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VOLUME – 2

Fundamental Processes & Cell Communication and Cell Signaling

SIERRA INNOVATIONS

Index

III UNIT

Fundamental Processes

DNA Replication - Mechanisms and Enzymes

1. Overview of DNA Replication

DNA replication is a fundamental process that duplicates the genome prior to cell division, ensuring each daughter cell receives an identical copy of the genetic material. It is semi-conservative, highly accurate, and tightly regulated to maintain genome integrity.

- **Key Features**:
  - **Semi-Conservative**: Each daughter DNA molecule contains one parental and one newly synthesized strand (Meselson-Stahl experiment, 1958).
  - **Bidirectional**: Replication proceeds in both directions from origins.
  - **Accurate**: Error rate ~10^{-10} due to proofreading and repair.
- **Biological Relevance**:
  - Essential for cell division, growth, and reproduction.
  - Errors cause mutations, leading to diseases (e.g., cancer, genetic disorders).
- **Applications**:
  - PCR amplifies DNA based on replication principles.
  - Replication inhibitors (e.g., aphidicolin) are anticancer agents.
  - Synthetic biology uses replication for genome engineering.

Table 1: Key Features of DNA Replication

Feature	Description	Biological Role	Example
Semi-Conservative	One parental, one new strand	Ensures genetic continuity	Meselson-Stahl experiment
Bidirectional	Two forks from each origin	Speeds up replication	E. coli OriC
High Fidelity	Error rate ~10^{-10}	Prevents mutations	DNA polymerase proofreading
Regulated	Origin firing, checkpoint control	Coordinates with cell cycle	CDK regulation

2. Unit of Replication

The unit of replication, or **replicon**, is a DNA segment replicated from a single origin, encompassing all necessary cis-acting elements and trans-acting proteins.

2.1 Replicon Structure

- **Components**:
  - **Origin of Replication (Ori)**: Specific DNA sequence where replication initiates.
    - Prokaryotes: Single origin (e.g., E. coli OriC, ~245 bp).
    - Eukaryotes: Multiple origins (~10^4 in human genome, ~100–1000 kb apart).
  - **Replication Fork**: Y-shaped structure where DNA unwinds and replicates.
  - **Terminus**: Region where forks converge, halting replication.
- **Prokaryotic Replicon**:
  - Circular chromosome, single OriC.
  - Example: E. coli ~4.6 Mb, replicates in ~40 min at 37°C.
- **Eukaryotic Replicon**:
  - Linear chromosomes, multiple origins.
  - Example: Human genome (~3.2 Gb) replicates in ~8 h during S phase.

- **Extrachromosomal Replicons**:
  - Plasmids, viral genomes with autonomous origins.
  - Example: pBR322 plasmid (~4 kb, ColE1 origin).

2.2 Origin Recognition

- **Prokaryotes**:
  - **OriC**: AT-rich, contains DnaA boxes (9 bp, TTATCCACA).
  - **DnaA**: Binds DnaA boxes, unwinds DNA, recruits helicase (DnaB).
- **Eukaryotes**:
  - **Autonomously Replicating Sequences (ARS)**: ~100–200 bp, AT-rich.
  - **Origin Recognition Complex (ORC)**: Six-subunit complex (Orc1–6), binds ARS.
  - **Pre-Replication Complex (pre-RC)**: ORC recruits Cdc6, Cdt1, MCM2–7 helicase.
- **Regulation**:
  - **Prokaryotes**: DnaA-ATP levels control initiation.
  - **Eukaryotes**: CDK2-Cyclin E, DDK (Cdc7-Dbf4) activate MCM, ensure once-per-cycle firing.
  - **Licensing**: Pre-RC assembly in G1, prevents re-replication.

2.3 Replication Fork Dynamics

- **Structure**:
  - Leading strand: Continuous synthesis (5'→3').
  - Lagging strand: Discontinuous synthesis (Okazaki fragments, ~100–200 nt in eukaryotes, ~1–2 kb in prokaryotes).
  - Y-shaped, with single-stranded DNA (ssDNA) exposed.
- **Components**:
  - **Helicase**: Unwinds DNA (e.g., DnaB in E. coli, MCM2–7 in eukaryotes).
  - **Single-Strand Binding Proteins (SSBs)**: Stabilize ssDNA (e.g., SSB in E. coli, RPA in eukaryotes).
  - **Primase**: Synthesizes RNA primers (e.g., DnaG in E. coli, Pol α-primase in eukaryotes).
  - **DNA Polymerase**: Synthesizes DNA (e.g., Pol III in E. coli, Pol δ/ε in eukaryotes).
  - **Clamp Loader**: Loads sliding clamp (e.g., γ-complex in E. coli, RFC in eukaryotes).
  - **Sliding Clamp**: Enhances processivity (e.g., β-clamp in E. coli, PCNA in eukaryotes).
- **Fork Movement**:
  - Rate: ~500–1000 nt/s in E. coli, ~50–100 nt/s in eukaryotes.
  - Bidirectional: Two forks per origin, converge at terminus.

2.4 Energetics

- **Origin Unwinding**:
  - DnaA/MCM ATP hydrolysis: ΔG ≈ -50 kJ/mol per event.
  - Helicase unwinding: ~1–2 ATP per bp, ΔG ≈ -10 kJ/mol/bp.
- **Primer Synthesis**:
  - Primase: ~10–20 nt/primer, ΔG ≈ -20 kJ/mol per nucleotide.
- **DNA Synthesis**:
  - Polymerase: ~50–100 nt/s, ΔG ≈ -20 kJ/mol per nucleotide.
- **Clamp Loading**:
  - RFC/γ-complex: ~2–4 ATP, ΔG ≈ -50 kJ/mol.

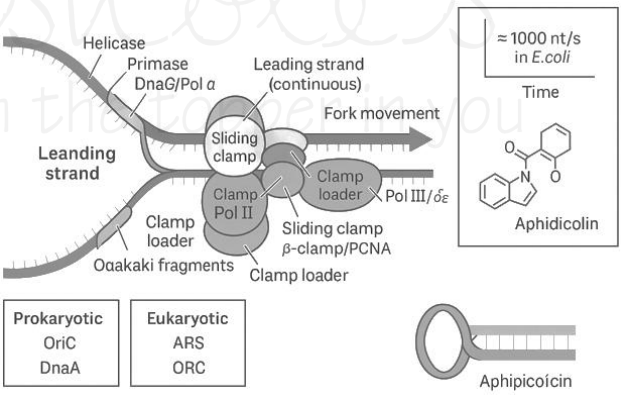

Diagram 1: Replication Fork Structure

[Description: A diagram showing a replication fork with leading (continuous) and lagging (Okazaki fragments) strands. Helicase (DnaB/MCM), SSB/RPA, primase (DnaG/Pol α), and polymerase (Pol III/δ/ε) are depicted. The sliding clamp (β-clamp/PCNA) and clamp loader (γ-complex/RFC) are shown. Prokaryotic (OriC, DnaA) and eukaryotic (ARS, ORC) origins are compared. A side panel illustrates fork movement (~1000 nt/s in *E. coli*) and aphidicolin inhibition, with biological roles (e.g., genome duplication).]

3. Enzymes Involved in DNA Replication

DNA replication requires a suite of enzymes working in concert to unwind, prime, synthesize, and process DNA.

3.1 Prokaryotic Enzymes

- **DnaA**:
  - Initiator protein, binds OriC DnaA boxes, unwinds AT-rich region.
  - ATP-dependent, recruits DnaB.
- **DnaB Helicase**:
  - Hexameric, unwinds DNA 5'→3', ~1000 bp/s.
  - Interacts with DnaG primase.
- **DnaG Primase**:
  - Synthesizes RNA primers (~10–20 nt) for lagging strand.
  - Recruited by DnaB.
- **DNA Polymerase III**:
  - Main replicative polymerase, high processivity (~500–1000 nt/s).
  - Subunits: α (polymerase), ε (3'→5' exonuclease), β (clamp).
  - Error rate: ~10^{-5}, improved by proofreading.
- **DNA Polymerase I**:
  - Removes RNA primers, fills gaps with DNA.
  - 5'→3' polymerase, 5'→3' exonuclease (nick translation).
- **DNA Ligase**:
  - Seals nicks between Okazaki fragments, uses NAD^+ (prokaryotes).
  - ΔG ≈ -30 kJ/mol per seal.
- **Single-Strand Binding Protein (SSB)**:
  - Tetramer, binds ssDNA, prevents reannealing/hairpins.
- **Clamp Loader (γ-Complex)**:
  - Loads β-clamp, ATP-dependent (5 subunits: γ, δ, δ').
- **Sliding Clamp (β-Clamp)**:
  - Dimeric ring, encircles DNA, tethers Pol III.
- **Topoisomerase**:
  - **Type I (TopA)**: Relieves negative supercoils, single-strand cuts.
  - **Type II (Gyrase)**: Introduces negative supercoils, ATP-dependent.

3.2 Eukaryotic Enzymes

- **Origin Recognition Complex (ORC)**:
  - Six subunits (Orc1–6), binds ARS, recruits pre-RC.
- **Cdc6, Cdt1**:
  - Load MCM2–7 helicase, license origins.
- **MCM2–7 Helicase**:
  - Hexameric, unwinds DNA, activated by CDK/DDK.
  - Rate: ~50–100 bp/s.
- **DNA Polymerase α-Primase**:
  - Synthesizes RNA-DNA hybrid primers (~10 nt RNA, ~20 nt DNA).
  - Low processivity, initiates leading/lagging strands.
- **DNA Polymerase δ**:
  - Main lagging strand polymerase, high processivity with PCNA.
  - 3'→5' exonuclease for proofreading.
- **DNA Polymerase ε**:
  - Main leading strand polymerase, associated with CMG (Cdc45-MCM-GINS).
- **Replication Protein A (RPA)**:
  - Binds ssDNA, stabilizes fork, recruits repair proteins.
- **Proliferating Cell Nuclear Antigen (PCNA)**:
  - Trimeric sliding clamp, enhances Pol δ/ε processivity (~1000 nt/s).
- **Replication Factor C (RFC)**:
  - Loads PCNA, 5 subunits, ATP-dependent.
- **Flap Endonuclease 1 (FEN1)**:
  - Removes RNA primers, cleaves 5' flaps on lagging strand.
- **DNA Ligase I**:
  - Seals nicks, uses ATP (eukaryotes).
- **Topoisomerase**:
  - **Topo I**: Relieves supercoils ahead of fork.
  - **Topo II**: Decatenates daughter strands, ATP-dependent.

3.3 Enzyme Functions

- **Unwinding**: Helicase separates strands, topoisomerase relieves torsion.

- **Priming**: Primase initiates synthesis, required for polymerase.
- **Synthesis**: Polymerases extend strands, clamps ensure processivity.
- **Processing**: Pol I/FEN1 remove primers, ligase seals nicks.
- **Stabilization**: SSB/RPA protect ssDNA, prevent secondary structures.

3.4 Regulation

- **Initiation**:
  - Prokaryotes: DnaA-ATP levels, SeqA prevents re-initiation.
  - Eukaryotes: CDK/DDK phosphorylate MCM, ORC licensing in G1.
- **Fork Progression**:
  - Polymerase switching: Pol α → Pol δ/ε in eukaryotes.
  - Checkpoint kinases (ATR, Chk1) monitor fork stability.
- **Termination**:
  - Prokaryotes: Tus protein binds Ter sites, halts forks.
  - Eukaryotes: Fork convergence, Topo II decatenation.
- **Inhibitors**:
  - **Aphidicolin**: Inhibits Pol α/δ/ε, arrests S phase.
  - **Ciprofloxacin**: Inhibits gyrase, blocks prokaryotic replication.
  - **Camptothecin**: Inhibits Topo I, induces DSBs.

3.5 Biological Applications

- **Genome Duplication**:
  - Ensures accurate replication for cell division (e.g., ~3.2 Gb in human S phase).
- **Biotechnology**:
  - Polymerases in PCR (e.g., Taq Pol, derived from Thermus aquaticus).
  - Plasmid replication in cloning (e.g., pUC19).
- **Disease**:
  - Replication stress in cancer (e.g., BRCA mutations).
  - Bloom syndrome: Defective helicase (BLM) causes genome instability.
- **Therapeutics**:
  - Topoisomerase inhibitors (e.g., etoposide) for leukemia.
  - DNA polymerase inhibitors (e.g., cytarabine) for AML.

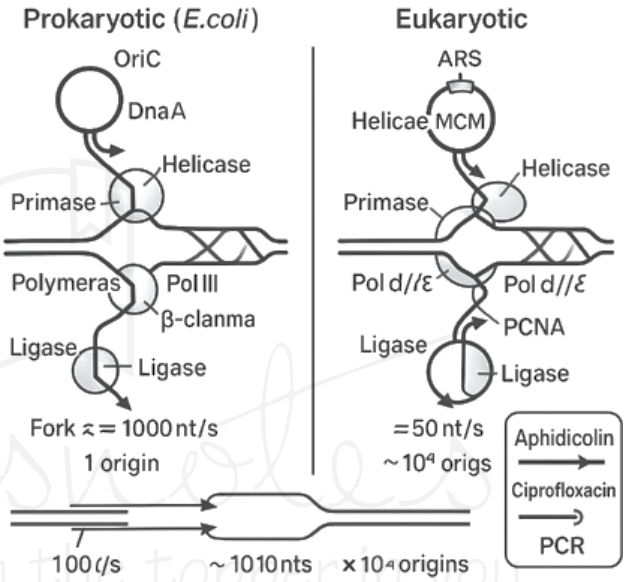

Diagram 2: Prokaryotic vs. Eukaryotic Replication

[Description: A diagram comparing prokaryotic (*E. coli*: OriC, DnaA, DnaB, Pol III, β-clamp) and eukaryotic (ARS, ORC, MCM, Pol δ/ε, PCNA) replication. Enzymes (helicase, primase, polymerase, ligase) and their roles (unwinding, priming, synthesis, sealing) are shown. Fork rates (~1000 nt/s vs. ~50 nt/s) and origin numbers (1 vs. ~10^4) are highlighted. A side panel shows aphidicolin and ciprofloxacin effects, with biological roles (e.g., PCR).]

Table 3: Replication Enzymes

Enzyme	Prokaryotic Example	Eukaryotic Example	Function
Initiator	DnaA	ORC, Cdc6, Cdt1	Origin recognition
Helicase	DnaB	MCM2–7	DNA unwinding
Primase	DnaG	Pol α-primase	RNA primer synthesis

Enzyme	Prokaryotic Example	Eukaryotic Example	Function
Polymerase	Pol III, Pol I	Pol δ, Pol ε, Pol α	DNA synthesis
SSB	SSB	RPA	ssDNA stabilization
Clamp	β-Clamp	PCNA	Processivity
Clamp Loader	γ-Complex	RFC	Clamp loading
Ligase	DNA Ligase (NAD^+)	DNA Ligase I (ATP)	Nick sealing
Topoisomerase	Gyrase, TopA	Topo I, Topo II	Supercoil relief

PYQ Analysis

Below are 20 PYQs from CSIR NET Life Sciences (2018–2024) related to DNA replication mechanisms and enzymes, with solutions and explanations.

(2018)

1. What is the mode of DNA replication?
(A) Conservative
(B) Semi-conservative
(C) Dispersive
(D) Random.

Solution: Semi-conservative (Meselson-Stahl).

Answer: B.

Tip: Semi-conservative = one old, one new strand.

2. Which enzyme unwinds DNA in E. coli?
(A) DnaA, (B) DnaB
(C) DnaG (D) Pol III.

Solution: DnaB helicase unwinds DNA.

Answer: B.

Tip: DnaB = helicase.

(2019)

3. What is the replication fork rate in E. coli?
(A) 10 nt/s (B) 100 nt/s
(C) 1000 nt/s (D) 10,000 nt/s.

Solution: ~500–1000 nt/s.

Answer: C.

Tip: E. coli = 1000 nt/s.

4. Which protein binds ssDNA in eukaryotes?
(A) SSB (B) RPA
(C) PCNA (D) ORC.

Solution: RPA stabilizes ssDNA.

Answer: B.

Tip: RPA = eukaryotic SSB.

(2020)

5. What initiates replication in E. coli?
(A) DnaB (B) DnaA
(C) DnaG (D) Pol I.

Solution: DnaA binds OriC.

Answer: B.

Tip: DnaA = initiator.

6. Which enzyme seals nicks in DNA?
(A) Helicase (B) Primase
(C) Ligase (D) Topoisomerase.

Solution: DNA ligase seals nicks.

Answer: C.

Tip: Ligase = sealing.

(2021)

7. What is the prokaryotic sliding clamp?
(A) β-Clamp (B) PCNA
(C) RPA (D) γ-Complex.

Solution: β-Clamp enhances Pol III processivity.

Answer: A.

Tip: β-Clamp = prokaryotic.

8. Which inhibitor blocks DNA polymerase?
(A) Rifampicin (B) Aphidicolin
(C) Tetracycline (D) Streptomycin.

Solution: Aphidicolin inhibits Pol α/δ/ε.

Answer: B.

Tip: Aphidicolin = polymerase.

(2022)

9. What synthesizes RNA primers in eukaryotes?
(A) Pol δ (B) Pol ε
(C) Pol α-primase (D) MCM.

Solution: Pol α-primase synthesizes primers.

Answer: C.

Tip: Pol α = primase.

10. How many origins are in the human genome?
(A) 1 (B) 10^2
(C) 10^4 (D) 10^6.

Solution: ~10^4 origins.

Answer: C.

Tip: Eukaryotes = multiple origins.

(2023)

11. What removes RNA primers in E. coli?

(A) Pol III (B) Pol I

(C) DnaG (D) Ligase.

Solution: Pol I removes primers via nick translation.

Answer: B.

Tip: Pol I = primer removal.

12. Which protein loads PCNA in eukaryotes?

(A) ORC (B) RFC

(C) MCM (D) RPA.

Solution: RFC loads PCNA.

Answer: B.

Tip: RFC = clamp loader.

(2024)

13. What is the error rate of DNA Pol III without proofreading?

(A) 10^{-2} (B) 10^{-5}

(C) 10^{-8} (D) 10^{-10}.

Solution: ~10^{-5} without proofreading.

Answer: B.

Tip: Pol III = 10^{-5}.

14. Which enzyme relieves DNA supercoils in E. coli?

(A) DnaB (B) Gyrase

(C) DnaG (D) Pol I.

Solution: Gyrase (Topo II) relieves supercoils.

Answer: B.

Tip: Gyrase = supercoil relief.

(2023):

15. What recognizes eukaryotic replication origins?

(A) DnaA (B) ORC

(C) SSB (D) PCNA.

Solution: ORC binds ARS.

Answer: B.

Tip: ORC = eukaryotic origin.

(2022):

16. Which strand is synthesized discontinuously?

(A) Leading (B) Lagging

(C) Both (D) Neither.

Solution: Lagging strand forms Okazaki fragments.

Answer: B.

Tip: Lagging = discontinuous.

(2021)

17. What is the role of the β-clamp?

(A) Unwinding (B) Priming

(C) Processivity (D) Sealing.

Solution: β-Clamp enhances Pol III processivity.

Answer: C.

Tip: β-Clamp = processivity.

(2020)

18. Which inhibitor targets gyrase?

(A) Aphidicolin (B) Ciprofloxacin

(C) Camptothecin (D) Etoposide.

Solution: Ciprofloxacin inhibits gyrase.

Answer: B.

Tip: Ciprofloxacin = gyrase.

(2019)

19. What is the size of Okazaki fragments in E. coli?

(A) 10–20 nt (B) 100–200 nt

(C) 1–2 kb (D) 10 kb.

Solution: ~1–2 kb in E. coli.

Answer: C.

Tip: E. coli Okazaki = 1–2 kb.

(2018)

20. Which disease is linked to defective helicase?

(A) PKU (B) Bloom syndrome

(C) Gout (D) Diabetes.

Solution: Bloom syndrome (BLM helicase).

Answer: B.

Tip: Bloom = helicase defect.

Exam Tips

1. **Memorize Key Facts**:
   - Replicon: Origin (OriC/ARS), fork, terminus.
   - Prokaryotic Enzymes: DnaA (initiator), DnaB (helicase), Pol III (main), β-clamp (processivity).
   - Eukaryotic Enzymes: ORC (origin), MCM (helicase), Pol δ/ε (main), PCNA (clamp).
   - Rates: E. coli ~1000 nt/s, eukaryotes ~50 nt/s.
   - Inhibitors: Aphidicolin (Pol), ciprofloxacin (gyrase), camptothecin (Topo I).
   - Disorders: Bloom syndrome (helicase), cancer (replication stress).

2. **Master Numericals**:
   - Calculate fork movement (e.g., 1000 nt/s × 40 min for E. coli).
   - Estimate error rates (e.g., 10^{-5} without proofreading).
   - Compute replicon sizes (e.g., human genome ÷ 10^4 origins).
3. **Eliminate Incorrect Options**:
   - For enzyme questions, rule out non-replication proteins (e.g., RNA pol ≠ DNA pol).
   - For fork questions, focus on lagging strand (discontinuous).
4. **Avoid Pitfalls**:
   - Don't confuse DnaA (initiator) vs. DnaB (helicase).
   - Don't mix up Pol III (replication) vs. Pol I (primer removal).
   - Distinguish prokaryotic (single OriC) vs. eukaryotic (multiple ARS).
5. **Time Management**:
   - Allocate 1–2 minutes for Part B questions (e.g., enzyme function).
   - Spend 3–4 minutes on Part C questions (e.g., fork rate calculations).
   - Practice sketching replication forks and origin structures.

DNA Replication - Fidelity and Extrachromosomal Replicons

1. **Overview of DNA Replication Fidelity and Extrachromosomal Replicons**

   DNA replication fidelity and extrachromosomal replicons are critical for maintaining genome integrity and enabling genetic manipulation in biotechnology.

- **Replication Fidelity**:
  - Ensures accurate DNA duplication, with an error rate of ~10^{-10} per nucleotide.
  - Achieved through polymerase selectivity, proofreading, and post-replication repair.
  - Critical for preventing mutations that cause diseases (e.g., cancer).
- **Extrachromosomal Replicons**:
  - Autonomous DNA molecules (e.g., plasmids, viral genomes) with independent replication origins.
  - Replicate outside chromosomal DNA, often in multiple copies.
  - Essential in biotechnology (e.g., cloning vectors) and pathogenesis (e.g., viral infections).
- **Biological Relevance**:
  - Fidelity prevents hereditary disorders and tumor formation.
  - Extrachromosomal replicons drive antibiotic resistance and viral propagation.
- **Applications**:
  - High-fidelity polymerases in PCR (e.g., Pfu).
  - Plasmids in gene therapy (e.g., AAV vectors).
  - Viral replication inhibitors as antivirals (e.g., acyclovir).

Table 1: Overview of Replication Fidelity and Extrachromosomal Replicons

Component	Definition	Key Feature	Biological Role	Example
Replication Fidelity	Accuracy of DNA synthesis	Error rate ~10^{-10}	Mutation prevention	Pol III proofreading
Proofreading	3'→5' exonuclease activity	Corrects misincorporations	Enhances accuracy	Pol ε exonuclease
Mismatch Repair	Post-replication error correction	Removes mismatches	Reduces mutations	MutS/MutL in E. coli
Extrachromosomal Replicons	Autonomous DNA molecules	Independent origins	Biotech, pathogenesis	pBR322, AAV

2. Replication Fidelity: Mechanisms Ensuring Accuracy

Replication fidelity is achieved through multiple layers of error prevention and correction, including polymerase selectivity, proofreading, and mismatch repair.

2.1 Polymerase Selectivity

- **Mechanism**:
  - DNA polymerases (e.g., Pol III in E. coli, Pol δ/ε in eukaryotes) select correct dNTPs based on base-pairing (A-T, G-C).
  - Active site geometry ensures Watson-Crick pairing.
- **Accuracy**:
  - Error rate: ~10^{-4} to 10^{-5} per nucleotide (without proofreading).
  - Example: Pol III rejects dCTP opposite adenine due to steric clash.
- **Factors**:
  - **Hydrogen Bonding**: 2–3 H-bonds per base pair (A-T: 2, G-C: 3).
  - **Base Stacking**: Hydrophobic interactions stabilize correct pairs.
  - **dNTP Concentration**: High [dNTP] reduces errors.
- **Energetics**:
  - Correct dNTP binding: ΔG ≈ -20 kJ/mol.
  - Incorrect binding: ΔG ≈ -10 kJ/mol, destabilized.

2.2 Proofreading (3'→5' Exonuclease Activity)

- **Mechanism**:
  - Polymerases have a 3'→5' exonuclease domain that removes misincorporated nucleotides.
  - Example: Pol III ε-subunit, Pol δ/ε in eukaryotes.
  - Process: If incorrect dNTP is added, polymerase pauses, transfers 3' end to exonuclease site, excises mismatch.
- **Accuracy**:
  - Improves error rate to ~10^{-7} (10^2-fold increase).
  - Example: Removes dCTP opposite adenine, replaces with dTTP.
- **Regulation**:
  - Proofreading active during replication stress (e.g., low dNTPs).
  - Inhibited in translesion synthesis (e.g., Pol IV, Pol V).
- **Energetics**:
  - Exonuclease cleavage: ΔG ≈ -20 kJ/mol per nucleotide.
  - dNTP reincorporation: ΔG ≈ -20 kJ/mol.

2.3 Mismatch Repair (MMR)

- **Mechanism**:
  - Post-replication system corrects base-pair mismatches and small insertion/deletion loops (IDLs).
  - **Prokaryotes**:
    - **MutS**: Binds mismatches (e.g., G-T, A-C).
    - **MutL**: Recruits MutH, activates endonuclease.
    - **MutH**: Nicks unmethylated strand at GATC (methyl-directed).
    - **UvrD**: Helicase unwinds DNA.
    - **Exonucleases**: ExoI, ExoVII remove mismatched strand.
    - **Pol III, Ligase**: Resynthesize, seal DNA.
  - **Eukaryotes**:
    - **MSH2-MSH6 (MutSα)**: Binds mismatches.
    - **MLH1-PMS2 (MutLα)**: Activates excision.
    - **EXO1**: Removes mismatched strand.
    - **Pol δ, Ligase I**: Resynthesize, seal.
    - Strand discrimination: PCNA, RFC (no methylation in eukaryotes).
- **Accuracy**:
  - Improves error rate to ~10^{-10} (10^3-fold increase).
  - Example: Corrects G-T to G-C post-replication.
- **Efficiency**:
  - Repairs ~99% of mismatches within minutes.
  - Processes ~1–10 nt loops (IDLs).

- **Regulation**:
  - **Prokaryotes**: Dam methylation (GATC) marks parental strand.
  - **Eukaryotes**: Nick-directed repair, PCNA polarity.
  - Upregulated by replication stress (e.g., ATR signaling).
- **Energetics**:
  - MutS binding: ΔG ≈ -20 kJ/mol per mismatch.
  - Excision: ATP-dependent, ΔG ≈ -50 kJ/mol.
  - Resynthesis: Pol δ, ΔG ≈ -20 kJ/mol per nucleotide.

2.4 Replication Stress and Error Handling

- **Replication Stress**:
  - Causes: dNTP depletion, DNA damage, secondary structures.
  - Response: ATR-Chk1 checkpoint stalls forks, recruits repair proteins.
  - Example: Stalled forks at hairpins resolved by Pol η.
- **Translesion Synthesis (TLS)**:
  - Low-fidelity polymerases (e.g., Pol IV, Pol V in E. coli; Pol η, Pol κ in eukaryotes).
  - Bypasses lesions (e.g., UV-induced thymine dimers).
  - Error rate: ~10^{-2} to 10^{-3}, increases mutations.
- **Fork Restart**:
  - RecA (prokaryotes), BRCA2 (eukaryotes) stabilize forks.
  - Homologous recombination repairs collapsed forks.

2.5 Biological Applications

- **Mutation Prevention**:
  - MMR defects cause microsatellite instability (MSI) in cancer (e.g., Lynch syndrome).
- **Genomics**:
  - High-fidelity replication ensures accurate sequencing (e.g., NGS).
- **Disease**:
  - MMR mutations in colorectal cancer (MSH2, MLH1).
  - TLS dysregulation in xeroderma pigmentosum (Pol η defects).
- **Therapeutics**:
  - MMR inhibitors (e.g., MLH1 silencers) sensitize tumors to chemotherapy.
  - TLS inhibitors (e.g., Pol η inhibitors) for UV-induced cancers.

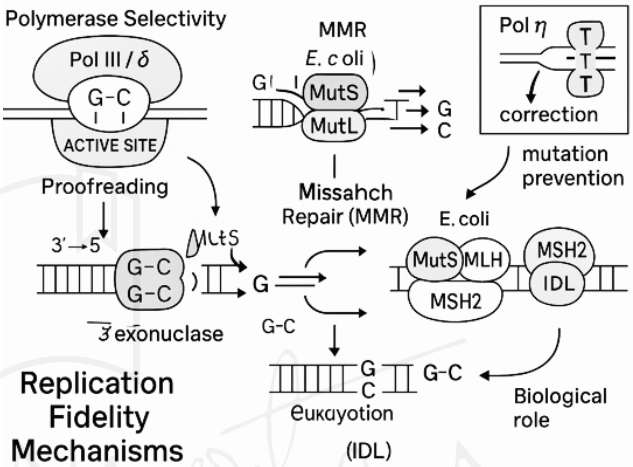

Diagram 1: Replication Fidelity Mechanisms

[Description: A diagram showing replication fidelity layers: polymerase selectivity (Pol III/δ active site, G-C pairing), proofreading (3'→5' exonuclease, ε-subunit), and MMR (MutS/MutL/MutH in *E. coli*, MSH2/MLH1 in eukaryotes). Mismatch correction (G-T → G-C) and IDL repair are depicted. A side panel illustrates TLS (Pol η bypassing thymine dimer) and MSI in cancer, with biological roles (e.g., mutation prevention).]

Table 2: Replication Fidelity Mechanisms

Mechanism	Key Proteins	Error Rate Improvement	Example
Selectivity	Pol III, Pol δ/ε	~10^{-4} to 10^{-5}	Correct dNTP pairing
Proofreading	Pol III ε, Pol δ/ε exonuclease	~10^{-7} (10^2-fold)	Removes G-T mismatch
Mismatch Repair	MutS/MutL (prok), MSH2/MLH1 (euk)	~10^{-10} (10^3-fold)	Corrects A-C mismatch
TLS	Pol IV/V, Pol η	~10^{-2} to 10^{-3} (error-prone)	Bypasses thymine dimer

3. Extrachromosomal Replicons

Extrachromosomal replicons are autonomous DNA molecules that replicate independently of chromosomal DNA, including plasmids and viral genomes.

3.1 Plasmids

- **Definition**:
  - Circular, double-stranded DNA molecules, ~1–200 kb.
  - Contain origins (e.g., ColE1 in E. coli), genes (e.g., antibiotic resistance).
- **Replication Mechanisms**:
  - **Theta Replication**:
    - Bidirectional, resembles chromosomal replication.
    - Example: pBR322 (~4 kb, ColE1 origin).
    - Process: RNA primer (RNA II), Pol III, DnaG primase.
  - **Rolling Circle Replication**:
    - Unidirectional, used by small plasmids (e.g., pSC101).
    - Process: Nicking by Rep protein, leading strand synthesis, lagging strand later.
  - **Strand Displacement**:
    - Used by some plasmids (e.g., R6K).
    - Process: Single-strand displacement, continuous synthesis.
- **Copy Number Control**:
  - **High Copy**: ~10–100/cell (e.g., pUC19, relaxed control).
  - **Low Copy**: ~1–2/cell (e.g., F plasmid, stringent control).
  - **Regulation**: Antisense RNA (e.g., CopA in R1 plasmid), iterons (e.g., RepA in pSC101).
- **Partitioning**:
  - ParA/ParB systems segregate low-copy plasmids (e.g., F plasmid).
  - Example: ParM forms actin-like filaments in R1 plasmid.
- **Energetics**:
  - Theta replication: ~10^4 kJ/plasmid, Pol III-dependent.
  - Rolling circle: ~10^3 kJ/plasmid, Rep protein ATP hydrolysis.

3.2 Viral Genomes

- **Types**:
  - **DNA Viruses**:
    - Double-stranded (e.g., SV40, herpesviruses).
    - Single-stranded (e.g., parvoviruses).
    - Replication: Host machinery (e.g., Pol δ in SV40) or viral polymerases (e.g., HSV Pol).
  - **RNA Viruses**:
    - Replicate via RNA-dependent RNA polymerase (RdRp).
    - Example: SARS-CoV-2, positive-sense ssRNA.
  - **Retroviruses**:
    - Reverse transcription (RNA → DNA) by reverse transcriptase.
    - Example: HIV, integrates into host genome.
- **Replication Mechanisms**:
  - **SV40 (DNA Virus)**:
    - T-antigen (viral helicase) binds origin, recruits host Pol α/δ.
    - Bidirectional, theta-like replication.
  - **Parvovirus (ssDNA)**:
    - Hairpin ends serve as primers, rolling hairpin replication.
  - **HIV (Retrovirus)**:
    - Reverse transcriptase synthesizes dsDNA from RNA.
    - Integrase inserts DNA into host genome.
- **Regulation**:
  - Viral proteins (e.g., T-antigen, E1 in HPV) control initiation.
  - Host checkpoints bypassed (e.g., p53 inhibition by SV40).
- **Energetics**:
  - SV40 replication: ~10^4 kJ/genome, host-dependent.
  - HIV reverse transcription: ~10^3 kJ/mol, RT-dependent.

3.3 Stability and Maintenance

- **Plasmids**:
  - **Addiction Systems**: Toxin-antitoxin (e.g., CcdB/CcdA in F plasmid) ensures retention.
  - **Multimer Resolution**: Site-specific recombination (e.g., Cer/Xer in ColE1).

IV UNIT

Cell communication and cell signaling

Host-Parasite Interaction - Bacterial and Viral Entry

1. Overview of Host-Parasite Interaction - Bacterial and Viral Entry

Host-parasite interactions involve complex molecular dialogues that enable pathogens to recognize, bind, and enter host cells, initiating infection.

- **Bacterial Entry**:
  - Bacteria use adhesins, pili, and secreted effectors to bind and invade animal or plant cells.
  - Entry mechanisms include phagocytosis, injection, and breach of cellular barriers.
- **Viral Entry**:
  - Viruses bind host receptors via envelope or capsid proteins, entering via fusion, endocytosis, or direct penetration.
  - Specificity depends on receptor-ligand interactions and host cell type.
- **Host Cells**:
  - **Animal Cells**: Epithelial, immune, and endothelial cells; targeted by diverse pathogens (e.g., Salmonella, HIV).
  - **Plant Cells**: Guarded by cell walls, targeted by specialized bacteria (e.g., Agrobacterium) and viruses (e.g., TMV).
- **Biological Relevance**:
  - Entry determines pathogen tropism, infectivity, and disease progression.
  - Host defenses (e.g., immune recognition, cell wall resistance) counter entry.
- **Applications**:
  - Antibiotics and antivirals target entry (e.g., enfuvirtide for HIV).
  - Vaccines mimic pathogen entry proteins (e.g., SARS-CoV-2 spike).
  - Biocontrol uses entry-disrupting agents in plants (e.g., RNAi against viruses).

Table 1: Key Features of Bacterial and Viral Entry

Feature	Bacterial Entry	Viral Entry	Biological Role	Example
Recognition	Adhesins, pili	Envelope/capsid proteins	Host cell targeting	E. coli FimH, HIV gp120
Entry Mechanism	Phagocytosis, injection	Fusion, endocytosis	Cell invasion	Salmonella T3SS, HIV fusion
Host Receptor	Integrins, sugars	ACE2, CD4	Specific binding	Plant lectins, animal integrins
Cellular Target	Epithelial, immune cells	Diverse cell types	Pathogen tropism	TMV in plants, influenza in animals

2. Bacterial Entry into Host Cells

Bacteria employ diverse strategies to recognize and enter animal and plant host cells, overcoming physical and immune barriers.

2.1 Recognition in Animal Cells

- **Adhesins**:
  - Surface proteins that bind host receptors (e.g., integrins, cadherins).
  - **Examples**:
    - Escherichia coli: FimH (type 1 pili) binds mannose on epithelial cells.
    - Staphylococcus aureus: Fibronectin-binding proteins (FnBPs) bind integrins.
  - **Specificity**: High-affinity interactions, K_d ~10^{-8} to 10^{-10} M.

- **Pili/Fimbriae**:
  - Filamentous structures for attachment.
    - Example: Pseudomonas aeruginosa type IV pili bind asialo-GM1 on lung epithelia.
    - **Regulation**:
  - Quorum sensing upregulates adhesin expression (e.g., Vibrio cholerae Tcp pili).
  - Host signals (e.g., pH, oxygen) modulate pili assembly.
- **Energetics**:
  - Adhesin-receptor binding: ΔG ≈ -30 to -50 kJ/mol.
  - Pili assembly: ATP-dependent, ΔG ≈ -50 kJ/mol per subunit.

2.2 Entry Mechanisms in Animal Cells

- **Phagocytosis**:
  - **Zipper Mechanism**:
    - Bacteria (e.g., Listeria monocytogenes) bind integrins, trigger actin remodeling.
    - InlA binds E-cadherin, induces engulfment.
  - **Trigger Mechanism**:
    - Bacteria (e.g., Salmonella enterica) inject effectors via type III secretion system (T3SS).
    - SipA/SipC induce membrane ruffling, engulf bacteria.
  - **Efficiency**: ~10^2–10^3 bacteria internalized/hour in macrophages.
- **Injection**:
  - T3SS delivers effectors into cytoplasm (e.g., Yersinia pestis Yops inhibit phagocytosis).
  - Example: Shigella flexneri IpaB/C disrupt actin, enable invasion.
- **Direct Penetration**:
  - Rare, used by Mycobacterium tuberculosis to breach alveolar epithelia.
- **Regulation**:
  - Effector secretion: Controlled by temperature, pH (e.g., 37°C for Salmonella).
  - Host immune evasion: Yops block NF-κB signaling.
- **Energetics**:
  - Actin remodeling: ATP-dependent, ΔG ≈ -50 kJ/mol per event.
  - T3SS injection: ~10^3 ATP/effector, ΔG ≈ -50 kJ/mol.

2.3 Recognition in Plant Cells

- **Adhesins**:
  - Bind cell wall components (e.g., cellulose, pectin).
    - Example: Agrobacterium tumefaciens VirB pili bind acetosyringone-induced receptors.
  - **Specificity**: Phenolic compounds (e.g., lignin) trigger adhesion.
- **Regulation**:
  - Plant signals: Wounding, phenolics induce vir genes.
  - Quorum sensing: Autoinducers (e.g., AHLs) upregulate adhesins.
- **Energetics**: Adhesin binding, ΔG ≈ -20 to -40 kJ/mol.

2.4 Entry Mechanisms in Plant Cells

- **T-DNA Transfer**:
  - Agrobacterium: Transfers T-DNA via VirB T4SS into plant nucleus.
    - VirD2 nicks T-DNA, VirE2 coats DNA, integrates into genome (e.g., crown gall).
  - **Efficiency**: ~10^2–10^3 T-DNA integrations/plant cell.
- **Cell Wall Breach**:
  - Xanthomonas: Secretes cellulases, pectinases via T2SS, degrades cell wall.
  - Example: Pseudomonas syringae uses T3SS to inject effectors (e.g., AvrPto), suppresses defense.
- **Regulation**:
  - Effector specificity: Avr genes match plant R genes (gene-for-gene resistance).
  - Host defenses: Callose deposition, HR (hypersensitive response).
- **Energetics**:
  - T4SS transfer: ATP-dependent, ΔG ≈ -50 kJ/mol per T-DNA.
  - Cellulase secretion: ΔG ≈ -20 kJ/mol per enzyme.

2.5 Biological Applications

- **Infection**:
  - Salmonella invades gut epithelia, causes gastroenteritis.
  - Agrobacterium transforms plant cells, induces tumors.
- **Disease**:
  - Yersinia (plague) evades phagocytosis, systemic spread.
  - Xanthomonas causes citrus canker, crop loss.
- **Therapeutics**:
  - T3SS inhibitors (e.g., salicylidene acylhydrazides) for Salmonella.
  - RNAi against Agrobacterium Vir genes in plants.
- **Biotechnology**:
  - Agrobacterium-mediated plant transformation (e.g., GM crops).
  - Bacterial adhesins in vaccine design.

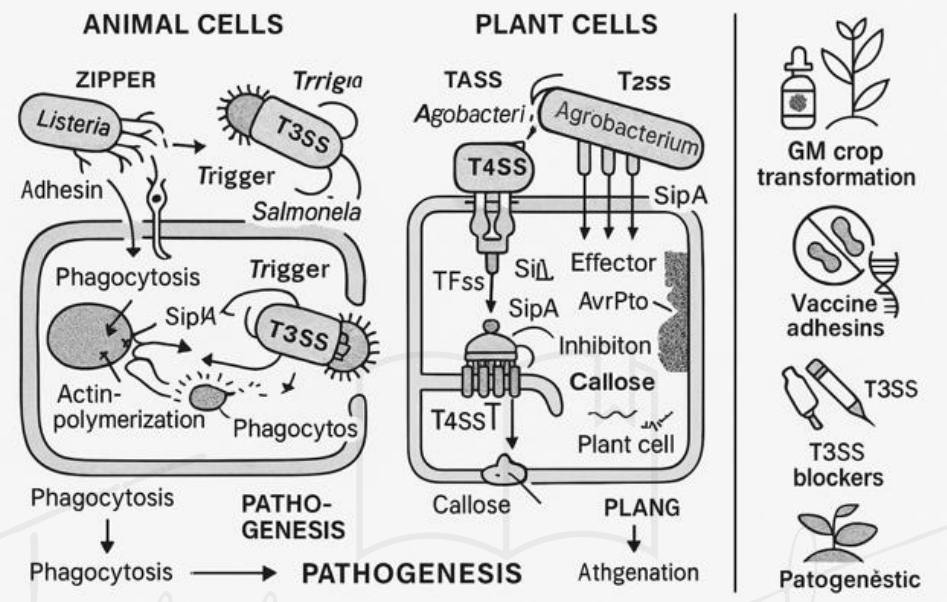

Diagram 1: Bacterial Entry Mechanisms

[Description: A diagram showing bacterial entry into animal (zipper: *Listeria* InlA, trigger: *Salmonella* T3SS) and plant (*Agrobacterium* T4SS, *Xanthomonas* T2SS) cells. Adhesins (FimH, VirB), receptors (integrins, pectin), and effectors (SipA, AvrPto) are depicted. Host defenses (phagocytosis, callose) and inhibitors (T3SS blockers) are shown. A side panel illustrates GM crop transformation and vaccine adhesins, with biological roles (e.g., pathogenesis).]

Table 2: Bacterial Entry Mechanisms

Mechanism	Example	Key Factors	Host Cell Type
Zipper	Listeria InlA	Integrins, actin	Animal epithelial
Trigger	Salmonella SipA/C	T3SS, membrane ruffling	Animal macrophage
T-DNA Transfer	Agrobacterium VirB/D2	T4SS, acetosyringone	Plant parenchyma
Cell Wall Breach	Xanthomonas cellulases	T2SS, pectinases	Plant mesophyll

3. Viral Entry into Host Cells

Viruses use specific receptor interactions and entry mechanisms to invade animal and plant cells, initiating replication.

3.1 Recognition in Animal Cells

- **Receptors**:
  - **Enveloped Viruses**:
    - HIV: gp120 binds CD4, CCR5/CXCR4 co-receptors on T-cells.
    - Influenza: Hemagglutinin (HA) binds sialic acid on respiratory epithelia.
  - **Non-Enveloped Viruses**:
    - Poliovirus: PVR (CD155) on neuronal cells.
    - Adenovirus: CAR (coxsackie-adenovirus receptor), integrins.
  - **Specificity**: K_d ~10^{-9} to 10^{-11} M, high affinity.

- **Regulation**:
  - Receptor density: High CD4 on T-cells enhances HIV tropism.
  - Host signals: Cytokines upregulate CCR5 (e.g., IL-2 in HIV).
- **Energetics**:
  - gp120-CD4 binding: ΔG ≈ -40 kJ/mol.
  - HA-sialic acid: ΔG ≈ -20 kJ/mol.

3.2 Entry Mechanisms in Animal Cells

- **Membrane Fusion**:
  - **Enveloped Viruses**:
    - HIV: gp41 mediates fusion with plasma/endosomal membrane, triggered by CD4/CCR5.
    - Influenza: HA undergoes pH-dependent conformational change in endosomes, fuses membrane.
  - **Efficiency**: ~10^3–10^4 virions enter/hour in T-cells (HIV).
- **Endocytosis**:
  - **Clathrin-Mediated**:
    - Adenovirus: CAR binding, clathrin-coated pits, endosomal escape via penton base.
  - **Caveolae-Mediated**:
    - SV40: Binds MHC-I, enters via caveolin vesicles.
  - **Macropinocytosis**:
    - Vaccinia virus: Induces actin-driven membrane blebs.
  - **Efficiency**: ~10^2–10^3 virions internalized/hour in epithelial cells.
- **Direct Penetration**:
  - Rare, used by some non-enveloped viruses (e.g., poliovirus injects RNA).
- **Regulation**:
  - pH: Low endosomal pH (e.g., pH 5.5) triggers HA fusion.
  - Proteases: Cleave HA (influenza) or gp120 (HIV) for activation.
- **Energetics**:
  - Fusion: ΔG ≈ -50 kJ/mol (gp41 conformational change).
  - Endocytosis: ATP-dependent, ΔG ≈ -50 kJ/mol per vesicle.

3.3 Recognition in Plant Cells

- **Receptors**:
  - **Cell Wall**: Viruses exploit plasmodesmata, wounds, or vector-mediated entry.
    - Example: Tobacco mosaic virus (TMV) binds cell wall proteins (e.g., pectin methylesterase).
  - **Vector-Dependent**:
    - Aphids transmit potyviruses, bind viral coat proteins (e.g., HC-Pro).
  - **Specificity**: Limited by cell wall, vector compatibility.
- **Regulation**:
  - Plant defenses: RNAi, R genes block receptor interactions.
  - Environmental cues: Temperature, humidity affect vector transmission.
- **Energetics**: Binding, ΔG ≈ -20 kJ/mol (TMV-pectin).

3.4 Entry Mechanisms in Plant Cells

- **Plasmodesmata**:
  - **Mechanism**: Movement proteins (MPs) enlarge plasmodesmata, allow viral RNA passage.
    - Example: TMV MP binds RNA, targets plasmodesmata, spreads cell-to-cell.
  - **Efficiency**: ~10–100 cells infected/day in tobacco.
- **Vector-Mediated**:
  - **Insects**: Aphids inject potyvirus virions into phloem.
    - Example: Potato virus Y (PVY) HC-Pro enhances aphid transmission.
  - **Fungi/Nematodes**: Deliver viruses via wounds (e.g., soil-borne wheat mosaic virus).
- **Direct Penetration**:
  - Mechanical wounds allow entry (e.g., TMV via leaf abrasion).
- **Regulation**:
  - RNAi: Silences viral RNAs, restricts movement.
  - Salicylic acid: Induces defense genes, closes plasmodesmata.

- **Energetics**:
  - MP-plasmodesmata interaction: ΔG ≈ -20 kJ/mol.
  - Vector injection: ΔG ≈ -10 kJ/mol per virion.

3.5 Biological Applications

- **Infection**:
  - HIV infects T-cells, causes AIDS.
  - TMV spreads in tobacco, reduces crop yield.
- **Disease**:
  - Influenza causes respiratory illness via sialic acid entry.
  - PVY leads to potato tuber necrosis.
- **Therapeutics**:
  - **Enfuvirtide**: HIV gp41 fusion inhibitor.
  - **Oseltamivir**: Inhibits influenza neuraminidase, blocks release.
- **Biotechnology**:
  - Plant viral vectors for gene delivery (e.g., TMV-based expression).
  - Antiviral RNAi in GM crops.

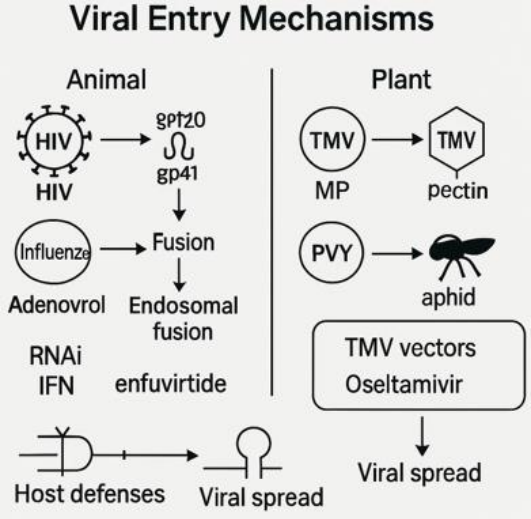

Diagram 2: Viral Entry Mechanisms

[Description: A diagram showing viral entry into animal (HIV: gp120-CD4 fusion, influenza: HA-endosomal fusion, adenovirus: clathrin) and plant (TMV: MP-plasmodesmata, PVY: aphid vector) cells. Receptors (CD4, sialic acid, pectin) and viral proteins (gp41, HC-Pro) are depicted. Host defenses (RNAi, IFN) and inhibitors (enfuvirtide) are shown. A side panel illustrates TMV vectors and oseltamivir, with biological roles (e.g., viral spread).]

Table 3: Viral Entry Mechanisms

Mechanism	Example	Key Factors	Host Cell Type
Membrane Fusion	HIV gp41	CD4, CCR5, low pH	Animal T-cells
Endocytosis	Adenovirus	CAR, clathrin, caveolin	Animal epithelial
Plasmodesmata	TMV MP	Cell wall, pectin	Plant mesophyll
Vector-Mediated	PVY HC-Pro	Aphids, phloem	Plant phloem

PYQ Analysis

Below are 20 PYQs from CSIR NET Life Sciences (2018–2024) related to bacterial and viral entry, with solutions and explanations.

(2018):

1. What mediates E. coli attachment to epithelial cells?
(A) FimH (B) SipA
(C) InlA (D) YopH

Solution: FimH (type 1 pili).

Answer: A.

Tip: FimH = E. coli adhesion.

2. Which receptor does HIV bind for entry?
(A) Sialic acid (B) CD4
(C) CAR (D) PVR

Solution: CD4 with CCR5/CXCR4.

Answer: B.

Tip: HIV = CD4.

(2019):

3. What injects effectors in Salmonella entry?
(A) T1SS (B) T3SS
(C) T4SS (D) T2SS

Solution: Type III secretion system.

Answer: B.

Tip: T3SS = Salmonella.

4. Which virus uses clathrin-mediated endocytosis?
(A) HIV
(B) Influenza
(C) Adenovirus
(D) TMV

Solution: Adenovirus (CAR).

Answer: C.

Tip: Adenovirus = clathrin.

(2020):

5. What facilitates Agrobacterium T-DNA transfer?

(A) T1SS (B) T2SS

(C) T3SS (D) T4SS

Solution: Type IV secretion system.

Answer: D.

Tip: T4SS = Agrobacterium.

6. Which influenza protein binds sialic acid?

(A) HA (B) NA

(C) M2 (D) NS1

Solution: Hemagglutinin (HA).

Answer: A.

Tip: HA = sialic acid.

(2021):

7. What blocks HIV entry?

(A) Oseltamivir (B) Enfuvirtide

(C) Ribavirin (D) Acyclovir

Solution: Enfuvirtide (gp41 inhibitor).

Answer: B.

Tip: Enfuvirtide = HIV fusion.

8. Which virus uses plasmodesmata for spread?

(A) HIV (B) Influenza

(C) TMV (D) Adenovirus

Solution: Tobacco mosaic virus.

Answer: C.

Tip: TMV = plasmodesmata.

(2022):

9. What mediates Listeria entry into epithelial cells?

(A) FimH (B) InlA

(C) SipA (D) VirB

Solution: InlA (E-cadherin).

Answer: B.

Tip: InlA = Listeria.

10. Which receptor does poliovirus bind?

(A) CD4 (B) PVR

(C) CAR (D) Sialic acid

Solution: PVR (CD155).

Answer: B.

Tip: Poliovirus = PVR.

Question (2023):

11. What degrades plant cell walls for Xanthomonas entry?

(A) Cellulases (B) Adhesins

(C) T3SS (D) T4SS

Solution: Cellulases via T2SS.

Answer: A.

Tip: Cellulases = Xanthomonas.

12. Which inhibitor targets influenza entry?

(A) Enfuvirtide (B) Oseltamivir

(C) Acyclovir (D) Ribavirin

Solution: Oseltamivir (neuraminidase).

Answer: B.

Tip: Oseltamivir = influenza.

(2024):

13. What induces Salmonella membrane ruffling?

(A) FimH (B) SipA/C

(C) InlA (D) YopH

Solution: SipA/C (T3SS effectors).

Answer: B.

Tip: SipA/C = ruffling.

14. Which virus enters via caveolae?

(A) HIV (B) SV40

(C) Influenza (D) TMV

Solution: SV40 (MHC-I).

Answer: B.

Tip: SV40 = caveolae.

(2023):

15. What transfers T-DNA in Agrobacterium?

(A) VirB (B) FimH

(C) SipA (D) InlA

Solution: VirB (T4SS).

Answer: A.

Tip: VirB = T-DNA.

(2022):

16. Which receptor does SARS-CoV-2 bind?

(A) CD4 (B) ACE2

(C) PVR (D) CAR

Solution: ACE2 (spike protein).

Answer: B.

Tip: SARS-CoV-2 = ACE2.

(2021):

17. What mediates Pseudomonas attachment to lung cells?

(A) Type IV pili (B) T3SS

(C) InlA (D) FimH

Solution: Type IV pili (asialo-GM1).

Answer: A.

Tip: Type IV pili = Pseudomonas.

(2020):

18. Which virus uses macropinocytosis?

(A) HIV (B) Vaccinia

(C) Adenovirus (D) Poliovirus

Solution: Vaccinia virus.

Answer: B.

Tip: Vaccinia = macropinocytosis.

(2019):

19. What disease is caused by Yersinia entry?

(A) Plague (B) AIDS

(C) Influenza (D) Canker

Solution: Plague (Yops).

Answer: A.

Tip: Yersinia = plague.

(2018):

20. Which plant virus is vector-transmitted?

(A) HIV (B) TMV

(C) PVY (D) Influenza

Solution: Potato virus Y (aphids).

Answer: C.

Tip: PVY = vector.

Exam Tips

1. **Memorize Key Facts**:
   - Bacterial Adhesins: FimH (E. coli), InlA (Listeria), VirB (Agrobacterium).
   - Bacterial Entry: Zipper (Listeria), trigger (Salmonella), T4SS (Agrobacterium).
   - Viral Receptors: CD4 (HIV), ACE2 (SARS-CoV-2), sialic acid (influenza).
   - Viral Entry: Fusion (HIV), endocytosis (adenovirus), plasmodesmata (TMV).
   - Inhibitors: Enfuvirtide (HIV), oseltamivir (influenza).
   - Diseases: Plague (Yersinia), AIDS (HIV), canker (Xanthomonas).
2. **Master Numericals**:
   - Calculate binding affinities (e.g., gp120-CD4 K_d ~10^{-9} M).
   - Estimate entry rates (e.g., ~10^3 HIV virions/hour).
   - Compute effector injection kinetics (e.g., ~10^3 ATP/s for T3SS).
3. **Eliminate Incorrect Options**:
   - For bacterial questions, match adhesin to species (e.g., FimH ≠ Listeria).
   - For viral questions, distinguish enveloped (fusion) vs. non-enveloped (endocytosis).
4. **Avoid Pitfalls**:
   - Don't confuse T3SS (animal pathogens) vs. T4SS (Agrobacterium).
   - Don't mix up HIV (fusion) vs. adenovirus (endocytosis).
   - Distinguish animal (receptor-driven) vs. plant (cell wall/vector) entry.
5. **Time Management**:
   - Allocate 1–2 minutes for Part B questions (e.g., viral receptor).
   - Spend 3–4 minutes on Part C questions (e.g., binding affinity calculations).
   - Practice sketching T3SS and viral fusion pathways.

Host-Parasite Interaction - Pathogen-Induced Alterations and Diseases

1. **Overview of Host-Parasite Interaction - Pathogen-Induced Alterations and Diseases**

   Pathogens manipulate host cell behavior, transform cells, induce diseases, and facilitate cell-cell fusion to establish infection and propagate disease.

- **Alteration of Host Cell Behavior**:
  - Pathogens (bacteria, viruses) hijack host signaling, metabolism, and cytoskeletal dynamics to promote survival and replication.
  - Mechanisms include effector injection, toxin production, and gene expression modulation.

- **Virus-Induced Cell Transformation**:
  - Viruses (e.g., HPV, EBV) integrate oncogenes or disrupt tumor suppressors, leading to uncontrolled cell growth.
  - Results in cancers (e.g., cervical cancer, lymphoma).
- **Pathogen-Induced Diseases**:
  - **Animals**: Bacterial (e.g., tuberculosis), viral (e.g., AIDS), and parasitic diseases (e.g., malaria).
  - **Plants**: Bacterial (e.g., citrus canker), viral (e.g., mosaic diseases), and fungal infections (e.g., rust).
- **Cell-Cell Fusion**:
  - **Normal**: Syncytium formation in muscle, placenta.
  - **Abnormal**: Pathogen-induced fusion (e.g., HIV syncytia, plant viral spread).
- **Biological Relevance**:
  - Host cell alterations drive pathogenesis and immune evasion.
  - Transformation and diseases impact health and agriculture.
  - Cell fusion facilitates pathogen spread and tissue damage.
- **Applications**:
  - Antibiotics/antivirals target pathogen effectors (e.g., rifampicin for TB).
  - Vaccines prevent transformation (e.g., HPV vaccine).
  - RNAi and biocontrol mitigate plant diseases.

Table 1: Overview of Pathogen-Induced Alterations and Diseases

Component	Definition	Key Feature	Biological Role	Example
Host Cell Alteration	Pathogen manipulation of host	Effectors, toxins	Promotes pathogen survival	Salmonella SopE
Virus-Induced Transformation	Viral oncogene activation	Disrupts cell cycle	Cancer development	HPV E6/E7
Animal Diseases	Pathogen-induced pathology	Immune evasion, tissue damage	Morbidity, mortality	TB (Mycobacterium)
Plant Diseases	Crop infections	Stunted growth, yield loss	Agricultural impact	TMV mosaic disease
Cell-Cell Fusion	Membrane merging	Syncytia, viral spread	Pathogen propagation	HIV gp41 syncytia

2. Alteration of Host Cell Behavior by Pathogens

Pathogens manipulate host cell signaling, metabolism, and structure to facilitate infection and replication.

2.1 **Bacterial Alteration in Animal Cells**

- **Effector Proteins**:
  - **Type III Secretion System (T3SS)**:
    - Salmonella enterica: SopE activates Rho GTPases, induces actin remodeling for invasion.
    - Yersinia pestis: YopH dephosphorylates FAK, inhibits phagocytosis.
  - **Type IV Secretion System (T4SS)**:
    - Legionella pneumophila: Dot/Icm effectors (e.g., RalF) recruit Arf1, form replication vacuoles.
  - **Efficiency**: ~10^2–10^3 effectors injected/cell, ~10^{-6} s/event.
- **Toxins**:
  - **Exotoxins**:
    - Clostridium botulinum: Botulinum toxin cleaves SNAREs, blocks neurotransmission (flaccid paralysis).
    - Vibrio cholerae: Cholera toxin ADP-ribosylates Gsα, elevates cAMP, causes diarrhea.
  - **Endotoxins**: LPS activates TLR4, induces inflammation (e.g., E. coli sepsis).
- **Regulation**:
  - Quorum sensing: Upregulates T3SS (e.g., Pseudomonas aeruginosa).
  - Host signals: pH, temperature modulate toxin expression (e.g., 37°C for Yersinia).