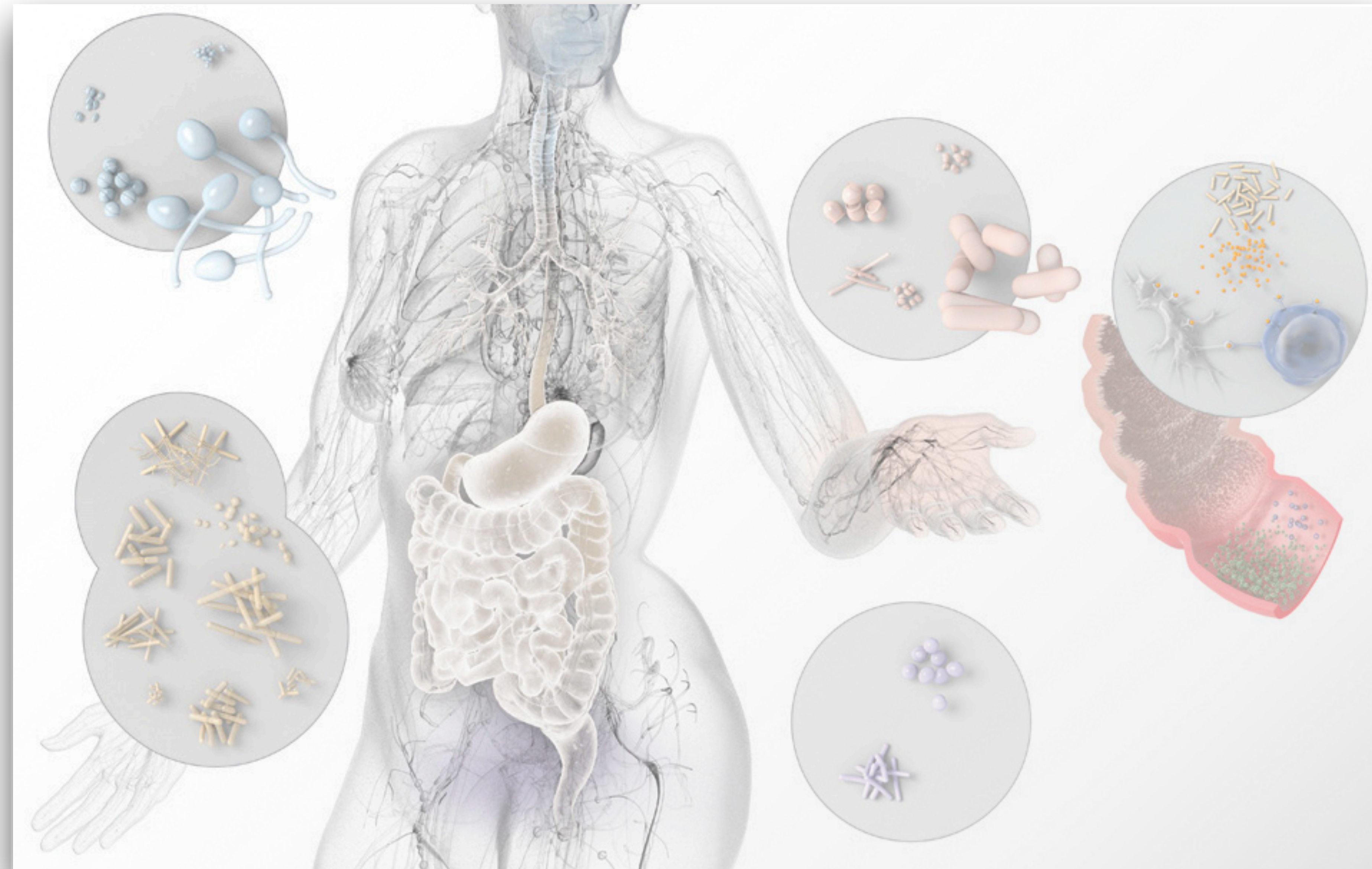
Gut Microbiota: Our Inner Ecosystem



Roderick (Chenmengxiao) Pan

MacMillan Group

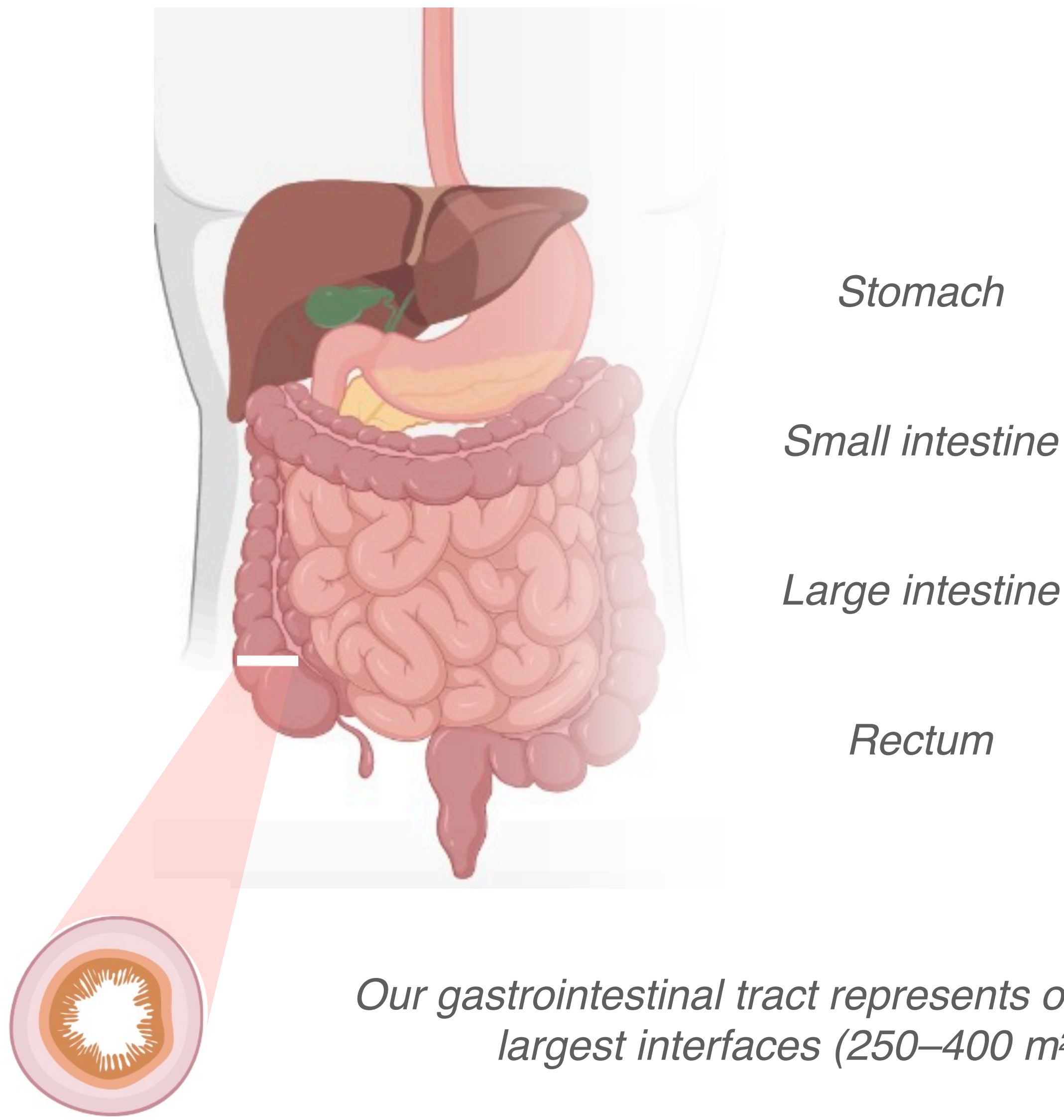
Princeton University

Sept. 27th, 2022


Who are they?

Gut microbiota, a crowded kingdom

Population, diversity, and distribution



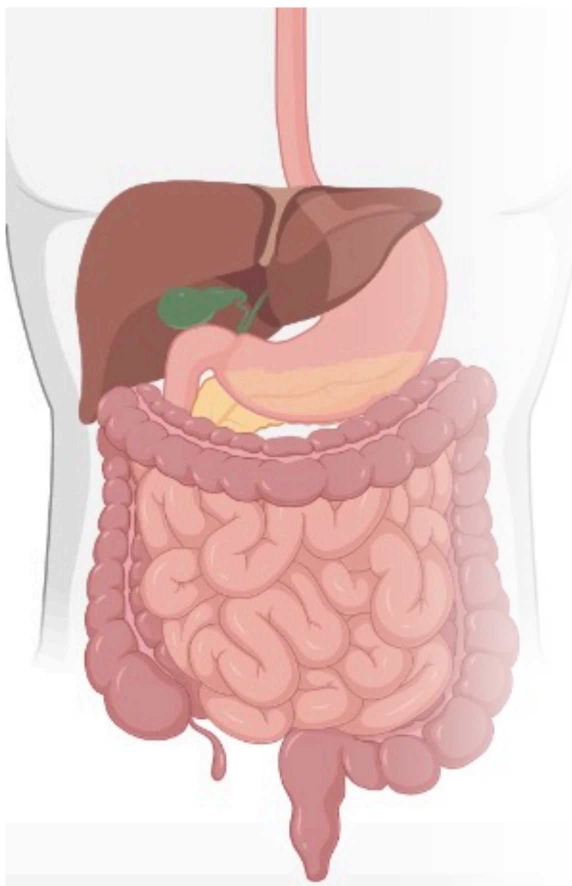
Our gastrointestinal tract represents one of the largest interfaces (250–400 m^2)

A total number of 30-400 trillion microbe species resides in our GI tract
More abundant than our cells of the body!

Adak A, Khan MR. *Cell Mol Life Sci*. 2019;76(3):473-493.

Gut microbiota, a crowded kingdom

Population, diversity, and distribution



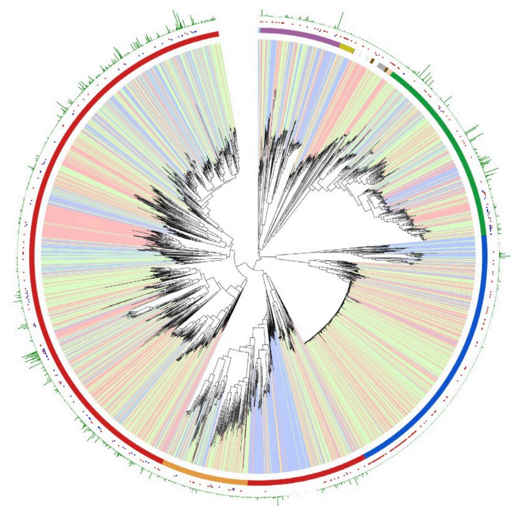
GI tract

Stomach

Small intestine

Large intestine

Rectum



Bacteria

Archaea

Eukarya, mostly yeast

Viruses, mostly phage

Parasites

...

Adak A, Khan MR. *Cell Mol Life Sci*. 2019;76(3):473-493.

Gut microbiota, a crowded kingdom

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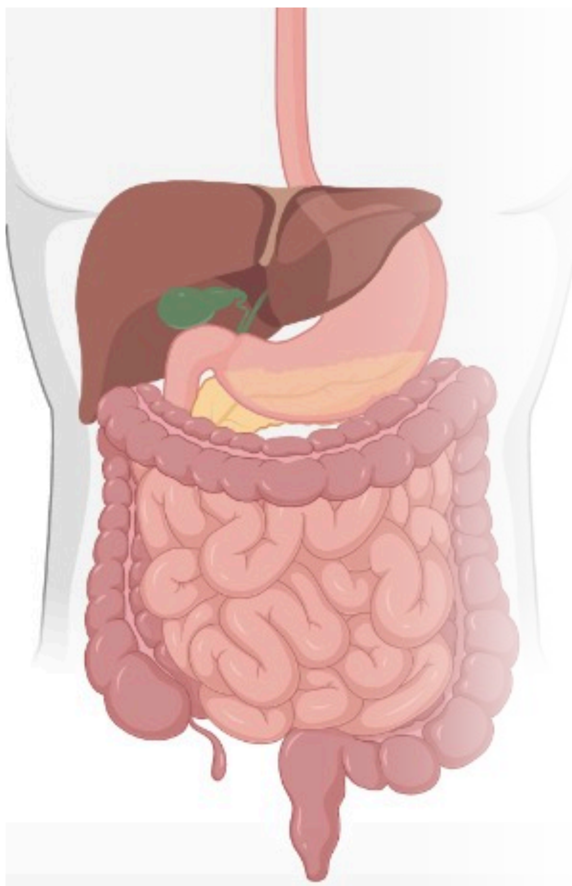

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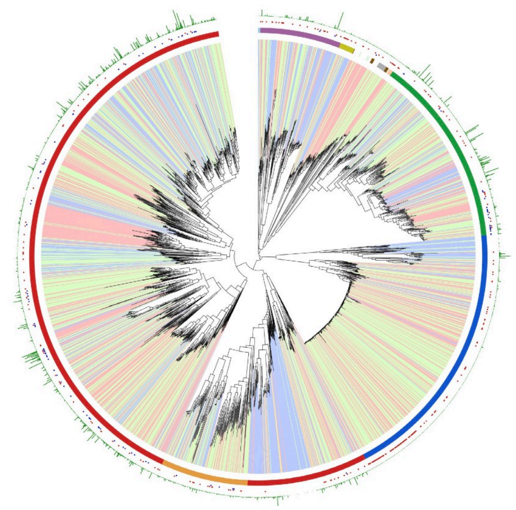


Bacteria

Archaea

Eukarya, mostly yeast

Viruses, mostly phage

Parasites

...

Firmicutes

Bacteroidetes

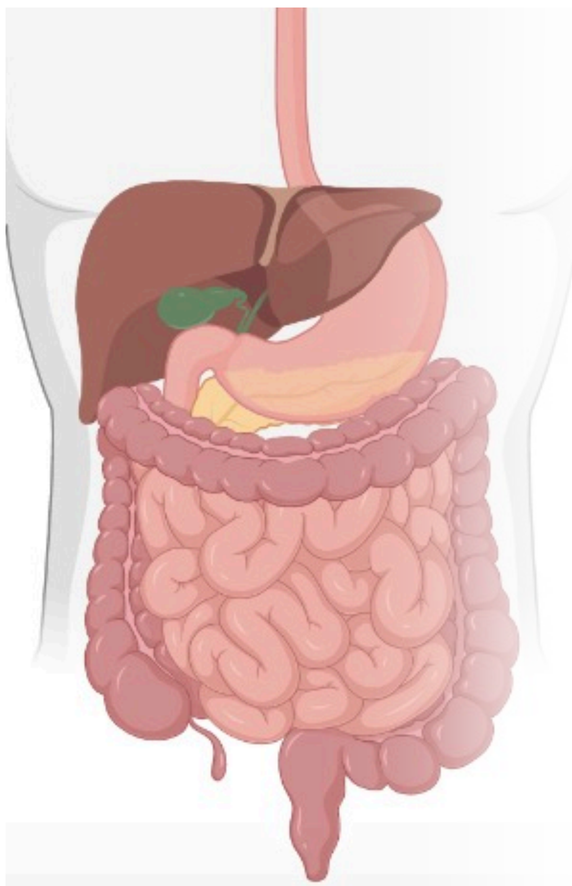
taking up to 90% of bacteria species

Over 1000 microbe species resides in our GI tract
Great genomic and functional diversity

Adak A, Khan MR. *Cell Mol Life Sci*. 2019;76(3):473-493.

Gut microbiota, a crowded kingdom

Population, diversity, and distribution



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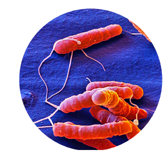
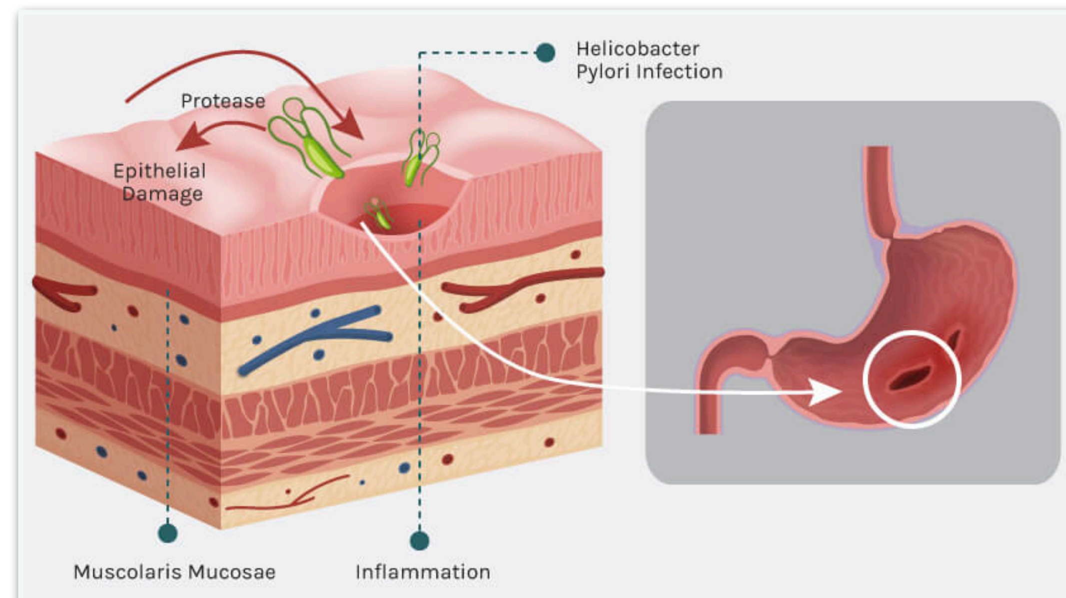


Heliobacter pylori

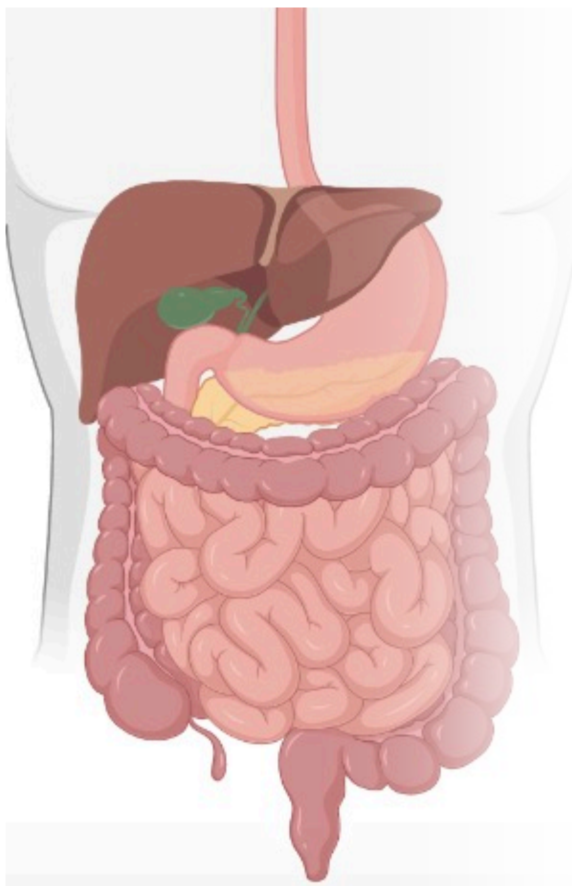
gastric acid resistant, damage mucus, cause severe ulcer



Diverse environment within our GI tracts leads to distribution of microbiota

Gut microbiota, a crowded kingdom

Population, diversity, and distribution



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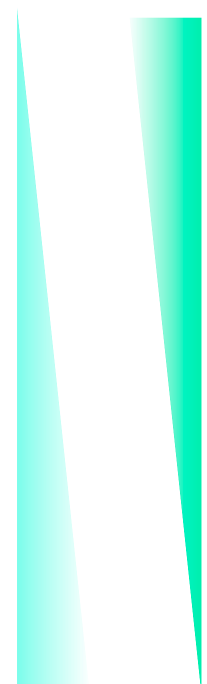


O^2 level

Aerobic species

digestion and absorption of foods and nutrients

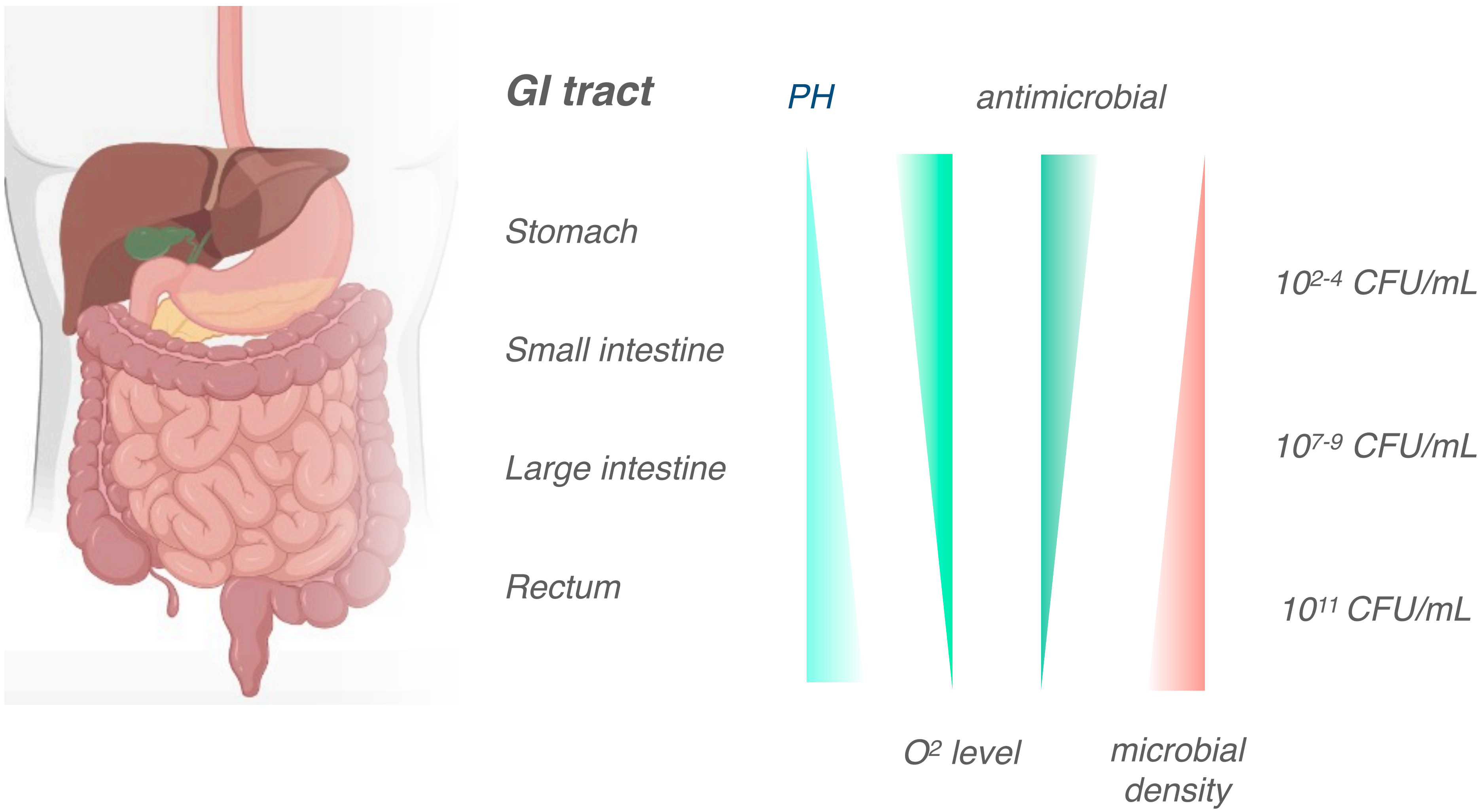
Anaerobic species

water absorption and fermentation

Diverse environment within our GI tracts leads to distribution of microbiota

Gut microbiota, a crowded kingdom

Population, diversity, and distribution



Diverse environment within our GI tracts leads to distribution of microbiota

Tools to interrogate gut microbiota

Culture-dependent

Robert Koch
1843-1910

pre-incubation
blood culture
rumen fluid

Culture
Different types of
agar media

builds up fundamental knowledge of gut microbiota

Uncultivable

Enhancing bias

1890s

Adak A, Khan MR. *Cell Mol Life Sci*. 2019;76(3):473-493.

Tools to interrogate gut microbiota

Culture-independent

Robert Koch
1843-1910

pre-incubation
blood culture
rumen fluid

Culture
Different types of
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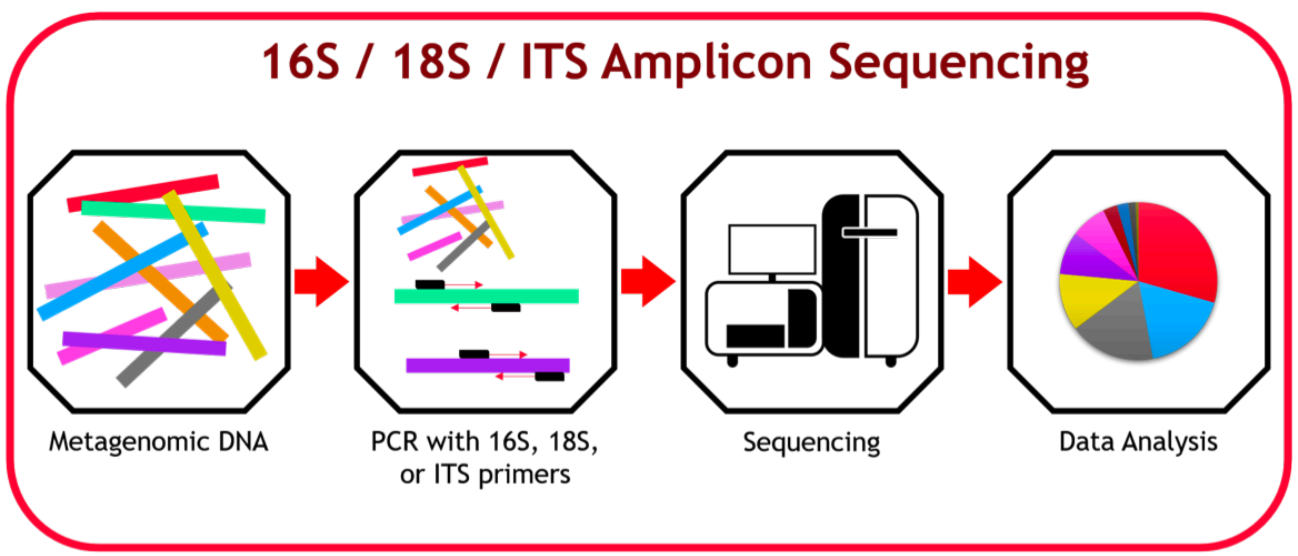
builds up fundamental knowledge of gut microbiota

Uncultivable ***Enhancing bias***

1990s-2010



Metagenomic analysis

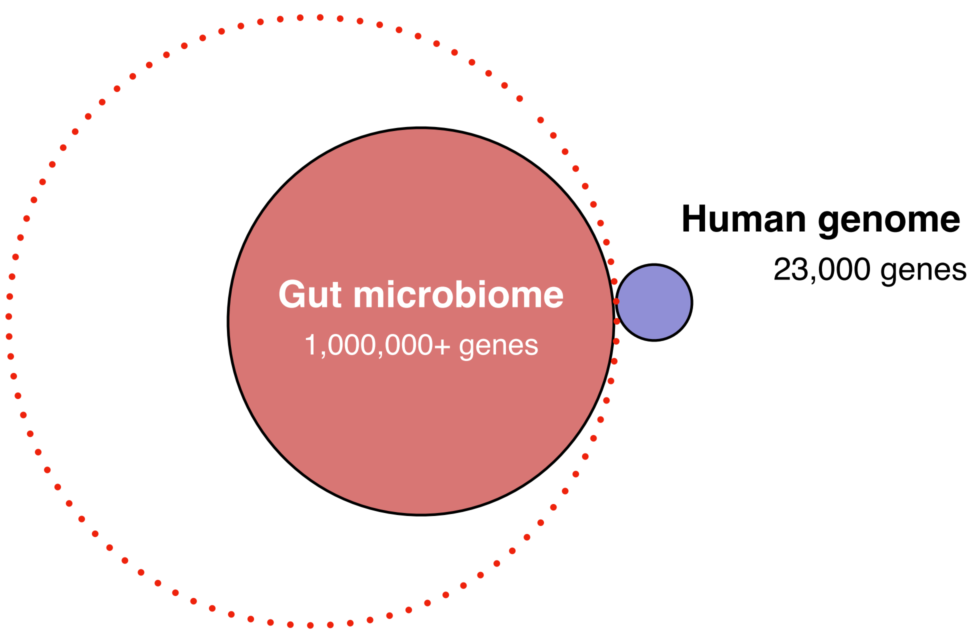
Advancing of PCR, 16s RNA, NGS…

Reconstitute diversity

Adak A, Khan MR. *Cell Mol Life Sci.* 2019;76(3):473-493.

Tools to interrogate gut microbiota

Evolution of our opinions



variability among groups
age, geographic…

NIH Human Microbiome Project

HMP1
2008 - 2013

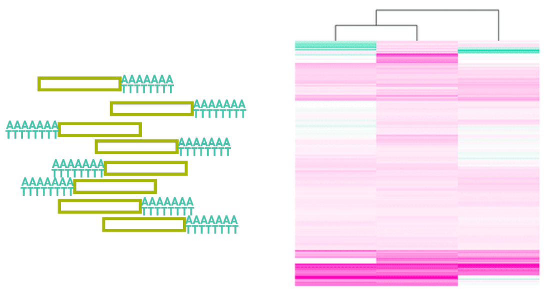
iHMP
2014 - Now

Yatsunenko T, Rey FE, Manary MJ, et al. *Nature*. 2012;486(7402):222

Tools to interrogate gut microbiota

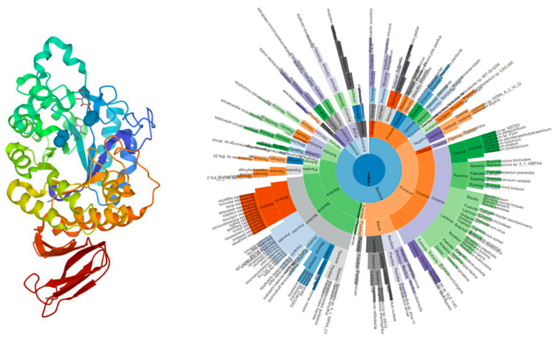
Multiomics - functional focus

Metatranscriptome



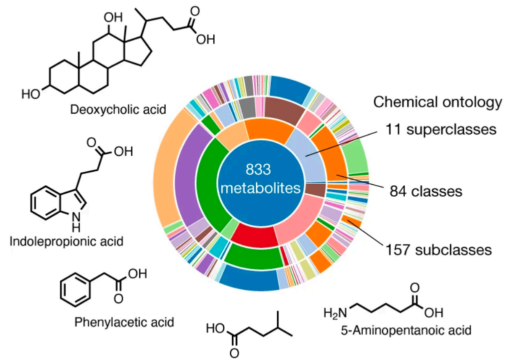
Expression profiles

Metaproteomics



Catalytic functions

Metabolomics



Metabolic activity

2010s-now

Metagenomic analysis

Advancing of PCR, 16s RNA, NGS…

Reconstitute diversity

3.3 million non-redundant genes from fecal microbes

Lai LA, Tong Z, Chen R, Pan S. *Methods Mol Biol*. 2019;1871:123-132.
Han S, Van Treuren W, Fischer CR, et al. *Nature*. 2021;595(7867):415-420.

Tools to interrogate gut microbiota

Multiomics - functional focus

Germ-free mice *Humanized germ-free mice*

Model systems in validation

2010s-now

Metagenomic analysis

Advancing of PCR, 16s RNA, NGS…

Reconstitute diversity

3.3 million non-redundant genes from fecal microbes

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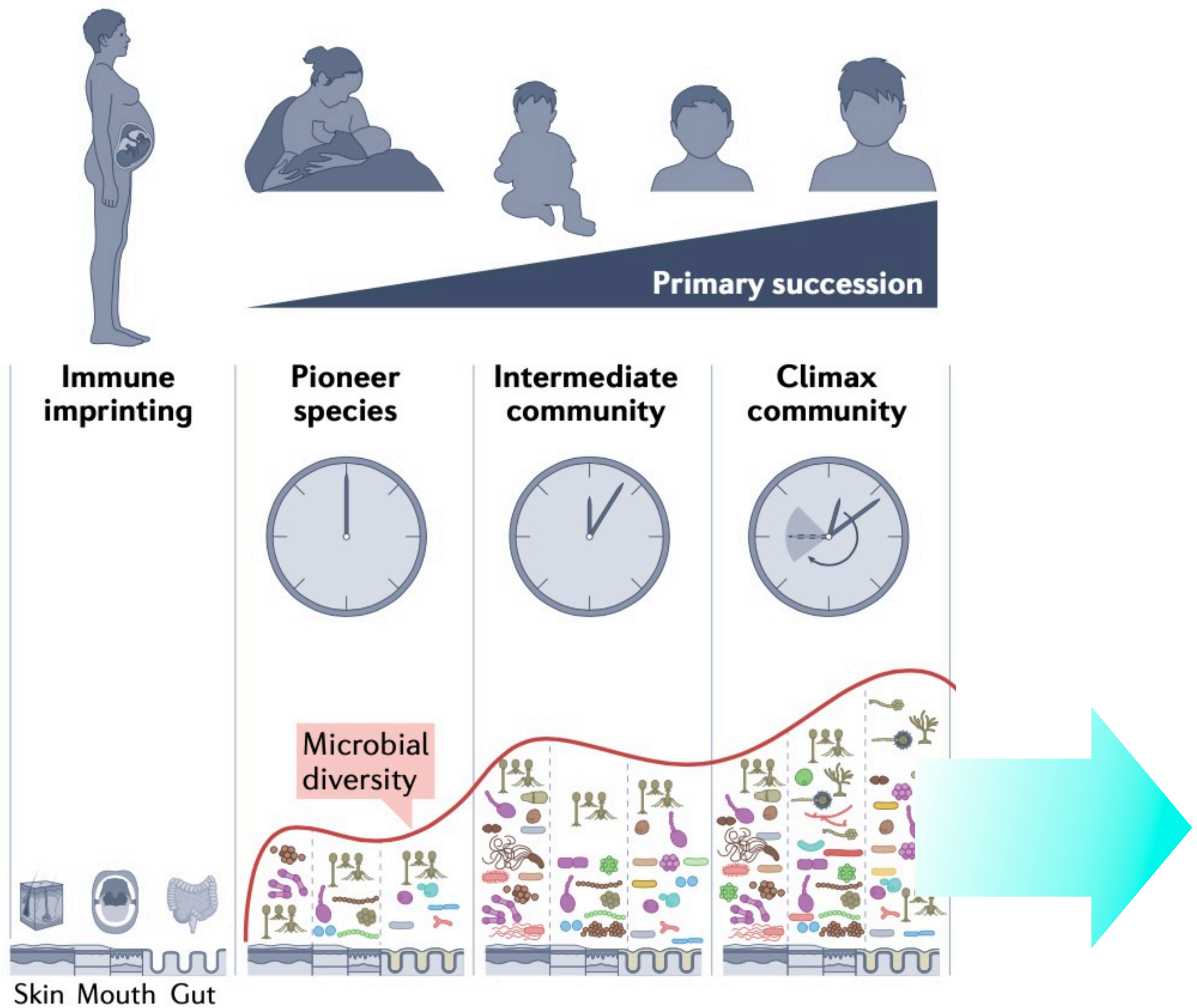
*How do **we** encounter?*

an evolution lasting one's lifespan

Gut microbiota evolution

Primary succession



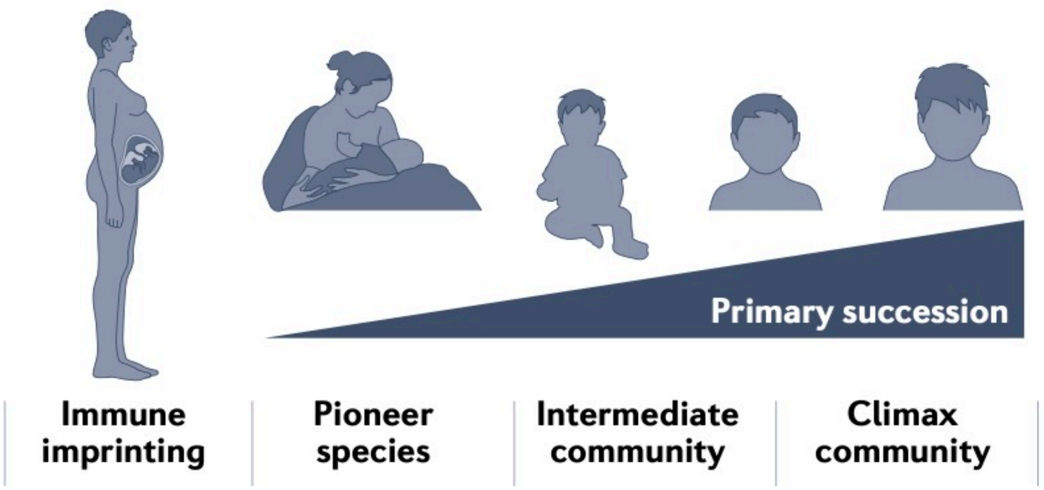
Dominated by keystone species
through adulthood

***Pioneer species** first establish the community, followed by rapid changes in one's childhood until a **stable, climax community** is finally reached*

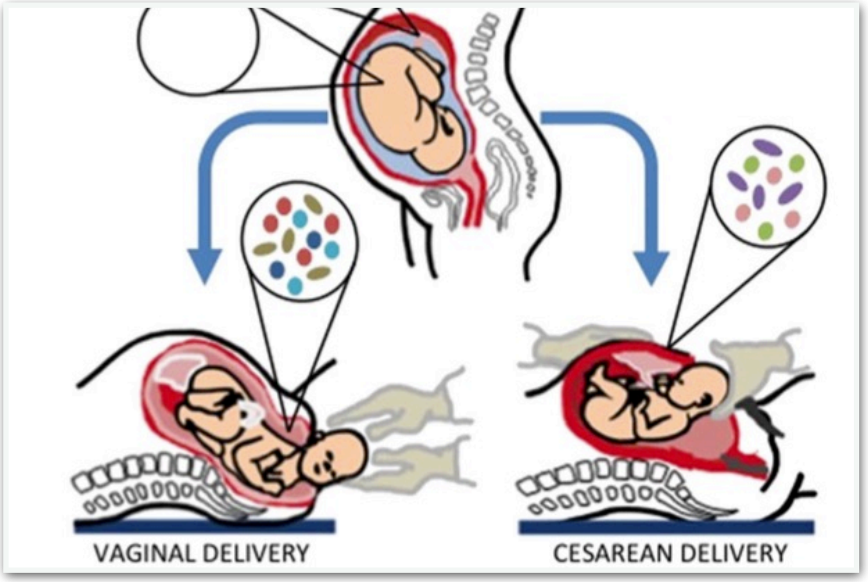
Martino C, Dilmore AH, et al. *Nat Rev Microbiol.* 2022

Gut microbiota evolution

Primary succession



Colonization during delivery

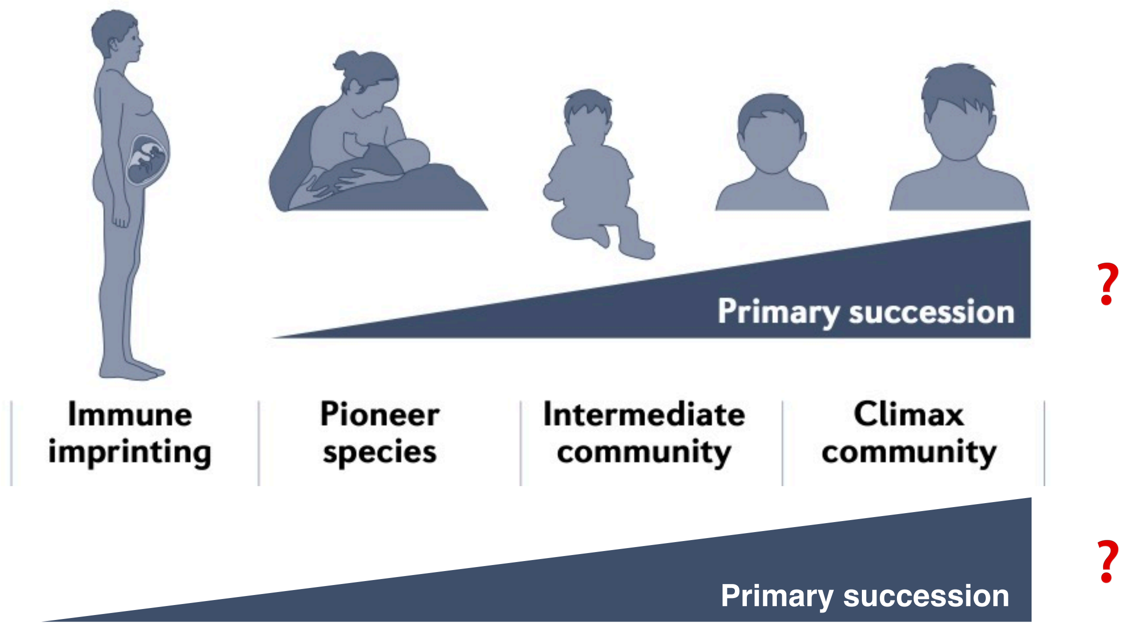


Delivery methods give fetus exposure to different sets of microbiota

Martino C, Dilmore AH, et al. *Nat Rev Microbiol.* 2022

Gut microbiota evolution

Primary succession



When does the first exposure take place?

Martino C, Dilmore AH, et al. *Nat Rev Microbiol.* 2022

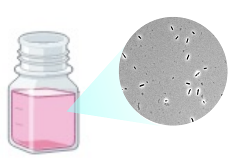
Gut microbiota evolution

First colonization debate

Fetal environment is sterile

No sign of live bacteria being cultivated

Physiological and immunological barriers prevents microbial invasion



Live bacteria / bacteria DNA contamination in commercial reagents

Low biomass
Statistical insignificance
Batch effects…

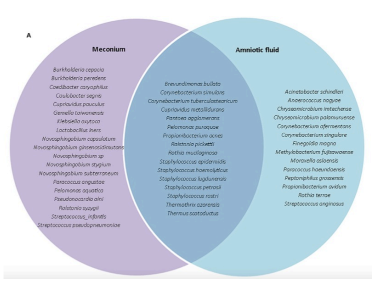
Fetal environment is not sterile

molecular techniques suggest bacterial communities in the ***placenta, amniotic fluid,*** *and* ***meconium*** *from healthy pregnancies*

Ensuring fresh sample and germ-free lab environment

Decontaminating commercial reagents

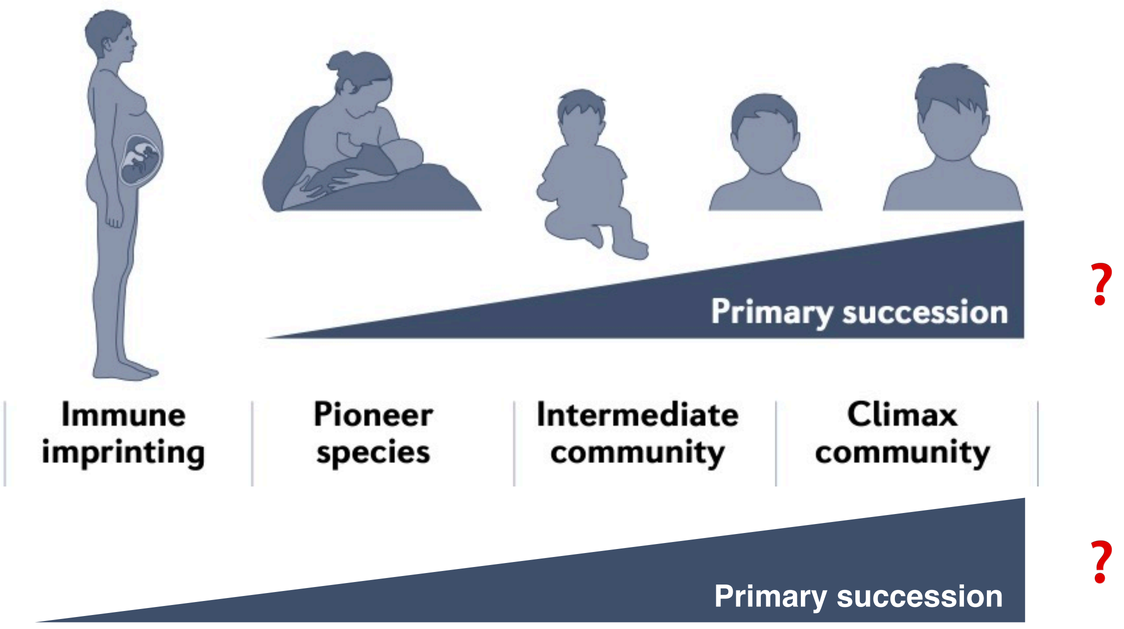
Multiple detections beyond 16s rRNA sequencing efforts
Immune-modulatory metabolites and fetal immunity response

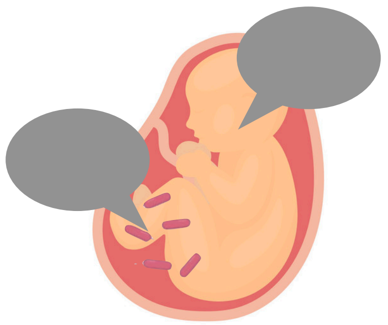


Stinson LF, Boyce MC, Payne MS, Keelan JA. *Front Microbiol*. 2019;10:1124.
Perez-Muñoz ME, Arrieta MC, Ramer-Tait AE, Walter J. *Microbiome*. 2017;5(1):48

Gut microbiota evolution

Primary succession





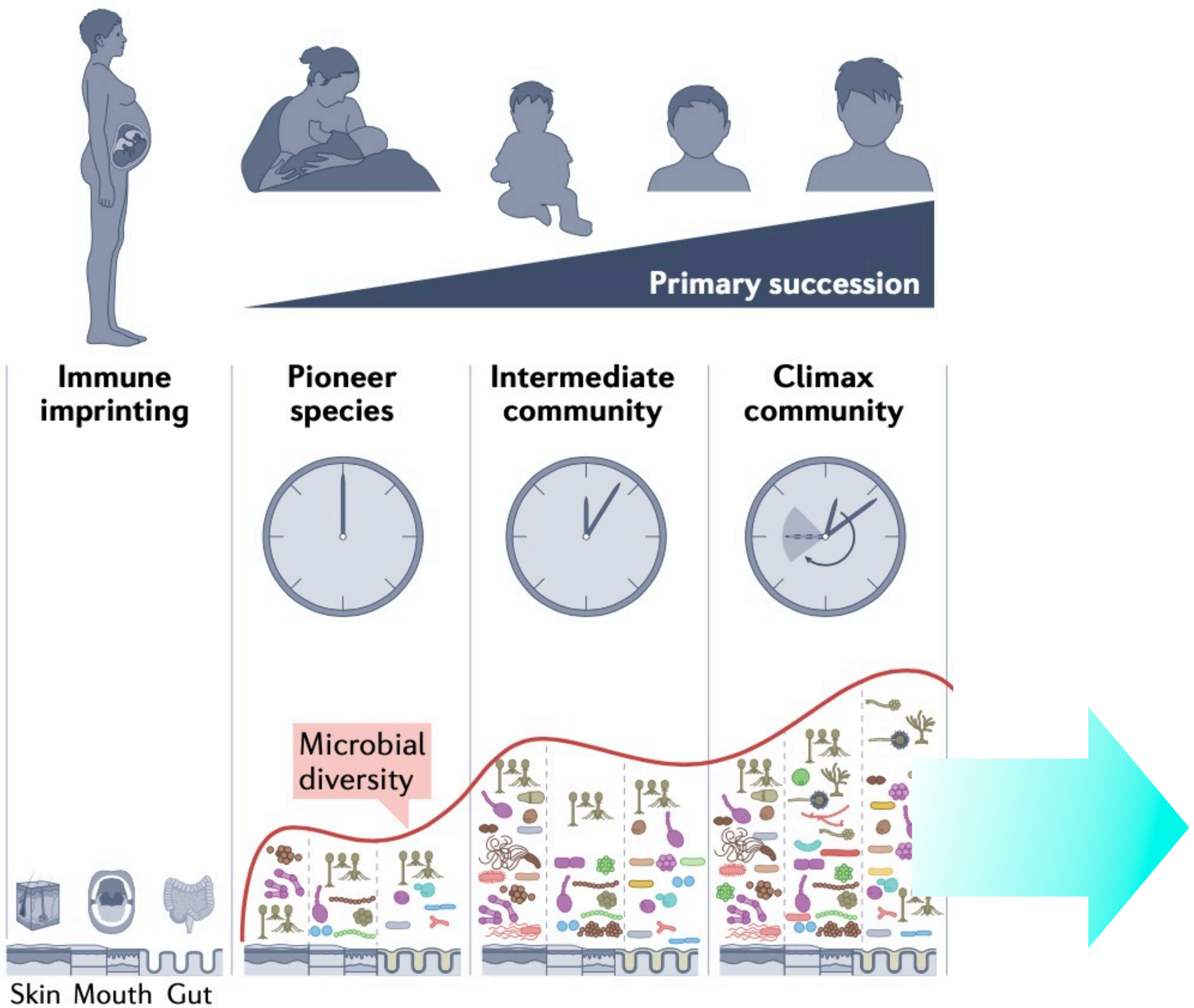
Mounting evidence showing womb as non-sterile place

The debate is still on…

Martino C, Dilmore AH, et al. *Nat Rev Microbiol.* 2022

Gut microbiota evolution

Primary succession



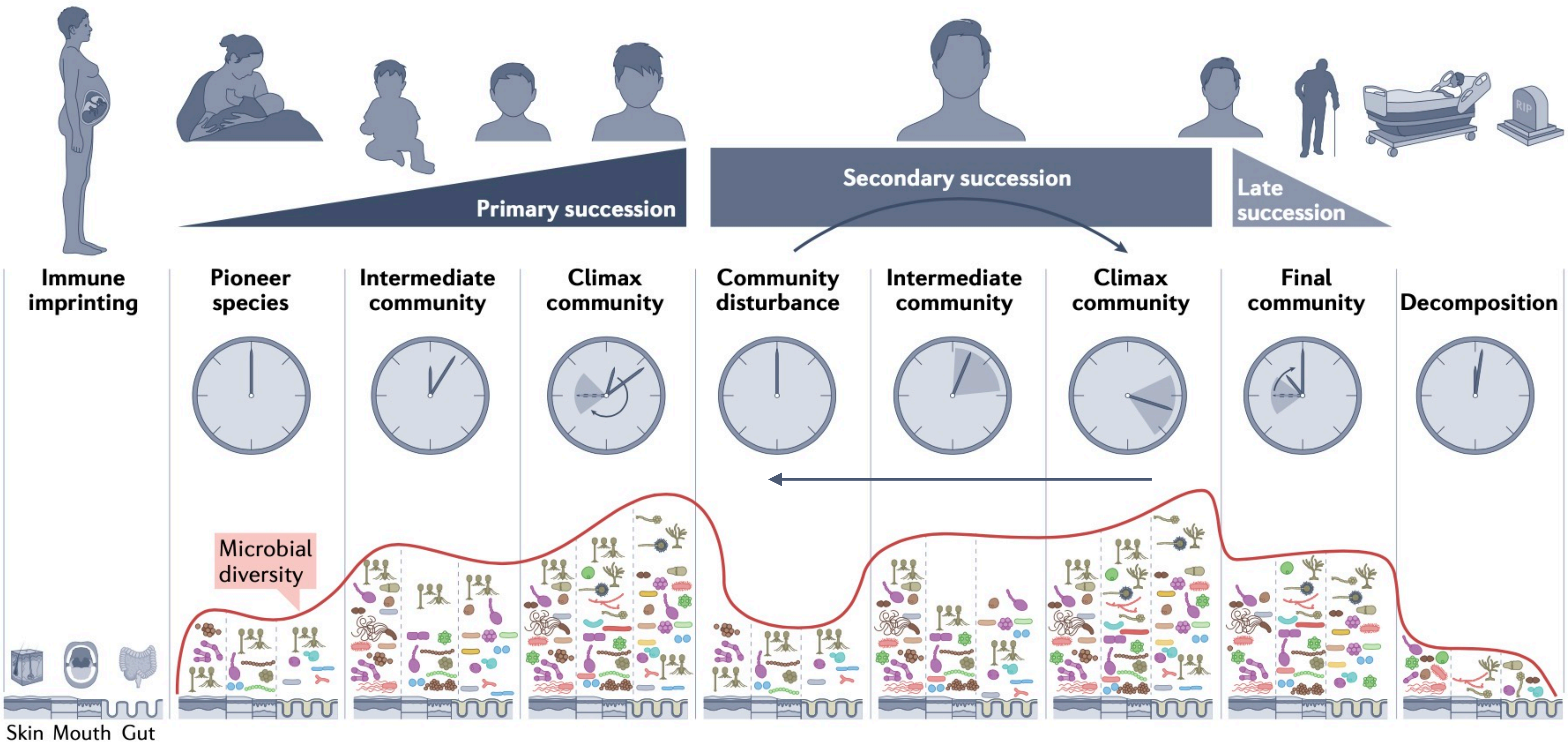
Dominated by keystone species

through adulthood

Martino C, Dilmore AH, et al. *Nat Rev Microbiol.* 2022

Gut microbiota evolution

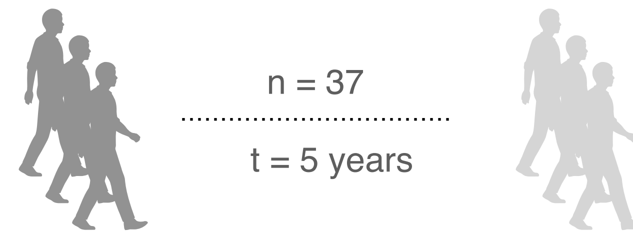
Secondary succession

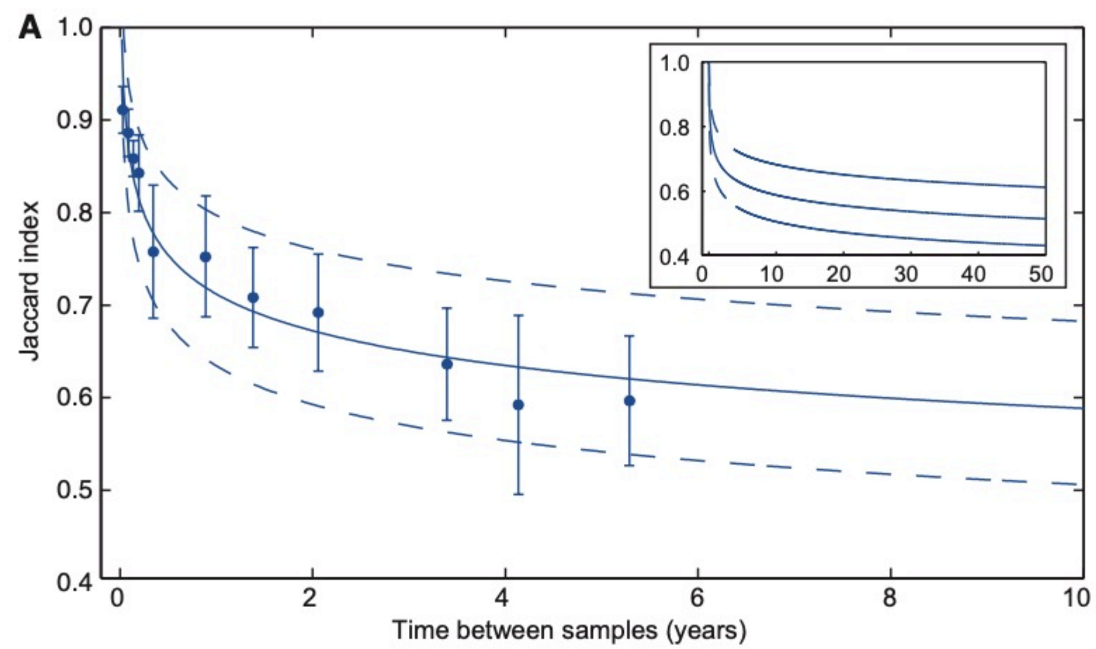


Rebound or rebuild *of the community after the relatively stable microbiota being* ***perturbed and pushed away from climax***

Martino C, Dilmore AH, et al. *Nat Rev Microbiol.* 2022

Gut microbiota stability and volatility



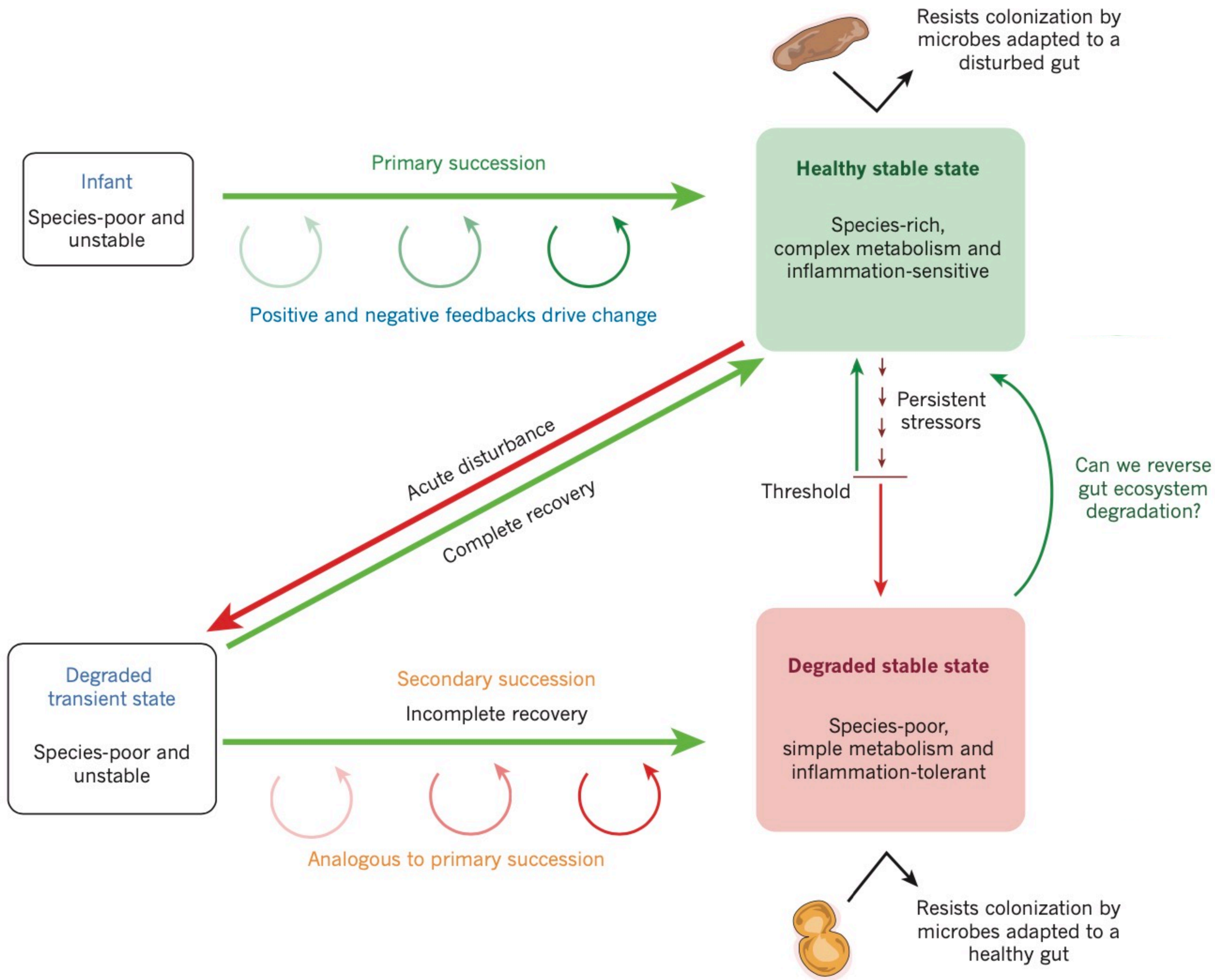


$$\text{Jaccard index}(\text{sample A, sample B}) = \frac{\text{sample A} \cap \text{sample B}}{\text{sample A} \cup \text{sample B}}$$

Gut microbiota composition is relatively stable across time for the same individual

Faith JJ, Guruge JL, Charbonneau M, et al. *Science*. 2013;341(6141)

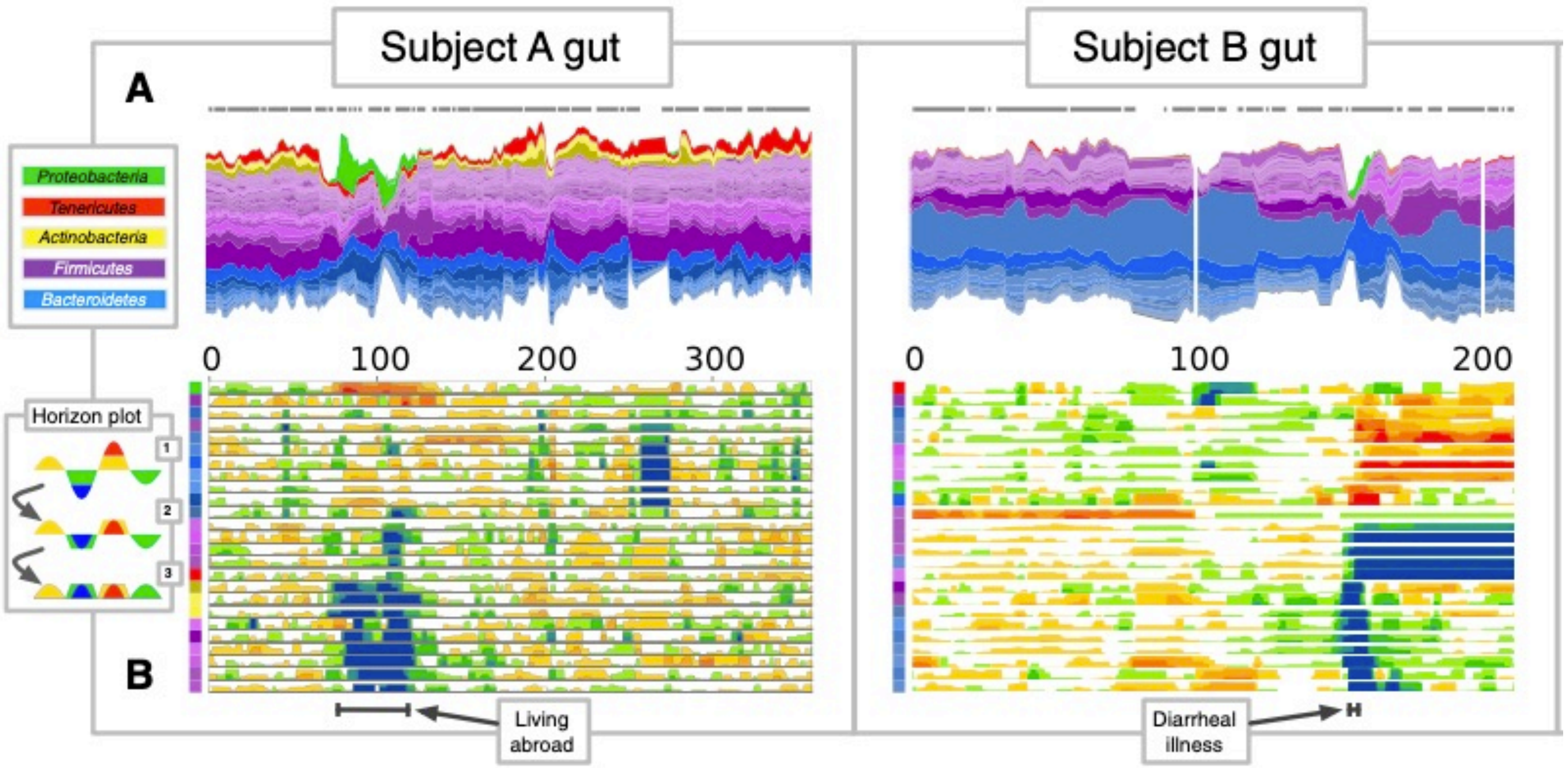
Gut microbiota stability and volatility



Lozupone CA, et al. *Nature*. 2012;489(7415):220

Gut microbiota stability and volatility

Volatility



Short-term perturbation

Nearly two-fold increase in the Bacteroidetes to Firmicutes ratio, which reversed upon return

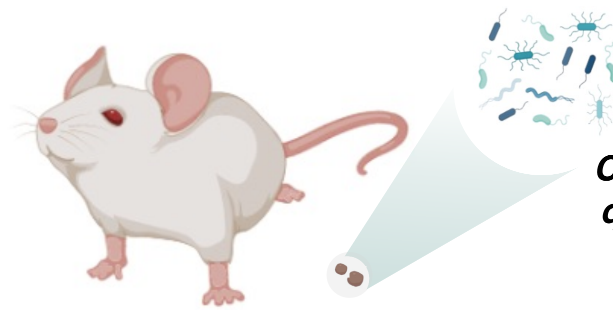
Long-term perturbation

permanent decline of most gut bacterial taxa, which were replaced by genetically similar species

David LA, Materna AC, Friedman J, et al. Genome Biol. 2014;15(7):R89

Gut microbiota stability and volatility

Daily diet and microbiota change



Composition responds quickly to diet change

humanized germ-free mice

Rich in fat and sugar but low in plant polysaccharides

Rich in plant polysaccharides but low in fat or sugar

Maslowski KM, Mackay CR. *Nat Immunol.* 2011;12(1):5

Gut microbiota stability and volatility

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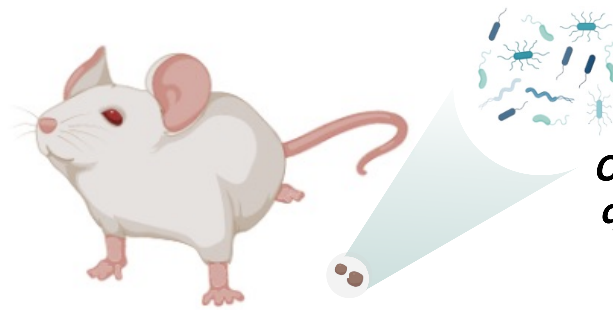
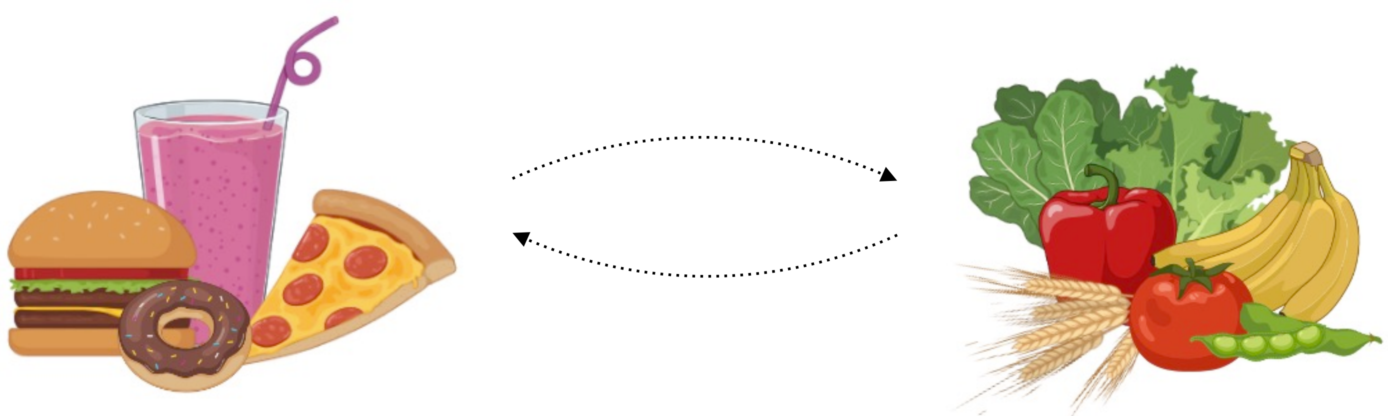


Composition responds quickly to diet change

humanized germ-free mice

We are feeding our gut microbiota with what we eat

Nutrients can directly interact with microorganisms to promote or inhibit their growth



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Gut microbiota stability and volatility

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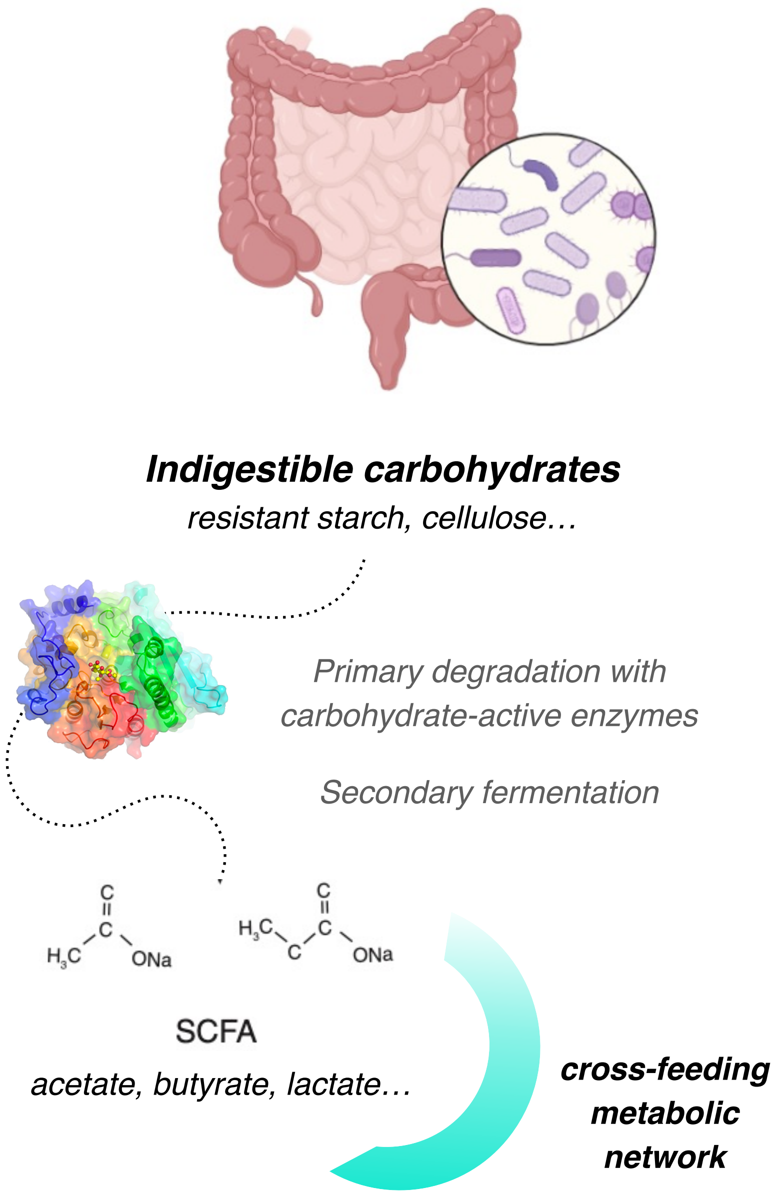
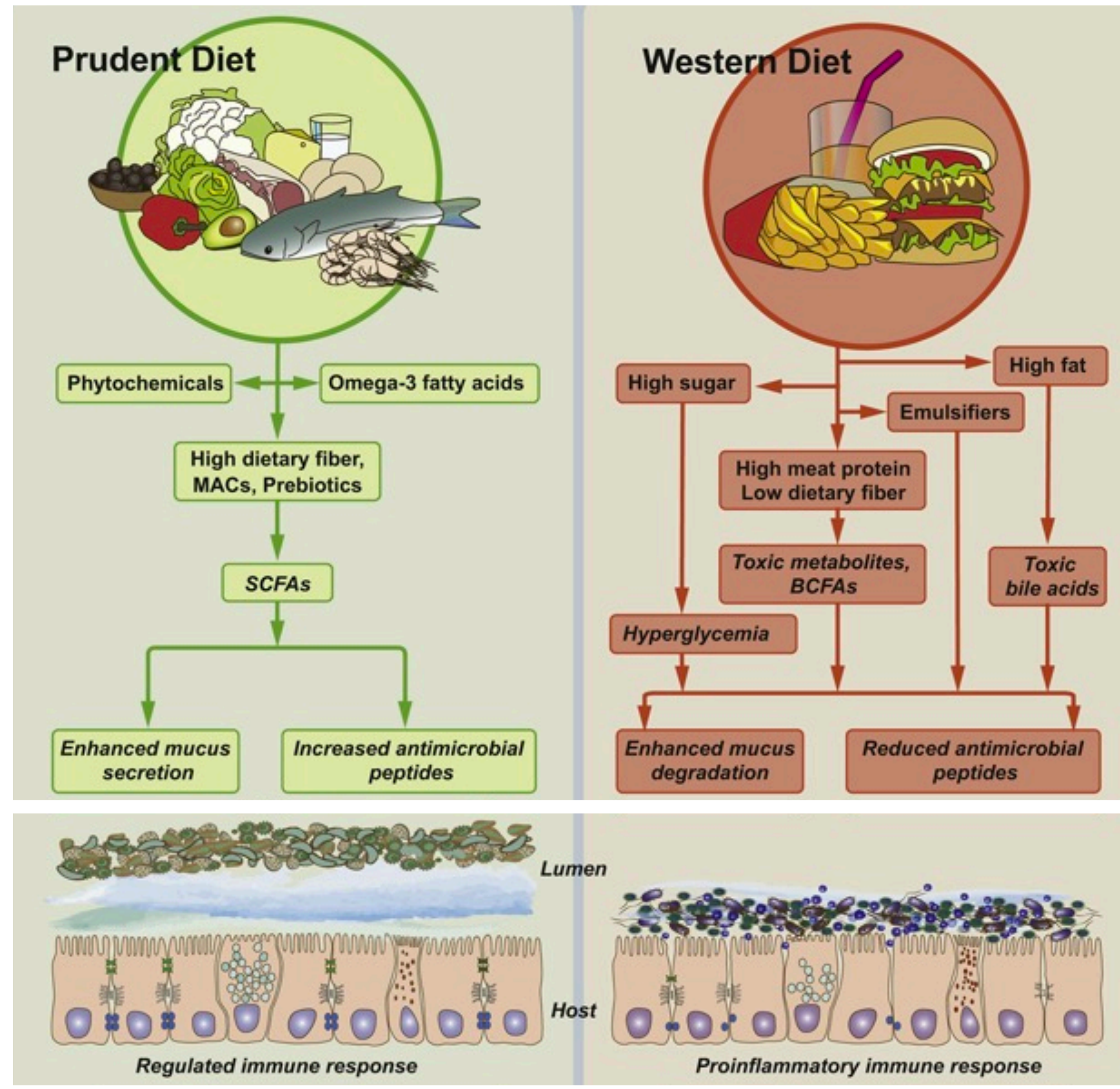


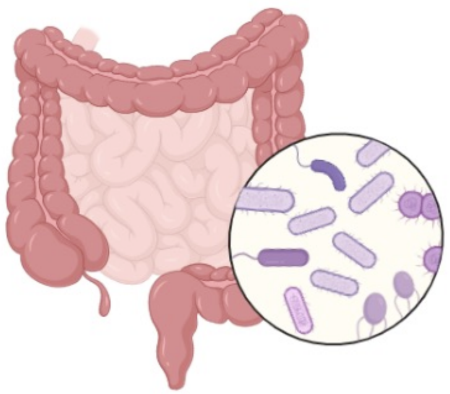


Healthy gut microbiota ***Microbiota dysbiosis***

Zmora N, Suez J, Elinav E. *Nat Rev Gastroenterol Hepatol.* 2019;16(1):35

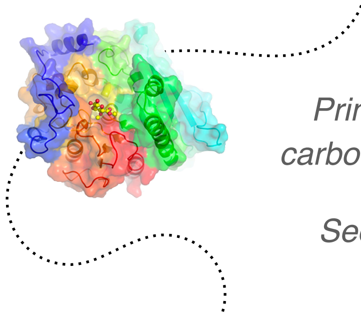
Gut microbiota stability and volatility

Daily diet and microbiota change



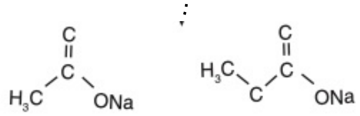
Indigestible carbohydrates

resistant starch, cellulose…



Primary degradation with carbohydrate-active enzymes

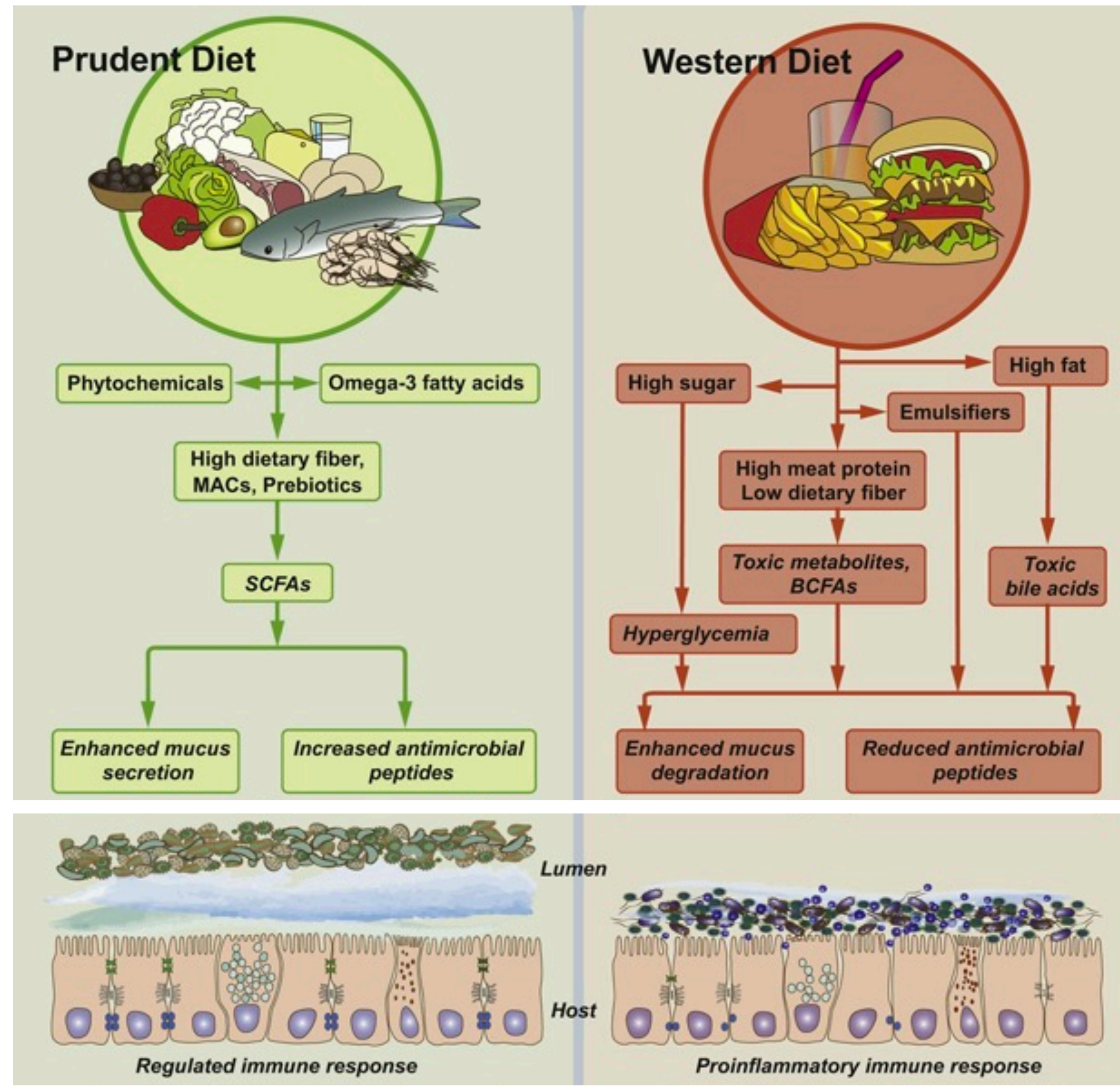
Secondary fermentation



SCFA

acetate, butyrate, lactate…

cross-feeding metabolic network



Healthy gut microbiota
intact barrier function

Microbiota dysbiosis
barrier dysfunction

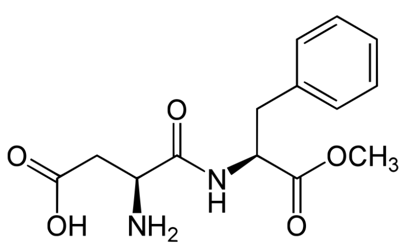
Zmora N, Suez J, Elinav E. *Nat Rev Gastroenterol Hepatol.* 2019;16(1):35

Gut microbiota stability and volatility

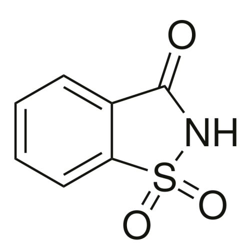
Drugs and antibiotics

Gut microbiota stability and volatility

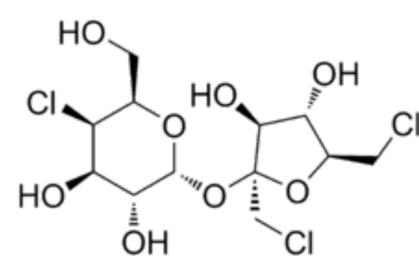
Sweetener vs added sugar



Aspartame



Saccharin



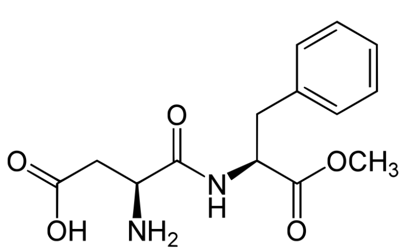
Sucralose

Stevia

Gut microbiota stability and volatility

Sweetener vs added sugar

Non-nutritive $\stackrel{?}{=}$ *biologically inert*



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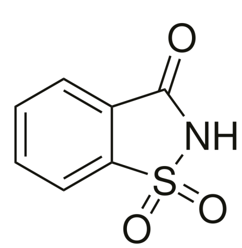

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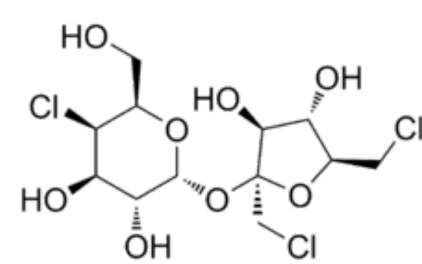

Sucralose

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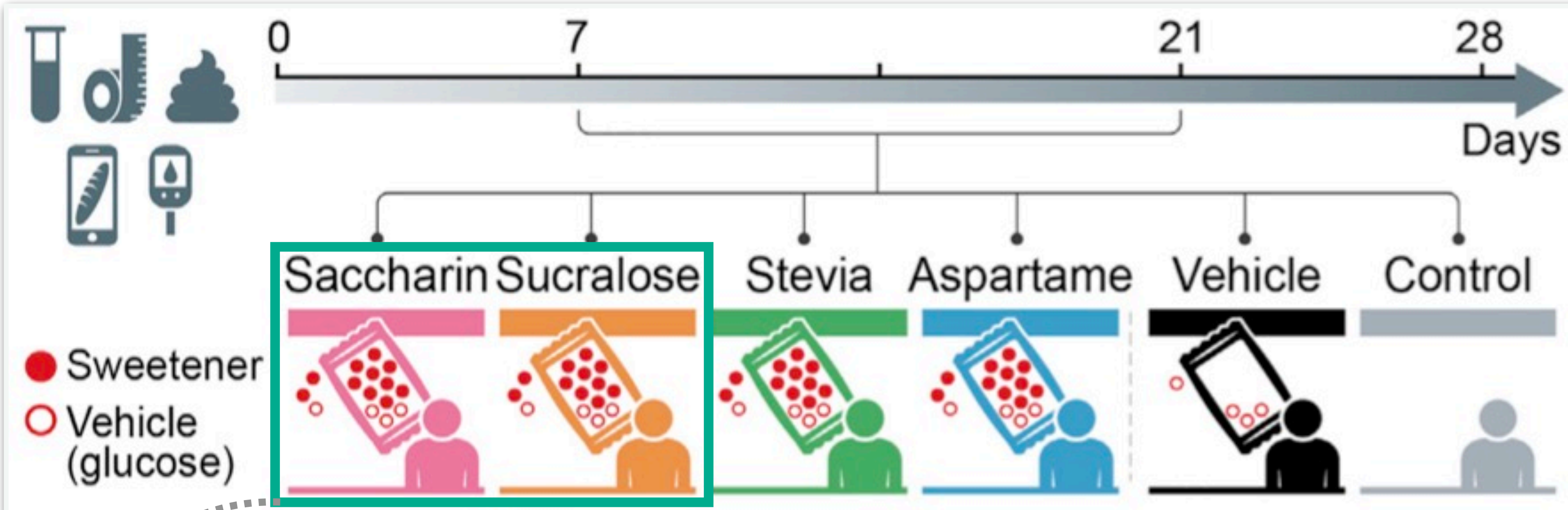
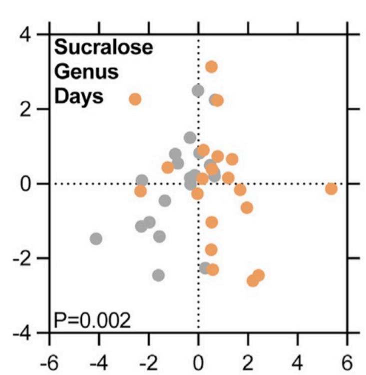
Gut microbiota stability and volatility

Sweetener vs added sugar

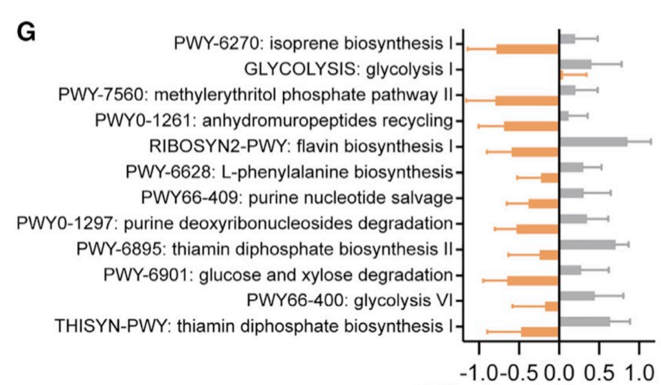


Supplementing specific sweeteners (8%-75% of daily intake guideline)

Gut microbiota change

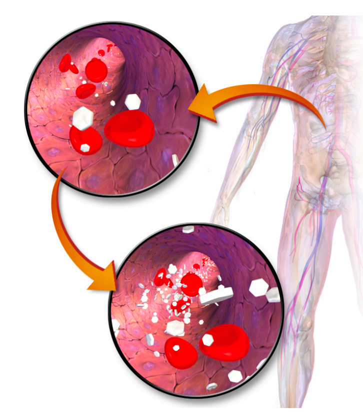


composition



Metabolism

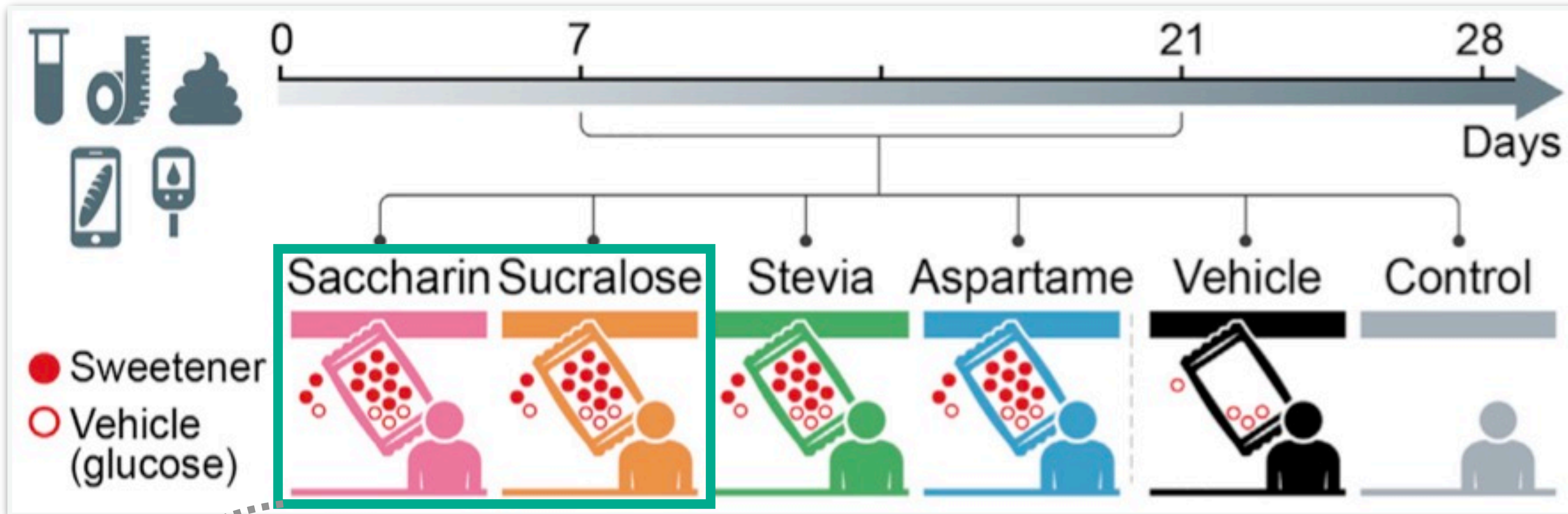
Glucose intolerance



Suez J, Cohen Y, Valdés-Mas R, et al. Cell. 2022;185(18):3307

Gut microbiota stability and volatility

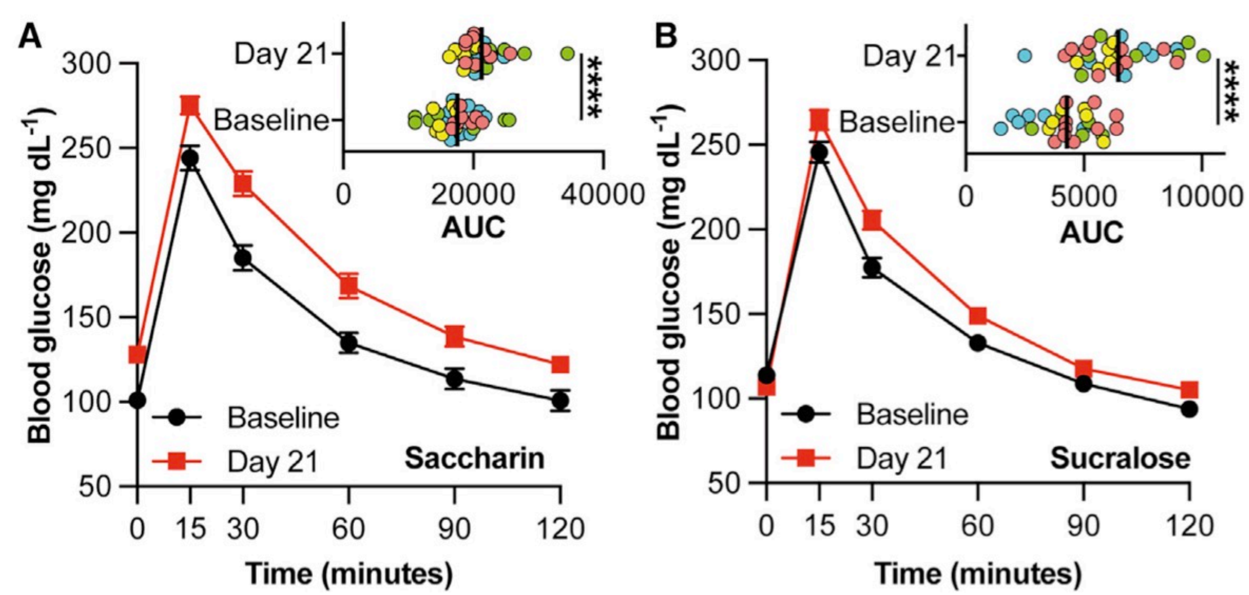
Sweetener vs added sugar



Supplementing specific sweeteners (8%-75% of daily intake guideline)

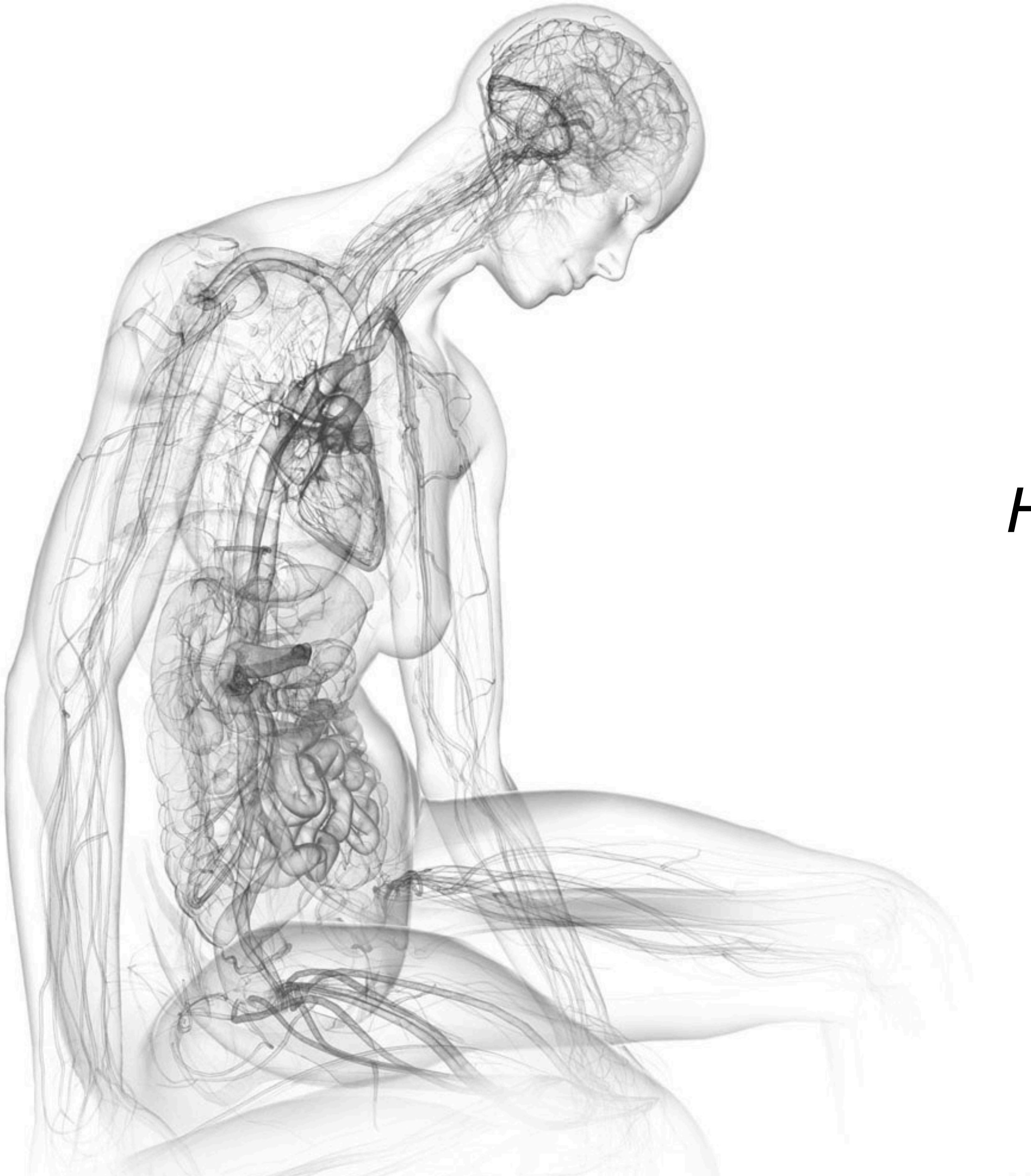
Stool sample Baseline / Day21

Germ-free mice conventionalized with participants gut microbial



Glucose intolerance linked with sweetener-altered gut microbiota

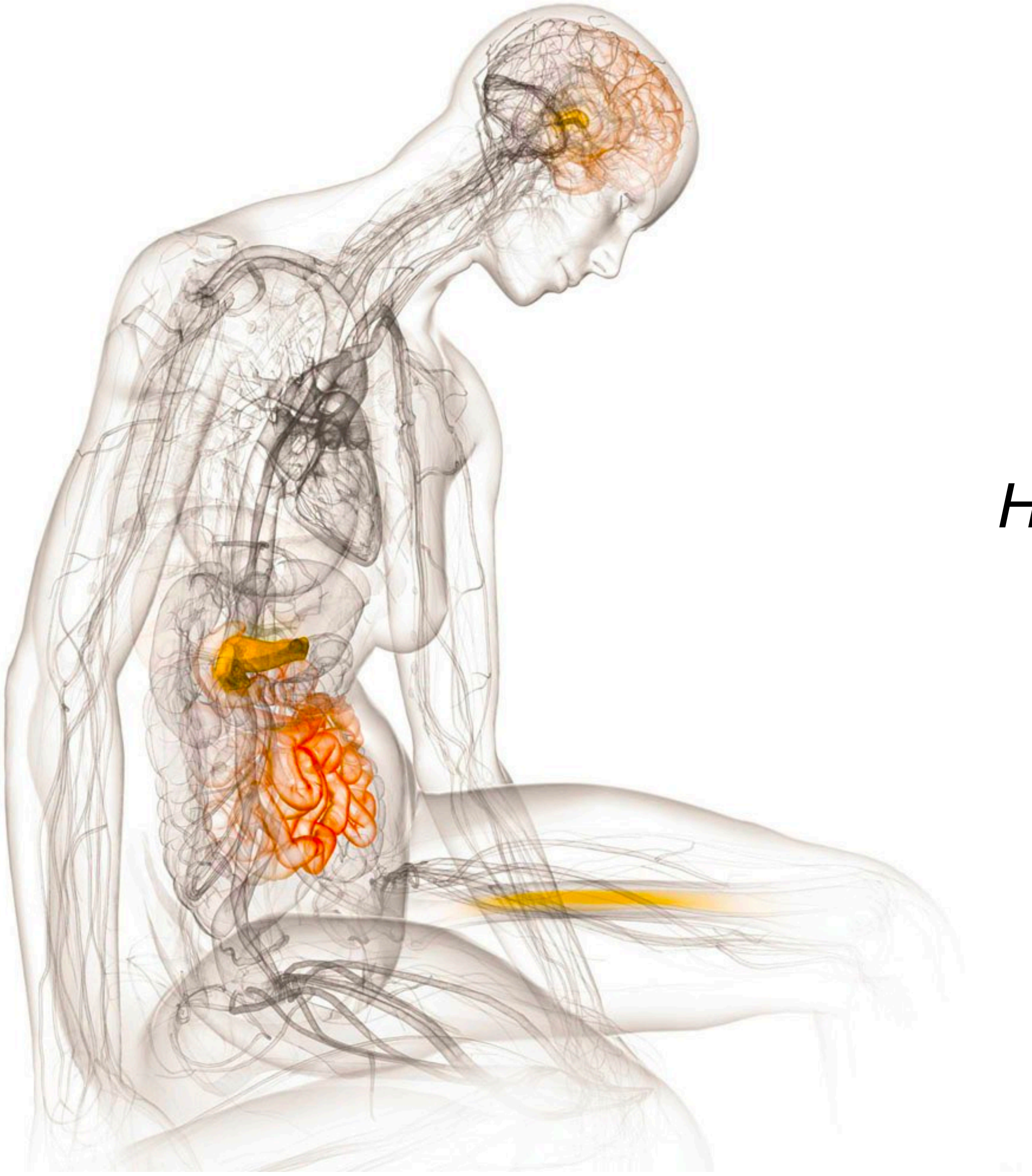
Suez J, Cohen Y, Valdés-Mas R, et al. Cell. 2022;185(18):3307



How do they affect us?

How do they affect us?

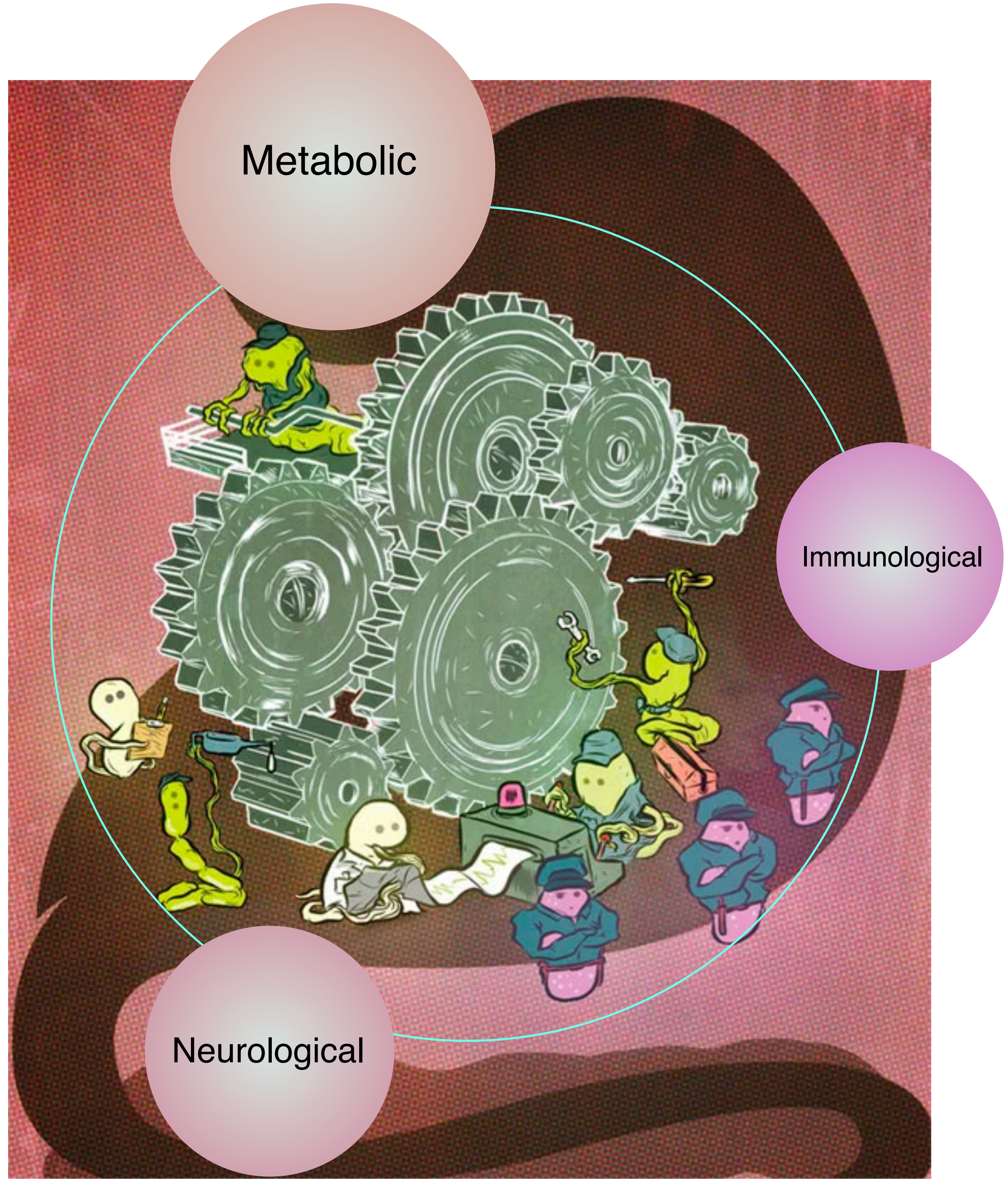
Locally…



How do they affect us?

Locally… and globally

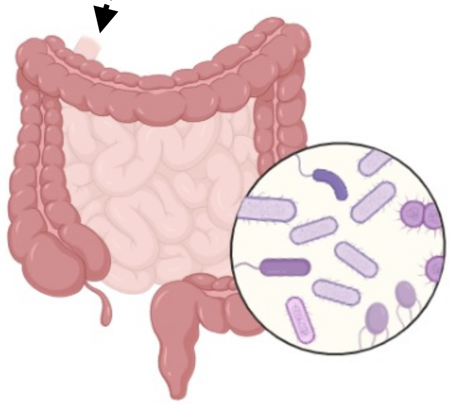
Gut microbiota and our health



Gut microbiota and our health

A biological rheostat

A healthy diet



after fermentation…

Secondary bile acid

Lipid metabolism, bile acid recycling and homeostasis

Short-chain fatty acids

Powering both microbiota diversity and mucosa integrity
Immune and inflammatory responses

Branched-chain amino acids

Essential amino acids

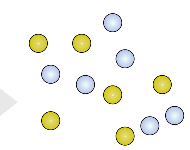
Tryptophan and indole-derivative

Intestinal 5-HT production, AHR ligands

Trimethyamine N-oxide

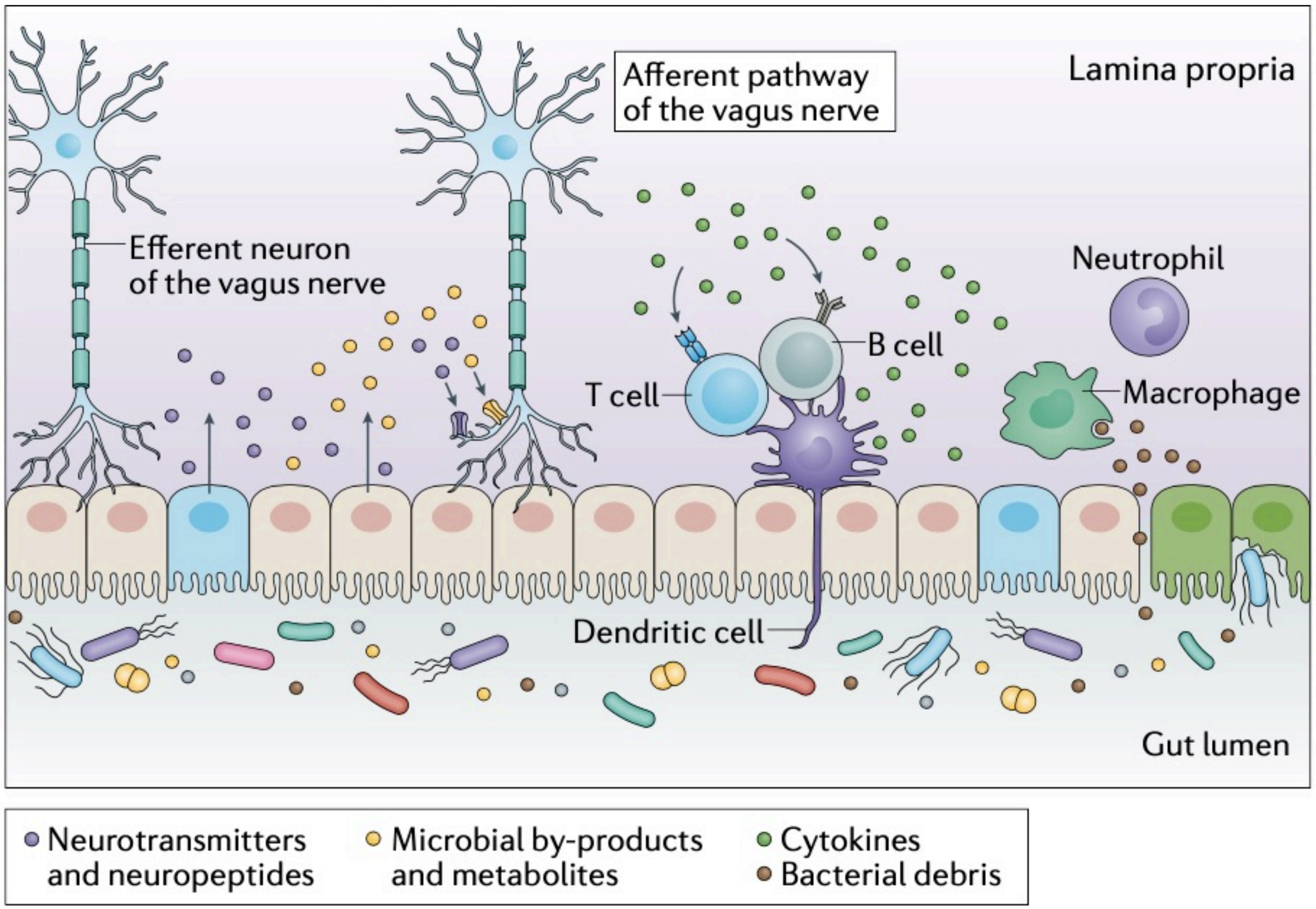
Imidazole propionate

…



Gut microbiota and our health

Cross talk with host cells



Healthy intestinal functioning

Diverse microbiota habitat

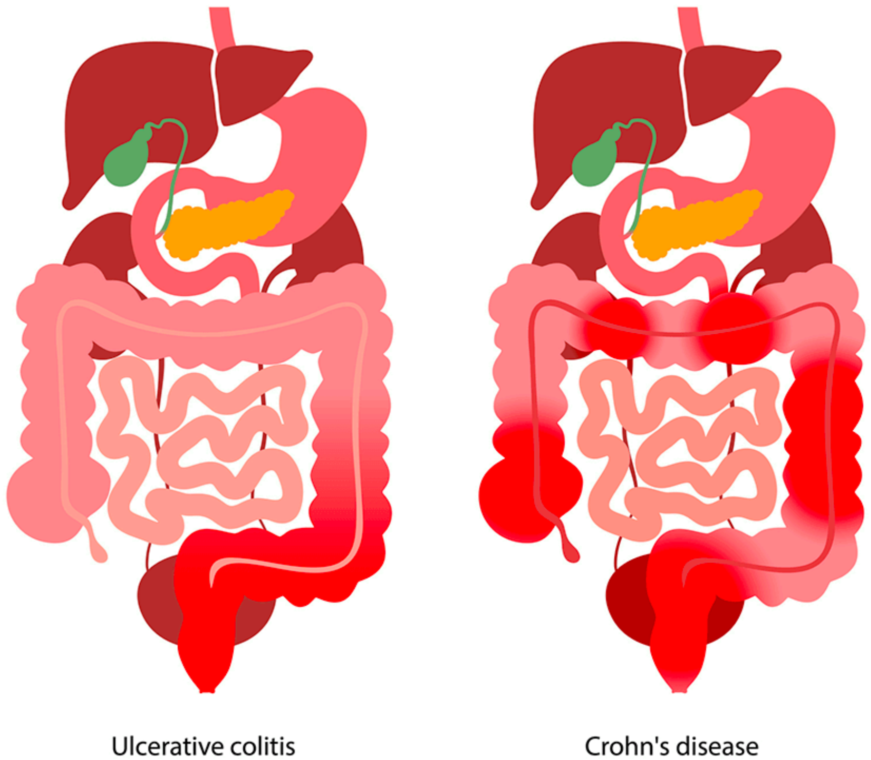
Metabolic syndrome

Depression and neurological disease

Inflammation and allergy

Gut microbiota and our health

Inflammatory bowel disease (IBD)



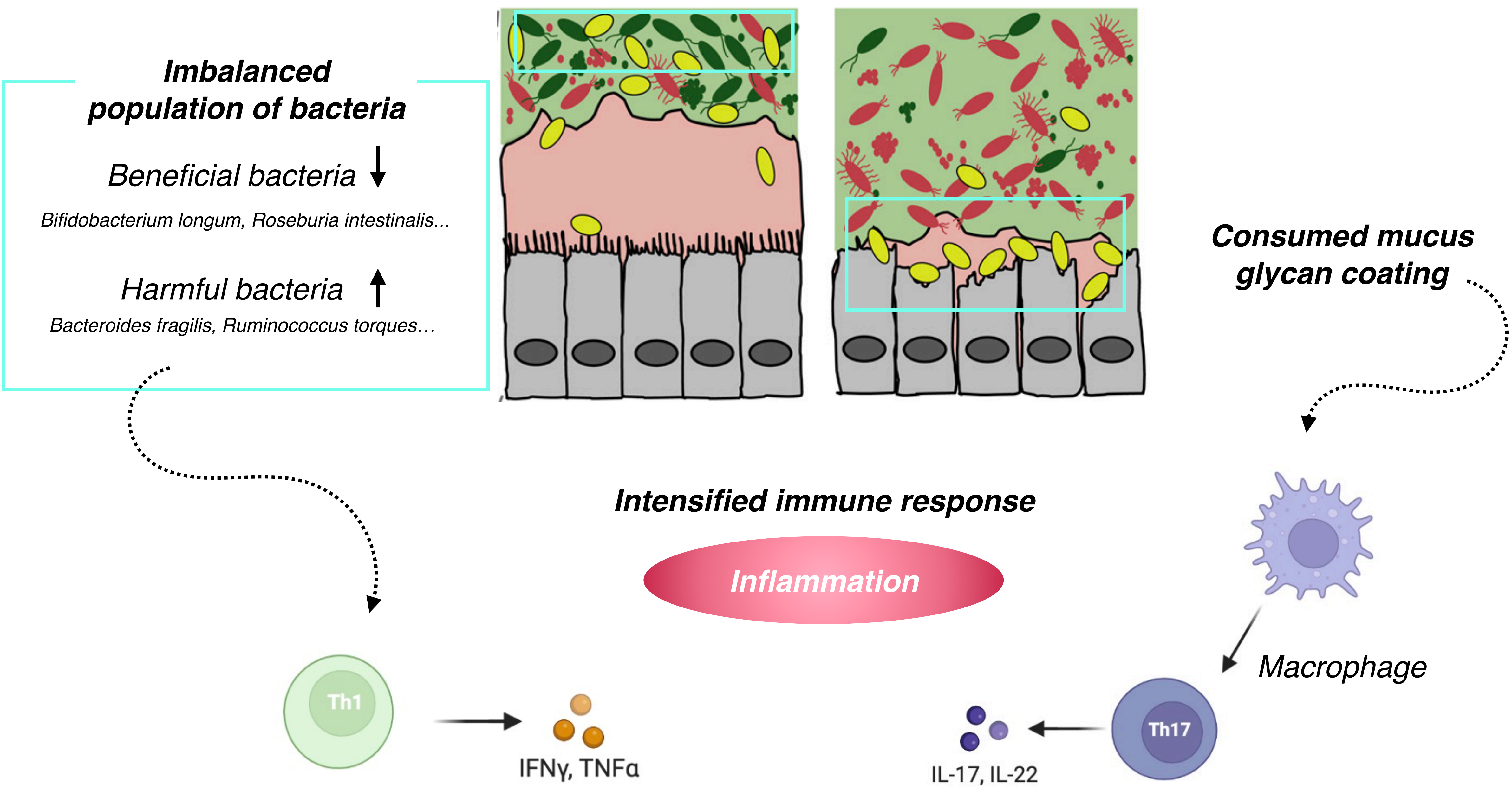
Chronic inflammation of the GI tract
associate with mucus damage and ulcers

0.3%–0.5% of the global population

Qiu P, Ishimoto T, et al. *Front. Cell. Infect. Microbiol.* 2020,12:733992.

Gut microbiota and our health

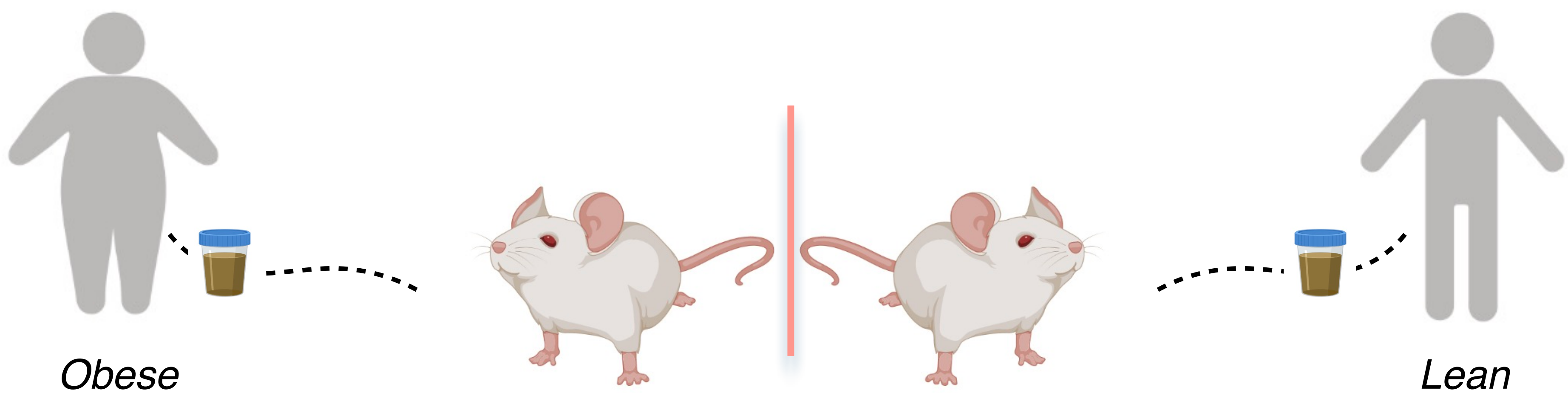
Inflammatory bowel disease (IBD)



Qiu P, Ishimoto T, et al. *Front. Cell. Infect. Microbiol.* 2020,12:733992.

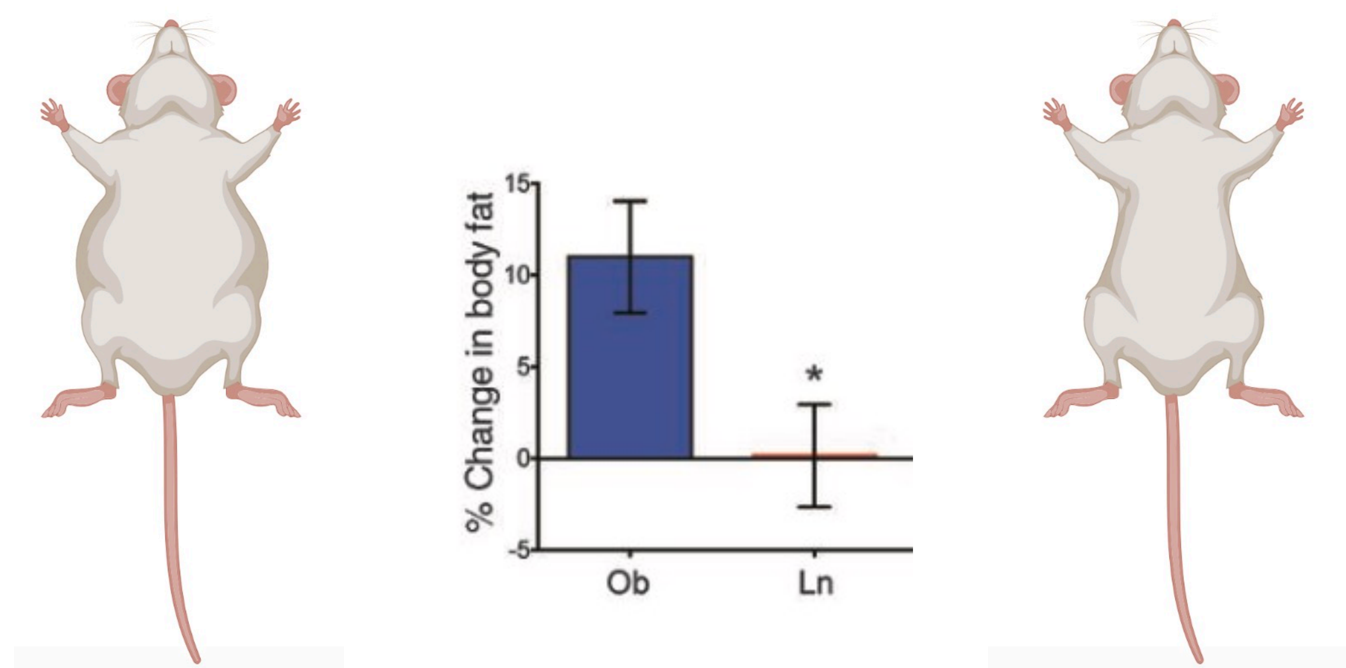
Gut microbiota and our health

Obesity



germ-free mice

fed with LF-HPP chow diet

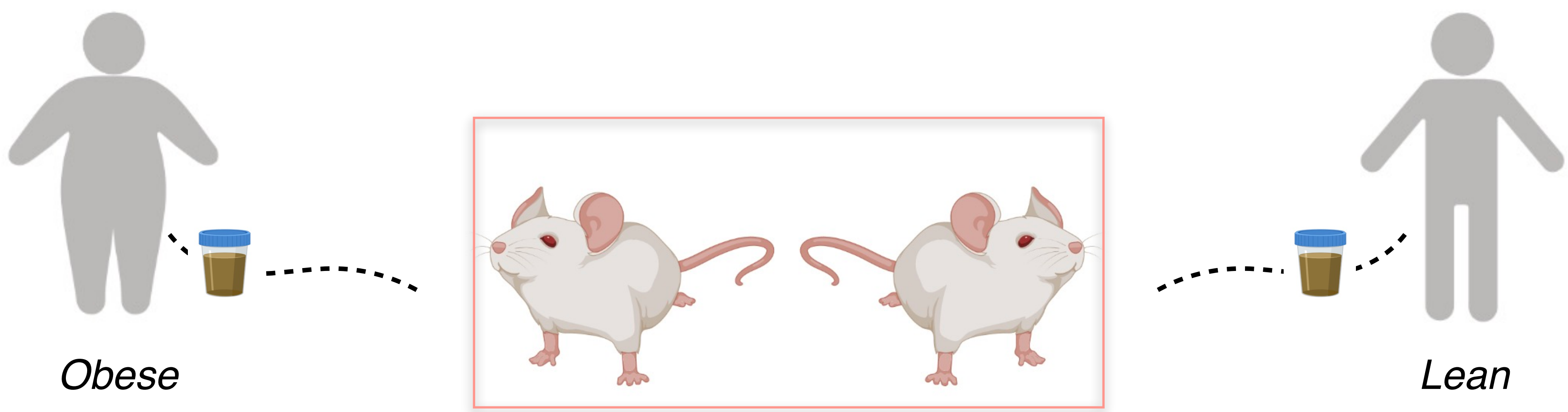


"Obese" gut microbiota utilized more energy from food

Ridaura VK, Faith JJ, Rey FE, et al. Science. 2013;341(6150):1241214.

Gut microbiota and our health

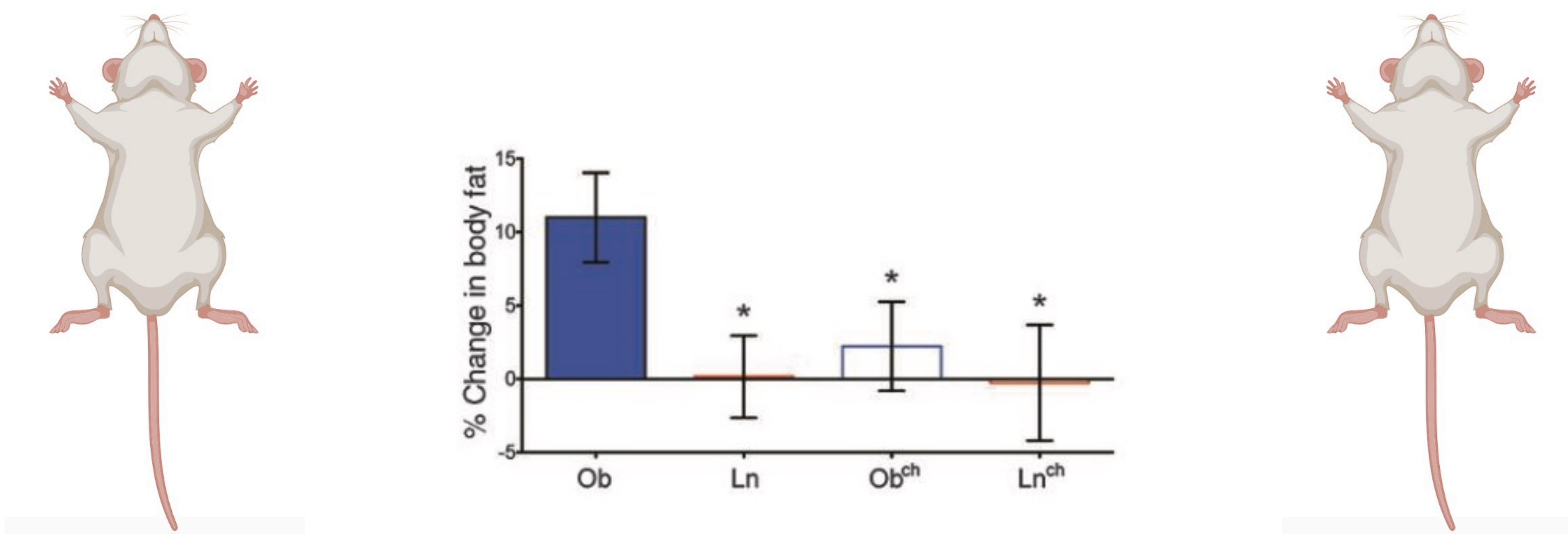
Obesity



Obese *Lean*

germ-free mice

fed with LF-HPP chow diet

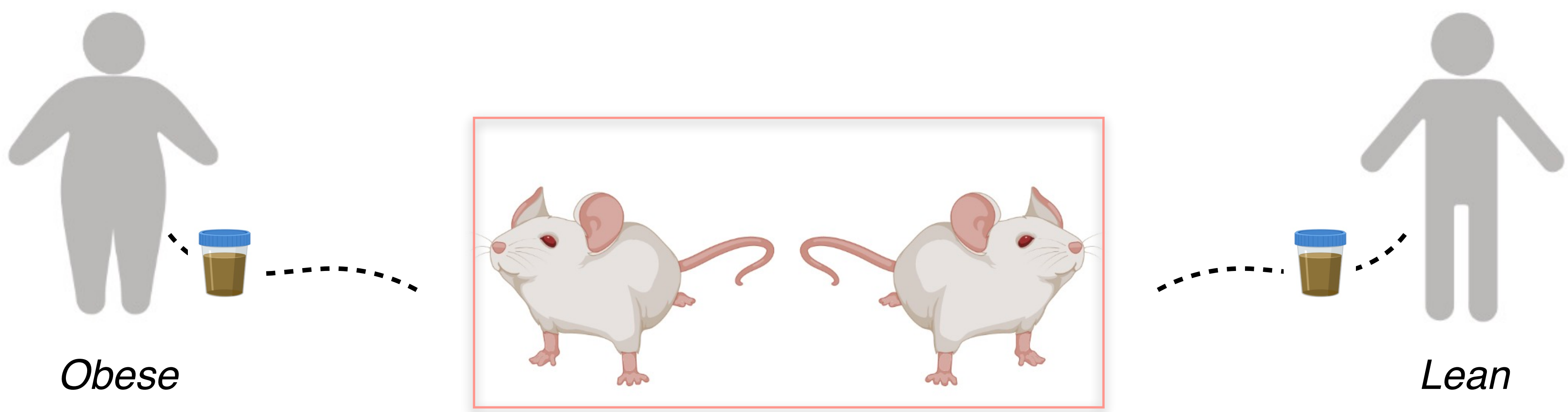


"Obese" phenotype ameliorated

Ridaura VK, Faith JJ, Rey FE, et al. Science. 2013;341(6150):1241214.

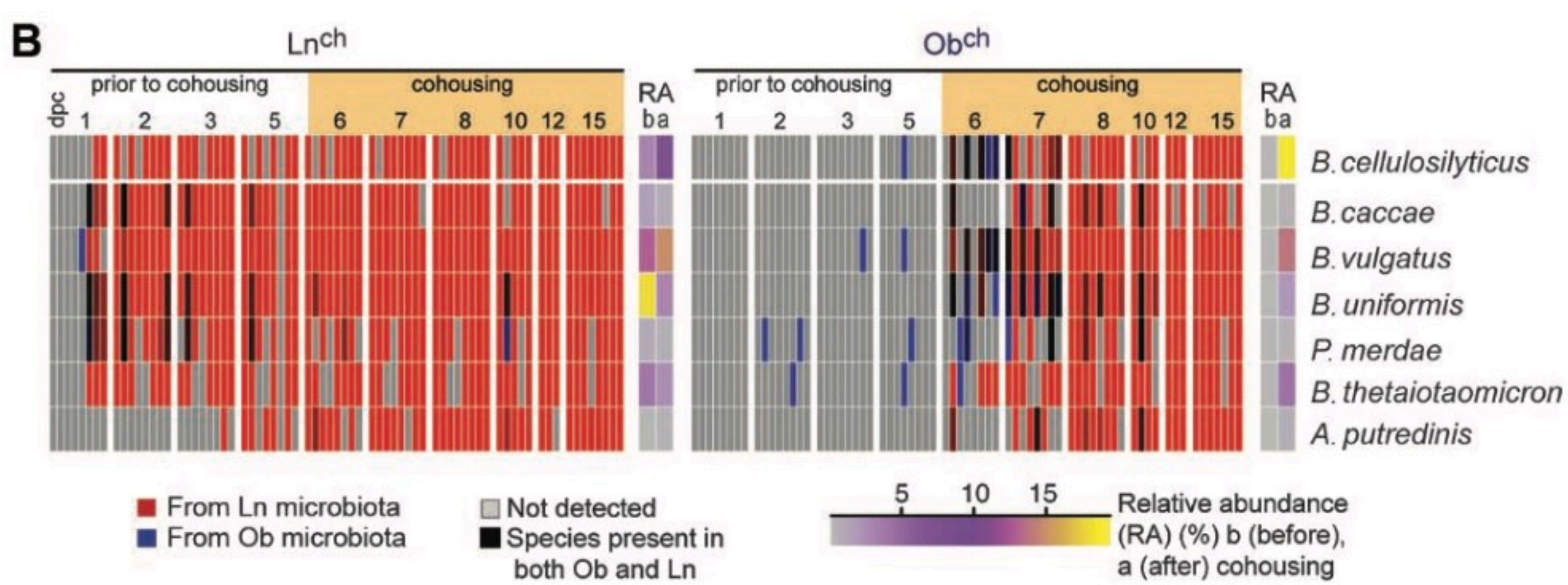
Gut microbiota and our health

Obesity



germ-free mice

fed with LF-HPP chow diet



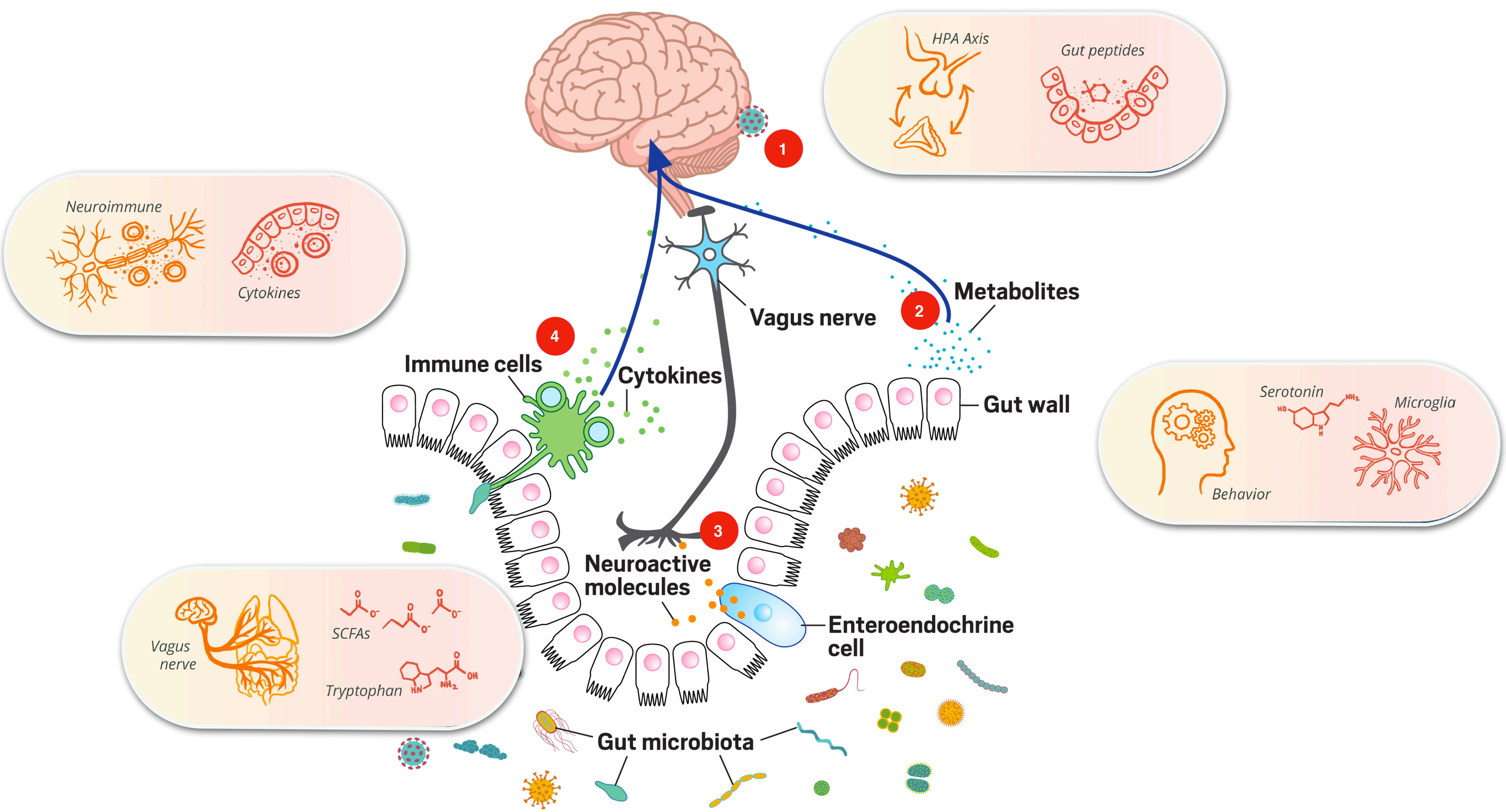
Reduced obesity phenotype due to "Lean" microbiota invasion

SCFAs (increased in Ln), branched-chain amino acids (increased in Ob), bile acid species (increased in Ln)

Ridaura VK, Faith JJ, Rey FE, et al. Science. 2013;341(6150):1241214.

Gut microbiota and our health

Depression and anxiety

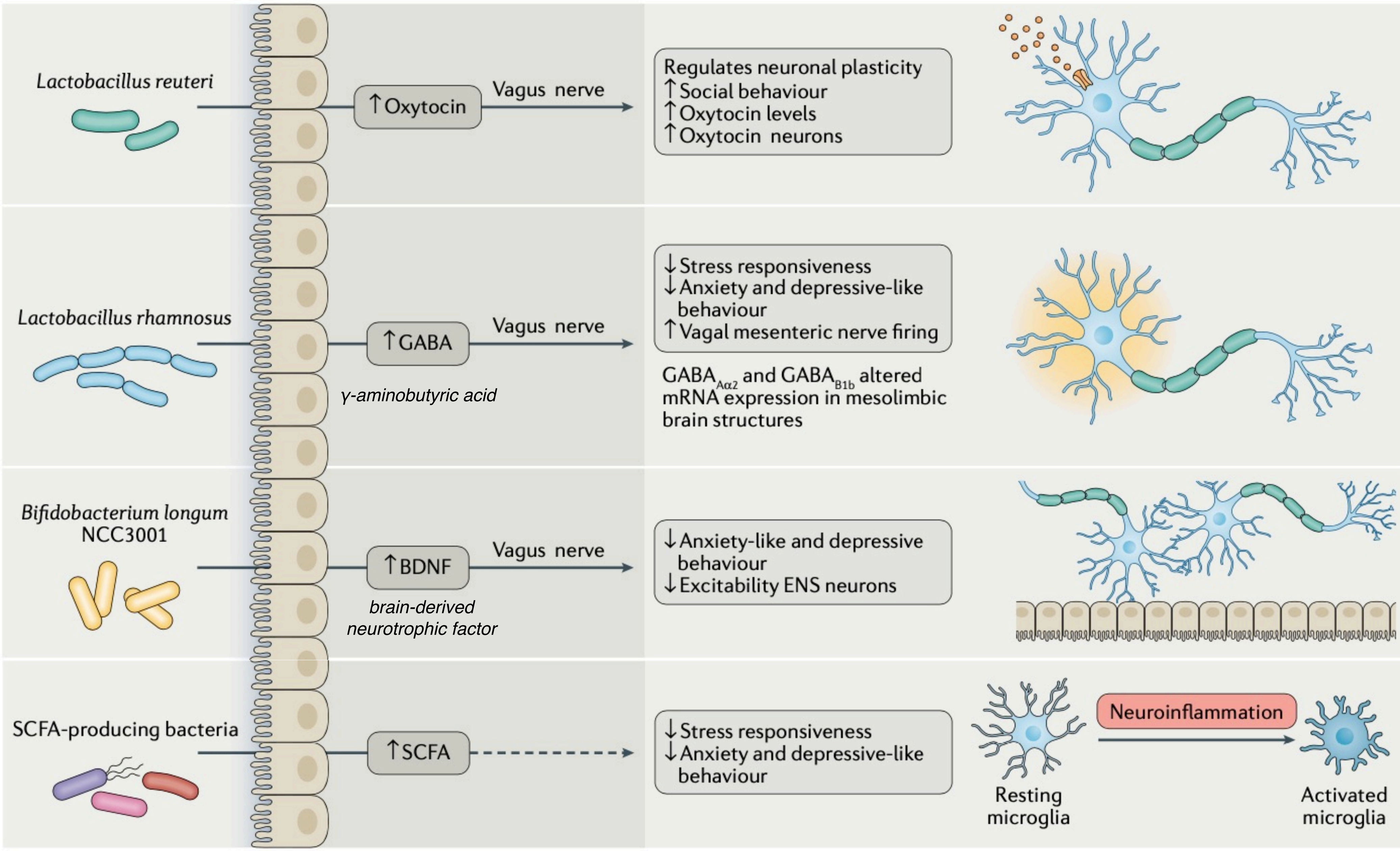


Moughnyeh MM, Brawner KM, et al. *J Surg Res*. 2021;266:33

Morais LH, Schreiber HL 4th, Mazmanian SK. Nat Rev Microbiol. 2021;19(4):241

Gut microbiota and our health

Depression and anxiety

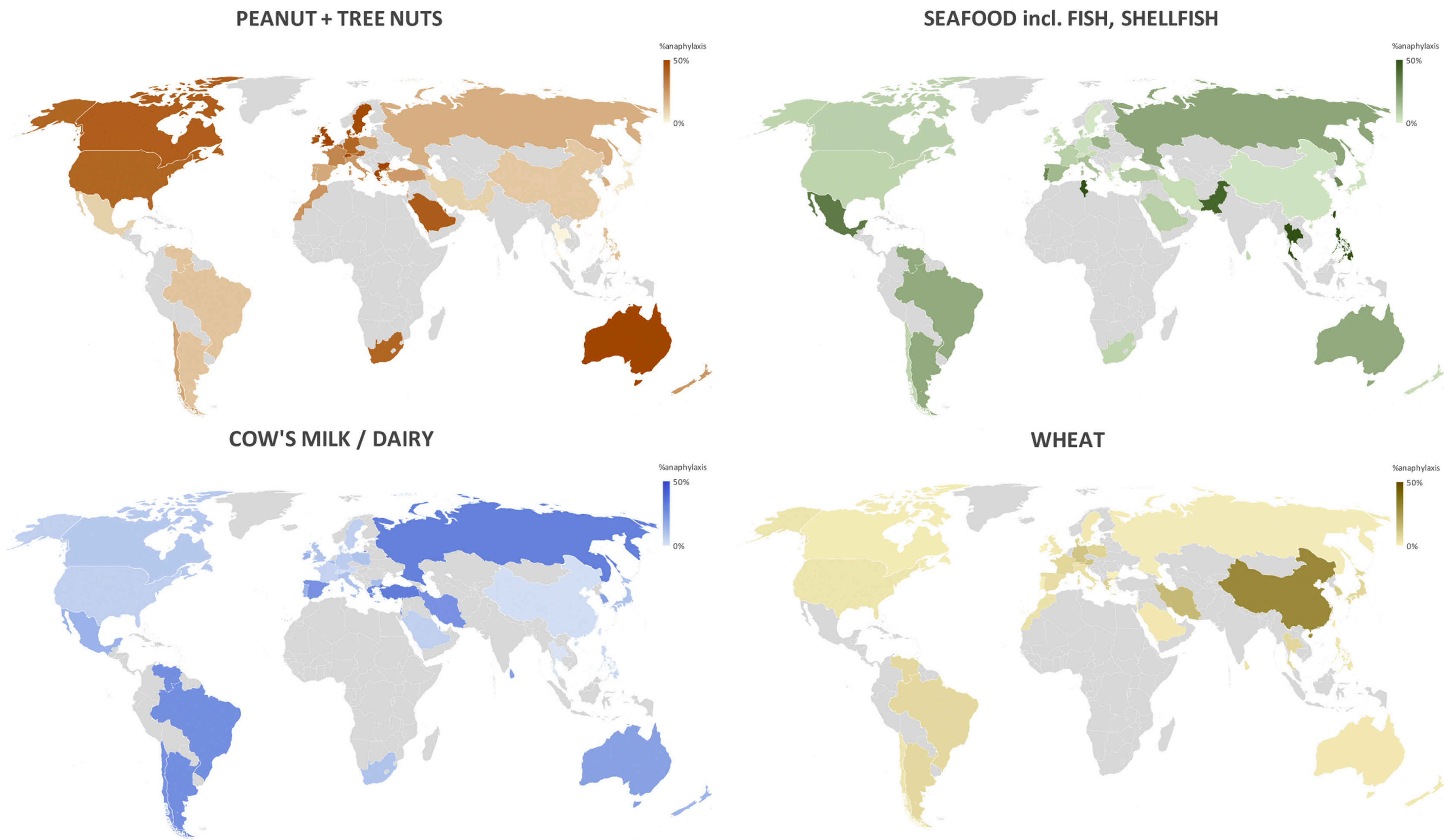


Moughnyeh MM, Brawner KM, et al. *J Surg Res*. 2021;266:33

Morais LH, Schreiber HL 4th, Mazmanian SK. Nat Rev Microbiol. 2021;19(4):241

Gut microbiota and our health

Allergic responses

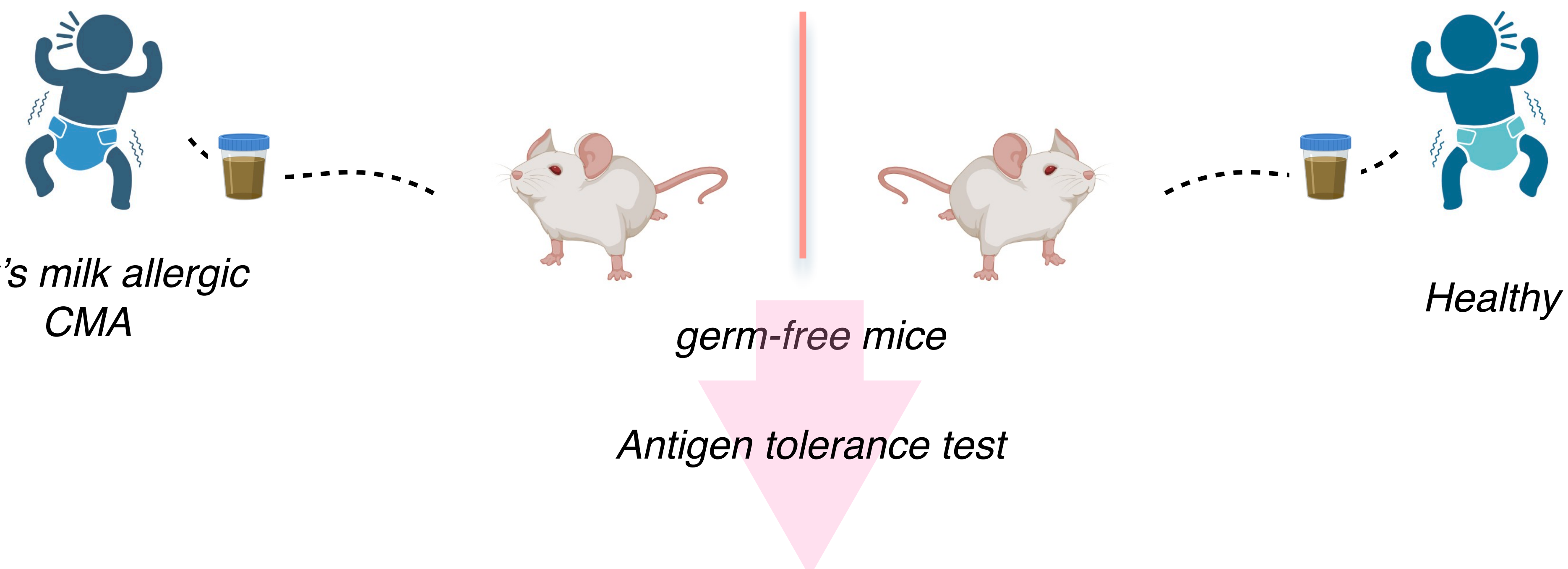


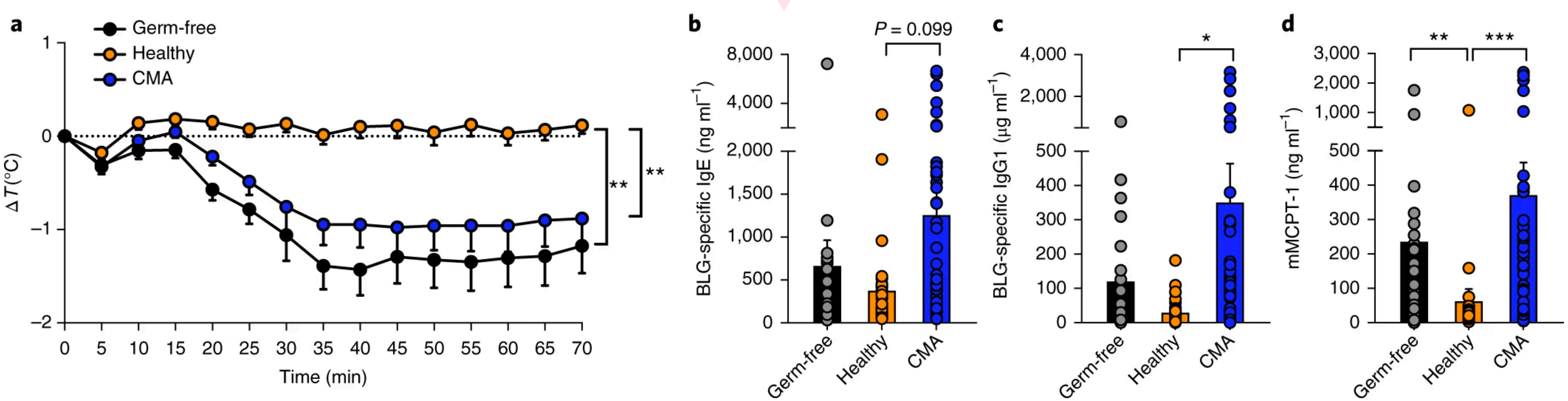
Genetic, environmental, social… Microbial !

Baseggio Conrado A, Patel N, Turner PJ. *J Allergy Clin Immunol.* 2021;148(6):1515

Gut microbiota and our health

Allergic responses



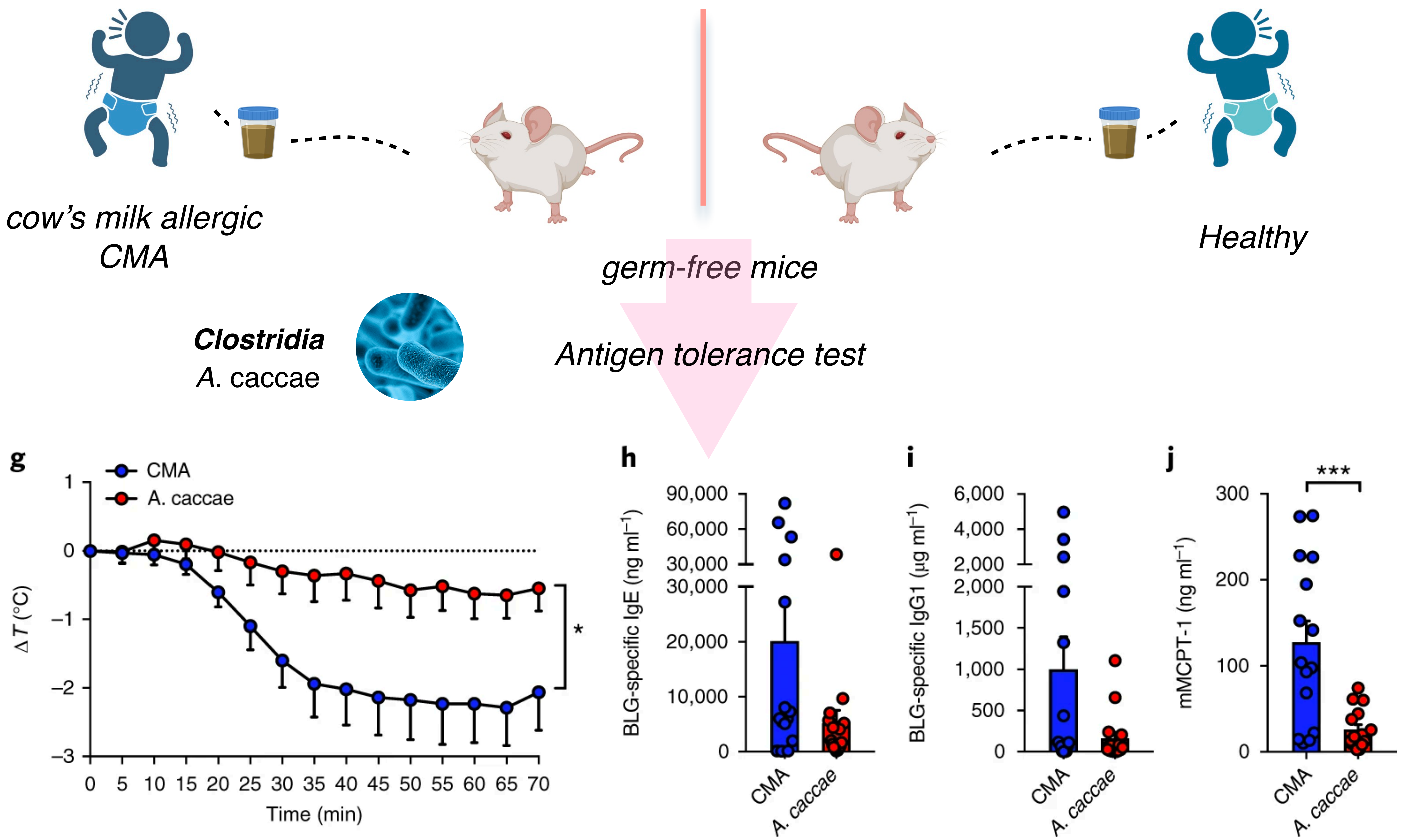


Healthy infants' gut microbiota associates with allergy prevention

Feehley T, Plunkett CH, Bao R, et al. *Nat Med.* 2019;25(3):448

Gut microbiota and our health

Allergic responses

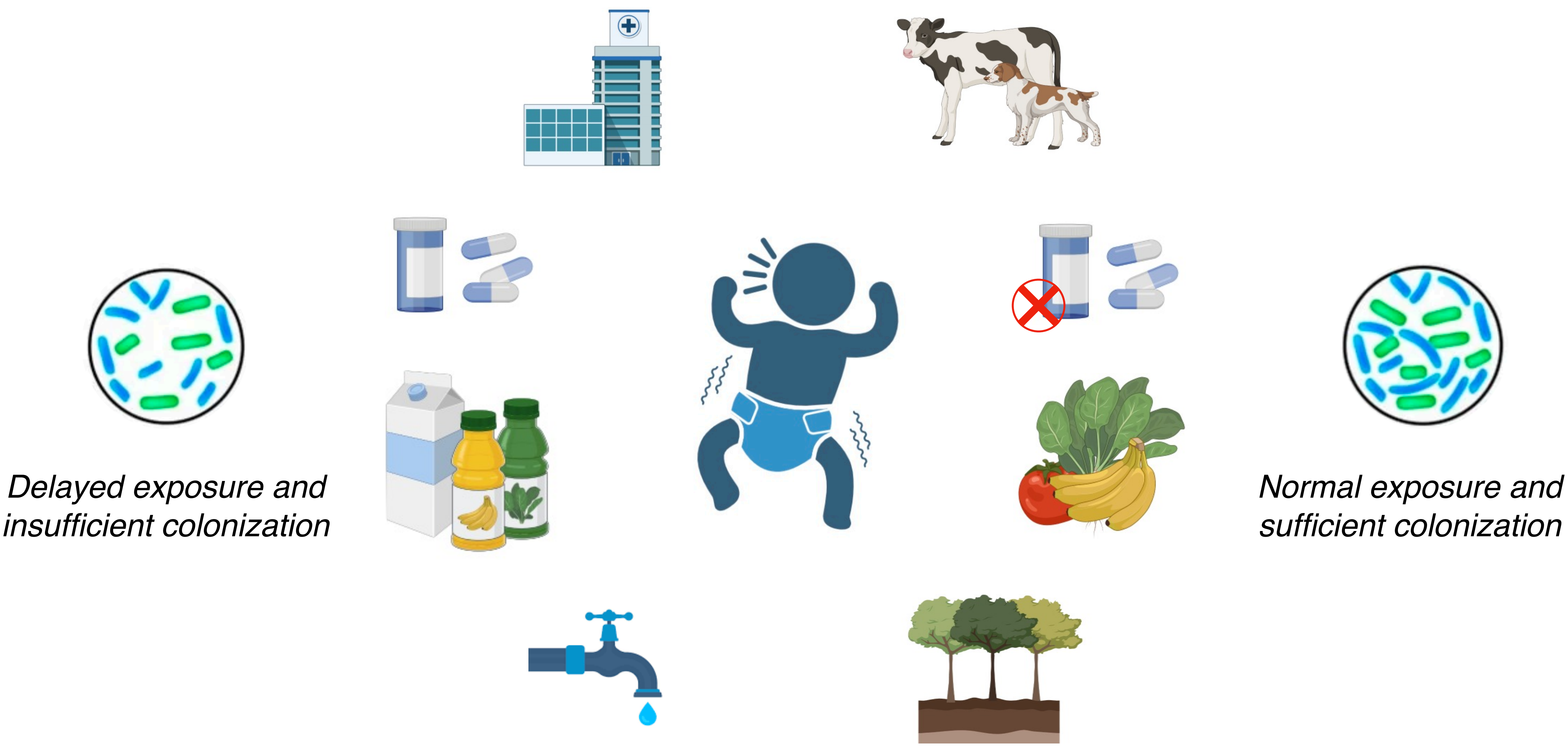


Clostridia-containing microbiota associates with allergy prevention

Stefka AT, Feehley T, Tripathi P, et al. *Proc Natl Acad Sci.* 2014;111(36):13145
Feehley T, Plunkett CH, Bao R, et al. *Nat Med.* 2019;25(3):448

Gut microbiota and our health

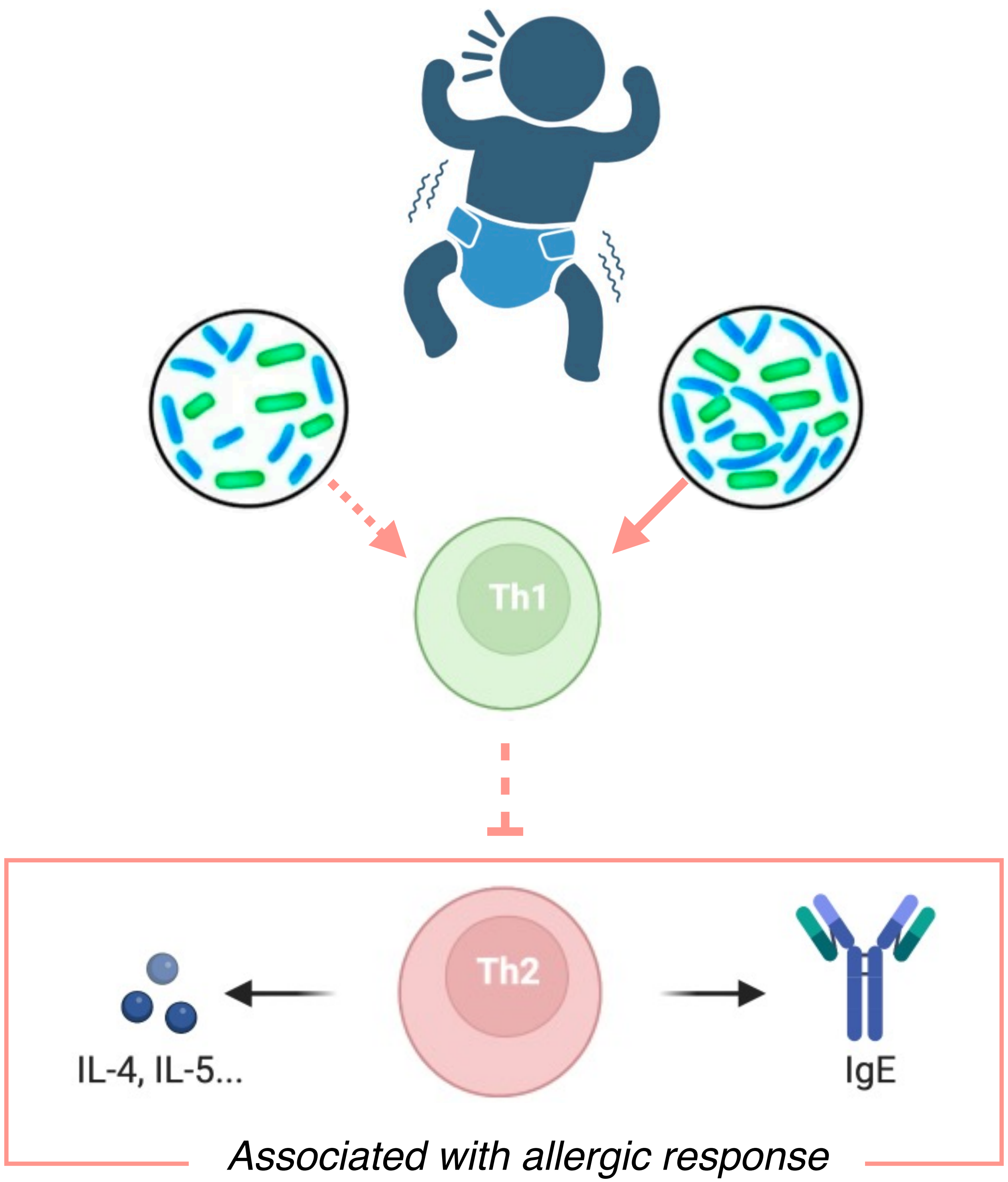
Hygiene hypothesis



Graham-Rowe D. Nature, 2011, 479(7374): S2-S4.

Gut microbiota and our health

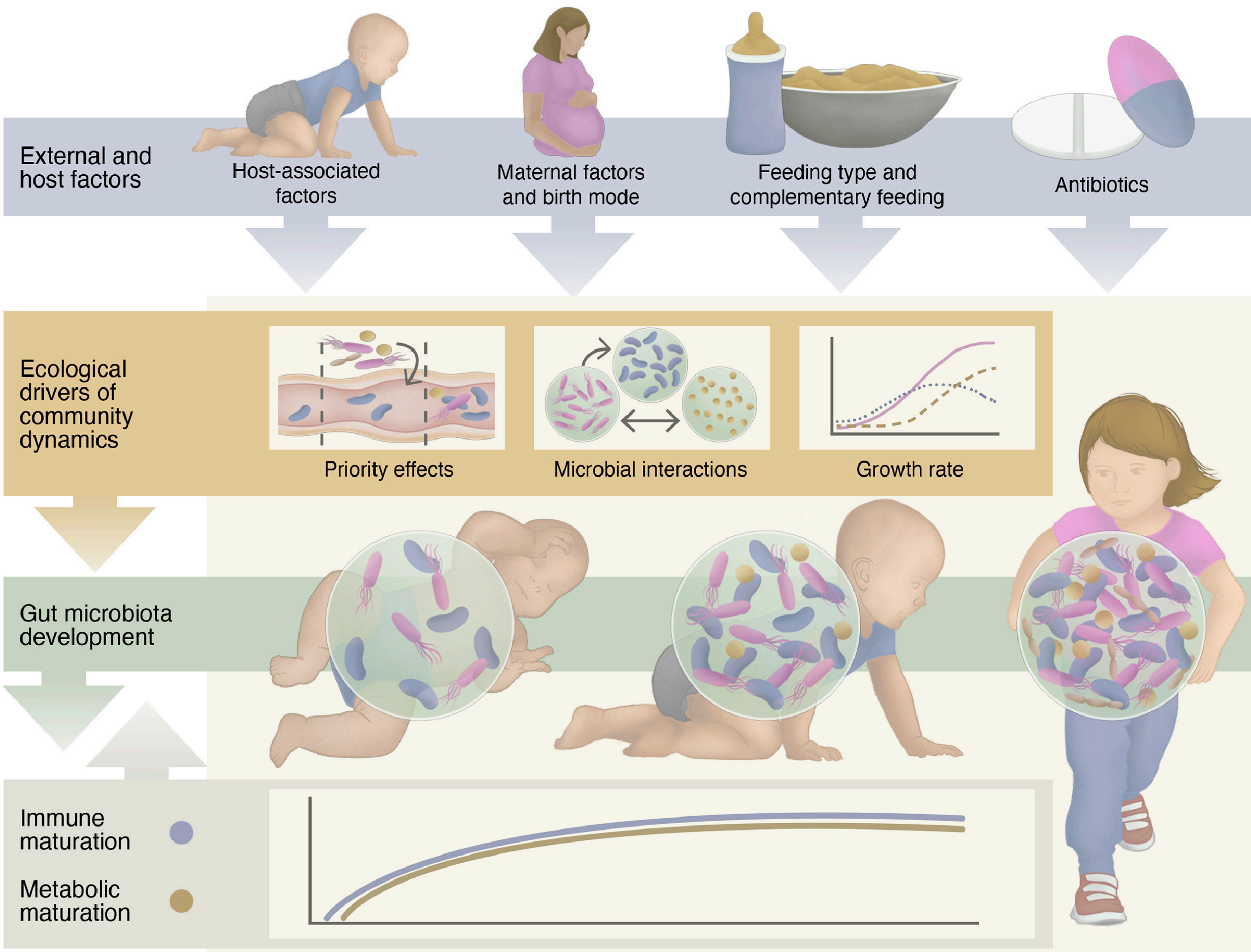
Hygiene hypothesis



Graham-Rowe D. Nature, 2011, 479(7374): S2-S4.

Gut microbiota and our health

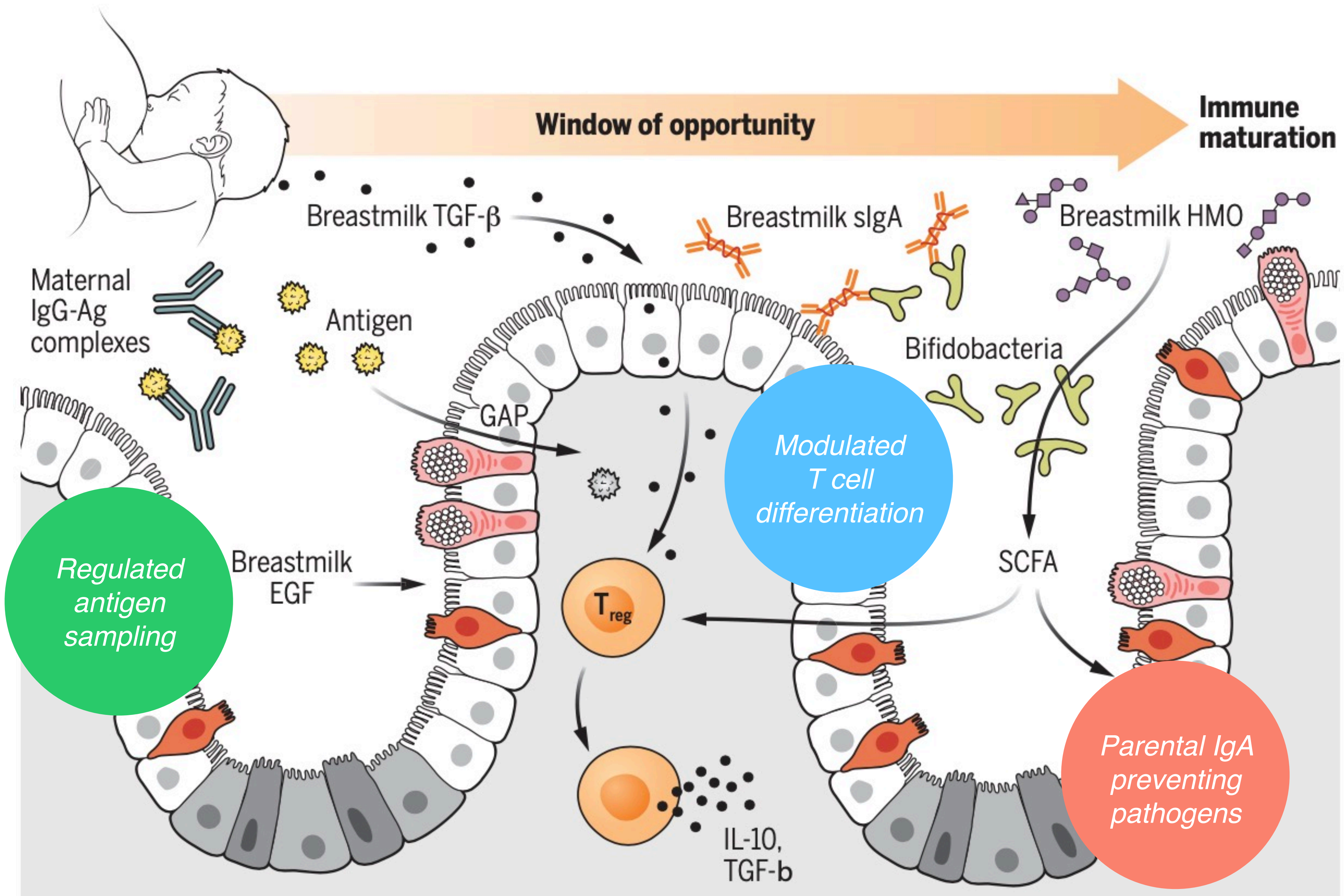
Maturation during infancy



Jian C, Carpén N, et al. EBioMedicine. 2021;69:103475.

Gut microbiota and our health

Maturation during infancy



Tolerance-promoting factors enabling a window of opportunity for intervention

Brodin P. *Science*. 2022;376(6596):945

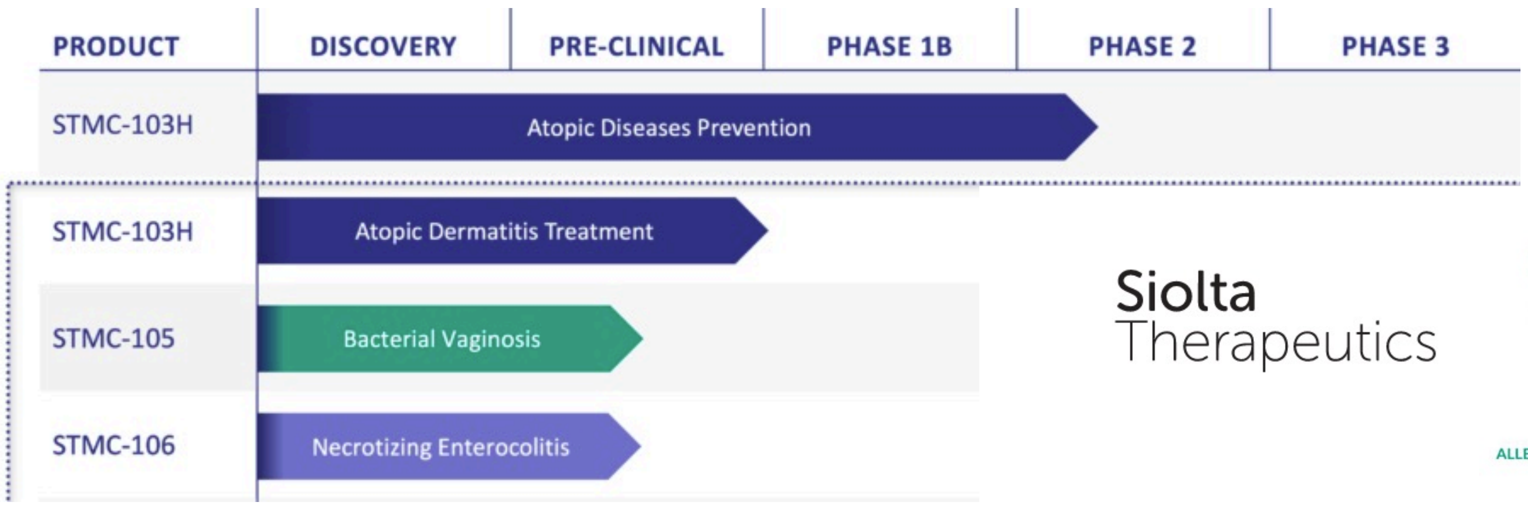
Manage, manipulate and design

better gut flora for better living

Targeting gut microbiota as bio-therapeutics

Early intervention

Combination of ***live bacteria*** *that are* ***isolated from healthy human microbiome*** *and manufactured as pharmaceutical biologics*



Siolta Therapeutics

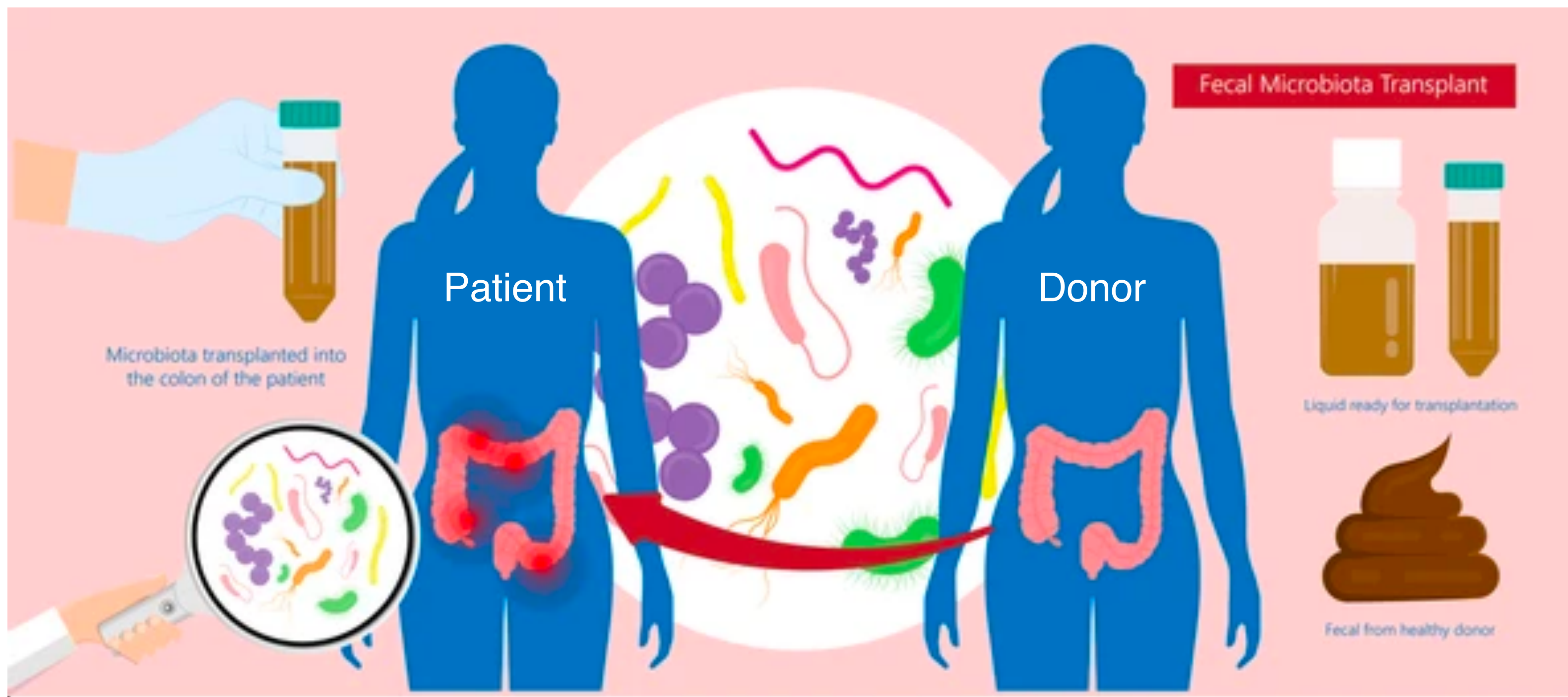
ALLERGIC DISEASE ONSET PREVENTION STUDY

allergic disease, bacterial vaginosis and necrotizing enterocolitis

Any methods/treatments for adults?

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)



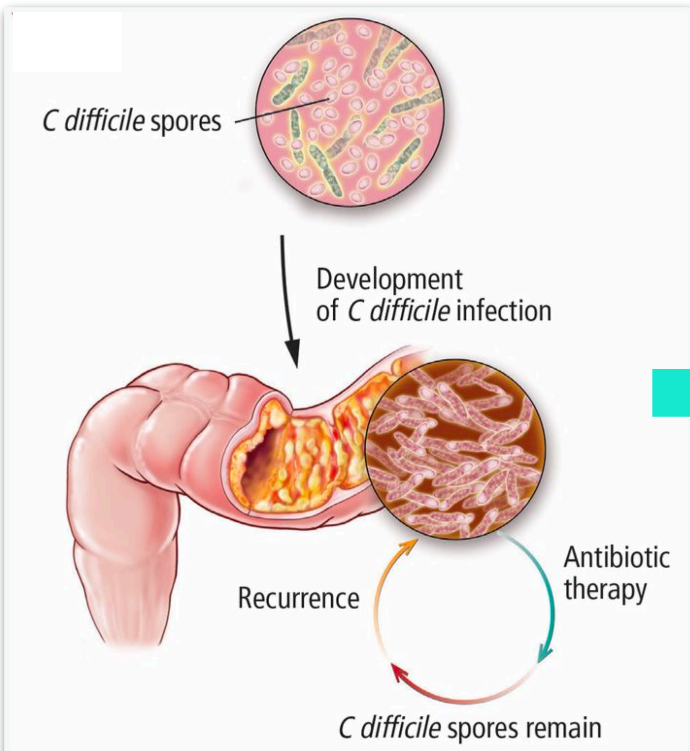
A well-established treatment for *Clostridioides difficile* infection

van Nood E, Vrieze A, Nieuwdorp M, et al. *N Engl J Med*. 2013;368(5):407

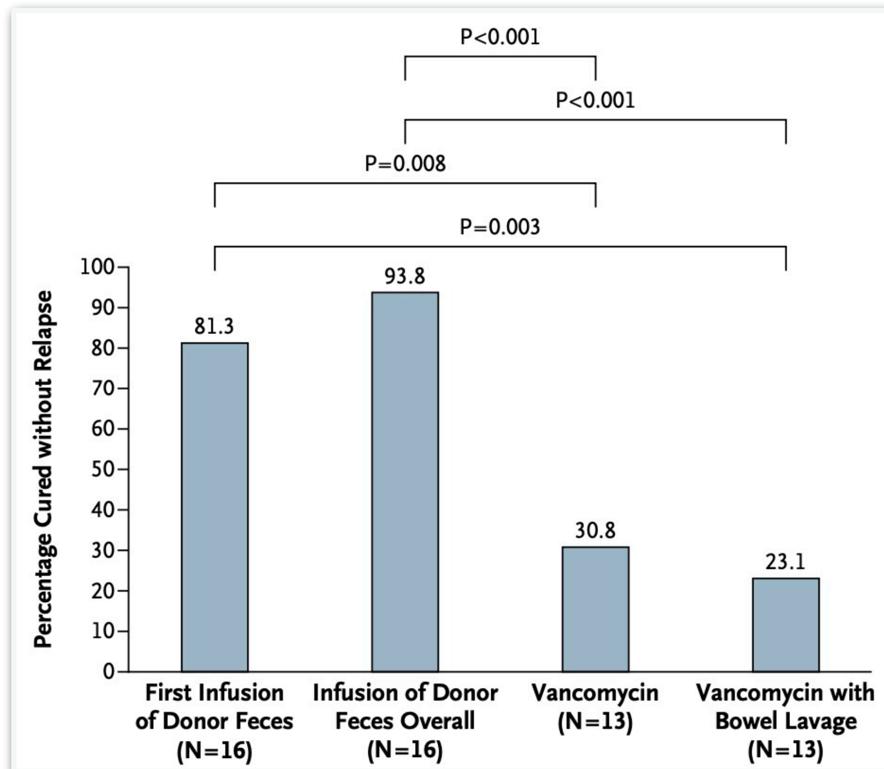
Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

A well-established treatment for *Clostridioides difficile* infection



Traditional antibiotic treatment fails to eradicate C. difficile



The infusion of donor feces was significantly more effective

van Nood E, Vrieze A, Nieuwdorp M, et al. *N Engl J Med.* 2013;368(5):407

How to carry out a fecal transplant?

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

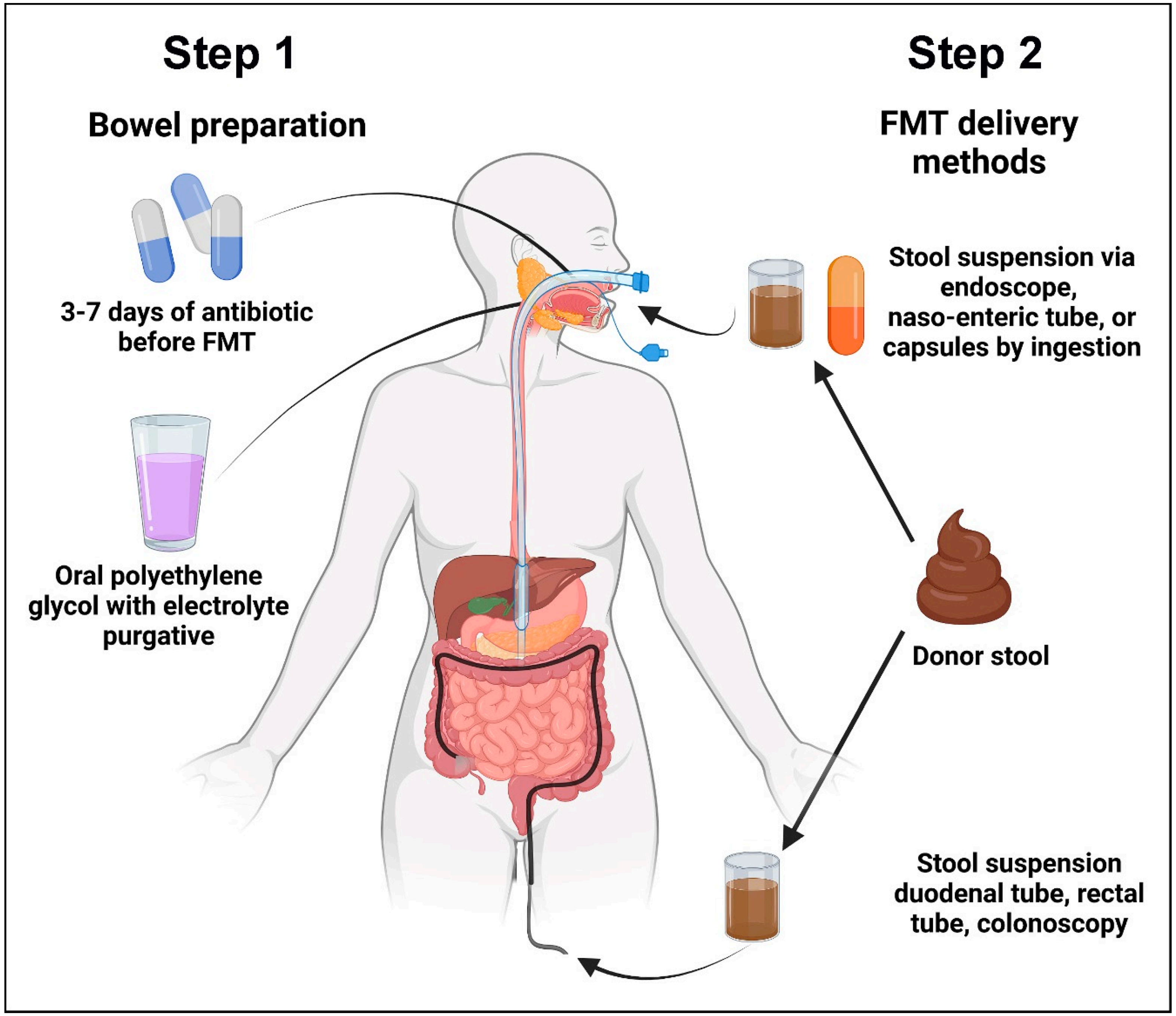
Capsules

Suspensions

Ooijevaar RE, Terveer EM, et al. Annu Rev Med. 2019;70:335

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)



Capsules

Suspensions

Ooijevaar RE, Terveer EM, et al. Annu Rev Med. 2019;70:335

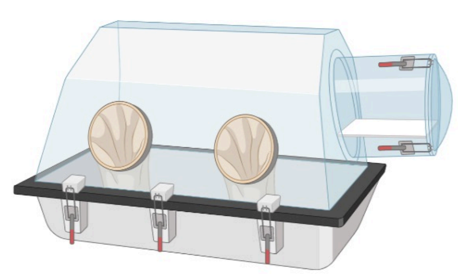
Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

Author	# patients	Mode and frequency of delivery	Outcome	Lesson Learned	
Rossen Gastro 2015	37	Nasoduodenal x 2	30% v. 20% clinical remission (SCAI≤2 + ≥1 decrease in Mayo endo)	Negative study, perhaps lower delivery preferable	☹️
Moayyedi Gastro 2015	70	Enema q week x 6 weeks	24% v. 5% remission (Mayo score ≤2, endo=0)	Superdonor effect	😀
Paramsothy Lancet 2017	85	Enema q day (M-F) x 8 weeks	27% v. 8% steroid-free clinical remission	Batched donors Numerous treatments	😀
Costello JAMA 2019	73	Colonoscopy + enema x 2	32% v. 9% achieved steroid free remission	Anaerobic preparation	😀
Haifer Lancet 2021	35	Abx + Capsule FMT	53% v. 15% steroid-free clinical remission	Antibiotic pretreatment	😀

The routes of delivery matters as contradictory outcomes been seen in the FMT attempts to cure ulcerative colitis (IBD)

A robust delivery frequency



Anaerobic preparation

Antibiotics pretreatment

Ooijevaar RE, Terveer EM, et al. Annu Rev Med. 2019;70:335

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

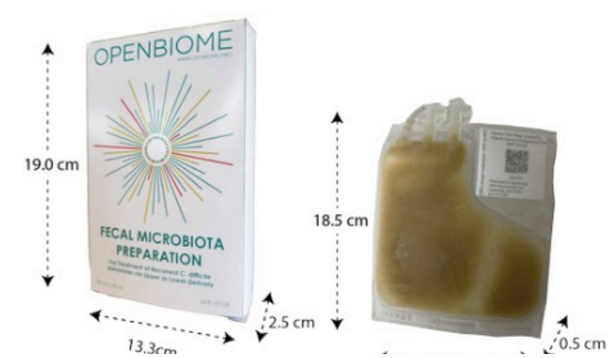
Stool banks

*high-quality, **ready-to-use** donor feces suspensions*
*from a **prescreened, well-defined** donor pool*

MTP-101LR

Manufacturer: University of Minnesota
Modality: Lower Delivery (colonoscopy, sigmoidoscopy, or enema) or Upper Delivery (Nasoenteric/gastric tube or EGD)
Pricing: $1,695 per dose (1 cryobag)

Information on storage, thawing, and administering MTP-101LR is located in our Clinician's Checklist.

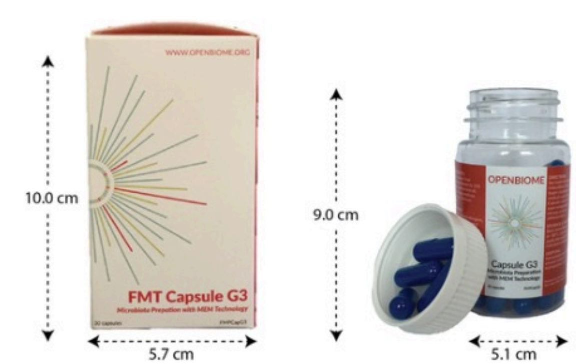


FMP CapDE

Manufacturer: OpenBiome
Modality: Oral administration
Pricing: $2,050 per dose (1 bottle=30 capsules)
**Includes 2 inert test capsules to assess patient's swallowing abilities*

Physician orientation is required before first order. Information on storage, thawing, and administering FMPCapDE is located in our Clinician's Checklist.

OPENBIOME



Ooijevaar RE, Terveer EM, et al. Annu Rev Med. 2019;70:335

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

serious adverse events

abdominal pain, followed by diarrhea

Oct. 30, 2019, 5:02 PM EDT

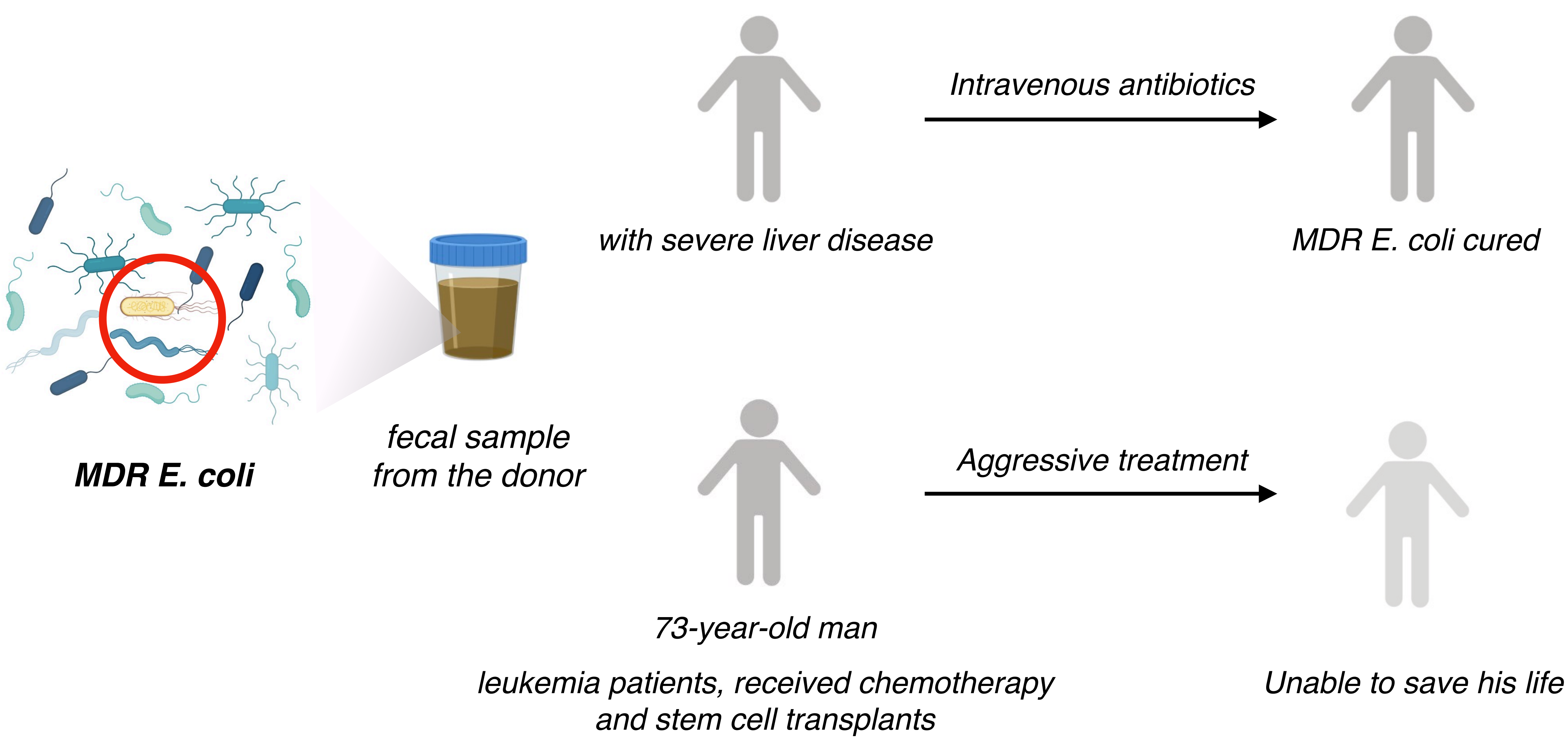
https://www.nbcnews.com/health/health-news/fecal-transplant-led-patient-s-death-here-s-what-happened-n1074081

Gut microbiota, new target for bio-therapeutics

Fecal microbiota transplant (FMT)

A fecal transplant led to a patient's death.

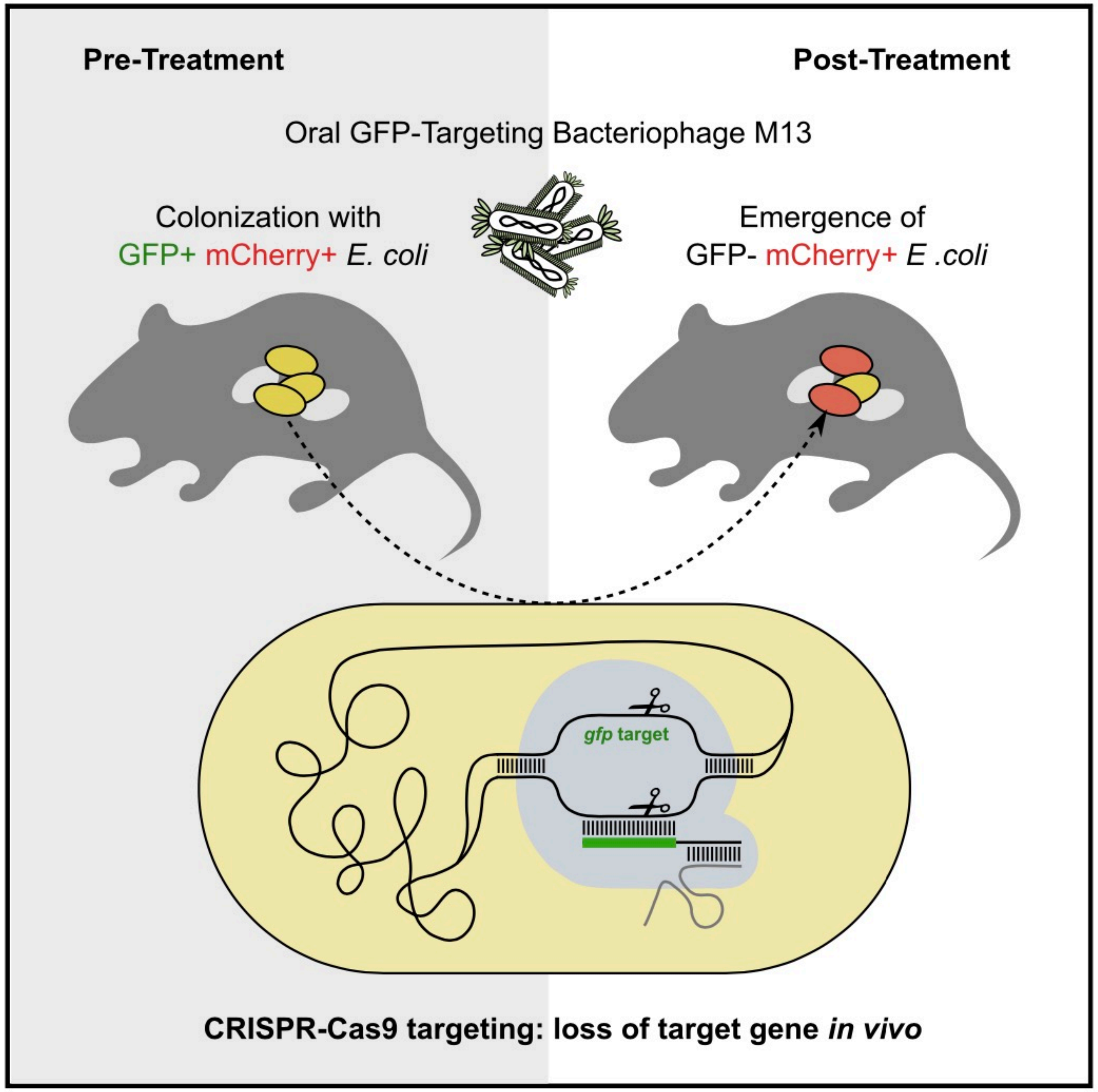
Oct. 30, 2019, 5:02 PM EDT



https://www.nbcnews.com/health/health-news/fecal-transplant-led-patient-s-death-here-s-what-happened-n1074081

Gut microbiota, new target for bio-therapeutics

Precision engineering



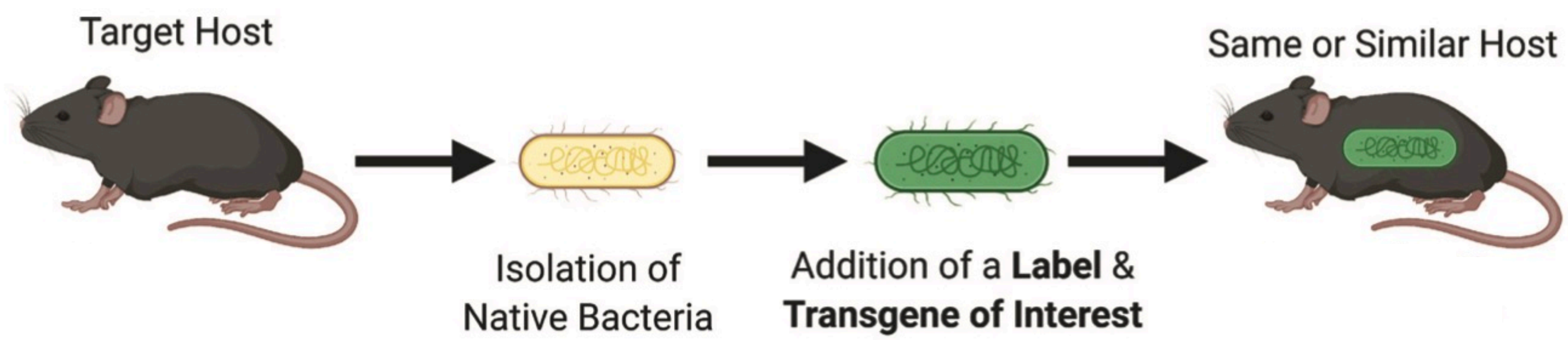
First example of precision editing of microbiome inside GI tract

(Though mice colonized with a single E. coli strain)

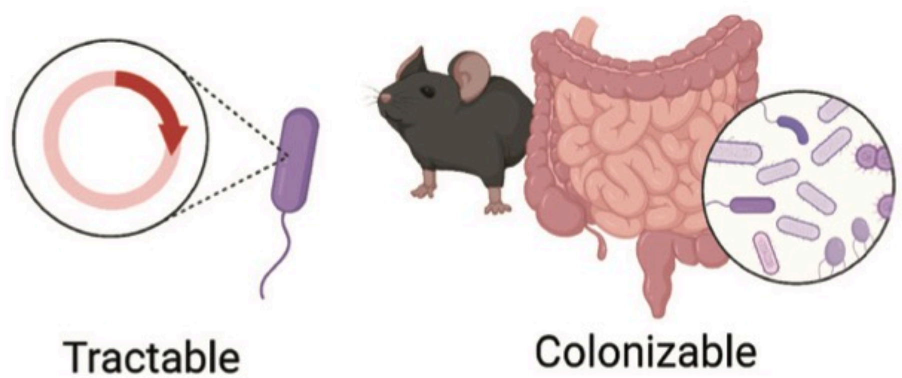
Lam KN, Spanogiannopoulos P, Soto-Perez P, et al. *Cell Rep.* 2021;37(5):109930.

Gut microbiota, new target for bio-therapeutics

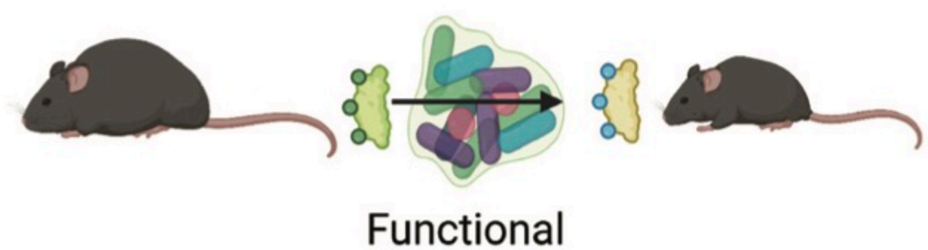
Precision engineering



Native E. coli from the murine and human gut can be engineered for transgene delivery



Bile salt hydrolase



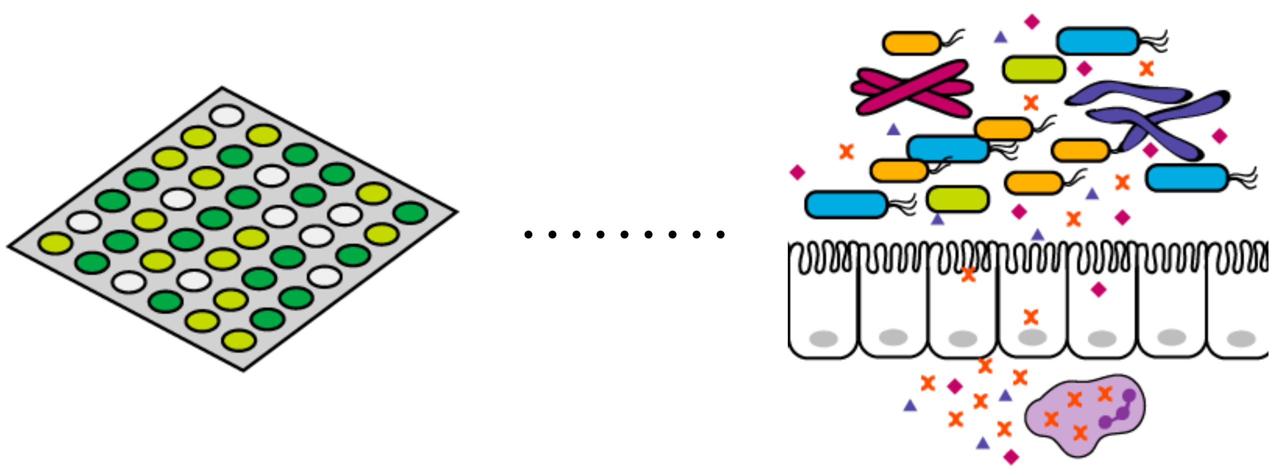
Single oral treatment in non-sterile conditions

Persistent improvement of bile acid metabolism

Russell BJ, Brown SD, Siguenza N, et al. *Cell*. 2022;185(17):3263

Gut microbiota, new target for bio-therapeutics

Modeling and designing

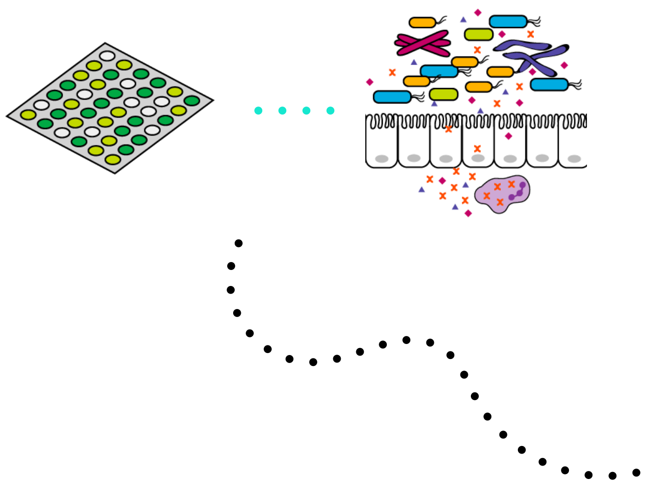


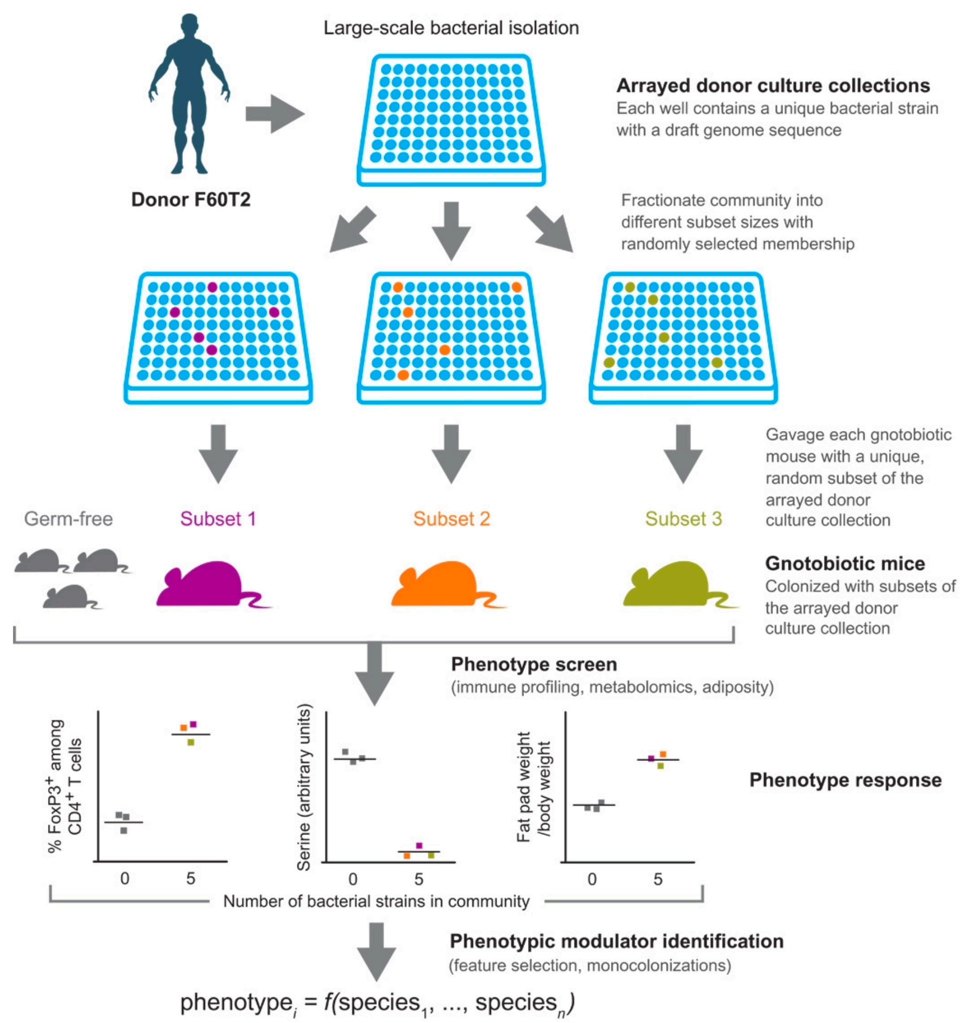
Can we design and synthesize a defined community?

that mimics phenotypes of a healthy native microbiota

Gut microbiota, new target for bio-therapeutics

Modeling and designing



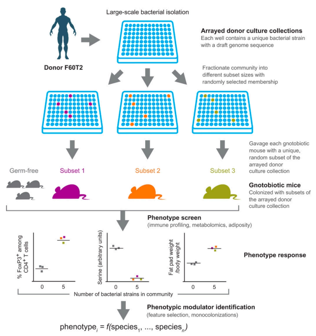


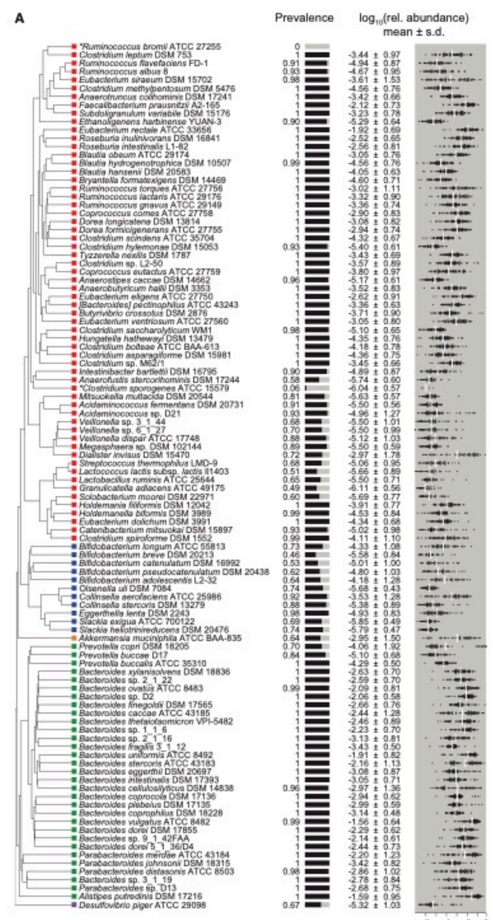
From late 2000s, separated low-complexity (<20 strains) gut microbiota have been used to study specific impacts on host

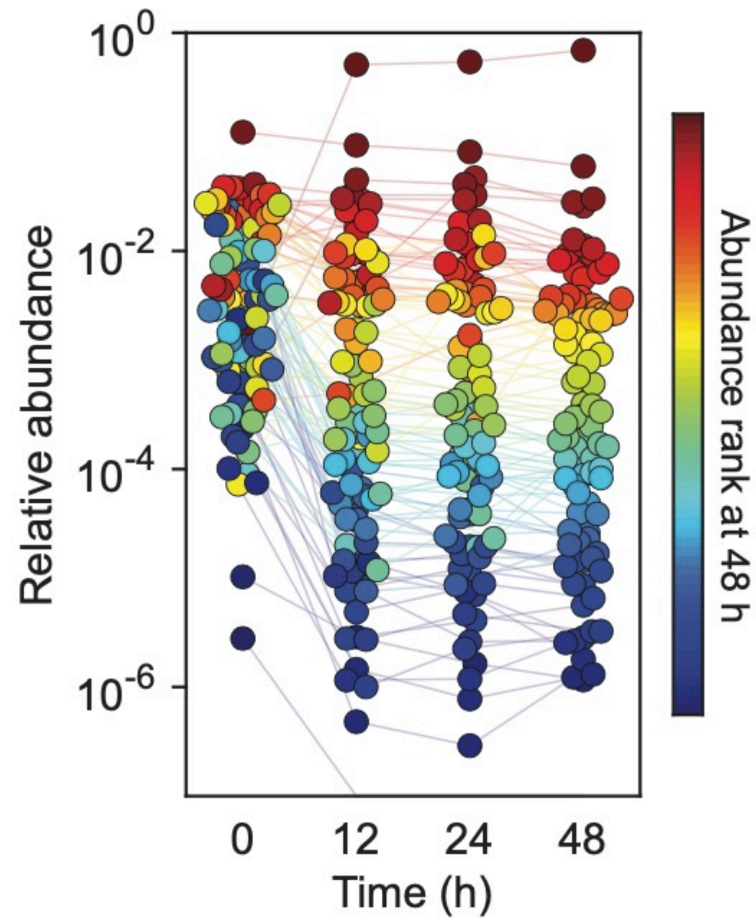
Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JI. *Sci Transl Med*. 2014;6(220):220

Gut microbiota, new target for bio-therapeutics

Modeling and designing







A defined community (stains=104) phenotypically similar to human fecal community

Build your own gut microbiota!

Cheng AG, Ho PY, Aranda-Díaz A, et al. *Cell*. 2022;185(19):3617

Gut microbiota, new target for bio-therapeutics

Daily care with healthy lifestyle

Be a good gardener if you want it flourish

Gut microbiota, new target for bio-therapeutics

Daily care with healthy lifestyle

Probiotics in fermented food

Probiotics supplements

One take-home massage…


Thank you!