During chain elongation each incoming aminoacyl-tRNA move through three ribosomal sites

Elongation factor (EFs): help ribosome move and tRNA move

Translocation: ribosome move

Correct base-pair → hydrolysis GTP → conformational change → tight bind aminoacyl-tRNA in A site and release EF1-GDP

The cleavage of the energy-rich anhydride bond in GTP enables the aminoacyl-tRNA to bind to codon at the A site.

Afterward the GDP still bound to eEF1α, is exchange for GTP as mediated by the eEF1βγ, can recycle.

The eEF1 α-GTP is now ready for the next cycle.

Subsequently a peptide linkage is form between the carboxyl group of methionine and the amino group of amino acid of the tRNA bound to A site.

Peptidyl transferase catalyzing the reaction. It facilitates the N-nucleophilic attack on the carboxyl group, whereby the peptide bond is formed with the released of water.

Ribosome contains two sites where the tRNAs can bind to the mRNA.

P (peptidyl) site allows the binding of the initiation tRNA to the AUG start codon.

The A (aminoacyl) site covers the second codon of the gene and the first is unoccupied.

On the other side of the P site is the exit (E) site where empty tRNA is released.

The elongation begins after the corresponding aminoacyl-tRNA occupies the A site by forming base pairs with the second codon.

Two elongation factors (eEF) play an important role. EF1 and EF2 eEF1α binds GTP and guides the corresponding aminoacyl-tRNA to the A site, during which GTP is hydrolized to GDP and P.

Peptide bond formation by large rRNA (peptidlytransferase reaction)
Accompanied by the hydrolysis of one molecule GTP to form GDP and Pi, the eEF2 facilitates the translocation of the ribosome along the mRNA to three bases downstream.

Free tRNA arrives at site E is released, and tRNA loaded with the peptide now occupies the P Site. The third aminoacyl-tRNA binds to the vacant A site and a further elongation.

An RNA-RNA hybrid of only three base pairs is not stable for normal physiological condition. Multiple interactions between the large and small rRNAs and general domains of tRNAs can stabilized the tRNA in the A and P site.

E. Coli 70S ribosomes

Translation is terminated by release factors when a stop codon is reached.

**Release factor RF:**
- In eukaryote, eRF1 like tRNA, can bind to A site of ribosome and eRF3 is GTP binding protein. Promote cleavage of the peptidyl-tRNA, and releasing the protein chain.
- In bacterial, RF1 and RF2 like eRF1, RF 3 GTP bind factor. Chaperone protect protein and help folding GTPase.

When A site finally binds to a stop codon (UGA, UAG, UAA), stop codons bind eRF accompanied by hydrolysis GTP to form GDP and Pi. Binding of eRF to the stop codon alters the specificity of the peptidyl transferase. Water instead amino acid is now the acceptor for the peptide chain. Protein released from the tRNA.
**Termination of translation**

When the ribosome reaches a stop codon in the A site, one of three releasing factors and initiate hydrolysis of the peptide chain from the tRNA in the P site

- RF-1 recognizes UAA and UAG
- RF-2 recognizes UAA and UGA
- RF-3 binds GTP and enhances the effects of RF-1 and RF-2

**Proteinsynthesis-4**

Polysomes and rapid ribosome recycling increase the efficiency of translation

Eukaryotic mRNA in circular form stabilized by interactions between protein bound at the 3' and 5' ends

Poly A binding protein (PABPI), interact with both mRNA poly A and Eif4g

Two ends is very close together, then ribosome subunit easy to bind.

Protein-protein and protein–mRNA interactions form a bridge.

The Synthesis of Protein

Polyribosomes — A cluster of ribosomes simultaneously translating an mRNA molecule
Polyribosomes are found in both prokaryotes and eukaryotes
The biological activity of proteins depends on a precise folding of the polypeptide chain into 3-D conformation
Some proteins must undergo post-translational modification before they become fully functional

DNA replication:
DNA-directed DNA polymerases
Semi-conservative
5' → 3'
Replication forks
Uni- or Bi-directional
Semi-discontinuous
Primers
DNA polymerase require a primer to initiate replication

![Diagram of DNA replication](image)

DNA replication direction: 5’ to 3’
DNA polymerase need a primer to initiation.

Action of DNA polymerase. DNA is elongated in its 5’ → 3’ direction.

Helicase: opens DNA double strand
Replication origin
DNA polymerase
Primase: provides a short primer
Replication fork
Topoisomerase I: releases the local unwinding of DNA produces torsional stress (supercoil)
DNA ligase
Single-stranded binding proteins
Leading strand
Lagging strand

Helicase: separates the two DNA strands, starting at replication origins (rich in A-T base pairs)
RNA primase: inserts a starter of RNA nucleotides at the initiation point
DNA polymerase binds a complementary leading strand of DNA nucleotides starting at the 3’ end of the RNA primer
Exonuclease removes RNA primer, which are replace with DNA nucleotides by DNA polymerase

Two strands are anti-parallel & DNA, polymerase synthesizes 5’ TO 3’
DNA synthesis is discontinuous on the lagging strand but continuous on the leading strand (Okazaki et al 1968).
The short DNA fragments on lagging strand are called Okazaki fragments.
DNA polymerase requires a primers so each Okazaki fragment must begin with a primer.

How are primers synthesized? First primer (starts strand synthesis) and primers for each Okazaki fragment
1. Like Helicase open DNA (unwind)
2. RPA (heterotrimeric protein) bind single DNA, Single-stranded binding proteins
3. Leading strand synthesis by DNA polymerase(s), PCNA and Rfc (replication factor) complex
4. Lagging strand synthesis by pol σ → Okazaki fragment
5. PCNA-Rfc-pol σ complex process each Okazaki fragment

DNA helicases - Separation of the Watson/Crick helix
DNA helicases utilize energy of ATP hydrolysis to cause disruption of hydrogen bonds in the double helix.
Helicases are necessary for movement of a replication fork. In E. coli, primary replicative helicase is dnaB
Helicases function by moving along ssDNA in one direction disrupting hydrogen bonds as they move.
Both 5' to 3' and 3' to 5' helicases exist.
Nomenclature - direction of helicase movement is defined on the strand the helicase binds. (A 5' to 3' helicase is shown at right).
Single-stranded DNA binding proteins (SSBs) bind tightly to ssDNA.
SSBs prevent formation of secondary structure, renaturation of ssDNA and non-specific interactions on ssDNA.
- SSBs usually bind cooperatively.
  - SSBs usually interact with other replication proteins; these interactions promote efficient replication

SSBs bind tightly to ssDNA.
- SSBs prevent formation of secondary structure, renaturation of ssDNA and non-specific interactions or ssDNA.
- SSBs usually bind cooperatively.
- SSBs usually interact with other replication proteins; these interactions promote efficient replication

DNA ligases form phosphodiester bonds; join strands of DNA

DNA helicases - Separation of the Watson/Crick helix
DNA helicases utilize energy of ATP hydrolysis to cause disruption of hydrogen bonds in the double helix.
Helicases are necessary for movement of a replication fork. In E. coli, primary replicative helicase is dnaB
Helicases function by moving along ssDNA in one direction disrupting hydrogen bonds as they move.
Both 5' to 3' and 3' to 5' helicases exist.
Nomenclature - direction of helicase movement is defined on the strand the helicase binds. (A 5' to 3' helicase is shown at right).
Single-stranded DNA binding proteins (SSBs) bind tightly to ssDNA.
SSBs prevent formation of secondary structure, renaturation of ssDNA and non-specific interactions on ssDNA.
- SSBs usually bind cooperatively.
  - SSBs usually interact with other replication proteins; these interactions promote efficient replication

SSBs bind tightly to ssDNA.
- SSBs prevent formation of secondary structure, renaturation of ssDNA and non-specific interactions or ssDNA.
- SSBs usually bind cooperatively.
- SSBs usually interact with other replication proteins; these interactions promote efficient replication

DNA ligases form phosphodiester bonds; join strands of DNA

Primases
starting nascent DNA chains
Primases synthesize short RNA (or RNA/DNA) oligonucleotides that act as primers for DNA polymerase.
Can initiate synthesis on ssDNA de novo (no 3'-OH needed).
Usually part of protein complex or need specific interactions with other replication proteins for efficient primer synthesis.
Most primases start synthesis at a random site; do not synthesize primers with a specific sequence.
Role of Topoisomerases

During DNA replication, Topoisomerases act to release the links between the parental DNA strands both during replication (swiveling) or after replication (decatenation).

**Type I** - change L by multiples of 1 by causing a transient ssDNA break.

**Type II** - change L by multiples to 2 by causing a transient dsDNA break.

Topoisomerases function by forming a covalent intermediate with the transiently broken end(s) of the DNA. Almost all topoisomerases relax both positively and negatively supercoiled DNA.

Topoisomerases

- **Topoisomerase I**
  - Lagging strand is used in discontinuous synthesis forms Okazaki fragments
  - Fragments joined by DNA ligase

  (a) RNA oligonucleotides (primer) copied from DNA.
  (b) DNA polymerase III chains RNA primers with new DNA.
  (c) DNA polymerase I removes 3' RNA at end of neighboring fragment and fills gap.
  (d) DNA ligase joins adjacent fragments.

  **Must supply a primer (i.e. 3'-OH) to start DNA synthesis**
  **This is the function of primase which makes RNA primers**
  **Must 'seal' the DNA fragments made on the lagging strand template**
  **This is the function of DNA ligase**

  After DNA is synthesized, RNA primer is being degraded and replaced by DNA (strand replacement synthesis).

The Okazaki fragment

Okazaki fragments are the short DNA fragments produced during lagging strand DNA synthesis. They will be ligated together by ligase shortly after completion.

Prokaryotes like *E. coli* has Okazaki fragment of 1000~2000 nucleotides long while eukaryotes like us has shorter Okazaki fragments (100~200 nucleotides).
In prokaryotes, the leading and lagging strand DNA replication machines are associated.
DNA replication generally occurs bi-directionally from each origin.

Mapping Using Electron-microscope
- Isolate partially replicated DNA (replication intermediates). Enrich using di-deoxynucleotides or density labeling.
- Compare location of replication bubble for a number of molecules (many).
- Orientation of DNA! Need reference point. Usually a restriction site.
- Very small bubbles identify location of origin.
- Movement of ends indicates number of active forks.

Eukaryotic chromosomal DNA contains multiple replication origins separated by tens to hundreds of kilobases.

ORC: origin recognition complex (6 subunits) combine with other factor (such as hexameric helicases) to start replication.

Prokaryote-Eukaryote Differences
Viruses: parasites of the cellular genetic system
Most viral host ranges are narrow
Viruses can not reproduced by themselves (no life without host)
RNA virus: replicate in the host cell cytoplasm
DNA virus: replicate in the host cell nucleus
Viral genomes has single or double stranded
Virion: entire infectious virus particle, consists nuclei acid and shell of protein
Bacteriophage (phage): infect only bacteria
Animal virus or plant virus

Physical Characteristics
Genetic Material
Nucleic acid
RNA (ssRNA, dsRNA, segmented)
DNA (ssDNA, dsDNA)
Protein coat (subunit structure)
Nucleoprotein
Capsid
Capsomers, Geometrical constraints
Envelope (some)

VIRUS STRUCTURE
Basic rules of virus architecture, structure, and assembly are the same for all families
Some structures are much more complex than others, and require complex assembly and disassembly
The capsid (coat) protein is the basic unit of structure; functions that may be fulfilled by the capsid protein are to:
- Protect viral nucleic acid
- Interact specifically with the viral nucleic acid for packaging
- Interact with vector for specific transmission
- Interact with host receptors for entry to cell
- Allow for release of nucleic acid upon entry into new cell
- Assist in processes of viral and/or host gene regulation

Nucleoprotein must be stable but dissociatable
Capsid is held together by non-covalent, reversible bonds:
hydrophobic, salt, hydrogen bonds
Capsid is a polymer of identical subunits
Terms:
- Capsid = protein coat
- Structural unit = protein subunit
- Nucleocapsid = nucleic acid + protein
- Virion = virus particle
Capsid proteins are compactly folded proteins which:
- Fold only one way, and robustly
- Vary in size, generally 50-350 aa residues
- Have identifiable domains
- Can be described topologically; similar topological features do not imply evolutionary relationships

There are two major structures of viruses called the naked nucleocapsid virus and the enveloped virus.
Virus Structure –
1. Helical: single coat protein, tobacco mosaic virus
2. Icosahedron: 20 faces

Helical symmetry

**Tobacco mosaic virus** is typical, well-studied example
Each particle contains only a single molecule of RNA
(6395 nucleotide residues) and 2130 copies of the coat protein subunit (158 amino acid residues)
TMV protein subunits + nucleic acid will self-assemble in vitro in an energy-independent fashion
Self-assembly also occurs in vitro

Function of the capsid/envelope

Protect nucleic acid from the host’s acid- and protein-digesting enzymes
Assist in binding and penetrating host cell
Stimulate the host’s immune system
Viral capsids are regular arrays of one or a few types of protein.

Capsid (protein coat): nucleic acid of a virion is enclosed, composed of multiple copies of one protein or few different protein.

Nucleocapsid: a capsid plus the enclosed nucleic acid, protect functions.

Two structure:
- Envelope: some viruses, symmetrically arranged nucleocapsid is covered by an external membrane (envelope), which consists mainly of a phospholipid but also contains one or two types of virus-encoded glycoproteins.

Enable pleomorphic (多形性) shape of the virus:
- Spherical (球形)
- Filamentous (丝形)

**Viral protein spikes protrude**

Influenza

**Plaque assay**

Clone: all the progeny birions in a plaque are derived from a single parental virus.

Plate → seeding host cell → virus add → infect host cell → host cell lysis → plaque
**Lytic viral growth cycles lead to death of host cell**

1. Adsorption
2. Penetration
3. Replication
4. Assembly
5. Release

**Degradation of host cell DNA**
- Provide nucleotides for synthesis of viral DNA

**Capsid & assembly protein**
- Expression of viral early proteins
- Replication of viral DNA

**Viruses vs. life cycle**

- Bacteriophages are viruses that infect bacteria. They reproduce by:
  - a) Lytic cycle
  - b) Lysogenic cycle

**Viral DNA is integrated into host cell genome in some nonlytic viral growth cycles**

Some viruses, nonlytic association with host cell (not kill) is called temperate phages.
- Prophage: integrated into the host cell chromosomes rather than being replicated.
- Lysogeny: Instead of destroying host to produce virus progeny, the viral genome remains within the host cell and replicates with the bacterial chromosome.

This relationship between phage and host is called lysogeny.
Progeny (後代) virions of enveloped viruses are released by budding from infected cells.

Viral DNA is integrated into the host cell genome in some nonlytic viral growth cycles

Retroviruses
- Such as HIV, use the enzyme reverse transcriptase
  - To copy their RNA genome into DNA, which can then be integrated into the host genome as a provirus

The lysogenic cycle
- Replicates the phage genome without destroying the host

Temperate phages
- Are capable of using both the lytic and lysogenic cycles of reproduction

Prophage: integrated viral DNA

1. Viral glycoprotein in envelop interact with specific host cell membrane → entry nucleocapsid into cytoplasm
2. Viral reverse transcriptase and protein → copy the ssRNA to ds DNA
3. ds DNA → transport into the nucleus → integrated HOST chromosomal DNA → leading to provirus

Retroviral life cycle

viral DNA is integrated into the host cell genome in some nonlytic viral growth cycles

Retroviruses
- Such as HIV, use the enzyme reverse transcriptase
  - To copy their RNA genome into DNA, which can then be integrated into the host genome as a provirus
end