Building a Translational Microbiome Toolbox

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http://dx.doi.org/10.1016/j.cell.2017.04.009

Designing successful microbiota-based therapies requires in-depth understanding of the ecological foundations of this community. In this issue, two studies by Whitaker et al. and Lim et al. provide refined genetic tools for dissecting the spatial organization and temporal dynamics of bacterial communities at the single-cell and -gene levels.

Progress in biomedical science has a long history of impacting translational research. This continuous cycle often begins with human clinical observations, moves onto mechanistic findings derived from animal models, and then circles back to development of novel therapeutics to ameliorate or treat human conditions. Microbiome research has launched onto this translational circle at a rapid pace with rich datasets demonstrating the existence of associations between dysbiotic microbial communities and human disorders such as obesity, diabetes, colitis, pathogen susceptibility, and colon cancer (Fischbach and Segre, 2016; Gilbert et al., 2016). The challenge now is to understand how changes in microbial communities impact disease states and then to use this knowledge to guide rational manipulations of these communities that could improve human health. Given the tremendous diversity, along with temporal, spatial, and interpersonal variation displayed by bacterial communities (Ursell et al., 2012), it is imperative for mechanistic insights to be sought within the context of microbial complexity. In this issue of *Cell*, Sonnenburg and Goodman's research groups report the development of powerful tools that will help to accelerate these mechanistic studies (Whitaker et al., 2017; Lim et al., 2017). Through different approaches, they achieve precise modulation of gene expression in the predominant human gut commensal Bacteroides in vivo, in the context of complex communities.

Distinct from the much-manipulated *E. coli*, Bacteroides evolved mechanisms for regulating gene expression at the level of transcription and translation. Both groups focus on developing modules that provide a dynamic range of gene expression and function across diverse commensal Bacteroides species. To achieve this, they identify broadly conserved consensus sequences computationally and functionally, and then, they use them to construct promoter-ribosomal binding site (RBS) combinations, which can be then tested with reporter assays (Figure 1).

Whitaker et al. (2017) developed clever modifications to gene transfer protocols, adapting the Golden Gate cloning technology, to introduce single-integration vectors into Bacteroides in a high-throughput fashion and achieve high constitutive expression of reporter genes. The researchers confirm that this ectopic expression does not come at the cost of bacterial fitness, even in the presence of a complex microbiota. Importantly, this gene expression is truly constitutive at all gut locations and under all culture conditions tested. A major breakthrough of this work is the adaptation of this method to express fluorescent proteins at high enough levels to allow in vivo imaging of fluorescently tagged Bacteroides strains. Sequence-based studies, especially of stool, suffer the drawback that they lose critical information about gut biogeography, which may hold important cues about bacterial functions. For example, distinct microbial signatures have been reported for mucosal versus lumen-associated gut communities, with nutrients, microbial interactions, and tolerance to various host factors thought to be key factors dictating this spatial heterogeneity (Donaldson et al., 2016).

The genetic system developed by Whitaker et al., 2017 opens the door for studying single-cell interactions and understanding spatial organization of the gut microbiota. The authors use this technique to provide direct, visual evidence of colonic crypt occupancy by Bacteroides, which confers species-specific colonization resistance against an incoming strain (Lee et al., 2013). Isogenic strains of *B. thetaiotaomicron* are labeled using red fluorescent protein or green fluorescent protein, which enables their differentiation with microscopy. These bacteria are then gavaged, either simultaneously or sequentially, in equal numbers, to germ-free mice. Co-gavaging results in equal abundance of the two strains in lumen and crypts; however, with sequential introduction, the strain gavaged at a later point is nearly absent from the gut lumen. This exclusion is even more pronounced in the epithelial crypts, indicating the importance of crypt colonization for niche establishment. While the previous studies established the ecologic principle, fine-tuned genetic manipulation demonstrated the biogeography of strain specificity, essential knowledge when considering probiotic or prebiotic therapies.

To study the function of a protein in its native environment, it is important to control and fine-tune its regulation by first uncoupling it from its native regulatory machinery and then placing it under the regulation of an exogenous inducer. Previous attempts at designing inducible gene expression systems in Bacteroides relied on native promoters that are responsive to dietary glycans (Hamady et al., 2008; Mimee et al., 2016). The system would be much improved if both the host and endogenous microbiota are essentially blind to the inducer molecule, so that phenotypic alterations could be more directly ascribed both temporally and kinetically to the altered gene's function. With this aim in mind, Lim et al. (2017) adopt the classical Tet expression system from *E. coli* into Bacteroides, which allows induction of native genes

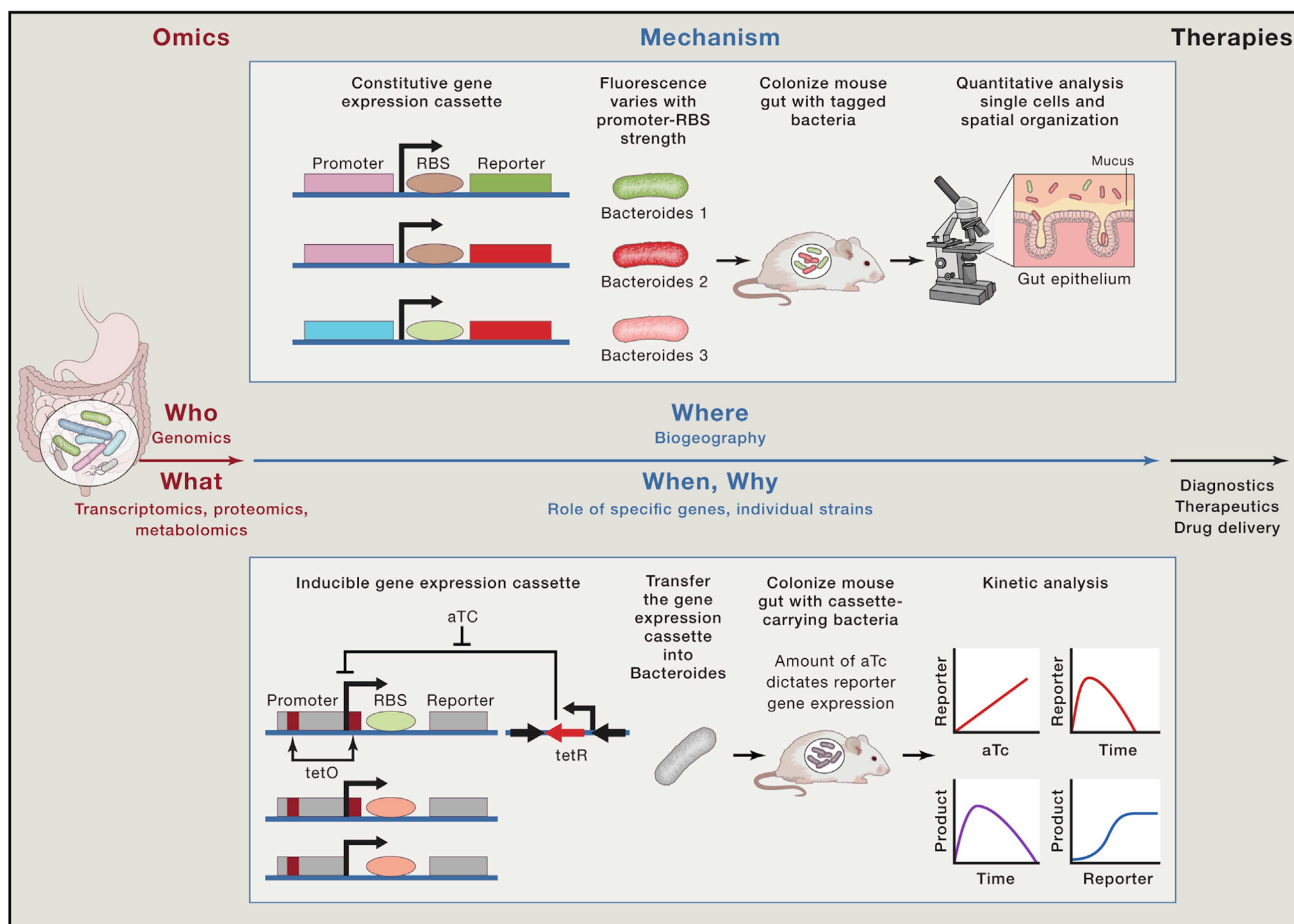

Figure 1. The Translational Microbiome Pipeline

Moving from large datasets associating alterations in microbial community to microbial-based therapeutics requires the foundational knowledge gained in animal models asking the 5 Ws: who, what, when, where, and why.

using synthetic anhydrotetracycline (aTc). Integrating modules with regulated gene expression into strains with the native gene deleted, one can achieve graded expression by varying aTc concentrations. Testing their system both in vitro and in vivo (in the mouse intestine colonized with complex microbiota), the authors convincingly show that their gene expression system truly functions as an independent unit. Furthermore, varying the levels of aTc modulates gene expression over four orders of magnitude, with full repression in the absence of aTc and increasing to wid-type levels with aTc. This fine-tuning of gene expression is especially important when studying genes whose products are either essential or toxic to the cell.

To demonstrate the utility of their inducible system, Lim et al. (2017) focus their attention on understanding the kinetics of sialic acid release from the intestinal epithelium in the presence of sialidase producing gut commensals. Free sialic acid is a crucial nutrient that enables gut establishment by pathogens upon antibiotic treatment (Ng et al., 2013). Understanding the enzyme kinetics that regulate sialic acid liberation and its consumption by the gut microbes in vivo will help in the identification of factors that govern this clinically relevant dynamic. For this purpose, the authors choose *B.thetaiotaomicron*, a bacterium that liberates, but does not utilize, sialic acid (using sialidase BT0455). An inducible cassette encoding BT0455 is transferred into a mutant strain lacking native sialidase. Sialidase activity can now be varied through aTc fed to gnotobiotic mice monocolonized with the inducible strain. Monitoring the levels of sialidase and free sialic acid in feces demonstrates a non-linear relationship, with free sialic acid levels plateauing at 25% of the enzyme activity. Conventional mice also show high sialidase activity; interestingly, the authors can readily detect free sialic acid, indicating limited sialic acid consumption by the native commensals, even when it is present in high quantities in the gut. The results reveal an imbalance that is exacerbated by antibiotics, which can decrease sialic acid catabolism without a proportional impact on sialic acid production. While the phenomenon could be studied with previous methods, the new tight temporal gene expression demonstrates when would be a crucial window of opportunity for future therapeutic intervention.

Despite the enormous excitement, the potential of the microbiota to benefit multiple aspects of human health is limited by deep mechanistic studies that demonstrate causal relationships. These resources open the door to studying the complexity of the who, what, where, when, and why of host-microbiome interactions (Figure 1).

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