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Biological Significance of Milk Protein Polymorphism

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Abstract

Milk protein polymorphism has been studied in depth since Aschaffenburg's discovery of genetically distinct forms of β -lactoglobulin in 1955. The significance of these various protein polymorphisms, as related to the synthesis and secretion of milk, is yet to be elaborated fully. However, certain areas in milk protein polymorphism warrant particular attention. These include: assignment of mRNA triplet codons to known amino acid substitutions; biosynthesis and primary structure of polymorphs; observations of micelle formation in vivo through electron microscopy; mode of inheritance, breed specificity, and linkage of these genes; and, finally, biological function of the milk protein variants.

Introduction

Synthesis and secretion of milk involve formation of lipids, membranes, proteins, and lactose. While this paper deals with genetic polymorphism of milk proteins, we found that the literature is nearing saturation on this subject. Therefore, we shall limit our discussion to those salient features which we believe warrant particular elaboration. Biosynthetic mechanisms responsible for formation of components of milk are currently being investigated in a number of laboratories. Many excellent data have been accumulated regarding lactose synthesis and lipid secretion; however, mechanisms of formation of the casein micelle in vivo have not been clearly defined. Consequently, our goals in this manuscript will be to discuss the significance of observed protein

polymorphisms as related to some aspects of protein synthesis, assemblage of the casein micelles, and milk production in general.

Assignment of triplet codons to milk protein variants and relation to genetic code. The central dogma of molecular biology, as enunciated by Watson and Crick, maintains that DNA serves as the repository of genetic information. While recent advances in the physiology of RNA tumor viruses have challenged the absolute role of DNA in all organisms, most organisms use the three base code of DNA for information storage (8).

The genetic code as elucidated in *Escherichia coli* (28) can be readily applied to the milk protein genetic polymorphs. Table 1 lists the variants where mRNA codons are reasonably certain. We shall also discuss the exception, that is, where more than a single point substitution is suggested. Some questions remain in the triplet codons assigned to the β -caseins because of the relatively large number of alleles and some uncertainties in the exact amino acid analyses. However, Groves and Gordon (17) have done much to clarify this situation. For α_{s1} -casein, all of the amino acid differences can be explained by a single base change, with the exception of α_{s1} -A which probably arose through the sequential deletion of eight amino acid residues (40) or 24 base pairs in the DNA. α_{s1} -D offers an interesting example of a variant, electrophoretically distinguishable from B, which does not differ in net charge (9).

β -Lactoglobulins A and B and κ -caseins A and B offer two interesting examples of not

one but two amino acid substitutions. With both of these proteins (Table 1) each substitution can be rationalized by an independent point mutation to which specific assignments of mRNA codons can be applied. While the existence of mutational intermediates can be postulated, they have never been found in the β -Lg family. The mutational intermediates in both β -Lg and κ -Cn would not be electrophoretically distinguishable from one of the recognized variants and could only be demonstrated by amino acid analysis. Thus, we reiterate our earlier statement that to assume variants are identical from identical electrophoretic mobilities is naive. With a few exceptions, as noted above, all the known phenotypes of α -lactalbumin (α -La^A and α -La^B), β -lactoglobulin (β -Lg^A, β -Lg^B, β -Lg^C, β -Lg^D), α_{s1} -casein (α_{s1} -Cn^A, α_{s1} -Cn^B, α_{s1} -Cn^C, α_{s1} -Cn^D), β -casein (β -Cn^{A1}, β -Cn^{A2}, β -Cn^{A3}, β -Cn^B, β -Cn^C, β -Cn^D), κ -casein (κ -Cn^A, κ -Cn^B) and the γ -caseins (γ -Cn^A, γ -Cn^B) are detectable by starch-gel or polyacrylamide-gel electrophoresis methods (39). No doubt phenotypes exist which cannot be detected by these conventional methods; that is, variants may (and do) exist which do not differ in charged amino acids.

Protein synthesis and the primary structure of milk protein polymorphs. As in all other cells, lactating mammary gland cells synthesize milk proteins on ribosomes of the endoplasmic reticulum. Heald and Saacke (18), with labeled amino acids and radioautography, have followed the course of protein synthesis in vivo, and Beitz et al. (6), with cell free systems,

TABLE 1. Assignment of mRNA triplet codons to some of the more common milk protein variants.

Protein	Amino acid substitution	Possible codons ^a
α -Lactalbumin		
B → A	Arg → Gln	CG ^{A/G} → CA ^{A/G}
β -Lactoglobulin		
B → C	Gln → His	CA ^{A/G} → CA ^{U/C}
B → D	Glu → Gln	GA ^{A/G} → CA ^{A/G}
B → A		
1.	1. Gly → Asp	GG ^{U/C} → GA ^{U/C}
2.	2. Ala → Val	GC ^{U/C/A/G} → CU ^{U/C/A/G}
α_{s1} -Casein		
B → C	Glu → Gly	GA ^{A/G} → GG ^{A/G}
B → D	Pro → Ser	CC ^{U/C/A/G} → UC ^{U/C/A/G}
B → A	Lys → Asn	AA ^{A/G} → AA ^{U/C}
κ -Casein		
B → A		
1.	1. Ala → Asp	GC ^{U/C} → GA ^{U/C}
2.	2. Ileu → Thr	AU ^{U/C/A} → AC ^{U/C/A}

^a Possible mRNA codons obtained by Nirenberg et al. (28) from *Escherichia coli* studies.

have demonstrated milk protein synthesis in vitro.

The physical and chemical properties of proteins are highly dependent upon the primary structure of the given protein because the linear array of amino acids put together on the ribosomes dictates the ultimate conformation of the protein. Therefore, a knowledge of the sequence of amino acids within the polypeptide chain(s) is of fundamental importance to understanding the mechanism and formation of micellar casein and to the function of such biologically active proteins as α -lactalbumin. The only milk protein whose complete amino acid sequence is known is α -lactalbumin, both the A and B variants (7). A partial amino acid sequence of β -lactoglobulin has been reported by Frank and Braunitzer (13).

Detailed amino acid sequences of the caseins are lacking although their end groups are known (Table 2). Several important features of these molecules can be described from data available. The first casein whose primary structure received any serious attention was β -casein. Peterson et al. (32) isolated the phosphopeptide from a trypsin digest of a sample of β -casein from pooled milk. (β -Casein variants were unknown at that time.) They observed that this peptide with 4 to 5 phosphorus atoms contained both N- and C-terminal arginine. Based on the specificity of trypsin, it can be deduced that the phosphorus-rich region of the molecule must be located in the N-terminal portion of the molecule. The empirical formula of this peptide follows: $\text{NH}_2 - \text{Arg Asp}_1, \text{Glu}_7, (\text{NH}_2)_8, \text{Gly}_1, \text{Val}_3, \text{Leu}_3, \text{Ileu}_2, \text{Ser}_4, \text{Thr}_1, (\text{PO}_4)_5, \text{Pro}_1, \text{Arg-OH}$.

Subsequently, genetic polymorphism of the β -casein variants has been detailed; however, only the His/Gln substitution of β -A²/ β -A³ has been localized (33). Kalan et al. (24) reported the C-terminal sequences of all known β -casein variants to be Ileu-Ileu-Val-OH. In addition, they discovered that the γ -caseins have identical end groups to the β -caseins, as well as similar peptide maps. Most recently,

Ribadeau-Dumas et al. (34) have reported additional tryptic peptides from β -A².

κ -Casein has received considerable attention as to its primary structure. The native κ -casein molecule contains C-terminal valine and an unknown N-terminal residue. Upon rennin action, two fragments of the protein appear; one fragment, soluble in water, is termed macropeptide whereas the other, para- κ -casein, is insoluble in water. The site of primary rennin action is located at a Phe-Met bond; therefore, upon rennin action two new end groups emerge—N-terminal methionine in the macropeptide, C-terminal phenylalanine in the para- κ -portion. Because of its insolubility, detailed sequences on the para- κ -casein-portion are unavailable; hence, we will limit our discussion of this protein to the macropeptide portion of the molecule (mole wt \sim 7,500), which constitutes the C-terminal third of the molecule. Significant advances in determination of its primary structure have been reported by Jollès et al. (22) and Hill and Wake (19). The latter authors have emphasized that κ -casein exhibits a high degree of charge polarization. The soluble macropeptide is highly hydrophilic whereas the insoluble para- κ is highly hydrophobic; thus, κ -casein is a soap-like molecule, and this may account for its micelle stabilizing capabilities.

α_{s1} -Caseins and α_s -like caseins have been studied as well. Caseins α_{s3} - and α_{s4} - can easily be distinguished from the dominant α_{s1} -caseins by amino acid composition (21) and end group analyses (1) since they both possess a C-terminal sequence of Leu-Tyr-OH.

Several details of the primary structure of the α_{s1} -caseins have been elucidated. The first worthy of comment is that α_{s1} -caseins like β - and γ - contain N-terminal arginine and a non-polar C-terminal sequence. These features are indicative of all casein molecules with the exception of κ -casein. The second point is that α_{s1} -casein A has a deleted sequence of 8 amino acids; the partial sequence of the deletion (compared with α_{s1} -casein B) is Leu-Leu-Arg-Phe-Phe. Location of the specific position of the Glu/Gly substitution in the α_{s1} - B and C variants has been accomplished in elegant research by Mercier et al. (26). The primary structure of the C-terminal peptide, determined by isolation of the peptide from a chymotryptic digest followed by sequencing is:

Glu
Asn-Ser- -Lys-Thr-Thr-Pro-Met-Leu-Trp-OH
Gly

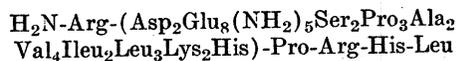
We have verified positioning of this peptide by first reacting native α_{s1} -casein B with car-

TABLE 2. End groups of the major caseins.

Casein	c-Terminal	N-Terminal	References
α_{s1} -	Met-Leu-Trp-OH	Arg	(26)
β -	Ileu-Ileu-Val-OH	Arg	(24)
κ -	Ala-Val-OH	?	(19,22)
γ -	Ileu-Ileu-Val-OH	Arg	(24)

boxypeptidase A to remove c-terminal tryptophan and penultimate leucine, followed by chromatography to remove residual carboxypeptidase and subsequent chymotryptic digestion. The resulting two-dimensional fingerprint showed a) the loss of tryptophan from the α_{s1} -B/C difference peptide and b) the repositioning of the difference peptide.

Recent studies (unpublished) in our laboratory lead us to the conclusion that the N-terminal sequence of the α_{s1} -caseins (deduced from analyses of a peptide from a chymotryptic digest of α_{s1} -B) is as follows:



This large peptide, while highly charged, carries a net charge of -1 and may be positioned adjacent to the phosphopeptide of Österberg (30), if the latter is located in the N-terminal third of the molecule. Evidence accumulated by Farrell and Thompson (11), from analyses of the N-bromosuccinimide cleavage of the internally located, second tryptophan confirms the suggestion that the phosphorus-rich region and the above charged peptide comprise the N-terminal region of the α_{s1} -molecule. Also from these data we have concluded that the c-terminal end of the α_{s1} -molecule is highly hydrophobic. These experiments have been substantiated by Mercier et al. (26), who sequenced the c-terminal region of the molecule, and by Grosclaude et al. (15) who reported the composition of the tryptic peptides of α_{s1} -casein.

From partial sequences (10, 15, 19, 26, 31) and from chemical and physical data on the caseins, one can now talk about charge distribution in the α_{s1} -, β - and κ - monomers. However, we are still hard pressed to describe how individual components are assembled into the unique package called the casein micelle. It is hoped that coupling of these data with information gained by in vivo observations will lead to a logical mechanism for micelle formation.

Observations of micelle formation in the lactating mammary gland. As previously noted, synthesis of the polypeptide chains of milk proteins occurs on ribosomes of the endoplasmic reticulum (ER). Following synthesis the proteins migrate to the vicinity of the Golgi apparatus by way of the ER lumen. It is in the Golgi region (or in Golgi vacuoles) that a variety of important events are believed to occur. Among these are the synthesis of lactose, addition of carbohydrate to κ -casein, and presumably the phosphorylation of the specific

casein components. While the lactose synthetase system undoubtedly occurs in the Golgi region (27), we cannot be certain at this time whether carbohydrate addition to κ -casein or the phosphorylation of caseins is occurring in the Golgi apparatus or in its vacuoles. However, Turkington and Topper (46) demonstrated a pool of unphosphorylated casein in the lactating cells; this presumably represents casein in transit from the ER to the Golgi region. Certain important features regarding casein micelle formation and structure appear conclusive. The first point is that Golgi vacuoles contain micelles in all degrees of development. Some vacuoles appear empty whereas others show strands (or threads) of caseins before micelle development (Fig. 1). As the Golgi vacuoles migrate from the region of their formation (the basal region of the cell) toward the apical region of the cell, the protein strands (threads) appear to "ravel up" into a porous micelle structure (Fig. 2). This "ravelling up" is likened to formation of the core of a golf ball. As the vacuoles approach the alveolar lumen, micelle formation nears completion, and the micelles become more electron dense. Figure 3 shows a Golgi vacuole at the juncture of the plasma membrane as it prepares to empty its contents into the alveolar lumen. Evidently the membrane of the vacuole replenishes the plasma membrane after contents of the vacuole enter the lumen. This appearance of long strands which aggregate to form the casein micelle seems to preclude micelle models based upon monomer nucleation about a core. We would like to emphasize at this point that all cells of the mammary gland are not performing the same function. That is, some cells appear to be exclusively involved in lipid biosynthesis, others in protein biosynthesis, while still others are performing both functions. Our studies confirm the contention of Saacke (35) that specific cells perform specific functions at various times.

Of interest to those readers concerned with lipid biosynthesis and secretion of fat droplets from the cell, we have inserted Figure 4. A fat globule (rat mammary gland) clearly can be seen passing through the plasma membrane by inverse pinocytosis. The edge of this fat globule shows an apparent double membrane, the outer layer of which is identical to the plasma membrane.

Mode of inheritance, breed specificity, and linkage of genes. The inheritance of all polymorphs of whey proteins and caseins can best be described as straightforward Mendelian transmission (2). Clearly, from the elegant

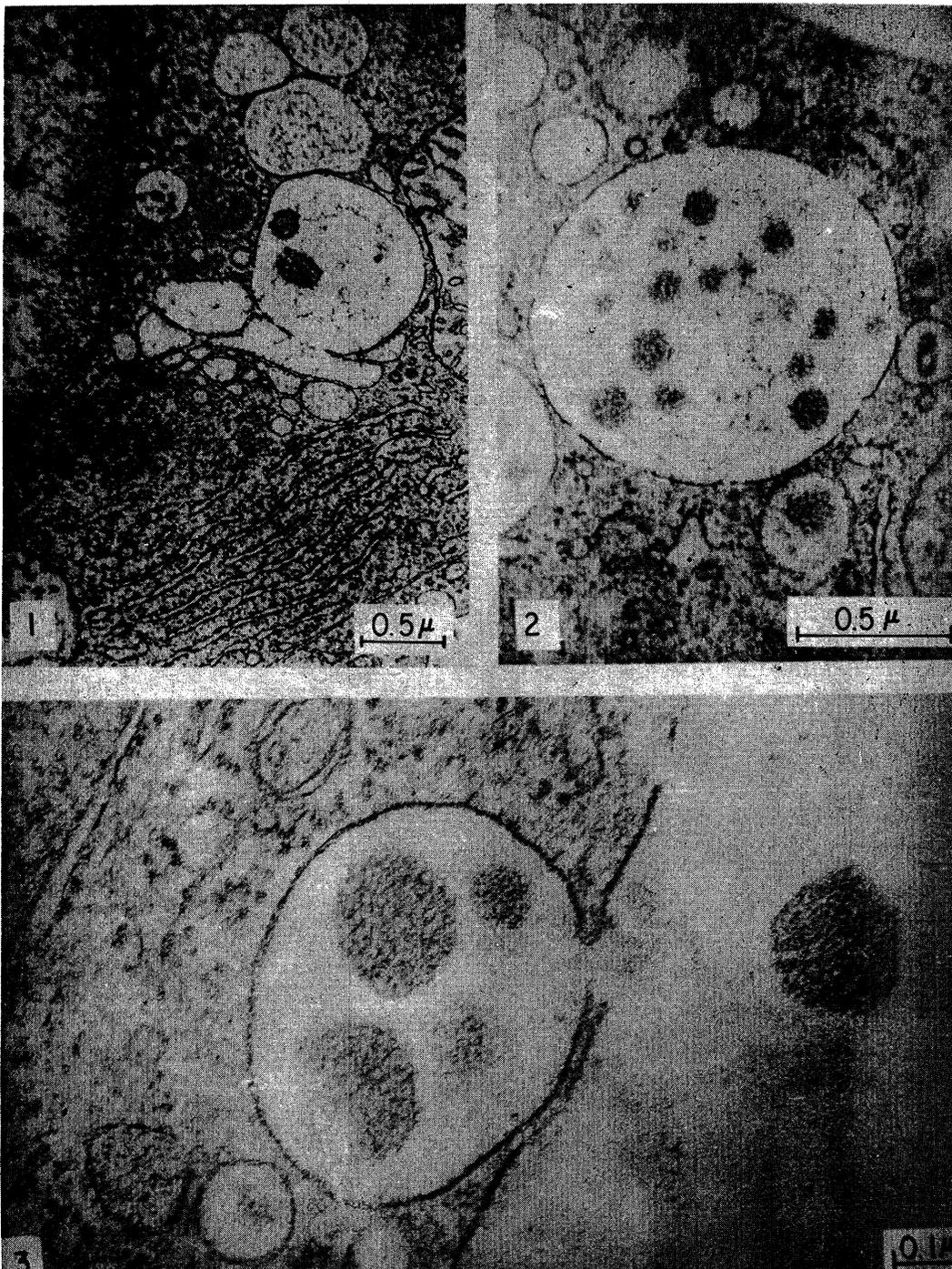


FIG. 1. Golgi complex from lactating rat mammary gland. The endoplasmic reticulum (ER) is clearly visible at the lower right. The caseins are synthesized on the ER, then migrate to the Golgi region, and are incorporated into the growing vacuoles. Initially, these vacuoles appear to be empty, while at later stages in development, thread-like structures appear. The tissue was fixed in buffered OsO₄, Epon embedded, and stained with uranyl acetate and lead citrate.

FIG. 2. Maturation of the casein micelles within the Golgi vacuoles. This vacuole and adjacent vacuoles (not shown) contain several micelles, apparently in various stages of development. Also apparent are the thread-like structures of the earlier stages.

FIG. 3. Golgi vacuole about to discharge its contents into the alveolar lumen. The Golgi vacuole shown appears to impinge upon the plasma membrane. A casein micelle is already present in the lumen; the micelles in the vacuole are now quite electron dense, and resemble those found in the lumen.

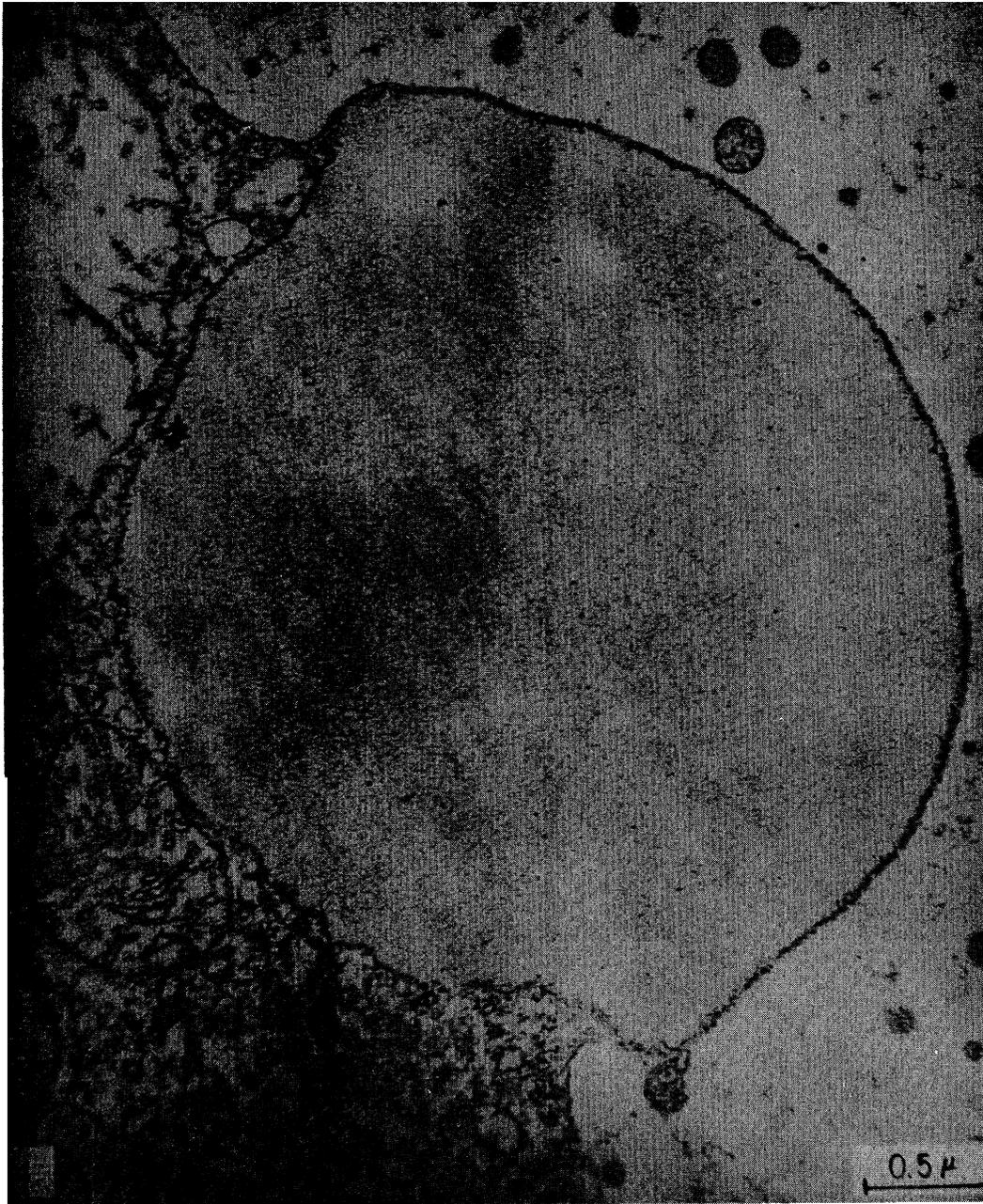


FIG. 4. Fat globule passing through the plasma membrane. The edge of the globule shows an apparent double membrane, which may be formed from the plasma membrane.

summation of available genetic data on numerous breeds of cattle by Aschaffenburg (3), breed specificity and gene frequency of particular phenotypes do occur. Nevertheless, large numbers of phenotypes do not exist unless one considers the β -Cn locus where six have been demonstrated.

Rare variants of note in *Bos indicus* are the α -La^A and β -Cn^D alleles; in *Bos taurus* the rarest variants are β -Cn^C (Guernsey and Brown Swiss) and α_{s1} -Cn^A. The α_{s1} -Cn^A allele was first reported in American Holstein cattle (42). Subsequently, Farrell et al. (12) confirmed the presence of the rare A allele in

Danish RDM cattle.

To summarize gene flow of the α_{s1} -Cn locus from East to West, Aschaffenburg (3) has pointed out that α_{s1} -Cn^C, which predominates in cattle of the subcontinent of India, gives way to the α_{s1} -Cn^B allele as the route of migration of cattle is traced through East Africa and finally into Western Europe. The reason for this, while not altogether clear, implies a selective advantage of the α_{s1} -Cn^B allele over the α_{s1} -Cn^C allele.

TABLE 3. Patterns of inheritance (contingency tables) of α_{s1} - and β -caseins (25) in western breeds. Absence of certain recombinant types can be seen, implying a close linkage of the α_{s1} - and β -casein loci.

		Jersey			
		β -Casein			
		A	AB	B	
α_{s1} -Casein	B	60	73	56	
	BC	45	82	
	C	35	
		Guernsey			
		β -Casein			
		A	AB	AC	C
α_{s1} -Casein	B	85	5	11	1
	BC	52	2	5	..
	C	9
		Brown Swiss			
		β -Casein			
		A	AB	B	AC
α_{s1} -Casein	B	146	69	4	2
	BC	22	5
	C	2

TABLE 4. Inhibition of the action of spleen phosphoprotein phosphatase on *p*-nitrophenyl phosphate by the genetic variants of β -lactoglobulin.

Protein added	Velocity ^{a,b}	Inhibition (%)
Buffer only	41.8 ± 0.8
β -Lactoglobulin A	25.4 ± 1.2	40
β -Lactoglobulin B	32.8 ± 1.2	22
β -Lactoglobulin C	36.6 ± 1.1	12

^a Velocity expressed as n moles of *p*-nitrophenyl liberated per milliliter per minute.

^b Average of four determinations ± S.

The matter of linkage of the α_{s1} -Cn and β -Cn loci first came to our attention in research reports published almost simultaneously by King et al. (25) Table 3, and Grosclaude et al. (16). The concept of close linkage implies that loci for α_{s1} -Cn and β -Cn are situated on the same chromosome; the distance between these two loci has been fixed at < 3.8 units of recombination (16). Phenotypic evidence for close linkage is the absence of certain recombinant types such as α_{s1} -Cn^C/ β -Cn^B which may be regarded as "forbidden types." While this recombinant type is indeed rare in western breeds, Aschaffenburg et al. (5) have demonstrated that α_{s1} -Cn^C seems to readily associate with the rare β -Cn^D allele. In western breeds the recombinant type from which all others appear to have arisen is α_{s1} -Cn^B/ β -Cn^A whereas in eastern breeds the recombinant (and dominant) type is α_{s1} -Cn^C/ β -Cn^A.

With regard to the close correlation of the α_{s1} -Cn, β -Cn loci with the κ -Cn loci, Grosclaude et al. (16) have estimated that the distance between these two separate pairs of loci on the chromosome is < 2.8 units of recombination.

Hines et al. (20) stated from a careful study of linkage of inherited characteristics that "... at any rate it seems evident that the DNA segments coding the amino acid sequences of the polypeptide chains of α_{s1} -, β -, and κ -caseins are very close to each other on the same chromosome either within the same locus (16) or in very closely linked loci. The order on the chromosome of these coding sequences, as indicated by this investigation, is α_{s1} -Cn, β -Cn and κ -Cn."

Biological function of milk protein variants. Preliminary analysis of the data (10, 31) on the primary structure of the α_{s1} - and β -caseins shows that the large number of proline residues of the proteins are evenly distributed throughout the molecules. This accounts for the lack of recognizable secondary structure (i.e. α -helix, β -structure) reported by Swaisgood and Timasheff (37) for α_{s1} - and Noelken and Reibstein (29) for β -casein. Hence, the α_{s1} - and β -caseins have an open structure and are readily attacked by the proteolytic enzymes, pepsin, trypsin, and chymotrypsin; the biological function of efficient nutrition is served. For the nutritional function of caseins, it is not unusual that the majority of the observed casein polymorphs have no apparent deleterious effects on the micellar system. However, α_{s1} -A represents the sequential deletion of ~ 4% of the α_{s1} -molecule, and altered properties of milks containing this variant might be expected.

Indeed α_{s1} -A milks are difficult to process (41) and yield poor cheeses (36). Occurrence of this allele, which is due to the sequential deletion of 24 base pairs in the DNA, in two unrelated breeds of cattle such as Danish (RDM) and American Holstein, indicates that this gene is of more ancient ancestry than originally postulated (12). This gene, which occurs in very low frequency, may have been selected against either by breeders or by nature because of a lack of digestibility of milks containing α_{s1} -A.

Whey proteins, α -lactalbumin and β -lactoglobulin, on the other hand, are highly organized proteins, and one might expect genetic polymorphism to play an important role. α -Lactalbumin occurs in two genetic forms which Gordon et al. (14) have shown differ by a single amino acid substitution (α -La^A/ α -La^B:Gln/Arg). The A gene occurs in Zebu and Australian Droughtmaster cattle while only the B gene occurs in Western cattle. Tanahashi et al. (38) have shown that α -lactalbumin functions as the B or "specifier protein" of the lactose synthetase system, and the net charge difference, generated by this mutation, does not alter the ability of either variant to participate in the lactose synthetase reaction.

Three major genetic variants of β -lactoglobulin have been reported in American and European cattle (β -lactoglobulins A, B and C). The more frequently occurring alleles A and B differ by two single amino acid substitutions per 18,000 mole wt (β -Lg^A/ β -Lg^B:Asp/Gly and Val/Ala) (23). β -Lg^C more closely resembles the B variant and differs from the latter by virtue of a single amino acid replacement (β -Lg^B/ β -Lg^C:Gln/His) (23). While these genetic changes in the β -Lg molecule appear as innocuous as those in α -lactalbumin, their effect on the physical properties of the molecule are profound. Most notably β -Lg^A undergoes a pH dependent tetramerization while the amino acid differences in B diminish the extent of aggregation and those of C do not permit the reaction to occur (43, 44, 45). These altered physical properties permit one to speculate that perhaps the molecules may behave differently in vivo. In this connection, Aschaffenburg and Drewry (4) indicated that a possible relationship might exist between the total amount of casein produced and the β -Lg genotype (A > B); however, no further correlations were made.

Gross influences on pooled milks by an altered biological activity of β -Lg would be difficult to discern because most breeds have a rather even distribution of β -Lg A and Lg B

with a high degree of heterozygosity (3). Nevertheless, the discovery of such a genetically determined biological role could lead to selective breeding for the optimally functioning genotype.

In our laboratory β -lactoglobulin produces a 35 to 40% inhibition of the hydrolysis of p-nitrophenyl phosphate (p-NPP) and o-carboxyphenyl phosphate by bovine spleen phosphoprotein phosphatase. Base denaturation of the β -lactoglobulin molecule markedly decreases the degree of inhibition. Other whey proteins (α -lactalbumin, γ -globulin, and serum albumin) do not inhibit the reaction. Kinetically, the inhibition of the phosphatase is defined as competitive, with a k_i equal to 1.98×10^{-5} M. The three genetic variants of bovine β -Lg (β -Lg A, B and C) were tested for their ability to inhibit the action of phosphoprotein phosphatase on p-NPP. The results in Table 4 indicate that β -Lg A > B > C in their inhibitory effect. The differences are related to the structural changes induced by genetic substitutions. The physical aggregation of β -Lg A does not occur to any great extent under the conditions of the enzymatic assay of phosphoprotein phosphatase. However, the carboxyl-rich region responsible for the tetramerization reaction (43) may play a role in the inhibition of p-NPP hydrolysis. Since the physical properties of β -Lg C are altered by the histidine/glutamine substitution (23), this genetic substitution may account as well for the drastically reduced ability of β -Lg C to inhibit the hydrolysis of p-NPP by the spleen enzyme.

β -Lactoglobulin does not inhibit the dephosphorylation of α_{s1} -casein, the major phosphoprotein of cow's milk. Thus, the inhibition appears to be limited to low molecular weight substrates and possibly is due to substrate binding by the β -lactoglobulin. Affinity for low molecular weight phosphates leads to the speculation that β -lactoglobulin may play a regulatory role in mammary gland phosphorus metabolism. β -Lactoglobulin homologues from goat and swine milk also inhibit hydrolysis of p-nitrophenyl phosphate by phosphoprotein phosphatase, thus, providing a type of enzymatic assay for studies in the comparative biochemistry of β -lactoglobulin.

Outlook for the future. The long range goal of research in animal genetics is increased productivity through selective breeding. This goal, in relation to biochemical genetics, has been realized only to the extent that undesirable forms can be recognized and culled from the gene pool. Actual positive correlations between

genetic polymorphism and productivity have not been realized. On the other hand, the subtleties of biochemical control mechanisms in higher animals are only beginning to be understood. Investigators may be able to find a clue to the ultimate potential of an animal by looking more closely at the amount of enzymes found in its milk. Furthermore, should β -lactoglobulin prove to be a regulator of mammary gland metabolism, and if the relative biological efficiency of the molecule can be correlated with either the A or the B variant, then a distinct possibility exists for genetic manipulation of the locus. Hence, the challenge still exists to be able to apply what we have learned to the practical goals of animal production. However, this can be done by gaining deeper insights into the metabolic pathways involved in the biosynthesis of milk, by studying the mechanisms which regulate lactation, and by the tedious application of this knowledge to the problem of selective breeding.

Addendum:

Since this manuscript was prepared, a more detailed sequence of α_{s1} -casein has been published.

Merciér, J. C., F. Grosclaude, and B. Ribadeau-Dumas. (1970). The primary structure of bovine α_{s1} -casein (in French). *European J. Biochem.*, 16: 453.

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