Chapter

1

Cell Biology

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QUESTIONS

1. Lysosomes are known as suicidal bags of the cell.
2. Mitochondria is known as power house of the cell.
3. ERAD is the cause of diabetes mellitus.
4. Mitochondrial diseases are transmitted to the offspring through their mother.
5. Cathepsins play a key role in tumor metastasis.
6. I cell disease is characterized by lack of enzymes in lysosomes.
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8. Biomembranes are fluid in nature.
9. Biomembranes are asymmetrical in nature.
10. Biomembranes are dynamic in nature.

QUESTIONS AND ANSWERS

Q1. Lysosomes are known as suicidal bags of the cell.

Ans: • Lysosomes are small, enzyme-filled cell organelles with an optimum pH of ~5. They contain numerous acid hydrolases that digest biomolecules.

When phagocytic vesicles containing foreign particles or endocytic vesicles carrying damaged organelles fuse with lysosomes, they form secondary lysosomes (digestive vacuoles).

Lysosomal Enzymes	
1. Carbohydrate hydrolyzing enzymes	α-Glucosidase, β-galactosidase, α-mannosidase, β-glucuronidase, hyaluronidase, aryl sulfatase
2. Lipid hydrolyzing enzymes	Lipase, Phospholipases, Fatty acyl esterases
3. Proteolytic enzymes	Peptidases, elastase, collagenase, cathepsins
4. Nucleic acid hydrolyzing enzymes	Ribonuclease, deoxyribonuclease
5. Other enzymes	Catalase, acid phosphatase
Acid Phosphatase is used as a marker enzyme for this organelle	

The contents are then broken down into simpler molecules, which are either utilized by the cell or released to the exterior as exosomes.
Lysosomes are central to autophagy, a controlled catabolic process that recycles damaged organelles and misfolded proteins.
Microautophagy—direct invagination of cytoplasmic contents by lysosomes.
Macroautophagy—fusion of lysosomes with autophagosomes containing unwanted cellular material.
Chaperone-mediated autophagy (CMA)—selective degradation of proteins recognized by specific chaperones.
Defective autophagy has been linked to diseases such as cancer, Alzheimer's disease, and neuromuscular disorders.
Lysosomes, the "suicidal bags" of the cell, mediate autophagy by digesting damaged organelles and proteins, with defects linked to cancer, Alzheimer's, and neuromuscular disorders.

Q2. Mitochondria is known as power house of the cell.

Ans: Mitochondria are called the **powerhouse of the cell** because they generate ~90% of cellular ATP through **oxidative phosphorylation**.
ATP serves as the universal energy currency, driving vital processes such as muscle contraction, nerve impulse transmission, protein synthesis, and DNA replication.

Mechanism of ATP production

- ***Stage 1: Pyruvate oxidation and citric acid cycle:*** Pyruvate is converted to acetyl-CoA, which enters the TCA cycle, producing reducing equivalents (NADH, $FADH_2$).
- ***Stage 2: Electron transport chain (ETC):*** Electrons from NADH and $FADH_2$ pass through complexes I–IV in the inner mitochondrial membrane, releasing energy to pump protons into the intermembrane space.
- ***Stage 3: Chemiosmosis:*** The proton gradient drives *ATP synthase (Complex V)*, converting ADP to ATP (Mitchell's chemiosmotic theory).

Structural adaptations: The ***inner mitochondrial membrane*** is highly folded into ***cristae***, greatly increasing surface area for ETC complexes and ATP synthase, thereby enhancing ATP output.

Quantitative yield

- ***Anaerobic glycolysis:*** 2 ATP/glucose
- ***Mitochondrial respiration:*** +32–34 ATP
  →~16–17 times higher energy yield with mitochondria.

Cell-specific adaptations

- ***Muscle and cardiac cells:*** Extremely rich in mitochondria (up to 40% of cell volume).
- ***Neurons:*** High mitochondrial density to support signaling.
- ***Ovum:*** Highest mitochondrial count among cells.
- ***Low-energy cells (e.g., RBCs):*** Few or no mitochondria.
- Thus, mitochondria are the ***powerhouse of the cell*** because they produce most of the cellular ATP, with structural and functional adaptations that match energy demands.

Mitochondria are the cell's powerhouse, generating ~90% of ATP via oxidative phosphorylation, with cristae structure and abundance adapted to cellular energy needs.

Q3. ERAD is the cause of diabetes mellitus.

Ans: Endoplasmic reticulum-associated degradation (ERAD) is a critical protein quality-control mechanism that eliminates misfolded or excess proteins from the endoplasmic reticulum (ER).

- In pancreatic β-cells, ERAD plays an essential role in maintaining the proper folding and processing of proinsulin.
- Deficiency of ERAD leads to diabetes mellitus by impairing β-cell function, despite preservation of β-cell mass.

Impaired insulin secretion

- ERAD-deficient β-cells exhibit markedly reduced glucose-stimulated insulin secretion (GSIS).
- The defect arises not from a loss of cell number but from functional disruption within the secretory machinery.

Mechanisms of dysfunction

a. ***Proinsulin entrapment***
   - In healthy β-cells, proinsulin is efficiently folded and processed, with minimal accumulation in the ER.
   - In ERAD-deficient cells, however, proinsulin becomes trapped within the ER, co-localizing with ER chaperones such as calnexin.
   - This entrapment blocks forward trafficking and results in ER congestion.

b. ***Calcium and mitochondrial dysfunction***
   - Intracellular calcium levels fall, compromising insulin granule exocytosis.
   - Mitochondria show reduced membrane potential and excess reactive oxygen species (ROS) generation, impairing ATP production and cellular viability.
   - Oxidative stress further accelerates mitochondrial decline.

c. ***ER stress and the unfolded protein response (UPR)***
   - Misfolded proinsulin activates ER stress pathways and enhances UPR gene expression.
   - Chronic ER stress worsens protein-folding defects, establishing a vicious cycle of dysfunction.
   - Chemical chaperones can partially restore function by suppressing ER stress signaling.

Interaction with autophagy: ERAD normally cooperates with autophagy to clear misfolded proinsulin. Deletion of both pathways in β-cells produces severe hyperglycemia, massive β-cell loss, and premature death, showing that these systems act synergistically to preserve cellular health.

Relevance to human diabetes

- ***Type 2 diabetes:*** Insulin resistance increases proinsulin production up to 50-fold. Even with normal misfolding rates (~20%), this overwhelms ERAD capacity, leading to toxic protein accumulation, ER stress, and secretory failure.
- ***Type 1 diabetes:*** ERAD deficiency makes β-cells more vulnerable to autoimmune attack. Misfolded proteins may serve as neo-antigens, amplifying immune-mediated β-cell destruction.

ERAD deficiency causes diabetes mellitus through a cascade of proinsulin misfolding, ER congestion, calcium and mitochondrial dysfunction, oxidative

stress, and unresolvable ER stress, leading to impaired insulin secretion, hyperglycemia, and enhanced vulnerability to autoimmune damage.

Q4. Mitochondrial diseases are transmitted to the offspring through their mother.

Ans: Mitochondrial diseases are transmitted exclusively through the mother because mitochondrial DNA (mtDNA) is maternally inherited. During fertilization, the oocyte contributes nearly all of the mitochondria to the zygote, while paternal mitochondria are actively destroyed.

Basis of maternal inheritance

- ***Maternal contribution:*** Each human oocyte contains ~100,000–200,000 mitochondria, each with multiple mtDNA copies.
- ***Paternal limitation:*** Sperm contribute only 50–100 mitochondria, with far fewer mtDNA copies. This creates at least a 1000-fold numerical dominance of maternal mitochondria.
- ***Elimination of paternal mitochondria:*** After fertilization, paternal mitochondria are targeted for mitophagy via ubiquitination, ensuring they are not transmitted.

Biological significance

1. ***Quality control***
   - *Prevents heteroplasmy:* Avoids mixing of maternal and paternal mtDNA, which could impair efficiency.
   - *Eliminates damaged mtDNA:* Sperm mitochondria are prone to oxidative stress and DNA mutations.
   - *Maintains uniformity:* Guarantees offspring inherit mitochondria from a single lineage.
2. ***Evolutionary advantages***
   - *Genetic bottleneck effect:* Maternal inheritance restricts mtDNA variation, helping purge deleterious mutations.
   - *Ancestry tracing:* Stable maternal lineages allow mtDNA to be used for studying human evolution and migration.

Clinical implications

- ***Inheritance pattern***
  - Mothers with mtDNA mutations can pass them to all children (male and female).
  - Fathers with mtDNA mutations do not transmit them.
  - Disease severity varies among siblings due to heteroplasmy (variable proportions of mutant vs normal mtDNA).
- ***Associated disorders:*** Mitochondrial myopathies, diabetes, cardiomyopathy, neurodegenerative diseases.
- ***Family histories:*** Pedigrees show strict maternal transmission, not Mendelian dominance or recessiveness.

Therapeutic considerations: Research into preventing maternal transmission has led to mitochondrial replacement therapies (MRT), where healthy donor mitochondria are introduced into oocytes or embryos to prevent inheritance of defective mtDNA.

Mitochondrial diseases follow maternal inheritance because paternal mitochondria are eliminated after fertilization by ubiquitin-mediated mitophagy, ensuring offspring inherit only maternal mitochondria and mtDNA.

Q5. Cathepsins play a significant role in tumor metastasis.

Ans: Cathepsins are lysosomal proteases primarily involved in intracellular protein degradation. In tumor cells, however, they are often produced in **high concentrations** and secreted into the **extracellular space**.

Mechanisms promoting tumor metastasis

a. ***Extracellular matrix (ECM) degradation***
   - Secreted cathepsins degrade ECM proteins such as *collagen, laminin, fibronectin, and elastin*, breaking down structural barriers that normally confine cells.
   - This facilitates *tumor cell invasion* into surrounding tissues.

b. ***Activation of other proteases:*** Cathepsins can activate *matrix metalloproteinases (MMPs)*, which further degrade ECM components, amplifying tissue remodeling and invasiveness.

c. ***Angiogenesis:*** Cathepsins contribute to the formation of new blood vessels, supplying nutrients to the tumor and supporting its growth and metastasis.

By degrading the ECM, activating MMPs, and promoting angiogenesis, cathepsins play a central role in tumor progression and metastasis.

Q6. I cell disease is characterized by lack of enzymes in lysosomes.

Ans:
- I cell disease is a disorder affecting lysosomal function. It is a protein targeting defect caused by mutations affecting the membrane proteins.
- Mutation in the gene encoding GlcNac phosphotransferase affects the normal transfer of GlcNac 1-P to specific mannose residues of enzymes destined for lysosomes.
- Consequently, these enzymes lack the Mannose-6-Phosphate (Man-6-P) label that is required to target them to their destination inside lysosomes. Hence these enzymes are rather secreted into the plasma without being targeted into lysosomes. The lysosomes are now deficient in the hydrolases required for digestion of cell debris. The partly digested cell material accumulates inside the lysosomes and manifests as inclusion bodies without being completely digested.
- Man-6-P serves as a chemical marker to target lysosomal enzymes into the organelle.
- There are two types of Man-6-P receptors that can recognise Man-6-P. These receptors are lectins involved in the intracellular sorting of lysosomal enzymes into clathrin coated vesicles that occurs in the trans-Golgi.
- GlcNac phosphotransferase located in the *cis*-Golgi synthesize Man-6-P. The vesicles then leave the golgi and fuse with the prelysosomal compartment. The low pH in this compartment cause the lysosomal enzymes to dissociate from their receptors and subsequently enter into lysosomes. The receptors are recycled and reused.
- Not all cells employ the Man-6-P receptor to target the lysosomal enzymes (hepatocytes use a different mechanism). Not all lysosomal enzymes are targeted by this mechanism.
- I cell disease is characterized by progressive severe psychomotor retardation with death often occurring in the first decade.
- Pseudo-Hurler polydystrophy is a milder condition closely related to I cell disease.

I cell disease is due to defect in the mannose-6-phosphate labeling of enzymes that is required for targeting them into lysosomes.

Q7. Biomembranes exist as bilayers.

Ans: Biomembranes are primarily composed of **lipids and proteins**, with lipids including **phospholipids, cholesterol, and glycolipids**. The main phospholipids are **phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol**.

Amphipathic nature of phospholipids: Phospholipids are amphipathic, having a hydrophilic head and a hydrophobic tail.

- ***Head:*** Phosphate group linked to choline, ethanolamine, serine, or inositol.
- ***Tail:*** Fatty acid chains.

Formation of bilayers: In aqueous environments (inside and outside the cell), phospholipids *spontaneously arrange* with:

- Hydrophilic heads facing water
- Hydrophobic tails facing inward, away from water

This arrangement:

- Minimizes free energy
- Forms a *thermodynamically stable bilayer*
- Provides the fundamental structure of biomembranes.

The amphipathic nature of phospholipids drives their spontaneous organization into bilayers, making this the most stable and energetically favorable structure in aqueous environments.

Q8. Biomembranes are fluid in nature.

Biomembranes are fluid in nature, and at 37ºC, they maintain this fluid state, as evidenced by the rotational movements and lateral diffusion of phospholipids.

Factors regulating membrane fluidity:

1. ***Fatty acids***
   - Saturated fatty acids decrease fluidity by allowing tight packing of hydrocarbon chains.
   - Unsaturated fatty acids increase fluidity by introducing kinks that prevent tight packing.
   - Chain length: Short-chain fatty acids increase fluidity, whereas long-chain fatty acids decrease it.
2. ***Cholesterol: Acts as a fluidity buffer***
   - At low temperatures, it prevents tight packing of phospholipids, increasing fluidity.
   - At high temperatures, it promotes tighter packing of phospholipids, decreasing fluidity.

At 37°C, biomembranes remain fluid, with fluidity regulated by fatty acid type and chain length—unsaturated and short-chain fatty acids increase fluidity, saturated and long-chain fatty acids decrease it—while cholesterol acts as a buffer, increasing fluidity at low temperatures and decreasing it at high temperatures.

Q9. Biomembranes are asymmetrical in nature.

Ans: Biomembranes are asymmetrical, meaning the phospholipid composition differs between the two leaflets.

- **Outer leaflet:** Mainly contains phosphatidyl choline and sphingomyelin.
- **Inner leaflet:** Contains phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidyl inositol.

This asymmetry is maintained by specific enzymes

- ***Flippases:*** ATP-dependent transporters that move phosphatidyl ethanolamine and phosphatidyl serine from the outer to inner leaflet.
- ***Floppases:*** ATP-dependent transporters that move phospholipids from the inner to outer leaflet.
- ***Scramblases:*** ATP-independent transporters that move phospholipids in either direction.

Biological significance

- The asymmetrical distribution is crucial for membrane function.
- For example, phosphatidyl serine is normally in the inner leaflet, but when exposed on the outer leaflet, it acts as a signal for apoptosis.
- Phosphatidyl inositol, present in the inner leaflet, serves as a second messenger in hormone signaling.

Biomembranes are asymmetrical, with distinct phospholipid distributions between leaflets maintained by flippases, floppases, and scramblases, which are crucial for functions like apoptosis and signal transduction.

Q10. Biomembranes are dynamic in nature.

Ans: Biomembranes are dynamic, with phospholipids exhibiting various movements:

- **Flexion:** Bending of fatty acid tails.
- **Rotation:** Spinning along their own axis.
- **Lateral diffusion:** Movement within the same leaflet.
- **Flip-flop:** Rare transfer between leaflets, often mediated by enzymes.

Membrane proteins are also in constant motion, though their movement is restricted by the cytoskeleton.

The FRAP assay (fluorescence recovery after photobleaching) is a classic technique to study membrane fluidity and molecular mobility:

Specific membrane proteins or lipids are tagged with a fluorescent dye.

A defined region is exposed to a strong laser, photobleaching the fluorescence there.

Over time, unbleached fluorescent molecules diffuse into the bleached area, and fluorescence recovers.

The rate and extent of recovery reflect how molecules are mobile within the membrane, demonstrating the dynamic nature of biomembranes.

Biomembranes are dynamic, with phospholipids and proteins constantly moving, as demonstrated by techniques like FRAP.