


DNA  
Genetic Code of Life




Entire Genetic Code  
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues  
and Future Consequences



Plants of Tomorrow

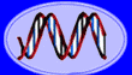
**HC70A, SAS70A, & PLSS599**  
**Winter 2022**  
**Genetic Engineering in Medicine,  
Agriculture, and Law**

**Professors Bob Goldberg, John Harada, &  
Channapatna Prakash**


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

**UCLA**      **TUSKEGEE**      **UCDAVIS**  
UNIVERSITY      UNIVERSITY OF CALIFORNIA

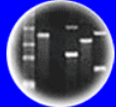
1




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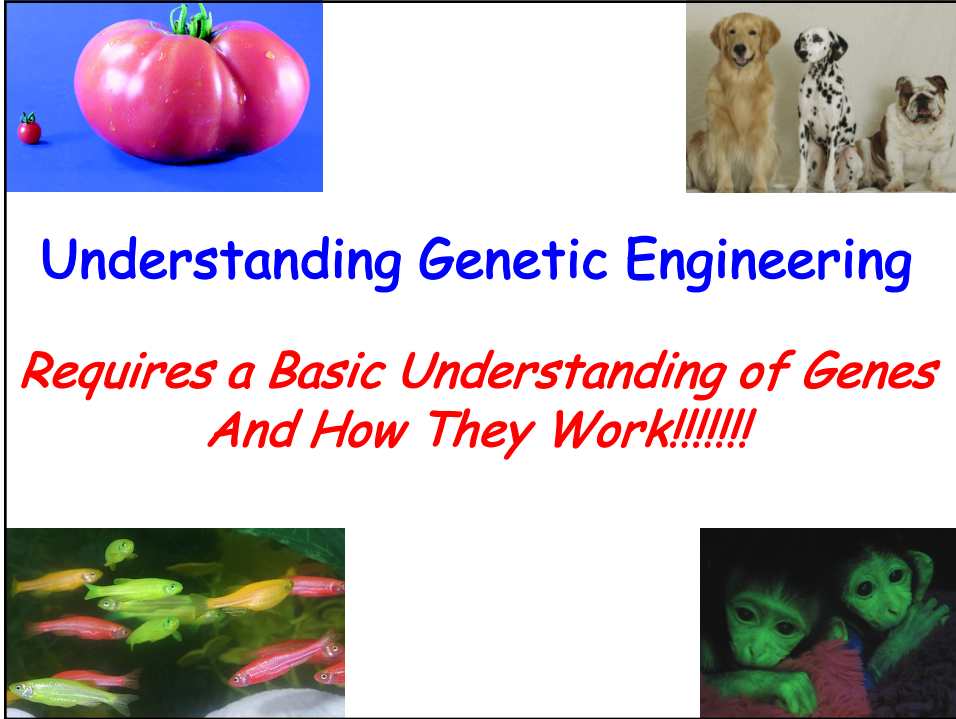


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**THEMES**  
**Gene Structure & Function**  
**Part One**

- 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



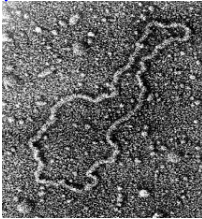
## Understanding Genetic Engineering

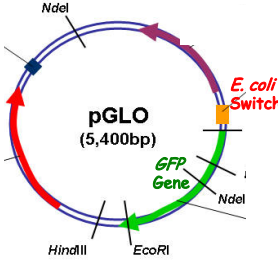
*Requires a Basic Understanding of Genes  
And How They Work!!!!!!*

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### What Are the *DNA Conceptual 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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### Other Experiments We Discussed Showing That DNA is the Genetic Material



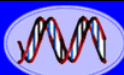



May 20, 2010


#### Researchers Say They Created a ‘Synthetic Cell’

By NICHOLAS WADE  
The genome pioneer J. Craig Venter has taken another step in his quest to create synthetic life, by synthesizing an

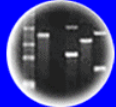
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### What Were Considered the Properties of a **Gene** **BEFORE** It Was Known That DNA Was the Genetic Material - In the 1920s to 1940s?

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

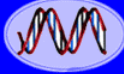
*For First Half of 20<sup>th</sup> Century Proteins Were Considered the Genetic Material*

- 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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DNA Fingerprinting

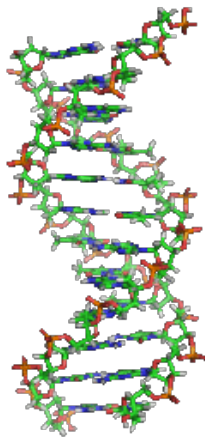


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## How Was DNA Shown to be the Genetic Material?



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

150 Years Ago





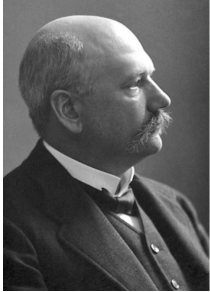




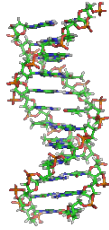
*But..... The Function of DNA Was Not Understood Until  
75 years Later in 1944!!!*

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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**

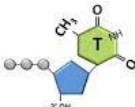


**Nobel Prize For Medicine in 1910**

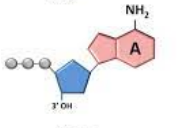





**G**



**T**

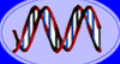


**A**




**C**

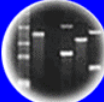
**Purine Deoxyribonucleotides**      **Pyrimidine Deoxyribonucleotides**




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**Evidence That DNA Is the Genetic Material Starts With Pneumonia**



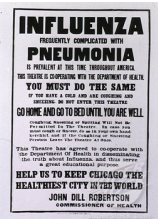
**TO STOP SPREAD OF INFLUENZA STAY AT HOME**  
Amuse Yourself With One of the Best **Player Pianos** We Sell for One Hundred Dollars Less Than Same Grade Offered Elsewhere

**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 prevent a further spread of the Spanish influenza epidemic, is a sweeping one. All clubs must close, including bowling alleys and pool rooms. No society, club or organization meeting can be held, not even at homes.

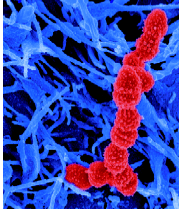
**Spanish Flu (H1N1) Killed 500 million People World-Wide from 1918 to 1920 & 675,000 in the US Most From Secondary Bacterial Infections**  
**No Vaccines or Antibiotics!!**

**Bacterial Pneumonia Caused Most Deaths in 1918 Influenza Pandemic**





*Streptococcus pneumoniae*



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## Frederick Griffith & The Transforming Principle

*The First Genetic Engineering Experiment (unintentional!)*

Frederick Griffith

1879-1941

*Streptococcus pneumoniae*

Smooth

Rough

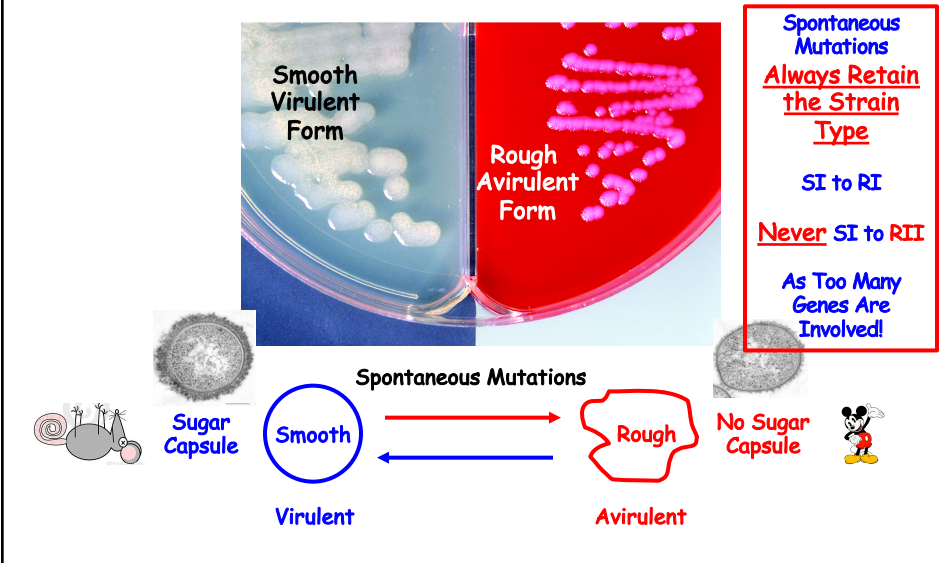
Note:  
Different Strains of *Streptococcus pneumoniae* Exist in Nature  
Type I, II, etc.  
Taken From Patients

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

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Griffith - J. Hygiene,  
1928

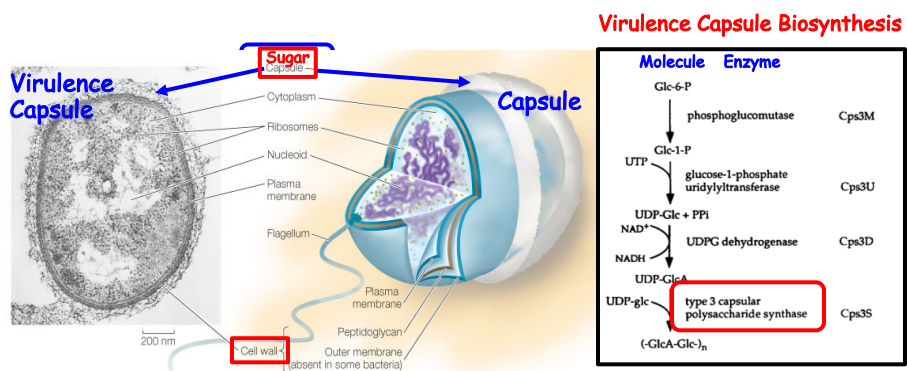
## Smooth Virulent Bacteria Can Mutate To Rough Avirulent Bacteria & Vice Versa!



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## Streptococcus pneumoniae

Flash Forward to 2021!  
50,000 Deaths/Year in the USA



**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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## Can Mutate Smooth to Rough But Never Smooth Strain X to Smooth Strain Y

**Smooth Virulent Form**

**Rough Avirulent Form**

**Smooth 8 & Smooth 3**

Type 8 →→3-β-D-GlcpA-(1→4)-β-D-Glcp-(1→4)-α-D-Galp-(1→

Type 3 →→3-β-D-GlcpA-(1→4)-β-D-Glcp-(1→

←←3-Cellobiuronic acid

**Have Different Sugars** 5 kb

**No Smooth 8 to Smooth 3**

**Spontaneous Mutations**

**Yes!**

**Only Smooth 8 to Rough 8**

**No Sugar Capsule**

**Virulent**      **Avirulent**

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## The Griffith Experiment (1928)

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

**Method:**

**CONTROLS**

**Live SI** (S)

Living S strain (virulent)

**Live RII** (R)

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

↓

Mouse dies

Has **living S strain**

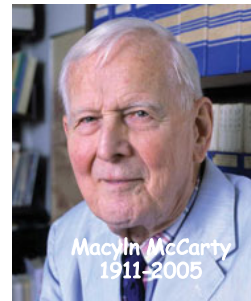
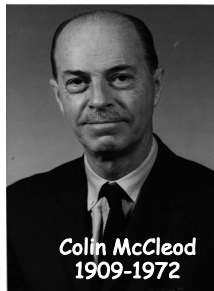
**RII Transformed Into SI**

**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?**

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



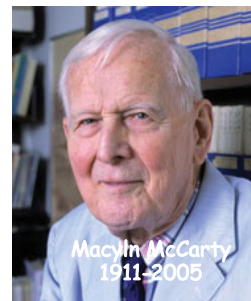
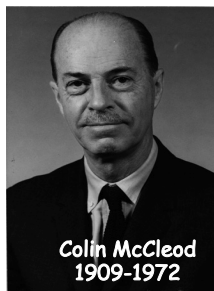
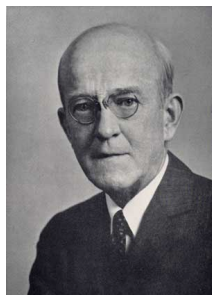
**DNA is the Genetic Material!**

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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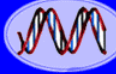


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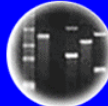
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STUDIES ON THE CHEMICAL  
NATURE OF THE SUBSTANCE  
INDUCING TRANSFORMATION  
OF PNEUMOCOCCAL TYPES

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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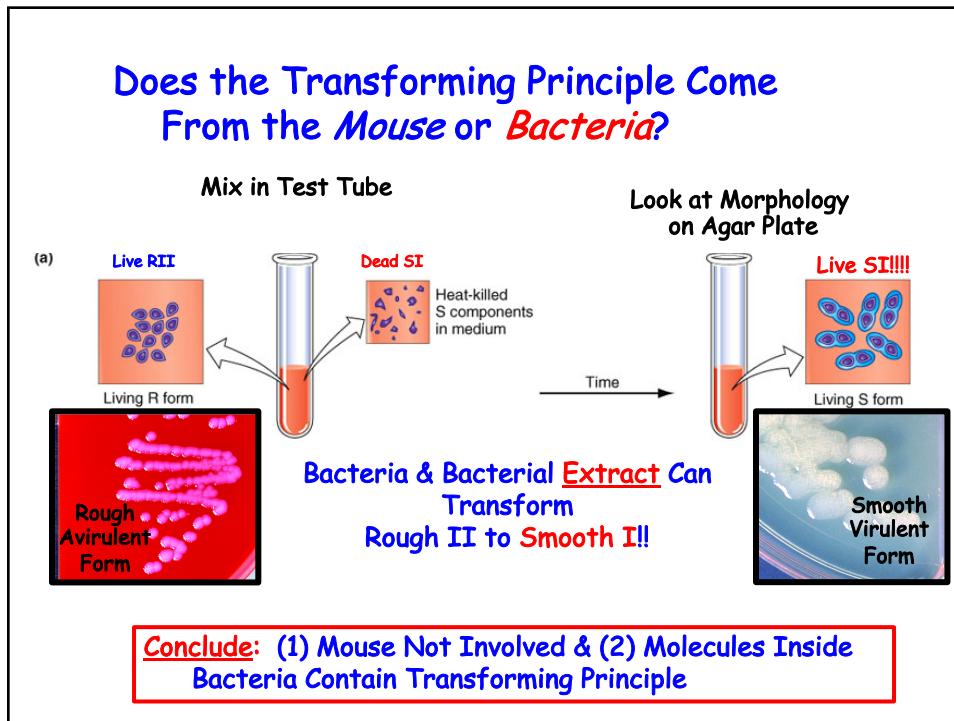
## Avery et al. Questions?

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!!!

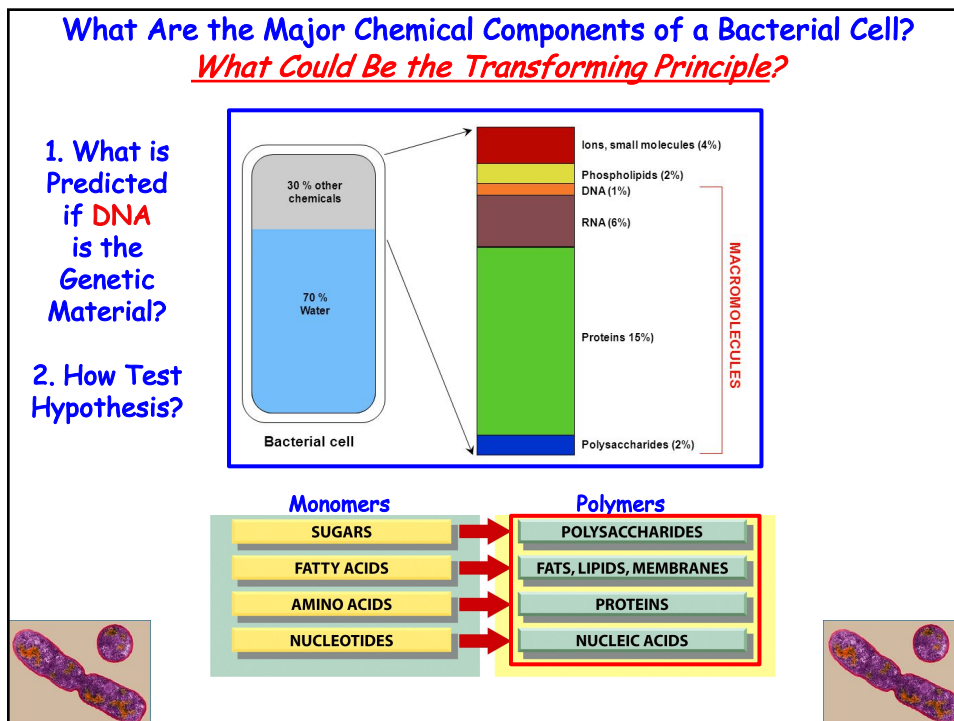
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## Does the Transforming Principle Come From the *Mouse* or *Bacteria*?



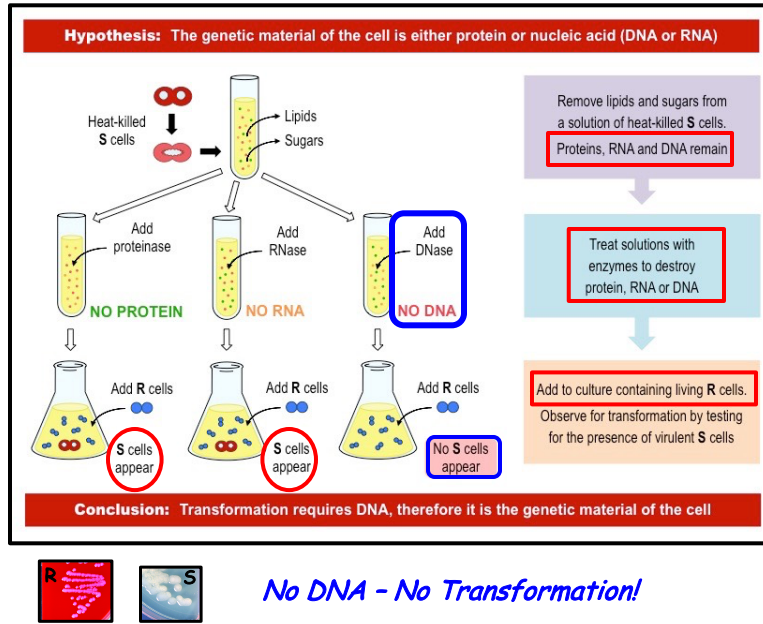
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## What Are the Major Chemical Components of a Bacterial Cell? *What Could Be the Transforming Principle?*



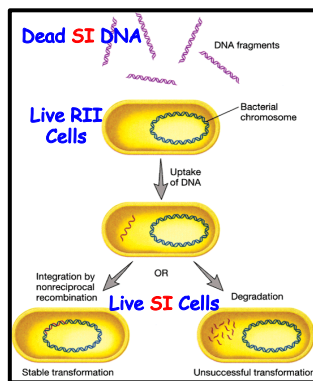
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## The Critical Experiment by Avery et al. Showing That DNA is the Genetic Material



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



<u>Predictions</u>	<u>Results</u>
Replication	Yes
Phenotype	Yes
Stable	Yes

### Cell Processes

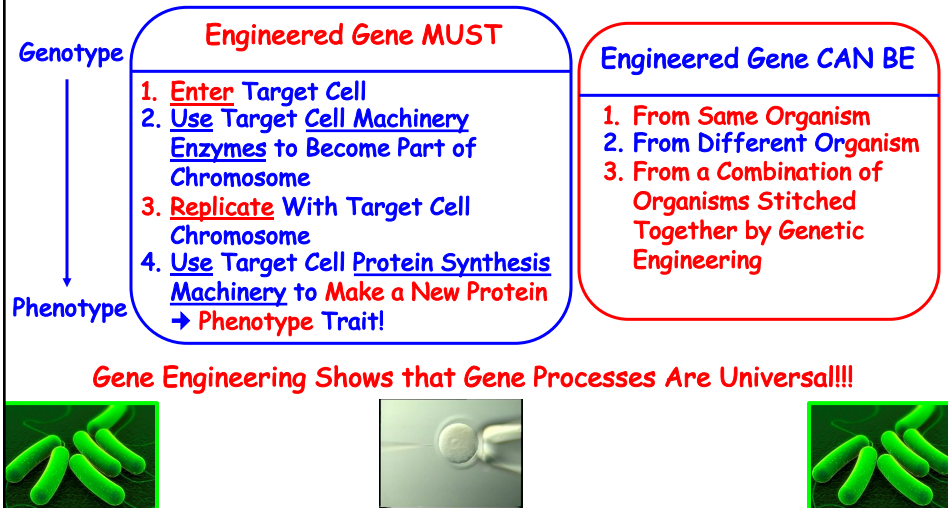
- SI DNA Taken Up By RII-Cells & Incorporated Into Chromosomes
- SI Genes Transcribed Into SI mRNAs
- SI mRNAs Translated Into Smooth I Proteins
- Smooth I Proteins Helps Construct Sugar Capsule and Protects Bacteria From Antibodies  
∴ Cells Virulent

- DNA Satisfies Criteria For Being the Genetic Material
- Replicates
- Directs Production of Strain/Capsule Type
- In All Progenitor Cells

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

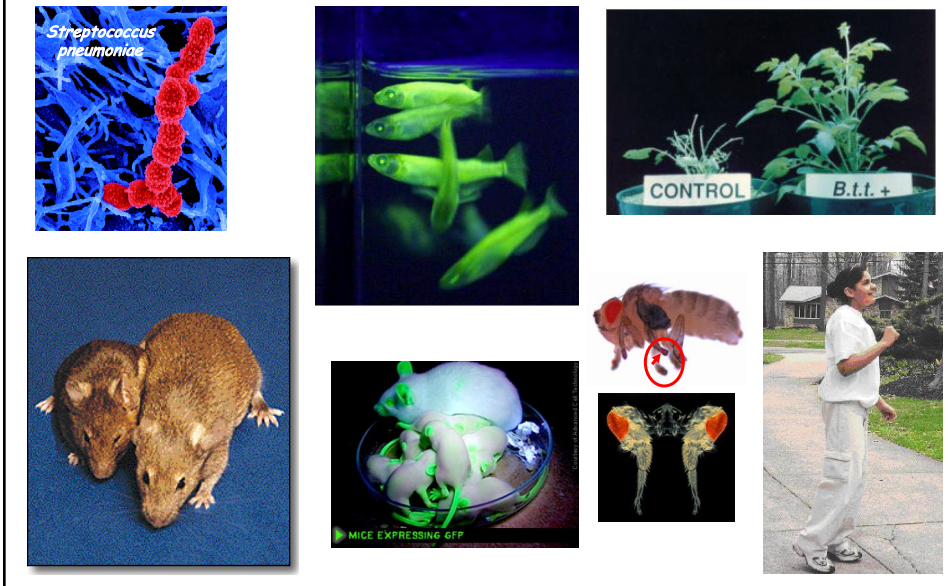
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## Genetic Engineering/Transformation Involves Incorporating Engineered DNA or Genes Into the Chromosomes of Different Organisms



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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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## What is A Gene?

**Begin** 5'

Sequence of Nucleotides Coding DNA Strand (Coding Strand)

```

TGAAGTCCAAAAAATAGCA
GTTTGGTGTGGGTTTAGG
TAGCAATATTTGGGCTTT
TTTAGGTTCCGGTTTGGGTT
ATTTGAGTGTTCACATTTGA
AATTTCCGGTTCATCTTCG
TGGGTGCCAGTGGCGTGA
GTTTCCCGGTTTCGTCAACT
TAGGTTTAGGGTTTACCAG
TTAGGTTTAGGGTTTGGAT
GGCGGCATTCTCATGTTTG
AATCAAGCCCTGAAATCAAA
TGGGTGCCGGTGGCGTGA
CGTCCCGGTTCCGTCAACT
ATCAGTACCCATGTTGGGA
TGACGTCATGACACGAAA
AAAAAATAGGAATCGACCC
AGAAAAGGGGGTGGCCATT
ACTATCCGTACACAAAAC
ATTTTTTGGCGGGTGGCC
ATAATAGATTTTTCCCTTGT
CCTTTCCATGTTCAAGTACC
TTTCTCATGTTTGAAGTCAA
CCTGAAATCCAAAAAATAG
CAGTGGCGTGAACATTTGG
GATACGTCATGACACGAAA
CATGTTTGGATTTTTTCCG
AGACCCAAAAAATAGTCT
GAATCGACCCTTTCCATGT
GGCGACCATTTCTCTGTTT
AAAAAAGCCGTAATATCTA
GTGAGTGTGCCAGTGGCGTA
TCGTTCCCGGTTCCCTCAC
GTTCAAGTACCCATGTTGGG
TTGGAGTCAAGAAACAAA
CAAAAAATAGCAATCGACC
AGAAAATGGAGGGCGCCAT
CTGACCGTAAAAAAGAGCT
TTTTTCCGCGGGTGGCCA
AAAAATAGTCCGTTCCCGTT
TTTTCCATGTTCAATACCCA
TCTCATATTTGACGTCAAAG

```

**End** 3'

**The  $\beta$ -Globin Gene**

Blood Protein Carries Oxygen to All Genes From Lungs  $\Leftrightarrow$  Energy

A Gene is a Unique Sequence of Nucleotides Specifying a Function

DNA Sequence = Biology!  
What If Sequence Changed?

SEQUENCE  $\rightarrow$  FUNCTION

Relative to Coding or Sense Strand of Gene

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## Genes and Genomes Can Be Sequenced!

**Water Gilbert**

**Fred Sanger**

DNA sequencing with chain-terminating inhibitors  
(DNA polymerase, nucleotide sequence, heterologous dNTP)  
F. SANGER, S. NICKLEN, AND A. B. COLEMAN  
**PNAS December, 1977**

**A new method for sequencing DNA**  
(DNA chemistry / Maxam's sulfate cleavage / Gilbert's pyrosequencing)  
ALLAN M. MAXAM AND WALTER GILBERT  
**PNAS February, 1977**

**The Nobel Prize in Chemistry 1980**

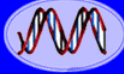
Paul Berg  
Prize share: 1/2

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..."


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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!**

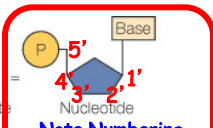
### Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

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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



Note Numbering  
& Polarity of Sugar

Pyrimidines

Purines

NC1=NC(=O)NC(=O)N1  
Cytosine (C)

CC1=CNC(=O)NC1=O  
Thymine (T)

O=C1NC=CC(=O)N1  
Uracil (U)

NC1=NC=NC2=C1N=CN2  
Adenine (A)

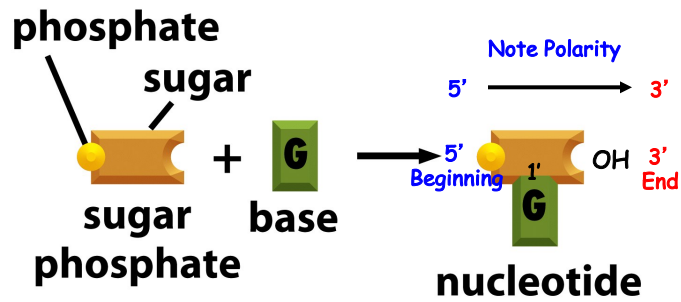
NC1=NC2=C(N=CN2)C(=O)N1  
Guanine (G)

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

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**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

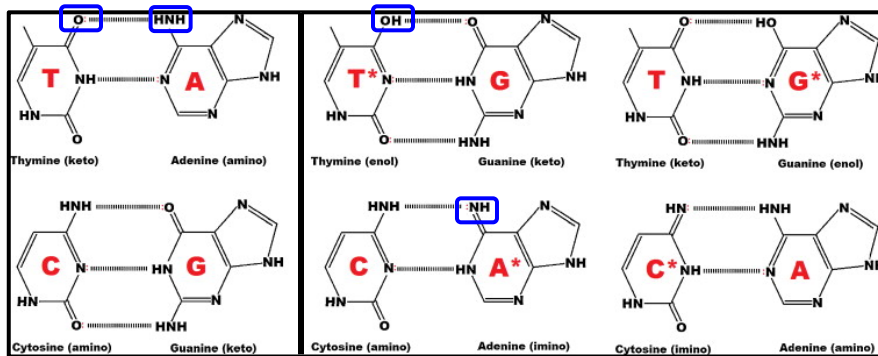
Figure 1-2a *Molecular Biology of the Cell*, Fifth Edition (© Garland Science 2008)

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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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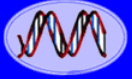


## Clues to the Double Helix-Chargaff's Rules


Purines = Pyrimidines



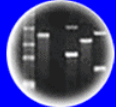
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of a Bacteria




DNA Fingerprinting



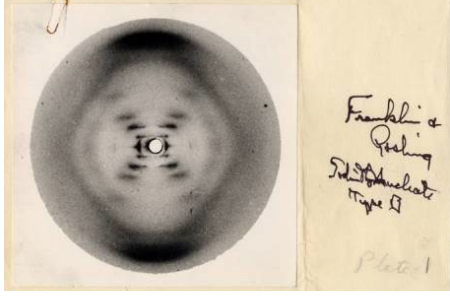
Cloning: Ethical Issues  
and Future Consequences



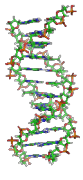
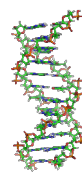
Plants of Tomorrow



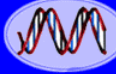
### Reflections on *The Double Helix*




Franklin &  
Wilkins  
type A  
Plate 1

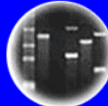
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DNA  
Genetic Code of Life




Entire Genetic Code  
of a Bacteria



DNA Fingerprinting



Cloning: Ethical Issues  
and Future Consequences



Plants of Tomorrow

## 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


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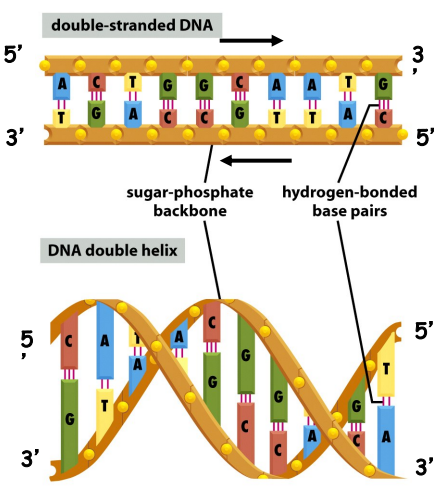
We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

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





DNA double helix

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  
( $4^n = n$  # base combinations = Diversity)

DNA has dimensions (Know # bp Know Length: 20Å diameter, 3.4Å/bp, 10bp/turn)

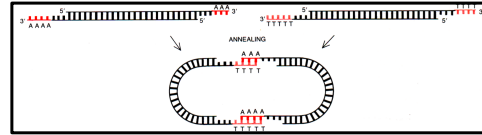
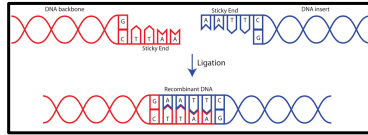
9. Sequence = Biology

Watson and Crick, Nature, 1953

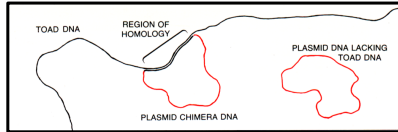
40

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

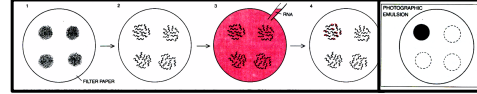
### 1. Annealing Two Two Molecules Together ("Cut & Splice")



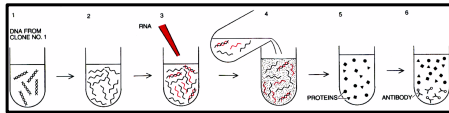
### 2. Heteroduplex Analysis



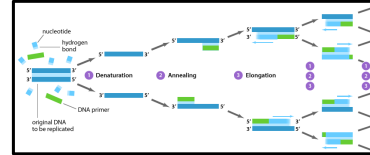
### 3. Colony Hybridization



### 4. Hybrid-Arrested Translation



### 5. Polymerase Chain Reaction

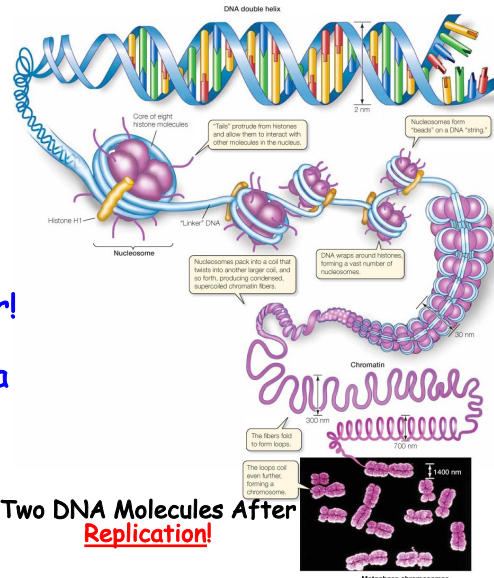


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## A Chromosome Contains One (or Two!!) Continuous DNA Molecule(s)

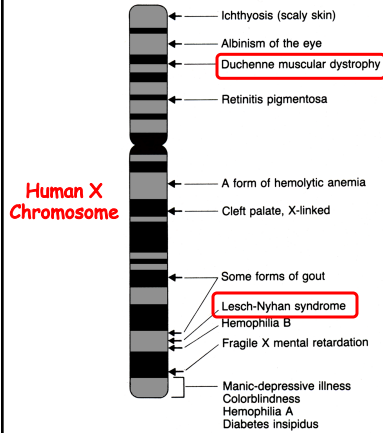
DNA in Human &  
Eukaryotic  
Chromosomes is **Linear!**

DNA in Most Bacteria  
is **Circular!**

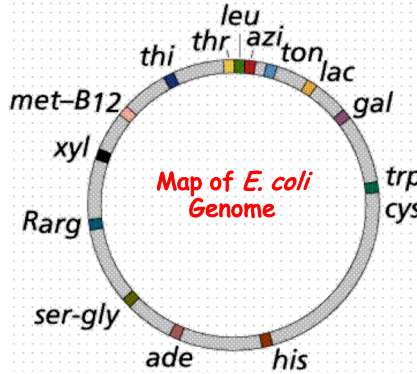


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## Genes Reside at Specific Locations That Can Be Mapped



Linear DNA  
How Know?

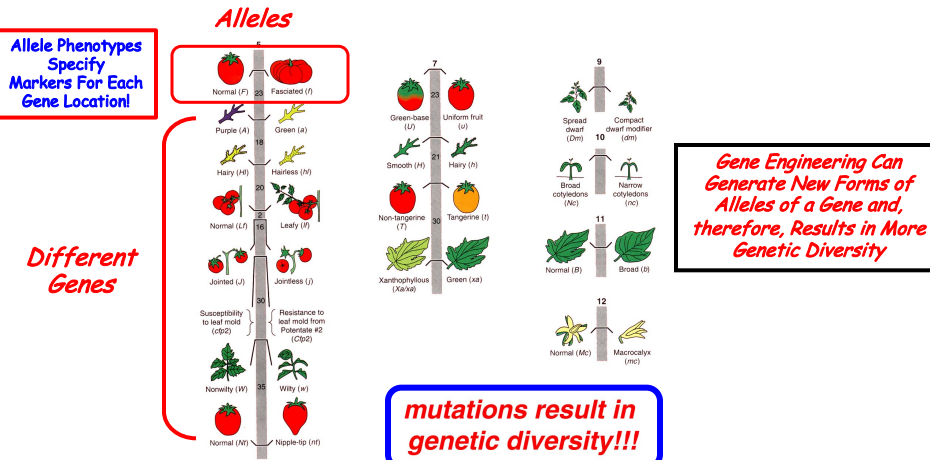


Circular DNA  
How Know?

- 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

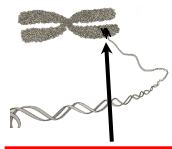


*Alleles Are Different Forms of the Same Gene That Arise By Mutation & Can be Made in a Laboratory By Modern Genetic Engineering!*

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## A Chromosome Contains Many Genes Operating Independently

### What is the Evidence?



**Position of Genes 1, 2, & 3 in chromosome**

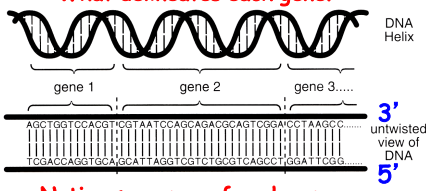
Discrete Units!

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

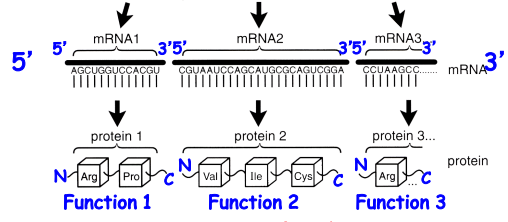
Central Dogma  
∴ Genes → Functions in Cells via Proteins  
Cells duplicate & stay the same → DNA replication

VERY IMPORTANT CONCEPT!  
COLINEARITY BETWEEN GENE SEQUENCE AND PROTEIN SEQUENCE

What delineates each gene?



Notice sequence of each gene

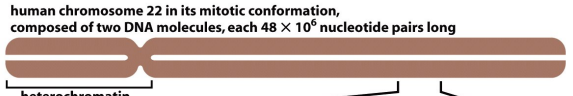


Note sequence of each protein

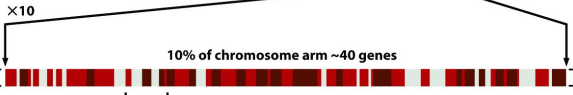
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## Organization of Genes on Human Chromosome 22


(A) human chromosome 22 in its mitotic conformation, composed of two DNA molecules, each  $48 \times 10^6$  nucleotide pairs long



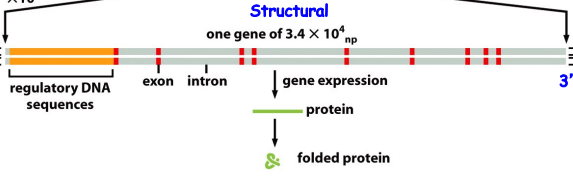
(B) 10% of chromosome arm ~40 genes



(C) 1% of chromosome containing 4 genes



(D) Structural one gene of  $3.4 \times 10^4$  np



250 genes

**Genes Are Defined/Precise Regions of DNA**

One Large Gene!

Genes Act As Individual Units?

How Know? GloFish Experiment! Genetic Engineering Antibiotic<sup>R</sup>

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

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## A Conceptualized Gene

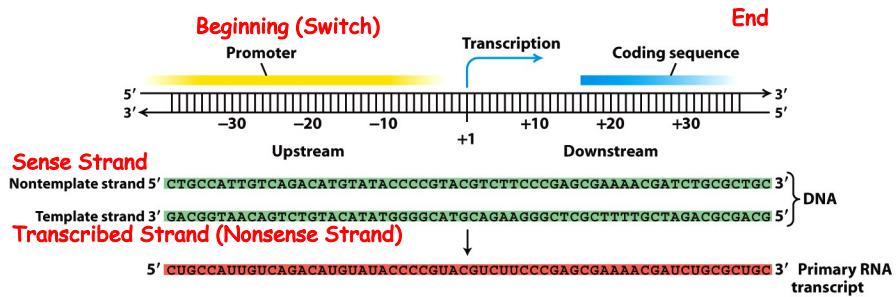


Figure 4-10b  
Molecular Cell Biology, Sixth Edition  
© 2008 W. H. Freeman and Company

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

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## 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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**DNA**  
Genetic Code of Life

**Entire Genetic Code of a Bacteria**

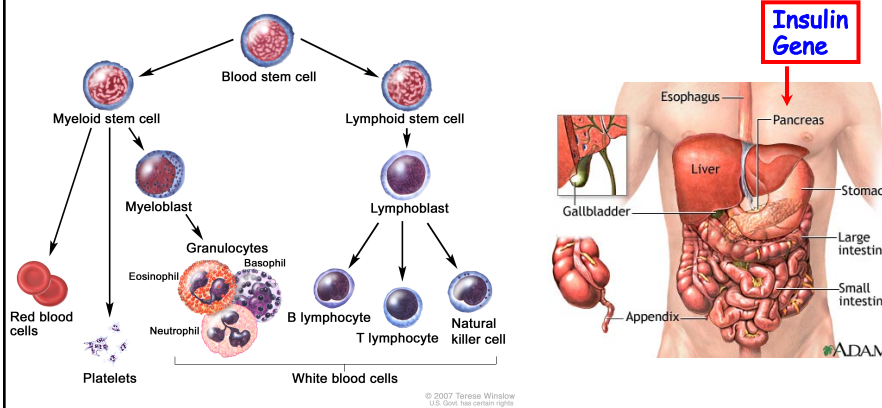
**DNA Fingerprinting**

**Cloning: Ethical Issues and Future Consequences**

**Plants of Tomorrow**



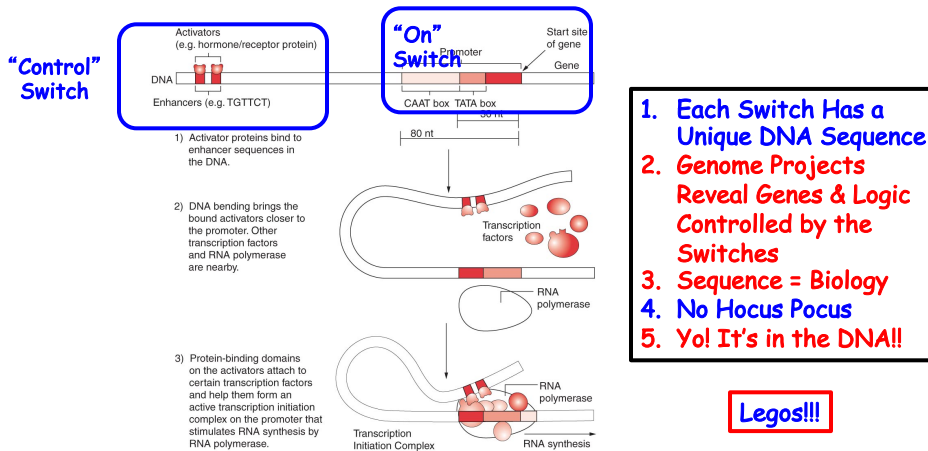
## Switches Control Where & When A Gene Is Active → Unique Functions → Unique Cells



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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

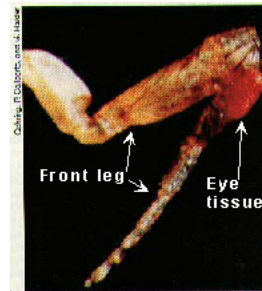
Eye Gene



Replace the Head Switch With the Leg Switch by Genetic Engineering

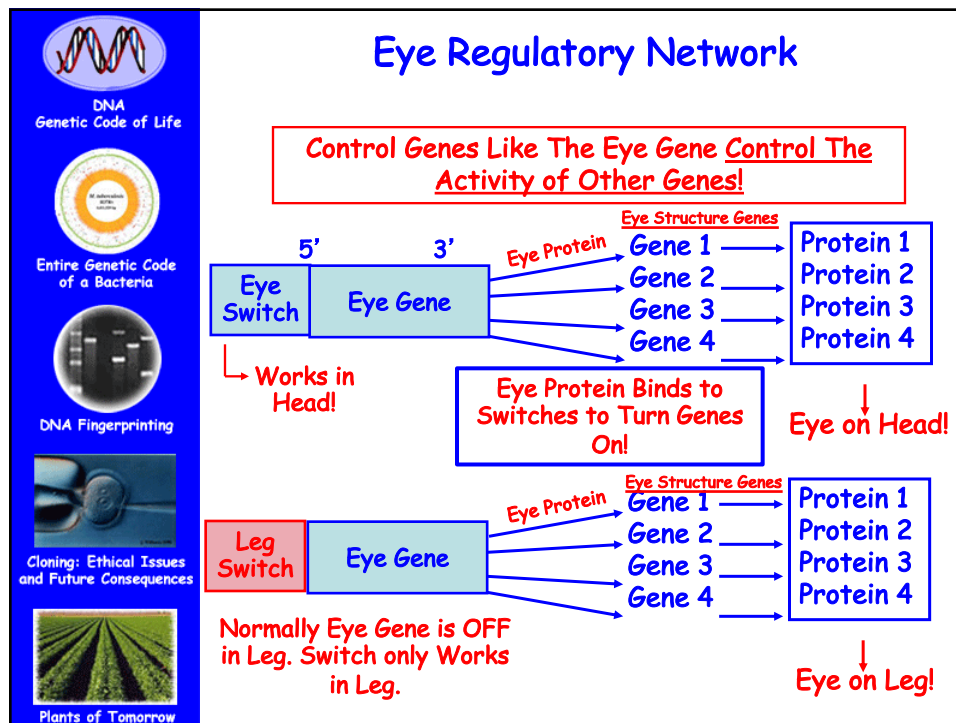


Eye Gene + Leg Switch

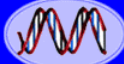


Abnormal activity of the eyeless gene has generated an eye on the leg of a fly.


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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

## 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!