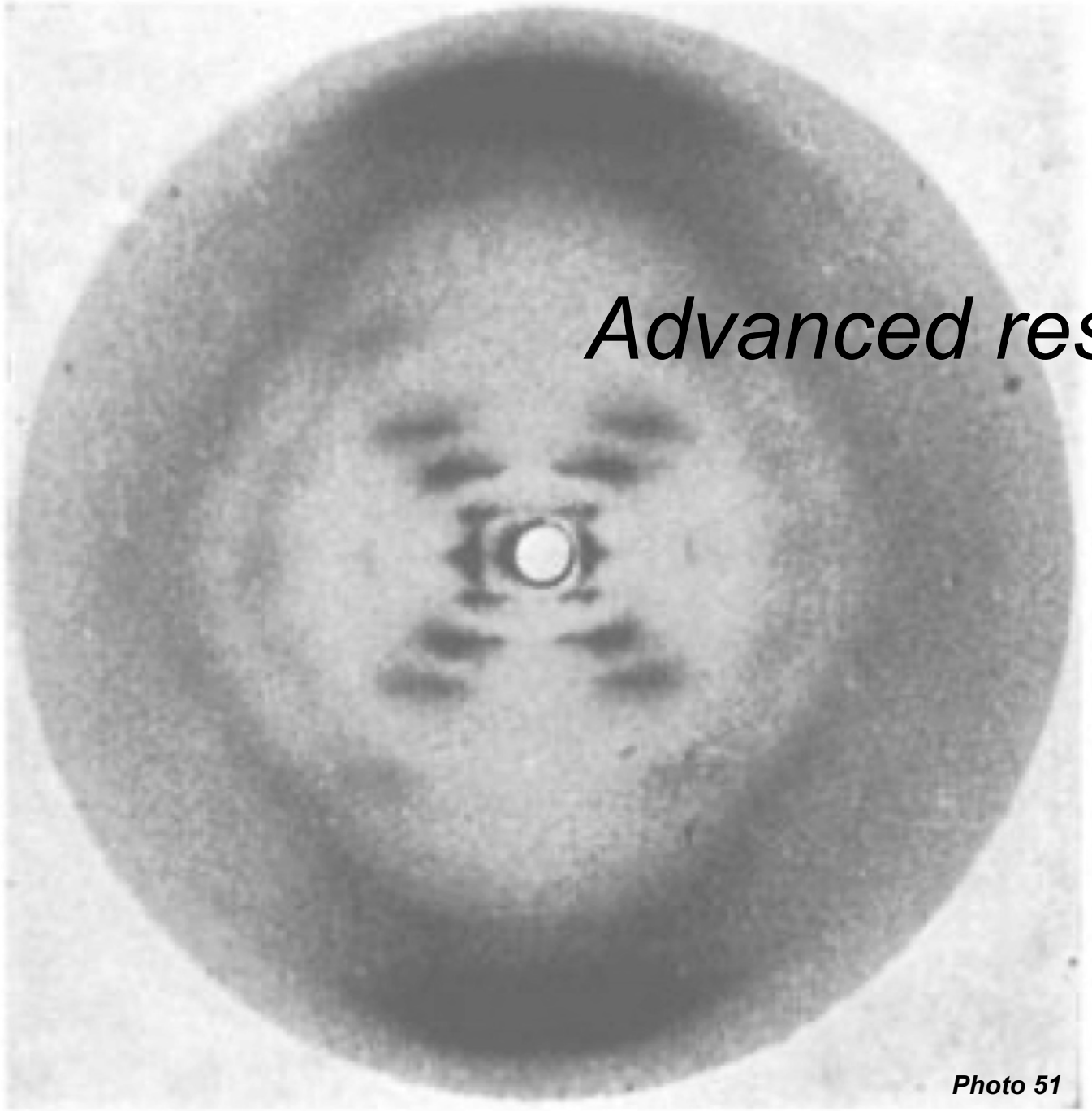


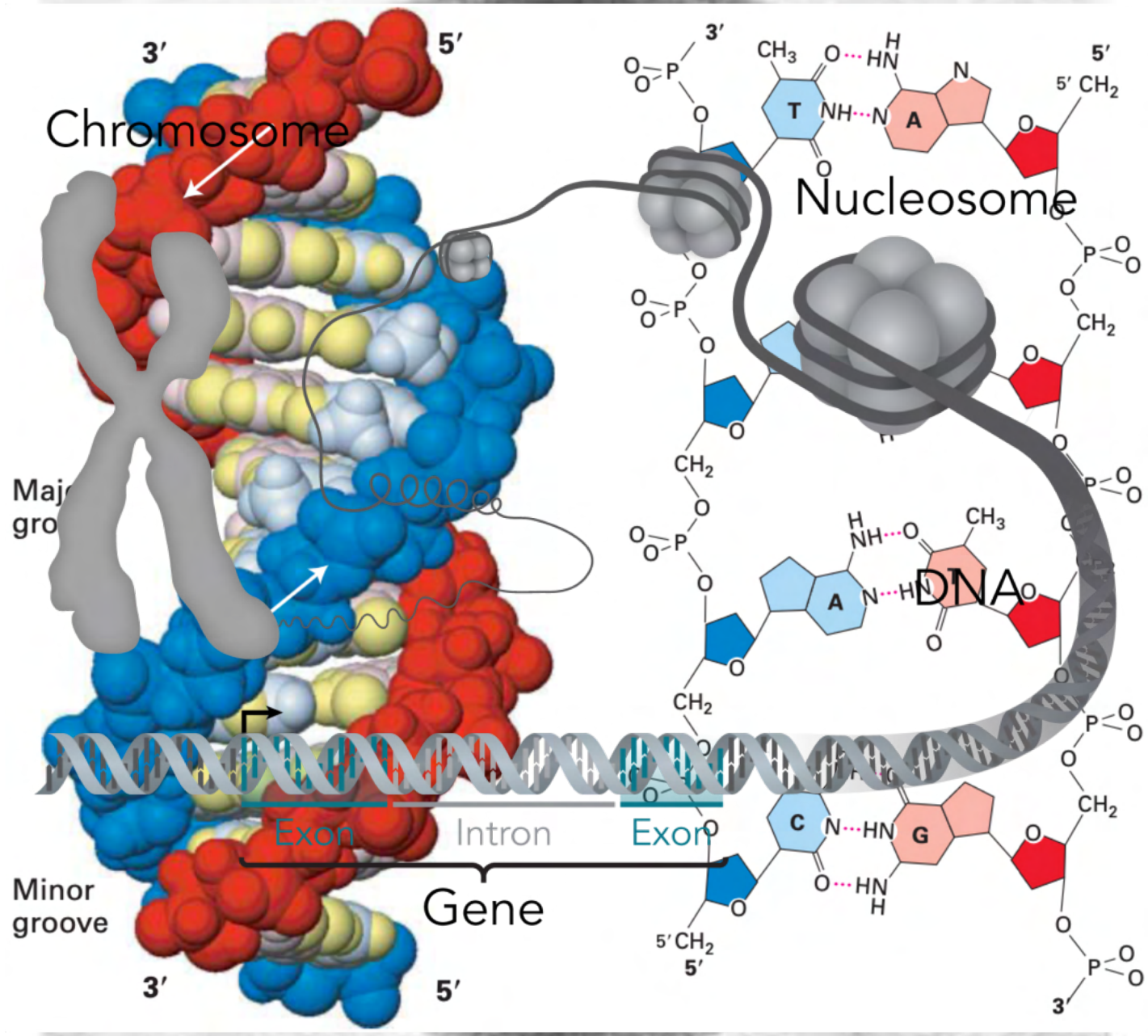
Photo 51

56901852
Advanced research techniques

introduction to PCR:
the DNA

DNA is the information molecule

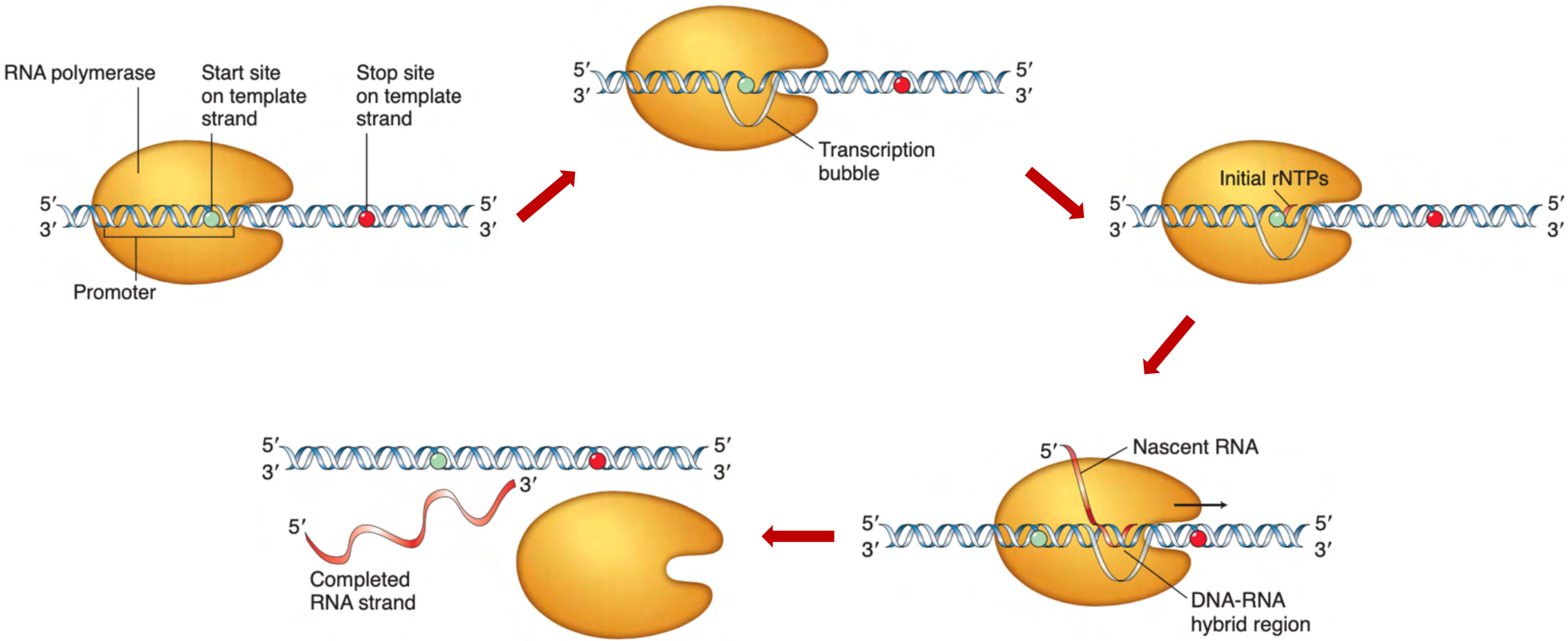
- **DNA** is an information molecule, within it's sequence carries the information required to produce all the proteins and RNAs.
- The exact replication of this information in any species ensures its genetic continuity from generation to generation.
- Virtually all forms of life use DNA to encode their genetic information and use a nearly identical genetic code.
- The information stored in DNA is arranged in hereditary units, known as genes.


Chromosome
Nucleosome
DNA
Exon
Intron
Exon
Gene
Minor groove
3′
5′
3′
5′
3′
5′
5′
3′

DNA is transcribed into RNA

- The information stored in DNA is copied/transcribed into **RNA.**
- **mRNA** directs the synthesis of a specific protein.
- **rRNA** and its associated proteins **& tRNA** which read the nucleotide sequence on mRNA and produce a specific protein.

Transcription of protein-coding genes and formation of functional mRNA



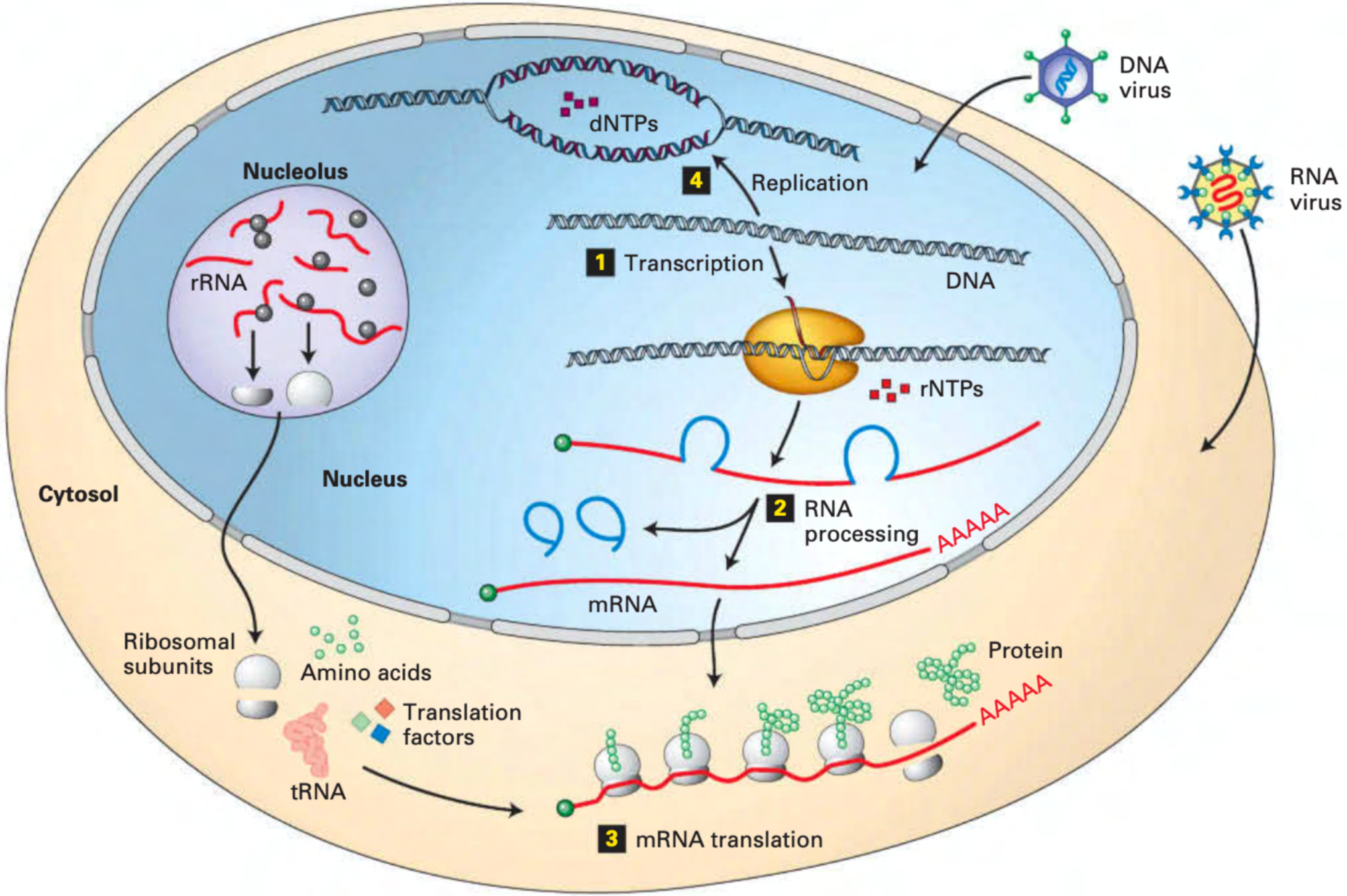
The organization of genes: eukaryotes vs prokaryotes

Prokaryotes:

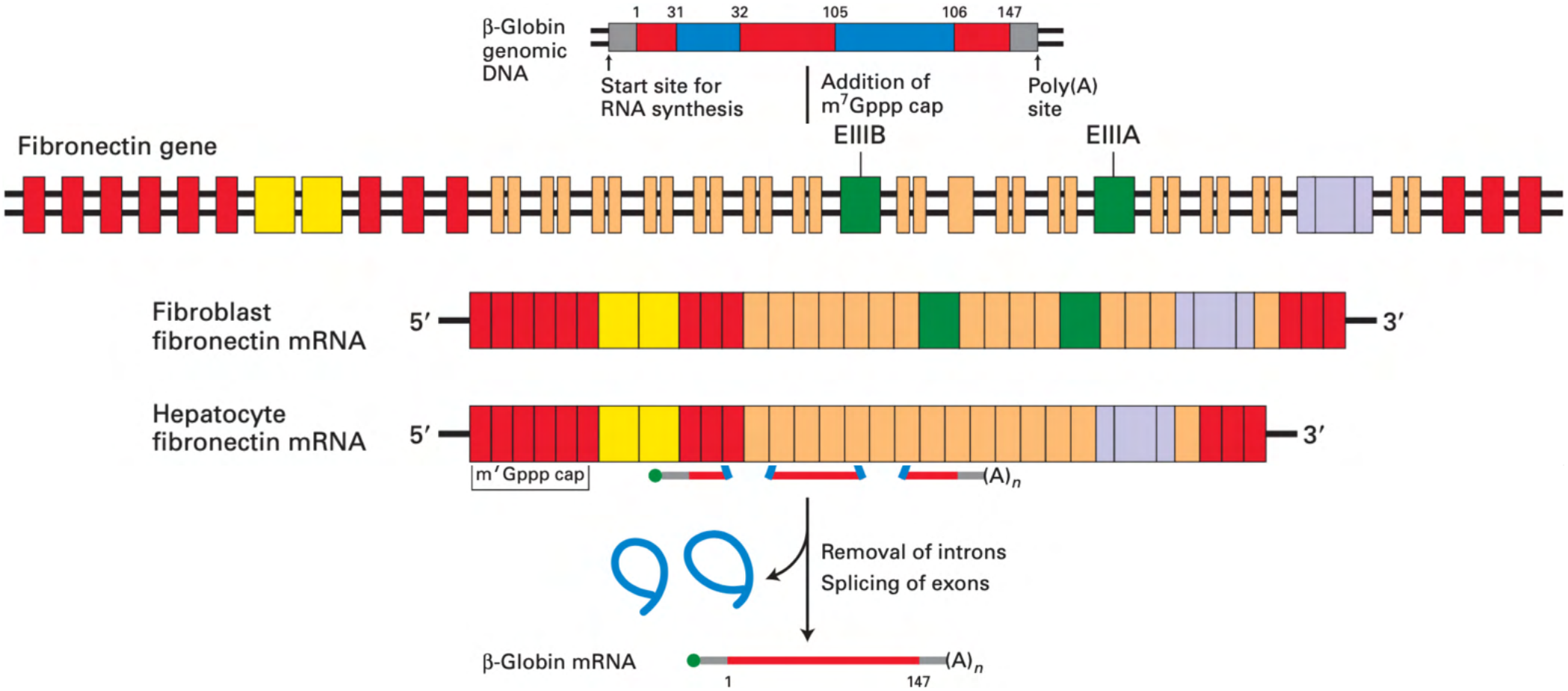
- Genes encoding proteins that function together are most often found in a contiguous array in the DNA.

Eukaryotes:

- Genes encoding proteins that function together are most often physically separated in the DNA. Each gene is transcribed from its own promoter, producing one mRNA, which is generally translated to yield a single polypeptide.


DNA virus
RNA virus
dNTPs
Nucleolus
4 Replication
rRNA
1 Transcription
DNA
rNTPs
Cytosol
Nucleus
2 RNA processing
AAAAA
mRNA
Ribosomal subunits
Amino acids
Translation factors
tRNA
Protein
AAAAA
3 mRNA translation

exons vs introns: RNA splicing, alternative splicing



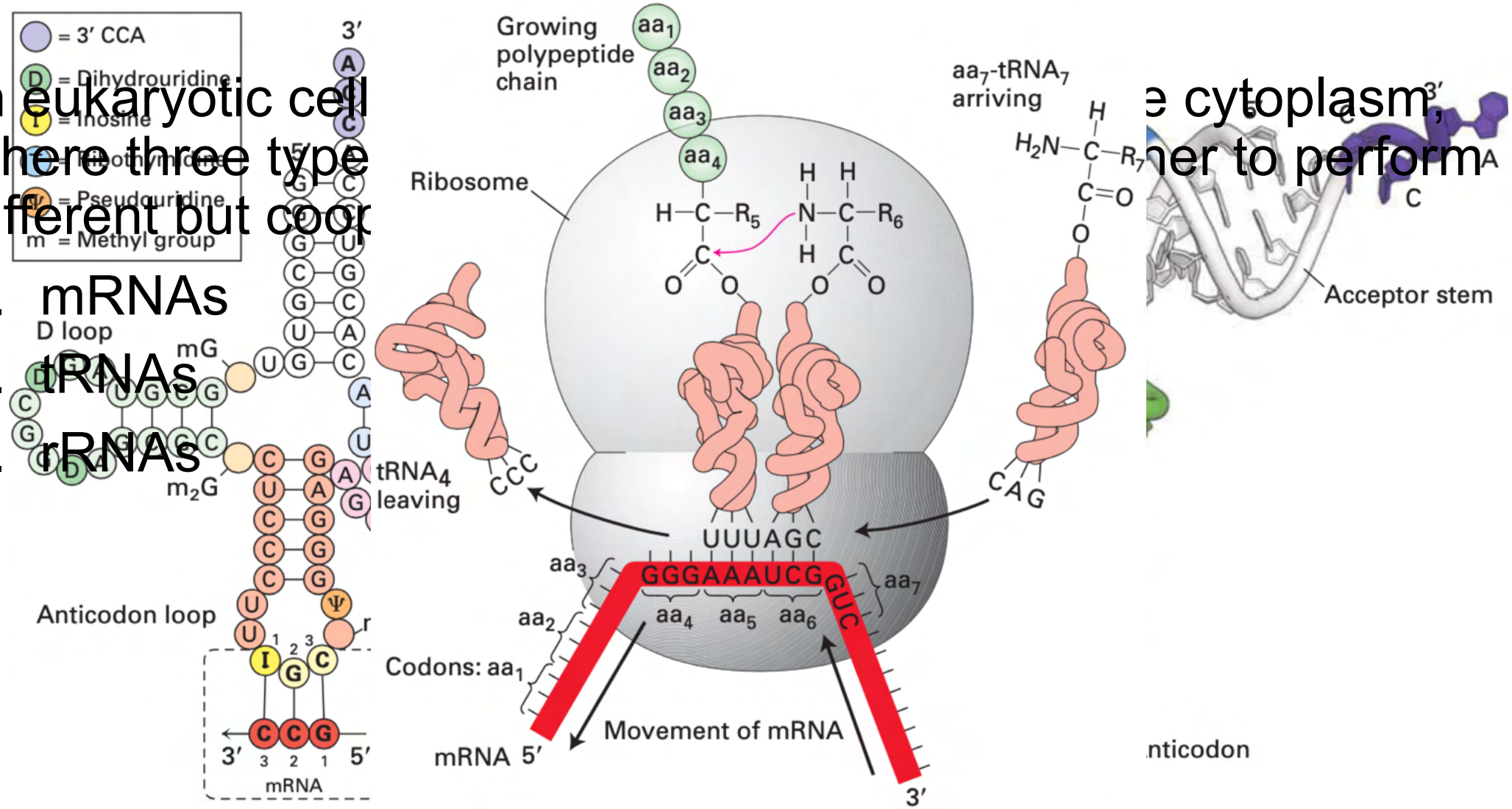
The three-letter genetic code

First position (5′ end)	Second position: U	Second position: C	Second position: A	Second position: G	Third positioin (3′ end)
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu (Met)*	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met (Start)	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val (Met)*	Ala	Glu	Gly	G

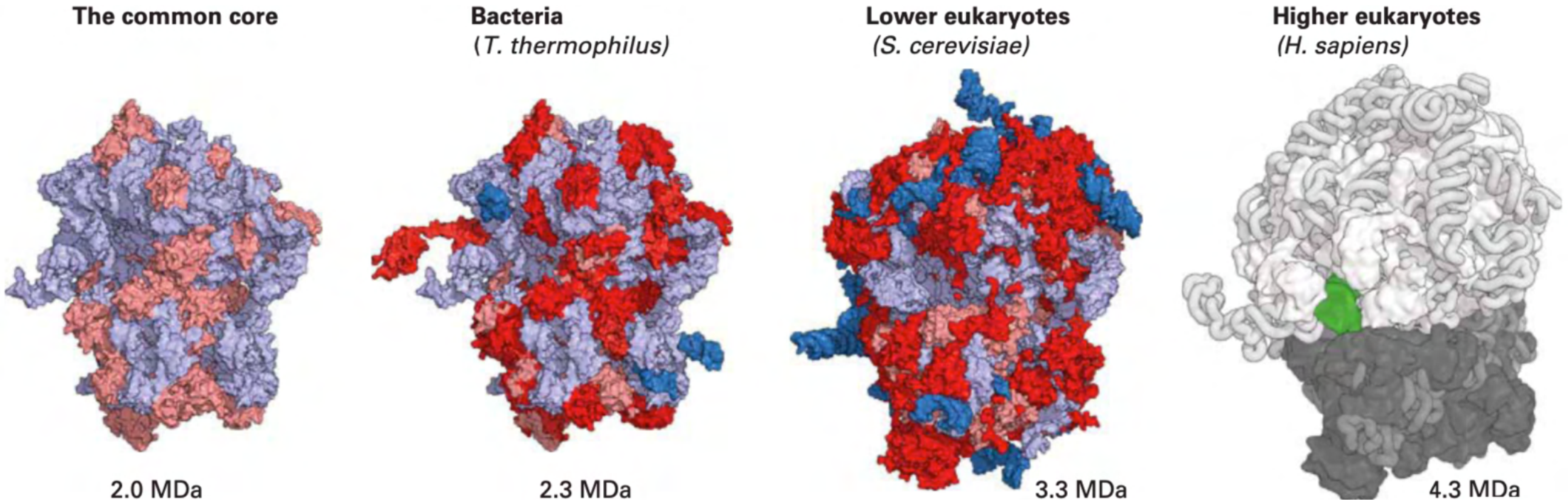
Decoding the mRNA with tRNAs

In eukaryotic cell e cytoplasm, where three type ner to perform different but coor

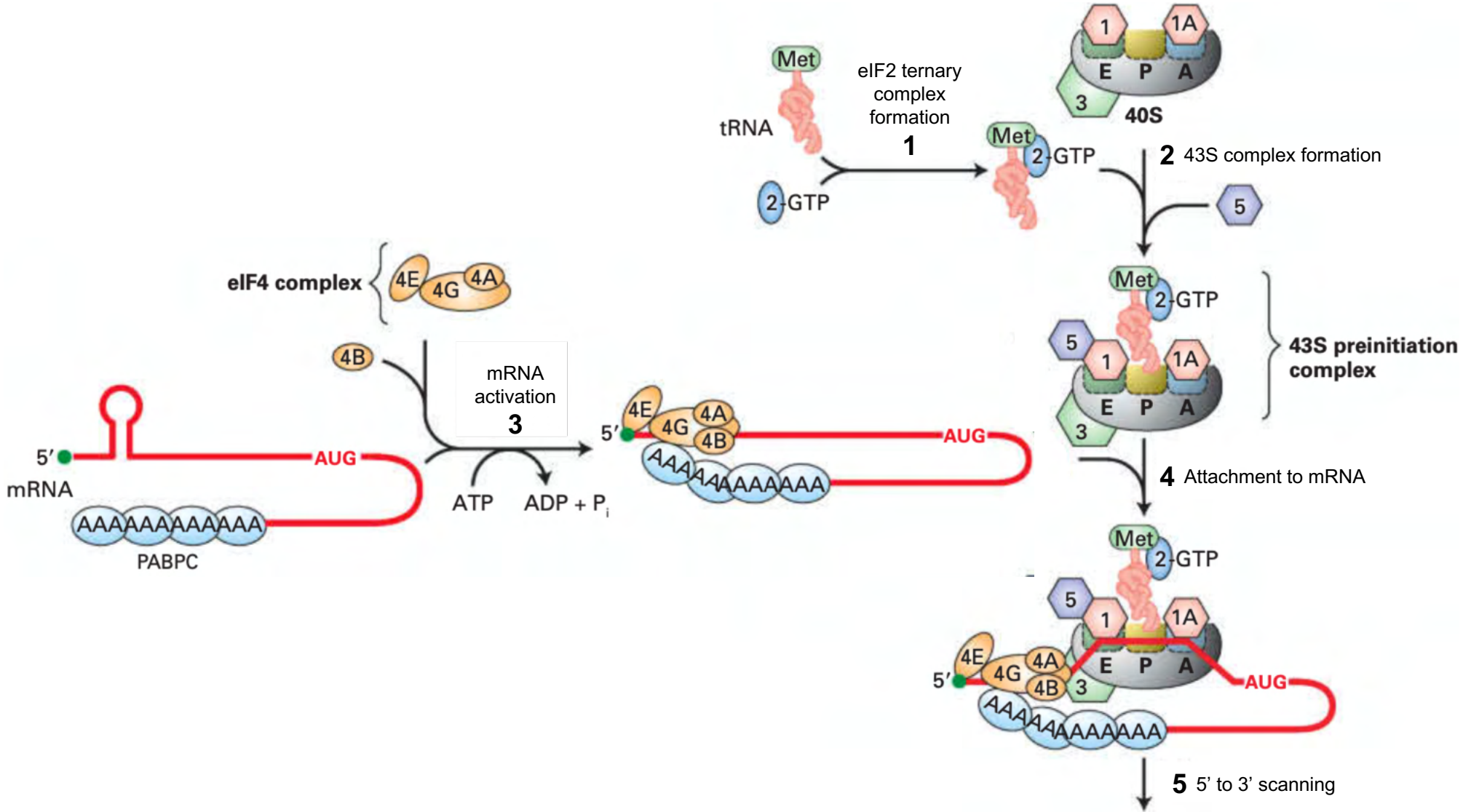
1. mRNAs
2. tRNAs
3. rRNAs

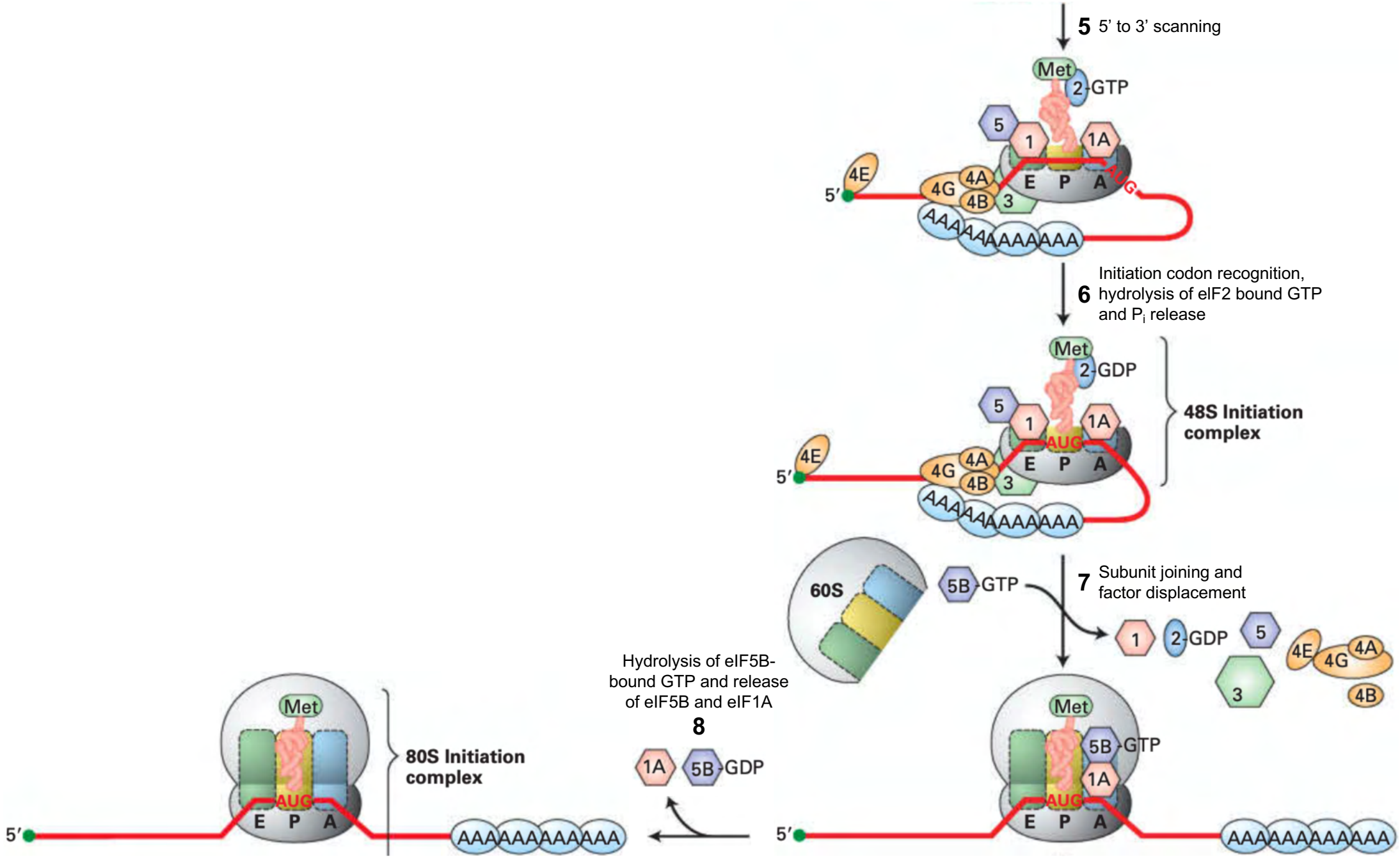


Synthesis of proteins on ribosomes

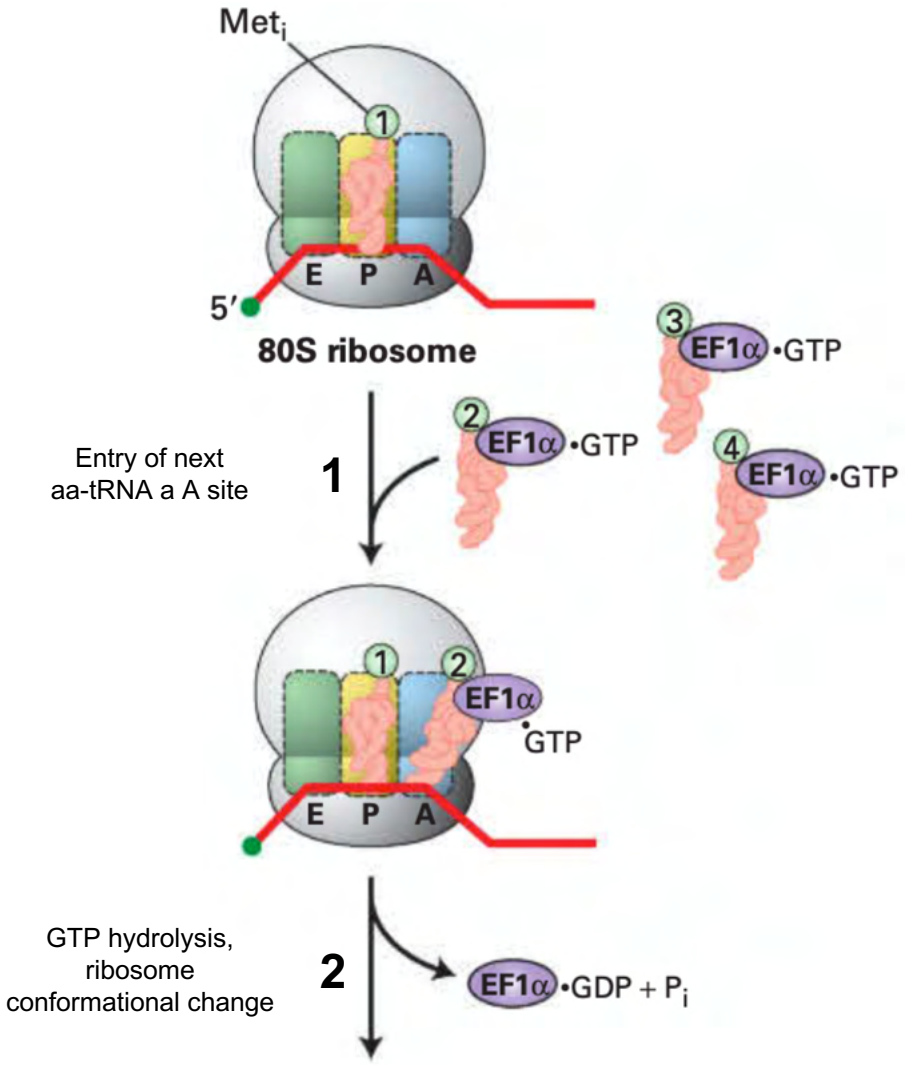


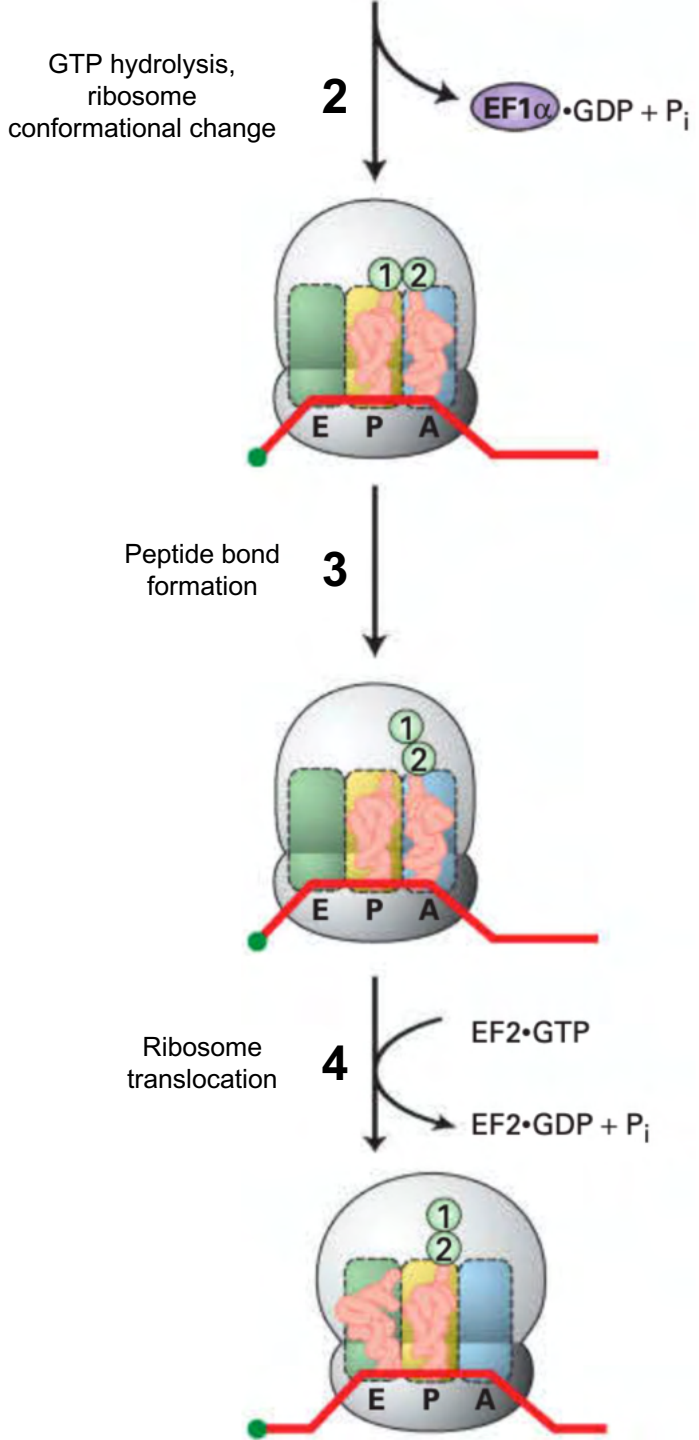
Synthesis of proteins on ribosomes



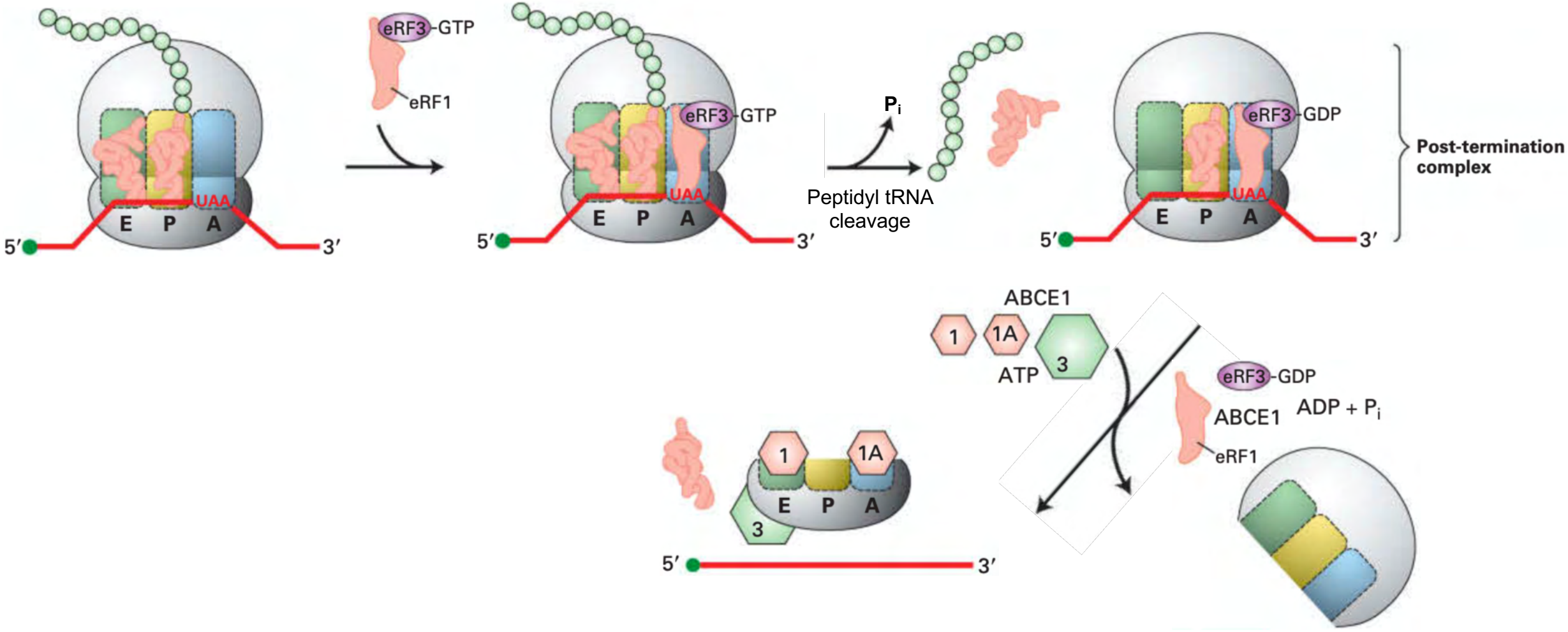

5 5' to 3' scanning
Met
2-GTP
5
1
1A
4E
5'
4G
4A
4B
3
E P A
AUG
AAAAAAAAAAA
6 Initiation codon recognition, hydrolysis of eIF2 bound GTP and P_i release
Met
2-GDP
48S Initiation complex
60S
5B-GTP
7 Subunit joining and factor displacement
1
2-GDP
5
4E
4G
4A
3
4B
Hydrolysis of eIF5B-bound GTP and release of eIF5B and eIF1A
8
1A
5B-GDP
80S Initiation complex

Chain elongation in eukaryotes

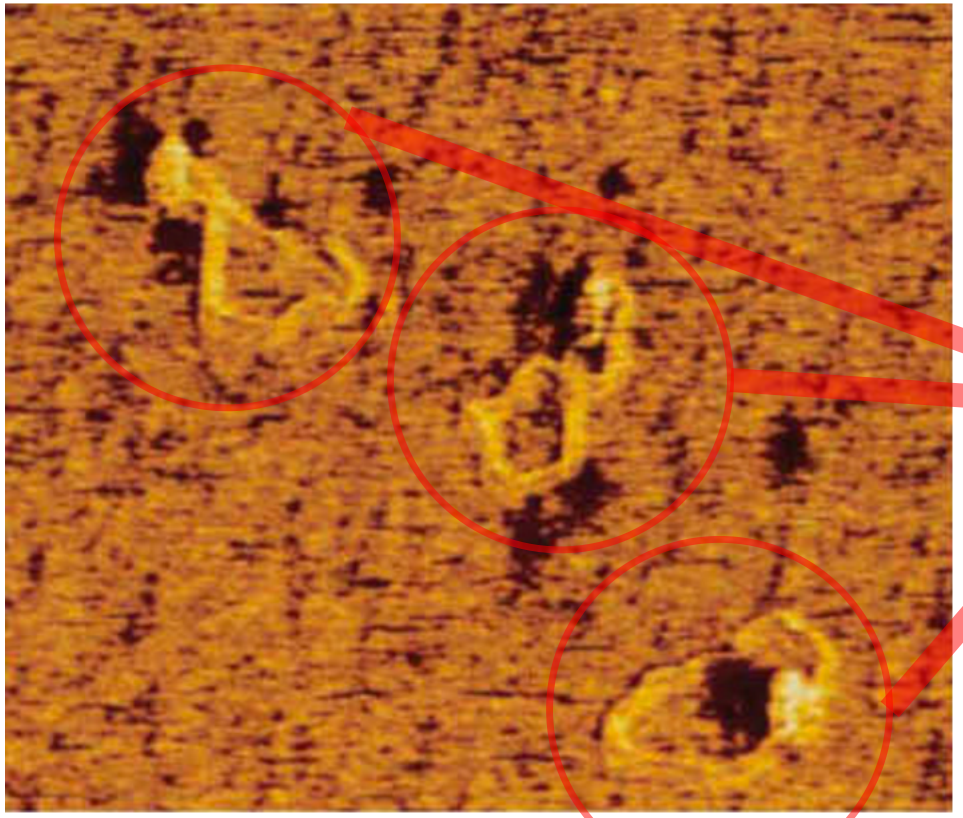


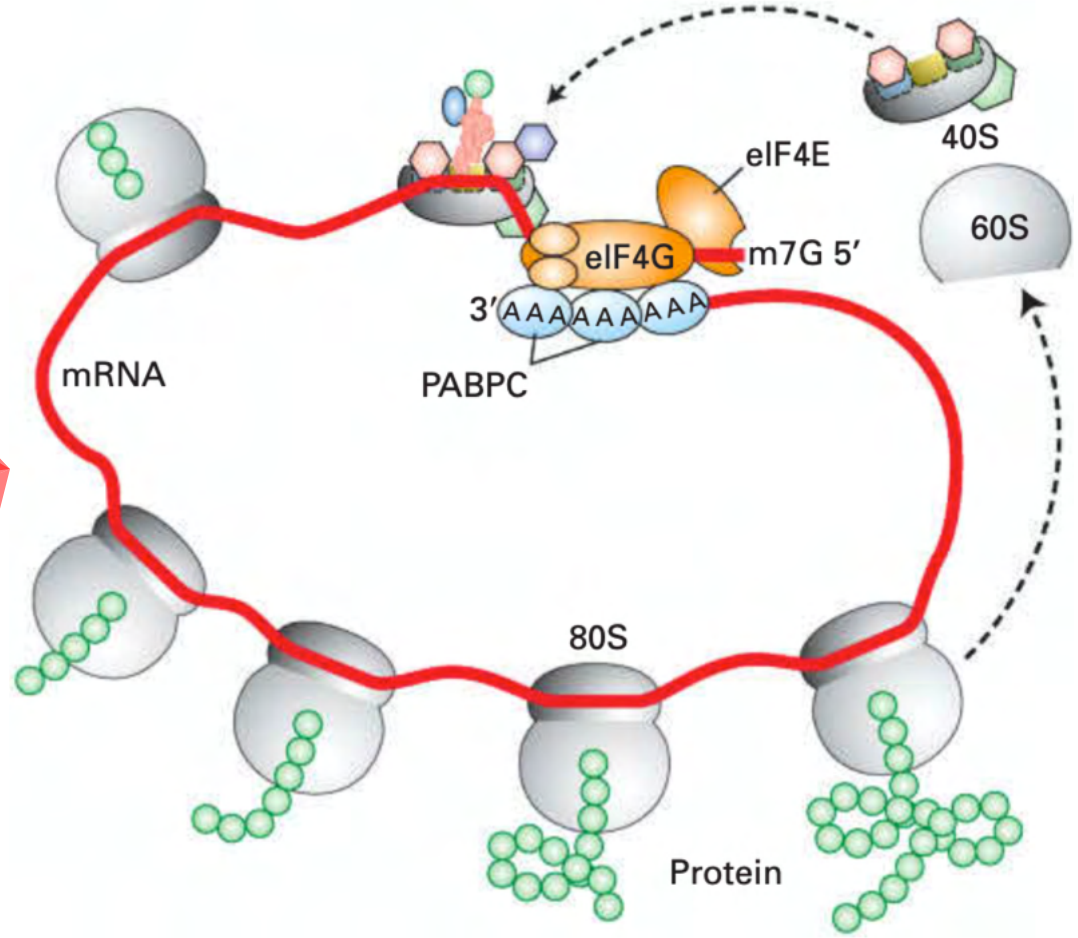

GTP hydrolysis,
ribosome
conformational change
2
EF1α•GDP + Pi
1
2
E
P
A
Peptide bond
formation
3
1
2
E
P
A
Ribosome
translocation
4
EF2•GTP
EF2•GDP + Pi
1
2
E
P
A

Termination of translation in eukaryotes

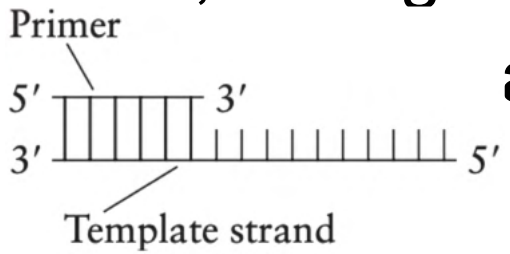


Polysomes and rapid ribosome recycling



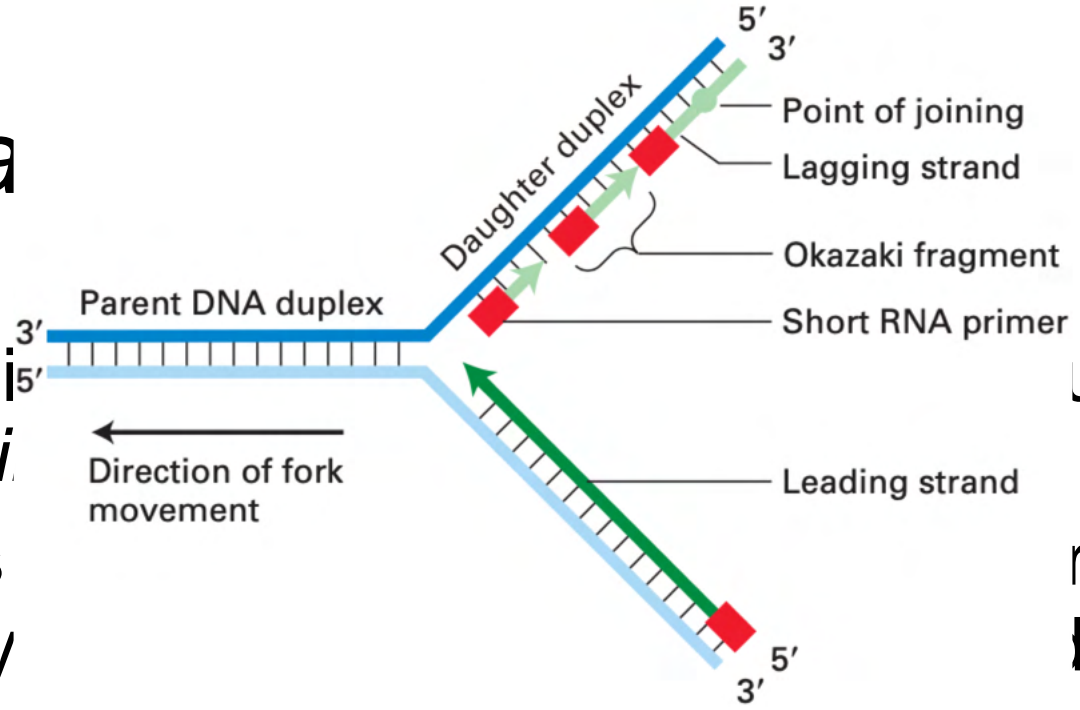


DNA replication

- Duplex DNA is replicated by a semiconservative mechanism.
- DNA is synthesized from deoxyribonucleoside 5′-triphosphate precursors (**dNTPs**).
- DNA synthesis always proceeds in the **5′→3′** direction.
- DNA polymerases require a short, preexisting RNA or DNA strand, called a **primer**, to begin chain growth.
- Unwinding of the 

ands is performed by enzymes called **helicases**.

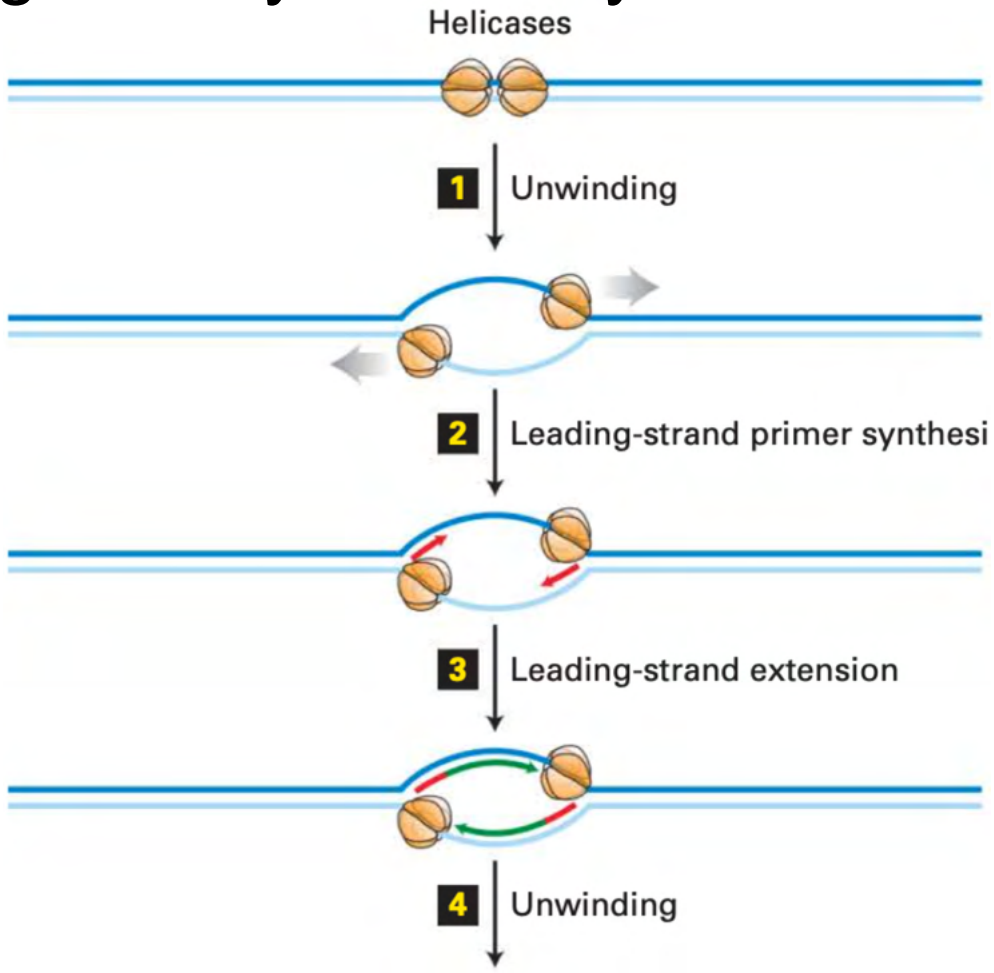
DNA replica

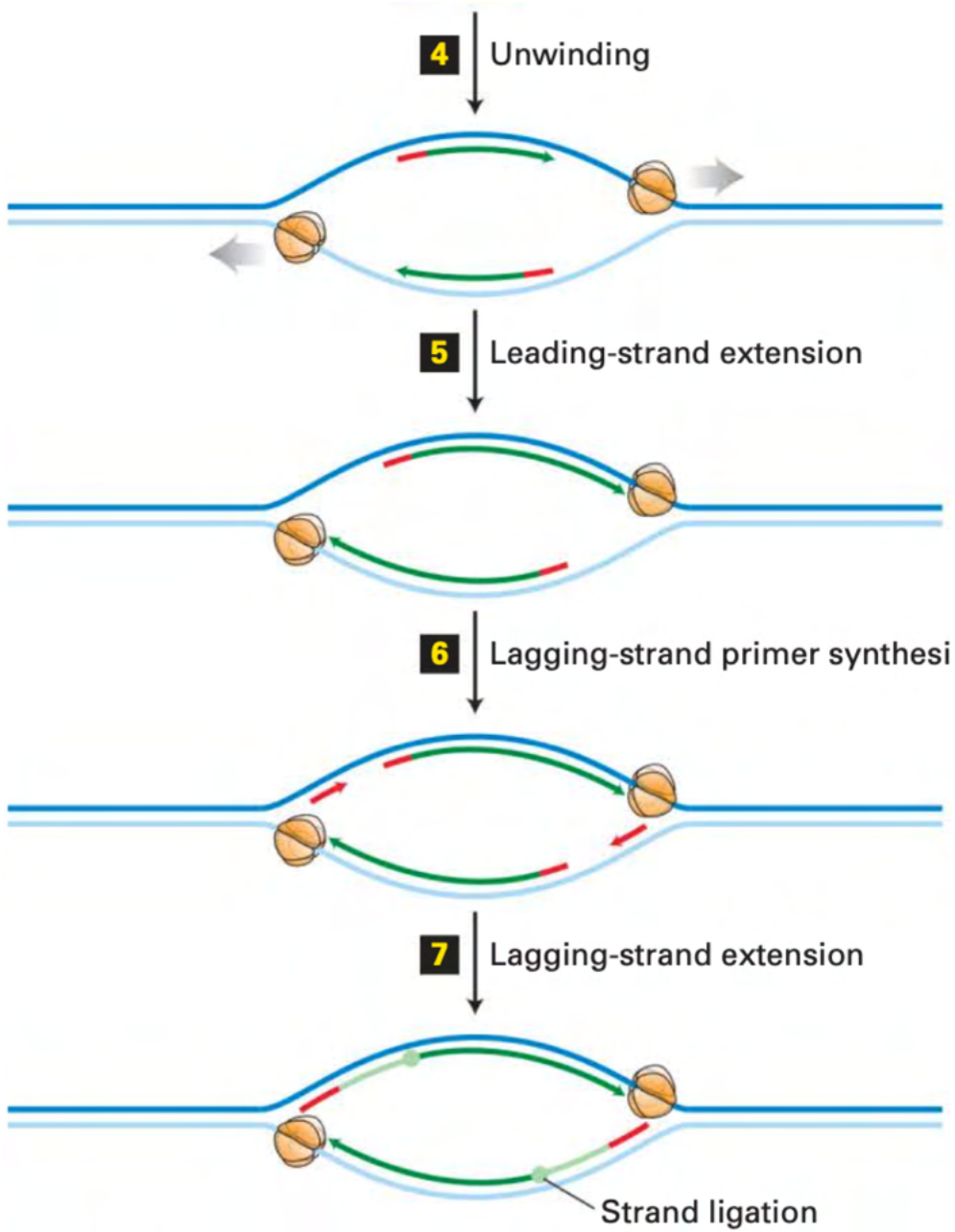


- Unwinding begi ule called *replication origi*
- **Primase** forms rimer complementary ls.
- Synthesis of one daughter strand, called the **leading strand**, can proceed continuously from a single RNA primer in the 5′→3′ direction.
- Growth of the **lagging strand** must also occur in the 5′→3′ direction, hence cell syntheses a new primer every 100 to 200 nucleotides on that template strand during unwinding.

DNA replication

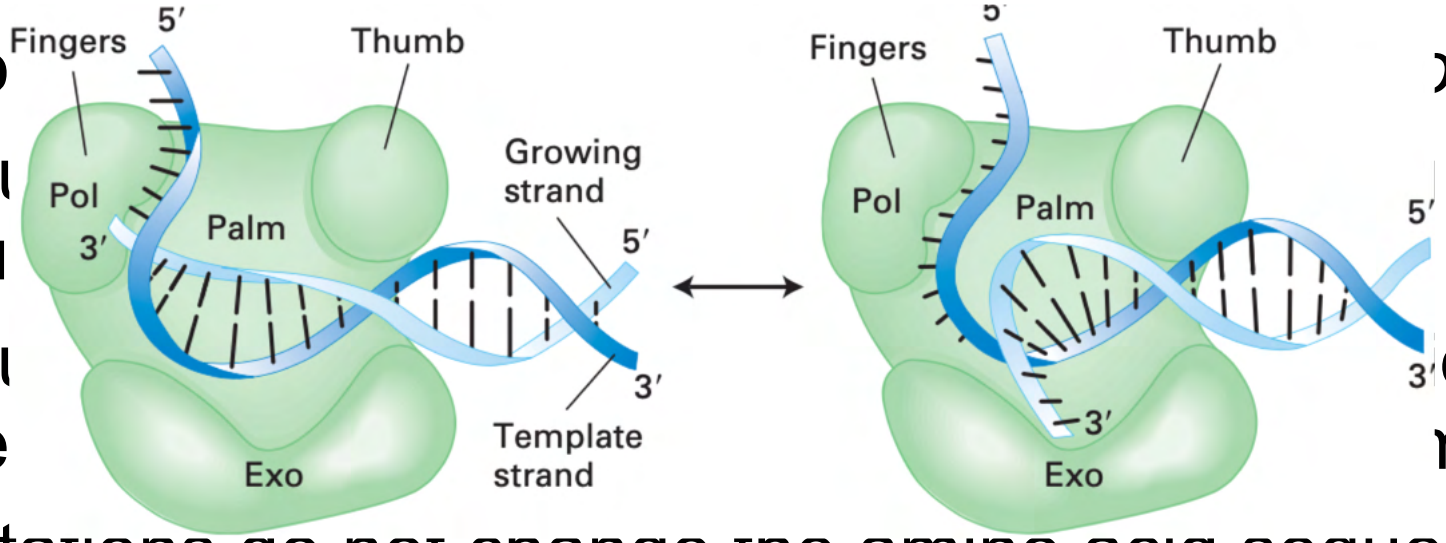
- DNA replication generally occurs by a bidirectional mechanism.



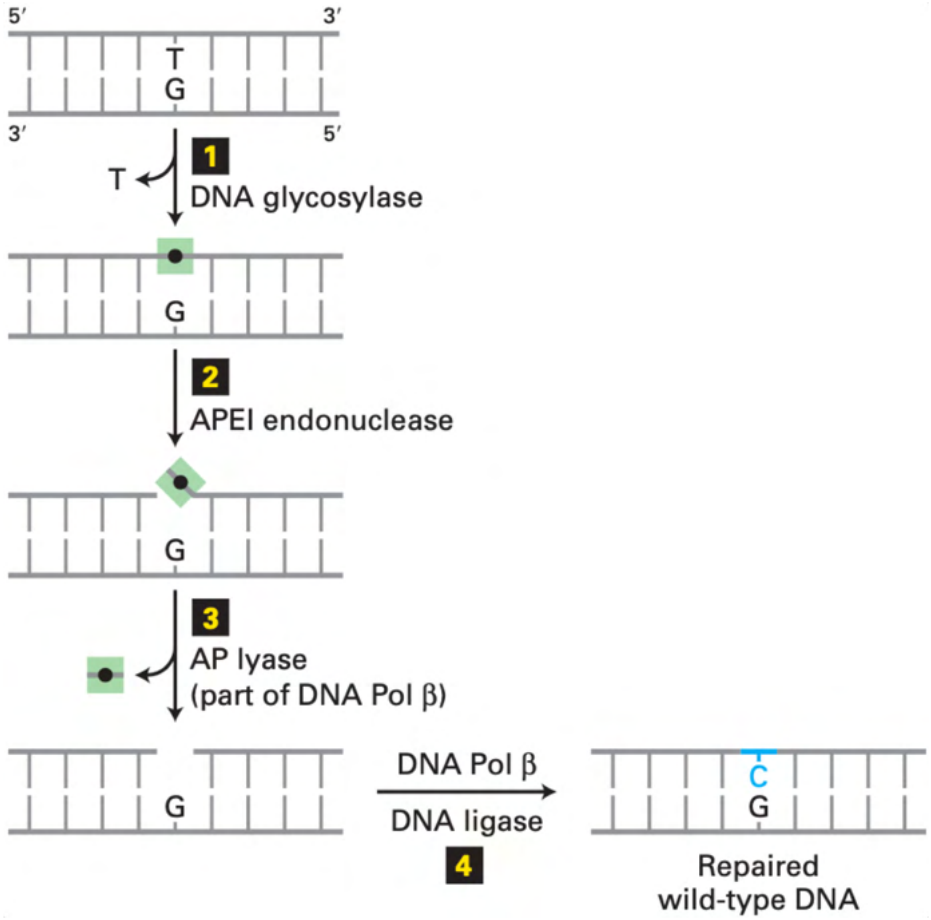

4
Unwinding
5
Leading-strand extension
6
Lagging-strand primer synthesi
7
Lagging-strand extension
Strand ligation

DNA repair

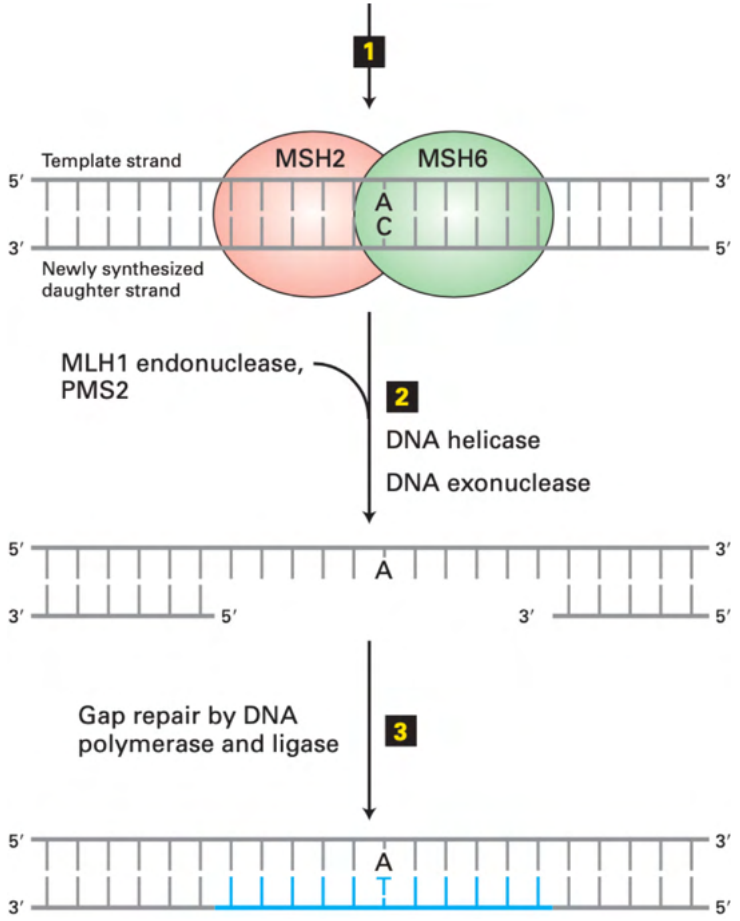
- Eukaryotic Pol δ and Pol ε have *proofreading* mechanism.
- Proofreading depends on the *3′→5′ exonuclease activity* of some DNA polymerases.
- Many spo ons.
- A point mu a *non-sense* mu
- A point mu d sequence nutation.
- *Silent* mutations do not change the amino acid sequence.



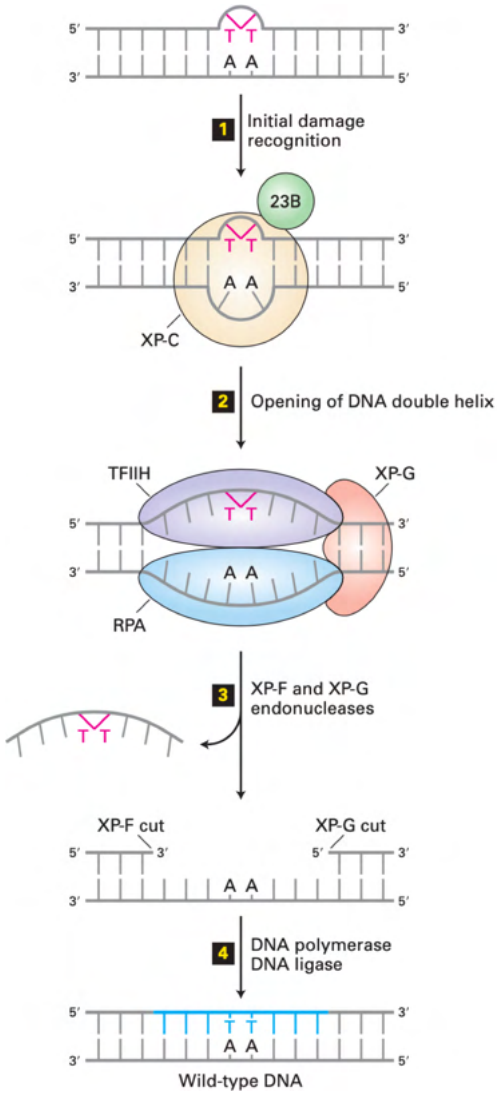
DNA excision repair



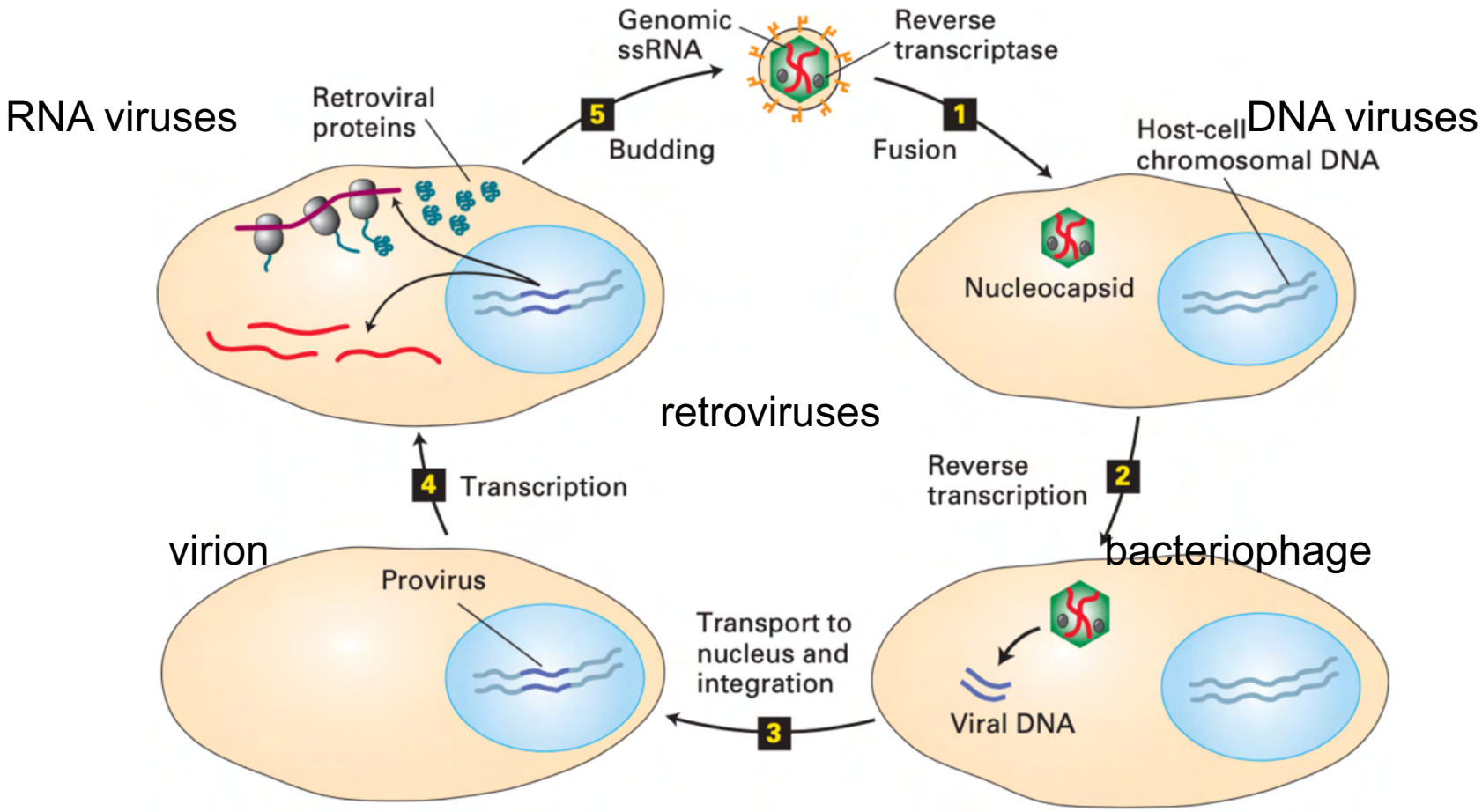
DNA mismatch excision repair



Nucleotide excision repair



Viruses…


RNA viruses
DNA viruses
retroviruses
bacteriophage
virion
Genomic ssRNA
Reverse transcriptase
Retroviral proteins
Host-cell chromosomal DNA
Nucleocapsid
Budding
Fusion
Transcription
Reverse transcription
Provirus
Transport to nucleus and integration
Viral DNA
1
2
3
4
5

Mobile DNA Elements: transposons