A BRIEF HISTORY OF MICROBIAL TECHNOLOGIES

UO-CHC 441H/431H: Microbes + Social Equity
Lecture 2
Dr. Sue Ishaq Pellegrini

Outline and learning objectives

- Extremely brief overview of microbiology and microbial ecology as pertains to this class and understanding journal articles

- How did we first discover microorganisms?
- Historical perspective of ecology and microbial ecology
- How ideas were shaped by emerging technology
- **TL;DR: Everything we know about microbes has always depended on available technology**

- Discussion: role of scientists in communicating science

The days before microorganisms were a thing

- Speculation there was some unseen cause for disease since BC
  - *Humors? Demons? "Seeds"? Something else?*

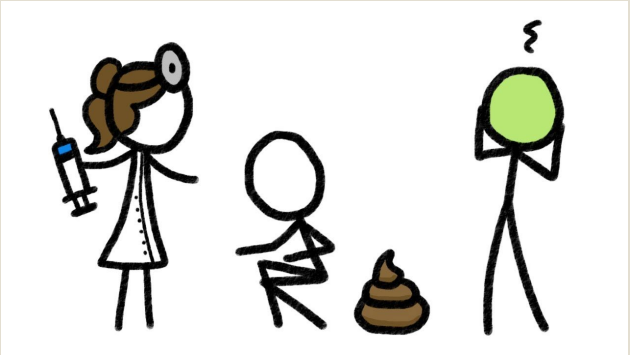
- We could see the effects of microbes, even if we didn't know it

PLATE LXXII.

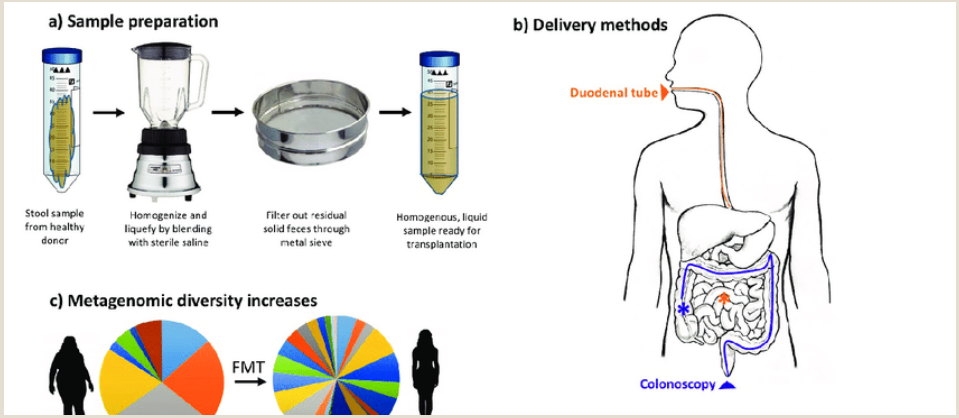
Obsolescent variolous lesions. On the hand the brown or black crusts were still adherent. On the forearm many of the crusts had become separated, the places from which they had fallen being stained pink.

Using microbes to treat microbial diseases, part 1

- Before we even knew about microbes, we were using them to treat microbial problems
- 300 A.D. China: used fecal transfers from infants to treat food poisoning and diarrhea
- Bedouins (middle east): used fresh camel feces to treat dysentery
- 1500s farmers use transfaunation
  - *transfer of rumen material/cud/feces to treat GI dysbiosis in livestock*



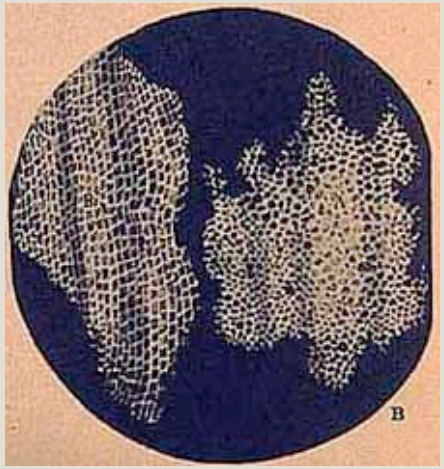
Poop Transplants! - YouTube



Fecal Microbiota Transplantation

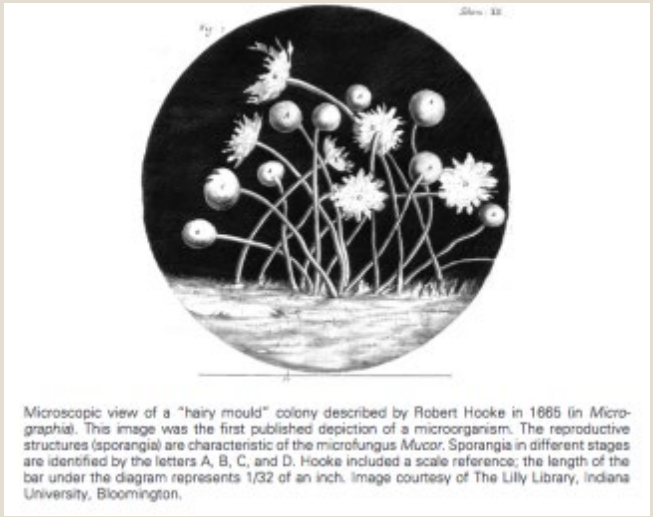
The first glimpse of cells

- 1665: Robert Hooke publishes Micrographia



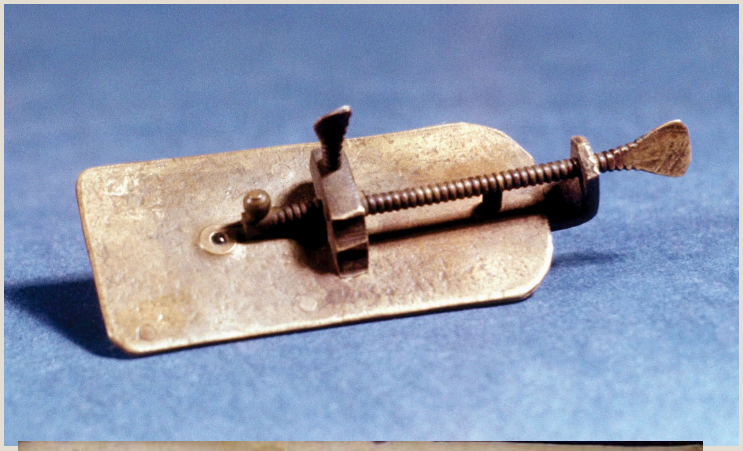
- Designed a compound microscope with illumination to look at plants up close
  - *allowed him to view **cells** in a slice of cork tree*
  - *So named because they looked like rooms in a monastery*

- **Also described first microorganism**
  - ***microfungus Mucor, a bread mold***



Microscopic view of a "hairy mould" colony described by Robert Hooke in 1665 (in *Micrographia*). This image was the first published depiction of a microorganism. The reproductive structures (sporangia) are characteristic of the microfungus *Mucor*. Sporangia in different stages are identified by the letters A, B, C, and D. Hooke included a scale reference; the length of the bar under the diagram represents 1/32 of an inch. Image courtesy of The Lilly Library, Indiana University, Bloomington.

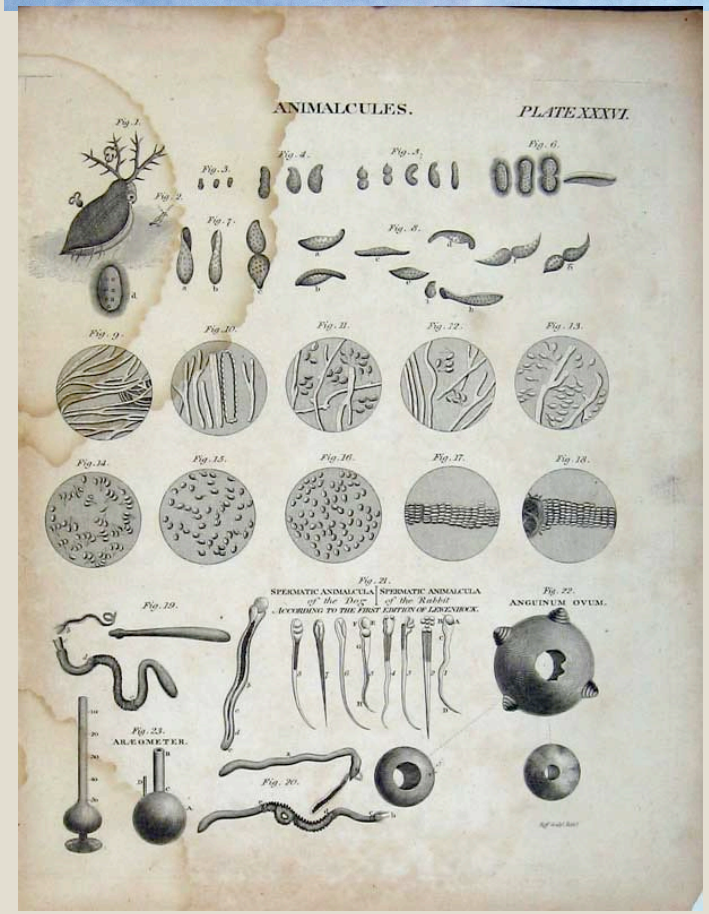
Antony van Leeuwenhoek discovers host-associated microorganisms by accident



"layu-wen-hook"

- 1678: A Dutch tradesman with no education was inspired by Hooke's Micrographia

- Decided to learn to grind lens and made microscopes
  - Viewed different substances and discovered **protozoa** ("animalcules"), muscle fibers, bacteria, blood cells, and sperm



Microbial ecology begins with regular ecology

- 1700s - 1900s Ecology was wrestling with many questions, which have all been answered elsewhere.
  - *Microbial ecology developed when we realized not all macro-ecology applies to microbes*

- WHY do organisms live where they do? How did they get there?
  - *Are our microbes incidental or adapted to live with us?*
  - *Do we need SPECIFIC microbes or ANY microbes to train our immune system?*
  - *How do we recruit "good" microbes?*
  - *Does it matter which microbe is there as long as the community is functional?*

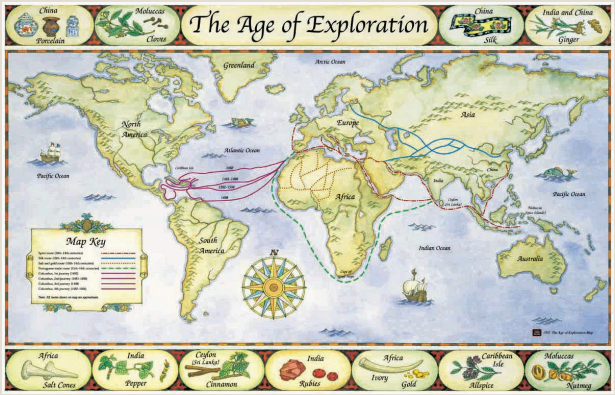
- Can you CHANGE a species to make it suitable for a certain habitat?
  - *Antimicrobial resistance*

THEORY OF MICROBIAL SPECIES' DISTRIBUTION

How do (micro)organisms end up where they are?

Are animal/plant species location-specific? Or can they disperse to new habitats?

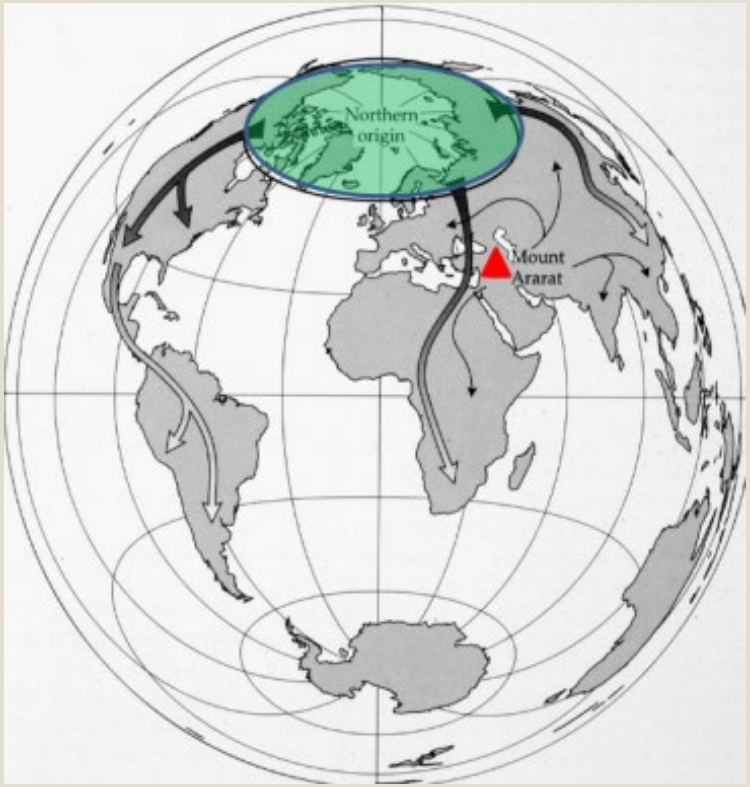
- Up to early 1800s
  - *Prevailing theory: there was a single speciation event*
  - *Species located in specific locations because they were +/- put there*
- The Age of Exploration 1600 - 1800s
  - *new information about species all over the globe*
  - *noticed similarities between animals on different continents*
  - *led to the idea that species started in one place and dispersed around the globe*



The beginnings of species distribution and speciation

- George-Louis Buffon (1707-1788) theories
  - *high waters and hot temperatures when Earth was created*
  - *animals/plants originated at the poles and moved towards the equator as the Earth cooled*

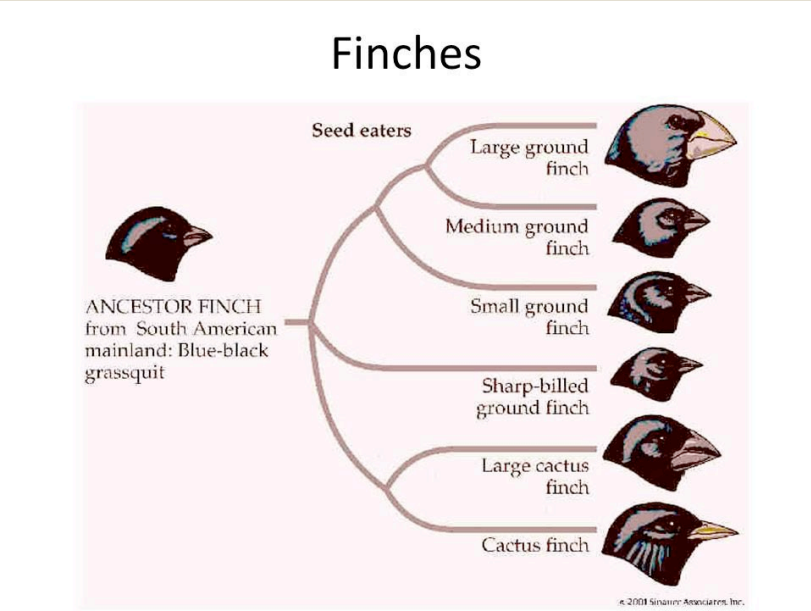
- **Wrong, but two important ideas hit the scientific community**
  - *Species could change to improve themselves in new environments*
  - *Species could degrade in new environments*
  - ***Contrary to the belief that species were unchanging***



Theory of Natural Selection

- Charles Darwin (1809–1882) went on a 5-year voyage aboard the HMS Beagle 1831-1836
  - *Was a geology mission*
  - *Puzzled by the slight variations of animals (finches and tortoises) on different islands*

- Began to think that maybe species can change over time
  - *Spent next 15 years working on it*



It all comes back to cows

- Darwin started suffering health problems and started taking time off in the UK countryside
  - *Noticed cattle breed <u>selection</u> by farmers*
  - *Selection: idea that certain traits can be increased in a population, either from human intervention or natural causes*

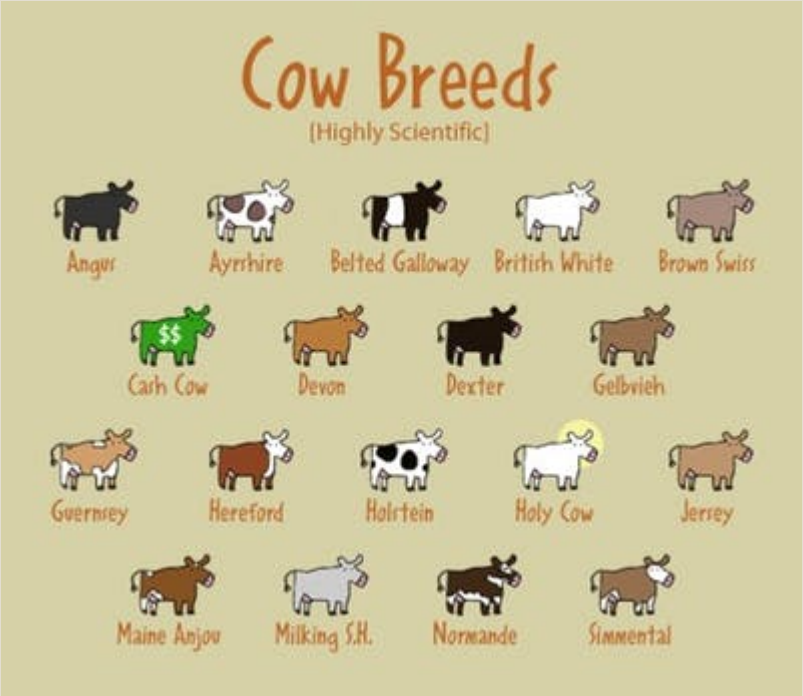


Image: Diet Doctor

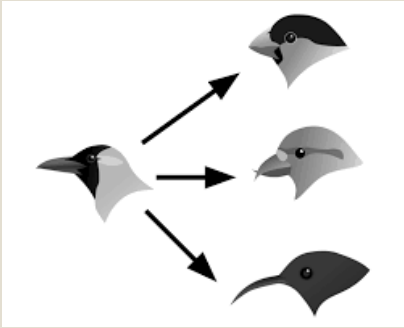
Speciation and evolution become a thing

- Alfred Wallace (1823 – 1913) was working on same theories on other side of the globe
  - *Contacted Darwin, started publishing together*
  - *Wallace's theory was closer than Darwin's, pushed Darwin to finish his work*
  - ***Darwin has social status/family money, age, proximity to other naturalists (Wallace was in the bush), and reputation on his side***

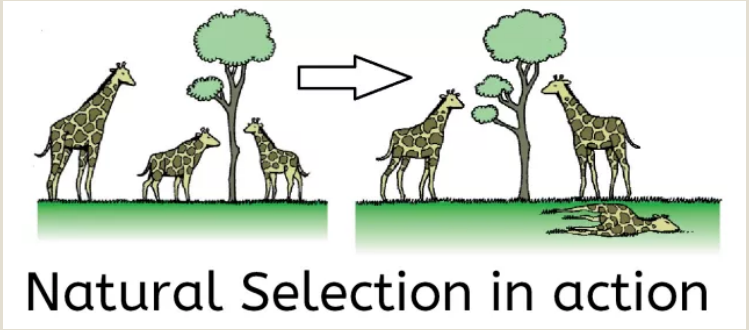
- Idea: Species continually disperse themselves to new locations, but they aren't suited to survive everywhere
  - **This leads to "speciation" or adapting to the local environment** (i.e. Galapagos finches)

Speciation and evolution become a thing

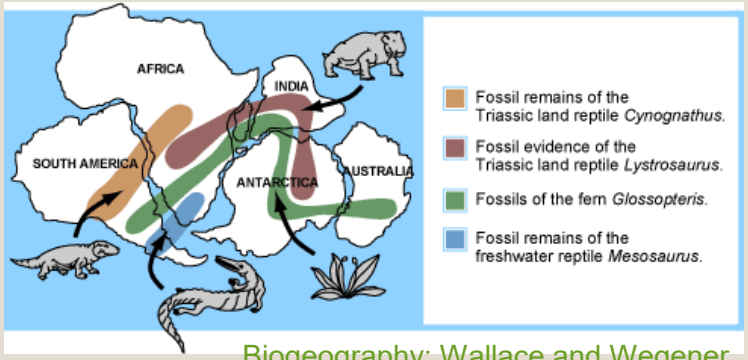
- **Speciation**
  - *adapting to the local environment*
  - *Led to theories of:*
- **Natural Selection**
  - *ability to survive an environment encourages the spread of genes that helped you survive*
- **Biogeography**
  - *geographic distribution of species*



EU 1.C - Biodiversity & speciation (practice) | Khan AcademyKhan Academy



Can Natural Selection Make Anything?Creation Science 4 Kids



Biogeography: Wallace and Wegener

“Everything is everywhere, but the environment selects”

- 1934 Becking
  - *Work on halophilic microbes in salt lakes, only few can survive there*
  - ***postulated that environmental factors select for particular biochemical abilities/taxa***

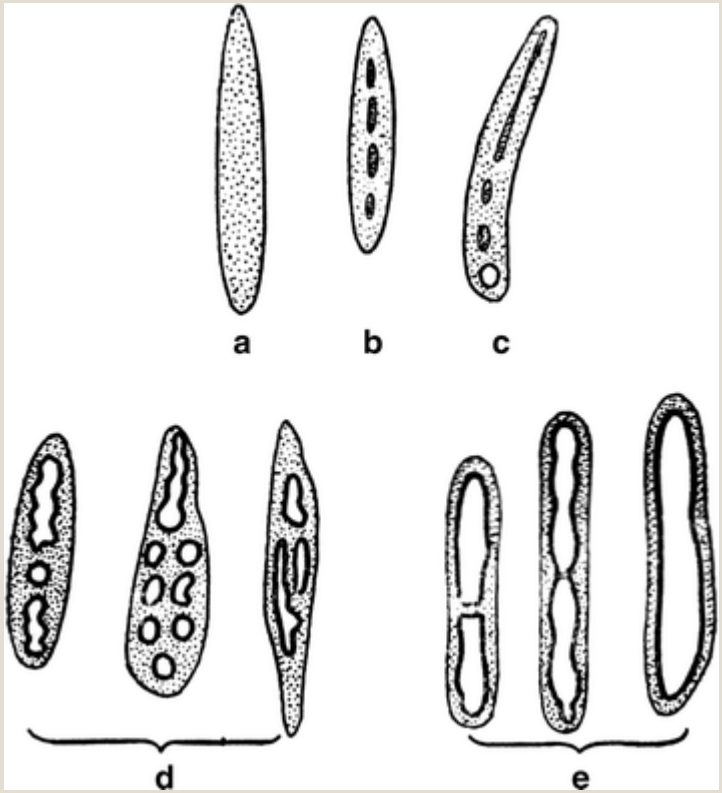


Image: The Halophilic World of Lourens Baas Becking

Two major theories about microbial distribution

Everything is everywhere

- **Microbes are easy to disperse, so they are the exception to biogeography**

- Supported by transient nature of the microbial community
  - *Some taxa pop in and out*
  - *Would mean than any microbes can be anywhere and we don't need certain ones*

Environment selects

- **The idea that environment is selecting for certain species (a.k.a. biogeography)**

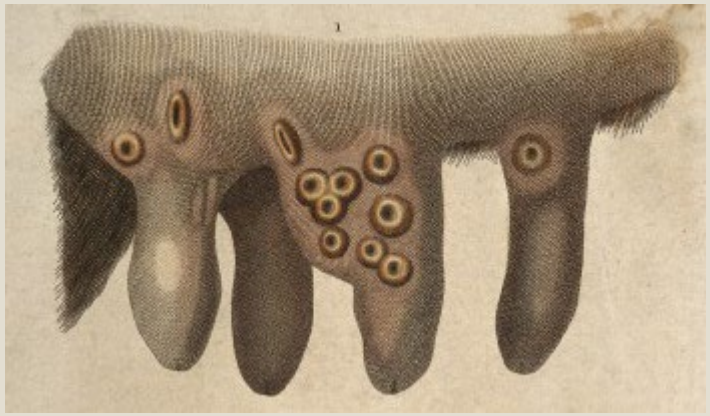
- Supported by body site specificity for the microbial community
- Means that there is a "correct" or "healthy" microbiota in a location

GERM THEORY

Now that we are thinking about how microbes move around – could we be transferring microbes that make us sick?

Using microbes to treat microbial diseases, part 2

- Observation: Milkmaids who previously caught cowpox did not catch smallpox
- Hypothesis: perhaps the milder infection of cowpox is protective against smallpox?



- Experiment: 1796 Jenner creates smallpox vaccine using pox scraped off cows
  - *Jenner called cowpox Variole vaccinae*
- Conclusion: yep

Images: Wikipedia, CDC

Wait, are we the vector?

- Prior to basic hand hygiene, most people died in hospitals

- 1850s Semmelweis
  - *advocates for hand washing to prevent the spread of disease*

- 1861 Pasteur
  - *Publishes news articles on **Germ Theory***
  - ***The idea that microorganisms are the causative agent for most diseases***

- 1867 Lister
  - *advocates for sterile surgery conditions*

Images: NPR, Method Quarterly

Microbes and Causative Agents

- Need to be able to grow it in the lab to study it
- 1881 Koch finally able to grow bacteria on solid media so we could see it
  - *Based on recommendation by cook Angelina Fanny Hesse (wife of Walthar Hesse his scientific illustrator) to use agar instead of gelatin*
  - *Agar is a polysaccharide made from seaweed*
    - heat-stable and able to withstand sterilization during prep

Culturing microorganisms in media (broth or agar) on purpose

- 1885 – 1890s Beijerinck
  - *Developed selective (enrichment) media to grow only certain kinds of microbes*
  - *Studied bacterial biochemistry*
- Culture-based identification is only as good as your media recipe!
  - *many microorganisms require very specific things they usually get from symbiotes*
  - *95% WON'T GROW IN CULTURE*

Microbes as Causative Agents

- We know that we can grow them, see them on plates, and under a microscope
  - *we think they might cause disease, but how do we KNOW?*

- **Causative agent is anything that is KNOWN to cause disease or illness**
  - *Usually refers to a biological pathogen, also a toxin or chemical*
  - *Must be proven*

Koch's Postulates on Causative Agents

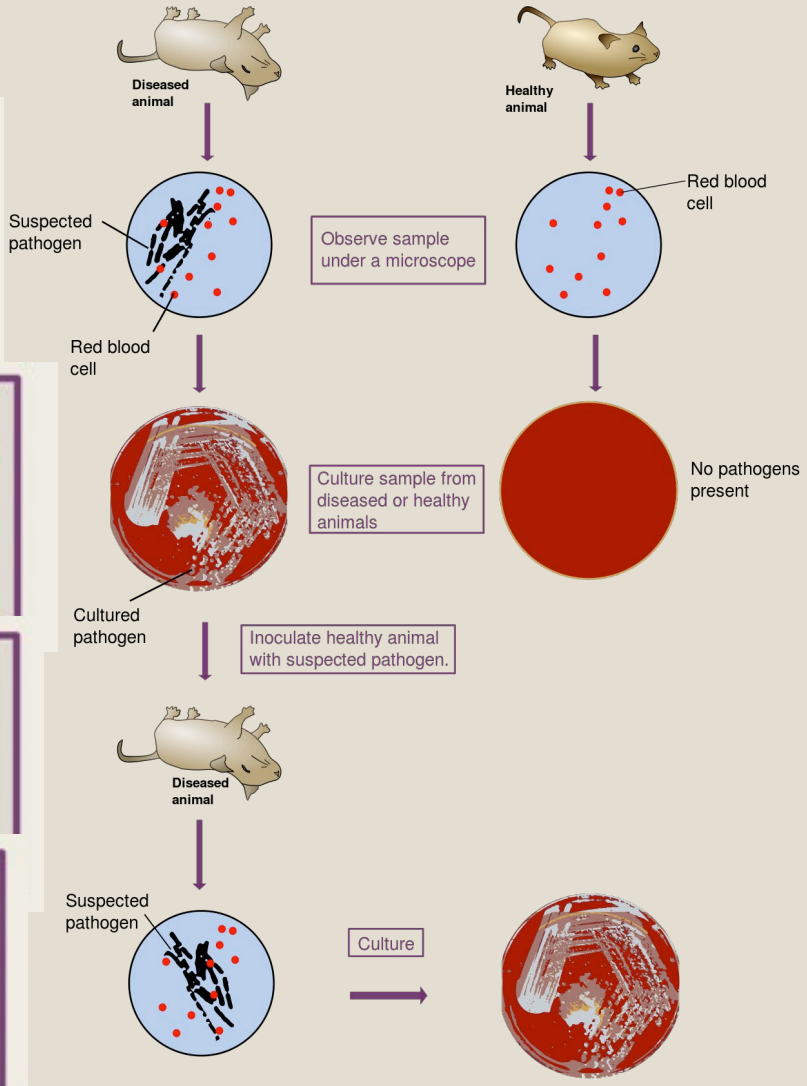
Koch's Postulates:

① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

② The microorganism must be isolated from a diseased organism and grown in pure culture.

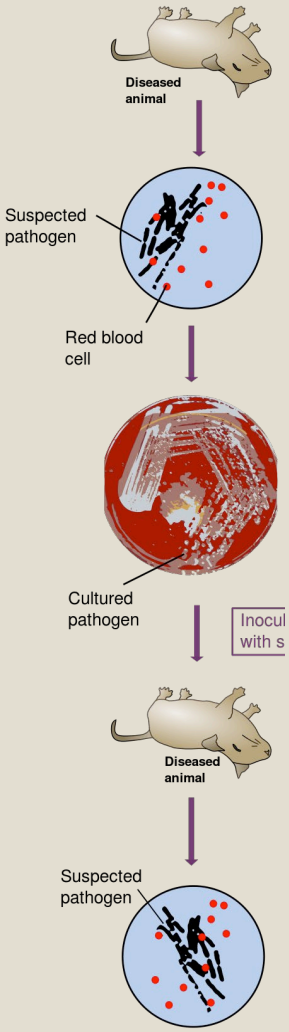
③ The cultured microorganism should cause disease when introduced into a healthy organism.

④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.



Koch's Postulates and human disease

- Many human diseases technically haven't been tested with all the Postulates
  - *Ethical to infect a person with a disease just to make sure it's the causative agent?*
  - *Disease is more nuanced than just crossing paths with a microbe*



Ex. Zika virus

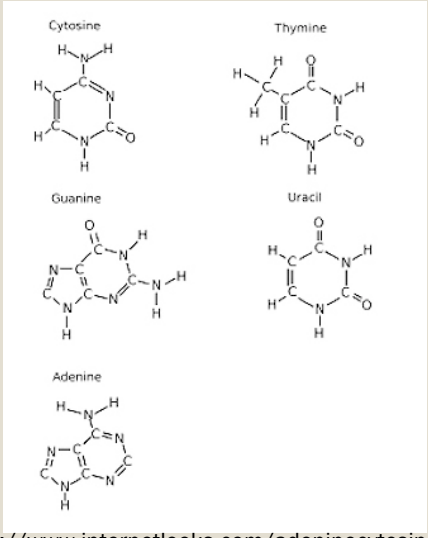
- Previously known, but not to cause such virulence or severe symptoms
- 2015 there was speculation about whether the virus caused microcephaly in infants or the mosquito insecticides did
  - *Backlash against trying to find a vaccine*

DNA BASICS

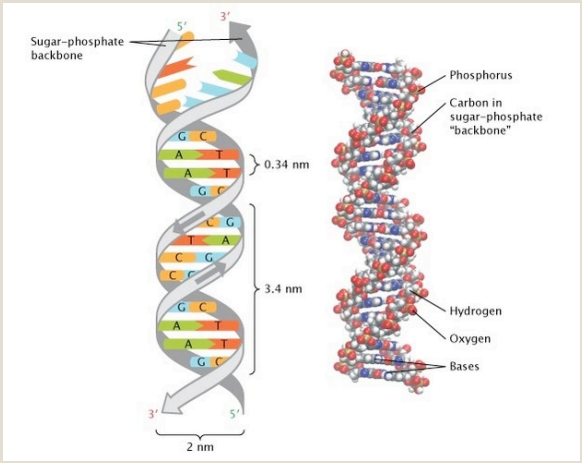
200 years of biochemistry in three slides

DNA basics

- 1860s: chemist Miescher broke cells open and precipitated DNA but didn't know what it was
- 1881: biochemist Kossel identified deoxyribonucleic acid (DNA) and its components:
  - *Deoxyribose sugar, phosphates*
  - *4 nucleotide bases: adenine (A), cytosine (C), guanine (G), thymine (T)*
- 1950s: Chargaff discovers
  - *Pattern to base quantities (a = t, c= g)*
    - Nucleotides always pair A-T and C-G across the 2 strands
  - *Nucleotide amount is different for different species but not within species*
    - **the genome length is the same within a species**
  - ***Nucleotide order is different for different species***
- 1953: Based on crystallography photos, Watson and Crick come up with double-helix structure
- 1957 Kornberg discovered a **DNA polymerase enzyme**
  - ***Helps build new DNA during replication***

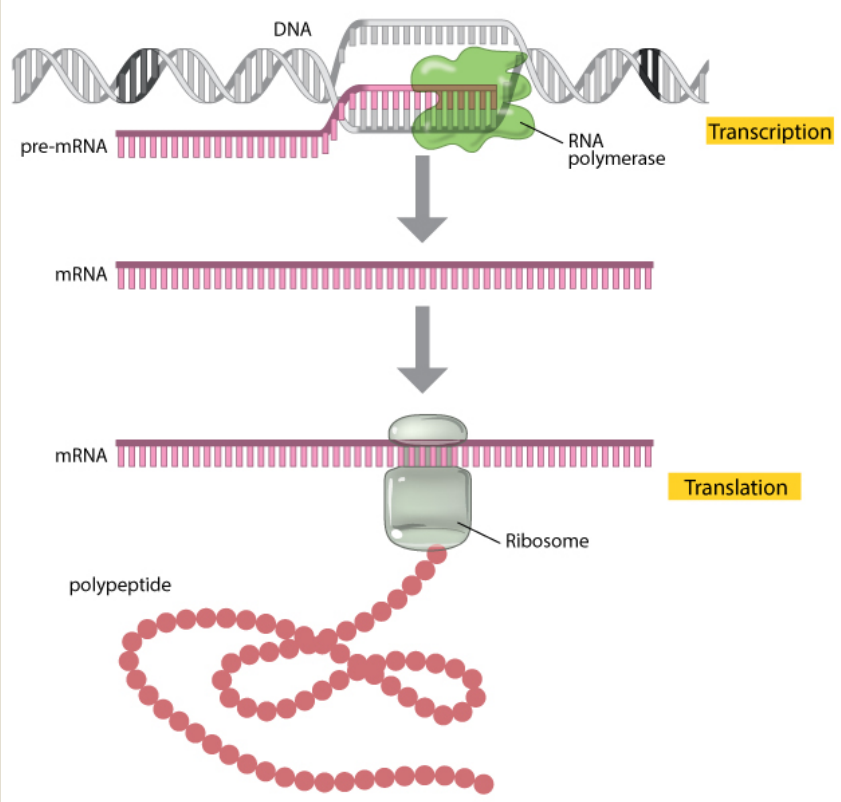


http://www.internetlooks.com/adeninecytosineguaninethymineuracil.jpg



Theory of Central Dogma and some thoughts

- 1958 Crick states Theory of Central Dogma (DNA -> RNA -> protein)
  - DNA code is translated to short-term RNA copies that are used to transcribe proteins
- DNA is the master code
  - *Ex. recipe book*
- RNA is the short-term mirror image copy
  - *Ex. tracing of the recipe, can be all or part of it*
- The ribosome reads the RNA and assembles amino acids into a protein
  - *Proteins (enzymes) give the cell functionality*
  - *Ex. the meal you make*



https://www.nature.com/scitable/topicpage/translation-dna-to-mrna-to-protein-393

Epigenetics,
the geneticist's version of Nature vs. Nurture

- The change in organisms based on **modification of gene expression, not changes to the genetic code**
  - *More common in eukaryotes*
  - *Ex. DNA methylation*
- Having a gene does not mean it gets translated
  - *Gene expression is how much RNA (and protein) are made*
- Slight differences in the DNA code can make very different proteins which might not work the same

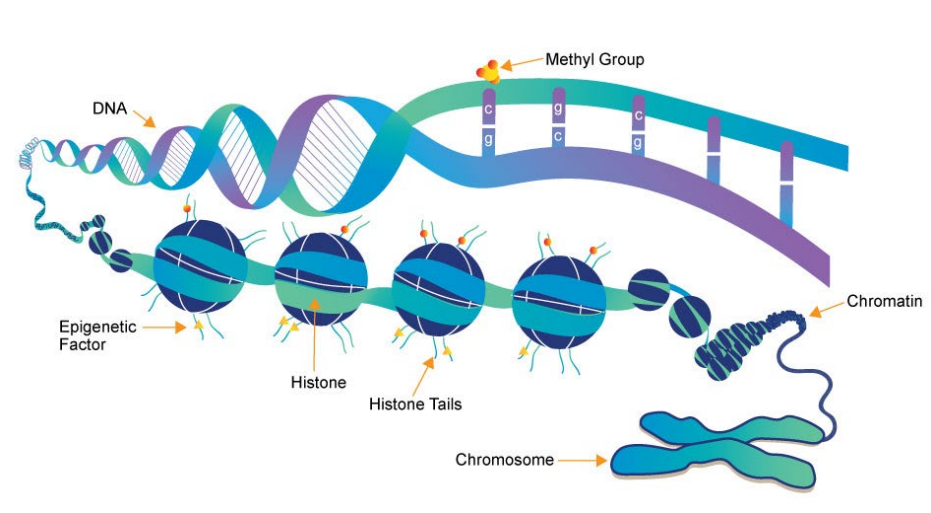


Image: whatisepigenetics.com

STRATEGIES FOR SEQUENCING TECHNOLOGY

The VERY short version

Sequencing tech mimics DNA replication so a computer can identify the nucleotides and their order

- Different technologies use different enzymes, reagents, or strategies
  - *Nuance is immaterial to this class*
  - *This info is a generalization to aid in reading comprehension*

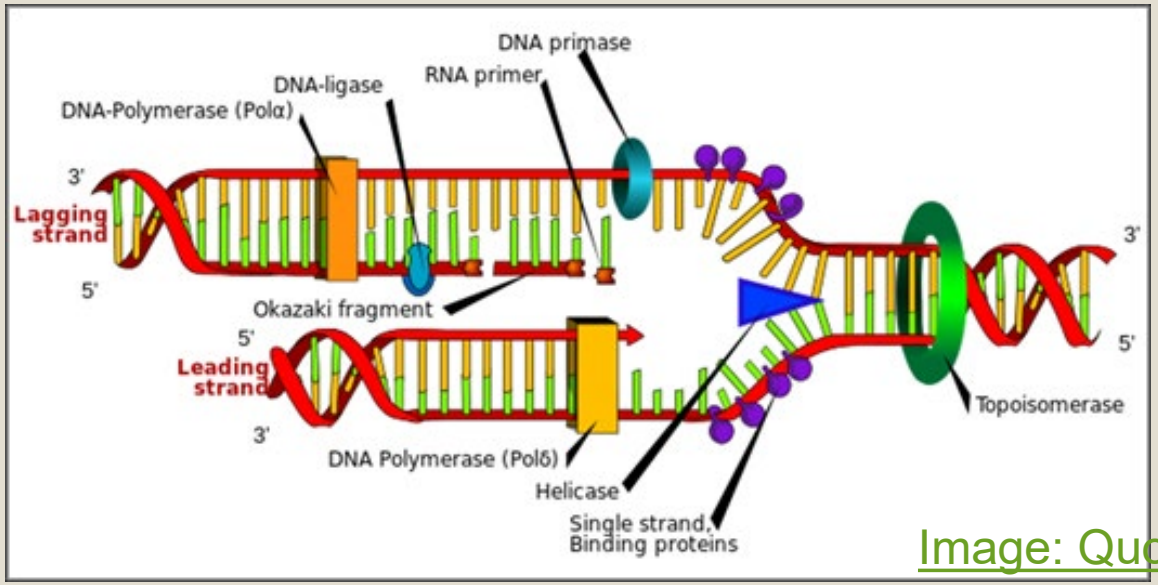
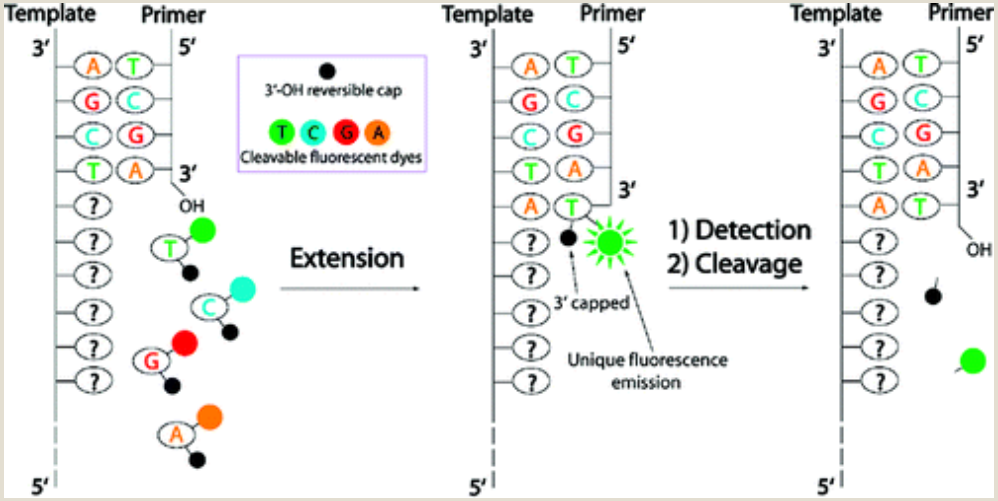


Image: Quora

Next Generation Sequencing strategies: mimicking DNA replication

DNA replication recipe:

- *The DNA of interest, extracted and cleaned from one organism or all the cells in a sample*
- *DNA polymerase enzyme that builds DNA*
- *Free DNA bases to add, that have fluorescent dye attached*



1. DNA polymerase adds the base to the new DNA sequence
    1. Will always add a base that pairs with the unknown template DNA
2. The dye gets removed from the base that was just added
3. Removing the dye causes a chemical reaction and a colored flash of light
4. The camera sees color flash and records what color/nucleotide base it was

Image: 454 Pyrosequencing
https://cdn-pubs.acs.org/doi/10.1021/ar900255c

Which part of the DNA do you want to sequence: random or targeted

- "Shotgun" using random DNA fragments
  - *take whole genomic DNA*
  - *create small fragments*
  - *ligate (paste) generic primers (scaffolds) on the end of all the pieces*
  - *sequence all pieces using scaffold as place for enzyme to attach*

One genome

many genomes - **metagenomics**

Which part of the DNA do you want to sequence: random or targeted

- "Amplicon"
  - *Use primers to **target** specific genes or parts of genes*
    - Creates scaffold so polymerase enzyme acts in certain areas of DNA
  - *16S rRNA in bacteria and archaea, 18S rRNA in protozoa, ITS 1 or 2 in fungi/plants*

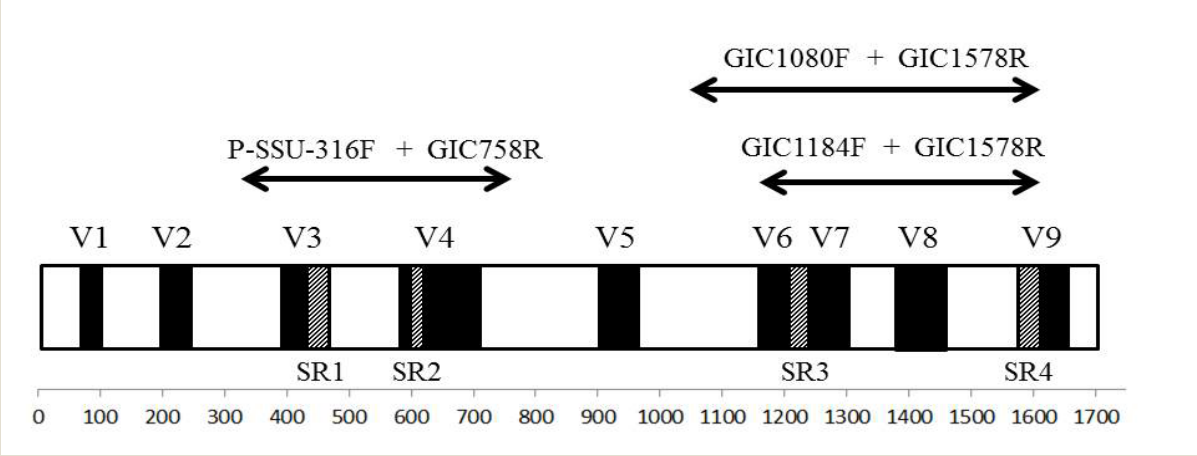


Image: Ishaq and Wright, 2014)

HOW ARE WE GOING TO COMPARE DNA SEQUENCES?

the emergence of rRNA-based techniques

Genetic Distance

- Mathematically quantify the number of nucleotide differences between two organisms (within a gene)

- **Genetic Distance:**
  - *Align 2+ sequences,*
  - *count the number of different bases they have out of the whole = **% divergence or distance***
  - *The number of bases they share out of the total is **% identity***

Comparing apples to apples

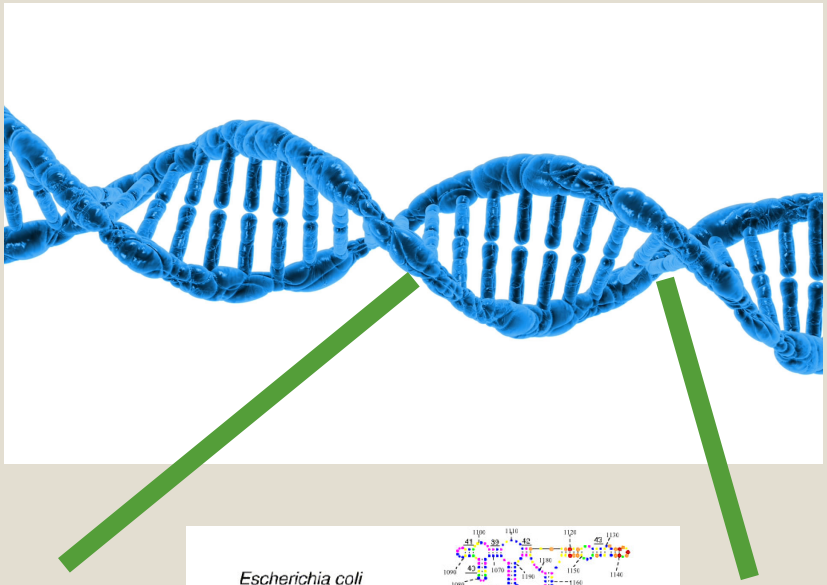
- We know that different species have different nucleotide order (Chargoff 1950s)

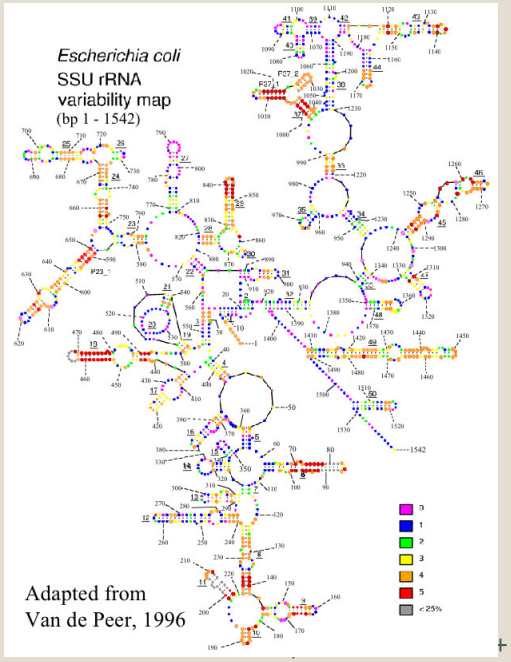
- How do we compare genetic code?
  - *Not all organisms have the same genes, especially microbes!*
  - *Prokaryotes vs. eukaryotes*
  - *Viruses*

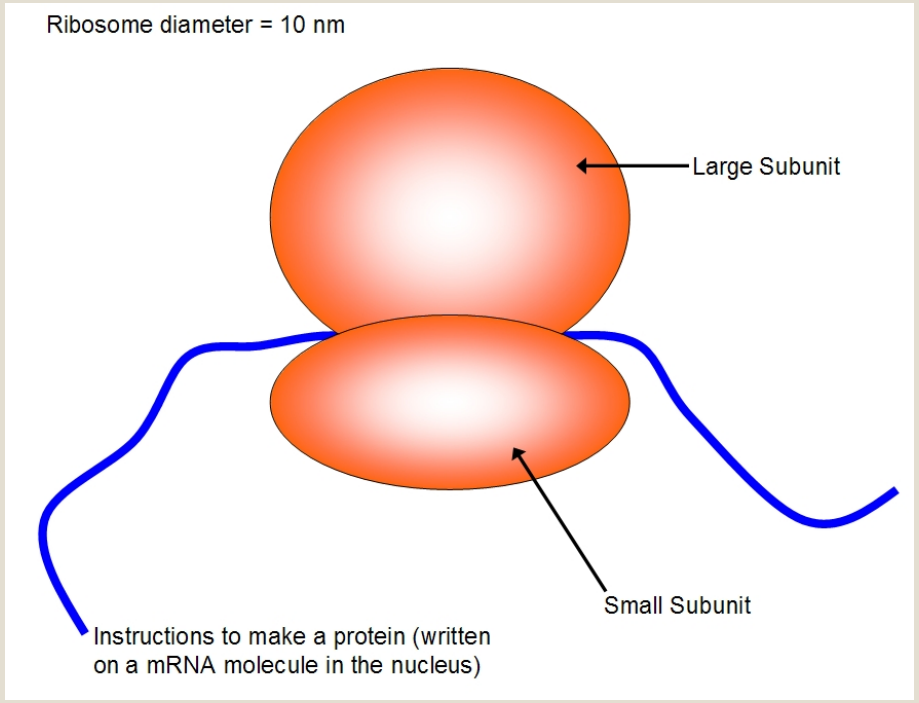
Picking an “identification” gene

http://www.publicdomainpictures.net/view-image.php?image=42718&picture=dna

rDNA genes that code for ribosomes







Ribosomal RNA gene (rDNA)

- Ribosomes are ubiquitous in living organisms
- Critical for reading RNA transcripts and creating amino acid chains for proteins
  - *If they are non-functional because of mutations, cell dies*

- **rDNA,** or ribosomal **gene** is DNA
  - Found in living, inactive, or dead cells

- rRNA, or ribosomal RNA is RNA and is the functional part in the cytoplasm
  - Found in active cells
  - In dormant cells in very low numbers

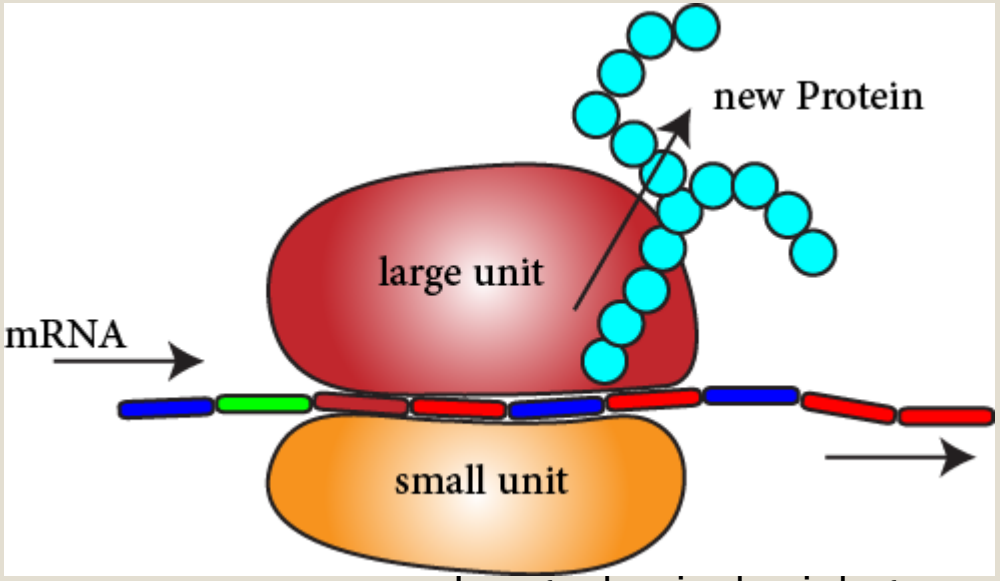
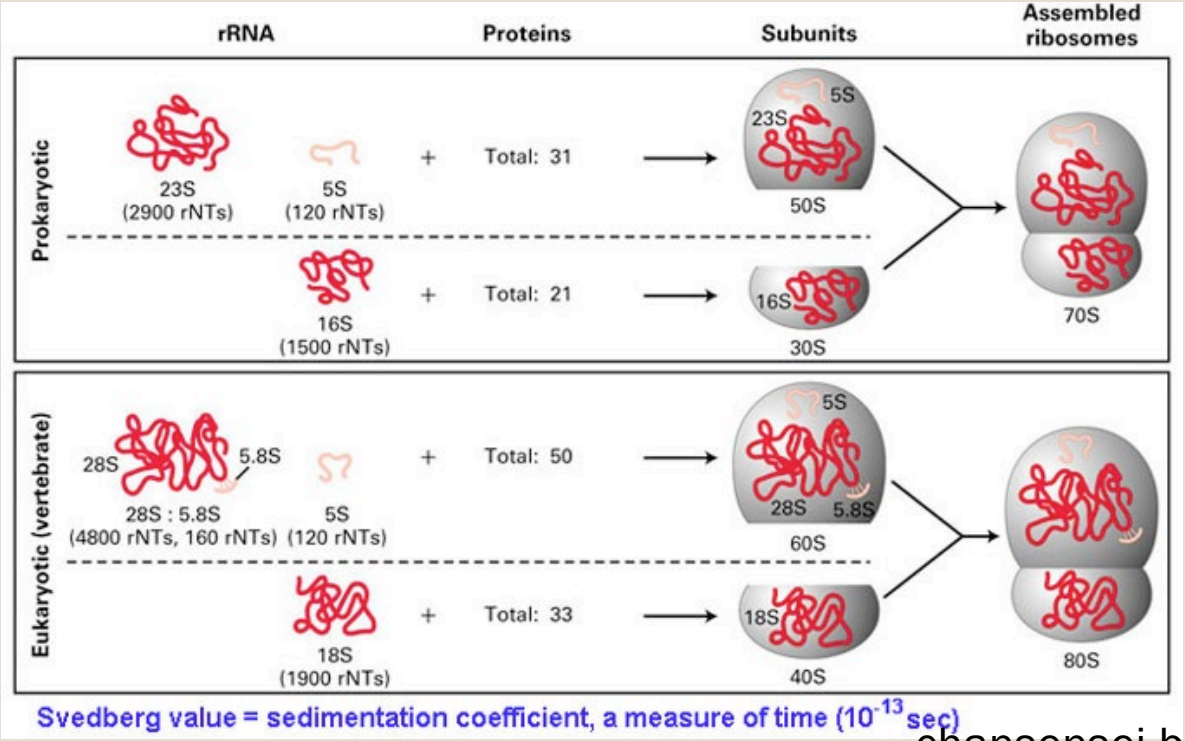


Image: basicphysiology.com

Ribosomal RNA gene (rDNA)

- Each ribosome has 1 small subunit, 1 large subunit
- All subunits made of ribosomal RNA and stabilized with proteins
- Subunits named by rough size, ex. 16S
  - *Svedburg units (S) are sedimentation rates, which roughly measure size of 3D molecules*



chansensei.blogspot.com

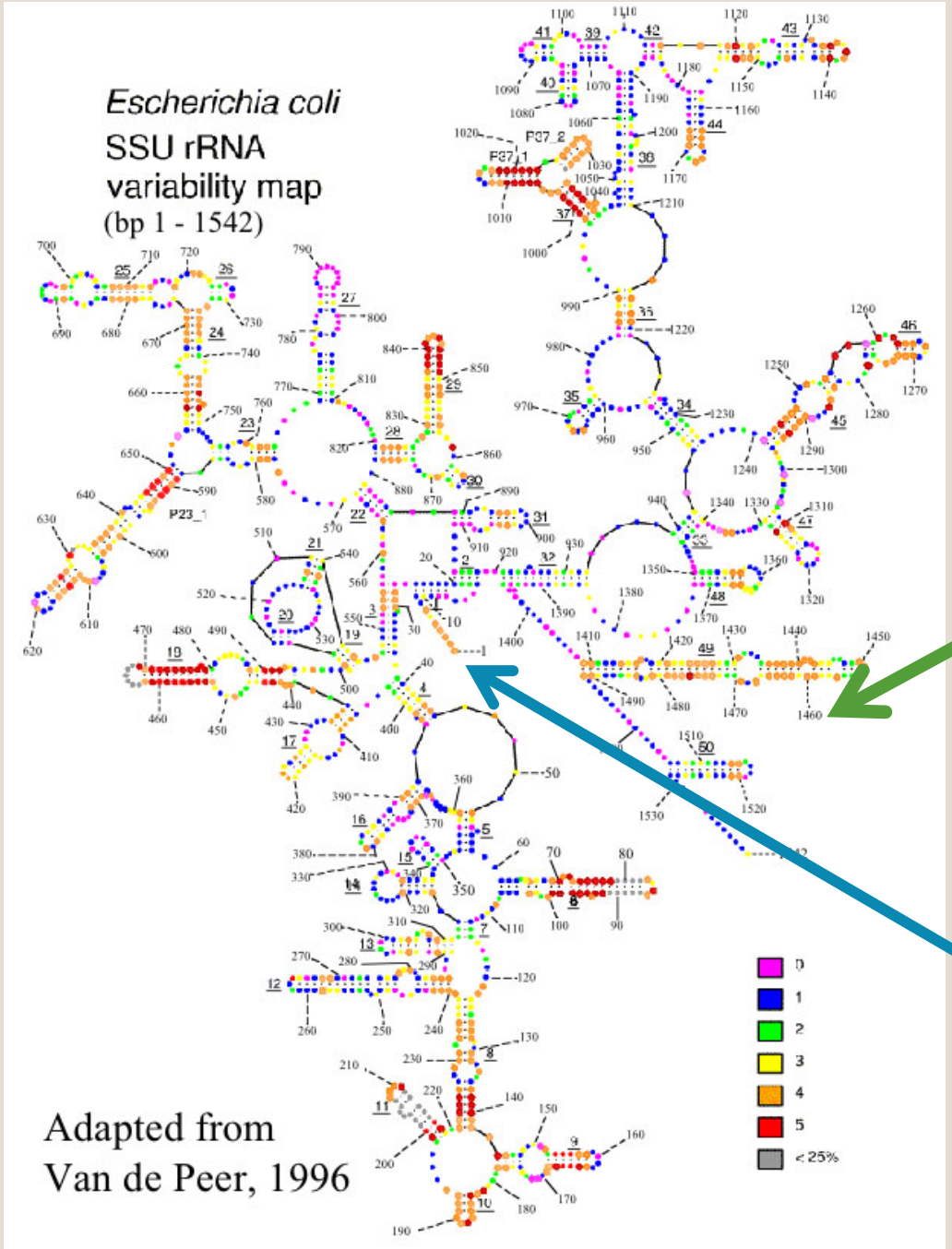
Prokaryotes vs Eukaryotes

Prokaryotes

- 16S, 23S, and 5S rDNA found together in genome as an operon (functional unit)
  - *Operon may have 1+ copies throughout genome*
- Highly variable between taxa

Eukaryotes

- rDNA copies in tandem repeats (one after the other)
  - *Ex. humans have upwards of 400 repeats in 5 locations*
- Also have mitochondrial RNA or chloroplast RNA
- Can be less variable between taxa, harder for identification



rDNA is a good ID because ribosomes have predictable evolution rates

Outer regions are **Variable** between multiple taxa because more exposed, prone to error/changes

Inner regions are **Conserved** across multiple taxa, if they mutate the ribosome might not work

Primer targets!!

Alternative gene for ID: Internal Transcribed Spacer Regions (ITS)

- Non coding regions that sit between ribosomal genes on RNA
- Can be used for taxonomic identification
  - *Ex. Fungal/eukaryotic taxonomy is more complicated and sometimes the ITS is more informative than 18S*
- Not great for phylogeny/evolution
  - *Length is highly variable*
  - *Very difficult to align the sequences and get idea of ancestry/change over time between taxa*

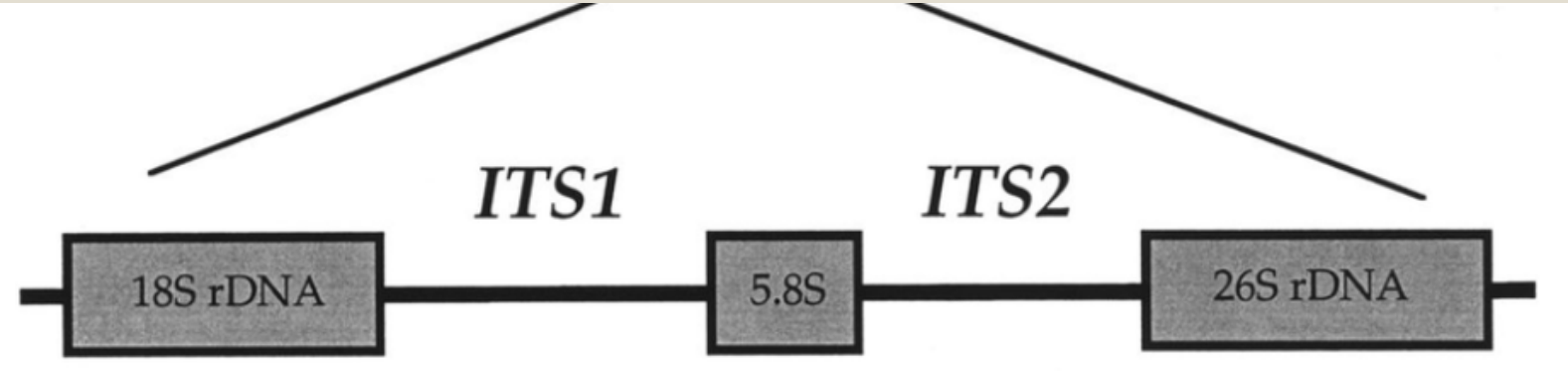


Image: Shaw et al. 2002

Taxonomy vs. function

- Viruses don't have ribosomes – require host cell machinery
- rRNA and ITS only tell you about who the microbe is
  - THEY CONTAIN NO INFORMATION ABOUT THE FUNCTIONALITY OF THAT MICROBE
- Many microbes have plastic genomes
  - Mobile genetic elements
  - Plasmids
  - "DNA expansion packs"

Horizontal gene transfer

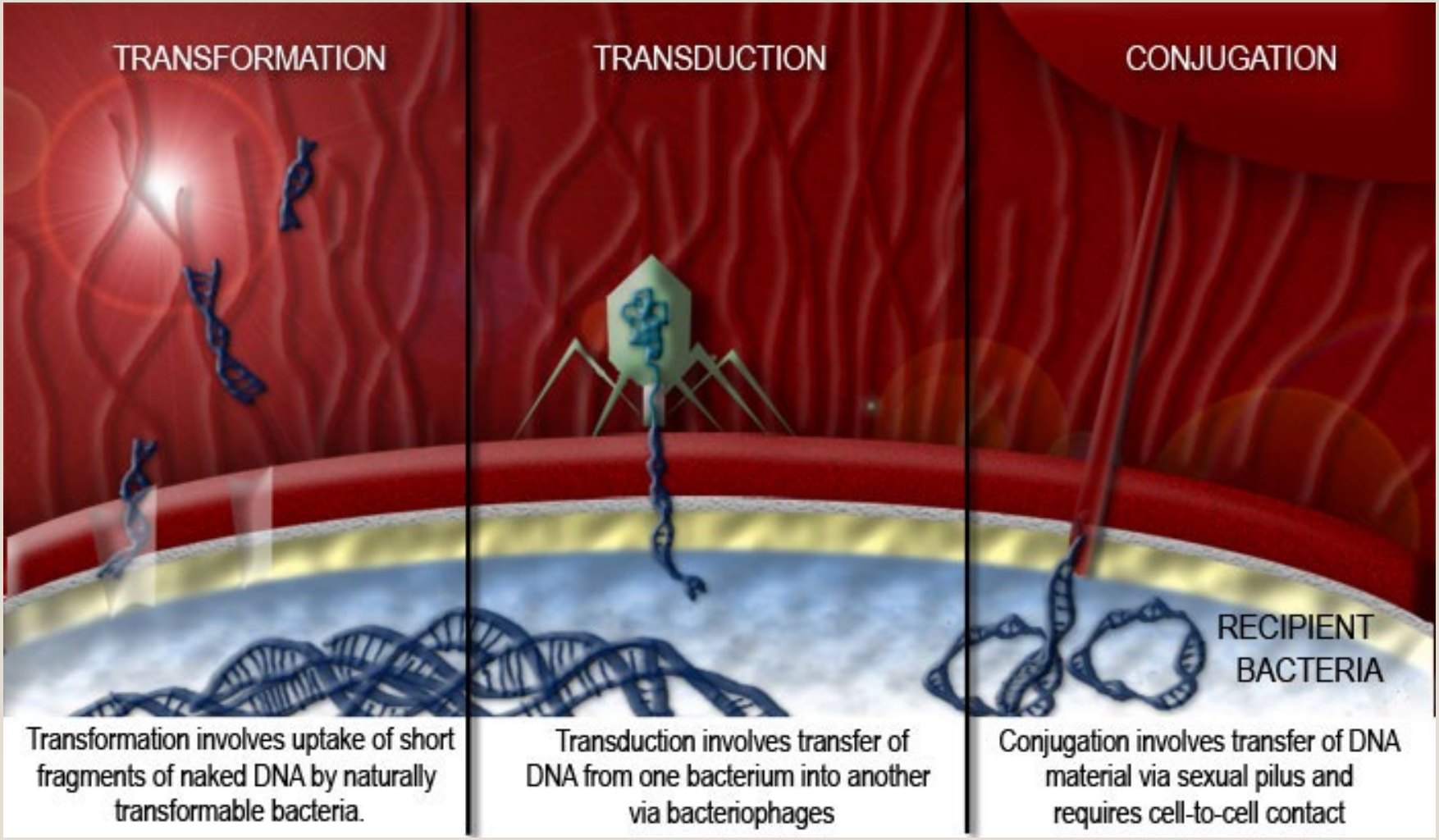


Image: Antimicrobial Resistance Learning Site - Michigan State University

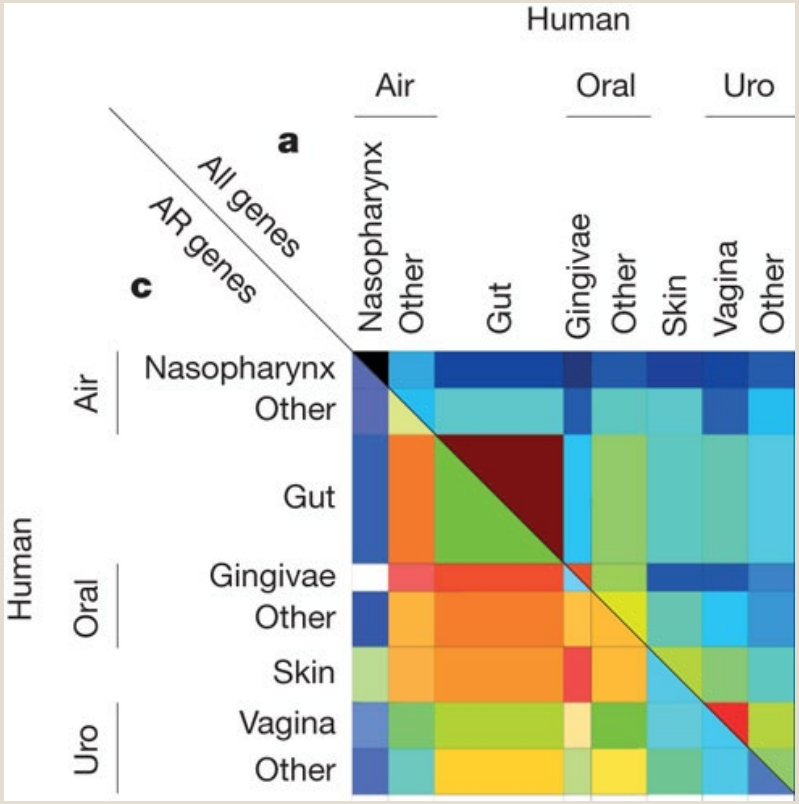
HGT

Across domains

Bacteria <-> fungi

Bacteria <-> protozoa?

Spreading antimicrobial resistance



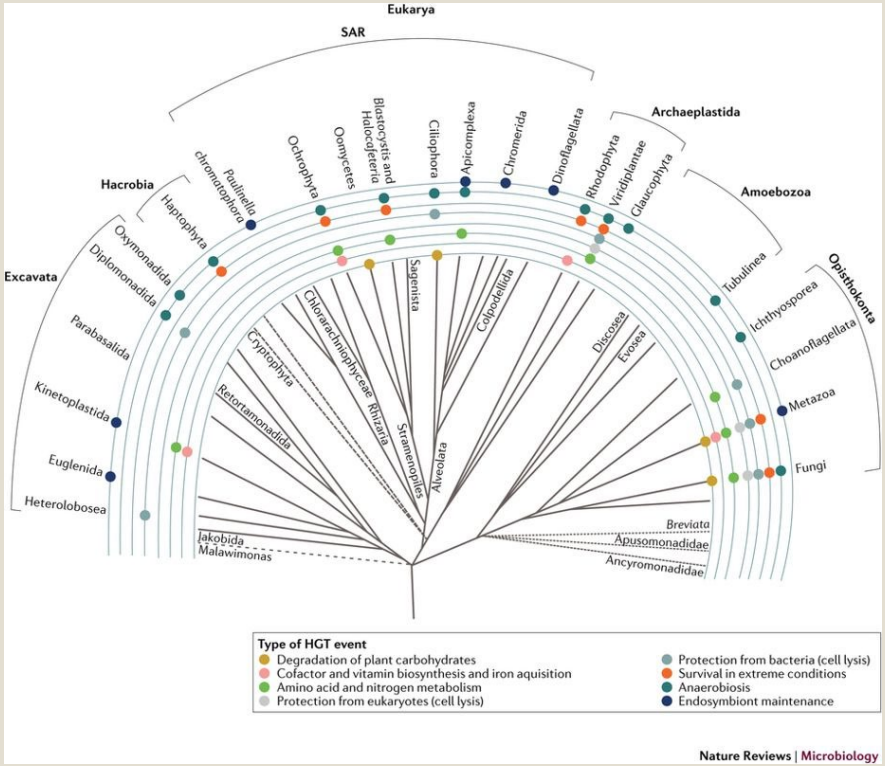


Figure right: Husnik and McCutcheon, 2018, Nat Rev Microbiol

Figure left: Smille et al. 2011 Nature

Measuring microbial ecosystems

Nucleic acid-based

- Amplicon
  - *Examine "who" from one type of microbe at a time*
- Microbiome
  - *Study of the genetic material of ALL the microorganisms in an environment*
  - *Ex. just studying bacteria DOES NOT count as microbiome work*
  - *Some people mean a specific gene, some people mean genomes*
- Transcriptome
  - *Study of the RNA transcripts produced by a microbiome*

Protein-based

- Proteome
  - *Study of the proteins produced by a microbiome*
- Metabolome
  - *Study of the metabolites produced by a microbiome*

DISCUSSION AND HOMEWORK

Discussion: science and society

- Today we accept the idea of beneficial microbes
- At one time we accepted that microbes made us sick and needed to be killed
- At one time we though flies spontaneously generated in rotten meat
- **Do scientists/researchers have an obligation to convince the general public?**
  - ***Should we be agnostic and option-free?***
  - ***Only publish our results, no editorials***

Homework

- **Reading**: Robinson_2010_structure to function in HAM
- **Assignment (4 pts):** Different types of scientific writing. Due 6/26
- More info:
- History of microbial ecology (lecture): IMM_2_microbial_ecology_2019
- Discovering DNA, related technology (lecture): IMM_3_DNAtechnology_2018
- DNA sequencing (lecture): IMM_4_Seq _Technology_2018_CHCversion
- Phylogeny (lecture): IMM_5_phylogeny_diversity_2018_CHCversion