Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

ISSN 0378-1119
GENEDB 61 (1) 1–122 (1987)

LINDA HALL
FEB 6 1988
LIBRARY

gene

an international journal focusing on gene cloning and gene structure and function

ELSEVIER

1987

volume 61 no. 1

© 1987, Elsevier Science Publishers B.V. (Biomedical Division)
All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior written permission of the Publisher, Elsevier Science Publishers B.V. (Biomedical Division), P.O. Box 1527, 1000 BM Amsterdam (Molenwerf 1, 1014 AG Amsterdam) The Netherlands.

No responsibility is assumed by the Publisher for any injury and/or damage to persons of property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of the rapid advances in the medical sciences, the Publisher recommends that independent verification of diagnoses and drug dosages should be made.

Although all advertising material is expected to conform to ethical (medical) standards, inclusion in this publication does not constitute a guarantee or endorsement of the quality or value of such product or of the claims made of it by its manufacturer.

Special regulations for authors. Upon acceptance of an article by the journal, the author(s) will be asked to transfer copyright of the article to the Publisher. This transfer will ensure the widest possible dissemination of information.

Submission of a paper to this journal entails the author's irrevocable and exclusive authorization of the publisher to collect any sums or considerations for copying or reproduction payable by third parties (as mentioned in article 17 paragraph 2 of the Dutch Copyright Act of 1912 and in the Royal Dutch Decree of June 20, 1974 (S. 351) pursuant to article 16b of the Dutch Copyright Act of 1912) and/or to act in or out of Court in connection therewith.

Special regulations for readers in the U.S.A. This journal has been registered with the Copyright Clearance Center, Inc. Consent is given for copying of articles for personal or internal use, or for the personal use of specific clients. This consent is given on the condition that the copier pays through the Center the per-copy fee stated in the code on the first page of each article for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. The appropriate fee should be forwarded with a copy of the first page of the article to the Copyright Clearance Center, Inc., 27 Congress Street, Salem, MA 01970, U.S.A. If no code appears in an article, the author has not given broad consent to copy and permission to copy must be obtained directly from the author. All articles published prior to 1980 may be copied for a per-copy fee of US $2.25, also payable through the Center. This consent does not extend to other kinds of copying, such as for general distribution, resale, advertising and promotion purposes, or for creating new collective works. Special written permission must be obtained from the Publisher for such copying.

Printed in Belgium

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

Vol. 61 No. 1

gene

1987

An International Journal Focusing on Gene Cloning and Gene Structure and Function

W. Szybalski – Editor-in-Chief

CONTENTS

Schardl, C.L., Byrd, A.D., Benzion, G., Altschuler, M.A., Hildebrand, D.F. and Hunt, A.G. 1–11
Design and construction of a versatile system for the expression of foreign genes in plants (GEN 02215)

Sánchez-Puelles, J.M., García, J.L., López, R. and García, E. 13–19
3′-End modifications of the *Streptococcus pneumoniae lytA* gene: role of the carboxy terminus of the pneumococcal autolysin in the process of enzymatic activation (conversion) (GEN 02216)

Mormeneo, S., Knott, R. and Perlman, D. 21–30
Precise nucleotide sequence modifications with bi-directionally cleaving class-IIS excision linkers (GEN 02217)

Rouhandeh, H. and Cohrs, R. 31–39
Cloning and physical mapping of *Herpesvirus sylvilagus* DNA (GEN 02219)

Rothstein, D.M. and Bertonis, J.M. 41–50
Transcription efficiency along the tissue-plasminogen-activator cDNA gene in *Escherichia coli* (GEN 02220)

Wu, J. and Yarbrough, L.R. 51–62
Expression of the alpha and beta tubulin genes of the African trypanosome in *Escherichia coli* (GEN 02221)

Takeshita, S., Sato, M., Toba, M., Masahashi, W. and Hashimoto-Gotoh, T. 63–74
High-copy-number and low-copy-number plasmid vectors for *lacZ* α-complementation and chloramphenicol- or kanamycin-resistance selection (GEN 02222)

Puente, J.L., Flores, V., Fernández, M., Fuchs, Y. and Calva, E. 75–83
Isolation of an *ompC*-like outer membrane protein gene from *Salmonella typhi* (GEN 02223)

Jamieson, A.C., Vandeyar, M.A., Kang, Y.C., Kinsella, J.E. and Batt, C.A. 85–90
Cloning and nucleotide sequence of the bovine β-lactoglobulin gene (GEN 02224)

Prentki, P., Pham, M.-H., Gamas, P., Chandler, M. and Galas, D.J. 91–101
Artificial transposable elements in the study of the ends of IS*1* (GEN 02225)

Lennick, M., Haynes, J.R. and Shen, S.-H. .. 103–112
High-level expression of α-human atrial natriuretic peptide from multiple joined genes in *Escherichia coli* (GEN 02226)

Short Communications

Ledley, F.D., Grenett, H.E., Bartos, D.P. and Woo, S.L.C. 113–118
Retroviral mediated transfer and expression of human $alpha_1$-antitrypsin in cultured cells (02214)

Hurley, W.L. and Schuler, L.A. 119–122
Molecular cloning and nucleotide sequence of a bovine α-lactalbumin cDNA (GEN 02218)

Cited in: Biological Abstracts; Current Contents (Life Sciences); Excerpta Medica (EMBASE); Index Medicus; Telegen; Pascal M.

This material may be protected by Copyright law (Title 17 U.S. Code)

Gene, 61 (1987) 85–90
Elsevier

GEN 02224

Cloning and nucleotide sequence of the bovine β-lactoglobulin gene

(Recombinant DNA; mammary gland; cDNA clone bank; whey protein; retinol)

Andrew C. Jamieson[a], Mark A. Vandeyar[a], Young C. Kang[b]*, John E. Kinsella[a] and Carl A. Batt[a]

[a] *Department of Food Science, Cornell University, Ithaca, NY 14853 (U.S.A.) Tel. (607)255-7902, and* [b] *Department of Food Science and Technology, University of California, Davis, CA 95616 (U.S.A.) Tel. (916)752-8945*

Received 10 July 1987
Revised 8 September 1987
Accepted 23 September 1987

SUMMARY

A clone coding for β-lactoglobulin A has been isolated from a cDNA bank constructed from poly(A^+)mRNA isolated from the bovine mammary gland. Its nucleotide sequence codes for the β-lactoglobulin A, from amino acid residues Leu-11 to Ile-162, as based on the amino acid sequence reported by Braunitzer et al. [Z. Physiol. Chem. 354 (1973) 867-878]. In addition to the 455-bp coding sequence, our clone pBβL4-10 contains a 3′-nontranslated region of approx. 270 bp.

INTRODUCTION

Bovine β-lactoglobulin (βLg) is a whey protein, found in the milk of animals including bovine species, sheep, deer, dogs, and pigs but not in humans (Pervaiz and Brew, 1985). It occurs in several different variants due to some genetic variations that exist in given animal species. It is a single polypeptide of 18 kDa which corresponds to 162 aa residues. The complete amino acid sequence of bovine βLg has been reported and genetic variations in the amino acid sequence have been identified (Creamer et al., 1983).

βLg is a globular protein and the primary sequence reveals two disulfide bonds between aa residues 66–160 and 106–119 and a free cysteine group at 121. On the basis of extensive analyses and calculations, the secondary structure of βLg is 15, 51, 17, and 17% α-helix, β-sheet, reversed turn and aperiodic structure, respectively (Creamer et al., 1983). In solution, above pH 4.0 βLg exists as a dimer in which the monomers appear to associate at the respective α-helical segments (aa 65–75 and 129–143) and the Cys-121 groups are close to the electron dense region of association. Hence dimerization protects the thiol groups. The β-sheet peptide sequence containing Cys-121 is relatively apolar and in the native monomer, Cys-121 is buried below the surface of the molecule.

Correspondence to: Dr. C.A. Batt, 107 Stocking Hall, Cornell University, Ithaca, NY 14853 (U.S.A.) Tel. (607)255-2896.
* Present address: Stauffer Chemical Co., Dobbs Ferry, NY (U.S.A.) Tel. (914)693-1200.

Abbreviations: aa, amino acids(s); βLg, β-lactoglobulin; bp, base pair(s); LB, Luria-Bertani (see Maniatis et al., 1982); nt, nucleotide(s); ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TBS, see MATERIALS AND METHODS, section **a**.

0378-1119/87/$03.50 © 1987 Elsevier Science Publishers B.V. (Biomedical Division)

The tertiary structure has been determined from x-ray crystallographic studies at a resolution of 2.8 Å (Sawyer et al., 1985; Papiz et al., 1986) and modelled using computer graphic programs (J. Brady, Dept. Food Science, Cornell University, personal communication). It possesses two antiparallel β-sheets, two disulfide bonds and one free cysteine residue.

The function of βLg is not clear especially since the milk of certain mammalian species appears to be devoid of it. Based upon its structural homology to retinol-binding protein, it is hypothesized that it may also bind and transport retinol (Papiz et al., 1986) to the neonate.

Although extensive information is available on the primary and tertiary structure of bovine βLg, very little has been reported on the cloning and characterization of its gene. A partial cDNA clone of bovine βLg has been isolated (Willis et al., 1982). The βLg clone obtained was 550 bp and a limited amino acid sequence inferred from nucleotide sequencing corresponded to aa residues 109–122.

In this study we have isolated a cDNA clone encoding for 93% of the structural gene and its 3′-nontranslated region extending to the poly(A^+)tail.

MATERIALS AND METHODS

(a) Identification of βLg cDNA clone

cDNA was synthesized from bovine poly-$(A)^+$mRNA isolated from mammary tissue of lactating cow, G-C tailed and cloned into the *bla* gene of pBR322 (Jimenez-Flores et al., 1987). This cDNA bank was screened using polyclonal anti-βLg serum obtained by repeated intramuscular injection of βLg (Sigma Chemical Co.) into Flemish Giant rabbits. Recombinant *Escherichia coli* RR1 colonies carrying the bovine cDNA bank were propagated overnight on LB agar containing tetracycline (15 μg/ml) and replicated onto 150-mm nitrocellulose filters (Biorad Inc., Richmond, CA). After incubation for 4–6 h at 37°C the colonies were lysed by suspending the filters in chloroform vapors for 20 min. The lysed colonies on the filters were incubated for 60 min in 50 mM Tris · HCl (pH 7.5), 150 mM NaCl, 5 mM $MgCl_2$ 3% gelatin, 1 μg/ml of DNase I, and 40 μg/ml of lysozyme. The filters were then washed twice in TBS buffer (20 mM Tris, pH 7.5; 500 mM NaCl) for 5 min. A 1:1000 dilution of rabbit anti-βLg antisera was added in TBS with 1% gelatin for 60 min and the filter was then washed twice with TBS. A goat anti-rabbit horseradish peroxidase conjugated antibody was then added for 60 min (1:3000 dilution in TBS + 1% gelatin) to detect the primary antibody-βLg complex. After washing the filter, bound anti-rabbit antibody was identified using a color-developing reagent (HRP color-developing reagent, Biorad Inc., with 0.02% hydrogen peroxide).

(b) Nucleotide sequencing and Western-blot analysis

The nucleotide sequence was determined by the method of Sanger et al. (1977) using M13mp18 or M13mp19 vectors.

Total cellular protein from *E. coli* RR1 alone and carrying pBβL4-10 was separated using 0.1% SDS–12.5% PAGE (Laemmli, 1970) transferred to nitrocellulose (Towbin et al., 1979) and developed using rabbit anti βLg serum as described before.

RESULTS

(a) Identification of βLg cDNA clone

A cDNA bank containing approx. 6000 clones was obtained from poly$(A)^+$mRNA isolated from the mammary tissue of a lactating cow. The screening of an initial 500 clones using rabbit polyclonal anti-βLg serum resulted in the identification of a immunoreactive clone designated pBβL4-10. pBβL4-10 contains an insert of approx. 800 bp and an internal *Pst*I site.

By virtue of its identification using polyclonal antibodies a fusion must exist between the N-terminal of β-lactoglobulin and β-lactamase encoded by the region 5′ to the *Pst*I site. Western-blot analysis of *E. coli* RR1 carrying pBR322 and pβBL4-10 was performed to determine the size of the fusion protein (Fig. 1). Although some cross-reactivity (or non-specific binding) is observed as common bands in both pBR322 and pBβL4-10 a unique band of 36 kDa is observed in pBβL4-10 extracts. Given the

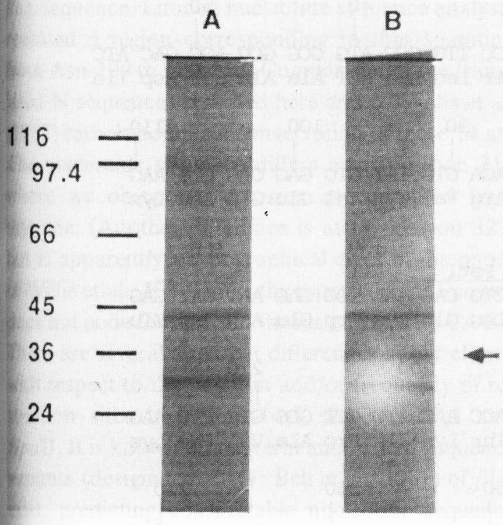

Fig. 1. Western-blot analysis of *E. coli* RR1 lysates using rabbit antiserum against βLg. Total cellular protein from the respective *E. coli* RR1 strains were separated on a 0.1% SDS–12.5% PAGE in 25 mM Tris · HCl, pH 8.8 at 30 mA overnight. The proteins were electrophoretically transferred to a nitrocellulose filter in transfer buffer (25 mM Tris · HCl, 192 mM glycine; 20% methanol, pH 8.3) at 30 V for 12 h. After blocking in NET buffer (150 mM NaCl, 5 mM EDTA, 0.25% gelatin, 0.05% Triton X-100, 50 mM Tris · HCl, pH 7.4) detection was performed using rabbit polyclonal antiserum against βLg (diluted 1:1000) The antibody-antigen complex was visualized using a secondary goat antirabbit antibody conjugated to horseradish peroxidase (diluted 1:3000; BioRad Inc.) and the blot developed according to the manufacturer's instructions. Lanes: A, pBR322 (control); B, pBβL4-10. The two bands common to both lanes are proteins which cross react with the anti-βLg antiserum, the unique band present in lane B is the βLg/β lactamase fusion protein. The positions of protein markers (in kDa) are shown in the left margin. The protein M_r markers (Sigma Co.) used were β-galactosidase (116 kDa), phosphorylase B (97.4 kDa), bovine albumin (66 kDa), egg albumin (45 kDa), glyceraldhyde-3-phosphate dehydrogenase (36 kDa) and trypsinogen (24 kDa). The arrow indicates the location of the unique βLg-β lactamase fusion protein.

size of the N terminus of β-lactamase to the *Pst*I site (20 kDa) approx. 16 kDa or 430 bp of the β-lactoglobulin-coding sequence should be present. β-lactoglobulin is 162 aa long (or a predicted 486 bp), therefore this clone should contain approx. 90% of the coding sequence for the mature protein.

A 600-bp *Pst*I insert from pBβL4-10 was used to probe the cDNA bank for additional βLg clones. Five colonies which hybridized to the 600-bp *Pst*I fragment were obtained and their plasmid inserts confirmed by Southern-blot analysis. Subsequent nucleotide sequence analysis revealed that they were in fact shorter at the 5′ end than pBβL4-10 (see further).

(b) Nucleotide sequence

The sequence for the entire 800-bp cDNA insert in pBβL4-10 was determined (Fig. 2). When translated, a single ORF of 452 bp or 151 aa was observed. This ORF corresponds to the published amino acid sequence of βLg from Leu-11 to Ile-162. A 270-bp 3′-nontranslated region is located after the translational stop (nt 456) followed by a poly(A^+) sequence which accounts for 36% of the predicted size of the full length message. A consensus polyadenylation site (nt 589; ATAAA) is also present upstream from the poly(A) sequence.

DISCUSSION

A cDNA clone coding for aa residues Leu-11 to Ile-162 of βLg has been isolated from a bank constructed from bovine poly$(A)^+$mRNA. The deduced amino acid sequence is in complete agreement with the reported sequence for βLg (Creamer et al., 1983). To date several cDNA clones coding for bovine mammary proteins have been identified including α_{s1}-casein, κ-casein (Stewart et al., 1984) and β-casein (Jimenez-Flores et al., 1987) and galactosyltransferase (Shaper et al., 1986). This is to our knowledge the first report on cloning of a virtually complete bovine βLg sequence.

We were not successful in locating any clones that coded for the region located 5′ to the Leu-11 codon. A 20-kDa protein was observed after in vitro translation of bovine poly$(A)^+$mRNA and subsequent immunoprecipitation with anti-βLg antiserum (Yoshikawa et al., 1978). This suggests that pre-βLg is approx. 2 kDa larger than the mature βLg. The signal sequence of the ovine βLg is 18 aa long and the sequence for the N terminus of the mature protein is almost identical to that of the bovine βLg (Mercier et al., 1978).

Willis et al. (1982) reported the identification of a cDNA clone coding for βLg from an Aryshire cow. It is estimated that it codes for approx. 60% of the

```
                10                  20                  30                  40                  50
AT ATC CAG AAG GTG GCG GGG ACT TGG TAC TCC TTG GCC ATG GCG GCC AGC GAC ATC
   Ile Gln Lys Val Ala Gly Thr Trp Tyr Ser Leu Ala MET Ala Ala Ser Asp Ile
                                   *                                   *
 60                  70                  80                  90                 100                 110
TCC CTG CTG GAC GCC CAG AGT GCC CCC CTG AGA GTG TAT GTG GAG GAG CTG AAG
Ser Leu Leu Asp Ala Gln Ser Ala Pro Leu Arg Val Tyr Val Glu Glu Leu Lys
                                                                   *
                120                 130                 140                 150                 160
                                                         PstI
CCC ACC CCT GAG GGC GAC CTG GAG ATC CTG CTG CAG AAA TGG GAG AAT GAT GAG
Pro Thr Pro Glu Gly Asp Leu Glu Ile Leu Leu Gln Lys Trp Glu Asn Asp Glu
            *                   *                   *                   *
                170                 180                 190                 200                 210
TGT GCT CAG AAG AAG ATC ATT GCA GAA AAA ACC AAG ATC CCT GCG GTG TTC AAG
Cys Ala Gln Lys Lys Ile Ile Ala Glu Lys Thr Lys Ile Pro Ala Val Phe Lys
                                                    *
220                 230                 240                 250                 260                 270
ATC GAT GCC TTG AAC GAG AAC AAA GTC CTT GTG CTG GAC ACC GAC TAC AAA AAG
Ile Asp Ala Leu Asn Glu Asn Lys Val Leu Val Leu Asp Thr Asp Tyr Lys Lys

                280                 290                 300                 310                 320
TAC CTG CTC TTC TGC ATG GAG AAC AGT GCT GAG CCC GAG CAA AGC CTG GTC TGC
Tyr Leu Leu Phe Cys MET Glu Asn Ser Ala Glu Pro Glu Gln Ser Leu Val Cys
                                                                   *
330                 340                 350                 360                 370                 380
CAG TGC CTG GTC AGG ACC CCG GAG GTG GAC GAC GAG GCC CTG GAG AAA TTC GAC
Gln Cys Leu Val Arg Thr Pro Glu Val Asp Asp Glu Ala Leu Glu Lys Phe Asp
                                        *   *
                390                 400                 410                 420                 430
AAA GCC CTC AAG GCC CTG CCA ATG CAC ATC CGG CTG TCC TTC AAC CCA ACC CAG
Lys Ala Leu Lys Ala Leu Pro MET His Ile Arg Leu Ser Phe Asn Pro Thr Gln
                                                    *
                440                 450                 460                 470                 480
CTG GAG GAG CAG TGC CAC ATC TAG AGC CCG GGA GCC TTG GCC CTC TGG CGC GTG
Leu Glu Glu Gln Cys His Ile ---

490                 500                 510                 520                 530                 540
TCA TCC CCG CCT GCC CCA CAT GGG ACC AGG AGG AAC CGG GAC CAT ATA TCC CTG

                550                 560                 570                 580                 590
TGG ACC AGG CCC TCA GGC CCT CTG CGT CTG CTT GGG CGC TCT CTT GAT AAA CTA

 600
ACT GA (A)51
```

Fig. 2. Nucleotide sequence and derived amino acid sequence of ORF from pBβL4-10. The nucleotide sequence for pBβL4-10 corresponds to aa Leu-11 to Ile-162 of mature bovine βLg. The nucleotide sequence was determined for nt 1-144 using the 160-bp *Pst*I fragment and for 550-bp *Pst*I fragment (nt 145-652) using the M13 −20 sequencing primer (New England Biolabs, Beverly, MA) and extended using synthetic oligodeoxynucleotide primers which bind to nt 353-368. Sequence analysis was performed using PC-Gene software (Intelligenetics Inc, Mountain View, CA). Asterisks mark the position of variable amino acids found in the different βLg derivatives, i.e., βLgB (Creamer et al., 1983). The underlined region corresponds to the sequence reported by Willis et al. (1982).

Case No.: IPR2023-00206
U.S. Patent No. 9,943,096

βLg sequence. Limited nucleotide sequence analysis revealed a region corresponding to that spanning from Asn-109 to Leu-122. Comparison of the translated N sequences reported here and by Willis et al. (1982) reveals complete conservation of these 14 aa. The nucleotide sequence differs at nt position 318 where we observe a cytosine as compared to a thymine. (Another difference is at nt position 327, but is apparently a typographical error in the paper of Willis et al., 1982, since the reported GAG codon does not code for Gln and thus is more likely CAG.) There are several apparent differences in our clones with respect to the position and/or frequency of restriction sites including *Ava*I, *Sau*3A, *Taq*I and *Hpa*II. It is known that several amino acid sequence variants (designated A–G; Bell et al., 1981) of βLg exist, predicting considerable nucleotide sequence divergence.

Our cDNA clone most probably codes for βLg A, as based on the presence of Asp-64 and Val-118 aa residues in the translated sequence (Braunitzer et al., 1973). Restriction polymorphisms would exist, for example, between βLgA and βLgC genes. The *Pst*I site (nt 141) present in our βLgA would be absent in the βLgC gene where the Gln-59 is replaced with a His residue, a substitution of C or T for G at nt 146. Furthermore, all of the aa substitutions in the βLg variants can result from single nucleotide substitutions. A new kind of mutation occurring in βLgDr (symbol Dr indicates a βLg variant isolated from *Bos indicus*, Droughtmaster) is the Asp-28 to Asn-28 change (Bell et al., 1981). The Asn-28 is glycosylated as the result of creating an Asn-X-Ser consensus glycosylation target, where the symbol X can be virtually any aa residue.

In conclusion, we have identified a cDNA clone coding for bovine βLg. We are presently attempting to express this gene at high levels in *E. coli* using a synthetic oligodeoxynucleotide coding for the missing 11 aa residues. Once accomplished, we intend to study the conformation and thermal stability of this recombinant βLg.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Dominic Wong and Mr. Steven Novak for their scientific contribution, and Dr. Tom Richardson for kindly sharing the cDNA bank. This work was supported by a grant from the Cornell Biotechnology Program which is sponsored by the New York State Science and Technology Foundation and a consortium of industries.

REFERENCES

Bell, K., McKenzie, A. and Shaw, D.C.: Bovine β-lactoglobulin E, F and G of Bali (Banteng) cattle, *Bos (Bibos) javanicus*. Aust. J. Biol. Sci. 34 (1981) 33–47.

Braunitzer, G., Chen, R., Schrank, B. and Stangl, A.: Die sequenzanalyse des β-lactoglobulins. Z. Physiol. Chem. 354 (1973) 867–878.

Creamer, L., Parry, D. and Malcolm, G.: Secondary structure of bovine β-lactoglobulin B. Arch. Biochem. Biophys. 227 (1983) 98–105.

Jimenez-Flores, R., Kang, Y.C. and Richardson, T.: Cloning sequence analysis of bovine β-casein cDNA. Biochem. Biophys. Res. Commun. 142 (1987) 617–621.

Laemmli, U.K.: Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 227 (1970) 680–685.

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

Mercier, J.-C., Haze, G., Gaye, P. and Hue, D.: Amino terminal sequence of the precursor of ovine β-lactoglobulin. Biochem Biophys. Res. Commun. 82 (1978) 1236–1245.

Papiz, M., Sawyer, L., Eliopoulos, E., North, A., Findlay, J., Sivaprasadarao, R., Jones, T., Newcomer, M. and Kraulis, P.: The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein. Nature 324 (1986) 383–385.

Pervaiz, S. and Brew, K.: Homology of β-lactoglobulin, serum retinol-binding protein, and protein HC. Science 228 (1985) 335–337.

Sanger, F., Nicklen, S. and Coulsen, A.R.: DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA 74 (1977) 5463–5467.

Sawyer, L., Papiz, M., North, A. and Eliopoulos, E.: Structure and fuction of bovine β-lactoglobulin. Biochem. Soc. Trans. 13 (1985) 265–266.

Shaper, N.L. Shaper, J.H., Meuth, J.L., Fox, J.L., Chang, H., Kirsch, I.R. and Hollis, G.F.: Bovine galactosyltransferase: identification of a clone by direct immunological screening of a cDNA expression library. Proc. Natl. Acad. Sci. USA 83 (1986) 1573–1577.

Yoshikawa, M., Mizukami, T., Sasaki, R. and Chiba, H.: Pre-β-lactoglobulin synthesis by mRNA from bovine mammary gland. Agric. Biol. Chem. 42 (1978) 2815–2186.

Stewart, A., Willis, I. and MacKinlay, A.: Nucleotide sequences of bovine α_{s1}-casein and κ-casein cDNAs. Nucl. Acids Res. 12, (1984) 3895–3907.

Towbin, H., Staehelin, T. and Gordon, J.: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA 76 (1979) 4350–4354.

Willis, I., Stewart, A., Caputo, A., Thompson, A. and MacKinlay, A.: Construction and identification by partial nucleotide sequence analysis of bovine casein and β-lactoglobulin cDNA clones. DNA 1 (1982) 375–386.

Communicated by R.E. Yasbin.