TODAY'S SUBJECT: NUTRITION AND METABOLISM

DKN

1.) What is needed for making new cells?
2.) What is needed for survival?
3.) What constrains metabolism?

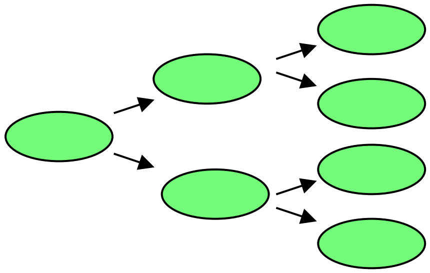
iClicker Quiz 1; Exam 1 (May 3) information

MMN

A.) What is the contribution of the gut microbiota to health?
B.) What happens when the microbiota is out of balance?

iClicker Quiz 2

All microbes want to do is divide & survive



Draw growth curve; explain lag, exp, stationary (Finkel)

Eq. $y = 2^X$ or $x = \log_2(y)$

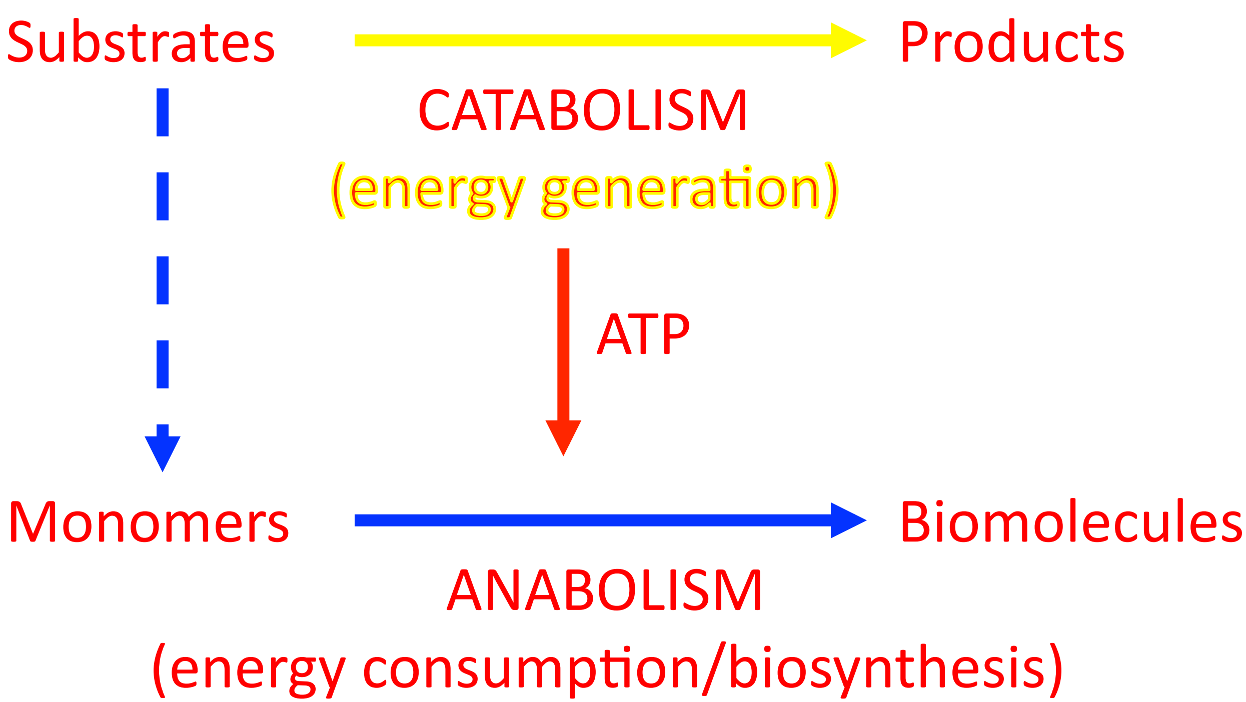
How do most bacteria exist in the environment? Not exp. growth!

1 cell = 10^{-18} m^3, Earth = 10^{21} m^3 : 10^{39} cell/Earth

→ $\log_2(10^{39}) = 130$ generations

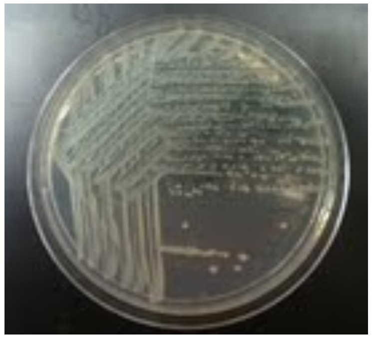
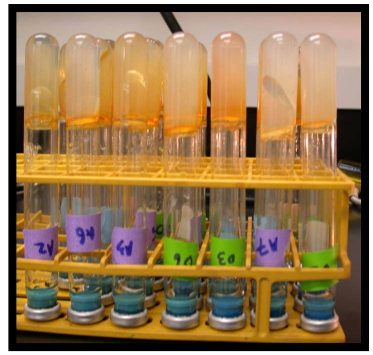
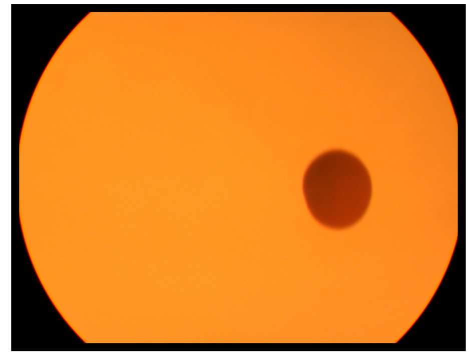
→ *E. coli* (20 min generation time) takes < 2 days to take over the Earth!

Metabolism is a combination of *catabolism* & *anabolism*:



How would you grow a bacterium *de novo?*

- Enrichment v. pure culture

Myth of the 1% culturable (*a.k.a.* "Great plate count anomaly")

Draw out environmental sample → DAPI count (microscope) vs. plate count

Problems here? → Get what you select for!

How could you get creative about culturing and solve the anomaly?

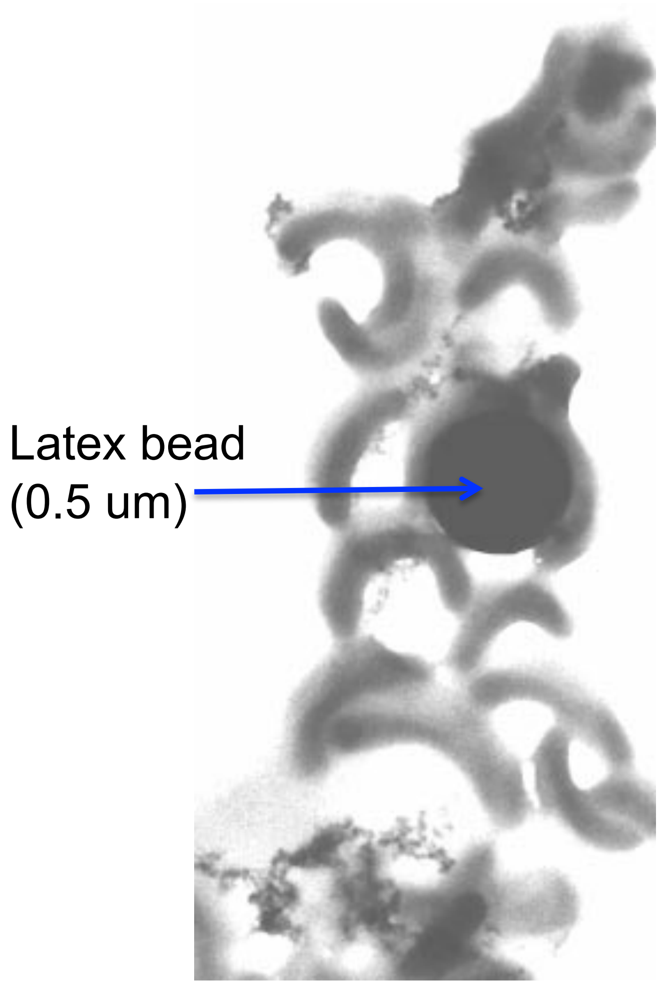
- Physically separate cells (separate weeds/slow growers)
- Using environmental waters (low nutrient)
- Appreciation for physical context (surface attached?)
- Design synthetic medium more thoughtfully:

    geochemically-informed

    symbiosis-informed

    metagenomically-informed

Story of SAR11



- SAR11 Discovered by 16SrRNA in 1986.
- Most successful group of bacteria in the oceans--can reach densities of 500, cells/ ml). Account for 25% of all microbial cells. Their combined weight exceeds that of all fish in the world's oceans!
- Evaded culture for decades!
- Dilution to extinction lead to isolation
- *Pelagibacter ubique*
- Oligotroph, proteorhodopsin pump
- Smallest free-living cell, smallest genome (1.3 million base pairs)

Rappe MS, Connon SA, Vergin KL, Giovannoni SJ. 2002.
Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature **418:**630-633.

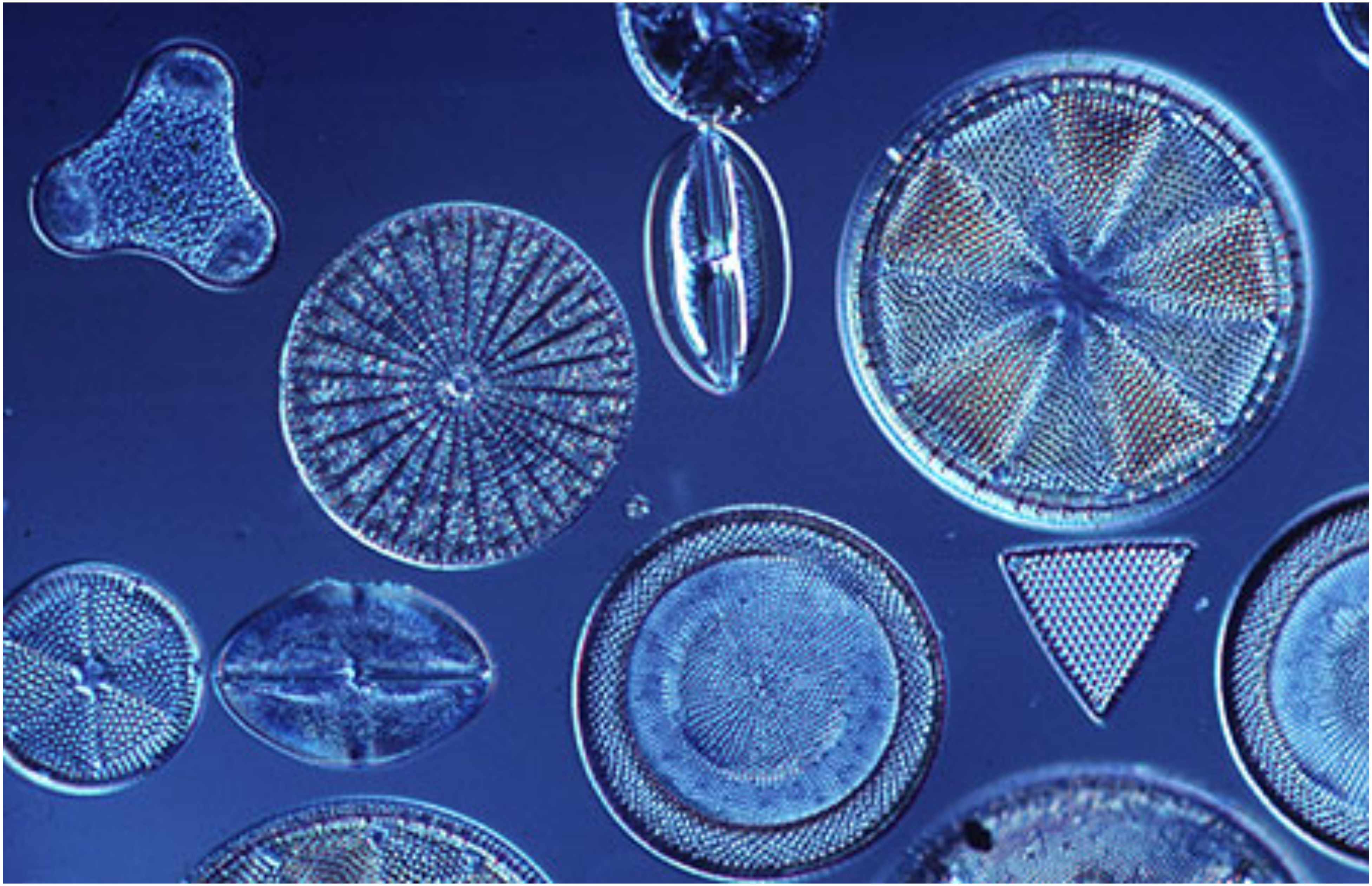
How do you design a growth medium?

COMPONENT	FUNCTION
CHNOPS (6 most important elements)	nuc acids, aa, phospholipids
trace elements	active sites, proteins (Cd example), QS molecules
vitamins	co-factors in proteins (Ex. Vit B by gut bacteria)
Salts (Na, K, Ca…)	membrane potential

Redfield ratio C:N:P = 100:16:1 : marine biomass, (Redfield, 1934)

This is an average value! N:P can vary by 10X (*e.g.* 6:1, 60:1)

Diatoms (glass houses): C:Si:N:P = 106:15:16:1



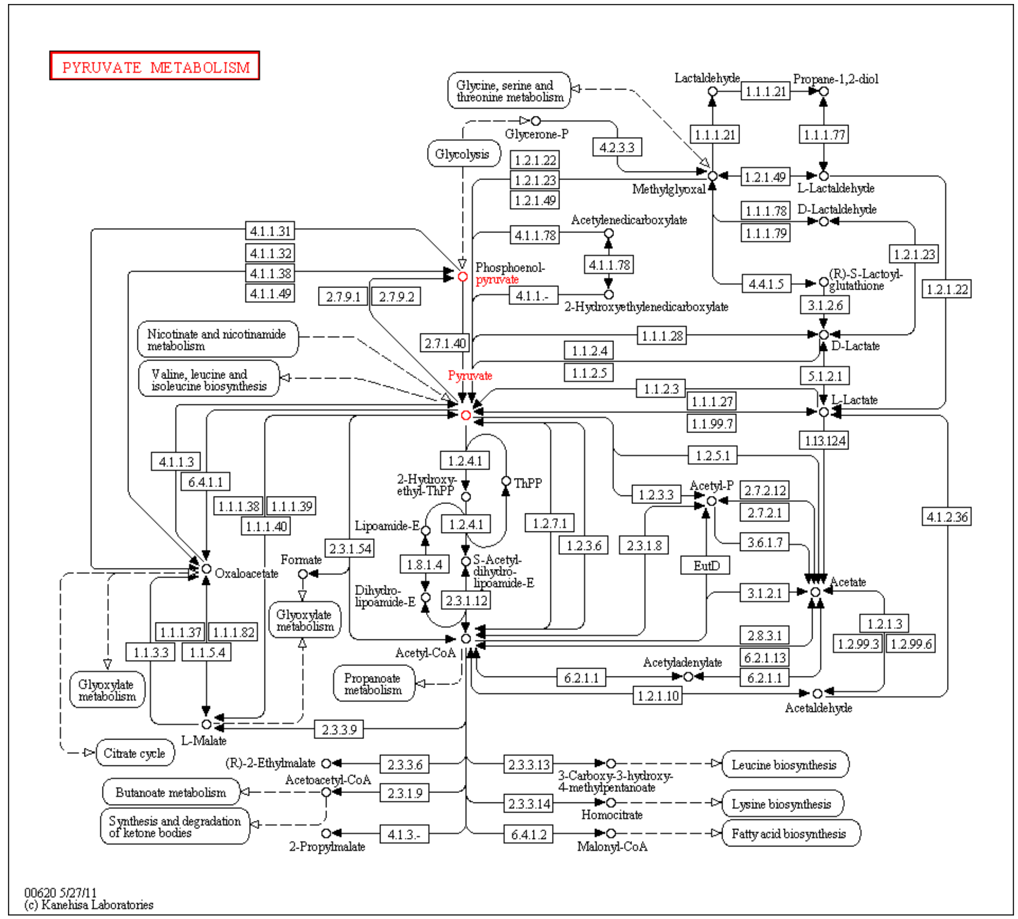
How do cells get things out & in ? (solubility, permeability, toxicity issues)

Pumps (delta p, ABC, secretory),
Channels (antiporters, symporters, uniporters)

Draw a comprehensive carton

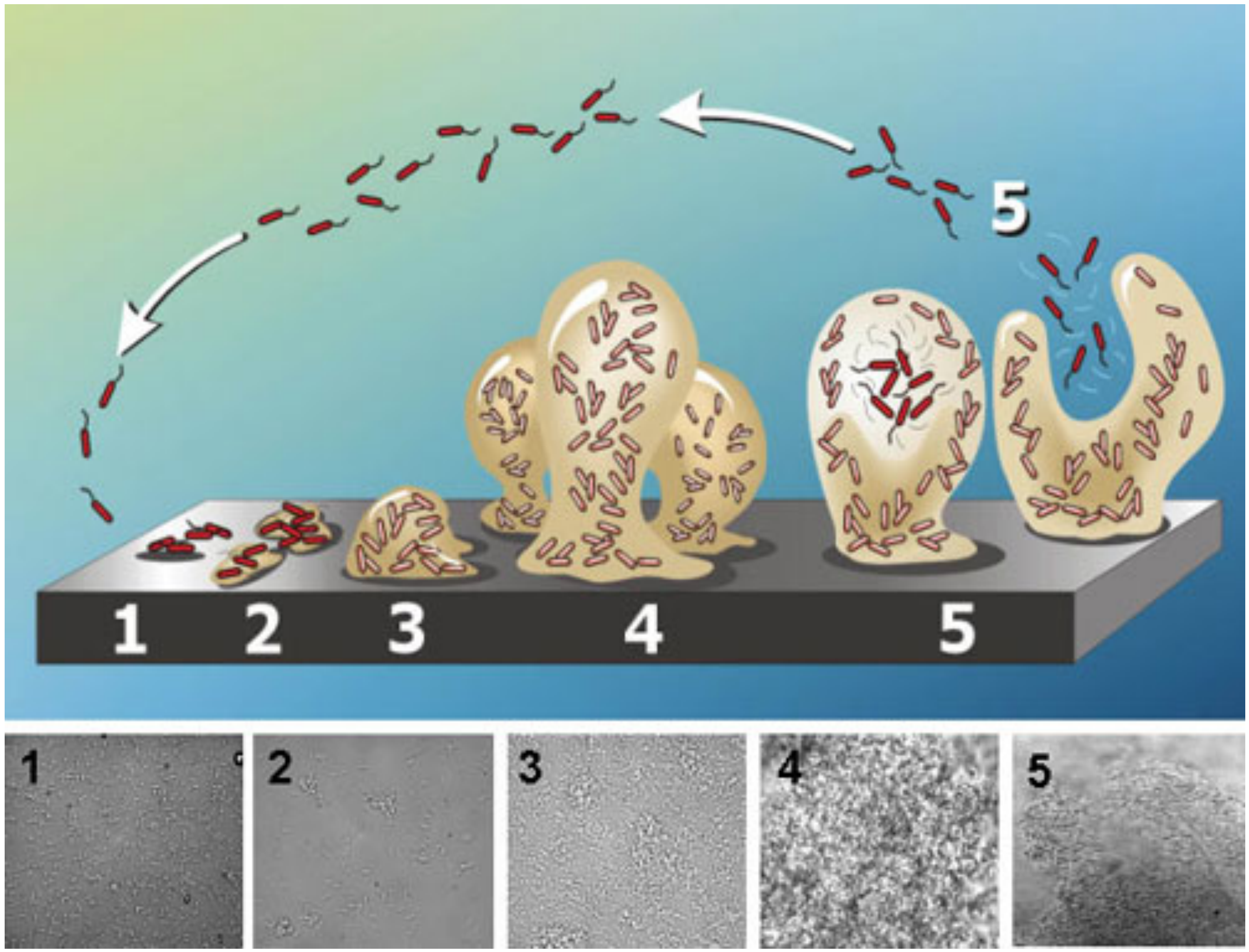
Once substrates are inside, what happens next?

KEGG Pathway Database



WILL LEARN IN RECITATION NEXT WEEK!

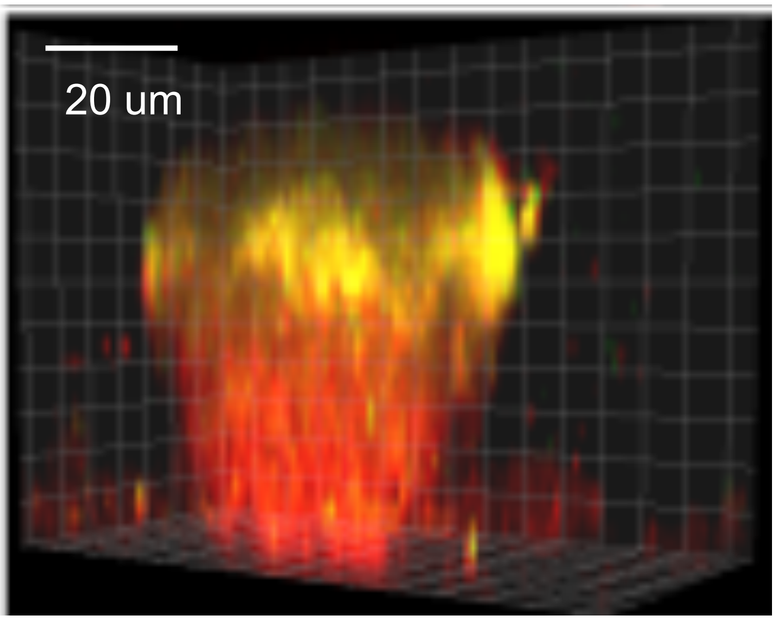
How do multicellular communities cope with nutrient acquisition/excretion?

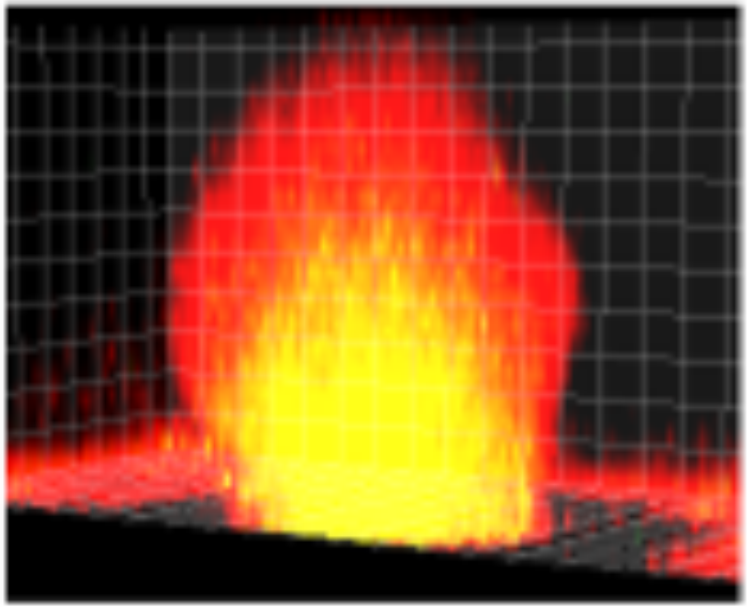


http://mathbio.colorado.edu/mediawiki/index.php/MBW:Role_of_Biofilm_Matrix_in_Structural_Development

Concept of metabolic differentiation - layered communites take II)

Regulated use of preferred energy sources (signal transduction)





Teal et al. (2005) Spatiometabolic stratification…, AEM

Catabolism: energy generation - broad classes (the “trophs”)

Carbon

Autotroph (uses inorganic C)

Heterotroph (uses organic C)

Energy

Phototroph (uses light)

Chemotroph (uses chemicals)

Organotroph (organics)

Lithotroph (inorganics)

lithos = rock)

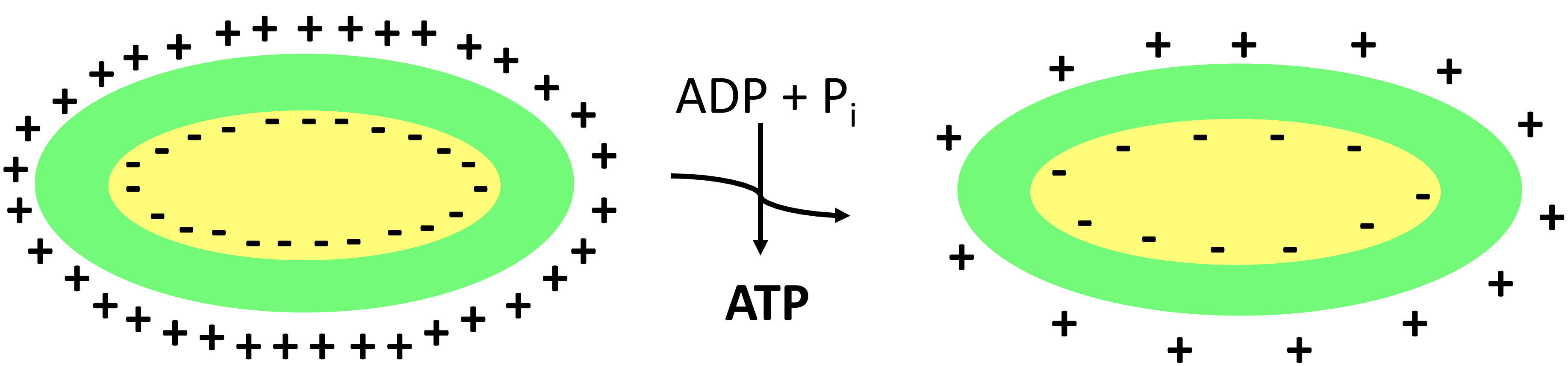
Catabolism: energy generation

2 strategies to make ATP:

- Substrate level phosphorylation (fermentation): performed inside cell

$$\mathbf{A} \longrightarrow \mathbf{B} \xrightarrow{P_i} \mathbf{B\text{-}P} \longrightarrow \mathbf{C\text{-}P} \xrightarrow{ADP} \mathbf{D + ATP}$$

- Oxidative phosphorylation (respiration, photosynthesis): performed in membrane -- proton motive force (PMF), Δp



What limits metabolism? (not including temp, pressure, pH, etc..)

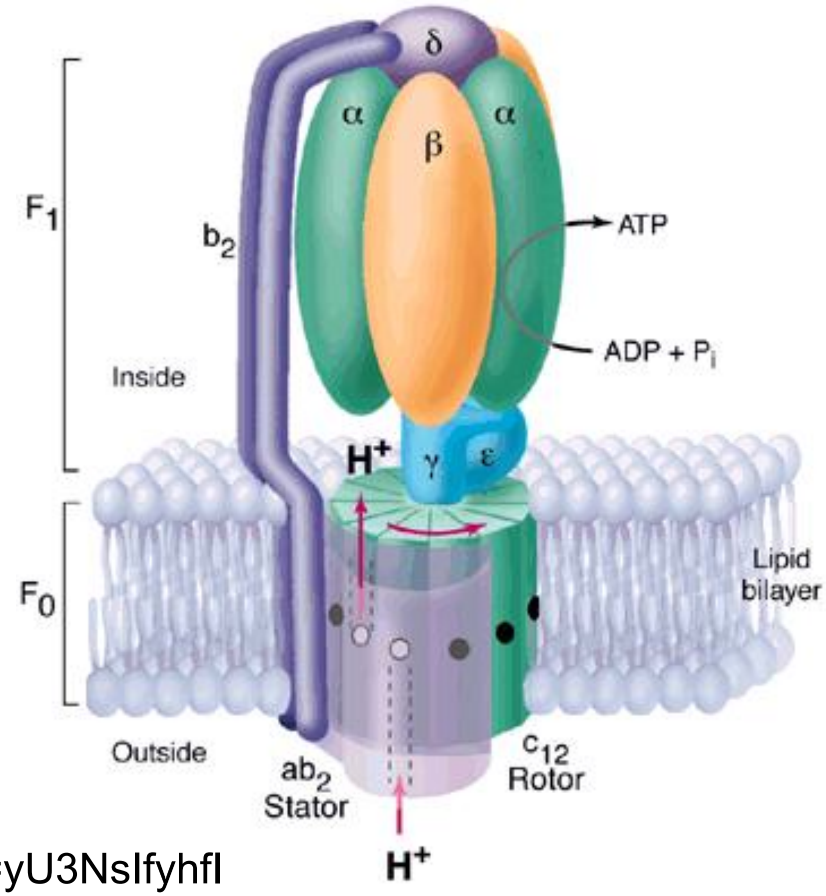
1.) minimum free energy? (thermodynamics)
2.) bioavailability of substrates (kinetics)
3.) toxicity of substrates/products (reactivity)

What is the minimum free energy that is stored in the membrane?

→ Energy released by 1 H+ translocation

→ 3 H^+/ATP synthesis
(average! could change by condition, organism!)

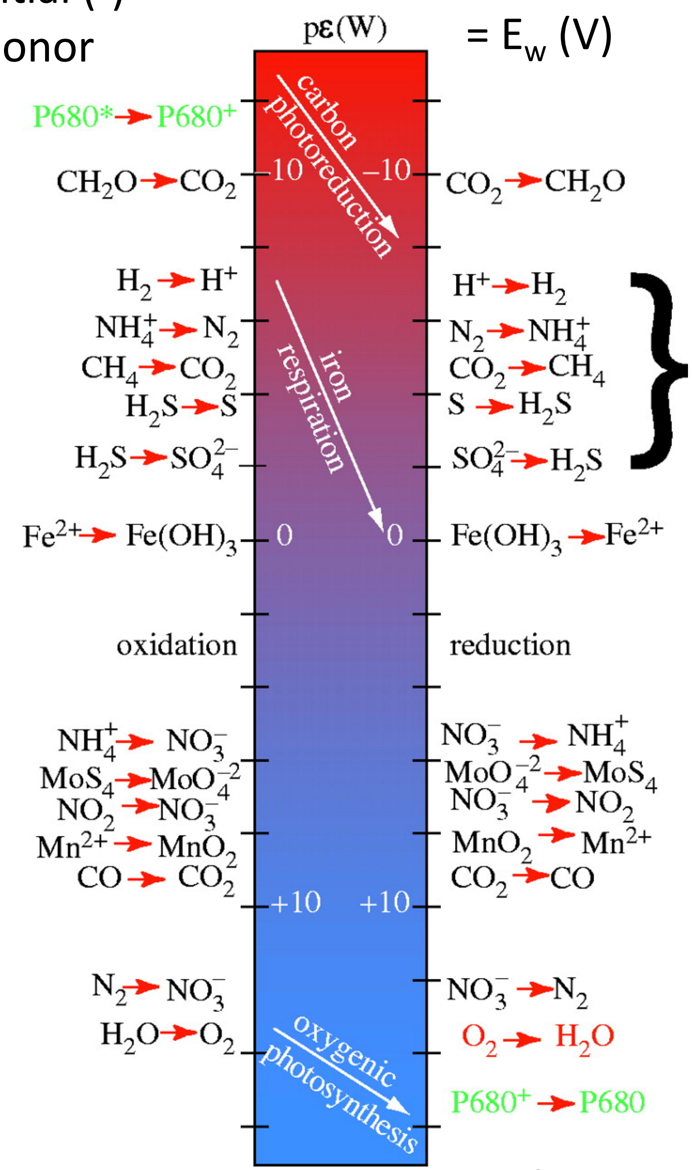
ATP synthase



http://www.youtube.com/watch?NR=1&feature=fvwp&v=yU3NsIfyhfI

Electron tower reprise:
how do you store energy?

Low potential (-)
Electron donor
reductant



High potential (+)
Electron acceptor
oxidant

Catabolic redox reaction:

$A_{red} + B_{ox} = A_{ox} + B_{red}$

$\Delta G = -nF\Delta E$ (kJ)

n = number of e^- transferred
F = Faraday constant
ΔE = difference in redox potential

$\Delta G = yF\Delta p$ (kJ)

y = number of H^+ translocated
F = Faraday constant
Δp = proton motive force

Let's use these relationships to calculate important things:

How large must Δp be to synthesize ATP?

Given: ΔG to make ATP = - 50 kJ (for an actively growing cell)
y = 3 (recall: $3H^+$/ATP in ATP synthase)
F = 96,000 C (charge for a mol of a monovalent ion: e^- or H^+)

$$\Delta G = yF\Delta p \qquad\qquad \Delta p = \frac{\Delta G}{yF} = -\,174 \text{ mV}$$

NOTE: most bacteria have Δp between -140 to -200 mV. *Why variation?*
depends on assumption for y, ΔG

What is the minimum potential difference (ΔE) to sustain this assuming $2e^-$ are transferred?

$$-nF\Delta E = yF\Delta p \qquad\qquad \Delta E = -\frac{y\Delta p}{n}$$

y is the critical tunable parameter!

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HOW DIVERSE IS THE HUMAN MICROBIOTA?

The 'second human genome project'

Human Organ Systems Interacting with Bacteria

		No. of species*
respiratory		
excretory		
digestive	*mouth, stomach, intestines*	~ 1,600**
integumentary		~ 200
endocrine		
circulatory		
reproductive		~ 500
immune		
nervous		
musculoskeletal		
	Total	~ 2,600

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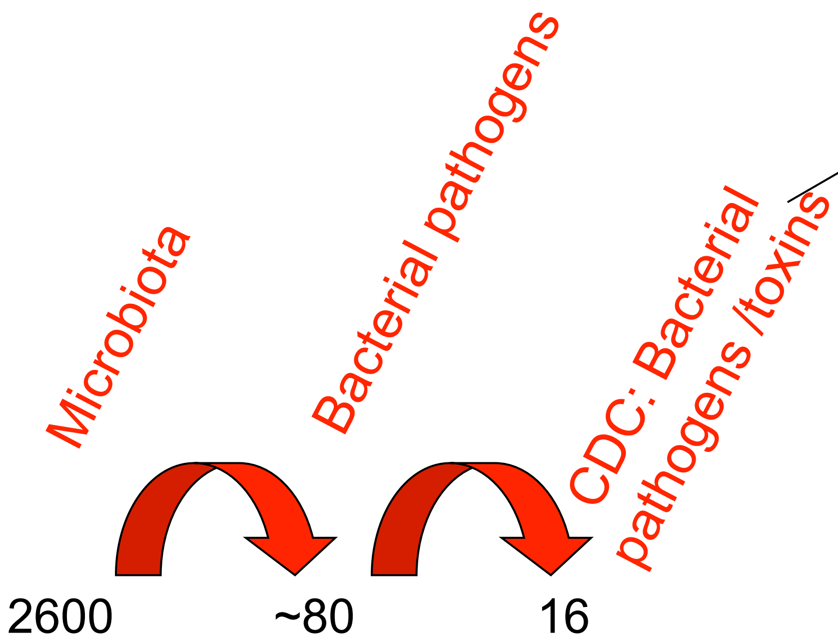
~ 80 bacterial pathogens of humans

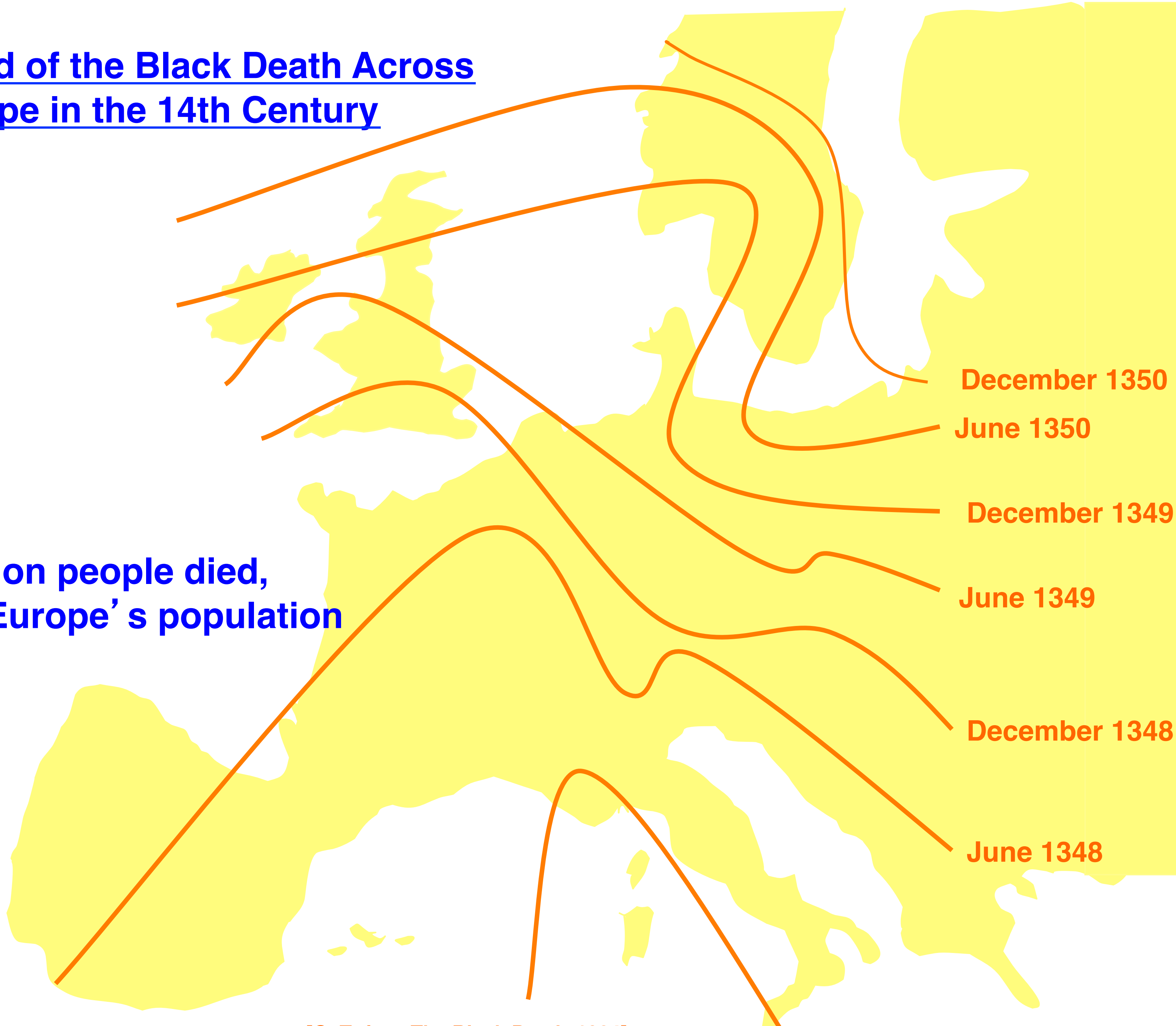
Are pathogens spies?



Bacillus anthracis
Brucella sp.
Burkholderia mallei
B. pseudomallei
Chlamydia psittai
Clostridium botulinum
C. perfingens
Coxiella burnetii
E. coli 0157:H7
Francisella tularensis
Rickettsia prowazekii
Salmonella typhi
Shigella flexneri
Staphylococcus aureus
Vibrio cholerae
Yersinia pestis

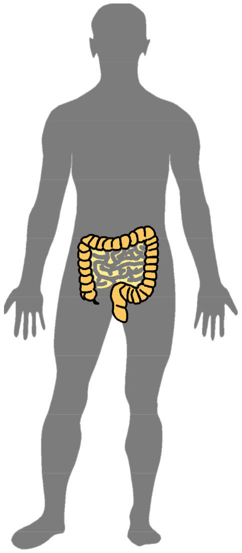
The Spread of the Black Death Across Europe in the 14th Century

20 million people died, 30-50% of Europe's population

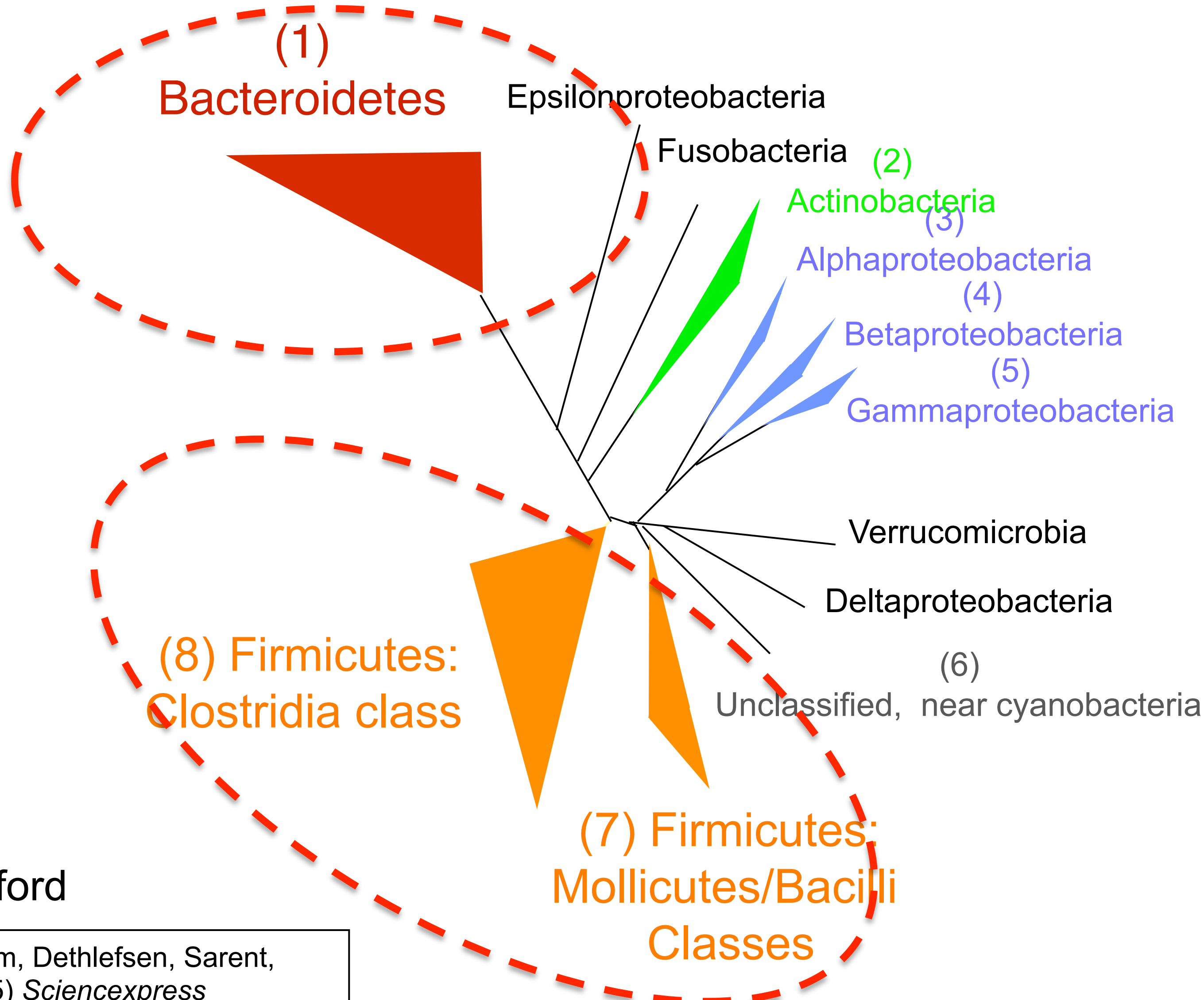


[G. Twigg, *The Black Death*, 1984]

HOW DIVERSE IS THE MICROBIOTA OF THE HUMAN GUT?



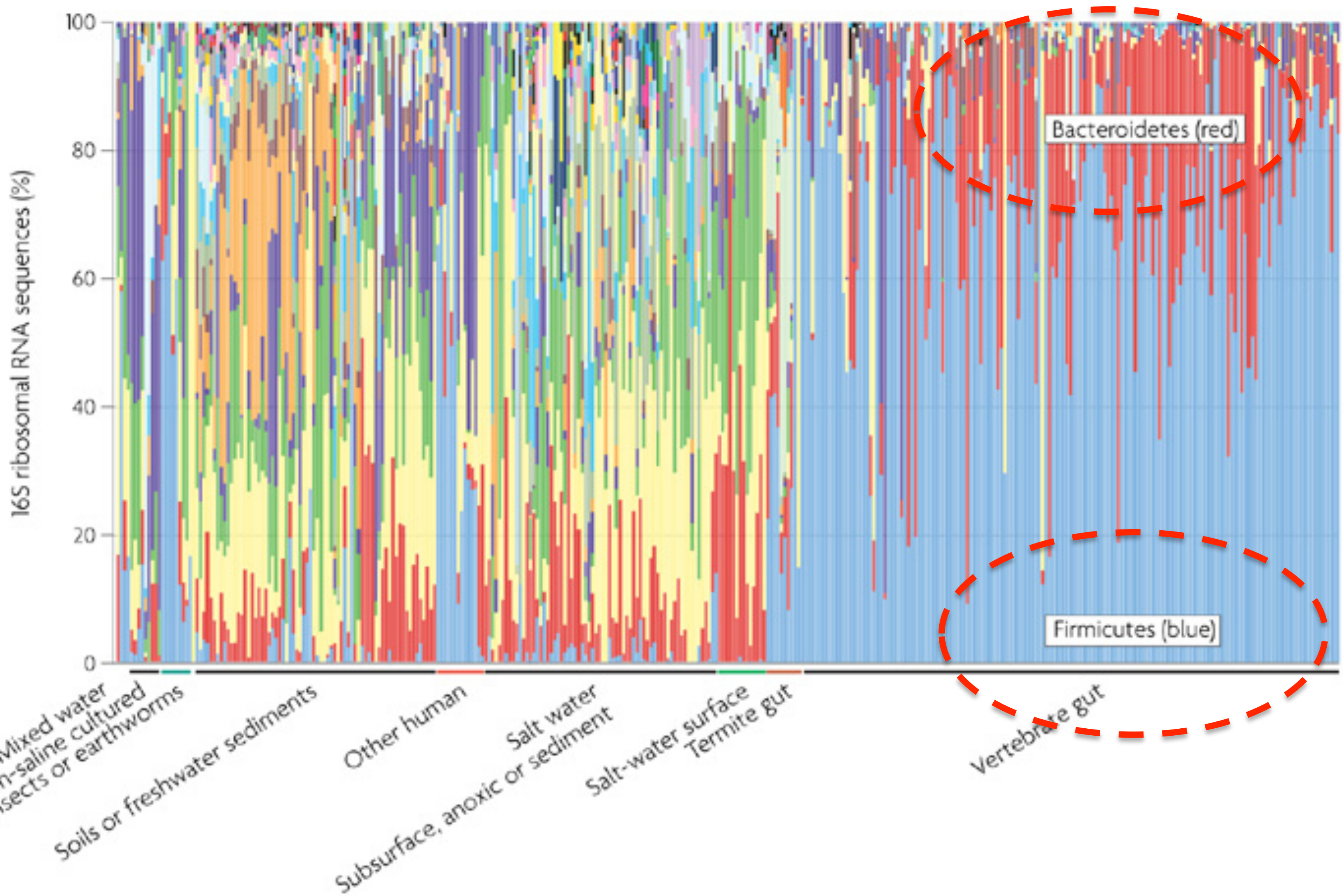
INTESTINE: 8 divisions, 800 species, 7000 strains



David Relman, Stanford

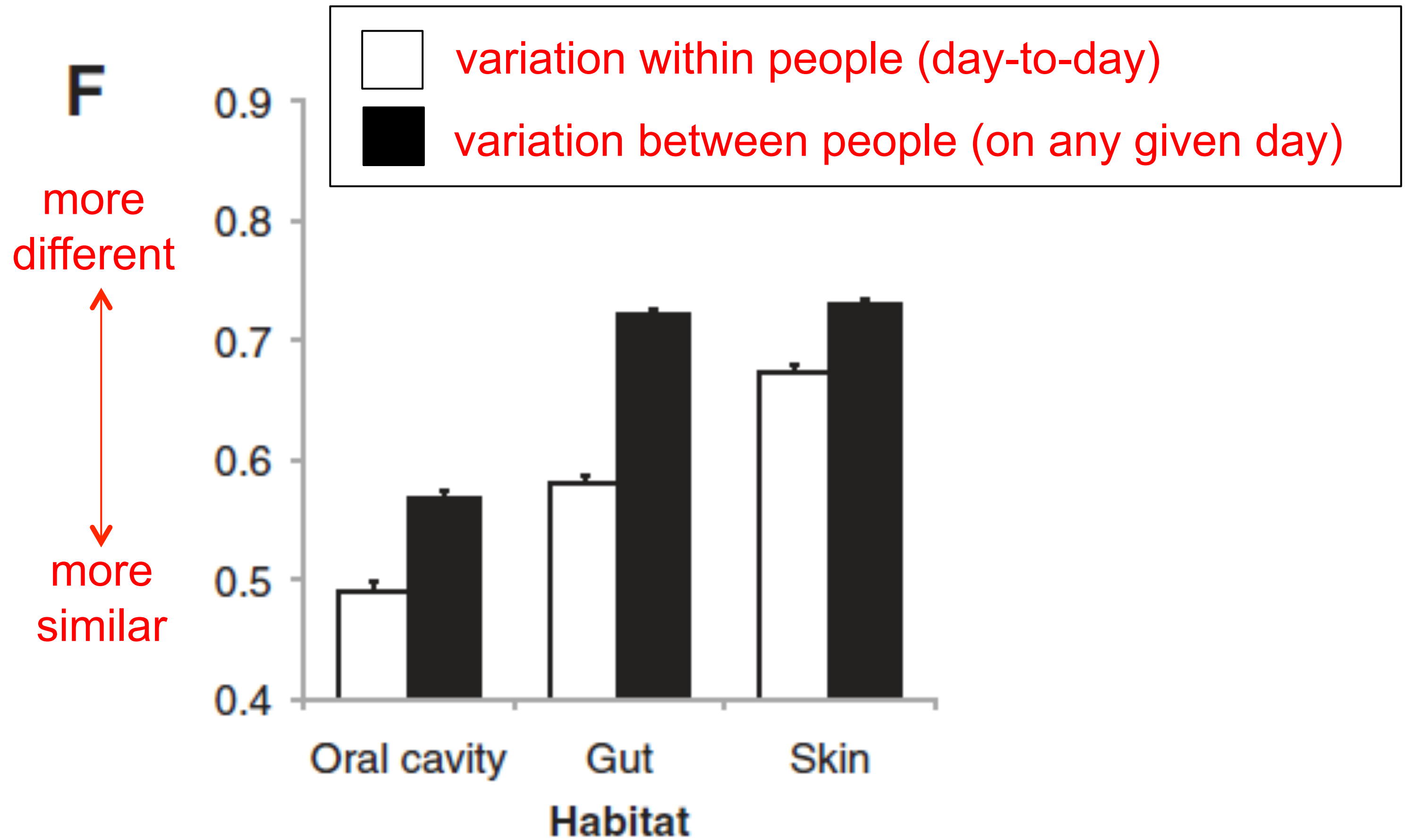
Eckburg, Bik, Bernstein, Purdom, Dethlefsen, Sarent, Gill, Nelson, & Relman (2005) *Sciencexpress*

HOW DOES DIVERSITY OF THE GUT COMPARE WITH THAT OF OTHER HABITATS?



Nature Reviews | Microbiology

Is there greater variation with a person over time or between individuals?



Costello et al. *Science* (2009) 326: 1694 - 1697

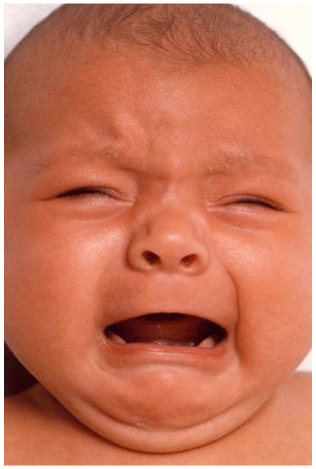
In the beginning…

What are the factors that affect early colonization of the gut?

Vaginal Delivery

Dominance of species in the genera *Lactobacillus* and *Prevotella*

Inheritance from mother high



C-section

Dominance of species in the genera:
Staphylococcus,
Corynebacterium
Proprionibacterium

Higher susceptibility to certain pathogens

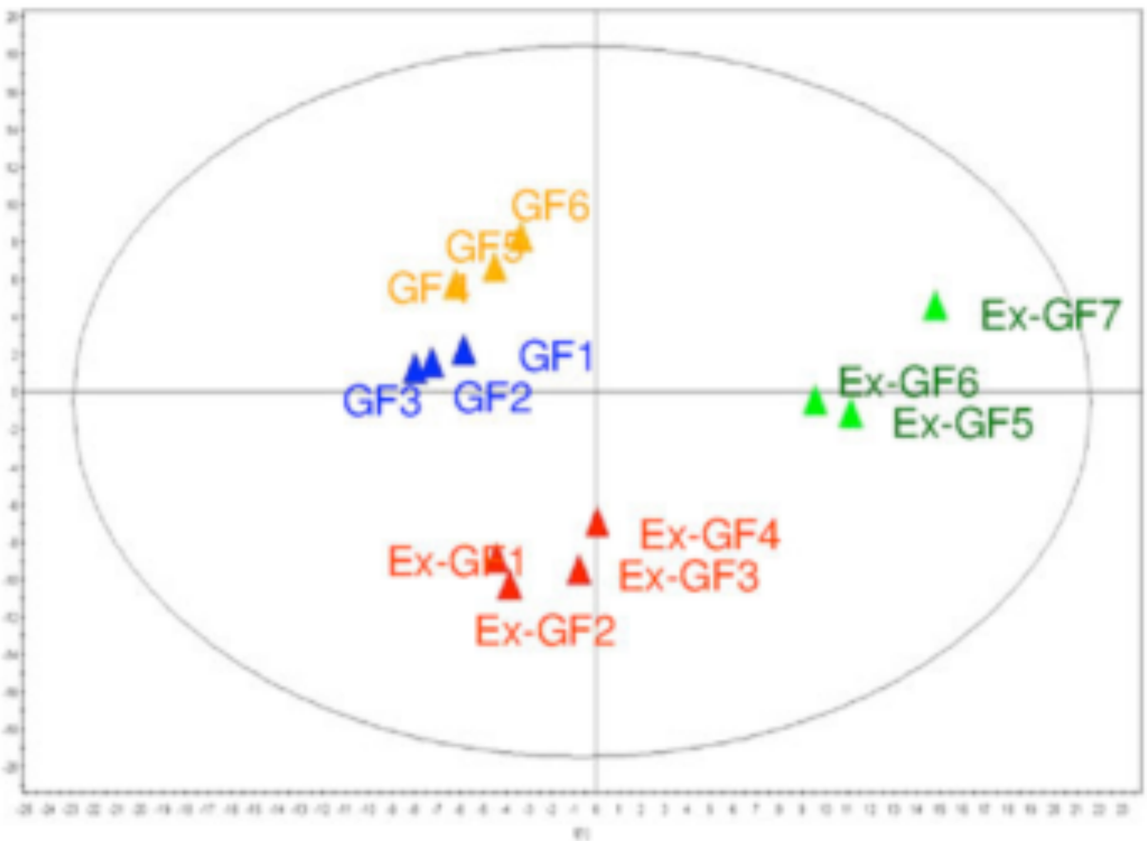
Higher risk of allergy

Metabolome = complete set of small-molecule metabolites

influences on digestion – your metabolism, the metabolism of your microbiota

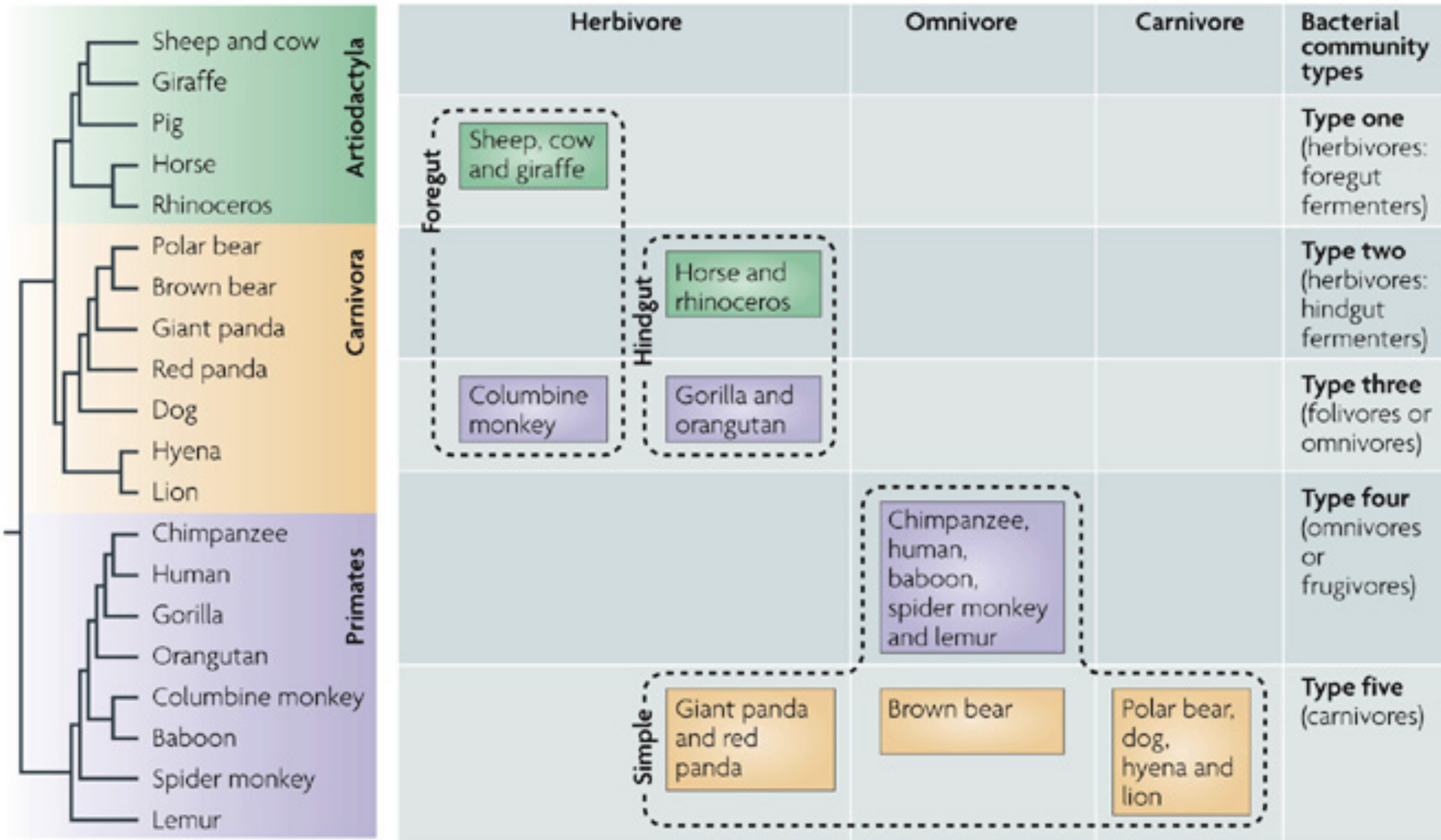
e.g., tylenol allergy

GF = Germ Free



Ruth Ley
Cornell U

Do we know about other animals?



Nature Reviews | Microbiology

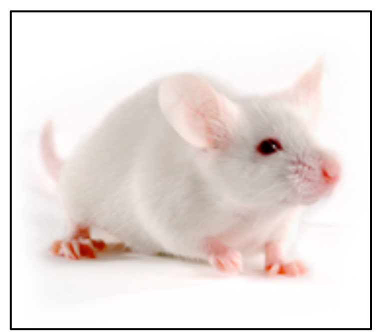
Jeff Gordon
Wash U St. Louis

John Rawls
[now UNC Chapel Hill]

A
B
B
F

B
F
A
P

A
B
B
F



B
F
A
P

Behavioral/Neurological Problems
(depression, sleep problems)

The Microbiota and Disease

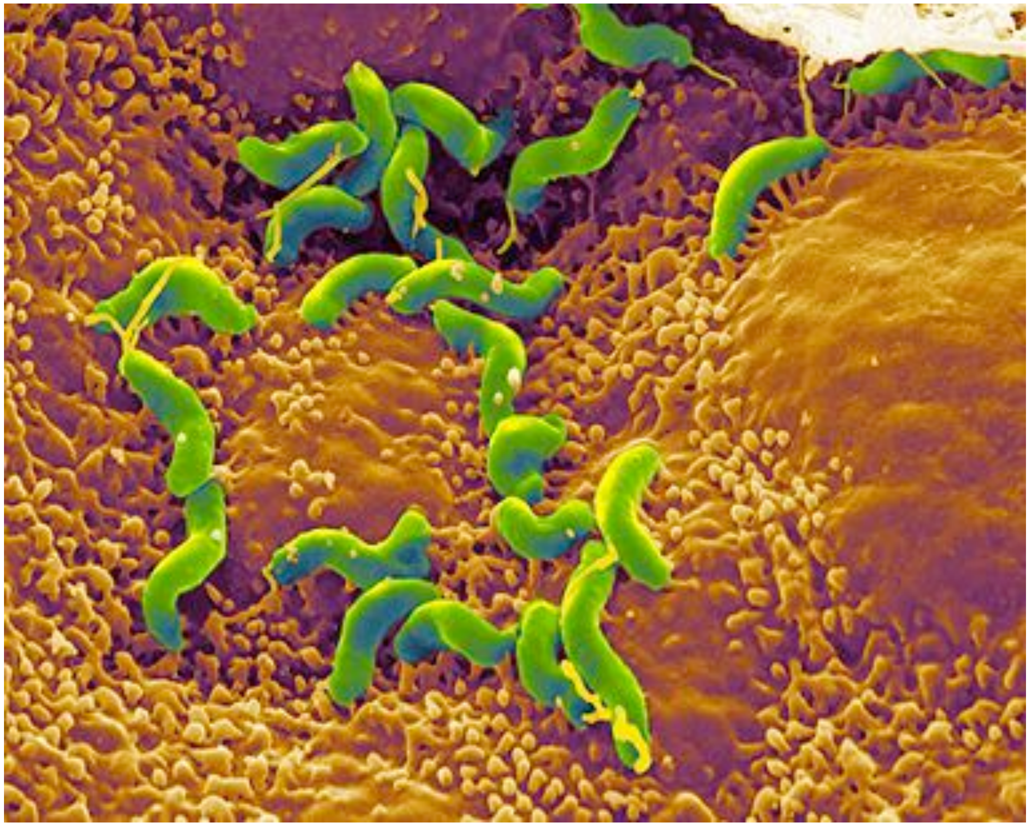
Obesity

Allergy
Autoimmune disease

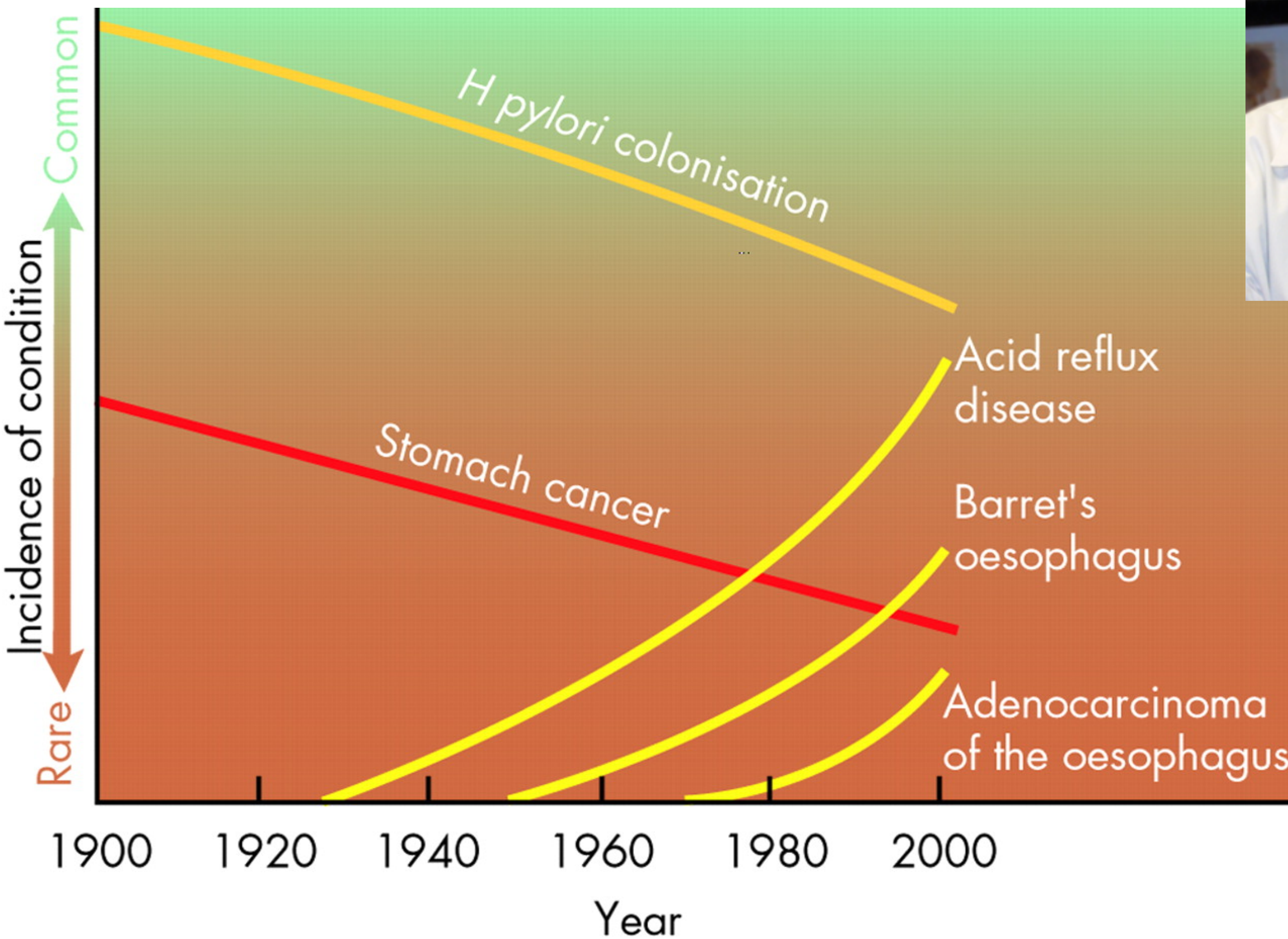
Communities interact and extend their influence by nerves/hormones -

Hygiene Hypothesis

The *Helicobacter pylori* enigma

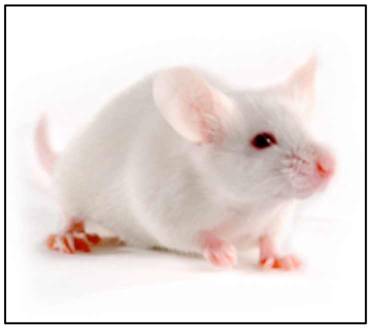


Causes ulcers – mainly in individuals post-reproductive


Common
Incidence of condition
Rare
H pylori colonisation
Stomach cancer
Acid reflux disease
Barret's oesophagus
Adenocarcinoma of the oesophagus
1900
1920
1940
1960
1980
2000
Year


NYU Medical Center
Attending Physician

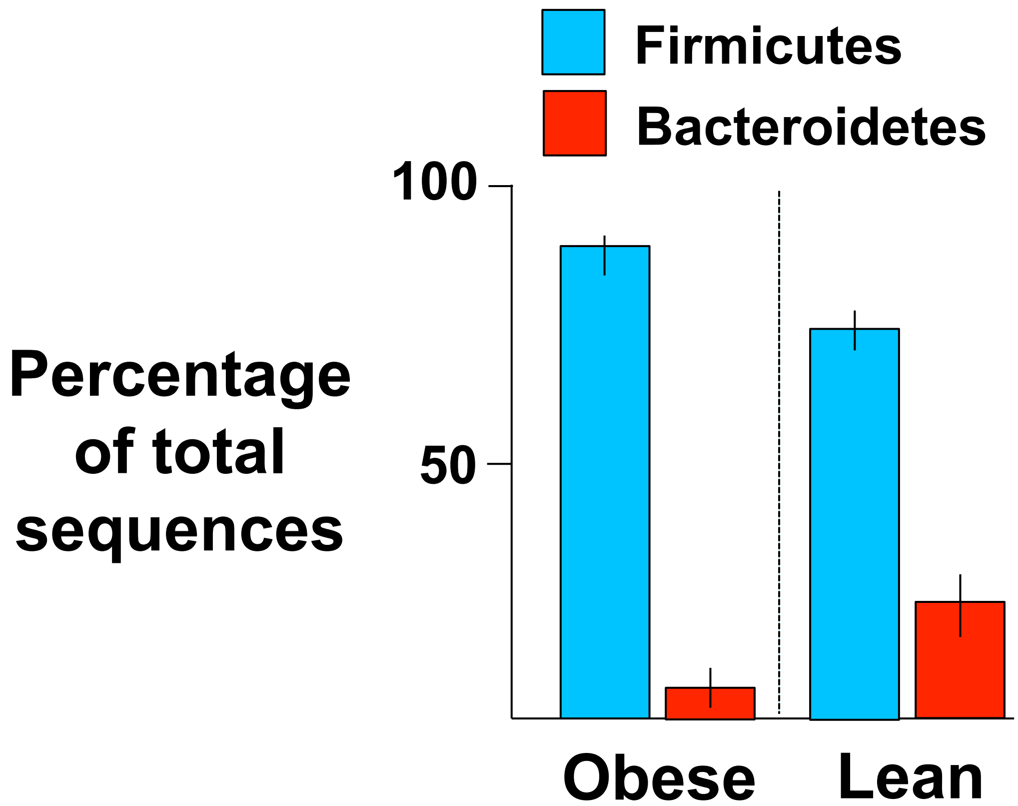
Carriage of *Helicobacter pylori* CagA



PROTECTIVE AGAINST ASTHMA

Should we infect all children?

Microbiota in disease: Is the the microbiome of obese individuals different?

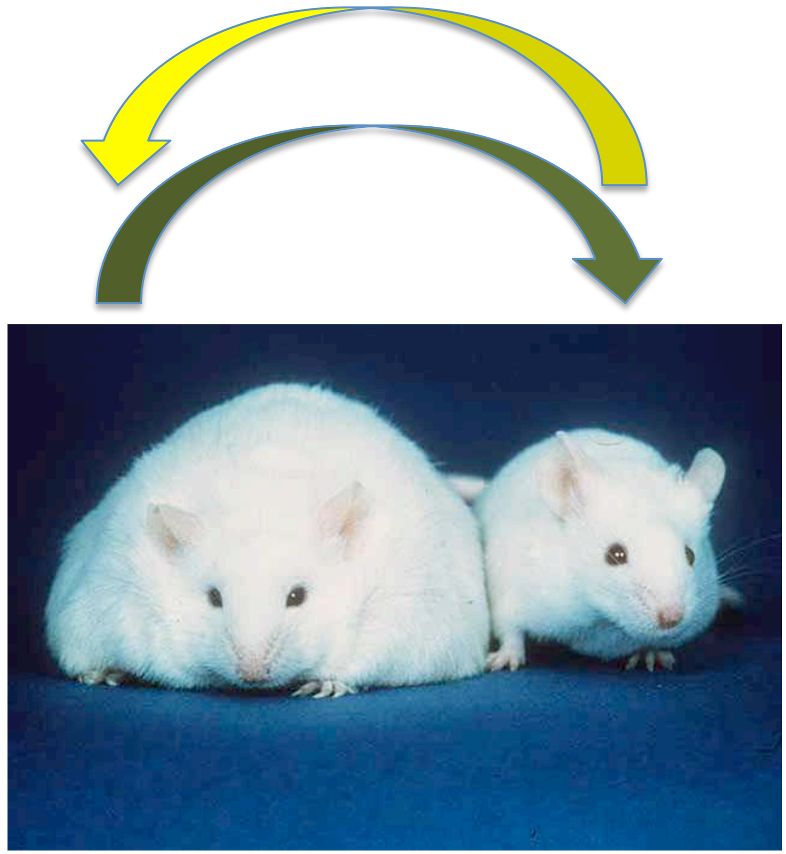


ob/ob mouse

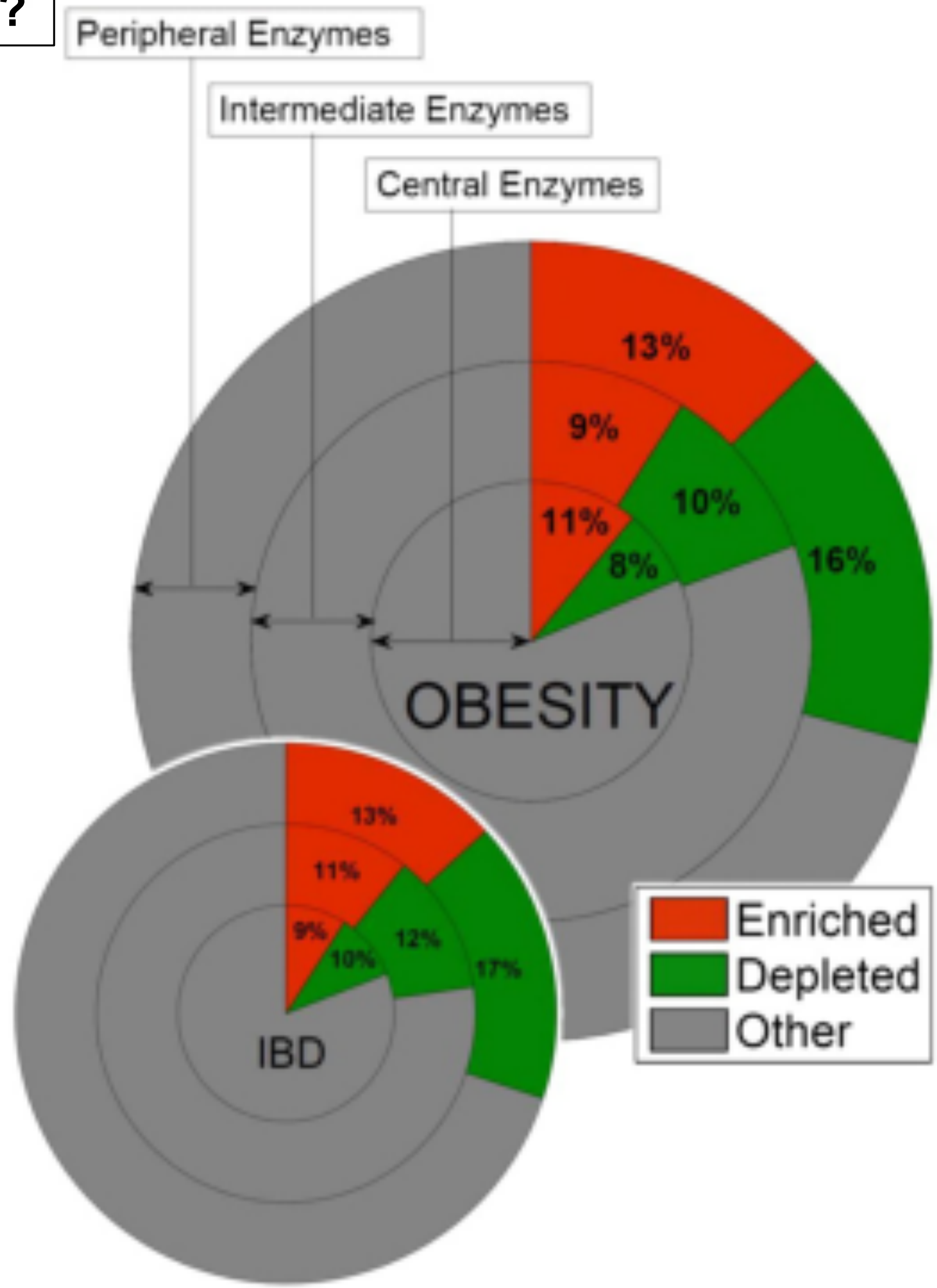
normal mouse

Mutant in gene that encodes leptin, a hormone that suppresses appetite.

Can the microbiota be manipulated?



What about other diseases of the gut?



Greenblum S et al. *PNAS* 2012;109:594-599

A balance of the gut microbiota is essential for human health; pathogens are unusual partners.

You are born relatively symbiont-free and then acquire your microbiota through a complex ecological succession.

Once your microbiota matures (early childhood), your microbiota is relatively stable in composition. Variations in ratios and proportions follow change or perturbation.

Two bacterial types, the Bacteroidetes and Firmicutes, are the most abundant in mammals, including humans.

Humans are losing their coevolved microbial partners, with significant consequences to health

Bacteroidetes are higher in proportion in lean individuals.

Both obesity and inflammatory bowel disease (IBD) show alterations in the ecology of the microbiota, as well as lower overall diversity.

Experimental models demonstrate that the microbiota ecology is affected by host genetics and the composition of the microbiota.