in various animal models indicate that translocation of bacterial components, including bacterial lipopolysaccharides, across a leaky gut barrier triggers low-grade inflammation, which contributes to insulin resistance. Mice deficient in TLR5 exhibit alterations in the gut microbiota and hyperphagia, and they develop features of metabolic syndrome, including hypertension, hyperlipidemia, insulin resistance, and increased adiposity.

The gut microbiota regulates biosynthesis as well as metabolism of host-derived products; these products can signal through host receptors to shape host physiology. An example of this symbiosis is provided by bile acids, which direct metabolic effects that are largely mediated through the farnesoid X receptor (FXR, also known as NR1H4). In leptin-deficient mice, FXR deficiency protects against obesity and improves insulin sensitivity. In mice with diet-induced obesity that are subjected to vertical sleeve gastrectomy, the surgical procedure results in elevated levels of circulating bile acids, changes in the gut microbiota, weight loss, and improved glucose homeostasis. However, weight reduction and improved insulin sensitivity are mitigated in animals with engineered FXR-deficiency.

**Xenobiotic Metabolism** Evidence is accumulating that pharmacogenomic studies need to consider the gene repertoire present in our *H. sapiens* genome as well as that in our microbiomes. For example, digoxin is inactivated by the human gut bacterium *Eggerthella lenta,* but only by strains with a cytochrome-containing operon. Expression of this operon is induced by digoxin and inhibited by arginine. Studies in gnotobiotic mice established that dietary protein affects (reduces) microbial metabolism of digoxin, with corresponding alterations in levels of the drug in both serum and urine. These findings reinforce the need to consider strain-level diversity in the gut microbiota when examining interpersonal variations in the metabolism of orally administered drugs.

**Characterizing the Effects of the Human Microbiota on Host Biology in Mice and Humans** Questions about the relationship between human microbial communities and health status can be posed in the following general format: Is there a consistent configuration of the microbiota definable in the study population that is associated with a given disease state? How is the configuration affected by remission/relapse or by treatment? If a reconfiguration does occur with treatment, is it durable? How is host biology related to the configuration or reconfiguration? What is the effect size? Are correlations robust to individuals from different families and communities representing different ages, geographic locales, and lifestyles?

As in all studies involving human microbial ecology, the issue of what constitutes a suitable reference control is extremely important. Should we choose the person himself or herself, family members, or age- or gender-matched individuals living in the same locale and representing similar cultural traditions? Critically, are the relationships observed between microbial community structure and expressed functions a response to disease state (i.e., side effects of other processes), or are they a contributing cause? In this sense, we are challenged to evolve a set of Koch's postulates that can be applied to whole microbial communities or components of communities rather than just to a single purified organism. As in other circumstances in which experiments to determine causality of human disease are difficult or unethical, Hill's criteria, which examine the strength, consistency, and biologic plausibility of epidemiologic data, can be useful.

Sets of mono- and dizygotic twins and their family members represent a valuable resource for initially teasing out relationships between environmental exposures, genotypes, and our own microbial ecology. Similarly, monozygotic twins discordant for various disease states enhance the ability to determine whether various diseases can be linked to a person's microbiota and microbiome. A twin-pair sampling design rather than a conventional unrelated case–control design has advantages owing to the pronounced between-family variability in microbiota/microbiome composition and the potential for multiple states of a community associated with disease. Transplantation of a microbiota from suitable human donor controls representing different disease states and communities (e.g., twins discordant for a disease) to germ-free mice is helpful in establishing a causal role for the community in pathogenesis and for providing insights relevant to underlying mechanisms. In addition, transplantation provides a preclinical platform for identifying next-generation probiotics, prebiotics, or combinations of the two (*synbiotics*). Obesity and obesity-associated metabolic dysfunction illustrate these points.

The gut microbiotas (and microbiomes) of obese individuals are significantly less diverse than those of lean individuals; the implication is that there may be unfilled niches (unexpressed functions) that contribute to obesity and its associated metabolic abnormalities. Le Chatelier and colleagues observed a bimodal distribution of gene abundance in their analysis of 292 fecal microbiomes: low-gene-count (LGC) individuals averaged 380,000 microbial genes per gut microbiome, while high-gene-count (HGC) individuals averaged 640,000 genes. LGC individuals had an increased risk for type 2 diabetes and other metabolic abnormalities, whereas the HGC group was metabolically healthy. When gene content was used to identify taxa that discriminated HGC and LGC individuals, the results revealed associations between anti-inflammatory bacterial species such as *Faecalibacterium prausnitzii* and the HGC group and between proinflammatory species such as *Ruminococcus gnavus* and the LGC group. LGC microbiomes had significantly greater representation of genes assigned to tricarboxylic acid cycle modules, peroxidases, and catalases—an observation suggesting a greater capacity to handle oxygen exposure and oxidative stress; HGC microbiomes were enriched in genes involved in the production of organic acids, including lactate, propionate, and butyrate—a result suggesting increased fermentative capacity.

Transplantation of an uncultured fecal microbiota from twins stably discordant for obesity or of bacterial culture collections generated from their microbiota transmits their discordant adiposity phenotypes as well as obesity-associated metabolic abnormalities to recipient germ-free mice. Co-housing of the recipient coprophagic gnotobiotic mice results in invasion of specific bacterial species from the transplanted lean twin's culture collection into the guts of cage mates harboring the obese twin's culture collection (but not vice versa), thereby preventing the latter animals from developing obesity and its associated metabolic abnormalities. It is noteworthy that invasion and prevention of obesity and metabolic phenotypes are dependent on the type of human diets fed to animals: prevention is associated with a diet low in saturated fats and high in fruit and vegetable content, but not with a diet high in saturated fats and low in fruit and vegetable content.

This approach provides evidence for a causal role for the microbiota in obesity and its attendant metabolic abnormalities. It also provides a method for defining unoccupied niches in disease-associated microbial communities, the role of dietary components in determining how these niches can be filled by human gut–derived bacterial taxa, and the effects of such occupancy on microbial and host metabolism. It also provides a way to identify health-promoting diets and next-generation probiotics representing naturally occurring members of our indigenous microbial communities that are well adapted to persist in a given body habitat.

A key to this approach is the ability to harvest a microbial community from a donor representing a physiology, disease state, lifestyle, or geography of interest; to preserve the donor's community by freezing it; and then to resurrect and replicate it in multiple recipient gnotobiotic animals that can be reared under conditions where environmental and host variables can be controlled and manipulated to a degree not achievable in clinical studies. Since these mice can be followed as a function of time prior to and after transplantation, in essence, a snapshot of a donor's community can be converted into a movie. Transplantation of intact uncultured human (fecal) microbiota samples from multiple donors representing the phenotype of interest, with administration of the donors' diets (or derivatives of those diets) to different groups of mice, is one way to assess whether transmissible responses are shared features of the microbiota or are highly donor specific. A second step is to determine whether the culturable component of a representative microbiota sample can transmit the phenotype(s) observed with the intact uncultured sample. Possession of a collection of cultured organisms that have co-evolved in a given donor's body habitat sets the stage