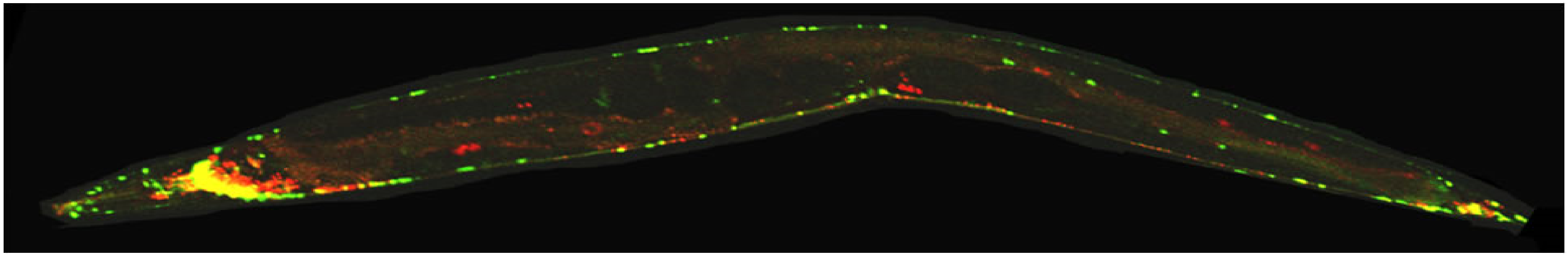
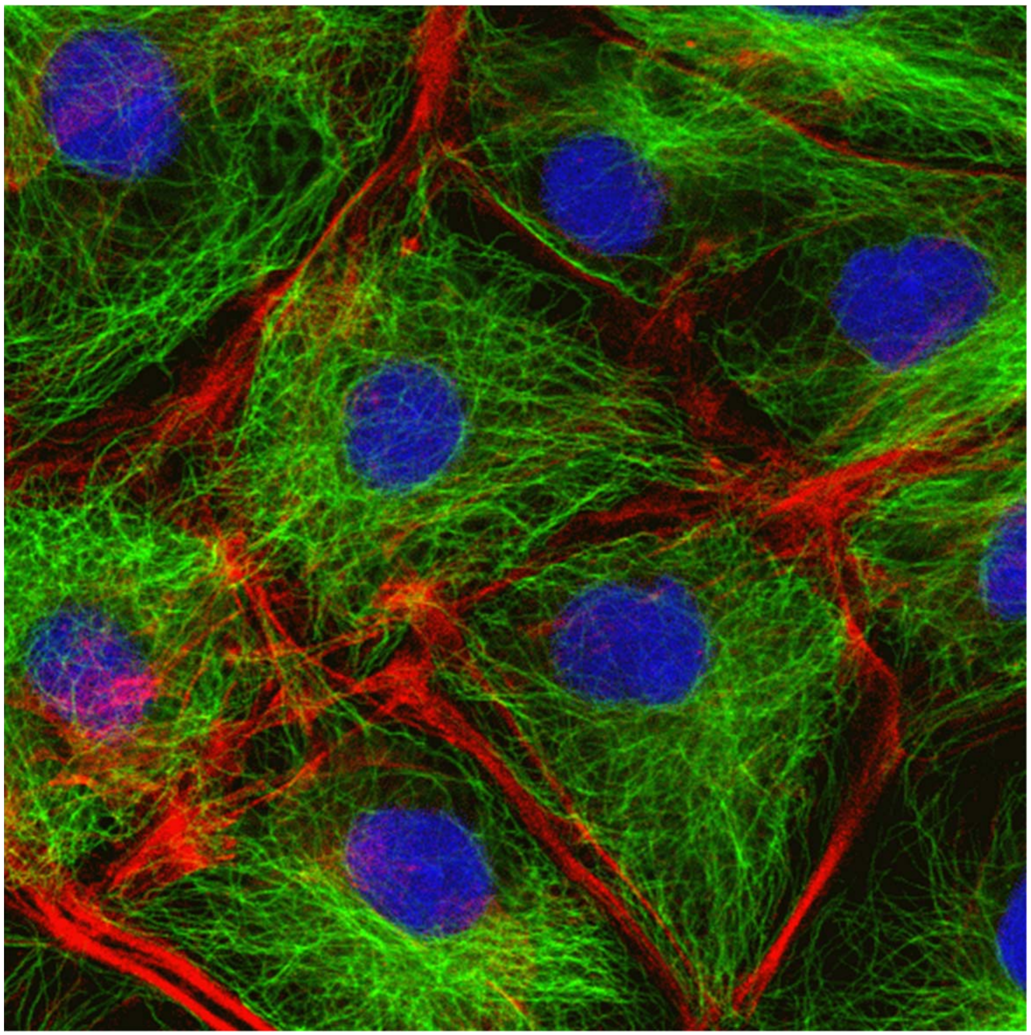


World of the Cell



Chapter 1:
A preview of the Cell

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National Tsing Hua University

Institute of Molecular & Cellular Biology

Department of Life Science

*http://life.nthu.edu.tw/~laboiw/

分子與細胞生物學一

Molecular and Cell Biology I

Spring, 2019

教科書(Textbook): **Becker's World of the Cell** (9th Edition) by Hardin, Bertoni, and Kleinsmith

上課時間 Time: T3T4R4. 學分 Credit: 3. 教室 Room: LSB II R109.

授課老師(Lecturers):

王歐力(Prof. Oliver Wagner), LSBI R507, 03-574-2487, owagner@life.nthu.edu.tw

TA: Odvogmed Bayansan, LSBI R509, 03-571-5131 ext. 33479, odgoob@yahoo.com

Course Schedule (Tuesdays marked bold/ Dr. Oliver Wagner's lecture offered in English):

Feb 19	王歐力	Chapter 1 (& Class Overview/Grading)
Feb 21	王歐力	Chapter 13
Feb 26	王歐力	Chapter 13 / **Quiz 1**
Feb 28	王歐力	*NO CLASS: Memorial Day*
Mar 05	王歐力	Chapter 14 / **Quiz 2**
Mar 07	王歐力	Chapter 14
Mar 12	王歐力	Chapter 15 / **Quiz 3**
Mar 14	王歐力	Chapter 15
Mar 19	王歐力	Chapter Appendix / **Quiz 4**
Mar 21	王歐力	Chapter Appendix
Mar 26	王歐力	*NO CLASS: Class Review and Exam Preparation*
Mar 28	王歐力	**FIRST EXAM**

成績評量 (Grading) 王歐力授課部分 (Lecture held by Prof. Wagner):

60% first exam, 40% quizzes

Language for exam and quizzes: **English ONLY**

FOR CLASS INQUIRIES PLEASE EMAIL TO THE RESPECTIVE TA. PLEASE AVOID EMAILING TO THE PROFESSORS FOR GENERAL QUESTIONS. THANKS.

Syllabus overview (Wagner part)

New text book: Artwork in this lecture only updated if important additional *content*!

TA: odgoob@yahoo.com

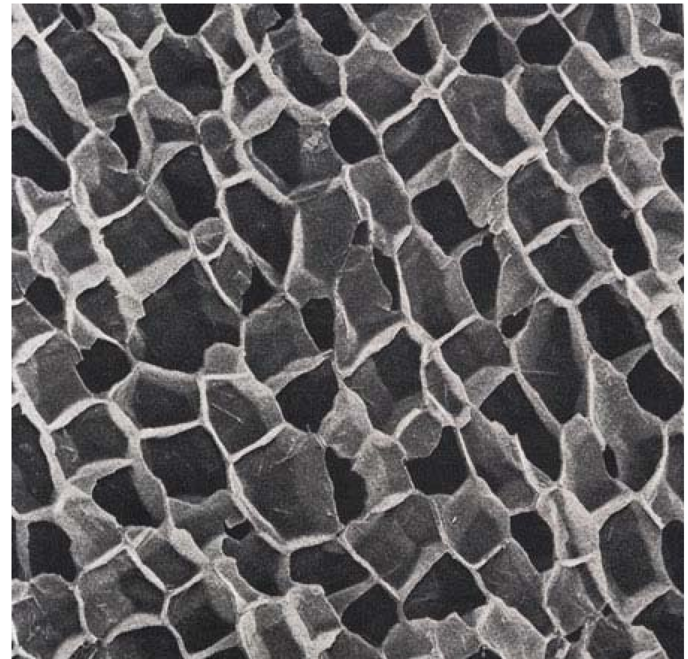
Tuesdays
10:10-11:00
11:10-12:00

Thursdays
11:10-12:00

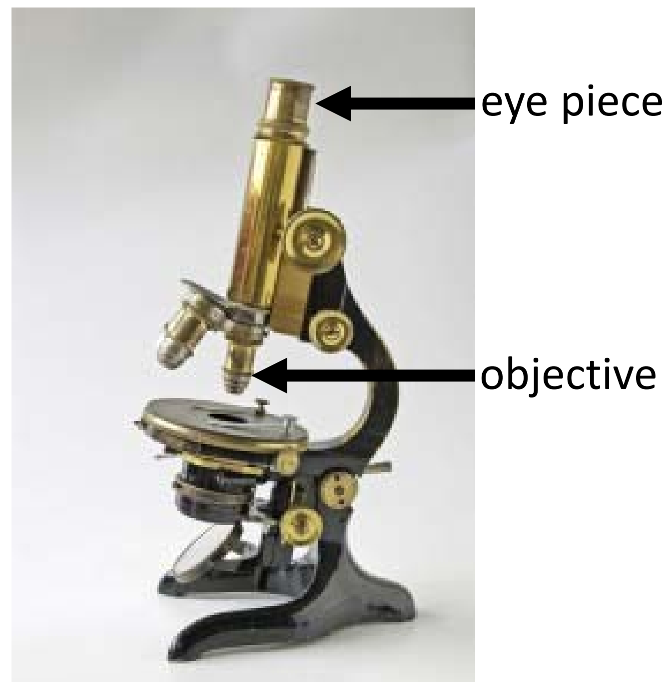
Quiz is 10 min

The Cell Theory: A Brief History

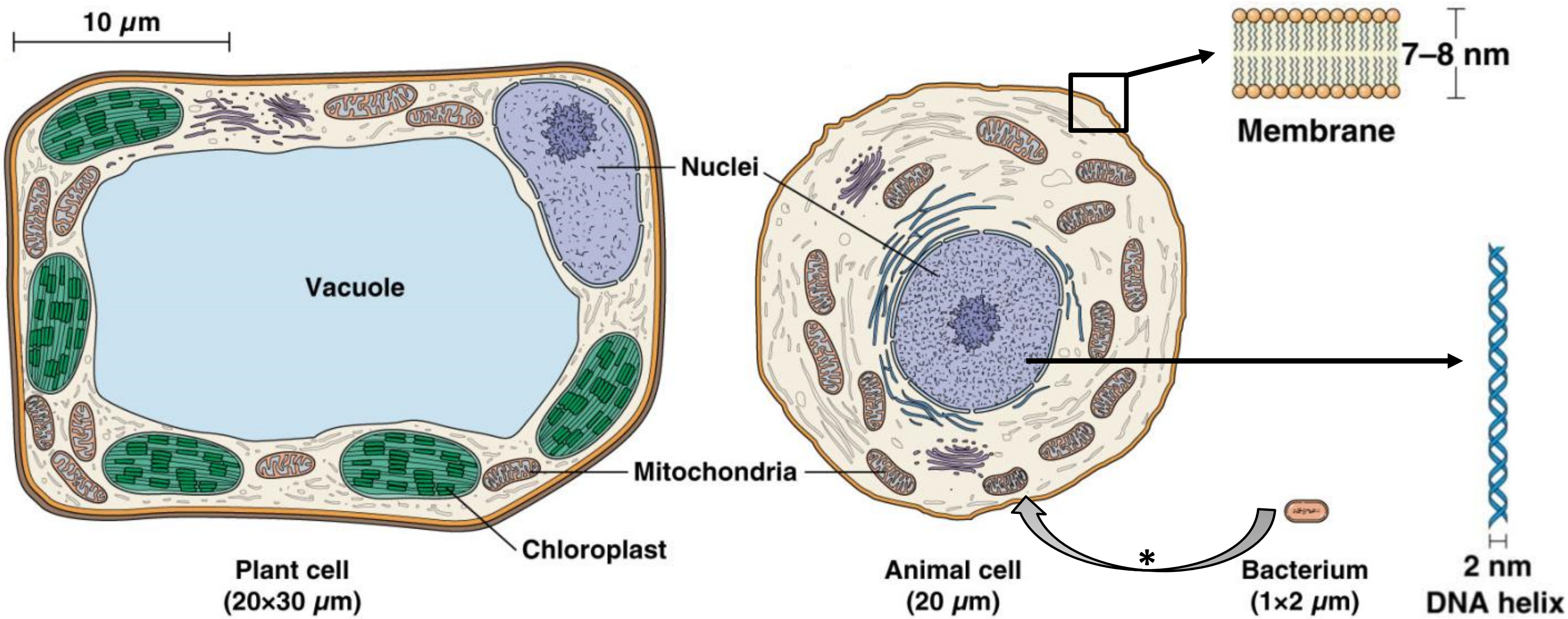
- The cell is the basic unit of biology: every organism consists of cells (e.g., **eukaryotes**) or is a cell itself (e.g., **prokaryotes**)
- The knowledge of the structure and function of cells has increased dramatically in the past decades
- But how do we know what we know? => **Cell Theory** ("**hypothesis driven research**")
- How does everything started? => With the **microscope**
- **Robert Hooke** 1665 examined plant tissue and found that the tissue consist of several small **compartments** = cells (cellula = "little room") (cork: 軟木)
- **Antonie van Leeuwenhoek** (late 1600s) developed a microscope with a much higher resolution (**300x**)
- He observed for the first time **living cells** (blood cells, sperm, bacteria, algae)
- Only much later in the 1830s the resolution ("ability to see fine details") of microscopes was largely improved: **compound microscopes** = one lens (**eyepiece**) magnifies the image created by a second lens (**objective**)
- Now structures around 1 micrometer (1 µm) can be seen
- But what is a micrometer?



Cork tissue (observed by Hook)



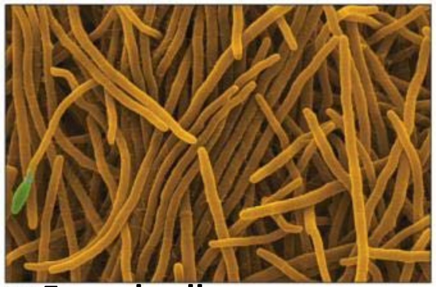
What is a micrometer? What is a nanometer?



- The size of many cells and organelles (mitochondria, nucleus and chloroplasts) is in the **micrometer** range (**10^{-6} m**). **Bacteria are of similar size as mitochondria** (*****"**endosymbiotic theory**": bacteria merged with cells and became mitochondria).
- Structures in the size of few nanometers are not possible to be seen by light microscopes due to the **limitations by the wavelength of light** (around 200 nm)
- One **nanometer 10^{-9} m**. One **angstrom (Å)** = **0.1 nm**.

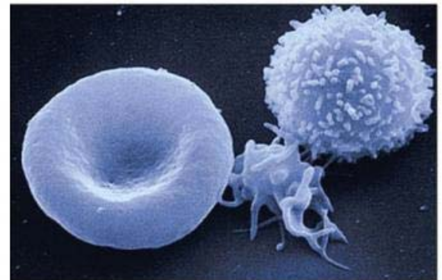
Cell theory postulated by Schwann and Virchow (around 1850)

- **Theodor Schwann** 1839:
  - *"All organisms consist of one or more cells"*
  - *"The cell is the basic unit of structure for all organisms"*
- **Rudolf Virchow** 1855 (observed cell divisions):
  - *"All cells arise only from preexisting cells"*



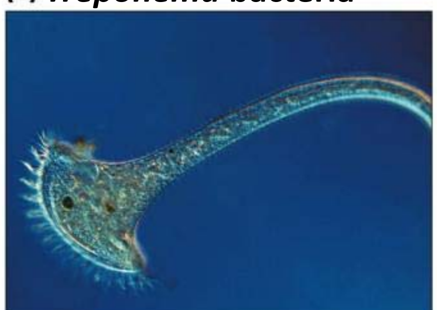
(a) Fungal cells

(b) *Treponema* bacteria



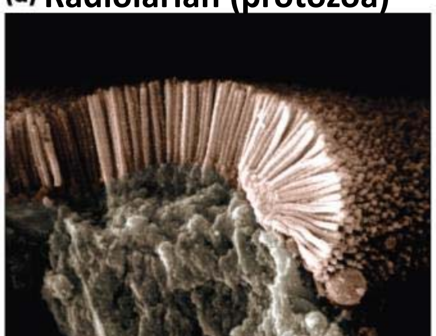
(c) Blood cells

(d) Radiolarian (protozoa)

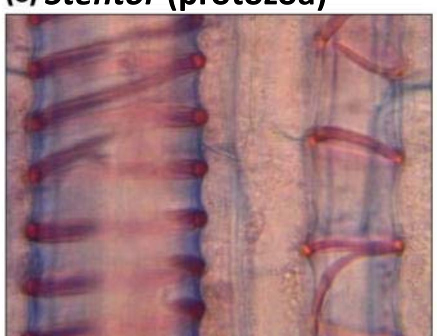


(e) *Stentor* (protozoa)

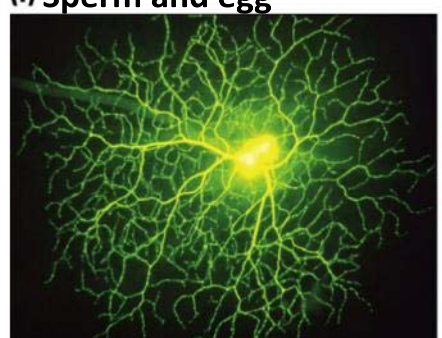
(f) Sperm and egg



(g) Intestinal cells



(h) Plant xylem cells



(i) Nerve cell (neuron)

- The **diversity** of **cell form** and **function** is **huge**
- In many cases **specialized cell shape** is also related to **specialized function** of the cell: "microvilli" in intestinal cells (surface enlargement) neuron "processes" (for networking)

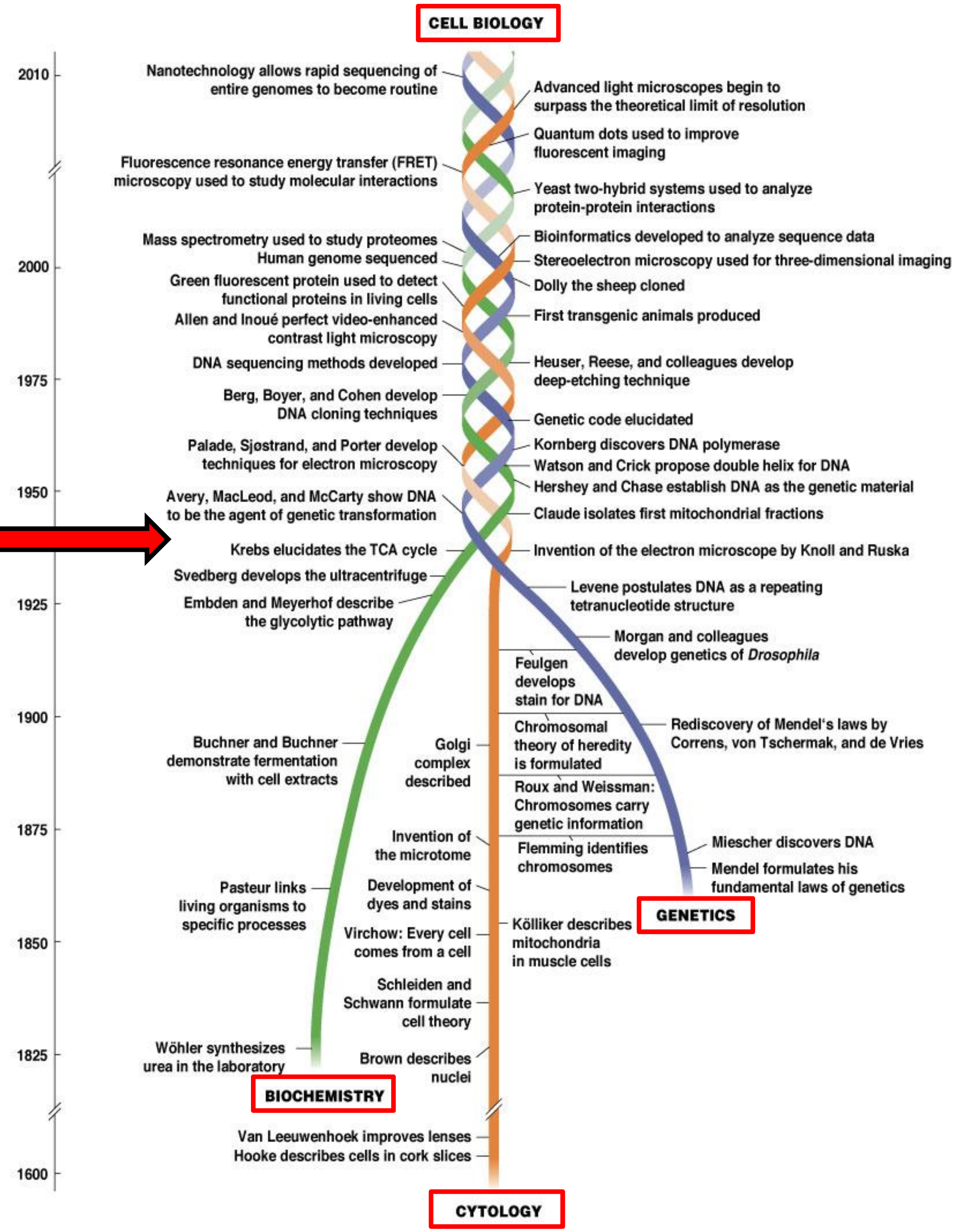
Cell Biology emerged from three different disciplines in the mid 1950s

- **Cell Biology** is a rather **modern discipline**. It is based on the emergence of three different biological majors: **Cytology**, **Biochemistry** and **Genetics**
  - **Cytology** is the oldest discipline back to the early 1600s. "Cyto" is from the Greek language and means "hollow vessel" (or cell). Mostly based on **observations of cell structures** ("*descriptive science*") employing optical techniques.
  - **Biochemistry** with its roots in the early 1800s. Investigates the **function of cell structures**. Techniques: Ultracentrifugation, electrophoresis, chromatography, mass spectrometry ("*separating and identifying cellular components*").
  - **Genetics** emerged in the late 1800s. **Gregor Mendel** (1860) established fundamental laws of genetics. **Watson and Crick** (1950) uncovered the double helix structure of DNA. Later, **cloning of mammals** and **sequencing of human genome**.

- Thus, modern cell biologists must acquire basic knowledge of all three strands as well as of the basic principles of **chemistry** and **physics** but also **computer science** and **engineering**.

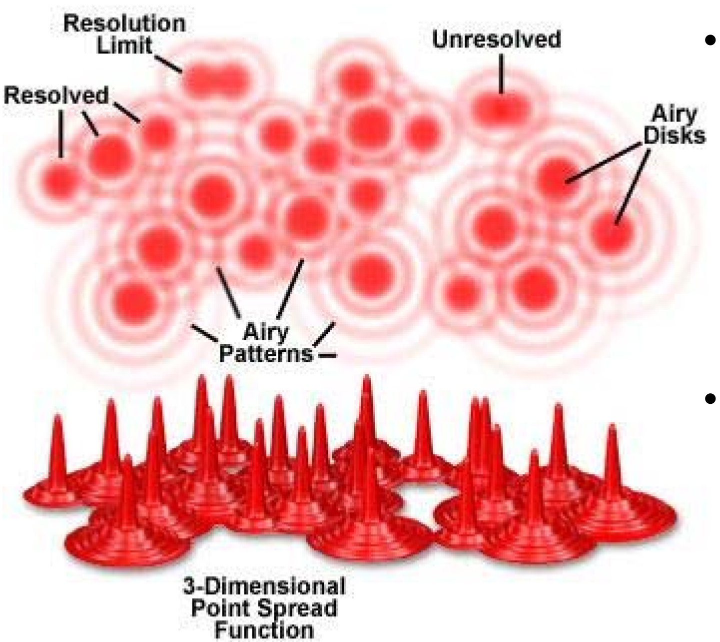
Cell Biology emerged from three different disciplines in the mid 1950s:
Cytology, Biochemistry and Genetics

Birth of cell biology

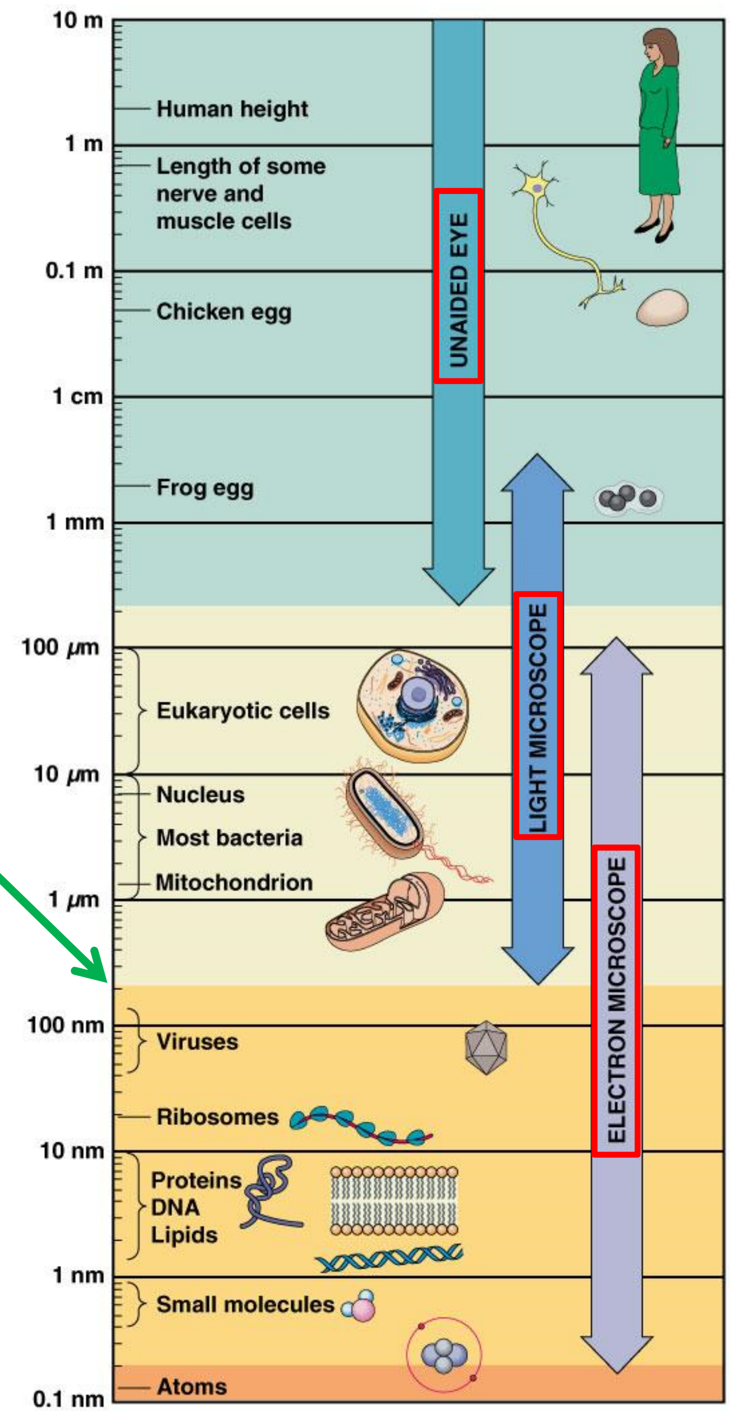


Going into details: The cytology branch

- Cytology is the study of cells. In earlier times is was restricted to the observation of cell structures by using limited optical techniques.
- Mid 1800s: **light microscopy** (can visualize organelles in the micrometer range), **dyes** and **stains** (to visualize specific organelles and structures) and **microtome** (thin slices of cells embedded in resin => histology = tissue analysis)
- **Limit of resolution** of a microscope = "*how far apart adjacent objects must be in order to be distinguished as separate units*". Light microscope: 200 nm



- Randomly distributed **point light sources** of a specimen appear as "airy disks" (light diffraction patterns around)
- Some of these single point light sources are well resolved and some not.



The diverse microscope techniques

Confocal vs. Non-Confocal

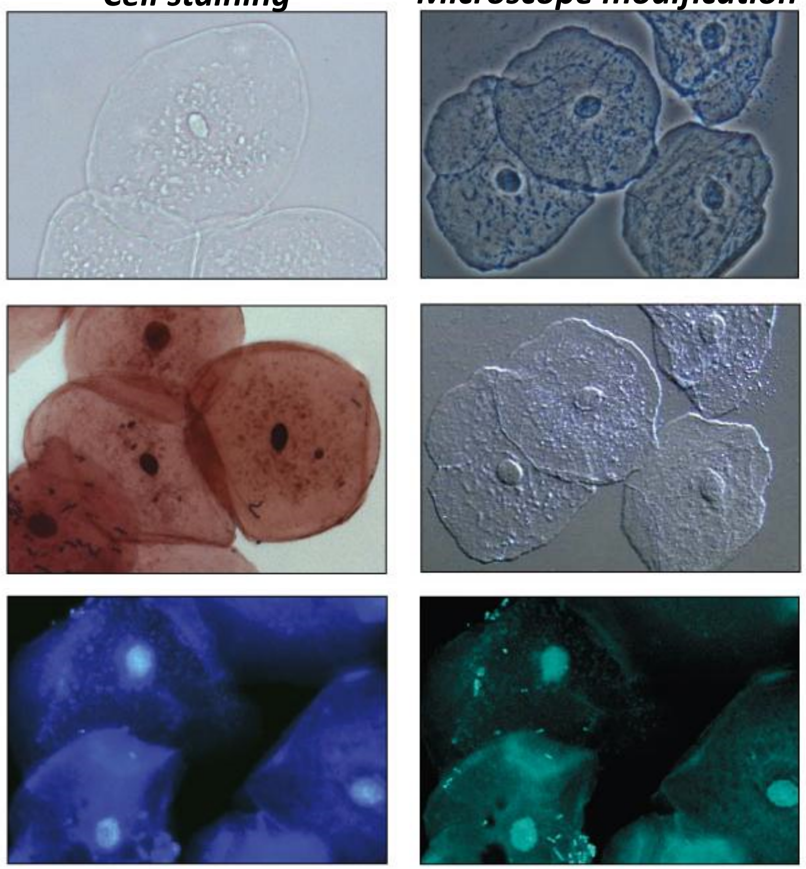
- The <u>greater</u> the **resolving power** of a microscope the <u>smaller</u> the <u>limit of resolution</u>.
- Resolving power can be <u>increased by</u> several **modifications of the microscopes** and by using different <u>specimen preparation techniques</u>
- **Specimen preparation** (chemical fixation for example), however, can lead to **artifacts** (a structure caused by preparation but not a legitimate/"real" cellular structure)

Brightfield (unstained specimen): Passes light directly through specimen; unless cell is naturally pigmented or artificially stained, image has little contrast.

Brightfield (stained specimen): Staining with various dyes enhances contrast, but most staining procedures require that cells be fixed (preserved).

Fluorescence: Shows the locations of specific molecules in the cell. Fluorescent substances absorb ultraviolet radiation and emit visible light. The fluorescing molecules may occur naturally in the specimen but more often are made by tagging the molecules of interest with fluorescent dyes or antibodies.

Cell staining | ***Microscope modification***



Human epithelial cells

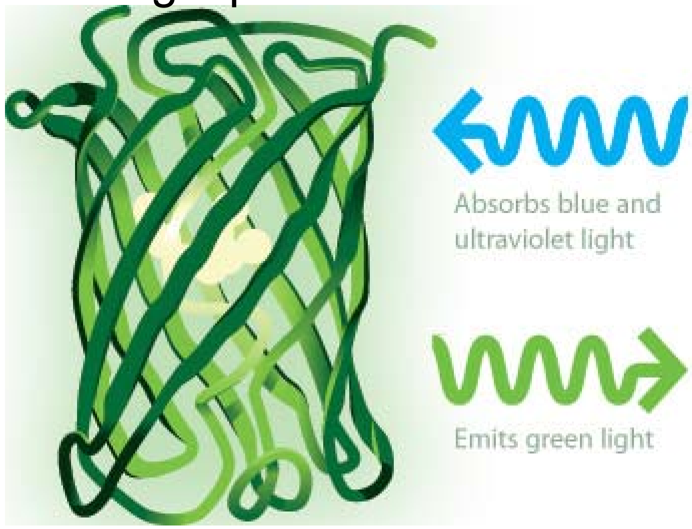
Phase contrast: Enhances contrast in unstained cells by amplifying variations in refractive index within specimen; especially useful for examining living, unpigmented cells.

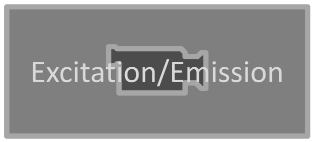
Differential interference contrast: Also uses optical modifications to exaggerate differences in refractive index.

Confocal: Uses lasers and special optics to focus illuminating beam on a single plane within the specimen. Only those regions within a narrow depth of focus are imaged. Regions above and below the selected plane of view appear black rather than blurry.

What is GFP?

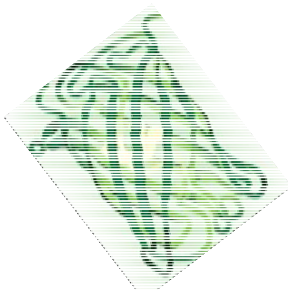
Single protein





Green fluorescent protein isolated from jellyfish *Aequorea victoria*

Engineering genes that <u>express GFP fused to specific proteins of interest</u> for visualizing in cells



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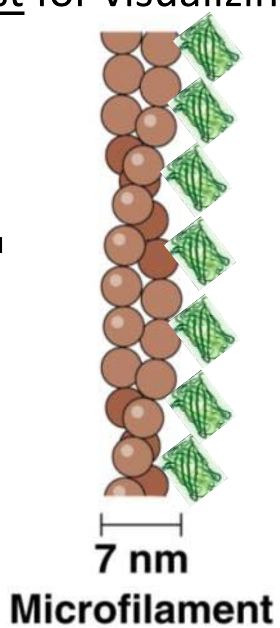

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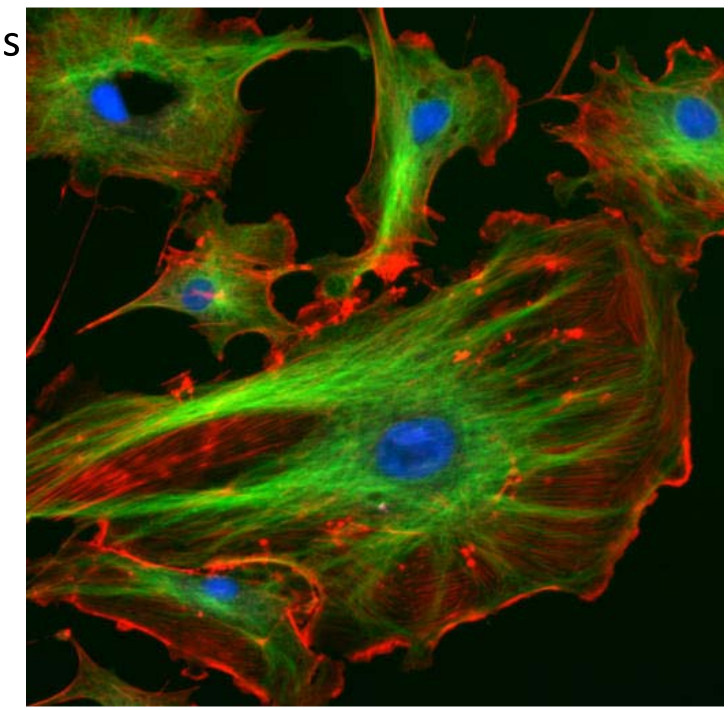


The Nobel Prize in Chemistry 2008

"for the discovery and development of the green fluorescent protein, GFP"

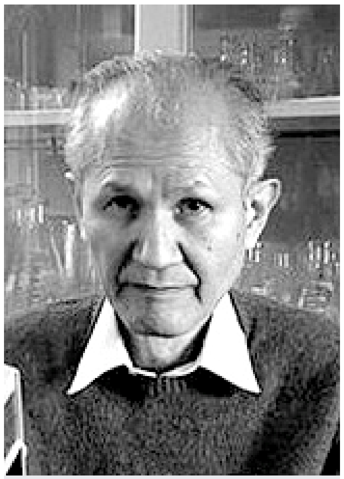


Photo: J. Henriksson/SCANPIX

Osamu Shimomura

1/3 of the prize

USA

Marine Biological Laboratory (MBL)
Woods Hole, MA, USA

b. 1928

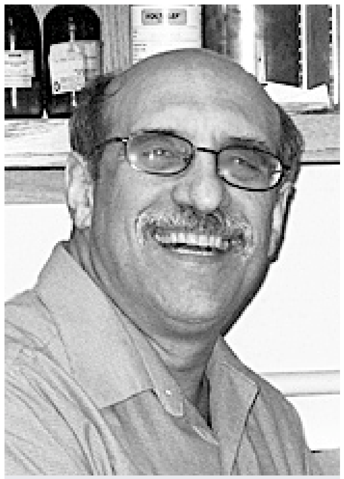


Photo: J. Henriksson/SCANPIX

Martin Chalfie

1/3 of the prize

USA

Columbia University
New York, NY, USA

b. 1947

Photo: UCSD

Roger Y. Tsien

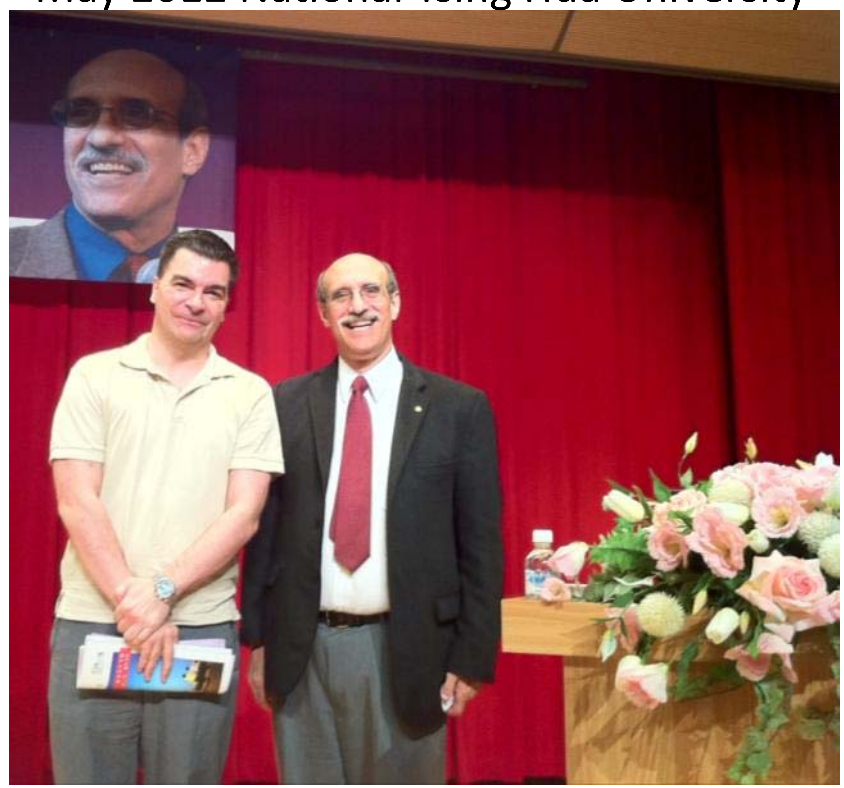
1/3 of the prize

USA

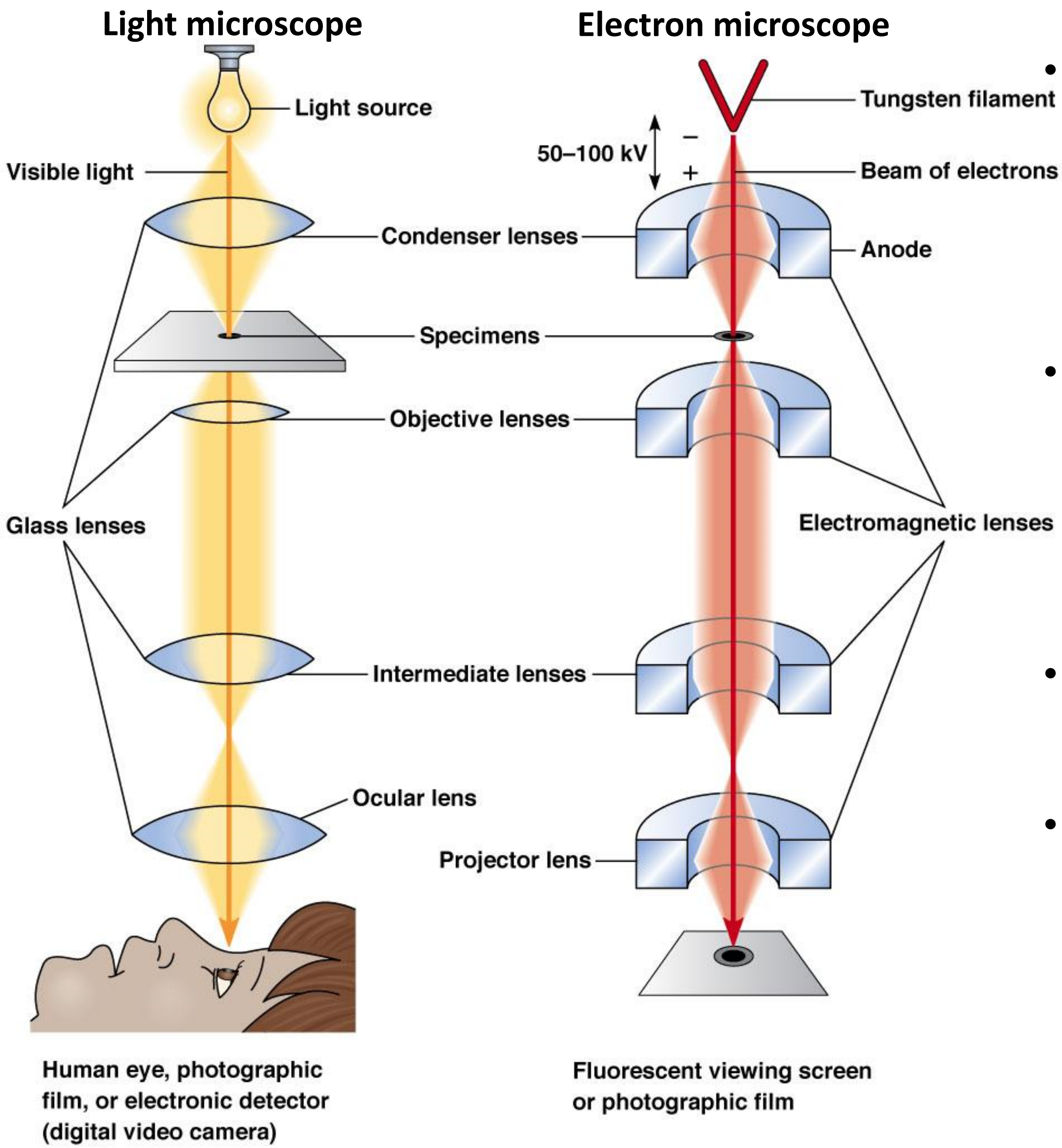
University of California
San Diego, CA, USA

b. 1952

May 2012 National Tsing Hua University



Breaking the micrometer resolution limit: The Electron Microscope



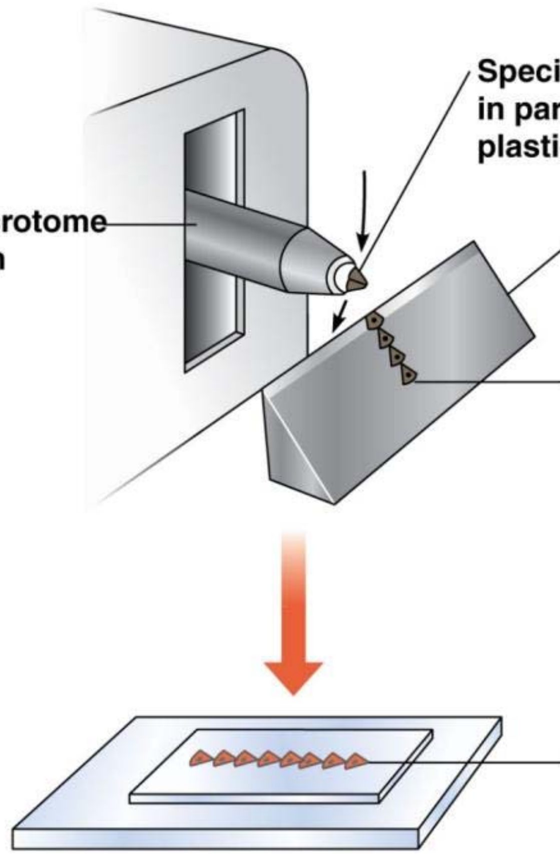
- Invented 1931 by Germans **Max Knoll** and **Ernst Ruska**: **visible light** is replaced by **electron beam** and **optical lenses** are replaced by **electromagnetic lenses**
- Wavelength of electrons is much shorter than wavelength of visible light. Thus, resolving power is very high with spatial resolution of **0.1 – 0.2 nm** (1-2 Å)
- Magnification up to 100,000X (compare to light microscope: 1000-1500X)
- **Requires demanding specimen preparation** as embedding, fixing, dehydrating and ultra-thin slicing of cells or tissues

Electron microscopy requires delicate specimen preparation

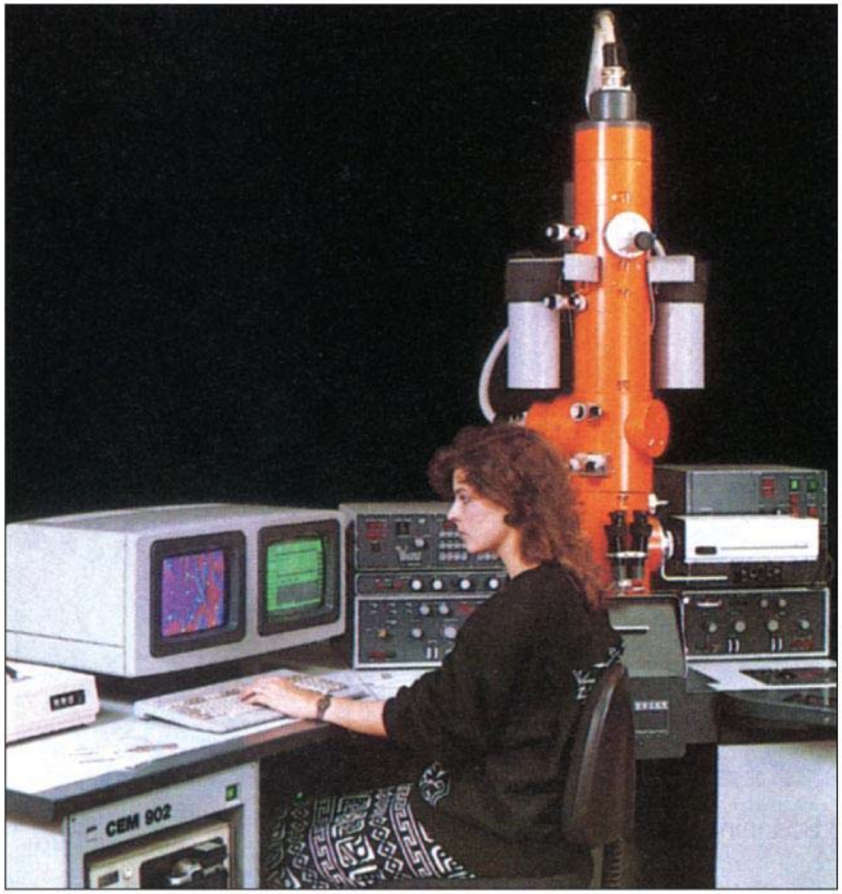
(a) Ultramicrotome

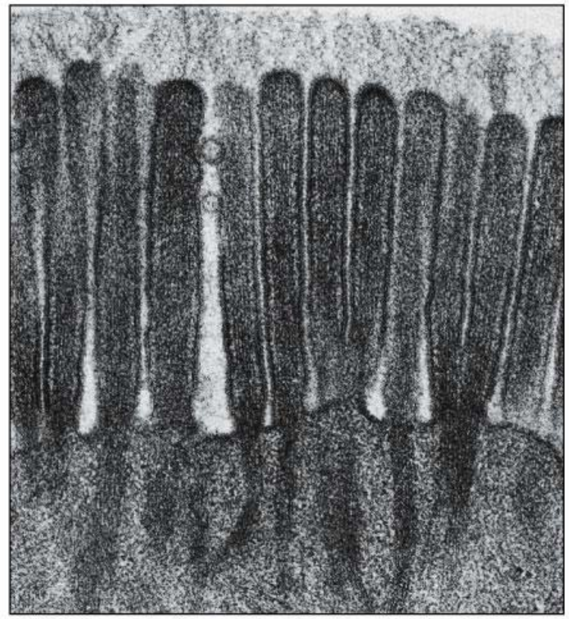
(b) Microtome arm of ultramicrotome

Cells **chemically fixed** (formaldehyde cross-links proteins) and **embedded** in **resin** (樹脂)

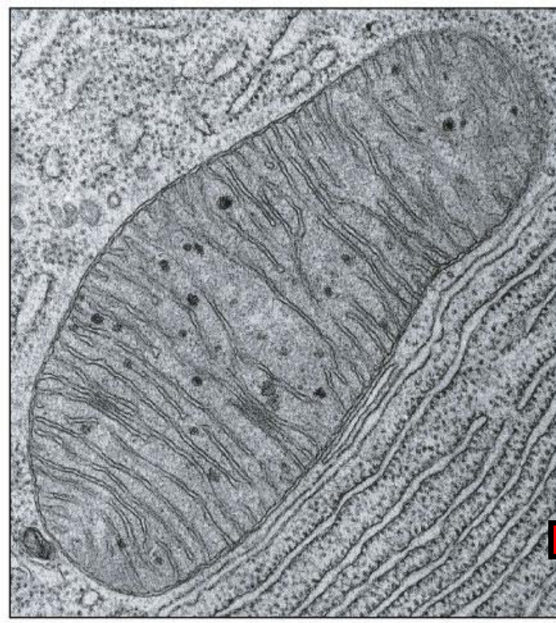


Transmission Electron Microscope (TEM)





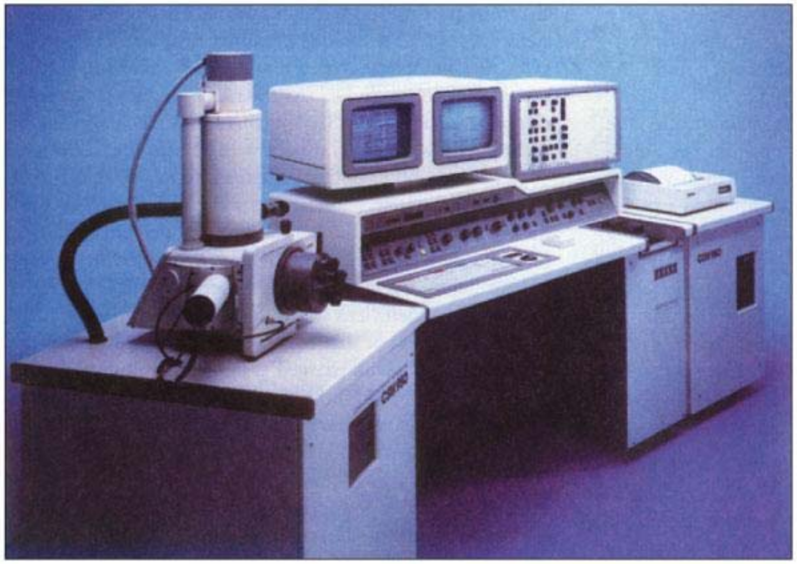
Intestinal cell with <u>microvilli</u> (increase surface for resorption of metabolites from the intestinal fluid)



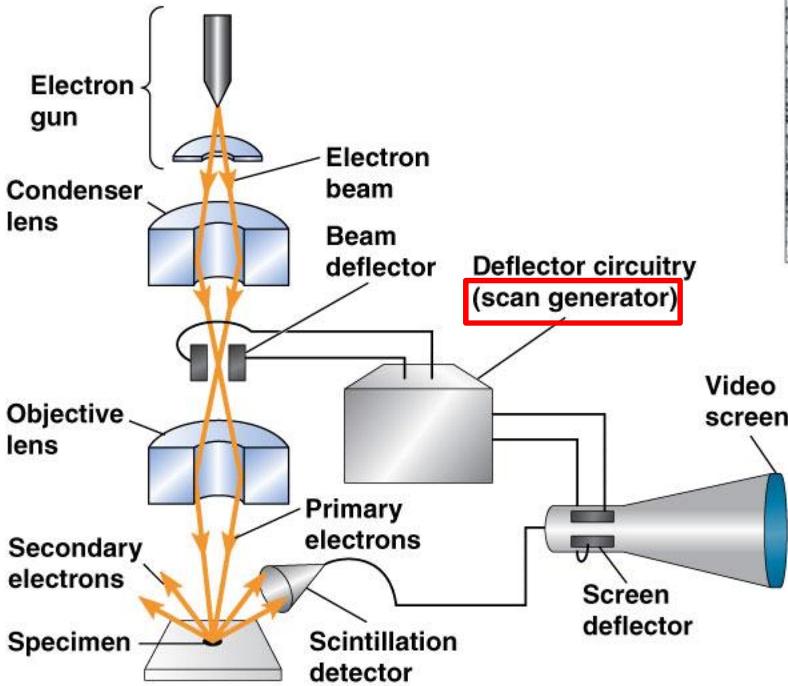
Mitochondria with <u>cristae</u> (membrane invaginations to increase surface for embedded ATP generating enzymes)

?

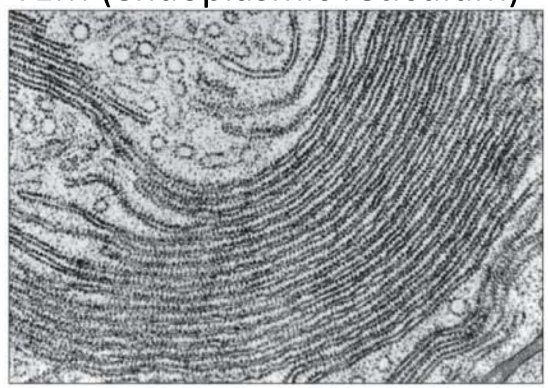
Scanning electron microscope (SEM): Providing depth of an TEM image



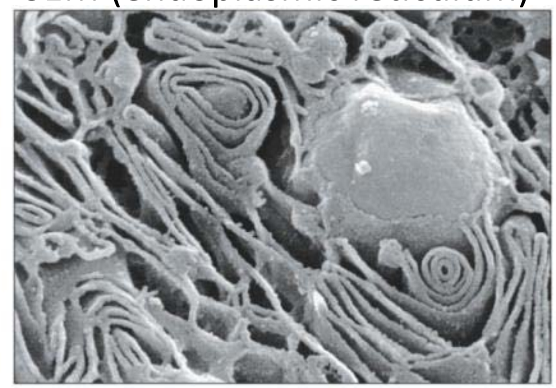
The principle of an SEM is similar to an TEM, however, it includes a **scan generator** for scanning on surfaces providing images with "**3D properties**"



TEM (endoplasmic reticulum)



SEM (endoplasmic reticulum)

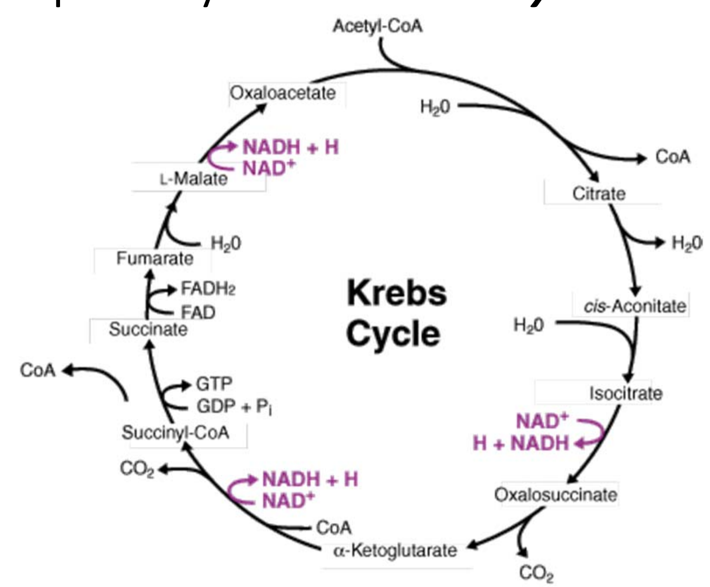


SEM: Human cancer cell

SEM: Pollen grains

Going into details: The biochemistry branch

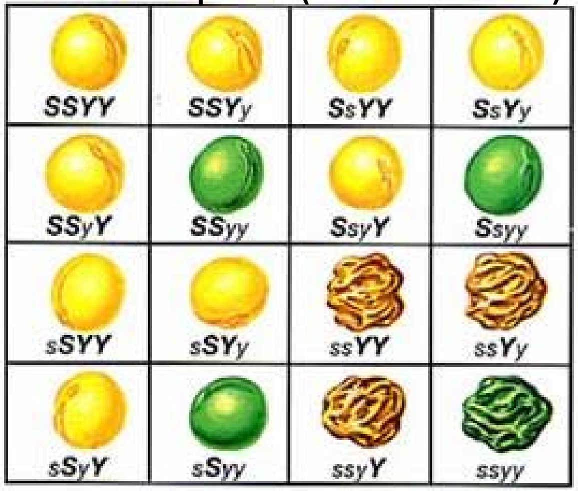
- Biochemistry developed almost parallel to Cytology
- In 1828 German chemist **Friedrich Wöhler** has shown that **an organic** ("vital") **compound** (urea) **can be made from an inorganic** ("dead") **compound** (ammonium cyanide): Before it was thought that organic compounds can only be made by "living things" (as cells). The strict distinction between the living and non-living world ("vitalists") was then lifted
- In 1870s French chemist **Louis Pasteur** has shown that **yeast** can **ferment sugar into alcohol** and in 1897 German scientists **Eduard** and **Hans Buchner** have shown that specific compounds (**enzymes**) are responsible for this process.
- In the 1920/30s German biochemists **Gustav Embden**, **Otto Meyerhof**, **Otto Warburg** and **Hans Krebs** have resolved the pathways of **glycolysis** and **aerobic respiration** (Krebs) **cycle**
- At the same time American biochemists **Fritz Lipmann** has uncovered the function of **ATP**
- In the 1940/50s the **radioisotope method** (e.g., ^{14}C) was used by American chemist **Melvin Calvin** to trace single molecules and atoms in complicated pathways as the ***Calvin cycle*** (carbon metabolism in plant cells)
- The development of **ultracentrifugation** technique by Swedish chemist **Theodor Svedberg** contributed enormously to the isolation of subcellular fractions (nucleus, ribosomes, mitochondria, membranes)
- **Chromatography** separates molecules by their sizes and charges. **Electrophoresis** separates DNA and proteins.
- **Mass spectrometry** analyses a protein's size and amino acid compositions (important for the *proteomics* field)



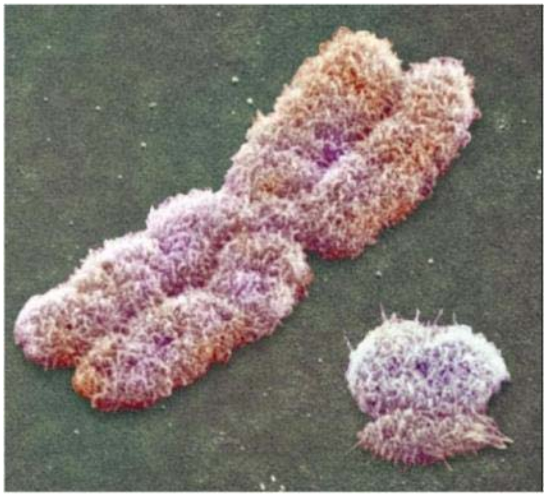
Going into details: The genetics branch

- In the 1860s Austrian **Georg Mendel** laid the foundation for genetics by his discovery of "**hereditary factors**" (now known as genes) when hybridizing pea plants
- In 1890 German **Walther Flemming** and Wilhelm Roux identified **chromosomes** during cell **mitosis**. Chromosomes were linked to the theory of heredity much later in the 1900.
- In 1869 Swiss biologist **Friedrich Miescher** discovered **DNA** as the chemical compound of inheritance but it was not clear how this "monotonous structure" could replicate.
- In 1953 **James Watson** and **Francis Crick** proposed their model of the DNA double helix.
- In the late 1960s the **genetic code** was unraveled (relation of the order of nucleotides in DNA/RNA to the order of amino acid composition in proteins).
- The discovery of **restriction enzymes** (that cleave/split DNA at defined positions) and **polymerases** (that synthesize DNA/RNA strands from nucleotides) lead to recombinant DNA technology and gene cloning

Mendel's peas (9:3:3:1 ratio)



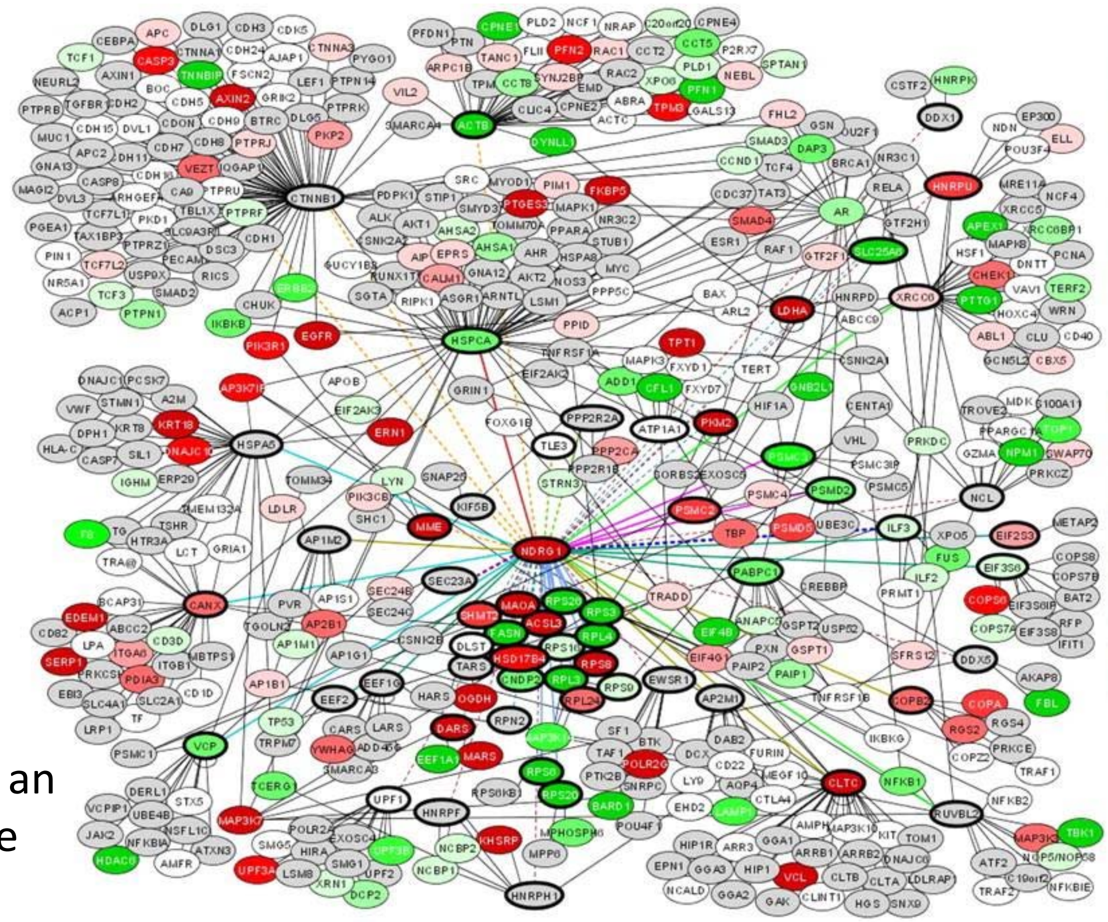
X and Y Chromosome



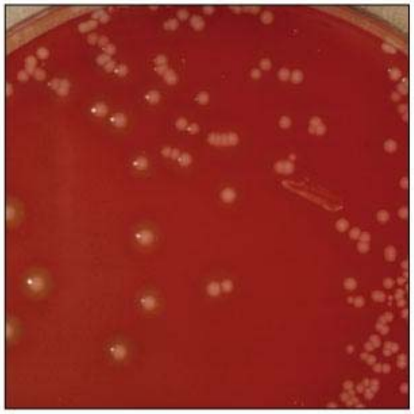
Dr. James Watson at NTHU

Going into details: The genetics branch

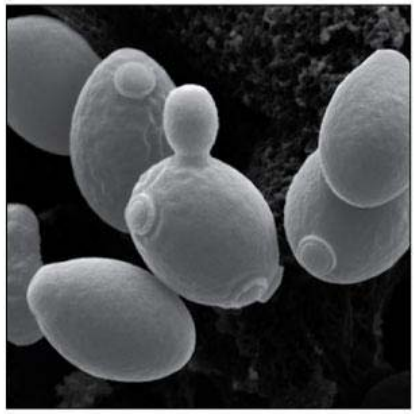
- **DNA sequencing** and **bioinformatics** allowed for the sequencing of **whole genomes** (total DNA content of a cell)
- **1998** whole genome of an animal (**nematode worm** *C. elegans*) was sequenced
- **2003** whole **human genome** was sequenced. **Surprise**: the number of protein coding genes in humans is almost similar to that of the worm (around **25,000**), though the human genome contains about 3.2 billion bases and the worm only 100 million bases (it is thus assumed that humans have **more regulatory genes** than worms).
- **Genomics** then lead to a new challenge: **Proteomics** with the goal to understand how all proteins function and interact in the cell (**interactome**)
- Computer-based **prediction tools**, advanced literature search (NCBI PubMed), **high-throughput yeast two-hybrid** (detects if two proteins interact in a cell) has helped to push proteomics forward



Example of an interactome

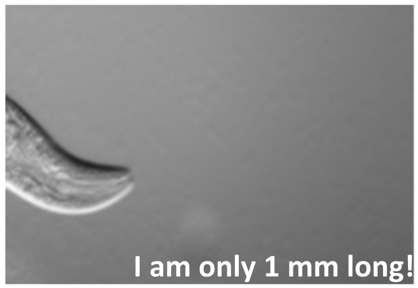


(a) *E. coli*



(b) *S. cerevisiae*

(c) *Drosophila*



(d) *C. elegans*

(e) *Mus musculus*

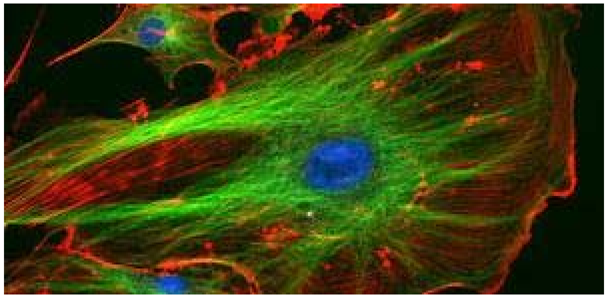
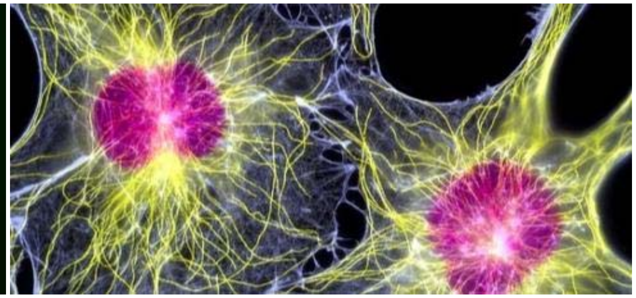
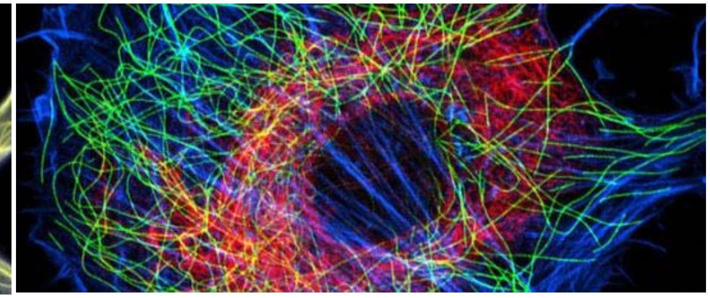
(f) *Arabidopsis*

From single cells to whole animal study

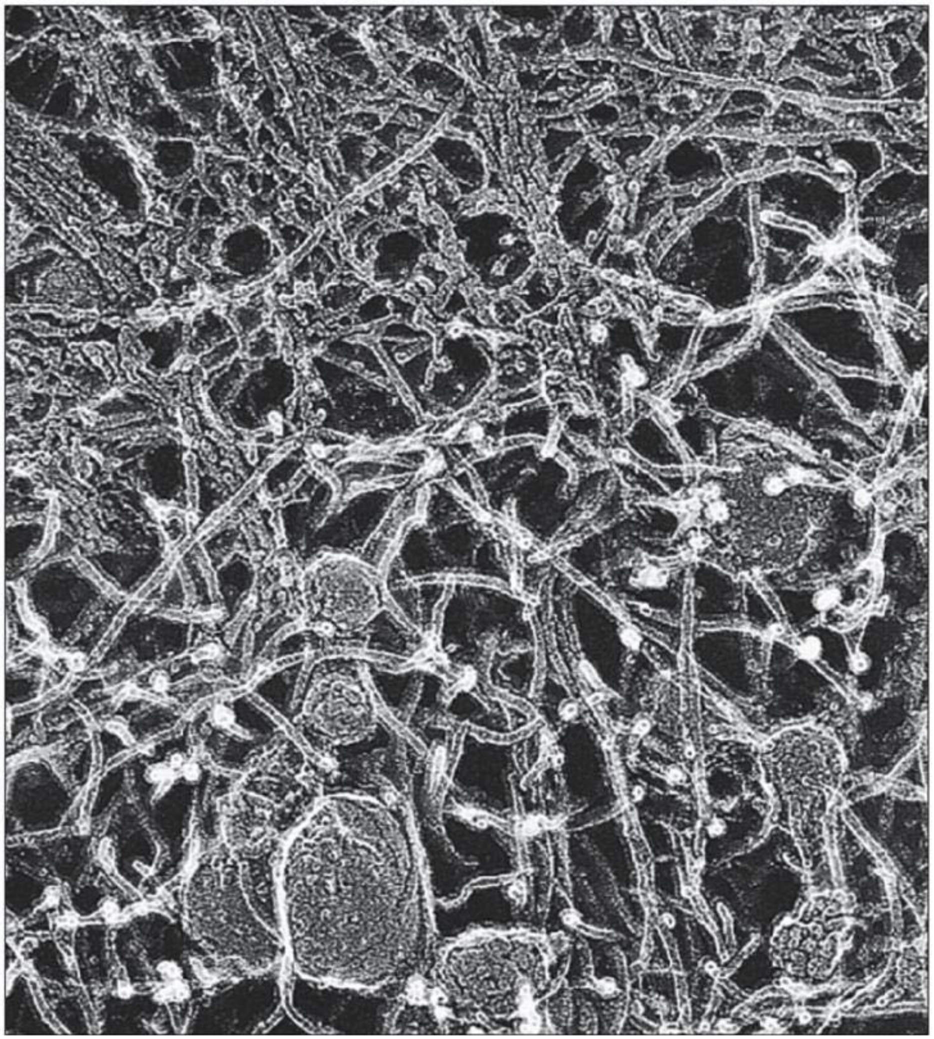
- To fully understand how cells work, we need to **study cells in their natural context** (tissues, organs or whole animals)
- **Model organisms** ideally have their whole genome sequenced and have a **fast reproduction** (life) **cycle** that makes crossing (breeding/hybridization) and mutagenesis easier
- In **mutagenesis** a mutagen (for example chemical) is used to randomly introduce mutations in the genome. **Screening** for new phenotypes (how the animals looks and behaves) leads to conclusions about the function of the affected gene (**forward genetics**)
- Much from what we now know about (e.g.):
  - DNA synthesis has come from ***E. coli***
  - Cell cycle has come from ***S. cerevisia***
  - Homeotic genes (control the development of body parts) comes from ***Drosophila*** (**Nobel prize 1995**)
  - Apoptosis (**Nobel prize 2002**), RNAi (**Nobel prize 2006**) and GFP expression (**Nobel prize 2008**) comes from ***C. elegans***

The scientific method: How do we know what we know?

- So called facts evolve from a theory, however, **facts** do not always reflect the truth and might be proven wrong in the future
- With increasing knowledge, facts might be recognized as **previous misconceptions**, thus altered and modified by **newer theories**
- A fact is simply an attempt to state **our best understanding** of, e.g., a cellular context
- A fact is **valid only until it is revised** or replaced by a better understanding based on more careful observations or sharper experiments
- **New** (and better) **information** becomes available with the **scientific method**:
  - After making observations and assessing prior studies a **hypothesis** is stated
  - This hypothesis (or tentative **model**) is then tested by a series of experiments
  - **Control experiments** are designed to exclude false explanations of the experiment
  - After data collection, statistical analysis, interpretation of the data and comparing previous studies, the **hypothesis is either accepted or rejected**
- Experiments using purified chemicals, proteins or cellular components are called ***in vitro*** (in the "glass"; in the test tube)
- Experiments using live cells or model organism are called ***in vivo*** ("in life")
- Computer-based experiments (e.g., simulations) are named ***in silico*** ("silicon" in chips)
- A **theory** is usually **stronger than a hypothesis** and evolves from many rounds of critically testing a hypothesis or a model (ideally by many different research groups)
- A **law** is **even stronger than a theory** but can be very seldom found in cell biology, based on the complex characters of cells. (Mendel's law of heredity is an example though.)

World of the Cell



The end of chapter 1!

Thank you!