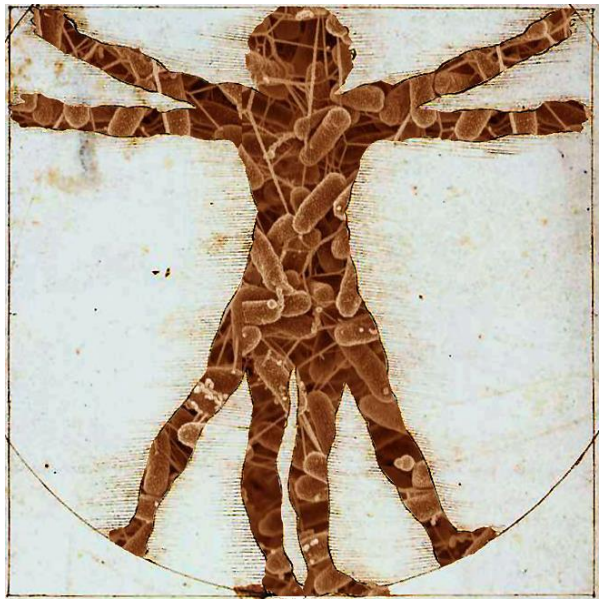
Microbiota and obesogens: environmental regulators of fat storage



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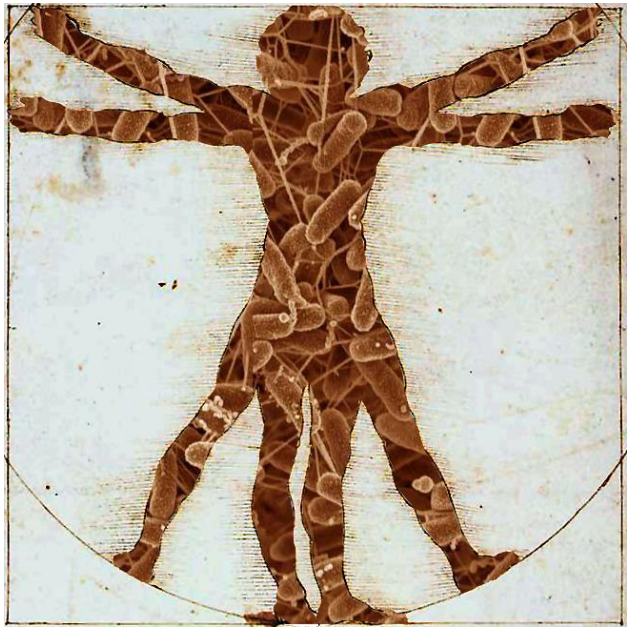


Seminar outline

1. Introduction to the microbiota
2. Microbiota as environmental factors in obesity
3. Obesogens as environmental factors in obesity

Seeing and understanding the unseen

Antonie van Leewenhoek

1620's: Described differences between microbes associated with teeth & feces during health & disease.

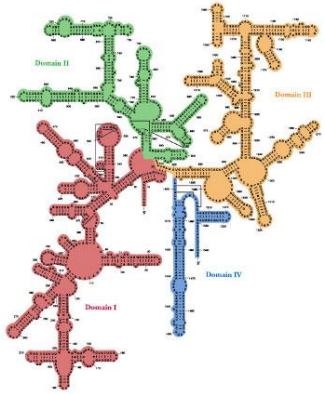
Louis Pasteur

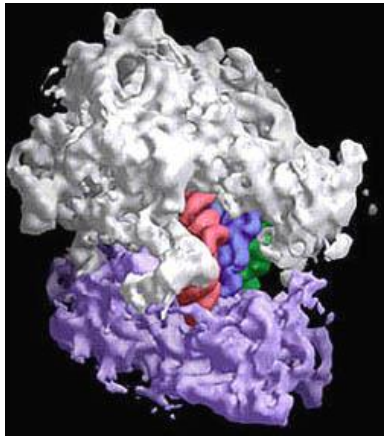
1850's: Helps establish the "germ theory" of disease.

1885: Predicts that germ-free animals can not survive without "common microorganisms".

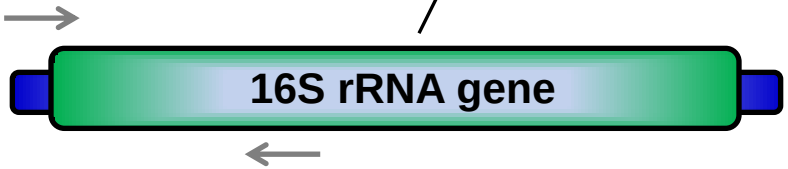
Culture-independent analysis of bacterial communities using 16S rRNA gene sequencing

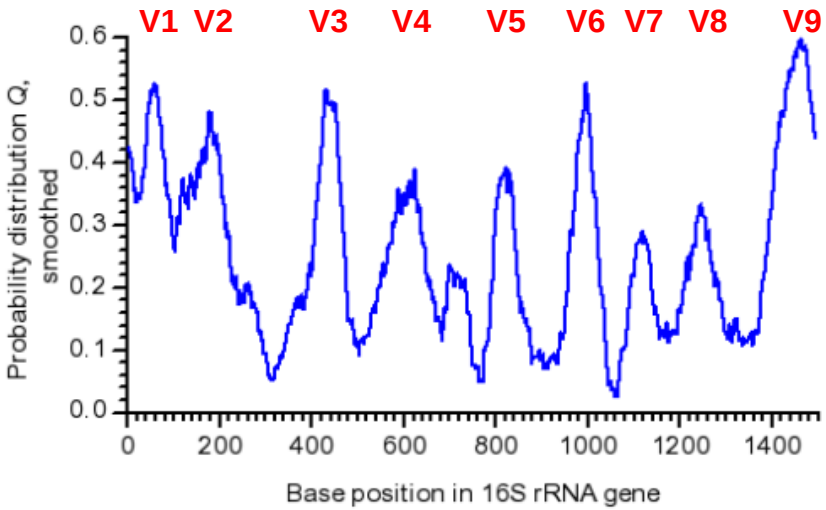
16S rRNA



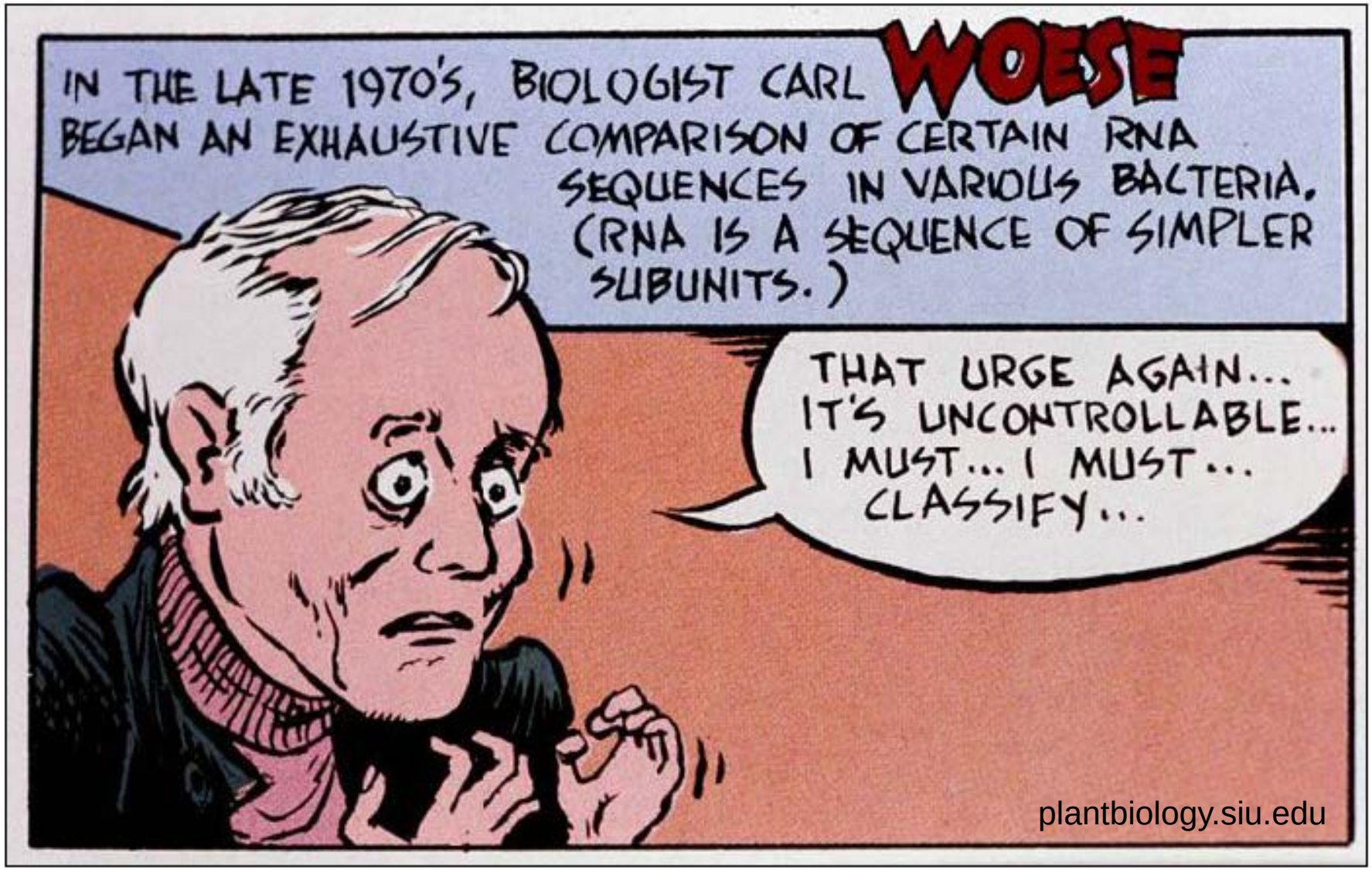


16S SSU rRNA

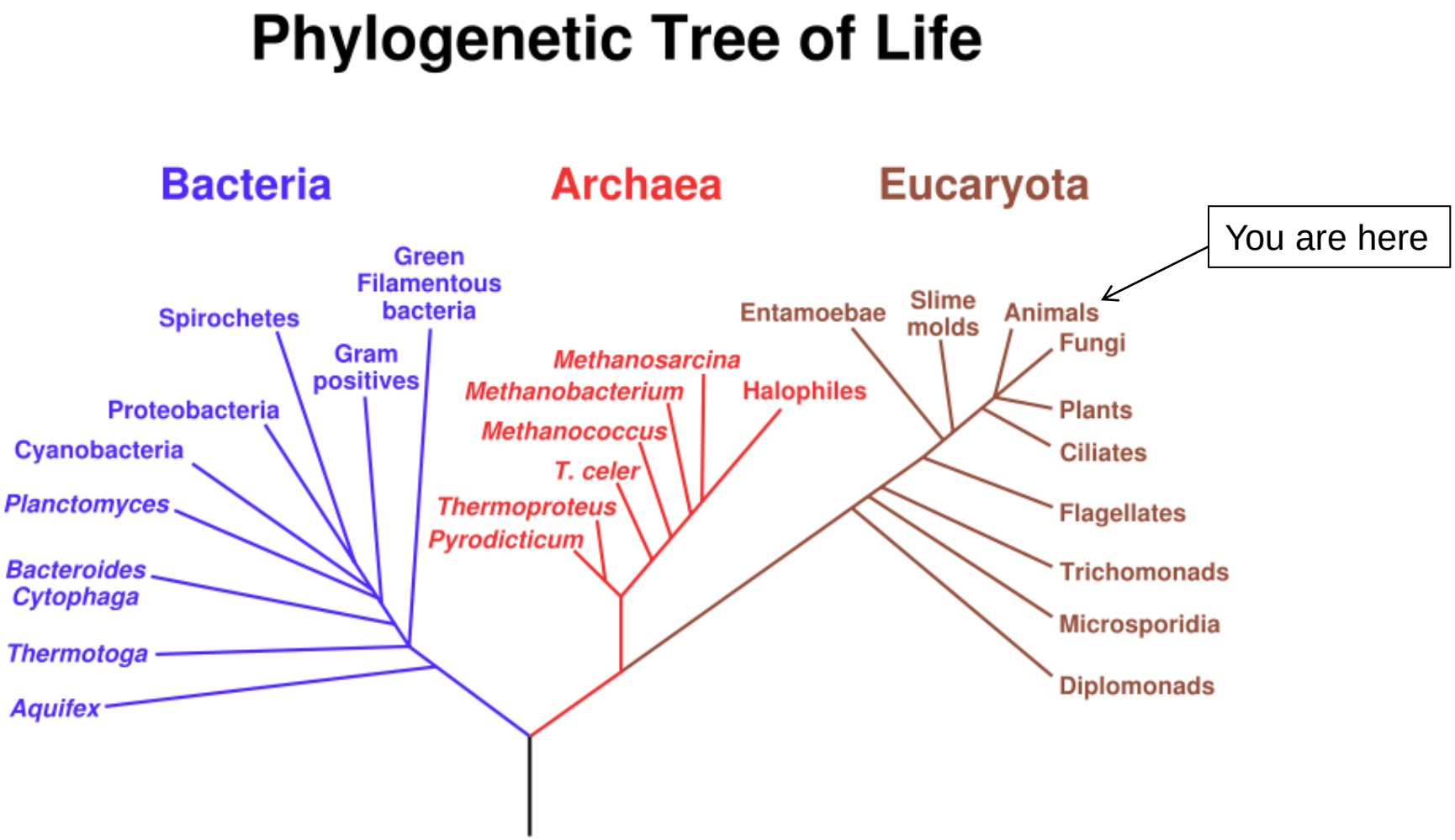




Culture-independent analysis of bacterial communities using 16S rRNA gene sequencing



Phylogenetic analysis of 16S rRNA genes revealed 3 domains and a vast unappreciated diversity of microbial life



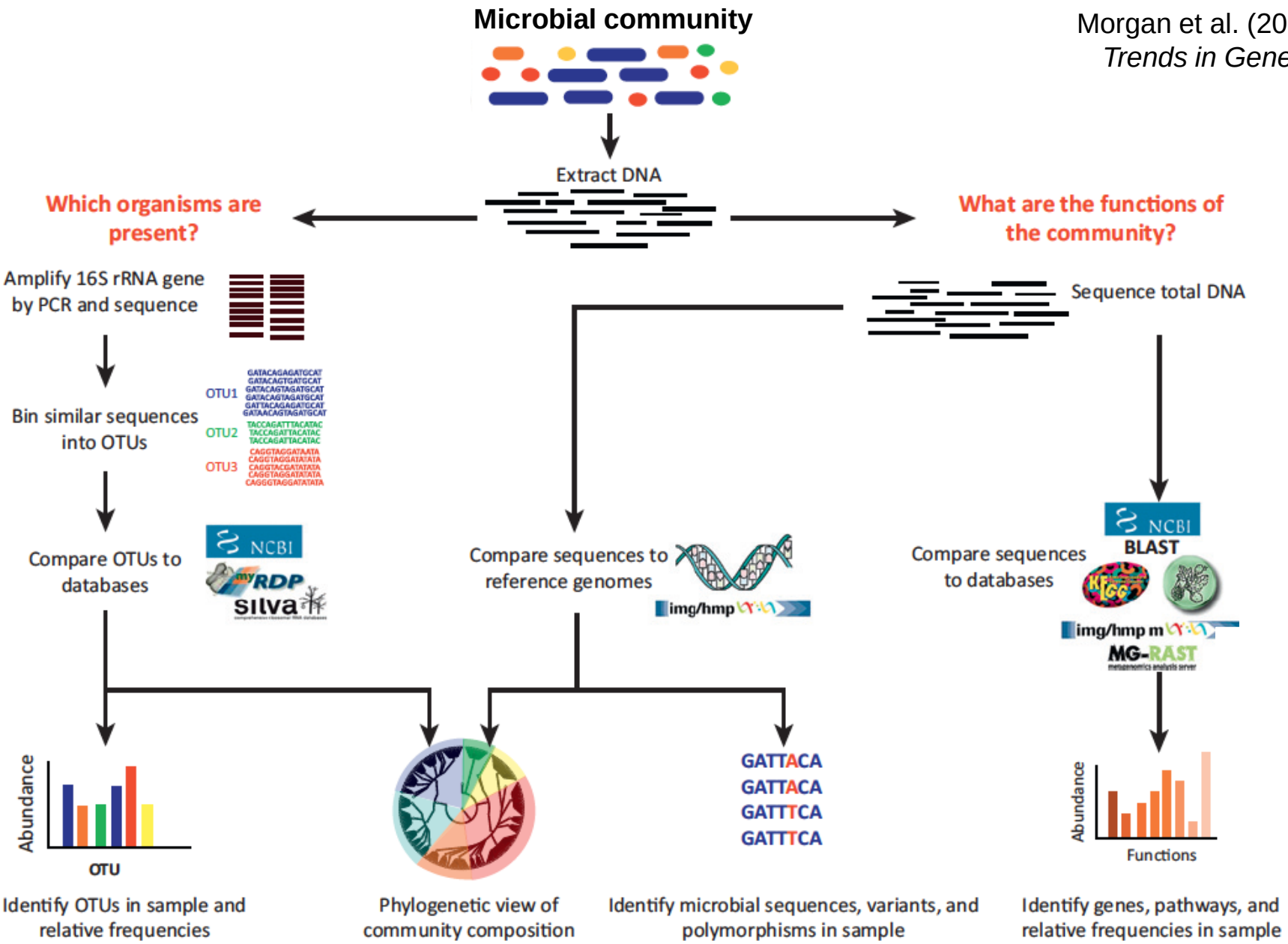
Carl Woese

Glossary

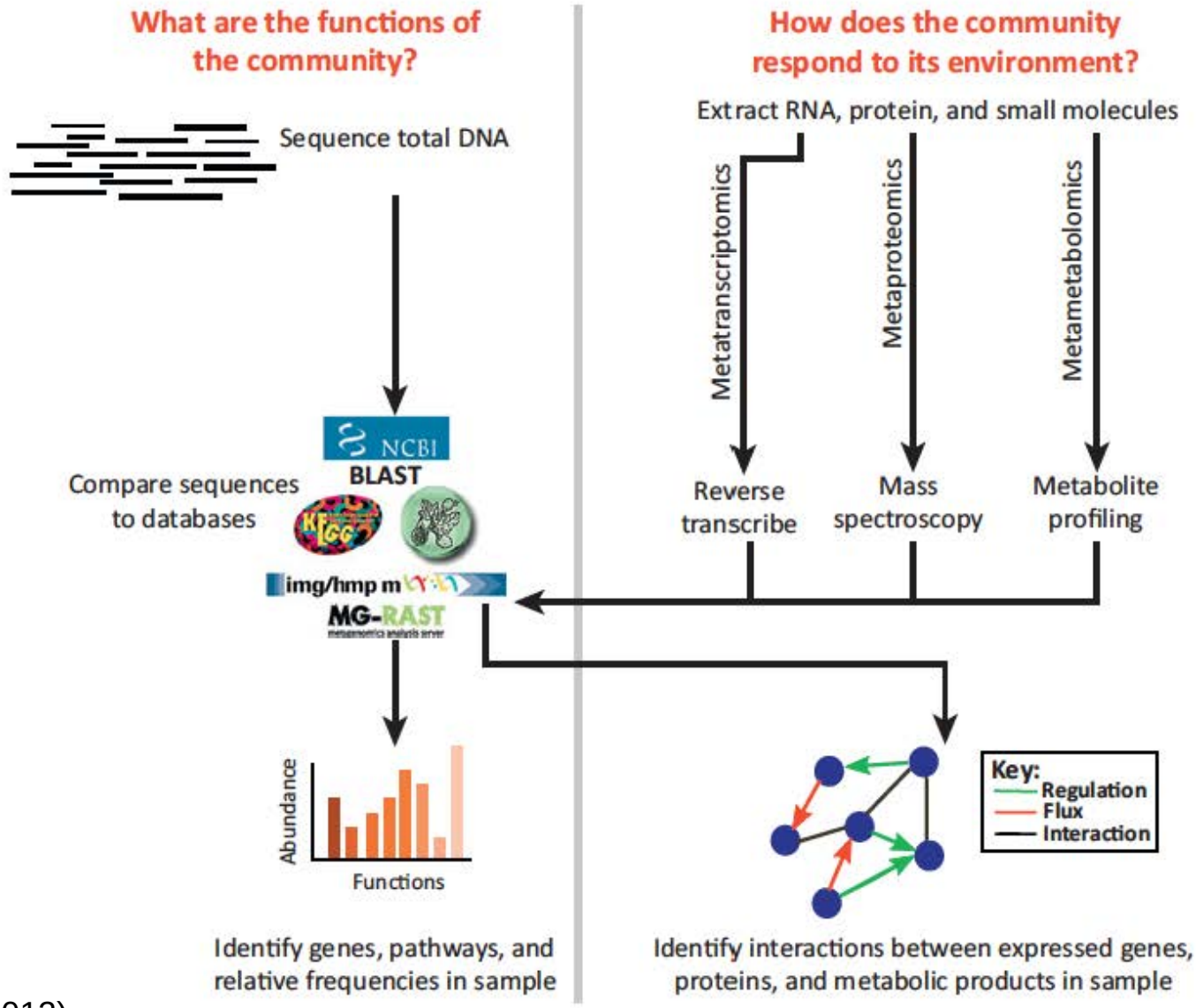
- **Microbiota / microflora**: the microorganisms of a particular site, habitat, or period of time.
- **Microbiome**: the collective genomes of the microorganisms within a microbiota.
- **Germ-free / axenic**: of a culture that is free from living organisms other than the host species.
- **Gnotobiotic**: of an environment for rearing organisms in which all the microorganisms are either excluded or known.

Current techniques for microbiome profiling

Morgan et al. (2012)
Trends in Genetics

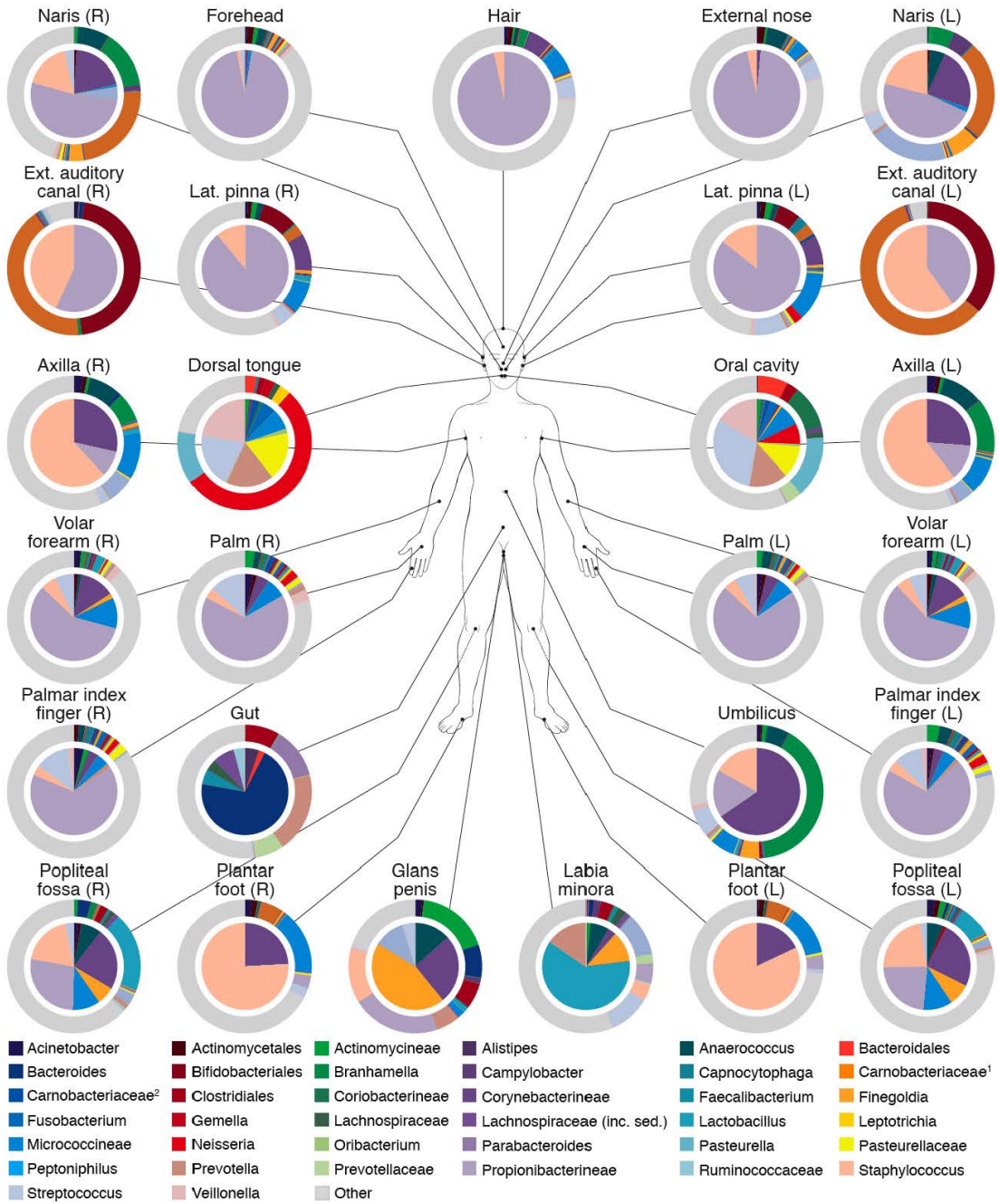


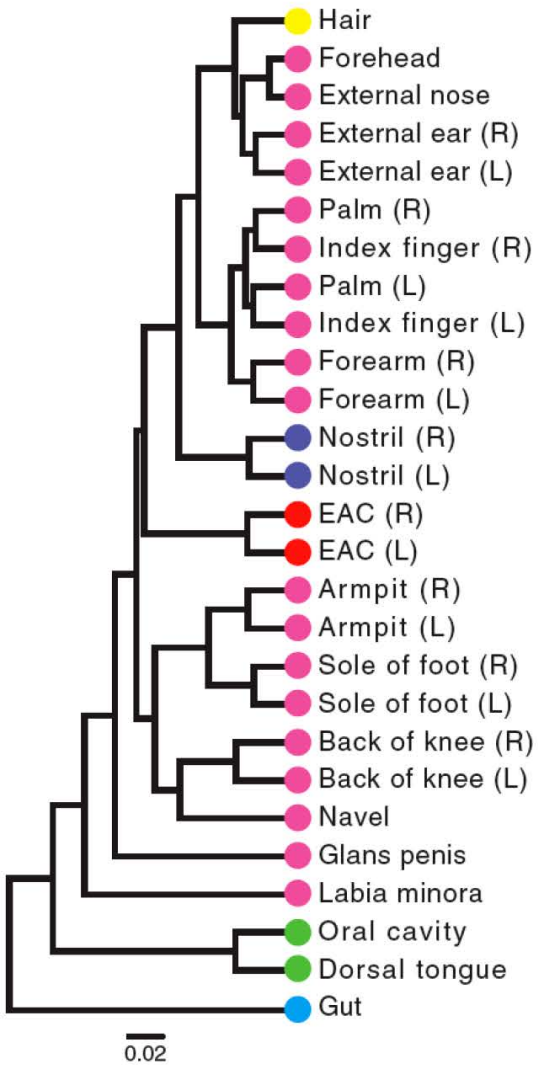
Current techniques for microbiome profiling



Morgan et al. (2012)
Trends in Genetics

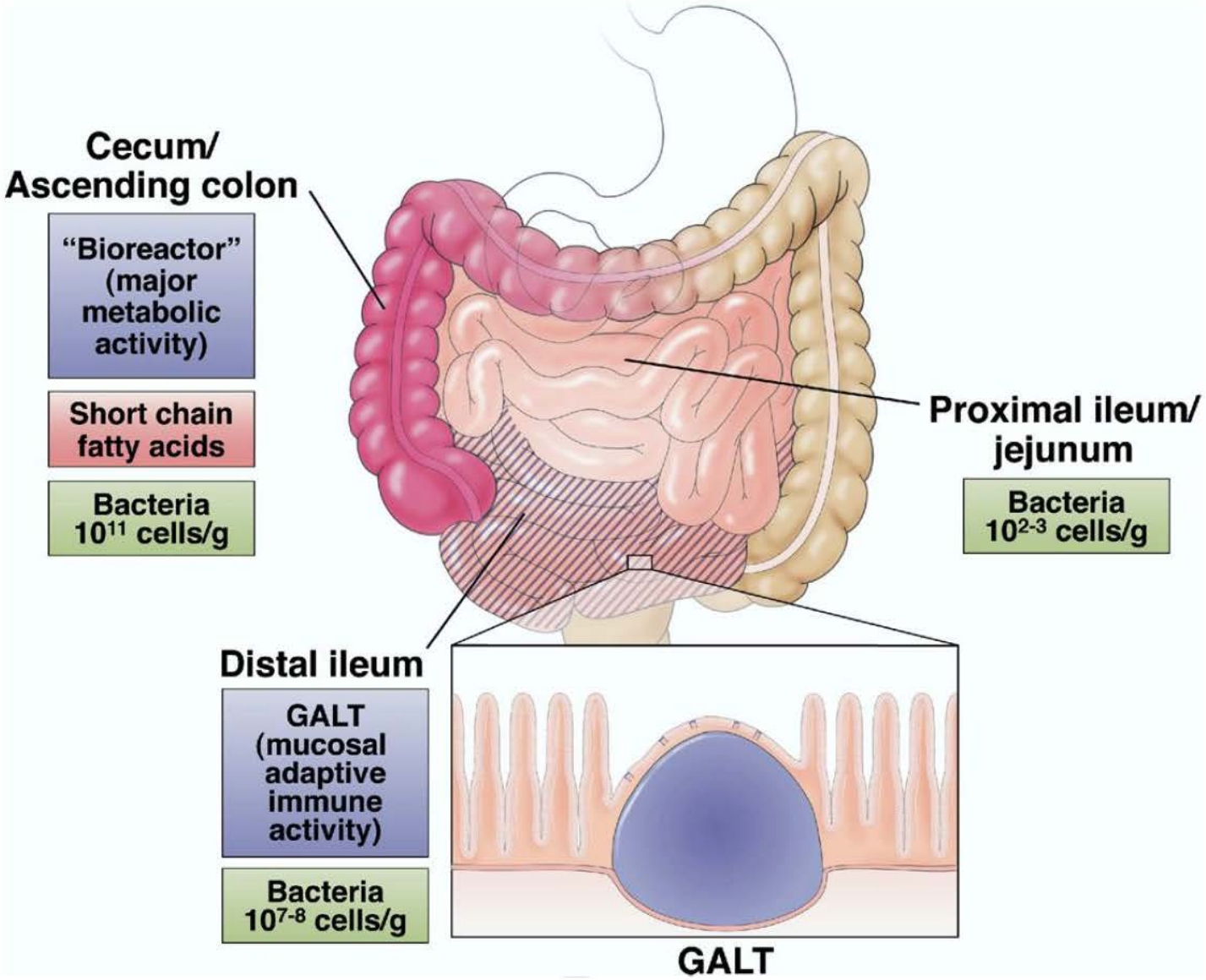
Different body habitats harbor distinct bacterial communities





Costello et al. (2009) *Science*

Sites of preferred microbial interaction in the gut



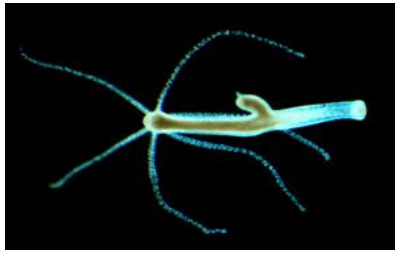
Neish (2009) *Gastroenterology*

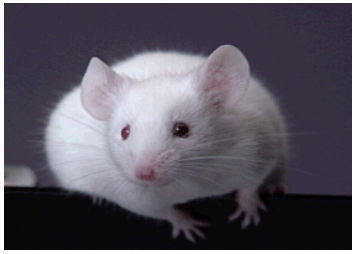
Microbiome profiling reveals membership and physiologic potential of host-associated microbial communities

<u>MetaHIT (EU)</u> Qin et al. (2010) *Nature*
124 European subjects, fecal samples only
Identified 1150 prevalent bacterial species and 3.3 million genes
Only 75 species and 294K genes shared by >50% of subjects!

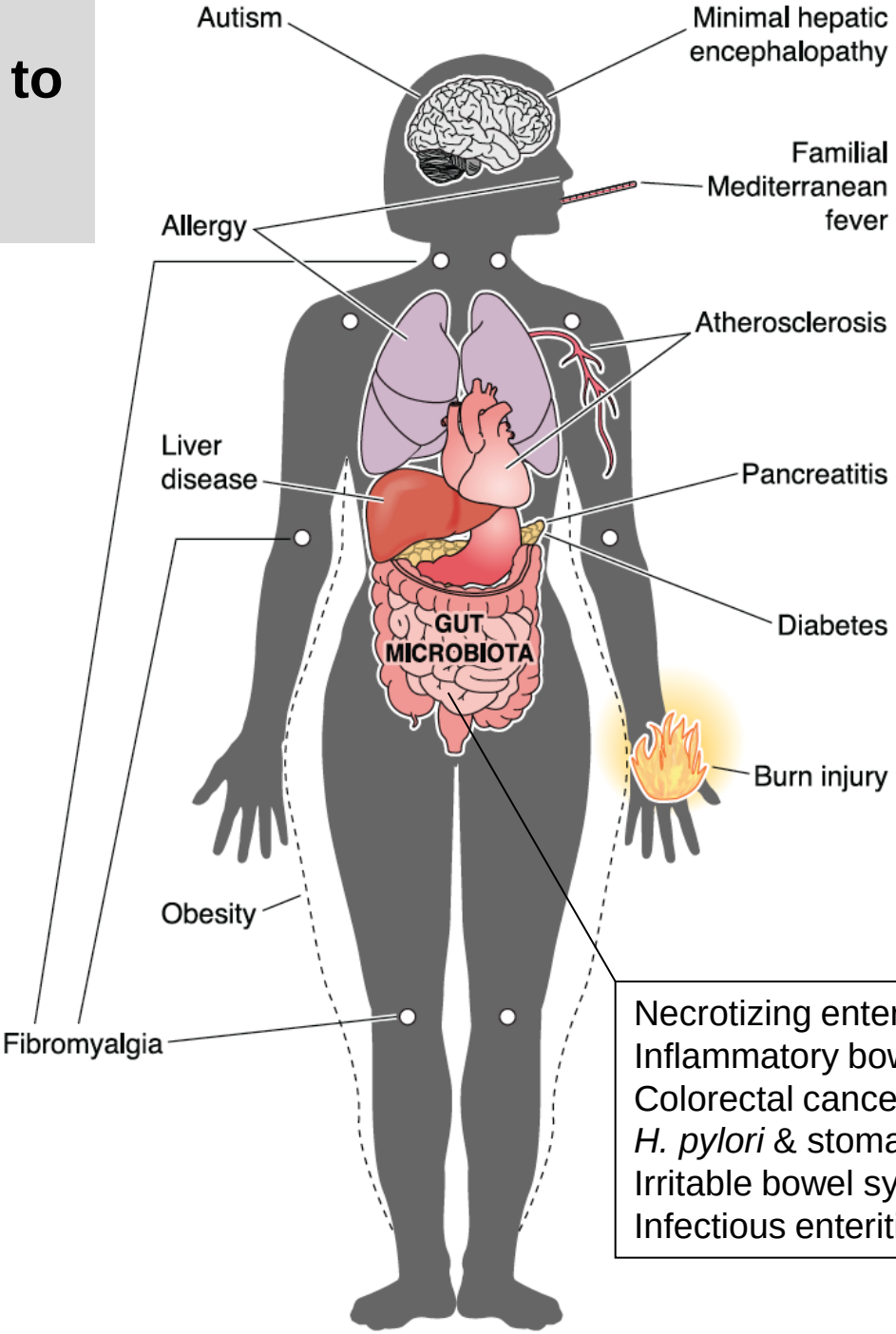
<u>Human Microbiome Project (NIH)</u> HMP (2012) *Nature*
250 <u>healthy</u> American subjects, 15-18 body sites
Identified >35K prevalent bacterial species and 10 million genes

<u>Many more studies of microbiomes in humans and other animals</u>





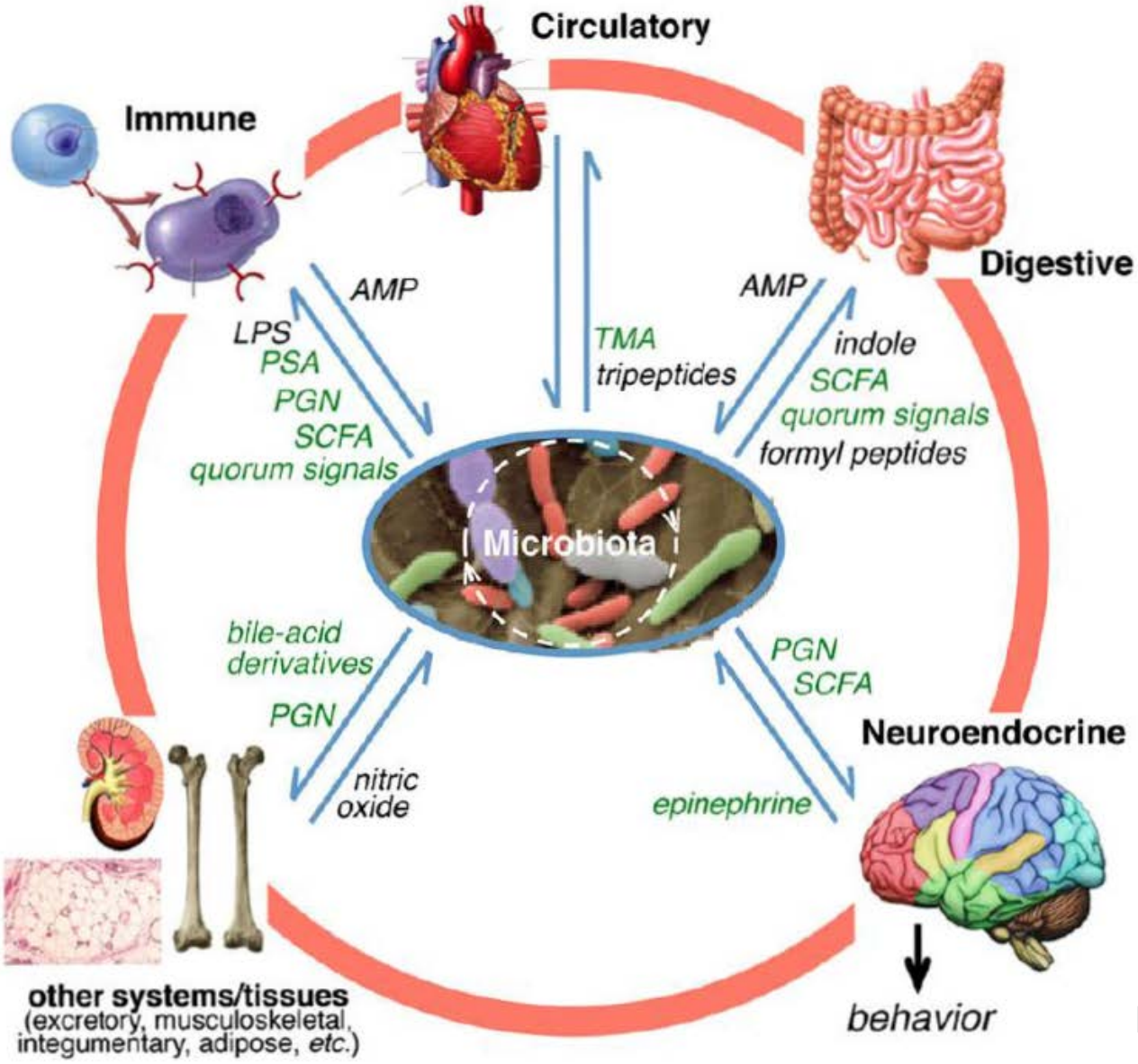
Human diseases linked to the gut microbiota



Necrotizing enterocolitis
Inflammatory bowel diseases
Colorectal cancer
H. pylori & stomach cancer
Irritable bowel syndrome
Infectious enteritis

Sekirov et al. (2010) *Physiol Rev*

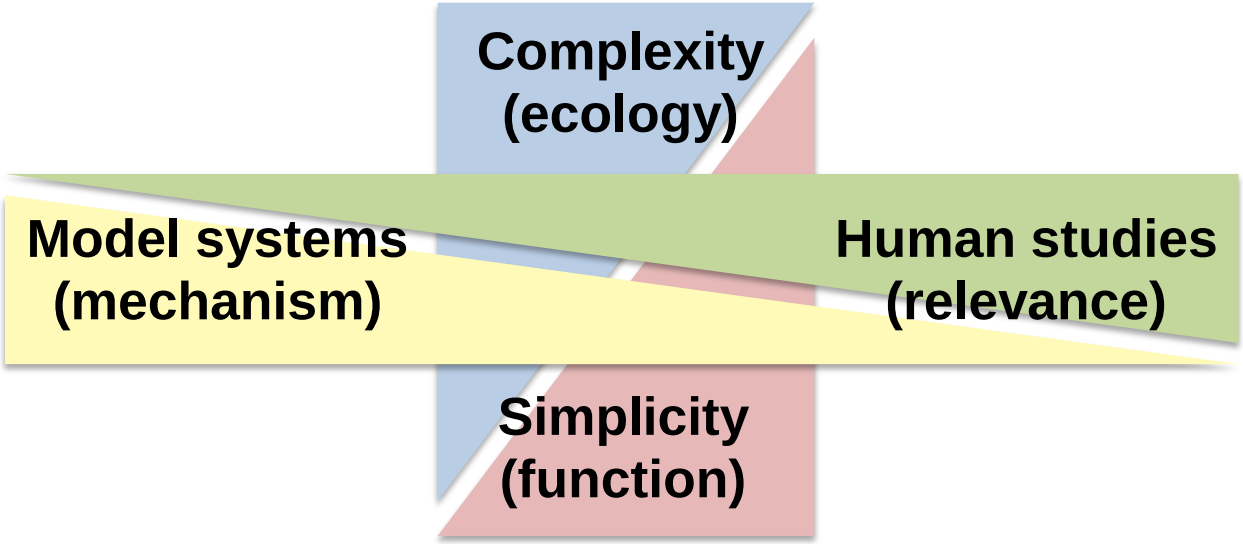
Signaling within and between the animal and its microbiota



McFall-Ngai et al. (2013) *PNAS*

Current challenges in microbiome research

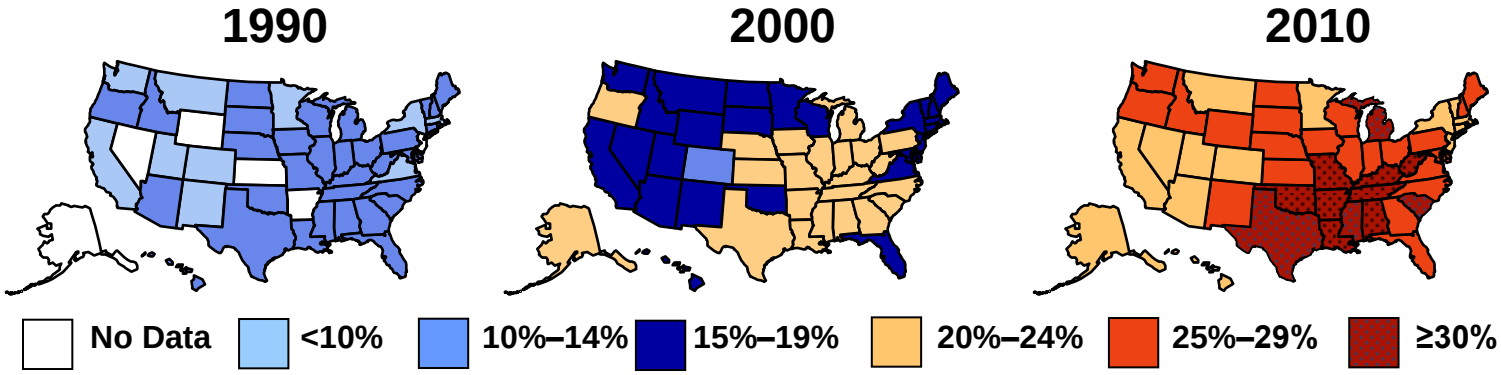
- Define how microbiomes vary as a function of host health & disease
- Define ecological principles governing microbial community assembly
- Define the function of microbial communities and individual members
- Define mechanisms by which hosts perceive and respond to microbiota
- Develop new microbiome-based therapeutic strategies for human health



Obesity: a major public health challenge of our time

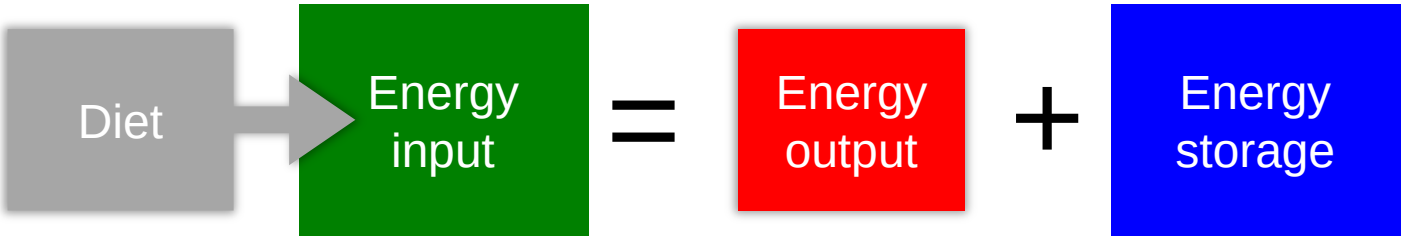
- 33% US adults are obese (BMI ≥ 30).
- 17% US children & adolescents are obese.
- Obesity is a risk factor for insulin resistance, type 2 diabetes and cardiovascular disease.
  - ~$147 billion/year direct medical costs

Prevalence of obesity among US adults:

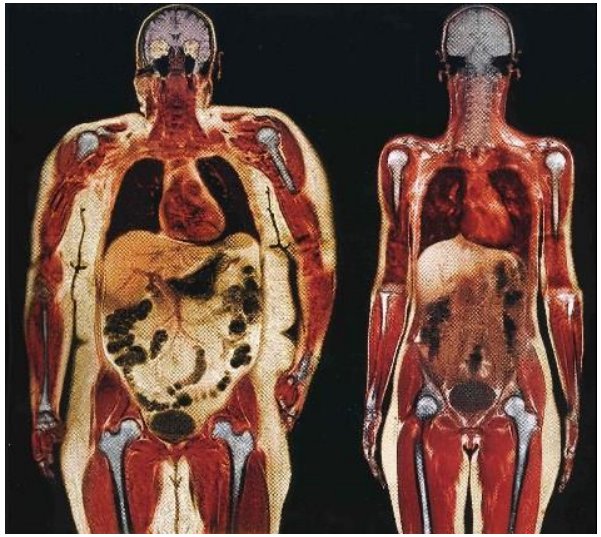


CDC BRFSS & CDC Weight of the Nation Press Briefing (27 July 2009)

Obesity is a disease of energy balance



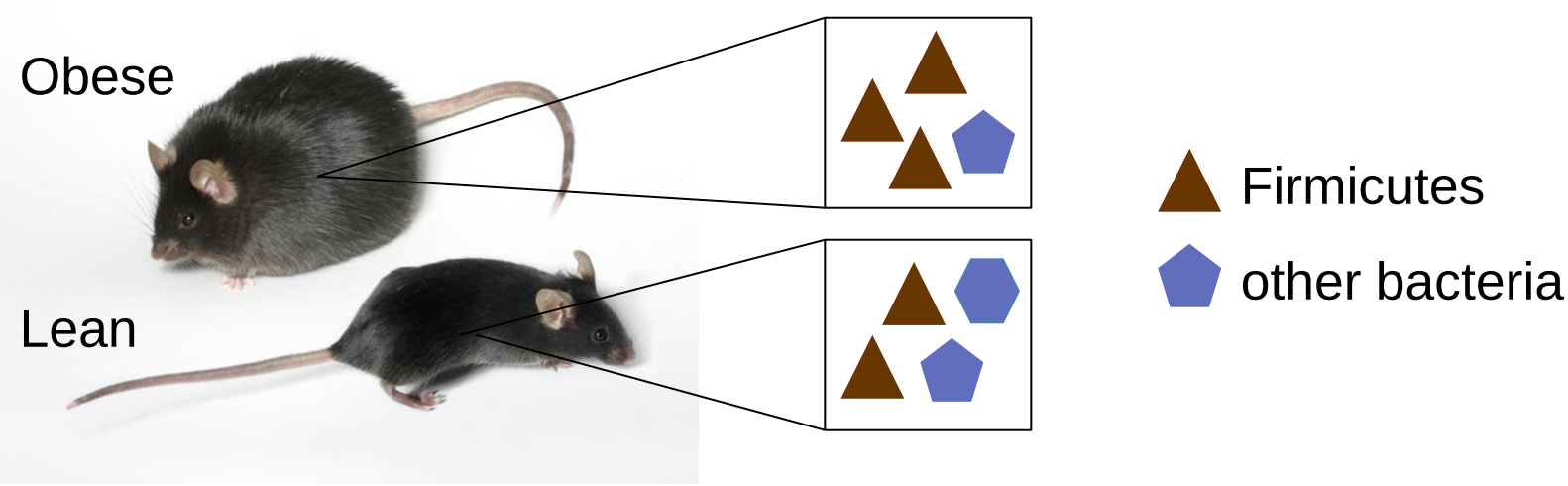
- Obesity is caused by prolonged “positive” energy balance leading to excessive energy storage.
- Excess energy is stored as fat in adipose tissues.
- Current obesity intervention strategies often fail to provide long-term weight reduction.
- Recent increases in obesity prevalence suggests strong contribution from environmental factors.
- Novel alternative approaches for obesity prevention and intervention are needed.



MRI (Heymsfield lab)

Evidence for microbiota roles in obesity

Obesity and dietary energy intake are associated with alterations in gut microbiota composition, often including positive correlation with the abundance of bacterial phylum Firmicutes.



Refs: obese humans (Ley, 2006; Turnbaugh, 2009), humans consuming high-calorie diet (Jumpertz, 2011), genetically obese mice (Ley, 2005, Vijay-Kumar, 2010), diet-induced obese mice (Turnbaugh, 2008; Hildebrandt, 2009; Murphy, 2010), humans after Roux-en-Y gastric bypass (Liou, 2013), fasting mice (Crawford, 2009)

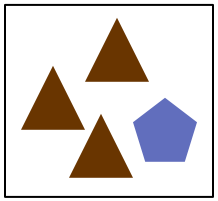
Evidence for microbiota roles in obesity

Subtherapeutic antibiotic treatment during early life stages is associated with increased adiposity and metabolic disease (Cho, 2012; Trasande, 2013)

Germ-free mice are protected from diet-induced obesity. (Backhed, 2007)

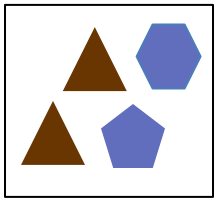
Gut microbiotas from obese individuals have physiological properties that can be transmitted to germ-free recipient mouse hosts. (Liou, 2013; Smith, 2013; Turnbaugh, 2006; Vijay-Kumar, 2010; Turnbaugh, 2008)

Obese



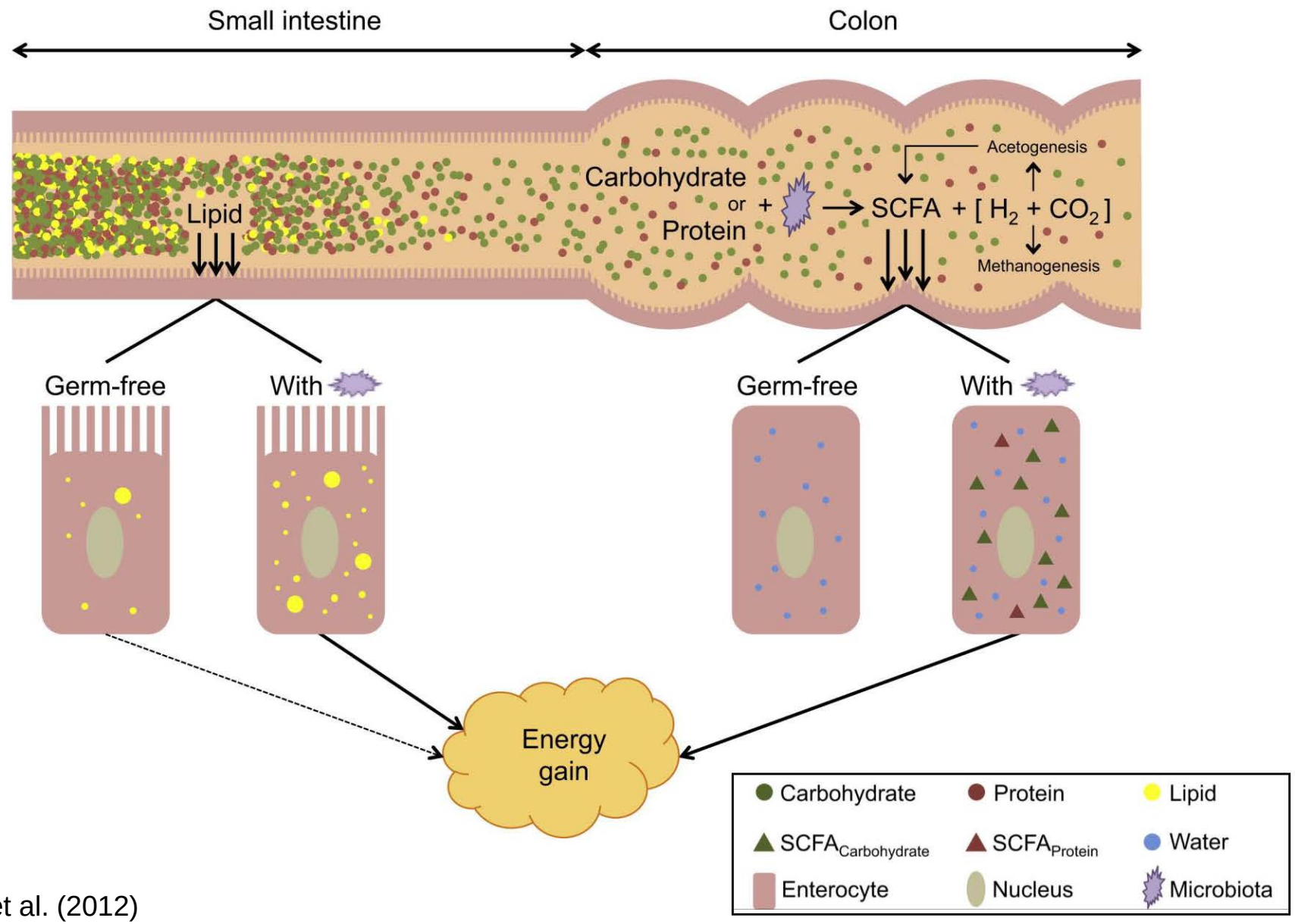
Colonize lean GF mice

Lean



Colonize lean GF mice

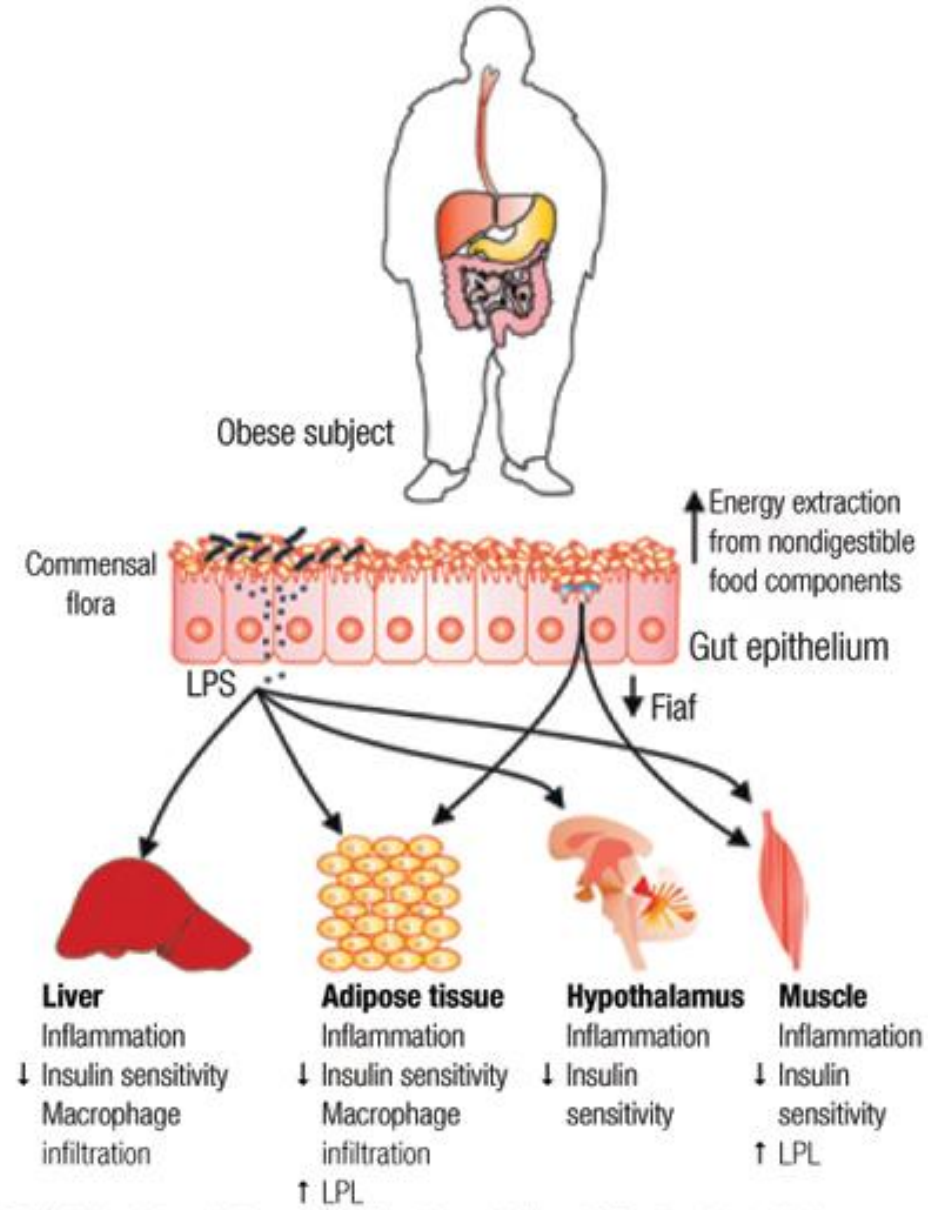
Microbiota enhance harvest of dietary carbohydrates, proteins, and lipids



Semova et al. (2012)
Carmody & Turnbaugh (2012)

Potential mechanisms by which microbiota contribute to obesity

- Microbiota enhance dietary energy harvest in the intestine.
- Microbiota regulate intestinal production of key endocrine factors (e.g., ANGPTL4/FIAF, GLP-1, PYY, FGF19).
- High-fat diet increases translocation of LPS leading to low-grade systemic inflammation and metabolic disorders (metabolic endotoxemia).



See Devaraj et al. (2013)
Figure from Tsukumo et al. (2009)

Evidence for xenobiotic chemical “obesogens” in obesity

- Low-dose chemical exposures are often associated with weight gain in animals (Baillie-Hamilton, 2002)

- Different obesogenic chemicals may have different mechanisms of action:
  - Adipocyte number and size
  - Endocrine control of appetite, satiety, food preferences, and metabolism
  - ??? Alterations in intestinal microbiota ???

- Numerous chemicals have been identified as obesogens, for example:
  - Organotins: Tributyltin (TBT) and Triphenyltin (TPT)
  - Bisphenol A (BPA)
  - Phytoestrogens: Genistein and Daidzein
  - Perfluorooctanoic acid (PFOA)
  - Perfluoroalkyl compounds (PFCs)

- ***An improved understanding of these obesogens’ mechanisms of action is an important research goal***

Grun & Blumberg (2007) *Rev Endocr Metab Disord*
Janesick & Blumberg (2012) *Int J Androl*

Tributyltin (TBT) as a model obesogen

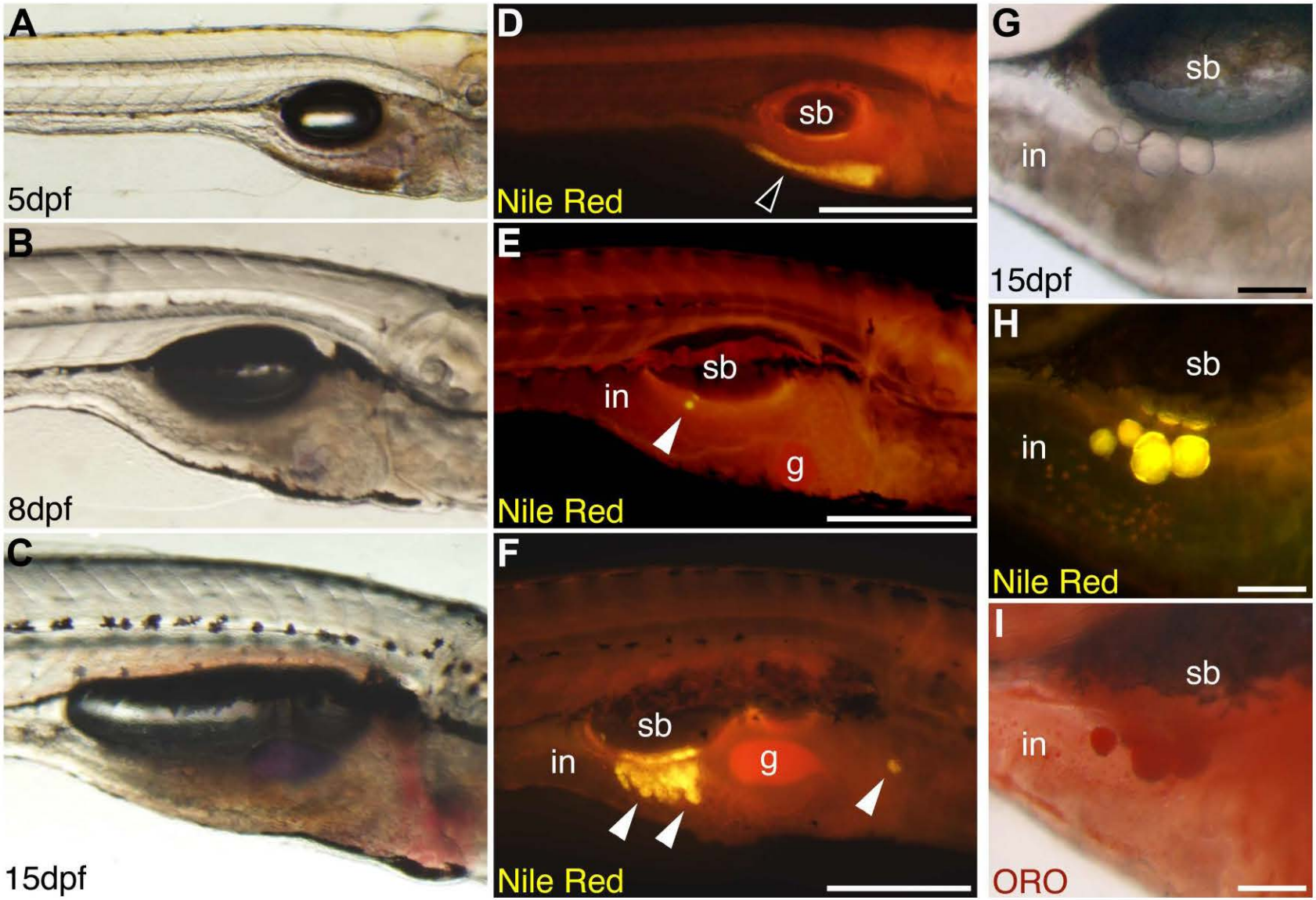
- TBT and other organotins have been used as agricultural pesticides, wood preservatives, plastics stabilizers, and antifouling agents in marine paints.

- Exposure to TBT and other organotins is toxic to bacteria and eukaryotes:
  - inhibition of amino acid uptake and cell growth in bacteria
  - interference with membrane function in bacteria and eukaryotic cells
  - disturbance of mitochondrial activity
  - inhibition of aromatase gene expression
  - other immunologic, hepatic, and neuronal toxicities in animals

- TBT has recently been identified as an obesogen:
  - directly induces adipogenesis in cell culture
  - agonist for pro-adipogenic transcription factor PPARγ and perhaps others
  - increases adiposity in vivo in mice, frogs, and zebrafish
    - Tingaud-Sequeira et al (2011) *JLR*, Riu et al. (2014) *Tox Sci*

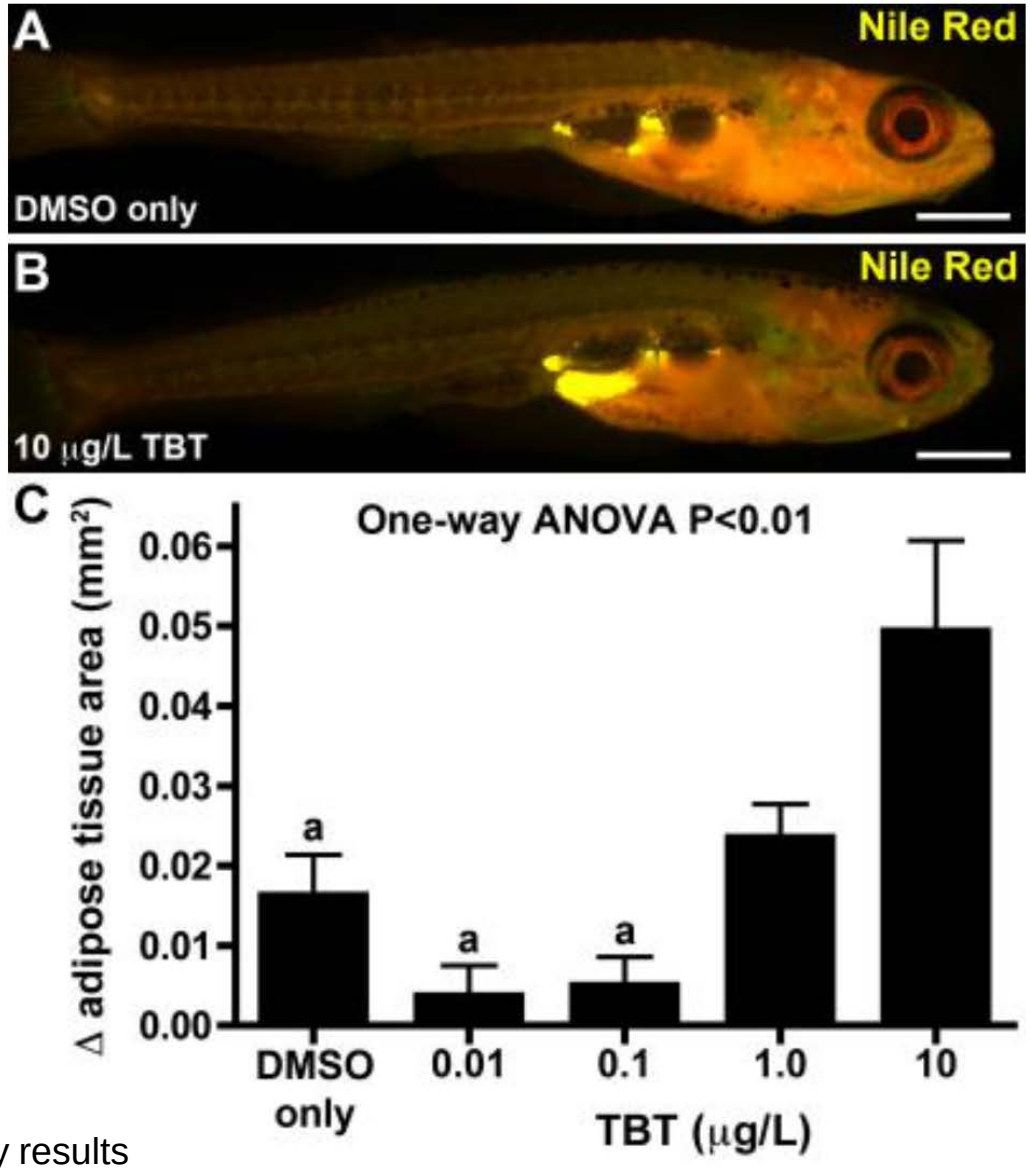
Grun & Blumberg (2007) *Rev Endocr Metab Disord*
Janesick & Blumberg (2012) *Int J Androl*

Neutral lipid stored in zebrafish adipocytes can be selectively labeled and visualized using vital lipophilic dyes



Flynn et al. (2009) *JLR*, Minchin & Rawls (2011) *Methods Cell Biol*

TBT induces dose-dependent increases in adipose tissue size



Edward Flynn, preliminary results
4d treatment starting 24dpf

Future Directions

- Despite the known capacity of TBT and other obesogens to directly impact microbes, their influence on animal-associated microbiotas is unknown.

- Exposure of gut microbiota antibiotics & host-targeted drugs results in microbial cell damage and specific transcriptional responses. (Maurice et al. 2013 *Cell*)

- Specific members of the gut microbiota modify host-targeted drugs and influence their activity on the host. (Haiseret al. 2013 *Science*)

- Microbial biotransformations already known to influence bioavailability and toxicity of some toxins (e.g. methylmercury). (Shao al. 2012 *Sci Total Environ*)

- ***Does obesogen exposure alters gut microbiota composition?***

- ***Do obesogen-induced alterations in gut microbiota composition confer increased obesogenic activity on the host?***

- ***What are the molecular, physiological, and ecological mechanisms underlying these host-microbiota-toxin interactions in animal hosts?***