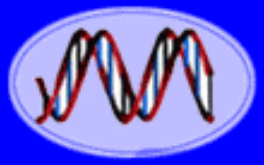
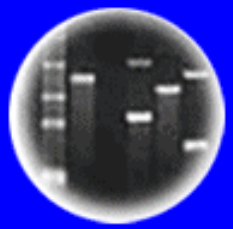


DNA
Genetic Code of Life

Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

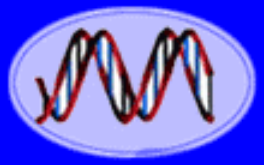
HC70A & PLSS059
Winter 2020
Genetic Engineering in Medicine, Agriculture, and Law

Professors Bob Goldberg & Channapatna Prakash

Lecture 3
What Are Genes & How Do They Work: Part One

UCLA

TUSKEGEE UNIVERSITY



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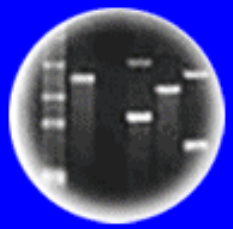

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THEMES
Gene Structure & Function
Part One

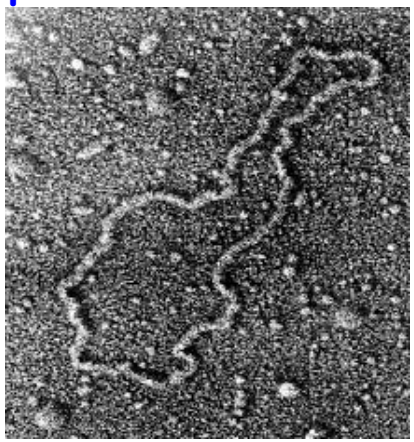
- What is the Function of a Gene?
- What are the Properties of Genes?
- How Was DNA Discovered?
- What is the Evidence That DNA is the Genetic Material (Griffith and Avery Experiments)?
- Is Transformation Universal?
- What is the Structure of DNA?
- What is the Structure of a Chromosome?
- What is the Colinearity Between Genes & Proteins (how does DNA→protein)?
- How Do We Know That Genes Function Independently of One Another?
- What is the Anatomy of a Gene?
- How Do Switches Work to Control Gene Activity?
- What Are the Possibilities For Manipulating Genes in the Future?

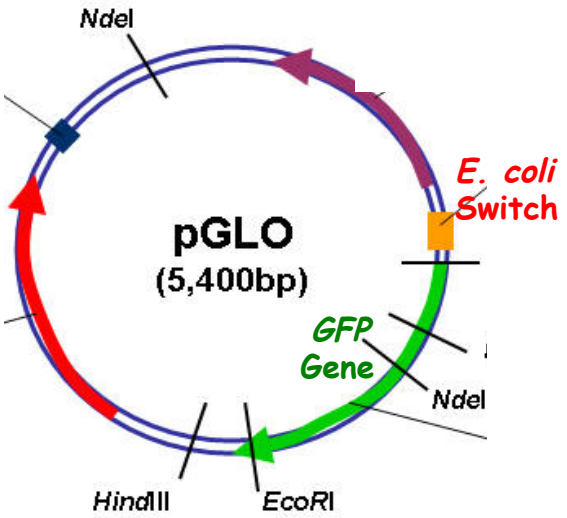
Understanding Genetic Engineering

Requires a Basic Understanding of Genes And How They Work

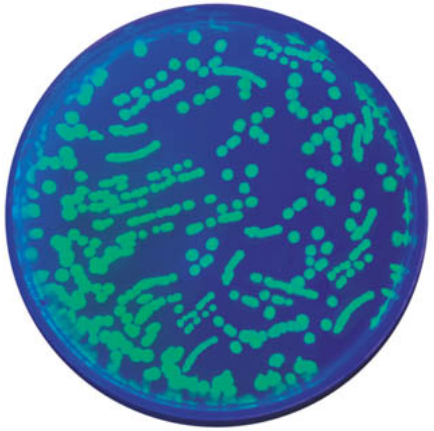
What Are the DNA Implications of Generating an E. coli Cell Producing GFP Protein?

pGLO Plasmid DNA

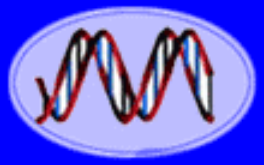




E. Coli Producing GFP Protein



1. DNA Replicates
2. DNA Directs the Cell to Produce a Specific Protein & Express a New Trait
3. DNA is Stable From Cell Generation to Generation – i.e. Cells Derived From the Original Transformed E. Coli Express the *GFP* Gene
4. The *E. coli GFP* Gene Transformation Experiment Shows Directly That DNA is the Genetic Material!



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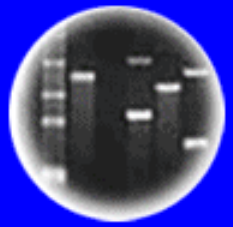


DNA Fingerprinting

Cloning: Ethical Issues and Future Consequences

Plants of Tomorrow

What Were Considered the Properties of a Gene BEFORE It was Known That DNA Was the Genetic Material?

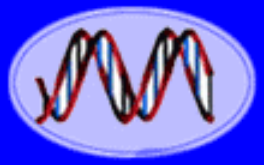
1. Replication – Transmitted With Chromosomes
2. Stability (Mutations)
3. Universality
   a) All Cells
   b) All Organisms
4. Direct Cell Function/Phenotype

For First Half of 20th Century Proteins Were Considered the Genetic Material

- How Can These Properties Be Tested Experimentally?
- What Predictions Follow From These Properties?

If DNA is the Genetic Material, THEN What……?

How Was DNA Shown to be the Genetic Material?



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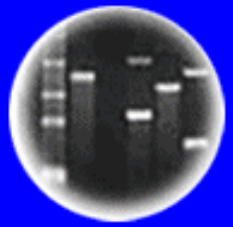

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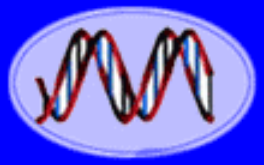

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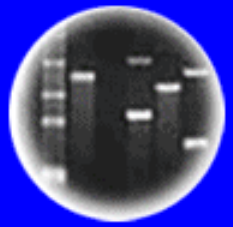

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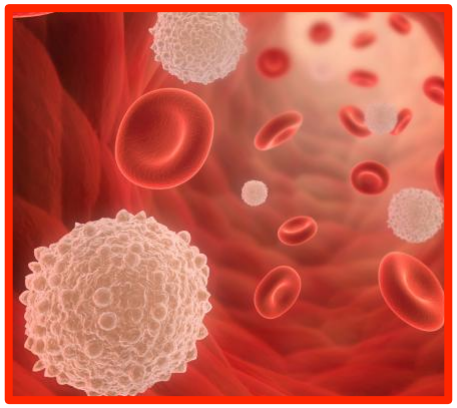
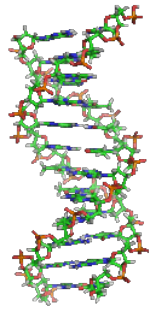
Frederick Miescher Discovered DNA in the Nuclei of White Blood Cells in 1869

150 Years Ago





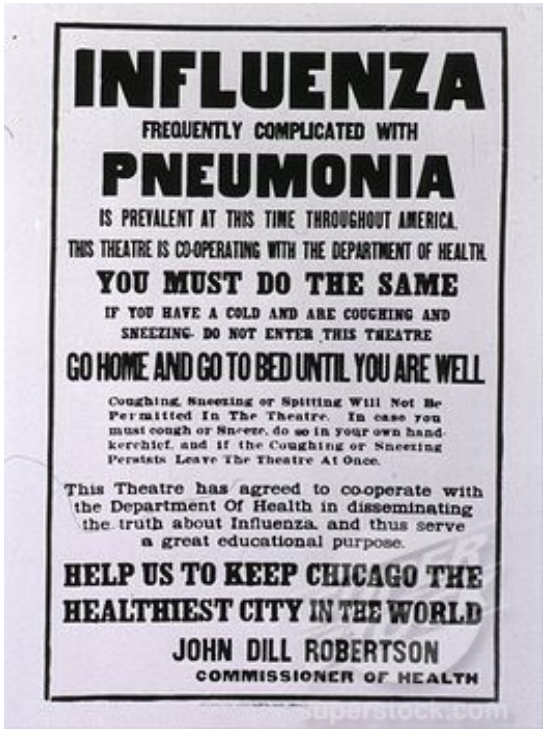
But…..The Function of DNA Was Not Understood Until 75 years Later in 1944!!!

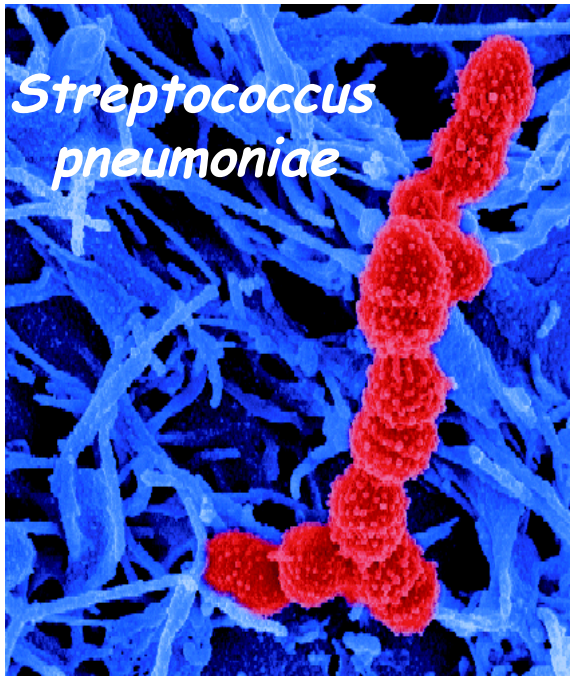
Evidence That DNA Is the Genetic Material Starts With Pneumonia

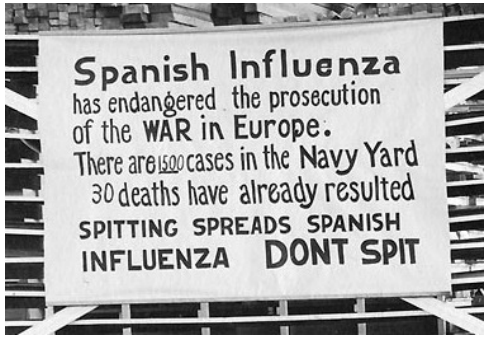
PNEUMONIA KILLS 990 IN CITY SINCE JAN. 1; Forty-Eight Die in Twenty-Four Hours, Four Fewer Than on Previous Day. 387 INFLUENZA CASES Six More Deaths Reported, but Copeland Sees Chief Danger in First-Named Disease.

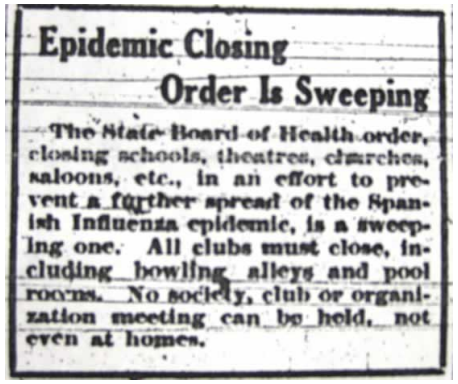
January 29, 1922 - New York City

Spanish Flu (viral) Was also "Killer" at This Time!







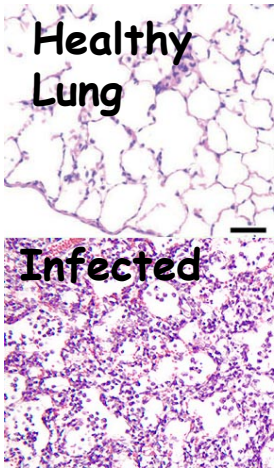


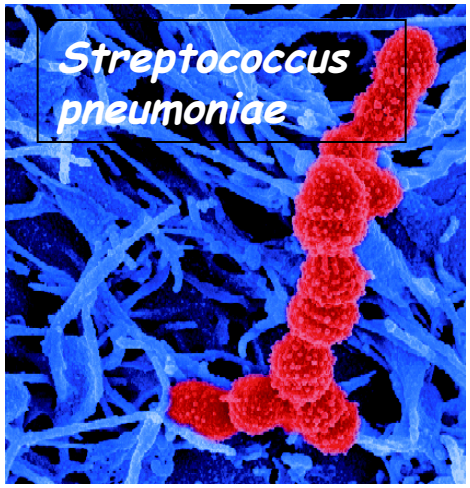
Spanish Flu Killed 50-100 million people world-wide from 1918 to 1920 – *Most From Secondary Bacterial Infections*

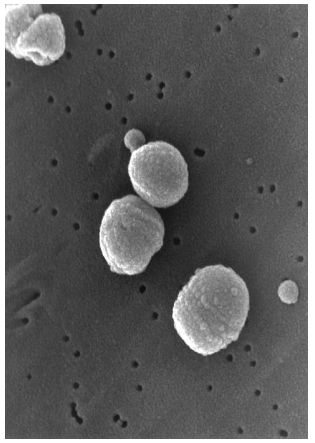
Frederick Griffith & The Transforming Principle

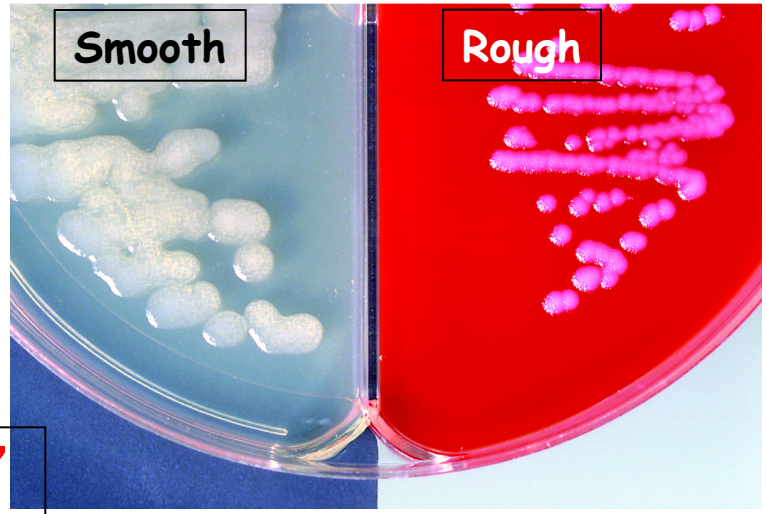
The First Genetic Engineering Experiment (unintentional!)

1879-1941





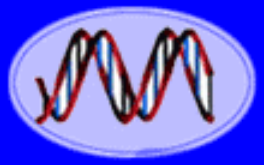




Note: Diffferent Strains of *Streptococcus Pneumoniae* Exist in Nature

Type I, II, etc.

Invented the Word "Transformation"
Not Understood For Another 50 Years



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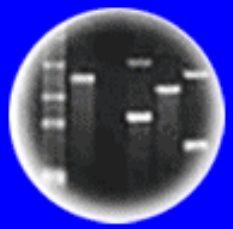

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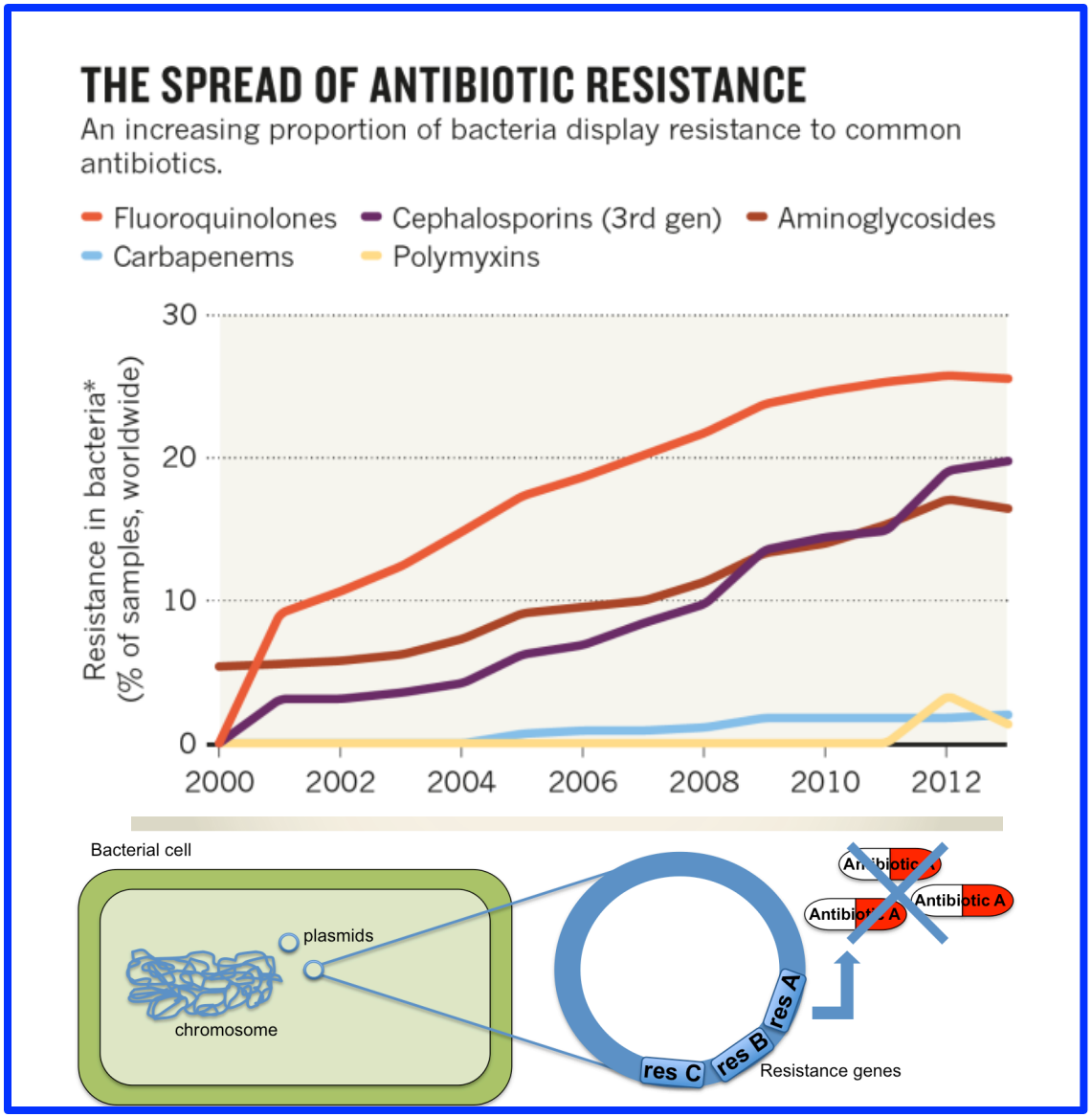
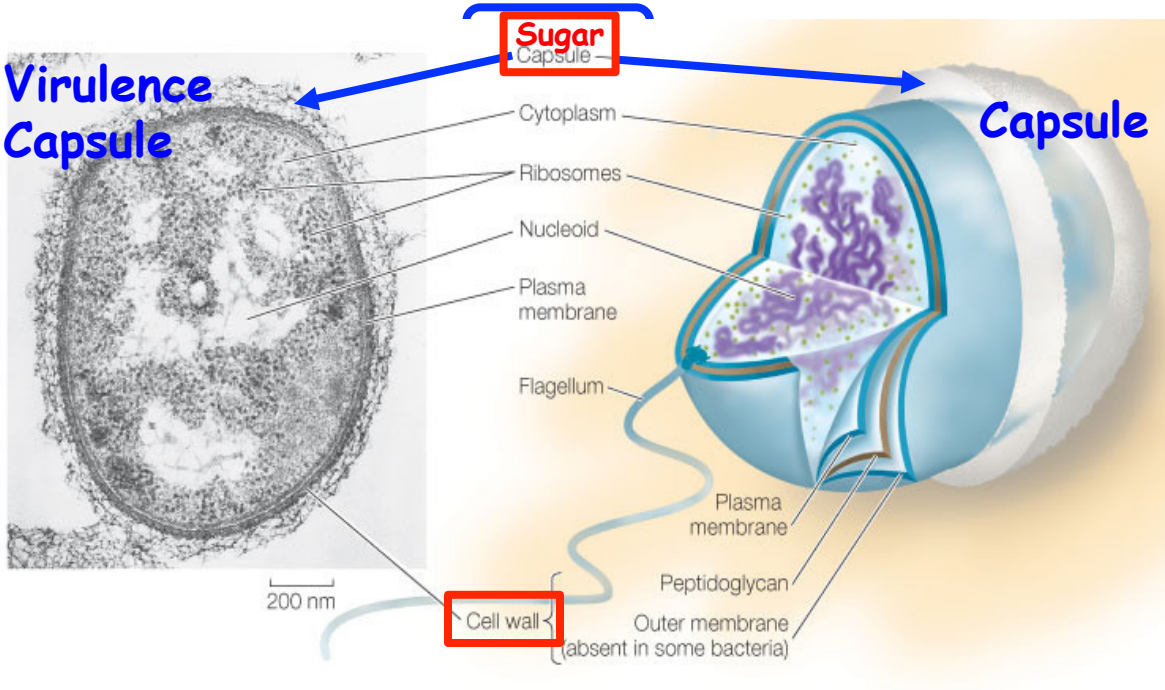
Pneumonia Can Be Treated With Antibiotics – but……



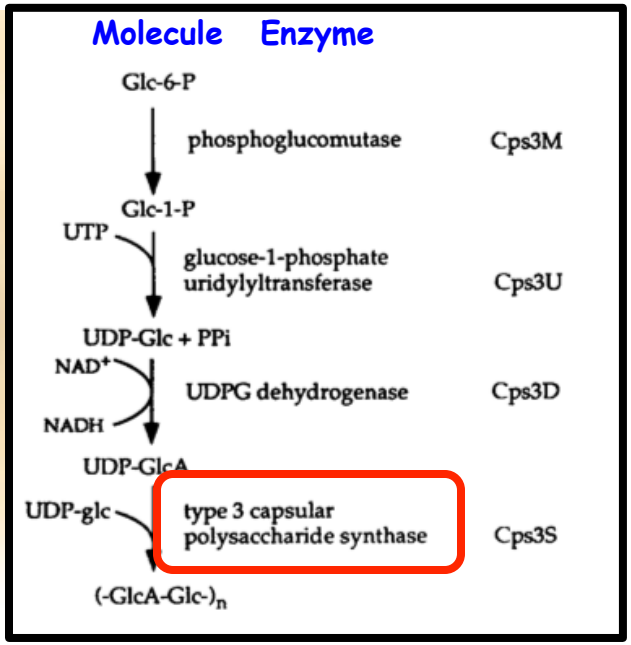
Streptococcus pneumoniae

Flash Forward to 2020!

50,000 Deaths/Year in the USA



Capsule Biosynthesis



J. Exp. Med. 181, 973, 1995

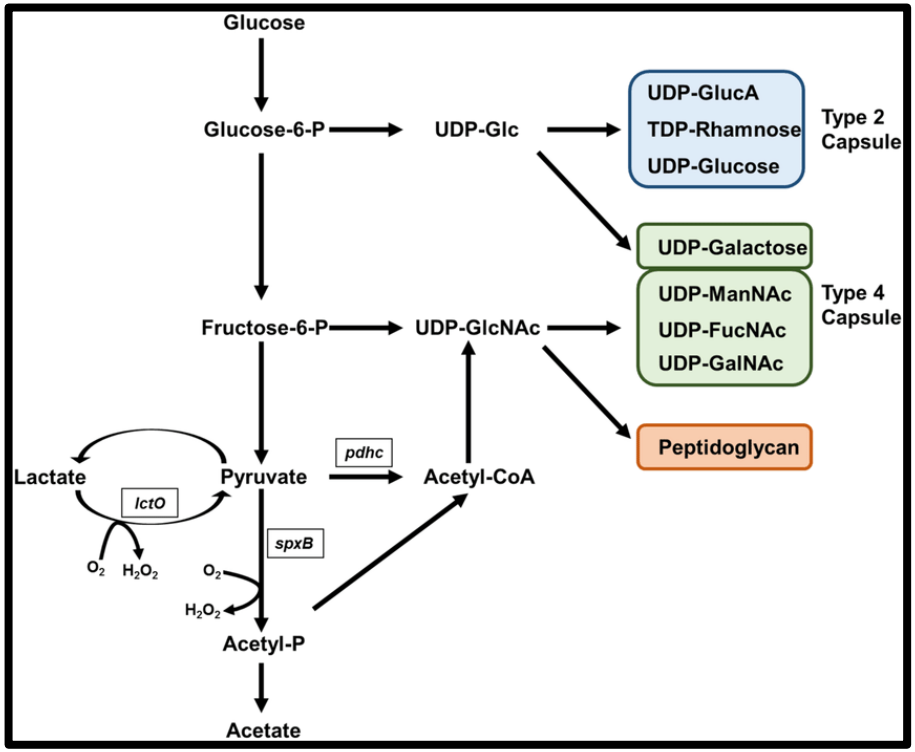
Streptococcus **Strains Depend On the Sugar Type in the Capsule – Which is a Product Of MANY Genes!**

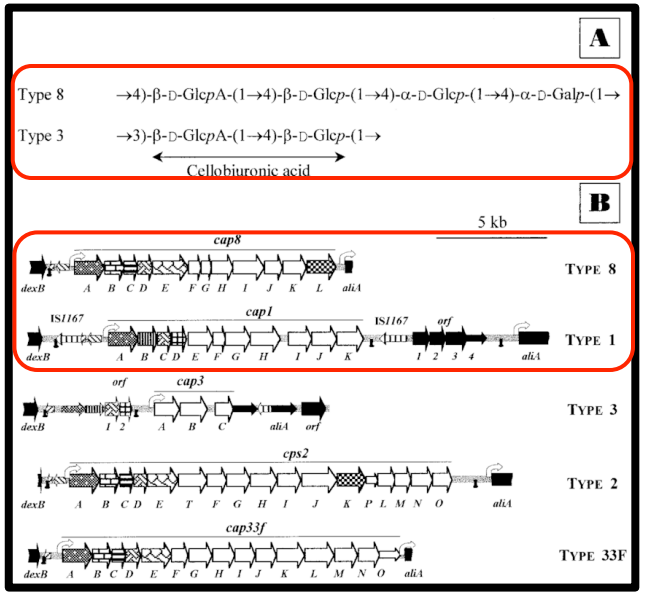
The Sugar Capsule Protects the Bacteria From Mammalian Host Antibodies

Capsule = Virulence **No Capsule = Avirulence**

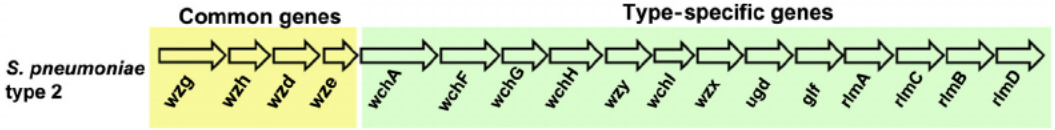
Streptococcus pneumoniae Virulence

Capsule Sugars – Different Strains Have Different Sugars





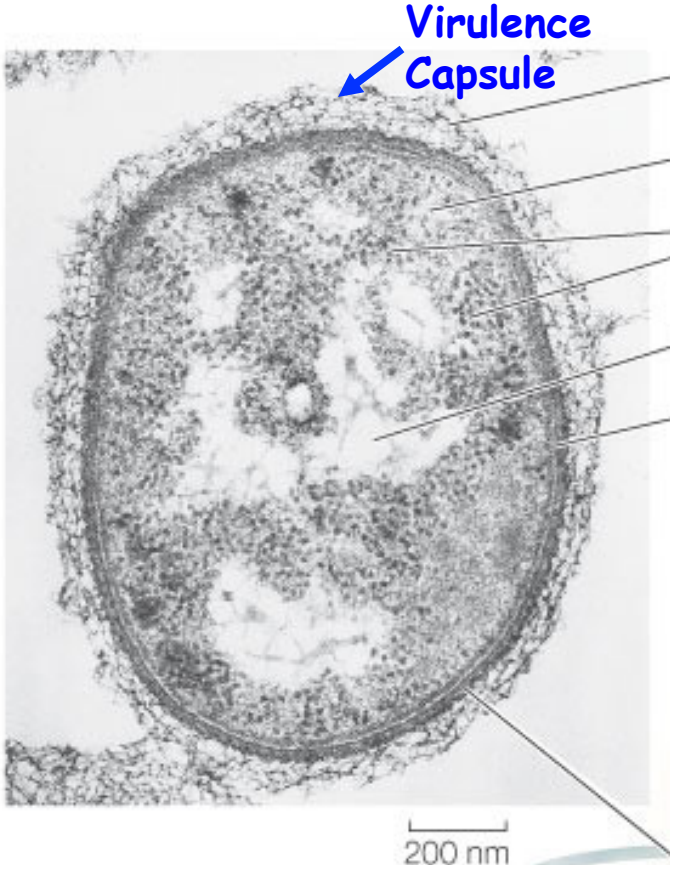
Different Sugars – Different Sugar Genes!

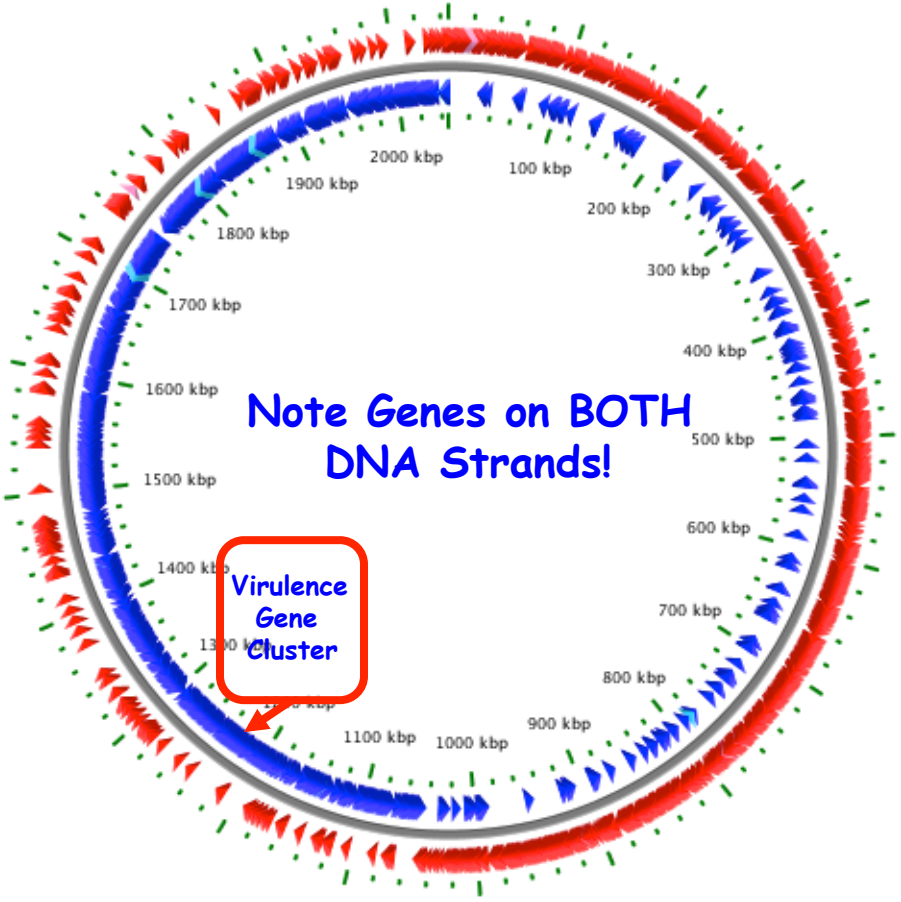


Different Strains Have Different Sugars Encoded By Shared & Distinct Genes Involved in Capsule Sugar Synthesis

Cannot Mutate From One Strain to Another – Too Many Genes involved

Streptococcus pneumoniae Genome Has Been Sequenced!





2,160,837 bp and 2,236 Genes

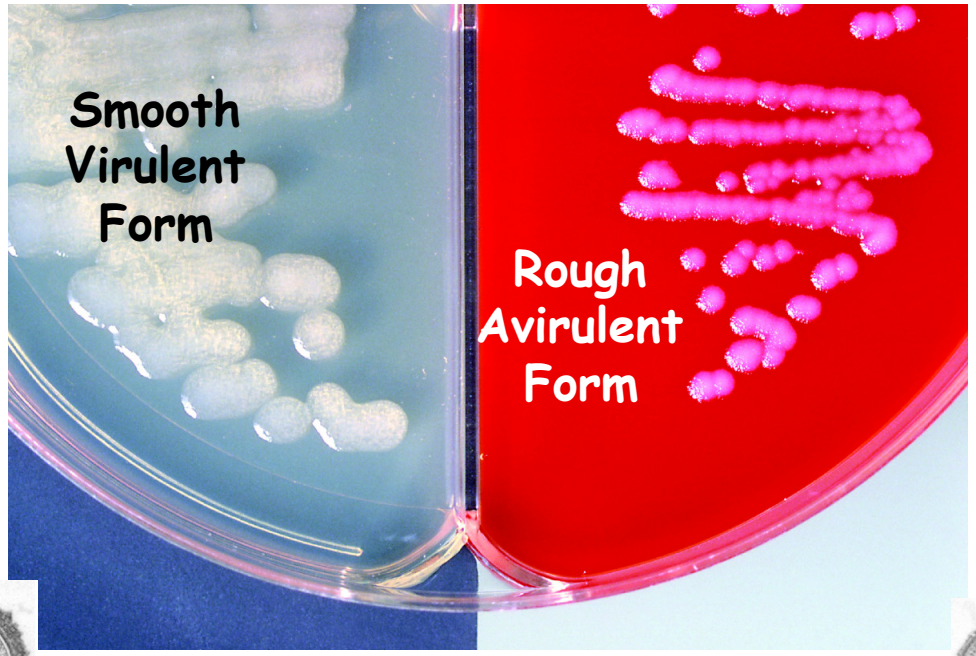
At Least 13 Genes Specify Capsule Formation

What Happens If One of These Genes Is Mutated?

Science 293,498 (2001)

J. Hygiene, 1928

The Griffith Experiment With Smooth and Rough Pneumonia Bacteria

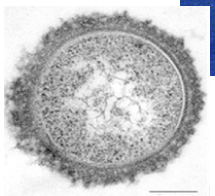


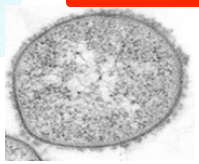
Spontaneous Mutations Always Retain the Strain Type

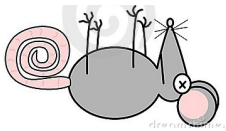
SI to RI

Never SI to RII

As Too Many Genes Are Involved!







Sugar Capsule

Spontaneous Mutations

Smooth

Rough

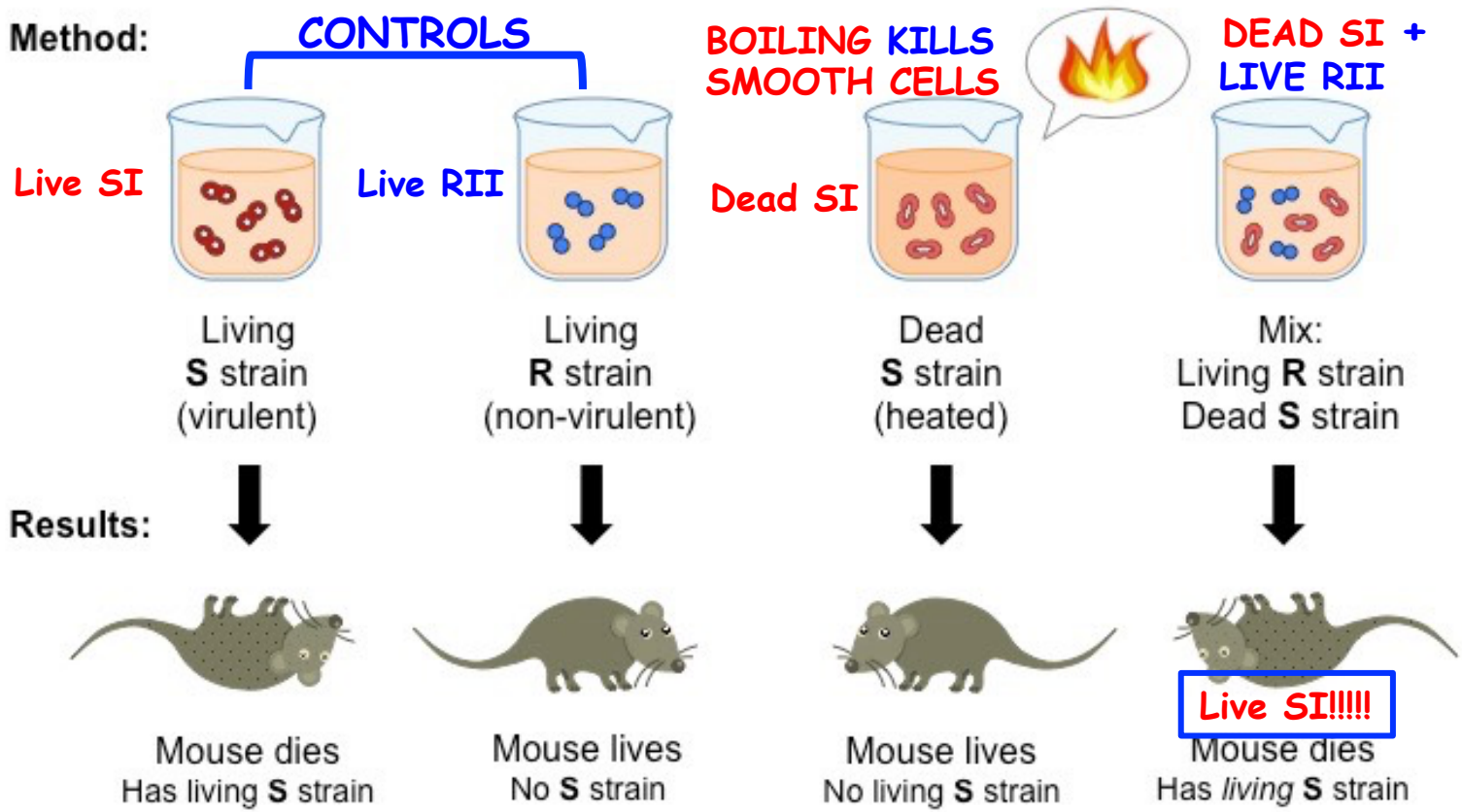
No Sugar Capsule

Virulent

Avirulent

The Griffith Experiment (1928)

Hypothesis: Material in dead bacterial cells can transform living bacterial cells



Conclusion: A chemical substance from one cell is genetically transforming another cell

LIVE Rough Cells TRANSFORMED by DEAD Smooth Cells!!!
HOW? What Was the Transforming Principle? Hypothesis?

Griffith, 1928, J. of Hygiene, 28 (2), 113-157

VOLUME XXVII JANUARY, 1928 No. 2

THE SIGNIFICANCE OF PNEUMOCOCCAL TYPES.

BY FRED. GRIFFITH, M.B.

(*A Medical Officer of the Ministry of Health.*)

(*From the Ministry's Pathological Laboratory.*)

Inoculation experiments with heated virulent Type I culture and attenuated R strains of Types I and II.

Conversion of R Type II into S Type I. In the experiment in Table VII two out of eight mice injected with heated virulent Type I culture together with an attenuated R culture derived from Type II died of pneumococcal septicaemia and yielded pure S colonies of Type I from the blood; plates from the lesions at the seat of inoculation showed a mixture of R and S colonies.

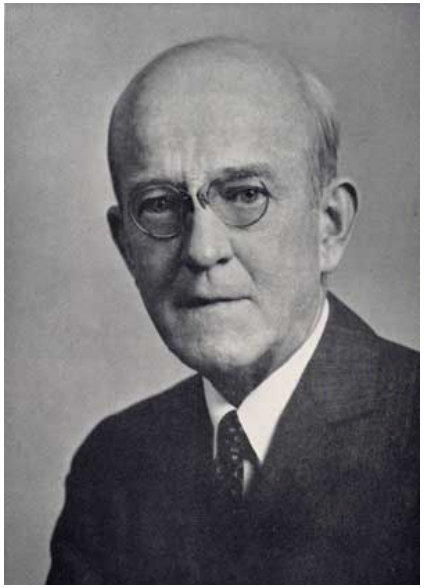
Table VII.

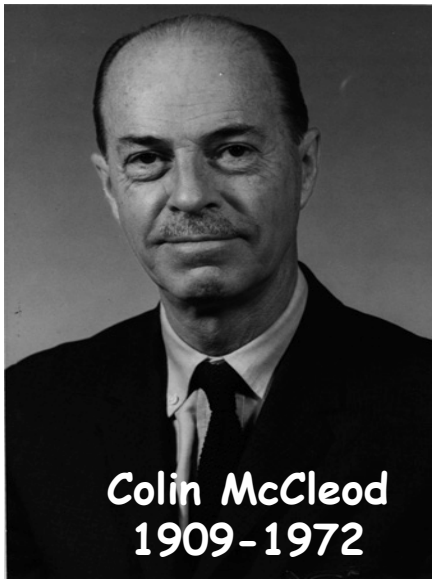
Killed S pneumococci	Living R pneumococci	No. of mouse	Result		Type of culture obtained from mouse
Type I heated 2 hours at 60° C. Dose = deposit of 50 c.c. of broth culture	None	641	Killed	5 days	None
	,,	642	,,	6 ,,	,,
	,,	643	,,	6 ,,	,,
	,,	644	,,	6 ,,	,,
As above +	R 4, Type II. Dose = 0·25 c.c. of blood broth culture	645	Died	3 days	S colonies, Type I
		646	Killed	5 ,,	R cols. from local lesion
		647	,,	6 ,,	,, ,,
		648	,,	6 ,,	,, ,,
As above	R 4, Type II, grown in the heated Type I deposit. Dose = 0·36 c.c.	649	Killed	5 days	R cols. from local lesion
		650	Died	4 ,,	S colonies, Type I
		651	Killed	6 ,,	None
		652	,,	6 ,,	One R colony

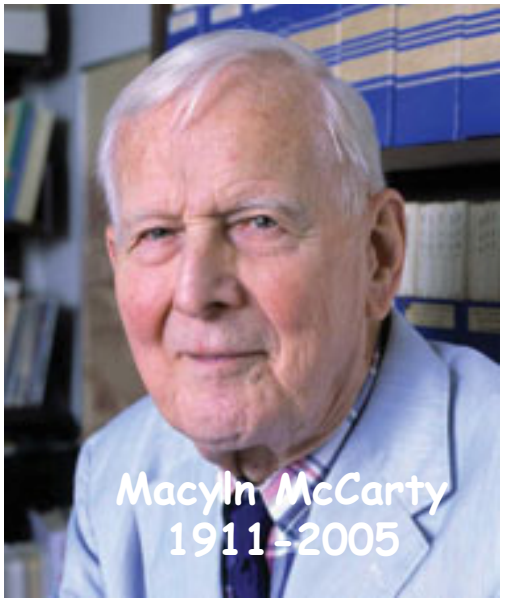
Note: R Strain II Transformed into Smooth Strain I

What Hypothesis Explains This Transformation?

What Was The Transforming Principle? Experiments of Avery, McCleod, & McCarty Fast Forward to the 1940s!





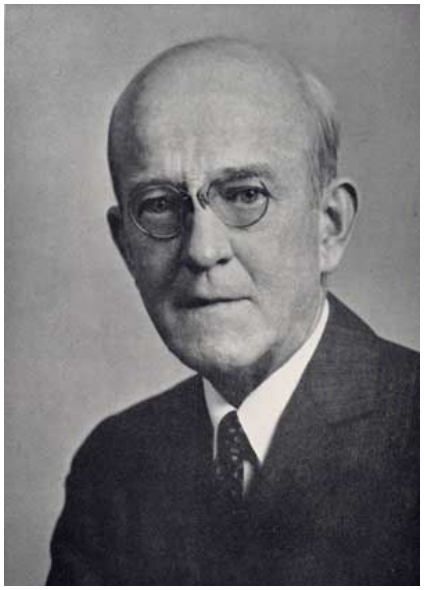


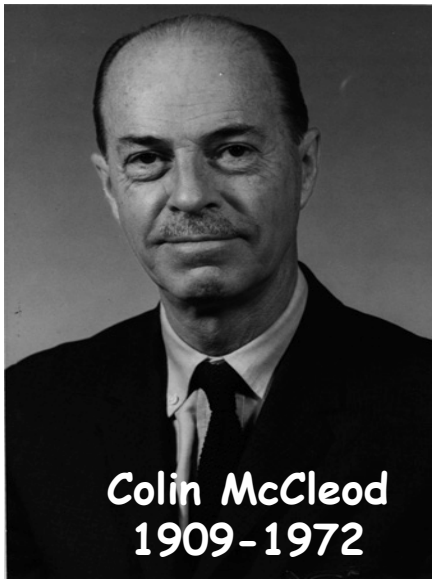
DNA is the Genetic Material!

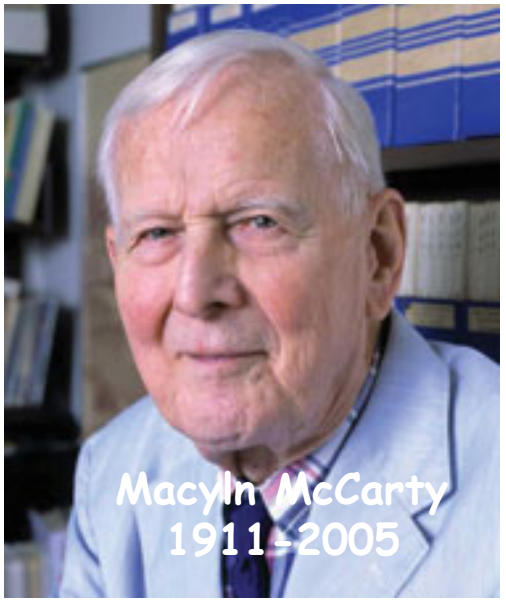
One of the Major Reasons Watson and Crick Considered DNA As the Genetic Material In Order to Solve DNA Structure

J. Exp. Med.,1944

What Was The Transforming Principle?
Experiments of Avery, McCleod, & McCarty
Fast Forward to the 1940s!







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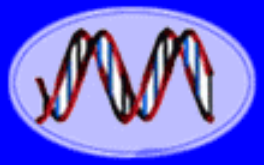
J. Exp. Med.,1944



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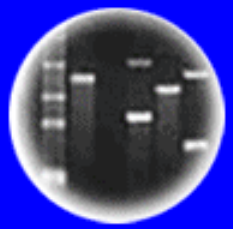


DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

OSWALD T. AVERY, COLIN M. MACLEOD, AND MACLYN MCCARTY

J. Of Experimental Medicine,79 (2), 137-158 (1944)

STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III

BY OSWALD T. AVERY, M.D., COLIN M. MACLEOD, M.D., AND MACLYN MCCARTY,* M.D.

Avery et al. Questions?

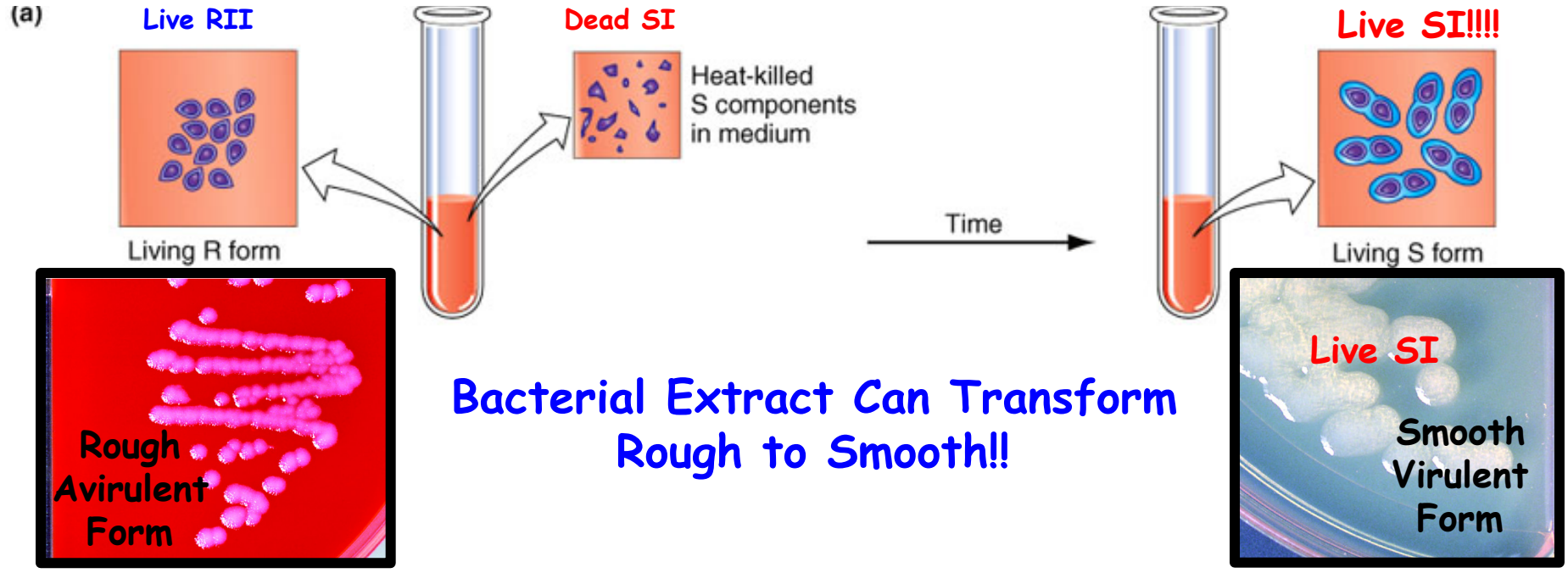
1. Does the *Transforming Principle* Come From the Mouse or Bacteria?
2. If From the Bacteria -- What Substance?
3. How Devise Techniques to Determine What the *Transforming Principle* is
   a) Transformation in Test Tube
   b) Isolation of Macromolecules
   c) Isolation of Enzymes (e.g., DNase, RNase)

Design Experiments To Show!!!

Does the Transforming Principle Come From the *Mouse* or *Bacteria*?

Mix in Test Tube

Look at Morphology on Agar Plate



Bacterial Extract Can Transform Rough to Smooth!!

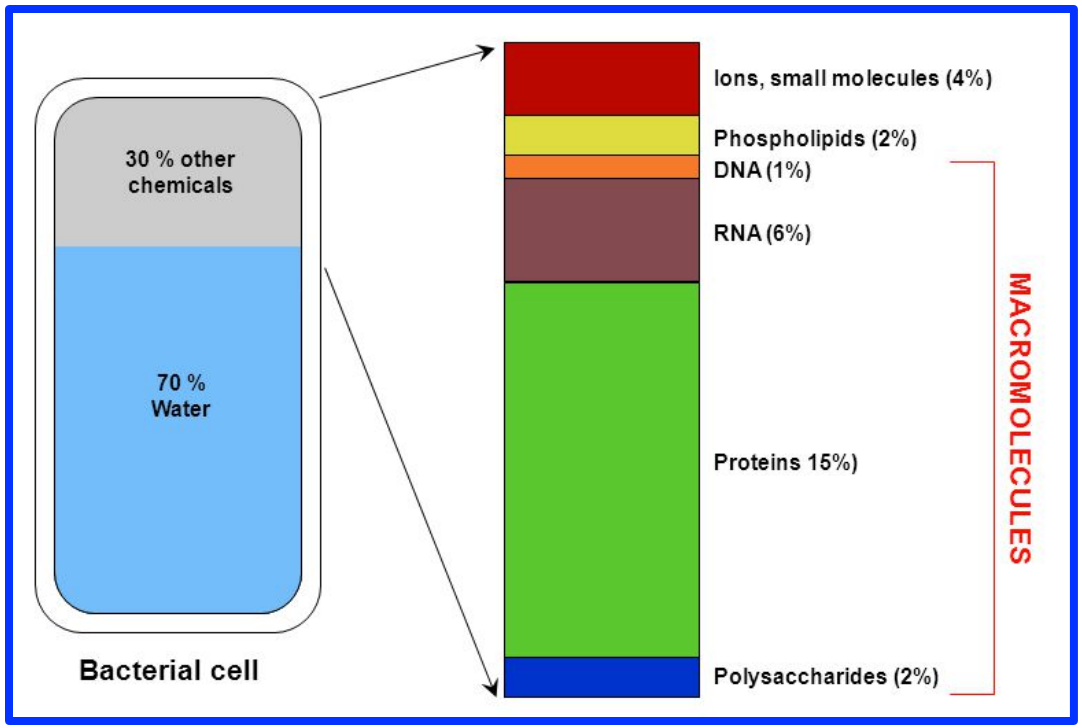
Hypothesis? Predictions? Experiment?

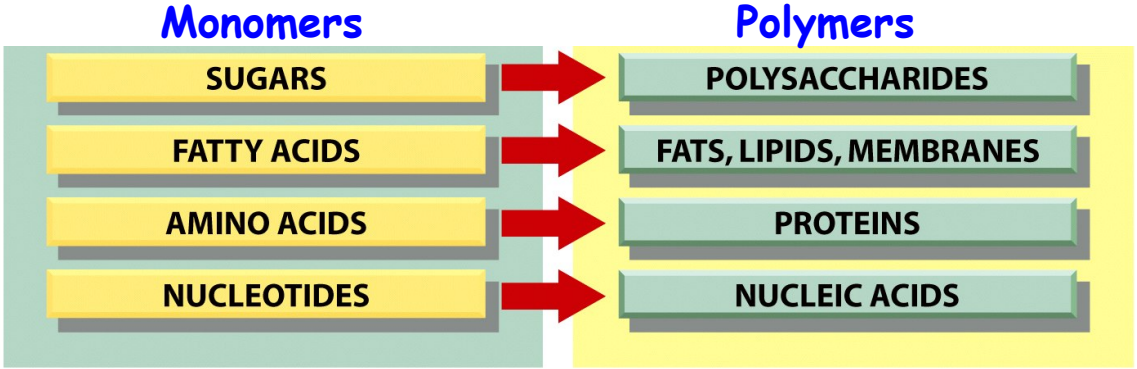
What Are the Major Chemical Components of a Bacterial Cell?

What Could Be the Transforming Principle?

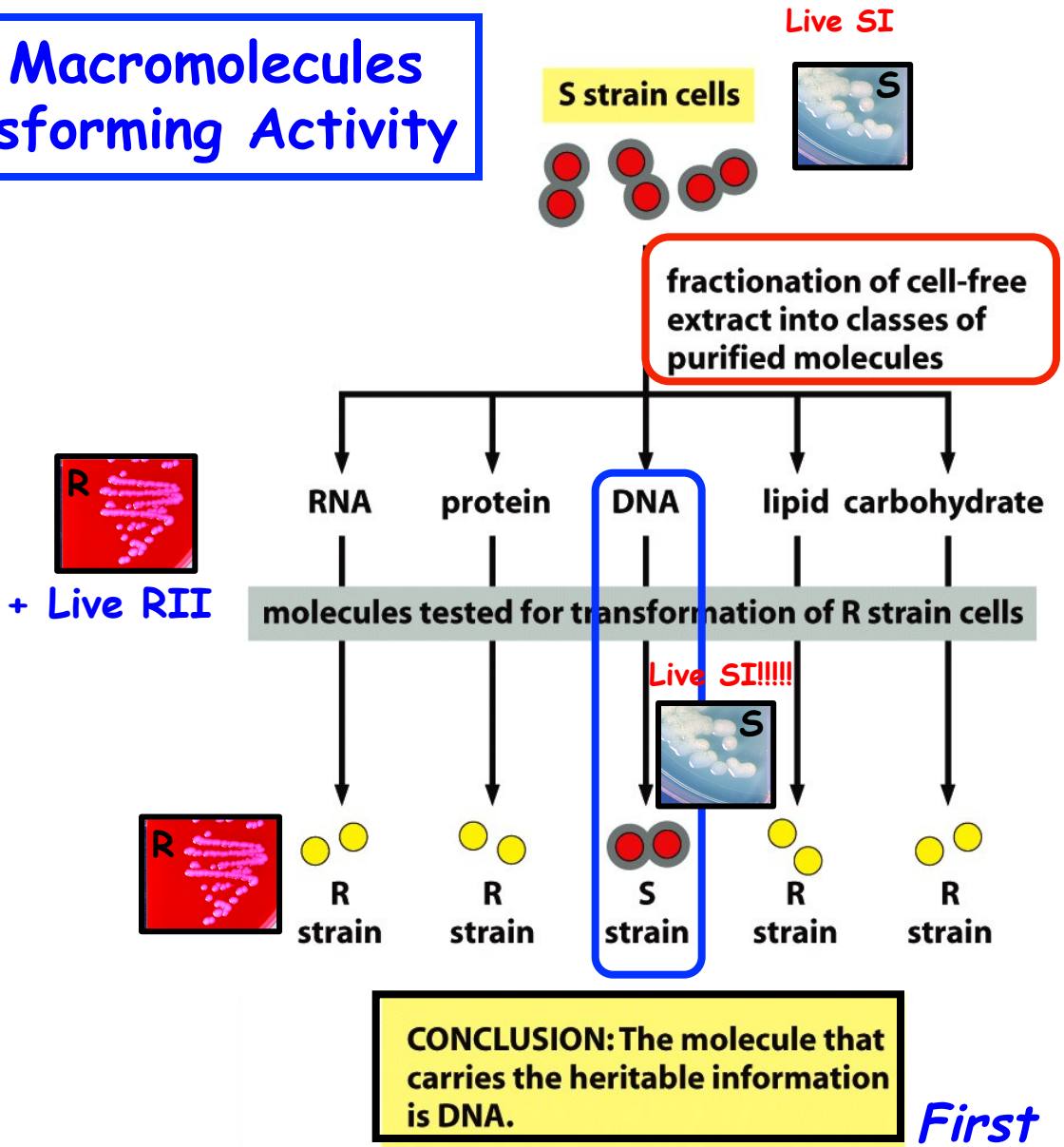
1. What is Predicted if DNA is the Genetic Material?

2. How Test Hypothesis?

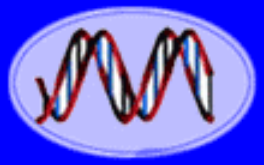




Testing Macromolecules For Transforming Activity



First Transformation Experiment With Purified Molecules!!



DNA
Genetic Code of Life

Entire Genetic Code
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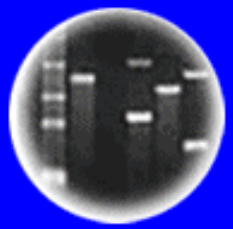

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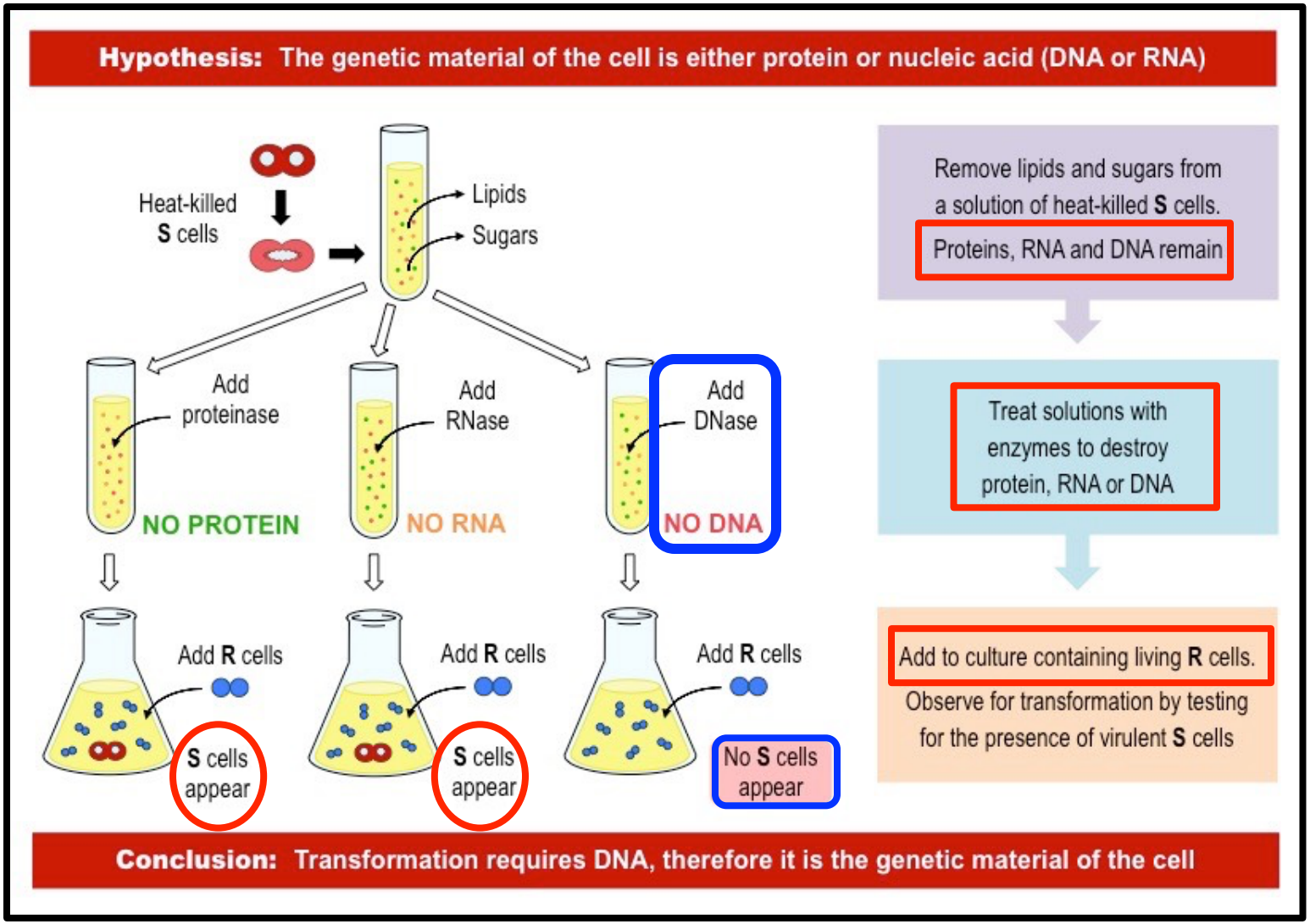
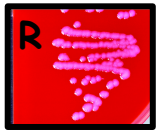
The Avery et al. Experiment Shows Conclusively that DNA is the Genetic Material?

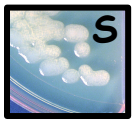
a. Yes
b. No

What is an Alternative Explanation?

The Critical Experiment by Avery et al. Showing That DNA is the Genetic Material

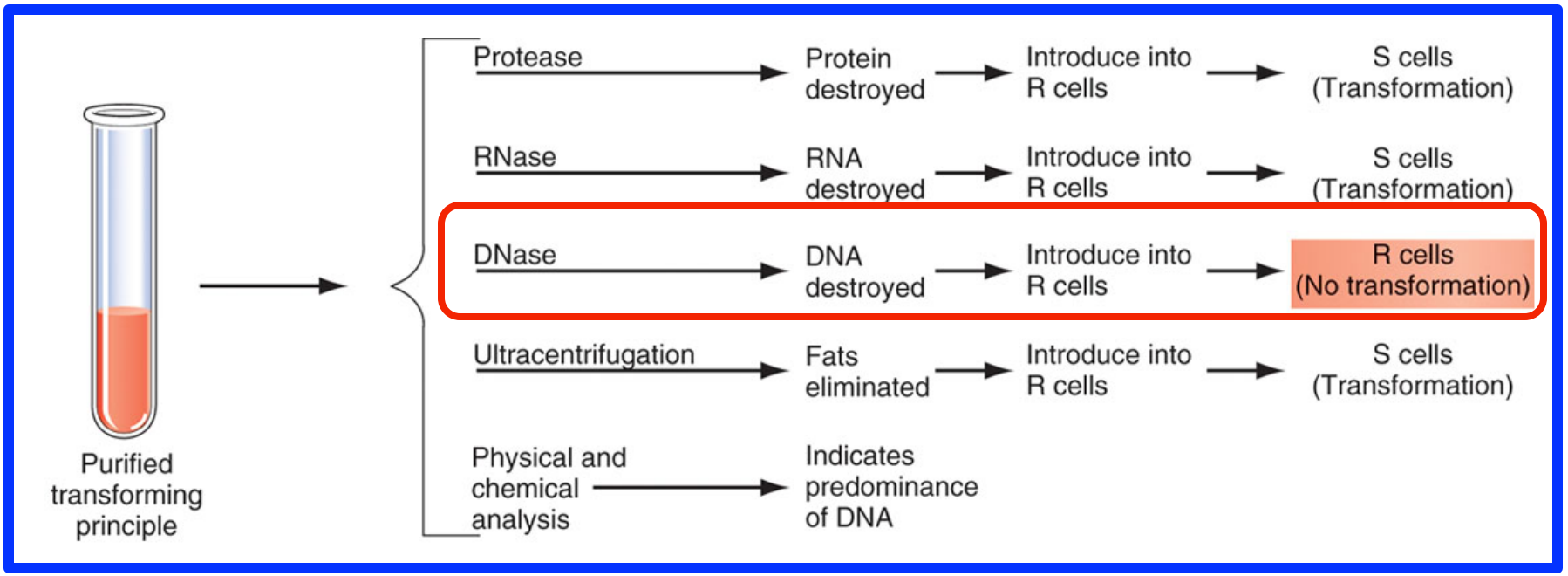






No DNA – No Transformation!

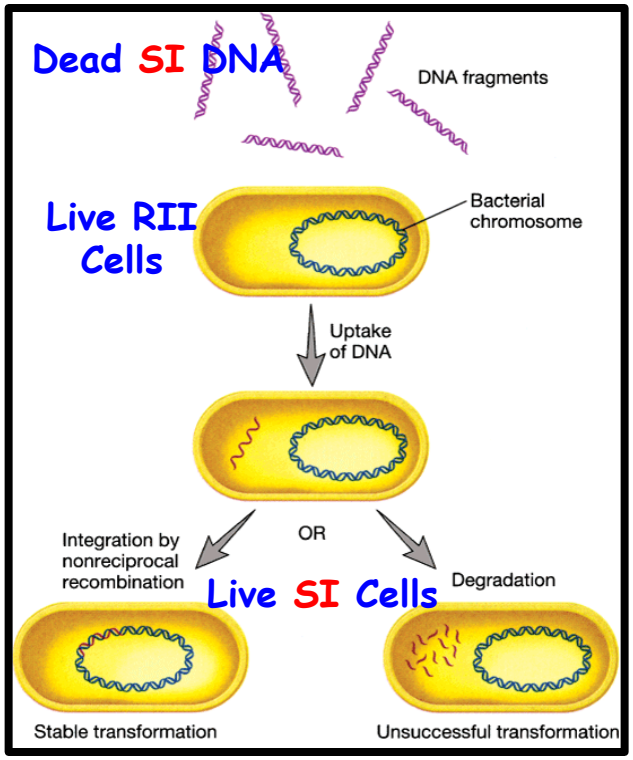
THE Critical Experiment by Avery et al.Showing That DNA IS THE Genetic Material



When DNase Destroyed DNA There Was No Transformation & Only Rough Cells Were Found in the Culture

If Smooth DNA Not Present, Rough Cells Cannot Be Transformed Into Smooth Cells!

How Did Avery et al. Experiments Verify the Hypothesis That DNA is the Genetic Material?



Predictions	Results
Replication	Yes
Phenotype	Yes
Stable	Yes

Cell Processes

1. SI DNA Taken Up By RII-Cells & Incorporated Into Chromosomes
2. SI Genes Transcribed Into SI mRNAs
3. SI mRNAs Translated Into Smooth I Proteins
4. Smooth I Proteins Helps Construct Sugar Capsule and Protects Bacteria From Antibodies ∴Cells Virulent

1. DNA Satisfies Criteria For Being the Genetic Material
2. Replicates
3. Directs Production of Strain/Capsule Type
4. In All Progenitor Cells

Transformation is a Basic Genetic Engineering Process Today!
Transformation=Ability of Cell Phenotype To Be Changed by DNA!

Genetic Engineering/Transformation Involves Incorporating Engineered DNA or Genes Into the Chromosomes of Different Organisms

Genotype

↓

Phenotype

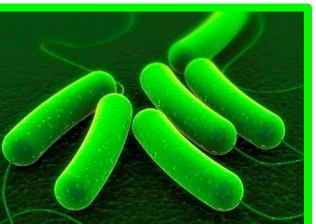
Engineered Gene MUST

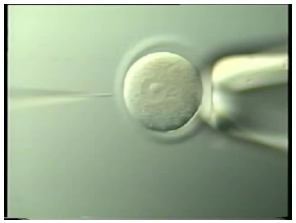
1. Enter Target Cell
2. Use Target Cell Machinery Enzymes to Become Part of Chromosome
3. Replicate With Target Cell Chromosome
4. Use Target Cell Protein Synthesis Machinery to Make a New Protein → Phenotype Trait!

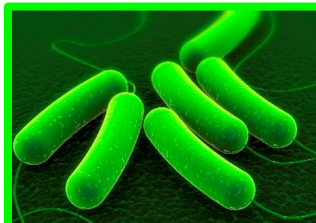
Engineered Gene CAN BE

1. From Same Organism
2. From Different Organism
3. From a Combination of Organisms Stitched Together by Genetic Engineering

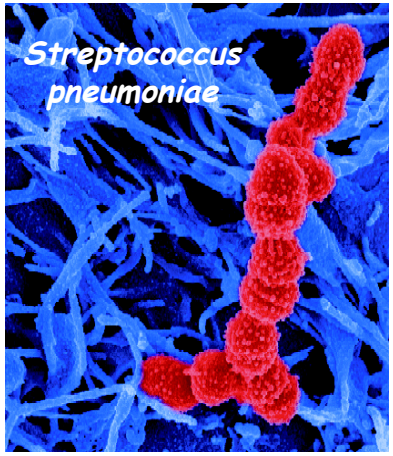
Gene Engineering Shows that Gene Processes Are Universal!!!

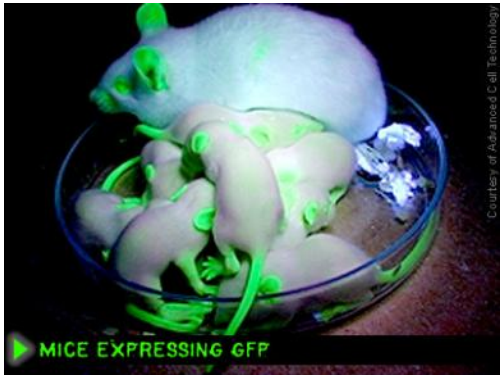


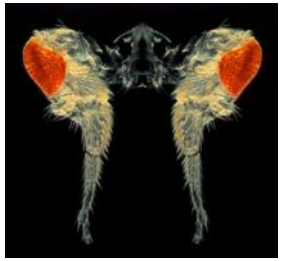




All Organisms Can Be Transformed!!
Genetic Engineering Has Come a Long Way Since Griffiths Experiments in 1928!!







What is A Gene?

Begin 5’

TGAAAATCCAAAAAAATAGGA
GTTTGGTGTTTGGGTTTTAGG
TAGGAAATAATTTGGGTCTTT
TTTAGGTTTCGGGTTTGGGTT
ATTTGAGTGTTTGACATTTGA
AATTTCGGTGTTTCATCTTCG
TGGGTGTGCCAGTGGCGTGAG
TGTTCCCCGGTTTCGTCAACT
TACGGTTTAGGGTTTACCAAG
TTAGGGTTTAGGGTTTGAGAT
GGCGGCCATTTCTCATGTTTG
AAACAAAGCCTGAAAATCAAA
TGGGTGTGCCGGTGGCGTGAG
CGTTCCCCGGTTCCGTCAACT
ATCAAGTACCCATGTTTGGGA
TGAACGTCAATGAACACGAAA
AAAAAAATAGGAAATCGACCC
AGAAAAGGGAGGGTGGCCATT
ACTATCACGTAACAACAAAAC
ATTTTTTTGCGTGGGTGTGCC
ATAAATAGATTTTTCCCTTGT
CCTTTTCCATGTTCAAGTACC
TTTCTCATGTTTTGAAGTCAA
CCTGAAAATCCAAAAAAATAG
CAGTGGCGTGAGACATTGGAG
GATACGTCAACTAACACGTAA
CATGTTTGGGATTTTTTTCCG
AGAACCCAAAAAAAATAGTCT
GAAATCGACCCTTTTCCATGT
GGGCAGCCATTTCTCTTGTTT
AAAACAAAGCCTGAATATCTA
GTGAGTGTGCCAGTGGCGTGA
TCGTTCCCCGGTTCCTTCAAC
GTTCAAGTACCCATGTTTGGG
TTGGACGTCAAAGAAACCAAA
CAAAAAAATAGGAAATCGACC
AGAAAATGGAGGGCGGCCAAT
CTGACACGTAAAAACAAAGCT
TTTTTTCGCGTGGGTGTGCCA
AAAATAGTCCCGTTCCCCGTT
TTTTCCATGTTCAATTACCCA
TCTCATATTTGGACGTCAAAG

End 3’

Sequence or Order of Nucleotides Coding DNA Strand (Coding Strand)

The β-Globin Gene

Blood Protein Carries Oxygen to All Genes From Lungs ⇨ Energy

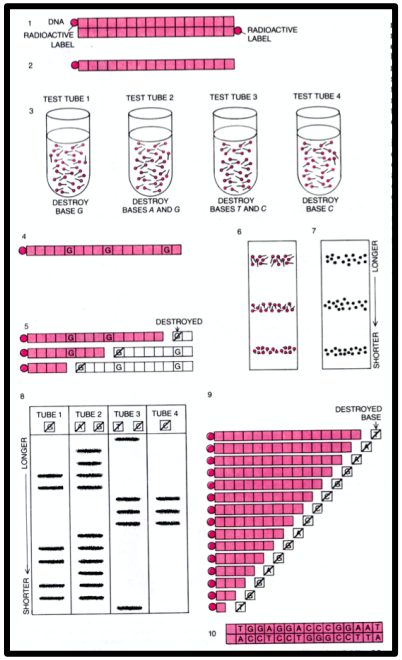
A Gene is a <u>Unique Sequence</u> of Nucleotides Specifying a Function

DNA Sequence = Biology!
What If Sequence Changed?

SEQUENCE → FUNCTION

Relative to Coding or Sense Strand of Gene

Genes and Genomes Can Be Sequenced!



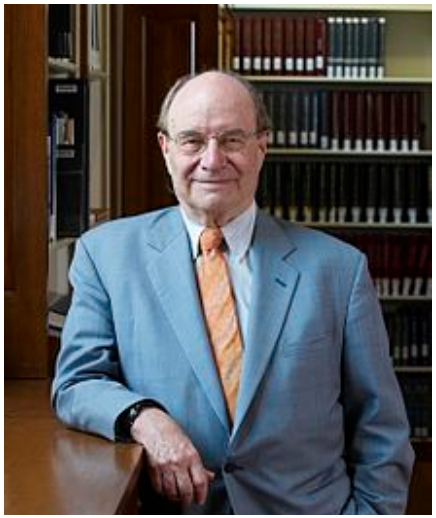
A new method for sequencing DNA

(DNA chemistry/dimethyl sulfate cleavage/hydrazine/piperidine)

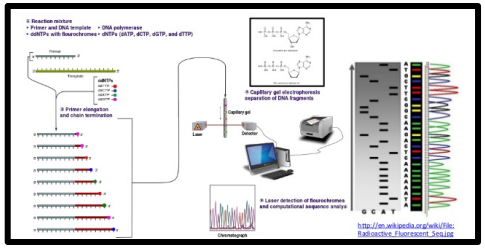
ALLAN M. MAXAM AND WALTER GILBERT

PNAS February, 1977

Water Gilbert



Fred Sanger



DNA sequencing with chain-terminating inhibitors

(DNA polymerase/nucleotide sequences/bacteriophage φX174)

F. SANGER, S. NICKLEN, AND A. R. COULSON

PNAS December, 1977

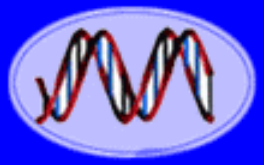
The Nobel Prize in Chemistry 1980

Paul Berg
Prize share: 1/2

Walter Gilbert
Prize share: 1/4

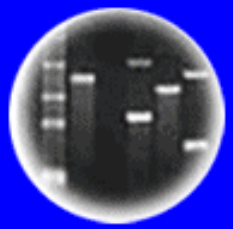
Frederick Sanger
Prize share: 1/4

- One half awarded to Paul Berg "*for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA*".
- The other half jointly to Walter Gilbert and Frederick Sanger "*for their contributions concerning the determination of base sequences in nucleic acids*".



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



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Genes & Genomes Differ Because the Sequence of DNA Differs

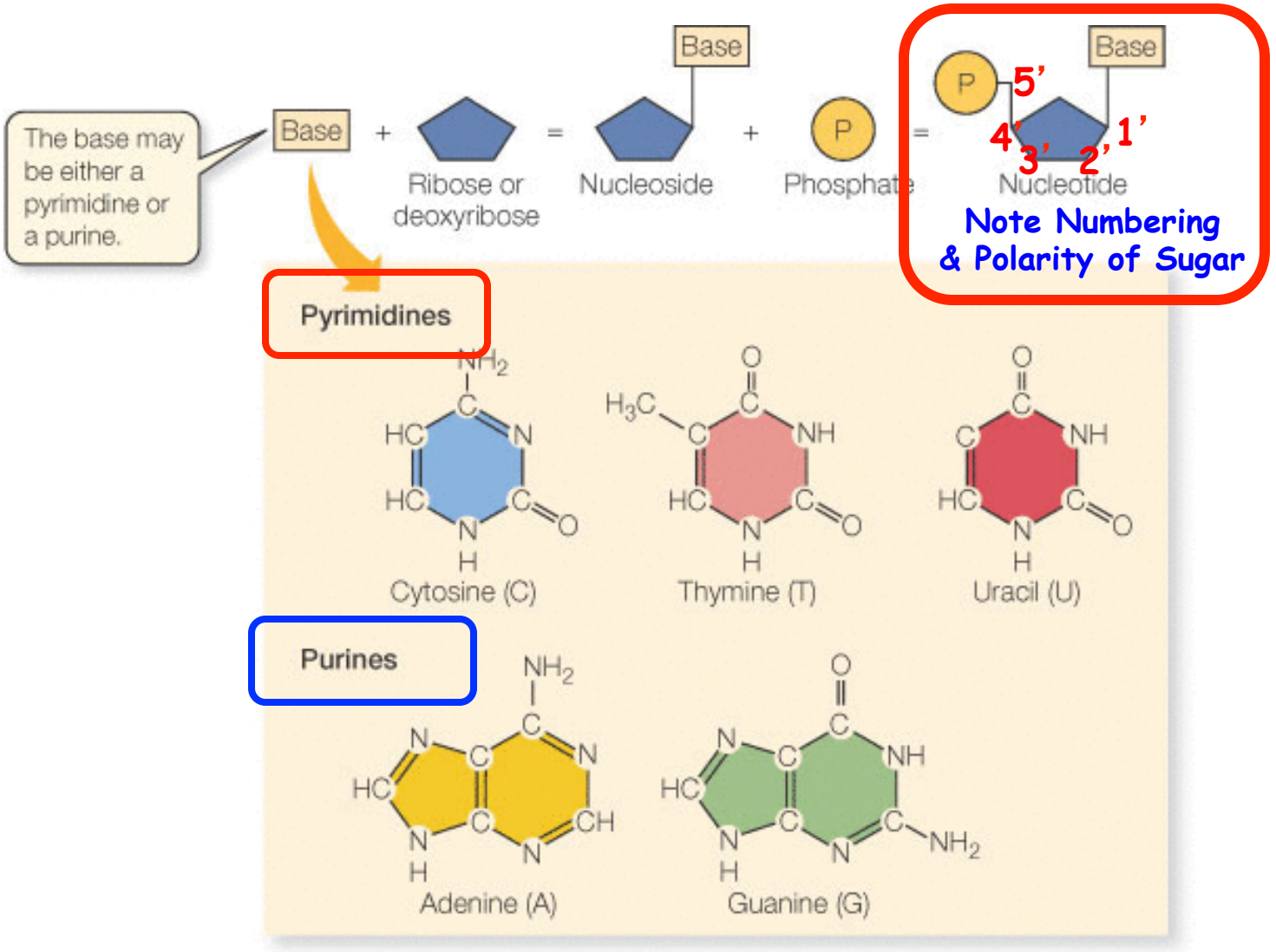
DNA Sequence
Beginning → End
5' 3'

→ Biological Uniqueness

If You Know the DNA Sequence, You Can Engineer Anything! Even Make New Genes & Genome!

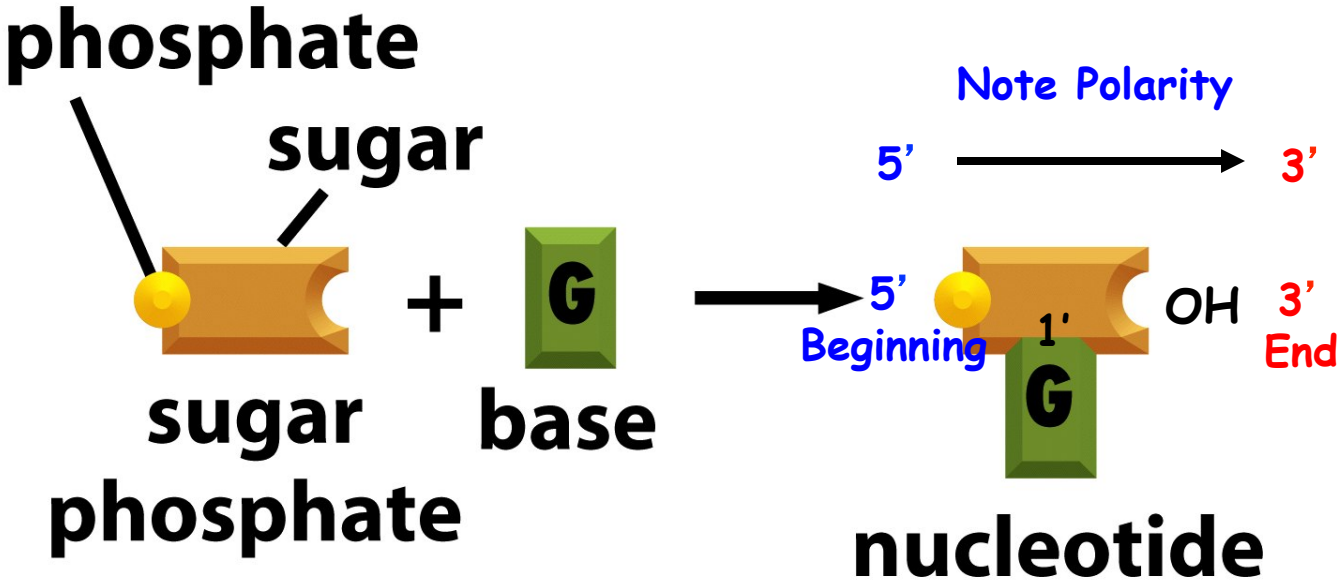
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

There Are Four Different Nucleotides in DNA



Note Chemical Differences in Bases -- Chemistry Leads to Biology!!

Nucleotides Have Polarity
Based on What is Bonded to the Five-Carbon Sugar
Phosphate on 5' Carbon and OH on 3' Carbon



The Sugar is the HUB

DNA Sequence Defined By Nucleotide Order

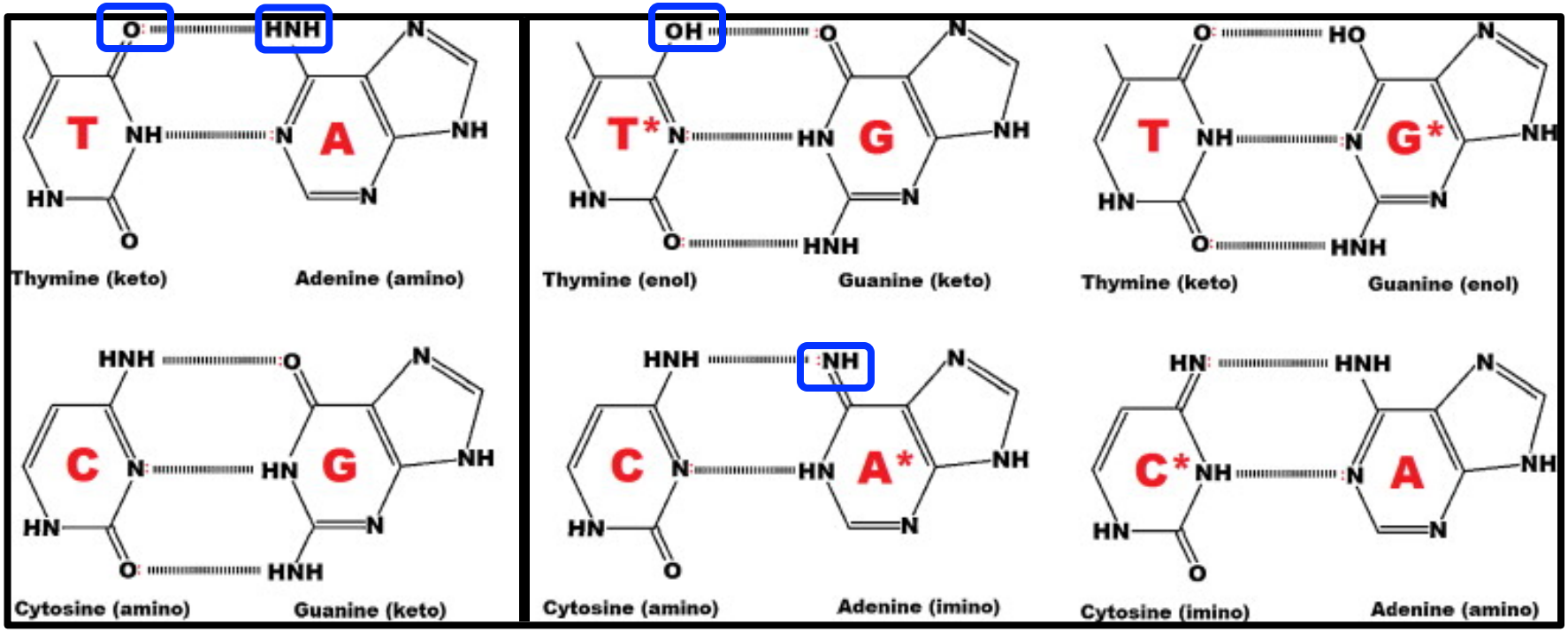
DNA Sequence = Functional Uniqueness = Biology

Figure 1-2a *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

Tautomers Change Base Pairing Rules

Normal Forms – Keto & Amino

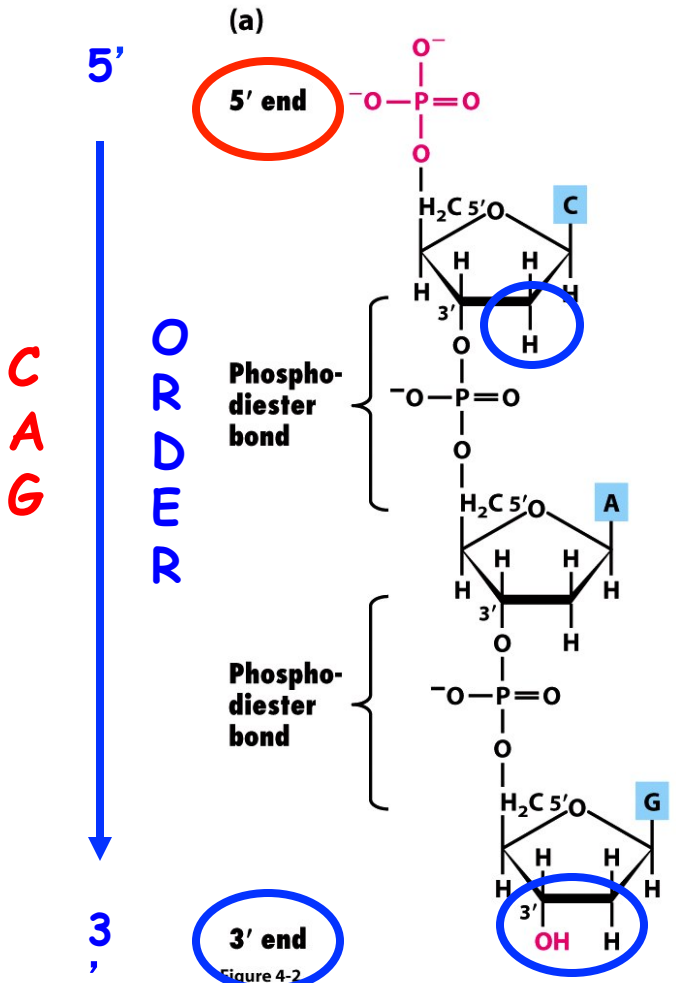
"Mutant" Forms – Enol & Imino



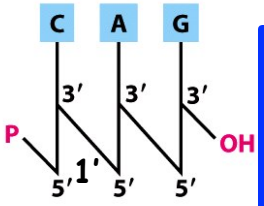
And Lead To Mistakes in DNA Replication & Mutations ➤ Genetic Diversity

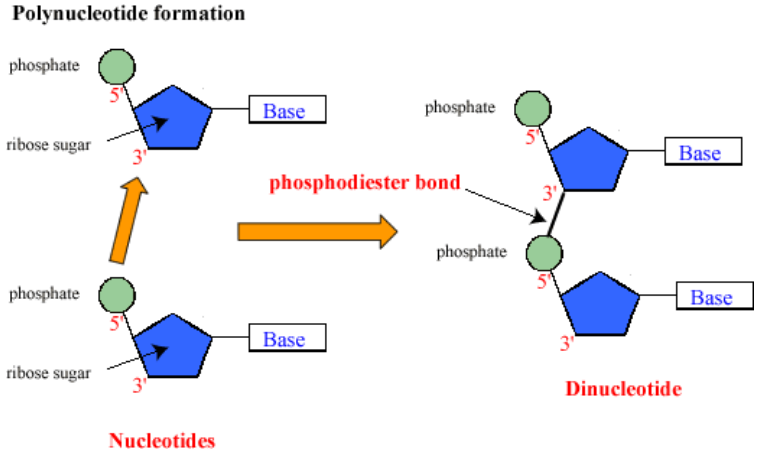
Chemistry Leads to Biology!!

Nucleotides Are Joined By 5' to 3' Phosphodiester Bonds



(b) Short-Hand Notation





1. Nucleotides That Join 5' to 3'
2. This is the Basis For All of Biology
3. Order is Maintained During DNA Replication
4. Basis of All Genetic Engineering

Pol[illegible]

Sugars & Order Specified By Bases

Clues to the Double Helix-Chargaff's Rules

STOPPED

Purines = Pyrimidines

TABLE 6.1 Chargaff's Data on Nucleotide Base Composition in the DNA of Various Organisms

	Percentage of Base in DNA				Ratios	
Organism	*A*	*T*	*G*	*C*	*A:T*	*G:C*
Staphylococcus afermentams	12.8	12.9	36.9	37.5	0.99	0.99
Escherichia coli	26.0	23.9	24.9	25.2	1.09	0.99
Yeast	31.3	32.9	18.7	17.1	0.95	1.09
*Caenorhabditis elegans**	31.2	29.1	19.3	20.5	1.07	0.96
*Arabadopsis thaliana**	29.1	29.7	20.5	20.7	0.98	0.99
Drosophila melanogaster	27.3	27.6	22.5	22.5	0.99	1.00
Honeybee	34.4	33.0	16.2	16.4	1.04	0.99
Mus musculus (mouse)	29.2	29.4	21.7	19.7	0.99	1.10
Human (liver)	30.7	31.2	19.3	18.8	0.98	1.03

*Data for *C. elegans* and *A. thaliana* are based on those for close relative organisms.

Note that even though the level of any one nucleotide is different in different organisms, the amount of A always approximately equals the amount of T, and the level of G is always similar to that of C. Moreover, as you can calculate for yourself, the total amount of purines (A plus G) nearly always equals the total amount of pyrimidines (C plus T).

What Would You Predict For a Single-Stranded DNA?

THE COMPOSITION OF THE DESOXYPENTOSE NUCLEIC ACIDS OF THYMUS AND SPLEEN*

ERWIN CHARGAFF, ERNST VISCHER,† RUTH DONIGER, CHARLOTTE GREEN, AND FERNANDA MISANI

J. Biological Chemistry, July, 1948

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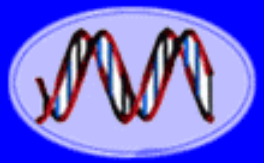

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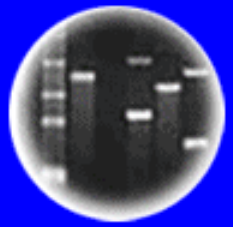

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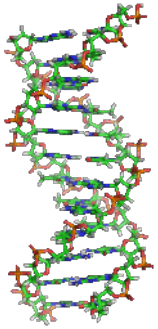
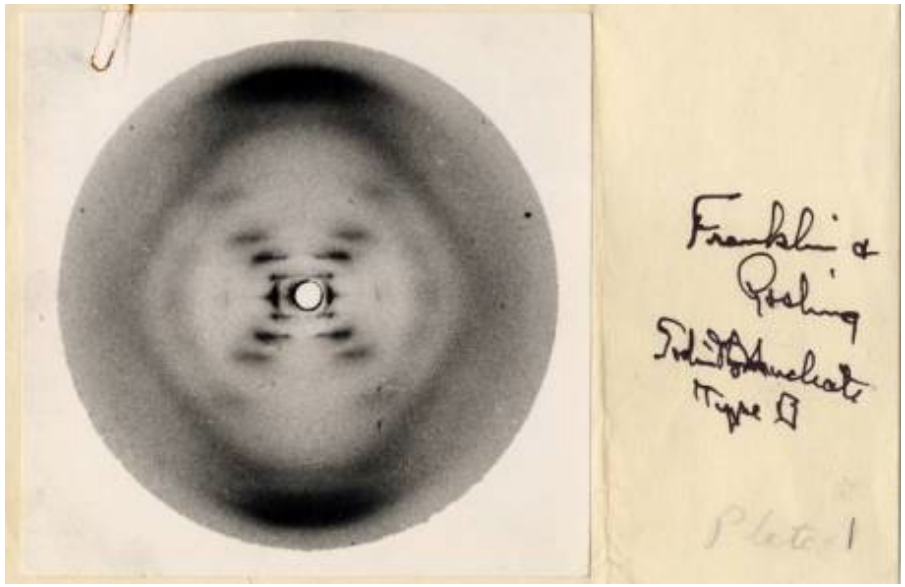
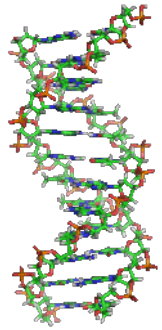
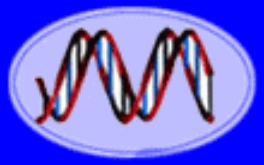
Reflections on *The Double Helix*









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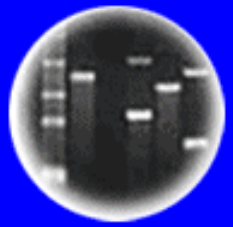

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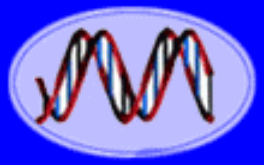
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

Nature, April 25, 1953

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at



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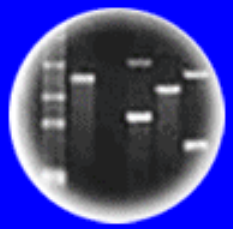
Entire Genetic Code
of a Bacteria



DNA Fingerprinting

Cloning: Ethical Issues
and Future Consequences

Plants of Tomorrow

Molecular Structure of Deoxypentose Nucleic Acids

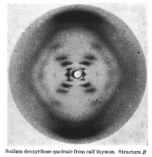
M. H. F. Wilkins
Medical Research Council Biophysics
Research Unit,

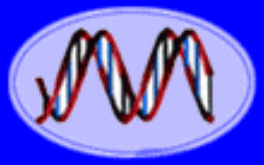
A. R. Stokes
H. R. Wilson
Wheatstone Physics Laboratory,
King's College, London.
April 2.
Nature, April 25, 1953

Molecular Configuration in Sodium Thymonucleate

Rosalind E. Franklin*
R. G. Gosling

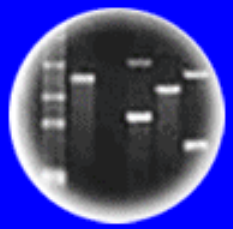
Wheatstone Physics Laboratory,
King's College, London.
April 2.
Nature, April 25, 1953





DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



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GENETICAL IMPLICATIONS OF THE STRUCTURE OF DEOXYRIBONUCLEIC ACID

By J. D. WATSON and F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge

Nature, May 30, 1953

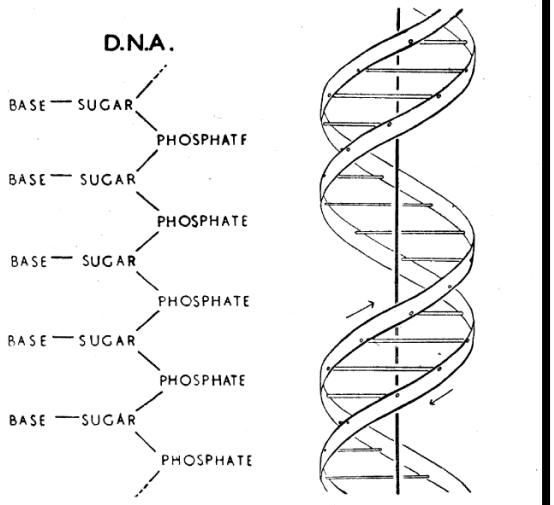


Fig. 1. Chemical formula of a single chain of deoxyribonucleic acid

Fig. 2. This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

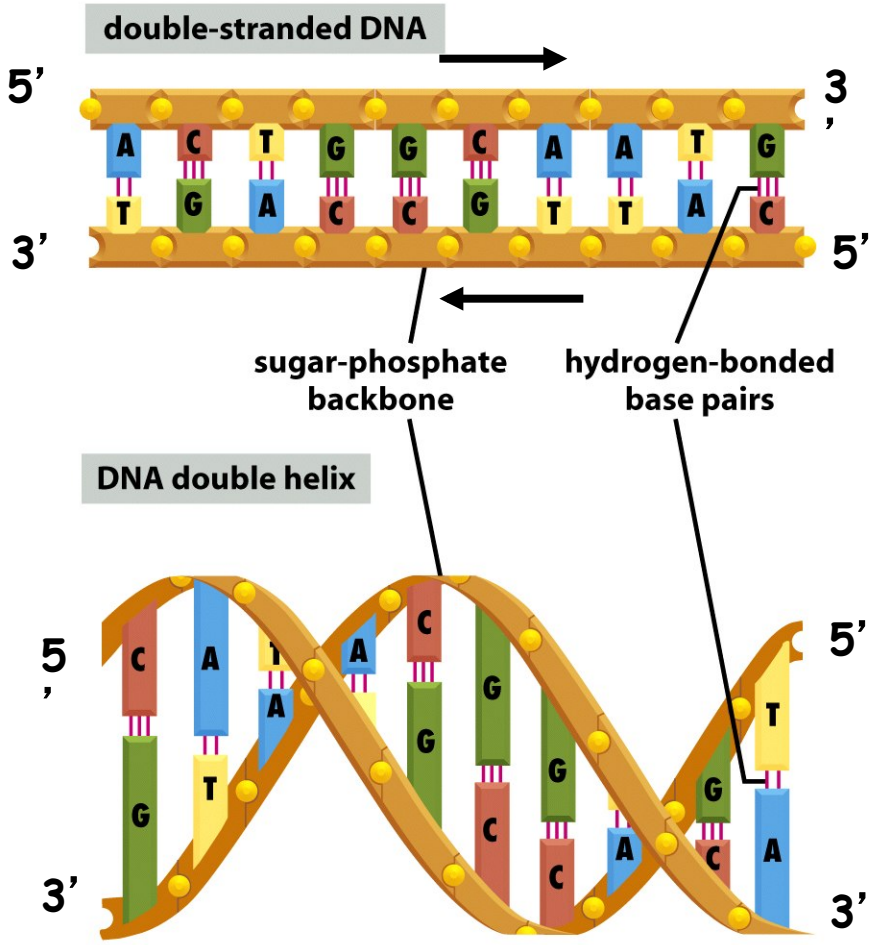
Our model suggests possible explanations for a number of other phenomena. For example, spontaneous mutation may be due to a base occasionally occurring in one of its less likely tautomeric forms. Again, the pairing between homologous chromosomes at meiosis may depend on pairing between specific bases. We shall discuss these ideas in detail elsewhere.

For the moment, the general scheme we have proposed for the reproduction of deoxyribonucleic acid must be regarded as speculative. Even if it is correct, it is clear from what we have said that much remains to be discovered before the picture of genetic duplication can be described in detail. What are the polynucleotide precursors? What makes the pair of chains unwind and separate? What is the precise role of the protein? Is the chromosome one long pair of deoxyribonucleic acid chains, or does it consist of patches of the acid joined together by protein?

Despite these uncertainties we feel that our proposed structure for deoxyribonucleic acid may help to solve one of the fundamental biological problems—the molecular basis of the template needed for genetic replication. The hypothesis we are suggesting is that the template is the pattern of bases formed by one chain of the deoxyribonucleic acid and that the gene contains a complementary pair of such templates.

Explained Replication
Explained Spontaneous Mutation

DNA is a Double Helix of Two Complementary Chains of DNA Wound Around Each Other

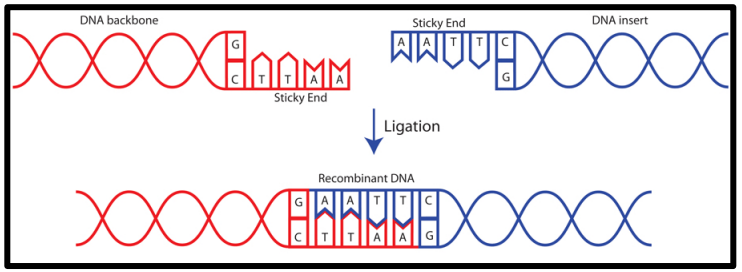


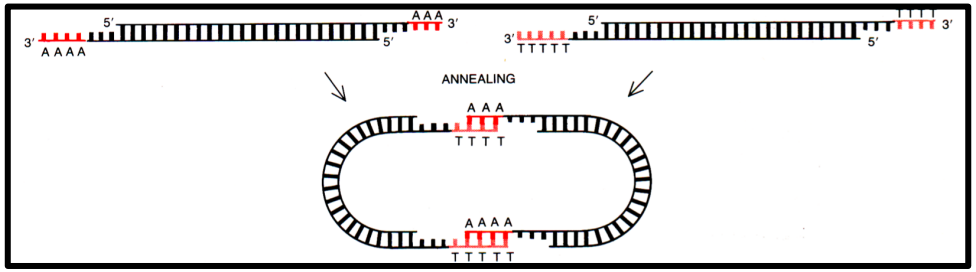
Watson and Crick, Nature, 1953

1. Complementary Strands
2. A=T and G=C (Four Bases)
3. Sequence of Strands Differ
4. Bases to Interior
5. Phosphate-Sugar Backbone on Exterior
6. DNA Strands in Opposite Direction (Only Way Helix Fits)
7. Sequence of One Chain Automatically Specifies Sequence of Complementary Chain (Basis of Replication!)
8. No Constraint on Sequence (4^n=n # sequences)
9. DNA has dimensions (Know # bp Know Length: 20Å diameter, 3.4Å/bp, 10bp/turn)
10. Sequence = Biology

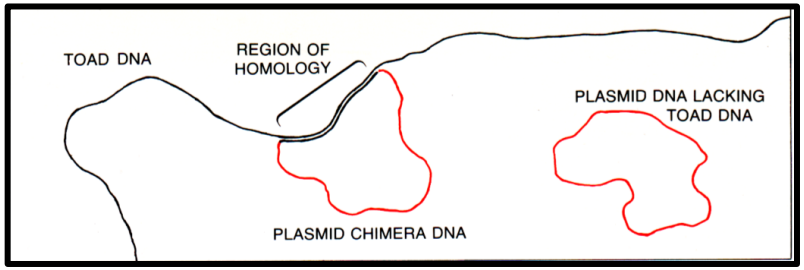
Complementary Base Pairs Are Essential For Genetic Engineering Engineering, Analysis of Recombinant Plasmids, and Polymerase Chain Reaction (PCR)

1. Annealing Two Two Molecules Together ("Cut & Splice")

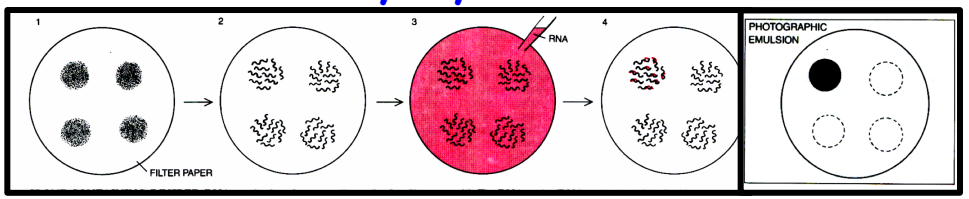




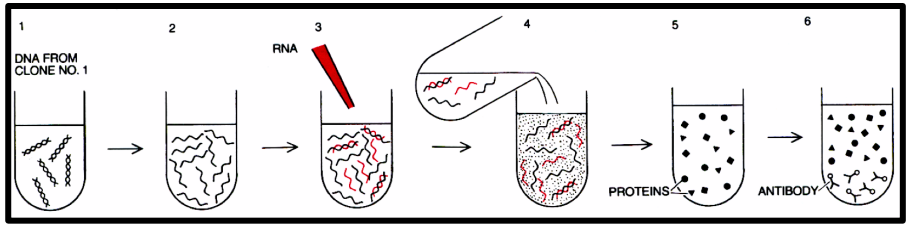
2. Heteroduplex Analysis



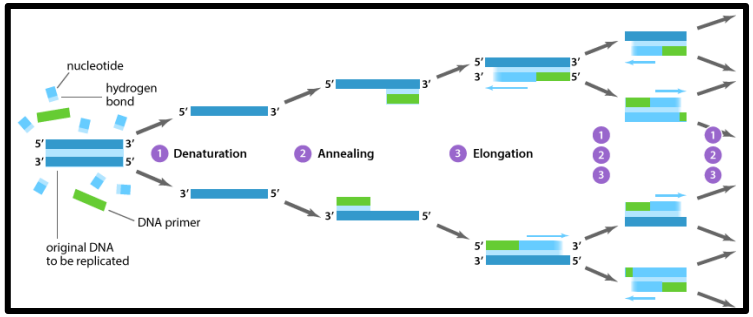
3. Colony Hybridization



4. Hybrid-Arrested Translation



5. Polymerase Chain Reaction

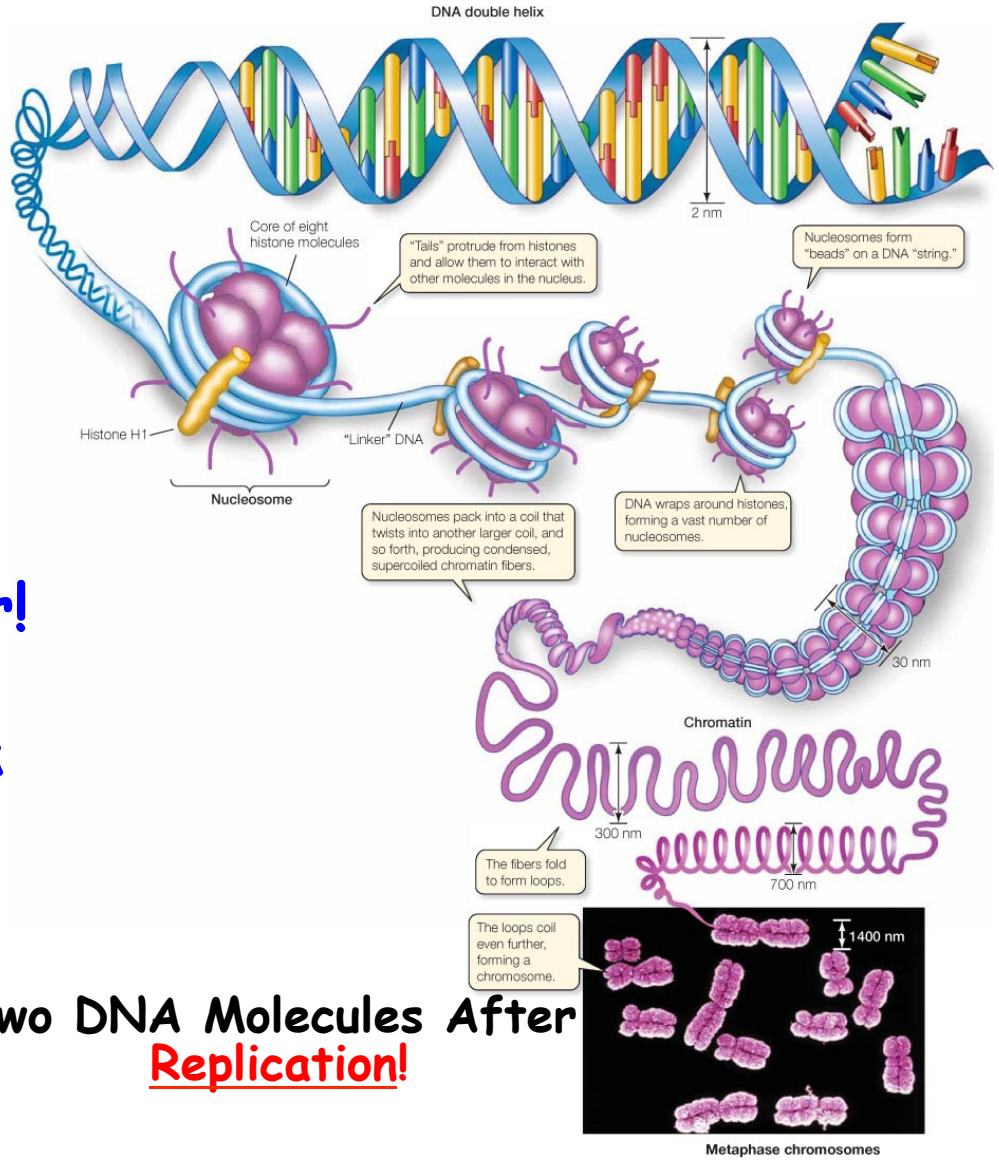


stop

A Chromosome Contains One (or Two!!) Continuous DNA Molecule(s)

DNA in Human & Eukaryotic Chromosomes is Linear!

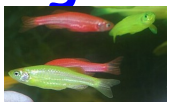
DNA in Most Bacteria is Circular!

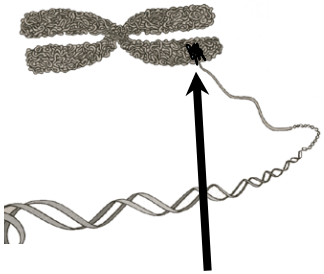


Two DNA Molecules After Replication!

A Chromosome Contains Many Genes Operating Independently

What is the Evidence?





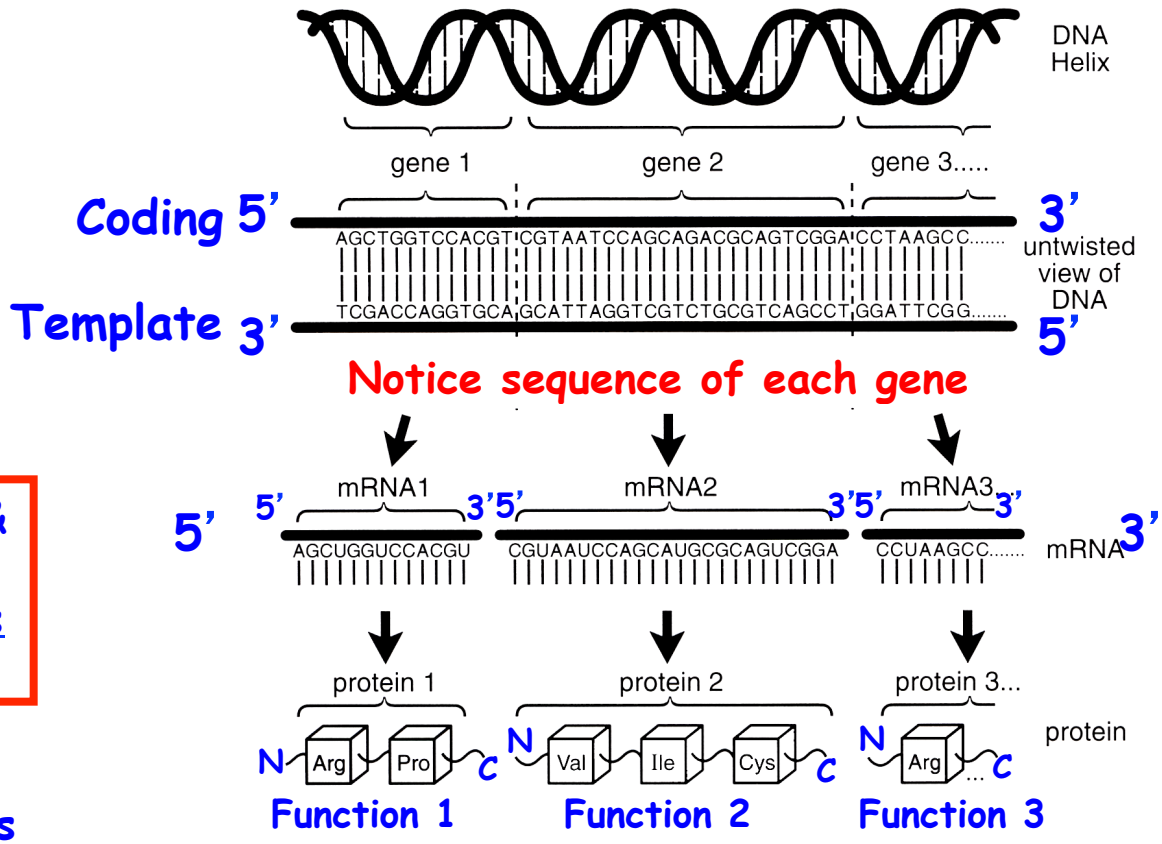
Position of Genes 1, 2, & 3 in chromosome

Discrete Units!

Notice- Each gene, mRNA, & protein has a <u>unique order/ sequence</u> of <u>monomeric units</u>

Central Dogma
∴Genes -> Functions in Cells via Proteins
Cells duplicate & stay the same -> DNA replication

What delineates each gene?

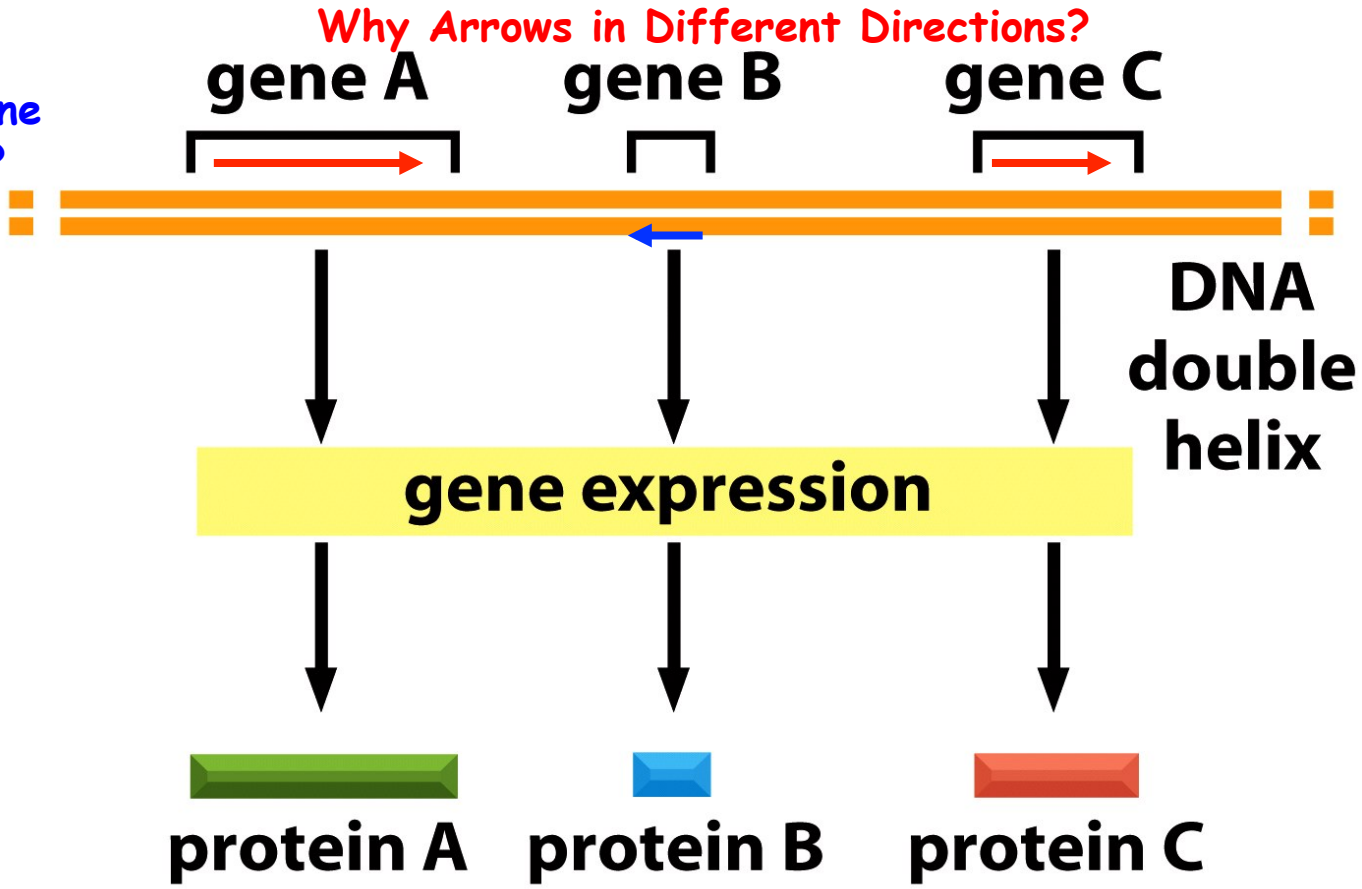


VERY IMPORTANT CONCEPT!

<u>COLINEARITY</u> BETWEEN GENE SEQUENCE AND PROTEIN SEQUENCE

A Chromosome Contains Many Genes That Reside at Specific Positions, or Loci, and Have Unique Functions

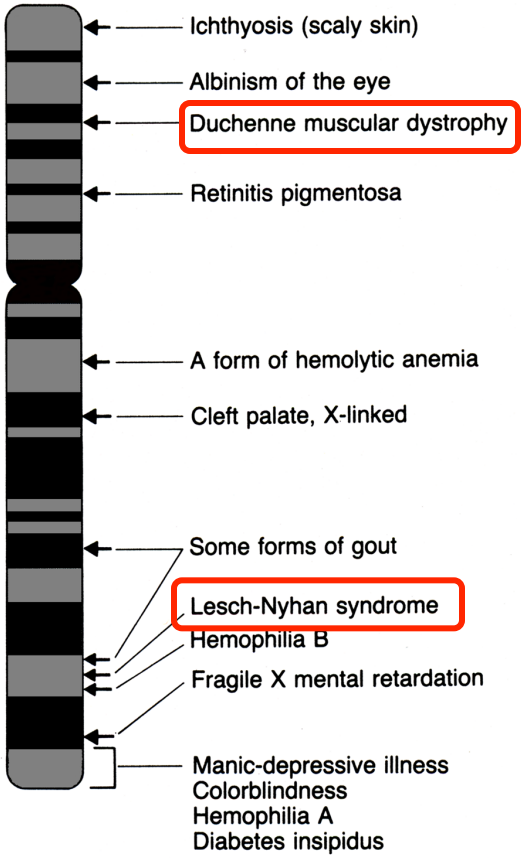
What Defines Gene Positions?



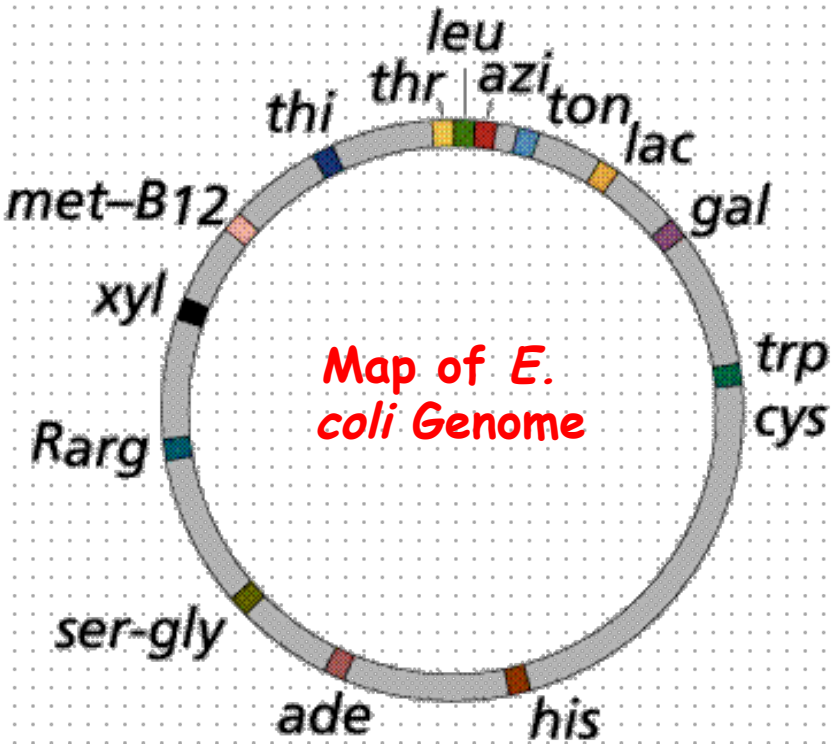
Because DNA Contains Two Strands--Genes Can Be Transcribed From Either Strand--But Only One Per Gene

Genes Reside at Specific Locations That Can Be Mapped

Human X Chromosome



Linear DNA
How Know?



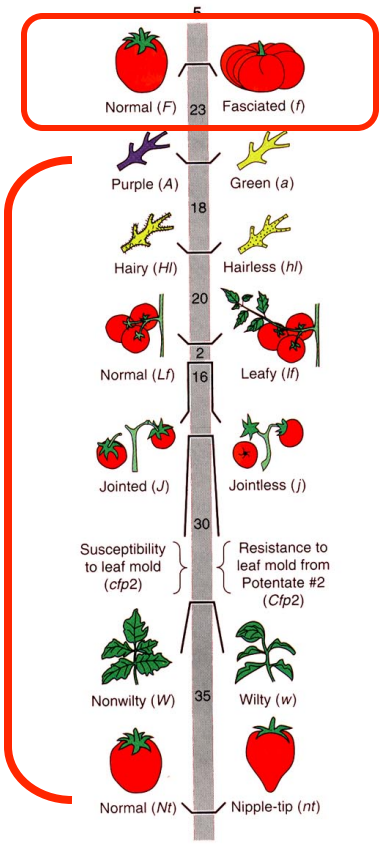
Circular DNA
How Know?

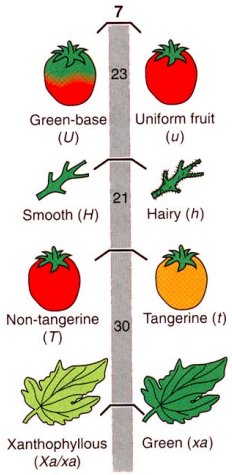
- Note Marker Bands - What are these? How are they useful?
- How Determine Gene Positions? Chromosome Number?

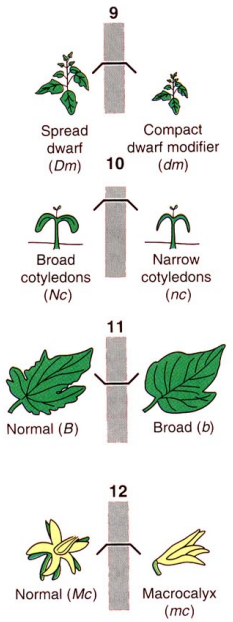
Alleles Reside at the Same Position on a Chromosome

Allele Phenotypes Specify Markers For Each Gene Location!

Alleles







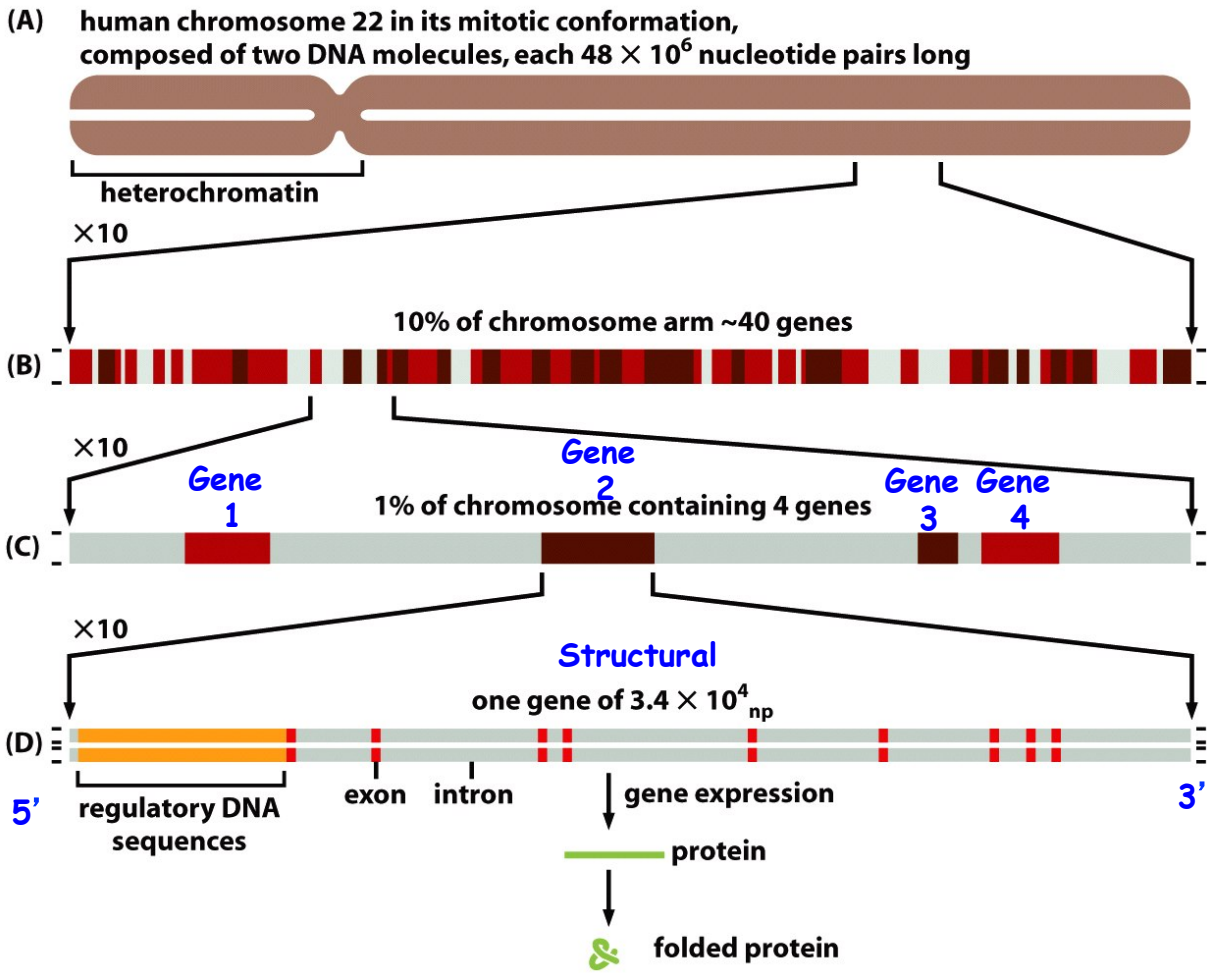
Different Genes

Gene Engineering Can Generate New Forms of Alleles of a Gene and, therefore, Results in More Genetic Diversity

mutations result in genetic diversity!!!

Alleles Are Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!

Organization of Genes on Human Chromosome 22



250 genes

Genes Are Defined/ Precise Regions of DNA

One Large Gene!

Genes Act As Individual Units?

How Know? GloFish Experiment! Genetic Engineering Antibiotic^R

Figure 4-15 *Molecular Biology of the Cell* (© Garland Science 2008)

A Conceptualized Gene

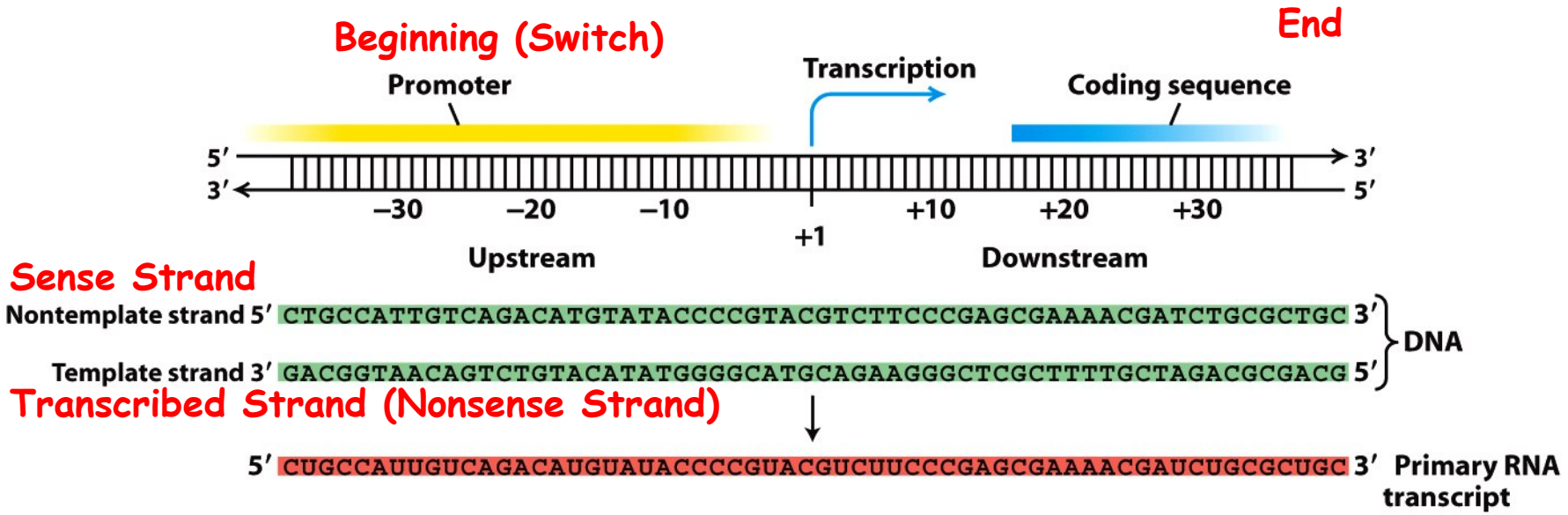
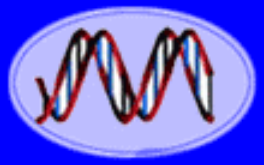


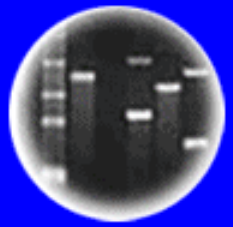
Figure 4-10b
Molecular Cell Biology, Sixth Edition

Major Concept in *"Making Proteins in Recombinant Bacteria"* Article by Gilbert



DNA
Genetic Code of Life

Entire Genetic Code of a Bacteria



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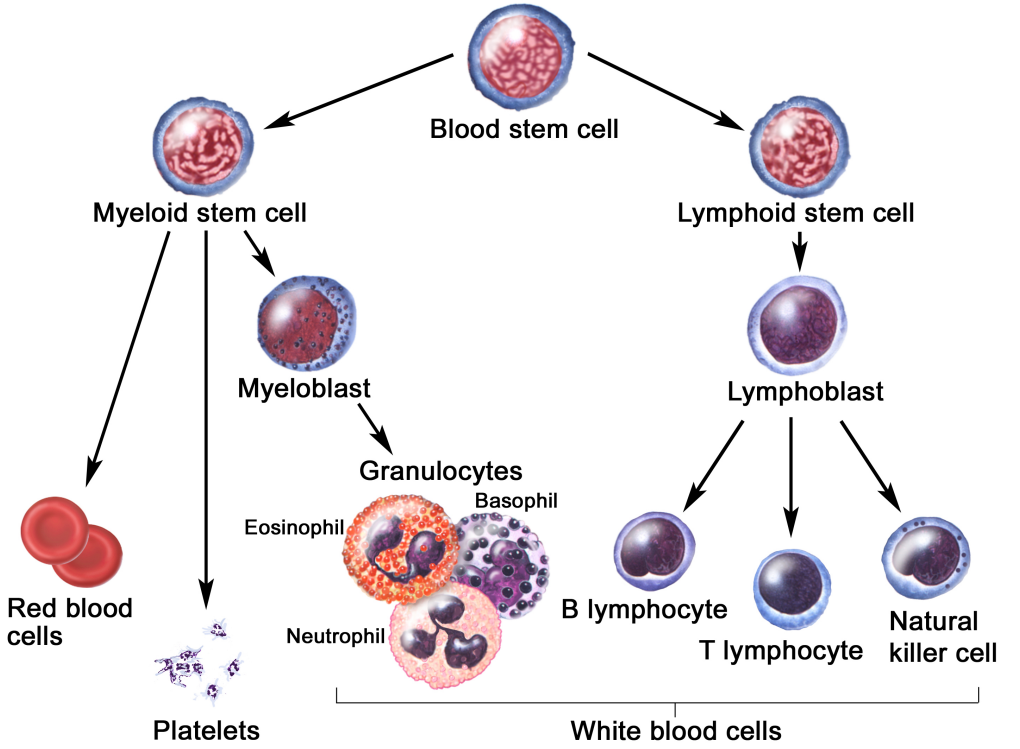
A "Simple" Gene Reviewed

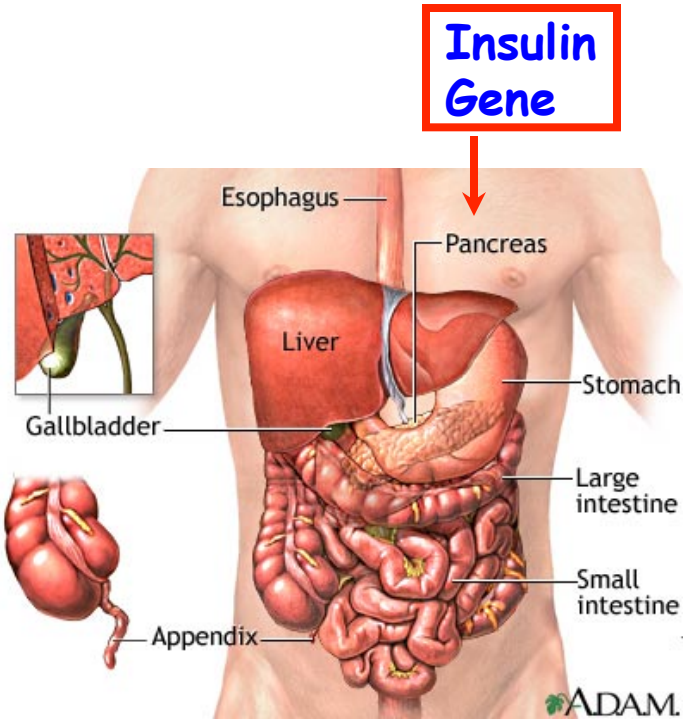
1. Sense Strand = Genetic Code
2. Sense Strand = 5' → 3' Direction (all DNA sequences specified 5' → 3')
3. Anti Sense Strand = Complement of Sense Strand & is Transcribed Strand
4. mRNA = Same Sequence As Sense Strand & Complementary to AntiSense Strand
5. mRNA = 5' → 3'
6. Switch Turns Gene On - Not Transcribed But Upstream of Coding Region

Genes Function As Independent Units! How Know? Design Experiment to Show!

"Everything" Follows the Double Helix & Its Rules - Anti-parallel Chains & Complementary Base Pairing!

Switches Control Where & When A Gene Is Active → Unique Functions → Unique Cells

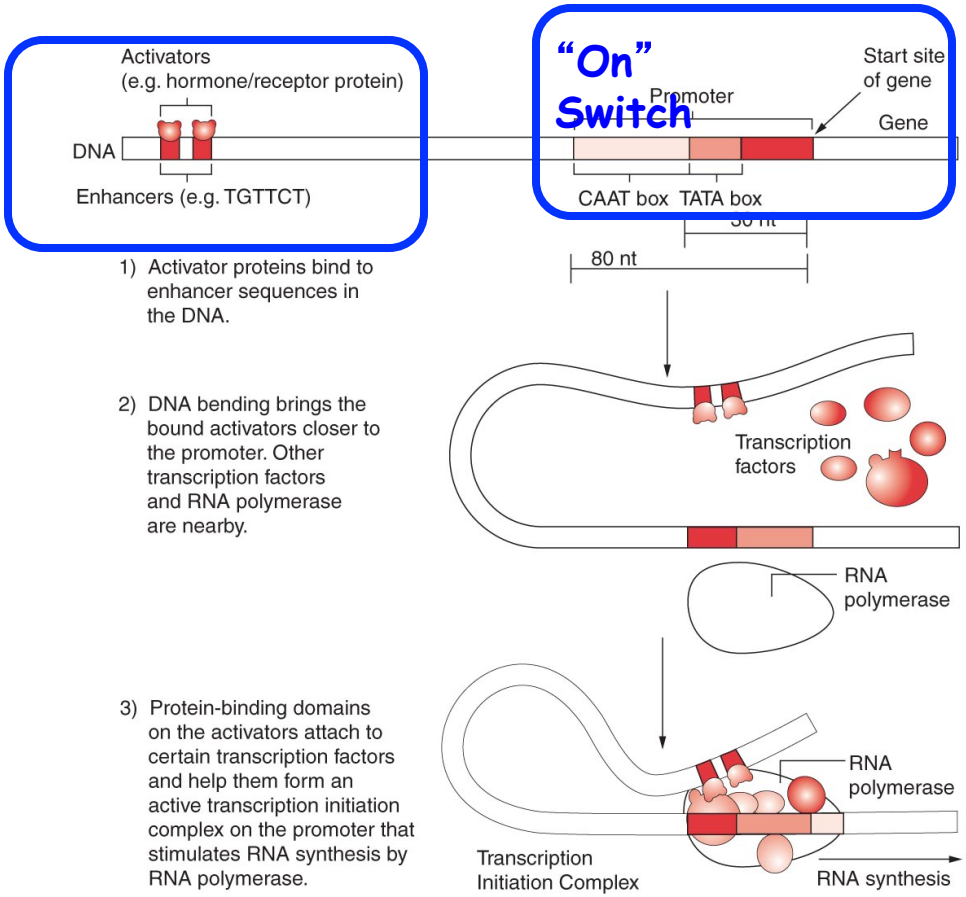




Control Switches Are Unique DNA Sequences & Can Be Cloned

AND used to Re-Engineer Organisms!!
Switches Act Independently of Gene!!

"Control" Switch



1. Each Switch Has a Unique DNA Sequence
2. Genome Projects Reveal Genes & Logic Controlled by the Switches
3. Sequence = Biology
4. No Hocus Pocus
5. Yo! It's in the DNA!!

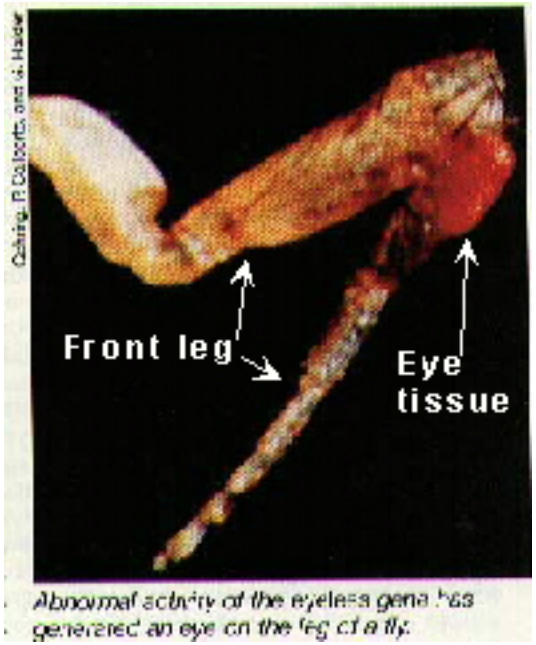
Legos!!!

The Eye Gene Can Be Expressed in Different Parts of the Fly by Engineering the Eye Switch

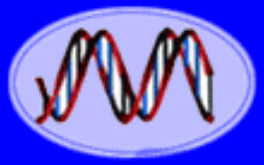
Eye Gene

Replace the Head Switch With the Leg Switch by Genetic Engineering

Eye Gene
+
Leg Switch



Abnormal activity of the eyeless gene has generated an eye on the leg of a fly.



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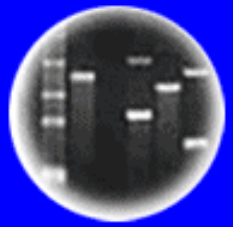

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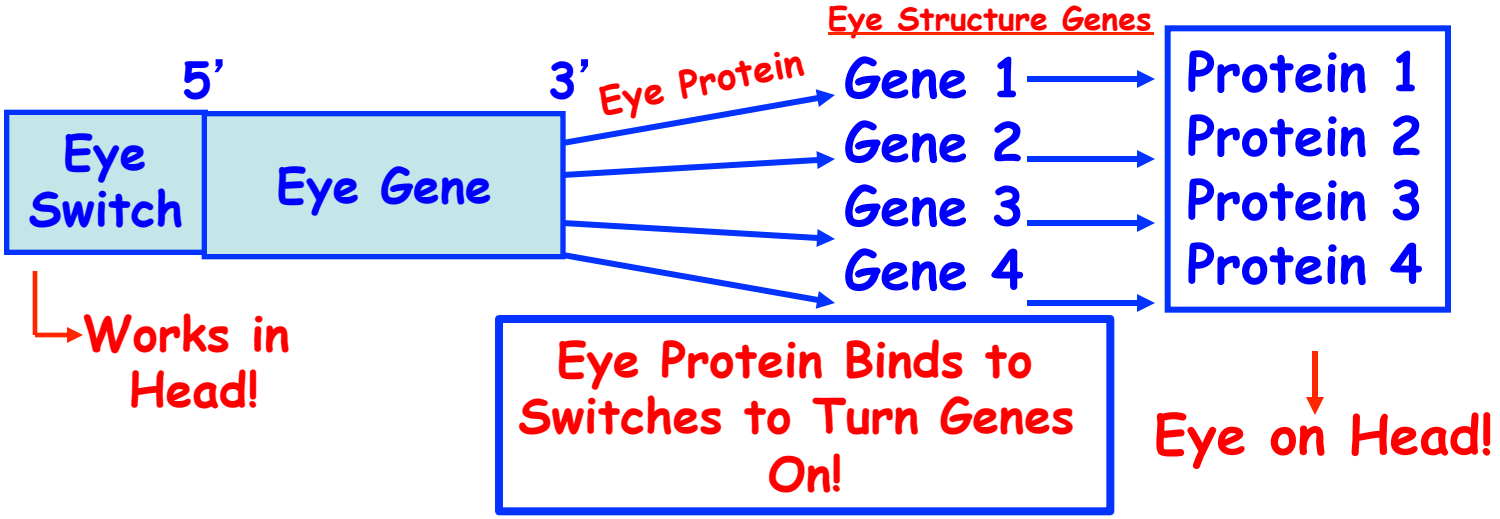
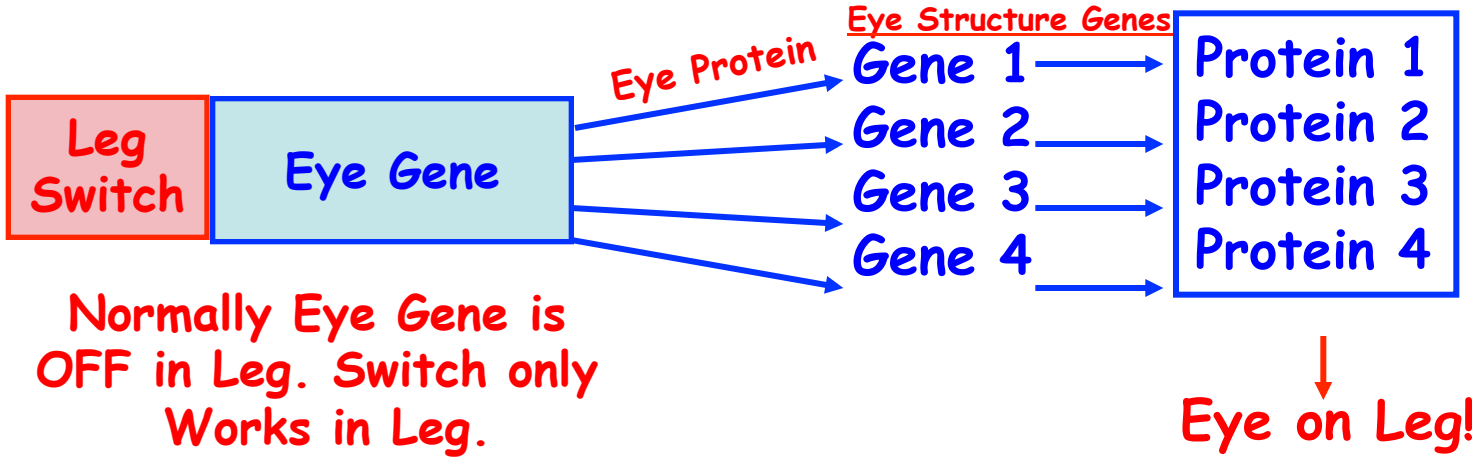
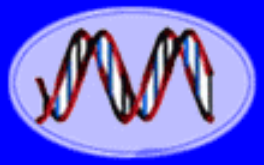
Eye Regulatory Network

Control Genes Like The Eye Gene Control The Activity of Other Genes!







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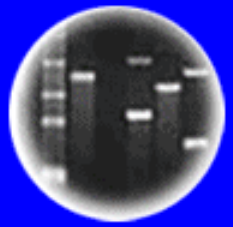

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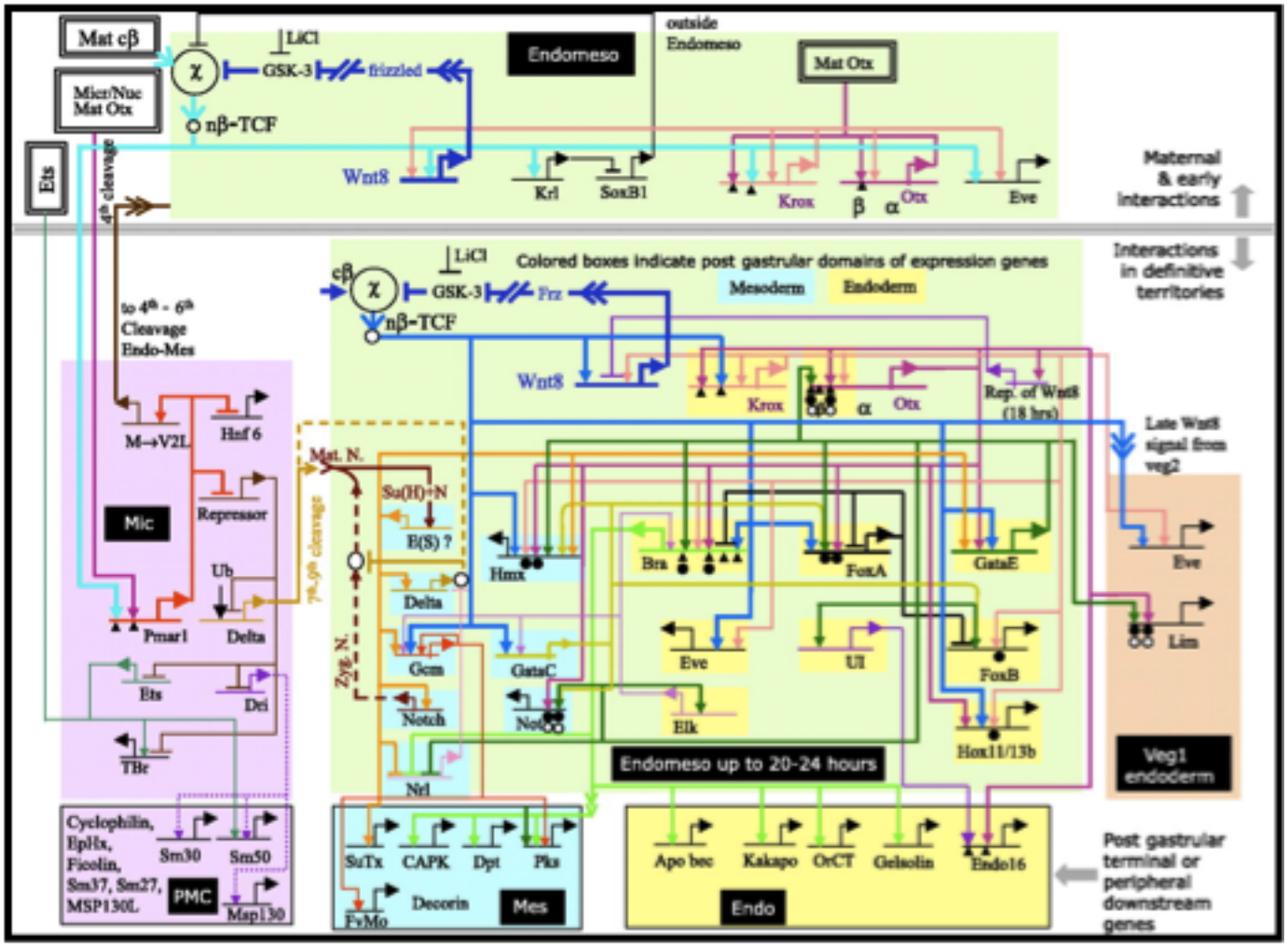
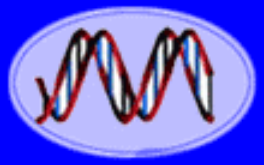
Ultimate Goal: To Dissect Genetic Regulatory Networks Programming Human Development From Birth to Death!



Genetic Networks Programming Early Sea Urchin Development



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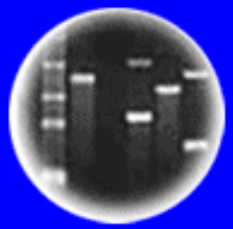

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100 Years Into The Future

1. If the Entire Human Genome is Sequenced?
2. If the Function/Protein of All Genes Are Known?
3. If All the Switches Are Identified & How They Go On & Off From Birth to Death?
4. If We Understand How Genes Are Choreographed & All the Sequences That Program them

What Does the Future Hold?

We Will Know at the DNA Level What Biological Information Programs Life to Death!

What Does This Mean For The Future of Humanity?

Remember - Mendel's Law Were Only Rediscovered 120 Years Ago & Look What We Can Do & Now!