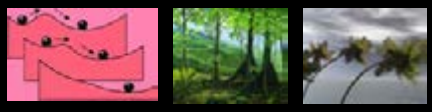
The Human Microbiome



David A. Relman, MD
Stanford University
18th International Congress on Infectious Diseases
Buenos Aires, 4 March 2018

The Human Microbiome

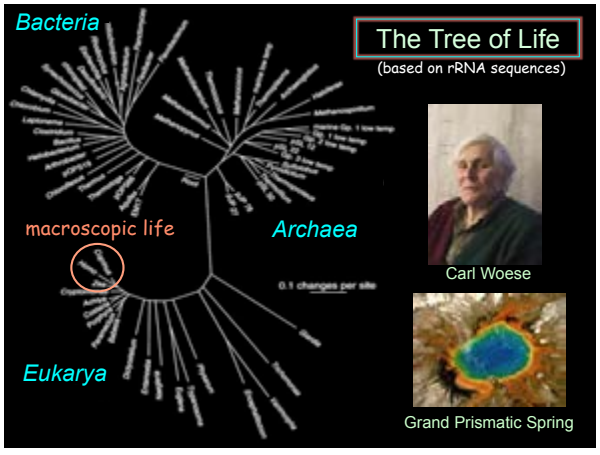
- Brief history; ecological perspective
- Disturbance; resilience
- Microbiome and adverse health outcomes (e.g., prematurity)
- Clinical impact; challenges

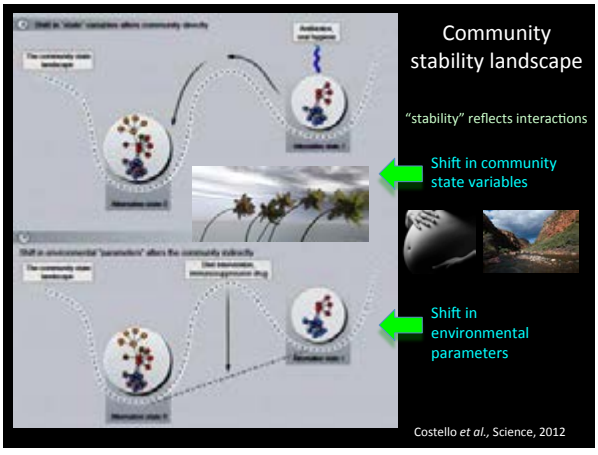
A bit of history: why now?

- Antony van Leeuwenhoek, 1683 ('animalcules')

"an unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time. The biggest sort. . . bent their body into curves in going forwards. . ."

- Razumov AS, 1932 ('Great plate anomaly')
  (Moore, Savage, 1975-7)
- Woese, Fox, Pace, 1977
- Convergence: disciplines, concepts





Attributes of 'stable' states in humans?

(Based on cell counts, we are ~1 part bacterial, 1 part human…and based on numbers of unique genes, we are 150 parts bacterial, 1 part human…)

Our benefits

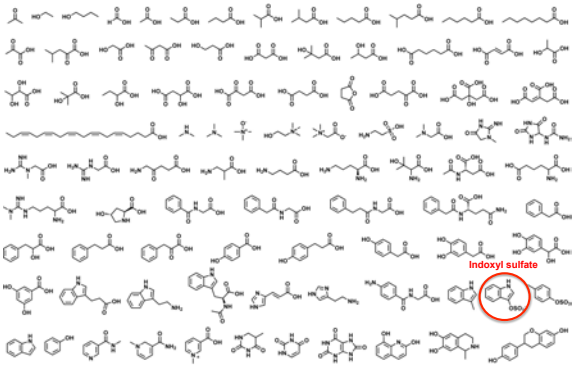
- Food digestion
- Nutrition (vitamins, energy)
- Xenobiotic processing
- Metabolic regulation, cometabolism
- Development: terminal differentiation of mucosa
- "Education", regulation of immune system
- Epithelial "homeostasis", barrier integrity
- Colonization resistance to pathogens

Turnbaugh P et al, Nature 449:804-810, 2007; Dethlefsen L et al, Nature 449:811-818, 2007

Gut Immune Maturation Depends on Colonization with a Host-Specific Microbiota

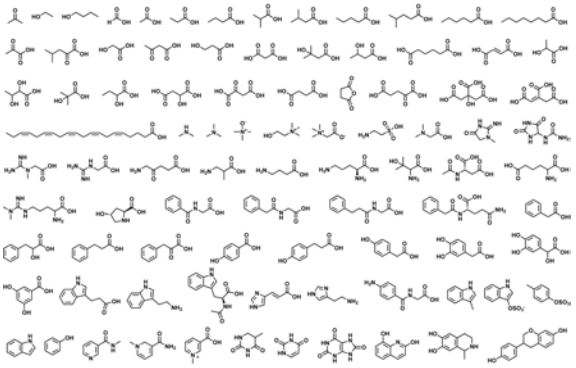
Cell 149:1578-93, 2012

Small molecules from the human microbiota



Michael Fischbach

Microbiota: Largest endocrine organ?



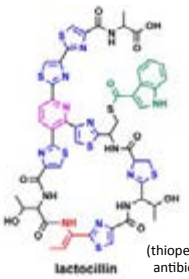
Michael Fischbach

Small molecules in the human microbiome

3,118 biosynthetic gene clusters from 755 metagenomic samples (100 healthy humans)

Average gut: 599 gene clusters
Average oral cavity: 1,061 gene clusters

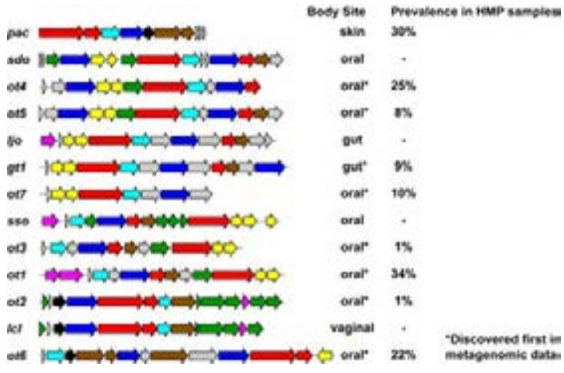
Dozens of gene clusters with unknown products present in >50% of healthy humans



Michael Fischbach, Stanford

Donia *et al*, *Cell* (2014), **158**, 1402

Thiopeptide clusters in the microbiota



Donia *et al*, *Cell* (2014), **158**, 1402

Disturbance

- Disturbances remove or kill some fraction of the community, creating opportunities for remaining community members or new colonists…effects directed at community and/ or host (e.g., diet shifts, diarrhea)
- Resilience: recovery of ecosystem services
- Increasingly prominent in "modern" societies? Problem of frequency, amplitude?
- Compounded, cumulative effects?

Disturbance

PERTURBATION EXPERIMENTS IN COMMUNITY ECOLOGY: THEORY AND PRACTICE[1]

Ecology 1984

EDWARD A. BENDER[2]
Department of Mathematics, C-012

TED J. CASE AND MICHAEL E. GILPIN
Department of Biology, C-016, University of California at San Diego, La Jolla, California 92093 USA

Physical, pulse disturbance: flash flood

Sycamore Creek, Maricopa County, AZ

Fisher et al., Ecol Monographs 1982; 52:93-110

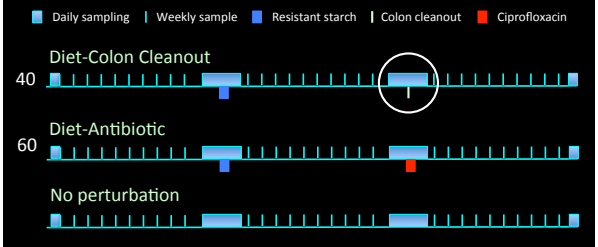
Understanding disturbance: flash flood in gut

Sycamore Creek, Maricopa County, AZ

Fisher et al., Ecol Monographs 1982; 52:93-110

Mixed, sequential pulse disturbance

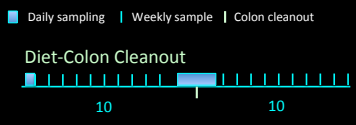
3 arms, 2 disturbance regimes, 36 wks



Stool & urine on each sampling day; 16S rRNA, shotgun metagenomes, metatranscriptomes, metabolites

Pilot: Clean-out pulse disturbance

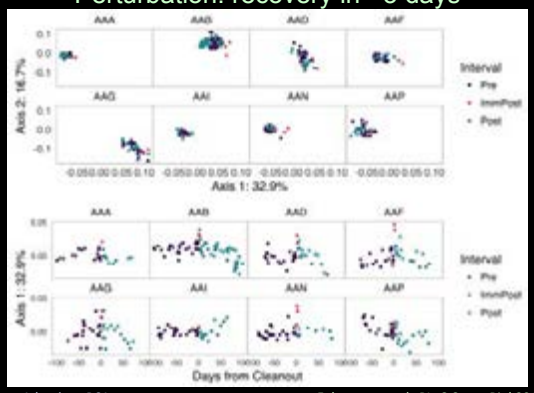
8 subjects, 20 wks



(Previous studies: few time points)

Fukuyama *et al.*, PLoS Comp Biol 2017

Perturbation: recovery in <3 days



Adaptive gPCA

Fukuyama *et al.*, PLoS Comp Biol 2017

Summary: cleanout disturbance

- Brief, discrete perturbation, rapid recovery (<3d) = resilience!
- Early enrichment of *Bacteroides*, depletion of *Ruminococcaceae*
- *Bacteroides*: versatile, mucus, rapid-growers; *Ruminococcaceae*: food particles, slow-growers
- Similarities to Fisher *et al.*: adaptation, historical precedents?
- Additional subjects, compare to other forms of disturbance…

Fukuyama *et al.*, PLoS Comp Biol 2017

Clinical problems associated with the indigenous microbiota

- Chronic periodontitis
- Crohn's disease
- Atherosclerosis
- Chronic renal insufficiency
- Environmental enteropathy
- Antibiotic-associated diarrhea
- Obesity, malnutrition
- Bacterial vaginosis
- Premature labor and delivery

"Community as pathogen"

that is,

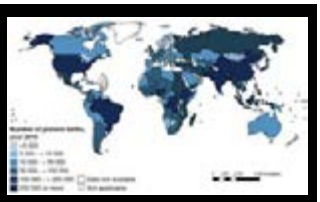
disease due to a community disturbance

"pathogenic states"

Clinical problems associated with the indigenous microbiota

Cause or effect?
Initiating or propagating?
Mono- or polyfactorial?

Microbiota and premature birth



- Worldwide incidence of preterm birth (PB) in 2010 was 11% (affecting 14.9 million babies)
- Direct complications of PB accounted for 1 million deaths
- Risks=bacterial vaginosis, chronic periodontitis; but only intra-amniotic infection has been causally linked to spontaneous preterm delivery

Blencowe et al. (2013) *Reproductive Health* 10 Suppl 1:S2
Romero et al. (2014) *Science* 345:760

Pregnancy, the microbiome, and premature birth

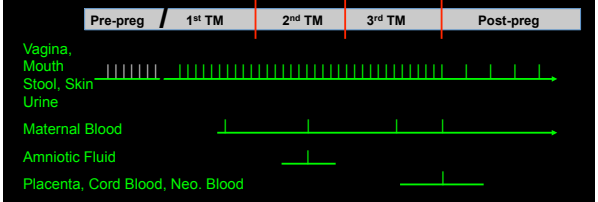
Hypotheses

- Microbiome of pregnancy contributes to fetal health, development. Perturbation of microbiome and associated host response contribute to preterm labor and delivery.

Approach

- Prospective longitudinal sampling of 4 body sites; taxonomic, gene/genomic, transcript, metabolite compositional analysis of microbiota; host gene expression & immune responses; multi-table methods for data integration, feature extraction

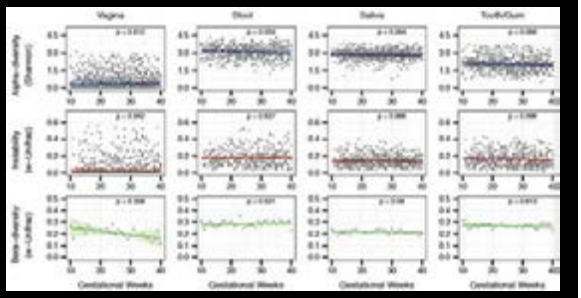
Sampling strategy and specimens collected to date



To date, 427 subjects enrolled (Stanford)…

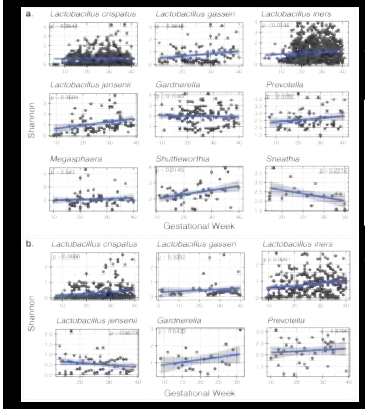
Self-collected specimens	~84,000
Blood specimens (maternal, cord, neonatal)	~6,000
Delivery specimens (placenta, amniotic fluid)	~1,500
Total specimens / time points	~91,500

Relative stability of phylogenetic composition during pregnancy



(?...absence of menstrual cycle)

DiGuilio *et al*, PNAS 2015

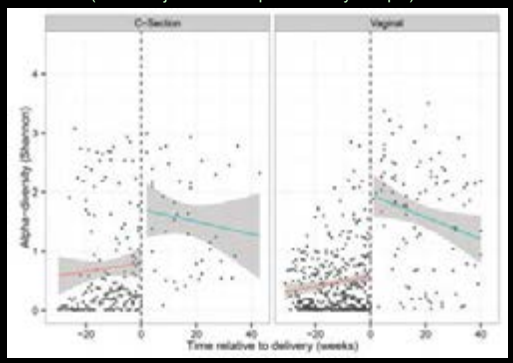


Lactobacillus iners – dominated communities become more diverse during pregnancy (2 studies)

Daniela Goltsman, Christine Sun

Postpartum perturbation of vaginal communities

(n=22 subjects with ≥1 post-delivery sample)



DiGuilio *et al*, PNAS 2015

Inverse relationship between interpregnancy interval and subsequent preterm birth?

THE INTERVAL BETWEEN PREGNANCIES AND THE OUTCOME OF SUBSEQUENT BIRTHS

Klebanoff MA, NEJM 1999; 340:643-4

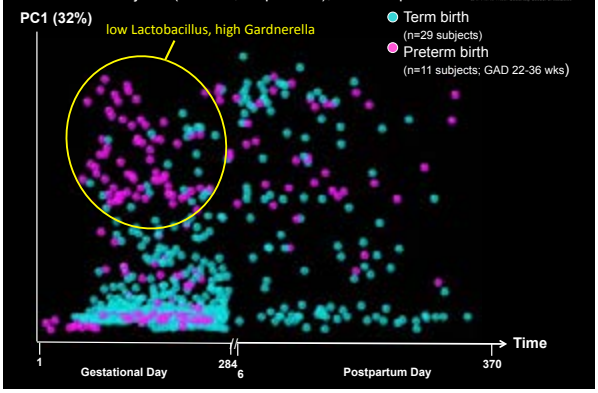
Interpregnancy Interval and Adverse Pregnancy Outcomes

An Analysis of Successive Pregnancies

Obstet Gynecol 2017; 129:408-15

Vaginal microbiota over time

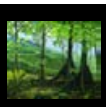
n= 40 subjects (29 term; 11 preterm), 996 samples



Vaginal microbiota and prematurity

- *Lactobacillus crispatus* (and not *L. iners*) associated with low risk of PTB; *Gardnerella vaginalis* subspecies associated with PTB high risk, in 2 racially diverse cohorts of U.S. women
- Ecological interactions may explain complex microbial community-wide attributes
- PTB in some women: loss of microbiome benefit and/ or gain of risk ?
- Mechanisms: Pro-inflammatory community? Breakdown of mucosal barrier? Invasion or bloom of 'pathogen'? Ascending infection (chorioamnionitis)?
- Identify women at risk? Mitigate risk?

Clinical Interventions?



- Promote or restore beneficial ecosystem
- Introduce strains, selectively support them
- Prepare local (cervico-vaginal) environment?
- Tailored to host or host population?
- Timing? (Pre-conception?)
- Need for monitoring?

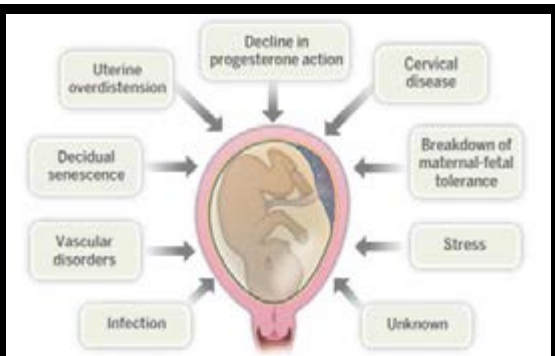


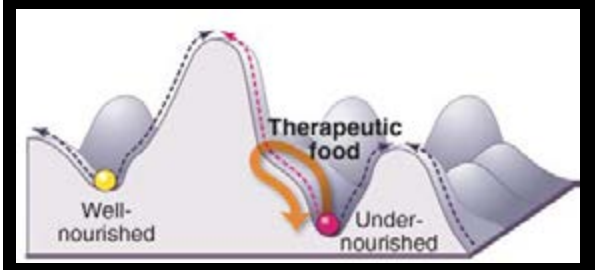
Fig. 2. Proposed mechanisms of disease implicated in spontaneous preterm labor. Genetic and environmental factors are likely contributors to each mechanism.

Science 2014; 345:760

Gut microbial ecosystem: Undernutrition

Science 339, 530 (2013)
Science 339, 548 (2013)

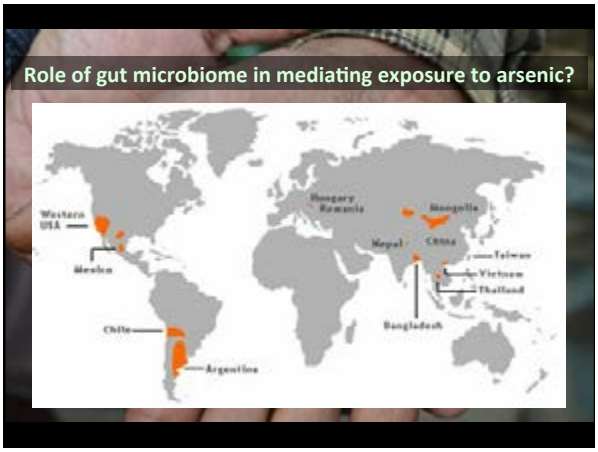
Gut microbial ecosystem: Undernutrition



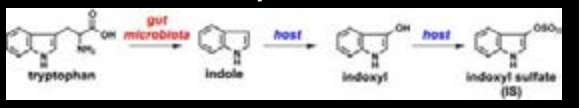
Science 339, 530 (2013)
Science 339, 548 (2013)

Role of gut microbiome in mediating exposure to arsenic?

Role of gut microbiome in mediating exposure to arsenic?



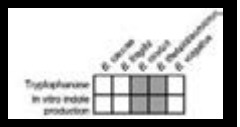
Role of gut microbiome in mediating chronic kidney disease?



indoxyl sulfate: uremic solute from gut microbiota

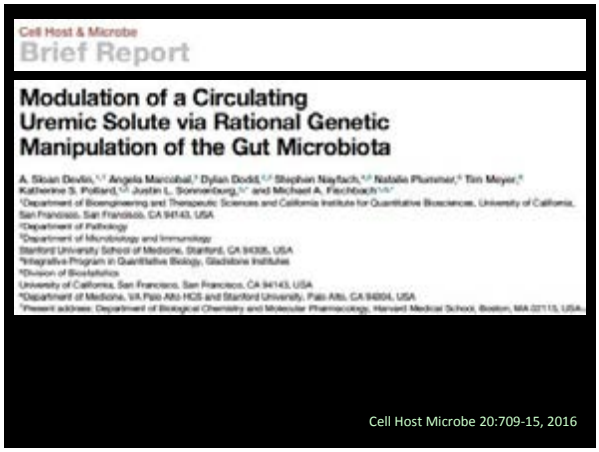
Level in humans: 10-200 mg/day

Correlated with disease



A single gene responsible for its production; its levels vary widely among people

http://www.usrds.org/atlas.aspx
Devlin *et al*, *Cell Host Microbe* (2016), **20**, 709

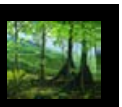

Cell Host & Microbe
Brief Report

Modulation of a Circulating Uremic Solute via Rational Genetic Manipulation of the Gut Microbiota

A. Sloan Devlin, Angela Marcobal, Dylan Dodd, Stephen Nayfach, Natalie Plummer, Tim Meyer, Katherine S. Pollard, Justin L. Sonnenburg, and Michael A. Fischbach

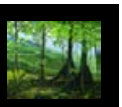
Cell Host Microbe 20:709-15, 2016

Clinical impact



- Great promise, exciting leads...unrealized; effect sizes in humans may be modest!
- Disease classification, predict health & disease; informed interventions
- Disturbance as a clinical tool: prognostics (assess resilience), therapeutics (establish alternative stable states = difficult)
- Maintenance, restoration of microbiota?
- First, do no harm: microbiota as Rx?

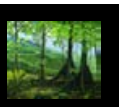
Study of human microbiome: challenges



- Tracking, understanding sources of variation: longitudinal study design
- Understanding, predicting function
- Assessing possible causation; role, relative contribution of microbiota
- Reproducibility
- Predictable, targeted manipulations of microbiome

Park Management Plan

(for human microbial ecosystem)



- Habitat restoration
- Promotion of native species
- Targeted removal of invasive species

- Ecosystem service providers? Community and host context?
- Adaptive management: system monitoring to inform decisions

Costello *et al.*, Science 336, 1255 (2012

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prematurity research center
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