Dietary sugar silences a colonization factor in a mammalian gut symbiont

Guy E. Townsend II[a,b], Weiwei Han[a,b], Nathan D. Schwalm III[a,b], Varsha Raghavan[a,b], Natasha A. Barry[a,b], Andrew L. Goodman[a,b], and Eduardo A. Groisman[a,b,1]

[a]Department of Microbial Pathogenesis, Yale School of Medicine, New Haven, CT 06536; and [b]Yale Microbial Sciences Institute, West Haven, CT 06516

Edited by Jeffrey I. Gordon, Washington University School of Medicine in St. Louis, St. Louis, MO, and approved November 16, 2018 (received for review August 9, 2018)

The composition of the gut microbiota is largely determined by environmental factors including the host diet. Dietary components are believed to influence the composition of the gut microbiota by serving as nutrients to a subset of microbes, thereby favoring their expansion. However, we now report that dietary fructose and glucose, which are prevalent in the Western diet, specifically silence a protein that is necessary for gut colonization, but not for utilization of these sugars, by the human gut commensal *Bacteroides thetaiotaomicron*. Silencing by fructose and glucose requires the 5′ leader region of the mRNA specifying the protein, designated Roc for regulator of colonization. Incorporation of the *roc* leader mRNA in front of a heterologous gene was sufficient for fructose and glucose to turn off expression of the corresponding protein. An engineered strain refractory to Roc silencing by these sugars outcompeted wild-type *B. thetaiotaomicron* in mice fed a diet rich in glucose and sucrose (a disaccharide composed of glucose and fructose), but not in mice fed a complex polysaccharide-rich diet. Our findings underscore a role for dietary sugars that escape absorption by the host intestine and reach the microbiota: regulation of gut colonization by beneficial microbes independently of supplying nutrients to the microbiota.

fructose | gene expression | glucose | leader mRNA | microbiota

The gut microbiota is critical to human health (1). The composition of the gut microbiota can be modified by diet (2–8). For example, complex polysaccharides commonly referred to as dietary fiber remain undigested in the small intestine, reach the microbiota in the distal gut, and promote colonization by beneficial microbes associated with lean and healthy individuals (3, 4, 7, 9, 10). Accordingly, polysaccharide-rich diets favor expansion of those organisms that can take up and break down dietary fiber (7, 11–13). Conversely, diets rich in simple sugars favor the expansion of organisms that utilize mucosal glycans because simple sugars are believed to be absorbed in the small intestine and, thus, are unavailable to the microbiota in the distal gut (12, 14).

The monosaccharide fructose can escape absorption in the small intestine and reach the microbiota in the distal gut (15, 16), where microbiota-derived products of fructose metabolism enter the host blood (15). Given the alarming increase in consumption of fructose and sucrose (a heterodimer of glucose and fructose) by Western populations (17, 18), we wondered how these simple sugars impact colonization by *Bacteroides thetaiotaomicron*, a member of the gut microbiota associated with lean and healthy individuals.

The *BT3172* gene, herein named *roc* for "regulator of colonization," is required for *B. thetaiotaomicron* colonization of germ-free mice fed a polysaccharide-rich diet (19). By contrast, the *roc* mutant exhibits no fitness defect in mice fed a simple sugar diet composed of glucose and sucrose (19). These findings suggested that Roc promotes gut colonization in a diet-dependent manner by mediating the utilization of a dietary component(s) present in the complex polysaccharide-rich diet but absent from the simple sugar chow. This is because Roc belongs to a class of proteins, designated hybrid two-component systems, that activate transcription of clustered polysaccharide utilization genes in response to a polysaccharide-derived ligand (19–24). Roc directly controls the adjacent polysaccharide utilization genes *BT3173* and *BT3174* (21), which likely facilitate the utilization of the Roc ligand. However, the mRNA abundance of *BT3173* was less than twofold lower in the *roc* mutant than in the wild-type strain in bacteria collected from the ceca of mice fed a polysaccharide-rich diet (19). These results imply that the ceca of mice fed a polysaccharide-rich diet contain very low amounts of the Roc-activating ligand, the identity of which remains unknown. How, then, does diet control Roc's ability to promote gut colonization by *B. thetaiotaomicron*?

We report that dietary glucose and sucrose silence Roc expression and that Roc is dispensable for utilization of glucose and sucrose-derived fructose. We establish that the mRNA leader preceding the *roc*-coding region is necessary and sufficient for Roc silencing by fructose and glucose. Furthermore, we engineered a strain that is refractory to silencing by these simple sugars and demonstrate that the engineered strain outcompetes wild-type *B. thetaiotaomicron* in mice fed glucose and sucrose. Our findings demonstrate how dietary simple sugars can suppress gut colonization in a commensal bacterium just by altering the levels of a colonization factor dispensable for the utilization of such sugars.

Results

Roc Is Required for Gut Colonization but Not for Expression of Its Regulated Genes in Mice Fed a Polysaccharide-Rich Diet. We determined that a strain deleted for the *roc* gene was dramatically outcompeted by wild-type *B. thetaiotaomicron* in germ-free mice

Significance

Diet is known to alter the gut microbiota composition by supplying nutrients that promote the expansion of particular microorganisms. However, we demonstrate that fructose, a common dietary additive in the Western world, decreases the abundance of a regulator of gut colonization in the human gut commensal *Bacteroides thetaiotaomicron*. Rendering the levels of this colonization factor refractory to silencing by fructose confers an advantage in mice fed a fructose-rich diet. We provide a singular example of the host diet controlling the amounts of a bacterial protein necessary for murine gut colonization by a beneficial gut commensal bacterium, but dispensable for growth on such dietary components.

Author contributions: G.E.T., A.L.G., and E.A.G. designed research; G.E.T., W.H., N.D.S., V.R., and N.A.B. performed research; G.E.T. and W.H. contributed new reagents/analytic tools; G.E.T., W.H., N.D.S., V.R., and E.A.G. analyzed data; and G.E.T. and E.A.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: eduardo.groisman@yale.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1813780115/-/DCSupplemental.

Published online December 17, 2018.

fed a polysaccharide-rich diet (*SI Appendix*, Fig. S1). However, *roc*-dependent activation of target genes was not observed in bacteria collected from the intestinal lumen of mice fed a polysaccharide-rich diet (*SI Appendix*, Fig. S2). Our findings, which are in full agreement with previous results (19), argue that Roc contributes to gut colonization additionally to or independently of regulating genes in the polysaccharide utilization locus (PUL) containing the *roc* gene.

Fructose and Glucose Decrease the Steady-State Levels of the Roc Protein. We examined Roc amounts in bacteria grown in the laboratory in the presence of various monosaccharides comprising the polysaccharide-rich or simple sugar diets. Both glucose and fructose reduced Roc amounts to a similar extent when supplied as sole carbon sources in laboratory media (Fig. 1*A*). This dramatic reduction in Roc amounts appears specific to these two monosaccharides because Roc was readily detectable in media containing arabinose, galactose, mannose, *N*-acetylglucosamine, rhamnose, or xylose (Fig. 1 *A* and *B*).

Fructose and glucose exert a dominant effect when supplied along with sugars that do not silence Roc. That is to say, fructose and glucose decreased Roc amounts within 1 h of addition to cultures growing in galactose-containing media (Fig. 1*C* and *SI Appendix*, Fig. S3*A*), and the decrease was more pronounced by 3 h (Fig. 1*C* and *SI Appendix*, Fig. S3*A*). By contrast, no changes in Roc abundance were observed when additional galactose was supplied to the cultures growing in galactose-containing media (Fig. 1*C* and *SI Appendix*, Fig. S3*A*). Shifting bacteria from fructose- or glucose-containing media to media lacking a carbon source triggered a rapid accumulation of Roc, resulting in a 17.2- and 20-fold increase by 60 min, respectively (Fig. 1 *D* and *E* and *SI Appendix*, Fig. S3 *B* and *C*). Fructose and glucose appear to act specifically on Roc because they did not alter the amounts of BT3334 and BT1635, which are Roc sequelogs (i.e., they exhibit sequence similarity) (*SI Appendix*, Fig. S4).

To determine if fructose could exert this effect as a component of a complex polysaccharide, we examined Roc levels during growth on the fructose homopolymers, fructo-oligosaccharides (fos) and levan. Roc levels were not silenced by fos, but the combination of monomeric fructose with fos reduced Roc levels to those in cells grown in fructose alone (Fig. 1*F*). Conversely, levan silenced Roc to levels similar to monomeric fructose alone (Fig. 1*F*), most likely due to the extracellular liberation of free fructose from levan by the secreted endo-levanase encoded by *BT1760* in *B. thetaiotaomicron* (23).

Cumulatively, these data demonstrate that monomeric glucose and fructose similarly reduce Roc abundance even in the presence of other carbohydrates. Moreover, the data provide a possible explanation for the wild-type strain not exhibiting a competitive advantage over the *roc* mutant in mice fed glucose and sucrose: by dramatically decreasing Roc abundance, these sugars render wild-type *B. thetaiotaomicron* effectively Roc-deficient.

Fructose and Glucose Reduce Roc Abundance at the Posttranscription Initiation Level. We determined that transcription of the *roc* gene starts from an identical position (Fig. 2*A*) and yields similar amounts of nascent transcript in bacteria grown in either glucose or galactose (*SI Appendix*, Fig. S5), sugars that either silence Roc or do not silence Roc, respectively (Fig. 1*A*). Identical transcription start sites were also observed under both conditions for the *BT3334* gene (Fig. 2*B* and *SI Appendix*, Fig. S5), which specifies a protein of the same family as Roc. The *roc* mRNA amounts changed less than 4-fold in bacteria grown in eight different carbohydrates (*SI Appendix*, Fig. S6), which is much less than the 20-fold change in Roc protein abundance (Fig. 1*A*).

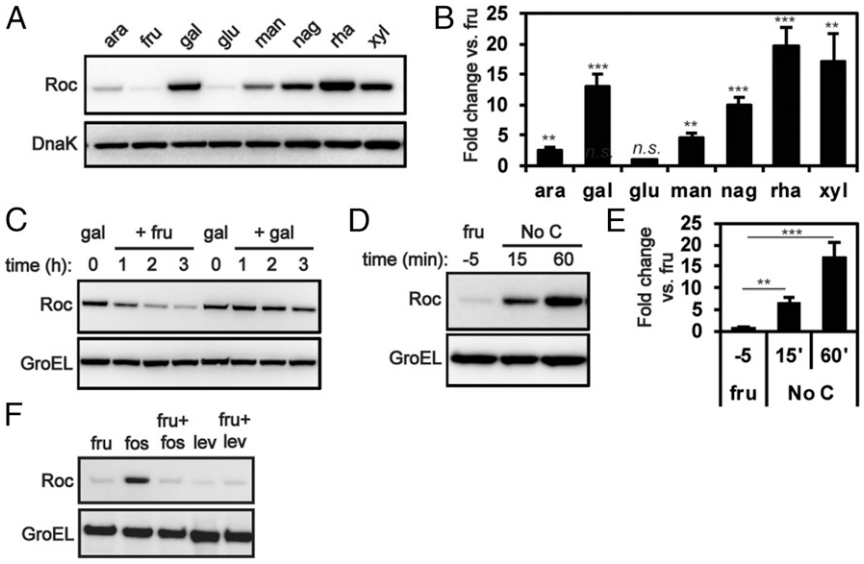

Fig. 1. Roc amounts are dramatically reduced by glucose and fructose. (*A*) Western blot analysis of crude extracts from *B. thetaiotaomicron* (GT593) grown to midexponential phase in minimal media containing arabinose (ara), fructose (fru), galactose (gal), glucose (glu), mannose (man), *N*-acetylglucosamine (nag), rhamnose (rha), or xylose (xyl). Blot was probed with anti-HA and anti-DnaK antibodies. (*B*) Fold difference in Roc amounts relative to bacteria grown in fructose calculated from Western blot quantification ($n = 7$ biological replicates; error bars represent SEM; *P* values derived from two-tailed Student's *t* test; n.s. indicates *P* values ≥ 0.05; $^{**}P < 0.01$; and $^{***}P < 0.001$). (*C*) Western blot analysis of crude extracts from *B. thetaiotaomicron* (GT593) grown in 0.25% galactose (0 h) and following addition of either 0.25% fructose or 0.25% galactose (1–3 h). Blot was probed with anti-HA and anti-GroEL antibodies. (*D*) Western blot analysis of crude extracts from *B. thetaiotaomicron* (GT593) grown in fructose (–5 min) and switched to media lacking a carbon source for 15 and 60 min. Blot was probed with anti-HA and anti-GroEL antibodies. (*E*) Roc amounts were calculated from quantified Western blots in *B. thetaiotaomicron* (GT593) grown in fructose to midexponential phase and subsequently shifted to carbon-source—deficient media ($n = 6$ biological replicates; error bars represent SEM; *P* values derived from two-tailed Student's *t* test; n.s. indicates *P* values ≥ 0.05; $^{**}P < 0.01$; and $^{***}P < 0.001$). (*F*) Western blot analysis of crude extracts from *B. thetaiotaomicron* (GT593) grown to midexponential phase in minimal media containing fructose (fru), fructose oligosaccharides (fos) with and without fructose, and levan (lev) with and without fructose. The blot was probed with anti-HA and anti-GroEL antibodies.

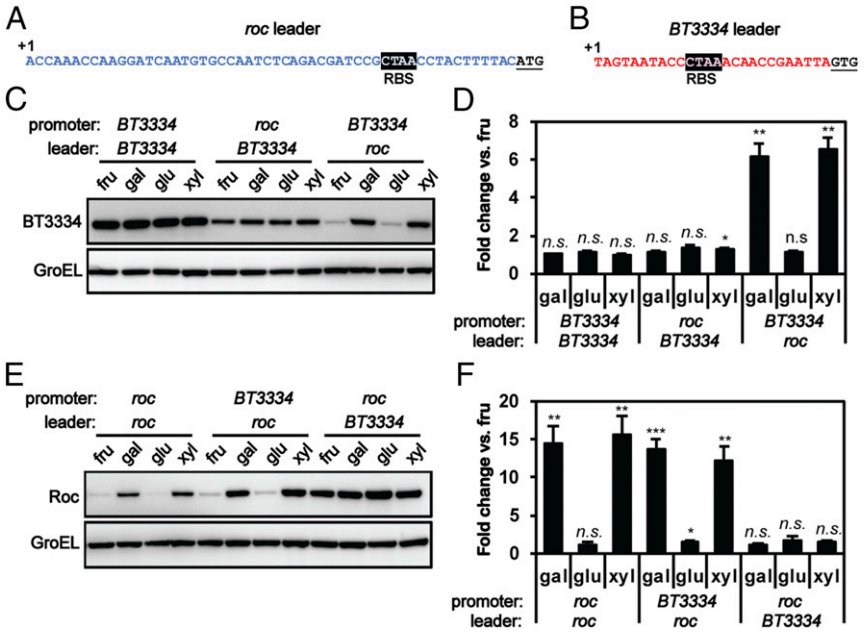

Fig. 2. The 5′ *roc* mRNA leader is necessary and sufficient for repression by glucose. (*A*) Nucleotide sequence of the *roc* 5′ mRNA leader (blue) up to the start codon (underlined) with the putative RBS shaded in black. (*B*) Nucleotide sequence of the *BT3334* 5′ mRNA leader (red) up to the start codon (underlined) with the putative RBS shaded in black. (*C*) Western blot analysis of crude extracts from *B. thetaiotaomicron* strains deleted for both the *BT3334* and *roc* genes and complemented with a single-copy plasmid harboring the *BT3334*-coding region preceded by its native promoter and leader (GT534), the *roc* promoter and its native leader (GT640), or its native promoter but the *roc* leader (GT663), grown as indicated in the figure. (*D*) Fold difference in BT3334 levels in *B. thetaiotaomicron* strains described in *C* grown in galactose (gal), glucose (glu), or xylose (xyl) relative to the same strains grown in fructose (fru) calculated from quantified Western blots ($n = 6$ biological replicates; error bars represent SEM; *P* values derived from two-tailed Student's *t* test). (*E*) Western blot analysis of crude extracts from *B. thetaiotaomicron* strains deleted for both the *BT3334* and *roc* genes and complemented with a single-copy plasmid harboring the *roc*-coding region preceded by its native promoter and leader (GT530), the *BT3334* promoter and its native leader (GT670), or its native promoter and the *BT3334* leader (GT663) grown as indicated in the figure. (*F*) Fold difference in Roc amounts in *B. thetaiotaomicron* strains described in *C* grown in galactose (gal), glucose (glu), or xylose (xyl) relative to the same strains grown in fructose (fru) calculated from quantified Western blots ($n = 6$ biological replicates, error bars represent SEM; *P* values derived from two-tailed Student's *t* test; *n.s.* indicates *P* values ≥ 0.05, $^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.001$).

That glucose and fructose control Roc abundance after the initiation of transcription is supported by two additional experimental results: first, the *roc* promoter failed to confer fructose- or glucose-dependent silencing to a heterologous gene (Fig. 2 *C* and *D* and *SI Appendix*, Fig. S7). And second, glucose reduced Roc protein amounts even when the *roc* gene was transcribed from a heterologous promoter (Fig. 2 *E* and *F* and *SI Appendix*, Fig. S8).

The *roc* mRNA Leader Is Necessary and Sufficient for Roc Silencing by Fructose and Glucose. To explore whether the 5′ leader region of the *roc* mRNA (Fig. 2*A*) is solely responsible for regulation of Roc amounts by glucose, we engineered two strains: one in which the 26-nt leader of the *BT3334* gene (Fig. 2*B*) was replaced by the 54-nt *roc* leader (Fig. 2 *C* and *D* and *SI Appendix*, Fig. S7); and another, in which the 54-nt *roc* leader region was replaced by the 26-nt leader from the *BT3334* gene (Fig. 2 *E* and *F* and *SI Appendix*, Fig. S8) (20).

The strain with the *roc* leader preceding the *BT3334*-coding region produced significantly less BT3334 protein when grown in fructose or glucose than cultures provided xylose or galactose (Fig. 2 *C* and *D*) and increased BT3334 levels following a switch to media lacking a carbon source (*SI Appendix*, Fig. S7). By contrast, the strain with the *BT3334* leader preceding the *roc*-coding region produced similar Roc amounts regardless of the carbohydrate provided (Fig. 2 *E* and *F*) and failed to increase Roc amounts following a switch from glucose to no-carbon conditions (*SI Appendix*, Fig. S8). As discussed above, glucose and fructose silence Roc (Fig. 1*A*) but not BT3334 (*SI Appendix*, Fig. S4 *A* and *B*) in wild-type *B. thetaiotaomicron*. These data establish that the 5′ leader region of the *roc* transcript is necessary and sufficient to control Roc abundance in response to glucose.

In agreement with the notion that the *roc* mRNA leader controls the response to glucose, the nucleotide sequence of the *roc* leader exhibits little shared identity with the nucleotides preceding the coding region of *BT1635* despite this gene specifying a protein exhibiting 74% amino acid identity to Roc but is not silenced by fructose or glucose (*SI Appendix*, Fig. S4 *C* and *D*).

An 11-nt Region in the *roc* mRNA Leader Is Critical for Silencing by Fructose and Glucose. To identify positions within the *roc* 5′ leader (Fig. 3*A*) necessary for Roc silencing by fructose and glucose, we constructed a library of plasmids harboring randomly generated mutations in the *roc* 5′ leader region preceding a *roc* gene specifying a C-terminally HA-tagged Roc protein. The plasmid library was then screened in a *roc*-deficient *B. thetaiotaomicron* strain using an implementation of a classic approach for this anaerobic microbe: directly immunoblotting colonies recovered from glucose-containing solid media using anti-HA antibodies (*SI Appendix*, Fig. S9).

Roc levels were 16.7- to 35.7-fold higher in strains with plasmids harboring single-nucleotide substitutions at four positions between the putative ribosome binding site (RBS) (25) and the start codon of *roc* than in the parental plasmid with the wild-type *roc* leader (Fig. 3 *B* and *C*). The four positions are critical for repression by glucose and fructose (as opposed to de-repressing Roc production under all conditions) because the mutations modestly altered Roc abundance (i.e., a 1.8- to 2.7-fold increase) when bacteria were grown in galactose (Fig. 3 *B* and *C*).

Comparison of the putative leader sequences from *roc* sequelogs in 18 other *Bacteroides* species reveals that the 11 nt

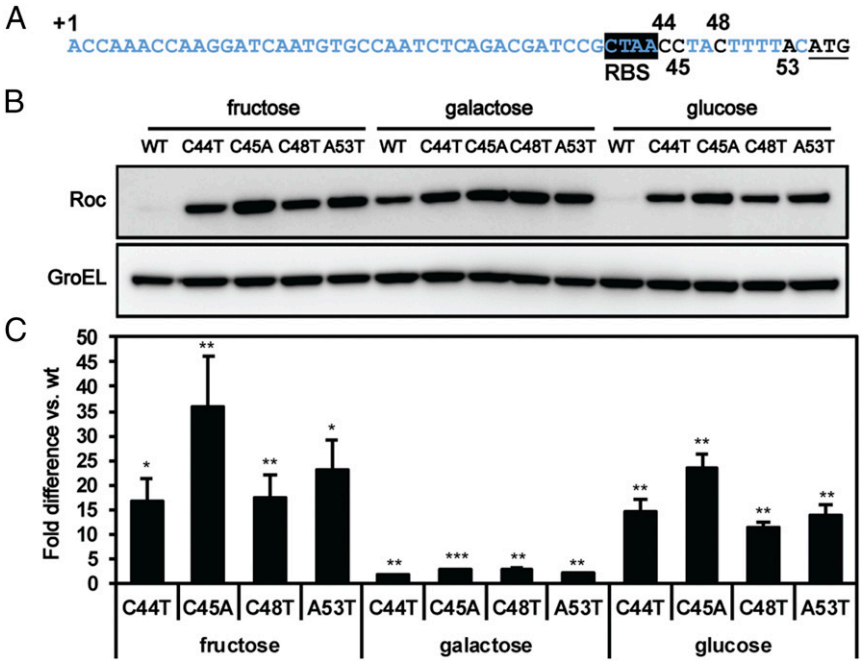

Fig. 3. Mutations in the *roc* leader alleviate Roc repression by fructose and glucose. (*A*) Nucleotide sequence of the *roc* 5′ leader with four positions required for repression by glucose. (*B*) Western blot analysis of crude extracts from *B. thetaiotaomicron* harboring plasmids encoding the wild-type *roc* sequence or the indicated single-nucleotide substitutions in the *roc* 5′ leader following growth in fructose, galactose, or glucose. (*C*) Fold difference in Roc amounts in *B. thetaiotaomicron* mutant strains described in *B* grown in fructose, galactose, or glucose relative to the wild-type strain grown in the same condition, calculated from quantified Western blots ($n = 6$ biological replicates; error bars represent SEM; P values derived from two-tailed Student's t test; $^{*}P < 0.05$; $^{**}P < 0.01$; and $^{***}P < 0.001$).

positioned between the putative RBS (25) and start codon are relatively well conserved, while the remainder of the leader has diverged (*SI Appendix*, Fig. S10). Furthermore, two of the four positions required to control Roc levels in response to glucose and fructose are conserved among all of the analyzed sequences upstream of Roc sequelogs of at least 83% amino acid identity. These data suggest that *roc* homologs are similarly regulated in other *Bacteroides* species.

Fructose and Glucose Are Present in the Cecum of Mice Fed a Glucose/Sucrose Diet. We determined that fructose abundance in the cecum of mice fed a glucose/sucrose chow was 2.6-fold higher than in animals fed a polysaccharide-rich chow (Fig. 4*A*). By contrast, glucose was not detected in the ceca of mice fed either diet (Fig. 4*B*), which likely reflects efficient glucose absorption by the gut epithelium (15). However, when cecal extracts were treated with invertase, an enzyme that liberates glucose assembled in oligo- and polysaccharides, we detected 2.7-fold more glucose in mice fed the glucose/sucrose diet relative to those fed the polysaccharide-rich diet (Fig. 4*B*). Given that sucrose hydrolysis is the sole source of fructose in the cecum of glucose/sucrose-fed mice, and that glucose is detected only after invertase addition, our results argue that sucrose is present in the distal gut and that sucrose hydrolysis exposes the microbiota to both fructose and glucose.

Rendering Roc Expression Refractory to Silencing by Fructose and Glucose Confers a Colonization Advantage to Mice Fed a Glucose/Sucrose Diet. If a glucose/sucrose diet decreases *B. thetaiotaomicron* fitness in the gut by reducing Roc abundance, a strain producing Roc in the presence of fructose or glucose (*SI Appendix*, Fig. S11) should exhibit a fitness advantage over both a strain lacking the *roc* gene and wild-type *B. thetaiotaomicron*. As hypothesized, the engineered strain with the *BT3334* leader preceding the *roc* coding region achieved five times greater abundance than wild-type *B. thetaiotaomicron* and the *roc*-deficient mutant in mice fed a glucose/sucrose diet by day 15 (Fig. 4*C*).

The colonization advantage exhibited by the engineered strain is specific to the gut of mice fed a glucose/sucrose diet because: first, the abundance of the engineered strain hardly changed in mice fed a polysaccharide-rich diet over 15 d (Fig. 4*D*), whereas the abundance of wild-type *B. thetaiotaomicron* and of the *roc* mutant increased and decreased, respectively (Fig. 4*D*), in agreement with previous results (*SI Appendix*, Fig. S1) (19). And second, the engineered strain grew similarly to wild-type *B. thetaiotaomicron* in laboratory media with fructose or glucose as the sole carbon source (*SI Appendix*, Fig. S12). These data demonstrate that the *roc* mRNA leader is necessary for *B. thetaiotaomicron*'s response to dietary sources of fructose, as it is for the response to fructose and glucose in laboratory media (Fig. 1).

Discussion

Our findings reveal how common dietary additives endemic to Western human populations can impact gut colonization by the microbiota independently of their ability to serve as nutrients (Fig. 4 and *SI Appendix*, Fig. S12). We establish that fructose and glucose silence a critical colonization factor, called Roc, in a widely distributed gut commensal bacterium *B. thetaiotaomicron* (Fig. 1). Furthermore, we demonstrate that fructo-oligosaccharides, which are used as prebiotics in humans (26), fail to silence Roc (Fig. 1*F*), indicating that the presence of monomeric fructose is required for this effect. Additionally, we determine that a short 5′ mRNA leader is necessary and sufficient for the silencing effects that fructose and glucose exert on the amounts of the associated coding region (Figs. 2 and 3). The identified mRNA leader may be used to engineer gut microbes to alter their behaviors in response to dietary components.

Roc is a transcriptional regulator that controls expression of genes in the PUL that also harbors the *roc* gene (21). However, Roc appears to promote gut colonization independently

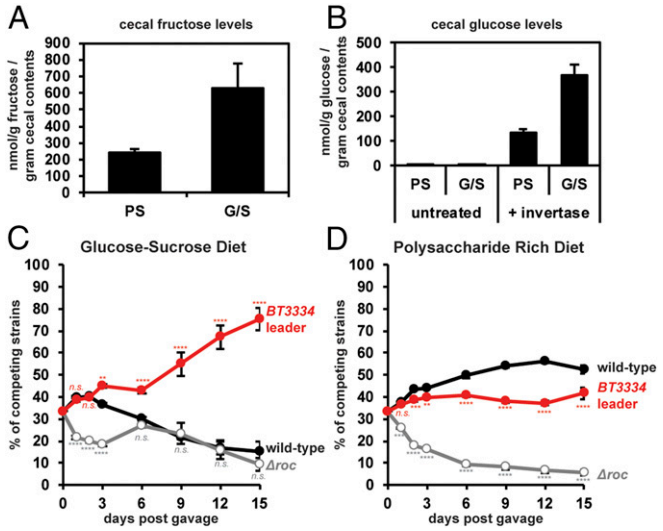

Fig. 4. A mutation that renders Roc production insensitive to fructose confers a fitness advantage to *B. thetaiotaomicron* in the gut of germ-free mice fed a glucose/sucrose-rich diet. (*A*) The amounts of fructose per gram of cecal material from mice fed either a polysaccharide-rich or glucose–sucrose diet (error bars represent SEM; *P* values were derived from a two-tailed Student's *t* test). (*B*) The amounts of glucose per gram of cecal material from mice fed either a polysaccharide-rich or glucose–sucrose diet before or after the addition of invertase (error bars represent SEM). (*C* and *D*) Relative abundance of wild-type *B. thetaiotaomicron* (GT480), a strain deleted in the *roc* gene (*Δroc*; GT490), and an engineered strain with the *roc* leader replaced by the *BT3334* leader (GT914) determined by qPCR from genomic DNA prepared from fecal samples recovered from ex-germ-free mice gavaged with an equivalent number of colony-forming units for each strain in mice were fed a diet rich in glucose and sucrose (*C*) or a standard chow polysaccharide-rich diet (*D*). Both panels represent the mean relative abundance of each strain from five mice (error bars represent SEM; *P* values were derived from two-way ANOVA with Dunnett correction; n.s. indicates $P \geq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; and $^{****}P < 0.0001$).

of its predicted role in mediating dietary carbohydrate utilization (19) (Fig. 4 *C* and *D*). The absence of Roc-dependent target gene activation in total cecal contents (*SI Appendix*, Fig. S2) may reflect a spatial and/or temporal distribution of the Roc ligand, as suggested for another *Bacteroides* species (27, 28). Alternatively, Roc may promote gut colonization by other means, such as the proposed interactions with glucose 6-phosphate isomerase and glucose 6-phosphate dehydrogenase (19). Although Roc interacting with these metabolic enzymes is an intriguing hypothesis, it is not clear how the cytoplasmic glucose 6-phosphate isomerase and glucose 6-phosphate dehydrogenase proteins would interact with Roc domains predicted to reside in the periplasm (22).

We have demonstrated that the *roc* leader is necessary and sufficient to confer glucose and fructose to a heterologous gene (Fig. 2 *C* and *D*) and that the *roc* promoter is dispensable for this regulation (Fig. 2 *E* and *F*). Furthermore, we identified four nucleotide positions in the short *roc* mRNA leader located between the putative ribosome-binding site (25) and start codon that are necessary for control of the downstream *roc*-coding region by glucose and fructose (Fig. 3) and conserved in leaders preceding *roc* homologs (*SI Appendix*, Fig. S10). Additionally, *roc* transcript levels vary less than 4-fold during growth in eight different carbon sources, while protein levels vary almost 20-fold (*SI Appendix*, Fig. S7). Similarly, the *roc* mRNA is present at similar levels, and transcription is initiated from an identical position in cells grown in glucose and galactose. By contrast, 13.7-fold more Roc protein is present in cells grown in galactose compared with glucose. The difference in Roc levels under these two conditions cannot be explained by *roc* mRNA stability, as *roc* transcript levels are indistinguishable in the presence of either glucose or galactose (*SI Appendix*, Fig. S13). These findings demonstrate that control of Roc occurs independently of transcription initiation or transcript stability and suggest that this regulation is exerted at the level of translation initiation or elongation.

We propose that a regulatory protein and/or small RNA acts on the *roc* leader to control Roc synthesis. This putative regulator is not likely encoded within the Roc-controlled PUL (21), as fructose silences Roc levels similarly in wild-type *B. thetaiotaomicron* and a strain lacking the region corresponding to the *BT3174–BT3180* genes (*SI Appendix*, Fig. S14 *A* and *B*). Furthermore, the *roc* leader is unlikely to operate as a glucose- and fructose-responding riboswitch due to its short length and the absence of regions that can adopt alternative structures that characterize riboswitches (29). The proposed regulator is likely to target the conserved portion of the *roc* mRNA leader between the predicted ribosome-binding site and start codon (*SI Appendix*, Fig. S10).

Finally, we demonstrated how colony blotting can be used to investigate the expression of any protein that can be epitope-tagged (*SI Appendix*, Fig. S9) in an organism with a limited number of reporter genes. Previous attempts to couple gene expression and reporter function in *Bacteroides* species have required the exposure to oxygen (30), the application of toxic reagents (31), or extremely high expression levels (32). We have developed a reporter to genetically dissect regulatory interactions that can be adapted to any *B. thetaiotaomicron* protein of interest assuming it can be tagged with an HA epitope. This approach enabled us to screen a library of strains harboring random mutations to identify and recover mutants of interest for further characterization.

Materials and Methods

Materials and methods including culturing conditions, strain construction, qPCR, Western blot analysis, mouse experiments, colony blotting, and sugar quantification are detailed in *SI Appendix*. Experiments using germ-free mice were approved by the Institutional Animal Care and Use Committee of Yale University.

ACKNOWLEDGMENTS. We thank Hubert Salvail, Bentley Lim, Liza-Marie Valle, and Diane Lazo for technical advice and support and Jennifer Aronson for comments on the manuscript. This research was supported with funds from Yale University and NIH Grant GM123798 (to E.A.G.).

1. Clemente JC, Ursell LK, Parfrey LW, Knight R (2012) The impact of the gut microbiota on human health: An integrative view. *Cell* 148:1258–1270.
2. Fontana L, Partridge L (2015) Promoting health and longevity through diet: From model organisms to humans. *Cell* 161:106–118.
3. Wu M, et al. (2015) Genetic determinants of in vivo fitness and diet responsiveness in multiple human gut Bacteroides. *Science* 350:aac5992.
4. Sonnenburg ED, et al. (2016) Diet-induced extinctions in the gut microbiota compound over generations. *Nature* 529:212–215.
5. Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI (2008) Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 3:213–223.
6. Turnbaugh PJ, et al. (2009) The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 1:6ra14.
7. David LA, et al. (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505:559–563.
8. Rothschild D, et al. (2018) Environment dominates over host genetics in shaping human gut microbiota. *Nature* 555:210–215.
9. Sonnenburg ED, Sonnenburg JL (2014) Starving our microbial self: The deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. *Cell Metab* 20:779–786.
10. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: Human gut microbes associated with obesity. *Nature* 444:1022–1023.
11. Koropatkin NM, Cameron EA, Martens EC (2012) How glycan metabolism shapes the human gut microbiota. *Nat Rev Microbiol* 10:323–335.
12. Desai MS, et al. (2016) A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 167:1339–1353.e21.

13. McNulty NP, et al. (2013) Effects of diet on resource utilization by a model human gut microbiota containing Bacteroides cellulosilyticus WH2, a symbiont with an extensive glycobiome. *PLoS Biol* 11:e1001637.
14. Earle KA, et al. (2015) Quantitative imaging of gut microbiota spatial organization. *Cell Host Microbe* 18:478–488.
15. Jang C, et al. (2018) The small intestine converts dietary fructose into glucose and organic acids. *Cell Metab* 27:351–361.e3.
16. Dahlqvist A, Thomson DL (1963) The digestion and absorption of sucrose by the intact rat. *J Physiol* 167:193–209.
17. Cordain L, et al. (2005) Origins and evolution of the Western diet: Health implications for the 21st century. *Am J Clin Nutr* 81:341–354.
18. Marriott BP, Cole N, Lee E (2009) National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. *J Nutr* 139:1228S–1235S.
19. Sonnenburg ED, et al. (2006) A hybrid two-component system protein of a prominent human gut symbiont couples glycan sensing in vivo to carbohydrate metabolism. *Proc Natl Acad Sci USA* 103:8834–8839.
20. Raghavan V, Lowe EC, Townsend GE, II, Bolam DN, Groisman EA (2014) Tuning transcription of nutrient utilization genes to catabolic rate promotes growth in a gut bacterium. *Mol Microbiol* 93:1010–1025.
21. Townsend GE, II, Raghavan V, Zwir I, Groisman EA (2013) Intramolecular arrangement of sensor and regulator overcomes relaxed specificity in hybrid two-component systems. *Proc Natl Acad Sci USA* 110:E161–E169.
22. Lowe EC, Baslé A, Czjzek M, Firbank SJ, Bolam DN (2012) A scissor blade-like closing mechanism implicated in transmembrane signaling in a Bacteroides hybrid two-component system. *Proc Natl Acad Sci USA* 109:7298–7303.
23. Sonnenburg ED, et al. (2010) Specificity of polysaccharide use in intestinal bacteroides species determines diet-induced microbiota alterations. *Cell* 141: 1241–1252.
24. Lynch JB, Sonnenburg JL (2012) Prioritization of a plant polysaccharide over a mucus carbohydrate is enforced by a Bacteroides hybrid two-component system. *Mol Microbiol* 85:478–491.
25. Wegmann U, Horn N, Carding SR (2013) Defining the bacteroides ribosomal binding site. *Appl Environ Microbiol* 79:1980–1989.
26. Slavin J (2013) Fiber and prebiotics: Mechanisms and health benefits. *Nutrients* 5: 1417–1435.
27. Lee SM, et al. (2013) Bacterial colonization factors control specificity and stability of the gut microbiota. *Nature* 501:426–429.
28. Donaldson GP, et al. (2018) Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* 360:795–800.
29. McCown PJ, Corbino KA, Stav S, Sherlock ME, Breaker RR (2017) Riboswitch diversity and distribution. *RNA* 23:995–1011.
30. Pudlo NA, et al. (2015) Symbiotic human gut bacteria with variable metabolic priorities for host mucosal glycans. *MBio* 6:e01282-15.
31. Coyne MJ, Weinacht KG, Krinos CM, Comstock LE (2003) Mpi recombinase globally modulates the surface architecture of a human commensal bacterium. *Proc Natl Acad Sci USA* 100:10446–10451.
32. Whitaker WR, Shepherd ES, Sonnenburg JL (2017) Tunable expression tools enable single-cell strain distinction in the gut microbiome. *Cell* 169:538–546. e12.