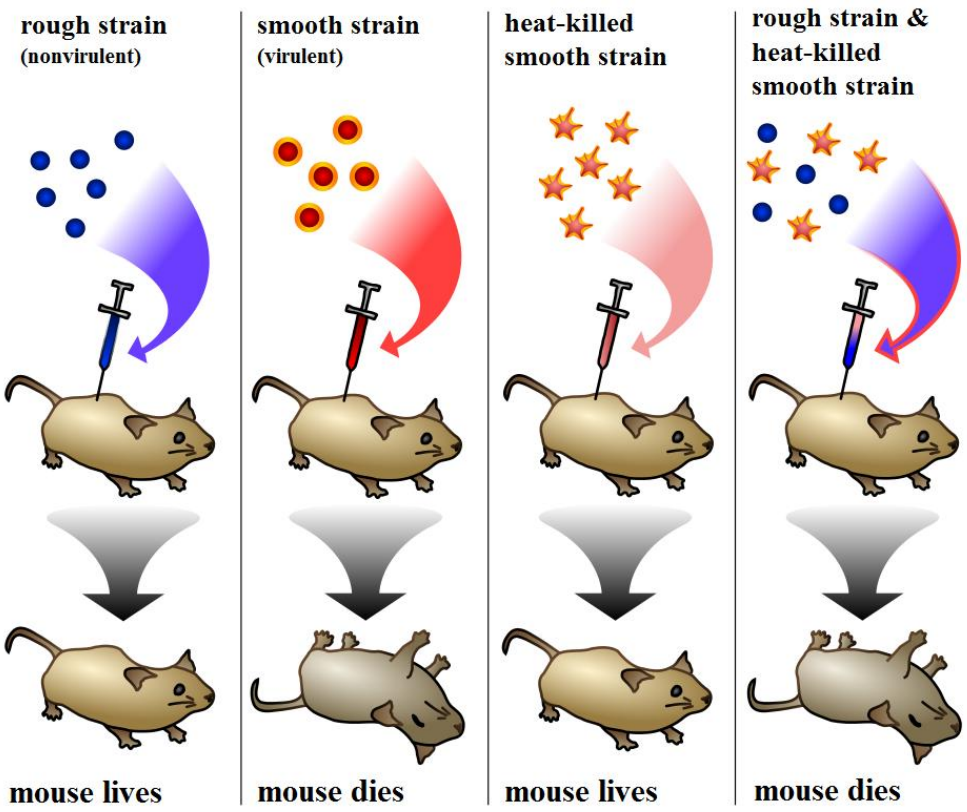
Genetics Notes

DNA as Genetic Material

Griffith's Experiment

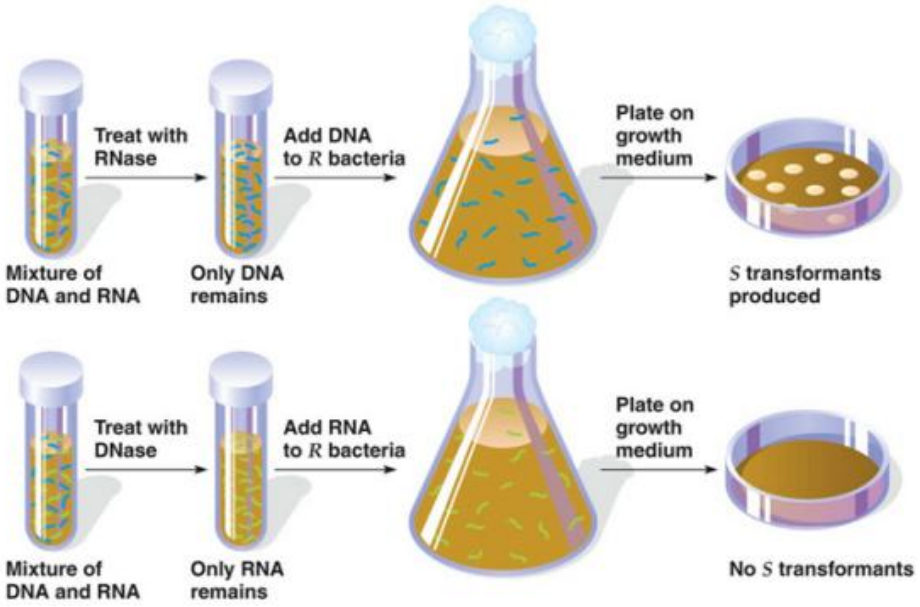
- Reported in 1928 by Frederick Griffith was one of the first experiments suggesting that bacteria are capable of transferring genetic information through a process known as transformation
- Transformation: a chemical compound within the dead bacteria was able to transform the non-pathogenic bacteria (rough strain) into pathogenic bacteria
- Today, we know that the "transforming principle" Griffith observed was the DNA of the III-S strain bacteria. While the bacteria had been killed, the DNA had survived the heating process and was taken up by the II-R strain bacteria. The III-S strain DNA contains the genes that form the protective polysaccharide capsule. Equipped with this gene, the former II-R strain bacteria were now protected from the host's immune system and could kill the host



Avery-MacLeod-McCarty Experiment

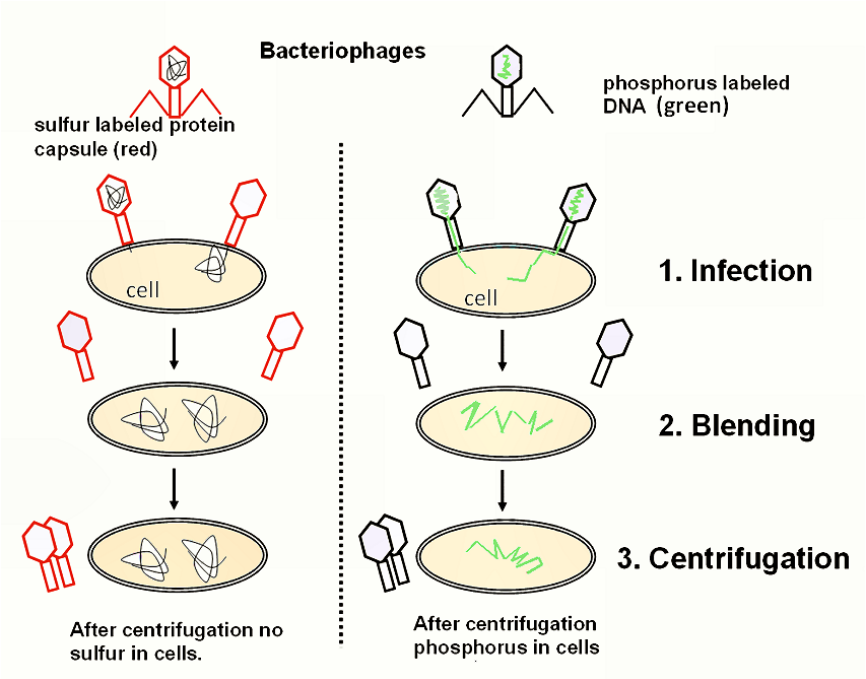
- Reported in 1944 by Oswald Avery, Colin MacLeod, and Maclyn McCarty, demonstrating that DNA is the substance that causes bacterial transformation
- They purified and treated heat killed bacteria to determine the chemical nature of the “transforming principle”
- They found that only DNA (not RNA or protein) transformed non pathogenic bacteria into pathogenic

- The experimental findings of the Avery–MacLeod–McCarty experiment were quickly confirmed, however there was considerable reluctance to accept the conclusion that DNA was the genetic material



Hershey and Chase Experiment

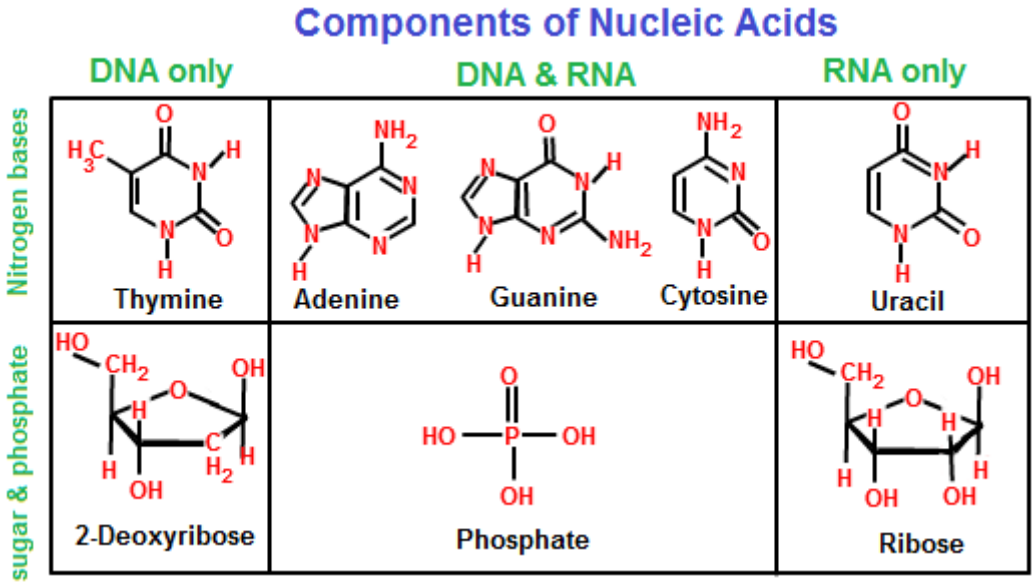
- A series of experiments conducted in 1952 by Alfred Hershey and Martha Chase that helped to confirm that DNA is the genetic material
- They wanted to find out if it was DNA or protein which entered the E. coli cells to produce new bacteriophage particles
- They grew the particles in radioactively labelled S labeled protein or P labeled DNA, as S is only found in proteins and P only in DNA
- They found that radioactivity was only found in the cell when DNA was labelled



Chargaff's Rules

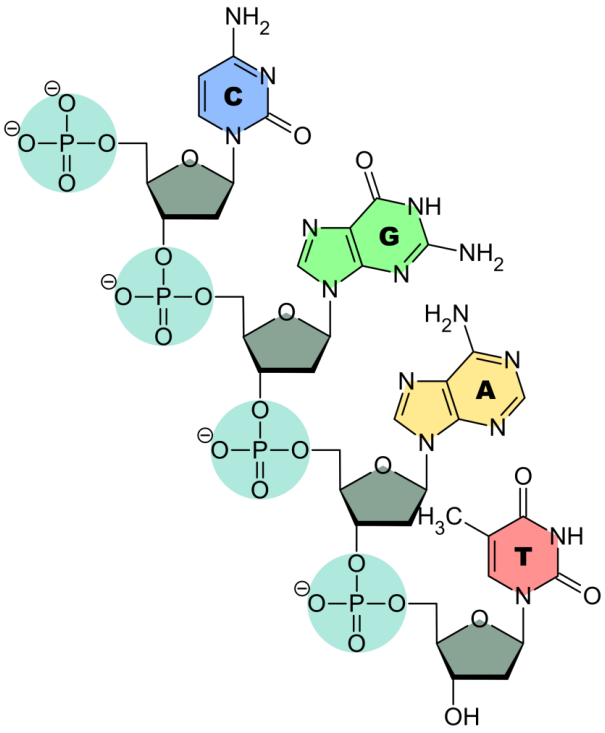
- Chargaff's rules state that DNA from any cell of all organisms should have a 1:1 ratio (base Pair Rule) of pyrimidine and purine bases and, more specifically, that the amount of guanine is equal to cytosine and the amount of adenine is equal to thymine
- The second of Chargaff's rules is that the composition of DNA varies from one species to another; in particular in the relative amounts of A, G, T, and C bases

Organism	%A	%G	%C	%T	A/T	G/C	%GC	%AT
φX174	24.0	23.3	21.5	31.2	0.77	1.08	44.8	55.2
Maize	26.8	22.8	23.2	27.2	0.99	0.98	46.1	54.0
Octopus	33.2	17.6	17.6	31.6	1.05	1.00	35.2	64.8
Chicken	28.0	22.0	21.6	28.4	0.99	1.02	43.7	56.4
Rat	28.6	21.4	20.5	28.4	1.01	1.00	42.9	57.0
Human	29.3	20.7	20.0	30.0	0.98	1.04	40.7	59.3
Grasshopper	29.3	20.5	20.7	29.3	1.00	0.99	41.2	58.6
Sea Urchin	32.8	17.7	17.3	32.1	1.02	1.02	35.0	64.9
Wheat	27.3	22.7	22.8	27.1	1.01	1.00	45.5	54.4
Yeast	31.3	18.7	17.1	32.9	0.95	1.09	35.8	64.4
E. coli	24.7	26.0	25.7	23.6	1.05	1.01	51.7	48.3



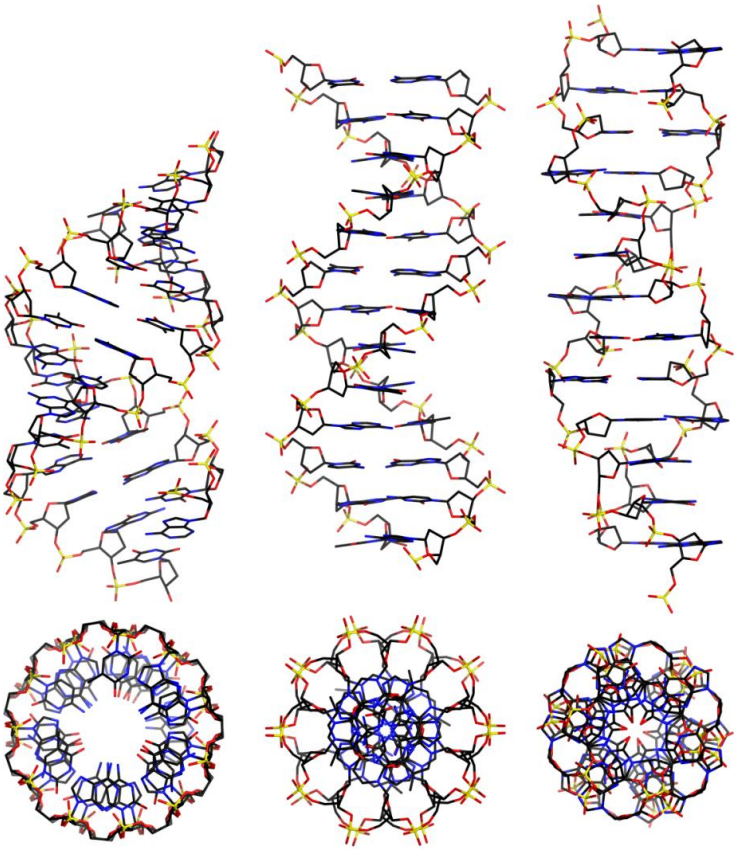
Watson and Crick

- Built models of DNA structure using crystallography data from Rosalind Fraklin
- They put sugar-phosphates on the outside, and nitrogenous bases on the inside
- Two polynucleotide chains (strands) wound around each other in right-handed double helix
- Two strands are anti-parallel (5' 3' : 3' 5')
- Bases are 0.34 nm apart, with 3.4 nm and 10 bases per turn
- Hydrogen bonds between A = T and G = C



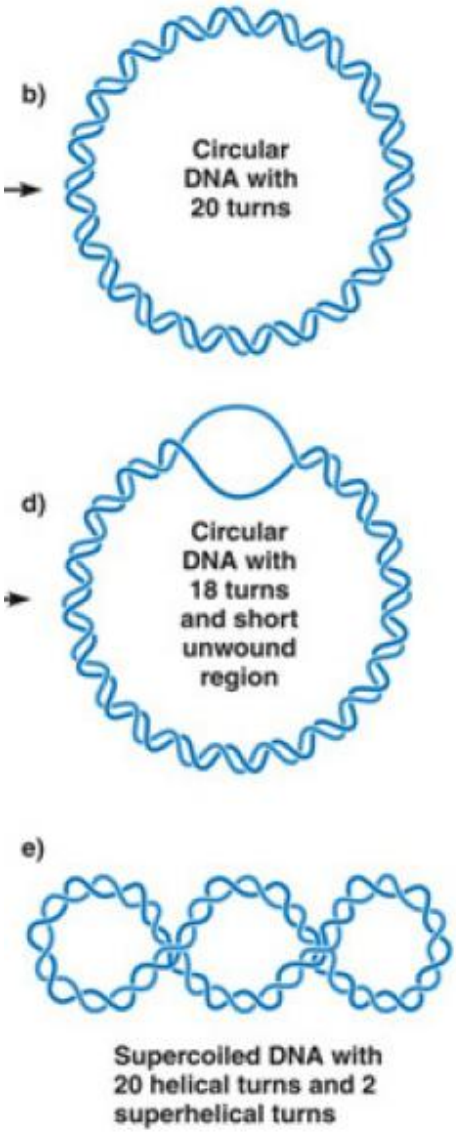
Different Forms of DNA

- A-DNA: right handed, more compact than B-DNA. Thought to only occur in dehydrated samples
- B-DNA: right handed, most common form
- Z-DNA: left handed, taller and thinner than A or B



Prokaryotic Chromosomes

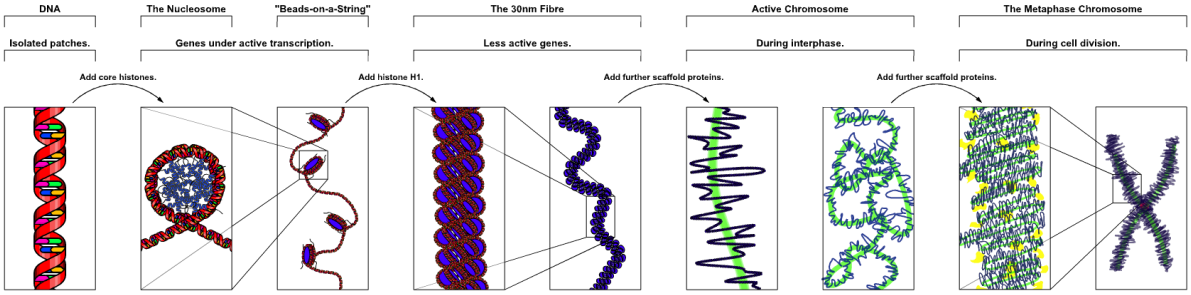
- Viral chromosomes can be double stranded DNA, single stranded DNA, double stranded RNA or single stranded RNA, with one or more chromosomes
- In bacteria, most contain a single, double stranded, circular chromosomes
- Chromosomes arranged in dense clump called nucleoid (pseudo nucleus)
- Supercoiling: additional winding and curling up of DNA strand to produce greater compaction, allowing it to fit into the cell

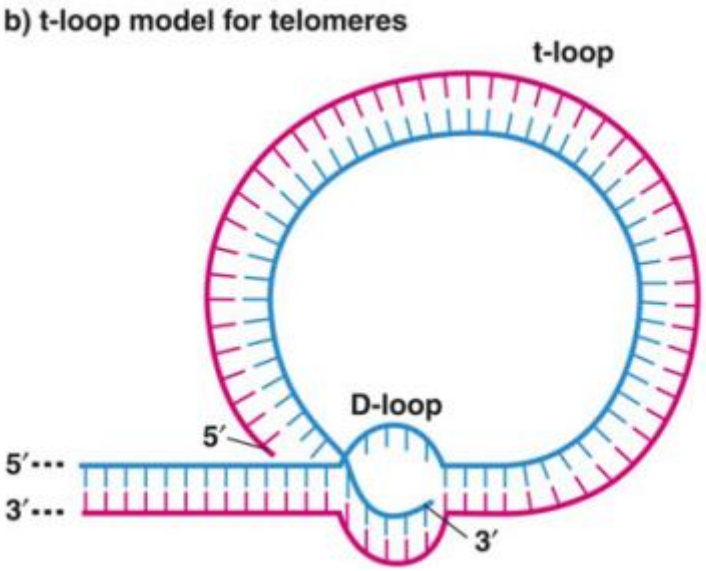


Eukaryote Chromosomes

- Chromatin: complex of macromolecules found in cells, consisting of DNA, protein and RNA. The primary functions of chromatin are 1) to package DNA into a smaller volume to fit in the cell, 2) to reinforce the DNA macromolecule to allow mitosis, 3) to prevent DNA damage, and 4) to control gene expression and DNA replication
- **Euchromatin**: a lightly packed form of chromatin that is rich in gene concentration, and is often under active transcription; 92% of the human genome is euchromatic

- **Heterochromatin**: a tightly packed form of DNA which stains more darkly than euchromatin, and consists of genetically inactive sequences - examples are centromeres and telomeres
- Centromeres: points of attachment of spindle fibers during mitosis, consist of three regions
- Telomeres: structures at the ends of the proteins which do not contain genes, but contain multiple repeats of short nucleotide sequence (hundreds or thousands of copies). Repeat sequences are evolutionarily conserved, and in humans and all vertebrates repeat sequence is 5'- TTAGGG -3'

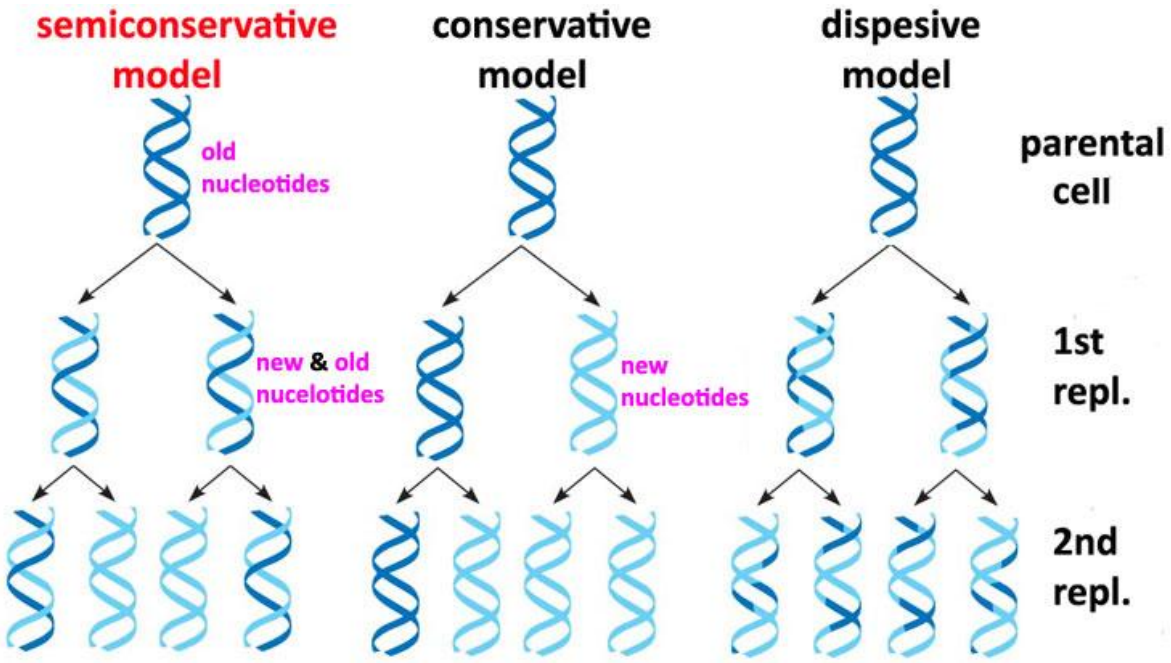


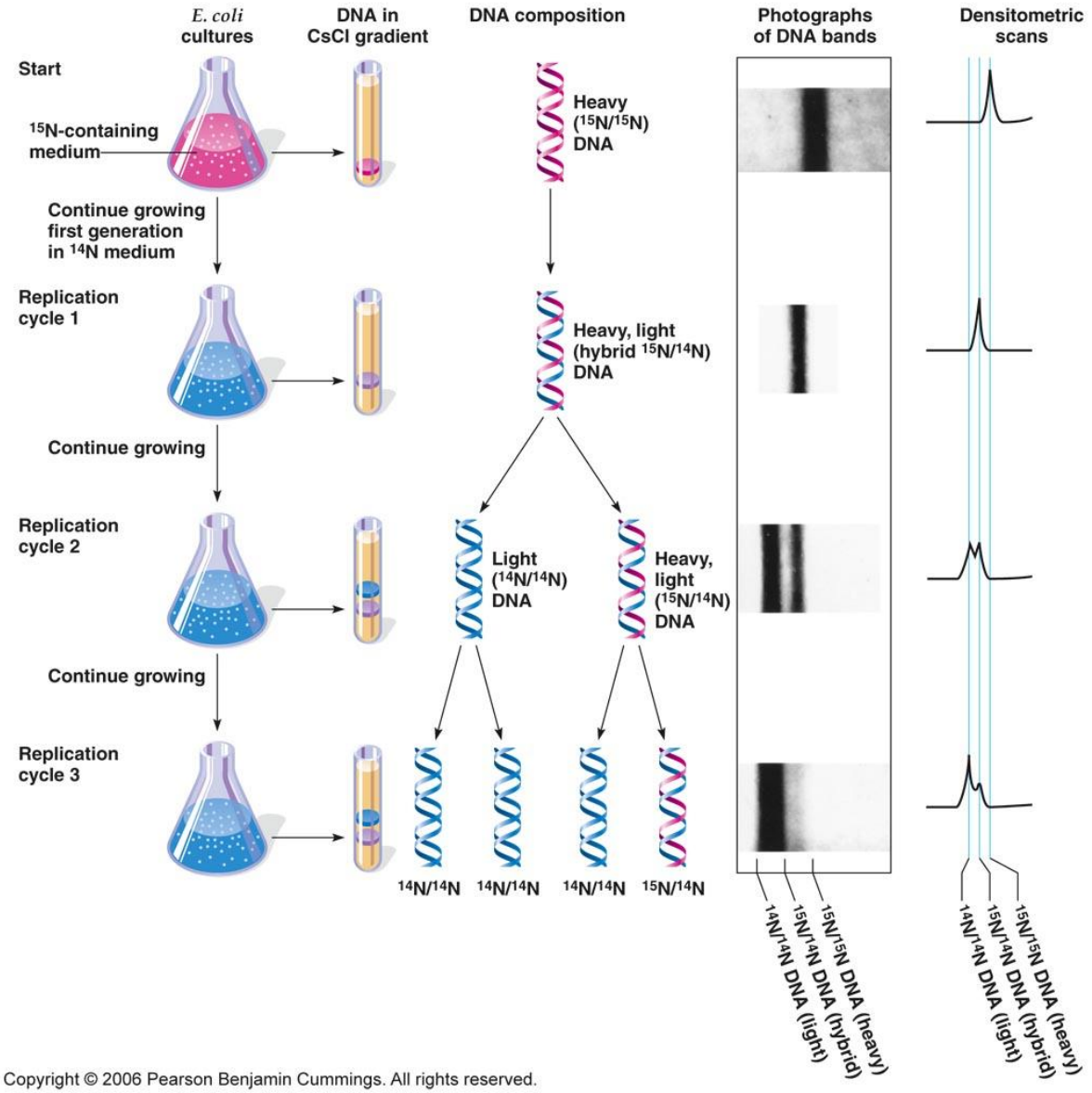


DNA Replication

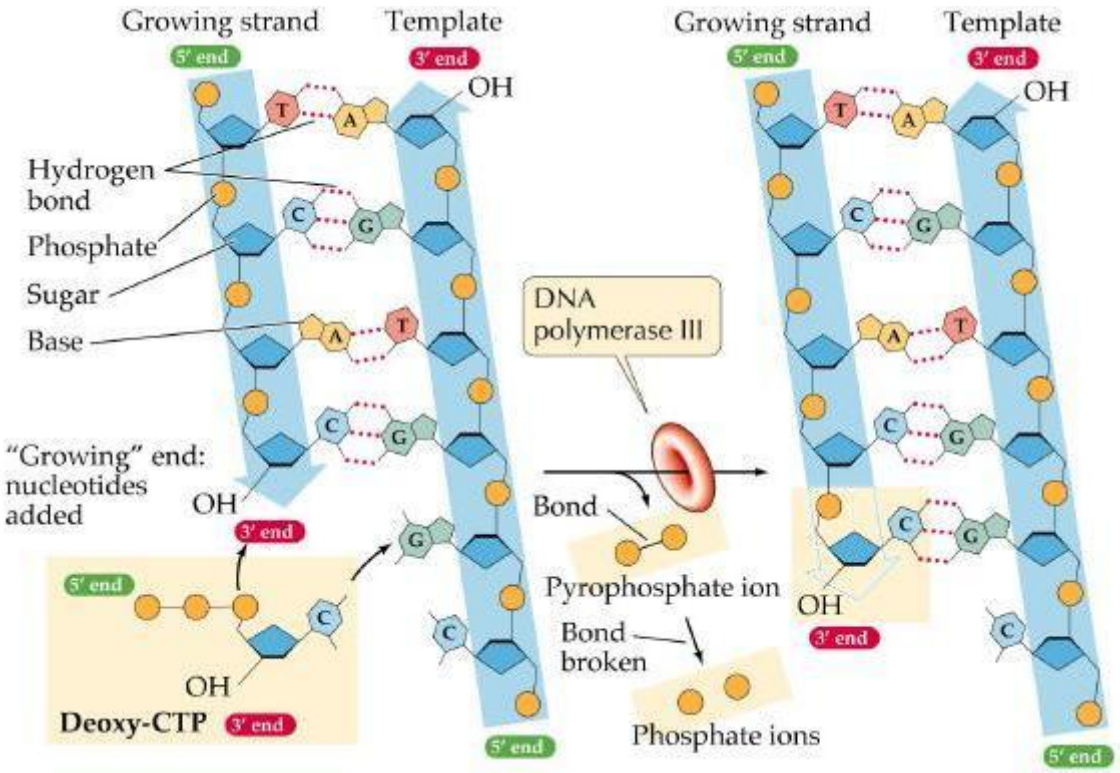
Meselson-Stahl experiment

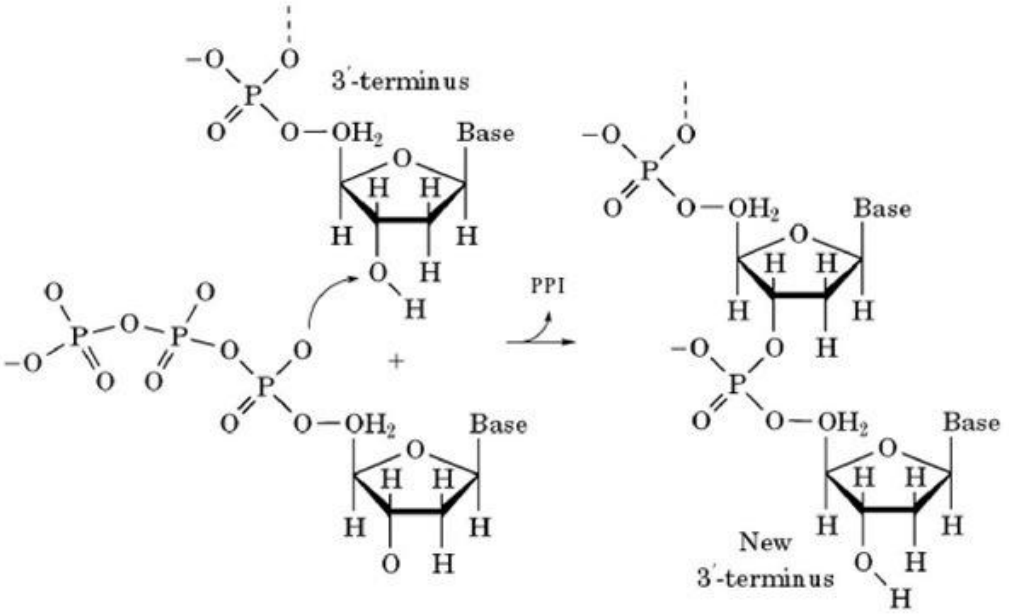
- The Meselson–Stahl experiment was an experiment by Matthew Meselson and Franklin Stahl in 1958 which supported the hypothesis that DNA replication was semiconservative
- Nitrogen is a major constituent of DNA. 14N is by far the most abundant isotope of nitrogen, but DNA with the heavier (but non-radioactive) 15N isotope is also functional
- *E. coli* cells with only ^{15}N in their DNA were transferred to a ^{14}N medium and allowed to divide; the progress of cell division was monitored by microscopic cell counts
- DNA was extracted periodically and was compared to pure ^{14}N DNA and ^{15}N DNA. After one replication, the DNA was found to have intermediate density

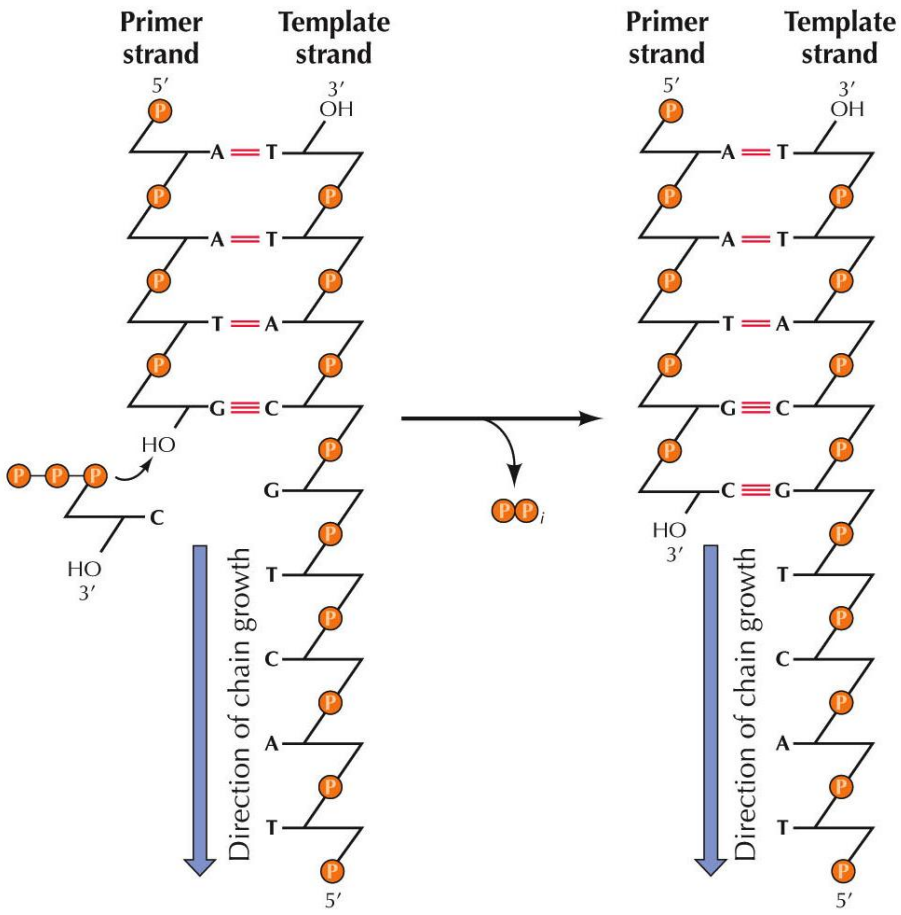




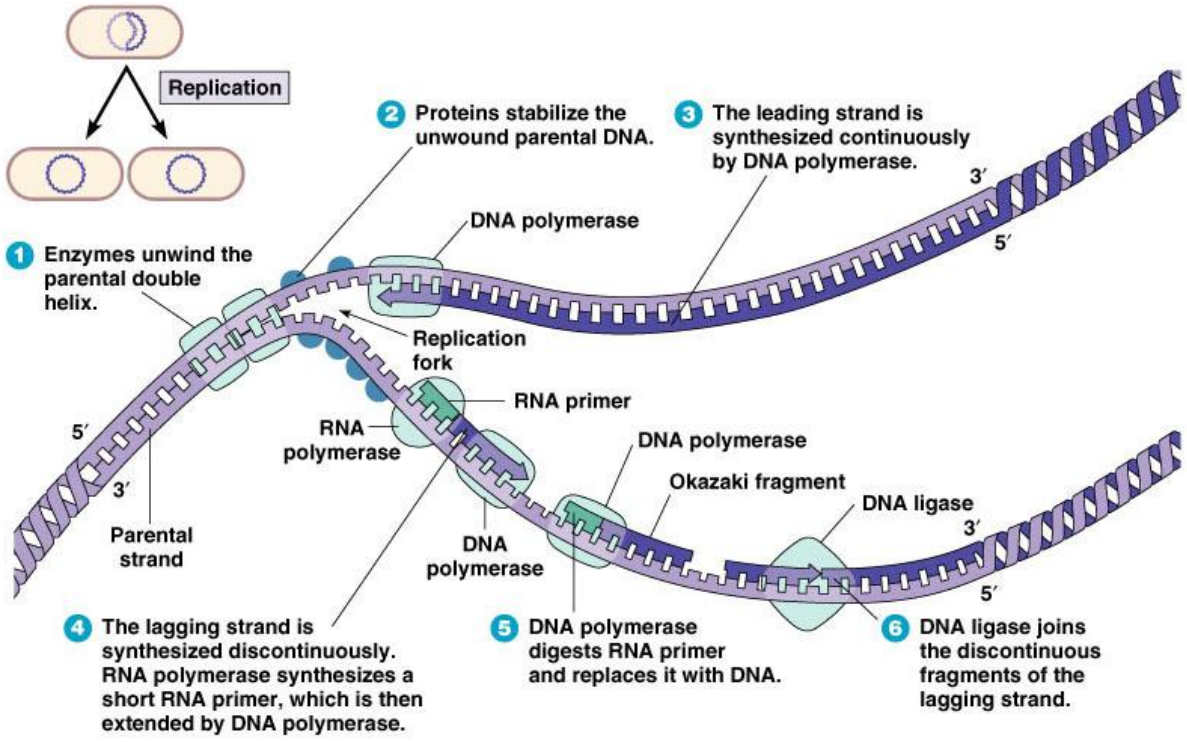
Replication Diagrams

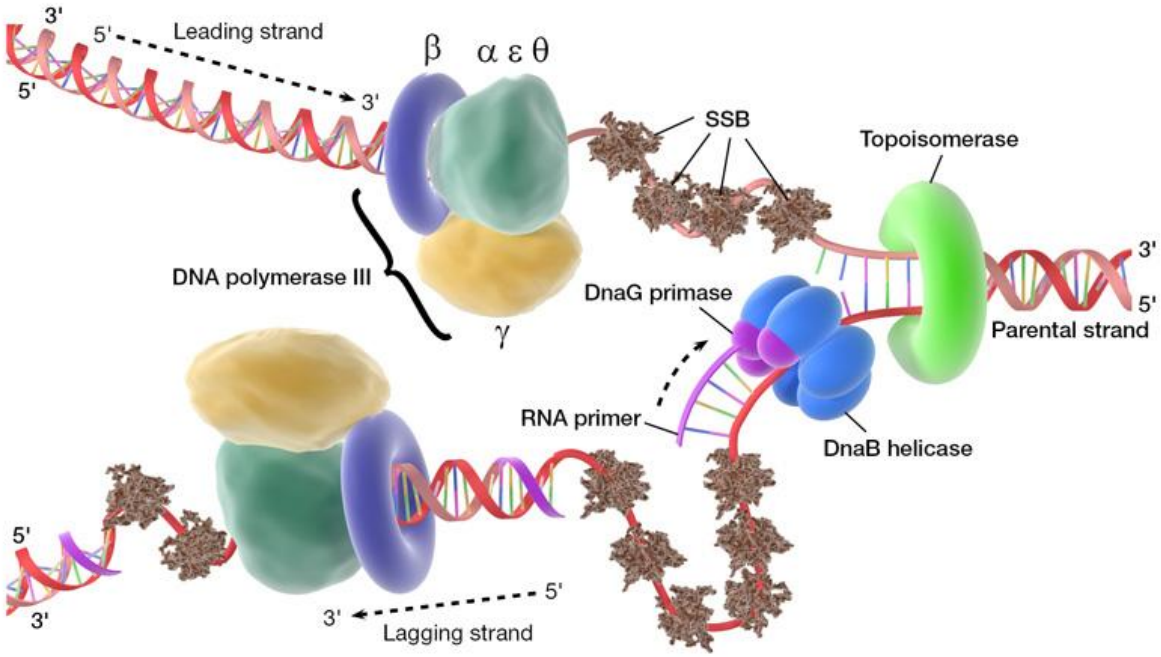






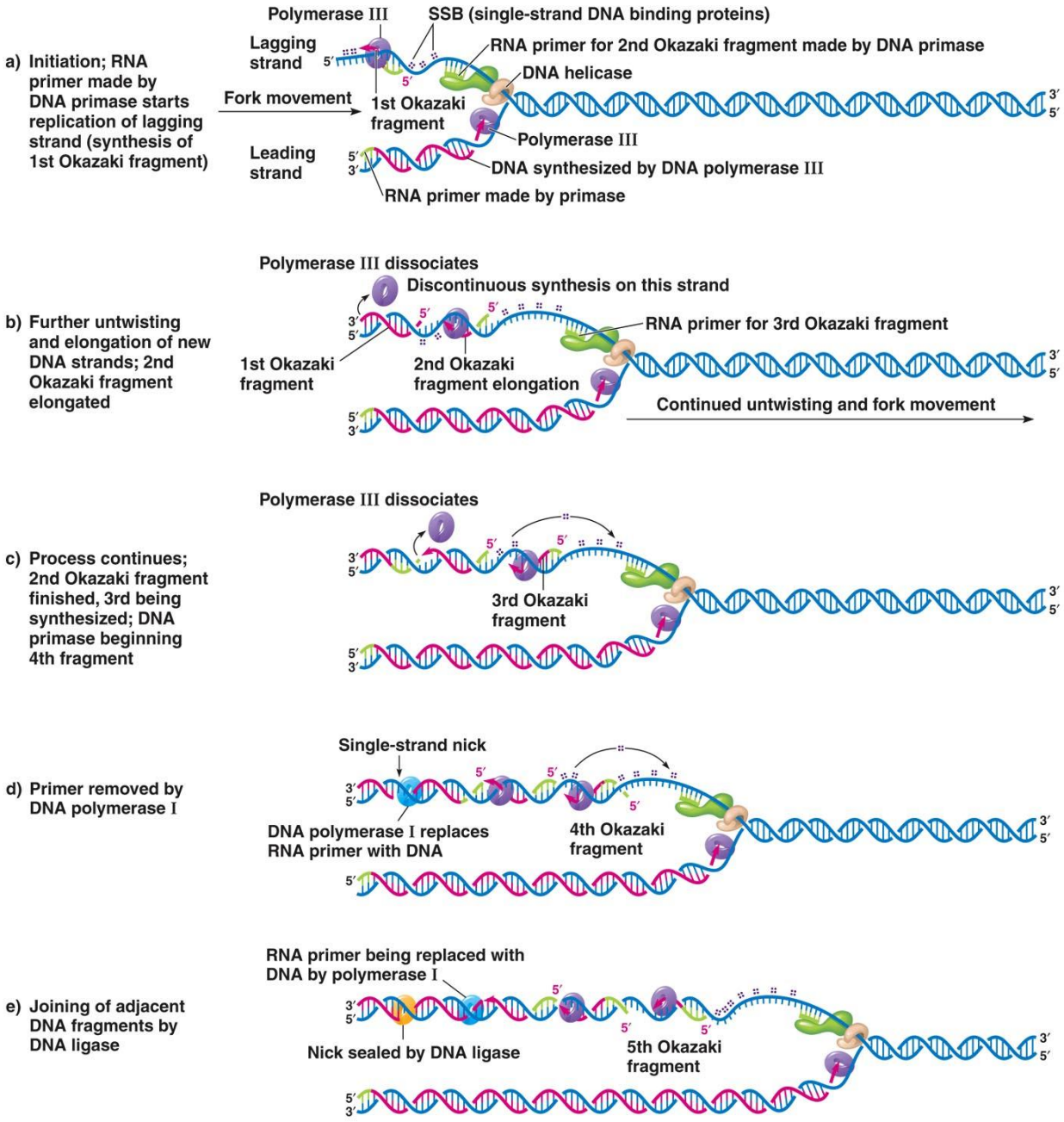
Replication Fork





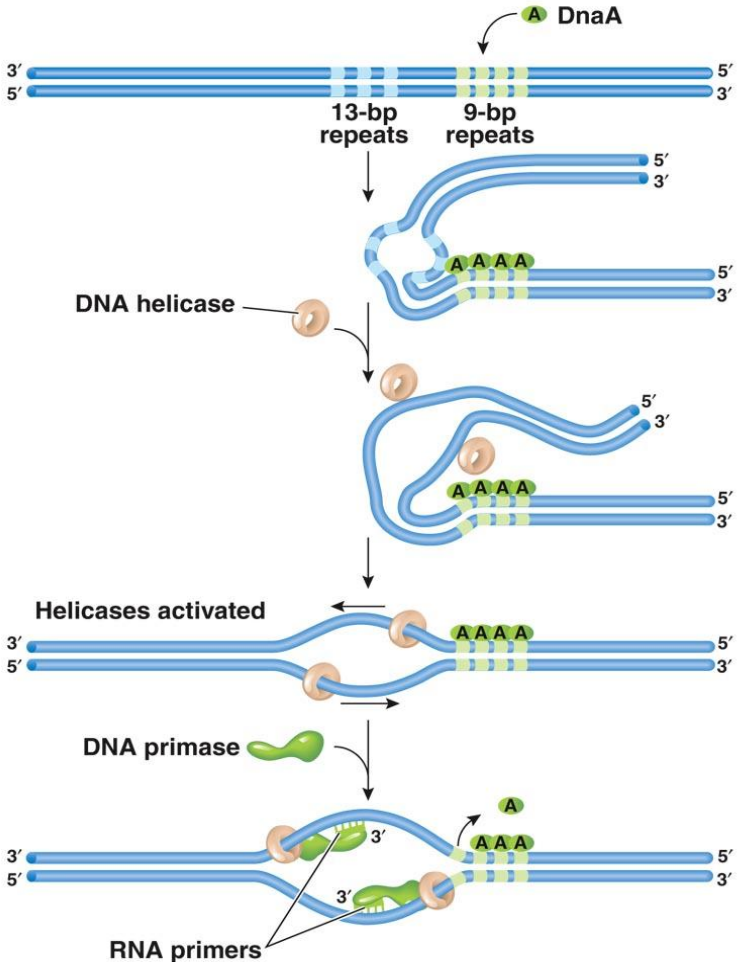
Replication Enzymes

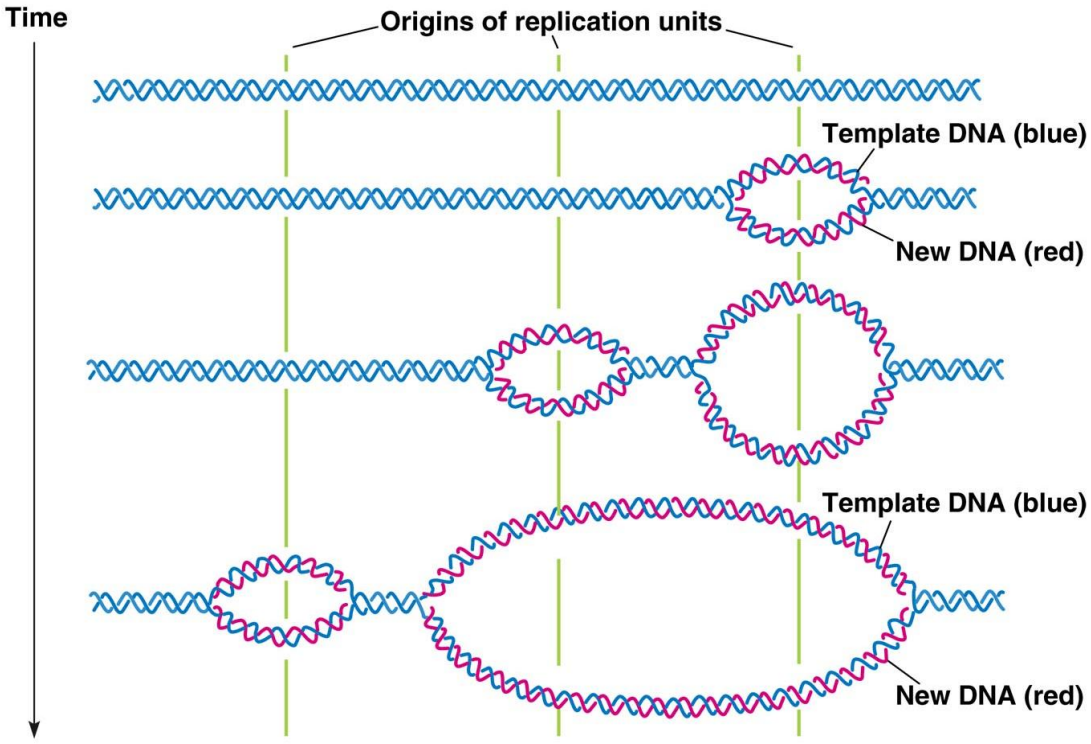
DNA Helicase	Also known as helix destabilizing enzyme. Unwinds the DNA double helix at the Replication Fork.
DNA Polymerase	catalyzes the formation of phosphodiester bond between 3'-OH group of the deoxyribose on the last nucleotide and 5'-phosphate of incoming dNTP. Cannot add nucleotides in other direction (3' -> 5'). Also performs proof-reading and error correction. DNA polymerase III extends the DNA chain in (5' -> 3'). DNA polymerase I replaces RNA primer with DNA, and has all functions (exo, both directions)
DNA clamp	A protein which prevents DNA polymerase III from dissociating from the DNA parent strand.
Single-Strand Binding (SSB) Proteins	Bind to ssDNA and prevent the DNA double helix from re-annealing after DNA helicase unwinds it, thus maintaining the strand separation.
Topoisomerase	Relaxes the DNA from its super-coiled nature.
DNA Ligase	Re-anneals the semi-conservative strands and joins Okazaki Fragments of the lagging strand.
Primase	Provides a starting point of RNA (or DNA) for DNA polymerase to begin synthesis of the new DNA strand.
DNA gyrase	Relieves strain while double-strand DNA is being unwound by helicase, prevents tangling



Origins of Replication

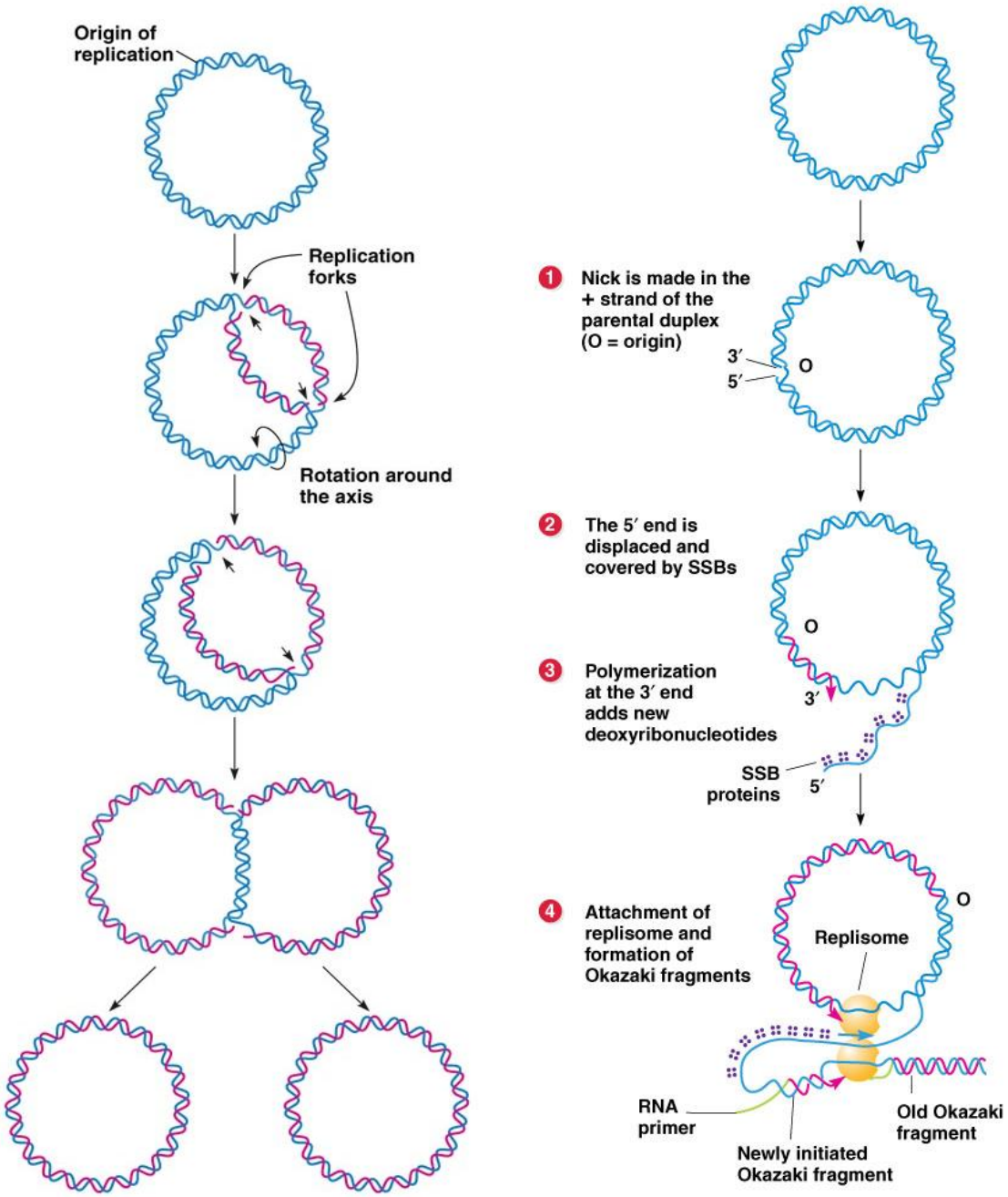
- The origin of replication (also called the replication origin) is a particular sequence in a genome at which replication is initiated
- In E. coli, the single origin of replication consists of three A-T rich 13-mer repeats and four 9-mer repeat
- The eukaryote chromosome has thousands of origins of replication, with each replication bubble having two replication forks
- Each bubble expands in both directions, and bubbles fuse when they meet


DnaA
3′
5′
5′
3′
13-bp repeats
9-bp repeats
5′
3′
A A A A
5′
3′
DNA helicase
5′
3′
A A A A
5′
3′
Helicases activated
3′
5′
A A A A
5′
3′
DNA primase
A
3′
A A A
3′
5′
3′
5′
3′
RNA primers


Time
Origins of replication units
Template DNA (blue)
New DNA (red)
Template DNA (blue)
New DNA (red)

Types of Circular Replication

- Rolling-circle replication (right) is initiated by an initiator protein which nicks one strand of the double-stranded, circular DNA molecule at a site called the double-strand origin, or DSO
- The initiator protein remains bound to the 5' phosphate end of the nicked strand, and the free 3' hydroxyl end is released to serve as a primer for DNA synthesis by DNA polymerase III
- Using the unnicked strand as a template, replication proceeds around the circular DNA molecule, displacing the nicked strand as single-stranded DNA, which can then be completed as a lagging strand
- Continued DNA synthesis can produce multiple single-stranded linear copies of the original DNA in a continuous head-to-tail series called a concatemer
- Commonly found in plasmids and bacteriophages

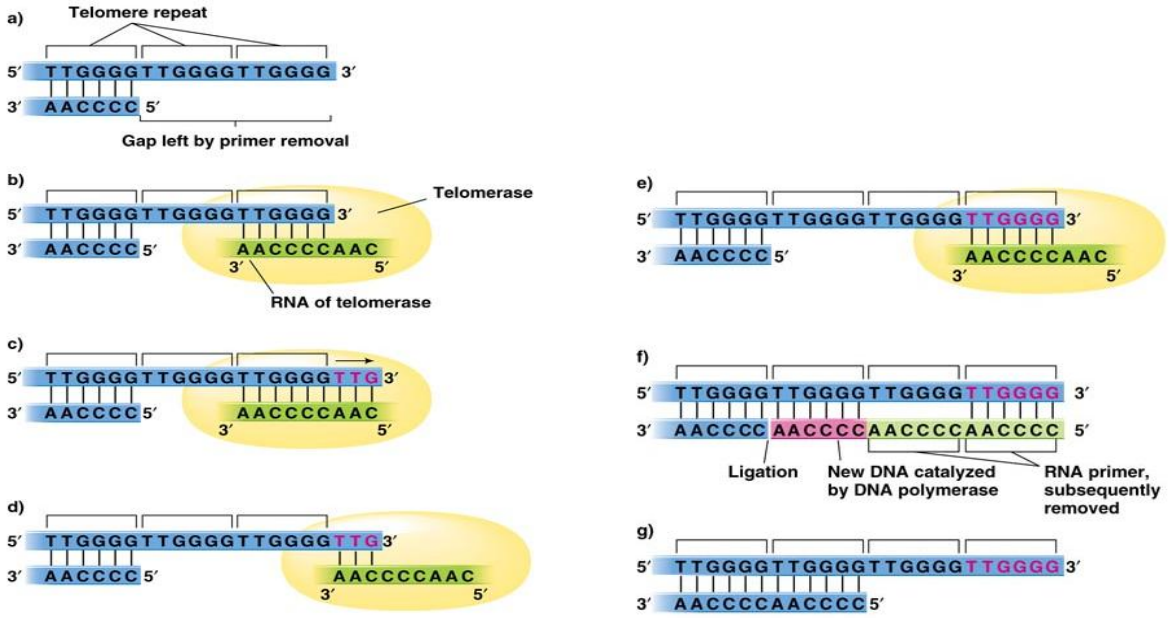


Time of Replication

- E. coli (bacteria): genome size 5 million bp with DNA polymerase adding 850 nucleotides/sec, so replicates in approximately 20 minutes
- Human nucleus: genome size 6 billion bp with DNA polymerase adding 60-90 nucleotides/sec, but replicates in only a few hours. This speedup is possible because of multiple origins of replications

Telomeres

- Normal DNA replication provides no way to complete the 5' end of the daughter strand
- If not fixed, this would result in shorter and shorter chromosomes
- A telomere is a region of repetitive nucleotide sequences at each end of a chromatid. For vertebrates, the sequence of nucleotides in telomeres is TTAGGG
- Telomerase is a reverse transcriptase, which is a class of enzyme that creates single-stranded DNA using single-stranded RNA as a template. It carries a template for synthesising an extension of the template strand of the DNA
- Telomerase activity is necessary for the immortality of many cancer types and is inactive in somatic cells, signifying that telomerase inhibition could selectively repress cancer cell growth with minimal side effects



Proof Reading

- Errors in base pairing: one in 10,000 bases
- Errors in the completed replication process: one in 1 billion bases
- Thus, there must be some repair/checking mechanism
- This is done by the DNA polymerase, which proof reads for incorrect pairing of bases
- If incorrect base added, it removes it by its 3'-> 5' exonuclease activity then it adds the right nucleotide

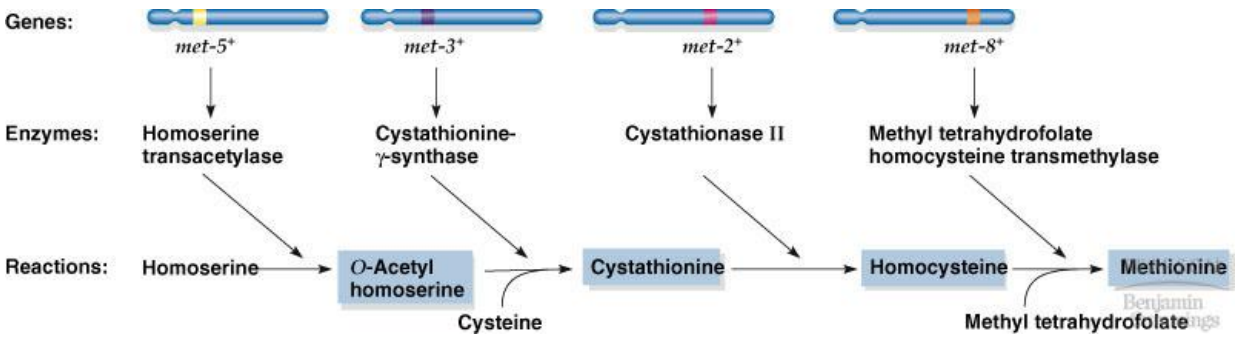
Gene Function

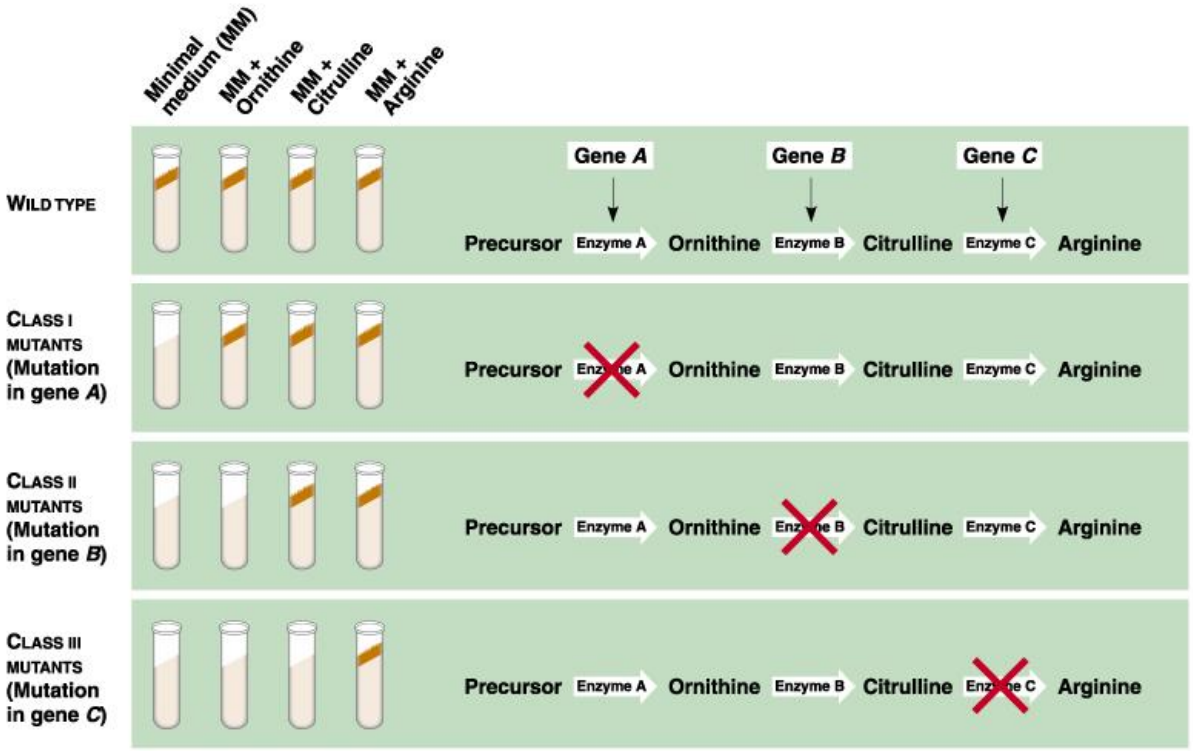
Archibald Garrod

- A British physician who in 1908 studied people whose urine turned black on exposure to air (alkaptonuria), and the inheritance of this trait in large number of families
- He found that it is genetically inherited disease, and postulated that symptoms were a reflection of the individuals inability to make a particular enzyme (homogentisic acid)
- He was the first to suggest that genes dictate phenotype through enzymes

One-Gene-One-Enzyme Hypothesis

- Beadle and Tatum in 1942 studied the bread mold *Neurospora crassa*, which reproduces asexually by producing haploid spores (conidia) and has two mating types, *A* and *a*
- Wild type can grow on minimal media (prototroph), but Beadle & Tatum realized it was possible to isolate nutritional mutants that could not survive on minimal medium (auxotroph)
- They treated conidia with X-rays (mutagen) and crossed the conidia with prototrophic strain of opposite mating type
- They then sub-cultured into different tubes having different amino acids added, thus strains that were mutants for particular amino acids were identified
- The strains that grew only when supplemented with particular amino acid were then sub-cultured into different tubes supplemented with individual components required to make that amino acid, and thus strains that were mutants for particular amino acid precursor were identified
- In their results, Beadle & Tatum could distinguish between different methionine auxotrophs
- They thus reasoned that different mutants must be blocked at different steps in the biosynthesis pathway, and each mutant lacked a different enzyme
- Thus they formulated the one-gene-one-enzyme hypothesis, whereby the function of a gene was to dictate production of a specific enzyme





Phenylketonuria

- Found in 1/12,000 Caucasian births
- Caused by a mutation in phenylalanine hydroxlase gene which prevents conversion of Phe to Tyr, so Phe accumulates and converted to phenylpyruvic acid
- Can be managed with dietary restrictions
- The PAH gene is located on chromosome 12

Lesch-Nyham Syndrome

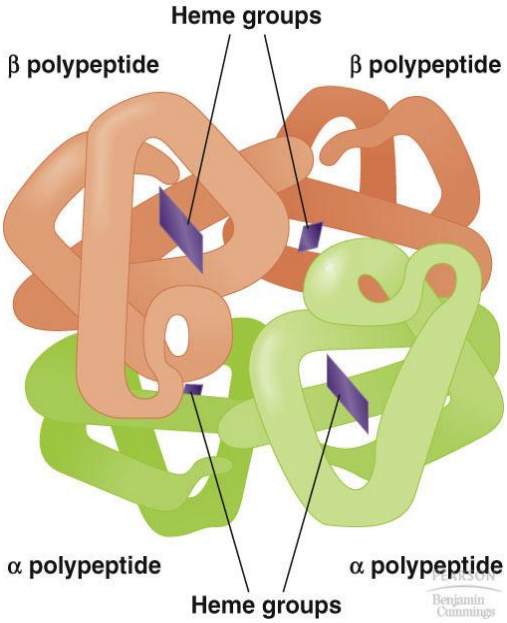
- Found in 1/10,000 males
- Caused by mutations on the HPRT gene located on the X chromosome
- Fatal recessive human trait caused by deficiency in hypoxanthine-guanine phosphoribosyl transferase
- As a result, purines accumulate and convert to uric acid, causing severe neurological symptoms

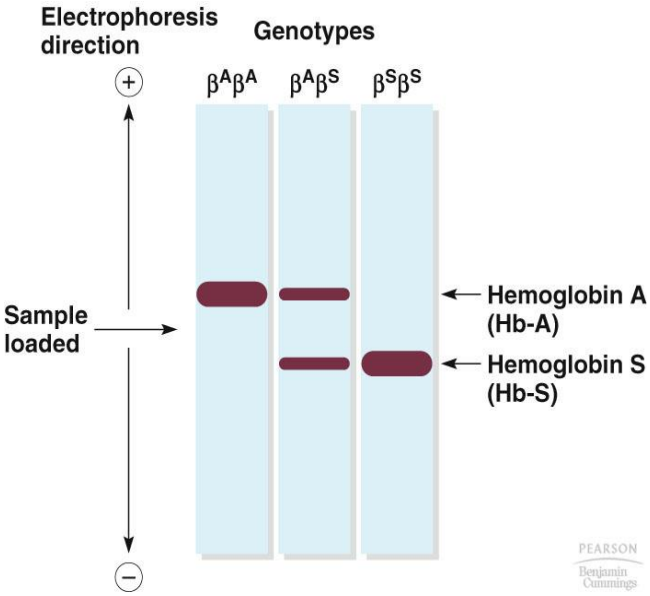
Tay-Sachs Disease

- Rare in general population but more frequent in Ashkenazi Jews: 1/3,600
- Gene defect in Lysosomal enzyme *hexA* which encodes N-acetylhexosaminidase A
- Symptoms – reaction to sharp sounds & cherry-coloured spot on retina
- Caused by a genetic mutation in the *HEXA* gene on chromosome 15

Sickle-Cell Anemia

- Recessive mutation in hemoglobin gene causing red blood cells to change shape
- Sickle-cell trait is a less severe form of the disease





Cystic Fibrosis

- Found in 1/2000 newborn Caucasions
- Causes pancreatic, pulmonary and digestive dysfunction, and very high viscosity mucus
- Caused by three base deletion in cystic fibrosis transmembrane conductance regulator (CFTR), which is a 1,480 amino acid long membrane protein which serves as a chloride channel

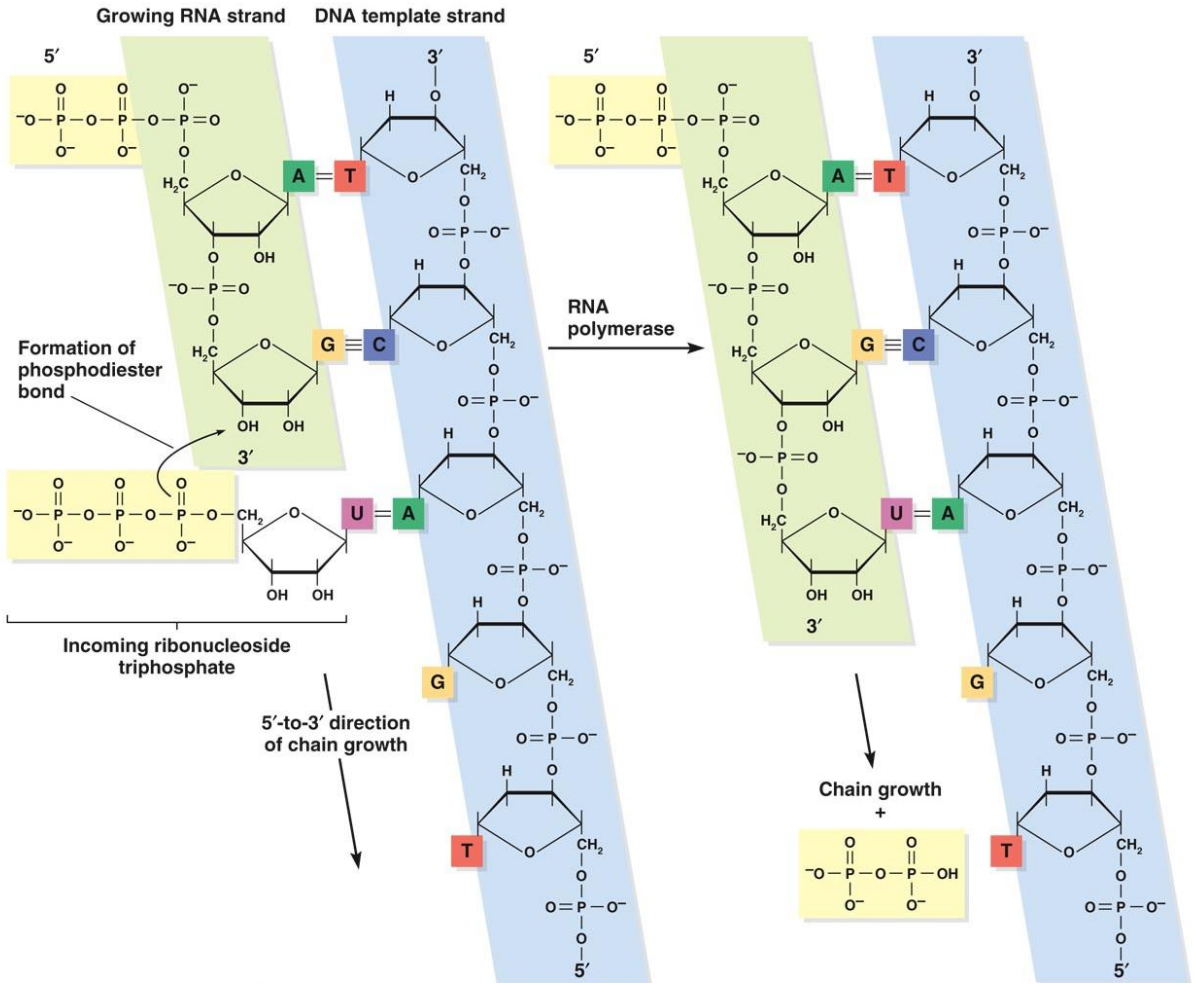
Transcription of RNA

The Structure of RNA

- Chemically similar to DNA
- Has 'ribose' instead of 'deoxyribose'
- Has 'uracil' instead of 'thymine'
- Single stranded

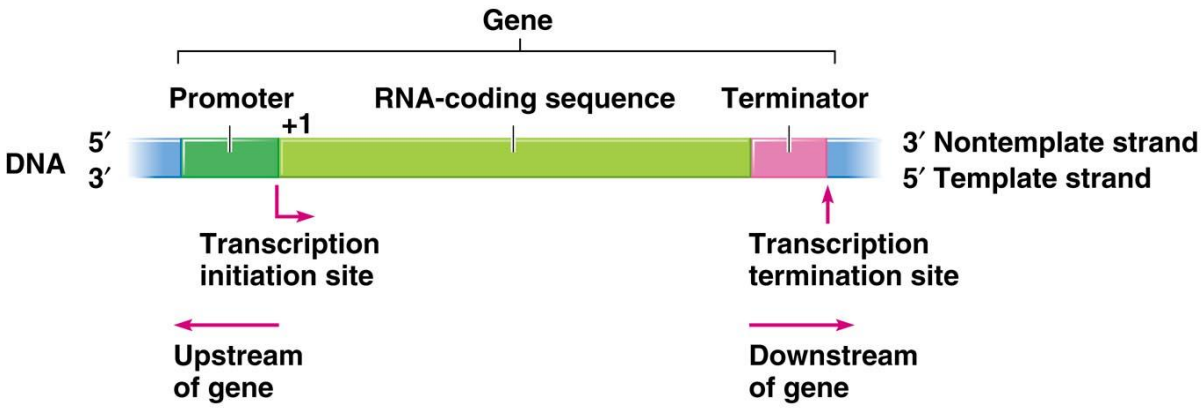
RNA Polymerase

- Pries the two strands of DNA apart
- Adds nucleotides to 3' OH only
- Energy comes from phosphodiester bond of incoming rNTP
- Does not require primer



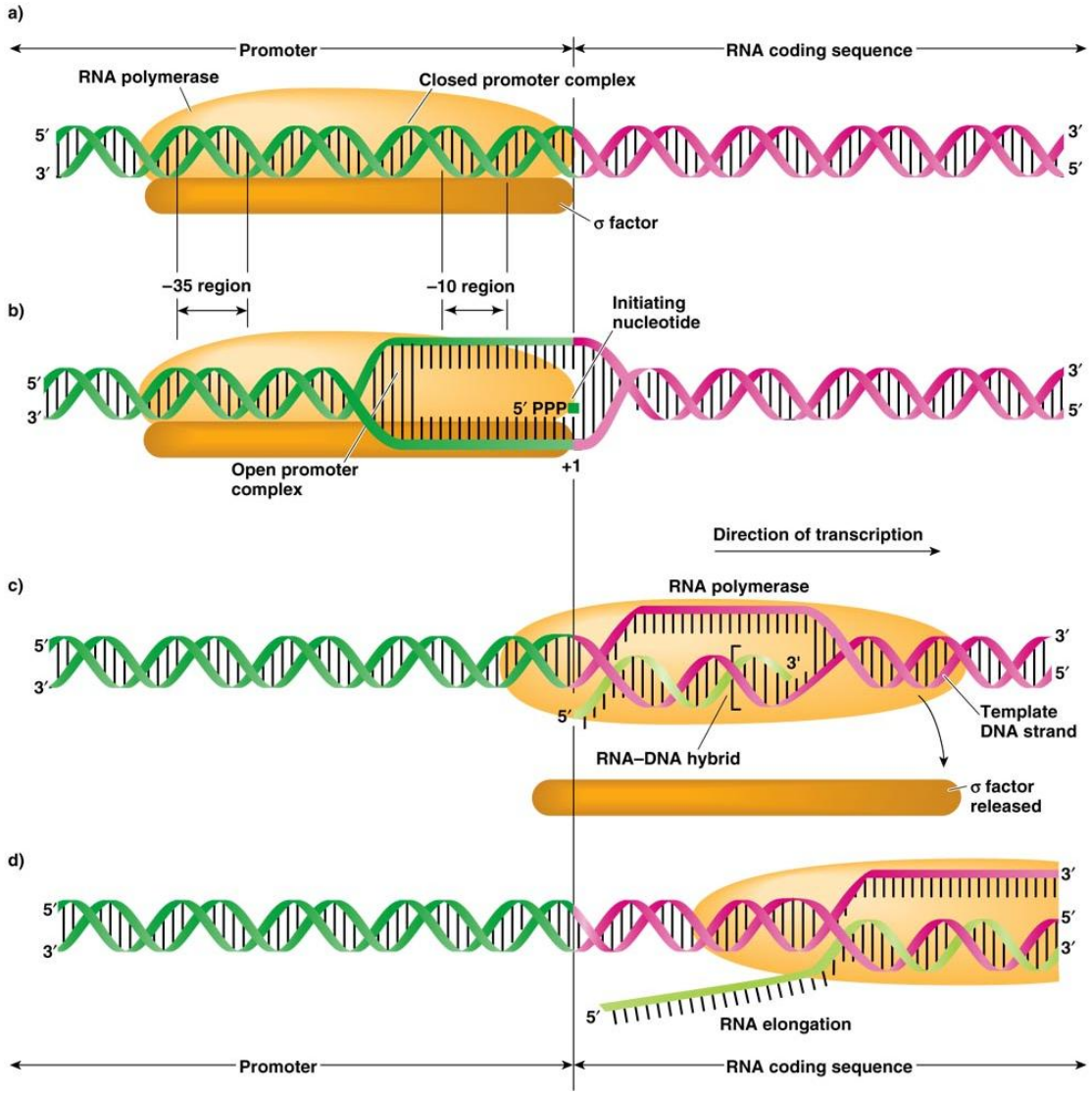
Promoters

- A region where RNA polymerase attaches & initiates transcription
- Determines which strand of the DNA is transcribed
- In prokaryotes RNA polymerase itself recognizes and binds promoter



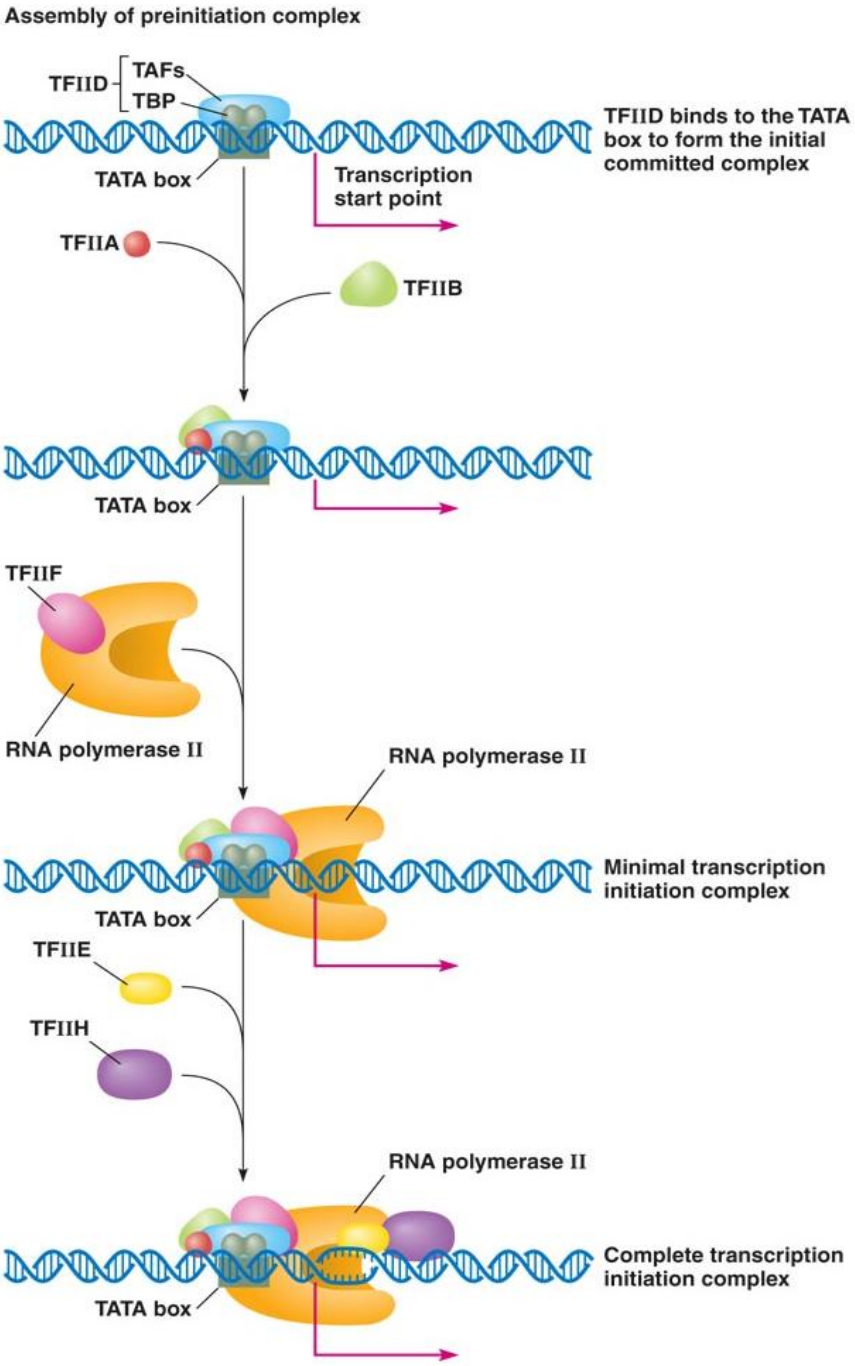
Initiation of Transcription in Prokaryotes

- Prokaryote promoter region consists of two conserved regions: -35 region (TTGACA) and -10 region (TATAAT)
- A **sigma factor** (**σ factor**) is a protein needed only for initiation of RNA synthesis. It is a bacterial transcription initiation factor that enables specific binding of RNA polymerase to gene promoters
- The specific sigma factor used to initiate transcription of a given gene will vary, depending on the gene and on the environmental signals needed to initiate transcription of that gene
- RNA polymerase then binds to the DNA strand at -35 and -10, forming the closed promoter complex
- The closed promoter complex consists of the RNA polymerase and/or accessory proteins attached to the promoter, before the DNA has been opened up by breakage of base pairs
- A holoenzyme then untwists DNA in -10 region, forming the open promoter complex
- The RNA polymerase then binds upsetream at -55 to +20 (75 bp)



Initiation of Transcription in Eukaryotes

- **Core Promoter** typically within 50bp upstream, includes binding site for RNA polymerase II (RNA I for rRNA and RNA III for tRNA) and general transcription factor binding sites, e.g. 'TATA' box
- **Proximal promoter located -**50 to -200 bp upstream of, and includes specific transcription factor binding sites, e.g. *cat* box (CAAT): -75bp and 'GC' box (GGGCGG): -90bp
- Transcription factors are a collection of proteins which mediate binding of RNA polymerase
- Only after binding of certain factors to the promoter does the RNA polymerase bind, forming the complete transcription initiation complex
- TATA box important in the formation of initiation complex



Elongation

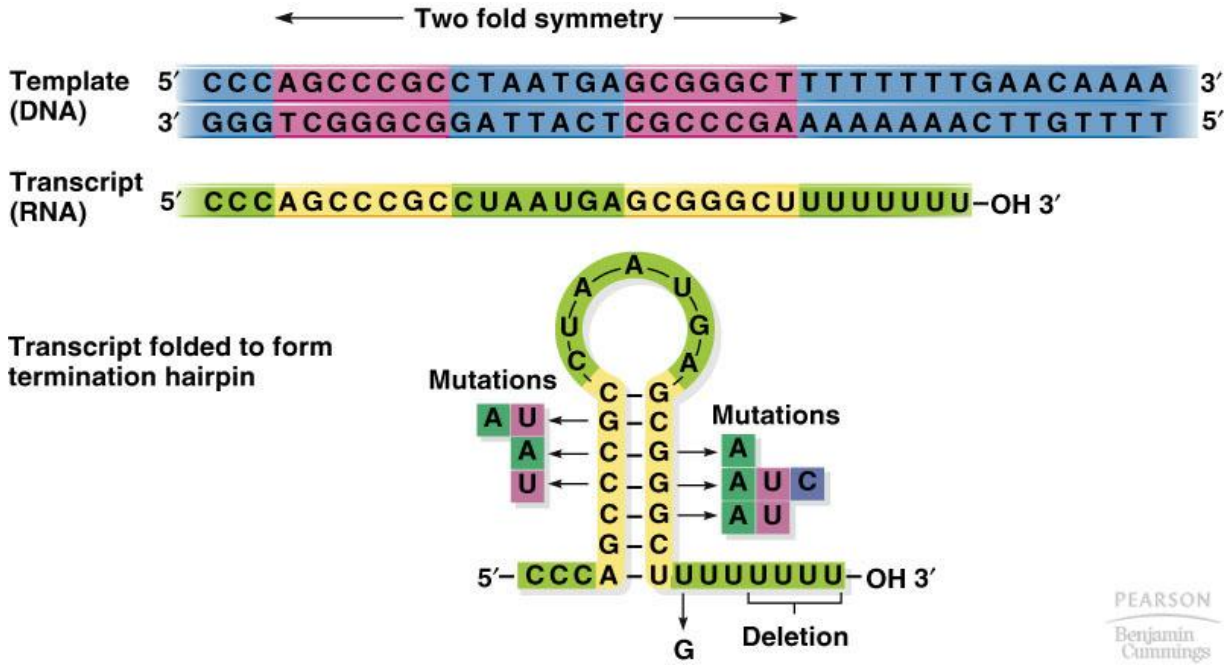
- RNA polymerase extends the RNA strand in 5’ to 3’ direction
- As RNA polymerase moves the σ factor dissociates
- *E. coli* synthesises RNA at 40 bases/sec
- In eukaryotes moves at about 60 nucleotides/sec
- As RNA polymerase moves forward, RNA behind dissociates and DNA double helix re-forms
- RNA polymerase proofreads as it moves forward

Types of RNA Polymerase

- In prokaryotes, one type of RNA polymerase synthesizes all types of RNAs: mRNA (messenger RNA), rRNA (ribosomal RNA), tRNA (transfer RNA), snRNA (small nuclear RNA)
- In eukaryotes there are three types of RNA polymerase. RNA polymerase II transcribes mRNA

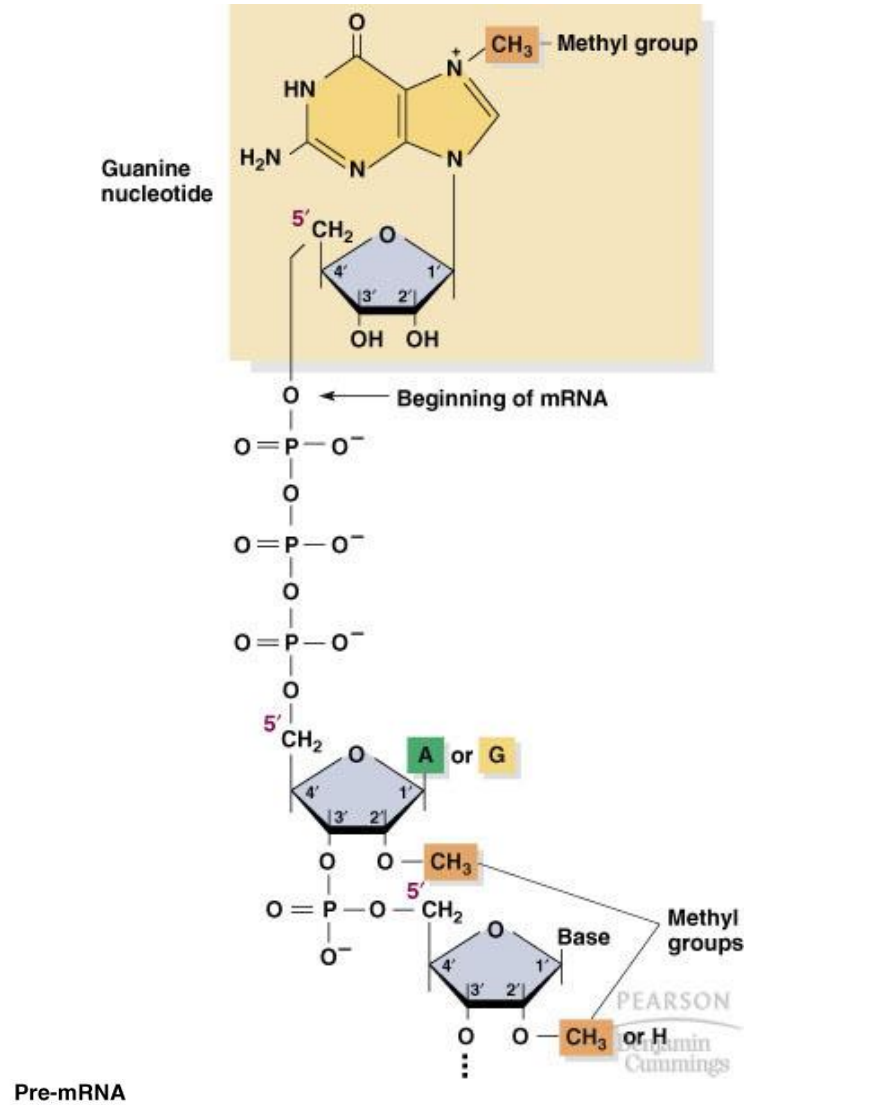
Termination

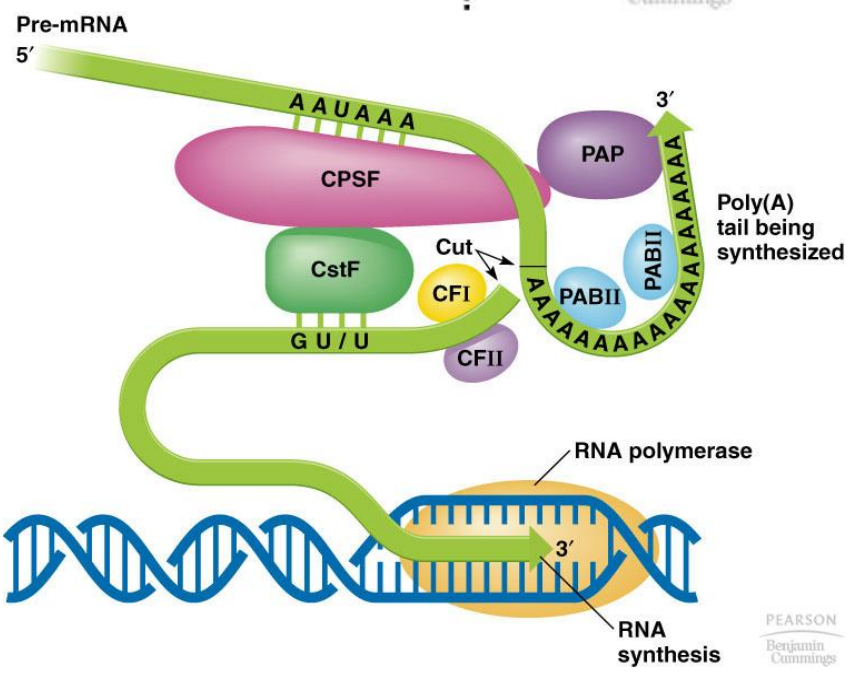
- Transcription continues until RNA polymerase reaches the termination signal
- In prokaryotes it stops immediately
- In eukaryotes RNA polymerase continues 10-35 nucleotides past the termination signal, the pre-mRNA is cut free and then a poly A tail is added

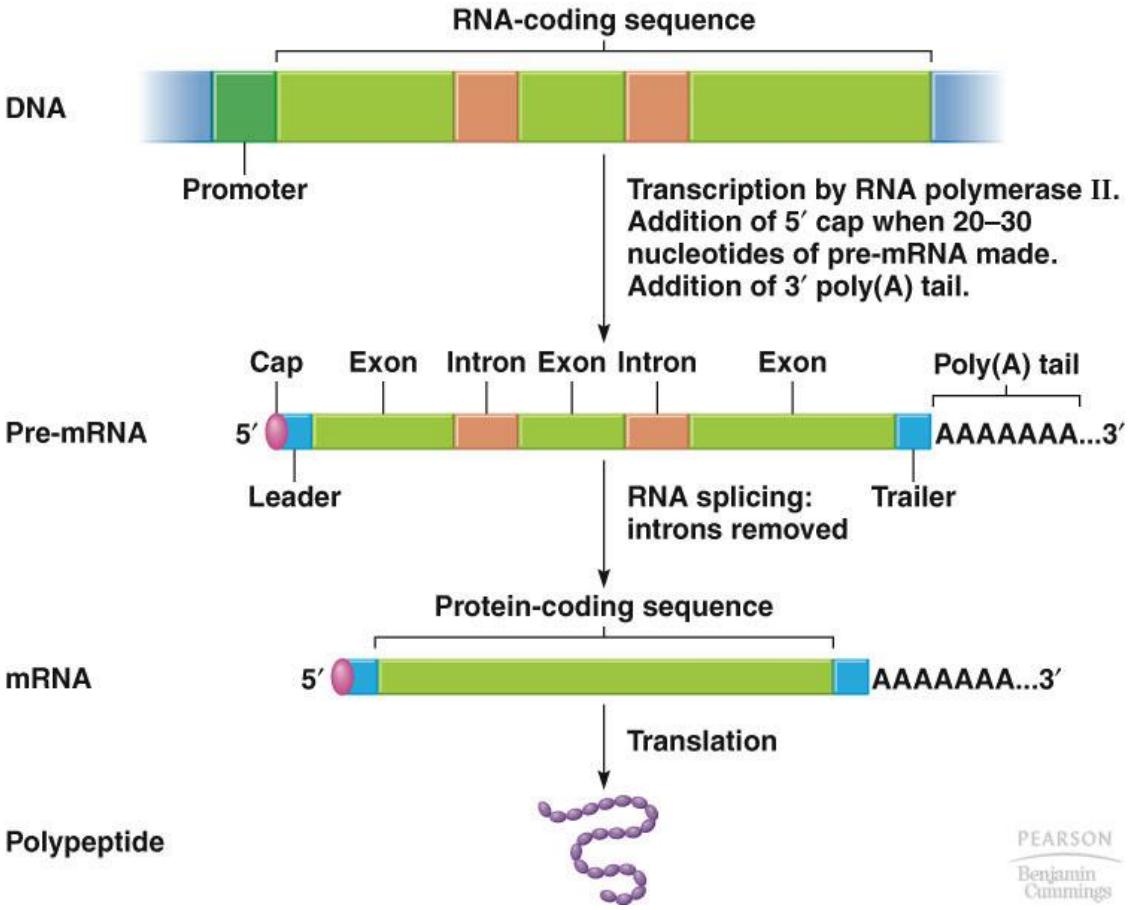


Modification of mRNA Transcripts

- 5' Cap: capping enzyme adds a 5’ cap of methylated guanine, which protects mRNA from degradation by exonucleases and facilitates ribosome binding
- 3' poly A tail: 50-250 adenine nucleotides added to 3’ end by poly (A) polymerase enzyme.This inhibits degradation and facilitates export from the nucleus
- Splicing: cut out introns and paste exons together. A lot to do, as average transcription unit 8000 bases but average protein only 400 amino acids

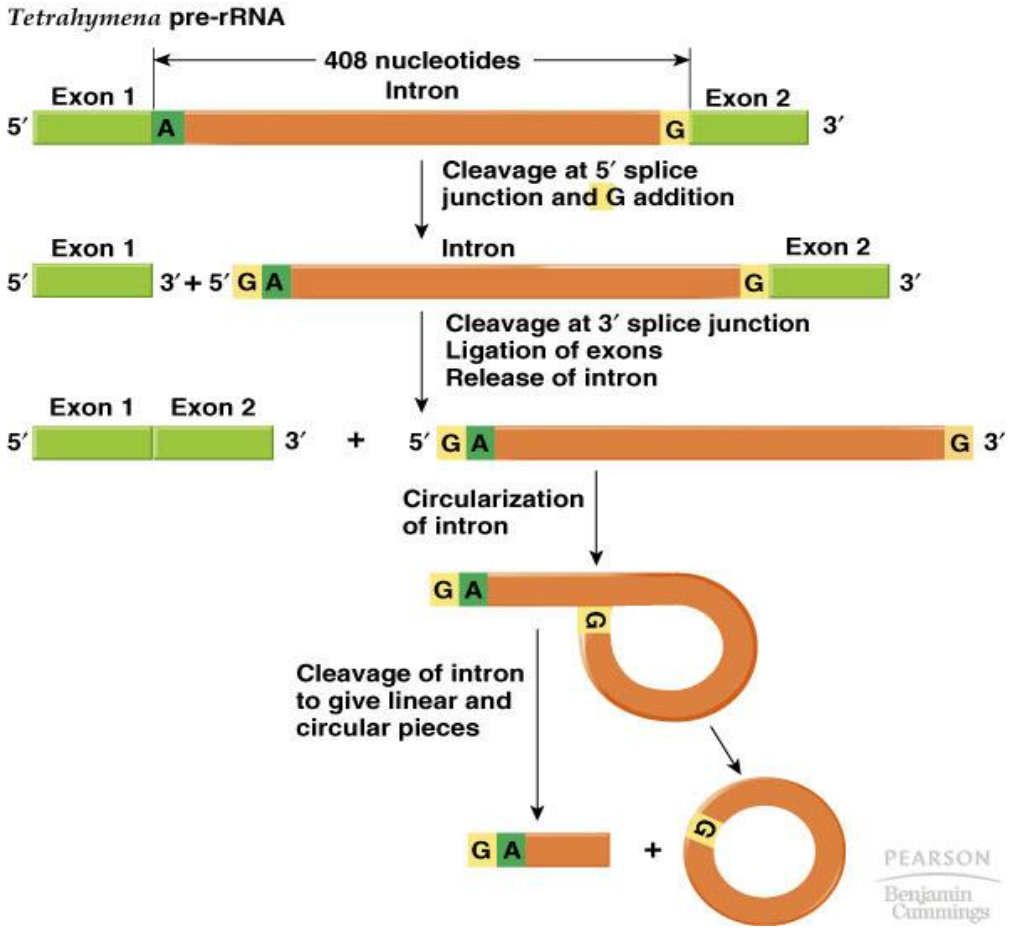

Guanine nucleotide
Methyl group
Beginning of mRNA
A or G
Methyl groups
Base
or H
PEARSON
Benjamin Cummings


Pre-mRNA
5′
AAUAAA
CPSF
PAP
3′
Poly(A) tail being synthesized
CstF
Cut
CFI
PABII
PABII
G U / U
CFII
RNA polymerase
3′
RNA synthesis
PEARSON
Benjamin Cummings

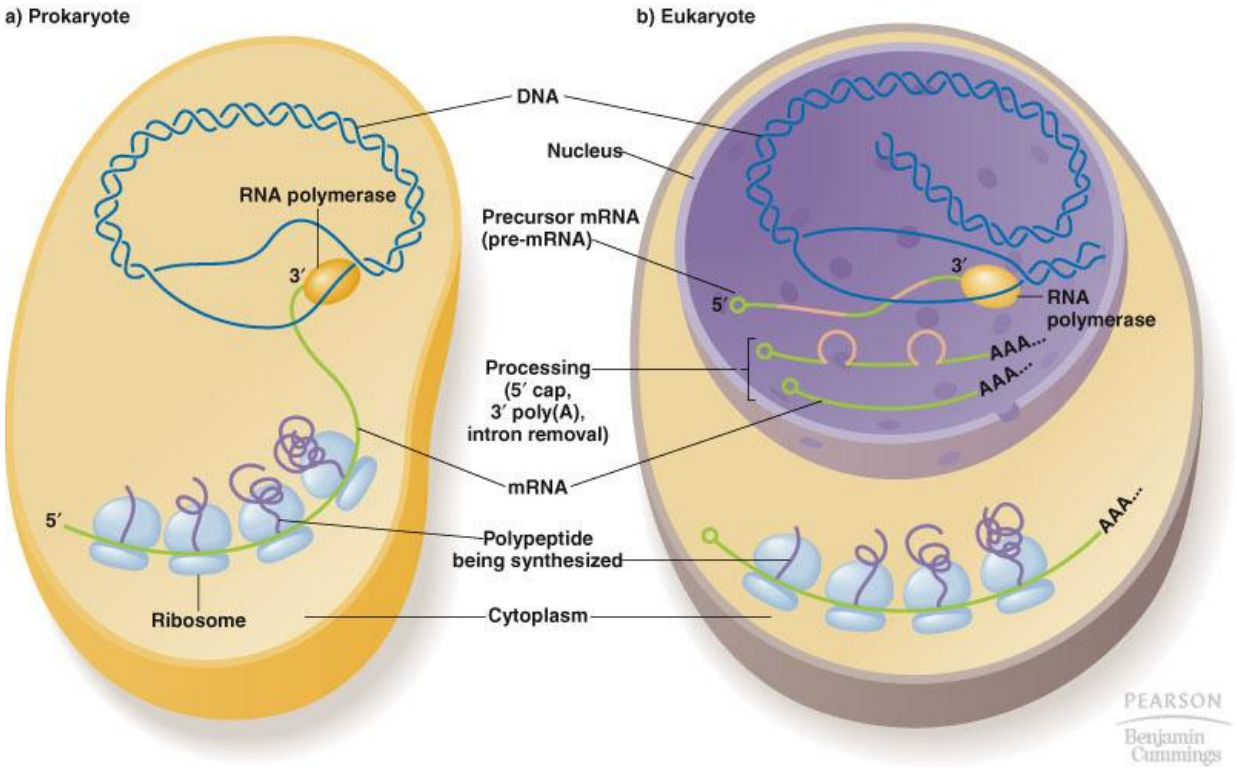


Intron Splicing

- Introns begin with 'GU' and end with 'AG'
- Small nuclear ribonucleoprotein particles called snRNPs (snurps) are RNA-protein complexes that combine with unmodified pre-mRNA and various other proteins to form a spliceosome, a large RNA-protein molecular complex upon which splicing of pre-mRNA occurs
- There are 5 principal snurps: U1, U2, U4, U5, U6
- Snurps join to from spliceosome , which cuts pre-mRNA at specific sites

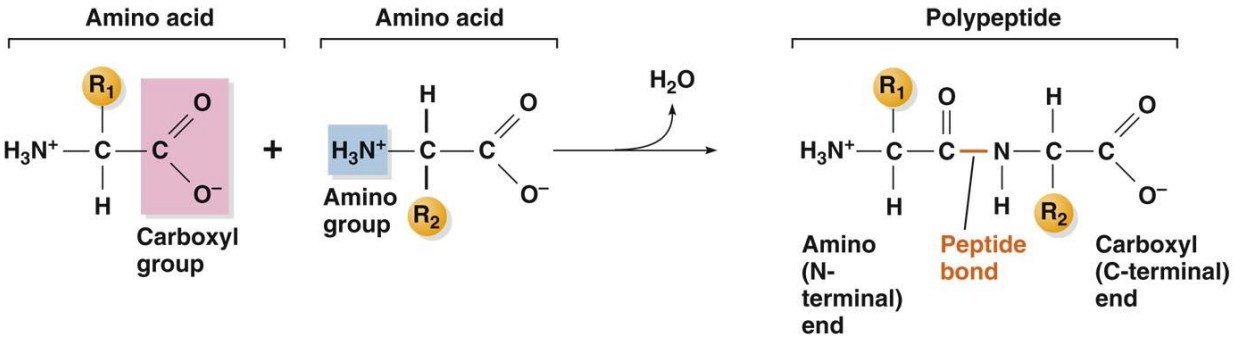


Difference in Transcription in Prokaryotes and Eukaryotes



Translation of Protein

Peptide Bond Formation

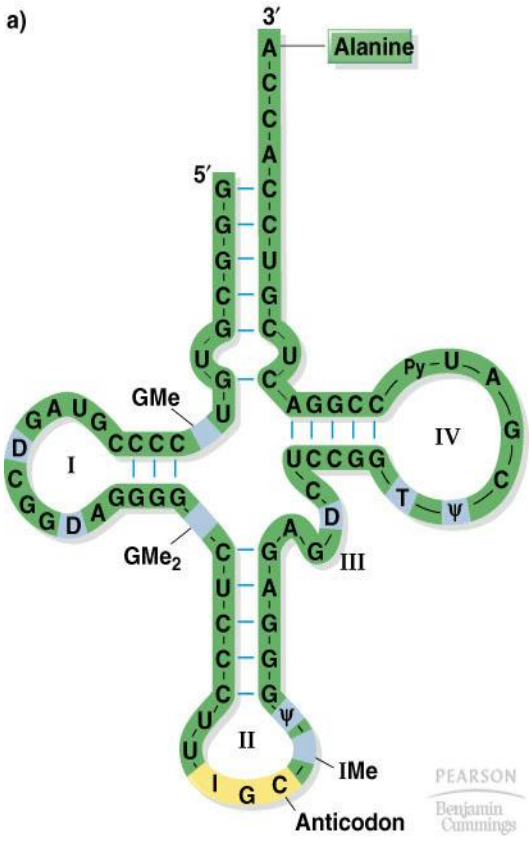


The Genetic Code

- The code is triplet, redundant, continuous, non-overlapping, and nearly universal
- First partly elucidated by the work of Nirenberg and Khorana in the 1960s

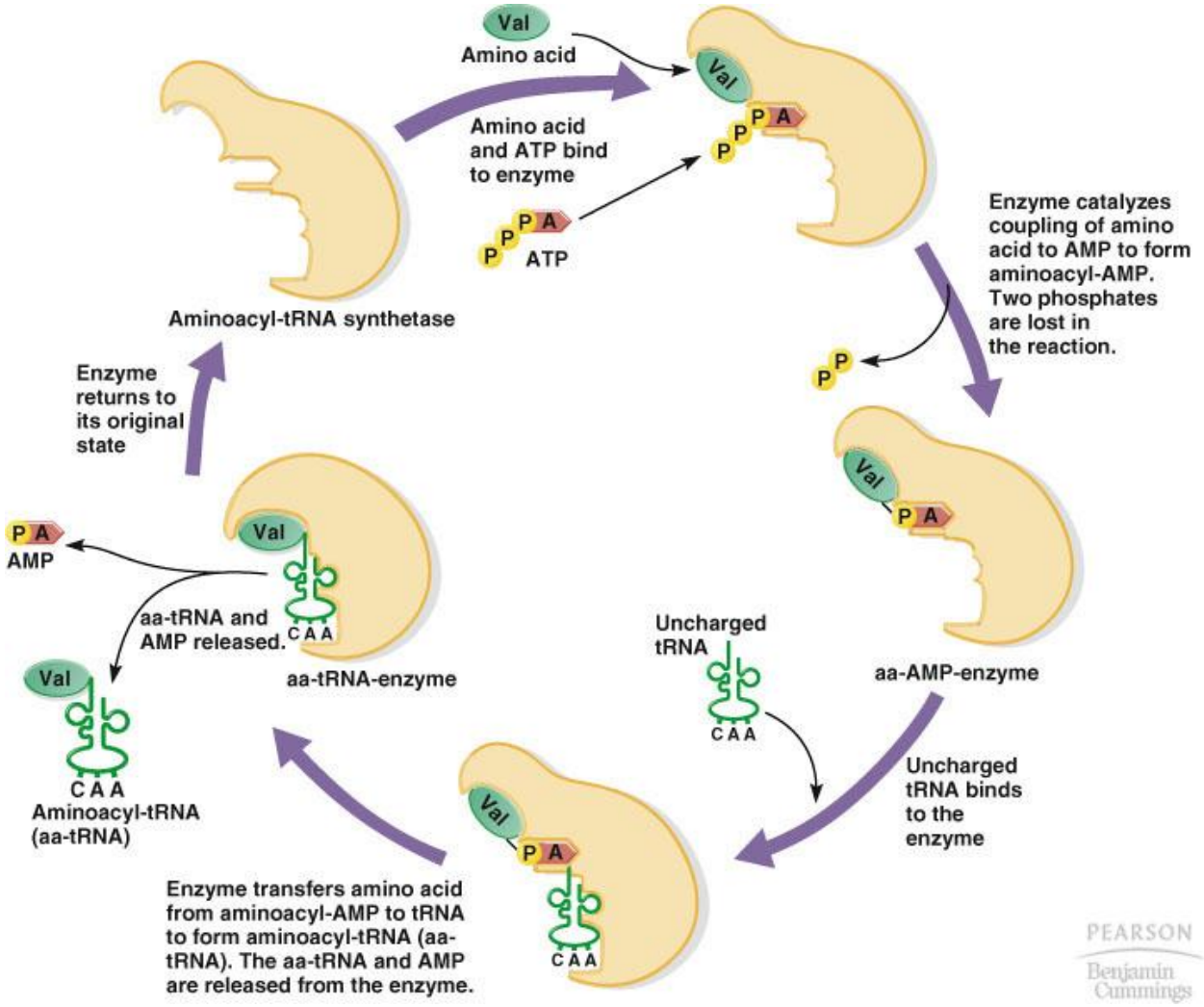
tRNA

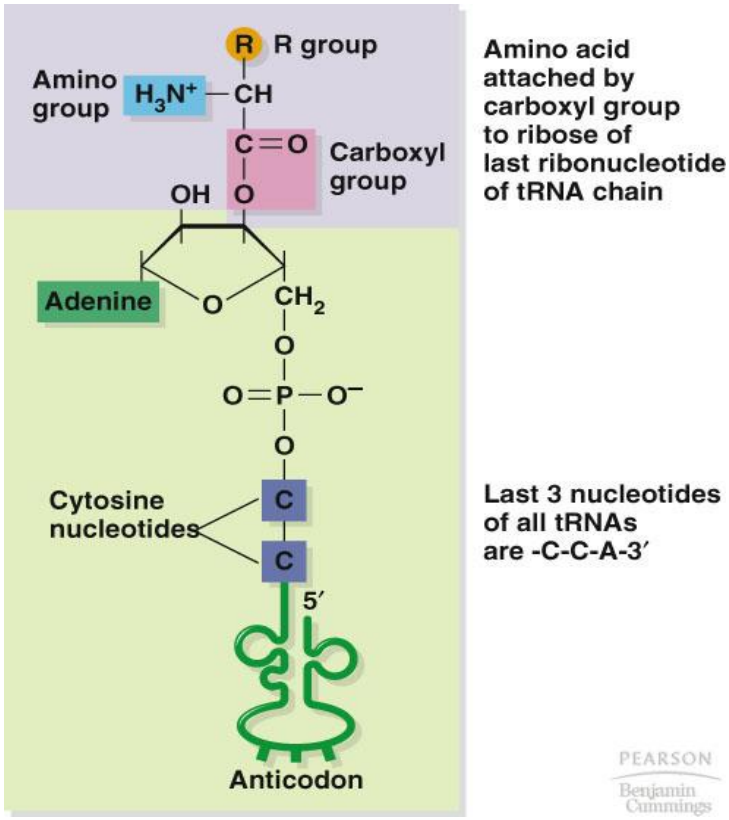
- tRNA transfers amino acids from the cytoplasm into the ribosome
- Cytoplasm is stocked with amino acids
- Ribosome adds each amino acid to the growing peptide
- It is single stranded, about 80 nucleotides long
- Each tRNA links a particular amino acid at one end, and with specific anticodon at the other
- Binding rules in the third position slightly relaxed, so for example inosine can bind to A, U, or C



Aminoacyl tRNA Synthetases

- An aminoacyl tRNA synthetase is an enzyme that catalyzes the esterification of a specific cognate amino acid or its precursor to one of all its cognate tRNAs to form an aminoacyl-tRNA
- The amino acid is attached by its carboxyl group to the ribose of the last ribonucleotide of the tRNA chain
- There are specific enzymes for each pairing, so 20 enzymes in the cell
- Energy from ATP hydrolysis



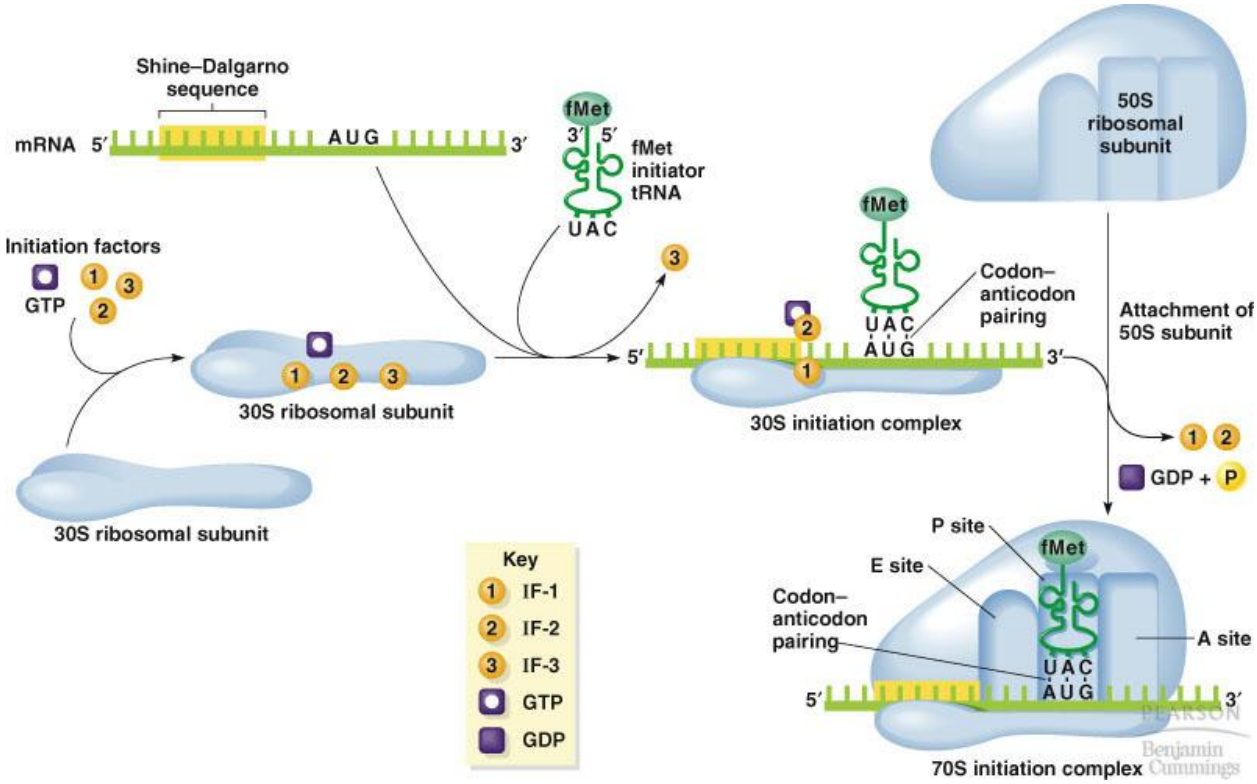


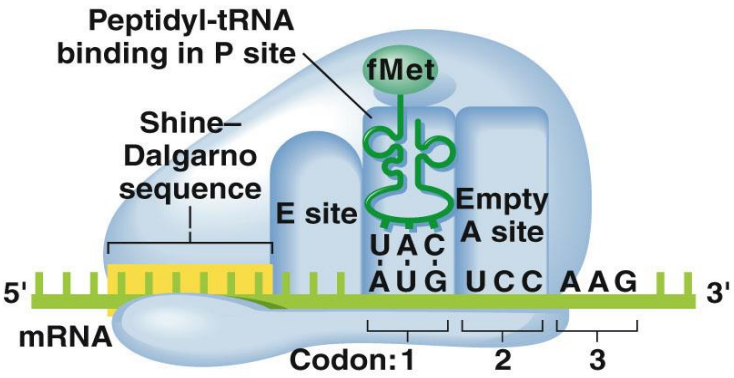
Ribosomes

- The ribosome is a large and complex molecular machine, found within all living cells, that serves as the site of biological protein synthesis (translation)
- It consists of 2 subunits - Large (50S): 23S rRNA & 5S rRNA and Small (30S): 16S rRNA
- Proteins and ribosomal RNA (rRNA) make up the ribosome
- In eukaryotes it is made in the nucleolus and exported to the cytoplasm
- Ribosome has 3 sites
    - E site: exit site
    - P site: peptidyl binding site
    - A site: aminoacyl-tRNA site
- The ribosome holds the tRNA and the mRNA together for the positioning of the amino acid to the growing peptide

Initiation of Translation

- Requires energy from GTP
- Brings mRNA, amino-acyl tRNA and the 2 ribosomal subunits together
- Small subunit binds to the mRNA upstream (5')
- Attachment of large subunit of the ribosome requires initiation factors, forming the 70S initiation complex

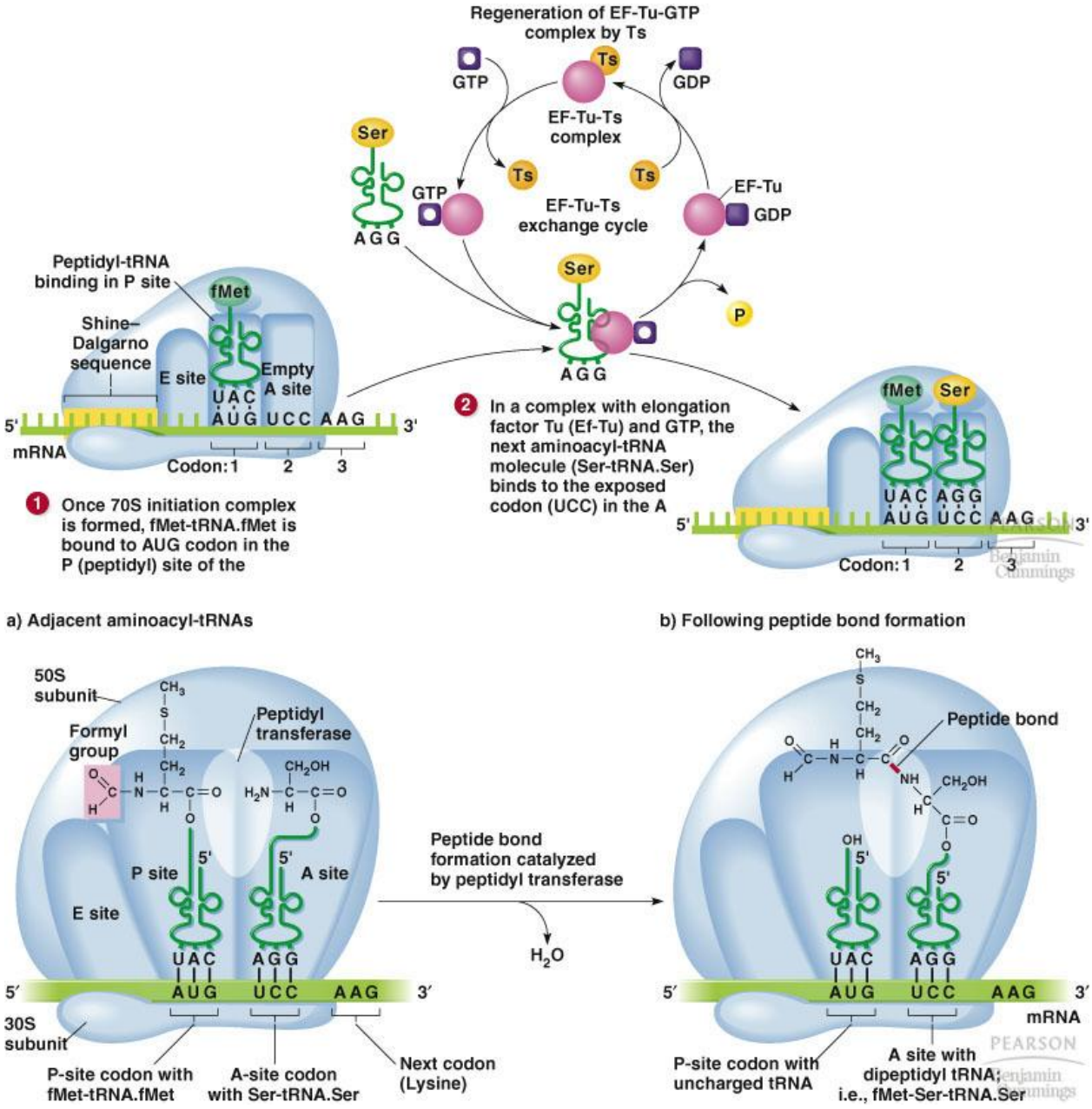




1 **Once 70S initiation complex is formed, fMet-tRNA.fMet is bound to AUG codon in the P (peptidyl) site of the**

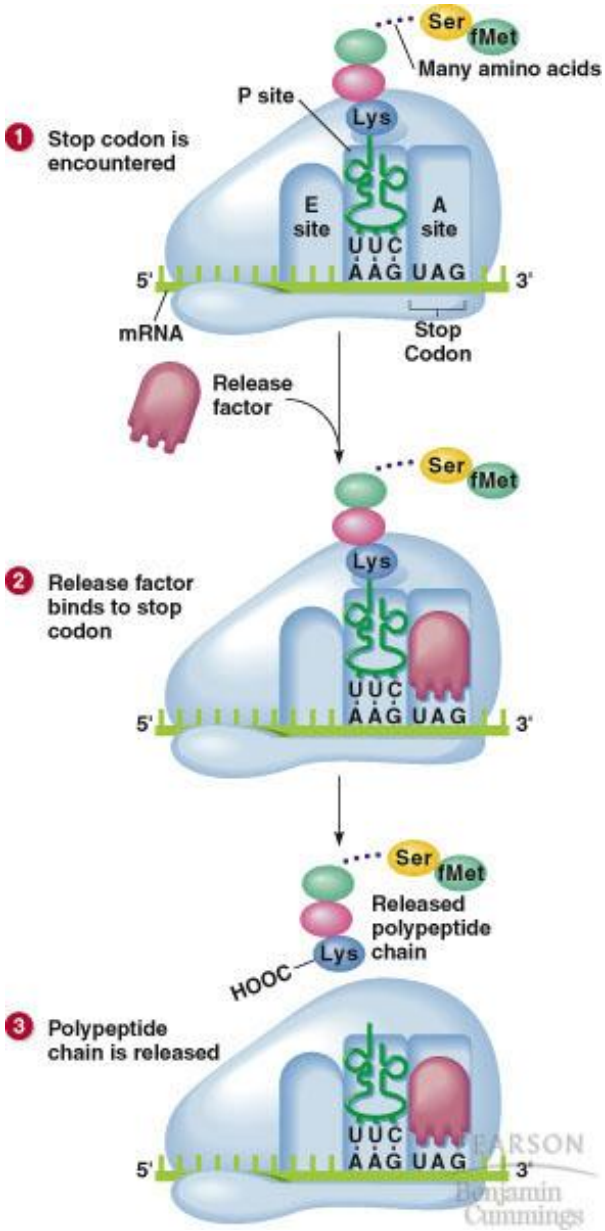
Elongation

- Amino acids added one at a time at the A site, aided by elongation factors
- Codon recognition occurs through hydrogen bonding between anticodon and codon
- Peptide bond formation is catalysed by *peptidyl transferase*, which joins newly arrived tRNA amino acid to the carboxyl end (COO^-) of the growing peptide
- In prokaryotes it proceeds at a rate of 15 to 20 amino acids added per second (about 60 nucleotides per second)
- In eukaryotes the rate is about two amino acids per second.
- Translocation involves the tRNA in the A site moving the P site, the next tRNA moving into the A site, and the tRNA in the P site moving to the E site
- mRNA moves 5’ to 3’ to the ribosome, codon by codon



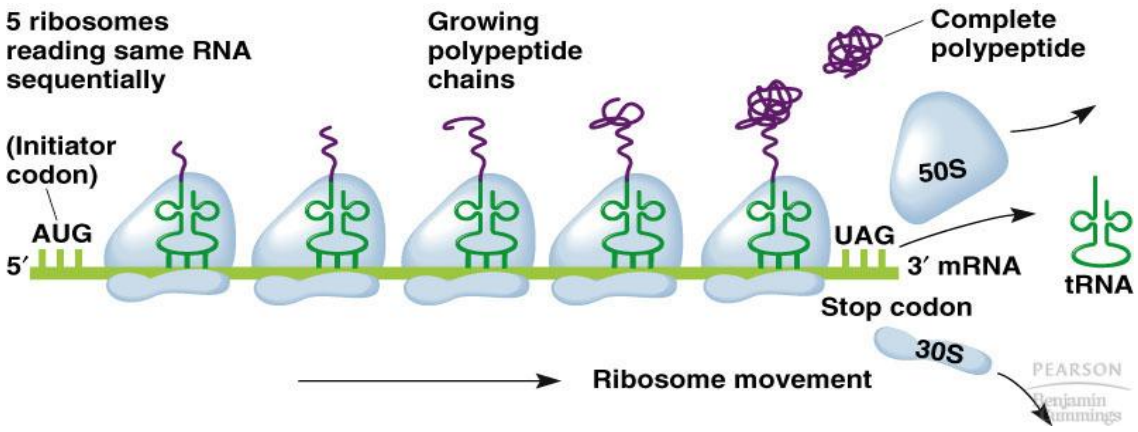
Termination of Translation

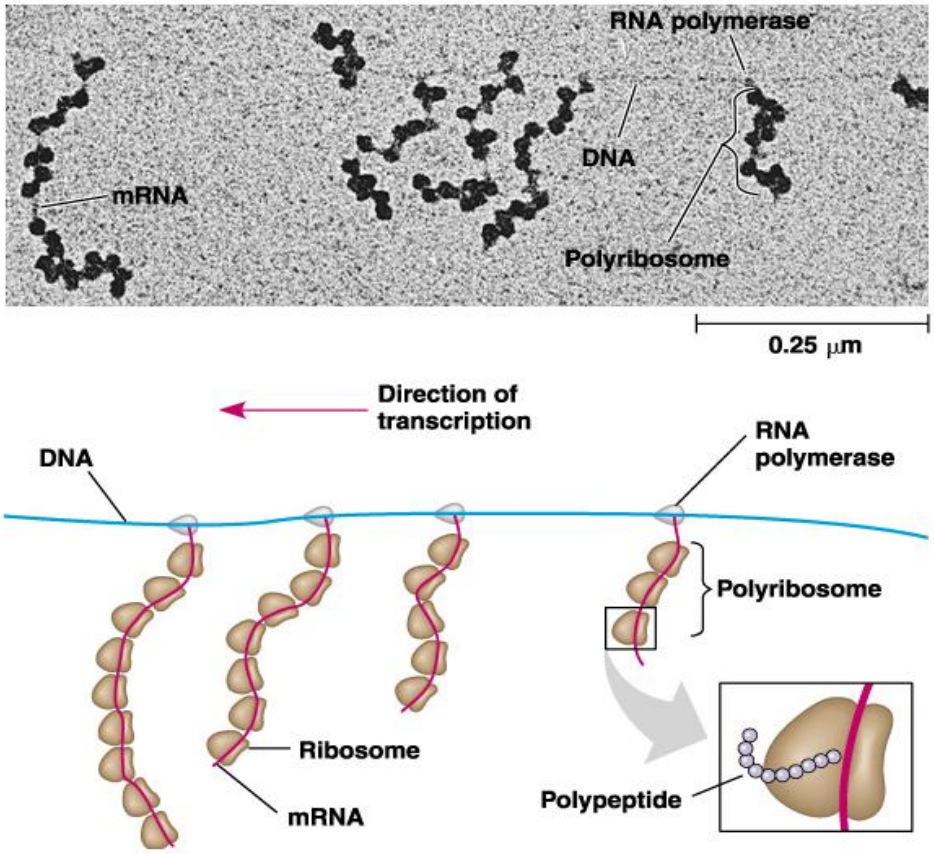
- Elongation continues until ribosome reaches stop codon
- Release factors bind to stop codon, which help ribosome to recognize stop codons
- *Peptidyl transferase* cleaves the polypeptide from P site
- Ribosome complex is dissembled; tRNA and ribosome are recycled
- Energy comes from GTP



Poly-Ribosomes

- mRNA can be translated by a number of ribosomes at once
- Makes several copies of the same polypeptide at once



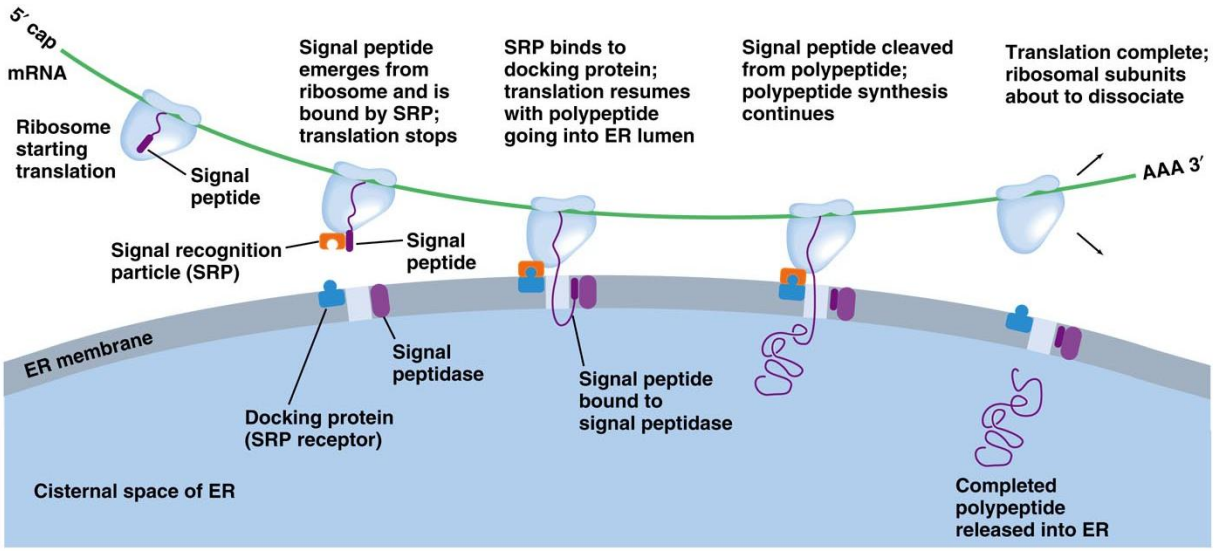


Post-Translational Modifications

- peptides fold into secondary/tertiary/ quaternary structures
- two or more peptides join together to form a protein (eg., hemoglobin)
- some amino acids are modified by attachment of particular groups – eg., phosphorylation

Sorting of Peptides

- In eukaryotes, proteins have to be transported to correct organelles
- 'Signal' or 'leader' sequences on proteins direct them to correct organelles
- A signal recognition particle (SRP) binds to leader sequence, and carries the peptide to endoplasmic reticulum (ER) where it binds to SRP receptor
- Peptide enters the cisternal space of ER where it is sorted



Mendelian Genetics

Genotype & Phenotype

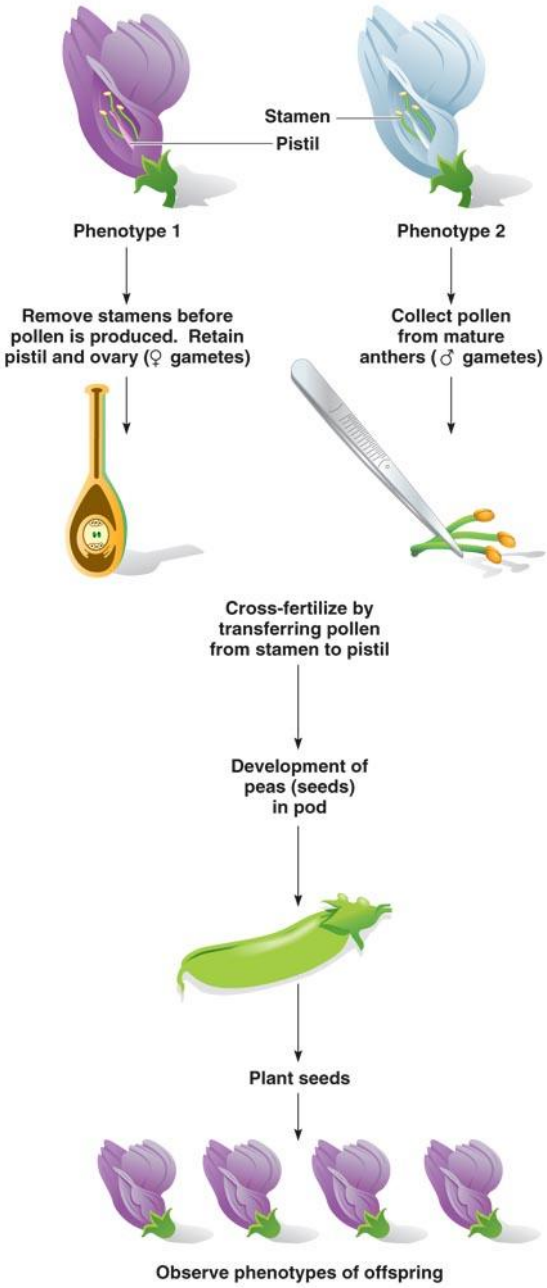
- **Traits** – characteristics of an individual
- Some traits heritable, others not
- **Genotype** – genetic constitution of an individual
- **Phenotype** – observable trait or set of traits of an individual
- Phenotype produced by interaction between genotype and environment

Factors Behind Mendel's Success

- Selected model genetic organism (peas - easy to grow, short life cycle, large number of seeds)
- Studied one trait at a time
- Simple interpretation of results
- Direct experiments to prove hypothesis
- Kept careful records
- Rigorous statistical analysis

Fertilisation in plants

- **Self fertilisation** – pollen from a flower fertilises ovary of the same flower
- **Cross fertilisation** – pollen from a flower fertilises ovary of a flower from another plant
- Repeated self-fertilisation for many generations produces a **true breeding** or **pure breeding strain**

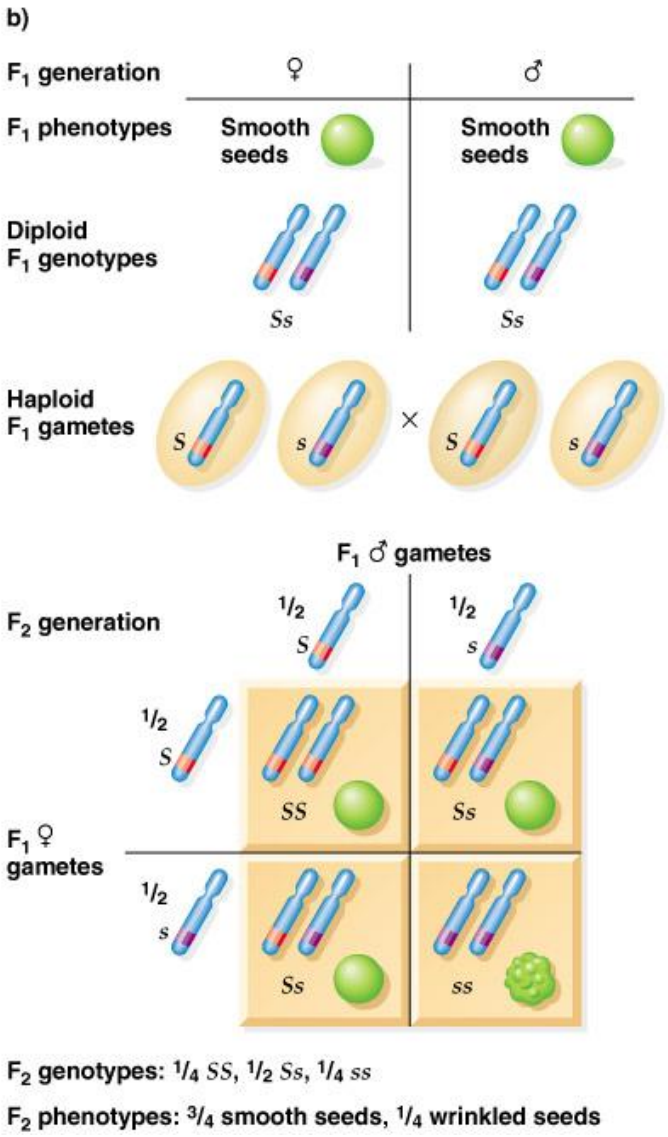


Character Traits Mendel Studied

- Mendel only studied '**either/or**' characters - no intermediate phenotypes
- Mendel began with **true-breeding** lines, with all offspring genetically identical
- **Parentals:** 'P' Generation
- **F_1** (the first filial generation): offspring of 'P' Generation
- **F_2** (the second filial generation): offspring produced by interbreeding 'F_1'individuals
- Similarly F_3, F_4, F_5…. produced by interbreeding individuals of previous generations
- Dominant allele represented by 'capital' letter and recessive allele by 'small' letter, e.g if you cross 'SS' homozygous with 'ss' homozygous every offspring will be 'Ss'

Findings

- 1. Alternate versions of traits (factors) account for variations in inherited characters. We now know that these 'factors' correspond to genes and each gene can have multiple alleles
- 2. For each character, an organism inherits 2 factors (genes) one from each parent. In true breeding varieties these factors were identical (same allele), while in hybrids they differ
- 3. If two alleles differ, one dominates the other to have its phenotype fully expressed
- 4. The two alleles for each character segregate during gamete production (meiosis produces haploid gametes)
- Law of segregation: Recessive traits, which are masked in the F_1 from a cross between two true breeding strains, reappear in a specific proportion in the F_2 at a 3:1 ratio
- Law of Independent Assortment: different traits sort independently during reproduction

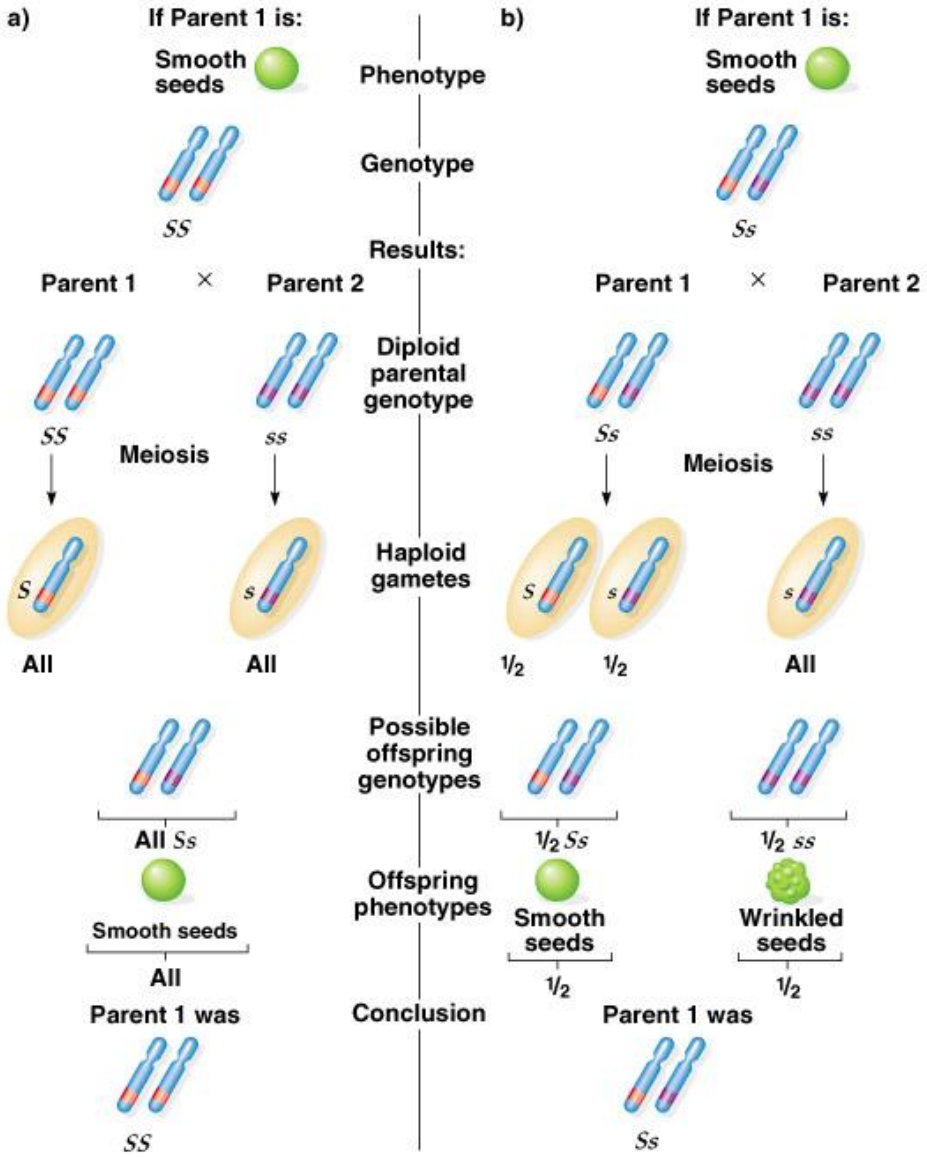


F_2 genotypes: $^1/_4$ *SS*, $^1/_2$ *Ss*, $^1/_4$ *ss*

F_2 phenotypes: $^3/_4$ smooth seeds, $^1/_4$ wrinkled seeds

Testcross

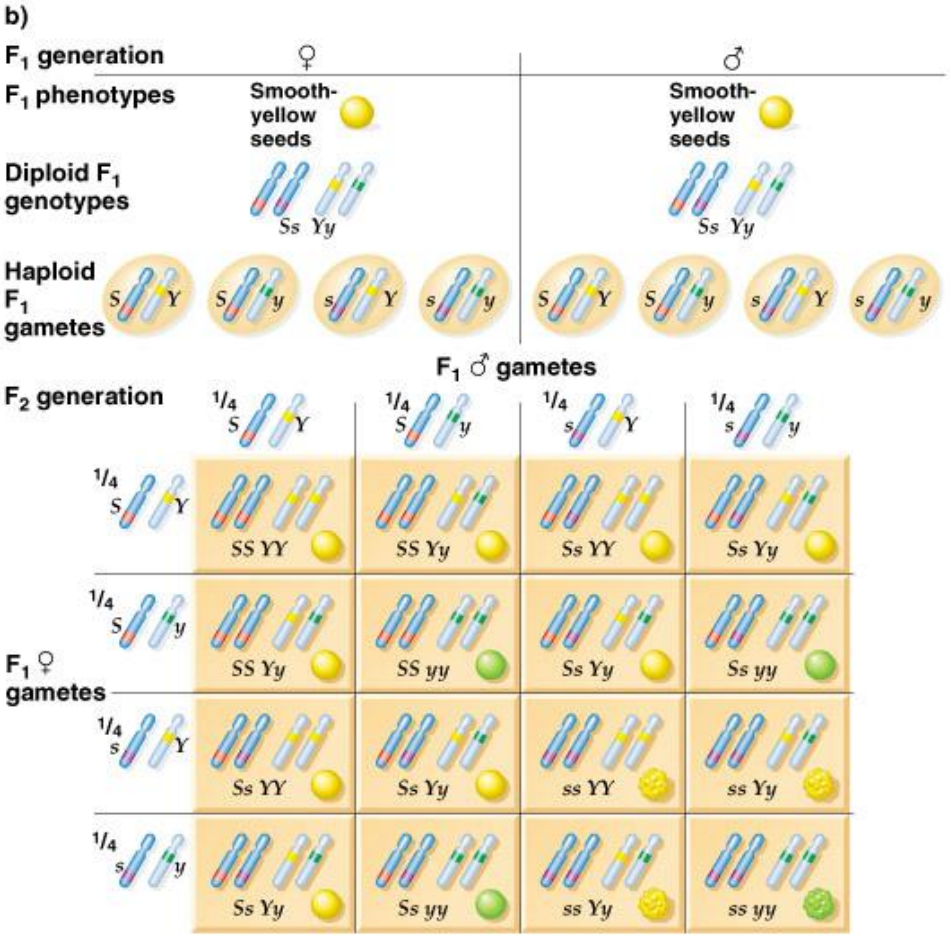
- Suppose you have an unknown gennotype. How can we determine what its genotype is? We can test cross it with a true breeding recessive
- For an individual displaying dominant characteristics, there are two possible outcomes:



Law of Independent Assortment

- What happens if we examine the joint variation of two traits?
- Consider an SY, sY, Sy and sy combinations of gametes in alleles, mating together two doubly heterozygous individuals
- The sperm and ovum produce 16 classes of allele combinations, grouped in 4 phenotypic classes
- Mendel found 315:108:101:32, very close to the 9:3:3:1 ratio expected from independence
- Thus he established his law of independent assortment: *Law of Independent Assortment*: The factors for different pairs of traits assort independently of one another
- In modern terms, pairs of alleles for genes on different chromosomes segregate independently in the formation of gametes

b)



F_2 genotypes: **F_2 phenotypes:**

$^1/_{16}$ (*SS YY*) + $^2/_{16}$ (*Ss YY*) + $^2/_{16}$ (*Ss Yy*) + $^4/_{16}$ (*Ss Yy*) = $^9/_{16}$ smooth-yellow seeds

$^1/_{16}$ (*SS yy*) + $^2/_{16}$ (*Ss yy*) = $^3/_{16}$ smooth-green seeds

$^1/_{16}$ (*ss YY*) + $^2/_{16}$ (*ss Yy*) = $^3/_{16}$ wrinkled-yellow seeds

$^1/_{16}$ (*ss yy*) = $^1/_{16}$ wrinkled-green seeds

$F_1 \times F_1$ *Ss Yy* (smooth, yellow) × *Ss Yy* (smooth, yellow)

F_2 phenotypes for $Ss \times Ss$	F_2 phenotypes for $Yy \times Yy$	F_2 phenotypic proportions
$^3/_4$ *S*– (smooth)	$^3/_4$ *Y*– (yellow)	$^9/_{16}$ *S*– *Y*– Smooth, yellow
	$^1/_4$ *yy* (green)	$^3/_{16}$ *S*– *yy* Smooth, green
$^1/_4$ *ss* (wrinkled)	$^3/_4$ *Y*– (yellow)	$^3/_{16}$ *ss Y*– Wrinkled, yellow
	$^1/_4$ *yy* (green)	$^1/_{16}$ *ss yy* Wrinkled, green

Chi-square Test

- Observed phenotypic ratios are rarely exactly same as expected
- Statistical analysis is needed to test if 'observed ratios' are significantly close to 'expected ratios'
- First set up a null-hypothesis: observed ratios = expected ratios
- Test 'goodness of fit': null hypothesis accepted/rejected based on result
- If, o = observed ratio; e = expected ratio, and d = deviation of observed from expected = (o - e)
- Then, Chi-square (χ^2) = $\Sigma\, d^2/e = \Sigma\, (o - e)^2/e$

Table 11.4 **Chi-Square Test Example**

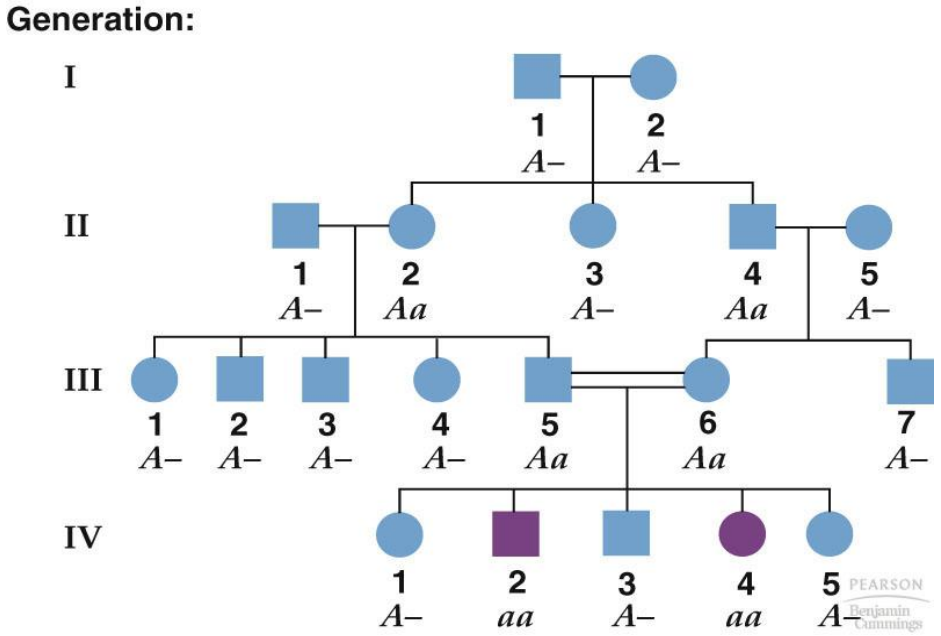
(1)	(2)	(3)	(4)	(5)	(6)
Phenotypes	**Observed Number (*o*)**	**Expected Number (*e*)**	***d*** **(= *o* : *e*)**	***d*²**	***d*²/*e***
Smooth, yellow	154	142	+12	144	1.01
Smooth, green	124	142	−18	324	2.28
Wrinkled, yellow	144	142	+2	4	0.03
Wrinkled, green	146	142	+4	16	0.11
Total	568	568	0		3.43

(7) $\chi^2 = 3.43$ (8) Degrees of freedom (df) = 3

Human Mendelian Genetics

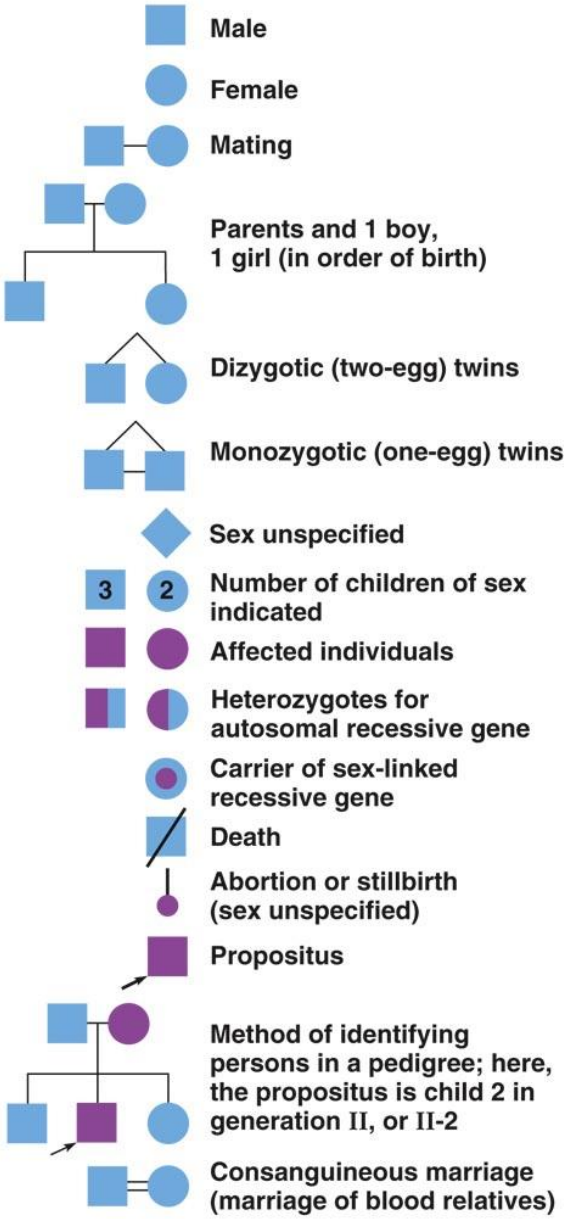
Pedigree Analysis

- We are unable to manipulate mating patterns in humans, therefore, we must look at the results of matings that have already occurred - i.e. the family's history for a specific trait
- A family pedigree is obtained by assembling a family tree across generations
- Family pedigrees are drawn up in a standard format, with different generations on separate lines, and individuals within a generation numbered in birth order



Symbols Used

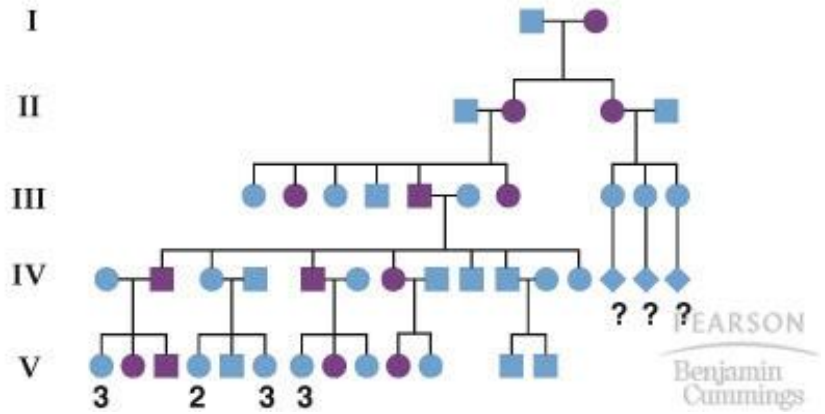
- Males - squares, Females - circles, Sex unknown - diamond
- Stillbirths, miscarriages- dots
- Deceased - line through
- Proband - the individual through whom the pedigree was discovered – denoted by arrow
- Affected individuals - solid symbols
- Unaffected individuals - open symbols
- Half solid symbols autosomal dominant - usually heterozygous
- Dot inside symbol - heterozygous carrier
- Sex-linked recessive gene
- Consanguineous matings, matings between related individuals, symoblised by a double line



Autosomal Dominant Trait

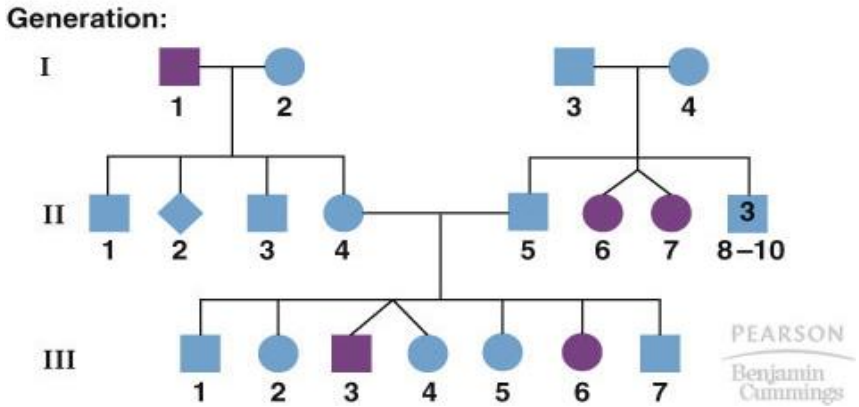
- Males and females affected in equal numbers
- All affected individuals have at least one affected parent
- Mating of affected (heterozygote) with an unaffected: 50% affected, 50% unaffected
- Trait does not appear in descendants of 2 unaffected individuals
- Trait may be more severe or extreme in homozygote than in the affected heterozygote

b) Generation:



Autosomal Recessive Trait

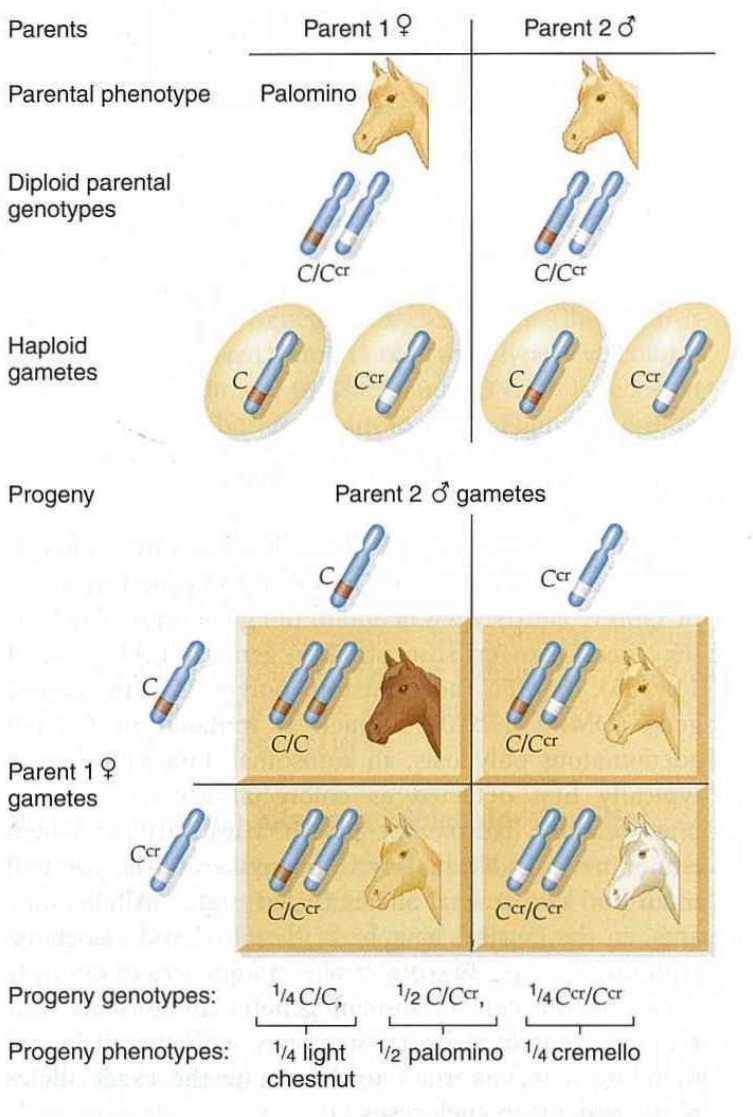
- trait expressed in both sexes
- most affected individuals have unaffected parents
- *i.e.,* parents are carriers (heterozygotes)
- Matings from unaffected individuals: 75% unaffected; 25% affected



Modification of Mendelian Ratios

Incomplete Dominance

- When one allele of a gene is not completely dominant to other allele of same gene
- The phenotype of heterozygote lies in a range between phenotypes of homozygotes, and so one can distinguish heterozygote from homozygotes
- Phenotypic ratio = genotypic ratio

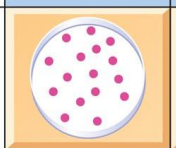
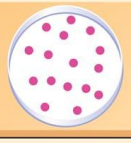
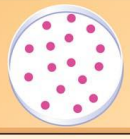
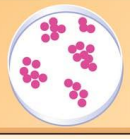
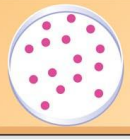
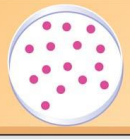


Codominance

- Heterozygotes exhibit phenotypes of both the homozygotes, *e.g.*, ABO blood groups in humans
- **H antigen**, also known as **substance H**, is a precursor to each of the ABO blood group antigens, apparently present in all people except those with the Bombay Blood phenotype (hh), which have no ABO type

Table 13.1 ABO Blood Groups in Humans, Determined by the Alleles I^A, I^B, and i

Phenotype (Blood Group)	Genotype
O	i/i
A	I^A/I^A or I^A/i
B	I^B/I^B or I^B/i
AB	I^A/I^B

Serum from blood type	Antibodies present in serum	Cells from blood type: O	A	B	AB
O	Anti-A Anti-B				
A	Anti-B				
B	Anti-A				
AB	—				

Epistasis

- One gene (pair of alleles) masks expression of another gene (pair of alleles)
- Therefore, number of modifications of 9:3:3:1 dihybrid cross are observed
- Read 'is epistatic to' as 'dominates', meaning that it alone determines the phenotype
- Recessive epistasis: a is epistatic to B and b, so 9:3:3:1 becomes 9:3:4
- Dominant epistasis: A is epistatic to B and b, so 9:3:3:1 becomes 12:3:1
- Dominant and recessive epistasis: A is epistatic to B and b, and b is epistatic to A and a, so 1 and 2 are the same, and 2 and 4 are the same, so 1, 2 and 4 are all the same, yielding a 13:3
- Duplicate recessive epistasis: a is epistatic to B and b, while and b is epistatic to A and a, so 9:3:3:1 becomes 9:7
- Duplicate dominant epistasis: A is epistatic to B and b, while B is epistatic to A and a, resulting in a 15:1 ratio

Table 13.3 Summary of Epistatic F_2 Phenotypic Ratios from an F_1 *A/a B/b* × F_1 *A/a B/b* Cross in Which Complete Dominance Is Shown for Each Allele Pair

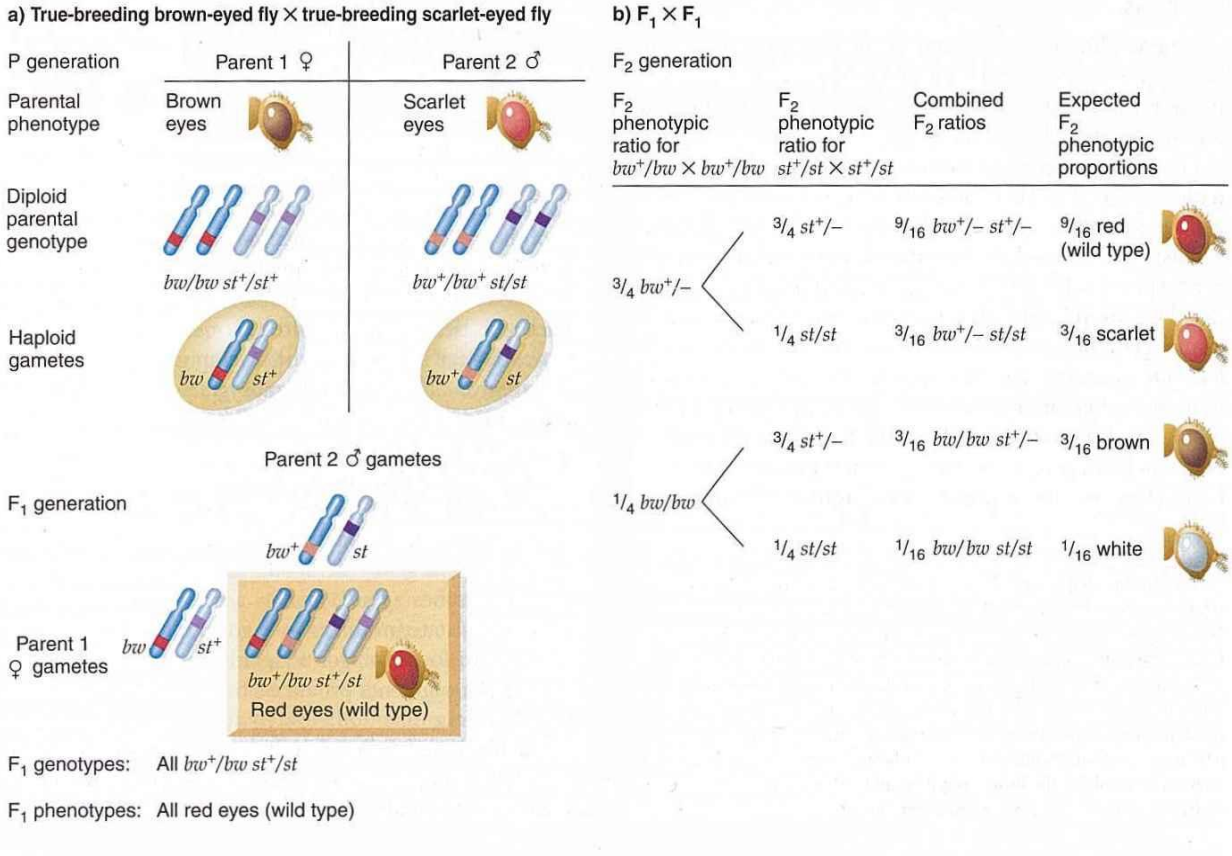
	F_2 Phenotypic Ratio from an *A/a B/b* × *A/a B/b* Cross			
Gene Interaction	***A/– B/–***	***A/– b/b***	***a/a B/–***	***a/a b/b***
None	9	3	3	1
Recessive epistasis *a/a* epistatic to *B* and *b*	9	3	4	
Dominant epistasis *A* epistatic to *B* and *b*	12		3	1
Duplicate recessive epistasis (complementary gene action) *a/a* epistatic to *B* and *b*; and *b/b* epistatic to *A* and *a*	9	7		
Duplicate dominant epistasis *A* epistatic to *B* and *b*; and *B* epistatic to *A* and *a*	15			1

Gene Interaction

- Two allelic pairs affect same phenotype, *e.g.*, eye colour in *Drosophila*

Figure 13.14

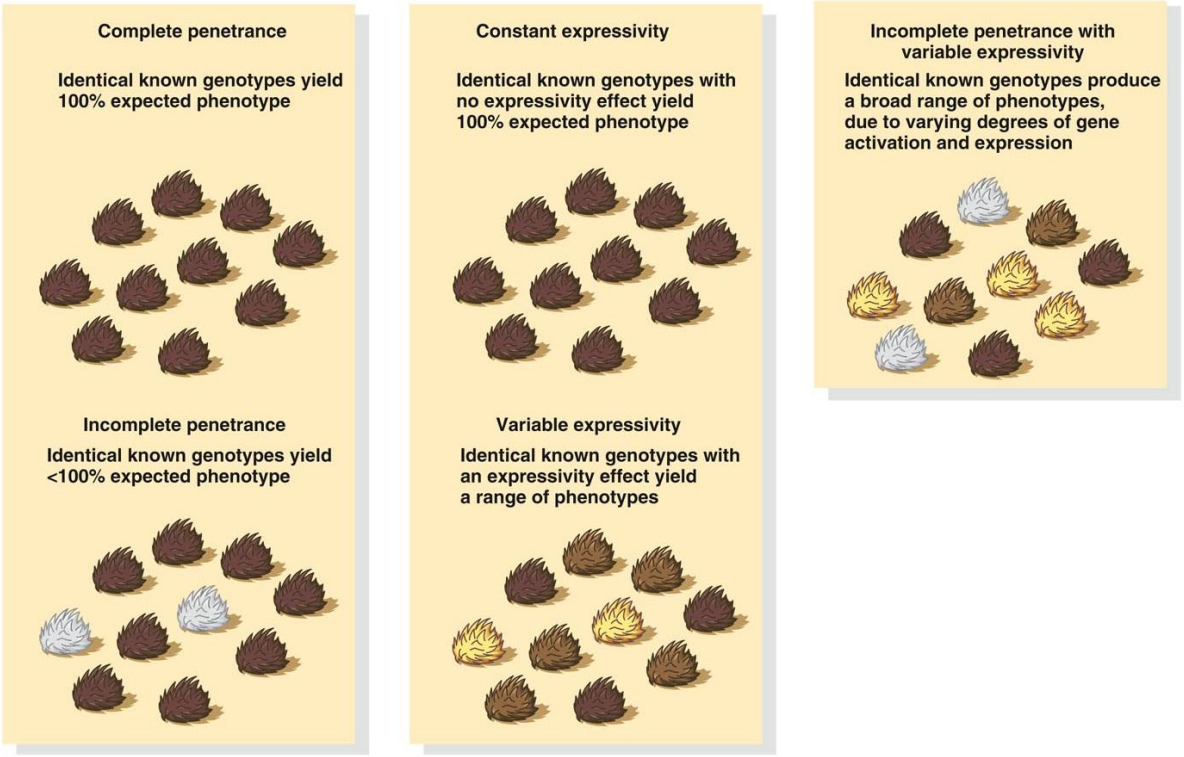
Gene interactions that produce new phenotypes. Genetic crosses show the interaction of genes for eye color in *Drosophila*.



Penetrance and Expressivity

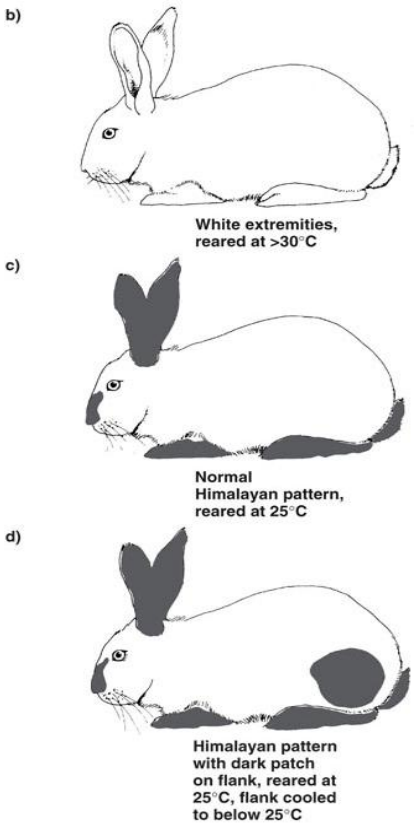
- In a population, many individuals may have the same genotype (example A/- B/-), however, based on their immediate environment, different individuals having the same genotype may produce different phenotypes

- **Penetrance:** the percentage of individuals with a genotype who exhibit the phenotype associated with the genotype
- **Expressivity:** degree to which a penetrant gene is phenotypically expressed in an individual
- Expressivity is for an *individual,* while penetrance is for a *population*



Effects of the Environment

- Genes provide only the potential for developing a particular phenotype
- The extent to which that phenotype is realised depend on the interaction between 'Genes and Environment'
- Many environmental factors effect penetrance and expressivity
- Age of Onset: some phenotypes expressed only at particular age
- Sex: sex-linked genes expressed only in particular sex
- Chemicals: phenotype of a genotype – protein (enzymes) can be modified by presence/absence of certain chemicals
- Temperature: phenotype of a genotype – protein (enzymes) can be modified by temperature



Sexed Traits

- Sex influenced traits: autosomal, expressed in both sexes but more pronounced in one than the other (e.g. male pattern baldness)
- Sex limited traits: autosomal, genes found in both sexes but only expressed in one gender (e.g. genes relating to lactation)
- Sex linked: gametes, differ in frequency and severity between the sexes because of different copies of sex chromosomes