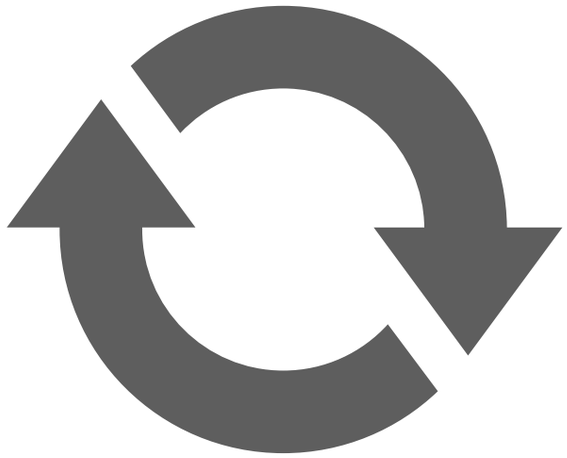
L08b

Symbiosis

- **Mutualism**
- **Parasitism**
- **Commensalism**

Dysbiosis



Holobiont

Symbiosis

- **Symbiosis:** An association between two dissimilar (or similar) (micro or macro-) organisms that have some degree of physical association, which is potentially long lasting, regardless of the implications for the fitness of either organism —> **living together**

Symbiosis

- **Parasitism:** An antagonistic symbiotic relationship in which one species is harmed, while the other benefits
- **Mutualism:** A symbiotic relationship in which both interacting species benefit, or are perceived to benefit. Benefit is often only confirmed empirically for the host symbiosis, in which the organisms are involved in a normal metabolic and immune signaling interactions
- **Commensalism**: A symbiotic relationship in which only one of interacting species benefit, or is perceived to benefit.

Dysbiosis

- ***Dysbiosis:*** *A status in which the relationship or interactions are heavily altered, possibly related to a major stress or infection event, are reversible (unhealthy state of the organisms), antonym is* ***Eubiosis***

- ***Dysbiosis*** *can cause/worsen a* ***disease***

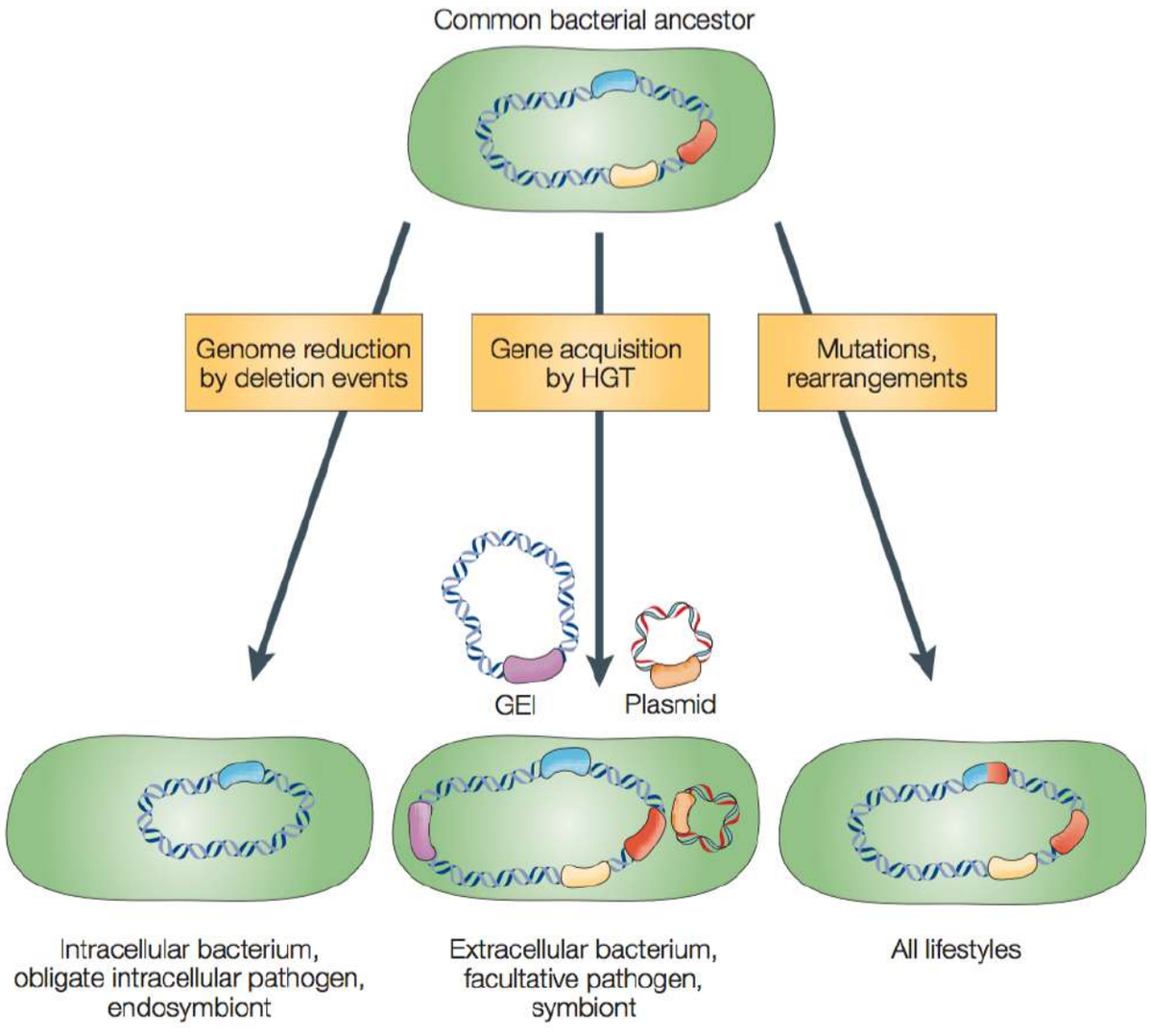
Holobiont

- From a non-biologist perspective, a holobiont can be thought of as a complex and interconnected system of organisms living in close association with each other
- *A **holobiont** is not a single organism but rather a collection of different species of organisms, including the host organism and various microorganisms (bacteria, archaea, viruses, fungi) that live within and on the host organism*
- *These microorganisms are not just passive inhabitants but are **actively** involved in various aspects of the **host biology**, including their digestion, immune responses, and even behaviour*

Symbiosis in the light of evolution

Origins many millions of years ago and have evolved to benefit the physiology of both partners, a process called **coevolution** —> **obligate symbiosis**

Obligate symbiosis: Streamlined genomes: retain only genes required for host fitness and essential molecular processes, such as translation, replication, and transcription



Dobrindt et al., 2004

Symbiosis lexicon

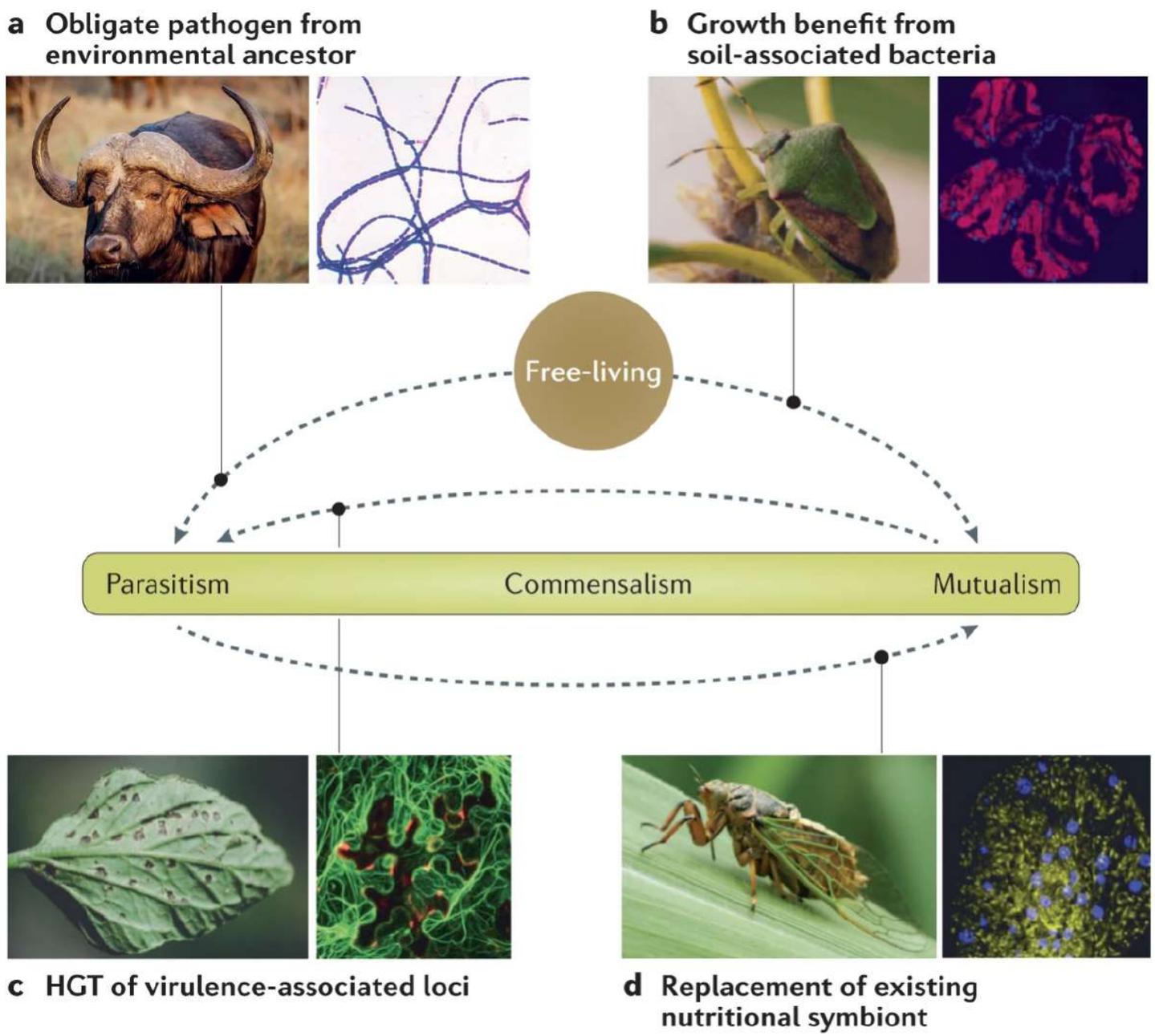
- **Parasites** are microorganisms that benefit **at some expense to the host**
- **Pathogens** actually cause a **disease** in the host
- **Commensals** have **no discernible effect** on the host
- **Mutualists are beneficial to the host**
- **Mutualistic microorganisms** as intimate **evolutionary partners** that influence both the evolution and physiology of their hosts
- **Pluricellular organisms have developed specialised structure to garden the symbiotic microbes**

Symbiosis, II

Trends in Ecology & Evolution

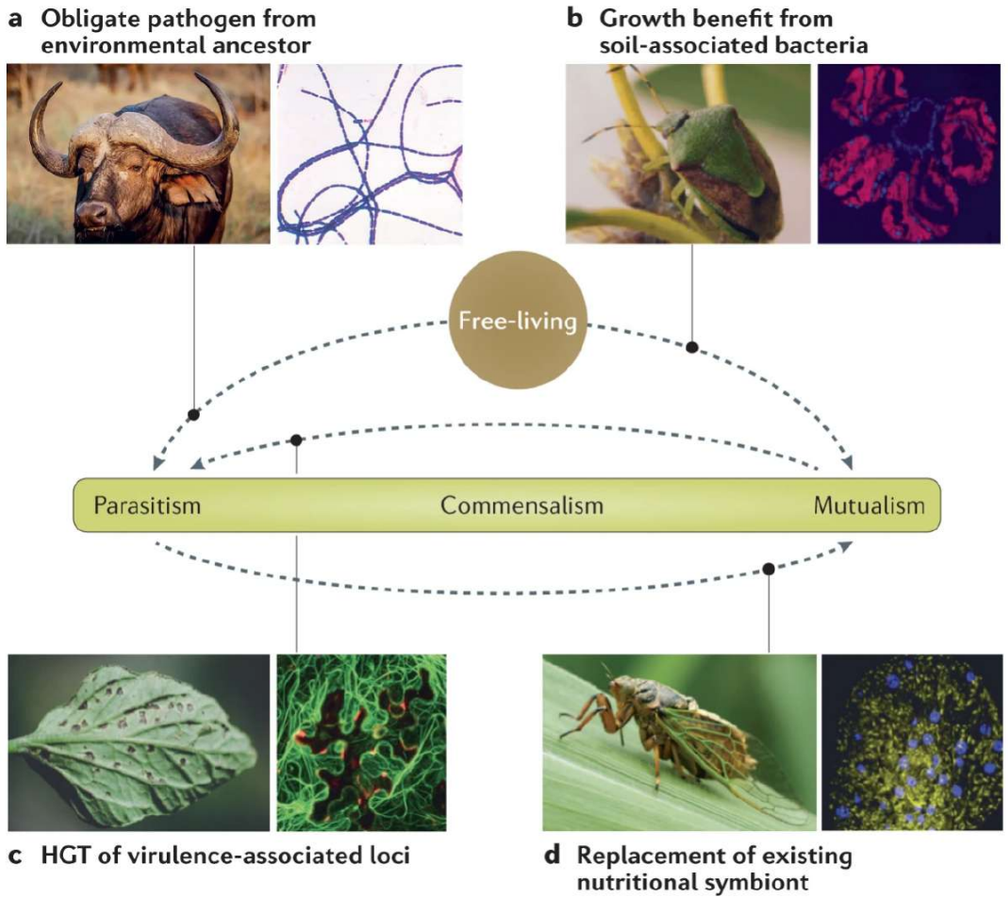
- Many hosts, both plants and animals, have **evolved specialized structures to filter and house beneficial microbes**
- Symbiotic organs share some core features linked to the **evolutionary maintenance of beneficial symbiosis**
- 'Joint phenotypes' have developed given the various selection pressures on symbiotic organs, including fitness feedbacks and conflicts between interacting genomes

Evolutionary transitions onto and along the parasite–mutualist continuum



Drew et al., 2021

Legend



a. Evolution of parasitic species in the *Bacillus cereus* group (for example, the causative agent of anthrax) from soil-dwelling ancestors

b. Environmental *Pantoea* bacteria evolving obligate mutualistic roles in stink bug growth and development

c. The widespread plant parasite *Pseudomonas syringae* likely evolving from mutualistic ancestors, driven by horizontal gene transfer (HGT) of type III secretion systems

d. Entomopathogens taking over the metabolic role of an ancient and degraded endosymbiont in cicadas

Drew et al., 2021

Holobiont

Coral
Sponge
Seagrass

Human
Mycorrhizae
Seagrass

Case studies:

1. Legume-Root Nodule
2. Lichen
3. Mycorrhizae
4. Hydrothermal vent chemolithotrophs and their animal hosts
5. Bobtail squid and *Aliivibrio fischeri*
6. Gut microbiota - Thermites and Mammals
7. Humans

U= nutritional
B= behaviour
Strawberry= gutless
Orange= light energy

Legume-Root Nodule Symbiosis
Lichen
Mycorrhizae

Legume-Root Nodule Symbiosis

- Partners in a symbiosis are called **symbionts, and most nitrogen-fixing bacterial symbionts of plants are collectively called rhizobia**, derived from the name of a major genus, *Rhizobium*
- Species of rhizobia are *Alpha*- or *Betaproteobacteria* that can grow freely in soil or infect leguminous plants and establish a symbiotic relationship
- The same genus (or even species) of legume can contain both rhizobial and non-rhizobial strains
- **Infection of legume roots by rhizobia** leads to the **formation of root nodules** in which the bacteria **fix gaseous nitrogen (N_2)**
- Nitrogen fixation in root nodules accounts for **a fourth of the N_2 fixed annually on Earth** and is of enormous agricultural importance, as it increases the fixed nitrogen content of soil
- Rhizobia can fix N_2 when grown in pure culture under microaerophilic conditions (a low-oxygen environment is necessary because the key nitrogen-fixing enzyme, called **nitrogenase**, is inactivated by high levels of O_2)
- In nodule, **O_2 levels** are precisely controlled by the **O_2-binding protein leghemoglobin** (Fe-containing protein induced through the interaction of the plant and bacterial partners)
- **Specificity in association**

Root Nodule Formation

1. **Recognition** of the correct partner by both plant and bacterium and attachment of the bacterium to the root hairs

2. **Secretion of oligosaccharide signalling molecules (Nod factors) by the bacterium**

3. **Bacterial invasion** of the root hair

4. **Movement of bacteria** to the main **root** by way of the **infection thread**

5. **Formation of modified bacterial cells (bacteroids) within the plant cells**, development of the **N_2-fixing state**, and continued plant and bacterial cell division forming the mature **root nodule**

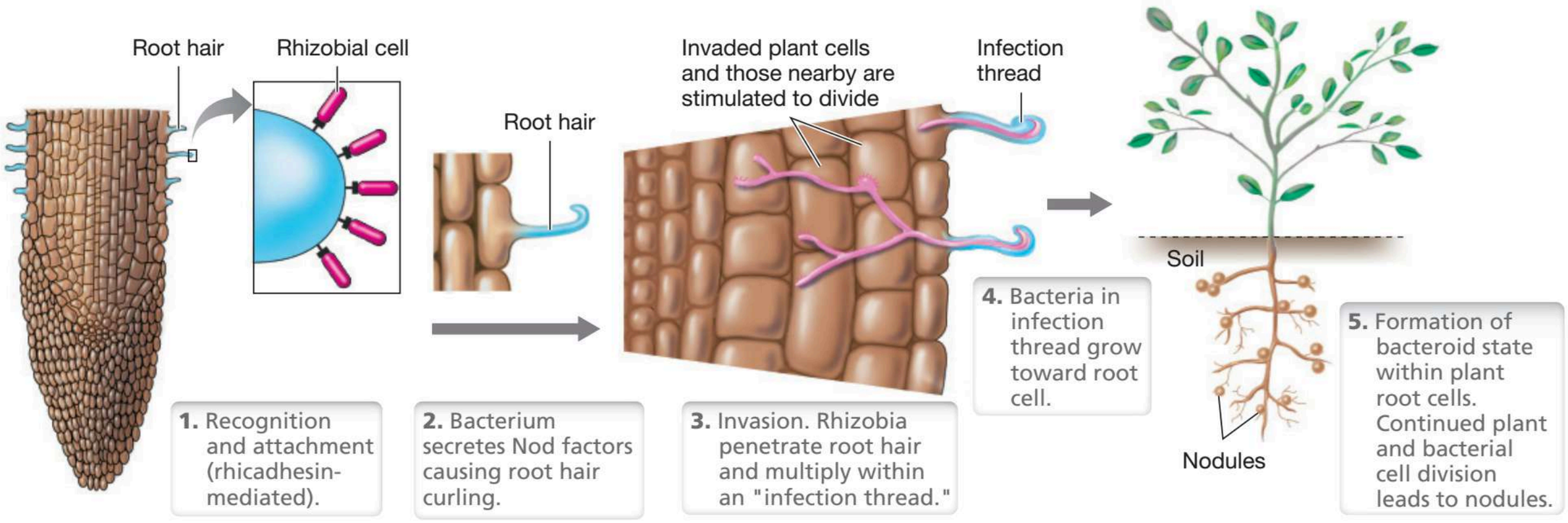
TABLE 23.1 Major cross-inoculation groups of leguminous plants

Host plant	*Nodulated by*
Pea	*Rhizobium leguminosarum* biovar *viciae*[a]
Bean	*Rhizobium leguminosarum* biovar *phaseoli*[a]
Bean	*Rhizobium tropici*
Lotus	*Mesorhizobium loti*
Clover	*Rhizobium leguminosarum* biovar *trifolii*[a]
Alfalfa	*Sinorhizobium meliloti*
Soybean	*Bradyrhizobium japonicum*
Soybean	*Bradyrhizobium elkanii*
Soybean	*Sinorhizobium fredii*
Sesbania rostrata (a tropical legume)	*Azorhizobium caulinodans*

[a]Several varieties (biovars) of *Rhizobium leguminosarum* exist, each capable of nodulating a different legume.

Madigan et al. 2018

Root Nodule Formation

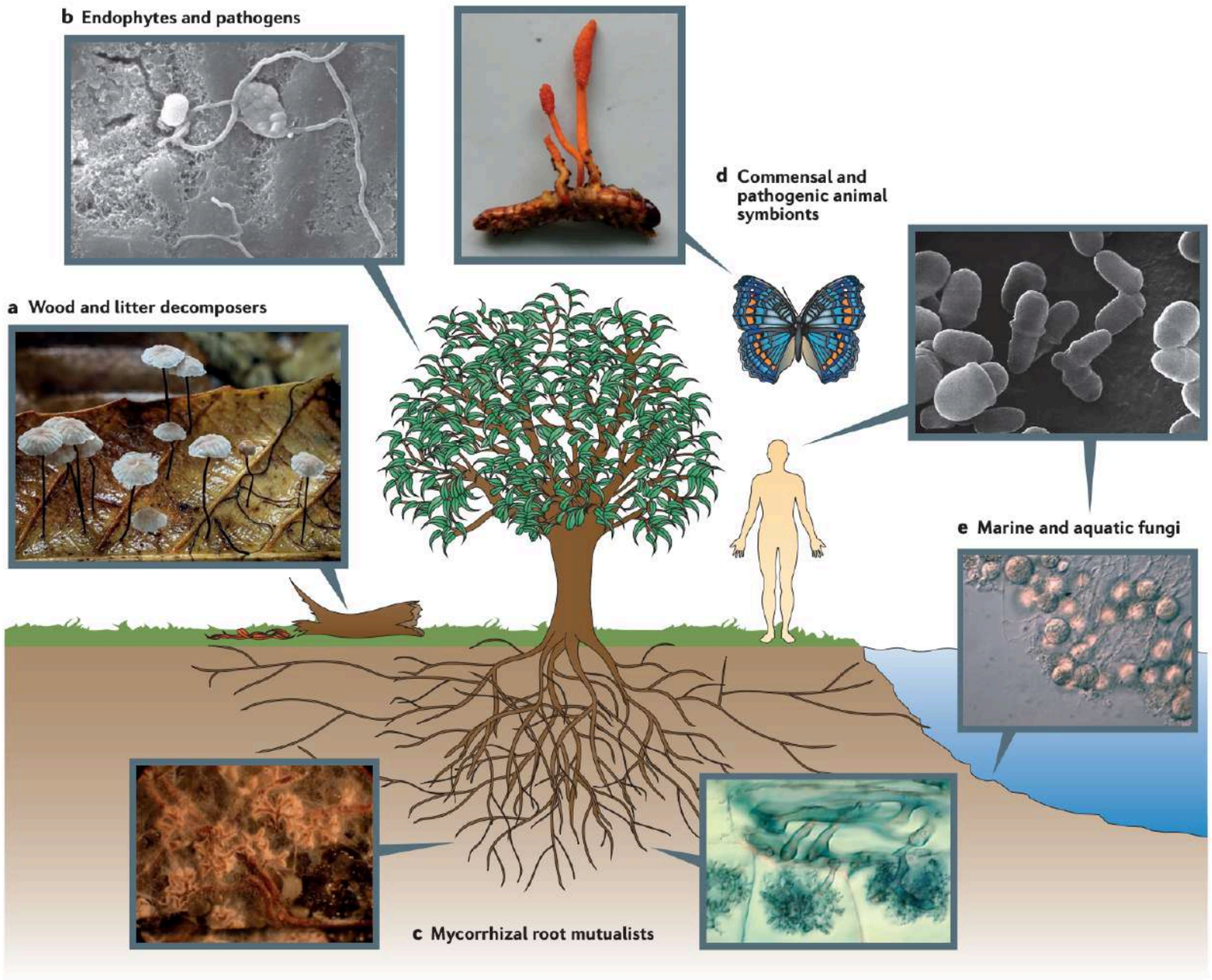


Madigan et al. 2018

Sugar from photosynthesis

Glutamine & Asparagine from N_2 fixation

Mycobiome

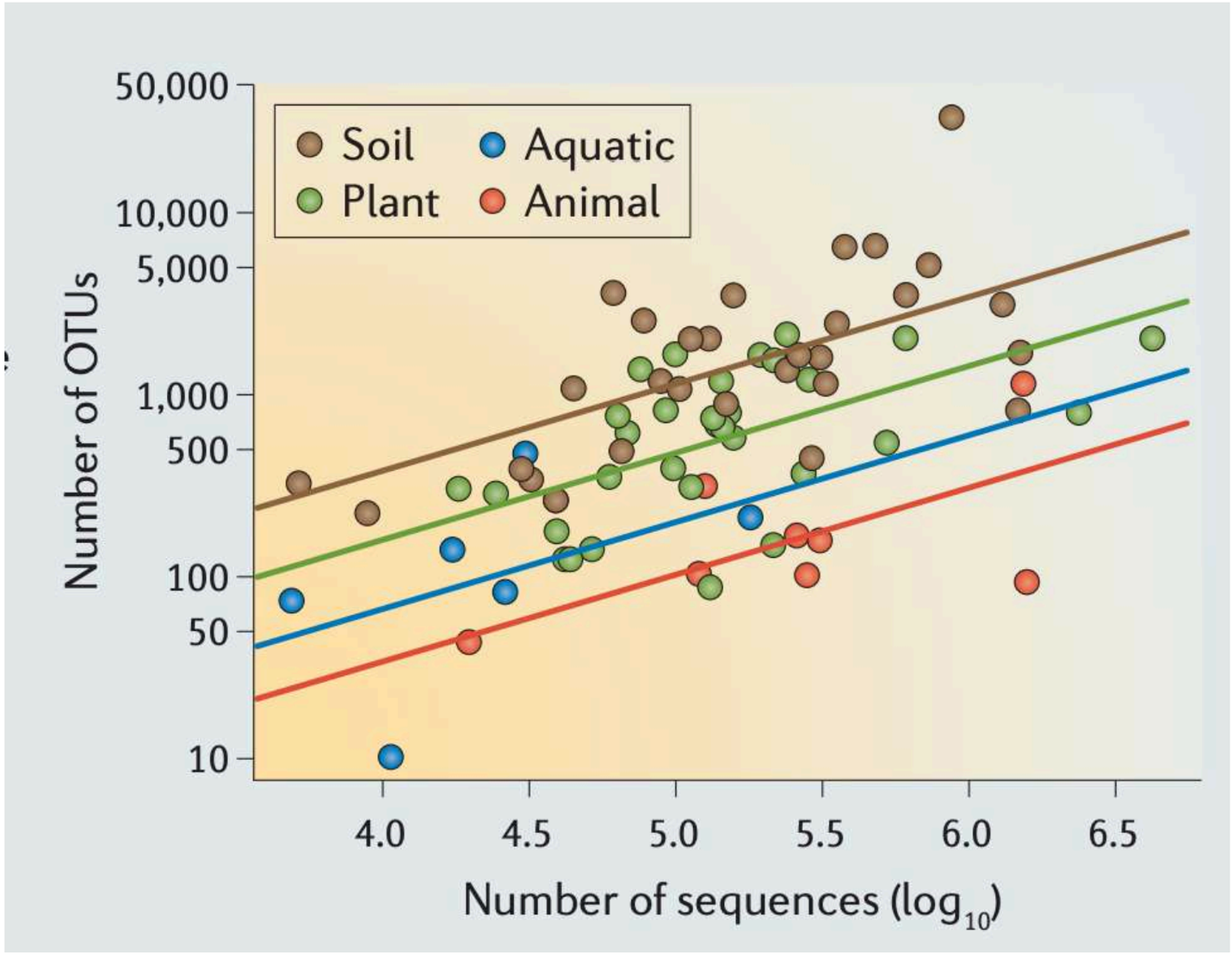


Peay et al., 2016

- **Fungi** are most **commonly associated** with terrestrial ecosystems, but can also be found growing on nearly any substrate on Earth, from deep ocean sediments to the human scalp

Mycobiome

- Majority of fungal species are **saprotrophs that are capable of decomposing complex polymers**, such as cellulose and chitin, although individual species can **vary** considerably in both the **substrates** that they decompose and the **enzymatic pathways** that they use
- In terrestrial and freshwater systems, fungi can dominate the decomposition of plant **necromass**

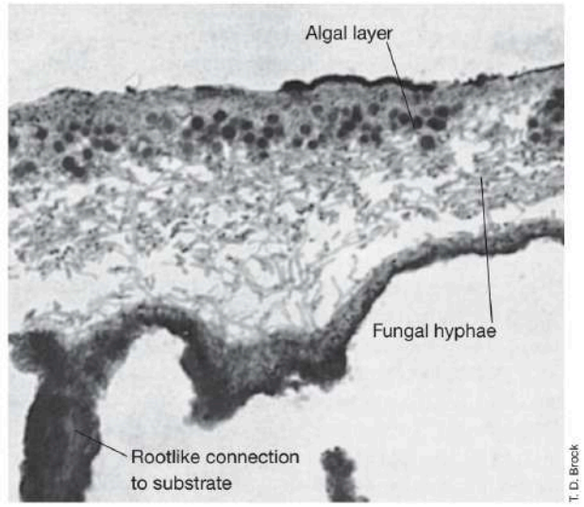


Peay et al., 2016

https://courses.lumenlearning.com/microbiology/chapter/lichens/



Lichen

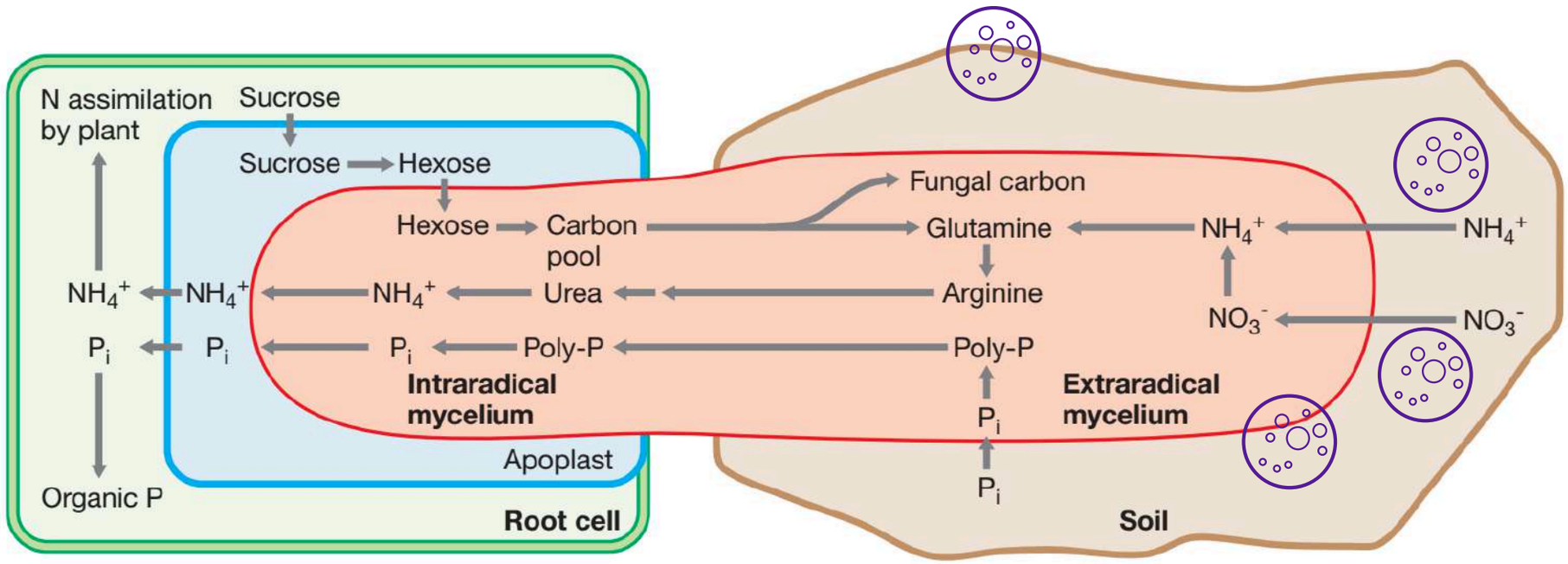


Madigan et al. 2018

- **A lichen** is a mutualistic association between two dominant microorganisms, a **fungus** (**constant humid environment, scavenging limiting elements by lichen complex organic acidic compounds**), usually an ascomycete **but many fungi** (**basidiomycete yeast**) and either an **alga or a cyanobacterium** (**photosynthesis**) but there are also **archaea** (**B12 and protection from toxic compounds**)
- **Morphology** of any given lichen is primarily **determined by the fungus**, and many fungi (more than 18,000 named species) are able to form lichen associations
- **Diversity among the phototrophs is much lower**, and thus many different kinds of lichens have the same phototrophic partner, some N_2 fixation
- **Lichen acids**, **complex organic compounds secreted by the fungus, promote the dissolution and chelation of inorganic nutrients from the rock or other surface** that are needed by the **phototroph**
- **Fungus protects** the phototroph from drying most of the habitats
- **Dry habitats**, fungi tolerate better than phototrophs
- The fungus actually **facilitates the uptake of water**
- Lichens typically **grow quite slowly**

Mycorrhizae

- **Mycorrhizae are symbiotic relationship between plant roots and fungi** in which nutrients are transferred in both directions, ~450 million years ago
- Over 80% of land plants (>250 000 plant species), from the Greek words for fungus and root
- Fungus and associated- microbes transfer inorganic nutrients—in particular, phosphorus and nitrogen—from soil to plant
- Plant transfers primarily carbohydrates to fungus and associated microbes
- Mycorrhizal fungi are crucial components of the plant microbiota and are key actors in terrestrial ecosystems, where they facilitate the exchange of carbon (C) and minerals
- Interacting with various plants mycorrhizal fungi provide multiple ecological services in natural and agricultural environments



Madigan et al. 2018

Mycorrhizae

- **Ectomycorrhizal fungi** engaged in a mycorrhizal symbiosis that is characterized anatomically by fungal hyphae that **wholly enclose the fine roots of the tree host**. Ectomycorrhizal fungi include diverse species from the Basidiomycota and Ascomycota phyla. **Some ectomycorrhizal fungi are involved in organic matter decomposition**.
- **Ectomycorrhizae, fungal cells form an extensive sheath (fungal mantle) around the outside of the root** with only a slight penetration into the root cellular structure (**roots of forest trees, especially conifers, beeches, and oaks, and are most highly developed in boreal and temperate forests**)—> **single species of tree** can form **multiple** mycorrhizal associations
- **Ectomycorrhizal mycelia to interconnect trees**, providing linkages for transfer of carbon and other nutrients between trees of the same or different species —> Nutrient transfer from well-illuminated overstory plants to shaded trees is thought to help **equalize resource availability**, subsidizing **young trees** and **increasing biodiversity** by promoting the **coexistence** of different species
- **Endomycorrhizae, a part of the fungus becomes deeply embedded within cells** comprising the root tissue, very **diverse**, some are **arbuscular mycorrhizae** (AM colonize **70–90% of all terrestrial plants**, including most **grassland** species and many **crop** species). AM Fungi that form a mycorrhizal symbiosis with a plant host. This is typical for certain trees and most non woody plants and is characterized **by fungal hyphae that penetrate plant cell walls, where they form highly branched structures known as arbuscules**. AM belong to a single **monopyhyletic** lineage of Glomeromycota. **They are not able to decompose biopolymers.**

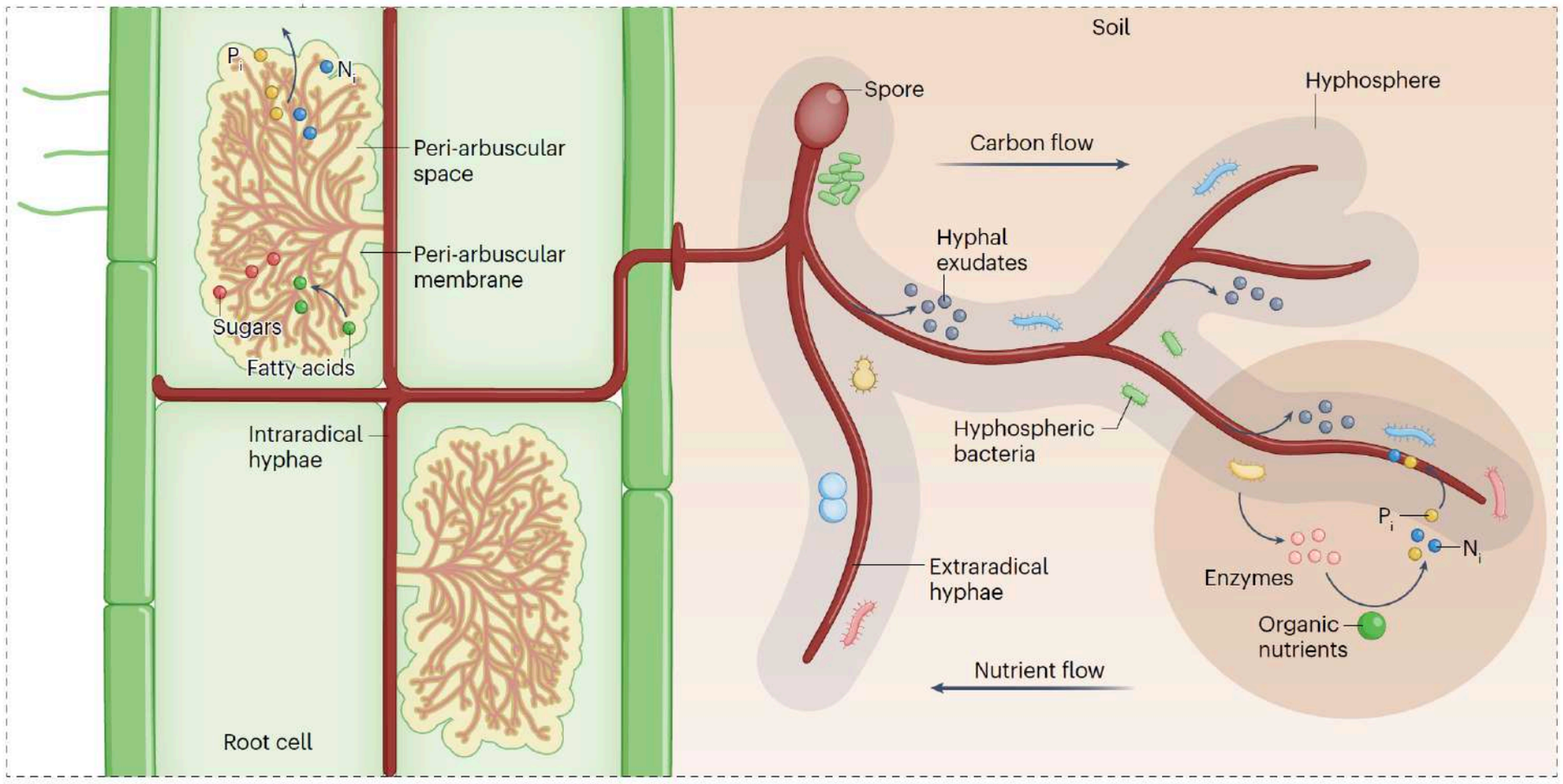
Chemical and spatial tight coupling in Plant-AM-microbe

- In AMF symbioses, **plants provide photosynthetically fixed C (including lipids and sugars) in exchange for minerals, especially phosphorus (P) and nitrogen (N)**

- **Moreover, AMF hyphae grow within and extend outside the plant root; the thin soil layer that surrounds these extraradical hyphae (ERH) forms a specialized niche called the' hyphosphere' .**

- This niche hosts a plethora of hyphospheric bacteria/microbes that feed on fungal metabolites that contain plant-derived C.

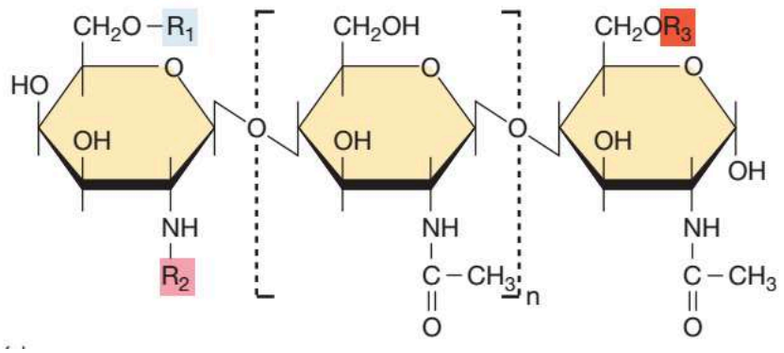
- **In return, the bacteria support the mineral nutrition of AMF by secreting extracellular enzymes, as AMF cannot decompose polymer nutrients owing to their limited saprotrophic capability**



Duan et al. 2024

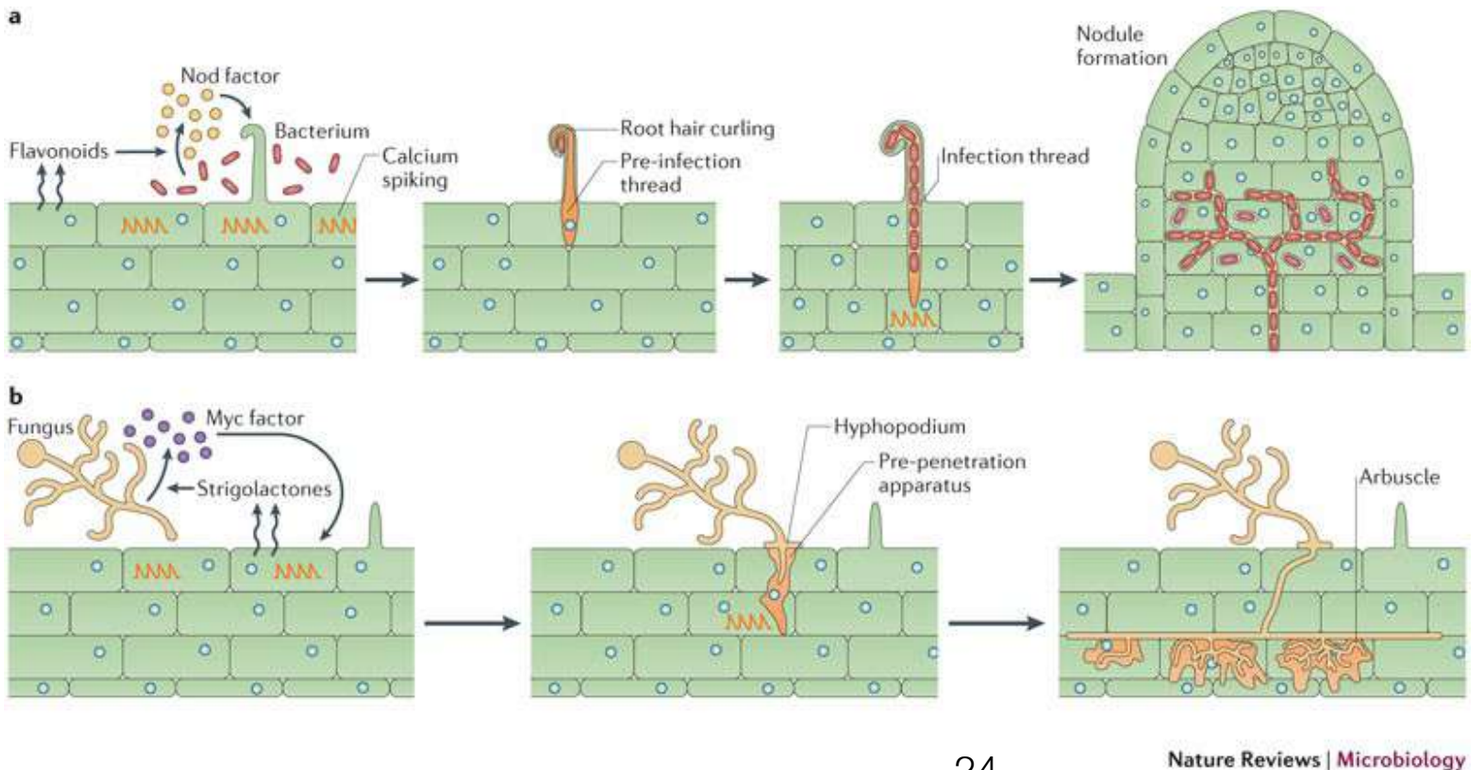
Nod & Myc Factors for chemical communication

Madigan et al. 2018



Rhizobial or AM fungus species	R_1	R_2	R_3
Sinorhizobium meliloti (alfalfa)	Ac	C16:2 or C16:3	SO_3H
Rhizobium leguminosarum biovar *viciae* (pea)	Ac	C18:1 or C18:4	H or Ac
Glomus intraradices (many agricultural crops)	H	C16 or C16:1 or C16:2 or C18 or C18:1Δ9Z	H or SO_3H

- **Nod & Myc factors are lipochitin** oligosaccharides to which various substituents are bonded that function as primary **rhizobial /mycorrhizal signaling molecules triggering** legumes/plant to develop either new plant organs: **root nodules** that host the bacteria as nitrogen-fixing bacteroids to allow the **physical interaction** with the **mycelium and formation arbuscules inter-or intra-cellularly**



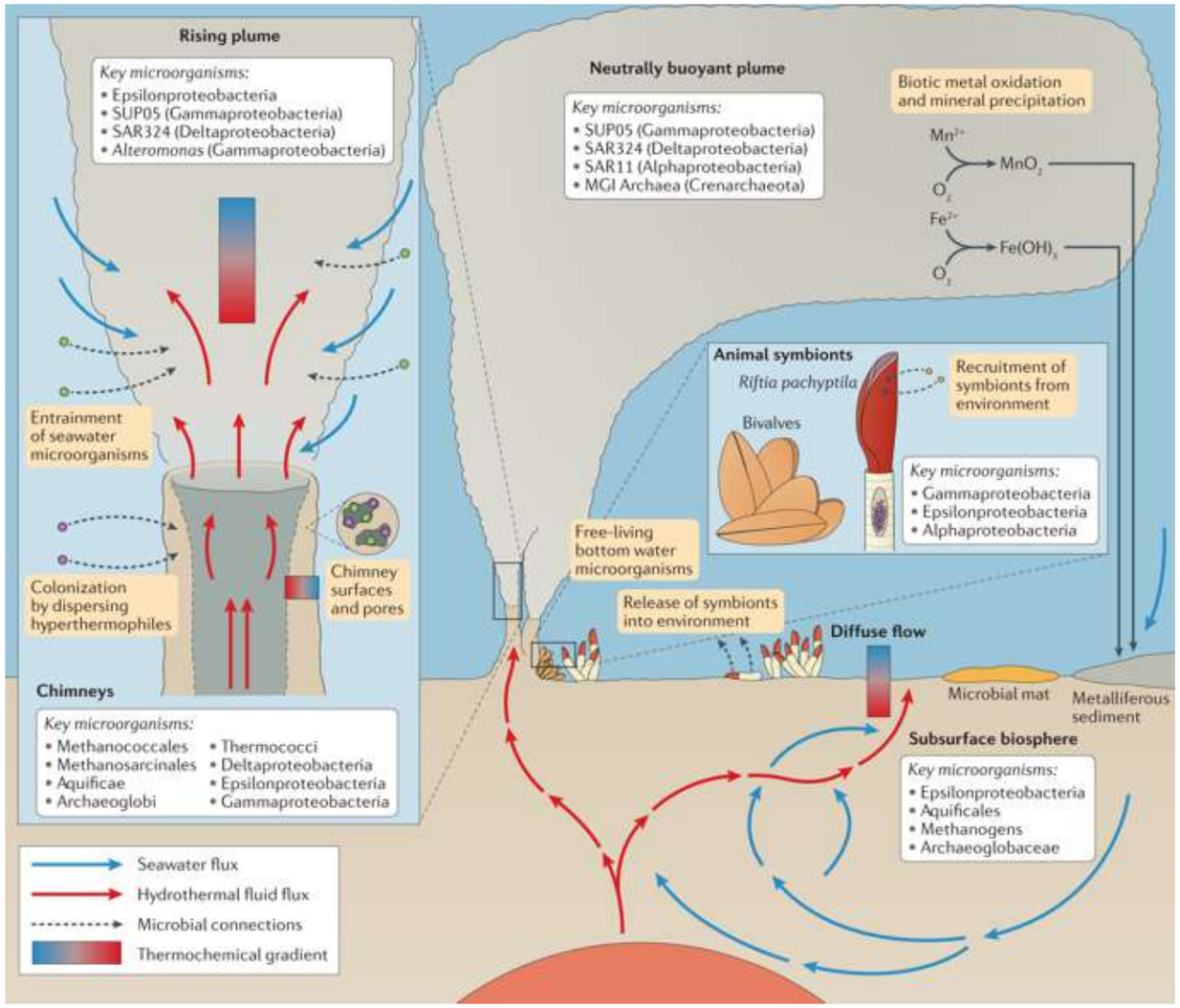
Oldroyd, 2013

Hydrothermal vent chemolithotrophs and their animal hosts

Bobtail squid and *Aliivibrio fischeri*

Hydrothermal vent chemolithotrophs and their animal hosts

- In a dark and cold ocean—> no photosynthetic C fixation —> **chemolithoautotrophy**
- Hydrothermal vents with **sharp contrasts in physical and chemical conditions** between these various habitats and their dynamic, **extreme** and **geographically isolated nature**
- **Hydrothermal fluids** contain large amounts of **reduced inorganic materials**, including H_2S, Mn^{2+}, H_2, and CO (carbon monoxide), and some vents contain high levels of ammonium (NH_4^+) instead of H_2S; all of these are **good electron donors for chemolithotrophs**
- **Mutualistic chemolithotrophs are either tightly attached to the animal surface (epibionts) or actually live within the animal tissues**, supplying organic compounds to the animals in exchange for a safe residence and ready access to the electron donors needed for their energy metabolism —> fix CO_2/biomass

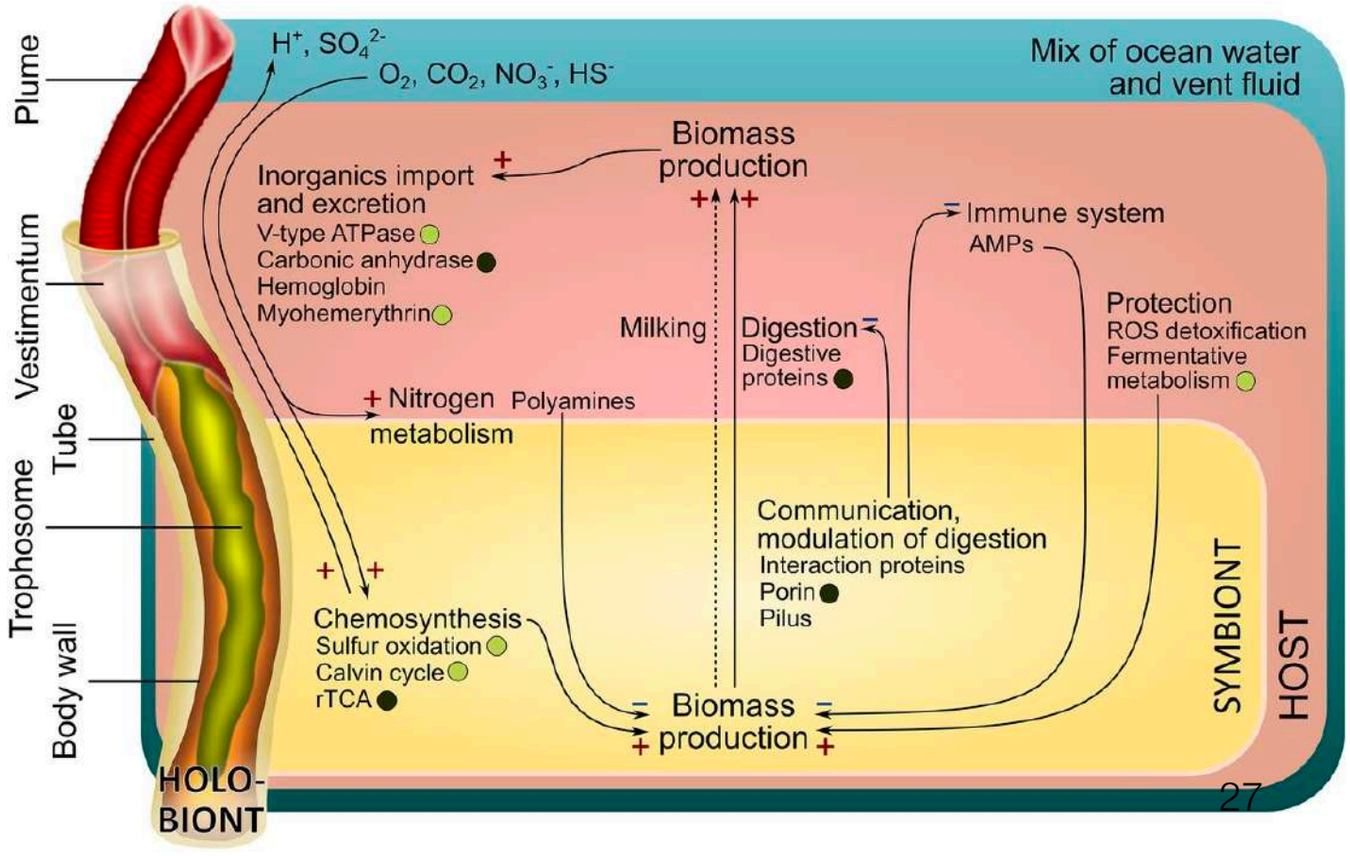


Dick, 2019

Biochemical REDOX coupling for primary production at vent sites

Temperature (°C)		Taxa	Energy metabolism		
			e⁻ donor	e⁻ acceptor	e⁻ donor/e⁻ acceptor
2 Seawater	2–10	Gammaproteobacteria (SUP05 and *Beggiatoa*)	S, H_2	O_2	Oxidizing
Psychrophiles		Epsilonproteobacteria (*Arcobacter*)	S	O_2	CH_2O/O_2
20	10–40	Epsilonproteobacteria (*Sulfurimonas* and *Sulfurovum*)	S	NO_3^-	HS^-/O_2
Mesophiles	20–60	Aquificales: Aquificae	H_2	O_2	Aerobic
55	40–70	Epsilonproteobacteria (*Caminibacter* and *Nautila*)	S, H_2	NO_3^-	H_2/O_2
		Methanosarcinales	CH_4	SO_4^{2-}*	HS^-/NO_3^-
Thermophiles	60–80	Aquificales: Desufurobacteriaceae	H_2	NO_3^-, S	Anaerobic
		Thermococcus	C_{org}, CH_4	S	H_2/SO_4^{2-}
80		Various archaea (DHVE2; *Archaeoglobus*) and bacteria	C_{org}, H_2	SO_4^{2-}, S, Fe(III)	Methanogenesis
Hyperthermophiles		*Methanococcus*, *Methanocaldococcus* and Methanosarcinales	H_2	CO_2	H_2/CO_2
121 Thermal fluid	>90	Methanopyri	H_2	CO_2	Reducing

Dick, 2019



Riftia pachyptila:

- ★ Energy is obtain from oxidation of reduced inorganic compounds O_2
- ★ Electron donor: reduced inorganic compounds HS^-
- ★ Carbon sources: inorganic CO_2
- ★ Specialized structure where the symbiotic microbes live, trophhosome

Hinzie et al., 2019

Biochemical diversity for C fixation

Chemolithoautotrophic growth
The growth of bacteria or archaea using an inorganic, chemical source of energy (for example, reduced forms of iron, sulfur, hydrogen and ammonia) to fix inorganic carbon into organic carbon

Microbial Primary productivity

Reductive tricarboxylic acid cycle
(rTCA). A metabolic pathway for carbon fixation in which two molecules of **carbon dioxide** are converted **into acetyl coenzyme A**; it uses most of the same enzymes as the oxidative tricarboxylic acid cycle but runs it in reverse by using three alternative enzymes: fumarate reductase, 2-oxoglutarate synthase and ATP citrate lyase.

Calvin–Benson–Bassham cycle
A carbon fixation pathway in which **carbon dioxide** is converted into **glyceraldehyde-3-phosphate** using the key enzyme Rubisco.

Wood–Ljungdahl pathway
A metabolic pathway for carbon fixation in which two molecules of **carbon dioxide** are converted into **acetyl coenzyme A** by the key enzyme carbon monoxide dehydrogenase–acetyl coenzyme A synthase.

Dicarboxylate–4-hydroxybutyrate pathway
A recently described carbon fixation pathway in Archaea in which a molecule of **bicarbonate** (HCO_3^-) is fixed onto **acetyl coenzyme A** via a combination of enzymes from the reductive tricarboxylic acid cycle and the 4-hydroxybutyrate part of the 3-hydroxypropionate–4-hydroxybutyrate cycle.

Bobtail squid and *Aliivibrio fischeri*

Bioluminescence

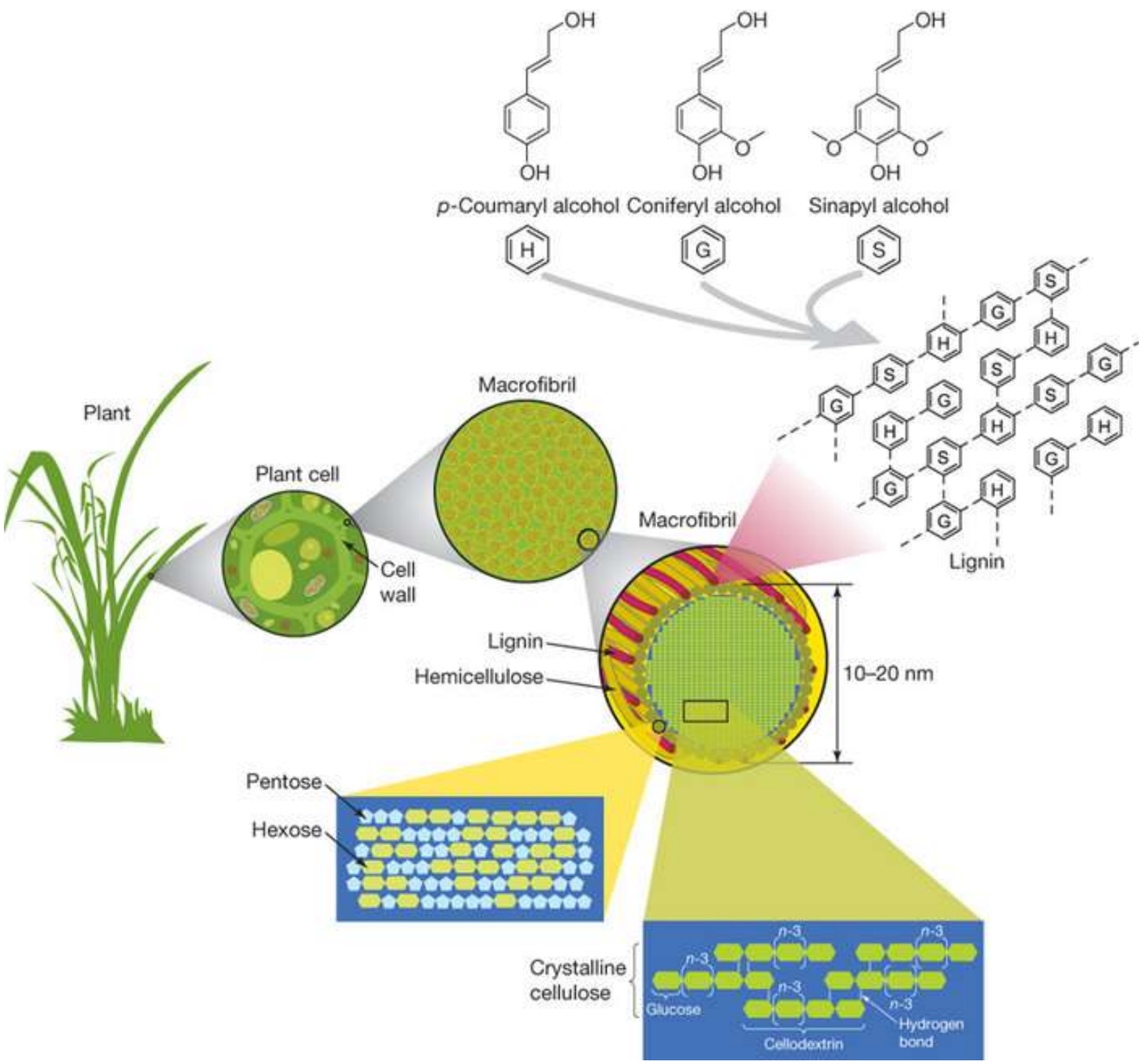
- Hawaiian bobtail squid, *Euprymna scolopes*, is a small marine invertebrate that harbors a large population of the bioluminescent gram-negative gammaproteobacterium *Aliivibrio fischeri* (unique species) in a light organ located on its ventral side
- Bacteria emit light that resembles moonlight penetrating marine waters, and this is thought to camouflage the squid from predators that strike from beneath
- Several other species of *Euprymna* inhabit marine waters near Japan and Australia and in the Mediterranean, w. *Aliivibrio* symbionts
- Transmission of bacterial cells to juvenile squid is a horizontal (environmental) rather than a vertical (parent to offspring) event
- Almost immediately after juveniles emerge from eggs, cells of *A. fischeri* in surrounding seawater begin to colonize them, entering through ciliated ducts that end in the immature light organ, 2h
- In light organ, 10^8-10^9 cells, coevolution in the presence of the microbes
- Animal in some way recognizes and accepts *A. fischeri* cells and excludes those of other species, lose flagellum
- Nitric oxide produced by the squid repel other bacteria
- Squid matures into an adult in ~ 2 months and then lives a strictly nocturnal existence in which it feeds mostly on small crustaceans; during the day, the animal buries itself and remains quiescent in the sand
- Each morning at dawn the squid nearly empties its light organ of *A. fischeri* cells and begins to grow a new population of the bacterium
- *A. fischeri* grows faster in the squid than in the ocean
- *A. fischeri* quorum sensing —> light production

Gut microbiota - Termites and Mammals

Termites

- Microorganisms are primarily responsible for the degradation of wood and cellulose in natural environments in tropical and subtropical
- Degradation of **lignocellulosic** materials
- **Insect gut provides a protective niche for microbial symbionts**, and in return, the **insect gains access to nutrients derived from an otherwise indigestible** carbon source
- Posterior alimentary tract of **higher termites** (most advanced, family Termitidae, ~3/4 of termite species) contains a dense and diverse community of **mostly anaerobic bacteria, including cellulolytic species**
- **Lower termites** (primitive) harbor diverse populations of both anaerobic bacteria and cellulolytic protists —> **Bacteria of lower termites participate little** or not at all in cellulose digestion; only the **protists phagocytize and degrade the wood particles ingested**
- **Higher vs lower** termites have **diverse gut architecture**
- Gut is **microbial bioreactor** that efficiently converts polymeric substrates to **acetate and variable amounts of methane, with hydrogen** as a central intermediate
- **Diverse food —> diverse gut microbiome (wood, fungus, soil)**

Structure of lignocellulose



https://www.nature.com/scitable/content/structure-of-lignocellulose-14464273/

The **main component** of lignocellulose is **cellulose, a beta(1–4)-linked chain of glucose molecules**. Hydrogen bonds between different layers of the polysaccharides contribute to the resistance of crystalline cellulose to degradation

Hemicellulose, the second most abundant component of lignocellulose, is composed of **various 5- and 6-carbon sugars such as arabinose, galactose, glucose, mannose and xylose**

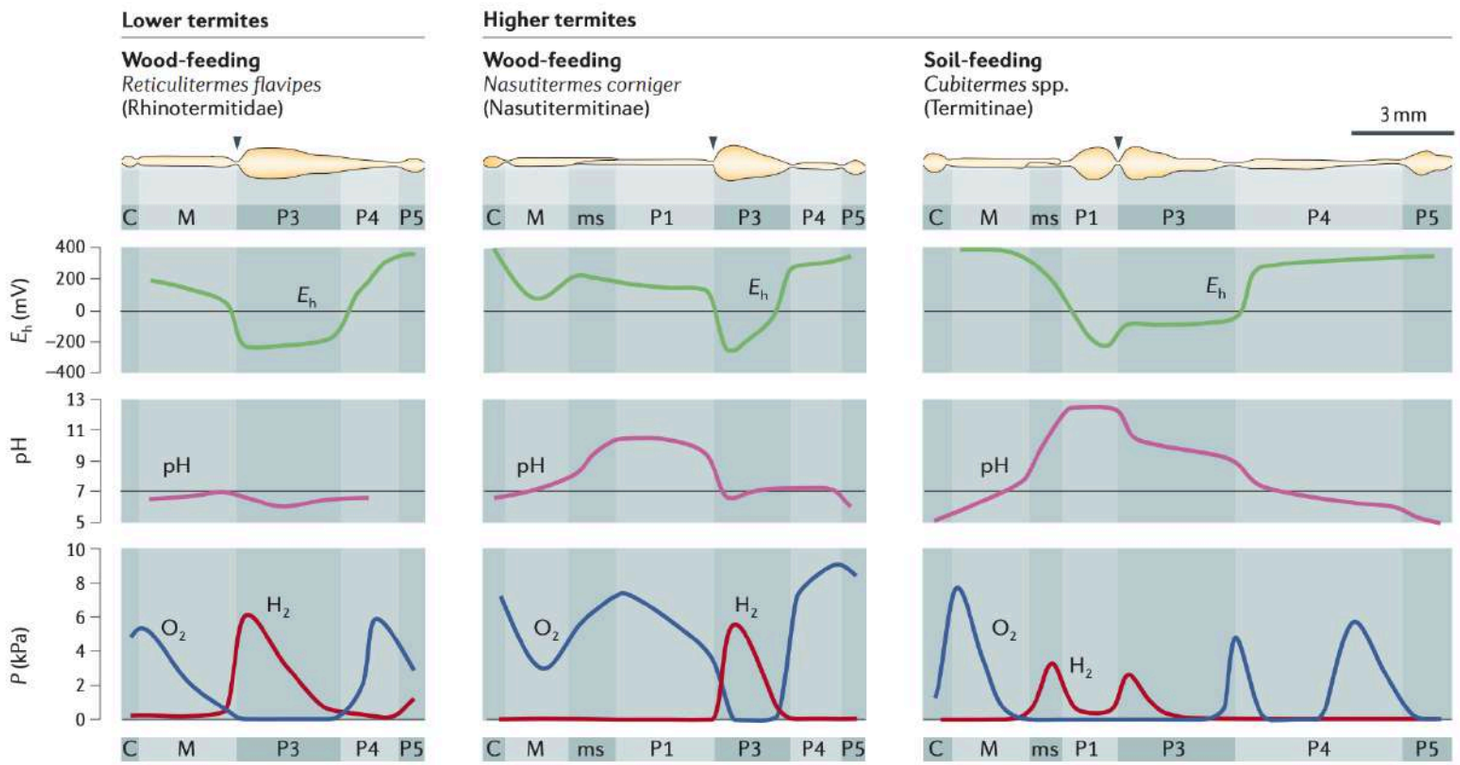
Lignin is composed of three major **phenolic** components, namely p-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (S)

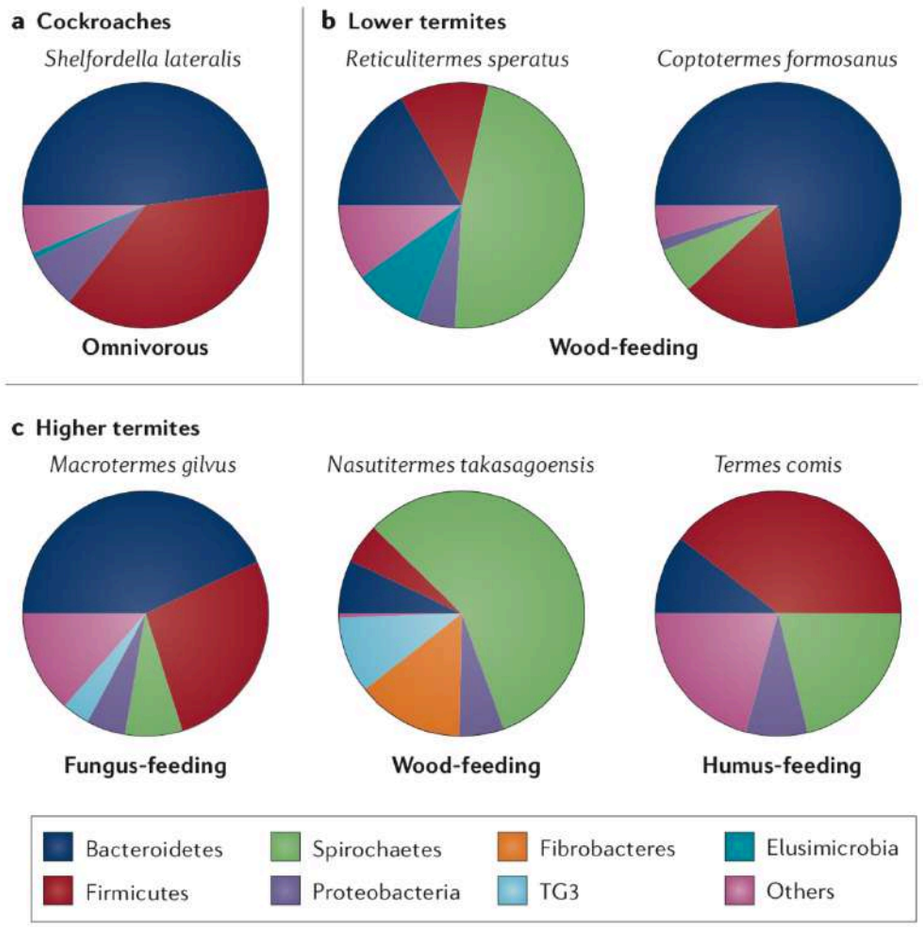
Lignin is synthesized by **polymerization** of these components and their ratio within the polymer varies between different plants, wood tissues and cell wall layers.

Cellulose, hemicellulose and lignin form structures called microfibrils, which are organized **into macrofibrils** that mediate structural stability in the plant cell wall

Termite gut

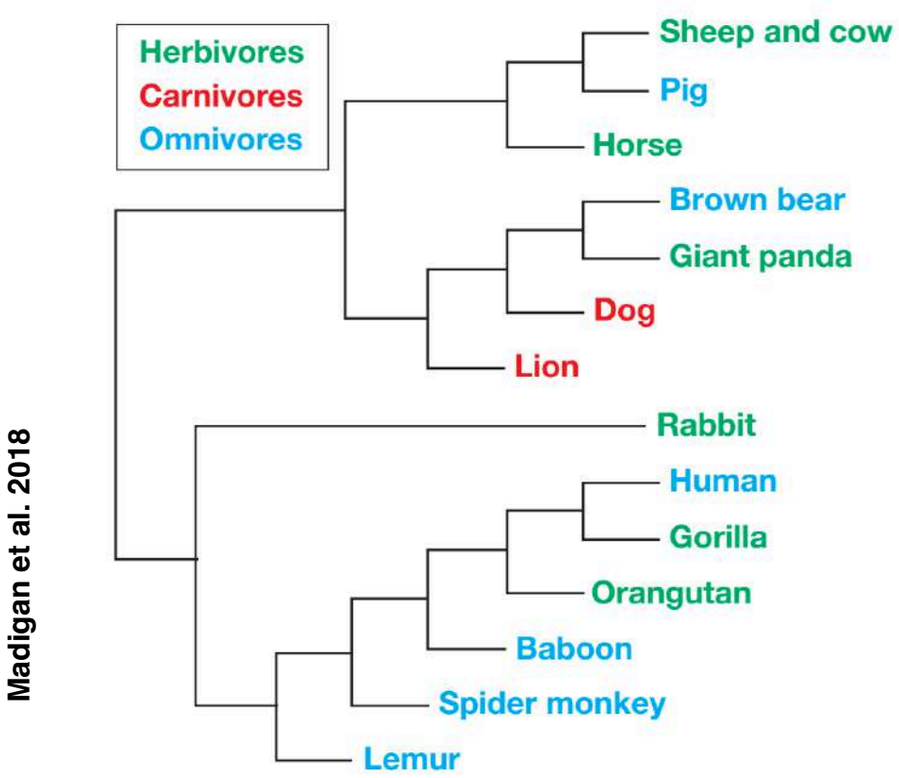
- **Metanogenesis** and **reductive acetogenesis** only in **absence of O_2**
- Within **gut local conditions select for microbial communities**

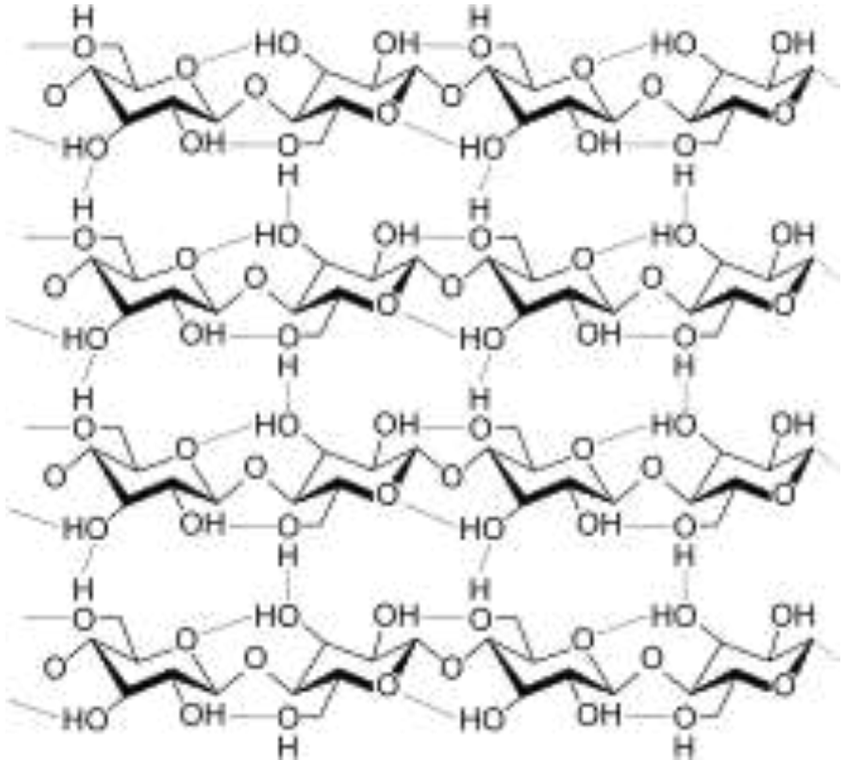




Brune, 2014

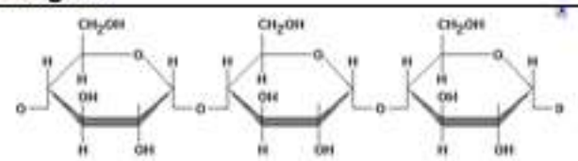
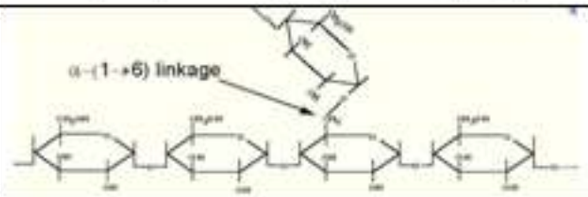
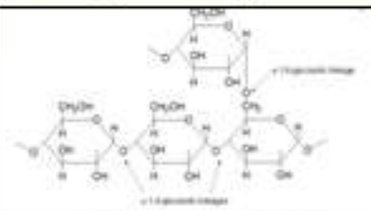
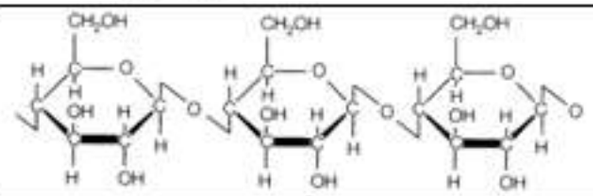
Gut microbes-Mammals symbiosis

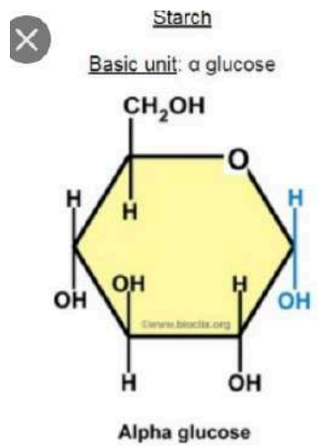


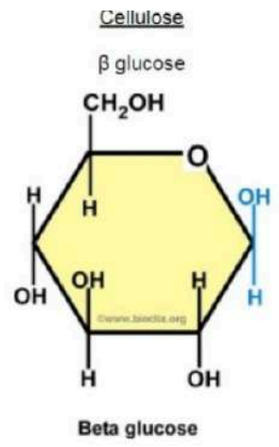


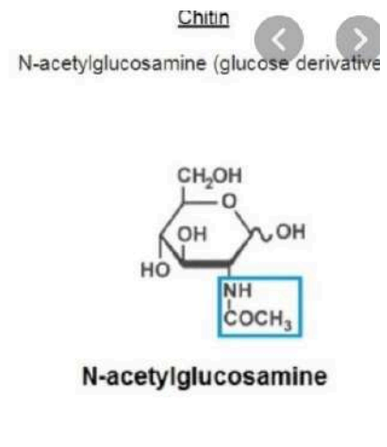
- Gut microbiome has evolved **strategy to utilize complex and insoluble polysaccharides** (e.g. cellulose, beta-glucose only unit)
- Microbes have genes encoding the glycoside hydrolases and polysaccharide lyases required to decompose these polysaccharides
- Most mammalian species evolved **gut structures that foster mutualistic associations with microorganisms**
- As anatomical differences evolved, microbial fermentation remained important or essential in mammalian digestion
- Herbivory has evolved many times in Mammals

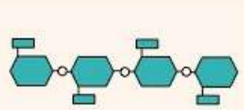
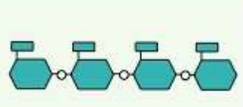
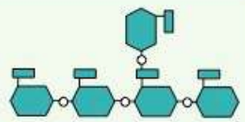
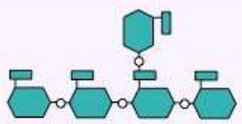
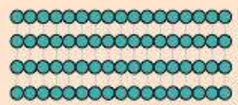
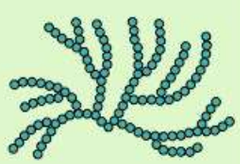
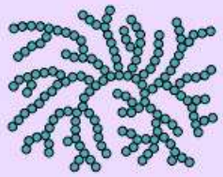
Sugar polymers

Polysacc	Monosac	Bonds	Diagram
Starch: Amylose	α - glucose	1-4	
Starch: Amylopectin	α - glucose	1-4 and 1-6	
Glycogen (**NOT starch!**)	α - glucose	1-4 and 1-6 (more 1-6 than amylo-pectin)	
Cellulose	β - glucose	1-4	



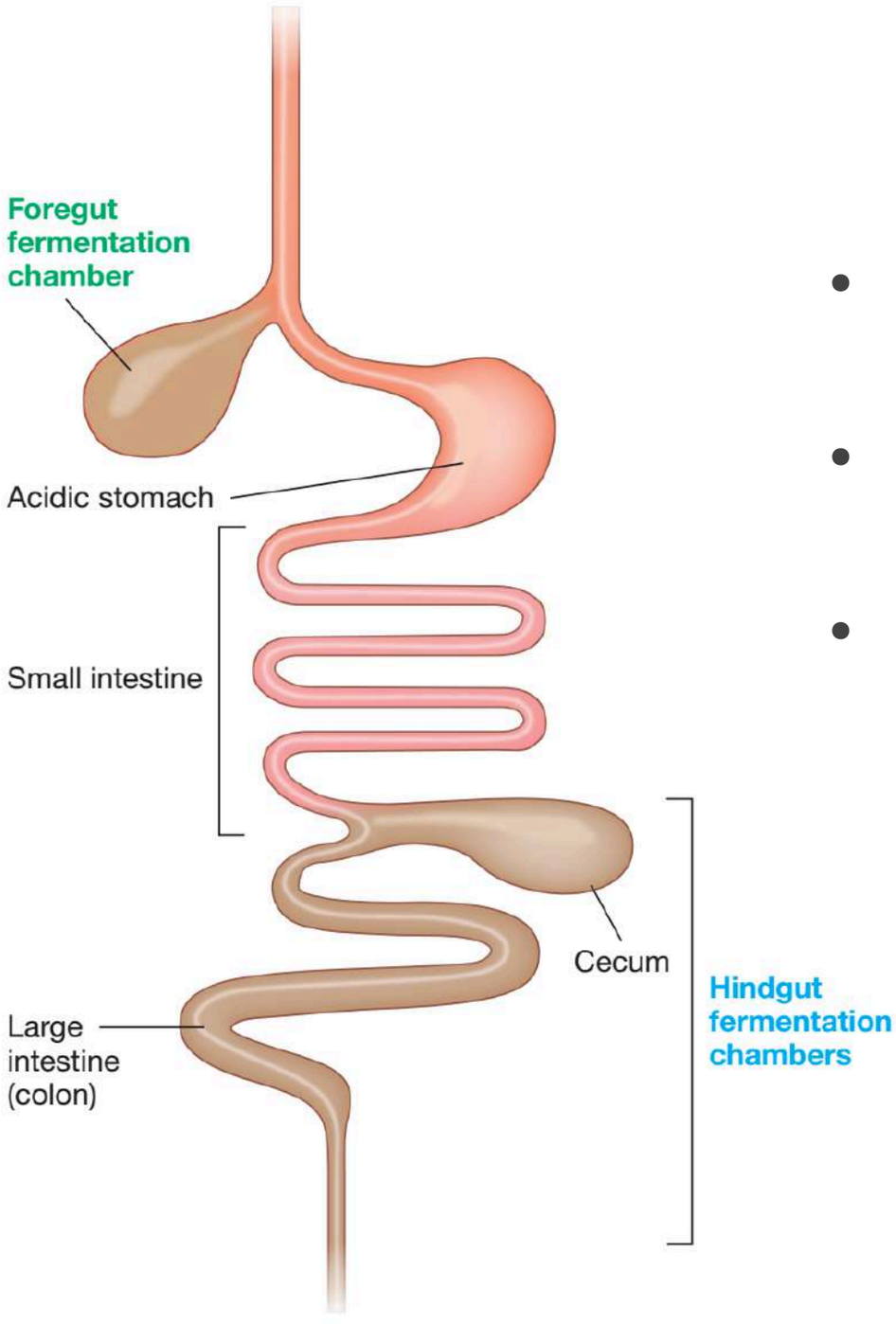




	Cellulose	Starch		Glycogen
		Amylose	Amylopectin	
Source	Plant	Plant	Plant	Animal
Subunit	β-glucose	α-glucose	α-glucose	α-glucose
Bonds	1-4	1-4	1-4 *and* 1-6	1-4 *and* 1-6
Branches	No	No	Yes (~per 20 subunits)	Yes (~per 10 subunits)
Diagram				
Shape				

Google search

Fermentation in the gut

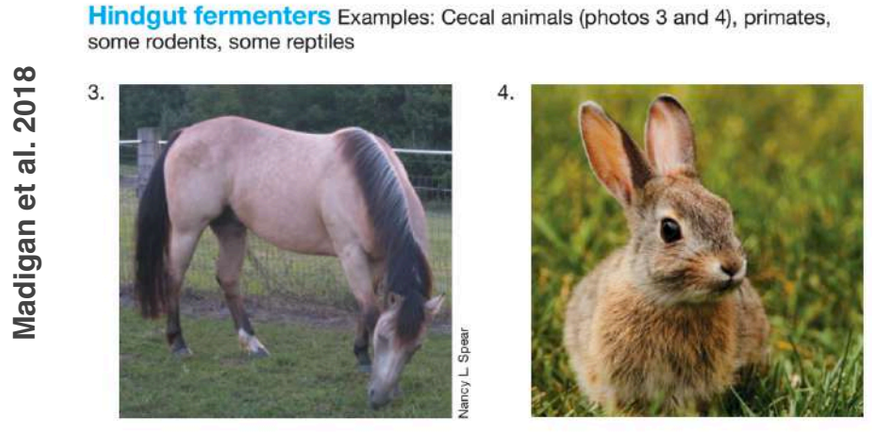


Madigan et al. 2018

Madigan et al. 2018

Cows

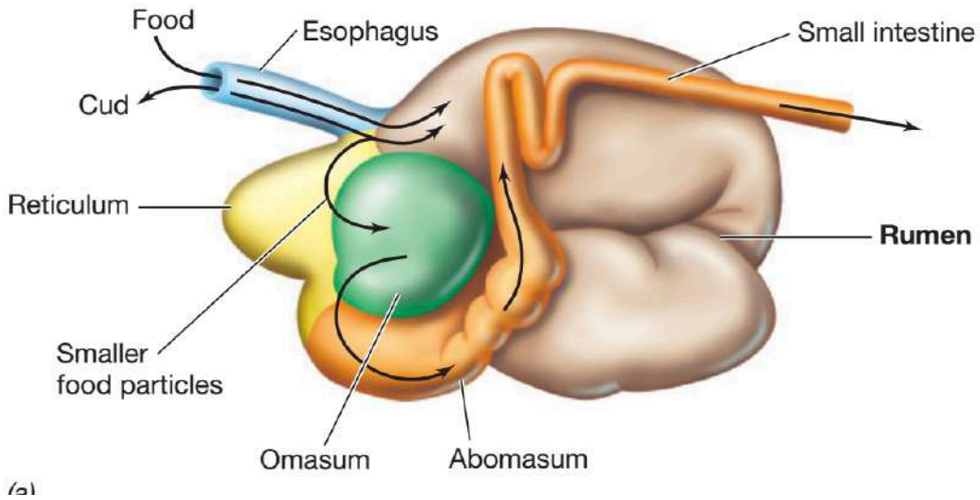
- (1) **Enlarged anoxic fermentation chamber** for holding ingested plant material
- (2) **Extended retention time**—the time that ingested material remains in gut
- A longer retention time allows for a longer association of microorganisms with ingested material and thus a more complete degradation of plant polymers



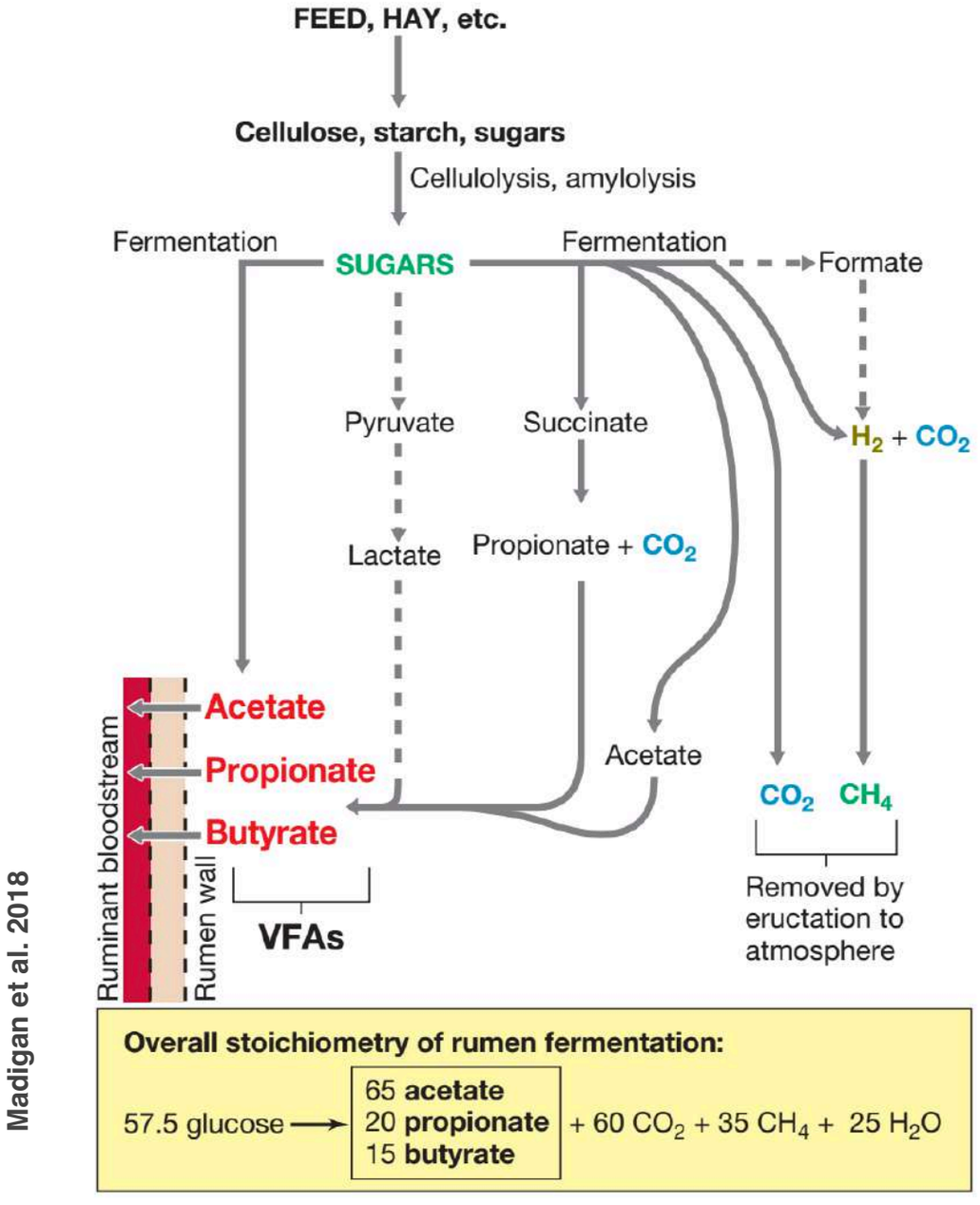
Madigan et al. 2018

Many chambers to maximize energy

Madigan et al. 2018



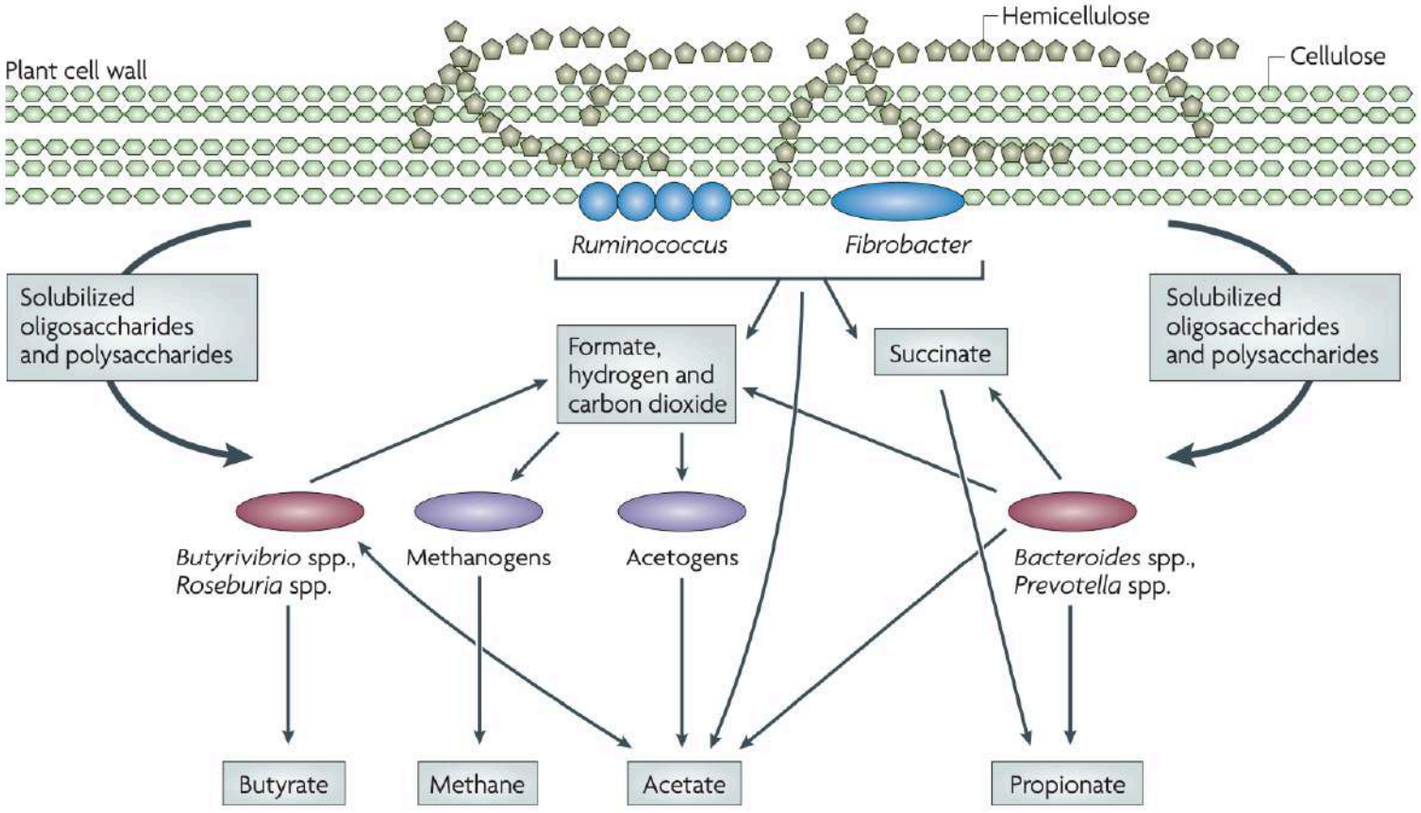
- 20-50 h food in the rumen
- Fermentation
- Anerobic bacteria dominate rumen
- Some anaerobic eukaryotes
- Cellulose diet: *Fibrobacter succinogenes, Ruminococcus albus*
- Starch diet: *Ruminobacter amylophilus, Succinomonas amylolitica*



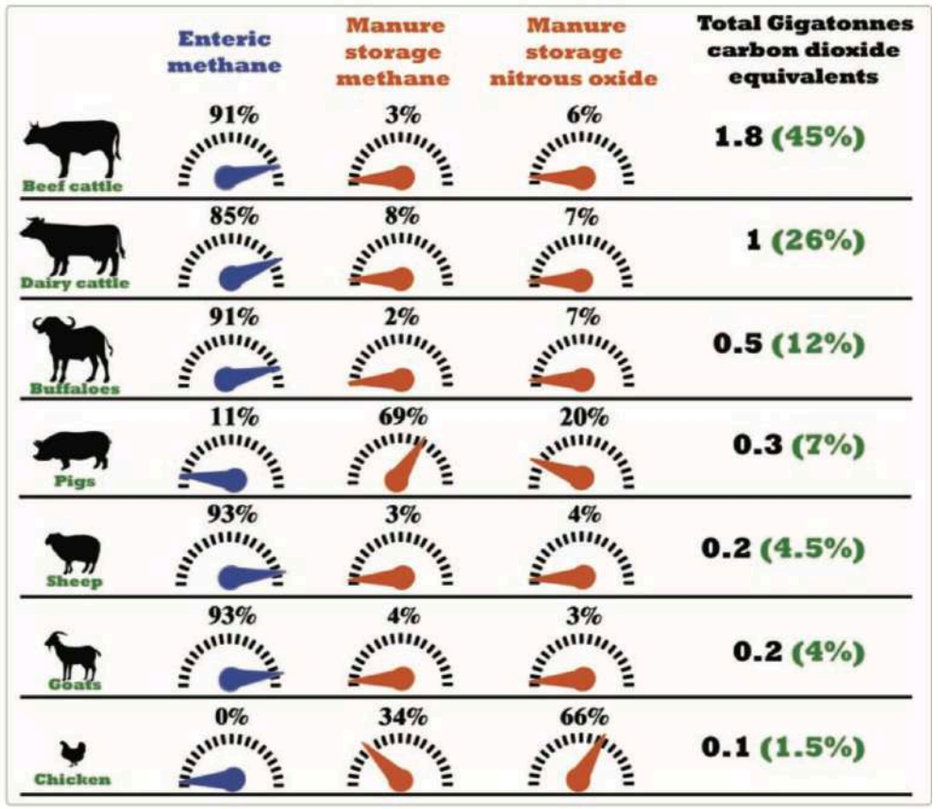
Madigan et al. 2018

Carbon budget

- Global emission ~ 37 Gt CO_2 in 2019 (https://www.globalcarbonproject.org/carbonbudget/19/highlights.htm)
- Livestock emission ~ 4.1 Gt CO_2 by FAO (http://www.fao.org/gleam/en/)



Flint et al., 2008



FAO, 2017

HUMANS

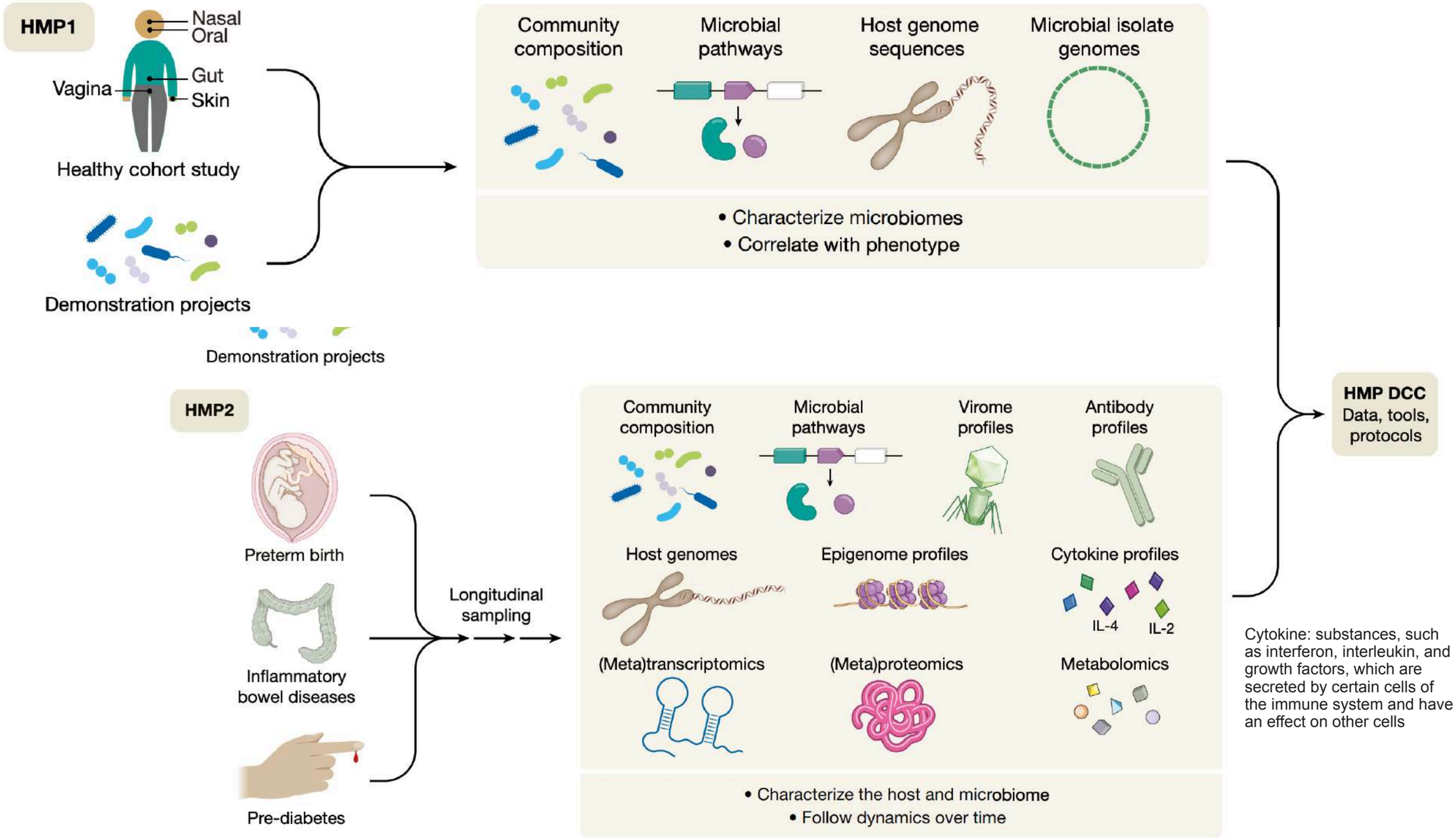
Ab initio

Prior 1983, knowledge based on culturable gut bacteria only and gut was considered as pretty axenic place

Humans are humans

Paradigm shift

HMP 1 & HMP 2

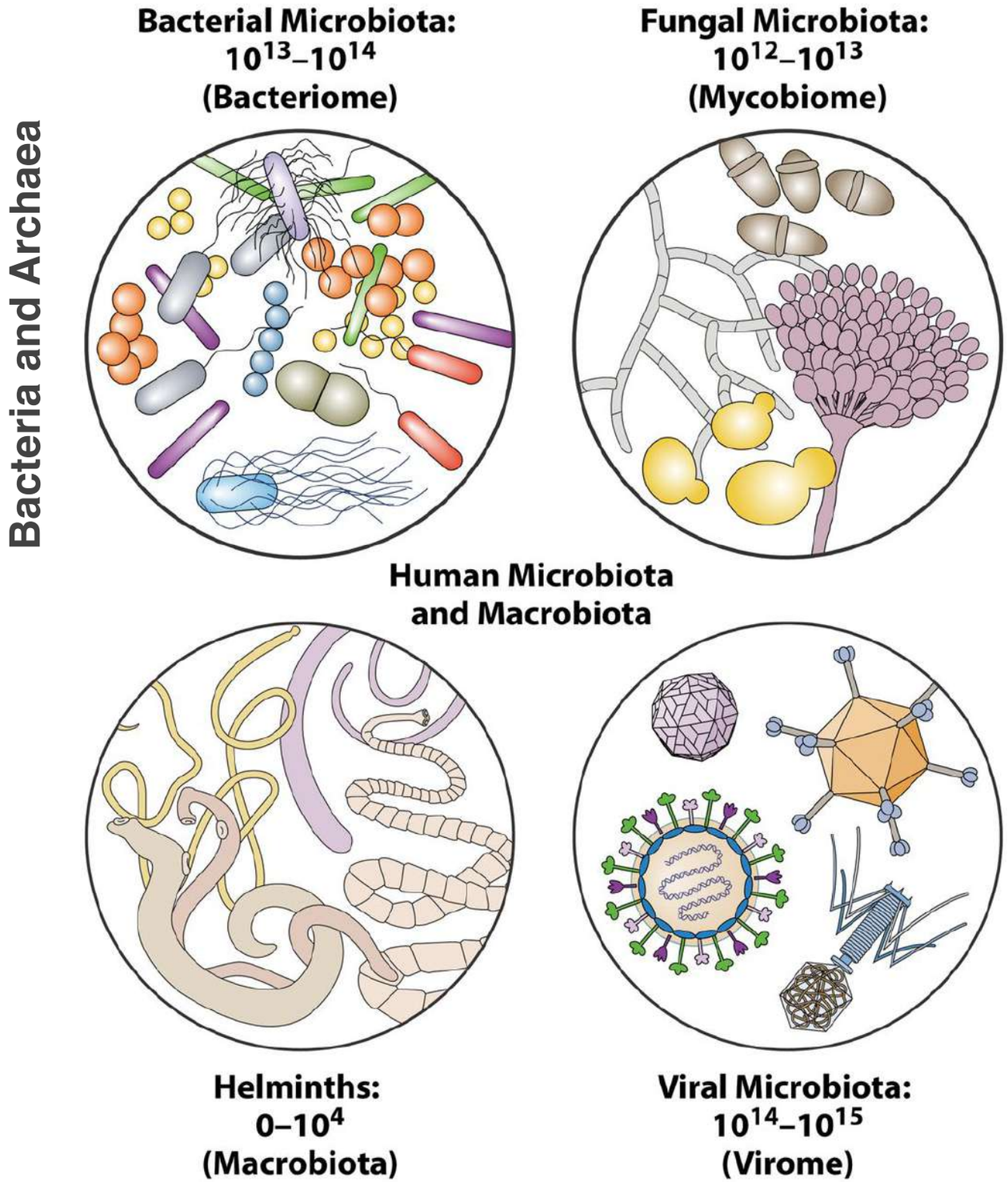


Cytokine: substances, such as interferon, interleukin, and growth factors, which are secreted by certain cells of the immune system and have an effect on other cells

iHMP, 2019

What is a human being?

- Complex ecosystem
- Cross-Domain and Viral Interactions
- Multitude of microrganisms
- Humans are microbial zoos
- Humans and microbes are interconnected for life



Rowan-Nash et al., 2019

Microbes on/in Humans

Major questions:

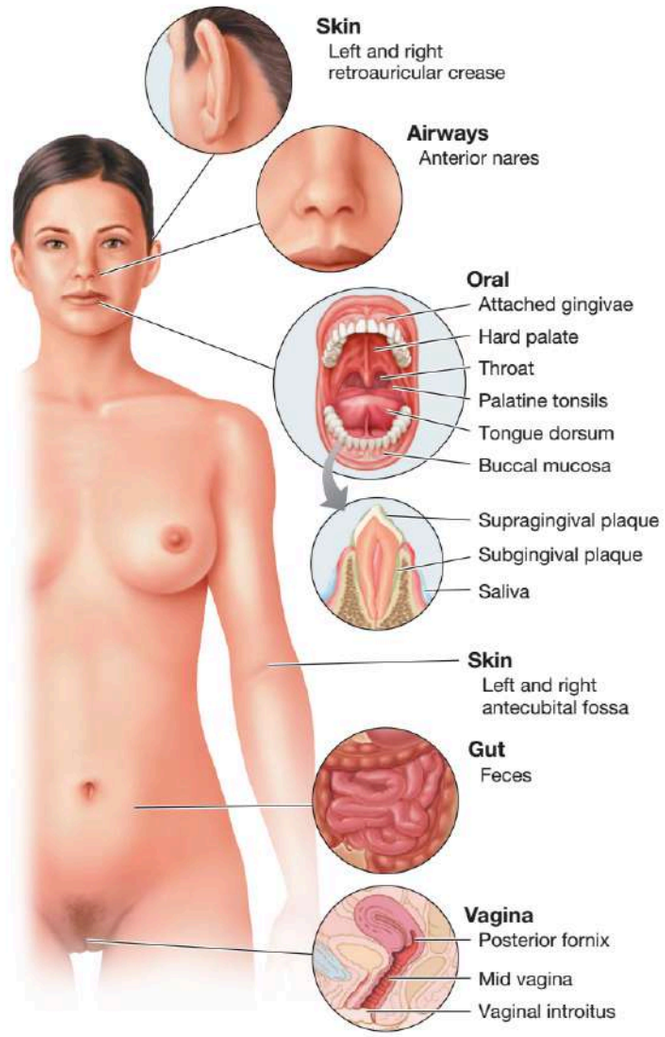
- Do individuals share a core human microbiome?
- Is there a correlation between the composition of microbiota colonizing a body site and host genotype?
- Do differences in human microbiome correlate with human health?
- Are differences in the relative abundance of specific bacterial populations important to either health or disease?

Now we now:

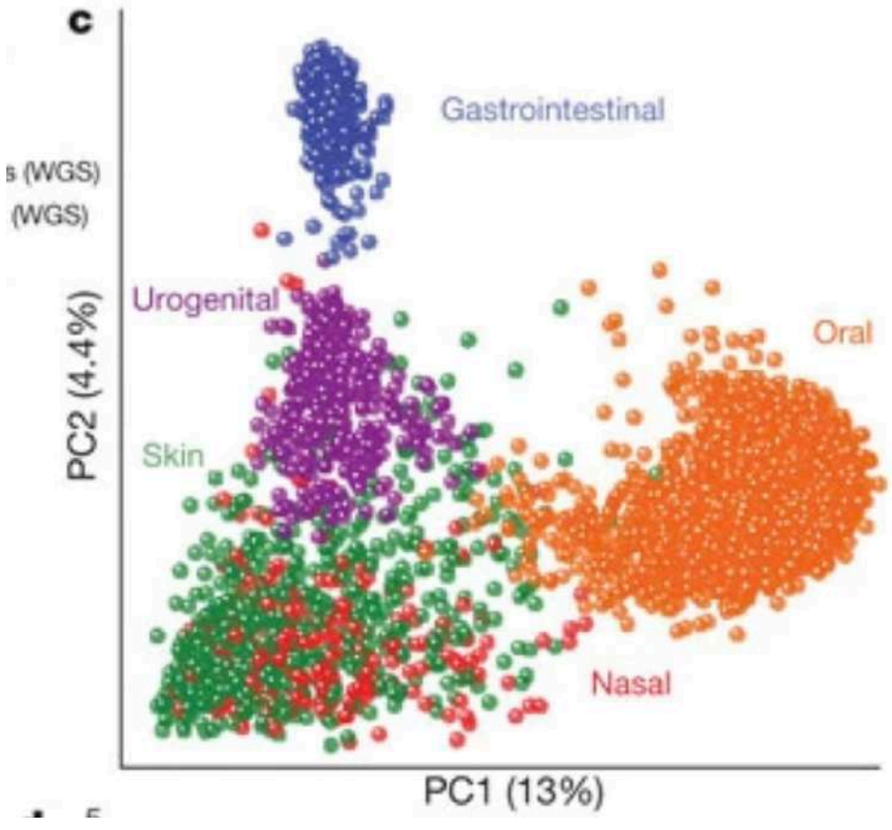
Microbial community (1) competes for and generates nutrients, (2) influences and is shaped by the host innate and adaptive systems, and (3) protects against pathogens and also triggers acute and chronic disease

Human-microbes association

- Microbiome: functional collection of different microbes in a particular environmental system
- Human microbiome formed by different microbiota (i.e. organisms living in a specific environment)
- Human holobiont and found an approximate ratio of only 1:1 of bacterial to human cells (Senders et al., 2016)
- Total number of **gut** bacteria of today's human population is between 3×10^{23} and 5×10^{23}, dental plaque: 8×10^{21} cells, skin:1×10^{21} cells



Madigan et al., 2018

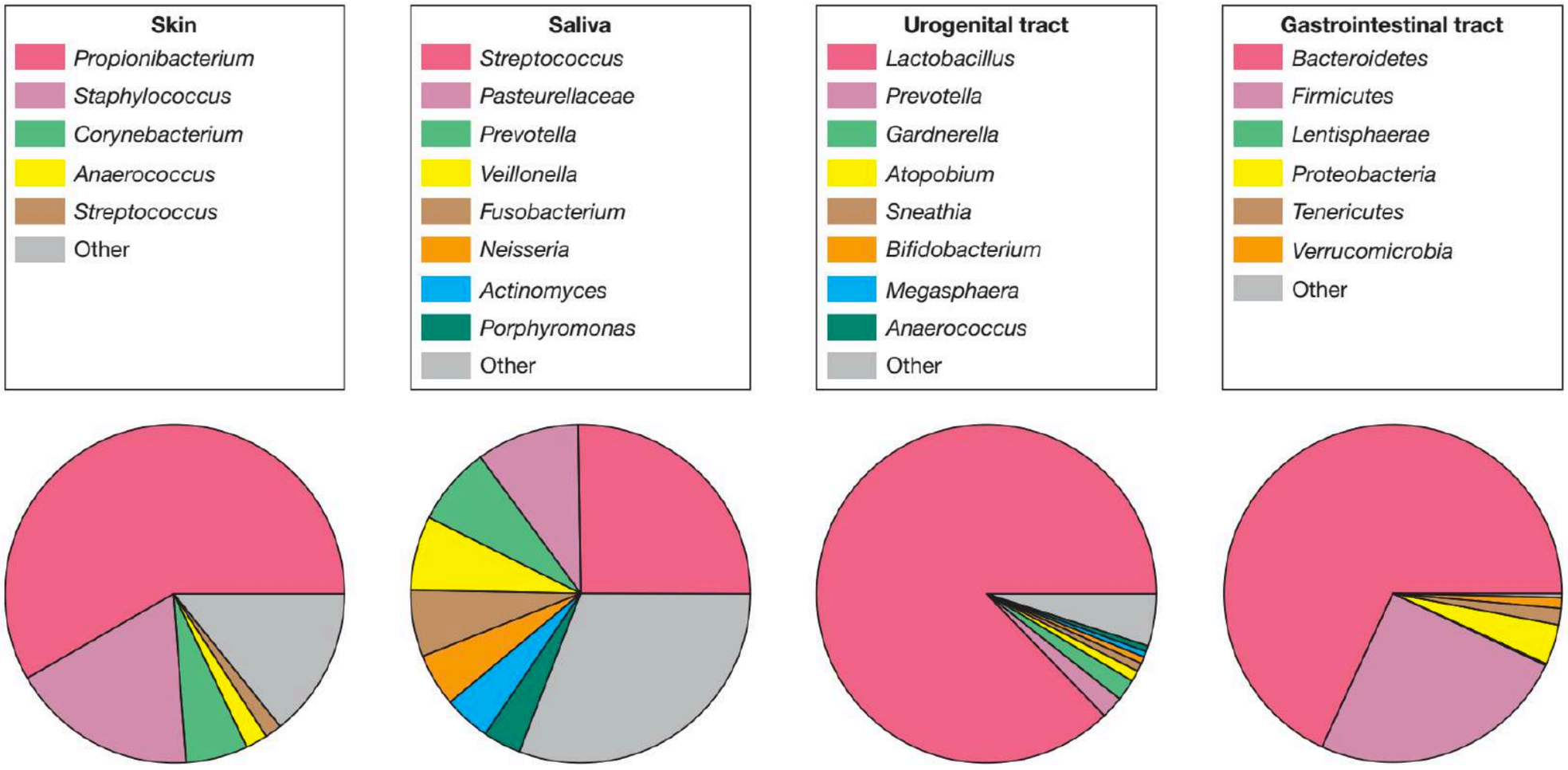


The Human Microbiome Project Consortium, 2012

What about viruses?

Human-microbes an ecosystem within ecosystems

- Microbial population based on cultured-dependent methods differ from culture-independent methods

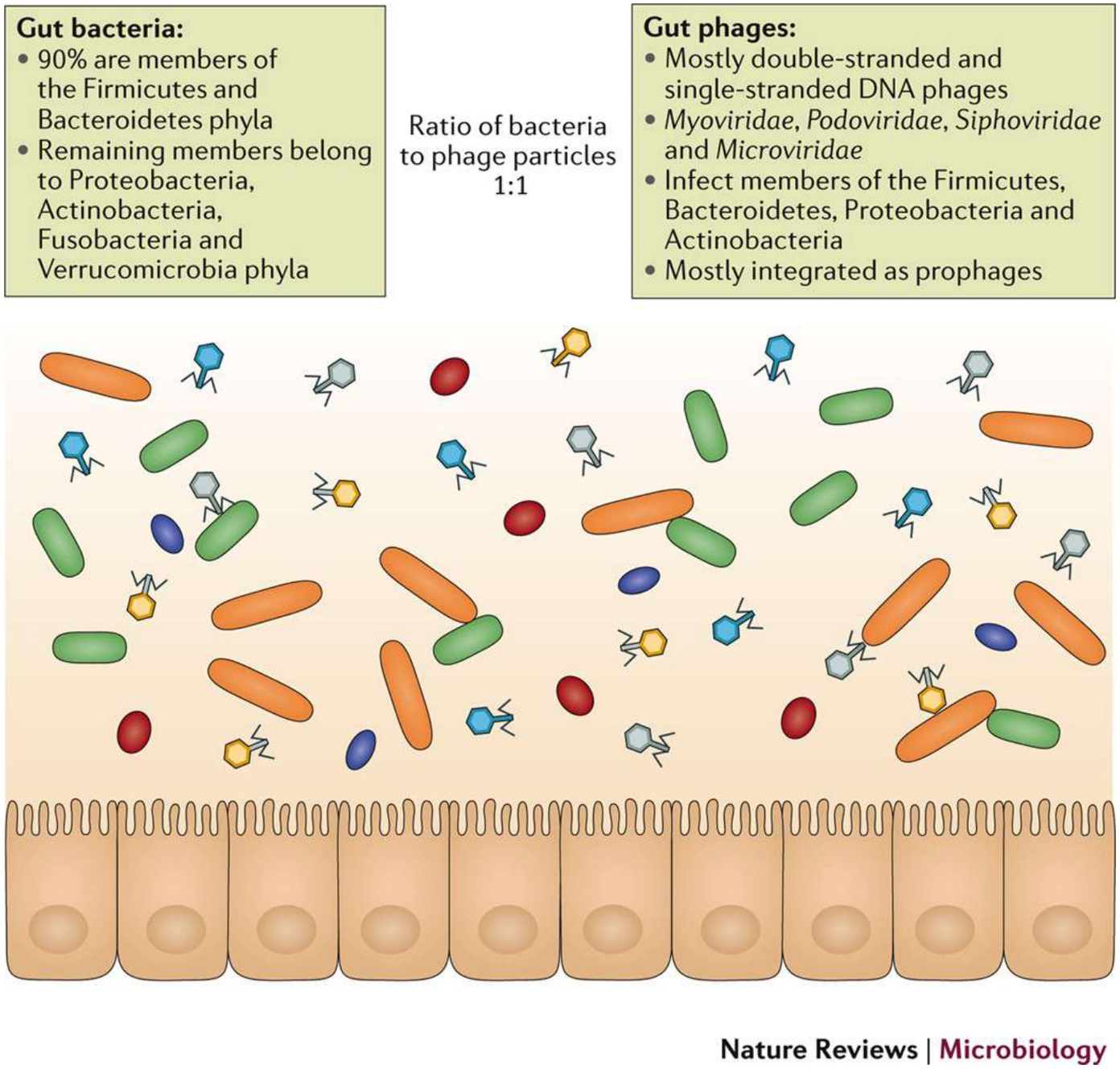


- 16S rRNA gene: species cluster with > 97% sequence similarity, culture-independent method

Madigan et al. 2018

Human Virome

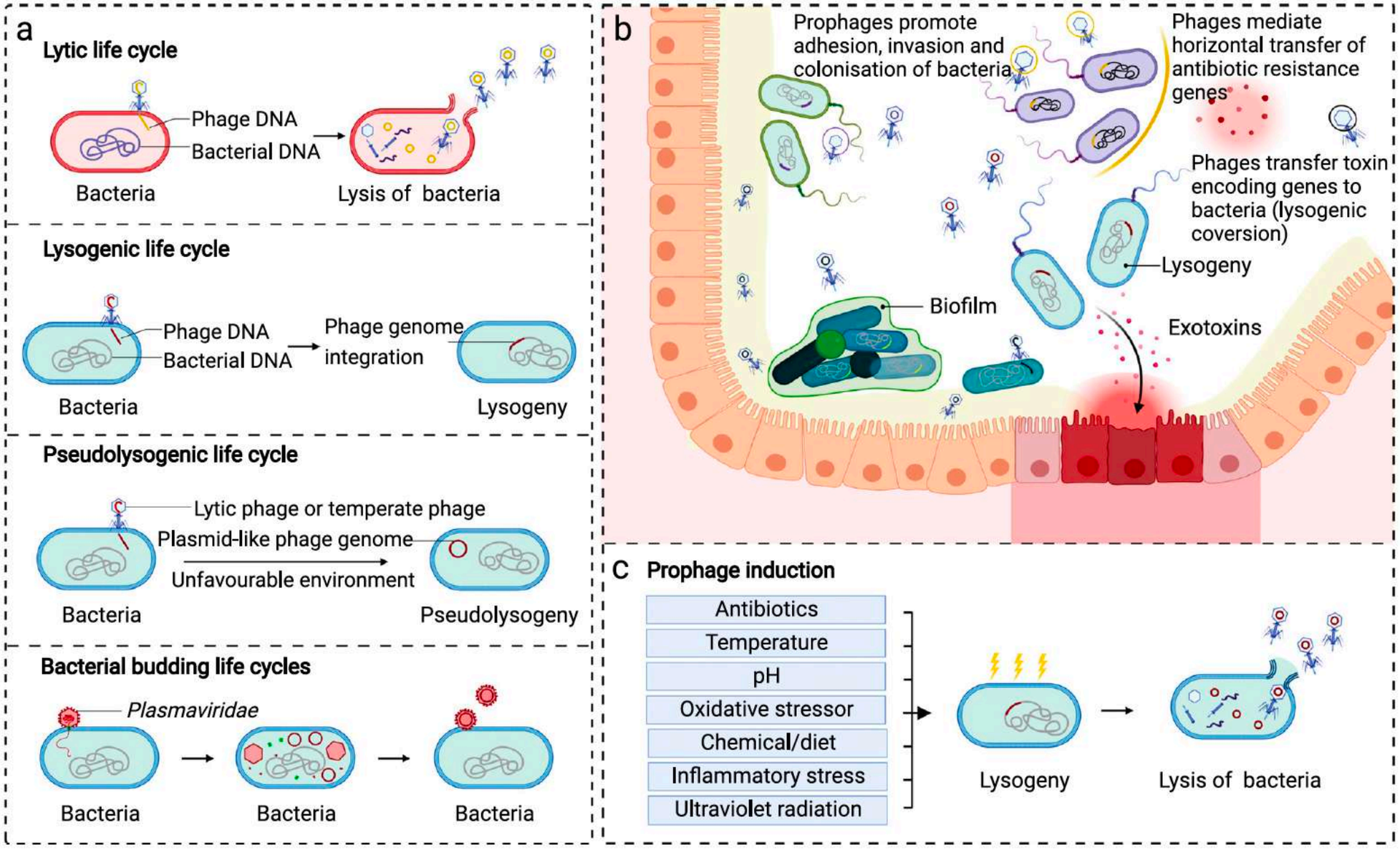
- The human gut is home to dense bacterial and **phage populations**
- —> regulating human health
- Phages regulate bacterial abundance, diversity and metabolism
- Phage effects in human gut remain largely unexplored
- Despite high bacterial abundance and metabolism
- Majority of described phages in the gut are integrated within their bacterial hosts (—> specific dynamic interactions)



Khan Mirzaei & Maurice, 2017

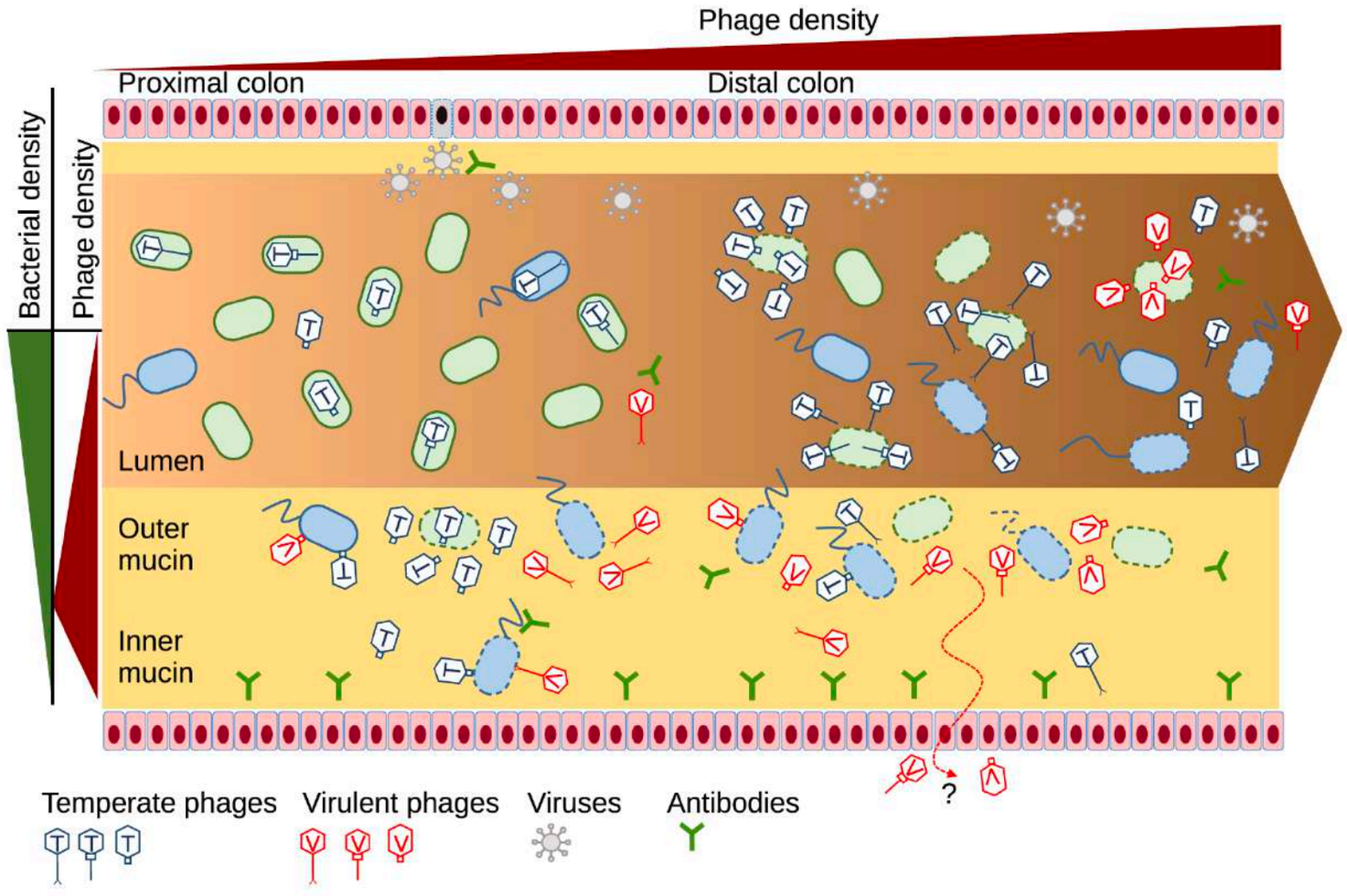
Viruses-Bacteria Interactions in the gut

Cao et al. 2022



Bacteriophage Production in the Human Gut

Shkoporov and Hill, 2019

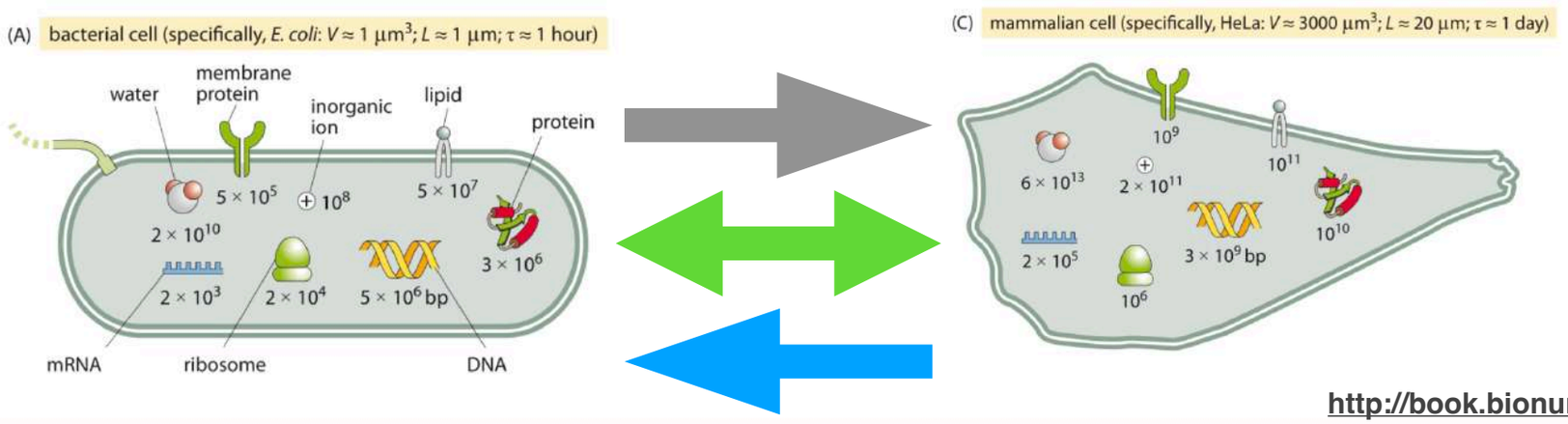


- The **luminal** contents contain **dense bacterial populations**, propelled in the distal direction by peristalsis and mass movement
- **Lysogeny if favored in the gut lumen over lytic cycle (''piggyback-thewinner'' model) resulting in low virus to microbe ratio**. Toward the terminal colon prophage induction is more likely due to nutrient **starvation** and, possibly, **oxidative stress**. In the thick mucin layer, bacterial density is kept to relatively low levels, causing a density-dependent switch to **lytic cycle in temperate bacteriophages** ("kill-the-winner" model)
- Large amounts of phage particles become attached to mucin where they provide BAM (bacteriophage adherence to mucus) immunity and could potentially translocate into lamina propria and sub-mucosal layer triggering an anti-phage immune response

Paradigm shift

Human-microbes interactions

- Many programs to study Prok-Euk interaction in the human ecosystems



http://book.bionumbers.org/

TABLE 24.1 Major human microbiome research programs

Research program	*Participating countries*	*Programmatic objectives*
MetaGenoPolis	France	Demonstrate the impact of the human gut microbiota on health and disease using metagenomics technology
International Human Microbiome Standards	European Commission	Optimize methods for the assessment of the effects of the gut microbiome on human health through the standardization of procedures and protocols
Korean Twin Cohort Project	Korea	Characterize microbiota associated with epithelial tissue in a twin cohort study group, with the goal of identifying targets for early disease diagnosis and prevention
NIH Human Microbiome Project (HMP)	USA	Characterize the microbes that live in and on the human body, and assess the ability to demonstrate correlations of changes of the human microbiome with health
Canadian Human Microbiome Initiative	Canada	Characterize the microorganisms colonizing the human body. Evaluate their relationship to health and examine compositional changes associated with chronic disease
NIH Jumpstart Program	USA	Generate the complete genome sequences of 200 bacterial strains isolated from the human body; recruit donors for securing samples from five body regions, and perform 16S rRNA and metagenomic sequence analysis of the sampled body regions
Integrative Human Microbiome Project	USA	Crowdsourcing model to secure fecal samples for 16S rRNA sequence analysis

Madigan et al. 2018

Representative studies linking human conditions to the microbiome

Condition or disease	Microbiome alteration	Potential or known mechanism	Comments	Refs
Obesity	Greater abundance of pathobionts and Firmicutes	Calorie harvesting, inflammation, modulating satiety, regulating adipogenesis	Controversial microbial links to complex, that is, multifactorial, disease	157
Type 2 diabetes	As for obesity, with signals related to *Prevotella copri* and *Akkermansia muciniphila*	Unclear; liver signalling, branched-chain amino acids?	Initial success with faecal microbiota transplantation not maintained in later studies	158
Inflammatory bowel disease	Reduced abundance of Christensenellaceae, *Coriobacteriaceae*, *Faecalibacterium prausnitzii*; higher abundance of Actinomyces, *Veillonella*, *Escherichia coli*	Products of colonic inflammation stimulate anaerobic respiration, driving microbiome further towards a pro-inflammatory type	Meta-analysis concedes lack of a unifying taxon signature for inflammatory bowel disease; once inflammation is triggered, the microbiome may be irrelevant for treating inflammatory bowel disease	159,160
Irritable bowel syndrome	*Ruminococcus gnavus* and Lachnospiraceae are more abundant, *Barnesiella intestinihominis* and *Coprococcus catus* depleted	Pathophysiology may involve a reduction of luminal pH by excessive fermentation and sensitization of the enteric nervous system by inflammation	Not all patients with irritable bowel syndrome have an altered microbiome; disruption of the diet–microbiome–metabolome connectivity is a feature of those who do	161,162
Colorectal cancer	Presence of *Fusobacterium nucleatum* and other oral biofilm-forming pathobionts is a feature of tumour microbiome	Inflammation, DNA breakage, mutagenesis	Microbiome alterations linked to colon cancer relate to known risk factors such as diet and inflammation; microbiome also influences the responsiveness of cancers to checkpoint immunotherapy	10
Cardiovascular disease	Bacterial taxa capable of generating trimethylamine from carnitine, choline and glycine betaine	Trimethylamine is a substrate for liver production of trimethylamine oxide, an atherogenic metabolite	Initial controversy due to inverse relationship between choline intake and cardiovascular disease but prospects for druggable targets	7,9,163
Cognitive function, behaviour and mood	Diverse observations and metabolites reported but a catalogue of gene products with neuroactive potential identified	Effects on neurodevelopment, neuroplasticity, degree of myelination, peptide binding to immune cells and vagus nerve endings, other brain signalling effects	Plausible leads but a paucity of compelling human studies	8,164

Gosh et al., 2022

How do microbes interact with human as a whole unit?

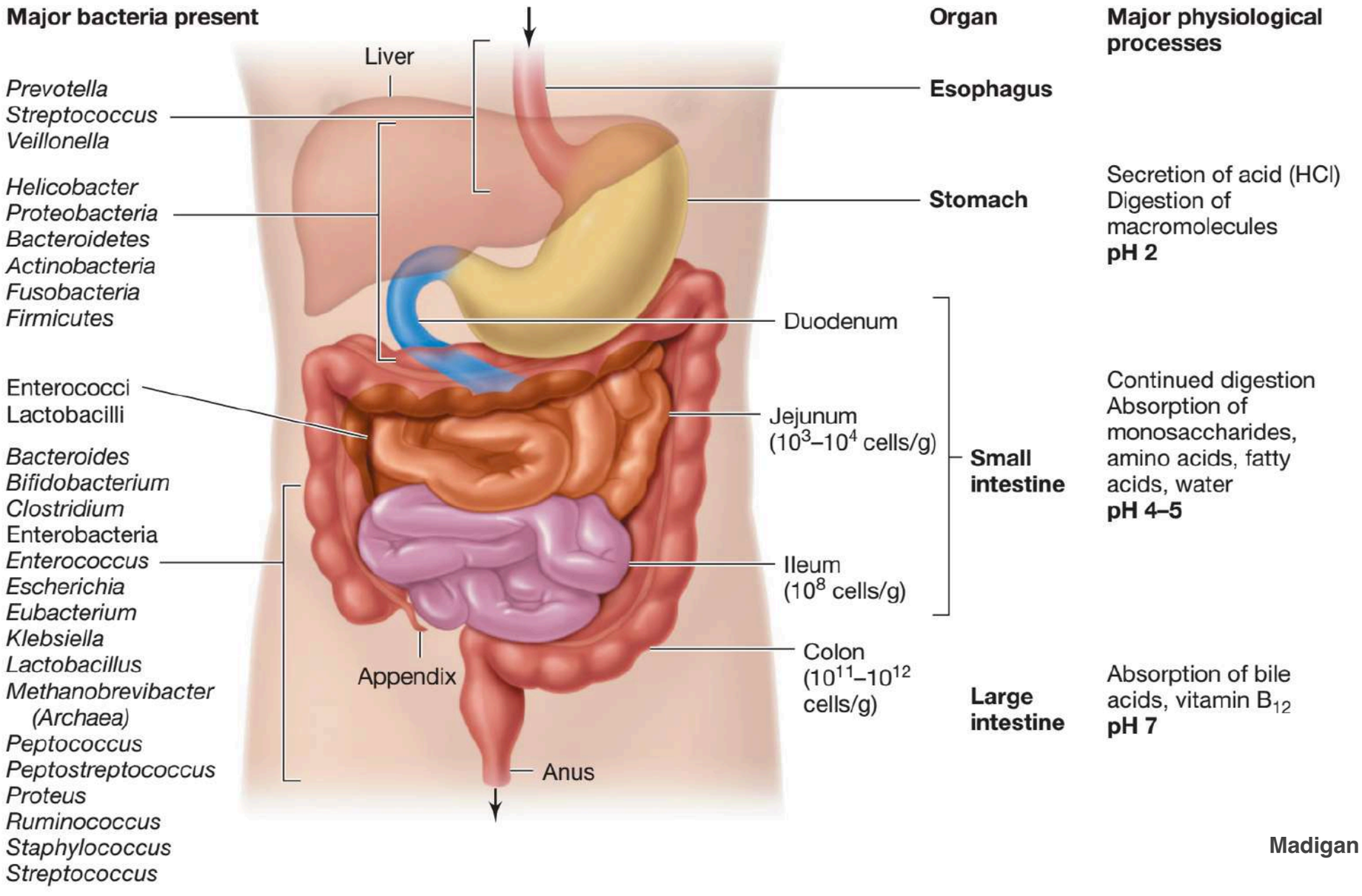
Let’s focus where there is the highest microbial abundance

THE GUT

Gut-microbes association

- Changing in space and time
- Changing with age host and health status
- Changing with food ingested and drugs

Ever-changing microbial communities and abundance



Madigan et al. 2018

Secondary metabolite production by gut microbiota association

TABLE 24.2 Biochemical/metabolic contributions of intestinal microorganisms

Process	*Product or enzyme*
Vitamin synthesis	Thiamine, riboflavin, pyridoxine, B_{12}, K
Amino acid synthesis[a]	Asparagine, glutamate, methionine, tryptophan, lysine, and others
Gas production	CO_2, CH_4, H_2
Odor production	H_2S, NH_3, amines, indole, skatole, butyric acid
Organic acid production	Acetic, propionic, butyric acids
Glycosidase reactions	β-Glucuronidase, β-galactosidase, β-glucosidase, α-glucosidase, α-galactosidase
Steroid metabolism (bile acids)	Esterified, dehydroxylated, oxidized, or reduced steroids

[a]Capacity for amino acid biosynthesis inferred from the identification of biochemical pathways encoded in gut metagenomic sequences (Sections 9.8 and 19.8).

Immune system does not properly develop in the **absence** of **microbial** stimulation and that early life exposure to a variety of microorganisms is essential for developing tolerance to beneficial microorganisms and recognizing pathogens as foreign

Window of opportunity for interactions in order to train the immune system

TABLE 24.3 Small bioactive molecules produced by bacteria in the large intestine

Class	*Compound*	*Example producer*	*Activity*
RiPP[a] (lantibiotic)	Ruminococcin A	*Ruminococcus gnavus*	Antibiotic
RiPP[a] (bacteriocin)	Ruminococcin C	*Ruminococcus gnavus*	Antibiotic
Amino acid metabolite	Indolepropionic acid	*Clostridium sporogenes*	Protective anti-oxidant
Amino acid metabolite	4-Ethylphenylsulfate	Undefined	Neuromodulatory
Amino acid metabolite	Tryptamine	*Ruminococcus gnavus*	Neurotransmitter
Volatile fatty acid	Propionic acid	*Bacteroides* spp.	Immunomodulatory[b]
Oligosaccharide	Polysaccharide A	*B. fragilis*	Immunomodulatory[b]

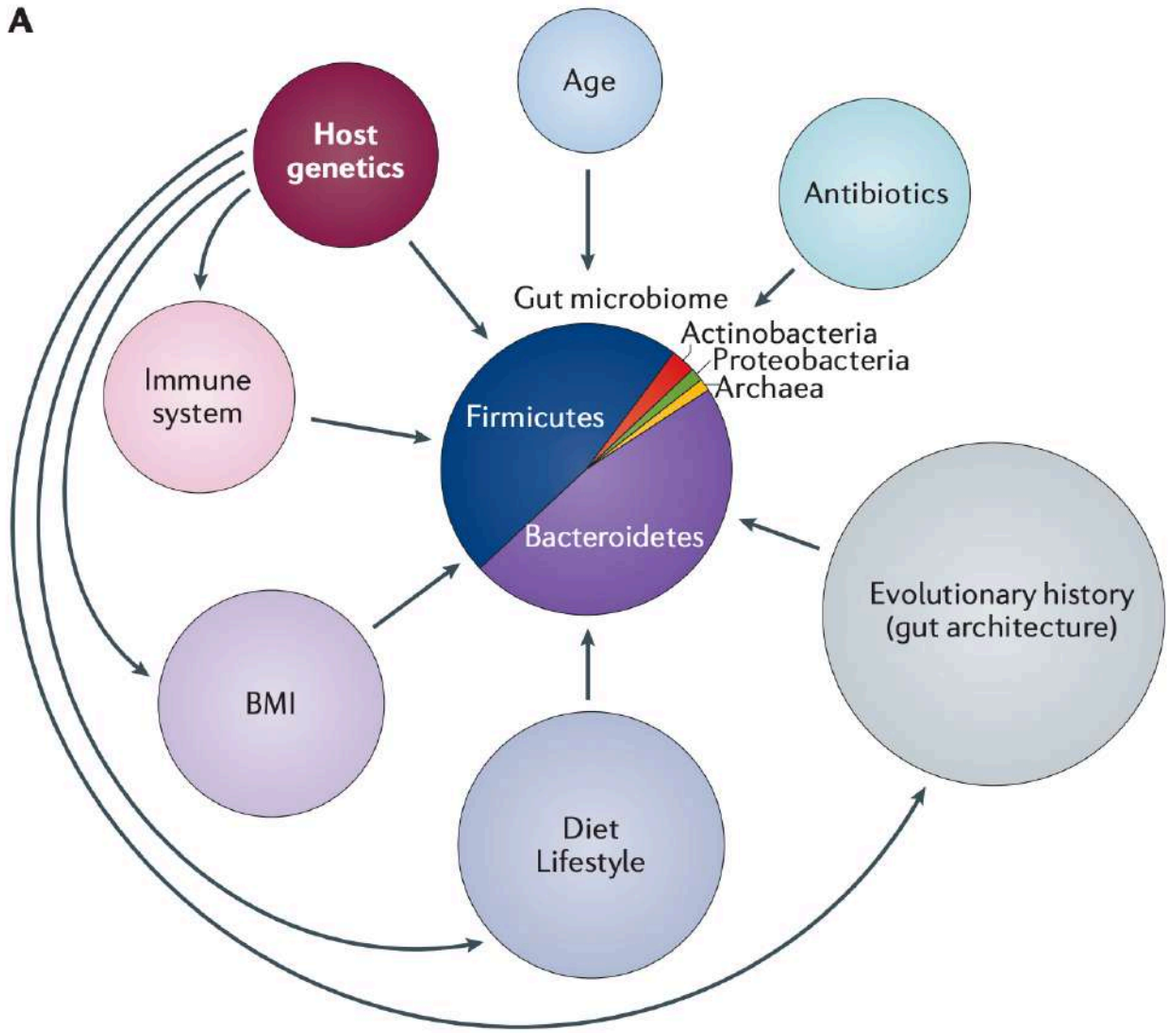
[a]Ribosomally synthesized and post-translationally modified peptides.
[b]These small molecules promote colonization by normal microbiota.

Gut as a second brain

Madigan et al. 2018

Dysbiosis, I

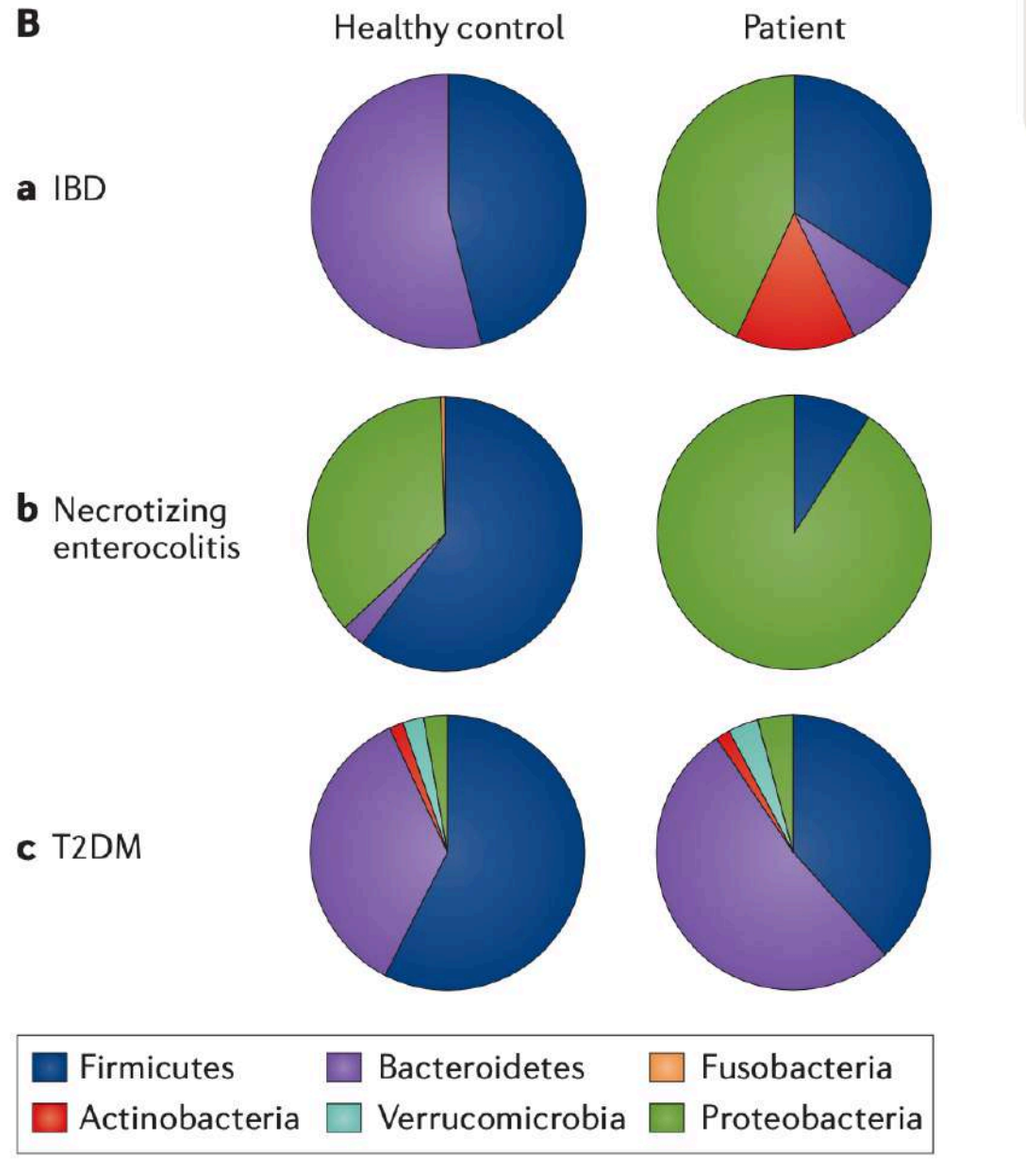
- D**ysbiosis** is defined as a **disruption or imbalance** in the normal microbial communities of the human body, which can contribute to disease
- Imbalance in symbiotic microbial community (changes in their functional composition and metabolic activities, or changes in their local distribution)
- In general, dysbiosis can be categorized into three different types: 1) Loss of beneficial organisms, 2) Excessive growth of potentially harmful organisms, and 3) Loss of overall microbial diversity. Changes of interactions among microbes due to changes in communities



Hall et al. 2017

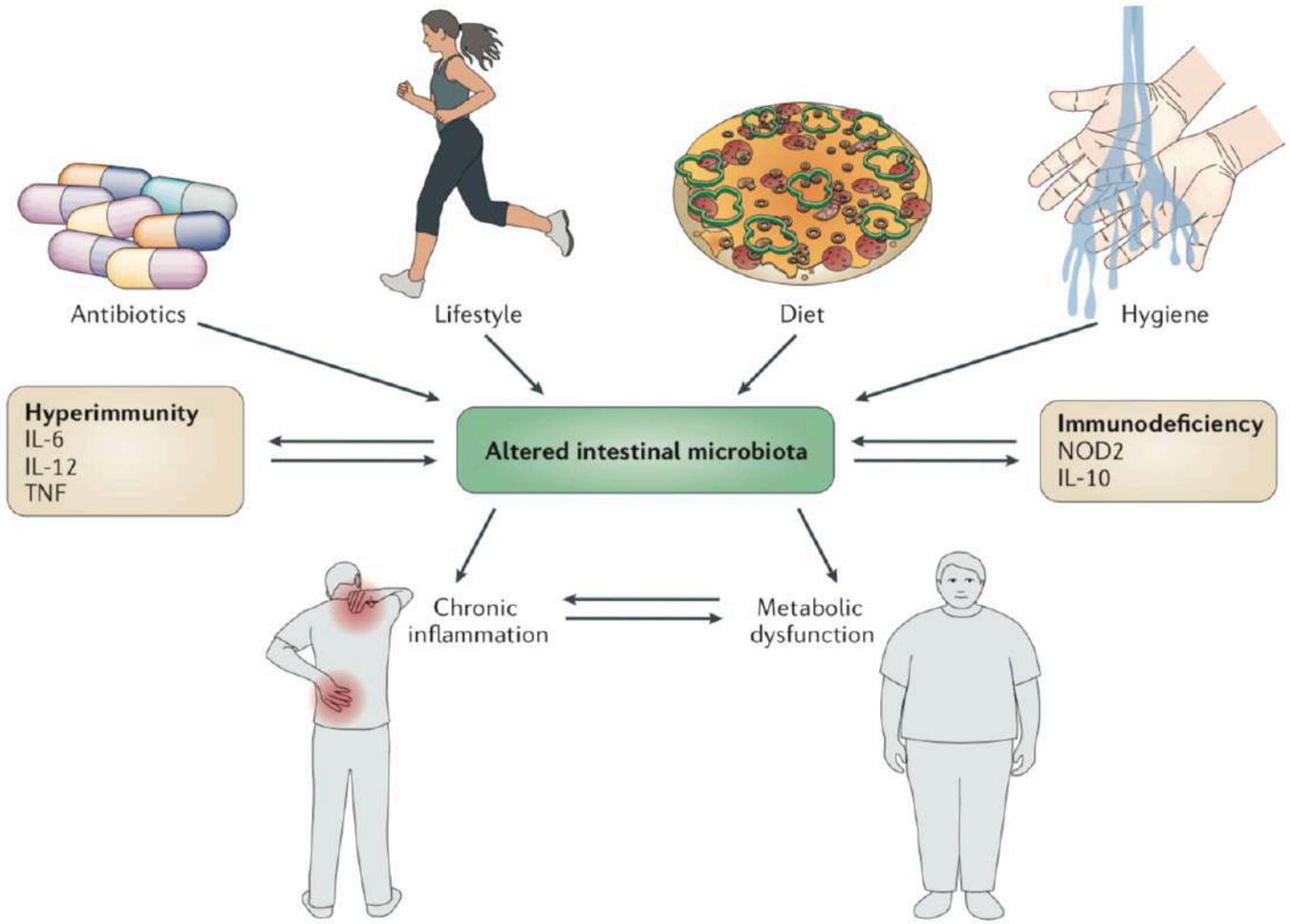
Dysbiosis, II

- **Dysbiosis (altered microbial community)** of the gut microbiome has been implicated in multiple diseases:
  - Inflammatory bowel disease (IBD)
  - Necrotizing enterocolitis (in premature infants)
  - Type 2 diabetes mellitus (T2DM)
  - Colorectal cancer



Hall et al. 2017

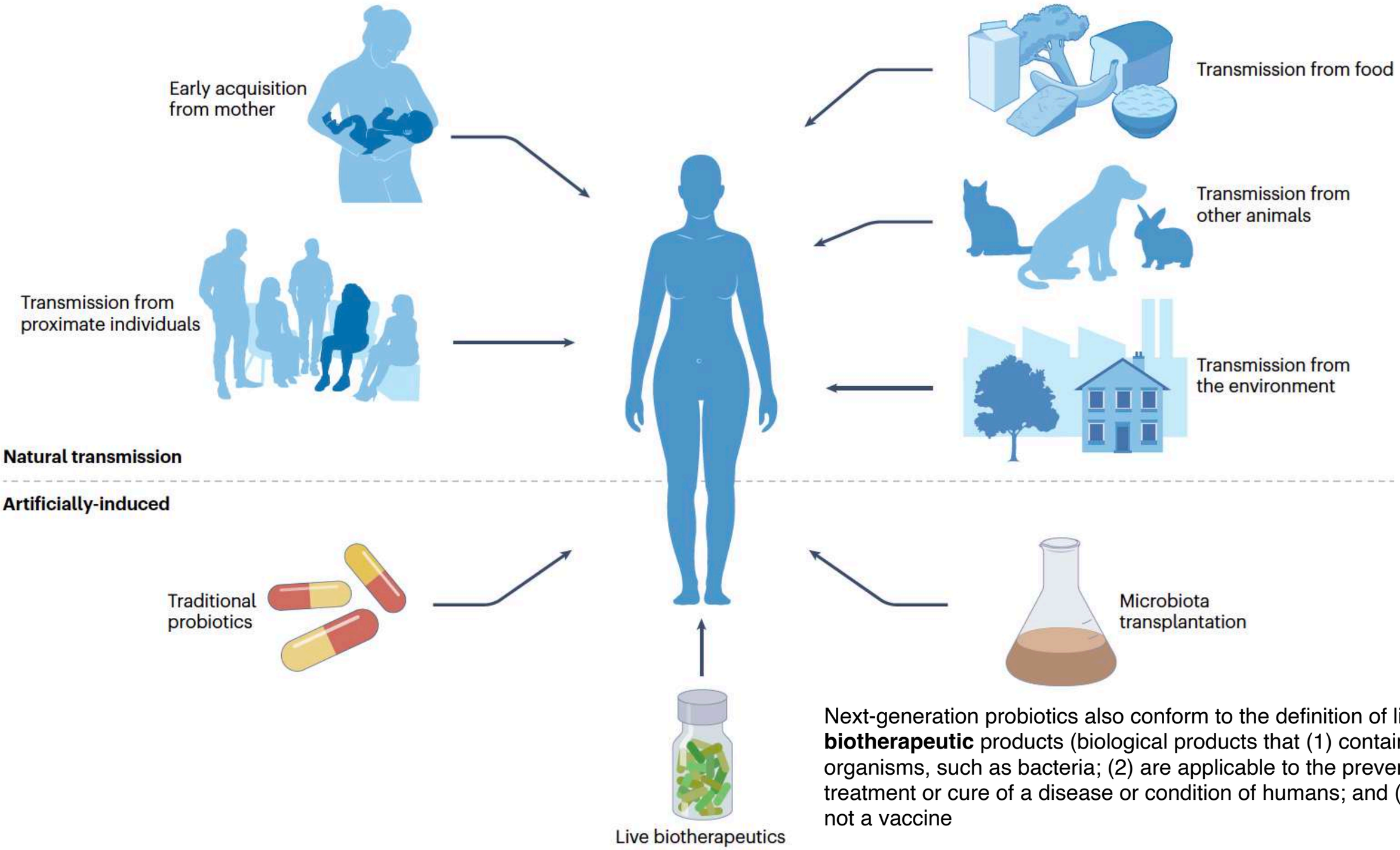
Factor shaping microbial gut community



- Environmental factors: **antibiotics, lifestyle, diet and hygiene preferences**
- Host's **genetic** disposition has a role in influencing gut microbiota composition
- Hyperimmunity (owing to over-representation of pro-inflammatory mediators such as interleukin-6 (IL-6), IL-12 or tumour necrosis factor (TNF))
- Immunodeficiency (owing to mutations in regulatory immune proteins such as NOD2 (nucleotide-binding oligomerization domain protein 2) or IL-10)
- Dysbiosis affects levels of immune mediators and induces both chronic inflammation and metabolic dysfunction

Sommer & Bäckhed, 2013

Diverse natural sources of human microbiome strains

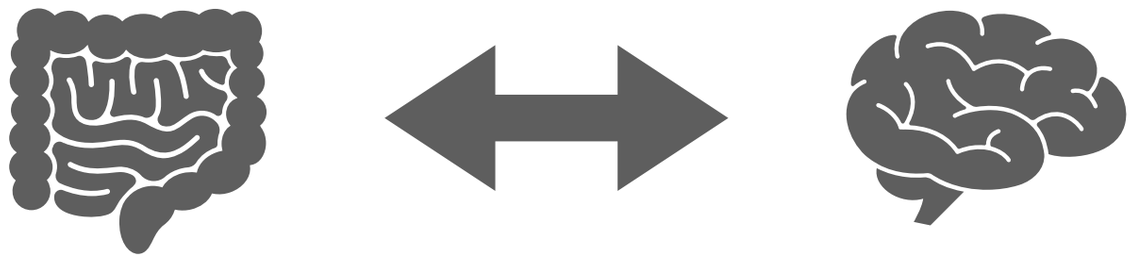


Next-generation probiotics also conform to the definition of live **biotherapeutic** products (biological products that (1) contain live organisms, such as bacteria; (2) are applicable to the prevention, treatment or cure of a disease or condition of humans; and (3) are not a vaccine

Heidrich et al., 2025

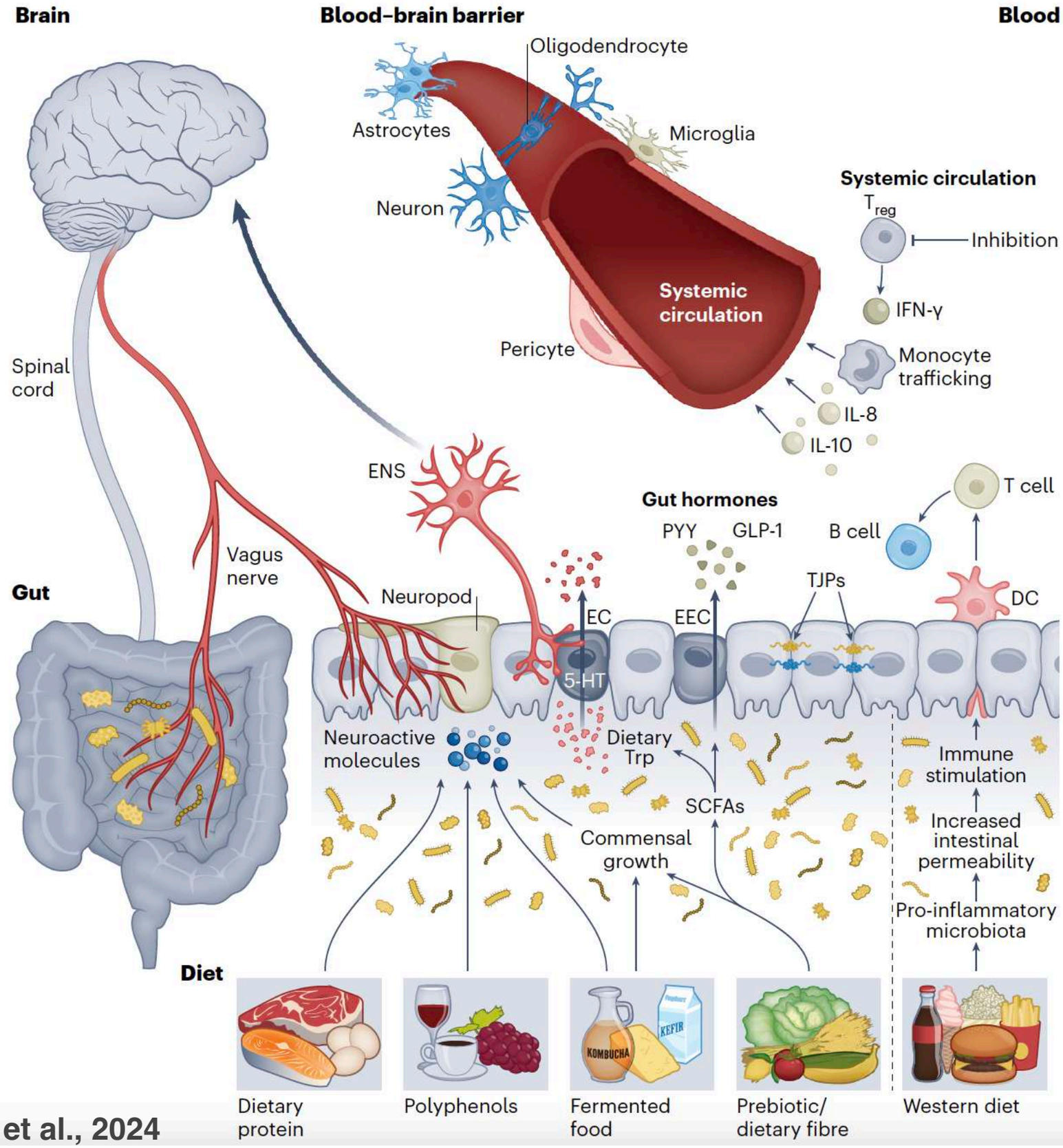
Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

Discovery: microbes play important roles in keeping us healthy and happy



https://www.who.int/about/accountability/governance/constitution#:~:text=Health%20is%20a%20state%20of,absence%20of%20disease%20or%20infirmity.

Mechanisms of action



DC, dendritic cell;
EC, enterochromaffin;
EEC, enteroendocrine cell;
ENS, enteric nervous system;
IFN-γ, interferon gamma;
Treg regulatory T cell;
TJPs, tight junction proteins;
Trp, trypotophan

Schneider et al., 2024

- Mechanisms of action: schematic overview of the several pathways of bidirectional communication through which the diet–microbiota–gut–brain axis impacts on cognition and emotion.

- Healthy dietary patterns, rich in fibre, fruits and vegetables, promote microbial diversity in the gut and the production of beneficial metabolites such as SCFAs.

- These metabolites can travel to the brain directly through the bloodstream, enhancing cognitive and emotional processing.

- Additionally, a healthy diet supports gut barrier integrity, preventing harmful substances from entering the bloodstream, and modulates the immune system to promote anti-inflammatory responses that benefit brain function.

- Neural pathways, such as signalling via the vagus nerve, also play a crucial role in transmitting signals from the gut microbiota to the brain.

- Conversely, the consumption of high-fat and high-sugar foods typical of the Western diet leads to a reduction in beneficial gut bacteria and increases the production of pro-inflammatory metabolites.

- This weakened gut barrier allows harmful substances to leak into the bloodstream, resulting in systemic inflammation that can negatively impact the brain.

- Inflammatory responses travel through the bloodstream and reach the brain, potentially affecting behaviour, cognition and emotional states.

- The schematic underscores the critical role of dietary choices in influencing mental health and cognitive function through these interconnected pathways involving gut microbiota and associated metabolic and inflammatory processes.

Short-Chain Fatty Acids

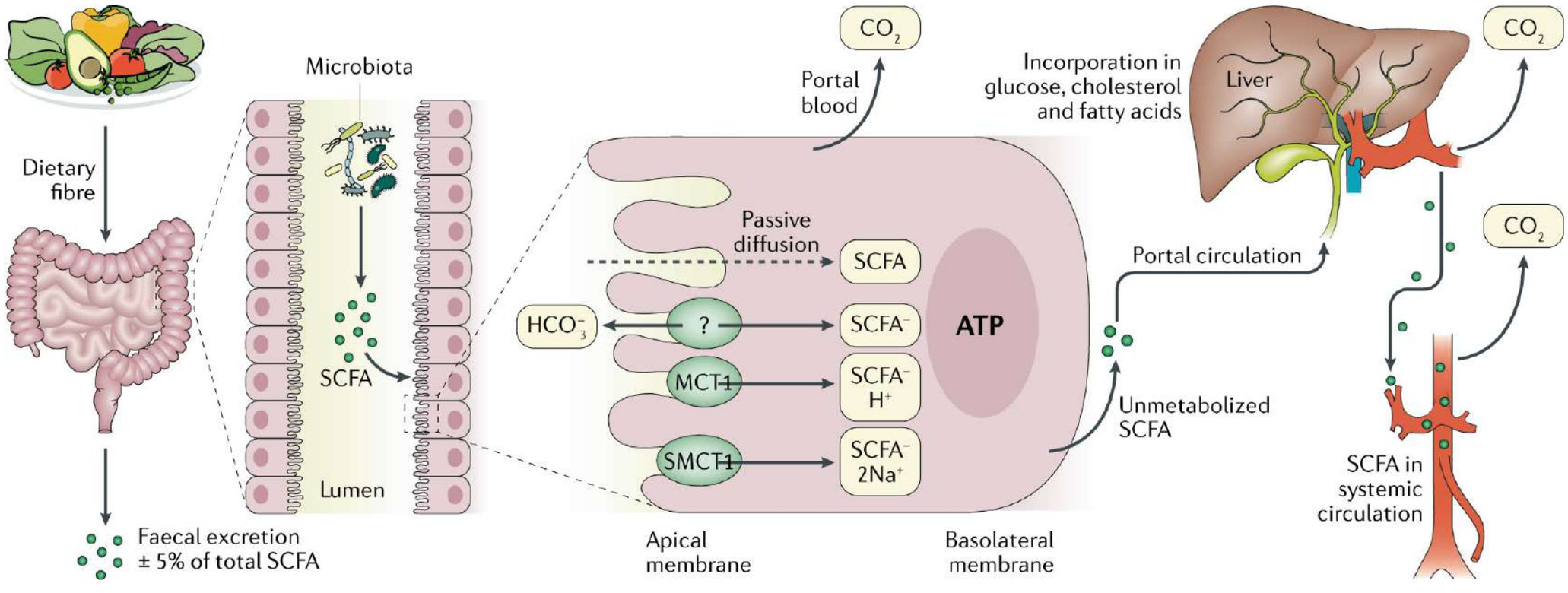
Short-chain fatty acids are volatile fatty acids produced mainly by microbial fermentation

Human physiology: SCFAs are produced in the proximal colon by microbial fermentation of oligo- and polysaccharides that have escaped digestion by mammalian enzymes further up the gastrointestinal tract

The major short-chain fatty acids are **acetic, propionic, and butyric**

They are very soluble and are absorbed easily from the gut lumen into the bloodstream

In humans, acetate is usually the major short-chain fatty acid present in the circulation



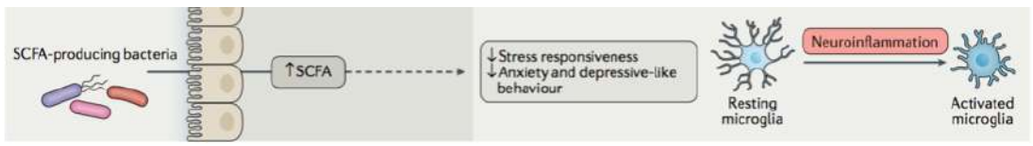
Dalile et al., 2019

Substrates	Dietary source	Fermenting genera
Resistant starch	Cashew, green banana, white beans, oat and potato	• *Ruminococcus* • *Bacteroides*
Cellulose	Seaweed and cereal bran	• *Bacteroides* • *Ruminococcus*
Hemi-celluloses (xylan and arabinoxylan)	Cereal bran	• *Bacteroides* • *Roseburia* • *Prevotella*
Pectin	Apples, apricots, cherries, oranges and carrots	• *Eubacterium* • *Bacteroides* • *Faecalibacterium*
Fructans (inulin and fructooligosaccharides)	Asparagus, leek, onions, banana, wheat, garlic, chicory and artichoke	• *Bacteroides* • *Faecalibacterium*
Milk oligosaccharides	Breast milk	*Bifidobacterium*
Lactose (only in lactose-intolerant people)	Milk, yogurt, buttermilk and cheese	*Bifidobacterium*
β-Glucan	Oat, barley, wheat, rye, mushrooms and seaweed	• *Eubacterium* • *Atopobium* • *Enterococcus* • *Lactobacillus* • *Prevotella* • *Clostridium* cluster XIVa
Gum arabic	Acacia tree and prepared food additive	• *Bifidobacterium* • *Lactobacillus* • *Ruminococcus*
Guar gum	Guar bean and prepared food additive	• *Bifidobacterium* • *Ruminococcus*
Laminarin	Seaweed	*Prevotella*
Galacto-oligosaccharides	Artichoke, beans, beetroot, broccoli, chickpeas, fennel, lentils, lettuce, radicchio and onion	*Bifidobacterium*
Raffinose and stachyose	Cottonseed flour, soy flour, onions, chickpeas, beans, peas and lentils	• *Bifidobacterium* • *Lactobacillus*

Dalile et al., 2019

Volatilomes of microbes in humans

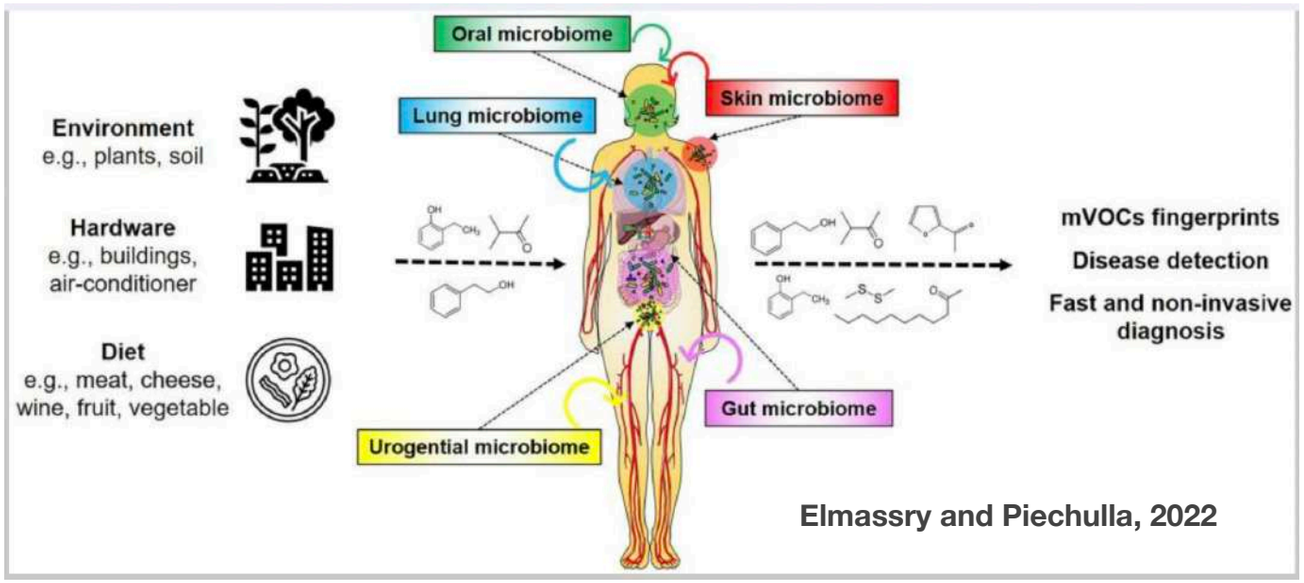
Microbes mediate human health



SCFAs can act on the central nervous system (CNS) by regulating neuroplasticity, epigenetic and gene expression, and the immune system in preclinical models

Neuroplasticity The ability of the nervous system to change activity by reorganizing its structure and function

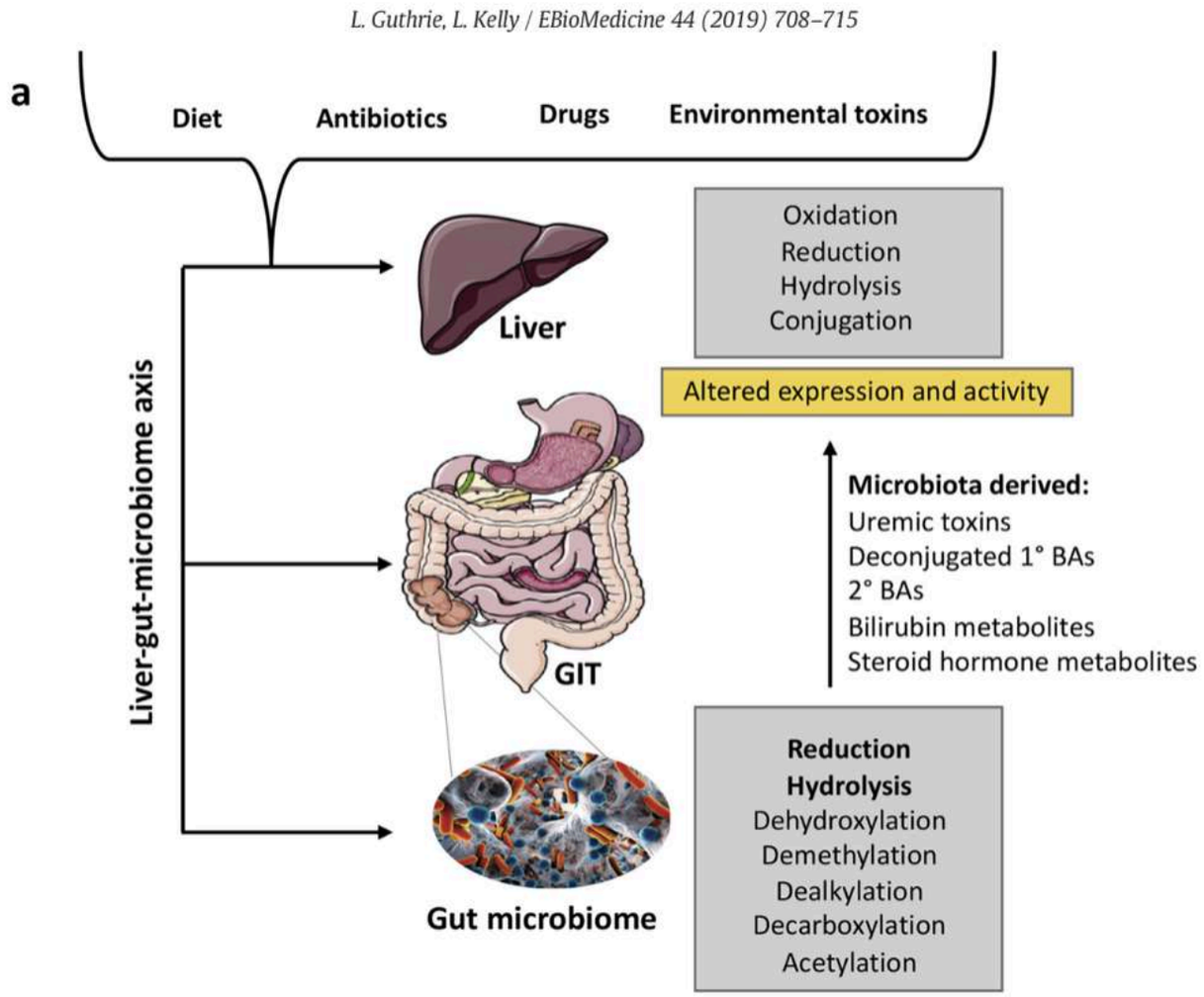
Microbial infections



- mVOCs: microbial volatile organic compounds
- Unique physico-chemical properties: they are small molecules (<300Da), with up to two functional groups and the ability to easily diffuse in air and water
- Microbes produces many compounds, including volatiles —> influence human health (SFCAs)
- Volatiles influence and affect humans
- mVOCs released of the human microbiomes are potential biomarkers for non-invasive diagnosis for pathogens

Microbiome to individual medicine

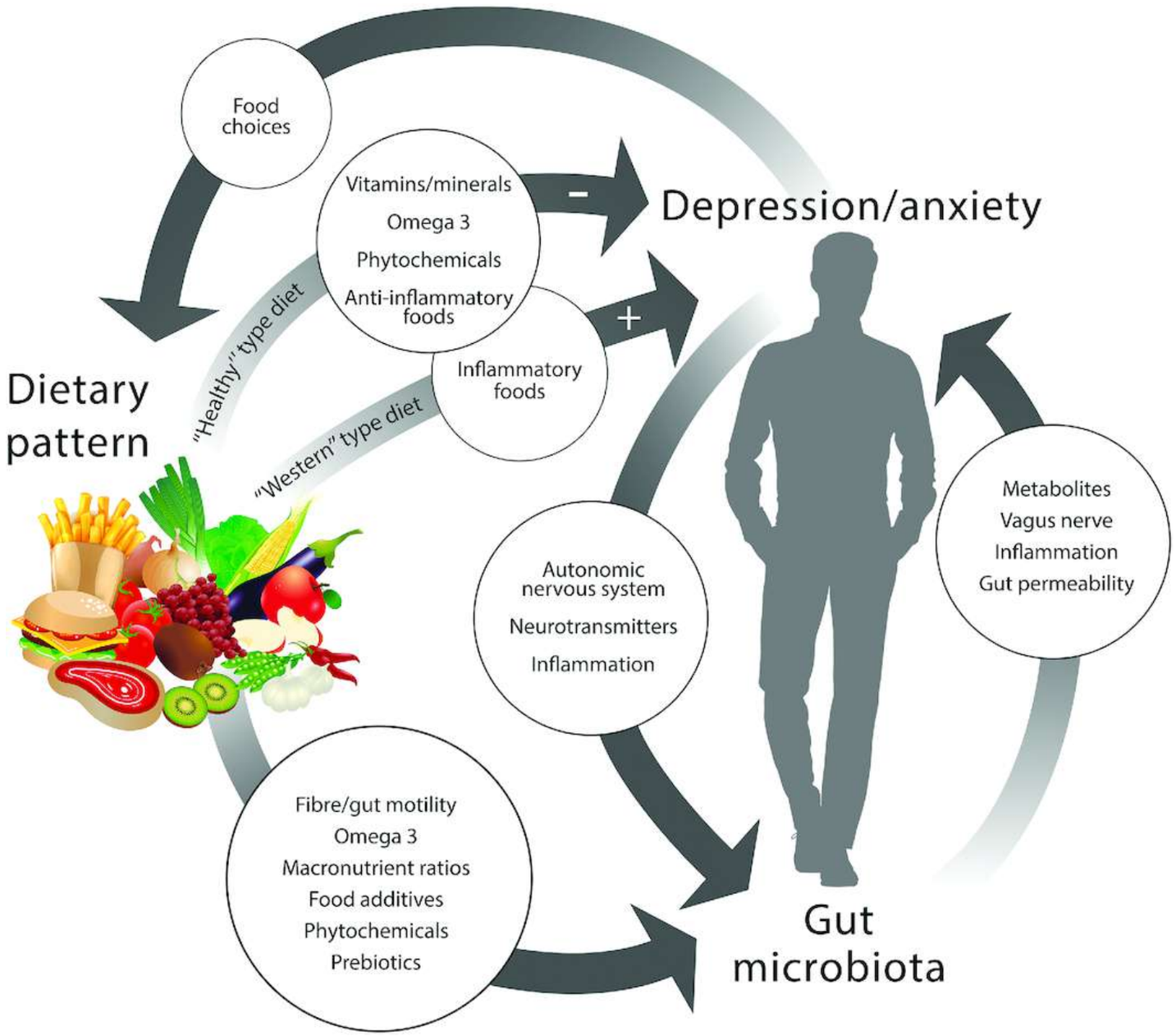
- Translating microbiome research into the clinic requires, in part, a **mechanistic and predictive understanding of microbiome-drug interactions**
- Human metabolism and individual variation in drug response
- Microbiome chemical mechanisms shape drug metabolism
- Microbiome modulation of drug metabolism enzymes
- Therapeutic drug influences on the gut microbiome



Guthrie & Kelly, 2019

Gut-Brain Axis

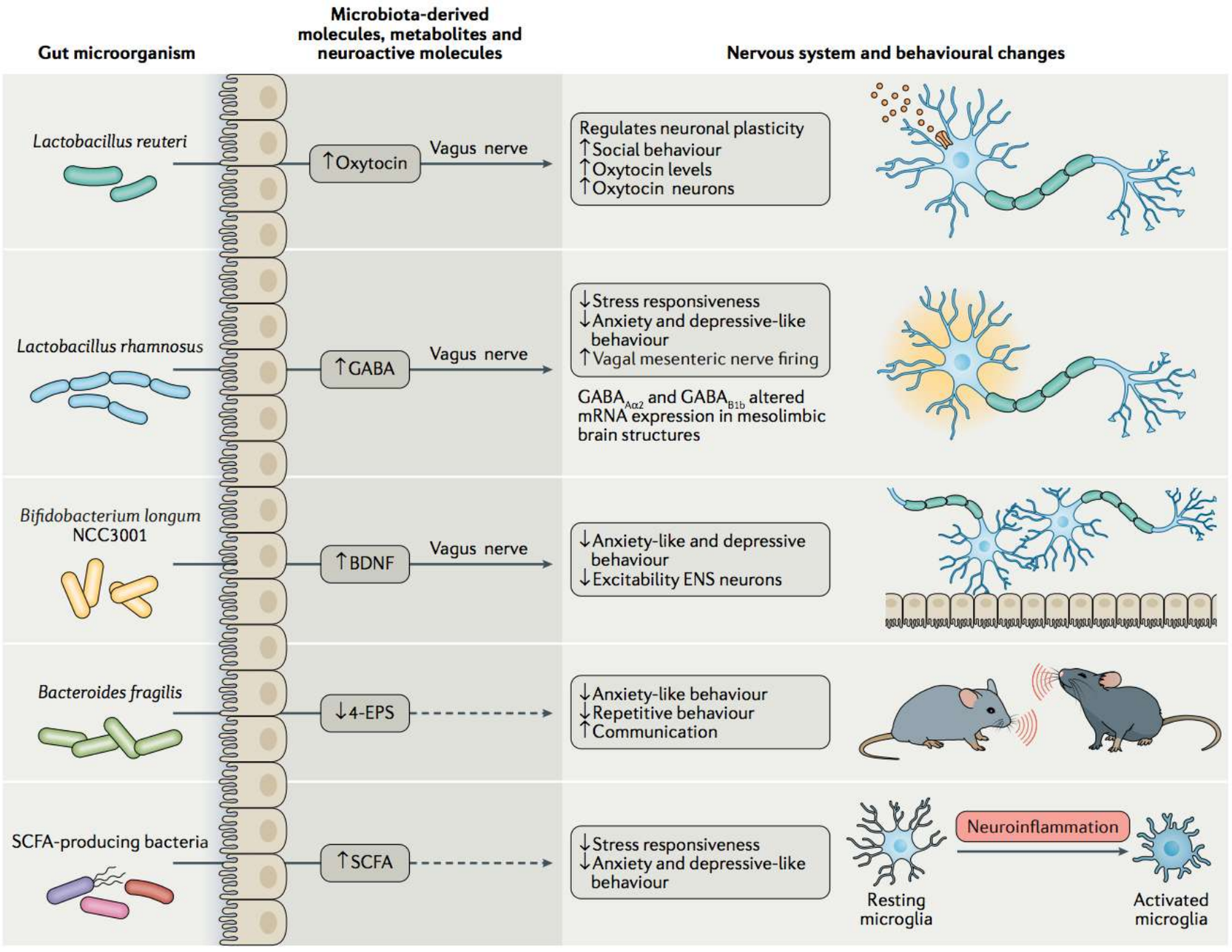
- **“Healthy”** dietary patterns**:** abundance of vegetables, fruits, cereals, nuts, seeds, and pulses, as well as moderate amounts of dairy, eggs, and fish and unsaturated fats, including the Mediterranean diet, Japanese diet, and Norwegian diet
- “**Western**” dietary pattern, consisting of sweet and fatty foods, refined grains, fried and processed foods, red meat, high-fat dairy products, and low fruit and vegetable intake, is associated with higher depression incidence
- **Food molecules influence brain via gut-brain axis**



Bear et al., 2020

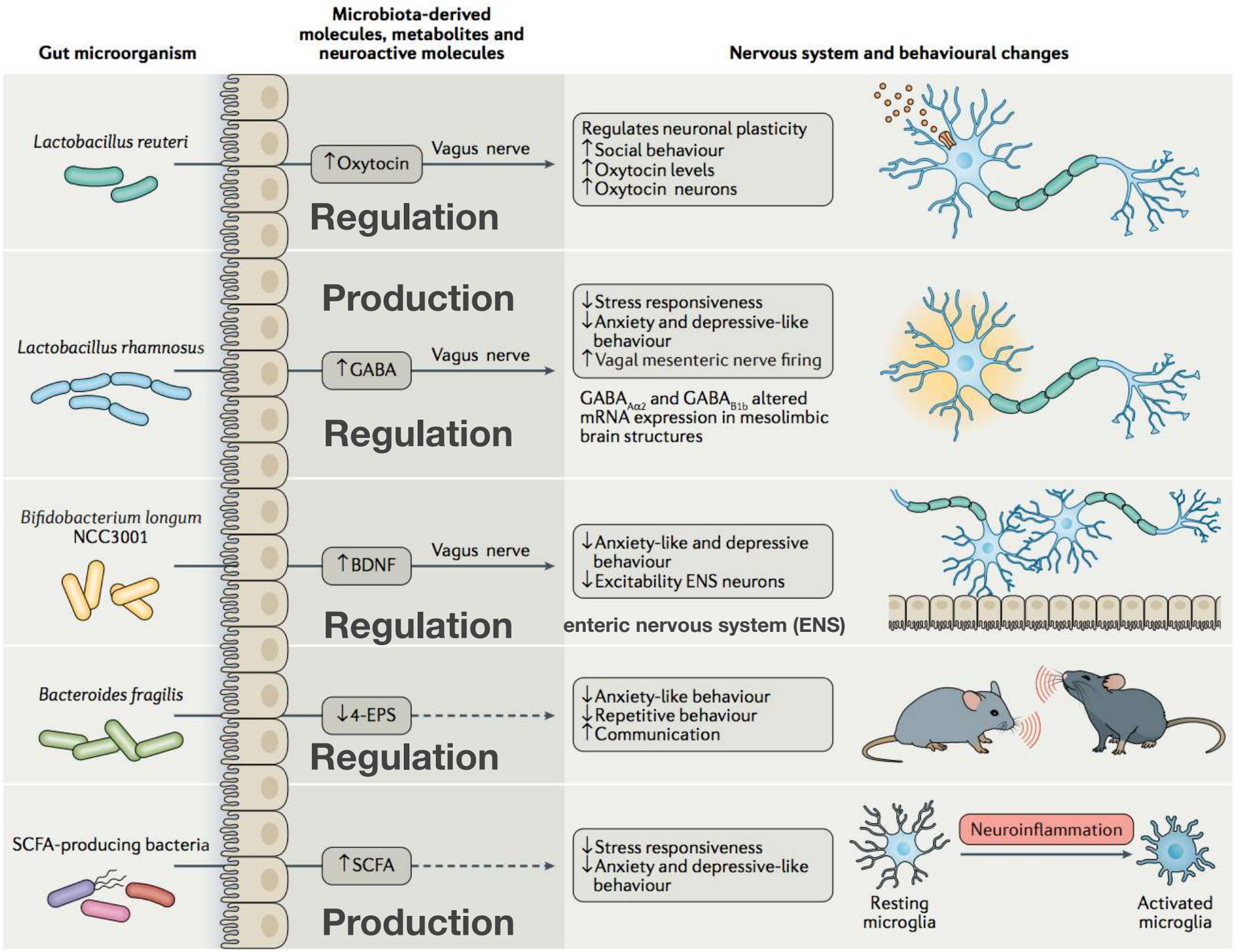
Microbiota and microbial-derived molecules modulate host behaviour and nervous system function

Culture and uncultured based-approach



Morais et al., 2020

Microbiota and microbial-derived molecules modulate host behaviour and nervous system function



γ-aminobutyric acid (GABA)

Brain-derived neurotrophic factor (BDNF)

4-ethylphenylsulfate (4-EPS)

Short-chain fatty acids (SCFAs)

Morais et al., 2020

TABLE 1 Examples of published literature evidence (from in vitro, animal, and human studies) of components within dietary patterns related to depression in humans or emotional behaviors in animals, which directly affect the host but also interact with the gut microbiota[1]

Dietary component	Effect	Summary	Subject	Ref.
Phytochemicals				
Cocoa polyphenols	Affected mood	In an RCT in adults, 500 mg supplement for 30 d increased self-rated calmness and contentedness compared with placebo.	Human	Pase et al. (183)
	Altered microbial growth	A 6-wk diet with 10% cocoa in rats caused a decrease in *Bacteroides*, *Clostridium*, and *Staphylococcus* genera in feces.	Animal	Massot-Cladera et al. (184)
		In vitro digestion with 1 g cocoa powder/60 mL water. 38.6% of phenols were solubilized, and an increase in *Bifidobacteria*, *Lactobacilli*, and butyrate was found.	In vitro	Fogliano et al. (185)
	Altered immune function	A 6-wk diet with 10% cocoa in rats caused an altered toll-like receptor pattern and increased gastrointestinal immunoglobin A secretion.	Animal	Massot-Cladera et al. (184)
Blueberry extract (anthocyanins)	Affected mood and cognition	In a BCT, in children and young adults, a single drink containing 253 mg anthocyanins increased positive but did not change negative affect scores using the "Positive and Negative Affect Scale" compared with a placebo drink.	Human	Khalid et al. (186)
		A 5% blueberry drink given to rats for 8 wk protected against cognitive impairment during chronic mild stress.	Animal	Guo et al. (187)
	Altered host metabolites	Decreased plasma norepinephrine and dopamine concentrations, and brain concentrations of antioxidant compounds due to 8 wk of chronic mild stress were attenuated by a 5% blueberry drink.	Animal	Guo et al. (187)
Fiber (prebiotic)				
GOS, PDX, and FOS	Attenuated stress-induced behaviors and mood, and gene expression in the brain	Male rats were fed diets containing GOS + PDX for 4 wk and then underwent inescapable stressors. The prebiotic reduced stress-induced exaggerated freezing and deficit in escape latency, and attenuated c-fos mRNA in parts of the brain.	Animal	Mika et al. (188)
		Male and female rats underwent early-life stress (maternal separation model). Prebiotic supplementation of GOS + FOS for 5 wk after the stress attenuated stress-induced deficits in spatial memory and locomotion, but not anxiety-like behaviors.	Animal	McVey Neufeld et al. (189)
		RCT, patients with depression: 8 wk supplementation with 5 g GOS resulted in decreases in scores on the Beck Depression Inventory compared with placebo.	Human	Kazemi et al. (190)
		Healthy volunteers given either FOS or GOS daily for 3 wk. Salivary cortisol awakening response and emotional bias (attention to negative information) were decreased after GOS but not FOS.	Human	Schmidt et al. (191)
	Altered the gut microbiota	Prebiotic diet of GOS + FOS increased *Lactobacillus rhamnosus* and also *Lactobacillus* spp.	Animal	Mika et al. (188)
		44 elderly subjects, given 5.5 g/d GOS or placebo for 10 wk in a double-blind, placebo-controlled, crossover study. Increase in *Bifidobacterium* spp., *Lactobacillus Enterococcus* spp., *Clostridium coccoides–Eubacterium rectale*, and a decrease in *Bacteroides* spp., *Clostridium histolyticum* group, *Escherichia coli*, and *Desulfovibrio* spp.	Human	Vulevic et al. (192)
	Altered immune function	Increases in immune function, including reduced proinflammatory cytokines and increased anti-inflammatory cytokines, phagocytosis, and NK cell activity.	Human	Vulevic et al. (192)
Wheat arabinoxylan	May counteract effects of high-protein diet on the gut microbiota	In pigs fed a 4-wk Western-type diet, added soluble fiber (wheat arabinoxylan) increased carbohydrate fermentation and reduced protein fermentation and fermentation products such as ammonia.	Animal	Williams et al. (193)

Dietary component	Effect	Summary	Subject	Ref.
Vitamins/minerals				
Vitamin D	Regulated gut physiological processes	Vitamin D receptors in the gut regulate processes including epithelial barrier function and immune processes.	Review	Barbáchano et al. (194)
	Associated with changes in the gut microbiota	Plasma 25-hydroxyvitamin D and vitamin D supplementation in women in their 36th week of pregnancy were measured, and compared with fecal samples in their 1-mo-old infants. Increased concentrations of both were associated with decreased *Bifidobacterium* spp. and *Clostridium difficile* and increased *B. fragilis*.	Human	Talsness et al. (182)
Magnesium	Dietary deficiency altered behavior	30 mice fed a magnesium-restricted diet for 6 wk had increased immobility in the forced swim test and increased hippocampal IL-6 compared with mice fed a normal diet.	Animal	Winther et al. (179)
	Associated with changes in the gut microbiota	The cecal gut microbiota was also altered, with cluster analysis showing significant differences between the diets.		
Vitamin A	Associated with changes in the gut microbiota and the gut mucosal barrier	A vitamin A–deficient diet in rats increased total bacteria, decreased *Lactobacillus* spp., and increased *Escherichia coli*. Mucin-producing goblet cells were altered and expression of toll-like receptors was increased.	Animal	Amit-Romach et al. (195)
		Vitamin A deficiency in children aged 1–12 mo with persistent diarrhea showed significantly different gut microbiota than in those with normal serum vitamin A concentrations.	Human	Lv et al. (196)
Macronutrients				
ω-3 fatty acids	Immunomodulatory	The metabolic and inflammatory effects in wild-type mice fed a diet with a high ratio of ω-6 to ω-3 were able to be prevented with antibiotic treatment, or by cohousing mice with *Fat-1* transgenic mice, which endogenously produce ω-3 fatty acids.	Animal	Kaliannan et al. (176)
	Increased endogenous antimicrobial defenses	*Fat-1* mice were found to produce increased intestinal alkaline phosphatase, an endogenous antimicrobial compound, which reduced gut permeability and LPS production.	Animal	Kaliannan et al. (176)
	Restored gut dysbiosis	*Fat-1* transgenic mice were found to be protected against gut dysbiosis and obesity caused by a Western-style diet after early-life antibiotic exposure.	Animal	Kaliannan et al. (197)
		Supplementation of 100–250 mg/d ω-3 FA (80% EPA, 20% DHA) for 12 wk to female rats reversed stress-induced gut dysbiosis.	Animal	Pusceddu et al. (177)
	Increased gut microbial metabolites (SCFAs)	An 8-wk open label trial using an EPA/DHA supplement drink or capsule in adult males and females reversibly increased SCFA-producing bacteria including *Bifidobacterium*, *Roseburia*, and *Lactobacillus*.	Human	Watson et al. (198)
	Deficiency affected mood as well as the gut microbiota	An ω-3 FA–deficient diet in pregnant mice and their male offspring resulted in an elevated ratio of *Firmicutes* to *Bacteroidetes* in the offspring, along with altered behavior and immune function.	Animal	Robertson et al. (178)
		Increased depressive behavior (immobility in forced swim test), decreased sociability (three chamber test), isolation-induced ultrasonic vocalizations in adulthood, and decreased memory (novel object recognition test) in both adolescence and adulthood. Increased contextual fear conditioning.		

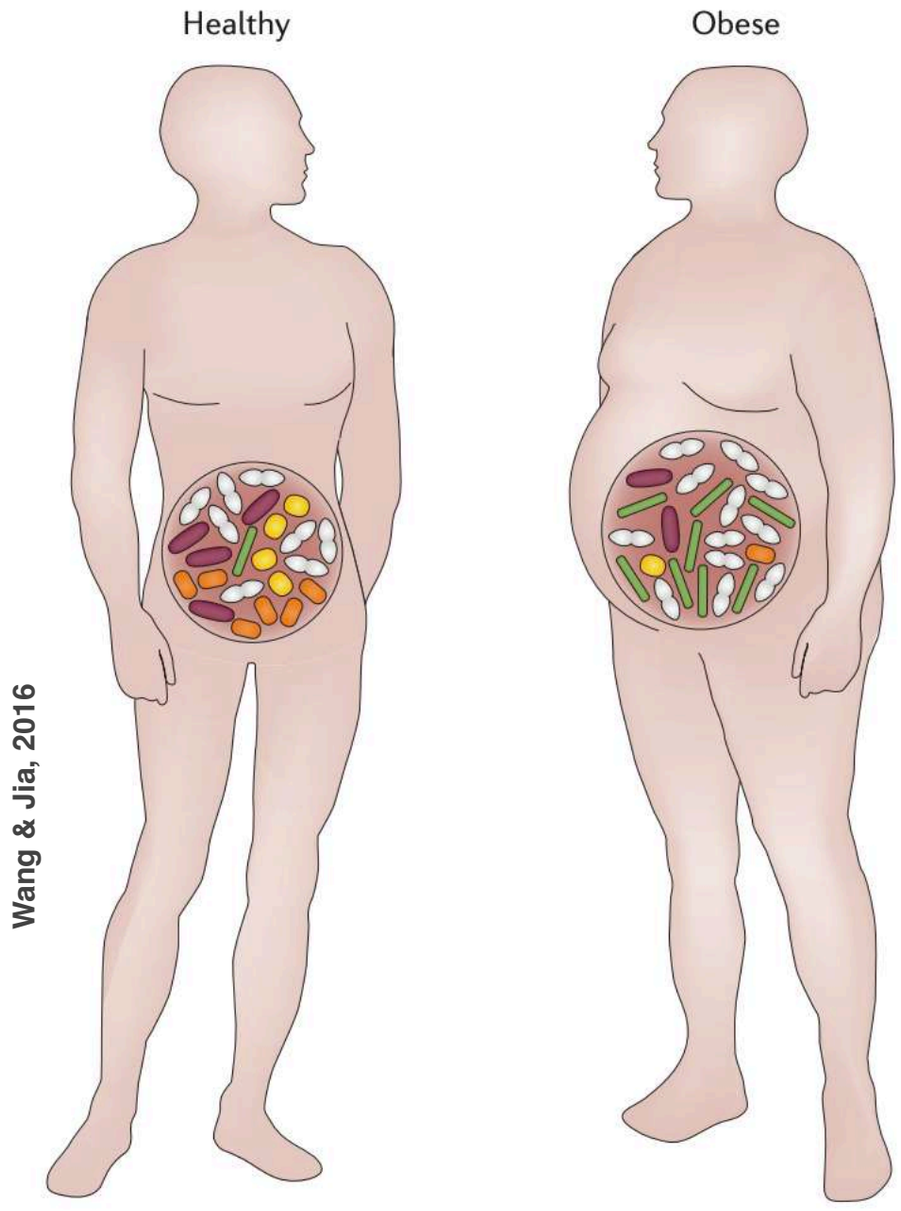
Dietary component	Effect	Summary	Subject	Ref.
High fat, particularly saturated fat	Altered microbiota composition	A high-fat diet in mice decreased *Ruminococcaceae* and increased *Rikenellaceae* compared with a carbohydrate diet.	Animal	Daniel et al. (199)
		Increase in *Firmicutes*, particularly the family *Erysipelotrichaceae*, and decrease in *Bacteroidetes* in mice fed a high-fat diet.	Animal	Fleissner et al. (200)
		Mice fed a low-fat diet who switched to a high-fat diet had a significant shift in microbiome composition within 1 d. Increased *Firmicutes*, particularly the *Erysipelotrichi* class, *Bacilli*, and decreased *Bacteroidetes*.	Animal	Turnbaugh et al. (201)
		BALB/c mice fed a high-fat diet showed alterations in the gut microbiota including an increase in *Firmicutes*, particularly in the families Rumunococcaceae and *Lachnospiraceae*, a decrease in the *Bacteroidetes* phylum, and a resulting decrease in the ratio of *Bacteroidetes* to *Firmicutes*.	Animal	Pyndt Jørgensen et al. (202)
	Altered anxiety-like behavior	Mice fed a high-fat diet displayed less burrowing (anxiety-like) behavior, and displayed reduced memory in the Morris water maze test compared with mice fed a control diet. The diets were not isocaloric, and the high-fat diet mice also gained more weight.	Animal	Pyndt Jørgensen et al. (202)
High-fat, high-sugar diet	Altered microbiota composition	A Western-style diet in humanized mice resulted in increased *Erysipelotrichi* class (mainly *Clostridium innocuum*, *Eubacterium dolichum*, and *Catenibacterium mitsuokai* genera) and *Bacilli* class (mainly *Enterococcus* spp. genera). The microbial shift occurred after only a single day.	Animal	Turnbaugh et al. (201)
High-sugar diet	Positive change in behavior when the gut microbiota was not altered	A high-sucrose diet did not alter the gut microbiota in BALB/c mice compared with a control diet and did alter some behaviors, but in a positive direction (increased latency to immobility in the forced swim test, less goal-orientated burrowing, and less anxiety-like behavior in the triple test).	Animal	Pyndt Jørgensen et al. (202)
Red meat	Modified gut microbiota composition	A comparison between a diet rich in red meat or whole grains (10-wk crossover trial) showed that increased red meat consumption increased the genera *Clostridium* spp. from the phylum *Firmicutes*.	Human	Foerster et al. (203)
	Microbial metabolism of heme-rich meat increases oxidative compounds	Comparison of meat types varying in heme content (beef, pork, chicken) in an in vitro digestion model showed that heme-rich meat caused higher concentrations of the nitrosoxide compound–derived DNA adduct O^6-carboxymethylguanine.	In vitro	Vanden Bussche et al. (204)
Food additives				
Emulsifiers CMC and P80	Altered gut microbiota composition	C57Bl/6J mice were given either CMC or P80 emulsifiers at 1% in their drinking water from weaning until 3 mo old. The gut microbiota was altered by the treatment. Interestingly the outcomes differed between males and females. In males, *Firmicutes* phylum and *Oscillospria*, *Coprococcus*, and *rc4_4* genera were reduced, as well as reduced *Dorea* with P80, and reduced *Bacteroides*, *Burkholderia*, *Clostridium*, and *Veillonella* with CMC. In females, *Bacteroides*, Sphingomonadales, *Sphingomonas*, and *Ruminococcus* were reduced, and there was an increase in *Anaeroplasma* with P80, and the *Proteobacteria* phylum and *Clostridium* and *Burkholderia* genera with CMC.	Animal	Holder et al. (205)
	Altered anxiety-like behavior	Treatment with emulsifiers decreased sociability in the 3-chamber test in females only, and increased locomotion in the Elevated Plus Maze in males only. No difference found in forced swim test or light-dark box.		

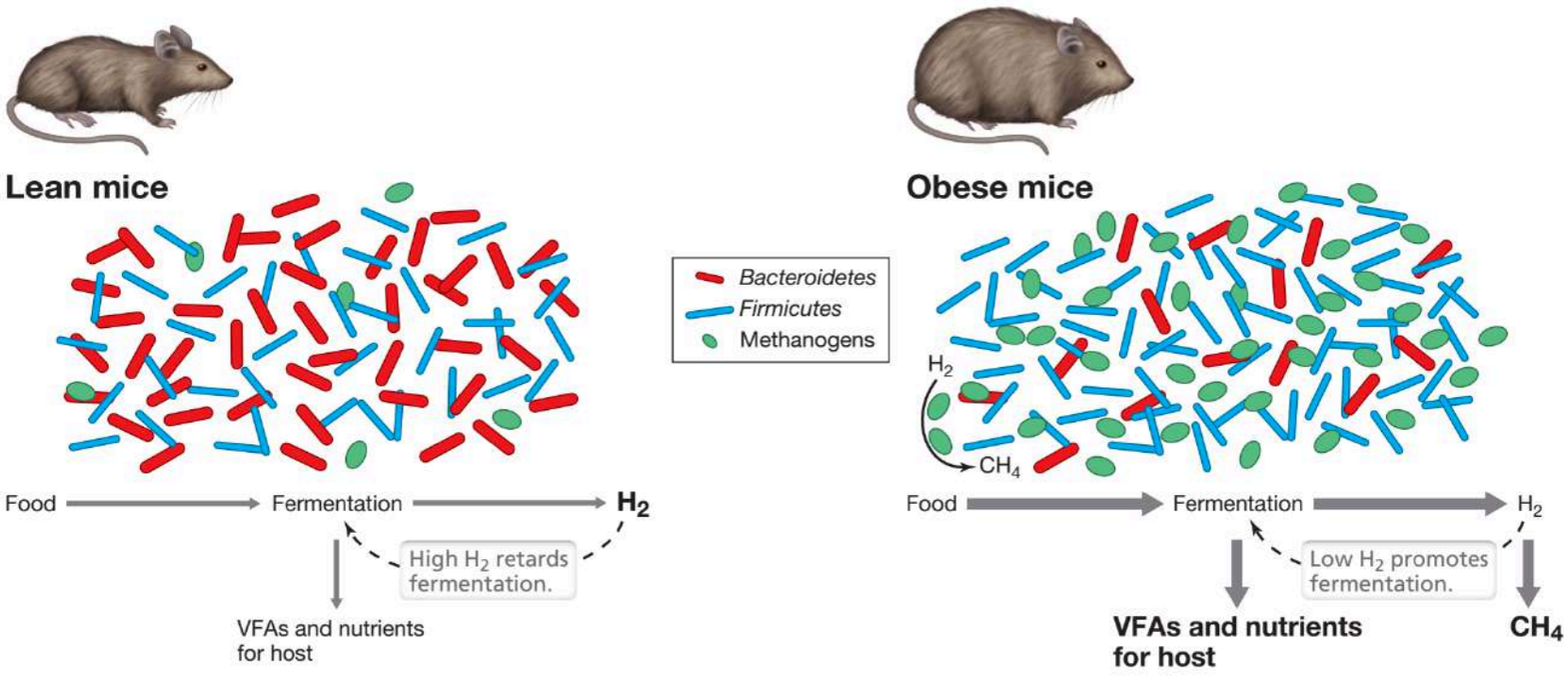
[1] BCT, blinded crossover trial; CMC, carboxylmethylcellulose; FOS, fructooligosaccharide; GABA, γ-aminobutyric acid; GOS, galactooligosaccharide; PDX, polydextrose; P80, polysorbate 80; RCT, randomized controlled trial.

Case studies:
Obesity
Pre-term baby
Athlete motivation
Neurodegenerative disease
Aging

Dysbiosis and Obesity

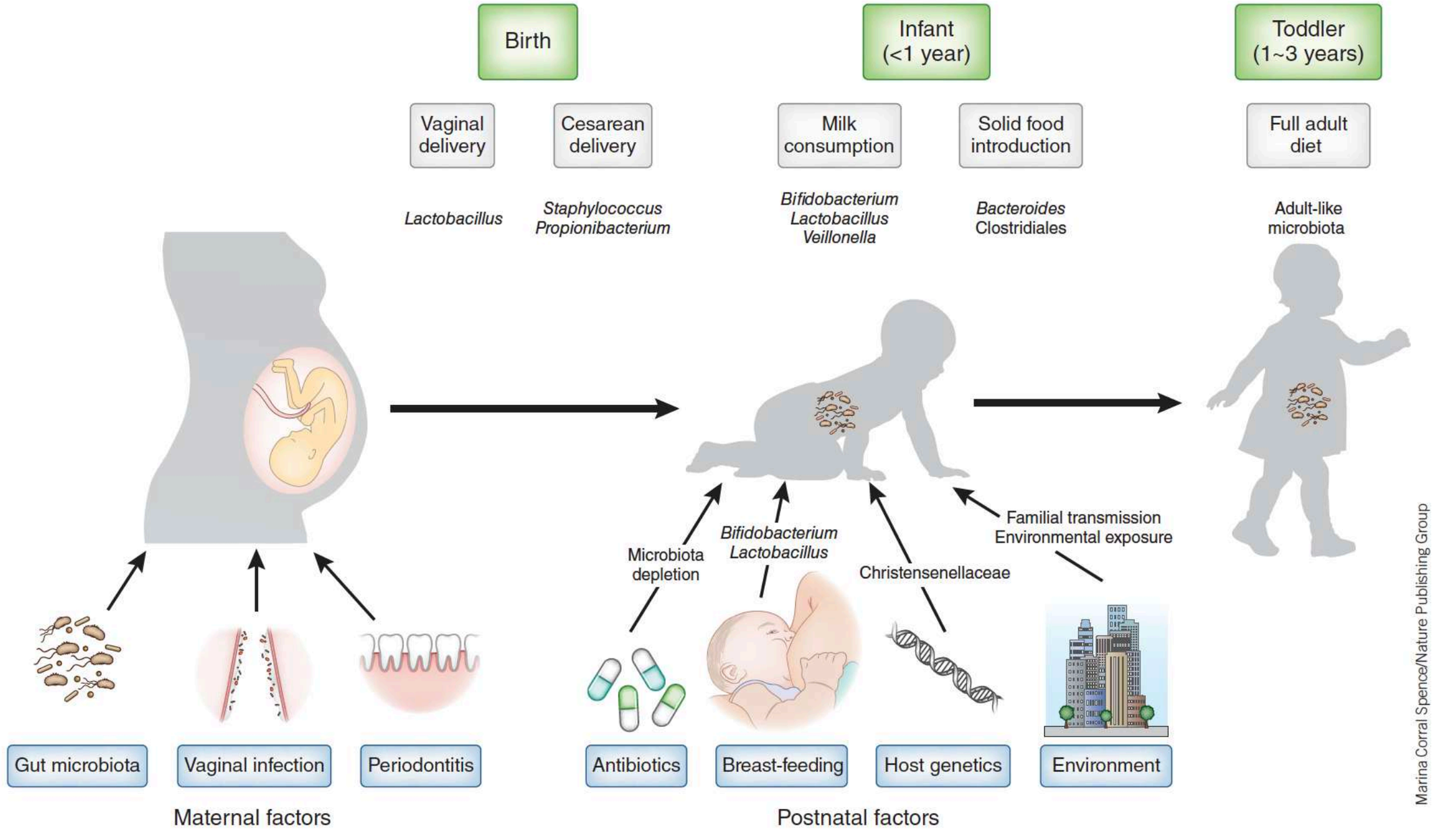
- Heritable species are partially responsible for the altered microbiome composition in obesity
- Obesity is associated with differential abundance of specific microbial species and metabolism
- *Christensenella minuta*, *Akkermansia muciniphila, Methanobrevibacter smithii* are under-represented in obesity
- *Blautia*, over-represented in obesity





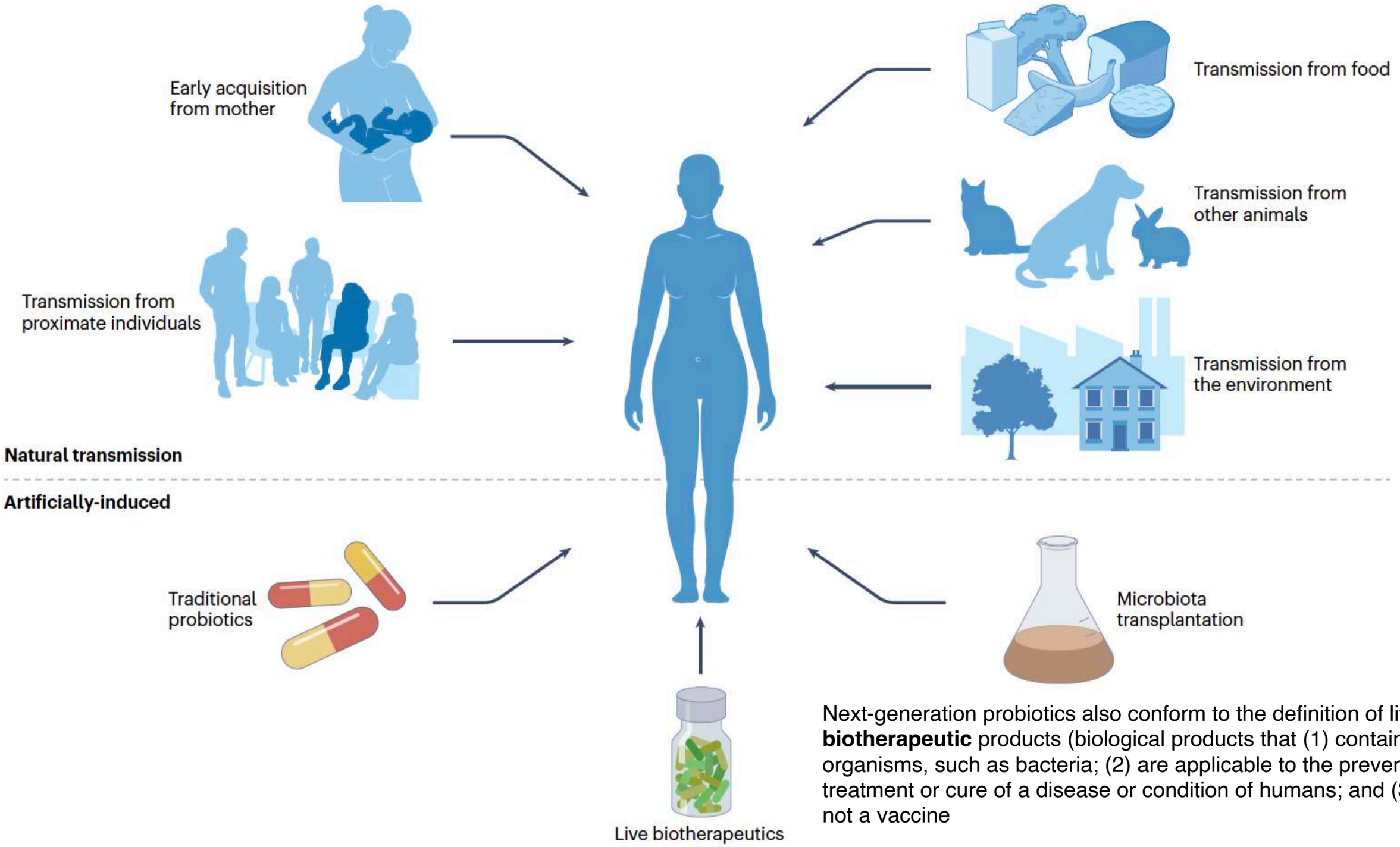
Madigan et al. 2018

Factors shaping the neonatal microbiome



Tamburini et al, 2016

Diverse natural sources of human microbiome strains



Next-generation probiotics also conform to the definition of live **biotherapeutic** products (biological products that (1) contain live organisms, such as bacteria; (2) are applicable to the prevention, treatment or cure of a disease or condition of humans; and (3) are not a vaccine

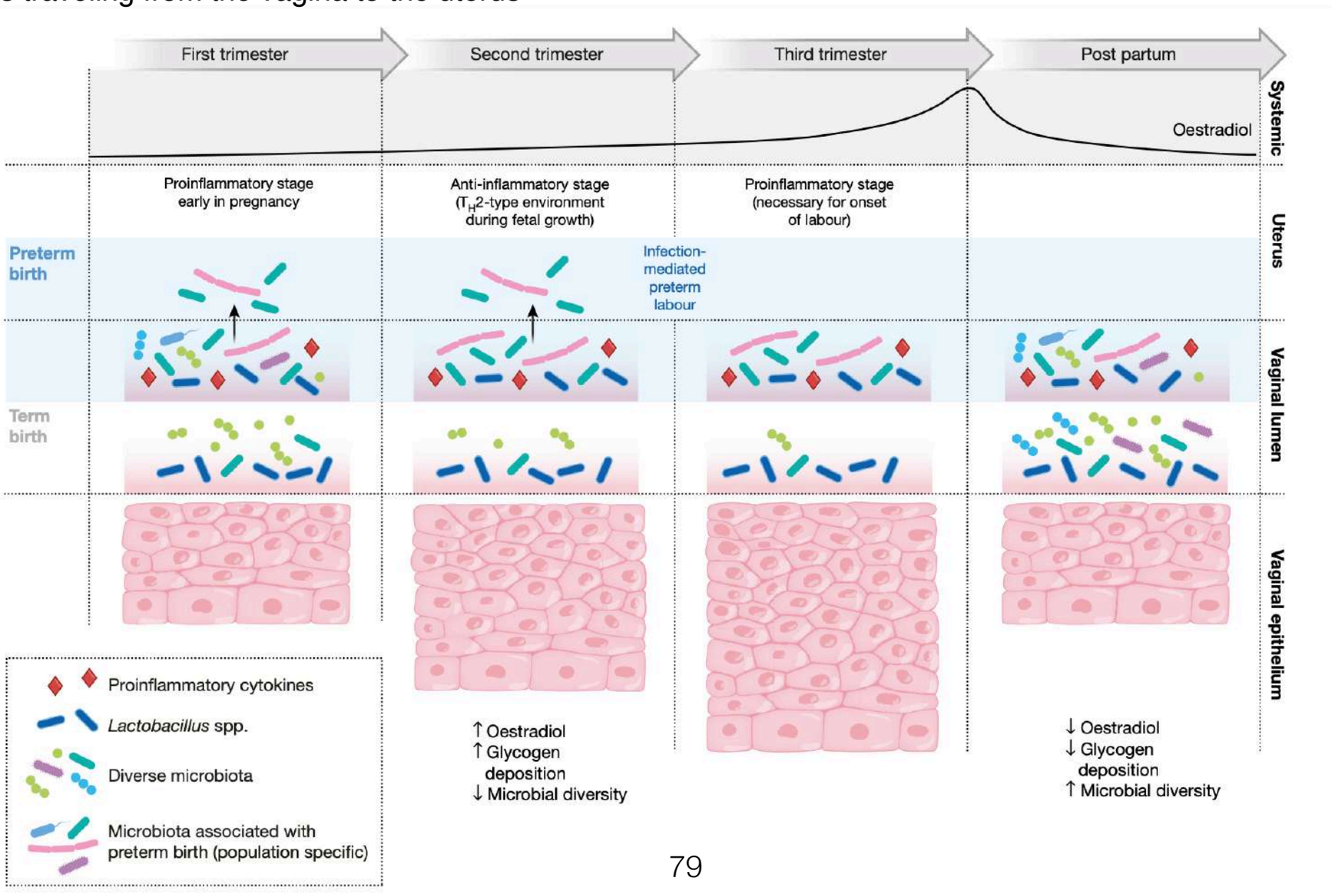
Heidrich et al., 2025

Vaginal microbiome in pregnancy and preterm birth

As pregnancy progresses, with predictable changes in systemic oestradiol levels, the uterine and vaginal environments undergo various changes

The uterus switches from an early pro-inflammatory condition to an anti- inflammatory condition in the second trimester, and then back to a pro- inflammatory condition before the onset of labour

Specific changes in the microbiome of the vaginal lumen can be associated with preterm birth, possibly through mechanisms involving microorganisms traveling from the vagina to the uterus



iHMP, 2019

Host colonization

Immune system 101

- Human babies are colonized during passage through the birth canal by environmental microorganisms (for example, from the **mother's vagina or skin**) and during **breast feeding** by microorganisms present in the milk

- Owing to the **highly oxidative environment** in the gastrointestinal tract of the newborn, primary colonizers are **facultative anaerobic bacteria** such as proteobacteria, which are thought to adjust the environmental conditions by decreasing the oxygen concentration to allow successive colonization by **anaerobic microorganisms** such as members of the genus *Bacteroides* and members of the phyla *Actinobacteria* and *Firmicutes*

- During the first year of life, the intestinal microbiota composition is simple and fluctuates widely between individuals and over time

- **Microbial signatures stabilize** and start to resemble the 'adult state' when the **infant reaches 1–2 years of age**

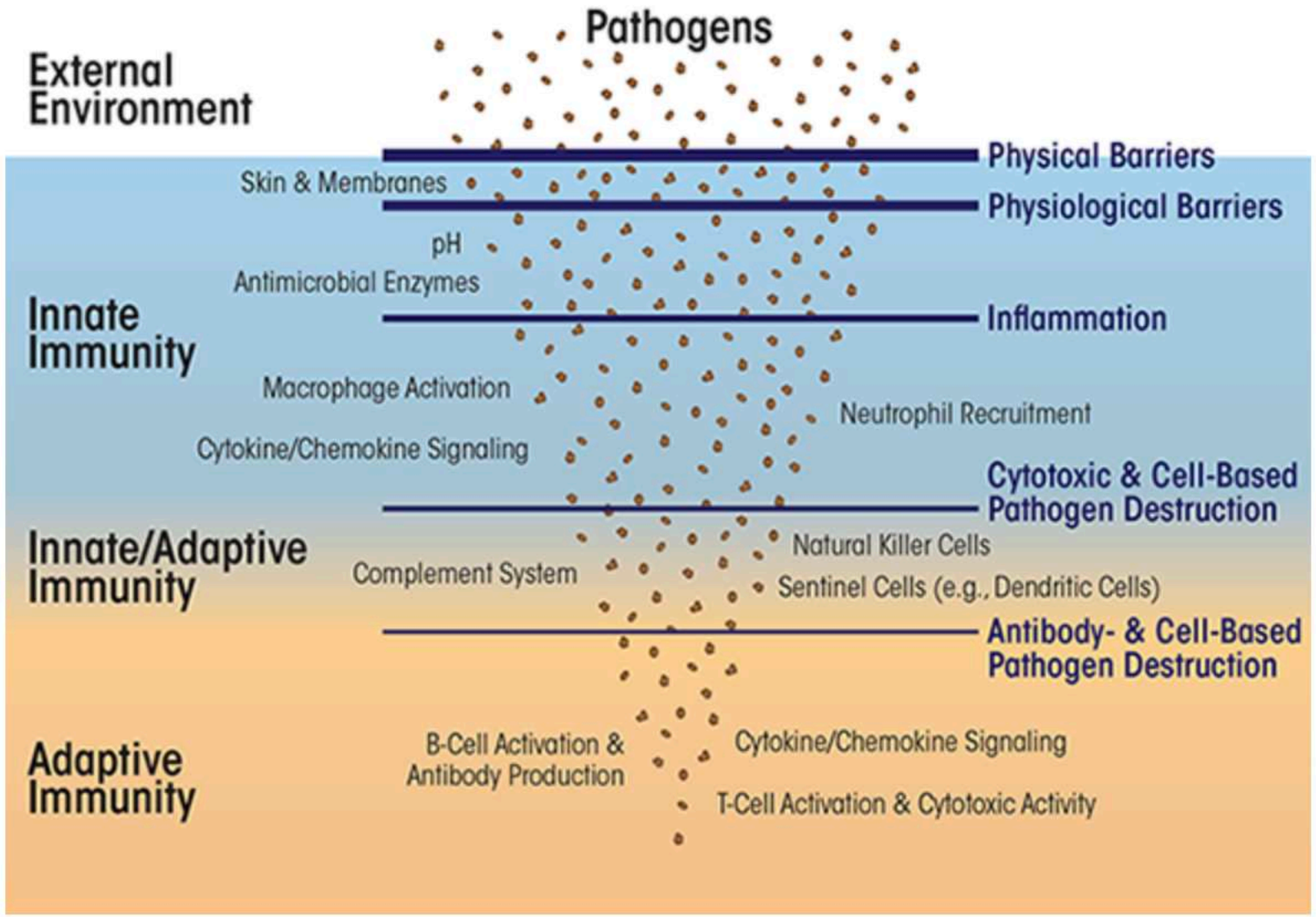
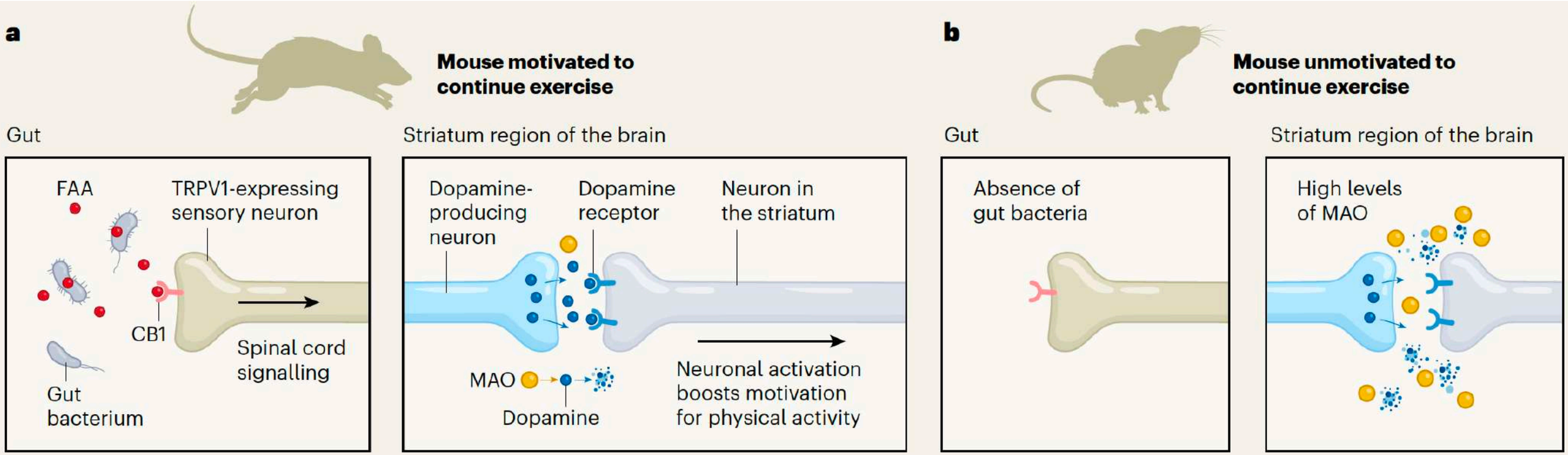


Figure Overview of the immune system. Innate immunity encompasses several non-specific protective mechanisms against infection, including physical and physiological barriers, cells (e.g., macrophages and neutrophils) that detect and attack other cells carrying pathogen-associated molecular patterns, and small proteins that signal pathogen invasion (i.e., cytokines and chemokines) or short peptides that directly attach to and restrict microbial pathogens. The adaptive immune system comprises specialized cells (e.g., B and T cells) and proteins (i.e., antibodies) that detect and eliminate specific pathogens and also uses cytokine/chemokine signaling to recruit additional immune cells. Several cells in adaptive immunity (i.e., memory B and T cells) can store immune memory of a pathogenic invasion. The complement system, along with natural killer cells and dendritic cells, straddles both innate and adaptive immunity.

Spiering, 2015

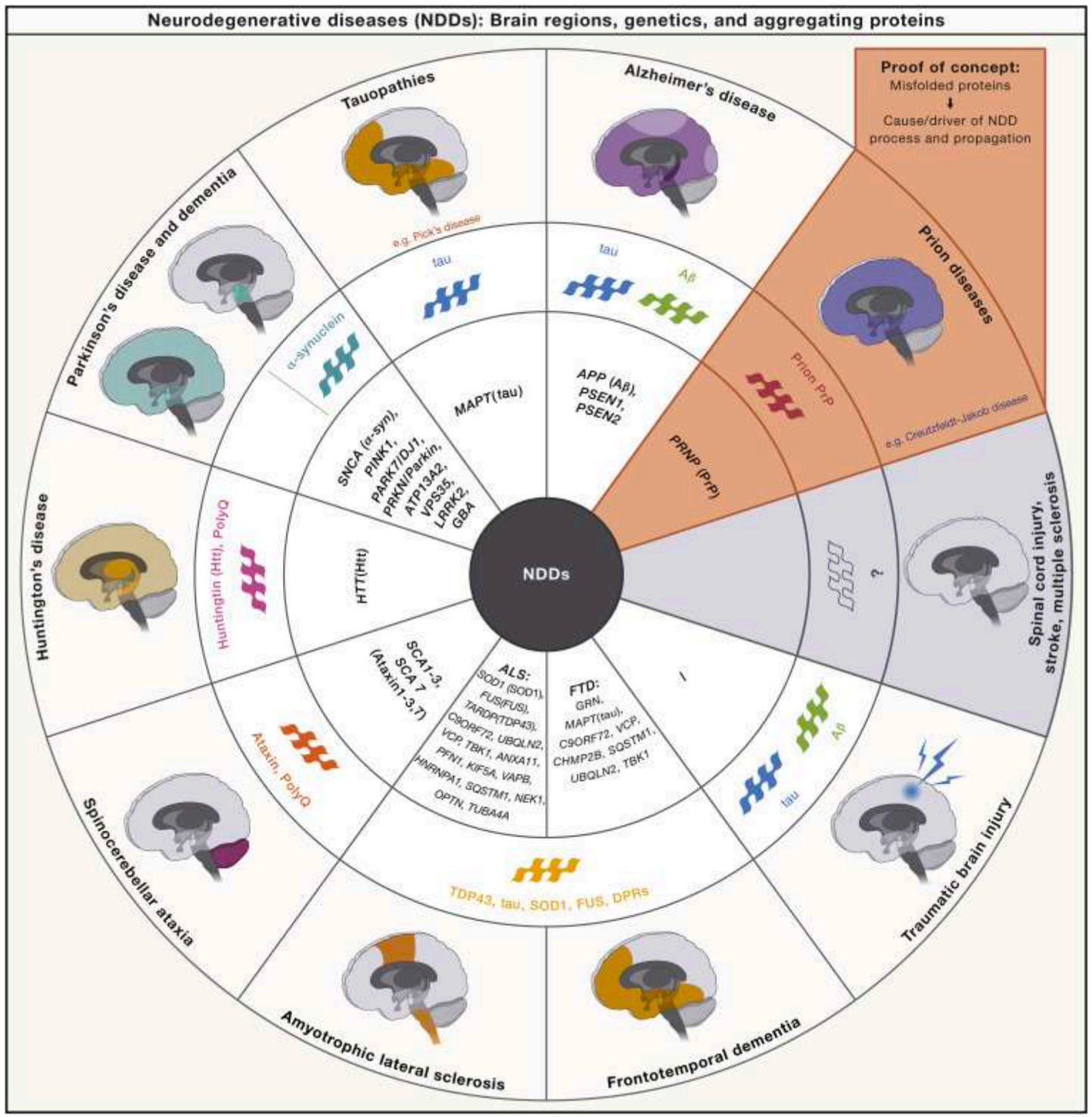
Gut microbes shape athletic motivation



Dohnalova' et al. 2023

- Certain **gut bacteria** in mice produce molecules called **fatty acid amides** (FAA), which bind to the cannabinoid 1 receptor (CB1) and thereby **activate sensory neurons** in the gut that express the protein TRPV1
- These neurons **connect to the brain through the spinal cord**. Activation of these neurons results in **decreased expression** of the enzyme monoamine oxidase (MAO) in the striatum region of the brain; this enzyme can **degrade dopamine** and other neurotransmitter molecules
- Dopamine-producing neurons induce an exercise-dependent surge of the molecule, which then activates neurons in the striatum that have dopamine receptors
- **This triggering of neuronal activity in the striatum aids the motivation for exercise**.
- In the absence of gut bacteria, the **sensory neurons in the gut are not excited**. The level of **MAO then remains high**, which **blunts dopamine** signalling in the striatum and results in a **premature termination of physical exercise**

Neurodegenertive disease and dysbiosis

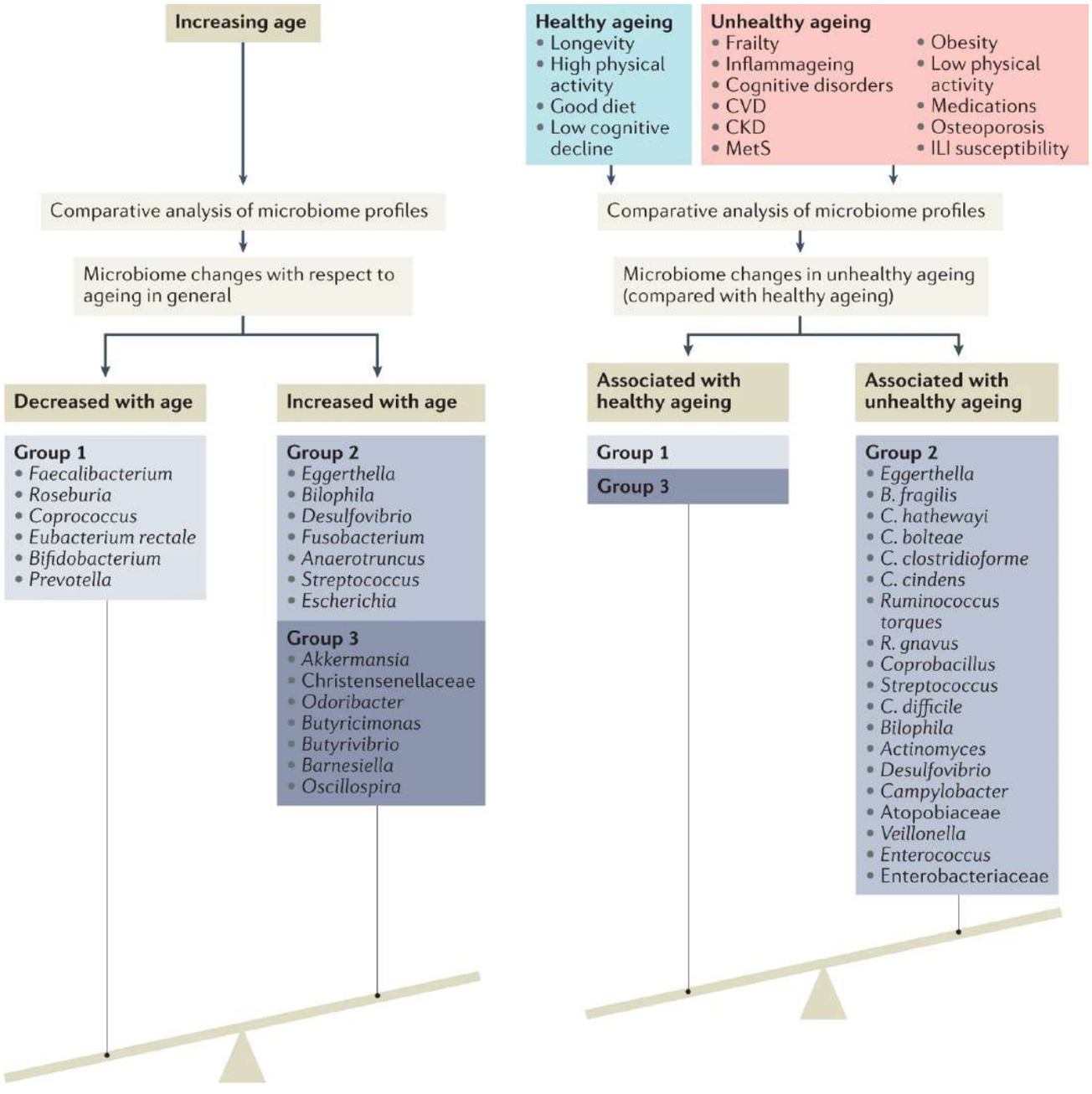


Willson III et al., 2023

Neurodegenerative diseases (NDDs) are a heterogeneous group of neurological disorders adversely affecting the lives of millions of people worldwide and entail the progressive loss of neurons in the central nervous system (CNS) or peripheral nervous system (PNS)

Characteristic aggregating proteins, genes linked to and affected brain regions in NDDs

The gut microbiome as a modulator of healthy aging

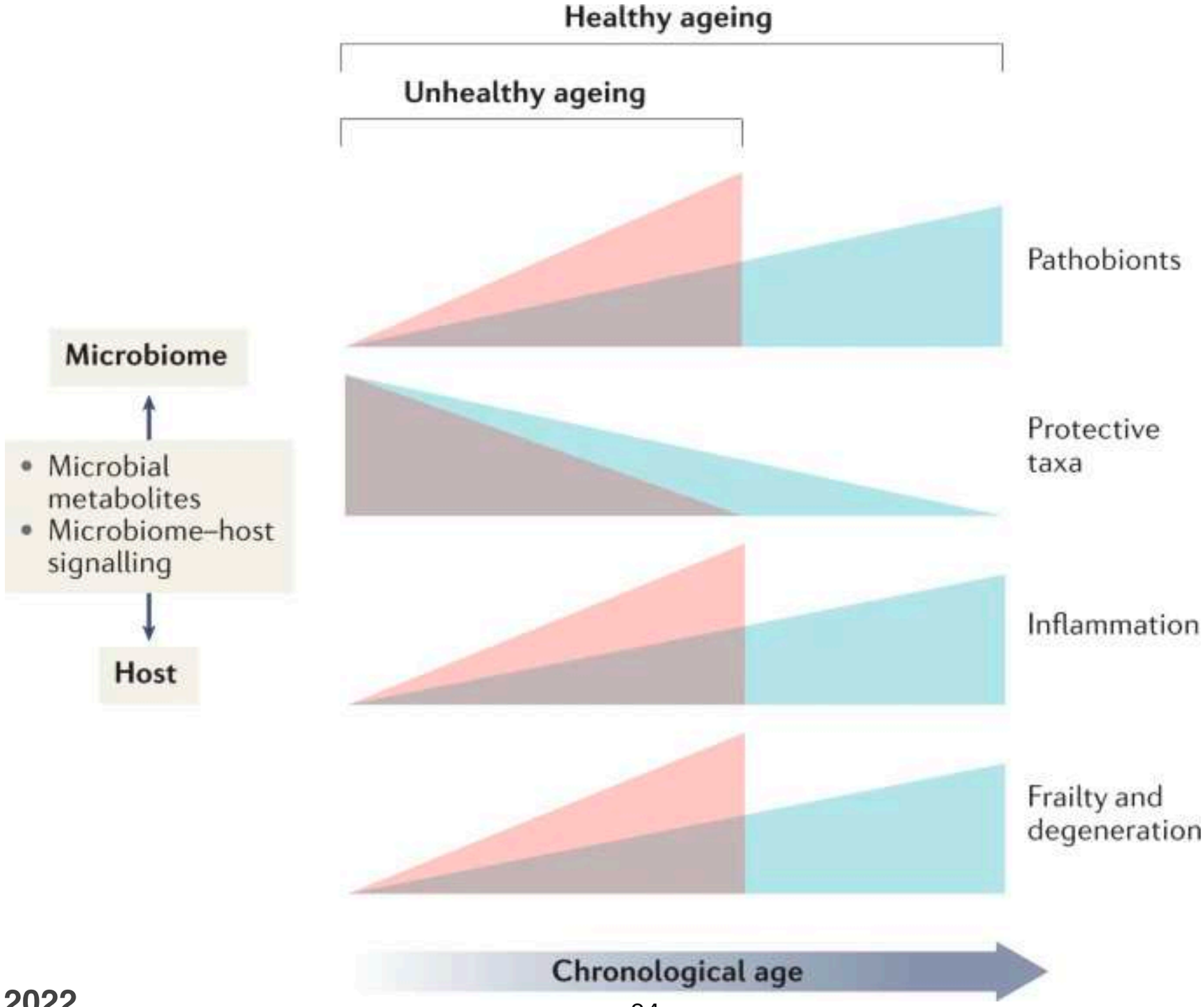


Gosh et al., 2022

- Group 1 taxa decreased with age and were associated with healthy aging
- Group 2 consisted of the pathobionts that increased with age and were associated with unhealthy aging
- Group 3 increased with age but were observed to be depleted in unhealthy aging

CKD, chronic kidney disease; CVD, cardiovascular disease; ILI, influenza-like illness; MetS, metabolic syndrome

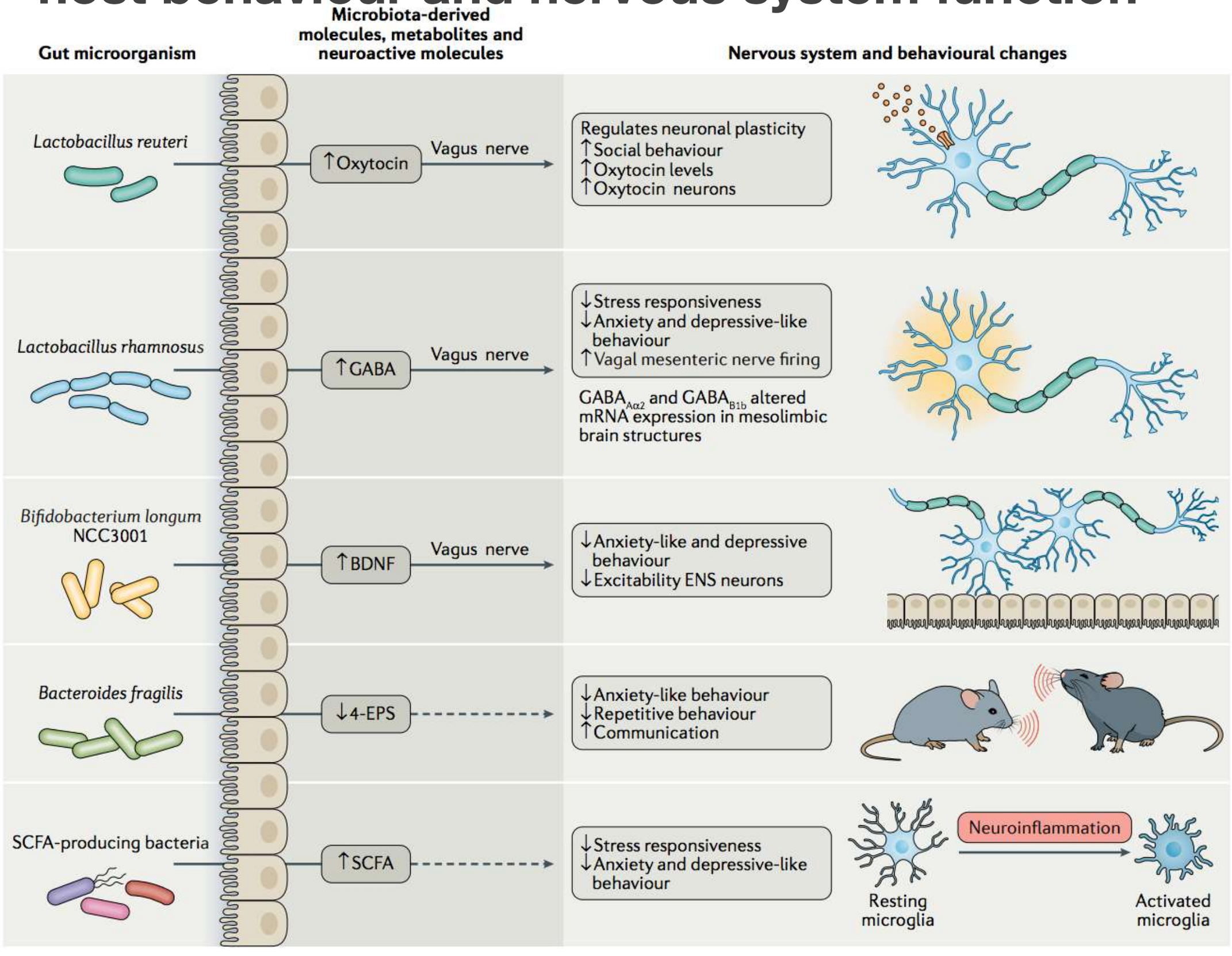
Microorganism–host signalling as a contributor to healthy or unhealthy ageing



Gosh et al., 2022

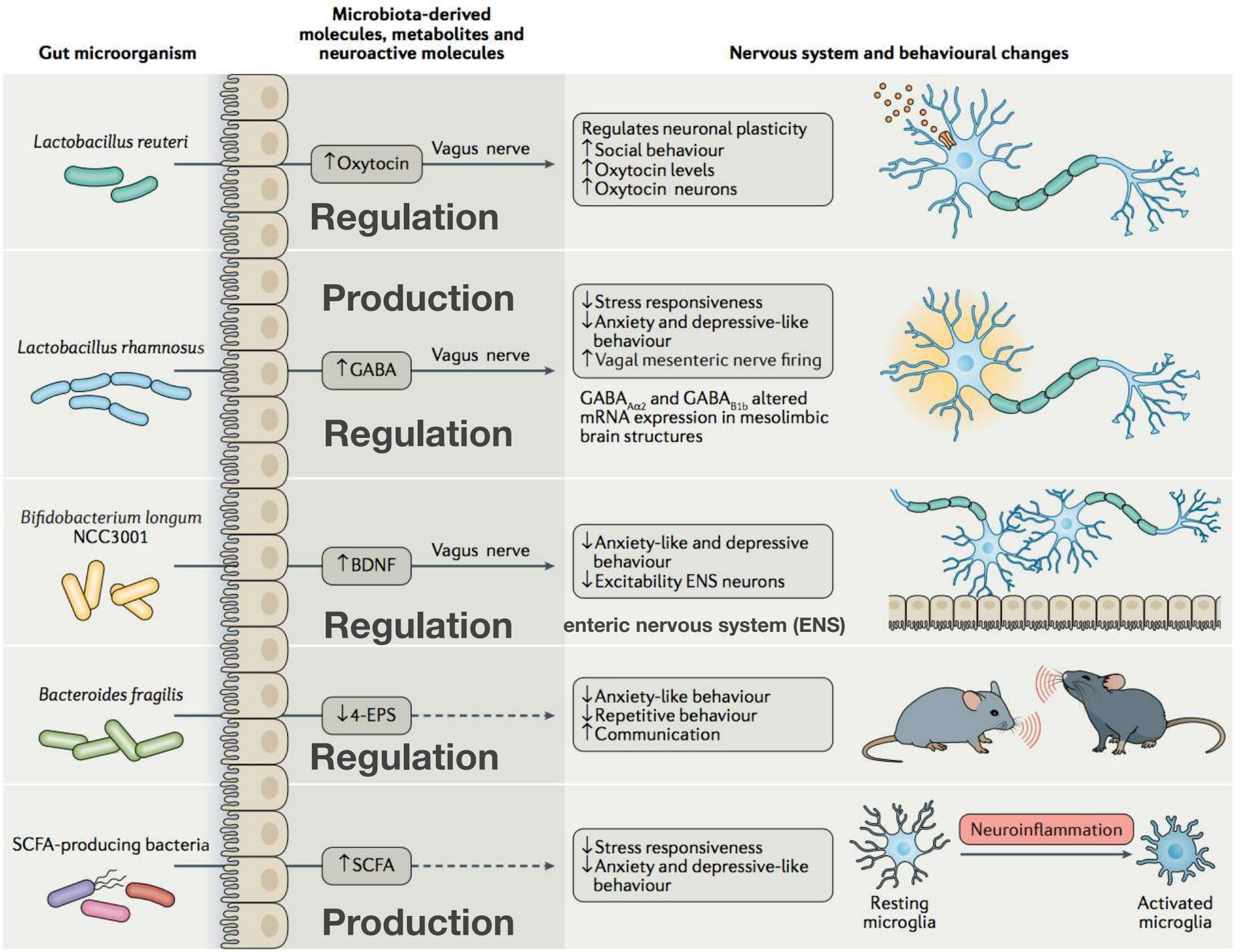
Comunications between microbes-human being

Microbiota and microbial-derived molecules modulate host behaviour and nervous system function



Morais et al., 2020

Microbiota and microbial-derived molecules modulate host behaviour and nervous system function



γ-aminobutyric acid (GABA)

Brain-derived neurotrophic factor (BDNF)

4-ethylphenylsulfate (4-EPS)

Short-chain fatty acids (SCFAs)

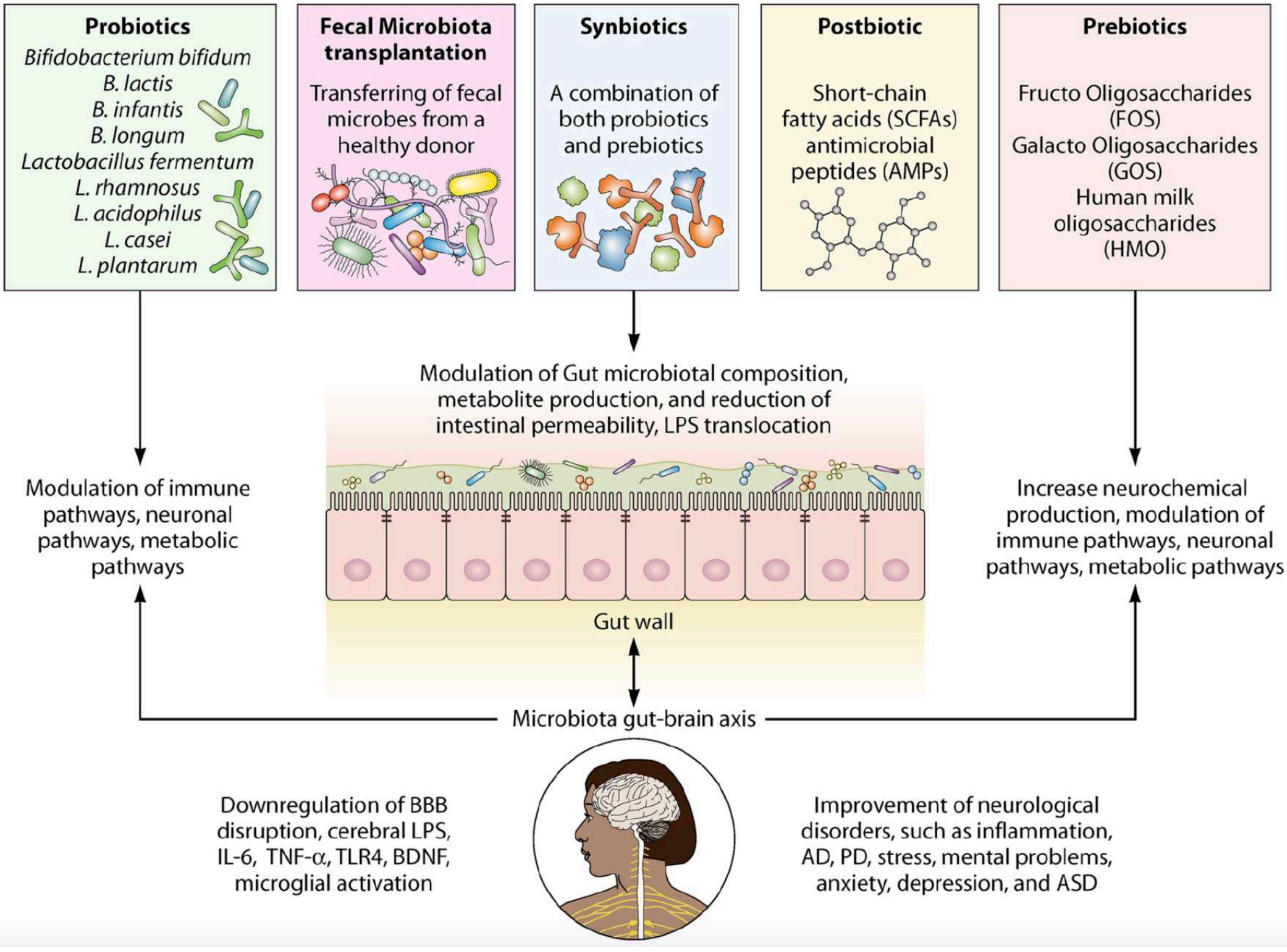
Morais et al., 2020

From Dysbiosis to Eubiosis

Fecal transplant

- The finding that thousands of bacterial species (as well as viruses and fungi) live in people, and are an integral part of human biology, has challenged medicine's view of microorganisms solely as agents of infectious disease
- The discovery that dietary fibre stimulates the particular groups of bacteria that produce key host-signalling molecules (such as short-chain fatty acids) is leading to the development of nutrition-based approaches to treating and restoring people's microbiomes —> **PROBIOTIC a "live microorganism which, when administered in adequate amounts, confer a health benefit on the host & PREBIOTIC APPROACH promotes the ingestion of certain plant compounds (e.g. carbohydrate for good fermenters in colon) as microbial growth stimulants with the idea that they will nurture healthy gut bacteria**
- The transplantation of gut microbiota from one person to another has been found to be more than 90% effective in the treatment of recurring *Clostridium difficile* infections (current care standard is repeated doses of antibiotics)
- Some cancer treatments activate the immune system —> new approach to these has emerged with the discovery that efficacy is related to specific members of the patient's gut microbiome

Modulation of gut microbiota by therapeutic microbial interventions



Sorboni et al., 2022

In sum the roles of the microbes in the human ecosystem are:

1. Digestion and Metabolism

- Helps break down complex carbohydrates, fiber, and proteins that the human body cannot digest alone
- Produces essential **short-chain fatty acids (SCFAs)** like butyrate, acetate, and propionate, which provide energy to gut cells and regulate metabolism
- Aids in the synthesis of **vitamins** (*e.g.*, B vitamins, vitamin K)

2. Immune System Regulation

- Plays a crucial role in **training and modulating the immune system**, helping to distinguish between harmful and harmless microbes
- Prevents infections by **competing with pathogens** for nutrients and space (colonization resistance)
- Produces **anti-inflammatory** and immune-modulating compounds

3. Protection Against Pathogens (Defense Mechanism)

- Maintains gut barrier integrity by strengthening **tight junctions** between intestinal cells
- Produces antimicrobial substances to prevent pathogen overgrowth

4. Influence on Brain and Behavior (Gut-Brain Axis)

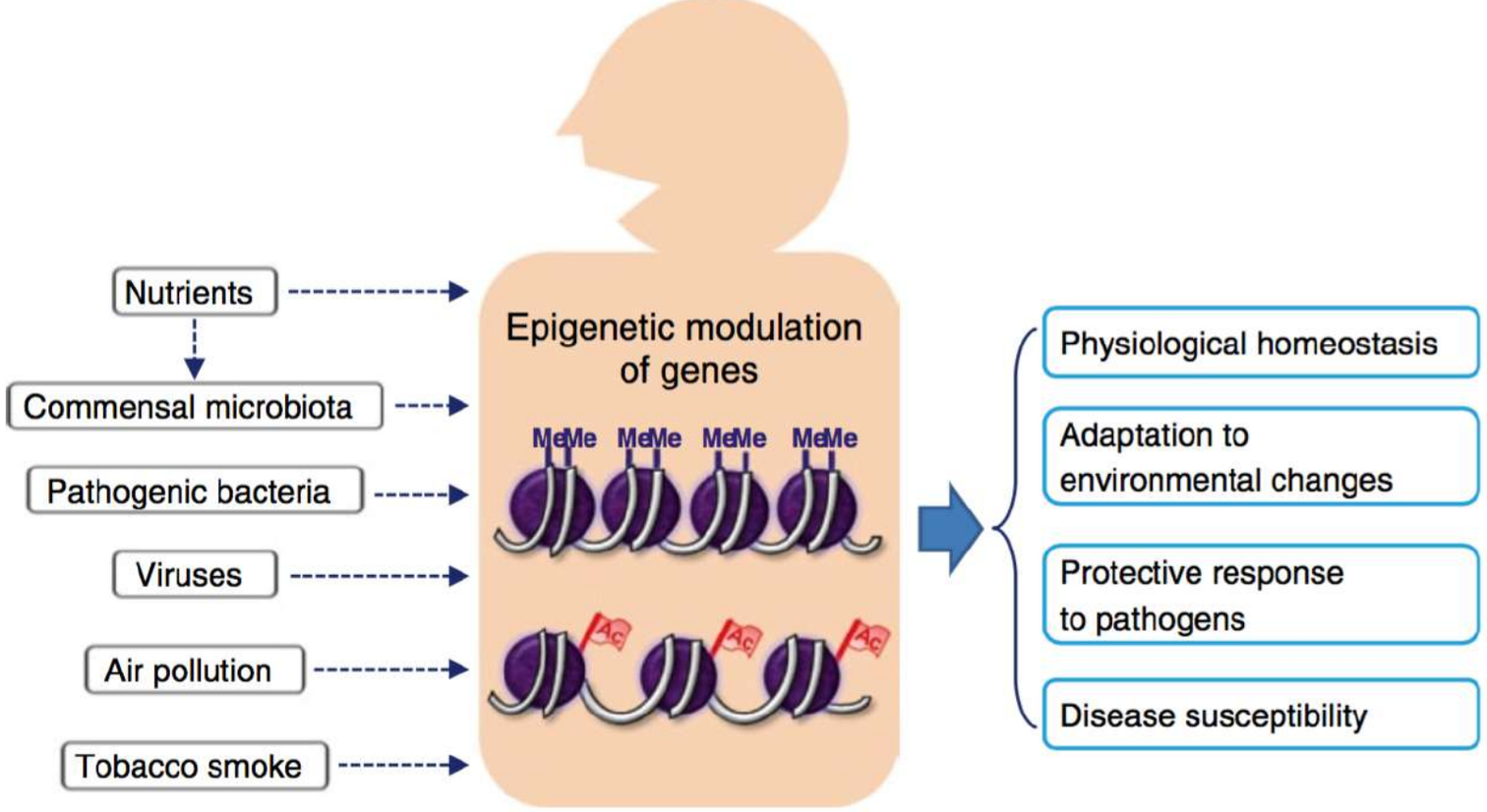
- Produces **neurotransmitters** like serotonin, dopamine, and GABA, which influence mood and cognitive functions
- Regulates the **gut-brain axis**, impacting mental health conditions like anxiety and depression

5. Role in Disease Prevention and Development

- **Dysbiosis (microbial imbalance)** is linked to diseases like **inflammatory bowel disease (IBD), obesity, type 2 diabetes, allergies, and even neurodegenerative disorders**
- **A healthy gut microbiome reduces inflammation and supports overall metabolic and immune balance**

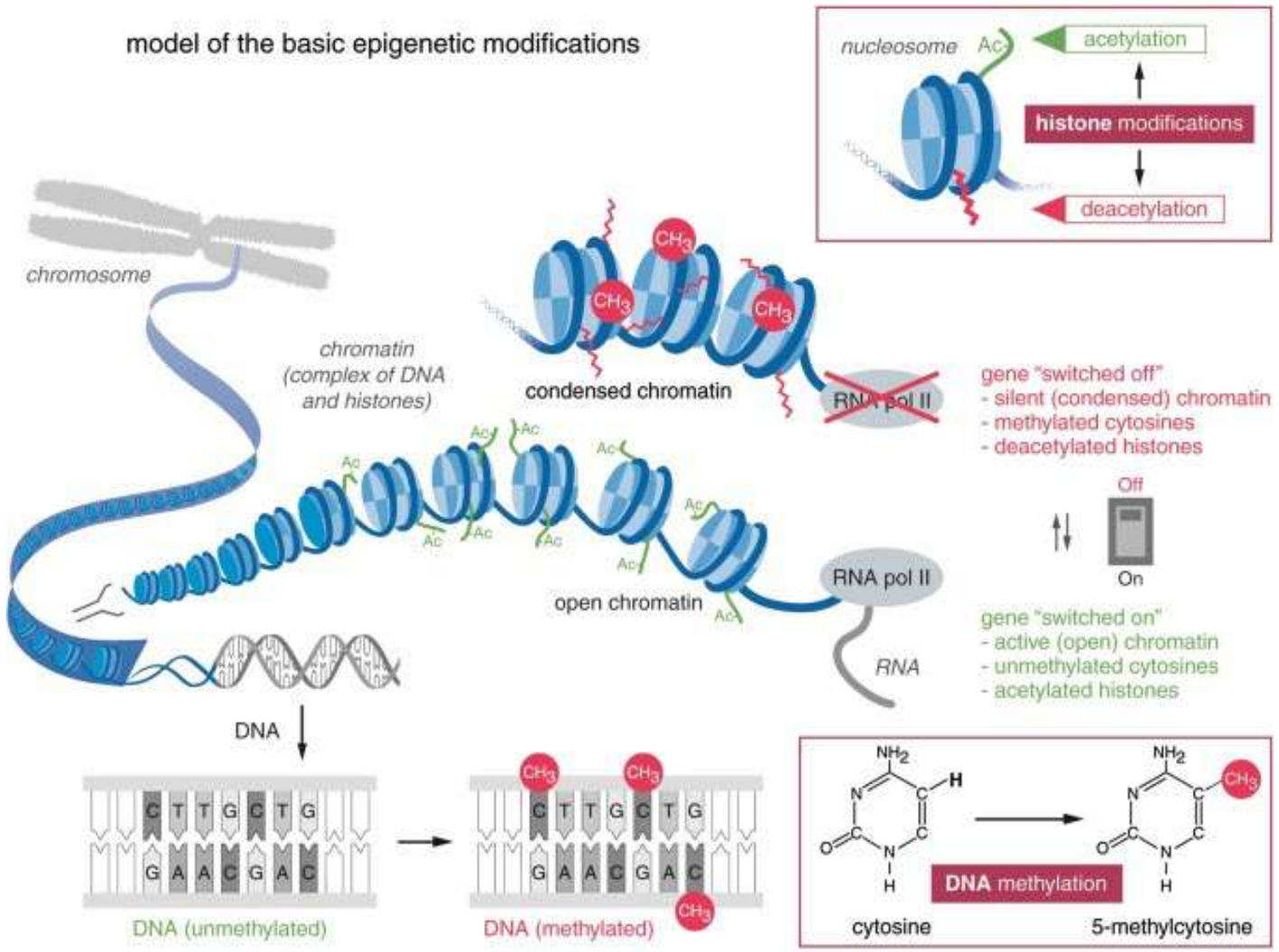
Epigenetics 101

- The word “epigenetics” was originally coined by Conrad Waddington in 1942, referring to how genotypes give rise to phenotypes during development
- Now we refer as the study of **phenomena and mechanisms that cause chromosome-bound, heritable changes to gene expression** that are **not dependent on changes to DNA sequence** (Deans and Maggert 2015)
- In Humans, gene expression is regulated prior to transcriptional initiation by the **chemical modification of DNA or the histone proteins** that together form chromatin



Takahashi, 2014

Epigenetic modifications of chromatin by DNA methylation and histone acetylation



Vilcinskas, 2015

- Methyl group transfer to cytosine —> 5-methylcytosine (m5C) pairs with guanosine
- m5C has different interactions with regulatory proteins
- **Chromatin structure** depends on net **charge** of core **histones**
- **Acetyl groups** promoting formation of *open* and **accessible** euchromatin vs **deacetylation** promoting the formation of *compact* and **inaccessible** heterochromatin

aDNA: Reconstructing ancient genomes and epigenomes

Orlando et al., 2015

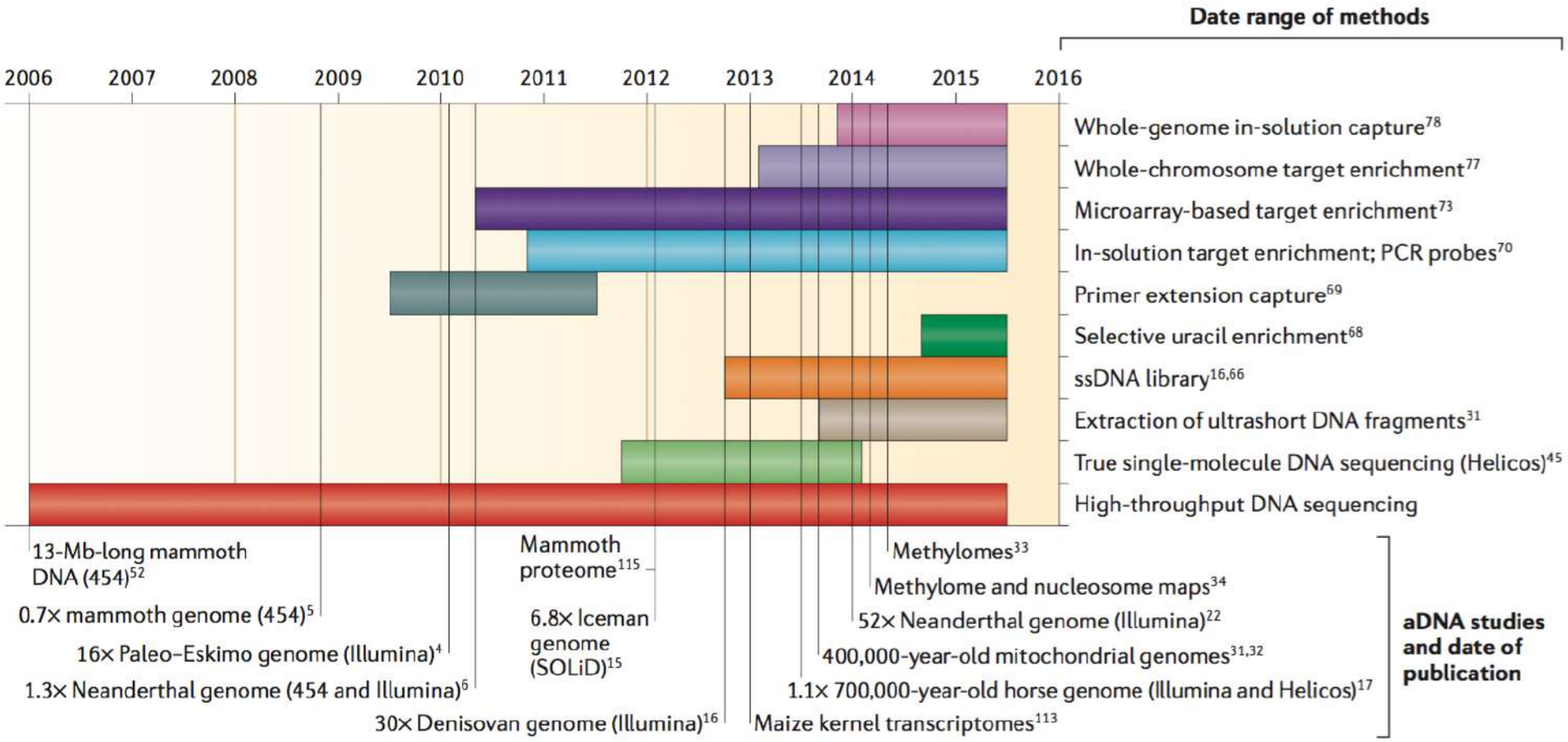
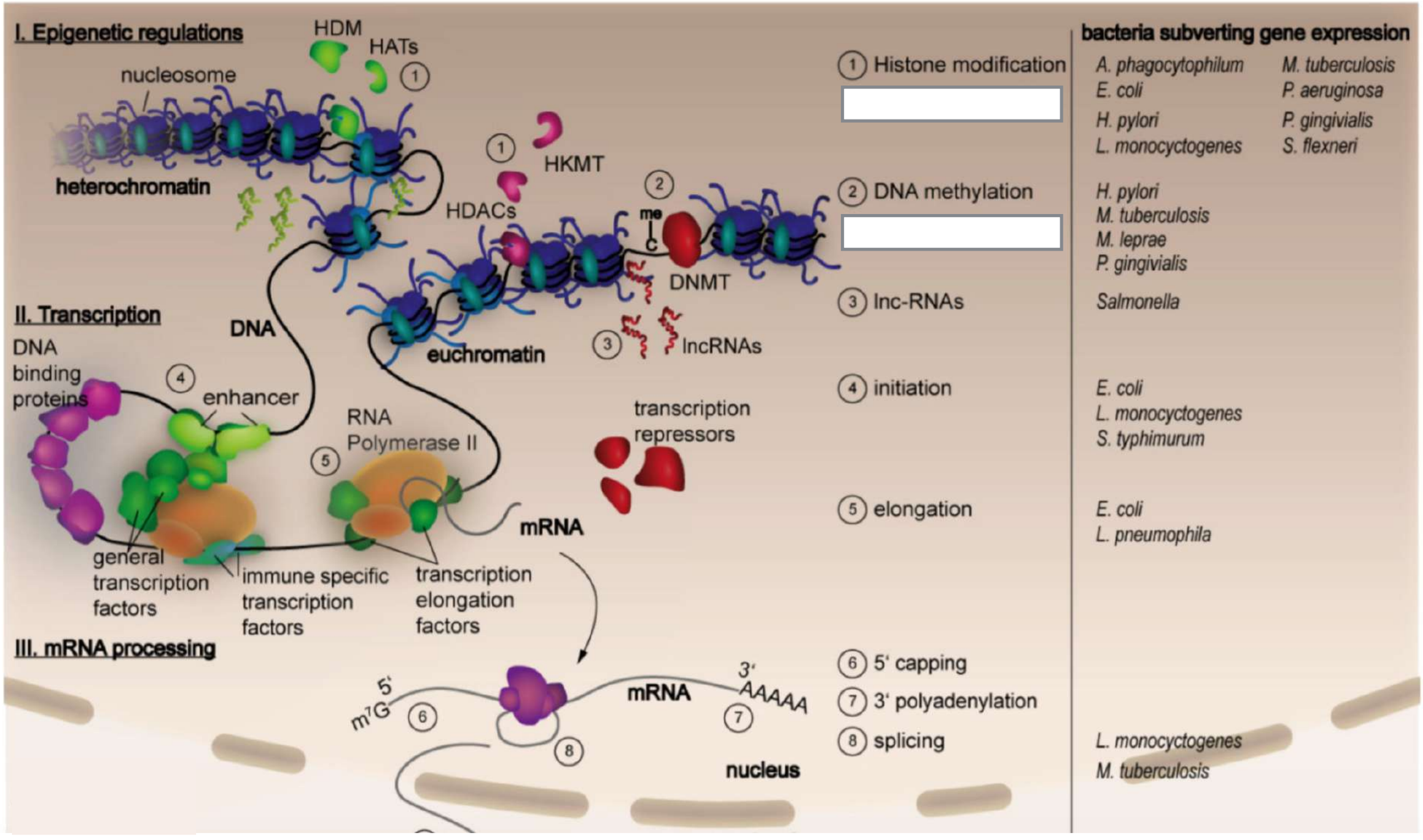


Figure 1 | **Major advances in ancient genomics.** The major methodological advances described in this Review are presented with respect to milestones in paleogenomics, including whole-genome sequencing and the characterization of transcriptomes, epigenomes and proteomes. Average genome fold-coverage (×) and sequencing platforms are indicated where applicable. aDNA, ancient DNA; ssDNA, single-stranded DNA.

- **Typical ancient DNA molecules:** diverse range of degradation reactions affect DNA post-mortem and result in extensive fragmentation (preferentially at purine nucleotides) and base modifications
- Most common base modification identified in high-throughput sequencing data sets is deamination of cytosines into uracils (red), or thymines (blue) when cytosines were methylated (^{m}C) —>deaminations occur much faster at overhanging ends
- Other modification: abasic sites and single-strand breaks

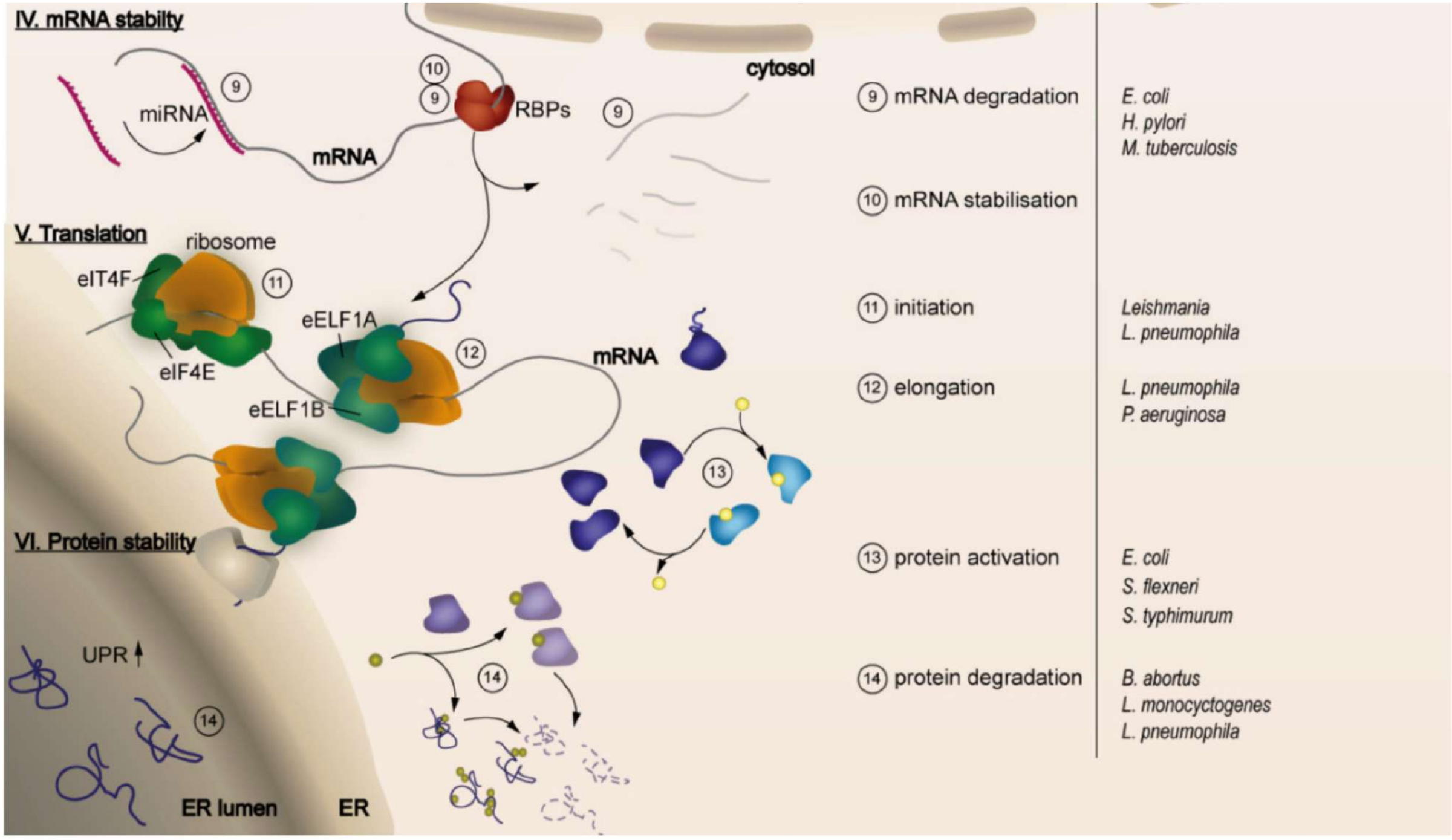
Bacteria manipulate host gene expression during infection, I



Denzner et al., 2020

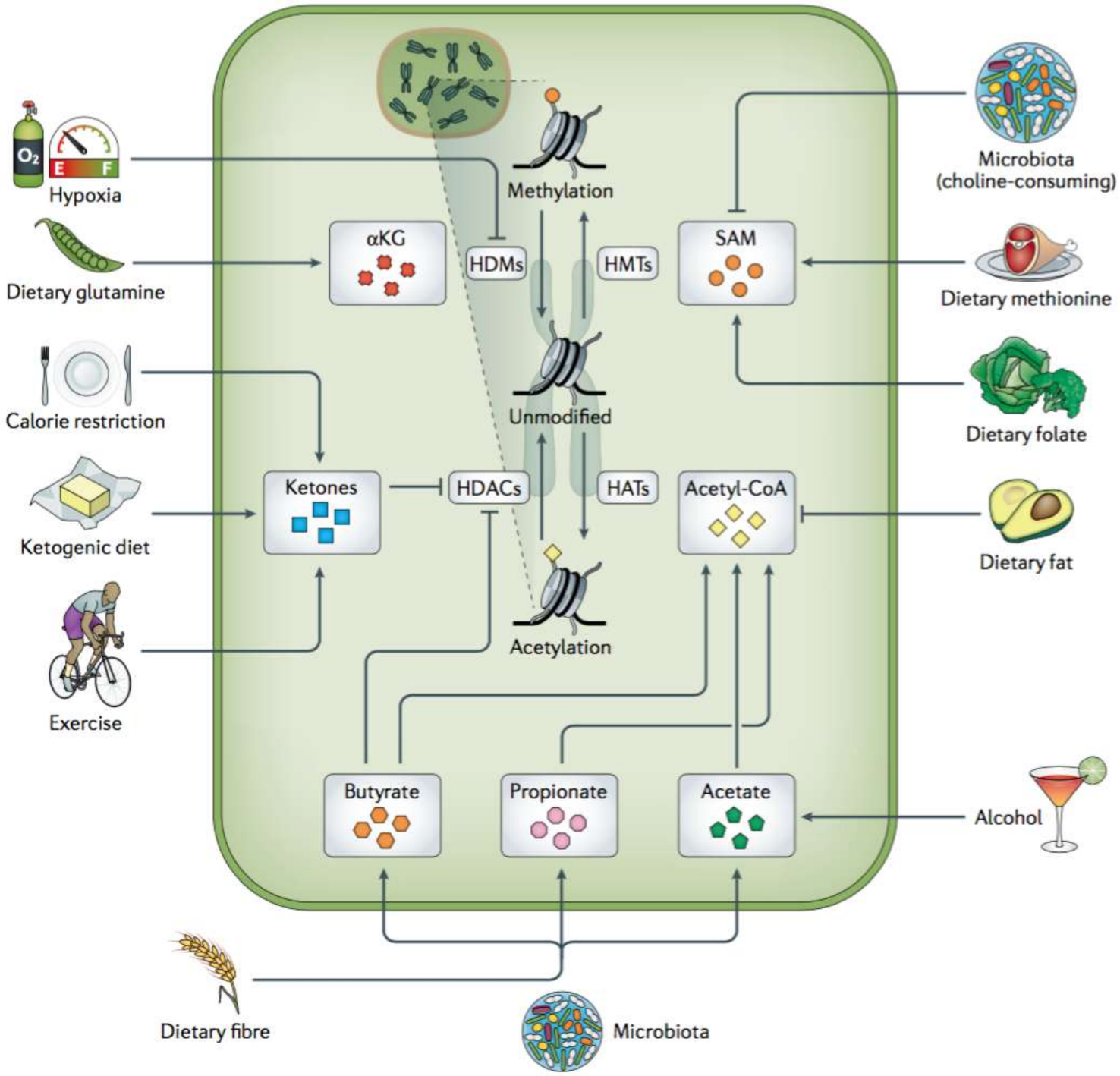
Bacteria evolved many strategies to survive and persist within host cells

Bacteria manipulate host gene expression during infection, II



Denzner et al., 2020

Influences of environmental factors on histone acetylation and methylation via microbiome



S-adenosylmethionine (**SAM**) and acetyl-CoA, that are used by histone methyltransferases (HMTs) and histone acetyltransferases (HATs)

The activity of histone demethylases (**HDMs**) is supported by α-ketoglutarate (αKG), which can be derived from dietary glutamine, and is inhibited by the limited oxygen availability during hypoxia

Ketone bodies and short-chain fatty acids (SCFAs) such as acetate, propionate and **butyrate** can provide **acyl-CoA** precursors for histone acylation, while also directly inhibiting the activity of histone deacetylases (HDACs)

Dai et al., 2020

Box 1 | **The epigenomes of eukaryotes and bacteria**

- In eukaryotes, epigenetic modification of the genome involves DNA methylation[3] and histone modification[4]. Bacteria lack histones, and epigenetic control relies on DNA methylation only[6].
- In eukaryotes, de novo and maintenance forms of DNA methylation are performed by separate enzymes[2]. Bacterial DNA methyltransferases have both de novo and maintenance activities[37].
- In eukaryotes, two main mechanisms exist to erase DNA methylation marks: active demethylation by dedicated proteins (Tet enzymes), and passive demethylation by the hindrance of DNA methylase activity upon DNA replication[35]. In bacteria, DNA demethylation is usually passive[66], and the relevance of active demethylation by DNA repair remains to be evaluated[82].
- In both bacteria and eukaryotes, transcriptional repression by DNA methylation is common[3,6]. Transcriptional activation of bacterial genes under DNA methylation control often involves demethylation (partial or complete, single- or double-stranded) of promoters or regulatory regions[57,72,89,90,94,158].
- The methylated base typically involved in the control of eukaryotic transcription is C^5-methyl-cytosine[3], whereas in bacteria it is often N^6-methyl-adenine[7,14]. However, direct control of bacterial transcription by C^5-methyl-cytosine has been demonstrated recently[126]. Transcriptional control by N^4-methyl-cytosine may also exist[130].
- In multicellular eukaryotes, the DNA methylation pattern of the genome is reprogrammed during gametogenesis and during early embryonic development[2]. In bacteria, reprogramming does not occur, and the DNA methylation pattern can be transmitted unaltered across generations. However, the acquisition and loss of DNA methyltransferase genes[41] and recombinational shuffling of DNA methyltransferase domains[27,33,143] can produce novel methylation patterns in bacterial genomes.
- In both bacteria and eukaryotes, DNA methylation controls the formation of phenotypic variants of genetically identical cells. However, DNA methylation-dependent formation of bacterial cell lineages can show programmed reversion (phase variation)[15,27,93,111].

Integrative approach for human health

Genome

Epigenome <-> Microbes

Life style <-> Microbes

Hygiene <-> Microbes <-> Disease

Diet <-> Microbes

Drugs <-> Microbes <-> Health

Age <-> Microbes

Health <->Microbes <-> Disease