Mammalian Milk Genomics: Knowledge to Guide Diet and Health in the 21st Century

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The life sciences are struggling with the challenges of diet and health. Agriculture wrestles to become both more productive and more sustainable in the face of the world's population growing past 6 billion. At the same time, in the most affluent parts of the world, diet-related diseases are considered to be the major threat to public health. The problems are complex; how can agriculture become simultaneously more productive and sustainable while foods become simultaneously safer, more nourishing and more delightful to our quality of life? The Foods for Health Institute was established at UC Davis to promote research and development across the campus. The goal of the institute is to develop multi-disciplinary and multi-collaborative approaches to address these challenges and deliver on the bold promise to improve health and prevent disease with food-based solutions. Promising to prevent disease (defined as not to cure disease or reverse the damage accumulated by disease, but to pre-emptively prevent disease from occurring) is indeed provocative. How can science address such an attractive yet seemingly insurmountable goal? One critical question for science and for food is at the core of the problem: what do we target to improve health in ways that ultimately reduce the risks of all diseases?

Preventing disease is a far-reaching goal. If healthcare approaches are truly preventative, then they act on individuals before diseases develop—any disease. If a food or food ingredient is developed to reduce the risk of one disease, but in so doing also increases the risk of any other disease, consumers aren't healthier. The current strategy for treating diseases is built on a mature scientific foundation. Laboratory bench breakthroughs identify the causes of disease which become targets for diagnostic development to identify those afflicted with the disease. The same targets become the objects of high-throughput screening programs to identify candidate chemicals that could act to reverse disease, which in turn leads to their evaluation, testing and validation as small-molecule drugs. These pharmaceuticals are subsequently rolled out into a regulated, world-wide pharmaceutical industry and prescribed by trained clinical professionals as curative solutions to disease. Yet, if the goal is to prevent disease, then we cannot rely on diseases to define the scientific

targets on which to act. What are the targets for prevention? What mechanism do we act upon? What ingredients, when regularly ingested, are capable of acting on these targets and achieving this beneficial goal of overall prevention?

Evolutionary Basis for Discovering Metabolic Targets for Improving Health

Determining the targets for improving health is the fundamental problem of prevention. What are the targets, mechanisms of action and ingredients that we can discover that would make healthy people healthier? The good news is, an arsenal of "omic" technologies is at our disposal to approach this problem. The global scientific enterprise of genomics has sequenced various life forms. Science has now built this knowledge as a magnificent public resource; the entire genomic sequences of hundreds of viruses and bacteria, dozens of plants and animals and, of course, of *Homo sapiens* have been elucidated (Karolchik *et al.*, 2003; Pruitt *et al.*, 2006; Liolios *et al.*, 2008). What can genomics tell us about diet and health? An animal's genome is its blueprint of evolution and the basis of each organism's multiple solutions to its Darwinian pressures (Gould, 2002). Understanding Darwinian pressures can reveal the basis for genetic outcomes. Plants exposed to a dry environment develop molecular strategies for water conservation. Animals exposed to predators develop strategies for camouflage, protection or escape. To understand how animals have developed mechanisms of diet and prevention, we need a genomic model in which the Darwinian pressure was for nourishment and prevention. This basic scientific logic has been used previously in fields from pharmaceuticals to building construction. For example, the genomics of plants are mined to identify candidate drugs (Oksman-Caldentey and Inzé, 2004). Pharmaceutical companies have purchased the genetic rights to specific rainforests, reasoning that the jungle is full of candidate drugs (Mendelsohn and Balick, 1995). Analyzing from the context of Darwinian pressure, it is clear that plants evolved in part to the threat of predation. Since they cannot run away, their defence has been to become chemical factories of selective toxicity (Xie and Lou, 2009). The secondary metabolism of plants is an ingenious result of this pressure. Yet, our goal is not treatments for disease, but food-based solutions to achieve health.

What could possibly have emerged from millennia of evolution under the constant Darwinian pressures to be nourishing, protective, and to act on targets that improve the overall health of healthy animals? Milk. Lactation is the remarkable biological invention of mammals as the sole source of nourishment for post-natal infants.

Discovering the Darwinian Pressure of Milk as a Model for Foods for Health

Through evolutionary experimentation, mammals have spent the last 120 million years developing "the most efficient, effective and adaptable means of postnatal nutrient provision that has ever arisen among vertebrates—lactation" (Blackburn, 1993). Milk and lactation are an appropriate model to guide scientific discovery for foods for health. Lactation is appropriate for a wider range of activities than simply the chemicals in milk. The mammary epithelial cell is, in turn, itself a marvel of engineering: a bioreactor that

synthesizes bioactive components and guides the self-assembly of supra-molecular structures (German *et al.*, 2002). The constant Darwinian pressure on lactation has been to deliver a complete, absorbable ensemble of chemicals and structures that, when ingested, acts upon metabolic and physiologic targets that promote the competitive success of healthy mammals (Ward and German, 2004). This Darwinian pressure has led to the elaboration of complex food in milk that not only delivers nutrients as complex supra-molecular structures (*i.e.* milk-fat globules, casein), but includes immune-modulatory, toxin-binding and growth factors and antimicrobial peptides and proteins that act within the intestine and beyond to promote health, growth and protection of the neonate. Furthermore, safety and efficacy of milk has been rigorously tested during these same millions of years of evolution. Thus, unlike pharmaceutical research that must in every case ask: is it healthy? With milk, the question researchers need to ask is, "How does milk mechanistically elicit its health effects?"

Shifting the Paradigm for R&D: Disease Treatment to Prevention

Traditional drug development requires up to 20 years from candidate drug discovery to market, with greater than 90% of drugs failing during human trials (Eliopoulos *et al.*, 2008). The entire process from target discovery to successful intervention typically takes 30 to 50 years. As a direct response to the length of time and poor success rate in which basic bench science is transformed into a breakthrough with clinical utility, the field of translational science under the NIH was initiated as a new multi-disciplinary field in which the stated objective is to bring science to practice (Zerhouni, 2007). Yet, the current model of bringing science to practice is for the scientific discovery of pharmaceutical solutions to disease. This serial process requires the identification of pathways that are affected and their causal mechanisms—whether pathogens, toxins or enzymes. These causal mechanisms become the target for pharmaceutical solutions. With *in-vitro* screening, thousands of molecules are tested for their ability to act upon the target. Subsequently, successful hits move to drug-development pipelines where their safety in animals and humans is tested and their efficacy is tested against the target and then the disease. Finally, the small molecule found to act on the target is tested for efficacy, safety, and commercialization (Tonkens, 2005). The process of bringing scientific discovery to practice requires a quarter of a century. To speed up this process, key steps in this system will have to be accelerated. Yet, what part of translation of drugs should we skip: the efficacy or the safety?

If the translation of drugs for targeting disease requires decades of testing, how long would it take to discover, test, validate and bring to practice the prevention of disease by foods? A major challenge to prevention is actually in the time to translation. Prevention means that disease is prevented in healthy people. Do we wait an additional 50 years to assess the effectiveness in preventing disease by a food? Fortunately, evolution has used 120 million years for testing the efficacy AND safety of milk. For preventing disease with foods, research on milk at the Foods for Health Institute has led to the adoption of a different translational model (Figure 1). Unlike use of serial development for discovering disease targets, health can be translated using parallel research and development. Based

on the basic evolutionary principle in which lactation evolved under the relentless pressure to be nourishing, supportive and protective, once a molecule in milk is identified, it is understood that it is going to be valuable. It is only to be known how and in what population will it be effective. This confidence of efficacy suggests we can immediately start the process of developing industrial scale sources of the molecule, processing strategies, analytics and diagnostics of its presence and actions. Once populations that could benefit from consuming the molecule are identified and metabolic benefit is clinically assessed, ingredients and products are already scaled up for commercialization.

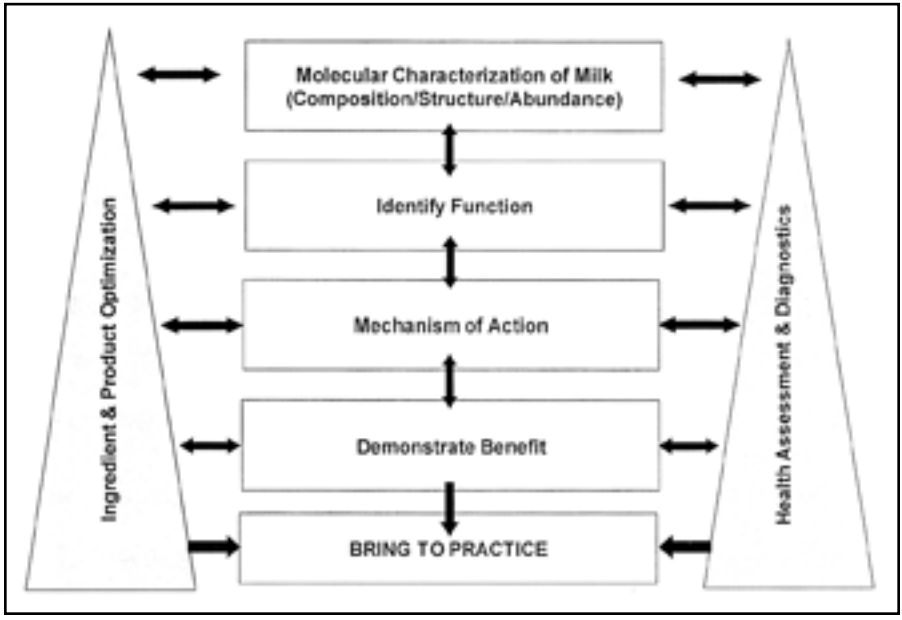

Figure 1. Parallel research and development for translating health.

Discovering the Mechanistic Targets of Milk Through Evolutionary Genomics

Identifying and annotating the milk genome is proving to be invaluable for the discovery of genes encoding molecules in human milk and their respective physiological targets. In efforts to provide a collaborative and interactive platform for researchers to accelerate the understanding of the biological processes underlying the mammalian milk genome, the International Milk Genomics Consortium (IMGC) was initiated in 2004. The IMGC has constructed a web-based portal as a public resource consisting of the genes of lactation and their annotation for the unique roles they play as molecules and structures for nourishment (www.milkgenomics.org). Research in this field extends across mammals and their varying lactation strategies, to provide insights into the diverse roles of milk (Bovine, 2009; Lemay *et al.*, 2009). IMGC has acquired lactation genomes across the entire evolution of mammals which permit the pursuit of a wide variety of scientific questions

about the origins, functions and distributions of lactation genes and their specific quality traits. For example, two animal species with contrasting lactation strategies have evolved in response to unique natural selective pressures: the black bear and the hooded seal. The black bear of the Northeastern United States and Canada gives birth during winter hibernation. The mother bear continues to hibernate for 2–3 months postpartum, during which time she does not exit the den to eat or drink despite nursing her cubs (Oftedal *et al.*, 2007). This example in which maternal stores solely support the first few months of lactation is a model for nutritional efficiency. At the other end of the spectrum is the hooded seal. This mammalian pup is born on an ice floe in the north Atlantic, in polar-bear country. The strategy for the neonate is to get off the ice floe and into the open sea quickly. During the 4 days of lactation, the mother seal transfers 26 kg of fat from her adipose stores and the pup gains 26 kg, of which three quarters is visceral fat. This most remarkable example of energy transfer in mammals supports the young seal through a post-weaning fasting period of 5–10 weeks (Oftedal *et al.*, 2007).

One over-arching question relates to the rate of evolution of lactation itself. Given the basic principle that mutations accumulate during evolution, the more identical the sequences are for a given protein, logic and prior experimentation have documented that the more important its sequence is to the survival of the organism. By taking such a quantitative perspective to evolution, lactation has been vital to the success of mammals. In Figure 2, the sequence identity of proteins expressed from genes in the entire genomes of seven mammals is distributed and compared with the proteins expressed by lactation genes of the same mammals. Most of the milk proteins are highly conserved across mammals, whereas a much smaller subset is highly divergent across mammals. There is less conservation among mammals for proteins of the liver, adipose, and brain than for lactation-related genes. This conservation throughout evolution emphasizes how important these genes have been to the survival of the species. Even evolution agrees that we have chosen the right model.

A Success Story for Foods For Health

Recall our model, the mother-infant pair. The mammalian mother actively dissolves her tissues to make milk. Everything in the milk is costly to the mother. If components found in milk do not enhance the survival of the infant, the cost to the mother would lead to its loss through evolution. However, if any component in milk, when consumed by the infant, provides it any competitive advantage over its genetic peers, it will be retained in evolution. This mother-infant pair is a Darwinian engine of nutrition. Considering this model, imagine to our surprise when we examined the various components in human milk and found that the third most abundant component class, at key stages of lactation more abundant than protein, were indigestible by the infant (Zivkovic *et al.*, 2010). How could this be?

Discovery

The presence of indigestible saccharides in milk led to a multi-disciplinary pursuit. Robert Ward, in Bruce German's laboratory, recognizing their paradoxical abundance, isolated these molecules from human milk and began to characterize their structures and func-

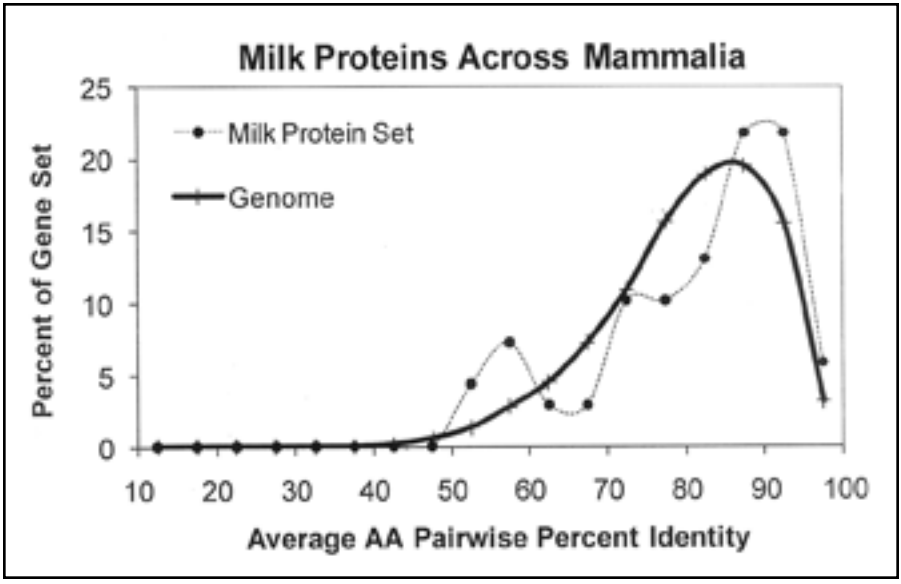

Figure 2. Distribution of proteins expressed by lactation-related and total genomes of seven mammals.

tions (Ward *et al*. 2007), although methods to characterize the chemical structures of these components were largely lacking. Their structural determination is one of the most complex problems for analytical chemistry. Carlito Lebrilla, a world leader in glycan analytics, and his team developed the tools to characterize the structures of the oligosaccharides in milk (Ninonuevo *et al.*, 2006, 2007, 2008; Tao *et al*., 2009, 2010). If the infant cannot digest oligosaccharides, what are their functions? David Mills, a leading microbiologist specializing in the bacteria of the intestine, and his team characterized one component of the intestinal microbiota—*Bifidobacterium infantis*—which digested and thrived on oligosaccharides purified from human milk as their only carbon source (Sela *et al*., 2008). This bacterial species was first isolated from the intestine of breast-fed infants. This is when the genius of milk became clear. Mothers are recruiting another life form: bacteria that protect their infants' health. These results have identified a unique target for foods; the intestinal microflora AND how to alter its population to derive a health benefit (Zivkovic *et al*., 2010).

Translation

One advantage of doing research at the University of California at Davis is the breadth and depth of scientific and clinical programs across campus. Mark Underwood, the head of the Neonatology Unit at the UC Davis Medical Center has been pursuing intestinal health as a target of success and failure for premature infants. Mark and his colleagues are now investigating the use of human milk oligosaccharides and *Bifidobacterium infantis* to protect premature infants at increased risk of intestinal disease.

Commercialization

The final test of translational science is bringing it to practice for appropriate consumers. From the beginning of the project we have been simultaneously developing sources and technologies to produce this unique class of oligosaccharides as a food ingredient. Sources such as dairy process streams to decorated oligosaccharides, are all candidates that could achieve structure-function benefits of human-milk oligosaccharides. The UC Davis Center for Entrepreneurship is working in parallel to define business models for this ingredient class to enter the food marketplace. The goal is to translate health to those who would most immediately benefit from consuming such ingredients.

Conclusions

The challenges facing the life sciences and the entire agriculture enterprise are to deliver on the promise of prevention. The models established for small molecules as drugs developed by the pharmaceutical industry and delivered through the clinical profession as curative therapeutics are not appropriate for foods for prevention. This failure does not mean that we compromise the quality of science, the rigors of regulation nor the expectations for efficacy. New models that are capable of enhancing safety, efficacy and personal benefits from foods constitute the 21st century's most vivid opportunity to improve the human condition. The University of California, Davis, has assembled the multiple disciplines to respond to such an opportunity.

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During her PhD program, Smilowitz discovered metabolic phenotypes associated with changes in body composition in a large multi-center study, and designed and executed several clinical studies probing specific lipid metabolic pathways in the inflammation cascade. Her current research focuses on the development of assessment technologies for the identification of metabolic phenotypes in response to food-based solutions for ameliorating metabolic diseases and optimizing health and performance. Other research interests include elucidating structure-function relationships of lipoproteins and protective functions of skin and human milk. She is the project coordinator for the UC Davis Lactation Study, a large multi-disciplinary and multi-collaborative prospective clinical trial collecting well-annotated human milk samples from lactating mothers.

Dr. Smilowitz is the contact person at the FFHI for investigators interested in obtaining structural support and guidance for initiating clinical research designed to assess metabolic, performance and health responses to food.