The central Dogma

Molecular Genetics and Genomics in Development and Disease

What is the central dogma and why is it so important? What are the important implications?

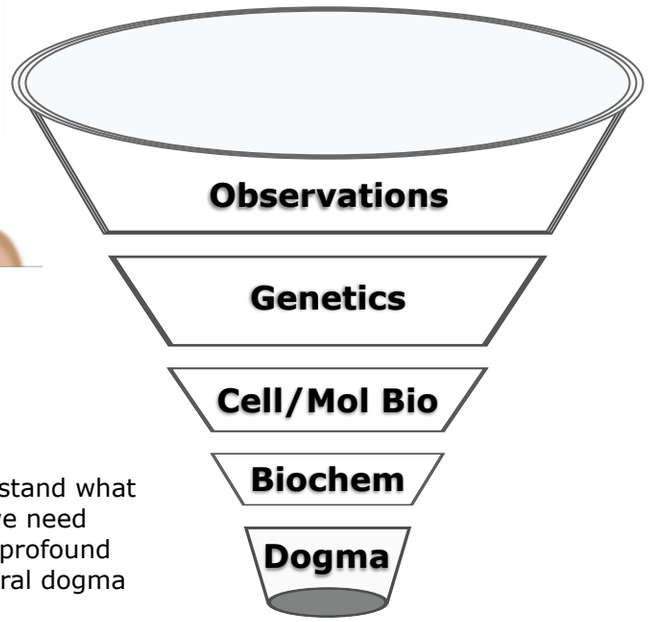
1) Molecular logic of a phenotype
2) Logical framework to understand molecular events in the cell and how they change in disease (e.g. if protein isn't made then it must be that DNA is missing or RNA isn't made)
3) Information on where and regulation would or could occur
4) Universally applicable to all organisms!
5) Logical framework to incorporate new species of regulatory elements (e.g. MicroRNA blocking translation shows the dogma flows in both directions)

Learning Objectives

To know the key logic and experiments that lead to the discovery of central dogma.

After this lecture, put yourself in 1900 and think of trying to write a grant on the idea of a central dogma. What key experiments led to solving the dogma? It wasn't just Jacob and Monod.

The Central Dogma : from Marvel to Molecules



Learning goals:
It is important to understand what each tells us and how we need them all to derive such profound insight such as the central dogma

Here, we are discussing the importance of the integration of Genetics, Cellular and Molecular Biology and Biochemistry to hone in on the molecule of inheritance. It is important to understand what each tells us and how we need them all to derive such profound insight such as the central dogma.

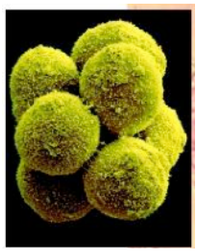
After lecture, imagine taking out one approach (i.e. genetics) and try to figure out the central dogma. (For instance, without genetics, we wouldn't have found the chromosome, which directed us to DNA)

Back to a Time Before DNA

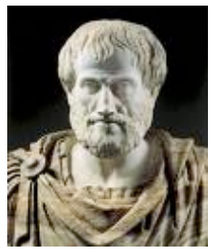
DNA → **RNA** → **Protein**

(300 BC — 1700) Epigenesis -vs- Preformation

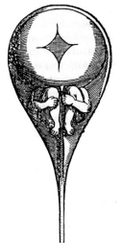
Epigenesis



Aristotle



Preformation



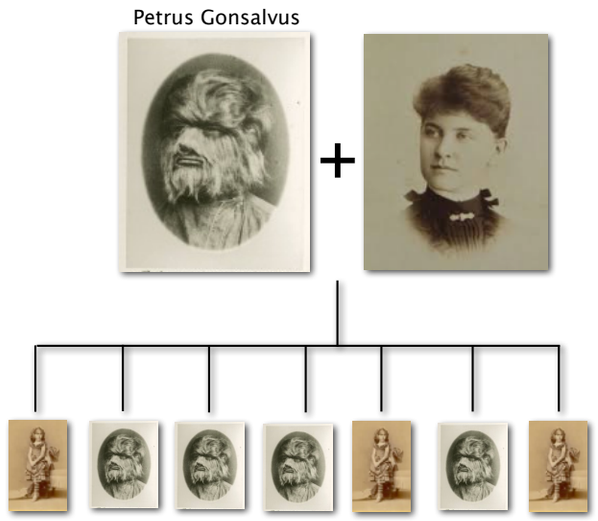
The embryo begins as an undifferentiated mass and that new parts are added during development

Here we are discussing how fascinated people were for over 2,000 years about how traits were passed down! One of the major efforts was breeding animals for domestication and farming.

This is an example of some of the first scientific events to figure out how traits are passed on. There was a fervent debate over whether “preformation” or “epigenesis” was the logic of inheritance (take time to think why this was important). If epigenesis wasn’t proven then, we may not have searched for the molecular traits or substance and chalked it up to “deity“ control of phenotypes.

Aristotle had a hobby of watching chickens develop in their egg and noticed that new structures emerged. He supported epigenesis although the evidence was not sufficient. Interestingly, he found support from people studying the solar system that it changed “epigenetically” in time !

1556 How are traits transferred ?



Distinct phenotypes are produced. What influences that?

The curious case of Petrus Gonsalvus (Italian name) (His Spanish name was Pedro Gonzalez). He was orphaned to the court of King Henry II of France. The king was interested in knowing whether Petrus Gonsalvus could learn or was a savage. Petrus Gonsalvus ended up being incredibly smart, married and had children. The king was intrigued and noted that not all children had Petrus Gonsalvus's traits. The king became suspicious that maybe a "diety" didn't curse Petrus Gonsalvus, but rather he had a special trait that could be passed down, which raised the question of what accounts for inheritance or how it happens.

Epigenesis & Regeneration (not preformation)

1759 : Caspar Wolff

Wolff's work rejected the idea of preformation and reinvigorated the theory of **epigenesis**.

Caspar Wolff used the plant root to prove Epigenesis. He was able to take differentiated tissues and regenerate a whole new plant! This was not possible under the preformation model and quickly turned focus to epigenesis. Also this example demonstrates the importance of technology (in this case the microscope) progression in addressing key biological questions of a given time.

This had a major implication that if it was epigenesis than phenotypes could be altered or engineered during the process.

What was the motivation to find out how traits are passed?

King Tut
Cleft Palate
Club Foot
Missing Bones in Feet
Scoliosis

Agriculture
Medicine
Deriving new traits

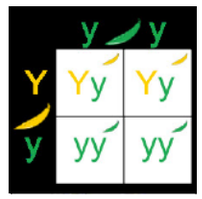
It is important sometimes to think back and ask: “what was driving/funding science at that time?” What are the driving factors today?

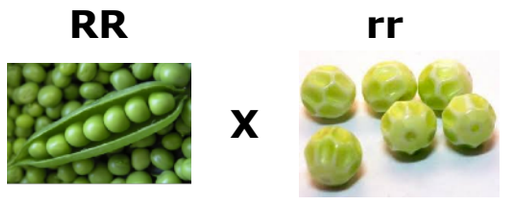
At the time many of the families in power had bizarre illnesses and phenotypes. Therefore, they were highly motivated to find out how traits get passed so they could fix them.

Farming and agriculture has always been a driving force of science, and in this case, was the original impetus behind understanding the “transforming principle” or rules of inheritance.

Genetics to the Transforming Principle

"In 1859 I obtained a very fertile descendant with large, tasty seeds from a first generation hybrid. Since in the following year, its progeny retained the desirable characteristics and were uniform....." (Gregor Mendel)





Mendel's ratio : 2.96 not 3

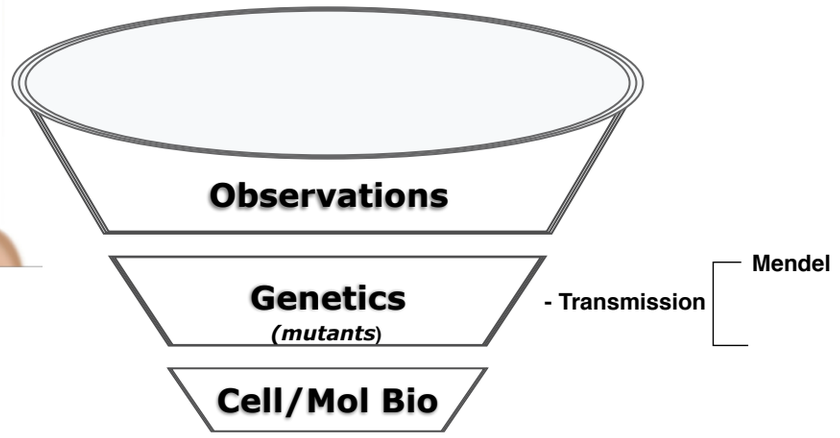
how was it occurring : need for molecular mechanism

Why is what Mendel did important? He gave a logic and framework for of how traits were passed down. Also thanks to this logic, we knew that the molecular substance of inheritance must follow these rules.

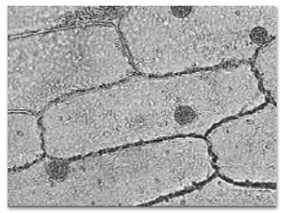
The race was on to find a substance that could alter or produce a phenotype. Why would that be important? We could engineer better food and have the ability to alter substances in cells to prevent disease.

Why was Mendel's ratio slightly off? What factor makes "independent assortment" not so independent?

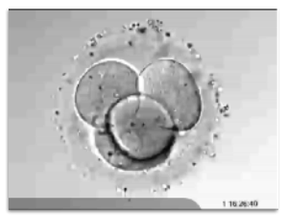
The Central Dogma : from Marvel to Molecules



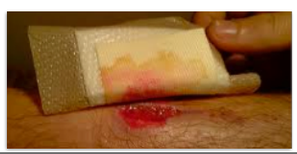
Find and Grind : Cell Biology



The Nucleus:
Robert Brown (1831)



Nuclei transfer of necleus in fertilization
Hermin Fol & Oskar Hertwig (1870)



1869 Fredric Meischer :
Discovers DNA in the Nucleus

Cell biology was in a prehistoric time, but it was very important in finding organelles and other cellular features. The main approach was “grind and find.” An example is a story of a doctor who discovered enzymes in the war through crude biochemistry by dangling meat and vegetables in soldiers stomach.

Robert Brown saw the nucleus, an opaque feature that is transferred into pollen and is consistent with Mendel’s ratios. Fol and Hertwig saw something similar with sperm nucleus being transferred to egg during fertilization. These observations led to the notion that whatever is in the nucleus must be the “transforming principle.”

Meischer was grinding up cells to find the parts inside cells. He found human nucleus in soldiers wounds and discovered that nucleus is mostly comprised of DNA.

Cytology : Chromatin

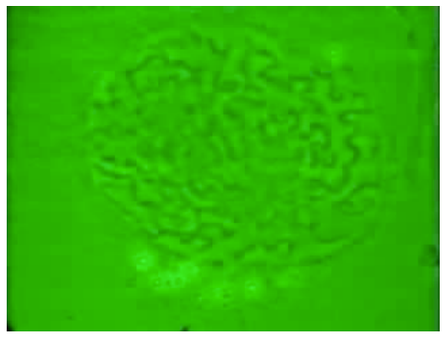
Walther Flemming (1880s) : Nuclear Threads

Importance of Cytology > Assay

Now the nucleus was the major suspect in the case, and the goal is to identify the substance inside the nucleus. There was a burgeoning chemical market of Aniline dyes and Flemming decided to try and see which dye stained which parts of the nucleus. One of his dyes revealed “nuclear threads” later to be coined chromosomes (colored body). He carefully watched these threads and noticed they lined up and split between dividing cells. This was a major clue that these nuclear threads behaved by rules needed for the “transforming principle.”

Cytology: Chromosomen

Heinrich Waldeyer-Hartz & William Ludwig Johansen



1891 : Herman Henning the X-cluded Chromosome

1900 : Sutton / E.B Wilson X splits between sperm

The chromosome became the clear substance of inheritance. The “Excluded” chromosome was observed during mitosis, where one chromosome didn’t have a partner and was termed the X chromosome. This only happened in male cells. Then, Sutton and Wilson found that the X chromosome was differentially loaded into sperm and thus could be the “sex” chromosome. Together with the splitting between normal cells, the writing was on the wall that the chromosome must be the transforming principle. It was also known that chromosomes are made of DNA, RNA and Protein. So the new race was to determine which one is responsible for inheritance of traits!

Medical story of Sutton: Wilson was very impressed with Sutton's abilities as an investigator. Unfortunately, Sutton never finished his doctorate. Sutton left research and entered medical school. He graduated from the College of Physicians and Surgeons at New York and became a surgeon. Sutton served in France during World War I and distinguished himself in treatments of wounded soldiers. Sutton died following an operation for appendicitis. He was only 39.

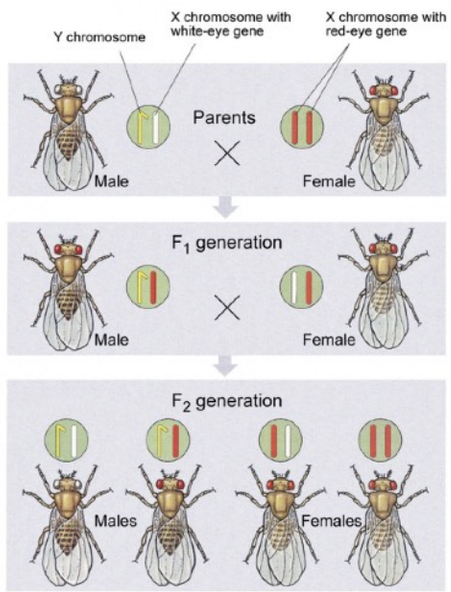
The proof : Back to Genetics

Thomas Hunt Morgan (1910)

Cell Bio & Genetics Prove Chromosome Guilty

We take a detour back to genetics that proves the chromosome contains information to transfer traits.

The proof : Back to Genetics



- The gene for eye color mutation is on the X chromosome
- In F2, while the ratio of 3:1 was obtained, all of the white-eyed second generation offspring were male flies. All females had red eyes.
- The first solid evidence associating a specific gene with a specific chromosome

Thomas Hunt Morgan's experiment showed that a sex specific trait was transferred on the X chromosome. Combined with the previous cell biology / cytology, his genetics proved the chromosome is the transforming principle. This is a good example of how neither approach could prove anything in isolation but together provide understanding beyond a shadow of a doubt.

Supplementary reading: http://www.nature.com/scitable/topicpage/each-organism-s-traits-are-inherited-from-6524917

Learning Catalytics Question

What if Males and Females had identical chromosomes:

(1) Would Hunt Morgan be able to make the same conclusion?

-True or False?

(2) How would this affect Sutton/Flemming conclusions?

(1) False : the traits would have been equally distributed between males and females and thus their serendipitous finding of males having the white eye phenotype in F2 but not females.

(2)Sutton and Flemming would not have seen a distinct chromosome or X chromosome in male cells that doesn't pair during mitosis or meiosis and thus wouldn't have known that chromosomes can segregate differently leading to sex determination.

Summary (I)

Trait Inheritance of a phenotype is 'quantifiable' logic

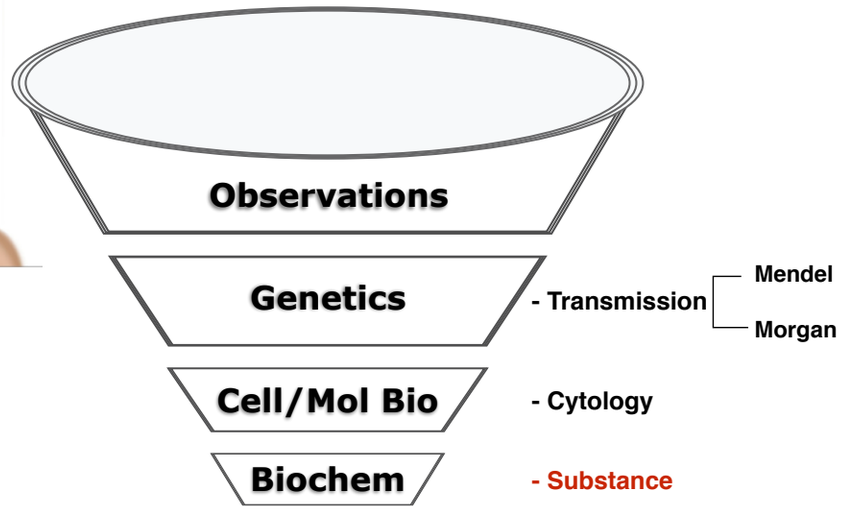
> What is the physical substance transferring this information

Clues:

(i) The Nucleus
(ii) Chromatin/Chromosomes

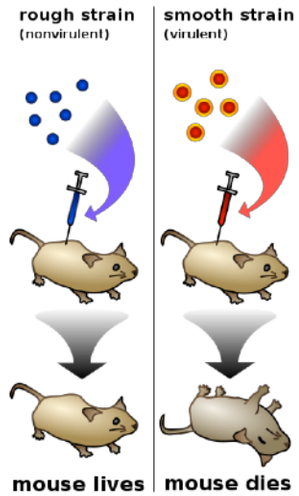
Now the Question is :
RNA/DNA/PROTEIN?

The Central Dogma : from Marvel to Molecules



Defining the Transforming Principle

Frederick Griffith 1928

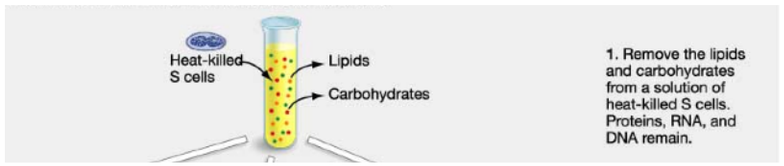


Something Transfers Traits Between Strains: Purify it!

The Griffith experiment was well known and important to use as an assay to see if DNA, RNA or Protein is transferred in this process. In other words, this finding was a great framework to take away each component and see which would make the mouse live again and thus must be the transforming principle. Oswald Avery had worked on this system and decided to use it to purify the transforming principle.

Biochemical Purification of the Transforming Principle

Oswald Avery



Collin MacLeod

- Conclusion: Transformation cannot occur unless DNA is present. Therefore, DNA must be the hereditary material.

In 1943, Avery and MacLeod removed DNA (DNase) from one sample of virulent strain and same for RNA (RNase) and Protein (Proteinase). Thus, in each case, two of the "transforming candidates" are there one is removed. DNase made the mice live, thus removed the ability of the virulent strain to "transform" the non-virulent! They later proceeded to isolate just the DNA and got the same result by just adding DNA to nonvirulent culture, that was considered conclusive proof by some and not by others (contamination of protein).

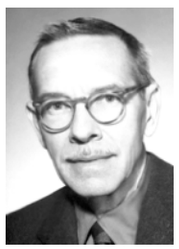
= DNA is transforming principle. Why wasn't this enough? What was going on around that time that may have made it get lost? World War II

Image source: http://biology.kenyon.edu/courses/biol114/KH_lecture_images/How_DNA_works/FG11_02.JPG

The Bacteriophage Revolution

- Bacteriophages: Viruses that infect bacteria.
- Bacteriophage was composed of only DNA and protein.
- Which one is the genetic material that is injected into the bacteria, DNA or protein?

DNA Transforming Principle 1952



Alfred Hershey* 1969

Martha Chase

Phage were discovered to be able to infect bacteria and kill them. It became the ‘popular’ biology reagent, sort of like siren or CRISPR today (actually CRISPR was discovered as a bacterial immune system to phage).
This technology lead to the “phage group” at cold spring harbor to understand how they work on a molecular level. Combined with radioactivity (new in the 50s), this developed to be the perfect reagent to determine the molecular basis of the “transforming principle”

Phage Fun Facts :

In 1915 Phagein, "to devour” (4-100 genes) were discovered // there are approximately 10 million per cubic centimeter of any environmental niche where bacteria or archaea reside

The dsDNA tailed phages, or Caudovirales, account for 95% of all the phages reported in the scientific literature

>> recombinant era , clone genes cut and paste inside torpedo capsule // Phage therapy // Phage display

DNA is the Transforming Principle (Hershey/Chase)

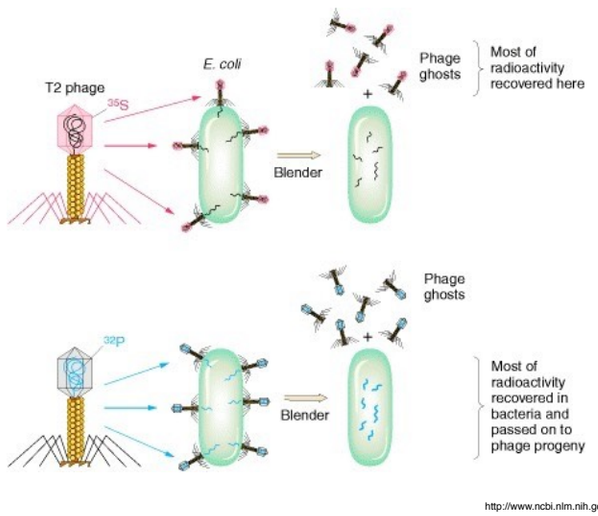
(1) They label either
Protein (S32) or **DNA (P32)**

(2) Incubate Phage in Bacteria

(3) Isolate what was transferred into bacteria by spinning down the infected bacteria.

> the label in pellet was inside bacteria

> Outside (supernatant) cannot be transferred



http://www.ncbi.nlm.nih.gov/books/NBK22104/

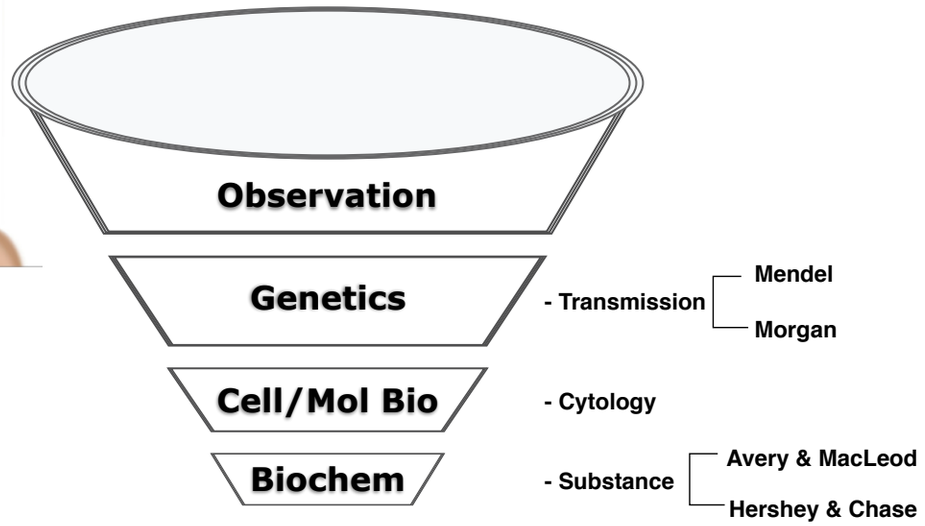
Conclusion: DNA is Transforming Principle

Hershey and Chase develop approach to label protein and DNA with radioactivity (a sensitive issue at the time of the cold war) within phage. Then expose to bacteria to see which is transferred from generation to generation = DNA

DNA is proven to be the “transforming principle.”

Supplementary reading: http://www.ncbi.nlm.nih.gov/books/NBK22104/

The Central Dogma : from Marvel to Molecules



The Structure of DNA

No. 4356 April 25, 1953 NATURE
737

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

Rosalind Franklin, c.a. 1953

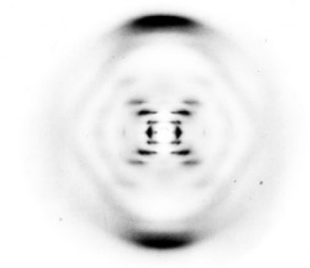


Figure 3-5 Fundamentals of Biochemistry, 2/e

The structure of DNA or the transforming principle is solved to be a double stranded helix !! This has a major implication of how DNA could replicate and transfer information. But did not lead to the understanding of how do we read the code of DNA or the code of life ?!

The Inconvenient Truths :
* X-ray fiber diffraction performed by Rosalind Franklin, working with Maurice Wilkins.
* The X pattern indicates a helix.
* The heavy black arcs at the top and bottom of the diffraction pattern indicates the spacing of the stacked bases (3.4 Å).

(Also evidence that bases stack perpendicular to helix.)

(Watson and Crick saw Franklin's data without her knowledge, and only acknowledged its influence after her death.)

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Discussion Question

Both Watson/ Crick and Hershey/Chase won separate Nobel Prizes

Why did Watson and Crick win Noble Prize for DNA?

Watson and Crick won the Nobel Prize for the implication of heredity. The structure of DNA had the implication that the two strands could be replicated and split between two cells. Thus, the profound conclusion of how DNA could be replicated and passed down. Note that the Nobel Prize for DNA being transforming principle or source of heredity was given to others such as Hershey, Delbruk and Luria for their work on viruses and showing DNA transferred properties. It is often confused that the structure of DNA lead to the central dogma but that Nobel Prize was given to Jacob and Monod.

Summary (II)

Biochemistry and Bacterial Genetics Demonstrate DNA is the Transforming Principle

Clues:

(i) DNA Purification / Transformation

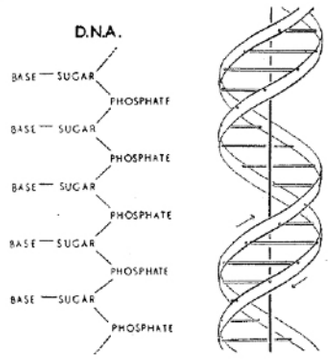
(ii) Implications from the Double Helical Structure of DNA

Structure of DNA

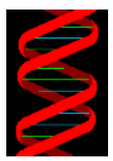
1953! The Structure of DNA Reveals Physical Mechanism of Inheritance

"It has not escaped our notice that the specific pairing that we have postulated immediately suggests a possible copying mechanism for the genetic material"

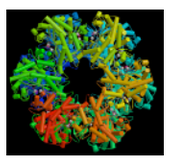
J.D. Watson and F.H Crick Nature 171, 964-967 (1953)



DNA



Protein



There is a missing link?
How is the Code Read?
-Insulin Mutant a Clue

1953

The structure of DNA was just the beginning of the central dogma. The key question became what is the gap in our knowledge of how DNA could encode specific amino acids in order.

* Insulin mutant with one amino acid change! This had a major implication that the code could have one base change (the most likely way a mutation could happen statistically) and produce a different amino acid.

Still Didn't Get the Message

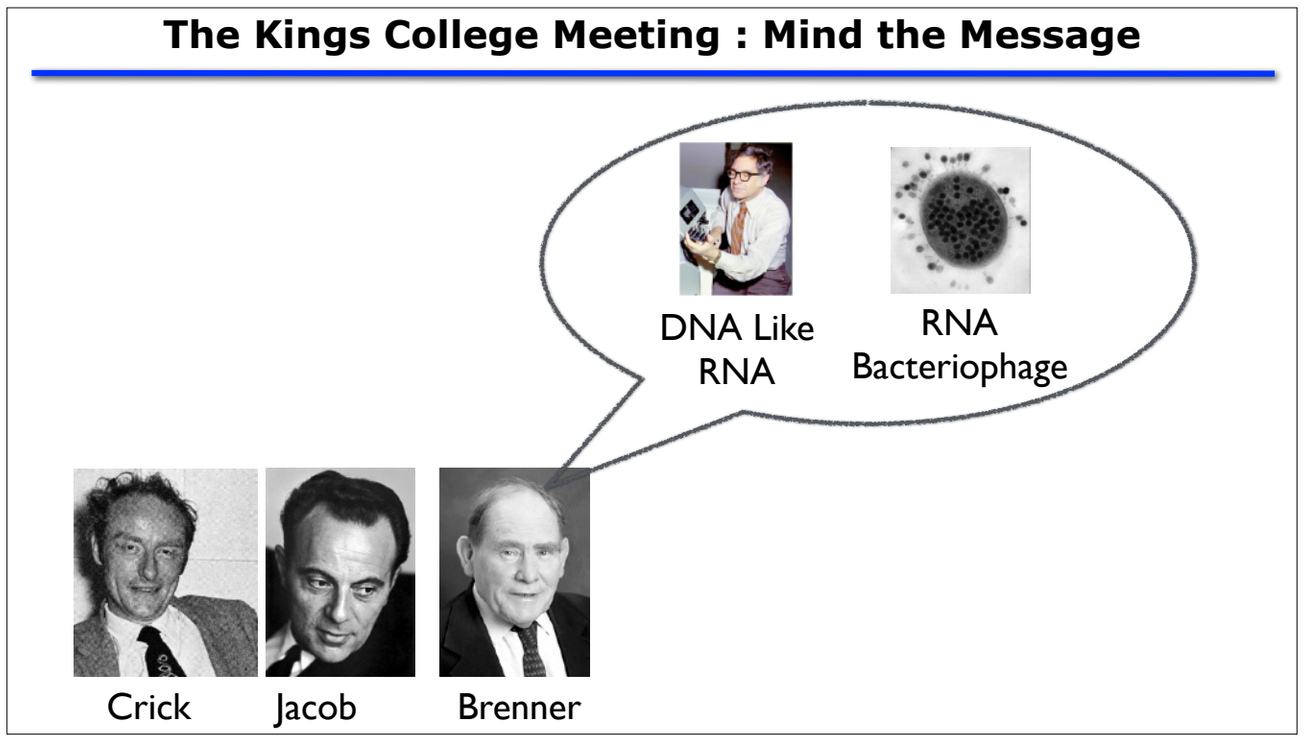
Ken Volkin/ Lazurus Astrachan: DNA Like RNA

Hershey: Small Fraction of RNA Produced Upon Viral Infection

The Ribosome is Not Specificity Factor for Translating DNA

The key experiments that pointed to RNA:

1)Upon infection with phage, DNA like RNA was produced from the phage DNA and couldn't have come from bacteria (only 1% of the total RNA so people thought it was an artifact), but Volkin/Astrachan proposed that the RNA was made from phage to instruct phage protein translation.
2)Hershey's phage experiments noticed some fraction of phage RNA being produced.
3)The PaJaMo experiment disproved a prevailing theory that maybe each ribosome was uniquely specified for each specific protein it produces, thus the RNA inside the ribosome is the code. They did an experiment, where old ribosomes were heavy labeled and then upon phage infection they determined if the phage proteins were translated from heavy or light ribosomes. If a new ribosome had to be made upon phage induction, translation would not occur from heavy labeled (or old) ribosomes. They found that phage proteins were translated from both and thus ribosomes are not unique specifiers of a given protein product.



The meeting of the minds at Kings College: the hypothesis of a messenger RNA!

The masterful enzyme kinetic studies of Jacob and Monod demonstrated a quick intermediate that can shut on and off protein synthesis. They knew about the “DNA like RNA” upon phage injection into bacteria. The RNA content has 1% that looks like Phage GC content and not bacterial host content. This observation led to the hypothesis that DNA like RNA may make phage proteins.

In 1961, RNA only bacteriophages were discovered by Loeb and Zinder in 1961. This had a major implication for RNA being the messenger. How else could the phage make its proteins if it only had RNA to work with? It couldn’t :). Thus, this was probably enough evidence that there had to be an RNA intermediate. Ok, it is possible that the RNA genome is reverse transcribed into DNA (but back then reverse transcriptase wasn’t known either)!

The Experiment! Meselson, Jacob and Brenner

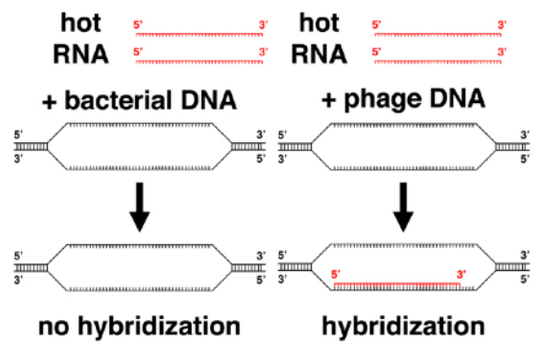
1) Let bacteria get exposed to phage

2) Add p32 to radio-label nucleic acids

3) After short incubation, isolate RNA

4) Hybridize radio-labeled RNA to bacterial DNA & phage DNA

*) Volkin/Astrachan similar experiment but GC content analysis



GC Content 1 GC Content 2

The first hybridization blot proves RNA as the messenger!

Volkin/Astrachan used GC content to determine the amount of phage RNA produced (since it was different than bacterial host GC content).

Meselson, Jacob and Brenner performed a definitive hybridization experiment. They radio-labeled RNA in a phage and hybridized the RNA to bacterial and phage genome DNA. The labeled RNA only stuck to the phage genome DNA. This observation indicates that the RNA from the phage was produced from the phage DNA and therefore was the messenger for the protein production! But really same proof as Volkin/Astrachan.

1961 The Message was Clear

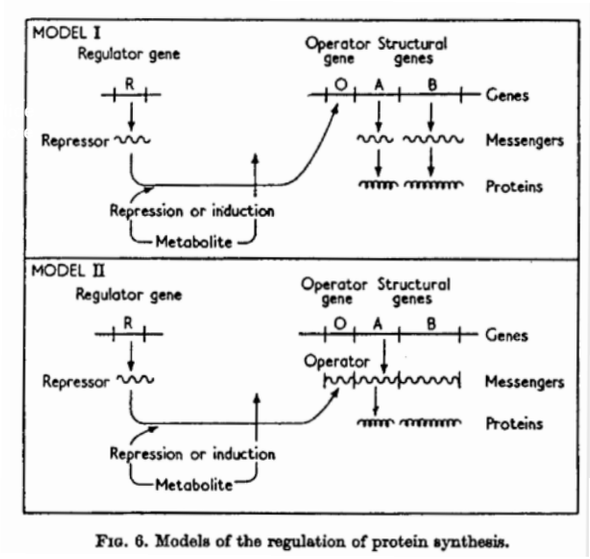


FIG. 6. Models of the regulation of protein synthesis.

-Jacob and Monod figured out how genes (A & B) are turned on and off

- They found that "O" the operator was what turned genes off

- Based on how fast genes could be turned on and off, they concluded a "message" is made under control of operator. That can be quickly shut off by a repressor.

- Note they had two models! What if the operator was found to be an RNA?

Gene expression is rapidly induced and repressed under different sugar conditions for bacteria. Jacob and Monod were able to show that after degrading DNA, they were still able to induce the expression of some genes upon switching sugars, a strong hint that there was an intermediate that wasn't DNA.

The key element was the operator that wasn't a gene product, but regulated the gene products. When sugar is present, the operator shuts genes off. In the absence of sugar, the operator turns genes on. Thus, "O" seemed to be bound by a repressor in the presence of sugar.

We now know today that O is a "promoter" and that a protein factor binds and represses A & B genes. But, at the time, Jacob and Monod also thought O could be part of the mRNA and that an RNA repressor could target "O" the operator. In other words, an RNA repressor could be a REGULATORY RNA that would bind and repress the messenger RNA for A & B (Model II).

Today, we know microRNAs regulate gene expression as shown in Model II. Thus, Jacob and Monod not only discovered the mRNA, but also suggested the mechanism by which microRNAs work 38 years earlier!

Learning Catalytics Question

Francois Jacob Jaques Monod

They won the Nobel Prize, but for what?

(a) How to read DNA
(b) DNA is transforming principle
(c) Enzyme Kinetics
(d) Messanger RNA

(C) They were the masters of understanding how genes turned on and off. It is often thought that they won the Nobel Prize for the mRNA, but their work on the lactose operon detailed the first gene locus and the elements that regulated it. It implied the messenger RNA, but they did the final experiment a year after they won the Nobel Prize!

How to Read DNA?

The RNA Tie Club

RNA Tie Club

	member	training	RNA Tie Club Designation	Officer designation
*	George Gamow	Physicist	ALA	Synthesizer
	Alexander Rich	Biochemist	ARG	Lord Privy Seal of the British Cabinet
	Paul Doty	Physical Chemist	ASP	
	Robert Ledley	Mathematical Biophysicist	ASN	
	Martynas Ycas	Biochemist	CYS	Archivist
*	Robley Williams	Electron Microscopist	GLU	
	Alexander Dounce	Biochemist	GLN	
*	Richard Feynman	Theoretical Physicist	GLY	
	Melvin Calvin	Chemist	HIS	
	Norman Simons	Biochemist	ISO	
	Edward Teller	Physicist	LEU	
*	Erwin Chargaff	Biochemist	LYS	
	Nicholas Metropolis	Physicist, Mathematician	MET	
	Gunther Stent	Physical Chemist	PHE	
*	James Watson	Biologist	PRO	Optimist
	Harold Gordon	Biologist	SER	
*	Leslie Orgel	Theoretical Chemist	THR	
*	Max Delbrück	Theoretical Physicist	TRY	
*	Francis Crick	Biologist	TYR	Pessimist
*	Sydney Brenner	Biologist	VAL	

The RNA tie club was formed to figure out the code of DNA and how it is read out to make specific amino acids. Each member was named after an amino acid.

The DNA or RNA?

George Gamow

DNA as the Template of Protein Synthesis.
Intrigued by 3.5A A.A. similar to b.p of DNA.
Thus, single overlapping code.
There are 20 amino acids => 4^n > 20 => 3 Base Triplet

Francis Crick

DNA not a good template.
Must be an adapter (or 20) that utilizes H-bonding.
Thus: The Adapter Hypothesis

"Is there anyone so utterly lost as he
that seeks a way where there is no way."

Kai Kā'ūs ibn Iskandar.

ON DEGENERATE TEMPLATES AND THE ADAPTOR HYPOTHESIS

A Note for the RNA Tie Club.

1955

Gamow had an elegant model called the diamond hypothesis that suggested a triplet codon with needed redundancy. The model was so perfect that everyone bough into it, except Francis Crick.

The diamond hypothesis took advantage of the fact that the major grove is the exact same size as an amino acid. So the amino acid could sit in the grooves and be connected in a triplet specific manner. However, Crick famously points out in his paper that there is no way this is possible. Crick was a structural biologist and knew that the structure of the major grove could not provide sequence-specific information to the amino acid. In other words, any amino acid would fall into any grove and some would never fit based on chemistry. This single letter eliminated this model from the history books :)!

Crick instead proposed that there must be an adapter that binds the DNA, reads it, and transfers it to protein. The tRNA had been discovered and was a likely candidate.

Cracking the Code

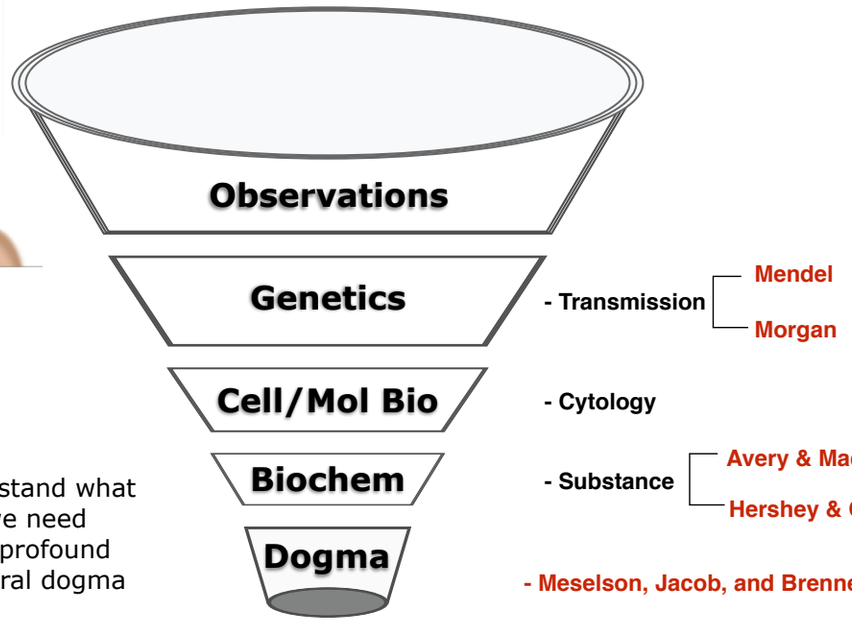
How Do We Read DNA?

1961: Brenner & Crick Perform Mutation Analysis > 3 bases > Degenerate
-Two suppressors don't rescue but 3 do
Genetics

Nirenberg/Leder/Khoran : Labeled Charged tRNA and Grid of Triplets 1964
Biochemistry

The code is solved to be in triplets. Brenner and Crick showed beautiful genetics that mutants could not be rescued with one mutation, but required three and sometimes 2 (degenerate codon). Using biochemistry, Nirenberg and colleagues showed which triplet sequences encoded which amino acid. Nirenberg's approach was to put triplet letters on a filter, label one amino acid and see which set of letters are labeled after washing off (translation of labeled amino acids will show up on one spot with a specified set of letters). Technically this was the first microarray with gridded test letters and radioactivity detected on the filter.

The Central Dogma : from Marvel to Molecules



Learning goals:
It is important to understand what each tells us and how we need them all to derive such profound insight such as the central dogma

END