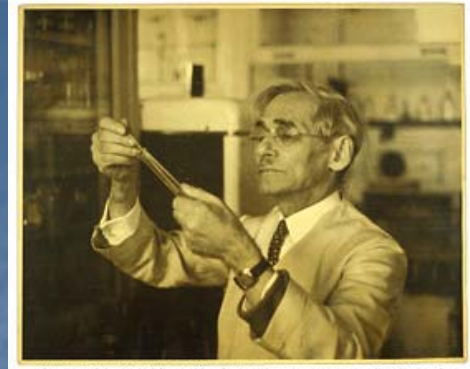
Genetics: From Mendel to Molecules


Friedrich Miescher

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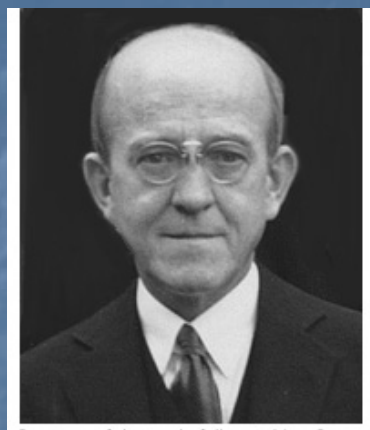
- In 1869 Friedrich Miescher isolated DNA from fish sperm and the pus of open wounds
  - Named it *nuclein* since it derived from the nucleus
- In 1914 Robert Feulgen discovered a test for it
  - fuchsin dye stained DNA
- In 1920s Phoebus Aaron Theodor Levene analyzed its composition and identified four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

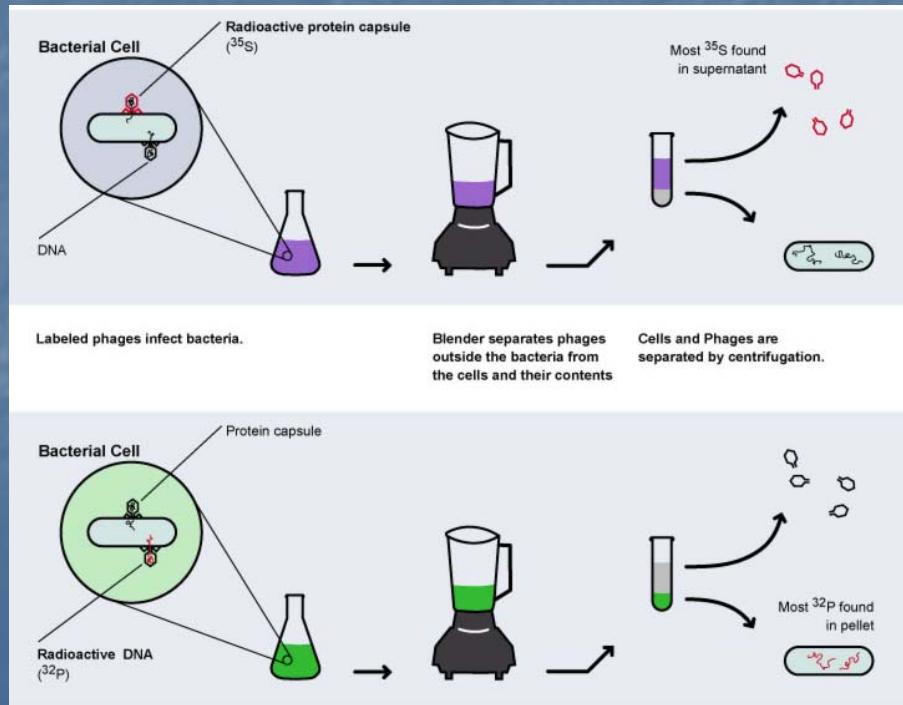
But what did DNA have to do with anything?

- Traditional view—DNA too simple to be the genetic material
  - The genetic material must be protein
- In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty concluded from experiments transfering new genetic traits between *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 which invade a bacterium, causing it to make new phage
  - First established exclusion principle: only one strain will infect a bacterium



Making the link

- 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



A Key Initially Ignored

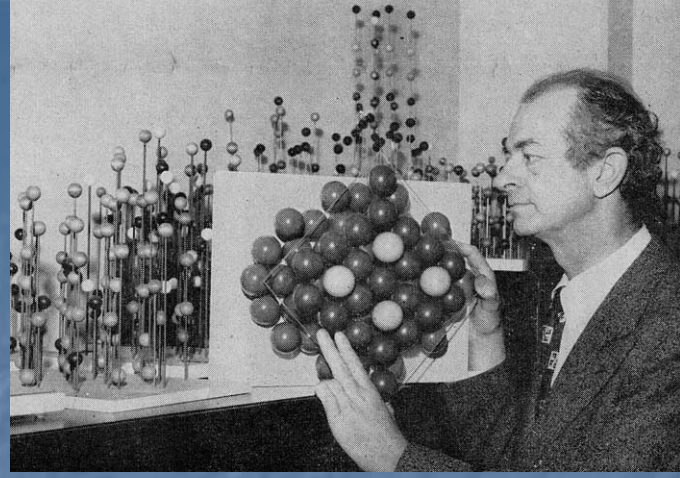
- Erwin Chargaff (1949) established that adenine and thymine were present in roughly the same amounts as were guanine and cytosine.
  - One of each of these pairs was a larger purine; the other, a smaller pyrimidine.

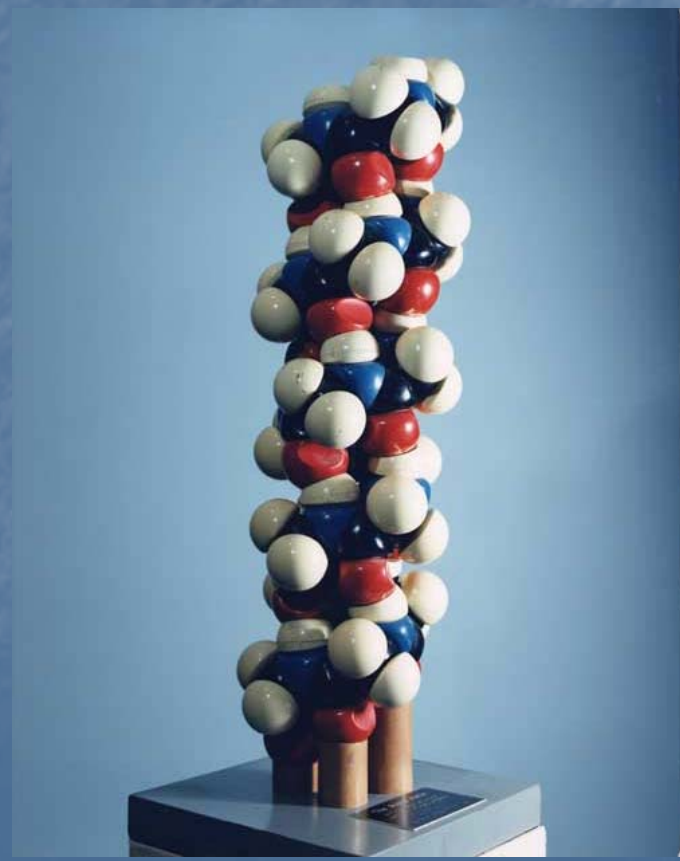
Linus Pauling

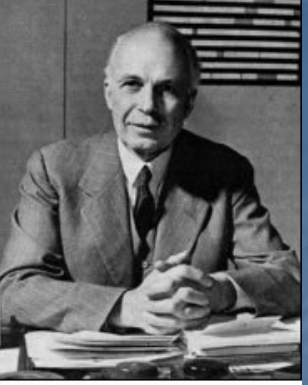
- Focused on protein as the genetic material:

  "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

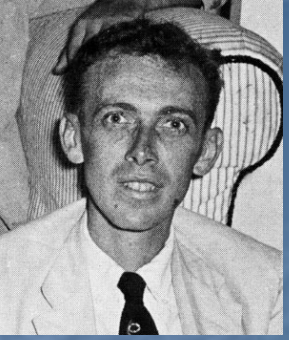





William Lawrence Bragg

Sir Laurence Bragg

- Won Nobel Prize for early work with his father on development of x-ray crystallography
- Head of the Cavendish Laboratory, which included eminent researchers such as Max Perutz and John Kendrew (in whose laboratory Watson, Crick were to work)
- Competitor with Pauling on structure of silicates and then on structure of proteins



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

Watson arrives at Cavendish

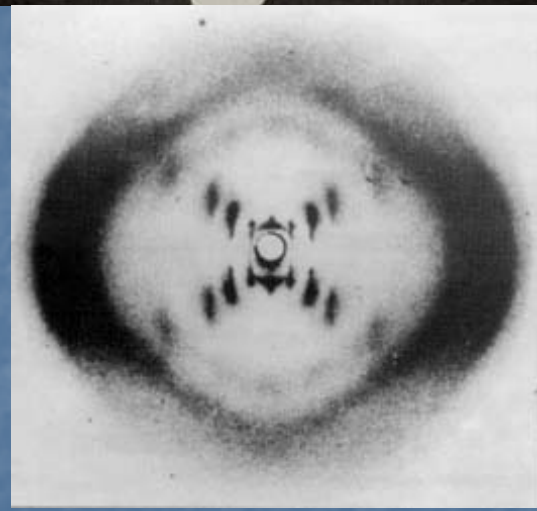
- Watson had a fellowship to study microbial metabolism in Europe when he heard a lecture by Maurice Wilkins presenting x-ray crystallography of DNA
- Wilkins refused to hire Watson at London
- Instead ended up at Cavendish to learn x-ray crystallography, where he was assigned to share an office with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

Early Proposal Watson & Crick

- At first Watson and Crick advanced a model with phosphates provide the core, nucleotides on the outside
  - three strands of DNA wound around each other
- But there was a problem—the phosphates would have a negative charge and repel each other
- Watson and Crick tried a fix—add 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
- She also showed Watson and Crick her x-ray crystallography results
- Watson and Crick ordered by Bragg to stop working on DNA

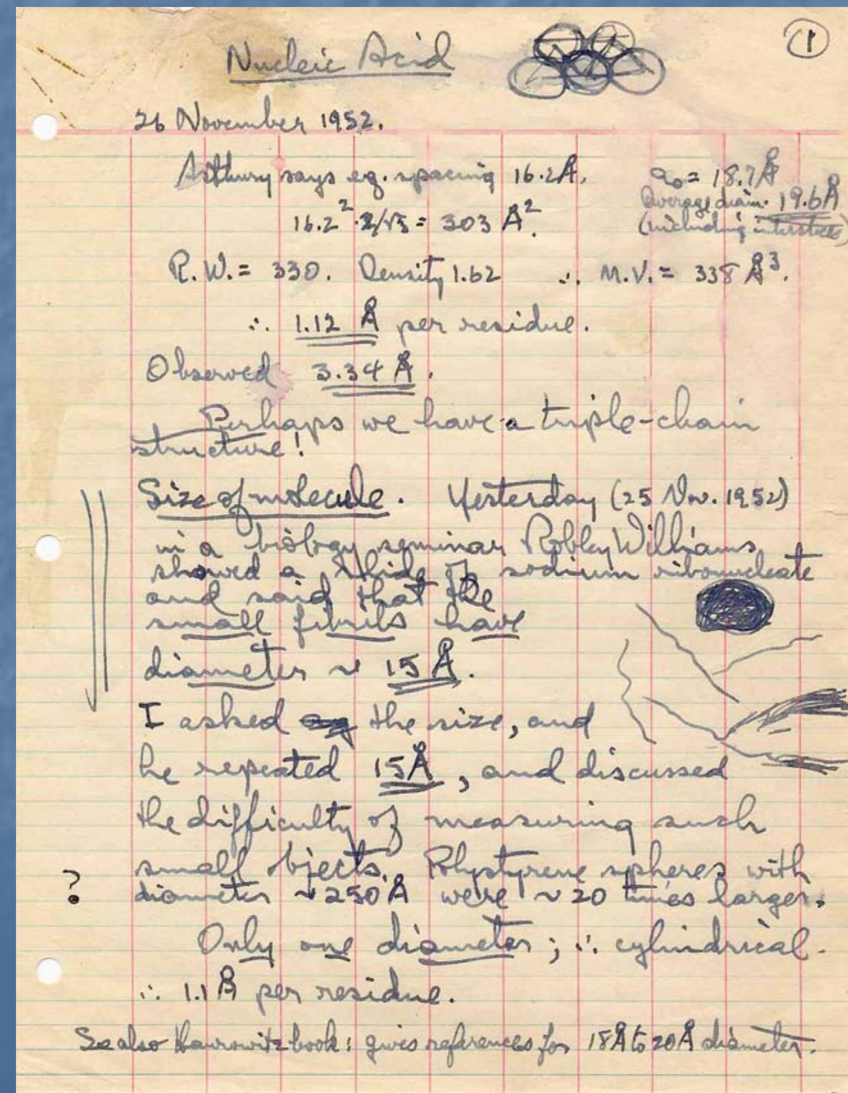


Pauling recognizes DNA

- Pauling learned of Hershey and Chase's results in 1952 and turned his attention to DNA
- Foresaw no competition
  - His colleague Max Delbruck 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

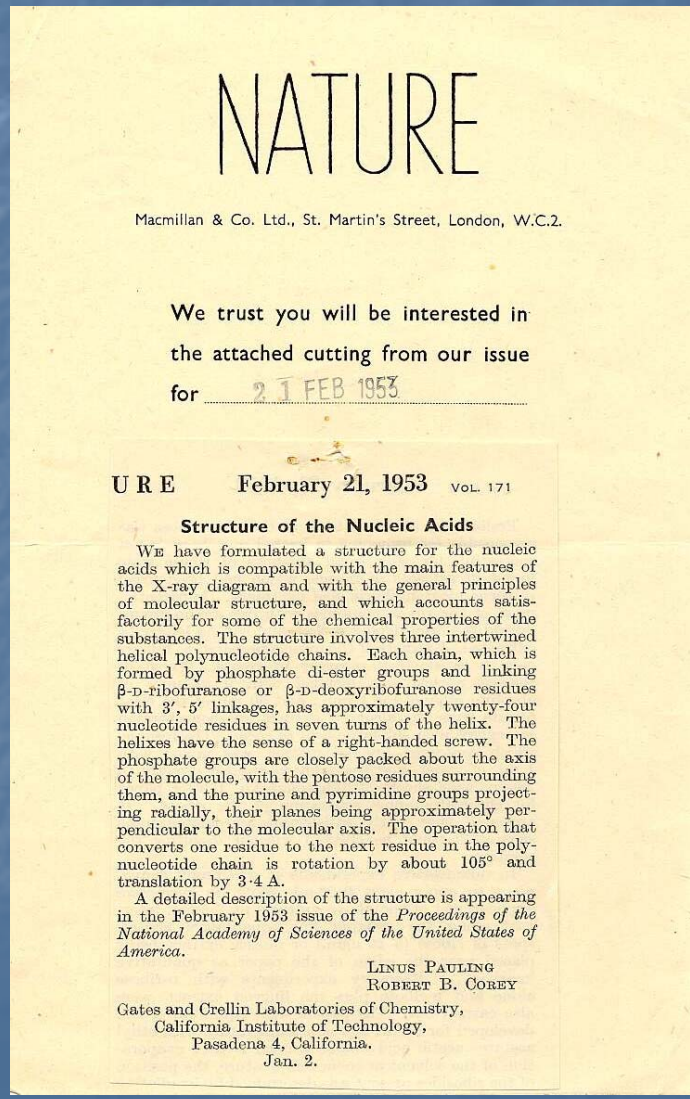
Pauling on a False Tack

- In November 1952 Pauling tried his hand at a model of DNA and came up with essentially the one Watson and Crick and 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."



Pauling Tries to Establish Priority

- On December 31, 1952 Pauling and Corey sent a paper to PNAS: "A Proposed Structure for the Nucleic Acids."
- 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."



NATURE

Macmillan & Co. Ltd., St. Martin's Street, London, W.C.2.

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

We have formulated a structure for the nucleic acids which is compatible with the main features of the X-ray diagram and with the general principles of molecular structure, and which accounts satisfactorily for some of the chemical properties of the substances. The structure involves three intertwined helical polynucleotide chains. Each chain, which is formed by phosphate di-ester groups and linking β-D-ribofuranose or β-D-deoxyribofuranose residues with 3′, 5′ linkages, has approximately twenty-four nucleotide residues in seven turns of the helix. The helixes have the sense of a right-handed screw. The phosphate groups are closely packed about the axis of the molecule, with the pentose residues surrounding them, and the purine and pyrimidine groups projecting radially, their planes being approximately perpendicular to the molecular axis. The operation that converts one residue to the next residue in the polynucleotide chain is rotation by about 105° and translation by 3·4 A.

A detailed description of the structure is appearing in the February 1953 issue of the *Proceedings of the National Academy of Sciences of the United States of America*.

Linus Pauling
Robert B. Corey

Gates and Crellin Laboratories of Chemistry,
California Institute of Technology,
Pasadena 4, California.
Jan. 2.

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

Chargaff's Key

- While Pauling was getting engaged, Crick and Watson met with Chargaff and he told them of his result that
  - adenine and thymine were present in roughly the same amounts
  - likewise were guanine and cytosine
  - one of each pair was a larger purine; the other, a smaller pyrimidine
- This lead and the suggestion from Franklin that the phosphates were on the outside suggested a new model

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!

- Watson and Crick put together a model, but a colleague showed an that they were using the wrong structures for guanine and thymine
- This pointed the way to 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 spend 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

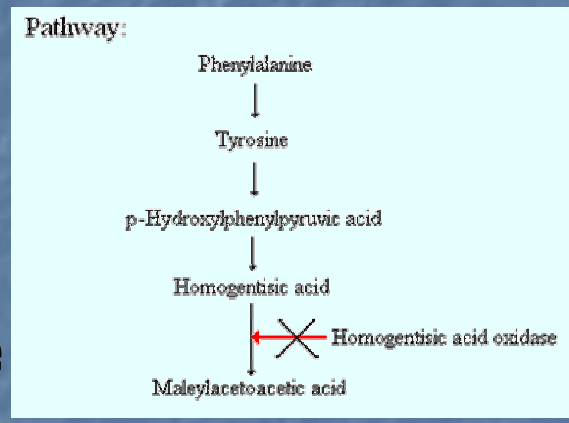
OK, but what does DNA do?

- Watson and Crick conclude: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

- Besides copying, DNA must do more if it is the genetic material
  - It must code for traits
  - There must be a mechanism by which it gets expressed as traits

Making traits molecular

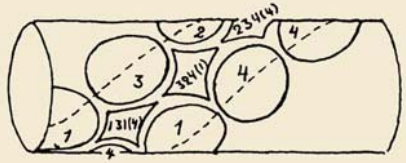
- Archibald Garrod (1909): "inborn errors of metabolism"
  - Alkaptonuria - an inherited condition in which the urine is colored dark red by alkaptons
  - Results from a single recessive gene, which causes a deficiency in the **enzyme** that normally breaks down alkapton

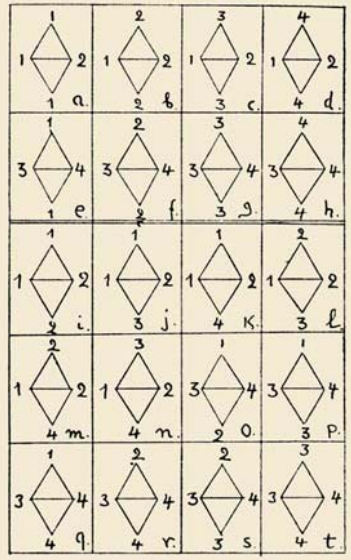


- Beadle and Tatum (1941): one gene=one enzyme
  - Strategy – find genetic mutants unable to carry out specific enzymatic reactions
  - Exposed Neurospora crassa (a bread mold) spores to X-rays or UV radiation and studied the resulting mutations.
  - Mutants required additions to their diets that their normal counterparts did not—e.g., thiamine or choline
- Revision: one gene, one polypetitde chain

Figuring out the code

- One base pair could not specify an amino acid—4 base pairs and 20 amino acids
- Two base pairs not enough—only 16 possibilities
- Three base pairs more than enough—64 possibilities



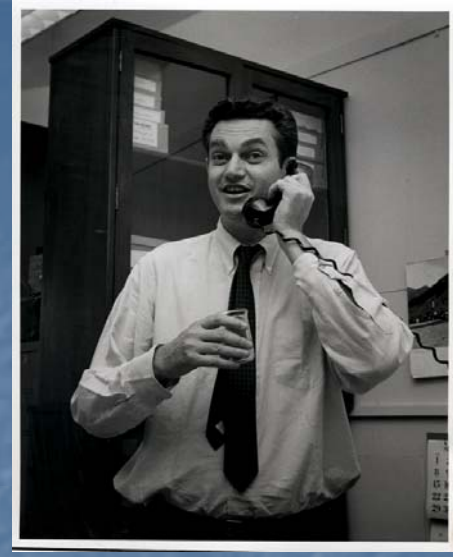


- physicist George Gamow proposed that the RNA polymerase read three-base increments of DNA while moving along the DNA one base at a time.
  - Prediction that certain bases should not occur side-by-side in nature (or else one triplet base sequence could code for more than one amino acid)

Crick: Reading Frames

- Addition or deletion of one or two nucleotides results in abnormal phenotype, but addition or deletion of three near to one another results in normal phenotype
- Supports
  - Three nucleotides comprise a unit
  - There is a reading frame from which reading starts

Code Breaking



- Marshall Nirenberg discovered that adding ribosomal RNA to disrupted cells enabled them to continue synthesizing protein
- Tried synthetic RNA polyuridylic acid, which they expected to protein synthesis, but it increased generation of phenylalanine sequences—PHE-PHE-PHE-
  - Indicated that UUU codes for phenylaline
- By 1964 Nirenberg and Har Gobind Khorana had succeeded in using radioactively labeled synthetic RNA to map the full code

The Genetic Code

UUU UUC phenyl alanine UUA UUG leucine	UCU UCC UCA UCG serine	UAU UAC tyrosine UAA UAG stop	UGU UGC cysteine UGA stop UGG tryptophan
CUU CUC CUA CUG leucine	CCU CCC CCA CCG proline	CAU CAC histidine CAA CAG glutamine	CGU CGC CGA CGG arginine
AUU AUC AUA isoleucine AUG methionine	ACU ACC ACA ACG threonine	AAU AAC asparagine AAA AAG lysine	AGU AGC serine AGA AGG arginine
GUU GUC GUA GUG valine	GCU GCC GCA GCG alanine	GAU GAC aspartic acid GAA GAG glutamic acid	GGU GGC GGA GGG glycine

But where is the machinery of making proteins?

- Albert Claude developed a procedure for separating cell organelles
  - Original focus on the mitochondria, an organelle known from light microscopy
  - Initially treated mitochondria as the small particles in his preparation, but soon discovered his mistakes
  - What were the small particles? A new, unknown constituent he called the microsome
- Particles soon found to be high in RNA content

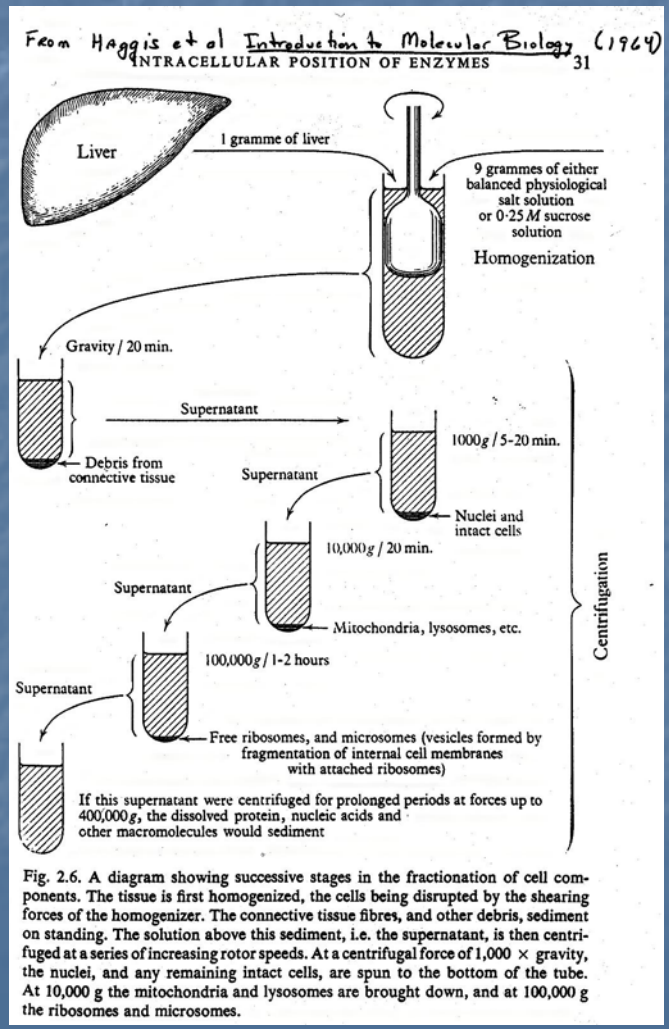
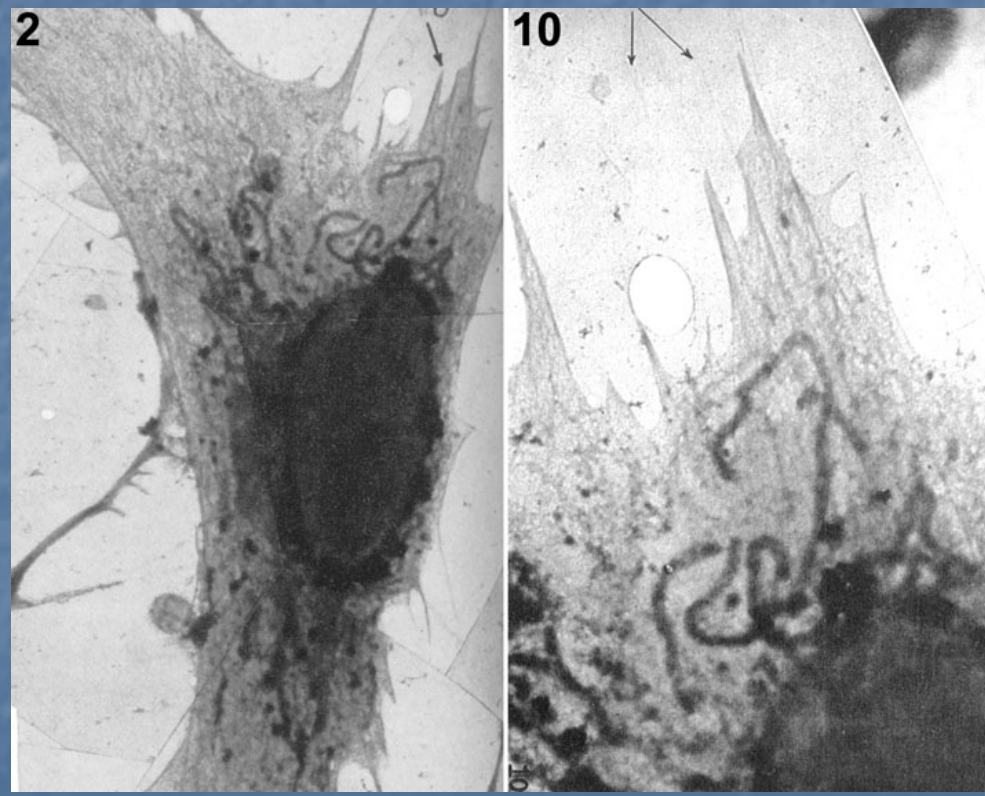


Fig. 2.6. A diagram showing successive stages in the fractionation of cell components. The tissue is first homogenized, the cells being disrupted by the shearing forces of the homogenizer. The connective tissue fibres, and other debris, sediment on standing. The solution above this sediment, i.e. the supernatant, is then centrifuged at a series of increasing rotor speeds. At a centrifugal force of 1,000 × gravity, the nuclei, and any remaining intact cells, are spun to the bottom of the tube. At 10,000 g the mitochondria and lysosomes are brought down, and at 100,000 g the ribosomes and microsomes.

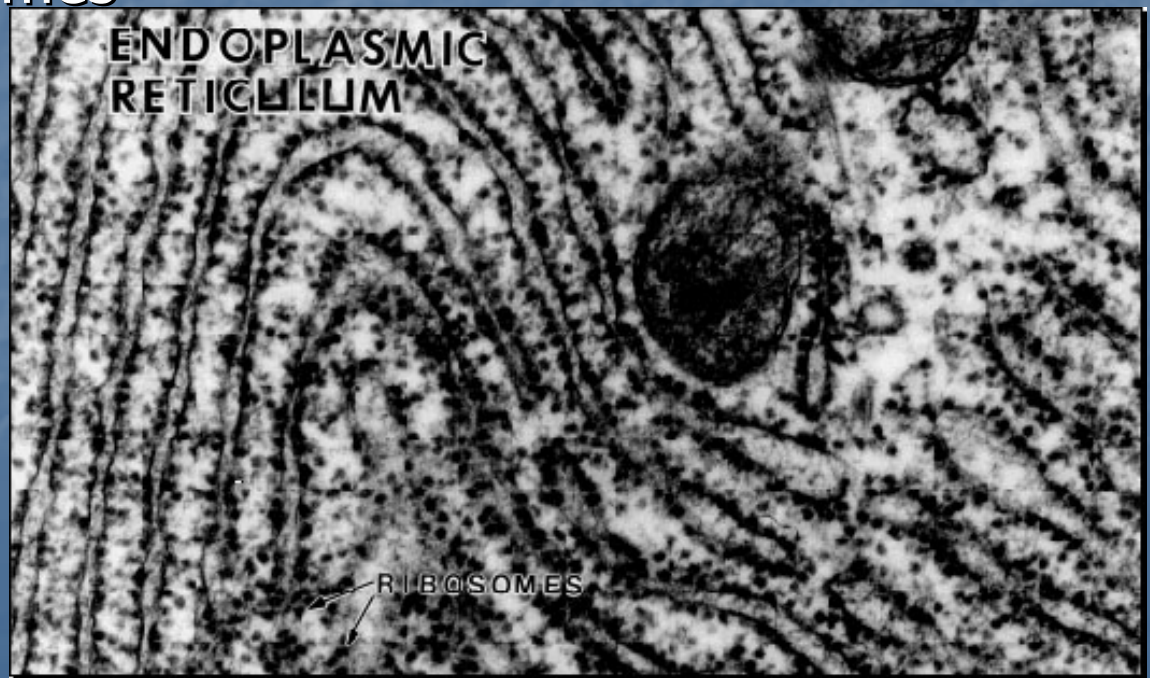
Electron microscopy and the Endoplasmic Reticulum

- Most internal cell structures too small to see with light microscope
- The hope of the electron microscope
- Needed thin specimens
- Porter, Claude, and Fullam succeed in developing a micrograph with tissue-cultured cells
- Mitochondria plus a "lace-like reticulum"



Rough Endoplasmic Reticulum and Ribosomes

- Advent of thin slicing techniques allows much greater resolution of cell structures
- Endoplasmic reticulum appears as ribbons
- Some portions appear to have particles attached--ribosomes



Ribosome the locus of Protein Synthesis

- Introduction of radioactive tracers by Philip Siekevitz while working with Paul Zamecnik at Harvard
- Collaboration with George Palade at Rockefeller provided evidence that the ribosomes were the locus of protein synthesis
- Newly created proteins then transported to the Golgi apparatus

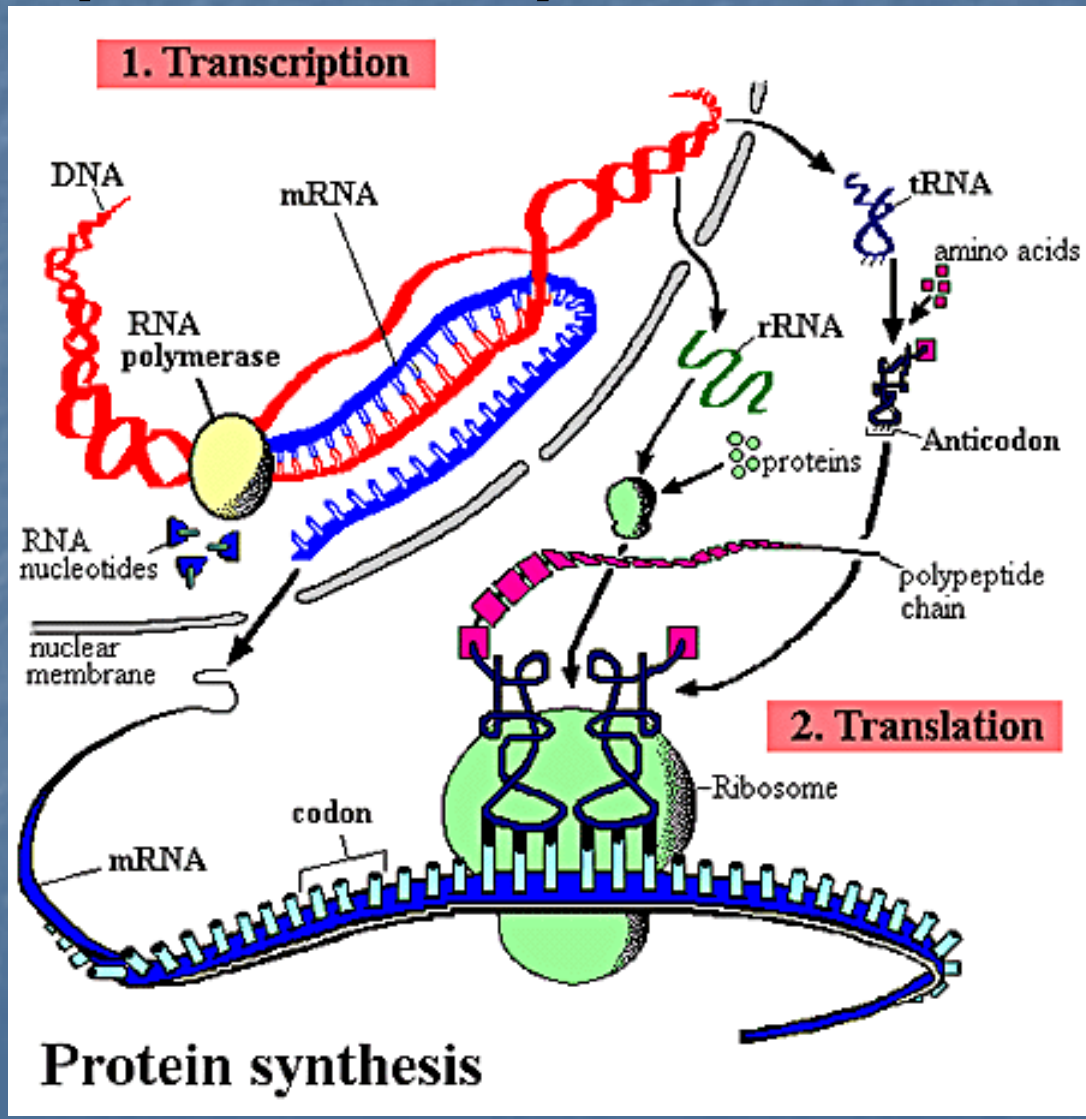
- Challenge: relate the DNA in the nucleus to protein synthesis in the ribosome

Multiplying RNAs

- Although it might seem plausible that ribosomal RNA (rRNA) directed protein synthesis, that could not explain the variability in the proteins produced
- 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
- Yet another RNA 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



Control Genes: The Lac Operon

- 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