

# Microbiome & Metabolomics

**Hui Wu, PHD**

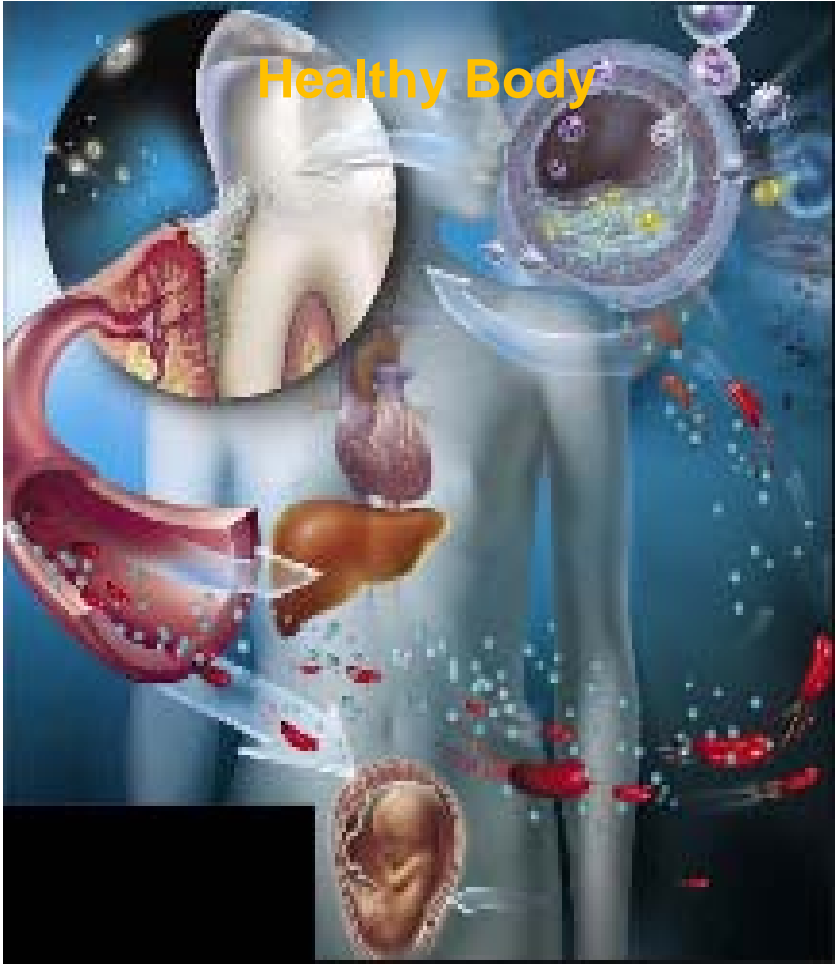
**Schools of Dentistry and Medicine**

**University of Alabama at Birmingham**

# Outline

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

# Microbial infection and systemic disease



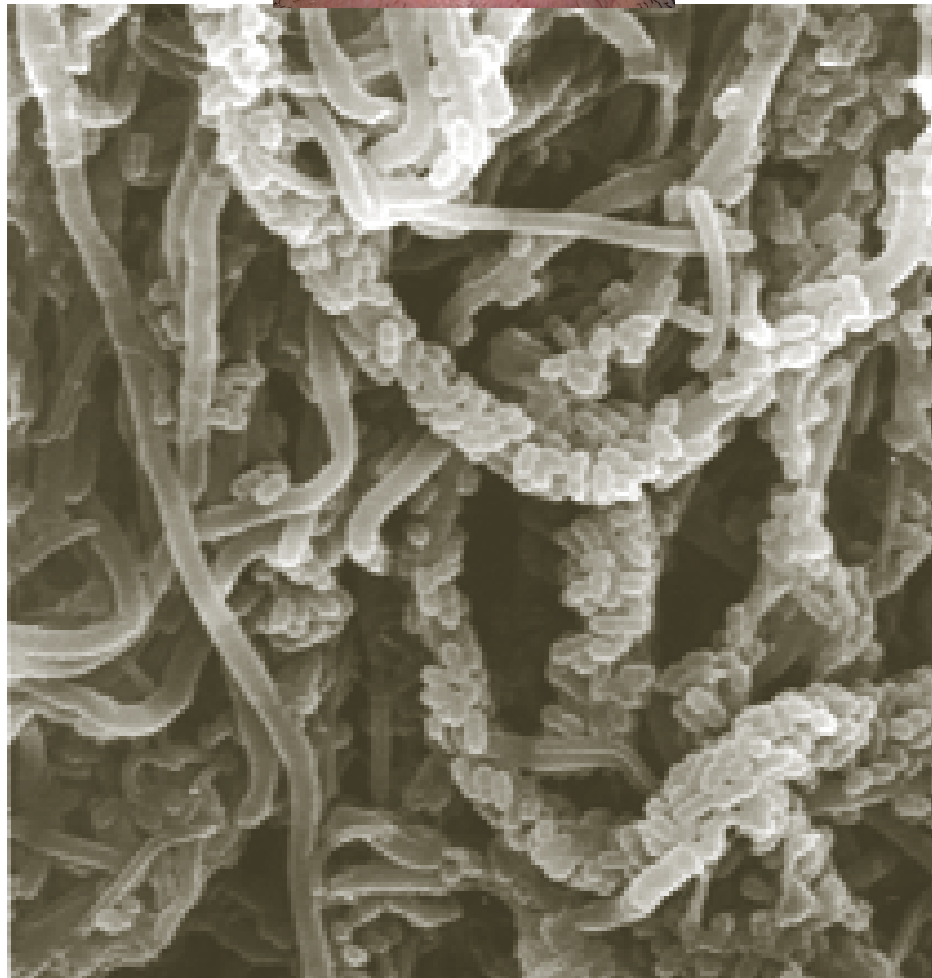
**Cardiovascular  
Complications**

**Respiratory  
infection**

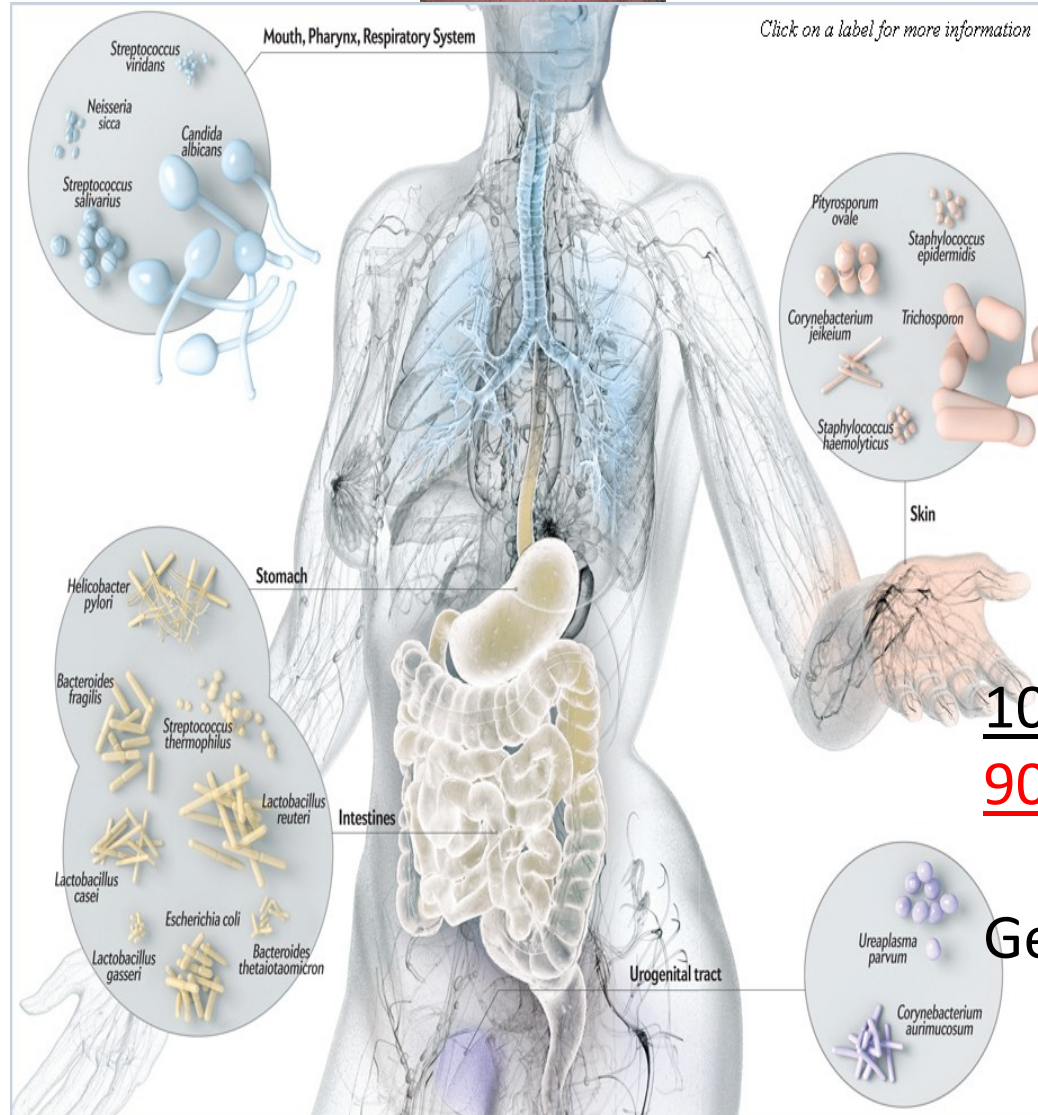
**Pregnancy  
complication**

**Colon  
cancer**

# Complex microbial communities-dental plaque



# Microbes are everywhere



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



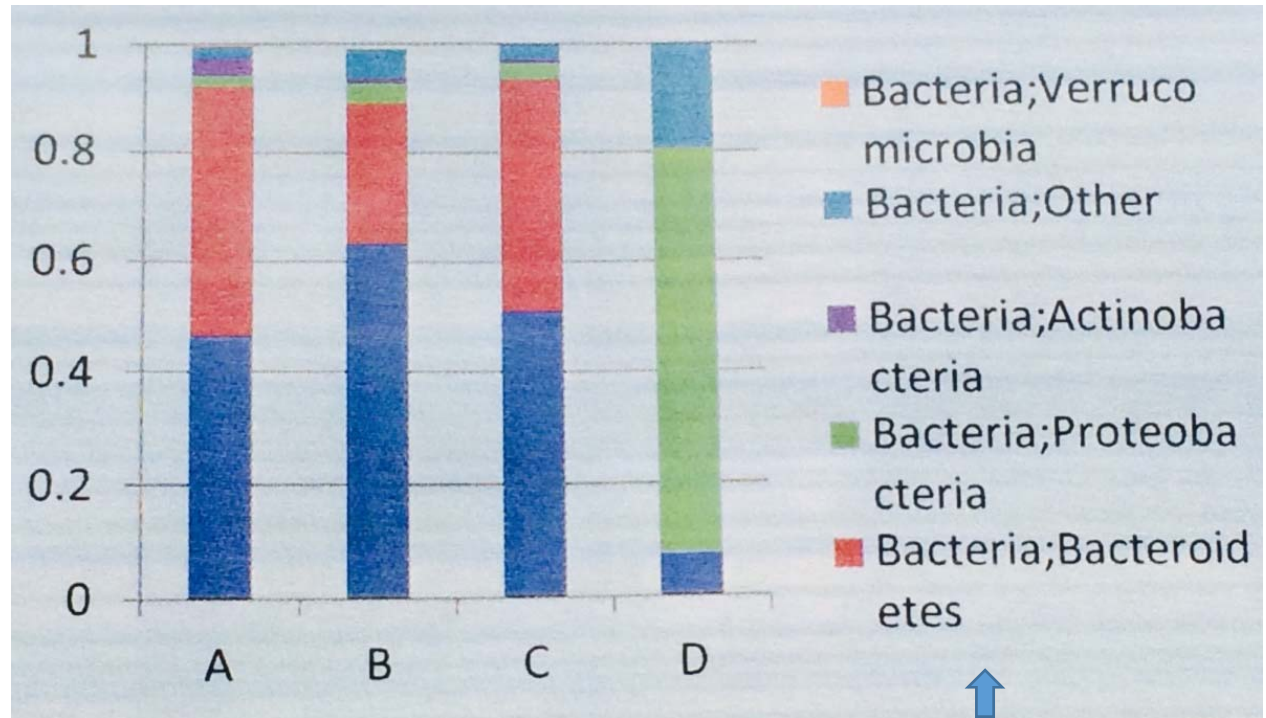
Plaque or saliva



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



Metagenomics



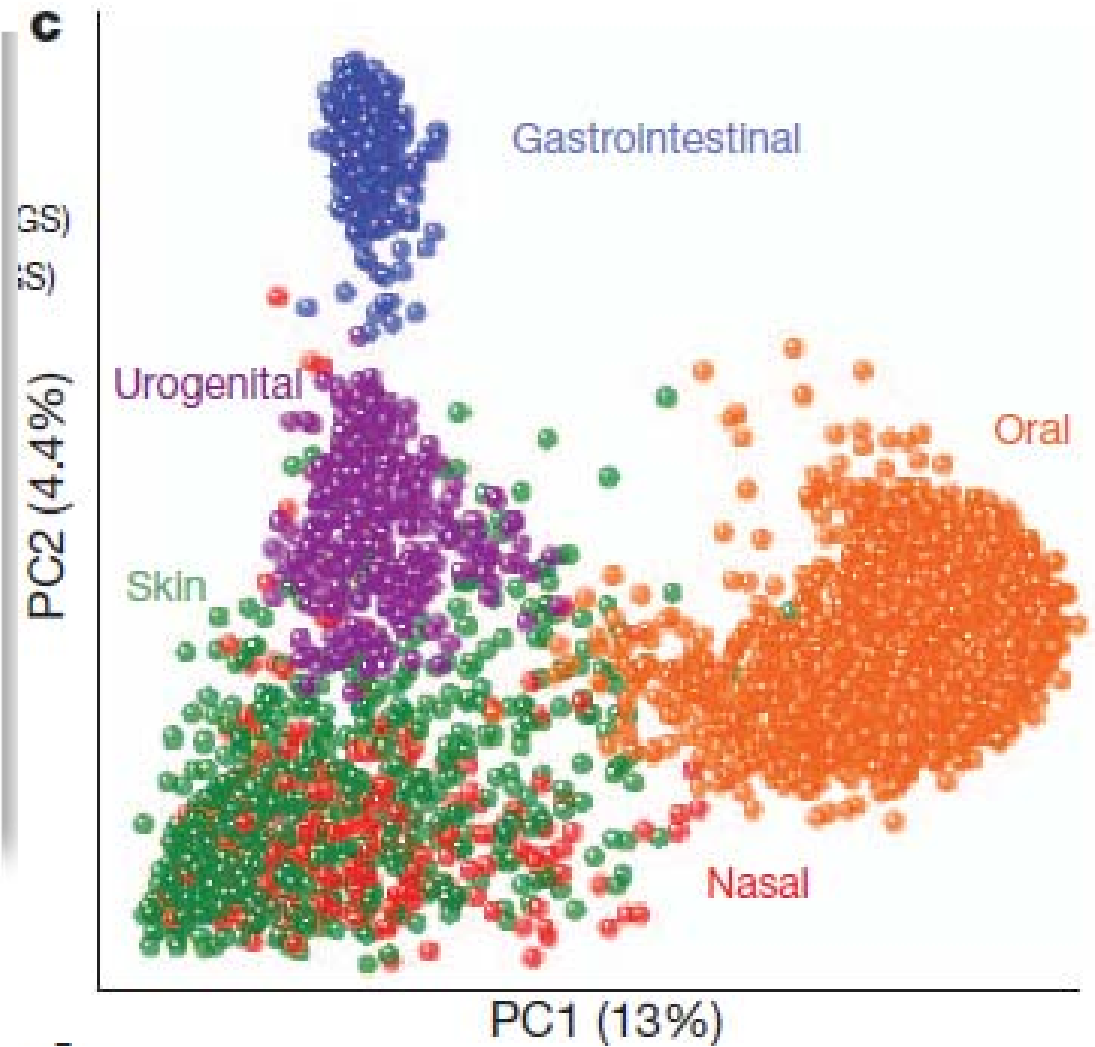
>GCACCTGAGGACAGGCATGAGGAA...  
>GCACCTGAGGACAGGGGAGGAGGA...  
>TCACATGAACCTAGGCAGGACGAA...  
>CTACCGGAGGACAGGCATGAGGAT...  
>TCACATGAACCTAGGCAGGAGGAA...  
>GCACCTGAGGACACGCAGGACGAC...  
>CTACCGGAGGACAGGCAGGAGGAA...  
>CTACCGGAGGACACACAGGAGGAA...  
>GAACCTTCACATAGGCAGGAGGAT...  
>TCACATGAACCTAGGGCAAGGAA...  
>GCACCTGAGGACAGGCAGGAGGAA...

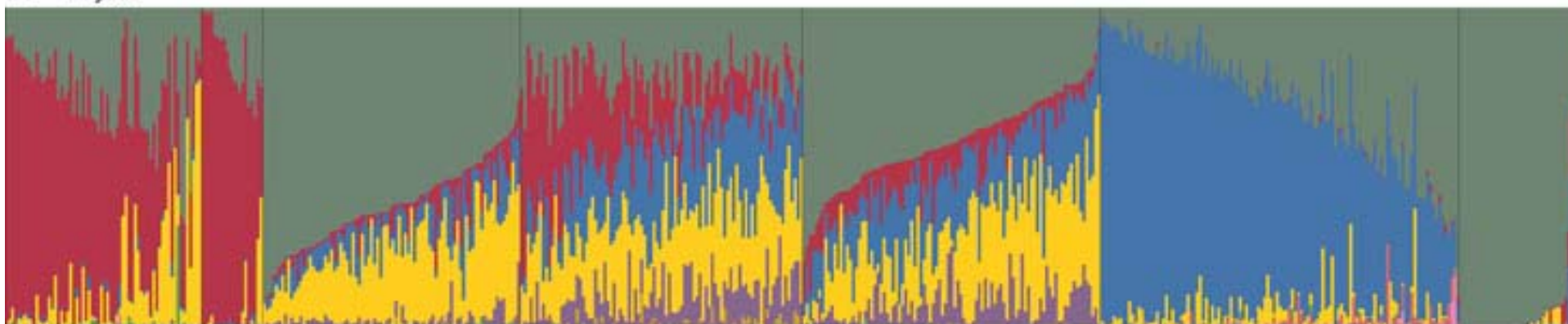


# Human Microbiome Project

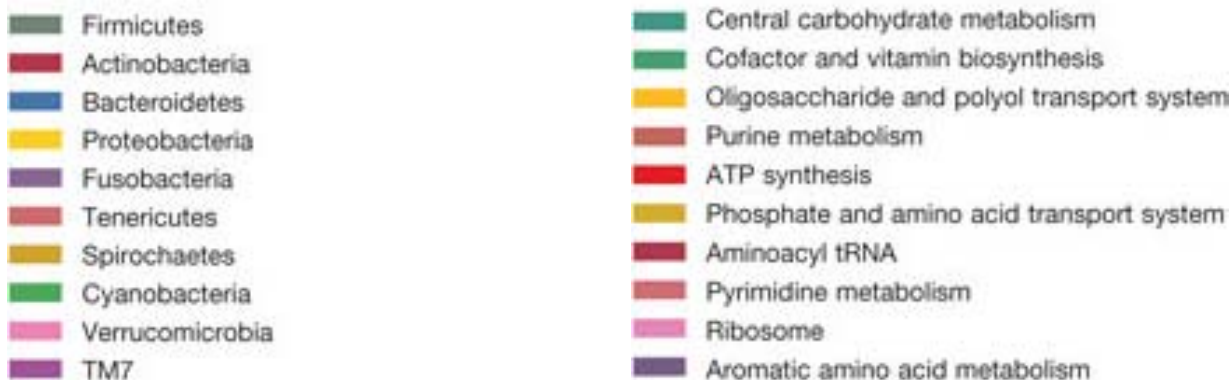
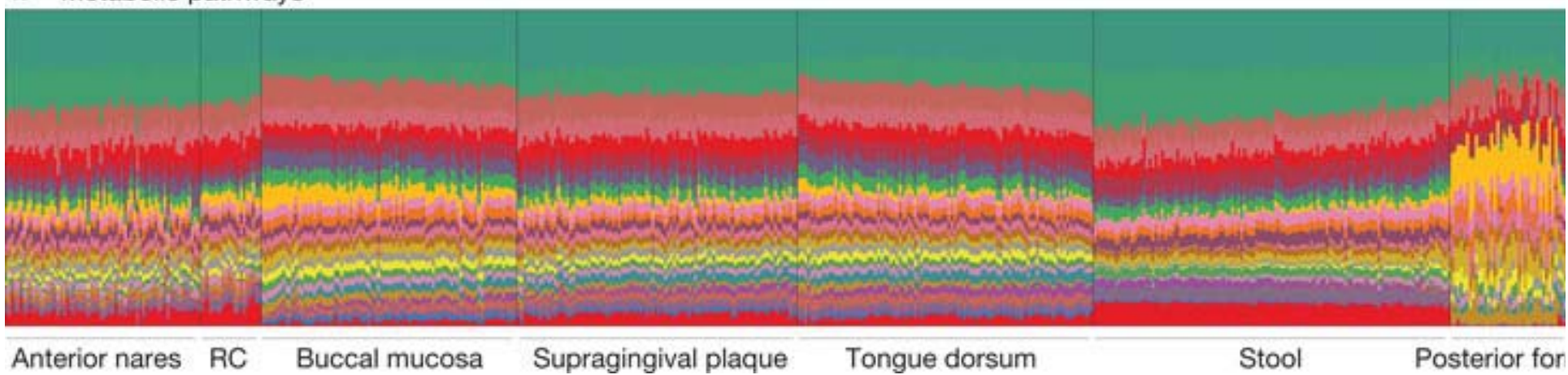


NIH HUMAN  
MICROBIOME  
PROJECT





**b** Metabolic pathways



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

# Transcriptomic Analysis-gene expression profiling



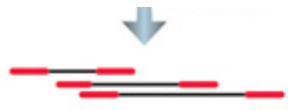
Plaque or saliva



RNA isolation

Microarray  
RNA-sequencing

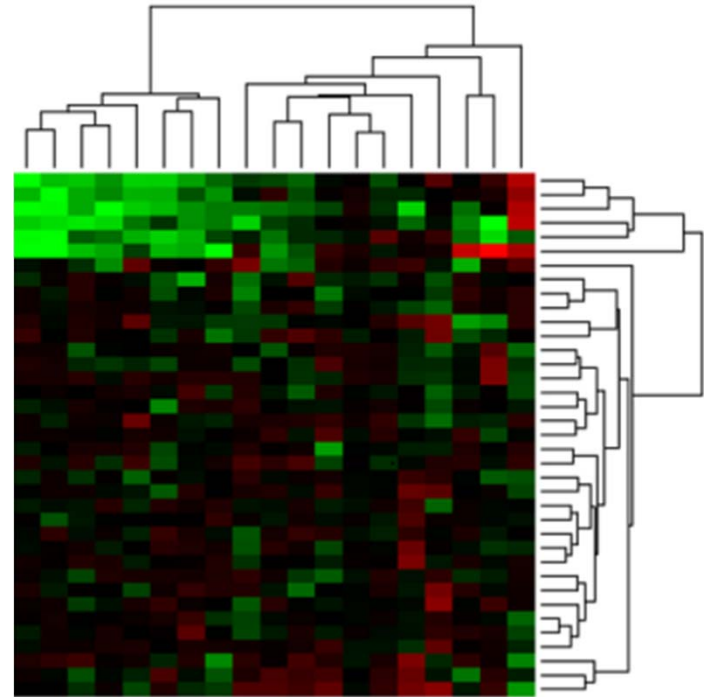
Add bar coded primers, DNA synthesis



Overlapping ds-cDNA Library



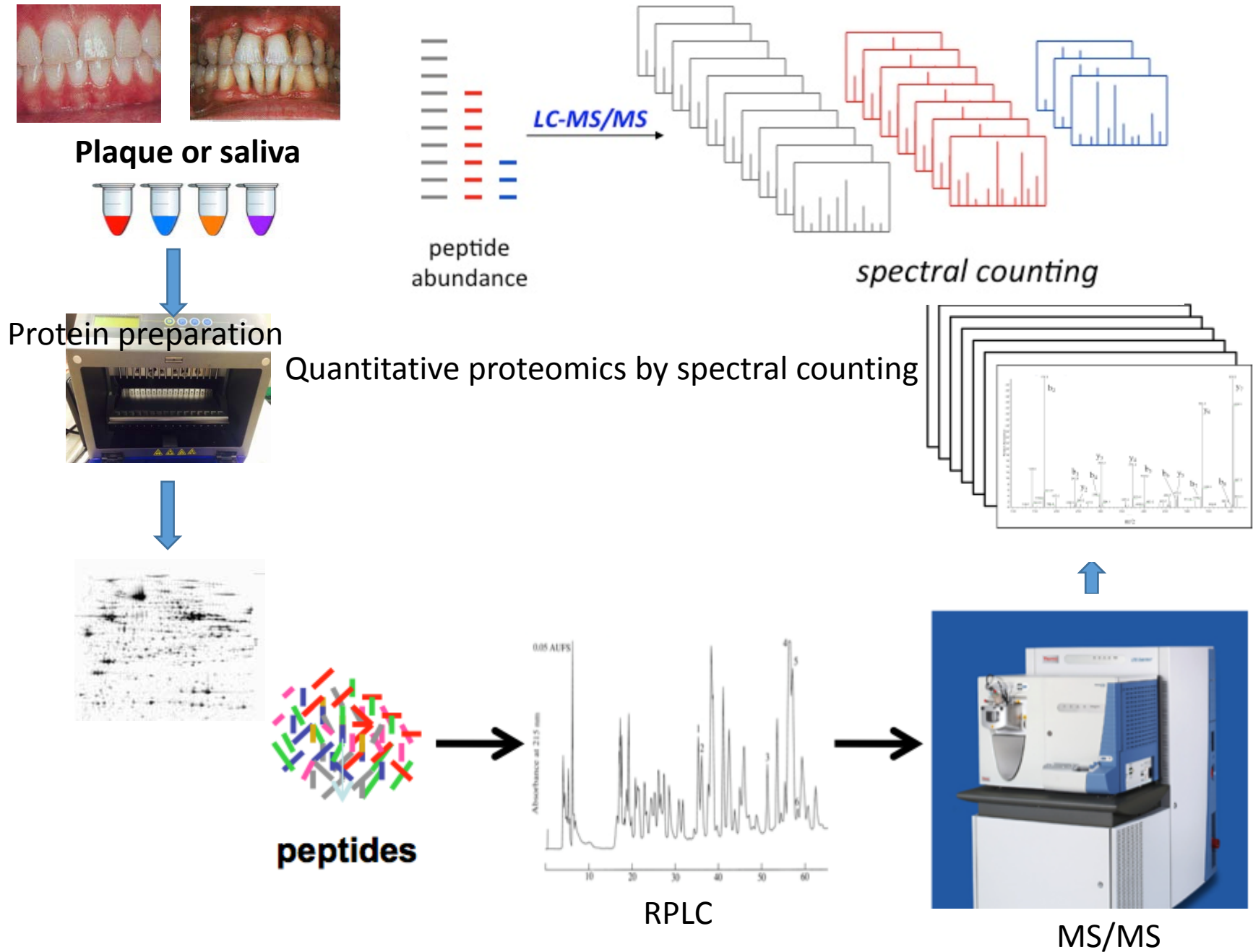
Amplified ds-cDNA  
Removal of primers



Data extraction  
and processing

NextSeq 500

# Proteomic Analysis-protein profiling

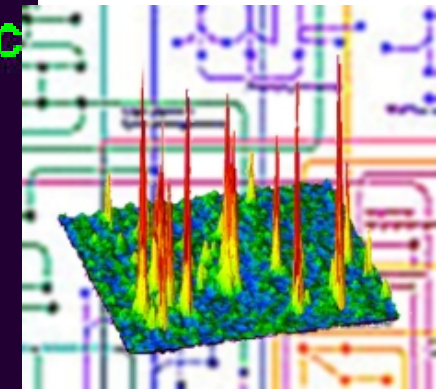
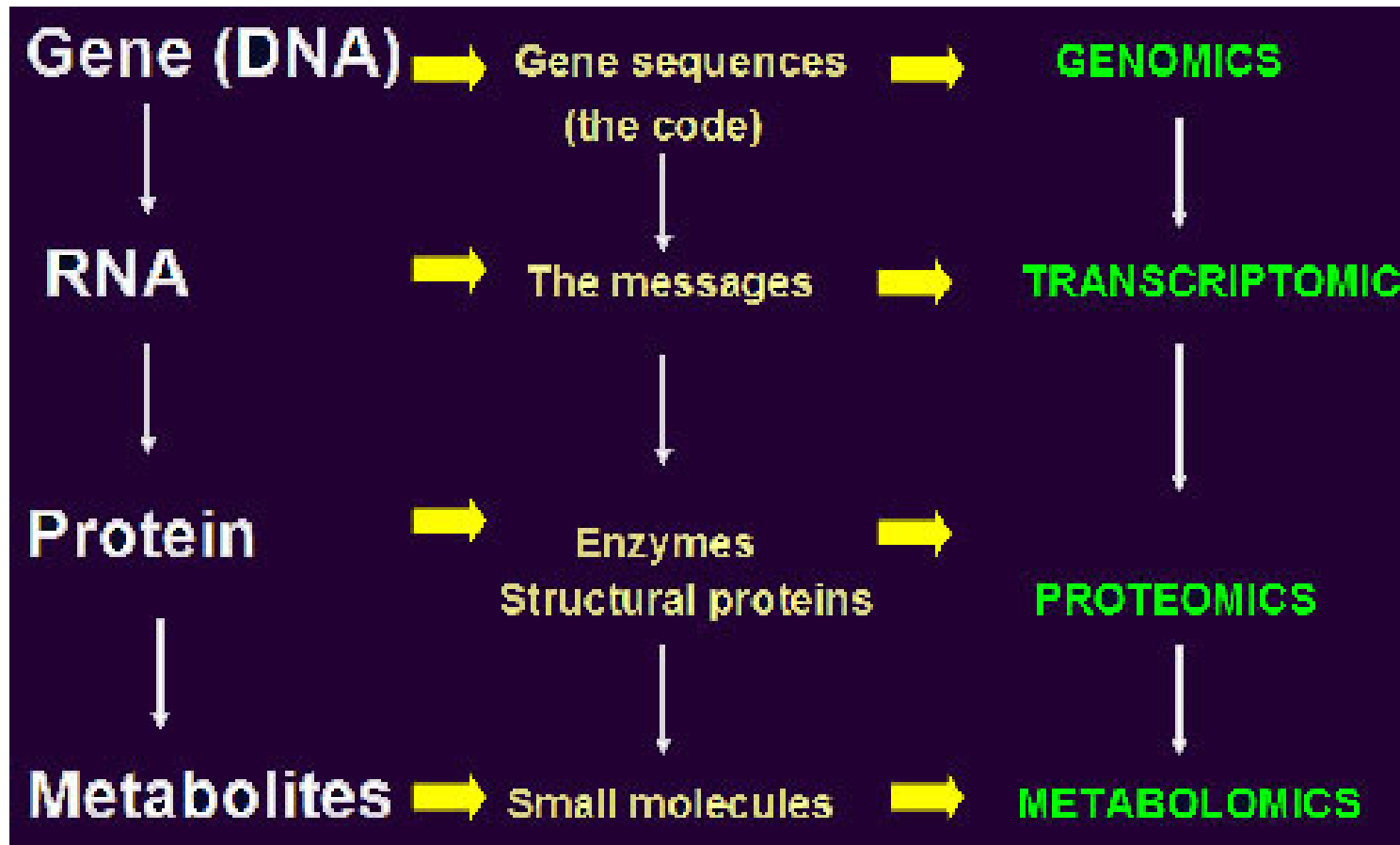


# Integration of Multi"Omics"

Biological samples



Microbiome



Networking pathways for metabolites

Environmental factors

Phenotypes





Fruit fly  
(*Drosophila melanogaster*)

## Microbiomes impact behaviors

Gut microbiota     Diet-specific microbiota influence mating preferences



Mosquito  
(*Anopheles gambiae*)

Human skin microbiota     Skin microbes of humans influence attraction to mosquitoes



Mouse

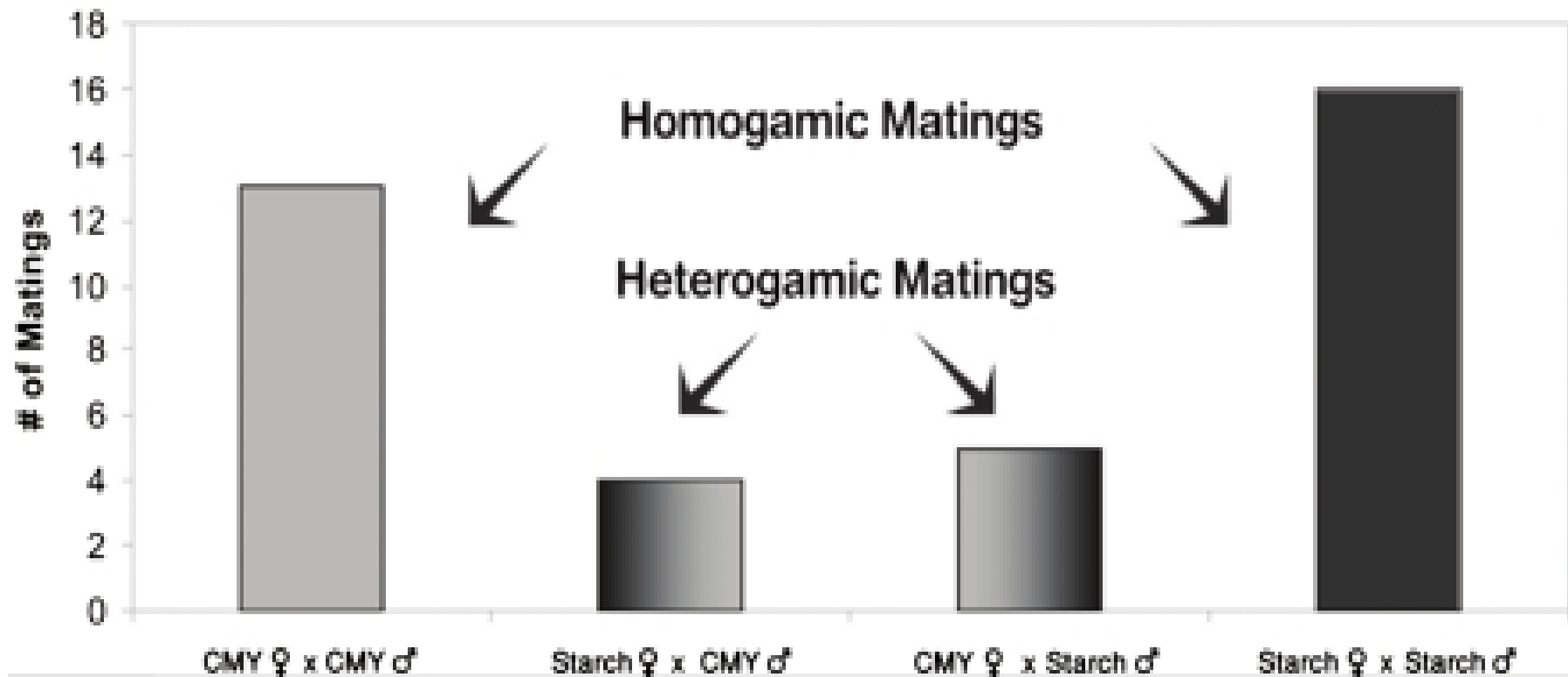
*Lactobacillus rhamnosus*     The probiotic *L. rhamnosus* decreases anxiety in mice

**Fighting microbes or Farming microbes?**



## Microbiomes impact behaviors

Gut microbiota     Diet-specific microbiota  
influence mating  
preferences



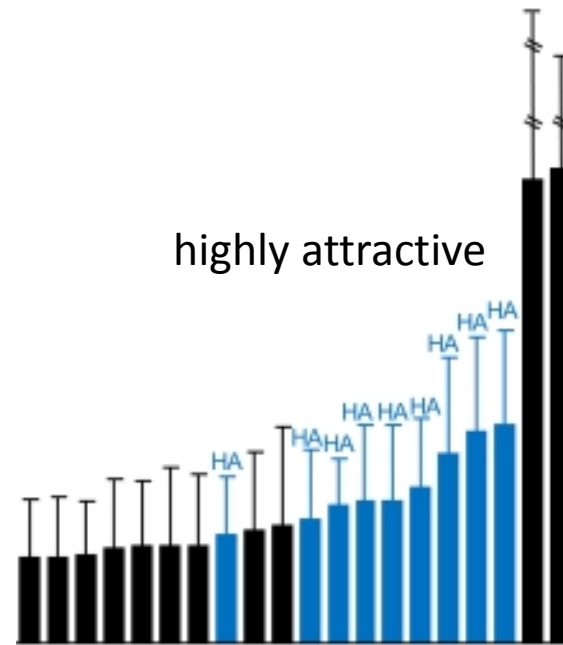
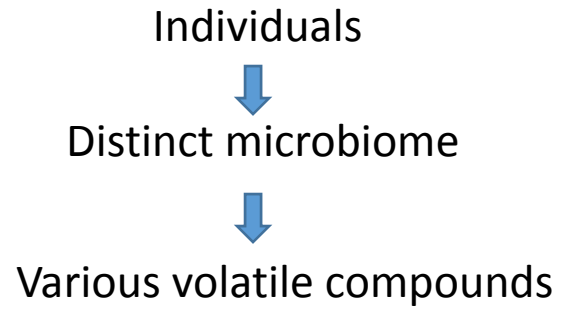
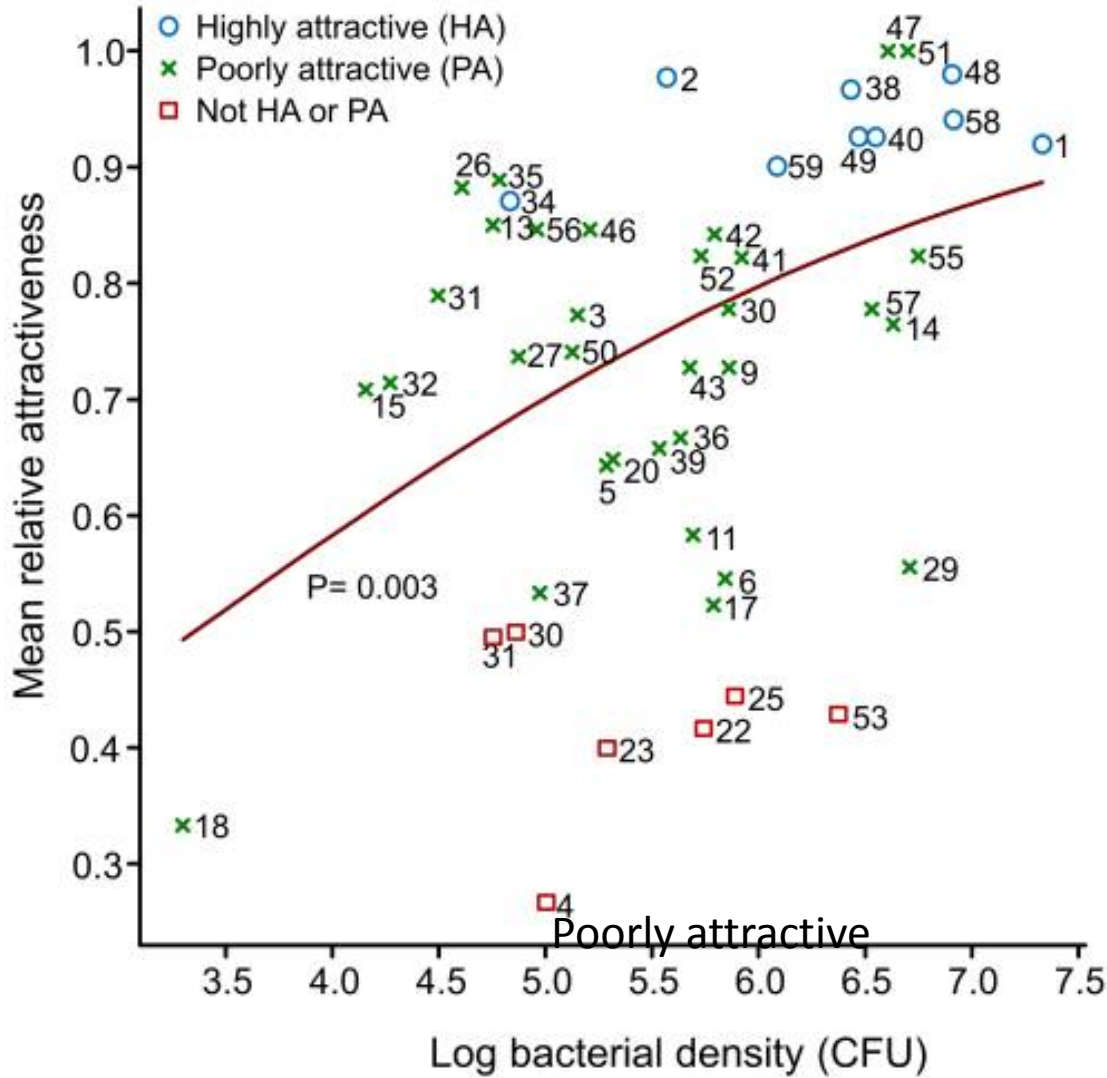
Sharon, et. al., Proc Natl Acad Sci U S A. 2010,107(46):20051-6.

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



Human skin  
microbiota

Skin microbes of humans  
influence attraction to



PLoS One. 2011;6(12):e28991

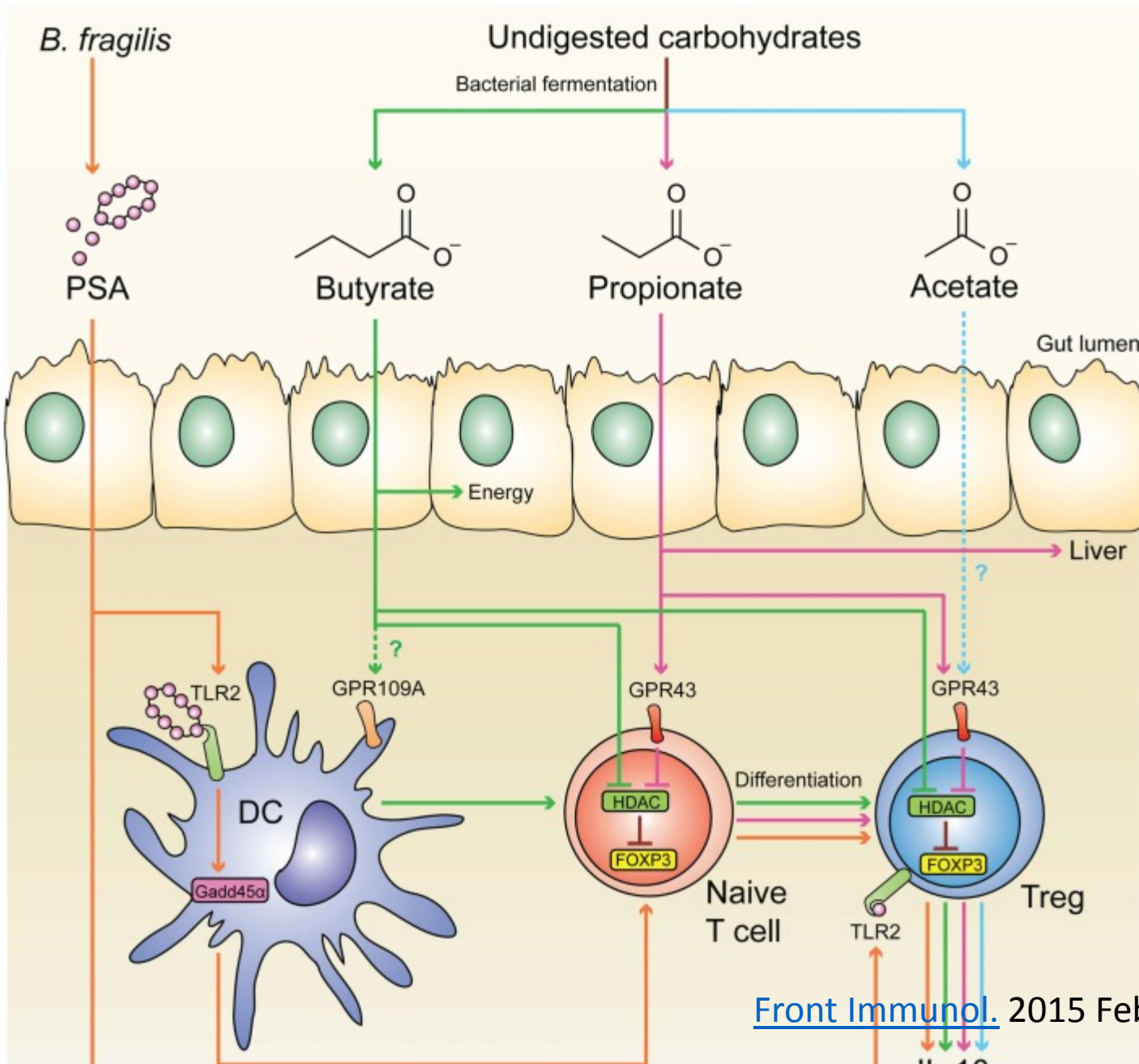
PLoS One. 2010 Dec 30;5(12):e15829



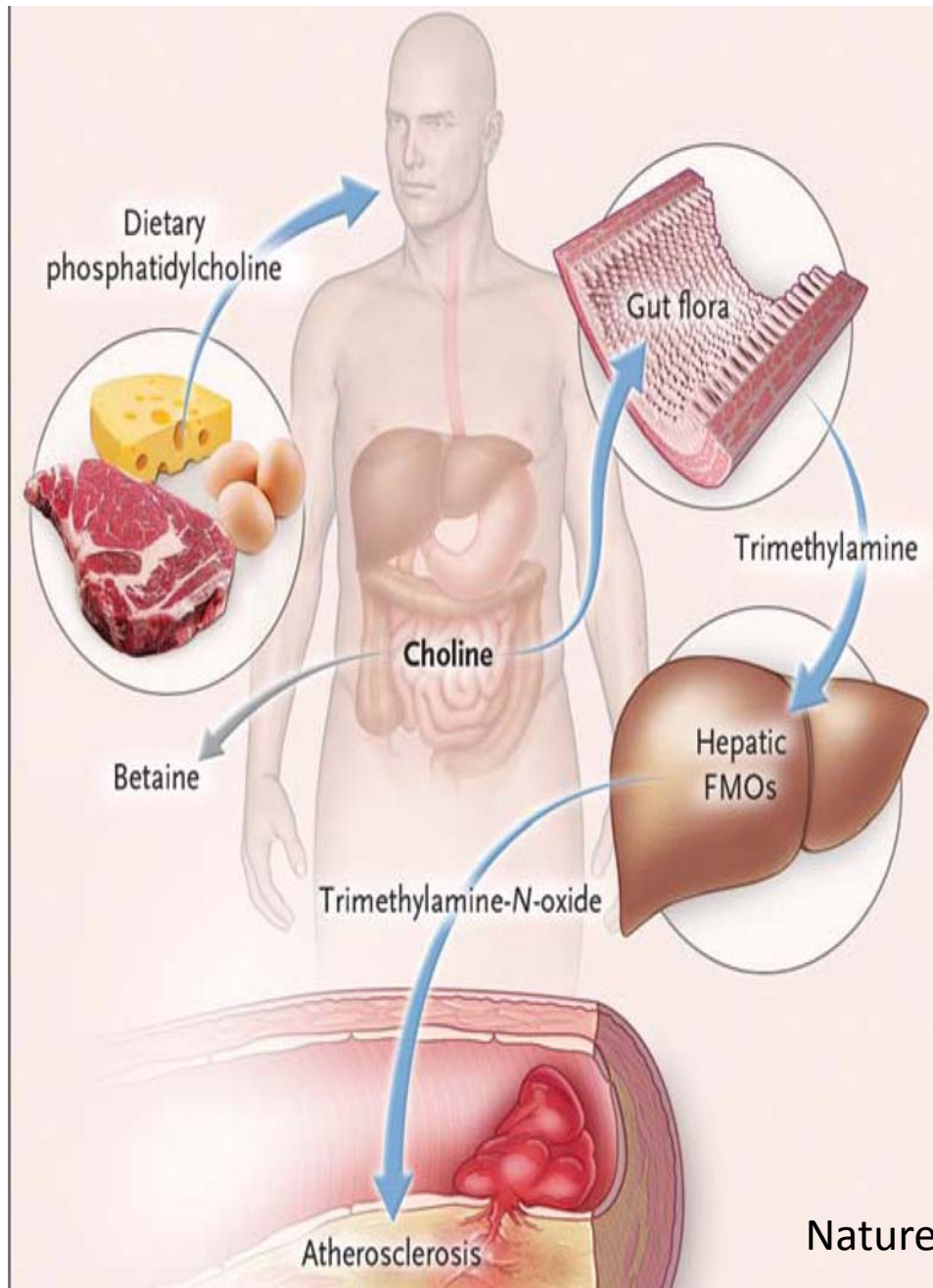
# Microbiome and Carb Metabolism

- ***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



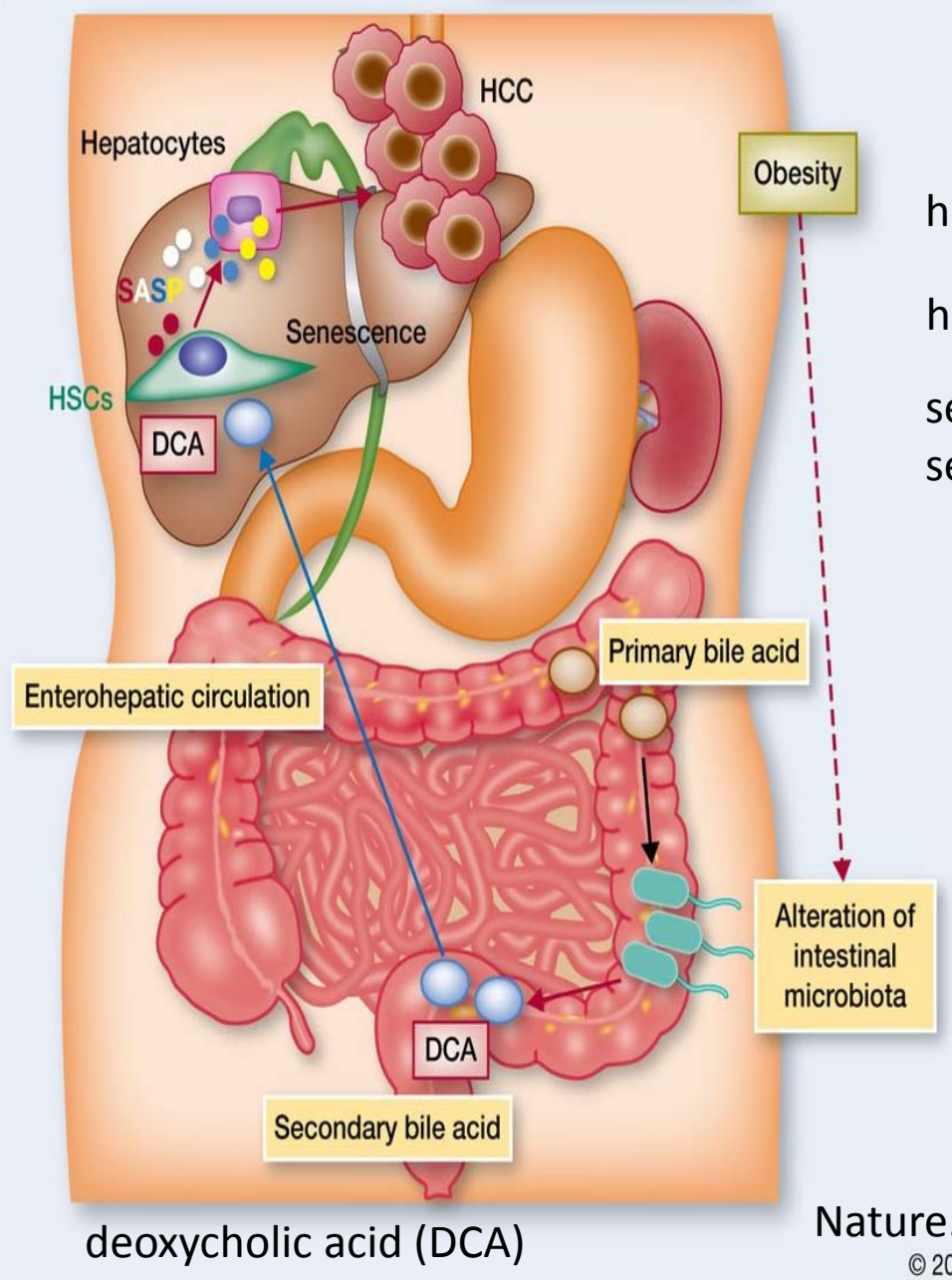
# Microbiome and Atherosclerosis



Flavin monooxygenases (FMOs)

Nature. Apr 7, 2011; 472: 57–63.

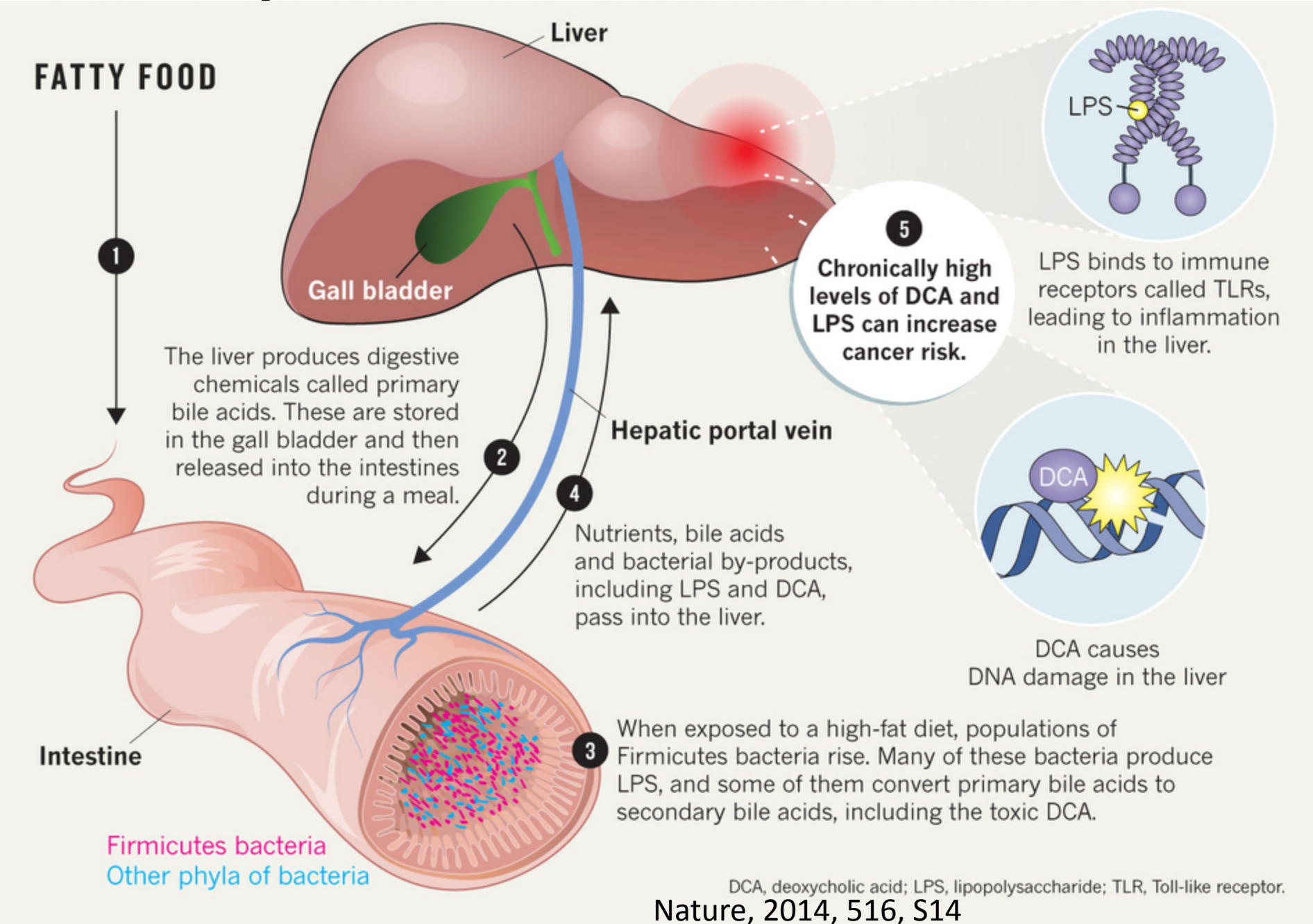
# Obesity and Cancer: a Microbial Connection



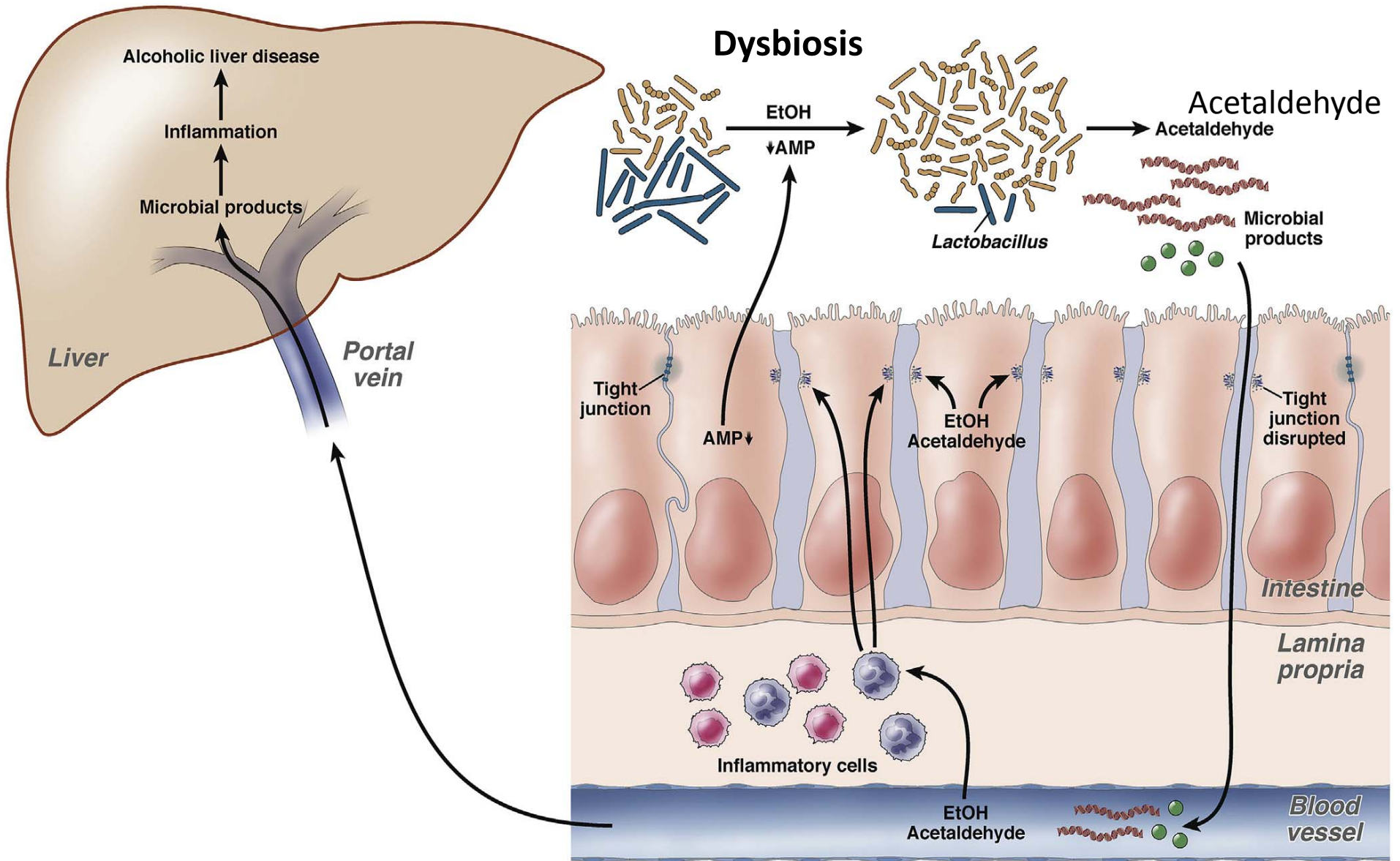
hepatic stellate cells (HSC)  
hepatocellular carcinoma(HCC)  
senescence-associated  
secretory phenotype (SASP)



# Obesity and Cancer: a Microbial Connection



# Microbiome & Alcoholic Liver Disease





# Gut Bacteria & Healthful Chocolate



Cocoa powder (polyphenols and Fibers)

Smaller molecules  
short fatty chain acids

2014 American Chemical Society meeting



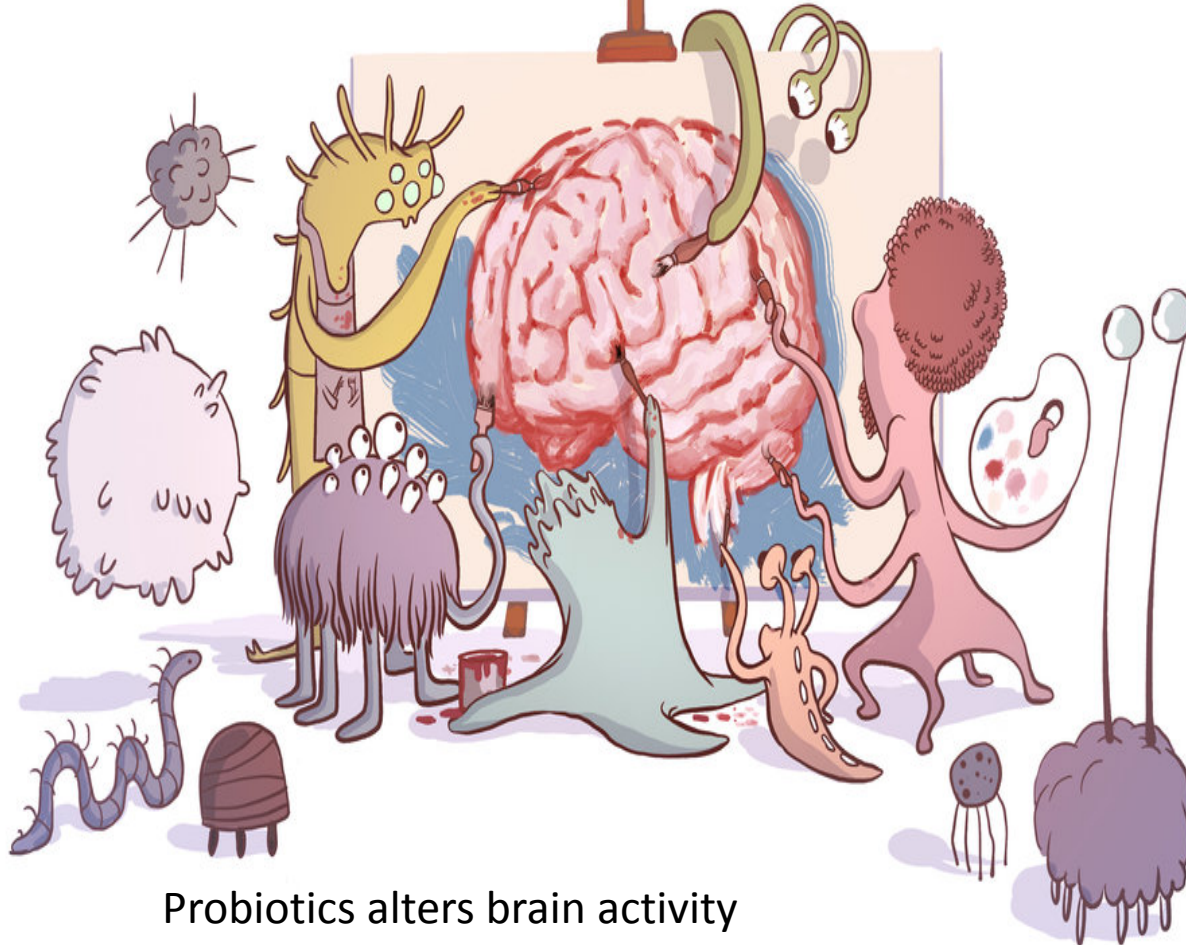
# Gut Bacteria and the Workings of Our Minds

## 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



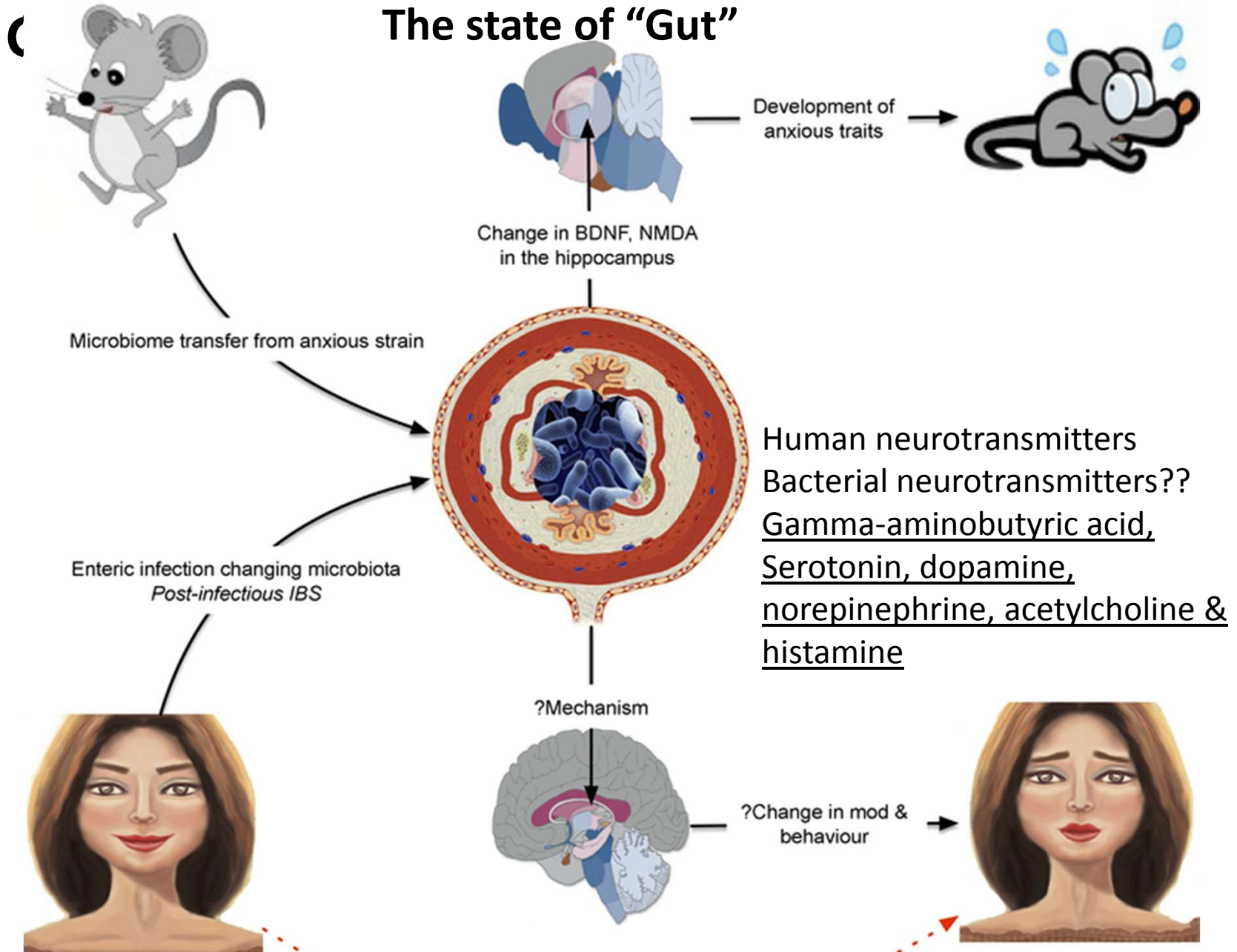
Probiotics alters brain activity

Gastroenterology, 144, 1394–1401. June 2013

Cell, 155,1451–63, 19 December 2013



# The state of "Gut"



Longitudinal Study needed of individuals before & after infectious insult occurs

J Physiol. 2014 , 592:2981-8

# Gut Microbes and Your Weight

**Slimming gut microbes?**

**Microbe transplants  
from obese humans**



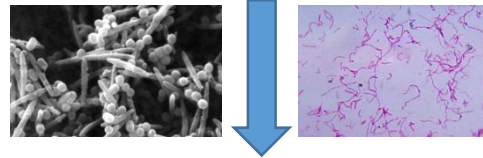
***Science*, 6 September 2013: 341(6150)**

# Oral bacteria in systemic conditions

## *Fusobacterium nucleatum*

- Gram-negative, rod-shape anaerobic
  - Butyric acid, a major metabolite
  - Periodontal disease and gingivitis
    - Systemic infections

# Fusobacterium & Colon Cancer

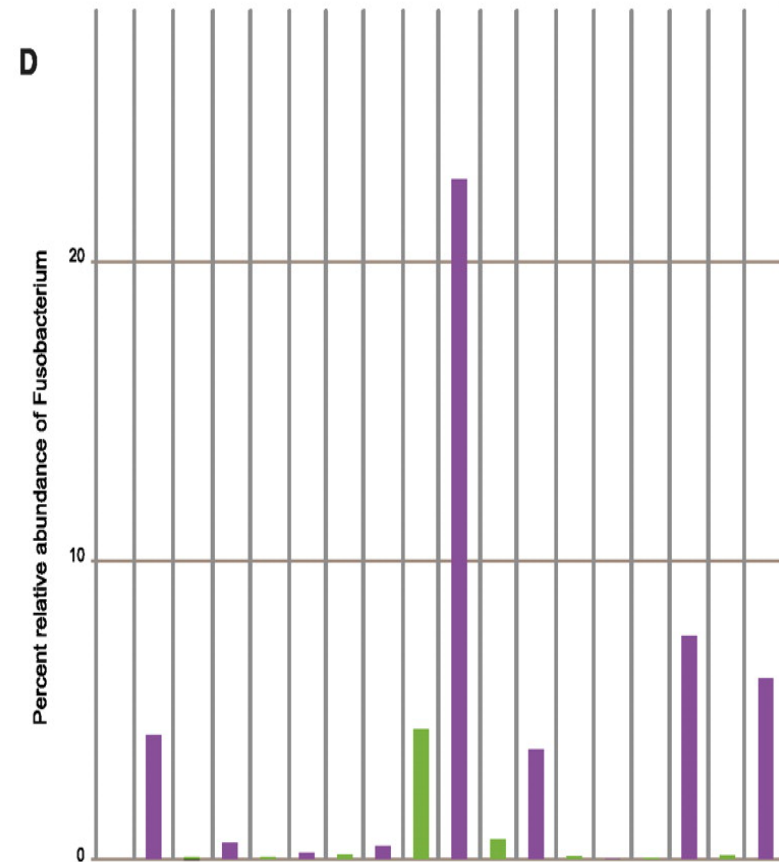
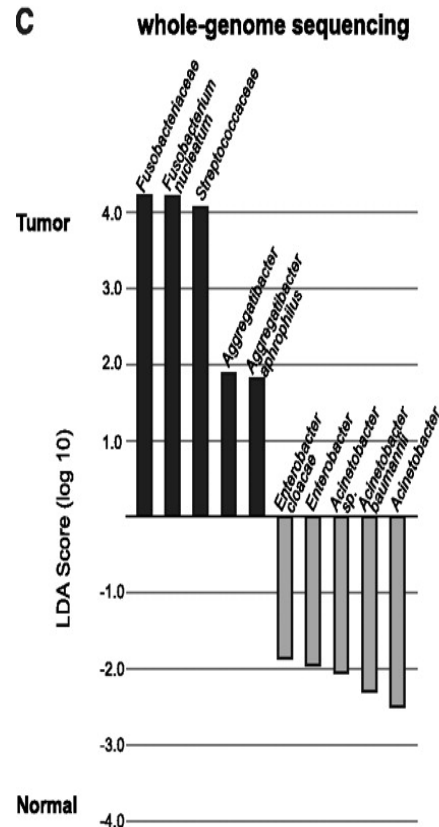
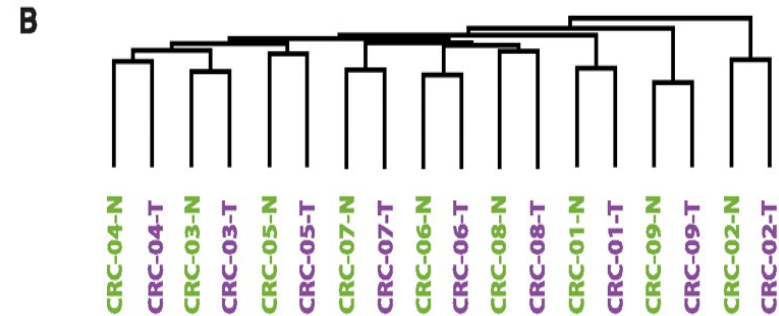
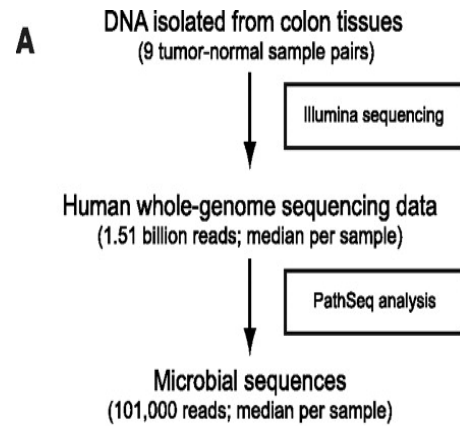


*Fusobacterium nucleatum*



- [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





# Bacteria and Colorectal Cancer

Colon luminal  
environment

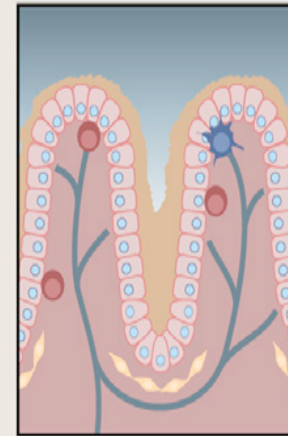
Host mucosal  
environment

Fusobacteria

Model 1  
Single microbes

Model 2  
Microbial community

Model 3  
Single microbes  
interacting with  
microbial  
community



Tumor  
microenvironment

Inflammation

Host genetics

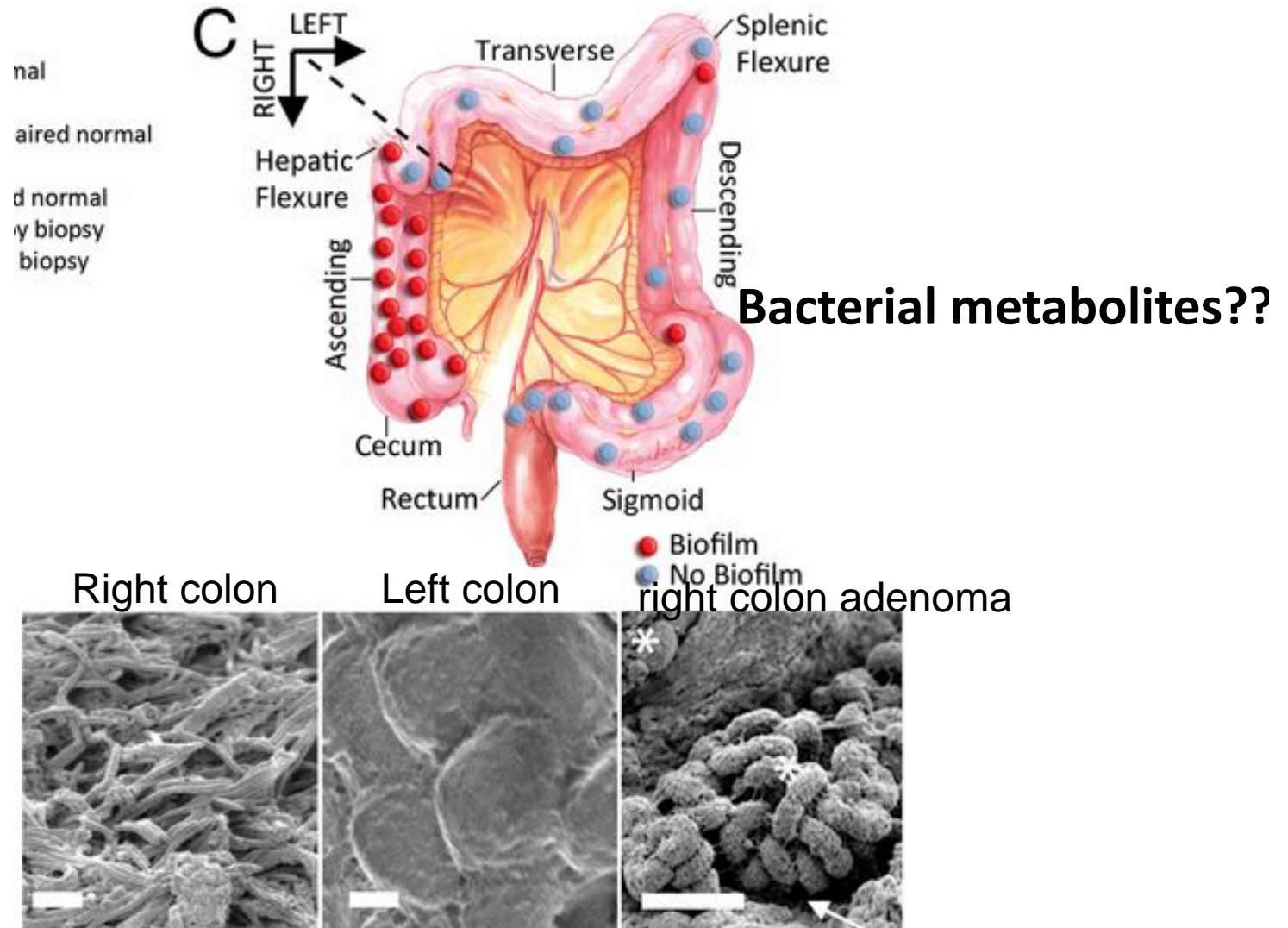


Colorectal Cancer

Cell Host Microbes, 2013  
Cell Host Microbes, 2014

# New Findings

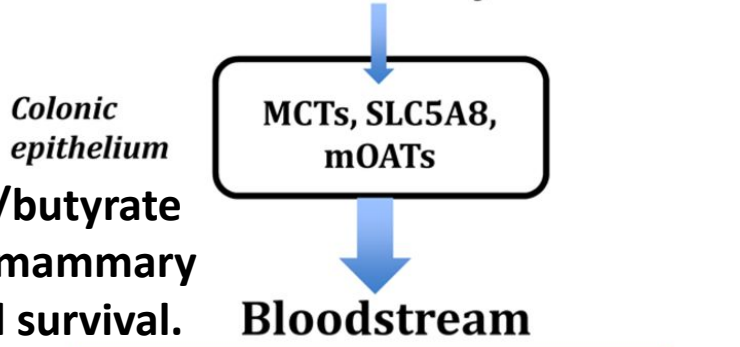
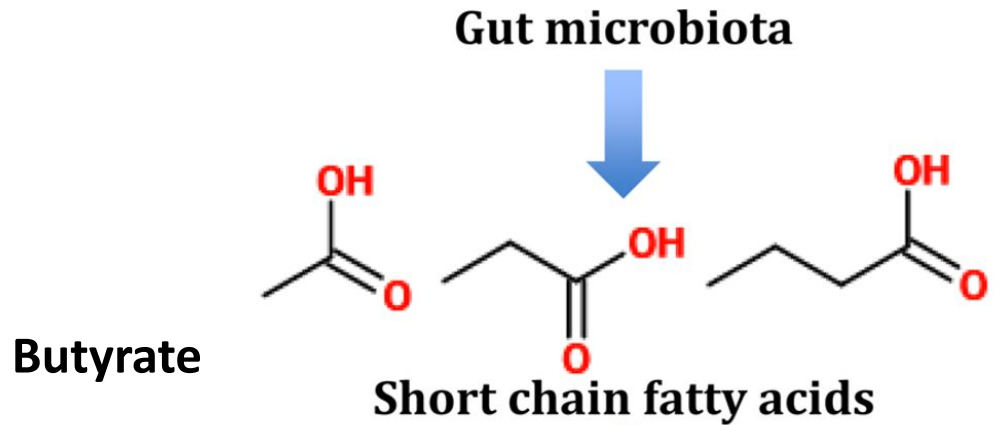
## Bacterial biofilms and cancer connection



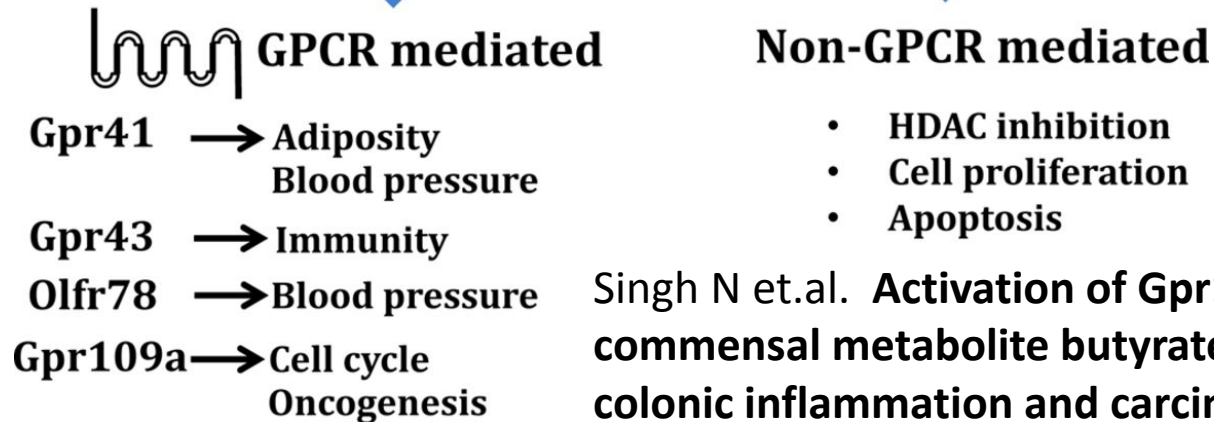
**Bacterial metabolites??**

microbial structural organization contributes to disease progression?  
Dejea, CM, et. al., Proc Natl Acad Sci U S A. 2014,111(51):18321-6.



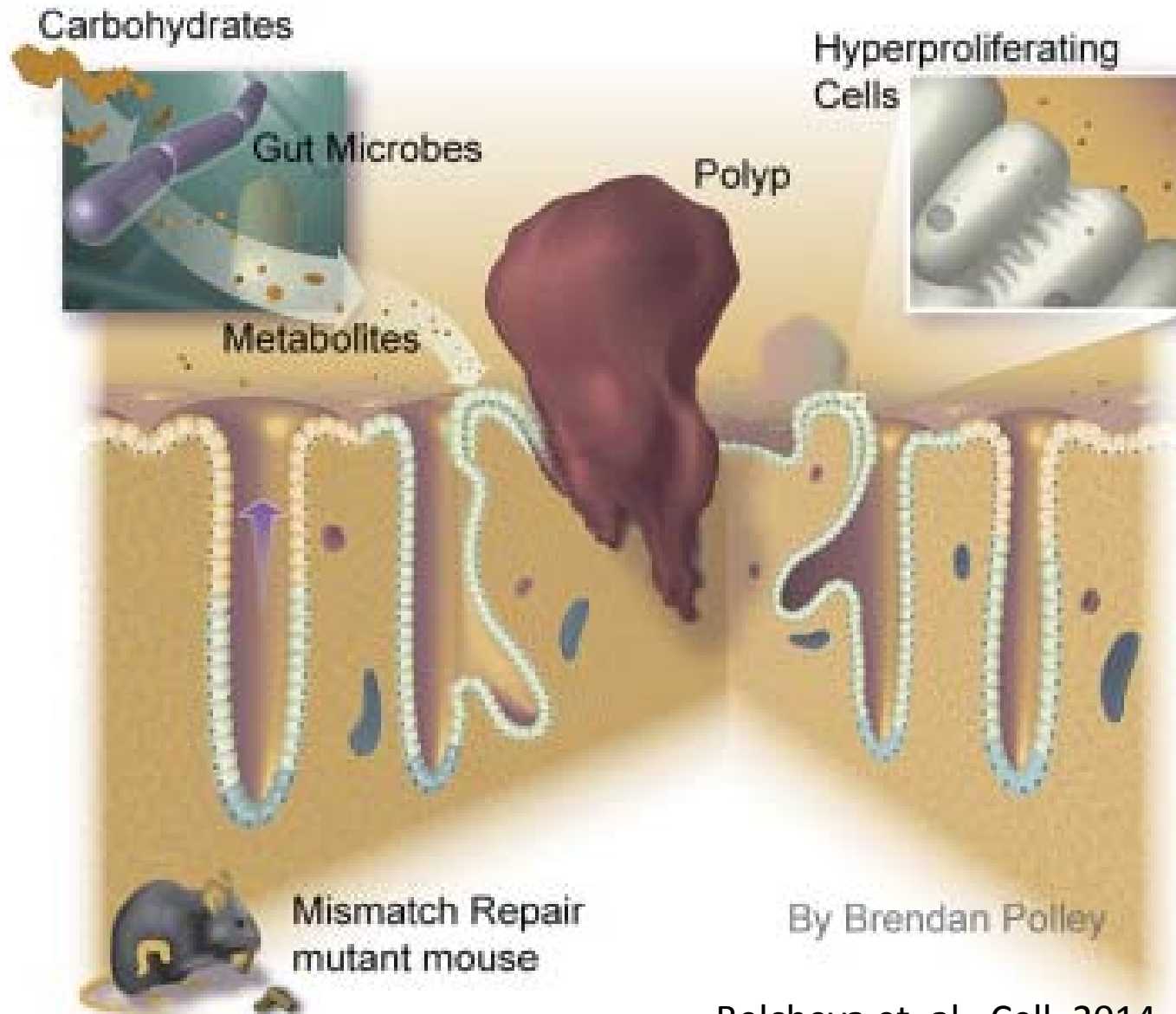


Elangovan S et. al., **The niacin/butyrate receptor GPR109A suppresses mammary tumorigenesis by inhibiting cell survival.** Cancer Res.2014,74:1166-78.

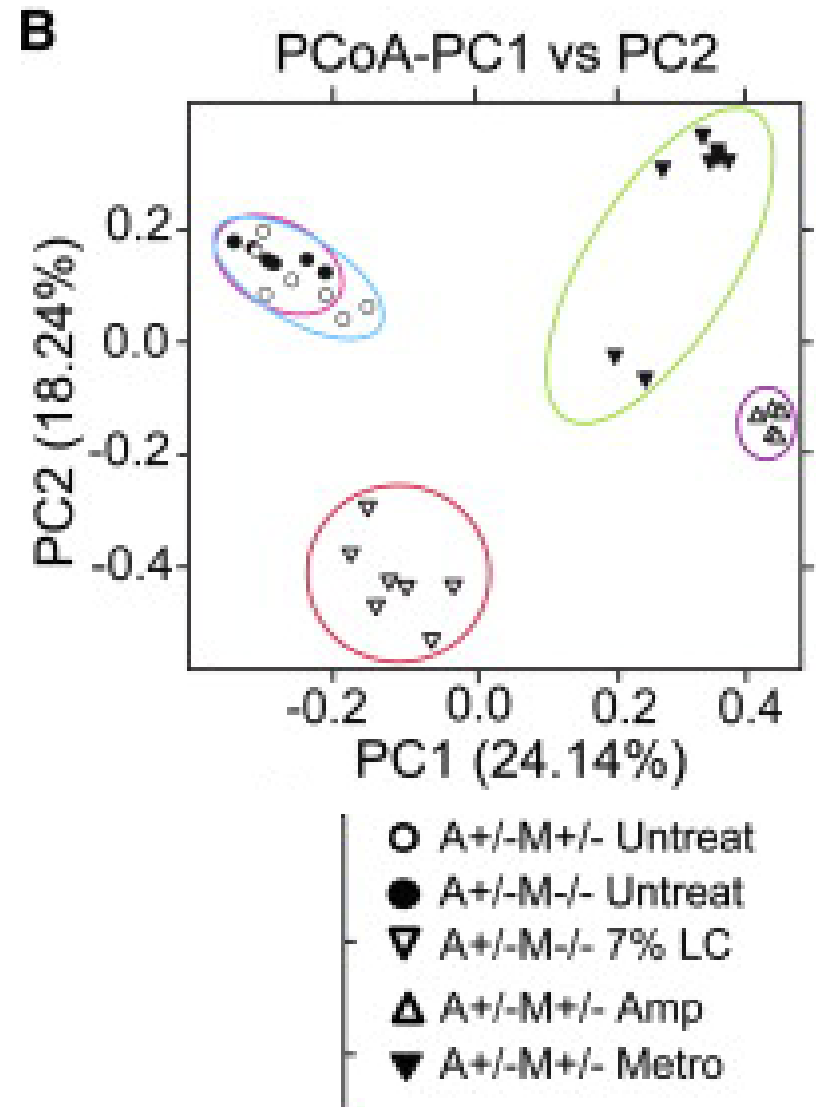
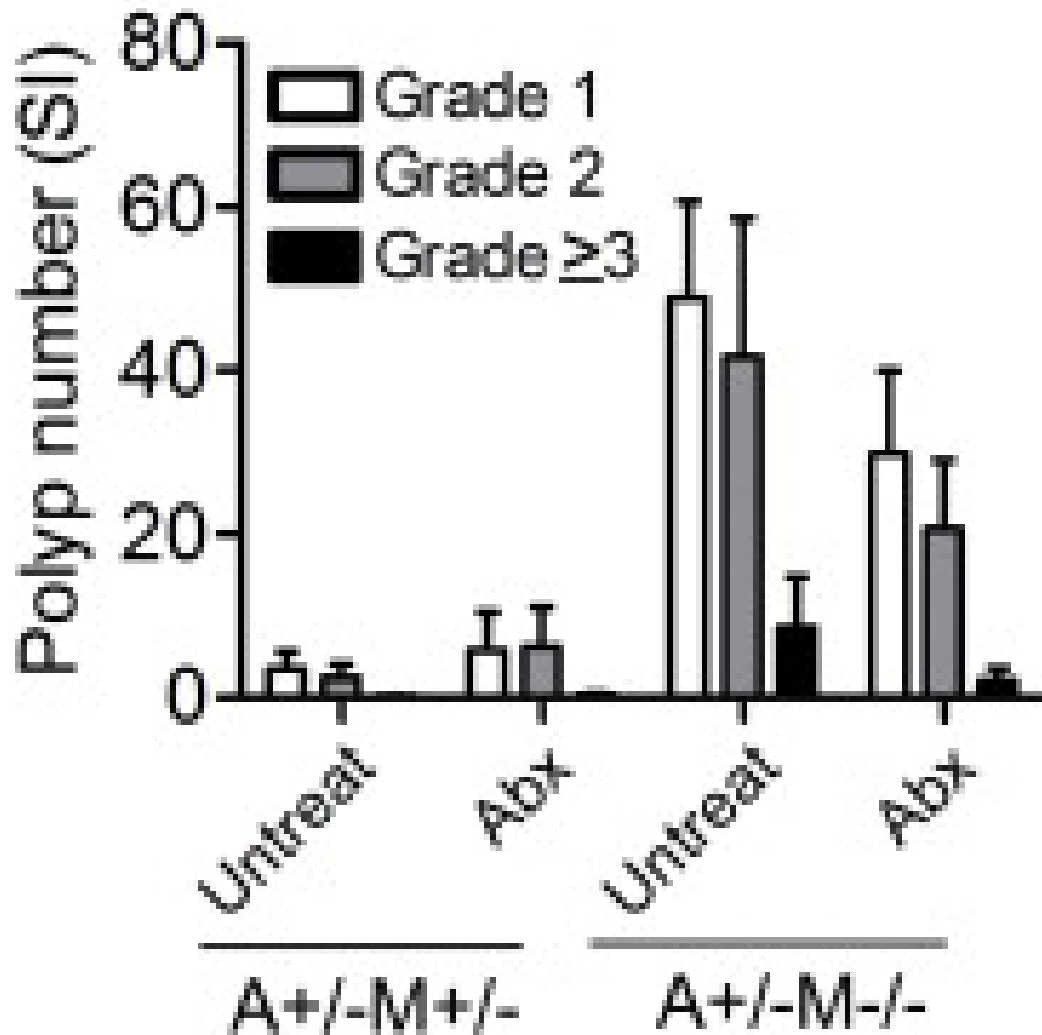


Singh N et.al. **Activation of Gpr109a by the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis.** Immunity. 2014 ,40(1):128-39.

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

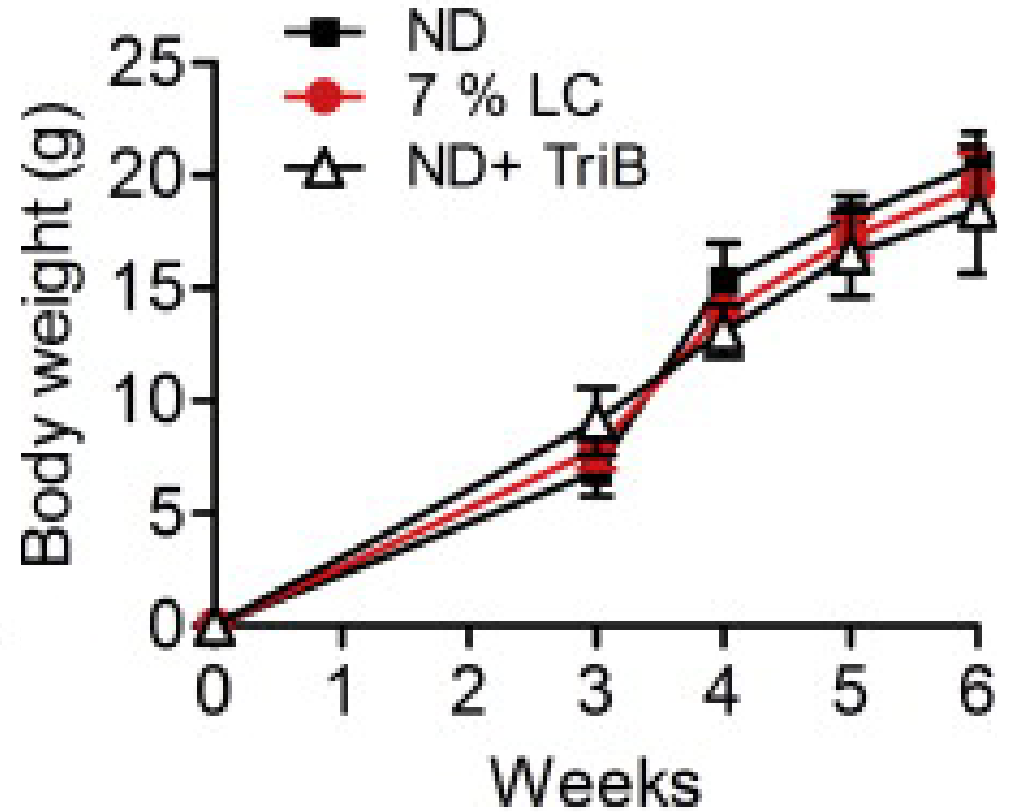
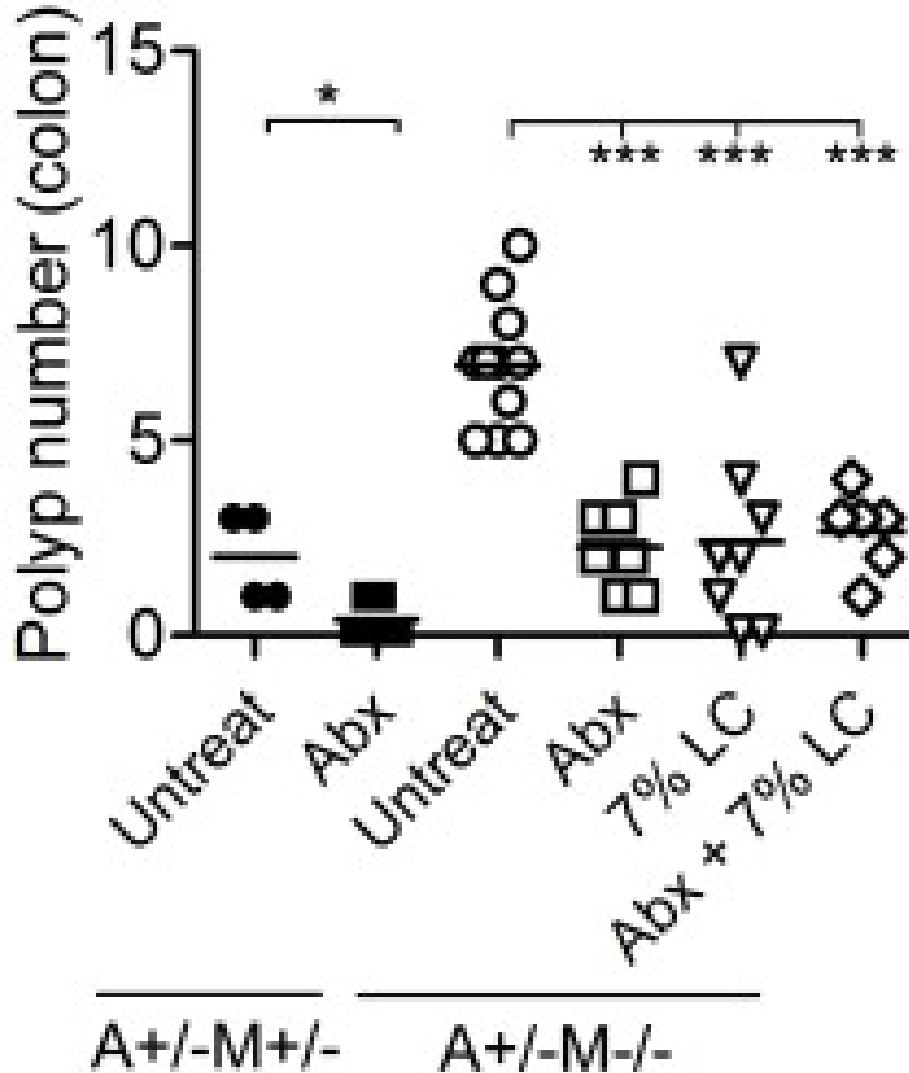


# Gut microbiota induce colon cancer in MSH2-deficient mice



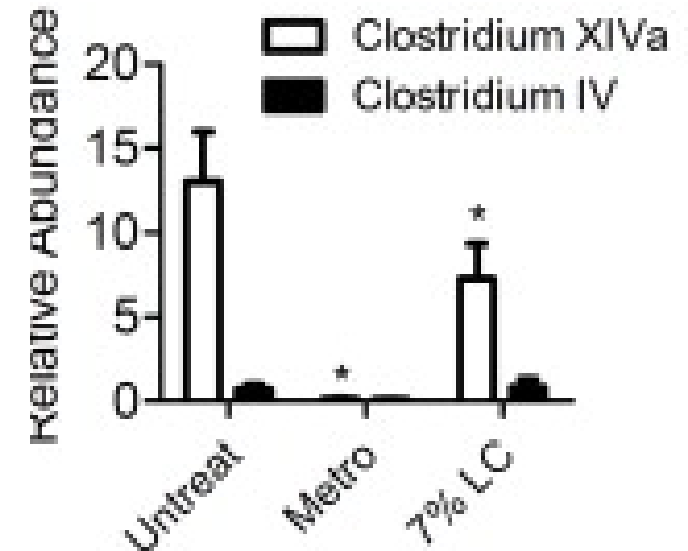
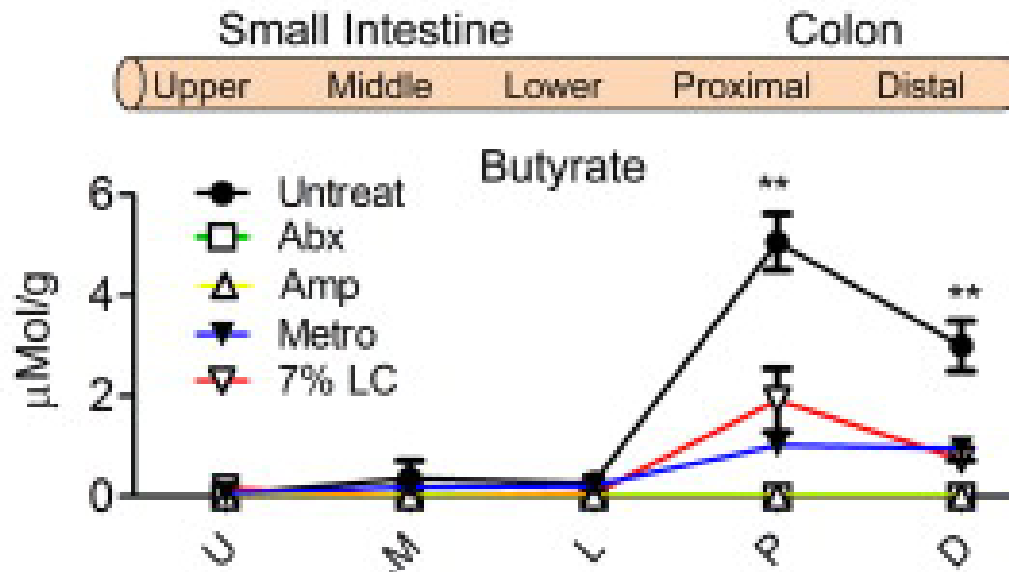
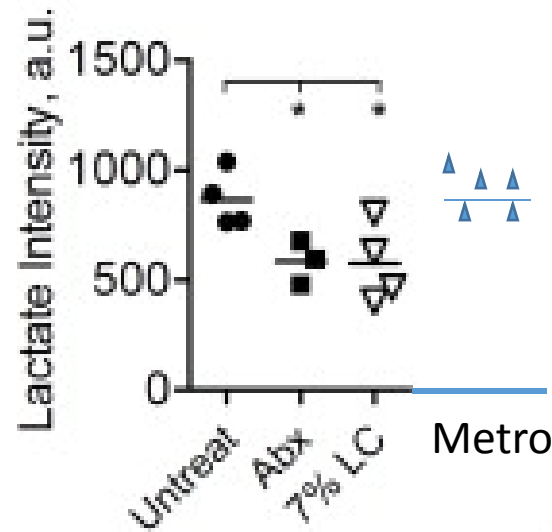
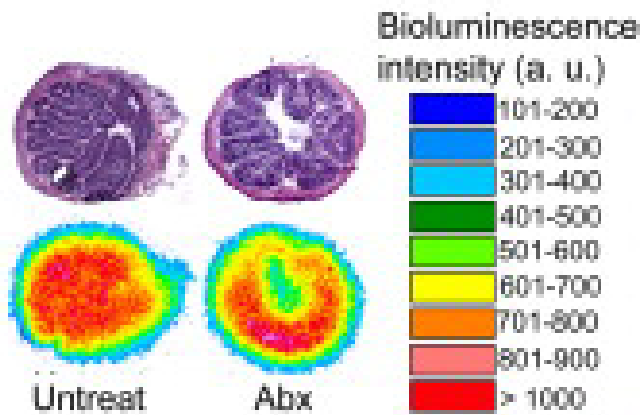
A/Adenomatous polyposis coli (APC); M/MutS homolog2 (MSH2)

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



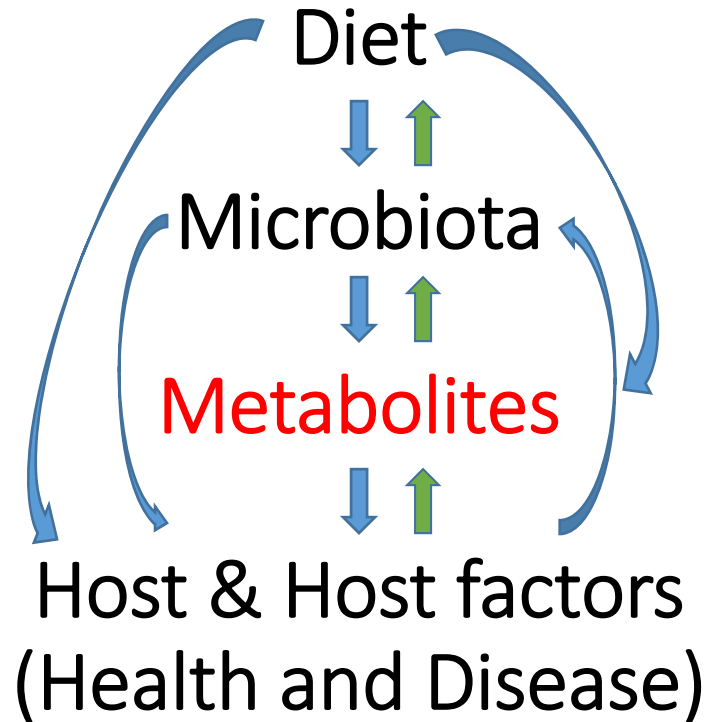
Tribuyrin (TriB)

# Butyrate & colon cancer in APC<sup>Min/+</sup>MSH2<sup>-/-</sup> mice



# Complex Interactions

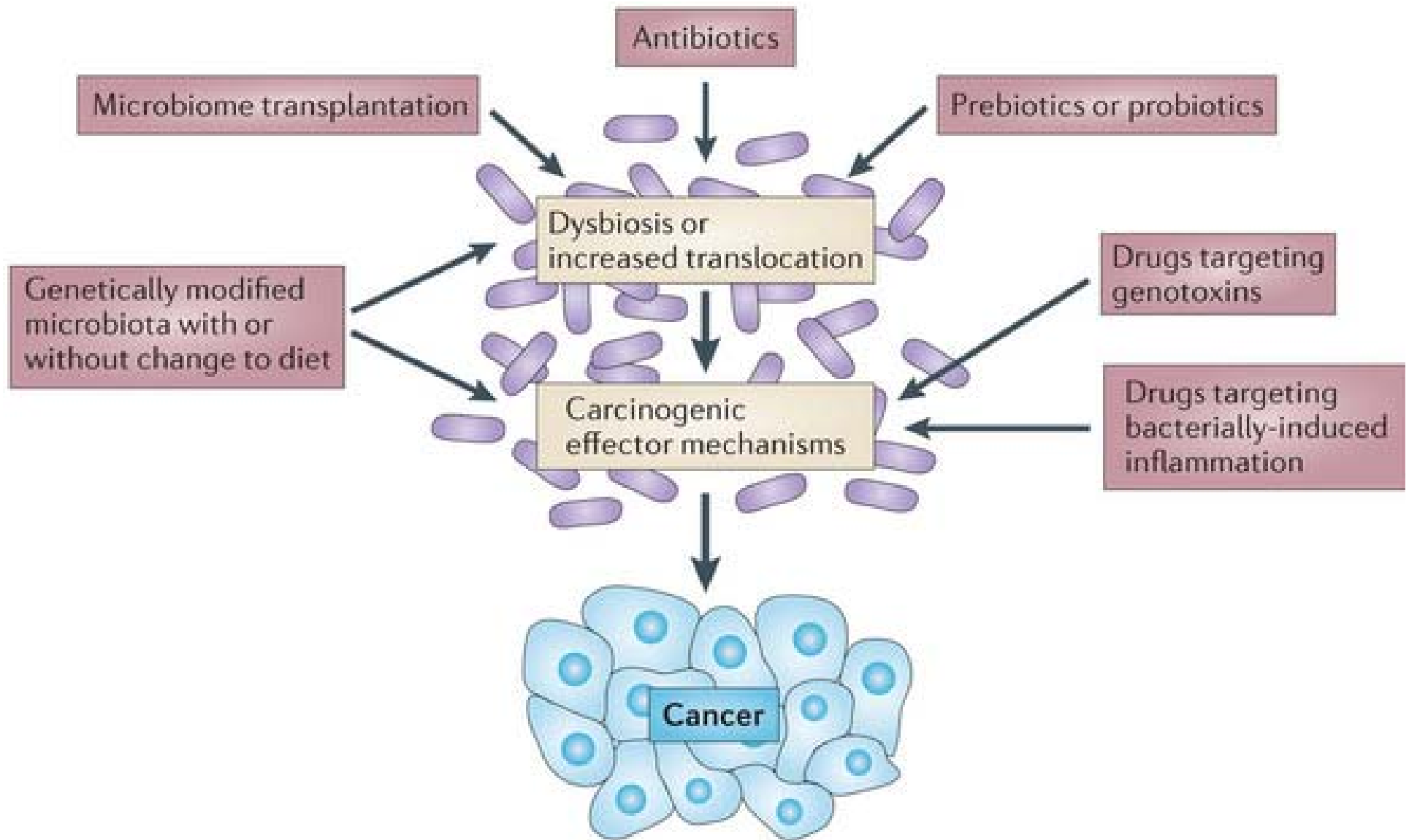
Genetic background matters!



# What's next?

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

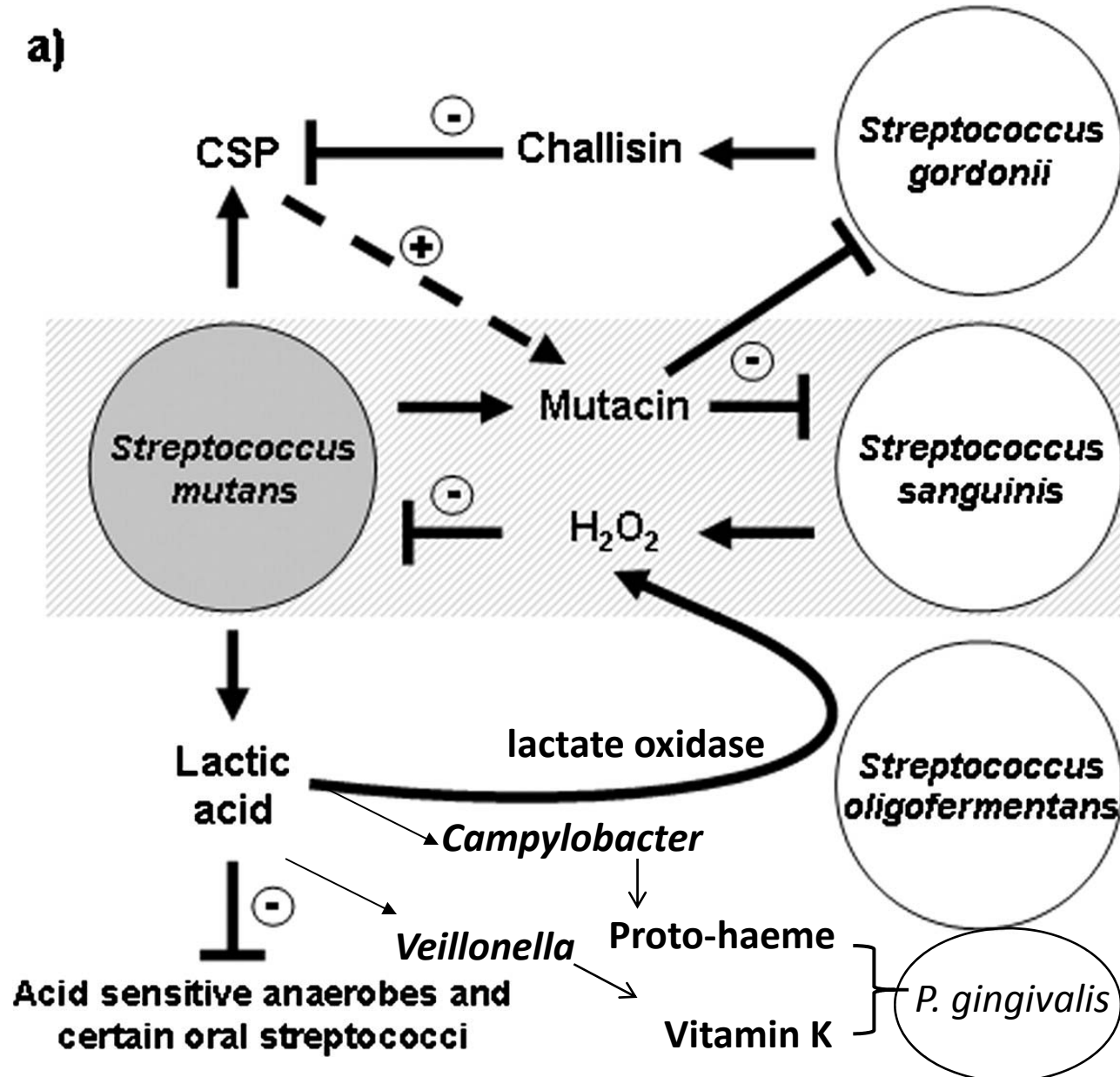
# Microbiome- and metabolites-targeted Therapies





# Metabolites-mediated Interspecies interactions within the oral biofilm community

a)



# Bacteria & Cystic Fibrosis

- ***Human genetic disease with a defect in chloride channel CFTR***
- **Chronic Lung Infections in Cystic Fibrosis Patients**
- ***Pseudomonas aeruginosa***
  - **Pathogen of multiple hosts (humans, plants, fungi, nematodes, and insects)**
  - **Leading gram-negative nosocomial pathogen (burn, wound, UTI, acute respiratory infections)**
  - **Genome contains over 6,000 genes that are involved in virulence, antibiotic resistance, nutrient acquisition, stress tolerance.**





**What is the mechanism that oral streptococci use to improve lung function?**

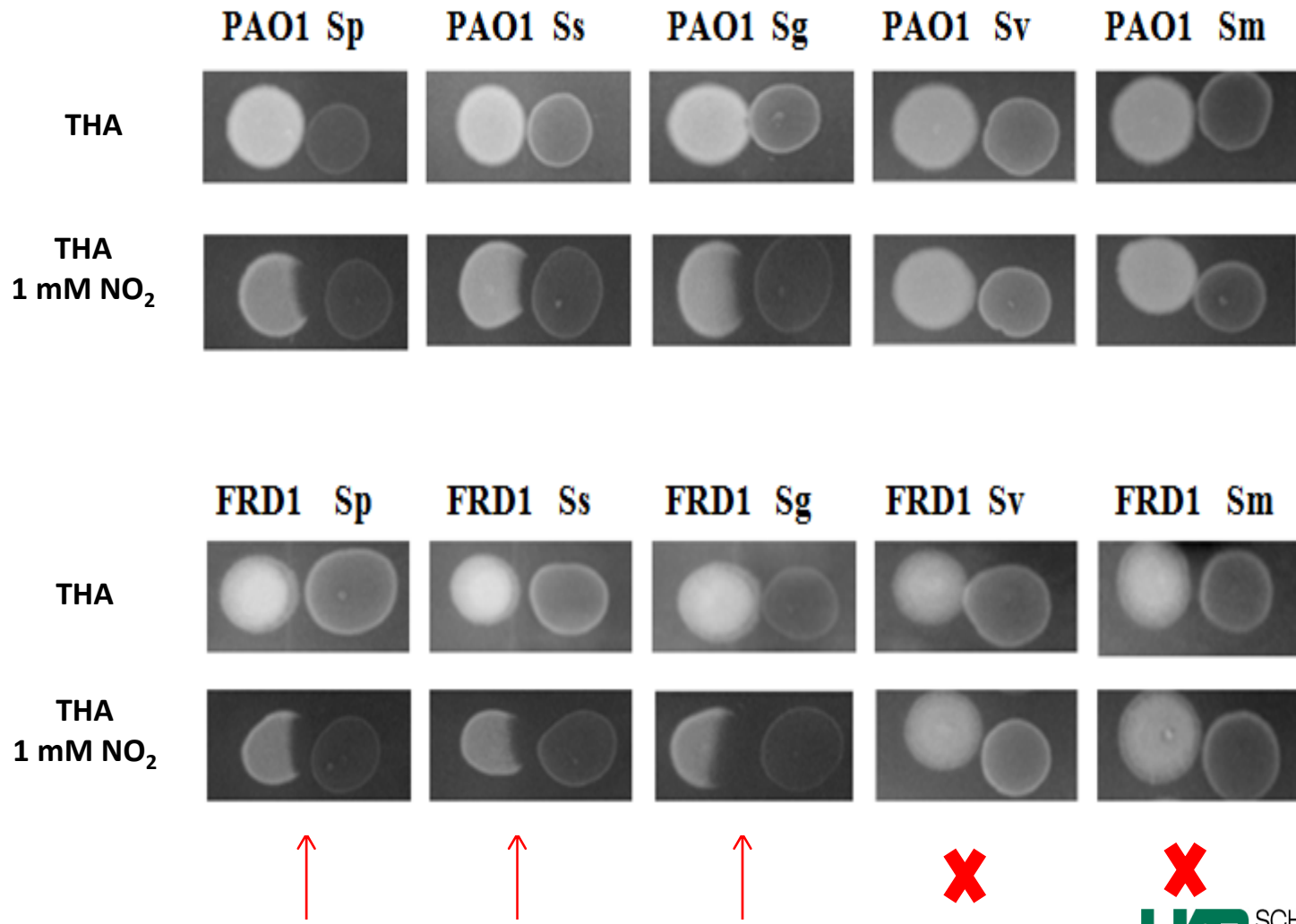
**Do oral streptococci inhibit *P. aeruginosa*?**

# Approach

- Assess commensal and pathogenic oral streptococci for their ability to inhibit *P. aeruginosa*
- **Commensal Streptococci**: *S. parasanguinis* FW213, *S. sanguinis*, *S. gordonii*, and *S. salivarius*
- **Pathogenic Streptococcus**: *S. mutans*
- *P. aeruginosa*: PAO1 (acute) and FRD1 (chronic CF strain)

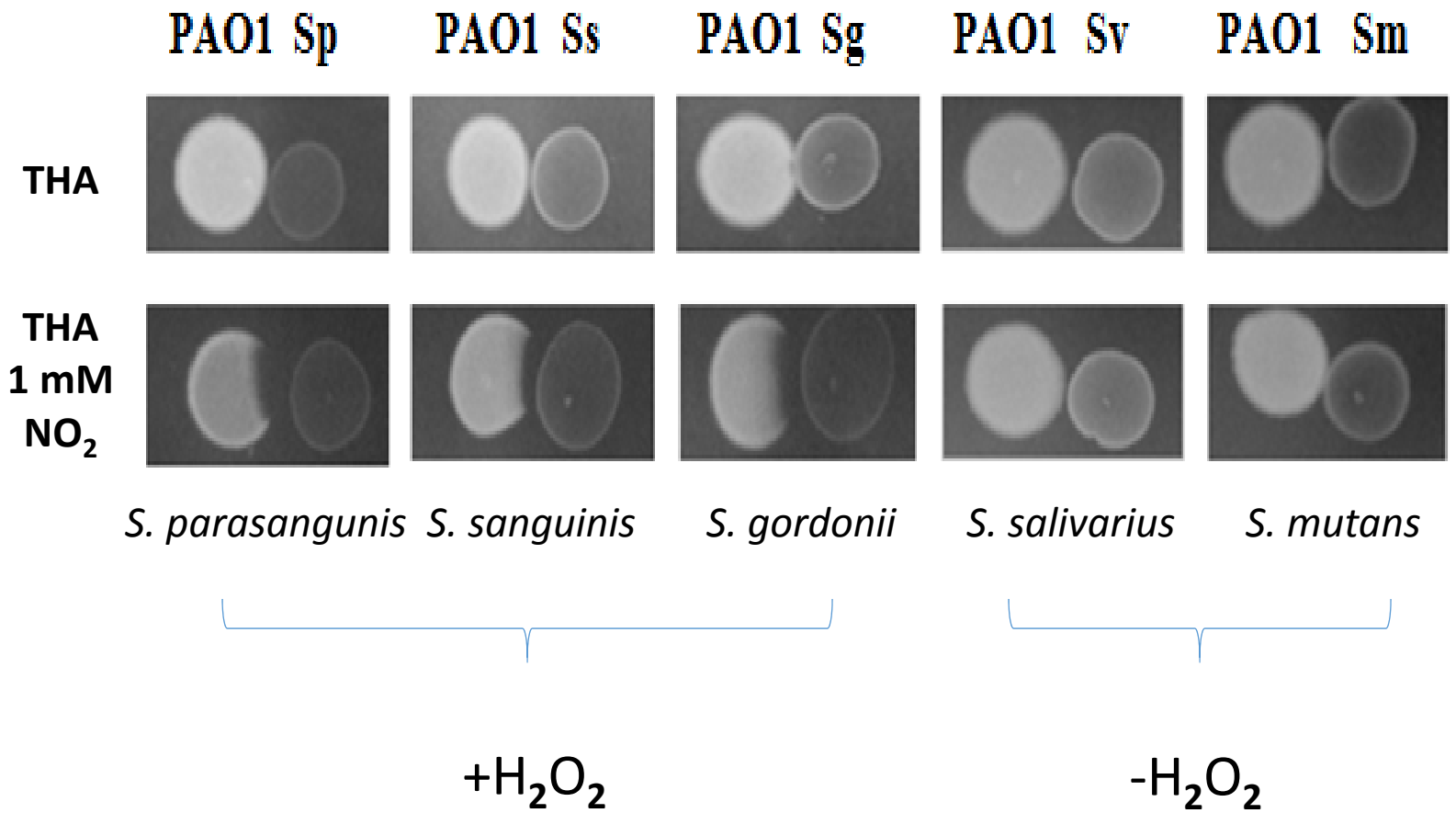


# Streptococci Inhibit *P. aeruginosa*

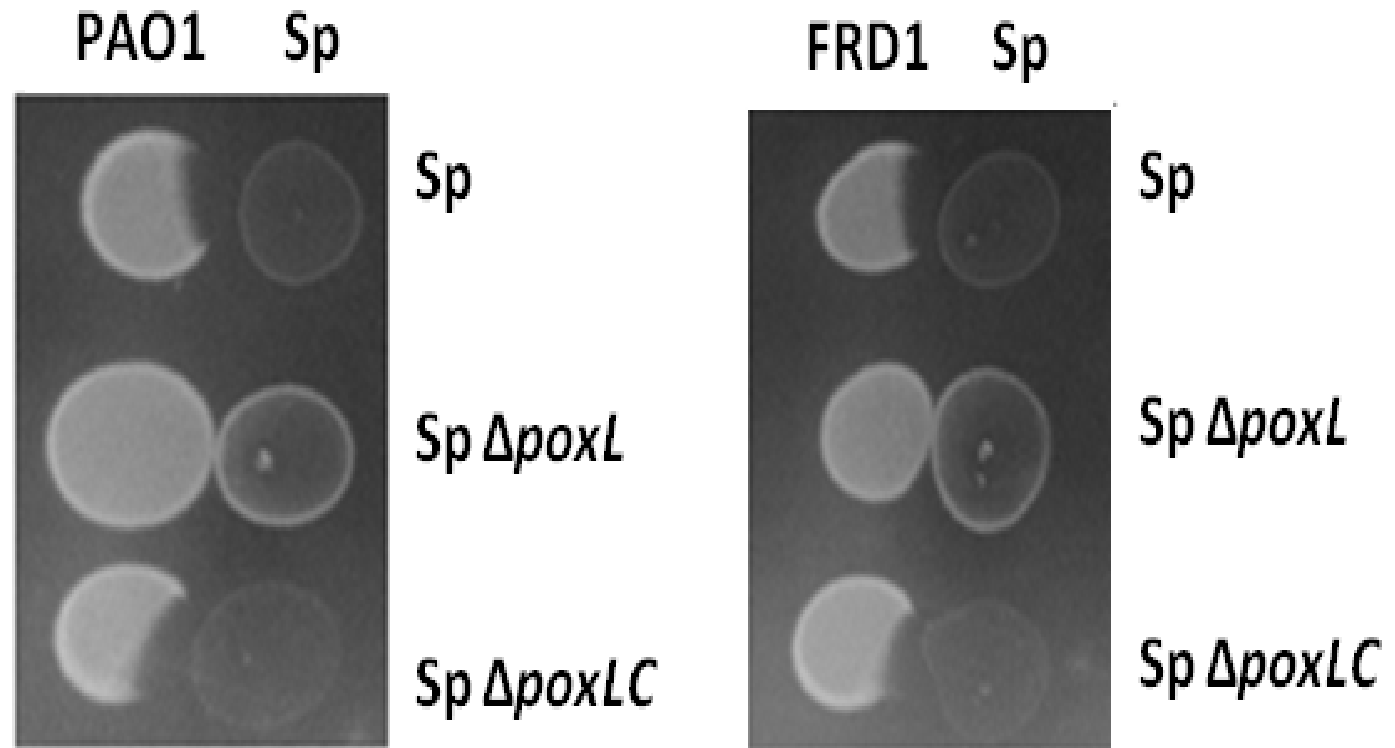




# What's in common among inhibitory streptococci ?

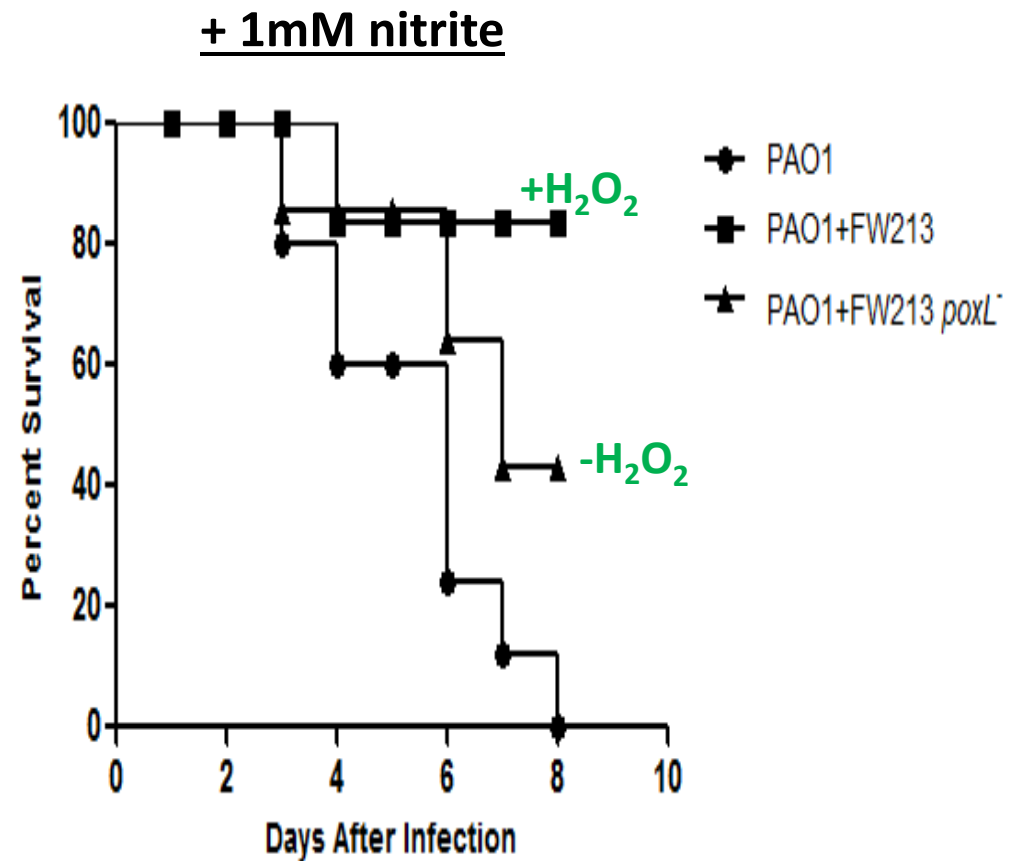
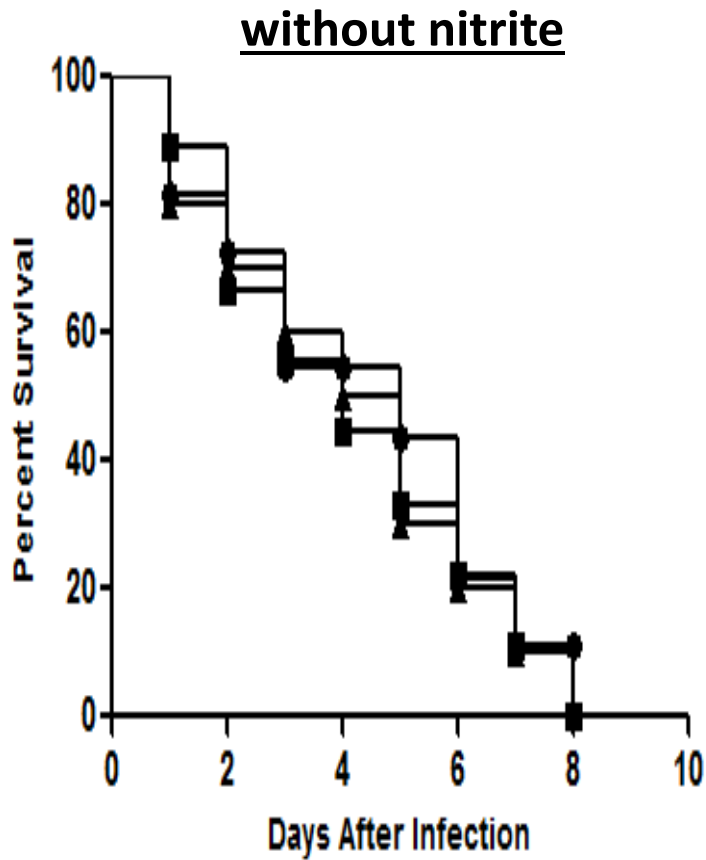


# Hydrogen Peroxide is Required for Killing

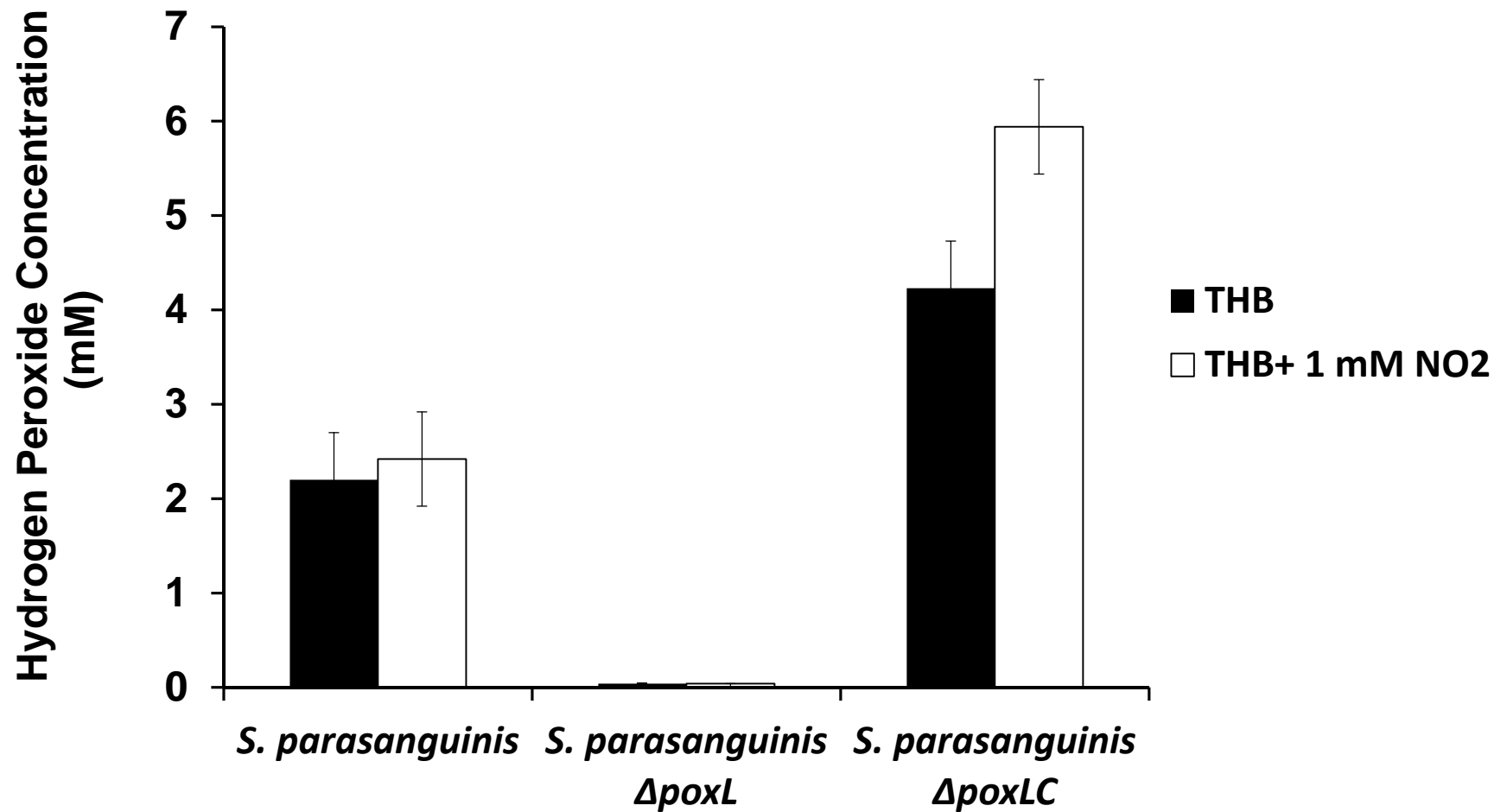


POXL Pyruvate oxidase responsible for production of H<sub>2</sub>O<sub>2</sub>

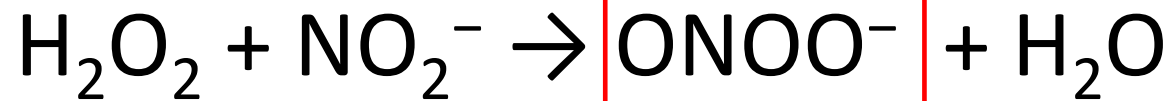
# *S. paransanguinis* Protects Flies from Killing by *P. aeruginosa*



# Nitrite does not Enhance Production of H<sub>2</sub>O<sub>2</sub>

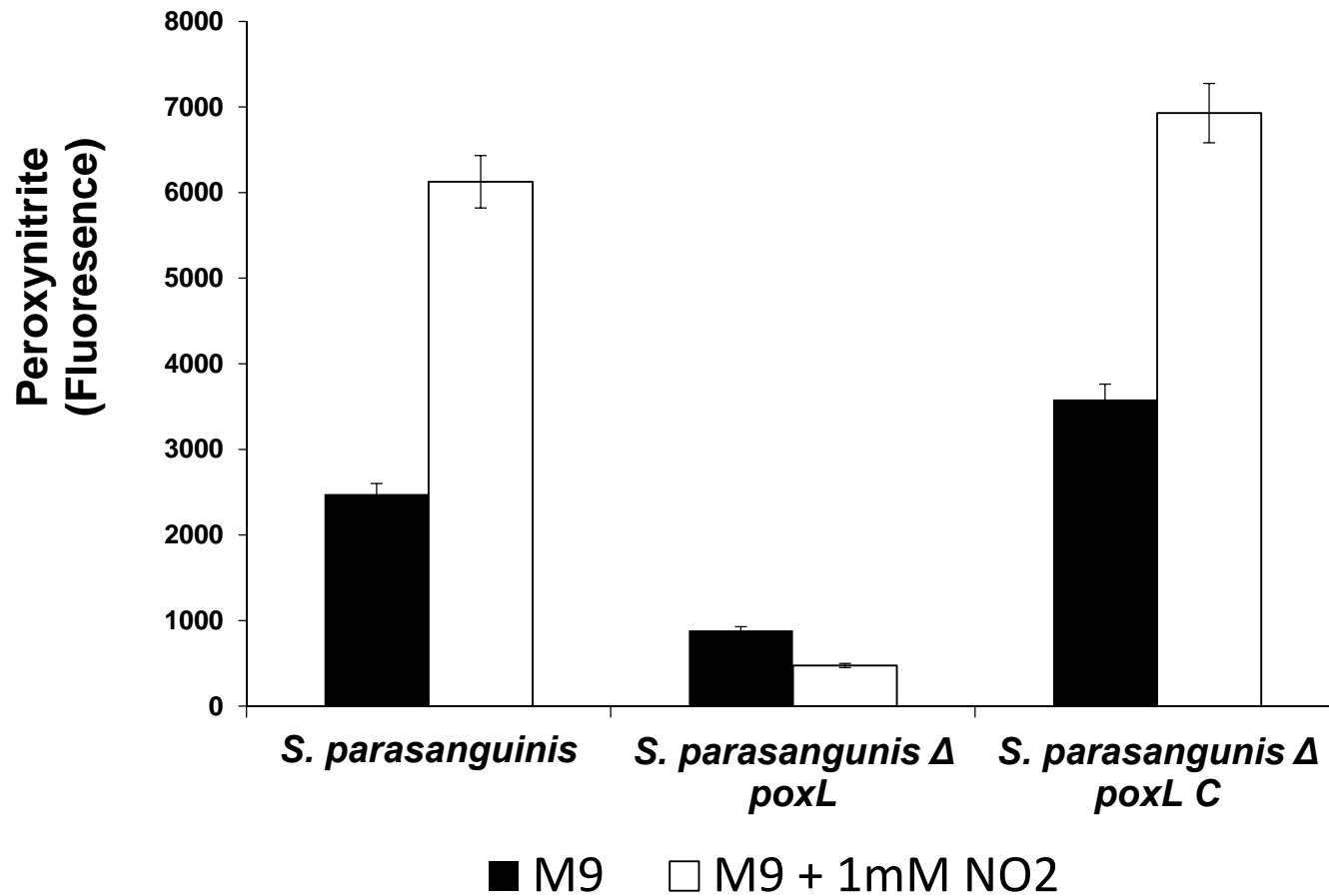


## Mechanism of Inhibition



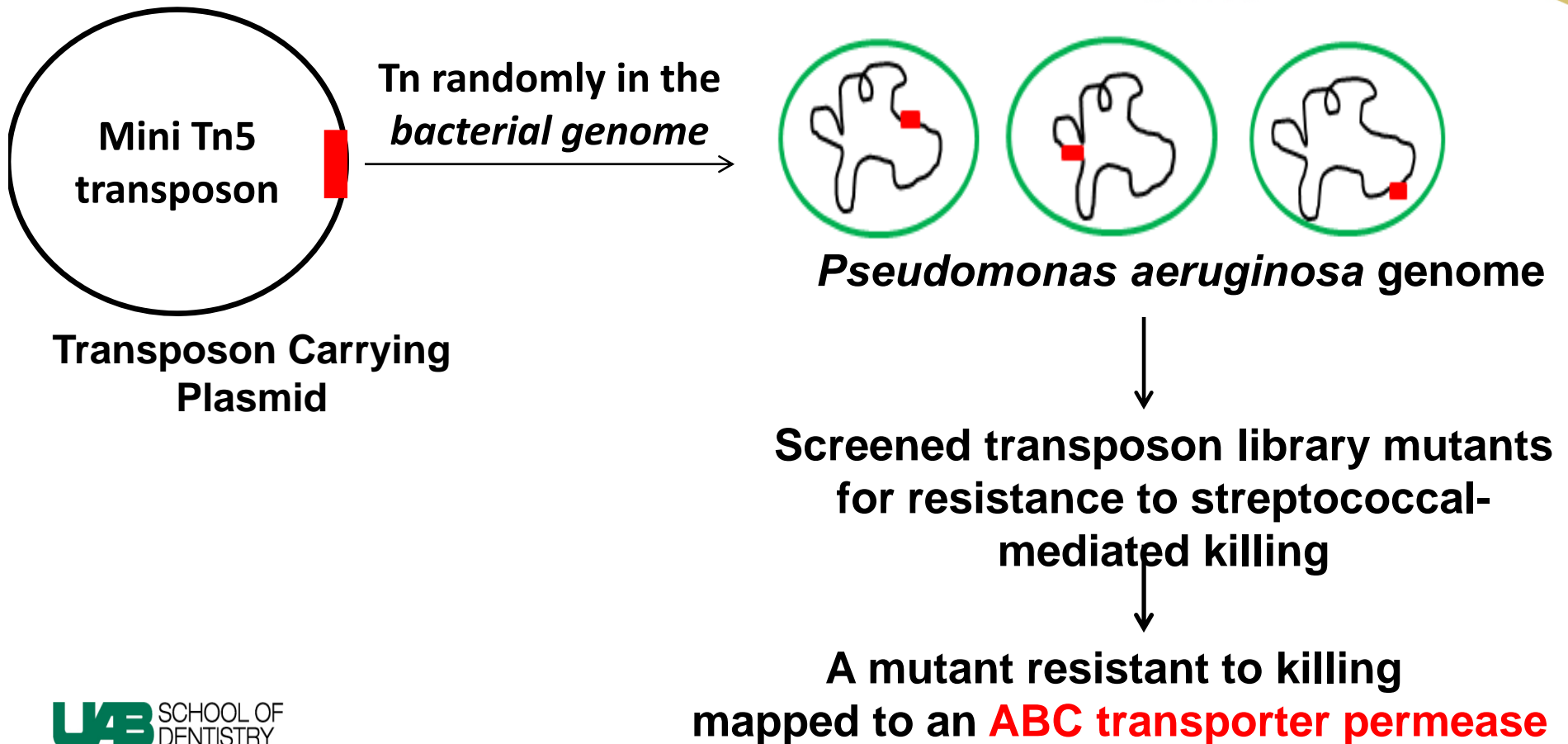
$\text{H}_2\text{O}_2$  and  $\text{NO}_2^-$  are precursors for the production of **peroxynitrite**

# Nitrite Promotes the Generation of Peroxynitrite

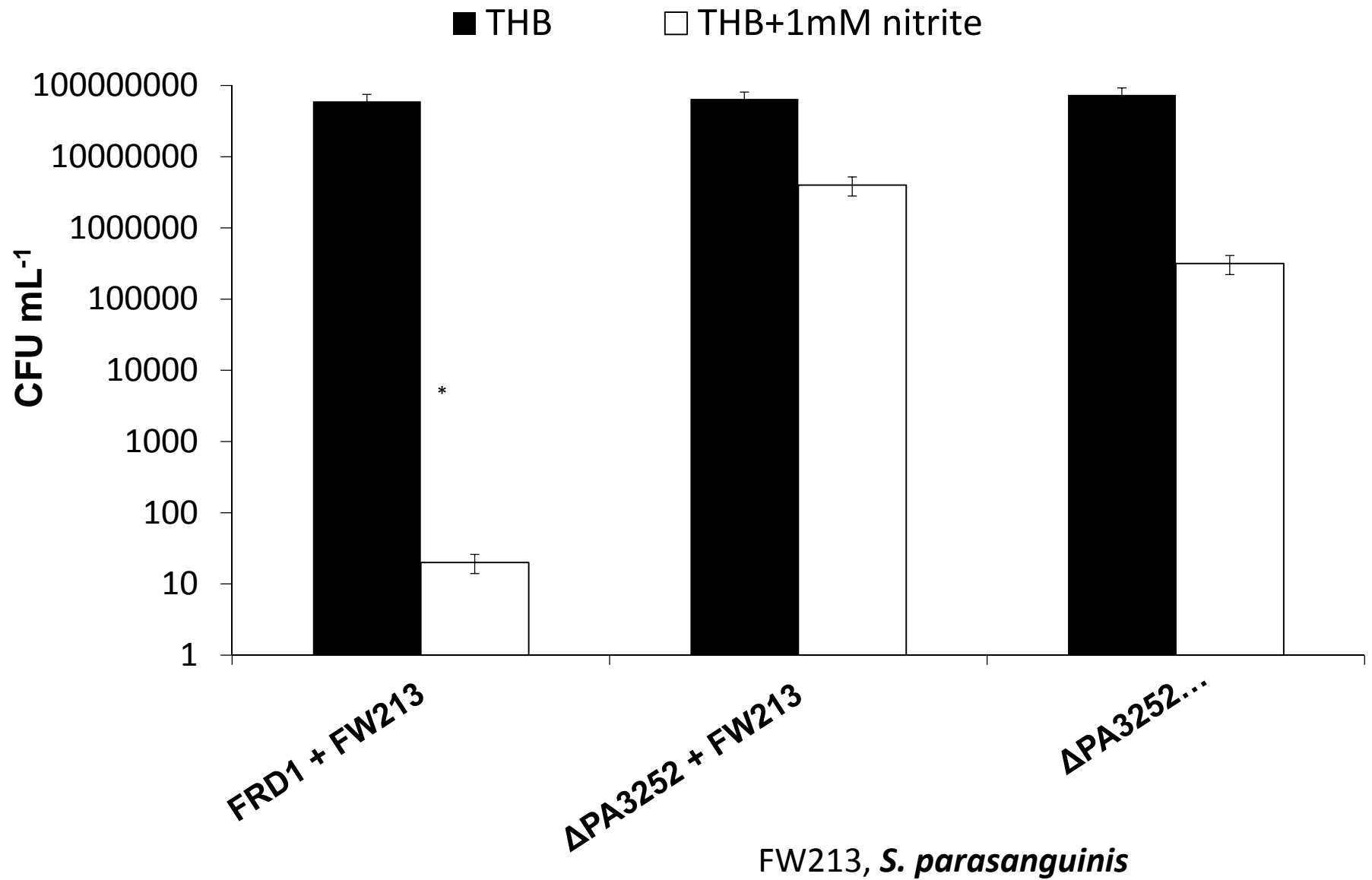




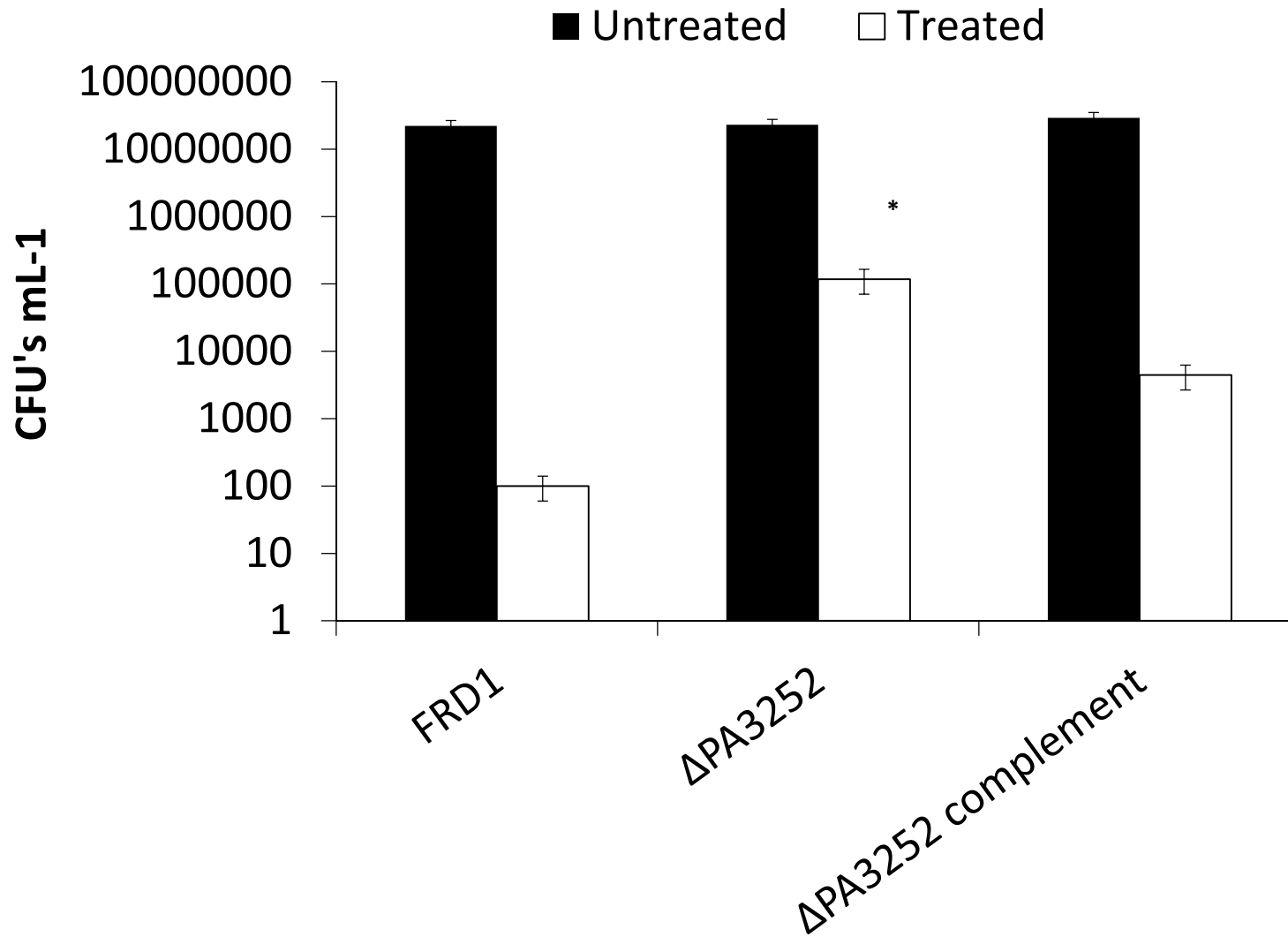
# Resistance to Streptococcus-mediated Killing



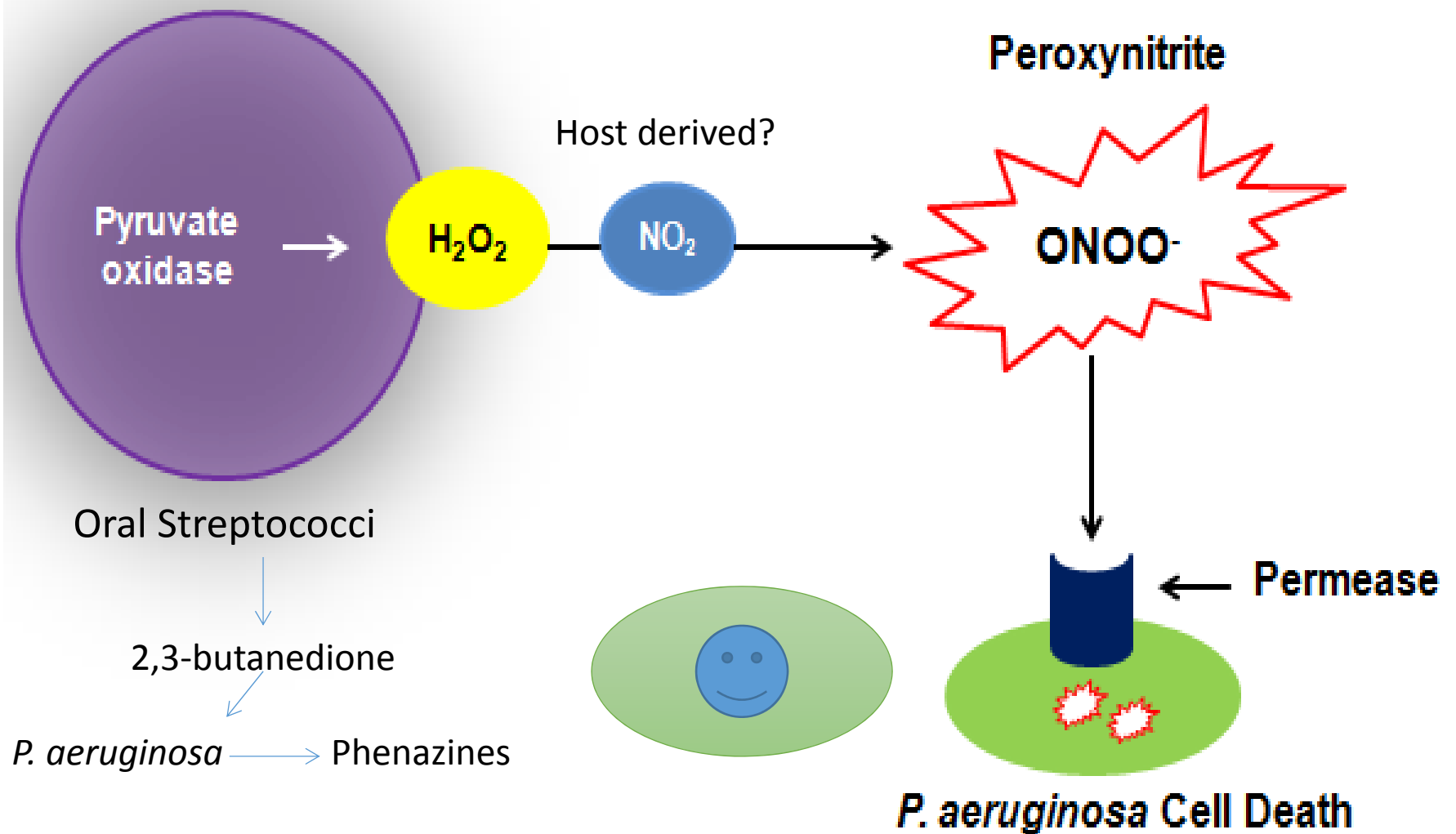
# An ABC Transporter Deficiency Confers *P. aeruginosa* Resistance to killing



# The ABC Transporter Mutant also Possesses Peroxynitrite Resistance



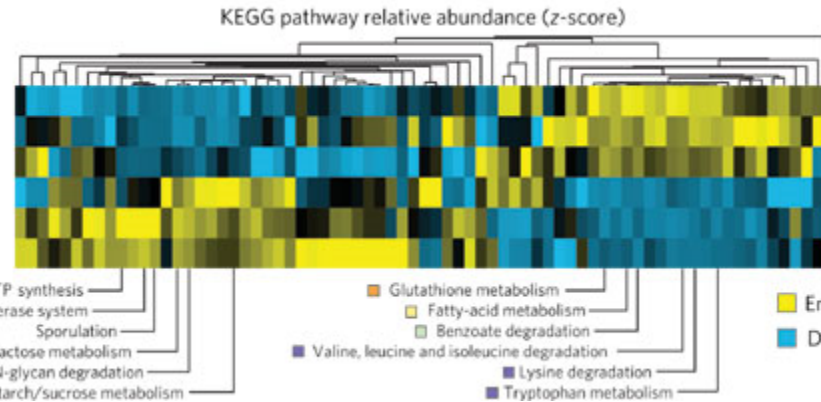
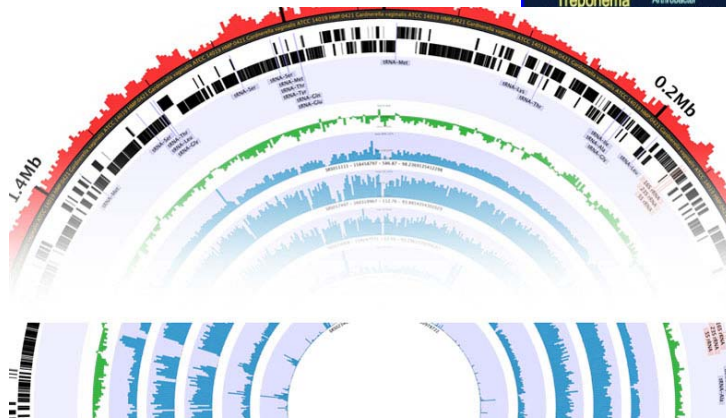
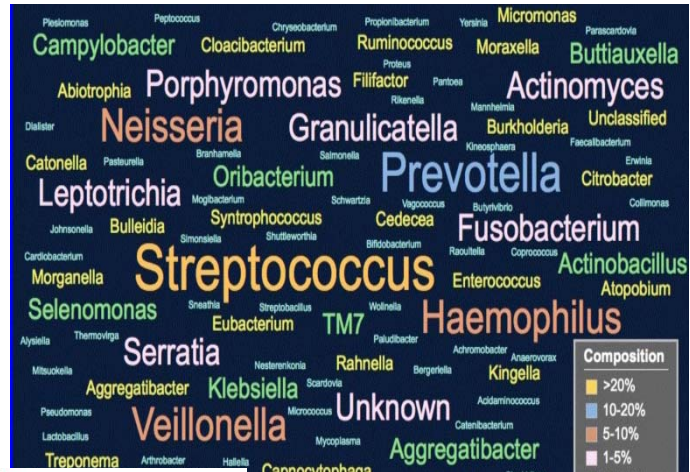
# Working Model of *S. parasanguinis* mediated killing and more



Sharon et. al. , Cell Metabolism, 2014, Nov.4

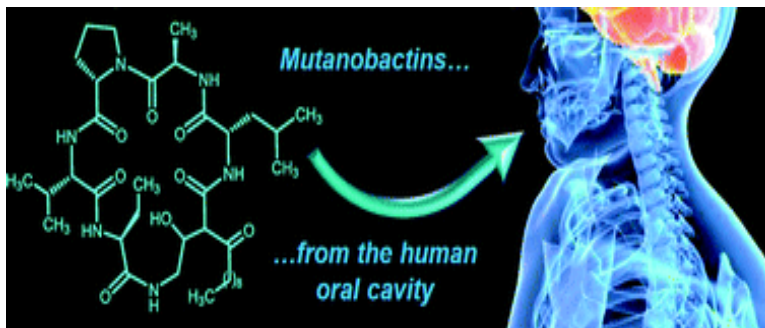
Jessica and Wu, Infect Immun, 2015, Jan.

# Microbiome & metabolites.....



Transcriptomics  
Proteomics  
Metabolomics  
Glycomics

Yellow Enrichment  
Blue Depletion



# Microbes rule the world



Cocoa powder (polyphenols and Fibers)

Smaller molecules  
short fatty chain acids

2014 American Chemical Society meeting





**Thank you!**