به نام خدا

Applications of Genetics to Cardiovascular Medicine

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Farzane Vafaeie

Structure of DNA

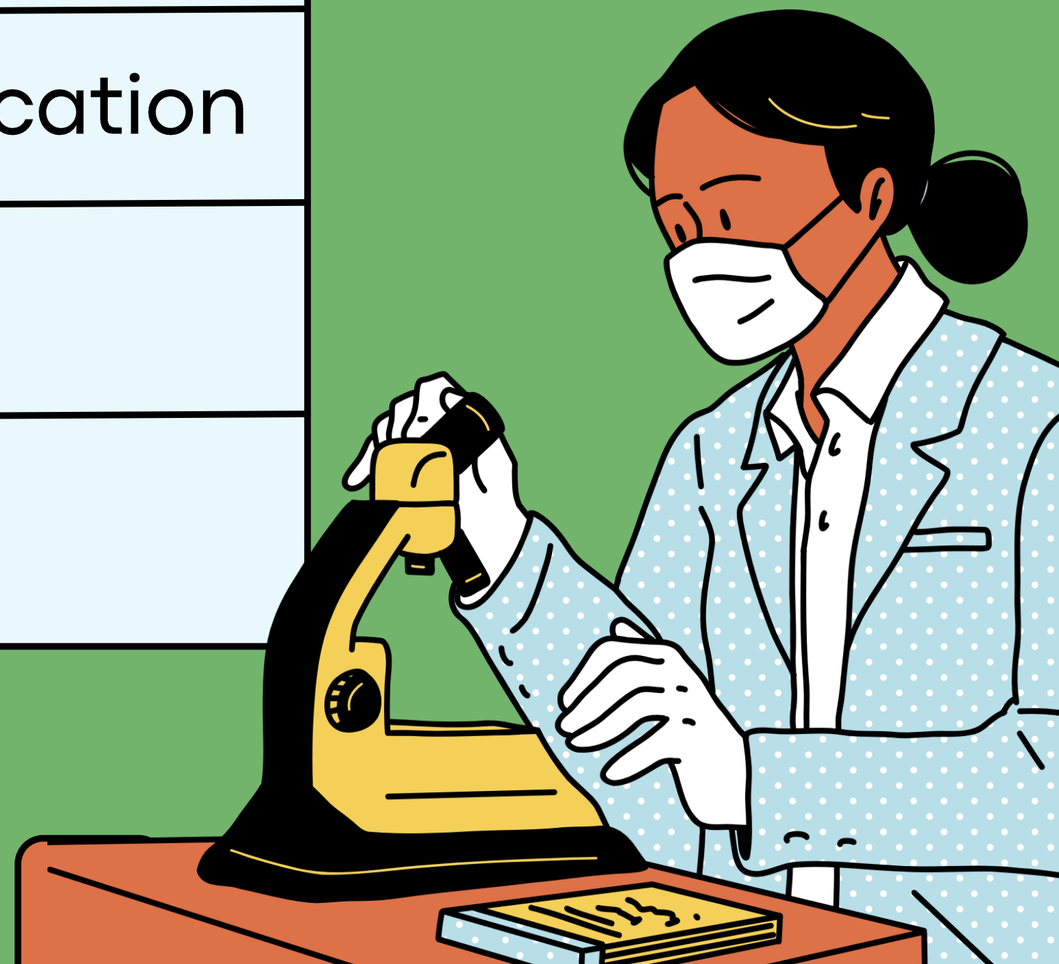
An Introduction to the Function and Characteristics of DNA

Lesson Outline

Composition, Structure, Replication

Chromosome Structure

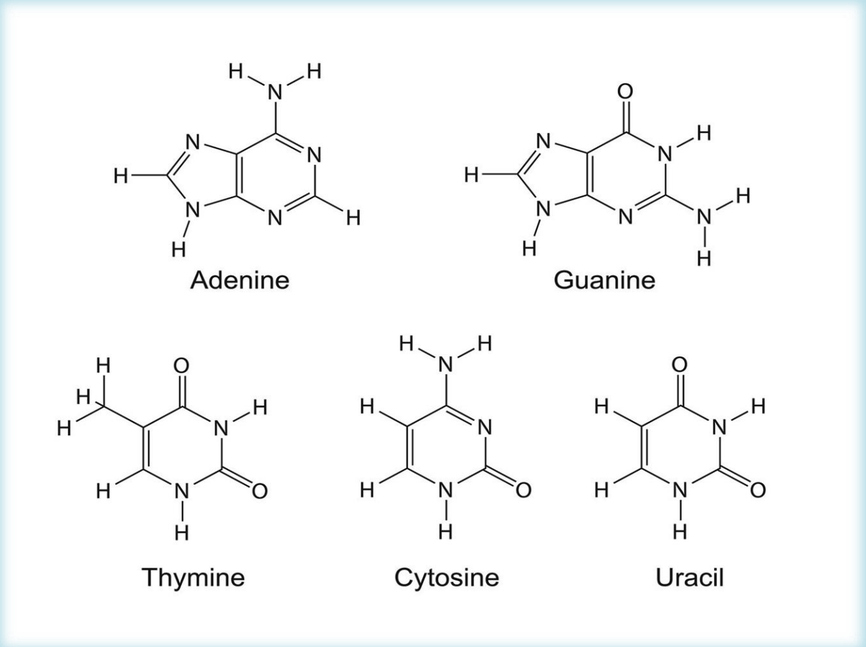
Types of DNA Sequence

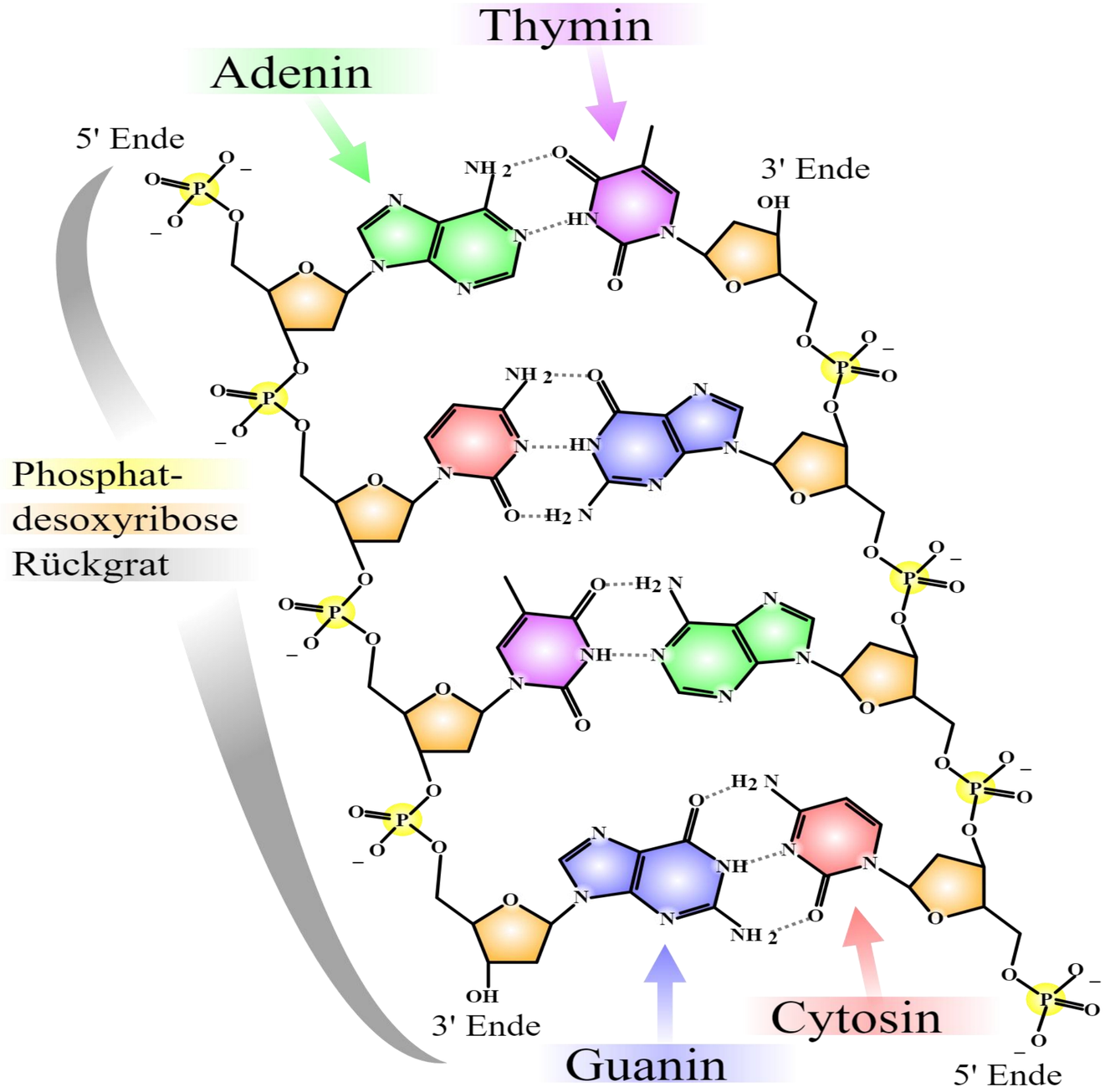


Composition

Each nucleotide is composed of a **nitrogenous base**, a **sugar molecule**, and a **phosphate molecule**.

The nitrogenous bases fall into two types, **purines** and **pyrimidines**. The purines include adenine (A) and guanine (G); the pyrimidines include cytosine (C), thymine (T), and uracil (U).



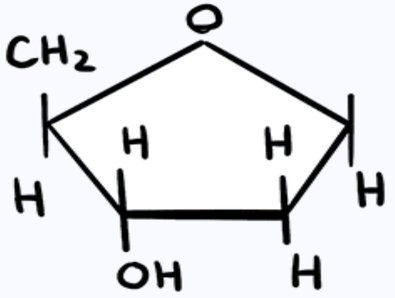

Thymin
Adenin
5' Ende
3' Ende
Phosphat-
desoxyribose
Rückgrat
3' Ende
Cytosin
Guanin
5' Ende

DNA vs. RNA

DEOXYRIBONUCLEIC ACID

DOUBLE-STRANDED
SUGAR* PHOSPHATE

* DEOXYRIBOSE



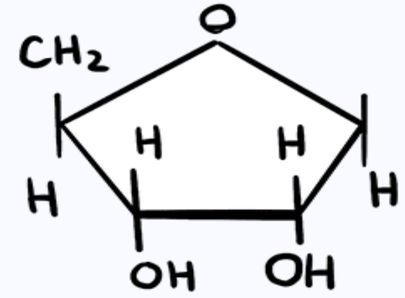
BASE PAIR

RIBONUCLEIC ACID

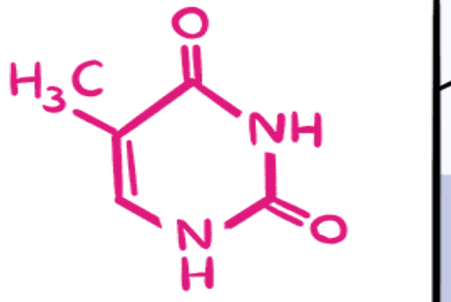
USUALLY SINGLE-STRANDED
SUGAR* PHOSPHATE

* RIBOSE

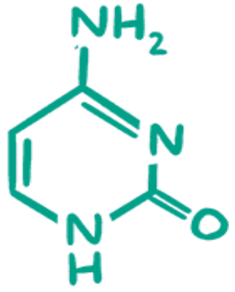
SINGLE
NUCLEOBASE



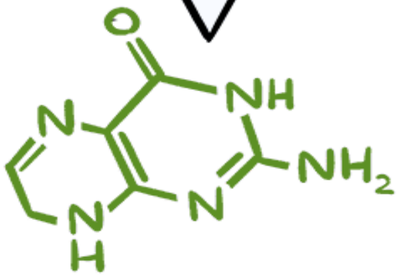
NUCLEOBASES



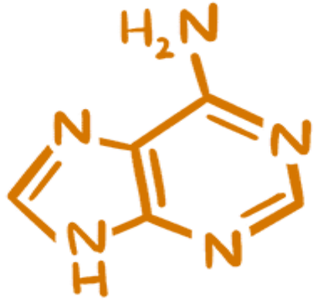
THYMINE



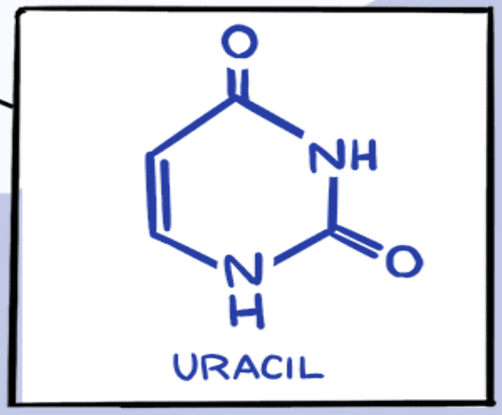
CYTOSINE



GUANINE



ADENINE



URACIL

ThoughtCo.

Structure

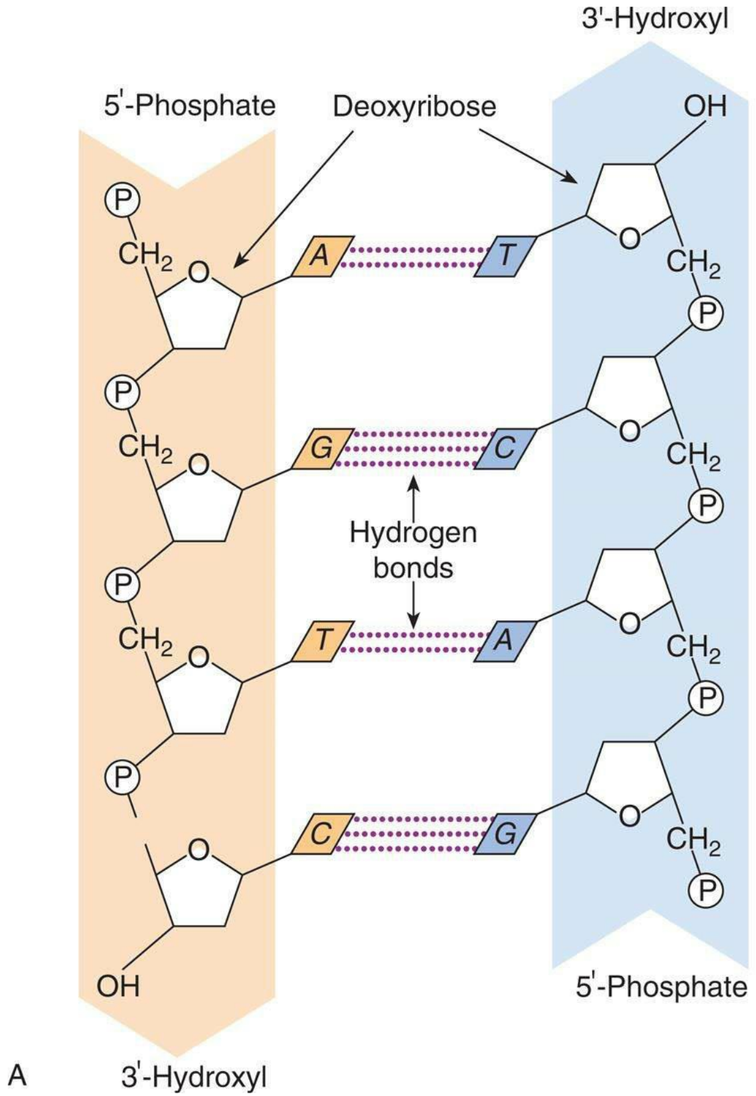
composed of two chains of nucleotides arranged in a double helix

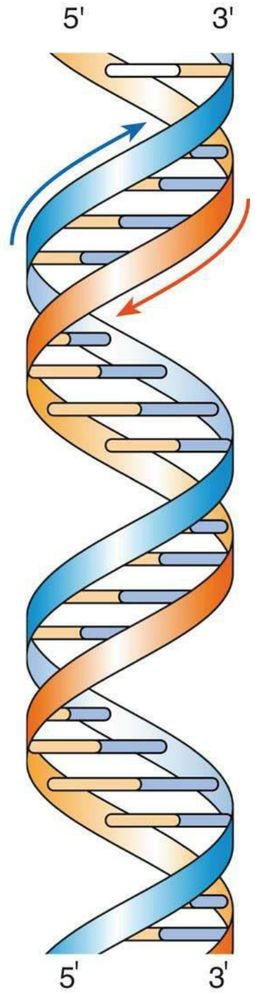
Phosphodiester bonds between the 3′ and 5′ carbons of adjacent sugars

two chains being held together by hydrogen bonds between the nitrogenous bases

they have
opposite orientations and are said to be antiparallel

A purine in one chain always pairs with a pyrimidine in the other chain





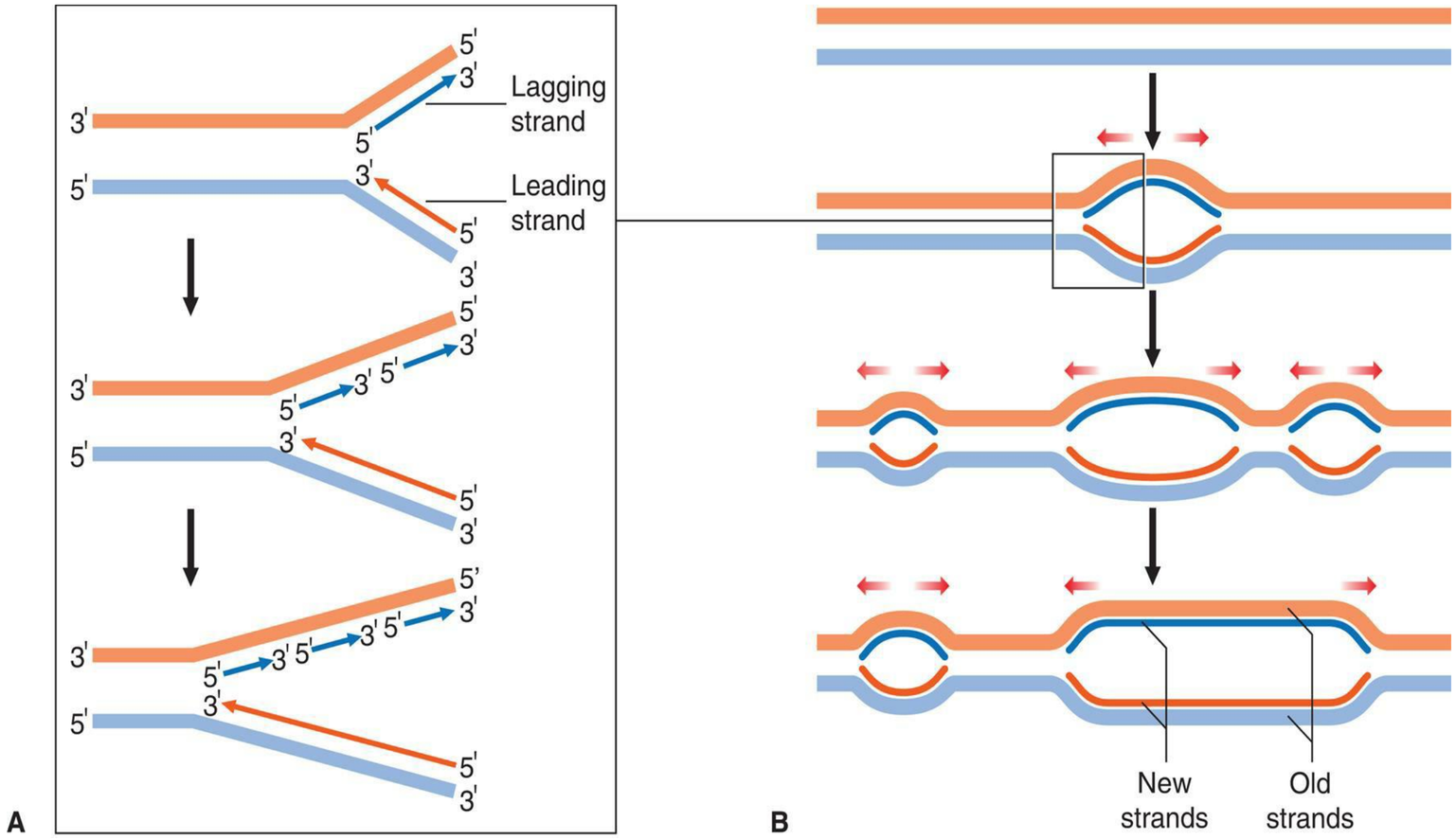
Replication

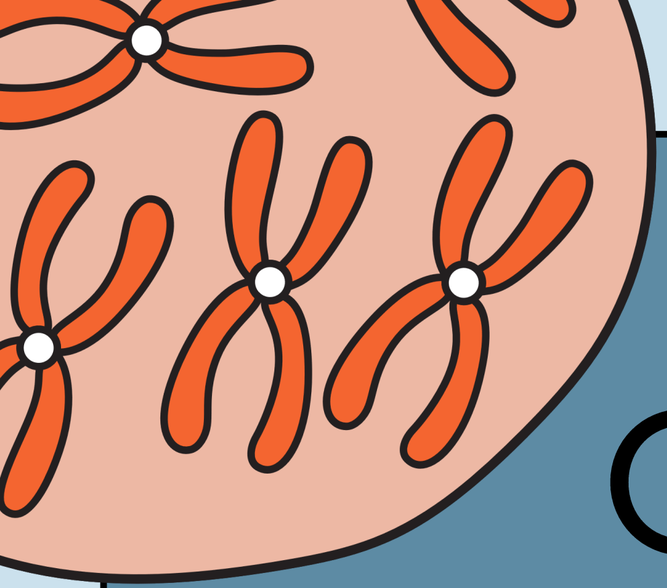
semiconservative

DNA synthesis occurs in the 5′ to 3′ direction.

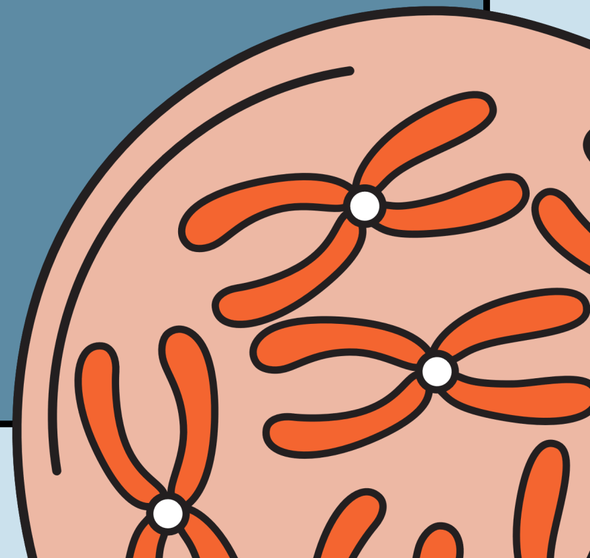
Okazaki fragments

DNA replication takes in the S phase of the cell cycle

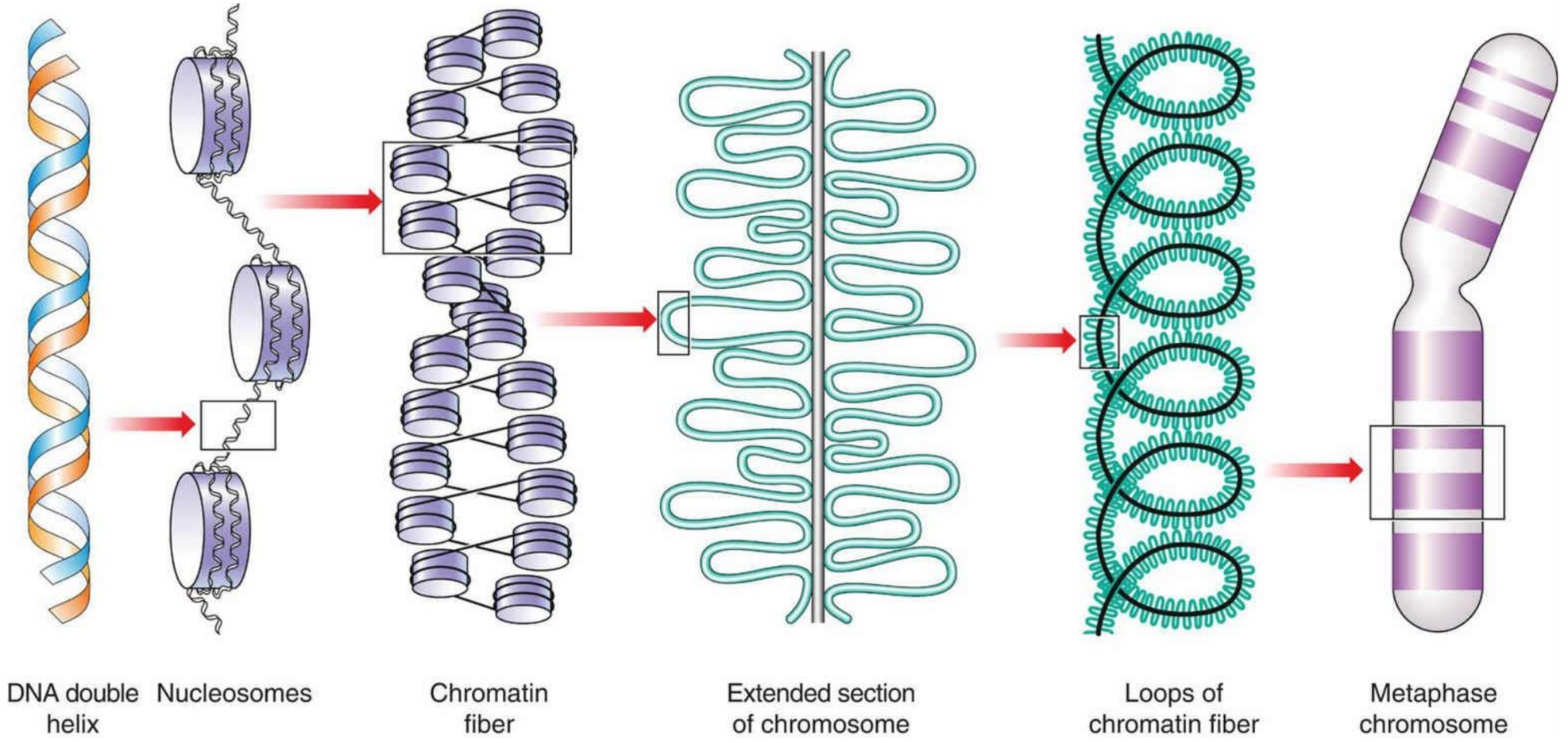




Chromosome structure



The packaging of DNA into chromosomes involves several orders of DNA coiling and folding.



Types of DNA Sequence

- 60% to 70% of the human genome consists of single- or low-copy number DNA sequences.

- The remainder of the genome, 30% to 40%, consists of either moderately or ***highly repetitive*** DNA sequences that are not transcribed.

- This latter portion consists of mainly satellite DNA and interspersed DNA sequences

Box 2.1

Types of DNA Sequences

Nuclear (~3×10^9 base pairs)

Genes (~20,000)
Unique, single-copy
Multigene families
Classic gene families
Gene superfamilies

Extragenic DNA (unique/low copy number or moderate/highly repetitive)

Tandem repeat
Satellite
Minisatellite
Telomeric
Hypervariable
Microsatellite
Interspersed
Short interspersed nuclear elements
Long interspersed nuclear elements

Mitochondrial (16.6 kilobases, 37 genes)

Two ribosomal RNA genes
22 transfer RNA genes

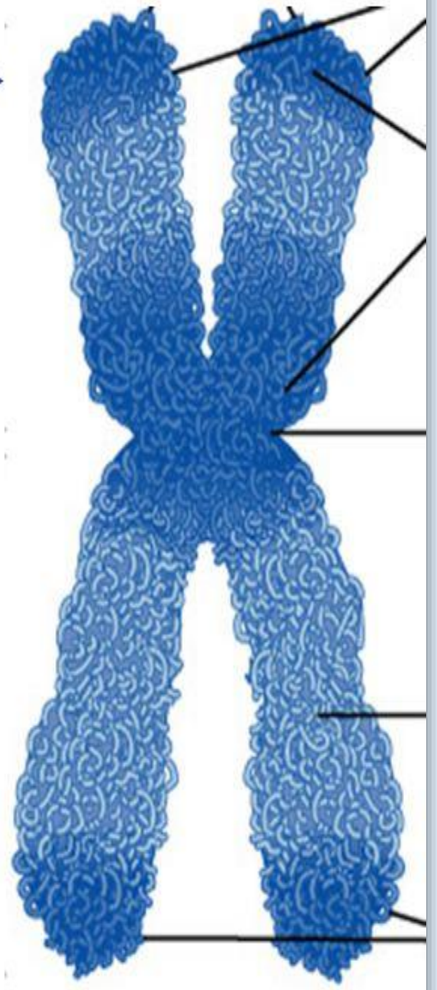
It is estimated that there are around 21,000 **protein-coding genes** in the nuclear genome.

– The distribution of these genes varies greatly between chromosomal regions.

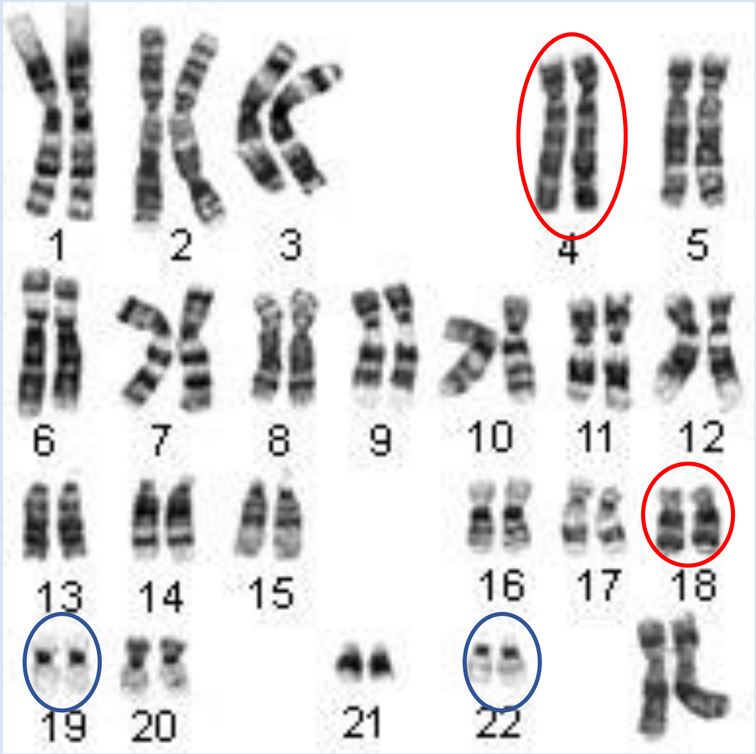
-Heterochromatic and centromeric regions are mostly **non-coding**, with the highest gene density observed in **sub telomeric** regions.

Chromosome Parts:

- Heterochromatin:
  - More condensed
  - Silenced genes (methylated)
  - Gene poor (high AT content)
  - Stains darker
- Euchromatin:
  - Less condensed
  - Gene expressing
  - Gene rich (higher GC content)
  - Stains lighter

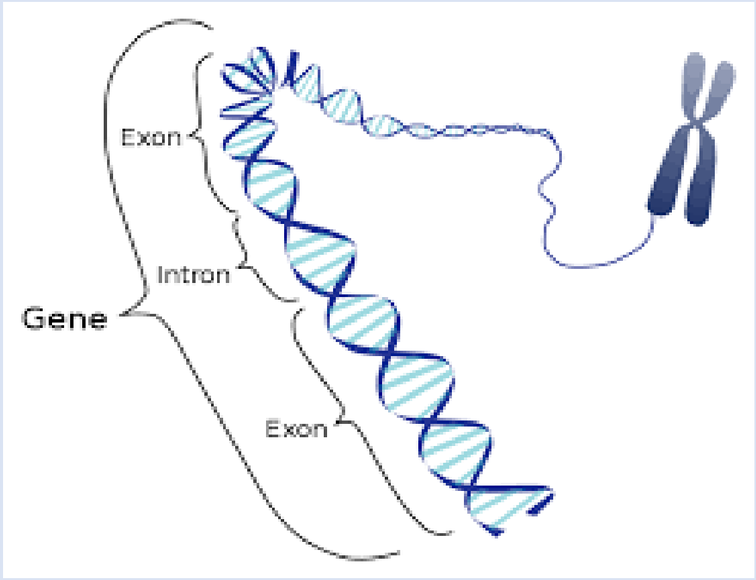


Chr. 19 and 22 are gene rich, whereas 4 and 18 are relatively gene poor.



The size of genes also shows great variability.

- small genes with single exons to the TTN gene, which encodes the largest known protein in the human body and has not only the largest number of exons (363), but also the single largest exon [17,106 base pairs (bp)]

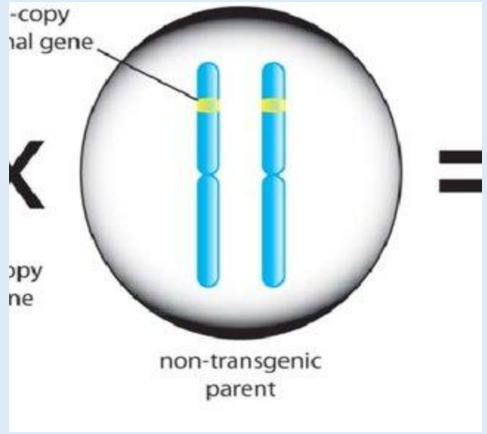


Nuclear Genes

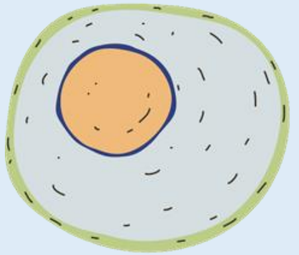
Unique Single-Copy Genes:

Most human genes are unique single-copy genes coding for polypeptides that are involved in or carry out a variety of cellular functions.

These include enzymes, hormones, receptors and structural and regulatory proteins.



Nuclear Genes



Multigene Families:

- **Classic gene families** — high degree of sequence homology
- **Gene superfamilies** — limited sequence homology

Many genes have **similar functions**, having arisen through **gene duplication** events with subsequent evolutionary divergence, making up what are known as multigene families.

Some are found physically *close together in clusters*, for example, the **α- and β-globin** gene clusters on chromosomes 16 and 11,

- Others are widely dispersed throughout the genome, occurring on different chromosomes, such as the **HOX homeobox** gene family.

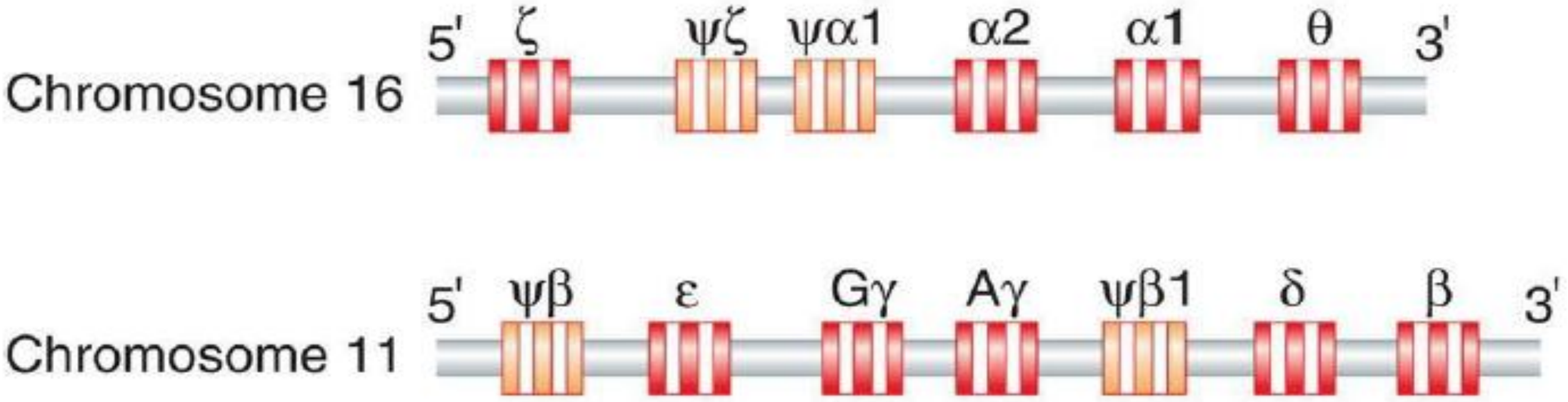


FIG. 2.5 Representation of the α- and β-globin regions on chromosomes 16 and 11.

Gene Structure

- Most human genes contain **introns**, but the number and size of both **introns** and **exons** is extremely variable.

- Individual introns can be far larger than the coding sequences, and some have been found to contain coding sequences for other genes (i.e., genes occurring within genes).

- Genes in humans do not usually overlap, being separated from each other by an average of 30 kb.

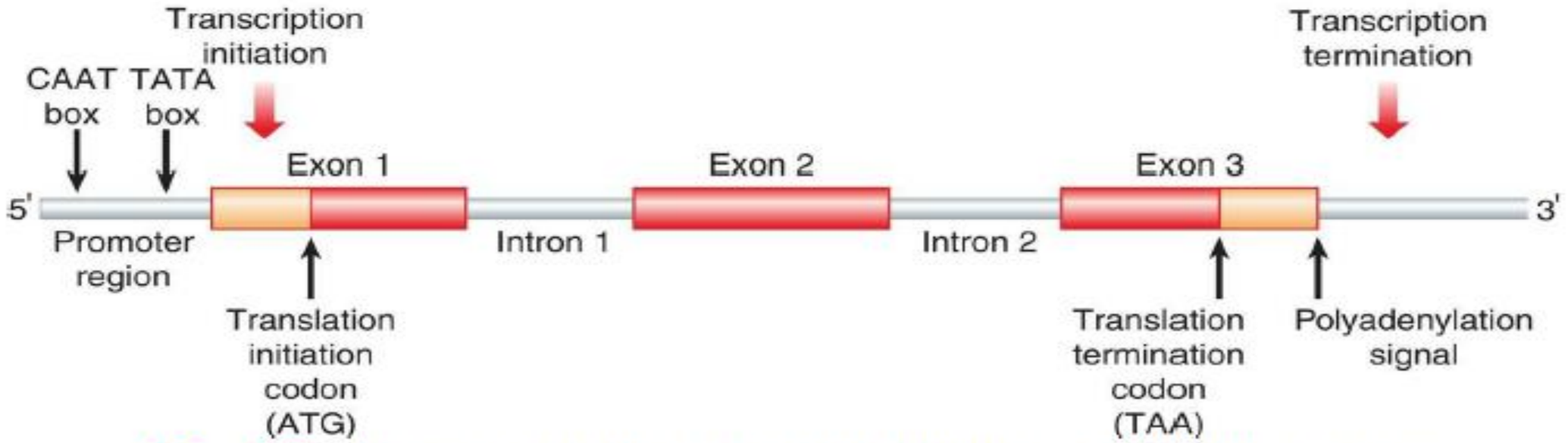
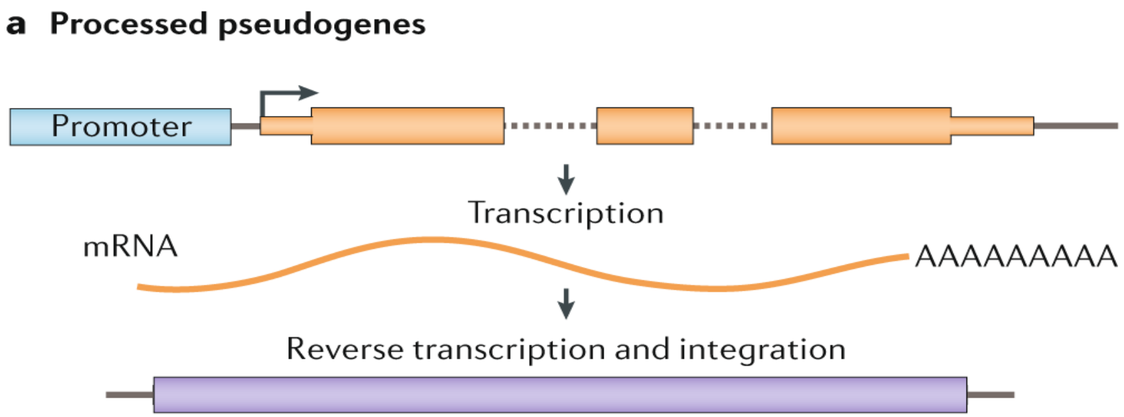
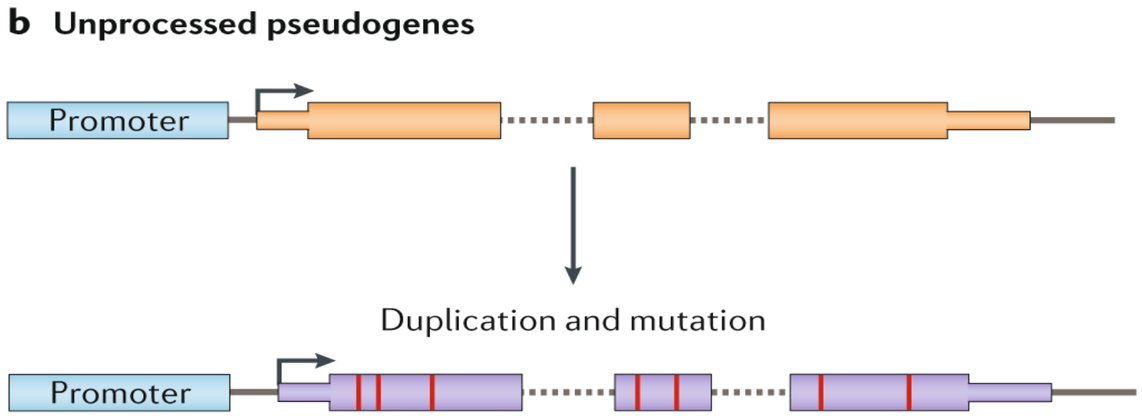


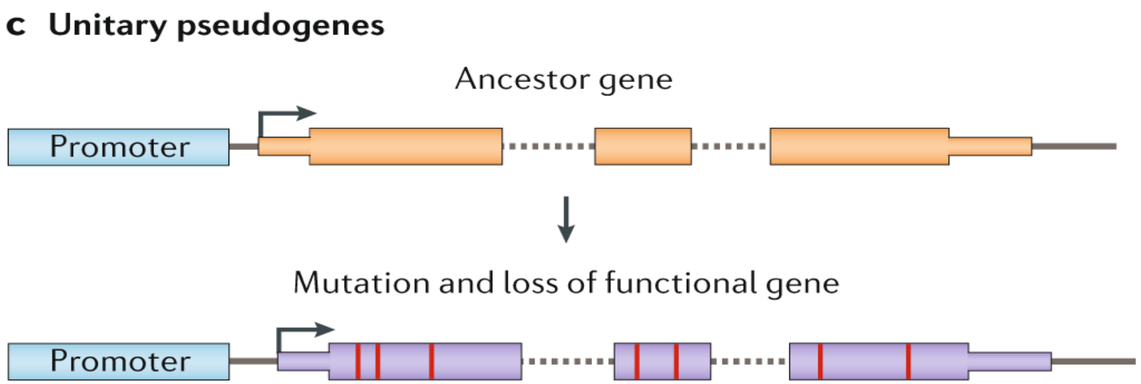
FIG. 2.6 Representation of a typical human structural gene.

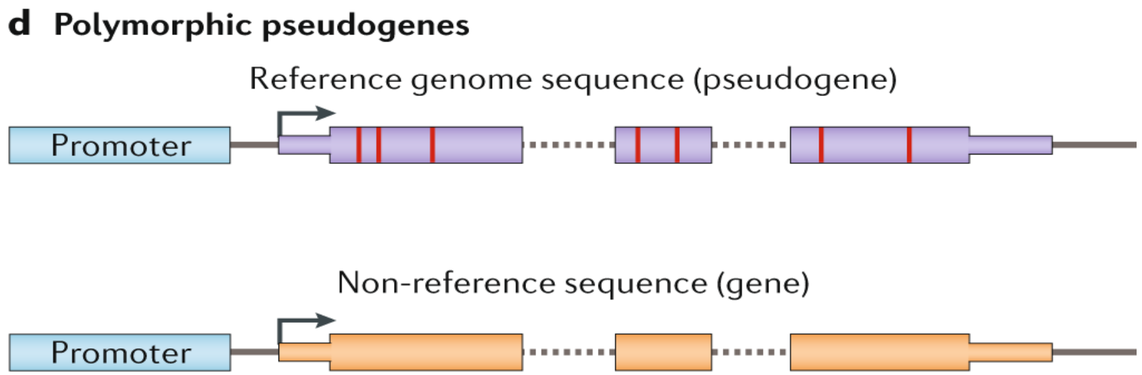
Pseudogenes

Particularly fascinating is the occurrence of genes that closely resemble known structural genes but which, in general, are not functionally expressed: so-called pseudogenes.









Extra-genic DNA

The estimated 20000 unique single-copy genes that encode proteins represent less than 2% of the human genome.

The remainder of the human genome is made up of repetitive DNA sequences that are predominantly transcriptionally inactive.

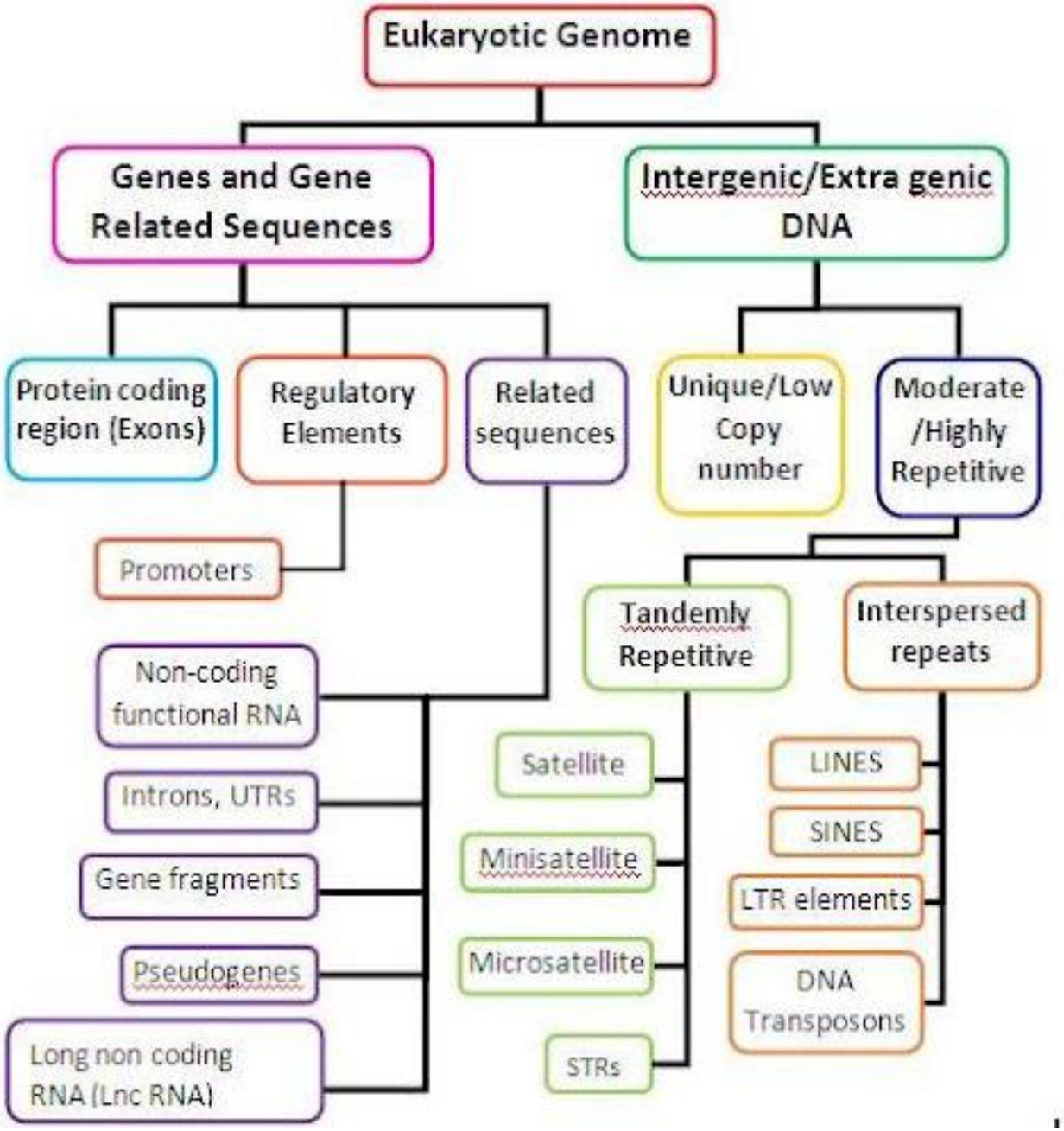
- This has been described as junk DNA, but some regions show evolutionary conservation and play a critical role in the **regulation** of temporal and spatial gene expression.

Extragenic DNA

- Tandemly Repeated DNA Sequences
  - Satellite DNA
  - Minisatellite DNA
  - Microsatellite DNA

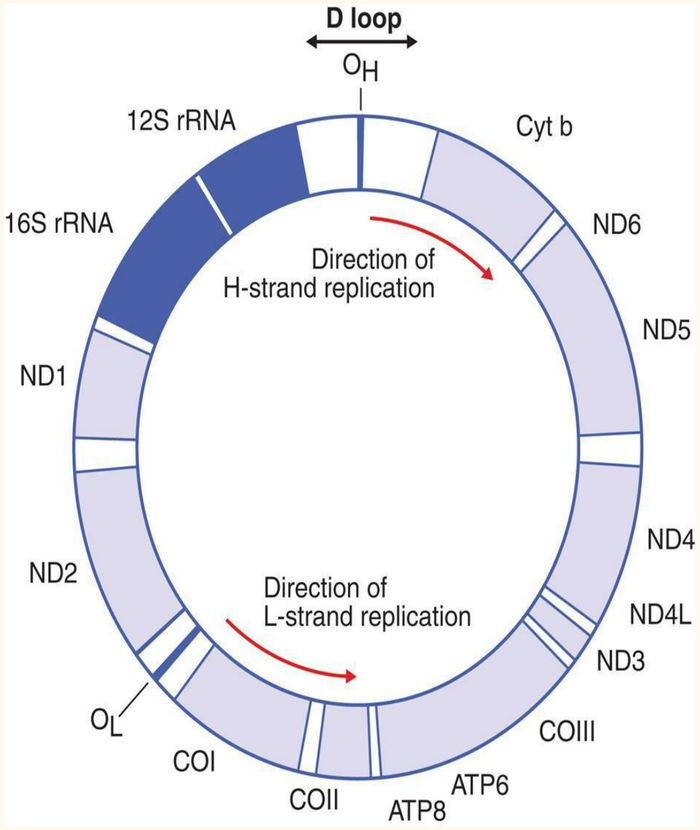
telomeric and hypervariable minisatellite DNA

- Highly Repeated Interspersed Repetitive DNA
  - Short Interspersed Nuclear Elements (**SINEs**)
  - Long Interspersed Nuclear Elements (**LINE**)


Eukaryotic Genome
Genes and Gene Related Sequences
Intergenic/Extra genic DNA
Protein coding region (Exons)
Regulatory Elements
Related sequences
Unique/Low Copy number
Moderate /Highly Repetitive
Promoters
Tandemly Repetitive
Interspersed repeats
Non-coding functional RNA
Introns, UTRs
Gene fragments
Pseudogenes
Long non coding RNA (Lnc RNA)
Satellite
Minisatellite
Microsatellite
STRs
LINES
SINES
LTR elements
DNA Transposons

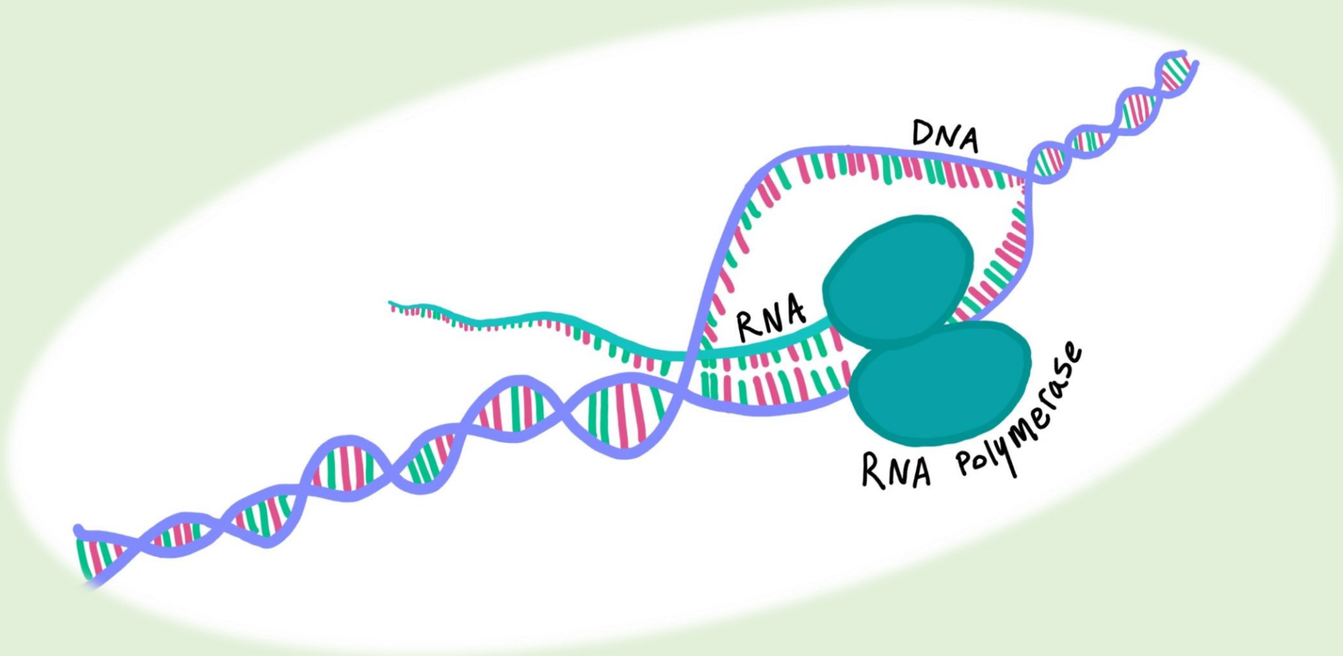
Mitochondrial DNA

In addition to nuclear DNA, the several thousand mitochondria of each cell possess their own 16.6-kb circular double-stranded DNA, mitochondrial DNA (mtDNA)

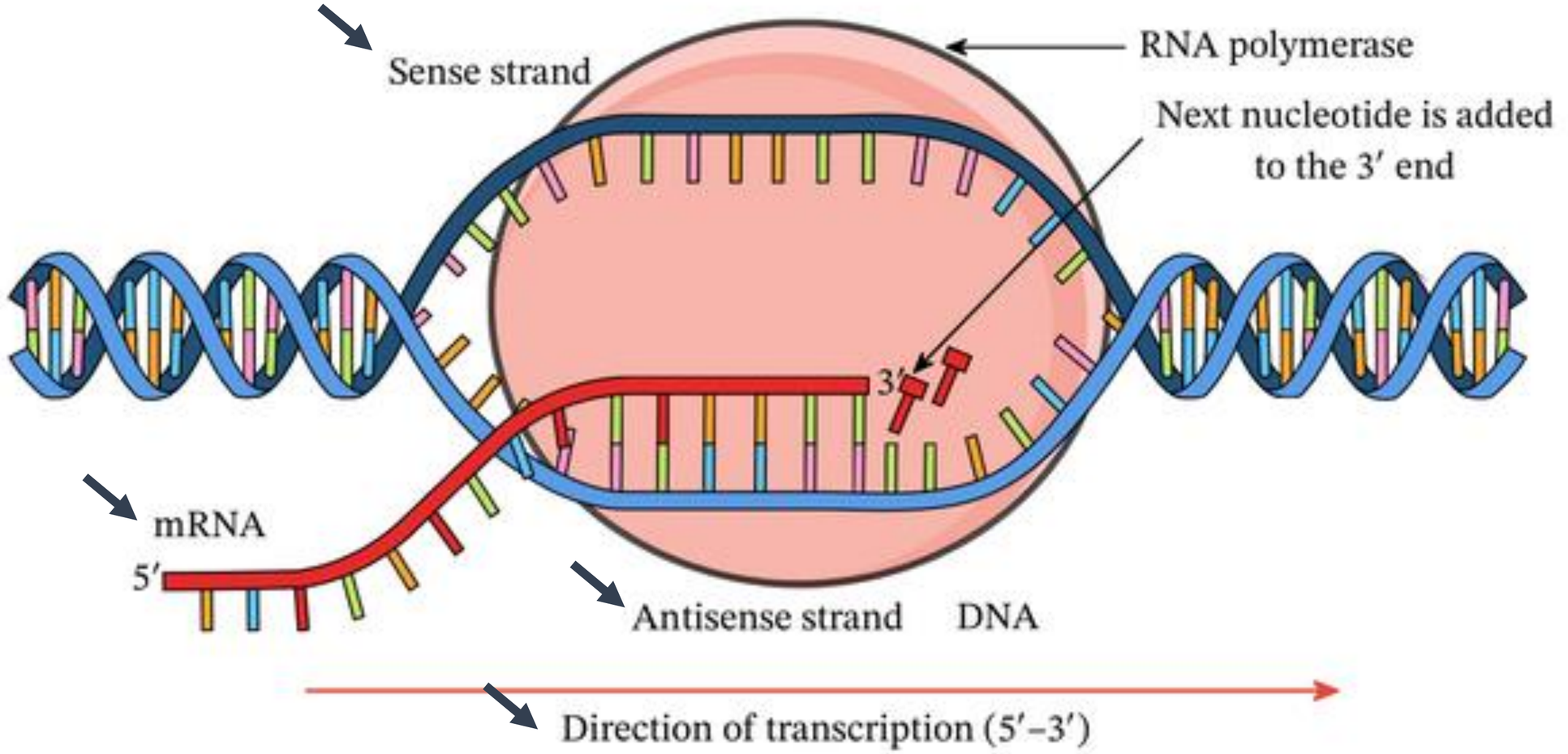


The mitochondrial genome is **very compact**, containing **little repetitive** DNA, and codes for **37** genes, which include two types of rRNA, 22 tRNAs and 13 protein subunits for enzymes, such as cytochrome b and cytochrome oxidase, which are involved in the energy-producing oxidative phosphorylation pathways.

Transcription



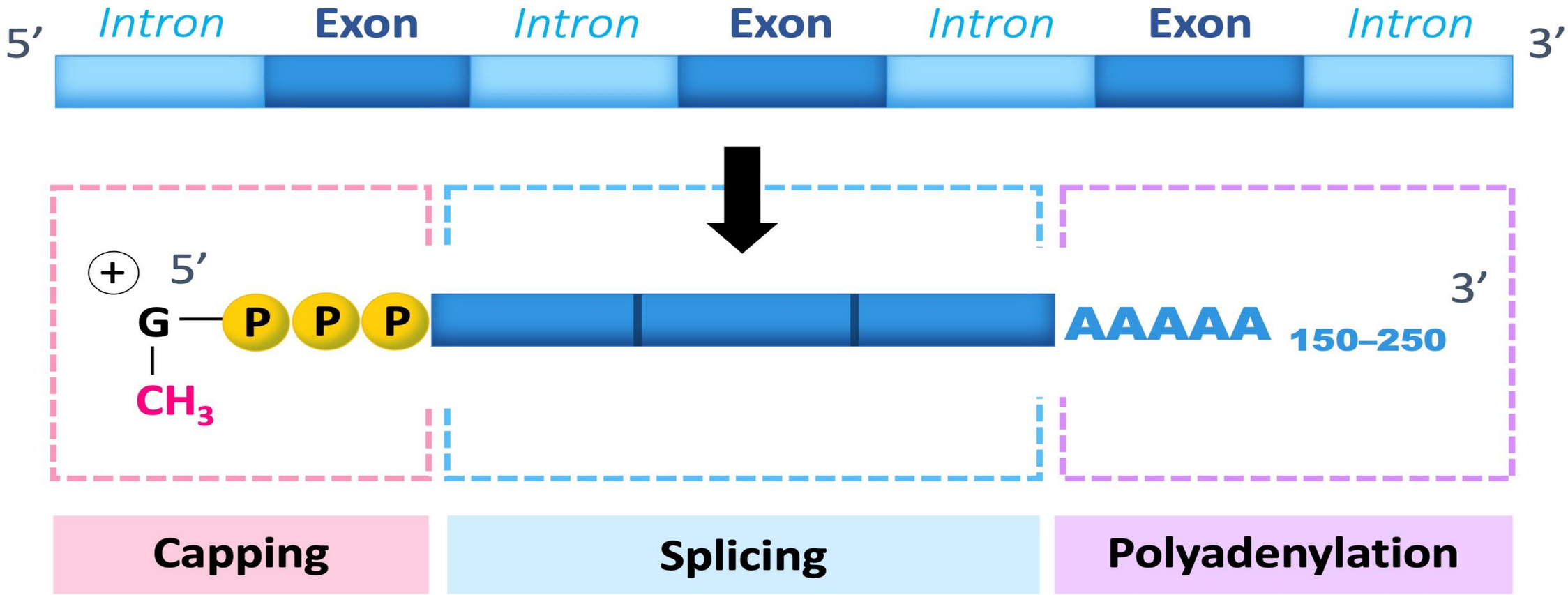
The process whereby genetic information is transmitted from **DNA** to **RNA** is called transcription.



RNA Processing

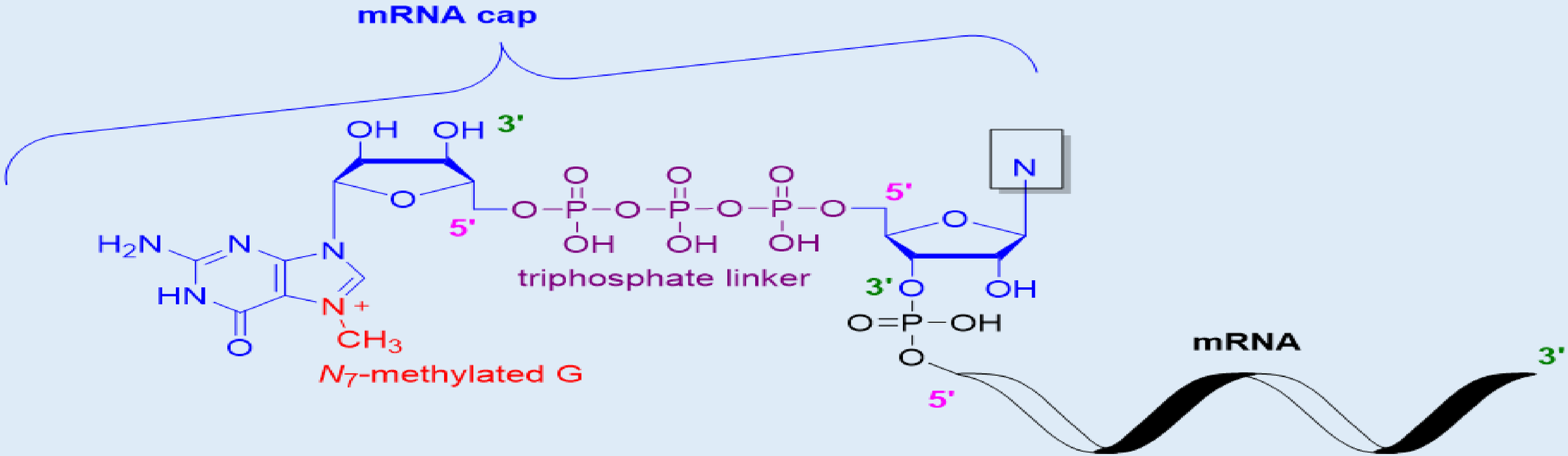
- Before the primary mRNA molecule leaves the nucleus, it undergoes a number of modifications, or what is known as RNA processing.

- This involves **splicing, capping, and polyadenylation**.



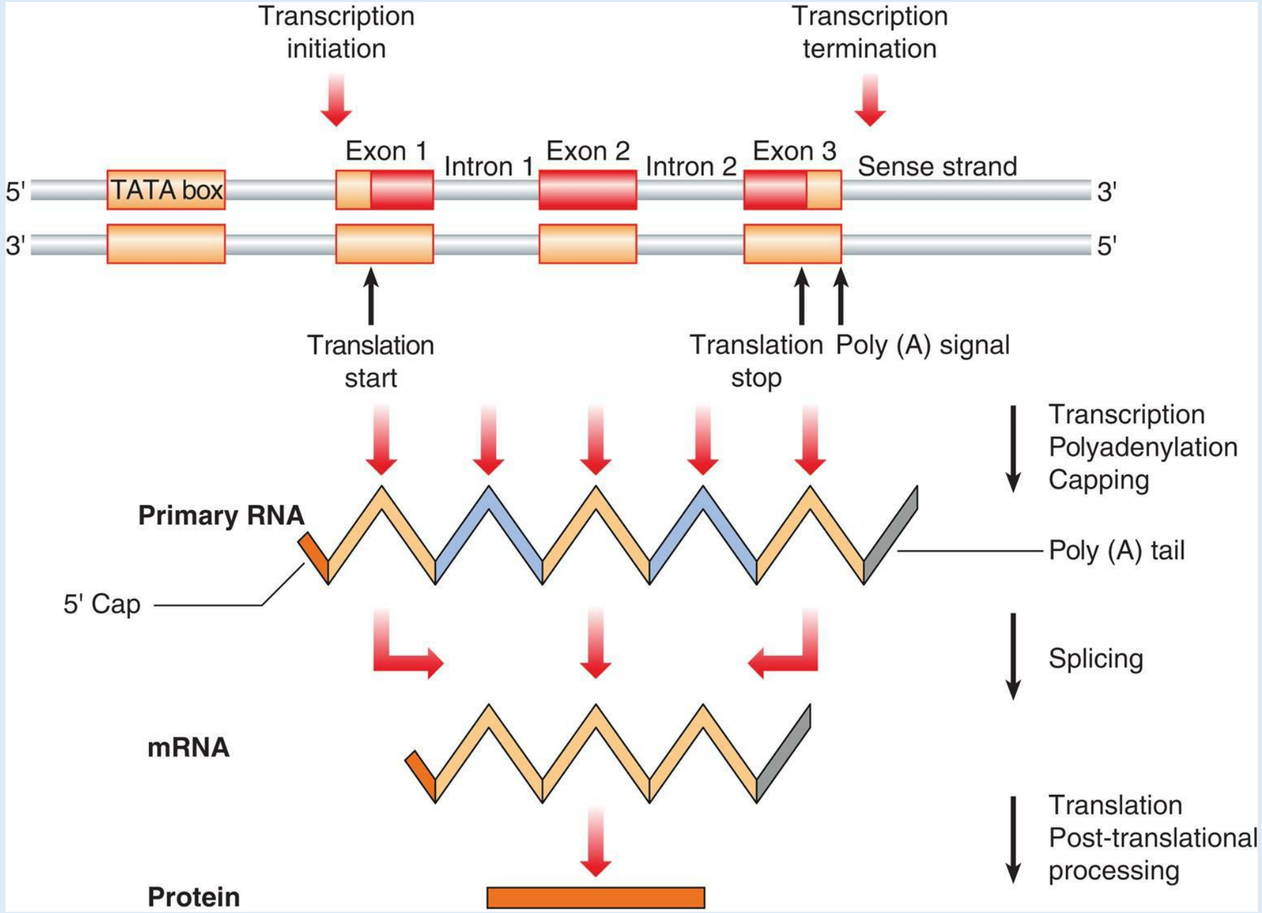
Capping

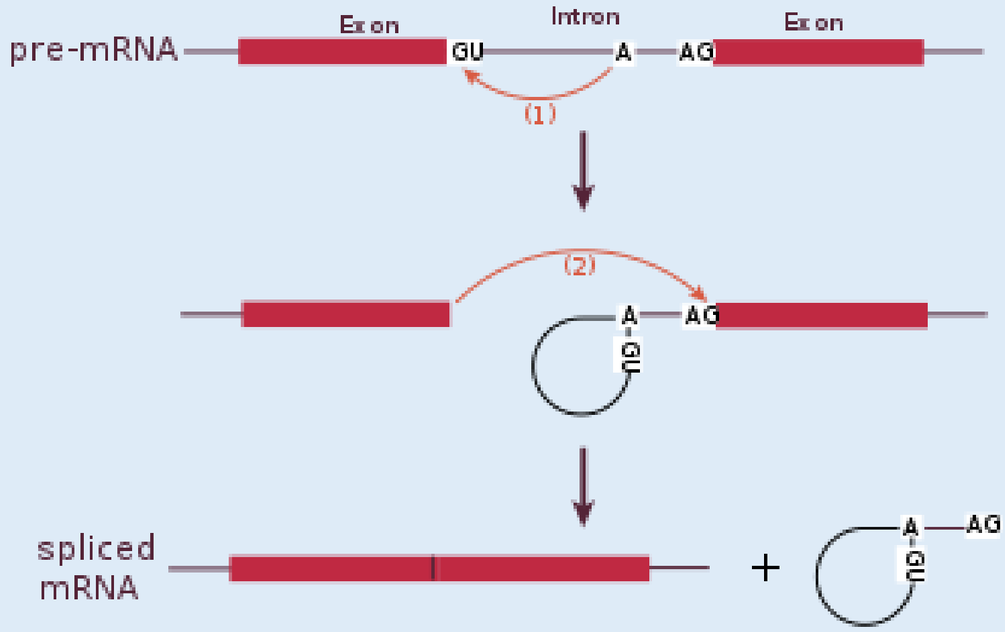
- The 5’ cap is thought to **facilitate <u>transport of the mRNA to the cytoplasm</u>** and **<u>attachment to the ribosomes</u>**, as well as to **<u>protect</u>** the RNA transcript from degradation by endogenous cellular exonucleases.
- After **20 to 30** nucleotides have been transcribed, the nascent mRNA is modified by the addition of a **<u>G nucleotide</u>** to the 5’ end of the molecule by an unusual 5’ to 5’ triphosphate linkage.
- A methyltransferase enzyme then methylates the N7 position of the G, giving the final 5’ cap.



mRNA Splicing

the non-coding introns in the precursor mRNA are excised, and the non-contiguous coding exons are spliced together to form a shorter mature mRNA **before its transportation to the ribosomes in the cytoplasm for translation**.

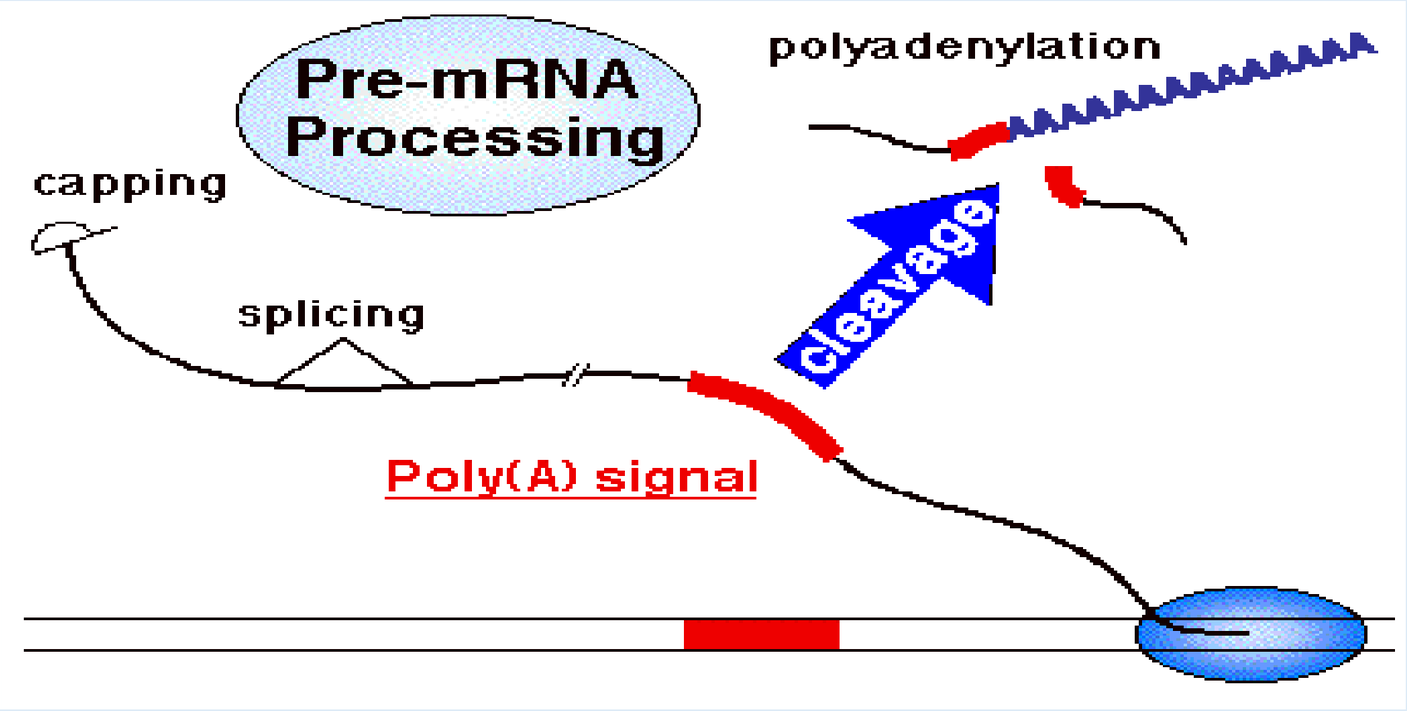




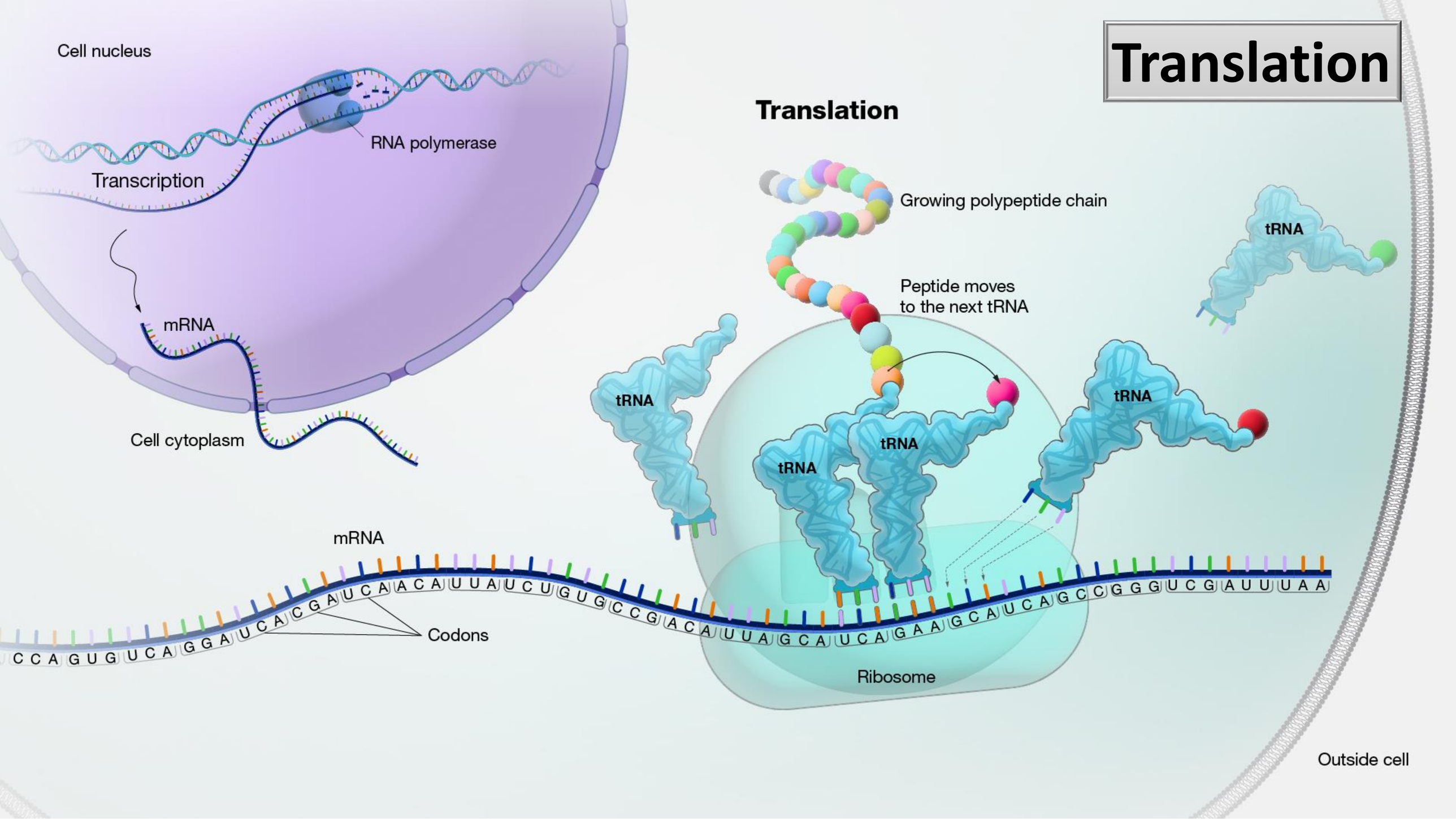
Polyadenylation

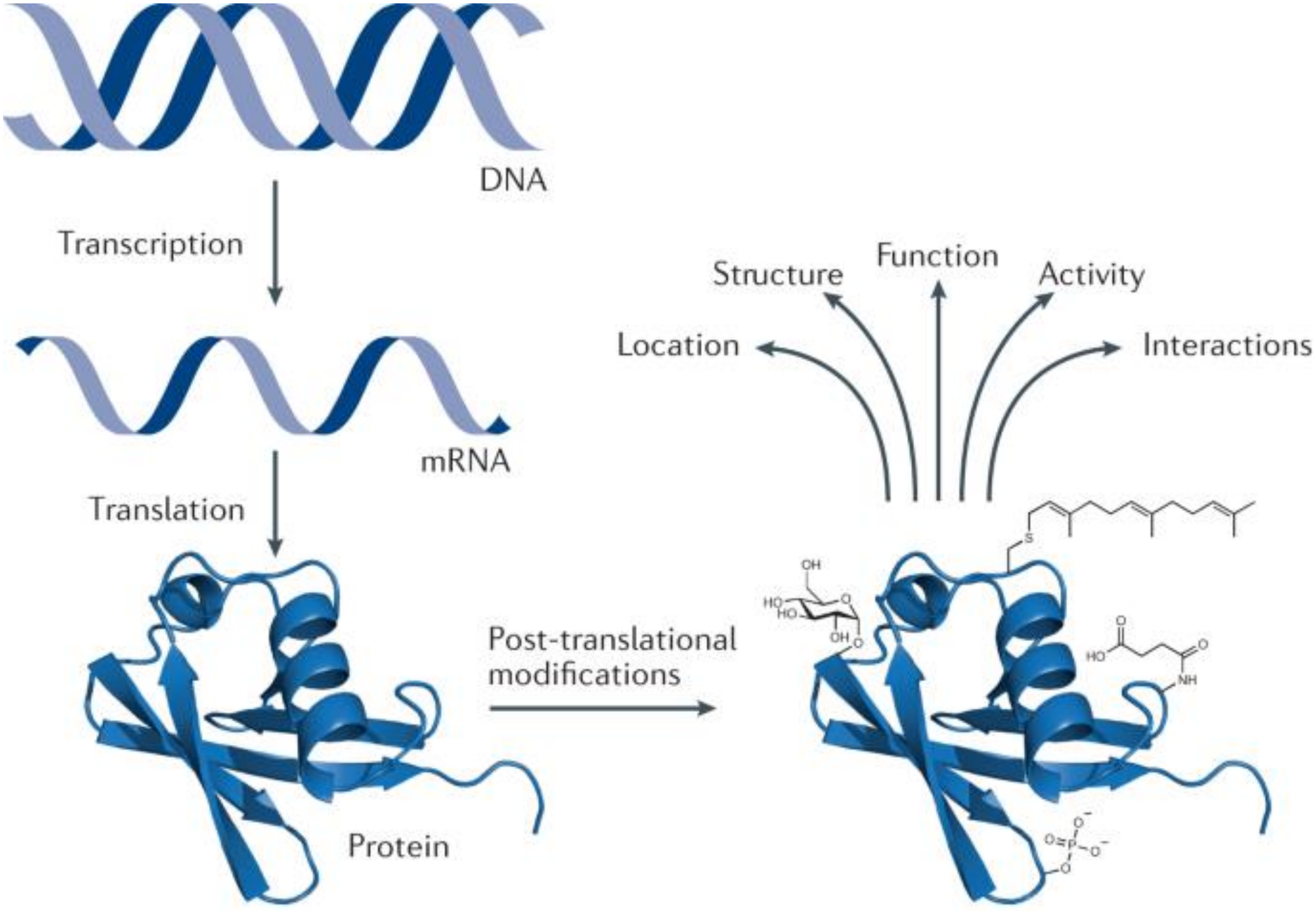
Transcription continues until specific nucleotide sequences are transcribed that cause the mRNA to be cleaved and RNA polymerase II to be released from the DNA template.

Approximately 200 adenylate residues—the so-called poly(A) tail—are added to the mRNA, which facilitates nuclear export and translation.



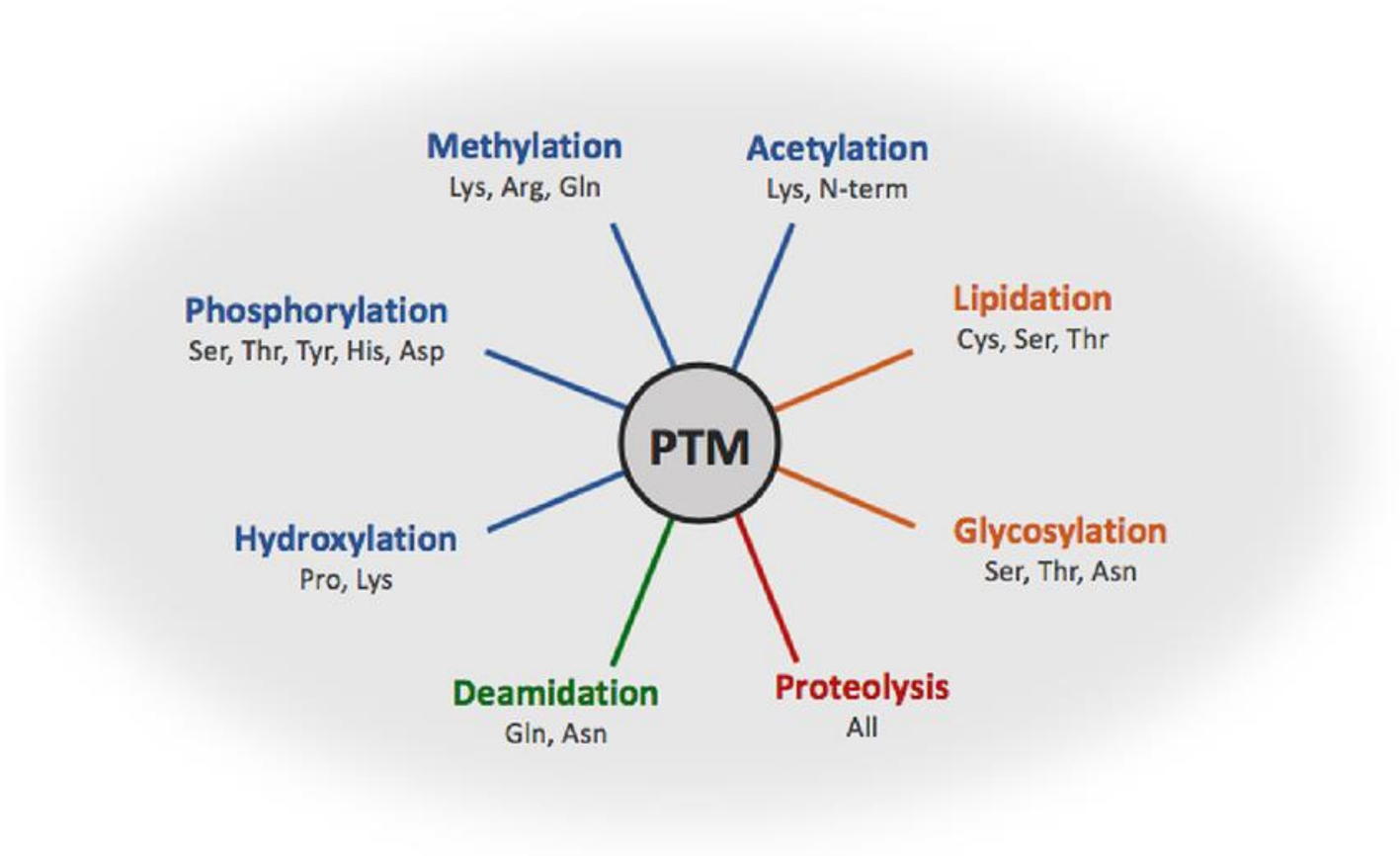
Translation




DNA
Transcription
mRNA
Translation
Protein
Post-translational
modifications
Structure
Function
Activity
Location
Interactions

Post-translational Modification

Many proteins**, before they attain their normal structure or functional activity**, undergo **post-translational modification**, which can include chemical modification of amino-acid side chains (e.g., hydroxylation, methylation), the addition of carbohydrate or lipid moieties (e.g., glycosylation) or proteolytic cleavage of polypeptides (e.g., the conversion of proinsulin to insulin).

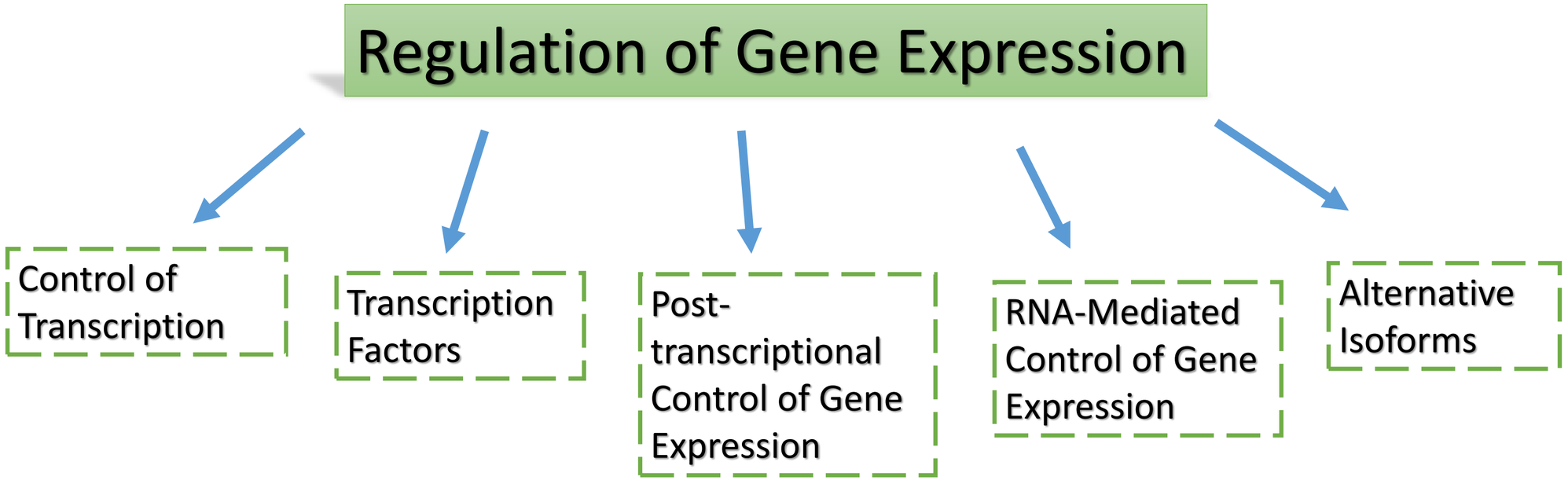


Regulation of Gene Expression

- Many cellular processes, and therefore the genes that are expressed, are common to all cells, for example ribosomal, chromosomal, and cytoskeleton proteins, constituting what are called the **housekeeping** genes.

- Some cells express large quantities of a specific protein in certain tissues or at specific times in development, such as hemoglobin in red blood cells.

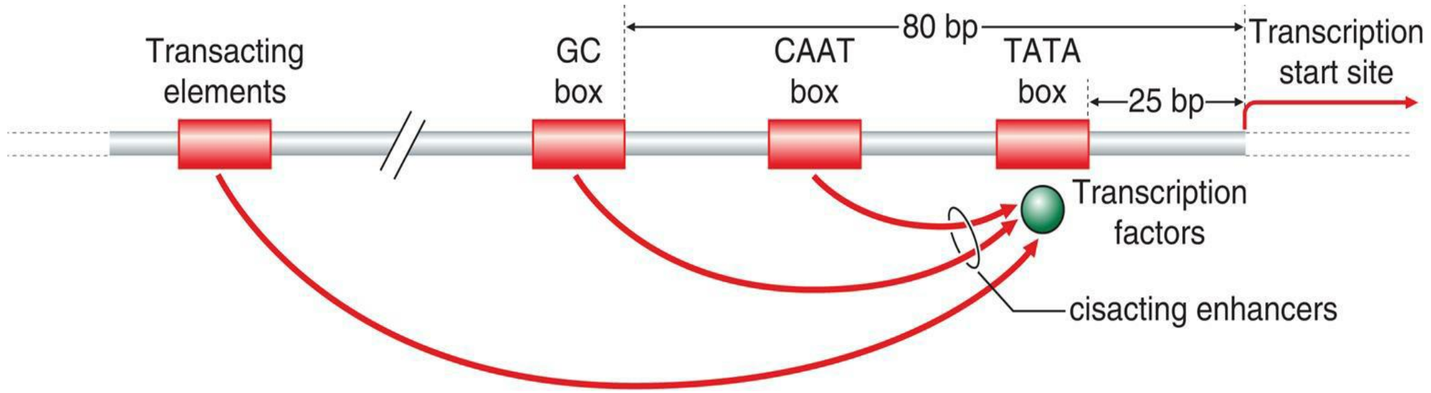
- This differential control of gene expression can occur at a variety of stages.


Regulation of Gene Expression
Control of Transcription
Transcription Factors
Post-transcriptional Control of Gene Expression
RNA-Mediated Control of Gene Expression
Alternative Isoforms

Control of Transcription

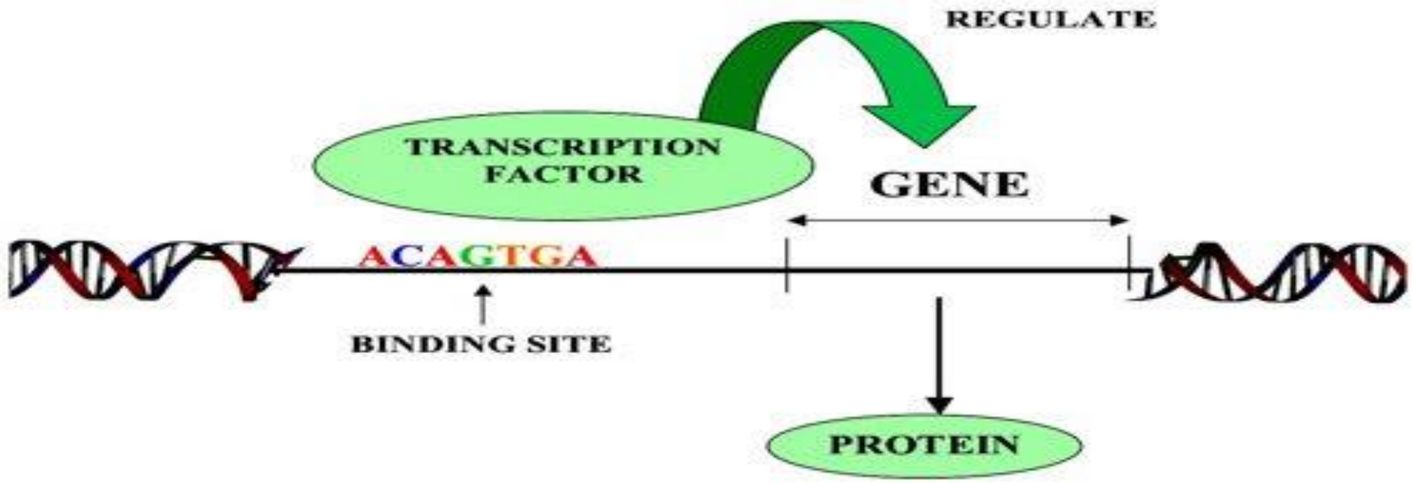
This occurs through a number of different mechanisms that include signaling molecules that bind to regulatory sequences in the DNA known as response elements, **intracellular receptors** known as **hormone nuclear receptors** and **receptors** for specific ligands on the cell surface involved in the process of signal transduction.

All of these mechanisms ultimately affect transcription through the binding of the general transcription factors to short specific DNA **promoter elements** located within 200 bp 5’ or upstream of most eukaryotic genes in the so-called core promoter region that leads to activation of RNA polymerase.



Transcription Factors

- A number of genes encode proteins involved in the regulation of gene expression.
- These proteins bind short nucleotide sequences, usually mediated through helical protein motifs, and are known as transcription factors.
- These gene regulatory proteins have a transcriptional activation domain and a DNA-binding domain.
- There are four types of DNA-binding domains, the most common being the **helix–turn–helix**, made up of two α helices connected by a short chain of amino acids that make up the “turn.”
- The three other types are the **zinc finger**, **leucine zipper** and **helix–loop–helix** motifs, so named as a result of specific structural features.



Post-transcriptional Control of Gene Expression

Regulation of the expression of most genes occurs at the level of transcription, but can also occur at the levels of :

- ❖RNA processing
- ❖RNA transport
- ❖mRNA degradation
- ❖mRNA translation.

For example, the G to A variant at position 20,210 in the 3′ untranslated region of the prothrombin-encoding gene increases the stability of the mRNA transcript, resulting in higher plasma prothrombin levels.

RNA-Mediated Control of Gene Expression

1.Small interfering RNAs (siRNAs):

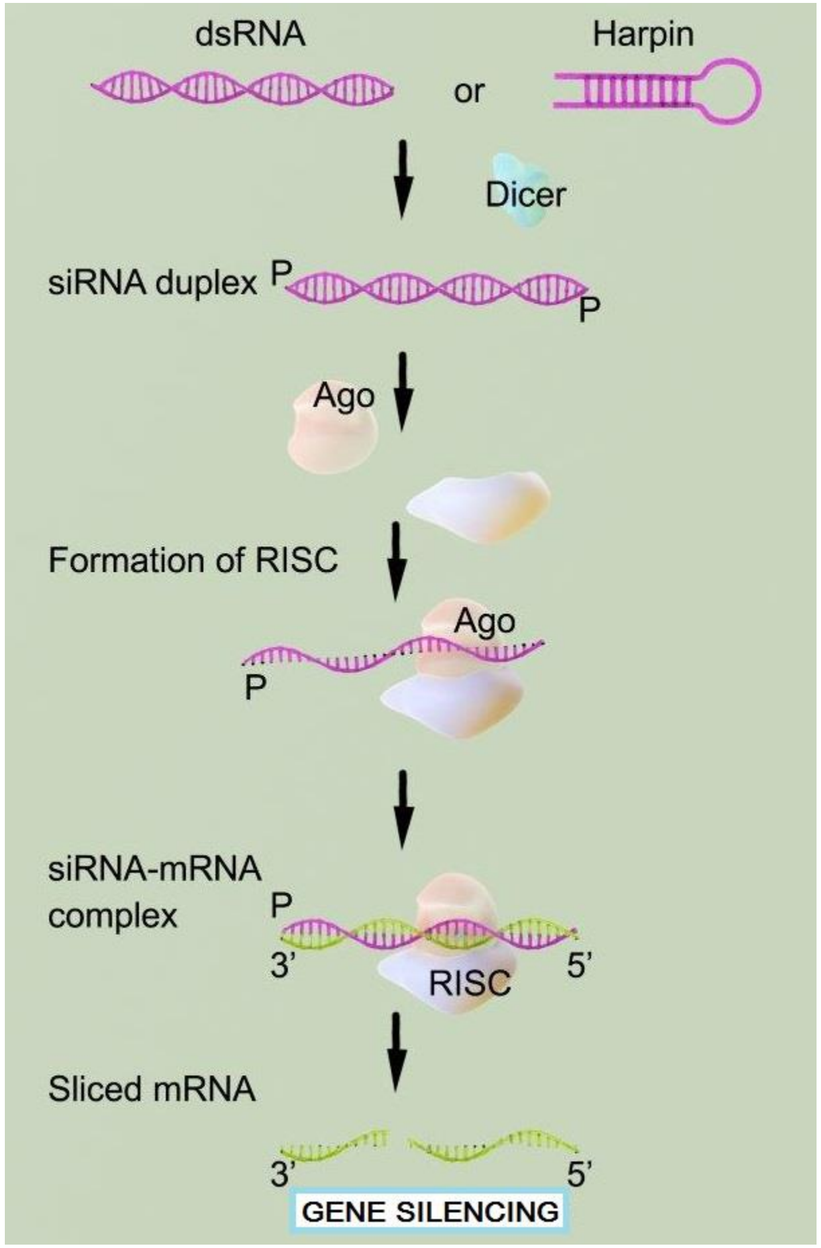
1. These molecules were discovered in 1998.
2. They play a crucial role as **effector molecules** in the **RNA interference (RNAi) pathway**.
3. RNAi is a cellular process that regulates gene expression by silencing specific genes.
4. siRNAs are **short double-stranded RNAs**, typically 21 to 23 nucleotides in length.

2.Mechanism of Action:

1. siRNAs **bind to messenger RNAs (mRNAs)** in a **sequence-specific manner**.
2. When bound, they trigger the **degradation of the target mRNA**.
3. This degradation occurs via a **ribonuclease-containing complex** called the **RNA-induced silencing complex (RISC)**.
4. Essentially, siRNAs prevent the production of proteins encoded by the targeted mRNA.

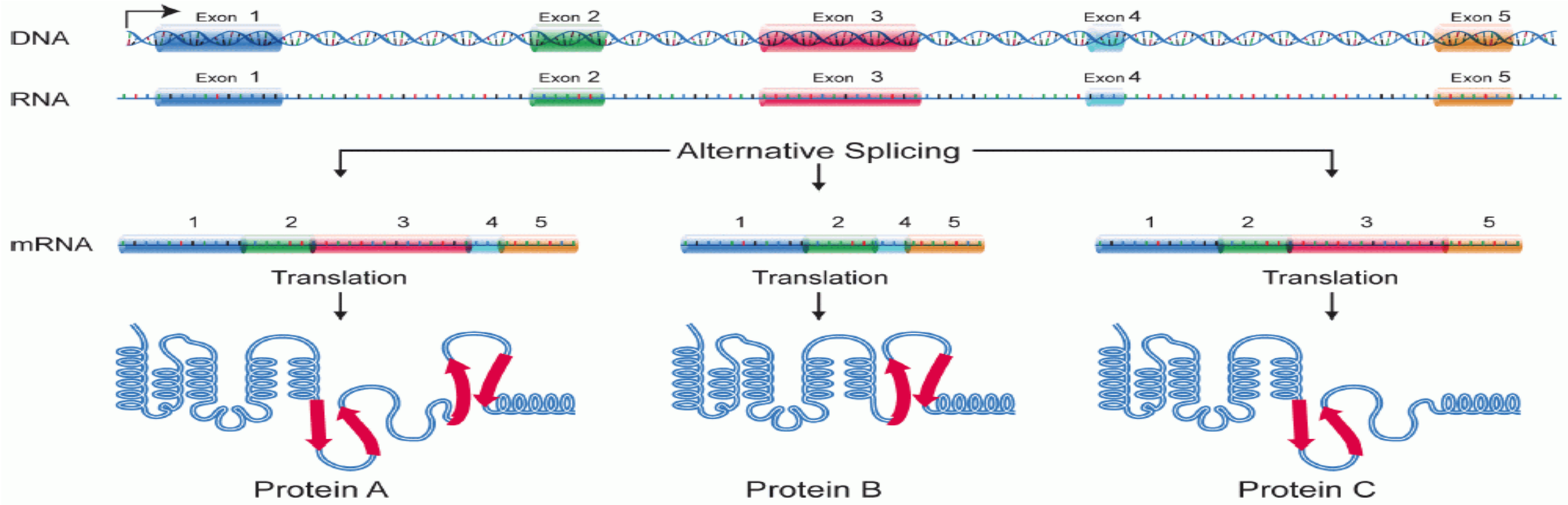
3.Two Modes of Action:

1. siRNAs can either:
   1. **Cause endonucleolytic cleavage** of the mRNA, breaking it into pieces.
   2. **Block translation**, preventing the mRNA from being used to synthesize proteins.



Alternative Isoforms

- Most (~95%) human genes undergo alternative splicing and **therefore encode more than one protein**.
- **Alternative polyadenylation** generates further diversity.
- Some genes have **more than one promoter**, and these alternative promoters may result in tissue-specific isoforms.
- **Alternative splicing of exons** is also seen with individual exons present in only some isoforms. The extent of alternative splicing in humans may be inferred from the finding that the human genome includes only approximately 20,000 genes, far fewer than the original prediction of more than 100,000.

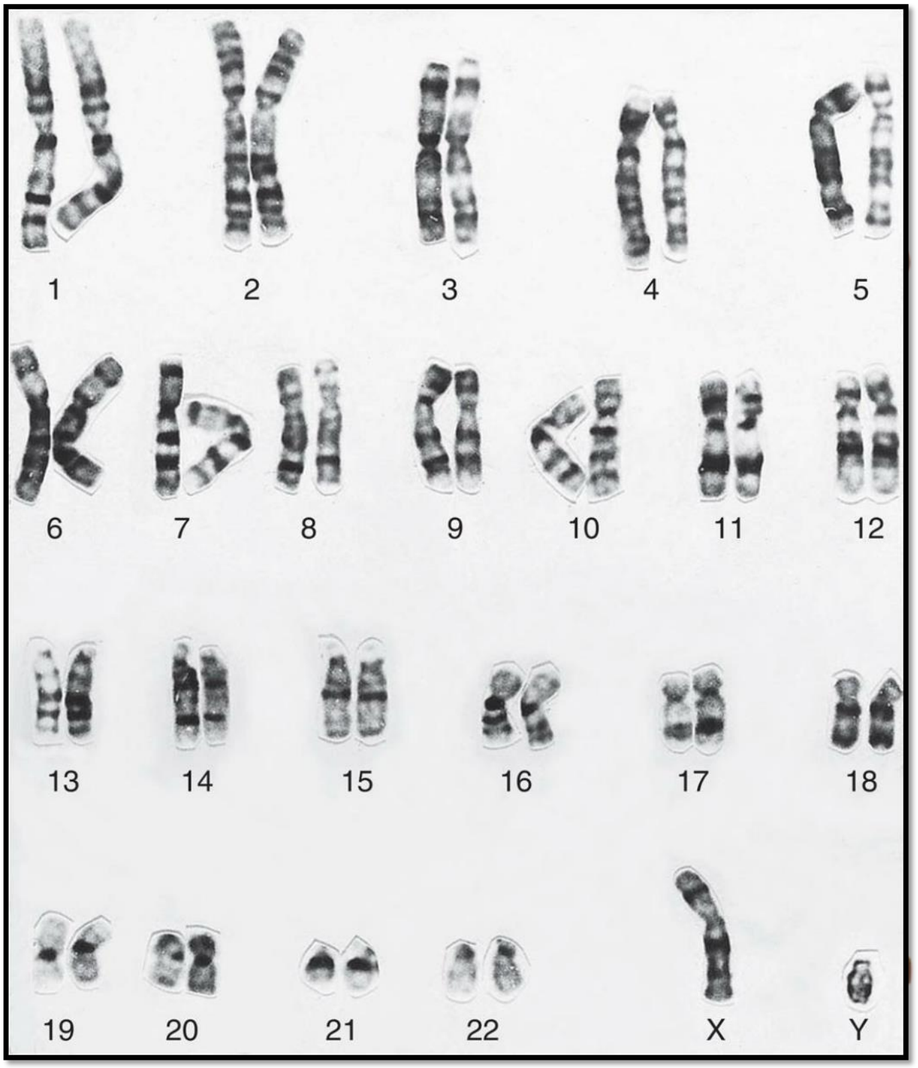


Mutations

A mutation is defined as alteration or change in the genetic material

Mutations drive evolution, but can also be pathogenic.

Sequence variants with no obvious effect on phenotype may be termed ***polymorphisms***.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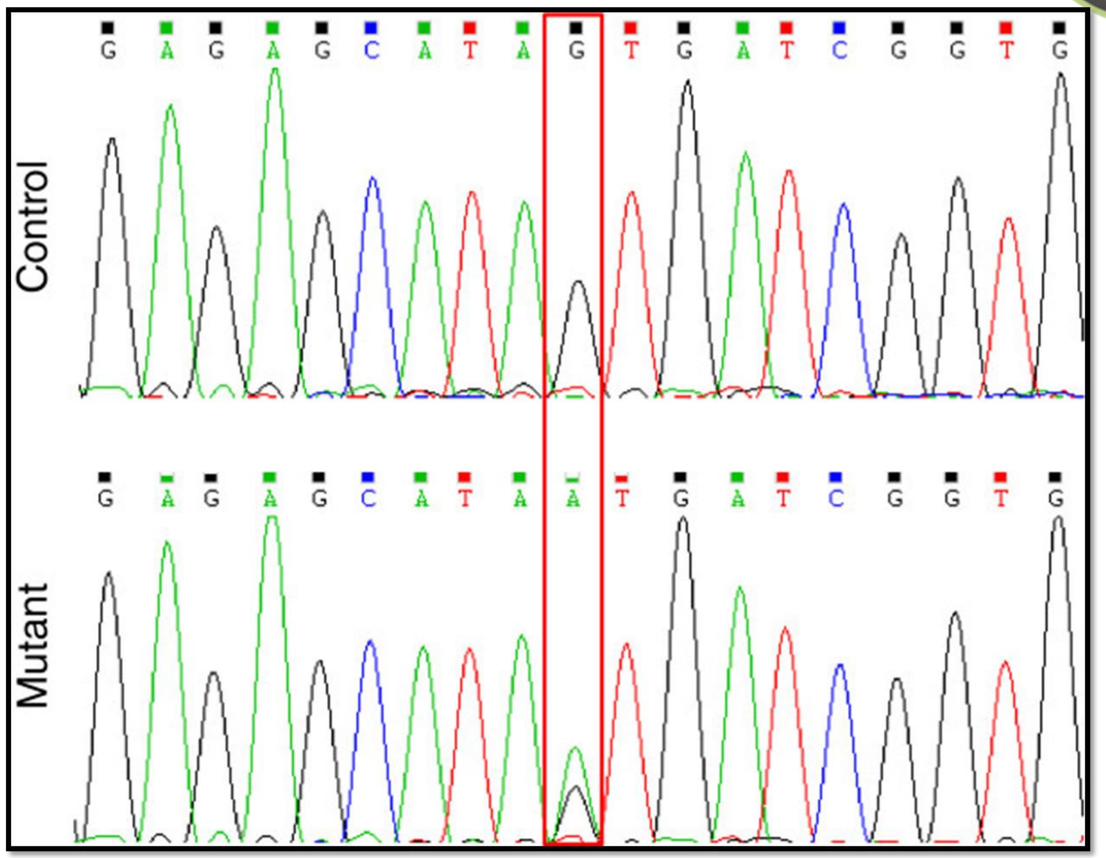
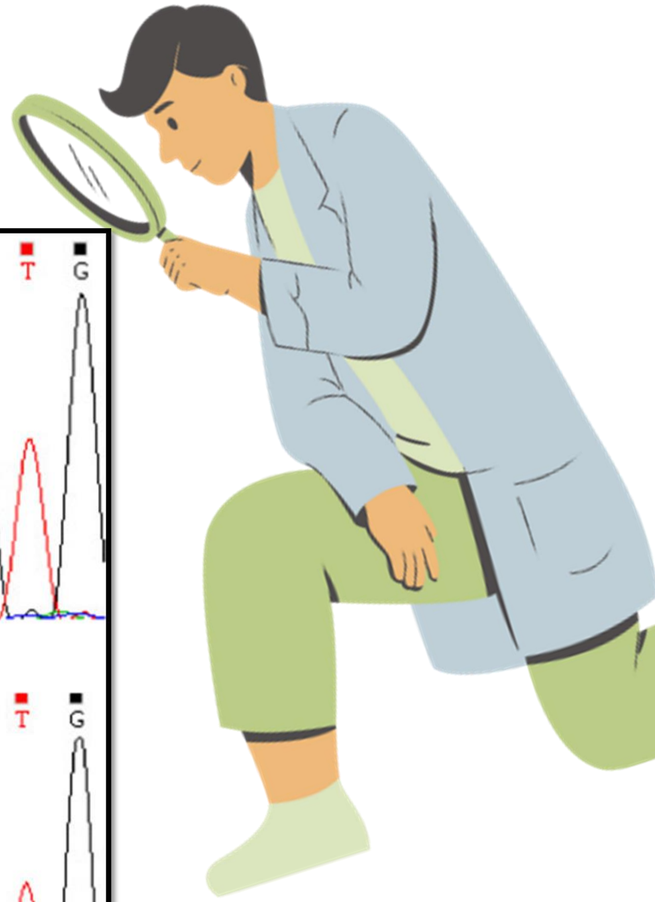
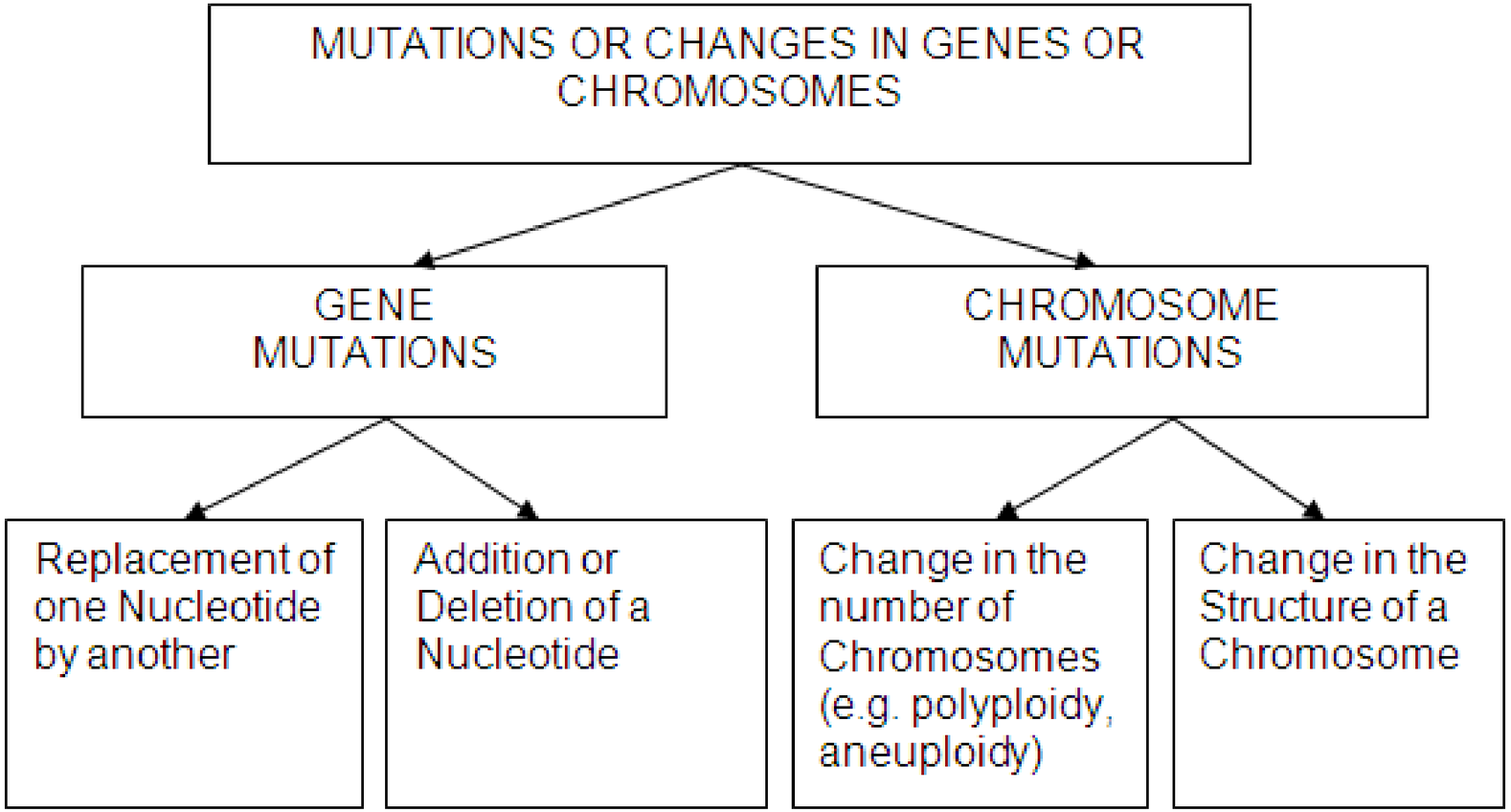

Control
G A G A G C A T A G T G A T C G G T G
Mutant
G A G A G C A T A A T G A T C G G T G



Types of Mutation

Mutations can range from loss or gain of **entire chromosomes** to **single base** substitutions, through insertions and deletions of single or multiple bases**.**



Loss or gain of entire chromosomes (aneuploidy)

Box 3.1

Types of Chromosome Abnormality

Numerical

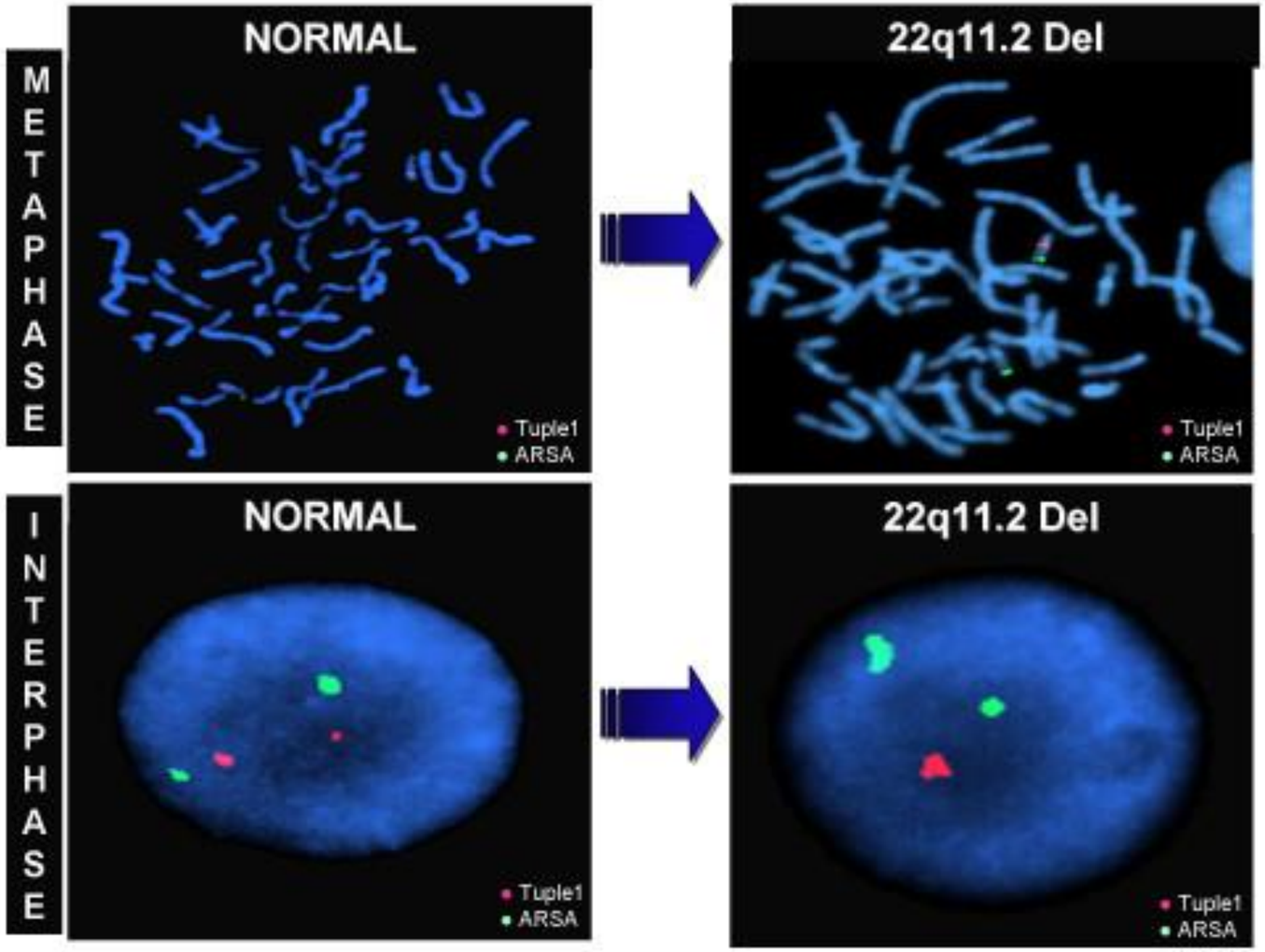
- Aneuploidy
- Monosomy
- Trisomy
- Tetrasomy
- Polyploidy
- Triploidy
- Tetraploidy

Structural

- Translocations
- Reciprocal
- Robertsonian
- Deletions
- Insertions
- Inversions
- Paracentric
- Pericentric
- Rings
- Isochromosomes

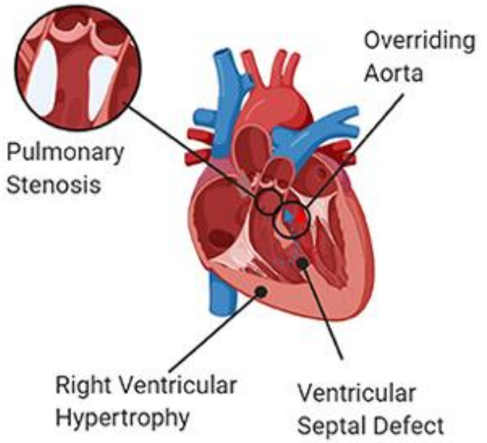
Different Cell Lines (Mixoploidy)

- Mosaicism
- Chimerism



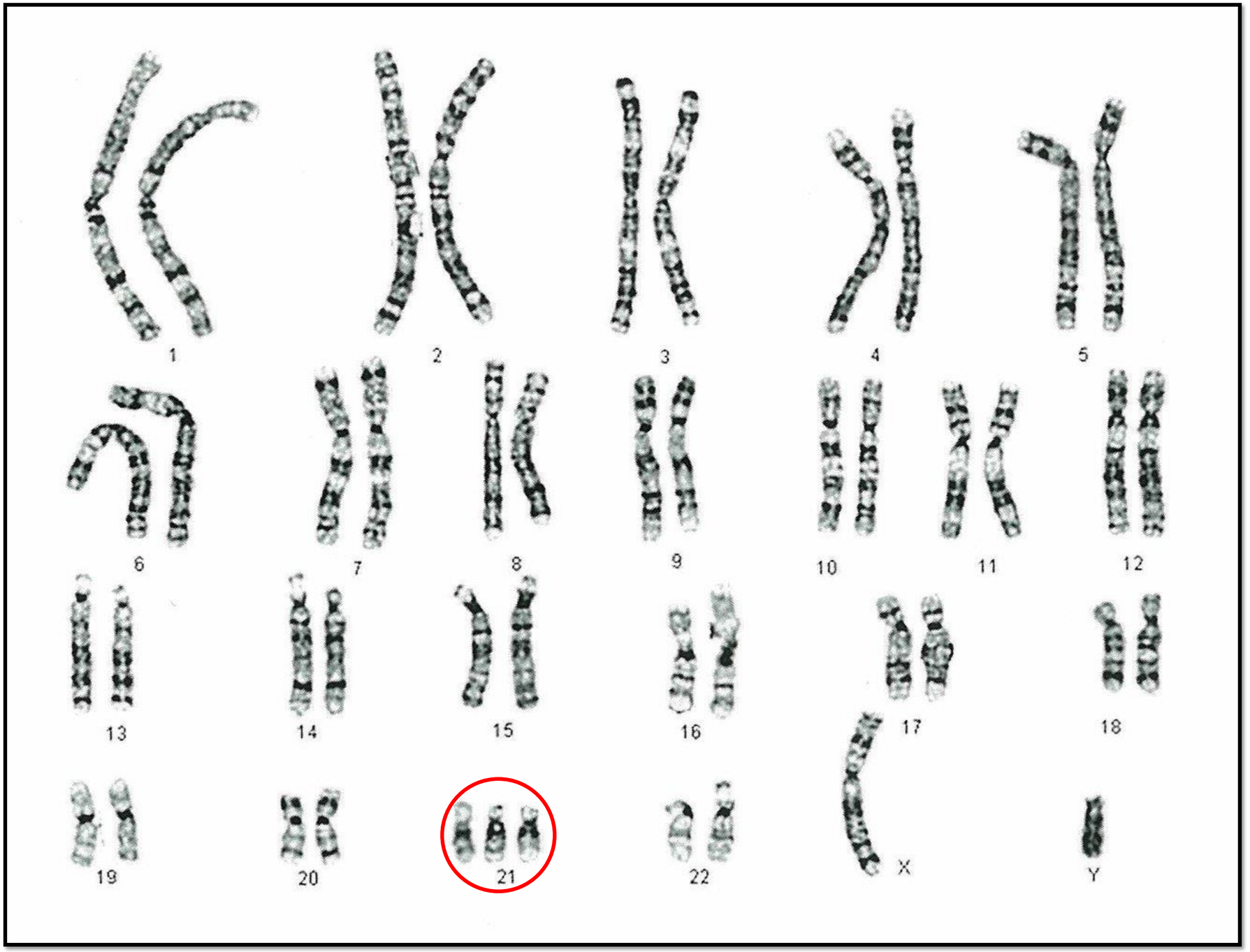
Digeorge syndrome

A DiGeorge Syndrome (22q11.2 deletion)

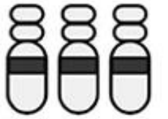


Thymic hypoplasia related immune defects (fewer mature T-cells, reduced B-cell response)

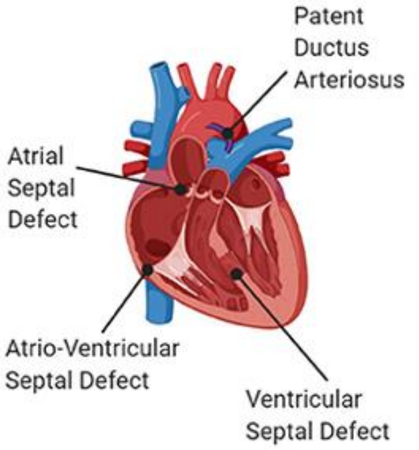
Down syndrome



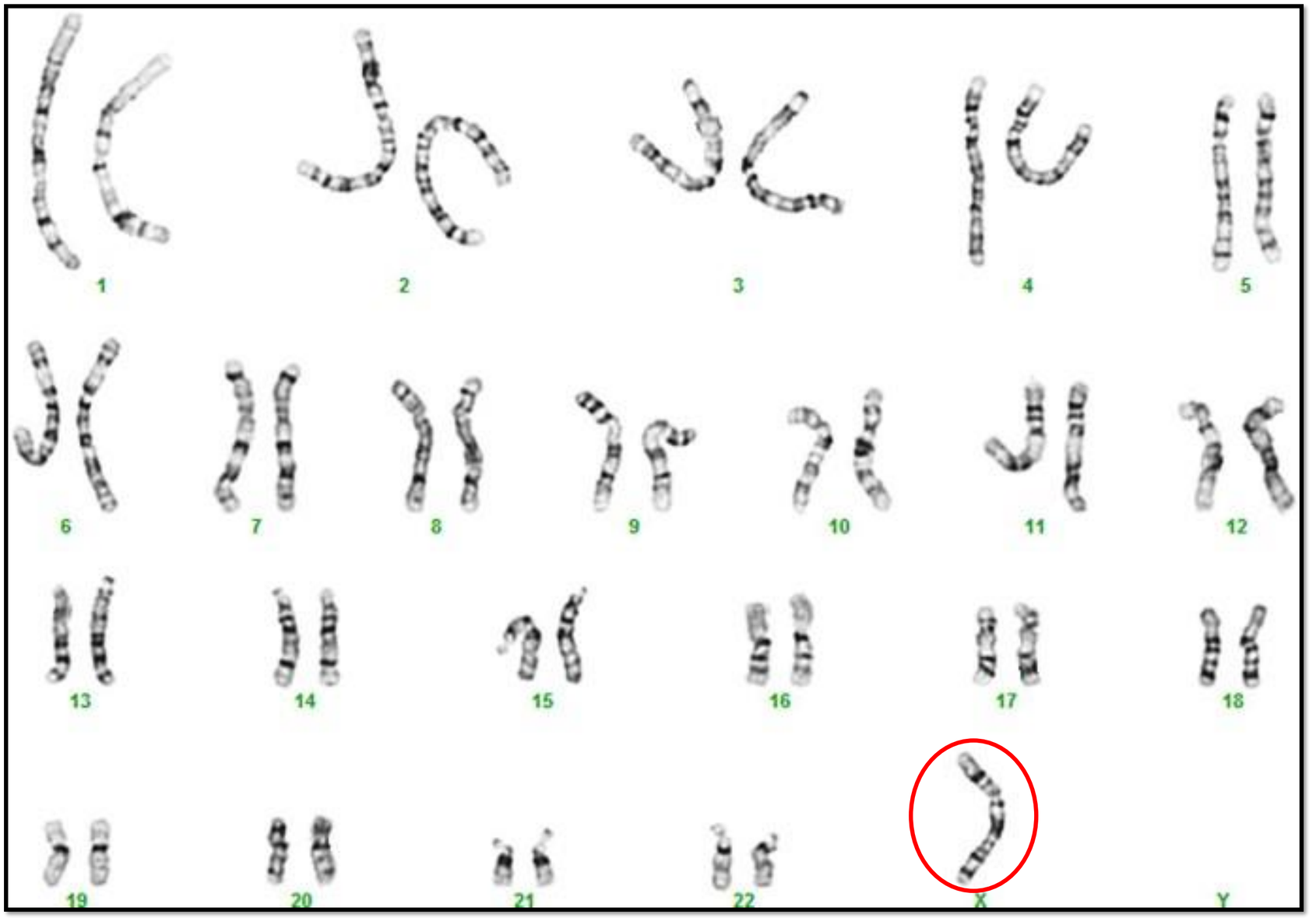
B **Down Syndrome (Trisomy 21)**



Extra chromosome 21



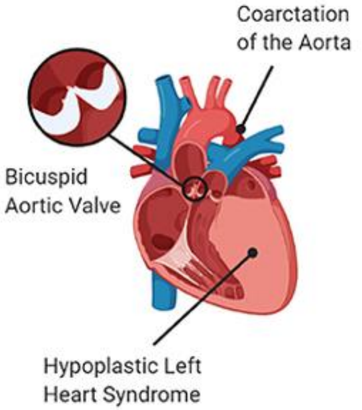
Increased sinopulmonary infections
Risk of immune cell malignancies



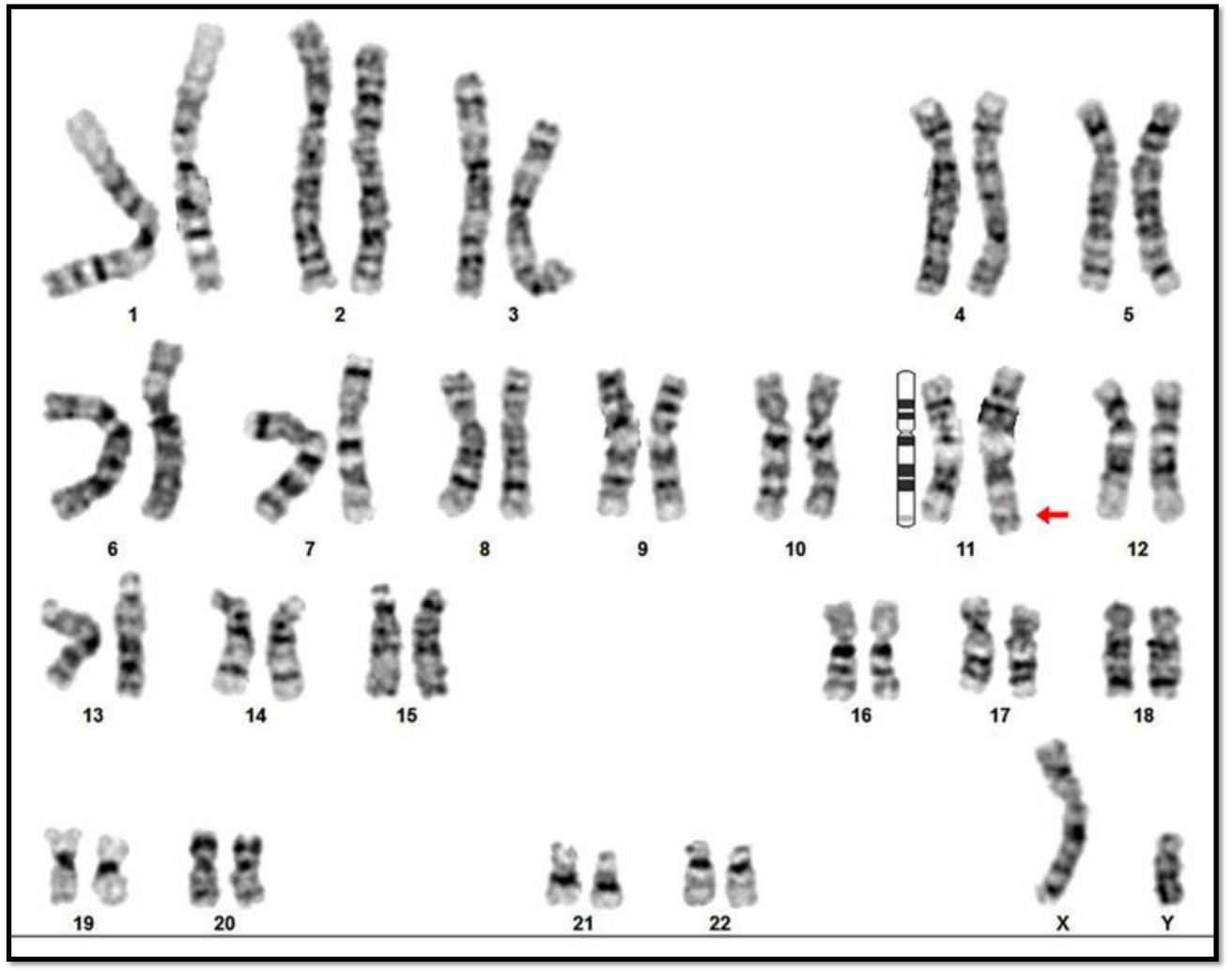
Turner syndrome

C **Turner Syndrome**

Missing X chromosome



Increased autoimmune disease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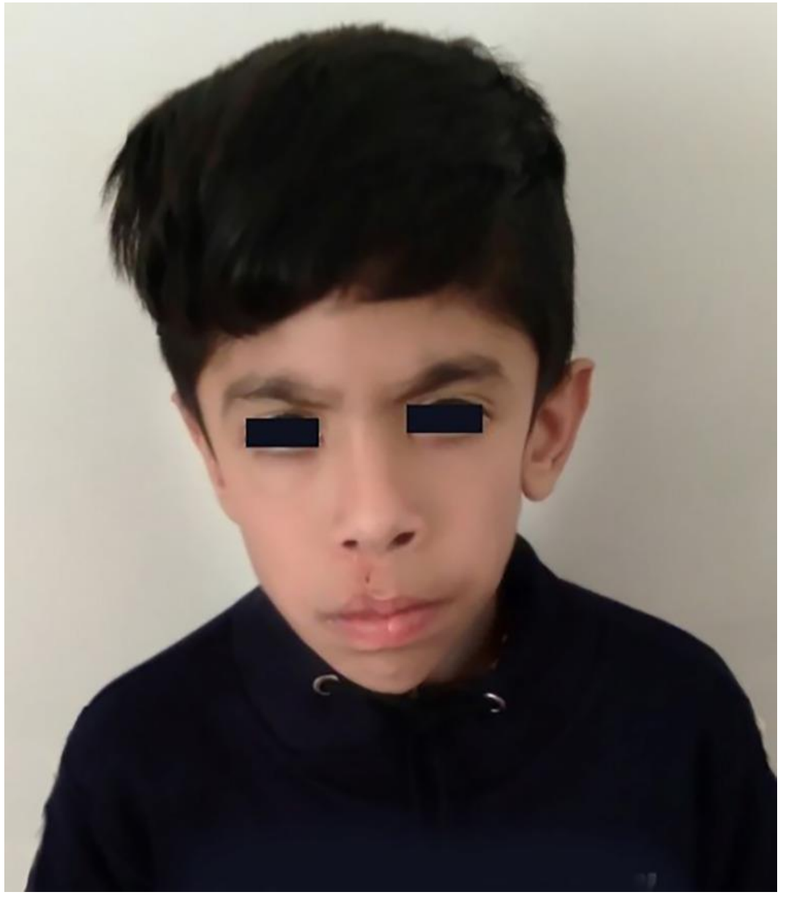

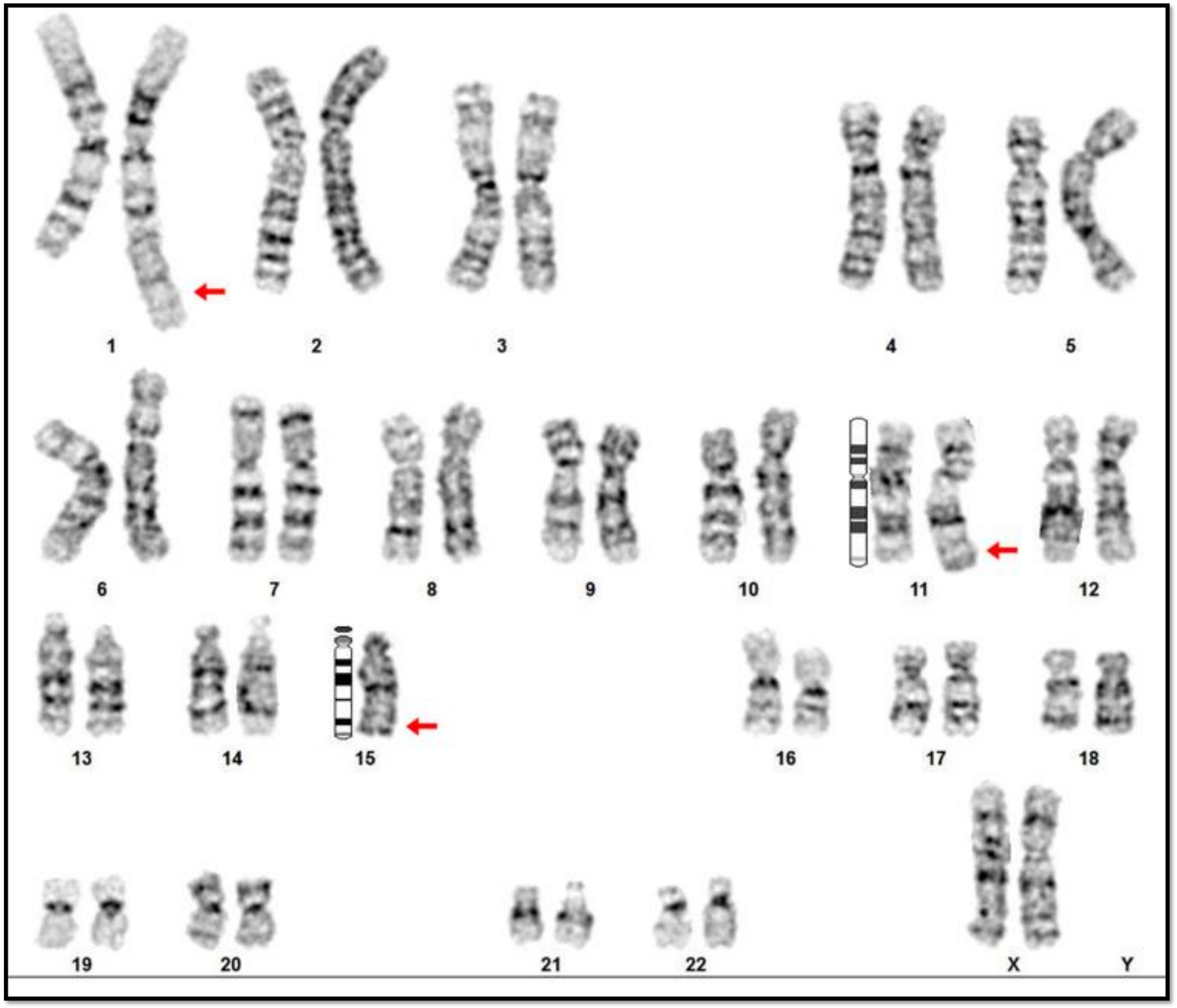
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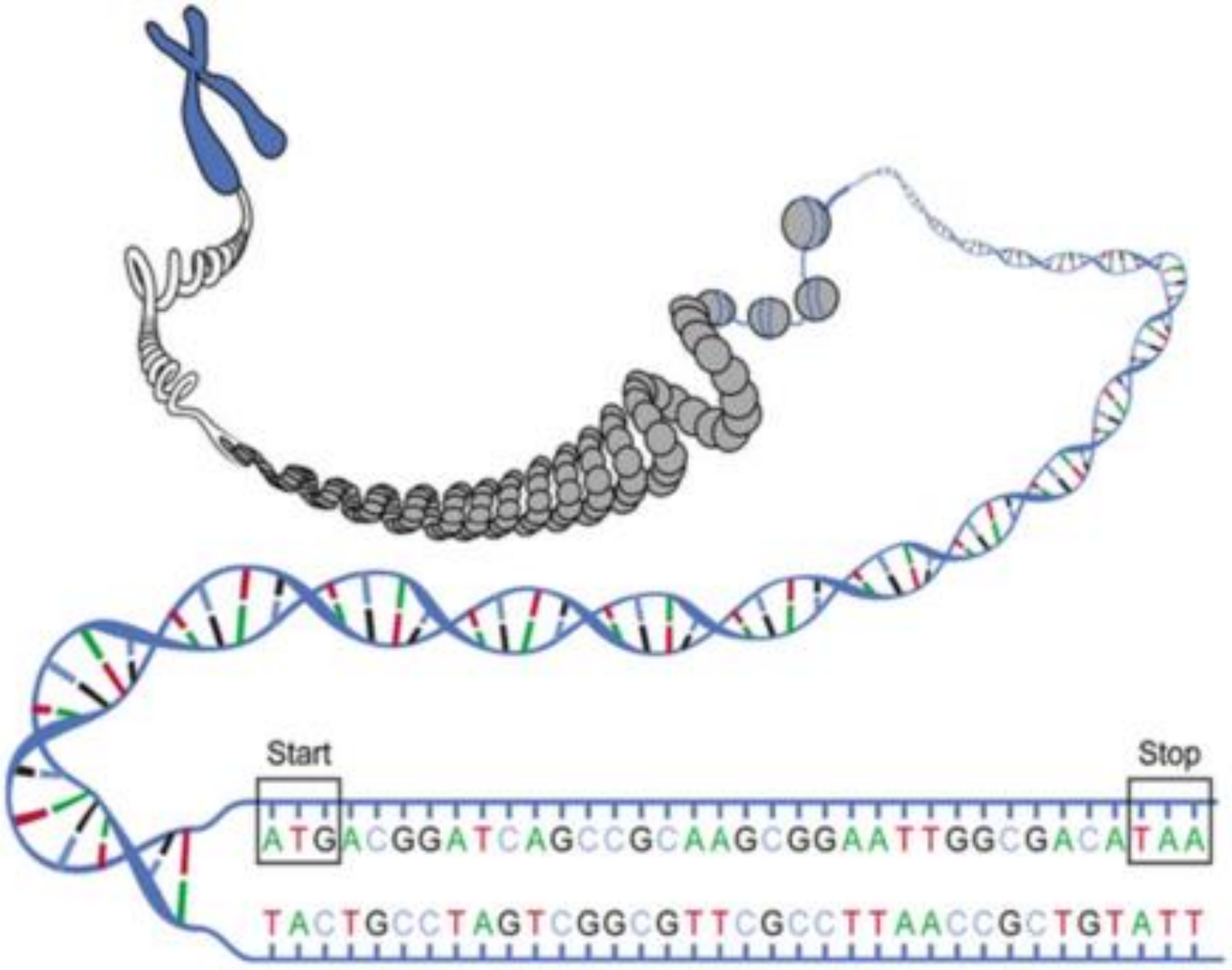
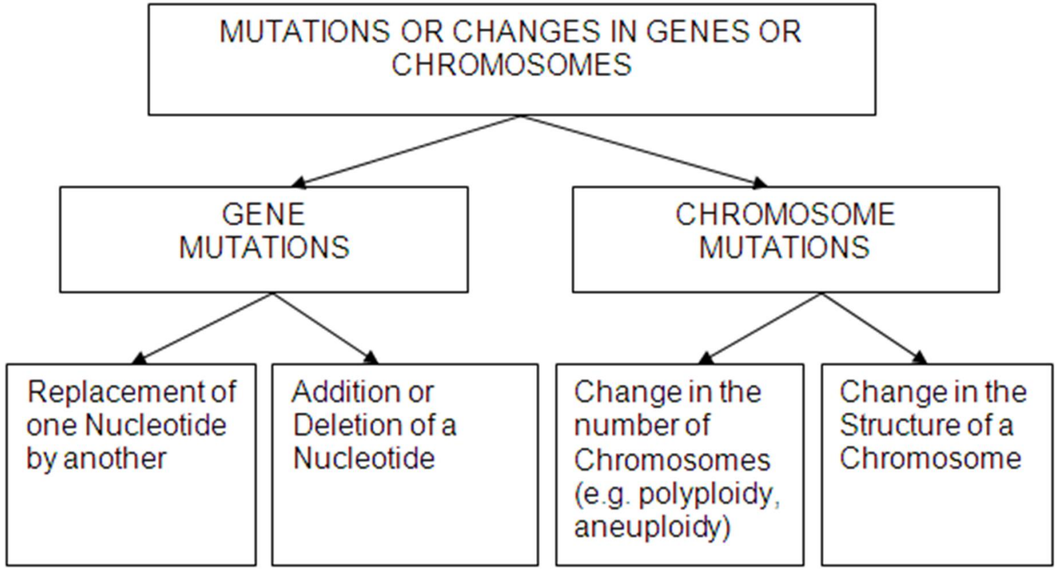
Genetics of Congenital Heart Disease: Defects Associated With Syndromes

CARDIOVASCULAR DISEASE	CHROMOSOMAL LOCATION	GENE(S) IMPLICATED*	COMMON CARDIAC DEFECTS
DiGeorge syndrome, velocardiofacial syndrome	22q11.2, 11p13p14	*TBX1*	TOF, IAA, TA, VSD
Familial ASD with heart block	5q35	*NKX2.5*	ASD, heart block
Familial ASD without heart block	8p22-23	*GATA4*	ASD
Alagille syndrome (bile duct hypoplasia, right-sided cardiac lesions)	20p12, 1p12	*JAGGED1*, *NOTCH2*	Peripheral pulmonary hypoplasia, PS, TOF
Holt-Oram syndrome (limb defects, ASD)	12q24	*TBX5*	ASD, VSD, PDA
Trisomy 21 (Down syndrome)	21q22	Not known	AVSD
Isolated familial AV septal defect (without trisomy 21)	1p31-p21, 3p25	*CRELD1*	AVSD
Familial TAPVR	4p13-q12	Not known	TAPVR
Noonan syndrome (PS, ASD, hypertrophic cardiomyopathy)	12q24, 12p1.21, 2p212, 3p25.2, 7q34, 15q22.31, 11p15.5, 1p13.2, 10q25.2, 11q23.3,17q11.2	*PTPN11*, *KRAS*, *SOS1*, *SOS2*, *RAF1*, *BRAF*, *MEK1*, *HRAS*, *NRAS*, *SHOC2*, *CBL*, *NF1*	PS, ASD, VSD, PDA, cardiomyopathy
Ellis–van Creveld syndrome (polydactyly, ASD)	4p16	*EVC*, *EVC2*	ASD, common atrium
Char syndrome (craniofacial, limb defects, PDA)	6p12-21.1	*TFAP2B*	PDA
Williams-Beuren syndrome (supravalvular AS, branch PS, hypercalcemia)	7q11.23	*ELN* (Elastin)	Supravalvular AS, peripheral PS
Marfan syndrome (connective tissue weakness, aortic root dilation)	15q21	Fibrillin	Aortic aneurysm, mitral valve disease
Familial laterality abnormalities	Xq24-2q7, 1q42, 9p13-21	*ZIC3*, *DNAI1*	Situs inversus, complex congenital heart disease

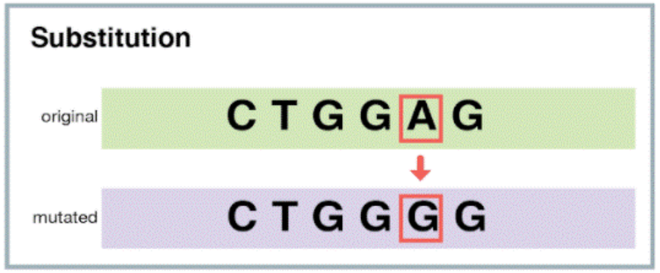
Molecular genetics





Main classes, groups and types of mutations and effects on protein products

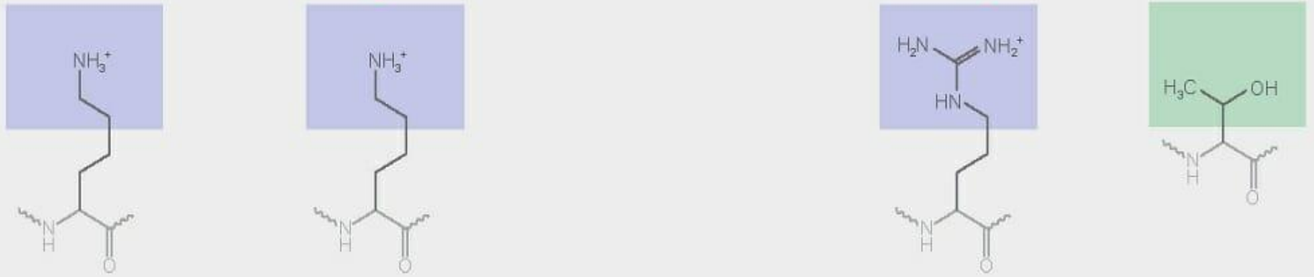
Class	Group	Type	Effect on Protein Product
Substitution	Synonymous	Silent[a]	Same amino acid
	Non-synonymous	Missense[a]	Altered amino acid—may affect protein function or stability
		Nonsense[a]	Stop codon—loss of function or expression because of degradation of mRNA
		Splice site	Aberrant splicing—exon skipping or intron retention
		Promoter	Altered gene expression
		Enhancer	Altered gene expression
Deletion	Multiple of three (codon)		In-frame deletion of one or more amino acid(s)—may affect protein function or stability
	Not multiple of three	Frameshift	Likely to result in premature termination with loss of function or expression
	Large deletion	Partial gene deletion	May result in premature termination with loss of function or expression
		Whole gene deletion	Loss of expression
Insertion	Multiple of three (codon)		In-frame insertion of one or more amino acid(s)—may affect protein function or stability
	Not multiple of three	Frameshift	Likely to result in premature termination with loss of function or expression
	Large insertion	Partial gene duplication	May result in premature termination with loss of function or expression
		Whole gene duplication	May have an effect because of increased gene dosage
	Expansion of trinucleotide repeat	Dynamic mutation	Altered gene expression or altered protein stability or function

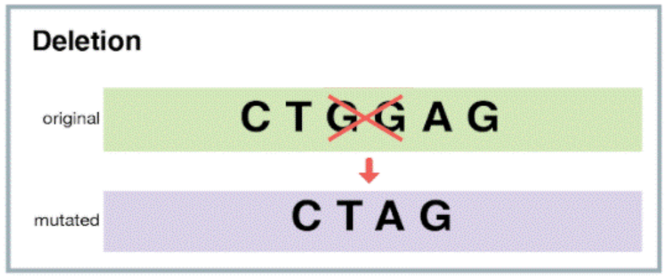


A substitution is the replacement of a single nucleotide by another.
This is the most common type of mutation.

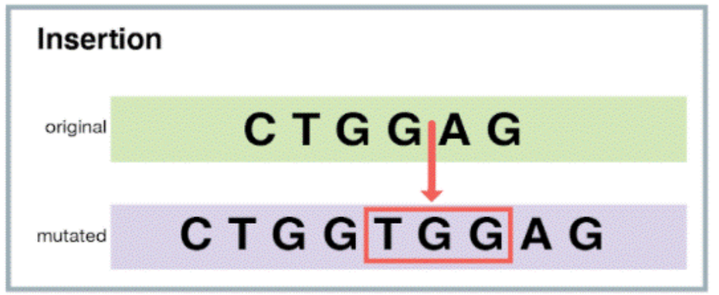
Transition
Transversion

	No mutation	Point mutations			
		Silent	Nonsense	Missense	
				conservative	non-conservative
DNA level	TTC	TTT	ATC	TCC	TGC
mRNA level	AAG	AAA	UAG	AGG	ACG
protein level	Lys	Lys	STOP	Arg	Thr

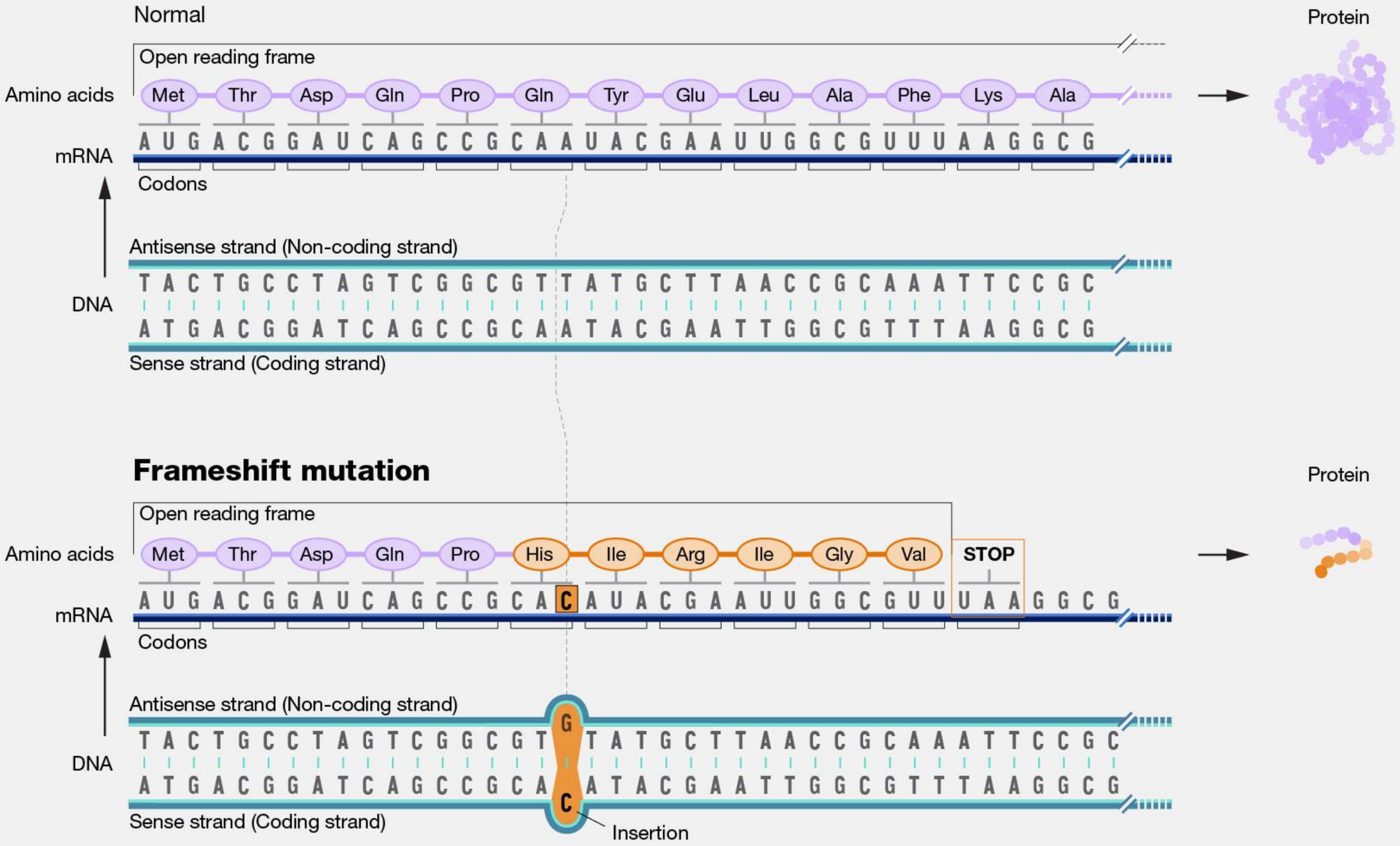




A deletion involves the loss of one or more nucleotides.
Frameshift Deletion of one or more residues



An insertion involves the addition of one or more nucleotides to a gene.
Frameshift insertion of one or more residues


Normal
Protein
Open reading frame
Amino acids
Met Thr Asp Gln Pro Gln Tyr Glu Leu Ala Phe Lys Ala
mRNA
A U G A C G G A U C A G C C G C A A U A C G A A U U G G C G U U U A A G G C G
Codons
DNA
Antisense strand (Non-coding strand)
T A C T G C C T A G T C G G C G T T A T G C T T A A C C G C A A A T T C C G C
A T G A C G G A T C A G C C G C A A T A C G A A T T G G C G T T T A A G G C G
Sense strand (Coding strand)
Frameshift mutation
Protein
Open reading frame
Amino acids
Met Thr Asp Gln Pro His Ile Arg Ile Gly Val STOP
mRNA
A U G A C G G A U C A G C C G C A C A U A C G A A U U G G C G U U U A A G G C G
Codons
DNA
Antisense strand (Non-coding strand)
T A C T G C C T A G T C G G C G T G T A T G C T T A A C C G C A A A T T C C G C
A T G A C G G A T C A G C C G C A C A T A C G A A T T G G C G T T T A A G G C G
Sense strand (Coding strand)
Insertion

Mutation nomenclature: examples of CFTR gene mutations

Type of Mutation	Nucleotide (Ref Seq NM_000492.3)	Protein Designation	Consequence Description
Missense	c.350G>A	p.Arg117His	Arginine to histidine
Nonsense	c.1624G>T	p.Gly542*	Glycine to stop
Splicing	c.489 + 1G>T		Splice donor site mutation
Deletion [1 base pair (bp)]	c.948delT	p.Phe316Leufs*12	Frameshift mutation
Deletion (3 bp)	c.1521_1523delCTT	p.Phe508del	In-frame deletion of phenylalanine
Insertion (1 bp)	c.3767dupC	p.Leu1258Phefs*7	Frameshift mutation

Mutations can be designated according to the genomic or complementary DNA (mRNA) sequence and are prefixed by "g." or "c.," respectively. The first base of the start codon (ATG) is c.1.

TEST REQUEST: Whole Exome Sequencing (WES)

CLINICAL INFORMATION: A 11-year-old symptomatic female with Muscular dystrophy, Movement disorders, no balance (until 5 years old), Developmental regression, Hearing and vision: normal, Heart muscle involvement, Echo: Diastolic Dysfunction, Mentally: normal. Her parents are relatives.

TEST RESULT:

GENE (OMIM)	VARIANT	EXON	ACMG CLASSIFICATION	ZYGOSITY	INHERITANCE	ASSOCIATED DISEASE (OMIM PHENOTYPE)
COL6A2 ***(120240)***	**NM_001849.4 c.2329T>C (p.Cys777Arg)**	**26**	**Likely Pathogenic**	**Homozygote**	**Autosomal Recessive**	**Ullrich congenital muscular dystrophy 1B (620727)**

INTERPRETATION & RECOMMENDATIONS:

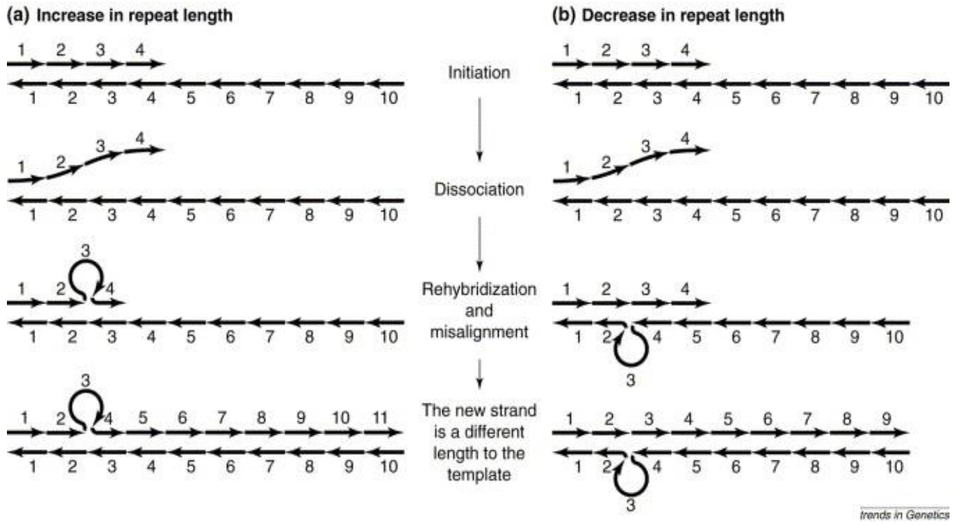
A homozygote likely pathogenic variant was identified in the COL6A2 gene. The genetic diagnosis of Ullrich congenital muscular dystrophy 1B is confirmed.

بر اساس نتایج این آزمایش، یک وریانت هموزیگوت شبه بیماریزا در ژن ***COL6A2*** شناسایی شد. وریانتهای بیماریزا در این ژن با ***Ullrich congenital muscular dystrophy 1B*** مرتبط است. جهت تایید یافته ی ژنتیکی فوق بررسی این وریانت در والدین توصیه می گردد. مشاوره ژنتیکی توصیه می گردد.

Ullrich congenital muscular dystrophy-1 (UCMD1) is characterized by generalized muscle weakness and striking hypermobility of distal joints in conjunction with variable contractures of more proximal joints and normal intelligence. Additional findings may include kyphoscoliosis, protruded calcanei, and follicular hyperkeratosis. Some patients manifest at birth and never achieve independent ambulation, whereas others maintain ambulation into adulthood. Progressive scoliosis and deterioration of respiratory function is a typical feature (summary by Kirschner, 2013).

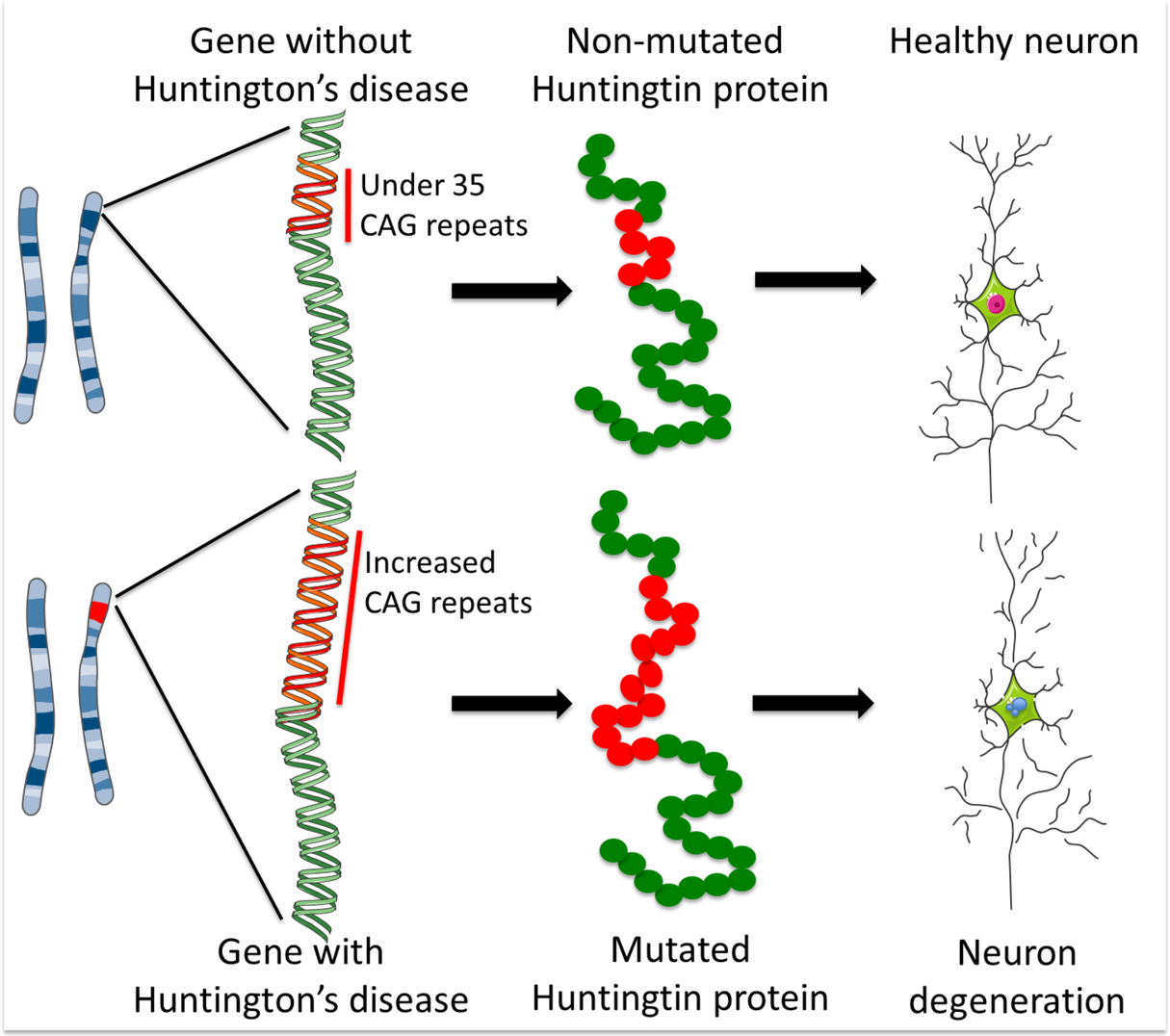
dynamic mutations

A number of single-gene disorders have subsequently been shown to be associated with triplet repeat expansions.



Disease (Gene)	Repeat Sequence	Normal Range (Repeats)	Pathogenic Range (Repeats)	Repeat Location
Huntington disease (*HTT*)	CAG	9–35	36–100	Coding
Myotonic dystrophy type 1 (*DMPK*)	CTG	5–35	50–4000	3′ UTR
Myotonic dystrophy type 2 (*CNBP*)	CCTG	11–26	75–>11000	Intron 1
Fragile X site A (*FMR1*)	CGG	10–50	200–2000	5′ UTR
Kennedy disease (*AR*)	CAG	13–30	40–62	Coding
Spinocerebellar ataxia 1 (*ATXN1*)	CAG	6–36	39–80	Coding
Spinocerebellar ataxia 2 (*ATXN2*)	CAG	13–31	32–79	Coding
Machado–Joseph disease/Spinocerebellar ataxia 3 (*ATXN3*)	CAG	14–44	52–86	Coding
Spinocerebellar ataxia 6 (*CACNA1A*)	CAG	4–18	19–33	Coding
Spinocerebellar ataxia 7 (*ATXN7*)	CAG	7–17	38–220	Coding
Spinocerebellar ataxia 8 (*ATXN8*)	CTG	15–50	71–1300	3′ UTR
Spinocerebellar ataxia 10 (*ATXN10*)	ATTCT	10–29	400–4500	Intron 9
Spinocerebellar ataxia 12 (*PPP2R2B*)	CAG	7–32	51–78	5′ UTR
Spinocerebellar ataxia 17 (*TBP*)	CAG	25–44	47–63	Coding
Dentatorubral-pallidoluysian atrophy	CAG	7–23	53–88	Coding

Huntington disease patients exhibit a high rate of cardiac events, with **heart failure** being the second leading cause of death among HD patients (accounting for 20–30% of HD deaths).

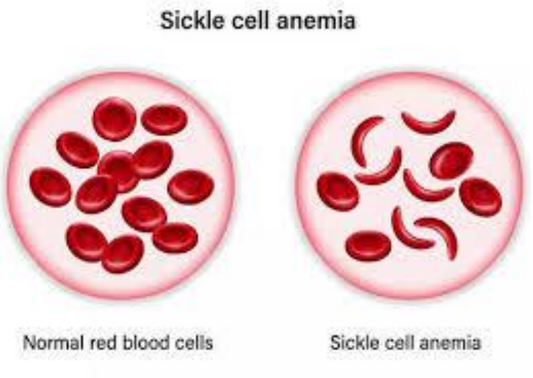


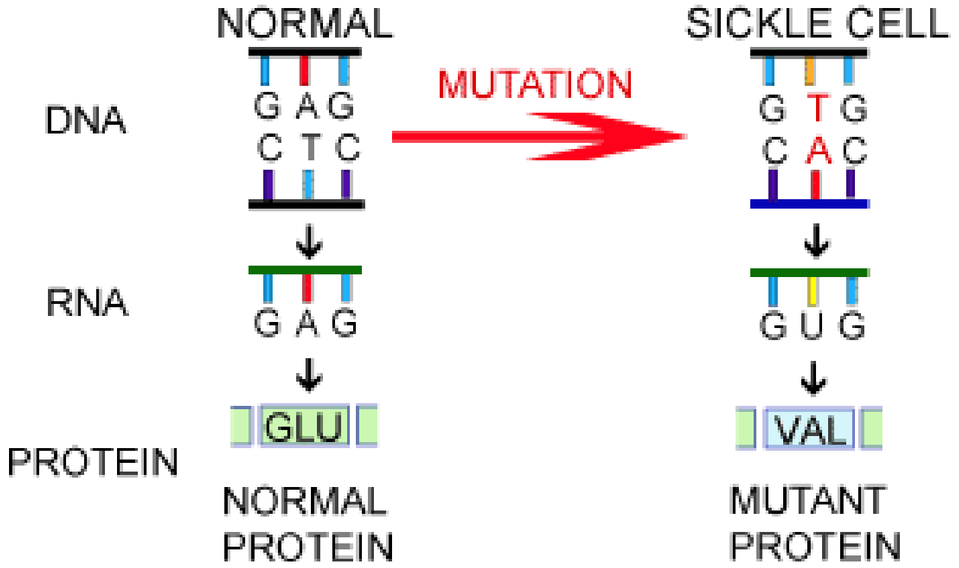
Frequency of different types of mutation

Type of Mutation	Percentage of Total
Missense or nonsense	56
Splicing	9
Regulatory	2
Small deletions, insertions or indels[a]	23
Gross deletions or insertions	9
Other (complex rearrangements or repeat variations)	<1

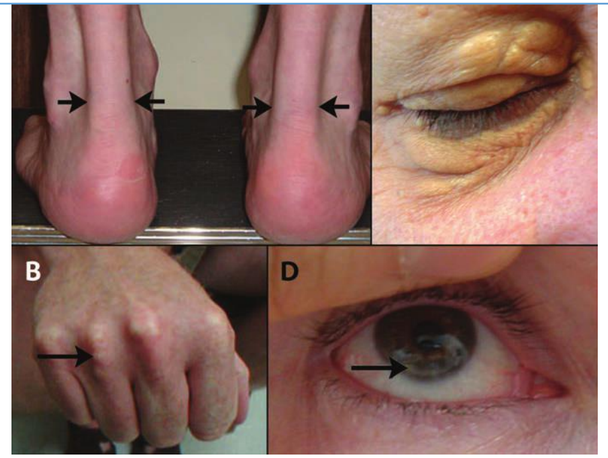
Example of Missense mutation

Sickle cell anemia





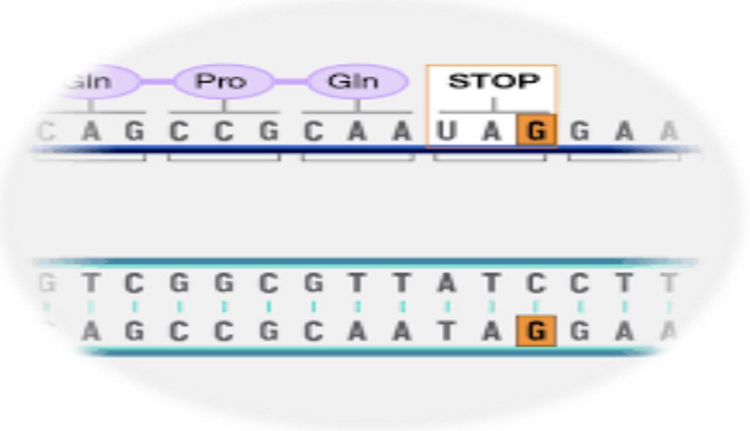
Familial hypercholesterolemia

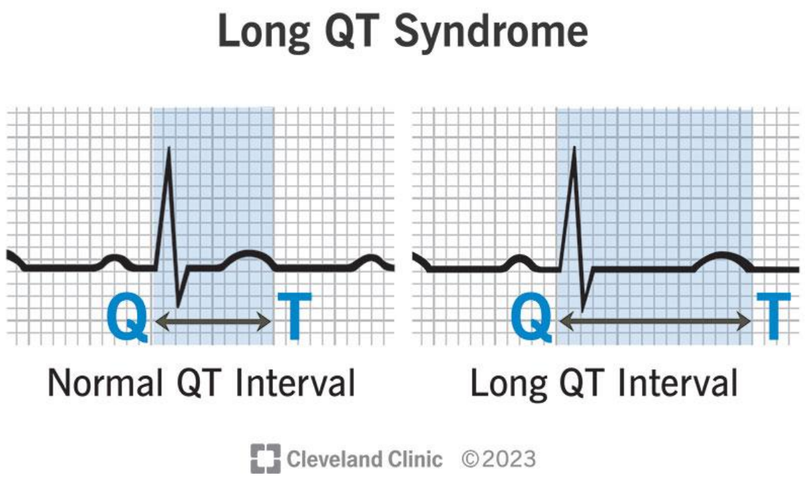


c.681G>A (p.Gly227Arg)

The most common missense mutation in the LDLR

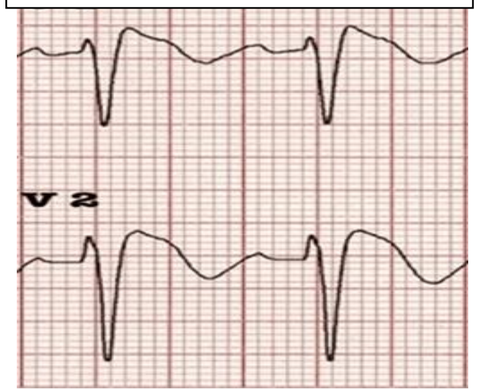
Example of Nonsense mutation





The R518X mutation in the KCNQ1 gene

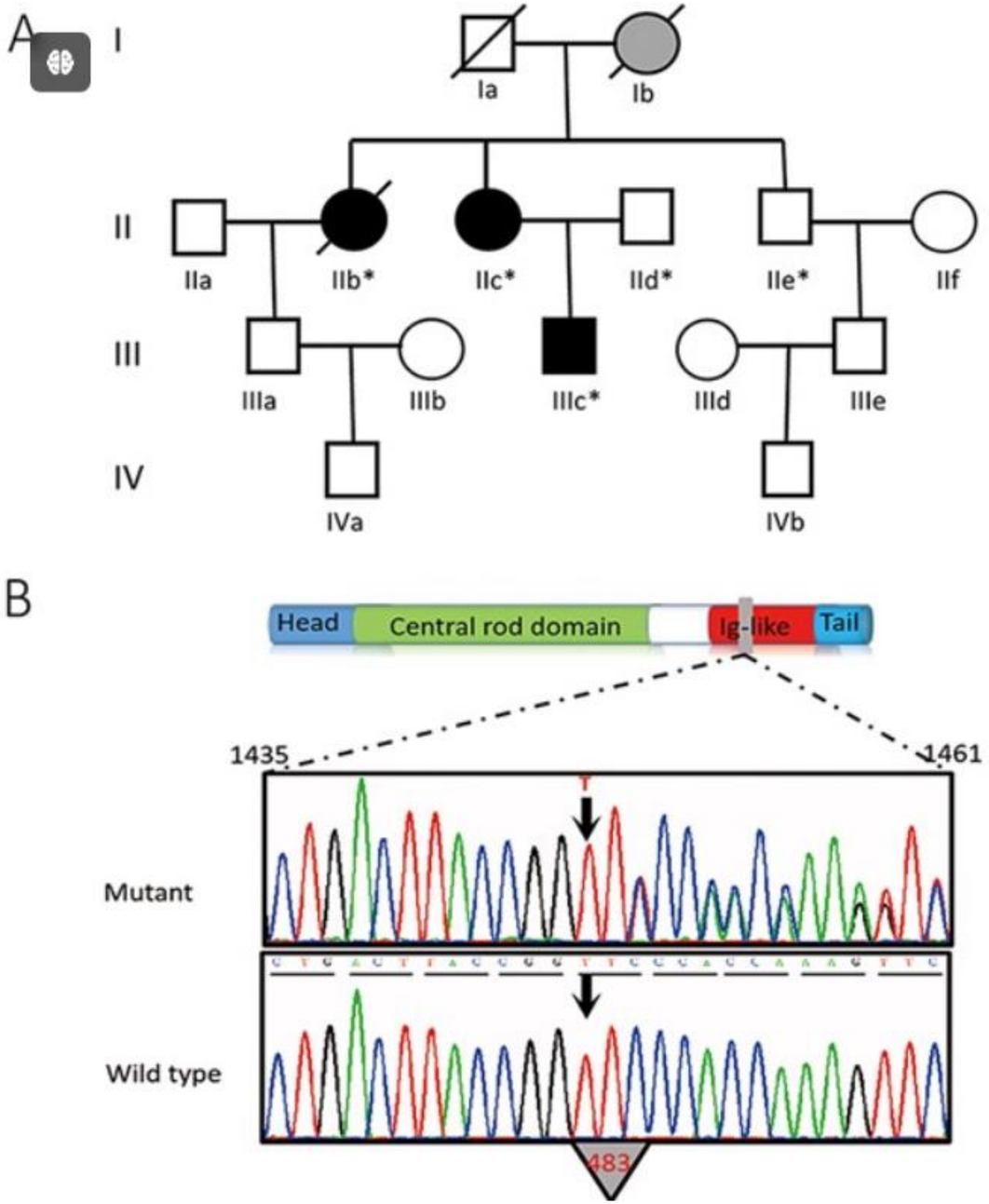
Brugada syndrome



The W156X mutation in the SCN5A gene

Example of Frameshift mutation

LMNA frameshift mutation, p.P485Tfs*67, from a patient with early-onset atrial disease.



THANK YOU