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provide evidence that profiling of variability in chromatin state is rapidly extending its reach both broadly across individuals and deeply into smaller amounts of primary tissues (Table 1). While measurement of sequence variation at both the individual and population levels is a necessary prerequisite for unraveling genetic contributions to diseases and traits, all genetic variation is ultimately interpreted in a cellular epigenetic context. The expanded application of chromatin profiling across cell-type, genetic, and cellular axes will surely offer novel insight into the precise molecular characterization of cellular diversity and its relationship to human traits and diseases.

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REFERENCES

Adey, A., Morrison, H.G., Asan, Xun, X., Kitzman, J.O., Turner, E.H., Stackhouse, B., MacKenzie, A.P., Caruccio, N.C., Zhang, X., and Shendure, J. (2010). Genome Biol. *11*, R119.

Buenrostro, J.D., Giresi, P.G., Zaba, L.C., Chang, H.Y., and Greenleaf, W.J. (2013). Nat. Methods *10*, 1213–1218.

Buenrostro, J.D., Wu, B., Litzenburger, U.M., Ruff, D., Gonzales, M.L., Snyder, M.P., Chang, H.Y., and Greenleaf, W.J. (2015). Nature. Published online June 17, 2015.

Buganim, Y., Faddah, D.A., Cheng, A.W., Itskovich, E., Markoulaki, S., Ganz, K., Klemm, S.L., van Oudenaarden, A., and Jaenisch, R. (2012). Cell *150*, 1209–1222.

Cusanovich, D.A., Daza, R., Adey, A., Pliner, H.A., Christiansen, L., Gunderson, K.L., Steemers, F.J., Trapnell, C., and Shendure, J. (2015). Science *348*, 910–914.

Davis, R.L., Weintraub, H., and Lassar, A.B. (1987). Cell *51*, 987–1000.

Degner, J.F., Pai, A.A., Pique-Regi, R., Veyrieras, J.-B., Gaffney, D.J., Pickrell, J.K., De Leon, S., Michelini, K., Lewellen, N., Crawford, G.E., et al. (2012). Nature *482*, 390–394.

Eldar, A., and Elowitz, M.B. (2010). Nature *467*, 167–173.

Gebhardt, J.C.M., Suter, D.M., Roy, R., Zhao, Z.W., Chapman, A.R., Basu, S., Maniatis, T., and Xie, X.S. (2013). Nat. Methods *10*, 421–426.

Graf, T., and Enver, T. (2009). Nature *462*, 587–594.

Gross, D.S., and Garrard, W.T. (1988). Annu. Rev. Biochem. *57*, 159–197.

Iliopoulos, D., Hirsch, H.A., and Struhl, K. (2009). Cell *139*, 693–706.

Kelly, T.K., Liu, Y., Lay, F.D., Liang, G., Berman, B.P., and Jones, P.A. (2012). Genome Res. *22*, 2497–2506.

Klein, A.M., Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Li, V., Peshkin, L., Weitz, D.A., and Kirschner, M.W. (2015). Cell *161*, 1187–1201.

Macosko, E.Z., Basu, A., Satija, R., Nemesh, J., Shekhar, K., Goldman, M., Tirosh, I., Bialas, A.R., Kamitaki, N., Martersteck, E.M., et al. (2015). Cell *161*, 1202–1214.

Maurano, M.T., Humbert, R., Rynes, E., Thurman, R.E., Haugen, E., Wang, H., Reynolds, A.P., Sandstrom, R., Qu, H., Brody, J., et al. (2012a). Science *337*, 1190–1195.

Maurano, M.T., Wang, H., Kutyavin, T., and Stamatoyannopoulos, J.A. (2012b). PLoS Genet. *8*, e1002599.

McDaniell, R., Lee, B.-K., Song, L., Liu, Z., Boyle, A.P., Erdos, M.R., Scott, L.J., Morken, M.A., Kucera, K.S., Battenhouse, A., et al. (2010). Science *328*, 235–239.

Ostuni, R., Piccolo, V., Barozzi, I., Polletti, S., Termanini, A., Bonifacio, S., Curina, A., Prosperini, E., Ghisletti, S., and Natoli, G. (2013). Cell *152*, 157–171.

Qu, K., Zaba, L.C., Giresi, P.G., Li, R., Longmire, M., Kim, Y.H., Greenleaf, W.J., and Chang, H.Y. (2015). Cell Syst. *1*, this issue, 51–61.

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B.B., Siddiqui, A., et al. (2009). Nat. Methods *6*, 377–382.

Thurman, R.E., Rynes, E., Humbert, R., Vierstra, J., Maurano, M.T., Haugen, E., Sheffield, N.C., Stergachis, A.B., Wang, H., Vernot, B., et al. (2012). Nature *489*, 75–82.

Voss, T.C., and Hager, G.L. (2014). Nat. Rev. Genet. *15*, 69–81.

A Toolbox for Microbiome Engineering

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Genetic tools to engineer a prominent member of the human gut microbiome represent initial steps toward cell-based diagnostics and therapeutics.

Obesity, diabetes, colon cancer, and inflammatory bowel disease have all been correlated with changes in the composition of the human gut microbiome (Cho and Blaser, 2012), but understanding, diagnosing, and therapeutically treating gut dysbioses will require more sophisticated tools than what we currently possess. In this issue, Mimee et al. (Mimee et al., 2015) develop a synthetic biology toolbox for engineering a non-model, prominent member of the human gut microbiome, *Bacteroides thetaiotaomicron,* to accurately detect and precisely respond to gut-localized signals. This work provides a platform for engineering this important bacterium to perform useful tasks, and an example of how synthetic biology tools developed in model organisms can be adapted for non-model, biologically relevant organisms.

Scientists studying the human microbiome have lately shifted their focus from describing its correlations with disease to understanding the underlying causes of these correlations at the molecular level. Elegant studies using microbiome transplantation in mice provide strong support for the hypothesis that changes in the

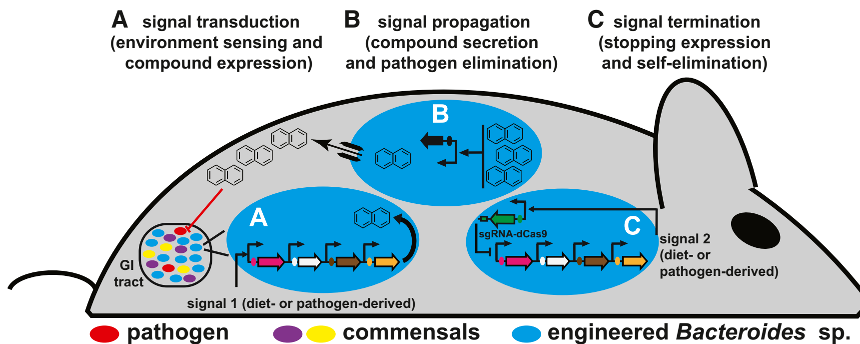

Figure 1. Engineering *Bacteroides* sp. to Eradicate a Pathogen through the Production of a Small Molecule Antibiotic

(A–C) In this hypothetical application of the toolbox described by Mimee et al., a biosynthetic gene cluster (red, white, brown, and yellow genes) for the synthesis and secretion of a small molecule antibiotic is engineered in a *Bacteroides* strain. (A) Signal transduction. The initial expression of the gene cluster is triggered after sensing a carbohydrate signal administered through diet or a pathogen-derived small molecule. Different combinations of promoters and ribosome binding sites control the expression of different genes in the gene cluster, to produce the antibiotic compound at a desired level. (B) Signal propagation. A circuit that activates after a specified amount of time, or a circuit that responds to high intracellular levels of the produced molecule, controls the expression of the export machinery (black gene). Once secreted, the small molecule antibiotic eliminates the pathogen. (C) Signal termination. Finally, a CRISPR interference circuit (green gene and green RNA) stops the expression of the gene cluster in response to a second diet-based carbohydrate signal, or to the absence of the pathogen-derived small molecule. The initiation of each of the steps in this example can be recorded using a genetic memory array (not shown).

microbiome play an active role in disease rather than simply being a consequence of it. For example, transfer of the microbiome alone from mice genetically induced to have ulcerative colitis to non-genetically predisposed recipients is sufficient to induce inflammation (Garrett et al., 2007). The growing evidence for a role of the microbiome in disease not only inspires detailed mechanistic studies to identify the specific microbes, genes, and molecules involved, but also highlights the potential value of manipulating the microbiome to diagnose and treat diseases.

Efforts to manipulate the composition and function of the microbiome have been ongoing for decades. These include the introduction of new members to the community through the use of probiotics and fecal transplantation, for example, and the removal of unwanted members by methods such as antibiotics and intestinal lavage. However, these manipulation strategies are nonspecific, and often result in unpredictable outcomes.

More recently, targeted strategies have been employed where one bacterium is engineered to perform a very specific function in the gastrointestinal tract. These functions range from secreting a therapeutic molecule (mostly peptides and small proteins) to detecting a particular signal, such as small molecules derived from other bacteria, food, or cancerous or inflamed tissues (Claesen and Fischbach, 2015).

Notably, almost all of these strategies have been used in model organisms that either transiently colonize the gastrointestinal tract (*Lactococcus lactis*), or are naturally present as minor members of the healthy microbiome (*Escherichia coli*). The drawback of using such organisms is the need to constantly administer the engineered bacterium if a long-term effect is sought and to introduce it in a relatively high titer. Although engineering common members of the microbiome is an attractive solution to these problems, the lack of genetic tools for these non-model organisms has hampered their development as alternative chassis for manipulating the microbiome.

A successful microbial diagnostic or therapeutic agent must to able to detect a particular signal with high fidelity, integrate this signal through precise intracellular circuitry, and respond to this signal at the appropriate level. Mimee et al. describe genetic tools that allow *B. thetaiotaomicron* to efficiently perform all three of these functions.

For signal detection, Mimee et al. exploit three transcriptional circuits naturally used by *Bacteroides* for detecting and responding to different carbohydrates and further determine the fold change in gene expression achieved upon their usage. The authors also engineer a LacO (operator)/LacI (repressor) pair, previously adapted from *E. coli* for use in *Bacteroides*, to obtain two IPTG-inducible promoters with different induction profiles. Importantly, Mimee et al. show that these four systems are orthogonal and that they can be induced in response to four different carbohydrates (rhamnose, chondroitin sulfate, arabinolgalactan, and IPTG) with no crosstalk between them. These inducible systems can elicit a transcriptional response that spans a range of 8- to 104-fold change in gene expression.

As an example of signal integration and propagation, Mimee et al. use CRISPR interference, in which an RNA-guided, catalytically inactive Cas9 protein binds to DNA and blocks transcription, to specifically knockdown the expression of certain genes in response to IPTG induction. In addition, they implement a carbohydrate-inducible memory switch using a DNA memory array that was previously constructed in *E. coli*. Briefly, this memory switch makes use of a series of serine integrases that catalyze DNA inversion events at cognate recognition sequences in a specific and unidirectional fashion. When inducible, these binary switches can efficiently record the exposure of the cell harboring the memory array to different cues in the gastrointestinal tract, allowing scientists to learn the molecular details of a bacterium's life.

Finally, for fine control over response to stimuli, the authors constructed and characterized a library of constitutive promoters and ribosome binding sites that spans 10^4-fold expression levels. Notably, they show that circuits integrating signal detection, genetic memory, and CRISPR interefence function as expected when engineered *B. thetaiotaomicron* is introduced into the gut microbiome of mice.

The applications of this set of tools are enormous and break ground for a new era of engineered chassis organisms that can be used to build microbiome-based diagnostics and therapeutics. In the future, one can imagine the use of different pairs of promoters and ribosome binding sites to tightly regulate the expression of different genes in a biosynthetic gene cluster for a small molecule therapeutic (e.g., an antibiotic), engineered in a microbiome-derived *Bacteroides* strain (Figure 1). The in vivo expression of this

gene cluster could be controlled by the level of a carbohydrate administered in the diet, or preferably, by a specific small molecule produced by the target pathogen itself. Decoupling of the synthesis and secretion of the small molecule (e.g., to reach an effective local therapeutic dose) can be achieved by putting the export machinery under the control of an inducible circuit that responds only to high intracellular levels of the small molecule, or by engineering a time delay between the synthesis and secretion of the molecule. Once the therapeutic effect has been achieved (e.g., the elimination of a pathogen), CRISPR interference can be used to knock down residual expression of the therapeutic genes or to eliminate the chassis itself by targeting an essential gene. This final step could be triggered by a second signal administered in diet, or by the absence of the pathogen-derived small molecule. This entire series of events could be recorded on memory switches and read through analysis of the *Bacteroides* genome in host feces, providing timely snapshots of what is happening in vivo.

Although it is still early days for its approval, using engineered commensals to produce therapeutic molecules (as in the aforementioned scenario) may be preferred over using oral or systemic drugs for several reasons. First, commensals naturally occupy specific niches in the gastrointestinal tract, allowing drug delivery to a very defined site. Subsequently, the dosage needed to obtain a local therapeutic effect would be much lower than needed if orally administered, and many adverse effects could in turn be eliminated. Second, because the production of a therapeutic molecule can be precisely controlled in engineered bacteria, long-term control of diseases can be achieved using a single organism that produces the drug only when needed. This is reminiscent to the currently sought after "artificial pancreas" for managing type 1 diabetes, in which a continuous glucose monitoring system is paired with an insulin pump and a small computer that determines and triggers the delivery of the needed insulin dose based on the current glucose level. Last, using an engineered bacterium to produce and deliver one or more therapeutic molecules could provide an economical alternative to the costly production, formulation, distribution, and storage of drugs. This is even more applicable in the cases where a drug is specially formulated or administered via intramuscular or subcutaneous injection to avoid degradation in the stomach.

Beyond diagnostics and therapeutics, there are clear advantages of using a cell-based delivery system for basic research. For example, there are no good models available for studying the precise effects of the in situ production of pathogen- or commensal-derived antibiotics on the composition of the microbiome. A microbial chassis that encodes an engineered biosynthetic gene cluster for the production of a small molecule antibiotic, and several circuits for sensing and recording its local environment, will enable scientists to measure these effects in a timely and localized manner.

More genetic tools will be needed in the future. For example, genetic circuits need to be engineered to detect non-carbohydrate signals. Systems that respond to pathogen-derived small molecules (Saeidi et al., 2011) and the inflammatory marker nitric oxide (Archer et al., 2012), were developed previously in *E. coli* and can possibly be adapted for use in *Bacteroides*. Additionally, self-elimination systems, working through lysis (Kong et al., 2008) or auxotrophy (Steidler et al., 2003), will also be important to satisfy safety concerns.

Genetic tools for other prominent gut bacteria also need to be developed. Notably, the two bacterial phyla that dominate the gut microbiome of healthy humans are the Bacteroidetes (the phylum in which *Bacteroides* sp. reside), and the Firmicutes. These two phyla occupy distinct niches of the human intestine, and perform different activities (Mahowald et al., 2009). Unlike the Bacteroidetes, most prominent Firmicutes, such as *Closridium* sp. and *Ruminococcus* sp., are not easily amenable to genetic manipulations and are not well studied with respect to regulation and signal transduction. Like *B. thetaiotaomicron* for the Bacteroidetes, developing a genetic toolbox for a member of the gut--derived Firmicutes is essential to gain access to the unique niches occupied by members of this phylum.

Finally, other than a vaginal strain of *Lactobacillus jensenii* engineered to produce the HIV-1 entry inhibitor protein cyanovirin-N (Lagenaur et al., 2011), most microbiome engineering attempts have focused on the gut microbiome. Ideally, one would want to develop tools for several bacterial chassis for each microbiome-rich body site (skin, gastrointestinal tract, urogenital tract, mouth, and respiratory tract). Engineering microbiome-based diagnostics and therapeutics supports the natural progression of microbiome research questions from "which bacteria are there?" to "what are they doing?" to "how can we manipulate them?"

REFERENCES

Archer, E.J., Robinson, A.B., and Süel, G.M. (2012). ACS Synth. Biol. *1*, 451–457.

Cho, I., and Blaser, M.J. (2012). Nat. Rev. Genet. *13*, 260–270.

Claesen, J., and Fischbach, M.A. (2015). ACS Synth. Biol. *4*, 358–364.

Garrett, W.S., Lord, G.M., Punit, S., Lugo-Villarino, G., Mazmanian, S.K., Ito, S., Glickman, J.N., and Glimcher, L.H. (2007). Cell *131*, 33–45.

Kong, W., Wanda, S.Y., Zhang, X., Bollen, W., Tinge, S.A., Roland, K.L., and Curtiss, R., 3rd. (2008). Proc. Natl. Acad. Sci. USA *105*, 9361–9366.

Lagenaur, L.A., Sanders-Beer, B.E., Brichacek, B., Pal, R., Liu, X., Liu, Y., Yu, R., Venzon, D., Lee, P.P., and Hamer, D.H. (2011). Mucosal Immunol. *4*, 648–657.

Mahowald, M.A., Rey, F.E., Seedorf, H., Turnbaugh, P.J., Fulton, R.S., Wollam, A., Shah, N., Wang, C., Magrini, V., Wilson, R.K., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 5859–5864.

Mimee, M., Tucker, A.C., Voigt, C.A., and Lu, T.K. (2015). Cell Syst. *1*, this issue, 62–71.

Saeidi, N., Wong, C.K., Lo, T.M., Nguyen, H.X., Ling, H., Leong, S.S., Poh, C.L., and Chang, M.W. (2011). Mol. Syst. Biol. *7*, 521.

Steidler, L., Neirynck, S., Huyghebaert, N., Snoeck, V., Vermeire, A., Goddeeris, B., Cox, E., Remon, J.P., and Remaut, E. (2003). Nat. Biotechnol. *21*, 785–789.