B.PHARM SEMESTER II | BP202T

BIOCHEMISTRY (THEORY)

UNIT V

Nucleic Acid Metabolism and Genetic Information Transfer

Short Notes for Quick Revision

What is covered in this unit

UNIT V TOPICS

Part A – Nucleotide metabolism: purine and pyrimidine biosynthesis, catabolism of purines and uric acid formation, hyperuricaemia and gout, and related disorders.

Part B – Genome and central dogma: organisation of the mammalian genome, DNA replication, transcription and translation.

Part C – Genetic code and regulation: properties of the genetic code, regulation of gene expression, inhibitors of transcription and translation (antibiotics and toxins).

Part D – DNA repair: types of DNA damage, repair mechanisms and disorders of faulty DNA repair.

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PART A: NUCLEOTIDE METABOLISM AND RELATED DISORDERS

1. Nucleotides and Nucleic Acids: Basic Structure

Nucleotides are the monomers of nucleic acids (DNA and RNA). Each nucleotide has three parts: a **nitrogenous base** (purine or pyrimidine), a **pentose sugar** (ribose in RNA, 2′-deoxyribose in DNA) and **one or more phosphate groups**. A **nucleoside** is a base plus a sugar (no phosphate); a **nucleotide** is a nucleoside plus phosphate attached at the 5′ carbon of the sugar. Nucleotides are linked by **3′→5′ phosphodiester bonds** to form a polynucleotide chain with a 5′ end and a 3′ end.

Base	Type	Nucleoside	Nucleotide	Found in
Adenine (A)	Purine	Adenosine / Deoxyadenosine	AMP / dAMP	DNA and RNA
Guanine (G)	Purine	Guanosine / Deoxyguanosine	GMP / dGMP	DNA and RNA
Cytosine (C)	Pyrimidine	Cytidine / Deoxycytidine	CMP / dCMP	DNA and RNA
Thymine (T)	Pyrimidine	Deoxythymidine	dTMP	DNA only (5-methyluracil)
Uracil (U)	Pyrimidine	Uridine	UMP	RNA only

DNA and RNA compared

Feature	DNA	RNA
Sugar	2′-Deoxyribose	Ribose (extra 2′-OH, makes RNA less stable)
Pyrimidines	Cytosine, Thymine	Cytosine, Uracil
Strands	Double-stranded antiparallel **double helix** (Watson and Crick, 1953)	Usually single-stranded (can fold, e.g. tRNA)
Base pairing	A = T (2 hydrogen bonds); G ≡ C (3 hydrogen bonds). **Chargaff's rule:** A = T and G = C	A = U, G ≡ C in folded regions
Location	Nucleus (chromatin), mitochondria	Nucleus, nucleolus, cytoplasm, ribosomes
Function	Storage of genetic information	Transfer of information and protein synthesis (mRNA, tRNA, rRNA); regulation
Types	Genomic, mitochondrial	mRNA (about 5%), tRNA (about 15%), rRNA (about 80%), snRNA, miRNA, siRNA, lncRNA

- **B-DNA (most common):** right-handed, 10 base pairs per turn, 3.4 nm pitch, 2 nm diameter, with major and minor grooves. **A-DNA** is shorter and wider (RNA-DNA hybrids); **Z-DNA** is left-handed (GC-rich, zig-zag).
- **Tm (melting temperature):** temperature at which half the DNA is single-stranded; higher with more G≡C pairs. DNA absorbs UV light at **260 nm** (proteins at 280 nm); purity is judged by the **A260/A280 ratio (about 1.8 for pure DNA, 2.0 for RNA)**. Single-stranded DNA absorbs more (hyperchromic effect).

Biological roles of nucleotides other than in nucleic acids

Nucleotide	Role
ATP, GTP	Energy currency; GTP drives protein synthesis and G-protein signalling
cAMP, cGMP	Second messengers of hormone action
NAD^+, $NADP^+$, FAD, coenzyme A	Nucleotide-containing coenzymes of redox and acyl transfer reactions
UDP-glucose, UDP-glucuronate, CDP-choline, SAM	Activated carriers in glycogen and phospholipid synthesis, conjugation and methylation
PRPP, PAPS	Activated ribose phosphate and activated sulphate
Allosteric regulators	ATP, ADP, AMP regulate glycolysis and TCA cycle enzymes

2. Biosynthesis of Purine Nucleotides

Purine nucleotides are made by two routes: the **de novo pathway** (from simple precursors, mainly in the **liver**) and the **salvage pathway** (re-use of preformed bases, in most tissues). The brain, bone marrow, erythrocytes and neutrophils depend largely on salvage. De novo synthesis is costly (about 6 high-energy phosphate bonds per purine), so salvage saves energy.

Sources of the atoms of the purine ring

Atom(s) of ring	Source
N-1	Aspartate (amino group)
C-2 and C-8	**N^{10}-Formyl-tetrahydrofolate (one-carbon units)**
N-3 and N-9	**Glutamine** (amide nitrogen)
C-4, C-5 and N-7	**Glycine** (whole molecule)
C-6	**Carbon dioxide (CO_2)**

De novo synthesis of purines (steps)

The purine ring is built **on ribose 5-phosphate**, not as a free base. The first completed purine nucleotide is **inosine monophosphate (IMP)**, which is converted to AMP and GMP.

Step	Reaction	Enzyme / remark
1	Ribose 5-phosphate + ATP → **5-Phosphoribosyl-1-pyrophosphate (PRPP)** + AMP	**PRPP synthetase** (activated by Pi, inhibited by ADP and GDP). Needed also for salvage and pyrimidine synthesis
2	PRPP + Glutamine → **5-Phosphoribosylamine** + Glutamate + PPi	**Glutamine-PRPP amidotransferase (GPAT)**: the **committed and rate-limiting step**; inhibited by IMP, AMP and GMP
3–9	Addition of glycine, formyl group (from N^{10}-formyl-THF), glutamine nitrogen, ring closure, CO_2, aspartate (then loss of fumarate), a second formyl group and final ring closure	Gives **IMP** (hypoxanthine nucleotide). Needs **glutamine, glycine, aspartate, CO_2, folate**; about 5 ATP
10a	IMP + Aspartate + **GTP** → Adenylosuccinate → **AMP** (+ fumarate)	Adenylosuccinate synthetase and lyase. **GTP drives AMP formation**
10b	IMP + NAD^+ → **Xanthosine monophosphate (XMP)** → **GMP**	**IMP dehydrogenase**, then GMP synthetase (glutamine, **ATP** drives GMP formation). IMP dehydrogenase is blocked by **mycophenolate**
11	AMP → ADP → ATP; GMP → GDP → GTP	Kinases (adenylate kinase, nucleoside diphosphate kinase)

Regulation of purine synthesis

- **Feedback inhibition** of PRPP synthetase and GPAT by the end products IMP, AMP and GMP (and ADP, GDP).
- **Reciprocal control of AMP and GMP:** GTP is needed for AMP synthesis and ATP for GMP synthesis, so the two nucleotides stay balanced.
- **PRPP level** (substrate availability) controls the rate: more PRPP increases purine output.

Salvage pathway

- Free purine bases from degradation of nucleic acids (and the diet) are re-attached to PRPP: **adenine + PRPP → AMP + PPi** by **adenine phosphoribosyltransferase (APRT)**; **hypoxanthine or guanine + PRPP → IMP or GMP + PPi** by **hypoxanthine-guanine phosphoribosyltransferase (HGPRT)**.
- Salvage uses up PRPP and restores nucleotides cheaply. When HGPRT is absent, PRPP accumulates and purines are over-produced and degraded to uric acid.

LESCH–NYHAN SYNDROME

Complete deficiency of HGPRT (X-linked recessive; boys). PRPP rises, GPAT is not inhibited, so **de novo synthesis is greatly increased** and uric acid is produced in excess. **Features:** hyperuricaemia, gout and uric acid stones, **self-mutilation (biting lips and fingers)**, choreoathetosis and spasticity, intellectual disability, aggression. Onset in infancy (orange 'sand' of uric acid crystals in diapers). **Treatment: allopurinol** controls uric acid but not the neurological signs. Partial HGPRT deficiency = **Kelley–Seegmiller syndrome** (gout, no neurological features).

Deoxyribonucleotide formation

- **Ribonucleotide reductase** converts ribonucleoside **diphosphates (ADP, GDP, CDP, UDP)** to deoxyribonucleoside diphosphates, removing the 2′-OH group. It needs **thioredoxin** (reduced by thioredoxin reductase using NADPH).
- **Regulation:** activity is switched on by ATP and off by dATP; substrate specificity is set by other nucleotides, giving a balanced supply of the four dNTPs for DNA synthesis. **Hydroxyurea** inhibits the enzyme (used in chronic myeloid leukaemia, sickle cell anaemia, polycythaemia vera).

Drugs acting on purine synthesis

Drug	Mechanism	Use
Methotrexate, aminopterin	Inhibit **dihydrofolate reductase** (DHFR), so less tetrahydrofolate for C-2 and C-8 of purines and for thymidylate	Cancer, rheumatoid arthritis, psoriasis
6-Mercaptopurine, azathioprine, thioguanine	Converted by HGPRT to a false nucleotide (thio-IMP) that blocks purine synthesis (feedback inhibition of GPAT, block of IMP conversion)	Leukaemia, immunosuppression (azathioprine; allopurinol raises its toxicity as xanthine oxidase breaks down 6-MP)
Azaserine, 6-diazo-5-oxo-norleucine (DON)	Glutamine analogues; inhibit glutamine-dependent steps	Experimental
Mycophenolic acid	Inhibits **IMP dehydrogenase** (GMP synthesis)	Immunosuppression in transplantation
Sulphonamides	Block bacterial folate synthesis (PABA analogues)	Antibacterial; human cells take folate from diet
Hydroxyurea	Inhibits ribonucleotide reductase	CML, sickle cell disease
Ribavirin, acyclovir, zidovudine	Nucleoside analogues that inhibit viral nucleic acid synthesis	Antiviral

3. Biosynthesis of Pyrimidine Nucleotides

In contrast to purines, the **pyrimidine ring is assembled first** (as orotate) and then attached to ribose 5-phosphate (from PRPP). The ring atoms come from **glutamine (N-3), CO_2 (C-2) and aspartate (N-1, C-4, C-5, C-6)**. De novo synthesis occurs in the **cytosol** except for one enzyme (dihydroorotate dehydrogenase) in the inner mitochondrial membrane.

Step	Reaction	Enzyme / remark
1	Glutamine + CO_2 + 2 ATP → **Carbamoyl phosphate**	**Carbamoyl phosphate synthetase II (CPS-II)** (cytosol): **regulated step** in animals; inhibited by **UTP**, activated by PRPP and ATP. (CPS-I is mitochondrial, uses ammonia, and serves the urea cycle.)
2	Carbamoyl phosphate + Aspartate → **Carbamoyl aspartate**	**Aspartate transcarbamoylase (ATCase)** (the regulated step in bacteria; inhibited by CTP)
3	Carbamoyl aspartate → **Dihydroorotate**	**Dihydroorotase**. Steps 1–3 are on one multifunctional protein, **CAD**
4	Dihydroorotate → **Orotate**	**Dihydroorotate dehydrogenase** (mitochondrial; inhibited by **leflunomide**, **teriflunomide**)
5	Orotate + PRPP → **Orotidine monophosphate (OMP)** → **UMP**	**Orotate phosphoribosyltransferase** and **OMP decarboxylase** (a single bifunctional protein, **UMP synthase**, in humans)
6	UMP → UDP → **UTP**; UTP + Glutamine + ATP → **CTP**	Kinases; **CTP synthetase** (inhibited by CTP)
7	UDP → **dUDP** → dUMP; **dUMP + N^5,N^{10}-methylene-THF → dTMP**	Ribonucleotide reductase; **thymidylate synthase** (uses folate; DHF made is recycled by DHFR)

Regulation

- In humans the main control point is **CPS-II** (feedback inhibited by UTP, activated by PRPP). **PRPP** and **ATP** raise the rate. CTP synthetase is controlled by CTP.
- Purine and pyrimidine syntheses are **coordinated**: both need PRPP, glutamine and aspartate, and the ATP/GTP and UTP/CTP pools adjust each other.

Pyrimidine salvage and degradation

- **Salvage:** uridine and thymidine are phosphorylated by **uridine and thymidine kinases**; **orotate** and uracil are reused with PRPP.
- **Degradation:** unlike purines, pyrimidine rings are **opened and broken down to soluble products**: cytosine and uracil → **β-alanine, CO_2 and NH_3**; thymine → **β-aminoisobutyrate**, CO_2 and NH_3. These dissolve in water, so pyrimidine breakdown does not cause gout. (β-Aminoisobutyrate is raised in leukaemia and after radiation.)

Disorders of pyrimidine metabolism

Disorder	Defect	Features
Hereditary orotic aciduria	**UMP synthase** (orotate phosphoribosyltransferase and OMP decarboxylase); autosomal recessive	Orotic acid in urine (crystals), **megaloblastic anaemia not responding to B_{12} or folate**, growth retardation. **Treatment: oral uridine** (bypasses the block, gives UMP and also feedback-inhibits CPS-II)
Orotic aciduria in OTC deficiency	Ornithine transcarbamoylase deficiency (urea cycle)	Carbamoyl phosphate accumulates in the mitochondria and enters pyrimidine synthesis; orotic acid rises with **hyperammonaemia**
Dihydropyrimidine dehydrogenase deficiency	Defect of pyrimidine degradation	**Severe toxicity from 5-fluorouracil** (cannot be broken down); neurological signs in some
Reye syndrome	Mitochondrial injury	Secondary orotic aciduria

Drugs acting on pyrimidine and thymidylate synthesis

Drug	Mechanism
5-Fluorouracil (5-FU)	Converted to FdUMP, which **irreversibly inhibits thymidylate synthase** (suicide inhibition), causing 'thymineless death'. Used in colon, breast cancers
Methotrexate	Inhibits DHFR, so dTMP synthesis falls (S-phase specific); leucovorin (folinic acid) rescues normal cells
Trimethoprim	Inhibits bacterial DHFR (given with sulphamethoxazole as co-trimoxazole); pyrimethamine for malaria and toxoplasmosis
Cytarabine, gemcitabine	Cytosine nucleoside analogues; inhibit DNA polymerase and ribonucleotide reductase
Leflunomide	Inhibits dihydroorotate dehydrogenase (rheumatoid arthritis)
Zidovudine (AZT), stavudine	Thymidine analogues; chain terminators for HIV reverse transcriptase

4. Catabolism of Purine Nucleotides and Uric Acid Formation

Dietary and tissue nucleic acids are hydrolysed by nucleases and nucleotidases to nucleosides, bases and finally **uric acid**, the end product of purine catabolism in humans and other primates (other mammals have **uricase**, which converts uric acid to the more soluble allantoin; birds and reptiles excrete uric acid). Degradation occurs mainly in the **liver, intestinal mucosa and, for the final step, tissues rich in xanthine oxidase (liver, intestine)**.

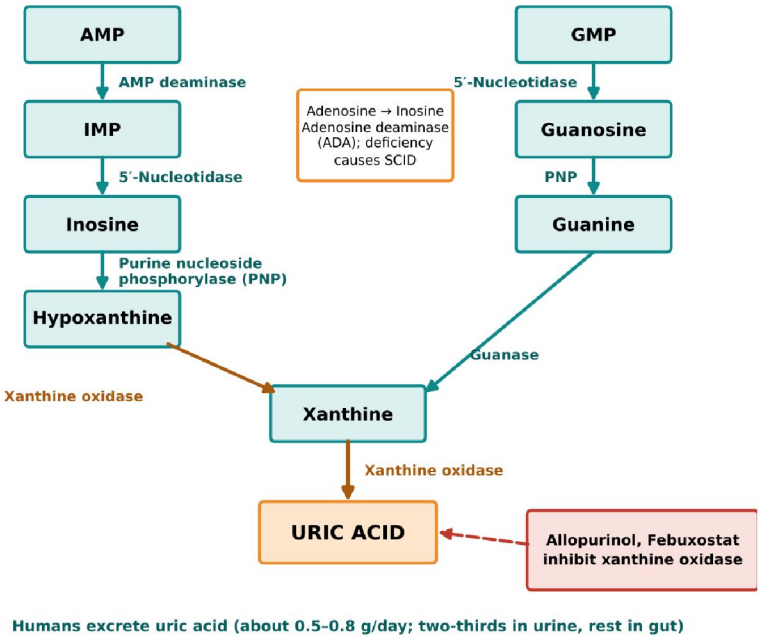

Figure 1: Figure 1: Catabolism of purine nucleotides to uric acid and sites of drug action

- **AMP** is deaminated to **IMP** (AMP deaminase) or dephosphorylated to adenosine, which **adenosine deaminase (ADA)** converts to inosine. **IMP** → inosine → **hypoxanthine** (purine nucleoside phosphorylase, PNP) → **xanthine** → **uric acid** (xanthine oxidase, a molybdenum- and FAD-containing enzyme that forms H_2O_2).
- **GMP** → guanosine → **guanine** (PNP) → **xanthine** (guanase) → **uric acid**.
- **Fate of uric acid:** filtered, largely reabsorbed and then secreted by the proximal tubule (URAT1, the main reabsorption carrier); about **two-thirds is excreted in urine** and one-third into the gut, where bacteria degrade it (uricolysis). Uric acid has a pKa of 5.8, so it is **poorly soluble in acid urine** (as undissociated uric acid) and crystallises easily.
- **Normal serum uric acid:** about **3.5–7.2 mg/dL in men** and **2.6–6.0 mg/dL in women** (solubility limit about 6.8–7 mg/dL). Urinary excretion is about 0.5–0.8 g/day. It is a powerful **antioxidant** in plasma.

Disorders due to enzyme defects in purine catabolism

Disorder	Defect	Features
Severe combined immunodeficiency (SCID)	**Adenosine deaminase (ADA)** deficiency; autosomal recessive	Adenosine and deoxyadenosine (dATP) accumulate and kill T and B lymphocytes. Recurrent severe infections from infancy. Treatment: bone marrow transplantation, **enzyme replacement (PEG-ADA)** and **first gene therapy** in humans (1990)
PNP deficiency	Purine nucleoside phosphorylase	T-cell immunodeficiency; low uric acid
Xanthinuria	Xanthine oxidase	Low uric acid, high xanthine; xanthine stones
Lesch–Nyhan syndrome	HGPRT (salvage)	See Section 2
von Gierke disease (GSD type I)	Glucose-6-phosphatase	Hyperuricaemia: lactic acid blocks uric acid excretion and more ribose 5-phosphate and PRPP is made

Hyperuricaemia and gout

Hyperuricaemia is a serum uric acid above the solubility limit (above about 7 mg/dL). **Gout** is the clinical disease in which monosodium urate crystals are deposited in joints and tissues and cause inflammation. Only a minority of hyperuricaemic people develop gout.

Causes of hyperuricaemia

Type	Causes
Primary (idiopathic, usually under-excretion; 90% of cases)	Inherited **reduced renal excretion** (URAT1/GLUT9 variants); **over-production** from enzyme defects: **PRPP synthetase superactivity** (increased PRPP), partial HGPRT deficiency (Kelley–Seegmiller), Lesch–Nyhan syndrome, GPAT feedback-resistance

Type	Causes
Secondary: over-production	**High cell turnover:** leukaemias, lymphomas, polycythaemia, psoriasis, haemolytic anaemia, **tumour lysis syndrome** (chemotherapy); high purine diet (organ meats, sardines, red meat, beer, fructose); glycogen storage disease type I; alcohol (raises ATP breakdown)
Secondary: under-excretion	**Chronic kidney disease**, lactic acidosis and ketoacidosis (organic acids compete for secretion), **drugs:** thiazide and loop diuretics, low-dose aspirin, pyrazinamide, ethambutol, cyclosporine, levodopa; lead poisoning; hypothyroidism, hyperparathyroidism; dehydration; obesity; hypertension

Clinical features of gout

- **Acute gouty arthritis:** sudden, severe pain, redness, swelling and heat, classically of the **first metatarsophalangeal joint (podagra)**, often at night, after alcohol, a heavy meal, injury or diuretics. Crystals are phagocytosed by neutrophils, which activate the **NLRP3 inflammasome and release IL-1β**.
- **Chronic tophaceous gout: tophi** (deposits of urate crystals) in ear helix, elbows, tendons and joints; joint destruction.
- **Uric acid kidney stones** (acid urine), urate nephropathy, and chronic kidney disease.
- **Diagnosis:** needle-shaped, **negatively birefringent** monosodium urate crystals in synovial fluid under polarised light (**pseudogout** = calcium pyrophosphate crystals, rhomboid and weakly positively birefringent); raised serum uric acid (may be normal during an attack); punched-out erosions on X-ray.

Treatment of gout

Aim / drug	Mechanism and notes
Acute attack: NSAIDs (indomethacin, naproxen), colchicine, glucocorticoids	Reduce inflammation. **Colchicine** binds tubulin and stops neutrophil migration (side effects: diarrhoea). **Aspirin is avoided** (low doses retain uric acid). Do not start urate-lowering drugs during an attack (may prolong it)
Xanthine oxidase inhibitors: allopurinol, febuxostat	Allopurinol (a hypoxanthine analogue) is converted by xanthine oxidase to **alloxanthine (oxypurinol)**, which stays bound to the enzyme (suicide inhibition). Hypoxanthine and xanthine are more soluble and excreted. Dose is reduced in renal failure; HLA-B 5801 carriers are at risk of severe skin reaction. Raises the toxicity of azathioprine and 6-MP (reduce their dose)
Uricosuric drugs: probenecid, sulfinpyrazone, benzbromarone, lesinurad	Inhibit renal tubular reabsorption (URAT1), raising urinary urate; need good hydration and alkaline urine. **Not effective in renal failure**; ineffective at low doses of salicylates which block them
Uricase therapy: rasburicase, pegloticase	Convert uric acid to allantoin; used in tumour lysis syndrome and refractory gout
Diet and lifestyle	Avoid alcohol (especially beer), organ meats, shellfish, sugary drinks; reduce weight; plenty of fluids; low-fat dairy; urine alkalinisation (potassium citrate) for stones

REMEMBER

Allopurinol is used for **prevention** (chronic), colchicine and NSAIDs for the **acute** attack. In **Lesch–Nyhan syndrome** allopurinol controls gout, but not the self-injury. Uric acid is made by **xanthine oxidase**, so inhibiting it raises hypoxanthine and xanthine and spares PRPP re-use through salvage.

PART B: GENOME STRUCTURE AND THE CENTRAL DOGMA

5. Organization of the Mammalian Genome

The **genome** is the complete set of DNA in a cell. The **human nuclear genome** has about **3.2 billion base pairs (3.2 Gb)** in **23 pairs of chromosomes** (22 autosomes and XY or XX) and about **20,000–25,000 protein-coding genes**. Only about **1.5–2% of the genome codes for proteins**; the rest includes introns, regulatory sequences, repetitive DNA and non-coding RNA genes. If stretched out, the DNA in one diploid cell is about 2 metres long, yet it fits in a nucleus about 6 µm wide because of **packaging into chromatin**.

Levels of DNA packaging (chromatin)

Level	Structure	Details
1	**DNA double helix**	2 nm wide
2	**Nucleosome** ('beads on a string')	About **147 base pairs of DNA wound 1.65 turns around a histone octamer** (two each of **H2A, H2B, H3, H4**); linker DNA of 20–60 bp joins nucleosomes; **histone H1** binds linker DNA and the entry and exit points. Diameter 11 nm. Histones are small and rich in **lysine and arginine** (positively charged) and bind the negative DNA
3	**30 nm chromatin fibre (solenoid)**	Nucleosomes coiled, stabilised by H1
4	**Loops and scaffold**	Looped domains (50–100 kb) attached to a nuclear scaffold (topoisomerase II, SMC proteins)
5	**Chromosome**	Maximally condensed at metaphase (about 10,000-fold compaction)

- **Euchromatin:** loosely packed, gene-rich, **transcriptionally active**, stains lightly. **Heterochromatin:** densely packed, gene-poor, **transcriptionally inactive** (constitutive at centromeres and telomeres; facultative, e.g. the inactive X chromosome or **Barr body**).
- **Histone modifications** form the **histone code**: acetylation of lysines (histone acetyltransferases, HAT) opens chromatin and activates genes; deacetylation (HDAC) closes it. Methylation can activate or repress. **DNA methylation** of cytosine in **CpG islands** silences genes (epigenetics; genomic imprinting, X inactivation). HDAC inhibitors (vorinostat) are used in lymphoma.
- **Chromosome features: centromere** (kinetochore attachment, tandem alpha-satellite repeats), **telomeres** (end repeats, TTAGGG in humans, protect ends) and **origins of replication**.

Types of DNA sequences in the genome

Class	Share of genome	Examples and features
Unique (single-copy) sequences	About 50%	Include protein-coding genes (exons) and introns; one copy per haploid genome
Moderately repetitive	About 25–30%	**Interspersed repeats: SINEs** (Alu elements, about 300 bp, 10% of genome) and **LINEs** (L1 retrotransposons, 17%); multi-copy genes for rRNA, tRNA, histones
Highly repetitive (satellite DNA)	About 10%	Tandem repeats at centromeres and telomeres; **microsatellites (short tandem repeats, STR)** and minisatellites (VNTR) are used for **DNA fingerprinting** and paternity tests; trinucleotide repeat expansion causes Huntington's disease (CAG) and fragile X (CGG)
Transposable elements (jumping genes)	About 45% in total	Retrotransposons and DNA transposons; can cause mutation and disease (McClintock)

Structure of a eukaryotic gene

- **Promoter and regulatory elements:** TATA box (about –25), CAAT box (–75), GC box; enhancers and silencers can be far from the gene.
- **Exons** (coding, are retained in mature mRNA; average human gene about 9 exons) and **introns** (non-coding, removed by splicing; make up most of a gene). Typical intron starts with **GU** and ends with **AG**.
- **5′ UTR, coding sequence (starting with AUG), 3′ UTR** and polyadenylation signal (AAUAAA). Human genes are **split genes and monocistronic** (one polypeptide per mRNA), unlike bacterial polycistronic mRNA.
- **Gene families and pseudogenes:** globin genes (α cluster on chromosome 16, β cluster on 11) arise by gene duplication; pseudogenes are non-functional copies. **Alternative splicing** lets one gene give several proteins (more than 90% of human genes).

Mitochondrial DNA (mtDNA)

- **Circular double-stranded DNA of 16,569 bp**, with 37 genes (13 protein subunits of oxidative phosphorylation, 22 tRNAs, 2 rRNAs), present in many copies per mitochondrion.
- **Maternal inheritance** (all mitochondria come from the ovum). High mutation rate. **Diseases:** Leber's hereditary optic neuropathy (LHON), MELAS, MERRF, Kearns–Sayre syndrome. Shows **heteroplasmy**.

Feature	Prokaryotic (bacterial) genome	Eukaryotic genome
Organisation	Single circular chromosome in nucleoid; plasmids	Multiple linear chromosomes within nucleus
Histones	Absent (HU proteins, supercoiling)	Present (nucleosomes)
Genes	Compact, **no introns**, polycistronic operons	Introns and exons, **monocistronic**
Non-coding DNA	Small (about 10%)	Large (about 98% in humans)
Transcription and translation	Coupled, in the same compartment	Separated (nucleus and cytoplasm)

6. Central Dogma and DNA Replication

The **central dogma of molecular biology** (Crick, 1958) states that genetic information flows **DNA → RNA → protein**. DNA is copied to DNA (**replication**), DNA is copied to RNA (**transcription**) and the RNA sequence is decoded into protein (**translation**). Later discoveries added **reverse transcription** (RNA → DNA, in retroviruses such as HIV and by telomerase) and RNA replication (RNA viruses).

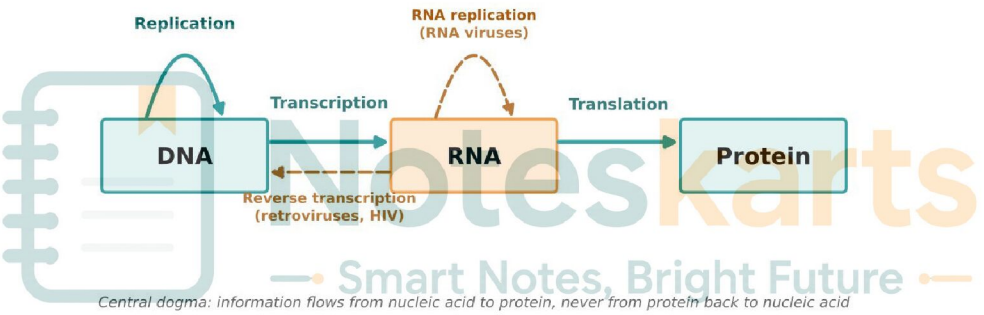

Figure 2: Figure 2: The central dogma of molecular biology

DNA replication

Replication is the process of copying DNA before cell division. It is **semi-conservative** (Meselson and Stahl, 1958): each daughter duplex has one old (parental) strand and one newly made strand. It is **bidirectional** from each origin, **semi-discontinuous** and always proceeds in the **5′→3′ direction** of the new strand. It occurs in the **S phase** of the cell cycle in eukaryotes.

- **Origin of replication:** bacteria have one (**oriC**); eukaryotes have **many origins** (about 30,000–50,000 in humans) so the huge genome is copied in hours. The origin binds the initiator complex (DnaA in bacteria; **ORC, Cdc6 and MCM helicase** in eukaryotes).
- **Replication fork:** the Y-shaped region where the parent DNA is unwound. The **leading strand** is made continuously towards the fork; the **lagging strand** is made discontinuously as short **Okazaki fragments** (1000–2000 nucleotides in bacteria; 100–200 in eukaryotes) away from the fork.

Enzymes and proteins of replication

Protein / enzyme	Function
Helicase (DnaB; MCM)	Unwinds the double helix using ATP, breaking hydrogen bonds
Single-strand binding proteins (SSB; RPA)	Keep strands apart and protect single-stranded DNA from nucleases and re-annealing
Topoisomerases (DNA gyrase = type II in bacteria; topoisomerase I and II)	Relieve the **supercoiling** (torsional strain) ahead of the fork by transient cutting. **Quinolones** (ciprofloxacin) block bacterial gyrase; **camptothecin and irinotecan** block topoisomerase I; **etoposide, doxorubicin** block topoisomerase II

Protein / enzyme	Function
Primase (an RNA polymerase)	Synthesises a short **RNA primer** (about 10 nt) with a free 3′-OH; DNA polymerases cannot start a chain de novo. Needed once on the leading strand and for each Okazaki fragment
DNA polymerase III (bacteria); Pol δ and Pol ε (eukaryotes)	Main replicative enzymes: add deoxynucleotides to the 3′ end of the primer using the template, in the 5′→3′ direction. Need dNTPs; release PPi. Have **3′→5′ exonuclease (proofreading)** activity. Error rate falls to about 1 in 10^{9}–10^{10}
DNA polymerase I (bacteria); Pol α (eukaryotes, initiates with primase)	Pol I removes the RNA primer (5′→3′ exonuclease) and fills the gap with DNA (nick translation). Pol α makes the RNA-DNA primer in eukaryotes
DNA ligase	Joins Okazaki fragments by sealing the nick (phosphodiester bond; uses ATP in eukaryotes, NAD^+ in bacteria)
Sliding clamp (PCNA, β-clamp) and clamp loader (RFC)	Hold the polymerase on the template (processivity)
Telomerase	A ribonucleoprotein reverse transcriptase with its own RNA template; adds **TTAGGG repeats** at the 3′ ends of linear chromosomes to solve the **end-replication problem**. Active in germ cells, stem cells and **about 90% of cancers**; most somatic cells lack it, so telomeres shorten with age (replicative senescence)

Steps of replication

Stage	Events
Initiation	Initiator proteins bind the origin; helicase opens the duplex into a replication bubble with two forks; SSB stabilises; primase lays down RNA primers
Elongation	Polymerase extends the primer: continuous on the leading strand, as Okazaki fragments on the lagging strand; topoisomerase removes supercoils
Primer removal and ligation	RNase H and Pol I (or FEN1 and Pol δ) remove RNA primers; gaps filled with DNA; ligase seals nicks
Termination	Forks meet (in bacteria at **Ter sites bound by Tus**); daughter molecules separated (topoisomerase IV in bacteria); telomeres completed by telomerase in eukaryotes

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Property	Prokaryotes	Eukaryotes
Origins	One (oriC)	Many
Rate of fork movement	About 1000 nucleotides/s	About 50 nucleotides/s
DNA polymerases	Pol III (main), Pol I (repair, primer removal), Pol II, IV, V	Pol α (primer), **δ and ε** (main), β (repair), γ (mitochondrial), η/ι/κ/ζ (translesion)
Okazaki fragment size	1000–2000 nt	100–200 nt
Ends	Circular, no end problem	Linear; telomerase

INHIBITORS OF REPLICATION

Quinolones (gyrase), **rifampicin** (bacterial RNA polymerase), **acyclovir and ganciclovir** (viral DNA polymerase, chain terminators), **zidovudine** (reverse transcriptase), **5-fluorouracil and methotrexate** (nucleotide supply), **cytarabine** (DNA chain termination), **hydroxyurea**, **etoposide and doxorubicin** (topoisomerase II), **mitomycin C and cisplatin** (cross-link DNA). **Dideoxynucleotides** (ddNTP) lack the 3′-OH, stop chain growth and are the basis of **Sanger DNA sequencing**.

7. Transcription: Synthesis of RNA

Transcription is the synthesis of RNA from a DNA template by **DNA-dependent RNA polymerase**. Only one strand (the **template or antisense strand**) is read in the 3′→5′ direction and RNA grows 5′→3′. The RNA has the same sequence as the **coding (sense) strand**, with U in place of T. No primer is needed, and no proofreading is available (error about 1 in 10^{5}). Substrates are ATP, GTP, CTP and UTP; PPi is released.

RNA polymerases

Organism	Enzyme	Product
Bacteria	One RNA polymerase (core $\alpha_2\beta\beta'\omega$ + **sigma (σ) factor** for promoter recognition = holoenzyme)	All RNAs. **Inhibited by rifampicin** (binds β subunit) and actinomycin D
Eukaryotes	**RNA polymerase I** (nucleolus)	Large rRNA (28S, 18S, 5.8S)
	RNA polymerase II (nucleoplasm)	**mRNA**, most snRNA and miRNA. Inhibited by **α-amanitin** (death-cap mushroom)
	RNA polymerase III	tRNA, 5S rRNA, small RNAs. Inhibited by high α-amanitin

Stages of transcription

Stage	Prokaryotes	Eukaryotes
Initiation	σ factor guides the polymerase to the **promoter**: **–10 (Pribnow box, TATAAT)** and **–35 (TTGACA)** sequences. DNA is unwound (open complex) and the first nucleotides joined	General transcription factors (**TFIID** with TATA-binding protein, TFIIA, B, E, F, H) assemble Pol II at the **TATA box** (–25) to form the preinitiation complex; TFIIH (helicase) opens DNA and phosphorylates the C-terminal domain of Pol II; **enhancers and activators** (via the Mediator complex) raise the rate
Elongation	Core polymerase moves along the template; σ is released after about 10 nucleotides. RNA-DNA hybrid is short (about 8 bp)	Pol II elongates; nucleosomes are displaced; RNA is processed during transcription
Termination	(1) **Rho-independent:** a GC-rich **hairpin** followed by a run of U's; (2) **Rho-dependent:** rho (helicase) releases RNA	Pol II continues past the polyadenylation signal, then the RNA is cleaved and the polymerase is released

Post-transcriptional processing of eukaryotic mRNA

The primary transcript (**heterogeneous nuclear RNA, hnRNA or pre-mRNA**) is processed in the nucleus into mature mRNA. Prokaryotic mRNA needs no processing.

Process	Details and significance
5′ Capping	**7-Methylguanosine (m^7G) cap** is joined 5′–5′ to the first nucleotide (guanylyl transferase; SAM provides methyl groups). Protects from exonucleases, helps nuclear export and **ribosome binding**
3′ Polyadenylation	Cleavage at AAUAAA signal and addition of **a poly-A tail** (about 200 A residues) by **poly(A) polymerase**. Gives stability and aids export and translation; histone mRNAs lack it
Splicing	**Introns are removed and exons joined by the spliceosome** (snRNPs U1, U2, U4, U5, U6 with proteins). Two transesterification steps form a **lariat** intermediate (branch point A). **Alternative splicing** gives protein diversity. Defects cause **β-thalassaemia** (splice-site mutations), some cancers and **systemic lupus (anti-Sm antibodies against snRNPs)**
RNA editing	Base changes after transcription, e.g. **apo B-100 → apo B-48** by cytidine deaminase in the intestine (C → U creates a stop codon)
Ribozymes	RNA with catalytic activity (self-splicing introns, RNase P, peptidyl transferase of the ribosome): Cech and Altman, Nobel 1989

Types of RNA

RNA	Function
mRNA (messenger)	Carries the genetic message from DNA to ribosomes; each three bases (codon) specify an amino acid. Short half-life (minutes to hours)
tRNA (transfer)	About 75–90 nucleotides; **cloverleaf secondary structure** and **L-shaped** 3D form; **3′-CCA end** carries the amino acid; **anticodon loop** reads the codon; many modified bases (pseudouridine, dihydrouridine, inosine). 20 or more types
rRNA (ribosomal)	Structural and catalytic part of ribosomes (about 60% rRNA, 40% protein). Prokaryote 70S = 30S (16S rRNA) + 50S (23S, 5S rRNA); eukaryote 80S = 40S (18S) + 60S (28S, 5.8S, 5S)
snRNA (small nuclear)	Splicing (U1–U6)

RNA	Function
miRNA, siRNA	**RNA interference:** bind mRNA through the RISC complex and block translation or cause cleavage (gene silencing); siRNA drugs (patisiran, inclisiran)
lncRNA	Long non-coding RNAs regulating chromatin, e.g. **XIST** (X inactivation)

REVERSE TRANSCRIPTION

Retroviruses (HIV, HTLV) carry RNA and the enzyme **reverse transcriptase (RNA-dependent DNA polymerase)**, which makes complementary DNA (cDNA) from RNA; **integrase** inserts it into the host genome. Reverse transcriptase is used in the laboratory to make cDNA (RT-PCR) and is the target of **NRTIs (zidovudine, tenofovir, lamivudine) and NNRTIs (nevirapine, efavirenz)**.

8. Translation: Protein Synthesis

Translation is the synthesis of a polypeptide on the **ribosome** from the sequence of codons on mRNA. It occurs in the **cytoplasm** (free ribosomes for cytosolic proteins; ribosomes on the rough endoplasmic reticulum for secreted and membrane proteins). Peptides are made from the **N-terminus to the C-terminus**; mRNA is read **5′→3′**. Protein synthesis is the most energy-costly process: at least **4 high-energy phosphate bonds per peptide bond** (2 for activation of the amino acid, 1 for delivery of tRNA, 1 for translocation).

Components required

Component	Role
mRNA	Template, read in triplet codons
Ribosomes	Two subunits with sites **A (aminoacyl)**, **P (peptidyl)** and **E (exit)**. Peptidyl transferase activity is in the large-subunit rRNA (ribozyme)
tRNA and aminoacyl-tRNA synthetases	Each of the 20 amino acids has its own **aminoacyl-tRNA synthetase** that joins it to the correct tRNA (2 steps: amino acid + ATP → aminoacyl-AMP + PPi; then amino acid is transferred to the 3′-CCA end), with proofreading. This 'second genetic code' decides the accuracy of translation
Initiation, elongation and release factors	Eukaryotic **eIF**, **eEF**, **eRF**; bacterial IF, EF-Tu, EF-G and RF
Energy	ATP and GTP; amino acids; Mg^{2+} and K^{+}

Steps of translation

Stage	Prokaryotes	Eukaryotes
Initiation	30S subunit binds the **Shine–Dalgarno sequence** of mRNA; **initiator fMet-tRNA (N-formylmethionine)** enters the P site at **AUG** (IF-1, 2, 3, GTP); 50S joins	40S subunit with **Met-tRNAi** and eIF2-GTP scans from the **5′ cap** to the first AUG (Kozak sequence); 60S joins (eIF5B); poly-A tail interacts with the cap through eIF4G and PABP (circularises mRNA). **eIF2 phosphorylation** shuts down translation in stress
Elongation	(1) **Aminoacyl-tRNA enters the A site** (EF-Tu-GTP, codon-anticodon match); (2) **peptide bond formation** by peptidyl transferase (the peptide moves to the A-site tRNA); (3) **translocation** of the ribosome by one codon (**EF-G**, GTP), moving the deacylated tRNA to the E site	Same steps with **eEF1α** (like EF-Tu) and **eEF2** (translocase). **Diphtheria toxin** ADP-ribosylates eEF2
Termination	Stop codon (**UAA, UAG, UGA**) in the A site is recognised by **release factors** (RF1, RF2, RF3); peptidyl-tRNA is hydrolysed, polypeptide, mRNA and subunits released (ribosome recycling)	**eRF1** (recognises all three stops) and eRF3-GTP

- **Polysomes (polyribosomes):** several ribosomes translate one mRNA at the same time, increasing yield.
- **Bacterial coupling:** translation starts while the mRNA is still being transcribed (no nucleus).
- **Signal peptide:** secreted proteins have an N-terminal signal sequence recognised by the **signal recognition particle (SRP)**, which brings the ribosome to the ER membrane (cotranslational translocation).

Post-translational modifications

Modification	Examples
Proteolytic cleavage	**Zymogens** (trypsinogen, pepsinogen, clotting factors); **proinsulin → insulin + C peptide**; removal of N-terminal Met and signal peptides
Folding and disulphide bonds	Chaperones (Hsp70, chaperonins), protein disulphide isomerase
Glycosylation	N-linked (Asn) and O-linked (Ser, Thr) sugars in ER and Golgi; glycoproteins; I-cell disease (mannose-6-phosphate tag missing)
Hydroxylation	Proline and lysine in collagen (vitamin C)
γ-Carboxylation	Glutamate in clotting factors II, VII, IX, X (vitamin K)
Phosphorylation	Kinases and phosphatases regulate enzymes and signalling
Acetylation, methylation, ubiquitination, lipidation	Histone code, protein turnover (proteasome), membrane anchoring (prenylation of Ras, myristoylation)

Comparison	Prokaryotic translation	Eukaryotic translation
Ribosome	70S (30S + 50S)	80S (40S + 60S)
Initiator amino acid	N-Formylmethionine	Methionine
mRNA	Polycistronic; Shine–Dalgarno sequence	Monocistronic; 5′ cap, poly-A tail
Coupling with transcription	Yes	No (nucleus vs cytoplasm)
Selective drug targets	Aminoglycosides, tetracyclines, macrolides, chloramphenicol	Cycloheximide, emetine, puromycin (both)

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PART C: GENETIC CODE AND REGULATION OF PROTEIN SYNTHESIS

9. The Genetic Code

The **genetic code** is the set of rules by which the nucleotide sequence of mRNA is translated into the amino acid sequence of a protein. It was deciphered by **Nirenberg, Matthaei, Khorana and Holley** (Nobel Prize 1968). The unit is the **codon**: a sequence of **three consecutive nucleotides**. Of the **64 possible codons** (4^3), **61 code for the 20 amino acids** and **3 are stop (termination) codons**.

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

Figure 3: Figure 3: The standard genetic code (mRNA codons, read 5′→3′)

Properties of the genetic code

Property	Meaning and examples
Triplet	Three bases make one codon (a doublet code could give only 16 combinations, too few for 20 amino acids)
Degenerate (redundant)	More than one codon for most amino acids (Leu, Ser, Arg have 6 each; Met and Trp have only one). Degeneracy is mostly at the **third base**, so many point mutations in the third position are **silent**
Non-ambiguous (specific)	Each codon codes for **only one** amino acid
Non-overlapping	Each base belongs to only one codon; the message is read in successive triplets
Commaless (continuous)	No punctuation between codons; reading frame is set by the start codon, so insertion or deletion of one or two bases causes a **frameshift**
Universal	Same code in nearly all organisms (bacteria to humans), which makes recombinant DNA technology possible. **Minor exceptions:** human **mitochondria** (UGA = Trp, AUA = Met, AGA/AGG = stop)
Start codon	**AUG** (methionine; fMet in bacteria); rarely GUG
Stop (nonsense) codons	**UAA (ochre), UAG (amber), UGA (opal)**; no tRNA reads them; release factors recognise them
Colinear	The order of codons corresponds to the order of amino acids
Wobble hypothesis (Crick)	The **third base of the codon pairs loosely** with the first base of the anticodon, so one tRNA can read several codons (e.g. inosine in the anticodon pairs with U, C or A). Fewer than 61 tRNAs are needed

Mutations and the code

Type	Change	Effect and example
Silent	Base change gives a synonymous codon	No change in protein (third-base wobble)
Missense	Codon for a different amino acid	Altered protein; **sickle cell anaemia (GAG → GUG, Glu → Val)**
Nonsense	Codon changed to a stop codon	Truncated protein; some β-thalassaemias, Duchenne muscular dystrophy
Frameshift	Insertion or deletion of bases (not in multiples of 3)	All codons after the change are altered; severe; Tay–Sachs (4-bp insertion), cystic fibrosis in some

Type	Change	Effect and example
In-frame deletion	Deletion of 3 bases	Loss of one amino acid: **ΔF508 in cystic fibrosis**
Point mutations	Substitution of one base: **transition** (purine ↔ purine) or **transversion** (purine ↔ pyrimidine)	Include silent, missense and nonsense
Trinucleotide repeat expansion	Increase in tandem repeats	Huntington's disease (CAG), fragile X syndrome (CGG), myotonic dystrophy

10. Regulation of Gene Expression

Cells contain the same genes but express only some of them, and at different levels. **Regulation of gene expression** allows adaptation to the environment (in bacteria) and differentiation and development (in higher organisms). Control can act at many levels (chromatin, transcription, RNA processing, mRNA stability, translation, post-translational), but **initiation of transcription is the most important**.

Prokaryotic regulation: the operon concept

Jacob and Monod (1961, Nobel Prize 1965) proposed the **operon model**. An **operon** is a cluster of structural genes transcribed together as one polycistronic mRNA, with a common **promoter (P)** and **operator (O)** controlled by a **regulator gene** (repressor).

Lac operon (inducible system)

- Contains **lacZ** (β-galactosidase, splits lactose into glucose and galactose), **lacY** (lactose permease) and **lacA** (thiogalactoside transacetylase). The regulator gene **lacI** makes the **repressor protein**.
- **No lactose:** the repressor binds the **operator** and blocks RNA polymerase, so the operon is **off**.
- **Lactose present:** a little lactose is converted to **allolactose (the inducer)**, which binds the repressor and changes its shape so that it leaves the operator; transcription is **on** (**negative control**, inducible).
- **Positive control by glucose (catabolite repression):** when glucose is low, cAMP rises and binds **CAP (catabolite activator protein, CRP)**; the cAMP–CAP complex binds near the promoter and **increases RNA polymerase binding**. When glucose is high, cAMP is low and the operon stays off even if lactose is present (so glucose is used first). **IPTG** is a gratuitous inducer used in the laboratory.

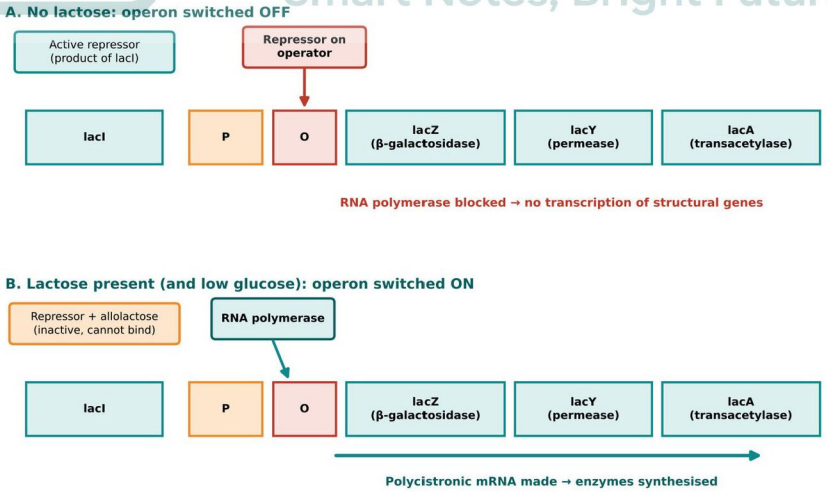

Figure 4: Figure 4: The lac operon: repressed (no lactose) and induced (lactose present)

Trp operon (repressible system)

- Genes for tryptophan synthesis. When **tryptophan is high**, it acts as a **corepressor**, binds the aporepressor and the complex attaches to the operator: operon **off**. When tryptophan is low, the repressor is inactive and the genes are transcribed.
- **Attenuation:** a second control by early termination of transcription, using a leader sequence, linking transcription to translation of a short peptide rich in Trp codons.

Feature	Lac operon	Trp operon
Type	**Inducible** (catabolic: breaks down lactose)	**Repressible** (anabolic: makes tryptophan)

Feature	Lac operon	Trp operon
Default state	Off	On
Small molecule	Allolactose (**inducer**) inactivates repressor	Tryptophan (**corepressor**) activates repressor
Other control	cAMP–CAP (positive)	Attenuation

Eukaryotic regulation

Level	Mechanism and examples
Chromatin	Histone acetylation and methylation, DNA methylation of CpG islands, chromatin remodelling (SWI/SNF): euchromatin is active, heterochromatin silent
Transcription factors	**General (basal)** factors (TFII) and **specific** factors (activators and repressors) bind **enhancers, silencers and response elements**. DNA-binding domains: **helix-turn-helix, zinc finger, leucine zipper, helix-loop-helix**. Mediator links enhancers to the promoter
Hormone response	**Steroid, thyroid hormone, vitamin D and retinoic acid** pass into the cell, bind intracellular receptors (zinc-finger proteins) and bind **hormone response elements (HRE)**, altering transcription. Cyclic AMP acts through **CREB** and the cAMP response element (CRE)
RNA processing	Alternative splicing and polyadenylation
mRNA stability	Half-life controlled by poly-A tail, AU-rich elements and **iron-responsive elements (IRE)**: low iron stabilises transferrin receptor mRNA and blocks ferritin mRNA translation. **miRNA and siRNA** (RNA interference) degrade mRNA
Translation	eIF2 phosphorylation (stress, heme deficiency in reticulocytes); regulation by mTOR
Post-translational	Phosphorylation, proteolysis, ubiquitination
Gene amplification and rearrangement	Immunoglobulin gene rearrangement (V(D)J recombination); dihydrofolate reductase gene amplification (methotrexate resistance)

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ONCOGENES AND TUMOUR SUPPRESSORS

Proto-oncogenes (growth factors, receptors, Ras, Myc) become **oncogenes** by mutation, amplification or translocation (e.g. BCR-ABL in chronic myeloid leukaemia, the Philadelphia chromosome). **Tumour suppressor genes** (**p53, Rb, BRCA1/2, APC**) restrain the cell cycle; **p53** (guardian of the genome) arrests the cycle or triggers apoptosis after DNA damage, and is mutated in over 50% of cancers.

11. Inhibitors of Transcription and Translation

Many **antibiotics and toxins** act by blocking steps of nucleic acid and protein synthesis. Antibiotics are useful because they are **selectively toxic**: they act on bacterial machinery (70S ribosome, bacterial RNA polymerase) with little effect on human cells.

Inhibitors of transcription

Inhibitor	Mechanism	Use / toxicity
Rifampicin (rifampin)	Binds the **β subunit of bacterial RNA polymerase** and blocks initiation (elongation not affected)	Tuberculosis, leprosy; turns body fluids orange; **induces CYP enzymes** (drug interactions); hepatotoxic
Actinomycin D (dactinomycin)	Intercalates in DNA (at G-C pairs) and blocks RNA chain elongation in both prokaryotes and eukaryotes	Wilms tumour, rhabdomyosarcoma; also inhibits replication
α-Amanitin	Binds eukaryotic **RNA polymerase II** (strongly) and III (weakly); toxin of the death-cap mushroom (*Amanita phalloides*)	Liver failure; poisoning; no antibiotic use
Rifamycin derivatives (rifabutin, rifapentine)	Same as rifampicin	TB and MAC
Cordycepin (3′-deoxyadenosine)	Chain terminator of poly-A tail synthesis	Research
Flavopiridol, DRB	Inhibit CDK9 (P-TEFb), blocking Pol II elongation	Experimental

Inhibitors of translation

Inhibitor	Target and mechanism	Notes
Streptomycin and aminoglycosides (gentamicin, amikacin, kanamycin, neomycin)	Bind **30S subunit (16S rRNA)**: block initiation and cause **misreading of mRNA**	Bactericidal; **ototoxicity and nephrotoxicity**; need oxygen for uptake
Tetracyclines (doxycycline, minocycline)	Bind **30S subunit** and block entry of aminoacyl-tRNA at the A site	Bacteriostatic; stain teeth, avoid in pregnancy and children; chelate Ca^{2+}
Chloramphenicol	Binds **50S subunit** and inhibits **peptidyl transferase**	Bacteriostatic; **aplastic anaemia, grey baby syndrome** (neonates lack glucuronidation)
Macrolides (erythromycin, azithromycin, clarithromycin)	Bind **50S subunit** and block **translocation** (exit tunnel)	Bacteriostatic; QT prolongation, CYP3A4 inhibition (erythromycin)
Clindamycin, lincomycin	Bind 50S subunit; block peptidyl transferase and translocation	**Pseudomembranous colitis** (*C. difficile*)
Linezolid	Binds 50S subunit; blocks formation of the initiation complex	MRSA, VRE; myelosuppression
Puromycin	Analogue of the aminoacyl end of tRNA; enters the A site and causes **premature chain termination**	Both prokaryotes and eukaryotes; research tool
Cycloheximide, emetine	Inhibit the **eukaryotic 60S** (translocation)	Research; emetine in amoebiasis (old)
Diphtheria toxin, Pseudomonas exotoxin A	**ADP-ribosylate eEF2** (diphthamide) and block translocation	Cell death; diphtheria
Ricin, shiga toxin	Remove an adenine from **28S rRNA** (a glycosidase) and inactivate the 60S ribosome	Castor bean poison; haemolytic uraemic syndrome
Interferon	Induces protein kinase that phosphorylates **eIF2α**	Antiviral response
Fusidic acid	Blocks EF-G release (translocation)	Staphylococcal infections

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MNEMONIC FOR RIBOSOMAL SUBUNIT TARGETS

'Buy AT 30, CCEL at 50': **A**minoglycosides and **T**etracyclines act at **30S**; **C**hloramphenicol, **C**lindamycin, **E**rythromycin (macrolides) and **L**inezolid act at **50S**. Aminoglycosides are **bactericidal**, whereas the others (except in some conditions) are bacteriostatic.

Process inhibited	Drugs (summary)
DNA replication	Quinolones (gyrase), nucleoside analogues, anthracyclines, irinotecan, etoposide, cisplatin
Nucleotide synthesis	Methotrexate, trimethoprim, 5-FU, 6-MP, hydroxyurea, mycophenolate, sulphonamides
Transcription	Rifampicin, actinomycin D, α-amanitin
Translation	Aminoglycosides, tetracyclines, chloramphenicol, macrolides, clindamycin, linezolid, puromycin, diphtheria toxin
Cell wall (for comparison)	β-Lactams, vancomycin

PART D: DNA REPAIR AND RELATED DISORDERS

12. DNA Damage: Types and Causes

DNA is the only biomolecule that is **repaired and not replaced**. Each human cell suffers an estimated **10,000 to 100,000 DNA lesions per day**. If damage is not repaired, it can cause **mutations, cell death, premature ageing and cancer**. DNA damage arises from internal (endogenous) processes and external (environmental) agents.

Source	Agents	Type of damage
Spontaneous (endogenous)	Hydrolysis, replication errors, oxidation	**Depurination** (loss of A or G, about 10,000 per cell per day, giving an **AP (apurinic) site**); **deamination** (C → U; 5-methyl-C → T; A → hypoxanthine); mismatches and slippage in repeats
Reactive oxygen species	Respiration, inflammation, radiation	**8-Oxoguanine** (pairs with A, causing G → T transversion), thymine glycol, single-strand breaks
Ultraviolet radiation (UV-B, 260–320 nm)	Sunlight	**Pyrimidine dimers**: **thymine (cyclobutane) dimers** and 6-4 photoproducts; distort the helix and block replication
Ionising radiation	X-rays, γ-rays, radon	**Single-strand and double-strand breaks** (most lethal), base damage
Chemical mutagens	Alkylating agents (nitrogen mustard, **methyl methanesulphonate**, nitrosamines), **aflatoxin B1** (adducts at guanine; liver cancer), **benzo[a]pyrene** (tobacco smoke), cisplatin	Alkylation (O^6-methylguanine pairs with T), bulky adducts, **inter-strand and intra-strand cross-links**
Base analogues	5-Bromouracil (pairs with G), 2-aminopurine	Mispairing during replication
Intercalating agents	Ethidium bromide, acridine orange, proflavin	Insert between bases, causing **frameshift** (insertion or deletion)
Nitrous acid	Nitrites in food	Deamination of A, C, G

TYPES OF MUTAGENIC LESIONS IN BRIEF

Base damage: deamination, oxidation, alkylation, loss of bases. **Distortion of helix:** thymine dimers, bulky adducts. **Strand breaks:** single or double. **Cross-links:** inter-strand or DNA–protein. **Mismatches:** wrong base inserted by polymerase.

13. DNA Repair Mechanisms

Cells have several repair systems, each specialised for one class of damage. Repair uses the **undamaged complementary strand as a template**, so the information can be restored. The **cell cycle checkpoint** (controlled by **p53, ATM and ATR kinases**) pauses division while repair occurs, or triggers apoptosis if damage is too heavy.

Mechanism	Damage repaired	Steps and key proteins
Direct reversal	Thymine dimers (bacteria, plants); **O^6-methylguanine**	**Photolyase** uses visible light to split pyrimidine dimers (absent in humans). **O^6-Methylguanine-DNA methyltransferase (MGMT)** transfers the methyl group to its own cysteine (suicide enzyme); high MGMT makes tumours resistant to temozolomide
Base excision repair (BER)	Single damaged bases (deamination, oxidation, alkylation); AP sites; single-strand breaks	(1) **DNA glycosylase** (uracil-DNA glycosylase, OGG1) removes the abnormal base, leaving an AP site; (2) **AP endonuclease** cuts the backbone; (3) **DNA polymerase β** inserts the correct nucleotide; (4) **DNA ligase III** seals. **PARP1** senses strand breaks
Nucleotide excision repair (NER)	**Bulky, helix-distorting lesions:** UV thymine dimers, benzo[a]pyrene adducts, cross-links	(1) Damage recognition (**XPC** in global-genome NER; stalled RNA polymerase in **transcription-coupled NER** with CSA and CSB); (2) **TFIIH helicase (XPB, XPD)** unwinds the DNA; (3) **endonucleases (XPF–ERCC1, XPG)** cut on both sides of the damage and remove a 24–32 nt oligomer; (4) **DNA polymerase δ/ε** fills the gap; (5) **ligase I** seals. Defective in **xeroderma pigmentosum**

Mechanism	Damage repaired	Steps and key proteins
Mismatch repair (MMR)	Mismatched bases and small insertion or deletion loops left after replication	**MutS** (MSH2, MSH6 in humans) recognises the mismatch; **MutL** (MLH1, PMS2) recruits endonuclease; the new strand is identified (nicks and, in bacteria, methylation of the old strand by Dam); exonuclease excises, polymerase fills, ligase seals. Increases replication fidelity 100-fold. Defective in **Lynch syndrome (HNPCC)**
Homologous recombination (HR)	**Double-strand breaks** (S/G2 phase), inter-strand cross-links, stalled forks	Error-free: uses the **sister chromatid** as a template. **MRN complex** and ATM detect; end resection; **BRCA1, BRCA2 and RAD51** form strand-invasion filaments; DNA synthesis and resolution of Holliday junctions. Defective in **BRCA1/2 breast and ovarian cancer**
Non-homologous end joining (NHEJ)	Double-strand breaks (G1 phase, main pathway in humans)	**Ku70/Ku80** bind the ends; **DNA-PKcs**, Artemis (trims ends) and **DNA ligase IV with XRCC4** join the ends. **Error-prone** (small deletions); also used in V(D)J recombination of immune genes. Defective in some SCID (Artemis)
Translesion synthesis (TLS)	Lesions that stall replication (not truly repair)	Special **low-fidelity polymerases (Pol η, ι, κ, ζ)** bypass the damage. **Pol η** inserts A across thymine dimers; its defect gives **xeroderma pigmentosum variant**
Proofreading (during replication)	Wrong nucleotide just inserted	3′→5′ exonuclease activity of DNA polymerase

- **Order of accuracy:** proofreading (about 100-fold) × mismatch repair (about 100-fold) → final error rate about 10^{-9} to 10^{-10} per base per replication.
- **Mutations arise** if repair is overwhelmed or defective, and can be inherited (germline) or give cancer (somatic). The **Ames test** (Salmonella reversion) screens chemicals for mutagenicity.
- **PARP inhibitors** (**olaparib, niraparib**) block BER; in cells lacking **BRCA1/2** (no homologous recombination) this is **synthetically lethal**, so they are used to treat BRCA-mutated ovarian, breast and prostate cancers.
- **Many anticancer drugs and radiotherapy** act by causing DNA damage; resistance occurs through increased repair.

14. Clinical Disorders Associated with Faulty DNA Repair

Inherited defects in repair genes cause syndromes of **hypersensitivity to DNA-damaging agents, genomic instability, premature ageing, neurological disease and a high risk of cancer**. Most are **autosomal recessive**, whereas the hereditary cancer syndromes are mostly **autosomal dominant**.

Disorder	Repair pathway / gene	Clinical features
Xeroderma pigmentosum (XP)	**Nucleotide excision repair** (XPA to XPG; XP-V is Pol η); AR	**Extreme sun sensitivity**: severe sunburn, freckling, **dry pigmented skin (parchment)**, ocular damage and **very high risk (1000-fold) of skin cancers (basal cell, squamous cell, melanoma)** at a young age; neurological deterioration in some. 'Children of the night'. Strict **sun protection**
Cockayne syndrome	Transcription-coupled NER (**CSA, CSB**); AR	Growth failure, **premature ageing**, microcephaly, deafness, retinopathy, photosensitivity, neurodegeneration; **no increase in cancer**
Trichothiodystrophy	NER (XPB, XPD, TTDA); AR	Brittle sulphur-deficient hair, ichthyosis, photosensitivity, intellectual disability
Hereditary non-polyposis colorectal cancer (Lynch syndrome)	**Mismatch repair** (MLH1, MSH2, MSH6, PMS2); AD	Early colorectal cancer (without numerous polyps), endometrial and ovarian cancers; **microsatellite instability** in tumours (detected by testing for MSI)
Hereditary breast and ovarian cancer (HBOC)	**Homologous recombination: BRCA1, BRCA2**; AD	Breast cancer (lifetime risk about 70%), ovarian cancer (BRCA1 about 40–60%), prostate and pancreatic cancers; treated by PARP inhibitors
Ataxia telangiectasia (AT)	**ATM kinase** (double-strand break response); AR	Progressive **cerebellar ataxia**, **telangiectasia** (dilated vessels on eyes and skin), **immunodeficiency**, sensitivity to ionising radiation, lymphoma and leukaemia; raised alpha-fetoprotein
Nijmegen breakage syndrome	NBS1 (MRN complex)	Microcephaly, immunodeficiency, lymphoma, radiosensitivity

Disorder	Repair pathway / gene	Clinical features
Fanconi anaemia	**Inter-strand cross-link repair** (FANC genes, including BRCA2/FANCD1); AR	Progressive **bone marrow failure (pancytopenia)**, **short stature, absent thumbs or radii**, café-au-lait spots, AML and squamous cancers; **chromosome breakage with mitomycin C or diepoxybutane** (diagnostic)
Bloom syndrome	**BLM helicase** (RecQ family); AR	Short stature, **sun-sensitive facial erythema (butterfly rash)**, immunodeficiency, **sister chromatid exchanges** greatly increased; many cancers
Werner syndrome (adult progeria)	**WRN helicase** (RecQ); AR	**Premature ageing** from the 20s: greying, cataracts, scleroderma-like skin, diabetes, osteoporosis, atherosclerosis; sarcomas
Rothmund–Thomson syndrome	RECQL4 helicase	Skin rash (poikiloderma), skeletal defects, osteosarcoma
Li–Fraumeni syndrome	**p53** (checkpoint control); AD	Early sarcomas, breast cancer, brain tumours, adrenocortical carcinoma, leukaemia
MUTYH-associated polyposis	**Base excision repair (MUTYH glycosylase)**; AR	Multiple colorectal adenomas, high colon cancer risk
Hutchinson–Gilford progeria	Lamin A mutation (nuclear architecture; DNA damage accumulates)	Dramatic premature ageing in children
SCID with radiosensitivity	NHEJ (Artemis, DNA ligase IV, Cernunnos)	T-cell and B-cell deficiency, radiosensitivity

Common features and laboratory diagnosis

- **Hypersensitivity** to specific agents: UV (XP), ionising radiation (AT), cross-linking drugs (Fanconi anaemia).
- **Genomic instability:** chromosomal breaks, rearrangements, **sister chromatid exchange**; detected by **cytogenetics** (chromosome breakage test), comet assay, **microsatellite instability** testing (Lynch), unscheduled DNA synthesis assay (XP) and **gene sequencing**.
- **Cancer predisposition**, premature ageing and neurological degeneration. Management includes **avoiding the damaging agent** (sunlight, X-rays), regular screening (colonoscopy, mammography, MRI), prophylactic surgery, **genetic counselling** and targeted therapy (PARP inhibitors, immune checkpoint inhibitors in MMR-deficient tumours).

QUICK LINK: DISORDER AND PATHWAY

NER → XP, Cockayne syndrome, TTD; **MMR → Lynch syndrome**; **HR → BRCA1/2, Bloom, Fanconi (cross-link repair), ataxia telangiectasia (damage sensing)**; **BER → MUTYH polyposis**; **NHEJ → radiosensitive SCID**; **RecQ helicases → Bloom, Werner and Rothmund–Thomson syndromes**.

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NOTE FOR STUDENTS

These notes are prepared for study and revision. Normal values and drug doses vary between laboratories and textbooks; always follow your prescribed textbook and the reference range of your laboratory.

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