Progetto Ematologia Romagna

Il microbioma umano: un target emergente per la salute dell'ospite

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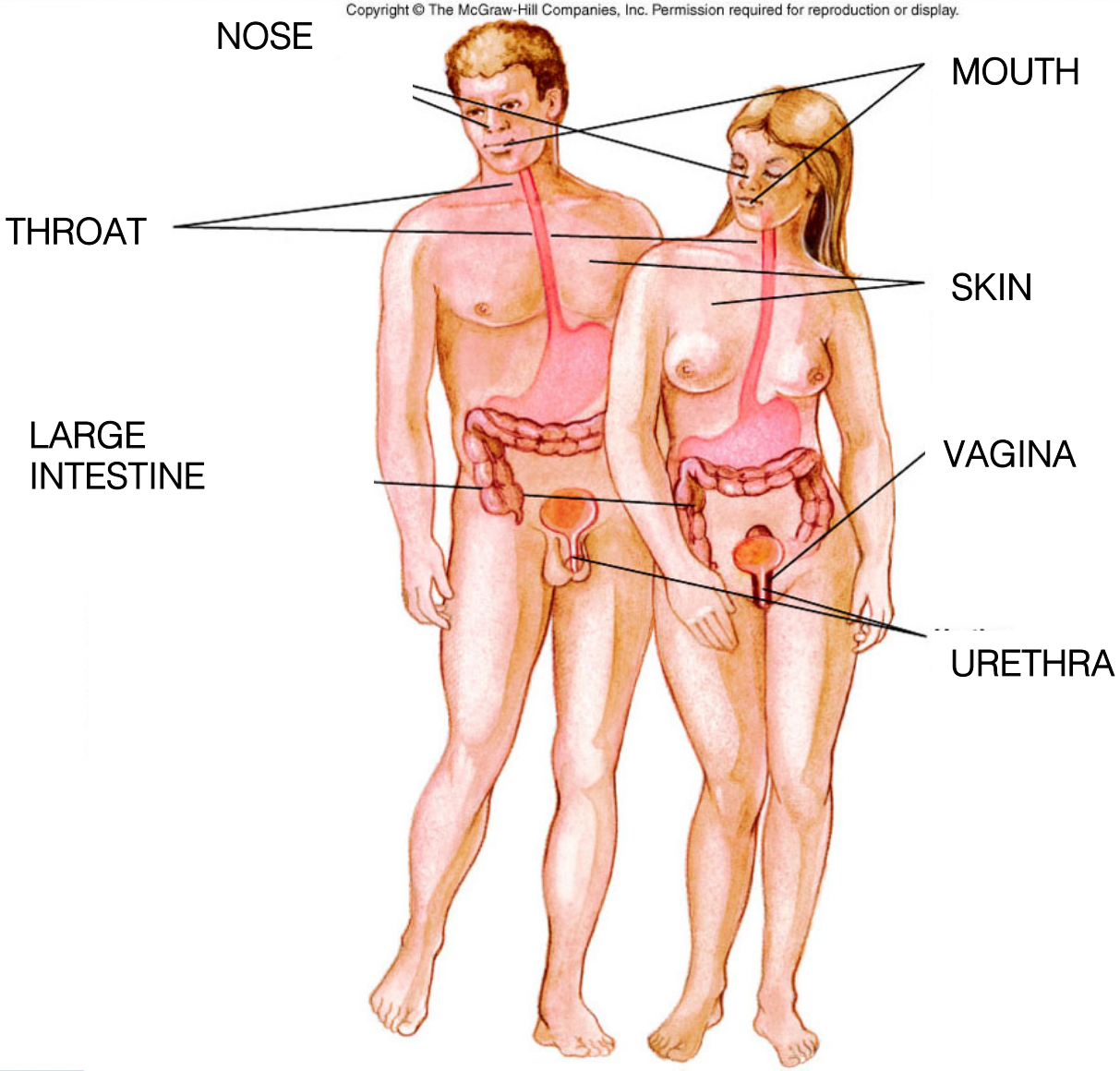
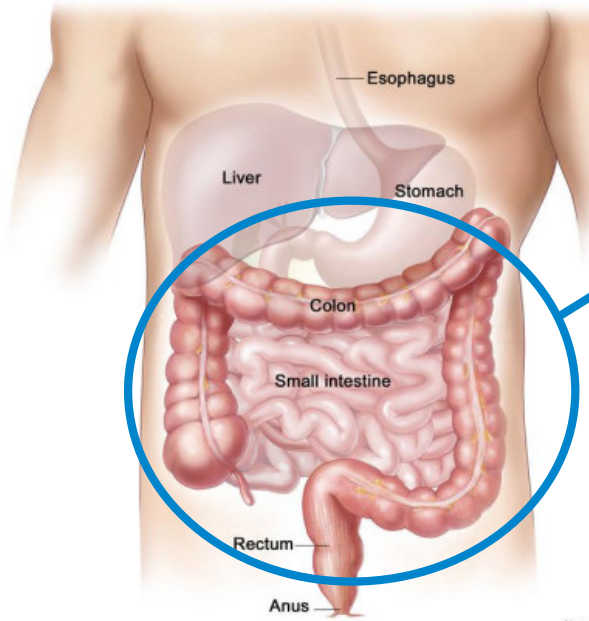
HUMAN MICROBIAL ECOSYSTEMS



THE HUMAN INTESTINAL MICROBIOTA

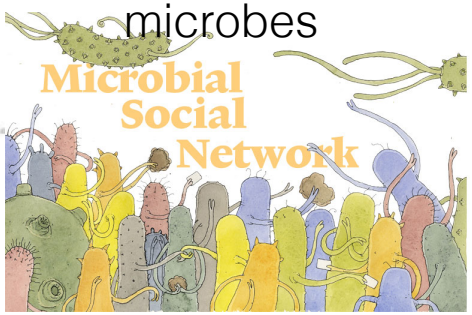
10^{13}–10^{14} habits our body and the great majority of these microorganisms is hidden in the gastrointestinal tract

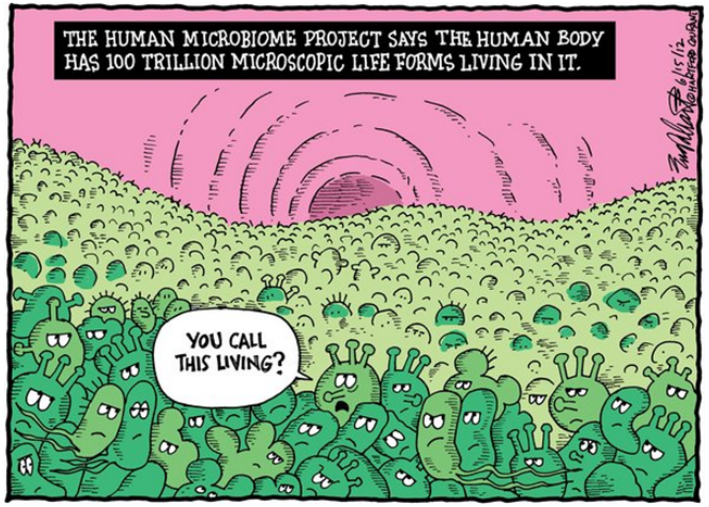


- 10^{12} CFU/g
- up to 1.5 kg

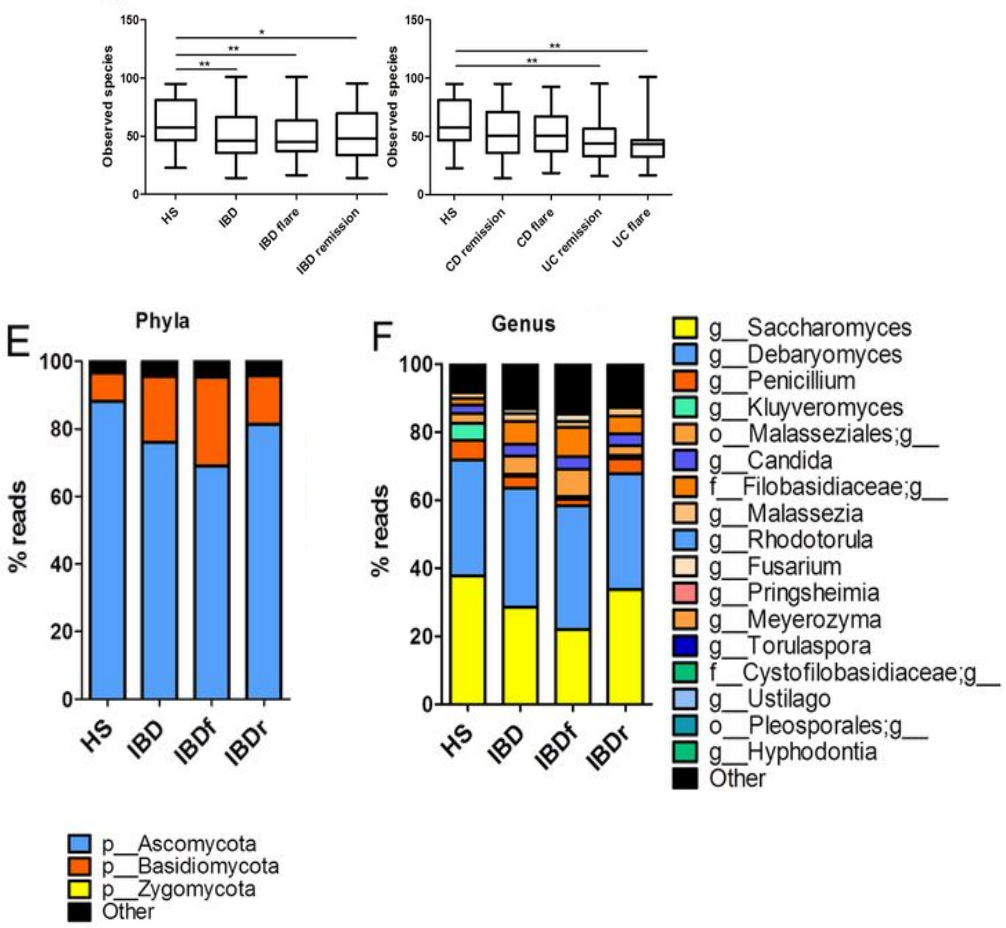
the most dense bacterial ecosystem on our planet

organized in a ecosystem of highly interconnected microbes

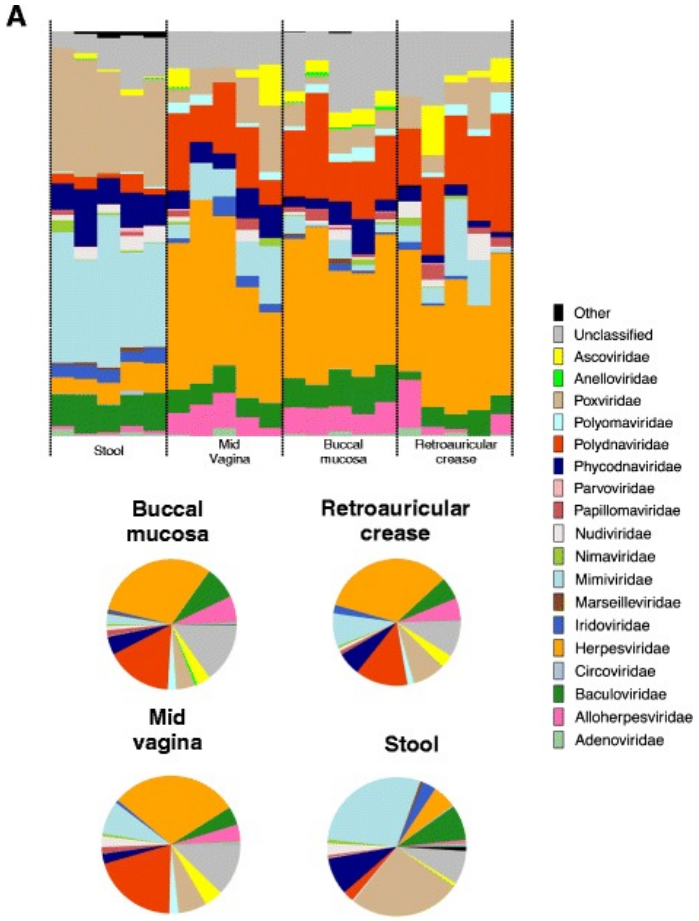




EUKARYOME AND VIROME: OVERLOOKED COMPONENTS OF THE



Sokol *et al.*, Gut 2015



Rampelli *et al.*, BMC Genomics 2016

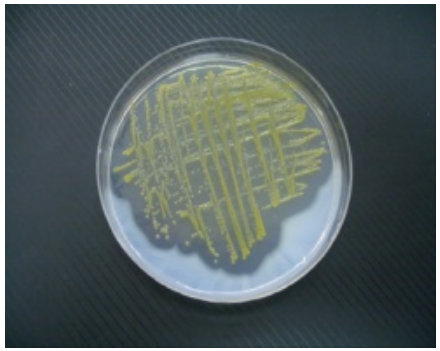
HUMAN INTESTINAL MICROBIOTA

our bacterial counterpart provides essential features we have not evolved

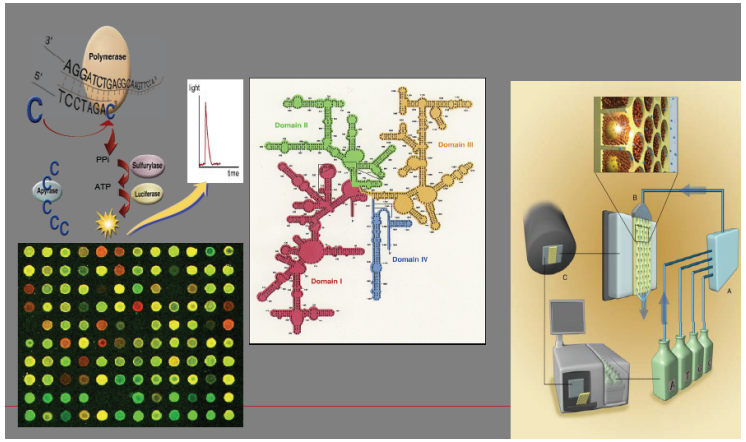
- **enhancement of the digestive efficiency and modulation of energetic homeostasis**
- vitamin synthesis
- **competitive barrier against colonization/invasion**
- **development, education and function of the immune system**
- strengthening of the GIT epithelium impermeability
- detoxification of xenobiotics
- **central nervous system modulation**
- endocrine system modulation

HOW DO WE KNOW THIS?

Culture-based methods allow to recover 20-30% of total microscopic counts

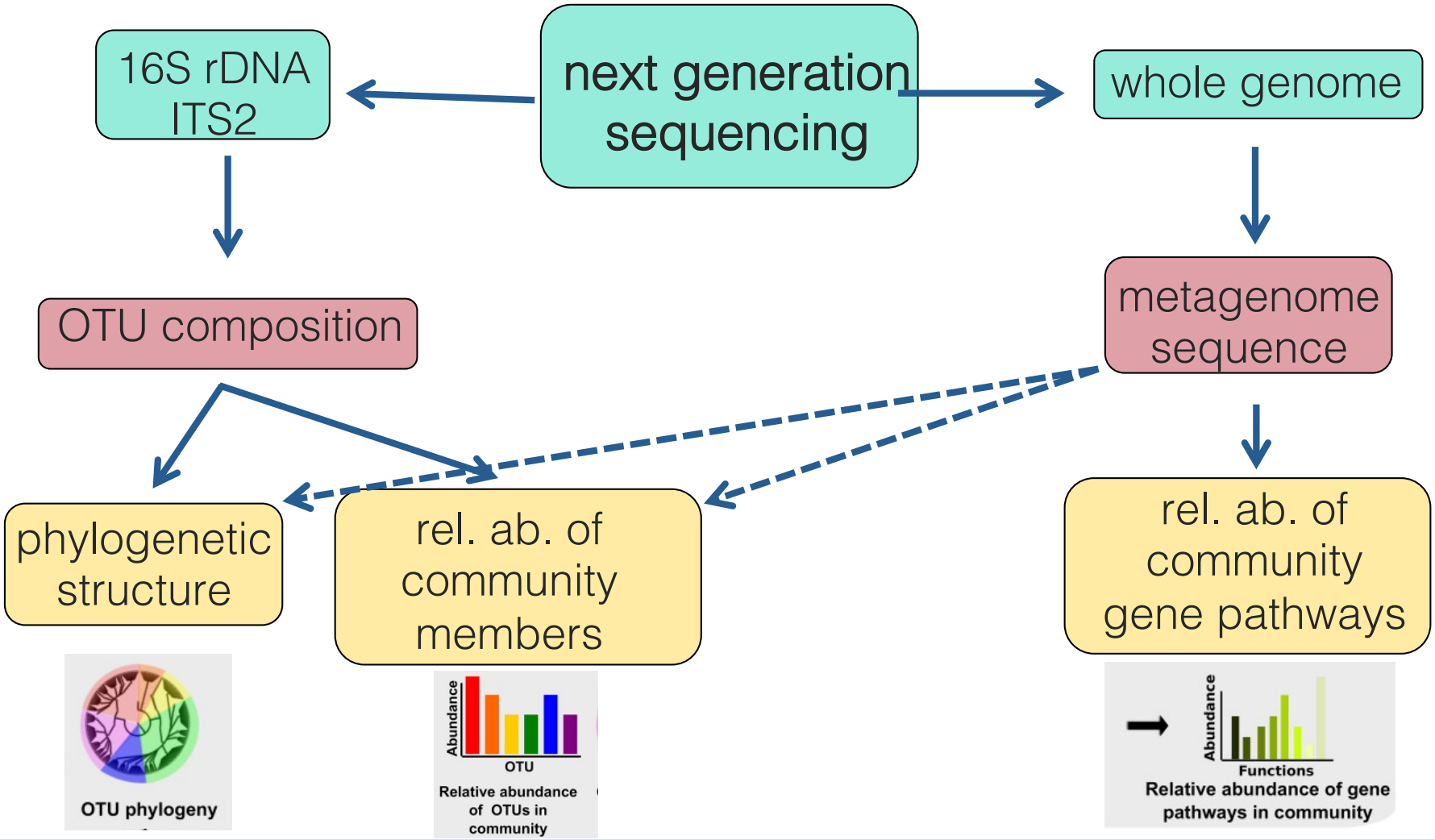


MOSTLY UNCULTURED!



Culture-independent molecular survey: NGS

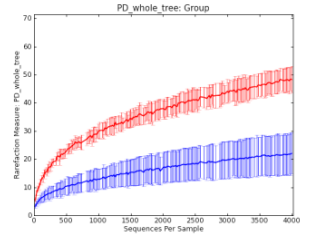
MICROBIOTA MOLECULAR ASSESSMENT



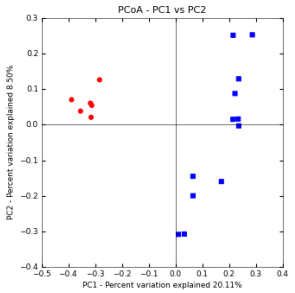
NEXT GENERATION SEQUENCING

- Possibility to sequence even 100 samples per run
- Obtain a large amount of infos simultaneously, thanks to BIOINFORMATICS

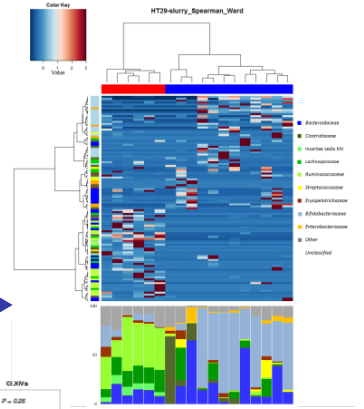
α-diversity *within subjects* → diversity index → rarefaction



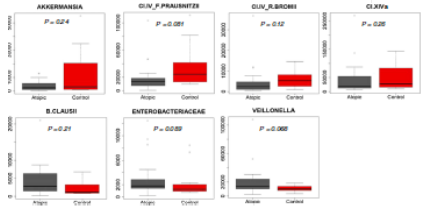
β-diversity *between subjects* → distance matrix → PCA-PCoA



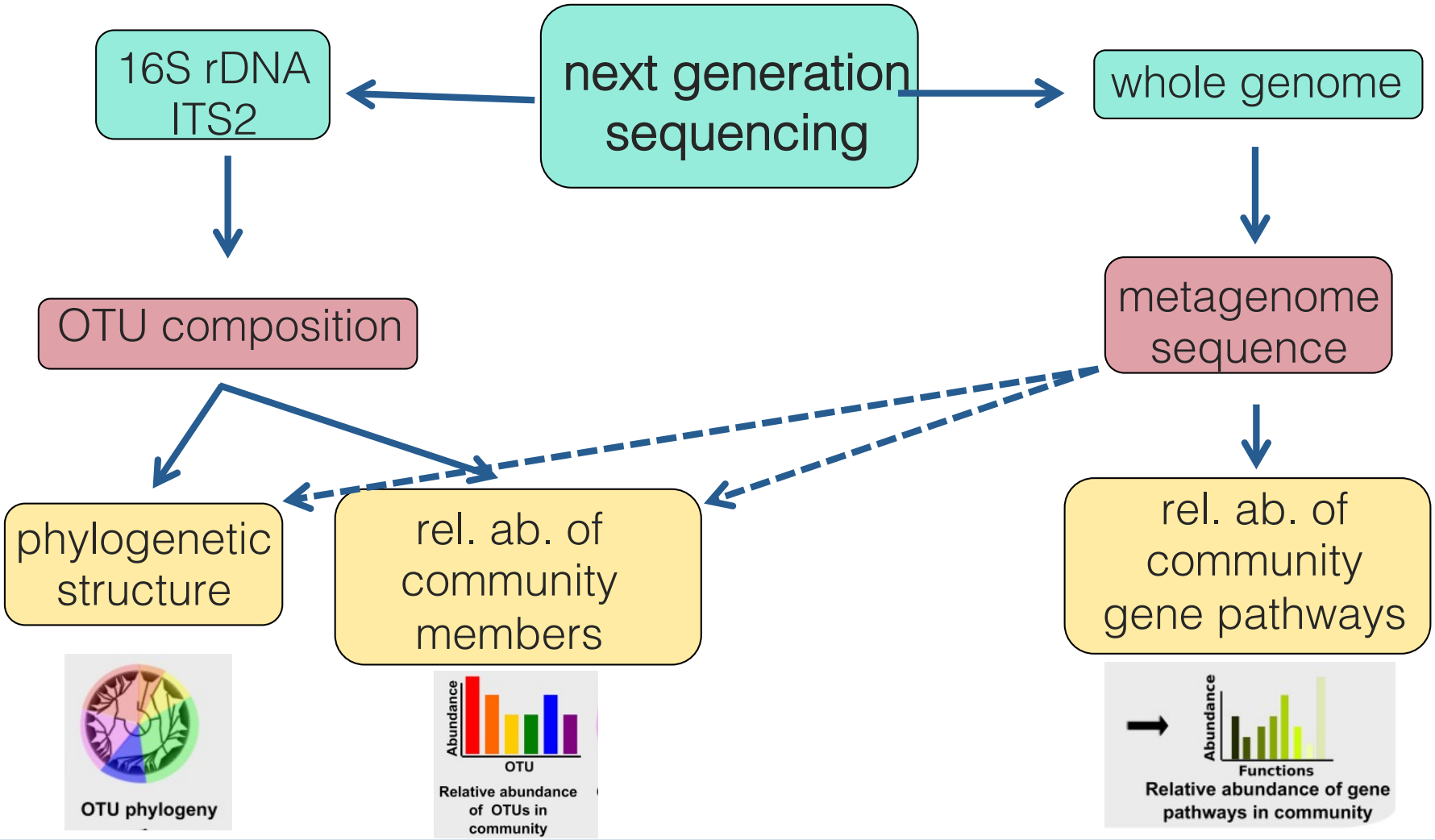
similarities → correlation matrix → clustering



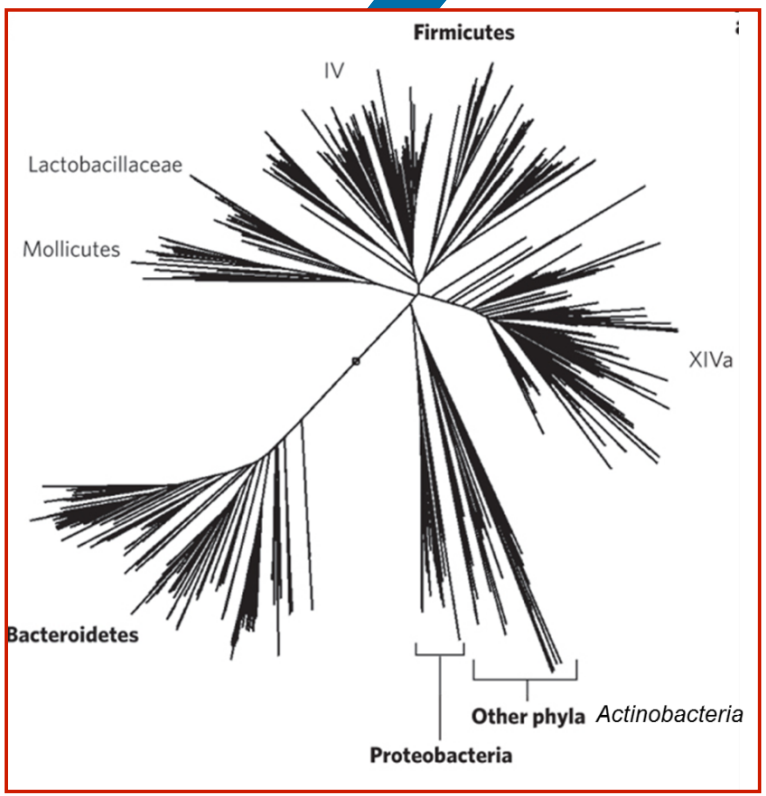
compositional differences → analysis of variance → box plots



MICROBIOTA MOLECULAR ASSESSMENT



PHYLOGENETIC DIVERSITY



> 1000 species

6 (out of 100) bacterial phyla

- Firmicutes, Bacteroidetes : 90%
- Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia : 10%

FUNCTIONAL DIVERSITY

MICROBIOME
(collective genome of the microbiota)

10^6 GENES

58% KNOWN

- carbohydrate metabolism (CAZymes)
- energy metabolism
- amino acid metabolism
- biosynthesis of secondary metabolites
- metabolism of cofactors and vitamins

42% UNKNOWN

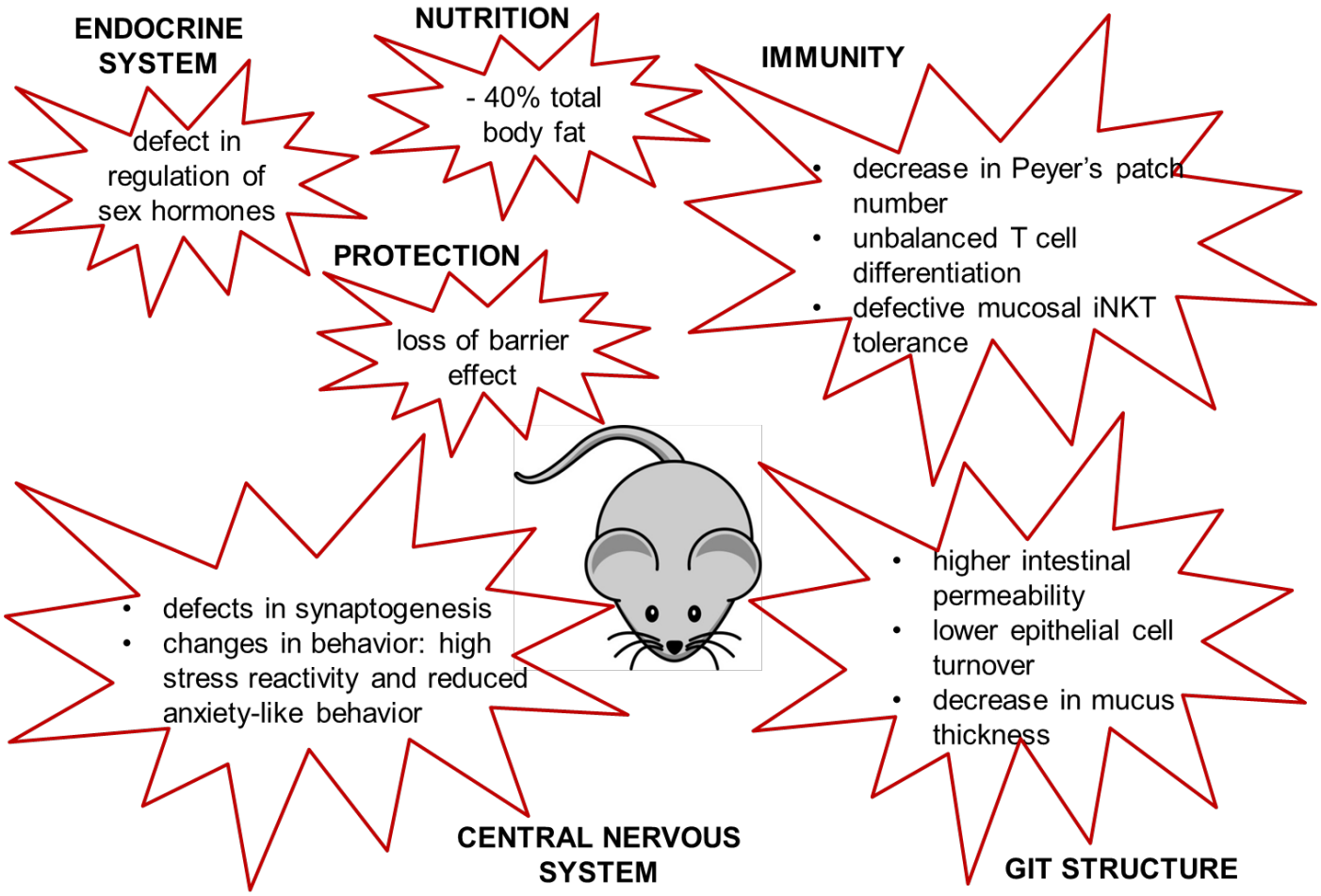
Schloissing et al, Nature 2013

HOW DO WE KNOW THIS?

- Microbiome sequence analysis
- Observation of human gut microbiota in different physiological/pathological condition
- In vitro studies using complex bacterial communities

THE ADOPTION OF GERM-FREE MICE ALLOWED TO MEASURE THE IMPACT OF GUT MICROBIOTA-HOST MUTUALISM ON SEVERAL PHYSIOLOGICAL PARAMETERS

WHAT IS MISSING IN GERM-FREE MICE



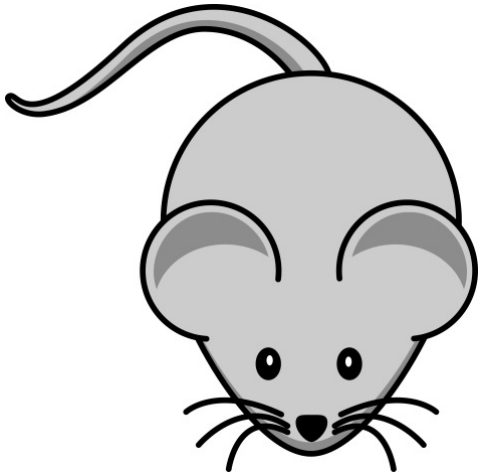
WHAT DO GERM-FREE MICE GAIN?

Effects associated to the colonization of specific bacterial groups

resistance to obesity in *ob/ob* model

resistance to certain metabolic diseases, such as: hepatic steatosis and type 2 diabetes

resistance to inflammation-associated intestinal diseases (IBD, CRC) in IL10-/- model

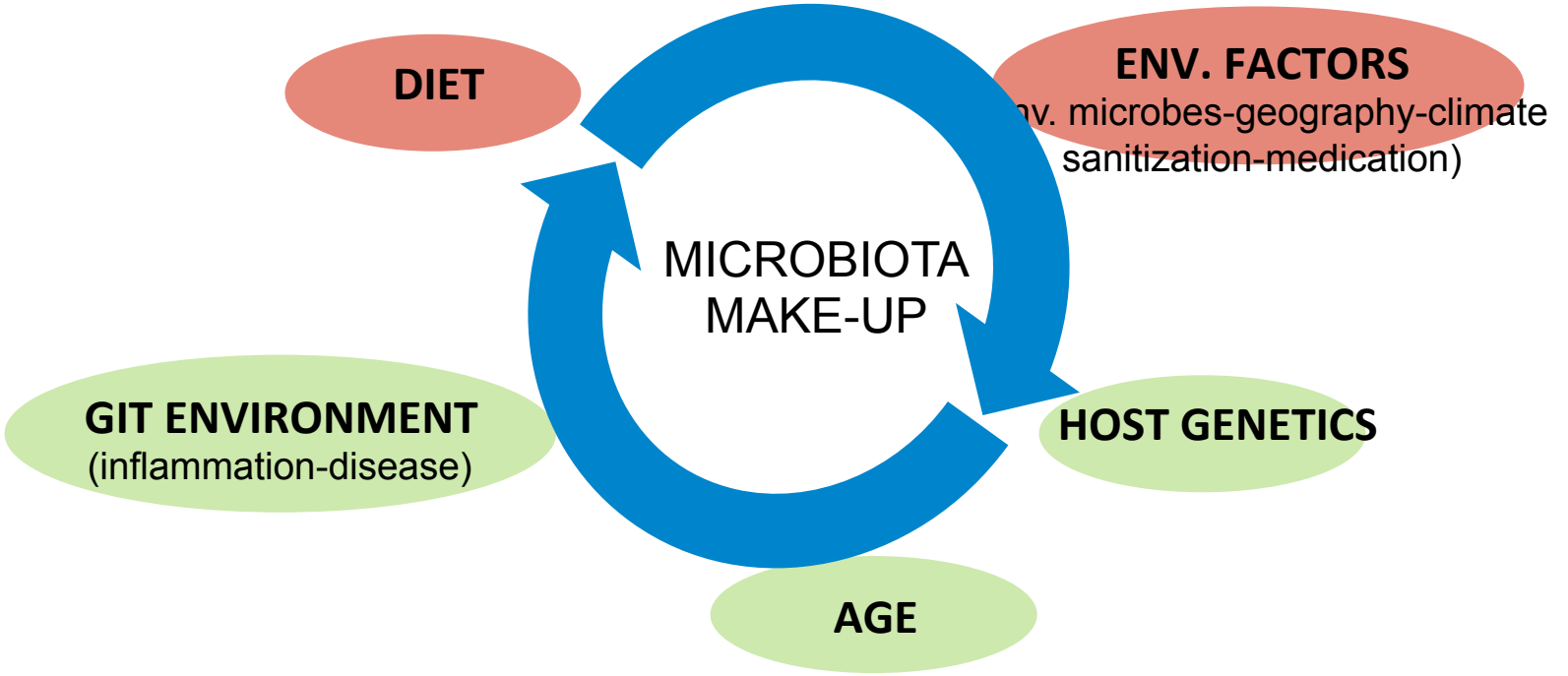


Resistance to certain retroviruses, e.g. poliovirus

Resistance to pathobionts -associated secondary infections

MICROBIOTA PLASTICITY

THE INDIVIDUAL MICROBIOTA COMPOSITION CONTINUOUSLY CHANGES IN RESPONSE TO **EXTRINSIC** AND **INTRINSIC** VARIABLES

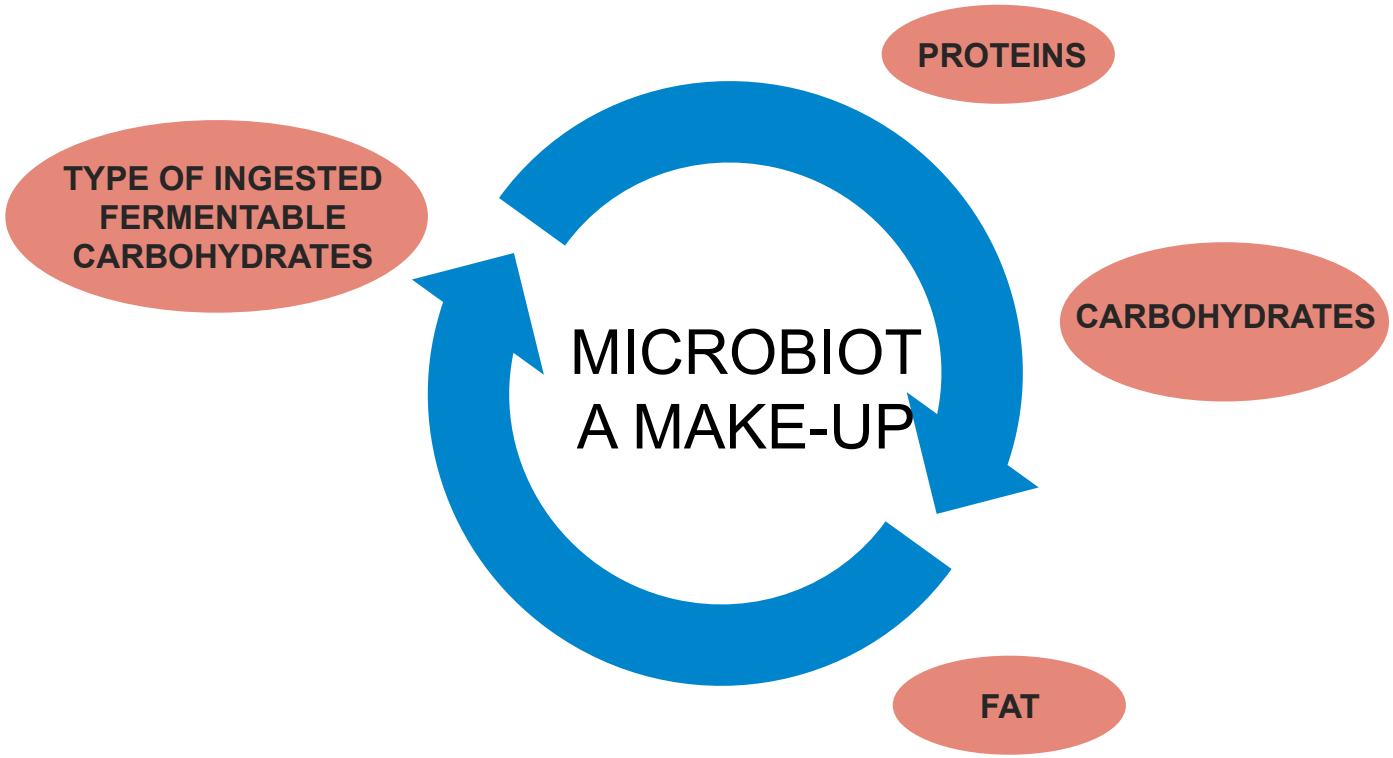


IN A MUTUALISTIC CONTEXT, THE PLASTICITY OF THE HUMAN MICROBIOTA GUARANTEES A RAPID ADAPTATION OF THE SUPER-ORGANISM IN RESPONSE TO DIET CHANGES, AGE, ETC

there is a strong selection towards a readily changeable individual microbiome profile

DIET AND MICROBIOTA DYNAMICS

THE MOST REMARKABLE EXAMPLE OF MICROBIOTA PLASTICITY IS ITS CAPACITY TO RAPIDLY RESPOND TO DIETARY CHANGES



IMPACT ON HOST NUTRITION

indigestible plant polysaccharides (xylan, pectin, arabinose containing-dietary carbohydrates as plant-derived pectin, cellulose, hemicellulose, resistant starches) reach unchanged the colon where they are metabolized by the intestinal microorganisms

indigestible plant polysaccharides

MICROBIOTA
CAZymes

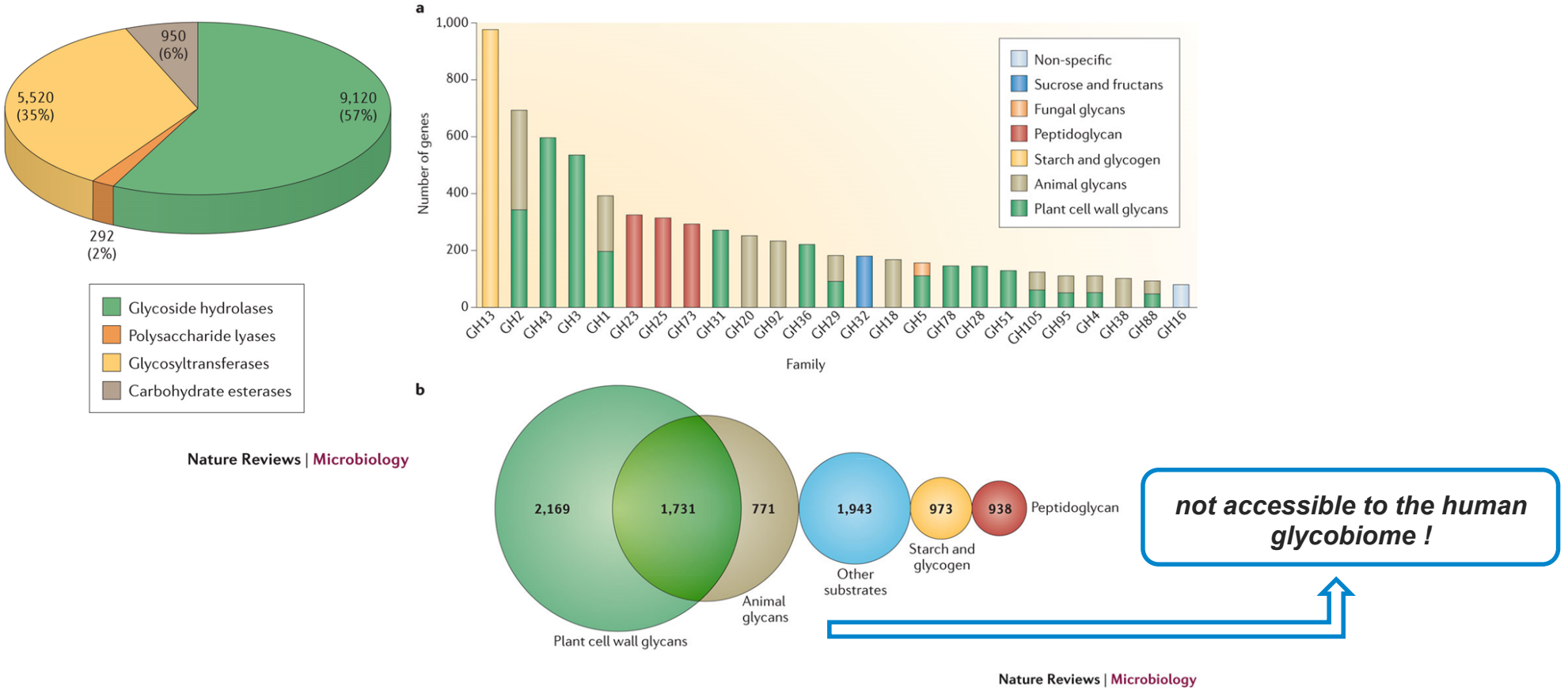
SCFA

key microbiota metabolites regulating the host nutritional status

equipped with a real arsenal of CAZymes – absent in human genome – intestinal microorganisms degrade plant polysaccharides to SCFA

SUBSTRATES OF THE GM CAZymes ARSENAL

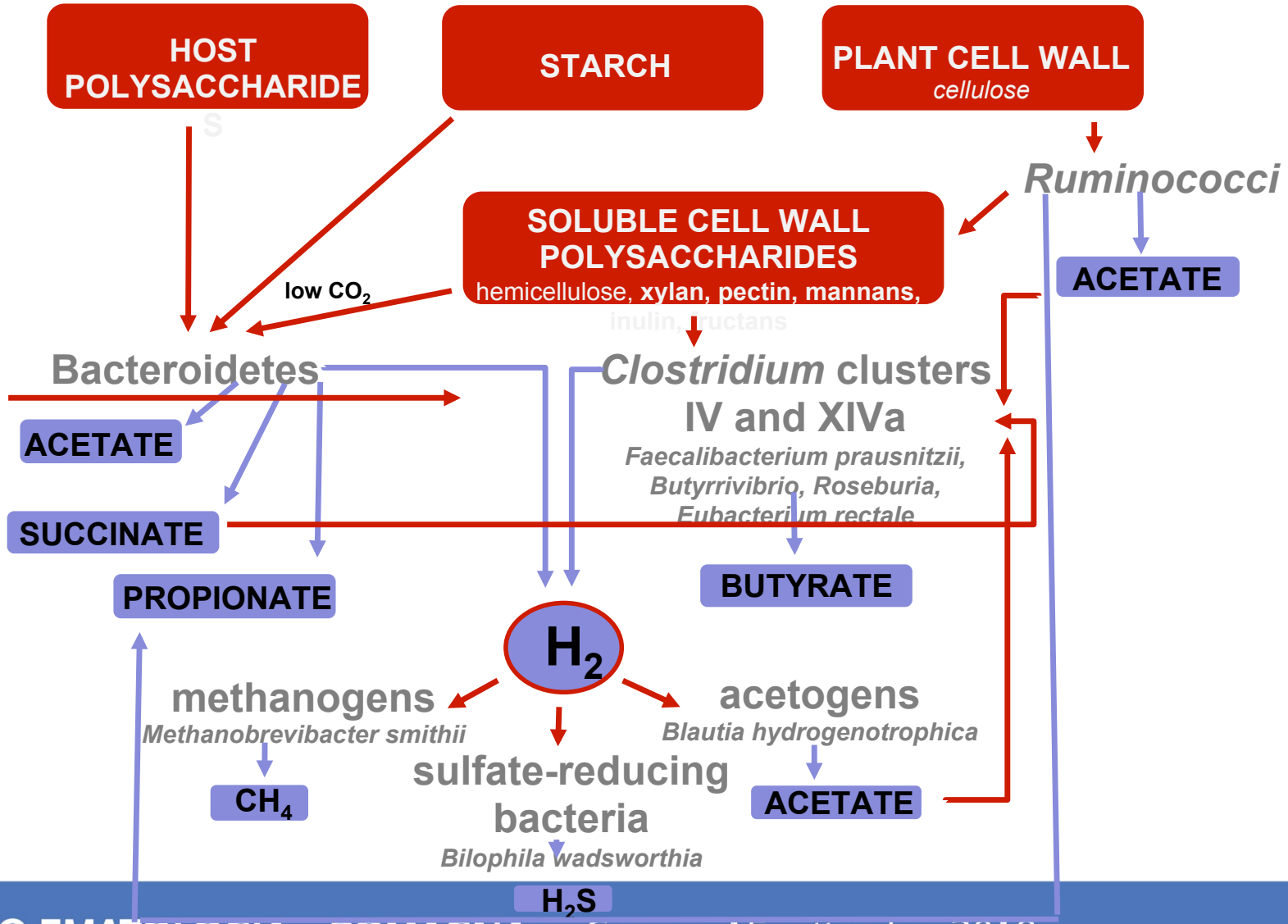
thousands of enzymes while we possess only 17



The GM possesses a broad glycobiome complexity, complementing the limited diversity of the human glycobiome and enhancing the host capacity to metabolize complex polysaccharides

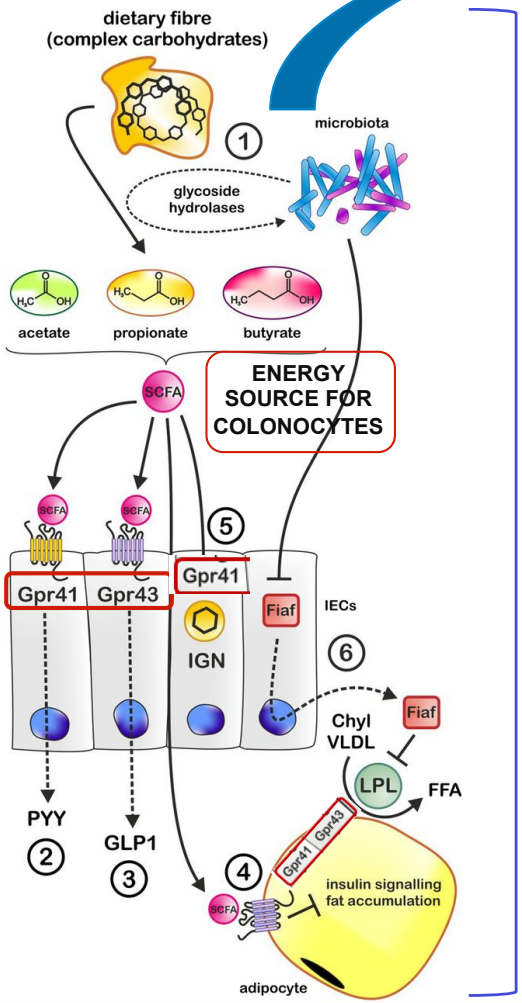
El Kaoutari *et al.*, Nat Rev Microbiol. 2013

DIETARY COMPONENTS: SYNTROPHIC MICROBIAL NETWORKS

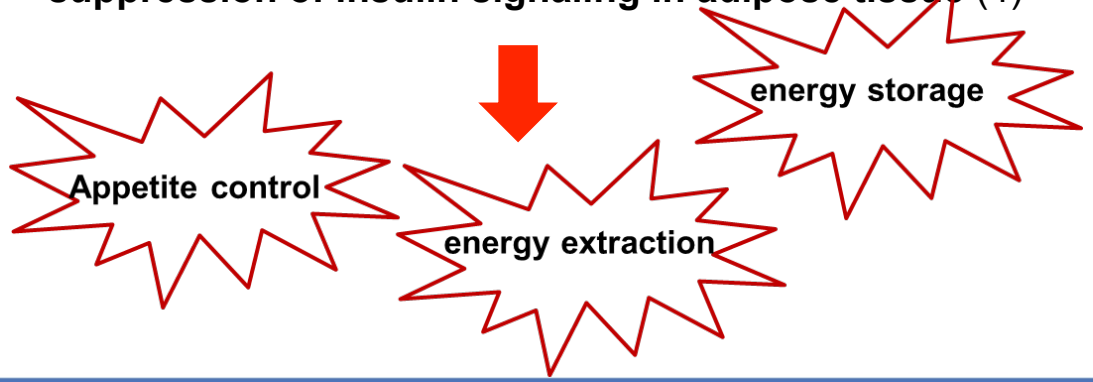


SCFA, MICROBIAL METABOLITES WITH A KEY MULTIFACTORIAL ROLE IN ENERGETIC HOMEOSTASIS

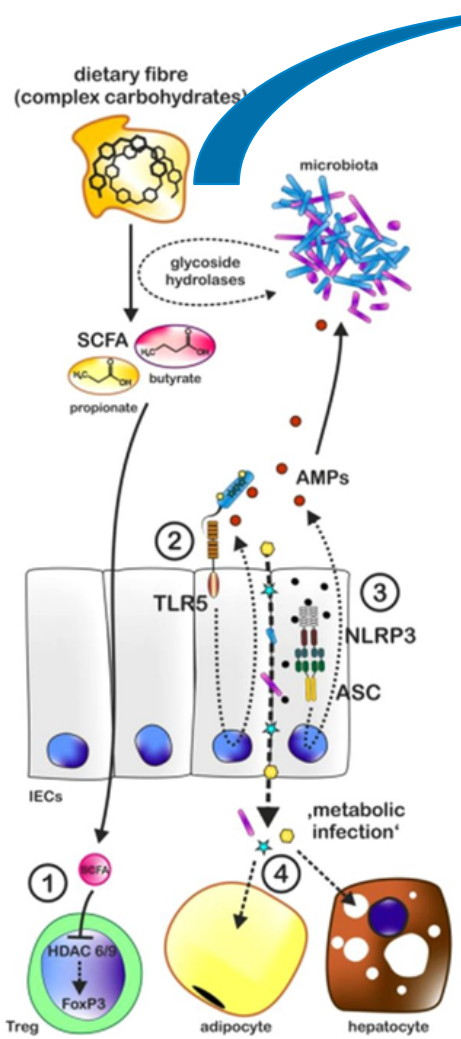
Tilg *et al.*, Gut 2014, Koh et al., Cell, 2016



- ✓ **peptide Pyy expression** (2)**:** inhibition of gut motility; increase of intestinal transit rate; reduction of energy harvest from diet
- ✓ **glucagon-like peptide 1 expression** (3)**:** increase of insulin sensitivity
- ✓ **intestinal gluconeogenesis activation** (5)**:** favors glucose control
- ✓ **expression of fasting-induced adipose factor** (6)**:** favors fat storage
- ✓ **suppression of insulin signaling in adipose tissue** (4)



SCFA POSSESS A KEY MULTIFACTORIAL ROLE IN REGULATION OF THE HOST IMMUNE FUNCTION

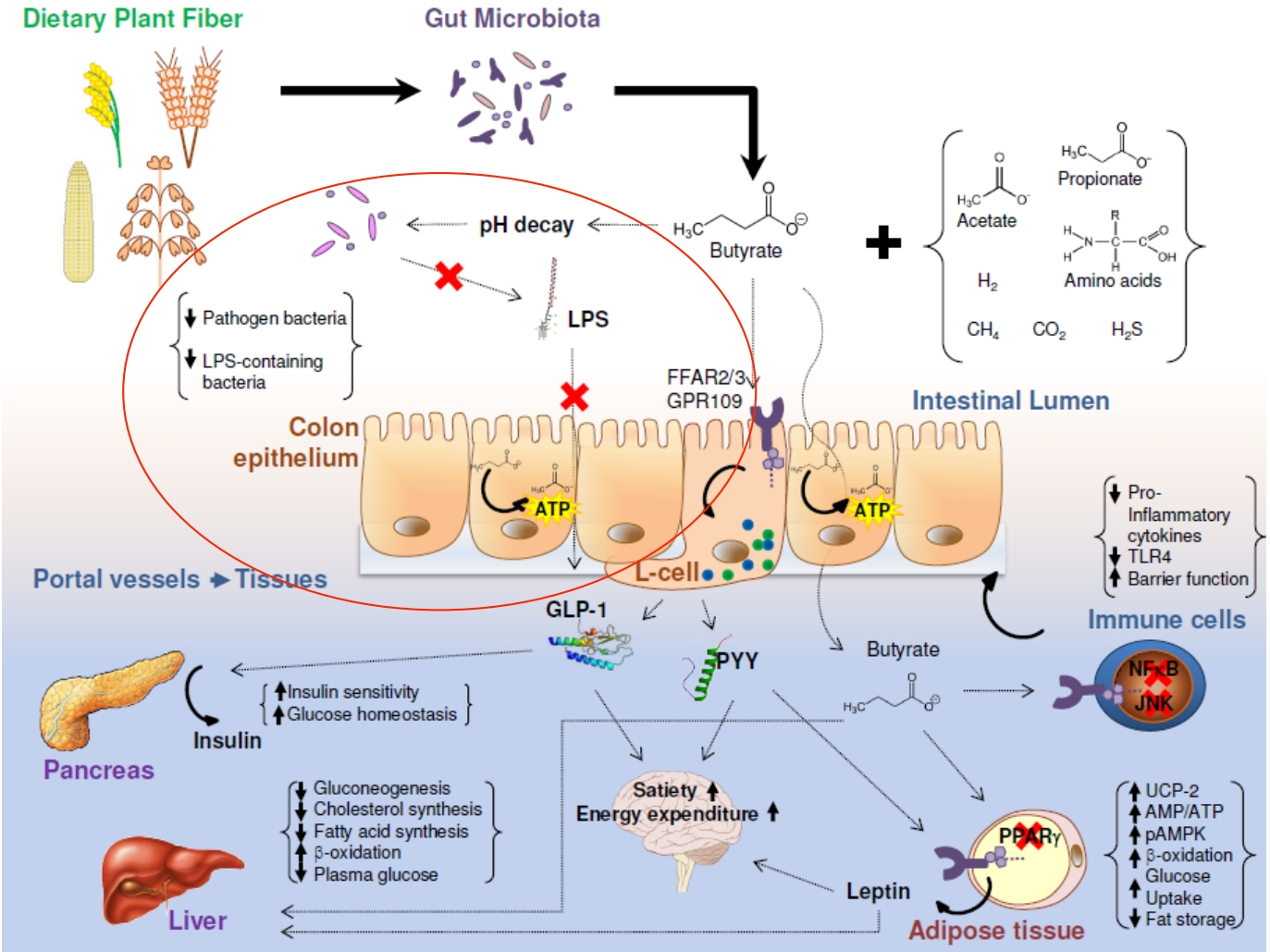


- ✓ **development of colonic and extrathymic Treg**
- ✓ **regulation of bone marrow hematopoiesis**
- ✓ **regulation of dendritic cell function**

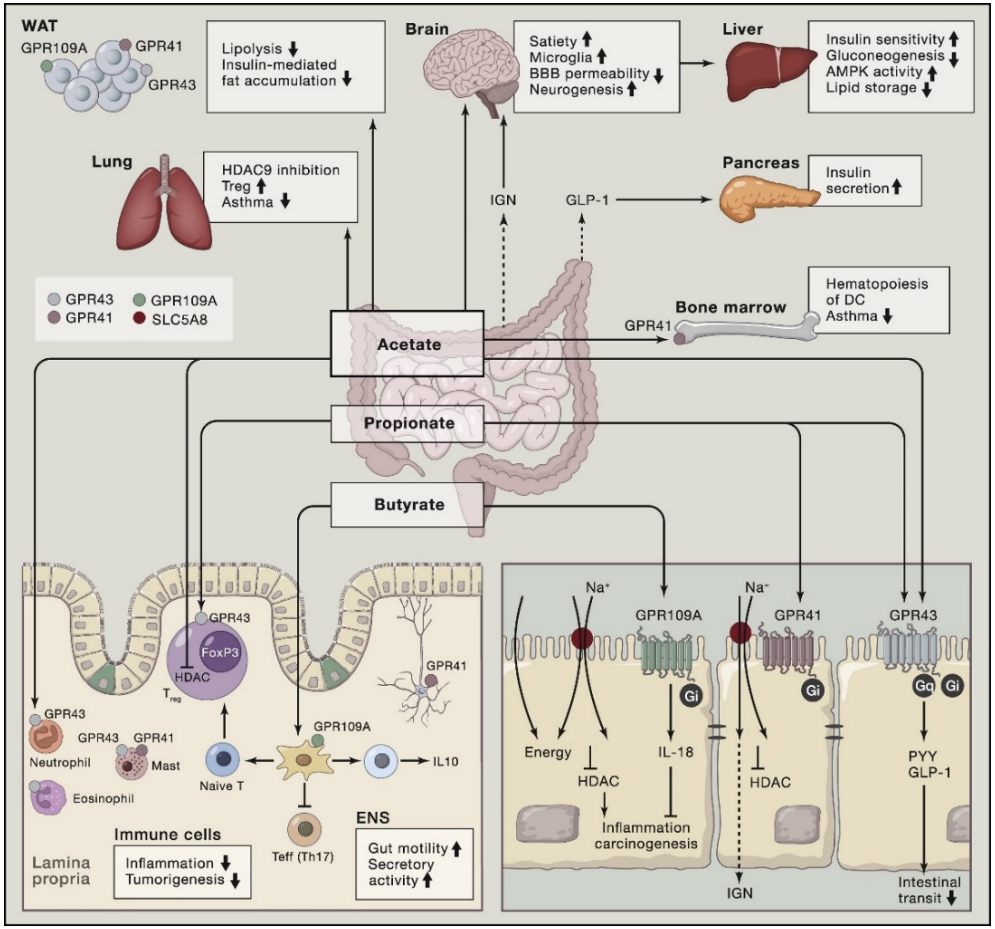
Immune homeostasis

Tilg *et al.*, Gut 2014, Koh et al., Cell, 2016

SCFA AND METABOLIC ENDOTOXEMIA



2018 SCFA, MICROBIAL METABOLITES WITH A KEY MULTIFACTORIAL ROLE IN HOST PHYSIOLOGY



SCFAs as Signaling Molecules

- *HDAC inhibitors*
- *GPCR (41,43,109A) ligands*

Serotonin biosynthesis

GABA production in CNS

CNS activity

Modulation of ENS and CNS function

energy source

Intestinal gluconeogenesis

Metabolic regulations

anti lipolytic activity (WAT)

release of major appetite and glucose regulatory peptides (PYY, GPL1) (L-cells)

leptin production (WAT)

Enhancement of IL-10 (DC)

Reduction of TNF-α, IL-6, IFN-γ (DC)

IL-18 biosynthesis (IEC)

Immune homeostasis

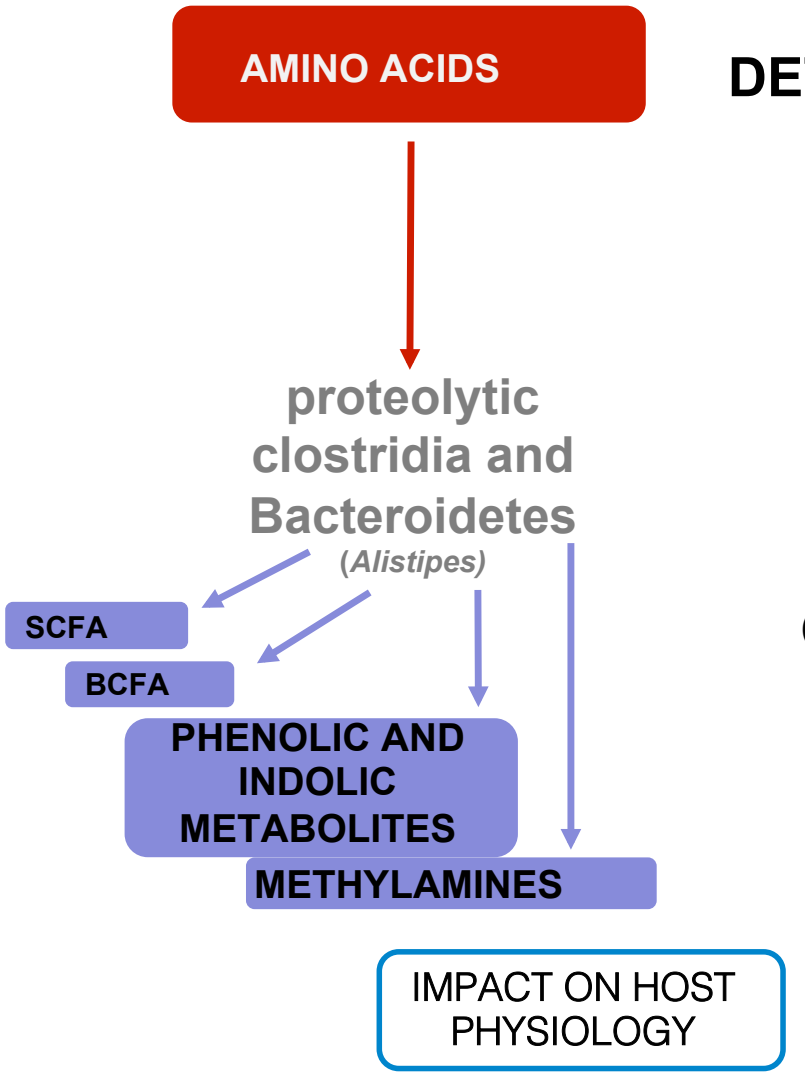
IEC barrier fortification

IgA promotion

Treg polarization

Nastasi *et al.*, Sci Rep. 2015; Koh *et al.*, Cell. 2016

SIDE PRODUCTS FROM GM PROTEIN FERMENTATION



DETRIMENTAL FOR THE HOST HEALTH

AROMATIC AMINO ACIDS

PHENOLIC AND INDOLIC METABOLITES
(indoles; p-cresol; phenols; phenylacetic acid)

LIVER STEATOSIS, OBESITY AND DIABETES

ALIPHATIC AMINO ACIDS

METHYLAMINES

IMMUNE ACTIVATION AND DIABETES

IMPACT ON HOST PHYSIOLOGY OF THE GM ADAPTATION TO FAT

DIETARY FATS → **BILE ACIDS**

Firmicutes ↑
Clostridia, Erysipelotrichi

Enterobacteriaceae ↑

MICROBIOTA MAKE-UP

Bacteroidetes ↓

sufate-reducing bacteria ↑
Bilophila wadsworthia

BIOACTIVE COMPOUNDS

SECONDARY BILE ACIDS

reabsorption and

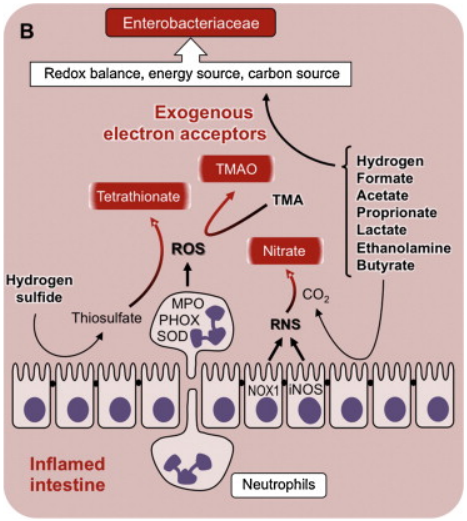
MODULATION OF FXR AND TGR5 RECEPTORS

REGULATION OF DIETARY FAT ABSORPTION

OBESITY, TYPE II DIABETES

H_2S

PRO-INFLAMMATORY DYSBIOSES OF GM

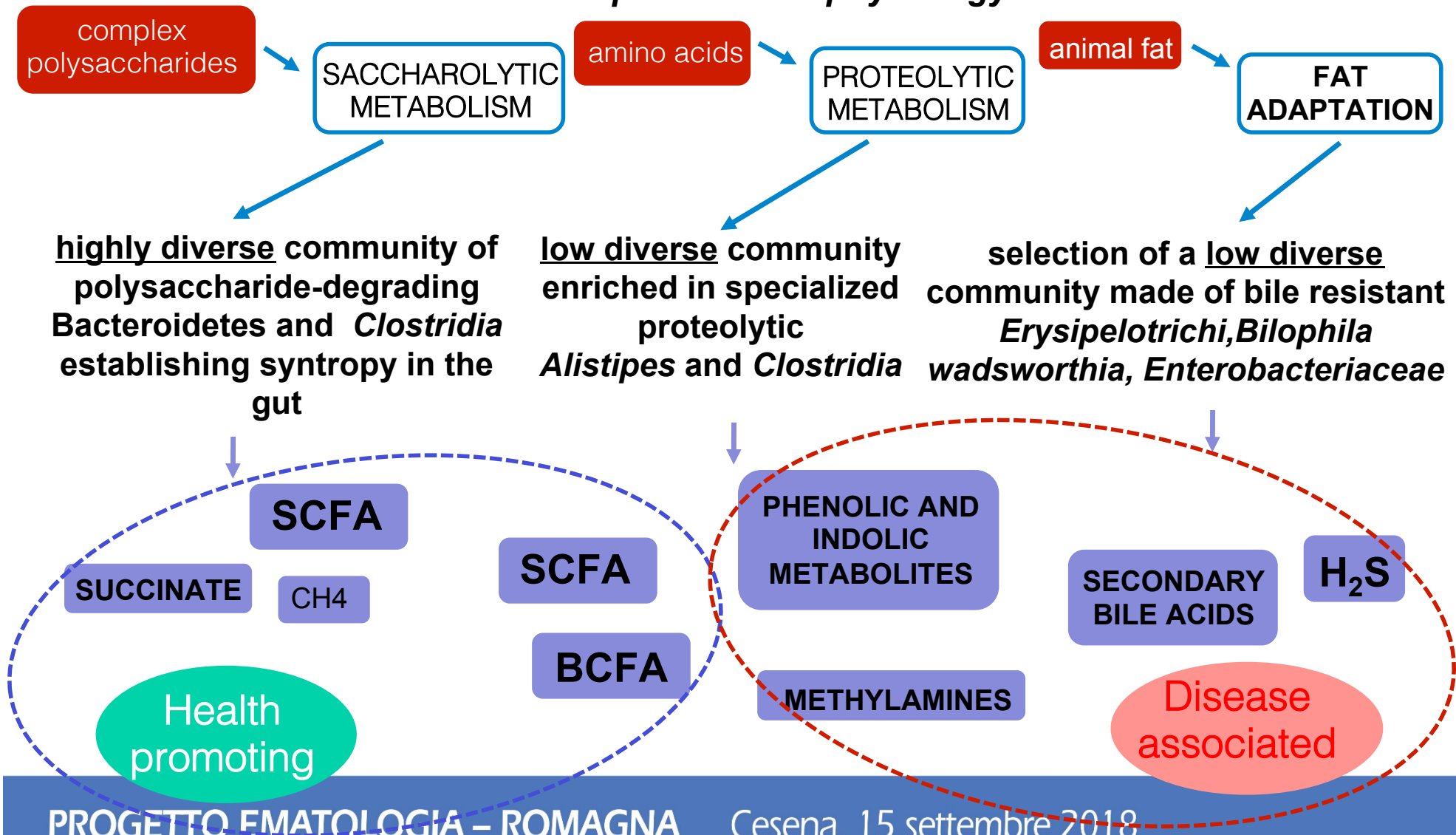


INCREASE OF TRANSEPITHELIAL PERMEABILITY

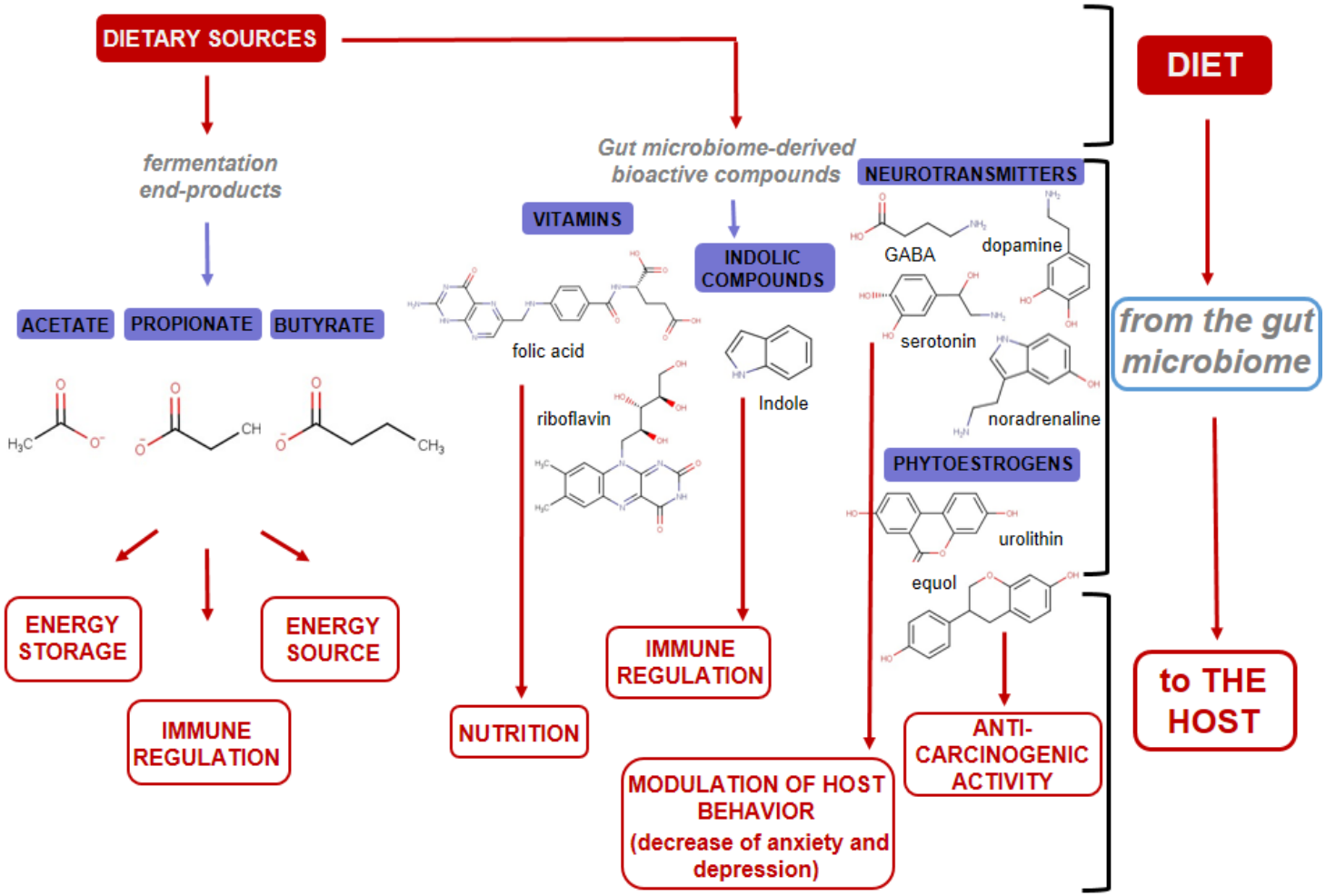
INFLAMMATION IN THE GUT

GM-HOST CO-METABOLIC LAYOUTS

diet regulates microbiota composition and metabolic output with a final impact on host physiology



THE DIET - FROM THE GUT MICROBIOME - TO THE HOST AXIS

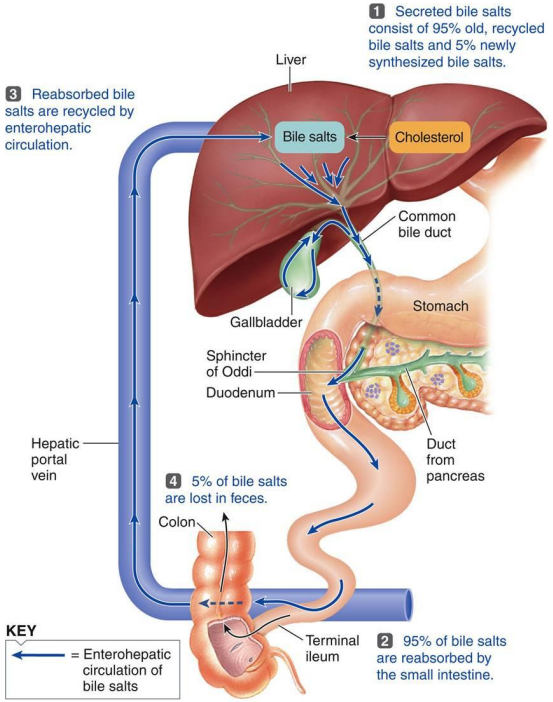


Turroni et al., JMC 2018

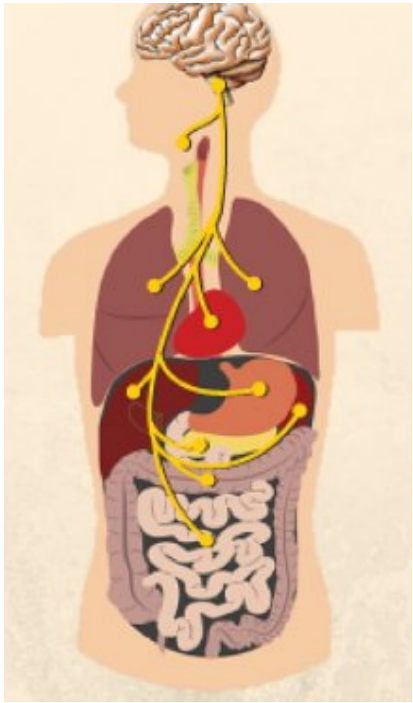
GM-HOST SYSTEM CONNECTION

Produced locally, molecular signals from the GM can reach extra-intestinal organs, establishing a system-level connection between the GM and the immune, endocrine, metabolic and nervous apparatus

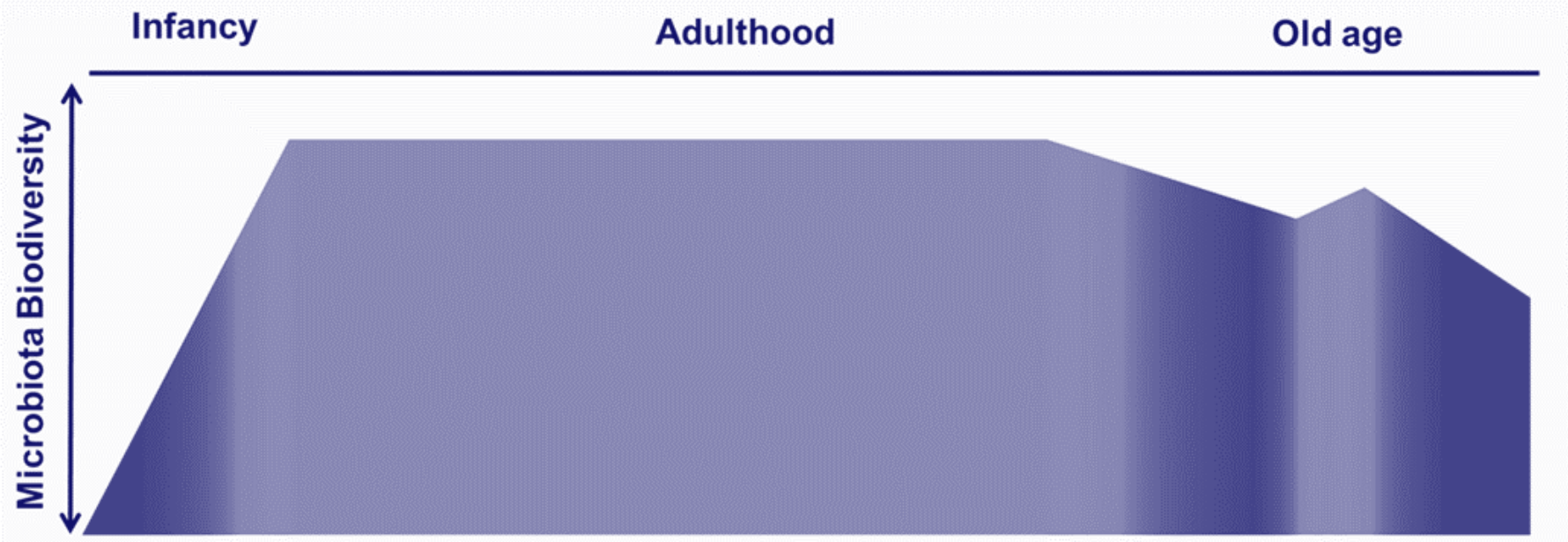
ENTEROHEPATIC CIRCULATION



VAGUS NERVE



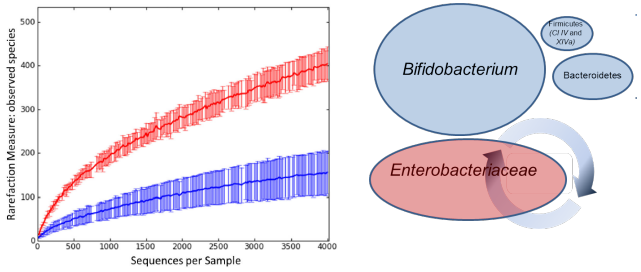
THE GUT MICROBIOTA DESCRIBES AN ADAPTIVE TRAJECTORY ALONG HUMAN AGING



AGE RELATED CHANGES OF THE GUT MICROBIOTA PROVIDE THE HOST WITH **ECOLOGICAL SERVICES CALIBRATED FOR EACH STAGE OF LIFE**

Candela et al., Critical Rev Microbiol, 2013

INFANT-TYPE MICROBIOTA: SIMPLE, READILY CHANGEABLE AND BIFIDOBACTERIUM-



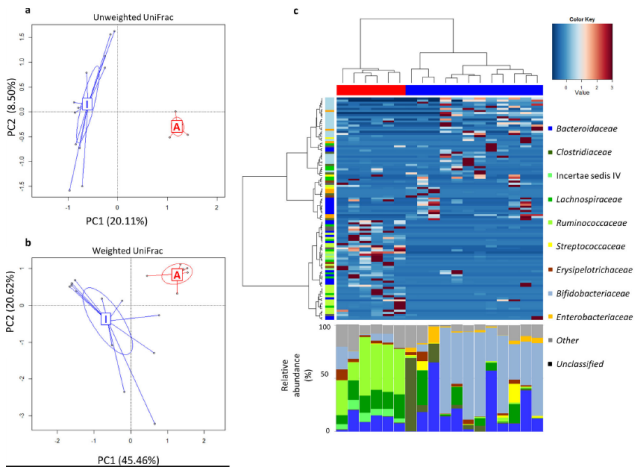


Table 3. TNF-α impact on the HT29 cell-associated microbiota fraction in breast-fed infants and adults.

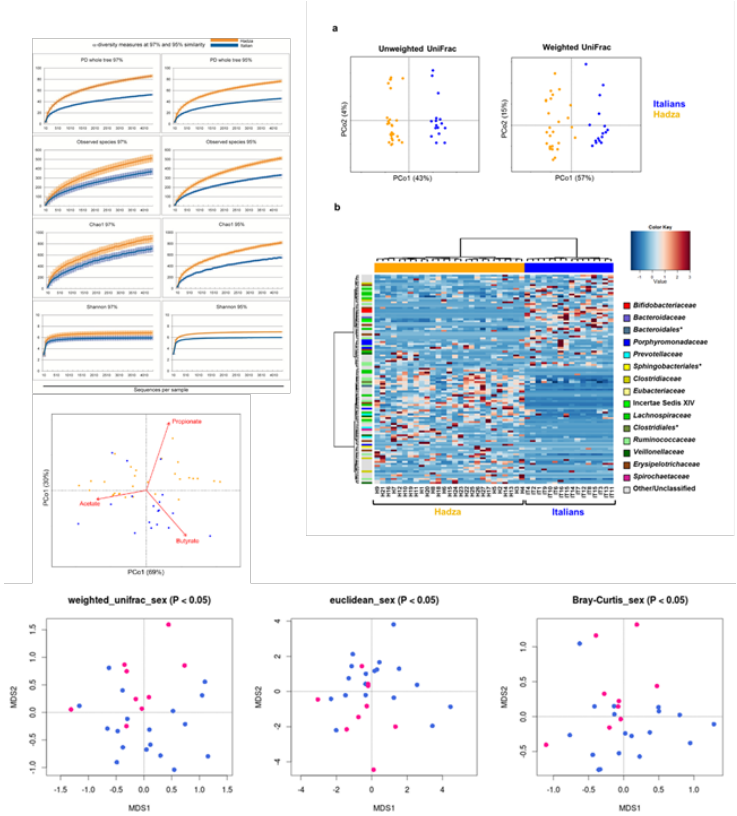
	Breast-fed infants			Adults		
Microbial group	**- TNF-α**	**+ TNF-α**	***FRD***	**- TNF-α**	**+ TNF-α**	***FRD***
Bacteroides - Prevotella	10.8	9.3	n.s.	23.5	15.4	**0.008**
***Clostridium* cluster IV**	1.7	2.5	0.520	21.5	18.2	n.s.
***Clostridium* cluster IX**	5.4	5.1	n.s.	5.2	3.4	n.s.
***Clostridium* cluster XIVa**	5.0	4.8	n.s.	28.7	37.5	0.154
***Clostridium* cluster XI**	0.5	0.6	n.s.	0.3	1.3	**<0.001**
***Clostridium* cluster I, II**	1.4	1.3	n.s.	1.2	1.8	0.24
Lactobacillaceae	6.9	7.4	n.s.	1.9	2.1	n.s.
Bifidobacteriaceae	14.4	13.1	n.s.	4.0	5.8	n.s.
Verrucomicrobiae	0.8	1.0	n.s.	3.3	1.5	n.s.
Bacillaceae	5.5	6.3	n.s.	1.8	4.0	**0.004**
Fusobacteriaceae	0.6	0.8	0.570	1.1	1.7	0.132
Enterococcales	10.9	10.4	n.s.	1.6	2.5	0.430
Enterobacteriaceae	35.3	36.8	n.s.	5.5	3.8	0.360
Campylobacteriaceae	0.7	0.8	n.s.	0.3	1.1	**<0.001**

For each microbial group, mean relative abundance (%) and statistical significance

INFANT-TYPE MICROBIOTRA IS STRUCTURED TO COPE WITH INFLAMMATION, BEING CO-EVOLVED TO PRIME THE EARLY IMMUNE SYSTEM IN THE CONTEXT OF TRANSIENT INFLAMMATORY RESPONSES; MILK DIGESTION; FOLATE BIOSYNTHESIS

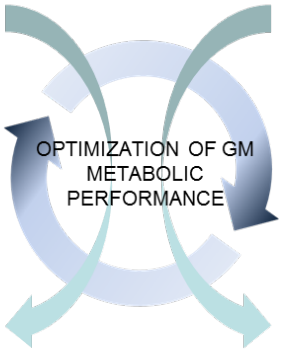
Centanni et al., PLoS ONE. 2013

ADULT-TYPE GM: COMPLEX AND ADAPTABLE ECOSYSTEM DOMINATED BY FIRMICUTES AND BACTEROIDETES



FUNCTIONALLY STRUCTURED TO PROVIDE THE HOST WITH SCFA FROM INDIGESTIBLE PLANT POLYSACCHARIDES

DIFFERENT DIETARY SUBSTRATES



EXTRASOMATIC ADAPTATION TO DIFFERENT DIETARY HABITS AND LIFESTYLE FACTORS

IMPROVEMENT OF ENERGY EXTRACTION

CONTROL OF ENERGETIC HOMEOSTASIS

OPTIMIZATION OF IMMUNOLOGICAL PERFORMANCE

Schnorr et al., Nature Communication, 2014

ELDERLY TYPE MICROBIOTA

Table 2. Genus-like bacterial groups that were found to differ significantly between centenarians (C), elderly (E) and young adults (Y).

		Relative contribution (%)[A]			Ratio[B]		P value[C]	
Phylum/order	**Genus-like phylogenetic group**	**C**	**E**	**Y**	**C/E**	**C/Y**	**C *vs* E**	**C *vs* Y**
Clostridium cluster XV	*Eubacterium limosum et rel.*	0.35	0.02	0.02	16	14.5	0.0009	0.01
Proteobacteria	*Klebsiella pneumoniae et rel.*	0.17	0.03	0.02	5.3	6.7	0.002	0.0009
	Vibrio	0.15	0.03	0.03	5.4	5.4	<0.0001	<0.0001
	Enterobacter aerogenes et rel.	0.05	0.03	0.02	1.9	2.1	0.03	0.04
Actinobacteria	*Eggerthella lenta et rel.*	0.11	0.06	0.04	1.8	2.7	0.02	0.0001
Bacilli	*Bacillus*	0.07	0.05	0.03	1.4	2.0	0.01	0.04
Clostridium cluster IV	*Clostridium leptum et rel.*	2.37	1.27	1.33	1.8	1.8	0.006	0.005
	Sporobacter termiditis et rel.	1.14	0.75	0.70	1.5	1.6	0.05	0.04
	Anaerotruncus colihominis et rel.	0.99	0.68	0.66	1.4	1.5	0.08	0.01
	Clostridium orbiscindens et rel.	1.52	1.05	1.16	1.4	1.3	0.03	0.08
	Faecalibacterium prausnitzii et rel.	2.01	4.05	4.24	0.5	0.5	0.01	0.006
	Papillibacter cinnamovorans et rel.	1.30	1.72	1.80	0.7	0.7	0.06	0.04
Clostridium cluster XIVa	*Clostridium colinim et rel.*	0.90	1.98	1.57	0.4	0.6	0.06	0.05
	Clostridium sphenoides et rel.	0.97	1.92	1.50	0.5	0.6	0.0002	0.003
	Eubacterium hallii et rel.	3.16	4.75	5.76	0.7	0.5	0.03	0.004
	Eubacterium rectale et rel.	1.68	3.61	3.02	0.5	0.5	0.001	0.004
	Eubacterium ventriosum et rel	1.21	2.77	2.62	0.4	0.4	0.0005	0.0002
	Lachnobacillus bovis et rel.	1.15	1.98	1.46	0.6	0.8	0.007	0.03
	Outgrouping *Clostridium* cluster XIVa	0.63	0.94	1.04	0.7	0.6	0.02	0.01
	Roseburia intestinalis et rel.	1.57	3.04	3.21	0.5	0.5	0.006	0.03
	Ruminococcus lactaris et rel.	0.65	1.07	0.87	0.6	0.7	0.002	0.01
	Ruminococcus obeum et rel.	1.73	2.79	2.65	0.6	0.6	0.003	0.01

[A]Relative contributions of genus-like phylogenetic group to the fecal microbiota was calculated as percentage of signal intensities the total signal intensity.
[B]Ratio of the average relative abundance of each genus-like phylogenetic group calculated for subjects belonging to C and E groups, and to C and Y groups. Bacterial groups showing C/E and C/Y ratio <1 decreased in the subjects of group C.
[C]In italic, relevant groups with P values ranging from 0.05 to 0.08 (italics) are reported.
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- LOW DIVERSITY
- DECREASE IN BIFIDOBACTERIA
- INCREASE IN PATHOBIONTS

  (*Fusobacteria, Bacillus, Staphylococcus, Enterobacteriaceae*)

SUBDOMINANT FRACTION

- REARRANGEMENT OF BUTYRATE PRODUCERS

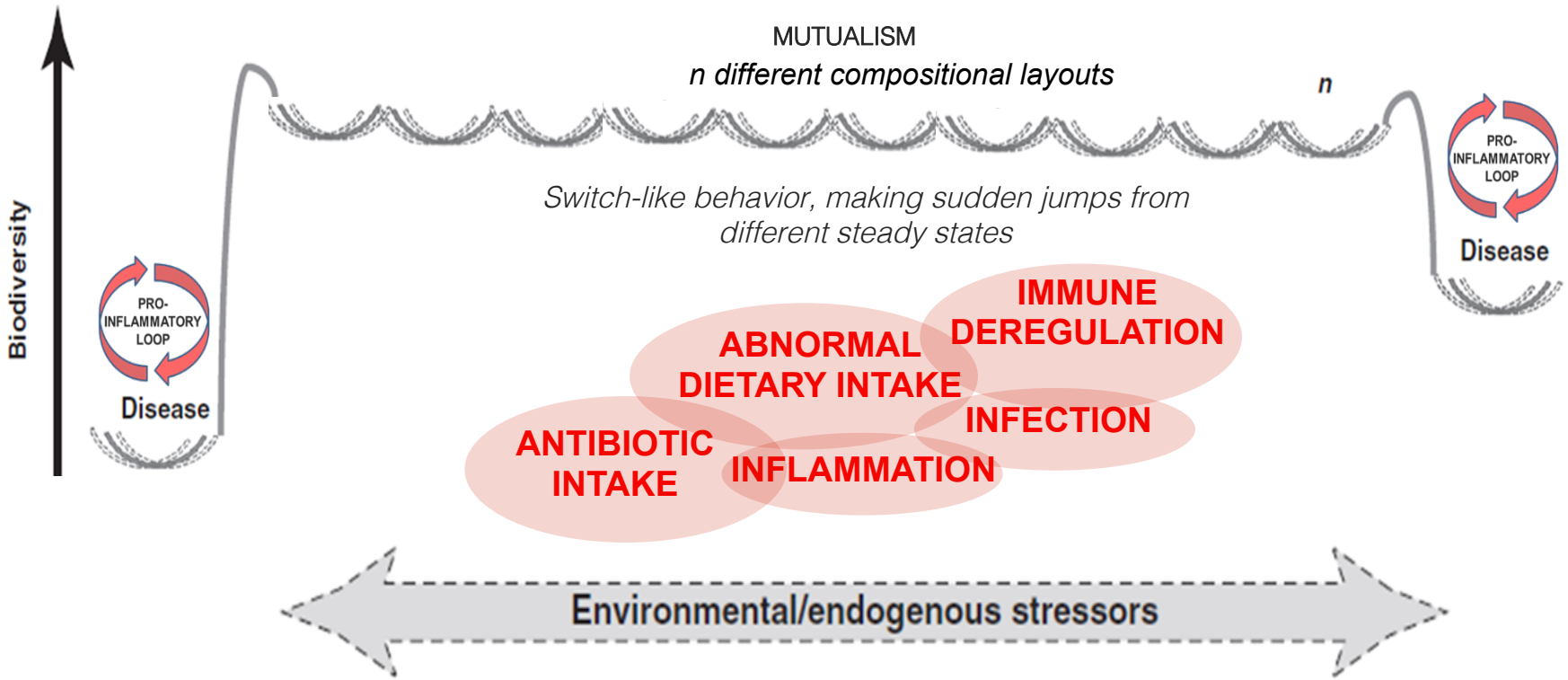
DOMINANT FRACTION

Ruminococcus obeum (Cl XIVa)
Roseburia intestinalis (Cl XIVa)
Eubacterium rectale (Cl XIVa)
Faecalibacterium prausnitzii (Cl IV)

Anaerotruncus colihominis (Cl IV)
Eubacterium limosum (Cl XV)

Biagi et al., Cur Biol, 2016

MUTUALISM BREAKDOWN



RUPTURE OF THE GM-HOST MUTUALISTIC AGREEMENT AND COMPROMISED HOST ENERGY BALANCE AND IMMUNE HOMEOSTASIS

INFLAMMATION AND MICROBIOTA

A non-controlled pro-inflammatory pathway can dramatically impact on the composition of GM