Unit 1: Mechanistic Cell Biology

4. Modern Cell and Molecular Biology

Two New Fields of Biology in Mid-20th Century

- Cell biology:
  - built through the use of the electron microscope and the ultracentrifuge
  - focusing on the cytoplasm of eukaryotic cells (e.g., liver cells from cattle, procured from slaughter houses)
- Molecular biology:
  - heavily influenced by physicists such as Max Delbrück
    - living organisms were a problem for physicists to solve
  - centrifugation with radioactive tracers
  - construction of models of physical components
  - focusing on viruses (phages) and bacteria
- Initially distinct communities, but over time have merged into "Cell and Molecular Biology"

Discussion Question

What determines how scientists differentiate themselves by discipline

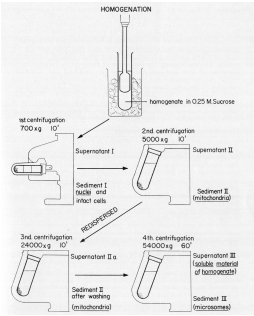
A. Phenomena on the world—different phenomena, different disciplines
B. Social connections between scientists—scientists divide into tribes of people who mostly like each other
C. Instruments—scientists divide according to the instruments they use to investigate nature
D. Administration—institutions divide science into units that can be reasonably administrated
E. Other

Cell Biology: Bridging Cytology and Biochemistry

- The study of cells in the 19th century had become known as cytology
  - and focused on what could be learned by (light) microscopy
- There was a gulf between cytology and biochemistry
  - Biochemists generally homogenated cell content to study chemical reactions in solution, without reference to cell structure
- Modern cell biology, established in the 1940s and 1950s, attempted to bridge biochemical and cell structure studies so as to link chemical reactions to cell structures, using two new research strategies
  - cell fractionation
  - electron microscopy

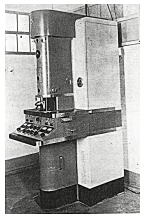
Cell Fractionation

- After homegenating cell contents, use the ultracentrifuge to separate material by mass
- A procedure developed by Claude separated three fractions and a supernate
- These fractions could be analyzed for their contents (enzymes)
  - Fraction 1: nuclear
  - Fraction 2: large particles
  - Fraction 3: smaller particles
  - Supernatant: Soluble enzymes



Electron Microscope

- Detect objects by refraction of electrons rather than transmission of light
  - Requires removing all water from the preparation
- The weak (50 kV) beam of the early electron microscopes required the preparations to be very thin



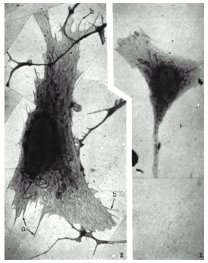
Clicker Question

What strategy did Keith Porter employ in 1945 to overcome the fact that the 50 kV electron beam could not penetrate eukaryotic cells

A. He cut the cell into thin slices and made micrographs of each separately
B. He first centrifuged cells and only imaged the factions that were extracted
C. He made the cell spread thinly so that at least the edges were thin enough to generate images

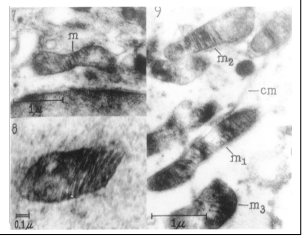
The First EM image of a eukaryotic cell

- In 1945 Keith Porter succeeded in making a micrograph of the edges of a tissue-cultured cell that spread out on the slide of the microscope revealing
  - mitochondria
  - what he took to be Golgi bodies
  - a new structure which he referred to as a *lace-like reticulum*
- By the 1950s researchers had developed microtomes enabling them to generate thin-slices of cells



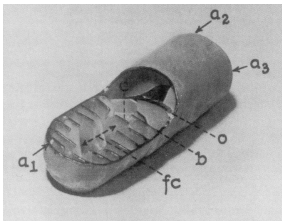
The First Bridge: Mitochondria

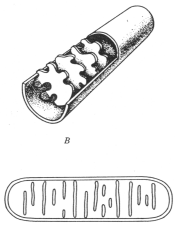
- In the late 1940s, a number of oxidative enzymes were identified in the large particles separated by cell fractionation
  - hypothesis that these belonged to the mitochondrion (large enough to be seen with the light microscope)
  - Confirmed by comparison of microscopic images
- Palade (1952) created electron micrographs of thin slices, using osmium as the stain
  - revealing inholdings of the mitochondria inner membrane: *cristae*



Competing Models of the Mitochondrion

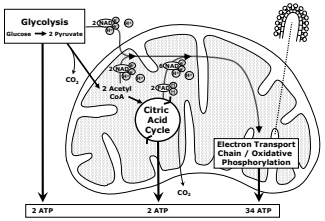
- Palade contended that what EM images showed were inholdings of the inner membrane
- Sjöstrand argued they the inner structures did not contact the membrane





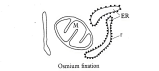
The Stable Bridge: Mitochondria —The Cell's Power Plant

- By the mid-1950s, cell biologists had developed a detailed account of where the chemical reactions in glucose metabolism took place



What's Real?

- Preparing objects for electron microscopy is brutal (as is cell fractionation)
- Different staining techniques produced different images
  - No one understood what the stains (heavy metals) attached to
- Controversies over the Golgi apparatus (first seen by Golgi in the 1890s with the light microscope)
  - Palade and Claude (1949) argued it was an artifact of osmium staining
    - they could produce similar structures in egg white
- Irony: after 10 years of denying its existence, Palade wins his Nobel Prize for work on the Golgi apparatus!



Discussion Question

Why is it so hard for scientists to figure out what is real?

A. They often have to rely on very indirect evidence it is up to them to figure out how the results relate to the phenomenon under study
B. Like any humans, different scientists start with different assumptions and this affects what studies they do and how they interpret the results
C. Science is a matter of story telling and from the same materials, different individuals can construct different stories
D. Other

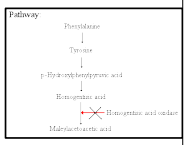

Friedrich Miescher

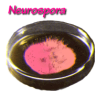
Prehistory of DNA

- In 1869 Friedrich Miescher isolated DNA from fish sperm and the pus of open wounds
  - Named it nuclein since it derived from the nucleus
- In 1914 Robert Feulgen discovered a test for it
  - fuchsin dye stained DNA
- In 1920s Phoebus Aaron Theodor Levene analyzed its composition and identified four nitrogenous bases—cytosine, thymine, adenine, and guanine—as well as deoxyribose sugar and a phosphate group
  - Base unit comprised a base attached to a sugar

Making traits molecular

- Mendel, and those who rediscovered Mendel's work in 1900, focused on observable **phenotypic** traits such as eye color
  - A crucial step in linking traits with genes was to **recharacterize** traits in chemical terms
- A potential link was provided by Archibald Garrod (1909) research on what he called "**inborn errors of metabolism**"
  - Alkaptonuria - an inherited condition in which the urine is colored dark red by alkaptons
  - Results from a single recessive gene, which causes a deficiency in the enzyme that normally breaks down alkapton
- Beadle and Tatum (1941): **one gene=one enzyme**
  - Strategy – find genetic mutants unable to carry out specific enzymatic reactions
  - Exposed Neurospora crassa (a bread mold) spores to X-rays or UV radiation and studied the resulting mutations.
  - Mutants required additions to their diets that their normal counterparts did not—e.g., thiamine or choline
- Subsequent revision: **one gene, one polypeptide** chain

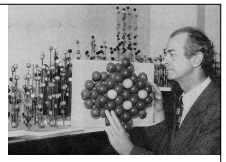


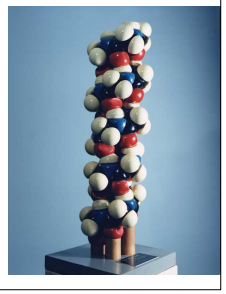


Does DNA have anything to do with genetics?

- We have all come to think of DNA as the material of genes
  - but this wasn't always obvious
- The relatively simple, repetitious structure of DNA did not suggest it as a candidate material out of which to make genes
- Proteins seemed a much better bet

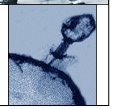
Linus Pauling





- Focused on protein as the genetic material:
  - "I believe that the same process of molding of plastic materials into a configuration complementary to that of another molecule, which serves as a template, is responsible for all biological specificity. I believe that the genes serve as the templates on which are molded the enzymes that are responsible for the chemical characters of the organisms, and that they also serve as templates for the production of replicas of themselves."
- Determined the molecular structure of proteins
  - Alpha helix model a result of folding experiments in which he wrote the structure of a polypeptide chain on a piece of paper and folded it until he found a way of creating an N-H-C-O bond

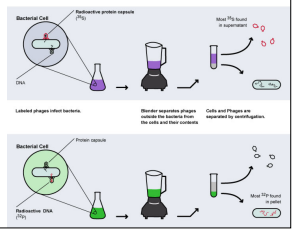
Clues to the Role of DNA



- In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty concluded from experiments transferring new genetic traits between dead and living Pneumococcus bacteria that DNA was the genetic material
- In 1940s: Max **Delbruck** and Salvador Luria began working with bacteriophage, which consist of a protein coat surrounding DNA
  - phages invade bacteria, causing the bacteria to make new phage
  - What in the phage directed the generation of new phage?

Making the link of DNA to genetics

- In 1952, Alfred D. Hershey and Martha Chase differentially labeled DNA and protein of phage to see which entered the bacterium
  - Only DNA entered the bacterium so it had to be the genetic material



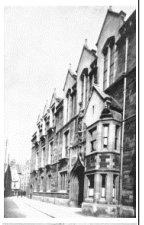
Enter James Watson

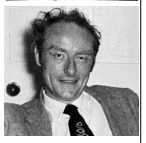
"To have success in science, you need some luck. Without it, I would never have become interested in genetics. I was 17, almost 3 years into college, and after a summer in the North Woods, I came back to the University of Chicago and spotted the tiny book *What is Life* by the theoretical physicist Erwin Schrodinger. In that little gem, Schrodinger said the essence of life was the gene. Up until then, I was interested in birds. But then I thought, well, if the gene is the essence of life, I want to know more about it. And that was fateful because, otherwise, I would have spent my life studying birds and no one would have heard of me."

James Watson (1993), "Succeeding in Science: Some Rules of Thumb", *Science*, 261, 24: 1812.

Watson arrives at Cavendish

- Watson had a fellowship to study microbial metabolism in Europe when he heard a lecture by Maurice Wilkins presenting x-ray crystallography of DNA
- Wilkins refused to hire Watson at London
- Instead Watson went to the Cavendish Laboratories at Cambridge to learn x-ray crystallography
  - He was assigned to share an office with Francis Crick
- Crick, by then a somewhat older graduate student, was attempting to solve the structure of hemoglobin from x-ray diffraction patterns
- The two set out to "imitate Linus Pauling and beat him at his own game."

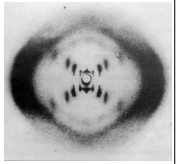




Early Proposal Watson & Crick

- Watson and Crick were model builders: they drew data from wherever they could get it and tried to put together a representation (physical, diagrammatic, etc.) of what was responsible
- Their first model model of DNA positioned phosphates in the center and nucleotides on the outside
  - three strands of DNA wound around each other
- There was a serious problem—the phosphates would have a negative charge and repel each other
  - Watson and Crick tried a fix—adding positive ions to cancel the charge

Rosalind Franklin



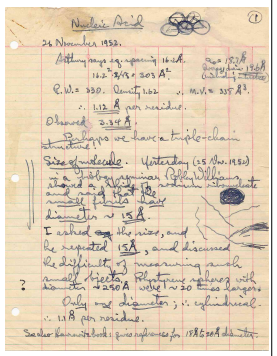
- Watson and Crick invited Maurice Wilkins and his assistant, Rosalind Franklin, up from London to look at their model
- Franklin ripped it apart: DNA soaked up water, indicating that the phosphates had to be on the outside of the molecule
- Wilkins also showed Watson and Crick Franklin's x-ray crystallography results
- Watson and Crick ordered by the director of the Cavendish Lab (Bragg) to stop working on DNA

Pauling recognizes DNA

- Pauling learned of Hershey and Chase's results in 1952 and turned his attention to DNA
- Foresaw no competition
  - His colleague Max Delbrück had received a letter from Watson mentioning his search for DNA
  - But Watson had been turned down for graduate school at Caltech. How serious a threat could he be?
  - Pauling's son Peter goes to Cavendish in September, 1952, and becomes an office-mate of Watson and Crick

Pauling on a False Tack

- In November 1952 Pauling tried his hand at a model of DNA and came up with essentially the one Watson and Crick had proposed and Franklin had shot down
- A week later he claimed: "I think now we have found the complete molecular structure of the nucleic acids."
- In December he wrote to Alex Todd at Cambridge "We have, we believe, discovered the structure of nucleic acids. I have practically no doubt. . . The structure really is a beautiful one."

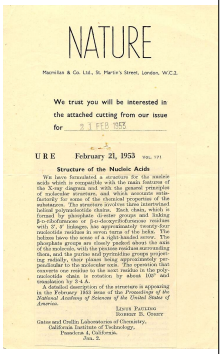

Nucleic Acid

24 November 1952.

Diameter ~ 15 Å.

Pauling Tries to Establish Priority

- On December 31, 1952 Pauling and Corey sent a paper to PNAS: "A Proposed Structure for the Nucleic Acids" (and a short note to *Nature*)
- Spoke of "a promising structure," but "an extraordinarily tight one" that accounted only "moderately well" for the x-ray data
- Gave only "reasonably satisfactory agreement" with theoretical values obtained by Crick
- Acknowledged the atomic positions were "probably capable of further refinement."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We trust you will be interested in the attached cutting from our issue for 21 FEB 1953

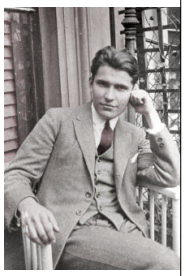
URE February 21, 1953 Vol. 171

Structure of the Nucleic Acids

Questioning the Master

- "At once I felt something was not right. I could not pinpoint the mistake, however, until I looked at the illustrations for several minutes. Then I realized that the phosphate groups in Linus' model were not ionized, but that each group contained a bound hydrogen atom and so had no net charge. Pauling's nucleic acid in a sense was not an acid at all. Moreover, the uncharged phosphate groups were not incidental features. The hydrogens were part of the hydrogen bonds that held together the three intertwined chains. Without the hydrogen atoms, the chains would immediately fly apart and the structure vanish.
"Everything I knew about nucleic-acid chemistry indicated that phosphate groups never contained bound hydrogen atoms. No one had ever questioned that DNA was a moderately strong acid. Thus, under physiological conditions, there would always be positively charged ions like sodium or magnesium lying nearby to neutralize the negatively charged phosphate groups. All our speculations about whether divalent ions held the chains together would have made no sense if there were hydrogen atoms firmly bound to phosphates. **Yet somehow Linus, unquestionably the world's most astute chemist, had come to the opposite conclusion**" James Watson, *The Double Helix*. 1968

A Key Finding that was Initially Ignored



- Erwin Chargaff (1949) established that adenine and thymine were present in roughly the same amounts
  - As were guanine and cytosine
  - One of each of these pairs was a larger purine; the other, a smaller pyrimidine.
- While Pauling was off getting engaged, Crick and Watson met with Chargaff and he related these findings

Chargaff on Crick and Watson

- "So far as I could make out, they wanted, **unencumbered by any knowledge of the chemistry involved**, to fit DNA into a helix. The main reason seemed to be Pauling's alpha-helix model of a protein.
  ...I told them all I knew. **If they had heard before about the pairing rules, they concealed it**. But **as they did not seem to know much about anything, I was not unduly surprised**. I mentioned our early attempts to explain the complementarity relationships by the assumption that, in the nucleic acid chain, adenylic was always next to thymidylic acid and cytidylic next to guanylic acid. ...I believe that the double-stranded model of DNA came about as a consequence of our conversation; but such things are only susceptible of a later judgment…."

  Erwin Chargaff, *Heraclitean Fire*. 1978.

Success!

- Putting together the insights from Chargaff and the clue from Franklin that the phosphates were on the outside, Crick and Watson generated a new model
- A colleague showed them that they were using the wrong structures for guanine and thymine
- One more revision, and voila—their celebrated model
- In April Pauling visited Cavendish and concluded that Watson and Crick had figured out the structure of DNA

Watson on Rosie Franklin

- "Rosalind Franklin was a very intelligent woman, but she really had no particular reason for believing that DNA was particularly important. She was trained in physical chemistry. I don't think she'd ever spent any length of time with people who thought DNA was important. And she certainly didn't talk to Maurice [Wilkins] or to John Randall, then the professor at Kings".

  James Watson quoted in *Nature*, 302, 21 (April 1983): 653.
- There's a myth which is, you know, that Francis and I basically stole the structure from the people at King's. I was shown Rosalind Franklin's x-ray photograph and, Whooo! that was a helix, and a month later we had the structure, and Wilkins should never have shown me the thing.
  I didn't go into the drawer and steal it, it was shown to me, and I was told the dimensions, a repeat of 34 angstroms, so, you know, I knew roughly what it meant and, uh, **but it was that the Franklin photograph was the key event. It was, psychologically, it mobilised us**…"

  James Watson, Center for Genomic Research
  Inauguration, Harvard. September 30, 1999. 1

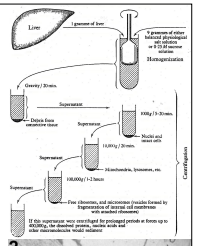
Discussion Question

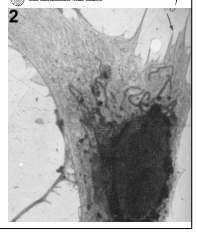
Was Rosalind Franklin treated unfairly (and if so, by whom)?

A. Yes
B. No
C. Other (specify)

Relating DNA to Cells: Where Do Proteins Get Synthesized?

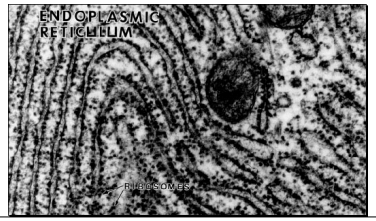
- When Claude developed the four step process of cell fraction one of the fractions consisted of small particles he called microsomes
  - found to be rich in RNA
- These were soon associated with the lace-like reticulum Porter had identified in his early micrograph



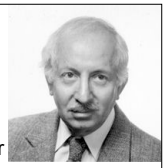


Rough Endoplasmic Reticulum and Ribosomes

- Advent of thin slicing techniques allowed Porter to examine this structure in much greater detail
- Endoplasmic reticulum appears as ribbons
- Some portions appear to have particles attached--ribosomes



Ribosome the locus of Protein Synthesis



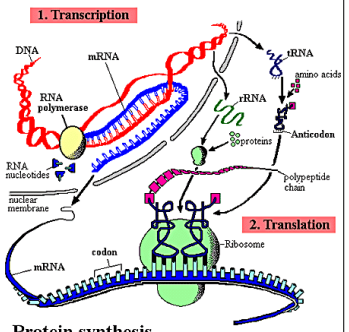
- Philip Siekevitz, while working with Paul Zamecnik at Harvard, learned the molecular biology technique of attaching radioactive tracers so as to trace steps in a process
- Working with Palade, he showed by tracing amino acids that new proteins were synthesized in the ribosome of the rough endoplasmic reticulum
- Palade's lab showed that these newly created proteins were then transported to the Golgi apparatus
- A remaining challenge: relate the DNA in the nucleus to protein synthesis in the ribosome

Multiplying RNAs

- Although early on it was recognized that ribosomes were rich in RNA, the RNA associated with the ribosome (rRNA) did not seem complex enough to directed the synthesis of multiple different protein
- 1961: Jacob and Monod proposed that a special type of RNA (messenger RNA or mRNA) might be synthesized directly from the DNA template of genes and transported to the ribosomes where it would provide the information for protein synthesis
  - Sydney Brenner, Jacob, and Matthew Meselson showed that when a T4 virus infects a bacterial cell, a virus-specific RNA is made that is rapidly associated with preexisting bacterial ribosomes
- Subsequently, yet another RNA was discovered, which binds both with amino acid and with mRNA: transfer RNA (tRNA)

Mechanism of protein synthesis

- All three types of RNA formed in the nucleus and migrate to the ribosome
- mRNA built on the DNA template and directs the order of amino acid binding
- tRNA binds with amino acids and deposits them onto the polypeptide chain



Protein synthesis

Control Genes: The Lac Operon

- All cells in a organism possess the same DNA
  - Some DNA gets expressed in different contexts
  - How is DNA expression controlled?
- In 1900, F. Dienert discovered that the enzymes needed for galactose metabolism were found in yeast only when the yeast used galactose as a carbon source
  - the presence of galactose had called forth or induced the specific enzymes (e.g., β-galactosidase) necessary to metabolize galactose
- Joshua Lederberg developed three mutant strains ($lacZ^-$, $lacY^-$, and $lacA^-$) that each lacked an enzyme needed to metabolize lactose and these were all mapped to the same region on the chromosome
  - This suggested the induction occurred at the level of the chromosome
  - Lederberg produced a different mutant ($lacI^-$) which always produced the enzymes, and it was located nearby

Basics of the Lac Operon: Jacob and Monod

- First influential account of control of DNA: explaining how enzymes for metabolizing different sugars are produced only when needed in bacteria