Finding funding

Given the new regulations, Watson agrees that it will certainly take a fair bit of money to get all disease candidates examined in multi-state pilots, but that the payoff is worth it. "Right now we're having a disproportionate discussion of the risks of research as compared to the benefits. The risks to privacy are pretty minimal. Each test ranges between $1 to $5 each, and the return on screening is really on the child not becoming sick and requiring very expensive health care."

To facilitate access to newborn blood spots, scientists are also contemplating how to make the consent process easier. One way to obtain consent from mothers and work with the new law is to explain what newborn screening is in the prenatal stage, where women will be "far more receptive" to that type of information, as opposed to when they are in labor and rushed to the hospital, or after they have just given birth, according to Watson.

Some lessons might be taken from Michigan and Texas, which both already had an opt-in process analogous to the one required by the reauthorization of the Newborn Screening Saves Lives Act when those changes went into effect. Michigan implemented its statewide consent policy in October 2010, according to Carrie Langbo, coordinator of Michigan BioTrust for Health. Based in Lansing, the BioTrust is a program that oversees the state's stored blood spots and their use in research. Langbo and the BioTrust worked extensively with prenatal care providers through discussion and on-site training to ensure the smooth adoption of consent regulations for newborn research.

Training and educating the birthing staff allowed the hospitals in Michigan to swiftly adopt opt-in consent. "Setting up the whole consent process was not a trivial matter," Langbo says. "It's optimal to receive education on newborn screening prenatally and then ensure that after delivery, all birthing attendants and staff have the information to provide prior to discharge." It took Michigan over two years to conduct this sort of training before the consent policies were adopted as law.

"We did really intensive training with the hospital staff back when we were first implementing the new [consent] regulations in Michigan, but it never really ends," says Jennifer Smith, a spokeswoman at the BioTrust for Health. "Continual reinforcement of the importance of newborn screening is really needed."

Wudan Yan

Microbiome models, on computers and in lab dishes, see progress

In the three years since the completion of the first phase of the Human Microbiome Project, the number of scientific papers linking the microbes that live in our gut to diseases ranging from diabetes and colitis to anxiety and depression has grown exponentially. Yet, these tantalizing connections have yielded few benefits from a therapeutics standpoint.

A major reason for this may be because researchers exploring the gut flora have struggled to find effective model systems within which to study the nature of these gut microbes. Now, however, mouse models and *in vitro* systems, along with new computational modeling, are being used by scientists to better observe how microbial populations influence the onset and progression of disease. Additionally, some models now look beyond bacterial populations to also include fungi and viruses that reside within hosts. The hope is that these updated models will shed light on microbiome mechanisms in a controlled laboratory environment and enable testing of therapeutics, ultimately leading to effective interventions in humans.

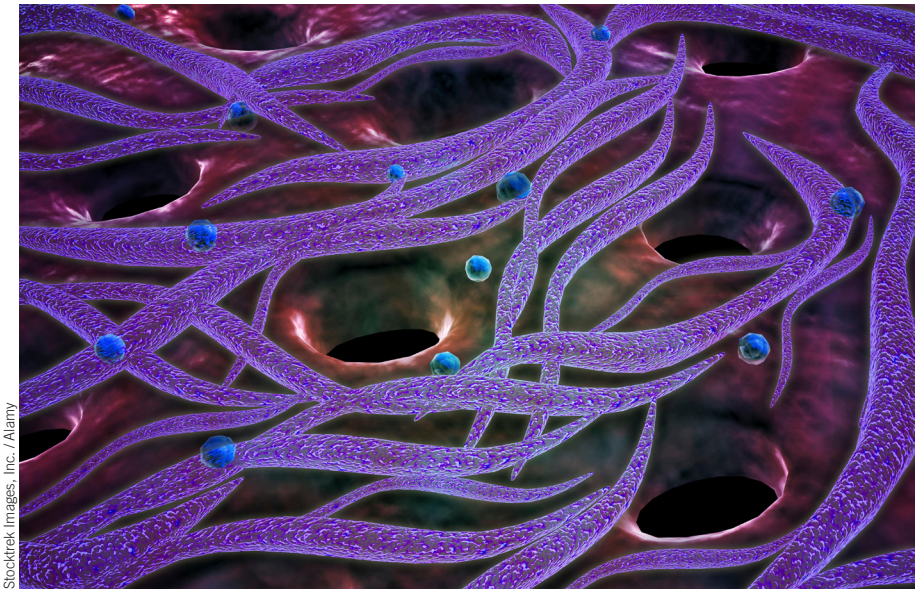
In recent years, the field of microbiome research has relied on metagenomics, which allows scientists to directly analyze genetic material from organisms without culturing in the lab. However, even though metagenomics gives researchers an impression of a microbial population's potential capabilities based on the genes the bacteria possess, it doesn't offer much information about when and where these genes produce proteins and how the bacteria actually interact with the human body.

One answer to this problem comes from the related field called integrated omics. Integrated omics combines metagenomics with metatranscriptomics, metaproteomics and metabolomics, which look at the relative abundance of RNA transcripts, protein products, and metabolites, respectively. "Integrated omics can put metagenomics results in a different light," says Willem de Vos, a microbiologist at Wageningen University in the Netherlands. He recalls a study in which the authors showed that obese people had a low abundance of *Bacteroidetes*, a major bacterial phylum[1]. "With metaproteomics we showed that these *Bacteroidetes* compensate their low abundance by being very metabolically active," says de Vos, about recent work that has yet to be published. This activity component is crucial, as the protein products and metabolites produced by microorganisms in the body largely mediate their influence.

To study causal effects between the gut microbes and their hosts, experimental models that can be manipulated and controlled are needed. For example, the Simulator of the Human Intestinal Microbial Ecosystem (SHIME), a model intestinal system consisting of glass bioreactors connected with tubes simulates the stomach, small intestine and three compartments of the large intestine. Developed at Belgium's Ghent University, it allows researchers to study how food compounds metabolize over a period of several weeks. Initially, SHIME, like other *in vitro* models, lacked human cells, ruling out the researchers' ability to study the interactions between the human host and its resident microbes, but recently a layer of human mucosal and intestinal cells was added to the system. The system is also seeded with bacteria from the human gut[2].

Another promising tool is the organoid model, a three-dimensional bud made from mouse or human cells that often mimics the function of a full-fledged organ, in this case the small intestine. As such, researchers are able to observe, albeit on a smaller scale, the mechanisms that govern how microbes influence the environment in which they live. Specific bacterial populations can be injected into the organoids to study the interaction of these microbes with the intestinal wall, as was done recently with two species of bacteria commonly found in the human gut[3]. The researchers measured which genes were switched on in the mouse organoid and found that one of the species, *Akkermansia muciniphila*, switched on fatty acid metabolism. This, they believe, gives this bacterial species potential value as a weight loss probiotic. Similarly, organoids can also be used to study the effects of pharmaceutical and nutritional compounds on the gut epithelium.

These models, however, lack full-scale blood circulation and an immune system within which to record responses. One tool being developed to address this issue comes from a team led by Paul Wilmes at the University of Luxembourg. They developed a microfluidics-based *in vitro* model that has three distinct culture chambers separated by semipermeable membranes: one for microbial cells, one for human epithelial cells and the third for human immune cell cultures. The system allows for control of environmental factors, including nutrient concentrations, pH and mucin compositions, in an attempt to mimic the conditions in the human body[4].



Stocktrek Images, Inc. / Alamy

Gut response: *Lactobacillus acidophilus* bacteria occur naturally in the human GI tract.

Mouse models

Almost all animal studies in microbiome research are conducted in germ-free mice. These mice, which are raised under sterile conditions, have guts that can be colonized with microbial populations obtained from healthy humans or individuals with specific diseases such as type 2 diabetes or rheumatoid arthritis. Introducing gut bacteria from obese individuals into these mice, for example, induced obesity in the animals, helping to provide some evidence for a causal link between the microbes and weight gain[5]. *In vivo* mouse models also allow researchers to study the effect of the microbiome on distant organs.

However, it remains difficult to translate results from mouse experiments to humans: the topology of the mouse gastrointestinal tract differs from that in humans, as does their diet and lifestyle, of course. Germ-free mice also have an underdeveloped immune system because gut microbes have a crucial role in the proper development of an immune system, notes Fredrik Bäckhed, a molecular biologist at University of Gothenburg in Sweden. Ultimately, to truly establish causality between an intervention that changes gut bacteria and its influence on human disease, "you have to move to human studies," he says.

Before making the crucial transition to clinical studies, however, researchers now also have the ability to make predictions using computational modeling. It took time to gather an appreciation for the importance of this expertise, says Elhanan Borenstein, a systems biologist at the University of Washington. "Computational models are often said to be incomplete and therefore not useful. But we have to start somewhere, and we can still learn a lot from such simple models. As systems biologists we are very aware of what we leave out."

Borenstein uses integrated omics in his models to gain a mechanistic understanding of the microbiome. "Our goal has always been not just to develop a predictive 'black-box', but to capture the mechanisms behind various processes that occur in the microbiome," he says. His team has published models describing the interaction between pairs of bacterial species in the gut microbiome. In one such example, Borenstein and his team demonstrated that bacterial species tended to co-occur more frequently with competing species, informing how networks of species are created across the microbiome[6]. He says that a new, unpublished model from his group that includes multiple bacterial species can predict changes in the composition of the gut community during perturbations such as dietary changes.

Beyond bacteria

In addition to getting a better sense of bacterial behavior in the body, microbiome models are also now being used to consider the influence of organisms beyond bacteria—such as viruses, fungi and other eukaryotic microbes. Until recently, owing to a lack of software, information databases to measure and identify various species as well as the know-how to extract, from a single sample, genetic material for species beyond bacteria, these nonbacterial pathogens were largely ignored. "As a field, we've gotten trapped in measuring what we can, rather than what we should," says Skip Virgin, a molecular pathologist at Washington University in St. Louis, who studies the human virome, the collection of viruses in and on our bodies. His team recently showed that viral infections can benefit mice, and that viruses interact with our bacteria in many ways[7]. "The tools are there now to study these viruses, so it's a mistake to dismiss them," Virgin says.

Similarly, Christen Rune Stensvold's group at State Serum Institute in Copenhagen is validating software for gut microbiota analysis including eukaryotic parasites. Stensvold's group is also contributing to the curation of databases for eukaryotic microbes, many of which are still incomplete, further delaying work in elucidating how these organisms influence diseases. "We now know that fungi and other parasites commonly make up stable microbial communities, but most scientists still ignore them in microbiota analyses," he says. Lean individuals, for instance, tend to carry the eukaryotic parasite *Blastocystis* more than obese people, suggesting that these organisms may prevent weight gain, though a causal link has not been established[8].

Despite these attempts at improving and expanding models to better study the microbiome, a remaining question is when a model is considered good enough. "Eventually, it should be accurate enough to predict the system's behavior under different perturbations," Borenstein says. "The microbiome is subject to manipulations. A successful microbiome model should be used to design targeted interventions."

A good way to move from a correlation to an intervention, Bäckhed suggests, would be to first colonize germ-free mice with a microbial population that mimics that seen in humans with a specific disease. "If the mice develop the disease, you set up *in vitro* studies to find out which signaling pathways are being triggered in the intestine. Then, you have to be bold enough to design an intervention in humans, such as a fecal transplantation or a more specific cocktail of microbes."

Many of the models put forth to study the microbiome remain in the experimental stage, and it's not yet clear which ones will be preferred as the field matures. No matter how well designed, one should never forget that a model is just a hypothesis, De Vos says. "A very good model, is still only a very good hypothesis. The ultimate proof of the pudding is in the eating."

Jop de Vrieze

1. Ley, R., Turnbaugh, P.J., Klein, S. & Gordon, J.I. *Nature* **444**, 1022–1023 (2006)
2. Marzorati, M. *et al. BMC Microbiology*, **14**, 133 (2014).
3. Lukovac, S. *et al. mBio*, **5**, e01438-14 (2014).
4. Fritz, J.V. *et al.* Microbiome, 1, 14 (2013).
5. Ridaura, V.K. *et al. Science,* **341**, http://dx.doi.org/10.1126/science.1241214 (2013).
6. Levy, R. & Borenstein, E. *Proc. Natl. Acad. Sci.*, **110**, 12804–12809 (2013).
7. MacDuff, D.A. *et al. eLife,* http://dx.doi.org/10.7554/eLife.04494 (2015).
8. Scanlan, P.D. *et al. FEMS Microbiol Ecol.*, **90**, 326–330 (2014).