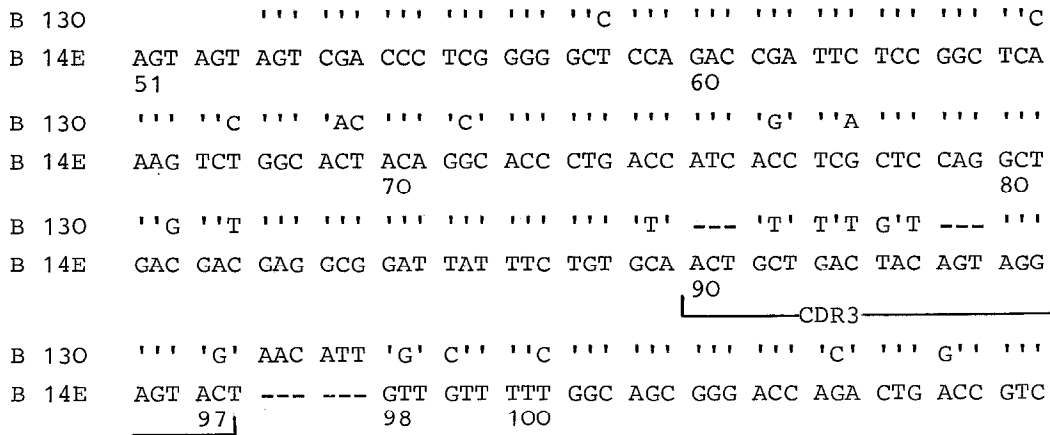


Fig. 6. A comparison of the nucleotide sequence of variable regions of bovine Ig λ chain cDNA fragments from clones 1-130 and 1-14E. The prime marks indicate nucleotides common to both sequences; dashes represent the gaps introduced to maximise homologies. The bovine nucleotide sequence codons (clone 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-

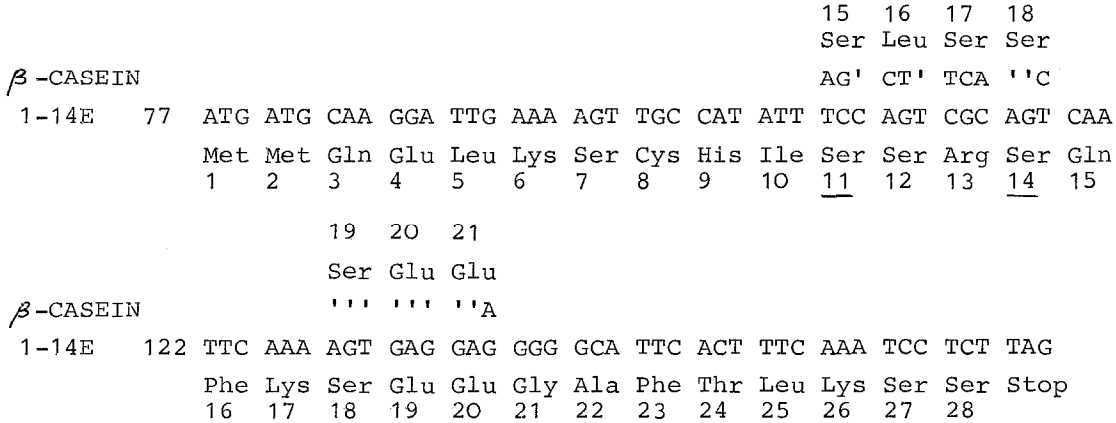


Fig. 7. Bovine Ig λ chain mRNA (clone 1-14E) read in a wrong frame starting from nt 77. The deduced amino acid sequence of polypeptide X is shown under the nucleotide sequence. The homology region with the polyserine cluster of bovine β-casein (Bayev et al., 1987) is underlined.

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 hyper-variability of these genes.

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