nature microbiology

LETTERS

PUBLISHED: 26 AUGUST 2016 | ARTICLE NUMBER: 16152 | DOI: 10.1038/NMICROBIOL.2016.152

Transcriptional interactions suggest niche segregation among microorganisms in the human gut

Damian Rafal Plichta[1,2], Agnieszka Sierakowska Juncker[1], Marcelo Bertalan[1], Elizabeth Rettedal[3], Laurent Gautier[1,4], Encarna Varela[5], Chaysavanh Manichanh[5], Charlène Fouqueray[6], Florence Levenez[6,7], Trine Nielsen[8], Joël Doré[7,9], Ana Manuel Dantas Machado[3], Mari Cristina Rodriguez de Evgrafov[3], Torben Hansen[8,10], Torben Jørgensen[11,12,13], Peer Bork[14], Francisco Guarner[5], Oluf Pedersen[8,11], Metagenomics of the Human Intestinal Tract (MetaHIT) Consortium†, Morten O. A. Sommer[3], S. Dusko Ehrlich[7,9,15], Thomas Sicheritz-Pontén[1], Søren Brunak[1,16]* and H. Bjørn Nielsen[1,2]*

The human gastrointestinal (GI) tract is the habitat for hundreds of microbial species, of which many cannot be cultivated readily, presumably because of the dependencies between species[1]. Studies of microbial co-occurrence in the gut have indicated community substructures that may reflect functional and metabolic interactions between cohabiting species[2,3]. To move beyond species co-occurrence networks, we systematically identified transcriptional interactions between pairs of coexisting gut microbes using metagenomics and microarray-based metatranscriptomics data from 233 stool samples from Europeans. In 102 significantly interacting species pairs, the transcriptional changes led to a reduced expression of orthologous functions between the coexisting species. Specific species–species transcriptional interactions were enriched for functions important for H_2 and CO_2 homeostasis, butyrate biosynthesis, ATP-binding cassette (ABC) transporters, flagella assembly and bacterial chemotaxis, as well as for the metabolism of carbohydrates, amino acids and cofactors. The analysis gives the first insight into the microbial community-wide transcriptional interactions, and suggests that the regulation of gene expression plays an important role in species adaptation to coexistence and that niche segregation takes place at the transcriptional level.

The gut microbiota is generally considered an ecosystem with many biological interactions[2–4]. Together with host effect[5], dietary habits[6], antibiotics[7] and other external factors[8,9], interactions between microbes may also be a defining factor for the gut microbiota. Known cooperative activities of microbial species include the formation of biofilms[10], fermentation of complex substrates[11] and exchange of metabolites[12], and antagonistic interactions that include competition for resources[13] or adhesion sites[14] and the production of antimicrobial compounds[15]. At the community level, microbes with shared genetic potential tend to coexist in the human GI tract, which suggests that the microbiome ensemble is driven by habitat filtering[4]. Classic population ecology theory, however, predicts that competition between two undifferentiated species eventually will lead to the extinction of one of them[16], which raises the question of how microbes with overlapping functional potential differentiate in the human GI tract. One possibility is that coexistence with other species invokes differentiation by transcriptional adaptations.

In vitro co-cultivations of selected species pairs have shown that microbes influence each other's gene expression[12,17]. Transcriptional interactions have also been observed in a co-inoculation of *Bacteroides thetaiotaomicron* and *Eubacterium rectale* in germ-free mice[13], which suggests that this may be a mechanism for avoiding or reducing competition and for niche segregation.

Although previous gut microbiome transcriptomics studies have provided a global overview of transcriptional activities[18], its variability between individuals[19] and the influence of external factors[7], they have not studied transcriptional interactions in a complex community.

Results

To study transcriptional activity in the gut we obtained microarray-based metatranscriptomics profiles from the 693,406 most-common gut microbiome genes[20] and abundance profiles for 741 metagenomic species[21] across 233 previously DNA-sequenced human-stool

[1]Department of Systems Biology, Center for Biological Sequence Analysis, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark. [2]Clinical-Microbiomics A/S, DK-2200 Copenhagen, Denmark. [3]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark. [4]Department of Systems Biology, DTU Multi-Assay Core, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark. [5]Digestive System Research Unit, University Hospital Vall d'Hebron, Ciberehd, 08035 Barcelona, Spain. [6]INRA, Institut National de la Recherche Agronomique, Metagenopolis, Cedex 78350 Jouy en Josas, France. [7]MGP, MetaGenoPolis, INRA, Université Paris-Saclay, Cedex 78350 Jouy en Josas, France. [8]The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark. [9]MICALIS, INRA, Université Paris-Saclay, Cedex 78350 Jouy en Josas, France. [10]Faculty of Health Sciences, University of Southern Denmark, DK-5230 Odense, Denmark. [11]Faculty of Health and Medical Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark. [12]Research Centre for Prevention and Health, Capital region, Glostrup Hospital, DK-2600 Glostrup, Denmark. [13]Faculty of Medicine, Aalborg University, DK-9220 Aalborg, Denmark. [14]European Molecular Biology Laboratory, 69117 Heidelberg, Germany. [15]King's College London, Centre for Host-Microbiome Interactions, Dental Institute Central Office, Guy's Hospital, London SE1 9RT, UK. [16]Faculty of Health and Medical Sciences, Novo Nordisk Foundation Center for Protein Research, Disease Systems Biology, University of Copenhagen, DK-2200 Copenhagen, Denmark. †A list of additional MetaHIT Consortium members and their affiliations is given in the Supplementary Information. *e-mail: hbjorn@clinical-microbiomics.com; soren.brunak@cpr.ku.dk

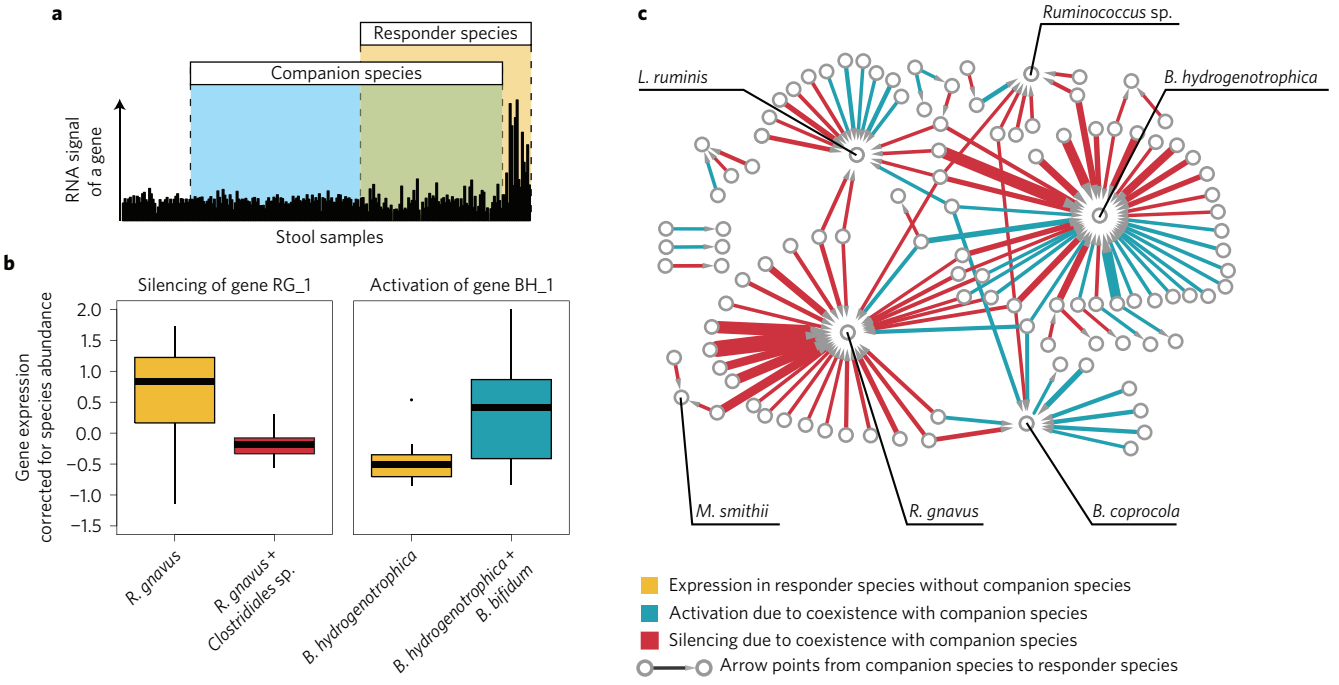

Figure 1 | Microbial co-occurrence in the GI tract results in transcriptional adaptations between coexisting species. a, Conceptual visualization of the experimental set-up for finding a companion species whose presence correlates with changes in a gene expression in a responder species. **b**, Examples of transcriptional interactions that result in reduced expression (left) of a *R. gnavus* gene during coexistence with a *Clostridiales* sp. (MGS:41, q value = 1.95×10^{-16}) and elevated expression (right) of a *B. hydrogenotrophica* gene during coexistence with *B. bifidum* (q value = 2.9×10^{-8}). The microarray signals were corrected for the abundances of the responder species and are shown as box plots with a horizontal line in the box that represents the median and with whiskers that indicate the lowest and highest point within 1.5 interquartile ranges of the lower or upper quartile, respectively. **c**, Microbial interactome in the human-gut microbiome. Significant species–species transcriptional associations were established with Fisher's exact test (P value <0.05 after Bonferroni correction) and are represented as a network in which species are depicted as circles. The arrows point from a companion to a responder species and their width increases with the number of genes with a changed gene expression in that pair. For simplicity, only interactions with at least ten modulated genes are shown. Species names are indicated for six responder species mentioned in the main text. Blue, majority of genes activated; red, majority of genes silenced.

samples[21,22] (Supplementary Table 1). We found significantly expressed genes from 322 species, which represented 86% of the species that occurred in at least 10% of the samples (Student's t-test, q value $\leq$0.05). Overall, we observed a tendency (Spearman correlation coefficient, $r = 0.37$) for abundant species to have more genes identified as significantly expressed than the less-abundant species (Supplementary Fig. 1), and that the number of expressed genes was robust to the reduced detection sensitivity of the metagenomics sequencing (Supplementary Fig. 2). In agreement with previous studies[7,18,19], we observed that the majority of the transcriptionally active species belonged to the Firmicutes and Bacteroidetes phyla with 231 and 51 species, and with 83 and 12% of all the expressed genes, respectively (Supplementary Fig. 3a), and that the archaea *Methanobrevibacter smithii* was among the top 5% of microbial species with the most expressed genes. Furthermore, we confirm that, although the species composition differed between individual samples, the overall distribution of expressed Kyoto Encyclopedia of Genes and Genomes (KEGG) functions is relatively constant across individuals[18] (Supplementary Fig. 3b).

However, any two species that coexist in an environment may affect each other's activity in a number of ways, for example, by direct inhibition or activation, by producing or consuming metabolites and by spatial competition. To identify potential interspecies transcriptional interactions, we tested for differential gene expression associated with pairwise species co-occurrence (Fig. 1a). This was done by comparing the expression of a given gene in a potential responder species across samples in which a companion species was either detected or absent (Fig. 1b (details in Methods)). In total, we identified 4,735 genes with transcriptional profiles significantly associated with the coexistence of specific species pairs (ANOVA, q value <0.1 (Supplementary Table 2)). The majority of these transcriptional adaptations (59%) were found in a small subset of the tested species–species pairs and showed significant enrichment in 249 species–species interactions (Fisher's exact test, $P < 0.05$, Bonferroni corrected). These encompassed 53 responder species and 142 companion species (Fig. 1c (Supplementary Information and Supplementary Table 3 give details)). The average number of genes affected in a species–species interaction was 31 (±4 s.e.m.), and the interaction that affected most genes was observed between *Ruminococcus gnavus* and the *Clostridiales* sp. (MGS:41, from Nielsen *et al.*[21]), with 542 *R. gnavus* genes (39% of the measured genes) expressed differentially. To verify these findings, we subsequently designed a series of co-cultivation experiments. In these, 11 out of 13 genes from five different responder–companion species pairs showed expression behaviour similar to that observed in the microbiome (Supplementary Fig. 4 and Supplementary Table 4).

What we observed as microbial transcriptional interactions could, to a large extent, be the consequences of environmental changes caused by activities of a companion organism, which in turn trigger transcriptional adaptation in the responder organism. The species pair *Catenibacterium mitsuokai* and *B. caccae* represents such a case. When observed independently, both species significantly expressed starch phosphorylase (EC 2.4.1.1), a gene that is important for starch degradation. The *C. mitsuokai* orthologue to this gene was, however, silenced during coexistence with *B. caccae*, possibly because *B. caccae*, a known specialist in polysaccharide metabolism[23], could have depleted the available starch resources.

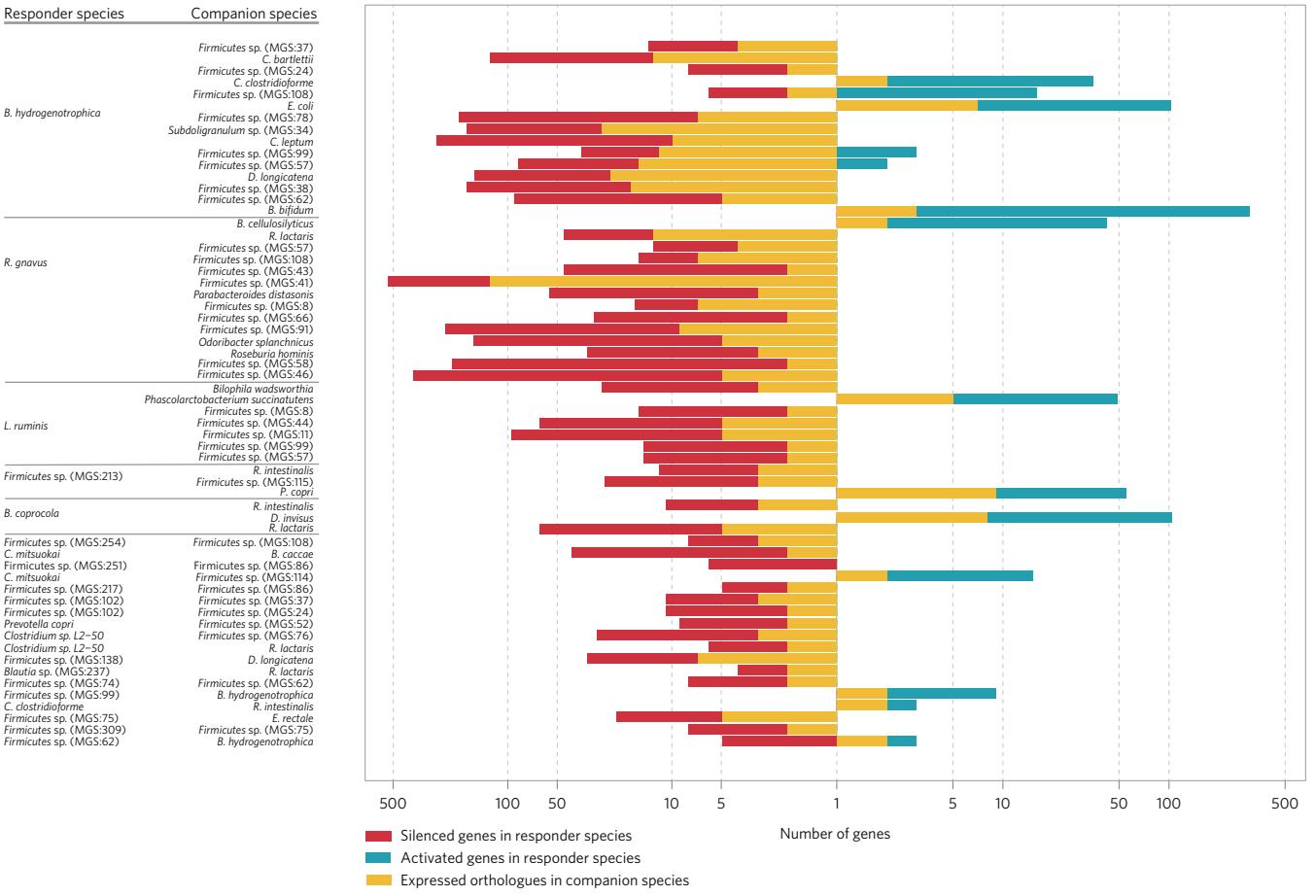

Figure 2 | Orthologous gene expression in a companion species coincides with modulation of a gene expression in a responder species. Gene orthology was derived based on KEGG annotation. Here we show 60 species pairs in which the companion species express at least two orthologous genes. The responder species are ordered according to the number of companion species; companion species are ordered according to the odds ratio of observing this number of orthologous genes expressed compared with the number of orthologous genes among non-expressed genes.

This may serve as an example of a phenomenon that we observed across 102 species pairs (41% of all interacting pairs) in which coexistence-associated expressed genes are significantly enriched for orthologues to genes that are significantly expressed in the companion species (Fisher's exact test, $P < 0.05$). Importantly, the majority of these orthologous genes (78%) were downregulated during coexistence (Fig. 2 and Supplementary Table 5). This suggests a decreased overlap in expressed functions in the interacting species pairs and indicates that species of the human-gut microbiome undergo niche segregation at the transcriptional level.

Among the transcriptionally modulated genes we observed a series of functions important for anaerobic fermentation, which is central to the colon-energy metabolism and results in incompletely oxidized nutrient substrates and H_2. Continued fermentation depends on removal of H_2 to stay energetically favourable and accumulation of H_2 and CH_4 has been associated with bloating and irritable bowel syndrome[24]. Part of the H_2 is excreted through flatus and breath, but a substantial part is anaerobically respired by a small subset of key hydrogenotrophic gut microbes, which include *M. smithii* and *Blautia hydrogenotrophica*[24,25], which both showed coexistence-associated regulation of their anaerobic respiration pathways (Supplementary Table 2). In *M. smithii* the expression of genes involved in the methanogenesis was reduced when it was observed together with either of two *Lachnospiraceae* sp. (MGS:45 and MGS:91). In *B. hydrogenotrophica* the Wood–Ljungdahl pathway, which yields acetate under H_2 and CO_2 consumption, was significantly activated during coexistence with *Bifidobacterium bifidum* (Fig. 3). The change in activity of the Wood–Ljungdahl pathway observed in *B. hydrogenotrophica* might be driven by a cross-feeding relationship with *B. bifidum*, as the latter species is a specialized carbohydrate-fermenting species[26] that produces the substrates for the CO_2 fixation by the Wood–Ljungdahl pathway. In contrast, the coexistence with each of five other species (*Clostridium bartlettii*, *C. leptum*, *Alistipes* sp., *B. pseudocatenulatum* and *B. dorei*) repressed the expression of the Wood–Ljungdahl pathway in *B. hydrogenotrophica* (Fig. 3). Particularly interesting is the relationship with *C. bartlettii*, which significantly expressed five orthologues from the Wood–Ljungdahl pathway (Fig. 3b), suggesting that *C. bartlettii* may substitute this activity.

In addition, we observed silencing of pathways for the biosynthesis of short-chain fatty acids, the key end products of anaerobic fermentation, significantly enriched in specific interacting pairs. This was, for example, observed in *B. hydrogenotrophica* and a *Lachnospiraceae* sp. (MGS:75) in response to coexistence with *Fusicatenibacter saccharivorans* (MGS:37) and *E. rectale*, respectively. Other transcriptional interactions were significantly enriched for nutrient-uptake functions, such as ABC transporters or phosphotransferase systems, flagella assembly and bacterial chemotaxis, and so on (Fisher's exact test, $P < 0.05$, Bonferroni corrected (Supplementary Fig. 5 and Supplementary Table 6)). The two most-frequent functional annotations across all the coexistence-associated differentially expressed genes (Supplementary Table 7) were the environment-sensing two-component systems[27] and the aminoacyl–transfer RNA biosynthesis pathway, which

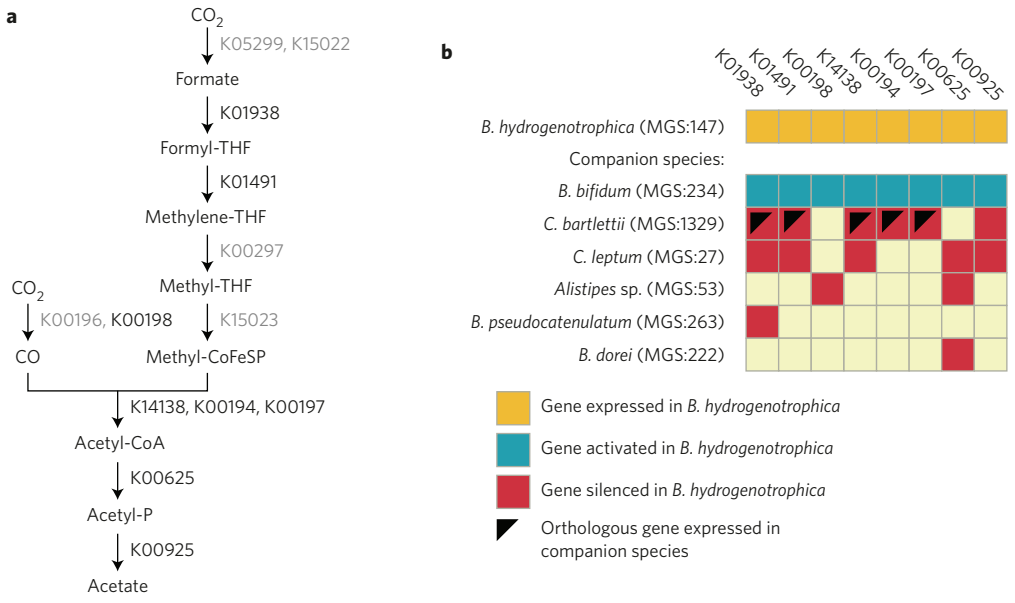

Figure 3 | Activity of the Wood–Ljungdahl pathway in *B. hydrogenotrophica* is influenced by other microbial species. a, The Wood–Ljungdahl pathway consists of two independent branches that fix CO_2 into acetyl-CoA, which subsequently can be transformed into acetate. KEGG orthology identifiers are assigned to all the steps in this transformation. **b**, Genes, indicated in black in the pathway graph (**a**), are up- or downregulated in *B. hydrogenotrophica* when it coexists with six companion species. Black triangles indicate orthologous genes that are expressed in the companion species.

could be responses to altered nutrient availability in the local environment. For instance, the expression of charged tRNA biosynthesis pathways is known to increases in response to low amino acid concentrations to better scavenge the remaining amino acids[28,29].

Discussion

All environments pose a selective pressure on the species that live in them, and hence enrich for species that share properties essential for survival in the given conditions. In the human-gut microbiome this leads to coexistence of microbial species with both complementary and overlapping functional properties[4] that may expose coexisting species to symbiotic or antagonistic interactions. In this study we utilized a microarray-based metatranscriptomics data set, which covers an unprecedented large set of 233 human-gut microbiome samples, to describe *in situ* transcriptional interactions by studying differential gene expression associated with coexistence between hundreds of specific gut microbial species. Interestingly, a significant part of the coexistence-associated differentially expressed genes shows a reduced expression when companion species express orthologous genes, and in consequence this shows that the functional overlap between species reduces. These observations stress that the activities of different microbes change in association with the community composition and, importantly, show that some microbes undergo niche segregation in the GI tract at the level of gene expression. This may, in turn, explain how closely related species can coexist over prolonged periods of time, rather than being outcompeted and excluded from the ecosystem. This mechanism can be beneficial to the host, as it sustains a diverse and rich gut microbial community with robustness to perturbations, a characteristic that is associated with metabolic health[20]. Furthermore, transcriptional adaptations may explain why the functional output of the gut microbiome is so consistent across individuals.

Although it is beyond the scope of a mere association analysis to determine causality and the mechanisms behind these adaptations, the bias towards reduced gene expression and enrichment of functional categories such as nutrient uptake or anaerobic respiration suggests that the mechanisms behind many of the observed transcriptional adaptations may be indirect through sensing the availability of local metabolites. The indirect effect of nutrient availability on the gene expression may also explain why two out of five coexistence-associated gene expressions observed *in situ* failed to reproduce in *in vitro* co-cultivations under rich nutrient conditions, in which critical nutrients may be in excess. This cross-feeding phenomenon is, however, less tangible as it requires a detailed understanding of metabolic pathways, their interconnections and the participating metabolites, whereas the identification of functional orthology required for our detection of niche segregation is entirely driven by sequence information.

This community-wide mapping of microbial transcriptional interactions was limited to detect only interactions between pairs of species. Therefore, our analysis may miss multispecies interactions (as, for example, is known from soil-biofilm formation) that have been shown to include up to four species[30]. In addition, we observe a weak tendency for the more-abundant species to have more-significantly expressed genes, which suggests that the analysis best describes the more-abundant species. However, the analysis was relatively robust to sensitivity differences between the microarray and the metagenomic sequencing, as shown in Supplementary Fig. 2. Together, these limitations suggest that the number of expressed genes and interactions presented here are on the conservative side.

In conclusion, we learn that the expression of some functions is less affected by coexistence (for example, DNA replication), whereas central metabolisms, which include the anaerobic respiratory pathway, environmental sensing and uptake of substrates, vary more with the specific community context. This and the observed specific species–species interactions is an important insight that may help constrain future metabolic network modelling and extend it to include species interactions. This study also adds to our understanding of how probiotics, faecal microbiota transplant and bacterial cocktail inocula may depend on the ability of species to adapt transcriptionally to the community context they are placed in. Finally, the insight that species transcriptionally adapt to each other further complicates microbiome-association analyses in that it highlights that species activities are context specific.

Methods

Microarray design. The human-gut microbiome microarray NimbleGen HD2 was designed to characterize transcriptional activity in the human faecal microbiome as described by Le Chatelier *et al.*[20] In short, it contains three probes for each of 693,406 human-gut microbial genes, which were selected to represent genes that were observed in 20 or more of 124 human-stool samples from Spanish and Danish individuals[22]. We chose microarrays over RNA sequencing because the great majority of the output from sequencing would probably originate from the rRNA of highly abundant species. This would render interrogation of mRNA from less-abundant species impractical. Although rRNA-depletion methods exist, such an approach could introduce biases in the resulting data. In contrast, the DNA microarray only interrogates the mRNA transcripts that are probed for.

Samples, RNA extraction and microarray hybridization. RNA was extracted from 233 human-stool samples (Supplementary Table 1) published in two previous metagenomic studies[22,21]. The frozen faeces (200 mg) were aliquoted into 2.0 ml microtubes using sterile spatulas. To each sample was added 400 µl of Tris-EDTA buffer (1×), 500 µl phenol-chloroform isoamyl alcohol (in a 5:1 mix of phenol (pH 4 from Eurobio) and chloroform:isoamyl alcohol (24:1 from Bioblock)), 25 µl of SDS (20% (Ambion)), 50 µl of sodium acetate (3 M, pH 4.8 (Sigma)) and 0.6 g of zirconia/silica beads (0.1 mm (BioSpec Product)), and then mixed by vortexing. Then, the mixture was shaken using a FastPrep FP220A (MP Biomedicals) at 5 m s^{-1} for 40 seconds, cooled on ice for 90 seconds and again shaken at 5 m s^{-1} for 20 seconds. After centrifugation at 13,000*g*, 400 µl of the supernatant was mixed with 500 µl of chloroform:isoamylalcohol (24:1) and homogenized by returning thoroughly to the tube. The homogenate was centrifuged at 13,400*g* at 4 °C for 15 minutes and 50 µl was transferred in a new 1.5 ml microtube. The following steps were performed using the 'High Pure Isolation' kit (Roche) according the manufacturer's recommendations. Residual genomic DNA was removed in two steps using the RNase-free DNase I (Promega) for 30 minutes at 37 °C, once as recommended in the High Pure Isolation kit and the second time after the elution step. RNA samples with a RNA integrity number over 5 (RIN, RNA Nano LabChip bioanalyzer from Agilent) were reverse transcribed to complementary DNA (Invitrogen, Superscript DS cDNA Synthesis kit protocol with random hexamer primers) and Cy3 labelled, hybridized to custom-designed NimbleGen HD2 arrays, washed and scanned according to the NimbleGen One Color Labeling Protocol for Expression Analysis v 3.0 (24 hours of incubation at 42 °C). The Ethical Committees of the Capital Region of Denmark (HC-2008-017) approved the study.

Microarray-data processing. The microarray data were background corrected and quantile normalized, and an expression index calculation at the gene level was done. To analyse the expression data in the context of the 3.9M gene catalogue generated from 396 stool samples by Nielsen *et al.*[21], we matched microarray design gene set to the new gene catalogue. Previously, the 3.9M gene catalogue was annotated with KEGG orthology and was structured into 741 metagenomic species[21], referred to as species in the majority of the manuscript. 201,071 genes could be assigned to one of the 741 species.

Significant gene expression. Transcriptionally active genes were inferred using a one-sided Student's *t*-test by comparing the microarray signal of each gene between samples in which a species that contained the gene was present or absent based on the metagenomics data described above. Genes from species with at least two genes assigned for species that were present and absent in at least 10% samples were tested. To fulfil the latter requirement for three species (MGS:6, MGS:9 and MGS:25), their abundance in samples below tenth quintile was set to zero. In total, we tested 186,231 genes from 375 species and accepted a gene to be significantly expressed at a *q* value <0.05 (ref. 31). We then summarized the expressions of genes across samples in matrix X by transforming the microarray signal for significantly expressed genes into Z scores:

$$X_{gs} = \frac{E_{gs} - M_{E_{ga}}}{SD_{E_{ga}}}$$

where E, M and SD stand for microarray signal, mean and standard deviation. Subscript *g* is the gene index, *s* is the sample index for samples in which the gene is present and *a* is the sample index for samples in which the gene is absent. Negative entries and entries for genes absent in a sample were set to 0.

The effect of the metagenomic sequencing depth on the detection of significantly expressed genes was tested by re-running the Student's *t*-test (described above) at downsizing levels below the original downsizing of 11M sequence reads (that is, from 10M to 1M with an interval of 1M sequence reads), which degenerated the species detection signal and increased the noise-to-signal ratio in calling significantly expressed genes.

Identification of coexistence-associated gene-expression changes. To identify gene-expression changes associated with coexistence between specific species pairs we tested each gene from potential responder species in a species pair, but only if the species was represented by 100 or more genes on the microarray ($n = 277$). Furthermore, only species pairs (consisting of a potential responder species and potential companion species) for which each species was found independently in ten or more samples, each was found together in ten or more samples and both were absent in ten or more samples (4×10) were tested ($n = 40{,}608$ species pairs). These filtering criteria were selected to ensure sufficient data for the statistical model to capture the microarray-background signal and the independent signal from the two species. Although the tested gene belongs to the responder species, modelling of the effect of the companion species was intended to capture potential cross-hybridization signals from that species. Each responder-gene expression level (the dependent variable) was tested independently in a linear two-way ANOVA model with the presence/absence of the companion and responder species as main factors, and the abundance of the responder species as an error factor. Importantly, this allowed for the inference of a companion/responder-species-interaction effect on the gene expression independently of the responder-species abundance. In other words, the statistical model corrects for the abundance of the responder species. Genes with a false discover rate[31] <0.1 for the interaction effect between the responder and companion factor were considered significantly coexistence associated.

Furthermore, each responder–companion species pair was tested for overrepresentation of genes with the expression changed associated with coexistence (Fisher's exact test, upper tail, $P < 0.05$, Bonferroni corrected) and significantly interacting species pairs were represented in the network in Fig. 1c using Cytoscape 3.0 (ref. 32).

Functional analysis. The gut microbial gene catalogue provided by Nielsen *et al.*[21] was annotated with KEGG orthology identifiers that we linked to the respective KEGG modules and pathways using an in-house version of the KEGG BRITE database. We tested significant interactions between microbial species for overrepresentation of functions belonging to KEGG modules or pathways (Fisher's exact test, upper tail $P < 0.05$, Bonferroni corrected).

Experimental validation. To verify the transcriptional interference observed between microbial species in the natural environment of the human gut, controlled experiments were set up for the following responder–companion bacterial pairs: (a) *E. sireaum* and *B. coprocola*, (b) *B. coprocola* and *Dialister invisus*, (c) *B. hydrogenotrophica* and *Dorea longicatena* and (d) *B. eggerthii* and *D. invisus*. Bacterial-type strains were obtained from the German Culture Collection (DSMZ). Bacteria were cultured overnight in 10 ml of brain heart infusion (BHI) broth (Fluka) at 37 °C under anaerobic conditions. The sterile BHI broth (2 ml) was placed in both the top and bottom wells of a six-well Transwell plate (0.4 um polyester membrane, 24 mm insert (Costar)). In triplicate, 20 µl of overnight culture of the companion bacteria was placed in the bottom well, and 20 µl of overnight culture from the responder bacteria was placed in the top well. The same bacteria type (20 µl) was inoculated into both the top and bottom wells of another Transwell plate to serve as a control. Cultures in Transwell plates were grown anaerobically for 18 hours at 37 °C. The responder bacteria culture was then collected, spun down with RNA protector and frozen at −80 °C for later RNA extraction. RNA was extracted using the RNeasy Mini Kit (Cat. No. 74104) from Qiagen. The RNA (200 ng) was used to synthesize cDNA using gene-specific primers and the RevertAid First Strand cDNA Synthesis Kit (#K1621 from Thermo Scientific). Gene expressions for selected genes were analysed by quantitative real-time PCR and differentail expression was tested using Student's *t*-test (Supplementary Table 4). Each sample was run in triplicate. The average threshold cycle number (*Ct*) values of the samples were obtained from each case. The relative gene expression was calculated using delta *Ct* (ΔCt) as an exponent of 2 ($2^{\Delta Ct}$). ΔCt was calculated with the average from the triplicates as follows: ΔCt = average *Ct* (control sample) – average *Ct* (co-culture sample).

Data availability. Microarray data can be found at the GEO database under accession code GSE76590.

Received 10 February 2016; accepted 22 July 2016; published 26 August 2016

References

1. Hayashi, H., Sakamoto, M. & Benno, Y. Phylogenetic analysis of the human gut microbiota using 16S rDNA clone libraries and strictly anaerobic culture-based methods. *Microbiol. Immunol.* **46,** 535–548 (2002).
2. Faust, K. *et al.* Microbial co-occurrence relationships in the human microbiome. *PLoS Comput. Biol.* **8,** e1002606 (2012).
3. Zelezniak, A. *et al.* Metabolic dependencies drive species co-occurrence in diverse microbial communities. *Proc. Natl Acad. Sci. USA* **112,** 6449–6654 (2015).
4. Levy, R. & Borenstein, E. Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules. *Proc. Natl Acad. Sci. USA* **110,** 12804–12809 (2013).
5. Kashyap, P. C. *et al.* Genetically dictated change in host mucus carbohydrate landscape exerts a diet-dependent effect on the gut microbiota. *Proc. Natl Acad. Sci. USA* **110,** 17059–17064 (2013).
6. David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505,** 559–563 (2014).

7. Maurice, C. F., Haiser, H. J. & Turnbaugh, P. J. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* **152,** 39–50 (2013).
8. Leone, V. *et al.* Effects of diurnal variation of gut microbes and high-fat feeding on host circadian clock function and metabolism. *Cell Host Microbe* **17,** 681–689 (2015).
9. Thaiss, C. A. *et al.* Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* **159,** 514–529 (2014).
10. Burmølle, M. *et al.* Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Appl. Environ. Microbiol.* **72,** 3916–3923 (2006).
11. Sproule-Willoughby, K. M. *et al. In vitro* anaerobic biofilms of human colonic microbiota. *J. Microbiol. Methods* **83,** 296–301 (2010).
12. Chassard, C. & Bernalier-Donadille, A. H_2 and acetate transfers during xylan fermentation between a butyrate-producing xylanolytic species and hydrogenotrophic microorganisms from the human gut. *FEMS Microbiol. Lett.* **254,** 116–122 (2006).
13. Mahowald, M. A. *et al.* Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. *Proc. Natl Acad. Sci. USA* **106,** 5859–5864 (2009).
14. Vollaard, E. J. & Clasener, H. A. Colonization resistance. *Antimicrob. Agents Chemother.* **38,** 409–414 (1994).
15. Donia, M. S. & Fischbach, M. A. Small molecules from the human microbiota. *Science* **349,** 1254766 (2015).
16. Hardin, G. The competitive exclusion principle. *Science* **131,** 1292–1297 (1960).
17. Samuel, B. S. & Gordon, J. I. A humanized gnotobiotic mouse model of host–archaeal–bacterial mutualism. *Proc. Natl Acad. Sci. USA* **103,** 10011–10016 (2006).
18. Gosalbes, M. J. *et al.* Metatranscriptomic approach to analyze the functional human gut microbiota. *PLoS One* **6,** e17447 (2011).
19. Franzosa, E. A. *et al.* Relating the metatranscriptome and metagenome of the human gut. *Proc. Natl Acad. Sci. USA* **111,** E2329–E2338 (2014).
20. Le Chatelier, E. *et al.* Richness of human gut microbiome correlates with metabolic markers. *Nature* **500,** 541–546 (2013).
21. Nielsen, H. B. *et al.* Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nat. Biotechnol.* **32,** 822–828 (2014).
22. Qin, J. *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464,** 59–65 (2010).
23. Wexler, H. M. Bacteroides: the good, the bad, and the nitty-gritty. *Clin. Microbiol. Rev.* **20,** 593–621 (2007).
24. Carbonero, F., Benefiel, A. C. & Gaskins, H. R. Contributions of the microbial hydrogen economy to colonic homeostasis. *Nat. Rev. Gastroenterol. Hepatol.* **9,** 504–518 (2012).
25. Rey, F. E. *et al.* Dissecting the *in vivo* metabolic potential of two human gut acetogens. *J. Biol. Chem.* **285,** 22082–22090 (2010).
26. Turroni, F. *et al. Bifidobacterium bifidum* as an example of a specialized human gut commensal. *Front. Microbiol.* **5,** 437 (2014).
27. Stock, A. M., Robinson, V. L. & Goudreau, P. N. Two-component signal transduction. *Annu. Rev. Biochem.* **69,** 183–215 (2000).
28. Putzer, H. & Laalami, S. Regulation of the expression of aminoacyl-tRNA synthetases and translation factors (Madame Curie Bioscience Database, 2000).
29. Condon, C., Grunberg-Manago, M. & Putzer, H. Aminoacyl-tRNA synthetase gene regulation in *Bacillus subtilis*. *Biochimie* **78,** 381–389 (1996).
30. Ren, D., Madsen, J. S., Sørensen, S. J. & Burmølle, M. High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation. *ISME J.* **9,** 81–89 (2015).
31. Storey, J. D. & Tibshirani, R. Statistical significance for genomewide studies. *Proc. Natl Acad. Sci. USA* **100,** 9440–9445 (2003).
32. Shannon, P. *et al.* Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13,** 2498–2504 (2003).

Acknowledgements

The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7-HEALTH-F4-2007-201052, MetaHIT and FP7-HEALTH-2010-261376: International Human Microbiome Standards. Additional funding was from the Metagenopolis grant ANR-11-DPBS-0001. E.R., A.N.D.M., M.C.R.E. and M.O.A.S. acknowledge funding from the Novo Nordisk Foundation and the Lundbeck Foundation. The Novo Nordisk Foundation Center for Basic Metabolic Research is an independent Research Center at the University of Copenhagen partially funded by an unrestricted donation from the Novo Nordisk Foundation (www.metabol.ku.dk).

Author contributions

All the authors are members of the MetaHIT Consortium. S.B. and H.B.N. managed the project. T.N., F.G., T.J., T.H. and O.P. performed clinical sampling. E.V., C.F., C.M. and F.L. performed RNA extraction. L.G. organized the microarray hybridizations. H.B.N., S.B., T.S.-P. and S.D.E designed the analyses. E.R., A.M.D.M., M.C.R.E. and M.O.A.S. did the co-cultivation and qPCR experiments. D.R.P., H.B.N., M.B. and A.S.J. performed the data analyses. D.P. and H.B.N. wrote the manuscript. D.R.P., H.B.N., S.B., E.R., A.S.J., C.M., S.D.E., O.P., J.D., M.O.A.S. and P.B. revised the manuscript. The MetaHIT Consortium members contributed to the design and execution of the study.

Additional information

Supplementary information is available online. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to S.B. and H.B.N.

Competing interests

The authors declare no competing interests.