

Unit 1—Who are the Microbes

Modern concept of a microbe has deepened through two major research tools:

- 1) Advanced microscopy
- 2) Sequencing of genomic DNA

Microbe: living organism that requires a microscope to be seen. Microbial cells range in size from millimeters (mm) down to 0.2 micrometer (μm), an virus may be tenfold smaller.

- **Super-size microbial cells:** some species of protists and algae, and even some bacterial cells, are large enough to see with the naked eye (i.e. the marine sulfur bacterium *Thiomargarita namibiensis*; a single cell plant: the “killer alga” *Caulerpa taxifolia*)
- **Microbial communities:** many microbes form complex multicellular assemblages, in these structure, cells are differentiated into distinct types that complement each other’s function
- **Viruses:** a non-cellular particle containing genetic material that takes over the metabolism of a cell to generate more virus particles.
 - o Some are only a few molecular parts or the size and complexity of cell
 - o Not fully functional cells, the mimivirus genome shows that it evolved from a cell

Microbial genomes are sequenced

Genome is the total genetic information contained in an organism’s chromosomal DNA.

- By determine the sequence of genes in a microbe’s genome, learn about microbe grows and associates with other species.
- By comparing DNA sequences, we can measure the degree of relatedness between different species base on the time since they diverged from a common ancestor
- The first organism whose genome sequence was determined was bacteriophage ΦX174 , a virus that grows in *E.coli*.
- Fred Sanger, who shared the 1980 Nobel Prize in Chemistry for devising the method of DNA sequence analysis that is the basis of genome sequencing.
- Claire Fraser-Liggett, past president of TIGR, which completed the sequences of *H.influenzae* (bacteria causes ear infection) and many other microbial genomes

Close relationships between microbes and higher organisms

- **Bioluminescence:** is the production and emission of light by a living organism.
- **Mycorrhiza:** is a symbiotic (generally mutualistic, but occasionally weakly pathogenic) association between a fungus and the roots of a vascular plant. Important for soil life and soil chemistry
- Cellulose digestion
- Food: baking bread with yeast, or making cheese with different microbes
- Waste recycling: waste water treatment plants use microbes

Sergei Winogradsky: first developed a system of enrichment culture, the Winogradsky column, to grow microbes from natural environments→microbes support natural ecosystems and showed importance of bacteria in

- **geochemical cycling (nitrogen cycle):** depends on bacteria and archaea that cycle nitrogen, phosphorus and other minerals throughout the biosphere by oxidative and reductive conversions.
- **Chemolithotrophs (or lithotrophs):** metabolize inorganic minerals such as ammonia, instead of the organic nutrients used by the microbes isolated by Koch.
- **Biofilms:** organized multispecies communities upon a surface. (i.e. colonic bacteria → E. Coli and Bacteroides). It plays a major roles in all ecosystems and within parts of the human body
- Biofuels:
 - o Plants use the energy of the sun to grow. Plant fiber, called cellulose, is the most abundant organic molecules on earth. Logen's EcoEthanol process **takes cellulose and using enzymes turns it into fermentable sugars and subsequently into ethanol. Using CO₂** absorbing plant material as an ethanol feedstock offers environmental advantages unequalled by other feedstock or fuels
 - o Fermentation: a process by which microbes gain energy by converting sugars into alcohol
 - o Best way of making biofuels is using microbes

Microbiology: study of microorganisms

Some current development in microbiology

1. The human microbiome project
 - a. Trivia → the longest bacteria discovered (up to 0.6mm) and live in the gut of sturgeon fish
 - b. there are 10 times more microorganisms colonizing the human body than the total number of the human cells in an individual
 - c. each person has a unique bacterial population in his/her gut
 - d. the composition of gut bacteria in each person generally remains constant throughout his/her life
 - e. human microbiota influence human physiology
 - i. late onset autism could be due to change in gut bacteria. Maybe from antibiotics
 - ii. stomach cramps from antibiotics :kills off beneficial bacteria. Can also causes other stomach problems.
2. Synthetic biology
 - a. Construction of new biological functions or systems not found in nature
 - b. Usually by genetic engineering of microorganisms
 - i. Photographic film using engineered bacteria that senses light
 - c. Synthetic cells: create cells with selected genes, grow life from non-living materials
3. Astrobiology/exobiology → life in other planet
 - a. Environments are hospitable (pleasant) for life
 - i. The chance finding human-like life on other planet is very small because of out intense requirements for living (temp/water etc)
 - ii. The chance for microbial life is higher because they can survive in: hot springs (yellow stone park), in very high salt concentration

environments (dead sea), desert conditions, very cold environments (Antarctic glacier)

4. Emerging (new) infectious diseases and antibiotic resistant microorganisms
 - a. With air travel, diseases are travelling seas and making their way across the whole world
 - b. WHO-world health organization → informs countries about possible diseases coming their way
 - c. US CDC-center for disease control and prevention
 - d. Public Health Agency of Canada

Classifying microorganisms

- Member of 3 domains (**diverged from a common ancestral cell**):
 - o **Prokaryotes** (cell lacking nucleus and free floating DNA, including **bacteria** and **archaea**)
 - o **Eukaryotes** (cells with a nucleus) that include simple multicellular forms: **algae and plants, fungi and animals, and protists**
 - **The formal names of the three domains are (common ancestor):**
 - **Bacteria**
 - **Archaea** → prokaryotic microorganisms including **hyperthermophiles** (can handle extreme temp.), **methanogens** (bacteria produce methane), **halophiles** (can handle high salt concentrations)
 - **Eukarya**
- Classifying higher organisms vs. microorganisms
 - o To construct a phylogenetic tree using DNA sequence homology:
 - gene must be found in all life forms and must have the same function
 - Commonly used sequence: gene encoding ribosomal RNA
 - Compare rRNA sequence to determine relatives

Naming a microorganisms

- binomial system: genus (capital) species (no capital)
- pyro=fire=survives in extreme heat
- lacto=milk=found in milk
- acido=acid environment
- rhizo=soil bacteria
- methano=methanogen
- Helicobacter pylori → creates ulcers(溃疡) in the stomach. Some cancers
- name after a person, no relation

Unit 2: Observing the small → from cells to molecules

Observing microbes

- the size at which objects become visible depends on the resolution of the observer's eye
- **Resolution** is the smallest distance by which two objects can be separated and still be distinguished

- The resolution of the human retina is about $150\mu\text{m}$ ($1/7\text{ mm}$) → means human normally cannot see anything less than this
- **When observing microbes, resolution and magnification are paramount (most important)**
- Microscopy for different size scales
 - Different microscopes are required to resolve various cells and subcellular structures
 - Human eye (10^{-3}m or 1mm)
 - Light microscopy (10^{-3} to 10^{-6}m)
 - Scanning electron microscopy (10^{-3} to 10^{-8}m)
 - Transmission electron microscopy (10^{-5} to 10^{-8}m)
 - Atomic force microscopy (10^{-6} to 10^{-9}m)
 - X-ray crystallography (10^{-8} to 10^{-9}m)
- Microbial Size
 - Microbes differ in size, over a range of a few orders of magnitude
 - Eukaryotic microbes
 - Protozoa, algae, fungi
 - $10\text{-}100\ \mu\text{m}$
 - Prokaryotes
 - Bacteria, Archaea
 - $0.4\text{-}10\ \mu\text{m}$
 - Virus 0.02 to $0.3\ \mu\text{m}$
 - There is a limit to the size of unicellular microorganisms because they need to have sufficient surface area, volume ratio for efficient nutrient uptake
- Optics and properties of light
 - Light is part of the spectrum of electromagnetic radiation
 - Wavelength of visible light = $400\text{-}750\text{nm}$ ($0.4\text{-}0.75\ \mu\text{m}$)
 - For electromagnetic radiation to resolve (see) an object, certain conditions must exist:
 - Contrast between object and its medium
 - Wavelength smaller than the object
 - A detector with sufficient resolution for the give wavelength
- Microscopy
 - **Bright-field microscopy → employs various stains**
 - **Generates a dark image of an object over a light background** (cannot see internal structures, background light, cells dark. Poses problems with non-pigmented specimens, therefore staining)
- **Fixation and staining** (kills cells)
 - The detection and resolution of cells under a microscope are enhanced by:
 - Fixation = cells are made to adhere to a slide in a fixed position
 - Staining = cells are given a distinct colour
 - Most stains have conjugated double bonds or aromatic rings, and one or more positive charges.

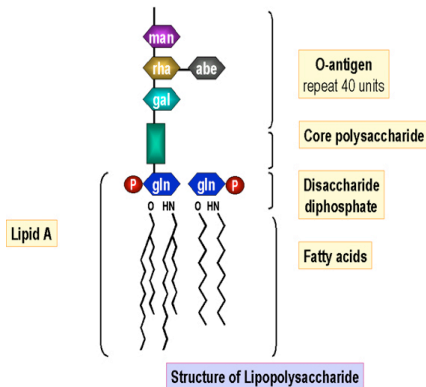
- A **simple stain** adds dark colour specifically to cells, but not to the external medium or surrounding tissue
- Most commonly used stain is **methylene blue**
- A **differential stain** stains one kind of cell but not the other
 - **Gram stain** differentiates between two types of bacteria
 - **Gram-positive** retain the crystal violet stain because of their thicker cell wall → dark purple
 - **Gram-negative** bacteria do not → colorless
 - 1. Add methanol to fix cells to surface air-dry
 - 2. Add crystal violet stains both change to purple
 - 3. Add iodine, which bind stain to gram-positive cells which increase the stain retention for positive
 - 4. Wash with ethanol no change for positive and stain is removed from negative
 - 5. Add safranin counterstain → positive remain purple and negative changes to pink
 - **Acid fast stain** = carbolfuchsin used to stain Mycobacterium species, wash with acid then counterstain with methylene blue
 - **Spore stain** = malachite green used to detect spores of Bacillus and Clostridium
 - **Negative stain** = colors the background, which makes capsules more visible
- **Dark-field Microscopy** → detects unresolved objects
 - Dark-field **optics enables microbes to be visualized as halos (光环) of bright light against darkness**
 - Object scatters light and is collected by the objective lens
 - Light that just pass through slide shine outside the objective lens and hence background is dark
- **Phase-Contrast Microscopy** → exploits differences in refractive indices (revolution is low and alive cell)
 - Superimposes refracted light (through cells) and **transmitted light --- half a wavelength out of phase = destructive (break) interference = dark**
 - Reveals (show) differences in refractive index as patterns of light and dark
 - Can be used to **view live cells and cellular organelles with sufficient contrast**
- **Fluorescence microscopy** → employs fluorophores for labeling

- In fluorescence microscopy, **incident light is absorbed by the specimen and reemitted (从新发出) at a lower energy, thus longer wavelength**
- **Need fluorescent stains** or tags for specific biomolecule, proteins, structures etc.
- Can visualize cells in **three dimensions** (confocal microscopy)
- Allows **observation of live microbes in real time**
 - i.e. brainbow- individual neurons expressing proteins that fluoresces at different wavelengths → each neuron fluoresces a different colour to distinguish them from each other
- **Electron Microscopy → use beam of electrons instead of light rays**
 - Electrons behave like light waves.
 - Very **high frequency**
 - Allows very great resolution
 - 0.0037nm wavelength
 - Sample must absorb electrons
 - **Coated with heavy metal** → cell don't absorb electron unless stained with heavy metal, but this kills the specimen
 - **Heavy metal staining and slicing can harm/change cells which changes their appearance under the microscope**
 - Electron beam and sample are **in a vacuum**
 - Lenses are **magnetic** fields
 - Two major types
 - Transmission electron microscopy (TEM)
 - Electrons **pass through the specimen**
 - Reveal **internal structure in 2-D**
 - Scanning electron microscopy (SEM) → can only see surface
 - Electrons **scan the specimen surface**
 - Reveals **external features in 3-D**
 - Sample preparation
 - The specimens for electron microscopy can be prepared in several ways
 - **Embedded(嵌入)** in a polymer for thin sections
 - **Microtome** is used to cut slices
 - **Sprayed** onto a copper grid
 - The specimen is then treated with a heavy-metal salt such as uranyl acetate
 - NOTE: for SEM, specimen is coated with heavy metal and it is not sliced
- **Cryo-electron Microscopy**
 - In **cryo-EM**, or **electron cryo-microscopy**, the specimen is **flash-frozen**
 - Suspended in water and frozen rapidly in a refrigerant

- Have the most extreme variation in phospholipid side-chain structures
 - Ether links between glycerol and fatty acids
 - Hydrocarbon chains are branched **terpenoids: a branched lipid derived from isoprene that is found in hydrocarbon chains of Archaea membranes**
 - Can only have a single layer of phospholipids in the Archaea
 - Membranes also include planar molecules that fill gaps between hydrocarbon chains.
 - In eukaryotic membranes, the reinforcing agents are **sterols**, such as cholesterol. →sterols helps makes the phospholipids less ridges and less fluid
 - In bacteria, the same function is filled by **hopanoids**, or **hopanes**→usually in between the phospholipid
- **Fluid mosaic model**
 - There are protein that involves
 - Fluid: protein and lipids is free to move in the membrane
 - Mosaic: membrane is pattern of protein (transporter protein and protein-driven ATP synthase) and phospholipids
- **Function**
 - **Permeability barrier**→prevents leakage and functions as a gateways for transport of nutrients into and out of the cell
 - **Site to anchor proteins**→site of many protein involved in transport, bioenergetics and chemotaxis
 - **For prokaryotes, the cytoplasmic membrane is important for energy generation for the cell (protein-motif force)**→site of generation and use of proton motive force
- Permeability of membranes to various molecules (good→poor)
 - Water→glycerol →tryptophan →glucose →chloride ion (Cl⁻)→potassium ion (K⁺) →sodium ion (Na⁺)
 - Permeability of the membrane to water maybe affected by aquaporin
- **Movement of substances across cell membrane**
 - **No expenditure of energy**
 - **Diffusion**→follow concentration gradient
 - Simple
 - Facilitated (requires channel forming proteins)
 - **Expenditure of energy –against concentration gradient**
 - **Active transport** →energy from proton gradient (i.e. ATP or PEP)
 - **ABC transporters:** the largest family of energy-driven transport system is the ATP-binding cassette superfamily or ABC transporters
 - **Found in all three domains of life**

- **Also required a channel to let molecules to go through**
- **Osmosis**
 - Movement of water molecules across membrane from a region with lower concentration of solutes to a region of higher concentration of solute → to balance the water activity, in order to reach equilibrium (diffusion of water)
 - Facilitated by aquaporins
 - Cytoplasm of cell is usually has more solutes than the environment the cell is in.
 - This leads to the water fluid into the cell, and the cell will be expand and cell content will lost and cell dies. In order to control, some of the cell that has cell wall, to prevent what go in and out
- **The Cell Wall**
 - Two kind of wall in domain bacteria
 - The gram stain
 - The structure of **the cell wall of the gram positive and negative are different**, therefore end up with different colour after wash with ethanol
 - **Both** gram positive and negative bacteria have **Peptidoglycan (made of amino acid and sugar) in the cell wall (prevent osmotic shock)** → an organic polymer that constitutes nearly 1% of the cell mass. It shows an importance of maintaining turgor pressure in dilute environments, where water would otherwise enter by osmosis, causing osmotic shock
 - Structure: peptide (glycan chain), in between there are rigid amino acid chain and peptide are cross-link
 - Composition between gram positive and gram negative bacteria cell wall
 - +ve
 - **thick peptidoglycan** is outside the membrane
 - **teichoic acid/lipoteichoic acids in Gram + bacteria maybe help important in maintaining cell wall structure, since they have phosphate attached**
 - -ve
 - **periplasm** is in between **outer membrane** (made of lipopolysaccharide and protein) and cytoplasmic membrane, and **thin peptidoglycan** is in the middle of periplasm
 - **easy to wash off during the gram staining procedure**

- **Gram-Positive Cell Envelope:**
 - **S-Layer** (not all species)
 - Made of **protein**
 - **Thick cell wall**
 - **Amino acid cross-links in peptidoglycan**
 - **Teichoic acids for strength**
 - **Plasma membrane**
- Gram-Negative cell wall



- The thin peptidoglycan layer consists of one or two sheets
- Covered by an outer membrane, which confers defensive abilities and toxigenic properties on many pathogens
- LPS: lipopolysaccharides, if in the blood, cause fever...

- Function of lipopolysaccharides
 - Lipid A is **toxic**
 - Forms a protective coat that **prevents recognition by host immune cells**

- The enzyme lysozyme breaks the covalent bonds between N-acetylglucosamine and N-acetylmuramic acid → **effects of degrading the peptidoglycan cell wall using lysozyme** (one of the way to helps cell of being attract by the bacteria pathogen)

- **The “Walless” bacterium –Mycoplasma**
 - The only exception of the cell that doesn’t have a cell wall
- **Cell wall of archaeobacteria**
 - Some archaeobacteria have protein or glycoprotein cell wall (S layer)
 - Others have **peptidoglycan-like structure, called pseudomurein**.
 - **Instead of N-acetylmuramic acids, pseudomurein has N-acetyltalosaminuronic acids** in the glycan chain → “false wall”

- **Eukaryotic microbes**

- **Eukaryotic microbes possess their own structures to avoid osmotic shock**
 - **Algae** form cell walls of **cellulose** (made of glucose)
 - **Fungi** form cell walls of **chitin** (homopolymer of N-acetylglucosamine)
 - **Diatoms** form **exoskeletons of silicate**
 - **Paramecia** (protist) **possess a contractile vacuole** to pump water out of the cell → no cell wall
 - For human, on our skin we have **chaperone** that prevent water goes into our body therefore, prevent the cell osmosis

- **Capsule and slime layer in bacteria** → polymer of polysaccharide, polypeptide or both
 - **Firmly** attached to cell wall: **CAPSULE**
 - **Loosely** attached to cell wall: **SLIME LAYER**
 - Function:
 - Protection from host immune cells
 - Adherence (依附) → attaching to surfaces
 - Nutrient reservoir (bank) → use as energy and carbon source
 - Protection from dehydration

Genome of microorganisms

- The nucleoid in prokaryotes
 - **Eukaryotes** have a membrane-bound **nucleus**
 - **Prokaryotes** have a **nucleoid region** (dense structure that makes up the DNA of the cell in prokaryotes) that extends throughout the cytoplasm
 - **Forms about 50 loops or domains, within each domain, the DNA is supercoiled by DNA-binding proteins**
- **Ribosomes**
 - **Central dogma: DNA(transcription) → RNA(translation) → Protein**
 - DNA has to convert to protein, in order to be function in the body, where ribosomes plays a major role in translating RNA to protein
 - Ribosomes are **fairly large molecules**, made of **protein** and **ribonucleic acid (RNA)**
 - **Can be separated into 2 parts**, one big and one small, after translation, it breaks up
 - Ribosomes from **prokaryotes has a smaller weight than eukaryotes**
 - **The Svedberg (S) unit** → a measure of sedimentation velocity (how fast the molecules are been pull down to the bottom)
 - **Svedberg (S) unit depends on:**
 - **Weight of object** = heavier objects have higher Svedberg value
 - **Ribosomes of prokaryotes = 70s (30s + 50s)**
 - **Ribosomes of eukaryotes = 80s (40s + 60s)**
 - **Surface area of object** → the smaller it gets, the faster it will go
 - **Svedberg unit cannot be simply added together, the final unit will be affect by surface area**
- **Motility**
 - Motile *Pseudomonas aeruginosa* using **flagella for movement in a liquid media (move longer distance faster)**
 - Each flagellum is a spiral filament of protein monomers called **flagellin**
 - The filament is rotated by a motor driven by the proton motive force
 - **Chemotaxis** → is the movement of a bacterium in response to chemical gradients

- **Attractants** cause **CCW** (counter close wise) rotation
 - **Flagella bundle together**
 - Push cell **forward** → "run"
 - **Repellents** cause **CW** rotation
 - **Flagella bundle falls apart**
 - "Tumble (倒塌)" = Bacterium **briefly stops**, then **changes direction** and shorter run
 - The alternating runs and tumbles cause a "**random walk.**" → more frequent changes in direction and shorter runs
 - **Receptors detect attractant concentrations**
 - Sugars, amino acids
 - **Attractant concentration increases and prolongs (longer) run**
 - This is termed a "biased (偏斜的) random walk."
 - Causes a net movement of bacteria toward attractant (or away from repellents)
 - The [nutrients] increase, tells bacteria to run longer and tumbles less and vice versa
 - Eukaryotic Flagella, Cilia
 - Some eukaryotic are motile microorganisms and some are not
- Spores
 - Bacterial ENDOSPORES
 - Endospore are not reproductive structure
 - Endospore are formed by one bacteria and that endospore have same # of bacteria that formed the endospore (germinal)
 - Usually formed in harsh condition to tender dormant state → survive extreme condition can survive long time without nutrients
 - Not multiplicative reproduction
 - When environment are favorable: spore germinate (grow) and reform bacteria
 - Eukaryotic spores → multiplicative reproduction
- ENERGY GENERATION
 - Eukaryotes – mitochondria
 - Invagination of IMM → combine protein that participate in ETC (respiration)
 - Photosynthesis → chloroplast
 - Absorbs light and converts light energy to ATP
 - Chloroplast have chlorophyll that are pigments when absorb
 - Prokaryotic: no mitochondria but protein of ETC are found in cytoplasmic membrane → **ETC residue in cytoplasmic membrane, still be able to respiration**

- **Prokaryotes also photosynthesis but don't contain chlorophyll**
 - **Prokaryotes –cyanobacteria**
- **Other structure present within cells**
 - **Food storage**
 - **Polysaccharide granules eg. Glycogen, starch**
 - **Lipid granules eg. Poly-p-hydroxybutyrate**
 - **Magnets- magnetosomes**
 - **Aquaspirillum magnetotacticum**
 - **Thylakoids, carboxysomes and gas vesicle in cyanobacteria**
 - **Thylakoids= extensively folded intracellular membrane**
 - **Carboxysomes = polyhedral bodies packed with the enzyme Rubisco for CO₂ fixation**
 - **Gas vesicles = to increase buoyancy**
- **Other membrane bound structure in Eukaryotic cells**
 - **Endoplasmic reticulum**
 - **Rough ER**
 - **Sorting and processing proteins (ribosomes)**
 - **Site of phospholipid synthesis**
 - **Smooth ER**
 - **Site of phospholipids, fats, and steroid synthesis**
 - **Golgi bodies**
 - **Modification of protein and precursor of secretory vesicles or storage vesicles**
 - **Lysosomes**
 - **contain digestive enzyme**