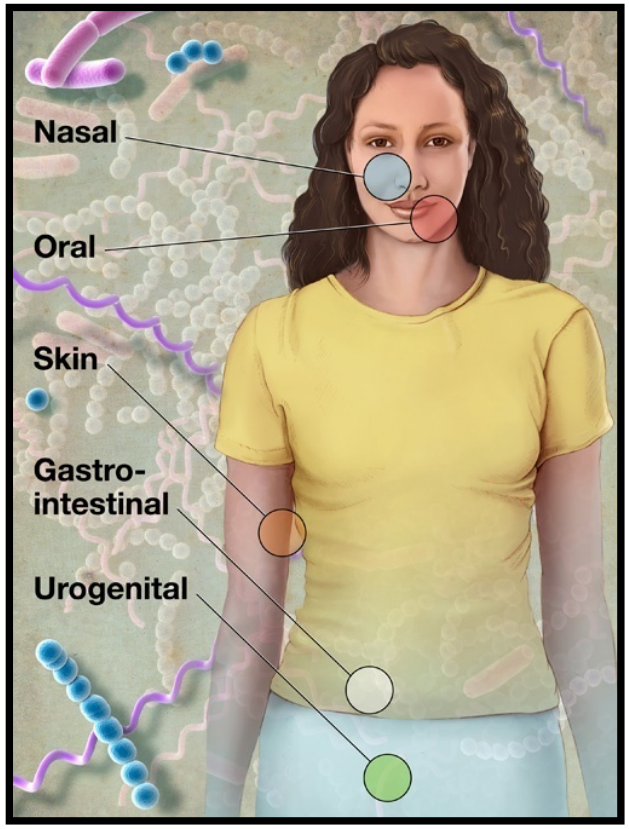
ASSISTENZA AL DIABETE IN LIGURIA:
INNOVAZIONE E SOSTENIBILITÀ

GENOVA
16/17 giugno
2017

Microbiota

The Human Bacteria

Human Microbiota



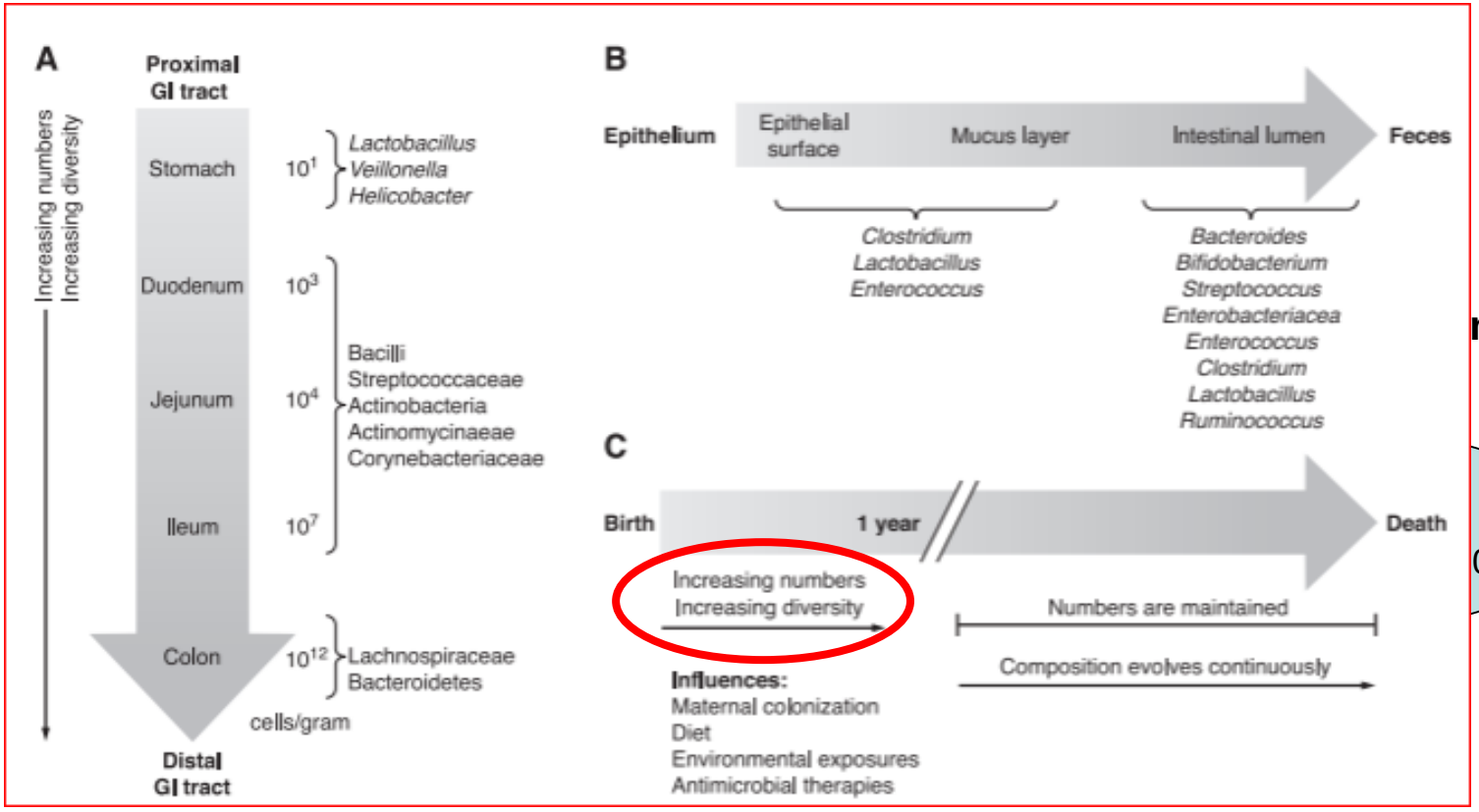
- **100 trillion microorganisms (10^{14})**
- **They colonize our gut, nasal, oral, airways, skin and urogenital system.**

  - **Bacteria**
  - **Fungi**
  - **Protists**
  - **Viruses?**

- **The great percentage of these are commensal and they are symbionts**
- **Only a small fraction are Pathogens**

Human microbiota

Your body has 10 - times more microbes cells than human cells

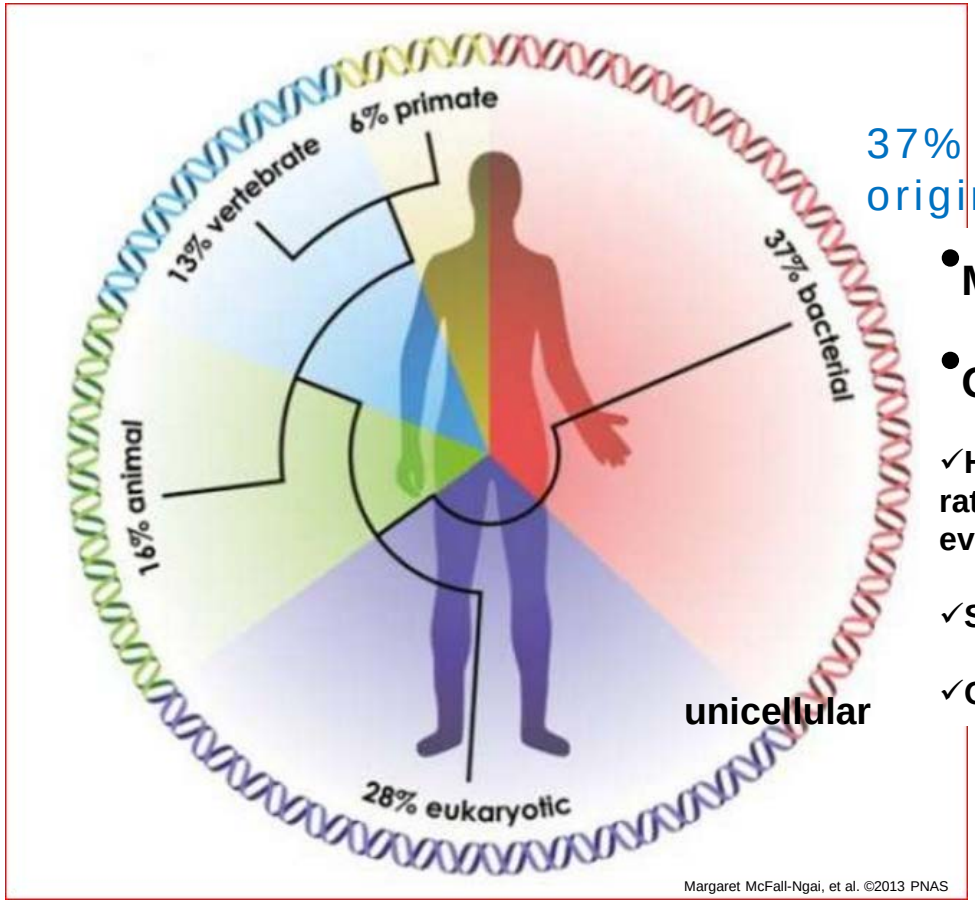


99% of the microbial mass of Human microbiota is within the gastrointestinal tract

We are living in a bacterial world

it's impacting us more than previously thought

Phylogenetic relationships indicate a deep evolutionary history shared by all living organisms



37% of human genes originated in bacteria.

- **Mostly derived by descent**
- **Others by gene transfer**

✓**Human-associated bacteria have a 25x higher rate of gene transfer compared to the other evironment**

✓**Some have genome reduction**

✓**Others have genome expansion (metabolic genes)**

Metagenomics

- Genomics, proteomics,Transcriptomics, Interactomics
  - **All focus on the whole of a single organism**
  - **However, organisms live in communities and interact**

- Metagenomics (study of metagenomes)
  - **genetic material sampled directly from the living communities present in the environments**
    - **(as opposed to lab cultured)**

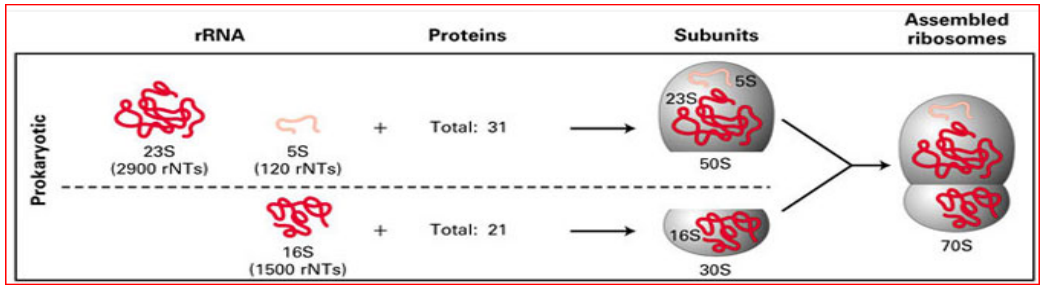
90% of gut microbial species are not culturable

Microbiota *vs* Microbioma

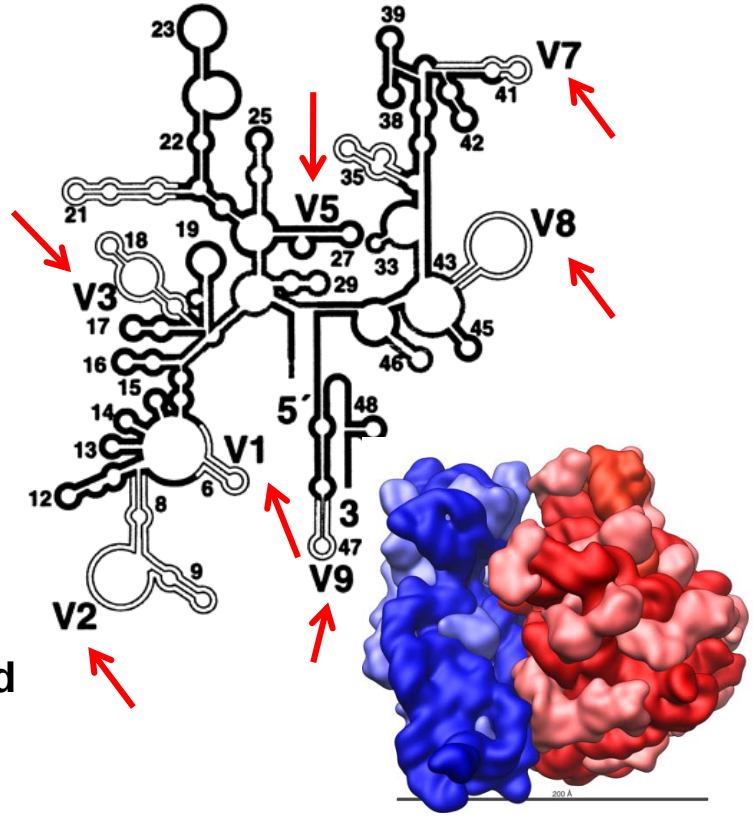
- Microbiota:
  - The microbes that inhabit a given ecosystem
- Microbioma:
  - The genomes of all microorganisms in the ecosystem

16S amplicon sequencing is a rapid method to assess microbial diversity

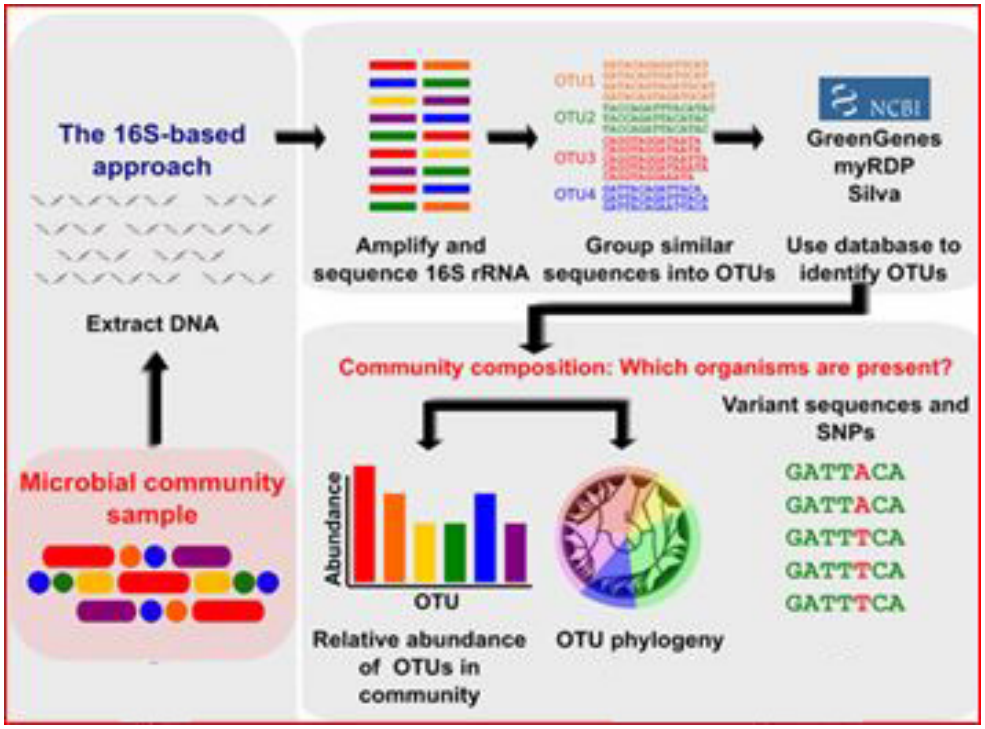
- **Structural component of 30S small subunit of prokaryotic ribosome**
- **Used as molecular clock to identify phylogeny**



- **Contains 9 variable regions flanked by conserved regions**
- **Well established marker for identifying and classifying microbes**



16S ribosomal RNA gene metagenomics



Used to define Bacterial taxonomy

- A rank-based classification of the bacteria

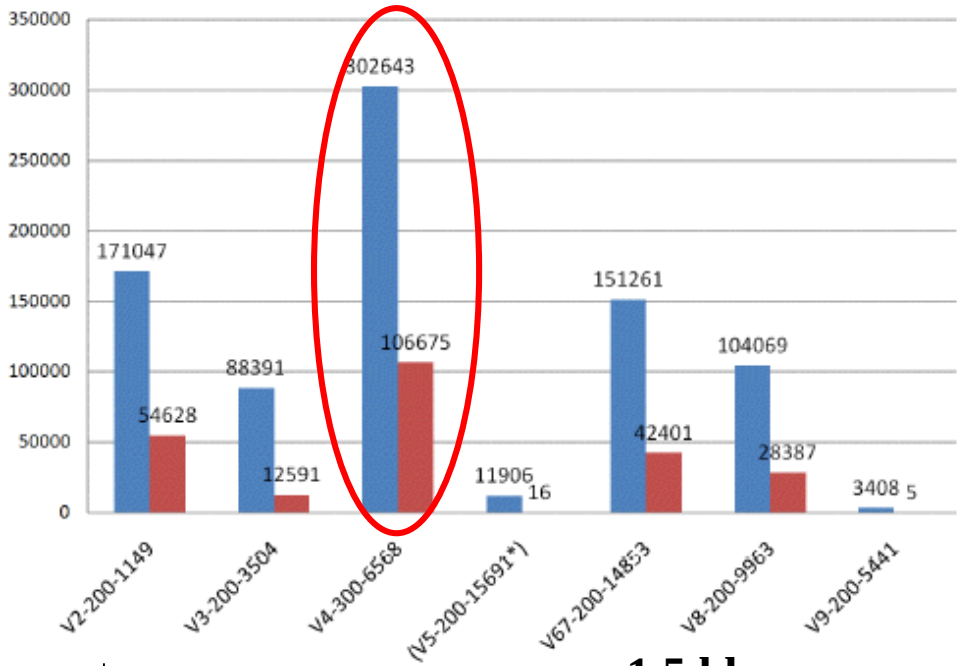
Example of *Akkermansias* taxonomic tree:

Phylum	Class	Order	Family	Genus	Species
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	*Akkermansia*	*A. muciniphila*

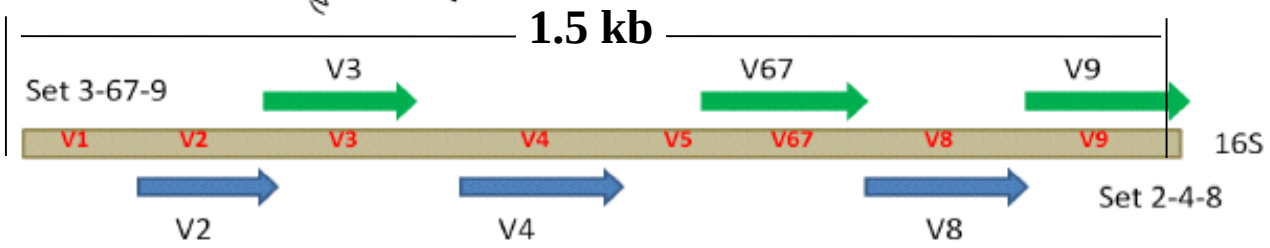
Morgan XC, Huttenhower C (2012) Chapter 12: Human Microbiome Analysis. PLoS Comput Biol 8(12): e1002808. doi:10.1371/journal.pcbi.1002808
http://journals.plos.org/ploscompbiol/article?id=info:doi/10.1371/journal.pcbi.1002808

16S Microbiome analysis

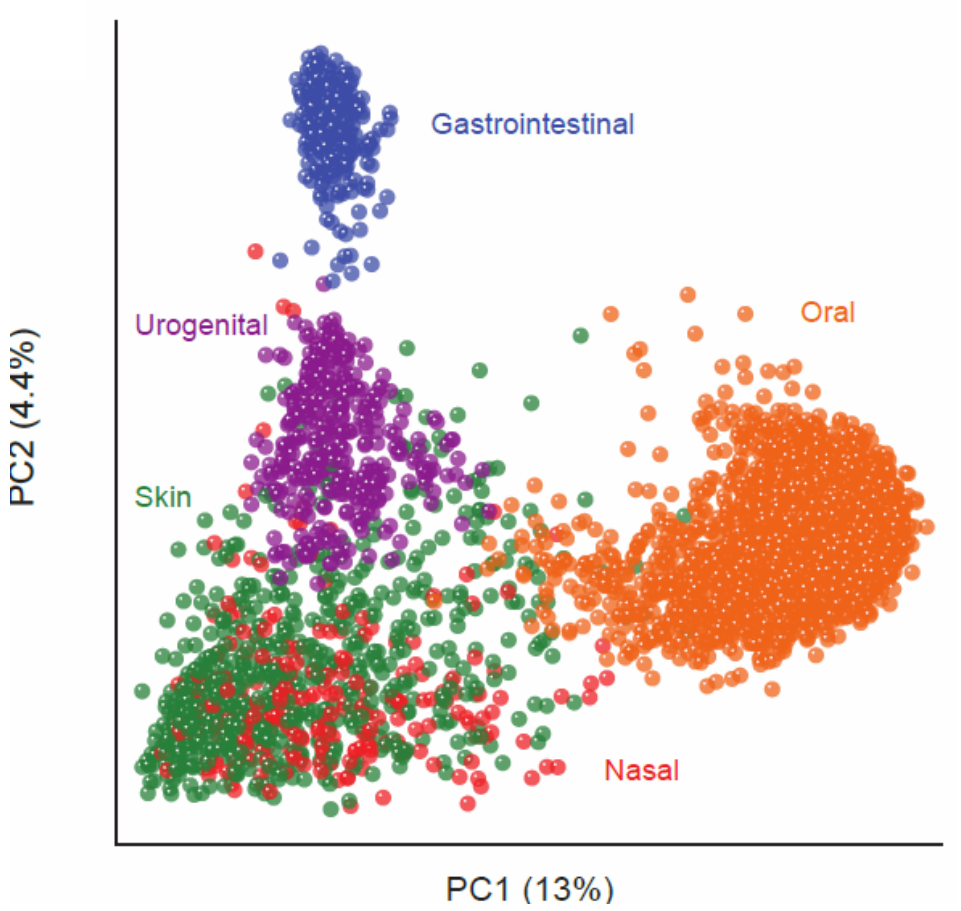
The majority of microbiome analysis published have been performed analyzing the 16S V4-region only



Detection of 16S sequences in GreenGenes database by a V-region primer sets (number of perfect matches only)

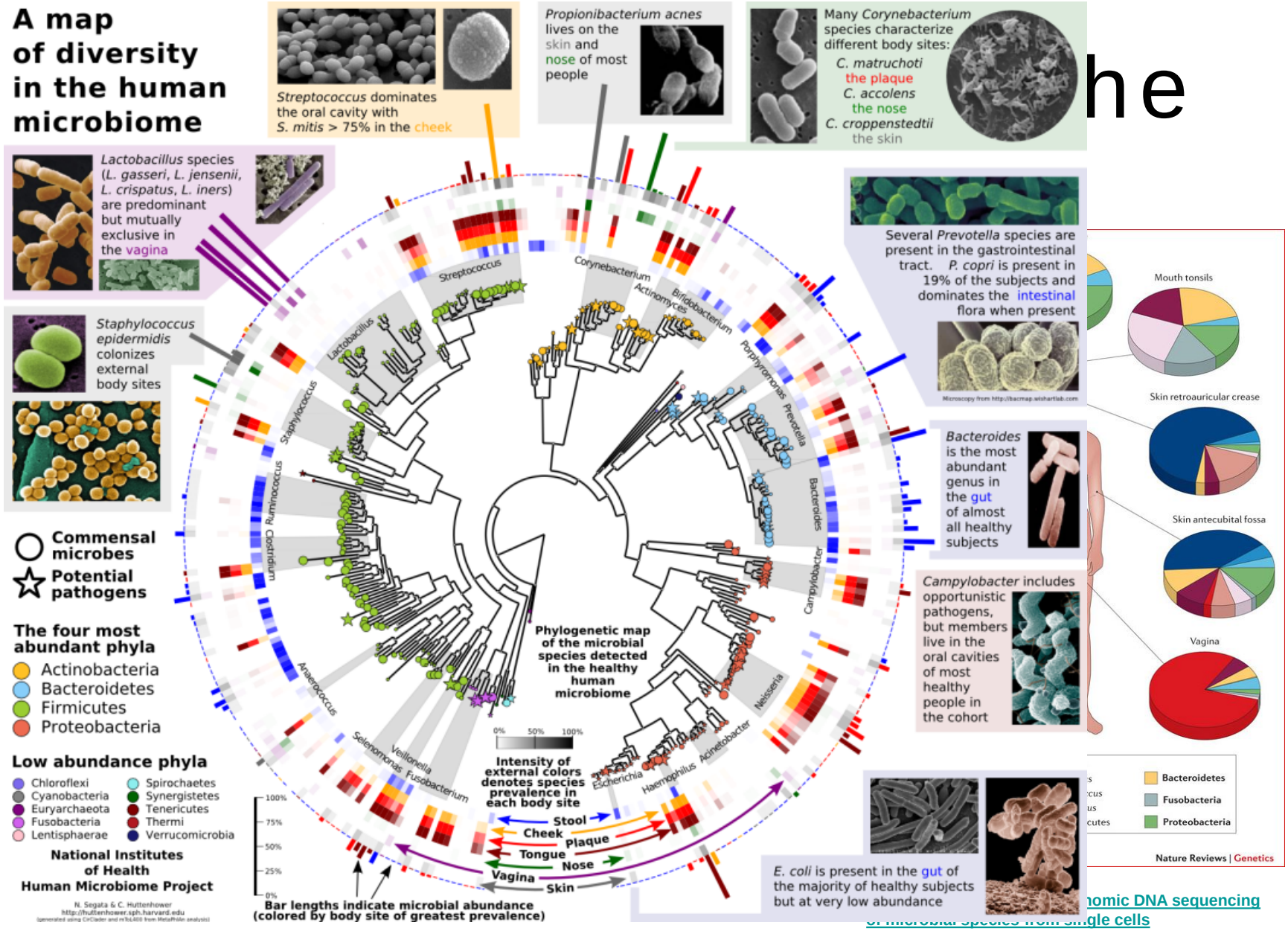


Diversity of the human microbiome



Structure, function and diversity of the healthy human microbiome The Human Microbiome Project Consortium Nature 486, 207–214 (14 June 2012)

A map of diversity in the human microbiome



[The Huttenhower Lab](#)
Department of Biostatistics, Harvard T.H. Chan School of Public Health

[omic DNA sequencing](#)
[gle cells](#)

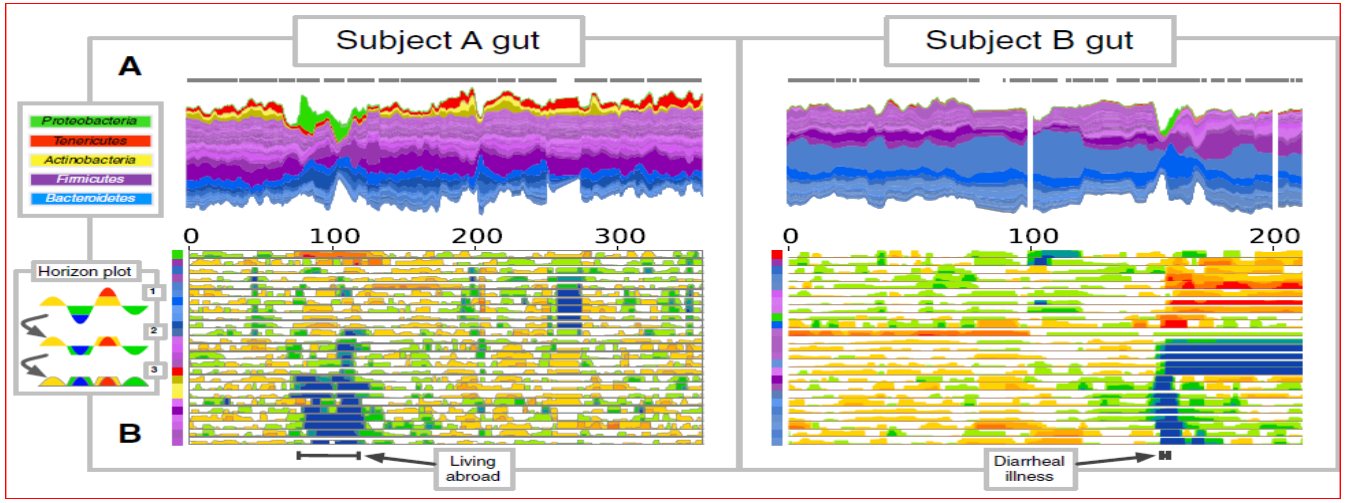
[Roger S. Lasken](#) & [Jeffrey S. McLean](#) *Nature Reviews Genetics* **15**, 577–584 (2014)

DYNAMIC ENVIRONMENTAL CONDITIONS INVOLVED IN GUT MICROBIOTA HOMEOSTASIS

- **HOST GENOTYPE**
- **DIET**
- **RAPID FLOW OF NUTRIENTS**
- **HOST IMMUNE SYSTEM**
- **OCCASIONAL INFECTIONS**
- **ANTIBIOTIC/CHEMOTHERAPEUTICS USE**

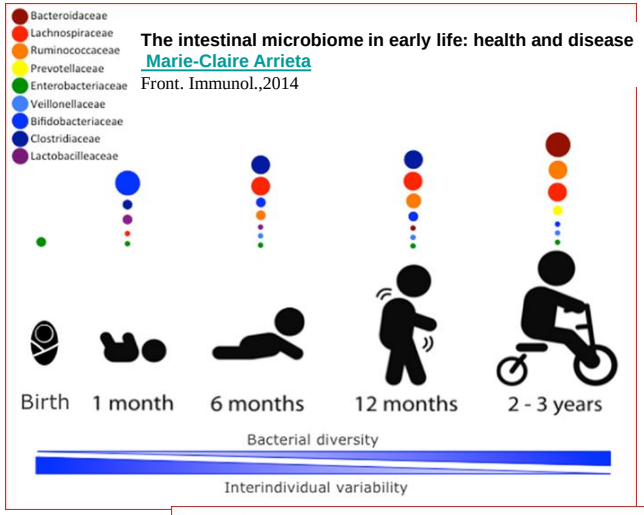
90% of the bacterial species found in termite gut are not found elsewere Hongoh Y 2010

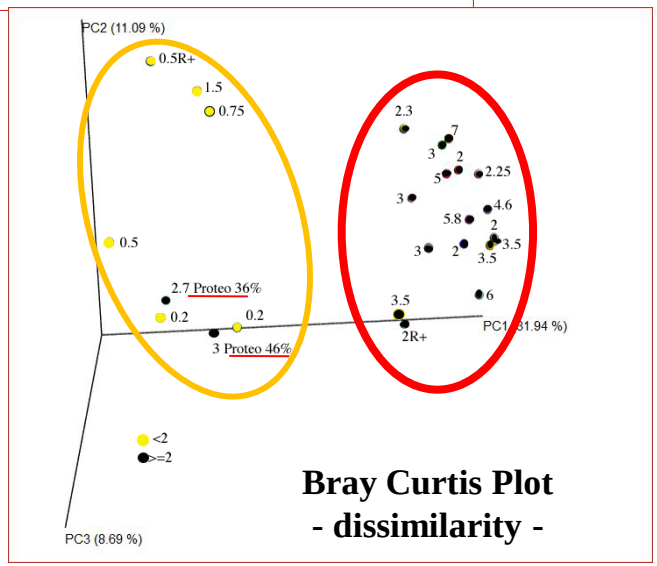
Different bacterial niche in the environments



Host lifestyle affects human microbiota on daily timescales.
David LA, Materna AC, Friedman J, Campos-Baptista MI, Blackburn MC, Perrotta A, Erdman SE, Alm EJ.
Genome Biol. 2014; 15(7): R89.

Stages of microbial colonization of the infant and child intestine.





Bacterial communities in the neonatal gut are **unstable due to its rapid temporal variation**. **Strict anaerobes dominate adult gut flora**

The microbiota **in the first week of life** are frequently **dominated by facultative anaerobes**, due to the abundance of oxygen in the neonatal gut[mainly **Proteobacteria species** (e.g., Escherichia, Klebsiella, and Enterobacter species)].

- Guaraldi, F. and Salvatori, G. (2012). Front. Cell. Infect. Microbiol. 2, 94

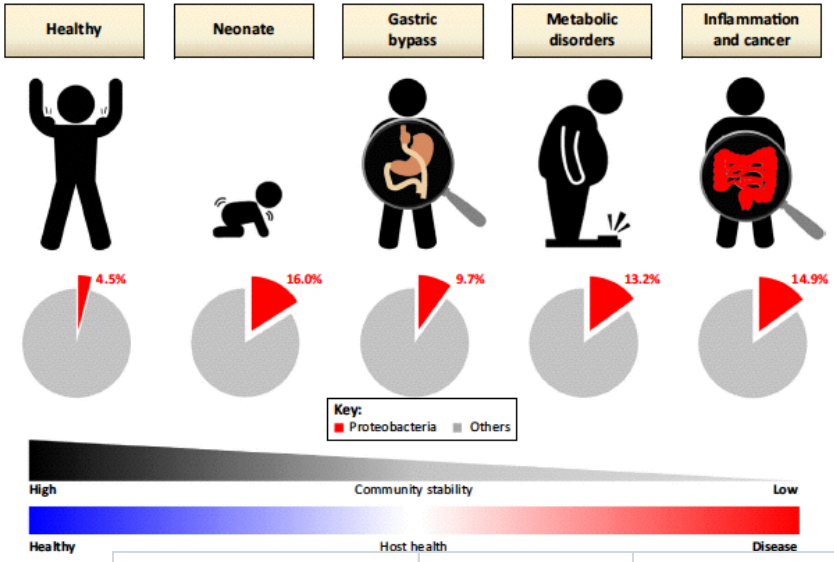
Facultative anaerobes make the habitat suitable for colonization by strict anaerobes, by **consuming oxygen**, altering the pH, lowering the redox potential, and producing carbon dioxide and nutrients.

- Chow, W.L. and Lee, Y-K. (2006), CRC Press
- Wilson, M. (2005), Cambridge University Press

Calprotectin assay

Proteobacteria in human microbiota

	Human gut	8 Samples >2 year sold: Healthy samples (2-7) Mean 4
Proteobacteria	4.5	7.3
Bacteroidetes	65.4	64.0
Firmicutes	24.4	26.8
Actinobacteria	2.2	1.82
Acidobacteria	0.0	0.0
Cyanobacteria	0.0	0.0
Verrucomicrobia	0.7	0.06
Others	2.8	0.02
F/B ratio	0.37	0. 42

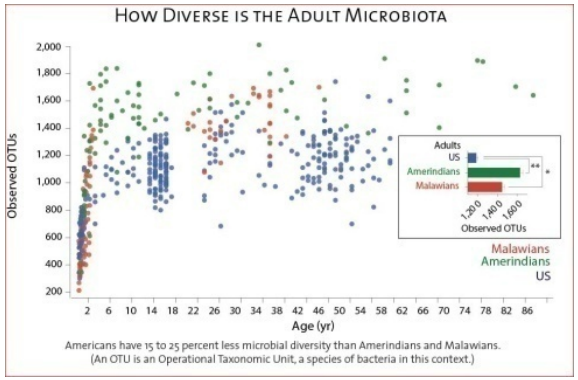


Literature	Healthy adult %	Neonate %
Proteobacteria	4,5	16,0

Trends in Biotechnology September 2015, Vol. 33, No. 9

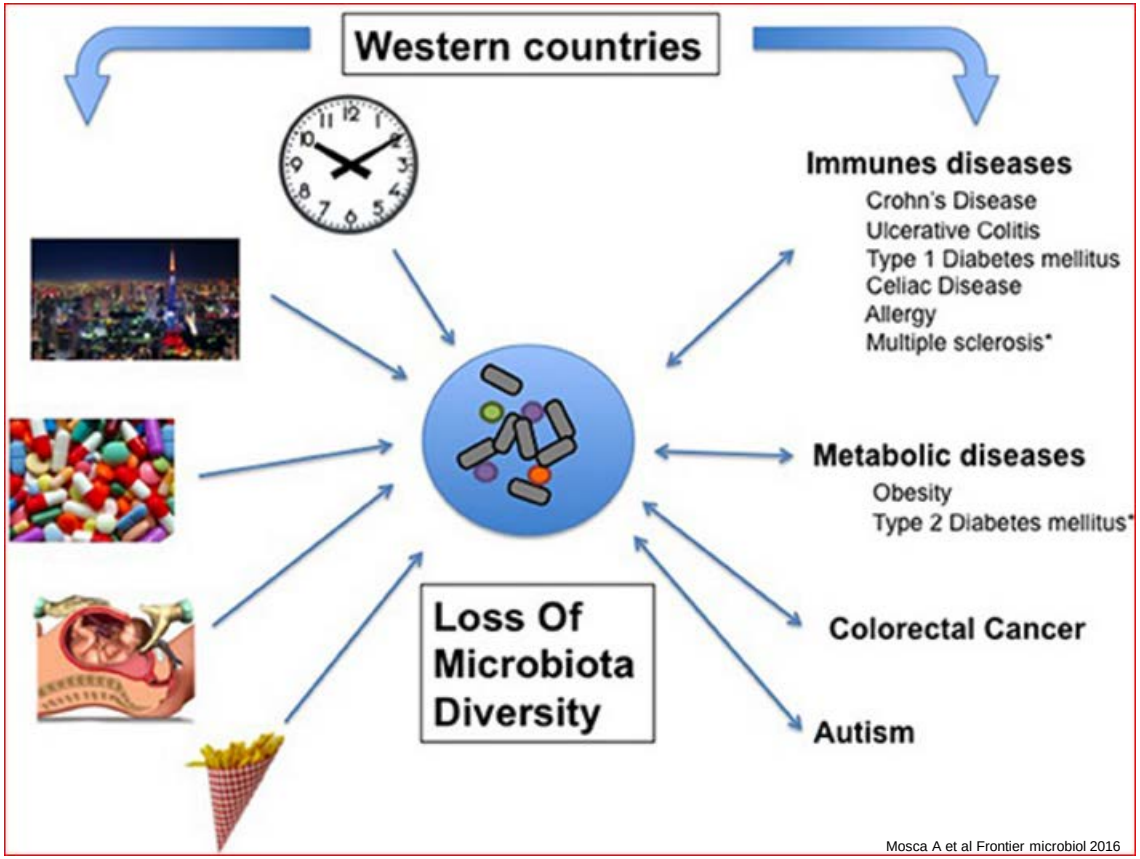
Enterotype I

Gut Microbiota Diversity



US 15-25% less species

Morgan XC, Huttenhower C (2012)



Mosca A et al Frontier microbiol 2016

Host nutrition and metabolic disorders

Transmissibility of the obese phenotype through fecal transplantation

Animal models suggests that an **altered gut microbial community**, as a primary trigger**.**

It is causative rather than consequential.

Turnbaugh, P.J. et al. (2006 Nature 444, 1027–1031

Turnbaugh, P.J. et al. (2008) Cell Host Microbe 3, 213–223

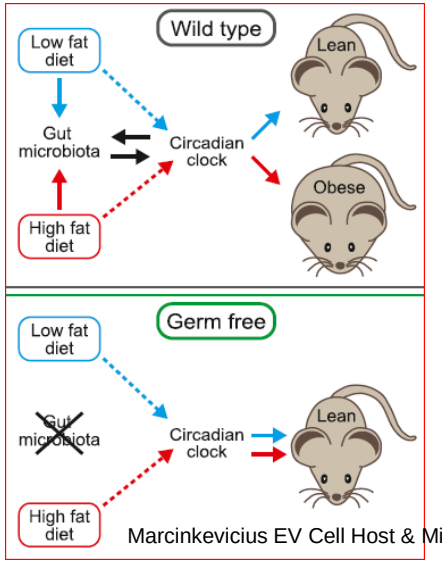
Dysbiosis, is well documented in **metabolic disorders**

Increase in abundance of **Firmicutes** respect to **Bacteroidetes** (**>F/B ratio**)

Loss of Bacteroidetes.

Ley, R.E. et al. (2005) Proc. Natl. Acad. Sci. U.S.A. 102, 11070–11075

Ley, R.E. et al. (2006). Nature 444, 1022–1023



Marcinkevicius EV Cell Host & Microbe 2015

More Proteobacteria in **European children** who **consumed a calorie-dense, high-fat, low-fiber diet** compared with children from **Burkina Faso** who were low-fat, high- fiber consumers.

De Filippo, C. et al. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 14691–14696

Noncaloric artificial sweeteners **and** emulsifiers, **impaired glucose control** and **induced a Proteobacteria bloom** and **elevations in the relative abundances** of the family **Enterobacteriaceae and class Delta-proteobacteria**,

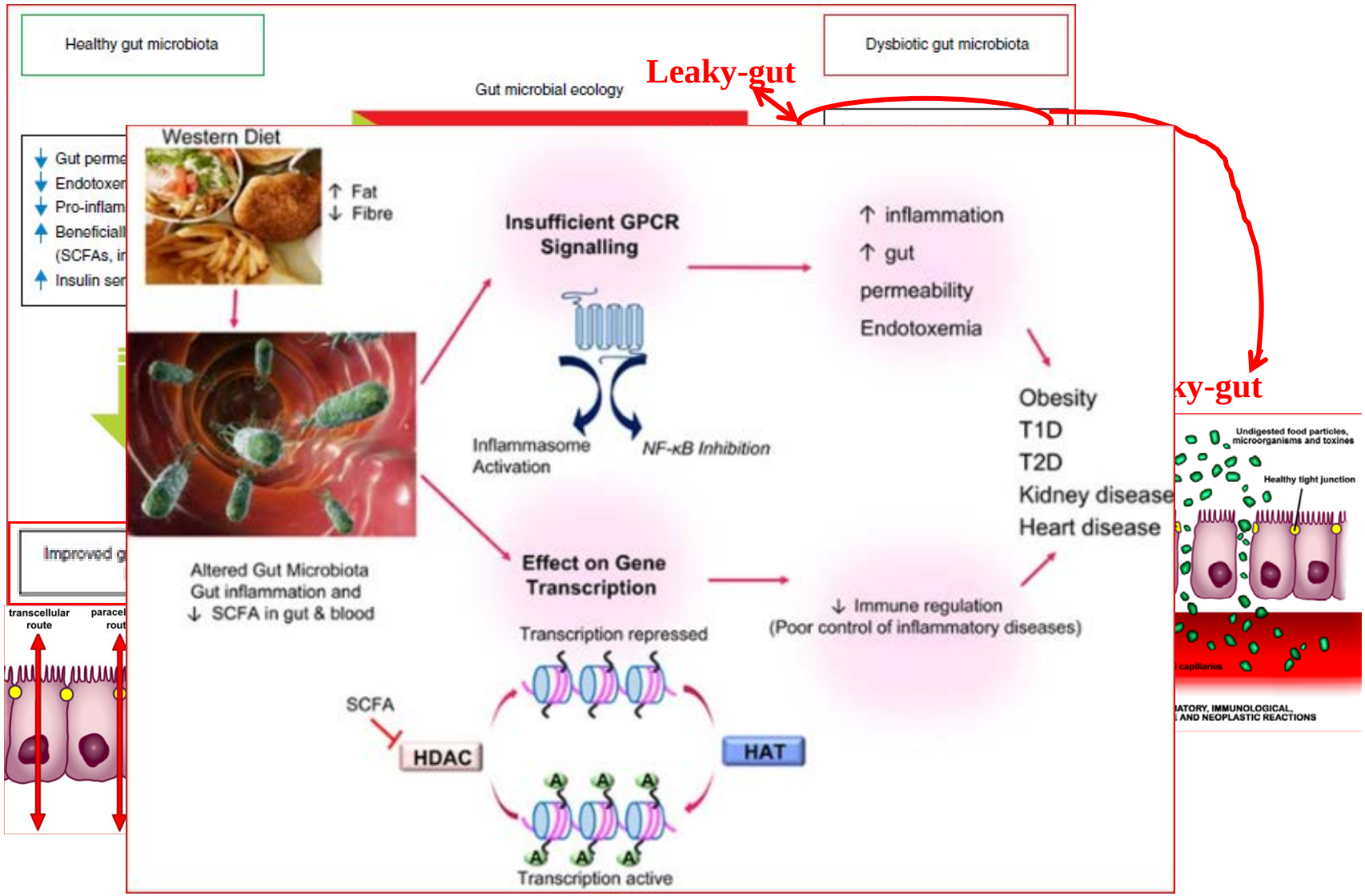
in line with results from patients with **type 2 diabetes mellitus**, suggesting a

link between glucose homeostasis and intestinal Proteobacteria.

Suez, J. et al. (2014) Nature 514, 181–186

Chassaing, B. et al. (2015) Nature 519, 92– 96

Gut microbiota inflammation



Immune disorders: inflammation

Commensals bacteria transmit a signal that induces a **tolerogenic response** of host immunity

- Mazmanian, S.K. et al. (2008). Nature 453, 620–625
- Atarashi, K. et al. (2013). Nature 500, 232–236

Host can discriminate between **beneficial** autochthonous microbes and **harmful pathogens.**

- Franchi, L. et al. (2012). Nat. Immunol. 13, 449–456

Gut-residing immune cell are hyporesponsive or display a mutualistic response to microbial stimulation

- Geuking, M.B. et al. (2011). Immunity 34, 794–806
- Manicassamy, S. et al. (2010). Science 329, 849–853

mucosal immune system is responsible for clearing pathogens, a process that **requires an active proinflammatory signaling cascade**.

Inappropriate immune response destroys the intestinal homeostasis, **triggers dysbiosis**, and contributes to local and systemic inflammation and metabolic dysfunction.

Gut microbiota is the prime suspect in IBD.

Es: Mice lacking Toll-like receptor (TLR)-5 flagellin receptor **[Tlr5-/-]** developed **transmissible spontaneous colitis** and dysbiosis, which was associated with an **abnormal expansion of Proteobacteria**.

- Carvalho, F.A. et al. (2012). Cell Host Microbe 12, 139–152

Concurrently with the Proteobacteria bloom, colitic Tlr5-/- mice exhibited a disorganized colonic mucous layer and had delayed clearance of infectious pathogens compared with their noncolitic Tlr5-/- siblings.

A Proteobacteria-dominated community, predisposes genetically susceptible mice to chronic colitis.

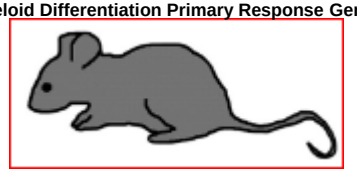
Diet and T1D

Patients with T1D have impaired gut barrier function

NOD mice model

Myd88$^{-/-}$ NOD

Myeloid Differentiation Primary Response Gene (88)



vs

NOD
(Non Obese Diabetic)

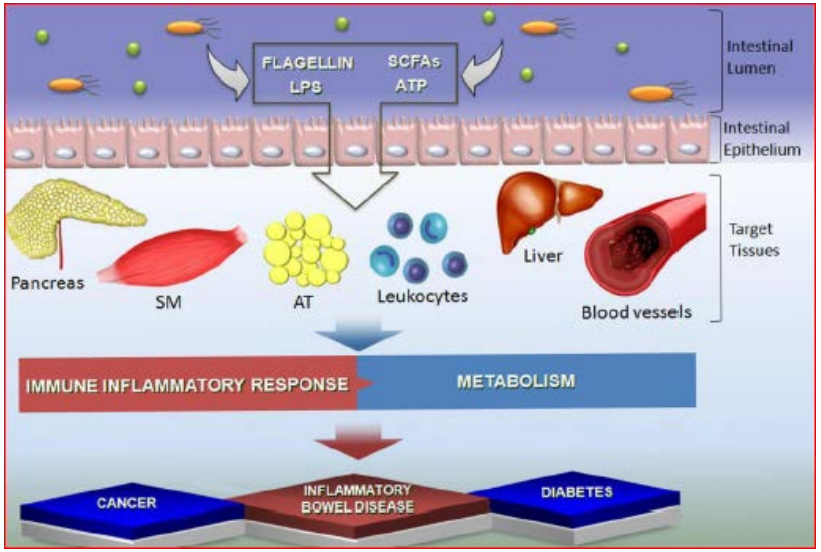
Protected from T1D
Rich in bacterial phylum *Bacteroidetes* in their gut
Lose this protection when housed in
"germ-free" condition

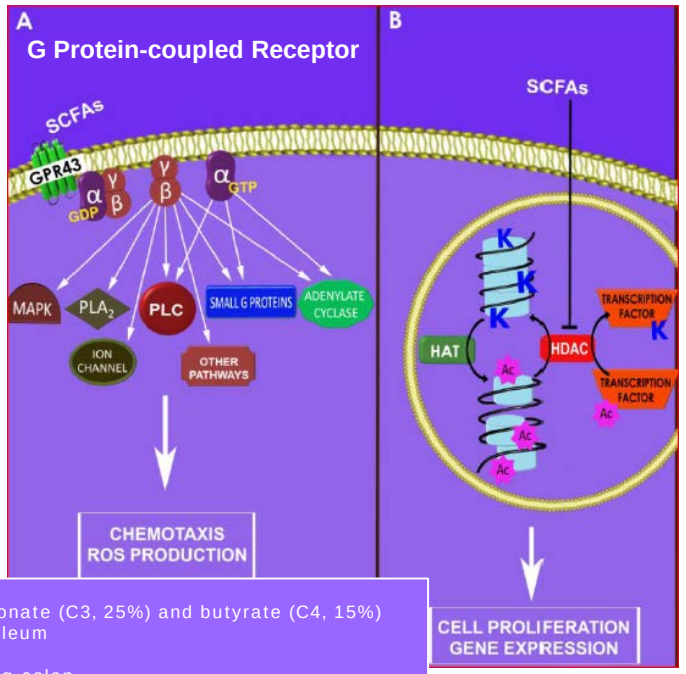
T1D is mediated by an immunomodulatory bacterial product??

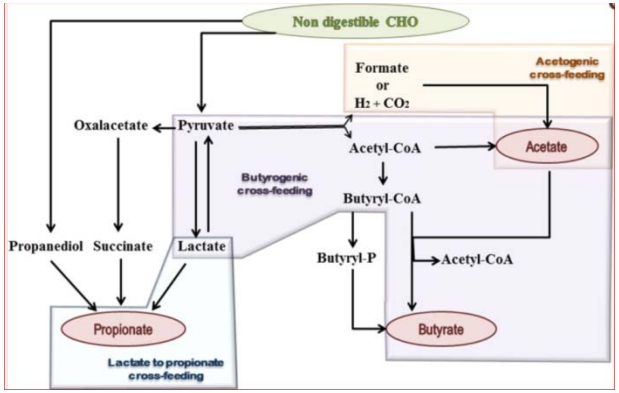
MyD88-/- NOD have higher blood levels of SCFA (acetate & butyrate)

Marino et al Nat Immunol 2017

Schematic representation of the interaction between gut microbiota and host tissues.

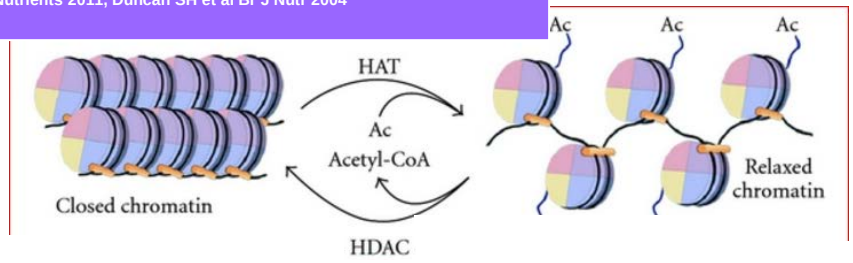






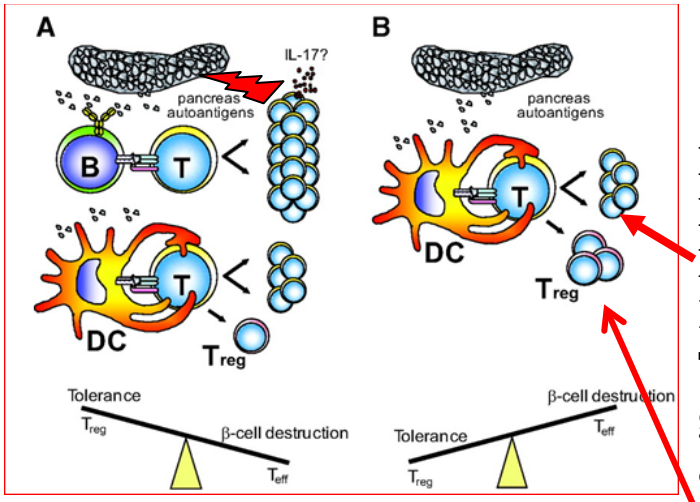
SCFAs
Acetate (C2, 60%), propionate (C3, 25%) and butyrate (C4, 15%)
~ 13 mM in the terminal ileum
~130 mM in the caecum
~80 mM in the descending colon

Vinolo M et al Nutrients 2011, Duncan SH et al Br J Nutr 2004



Open
conformation transcriptionally active

Dietary supplement as treatment for T1D??



Adapted from Susan H. Smith, and Thomas F. Tedder Diabetes 2009;58:1479-1481

Fed with acetate rich diet

Less T1D autoreactive T cells
Less B cells
Less B-mediated T1D autoreactive T cells expansion
Increase Bacteroidetes number
Transfer of microbiota from acetate-fed mice to germ-free NOD mice fed with normal diet protect against diabetes for 30 days

Fed with butyrate rich diet

Increased number T_{reg} T1D protected
Promote naive T cells → T_{reg}
by affecting histone acetylation upregulating FOXP3

NOD mice fed with special diet that release large amounts of acetate & butyrate after bacterial fermentation in the gut have a complete protection from T1D

Marino et al Nat Immunol 2017

Microbioma & T2D

Current Human evidence

Dysbiotic microbiota

Slighly altered overall bacterial composition
↓Butyrate-producing bacteria
↓*A.muciniphila*
↑Serum Branched-chain AAs via P.copri and B.vulgatus in insulin-resistant subjects
↑CH_4 METABOLISM
↑H_2S biosynthesis
↑Resistance to oxidative stress

Glucose-lowering medication (metformin)

↑ ***Lactobacillus* and *Escherichia* species**

Diet

↑ **Prevotella w high fiber diet, <u>in some individual</u>**

Gut microbial composition maybe used to identify Responders to dietary intervention

Bariatric surgery

Future Possibilities

Personalized nutrition and probiotic use

Synergistic approach: diet, probiotics and microbiota
Need for further studies of:
(1) impact of habitual dietary intake on response;
(2) single *vs* multiple probiotic strain effects;
(3) use as an adjunct to glucose-lowering drugs

Targeted colonic delivery of SCFAs

Targeted delivery of **propionate** decreases energy intake and improves glucose metabolism

Pasteurised probiotics

Pasteurised ***A. muciniphila*** *improves glucose metabolism in mice:* human studies needed

Genetically modified bacteria

L. lactis *<u>modified to produce GLP-1</u>, leading to improved glucose* metabolism in mice: human studies needed

FMT (Faecal microbiota transplantation)

Little evidence for improved glycaemic control
Can potential risks be eliminated?

Louise Brunkwall L & Orho-Melander M, Diabetologia 2017

Metformin alters gut microbiome in T2D contributing to the therapeutic effects.

genera level

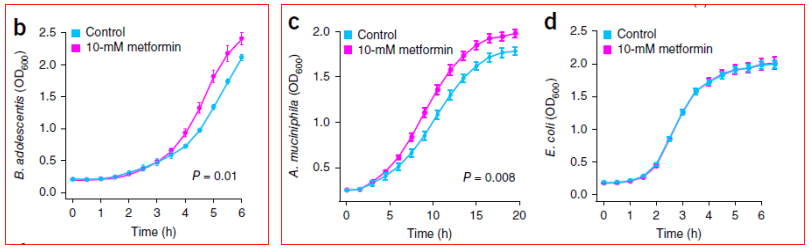
↑ ***Escherichia*** *2-4 month*

↓ ***Intestinibacter*** *2-4 month*

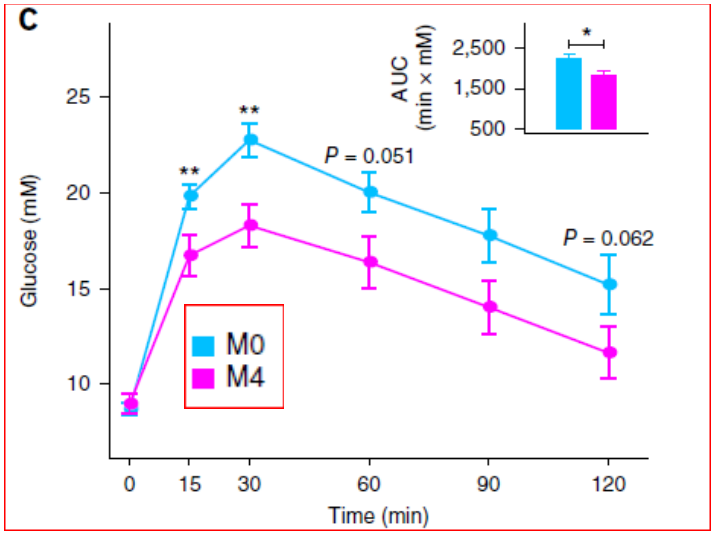
↑ ***A.muciniphila*** *4 month*

↑ ***B.adolescentis*** **switched** *6 month*

Effect on microbial growth



Metformin-altered microbiota improves glucose tolerance.



Germ-free mice after colonization with fecal microbiota obtained from three individuals with T2D.

Negative correlation between the of ***B. adolescentis and*** ***%Hb A1c, suggests that*** **increased growth of this bacterial species could potentially contribute to the antidiabetic effect of metformin.**

Wu H et al Nat Med 2017- doi:10.1038/nm.4345

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GENOVA
16/17 giugno
2017

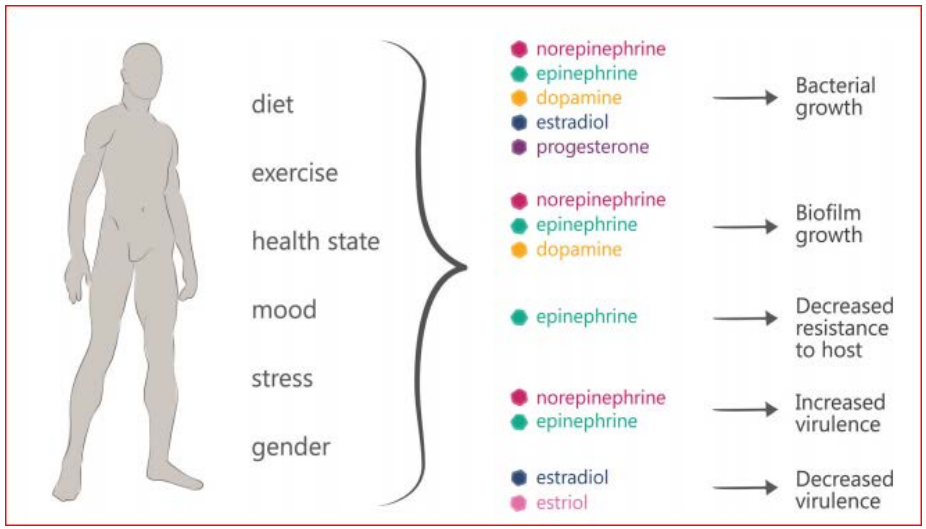
- Elisabetta Ugolotti
- Eddi Di Marco
- Roberto Biassoni

- Cinzia Gatti
- Rodolfo Pessina

Il dr. Roberto Biassoni dichiara di NON aver ricevuto negli ultimi due anni compensi o finanziamenti da Aziende Farmaceutiche e/o Diagnostiche

The crosstalk between microbes and hormones can affect host metabolism, immunity and behavior

Host effects on the microbiota



Neuman H et al. FEMS Microbiology Reviews, 39, 2015, 509–521

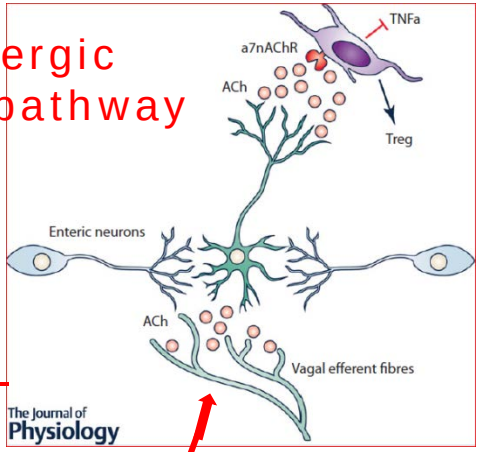
- **Neurohormones,**
  - **serotonin**
  - **catecholamines**
    - **dopamine**
    - **epinephrine (adrenaline)**
    - **norepinephrine (noradrenaline)**
- **stress hormones,**
  - **cortisol**
  - **corticosterone**
  - **adrenocorticosterone**
  - **corticotropin**

- **Bacteria use quorum sensing (QS).**
  - regulate bacterial growth, motility and virulence (Fuqua, Winans and Greenberg 1996).
- **Host hormones affect bacterial gene expression** (Sperandio *et al., 2003)*.
  - **Catecholamines enhance** bacterial attachment to host tissues, and affect **growth and virulence of bacteria** (Freestone and Lyte 2008; Hegde, Wood and Jayaraman 2009).
  - In contrast, the human sex hormones estriol and **estradiol decrease** bacterial virulence **by inhibiting QS** (Beury-Cirou *et al., 2013).*

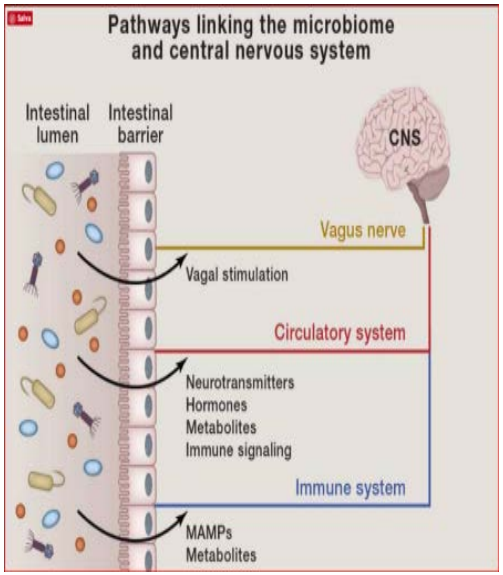
Microbiota & CNS

The gut-microbiota interact with the host through

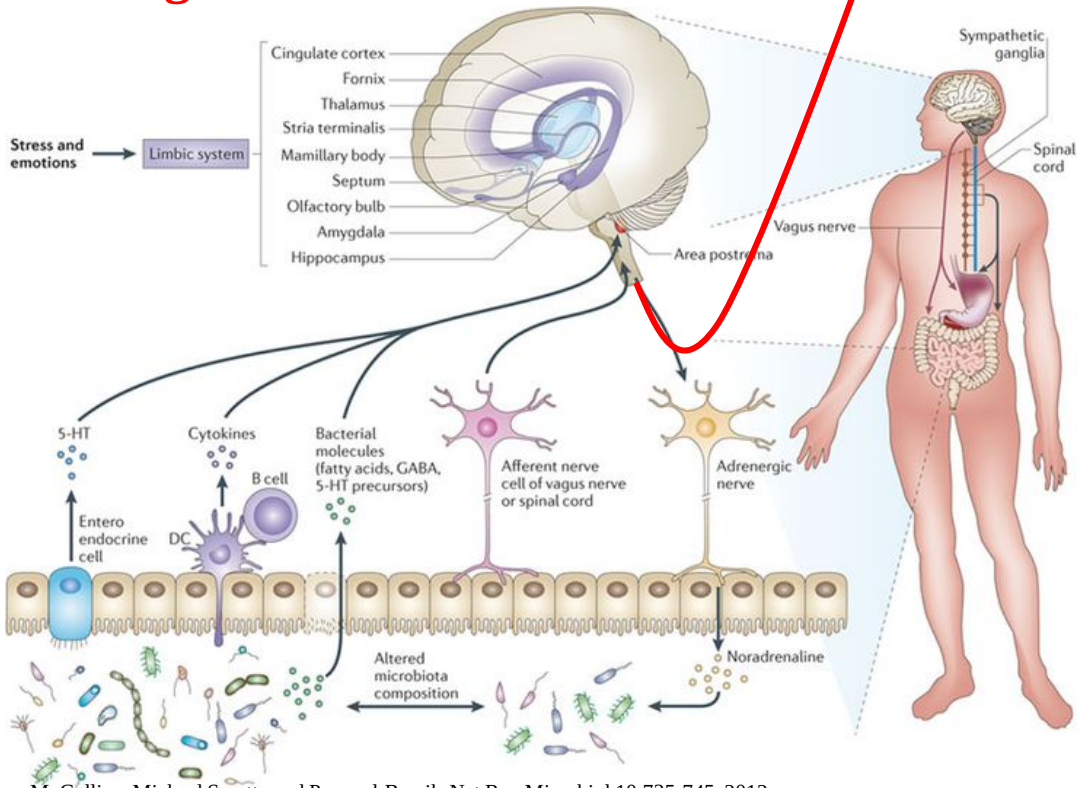
- **immune,**
- **neuroendocrine**
- **neural pathways**



The bidirectional microbiota–gut–brain axis.



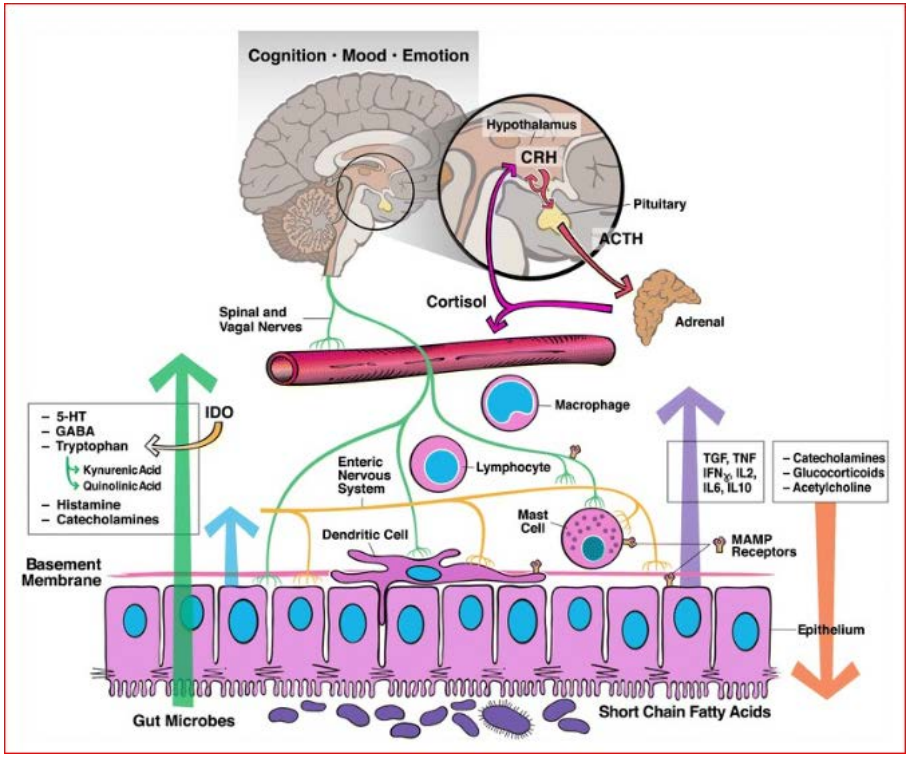
Samposon TR et al Cell host & microbes 2015



Transient changes in the microbiota influence brain chemistry and behaviour in mice.

Stephen M. Collins, Michael Surette and Premysl Bercik Nat Rev Microbiol 10:735-745, 2012.

Gut microbiota
and psychiatric disorders such as anxiety, depression, schizophrenia and autism.



Forsythe–P et al BMC 2016

- **transplantation of the gut microbiota from depressed patient into microbioma-depleted rats induced behavioural and physiological features characteristics of depression.**

Microbiota has a causal role in the development of depression

Campylobacter jejuni induce stress in animal models

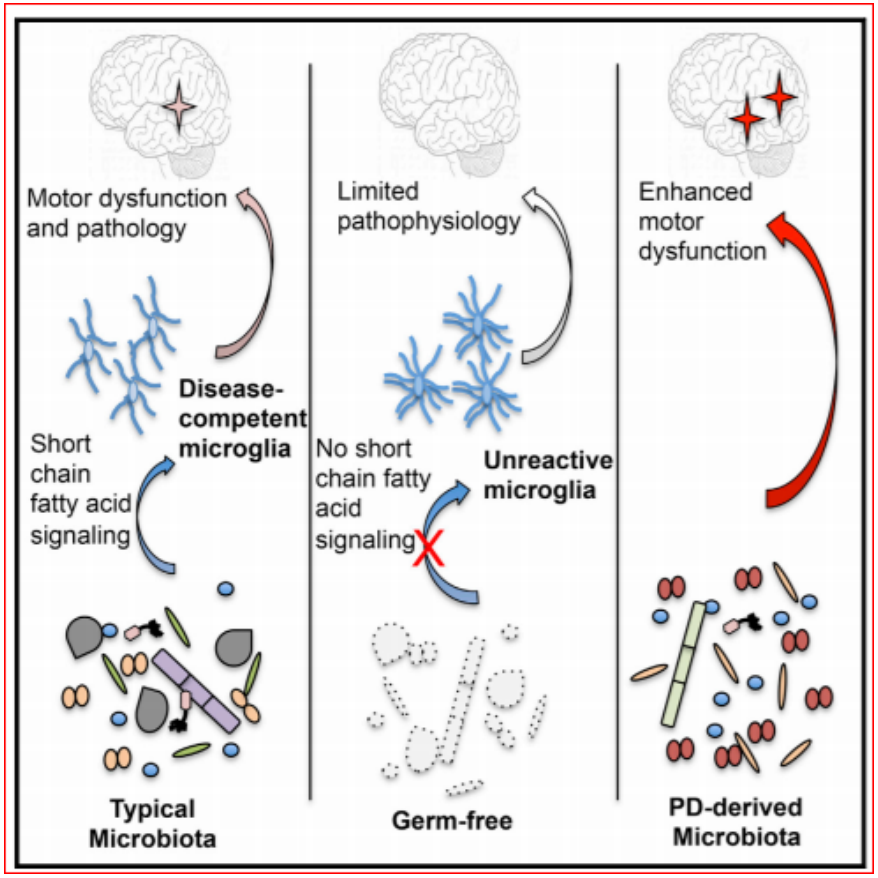
The use of probiotics like **Lactobacillus Rhamnosus** reduce stress via GABA

Autism spectrum disorder

Inconsistent Altered microbioma composition reported

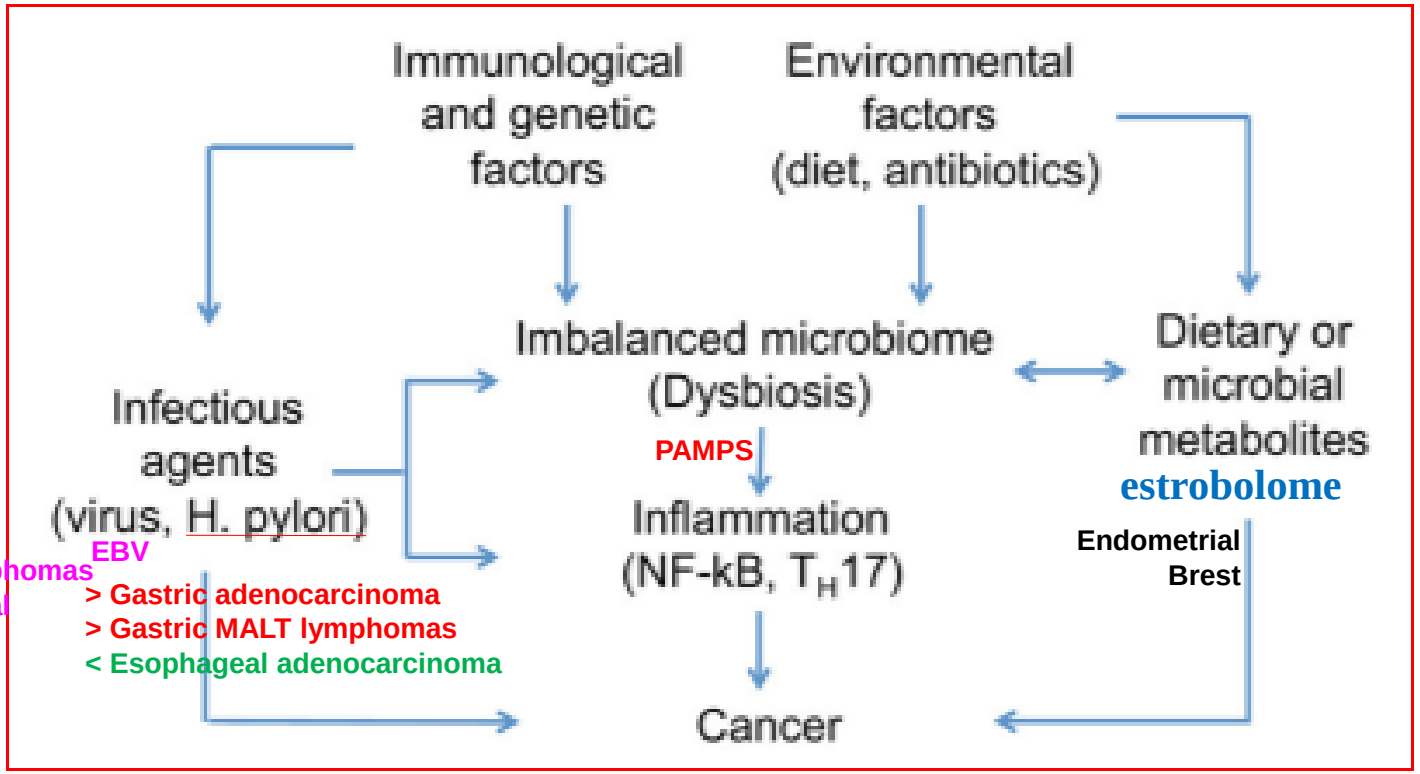
Fineglod et al 2010, Williams et al 2011,
Khang et al 2013, Son et al 2015,
Parracho et al2012, Gondalia et al 2012

Parkinson and Microbiota



- **Gut microbes promote a-synuclein-mediated motor deficits and brain pathology**
- **Depletion of gut bacteria reduces microglia activation**
- **SCFAs (butyrate?) modulate microglia and enhance PD pathophysiology**
- **Human gut microbiota from PD patients induce/enhanced motor dysfunction in mice**
- **Microbiota's metabolites may enter the circulation and impact neurological function.**
- **Identification of bacterial taxa or metabolites altered in PD as disease biomarkers or even drug targets,**
- **interventions that correct dysbiosis as effective treatments to slow or halt the progression motor symptoms.**

Mechanisms by which the bacterial microbiome modulates carcinogenesis.

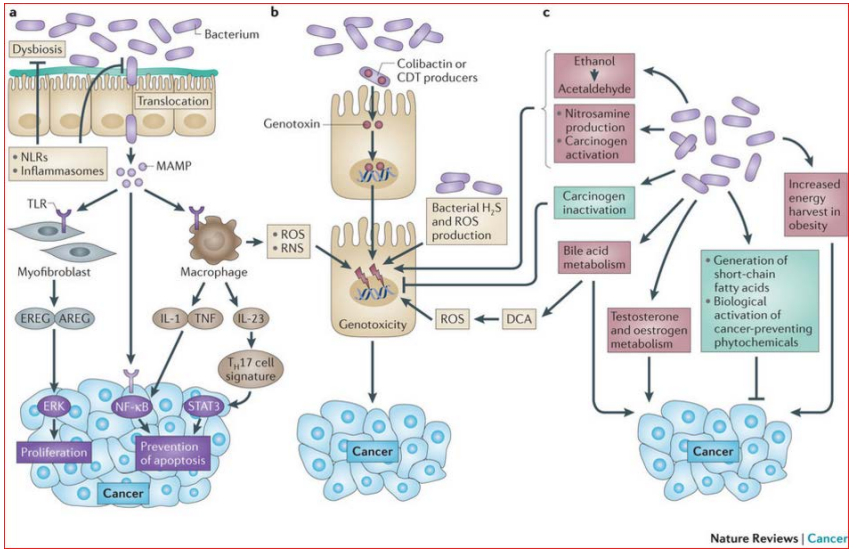


Contreras et al Frontiers in Physiology 2016 | Volume 7 | Article 606

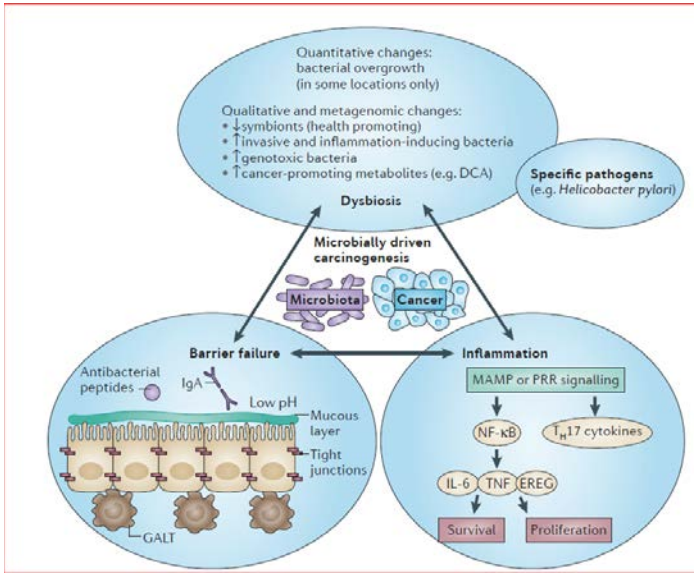
Higher rate of cancer in the large intestine, where microbial densities are much higher than in the small intestine

Plottel CS 2011 Cell Host Microbe

Mechanisms by which the bacterial microbiome modulates carcinogenesis.



Schwabe RF and Jobin C Nature Rev Cancer 2013; 13, 800



Rajapopala SV Cancer Prev Res; 2017

In murine models commensal microbiota modulate the efficacy of anticancer therapy

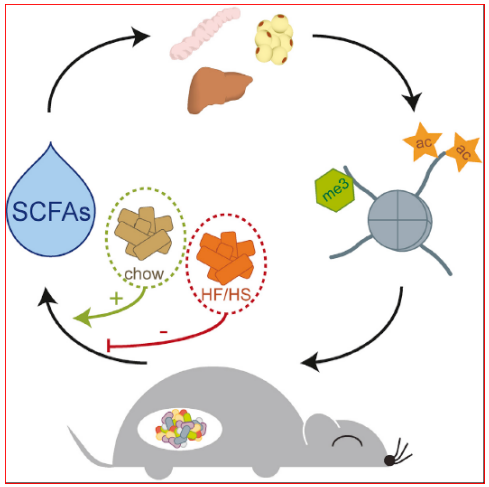
Efficacy of the treatment maybe improved through combined cancer therapy with probiotics

Type of cancer related to microbiome dysbiosis

- **Colorectal cancer**
  - **SCFA protective, Harmful metabolites phenols, nitroso compounds, HS, microbial derived-secondary bile salts (DCA, LCA), ROS reactive nitrogen species,ethanol oxidative products acetaldehyde**
- **Hepatocellular carcinoma**
  - **Gut-Liver axis - leaky gut, LPS, metabolites, inflammation (NFkB pathway), TLR4, oxidative damage via portal vein**
  - **No iniziation - support HCC development**
- **Pancreatic cancer**
  - **Inflammation, (oral microbiome & periodontitis, pathobionts into circulation), leaky-gut, pamps, LPS and its receptor TLR4**
- **Lung cancer**
  - **Lung microbiota (S.pneumoniae, H.influenzeae, M.catarrhalis infections associated with 50% COPD, cronic inflammation**
- **Squamous cell cancer**
  - **oral microbiome & periodontitis, inflammatory markers (saliva, blood)**

J med Microbiol 2016: 65, 1347-62

Gene methilation and acetylation



Microbiota has **methyl-donor capacity** for epigenetic methylation

Circadian oscillations of serum metabolites are regulated by the microbiota.

Microbiota rhythms program the circadian epigenetic and transcriptional landscape

It is critical for maintaining the homeostatic rhythmic transcription in the liver.

- **Gut microbiota alter host histone acetylation and methylation in multiple tissues**

- **Western diet suppresses microbiota-driven SCFA production and chromatin effects**

Effect on histone acetylation. more than source of energy, **SCFA** can be either directly converted (acetate) or oxidized (propionate and butyrate) to acetyl-CoA, substrate for Histone acetyltransferases (HAT) enzymes, butyrate is a known HDAC inhibitor.

- **SCFAs recapitulate microbiota-driven chromatin and transcriptional effects**