Microbiome & Metabolomics

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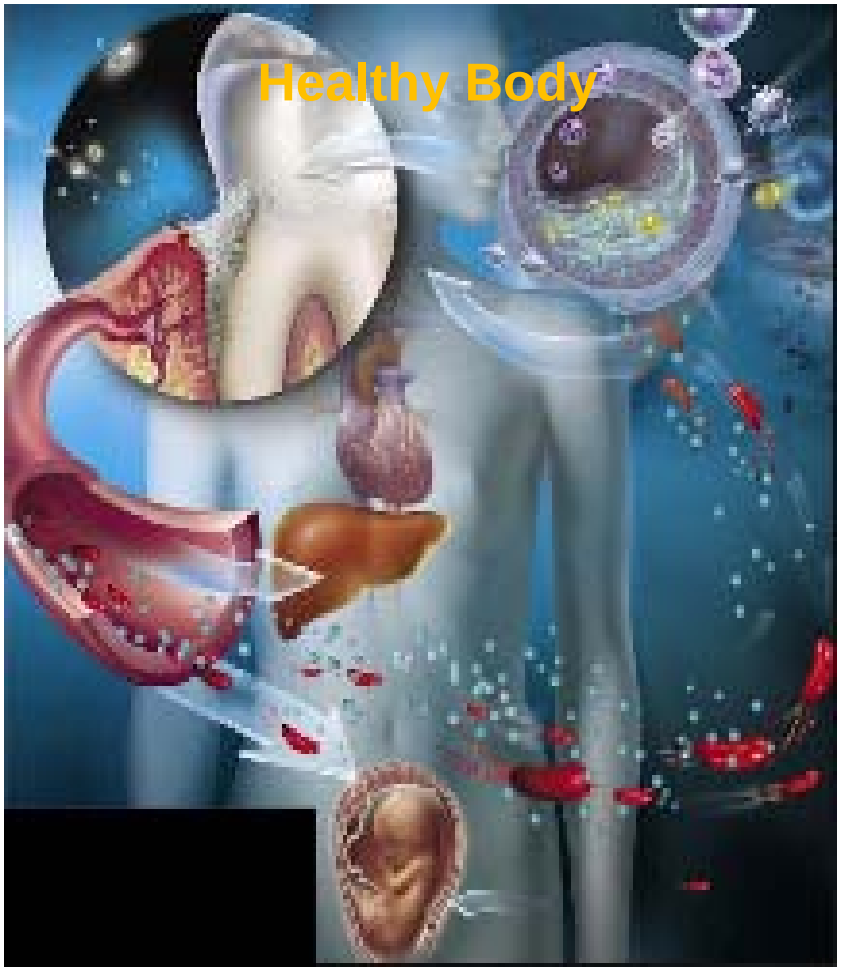
Outline

- **Microbe rules the world**
- **Microbial product matters**
- **Examples of microbiome in health and disease**
- **Examples of metabolites in health and disease**

Microbial infection and systemic disease



Cardiovascular Complications

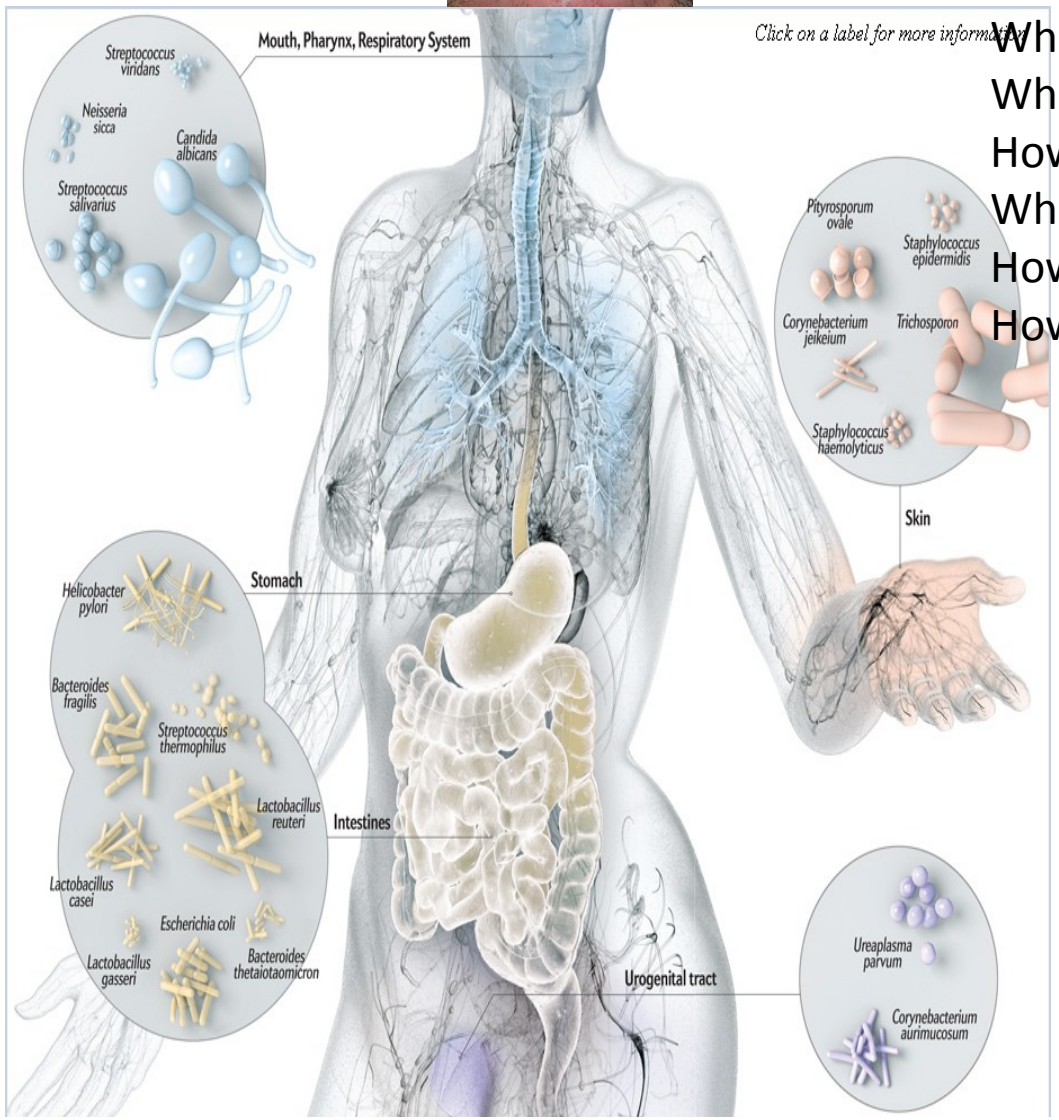
Respiratory infection

Pregnancy complication

Colon cancer

Complex microbial communities-dental plaque

Microbes are everywhere



Who are they?
What are they doing?
How is the host responding?
What maintains the equilibrium?
How do we differ?
How can we manipulate microbes?

10 % human cells
90 % microbial cells

Genetic info > 100

The Human Microbiome Project

- Microbial components of the human genetic and metabolic landscape, and how they contribute to health and disease
- The genomes of microbial symbionts provide traits that humans did not need to evolve on their own
- Humans, a composite of microbial and human cells
- Human genetic landscape dictated by the genes in the human genome and the microbiome
- Human metabolic features, a blend of human and microbial traits

A human ‘supraorganism’

Nature **449**, 804-810, 2007

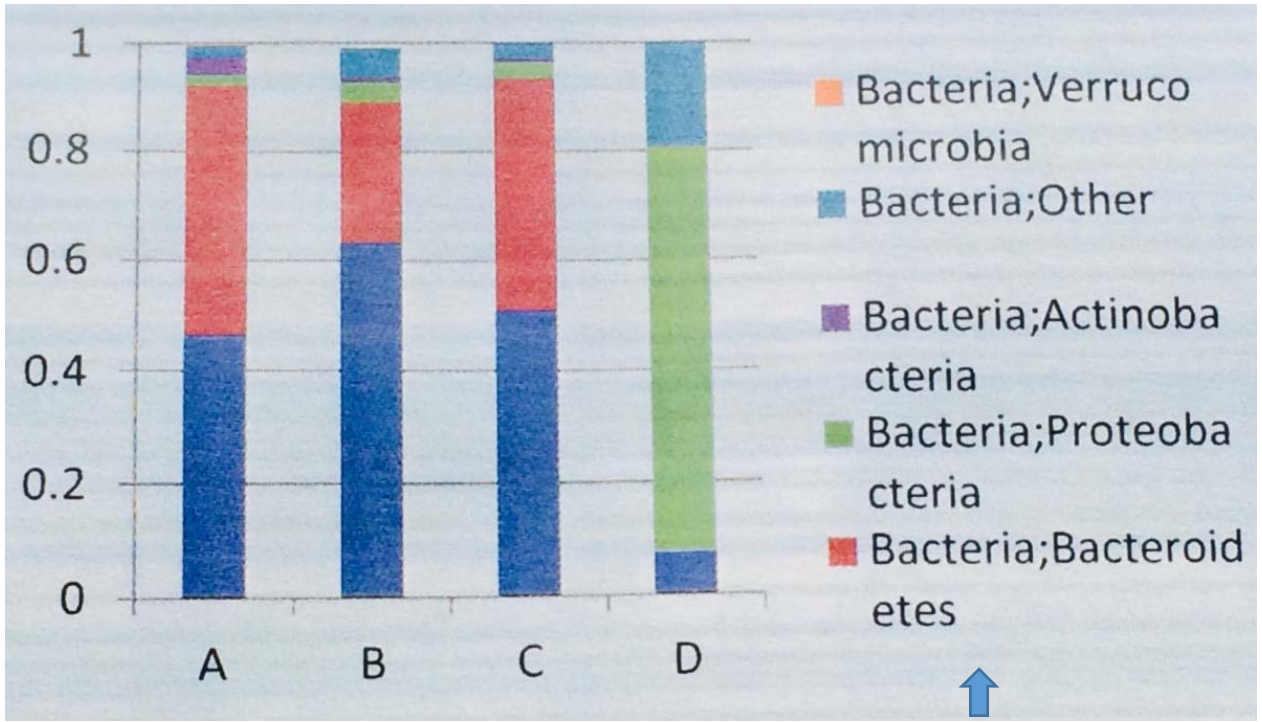
The Human Genome Project

The project funded by the US government in 1990, and declared complete in 2003.
A parallel project by the Celera Genomics in 1998.
Capacity-3 billion bps
Major advance in DNA sequencing

versus **the Human Microbiome Project**

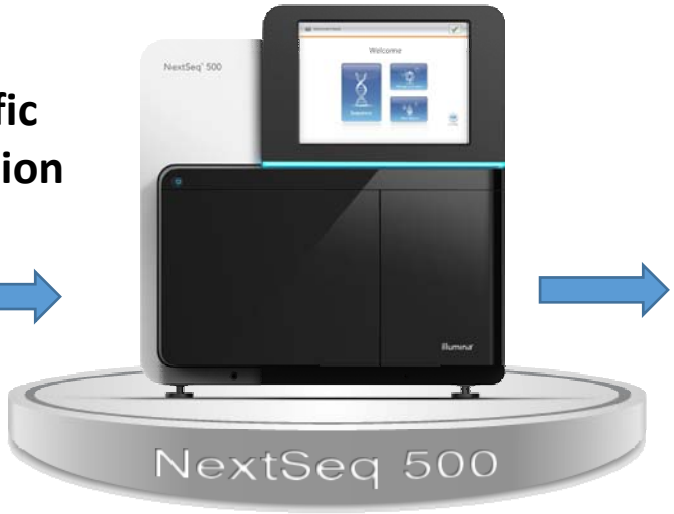
Microbiome Analysis-microbial profiling/genomics

Plaque or saliva



PCR with bar coded primers specific for 16S rDNA region for amplification

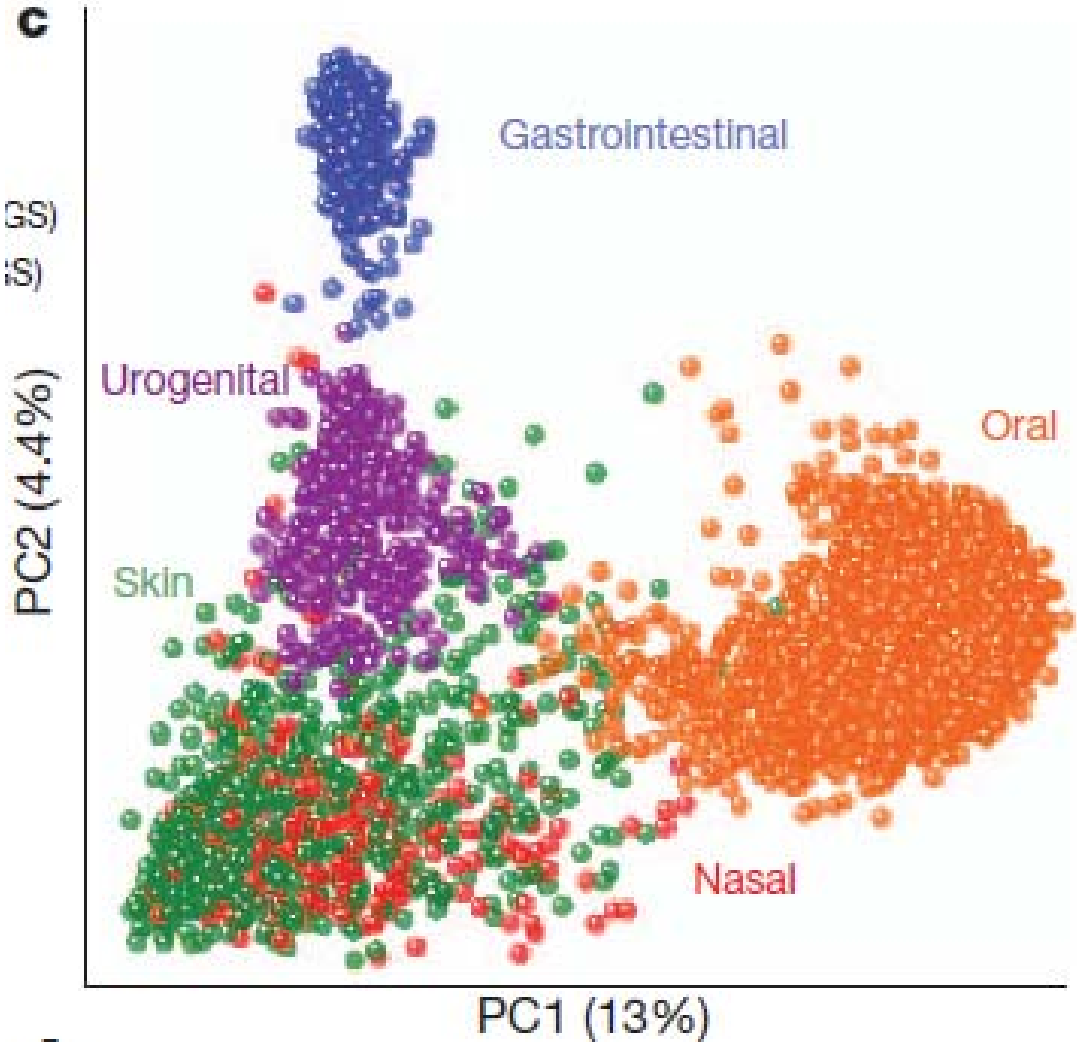
Metagenomics

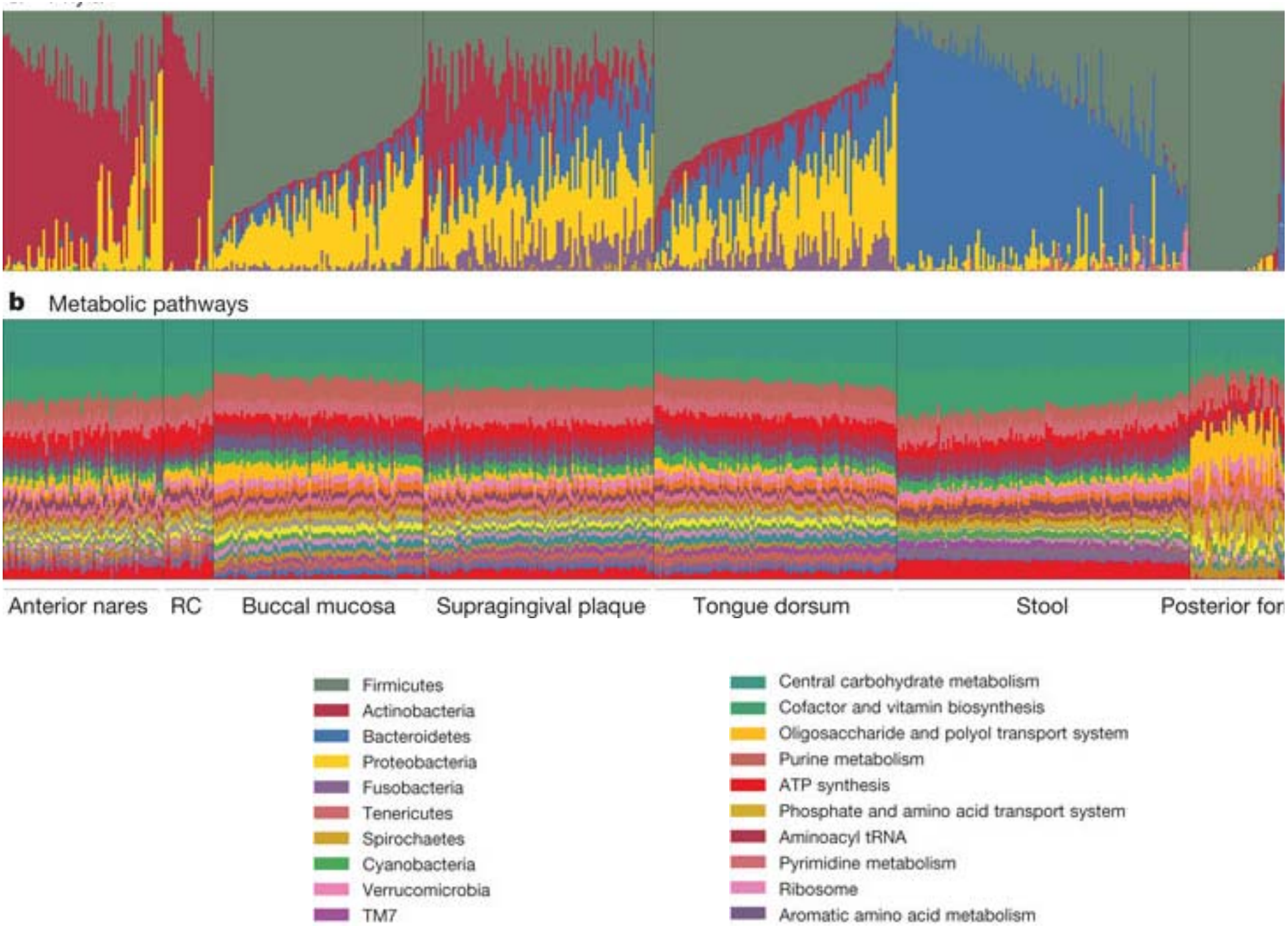


>GCACCTGAGGACAGGCATGAGGAA…
>GCACCTGAGGACAGGGGAGGAGGA…
>TCACATGAACCTAGGCAGGACGAA…
>CTACCGGAGGACAGGCATGAGGAT…
>TCACATGAACCTAGGCAGGAGGAA…
>GCACCTGAGGACACGCAGGACGAC…
>CTACCGGAGGACAGGCAGGAGGAA…
>CTACCGGAGGACACACAGGAGGAA…
>GAACCTTCACATAGGCAGGAGGAT…
>TCACATGAACCTAGGGGCAAGGAA…
>GCACCTGAGGACAGGCAGGAGGAA…

Human Microbiome Project

NIH HUMAN MICROBIOME PROJECT



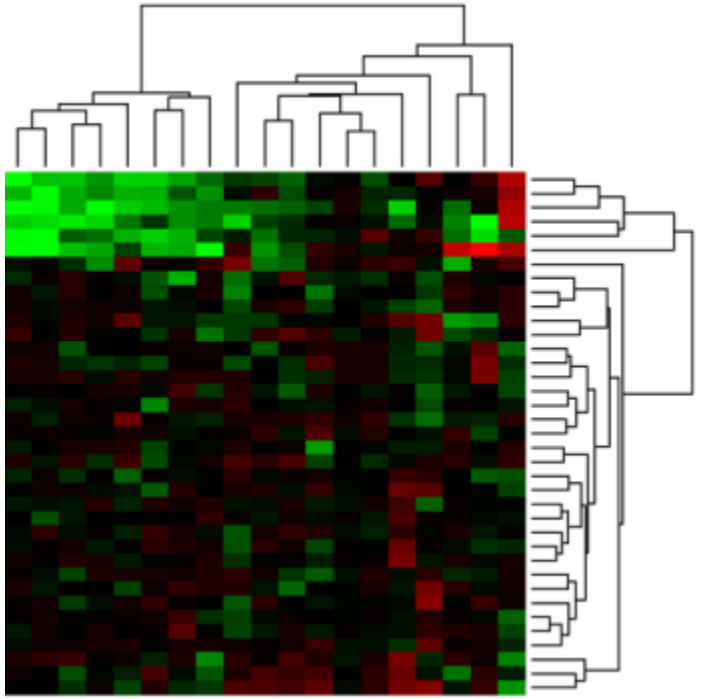


Structure, function & diversity of the healthy human microbiome, Nature, 2012, 486, 207

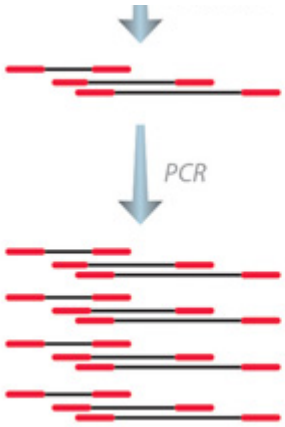
Transcriptomic Analysis-gene expression profiling

Plaque or saliva

Microarray
RNA-sequencing



Add bar coded primers, DNA synthesis



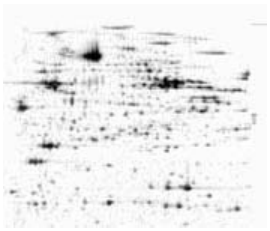
Removal of primers

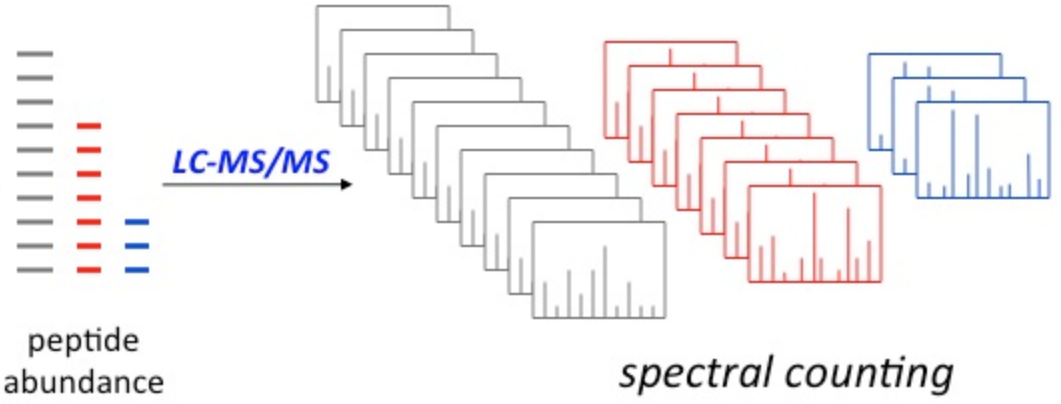
Data extraction
and processing

Proteomic Analysis-protein profiling

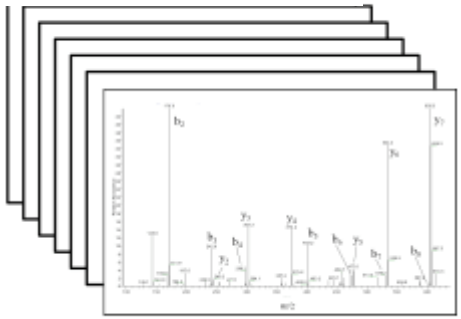
Plaque or saliva

Protein preparation

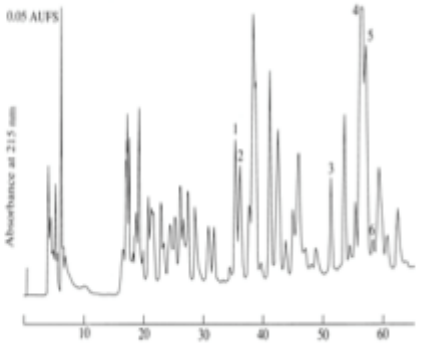




Quantitative proteomics by spectral counting



peptides



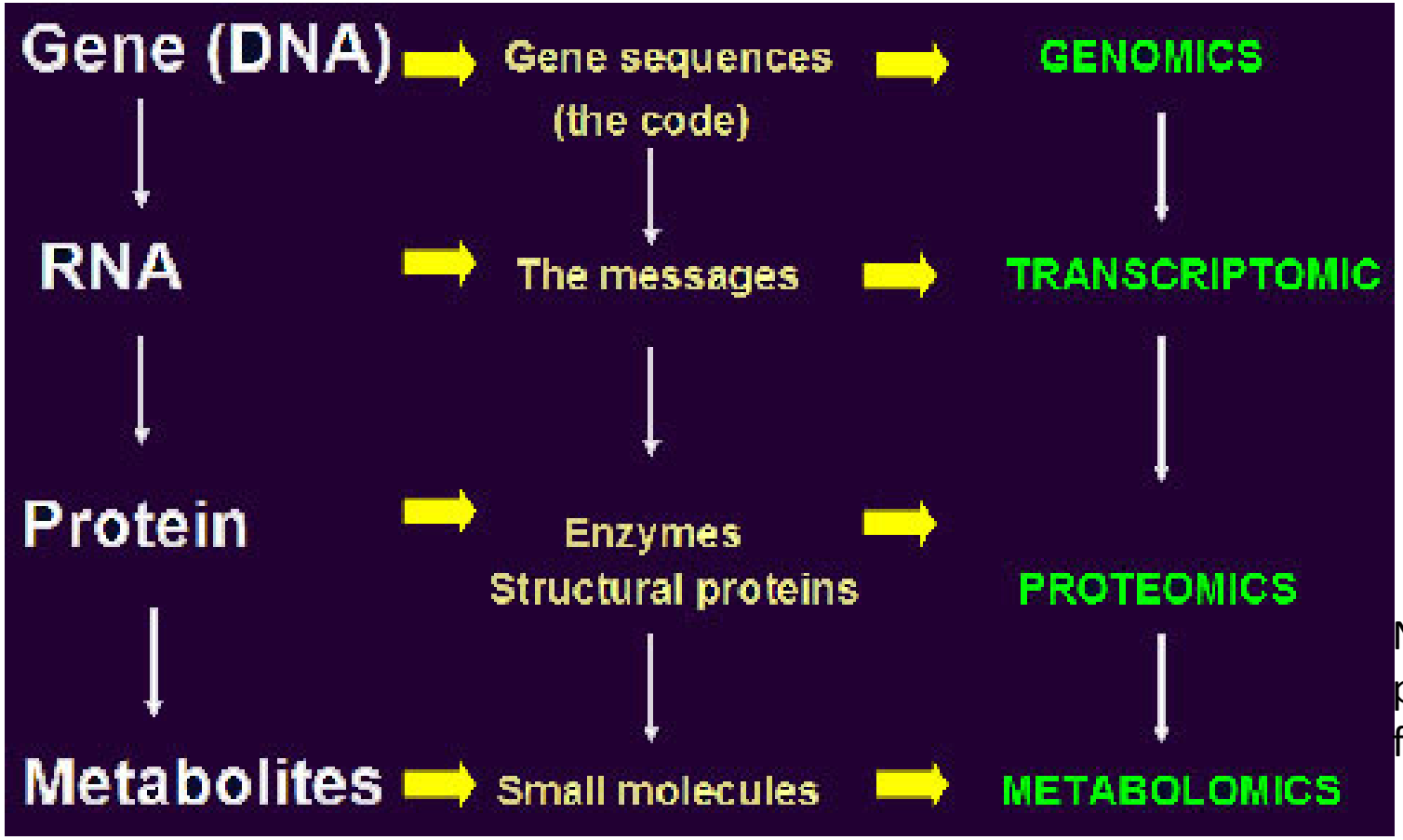
RPLC

MS/MS

Integration of Multi"Omics"

Biological samples

Microbiome



Networking pathways for metabolites

Microbiomes impact behaviors

Gut microbiota — Diet-specific microbiota influence mating preferences

Human skin microbiota — Skin microbes of humans influence attraction to mosquitoes

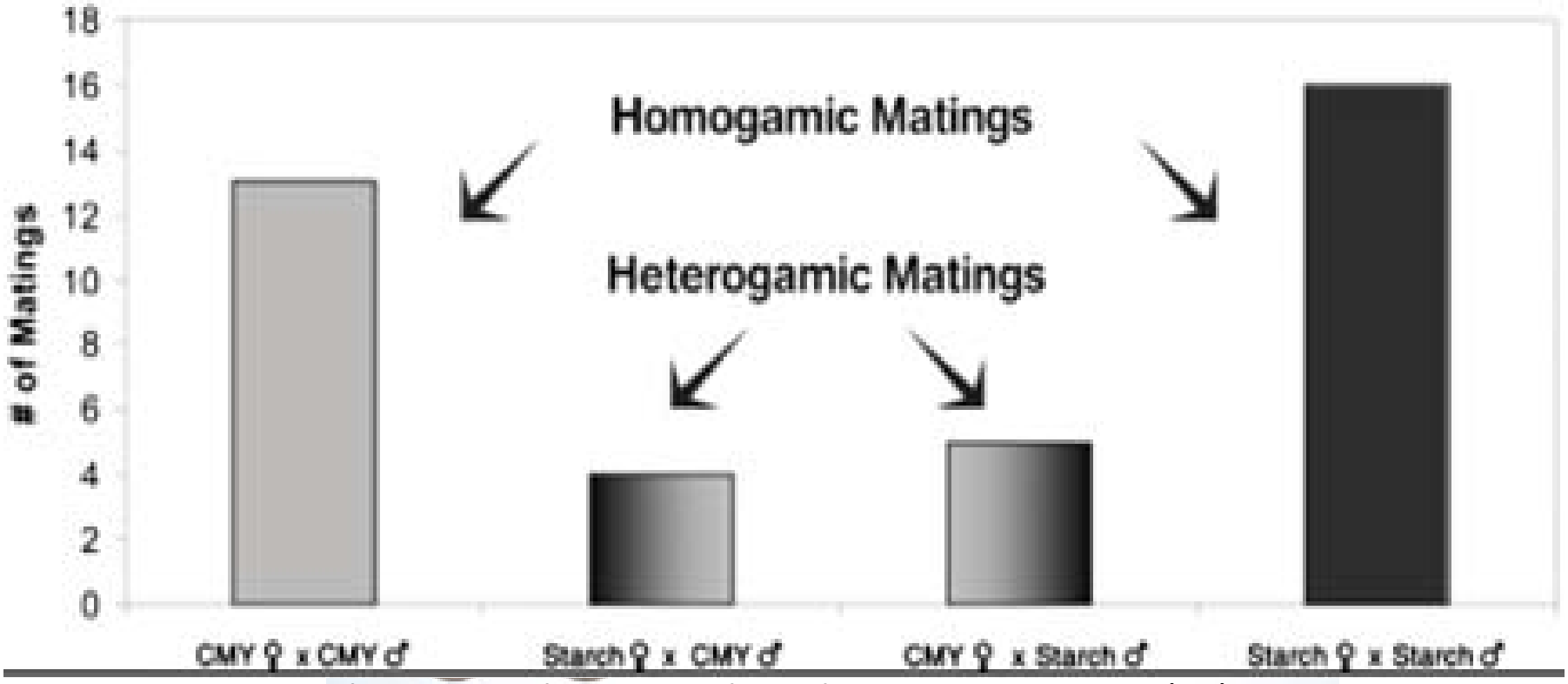
Lactobacillus rhamnosus — The probiotic *L. rhamnosus* decreases anxiety in mice

Fighting microbes or Farming microbes?

Microbiomes impact behaviors

Gut microbiota

Diet-specific microbiota influence mating preferences



Sharon, et. al., Proc Natl Acad Sci U S A. 2010,107(46):20051-6.

Lactobacillus plantarum strain IMAU:10272
cuticular hydrocarbon sex pheromones
the hologenome theory of evolution

Microbiomes impact behaviors

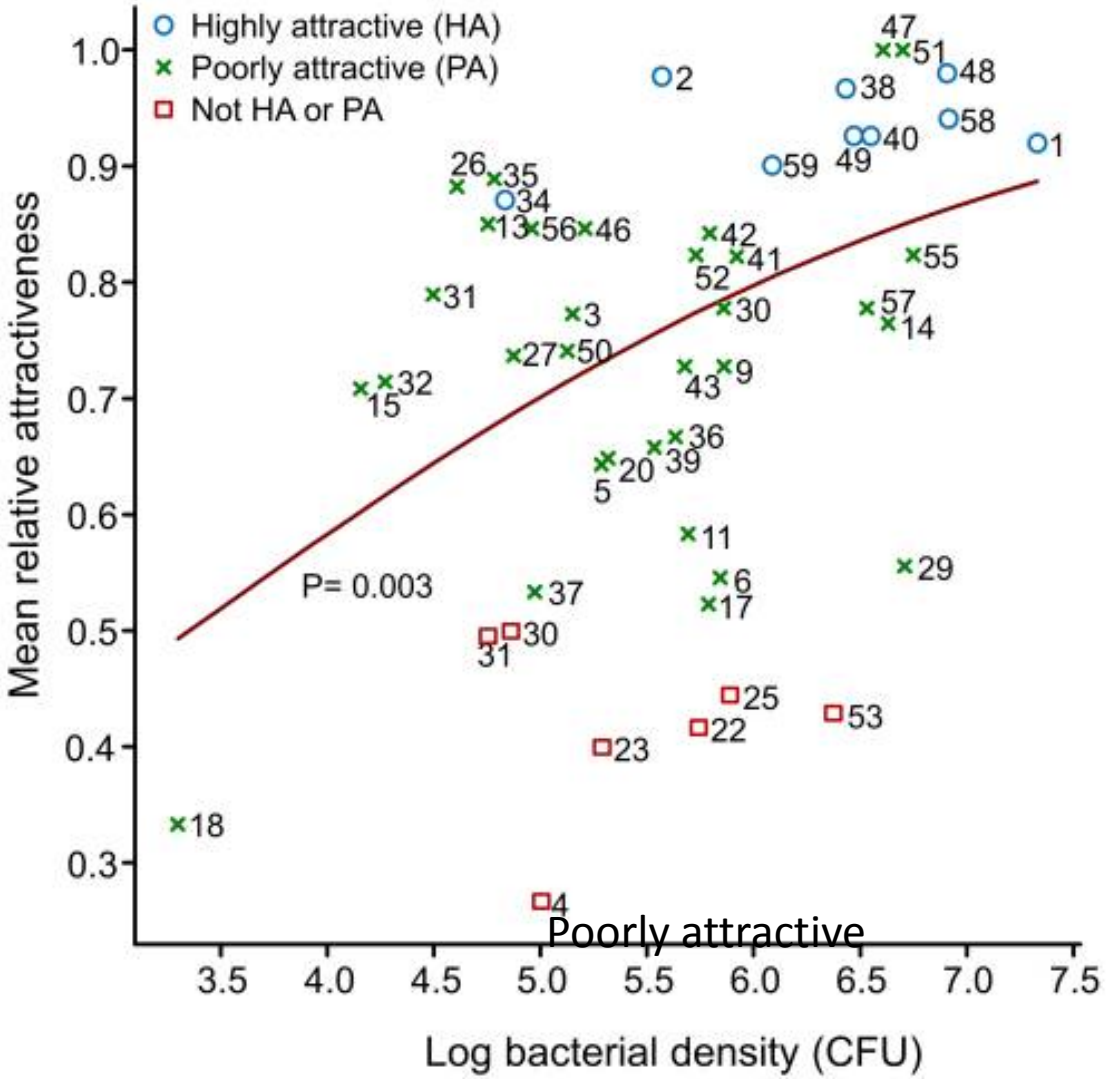
Individuals

⬇

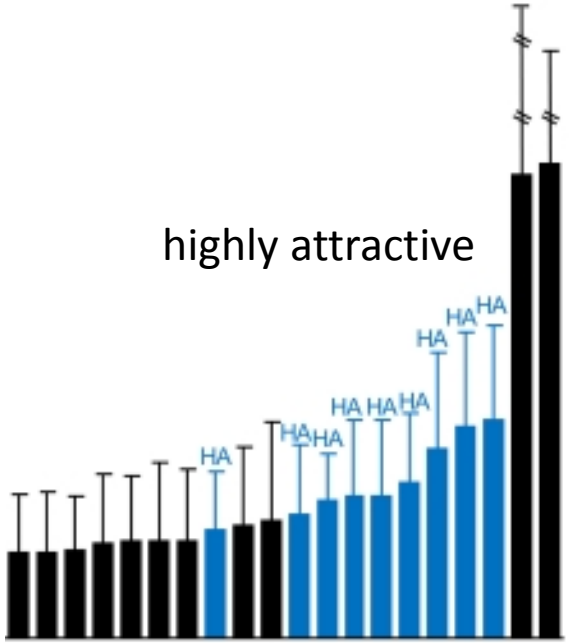
Distinct microbiome

⬇

Various volatile compounds



Poorly attractive



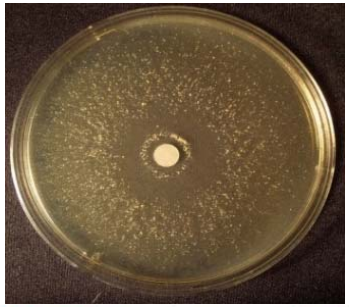
highly attractive

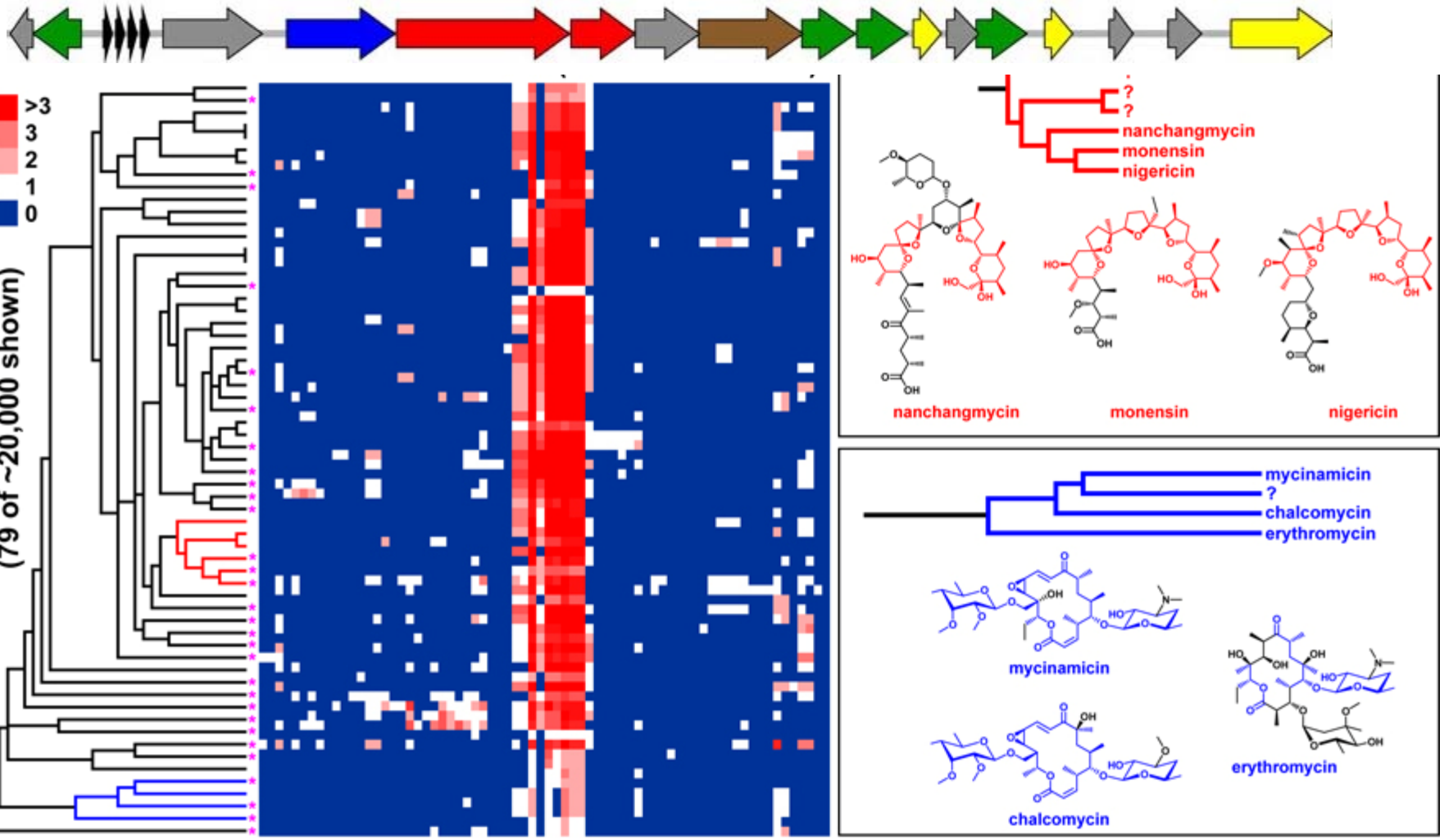
PLoS One. 2011;6(12):e28991

41 55 52 42 46 56 13 34 26 35 59 1 40 49 58 38 2 48 51 47

PLoS One. 2010 Dec 30;5(12):e15829

Do metabolites matter???



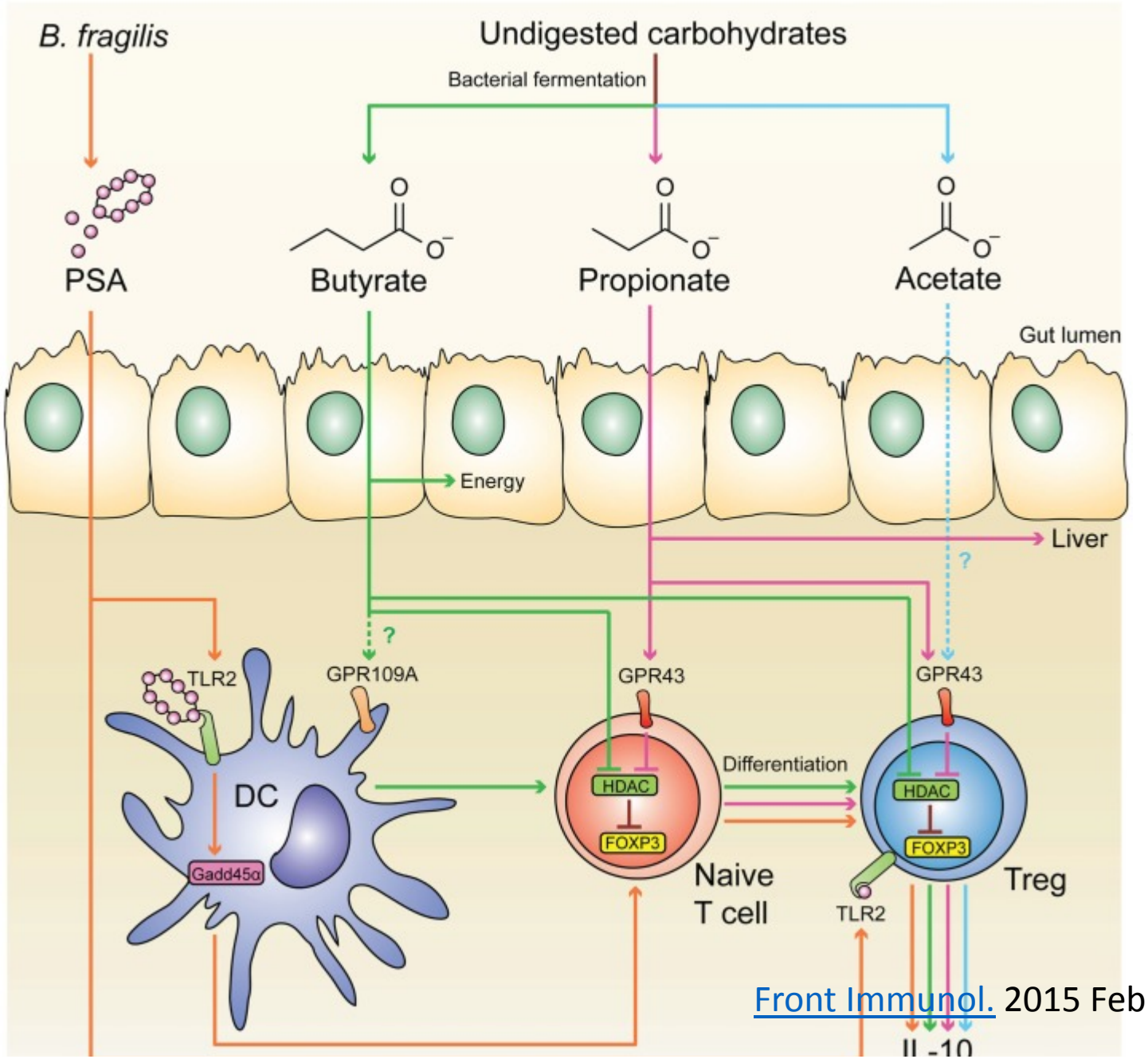


Microbiome and Carbohydrate Metabolism

- **Carbohydrates: humans and bacterial nutrients**
- ***Human and mammals*: disaccharides and starches**
- ***Microbes*: complex polysaccharides by Carohydrate-active enzymes: glycoside hydrolases, carb esterases, glycosyltransferases and polysaccharide lyases**
- **Biogeographical distribution of microbiome/ genes/pathways such as simple Carb transport PTS**

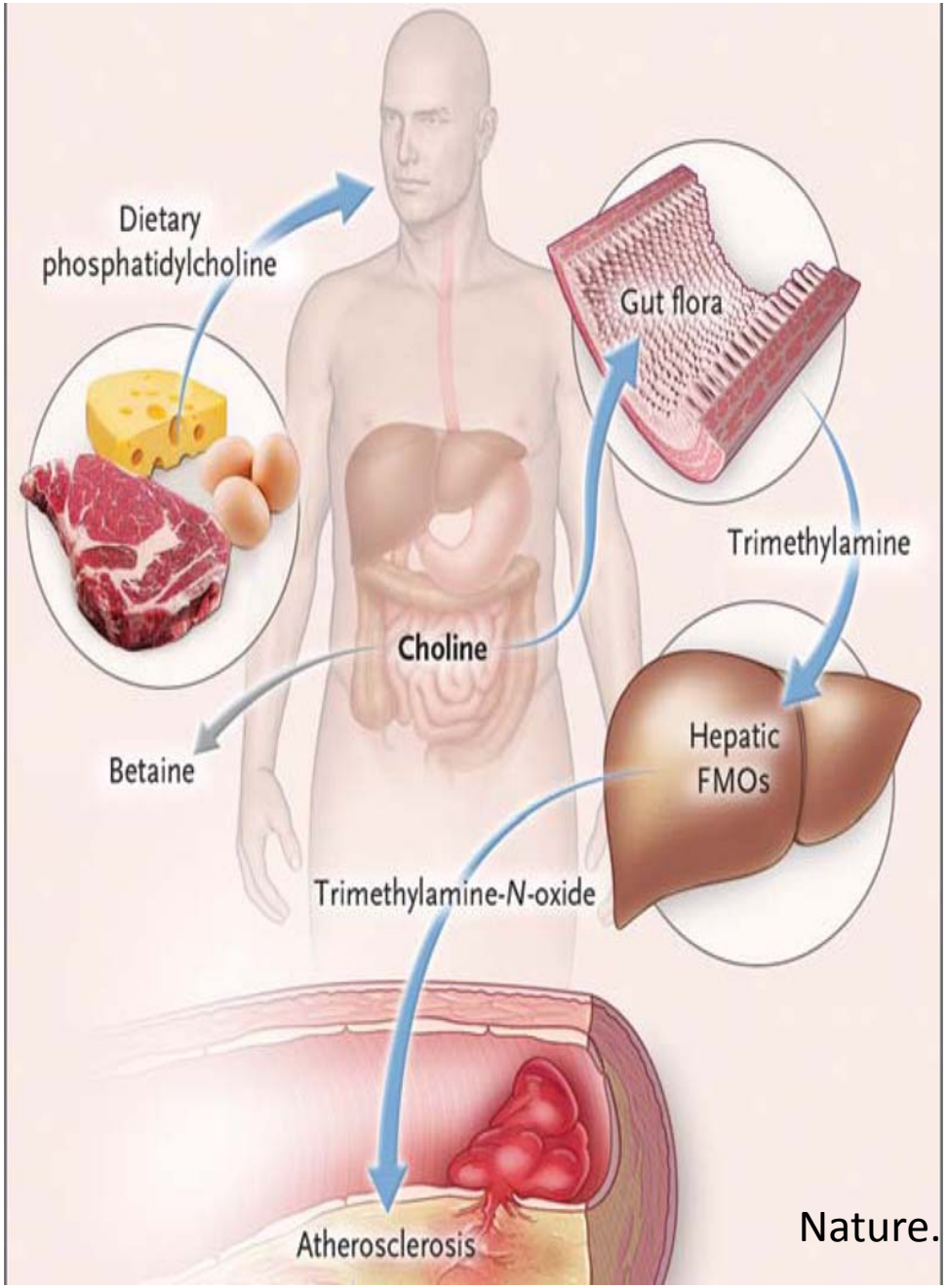
  small intestine>colon
- **Probing microbe altered pathways in the development of metabolic disorders in humans**

Microbiome and Fatty Acids Metabolism



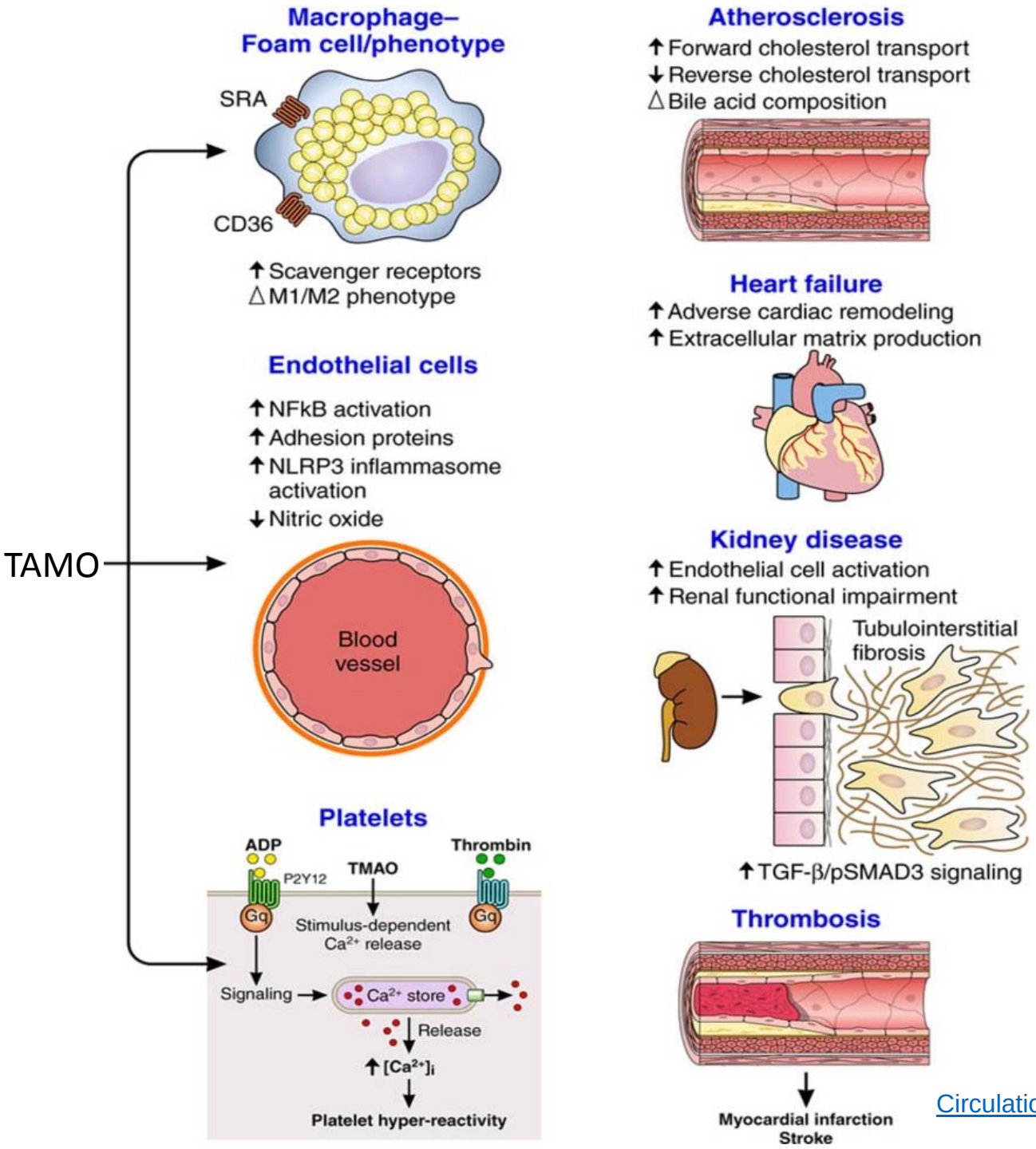
Front Immunol. 2015 Feb 18;6:61.

Microbiome and Atherosclerosis



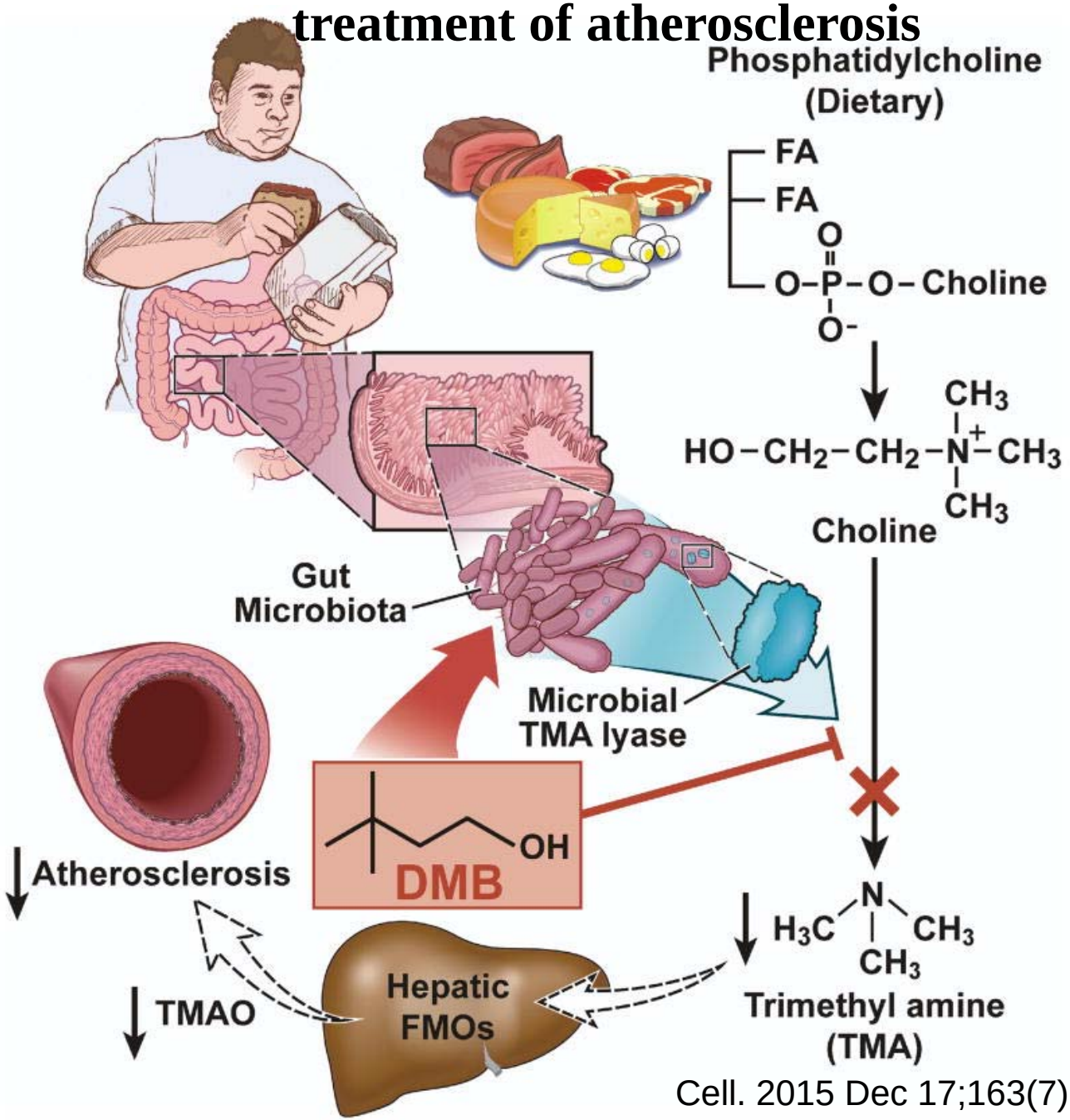
Flavin monooxygenases (FMOs)

Nature. Apr 7, 2011; 472: 57–63.



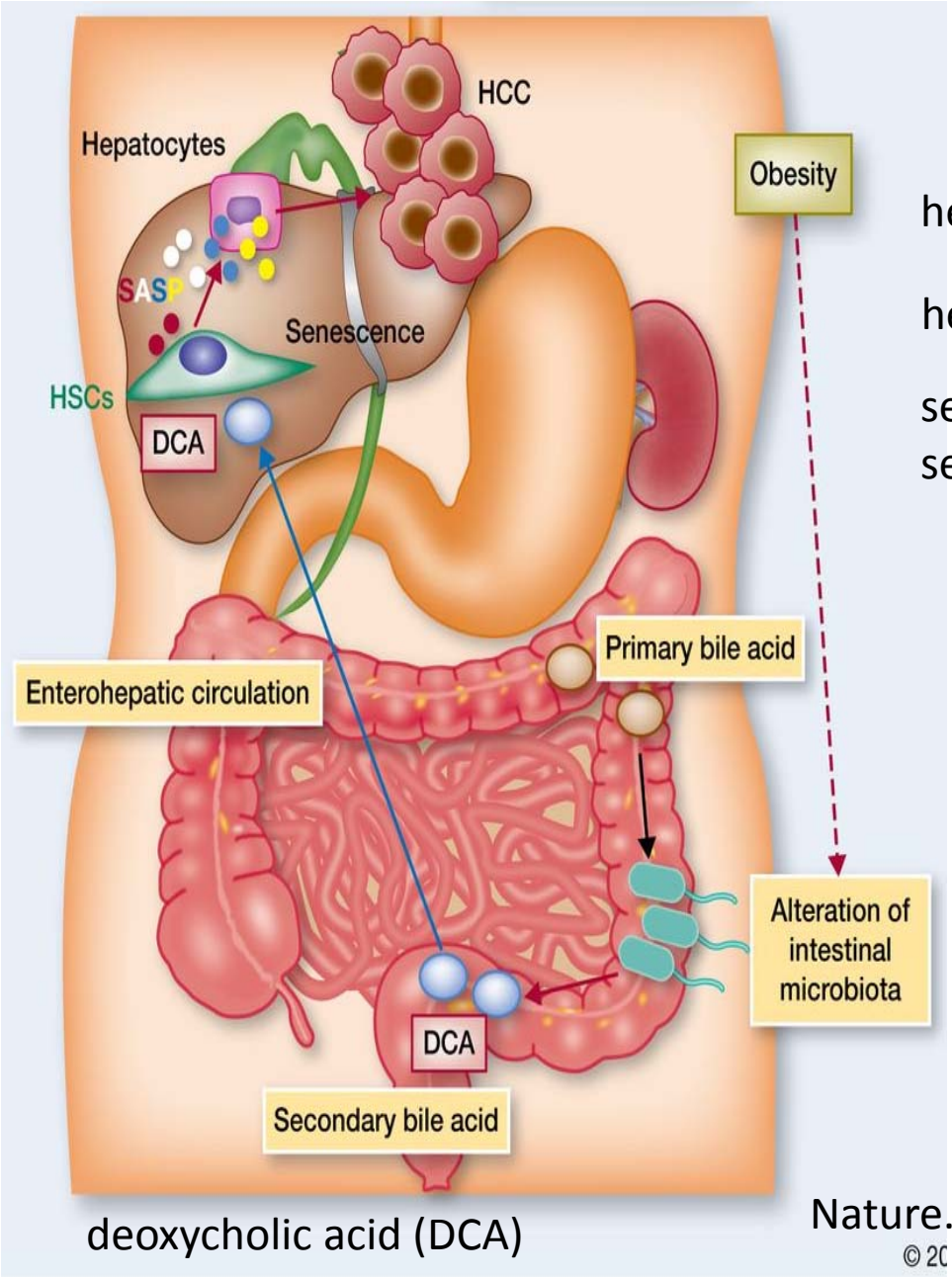
Circulation. 2017 Mar 14;135:1008-1010.

Inhibition of trimethylamine production by microbiome for the treatment of atherosclerosis



Cell. 2015 Dec 17;163(7):1585-95.

Obesity and Cancer: a Microbial Connection



deoxycholic acid (DCA)

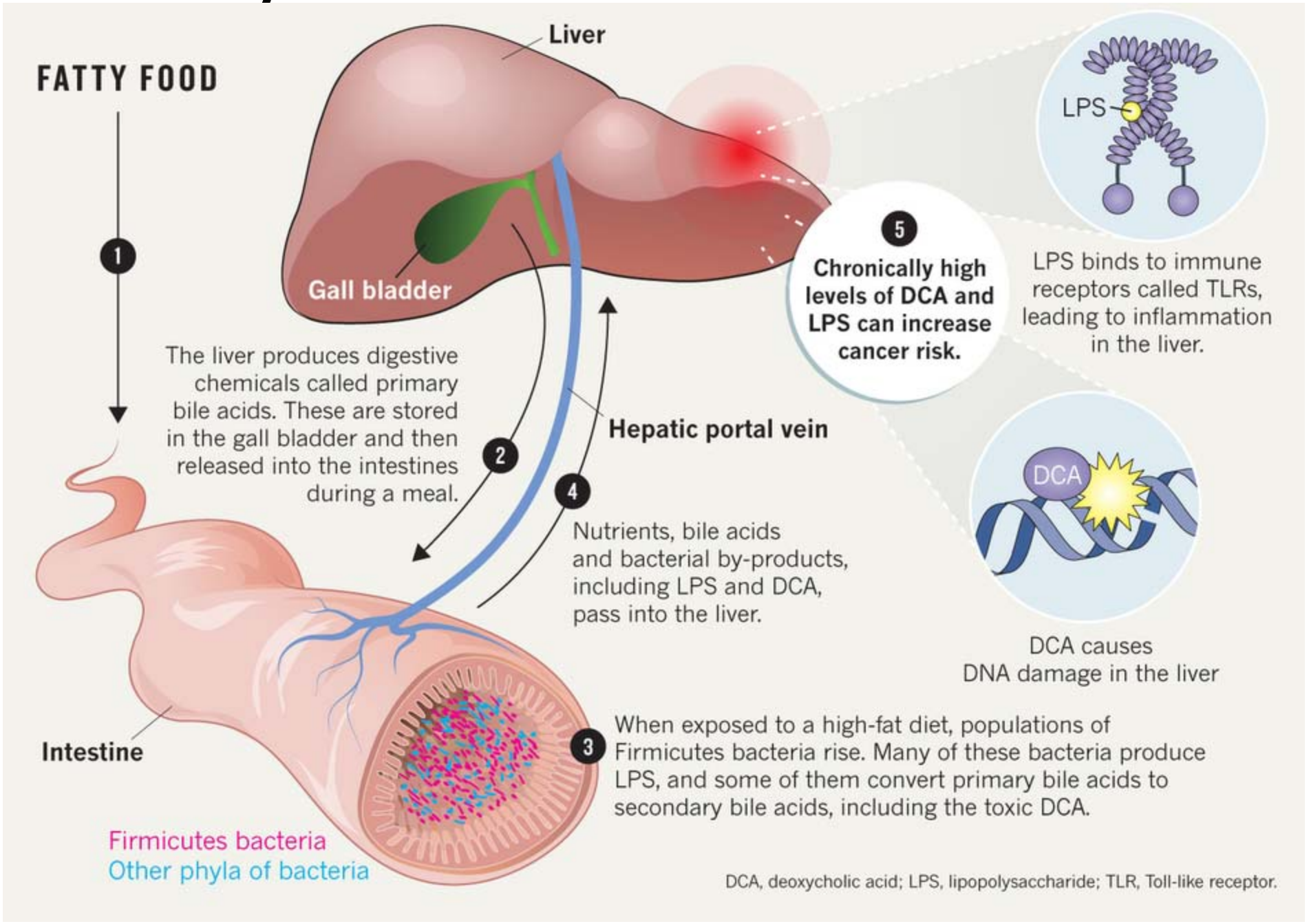
hepatic stellate cells (HSC)

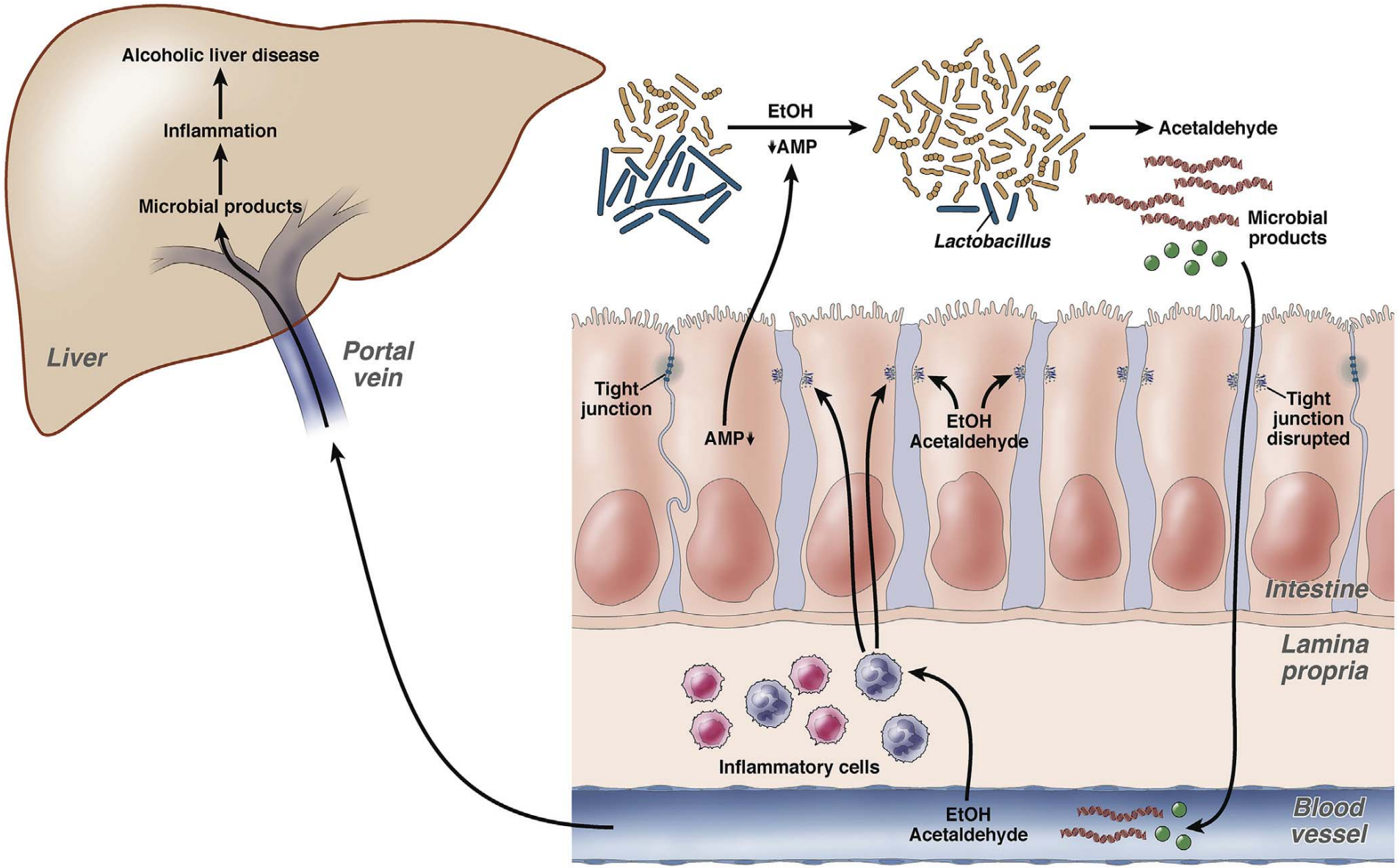
hepatocellular carcinoma(HCC)

senescence-associated secretory phenotype (SASP)

Nature. 2013 Jul 4;499:97-101

Obesity and Cancer: a Microbial Connection

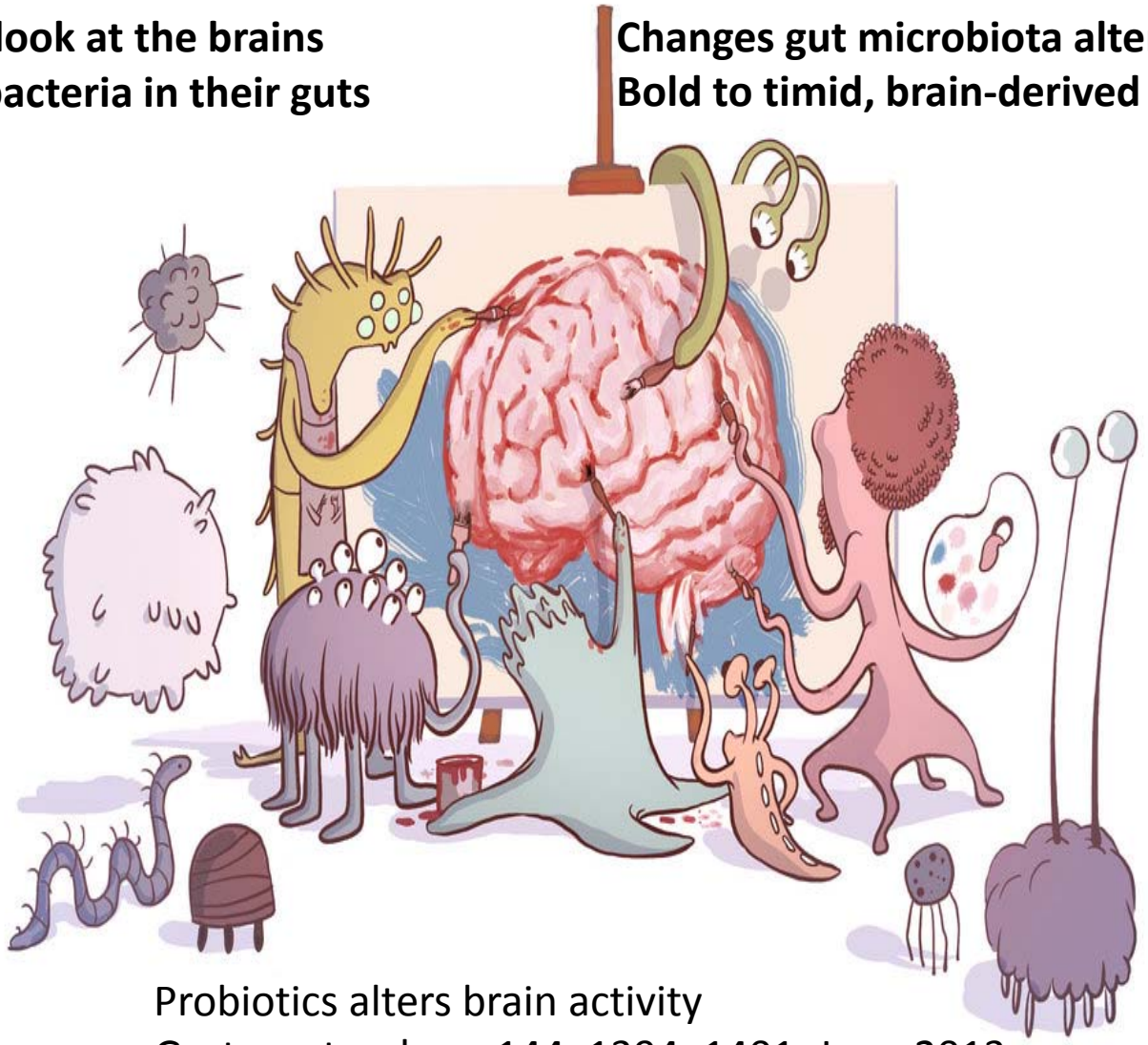



Alcoholic liver disease
Inflammation
Microbial products
Liver
Portal vein
EtOH
AMP
Lactobacillus
Acetaldehyde
Microbial products
Tight junction
AMP
EtOH
Acetaldehyde
Tight junction disrupted
Intestine
Lamina propria
Inflammatory cells
EtOH
Acetaldehyde
Blood vessel

Gut Bacteria and the Workings of Our Minds

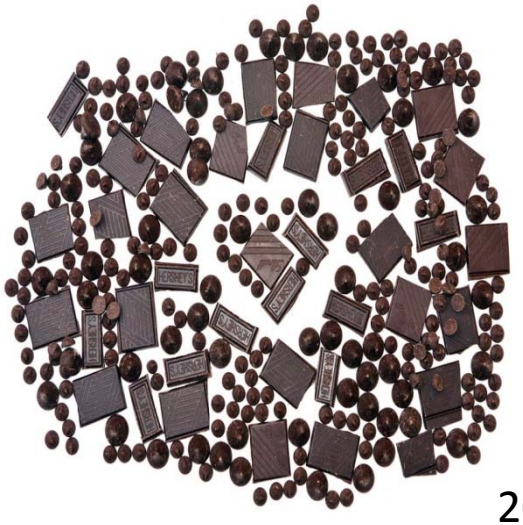
Of Humans
MRI scans to look at the brains
the types of bacteria in their guts

Of Mice
Changes gut microbiota alter mouse behavior
Bold to timid, brain-derived neurotrophic factor



Probiotics alters brain activity
Gastroenterology, 144, 1394–1401. June 2013
Cell, 155,1451–63, 19 December 2013

Gut Bacteria & Healthful Chocolate



Cocoa powder(polyphenols and Fibers)

Smaller molecules
short fatty chain acids

2014 American Chemical Society meeting

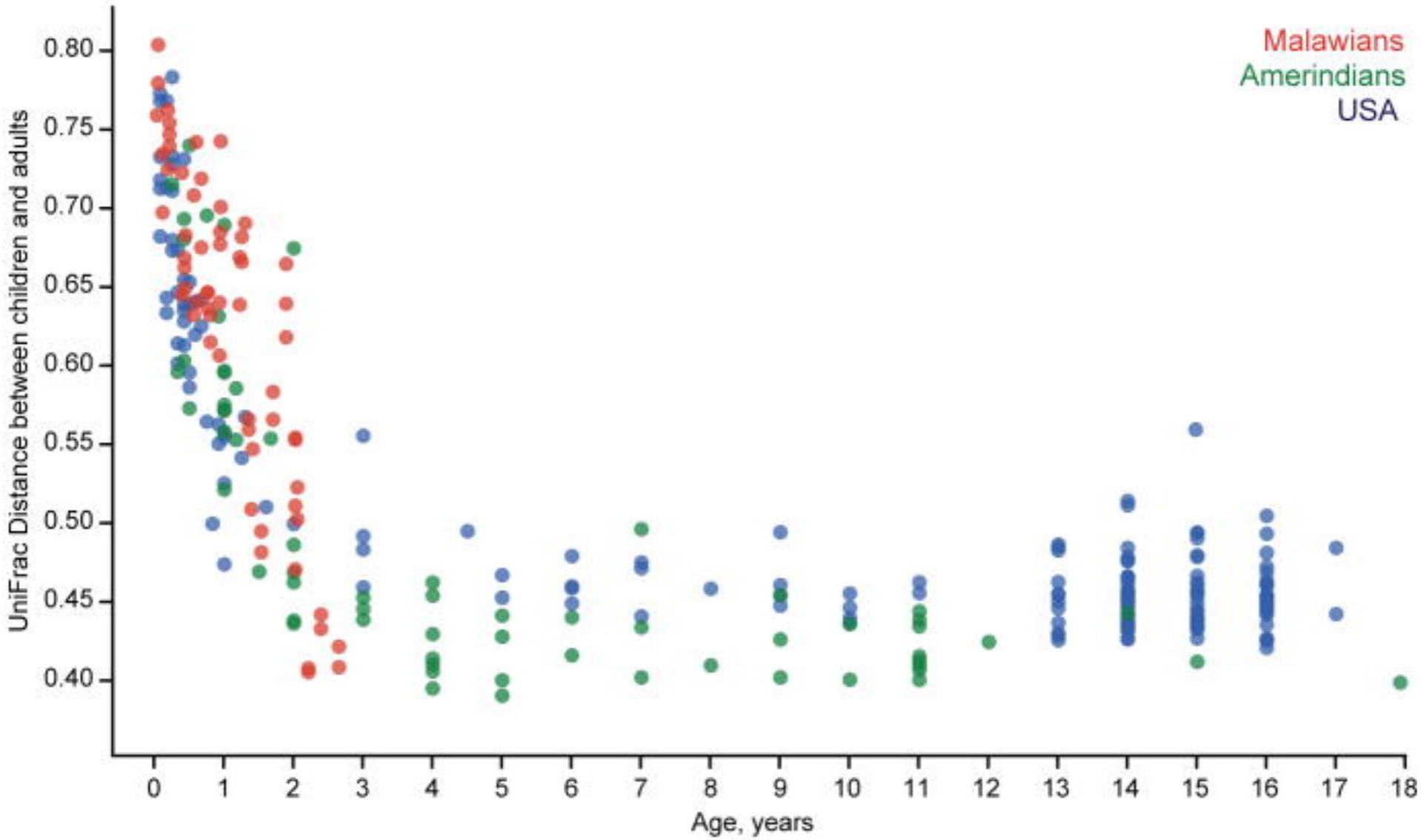
Gut Microbes and Your Weight

Slimming gut microbes?

Microbe transplants from obese humans

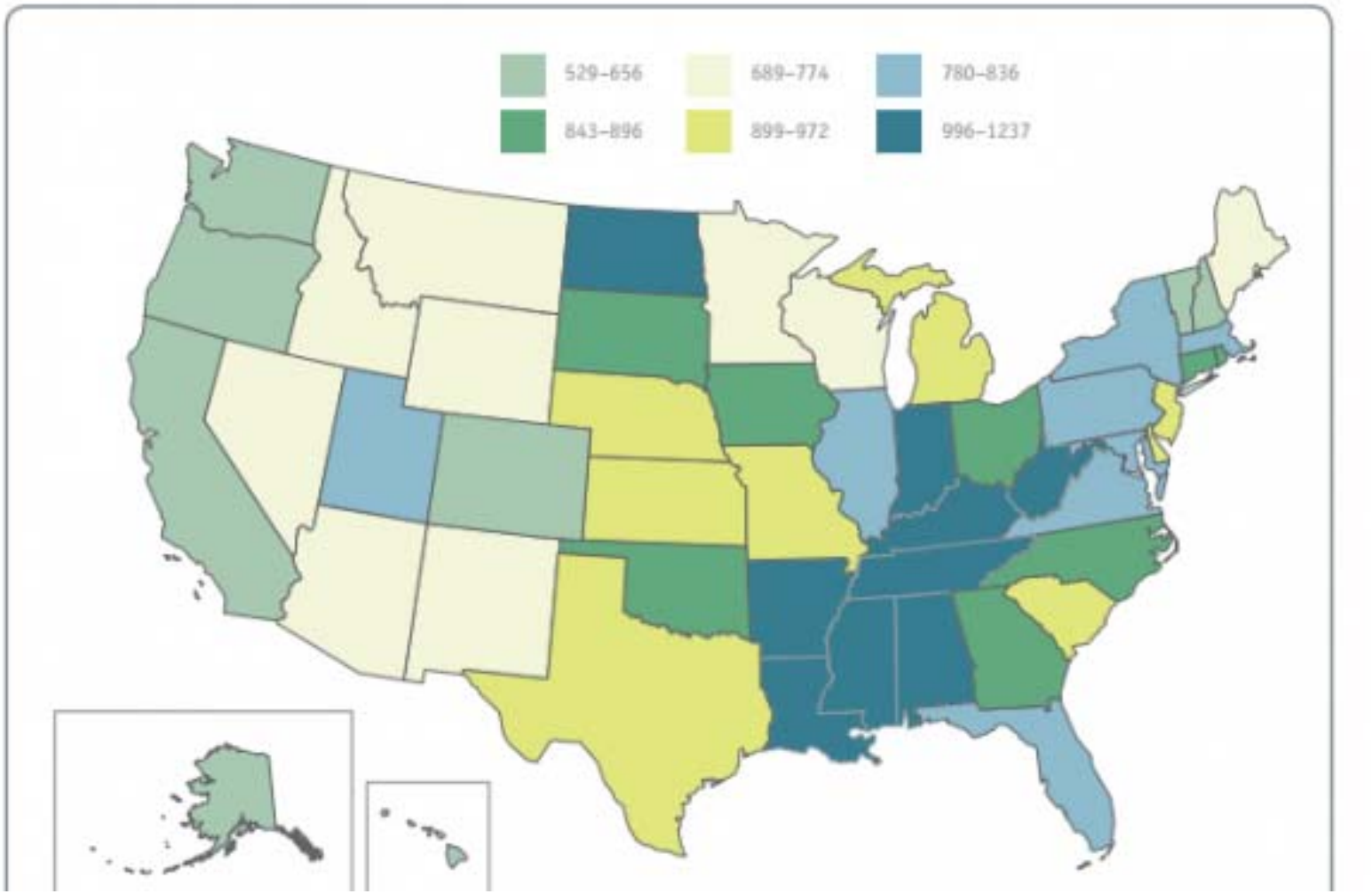
Science, *6 September 2013:* ***341(6150)***

When do our microbiome established?

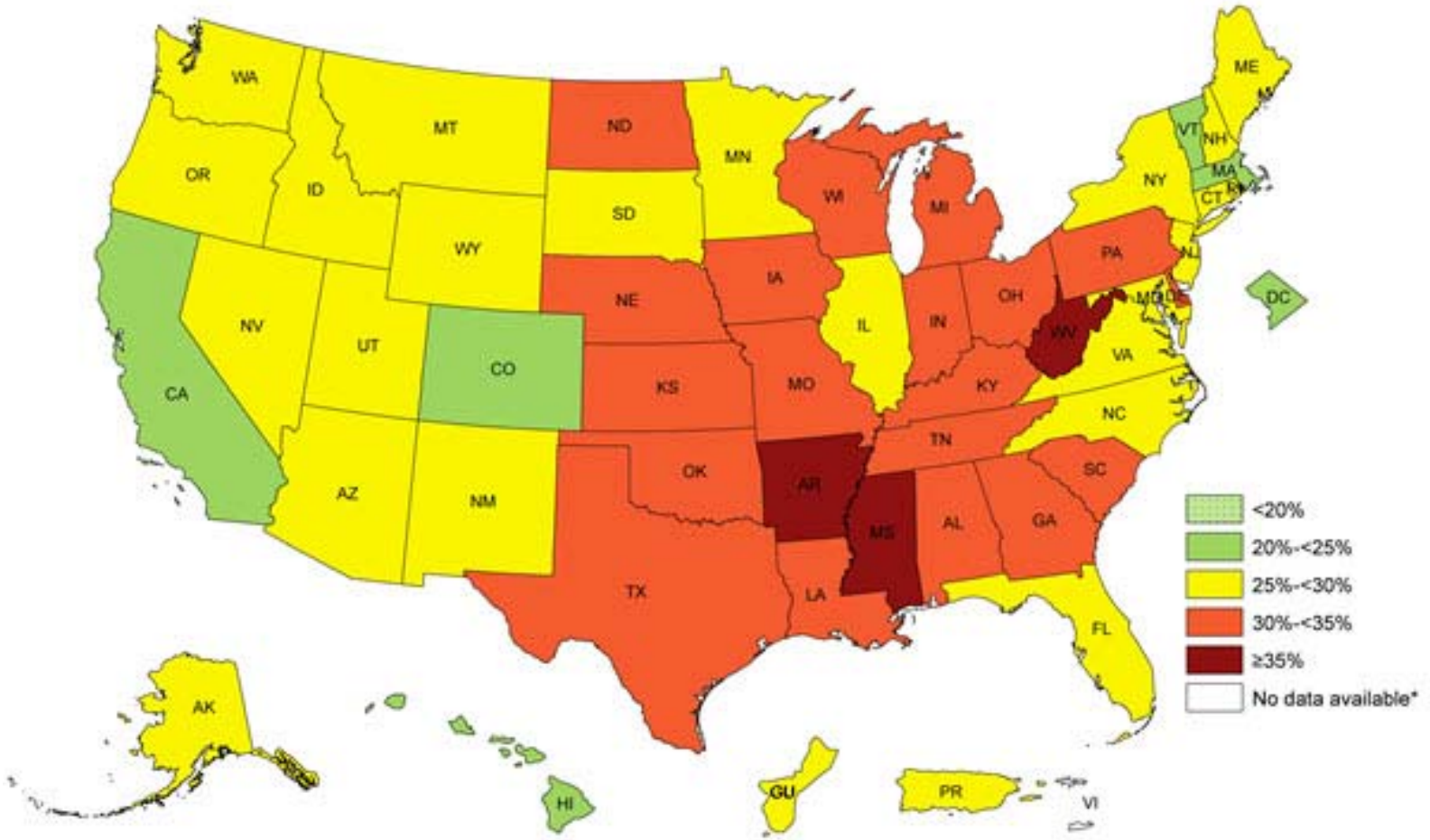


Antibiotic use and growth promotion in farming animals

The Use of Antibiotics prescriptions

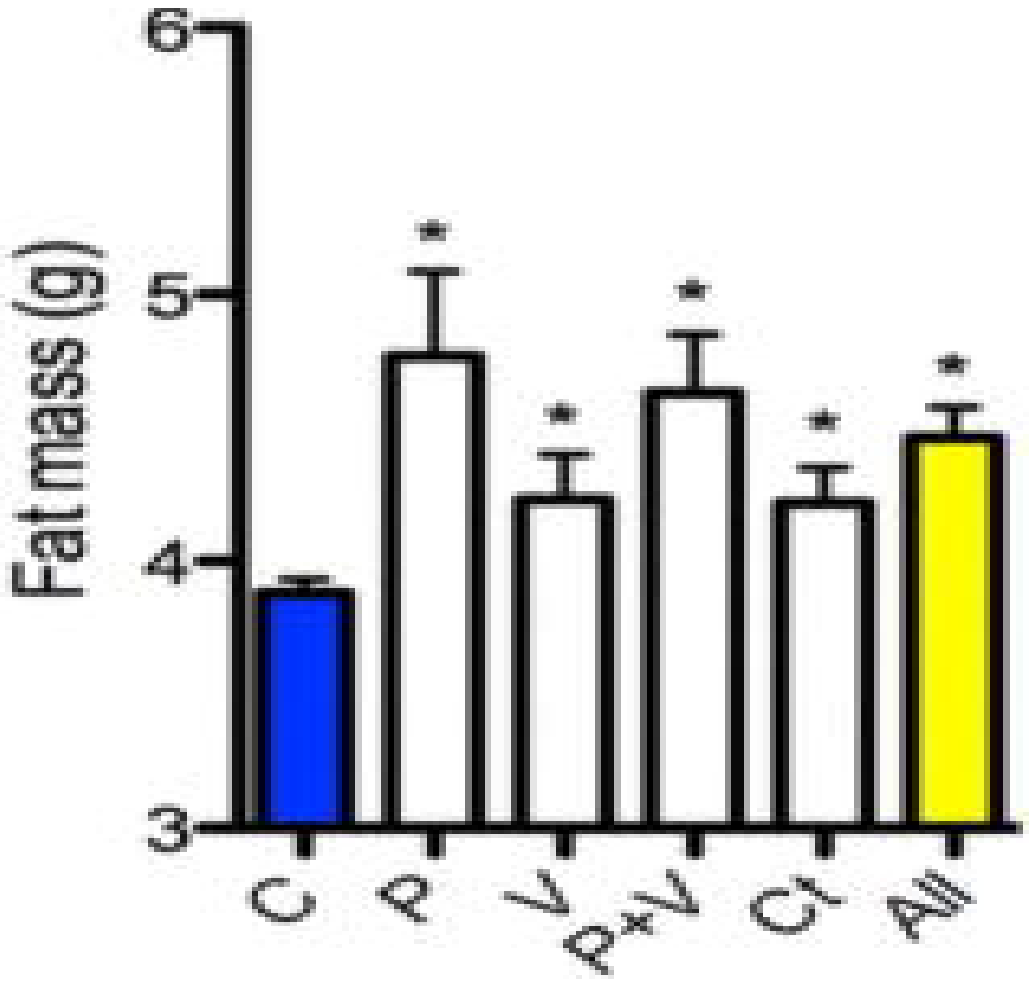


Obesity Among U.S. Adults by State and Territory

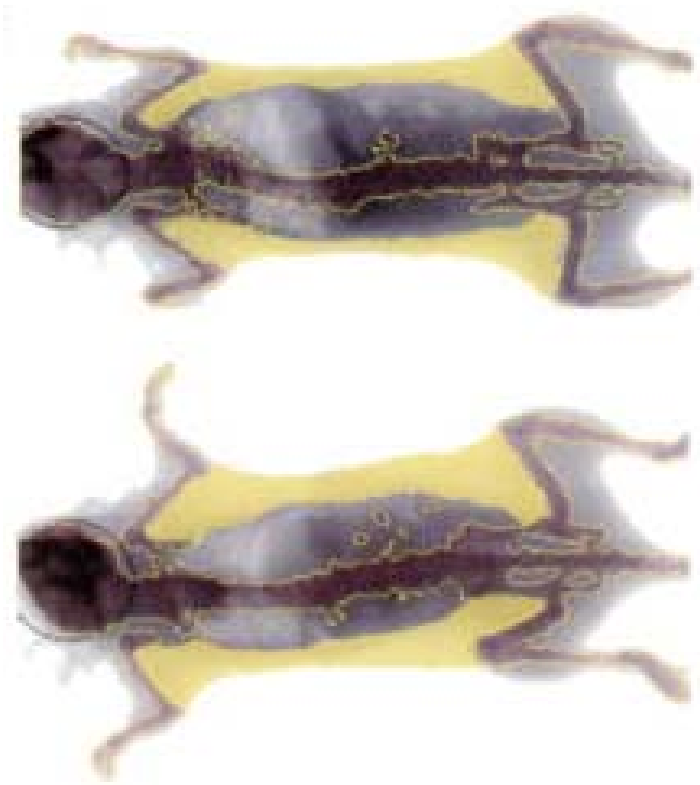


2014

Increased body fat by the use of antibiotics



Increased body fat by the use of antibiotics



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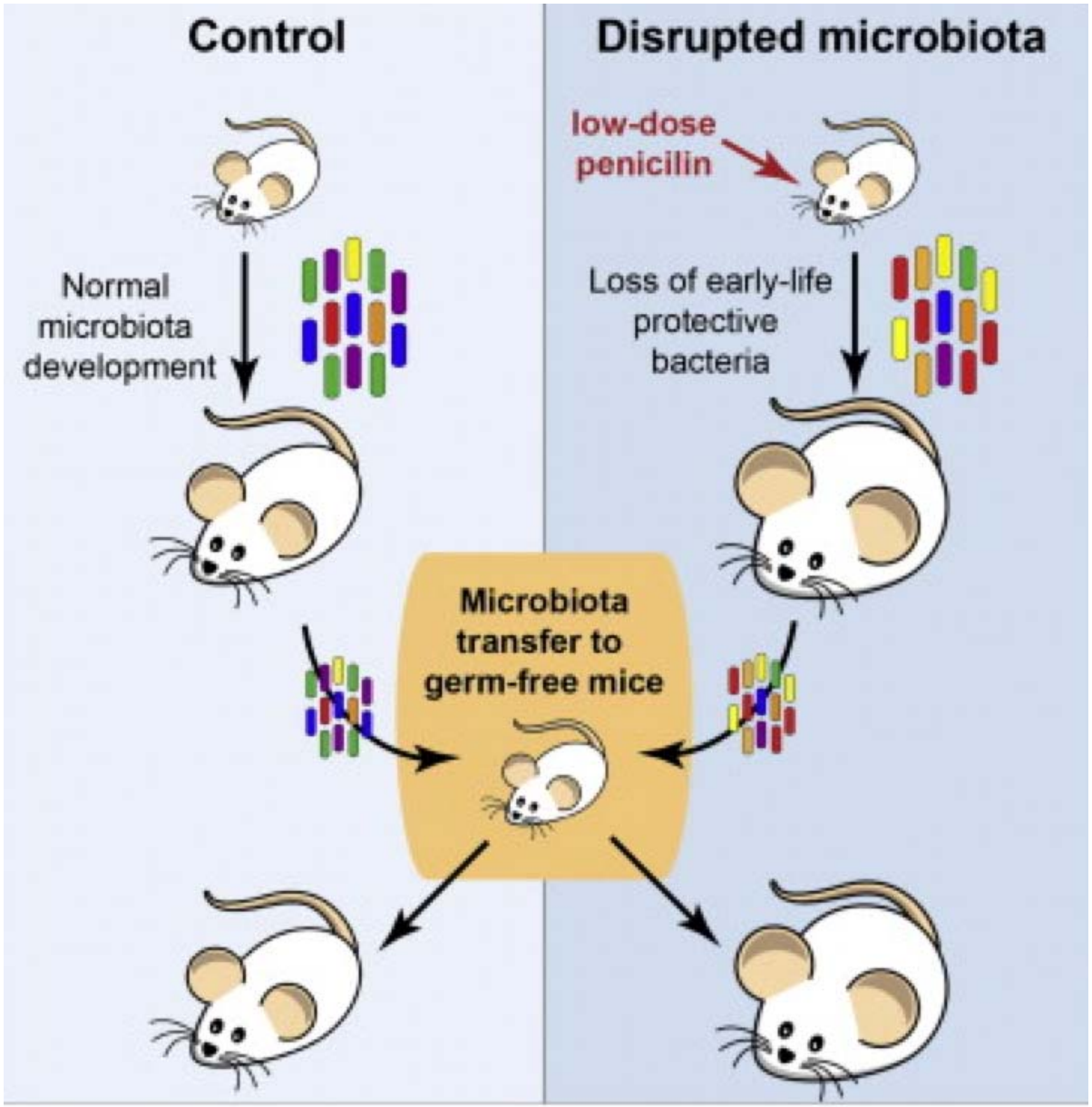
Cho I et. al., Nature. 2012 Aug 30;488(7413):621-6.

Dysbiosis in Gut and Obesity



Cox et al., Cell *158*, 705–721, August 14, 2014

Antibiotics in Infancy and Early Childhood Obesity

- 69% of children exposed to antibiotics before end of first year
- Increase in the antibiotics use associated with increased risk to obesity
- Asthma and wheezing also predicted obesity

Bailey et al., *JAMA Pediatr*. Published online September 29, 2014

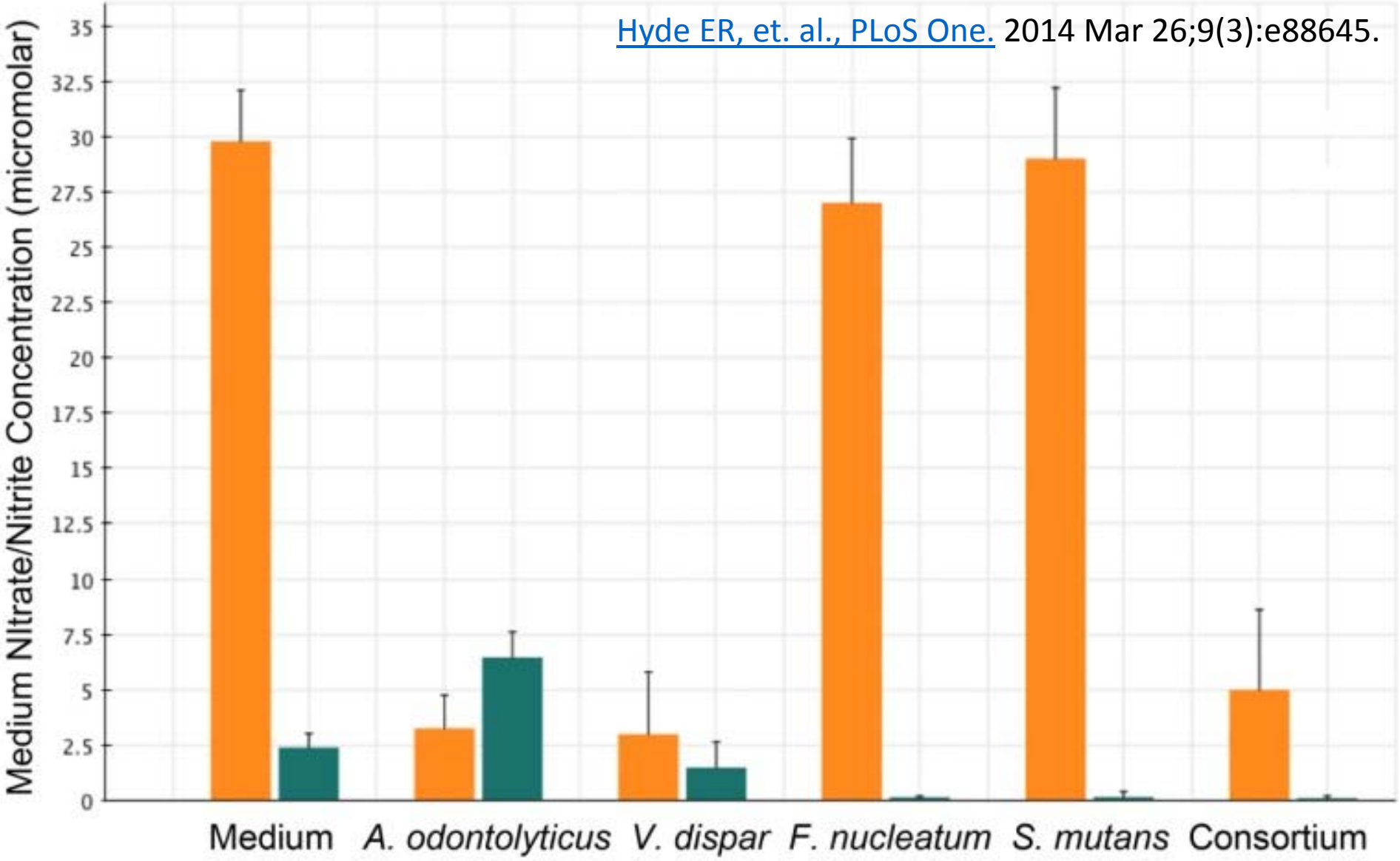
Antiseptic Mouthwash and Blood Pressure

- Bacteria in the mouth reduce nitrates to nitrites
- Nitrite reduced to **NO**– relaxes vessels and lowers blood pressure
- Antiseptic mouthwash reduced oral nitrite production by 90% and plasma nitrite levels by 25% ($p<0.001$)
- Systolic and diastolic blood pressure increased by 2–3.5 mm Hg

Kapil et al., Free Radic Biol Med. Feb 2013; 55(C): 93–100.

The nitrate- and nitrite-reducing capacity of oral bacteria

Hyde ER, et. al., PLoS One. 2014 Mar 26;9(3):e88645.



Oral bacteria in systemic conditions

Fusobacterium nucleatum

Fusobacterium & Colon Caner

Fusobacterium nucleatum

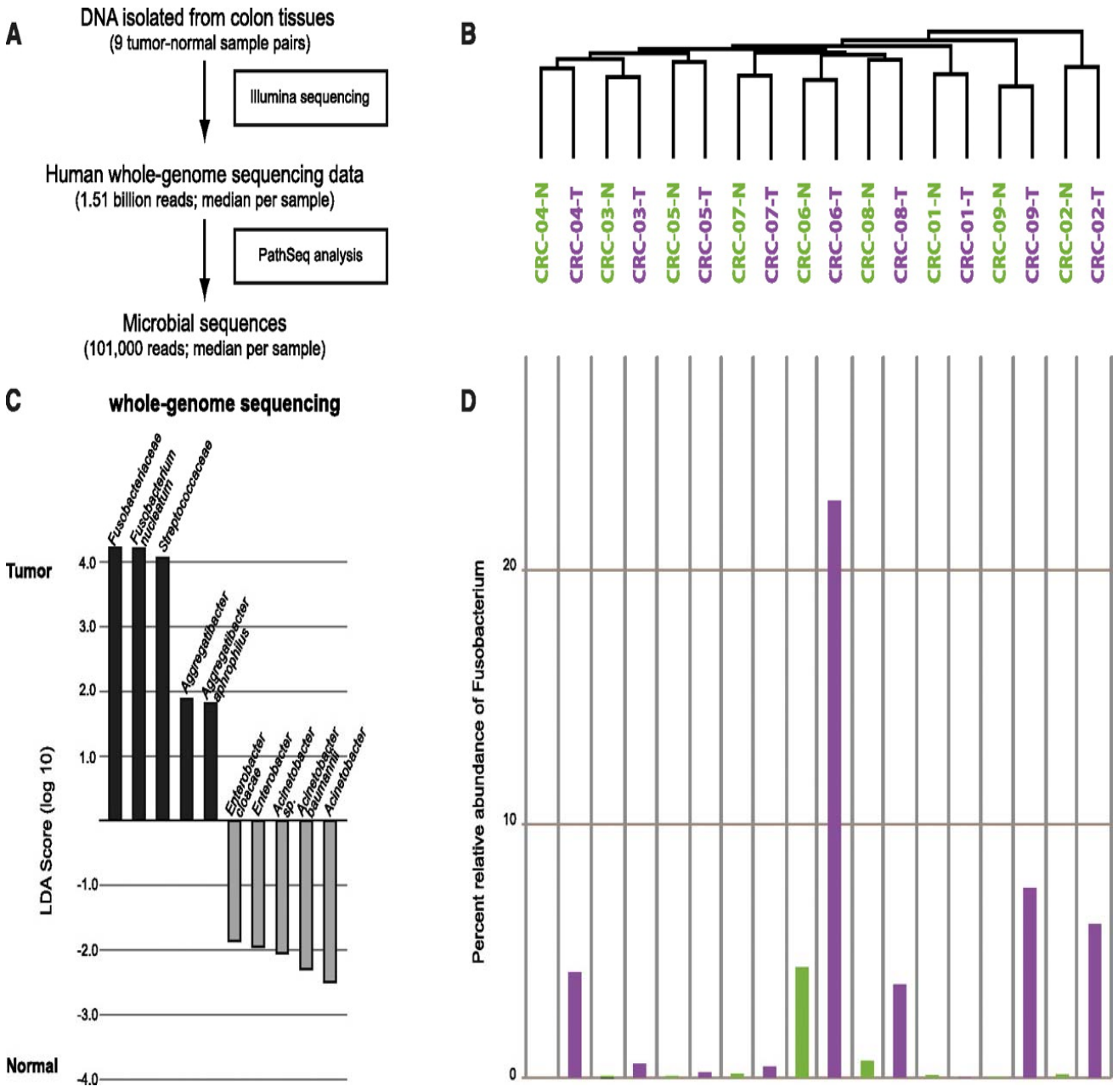
Kostic AD et. al., Cell Host Microbe 2013, 14:207-15.
Rubinstein MR et. al., Cell Host Microbe. 2013, 14:195-206.
Castellarin M et. al., Genome Res. 2012, 22:299-306.
Kostic AD et. al., Genome Res. 2012, 2:292-8.

Whole-genome analysis of the colorectal cancer microbiome

Fusobacterium

Streptococcus

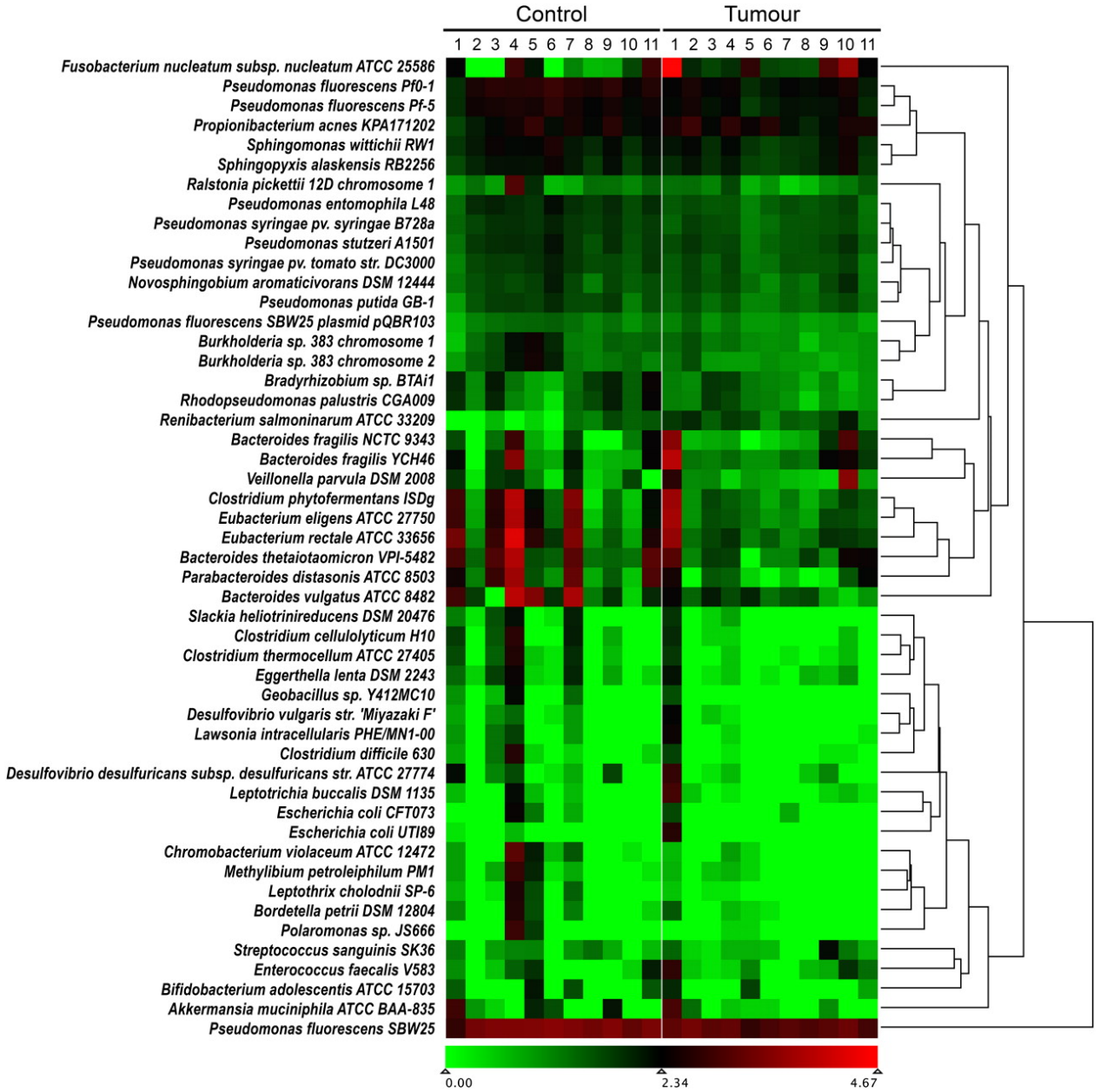
Aggregatibacter



Genome Res. 2012;22:292-298

Bacterial abundance in colon cancer

Fusobacterium nucleatum



Castellarin M et al. Genome Res. 2012;22:299-306

Bacteria and Colorectal Cancer

Colon luminal environment

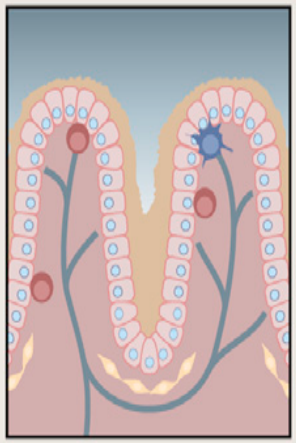
Host mucosal environment

Fusobacteria

Model 1
Single microbes

Model 2
Microbial community

Model 3
Single microbes interacting with microbial community



Tumor microenvironment

Inflammation

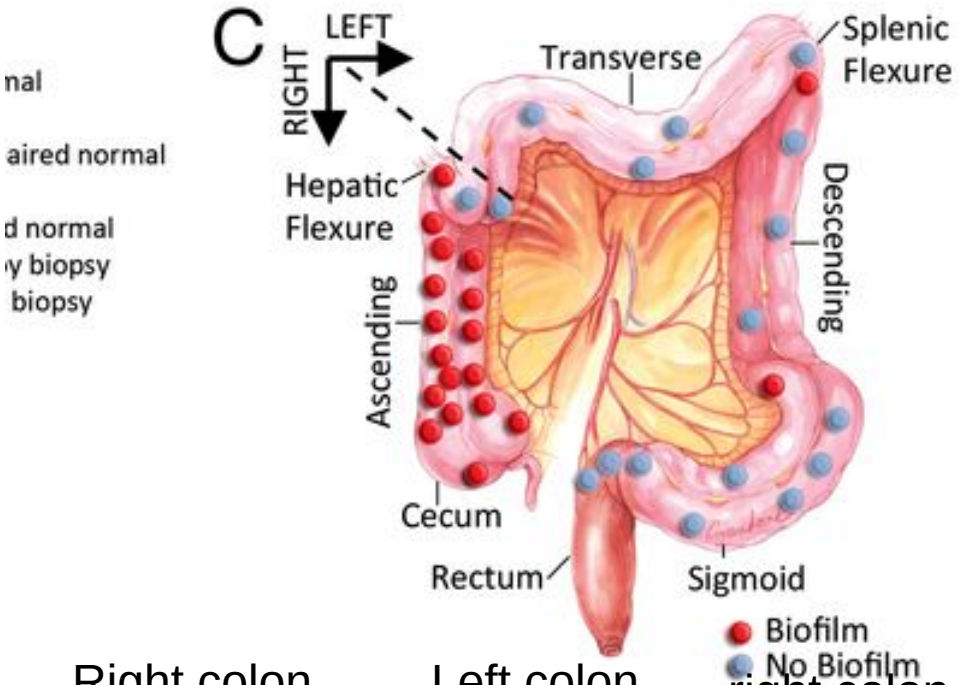
Host genetics

Colorectal Cancer

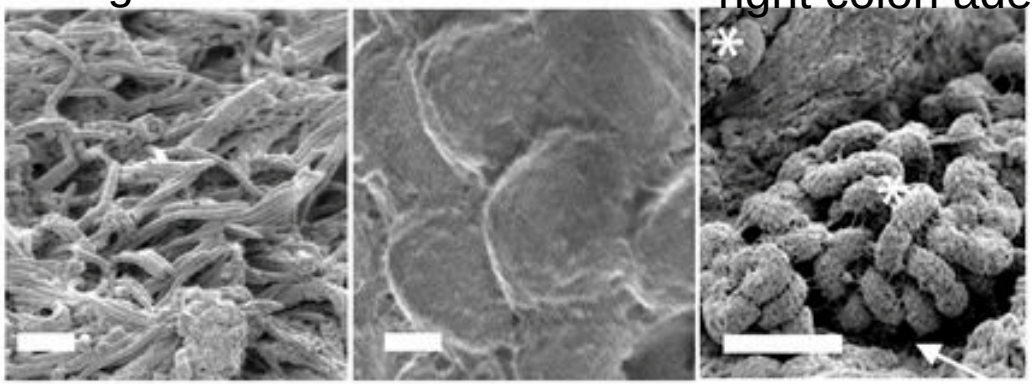
Cell Host Microbes, 2013
Cell Host Microbes, 2014

New Findings

Bacterial biofilms and cancer connection

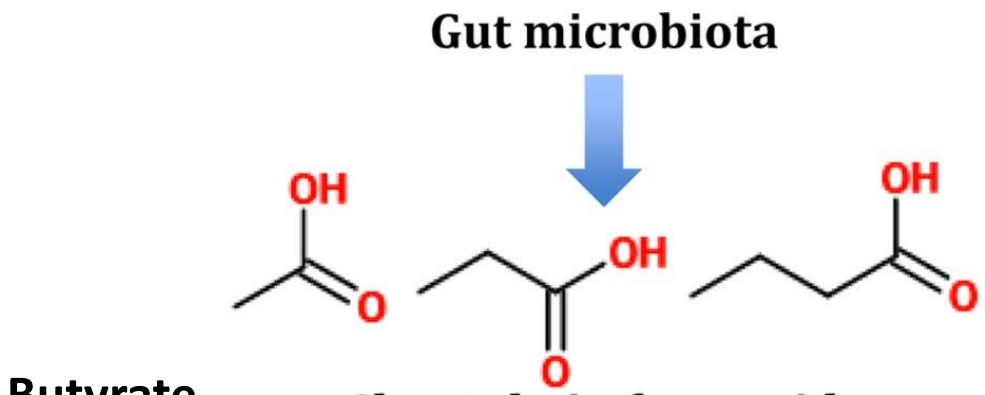


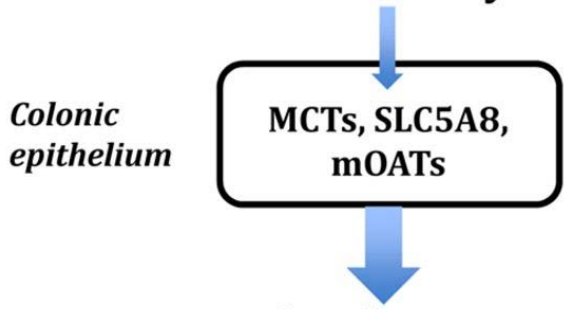
Right colon Left colon right colon adenoma



microbial structural organization contributes to disease progression?

Dejea, CM, et. al., Proc Natl Acad Sci U S A. 2014,111(51):18321-6.





Elangovan S et. al., **The niacin/butyrate receptor GPR109A suppresses mammary tumorigenesis by inhibiting cell survival.** CancerRes.2014,74:1166.

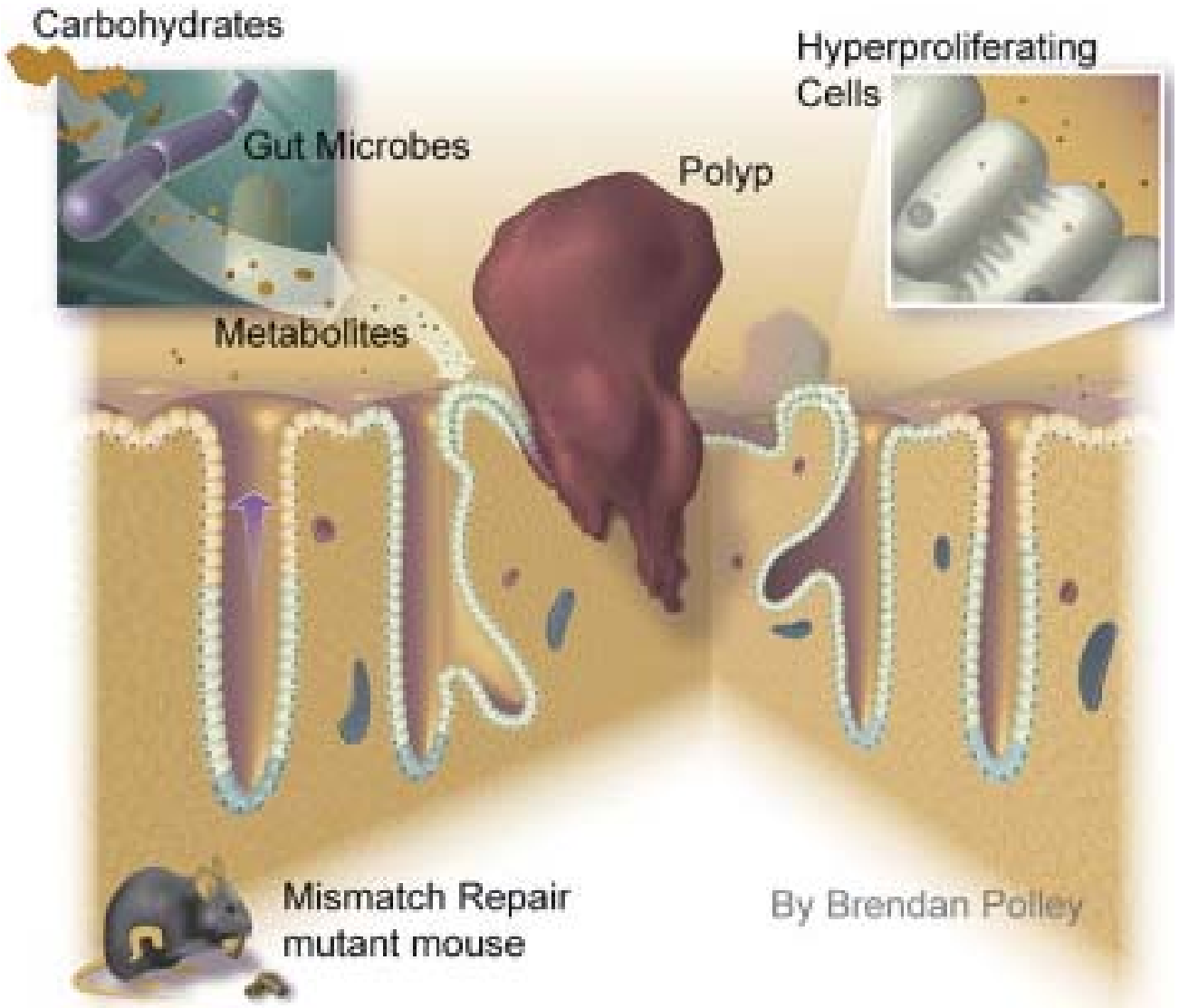
Gpr41 → Adiposity, Blood pressure
Gpr43 → Immunity
Olfr78 → Blood pressure
Gpr109a → Cell cycle, Oncogenesis

Non-GPCR mediated

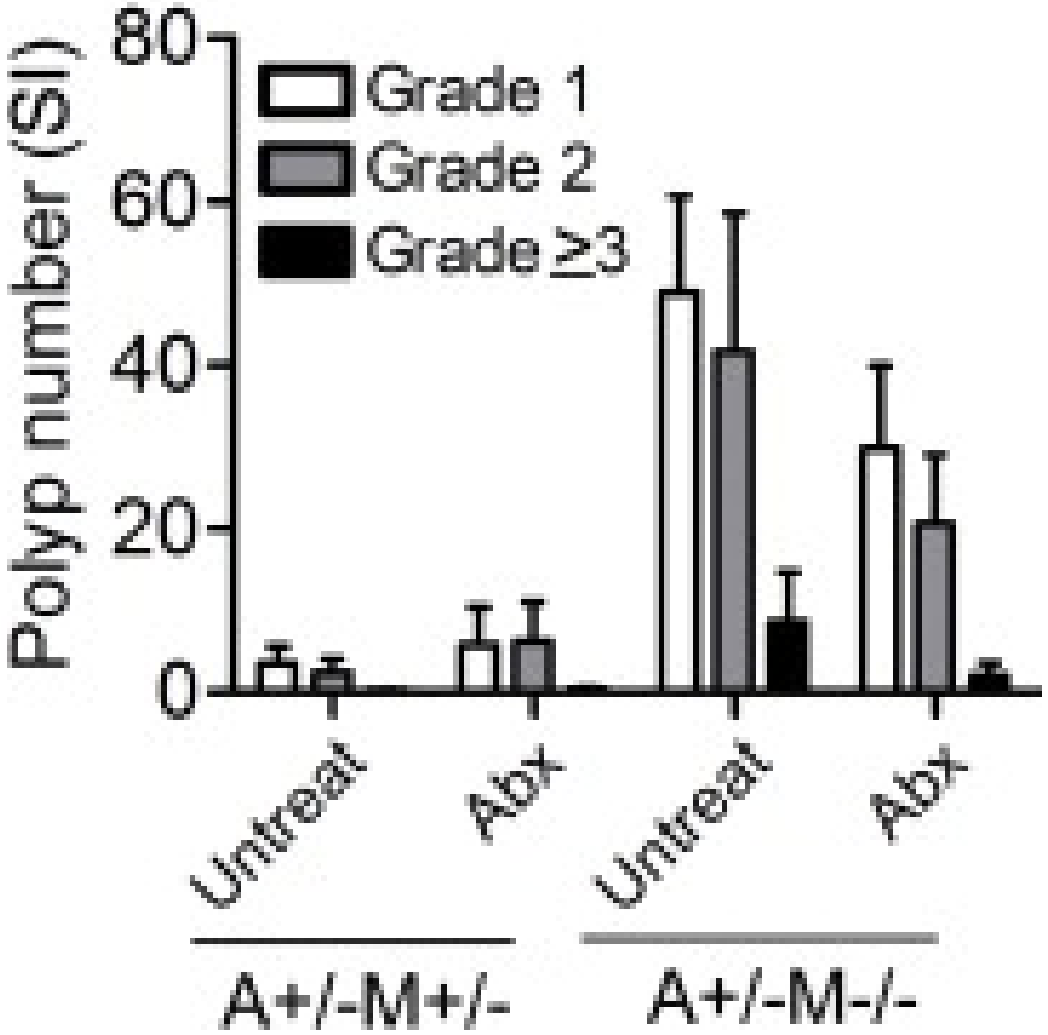
- HDAC inhibition
- Cell proliferation
- Apoptosis

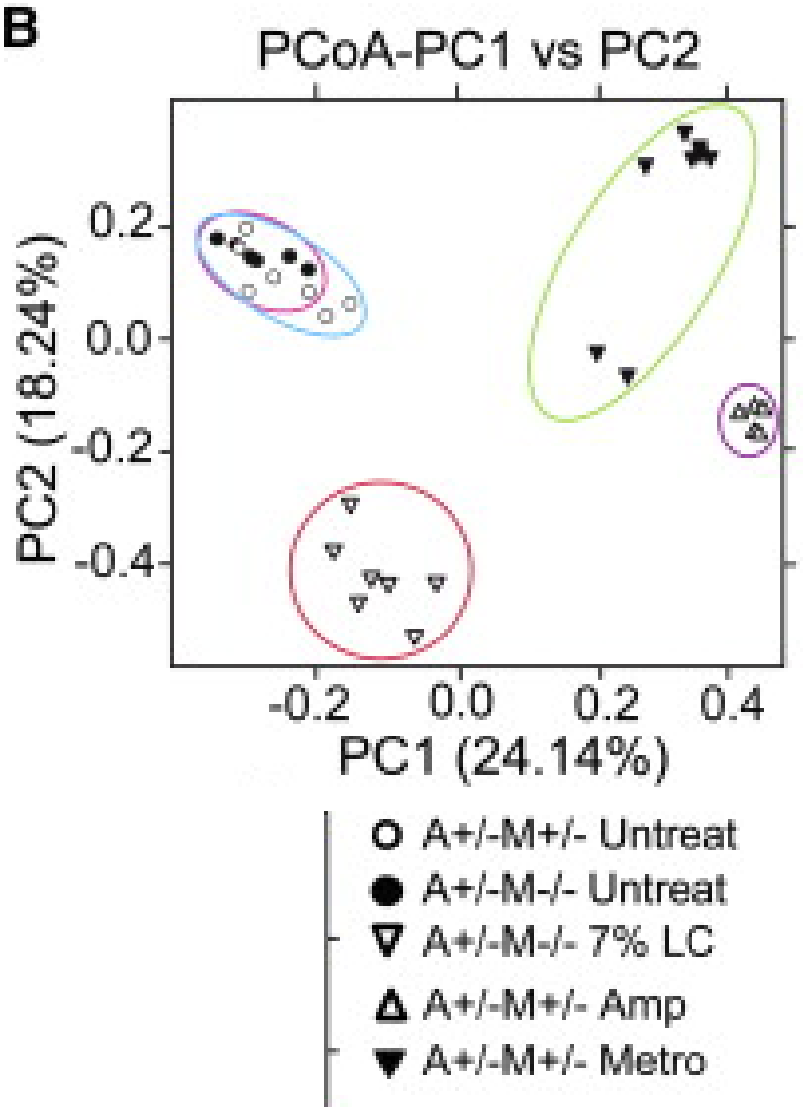
Singh N et.al. **Activation of Gpr109a by the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis.** Immunity. 2014 ,40(1):128-39.

Microbial Metabolism Drives Transformation of Colon Epithelial Cells(Msh2-deficient)

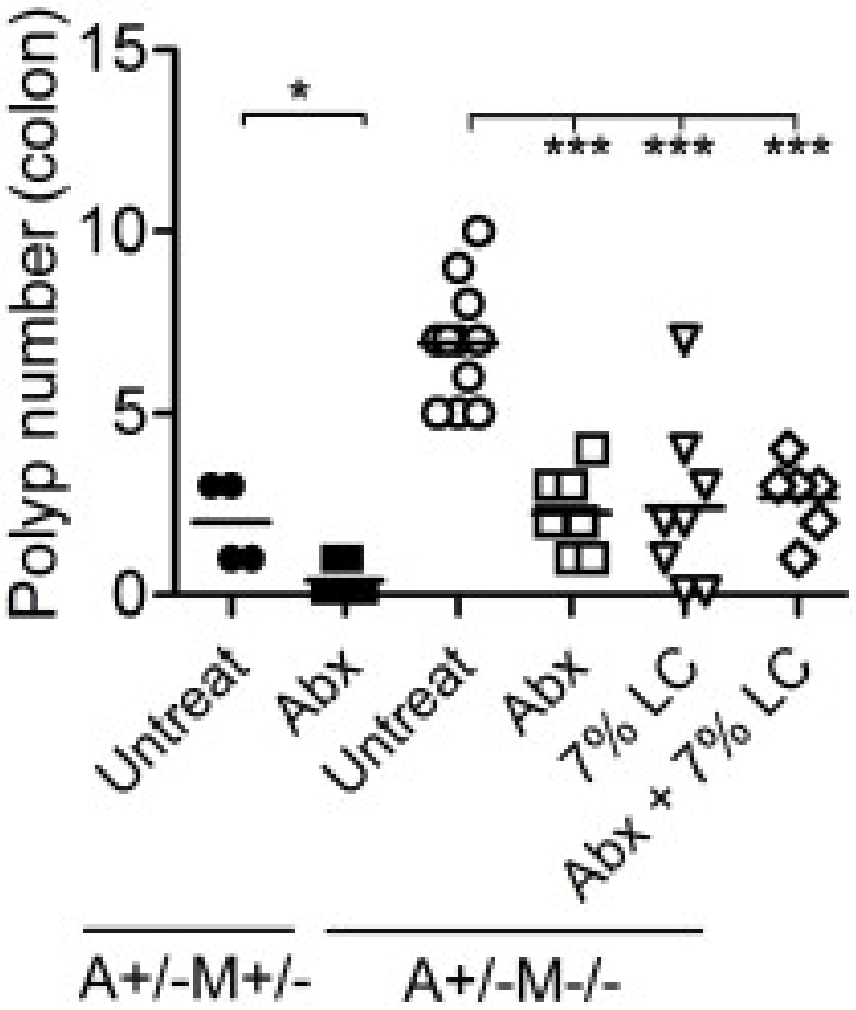


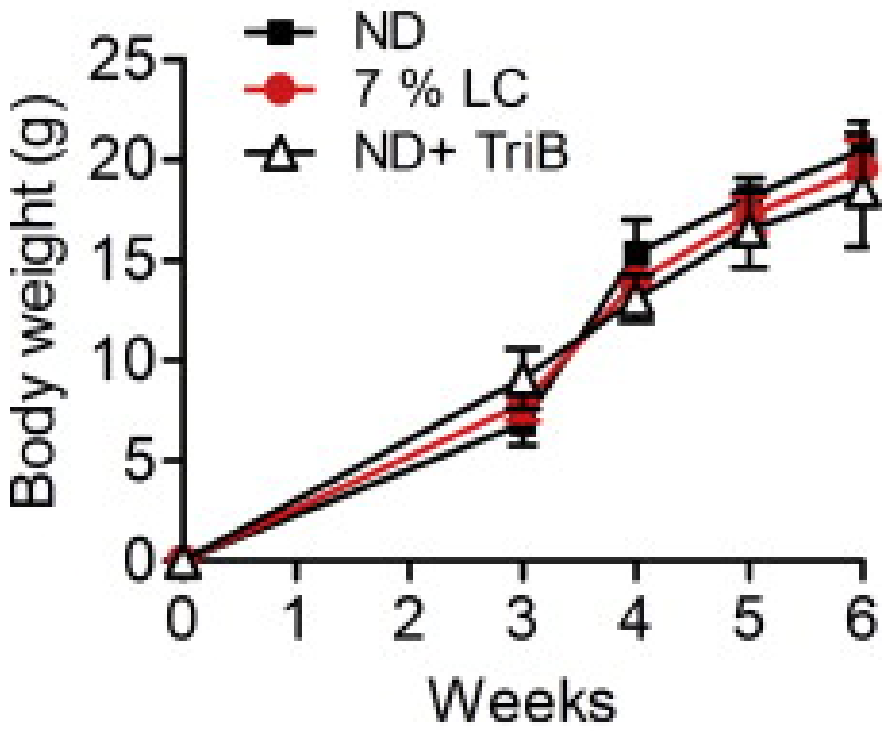
Gut microbiota induce colon cancer in MSH2-deficient mice



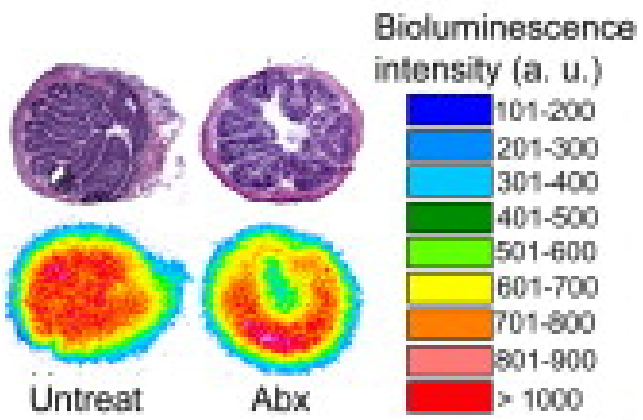


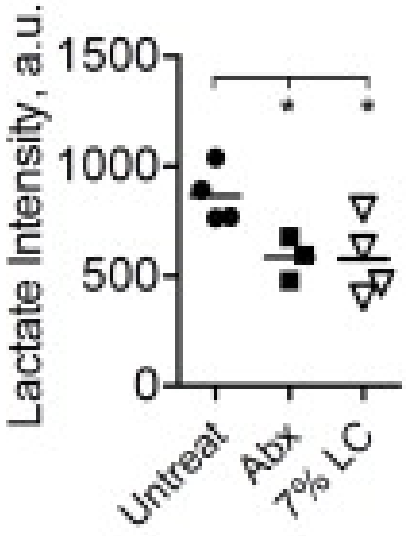
Reduced dietary carbohydrates decreases polyp frequency in $APC^{Min/+}MSH2^{-/-}$ mice

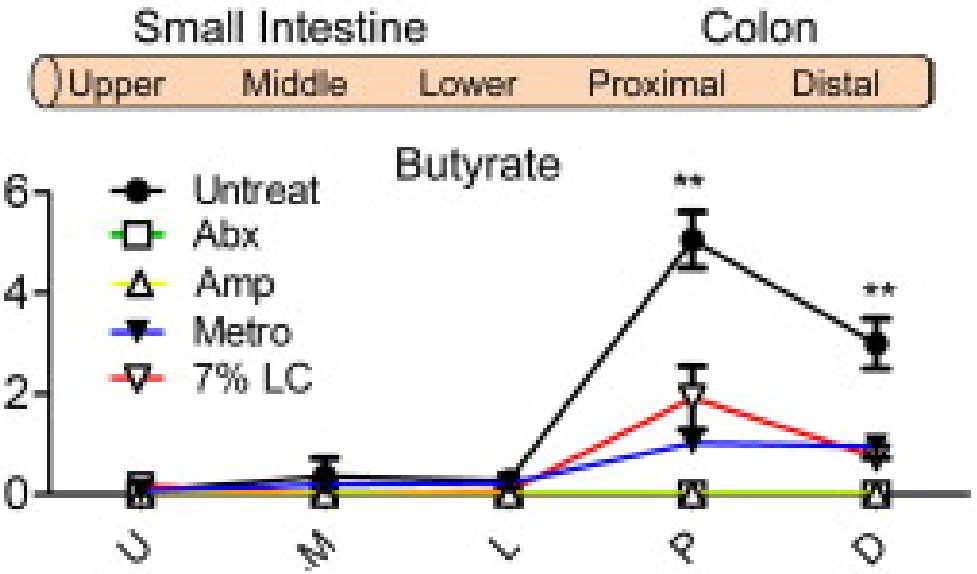


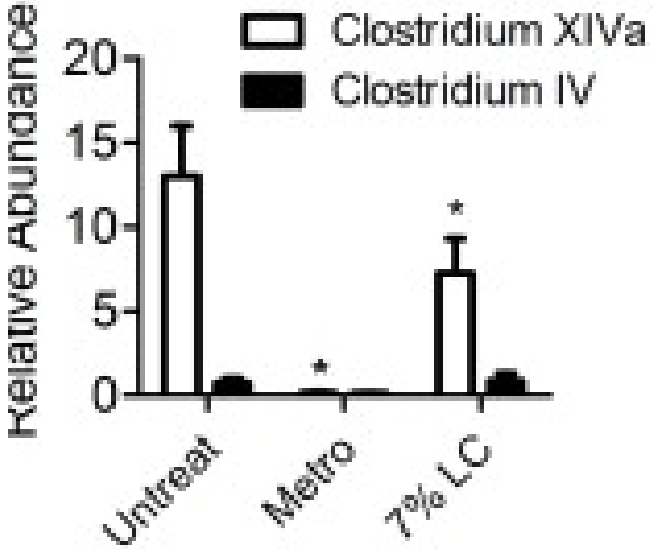


Butyrate induces colon cancer in $APC^{Min/+}MSH2^{-/-}$ mice



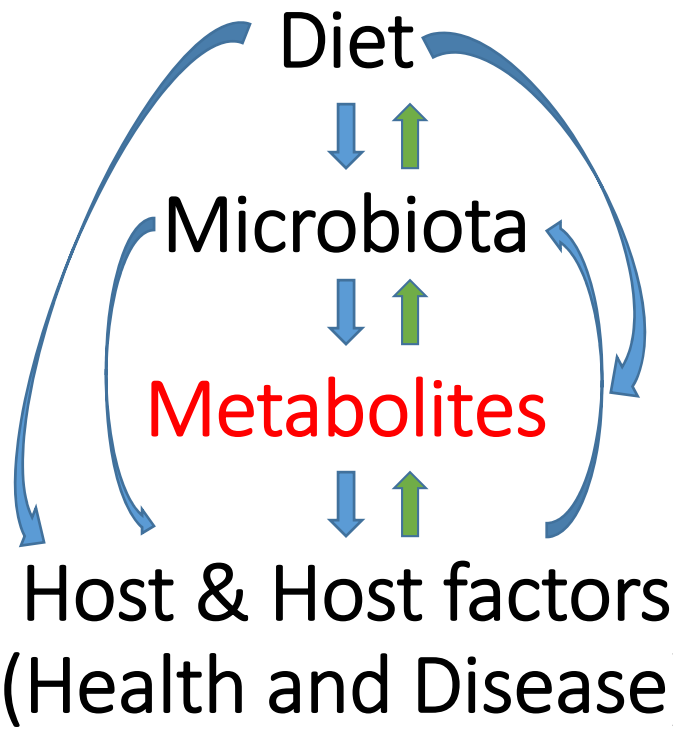






Complex Interactions

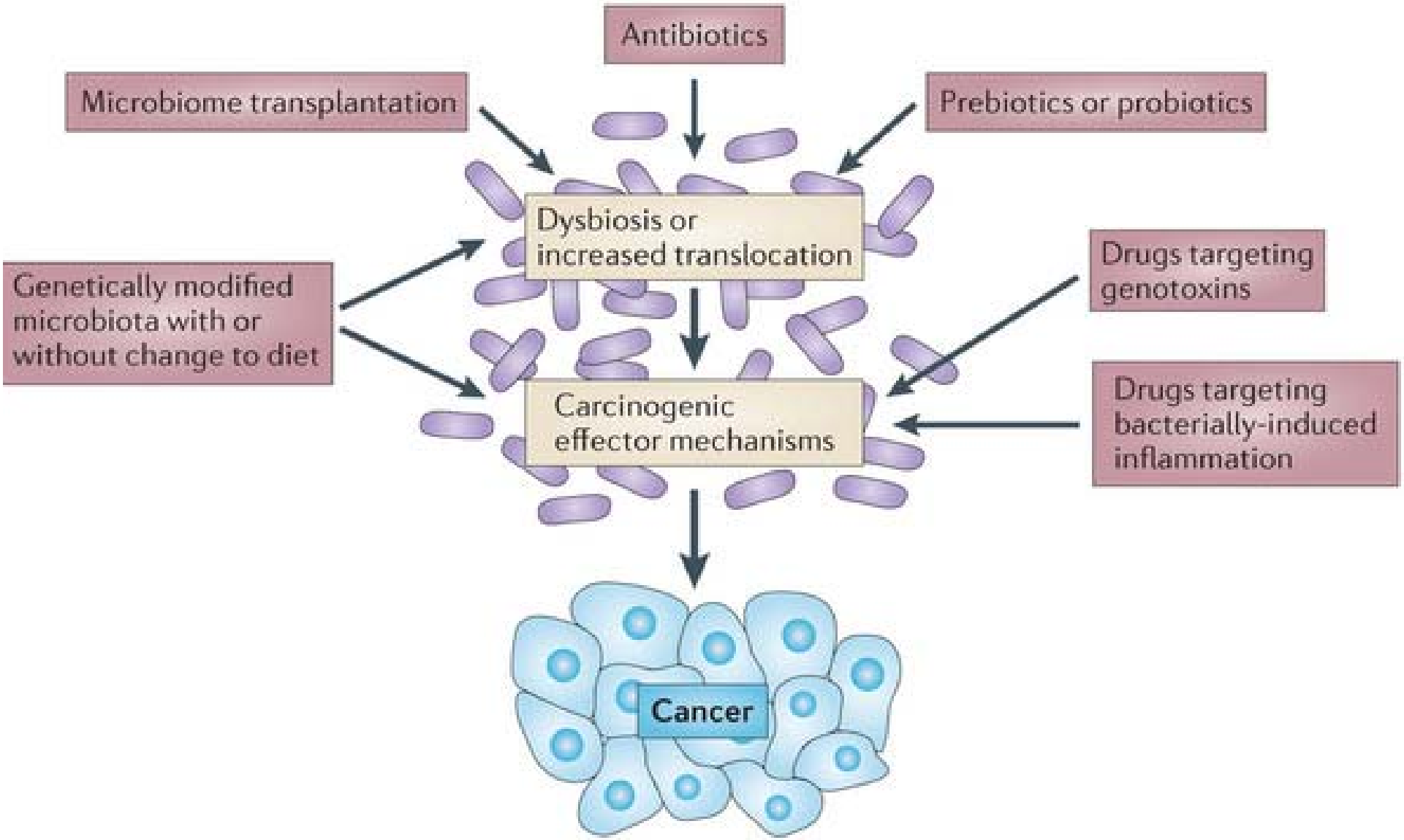
Genetic background matters!



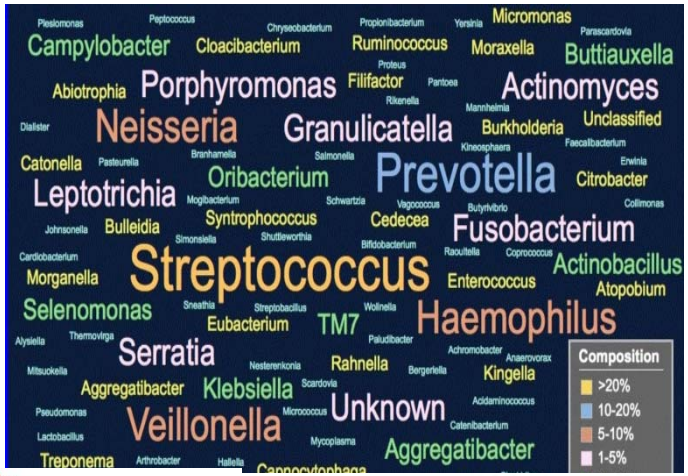
What's next?

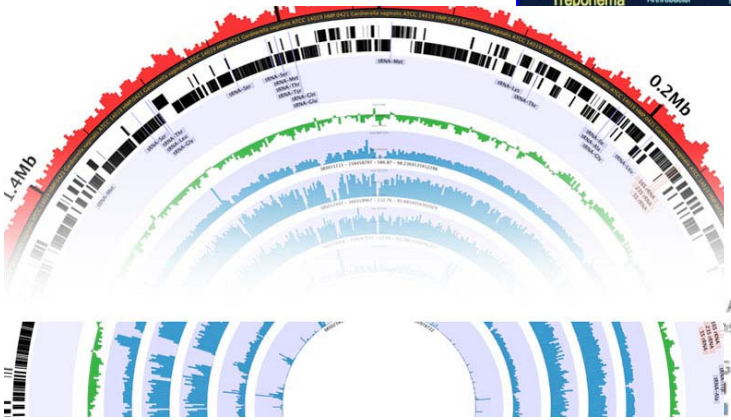
- Presence in the tumor environment
- Progression of tumor and bacterial infection
- Role in tumorigenesis via inflammation mechanisms
- Tumor diagnostics
  - Fusobacterium biomarkers
- Bacterial cancer therapy

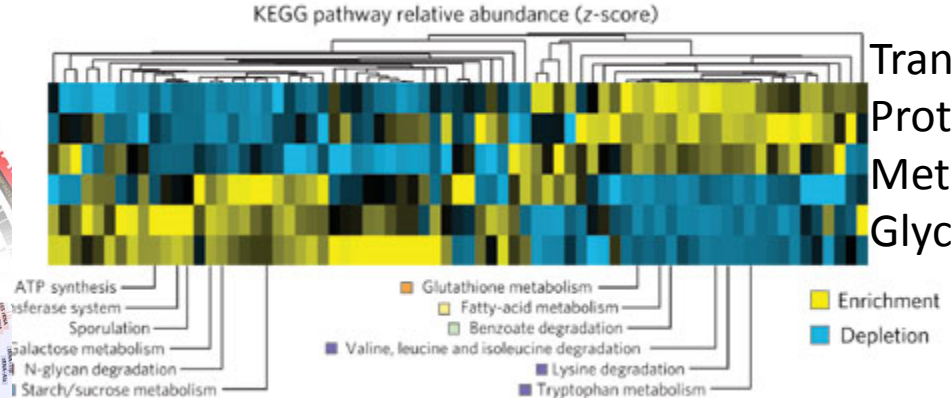
Microbiome- and metabolites-targeted Therapies



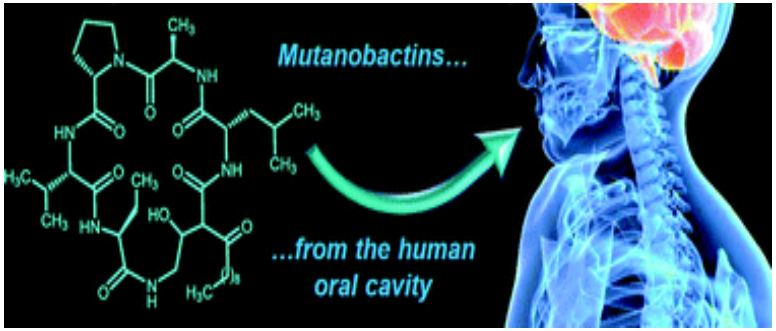
Microbiome & metabolites……



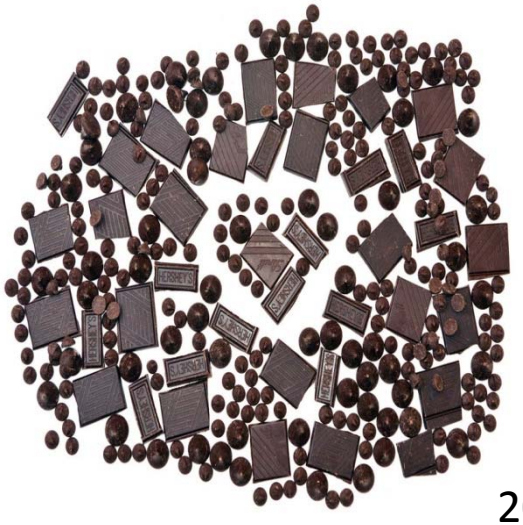




Transcriptomics
Proteomics
Metabolomics
Glycomics



Microbes rule the world



Cocoa powder(polyphenols and Fibers)

Smaller molecules
short fatty chain acids

2014 American Chemical Society meeting

Thank you!