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The gut microbiota, food science and human nutrition; a timely marriage

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Abstract

Analytic advances are enabling more precise definitions of the molecular composition of key food staples incorporated into contemporary diets and how the nutrient landscapes of these staples vary as a function of cultivar and food processing methods. This knowledge, combined with insights about the interrelationship between consumer microbiota configurations and biotransformation of food ingredients, should have a number of effects on agriculture, food production and strategies for improving the nutritional value of foods and health status. These effects include decision-making about which cultivars of current or future food staples to incorporate into existing and future food systems, and which components of waste streams from current or future food manufacturing processes have nutritional value that is worth capturing. They can also guide which technologies should be applied, or need to be developed, to produce foods that support efficient microbial biotransformation of their ingredients into metabolic products that sustain health.

Introduction

Historical analyses have emphasized how improved nutrition is a major contributor to the economic growth of societies, and have underscored the synergism between physiologic and technical improvements (Fogel, 2004). The rapid expansion of our human population, and the need to produce more food, more sustainably (e.g., Eshel et al., 2014), highlights a critical and present need: improve knowledge of what to eat and develop technologies to make affordable foods that promote wellness. Disappointingly, nutrition has been a neglected area of global health and development, accounting for less than 1 percent of global foreign aid (Action Against Hunger, 2013).

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The gut microbiota contains tens of trillions of microbial cells. Development of this microbial ‘organ’ is initiated at birth and in healthy individuals is largely completed within three years (e.g., Yatsunenko et al., 2012; Subramanian et al., 2014). A major function of the microbiota is to process food ingredients so that the products of their biotransformation can be utilized in beneficial ways to support myriad aspects of our human biology (Sharon et al., 2014; Schroeder and Bäckhed, 2016; Koh et al., 2016). Well known examples include short chain fatty acids generated from fermentation of dietary polysaccharides, bioactive indole derivatives resulting from tryptophan metabolism, and modification of the pharmacologic properties of plant flavonoids (Zhang and Davies, 2016; Hubbard et al., 2015; Cassidy and Minhane, 2017). Of course, the relationship between microbiota and host should not be construed in an entirely host-centric fashion; members of the microbiota derive benefit from the products of one another's metabolism and from the processing of host-derived biomolecules (e.g., mucus glycans; bile acids) (Degnan et al., 2014; Pudlo et al., 2015; Wahlström et al., 2015; Wu et al., 2015).

For the past 10 years, *Cell Host and Microbe* has served as an important home for interdisciplinary studies describing interactions between diet and the gut microbiota. We believe that it is timely for the readers of the journal to now look forward to the next decade and envision how studies of the gut microbiota could provide a way to promote discovery and development efforts required to identify safe, affordable food products that enhance health. This ambitious goal requires interdisciplinary approaches focused on (i) identifying the specific members and expressed functions of the microbiota that are essential contributors to the health status of humans at different stages of life; (ii) determining whether these functional effects can be generalized within and across populations with different anthropologic features, and (iii) deciphering the biological impact of perturbations of these microbiota functions (Schroeder and Bäckhed, 2016; Sharon et al., 2014). Achieving this goal necessitates delineating the effects of current foods on the microbiota of its consumers. More fundamentally, it requires detailed biochemical characterization of components represented in the world's major food staples, including cultivars designed to have enhanced nutritional content, the effect of food processing and preparation on this component profile, and how these components, singly and in combination, alter properties of gut microbial communities (David et al., 2014; Hibberd et al., 2017).

We propose that defining these microbiota ‘structure-function’ relationships will enable the creation of ‘microbiota-directed’ foods (MDFs). In principle, MDFs can operate by altering the functional configurations of a consumer's gut microbial community, providing substrates for microbial transformation to biomolecules necessary for a healthy state, or by acting through a combination of these mechanisms. Designation as an MDF would reflect the fact that it contains components designed to *deliberately* manipulate a microbiota in a selective manner so as to benefit one or more facets of host biology, with resulting improvements in health status. A ‘prebiotic’ has been defined as “a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” (Gibson and Roberfroid, 1995; see also Hutkins et al., 2016). Here, we use the term MDF to represent a ‘food’ composed of a variety of ingredients including one or more prebiotic components capable of being metabolized by microbes alone, and/or nutrients whose transformation by

microbes makes them available to the host for direct use or for further biotransformation, as well as components that do not require microbial metabolism for their effects on the host.

Developing MDFs should provide opportunities to forge (i) new types of alliances between microbial scientists and food scientists that extend far beyond traditional considerations/definitions of nutritional content and food safety, (ii) new types of collaborative interactions between microbial and nutritional scientists that expand and deepen our understanding of the determinants of host biologic phenotypes, and (iii) new relationships between microbiologists, agricultural stakeholders, and policy makers that fundamentally change how food systems are designed and overseen. Perhaps, the most durable impact will be to catalyze new interdisciplinary educational programs for students so that they can contribute, in their unique ways, to solving the problem of how we and other inhabitants of our planet can survive and flourish in this phase of the Anthropocene epoch.

One approach for developing ‘microbiota-directed’ foods

One approach for discovering and developing MDFs is illustrated in Figure 1 and discussed here. A ‘preclinical gnotobiotic animal arm’ utilizes formerly germ-free animals colonized with human gut microbes - either sequenced collections of cultured microbial strains or intact, uncultured microbiota from donors representing biological phenotypes and consumer populations of interest. This arm is used to identify food ingredients that affect the representation and expressed functions of community members. Moreover, as knowledge of interpersonal variations in community structure/functional relationships expands, these preclinical models can help develop more refined views of what configurations provide benefit to specified host functions in specified dietary and microbiota contexts.

A ‘food science technology arm’ uses advanced analytical techniques to characterize and quantify the biochemical components of food ingredients before and after incorporation into food prototypes. These food prototypes are fashioned based on considerations of the biochemical characteristics, availability and affordability of their ingredients, how the ingredients might be processed in ways that do not deleteriously affect the integrity/bioactivity of key nutrients, and whether the manufactured food products will have acceptable organoleptic properties. Food prototypes are then tested in the gnotobiotic animal models colonized with microbial communities sampled from members of the target human population so that the effect size on features of community and host biology, and their mechanisms of action, can be delineated and relevant biomarkers identified for follow-up human studies. Germ-free animals that are not colonized provide a reference control for definitively assigning microbiota and host contributions to the biotransformation of the food prototype and the effects of its metabolic products on host biology.

A ‘human studies arm’ captures the output of the gnotobiotic and food science arms to validate the effects of lead food prototypes in members of the very target population whose gut microbes were incorporated into the preclinical model. Put in a broader context, this arm helps address an important need in the field of human microbial ecology research; to directly assess the translatability of results from studies of microbiota in animal models to humans. For example, replication of human donor microbiota, while reproducible across recipient

gnotobiotic animals in a given treatment group, is not fully representative even for members of the domain Bacteria, with considerably less information available about capture of members of Archaea and Eukarya (and their viruses). Mice and humans have different nutrient requirements. The biochemical features of their mucus, which serves as a microhabitat for components of the microbiota, varies between humans and mice. Mucus provides glycans that community members can forage, a means to avoid their washout from the gut bioreactor, and opportunities to exchange metabolites with their syntrophic partners as well as to signal host mucosal epithelial and immune cell lineages. The immune systems of germ-free animals are incompletely developed and the extent of maturation that does occur is affected by the timing and duration of colonization. Consideration of these and other factors suggest that the selective pressures applied to a transferred human microbiota may be different from those experienced by the community in its native host habitat. The qualitative and quantitative effects of these differences on biotransformation of food ingredients can be ascertained through comparative studies of gnotobiotic animal models harboring the very microbial communities of the humans that are subjects of clinical studies.

We believe that consummating this marriage of preclinical models, food science and human studies is necessary and timely given the rapidly changing patterns of food preferences brought about by economic development/globalization, changes in food technology and food distribution systems that have produced dramatic changes in how and what we eat, as well as ongoing efforts to deploy various technologies to create new more nutritious foods or identify ways of capturing discarded nutrients from food waste streams (Parfitt et al., 2010).

An important consideration is how this pipeline can be linked to ongoing agricultural initiatives that focus on current food staples (e.g., yield improvements from selective breeding, genetic engineering, new fertilizers, or manipulations of the rhizophere), as well as potential future staples. In developed countries, the availability of animal sources of protein can provide sufficient amounts of essential amino acids for human diets. In developing countries, plants may account for a significant fraction of the protein intake (de Bruyn et al., 2016; Schönfeldt and Gibson Hall, 2012). However, many of the staple cereal and legume crops grown in developing countries are deficient in essential amino acids; tryptophan, and methionine are most limiting in cereals and while methionine is deficient in legumes (Ufaz and Galli, 2008). In parts of sub-Saharan Africa where maize is the principal food staple accounting for up to 50% of daily energy intake (Nuss and Tanumihardjo, 2011), symptoms of tryptophan deficiency, including reduced growth, impaired bone development and neurological abnormalities can occur at intakes as little as 25% below the minimum requirement (Moehn et al., 2012). Tryptophan deficiency has also been associated with growth deficits and vaccine underperformance in children with environmental enteropathy (Kosek et al., 2016). Therefore, there is significant humanitarian and economic interest in bio-fortification approaches to enrich crops in these essential amino acids (Nuss and Tanumihardjo, 2011), as well as to address the ‘hidden hunger’ associated with wide-scale micronutrient deficiencies (Tang et al., 2009).

Another approach is illustrated by the African Orphan Crops Consortium (AOCC), whose goal is to sequence the genomes of crops that are not traded internationally and thus little studied by science but relied on by the 600 million people who live in rural Africa (http://

africanorphancrops.org/). The consortium conducted an Africa-centric survey, with participation from African plant breeders, sociologists, anthropologists, nutritionists, policy makers, farmers, and various other stakeholders to identify 101economically important but also socio-culturally relevant crop and tree species that are important components of local diets (http://africanorphancrops.org/meet-the-crops/). The consortium's mission is to sequence, assemble and annotate the genomes of these crops; the resulting data will be available in open access format and offered free to plant breeders, seed companies and farmers on condition that it is not patented. It is anticipated that providing African plant scientists with the knowledge and tools to assess genetic diversity in crops and to support breeding programs through the AOCC's African Plant Breeding Academy will spawn biotechnology and breeding initiatives to develop more nutritious crops with superior yields, plus greater resiliency to pests and changing climate. Furthermore, the AOCC partnership seeks to mainstream orphan crops into food supply systems by making improved cultivars available to smallholder farmers throughout Africa, developing new markets, and supporting promotional activities to boost their wider production and consumption. If successful, this initiative provides a blueprint that could be expanded to other parts of the world.

The pipeline described in Figure 1 provides an opportunity to make a contemporaneous investment in determining how orphan crops themselves and various food prototypes constructed from them are processed by the gut microbiota of consumers representing different ages and different health states in the populations to which these foods might be distributed. These preclinical models could also be used to examine how the food prototypes derived from these staples affect various aspects of host physiology and metabolism, as a prelude to future human studies. In addition to providing practical knowledge for development of affordable foods that improve the nutritional status of consumers, this type of investment could yield basic knowledge. For example, which gut microbial proteases are expressed in response to exposure to foods containing different plant-derived proteins, and to what extent are levels of amino acids present in different regions of the gut and blood of these preclinical models a reflection of microbial proteolytic activity? Importantly, in the case of undernutrition, do distal gut microbial enzymatic activities have the potential to provide the host with a bioavailable source of essential amino acids from glycoproteins/peptides that escape absorption in the upper gastrointestinal tract?

Defining the nutrient content of food: a key missing ‘ingredient’

Advances in biotechnology that are enabling characterization and manipulation of the genomes of cultivars of various crops are occurring contemporaneously with advances in analytical chemistry; as such, there is an opportunity to gain unprecedented insights into their biochemical composition. In particular, details of the carbohydrate structures in staple crops and how these vary as a function of cultivar and food processing methods have remained largely enigmatic until recently. However, analytical advances originally developed to study the human milk glycome (De Leoz et al., 2013; Totten et al., 2014) are poised to address this gap in knowledge.

Milk is a complex mixture of macronutrients and bioactive molecules, many of them extensively glycosylated (Ballard and Morrow, 2013). As nature's first food, human milk has

provided a blueprint to guide our understanding of the fundamental relationships between indigestible carbohydrates and the gut microbiota (Davis et al., 2016). Human milk oligosaccharides (HMOs), on a dry weight basis, are the third most abundant component of milk (5-15g/L), after lactose (70g/L) and lipids (40g/L) (Zivkovic et al., 2011). HMOs remain largely intact and unabsorbed during transit through the proximal gut (Engfer et al., 2000; Bode, 2012), although recent reports have identified certain HMOs in serum and urine (De Leoz et al., 2013). HMOs have evolved to support the establishment of a select group of beneficial gut bacteria that are exquisitely adapted to consume these oligosaccharides in the gut environment of breastfed infants (Pacheco et al., 2015; De Leoz et al., 2015). Among these specialized, breast milk-adapted, early colonizers of the infant gut, *Bifidobacterium longum* subspecies *infantis* is unique in its prodigious capacity to digest and consume all of the known HMO structures; this capacity reflects its large repertoire of bacterial genes encoding an array of glycosidases and oligosaccharide transporters that are not found in other bacterial species (Underwood et al., 2015).

The ability to monitor, with quantitation, specific HMO structures has led to new understanding of the unique relationship between HMOs and the nascent gut microbiota of infants. Methods such as nano-liquid chromatography chip time-of-flight mass spectrometry (nano-LC chip-TOF MS), coupled with the development and implementation of comprehensive libraries containing fully characterized structures have enabled hundreds of structures to be identified and quantified in a single analysis (Wu et al., 2010, 2011; Totten et al., 2014). These analytical tools have re-defined the scope of HMO structural diversity from the millions of structures previously believed to exist, to a few hundred molecules whose abundances range over 4 to 5 orders of magnitude in human milk (Totten et al., 2014). Although HMOs are composed of a relatively small number of monosaccharides including glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc), and sialic acid (NeuAc/Sia), the linkages and branching create a large diversity of structures. HMOs vary in size from three to 20 monosaccharides, built by the action of competing glycosyltransferases that produce branched and linear structures composed of lactose core, elongated by GlcNAc and Gal, and potentially capped by Fuc and Sia (Figure 2).

During the weaning process, the source of dietary carbohydrate shifts from breast milk-derived oligosaccharides and glycoproteins to polysaccharides originating from cereals, fruits and vegetables that comprise complementary foods. The monosaccharide composition, glycosidic linkages and degree of polymerization of plant-derived polysaccharides exhibit tremendous diversity. Like HMOs, plant polysaccharides are often built from glucose and galactose monomers, but they generally lack fucose and sialic acid residues that are common in HMOs. In addition, plant polysaccharides contain fructose, arabinose, rhamnose, hexuronic acids, and other less abundant monosaccharides. Compared to HMOs, with a few exceptions, surprisingly little is known about many of the plant-derived carbohydrate structures that we consume in our diets. Unlike human milk, plants contain mixtures of polysaccharides that even when pure are more difficult to fully structurally elucidate because of their large size, linkages, branching and variable polymeric side chains. Plant polysaccharides are broken down in the distal intestine by microbes in the (weaning) gut microbiota whose genomes encode a large and diverse collection of carbohydrate esterases,

glycoside hydrolases, and polysaccharide lyases (collectively referred to as **c**arbohydrate-**a**ctive en**zymes** or CAZymes; Bhattacharya et al., 2015) that are not encoded in our human genome. Henrissat and coworkers have developed, and are continuing to evolve, methods for predicting gene loci involved in glycan utilization by members of Bacteroidetes, one of the two most prominent human gut bacterial phyla (Terrapon et al., 2015), and the carbohydrate structures targeted by these different gene clusters. They, and others, are extending this work to glycan-utilization clusters in other bacterial taxa represented in the microbiota.

Coupling these *in silico* efforts with advances in analytic methods and the pipeline described in Figure 1, sets the stage for defining the glycan structures present in major food staples, determining how these structures are affected by food processing and by different consumers' microbiota, and characterizing how glycan processing influences the expressed functional properties of gut communities. Nonetheless, the complexity of the analytic challenge is great. To illustrate this point, Figure 3 shows the major polysaccharides that make up two important food staples; rice and potato. Rice is composed primarily of glucose polymers including amylose and amylopectin. Potato contains these two polymers but also includes arabinogalactan and rhamnogalacturonan. Amylose and amylopectin are composed of glucose polymers in a combination of mainly α(1-4) glucose linkages and some α(1-6) linkages. However, arabinogalactan and rhamnogalacturonan I contain other monosaccharides including rhamnose, arabinose, and galacturonic acids with their respective linkages. For a healthy gut microbiota to utilize these polysaccharides, appropriate CAZymes need to be available to process each monosaccharide with its associated linkage(s). Furthermore, the ability to *quantitate* each monosaccharide and its linkages, which significantly advanced our understanding of the structure and function of HMOs, is currently unavailable for polysaccharides, even those in rice and potato. In addition, carbohydrate structures in many other foods are poorly characterized, if at all. Adding to the analytic challenge is the critical impact of food processing technologies and methods of food preparation that result in various enzymatic and non-enzymatic structural modifications to the food matrix that can affect carbohydrate structure.

Processing may further increase the structural diversity by, for example, affecting the representation of oligosaccharides (di, tri- and tetrasaccharides), thereby altering the bioavailability of these compounds to members of the microbiota and host (Christiaens et al., 2016; Fardet, 2015). Therefore, development of more advanced analytical tools to characterize polysaccharides with improved quantitation, sensitivity, and significantly greater throughput is critical. Moreover, the general approach to food carbohydrate analysis should be realigned to focus on biological functions; since these functions are defined by monosaccharide compositions and linkages, a priority should be to elucidate these structural features with accurate quantitation.

Another facet of considering how microbes process plant glycans in foods involves the ‘universe’ of small particles, representing fragments of plant-derived material (e.g. fibers), that exist in the distal gut. Development of higher throughput methods for imaging and quantifying the spatial distribution of different taxa within the gut (Tropini et al., 2017) would allow tests of the following concepts. These particles serve as an important site of attachment for saccharolytic organisms so that they can harvest particle-associated glycans

and share the products of their digestion with syntrophic partners who also reside on these particles. The physical proximity of primary and secondary consumers on the same particle facilitates nutrient sharing and metabolic exchange; as such, the food particle can be viewed as a functional unit in the gut ecosystem (a ‘center of commerce’). Other key parameters to explore from both a microbial community and food science perspective include; (i) the degree to which these food particles provide other nutrient resources to different members of attached microbes and the food webs that they support, (ii) the relationship between particle size, nutrient content, the on and off rates of organisms that have the adhesive apparatus needed to adhere to particles, and (iii) the size of the microbial population that a particle can support, and how this varies as a function of particle degradation. These questions encompass both the glycan and protein constituents of particles and prompt consideration of how particles of defined size and composition might be deliberately fabricated to promote processing by members of a consumer's microbiota. Comparing processing of these particles or other components of a diet in colonized versus germ-free animals provides a direct test of which products are generated through the metabolic activity of gut microbes, versus those that result from host digestive processes alone.

A holistic approach for developing more affordable nutritious foods

The discovery and development platform outlined in Figure 1 could fundamentally change the way we define the nutritional value of foods, how next generation food products are designed/ tested and, ultimately, national policies on nutritional recommendations. While we have focused our attention on human consumers and their microbiota, in principle the approach described could be applied to the microbiota of other species (for example, those that are important sources of animal protein such as fish), and involve different gnotobiotic host species, including those capable of supporting higher throughput early stage screens (e.g. zebrafish; Melancon et al., 2017). The pipeline could also be expanded to encompass an ‘abiotic’ component based on advances in *in vitro* bioreactor systems that support growth of gut microbial consortia (e.g., Kettle et al., 2015; Auchtung et al., 2015; Chung et al., 2016; Kim et al., 2016).

A grand challenge will be to quantify the nutrient/biochemical content of the world's major food staples using advanced analytic methods of the type discussed above, with initial priority placed on food ingredients commonly consumed across populations, and the impact of commonly used procedures for processing these ingredients. To provide maximum utility, the results will need to be assembled in a systematically annotated, readily searchable, publicly accessible database that is regularly updated. This effort could be expanded to include different cultivars of a staple, prioritized according to their current or envisioned/ predicted incorporation into food systems, the sustainability of their supply chains, and the adoption of new processing technologies. The effort could be linked to the work of both governmental and nongovernmental organizations in monitoring and forecasting changes in patterns of food consumption, so that microbiota from representative consumer populations, both current and anticipated, can be incorporated into the pipeline described above. An additional focus would be on waste streams generated during food manufacture that, through the lens of the microbiota, may contain ingredients with high potential nutritive value (Gullon et al., 2013). Ideally, the outcome of applying this ‘choreography’ will be better

informed decisions about food ingredients and food manufacturing as they relate to the nutritional status and health of consumers. This capability, if implemented so that it could be deployed in a nimble fashion, would be timely based on the pressing challenges posed by population growth and climate change. However, until the 'value proposition' of this approach is established through human studies, economic and geopolitical factors may conspire to limit enthusiasm (and funding) for its adoption (e.g., Blanton et al., 2016).

In addition, development of microbiota-directed foods requires a holistic approach that proactively considers and thoughtfully addresses a number of biological, educational, cultural, and regulatory issues (Blanton et al., 2016); the consequence of failing to do so is vividly illustrated by the problems that have arisen with acceptance of genetically engineered food products. For example, the short- and long-term safety and efficacy of MDFs need to be established through rigorous clinical studies. Culturally-sensitive educational approaches will be needed not only to perform such studies, but also to inform the population, through a readily understood narrative, about the microbiota, what is known about its interaction with foods, and what this means to the health of consumers. The claims made for a MDF, including evidence for its safety and efficacy, will have regulatory implications, including whether that MDF is classified as a food, dietary supplement, medical food or drug (discussed further in Green et al., 2017). Classification, in turn, will affect labeling and advertising/marketing strategies. We hope that the readers of *Cell Host and Microbe* will not only observe with interest how these interdisciplinary studies and discussions take form and evolve, but will be active participants in guiding them.

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In this Perspective, Barratt et al. propose the integration of preclinical models, food science technologies and human studies to generate detailed knowledge of the biotransformation of food ingredients by consumers' gut microbiota. This could alter traditional definitions of nutrient content and inform global efforts designed to produce affordable, healthier foods.

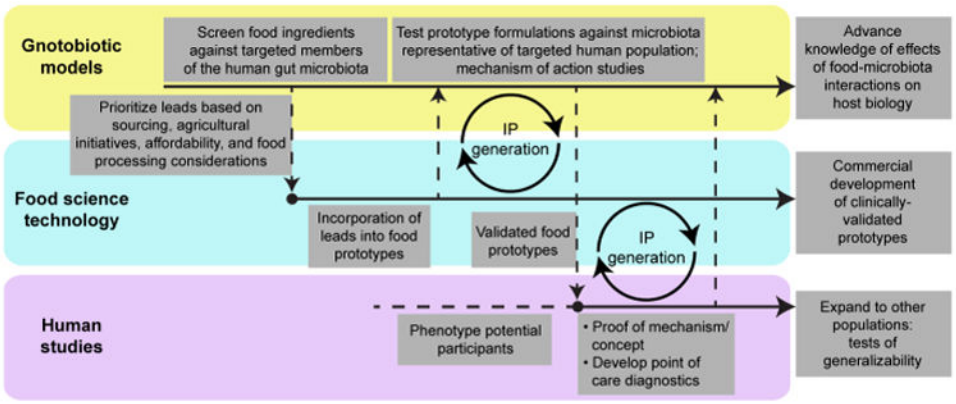

Figure 1. Pipeline for developing microbiota-directed foods (MDFs)

A ‘preclinical arm’ (highlighted in yellow) uses gnotobiotic animals colonized with members of microbial communities from the target human population; animals are fed complementary food ingredients to identify those combinations that affect the abundances and expressed beneficial functions of targeted microbial taxa. Effects of these MDFs on various facets of host biology are defined. A ‘food science arm’ (blue) incorporates affordable lead food ingredients with sustainable supply sources into MDF prototypes that have desired nutritional content and organoleptic properties. The ‘clinical arm’ (purple) begins with a series of pilot studies designed to validate the activities of various MDF prototypes observed in/forecast by the preclinical arm. Dashed lines indicate key points of interaction/feed-back/decision-making between the three arms of this dynamically operating pipeline.

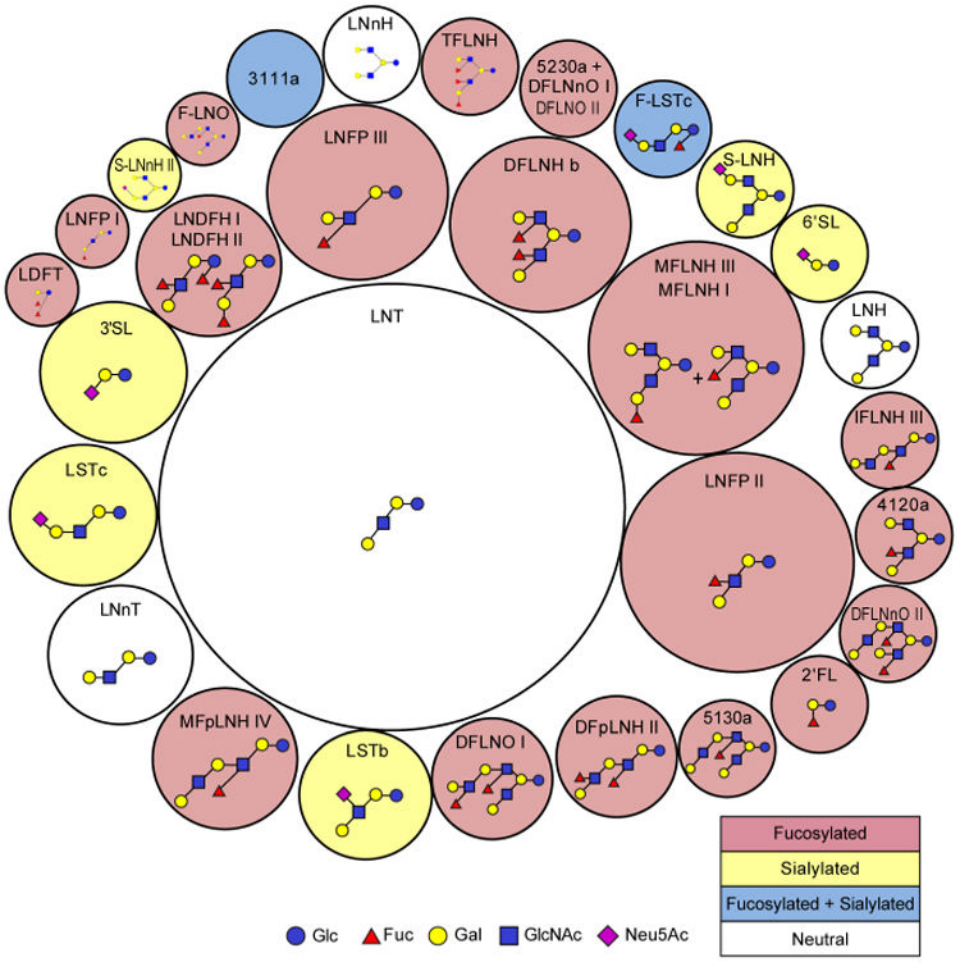

Figure 2. Structural compositions of human milk oligosaccharides (HMOs) in a 'secretor individual'

Two HMO 'phenotypes' are apparent based on the presence/absence of specific fucosylated structures. Mothers that produce HMOs decorated with α(1,2)-fucose (Lewis b antigen) are termed 'secretors' and those that do not are termed 'non-secretors'. While genetics underlies these differences in milk HMO structural composition, recent work has revealed differences in structural profiles and abundances associated with geography, maternal age, stage of lactation and body mass index (BMI) (McGuire et al., 2017). In this figure, the size of the circle designates the relative abundances of HMO structures. The colors correspond to subclasses of fucosylated (containing at least one fucose), sialylated (containing at least one sialic acid), and fucosylated and sialylated (at least one of each) and neutral (contains neither fucose nor sialic acid) HMO structures. Abbreviations of structures shown are based on Totten et al. (2014). LNT: lacto-N-tetraose, LNFP II: lacto-N-fucopentaose II, MFLNH III + MFLNH I: monofucosyllacto-N-hexaose III/ monofucosyllacto-N-hexaose I, DFLNH b: difucosyllacto-N-hexaose b, LNFP III: lacto-N-fucopentaose III, 3120: Hexaose(3) N-Acetylglycosamine(1) Fucose(2), 3′SL: 3′-sialyllactose, LSTc: sialyllacto-N-tetraose c, LNnT: lacto-N-neotetraose, MFpLNH IV: monofucosyl-para-lacto-N-hexaose IV, LSTb: sialyllacto-N-tetraose b, DFLNO I: difucosyl-lacto-N-octose I, DFpLNH II: difucosyl-para-lacto-N-hexaose II, 5130a: Hexaose(5) N-Acetylglycosamine(1) Fucose(3), 2′FL: 2′-

fucosyllactose, DFLNnO II: difucosyl-lacto-N-neooctase II, 412Oa: Hexaose(4) N-Acetylglycosamine(1) Fucose(2) a, IFLNH III: isofucosyl-lacto-N-hexaose III, LNH: lacto-N-hexaose, 6′SL: 6′-sialyllactose, S-LNH: sialyl-lacto-N-hexaose, F-LSTc: fucosyl-sialyllacto-N-tetraose c, 5230a + DFLNnO I/DFLNO II: Hexaose(5) N-Acetylglycosamine(2) Fucose(3) a + difucosyl-lacto-N-neooctase I/ difucosyl-lacto-N-octase II, TFLNH: trifucosyllacto-N-hexaose, LNnH: lacto-N-neohexaose, 3111a: Hexaose(3) N-Acetylglycosamine(1) Fucose(1) N-Acetyleneuraminic acid(1) a, F-LNO: fucosyl-lacto-N-octaose, 4021a + S-LNnH II: Hexaose(4) Fucose(2) N-Acetyleneuraminic acid(1) a + sialyl-lacto-N-neohexaose II, LNFP I: lacto-N-fucopentaose I, LDFT: lactodifucotetraose.

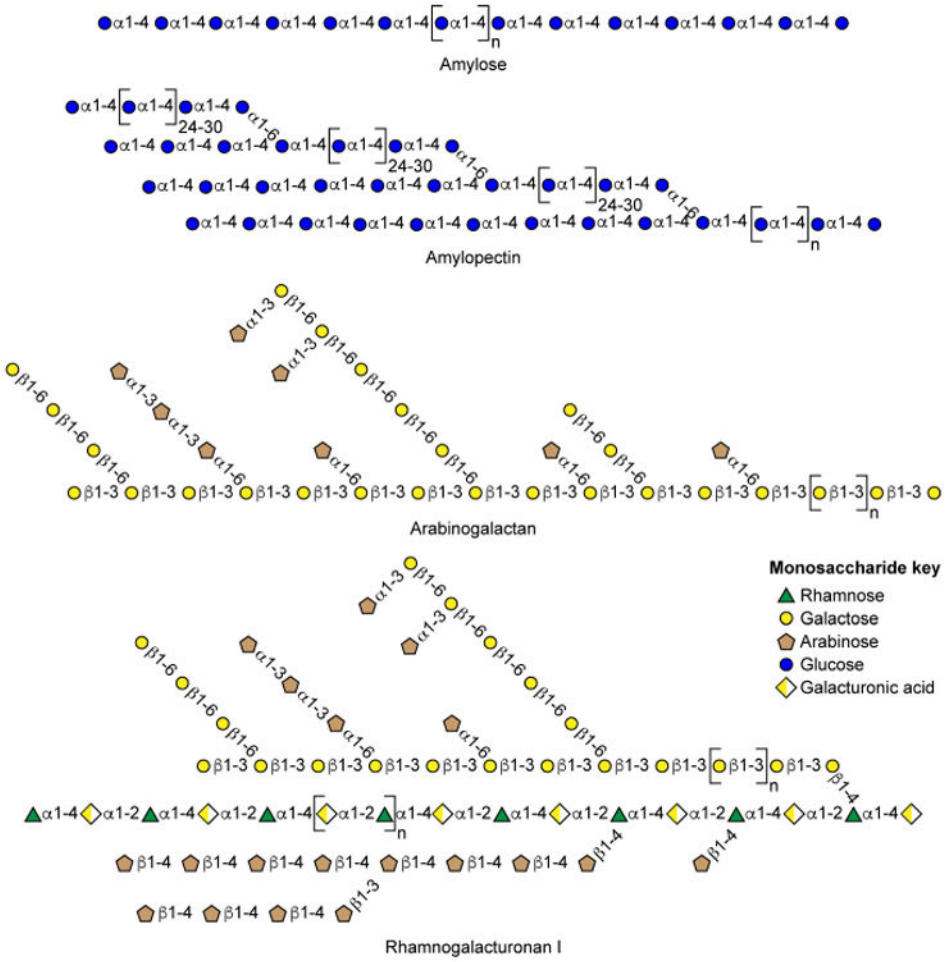

Figure 3. Structural representation of polysaccharides found in rice and potatoes

Rice contains primarily amylose and amylopectin. Potatoes contain the same polymers but additionally arabinogalactan and rhamnogalacturonan I. These structures illustrate the challenge in characterizing food polysaccharides. The large sizes of the polymers (millions of Daltons), the heterogeneity in their monosaccharide compositions and linkages make analysis unlike any other biopolymer.