Theor Appl Genet (1994) 89: 121–126

V. A. Wagner · T. A. Schild · H. Geldermann

DNA variants within the 5′-flanking region of milk-protein-encoding genes. II. The β-lactoglobulin-encoding gene

Received: 27 November 1993 / Accepted: 2 Febuary 1994

Abstract For the detection of polymorphisms within the 5′-flanking region of the β-lactoglobulin (-LG) -encoding gene a nucleotide sequence containing 795 bp of the promoter and 59 bp of exon I was cloned and sequenced. After comparing the sequence from the DNA of 11 diverse cows (different breeds and milk-protein yields), 14 single-bp substitutions were identified within the 5′-flanking region and two in the 5′-untranslated region (5′-*UTR*) of exon I. Some of the variants are located in potential binding sites for *trans*-acting factors or in the 5′-*UTR*. A PCR-based RFLP analysis was performed, and the genotypes of an additional 60 cows were identified at five variable 5′-flanking sites. The results reveal three frequent combinations between the A and B alleles of the protein-coding region and the novel 5′-flanking DNA variants. This finding may explain the differences of the protein-variant-dependent β-LG synthesis (A>B) observed in vivo. A sequence comparison of the bovine and ovine promoters reveals an homology of 92.8% and shows a higher degree of conservation between positions –600 and –300.

Key words β-lactoglobulin-encoding gene
Comparative DNA sequencing
Restriction-fragment length polymorphisms (RFLP)
Regulatory DNA sequences · DNA variants · Bovine

Introduction

β-lactoglobulin (β-LG), one of the best studied proteins, is found in the whey of many mammals (ruminants, pigs, horses, dolphins, etc.) but not in rodents and humans. Whereas its physiological role remains unclear, a biological function in the intestinal uptake and transport of retinol has been suggested from its structural homologies with the retinol-binding protein (Papiz et al. 1986; Said et al. 1989).

Up to now variants in the β-LG gene have been described only for the protein-coding region (Eigel et al. 1984). Associations between β-LG variants A and B and milk composition, milk yield, and cheese production, have been extensively studied (McLean et al. 1984; Ng-Kwai-Hang et al. 1986, 1990; Lin et al. 1989; Aleandri et al. 1990; Bech and Kristiansen 1990; De Lange et al. 1990; Mao et al. 1992). Although the results are somewhat contradictionary the β-LG genotype BB seems to be associated with a higher casein content, fat yield and content, whereas the β-LG genotype AA is connected with high whey protein and milk-protein quantities. In heterozygous cows marked differences in allele-dependent β-LG synthesis have been described with A>B (Graml et al. 1989). The reason for such differences remains unclear, but genetic polymorphisms within the 5′-flanking region of the human *cytochrome P450IIE1* gene (Hayashi et al. 1991) or the human *haptoglobin 2-1* gene (Grant and Maeda 1993) are known to cause differences in transcription activity. Thus, screening of polymorphisms in the 5′-flanking region of the β-LG-encoding gene may help to detect allele combinations with effects on the gene regulation of milk proteins.

Materials and methods

Selection of animals

About 2000 cows of dairy breeds (Jersey, German Friesian, German Brown Swiss, German Simmental) were selected within each breed according to extremely high and low milk-protein yield. Milk samples of the cows were analysed for milk-protein genotypes by isoelectric focusing. Subsequently, 300 cows with maximal differing milk-protein-genotype combinations were chosen. From these, two individuals per breed with the most extreme milk-protein yield were selected for DNA-analysis. Furthermore, one cow of the Scottish Highland breed and two cows of the Galloway breed were added in order to obtain a divergent group of 11 cows.

Cloning and sequencing of the PCR-amplified 5′-flanking region

Genomic DNA of the 11 selected cows was isolated from blood samples according to Miller et al. (1988). The polymerase chain reac-

Communicated by E. J. Eisen

V. A. Wagner · T. A. Schild · H. Geldermann (✉)
Fachgebiet Tierzüchtung, Institut für Tierhaltung und Tierzüchtung (470), Universität Hohenheim, Garbenstraße 17, D-70599 Stuttgart, Germany

Table 1 DNA-variants within the 5′-flanking region of the bovine *β-LG* gene

Allele[a]	Position[b]	nt[c]	RFLP[d]
R1A/B	+ 31	A/G	*Msp*I
R2A/B	+ 27	C/T	*Sau*3AI
R3A/B	− 22	G/A	*Ava*I
R4A/B	−103	G/C	*Hae*III
R5A/B	−209	G/C	*Rsa*I
R6A/B	−221	C/T	–
R7A/B	−367	T/C	–
R8A/B	−422	G/A	–
R9A/B	−424	T/G	–
R10A/B	−435	C/G	*Fnu*4HI
R11A/B	−462	C/G	*Mae*II
R12A/B	−601	A/C	*Aci*I
R13A/B	−634	C/T	*Nla*III
R14A/B	−662	G/C	*Bfa*I
R15A/B	−691	G/A	–
R16A/B	−765	A/G	*Aci*I

[a] Number of polymorphic sites (R1, R2 ...) with allele A or B
[b] Position of polymorphic sites relative to the transcription start point
[c] Nucleotides at corresponding positions
[d] Possible restriction enzyme for RFLP analysis

tion (PCR) (Saiki et al. 1988) was used to amplify an 854-bp fragment containing 795 bp of the promoter region and 59 bp of exon I. PCR-primer sequences were deduced from the bovine cDNA (Alexander et al. 1989) and the genomic sequence of the ovine β-LG gene (Harris et al. 1988). Enhanced cloning efficiency was achieved by adding *Eco*RI and *Hin*dIII restriction sites at the 5′-end of the primers. After restriction digestion the whole fragment was cloned into phage M13mp18 (Yanish-Perron et al. 1985). Single-stranded DNA was used for sequencing by the dideoxy method (Sanger et al. 1977) with a Sequenase kit (United States Biochemicals). To avoid misinterpretation, due to amplification-mediated errors by the *Taq*-Polymerase, 3–5 independent clones were analysed for each animal (Furthermore, the vaviable sites were verified in a larger number of individuals by the RFLP technique; see below).

Identification of DNA variants

Sequence analysis was performed with the HUSAR programme package of the German Cancer Research Center (Heidelberg, Germany), and the sequences of the 11 cows were aligned to detect DNA variants.

Results and discussion

DNA variants in the 5′-flanking region

Fourteen single-point mutations, which lead in some cases to detectable RFLPs (Table 1), were found in the −795 bp flanking region and two in the 5′-UTR of exon I. The most frequent nucleotide at each variable site was named A, the other B. The variable sites in the 5′-flanking region

Fig. 1 DNA-sequence variants within the 5′-flanking region of the bovine β-LG gene derived from 11 cows of different breeds. For the basic sequence of the gene see accession No. X6313, EMBL data library. The *arrows* indicate the positions of point mutations. *Numbers* to the left of the figure refer to nucleotides relative to the transcription start point (+1). The coding region is marked in a *shaded background*, and consensus sites for *trans*-acting factors containing polymorphic sites are *underlined*. Abbreviations: *MPBF*, milk protein-binding factor; *TRE*, thyroid receptor element; *PRE*, progestrone receptor element; *AP*, activator protein; *MAF*, mammary cell-activating factor; *MGF*, mammary gland factor; *RC*, reverse complement; *R*, A/G; *S*, G/C; *Y*, C/T; *M*, A/C; *K*, T/G. Sequence alignment and search for *cis*-acting elements were performed using the programme MULTALIGN and FIND from the HUSAR programme package of the German Cancer Research Center, Heidelberg

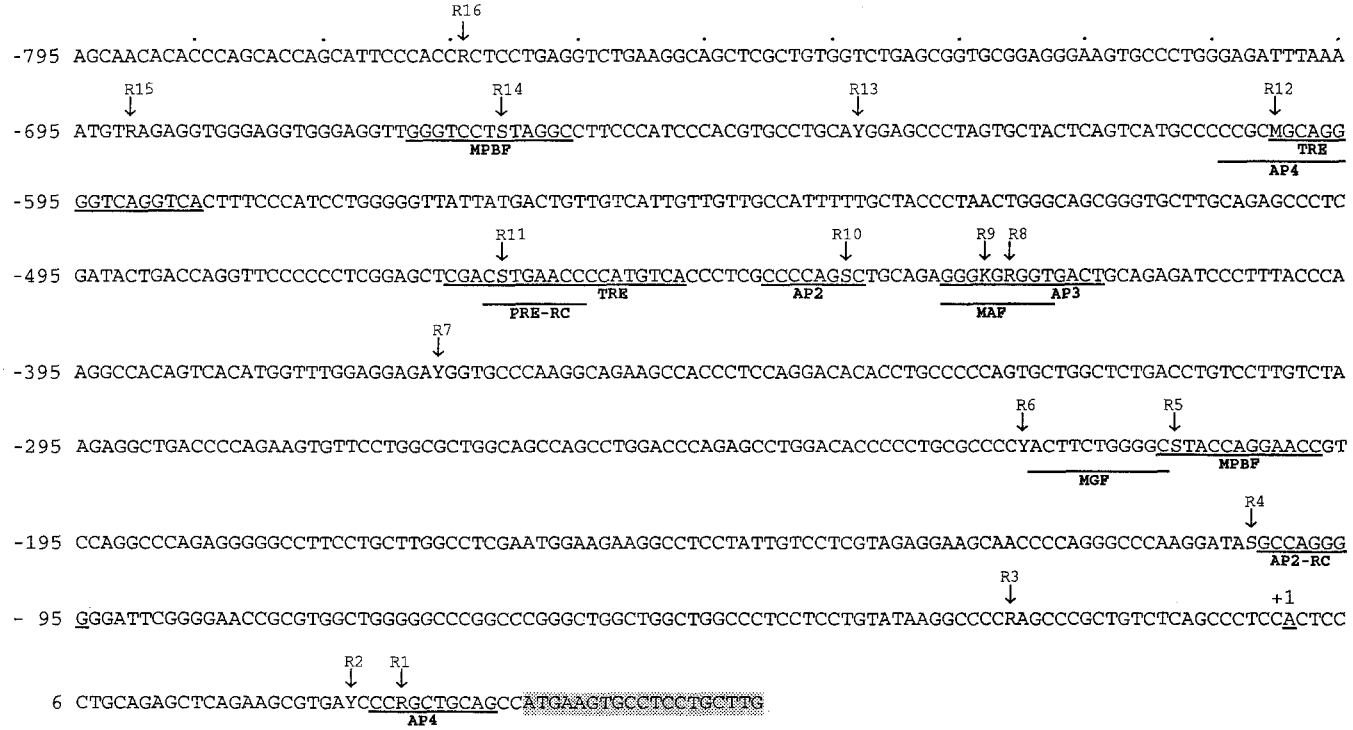


Table 2 Potential binding sites for *trans*-acting factors within the 5′-flanking region of the bovine β-LG gene

Factor	Consensus-sequence[a]	Sequence in the β-LG gene[b]	Position[c]	Homology[d] (%)
MPBF	GGTTCCNGGAACC	GGgTCCTStAggC	−669	69
		cSTaCCAGGAACC	−210	85
MAF	GA/GA/GGC/GAAGG/T	GGGKGRgGT	−427	89
MGF	ANTTCTTGGNA	ACTTCTgGGGc	−220	82
GR	GGTACAN$_3$TGTT/CCT	AGGACACACctgcCC (RC)	−340	73
		GcTctgACCTGTCCT	−316	73
		GcctCcTATTGTCCT	−150	73
PR	TGTTCACT	aGgTCACT	−591	75
		AcTGAcCA (RC)	−492	75
		cSTGAACc (RC)	−463	75
	AAAATG/TGG/AC	GCCAtTTTT (RC)	−542	89
TR	AGGTCAN$_4$AGGTCA	MGcaggGGTCAGGTCA	−601	75
		TGACCTGTCCTtgtCT (RC)	−312	81
	TGACCTGAN$_3$TCAGGTCA	cGACSTGAACCcCAtGTCA	−466	84
	GGTCAN$_3$TGACC	GGTtATTATGACt	−570	85
	TCAGGTCA	TCAGGTCA	−593	100
		TGACCTGt (RC)	−312	88
	(TGACC)$_2$	GGTCAGGTCA (RC)	−595	100
AP2	CCCCAGGC	CCCCAGSC	−441	100
		GCCaGGGG (RC)	−102	88
AP3	GGGTGTGGAAAGT	GGGKGrGGtgAcT	−427	70
AP4	T/CCAGCTGT/CGG	CCGCMGCaGG (RC)	−605	90
		CCRGCTGCaG	+ 29	90

[a] References for the consensus-binding sites of MPBF, MAF and MGF are given in the text; GR, glucocorticoid receptor (Karin et al. 1984; Beato 1989); PR, progesterone receptor (Dean et al. 1983; Bailly et al. 1986); TR, thyroid receptor (Beato 1989; Näär et al. 1991; Umesono et al. 1991; Adan et al. 1992); AP2, AP3, AP4, activator proteins (Chiu et al. 1987; Mitchell et al. 1987; Mermod et al. 1988)
[b] Putative binding sites within the 5′-flanking region. Ambiguities: S, G/C; K, T/G; R, A/G; M, A/C; RC, reverse complement. Nucleotides differing from the consensus sites are indicated in lower case letters
[c] Position of the elements relative to the transcription start point
[d] Homology relative to the consensus site

were marked R1, R2, R3 ... (R=regulatory region), beginning at the coding region of the signal peptide. In contrast to a recently described breed-specific single-bp polymorphism in the 5′-UTR of the bovine α-lactalbumin gene (Bleck and Bremel 1993), no breed-specific variant was found.

Potential binding sites for *trans*-acting factors

Milk-protein synthesis is positively regulated by insulin, prolactin, glucocorticoids and thyroid hormone (Topper and Freeman 1980; Ziska et al. 1988) and negatively by progesterone (Rosen et al. 1978). Recently, mammary-gland nuclear factors, such as MGF (mammary gland factor, Schmitt-Ney et al. 1991), MPBF (milk protein-binding factor, Watson et al. 1991), PMF (pregnancy-specific mammary nuclear factor, Lee and Oka 1992) and MAF (mammary cell-activating factor, Mink et al. 1992), have been described which bind site-specifically in the 5′-flanking regions of milk-protein genes. Point mutations in *cis*-acting elements may affect the finding of such *trans*-acting factors and hence the activity of the gene (Watson et al. 1991; Groenen et al. 1992). With the help of a computer-aided search, several point-mutations were found within such binding-motives for regulatory factors (Fig. 1; Table 2).

Detection of allele combinations within the β-LG gene

Sequence data of the 5′-flanking regions and the protein genotypes indicate that the A allele of the β-LG coding region is mostly associated with the B alleles within the promoter sequence, whereas the B allele of the coding region is often combined with the A alleles of the flanking region. As given in Table 3, RFLP analysis of the variants at positions R1, R2, R3, R5 and R14 (Fig. 2) was carried out to confirm these associations for 60 cows. Eighty percent of the individuals show the common genotype combination with the exception of the rare variant R3 which most frequently occurs as genotype AA. This observation suggests that the alleles A or B in the coding regions are connected with distinct promoter variants. These kinds of haplotype associations within the gene may explain the observed differences in β-LG protein synthesis (A>B) in heterozygous cows (Graml et al. 1989). To verify this hypothesis, ex-

Table 3 Genotype combinations within the bovine β-LG gene using data of 60 cows from different breeds

Genotypes[a]						Frequency[b]		Breed[c]			
β-LG	R1	R2	R3	R5	R14	n	%	J	BS	GF	GS
AA	BB	BB	AA	BB	BB	16	26.7	0	7	6	3
AB	AB	AB	AA	AB	AB	13	21.7	3	4	2	4
BB	AA	AA	AA	AA	AA	19	31.7	3	7	5	4
AA	AA	AA	AA	BB	BB	1		1	0	0	0
AA	AB	AB	AA	AB	AB	1		0	0	0	1
AA	BB	BB	AB	BB	AB	2		0	2	0	0
AA	BB	BB	AA	BB	AB	1		0	1	0	0
AB	BB	BB	AB	BB	AB	3	20.0	0	3	0	0
BB	AB	AB	AB	AB	AA	2		0	1	0	1
BB	AB	AB	AA	AA	AA	1		0	0	0	1
BB	BB	BB	BB	BB	AA	1		1	0	0	0

[a] Genotypes of the protein-coding region *β-LG* and the 5′-flanking region at positions R1, R2, R3, R5 and R14
[b] Number (n) and frequency (%) of the genotype combinations within the animals investigated
[c] J, Jersey; BS, Brown Swiss; GF, German Friesian; GS, German Simmental

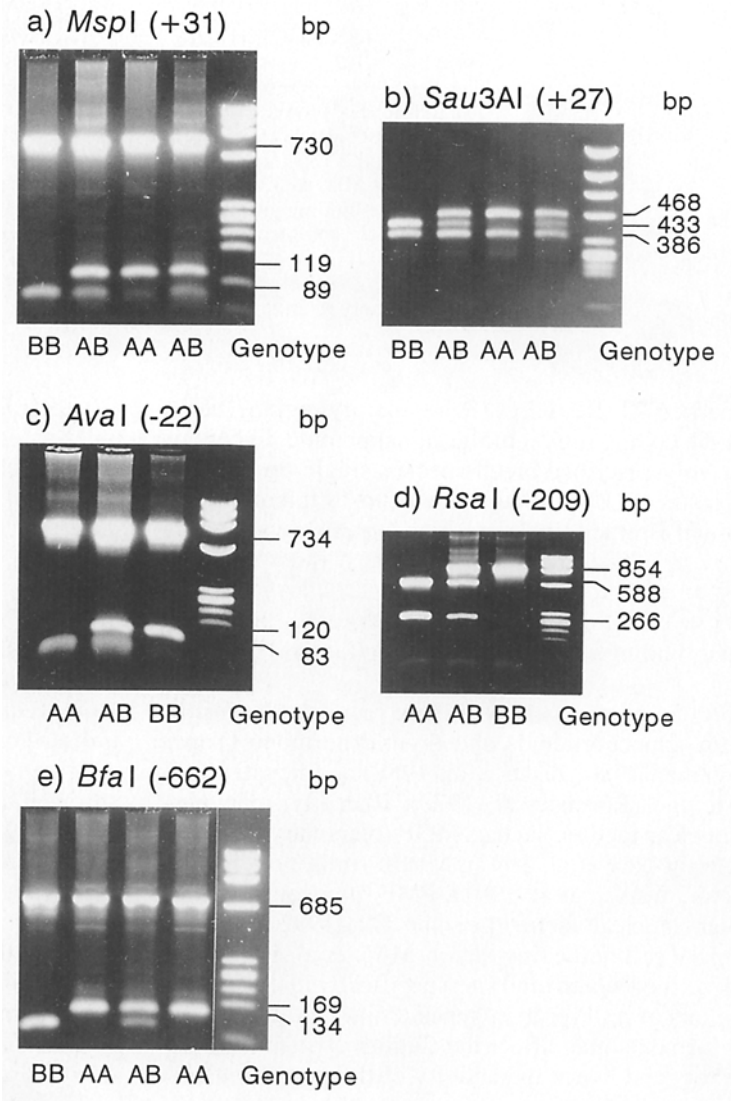


Fig. 2 a–e RFLP analysis of the bovine *β-LG* gene at 5′-flanking positions +31 (R1), +27 (R2), –22 (R3), –209 (R5) and –662 (R14). Amplification of the 854-bp fragment (–795/+59) was performed on a TRIO Thermoblock (Biometra, Göttingen, Germany) using the upstream primer 5′-AGCAACACACCCAGCACCAG-3′ and downstream primer 5′-CAAGCAGGAGGCACTTCATG-3′. PCR conditions were 94°C for 3 min, multiple cycles of 94°C for 1.30, 58°C for 1.30 min and 72°C for 3 min. Extension time in the last cycle was 5 min at 72°C. The reaction mix contained 1 µM of each primer, 200 µM of each dNTP, 0.5 µg of genomic DNA, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatine and 2.5 U of *Taq* Polymerase (Stratagene, La Jolla, Calif.). The PCR products were digested with the restriction enzymes indicated at the top of each figure. Expected fragment sizes for the *Msp*I-RFLP: allele A 730 bp, 119 bp, 5 bp and allele B 730 bp, 89 bp, 30 bp, 5 bp; *Sau*3AI-RFLP: allele A 468 bp, 386 bp and allele B 433 bp, 386 bp, 35 bp; *Ava*I-RFLP: allele A 734 bp, 83 bp, 37 bp abd allele B 734 bp, 120 bp; *Rsa*I-RFLP: allele A 588 bp, 266 bp and allele B 854 bp; *Bfa*I-RFLP: allele A 685 bp, 169 bp and allele B 685 bp, 134 bp, 35 bp. The fragments < 50 bp are not relevant for genotyping. The digestion products were seperated in 3.5% agarose gels and visualized with ethidium bromide. The size standard is ϕX174 *Hinc*II for the *Sau*3AI-RFLP and ϕX174 *Hae*III in the other cases

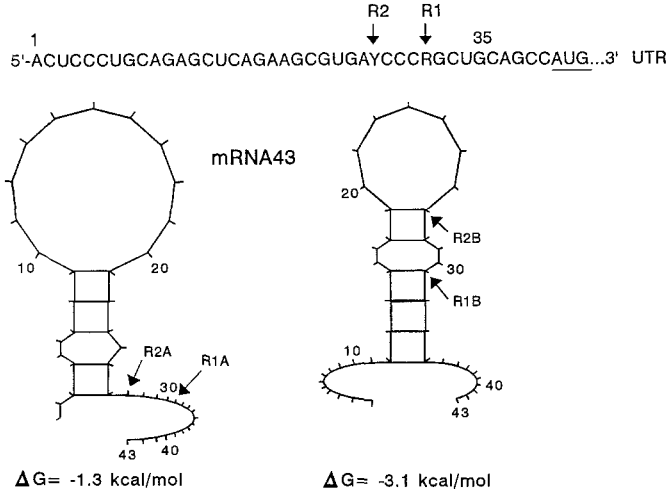


Fig. 3 Computer-calculated effects of the variants R1A/B and R2A/B on secondary structures in the 5′-untranslated region (UTR) of the bovine β-LG mRNA. The secondary structure between +1 and the AUG codon was predicted by using the programme FOLD (HUSAR programme package; German Cancer Research Center, Heidelberg). Minimal free energies (*ΔG*) and hairpins are given for the alleles A or B. *R*, A/G; *Y*, C/U; alleles: *R1A*, A; *R1B*, G; *R2A*, C; *R2B*, U; *mRNA43*, 5′-UTR with the start codon AUG

pression experiments are currently being carried out in cell cultures with different promoter-haplotypes.

Influence of DNA variants on potential secondary structures within the 5′-UTR

The occurence of hairpin structures within the 5′-UTR can modulate translation efficiency (Pelletier and Sonenberg 1985; Kozak 1986). Possible effects of R1 and R2 alleles on hairpin formation within the 5′-UTR are shown in Fig. 3. The presence of a different secondary structure caused by the different alleles may alter the stability of the processed mRNA or the binding of particular initiation factors. However, it seems unlikely that the described structures can influence translation initiation by halting the downstream migration of 40 *s* ribosomal subunits, because there is evidence that the 40 *s* subunit-factor complex has the ability to penetrate secondary structures with a free energy of –30 kcal/mol (Kozak 1989).

Comparison of the 5′-flanking region of the bovine and ovine β-LG gene

Alignment of the 5′-flanking regions (Fig. 4) display differences between the bovine and ovine sequence (Harris et al. 1988). After introducing a small number of gaps in order to maximize alignment, 65 single-bp differences were found in the -795 promoter region and two single-bp substitutions in the 5′-UTR, giving rise to nucleotide homologies of 91.8% and 95%, respectively. One striking feature is that the sequences between position –795/–600 (17 differences) and –300/–1 (31 differences) apparently have evolved more rapidly than the sequence between position –600/–300 (17 differences). Interestingly, the –600/–300 region contains nearly all of the potential binding sites for hormone receptors (see Table 2). Although Whitelaw et al. (1992) have previously shown that the proximal 406 bp of the ovine 5′-flanking region are sufficient for tissue-specific and developmental expression of the β-LG gene in transgenic mice, the higher conservation of the –600/–300 region compared with the upstream and downstream sequences might reflect a particular influence for hormonal stimulation on β-LG synthesis in the mammary glands of ruminants. The five point mutations (see Fig. 1; Table 1) found in this region may be of special significance for functional effects.

Acknowledgements This work was supported by the 'Deutsche Forschungsgemeinschaft'.

References

Adan RAH, Cox JJ, van Kats JP, Burbach JPH (1992) Thyroid hormone regulates the oxytocin gene. J Biol Chem 267:3771–3777

Aleandri R, Buttazzoni LG, Schneider JC, Caroli A, Davoli R (1990) The effects of milk protein polymorphisms on milk components and cheese-producing ability. J Dairy Sci 73:241–255

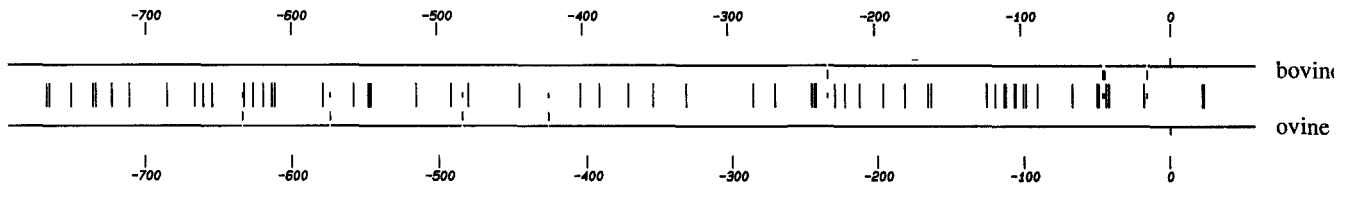
Alexander LJ, Hayes G, Pearse MJ, Beattie CW, Stewart AF, Willis IM, Mackinlay AG (1989) Complete sequence of bovine β-lactoglobulin cDNA. Nucleic Acids Res 17:6739

Bailly A, Le Page C, Rauch M, Milgrom E (1986) Sequence-specific DNA binding of the progesterone receptor to the uteroglobin gene: effects of hormone, antihormone and receptor phosphorylation. EMBO J 5:3235–3241

Beato M (1989) Gene regulation by steroid hormones. Cell 56:335–344

Bech A-M, Kristiansen KR (1990) Milk protein polymorphism in Danish dairy cattle and the influence of genetic variants on milk yield. J Dairy Res 57:53–62

Fig. 4 Sequence diffenrences between the bovine and ovine 5′-flanking region (–795/+59) of the β-LG gene. The alignment was obtained using the GAPSHOW programme (HUSAR programme package; German Cancer Research Center, Heidelberg). The positions of each base-substitution are marked with *vertical bars* and gaps with *interrupted vertical bars*



Bleck GT, Bremel RD (1993) Sequence and single-base polymorphisms of the bovine α-lactalbumin 5′-flanking region. Gene 126:213–218

Chiu R, Imagawa M, Imbra RJ, Bockoven JR, Karin M (1987) Multiple *cis*- and *trans*-acting elements mediate the transcriptional response to phorbol esters. Nature 329:648–651

Dean C, Brian J, Riser ME, O'Malley BW (1983) A 5′-flanking sequence essential for progesterone regulation of an ovalbumin fusion gene. Nature 305:551–554

De Lange DJ, Meyer EHH, van Rensburg L, Swanepoel A (1990) The polymorphic protein system in cow's milk. I. The association with the production of milk protein and fat during lactation. S Afr J Dairy Sci 22:67–71

Eigel WN, Butler JE, Ernstrom CA, Farrell jr HM, Harwalkar VR, Jenness R, Whitney R Mcl (1984) Nomenclature of proteins of cow's milk: fifth revision. J Dairy Sci 67:1599–1631

Graml R, Weiss G, Buchberger J, Pirchner F (1989) Different rates of synthesis of whey protein and casein by alleles of the β-lactoglobulin and α^{S1}-casein locus in cattle. Genet Sel Evol 21:547–554

Grant DJ, Maeda N (1993) A base substitution in the promoter associated with the human haptoglobin 2-I-modified phenotype decreases transcriptional activity and responsiveness to interleukin-6 in human hepatoma cells. Am J Hum Genet 52:974–980

Groenen MAM, Dijkhof RJM, van der Poel JJ, van Diggelen R, Verstege E (1992) Multiple octamer binding sites in the promoter region of the bovine α^{S2}-casein gene. Nucleic Acids Res 20:4311–4318

Harris S, Ali S, Anderson S, Archibald AL, Clark AJ (1988) Complete nucleotide sequence of the genomic ovine β-lactoglobulin gene. Nucleic Acids Res 16:10379–10380

Hayashi S, Watanabe J, Kawajiri K (1991) Genetic polymorphisms in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIEI gene. J Biochem 110:559–565

Karin M, Haslinger A, Holtgreve H, Richards RI, Krauter P, Westsphal HM, Beato M (1984) Characterization of DNA sequences through which cadmium and glucocorticoid hormones induce the human metallothionein-II_A gene. Nature 308:513–519

Kozak M (1986) Influences of mRNA secondary structure on initiation by eukaryotic ribosomes. Proc Natl Acad Sci USA 83: 2850–2854

Kozak M (1989) Circumstances and mechanisms of inhibition of translation by secondary structure in eukaryotic mRNAs. Mol Cell Biol 9:5134–5142

Lee CS, Oka T (1992) A pregnancy-specific mammary nuclear factor involved in the repression of the mouse β-casein gene transcription by progesterone. J Biol Chem 267:5797–5801

Lin CY, McAllister AJ, Ng-Kwai-Hang KF, Hayes JF, Batra TR, Lee AJ, Roy GL, Vesely JA, Wauthy JM, Winter KA (1989) Relationships of milk protein types to lifetime performance. J Dairy Sci 72:3085–3090

Mao IL, Buttazzoni LG, Aleandri R (1992) Effects of polymorphic milk protein genes on milk yield and composition traits in Holstein cattle. Acta Agric Scand, Section A, Anim Sci 42:1–7

McLean DM, Graham ERB, Ponzoni RW, McKenzie HA (1984) Effects of milk protein genetic variants on milk yield and composition. J Dairy Res 51:531–546

Mermod N, Williams TJ, Tjian R (1988) Enhancer binding factors AP-4 and AP-1 act in concert to activate SV40 late transcription in vitro. Nature 332:557–561

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Mink S, Härtig E, Jennewein P, Doppler W, Cato ACB (1992) A mammary cell-specific enhancer in mouse mammary tumor virus DNA is composed of multiple regulatory elements including binding sites for CTF/NF1 and a novel transcription factor, mammary cell-activating factor. Mol Cell Biol 12:4906–4918

Mitchell PJ, Wang C, Tjian R (1987) Positive and negative regulation of transcription in vitro: enhancer-binding protein AP-2 is inhibited by SV40 T Antigen. Cell 50:847–861

Näär AM, Boutin J-M, Lipkin SM, Yu VC, Holloway JM, Glass CK, Rosenfeld MG (1991) The orientation and spacing of core DNA-binding motifs dictate selective transcriptional responses to three nuclear receptors. Cell 65:1267–1279

Ng-Kwai-Hang KF, Hayes JF, Moxley JE, Monardes HG (1986) Relationships between milk protein polymorphisms and major milk constituents in Holstein-Friesian cows. J Dairy Sci 69:22–26

Ng-Kwai-Hang KF, Monardes HG, Hayes JF (1990) Association between genetic polymorphism of milk proteins and production traits during three lactations. J Dairy Sci 73:3414–3420

Papiz MZ, Saywer L, Eliopoulos EE, North ACT, Findlay JBC, Sivaprasadarao R, Jones TA, Newcomer ME, Kraulis PJ (1986) The structure of β-lactoglobulin and its similarity to plasma retinol-binding protein. Nature 324:383–385

Pelletier J, Sonenberg N (1985) Insertion mutagenesis to increase secondary structure within the 5′ noncoding region of a eukaryotic mRNA reduces translational efficiency. Cell 40:515–526

Rosen JM, O'Neal DL, McHugh JE, Comstock JP (1978) Progesterone-mediated inhibition of casein mRNA and polysomal casein synthesis in the rat mammary gland during pregnancy. Biochem 17:290–297

Said HM, Ong DE, Shingleton JL (1989) Intestinal uptake of retinol: enhancement by bovine milk β-lactoglobulin. Am J Clin Nutr 49:690–694

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA Polymerase. Science 239:487–491

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74: 5463–5467

Schmitt-Ney M, Doppler W, Ball RK, Groner B (1991) β-casein gene promoter activity is regulated by the hormone-mediated relief of transcriptional repression and a mammary gland-specific nuclear factor. Mol Cell Biol 11:3745–3755

Topper YJ, Freeman CS (1980) Multiple hormone interactions in the developmental biology of the mammary gland. Physiol Rev 60:1049–1106

Umesono K, Murakami KK, Thompson CC, Evans RM (1991) Direct repeats as selective response elements for the thyroid hormone, retinoic acid, and the vitamin D_3 receptor. Cell 65:1255–1266

Watson CJ, Gordon KE, Robertson M, Clark AJ (1991) Interaction of DNA-binding proteins with a milk protein gene promoter in vitro: identification of a mammary gland-specific factor. Nucleic Acids Res 19:6603–6610

Whitelaw CBA, Harris S, McClenaghan M, Simons JP, Clark AJ (1992) Position-independent expression of the ovine β-lactoglobulin gene in transgenic mice. Biochem J 286:31–39

Yanish-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of M13mp18 and pUC19 vectors. Gene 33:103–119

Ziska SE, Bhattacharjee M, Herber RL, Qasba PK, Vonderhaar BK (1988) Thyroid hormone regulation of α-lactalbumin: differential glycosylation and messenger ribonucleic acid synthesis in mouse mammary glands. Endocrinology 123:2242–2248