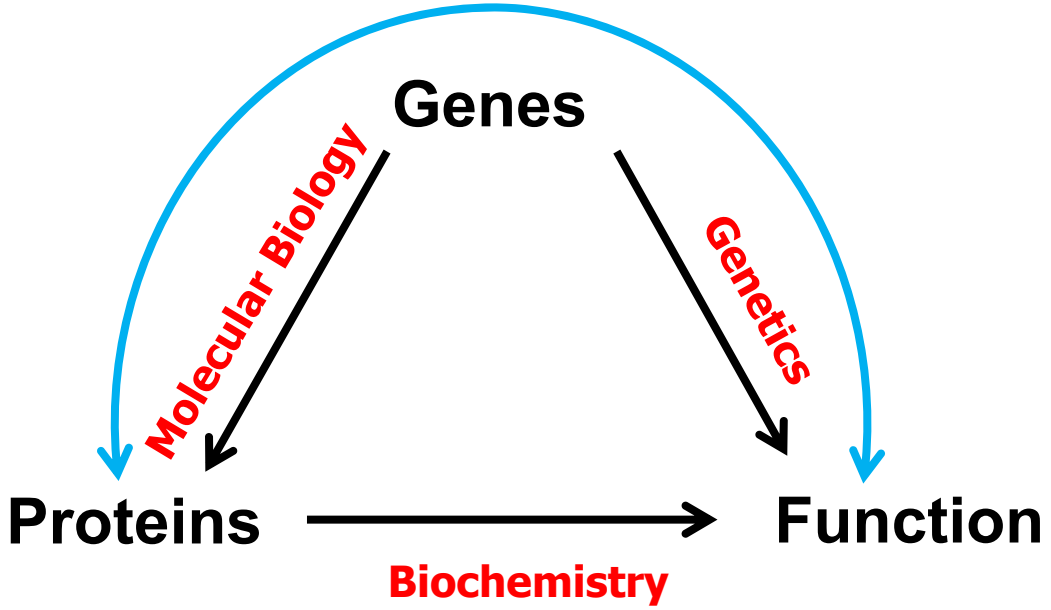
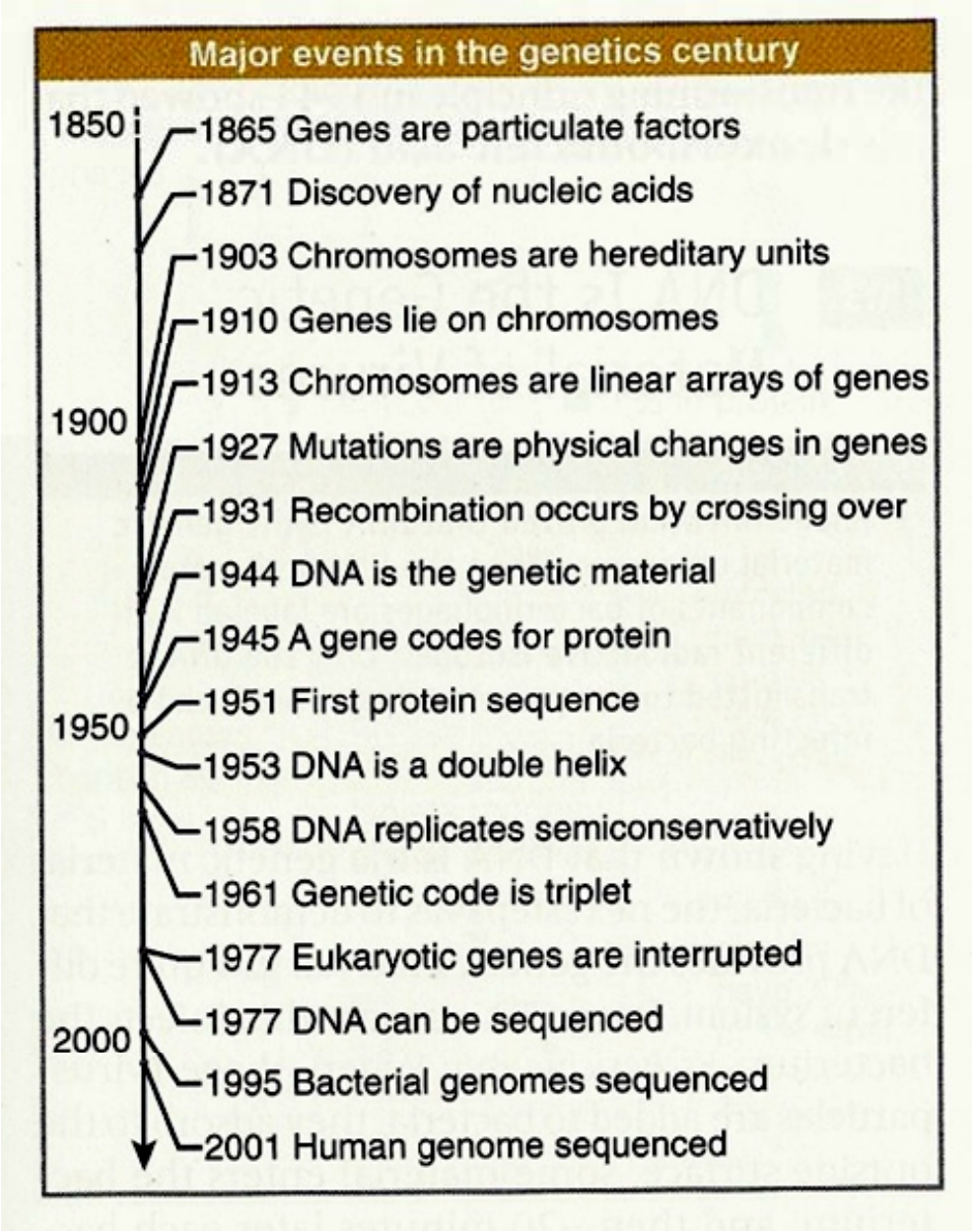
Introduction to Molecular Biology

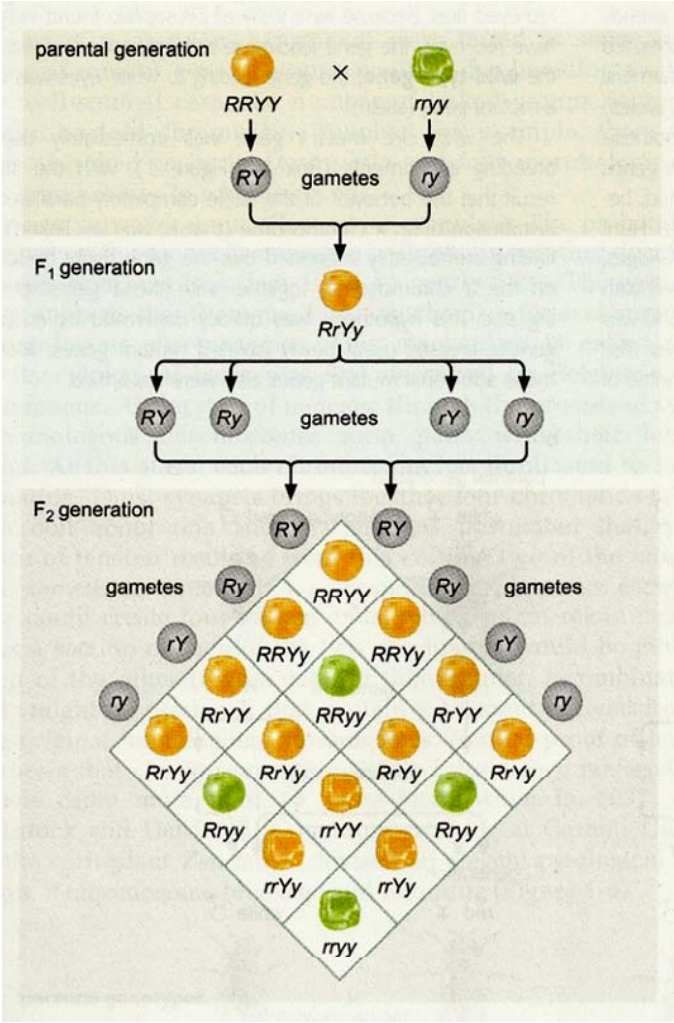


- **The study of chemical and physical structure of macromolecule was first referred as Molecular Biology by William Astbury.**
- **The discipline is enormously complex and crosses traditional boundaries between genetics, biochemistry, cell biology and biophysics.**

Introduction to Molecular Biology

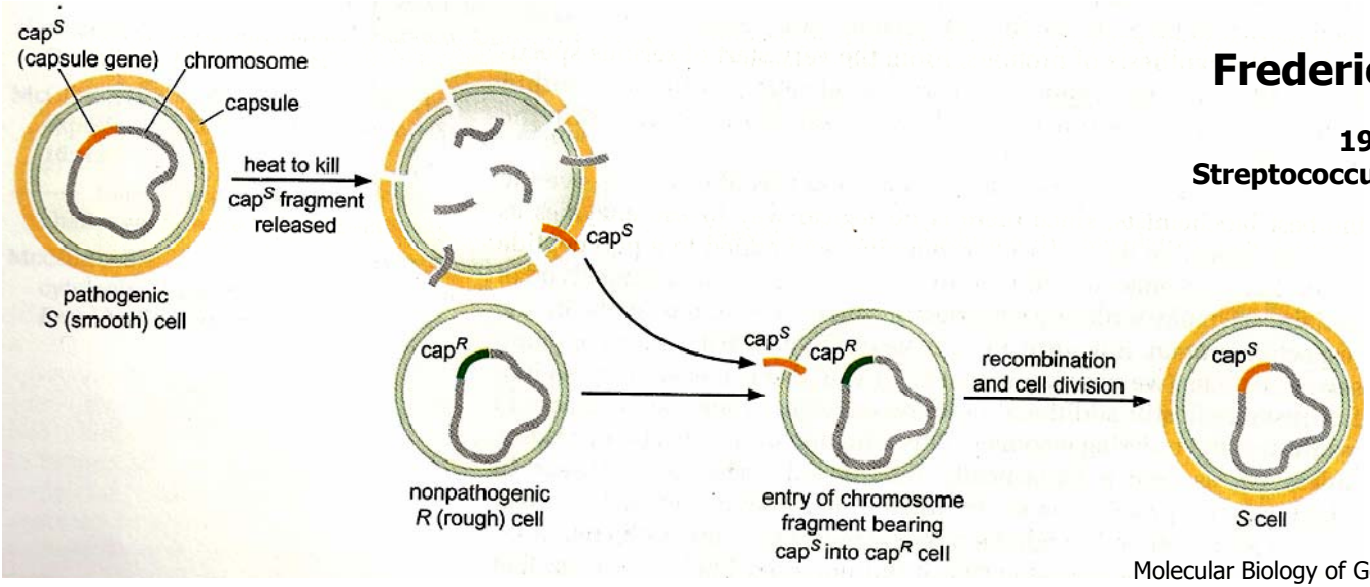


Mendel's Pea experiment



Molecular Biology of Gene *Watson et al* ©

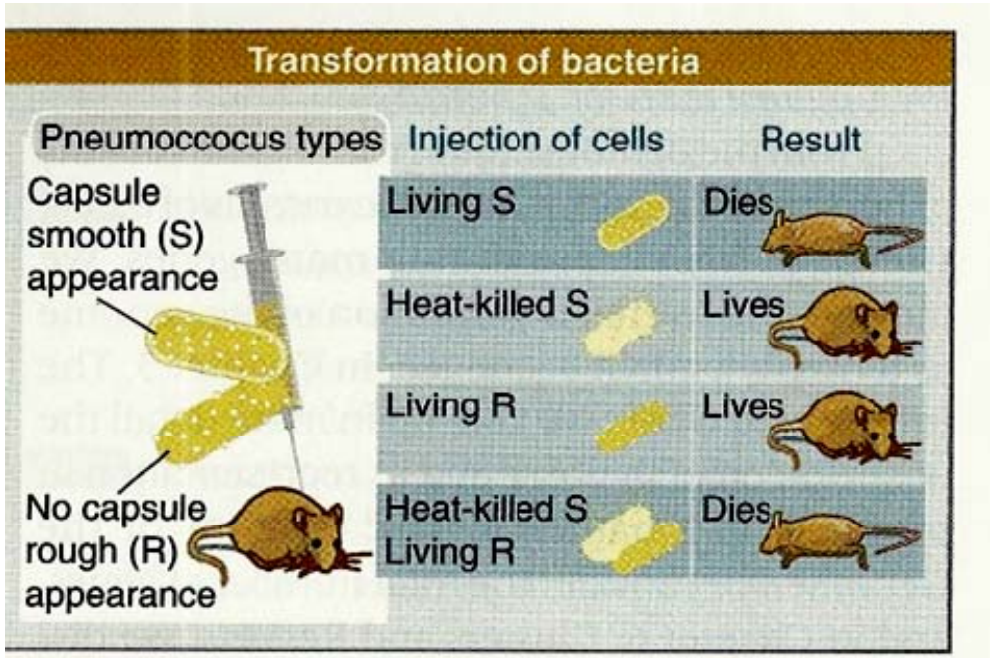
Nucleic acid carry genetic specificity

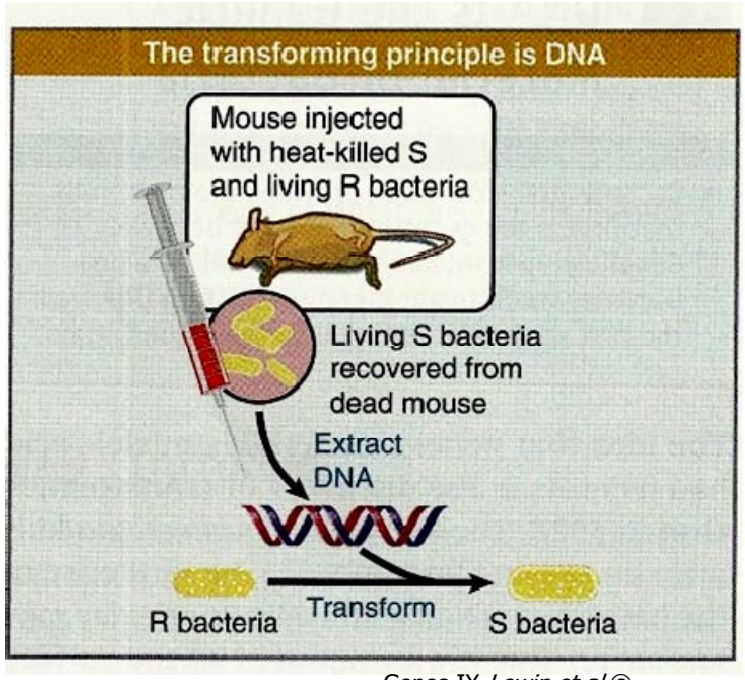


Frederick Griffith

1928
Streptococcus pneumonia

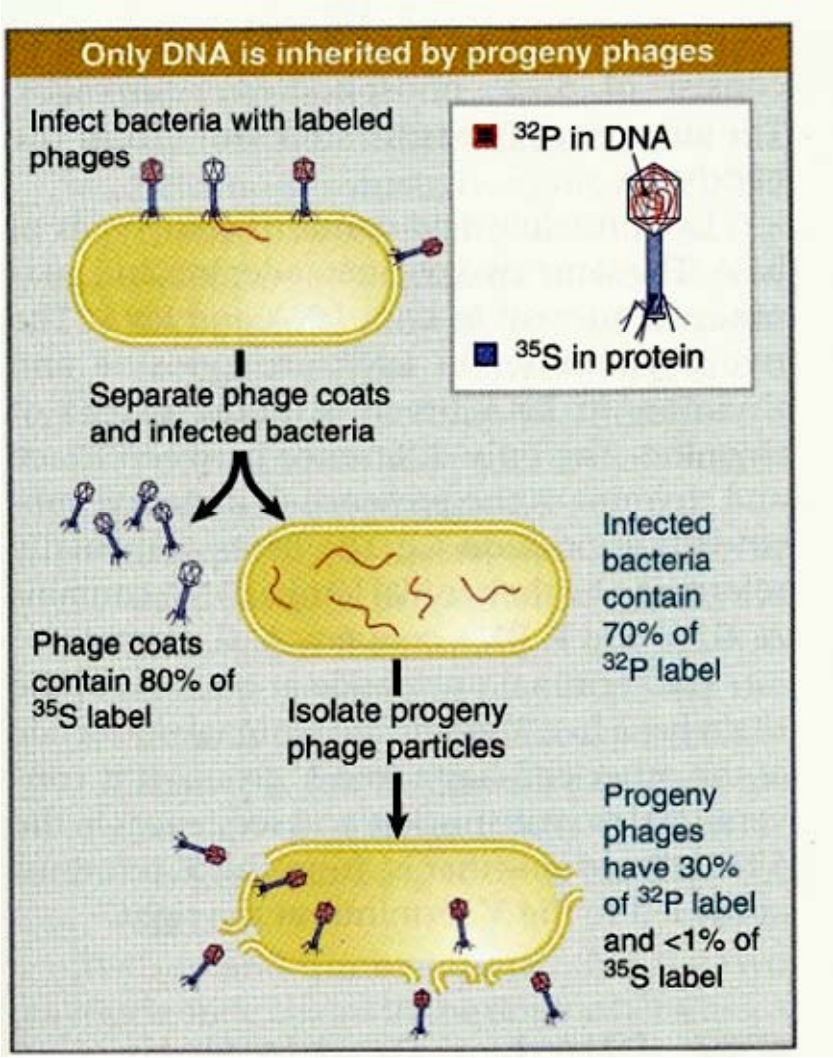
Molecular Biology of Gene *Watson et al* ©





Genes IX *Lewin et al* ©

Nucleic acid is inherited in next generation



Genes IX *Lewin et al* ©

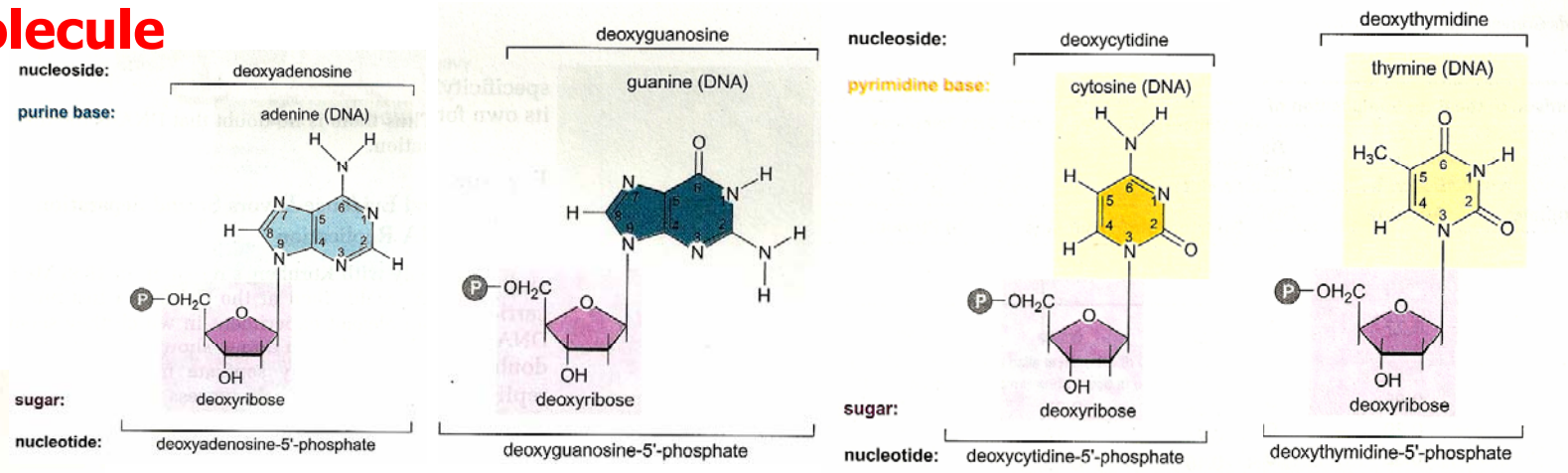
Nucleic acid controls Amino acid sequence in proteins

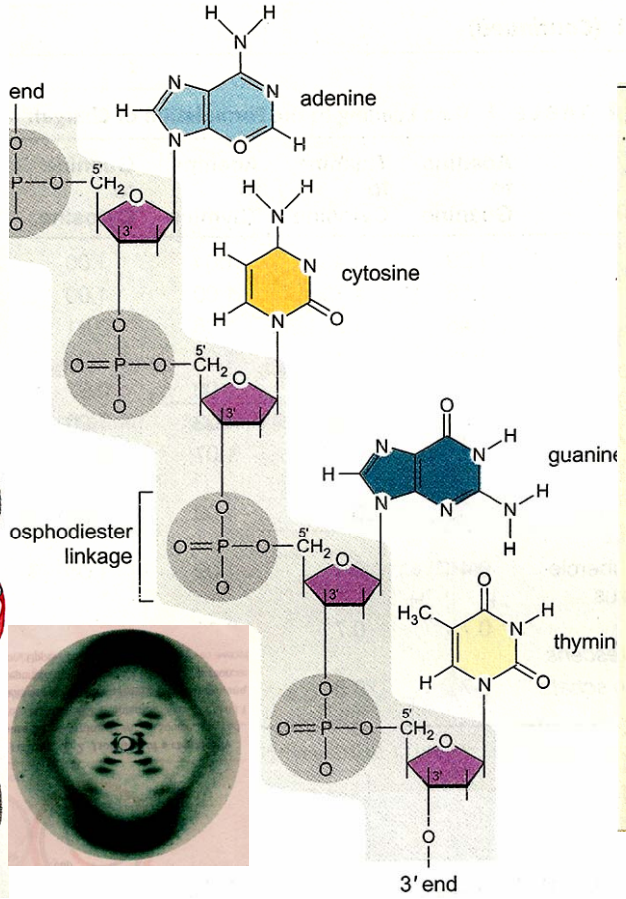
The first evidence that DNA controls AA sequence came from the study of genetic disease sickle cell anemia

When the protein was analyzed in these patients. Most of them found to contain change in Amino acid sequence at 6th position Glu=>Val.

The DNA molecule

Francis Crick,
James Watson
Maurice Wilkins
Rosalind Franklin





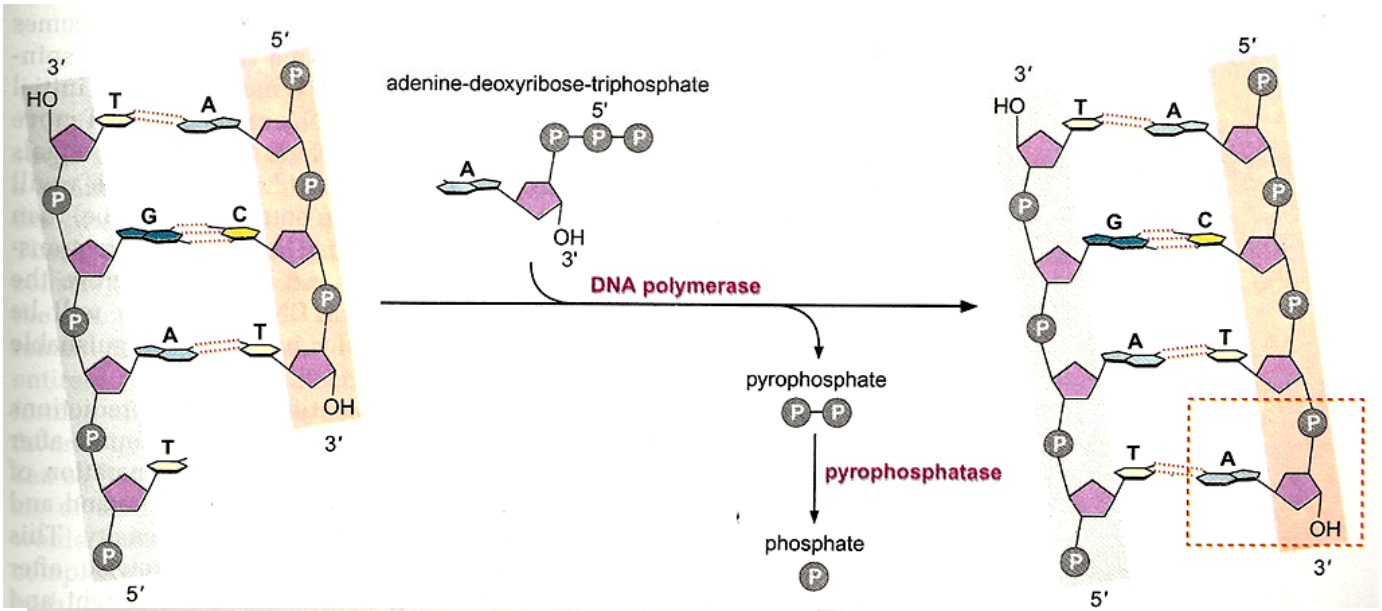
BOX 2-1 TABLE 1 Data Leading to the Formulation of Chargaff's Rules

Source	Adenine to Guanine	Thymine to Cytosine	Adenine to Thymine	Guanine to Cytosine	Purines to Pyrimidines
Ox	1.29	1.43	1.04	1.00	1.1
Human	1.56	1.75	1.00	1.00	1.0
Hen	1.45	1.29	1.06	0.91	0.99
Salmon	1.43	1.43	1.02	1.02	1.02
Wheat	1.22	1.18	1.00	0.97	0.99
Yeast	1.67	1.92	1.03	1.20	1.0
Hemophilus influenzae	1.74	1.54	1.07	0.91	1.0
Escherichia coli K2	1.05	0.95	1.09	0.99	1.0
Avian tubercle bacillus	0.4	0.4	1.09	1.08	1.1
Serratia marcescens	0.7	0.7	0.95	0.86	0.9
Bacillus schatz	0.7	0.6	1.12	0.89	1.0

Source: After Chargaff E. et al.1949. *J. Biol.Chem.* 177: 405.

DNA replication

Arthur Kornberg: discover first DNA polymerase enzyme (Pol I) . It works only in presence of DNA template.

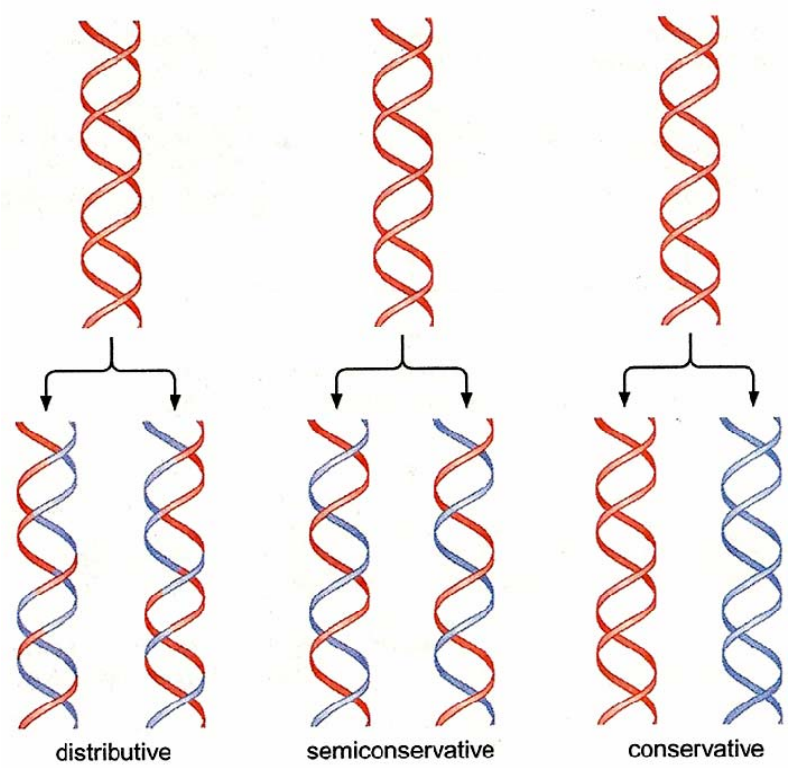


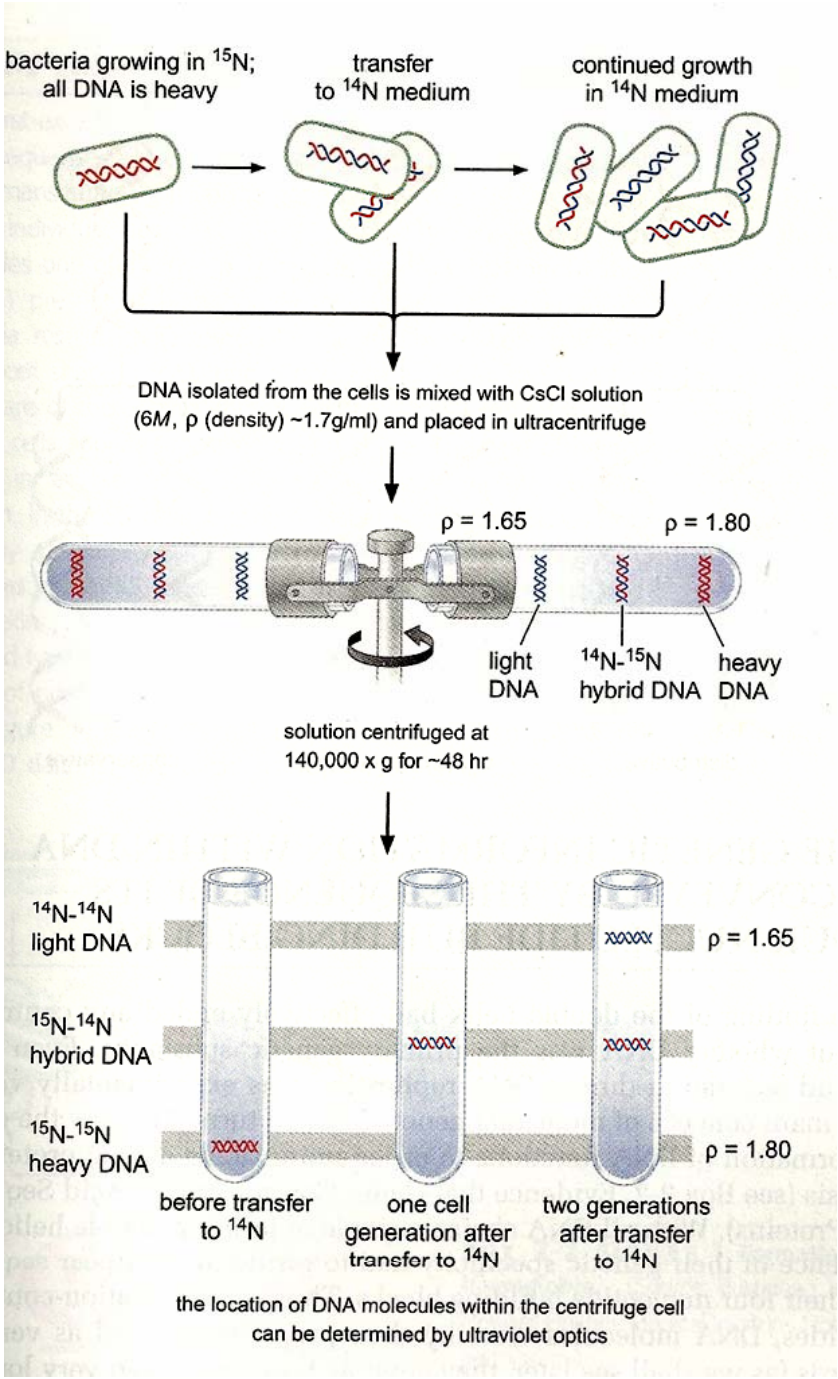
Source of DNA Template	Base Composition of the Enzymatic Product				$\frac{A+T}{G+C}$	$\frac{A+T}{G+C}$
	Adenine	Thymine	Guanine	Cytosine	In Product	In Template
Micrococcus lysodeikticus (a bacterium)	0.15	0.15	0.35	0.35	0.41	0.39
Aerobacter aerogenes (a bacterium)	0.22	0.22	0.28	0.28	0.80	0.82
Escherichia coli	0.25	0.25	0.25	0.25	1.00	0.97
Calf thymus	0.29	0.28	0.21	0.22	1.32	1.35
Phage T2	0.32	0.32	0.18	0.18	1.78	1.84

Molecular Biology of Gene *Watson et al* ©

DNA replicate by semi-conservative mode

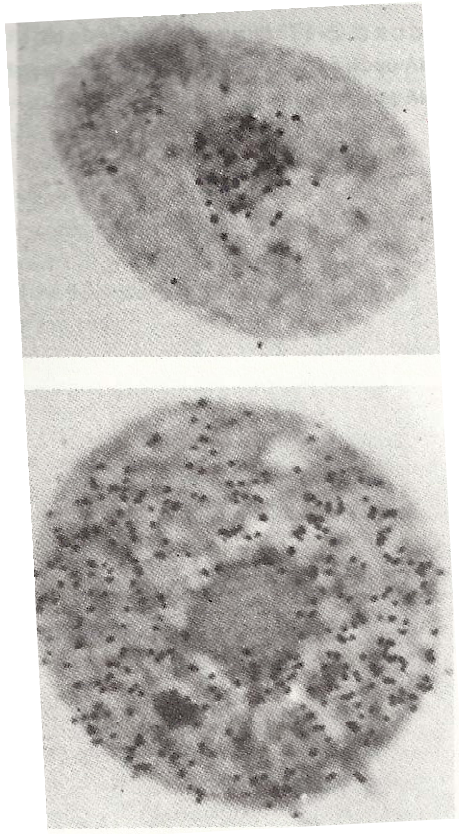
Meselson and Stahl @CalTech

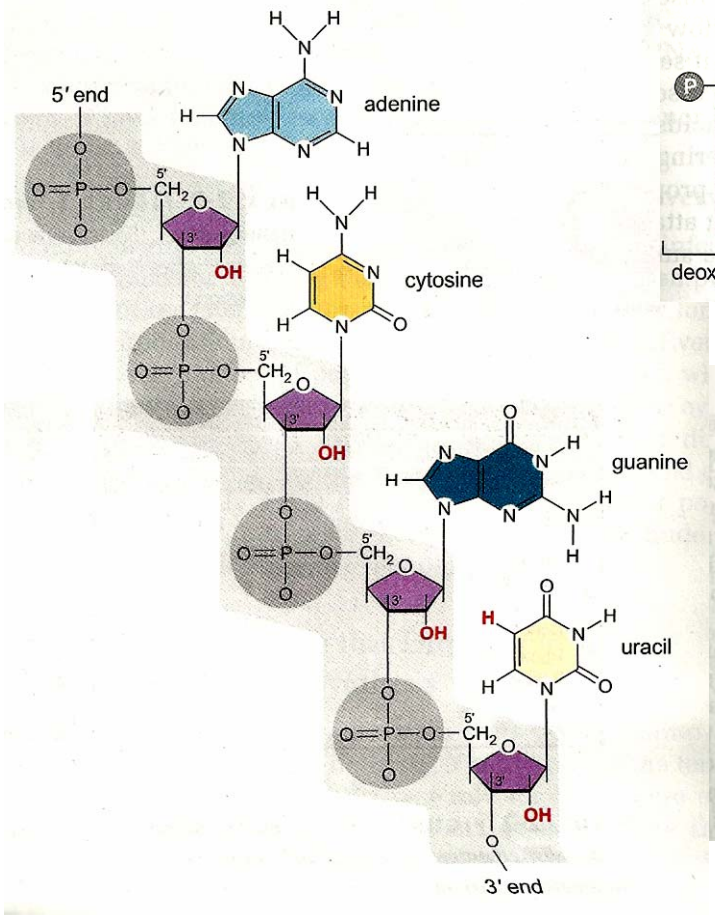


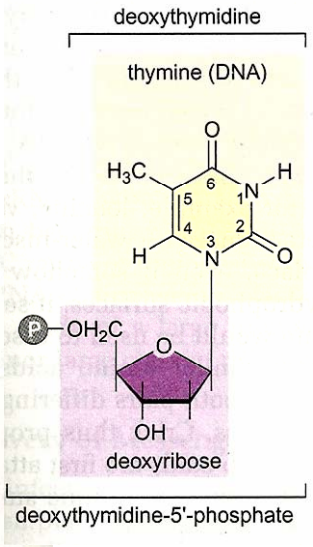


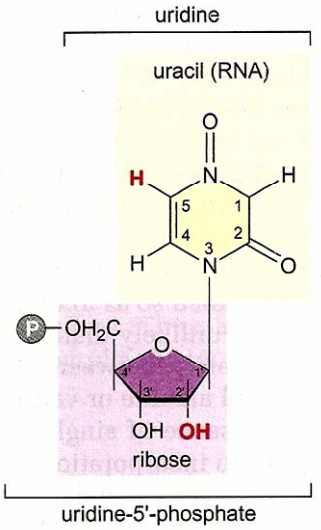
Molecular Biology of Gene *Watson et al* ©

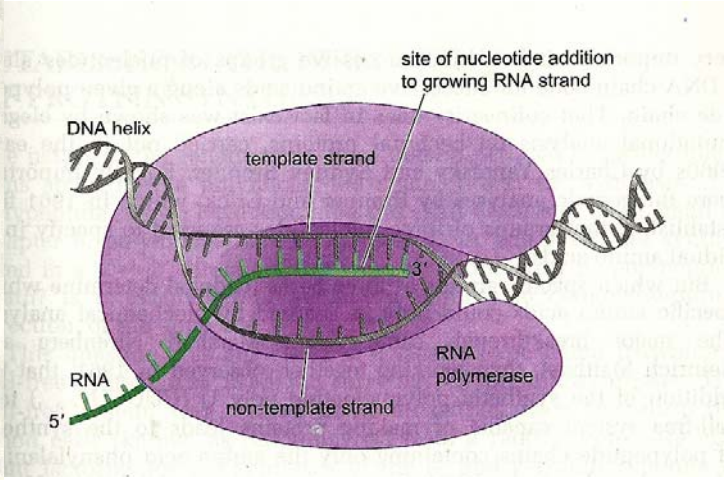
Experiment demonstrating that RNA is synthesized it nucleus and move to cytoplasm





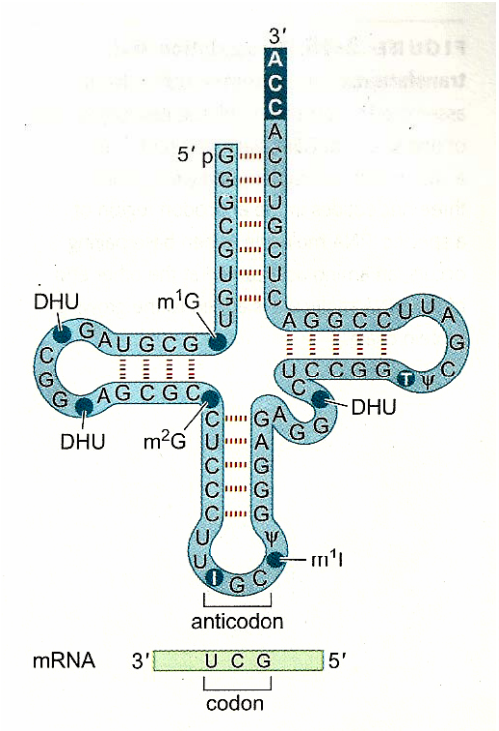






Molecular Biology of Gene *Watson et al* ©

RNA dominate the information decoding pathway



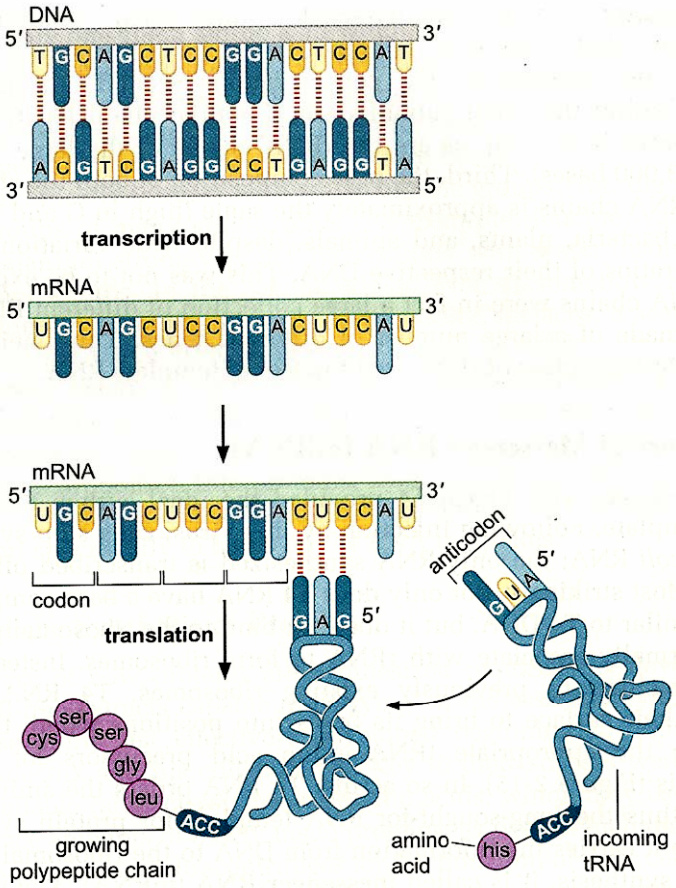
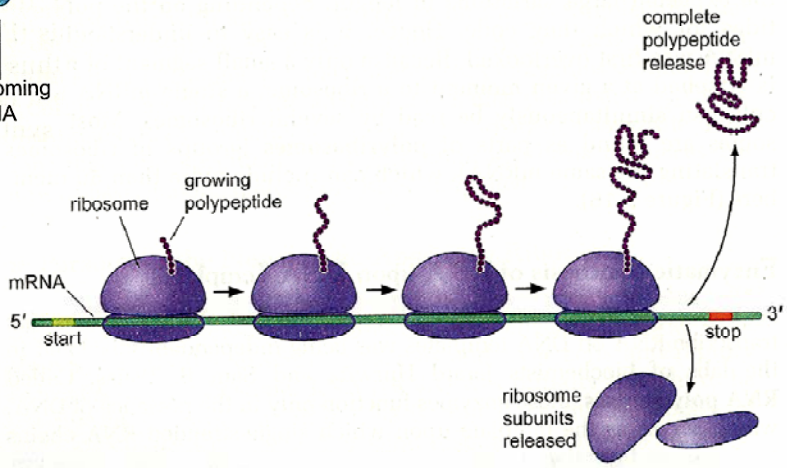
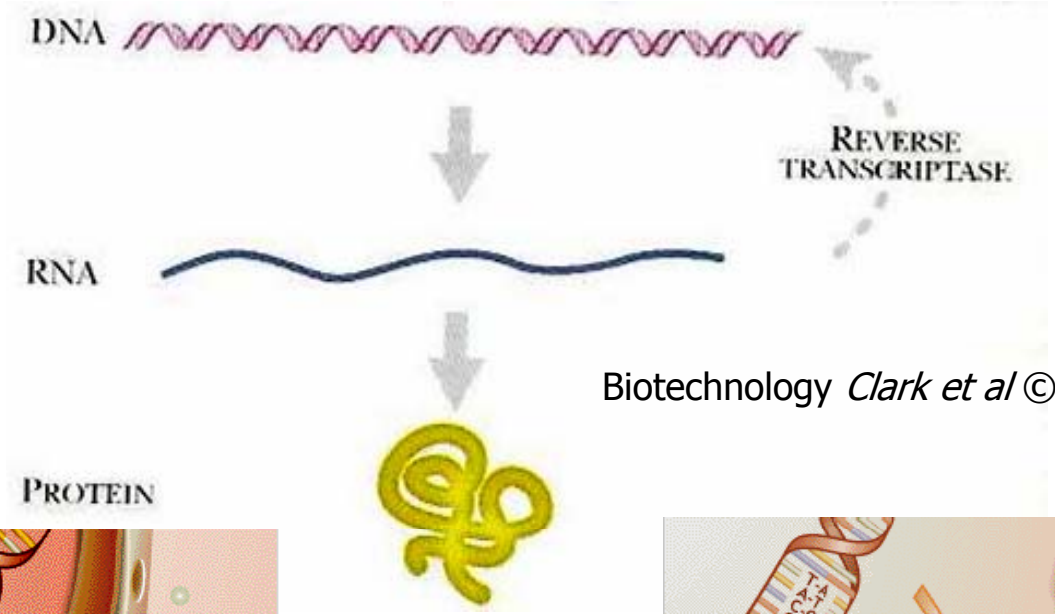


TABLE 2-3 The Genetic Code

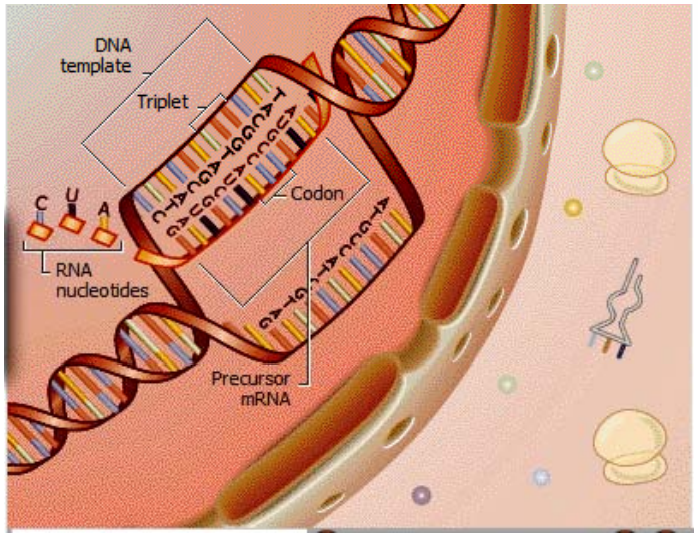
first position	second position: U	C	A	G	third position
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA stop	UGA stop	A
	UUG Leu	UCG Ser	UAG stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GAA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G



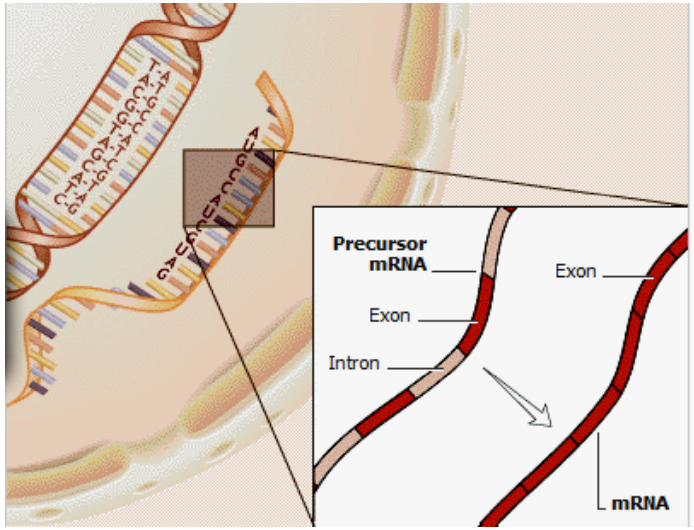
Central Dogma of Molecular Biology



Biotechnology *Clark et al* ©

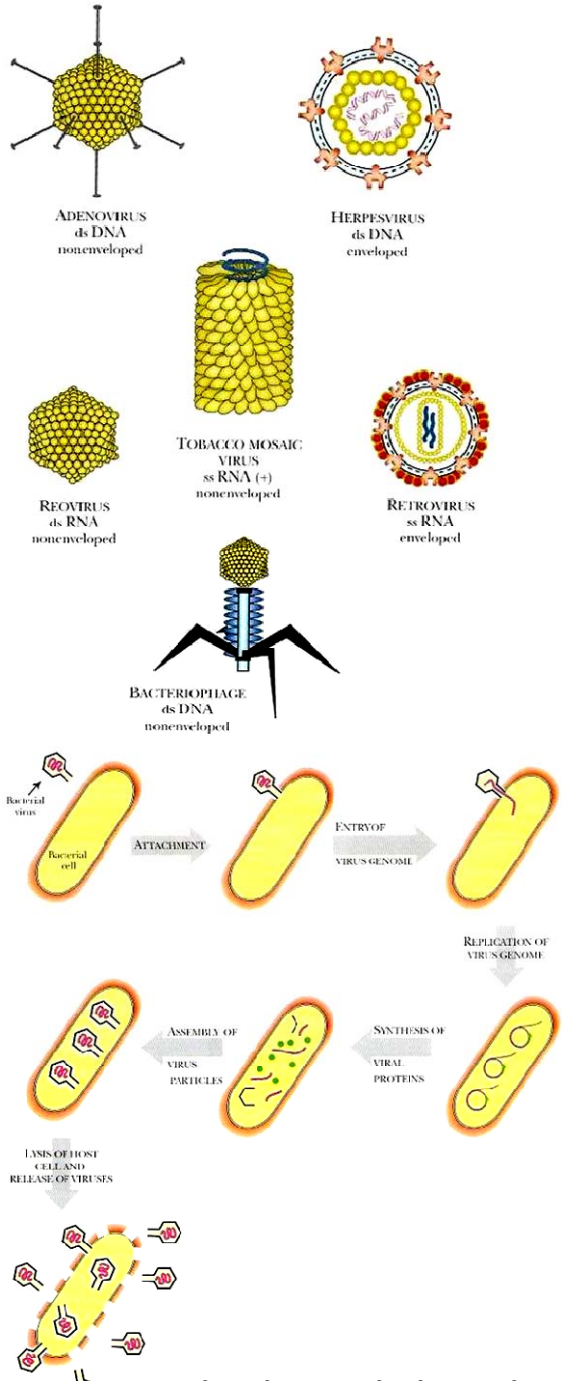


Encarta ©



Encarta ©

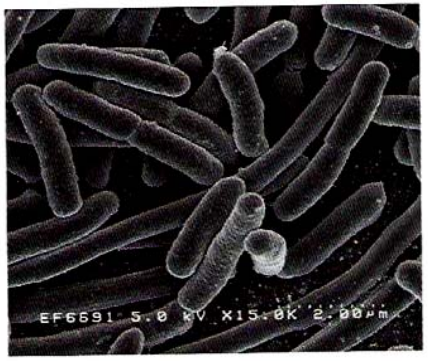
Virus



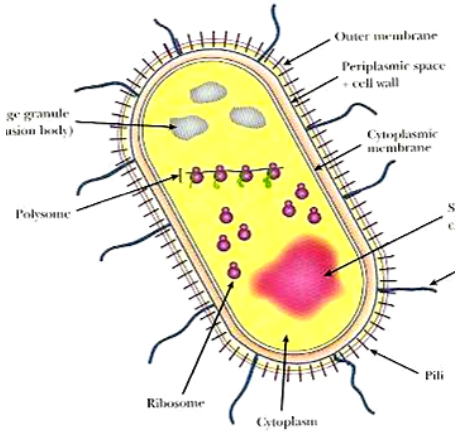
- Viruses are the entities that border on living
- Made up of outer protein coat (capsid) and a genetic material ss/dsDNA or ss/dsRNA.
- Devoid of cellular machinery and cannot make energy or duplicate it genome.
- Virus are obligatory parasites.
- Most of the understanding of genetic material and genes functions comes from the study of Bactriophages.
- T4, lambda, Pl and Mu were commonly used for the study.
- The genetic material of certain viruses (QB) are as small as 3500 bp consist of only 4 genes. Some viruses genome is complex and may contains around 200 genes.
- The genes are categorized as early or late depending upon their action in time of infection.
- The virus hijacks host translation machinery to synthesis its proteins and DNA for survival.
- This is good system to study DNA replication and RNA transcription
- Virus inserts/ integrate it DNA into host genome and modify host genome.
- To alter genome of host organism (Lenti virus).
- For gene therapy on humans.

Biotechnology *Clark et al* ©

Escherichia. coli



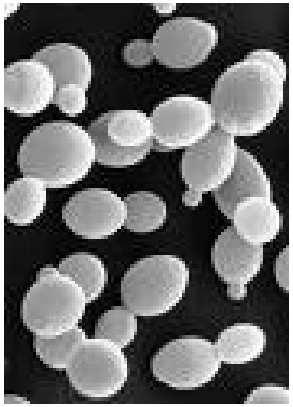
GRAM-NEGATIVE (e.g., *E. coli*)

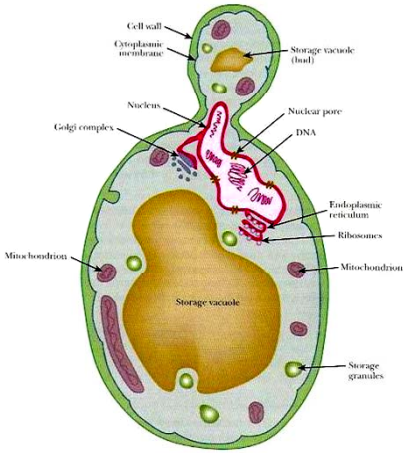


Biotechnology *Clark et al* ©

- Most important organism of Modern biotechnology(work horse)
- Prokaryote, Gram –ve, rod-shaped and about 1x2.5 micron in size. with 10-12 flagella for locomotion and numerous pili.
- No nucleus and single circular chromosome. It has around 4000 genes.
- Usually found in the gut, but are generally harmless. Occasional strains are pathogenic that secrete enterotoxin causing diarrhea.
- They grow on simple liquid or solid nutrient. The doubling time is 20 min. It can be stored in a refrigerator for weeks and can be maintained forzen at -70°C for 20 or more years.
- The have extra chromosomal genetic material called colicin plasmid, which harbors genes to kill neighboring cells. This plasmid found pivotal role in modern biotechnology after removal of toxin genes.

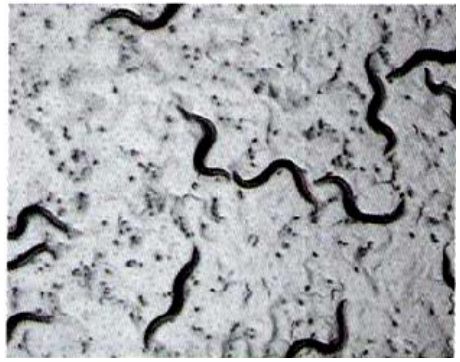
Fungi : *Saccharomyces cerevisiae* (baker's yeast)

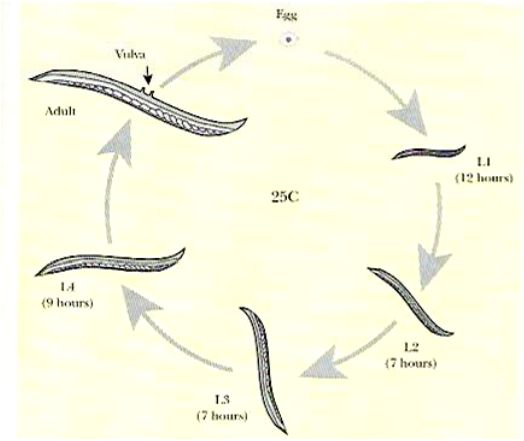




- Unicellular eukaryote with diploid or haploid chromosome.
- Distinct nucleus, cellular components are compartmentalized.
- Cellular organelles includes endoplasmic reticulum, Golgi and mitochondria.
- S. cerevisiae has 16 linear chromosomes with telomeres and centromeres, the features absents in bacteria.
- The genome is 12 Mb of DNA with 6000 genes.
- Like bacteria, yeast is also easy to maintain in simple media. Their doubling time is 90 min (compare to 20 min in *E. coli*).
- Can be store frozen at -70°C for years.
- Yeast also has extra-chromosomal plasmid called 2-micron circle. It is very useful in yeast manipulation.
- Yeast grow by budding to give one daughter and mother cell.
- It also shows sexual mating: with a and α type haploid cells mate to form genetically unique diploid cells
- Under poor environment spore formation takes place called Ascus, subsequent meiosis give 4 haploid spores.
- The organism is very easy to manipulate genetically.
- Useful to express proteins from higher eukaryotes with post-translational modifications.

Caenorhabditis elegans



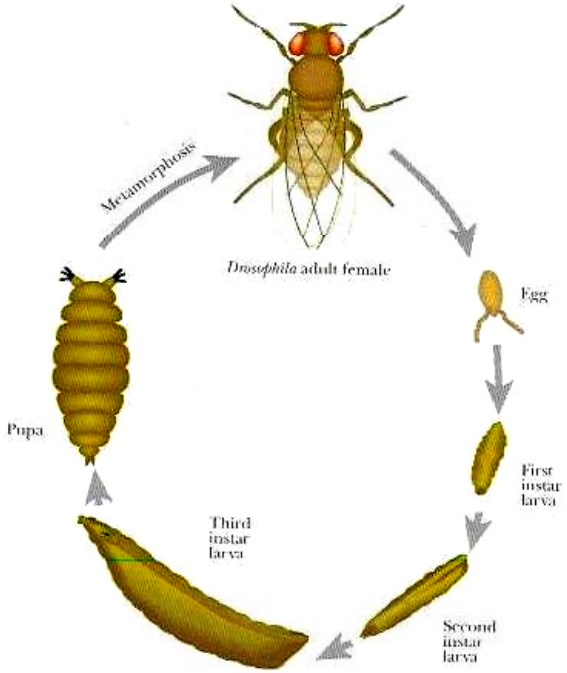


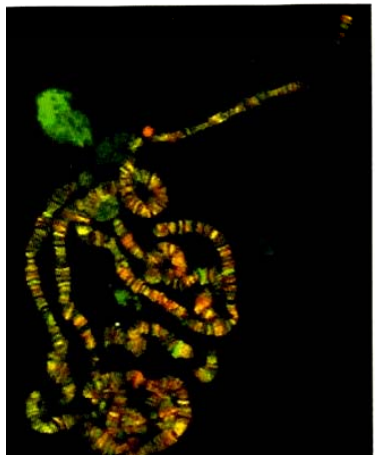
Biotechnology *Clark et al* ©

- C. elegans is a small round worm found in rotting vegetation and feeds on bacteria.
- They are self fertilizing hermaphrodite (and separate male).
- There are exactly 959 somatic cells in C. elegans.
- Life cycle of this worm last for 3 day (Fast reproduction).
- The organism is transparent and hence very useful in study real time cellular physiology with the fluorescent labeling.
- The advantage of using C. elegans is to study development, aging, sexual dimorphism, alcohol metabolism and other phenomena that apply to humans.

Easy to grow and make genetic clones of itself

Drosophila melanogastrer (fruit fly)





Biotechnology *Clark et al* ©

- Multi cellular invertebrate commonly found around rotting fruit.
- Small insect to handle, simple food source, and easy to maintain.
- Life span is 2 weeks.
- Genome is sequenced (165 Mb DNA) divided into 3 autosome and x/y sex chromosome.
- 12,000 predicted genes.
- Very versatile model to study genetic.
- Visible marker for genetic study includes wings, legs, antenna, eyes, hair formation, etc.
- True sexual reproductions.
- Easy genetic crosses.

Polytene chromosomes: While Drosophilla's larvel development the number of cells remains constant while the size of cells increase dramatically. In order for the cells to work properly there need lot of mRNA and proteins. This is accomplished by duplication of genome hundreds of times to give giant polytene chromosome.

Zibra fish (*Danio rerio*)

Biotechnology *Clark et al* ©

- Simple vertebrate, easy to maintain and breed in home made aquarium
- Great model system to study genetic.
- Adult lays about 200 eggs, great numbers of offspring to study in subsequent generation.
- Embryo develop out side the mother and transparent, good system to study the embryo development.
- Cells inside the embryo can be manipulated to study the effect of position on cellular development.
- 75% of zebra fish DNA is homologous to humans thus the finding from zebra fish gene study can be extrapolated to human genes.

Biotechnology *Clark et al* ©

Arabidopsis thaliana (wild mustard seeds)

- ❑Excellent model system for plant biotechnology.
- ❑Easily grown and maintained in laboratory settings.
- ❑Entire generation is finished in 6-10 weeks.
- ❑It has small genome (125Mb) and 25,000 genes as compare to 40-50,000 genes in rice.
- ❑This plant can be maintained in haploid state like yeast.

Mus musculus (Mouse)

- ➢This model is most closely related to humans
- ➢20 different chromosome, and less than 1% genes has no human counterpart.
- ➢Very easy to manipulate genetically by creating knockout mouse or transgenic mouse (adding extra genes)
- ➢Ideal to study growth, development and physiology

Tissue culture

Biotechnology *Clark et al* ©

- Mammalian cell culture is another way to study in-vitro effects.
- No ethical issues to address.
- Different human, monkey, hamster cell lines are available (kidney, liver, heart, NIH3T3, Hella, HK213, etc).
- These are immortal cell lines and can be grown forever with proper maintenance.
- Direct genetic manipulation (deletion or mutations) is possible.
- Recombinant protein from human origin can purified for medical use.
- Every expensive to maintain. Need fetal bovin serum and CO_2 incubator.
- Insect cell lines are also available for heterologous protein expression.
- Advantage: Can be maintain on serum free media, grow on lower temperature and CO_2 free condition.