DNA Replication

Jason Ryan, MD, MPH

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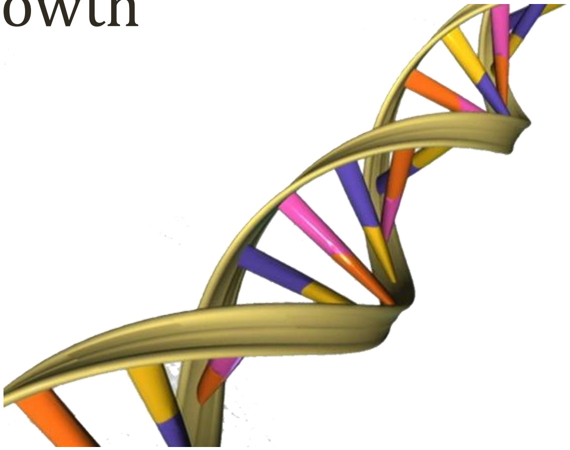
DNA

- Contains genetic code
- Nucleus of eukaryotic cells
- Cytoplasm of prokaryotic cells
- **Replicated** for cell division/growth



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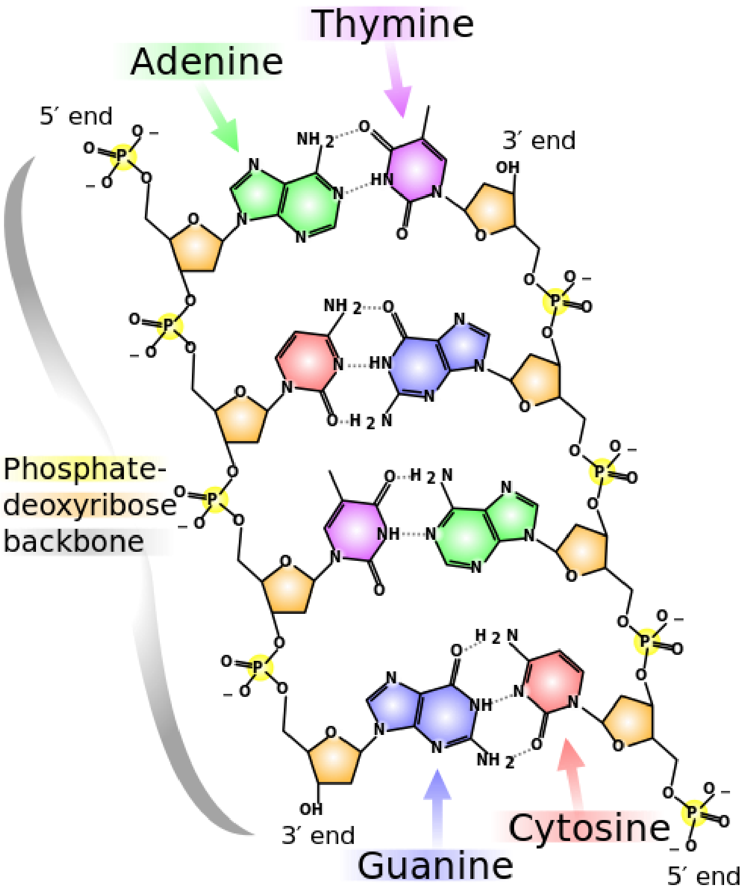
DNA Structure

1. Sugar (ribose) backbone
2. Phosphate
3. Nitrogenous base



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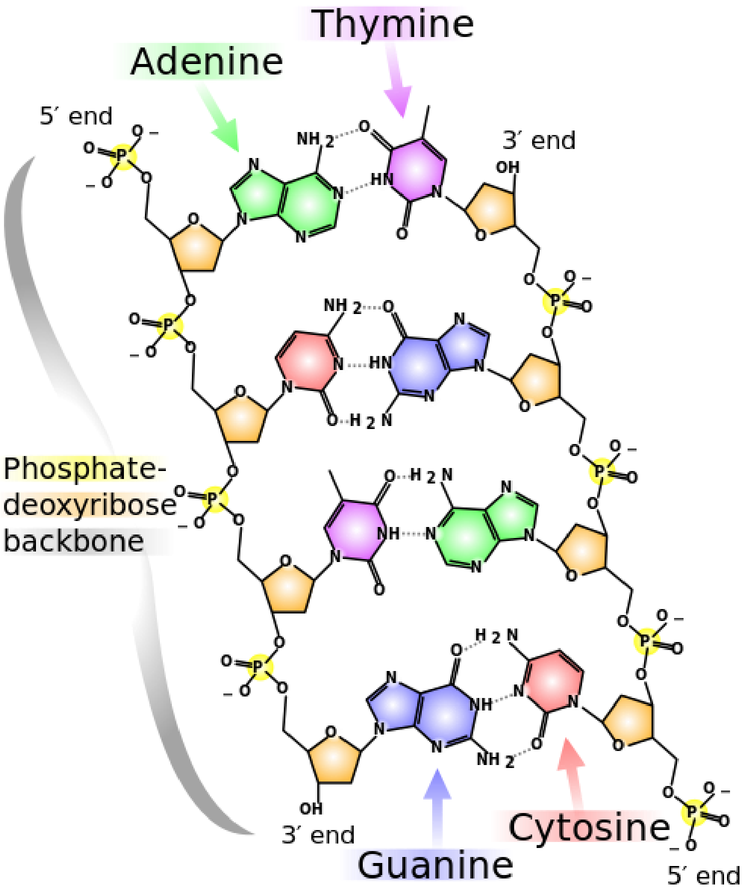
Base Pairing

- DNA
  - Adenine-Thymine
  - Guanine-Cytosine
- RNA
  - Adenine-Uracil
  - Guanine-Cytosine
- Antiparallel



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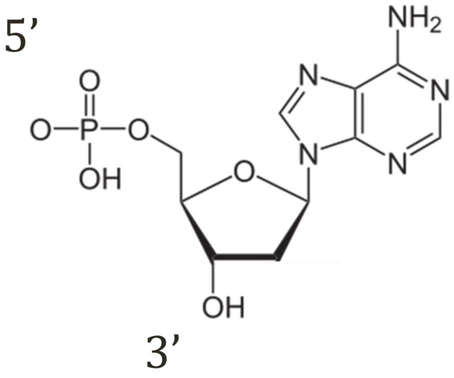
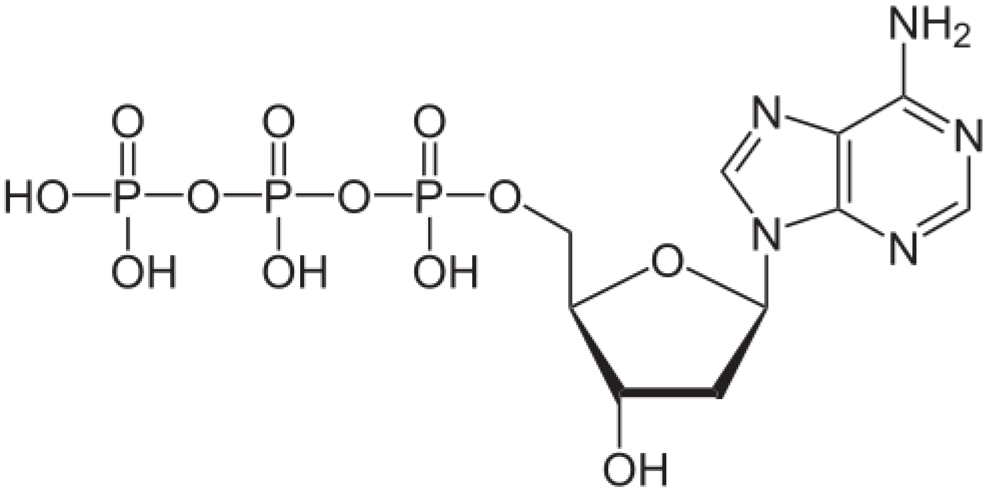
Nucleotides

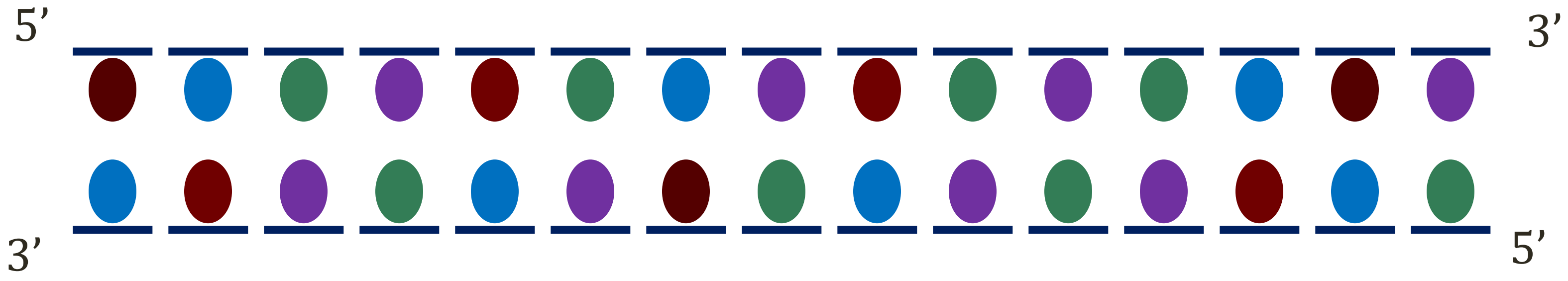


- Synthesized as monophosphates
- Converted to triphosphate form
- Triphosphate form added to DNA



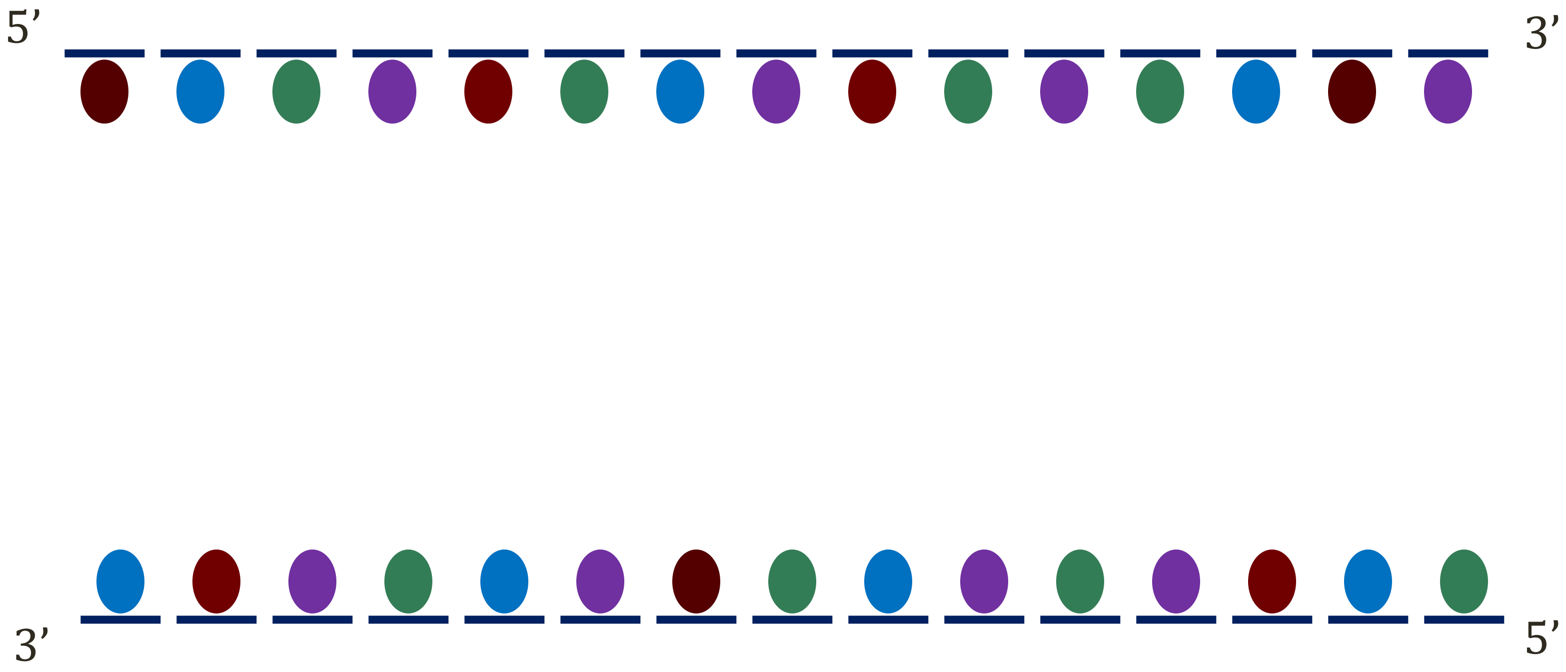
Deoxy-adenosine Triphosphate

DNA Replication



● Adenosine
● Cytosine
● Thymidine
● Guanosine

DNA Replication



● Adenosine

● Cytosine

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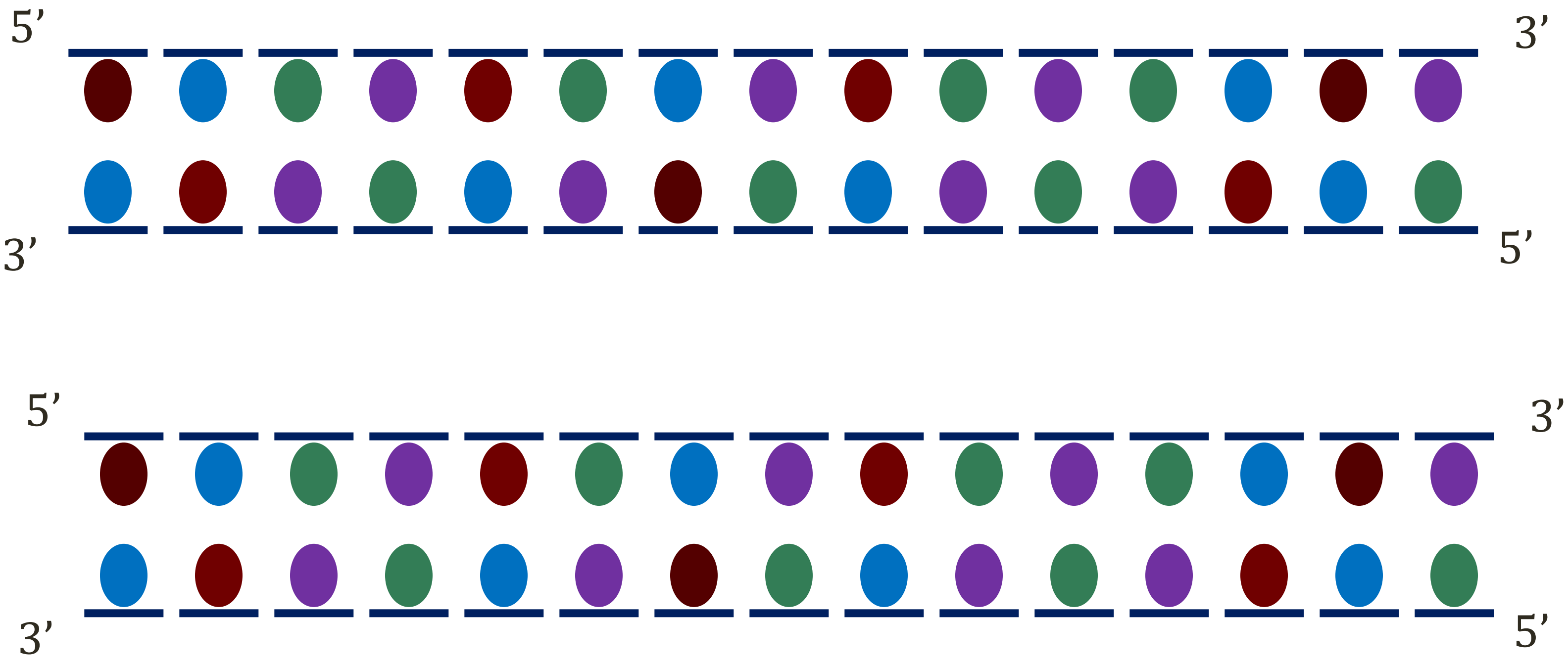
DNA Replication



● Adenosine

● Cytosine

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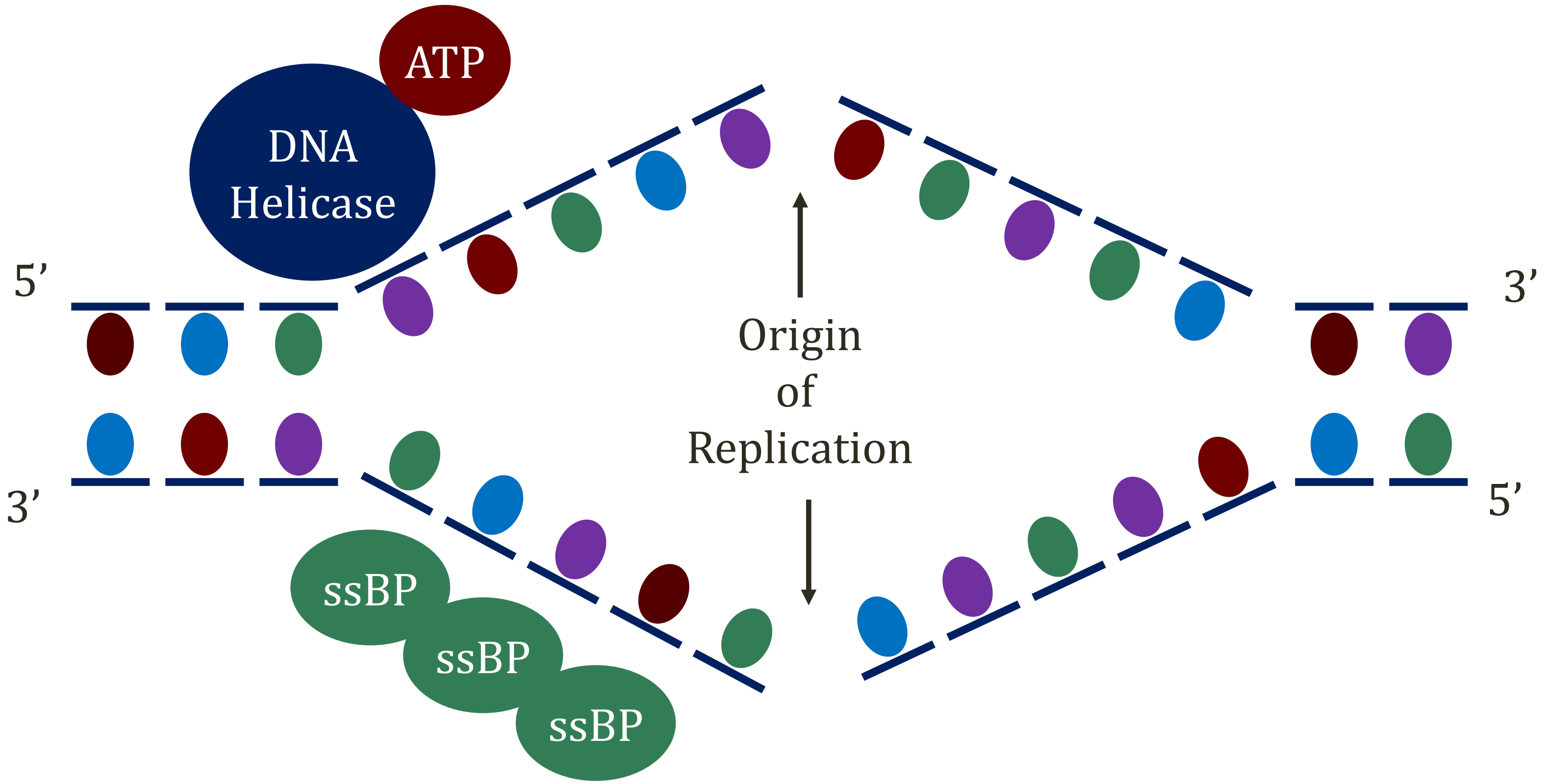
DNA Replication



ATP

DNA Helicase

5’

3’

3’

5’

Origin of Replication

ssBP

ssBP

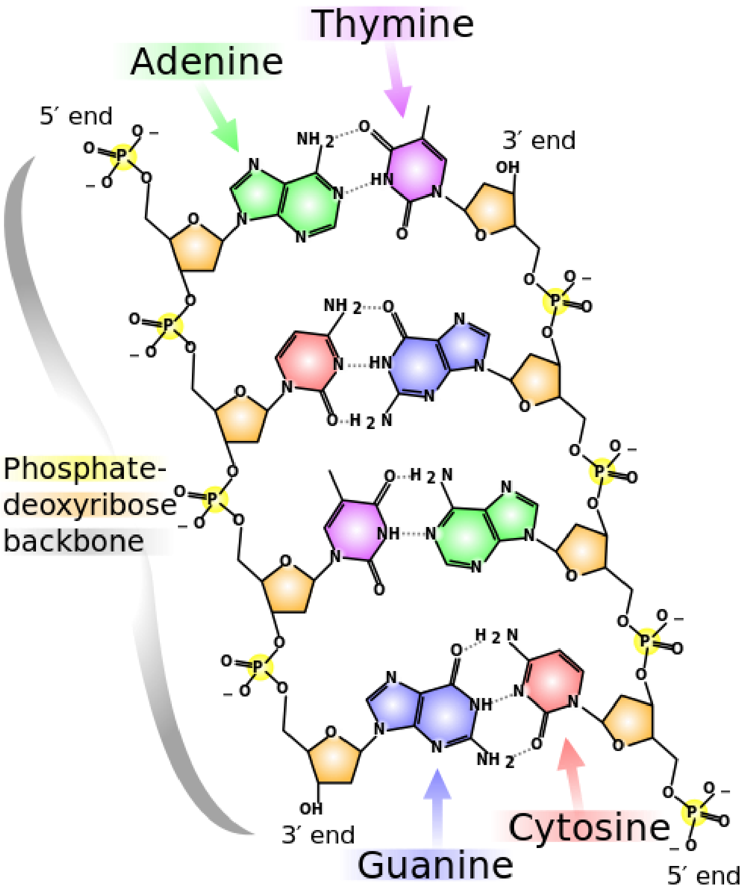
ssBP

DNA Replication

- **Helicase**
  - Unwinds/opens double helix
  - Hydrolyzes ATP
- **Single strand binding proteins**
  - Assist helicase
  - Stabilize and straighten single strands of DNA

Origin of Replication

- Specific DNA sequences
  - Attract initiator proteins
  - Easy to unwind/open
- Fewer bonds A-T
  - **"AT rich" sequences**
  - Easy to open



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DNA Polymerases

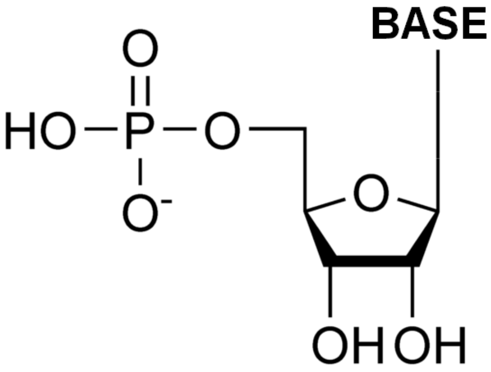
- Bacteria (prokaryotes)
  - DNA polymerase I-IV
  - Polymerase III: Major DNA polymerase
  - Polymerase I: Removes RNA primers
- Eukaryotes
  - DNA polymerase α, β, γ, δ, and ε
  - Polymerase γ: located in mitochondria

Primers

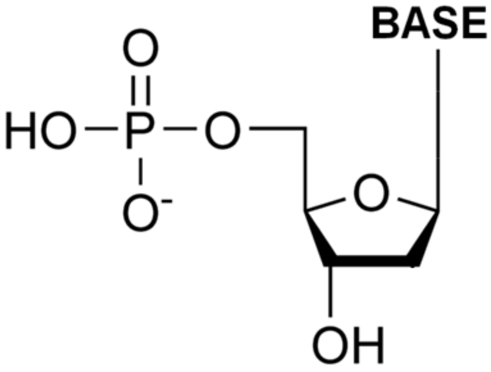
- DNA polymerase **cannot initiate replication**
- Primers: short nucleotide sequences
- Formed at point of initiation of new chain
- Required by DNA polymerase to function

Primers

- **DNA Primase**: Makes primers
- Primers contain **RNA**
  - Ribonucleotides (not deoxy-ribonucleotides)
  - Uracil instead of thymine
  - Eventually removed and replaced with DNA

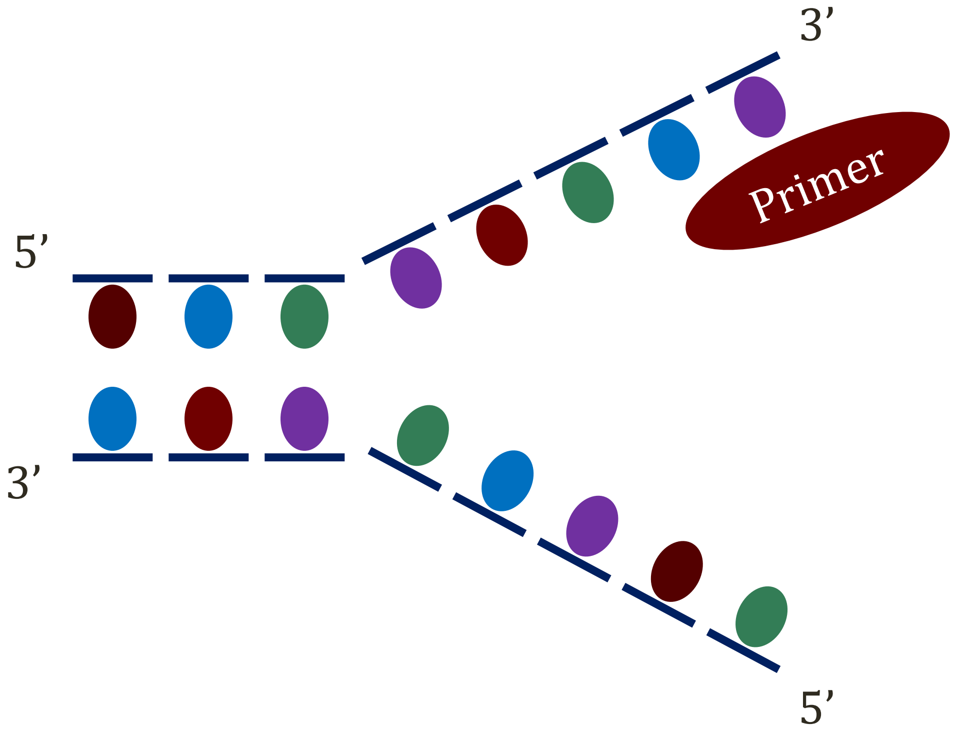


Ribonucleotide



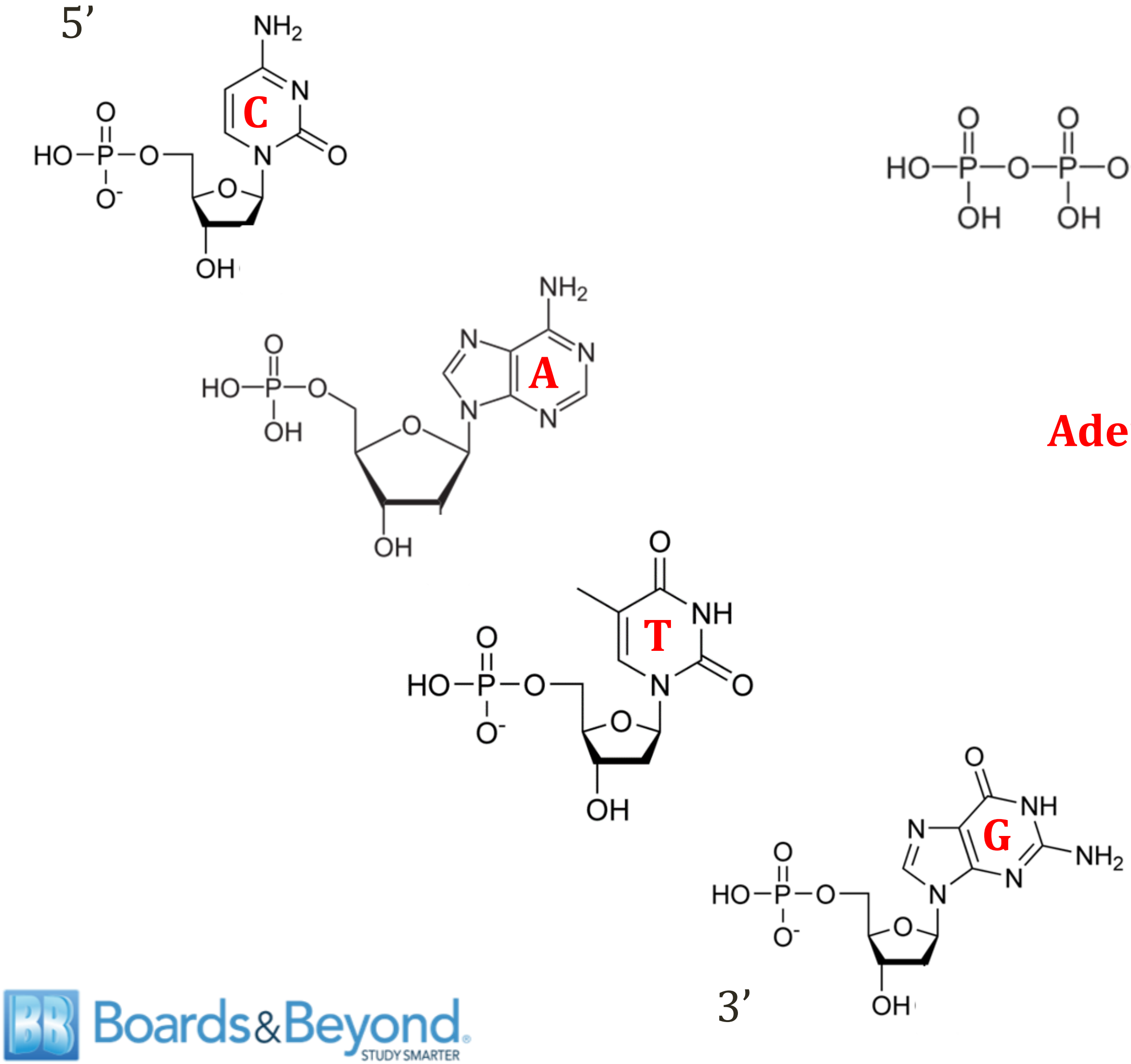
Deoxyribonucleotide

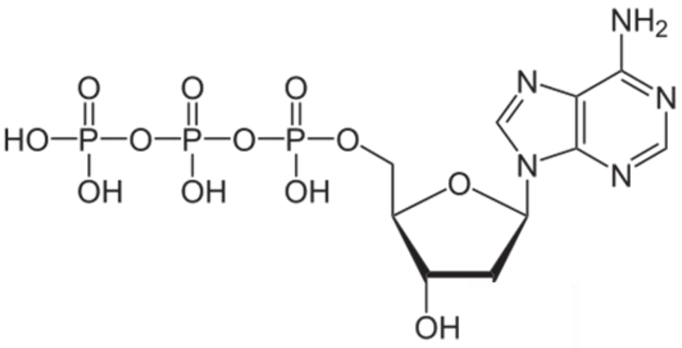
Replication Fork



DNA Replication

Directionality

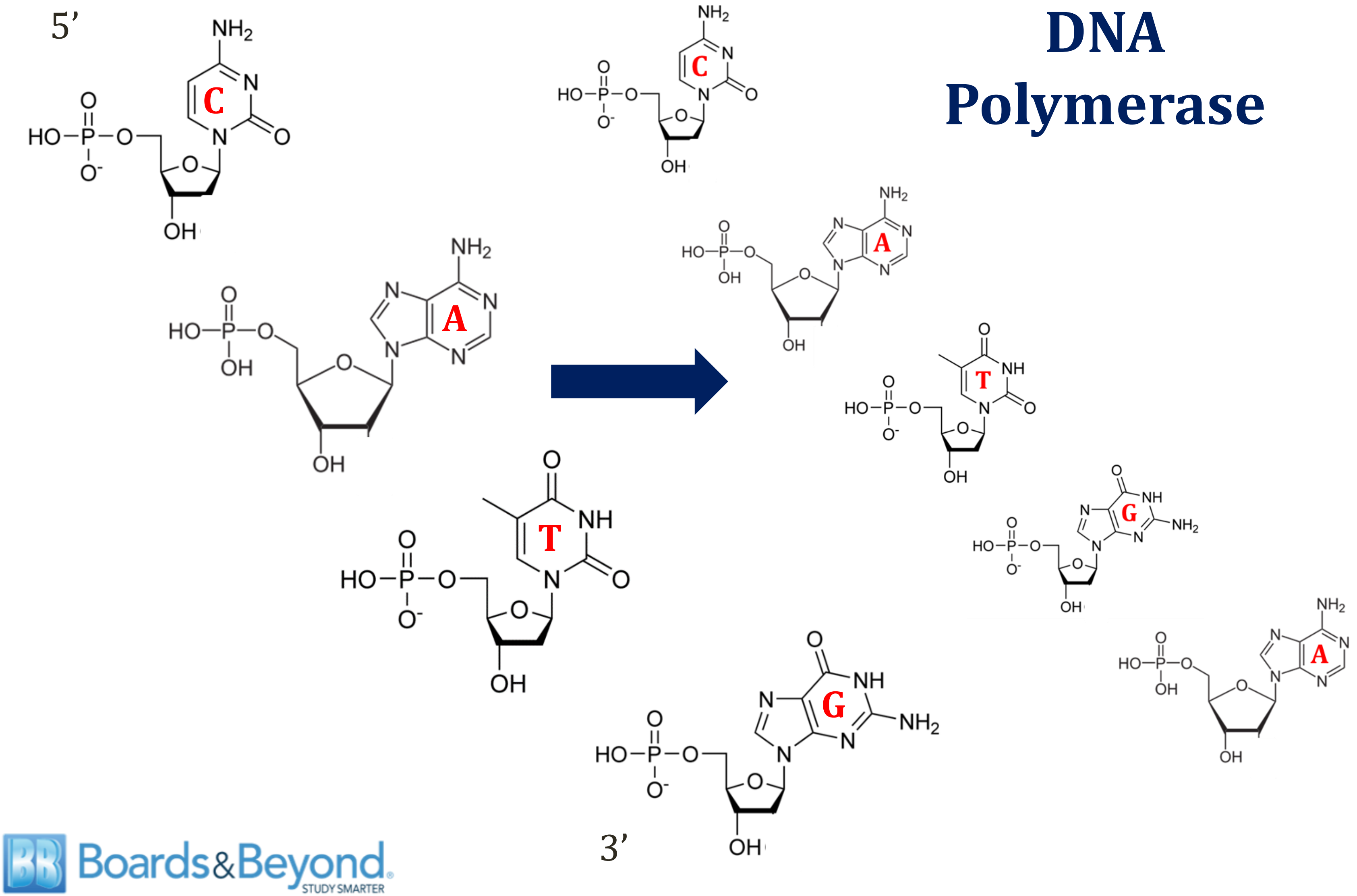




Adenosine-TP

DNA Replication

Directionality



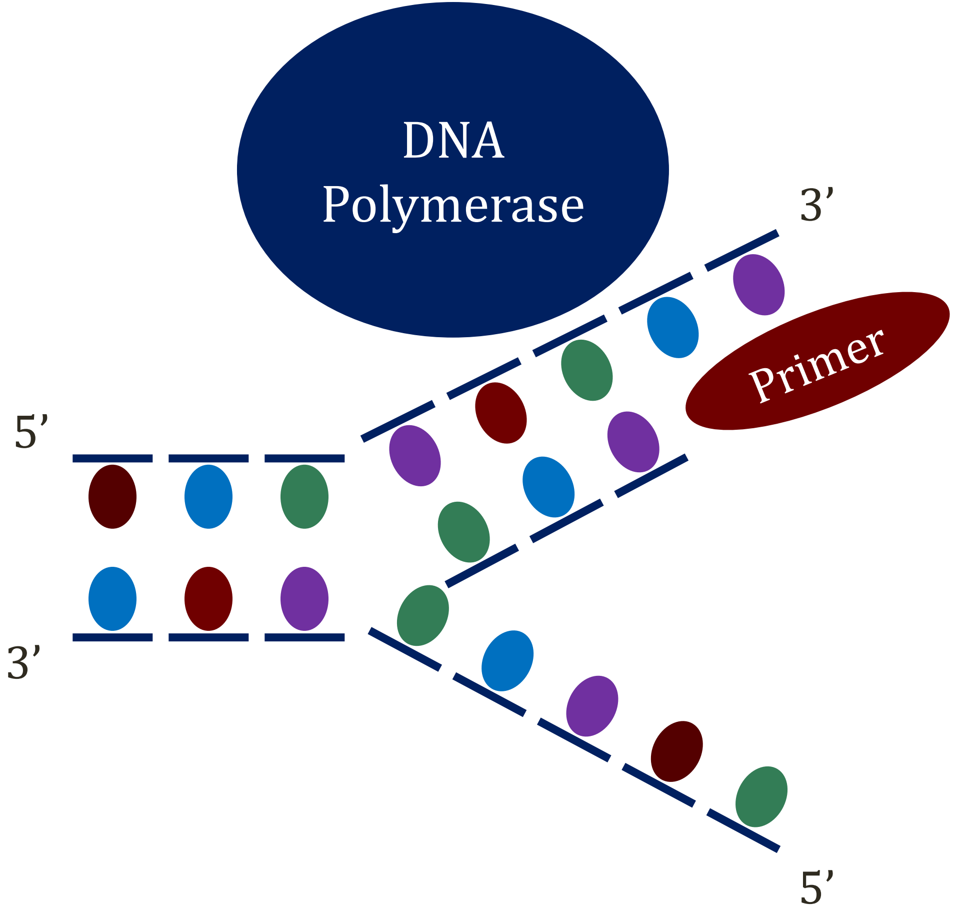
DNA Polymerase

DNA Replication

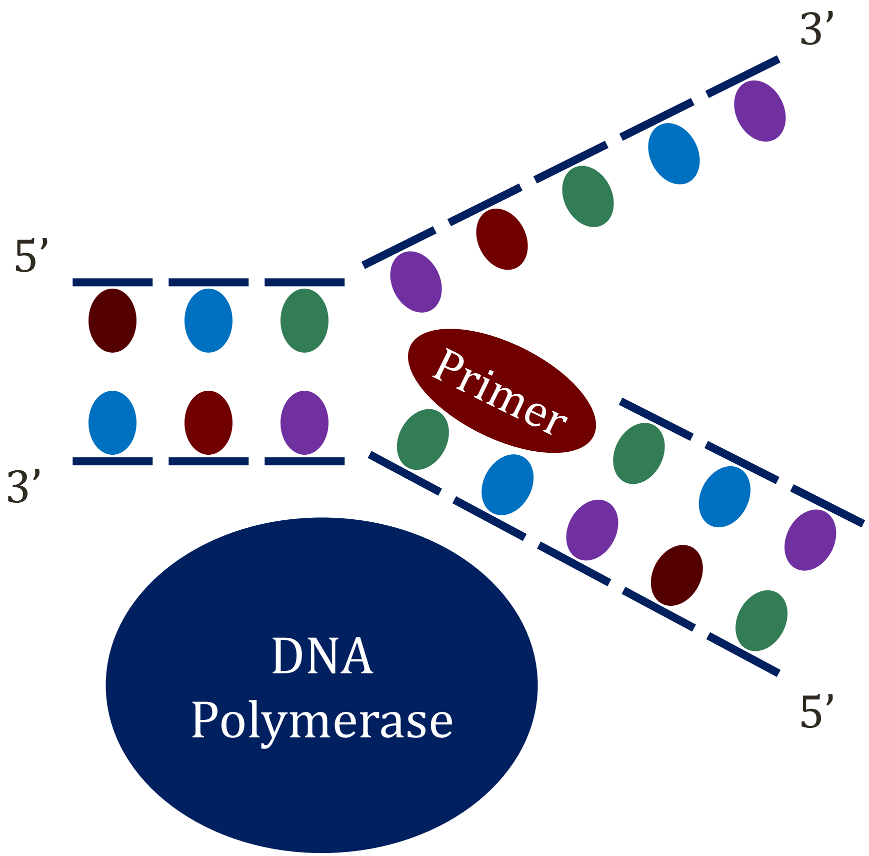
Directionality

- **Always occurs in 5’ to 3’ direction**
- Nucleotides added to **3’ end** of growing strand

Replication Fork



Replication Fork



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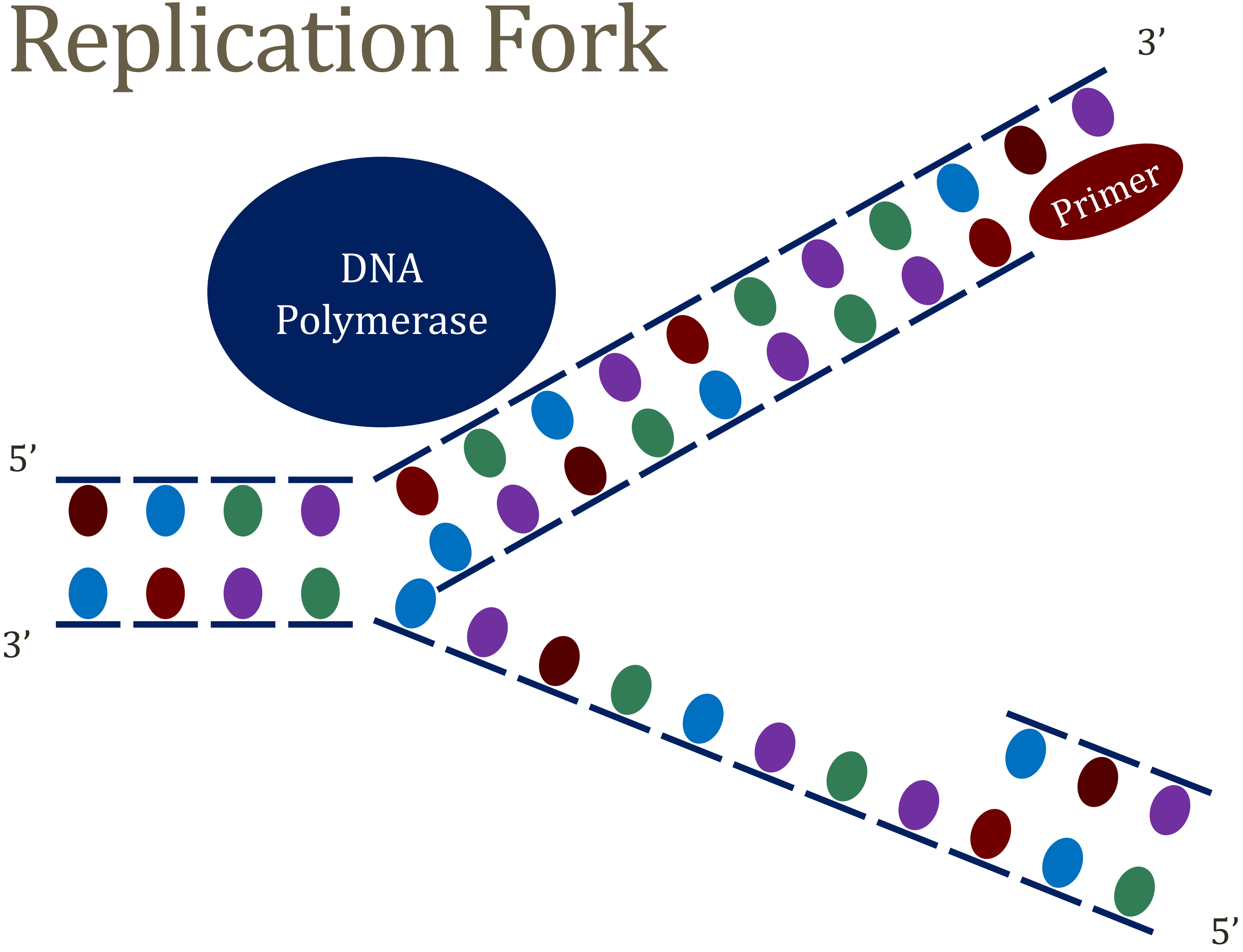

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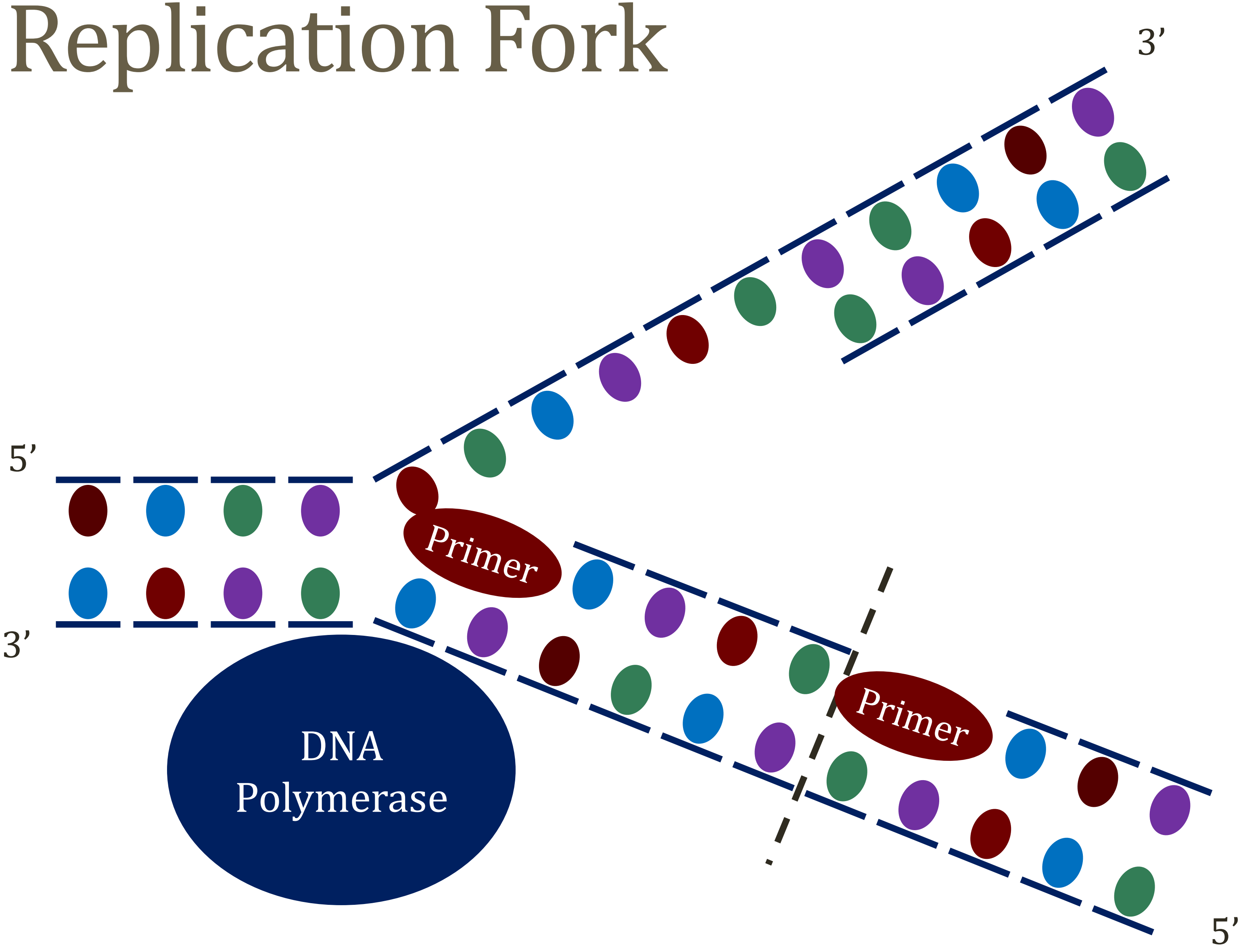
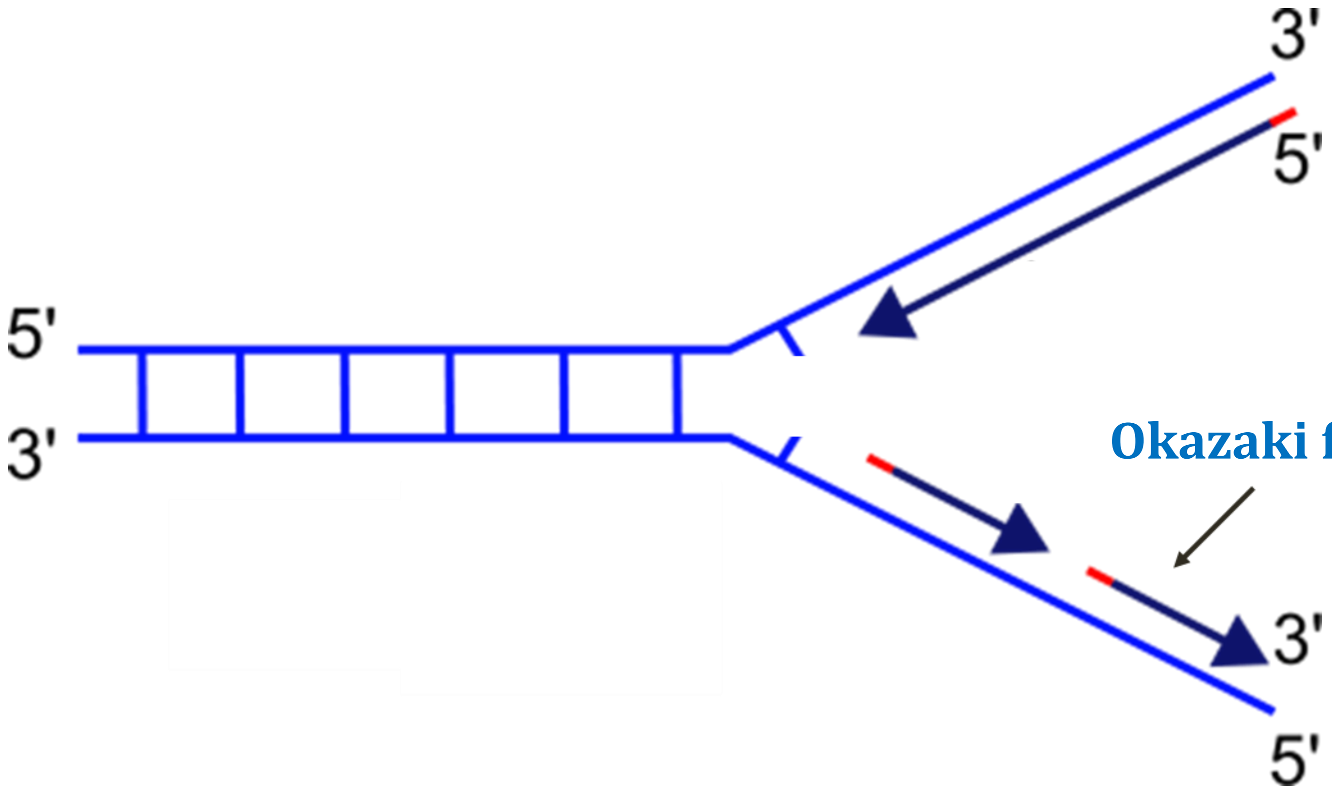


Replication Fork



Leading Strand

Okazaki fragments

Lagging Strand

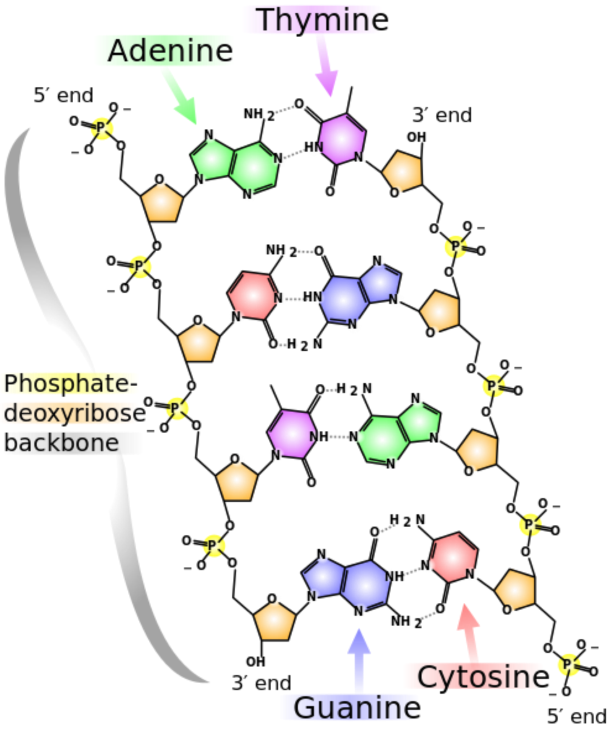
Masur/Wikipedia

Primer Removal

- Okazaki fragments synthesized until primer reached
- RNA primer removed and replaced with DNA
- Prokaryotes: **DNA polymerase I**
- Eukaryotes: **DNA polymerase delta**

DNA Ligase

- Joins Okazaki fragments
- Creates **phosphodiester bonds**



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Topoisomerase

- Prevent DNA tangling
- Break DNA then reseal to relieve tension/twists
- Topoisomerase I
  - Break single strands of DNA then reseal
- Topoisomerase II
  - Break double strands then reseal

Topoisomerase

Clinical Correlations

- Quinolone antibiotics
  - Prokaryotic topoisomerases
- Chemotherapy agents
  - Eukaryotic toposiomerases
  - Etoposide/teniposide
  - Irinotecan, topotecan
  - Anthracyclines

DNA Replication

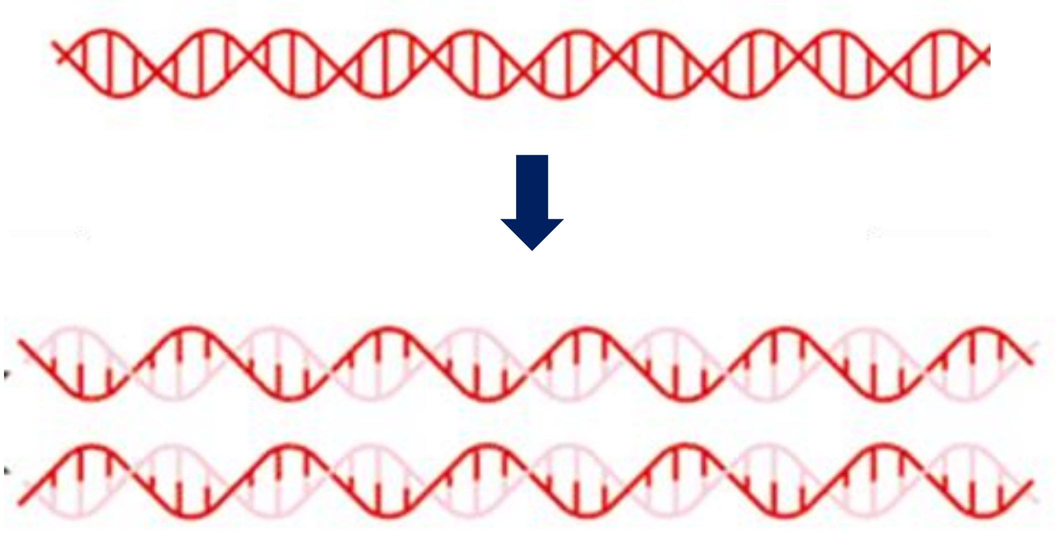
Key Points

- Leading strand replication is **continuous**
- Lagging strand replication is **discontinuous**
  - Okazaki fragments
  - DNA ligase

DNA Replication

Key Point

- Semi-conservative
  - New DNA: one old and one new strand

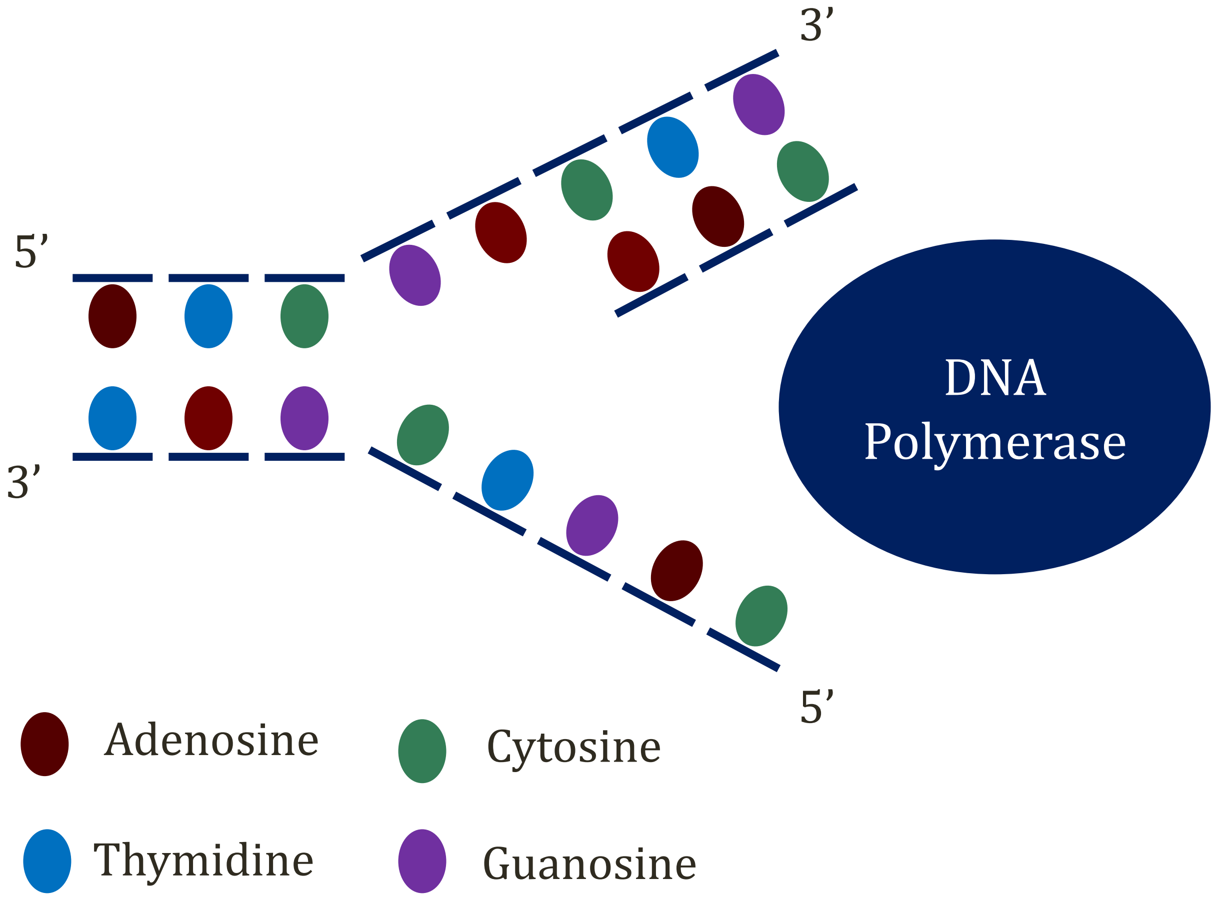


Adenosine/Wikipedia

Proofreading

- DNA polymerase can correct errors
- Synthesizes in new strand 5’ to 3’ direction
- Wrong nucleotide added: Can move backwards
  - 3’ to 5’ direction
  - Correct error
- **Exonuclease activity**: remove incorrect nucleotide
- DNA polymerase: **“3’ to 5’ exonuclease activity”**
- Significantly reduces error rate

Replication Fork



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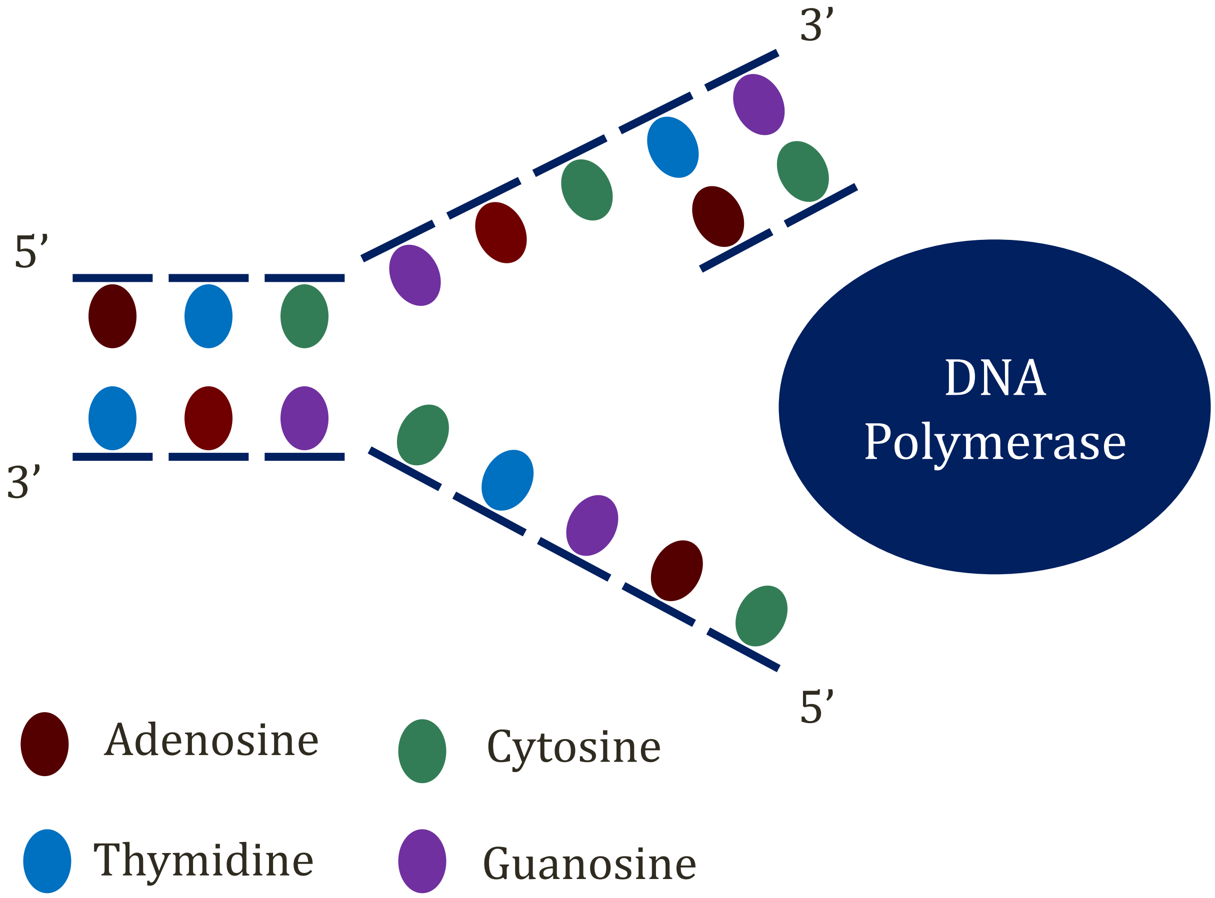
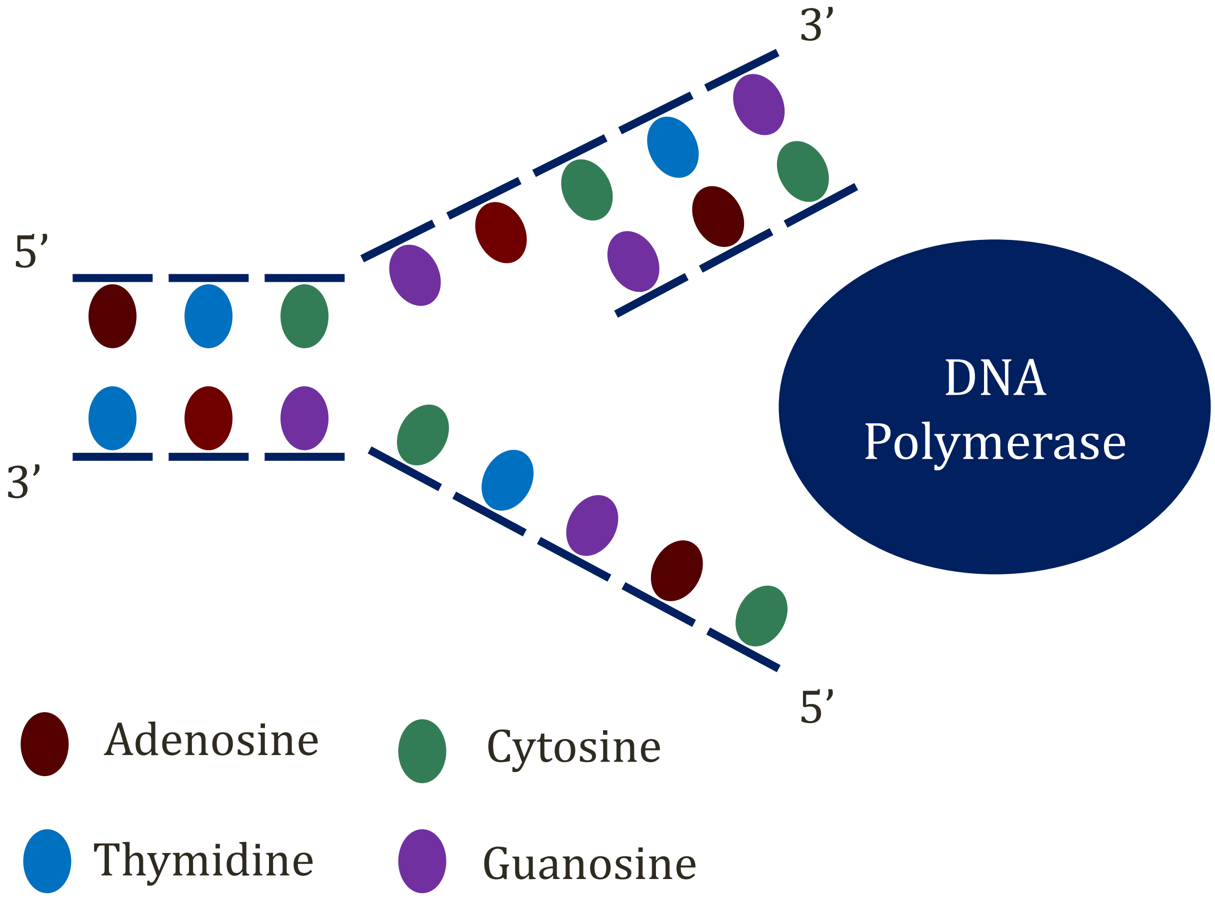


Replication Fork

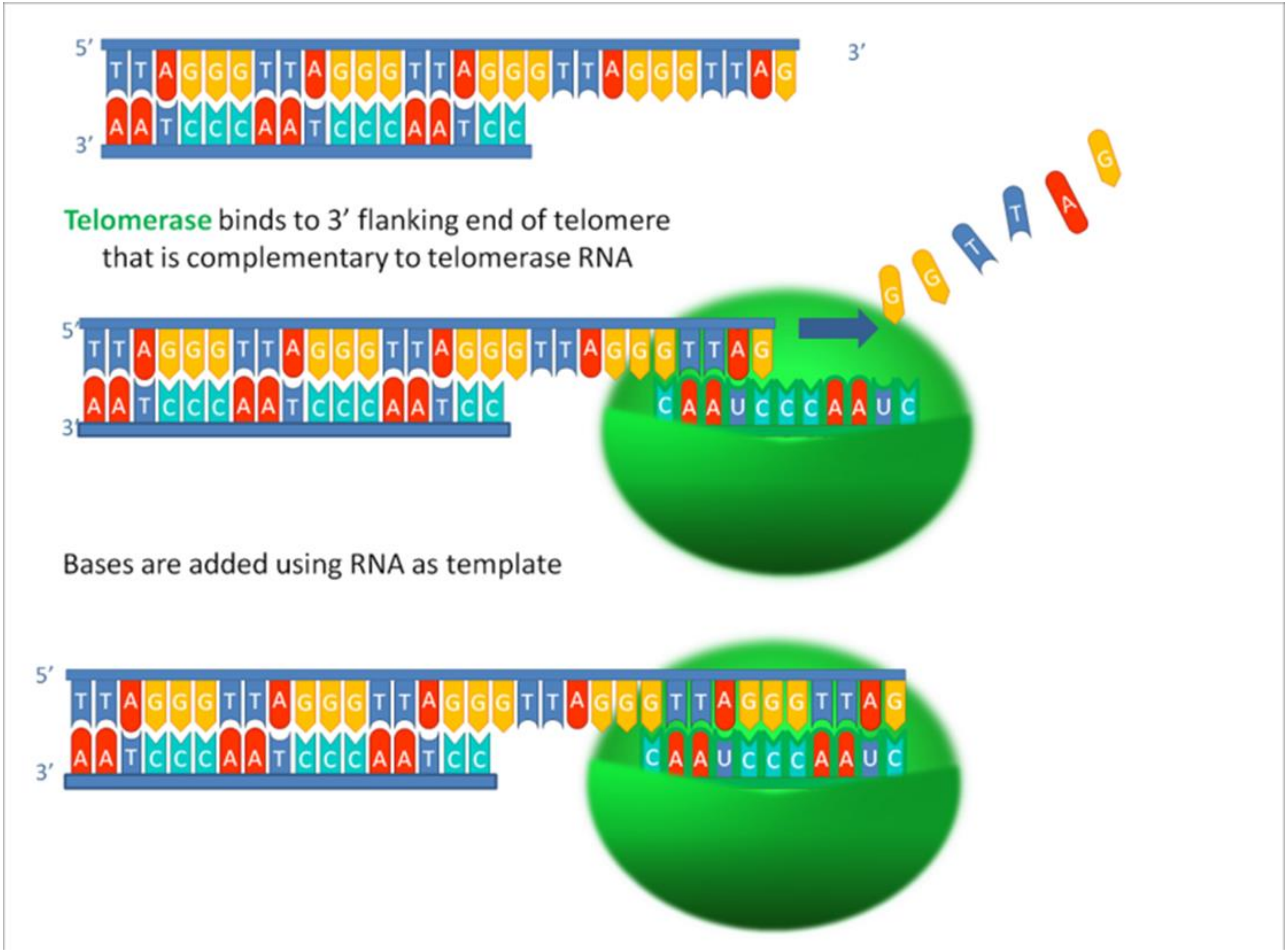


Telomerase

- Telomeres: nucleotides at end of chromosomes
- Contain T-T-A-G-G-G sequences
- No place for RNA primer on lagging strand
- Major problem eukaryotic cells (non-circular DNA)
- **Telomerase enzyme**
  - Recognizes telomere sequences
  - Adds these sequences to new DNA strands

Telomerase

- Contains an **RNA template**
- Uses template to synthesize telomere DNA
- "RNA-dependent DNA polymerase"
- Similar to reverse transcriptase



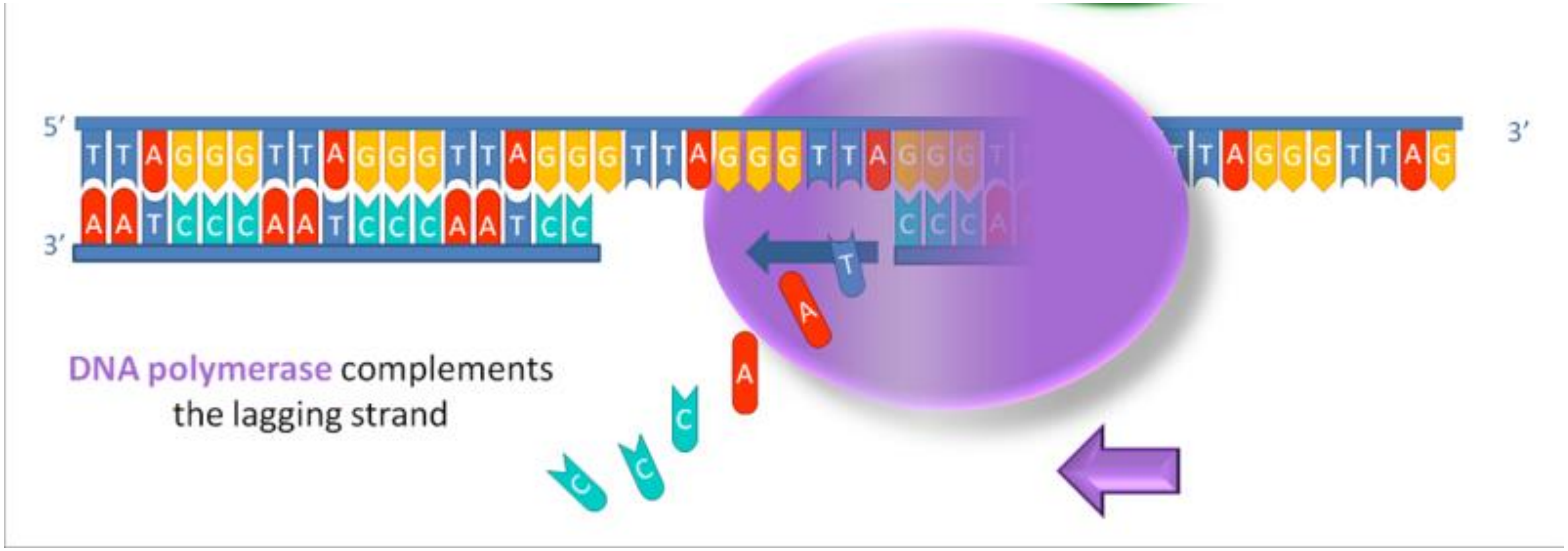
Telomerase binds to 3′ flanking end of telomere that is complementary to telomerase RNA

Bases are added using RNA as template

Uzbas, F/Wikipedia

Telomerase

- Extends 3' end of DNA
- Allows DNA polymerase to complete lagging strand
- Avoids loss of genes with duplication



Uzbas, F/Wikipedia

Telomerase

- Found in hematopoietic stem cells
  - Allows controlled indefinite replication
- Other cells that divide indefinitely
  - Epidermis, hair follicles, intestinal mucosa
- Implicated in many cancers
  - Allows immortality

DNA Mutations

Jason Ryan, MD, MPH

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Protein Synthesis

DNA

↓ Transcription

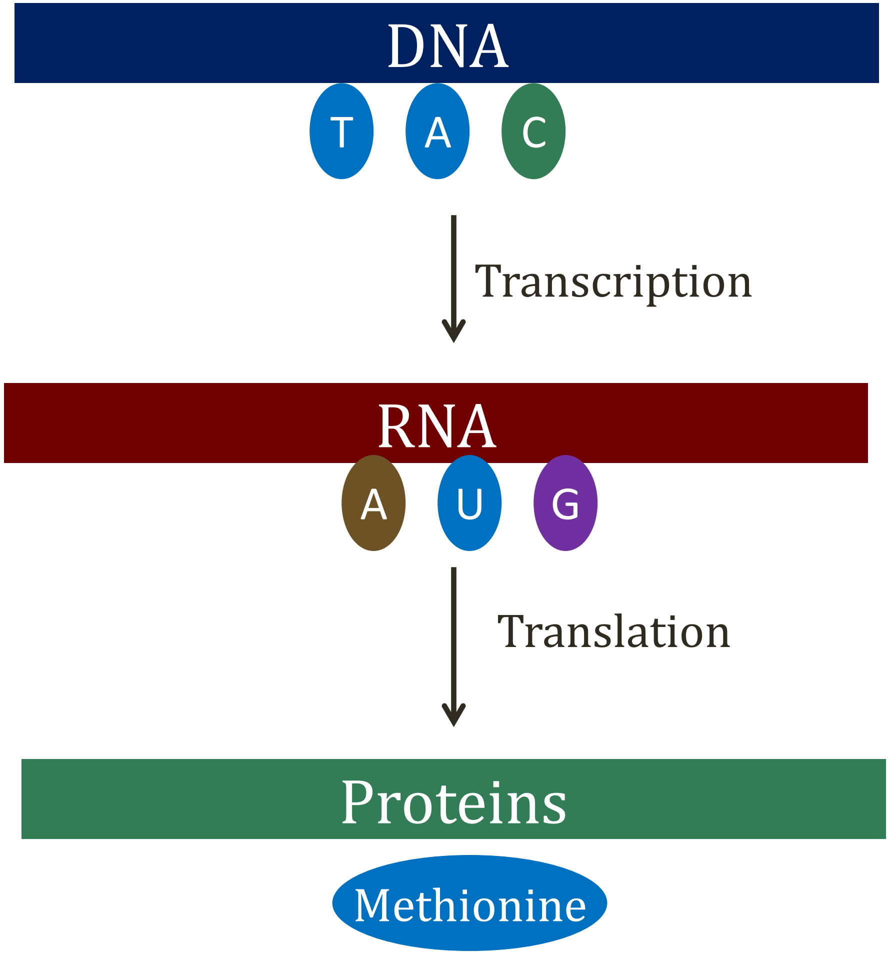
RNA

↓ Translation

Proteins

Codons

3 Nucleotide Sequences



DNA

T A C

Transcription

RNA

A U G

Translation

Proteins

Methionine

Genetic Code

Standard genetic code

1st base	2nd base								3rd base
	U		C		A		G		
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

DNA Mutations

- Errors in DNA
- Simple: One/few base(s) abnormal
- Complex: Gene deletions, translocations

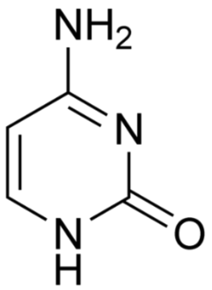
DNA Mutations

- **Germ line** mutations
  - DNA of sperm/eggs
  - Transmitted to offspring
  - Found in every cell in body
- **Somatic** mutations
  - Acquired during lifespan of cell
  - Not transmitted to offspring

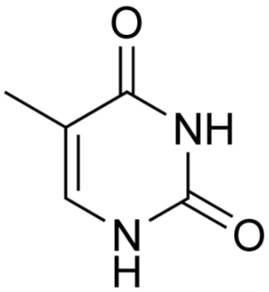
Point Mutations

- **Transition** (more common):
  - Purine to purine A to G
  - Pyrimidine to pyrimidine (C to T)
- **Transversion**:
  - Purine to pyrimidine (A to T)
  - Pyrimidine to purine (C to G)

Pyrimidines

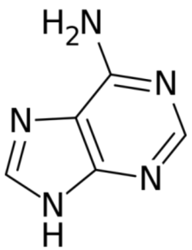


Cytosine

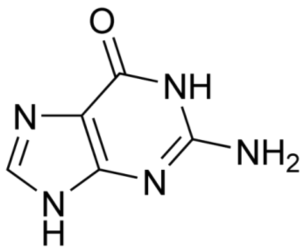


Thymine

Purines



Adenine

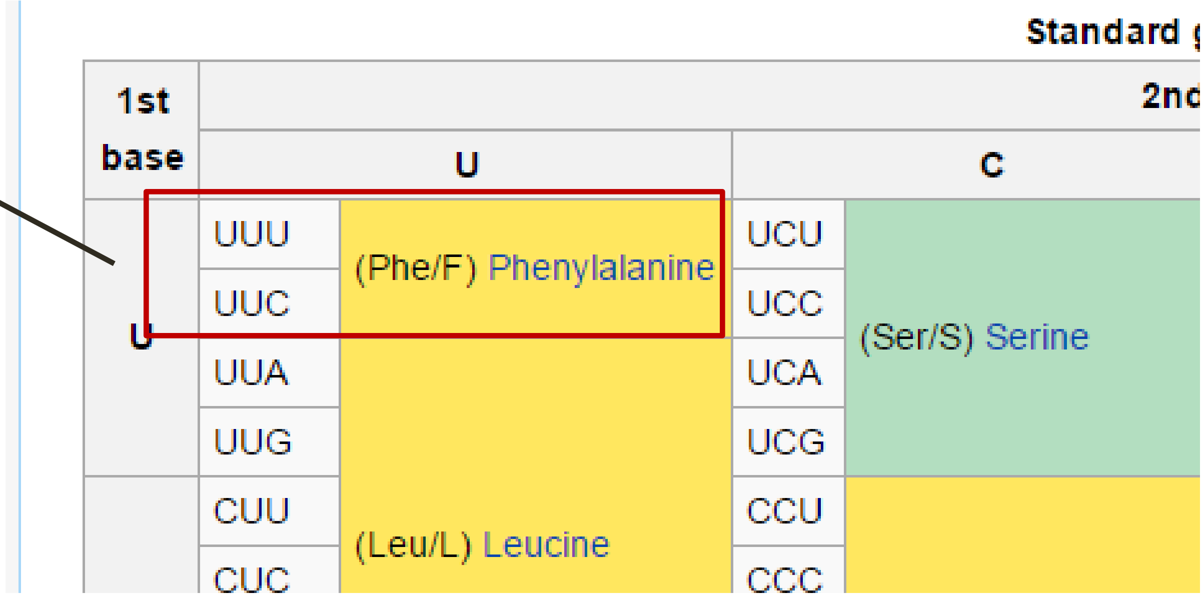


Guanine

Wobble

- Some transitions less likely to alter amino acids
- Genetic code: often same AA with altered base

UU-Pyrimidine
Same AA



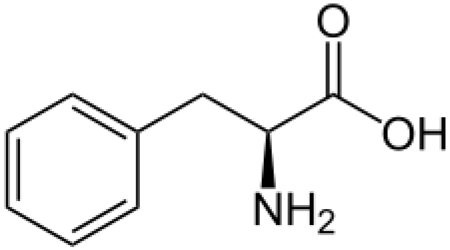
Standard

1st base	2n			
	U		C	
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine
	UUC		UCC	
	UUA	(Leu/L) Leucine	UCA	
	UUG		UCG	
	CUU		CCU	
	CUC		CCC	

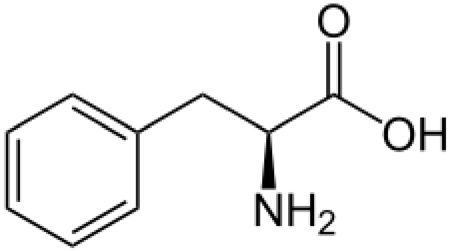
Silent Mutation

- Nucleotide substitution codes for **same amino acid**
- Often base change in 3rd position of codon

DNA	**A – A – A**		**A – A – G**
RNA	**U – U – U**		**U – U – C**
	PHE	→	PHE



Phenylalanine



Phenylalanine

Nonsense Mutation

- Nucleotide substitution
- Result: **Early stop codon**
  - Nucleotide triplet
  - Signals termination of translation of proteins
  - UGA, UAA, UAG

DNA A – C – C
RNA U – G – G

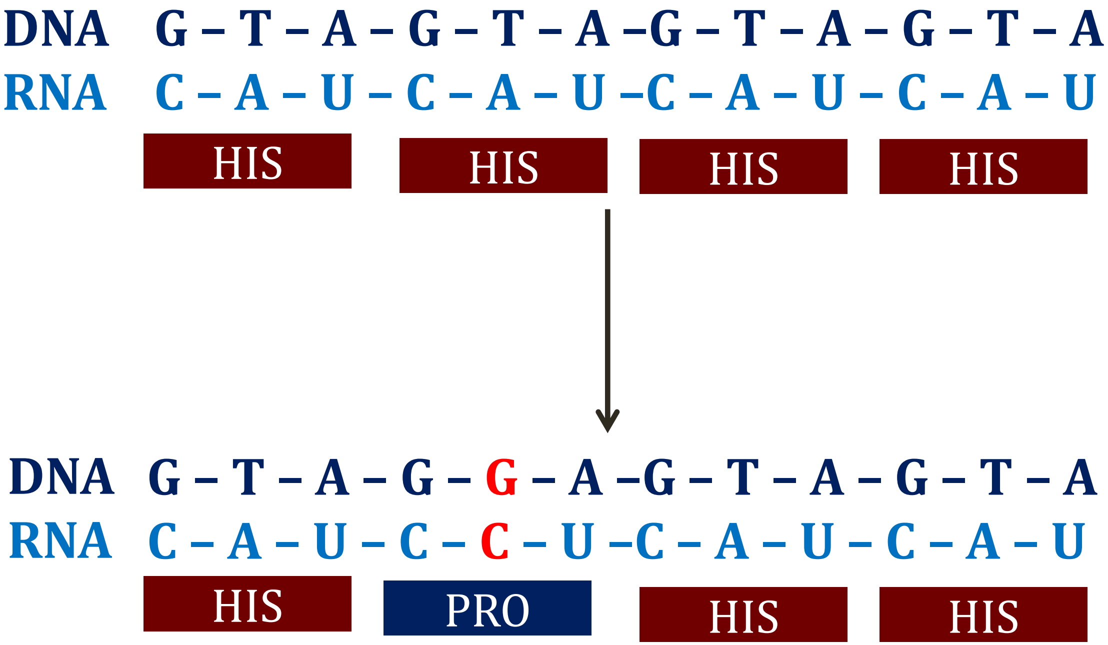
TRY

→

A – C – T
U – G – A

Missense Mutation

- Nucleotide substitution
- Result: **Different amino acid**



DNA G – T – A – G – T – A –G – T – A – G – T – A

RNA C – A – U – C – A – U –C – A – U – C – A – U

HIS HIS HIS HIS

DNA G – T – A – G – G – A –G – T – A – G – T – A

RNA C – A – U – C – C – U –C – A – U – C – A – U

HIS PRO HIS HIS

Sickle Cell Anemia

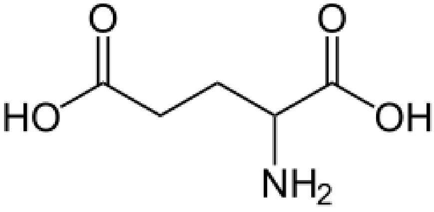
- Root cause: **Missense mutation** beta globin gene
- Single base substitution 6th codon of β gene
  - Adenine changed with thymine
  - Substitution of valine for glutamate in beta chains

DNA **G – A – G** → **G – T – G**

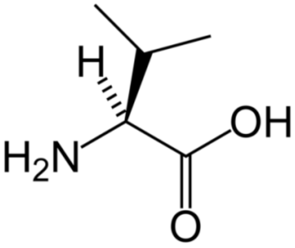
C – T – C → **C – A – C**

RNA **G – A – G** → **G – U – G**

GLU → VAL



Glutamate



Valine

Insertions and Deletions

- Addition/subtraction of nucleotides
- Can alter the protein product of a gene
- Cystic fibrosis
  - Most common mutation: delta F508
  - **Deletion** of 3 DNA bases
  - Loss of phenylalanine
  - Abnormal protein folding

Insertions and Deletions

- Addition/subtraction of nucleotides
- Can alter the protein product of a gene
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  - Most common mutation: delta F508
  - **Deletion** of 3 DNA bases
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Frameshift Mutation

- Insertion or deletion of nucleotides/bases
- **Alters the reading frame**

DNA G – T – A – G – T – A – G – T – A – G – T – A

HIS HIS HIS HIS

Point Mutation ↓

DNA Size Unchanged

G – T – A – G – C – A – G – T – A – G – T – A

HIS ARG HIS HIS

G – T – A – G – T – A – G – T – A – G – T – A

HIS HIS HIS HIS

Frameshift Mutation ↓

DNA Size Changed

A – G – T – A – G – T – A – G – T – A – G – T – A

SER SER SER SER

Frameshift Mutation

- Deletion/insertion not multiple of 3
- Misreading of nucleotides downstream
- **Significant change to protein**
  - Many amino acids may change
  - Early stop codon → truncated protein
  - Loss of stop codon → elongated protein

Frameshift Mutation

- Described in **Tay Sachs disease**
  - Frameshift mutations (insertions/deletions)
  - Gene for hexosaminidase A
- **Duchenne muscular dystrophy**
  - Dystrophin gene
  - Frameshift deletions → absence of functional dystrophin

Slipped-Strand Mispairing

DNA Slippage

- Occurs in areas of **repeated nucleotide sequences**
- Occurs with inadequate mismatch repair
- Insertions/deletions → frameshift mutations

Slipped-Strand Mispairing

DNA Slippage

Slippage in template strand → deletion (DNA not replicated)
Slippage in replicated strand → insertion (replicated strand longer)

Trinucleotide Repeat Disorders

- Occur in genes with repeat trinucleotide units
  - Example: CAGCAGCAGCAG
- Extra repeats in gene → disease
- Key examples
  - Fragile X syndrome
  - Friedreich's ataxia
  - Huntington's disease
  - Myotonic dystrophy

Microsatellite Instability

- Microsatellite
  - Short segments of DNA
  - Repeated sequence (i.e. CACACACA)
- Mismatch repair enzyme failure → instability
  - Variation (instability) in size of segments among cells
- Seen in colon cancer

DNA Repair

Jason Ryan, MD, MPH

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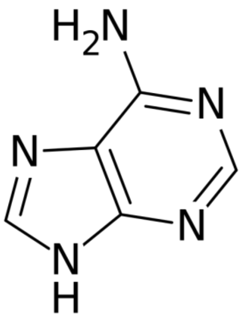
DNA Damage

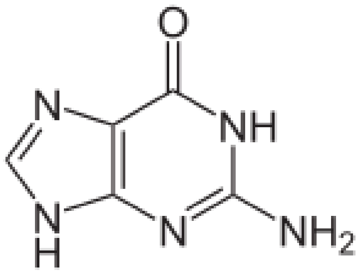
- Occurs frequently in life of a cell
  - Heat, UV radiation, chemicals, free radicals
- Rarely leads to permanent damage
- Numerous repair enzymes/mechanisms exist
- Without repair, genetic material quickly lost

Types of DNA Damage

- **Depurination**
  - Occurs spontaneously thousands of times per day
  - Results in loss of purine bases (guanine and adenine)
- **Deamination**
  - Occurs spontaneously hundreds of times per day
  - Base loses amine group (cytosine)



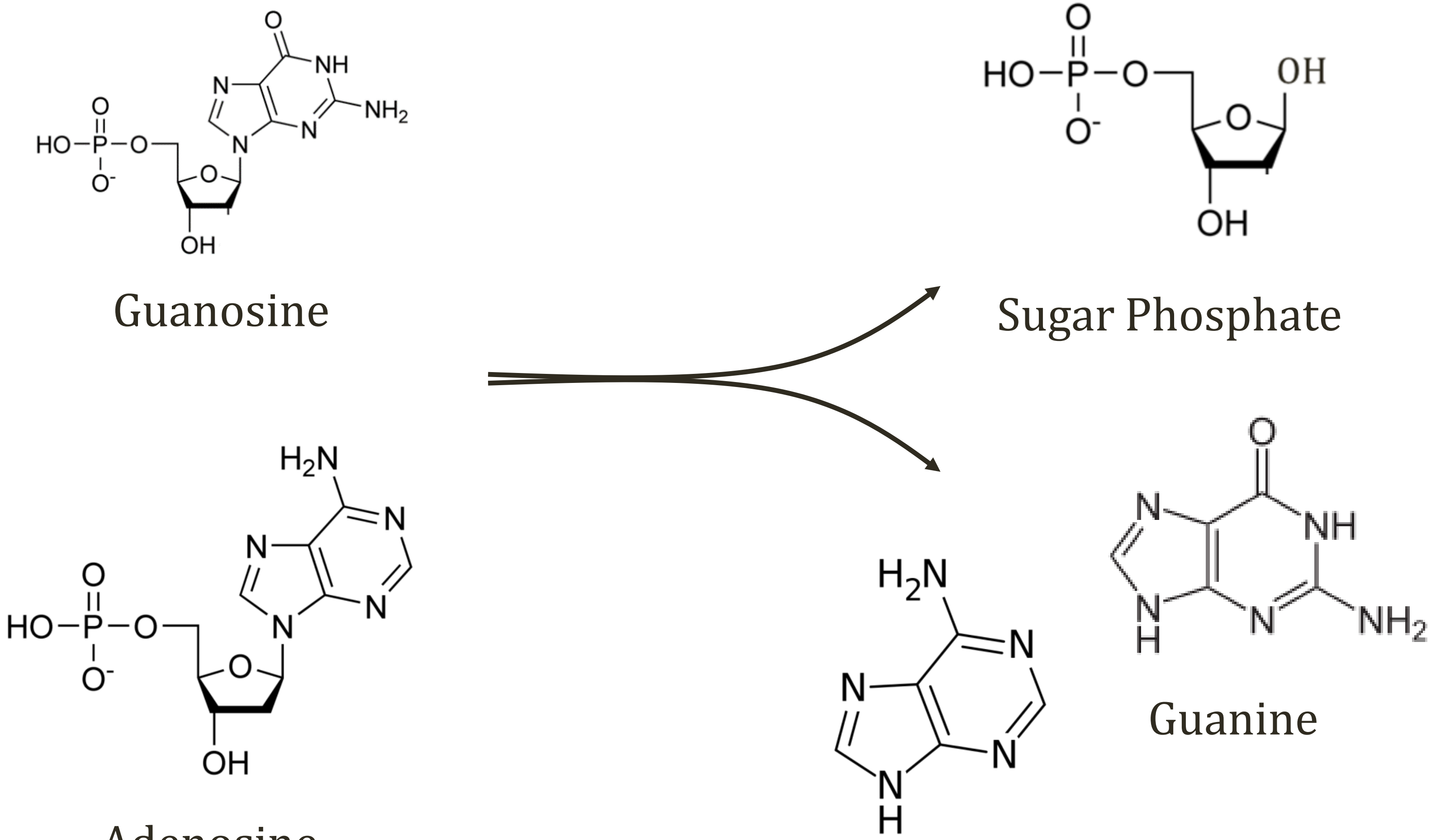
Adenine



Guanine

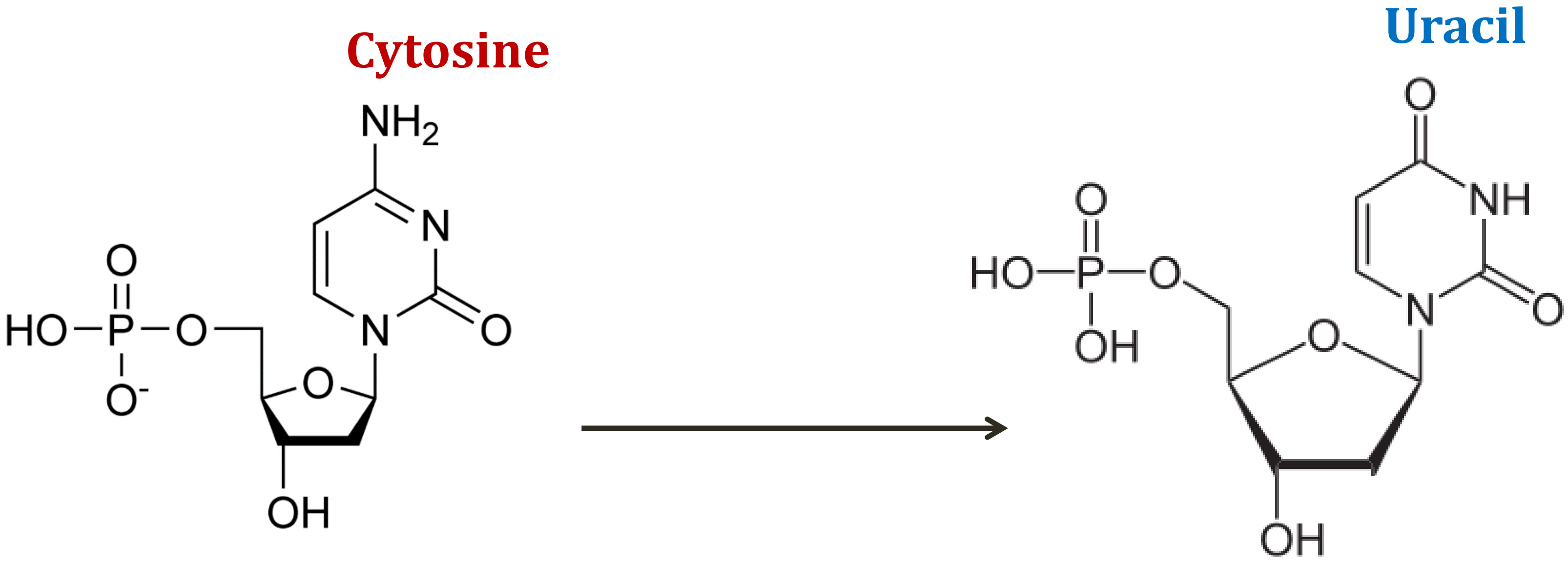
Types of DNA Damage

Depurination



Types of DNA Damage

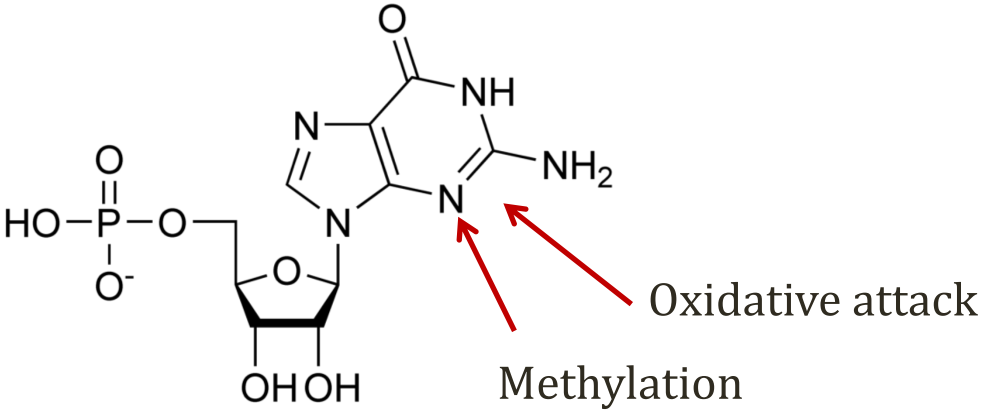
Deamination



Cytidine

Types of DNA Damage

- Free radicals or radiation damage base rings
- Oxidative damage, methylation, hydrolysis



Guanosine

Repair Mechanisms

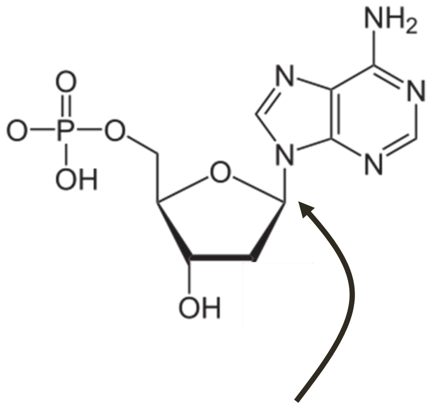
- Single strand
  - Base excision
  - Nucleotide excision
  - Mismatch repair
- Double strand
  - Homologous end joining
  - Non-homologous end joining

Base Excision Repair

- Pathway for damaged DNA repair
- Recognize specific base errors
    - Deaminated bases, oxidized bases, open rings
- Numerous variations/enzymes used by cells
- Functions throughout the cell cycle (all phases)

Base Excision Repair

- **DNA glycosylases**
  - Several different enzymes
- Remove damaged bases
- Creates a baseless nucleotide
- “Apurinic” or “apyrimidic” nucleotide

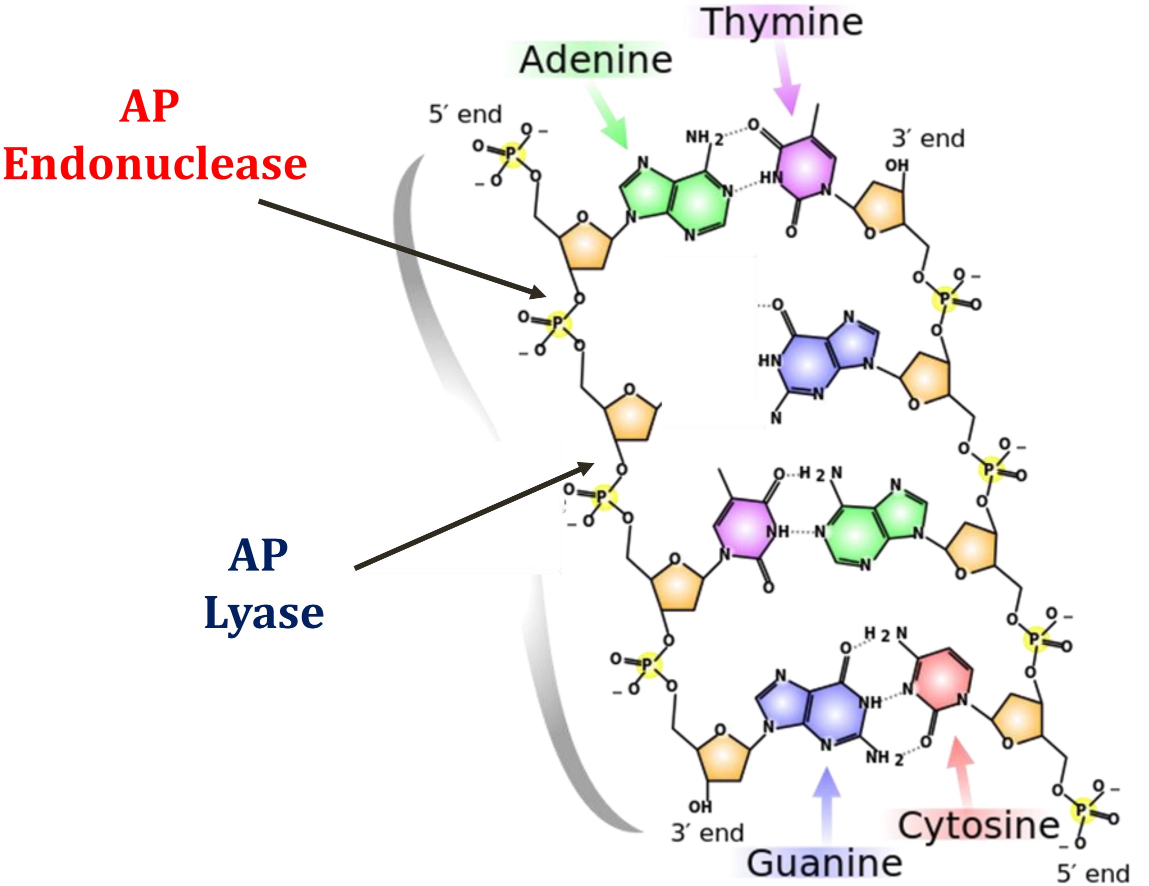


Glycosidic Bond

Base Excision Repair

- **AP endonuclease**
  - Recognizes nucleotides without a base
  - Attacks 5’ phosphate end of DNA strand
  - “Nicks” damaged DNA upstream of AP site
  - Create a 3'-OH end adjacent to the AP site
- **AP lyase**
  - Some DNA glycosylases also possess AP lyase activity
  - Attack 3’ hydroxyl end of ribose sugar

Base Excision Repair



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Base Excision Repair

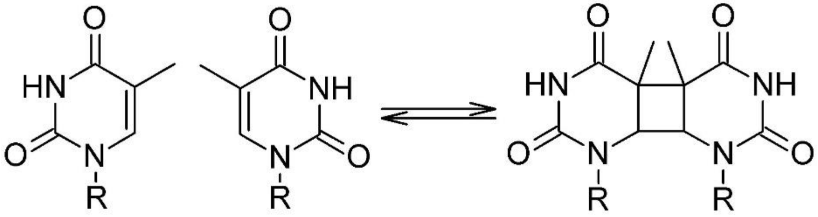
- **DNA polymerase**
  - Adds new nucleotide (complementary to opposite base)
  - Extends 3'-OH terminus
- **DNA ligase** seals strand

Nucleotide Excision Repair

- Removes **"bulky"** DNA damage
  - Multiple bases
  - Often **pyrimidine dimers**
  - Commonly caused by UV radiation (sunlight)
- G1 phase (prior to DNA synthesis)
- **Endonucleases** removed multiple nucleotides
- DNA polymerase and ligase fill gap

Public Domain

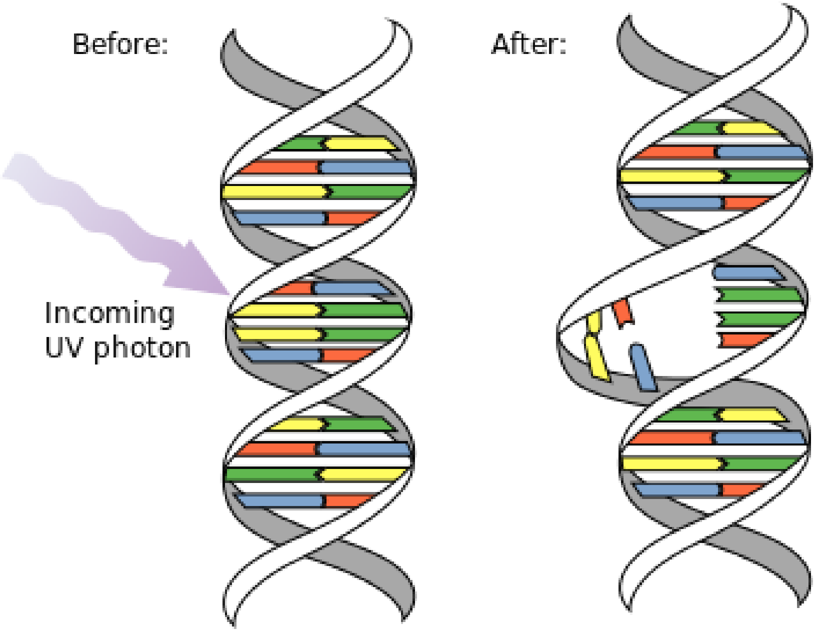
Nucleotide Excision Repair



Thymidine

Thymidine

Cyclobutane
Pyrimidine
Dimer



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Xeroderma Pigmentosum

- Defective **nucleotide excision repair**
- Extreme sensitivity to **UV rays from sunlight**
- Signs appear in infancy or early childhood
- Very easy sunburning
- Freckling of skin
- Dry skin (xeroderma)
- Changes in skin pigmentation
- Very high risk of **skin cancer**
  - May develop in childhood

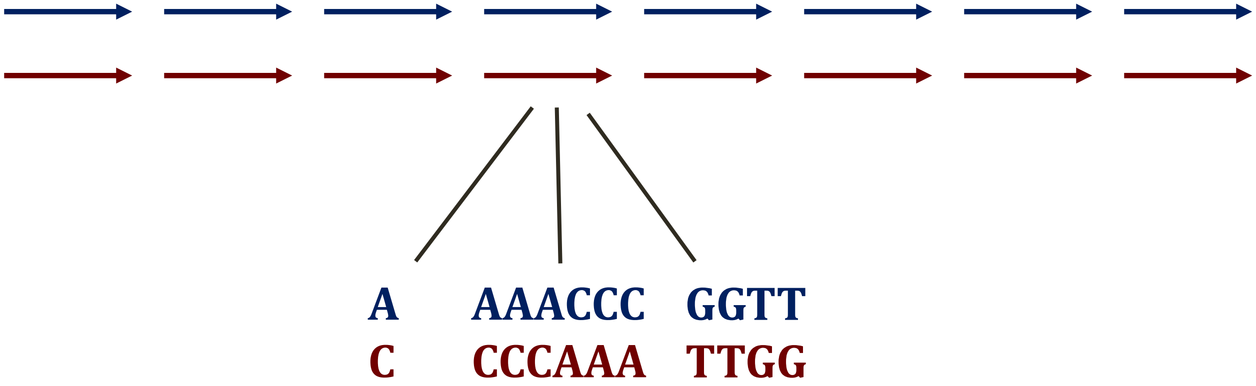
James Halpern, Bryan Hopping and Joshua M Brostoff

Mismatch Repair

- Identifies **incorrectly placed bases/nucleotides**
  - Insertions, deletions, incorrect matches
  - Occurs when proofreading misses errors
- No damage to base – not recognized by repair systems
- Occurs in **S/G2 phase** (after DNA synthesis)
- Newly synthesized strand compared to template
- Nucleotide errors removed and resealed

Mismatch Repair

- Important for **microsatellite stability**
- DNA has many repeating segments
- "Microsatellites"



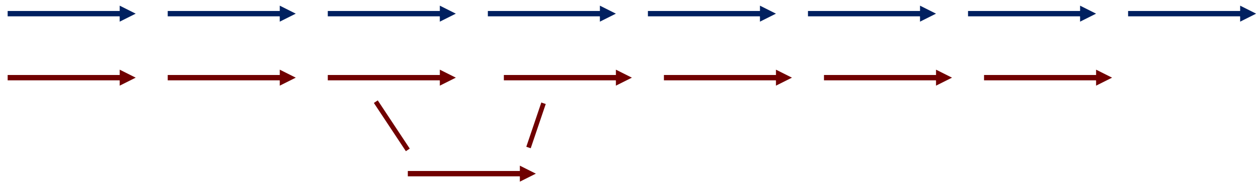
A **C**

AAACCC **CCCAAA**

GGTT **TTGG**

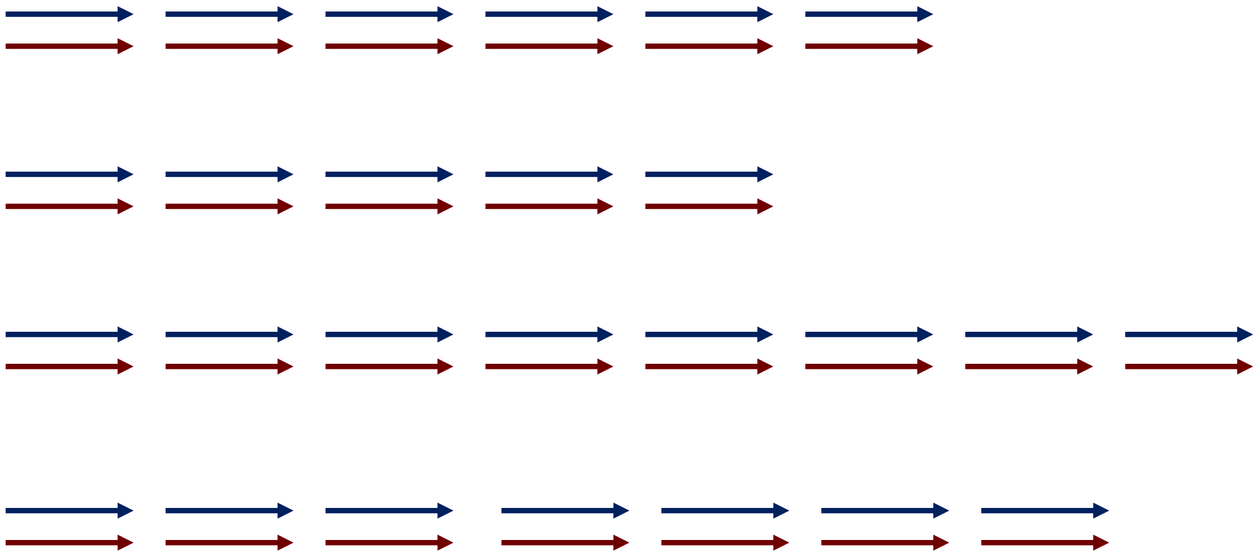
Mismatch Repair

- **DNA slippage** can occur at repeats
- Results in a mismatch
- Repaired by MMR systems
- Result: number of repeats (microsatellites) stable



Mismatch Repair

- Microsatellite instability
    - Results if **MMR systems deficient**
    - Seen in cancers cells (**colon cancer**)



HNPCC

Hereditary Non-Polyposis Colorectal Cancer/Lynch Syndrome

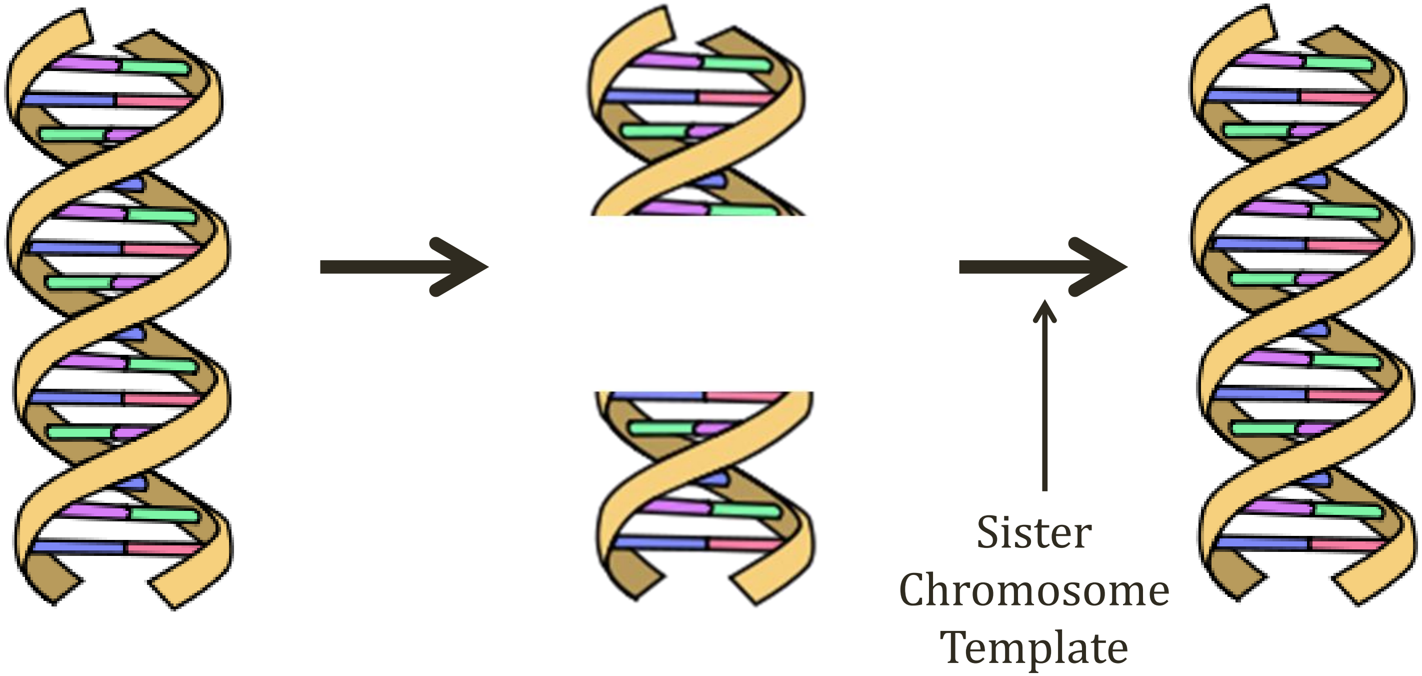
- **Germline mutation** of DNA mismatch repair enzymes
  - About 90% due to **MLH1 and MSH2 mutations**
- Leads to colon cancer via microsatellite instability
  - About 80% lifetime risk
- Hallmark: cancer cells with **microsatellite instability**

Double Strand Damage

- Commonly result from exogenous sources
    - Ionizing radiation
- Caused by radiation therapy (cancer)

Homologous End Joining

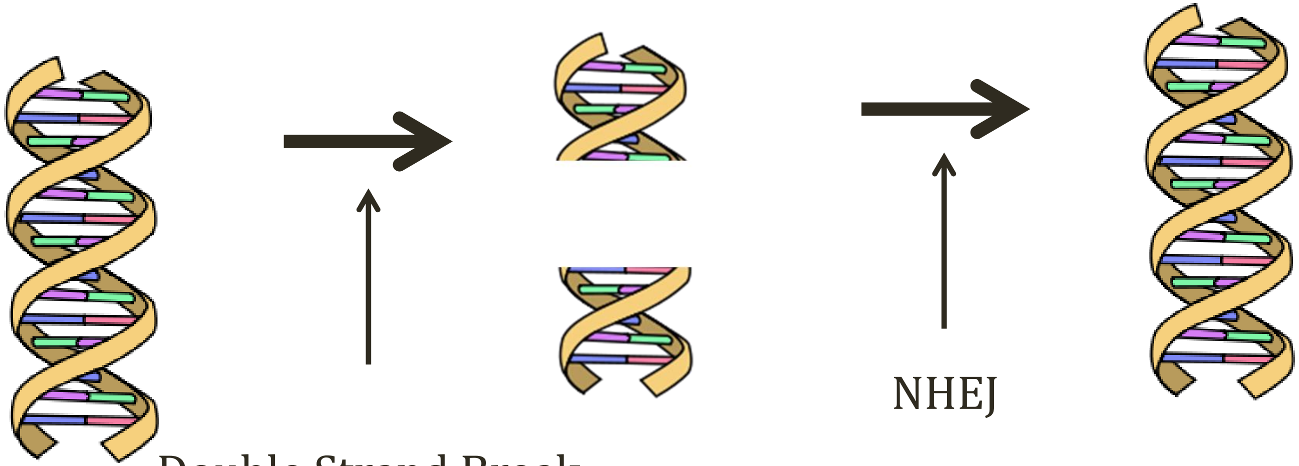
- Homology = similar structure
- HEJ = uses sister chromosome template



Sister Chromosome Template

Non-Homologous End Joining

- Uses many proteins to re-join broken ends
  - DNA pol λ and μ re-extend the ends
  - Many other enzymes
- No template used (non-homologous)
- Highly error-prone



Double Strand Break
(ionizing radiation)

NHEJ

Fanconi Anemia

- Inherited aplastic anemia
- More than 13 genetic abnormalities identified
- Many involve **DNA repair enzymes**
  - Hypersensitivity to DNA damage
  - Cells vulnerable to DNA strand cross-links
  - Also impaired **homologous recombination**

Ataxia Telangiectasia

- Defective **Nonhomologous end-joining (NHEJ)**
  - Mutations in ATM gene on chromosome 11
  - Ataxia Telangiectasia Mutated gene
  - Repairs double stranded DNA breaks via NHEJ
- **DNA hypersensitive to ionizing radiation**
- CNS, skin, immune system affected

Ataxia Telangiectasia

Clinical Features

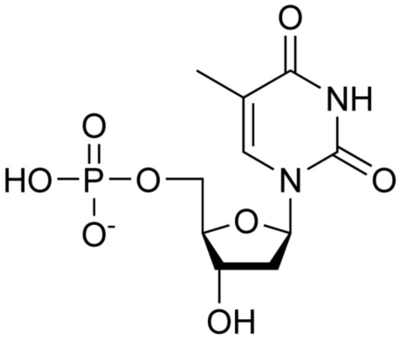
- Most children healthy for first year
- Begin walking at normal age but slow development
- Progressive motor coordination problems
- By 10 years old, most in wheelchairs
- Other symptoms
  - Recurrent sinus/respiratory infections (immune system)
  - Telangiectasias (skin)
- **High risk of cancer**

Transcription

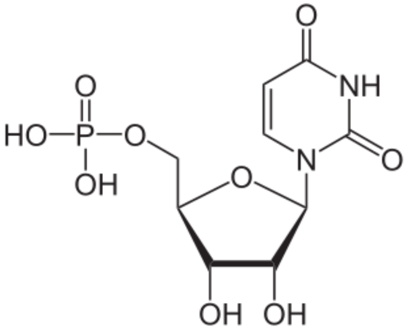
Jason Ryan, MD, MPH

Transcription

- Synthesis of RNA
  - Ribonucleotides (not deoxyribonucleotides)
  - Uridine (not thymidine)
- DNA used as template

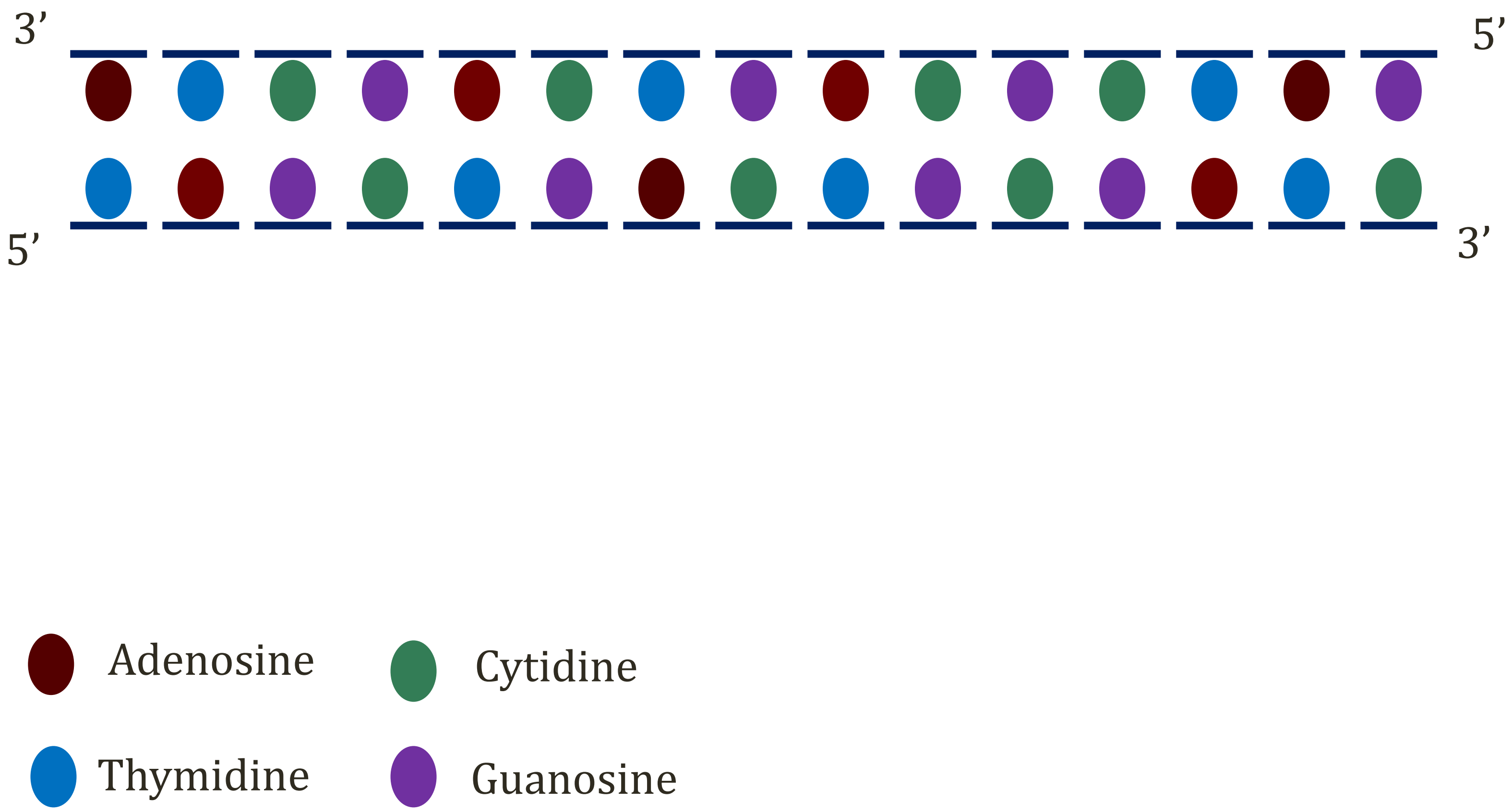


Thymidine

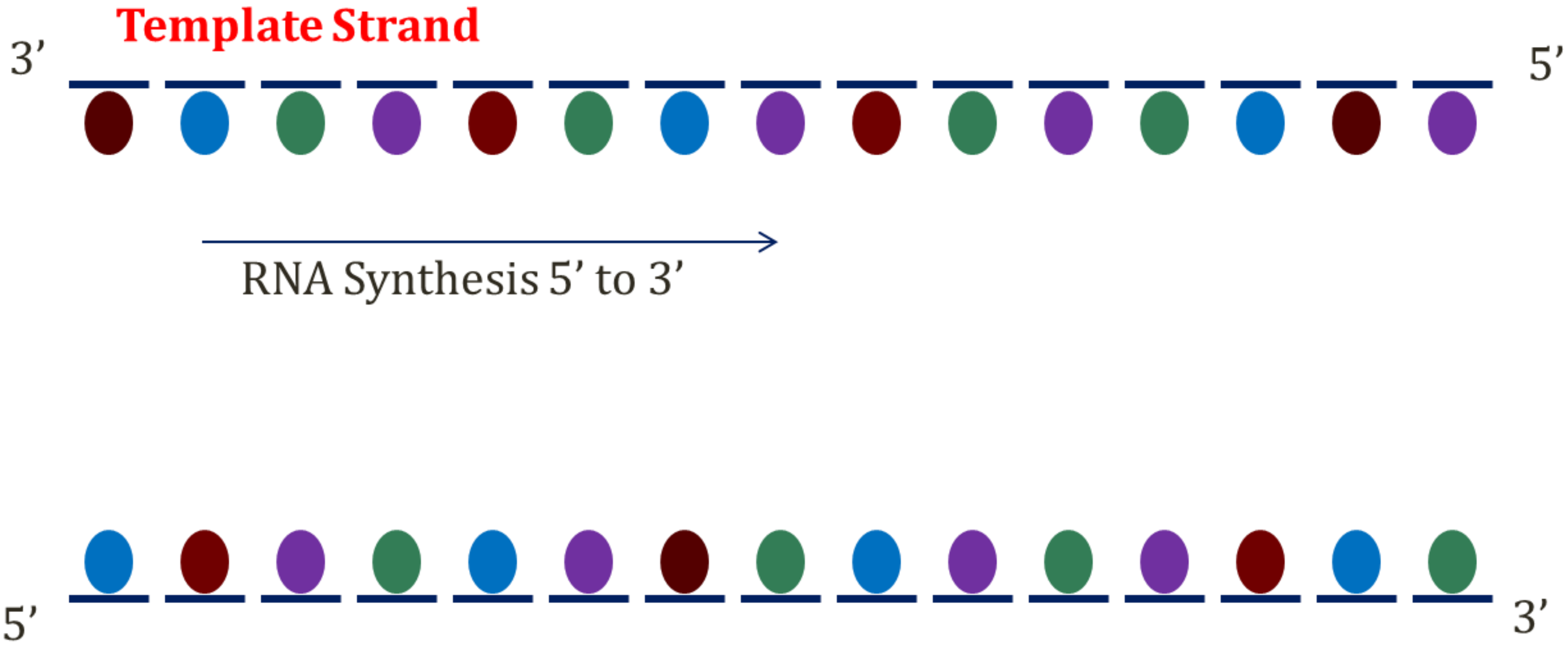


Uridine

Transcription



Transcription



Template Strand

3’ 5’

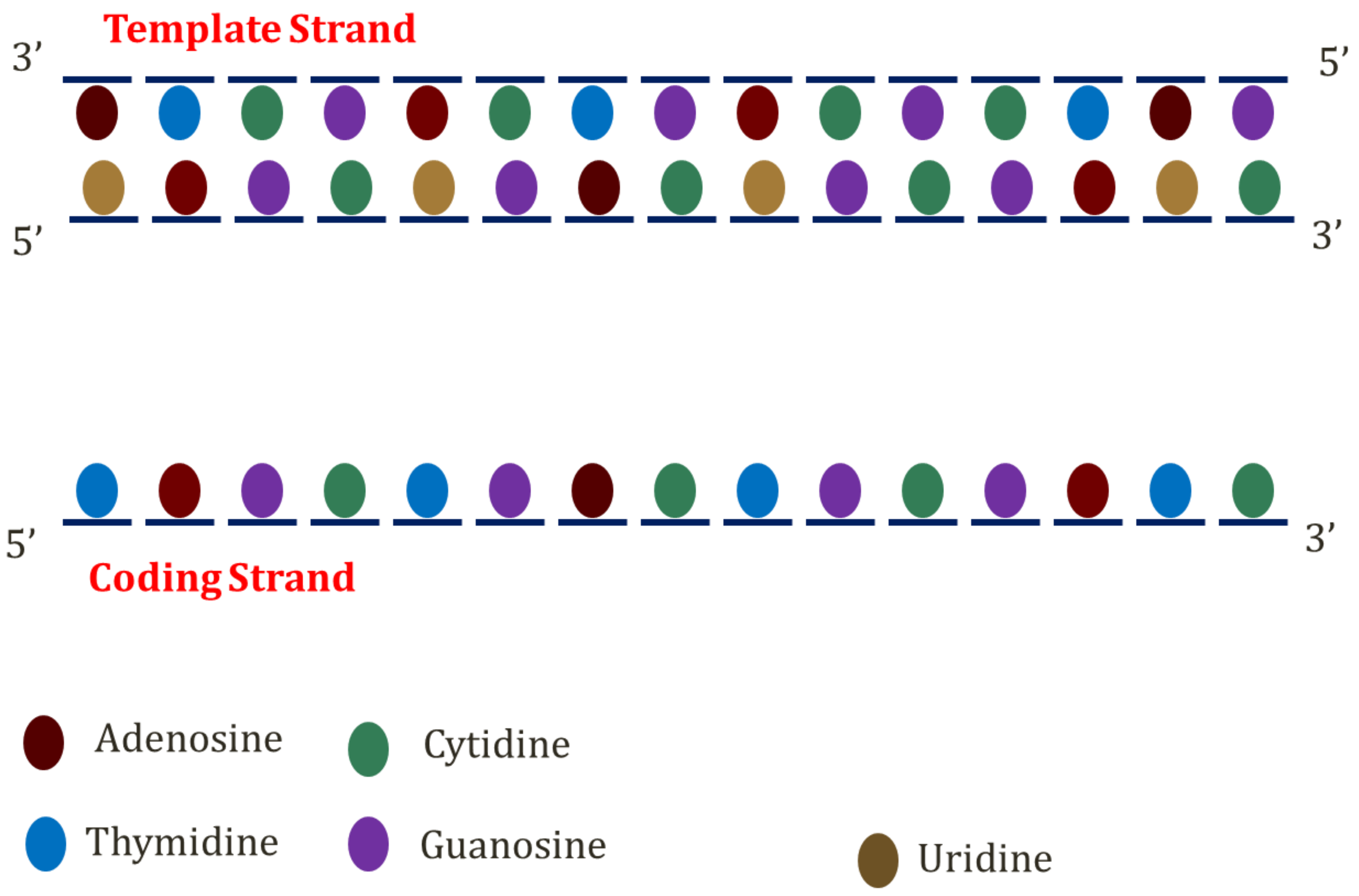
RNA Synthesis 5’ to 3’

5’ 3’

● Adenosine ● Cytidine

● Thymidine ● Guanosine

Transcription



Template Strand

3'

5'

5'

3'

5'

3'

Coding Strand

Adenosine

Cytidine

Thymidine

Guanosine

Uridine

Transcription

DNA GTCA

↓ Transcription

RNA GUCA

Types of RNA

- Messenger RNA
  - Longest chains of RNA
  - Nucleotides specify **amino acids**
  - Used the synthesize **proteins**
- Ribosomal RNA
  - Form ribosomes
- Transfer RNA
  - Transfer amino acids to proteins

Types of RNA

- Micro RNA (miRNA)
  - Regulate gene expression
  - Target mRNA molecules → bind via base pairing
  - Block translation into protein
- Small interfering RNA (siRNA)
  - Also regulate gene expression
  - Cause degradation of mRNA
- Small nuclear RNA (snRNA)
  - Splicing of pre-mRNA

RNA Polymerase

- Synthesizes RNA from DNA template
- **Does not require a primer** (like DNA polymerase)
- Binds **promoter regions** of DNA
- Requires **transcription factors** (proteins)
- Binds DNA → opens double helix

RNA Polymerase

- Prokaryotes: One RNA polymerase
  - Multi-subunit complex
  - Makes all types of RNA
- Eukaryotes: multiple RNA polymerase enzymes
- RNA polymerase I: most **rRNA** (5.8S, 18S, 28S)
- RNA polymerase II: **mRNA**
- RNA polymerase III: rRNA (5S), other RNAs

RNA Polymerase Inhibitors

- Alpha amanitin
  - Protein found in *Amanita phalloides* (death cap mushrooms)
  - Powerful inhibitor of RNA polymerase II
  - Liver failure (taken up by liver cells)

Newnam/Wikipedia

RNA Polymerase Inhibitors

- Rifampin
  - Inhibits ***bacterial*** RNA polymerase
  - Used to treat tuberculosis
- Actinomycin D
  - Used as chemotherapy
  - Inhibits RNA polymerase

Transcription Factors

- Additional **proteins** required to initiate transcription
- Prokaryotes
  - Protein factor (σ factor)
- Eukaryotes
  - Multiple factors ("**transcription factors**")
  - Many bind RNA polymerase II
  - TFIID, TFIIB, TFIIE, etc

Promoters

- DNA regions
- Not transcribed
- **Bind RNA polymerase** and transcription factors
- Bound RNA polymerase opens double helix


Transcription Start Point

Promoter

Gene

5’

3’

Promoters

- **TATA Box**
  - Very common eukaryotic promoter
  - TATAAA
  - Binds transcription factors (TFIID)
- **CAAT Box**
  - CCAAT sequence
- GC Box
  - GGGCGG

Enhancers

- DNA sequences that **increase** rate of transcription
- May be upstream or downstream of gene they regulate
- Binds transcription factors called **activators**
- Because of DNA coiling, many are geometrically close but many nucleotides away from gene
- Stabilize transcription factors/RNA polymerase

Silencers

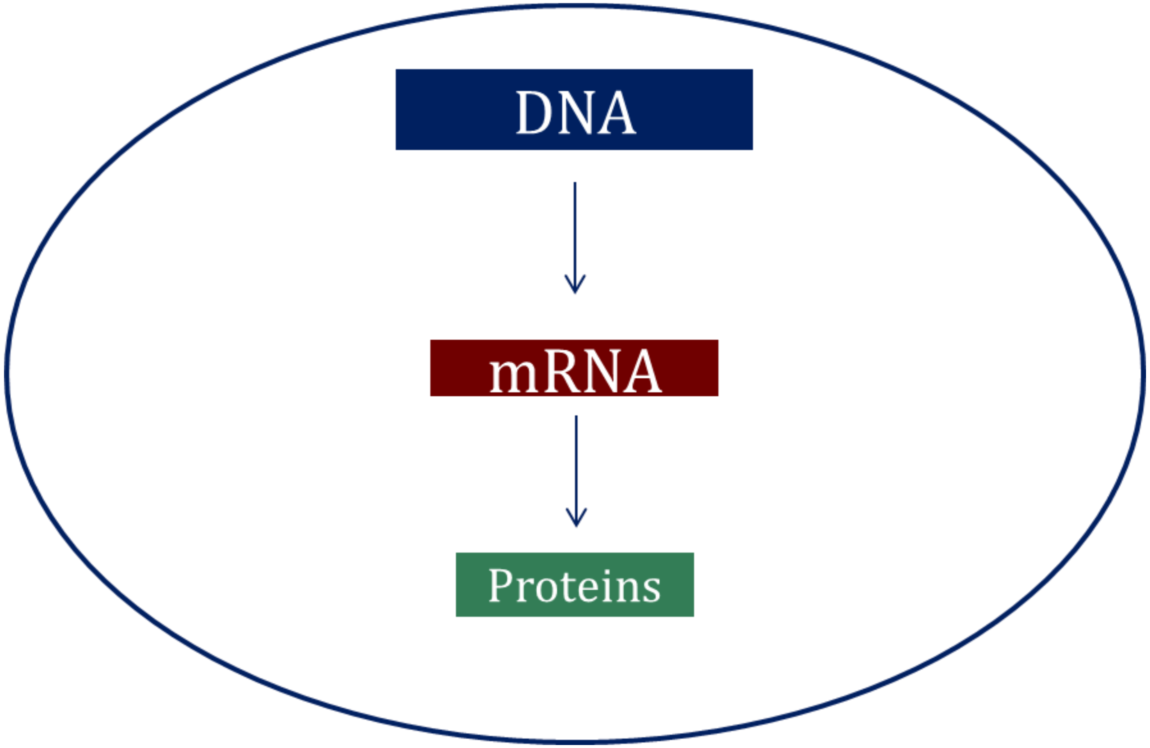
- DNA sequence that **decreases** rate of transcription
- May be upstream or downstream of gene they regulate
- Binds transcription factors called **repressors**
- Repressors prevent RNA polymerase binding

Untranslated Regions

- Portions of mRNA at 5’ and 3’ ends
- Not translated into protein
- 5’ UTR upstream from coding sequence
  - Recognized by ribosomes to initiate translation
- 3' UTR found following a stop codon
  - Important for post-transcriptional gene expression

Protein Synthesis

Prokaryotes



DNA

↓

mRNA

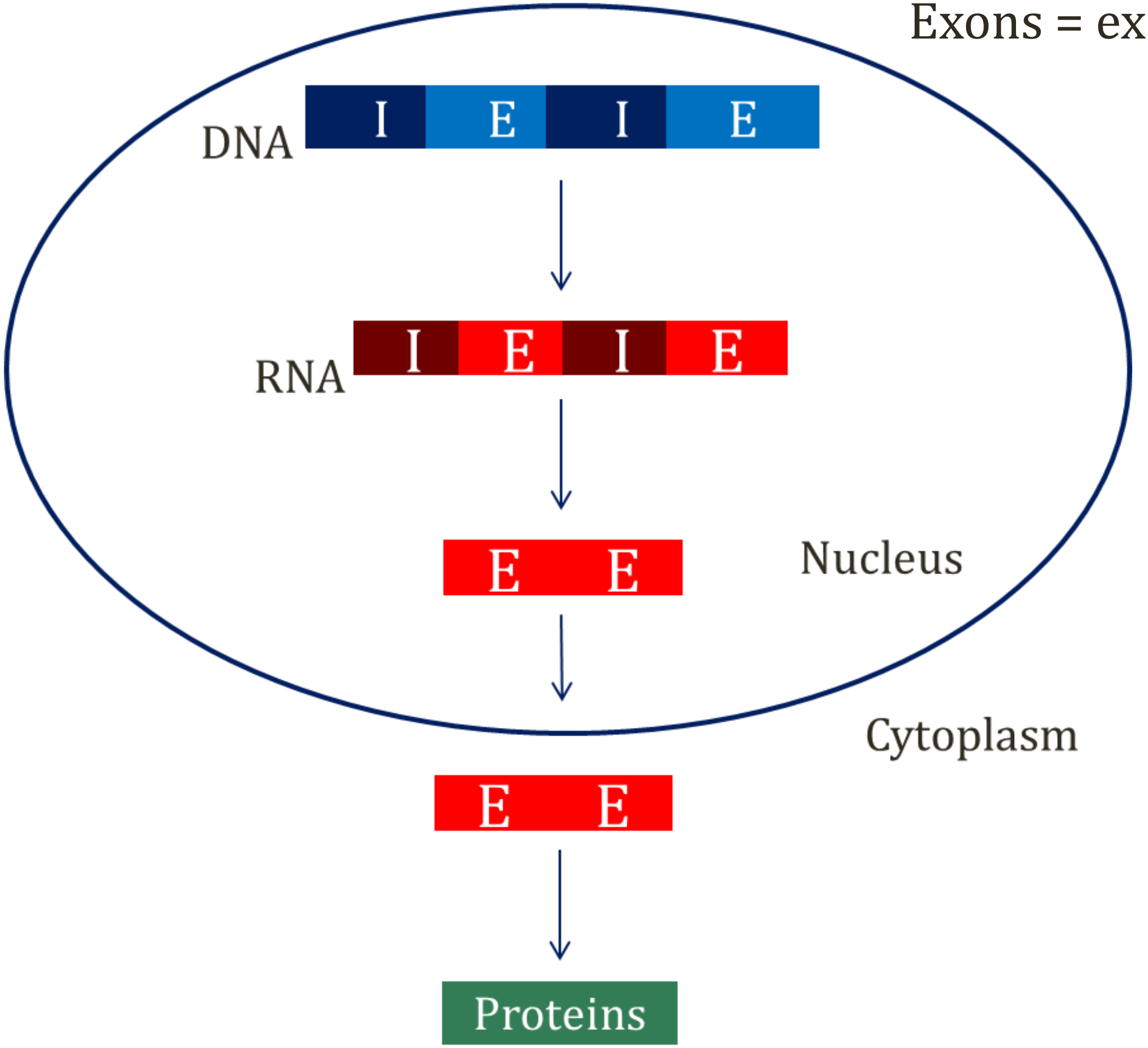
↓

Proteins

Protein Synthesis

Eukaryotes

Introns = stay IN nucleus
Exons = exit nucleus

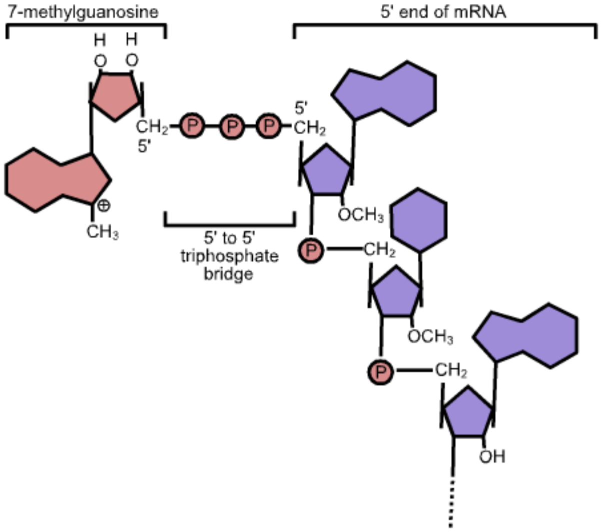


mRNA in Eukaryotes

- Initial transcript: hnRNA
  - Heterogeneous nuclear RNA
  - Also called pre-mRNA
- hnRNA modified to become mRNA
- **Three key modifications** before leaving nucleus
  - 5’ capping
  - Splicing out of introns
  - 3’ polyadenylation

5’ Capping

- Addition of 7-methylguanosine to 5’ end
- Added soon after transcription begins
- Distinguishes mRNA from other RNA



Zephyris/Wikipedia

RNA Splicing

- Occurs during transcription
- Introns removed from mRNA in nucleus
- Introns always have two nucleotides at either end
- 5' splice site: GU
- 3' splice site: AG

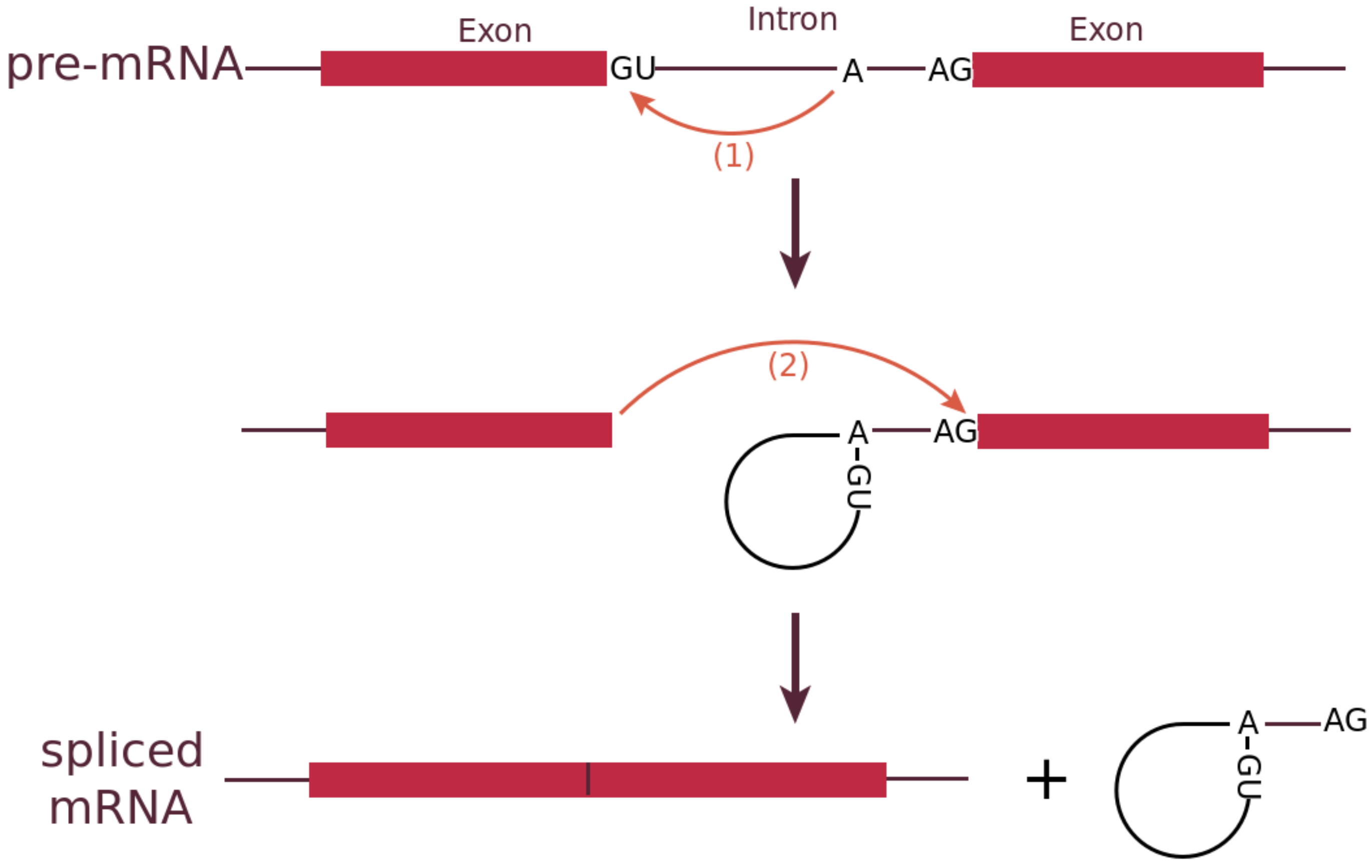
RNA Splicing

- Primary transcript combines with snRNPs
  - **Small nuclear ribonucleoproteins (snRNPs)**
  - Short RNA polymers complexed with proteins
  - RNAs contain high content of uridine (U-RNAs)
  - Five different U-RNAs defined: U1, U2, U4, U5, and U6

RNA Splicing

- snRNPs and mRNA forms “**spliceosome**”
- Loop of mRNA with intron is formed (“lariat”)
- Lariat released → removes intron
- Exons joined

RNA Splicing



BCSteve/Wikipedia

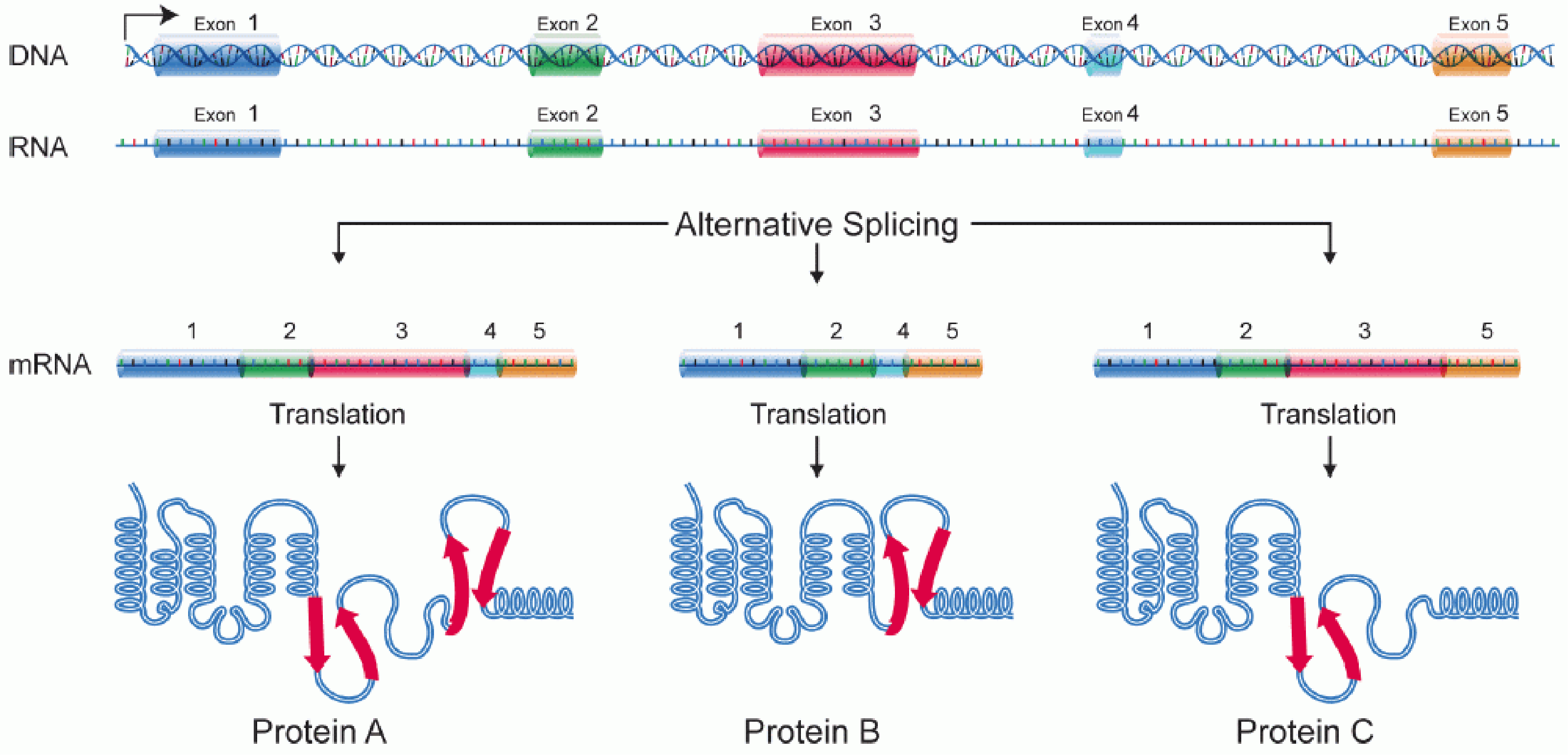
Antibodies

- Anti-Sm (anti-smith)
    - Antibodies against proteins in snRNPs
    - Seen in patients with **SLE**
- Anti-RNP
    - Antibodies against proteins associated with **U1 RNA**
    - Strongly associated with **Mixed Connective Tissue Disease**
    - Also seen in SLE, Scleroderma

Alternative Splicing

- Allows **many proteins from same gene**
- DNA: Exon1 – Exon 2 – Exon 3 – Exon 4 … Exon 10
- Protein 1: Exon1 – Exon 3 – Exon 7
- Protein 2: Exon 2 – Exon 5 - Exon 10

Alternative Splicing



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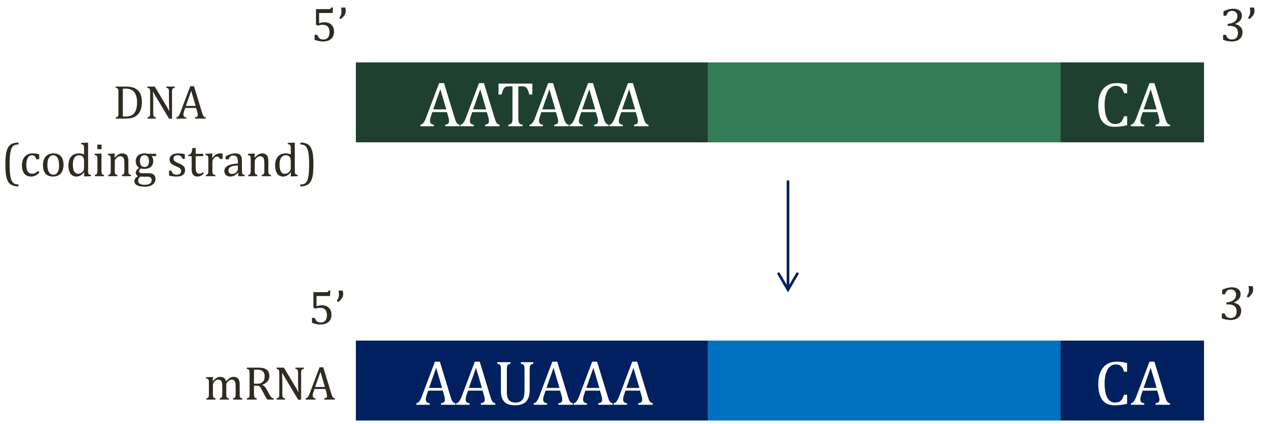
Splicing Errors

- Can lead to disease
  - Loss of exons, retention of introns
  - Incorrect joining of introns
- Beta thalassemia
  - Many mutations described
  - Some involve splice sites
- Oncogenesis
  - Many splice site mutations/errors described

3’ Polyadenylation

- Occurs at termination of mRNA transcription
- Triggered by specific DNA/RNA sequences
- “Polyadenylation signal:” AAUAAA
- AAUAAA followed by 10-30 nucleotides then CA

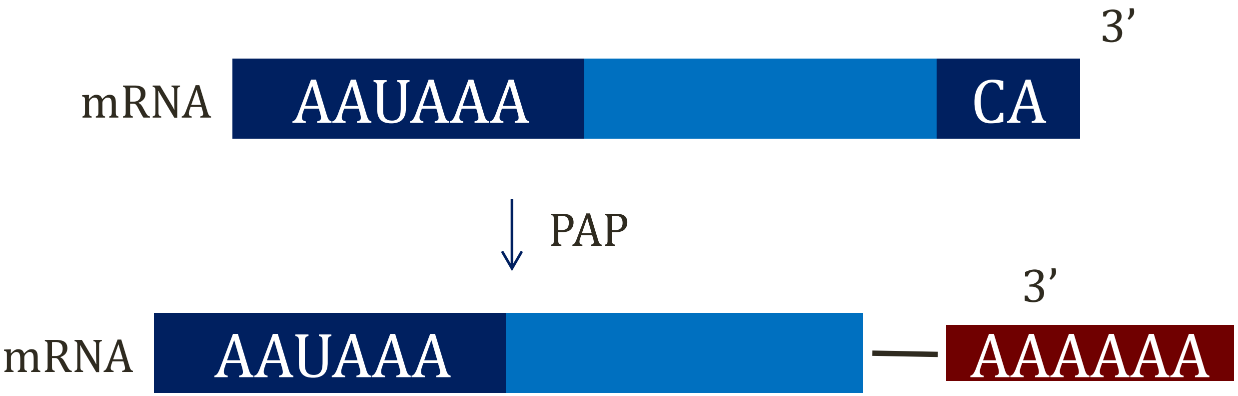


3’ Polyadenylation

- Requires several **RNA binding proteins**
- Cleavage and polyadenylation specificity factor (CSF)
  - Binds AAUAA
- Cleavage stimulation factor (CstF)
  - Binds CA sequence
- Leads to termination of DNA transcription

3’ Polyadenylation

- Enzyme: Poly-A polymerase (PAP)
- Adds ~200 adenosine nucleotides to 3’ end mRNA
- No template



3’

mRNA AAUAAA CA

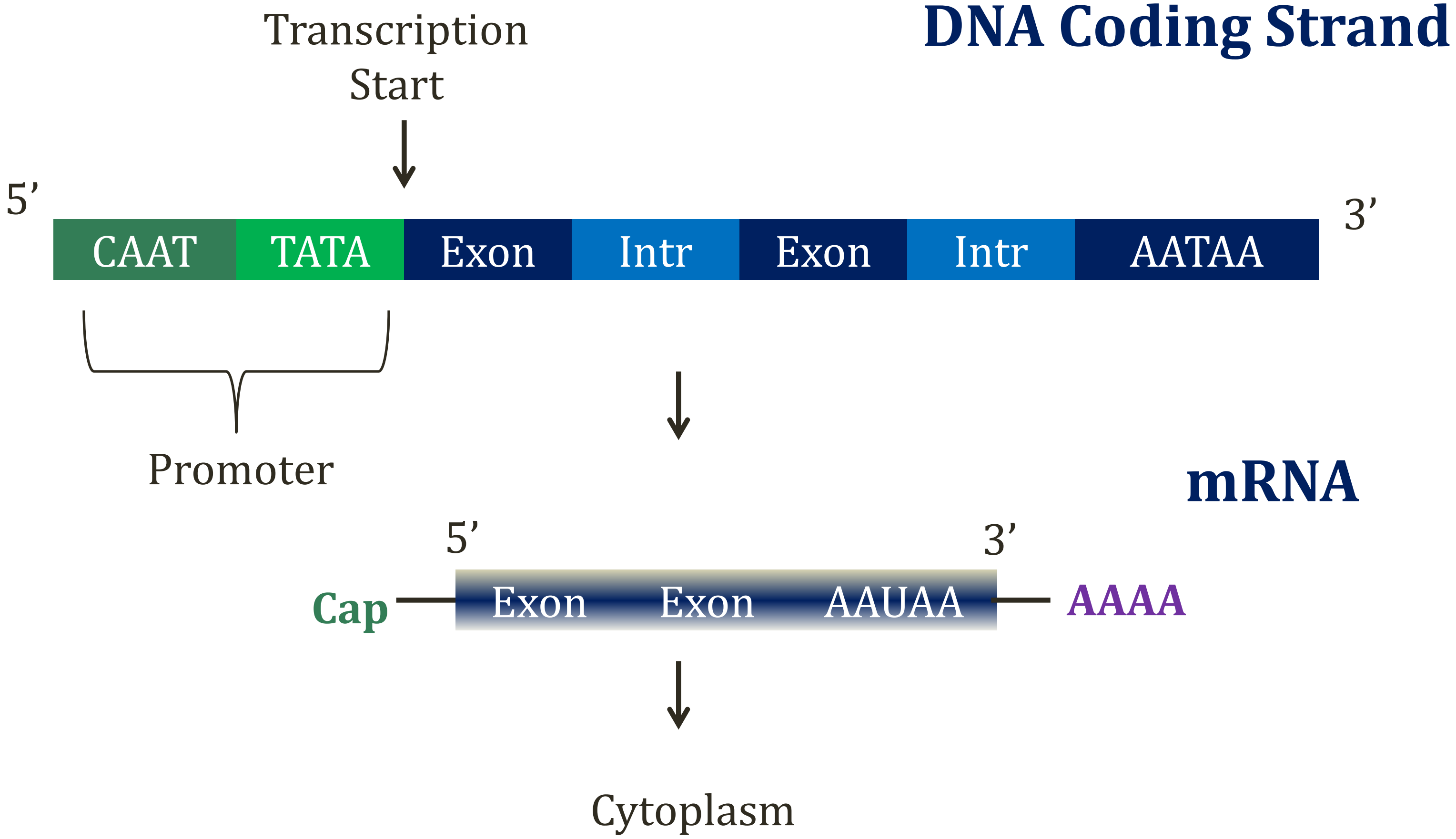
PAP

3’

mRNA AAUAAA AAAAAA

Transcription

Summary



Transcription
Start

DNA Coding Strand

5’

CAAT	TATA	Exon	Intr	Exon	Intr	AATAA

3’

Promoter

mRNA

5’ 3’

Cap — Exon Exon AAUAA — **AAAA**

Cytoplasm

MicroRNA

miRNA

- Important regulatory molecules for mRNA
- Regulate mRNA expression to proteins
- Bind mRNA via base pairing
- Extensive binding can remove poly-A tail
- Exposes mRNA to degradation by endonucleases
- **Modifies gene expression** at mRNA level

Processing Bodies

P-bodies

- Some mRNA moved to P-bodies in cytoplasm
  - Seen with less extensive miRNA binding
- mRNA sequestered from ribosomes
- Often degraded
- Some evidence that mRNA may later be translated

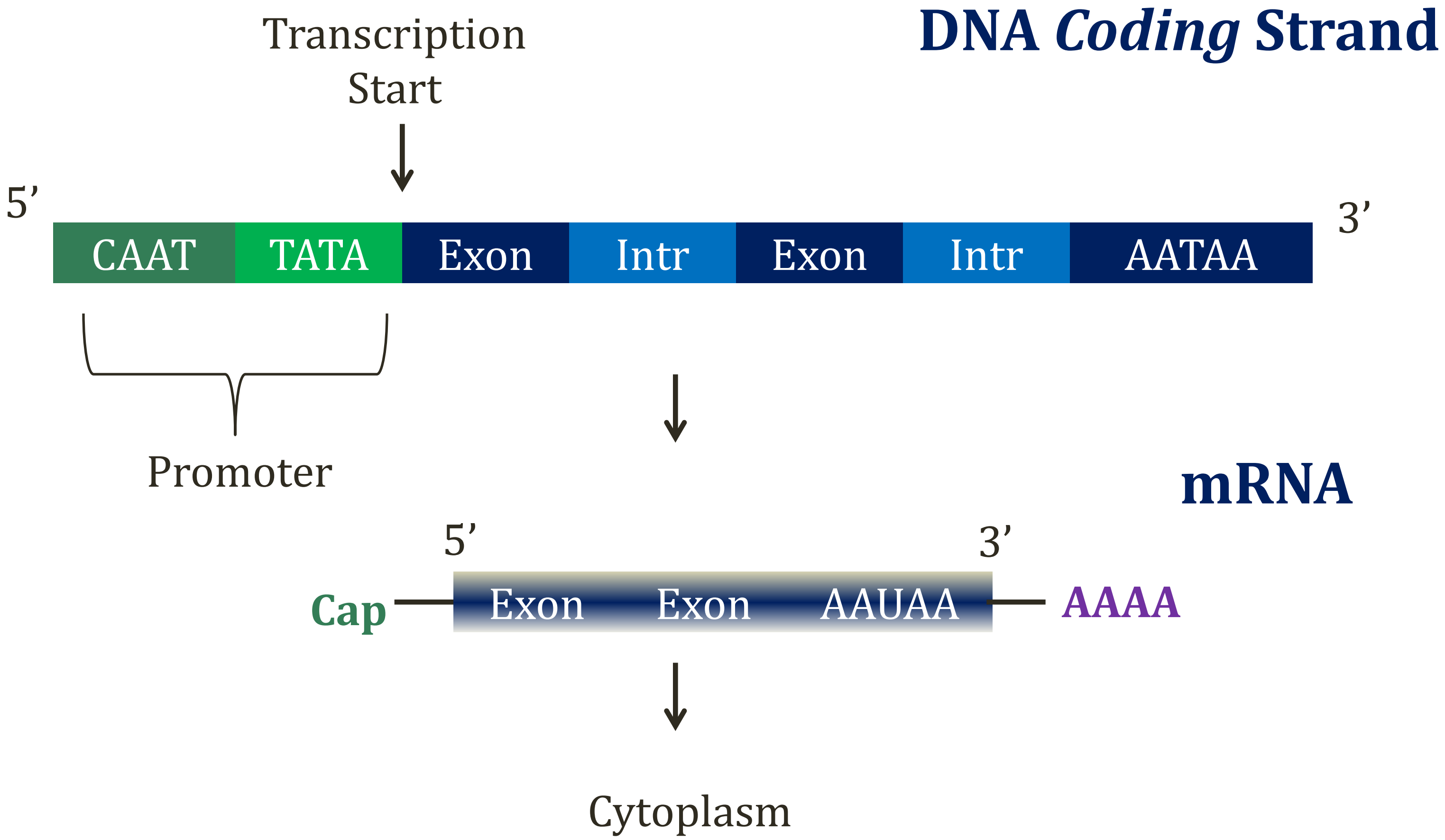
Translation

Jason Ryan, MD, MPH

Boards&Beyond
STUDY SMARTER

Transcription

Summary



DNA *Coding* Strand

Transcription Start

5’

CAAT | TATA | Exon | Intr | Exon | Intr | AATAA

3’

Promoter

mRNA

5’

3’

Cap — Exon Exon AAUAA — **AAAA**

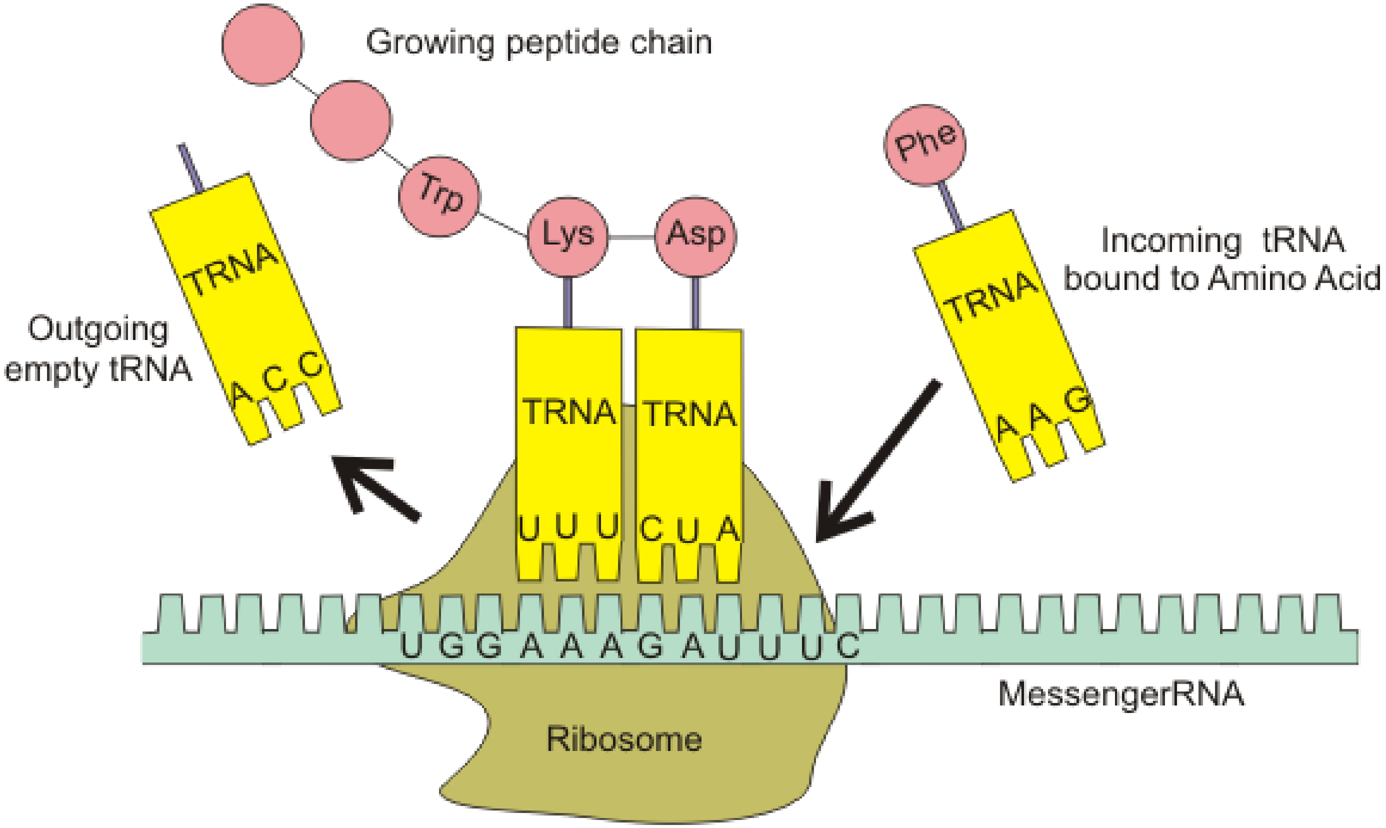
Cytoplasm

mRNA read 5’ to 3’

Translation

- Synthesis of protein using mRNA as template
- Occurs in cytoplasm on ribosomes
- tRNA brings amino acids to ribosome for assembly

Translation



Boumphreyfr /Wikipedia

Ribosomes

- Some are “free” in cytoplasm
- Also bound to the endoplasmic reticulum
  - Forms rough ER
- Contain rRNA and proteins
- Arranged as a large and small subunit
- Size measured in Svedberg units
  - Measure of rate of sedimentation by centrifugation

Ribosomes

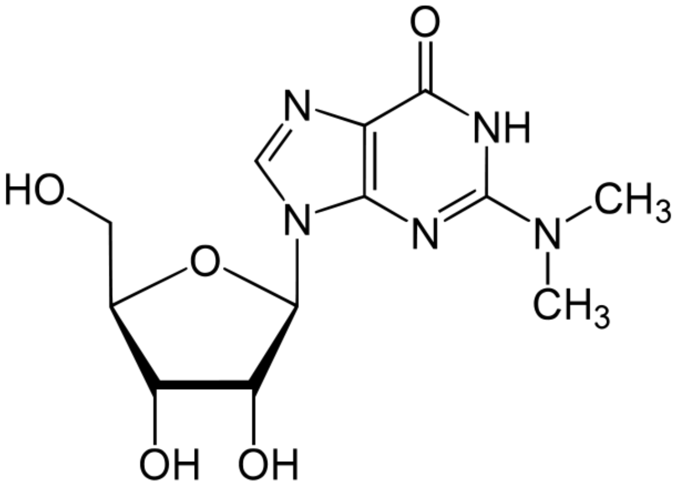
- Prokaryotes
  - 70S ribosomes
  - Small (30S) and large (50S) subunit
  - Small subunit: 16S RNA plus proteins
  - Large subunit: 5S RNA, 23S RNA, plus proteins
- **Protein synthesis inhibitor antibiotics**
  - Aminoglycosides, others
  - Target components of bacterial ribosomes

Ribosomes

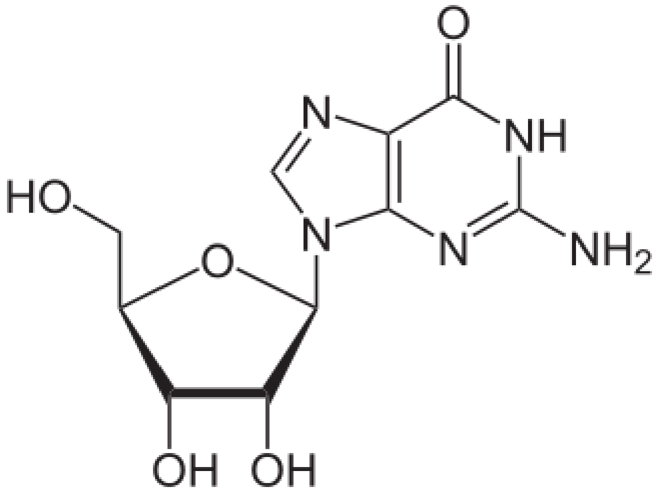
- Eukaryotes
  - 80S ribosomes
  - Small (40S) and large (60S) subunits
  - Small subunit: 18S RNA plus proteins
  - Large subunit: 5S RNA, 28S RNA, 5.8S RNA plus proteins

tRNA

- Transfers amino acids to protein chains
- Synthesized by RNA polymerase III
- Many bases are **chemically modified**

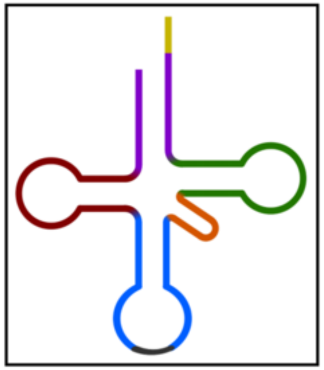


N,N dimethyl Guanosine

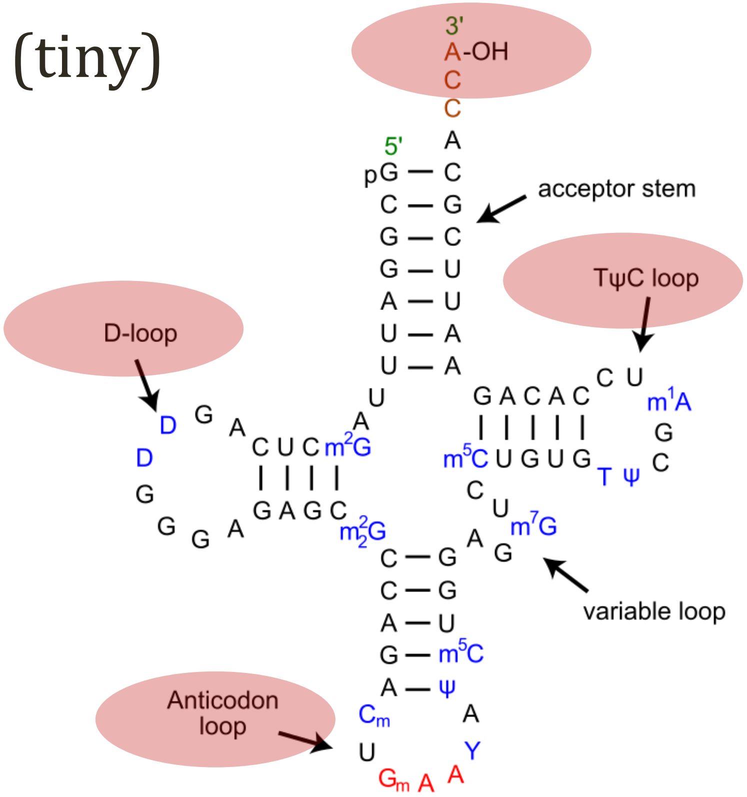


Guanosine

tRNA



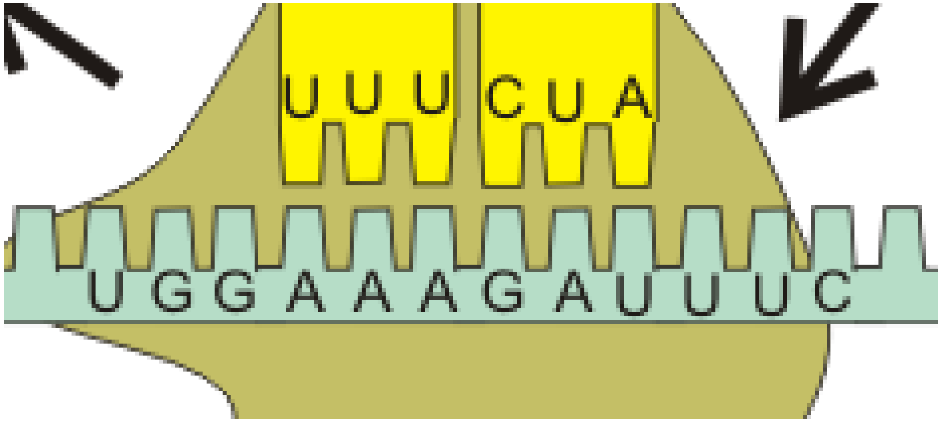
- Cloverleaf shape (secondary structure)
- Base pairing within molecule
- 70-90 nucleotides in length (tiny)
- Key portions
  - Anticodon
  - D loop (part of D arm)
  - T loop (part of T arm)
  - 3’ end



Yikrazuul

Anticodon

- 3 nucleotides on tRNA
- Pairs with complementary mRNA
- Correct pairing → correct protein synthesis



Boumphreyfr /Wikipedia

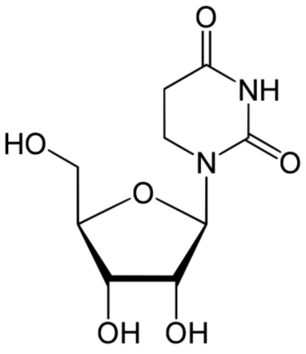
Genetic Code

Standard genetic code

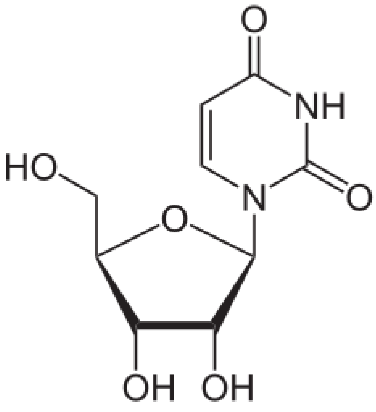
1st base	2nd base								3rd base
	U		C		A		G		
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[A]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

D loop

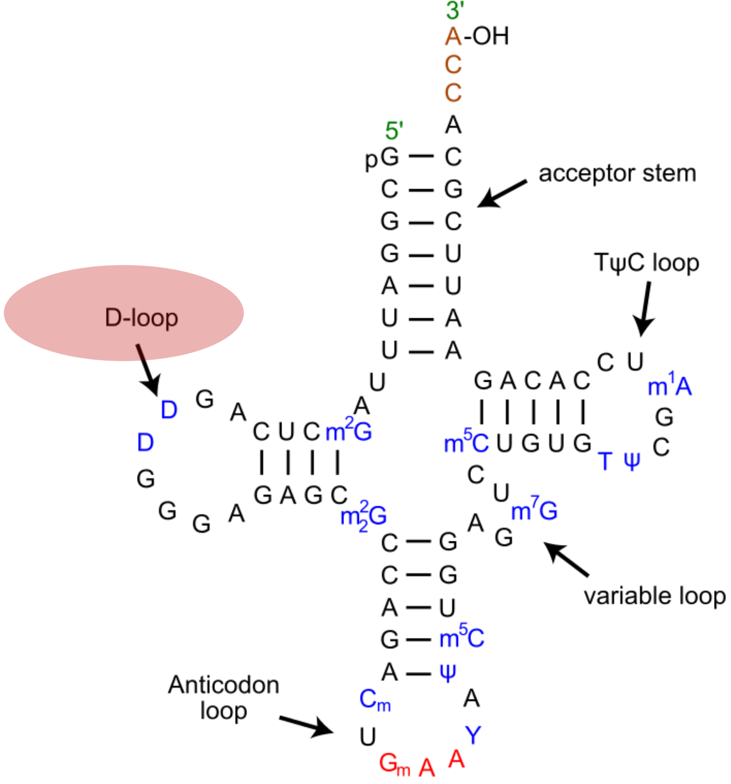
- Contains **dihydrouridine**
- tRNA recognition by aminoacyl-tRNA synthetase



Dihdydrouridine

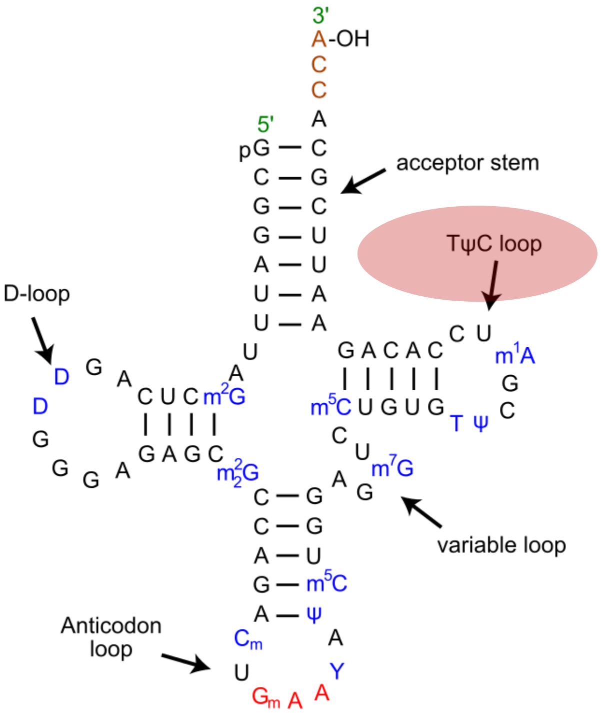


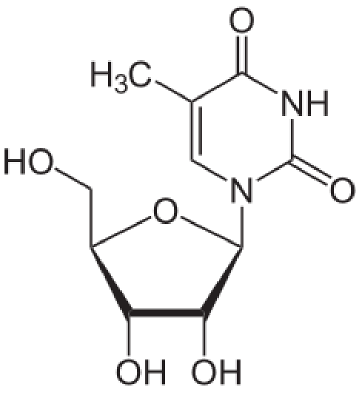
Uridine



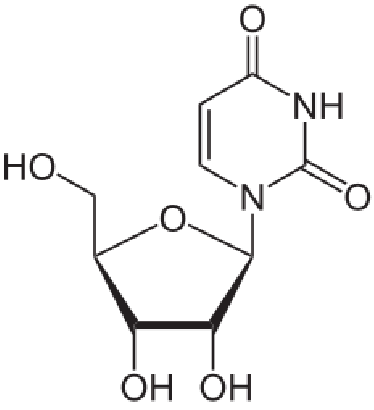
T loop

- Contains a **TΨC sequence**
  - T = Ribotymidine
  - Ψ = Pseudouridine
  - C = Cytidine
- Needed for tRNA ribosome binding

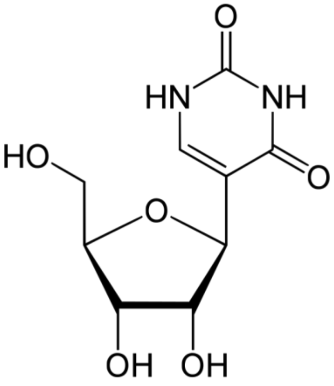




Ribothymidine



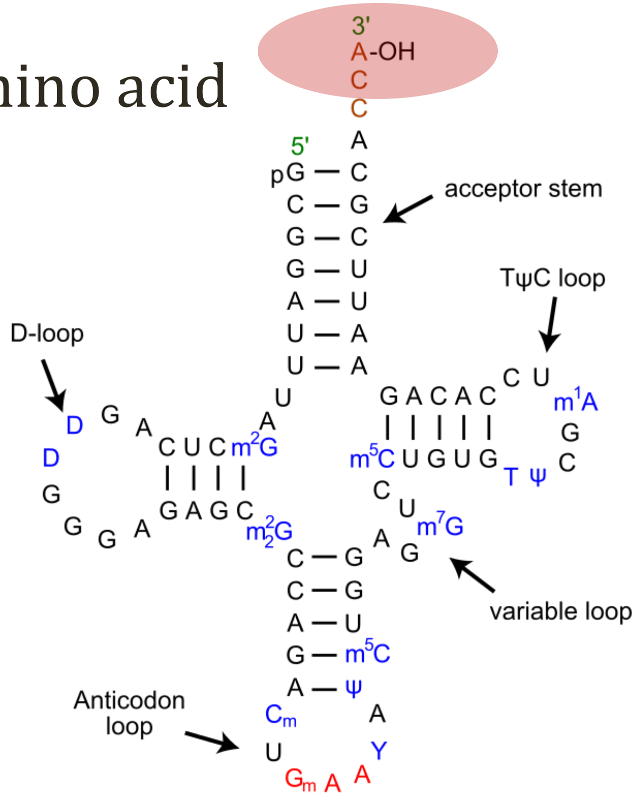
Uridine



Pseudouridine

3’ End

- Always ends in CCA
- Hydroyxl (OH) of A attaches to amino acid

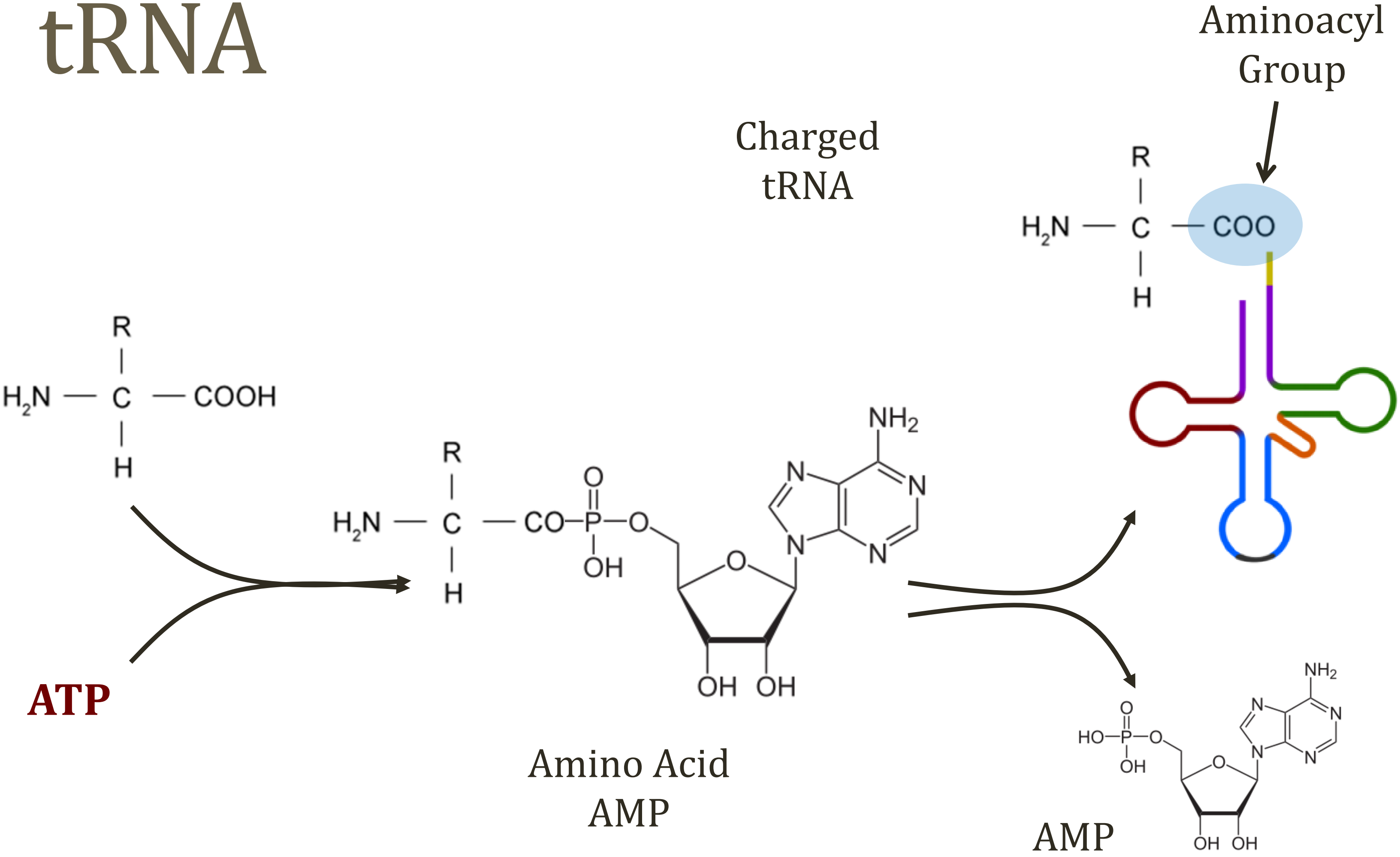


Yikrazuul

Charging

- Process of linking amino acids to tRNA
- Each tRNA linked to one amino acid
- Catalyzed by **Aminoacyl-tRNA synthetase**
- Adds amino acid to tRNA
- Requires ATP

tRNA



Aminoacyl Group

Charged tRNA

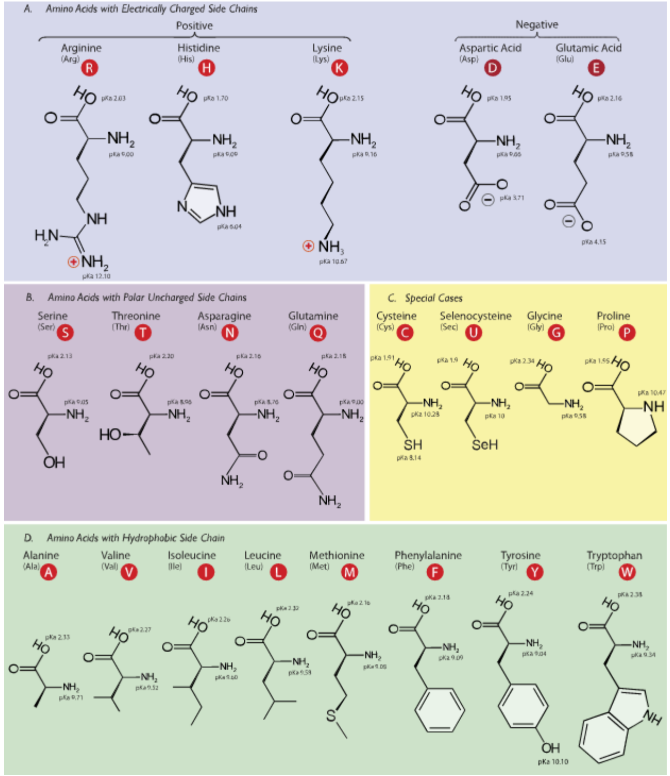
ATP

Amino Acid AMP

AMP

Aminoacy-tRNA synthetase

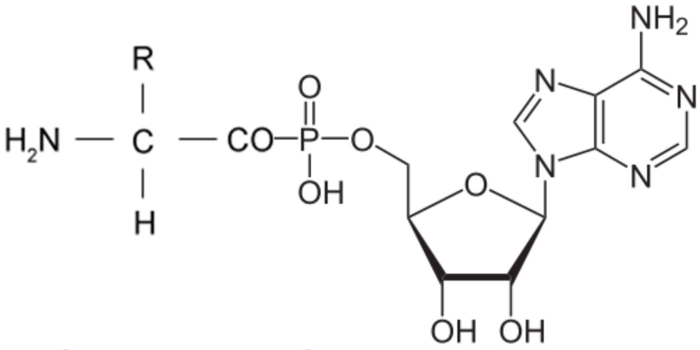
- One enzyme per amino acid in most eukaryotic cells
  - i.e. one enzyme attaches glycine to correct tRNA

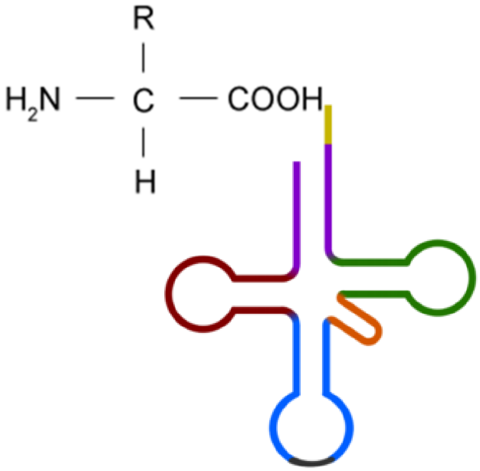


Dancojocari/Wikipedia

tRNA

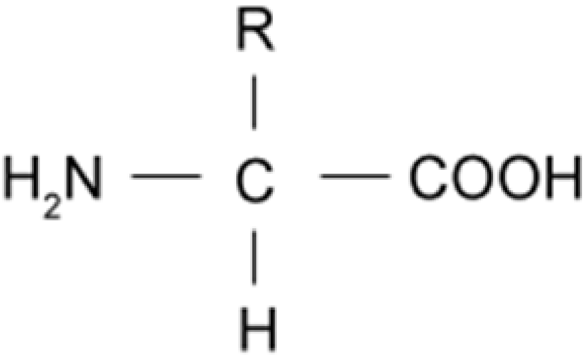
- Many amino acids have similar structures
- Mischarged tRNA → wrong AA for mRNA codon
- **Hydrolytic editing**
  - Aminoacy-tRNA synthetase scrutinizes amino acid
  - If incorrect → hydrolyzes from AMP or tRNA
- Increases accuracy of charging tRNA





Protein Synthesis

- Amino acids: N-terminal and C-terminal ends
- Proteins synthesis: addition to C-terminal end



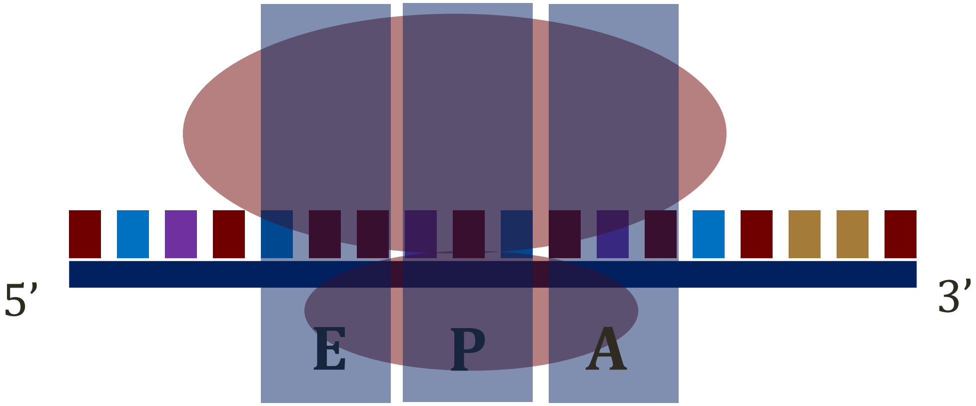
$$H_2N-\underset{\displaystyle H}{\overset{\displaystyle R}{C}}-COOH$$

Protein Synthesis

- Three stages:
  - Initiation
  - Elongation
  - Termination

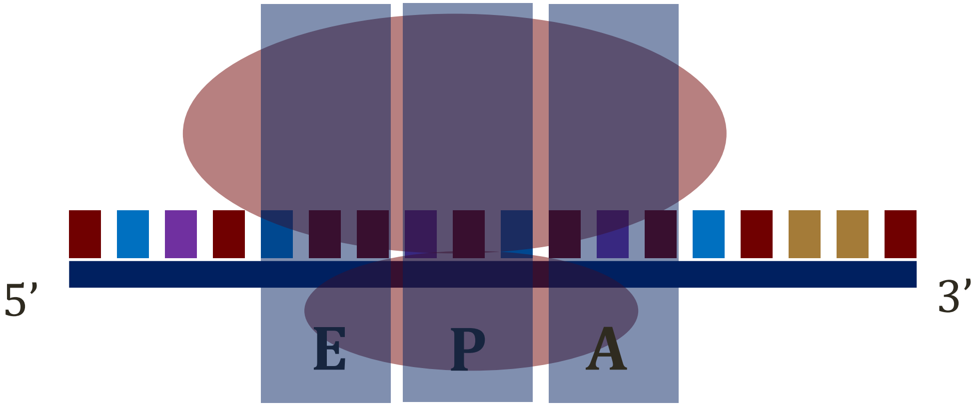
Protein Synthesis

- Ribosomes: Four binding sites
  - One for mRNA
  - Three for tRNA: A-site, P-site, E-site



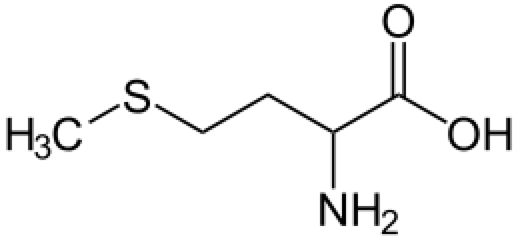
Protein Synthesis

- A-site: Amino acid binding (charged tRNA)
- P-site: tRNA attached to growing protein chain
- E-site: Exit of tRNA

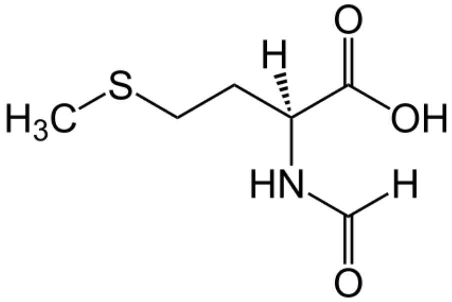


Initiation

- Begins with AUG on mRNA
- Codes for methionine or N-formylmethionine (fMet)
- Binds directly to P-site
- Usually removed later by protease enzymes
- fMET = chemotaxis of neutrophils (innate immunity)



Methionine



N-formylmethionine

Initiation

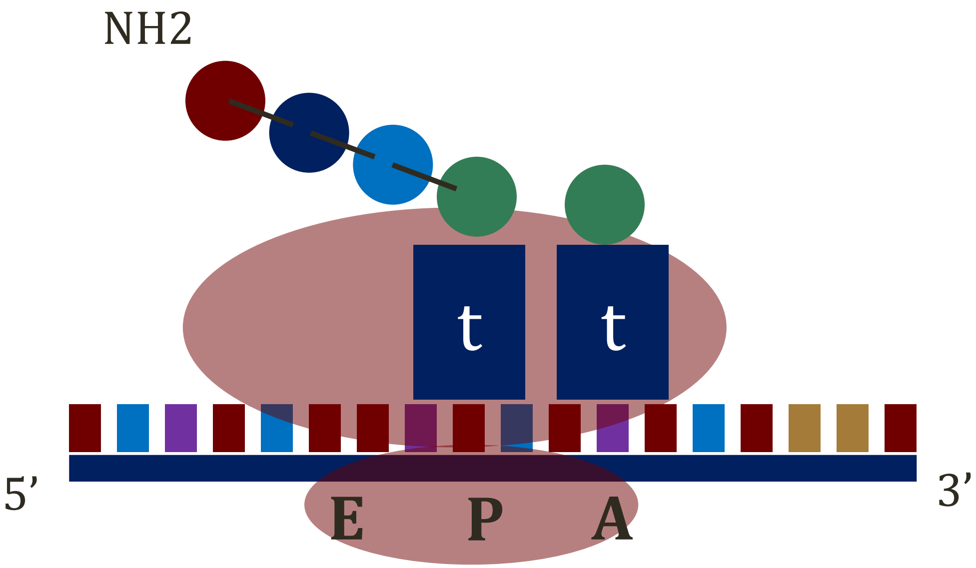
- Uses GTP hydrolysis
- In eukaryotes require **initiation factors** (proteins)
  - Assemble ribosomes and tRNA

Elongation

- Usually divided into a sequence of four steps
- Uses **elongation factors** (proteins)
    - Bacteria: EF-Tu and EF-G
    - Eukaryotes: EF1 and EF2
    - Hydrolyze GTP to GDP
- **EF2**: Target of bacterial toxins
    - Diphtheria toxin (Corynebacterium diphtheriae)
    - Exotoxin A (Pseudomonas aeruginosa)
    - Inhibits protein synthesis

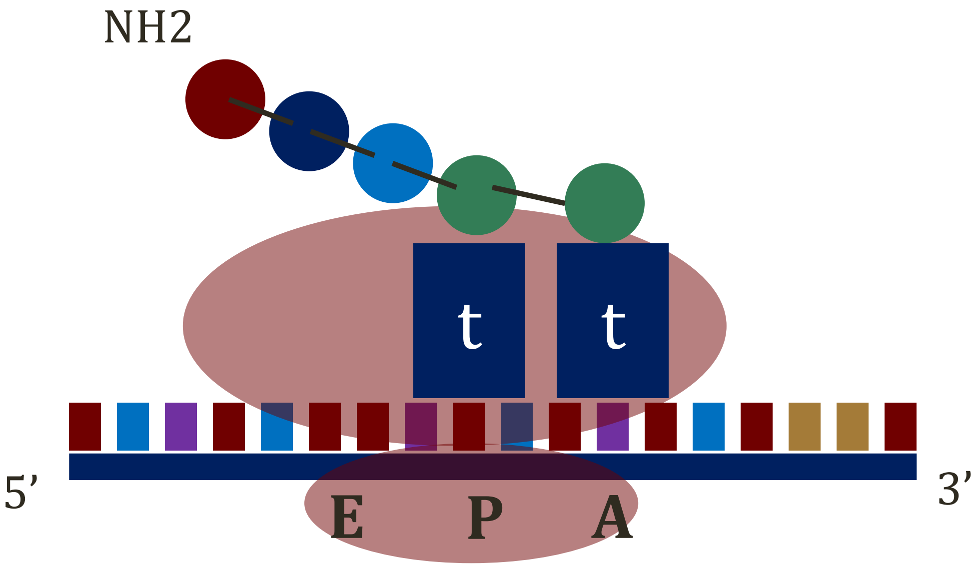
Protein Synthesis

- Step 1: Charged tRNA binds A-site
  - P-site and A-site next to one another



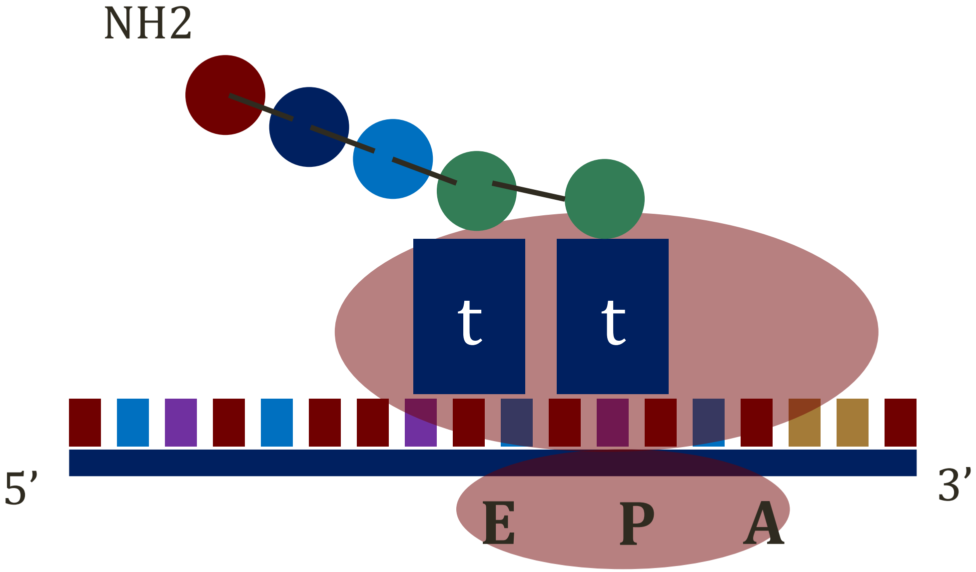
Protein Synthesis

- Step 2: Amino acid joined to peptide chain
  - Catalyzed by ribosome ("ribozyme")
  - **Peptidyl transferase**: Part of large ribosome (made of RNA)
  - Protein attached to A-site



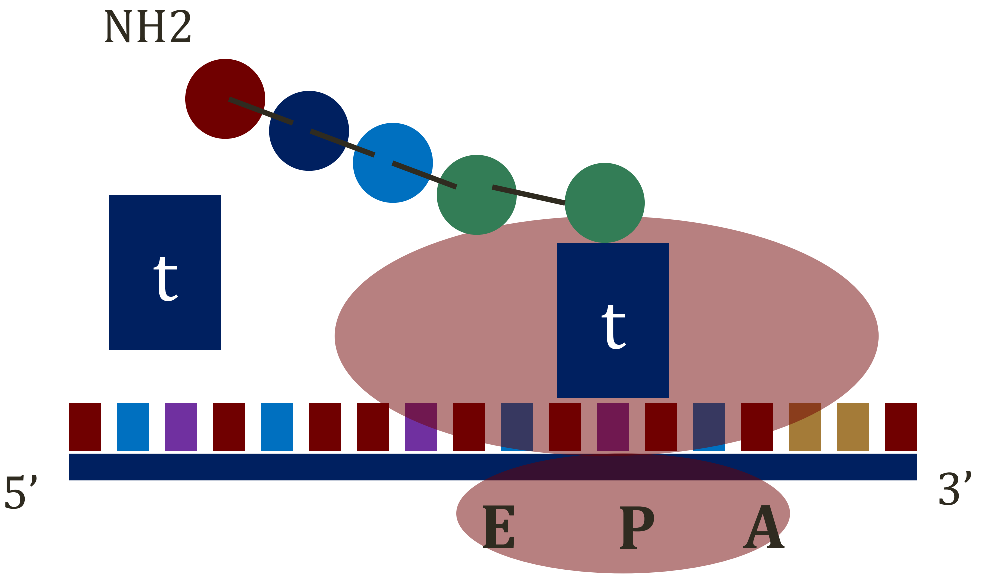
Protein Synthesis

- Step 3: Ribosome moves down mRNA toward 3’ end
  - “Translocation”
  - Protein moves to P-site



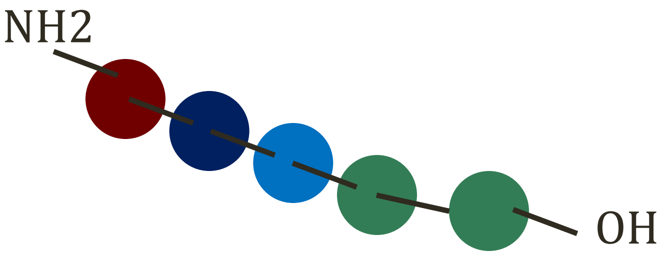
Protein Synthesis

- Step 4: tRNA leaves E-site



Termination

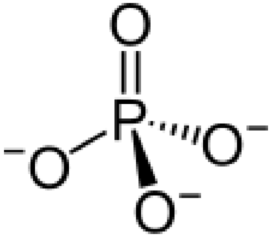
- Translation ends at mRNA stop codons
  - UAA, UAG, UGA
- Not recognized by tRNA
- Do not specific an amino acid
- **Releasing factors** bind to ribosome at stop codons
- Catalyze water added to protein chain



Posttranslational Modifications

- Creates functional protein
- Folding
- Addition of other molecules

Posttranslational Modifications



- Phosphorylation
  - Amino acid residue phosphorylated
  - Protein kinase enzymes add phosphate group
- Glycosylation
  - Formation of the sugar–amino acid linkage
  - Many linkages: N-, O-, C-linked glycosylation
  - Creates glycoproteins

Posttranslational Modifications

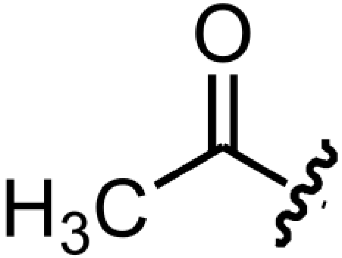
- Hydroxylation
  - Addition of hydroxyl (OH) groups
  - Important for **collagen synthesis**
  - Hydroxylation of proline and lysine residues

Proline

Hydroxyproline

Posttranslational Modifications

- Methylation
  - Addition of methyl (CH3) groups
- Acetylation
  - Addition of acetyl (CH3CO) group
- Ubiquitination
  - Addition of ubiquitin (small protein)
  - Tags proteins for destruction in proteasome



Acetyl Group

Chaperones

- Proteins that facilitate folding
- Bind to other proteins → ensure proper folding
- Classic example: **Heat shock proteins**
  - Family of proteins
  - Also called stress proteins
  - Constitutively expressed
  - Increased expression with heat, pH shift, hypoxia
  - Stabilize proteins; maintain protein structure
  - Help cells survive environmental stress

Polymerase Chain Reaction

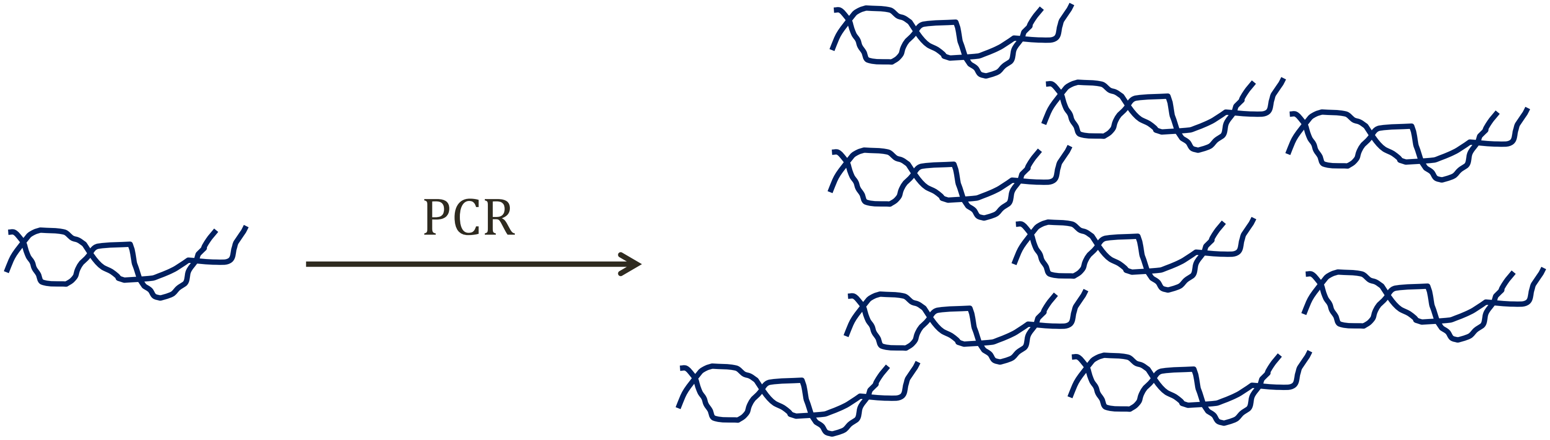
Jason Ryan, MD, MPH

Boards&Beyond
STUDY SMARTER

PCR

Polymerase Chain Reaction

- Laboratory technique
- Amplifies (copies) DNA molecules in a sample
- Uses:
  - Make more DNA from small amount
  - Determine if DNA is present (i.e. does it amplify?)
  - Determine amount of DNA (i.e. how quickly does it amplify?)



PCR

Ingredients

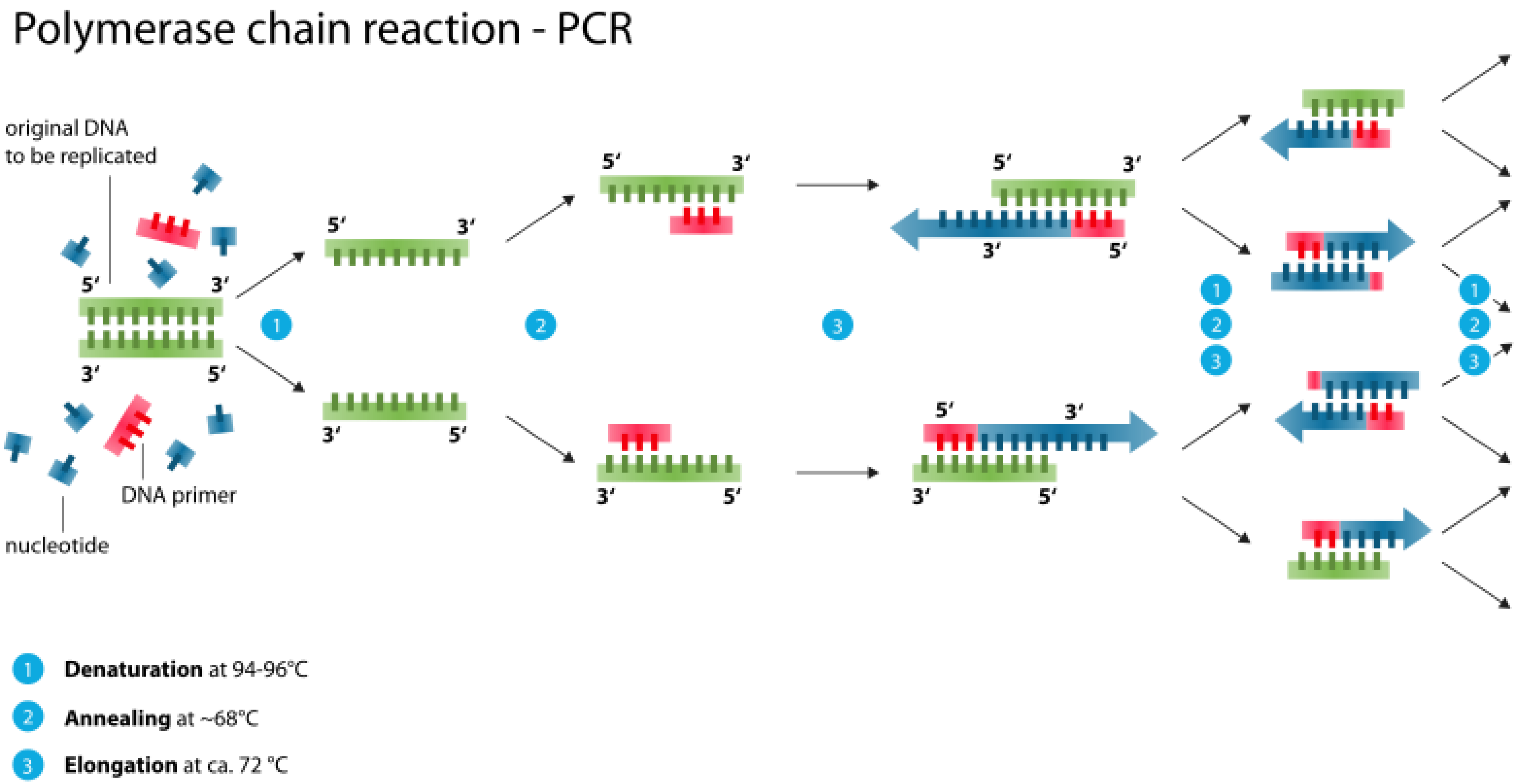
- Sample (DNA)
- DNA polymerase
- Primer
  - Single-stranded DNA segment
  - Complementary to DNA under evaluation
- Nucleotides

PCR

Technique

- Heat sample
  - DNA **denatures** into single strands
- Cool sample
  - Primer **anneals** (binds) complementary DNA (if present)
- Warm sample
  - DNA polymerase **elongates** from primer
- Process repeated in cycles
- Each cycle generates more DNA

PCR



Enzoklop/Wikipedia

Real Time PCR

Quantitative PCR

- PCR done in presence of **fluorescent dye**
- Amount of dye proportional to amount of DNA
- More DNA = more fluorescence
- Fluorescence detected as PCR ongoing
- Rapid increase florescence = more DNA in sample

PCR

Uses

- **Herpes simplex virus encephalitis**
  - DNA in CSF
- **HIV Viral Load**
  - Uses reverse transcriptase to make cDNA
  - Amplification of cDNA
  - Amount of cDNA produced over time indicates viral load
  - Standard tool for monitoring viral load

Blotting

Jason Ryan, MD, MPH

Blotting

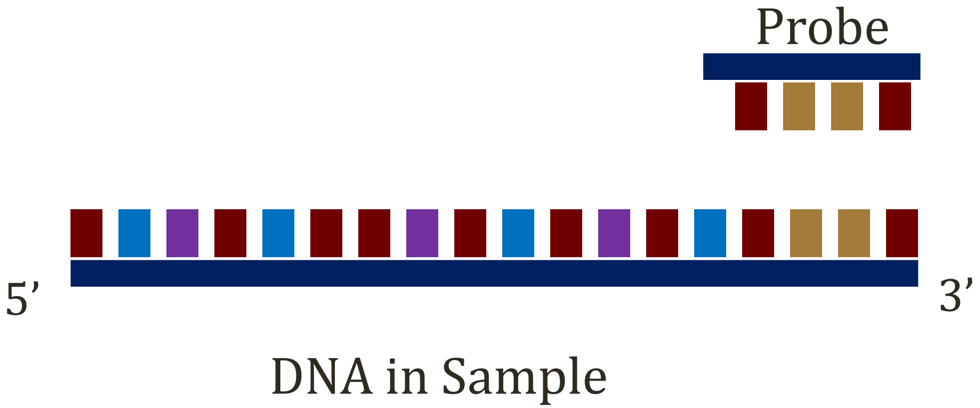
- Laboratory techniques
- Southern blot: Identifies DNA
- Northern blot: Identifies RNA
- Western blot: Identifies proteins

Southern Blot

- Named for inventor (Edward Southern)
- Uses a probe to identify presence of **DNA** in a sample

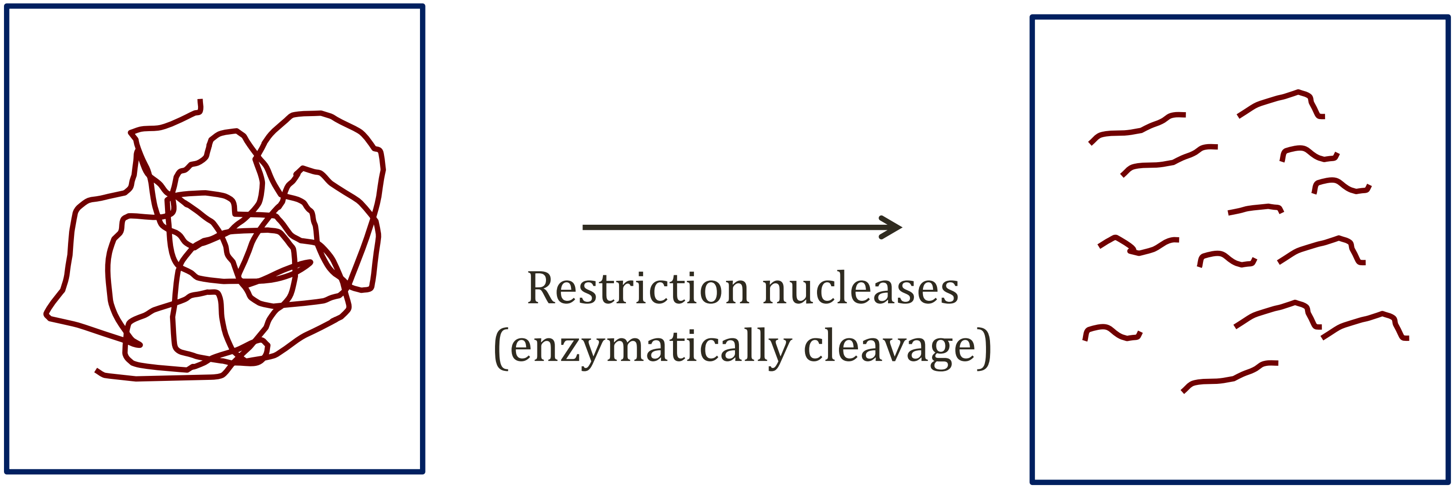
Probe

- Single-stranded DNA molecule
- Carries radioactive or chemical markers
- Binds complementary sequences
  - Probe called "cDNA"
  - "Hybridization"
- Once bound, markers reveal DNA in sample



Southern Blot

Step 1

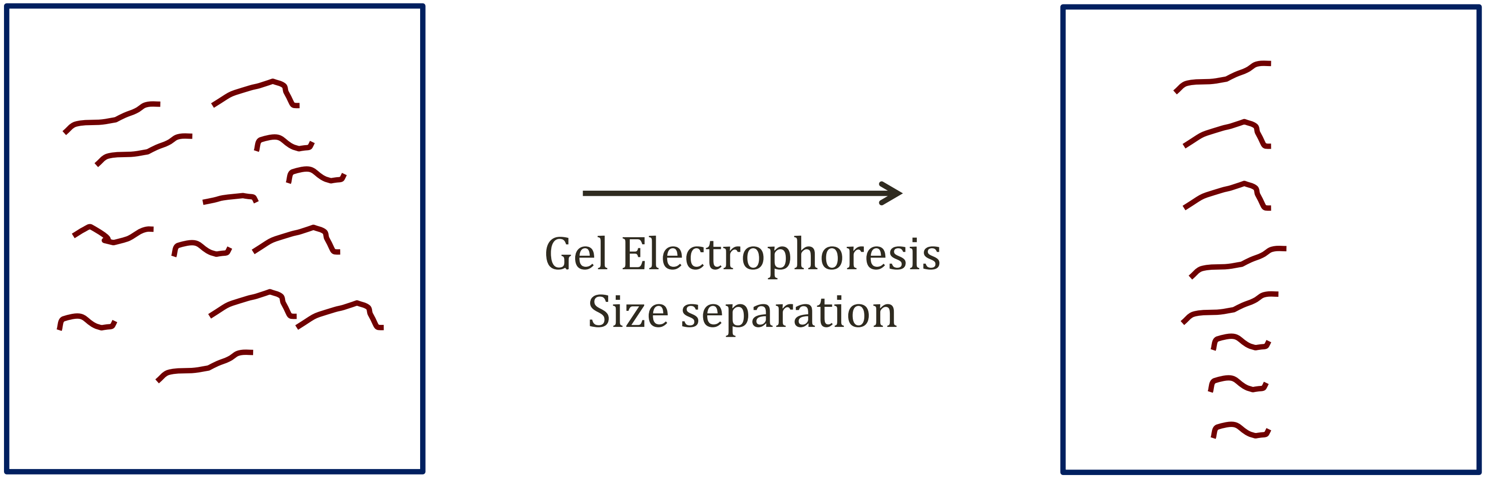


Restriction nucleases
(enzymatically cleavage)

DNA
Sample

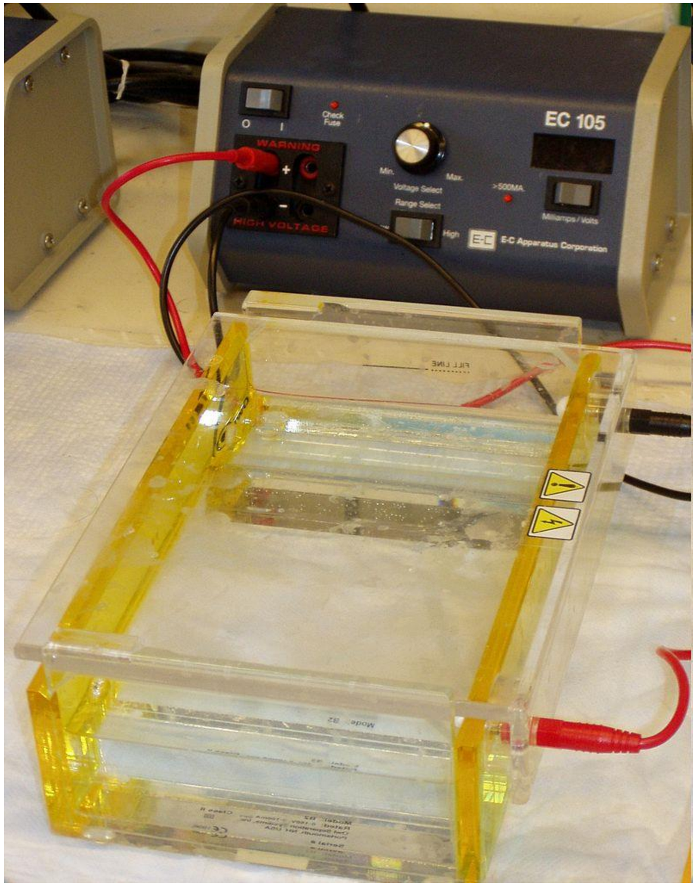
Southern Blot

Step 2



Gel Electrophoresis
Size separation

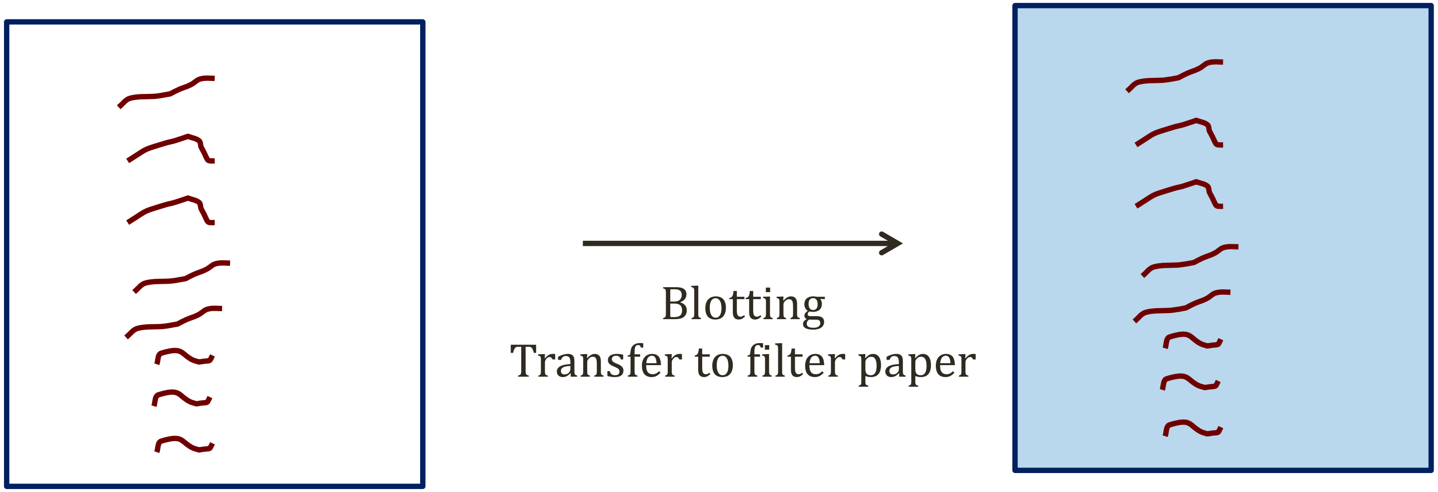
Gel Electrophoresis



Jeffrey M. Vinocur

Southern Blot

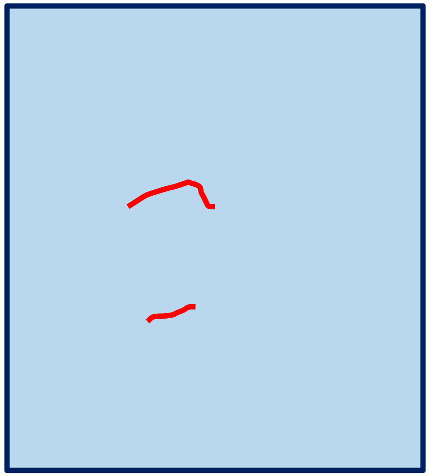
Step 3


Blotting
Transfer to filter paper

Southern Blot

Step 4

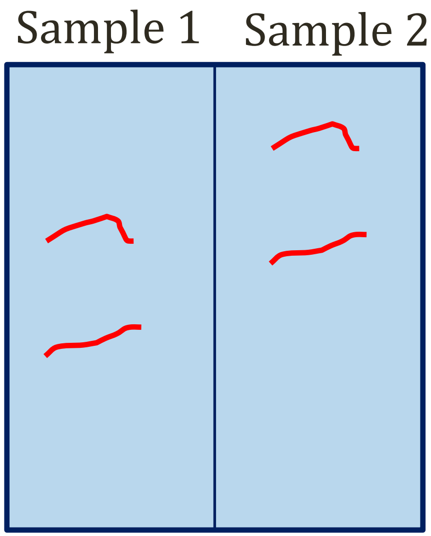
- Add probe
- Wash away unbound probe
- Only bound probe remains
- Filter paper exposed to film → bound DNA revealed



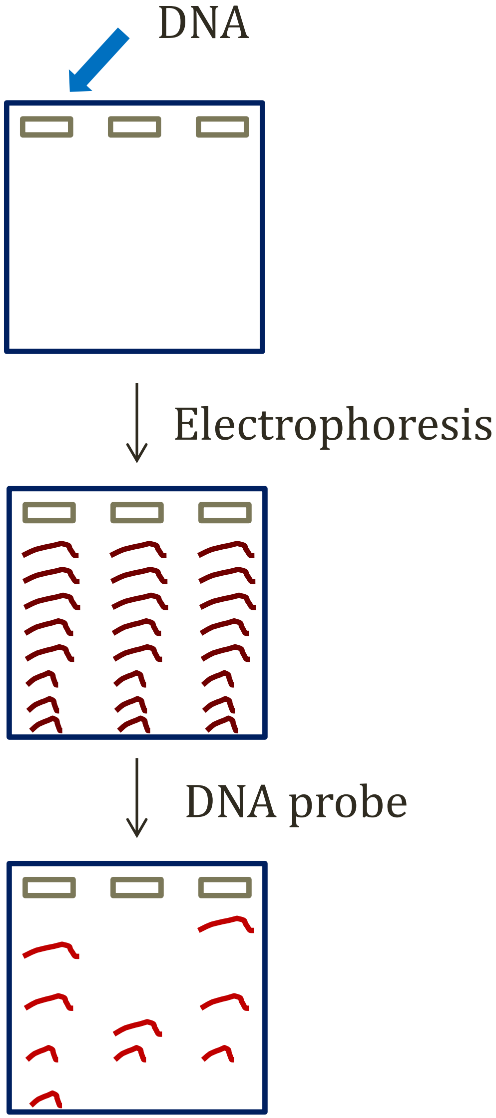
Southern Blot

Step 4

- Often done with multiple samples



Southern Blot



Southern Blot

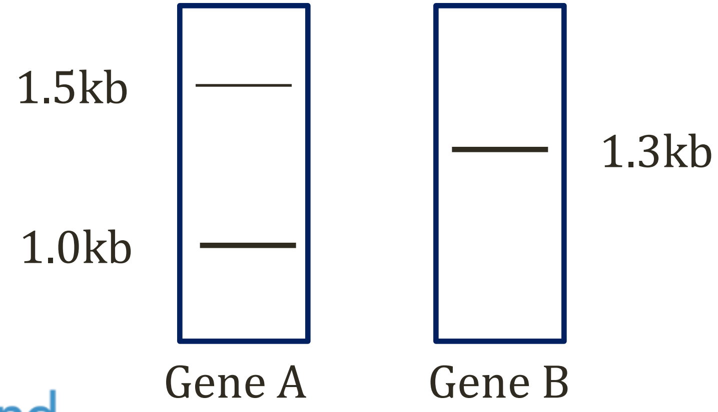
Clinical Uses

- Restriction fragment length polymorphisms
- Sickle cell anemia

RFLP

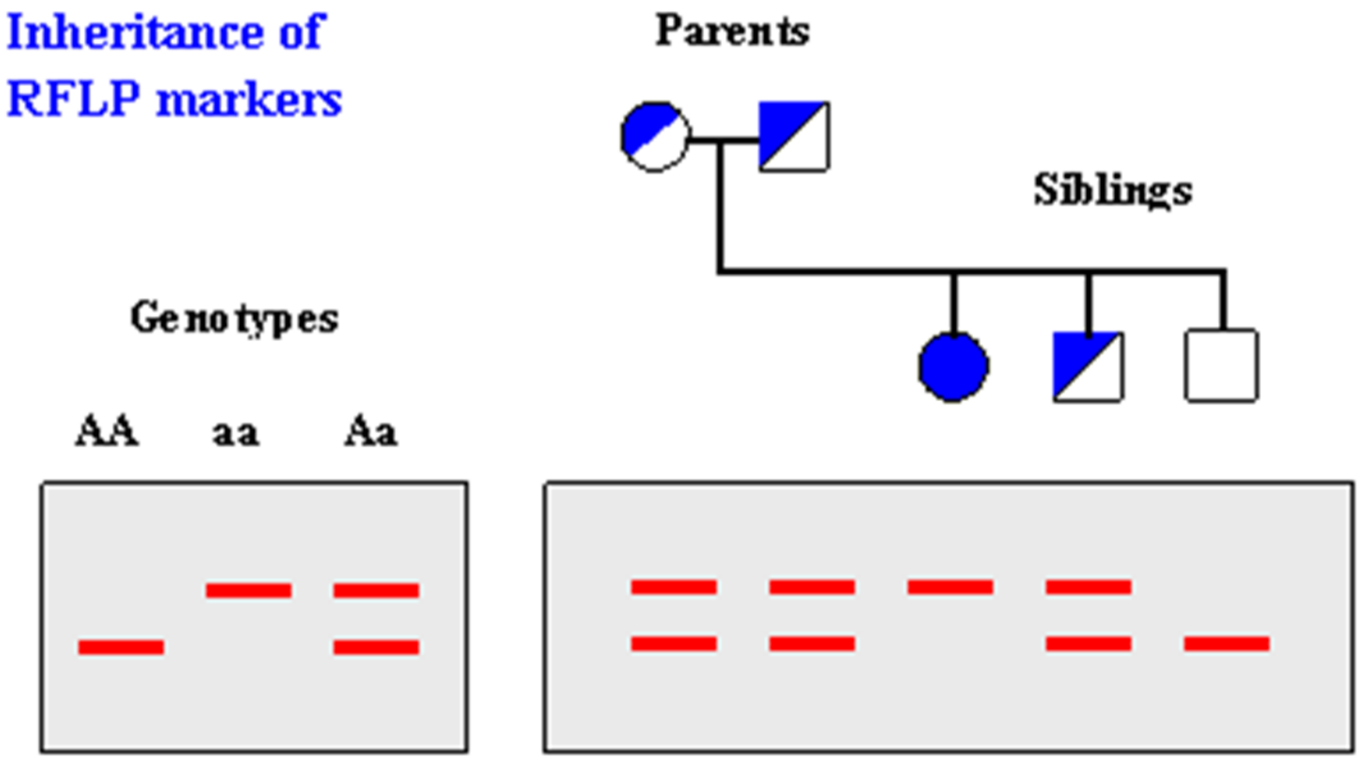
Restriction fragment length polymorphisms

- **Restriction nucleases**
  - DNA cutting enzymes
  - Cut DNA at specific base sequences (i.e. GTGCAC)
- Restriction fragment length polymorphisms
  - Analysis of **fragments** of DNA from restriction nucleases
  - Different genes = different length of fragments
  - Southern blotting to detect lengths after fragmentation



RFLP

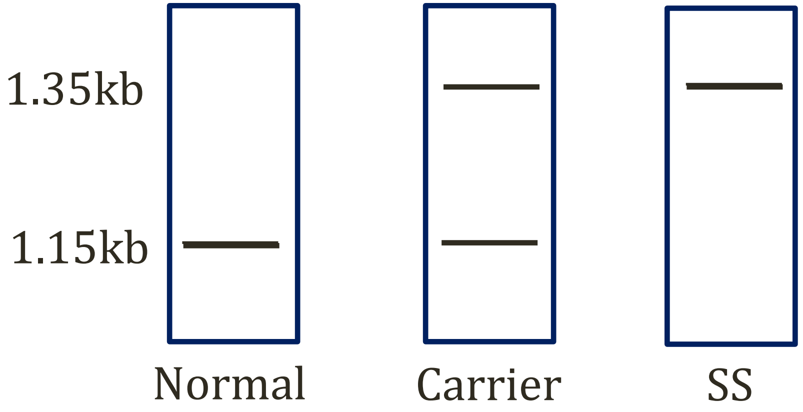
Restriction fragment length polymorphisms



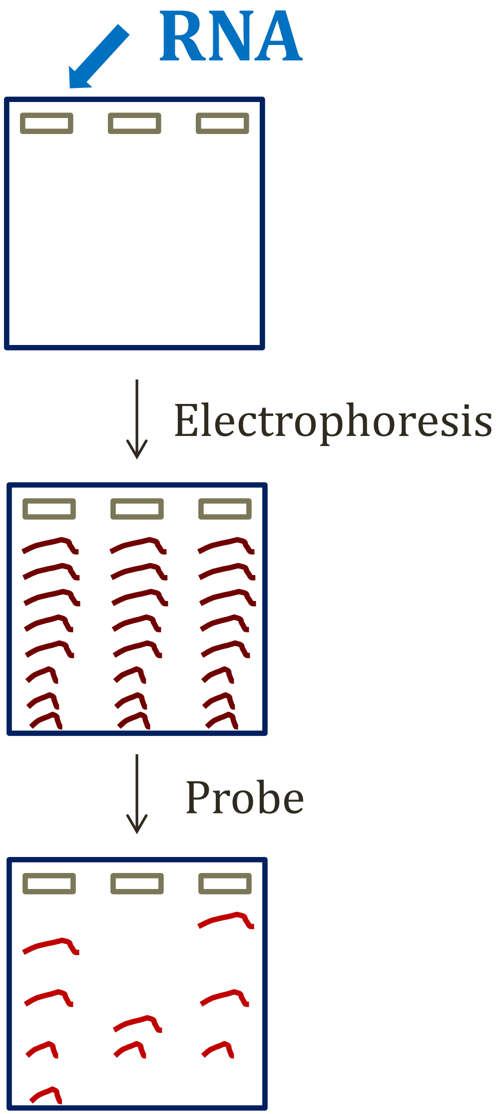
Wikipedia/Public Domain

Sickle Cell Anemia

- Normal β-globin gene: Two fragments
  - 1.15kb and 0.2kb
- Sickle cell: One fragment
  - 1.35kb
  - This fragment seen only with HbS gene

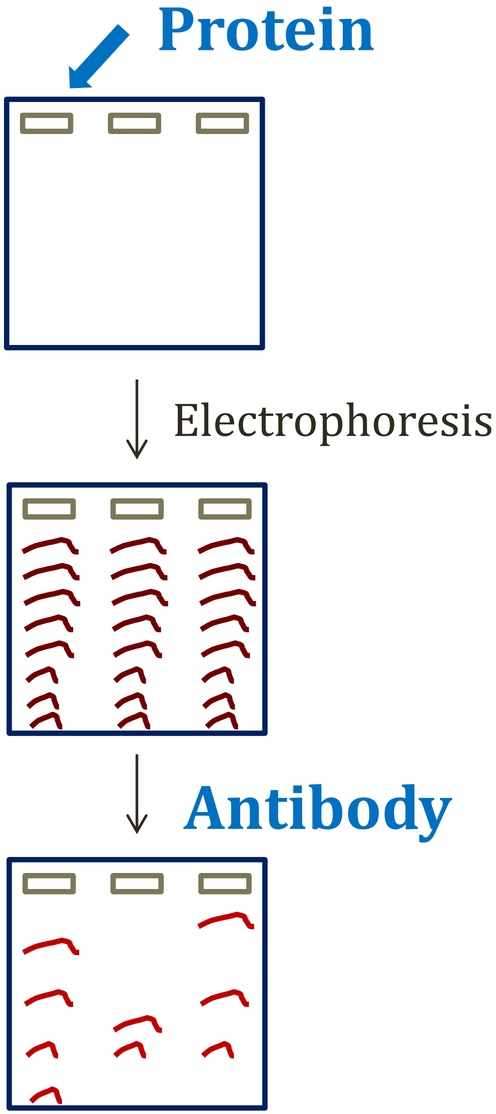


Northern Blot



Useful for assessing
mRNA levels
(gene expression)

Western Blot



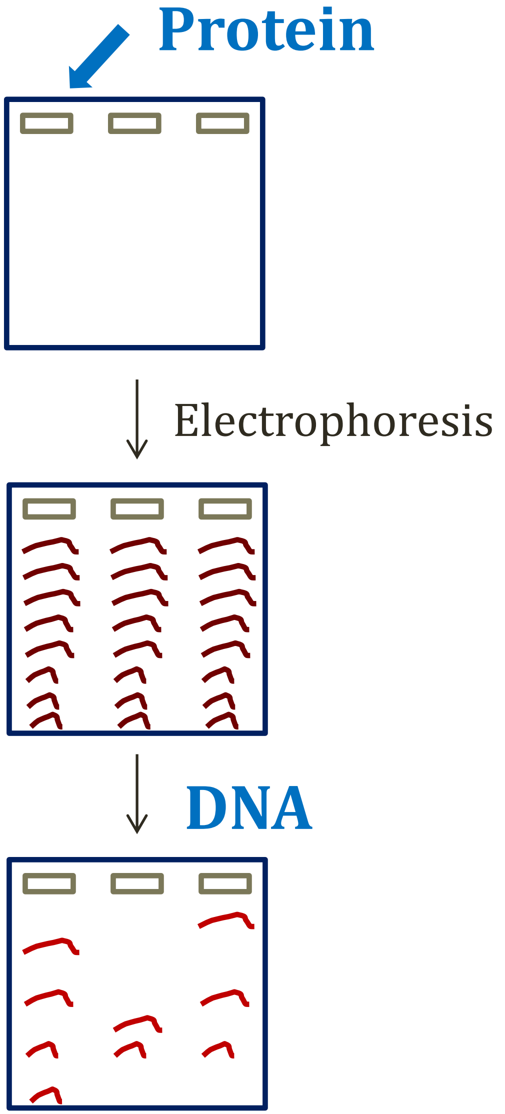
Western Blot

- Detection of **antibodies**
  - IgG or IgM in Lyme disease
  - IgG HIV-1

Southwestern Blot

- Used to study **DNA-protein interaction**
- Combines features of Southern and Western blots
- Proteins separated by electrophoresis (Western)
- DNA probe added (Southern)
- Used for studying DNA-binding proteins
- Especially transcription factors

Southwestern Blot



Protein

Electrophoresis

DNA

Flow Cytometry

Jason Ryan, MD, MPH

Flow Cytometry

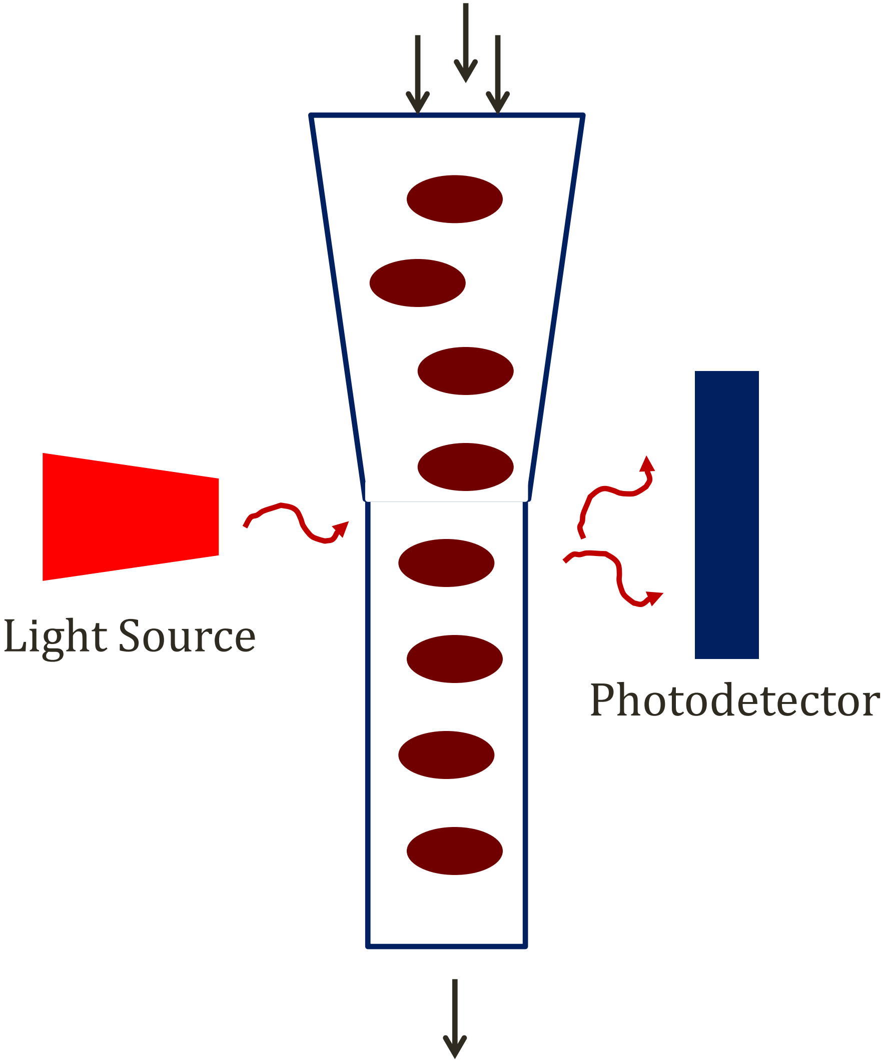
- Flow = motion of fluid
- Cytometry = measurement of cells
- Flow cytometry = Analysis of cells as they flow in a liquid through a narrow steam
- Key point: **Used to analyze cells**
  - By size
  - By surface proteins

Flow Cytometer

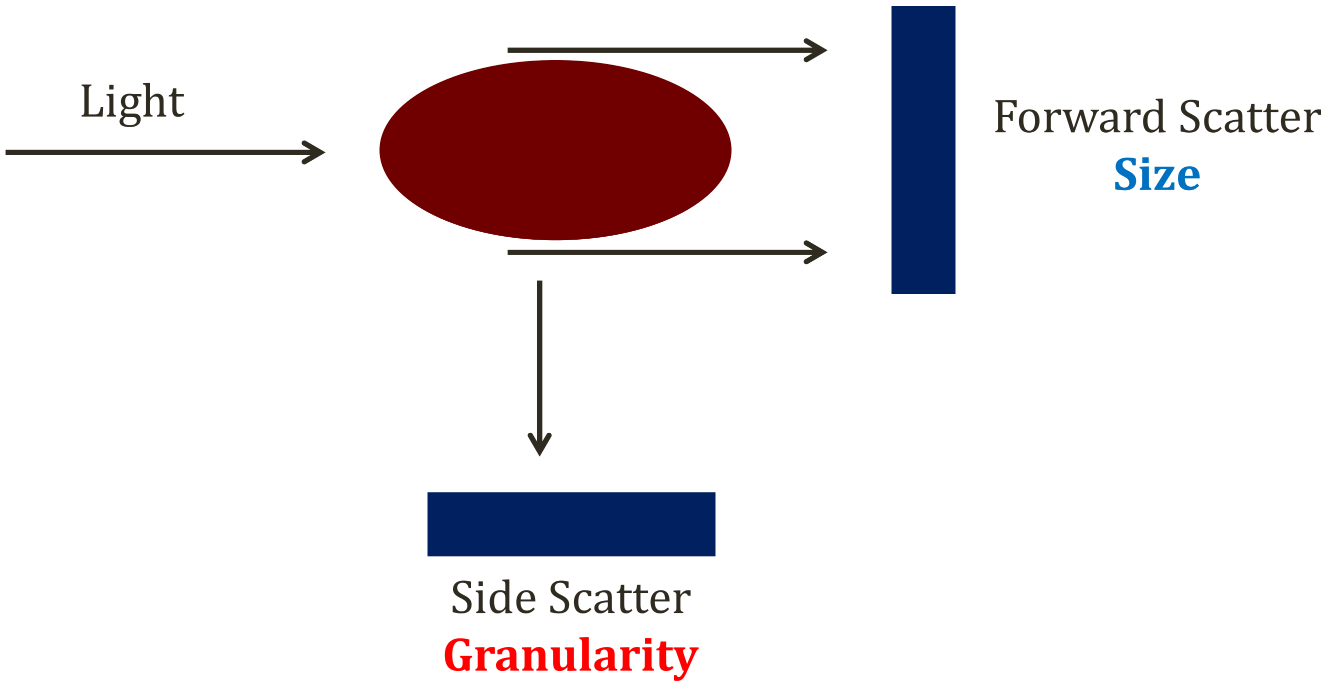
- Key components:
  - Flow cell: moves cells through machine
  - Laser: light scattered by cells
  - Photodetector: detects light scatter

Biol/Wikipedia

Flow Cytometer



Flow Cytometer

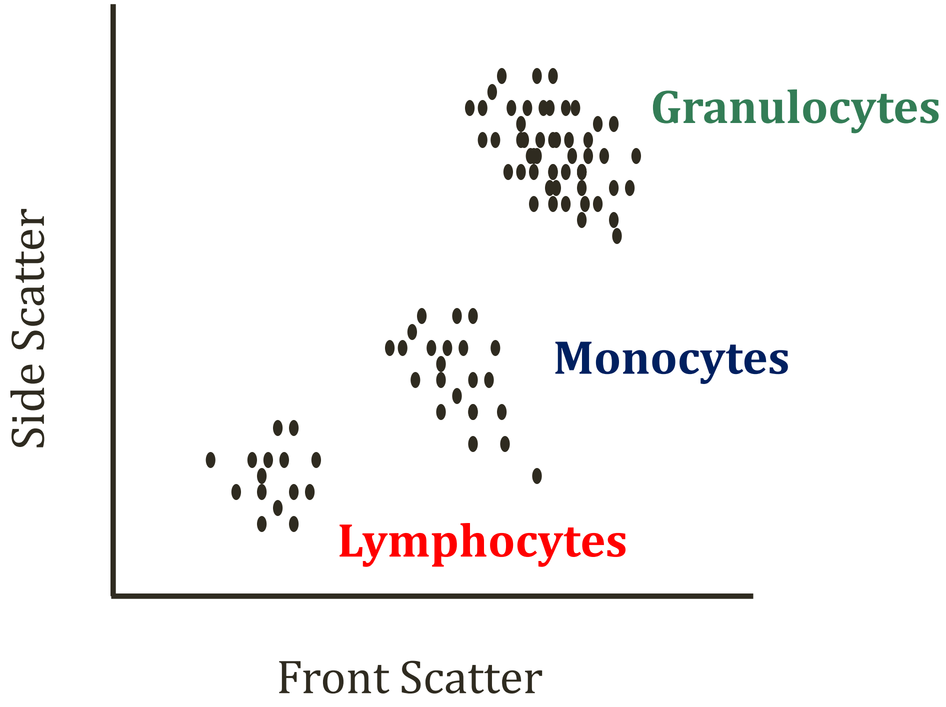


Light

Forward Scatter
Size

Side Scatter
Granularity

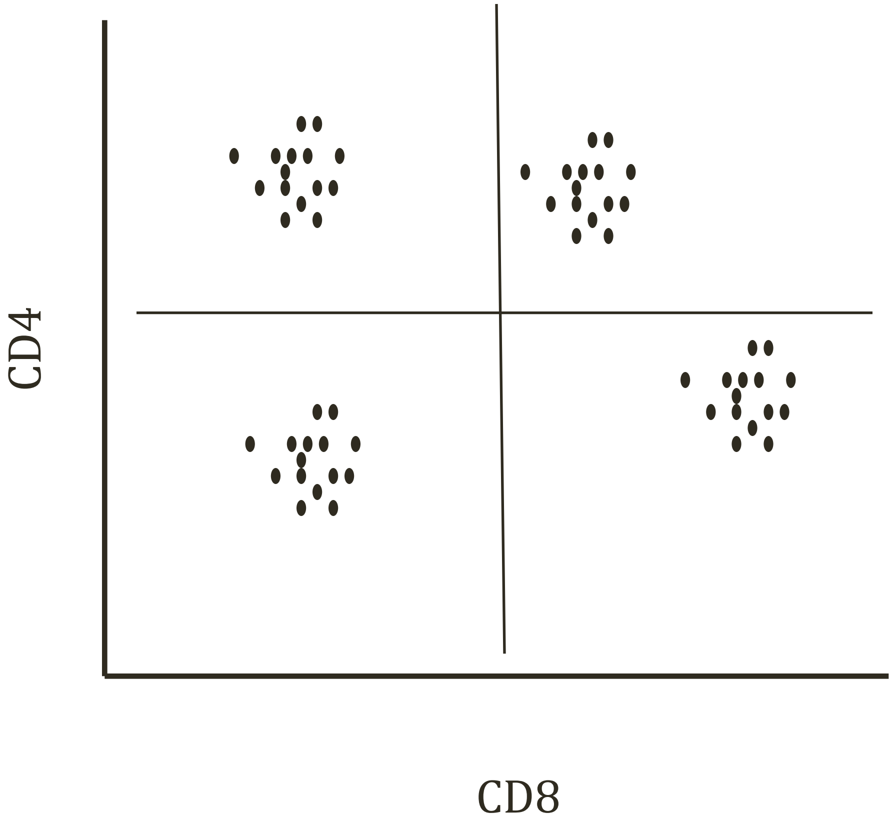
Flow Cytometry



Antibody Staining

- Specific antibodies to surface/intracellular proteins
- Tagged with unique fluorochrome
- Flow cytometer detects fluorochrome
- Indicates presence of protein in cells

Antibody Staining



Flow Cytometry

Clinical Uses

- **Fetal maternal hemorrhage**
  - Fetal red cells cross placenta to maternal blood
  - Seen with placental failure/trauma
  - Presents as decreased fetal movement, abnormal fetal HR
  - Can cause stillbirth
  - Flow cytometry: monoclonal antibody to hemoglobin F
  - Detects fetal hemoglobin in red cells

Øyvind Holmstad/Wikipedia

Flow Cytometry

Clinical Uses

- **Paroxysmal nocturnal hemoglobinuria**
  - Fluorescently-labeled monoclonal antibodies
  - Bind glycosylphosphatidylinositol (GPI) anchored proteins
  - Decay Accelerating Factor (DAF/CD55)
  - MAC inhibitory protein (CD59)
  - Reduced or absent on red blood cells in PNH

Databese Center for Life Science (DBCLS)

ELISA

Jason Ryan, MD, MPH

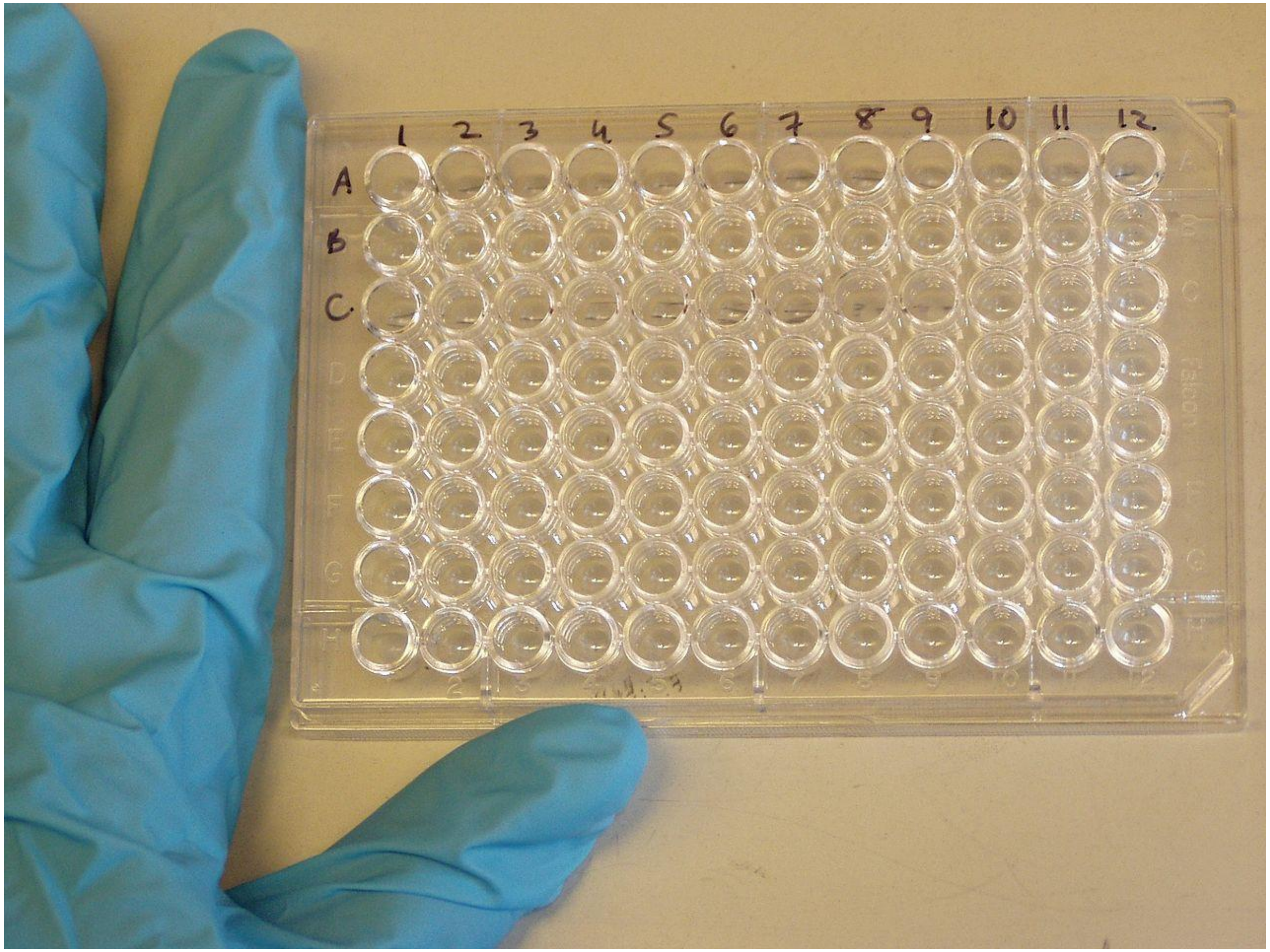
ELISA

Enzyme-linked immunosorbent assay

- Detects **antigens** and **antibodies** in serum
- Based on enzymatic **color change** reaction
- Several forms
  - Direct
  - Indirect
  - Sandwich
  - Competitive

ELISA

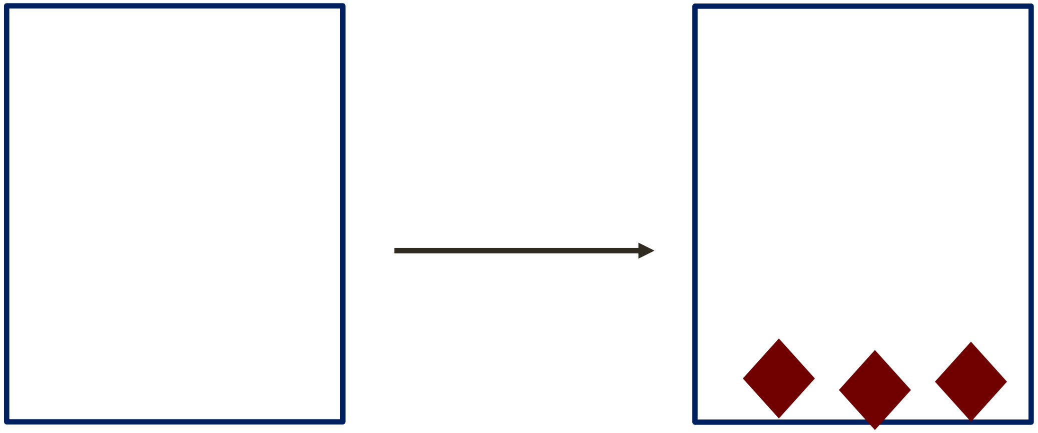
Enzyme-linked immunosorbent assay



Jeffrey M. Vinocur

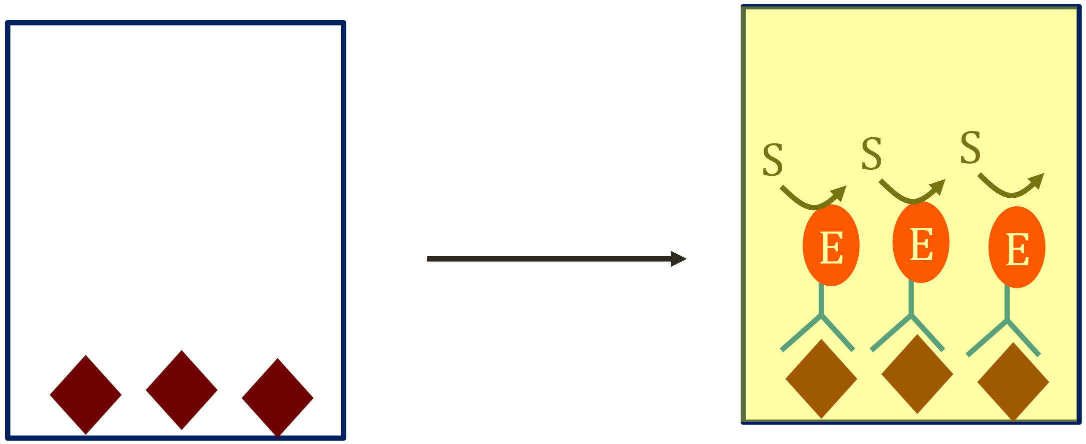
Direct ELISA

- Add serum to be tested
- Serum coats plate → antigen secured to surface
- Wash away fluid



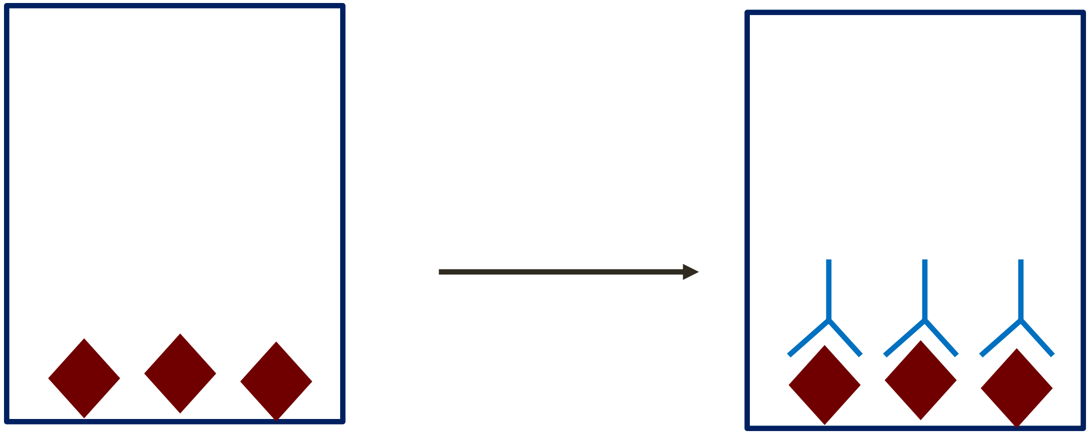
Direct ELISA

- Add enzyme-labeled **antibody specific to antigen**
- Wash away unbound antibodies
- Add substrate → color change
- Enzyme-linked antibodies **directly** bind antigen



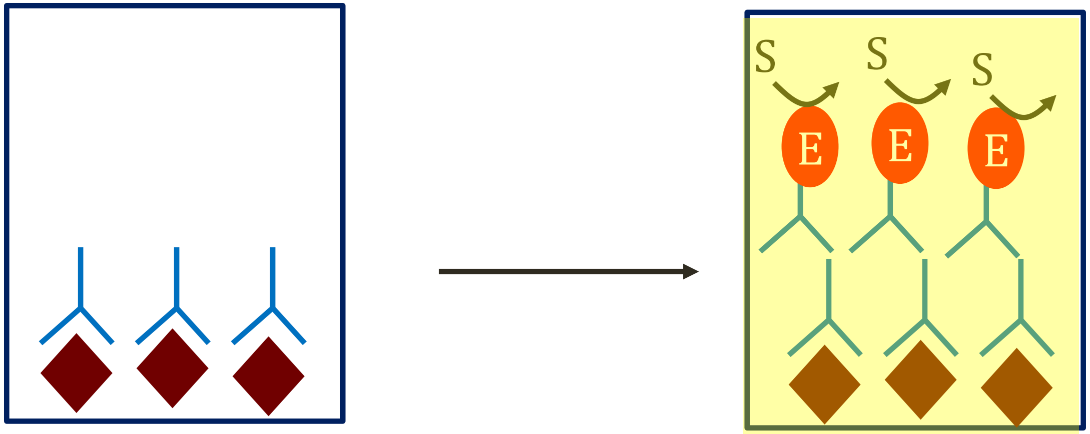
Indirect ELISA

- Add serum for analysis (like direct)
- Add antibody to antigen of interest
- **Antibody not enzyme linked**
- Wash away unbound antibody



Indirect ELISA

- Add enzyme-labeled **secondary** antibody
- Substrate → color change → identification of antigen
- Result: Identifies presence of **antigen** in serum
- Enzyme-linked antibodies **indirectly** bind antigen



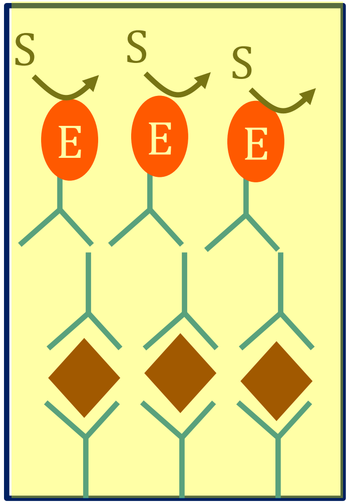
ELISA

Direct vs. Indirect

- Direct
  - Fewer steps
  - Specific antibody must be enzyme-linked
  - Time-consuming to label antibodies to unique antigens
- Indirect
  - More steps
  - Specific antibody NOT enzyme-linked
  - Specific antibody easier to acquire (i.e. mouse antibody)
  - Secondary antibody easier to acquire (i.e. anti-mouse IgG)

Sandwich ELISA

- Plate coated with capture antibody
- Sample added → any antigen present binds
- Detecting antibody added → binds to antigen
  - Direct: detecting antibody enzyme linked
  - Indirect: secondary enzyme-linked antibody added
- Substrate added → color change

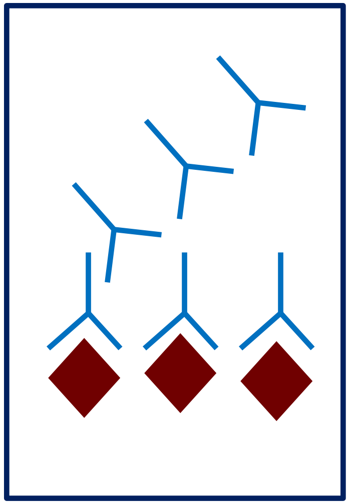


Sandwich ELISA

- High specificity
  - Two antibodies used
  - Unlikely to bind wrong antigen
- Works with complex samples
  - Antigen does not require purification
- Can use secondary antibody like indirect

Competitive ELISA

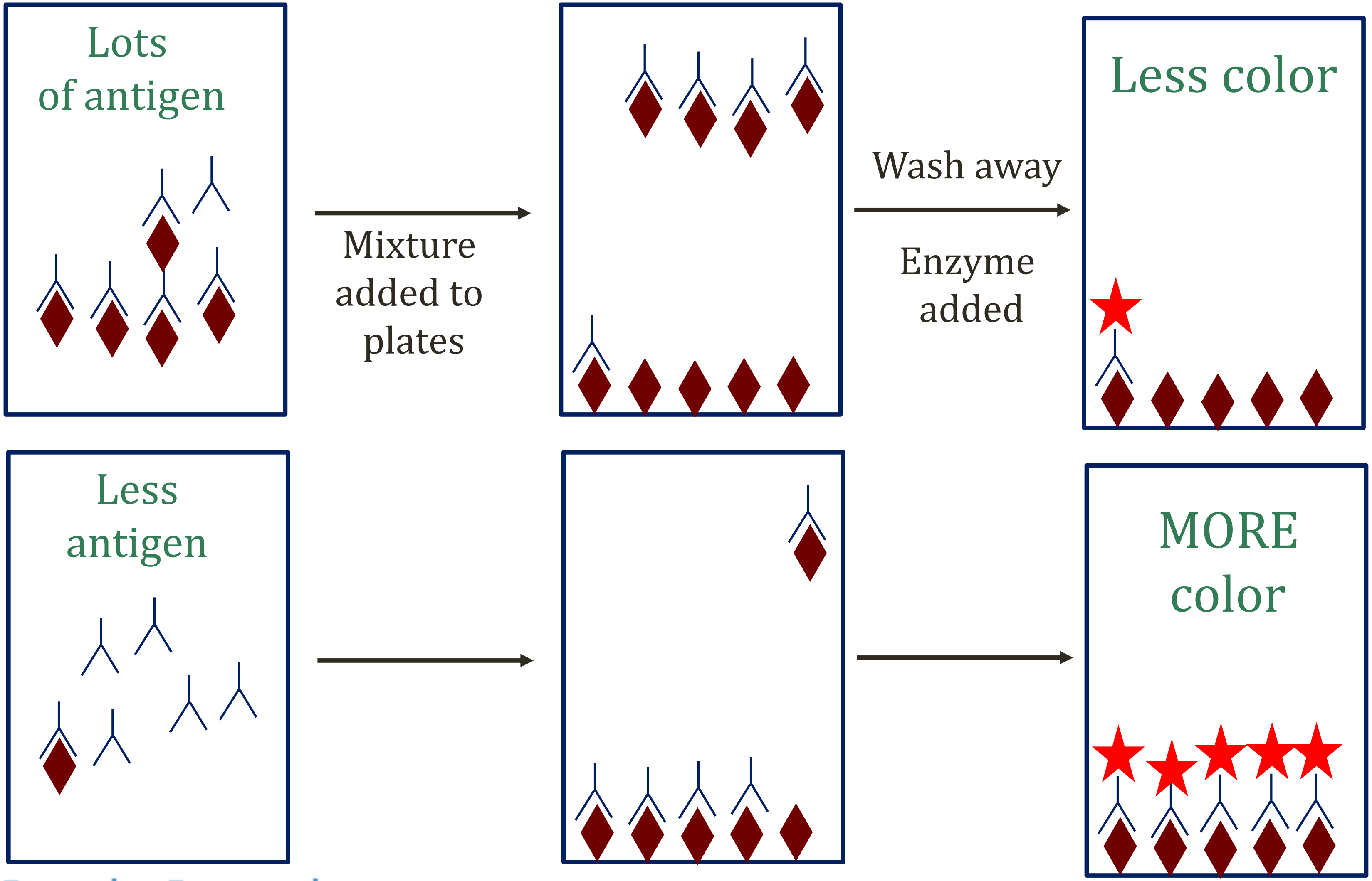
- Primary antibody incubated with sample
- Antigen-antibody complexes form
- More antigen = more binding = less free antibody



Competitive ELISA

- Mixture added to antigen coated plates
- Unbound antibody binds antigen
- Wash away antigen-antibody complexes
- Secondary antibody and substrate added
- More color change = LESS antigen in sample

Competitive ELISA



Lots of antigen

Mixture added to plates

Wash away

Enzyme added

Less color

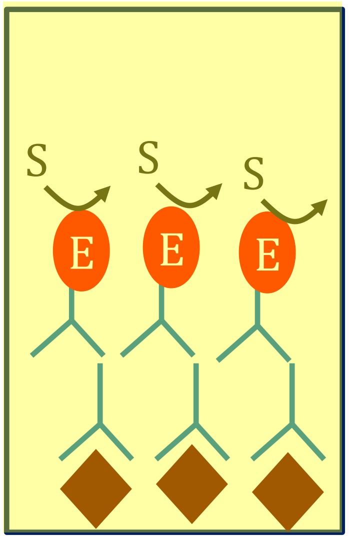
Less antigen

MORE color

ELISA

Uses

- **HIV antibody detection**
  - Indirect method (many variants used)
  - HIV antigen attached to well
  - Sample reacts with antigen-coated plate
  - 2° antibody: antihuman immunoglobulin with bound enzyme
  - Addition of substrate results in color change



ELISA

Uses

- **HIV p24 antigen detection**
  - Often sandwich ELISA used (many variants)

Microarrays and FISH

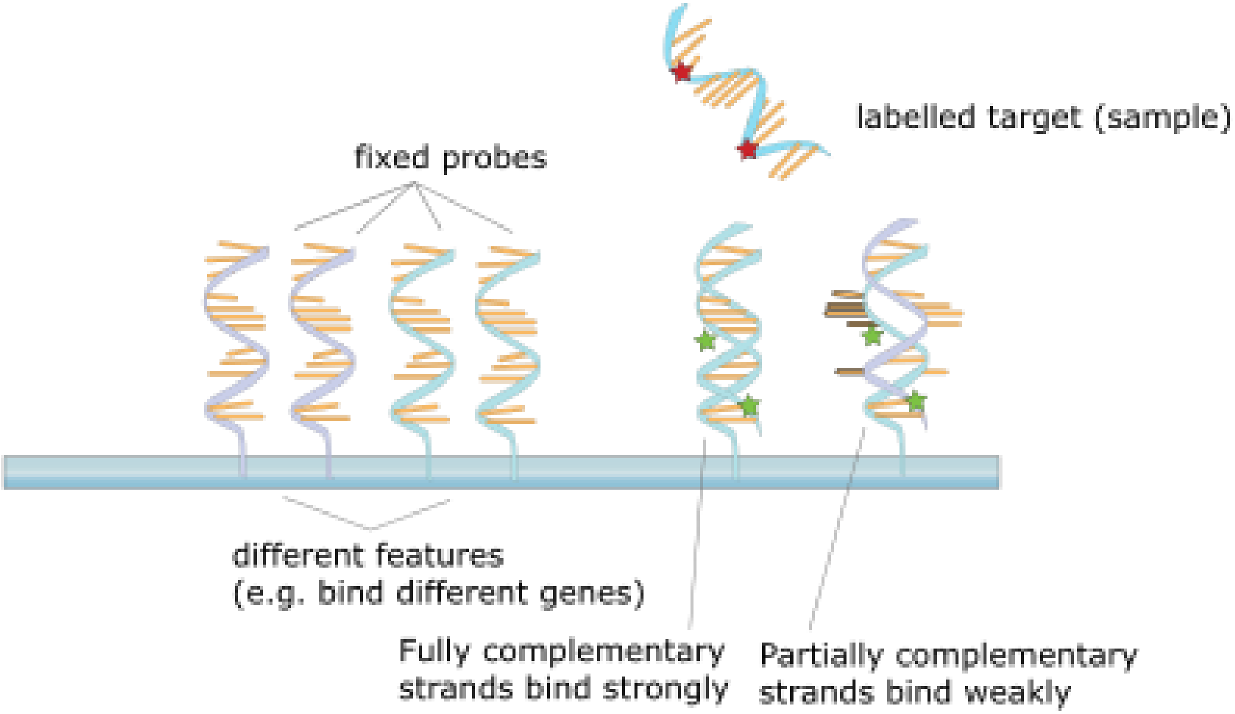
Jason Ryan, MD, MPH

Boards&Beyond
STUDY SMARTER

DNA Microarray

- Also called DNA chip or biochip
- Solid structure: glass, plastic, or silica
- Thousands of DNA sequences (probes) attached
- Used to test a sample DNA with fluorescent markers
- Sample **hybridizes** with complementary bases
- Computer detects which probes bind sample

DNA Microarray



Wikipedia/Public Domain

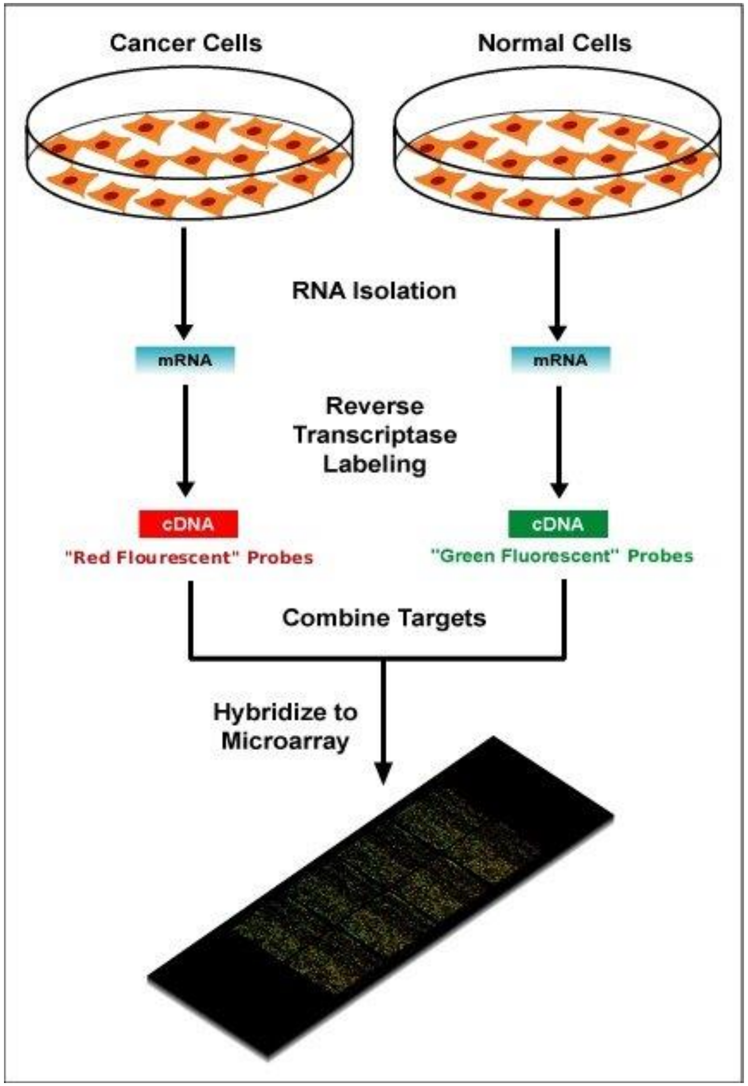
DNA Microarray

Schutz/Wikipedia

DNA Microarray

- **Gene expression**
  - Which genes active/inactive
  - Example: cancer cells versus normal cells
- Cellular mRNA collected → cDNA
- cDNA tested using microarray
- Determines gene expression

DNA Microarray



Cancer Cells

Normal Cells

RNA Isolation

mRNA

mRNA

Reverse Transcriptase Labeling

cDNA

"Red Flourescent" Probes

cDNA

"Green Fluorescent" Probes

Combine Targets

Hybridize to Microarray

Wikipedia/Public Domain

DNA Microarray

- **Copy number variation**
  - Some cells contain ↓/↑ copies of genes/DNA
  - Increased/decreased copies linked to disease
- Cellular DNA collected → microarray testing
- Reference sample also tested
- Results (fluorescence intensities) compared
  - Sample = reference (no extra copies)
  - Sample > reference (more copies)
  - Sample < reference (fewer copies)

DNA Microarray

- **Single nucleotide polymorphisms (SNPs)**
  - Genes exist with variations in a single nucleotide
  - Variations represented in the microarray
- Cellular DNA collected → tested using microarray
- Binding indicates which SNP present in sample gene
- Many SNPs associated with disease
- Many SNPs preserved within families

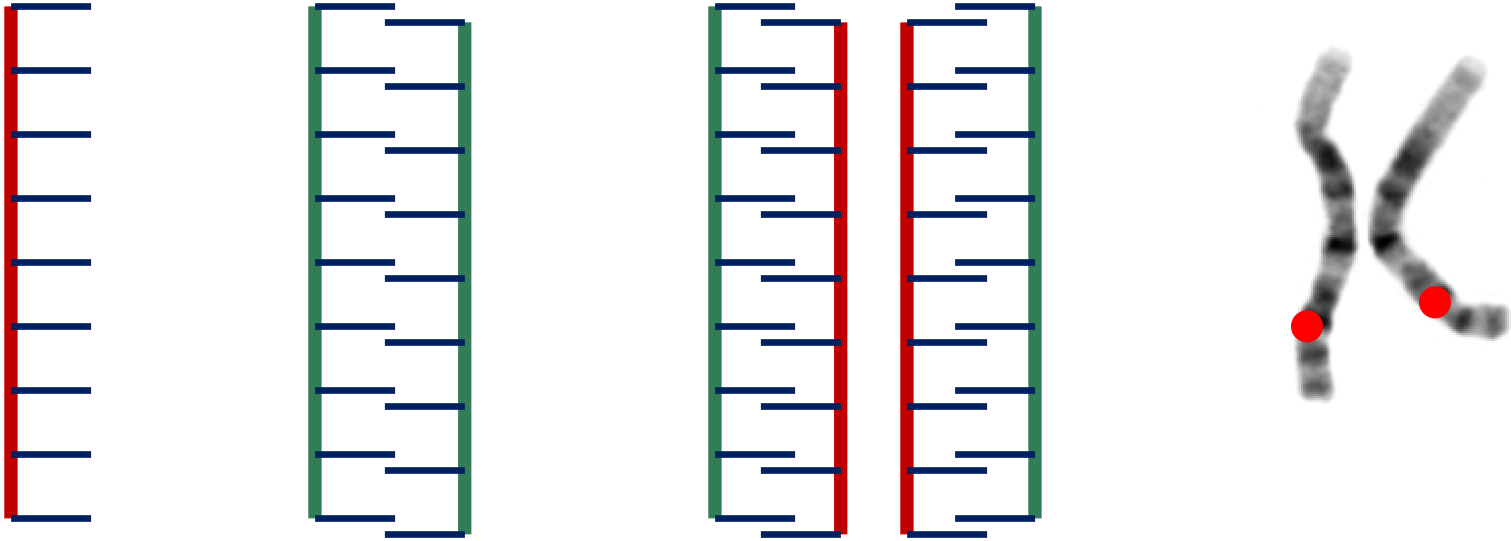
FISH

Fluorescence in situ hybridization

- Fluorescent DNA probe binds to specific gene site
- Localizes genes to a chromosome
- Determine which chromosome contains gene

FISH

Fluorescence in situ hybridization



Fluorescent DNA Probe

DNA of Interest

Hybridization

FISH

Fluorescence in situ hybridization

- Often done on cells in **metaphase**
  - Cells arrested in mitosis
  - Chromosomes visible individually
  - Fixed to glass slide
  - DNA probes used that match regions of known chromosomes
  - Probes hybridized to chromosomes on slide
    - “in situ” hybridization
  - Probes visualized with fluorescence microscopy

FISH

Fluorescence in situ hybridization

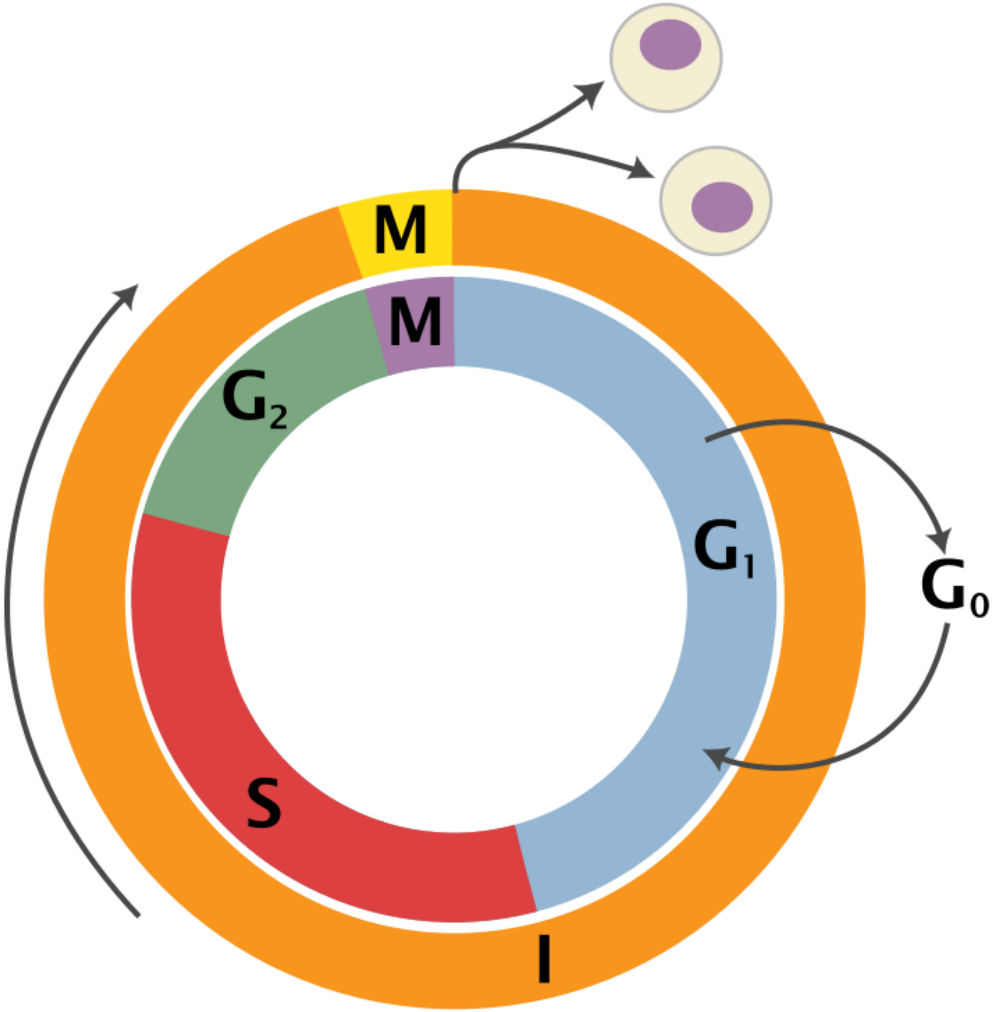
- Often used to compare test cell to normal cells
  - Locate gene in test cells
- **Microdeletion**: no fluorescence of chromosome
  - 22q11(DiGeorge syndrome)
- Translocation: fluorescence on different chromosome
- Duplication: extra site of fluorescence

Cell Cycle

Jason Ryan, MD, MPH

Boards&Beyond
STUDY SMARTER

Cell Cycle



Richard Wheeler (Zephyris) 2006

Cell Cycle

Interphase
(Growth)

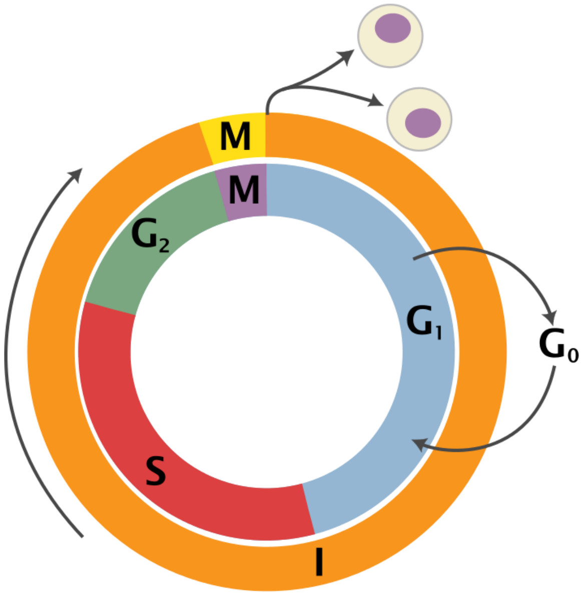
→

M phase
(Mitosis)

G1 (growth)
S (synthesis)
G2 (growth)

⇄

G0
(resting)



Richard Wheeler (Zephyris) 2006

Cell Cycle

- G1 phase
  - Synthesis of proteins, organelles
  - **Length varies** depending on conditions
- **Mitogens**:
  - Extracellular signaling molecules, usually proteins
  - Stimulate **cell division**
  - Function via **cyclin dependent kinases (Cdks)**
- Growth factor: Stimulates growth in size
- Some molecules both mitogens and GFs
- Terms sometimes used interchangably

Cell Cycle

- S phase
  - Synthesis of DNA
  - Chromosomes → two sister chromatids
- G2 phase
  - Growth in preparation for mitosis

G_0 Phase

- May occur in absence of mitogen stimulation
- Specialized non-dividing state
- Most cells in our body are in G_0
- Some permanent G_0
- Others go in/out

G_0 Phase

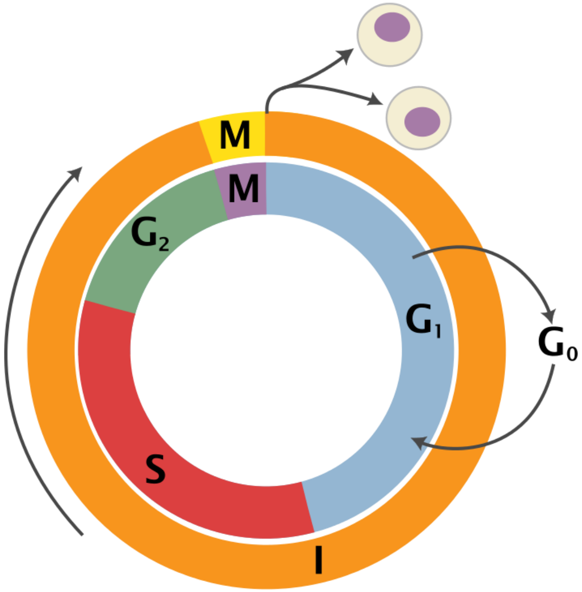
- Neurons, skeletal muscle cells
  - Permanent G_0 state (“terminally differentiated”)
- Liver cells
  - Often in G_0 but may divide if stimulated
- Fibroblasts, lymphocytes
  - Enter and exit G_0 many times in their lifespan

G_0 Phase

- Bone marrow cells, GI epithelial cells, hair follicles
  - “Labile cells”
  - Rapidly dividing
  - Rarely/never enter G_0
  - Most effected by many forms of **chemotherapy**

Mitosis

- Shortest (most rapid) portion of cell cycle
- Divided into phases
  - Prophase
  - Prometaphase
  - Metaphase
  - Anaphase
  - Telophase

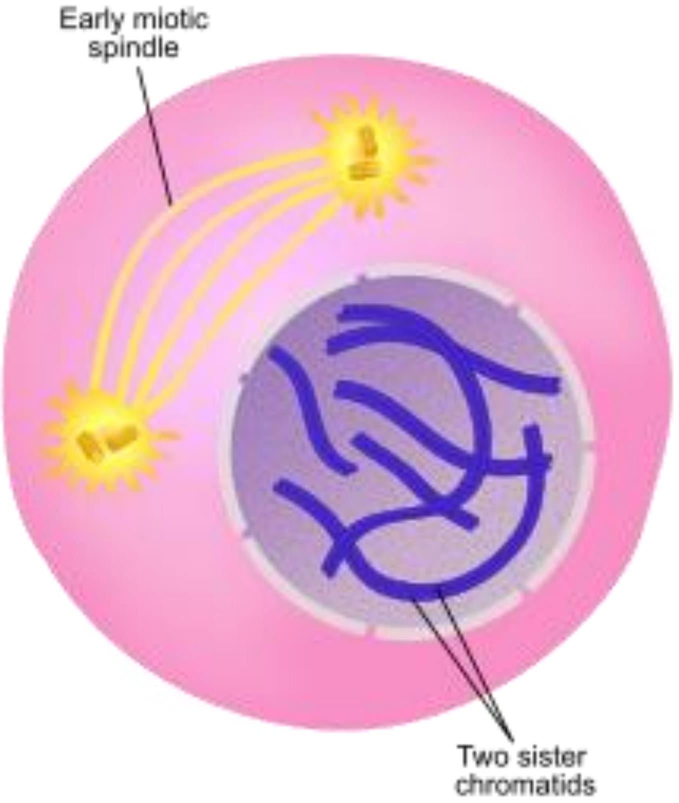


Richard Wheeler (Zephyris) 2006

Mitosis

Prophase

- Chromosomes condense
- Spindle fibers forms

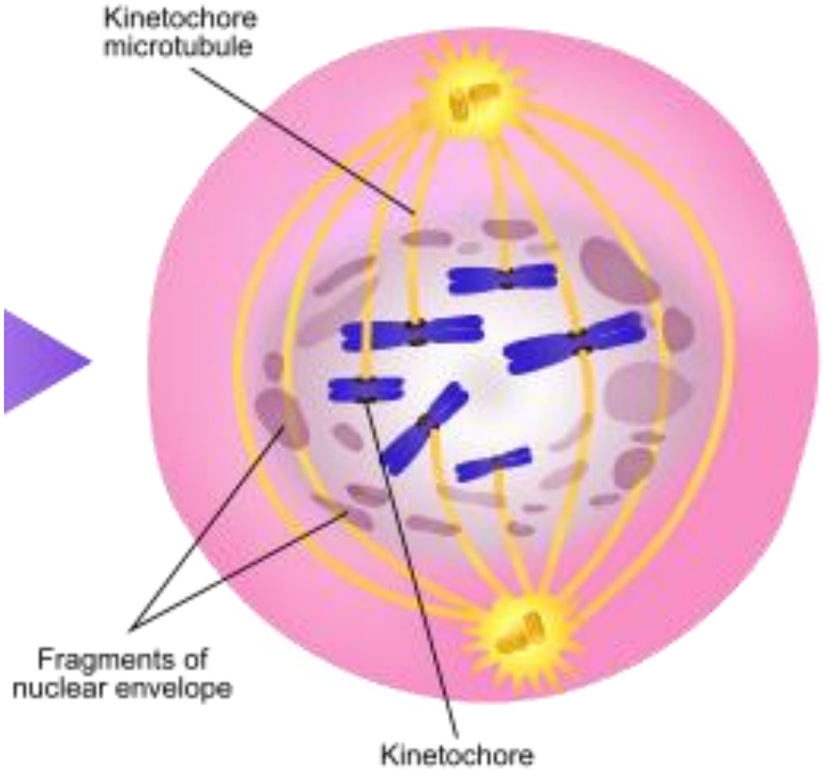


Ali Zifan/Wikipedia

Mitosis

Prometaphase

- Chromosomes organize on mitotic spindle

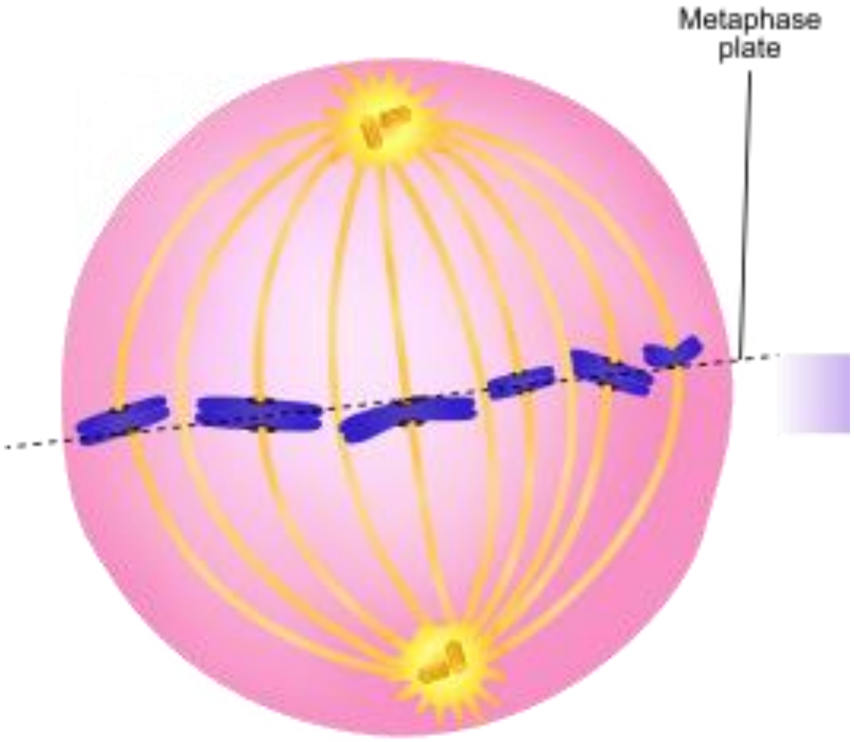


Ali Zifan/Wikipedia

Mitosis

Metaphase

- Chromosomes line up on metaphase plate



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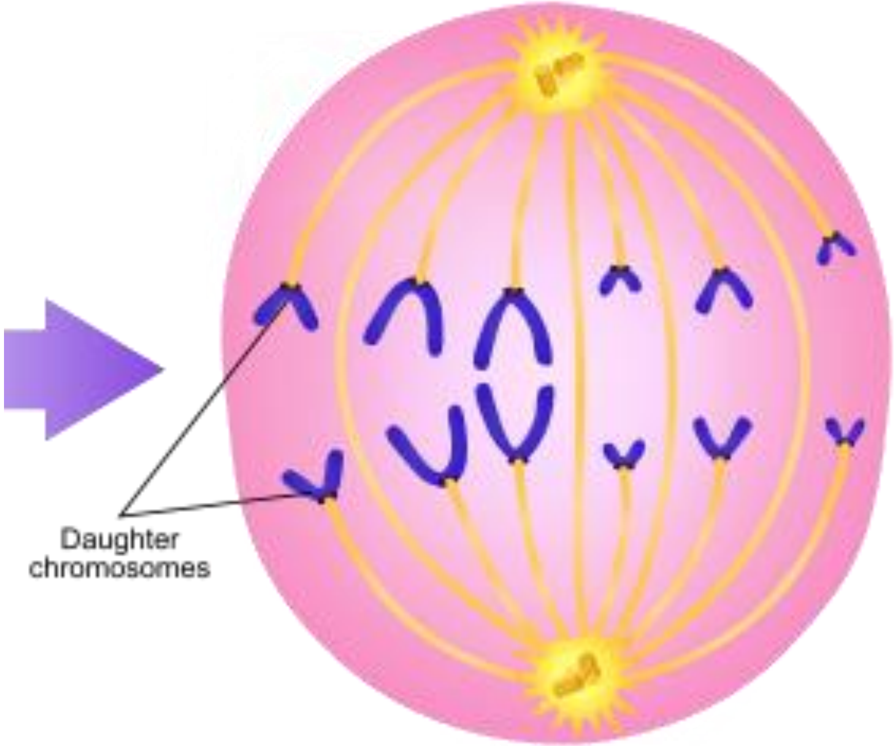
Mitosis

Anaphase

- Chromosomes separate



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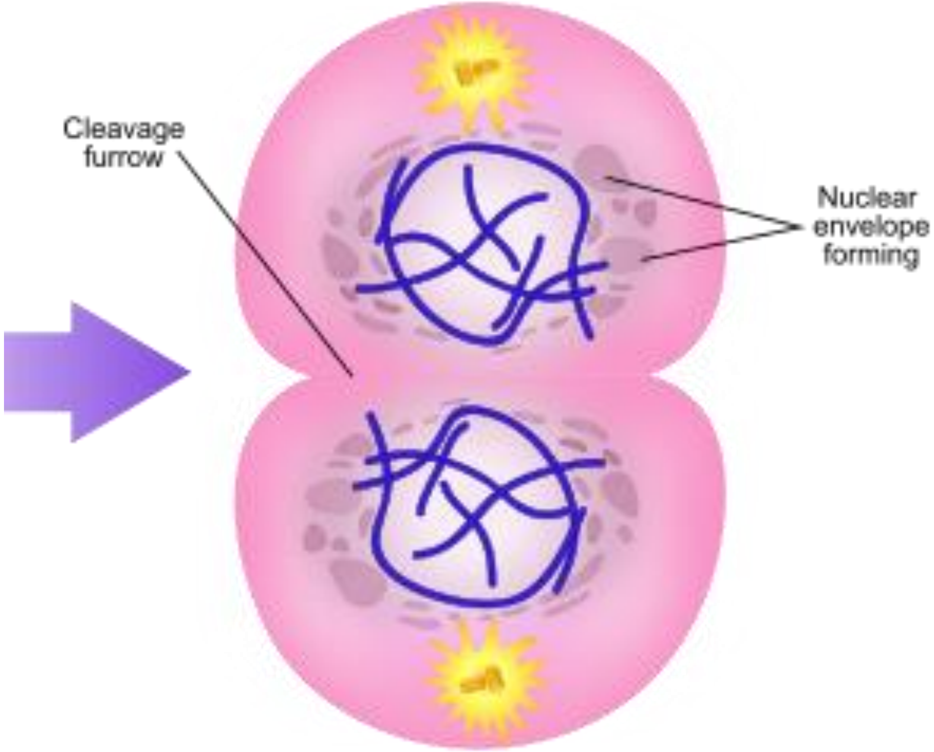
Mitosis

Telophase/Cytokinesis

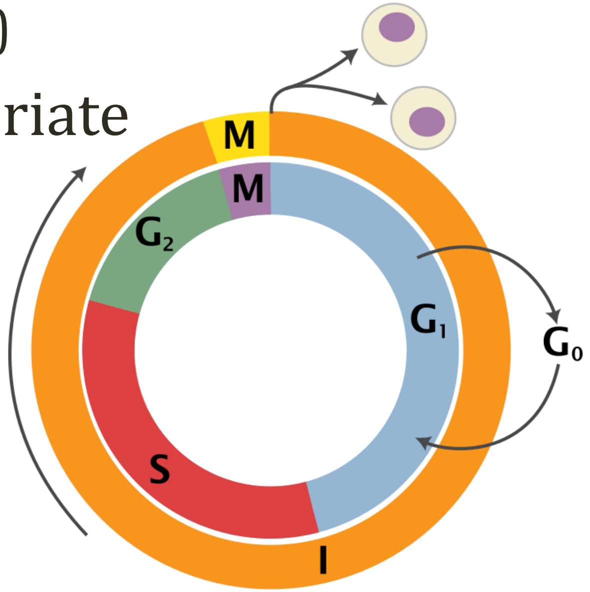
- Spindle breaks down
- Cell divides



Ali Zifan/Wikipedia

Cell Cycle Control

- Cells regulate progression through "**checkpoints**"
  - Also called "restriction points"
  - G1-S (prior to S phase entry)
  - G2-M (prior to mitosis)
  - M phase (prior to anaphase/cytokinesis)
- Arrests cell if conditions not appropriate
- First checkpoint: Late G1 (G1-S)
  - Cell commits to cell cycle/growth



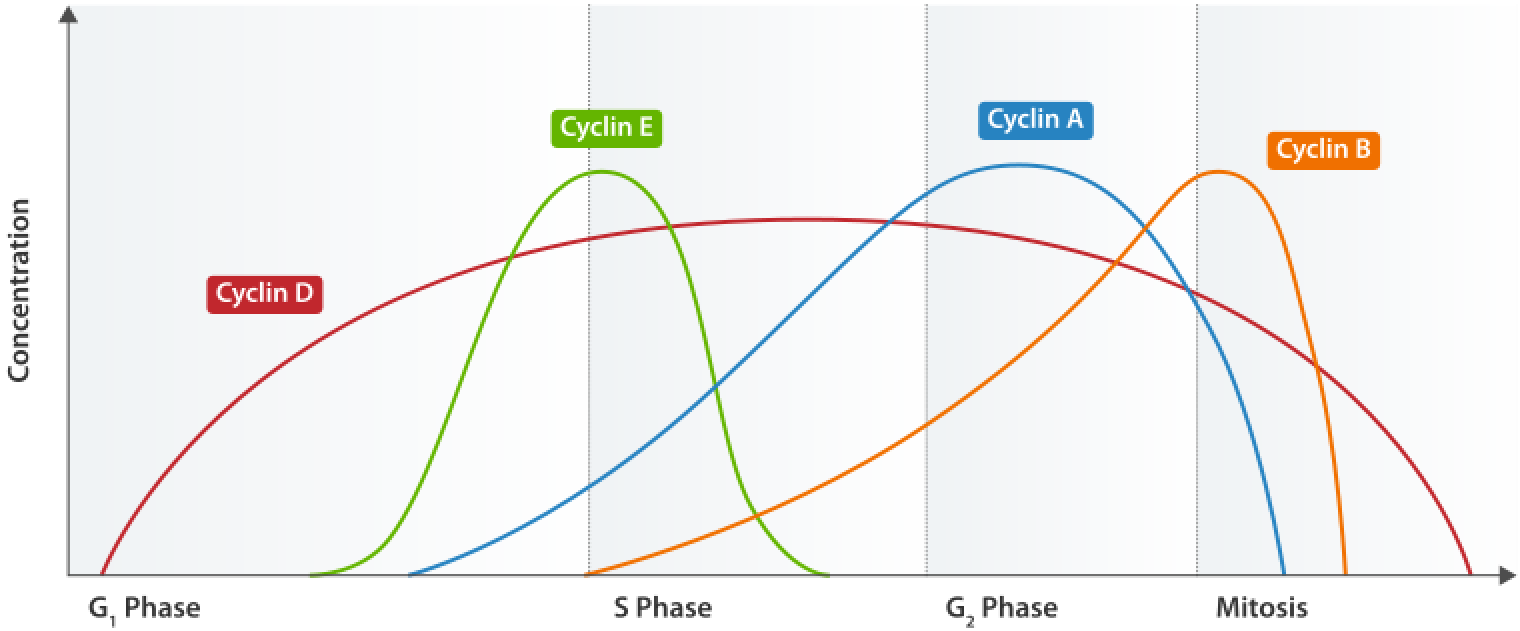
Richard Wheeler (Zephyris) 2006

Cell Cycle Control

- **Cyclin Dependent Kinases** (Cdks)
  - Central components of cell cycle control
  - Kinase enzymes (lead to **phosphorylation** of other proteins)
  - Always present in cells but inactive
  - Depend on cyclins to activate
- **Cyclins**: regulatory proteins – activate Cdks
- **Cyclin-Cdk complexes**
  - Phosphorylate regulatory proteins
  - Allow progression through cell cycle

Cyclins

- Many classes/subtypes
- Levels vary during cell cycle



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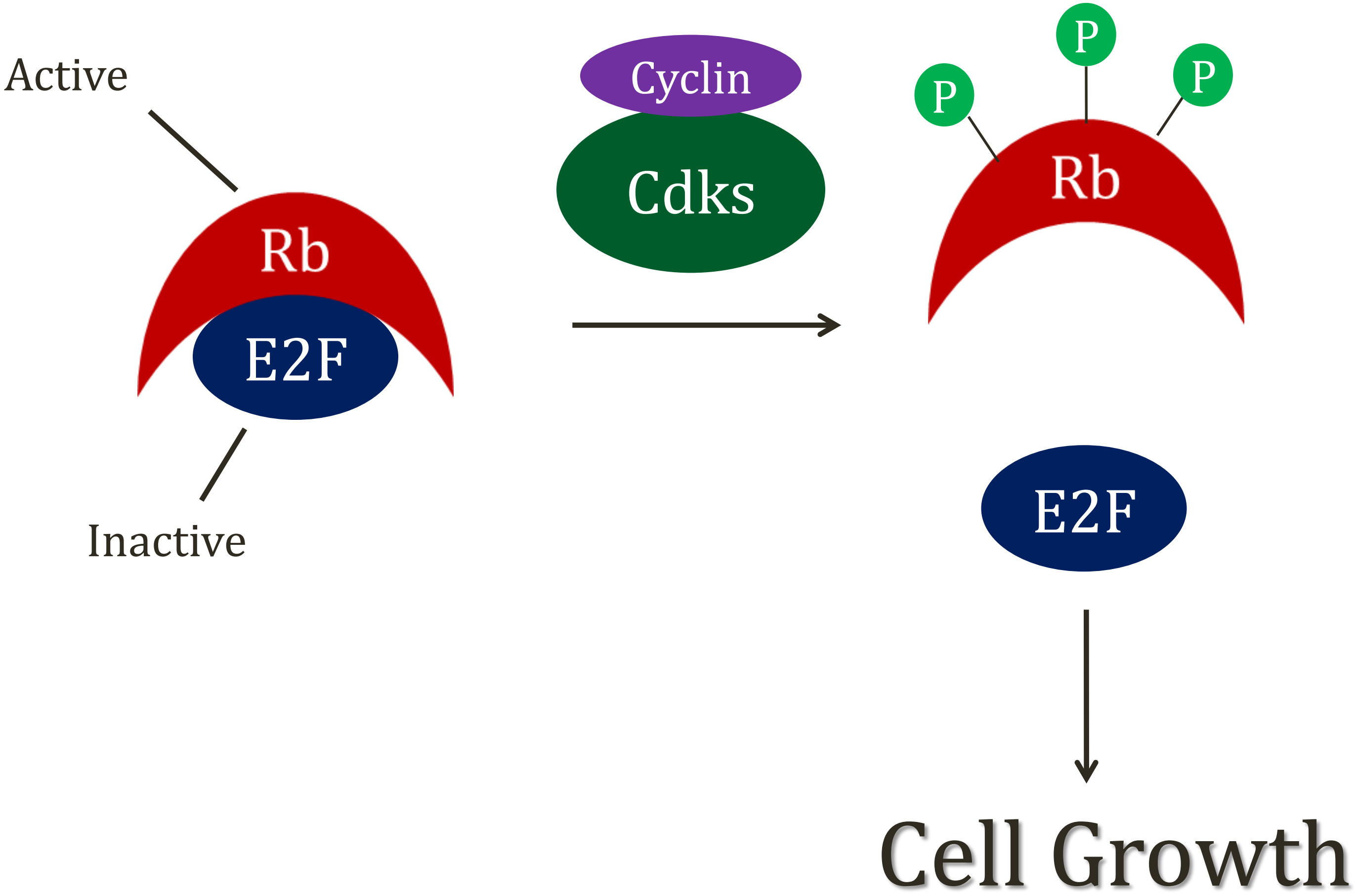
G1-S Checkpoint

- During G1 phase → Cdk activity suppressed
- **Mitogens** activate Cdk → entry into S phase
  - Interact with cell surface receptors
  - Activate intracellular pathways
  - Increase G1 **cyclin levels**
  - Increase Cdk activity

G1-S Checkpoint

- Cyclin-Cdk complexes activate **E2F proteins**
  - Transcription factors
  - Bind to DNA promoter regions
  - Activate genes for S phase
- E2F normally inhibited
  - Inhibited by E2F binding to **retinoblastoma proteins (Rb)**
  - Inhibition released by G1-S-Cdk phosphorylation of Rb
- Rb regulates cell growth
  - "Tumor suppressor"

G1-S Checkpoint



G1-S Checkpoint

- **DNA damage** can arrest cell division
    - Allows for repair
    - Prevents development of mutant cells/cancer
- DNA damage initiates signaling pathways

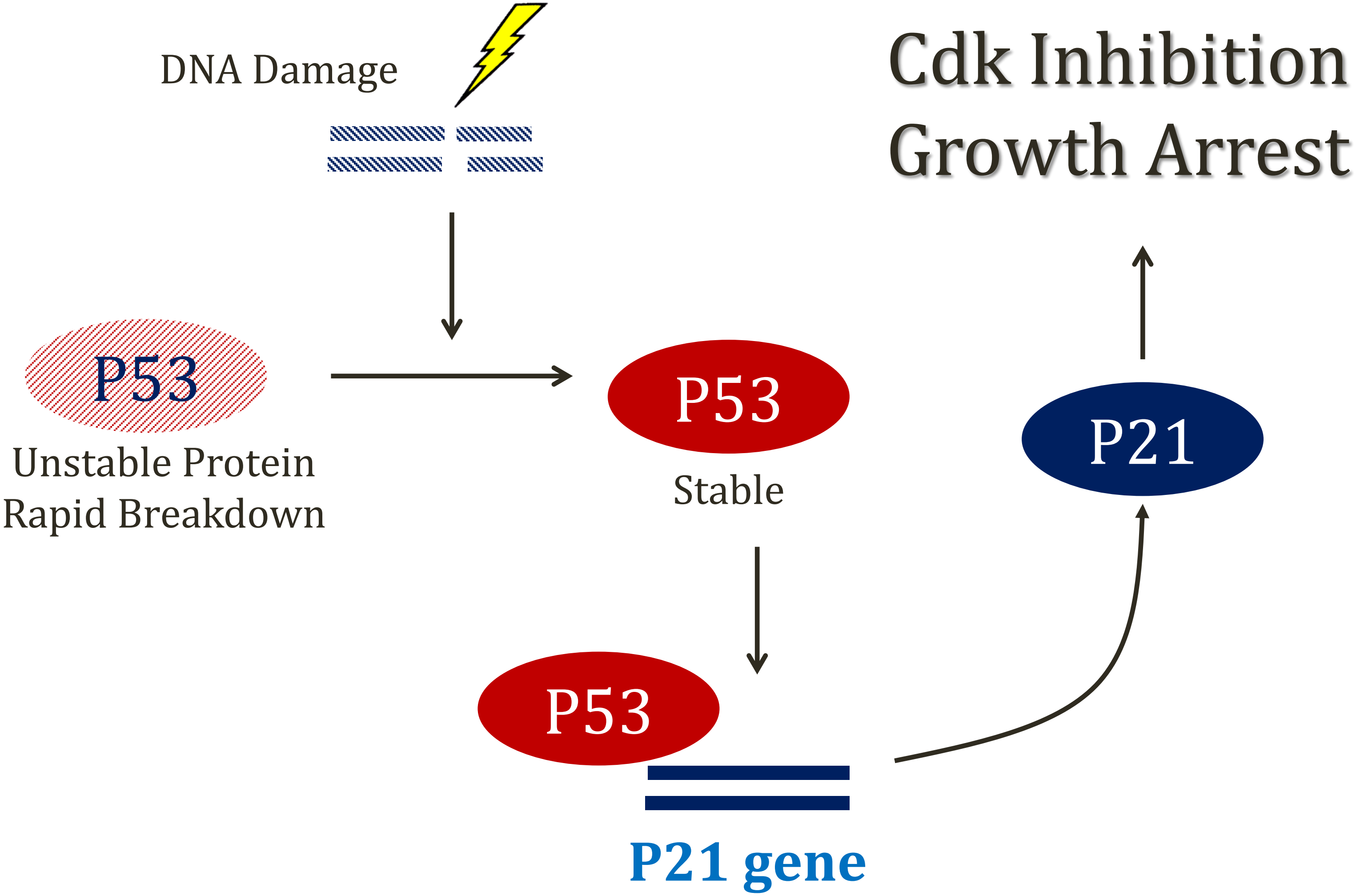
G1-S Checkpoint

- **ATM pathway**: Activated by double strand breaks
  - ATM: Ataxia Telangiectasia Mutated
  - ATM gene mutation → Ataxia Telangiectasia
- **ATR pathway**: Single stranded breaks
- Both lead to phosphorylation of proteins
- Causes cell cycle/growth arrest

P53 Protein

- **Major target of ATM/ATR systems**
- Phosphorylated after DNA damage
  - Prevents p53 breakdown
  - Increases levels/activity
- p53 induces transcription of **p21 protein**
- p21 binds to Cdks → inhibits Cdk activity
- Blocks cell progression through cell cycle
- p53/p21 = tumor suppressors

P53 Protein



DNA Damage

P53

Unstable Protein
Rapid Breakdown

P53

Stable

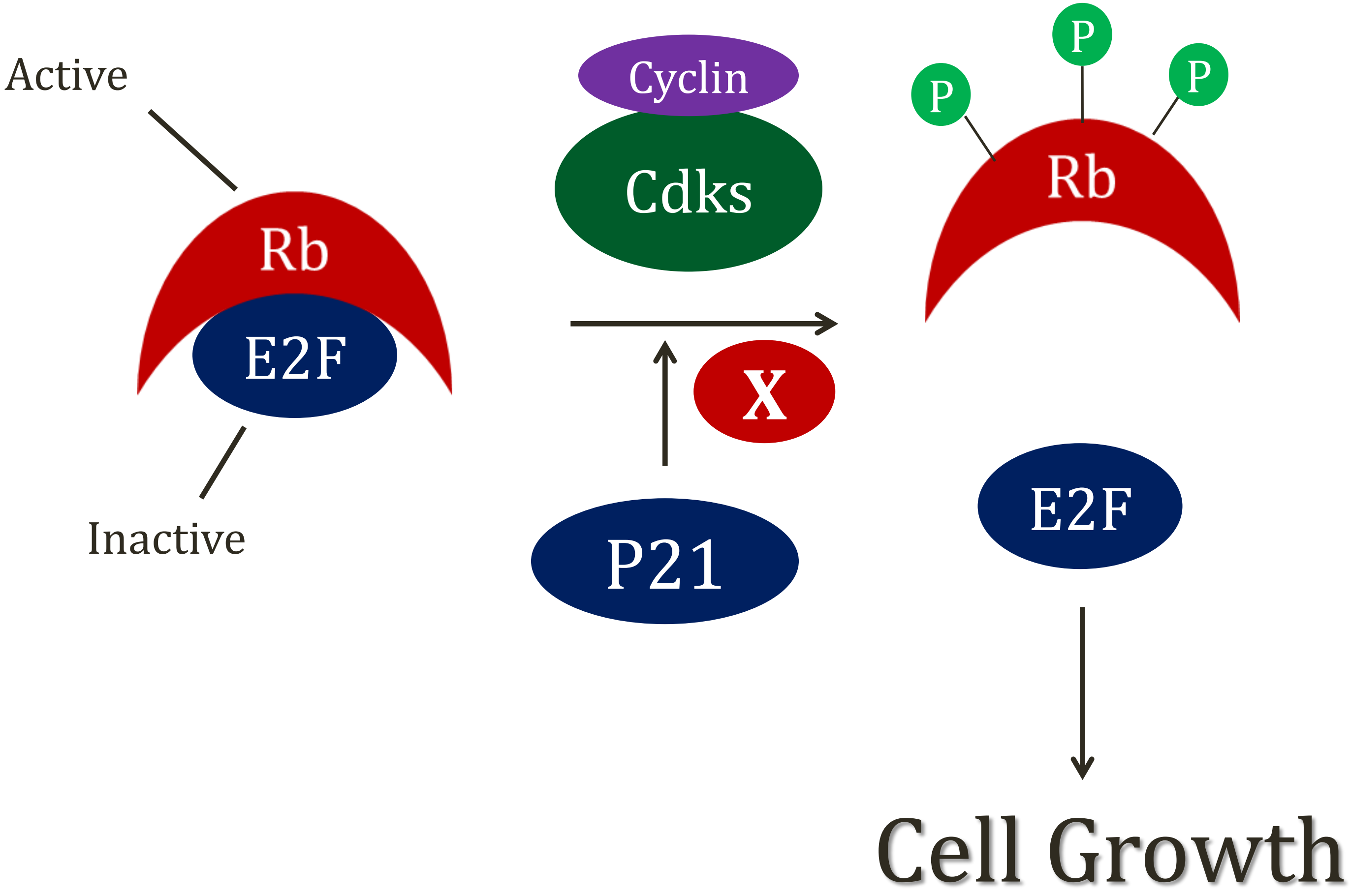
P53

P21 gene

P21

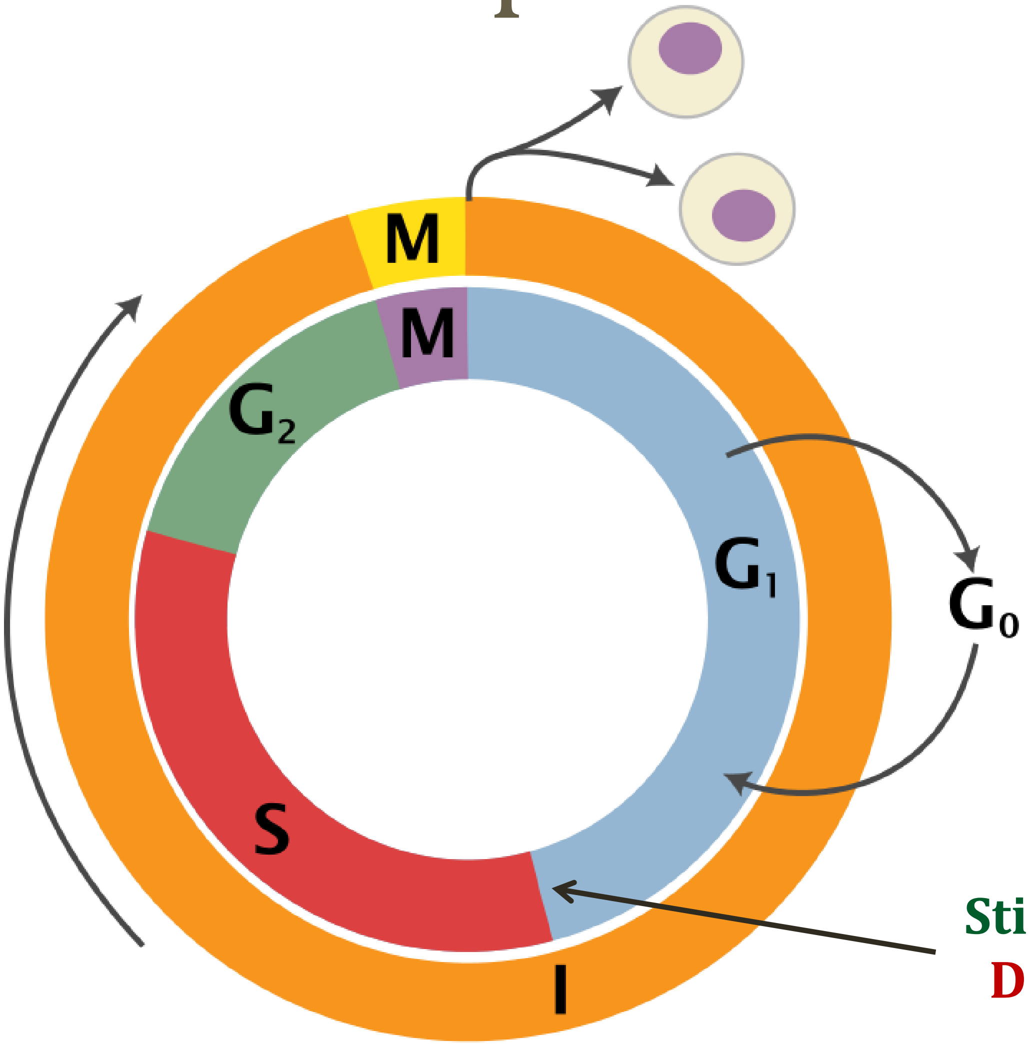
Cdk Inhibition
Growth Arrest

G1-S Checkpoint



Cell Growth

G1-S Checkpoint



Stimulation (Rb-E2F)
DNA Damage (p53)

Richard Wheeler (Zephyris) 2006

Retinoblastoma

- Rare childhood eye malignancy
- Mutations in RB1 gene
- Codes for Rb protein
- Abnormal Rb → Unregulated cell growth (via E2F)

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Li-Fraumeni Syndrome

- Syndrome of multiple malignancies at an early age
  - Sarcoma, Breast, Leukemia, Adrenal Gland
  - "SBLA" cancer syndrome
- Mutation in tumor suppressor gene TP53
- Codes for p53 protein
- Mutation: Cycle not arrested to allow for DNA repair
- Accumulation of damage → malignancy

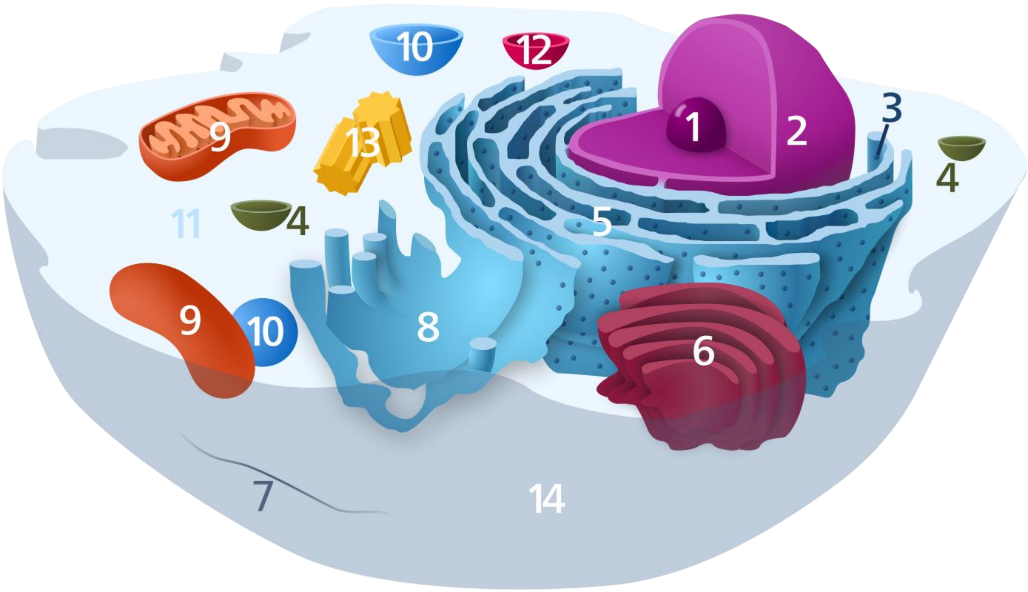
Cell Structure

Jason Ryan, MD, MPH

Boards&Beyond
STUDY SMARTER

Endoplasmic Reticulum

- Found in all eukaryotic cells
- Folded membrane of sacs/tubules
- Continuous with nuclear membrane
- Site of synthesis of proteins and lipids



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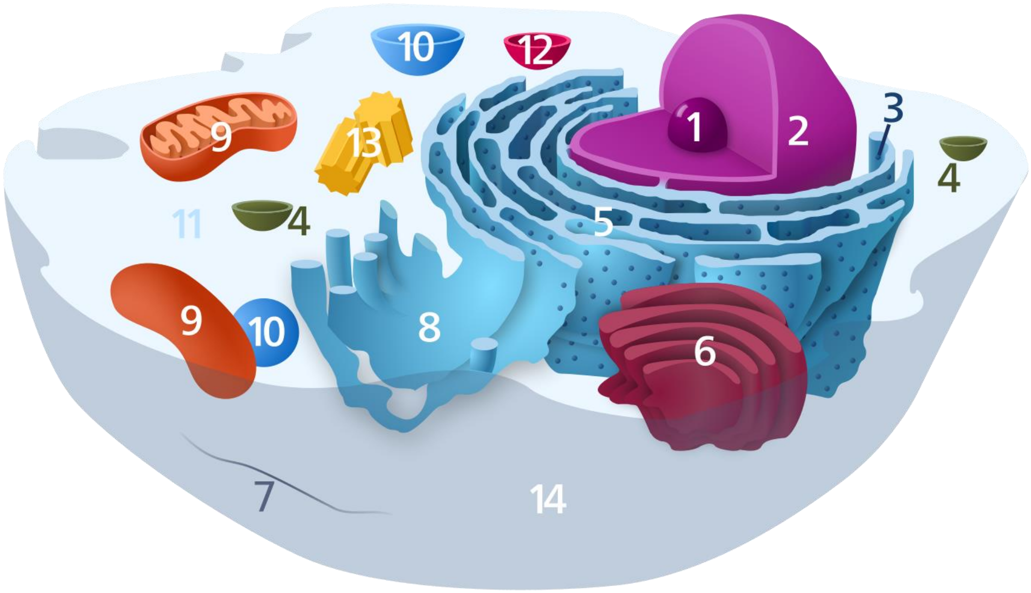
RER

Rough Endoplasmic Reticulum

- Surface of ER covered with ribosomes
- Gives granular or "rough" appearance
- Site of protein synthesis



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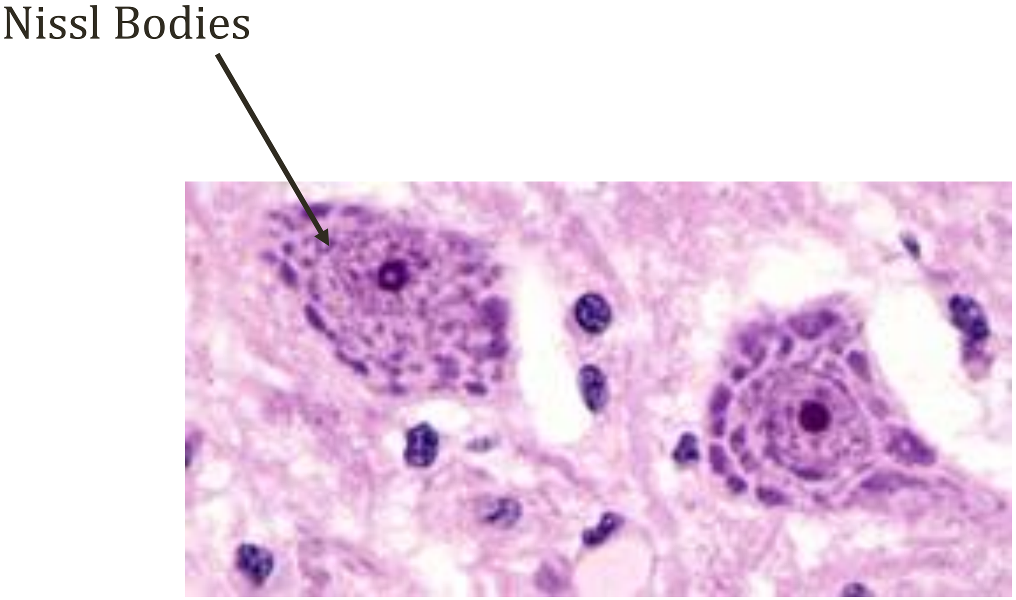
RER

Rough Endoplasmic Reticulum

- **Membrane bound ribosomes**
  - Found in RER
  - Produce proteins mostly for secretion from cell
  - Protein hormones, digestive enzymes
- **Free ribosomes**
  - Found “free” in cytosol
  - Produce proteins mostly used by cell
  - Metabolism, structure

Nissl Bodies

- Rough endoplasmic reticulum in neurons
- Synthesize neurotransmitters

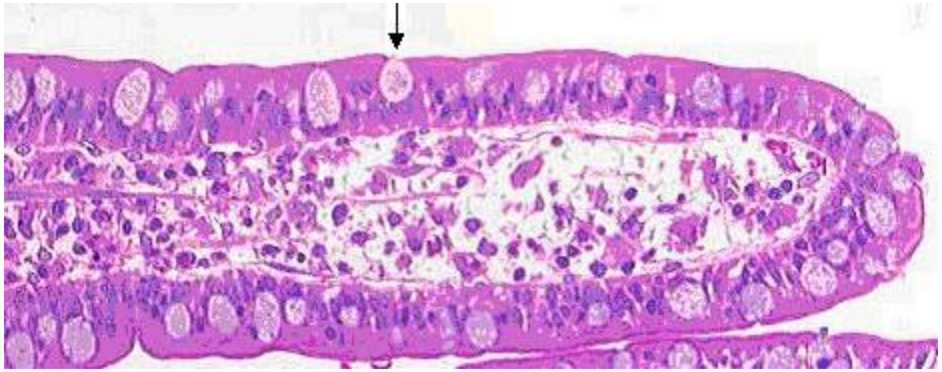


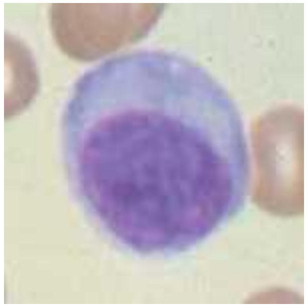
Dr. Dimitri Agamanolis
neuropathology-web.org

RER

Rough Endoplasmic Reticulum

- Abundant in cell that secrete proteins
  - Goblet cells of intestines (mucus)
  - Plasma cells (antibodies)
  - Pancreatic beta cells (insulin)





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SER

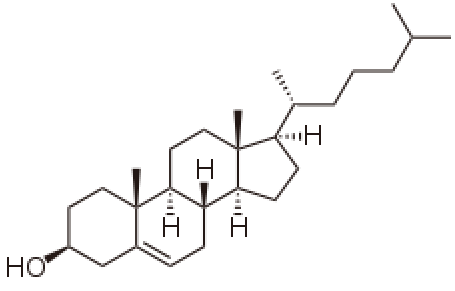
Smooth Endoplasmic Reticulum

- Portions of ER without ribosomes
- Important for **lipid/steroid synthesis**
- Also **detoxification** of drugs and toxins
- Sarcoplasmic reticulum = SER in myocytes
  - Stores calcium for muscle contraction

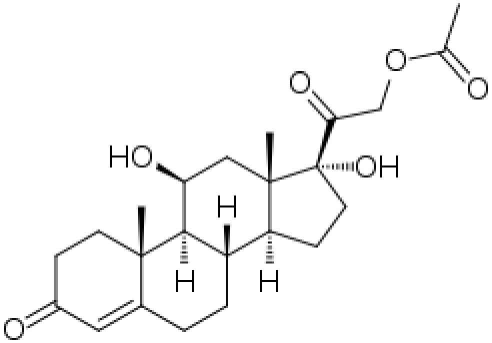
SER

Smooth Endoplasmic Reticulum

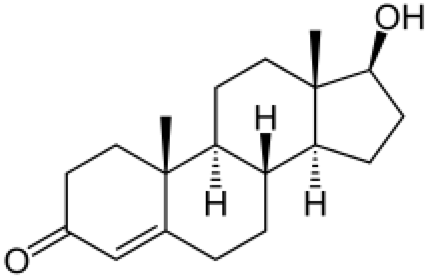
- Lots of SER found in **hepatocytes**
  - Synthesis of cholesterol/lipoproteins
  - Many detoxification enzymes
  - Cytochrome P450 family of enzymes
- Also found in **steroid producing organs**
  - Adrenal glands
  - Gonads



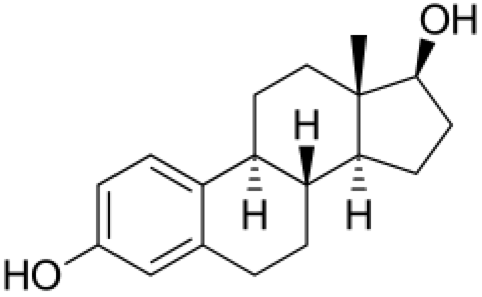
Cholesterol



Cortisol



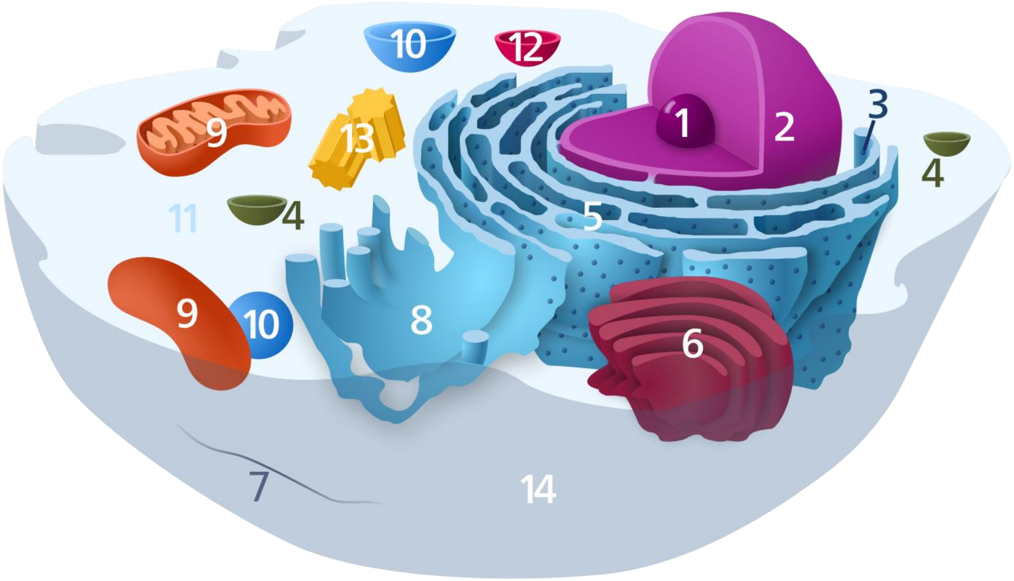
Testosterone



Estradiol

Golgi Apparatus

- Proteins leave ER in vesicles → transported to Golgi
- Fuse with Golgi membrane → empty their contents
- In Golgi → proteins modified
- Sorted for transport to next destination



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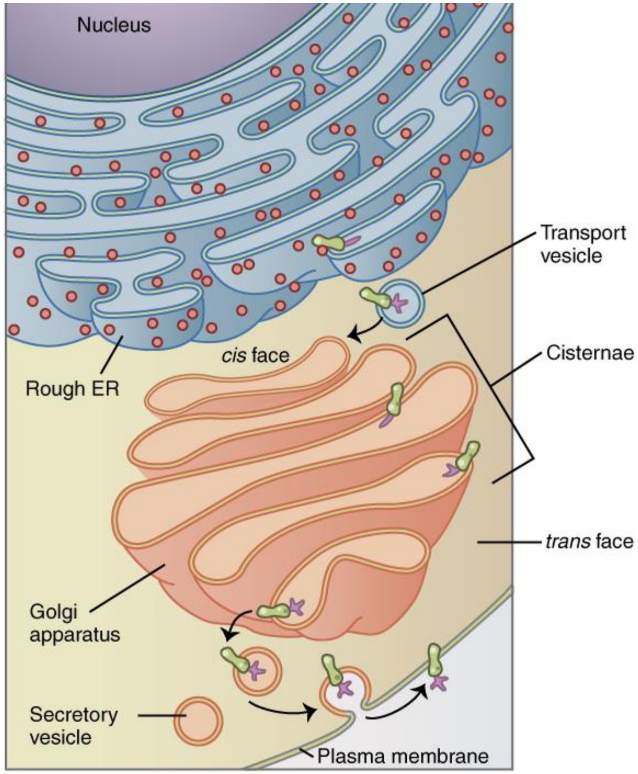
Golgi Apparatus

- Cis Golgi network
  - Vesicles come into *cis face* from RER
- Trans Golgi network
  - Vesicles leave from *trans face*
- Proteins sorted/shipped by adding signal sequences



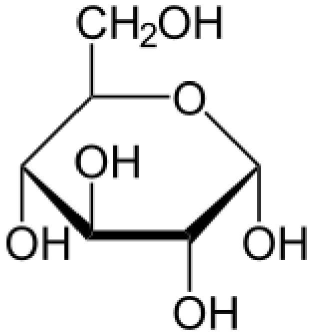
OpenStax College

Golgi Modifications

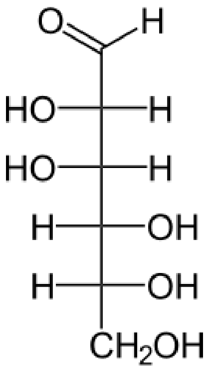
- Modifies N-oligosaccharides on asparagine
- Adds O-oligosaccharides to serine and threonine
- Adds mannose-6-phosphate to lysosomal proteins
- Likely serves many purposes:
  - Protects proteins from degradation
  - Directs proteins to target location
  - Allows protein recognition by receptors

Oligosaccharides

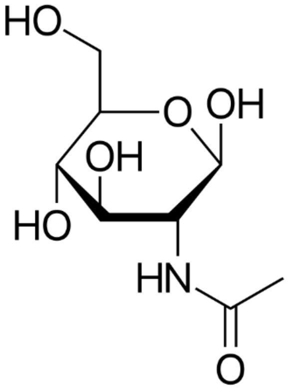
- Polymers (chains) of sugar molecules



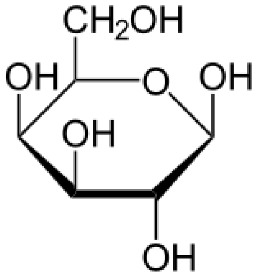
Glucose



Mannose



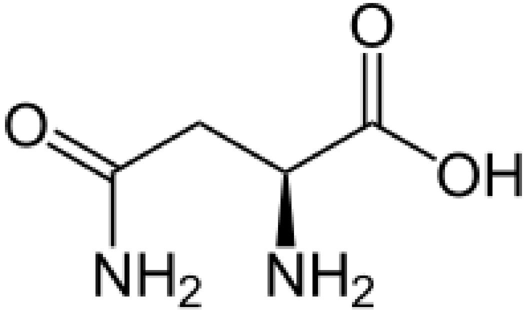
N-acetyl-glucosamine



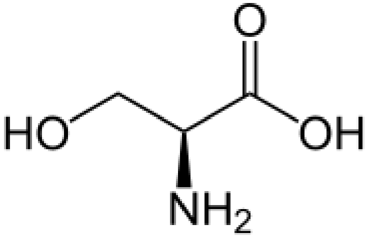
Galactose

Oligosaccharides

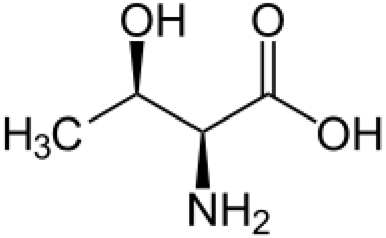
- N-linked: Attached to nitrogen
  - Often attached to asparagine (extra N molecule)
- O-linked: Attached to oxygen
  - Often attached to serine/threonine (extra O molecule)



Asparagine



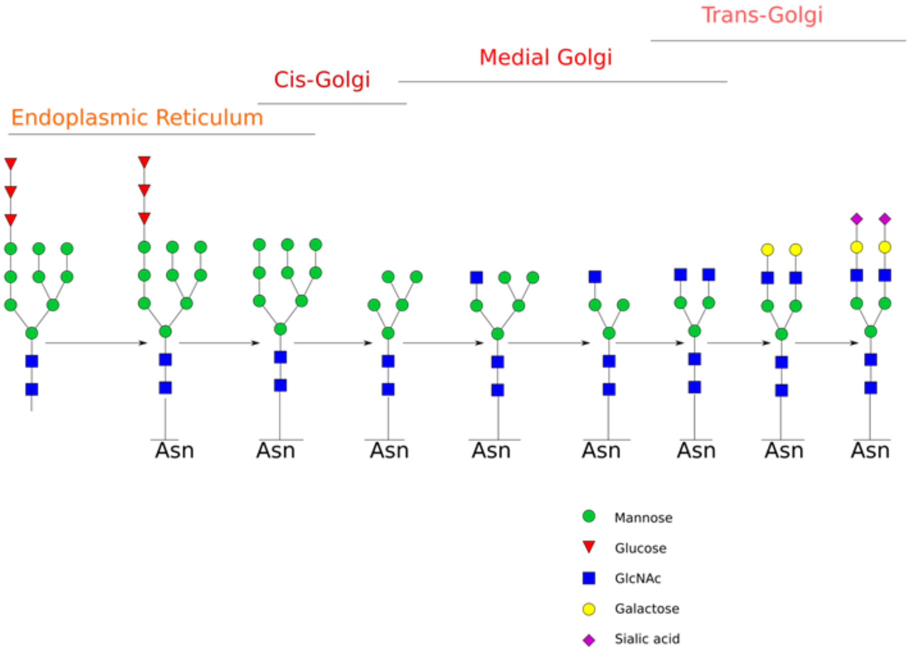
Serine



Threonine

N-linked Oligosaccharides

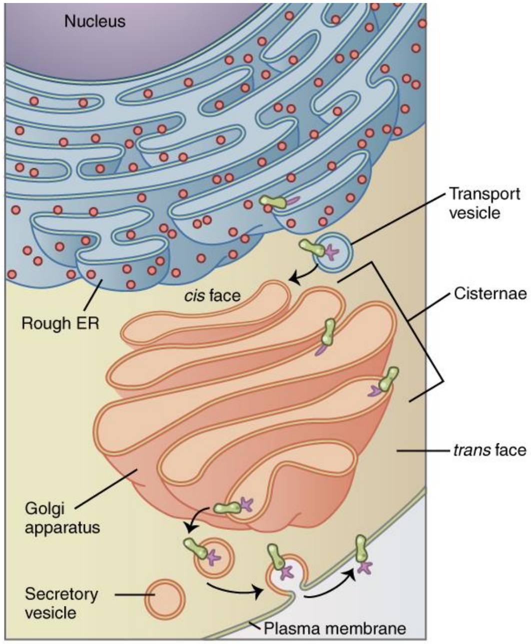
- Synthesized in endoplasmic reticulum
- Sugars added to asparagine (extra N molecule)
- Modified in Golgi apparatus (trimmed, sugars added)



Dna 621/Wikipedia

O-linked Oligosaccharides

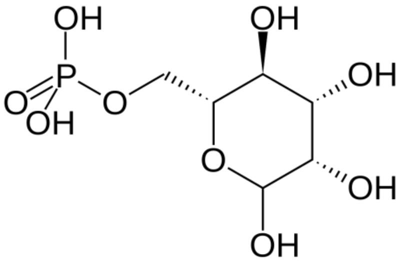
- Occurs in Golgi apparatus
- Sugars added to serine/threonine (extra O molecule)
- Example: Mucins heavily O-glycosylated



OpenStax College

Mannose-6-Phosphate

- Added to proteins destined for **lysosomes**
  - Acid hydrolase enzymes
  - Added to N-linked oligosaccharides
- Triggers packaging in trans-Golgi → lysosomes
- Process disrupted/abnormal in **I-cell disease**



Mannose-6-Phosphate

I-cell Disease

Inclusion Cell Disease

- Rare autosomal recessive metabolic disorder
- Lysosomal storage disease (mucolipidosis)
- Onset in 1st year of life
  - Growth failure
  - Coarse facial features
  - Hypotonia/Motor delay

I-cell Disease

Inclusion Cell Disease

- Failure of processing in **Golgi** apparatus
  - **Mannose-6-phosphate** NOT found on lysosome proteins
  - Deficiency: ***N*-acetylglucosaminyl-1-phosphotransferase**
  - Phosphate not added to mannose due to missing enzyme
- Result: enzymes secreted outside of cell
  - Hydrolases missing from lysosomes
  - Can be detected in blood/urine (outside cell)
- Lysosomes contain *inclusions* of undigested glycosaminoglycans and glycolipids

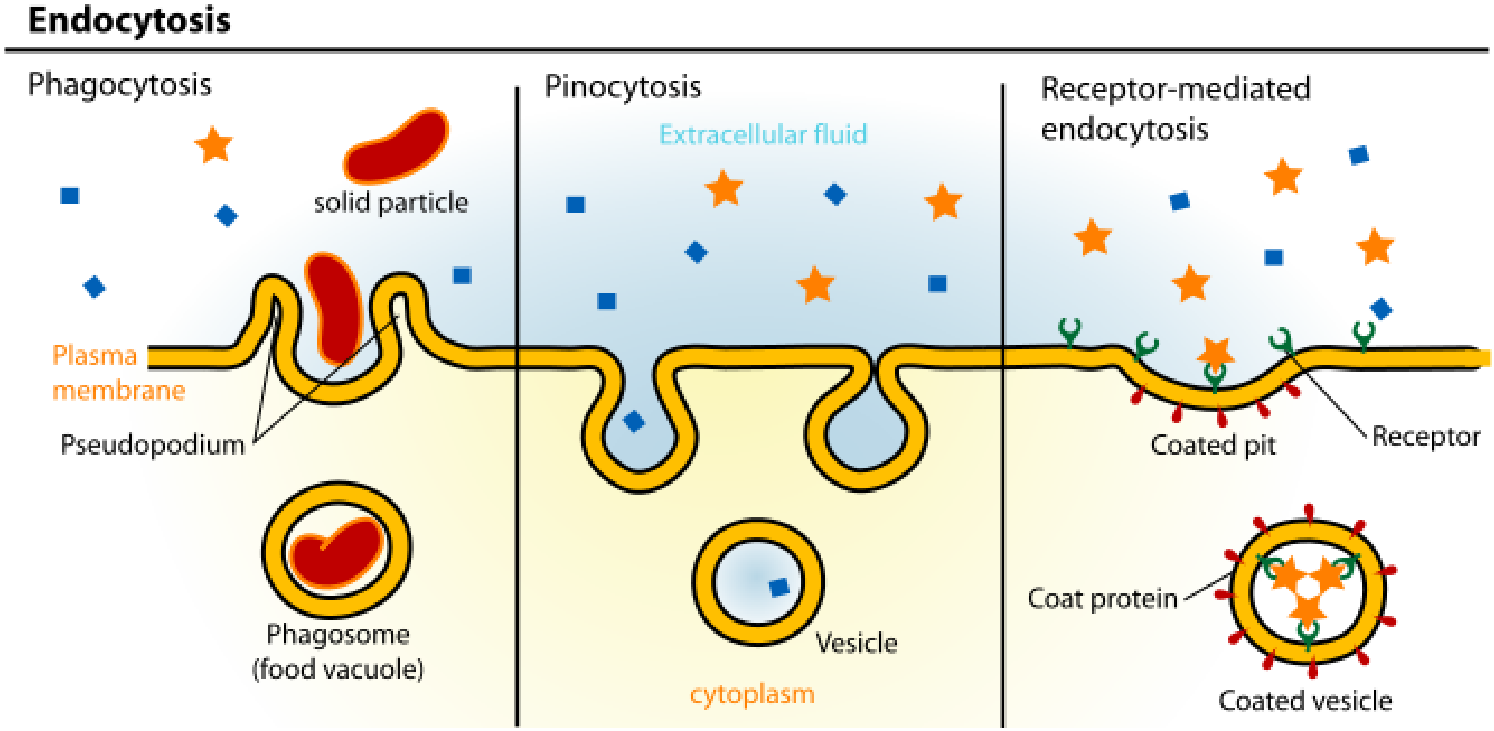
Endosomes

- Membrane-bound compartments in cells
- Formed by **endocytosis**
  - Invagination of plasma membrane to surround molecules
  - Pinching off of membrane to form enclosed structure

Endocytosis

- Receptor-mediated endocytosis
  - Cells take up specific molecules (ligands) that bind receptors
  - Receptors often located in coated pits
- Pinocytosis
  - Cells ingest droplets of liquid from extracellular space
- Phagocytosis
  - Cells extends pseudopods
  - Encircle particles
  - Important part of **immune defense**
  - Macrophages, Neutrophils = professional phagocytes

Endocytosis



Endosomes

- Transport contents to **lysosome**
  - Often fuse (join together) with membrane of lysosome
  - Lysosome digests materials
- Sometimes transport back to cell membrane

Lysosomes

- Acidic (pH ~4.8)
- Many **acid hydrolase** enzymes (40+ types)
  - Require acidic environment
  - Breakdown substrates by addition of water molecules
- Breakdown cellular waste
- Also fats, carbohydrates, proteins
- Generate simple compounds
- Returned to cytoplasm to be used by cell

Lysosomes

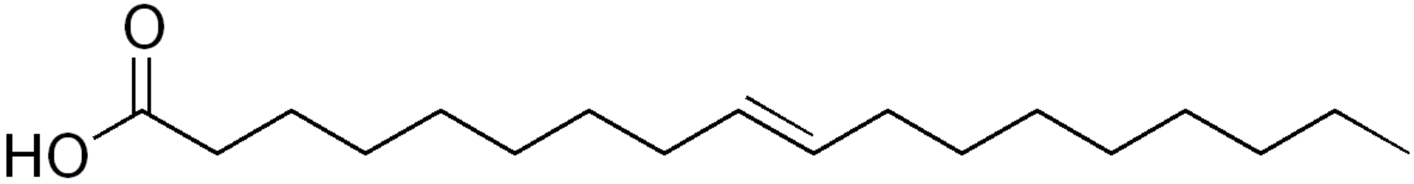
- Enzyme deficiency → lysosomal storage disease
- Cellular buildup of macromolecule → disease

Peroxisomes

- Cellular organelles (membrane-enclosed)
- Contain oxidative enzymes
- Can generate hydrogen peroxide (H_2O_2)
- **Catalase**
  - Oxidizes substances with H_2O_2
  - Detoxifies many substances in liver cells
  - Can metabolize ethanol (alternative, minor pathway)

Peroxisomes

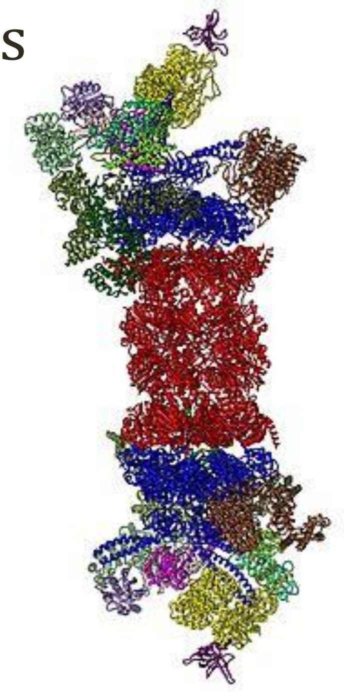
- **Beta oxidation fatty acids**
  - Occurs in mitochondria but also peroxisomes
  - Peroxisomes preferentially oxidize longer fatty acids



Fatty Acid

Proteasomes

- **Destroy aberrant proteins**
    - Misshaped/misfolded
- Barrel-shaped structure
- Protein “complex”: multiple protein subunits
- Requires ATP



FridoFoe/Wikipedia

Proteasomes

- Mostly destroys proteins "marked" by **ubiquitin**
    - Small protein
    - Tags damaged proteins
- May play a role in **Parkinson's disease**
    - Reduced ubiquitin-proteasome activity
    - Toxic accumulations of proteins in neurons

Secretory Pathway

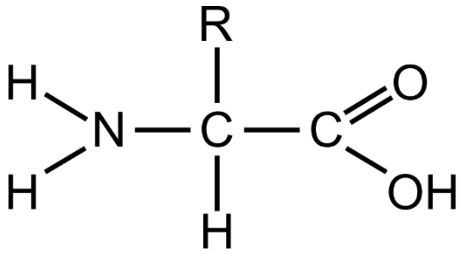
- Series of steps for secretory proteins
- Begins with translation of mRNA in cytosol
- Protein enters endoplasmic reticulum lumen
- Transferred to Golgi
- Exits Golgi in vesicle
- **Exocytosis** at plasma membrane → secretion

Signal Sequences

- Found on proteins undergoing synthesis (translation)
- Used to pull free ribosomes to ER membrane
  - Creates rough ER
  - Leads to proteins entry into ER lumen
  - Many will ultimately be secreted (via secretory pathway)
  - Some will go to ER, other organelles

Signal Sequences

- Short peptides (proteins)
- Found on N-terminal of protein
- Directs protein-ribosome to endoplasmic reticulum



Amino Acid

Signal Sequences

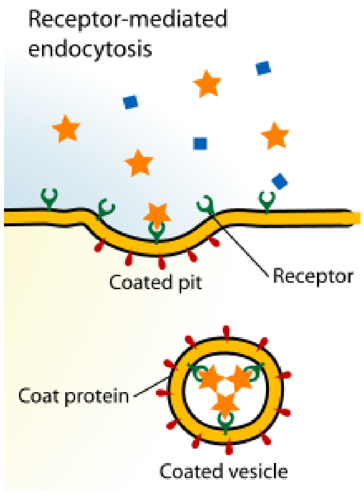
- **Signal Recognition Particle (SRP)**
  - Ribonucleoproteins found in cytosol
  - Complex particle with many proteins and RNA
  - Recognize signal sequences
  - Moves proteins from cytosol to ER
- SRP Receptor
  - Found on ER membrane
  - Binds SRPs
- Protein translocated through pore into ER lumen

Coated Vesicles

- Vesicles with protein coat on surface
- Formed from specialized portions of membranes
- Different coats in different forms of traffic
- Important for secretory pathway
- Also important in transport from cell surface
- Three well-characterized coats
  - Clathrin
  - COPI
  - COPII

Clathrin-Coated Vesicles

- Transport between plasma membrane and Golgi
- Also to/from endosomes in cytoplasm
- Major vesicle: **receptor-mediated endocytosis**
  - Uptake of extracellular component into vesicle
  - Receptors found in “clathrin-coated pits”
  - LDL-receptor
  - Growth factor receptors



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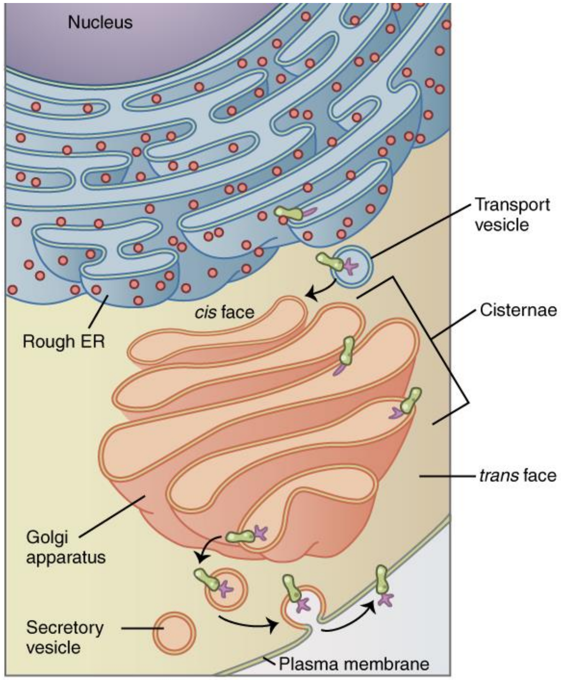
COPI and COPII Vesicles

- COPI: Golgi to ER (retrograde)
- COPII: ER to Golgi (anterograde)



OpenStax College

Cytoskeleton

Jason Ryan, MD, MPH

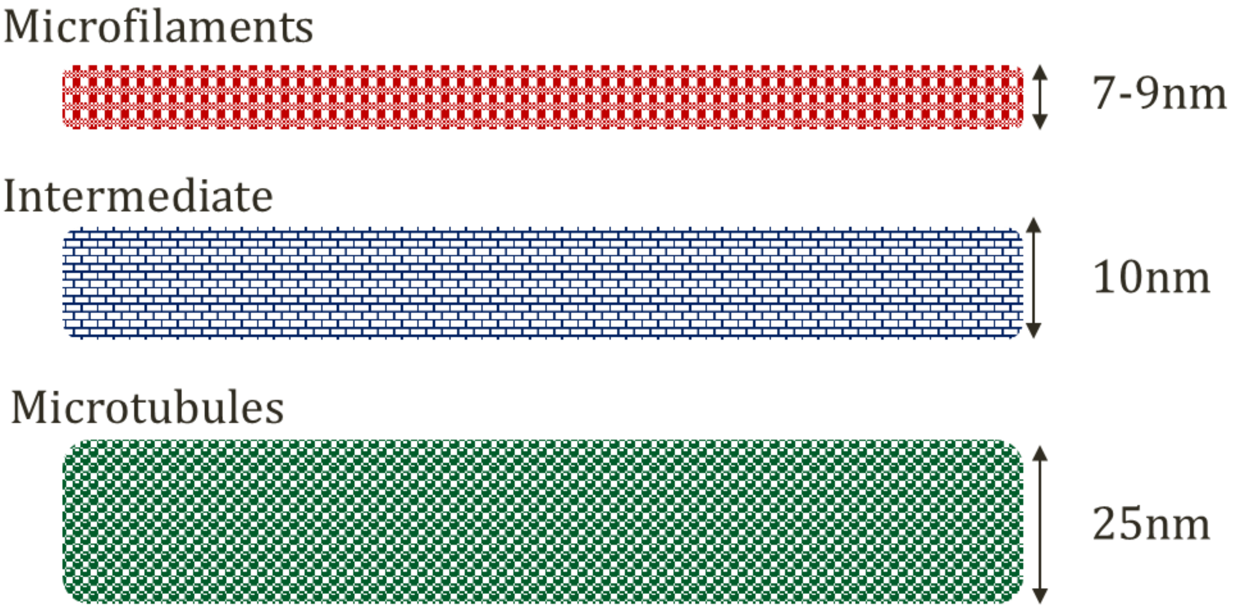
Boards&Beyond
STUDY SMARTER

Cytoskeleton

- System of filaments (Latin = thread)
- All constructed from smaller protein subunits
- Maintains shape of cells
- Moves intracellular traffic
- Pulls chromosomes apart in mitosis

Types of Filaments

- Microfilaments (actin filaments)
- Intermediate filaments
- Microtubules



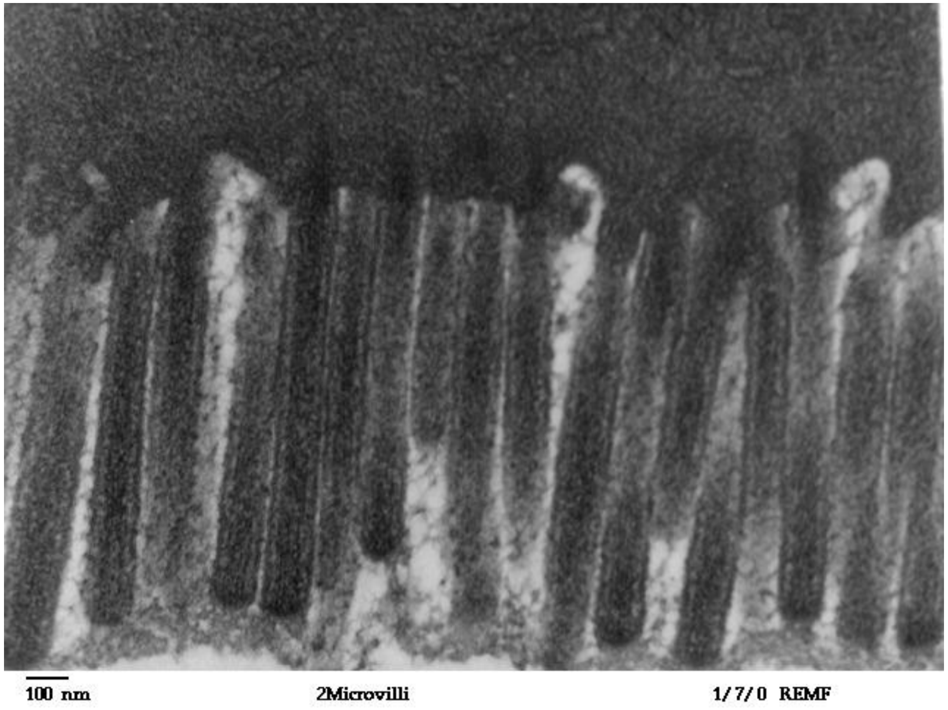
Microfilaments

Actin Filaments

- Polymers of protein **actin**
- Often found under cell membrane
- Many roles: cell shape, cell movement

Microvilli

- Extensions of **intestinal cell membranes**
- Formed from actin filaments



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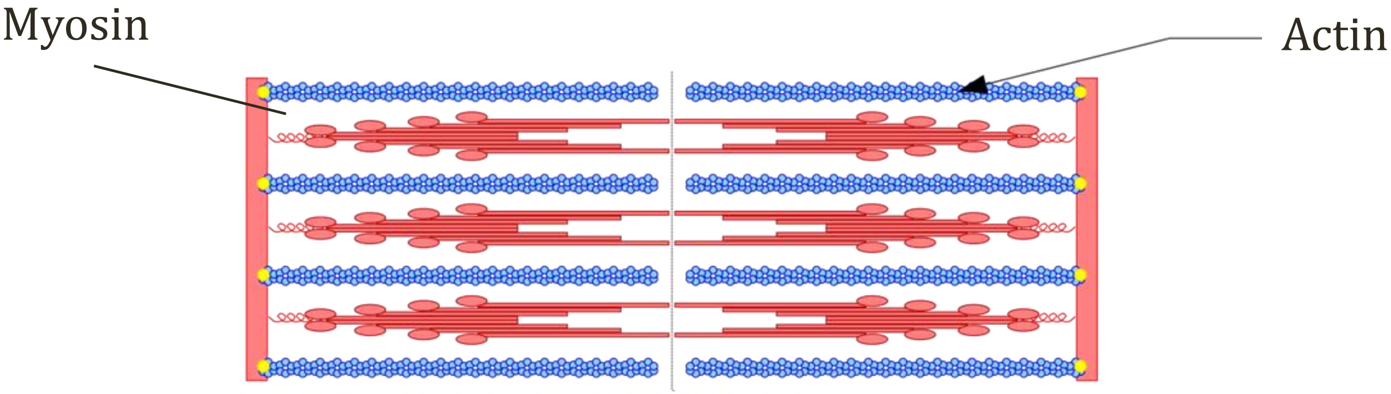
Muscle Fibers

- Basic unit: **Sarcomere**
- Overlapping thin and thick filaments
- Thin filaments: actin and associated proteins
- Thick filaments: myosin
- Myosin filaments slide past actin → contraction



Intermediate filaments

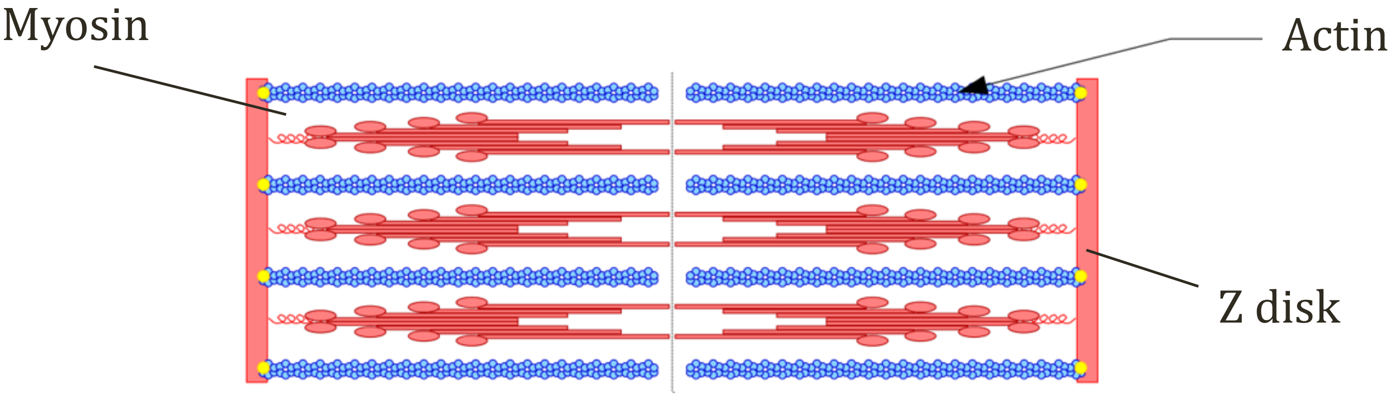
- Maintain cell shape/structure
- Many different types found in variety of cells
- Often used as tumor markers
- **Immunohistochemical staining**
    - Antibodies against intermediate filament proteins
    - Specific filaments associated with certain tumors
    - Various methods for detecting antibody binding
    - "Positive staining" suggests tumor origin/type

Vimentin

- Found in **mesenchymal** tissue
  - Cells/tissue derived from mesoderm in embryo
  - Mostly connective/soft tissue (i.e. not organs)
  - Fibroblasts
  - Skeletal muscle
  - Mesothelium lining of peritoneum, synovial joints
  - Endothelium
  - Adipocytes
  - Osteoblasts

Vimentin

- Z-disks in sarcomeres
  - Contain vimentin and desmin

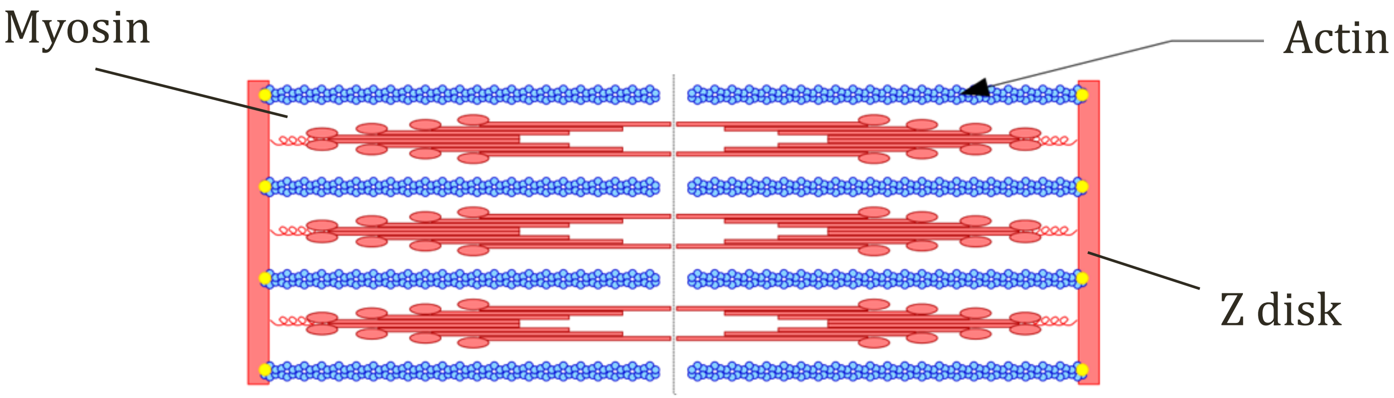


Vimentin

- **Sarcoma**
  - Tumor of mesenchymal origin
  - Positive for vimentin
  - Many subtypes
  - Liposarcoma (adipocytes)
  - Leiomyosarcoma (smooth muscle)
- Also found in other non-sarcoma tumors
  - Used to distinguish from other tumors
  - Renal cell carcinoma
  - Some CNS tumors (meningioma)
  - Endometrial carcinoma

Desmin

- **Muscle** filament
- Part of Z-disks in sarcomeres (vimentin and desmin)
- Marker for **muscle tumors**
- Rhabdomyosarcoma
- Leiomyoma and leiomyosarcoma



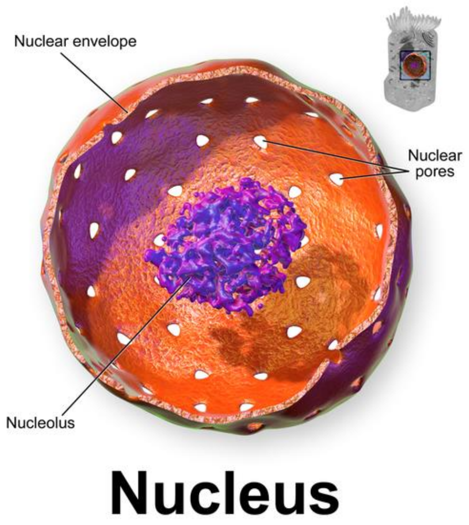
Keratin

Cytokeratin

- **Epithelial** cell filaments
- Found in cytoplasm (intracellular)
- Many subtypes (i.e. cytokeratin 8, 18, 19)
- Used to diagnose epithelial tumors (cytokeratin+)
- Useful in **squamous cell carcinoma**
  - Cervical cancer
  - Head and neck
  - Lung
  - Skin
  - Esophagus

Lamins

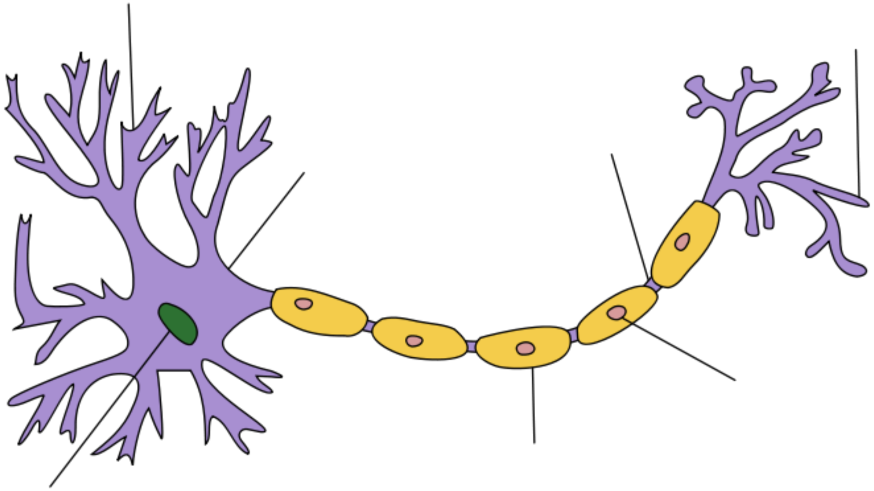
- Forms nuclear envelope
  - Separates nucleus from cytoplasm
  - Outer membrane, inner membrane, intermembrane space
  - "Nuclear lamins"
- Note: Laminin = extracellular proteins



BruceBlaus/Wikipedia

Neurofilaments

- Found in neurons (especially **axons**)
- Positive staining in many CNS tumors
  - Neuroblastoma
  - Medulloblastoma
  - Retinoblastoma

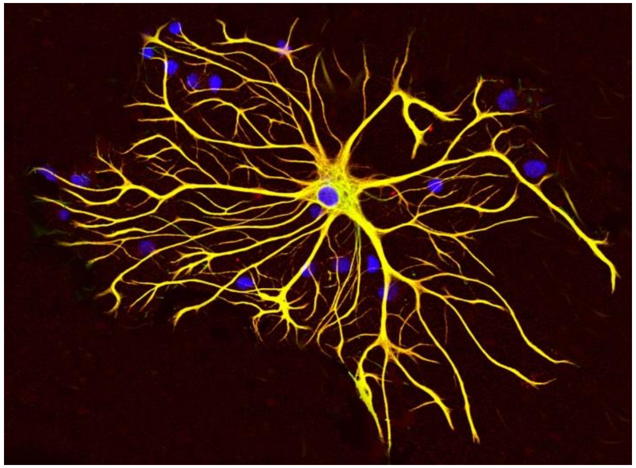


Quasar Jarosz/Wikipedia

GFAP

Glial fibrillary acidic protein

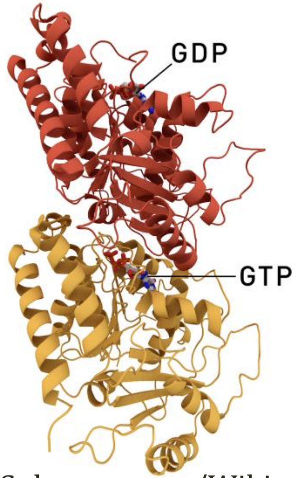
- Major intermediate filament for **astrocytes**
- Also found in some other CNS glial cells
- Seen in CNS tumors
  - Astrocytoma
  - Glioblastoma



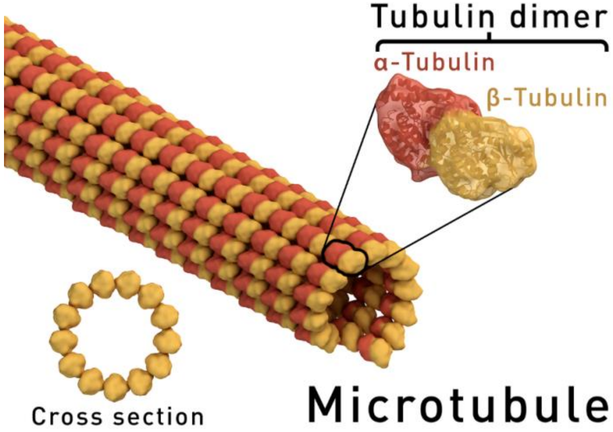
GerryShaw /Wikipedia

Microtubules

- Polymers of alpha and beta tubulin
- "Heterodimer" units: one alpha, one beta
- Polymerize into a long "protofilament"
- Each dimer has 2 GTP
  - Alpha GTP: part of structure
  - Beta GTP: can be hydrolyzed



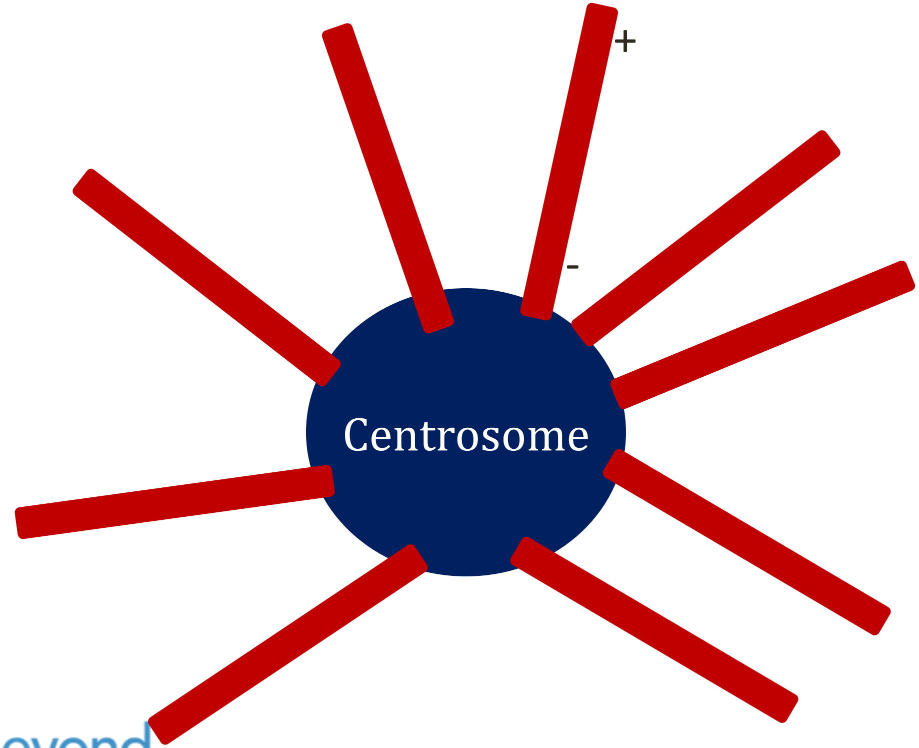
Thomas Splettstoesser/Wikipedia



Thomas Splettstoesser (www.scistyle.com)

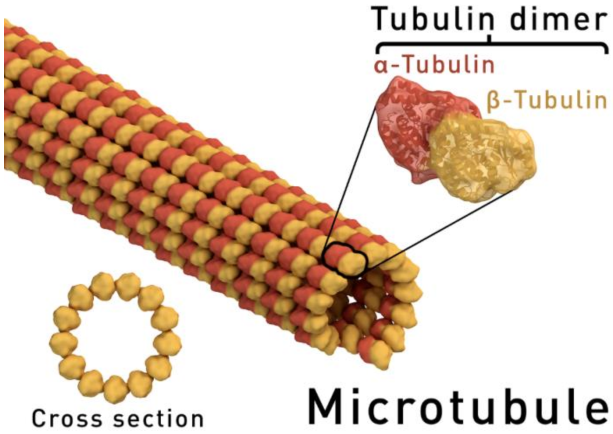
Microtubules

- Grow from a **centrosome** near nucleus
- Have a (-) and (+) end
- Emanate in a star pattern in cell



Dynamic Instability

- Microtubules **grow slowly**
- **Rapidly disassemble** (~100x faster)
- "Dynamic instability"



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Molecular Motor Proteins

- Bind and move along filaments
- Often carry "cargo"
  - Organelles (mitochondria)
  - Secretory vesicles

Dynein and Kinesin

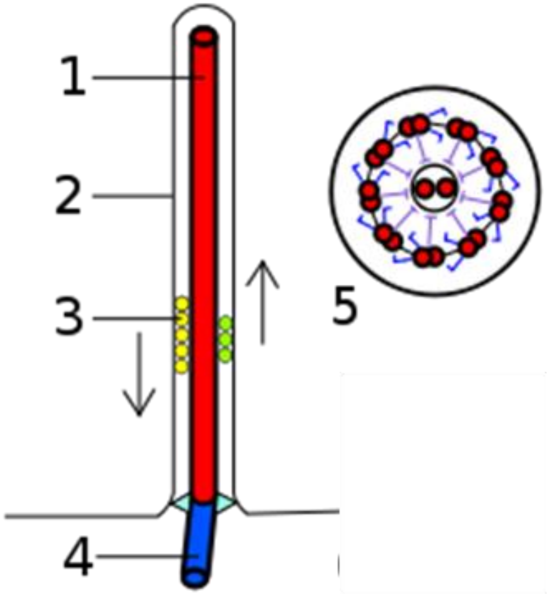
- Microtubule motor proteins
- **Kinesin moves toward (+) end**
  - Away from nucleus/cell body
  - Important for axonal transport (toward terminal)
- **Dynein moves toward (-) end**
  - Movement of vesicles
  - Localization of Golgi apparatus near cell center

Cilia and Flagella

- Motility structures
- Built from **microtubules and dynein**
- Cilia (shorter): Move mucus in respiratory tract
- Flagella (longer): Sperm motility

Cilia and Flagella

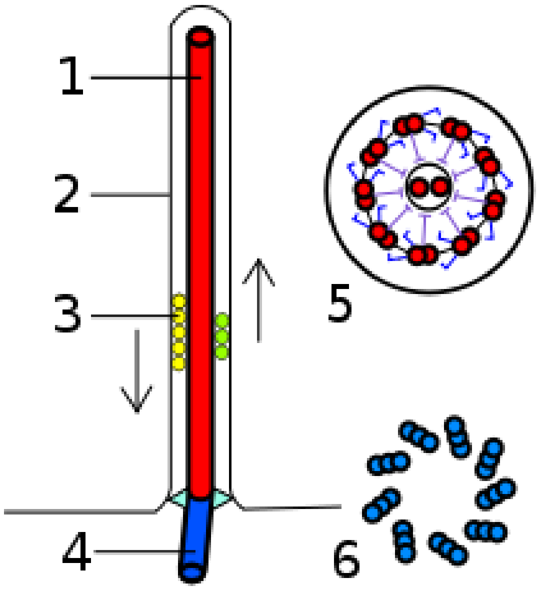
- Microtubules/proteins formed into an "axoneme"
- Structures arranged in special pattern ("9 x 2")
  - 9 **doublet** microtubules in ring
  - Surround a **pair** ("2") microtubules



Franciscosp2/Wikipedia

Cilia and Flagella

- Secured by “basal body” root in cell surface
- Nine groups of fused triplets of microtubules
- No central pair



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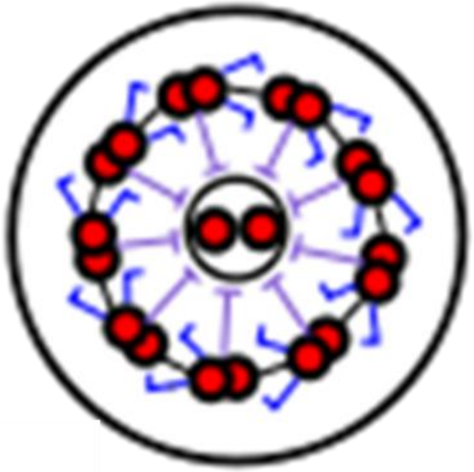
Cilia and Flagella

- Axonemal dynein: forms bridges between microtubules
- Activated dynein→ pulls on neighboring doublets
  - Requires ATP ("microtubule dependent ATPase")
- Sliding of doublets → bending of cilia/flagella



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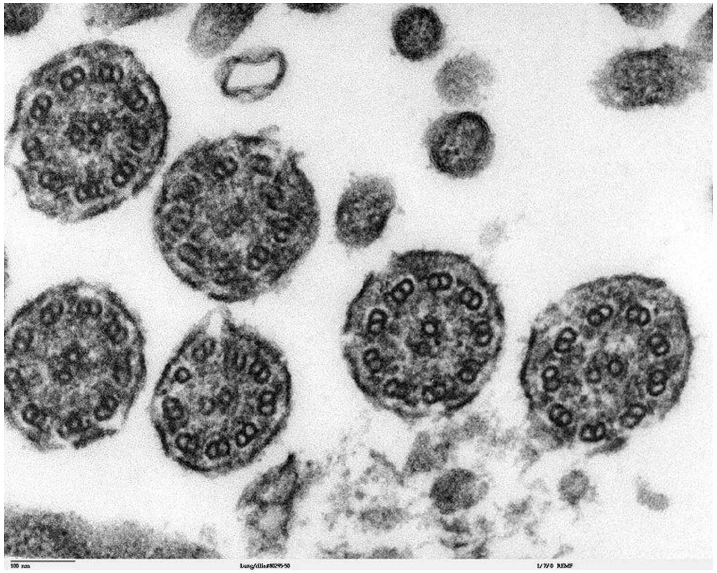
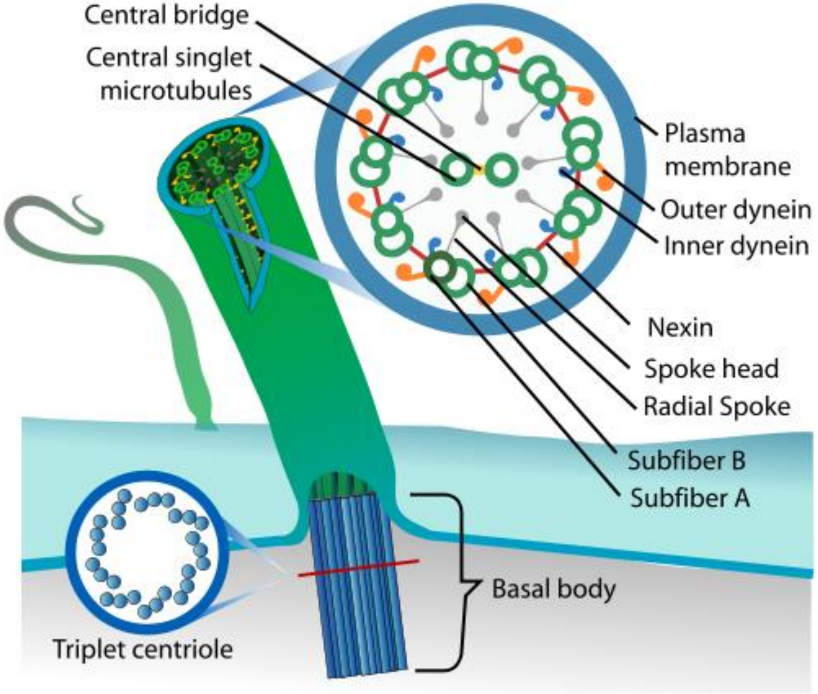
Cilia



Louisa Howard, Michael Binder



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Primary Ciliary Dyskinesia

Immotile-cilia syndrome

- Cilia unable to beat, beat normally, or absent
- Inherited (autosomal recessive)
- **Dynein** gene mutations

Primary Ciliary Dyskinesia

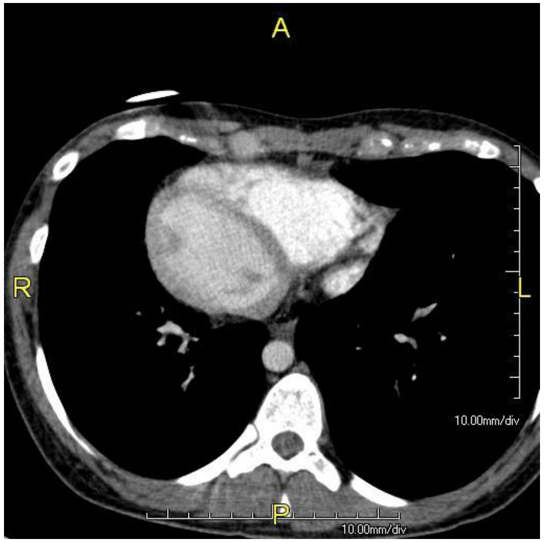
Clinical Features

- Rhinosinusitis
  - Lining of sinuses irritated, swollen
  - Excessive mucus production
- Infertility
  - Immotile sperm (sperm still viable)
  - Dysfunctional fallopian tube cilia (↑ risk ectopic)

Kartagener's syndrome

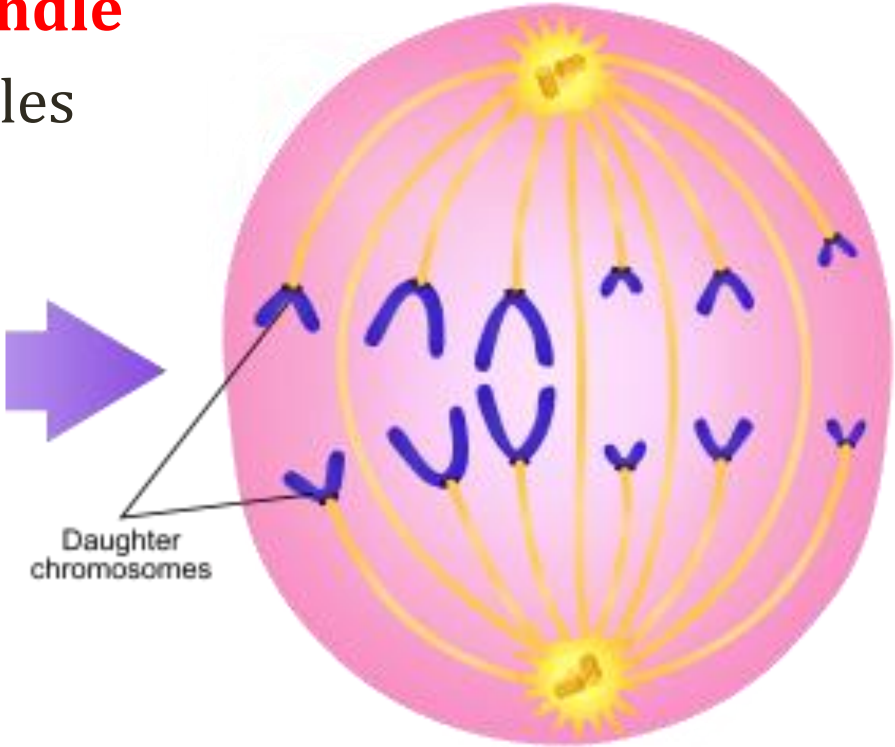
Manifestation of PCD

- Triad:
  - Chronic sinusitis
  - Bronchiectasis (chronic cough, recurrent infections)
  - Situs inversus



Mitosis

- Chromosomes separate
- Depends on **mitotic spindle**
- Composed of microtubules



Ali Zifan/Wikipedia

Microtubule Drugs

- Cancer drugs
  - Vincristine/Vinblastine (inhibit polymerization)
  - Paclitaxel (enhance polymerization – block breakdown)
- Colchicine (gout)
  - Prevent microtubule assembly
  - Disrupts chemotaxis, generation of cytokines, phagocytosis
- Griseofulvin (fungi)
- Mebendazole (helminths)

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Connective Tissue

Jason Ryan, MD, MPH

Connective Tissue

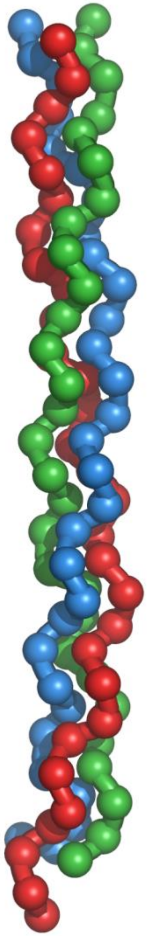
- Supports/connects organs and other structures
- Key components:
  - Collagen
  - Elastin
  - Fibrillin

Collagen

- Family of fibrous proteins
- Most abundant proteins in human body
- 25% of total protein mass
- Synthesized/secreted by connective tissue cells

Collagen

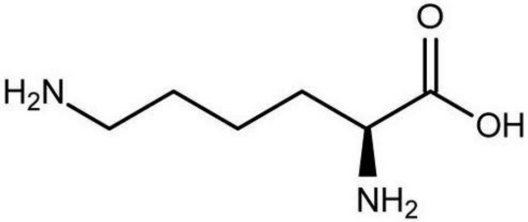
- Contains three long **α chains**
- Basic unit: “triple helix”
- 42 different genes for alpha chains
- Combinations → different collagen types



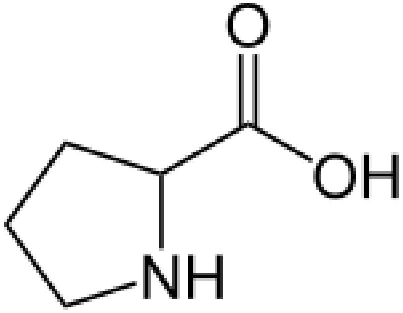
Vossman

Collagen

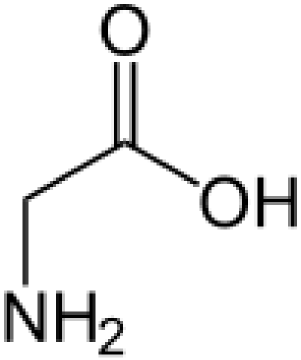
- Large amounts of **proline, lysine, and glycine**
- Repeating units: Gly-X-Y



Lysine



Proline



Glycine

Collagen Types

- Type I (most common – 90% of collagen)
  - **Bone**
  - Skin
  - Tendons, ligaments
  - Cornea
  - Internal organs
- Defective production: **Osteogenesis imperfecta**

Collagen Types

- Type II
  - Cartilage
  - Intervertebral discs
  - Vitreous humor (eye)
- Type III
  - Skin
  - Blood vessels
  - Abnormal in some forms of **Ehlers-Danlos syndrome**
- "Fibrillar collagens": Types I, II, and III
  - Collagen molecules assemble into polymers (fibrils)

Collagen Types

- Type IV
  - **Basement membranes**
  - Basal lamina (beneath epithelial layer)
  - Lens
  - Cochlea

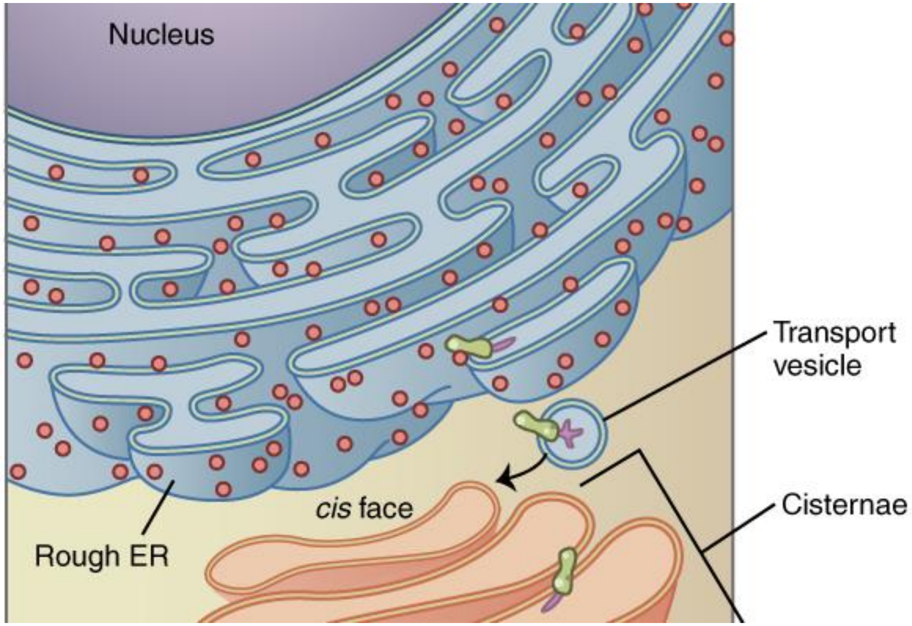
Alport Syndrome

Hereditary Nephritis

- Genetic **type IV collagen** defect
  - Mutations in **alpha-3, alpha-4, or alpha-5** chains
- Most commonly X-linked
- Classic triad:
  - Hematuria
  - Hearing loss
  - Ocular disturbances

Collagen Synthesis

- Extensive **post-translational modification**
- Alpha chains synthesized in rough ER
  - Contain signal molecules
  - “Pre-procollagen”
- Enter ER lumen
  - **Pro-alpha chains**



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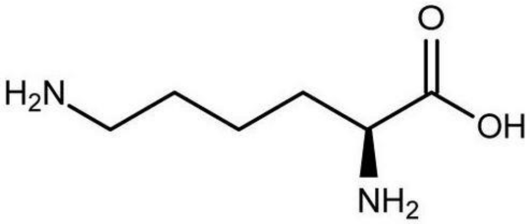
Collagen Synthesis

Endoplasmic Reticulum Modifications

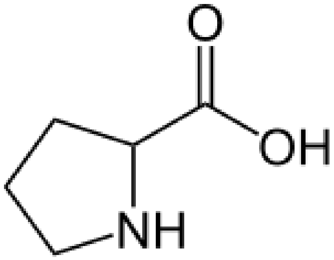
- Some prolines and lysines are **hydroxylated**
  - Form “hydroxyproline” and “hydroxylysine”
  - Requires **vitamin C** (cofactor for hydroxylase enzymes)
  - Deficiency of vitamin C → scurvy
- Some hydroxylysines are **glycosylated**
  - Sugar molecules added

Collagen Synthesis

Endoplasmic Reticulum Modifications



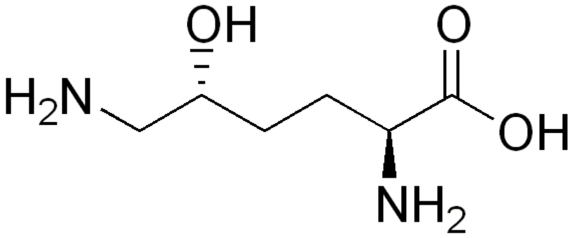
Lysine



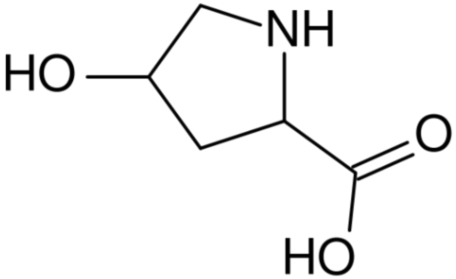
Proline

Hydroxylation

(Vitamin C)



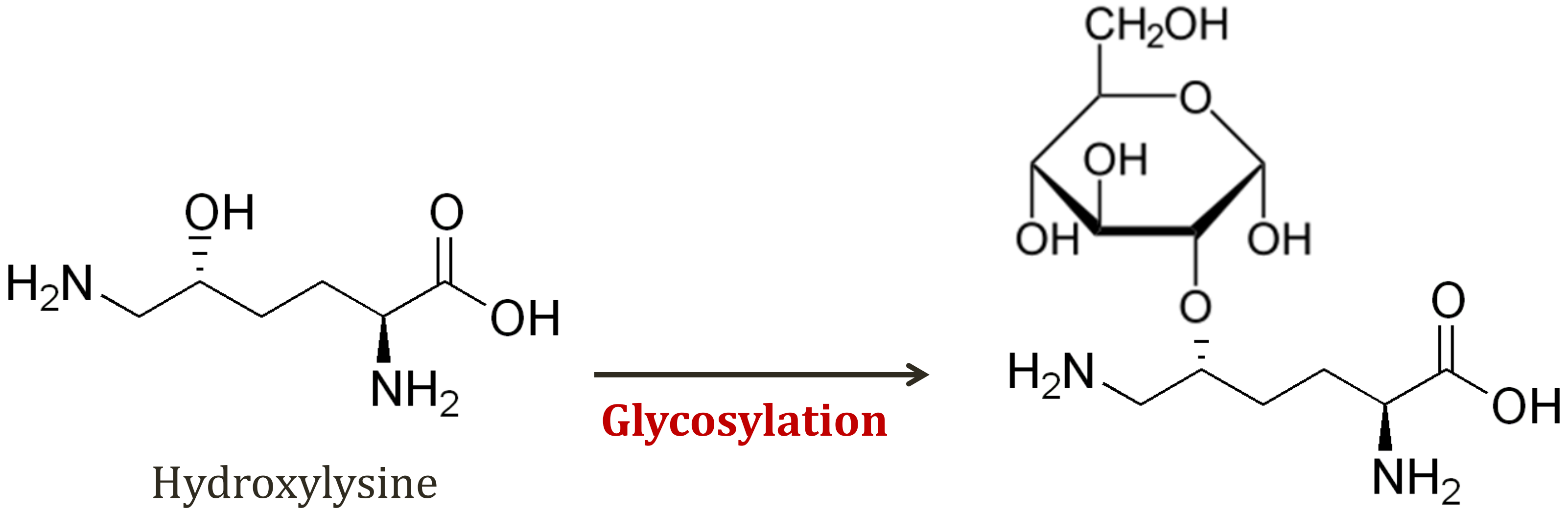
Hydroxylysine



Hydroxyproline

Collagen Synthesis

Endoplasmic Reticulum Modifications



Glycosylation

Hydroxylysine

Scurvy

- **Vitamin C deficiency**
- Defective pro-alpha chains
- Do not form triple helix
- Degraded in cell (not secreted)
- Fragile blood vessels (bleeding/bruising)
- Loss of teeth
- Loss of wound healing

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Collagen Synthesis

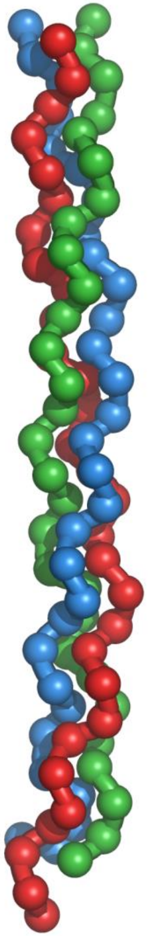
Endoplasmic Reticulum

- **Propeptides**
  - Extra amino acids at N and C ends of pro-alpha chains
  - Form in fibrillar collagen alpha chains (Type I, II, III)
  - Form **disulfide bonds** that stabilize alpha chains
- Three pro-alpha chains combine: **procollagen**
  - Triple helix formation

Collagen Synthesis

Extracellular Modifications

- Moves through Golgi
- Procollagen excreted by **exocytosis**
- Propeptides (N and C terminal) cleaved
- **Tropocollagen** formed
  - Individual triple helix alpha chain molecules
  - No propeptides (removed)
  - Not yet crosslinked

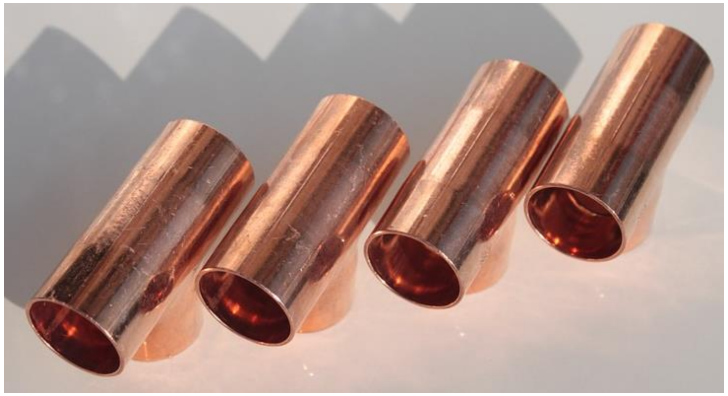


Vossman

Collagen Synthesis

Extracellular Modifications

- **Collagen fibrils** form
  - Tropocollagen much less soluble than procollagen
  - Fibrils self assemble
  - Strengthened by **lysine crosslinking**
  - Extracellular enzyme: **lysyl oxidase**
  - Requires **copper** as cofactor
- Collagen **fibers**: bundles of triple helices



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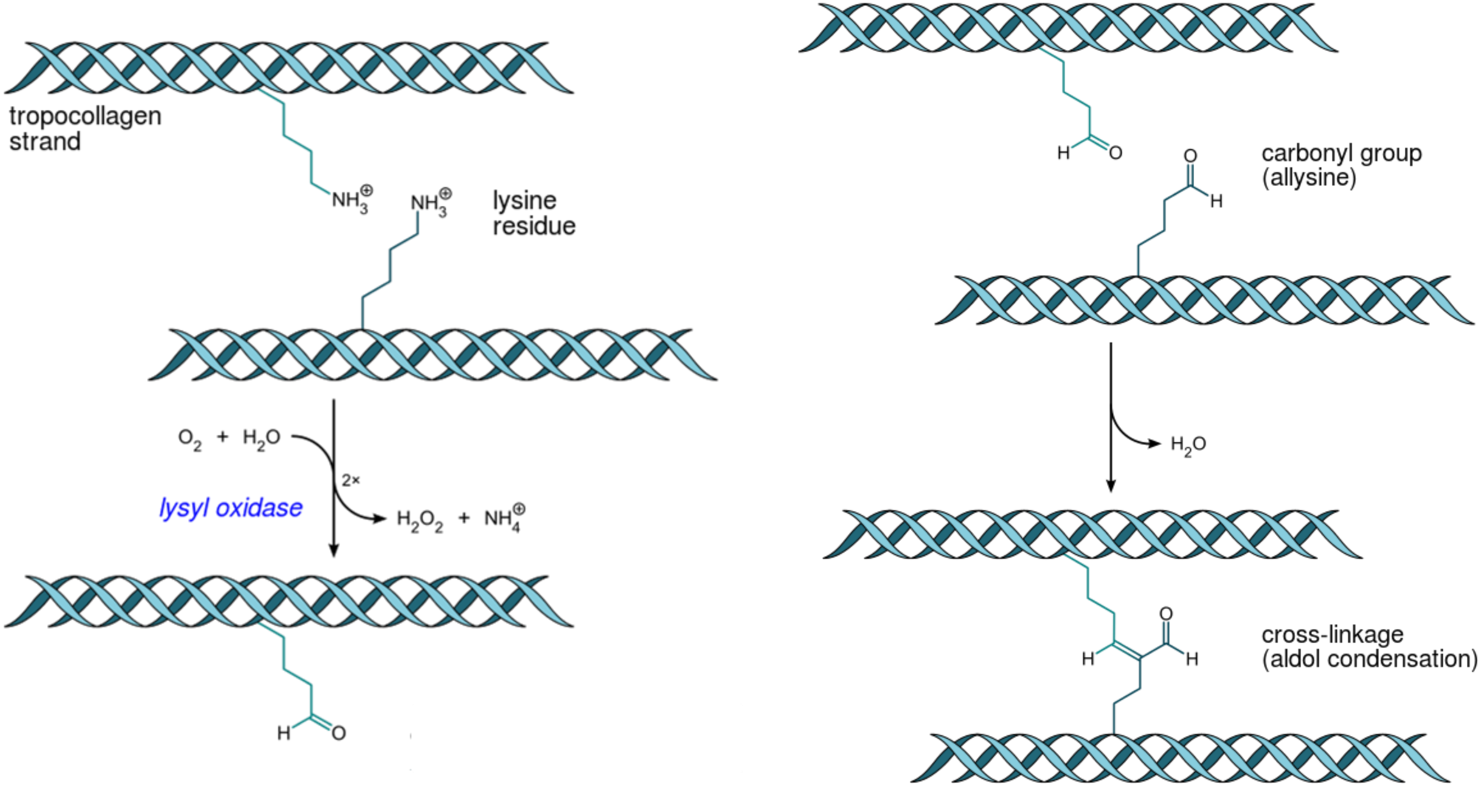
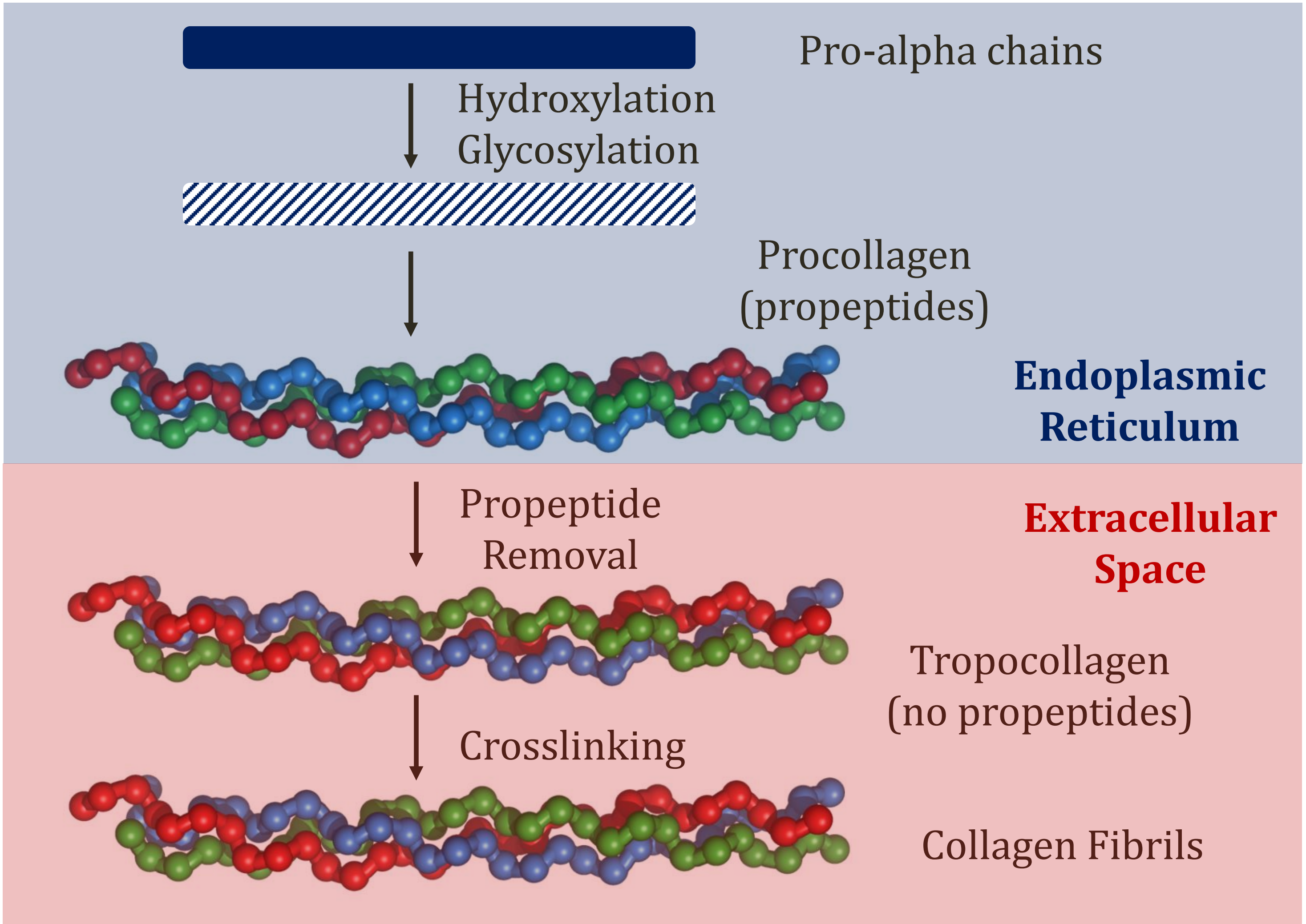
Crosslinking



Collagen Synthesis

Summary



Pro-alpha chains

Hydroxylation
Glycosylation

Procollagen
(propeptides)

Endoplasmic Reticulum

Propeptide
Removal

Extracellular Space

Tropocollagen
(no propeptides)

Crosslinking

Collagen Fibrils

Aging Wrinkles

- ↓ production of elastin and collagen in dermis
- Also collagen/elastin fibers thicken and clump

Wikipedia/Public Domain

Scleroderma

Systemic Sclerosis

- Autoimmune disorder
- Stiff, hardened tissue (sclerosis)
- Skin, other organ systems involved
- Caused by **fibroblast** activation
- Excess **collagen** deposition

Osteogenesis Imperfecta

“Brittle bone disease”

- Family of genetic bone disorders
- Range of severity (some forms lethal in utero)
- All involve **osteoporosis and fractures**
- Defective/deficient **collagen** production

Xiong/Wikipedia

Osteogenesis Imperfecta

“Brittle bone disease”

- Type I: most common form
- Autosomal dominant
- Mutation in **COL1A1 or COL1A2 genes**
  - Encode alpha chains for type I collagen
  - Abnormal/absent alpha chains
  - Triple helix not formed normally
- Decreased production of **type I collagen**

Osteogenesis Imperfecta

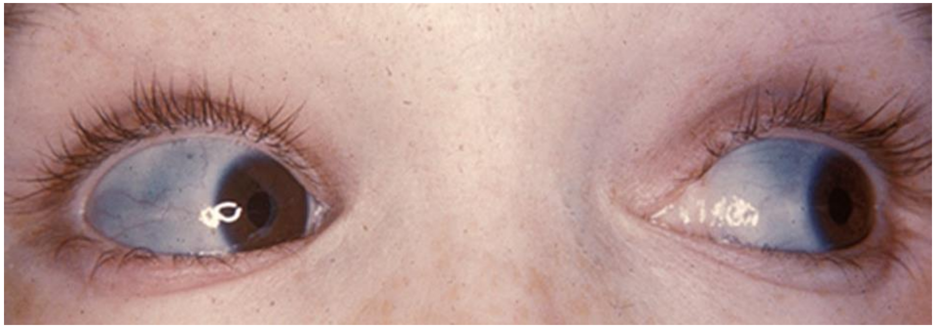
“Brittle bone disease”

- Type II
  - Lethal in utero
- Type III and IV
  - More severe than type I
- Severity: II, III, IV, I

Osteogenesis Imperfecta

Clinical Features

- Multiple, recurrent **fractures** with minimal trauma
  - May be confused with **child abuse**
- **Blue sclera**
  - Clear connective tissue over veins
- Hearing loss
  - Abnormal malleus, incus, and stapes (ossicles)



Herbert L. Fred, MD and Hendrik A. van Dijk

Osteogenesis Imperfecta

Other Features

- **Dentinogenesis imperfecta**
  - Rarely seen in type I
  - Common in types III, IV
  - Discolored teeth (blue-gray or yellow-brown color)
  - Teeth translucent or shiny
  - Weak teeth, easily fall out or break
- Bony deformity
- Short stature

BMC Med Genet. 2007 Aug 8;8:52

Ehlers Danlos Syndrome

- Family of genetic connective tissue disorders
  - Range of severity
  - Range of inheritance patterns
- All caused by **defective collagen synthesis**
- Predominantly affects joints and skin

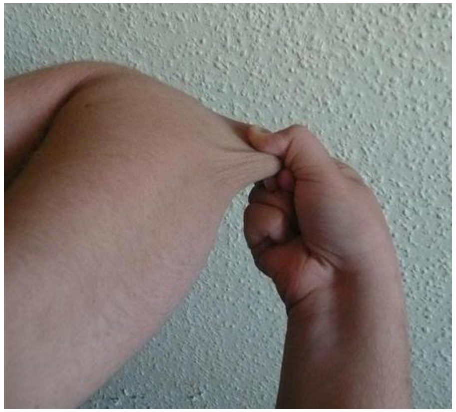
Ehlers Danlos Syndrome

- Classic type
  - Autosomal dominant (often de novo mutation)
  - COL5A1 or COL5A2 genes (type V collagen)
  - Type V interacts with other collagens
- Vascular type
  - Autosomal dominant
  - COL3A1 gene (type III collagen)
  - Skin, blood vessels

Ehlers Danlos Syndrome

Classic Type (type V collagen)

- Joint hypermobility
- Hyperextensible skin (“velvety” skin)
- Easy bruising
- Thin, wide scars ("cigarette paper" scars)
- Mitral valve prolapse
- Same features in many subtypes (varying degrees)

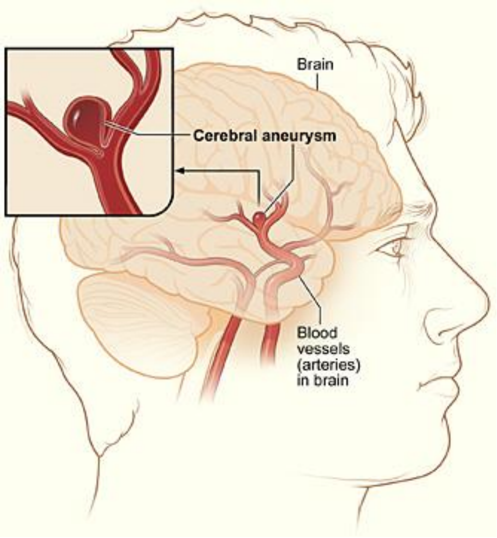


Piotr Dołżonek/Wikipedia

Ehlers Danlos Syndrome

Vascular Type (type III collagen)

- Thin skin, easy bruising
- Rupture of large arteries
  - CNS ("berry") aneurysms
- Rupture of "hollow" organs
  - Intestinal perforation
  - Uterus during pregnancy
- **Life-threatening** form of EDS
  - 80% have vascular event or rupture by 40 years old
  - Median age of death: 48 years old



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Menkes Disease

- X-linked recessive disorder
- Mutations in the ATP7A gene
  - ATPase involved in intestinal copper uptake/transport
- Impaired **copper** absorption → deficiency
  - Contrast with Wilson's disease (copper excess)
  - Wilson's ATP7B gene
- ↓ lysyl oxidase activity

Menkes Disease

- Classic features: **Sparse, brittle ("kinky") hair**
- Low body temperature
- CNS features
  - Hypotonia
  - Seizures
- Poor growth
- Developmental delay
- Osteoporosis/fractures
- Usually fatal in childhood

Datta AK, Ghosh T, Nayak K, Ghosh M.

Elastin

- Connective tissue protein
- Main component of **elastic fibers**
  - Allows **stretching/recoil**

Elastin

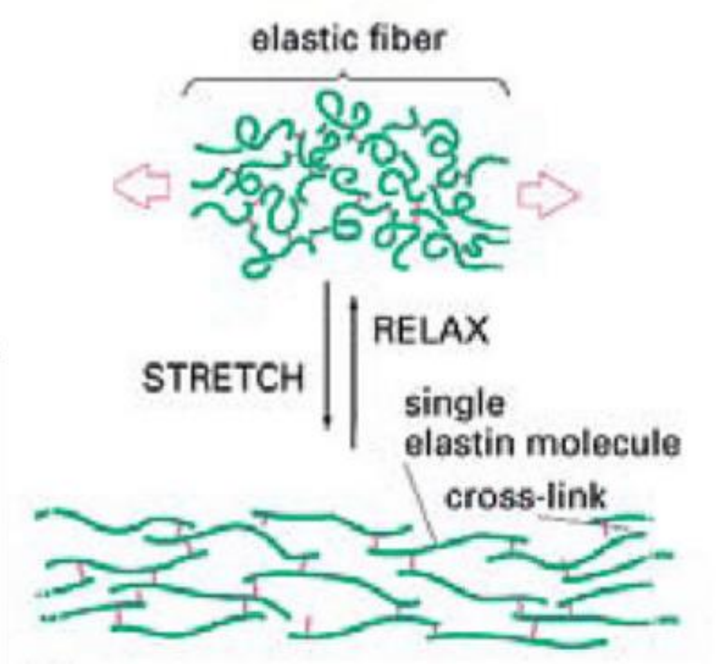
- Arteries
  - Dominant elastic protein
  - Makes up 50% of aortic tissue
- Skin
- Lungs
- Ligaments
- Vocal cords
- Spinal ligaments (ligamenta flava)

Elastin

- Contains glycine, lysine, and proline (like collagen)
- Mostly non-hydroxylated amino acids
  - No hydroxylysine
  - Some hydroxyproline (less than collagen)
- Not glycosylated

Elastin

- Secreted as tropoelastin
- Assembled into elastin fibers with crosslinking



sportEX journals/Flikr

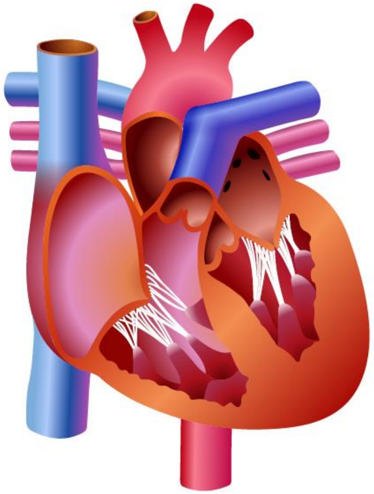
α1 Anti-trypsin Deficiency

- Inherited (autosomal co-dominant)
- Decreased or dysfunctional AAT
  - Inhibitor of enzyme **elastase**
- Excessive breakdown of elastin
- Result: **Emphysema**
  - Lung damage
  - Imbalance between neutrophil elastase (destroys elastin) and elastase inhibitor AAT (protects elastin)

Williams Syndrome

Williams-Beuren syndrome

- **Partial deletion** on long arm of chromosome 7
- Deleted portion includes gene for **elastin**
- Elfin appearance, intellectual disability
- **Supravalvular** aortic stenosis
  - Constriction of ascending aorta above aortic valve
  - High prevalance among children with WS
  - Histology: Loss of elastin



Fibrillin

- Glycoprotein
- Major component of **microfibrils**
- Sheath that surrounds elastin core
- Elastic fibers: Elastin, microfibrils, other molecules
- Abundant in the aorta
- Deficient fibrillin: **Marfan syndrome**

Marfan Syndrome

- Genetic connective tissue disorder
- Abnormal **fibrillin**
- Mutations in **FBN1 gene** (chromosome 15)
  - Codes for fibrillin-1
- Affects bones, joints, heart, eyes

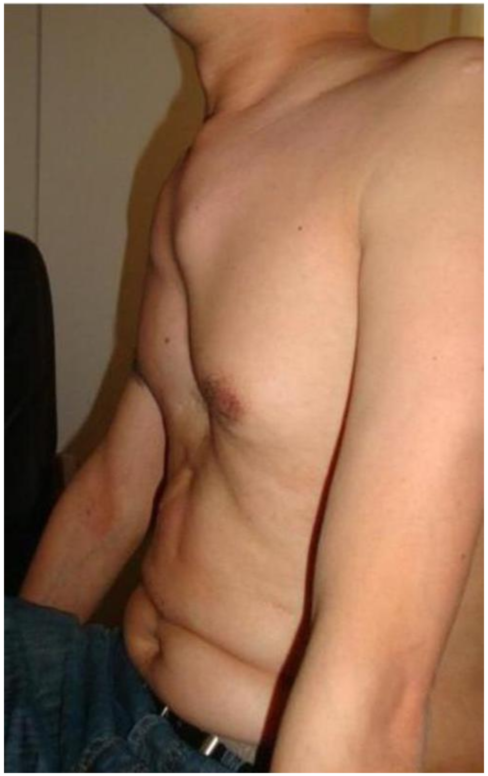
Marfan Syndrome

- Classic appearance: **Tall with long wingspan**

Wikipedia/Public Domain

Marfan Syndrome

- Classic finding: **Pectus Excavatum** (sunken chest)



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Marfan Syndrome

- **Extremities**:
  - Hypermobile joints
  - Long fingers and toes
  - "Arachnodactyly": Long, curved finger (like a spider)
- Wrist sign:
  - Tip of thumb covers entire fingernail of fifth finger
- Thumb sign:
  - Thumb protrudes beyond ulnar border

Marfan Syndrome

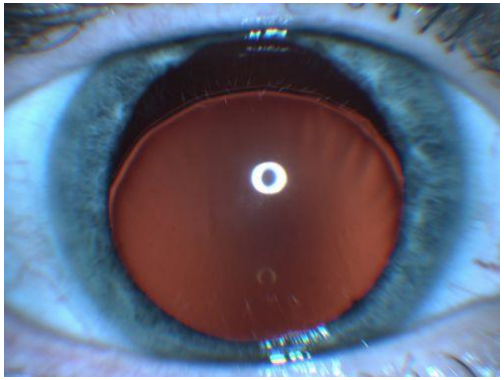
Pixabay/Public Domain

Wikipedia/Public Domain

Marfan Syndrome

Eye

- **Cataracts** at early age ("pre-senile")
- **Dislocation** of lens
  - Commonly due to trauma
  - Can be associated with systemic condition
  - Marfan most common
  - Classically upward/outward lens dislocation

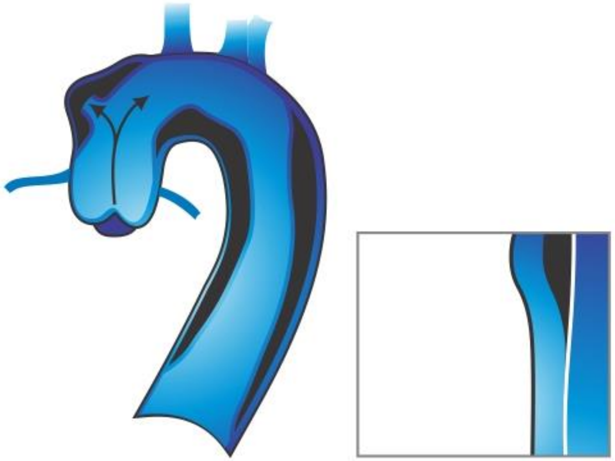


Retina Gallery

Marfan Syndrome

Cardiovascular

- Mitral valve prolapse
- Thoracic **aortic aneurysms and dissection**
  - Cystic medial necrosis
  - Cysts and necrosis in medial layer



Marfanoid Habitus

- Tall with long wingspan
- Long fingers
- Seen in some rare systemic disease
  - Homocystinuria
  - MEN 2B
  - Rare forms of Ehlers Danlos