CHAPTER 1: THE CELL AS A UNIT OF HEALTH AND DISEASE

- **Patholog**y - pathos (suffering); logos (study); study of disease
- **Cellular pathology** – all disease originate from the cellular level
- **Modern pathology** – basically the study of cellular abnormalities

THE GENOME

- Sequencing of the human genome served as a landmark achievement of biomedical science

Noncoding DNA

- Human genome contains **3.2 billion DNA base pairs**
  - 20,000 protein-encoding genes (1.5% of the genome) → function as enzymes, structural components, and signalling molecules; used to assemble and maintain all of the cells in the body
  - 98.5% of human genome does not encode proteins
- Protein coding genes in higher organisms are separated by long stretches of DNA → denoted as "**dark matter**" of the genome
- 80% of the human genome binds proteins → involved in regulating gene expression
- Proteins provide building blocks and machinery required for assembling cells
- **Noncoding regions of the genome** – provide the critical "**architectural planning"**

Major classes of functional non-protein coding sequences:	
Promoter and enhancer regions	- provide binding sites for transcription factors
Binding sites for factors	- organize and maintain higher order chromatin structures
Noncoding regulatory RNAs	- 60% of genome is transcribed into RNAs that are never translated into protein but can regulate gene expression - Two best studied varieties: o **Micro RNAs** o **Long noncoding RNAs**
Mobile genetic elements (e.g. transponsons)	- 1/3 of genome is composed of these elements - **"jumping genes"** - Can move around the genome, exhibiting wide variation in number and positioning in closely related species - They are implicated in gene regulation and chromatin organization
Special structural regions of DNA	- **Telomeres –** chromosome ends - **Centromeres –** chromosome "tethers"

- **Polymorphism**s – genetic variations; many are associated with diseases
- Variation in gene regulation is more important in disease causation than structural changes in specific proteins
- Two most common forms of DNA variation in the human genome are:
  - **Single-nucleotide polymorphism (SNP)**
  - **Copy number variations (CNV)**
- **SNPs –** variants at single nucleotide positions; almost always **biallelic** (only two choices exist at a given site w/in the population; such as A or T)
  - 6 million human SNP identified;
  - Occur across the genome – w/in exons, introns, intergenic regions, and coding regions
  - SNP in non coding regions are regulatory elements in the genome → alters gene expression
  - May be "neutral" → no effect; useful markers if co-inherited w/ a disease-associated gene
  - SNP and the causative genetic factor are in **linkage equilibrium**
  - May serve as **markers** of risk of multigenic complex diseases such as DM II and hypertension
  - Effect on disease susceptibility is weak
- **CNVs –** consist of different numbers of large contiguous stretches of DNA from 1000 base pairs to millions of base pairs
  - Also **biallelic** and simply duplicated or deleted in a subset
  - Responsible for 5-24 million base pairs of sequence difference between two individuals
  - 50% involve gene-coding sequences → phenotypic diversity
- **Epigenetics –** heritable changes in gene expression that are not caused by alterations in DNA sequence

Histone Organization

- Cell type-specific differences in DNA transcription and translation depend on **epigenetic factors** ("above genetics")

Histone and histone modifying factors	▪ **Nucleosomes** – consist of DNA segments 147 base pairs and wrapped around a central core of low molecular weight proteins called histones ▪ DNA-histone complex → resembles "beads" joined by DNA linkers → called **chromatin** ▪ DNA is **not uniformly** and compactly wound ▪ Nuclear chromatin exist in two basic forms at LM: 1. **Heterochromatin** – cytochemically dense and transcriptionally inactive 2. **Euchromatin** – cytochemically dispersed and transcriptionally active ▪ "Unwound" portion of nuclear chromatin → regulates gene expression and dictates cellular identity and activity ▪ Histones are **not static** but are highly dynamic ▪ **"Chromatin remodelling complexes"** → reposition **nucleosomes** on DNA → exposes gene regulatory elements (promoters) ▪ **"Chromatin writer complexes"** → carry out >70 different histone modifications denoted as **marks** o Alterations include methylation, acetylation, phosphorylation ▪ Active genes in euchromatin are asso.w/ histone marks that make the DNA accessible to **RNA polymerase** ▪ Inactive genes have histone marks that enable DNA compaction into heterochromatin

	▪ Histone marks are reversible through the activity of **"chromatin erasers"** ▪ **"Chromatin readers"** – bind histones that bear marks and regulate gene expression
Histone methylation	▪ **Lysines** and **arginines –** can be methylated by specific writer enzymes ▪ **Methylation of lysine** residues in histone is asso.w/ either transcriptional activation or repression
Histone acetylation	▪ **Lysine** residues are acetylated by **histone acetyl transferase (HAT)** → modifications tend to open up the chromatin and increase transcription ▪ **Histone deacetylases (HDAC)** → reverses changes; lead to **chromatin condensation**
Histone phosphorylation	▪ Serine residues can be modified by **phosphorylation** ▪ Depending on the specific residue, the DNA may be opened up for transcription or condensed to become inactive
DNA methylation	▪ High levels of DNA methylation in gene regulatory elements result in **transcriptional silencing** ▪ Tightly regulated by **methyltransferases**, **demethylating enzymes** and **methylated-DNA-binding proteins**
Chromatin organizing factors	▪ Bind to noncoding regions and control long-range looping of DNA → important in regulating the spatial relationships between gene enhancers and promoters that control gene expression

Micro-RNA and Long Noncoding RNA

- Another mechanism of gene regulation depends on the functions of noncoding RNAs → encoded by genes that are **transcribed but not translated**
  - *microRNAs (small RNA molecules)*
  - *long noncoding RNAs (>200 nucleotides in length)*

Micro-RNA (miRNA)

- **miRNAs do not encode proteins** →function primarily to **modulate the translation of target mRNAs into their corresponding proteins**
- Posttranscriptional silencing of gene expression by miRNA is a **fundamental and well-conserved mechanism of gene regulation present in all eukaryotes**
- Human genome encodes approx 1000 miRNA genes
- miRNAs appear to regulate multiple protein-coding genes, allowing each miRNA to co-regulate entire programs of gene expression
- Transcription of miRNA genes produces **primary miRNA** → processed and trimmed by the enzyme **DICER** → generated mature single-stranded miRNA asso.w/ multiprotein aggregate called **RNA-induced silencing complex (RISC)**
- Base pairing between the miRNA strand and its target mRNA directs RISC to either induce mRNA cleavage or repress its translation
- **Seed sequence** – found in all mRNA in their **3-untranslated region (UTR)** that determines the specificity of miRNA binding and gene silencing
  - Target mRNA is posttranscriptionally silenced
- **Small interfering RNAs (siRNAs)** – short RNA sequences that can be introduced into cells
  - Serves as **substrates** for Dicer and interact w/ RISC complex
- **Knockdown technology** – synthetic siRNAs targeted against specific mRNAs to study gene function

Long Noncoding RNA (lncRNA)

- May exceed number of coding mRNAs by 10 to 20-fold
- **lncRNAs modulate gene expression in many ways**:
  - e.g., bind to regions of chromatin, restricting RNA polymerase access to coding genes w/in the region
- **XIST** – repressive function; transcribed from the X chromosome; plays essential role in physiologic X chromosome inactivation
  - XIST escapes X inactivation → forms a repressive cloak on the X chromosome resulting in gene silencing
- **Enhancers** – sites of lncRNA synthesis

CELLULAR HOUSEKEEPING

- Fundamental cellular housekeeping functions:
  - *Protection from environment*
  - *Nutrient acquisition*
  - *Communication*
  - *Movement*
  - *Renewal of senescent molecules*
  - *Molecular catabolism*
  - *Energy generation*
- Many normal housekeeping functions are **compartmentalized** w/in the membrane-bound intracellular organelles
  - *Potentially injurious degradative enzymes or reactive metabolites can be concentrated or stored at high concentrations in specific organelles w/o risking damage to other cellular constituents*
  - *Creates unique intracellular environments → regulates function*
- **New proteins (destined for plasma membrane) are synthesized in the rER and physically assembled in the Golgi apparatus**
  - **Proteins intended for the cytosol** are synthesized on **free ribosomes**
- **sER** – abundant in gonads and liver
  - used for steroid hormone and lipoprotein synthesis
  - used for modification of hydrophobic compounds (e.g., drugs) into water-soluble molecules for export
- Catabolism of constituents takes place at three different sites and serves different functions:
  - **Lysosomes –** intracellular organelles; contain degradative enzymes that permit the digestion of a wide-range of macromolecules including proteins, polysaccharides, lipids, nucleic acids
  - **Proteasomes** – "grinder"; selectively chews up denatured proteins, releasing peptides; in some cases peptides can be presented as MHC I molecules; signalling molecules trigger the proteasomal degradation of negative regulatory proteins, leading to activation of pathways that alter transcription
  - **Peroxisomes** – play specualized role in the breakdown of fatty acids, generating hydrogen peroxide in this process
- **Endosomal vesicles** – shuttle internalized material to intracellular sites or direct newly synthesized materials to cell surface or targeted organelle
- Cell movement is accomplished through the **cytoskeleton**

- Also maintain basic cellular shape and intracellular organization → requisite for maintaining cell polarity
- **Mitochondria** – where most ATP are made through oxidative phosphorylation
  - Also serve as an important source of metabolic intermediates that are needed for anabolic metabolism
  - Sites of **synthesis of certain macromolecules** (e.g., heme)
  - Contain important sensors of cell damage that can initiate and regulate the process of programmed cell death
  - **Lifespan: 10 days**
- **Organellar biogenesis** – replication of organelles

Plasma Membrane: Protection and Nutrient Acquisition

- **Plasma membrane** – fluid bilayers of amphipathic phospholipids w/ **hydrophilic head** groups (face aqueous part) and **hydrophobic lipid tails** (interact w/ each other to form a barrier to passive diffusion of large or charged molecules
- Bilayer is composed of heterogenous collection of different phospholipids → distributed **asymmetrically**
- Asymmetric partitioning of phospholipids is important in other cellular processes:

Phosphatidylinositol	▪ Inner membrane leaflet ▪ Can be phosphorylated → **serve as electrostatic scaffold for intracellular proteins** ▪ **Phosphatidylinositides** → can be hydrolyed by **phospholipase C** to generate intracellular signals like **DAG** and **IP3**
Phosphatidylserine	▪ Normally restricted to inner face; **confers negative charge for electrostatic protein interactions** ▪ When it flips to the extracellular face → during **apoptosis** → "**eat me**" signal for phagocytes ▪ Serve as **cofactor** in the clotting of blood for platelets
Glycolipids and sphingomyelin	▪ Expressed on extracellular face ▪ Glycolipids – important in cell-cell and cell-matrix interactions (inflammatory cell recruitment and sperm-egg interactions)

- **Lipid rafts** – lipid domains created from certain membrane components that have interacted horizontally in bilayer
- Plasma membrane is liberally studded w/ a variety of proteins and glycoproteins involved in:
  1. *Ion and metabolic transport*
  2. *Fluid-phase and receptor-mediated uptake of macromolecules*
  3. *Cell-ligand, cell-matrix, and cell-cell interactions*
- Proteins associate w/ lipid bilayer by one of four general arrangements;
  - *Most proteins are integral or transmembrane proteins. Integral membrane proteins contain positively charged aminoacids in their cytoplasmic domains w/c anchor the proteins to the negatively charged head groups of membrane phospholipids*
  - *Proteins may be synthesized from cytosol and posttranslationally attached to prenyl groups or fatty acyds*
  - *Insertion into membrane occur through GPI anchors on the extracellular surface*
  - *Peripheral membrane proteins may be noncovalently associate w/ true transmembrane proteins*
- **Many plasma membrane proteins function together as large complexes; these may either be aggregated under the control of chaperone molecules in the rER or by lateral diffusion in the plasma membrane followed by complex formation in situ**
- Extracellular surface of PM is studded w/ **carbohydrates** attached to integral membrane proteoglycan
  - **Glycocalyx** – functions as a chemical and mechanical barrier; also involved in cell-cell and cell-matrix interactions

Passive Membrane Diffusion

- **Small, nonpolar molecules (O2 and CO2)** → readily dissolve in lipid bilayers and rapidly diffuse cross them
- **Hydrophobic molecules** (steroid-based molecules, estradiol or vit D) → cross lipid bilayers w/ relative impunity
- **Polar molecules <75 daltons** → also readily cross membranes **(water, ethanol, urea)**
- **Aquaporins** – special integral protein that augment passive water transport in large volumes (renal tubular epithelium)
- Lipid bilayer is an effective barrier to the passage of polar molecules of >75 daltons in mass **(glucose)**
- Lipid bilayer is also **impermeant to ions**, no matter how small → **due to their charge and high degree of hydration**

Carriers and Channels

- For **low molecular weight species: ions and small molecules up to 1000 daltons**

Channel Proteins	▪ Create hydrophilic pores, w/c. When open, permit rapid movement of solutes (usually restricted by size and charge)
Carrier Proteins	▪ Bind their specific solute and undergo a series of conformational changes to transfer the ligand across the membrane ▪ Transport is relatively **slow**

- **Passive transport** – movement that involves a concentration and/or electrical gradient between in and out of the cell
- **Active transport** – of certain solutes against a concentration gradient is accomplished by carrier molecules (not channels)
  - Uses energy by ATP hydrolysis or a coupled ion gradient
- **Multidrug resistance (MDR) protein** – pumps polar compounds (chemo drugs) out of cells and may render cells resistant to treatment
- Plasma membranes are freely permeable to water → moves through osmosis
  - **Hypertonicity** – extracellular salt excess of that in the cytosol; causes a net movement of water out of cells
  - **Hypotonicity** – causes a net movement of water into cells
- **Cytosolic enzymes work at pH 7.4; lysosomal enzymes function best at pH5 or less**

Receptor-Mediated and Fluid-Phase Uptake

- **Endocytosis** – uptake of fluids or macromolecules by the cell; occurs by two mechanisms
- **Caveolae** – invaginations of plasma membrane; for take up of small molecules (vitamins)
- For bigger molecules, uptake occurs after binding to specific cell-surface receptors
  - Internalization occurs through a membrane invagination process driven by an intracellular coat of **clathrin** proteins
- **Clathrin** – hexamer of proteins that spontaneously assembles into a basket-like lattice to drive the invagination process
- **Exocytosis** – process where large molecules are exported from cells
  - Proteins synthesized and oackaged w/in the rER and GA are concentrated in secretory vesicles → then fuse w/ the plasma membrane and expel their contents
- **Transcytosis –** movement of endocytosed vesicles between the apical and basolateral part of cells
  - Mechanism for transferring large amounts of intact proteins across epithelial barriers (e.g., ingested antibodies in maternal milk across intestinal epithelia) or for rapid movement of large volume of solutes
  - **Increased transcytosis** → increased vascular wall permeability (healing wounds and tumor)
- Two forms of endocytosis:

Caveolae-mediated endocytosis	▪ **Caveolae** ("little caves") – noncoated PM invaginations asso.w/ GPI-linked molecules, cAMP binding proteins, SRC-family kinases, and folate receptor ▪ **Caveolin** – major structural protein of caveolae ▪ Potocytosis – internalization of caveolae and associated extracellular fluid; "**cellular sipping"** ▪ Caveolae participate in transmembrane delivery of molecules (folate); also regulate transmembrane signalling and/or cellular adhesion via the internalization of receptors and integrins
Pinocytosis and receptor-mediated endocytosis	▪ **Pinocytosis – "cellular drinking";** describes a fluid-phase process where the PM invaginates and is pinched off to form a cytoplasmic vesicle ▪ Endocytosed vesicles may recycle back to PM (exocytosis) ▪ Both process begin at a specialized region of the PM called the **clathrin-coated pit,** w/c rapidly invaginates and pinches off to form a **clathrin-coated vesicle** ▪ Vesicles uncoat and fuse w/ early endosome where they discharge their contents for digestion and further passage to the lysosome ▪ **Receptor-mediated endocytosis** – major uptake mechanism for certain macromolecules (e.g., transferring, LDL) o These macromolecules bind to receptors that are localized in clathrin coated pits o Then endocytosed and fuse w/ lysosome o w/in lysosome, they release their cargo and taken up in cytoplasm

Cytoskeleton and Cell-Cell Interactions

- The ability of cells to adopt a particular shape, maintain polarity, organize the relationship of intracellular organelles, and move about depends on the intracellular scaffolding of proteins called **cytoskeleton**
- Three major classes of cytoskeletal proteins (eukaryotes):

Actin microfilaments	▪ **5-9nm** dm fibrils from G-actin ▪ Most abundant cytosolic protein in cells ▪ G-actin are polymerize into long F-actin → form double stranded helices ▪ In muscle cells, myosin binds to actin and moves along it, driven by ATP hydrolysis ▪ In non-muscle cells, F-actin assembles via an assortment of actin-binding proteins to well-organized bundles and networks that control shape and movement
Intermediate filaments	▪ **10nm dm** fibrils; comprise a large and heterogenous family ▪ Have cxc tissue-specific patterns of expression; useful for assigning a cell for poorly differentiated tumors: o **Laminin A, B, C** – nuclear lamina of all cells o **Vimentin** – mesenchymal cells (fibroblast, endothelium) o **Desmin –** muscle cells, forming the scaffold on w/c actin and myosin contract o **Neurofilaments** – axons of neurons, imparting strength and rigidity o **Glial fibrillary acidic protein** – glial cells around neurons o **Cytokeratins –** at least 30 varieties; subdivided into acidic **(type I)** and neutral/basic **(type II);** used as cell markers ▪ Found predominantly in a polymerized form w/in cells ▪ **Do not actively reorganize** like actin and microtubules ▪ Impart tensile strength and allow cells to bear mechanical stress; also form the major structural proteins of skin and hair ▪ **Nuclear membrane lamins** – maintain nuclear morphology and regulate normal nuclear transcription
Microtubules	▪ **25 nm thick fibrils**; noncovalently polymerized dimmers of α and β-tubulin arrayed in elongating or shrinking hollow tubes ▪ Ends are designated as **"+" or "-"** ▪ **"-" end** → embedded in a MTOC or centrosome near nucleus asso.w/ paired nucleus ▪ **"+" end** → elongates or recedes in response to stimuli by addition or subtraction of tubulin dimmers ▪ Microtubules serve as connecting cables for **"molecular motor"** proteins ; also participate in sister chromatid separation during mitosis ▪ Adapted to form motile cilia or flagella ▪ Two motor proteins: o **Kinesins** – anterograde (- to +) transport o **Dyneins** – for retrograde (+ to -) transport

Cell-Cell Interactions

- Cells interact and communicate w/ one another by forming junctions that provide mechanical links and enable surface receptors to recognize ligands on other cells
- Cell junctions are organized into three basic types:

Occluding junctions (tight junctions)	▪ Seal adjacent cells to create a **continuous barrier** → restricts paracellular (between cells) movement of ions and other molecules ▪ Form a tight meshlike network of macromolecular contacts between neighboring cells ▪ Transmembrane proteins that mediate cell-cell interactions: o Occludin; Claudin; Zonulin; Catenin ▪ Form a high resistance barrier to solute movement ▪ Represents the **boundary** that allows the segregation of apical and basolateral domains of cells → maintain polarity ▪ Can dissociate and reform
Anchoring junctions (desmosomes)	▪ Mechanically attach cells to other cells or ECM ▪ **Cadherins** – transmembrane glycoproteins found in desmosomes ▪ **Spot desmosome or macula adherens** – adhesion focus is between cells and is small and rivet-like o Cadherins are called **desmogleins** and **desmocollins** ▪ **Hemidesmosome** – adhesion attahes the cell to the ECM o **Integrins** – transmembrane connector proteins o **Focal adhesion complexes** – large (>100 proteins) macromolecular complexes; localized at desmosomes; can generate intracellular signals when cells are in shear stress ▪ **Belt desmosomes** – occur as broad bands between cells o **E-cadherins** – transmembrane adhesion molecules; asso.w/ intracellular actin microfilaments; influence cell shape and motility
Communicating junctions (gap junctions)	▪ Mediate the passage of chemical or electrical signals from one cell to another ▪ Consists of a dense planar array of 1.5-2 nm pores called **connexons** formed by **connexins** ▪ **Connexons** → permit the passage of ions, nucleotides, sugars, amino acids, vitamins, and other small molecules ▪ Permeability of junction is rapidly reduced by lowered intracellular pH or increased intracellular calcium ▪ Play critical role in cell-cell communication: cardiac myocytes

Biosynthetic Machinery: Endoplasmic Reticulum and Golgi

- **Endoplasmic reticulum (ER**) – site for synthesis of all transmembrane proteins and lipids for plasma membrane and cellular organelles, including ER itself
  - Initial site for the synthesis of all molecules destined for export out of cell
  - Meshlike interconnected maze of branching tubes and flattened lamellae → form continuous sheet
  - Composed of contiguous but distinct domains → distinguished by the presence **(rough ER)** or absence **(smooth ER)** of **ribosomes**
- Membrane-bound ribosomes on cytosolic face of rER translate mRNA into proteins that are extruded into ER lumen or integrated into ER membrane
  - Directed by signal sequences on N-termini of nascent proteins
  - Lack of signal sequence → translation occurs on free ribosomes in the cytosol
  - Transcripts are read by **polyribosomes**
- Proteins inserted in ER fold can **oligomerize** (form polypeptide complexes) and **N-linked oligosaccharides** are added
- **Chaperone molecules** – retain proteins in the ER until modifications are done
  - If protein fails to fold or oligomerize → retained and degraded in ER
- **ER stress response** – aka **unfolded protein response (UPR)**
  - Triggers cell death through apoptosis that resulted from excess accumulation of misfolded proteins and exceeds the capacity of ER to edit and degrade them
- **From RER, proteins and lipuds are shuttled into golgi apparatus**
- **Golgi apparatus** – consist of stack cisternae that progressively modify proteins in an orderly fashion → from **cis (near the ER) to trans (near the PM)**
  - **N-linked oligosaccharides** – pruned and further modified
  - **O-linked oligosaccharides** (sugar moieties linked to serine or threonine)
  - **Glycosylation** – important in directing molecules to lysosomes
- **Cis Golgi network** – recycle proteins back to the ER
- **Trans Golgi network** – sorts proteins and lipids and dispatches them to other organelles (including PM) or to secretory vesicles destined for release
- **Golgi complex** – prominent in cells specialized for secretion (goblet cells, bronchial epithelium, and plasma cells)
- **sER –** sparse and exist as the transition zone from RER to transport vesicles moving to the Golgi
  - conspicuous in cells that synthesized steroid hormones (gonads, adrenals) or that catabolise lipid-soluble molecules (liver)
  - repeated exposure to compounds that are metabolized by sER (Phenobarbital) → **reactive SER hyperplasia**
  - responsible for sequestering intracellular calcium
  - muscle cells: **sacroplasmic reticulum (**specialized sER) – responsible for cyclical release and sequestration of calcium ions → regulate contraction and relaxation

Waste Disposal: Lysosomes and Proteasomes

- Cellular waste disposal depends on the activities of **lysosomes** and **proteasomes**

Lysosomes

- Membrane-bound organelles containing 40 different acid hydrolases → **best function at pH <5** (proteases, nucleases, lipases, glycosidases, phosphatases, sulfatases)
- Lysosomal enzymes are initially synthesize in the ER lumen and then tagged w/ a **M6p residue w/in GA**
- Other molecules destined for catabolism in the lysosomes arrive by one of three other pathways:
    - **Fluid-phase pinocytosis or receptor mediated endocytosis**
        - Material internalized passes from PM to early endosome to late endosome and lastly to lysosome
        - Maturation → organelle becomes more acidic
    - **Autophagy** – shuttles senescent organelles and large, denatured protein complexes into lysosomes
        - Autophagosome fuses w/ lysosomes and contents are catabolise
        - Use to preserve cell viability during nutrient depletion
    - **Phagocytosis** – occurs primarily in professional phagocytes (macrophage or neutrophils
        - Material is engulfed to form a phagosome that fuses w/ lysosome

Proteasomes

- Play important role in **degrading cytosolic proteins** (denatured or misfolded proteins, macromolecules)
- Proteins destined for destruction are identified by covalently binding to **ubiquitin**
- **Proteasomes digest proteins into small (6-12 amino acids) fragments that can subsequently be degraded to amino acids and recycled**

CELLULAR METABOLISM AND MITOCHONDRIAL FUNCTION

- Mitochondria contain their own DNA
    - Can carry out all steps of DNA replication, transcription, and translation
    - Mitochondrial DNA is entirely **maternally inherited**
- Mitochondrial disorders may be: X-linked, autosomal, or maternally inherited
- Mitochondria provide enzymatic machinery for oxidative phosphorylation
    - Also have a role in anabolic metabolism
    - Play a fundamental role in regulating apoptosis

Energy Generation

- Mitochondria has two separate membranes
- **Inner membrane** contains the enzyme of the respiratory chain folded into **cristae**
    - Encloses a core **matrix space** → contains most of the enzyme of citric acid cycle
- **Intermembrane space** – outside the inner membrane
    - Site of ATP synthesis; enclosed by outer membrane
- **Outer membrane** – encloses the intermembrane space
    - Studded w/ **porin** proteins → forms aqueous channels permeable to small (<5000 daltons) molecules
- Oxidation of various metabolites drives hydrogen ion (proton) pumps that transfer H+ from the core matrix in the intermembrane space
    - As H+ ions flow back, the energy released is used on the synthesis of ATP
- **Thermogenin** – inner membrane protein; energy can be used to generate heat
    - Brown fat → nonshivering thermogenesis
- **Lifespan of mitochondria → 1-10 days**

Intermediate Metabolism

- Oxidative phosphorylation produce ATP but also burns glucose to CO_2 and H_2O
    - No carbon moieties left to use as building blocks for lipids or proteins
    - Leads to **Warburg effect** → rapidly growing cells upregulate **glucose** and **glutamine** uptake and decrease their production of ATP per glucose molecule
    - Glutamine and glucose provide carbon moieties that prime mitochondrial TCA cycle → intermediates are spun off to make lipids, nucleic acids and proteins

Cell Death

- Mitochondria also regulate the balance of cell survival and death
- Two major pathways of cell death:

Necrosis	▪ External cellular injury (toxin, ischemia, trauma) can damage mitochondria → formation of **mitochondrial permeability transition pores in the outer membrane** ▪ Channels allows dissipation of protein potential → ATP generation fails → cell death
Apoptosis	▪ **Programmed cell death;** central feature of normal tissue development and turnover ▪ Triggered by extrinsic signals (cytotoxic T cells, inflammatory cytokines) or intrinsic pathways (DNA damage, intracellular stress) ▪ Mitochondria play a central role in the intrinsic pathway of apoptosis o Damaged mitochondria → cell cannot synthesize proteins → **leaky mitochondria** o Cytochrome C leaks into cytosol → activate caspase → induce apoptosis

CELLULAR ACTIVATION

Cell Signaling

- Signals that most cells respond to can be classified into several groups:

✓ *Damage to neighboring cells and pathogens* → "danger signals"
✓ *Contact with neighboring cells*
  - Through adhesion molecules or gap junctions
  - Gap junction signalling – accomplished between adjacent cells via hydrophilic connexons that permit movement of small ions, metabolites, second messengers (cAMP) but not larger molecules
✓ *Contact w/ ECM* → mediated through integrins
✓ *Secreted molecules* → includes growth factors, cytokines, hormones

- Extracellular cell-cell signalling parthways are classified as:
  - **Paracrine signalling** – cells at immediate vicinity are affected
  - **Autocrine signalling** – affects the same cell that secretes molecules
  - **Synaptic signalling** – neurotransmitters at synapse
  - **Endocrine signalling** – mediator is released into blood stream and acts on target cells at a distance
- Regardless of the nature of an extreacellular stimulus, the signal it conveys is transmitted to the cell via a specific receptor protein
- Receptors may be present on the cell surface or located w/in the cell

✓ **Intracellular receptors**
  - Transcription factors that are activated by lipid-soluble ligands that easily cross PM
  - E.g., vitamin D and steroid hormones → activate nuclear hormone receptors
  - Nitric oxide – directly activate guanylyl cyclase → cGMP
✓ **Cell-surface receptors**
  - Transmembrane proteins w/ extracellular domains that bind soluble ligands
  - Depending on receptor, ligand binding can then:
    1. *Open ion channels*
    2. *Activate G protein*
    3. *Activate endogenous or associated enzyme (e.g., tyrosine kinase)*
    4. *Trigger a proteolytic event or change in protein binding or stability that activates latent transcription factor*
  - Activities (2) and (3) associated w/ growth factor signalling pathways that drive cell proliferation
  - Activity (4) common feature of multiple pathways (e.g., Notch, Wnt, Hedgehog) that regulate normal development
  - Signal transducer by cell surface receptors are often deranged in developmental disorders and in cancers

SIGNAL TRANSDUCTION PATHWAYS

- Cellular receptors are grouped into several types based on the signalling mechanisms they use and the intracellular biochemical pathways they activate
- Receptor signalling leads to formation or modification of biochemical intermediates or activation of enzymes →generation of active transcription factors that enter nucleus and alter gene expression

✓ **Receptor associated w/ kinase activity**
  - Downstream phosphorylation is a common pathway of transducing signals
  - Alteration in receptor geometry → elicit receptor protein kinase activity or promote enzymatic activity → addition of charged phosphate residues to target molecules
  - **Tyrosine kinase** – phosphorylate specific tyrosine residues
  - **Serine/threonine kinase** – add phosphates to distinct serine or threonine residues
  - **Lipid kinases** – phosphorylate lipid substrates
  - **Phosphatases** – enzyme involve in phosphorylation
    - Enzyme that can remove the phosphate residue and thus modulate signalling
    - Play an inhibitory role in signal transduction

    1. **Receptor tyrosine kinase (RTKs)** – integral membrane proteins (receptors for insulin, epidermal growth factor, PDGF); ligan-induced cross-linking activates intrinsic tyrosine kinase in cytoplasmic tails
    2. **Nonreceptor tyrosine kinase** – no intrinsic catalyric acitivity (immune receptors, some cytokine receptors, integrins); intracellular protein that phosphorylates specific motifs on the receptor or other proteins (e.g., Src-family kinases)

✓ **G-protein coupled receptors (GPCR)**
  - Polypeptides that characteristically traverse the PM seven times → seven-transmembrane or serpentine receptors
  - After binding, receptor dissociates w/ a GTP-binding protein (G-protein) that contains GDP
  - G-protein interaction w/ receptor-ligand complex results in activation through exchange of GDP for GTP
  - Downstream receptor mediated signalling events leads to generation of cAMP and IP3 (releases calcium from the ER)

✓ **Nuclear receptors**
  - Lipid-soluble ligands can diffuse into cells and interact w. Protein to form a receptor-ligand complex that directly binds to nuclear DNA → activate or repress gene transcription

- ✓ **Other classes of receptors**
  - Originally recognized as important for embryonic development and cell fate determination
  - Also participate in mature cells (immune system)
  - **Notch family receptor proteins** – ligand binding to Nitch receptors leads to proteolytic cleavage of the receptor and nuclear translocation of the cytoplasmic piece (intracellular Notch) to form a transcription complex
  - **Wnt protein ligands** – influence cell development through **Frizzled family receptors,** w/c regulate intracellular **β-catenin** (targeted for ubiquitin-directed proteasome degradation);
    - Wnt binding to Frizzled recruits **Disheveled** that leads to disruption of the degradation-targeting complex

Modular Signaling Proteins, Hubs, and Nodes

- Specific phosphoryation of a protein can allow it to associate w/ a host of other molecules, resulting in multiple effects:
  - *Enzyme activation (or inactivation)*
  - *Nuclear (or cytoplasmic) localization of transcription factors*
  - *Transcription factor activation (or inactivation)*
  - *Actin polymerization (or depolymerisation)*
  - *Protein degradation (or stabilization)*
  - *Activation of feedback inhibitory (or stimulatory) loops*
- **Adaptor proteins** – play a key role in organizing intracellular signalling pathways
  - Functions as molecular connectors that physically link enzymes and promote assembly of complexes → can determine downstream signalling events
  - Can be integral or cytosolic proteins
  - May contain few specific domains that mediate protein-protein interactions
  - **Nodes** – protein-protein complexes
  - **Hubs** – biochemical events feeding into or emanating from these nodes

Transcription Factors

- **Most signal transduction pathways ultimately influence cellular function by modulating gene transcription through the activation and nuclear localization of transcription factors**
- Changes allow translocation into nucleus or expose specific DNA or protein binding motifs
- **MYC** and **JUN** – transcription factors that regulate the expression of genes needed for growth
- **p53** – transcription factor that triggers the expression of genes that lead to growth arrest
- Transcription have modular design containing domains that bind DNA and interact w/ other proteins, such as components of RNA polymerase complex, that are needed to drive transcription.
  - DNA-binding domain permits specific binding to short DNA sequences
  - To induce transcription, it must also possess protein:protein interaction domains that directly or indirectly recruit histone modifying enzymes, chromatin remodelling complexes, and RNA polymerase (most important)

GROWTH FACTORS AND RECEPTORS

- A major role of growth factors is to **stimulate the activity of genes** that are required for cell growth and cell division
- Activity is mediated through binding to specific receptors, ultimately influencing the expression of genes that can:
  - *Promote entry of cells into the cell cycle*
  - *Relieve blocks on cell cycle progression* (promote replication)
  - *Prevent apoptosis*
  - *Enhance biosynthesis of cellular components* (nucleic acids, proteins, lpids, carbohydrates) required for a mother cell to give rise to two daughter cells
- **Proto-oncogenes** → gain-of-function mutations in these genes can convert them into oncogenes capable of driving unfettered cell proliferation and tumor formation
- Table 1-1

Epidermal Growth Factor and Transforming Growth Factor-α	• Both belong to EGF family • Both are produced by: **macrophages, variety of epithelial cells** • Mitogenic for heptaocytes, fibroblasts, and a host of epithelial cells • **EGF receptor family →** includes four membrane receptors w/ intrinsic tyrosinase activity: ○ **EGFR1** (aka ERB-B1 or EGFR) • **EGFR1 mutations** → cancers of the lung, head, neck, breast, brain • **ERBB2 receptor (aka HER2**) → breast cancers
Hepatocyte Growth Factor	• HGF; aka **scatter factor** • Has mitogenic effects on hepatocytes and most epithelial cells, including biliary, pulmonary, renal, mammary, and epidermal • Acts as **morphogen** in embryonic development → influences tissue differentiation • Promotes cell migration → **"scatter factor"** • Enhances heaptocyte survival • Produced by **fibroblasts, mesenchymal cells, endothelium and nonhepatocyte liver cells** • Synthesized as an inactive precursor (**pro-HGF**) → activated by **serine proteases** released at site of injury • **MET** – receptor for HGF; has intrinsic tyrokinase activity; overexpressed or mutated in tumors (renal and thyroid papillary carcinomas)
Platelet-Derived Growth Factor	• Family of several closely related proteins; each consisting of two chains • Three active isoforms → **PDGF-AA, AB, BB** • **PDGF-CC** and **PDGF-DD** → must be activated by proteolytic cleavage • **PDGF –** stored in platelets and released on platelet activation • Produced by: **platelets, macrophages, endothelium, smooth muscle cells and variety of tumors** • All PDGF isoforms exert their side effects by binding to two cell surface receptors (**PDGFR α & β),** both have tyrosinase kinase activity

	• PDGF induce fibroblast, endothelial and smooth muscle cell proliferation and matrix synthesis, and is **chemotactic** for these cells (and inflammatory cells) • Promote recruitment of the cells into areas of inflammation and tissue injury
Vascular Endothelial Growth Factor	• **VEGFs (A,B,C,D)** and **PIGF (placental growth factor)** – family of homodimeric proteins • **VEGF-A** – aka VEGF; major angiogenic factor after injury and tumors • **VEGF-B** and **PIGF** – involved in embryonic vessel development • **VEGF-C** and **D** – stimulate both angiogenesis and lymphatic development (lymphangiogenesis) • Involved maintenance of normal adult endothelium, w/ highest expression in epithelial cells adjacent to fenestrated epithelium (e.g., podocytes, epith in retina, choroid plexus in brain) • Induces angiogenesis by promoting endothelial cell migration, proliferation (capillary sprouting), and formation of the vascular lumen • Induce vascular dilation and increased vascular permeability • **Hypoxia** – most important inducer of VEGF thru HIF-1 • **PDGF** and **TGF-α** – also induce VEGF • Bind to family of receptor tyrosine kinases (VEGFR-, 2, 3) • **VEGFR-2** – highly expressed in endothelium; most important for angiogenesis • **Anti-VEGF antibodies** – used for ophthalmic diseases (e.g., AMD) • Increased levels of soluble VEGFR-1 (s-FLT-1) in pregnant women may cause preeclampsia (hypertension and proteinuria) by "sopping up" the free VEGF required for maintaining normal endothelium
Fibroblast Growth Factor	• Family of growth factors w/ more than 20 members • **Acidic FGF** → aFGF or FGF-1 • **Basic FGF** → bFGF or FGF-2 • **FGF-7** – referred to as keratinocyte growth factor (KGF) • **Released FGFs asso.w/ heparan sulfate in ECM** → serves as reservoir for inactive factors that can be released by proteolysis (e.g., wound healing) • Contribute to wound healing process, hematopoiesis, and development • bFGF has all the activities necessary for angioegenesis

Transforming Growth Factor-β	• **TGF-β1, TGF-β2, TGF-β3**; belong to a family of 30 members; includes BMPs, activins, inhibins, mullerian inhibiting substance
	• **TGF-β1** – has most widespread distribution; more commonly referred to as TGF-β
	• Homodimeric protein; produced by multiple cell types: platelets, endothelium, mononuclear inflammatory cells
	• Secreted as precursors that requires proteolysis to activate
	• Has two receptors (type I and II) → both w/ **serine/threonine kinase activity** → induce phosphorylation of transcription factors called **Smads**
	• Phosphorylated Smads form heterodimers w/ Smad4 → allow nuclear translocation and association w/ other DNA-binding proteins to activate or inhibit gene transcription
	• TGF-β has multiple opposing effects; **pleiotropic w/ a vengeance**
	• **Pleiotropic** – agents w/ multiple effects
	• TGF-β drives scar formation and applies brakes on the inflammation that accompanies wound healing
	  ○ **TGF-β** stimulates production of collagen, fibronectin and proteogylcans
	  ○ Inhibits collagen degradation by both decreasing MMP acitivity and increasing activity of TIMPS
	  ○ Involve in scar formation and drives fibrosis in lung, liver, kidneys in chronic inflammation
	  ○ TGF-β is an anti-inflammatory cytokine that serves to limit and terminate inflammatory responses by inhibiting lymphocyte proliferation and other leukocytes
	  ○ **Absence of TGF-β** → widespread and persistent inflammation

INTERACTION WITH THE EXTRACELLULAR MATRIX

- **ECM** – network of interstitial proteins with significant proportion of tissues; "simple filler"
- Cell interactions w/ ECM are critical for development and healing, as well as for maintain normal tissue architecture
- Functions:
  - *Mechanical support*
  - *Control of cell proliferation*
  - *Scaffolding for tissue renewal*
  - *Establishment of tissue microenvironments*
- ECM is constantly being remodelled; its synthesis and degradation accompany morphogenesis, tissue regeneration and repair, chronic fibrosis and tumor invasion and metastasis
- ECM occurs in two basic forms: interstitial matrix and basement membrane:

Interstiital matrix	• Present in the spaces between cells and CT, and between parenchymal epithelium and vascular and smooth muscle structure • Synthesized by **mesenchymal cells** (e.g., fibroblasts); forms a three-dimensional amorphous gel • Major constituents**: fibrillar and nonfibrillar collagens, fibronectin, elastin, proteoglycans, hyaluronate,** etc
Basement membrane	• Highly organized matrix • Synthesized by contributions from overlying epithelium and underlying mesenchymal cells, forming a flat lamellar "chicken wire" mesh (porous) • Major constituents: **amorphous nonfibrillar type IV collagen and laminin**

Components of ECM

Fall into three groups:

1. **Fibrous structural proteins** – such as collagens and elastins that confer tensile strength and recoil
2. **Water-hydrated gels** – such as proteoglycan and hyaluronan that permit compressive resistant and lubrication
3. **Adhesive glycoproteins** – that connect ECM elements to one another

<table>
<tr><td>Collagen</td><td>
Composed of three separate polypeptide chains braided into a ropelike helix; 30 types are known
<table>
<tr><td>Fibrillar collagens</td><td>Form linear fibrils stabilized by interchain hydrogen bonding → e.g., types I, II, III, V collagens
Major proportion of the CT in structures such as bone, tendon, cartilage, blood vessels and skin, also in healing wounds and scars
Tensile strength came from lateral cross-linking of triple helices by lysyl oxidase
Process is dependent in vitamin C
Ascorbate deficiency in children → skeletal deformities
Any age → bleed easily
Genetic defects in collagen → osteogenesis imperfect and Ehlers-Danlos syndrome</td></tr>
<tr><td>Nonfibrillar collagens</td><td>Contribute to the structures of planar basement membrane (type IV)
Help regulate collagen fibril diameters or collagen-collagen interactions via FACITS (e.g., type IX)
Provide anchoring fibrils to the basement membrane beneath SSE (type VII)</td></tr>
</table>
</td></tr>
<tr><td>Elastin</td><td>Confers the ability of tissues to recoil and recover their shape after physical deformation
Important in cardiac valves and large vessels, uterus, skin, ligaments
Elastic fibes consist of a central core of elastin w/ am associated meshlike network composed of fibrillin
Fibrillin synthetic defects lead to skeletal abnormalities and weakened aortic walls → Marfan syndrome</td></tr>
<tr><td>Proteoglycans and hyaluronans</td><td>Proteoglycans – form highly hydrated compressible gels that confer resistance to compressive forces
Joint cartilage → proteoglycans provide a layer of lubrication between adjacent boney surfaces
Consist of link polysaccharides called glycosaminoglycans (e.g., keratan sulfate and chondroitin sulfate) attached to a core protein → linked to long hyaluronic acid polymer called hyaluronan → attracks water→ gelatine-like matrix
Serve as reservoir for growth factors secreted into ECM
Some are integral cell membrane proteins involve in cell proliferation, migration and adhesion</td></tr>
</table>

<table>
<tr><td>Adhesive glycoproteins and adhesion receptors</td><td>
Structurally diverse; involved in cell-cell adhesion, linking cells to ECM and interactions between ECM components
Fibronectin – major components of interstitial ECM
Laminin – major constituent of basement membrane
Integrins – representative of the adhesion receptors aka CAMs;
CAMs also includes Ig, cadherins and selectins
<table>
<tr><td>Fibronectin</td><td>Large; 450kD disulfide-linked heterodimer; exist in tissue and plasma forms
Synthesized by fibrobalsts, monocytes and endpthelium
Has specific domains that bnd to ECM components (collagen, fibrin, heparin, proteoglycans) and integrins
In healing wounds, tissue and plasma fibronectin provide the scaffolding for subsewuent ECM deposition, angiogenesis, and reepithelialisation</td></tr>
<tr><td>Laminin</td><td>Most abundant glycoprotein in BM
820kD cross-shaped heterotrimer that connects cells to underlying ECM components such as type IV collagen and heparan sulfate
Also modulate cell proliferation, differentiation and motility</td></tr>
<tr><td>Integrin</td><td>Large family of transmembrane heterodimeric glycoproteins composed of α and β subunits
Allow cells to attach to ECM constituents such as laminin and fibronectin
Functionally and structurally links the intracellular cytoskeleton w/ the outside world
Integrin on the surface of WBC are essential in mediating firm adhesion and transmigration across endothelium at sites of inflammation, and play a role in platelet aggregation
Integrin attach to ECM components via a tripeptide arginine-glycine-aspartic acid motif (RGD)
Also influence cell locomotion proliferation, shape and differentiation</td></tr>
</table>
</td></tr>
</table>

MAINTAINING CELL POPULATIONS

Proliferation and the Cell Cycle

- Cell proliferation is fundamental to development, maintenance of steady state tissue homeostasis, and replacement of dead or damaged cells
- Key elements of cellular proliferation:
  - *Accurate DNA replication*
  - *Equal apportionment of DNA through mitosis and cytokinesis*
- **Cell cycle** – sequence of events that results in cell division; consists of:
  - *G1 phase* (presynthetic growth)
  - *S phase* (DNA synthesis)
  - *G2 phase* (premitotic growth)
  - *M phase* (mitotic)
- **G0 state** – where quiescent cells are not actively cycling
- The cell cycle is regulated by activators and inhibitors
- **Cyclins and CDKs** – proteins that drives cell cycle progression
  - CDKs acquire ability to phosphorylate by forming complexes w/ cyclins
  - Cyclins D, E, A , B → appear in cell cycle and bind to CDK
- Transient increase in synthesis of cyclin leads to increase kinase activity
  - After CDK completes phosphorylation, asso. cyclin is degraded and CDK activity abates
- Quality control checkpoints serve as surveillance mechanisms that sense DNA or chromosomal damage
  - Cells w/ genetic imperfections do not complete replication
- **G1-S checkpoint** – monitors the integrity of DNA before irreversibly committing cellular resources to DNA replication
- **G2-M restriction point** – ensures that there has been accurate genetic application before the call actually divides
- When cells detect DNA irregularities:
  - Checkpoint activation delays cell cycle progression and triggers DNA repair mechanism
  - If too severe → **apoptosis**
  - Nonreplicative state → **senescence** → through p53-dependent mechanisms
- **CDK inhibitors (CDKIs)** – enforces cell cycle checkpoints
  - Modulate CDK-cyclin complex activity
- Several different CDKIs:
  - One family: composed of three proteins that broadly inhibits multiple CDKs
    - **P21 (CDKN1A)**
    - **P27 (CDKN1B)**
    - **P57 (CDKN1C)**
  - Other family that selectively effects cyclin CDK4 and cyclin CDK6
    - **P15 (CDKN2B)**
    - **P16 (CDKN2A)**
    - **P18 (CDKN2C)**
    - **P19 (CDKN2D)**

Stem cells

- **During development, stem cells give rise to all the various differentiated tissues; in the adult organism, stem cells replace damaged cells and maintain tissue populations as individual cells w/in them undergo replicative senescence due to attrition of telomeres**
- Stem cells are characterized by two important properties:
  - **Self renewal** – permits stem cells to maintain their numbers
  - **Asymmetric division** – one daughter cell enters a differentiation pathway and give rise to mature cells, while other remains undifferentiated and retains its self-renewal capacity
- **Two varieties:**

Embryonic stem cells (ES cells)	• **Most undifferentiated** • Present in **inner cell mass of the blastocyst** • Have **limitless cell renewal capacity** and can give rise to every cell in the body → **totipotent** • Can be maintained for extended periods w/o differentiating • Can be induced under appropriate culture conditions to form specialized cells of all three germ cell layers (neurons, cardiac muscle, liver cells and pancreatic islet cells)

Tissue stem cells (adult stem cells)	• Found in intimate asso.w/ the differentiated cells of a given tissue
	• Normally protected w/in specialized tissue microenvironments called **stem cell niche**
	  ○ Brain: subventricular zone and dentate gyrus
	  ○ Skin: hair follicle
	  ○ Cornea: limbus
	• Limited repertoire of differentiated cells that they can generate
	• Can maintain tissues w/ high (skin, GIT) or low (heart and brain) cell turnover
	• Can only produce cells that are normal constituents of that tissue
	• E.g., hematopoietic stem cells, mesenchymal stem cells

Regenerative Medicine

- The ability to identify, isolate, expand and transplant stem cells has given birth to the new field of regenerative medicine
- Difficulties encountered in introducing and functionally integrating the replacement cells into sites of damage:
  - **Immunogenicity** – some express HLA
- **Induced pluripotent stem cells (iP cells)** – derived from patient; their differentiated progeny can be engrafted w/o eliciting a rejection reaction
- **Genomic editing** – process using a nuclease called **Cas9** ; selectively alter or correct DNA sequences