



DNA
Genetic Code of Life



Entire Genetic Code
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues
and Future Consequences



Plants of Tomorrow

HC70A & SAS70A

Winter 2023

Genetic Engineering in Medicine, Agriculture, and Law

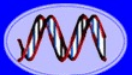
Professors Bob Goldberg & John Harada

Lecture 3


What Are Genes & How Do They Work: Part One



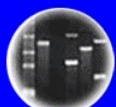

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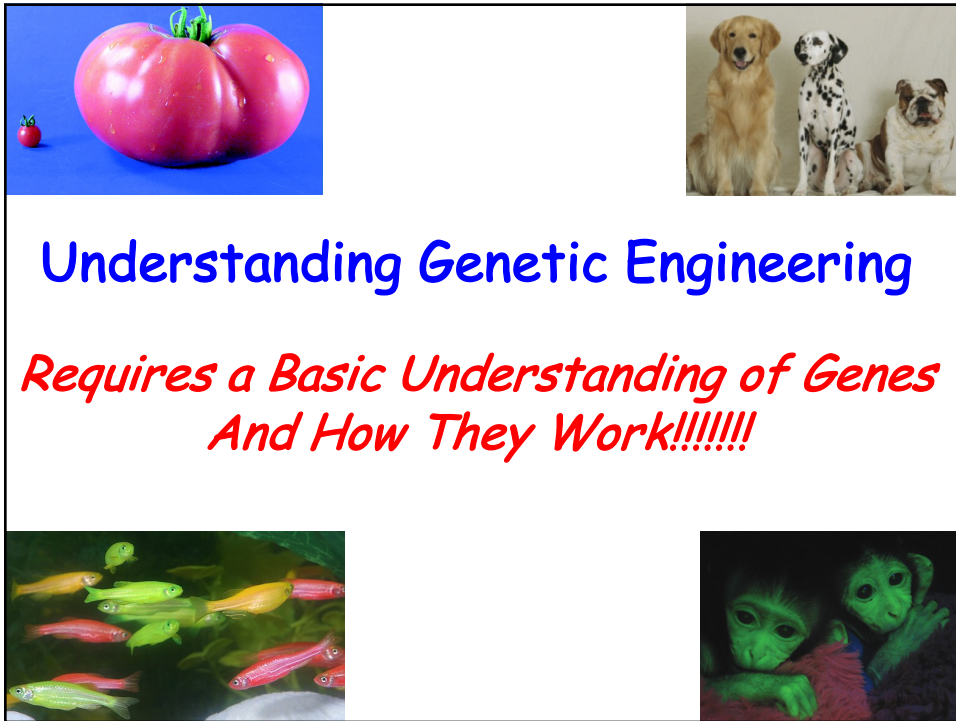
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THEMES

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?

2



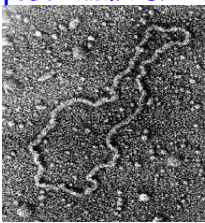
Understanding Genetic Engineering

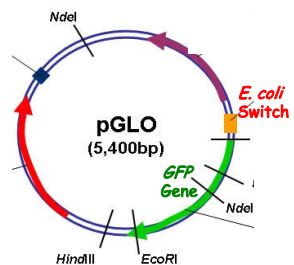
*Requires a Basic Understanding of Genes
And How They Work!!!!!!*

3

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

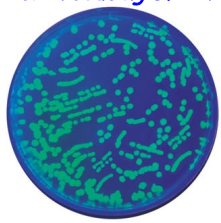
pGLO Plasmid DNA





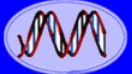
pGLO (5,400bp)

***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!

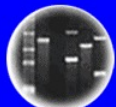
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What Were Considered the Properties of a Gene BEFORE It Was Known That DNA Was the Genetic Material - In the 1920s to 1940s?

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

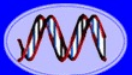
For First Half of 20th Century Proteins Were Considered the Genetic Material

- 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?

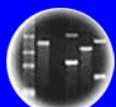
5




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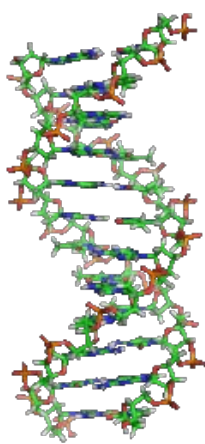


Cloning: Ethical Issues
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How Was DNA Shown to be the Genetic Material?



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

150 Years Ago Franco-German War



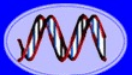







*But..... The Function of DNA Was Not Understood Until
75 years Later in 1944!!!*

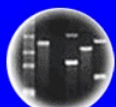
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Albrecht Kossel Discovered the Five Nucleotide Molecules That Make Up DNA & RNA in 1881

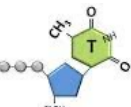
140 Years Ago



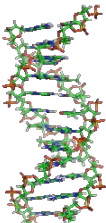
He Named
Nuclein as
Deoxyribnucleic
Acid or DNA &
Its Nucleotides




Purine
Deoxyribonucleotides



Pyrimidine
Deoxyribonucleotides



Nobel Prize
For
Medicine in
1910



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Evidence That DNA Is the Genetic Material Starts With Pneumonia



TO STOP SPREAD OF
INFLUENZA
STAY AT HOME
Amuse Yourself With One of the
Best
Player Pianos
We Sell for One Hundred Dollars
Less Than Same Grade Offered
Elsewhere

FIGHT THE 'FLU'
Rigid Quarantine Rules Established
**YOU ARE ASKED TO AID
IN ENFORCING THEM**

**Epidemic Closing
Order Is Sweeping**
The State Board of Health order,
closing schools, theatres, churches,
saloons, etc., in an effort to pre-
vent a further spread of the Span-
ish Influenza epidemic, is a sweep-
ing one. All clubs must close, in-
cluding bowling alleys and pool
rooms. No society, club or organi-
zation meeting can be held, not
even at homes.

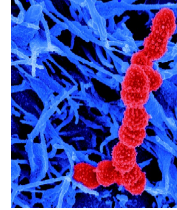
**Spanish Flu (H1N1) Killed 500 million People World-Wide from 1918 to 1920
& 675,000 in the US Most From Secondary Bacterial Infections
No Vaccines or Antibiotics!!**

Bacterial Pneumonia Caused Most Deaths in 1918
Influenza Pandemic

INFLUENZA
Frequently Complicated With
PNEUMONIA
A PRESENT AT THE TIME OF THE PANDEMIC
THE NATURE OF THE DISEASE WAS SUCH
YOU MUST DO THE SAME
IF YOU HAVE BEEN AND ARE SUFFERING AND
BECAUSE OF THE DANGER OF THE
GO HOME AND GO TO BED UNTIL YOU ARE WELL
The American People are advised to
be especially careful of their health
and to avoid all public places
where the disease is prevalent
**HELP US TO KEEP CHICAGO THE
HEALTHIEST CITY IN THE WORLD**
JOHN BILL ROBERTSON
COMMISSIONER OF HEALTH



Streptococcus pneumoniae

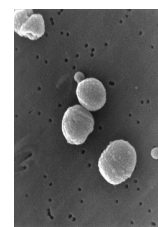
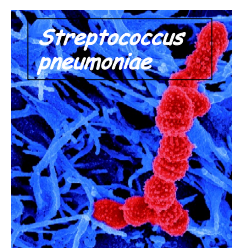
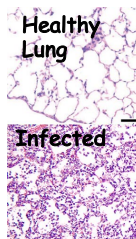


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Frederick Griffith & The Transforming Principle *The First Genetic Engineering Experiment (unintentional!)*



1879-1941



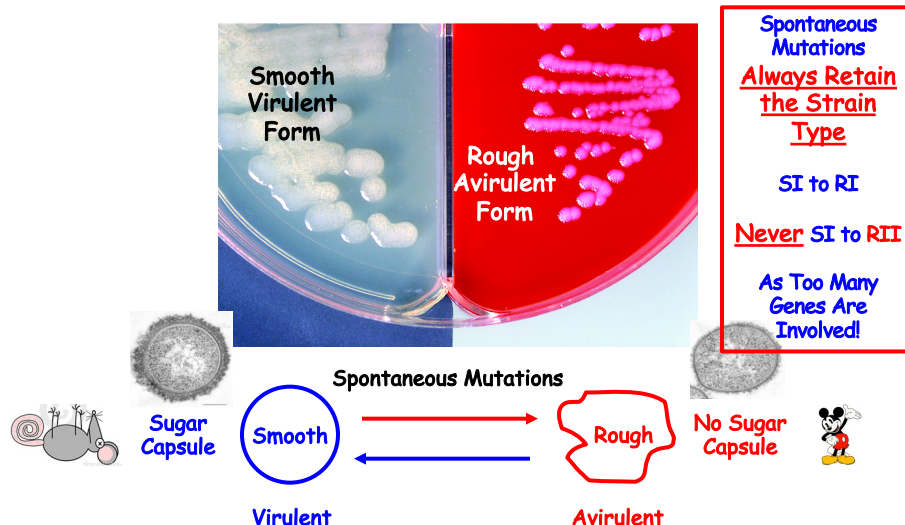
Note:
Different
Strains of
*Streptococcus
pneumoniae*
Exist in
Nature
Type I, II,
etc.
Taken From
Patients

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

10

Griffith - J. Hygiene,
1928

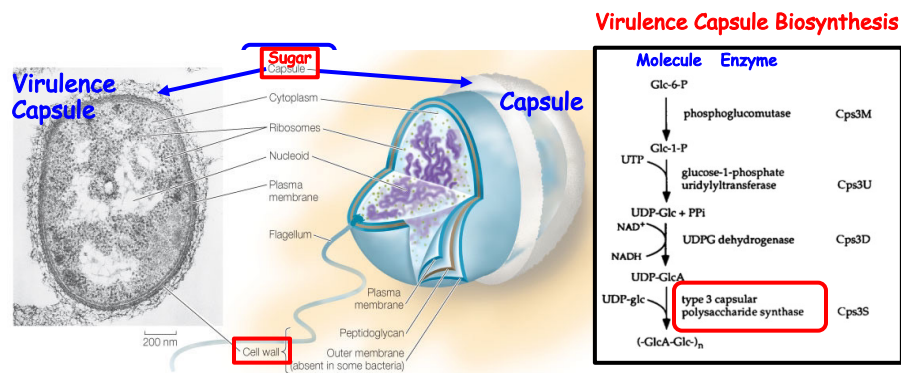
Smooth Virulent Bacteria Can **Mutate** To Rough Avirulent Bacteria & Vice Versa!



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Streptococcus pneumoniae

Flash Forward to 2023!
50,000 Deaths/Year in the USA



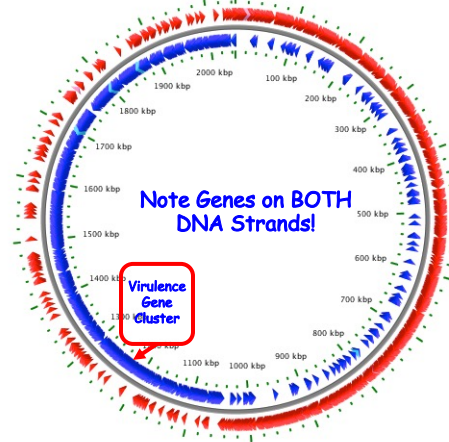
J. Exp. Med. 181, 973, 1995

***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

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Streptococcus pneumoniae Genome Has Been Sequenced!

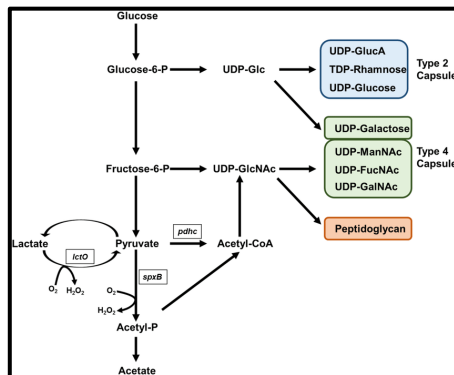


2,160,837 bp and 2,236 Genes
At Least 13 Genes Specify Capsule Formation
What Happens If One of These Genes Is Mutated?

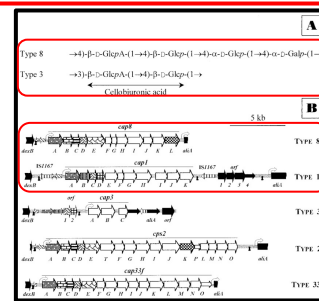
Science 293,498 (2001)

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Streptococcus pneumoniae Virulence Capsule Sugars - Different Strains Have Different Sugars



Genes Clustered Next to Each Other in the Genome



Different Sugars - Different Sugar Genes!



Mutations in any one gene prevents capsule from forming S to R but strain remains the same

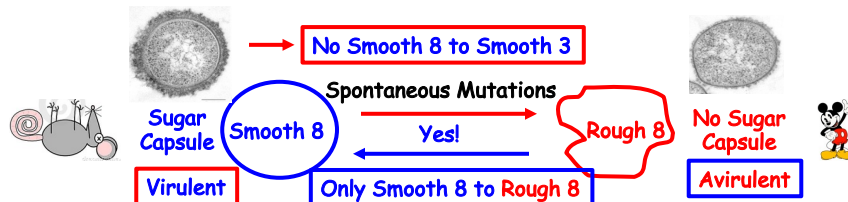
1. Different Strains Have Different Sugars Encoded By Shared & Distinct Genes Involved in Capsule Sugar Synthesis
2. Cannot Mutate From One Strain to Another - Too Many Genes Involved
3. All Capsule Sugar Genes (i.e., the Entire Cluster) Must Be Transferred For One Strain to Change In to a Different Strain (i.e., a Large Piece of DNA to change SI to SII)

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Figure 1. Phenotypic and genomic differences between Type 8 and Type 3.

(A) Phenotypic differences: Type 8 (Smooth Virulent Form) shows a smooth, virulent form, while Type 3 (Rough Avirulent Form) shows a rough, avirulent form.

(B) Genomic differences: The genomic maps show the capsular polysaccharide (cap) gene cluster. Type 8 has a 5 kb cluster with genes *cap1*, *cap2*, and *cap3*. Type 3 has a 5 kb cluster with genes *cap1*, *cap2*, and *cap3*. The *cap1* gene in Type 3 is truncated compared to Type 8.




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

Method:


CONTROLS

Live SI



Living S strain (virulent)


Live RII



Living R strain (non-virulent)


BOILING KILLS SMOOTH CELLS

Dead SI




Dead S strain (heated)

DEAD SI + LIVE RII




Mix: Living R strain
Dead S strain


Results:




Mouse dies
Has living S strain



Mouse lives
No S strain



Mouse lives
No living S strain

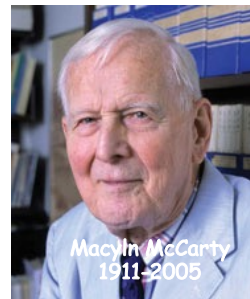
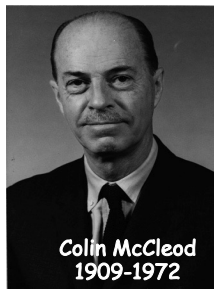


Live SI!!!!
Mouse dies
Has living S strain

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

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



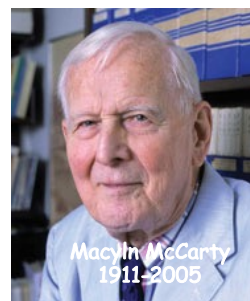
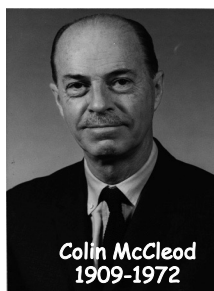
DNA is the Genetic Material!

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

J. Exp. Med., 1944

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

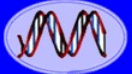


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J. Exp. Med., 1944

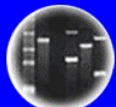
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STUDIES ON THE CHEMICAL
NATURE OF THE SUBSTANCE
INDUCING TRANSFORMATION
OF PNEUMOCOCCAL TYPES

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.

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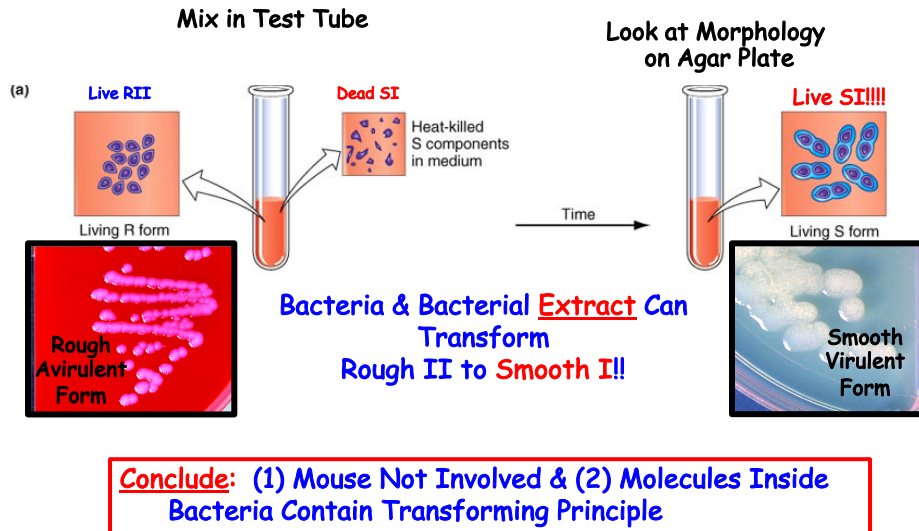
Avery et al. Questions?

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!!!

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

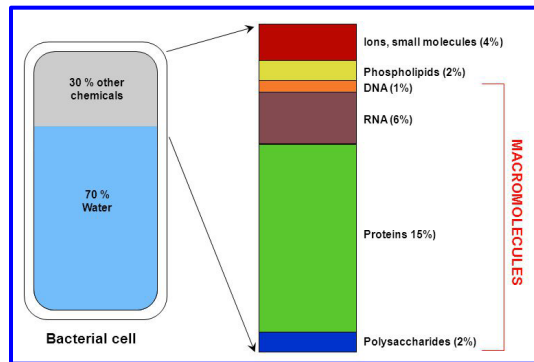


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What Are the Major Chemical Components of a Bacterial Cell? *What Could Be the Transforming Principle?*

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

2. How Test Hypothesis?

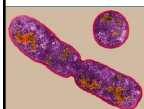


Monomers

SUGARS
FATTY ACIDS
AMINO ACIDS
NUCLEOTIDES

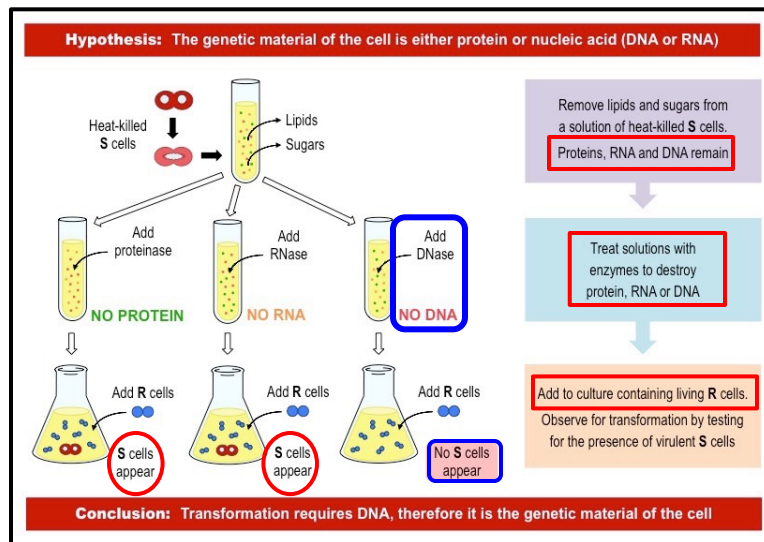
Polymers

POLYSACCHARIDES
FATS, LIPIDS, MEMBRANES
PROTEINS
NUCLEIC ACIDS



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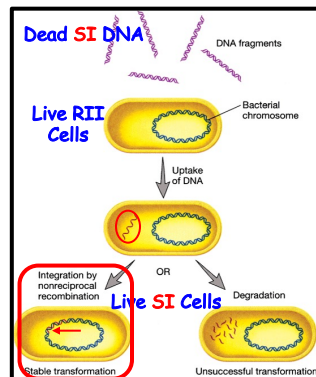
The Critical Experiment by Avery et al. Showing That DNA is the Genetic Material



No DNA - No Transformation!

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



Predictions	Results
Replication	Yes
Phenotype	Yes
Stable	Yes

Cell Processes

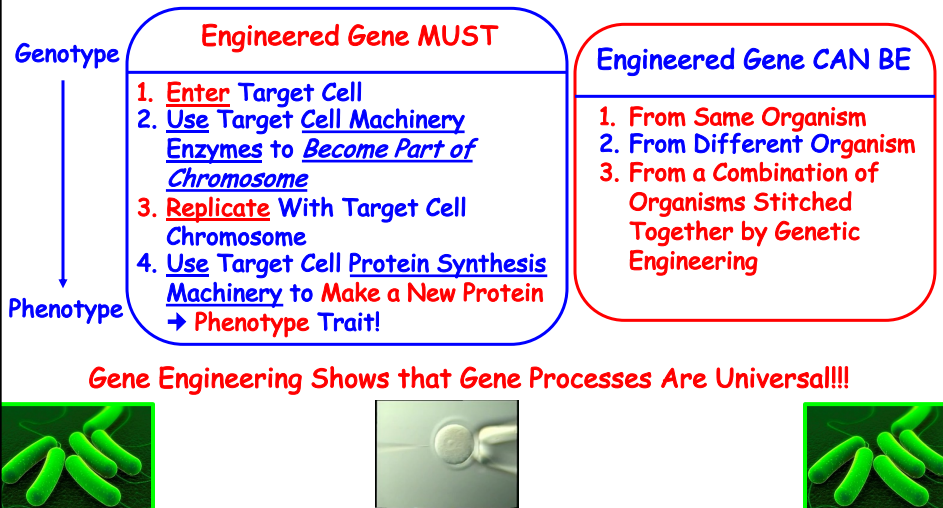
1. Large SI DNA Taken Up By RII-Cells & Incorporated Into Chromosomes
2. SI Gene Cluster Transcribed Into SI mRNAs
3. SI mRNAs Translated Into Smooth I Proteins
4. Smooth I Proteins Helps Construct Sugar Capsule and Protects Bacteria From Antibodies
∴ Cells Virulent

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!

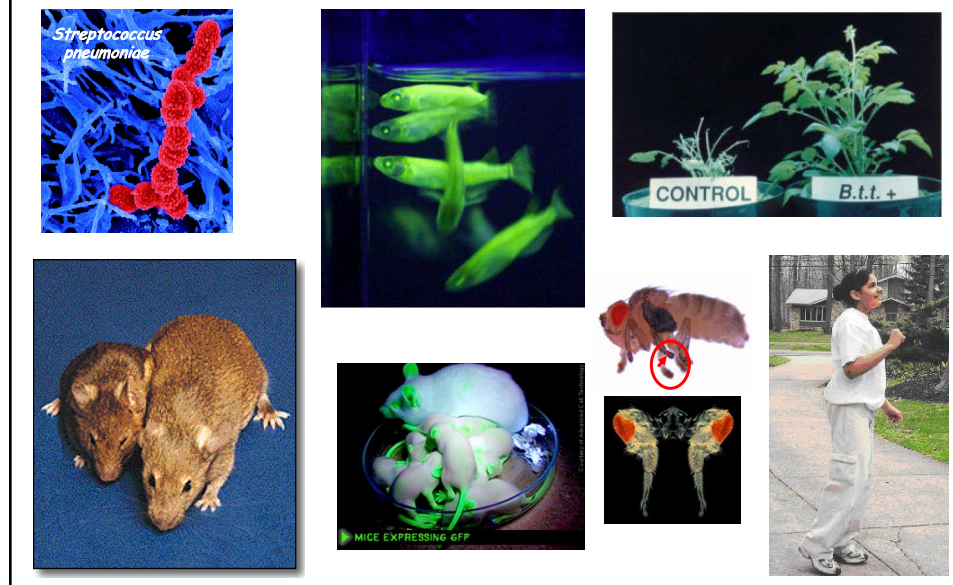
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Genetic Engineering/Transformation Involves Incorporating Engineered DNA or Genes Into the Chromosomes of Different Organisms

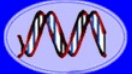


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All Organisms Can Be Transformed!! Genetic Engineering Has Come a Long Way Since Griffiths Experiments in 1928!!



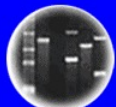
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Plants of Tomorrow

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

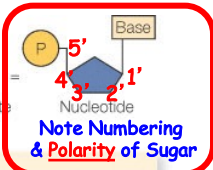
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There Are Four Different Nucleotides in DNA

The base may be either a pyrimidine or a purine.

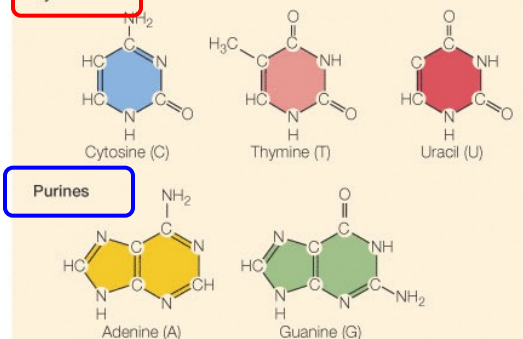
Base + Ribose or deoxyribose = Nucleoside + Phosphate = Nucleotide

Pyrimidines



Note Numbering
& Polarity of Sugar

Purines



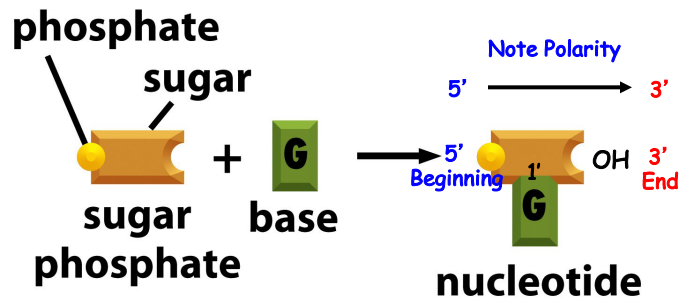
Cytosine (C) Thymine (T) Uracil (U)

Adenine (A) Guanine (G)

Polarity of Sugar Leads to Polarity of DNA (and RNA) - 5' to 3'

30

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

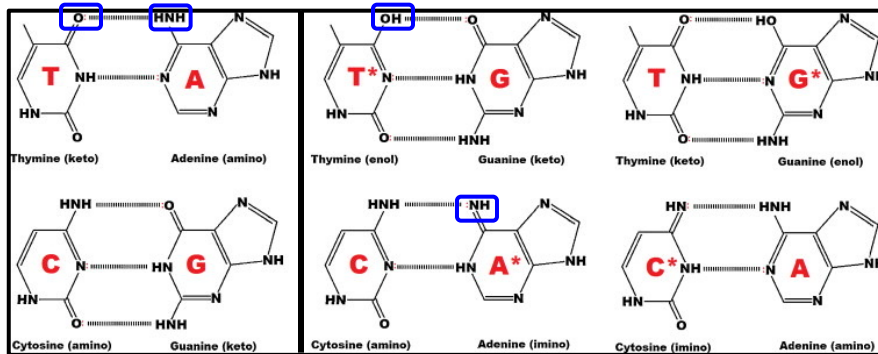
Figure 1-2a *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

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Tautomers Change Base Pairing Rules!!!

Normal Forms - Keto & Amino

"Mutant" Forms - Enol & Imino

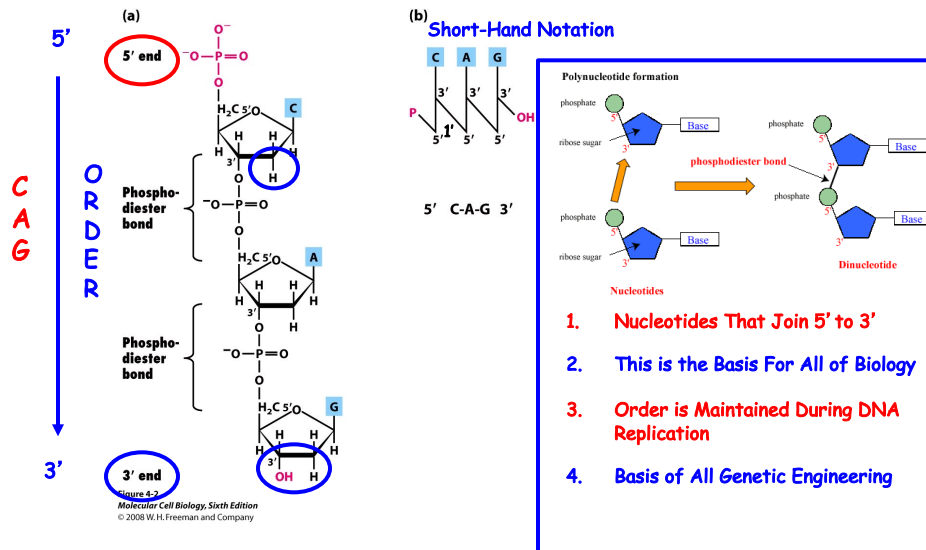


And Lead To Mistakes in DNA
Replication & Mutations → Genetic
Diversity
Chemistry Leads to Biology!!



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Polarity Defined By Sugars & Order Specified By Bases



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Clues to the Double Helix-Chargaff's Rules

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

Organism	Percentage of Base in DNA				Ratios	
	A	T	G	C	A:T	G:C
<i>Staphylococcus afermentans</i>	12.8	12.9	36.9	37.5	0.99	0.99
<i>Escherichia coli</i>	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
<i>Caenorhabditis elegans</i> *	31.2	29.1	19.3	20.5	1.07	0.96
<i>Arabidopsis thaliana</i> *	29.1	29.7	20.5	20.7	0.98	0.99
<i>Drosophila melanogaster</i>	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
<i>Mus musculus</i> (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

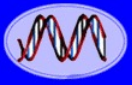
34

Clues to the Double Helix-Chargaff's Rules


Purines = Pyrimidines




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DNA
Genetic Code of Life




Entire Genetic Code
of a Bacteria



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

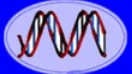
J. Of Experimental Medicine, 79 (2), 137-158 (1944)

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.

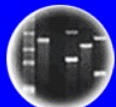
36




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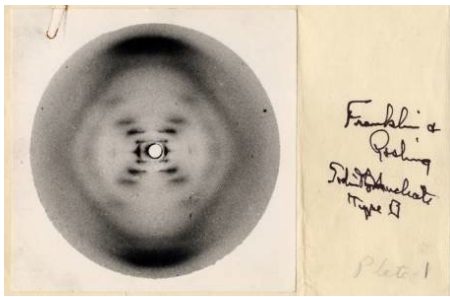
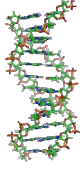
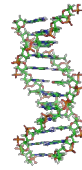
Cloning: Ethical Issues
and Future Consequences



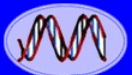
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Reflections on *The Double Helix*

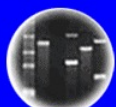
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DNA Fingerprinting



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and Future Consequences



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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 inter-atomic 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

38

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

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
($4^n = n \text{ \# base combinations} = \text{Diversity}$)
- DNA has dimensions (Know # bp Know Length: 20Å diameter, 3.4Å/bp, 10bp/turn)
9. Sequence = Biology

Watson and Crick, Nature, 1953

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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

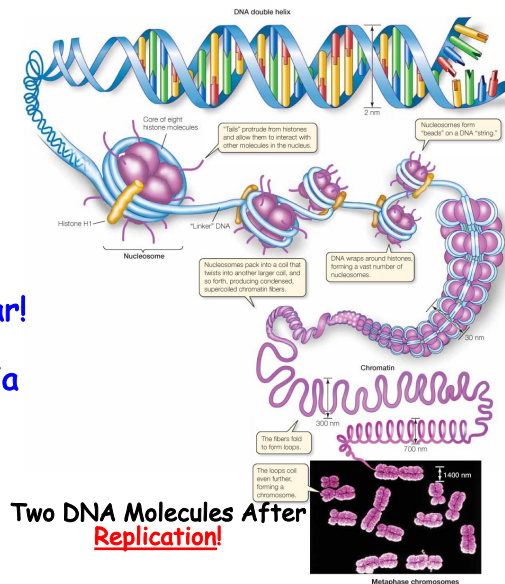
5. Polymerase Chain Reaction

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

DNA in Human & Eukaryotic Chromosomes is Linear!

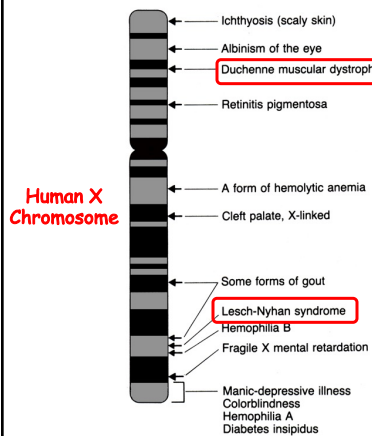
DNA in Most Bacteria is Circular!



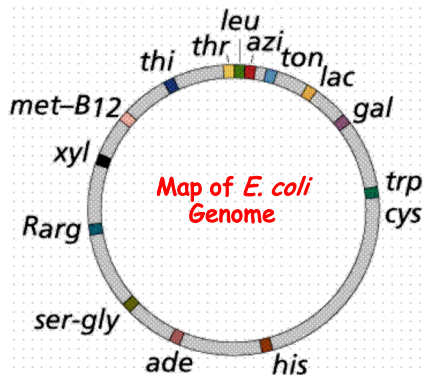
Two DNA Molecules After Replication!

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Genes Reside at Specific Locations That Can Be Mapped



**Linear DNA
How Know?**

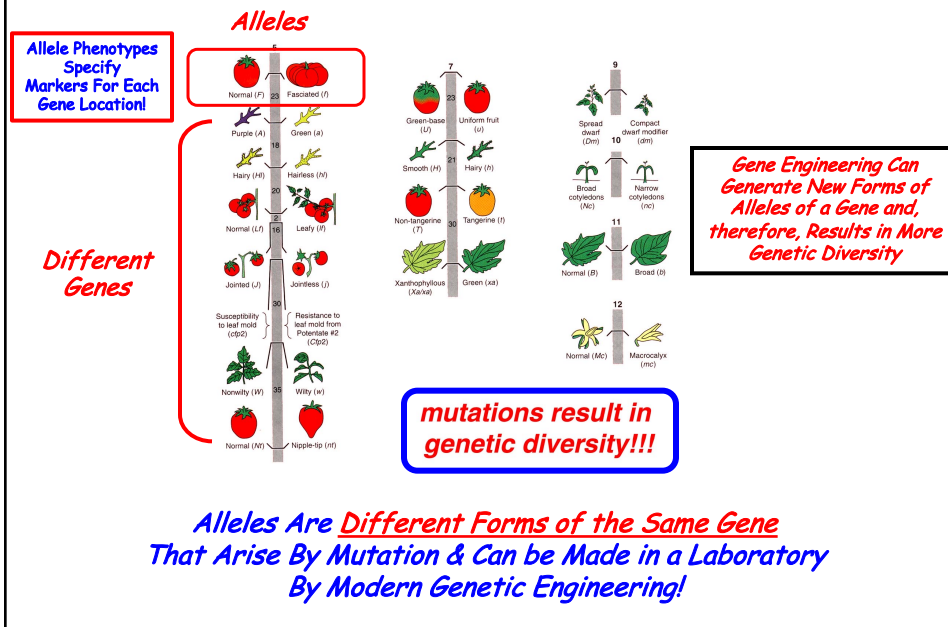


**Circular DNA
How Know?**

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

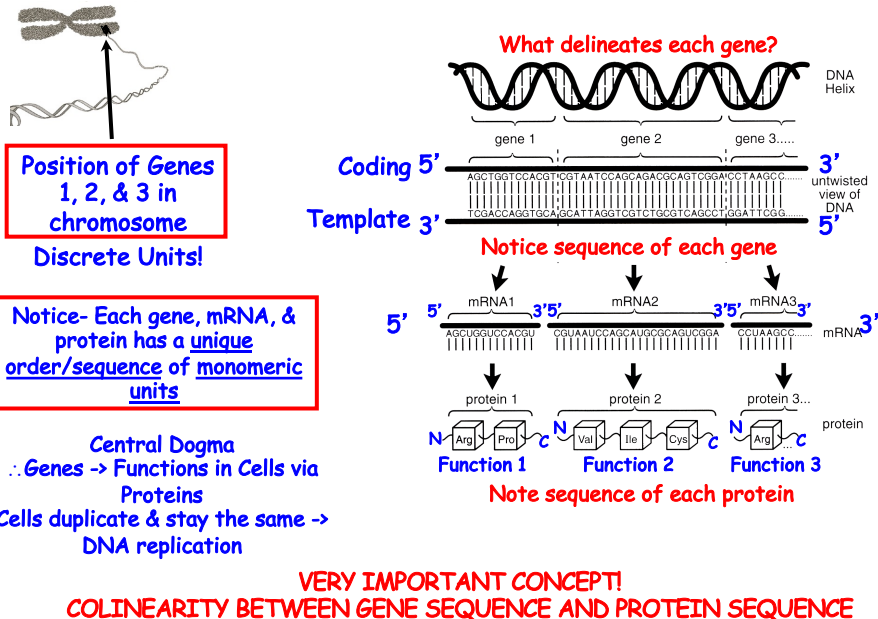
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Alleles Reside at the Same Position on a Chromosome



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A Chromosome Contains Many Genes Operating Independently What is the Evidence?



44

Organization of Genes on Human Chromosome 22

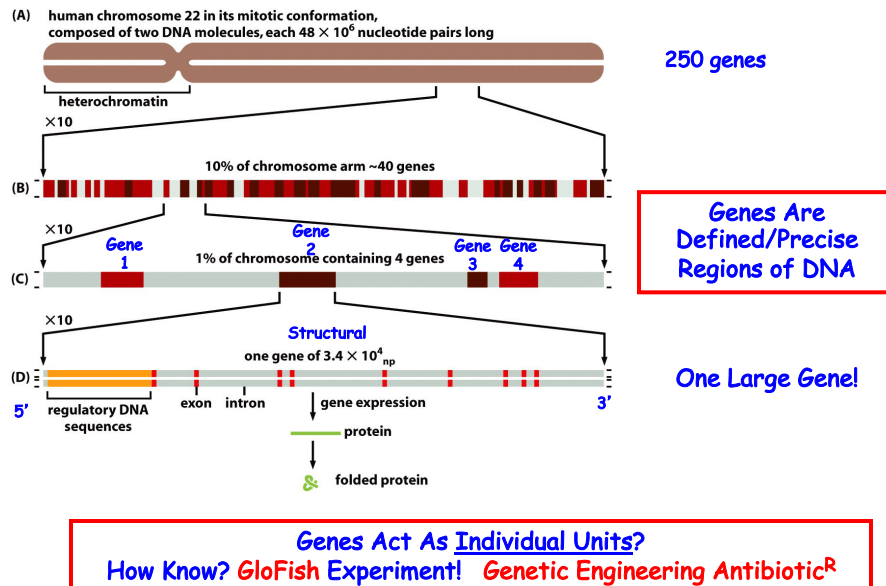


Figure 4-15 Molecular Biology of the Cell (© Garland Science 2008)

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A Conceptualized Gene

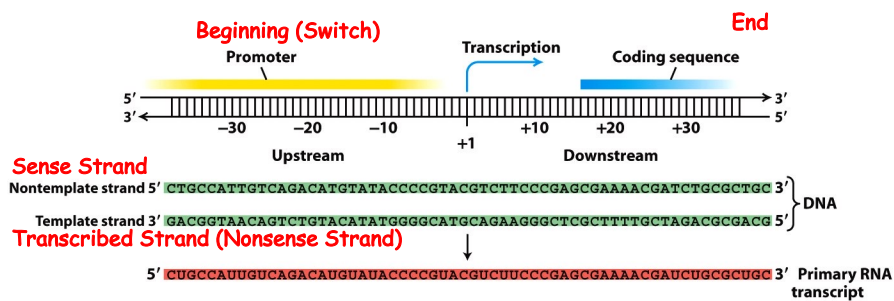
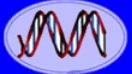



Figure 4-10b
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company

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

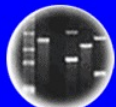
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DNA
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DNA Fingerprinting



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Plants of Tomorrow

A "Simple" Gene Reviewed

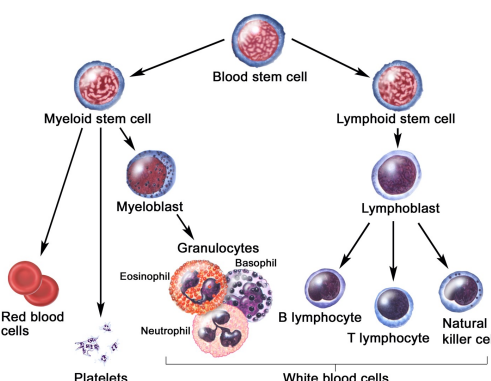
1. Sense Strand = Genetic Code
2. Sense Strand = 5' → 3' Direction (all DNA sequences specified 5' → 3')
3. Anti Sense Strand = Complement of Sense Strand & is Transcribed Strand
4. mRNA = Same Sequence As Sense Strand & Complementary to AntiSense Strand
5. mRNA = 5' → 3'
6. Switch Turns Gene On - Not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

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

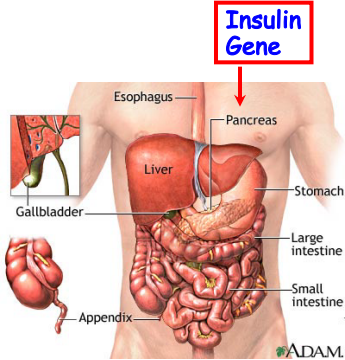
"Everything" Follows the Double Helix & Its Rules - Anti-parallel Chains & Complementary Base Pairing!

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



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All Good has certain rights

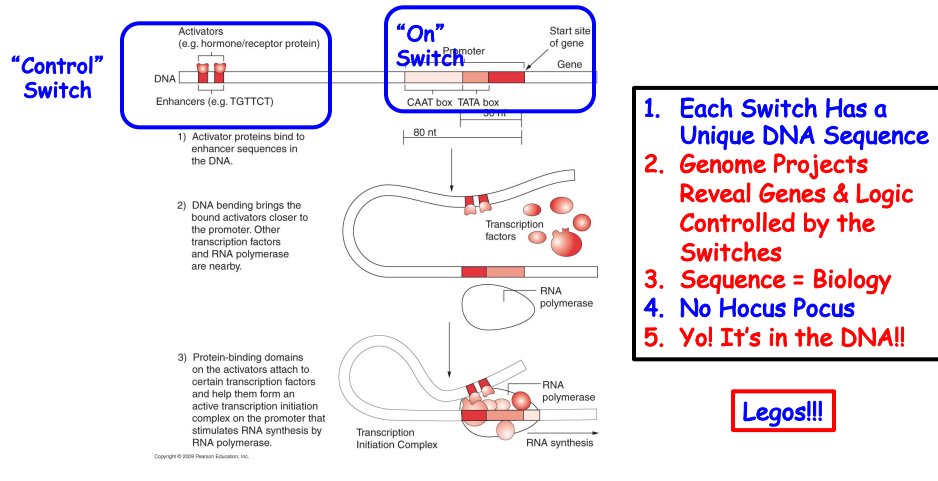


Insulin Gene

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Control Switches Are Unique DNA Sequences & Can Be Cloned

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



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

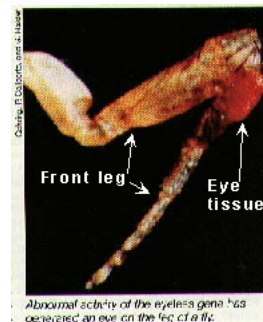
Eye Gene



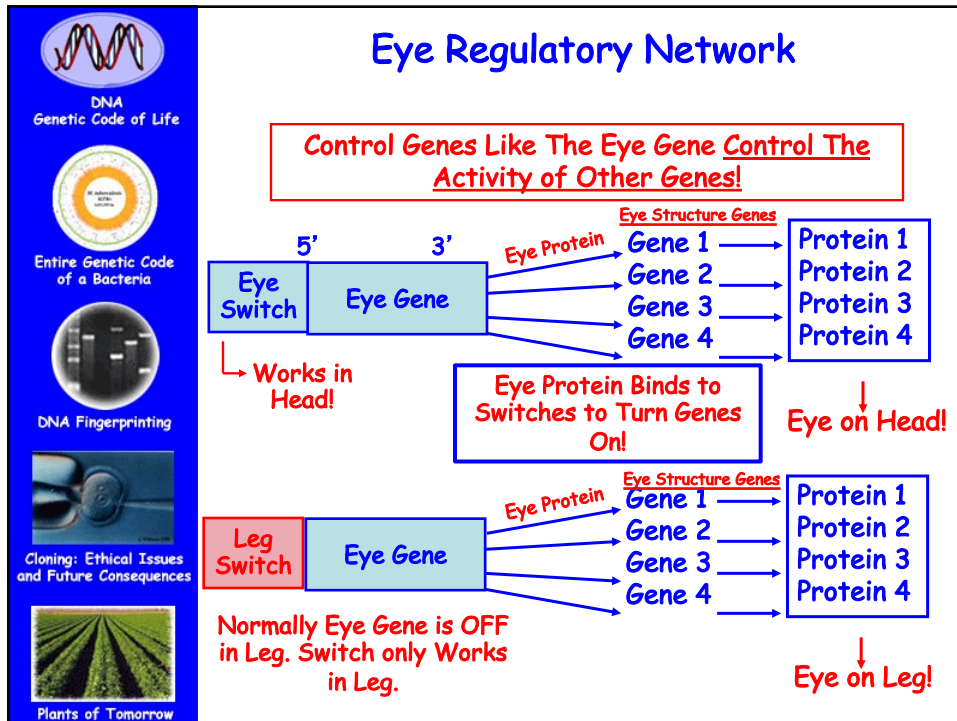
Replace the Head Switch With the Leg Switch by Genetic Engineering



**Eye Gene
+
Leg Switch**



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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!

Left Sidebar:

- DNA Genetic Code of Life
- Entire Genetic Code of a Bacteria
- DNA Fingerprinting
- Cloning: Ethical Issues and Future Consequences
- Plants of Tomorrow

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