Gut microbiota composition impacts host gene expression by changing chromatin accessibility

Allison L Richards [1], Amanda L Muehlbauer [3,4], Adnan Alazizi [1], Michael B Burns [3,4], Trevor J Gould [3,4], Camilla Cascardo [1], Roger Pique-Regi [1,2,5], Ran Blekhman [3,4,5], Francesca Luca [1,2,5]

[1]Center for Molecular Medicine and Genetics, and

[2]Department of Obstetrics and Gynecology, Wayne State University, Detroit, Michigan 48201, USA

[3]Department of Genetics, Cell Biology and Development, and

[4]Department of Ecology, Evolution and Behavior, University of Minnesota, Minneapolis, Minnesota, USA

[5]To whom correspondence should be addressed.

Email: fluca@wayne.edu, blekhman@umn.edu, rpique@wayne.edu

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Abstract

Variation in gut microbiome is associated with wellness and disease in humans, yet the molecular mechanisms by which this variation affects the host are not well understood. A likely mechanism is through changing gene regulation in interfacing host epithelial cells. Here, we treated colonic epithelial cells with live microbiota from five healthy individuals and quantified induced changes in transcriptional regulation and chromatin accessibility in host cells. We identified over 5,000 host genes that change expression, including 588 distinct associations between specific taxa and host genes. The taxa with the strongest influence on gene expression alter the response of genes associated with complex traits. Further, using ATAC-seq, we show that these changes in gene expression are likely the result of changes in host chromatin accessibility induced by exposure to gut microbiota. We then created a manipulated microbial community with titrated doses of *Collinsella*, demonstrating that both natural and controlled microbiome composition leads to distinct, and predictable, gene expression profiles in the host. Together, our results suggest that specific microbes play an important role in regulating expression of individual host genes involved in human complex traits. Our work also supports the hypothesis that one of the mechanisms by which the microbiome regulates host gene expression is through changes in chromatin accessibility in host cells. Finally, the ability to fine tune the expression of host genes by manipulating the microbiome suggests future therapeutic routes for human wellness.

Introduction

The microbial community in the human digestive tract, the gut microbiota, is highly complex, and also displays strong variation across individuals [21, 30, 39]. Variability in gut microbiota composition is related to many factors, including medication, diet and genetics [3, 4, 12, 14, 22, 28, 31, 33, 43, 46, 48,

49, 51]. The gut microbiota has a variety of functions within the host, such as metabolism of certain compounds [1, 17, 20, 41], and its composition is correlated with several diseases, such as Crohn's disease and colorectal cancer [6, 18, 27, 38, 42, 44]. In mouse, certain microbial communities can lead to changes in the host's weight and overall health, suggesting that there is a reciprocal effect between the host and the gut microbiota [19]. Recent work in mice has explored host gene expression [7, 10, 15] and gene regulation in response to microbiome exposure. These studies suggested that the microbiome doesn't induce gross changes in chromatin accessibility but rather influences expression and binding of specific transcription factors [7, 10]. However, in humans, our ability to study the effects and mechanism of the microbiome *in vivo* are severely limited. Recently, we have described an in vitro approach based on human epithelial cells inoculated with live microbial communities [40] that is well suited to study the effects of the microbiome on human gene regulation. Here we seek to use this in vitro system to determine the extent and mechanism by which variation in microbiome composition drives differences in gene expression in the host cells. We also seek to determine if specific microbial taxa drive gene expression variation, and if these changes are predictable, i.e. they can be recapitulated by manipulating the composition of the microbiome. These open question are crucial for understanding the causal role of the microbiome in host physiology and designing targeted therapies revolving around interventions on the gut microbiome.

Results

Exposure to Microbiota Influences Host Gene Expression

To determine the impact of variation in the gut microbiota on host cells, we treated human colonic epithelial cells with live gut microbiota extracted from 5 healthy, unrelated, human individuals (Figure 1A). These samples are representative of other healthy gut microbiome samples from the Human Microbiome Project (Figure S4) [21, 34]. We then assessed changes in gene expression and microbial composition following 1, 2 and 4 hours of exposure separately. The overall changes in gene expression between each microbiome treatment and control cluster first by time-point (Figure 1B, Table S3) where the strongest response occurs at 2 hours following exposure (3,260 genes across any of the five microbiota samples, BH FDR $< 10\%$, $|\log_2 \mathrm{FC}| > 0.25$). Among these, we identified 669 transcripts (188 genes) that are differentially expressed in all five treatments following 2 hours of treatment (Figure 1C, 1 and 4 hour comparisons in S1A and B Figs). We used meta-analysis to identify genes that change consistently across the treatments and time points (examples in Figure 1D, S2A and B Figs, Table S1) and found that they are enriched for genes that function in protein translation, as well as those on the cell surface, such as in adherens junctions (BH FDR $< 10^{-4}\%$) (Table S2, Figure S3), suggesting a biological function for consistent changes in gene expression that may relate to the host cell's interaction with the microbiota.

Each microbiota sample is derived from a different individual with unique diet and genetic makeup. Therefore, we expect that the microbial composition and diversity of each sample differs. When we considered the uncultured microbiome, we found variability in their microbial composition and diversity (Simpson's index range between 0.94 to 0.98). When we considered how the human colonocytes influenced microbial composition, we found that most taxa were unaffected by the presence of human cells, while 13 taxa showed varying abundance dependent on the presence of host cells (likelihood ratio test, BH FDR $< 10\%$, examples in S5, Table S5). In order to determine how the microbiota composition of each sample influences host gene expression differently, we utilized a likelihood ratio test to compare models including or excluding the individual microbiota effect. We identified 409 genes (1,484 transcripts, BH FDR $< 10\%$, Table S4) with gene expression responses significantly different across colonocytes treated with the five microbiota samples (examples in Figure 1E). These data demonstrate that both the host

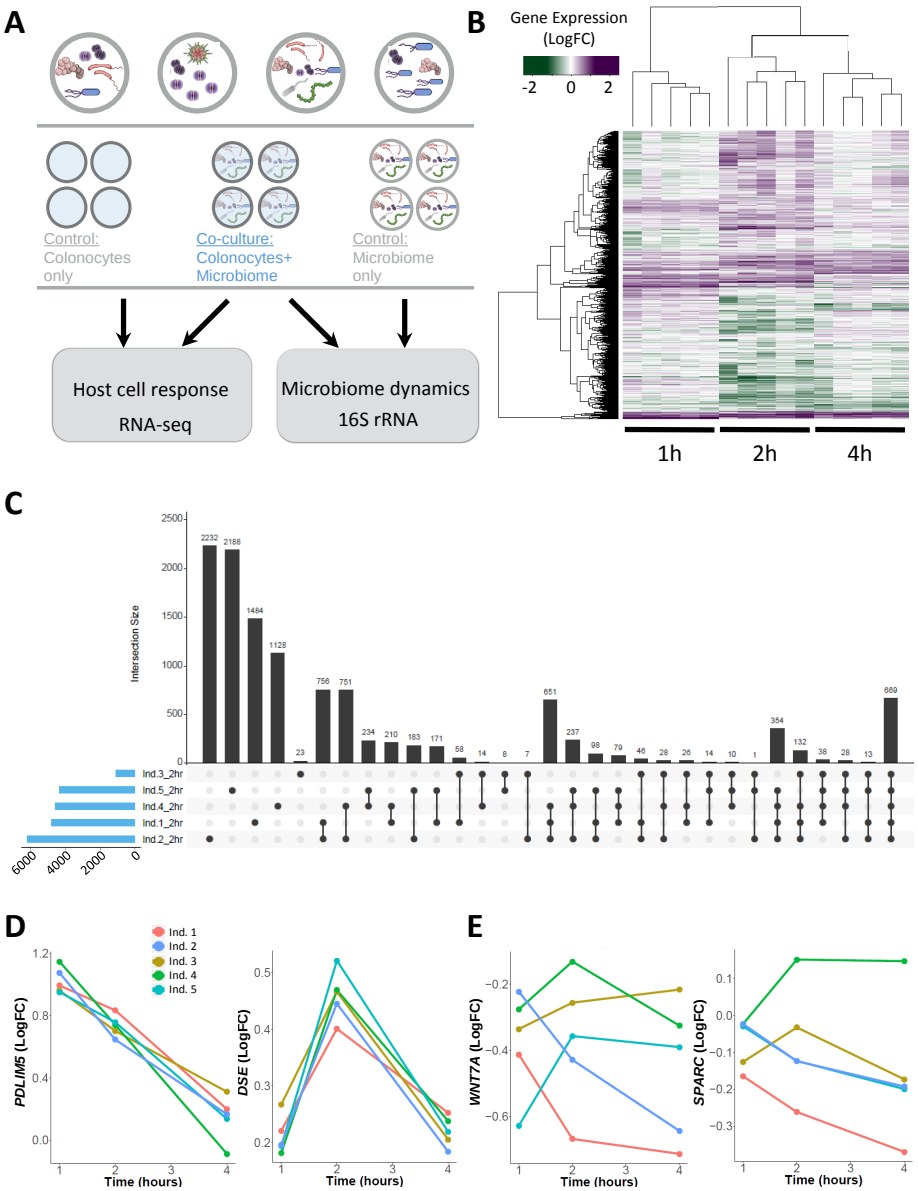

Figure 1: Gene expression changes in Colonocytes Treated with Microbiota from Five Unrelated Individuals. **A** Study design. Human colonocytes were inoculated separately with five microbiota samples from unrelated individuals. **B** Heatmap of gene expression changes induced at each time point by the individual microbiota samples. Purple denotes an increase in gene expression (green shows a reduction) compared to the gene expression in the control (colonocytes cultured alone). Only genes that are differentially expressed in at least one sample are shown. **C** Comparison of transcripts differentially expressed at 2 hours across the five treatments. The blue bars to the left show the total number of differentially expressed transcripts in the given set. The gray vertical bars show the number of transcripts that are in the set denoted below them. Sets with a single dark gray circle show the number of differentially expressed transcripts unique to that sample. **D** Examples of genes (*PDLIM5* and *DSE*) whose changes in expression are consistent across treatments with the five different microbiota. Changes in expression (y-axis) are shown as $\log_2$ fold change as compared to control. **E** Examples of genes (*WNT7A* and *SPARC*) whose changes in expression are significantly different across treatments with the five microbiota samples.

and the microbiota influence each other and that inter-individual variation in the microbiome can lead to different gene expression responses in interacting host cells.

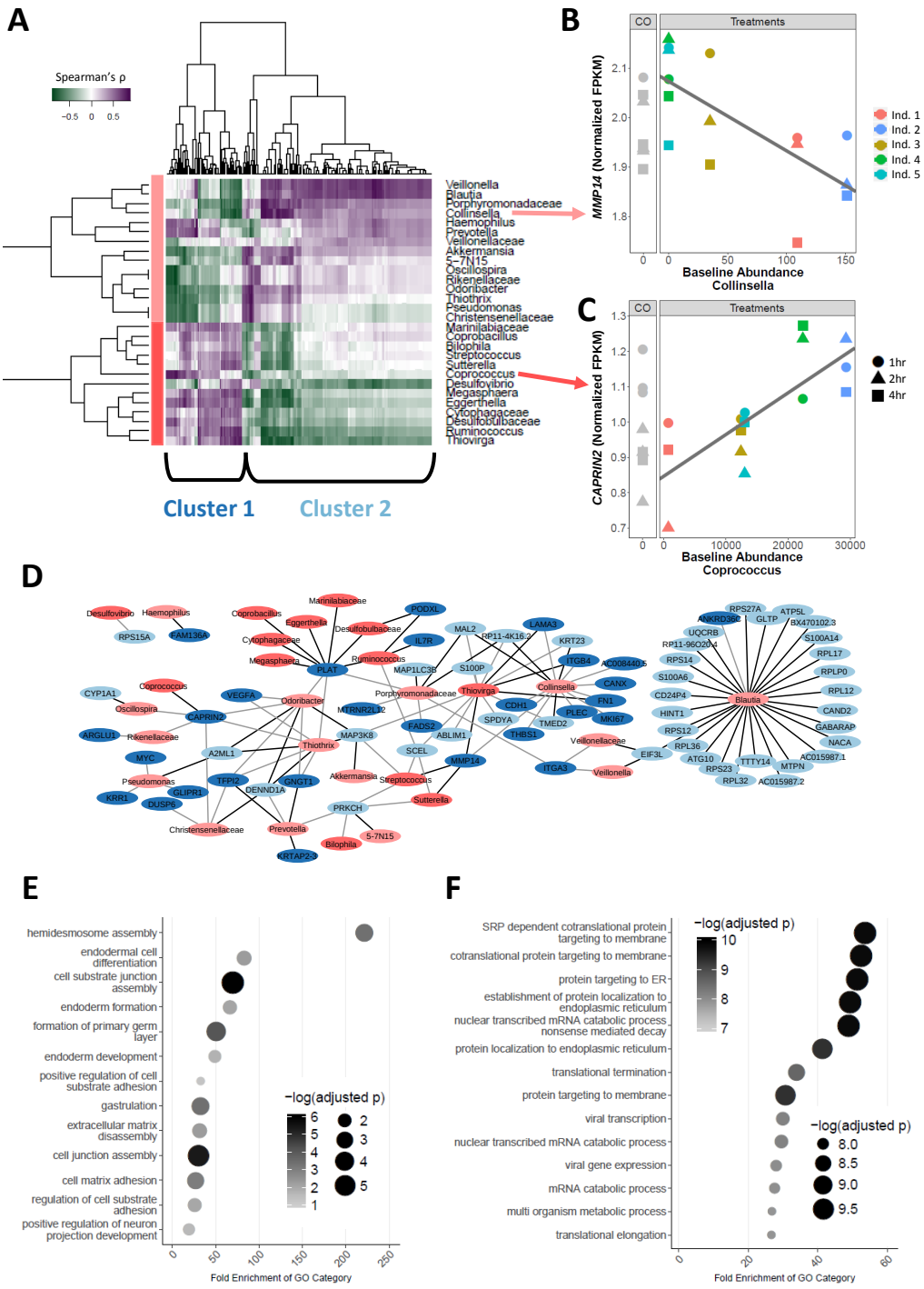

Figure 2: Abundance of microbiome taxa is associated with specific host gene expression changes. A Heatmap of microbiota taxa and colonocyte gene expression correlation (Spearman's ρ). Columns correspond to 28 microbiota taxa and rows correspond to 219 transcripts (70 genes) that had at least one significant correlation in the model. Taxa and transcripts are each clustered via hierarchical clustering showing two major groups indicated by a different shade of red (taxa) / blue (transcripts). **B and C** Examples (*MMP14*, and *CAPRIN2*) of significant association (BH FDR = 7% for both genes) between host gene expression (FPKM quantile normalized) and baseline abundance of specific taxa. **D** Network of associations between taxa and genes from the heatmap in **A**. Nodes in blue denote genes while nodes in red denote microbial taxa. Color shading indicates clusters of genes or taxa from **A**. Black edges indicate a positive correlation while light gray indicates a negative correlation. **E and F** Gene ontology enrichment for cluster 1 and 2 respectively, defined in **A**.

Specific Microbes Influence Unique Host Genes

We hypothesized that the differences in gene expression response to each microbiome could be attributed to specific microbiota features, such as the abundance of specific taxa. For this reason we studied the association between host gene expression (147,555 transcripts) and the abundance of microbial taxa (62 taxa that pass filtering criteria; see Methods and Materials) at the time of treatment. Across all possible associations (9,125,927 tests) we identified 588 transcript-by-taxon pairs with a significant association (BH FDR = 10%, Table S6), corresponding to 121 host genes with changes in expression associated with the abundance of 46 taxa. 35 of these taxa were associated with the expression of more than one host gene (BH FDR = 10%) demonstrating that a single microbe may affect the regulation of many genes, and suggesting that microbes may influence a particular trait in a polygenic manner.

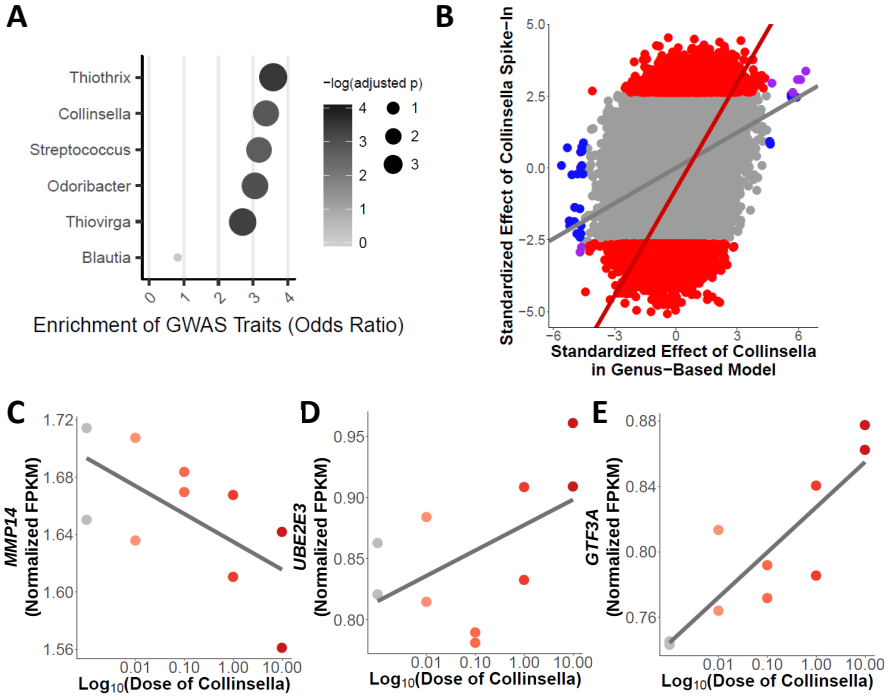

Figure 3: Manipulation of the Microbial Community Induces Predictable Gene Expression Changes in the Host. **A** Enrichment for complex traits across genes with changes in expression associated with microbial taxa (BH FDR < 20%). **B** Scatterplot of the effect of *Collinsella* abundance from the five microbiome samples (x-axis) and effect of *Collinsella* aerofaciens from the spike-in validation experiment (y-axis). Plotted are $\log_2$ fold changes normalized by the standard error. The red points and line highlight the transcripts that are DE in the spike-in experiment. There is a correlation across all points (p-value $< 10^{-20}$, $\rho = 0.29$) and across transcripts differentially expressed in the spike-in experiment (p-value $< 10^{-20}$, $\rho = 0.46$). **C-E** Examples (*MMP14*, *UBE2E3*, and *GTF3A*) of significant association (BH FDR = 9%, 6% and 7%, respectively) between host gene expression (FPKM quantile normalized) and abundance of *Collinsella* aerofaciens from spike-in validation experiment.

Of the 121 host genes whose expression is associated with abundance of microbial taxa, 70 genes (219 transcripts) were also differentially expressed when each microbiota treatment was compared to control conditions, and formed two clusters with distinct functions (Figure 2A and D, examples in Figure 2B and C). Genes in the first cluster are positively correlated with genera *Ruminococcus*, *Coprococcus* and *Streptococcus*, and have functions in cell junction assembly (BH FDR $< 10^{-3}$%, Figure 2E), while the second cluster of genes, positively correlated with microbial genera including *Odoribacter*, *Blautia* and *Collinsella*, function in protein targeting to the endoplasmic reticulum (BH FDR $< 10^{-7}$, Figure 2F).

These results suggest that microbial consortia may work in concert to affect specific functions through changes in host gene regulation.

We then focused on microbial taxa associated with changes in expression for a large number of genes, since these microbes are more likely to impact host traits. To test this hypothesis, we focused on the six microbial genera that were associated with the largest number of host genes (at least 30 host genes at p-value $< 3.5 \times 10^{-5}$): *Odoribacter*, *Streptococcus*, *Blautia*, *Thiovirga*, *Thiothrix*, and *Collinsella*. Indeed, all taxa, except for *Blautia*, led to expression changes in genes enriched for complex traits (p-value < 0.005, OR > 2.7, Figure 3A, Table S7) [50]. Moreover, we identified 21 genes that were associated with traits already linked to the gut microbiome, including colorectal cancer [6, 44], obesity [19, 47], and Inflammatory Bowel Disorder (IBD) [2, 16, 26]. Half of the genes (15/30) associated with the genus *Collinsella* are associated with a trait in GWAS (p-value $= 0.001$, OR $= 3.4$, Figure 3A). Previous studies have found that the abundance of *Collinsella* is correlated with several diseases, including colorectal cancer [35], Type 2 Diabetes [8] and irritable bowel syndrome [24]. Interestingly, we identified a gene, *GLTP*, that is involved in glycolipid transfer and has been associated with metabolic syndrome [52], whose expression is influenced by the abundance of the genus *Collinsella* in each of the five microbiota samples (BH FDR = 12.6%). This suggests that microbes of the genus *Collinsella* may influence metabolic syndrome in the host through regulation of genes in the colon, such as *GLTP*. These data also suggest that specific microorganisms, and not simply general exposure to the entire gut microbiota, can lead to changes in many genes' expression. Furthermore, these results support the hypothesis that variation in the abundance of members of the microbiota may influence complex traits.

Validation of Changes in Host Gene Expression Due to Specific Microbiota

To validate and further demonstrate the effect of specific microbes on host gene expression, we treated colonocytes with a microbiota sample without any detectable *Collinsella aerofaciens*, and supplemented it with titrated abundances of this bacterium relative to the whole microbiota sample: 0.01%, 0.1%, 1% and 10%. We used RNA-sequencing to study the resulting changes in gene expression, and identified 1,570 genes that change expression (BH FDR = 10%, Table S8) depending on the abundance of *Collinsella aerofaciens*. When we consider the changes in gene expression associated with *Collinsella* abundance in the five microbiota treatments, we found that the effects of *Collinsella* in both experiments are correlated (Figure 3B). We validate 19 out of 29 genes (p-value $= 0.0002$, OR $= 4.1$), originally identified (BH FDR = 20%), including *GLTP* and *MMP14* (ENST00000547279, original BH FDR = 7%in Figure 2B, spike-in validation BH FDR = 9% in Figure 3C), demonstrating that *Collinsella* is responsible for changes in the expression of these genes. The large number of genes that change expression in this experiment could be due to several factors, including the increase in power from a larger number of samples. These 1,570 genes are enriched for genes associated with complex traits from GWAS (p-value $= 10^{-10}$, OR $= 1.5$, examples in Figure 3D and E) and specifically enriched for genes associated with HDL cholesterol (bonferroni-corrected p-value $= 0.018$, OR $= 2.75$) . This spike-in experiment shows that host gene expression can be modulated by changing the abundance of a single bacterial species within the microbiome.

Chromatin Accessibility Explains Changes in Gene Expression Following Microbiota Exposure

In order to investigate the regulatory mechanism whereby the microbiome induces changes in host gene expression, we performed ATAC-seq in colonocytes inoculated for two hours with each of the five microbiota samples. We then used DESeq2 to characterize regions of differential chromatin accessibility and identified 234 regions that were differentially accessible between the treated samples and untreated controls (BH

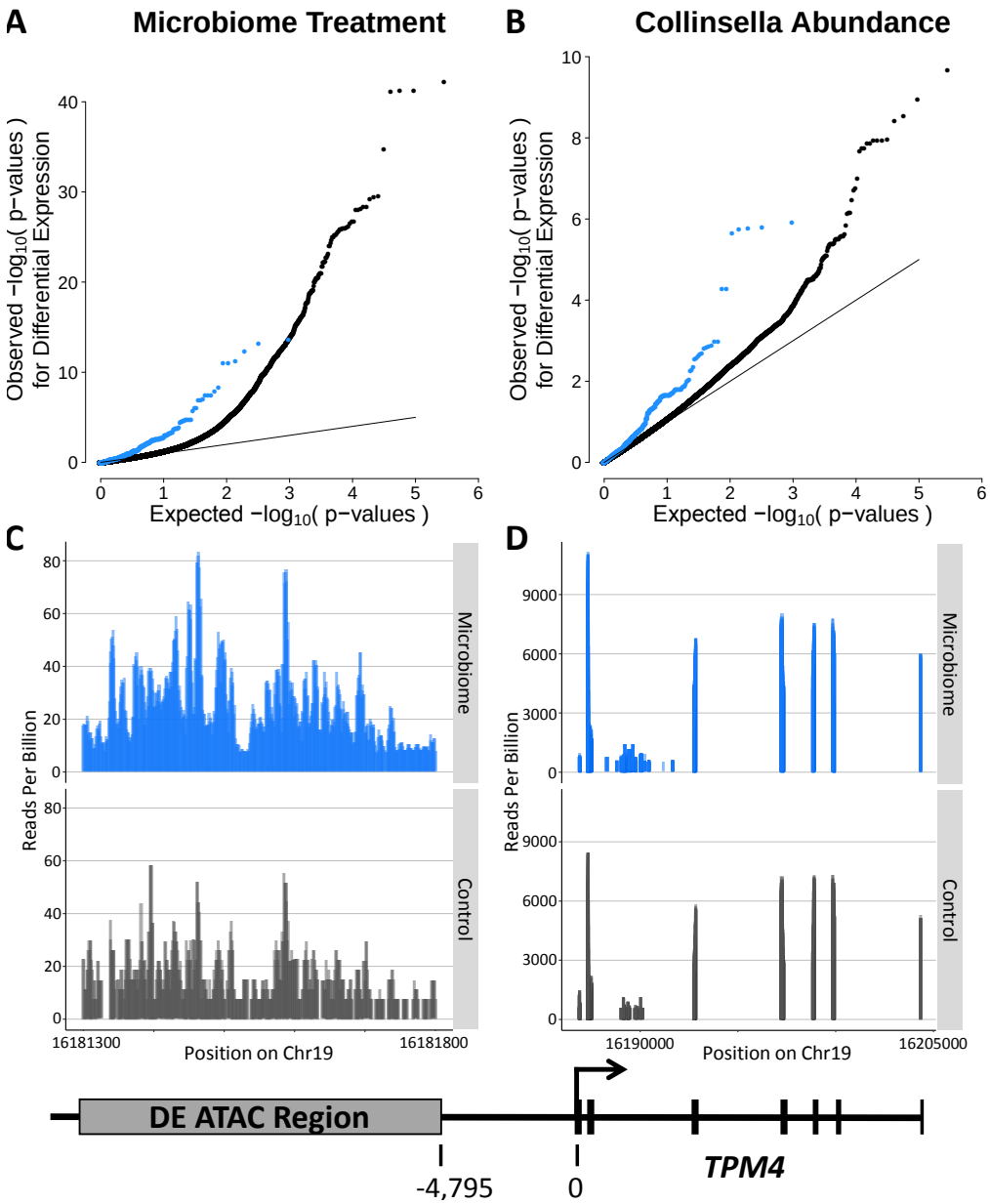

Figure 4: The microbiome induces changes in gene expression through changes in chromatin accessibility. **A** QQ-plot of p-values from the meta-analysis of differential gene expression in colonocytes treated with five microbiota samples for 4 hours. The bluepoints indicate the p-values for transcripts that are within 10kb of a differentially accessible region identified through ATAC-seq. The gray points are for transcripts not within 10kb of a differentially accessible region. **B** QQ-plot of p-values from the genus-based model of differential gene expression analysis of colonocytes. The blue points indicate the p-values for transcripts that are within 10kb of a differentially accessible region identified through ATAC-seq. The gray points are for transcripts not within 10kb of a differentially accessible region. **C** ATAC-seq profile centered on the 300bp window (with 100bp on either side) that is differentially accessible following 2 hours of treatment with the microbiome (BH FDR = 12%). This region is found 4,795bp from the differentially expressed gene *TPM4*. **D** RNA-seq profile of *TPM4* (ENST00000586833) which is differentially expressed following 4 hours of treatment with the microbiome (BH FDR = 1.9%). The gene model is shown below.

FDR = 20%). As expected, we found an enrichment for differentially accessible regions within 10kb of genes differentially expressed at 4 hours (Figure 4A, p-value = 0.013, OR = 2.22). Interestingly, we did not find the same enrichment when we examined genes differentially expressed at 1 and 2 hours following

exposure to gut microbiota (p-value > 0.15), suggesting that the changes in chromatin accessibility that we identified occur first (at 2 hours), and then lead to subsequent changes in gene expression by 4 hours post-inoculation. In addition to changes in chromatin openness that are a result of the presence of the total microbiota sample, we wondered whether particular microbes can induce specific changes in chromatin accessibility. To answer this question, we focused on *Collinsella aerofaciens* and observed an enrichment for regions of differential chromatin accessibility near genes differentially expressed relative to the abundance of this microbe (Figure 4B, see Table S9 for the full list of genes). One interesting example of a gene that is regulated by the microbiome through changes in chromatin accessibility is *TPM4* which binds actin filaments and shows increased expression at 4 hours following treatment with the five microbiota samples (Figure 4C and D). We identified the chromatin change at a region located at 4,700 bp upstream of the transcription start site of *TPM4*. This example, and others (Table S9), support the hypothesis that the microbiota influences host gene expression through modulation of chromatin accessibility.

Discussion

The gut microbiota has recently been associated with several different diseases and disorders [2, 6, 16, 19, 26, 44, 47]. However, the mechanism of action is not well understood, and we know little about how microbiome risk factors interact with host genes that have been linked to the same disorders. Here, we investigated how inter-individual variation in microbiome composition affects host gene regulation. Previous work in mice has shown that transplantation with microbiomes derived from mice with different phenotypes, e.g. obese versus lean, can lead to differences in organismal phenotypes [47]. In contrast, our work focuses on variable responses when considering microbiomes derived from healthy humans. We illustrate that while there are thousands of genes that show consistent response induced by the five microbiome treatments, there are several hundreds that respond differently to each microbiome. Furthermore, some of the differences in response can be understood through the variation in microbiome composition of the five samples. Though we focus on healthy microbiomes, our results highlight microbes and host genes interactions that may play roles in various host traits. For example, we identify hundreds of genes that respond to particular microbes in a predictable way, such as the gene *GLTP* for which the response is correlated with *Collinsella*. Both *GLTP* and *Collinsella* have been associated with metabolic syndrome traits [8, 52] and our results now suggest that *Collinsella* may impact complex traits through regulation of host gene expression. While we closely examined the effect of *Collinsella* on host gene expression, there were many other relevant microbes that may also play a role in host gene regulation. For example, the abundance of *Prevotella*, which has been associated with colorectal carcinoma [45] and ileal Crohn's disease [32], induces changes in the expression of 6 genes (BH FDR = 10%). Further studies with a wider panel of microbiome samples will allow for increased power to detect more genes that are differentially expressed across microbiome treatments.

Our study of chromatin accessibility changes after 2 hours of microbiome exposure in 12 samples identifies over 200 differentially accessible regions that were enriched near differentially expressed genes. In contrast, previous work in conventionalized mice do not show broad changes in chromatin accessibility compared to germ-free mice, perhaps because of limited statistical power and chromatin accessibility assays requiring bigger sample sizes [7, 10]. Furthermore, regions that are differentially accessible are enriched near genes that change their expression following treatment, suggesting that microbiome-induced changes in chromatin accessibility may be one of the mechanisms by which the microbiota drives host gene expression. We expect that a larger sample size would yield more power to detect a larger number of microbiome-controlled chromatin accessible regions and affected genes.

Conclusion

Our results suggest that specific microbes in the microbiome may be important in regulating host gene expression in the gut, and that microbes can induce changes to a large number of genes. Furthermore, our data support the hypothesis that changes in chromatin accessibility in host cells is one of the mechanisms by which the microbiome induces changes in expression of host genes that are involved in complex traits, including those that have already been associated with microbiome composition. Finally, our work suggests a molecular mechanism by which supplementing the microbiome can influence human health through changes in host cellular response. We have shown that by manipulating microbiome composition by supplementing a single microbe, we can influence the host cell regulatory response in a predictable way. This work and future research will help to determine which microbes may be most beneficial as interventional therapy to improve one's health.

Methods

Extended Materials and Methods can be found in the supplement.

Cell culture and treatment

Experiments were conducted using primary human colonic epithelial cells (HCoEpiC, lot: 9810) which we also term, colonocytes (ScienCell 2950). Fecal microbiota was purchased from OpenBiome as live microbial suspension in 12.5% glycerol. On the day of the experient, media was removed from the colonocytes and fresh antibiotic-free media was added, followed by inoculation with microbial extract for a final microbial ratio of 10:1 microbe:colonocyte in each well. Additional wells containing only colonocytes were also cultured in the same 6-well plate to be used as controls. This experimental protocol was already described in [40].

Following 1, 2 or 4 hours, the wells were scraped on ice, pelleted and washed with cold PBS and then resuspended in lysis buffer (Dynabeads mRNA Direct Kit) and stored at -80°C until extraction of colonocyte RNA.

Collinsella Spike-in Experiment

Collinsella aerofaciens was purchased from ATCC (cat#: 25986) and grown in Reinforced Clostridial Medium (BD Biosciences, cat#: 218081) following manufacturer's protocol, in anaerobic conditions. We verified that we were utilizing *Collinsella aerofaciens* by extracting the DNA with the PowerSoil kit as described below. We then PCR amplified the 16S region using primer specific to *Collinsella aerofaciens* [23] (Figure S8).

Cell culturing conditions for this experiment were the same as described above. On the day of treatment, solutions were made with the microbiota sample (from individual 4) at 10:1 to the number of colonocytes and the *Collinsella aerofaciens* was spiked into the microbiota sample at 4 dilutions: 10%, 1%, 0.1% and 0.01%. Additional wells containing only colonocytes and colonocytes with the microbiota sample (0% *Collinsella aerofaciens*) were cultured as controls on the same 12-well plate. Each treatment was performed in duplicate.

Following 2 hours of culturing, the wells were scraped on ice, pelleted and washed with cold PBS, re-suspended in lysis buffer (Dynabeads mRNA Direct Kit) and stored at -80°C until extraction of colonocyte RNA (as described above).

RNA-library preparation from colonocytes

Poly-adenylated mRNAs were isolated from thawed cell lysates using the Dynabeads mRNA Direct Kit (Ambion) and following the manufacturer's instructions. RNA-seq libraries were prepared using a protocol modified from the NEBNext Ultradirectional (NEB) library preparation protocol to use Barcodes from BIOOScientific added by ligation, as described in [36]. The libraries were then pooled and sequenced on two lanes of the Illumina Next-seq 500 in the Luca/Pique laboratory using the high output kits for 75 cycles to obtain paired-end reads for an average of over 40 million total reads per sample.

RNA sequencing and Alignment

Reads were aligned to the hg19 human reference genome using STAR [13] (`https://github.com/alexdobin/STAR/releases`, version `STAR_2.4.0h1`), and the Ensemble reference transcriptome (version 75). We further removed reads with a quality score of < 10 (equating to reads mapped to multiple locations) and removed duplicate reads using `samtools rmdup` (`http://github.com/samtools/`).

Differential Gene Expression Analysis

To identify differentially expressed (DE) genes, we used `DESeq2` [29] (`R` version `3.2.1`, `DESeq2` version `1.8.1`). Gene annotations from Ensembl version 75 were used and transcripts with fewer than 20 reads total were discarded. `coverageBed` was utilized to count reads with `-s` to account for strandedness and `-split` for BED12 input. The counts were then utilized in DESeq2 with several models to determine changes in gene expression under various conditions. A gene was considered DE if at least one of its transcripts was DE. In order to identify genes that changed at each time point following co-culturing, we used each microbiota treatment as a replicate. With this model, we identified 1,835 genes that change after 1 hour (70% of genes increase in expression), 4,099 genes after 2 hours (53% of genes increase in expression) and 1,197 genes after 4 hours (56% increase) with BH FDR $< 10\%$, $|\text{logFC}| > 0.25$ (Figure S2).

In order to identify genes that were differentially expressed at a given time point after co-culturing with a specific microbiota sample we used an alternative model which allows for a different effect to each microbiome. With this model 1,131 genes changed after 1 hour with any of the 5 samples, 3,240 after 2 hours and 1,060 after 4 hours with BH FDR $< 10\%$, $|\text{logFC}| > 0.25$ (Figure 1B).

We next used the likelihood ratio test that is a part of DESeq2 to compare the 2 models above in order to identify genes whose expression changes over time are determined by the individual from which the microbiota sample was taken. In this way, we identified 409 genes at BH FDR $< 10\%$.

In order to identify components of the microbiota samples that affect gene expression we used a model that included baseline abundance of a given taxon. Baseline abundance is the number of reads (after all samples have been rarified to the sample with the lowest read count of 141,000) for a given taxon in each of the uncultured samples. Each of the time points had the same baseline abundance. This model was run for 62 taxa that had at least 141 reads (0.1% of the total reads in a sample) in at least one of the five uncultured samples. Comparing each taxon to all genes expressed in the colonocytes, we had 9,125,927 tests. We identified 588 significant comparisons (BH FDR $< 10\%$) comprising of 46 taxa and 121 genes (Table S6).

Finally, we analyzed the validation experiment with the spike-in of *Collinsella aerofaciens*. In order to identify genes that were differentially expressed because of the *Collinsella aerofaciens*, we used the a model that included the increasing abundances of *Collinsella aerofaciens*. We identified 1,570 genes that change expression (BH FDR $= 10\%$, Table S8, Figure S6) depending on the abundance of *Collinsella aerofaciens*.

16S rRNA gene sequencing and analysis of the microbiome

Half of each culturing well and the full volume of wells with microbiota samples cultured alone were used for extraction of microbial DNA using the PowerSoil kit from MO BIO Laboratories as directed, with a few modifications (found in the supplement). Microbial DNA was also extracted from the uncultured microbial samples. 16S rRNA gene amplification and sequencing was performed at the University of Minnesota Genomics Center (UMGC), as described in Burns et al. [6].

The trimmed 16S rRNA gene sequence pairs were quality filtered (q-score ¿ 20, using QIIME 1.8.0) resulting in 1.41, 1.06, and 1.53 million high quality reads for sample replicates 1, 2, and 3, respectively [9, 37]. OTUs were picked using the closed reference algorithm against the Greengenes database (August, 2013 release) [6, 9, 11, 37]. The resulting OTU table was analyzed to determine microbial community diversity using QIIME scripts and rarefying to 141,000 reads.

We verified that the fecal samples we utilized were similar to other healthy samples by comparing the OTUs detected to the Human Microbiome Project data [21, 34]. 16S V4 OTU and HMP V1V3 OTU tables (`https://www.hmpdacc.org/HMQCP/`, final OTU table) were run through QIIME's `summarize_taxa.py` and consolidated at the L3 class level.

Determining Effect of Colonocytes on Microbiota Composition

OTUs were collapsed to the genera level using scripts in QIIME 1.9.1. In total, 292 taxa were detected across all samples and treatments. After filtering on the relative abundances of each taxon, we focused on 112 taxa.

To assess how each taxon changed in response to culturing with colonocytes, we ran two linear models (including or excluding the effect of colonocytes) and compared the goodness of fit using a likelihood ratio test. The model yielded 13/112 taxa that change significantly due to treatment with a BH FDR $< 10\%$ (Table S5).

ATAC-seq in Colonocytes Exposed to Gut Microbiota

Colonocytes were treated as described above. Each of the 5 microbiota samples were used for treatment, in replicate. Two wells of colonocytes were untreated for 2 hours under the same culturing conditions, as controls. Following 2 hours of exposure to the microbiome, cells were collected by scraping the plate on ice. Each well was counted in order to remove 50,000 cells to be used for ATAC-seq. We followed the protocol by [5] to lyse 50,000 cells and prepare ATAC-seq libraries, with the exception that we used the Illumina Nextera Index Kit (Cat #15055290) in the PCR enrichment step and the cells were not lysed with 0.1% IGEPAL CA-630 before adding the transposase to begin the ATAC-seq protocol. Individual library fragment distributions were assessed on the Agilent Bioanalyzer and pooling proportions were determined using the qPCR Kapa library quantification kit (KAPA Biosystems). Library pools were run on the Illumina NextSeq 500 Desktop sequencer in the Luca/Pique-Regi laboratory. Barcoded libraries of ATAC-seq samples were pooled and sequenced in multiple sequencing runs for 130M 38bp PE reads (max: 159M, min: 105M). One sample, derived from treatment of the colonocytes with microbiota from individual 3 was removed from later analysis due to low sequencing coverage (63M reads).

Reads were aligned to the hg19 human reference genome using HISAT2 [25] (`https://github.com/`, version `hisat2-2.0.4`), and the Ensemble reference transcriptome (hg19) with the following options:

```
HISAT2 -x <genome> -1 <fastq_R1.gz> -2 <fastq_R2.gz>
```

where `<genome>` represents the location of the genome file, and `<fastqs_R1.gz>` and `<fastqs_R2.gz>` represent the fastq files.

The multiple sequencing runs were merged for each samples using samtools (version 2.25.0). We further removed reads with a quality score of < 10 (equating to reads mapped to multiple locations) and removed duplicate reads using `samtools rmdup` (`http://github.com/samtools/`).

Identification of Differentially Accessible Region Following Inoculation with Microbiota

To identify differentially accessible regions, we used `DESeq2` [29] (`R` version `3.2.1`, `DESeq2` version `1.8.1`). We separated the genome into 300bp regions and `coverageBed` was used to count reads in these regions. These counts were then utilized in DESeq2 using the following model:

$$\begin{aligned} &\text{Gene expression} \sim \text{treatment} \\ &Y_{jn} = \sum_t \beta_{jt}^M M_{tn} \end{aligned} \tag{1}$$

where Y_{jn} represents the internal DEseq mean accessibility parameter for region j and experiment n, M_n is the treatment indicator (control or microbiome), and β_{jt}^M parameter is the microbiome effect. With this model, we identified 234 regions that change in the host following exposure to the gut microbiota with BH FDR $< 20\%$. These regions were then compared to gene annotations from Ensembl version 75 to identify those that were within 10kb of a transcription start site. We found enrichment for differentially accessible regions close to DE genes at 4 hours (p-value $= 0.013$, OR $= 2.22$) (Figure 4A). We also compared differentially accessible regions to gene DE relative to *Collinsella aerofaciens* abundance (Figure 4B).

Additional Files

Supplementary File 1 — Supplemental Methods and Results.

Supplementary text for materials and methods and additional results.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

FL, RB, RP-R, and ALR conceived the study, designed the data analysis and interpreted the results. ALR, FL, RB and RP-R wrote the manuscript with input from ALM. ALR, ALM. MBB and TJG performed the computational analyses. ALR generated all figures. ALR, AA and CC performed experiments including extraction of RNA and DNA and preparation and sequencing of RNA-seq and ATAC-seq libraries. All authors read and approved the final manuscript.

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Availability of data and materials

Submission of 16S rRNA sequencing data and RNA sequencing data of colonocytes in all conditions to Short Read Archive (SRA) is pending.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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