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Viewpoint

International milk genomics consortium

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The first international symposium on Milk Genomics & Human Health brought scientists from around the world and across the milk research spectrum to the task of annotating the subsets of mammalian genomes responsible for milk and the nutritional functions of its constituents as food for mammalian offspring. These scientists are a part of The Milk Genomics Consortium, an international consortium for milk genomic research. The general goals of the Milk Genomics Consortium are to link the scientific community through milk and genomics to understand the biological and health-conferring values of milk, to create tools for interactive web-based data exchange, and to coordinate pre-competitive and post-competitive research on the components, functions and health benefits of milk and its components.

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Introduction

Milk is the only biomaterial that evolved to nourish growing mammals. Survival of mammalian offspring consuming milk as their sole food exerted a strong Darwinian selective pressure on the biochemical and genetic evolution of the lactation process, leading to the appearance of components that promote health, strength and survival (Goldman, Chheda, & Garofalo, 1998). This evolutionary pressure led to the elaboration of a whole food that contains proteins, peptides, complex lipids and oligosaccharides in higher order structures coming together as a complex, multi-component—yet highly organized—food. Research to date has recognized that these compounds, structures and configurations act as growth factors, toxin-binding factors, antimicrobial peptides, prebiotics and immune regulatory factors within the mammalian intestine (Walzem, Dillard, & German, 2002). Importantly, these trophic macromolecules deliver nutritional functions that, although not essential, provide biological advantages within the intestine and throughout the body that contribute to neonatal mammalian survival (Newburg, 2001). However, because not all of the compounds and their functions are essential for all infants under all circumstances, their activities have proven exceedingly difficult to recognize, much less study, and it is generally agreed that only a very small subset of the total biological value of milk's components is known (German, Dillard, & Ward, 2002). The scientific challenge, therefore, is to find new ways to identify the molecular actions and nutritional benefits that were the basis by which mammalian genes having a relation to milk and their products evolved and persisted.

Genomics is defined as the molecular characterization of all the genes in a species. Genomics studies have expanded into research focused on understanding the functions and effects of genes—functional genomics. Functional genomics entails research on the protein function (proteomics) or, even more broadly, the whole metabolism (metabolomics) of an organism (International Council for Science, 2006). Current approaches of genomics research are building and applying the new techniques of systems biology by integrating structural and comparative genomics, molecular

structure and biological networks to understand genomes, evolution and gene functionality (Hood, 2003). These same tools provide the means to apply innovative strategies to accelerate our understanding of the functionality of the subsets of genes that are the products of evolutionary pressure on lactation—those expressed in milk (German *et al*., 2002; Ward & German, 2004). Genomic tools utilize microarrays for gene expression profiling and proteomics utilizes mass spectrometry and chromatography (Takahashi, Kaji, Yanagida, Hayano, & Isobe, 2003; Tyers & Mann, 2003). The main analytical tools used for metabolic fingerprinting—also termed metabolic profiling (Fiehn *et al*., 2000)—in complex systems are nuclear magnetic resonance spectroscopy and mass spectrometry. Metabolomics measures small molecules generated in the process of metabolism that represent the sum of all the metabolic pathways in an organism. Metabolomics is concerned with the identification of each pathway and its role in an organism's function (German, Watkins, & Fay, 2005). These tools are all aided by bioinformatics—the science of managing and analyzing biological data using advanced computing techniques and computational biology to establish the statistical and functional differences in protein patterns among samples.

Rapid growth in the field of genomics is on pace to develop an unprecedented biological information base and tool set. The International Milk Genomics Consortium recognizes the opportunities to use these same approaches to dramatically increase the understanding of the biological properties of milk, and transform this understanding into actionable intelligence as a guide for the improvement of human health. This consortium of investigators from around the world is addressing major questions with the intent of being instrumental to increasing the knowledge on milk and its health benefits: "What is the current state of research on milk—what is currently known about its nutritional, bioactive and therapeutic benefits? What are the important areas of future research to pursue that will increase our understanding of the key molecular and nutritional principles that underlie these properties, and how can discovery be accelerated by studies based on the genomics of milk?"

The genetic basis of milk has been pursued for decades to gain an understanding of the basic processes of lactation in mammals, mammary tissue physiology and the basis of milk composition and yield. The goals of the International Milk Genomics Consortium are to leverage this existing knowledge with the knowledge emerging from genomics to understand the evolutionary selective advantage that was the basis for the production of molecules in the mammary gland—or secretion into milk—and the nutritional and functional benefits that they provide. The general aims of the Milk Genomics Consortium are to link the entire scientific community in order that researchers achieve a better understanding of the biological and health-conferring values of milk, to create tools for interactive web-based data exchange, and to coordinate pre-competitive and post-competitive research on the components, functions and health benefits of milk. Clearly, these objectives cannot be achieved by a single group of researchers, and their fulfillment necessitate recruiting the international scientific community to pursue avenues of research that will lead to an understanding of the biological values of milk's many components. Thus, research by participants in the International Milk Genomics Consortium will focus on a web-accessible database and will use a common set of bioinformatics tools to query this resource in search of biological functions, actions and benefits (Ward & German, 2004).

The state of the science on the molecules in milk and their functions, and new approaches to studies of the genetics of milk and lactation are reviewed and summarized, and the efficiencies of scale that can be gained through the International Milk Genomics Consortium are explored.

The biology of lactation—what can Mother Nature tell us about milk genomics?

The biology of lactation has been an active area of research for decades. This research has produced substantial knowledge of mammary epithelial cell biology through the various stages of lactation, the physiology of the mammary gland, the physiology and supporting role of non-epithelial tissues, the energetics of lactation, the regulation of genes and proteins expressed during lactation, and the biochemical processes that occur within lactating mammary epithelial cells and the mammary gland (Fig. 1; Schanbacher, personal communication).

The part of the genome related to the components in milk represents the genetic basis for the evolution and function of milk; the evolution and regulation of milk synthesis and secretion by the mammary gland; the evolution and regulation of the mammary gland itself; the co-evolution of infant development; and reproductive strategies. To understand a mammal's genome related to lactation strategies it is necessary to understand what milk is—a secretion made by the differentiated mammary gland. This essential food of all mammalian life is principally composed of water, fat, protein (largely casein and whey), and the carbohydrate lactose. Milk composition, mammary function and the genes associated with lactation show significant variation across species. The great variation in the composition of milk and in the functions of milk across species reflects the need to adapt milk composition to the environmental niche, reproductive strategy, and nutrient and growth requirements of the neonate.

The tools of genomics and systems biology will be used to integrate data obtained on the various cellular processes of lactation with the nutritional functions of the molecules and structures produced through the process of lactation. The evolution of the mammary gland shown in Fig. 1 illustrates the context in which the mammary gland led to the successful survival of offspring (Schanbacher, Talhouk, & Murray, 1997).

Successive cycles through lactation are the rule for mammalian mothers. Such repeated cycling puts

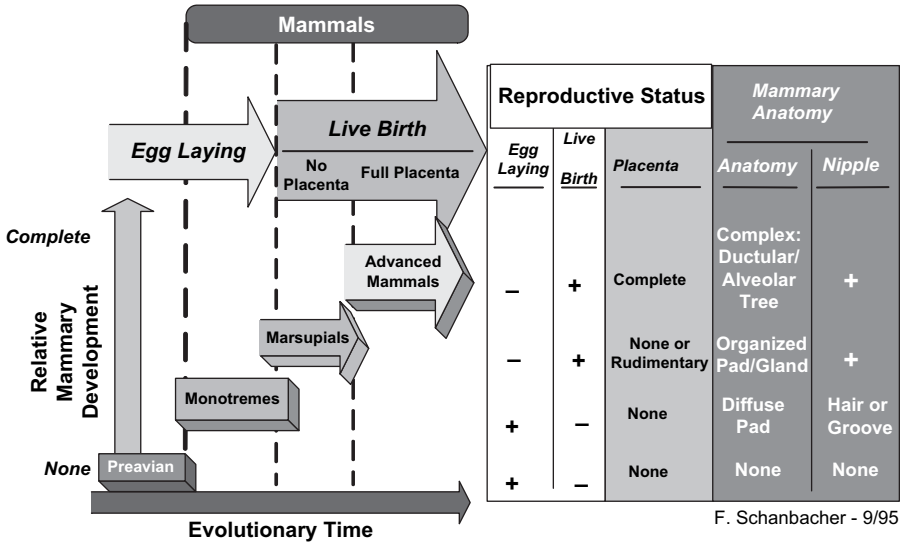

Fig. 1. Origins of the genomics of milk: evolutionary relationship of mammary development and reproductive strategy (F. Schanbacher).

astonishing demands on the plasticity of all aspects of mammary tissue—from structures to functions. The processes of lactation have been subdivided into discrete stages, and the functionality during each has been described—from the immature gland, developing gland (Holland & Holland, 2005), colostrum formation (Fetherston, Lee, & Hartmann, 2001), secretory activation (lactogenesis) (McManaman & Neville, 2003; McManaman, Palmer, Anderson, Schwertfeger, & Neville, 2004; Neville, McFadden, & Forsyth, 2002), involution and senescence (Stefanon, Colitti, Gabai, Knight, & Wilde, 2002) (Fig. 2) Each of these functional stages is distinguishable structurally, and various strategies have been initiated to catalog the genetic regulation necessary to accomplish the sequential transitions. The overall process of lactation requires periods in which neither actual secretion by the gland nor consumption by the neonate takes place and instead physiological, structural and metabolic remodeling of the tissue is taking place to enable it to acquire lactation capabilities and subsequently to return to the non-lactating state after weaning. Considerable regulation of mammary epithelial gene expression leads to cell differentiation and tissue remodeling, leading initially to milk secretion and, subsequently, to milk production, involution and senescence.

Functions of milk

The evolutionary origins of milk proteins and mammary regulation define the key functions of milk and the mammary gland. The evolution of the mammary gland likely involved adaptive recruitment of existing precursor genes through alteration of regulatory sequences to allow expression in primitive mammary glands, and duplication and mutation of structural sequences to acquire new functions from pre-existing primitive proteins. The earliest mammary function after provision of nutrition was possibly the passing of protective advantages on to offspring by immunoglobulins, thus aiding selection for survival. This paved the way for the elaboration of myriad protective functions that we are only now beginning to appreciate.

The functions of milk generally can be considered supportive of both mammalian mothers and infants through several mechanisms. Milk provides nourishment to infant offspring; disease defense for the infant; disease defense for the mother; regulation or stimulation of infant development, growth or function; regulation or stimulation of maternal mammary tissue development, growth or function; inoculation, colonization, nourishment, regulation and elimination of infant microflora; and inoculation, colonization, nourishment, regulation and elimination of maternal mammary microflora.

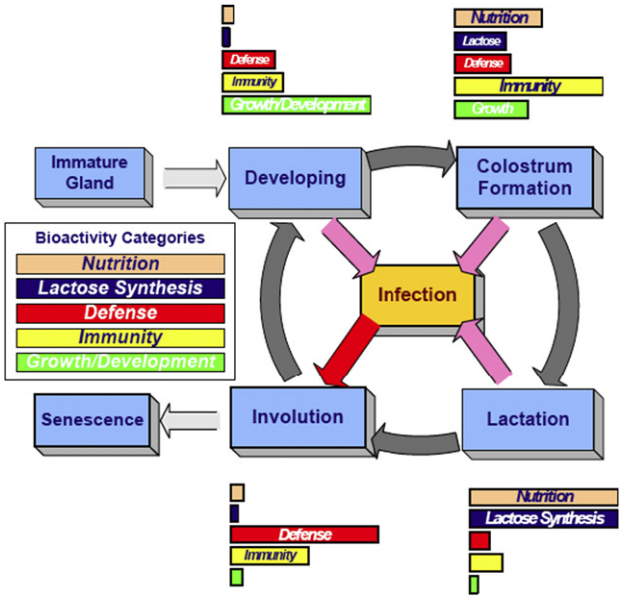

Fig. 2. Development and lactation cycle of the mammary gland: change in secretion functions vs. mammary state (F. Schanbacher).

Infant nourishment

Nourishing the mammalian neonate is the most obvious role of milk, and the success of mammals attests to the values of milk as an initial food source for the young of these species. The demands on milk as a sole source of nutrition are remarkable. All of the essential macronutrients, water, vitamins, minerals, amino acids and fatty acids, plus the basic structural and energetic intermediates needed to sustain life, must be delivered to the neonate in a highly absorbable form that is appropriate to the species and the stage of development—all at minimal energy cost to the mother. Lactation research has illuminated many of the biological processes needed to mobilize the essential biomolecules from maternal stores and to convert them into dispersed, transportable and bioavailable structures in milk.

Milk also provides myriad benefits to the growth, development and health-supporting processes of young and mother beyond the essential nutrients. The non-essential components of milk are not understood as well as those of essential nutrients, but research is now beginning to focus on their roles in the well-being of neonates (German *et al*., 2002). The research strategies needed to discover these properties are different from those used to discover the properties and roles of essential nutrients. Essential nutrients can be studied with relative ease because their elimination from the diet of animals leads to overt signs of deficiency in each individual. Non-essential nutrients and their functions, however, are only valuable within a particular context, thus investigations of benefits of non-essential nutrients must first recognize the context in which they are valuable.

Bioactive molecules in milk

Milk is not only an excellent source of nutrients—high quality protein, water-soluble and fat-soluble vitamins, calcium, phosphorus, magnesium, etc.—but also a source of proteins that have biological activities that have been demonstrated in vitro, in animal models and in infant and adult humans (Lönnerdal, 2003, 2004). Human infants fed formula exhibit different growth patterns (Dewey, 1998), different nutritional status (Castillo, Atalah, Riumallo, & Castro, 1996), more infections of longer duration (Oddy, 2001) and different gut microflora from infants fed breast milk (Rubaltelli, Biadaiol, Pecile, & Nicoletti, 1998). The physiological activities provided by milk proteins in the gastrointestinal tract include enhancement of nutrient absorption, inhibition of enzymes, enzyme activity, growth stimulation, modulation of the immune system and defense against pathogens (German *et al*., 2002).

Total protein makes up approximately 3.2 and 0.9% of bovine and human milk, respectively. The classes of milk proteins are the whey proteins, caseins and mucins. Caseins (α_{s1}, α_{s2}, β, κ) make up 80% of bovine milk protein and 20–40% of human milk protein. In cows, caseins are produced in highest amounts during peak lactation, lactoferrin is highest during involution, immunoglobulins highest during colostrum production, growth promoters are highest during development phase and proteases are at their lowest at lactation.

Defense against infections is provided by many milk components, including immunological factors (antibodies, cells, cytokines), proteins (lactoferrin, enzymes, e.g., lysozyme, oligosaccharides and glycoproteins), gut microflora (prebiotics) and nutrients to optimize the infant's immune system (for reviews see Baldi *et al*., 2005; Meisel, 2005; Rutherfurd-Markwick & Moughan, 2005; Severin & Wenshui, 2005). Antimicrobial activity is provided by iron-dependent bacteriostatic and iron-independent bactericidal factors; antiviral factors inhibit virus binding to cells and inhibit virus infection; immunoregulatory factors modulate lymphocyte and monocyte responses; opioid agonists inhibit gut motility and reduce digesta passage rate; opioid antagonists block opioid effects on gut; antihypertensive factors inhibit angiotensin-converting enzyme, reduce vasoconstriction and increase blood flow; antithrombotic agents inhibit platelet activation and reduce clot formation; cell regulation factors modulate cell proliferation and induce apoptosis; and pro-microbial (prebiotic) factors stimulate growth of beneficial bacteria. Antimicrobial proteins, such as lactoferrin, lysozyme and haptocorrin, a vitamin B_{12}-binding protein in human milk (Adkins & Lönnerdal, 2003), may exert a host defense function against pathogens in the gastrointestinal tracts of breastfed infants.

Many bioactivities of milk are latent, i.e., they are inactive while "encrypted" within the intact milk protein (Meisel & FitzGerald, 2000) and become active once released from the parent milk protein by proteolytic activity. An important aim of the Milk Genomic Consortium is to identify these bioactive molecules and their health benefits through functional genomics studies of genes involved in lactation. Among the proteins and bioactive peptides within milk are β-casein, a phosphorylated protein (β-casomorphin and antibacterial peptides); α_{S1}-casein (α-casomorphin, an ACE inhibitor); κ-casein, about 40% glycosylated (glycomacropeptide, an antithrombotic peptide formed during proteolysis); β-lactoglobulin (β-lactorphin); α-lactalbumin (α-lactorphin); lactoferrin (lactoferroxins—bactericidal peptides, including an immunoregulatory peptide and an antibacterial peptide).

The tools of gene expression

Two systems of genetic databases, one containing expression data and one containing annotation data, are quickly becoming essential knowledge repositories of the research community (Anderle *et al*., 2003). The availability of gene expression arrays has led to studies designed to identify the genes expressed during lactation in mammary epithelial cells. These represent the closest link currently available to sample the spectrum of genes responsible for milk. Microarray studies search for regulatory genes for metabolic pathways, such as those of fatty acid synthesis (McManaman *et al*., 2004), and for hormonal influences on mammary differentiation and milk secretion (Neville *et al*., 2002). The

microarray data are pared to the significant genes, clustered to find similar expression patterns, classified to ask what the genes are, classification is mapped on the clusters and pathway analysis is used to find biological meaning.

Microarray data are validated by real-time polymerase chain reaction (PCR) analysis of any short sequence of DNA (or RNA) (Wong & Medrano, 2005). Nuclear magnetic resonance spectroscopy, mass spectroscopy and chromatographic systems are the high-throughput analytical platforms becoming the essential tools of metabolomics (global analysis of small molecules generated in the process of metabolism) and proteomics (study of identification and function of proteins coded by an organism's genetic material). These technologies are capable of simultaneously producing highly quantitative data on important metabolites, including lipids, simple sugars, amino acids, organic acids, steroids, peptides, etc. Milk Genomics Consortium participants will utilize the power of 'omics technologies to gain an understanding of the metabolic processes involved in mammary physiology and milk secretion (McManaman & Neville, 2003) in the process of lactation.

Genomic knowledge management tools

The Milk Genomics Consortium aims to develop a new approach to nutritional research that uses knowledge of the genes involved in lactation, the functions of milk-related genes, and the biology and physiology of milk consumption. Such an approach will also focus on the discovery of the evolutionary basis for delivering health through food. In short, the genes that code the composition of milk have evolved under evolutionary pressure to nourish for optimal growth, development, maturation, metabolism and protection of mammalian offspring. Hence, there is information embedded in the sequence of the genome that describes the principle biological definition of mammalian nutrition.

Bioinformatics is enhancing the capabilities of virtually all aspects of scientific research, from analytical technologies to database mining. The downside of the new genomics and bioinformatics technologies is that they are providing such unprecedented amounts of information about genomes and phenomes (proteomes, metabolomes, etc.) that they are outpacing our ability to transform that information into actionable knowledge. Access to tools that allow researchers to mine this information is similarly daunting, with steep learning curves both for choosing available tools and learning how to use them. Precisely because of these constraints, knowledge management is a key focus area for the Milk Genomics Consortium (Fig. 3).

Bioinformatics will be used to not simply build a library of the genes within the mammalian genome but to develop an interpretive tool for annotation of functionality and health. From online database polling to annotation, curation and contextualization of biological information, the development and assembly of a set of software tools is necessary to access various databases, such as those that contain the massive amounts of data being produced in studies of the human genome, proteomes, etc. (Kell & King, 2000). These tools are being used to build a database of genes related to expression in mammary epithelia cells, and regulatory sequences of genes expressed during lactation. Obtaining subsets of mammalian genomes responsible for the production of milk will facilitate research into nutritional bioactivity of milk components and will also enable investigations into what was the evolutionary origin of specific genes and how such genes are expressed in milk.

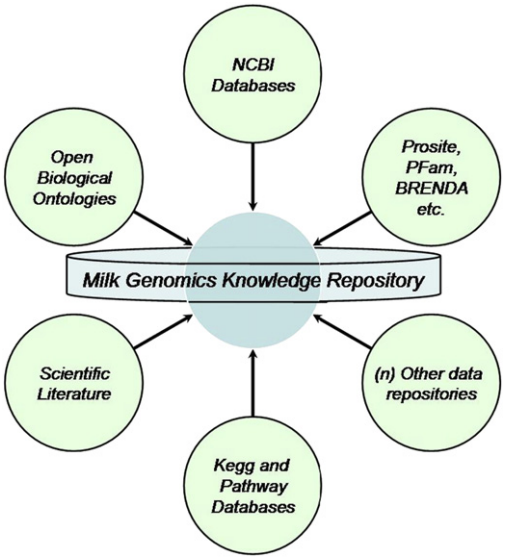

Fig. 3. Simplified architecture of the milk genomics knowledge repository.

Knowledge management tools are being developed at the University of California, Davis that will provide access to the milk genomics databases, allow researchers to annotate specific information residing within and across these databases, integrate the same information with the researchers' own intrinsic knowledge of mammary biology, and then coordinate ongoing research using a world-wide accessible website environment. These bioinformatic tools will be used to poll various databases—including that of the human genome—to eventually build databases of genes expressed during lactation in the mammary gland, thus it will be possible to obtain the subset of the human genome that is responsible for milk. The software will allow researchers to explore the structure and regulation of genes that are either induced or repressed by lactation within mammary epithelial cells, and to begin the process of assigning these genes to their metabolic activities. The software infrastructure being assembled can be used to manage data sets produced by all who wish to share genomics capabilities for studying milk.

Great strides have already been achieved in building a scientific understanding of the process of lactation. However, with the sequencing of several mammalian genomes, the ability to acquire massive amounts of biological data, to store that data on computers and to query it using bioinformatic tools, that understanding can now be leveraged to achieve a deeper understanding of the lactation process

and the evolutionary knowledge contained therein. The Milk Genomics Consortium is bringing this idea to practice by developing a website through which genomic, proteomic, transcriptomic and metabolomic data related to lactation and milk can be accessed. The depth of this resource, combined with the utility to access it from points around the globe, will bring together milk researchers towards a common goal—understanding the nutritional and functional benefits of the lactation process. The 'omic data sets are massive and disparate, and few individual research groups have the means to produce them. Yet, making these databases electronically accessible to interested researchers and educators, together with the tools to query them, will dramatically increase the value and power of the investigation of the genomics of milk. Educators will be enabled to introduce students, as the next generation of scientists, to genomics in an easily understandable and relevant way. Specific benefits from consuming human milk and dairy products are also likely to be identified and validated through this initiative.

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References

Adkins, Y., & Lönnerdal, B. (2003). Potential host-defense role of a human milk vitamin B-12-binding protein, haptocorrin, in the gastrointestinal tract of breastfed infants, as assessed with porcine haptocorrin in vitro. *American Journal of Clinical Nutrition, 77*, 1234–1240.

Anderle, P., Duval, M., Draghici, S., Kuklin, A., Littlejohn, T. G., Medrano, J. F., *et al.* (2003). Gene expression databases and data mining. *Biotechniques, Suppl.*, 36–44.

Baldi, A., Ioannis, P., Chiara, P., Eleonora, F., Roubini, C., & Vittorio, D. (2005). Biological effects of milk proteins and their peptides with emphasis on those related to the gastrointestinal ecosystem. *Journal of Dairy Research, 72*(Spec No.), 66–72.

Castillo, C., Atalah, E., Riumallo, J., & Castro, R. (1996). Breast-feeding and the nutritional status of nursing children in Chile. *Bulletin of the Pan American Health Organization, 30*, 125–133.

Dewey, K. G. (1998). Growth characteristics of breast-fed compared to formula-fed infants. *Biology of the Neonate, 74*, 94–105.

Fetherston, C. M., Lee, C. S., & Hartmann, P. E. (2001). Mammary gland defense: the role of colostrum, milk and involution secretion. *Advances in Nutrition Research, 10*, 67–98.

Fiehn, O., Kopka, J., Dormann, P., Altmann, T., Trethewey, R. N., & Willmitzer, L. (2000). Metabolite profiling for plant functional genomics. *Nature Biotechnology, 18*, 1157–1161.

German, J. B., Dillard, C. J., & Ward, R. E. (2002). Bioactive components in milk. *Current Opinion in Clinical Nutrition and Metabolic Care, 5*, 653–658.

German, J. B., Watkins, S. M., & Fay, L.-B. (2005). Metabolomics in practice: emerging knowledge to guide future dietetic advice toward individualized health. *Journal of the American Dietetics Association, 105*, 1425–1432.

Goldman, A. S., Chheda, S., & Garofalo, R. (1998). Evolution of immunologic functions of the mammary gland and the postnatal development of immunity. *Pediatric Research, 43*, 155–162.

Holland, M. S., & Holland, R. E. (2005). The cellular perspective on mammary gland development: stem/progenitor cells and beyond. *Journal of Dairy Science, 88*(Suppl. 1), E1–E8.

Hood, L. (2003). Systems biology: integrating technology, biology, and computation. *Mechanisms of Ageing Development, 124*, 9–16.

International Council for Science (2006). New genetics, food & agriculture: scientific discoveries—societal dilemmas. http://www.doylefoundation.org/icsu/glossary.htm Accessed 13.03.06.

Kell, D. B., & King, R. D. (2000). On the optimization of classes for the assignment of unidentified reading frames in functional genomics programmes: the need for machine learning. *Trends in Biotechnology, 18*, 93–98.

Lönnerdal, B. (2003). Nutritional and physiologic significance of human milk proteins. *American Journal of Clinical Nutrition, 77*, 1537S–1543S.

Lönnerdal, B. (2004). Human milk proteins—key components for the biological activity of human milk. *Advances in Experimental Biology and Medicine, 554*, 423–426.

McManaman, J. L., & Neville, M. C. (2003). Mammary physiology and milk secretion. *Advanced Drug Delivery Reviews, 55*, 629–641.

McManaman, J. L., Palmer, C. A., Anderson, S., Schwertfeger, K., & Neville, M. C. (2004). Regulation of milk lipid formation and secretion in the mouse mammary gland. *Advances in Experimental Medicine and Biology, 554*, 263–279.

Meisel, H. (2005). Biochemical properties of peptides encrypted in bovine milk proteins. *Current Medicinal Chemistry, 12*, 1905–1919.

Meisel, H., & FitzGerald, R. J. (2000). Opioid peptides encrypted in intact milk protein sequences. *British Journal of Nutrition, 84*(Suppl. 1), S27–S31.

Neville, M. C., McFadden, T. B., & Forsyth, I. J. (2002). Hormonal regulation of mammary differentiation and milk secretion. *Journal of Mammary Gland Biology and Neoplasia, 7*, 49–66.

Newburg, D. S. (Ed.). (2001). *Advances in experimental medicine and biology: bioactive components of human milk.* New York: Kluwer Academic/Plenum Publishers.

Oddy, W. H. (2001). Breastfeeding protects against illness and infection in infants and children: a review of the evidence. *Breastfeeding Review, 9*, 11–18.

Rubaltelli, F. F., Biadaiol, R., Pecile, P., & Nicoletti, P. (1998). Intestinal flora in breast- and bottle-fed infants. *Journal of Perinatal Medicine, 26*, 186–191.

Rutherfurd-Markwick, K. J., & Moughan, P. J. (2005). Bioactive peptides derived from food. *Journal of AOAC International, 88*, 955–966.

Schanbacher, F. L., Talhouk, R. S., & Murray, F. A. (1997). Biology and origin of bioactive peptides in milk. *Livestock Production Science, 50*, 105–123.

Severin, S., & Wenshui, X. (2005). Milk biologically active components as nutraceuticals: review. *Critical Reviews in Food Science and Nutrition, 45*, 645–656.

Stefanon, B., Colitti, M., Gabai, G., Knight, C. H., & Wilde, C. J. (2002). Mammary apoptosis and lactation persistency in dairy animals. *Journal of Dairy Research, 69*, 37–52.

Takahashi, N., Kaji, H., Yanagida, M., Hayano, T., & Isobe, T. (2003). Proteomics: advanced technology for the analysis of cellular function. *Journal of Nutrition, 133*(6 Suppl. 1), S2090–S2096.

Tyers, M., & Mann, M. (2003). From genomics to proteomics. *Nature, 422*, 193–197.

Walzem, R. L., Dillard, C. J., & German, J. B. (2002). Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking. *Critical Reviews in Food Science and Nutrition, 42*, 353–375.

Ward, R. E., & German, J. B. (2004). Understanding milk's bioactive components: a goal for the genomics toolbox. *Journal of Nutrition, 134*, 962S–967S.

Wong, M. I., & Medrano, J. F. (2005). A primer on real time PCR for mRNA quantitation. *Biotechniques, 39*, 75–85.