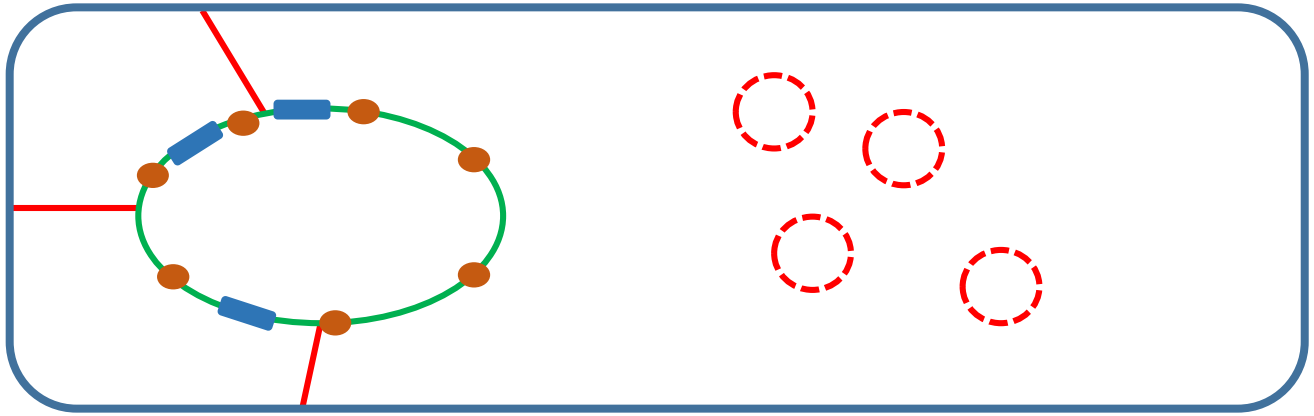
Replication and gene expression in prokaryotes

Molecular Biology
FaF MU Brno
24.9.2021

Structure of prokaryotic genome



NUCLEOID

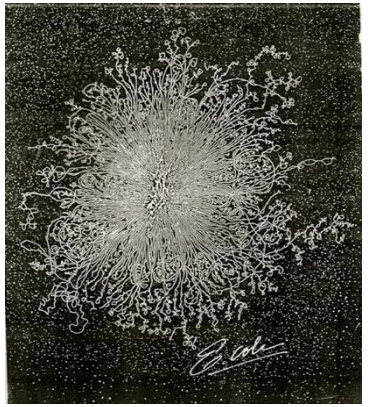
PLASMIDS

- Not designated by a nuclear membrane
- **DNA**, HLP (histone-like proteins), non-histone proteins
- Prokaryotic chromosome - DNA is one circular molecule of dsDNA
- *E. coli* – 4,7 Mbp - >4000 genes
- Attached to cellular membrane in several places
- Origin of replication *oriC* and termination region *ter*

- Small circular molecules of dsDNA (1-200 kbp) – up to several thousands per cell (usually 100s)
- They carry genes that are essential for survival (resistence to antibiotics e.g.)
- Every plasmid is a replicon (has its own *oriC*) and replicates independently of the genomic DNA
- Bacteria can acquire plasmids by conjugation or from the environment
- Great importance in biotechnology

Prokaryotic chromosome

- Circular molecule
- Organized into looped domains
- Each loop is independently supercoiled



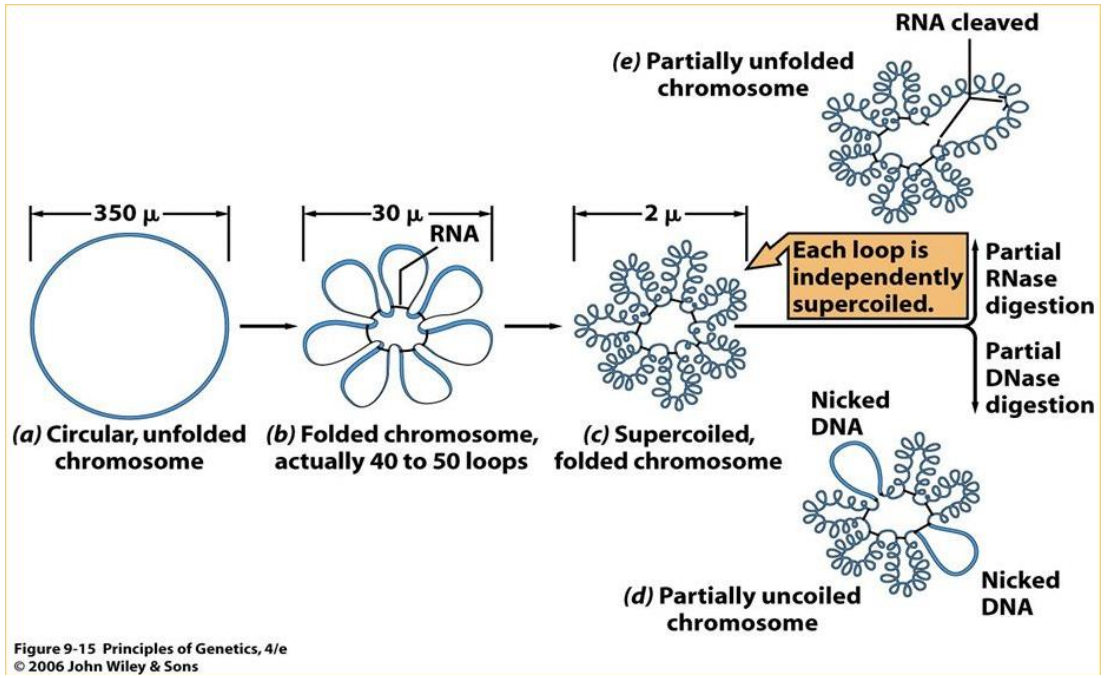


Figure 9-15 Principles of Genetics, 4/e
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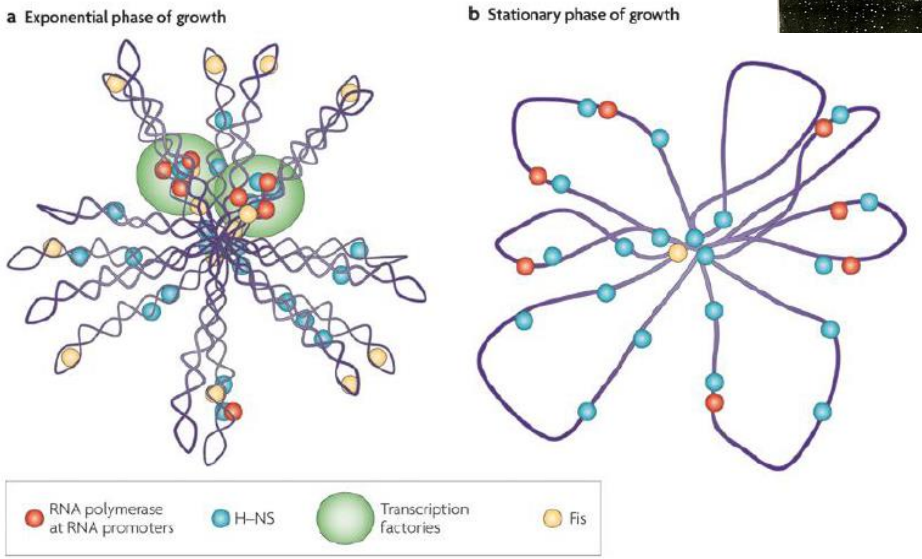


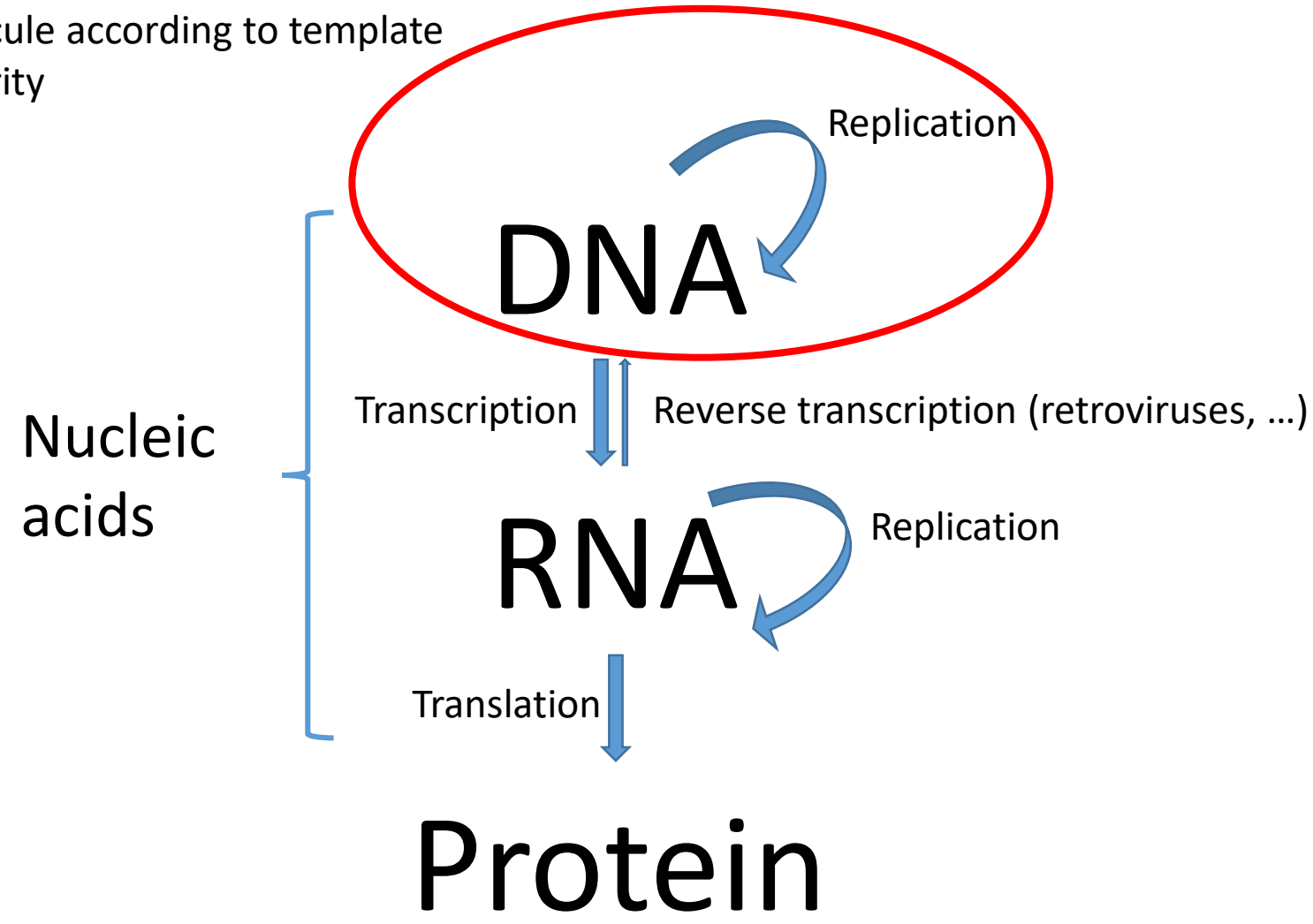
doi:10.1038/nrmicro2261

Nature Reviews | Microbiology

a | The folded chromosome is organized into looped domains that are negatively supercoiled during the exponential phase of growth. In this phase, the abundant nucleoid-associated proteins histone-like nucleoid-structuring protein (H-NS) and factor for inversion stimulation (Fis) bind throughout the nucleoid and are associated with the seven ribosomal RNA operons. As shown here in two cases, these are organized into superstructures called transcription factories. **b** | In stationary phase the rRNA operons are quiescent and Fis is almost undetectable. The chromosome has fewer looped domains, and those that are visible consist of relaxed DNA.

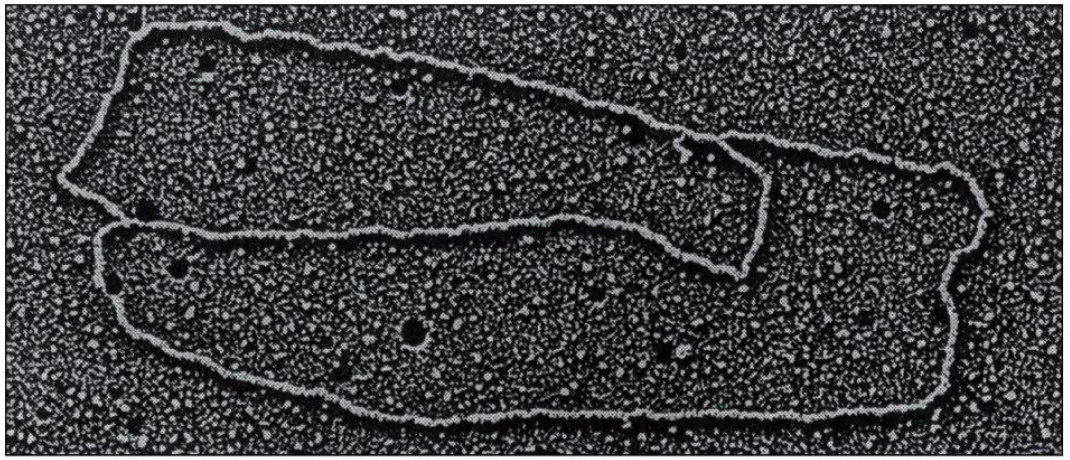
DNA replication

- Synthesis of new DNA molecule according to template
- Rules of base complementarity
- Key element of cell division



Replication goes in both directions

- Bacterial chromosome has one origin of replication (*oriC*)
- Replication goes in both directions from *oriC*
- Replication ends in *ter* region



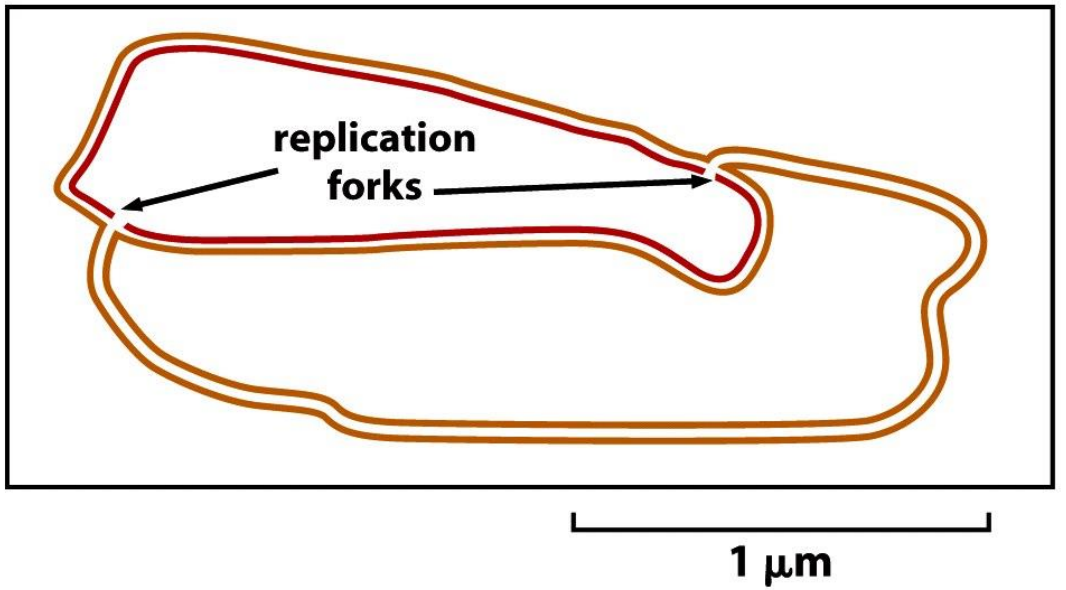
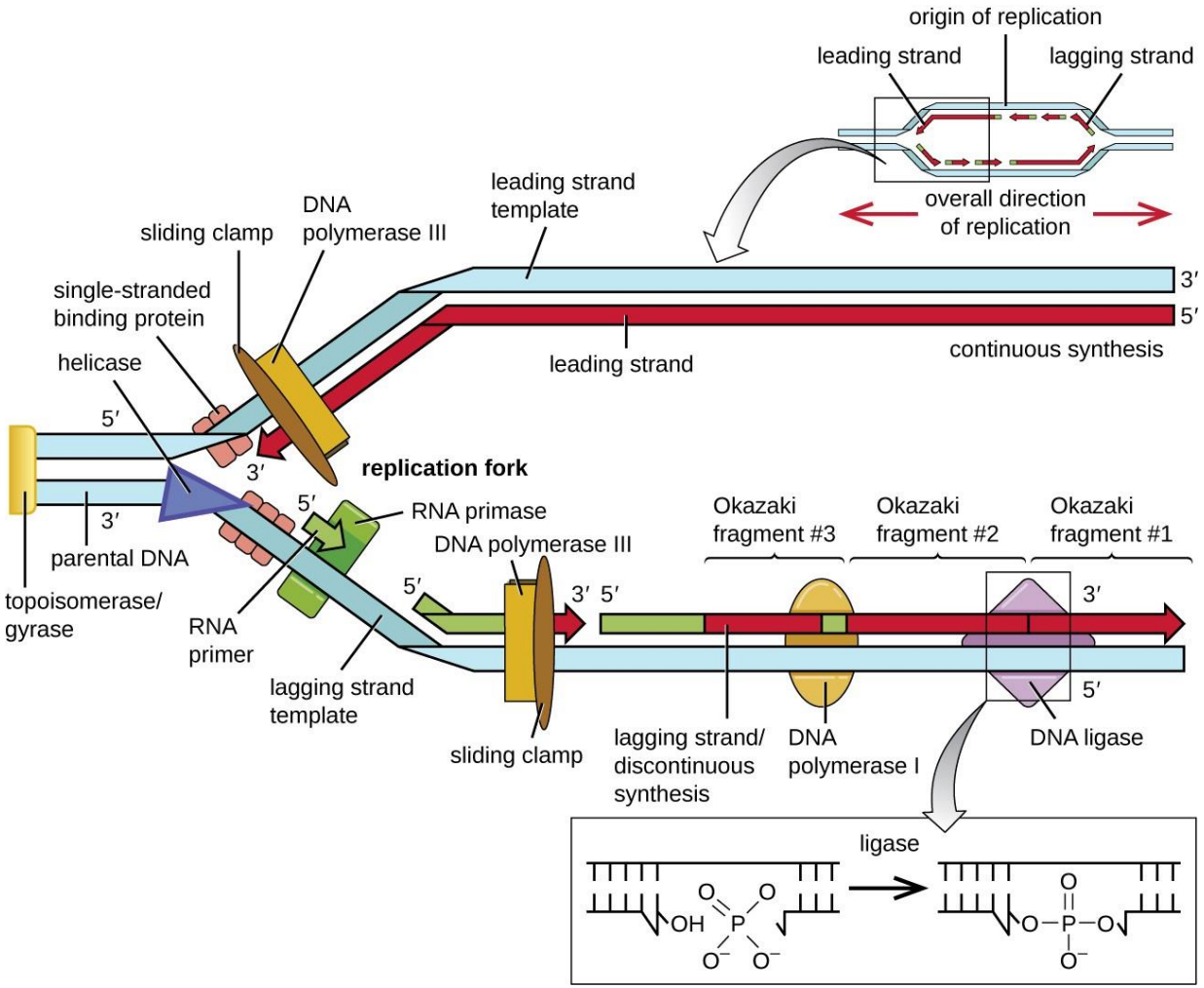


Figure 5-6 Molecular Biology of the Cell 5/e (© Garland Science 2008)

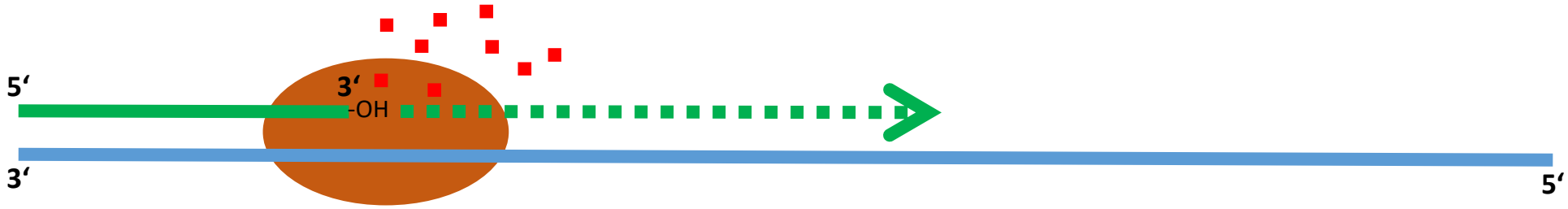
Replication fork



https://courses.lumenlearning.com/microbiology/chapter/dna-replication/

DNA replication rules

- Template - DNA strand used for synthesis
- Primer – free 3‘OH end
- Replication proteins – DNA polymerase, primase
- dNTP – deoxyribonucleosidtriphosphates (dATP, dGTP, dCTP, dTTP)



DNA replication is 5‘-3‘ directed

- Strand growth can occur in both directions - 5‘-3‘ or 3‘-5‘ from template
- 3‘-5‘ – different polymerase (artificial)
- Problem is in the proofreading mechanism – repair of wrongly incorporated base: in the case of 3‘-5‘ polymerization – termination of replication

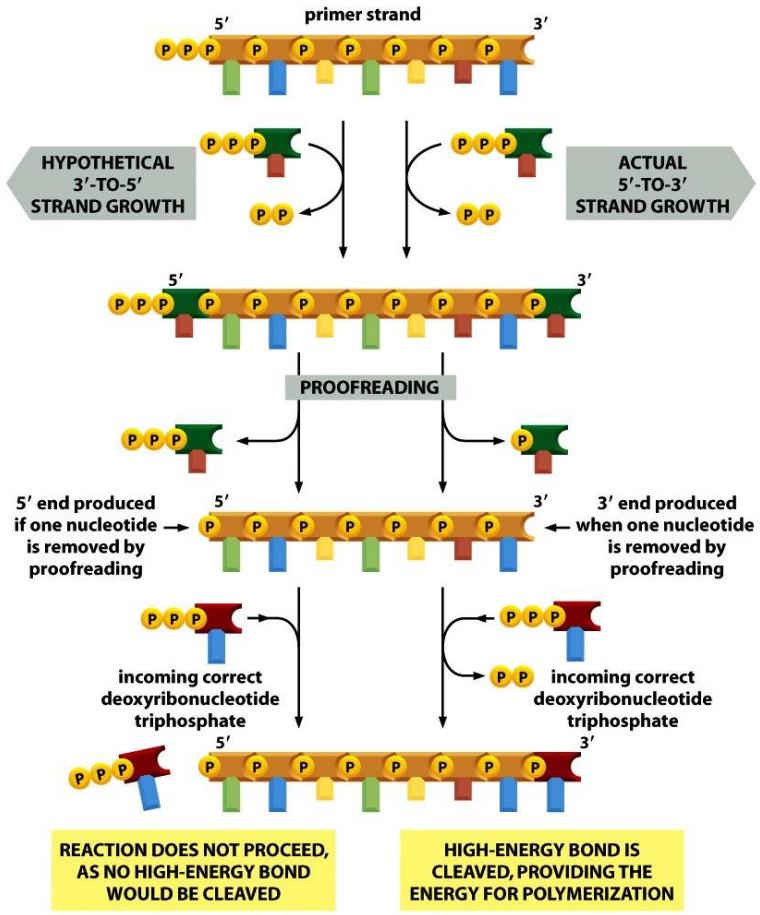


Figure 5-10 Molecular Biology of the Cell 5/e (© Garland Science 2008)

DNA replication is SEMI-DISCONTINUOUS

- Replication proceeds on both strands simultaneously – template strands have opposite polarity (5‘-3‘ and 3‘-5‘) – they are antiparallel:
  - leading strand - continuous synthesis from one primer
  - lagging strand - discontinuous, Okazaki fragments from multiple different primers

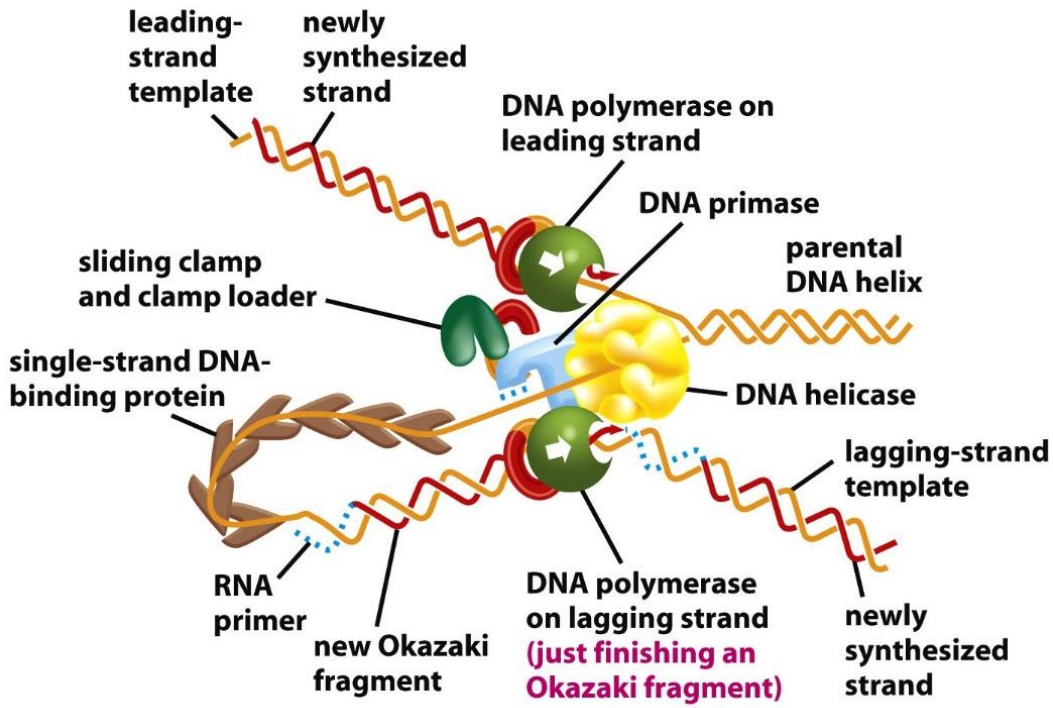
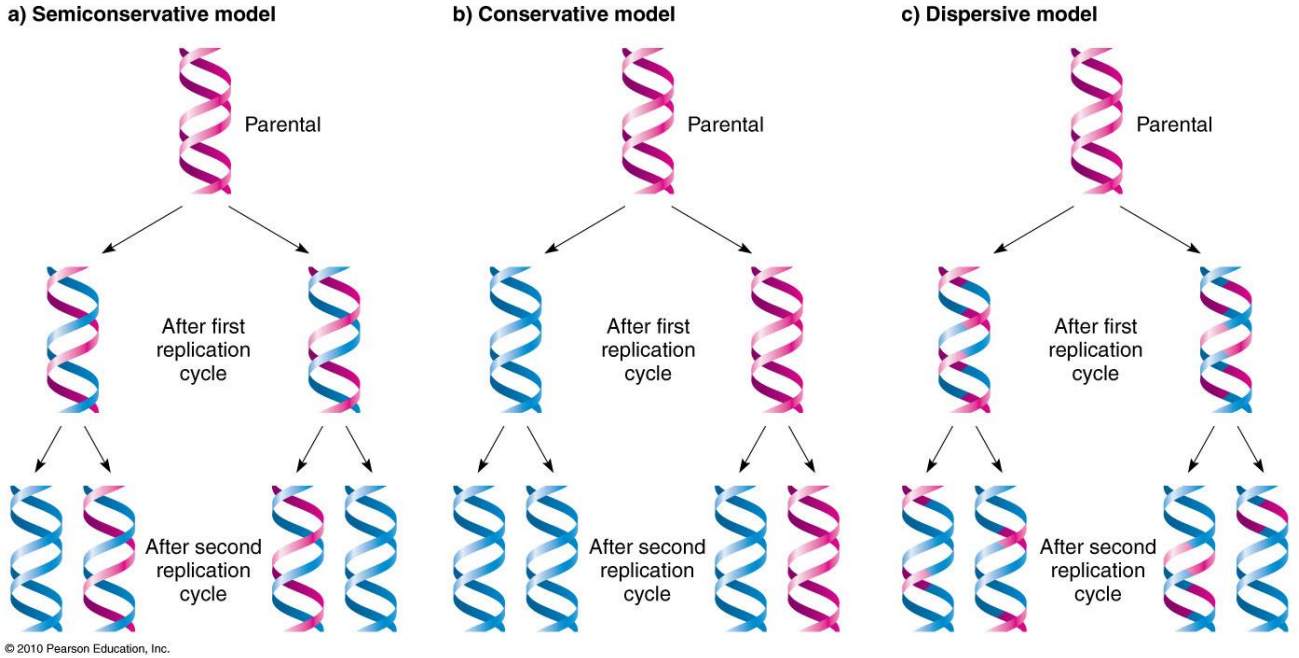


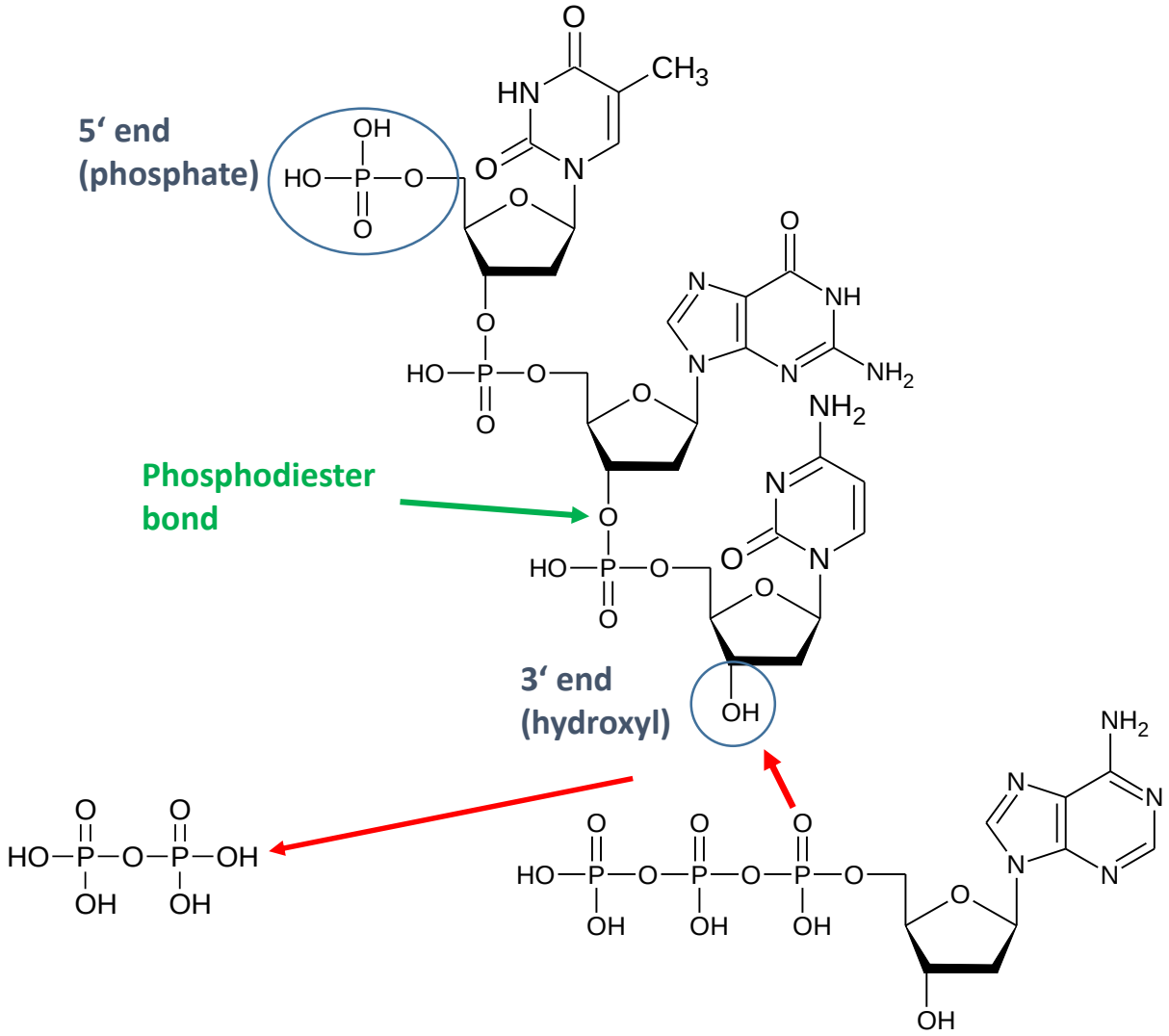
Figure 5-19a Molecular Biology of the Cell 5/e (© Garland Science 2008)

DNA replication is SEMI-CONSERVATIVE

- New molecule of dsDNA contains one strand from template DNA and one strand of newly synthesised DNA
- Meselson-Stahl experiment – ^{15}N labeled DNA in bacteria further cultivated in ^{14}N environment
- www.youtube.com/watch?v=JcUQ_TZCG0w



DNA synthesis during replication



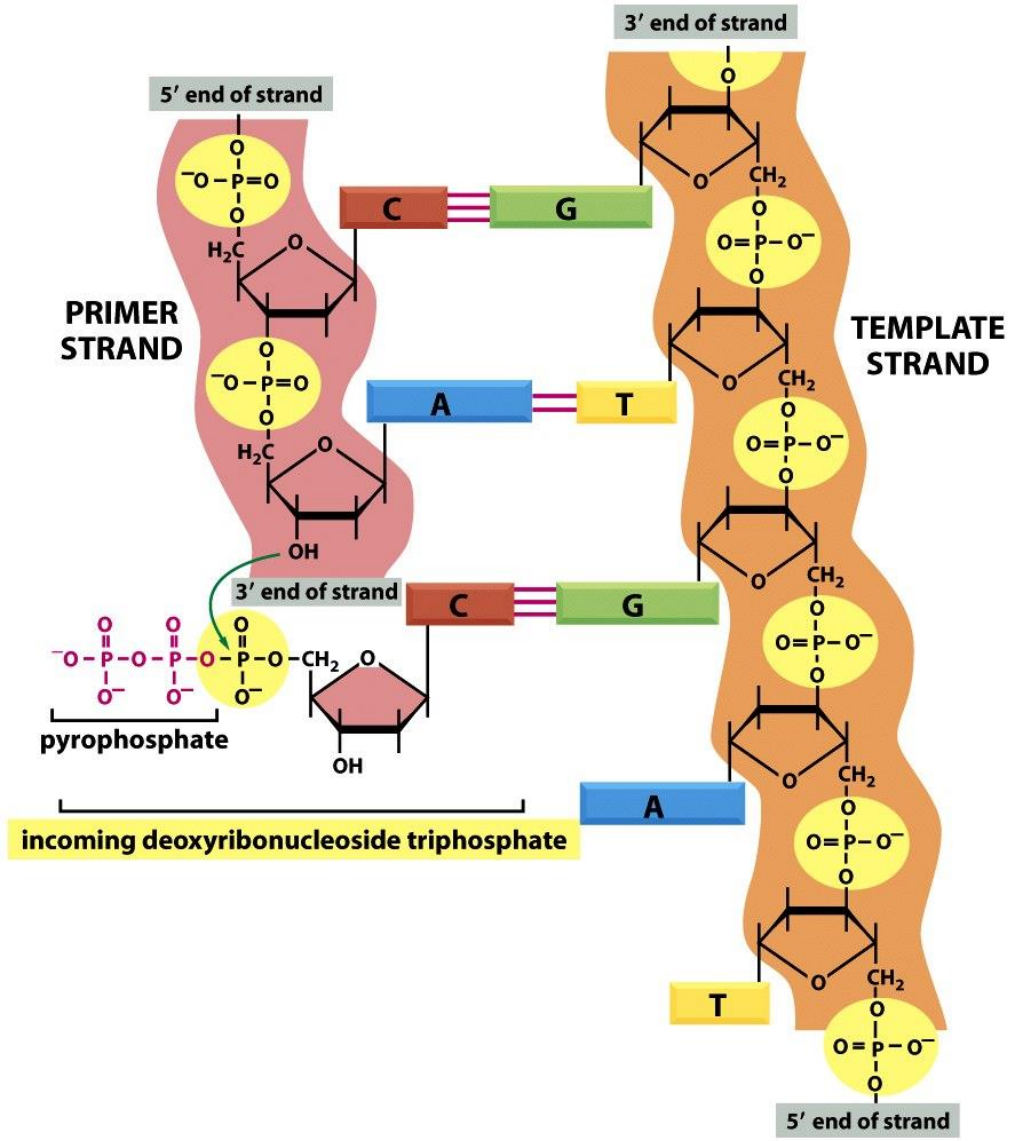
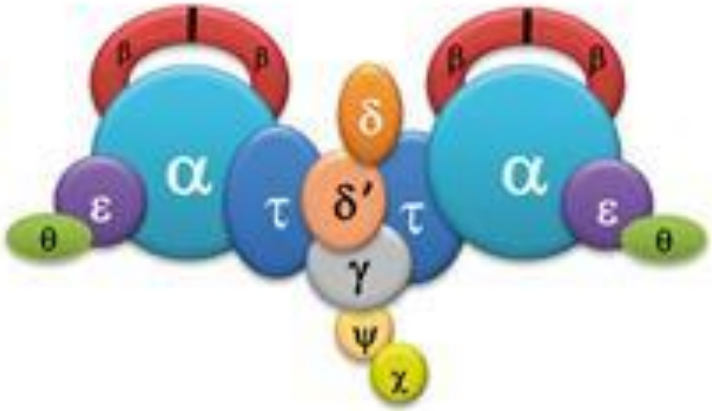


Figure 5-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Replication proteins - polymerases

	Pol I	Pol II	Pol III	Pol IV	Pol V
DNA polymerase family	A	B	C	Y	Y
Activity	5'-3' polymerase 3'-5' exonuclease 5'-3' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase 3'-5' exonuclease	5'-3' polymerase	5'-3' polymerase
	PolA	PolB		DinB	UmuC UmuD' UmuD'
Number of molecules/ cell					
– SOS	400	50 - 75	10 - 20	150 - 250	< 15
+ SOS	400	350 - 1000	10 - 20	1200 - 2500	200
Biological functions in the cell	DNA replication, Okazaki fragment maturation, DNA repair	DNA replication (backup DNA polymerase), DNA repair, TLS	DNA replication DNA repair	TLS	TLS

{Fijalkowska J. et al., FEMS Microbiology Reviews, 2012}

Replication proteins - polymerases

Processivity

- Ability to synthesize the reaction without releasing the template – in case of DNA synthesis it is incorporation of nucleotides – measured in nucleotides, without dissiciation of polymerase - high processivity (leading strand synthesis), low (synthesis of spacers between Okazaki fragments)

Fidelity

- Ability of DNA polymerase to copy template strand, how it incorporates complementary bases – relates to polymerase errors (e.g. 10^5 correctly incorporated nucleotides to 1 error) - higher because of proofreading activity of polymerase = ability to cut out incorrectly incorporated nucleotide (3‘-5‘ exonuclease activity)

Speed

- Average amount of nucleotides incorporated per second – connected to processivity

Replication proteins - polymerases

DNA polymerase I

- Globular protein; Mw = 109 000
- Multidomain structure – own polymerase activity, 3‘-5‘ exonuclease (proofreading) and 5‘-3‘ exonuclease activity
- Replication in spaces between Okazaki fragments
- 5‘-3‘ exonuclease activity ensures degradation of RNA primers during ligation of Okazaki fragments
- Low processivity (20-25 nt), low synthesis speed (10-20 nt/s), high fidelity (10^4-10^5)

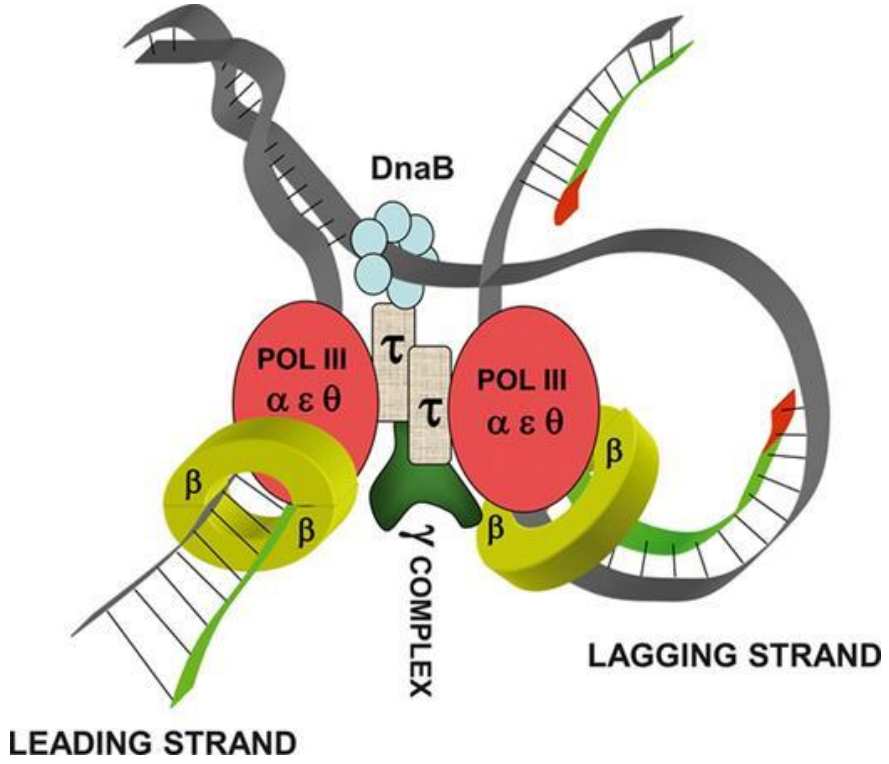
DNA polymerase II

- Monomer; Mw = 90 000
- Polymerase and 3‘-5‘ exonuclease (proofreading) activity
- DNA repair

DNA polymerase III

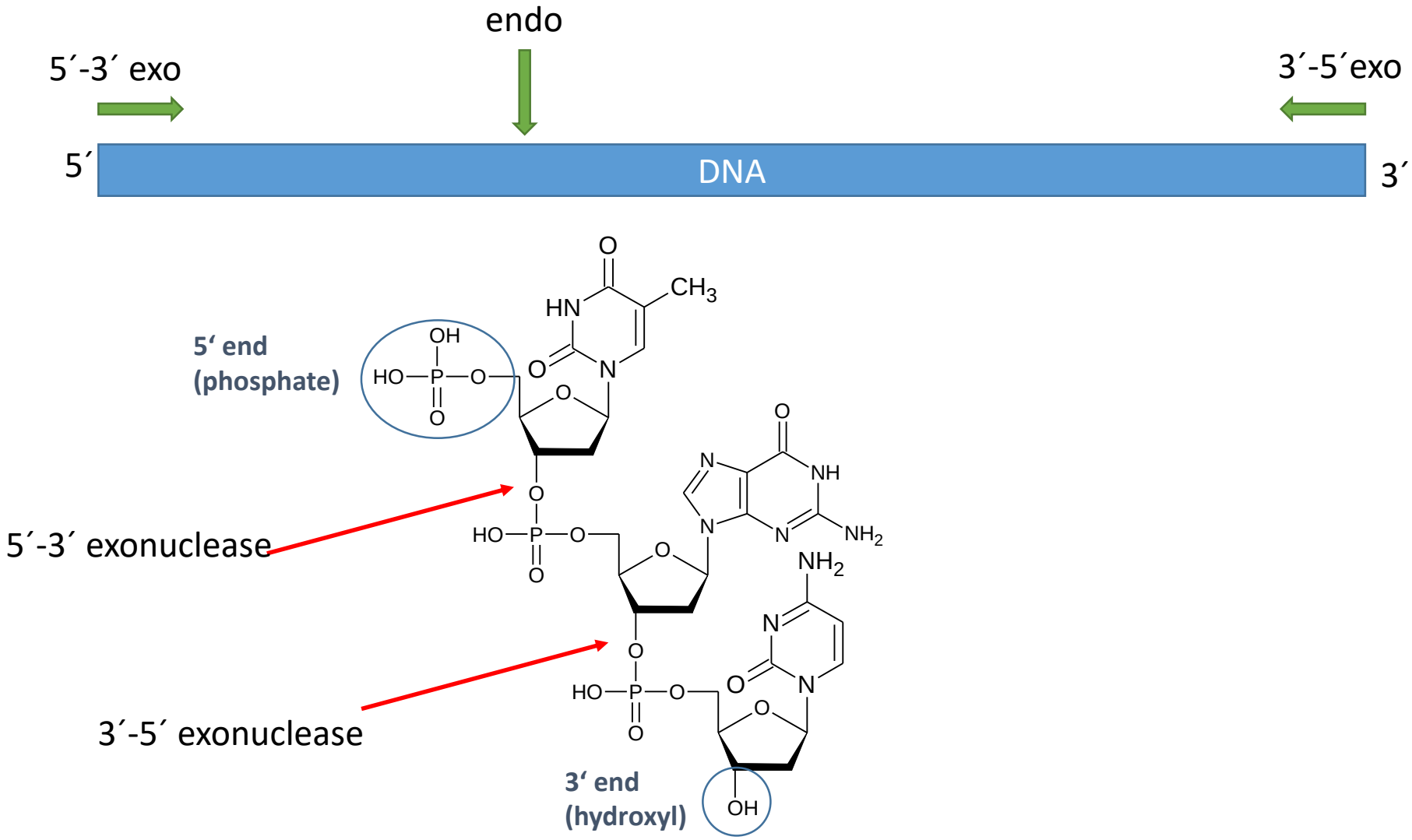
Polymerase III

- Complex of several proteins ; Mw = 900 000
- Basic complex of subunits α, ε a θ, through τ subunits form a dimer, further associates with subunits γ and β clamps which raise the processivity of enzyme
- Replication of leading and lagging strand
- Processivity and speed of synthesis depends on the structure of complex (monomer x dimer; presence of β clamp) – whole complex syntesizes DNA 1000 nt/s with high processivity
- Polymerase and 3‘-5‘ exonuclease (proofreading) activity



{Fijalkowska J. et al., FEMS Microbiology Reviews, 2012}

Exo- and endo-nuclease activity



Replication proteins - other

DNA ligase

- Creates phosphodiester bond between 5‘ end and 3‘ end of two polynucleotide strands
- Connects Okazaki fragments

DNA primase

- DNA-dependent RNA-polymerase
- Syntesizes RNA primers (one for leading strand and one for each Okazaki fragment)

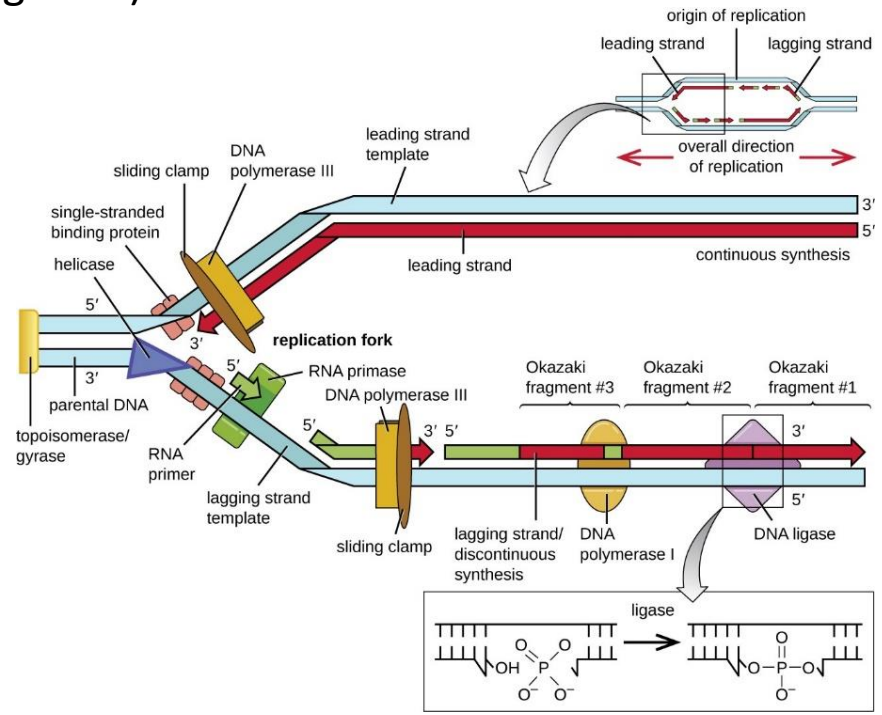
DNA helicase

- Unwinds DNA strands in duplex

DNA gyrase (topoisomerase II)

- Unwinds superhelical twists created by replication fork progression
- Converts positive superhelicity to negative

Replication proteins form complex – **replisome**

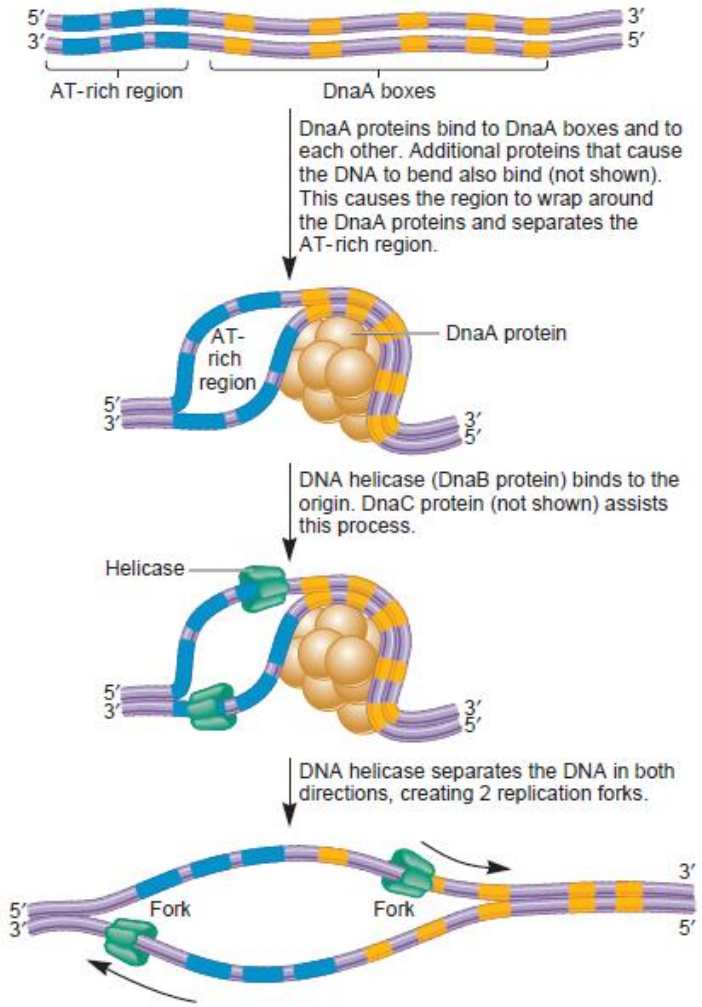


Replication progress

- **initiation:** requires primer in replication origin: short RNA synthesized by primase
- **elongation:** DNA polymerase creates new strand in 5'—>3' direction
- **termination:** in *ter* region

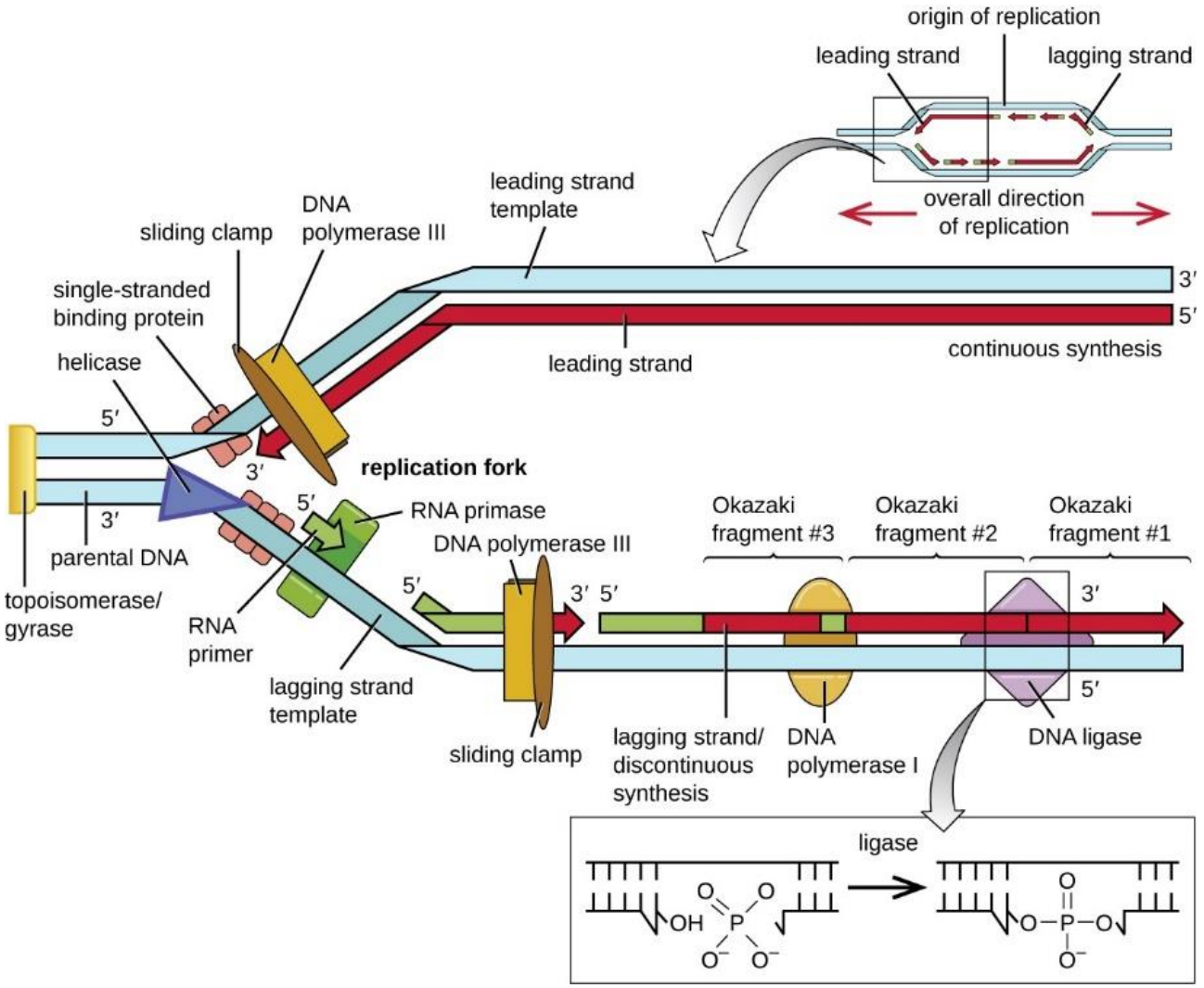
Replication initiation

1. Recognition of replication origin (oriC) by **DnaA proteins**, relaxation of hydrogen bonds at oriC
2. Binding of helicase to unwound DNA strands – unwinding of duplex DNA in 5‘-3‘ direction – creates replication fork
3. SSB proteins bind to ssDNA (single-strand binding), they keep DNA in unwound state



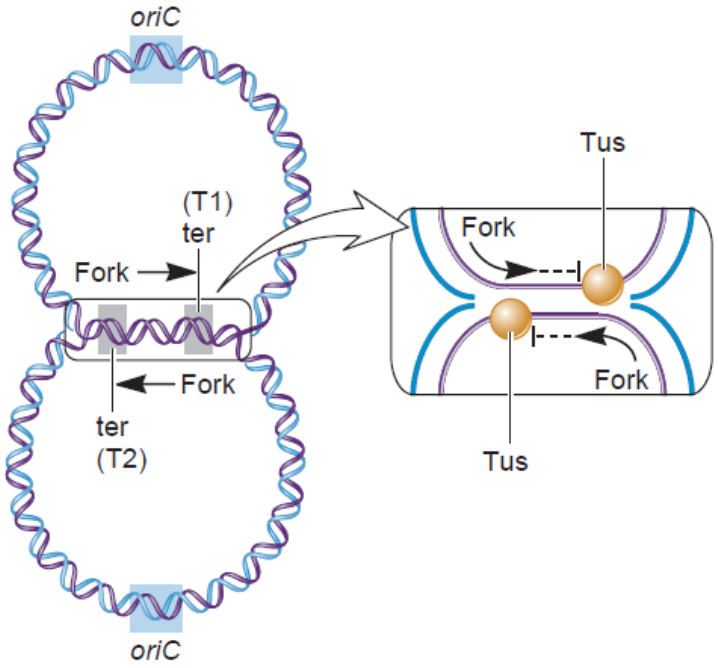
Replication progress

- Unwinding of dsDNA by **helicase**
- Relaxation of superhelicity by **topoisomerase**
- Binding of **SSB proteins** to ssDNA
- Synthesis of RNA primers on the lagging strands in Okazaki fragments by **RNA primase**
- Synthesis of DNA on leading and lagging strands by **DNA polymerase III**
- Cleaving of RNA and synthesis of spacers in Okazaki fragments by **DNA polymerase I**
- Ligation of DNA segments on lagging strand by **DNA ligase**



Replication termination

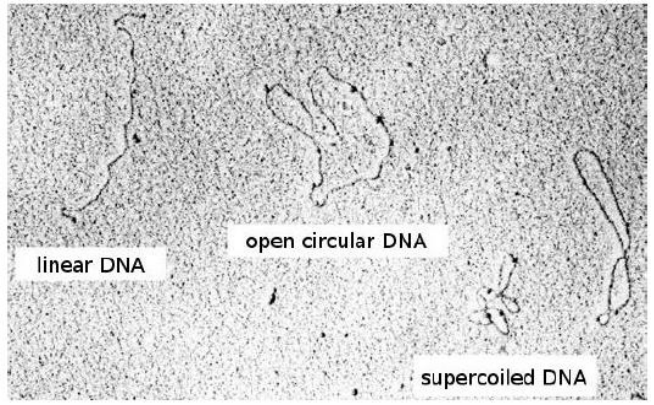
- Termination region = ***ter***
- **Tus protein** binds to ***ter***
- Tus – inhibits the activity of helicases (DnaB proteins)



http://reasonandscience.heavenforum.org/t1849p25-dna-replication-of-prokaryotes

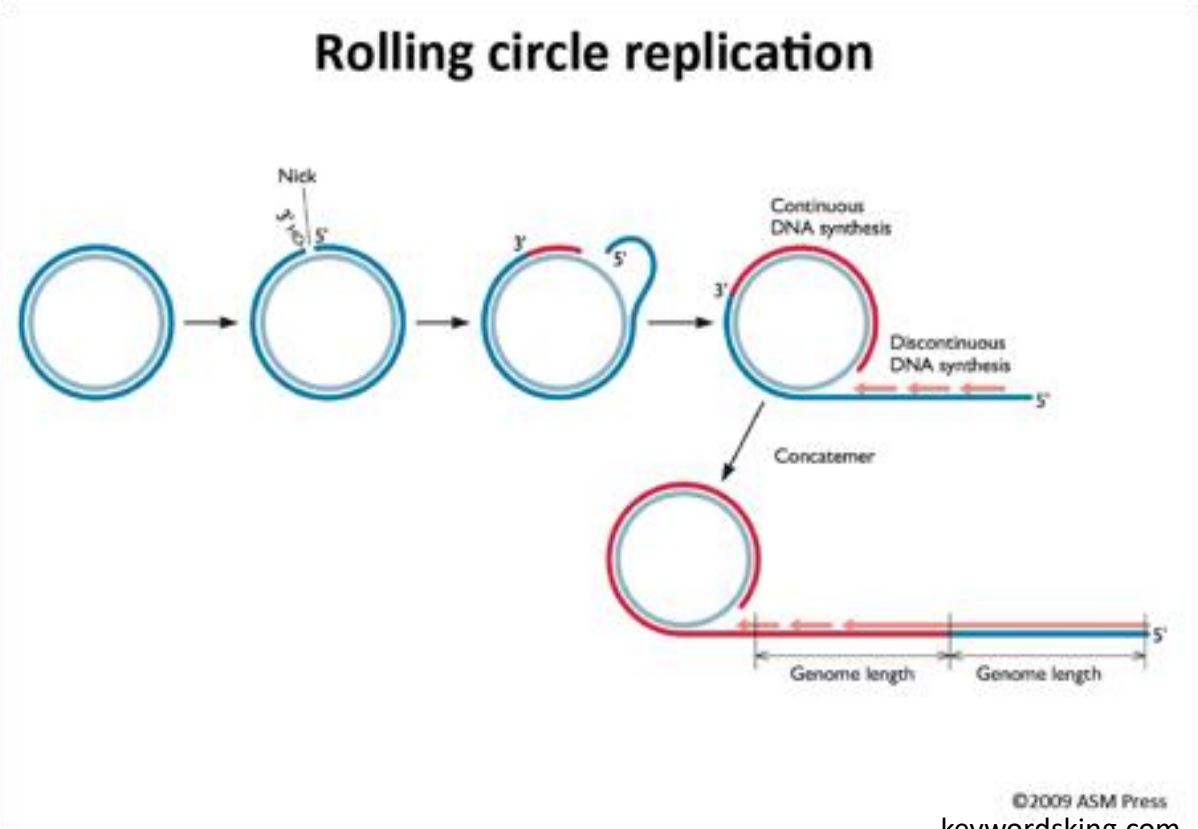
Replication of plasmid DNA

- Plasmids are circular molecules of double-stranded DNA
- Plasmid = circular replicon (origin of replication)
- Replication by rolling circle mechanism – can result in product containing multiple copies of DNA – concatemer
- Smaller plasmids are replicated by host cell replication machinery
- Different types/lengths of plasmids



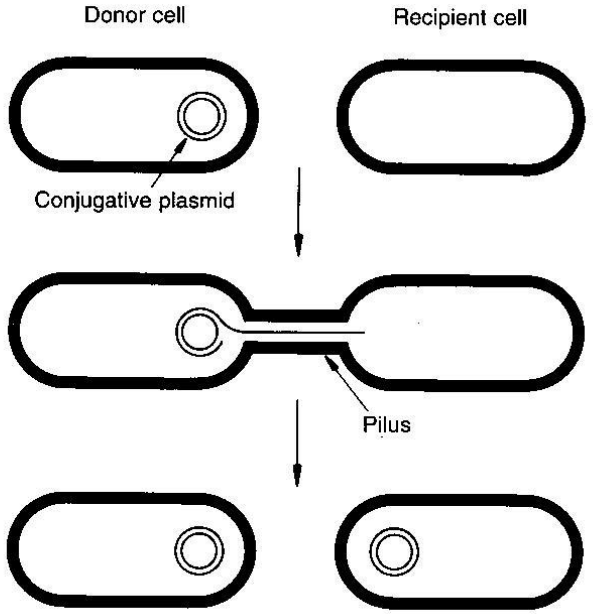
https://**www.youtube.com**/watch?v=z0PMLofObxk

Rolling circle replication



keywordsking.com

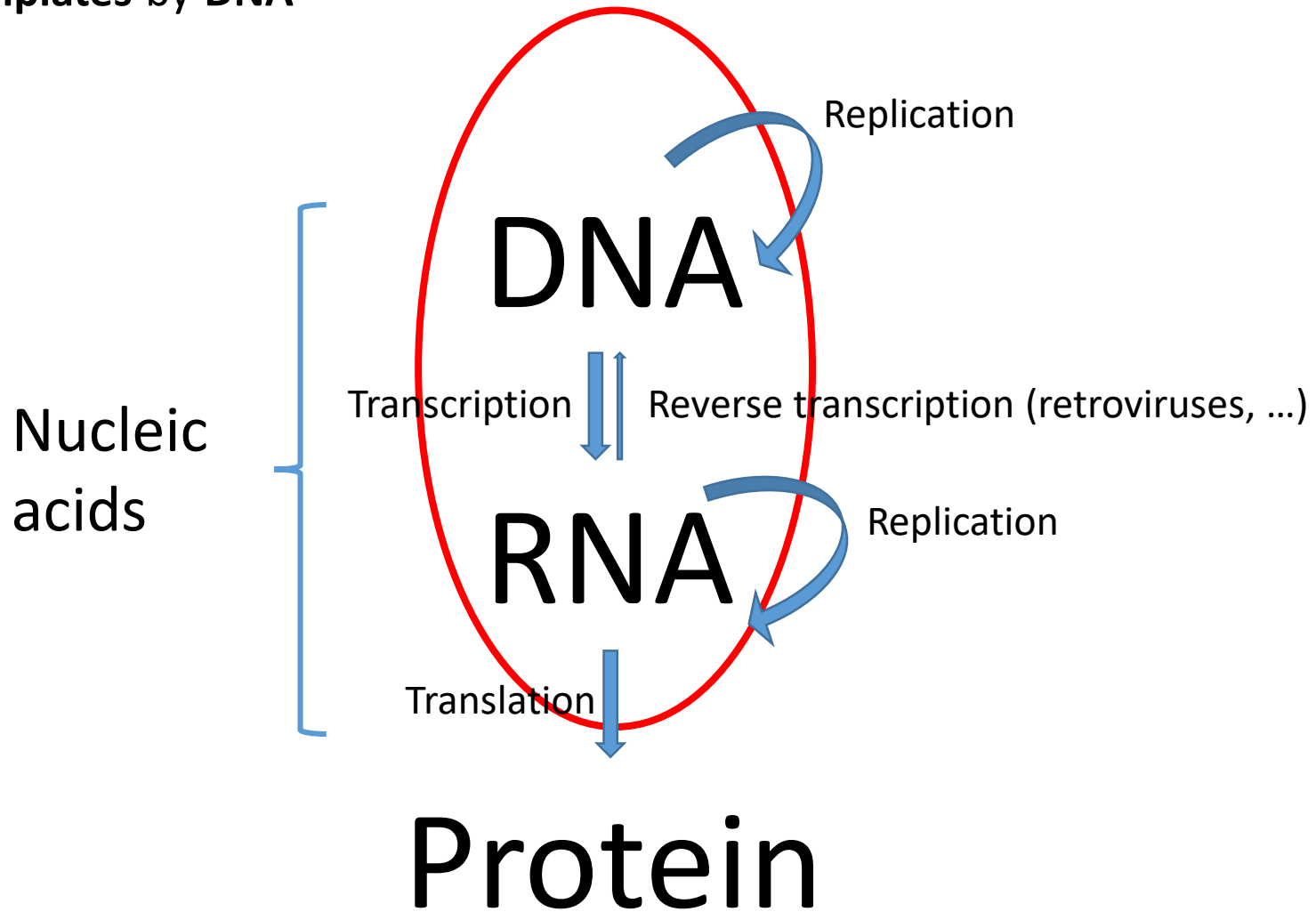
Conjugative plasmid



learning.uonbi.ac.ke

Transcription

- **RNA** synthesis from **rNTP** on **DNA templates** by **DNA-dependent RNA-polymerase**
- Rules of base complementarity
- Essential for life – gene expression



Transcription

Messenger RNA (mRNA)

- Transcription of genetic information in structural genes (then translated to amino acids in proteins)

Precursor ribosomal RNA (pre-rRNA)

- Primary transcript for rRNA, subsequently posttranscriptionally edited to rRNA

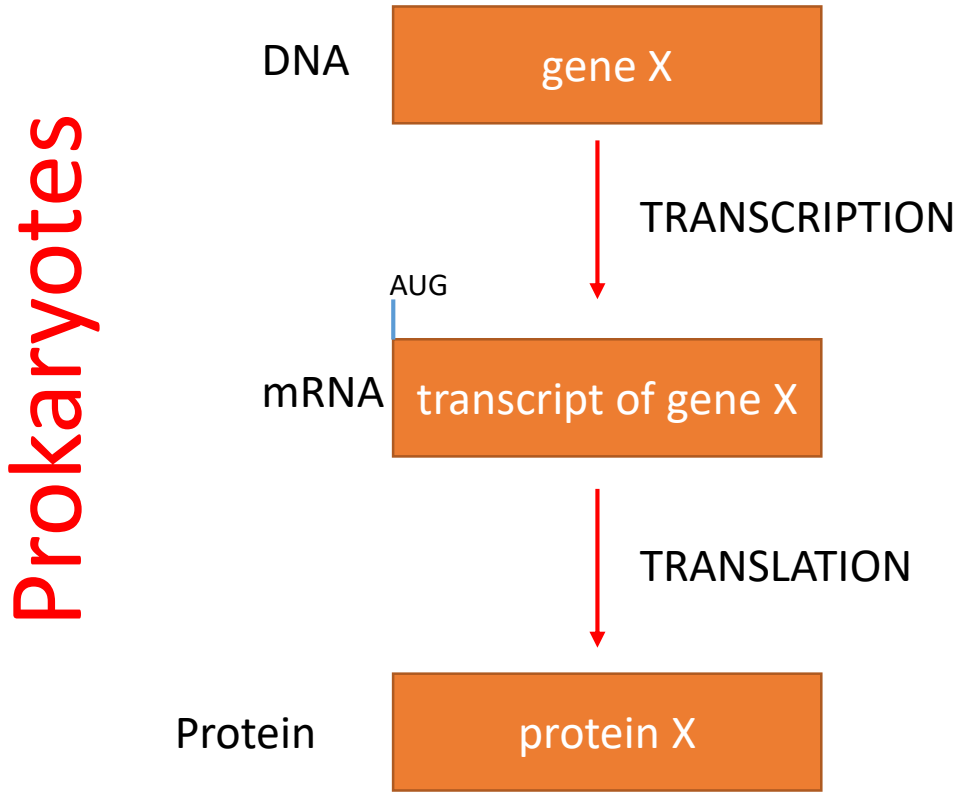
Precursor transfer RNA (pre-tRNA)

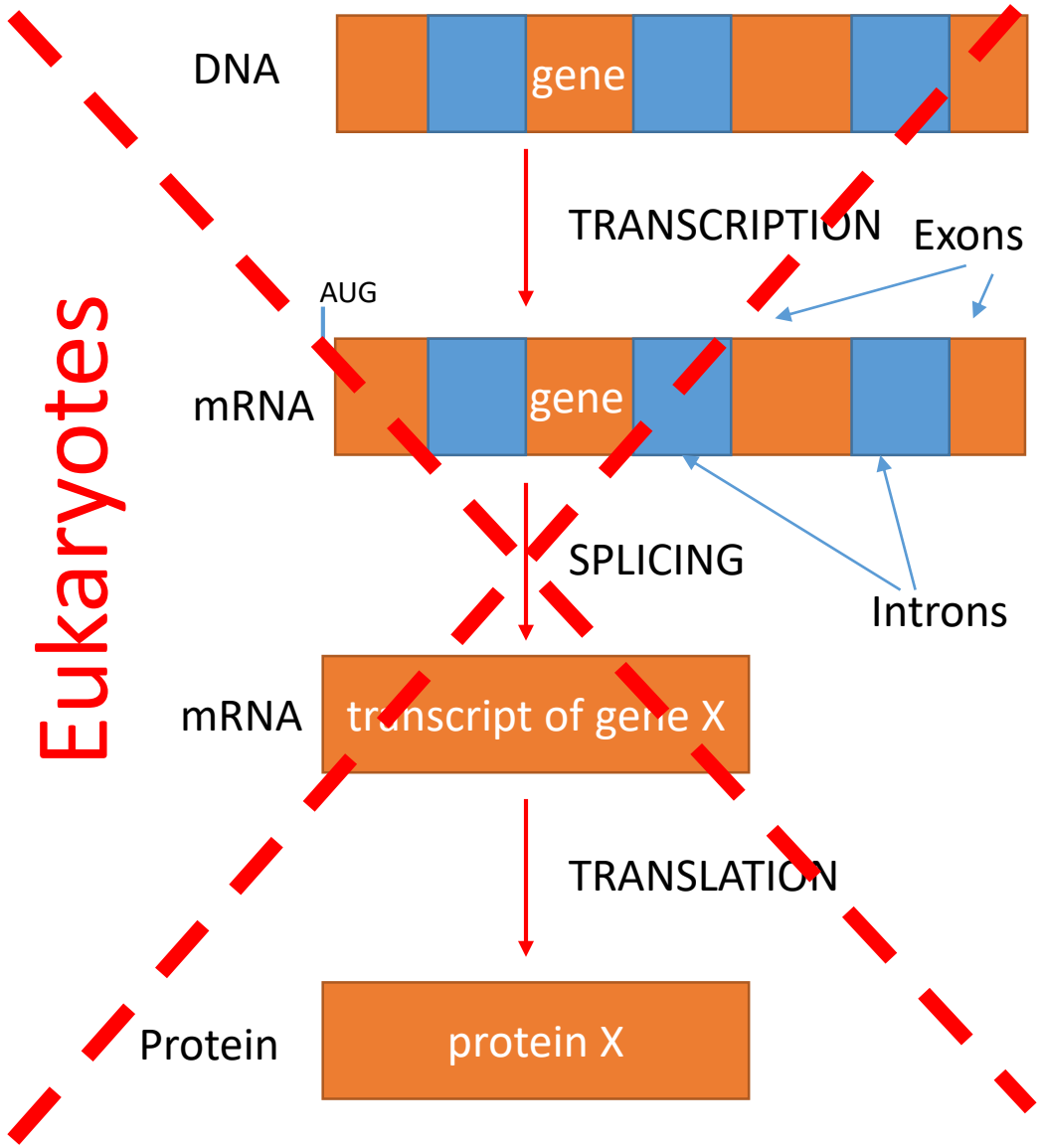
- Primary transcript for tRNA, subsequently posttranscriptionally edited to tRNA

Primary transcripts of regulatory RNAs

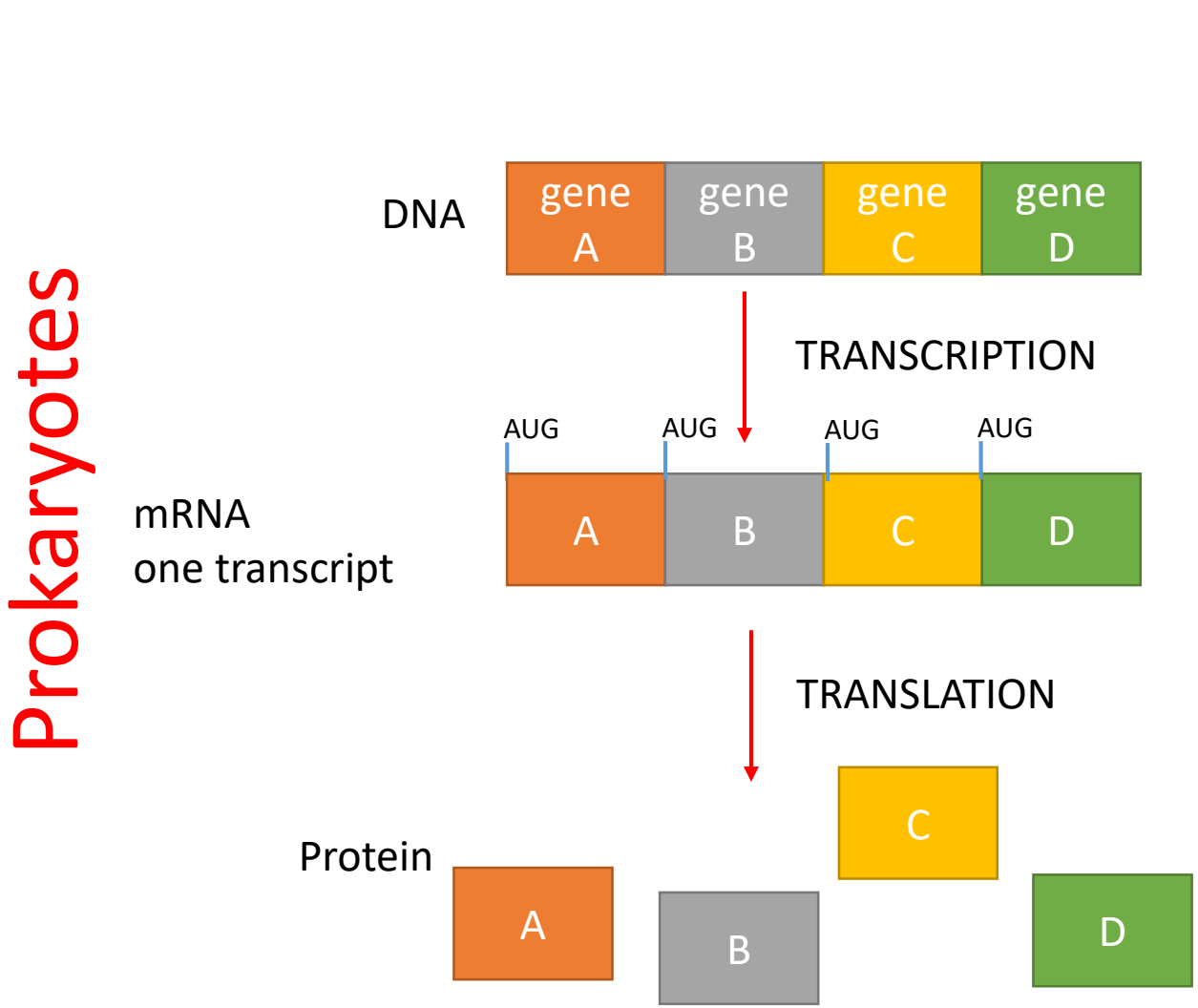
Different types of **transcripts** are synthesized in **transcriptional units**

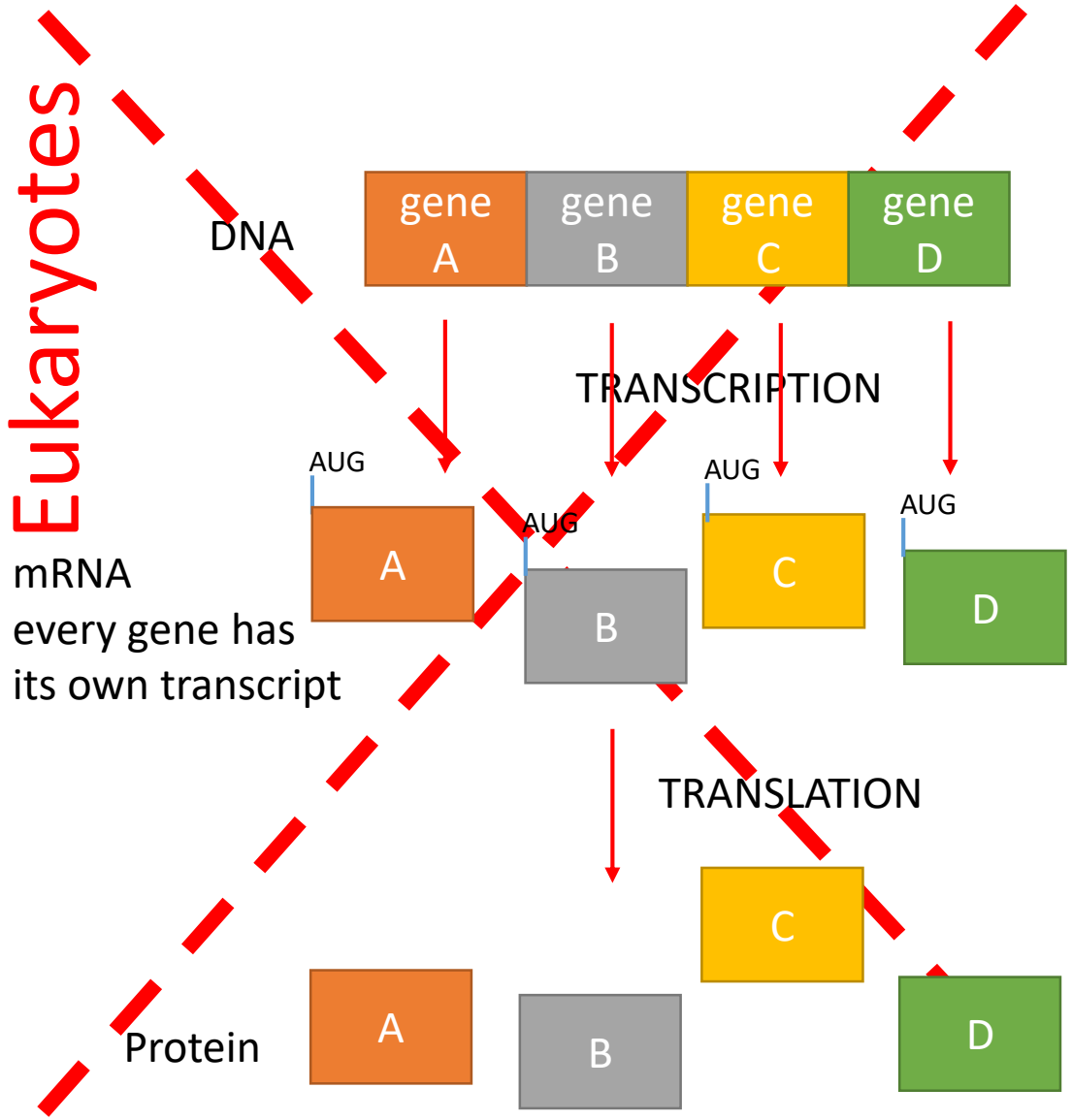
Prokaryotic genes do not contain introns





Prokaryotic genes are polycystronic





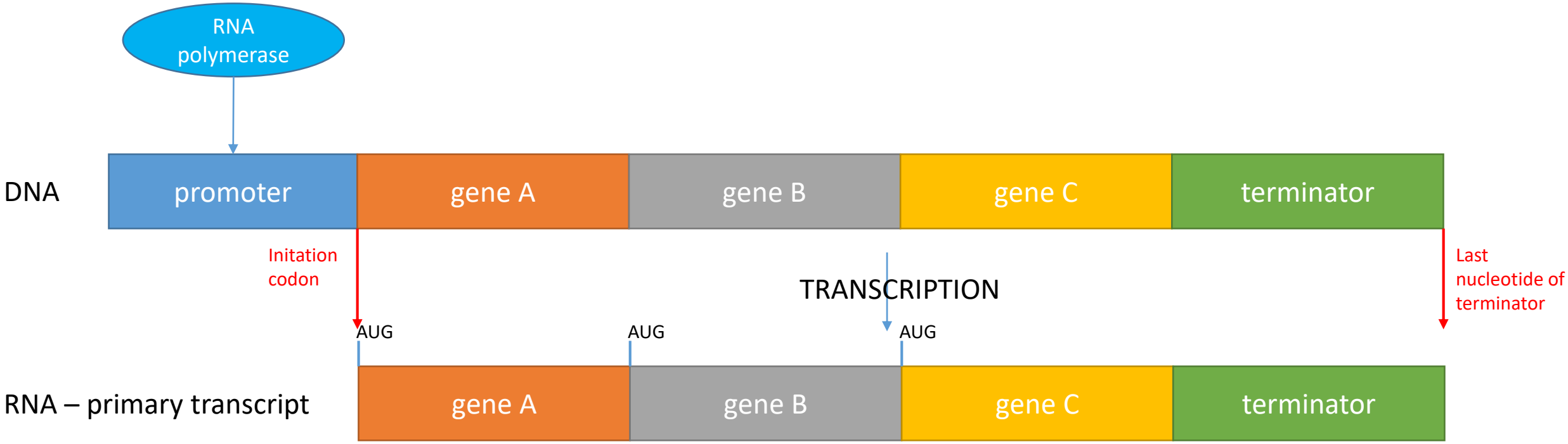
Cistron ~ gene

Transcriptional unit

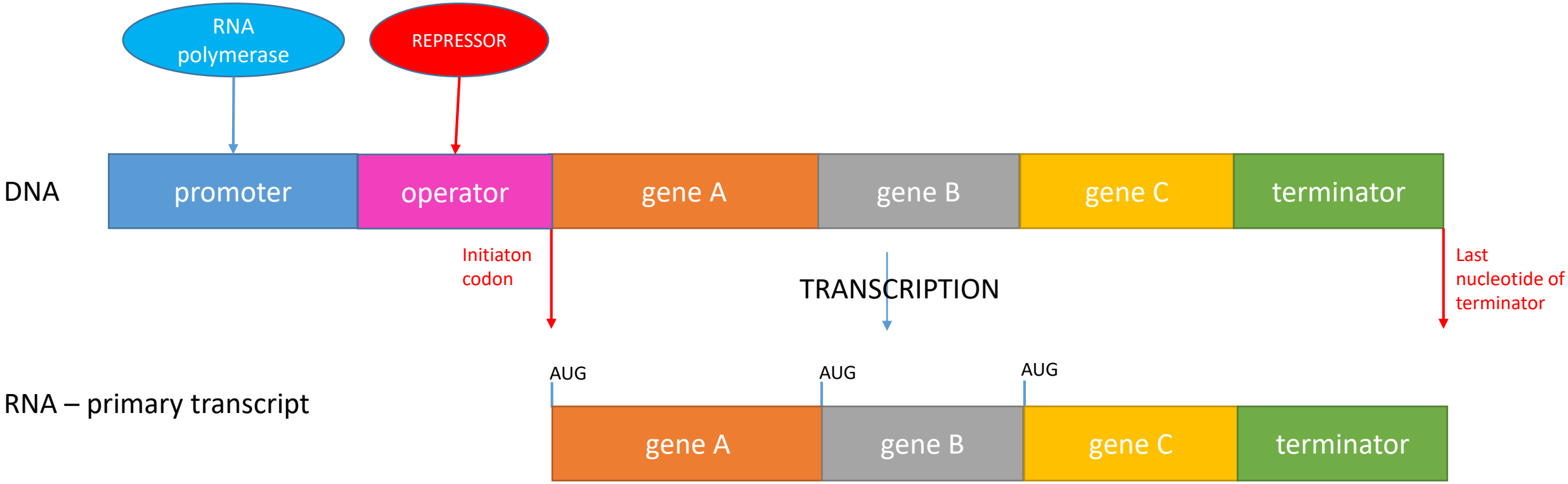
Transcription occurs in forms of transcriptional units – polycystronic RNA

- **Non-operon transcriptional units**
- **Operons**

Non-operon transcriptional unit

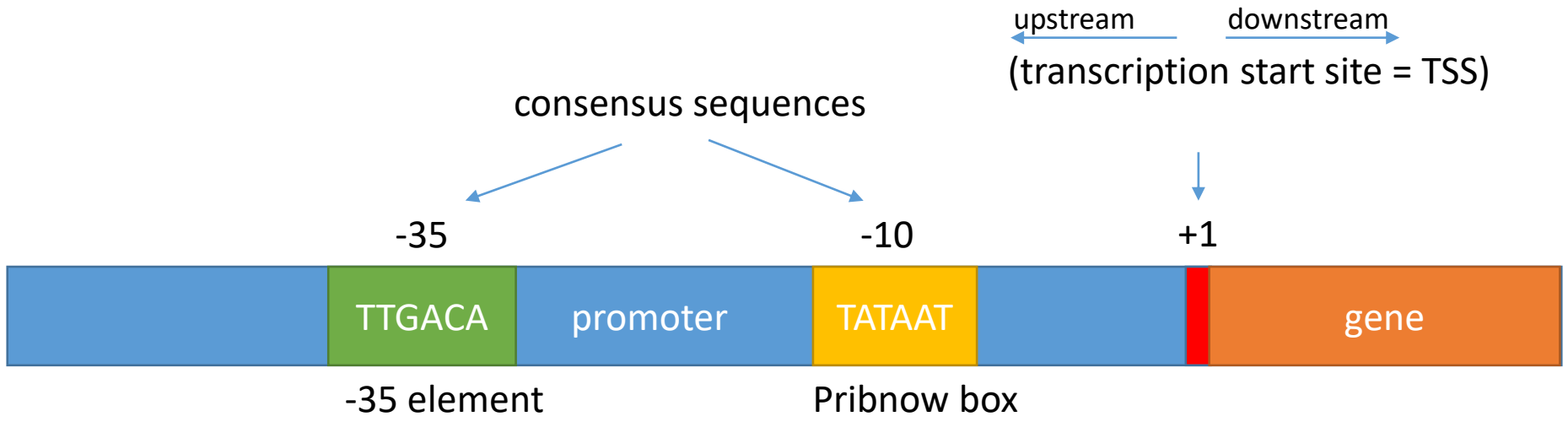


Operon



- PROMOTER CAN OVERLAP WITH OPERATOR

Prokaryotic promoter



- Consensus sequences are „average“ of all promoters
- Promoter similarity ensures affinity to **one** RNA polymerase
- Differences in consensus sequences modify affinity to RNA polymerase = promoter strength = how often will transcription be initiated from the promoter – accurate consensus sequences are rare
    - Strong bacterial promoter = more similar with consensus
    - Weak bacterial promoter = differs more from consensus
- More detailed regulation – substitutions in -35 and -10 elements, distance of -35 and -10 elements, additional elements in some promoters

Prokaryotic RNA polymerase

- **Recognizes promoters of all transcriptional units**
- Consists of 5 types of subunits

2x α

- 40 kDa – maintains the stability of complex

1x β

- 155 kDa – key subunit for rNTP binding to enzyme

1x β‘

- 160 kDa – key subunit for promoter binding

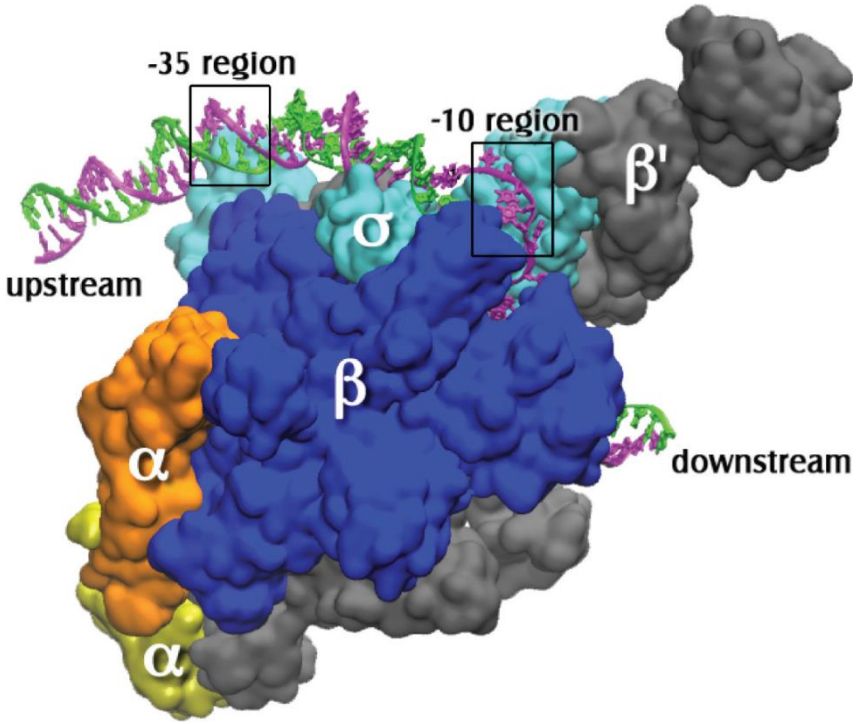
1x ω

- 160 kDa – regulation and stability

1x σ

- 85 kDa – σ-factor (sigma factor) – key subunit for promoter binding - few „exchangeable“ variants, for different types of promoters

- Polymerization speed cca 15-20 nt/s – depends on σ-factor



Transcription progress

Initiation of transcription

Binding of RNA polymerase to promoter – template DNA strand

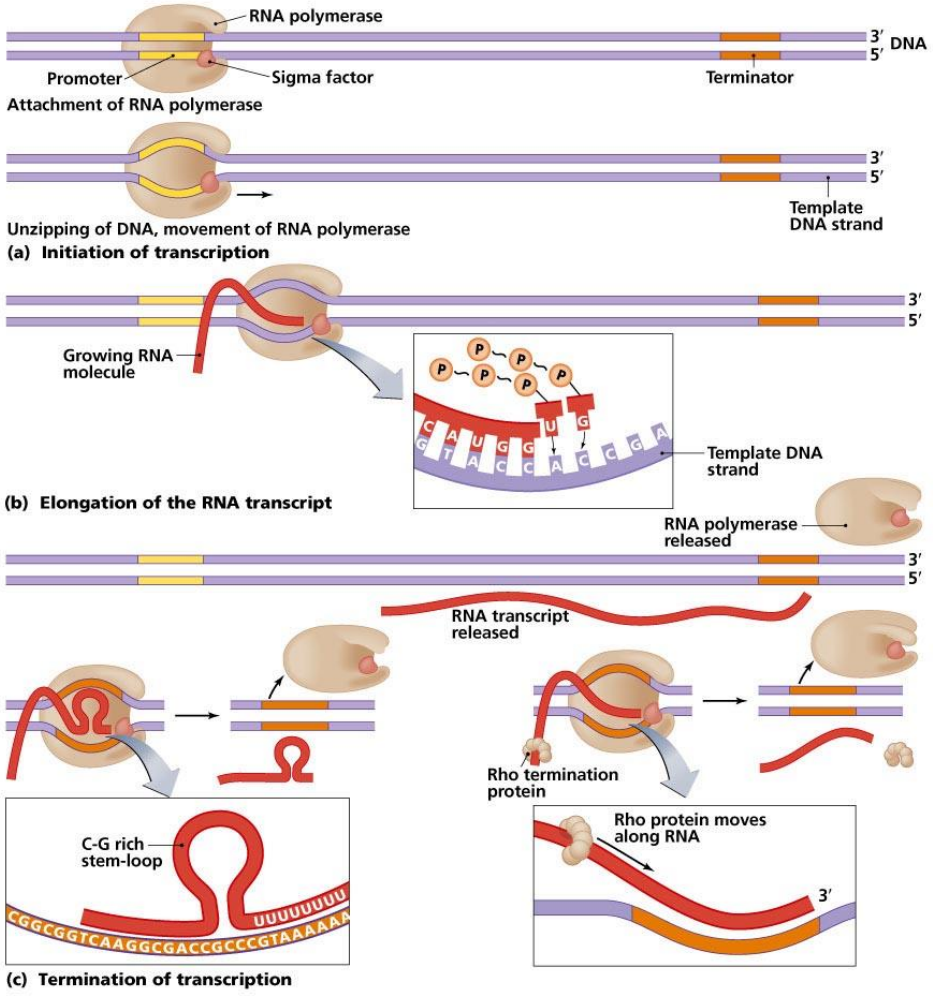
- Binding of first and second NMPs – rules of complementarity

Elongation

- Begins by creating of first phosphodiester bond
- Connection of nucleoside monophosphates to growing RNA strands

Termination of transcription

- RNA polymerase stalling
- Release of RNA
- Release of RNA polymerase from DNA

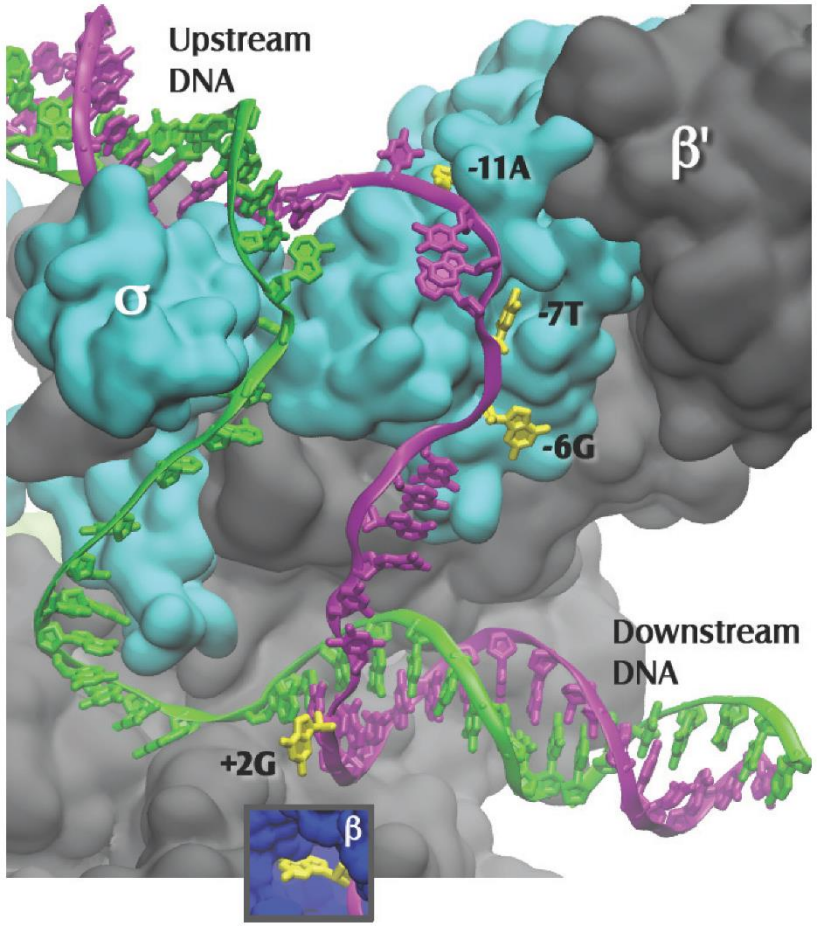


Initiation of transcription

1. **Binding of RNA polymerase on -35 element and Pribnow box (closed transcriptional binary complex)**

2. **Abolishment of hydrogen bonds in Pribnow box (open transcriptional binary complex) – transcriptional bubble**

3. **Transcription of first two nucleotides (open transcriptional ternary complex)**

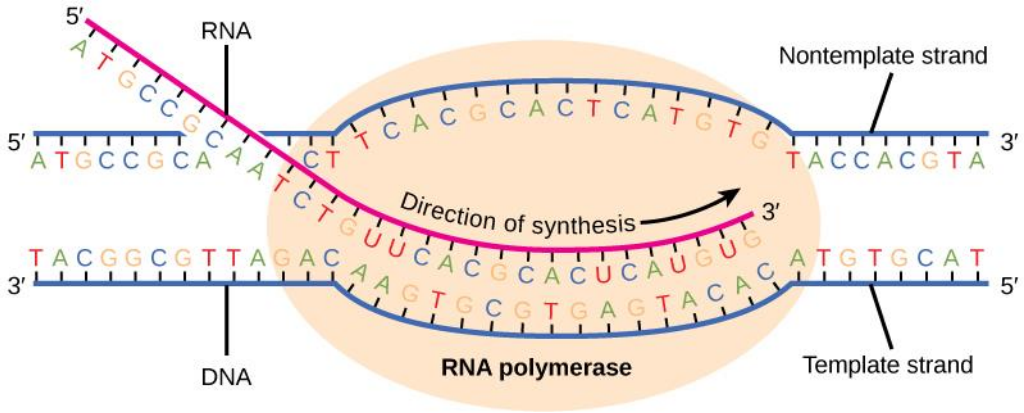


The β subunit removed to reveal the transcription bubble and the flipped bases in their pockets. Template DNA is in green and nontemplate DNA is in magenta, with the flipped-out bases in yellow. Bases −11A and −7T interact solely with the σ subunit. Base −6G is at the σ-β subunit interface. Base +2G interacts solely with the β subunit (insert).

(Karpen a deHaseth, Biomolecules, 2015)

Elongation

1. Formation of the first phosphodiester bond
2. Connecting of nucleoside monophosphates to the 3 'end of the newly synthesized RNA molecule - energy from rNTP cleavage to rNMP + PP
3. The nucleotides are joined according to the rules of complementarity of the bases according to the sequence of the template strand (similar to replication)
4. After the first 10 nucleotides are added, the σ-factor will be released - the stability of the polymerase and matrix DNA complex will increase



Termination of transcription

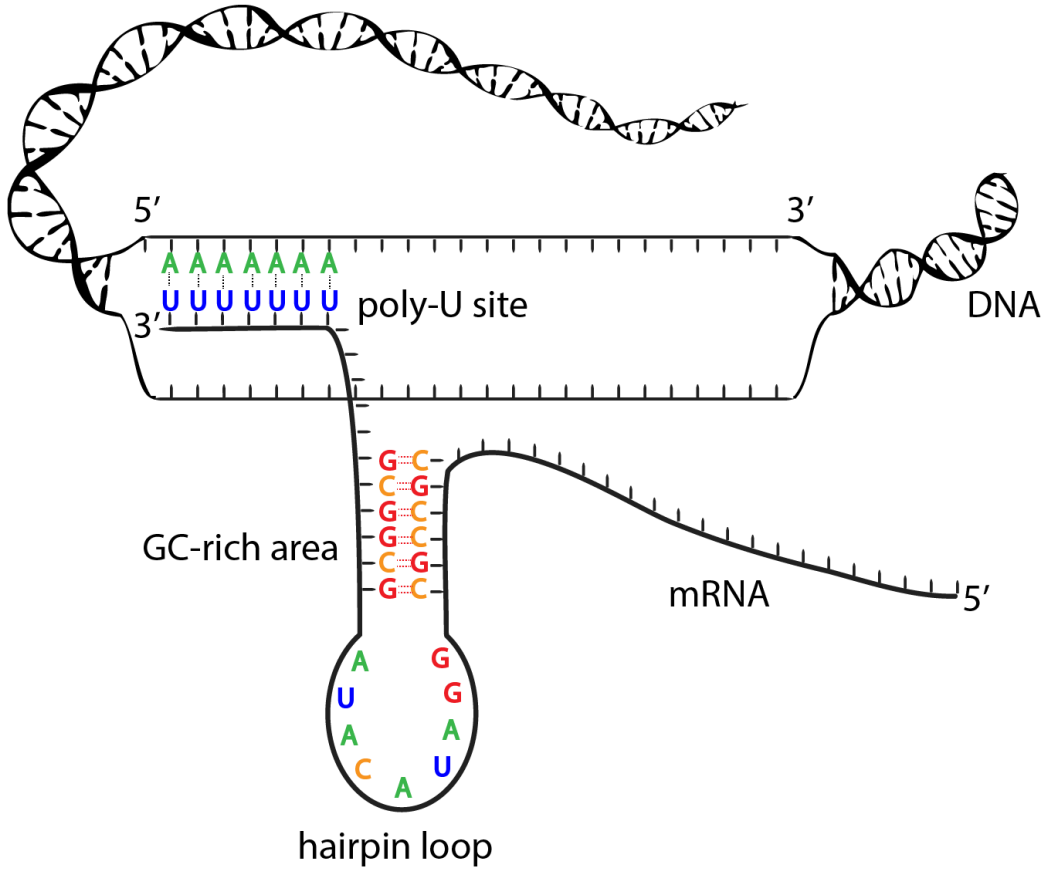
1. RNA polymerase stalling
2. Release of synthesized RNA
3. Release of RNA polymerase from DNA

Termination of transcription occurs in terminator of transcription:

1. Terminator independent of ρ-factor (rho) - terminator type 1
2. Terminator dependent on ρ-factor (rho) – terminator type 2

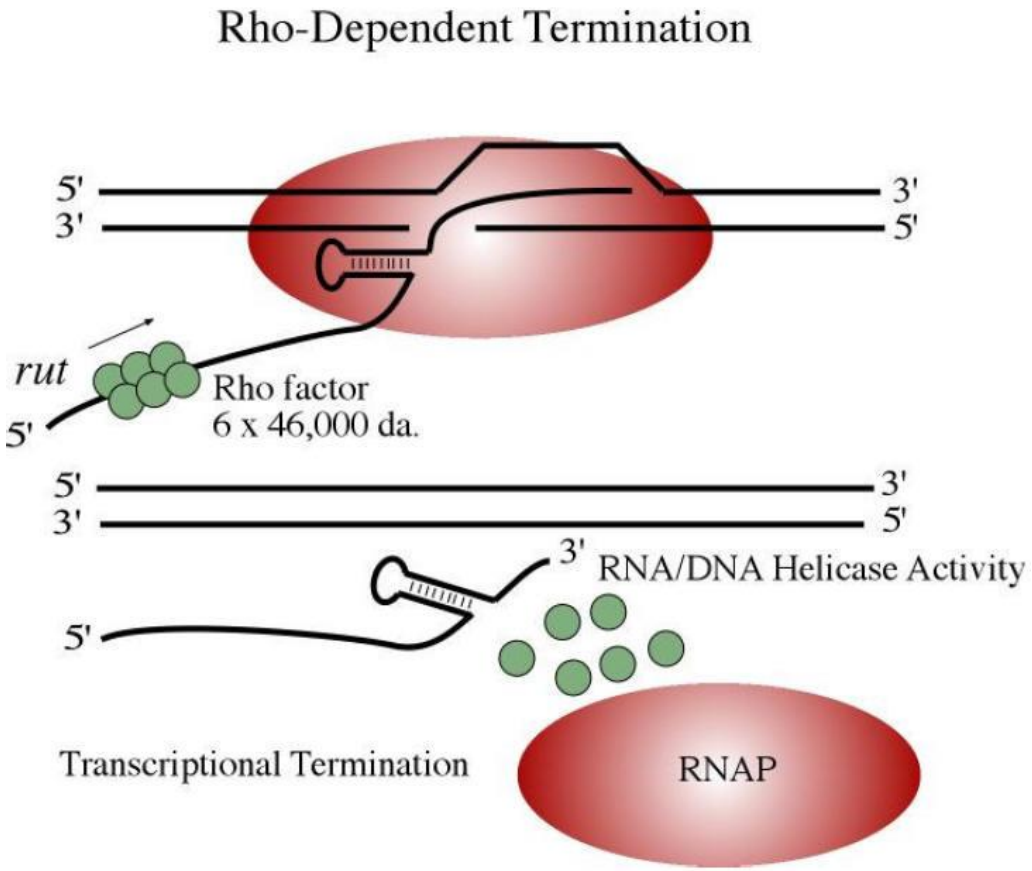
Terminator independent of ρ-factor

- The DNA matrix and therefore the terminator transcript contains palindromic GC-rich followed by poly-U sequences
- After transcription, synthesized RNA results in the formation of a hairpin with a loop that slows the RNA polymerase
- The RNA polymerase synthesizes the poly-U region and drops out of the DNA
- Free RNA polymerase is re-associated with σ-factor



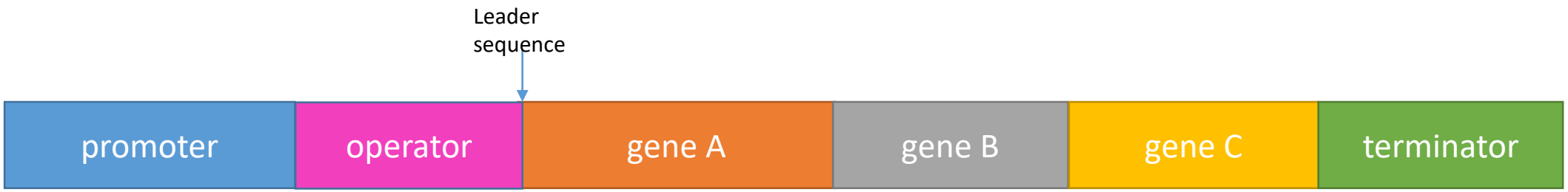
Rho-dependent transcription termination

- Structure similar to a ρ-factor independent termination
- The DNA matrix and therefore the transcription terminator contains palindromic GC-rich region
- After transcription, synthesized RNA results in the formation of a hairpin with a loop that slows the RNA polymerase
- The poly-U sequence is replaced by another - the polymerase has no release signal
- The newly generated RNA is bound by ρ-factor at the *rut* site , which slides towards the end of the RNA and RNA polymerase
- After contact of the ρ-factor with the RNA polymerase, the polymerase is released
- Free RNA polymerase is re-associated with σ-factor



Transcription of structural genes

- The structural genes contain the so-called leader sequence (structural genes only) - before the first structural gene within the transcription unit - behind the promoter / operator
- The leader sequence (Shine-Delgarn sequence) AGGA (GGU) (RNA) sequence - facilitates binding of RNA to ribosome to the 3'-end of 16S rRNA



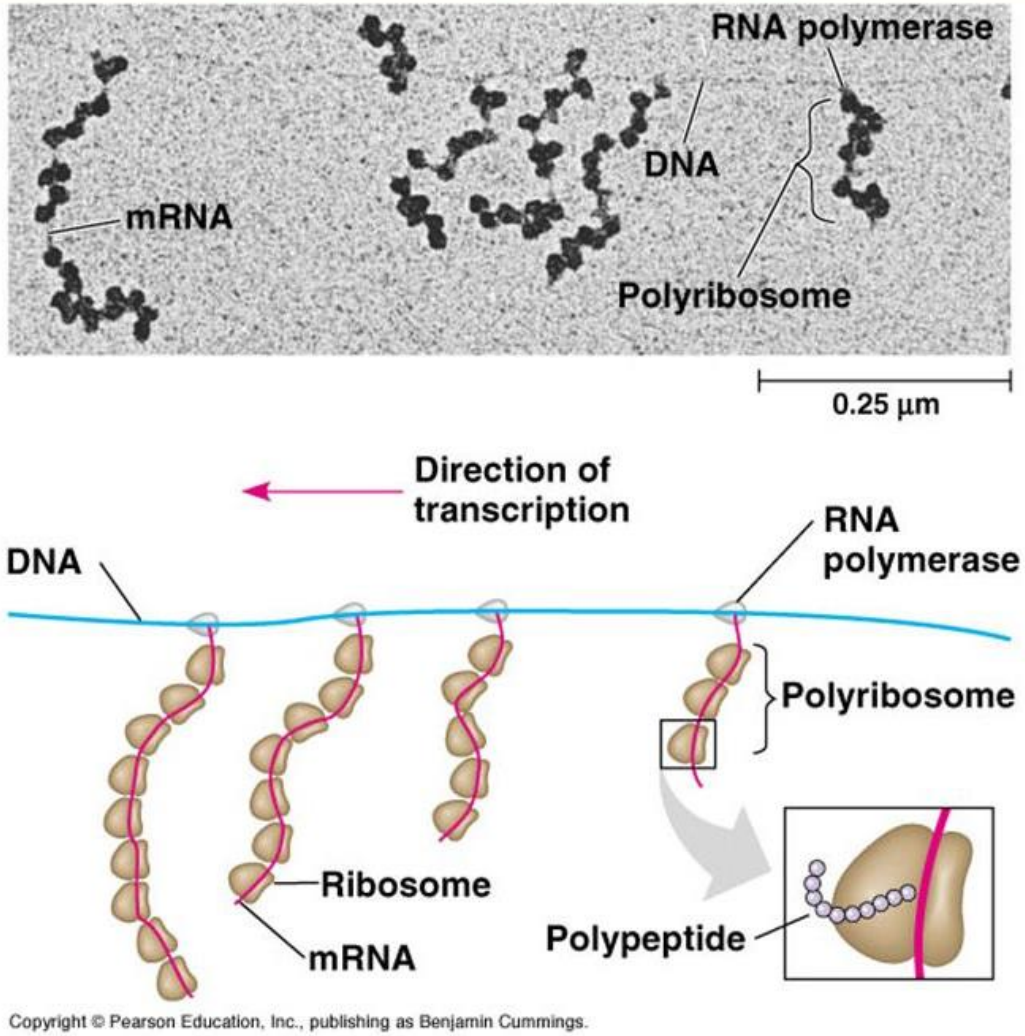
Transcription of mRNA - summary

- The primary transcript of the transcription unit containing the structural genes:
- Contains a leader sequence at the 5 'end
- It contains transcripts of structural genes that are translated into the polypeptide chain
- At the 3'-end UUUUUUUU = end sequence
- Contains transcriptions of several genes = polycistronic (multigenic) mRNA
- Posttranscriptionally not modified - does not contain introns = no splicing
- Life span for several minutes, ribonuclease decomposition in 5'-3'
- At any given time, it accounts for 3% of total RNA in the cell

Coupling of transcription with translation

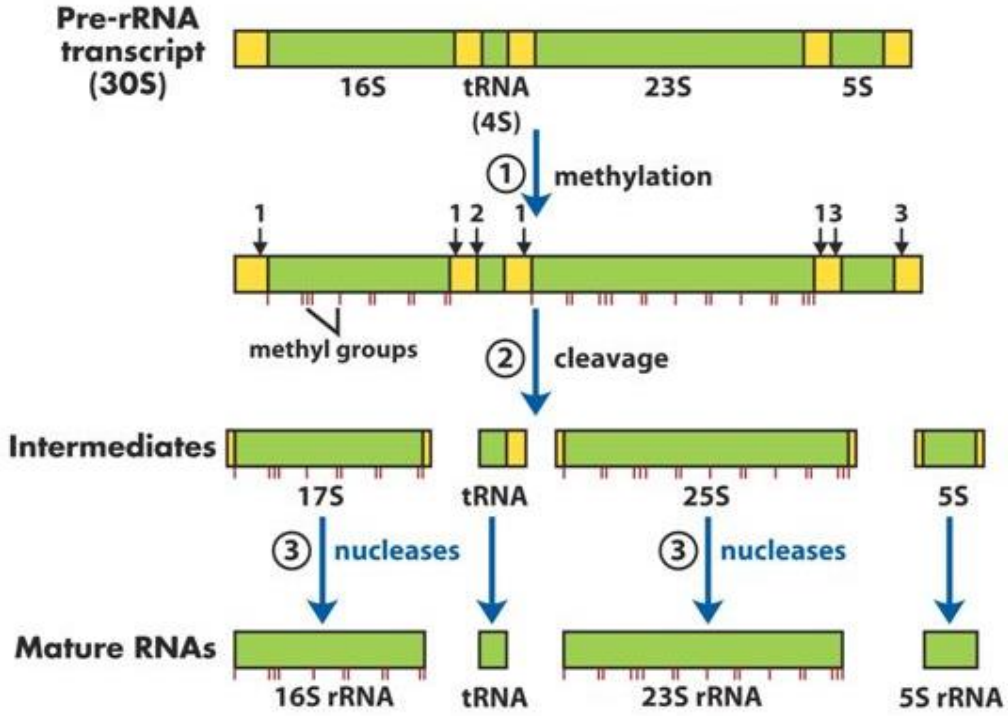
- Coupled synthesis
- Ribosomes are already attached to mRNA during transcription
- On the same mRNA molecule both processes (transcription + translation) are simultaneous + degradation of mRNA from the beginning of transcription
- For some transcription units up to 15 transcription initiations per minute = 15 new mRNA molecules
- For each mRNA up to 30 ribosomes = 30 new polypeptide chains
- Binding and sliding of ribosomes along mRNA increases the synthesis rate of RNA polymerase
- Absence of ribozomes, on the other hand, causes slowing of RNA polymerase, waiting for ribosomes

Figure 17.22 Coupled transcription and translation in bacteria



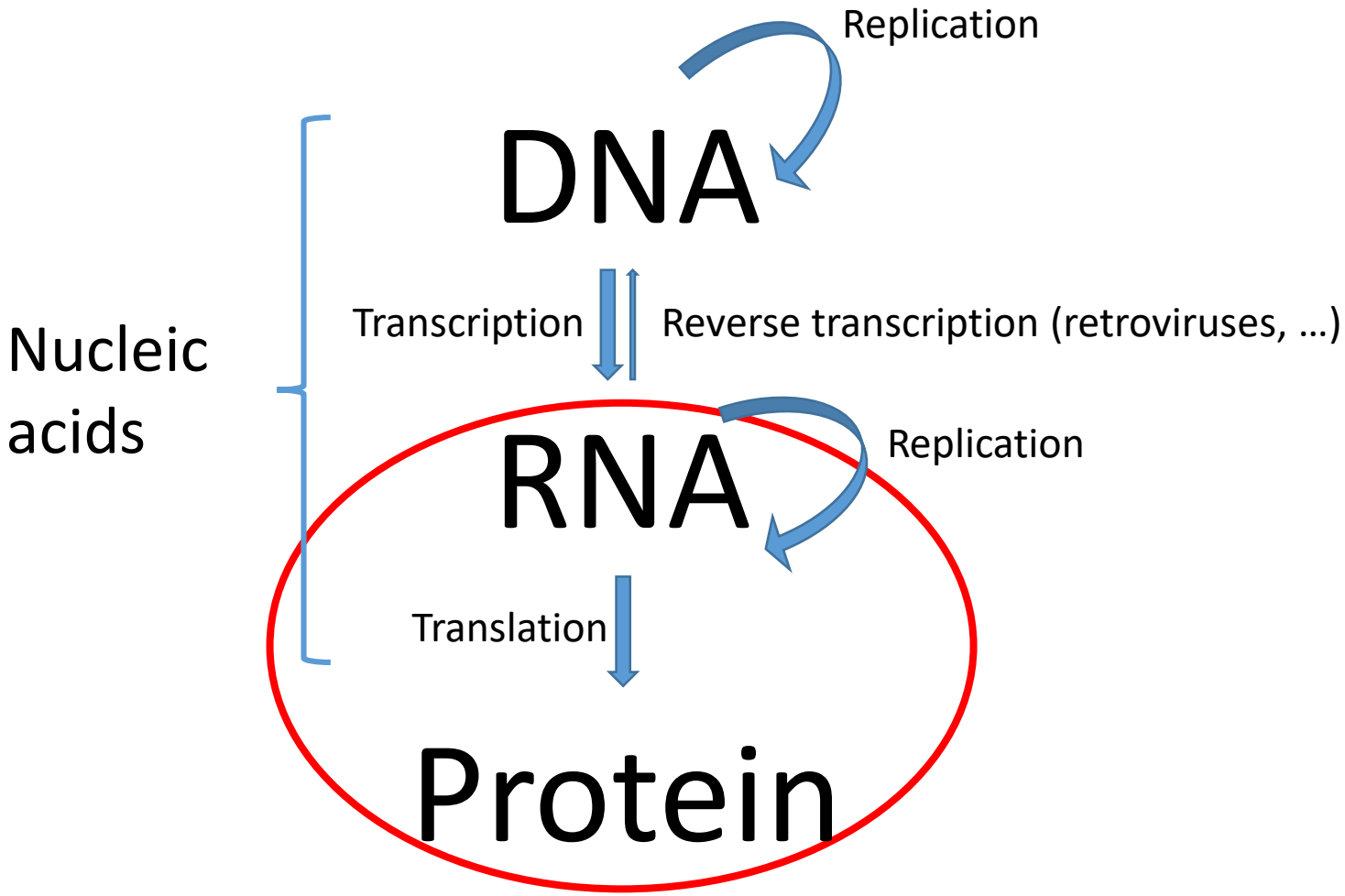
Transcription of functional RNAs

- Transcription proceeds from several rRNAs (7 in E. coli) to form precursor rRNA (pre-rRNA)
- Each transcript contains 16S, 23S and 5S rRNA along with several tRNAs
- Pre-rRNA is posttranscriptionally methylated and cleaved by RNase (RNase III) to the respective rRNA and tRNA



Translation

- Synthesis of the polypeptide chain (protein, amino acid sequence) according to the nucleotide sequence in mRNA



Genetic code

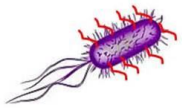
• the genetic code is a **triplet** code - one amino acid in the protein is encoded by a sequence of three nucleotides

mRNA CGUGGUACGAUUGGAUGU

Protein Arg Gly Thr Ile Gly Cys

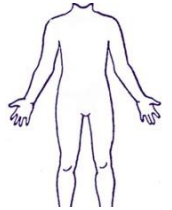
Triplet – Codon x anticodon = complementary sequence on tRNA carrying aminoacid

• the genetic code is **universal** - the meaning of individual triplets is almost always universal and therefore independent of the species of organism



CGU = **Arginine**

CGU = **Arginine**



CGU = **Arginine**

• the genetic code is **degenerate** - one amino acid can be encoded by several different triplets (but not vice versa)

Genetic code

	Second nt				
First nt	U	C	A	G	Third nt
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP/Sel	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	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	Ala	Glu	Gly	G

Translation progress

Activation of aminoacids

- Binding of amino acids to tRNA by aminoacyl-tRNA synthetases

Initiation of translation

- A sequence of actions to create the initiation complex - 70S ribosome, mRNA, initiation tRNA - initiation factors

Elongation

- Polypeptide chain extension - elongation factors

Termination of translation

- Termination of synthesis on a senseless codon, release of the polypeptide from the ribosome - termination factors

Translation conditions in prokaryotes

- mRNA template
- 22 standard aminoacids
- tRNA
- Aminoacyl-tRNA-synthetase
- Ribosomes
- Translation factors
- ATP and GTP

tRNA

- 74 to 95 nucleotides
- MW = 80 000
- 3´- end = CCA
- part of the sequence are unusual bases arising from enzyme modification after transcription (dihydrouridine, pseudouridine)
- names: tRNA: $tRNA^{Ala}$, $tRNA^{Leu}$

  tRNA + AA: Ala ~ $tRNA^{Ala}$, Leu ~ $tRNA^{Leu}$

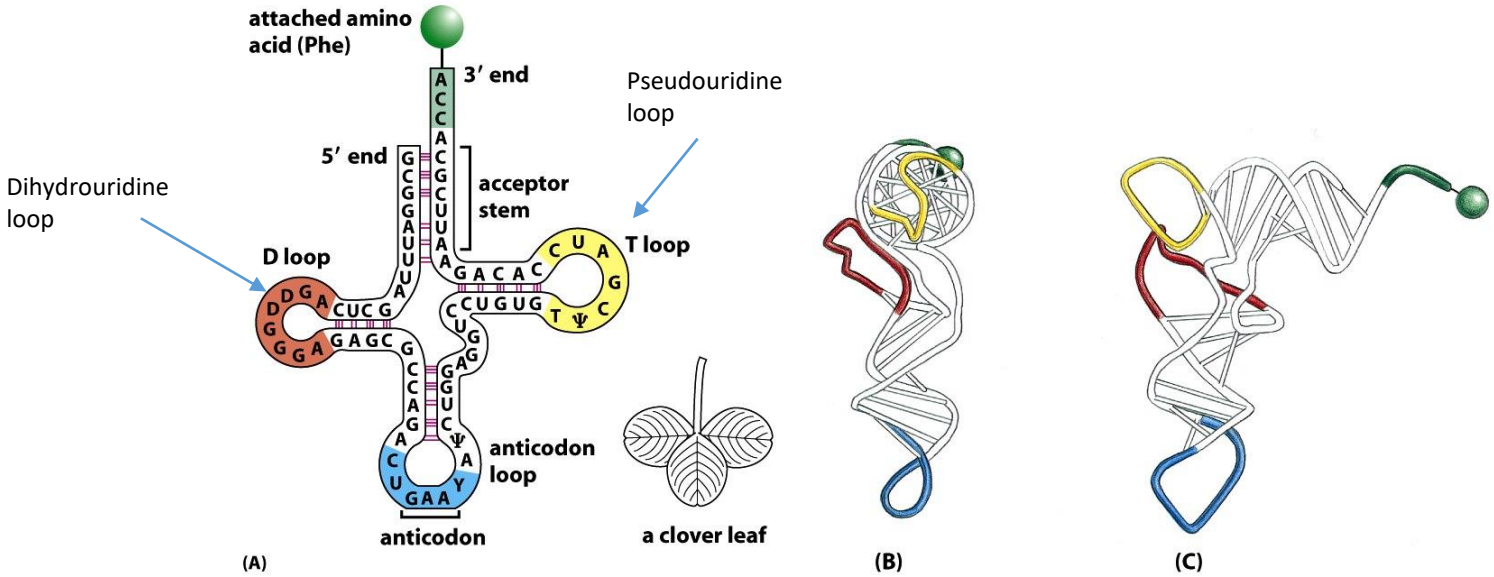


Figure 6-52 Molecular Biology of the Cell 5/e (© Garland Science 2008)

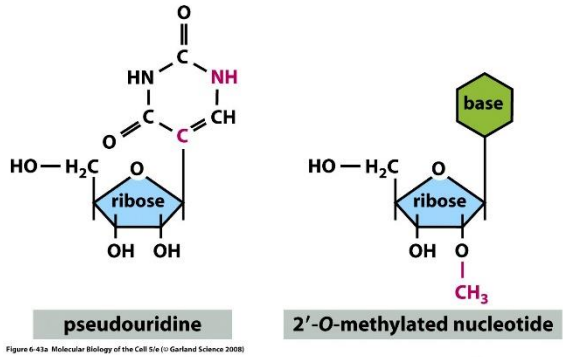


Figure 6-43a Molecular Biology of the Cell 5/e (© Garland Science 2008)

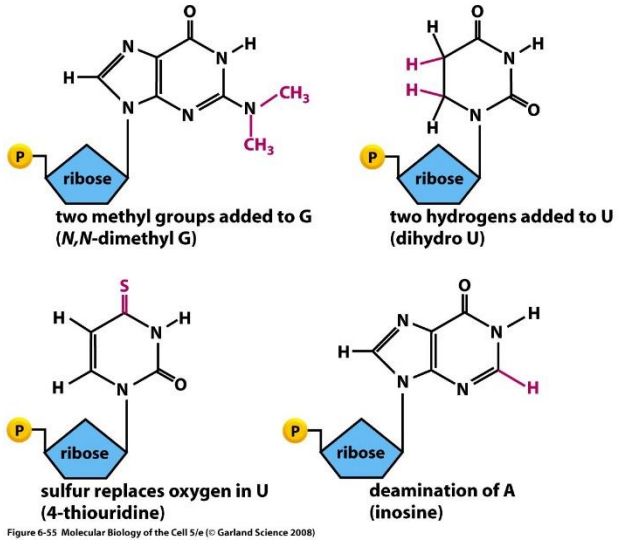


Figure 6-55 Molecular Biology of the Cell 5/e (© Garland Science 2008)

CCA end of tRNA

- this triplet of nucleotides is present at the 3-end of each tRNA
- on the last A the amino acid is bound during activation
- CCA is added by nucleotidyltransferases using ATP and CTP in post-transcriptional modifications of the primary transcript
- nucleotidyltransferases are in two classes:
  - class I – Archae
  - class II - prokaryotes a eukaryotes
- Nucleotidyltransferases recognize immature tRNA (does not have CCA) from mature (CCA-terminated) and catalyze only immature tRNA

Aminoacids

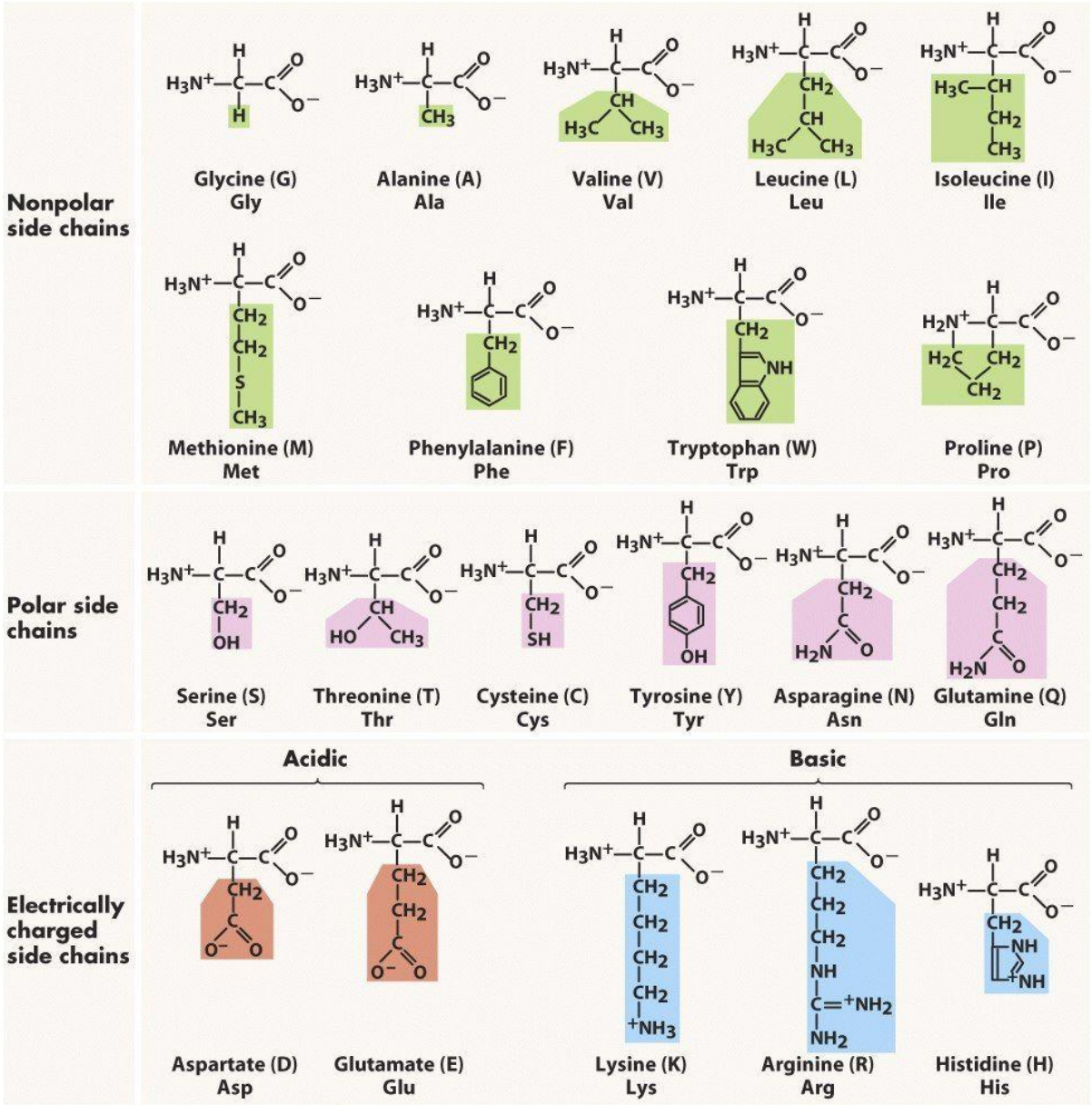
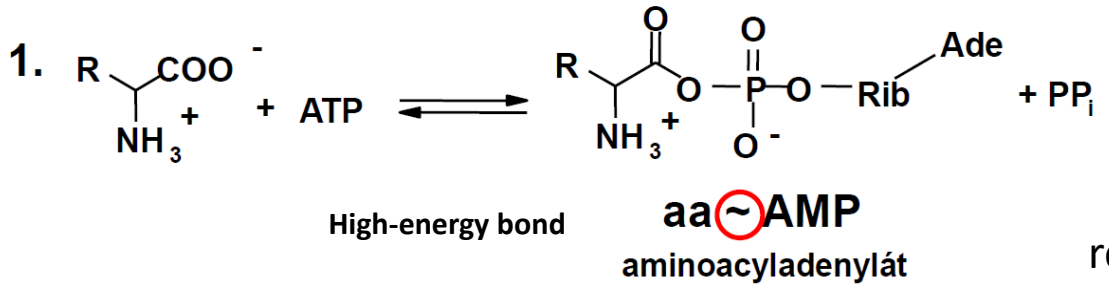


Figure 3-5 Biological Science, 2/e

Aminoacyl-tRNA-synthetases

- Enzymes catalysing the covalent binding of the amino acid to the corresponding tRNA
- M = 40 to 100 kDa
- low homology in the primary structure, only a few conservative sequences
- binding site for amino acid, tRNA and ATP
- tRNA binding site binds related tRNAs
- generally, each amino acid has its aa-tRNA synthetase
- in some bacteria there is one synthetase that binds more amino acids to the corresponding tRNA
- each tRNA is specific for a single amino acid (not vice versa - each amino acid can be linked to more than one tRNA)

Activation of AA - Aminoacyl-tRNA



reaction is catalyzed by aminoacyl-tRNA synthetases

2. aa ~ AMP + tRNA → aa ~ tRNA + AMP

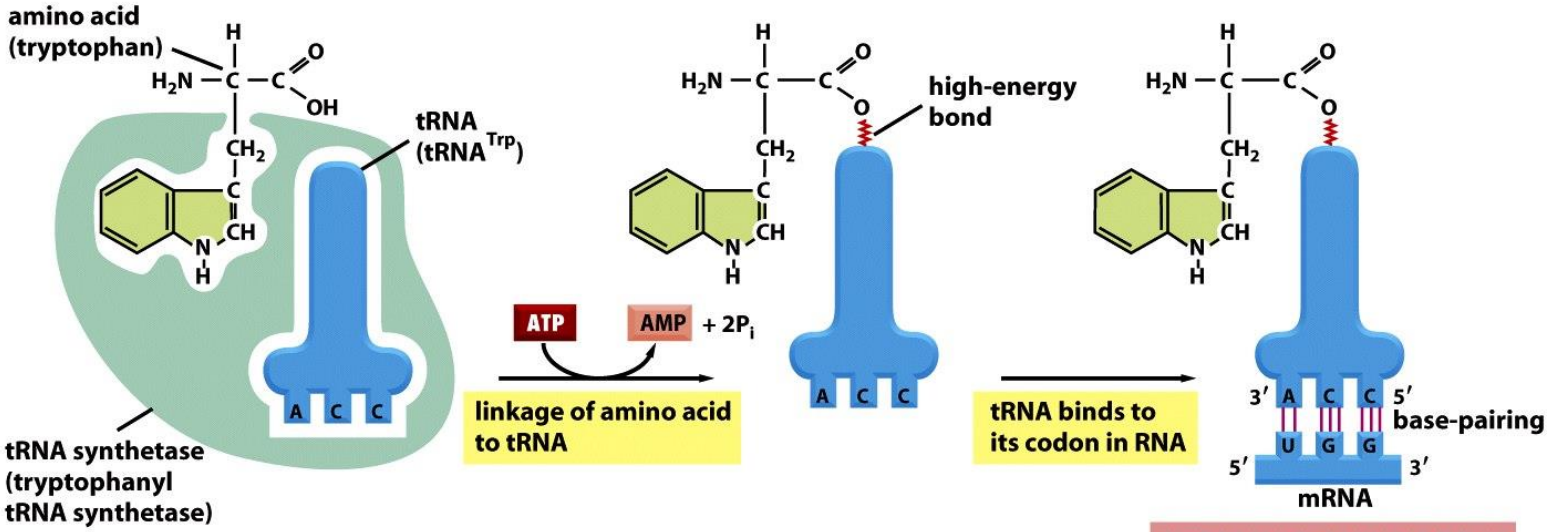
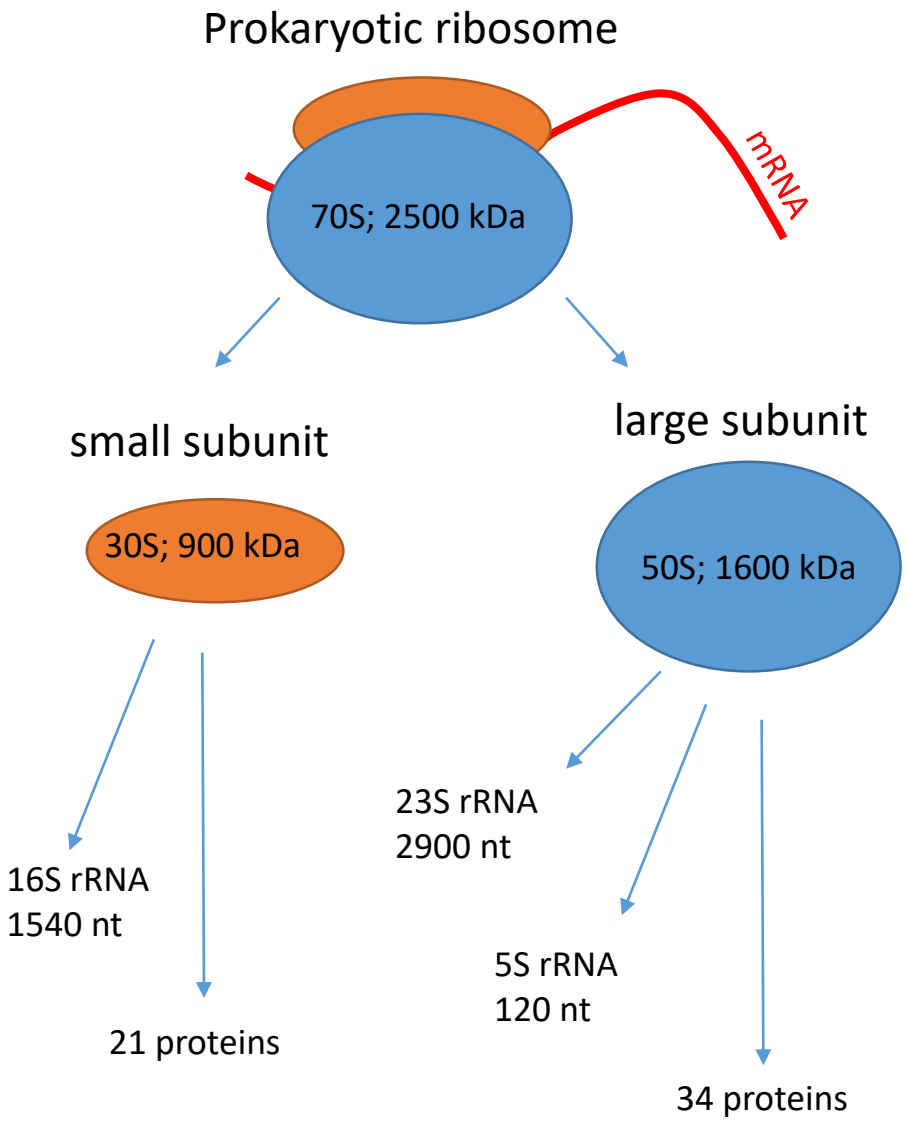


Figure 6-58 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Prokaryotic ribosome



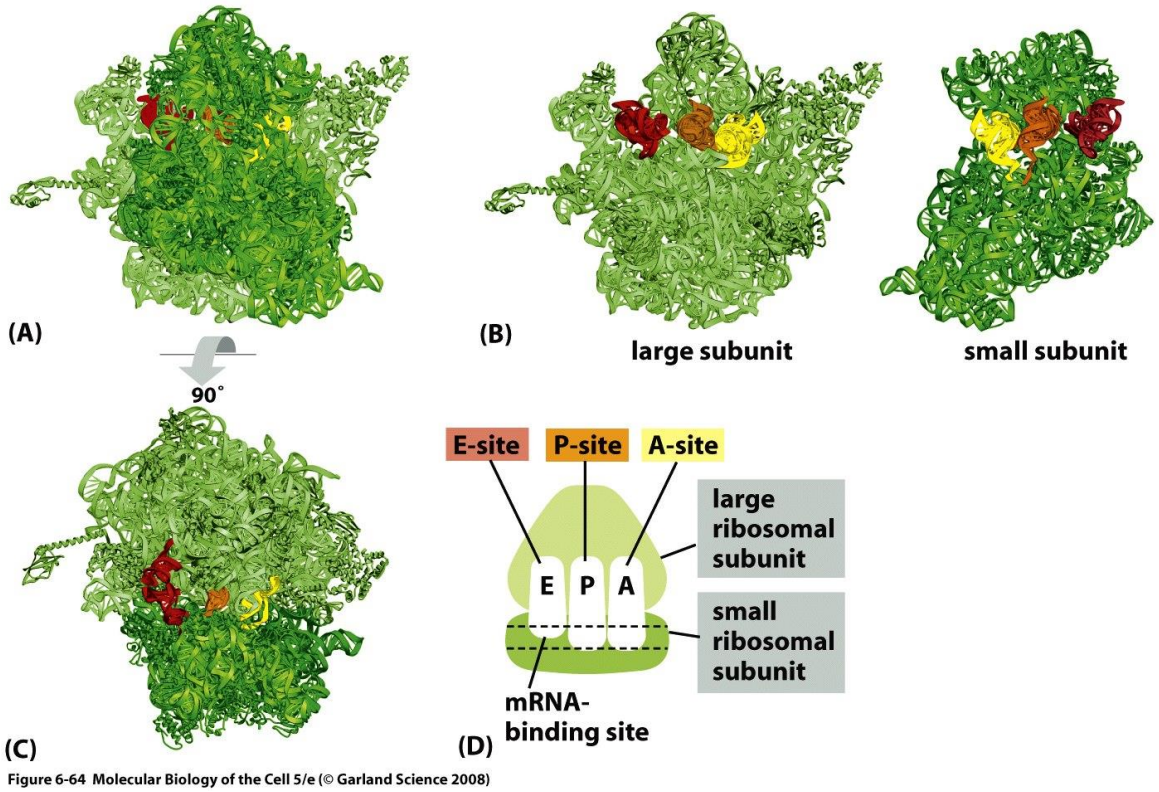


Figure 6-64 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Prokaryotic ribosome

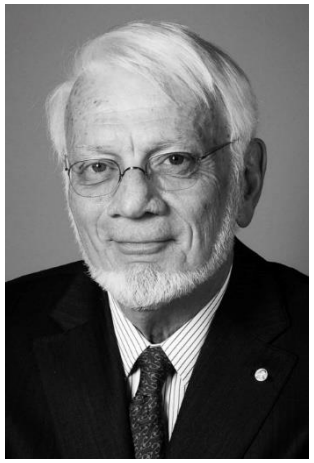
- The ribosomal subunits are composed separately after the synthesis of the individual components
- The subunits associate together with the synthesis of the protein by binding to mRNA near the 5'-end of the mRNA
- The small subunit provides pairing of anticodon (tRNA) - codon (mRNA)
- The large subunit provides the synthesis of peptide bonds
- The key catalytic activities of the ribosome are assured by rRNA - ribozyme
- Bacterial ribosomes synthesize a binding of approximately 20 amino acids per second
- 4 key binding sites:
  - 1x for mRNA
  - 3x for tRNA – A (acceptor / aminoacyl), P (peptidyl) a E (exit) site
- Additional binding sites for translational factors (initiation and elongation)

Nobel Prize in Chemistry

The Nobel Prize in Chemistry 2009 was awarded jointly to Venkatraman Ramakrishnan, Thomas A. Steitz and Ada E. Yonath "for studies of the structure and function of the ribosome."

Venkatraman Ramakrishnan

Affiliation at the time of the award: MRC Laboratory of Molecular Biology, Cambridge, United Kingdom



Thomas A. Steitz

Affiliation at the time of the award: Yale University, New Haven, CT, USA , Howard Hughes Medical Institute

Ada E. Yonath

Affiliation at the time of the award: Weizmann Institute of Science, Rehovot, Israel

<https://www.nobelprize.org/prizes/chemistry/2009/summary/>

Prokaryotic translation

Initiation of translation

- Creation of a ternary complex : fMet (*N*-Formylmethionine) ~ $tRNA^{fMet}$ + GTP + IF2
- Identification of the Shine-Delgarno mRNA sequence associated with the IF3, the 30S ribosomal subunit - the exact localization of the AUG codon mRNA into the ribosome P site by the complementarity of the Shine-Delgarno sequence and the 16S rRNA region
- Binding of the ternary complex via tRNA-fMet to the ribosome P-site - IF1-containing formation of the pre-initiation complex
- Cleavage of GTP to GDP + Pi, releasing IF1-3, and initiation complex formation with the 50S subunit where fMet ~ tRNA-fMet is at the ribosome P site

Elongation

- Repeated binding of tRNAaa to the ribosome A site with EF-Tu and GTP, creation of a peptide bond between aa at the P and A site, ribosome translocation (content of A to P, P to E) with EF-G and GTP

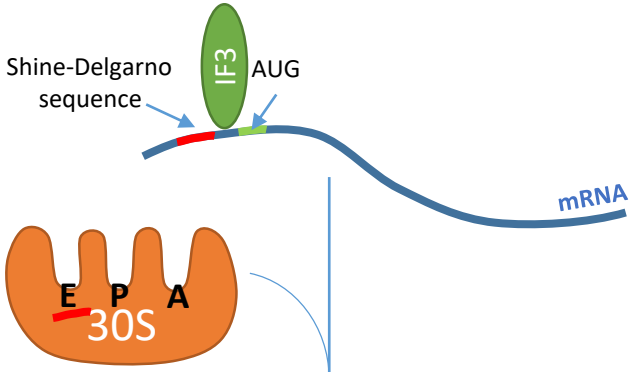
Termination of translation

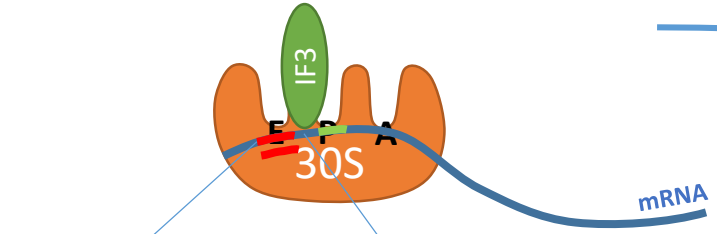
- Recognizing a senseless codon at the A site by RF factor
- Cleavage of the synthesized peptide
- Release of tRNA, mRNA and dissociation of ribosomal subunits

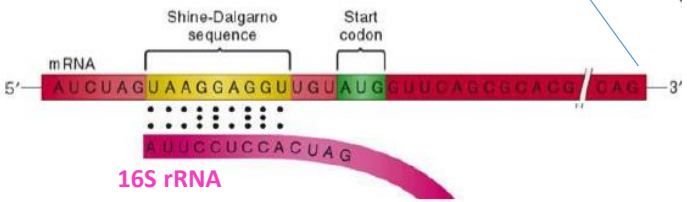
https://www.youtube.com/watch?v=KZBljAM6B1s

Prokaryotic translation - initiation

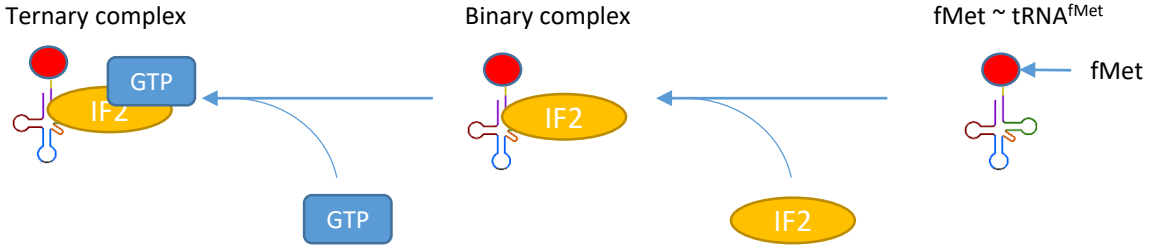
Identification of the Shine-Delgarno mRNA sequence associated with the IF3, the 30S ribosomal subunit - the exact localization of the AUG codon mRNA into the ribosome P site by the complementarity of the Shine-Delgarno sequence and the 16S rRNA region

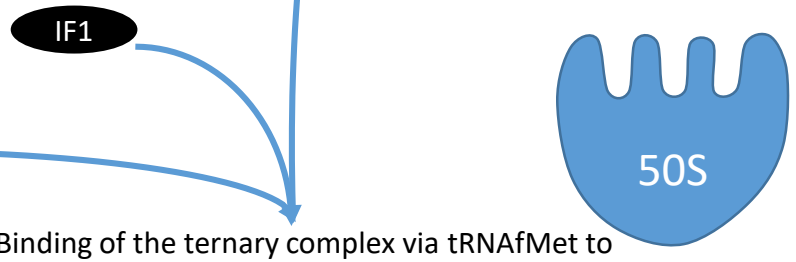




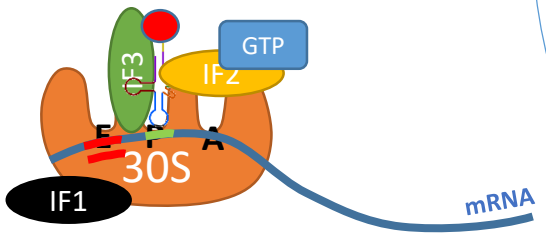


Creation of a ternary complex: fMet ~ $tRNA^{fMet}$ + GTP + IF2



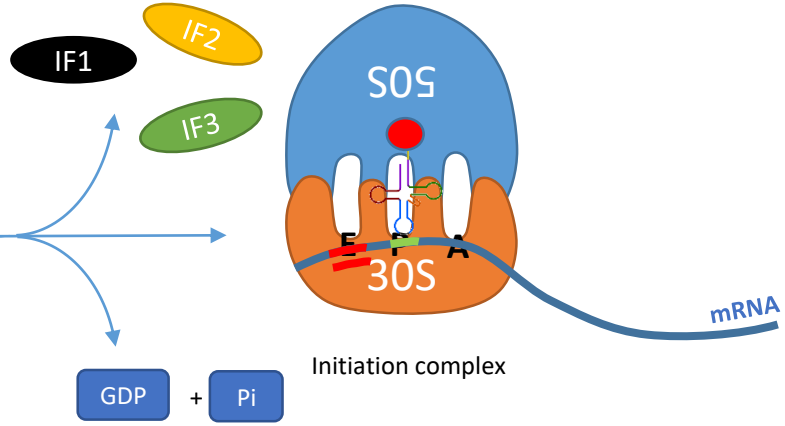


Binding of the ternary complex via tRNAfMet to the ribosome P-site - IF1-containing formation of the pre-initiation complex



Preinitiation complex

Clearing GTP to GDP + Pi, releasing IF1-3, and initiating complex formation with the 50S subunit where fMet ~ tRNAfMet is at the ribosome P site

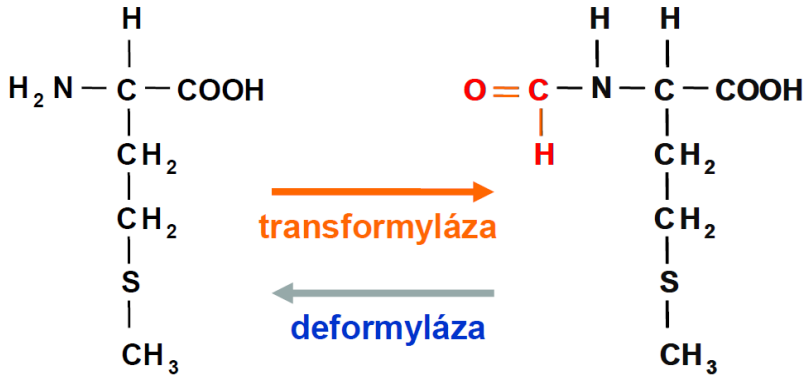


Initiation complex

AUG codon and formylmethionin

AUG codon

- It encodes the first amino acid in the peptide
- It also occurs within the mRNA chain
- AUG encodes for methionine, but at the beginning of the polypeptide is formylmethionine
- There are 2 different methionine tRNAs – $tRNA^{Met}$ a $tRNA^{fMet}$



- At the **beginning** of mRNA **Met ~ $tRNA^{fMet}$** binds AUG codon which is formylated to **fMet ~ $tRNA^{fMet}$** by **IF2**
- **Inside** mRNA **Met ~ $tRNA^{Met}$** binds AUG codon via **EF-Tu**

Elongation of polypeptide chain

1. aminoacyl-tRNA binds to the A site and the tRNA used is released from the E site
2. A new peptide bond is created between the amino acids at the E and at the A site
3. The large subunit of the ribosome moves and the so-called hybrid sites P / A and E / P are created
4. The small subunit moves one codon – opens the A site, tRNAs are shifted from P to E and from A to P

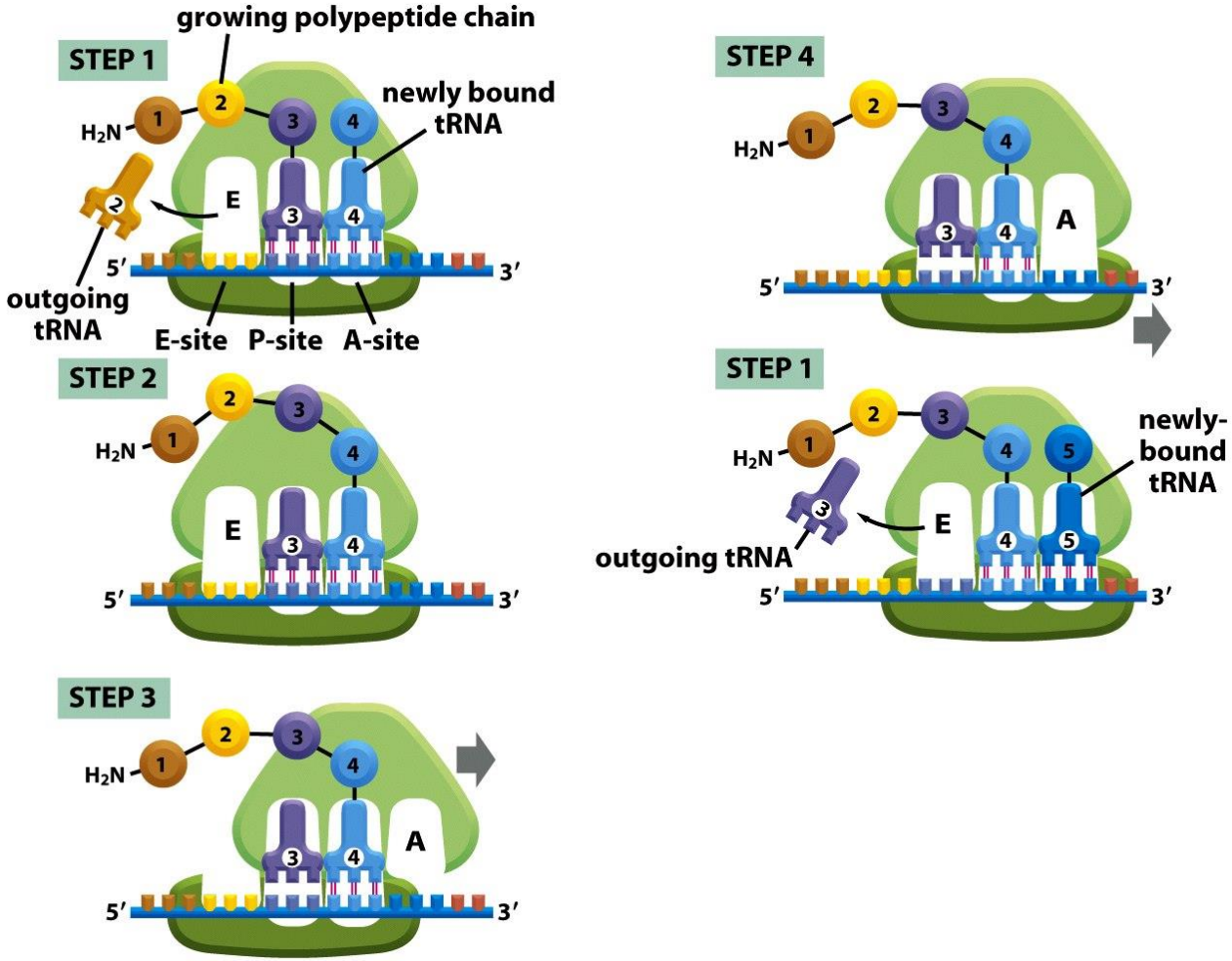


Figure 6-66 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Elongation of polypeptide chain

- Binding of aminoacyl-tRNA to site A is mediated by the elongation factor ET-Tu, which is "driven" by hydrolysis of GTP to GDP (hydrolysis catalyses conformational protein change)
- EF-Tu increases the speed and accuracy of translation - proofreading mechanisms
- EF-G elongation factor catalyses large ribosome subunits movement - energy from GTP hydrolysis

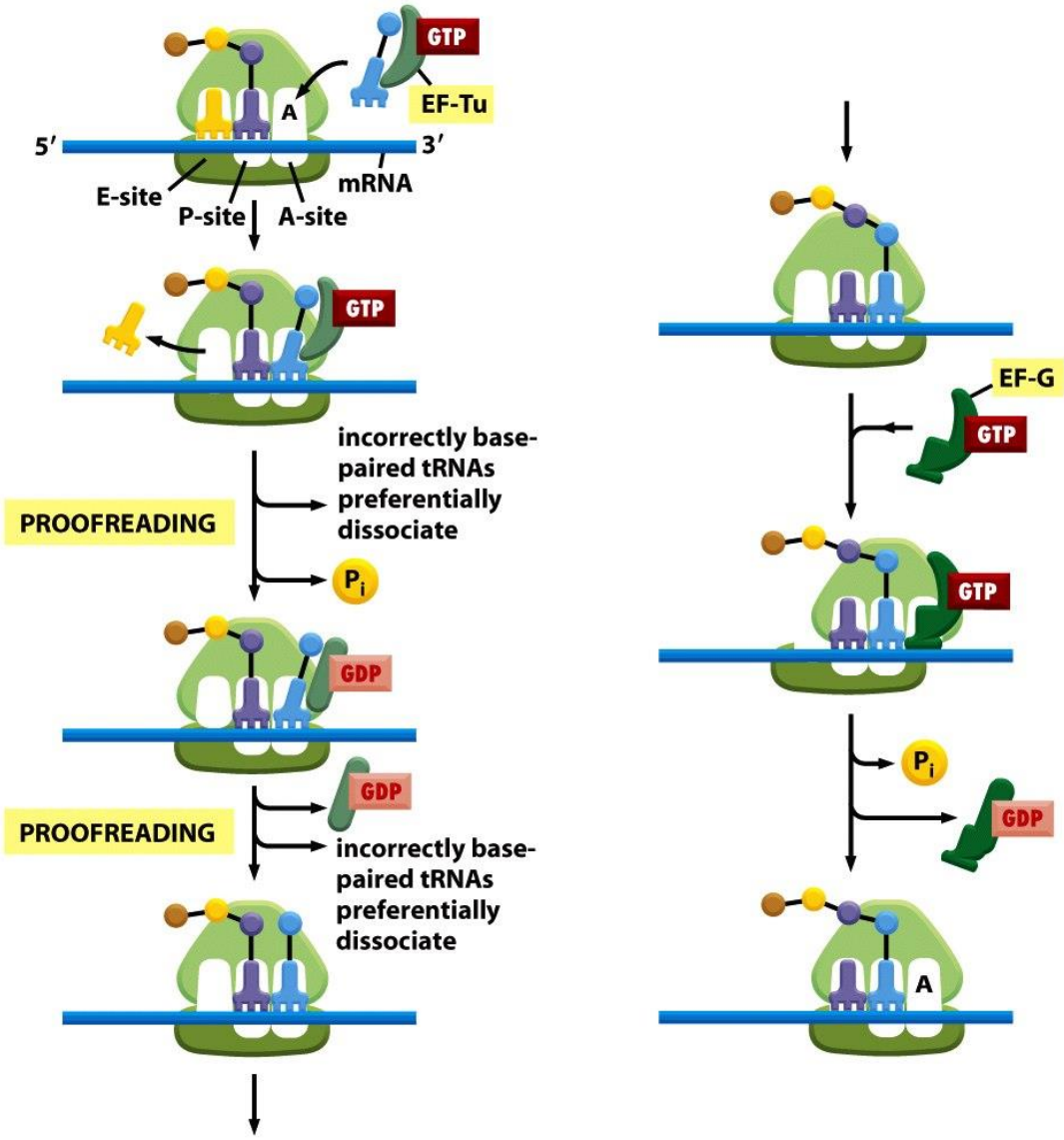
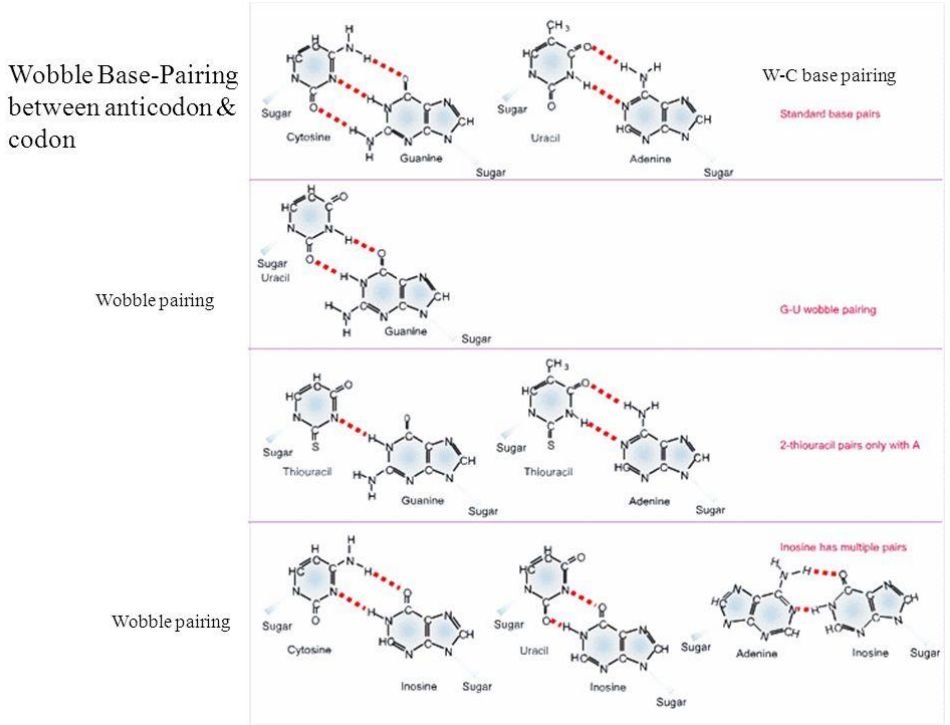


Figure 6-67 Molecular Biology of the Cell 5/e (© Garland Science 2008)

Wobble base pairing

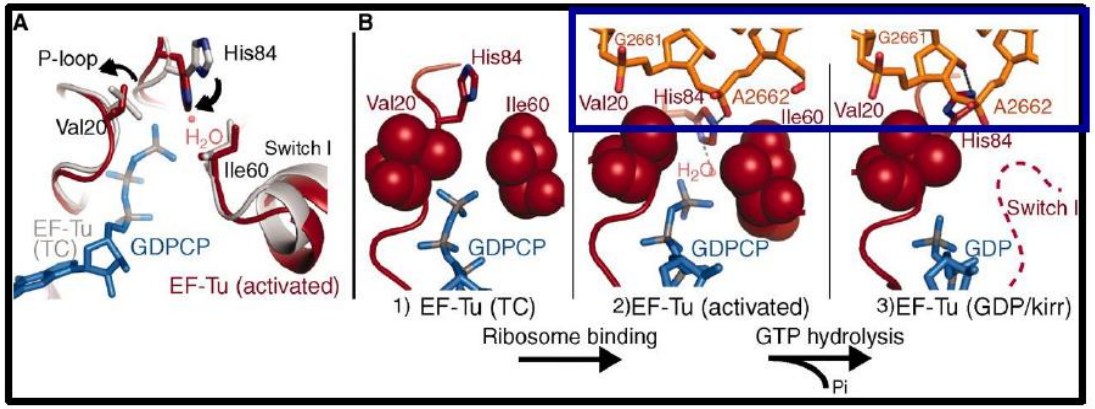
- 22 AA – 64 codons – 40 tRNA
- Some tRNAs bind to multiple codons
- In 1966 F. Crick postulated the wobble hypothesis
- The 1st and 2nd nucleotide (5'-XXo-3 ') are strictly bound by Watson-Crick pairing to tRNA
- 3rd nucleotide (5'-ooX-3 ') binds alternatively to non-Watson-Crick pairing
- tRNAs carrying the same AA but recognizing the different codons are called isoacceptor

Codon	Anti-Codon
A	U or I
G	C or U or I
C	G or I
U	A or G or I



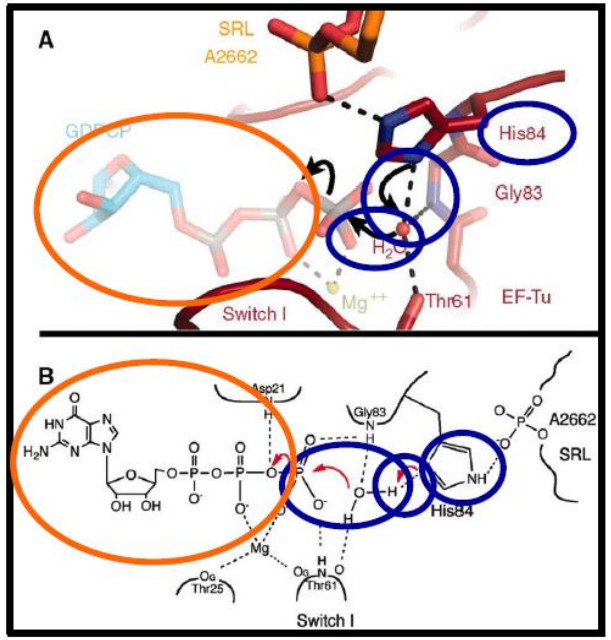
GTPase and GTP

- The process involves GTPase, also a part of the EF-Tu factor
- The GTP-binding domain is evolutionarily highly conserved and occurs from bacteria to higher eukaryotes
- GTP hydrolysis is associated with a conformational change involving A2662 from 23S-rRNA



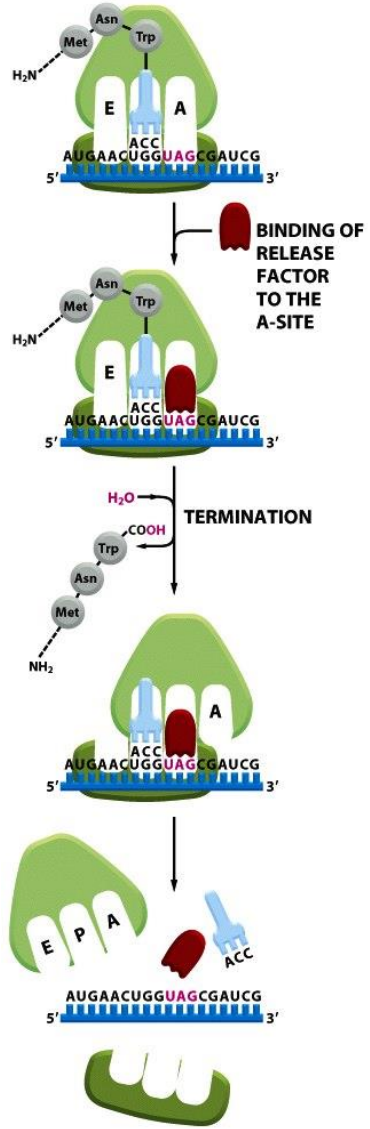
R. M. Voorhees et al., Science 330, 835-838 (2010)

- His84 acts as a base that removes the proton from H2O
- OH- attacks γ-phosphate in GTP
- GDP is released

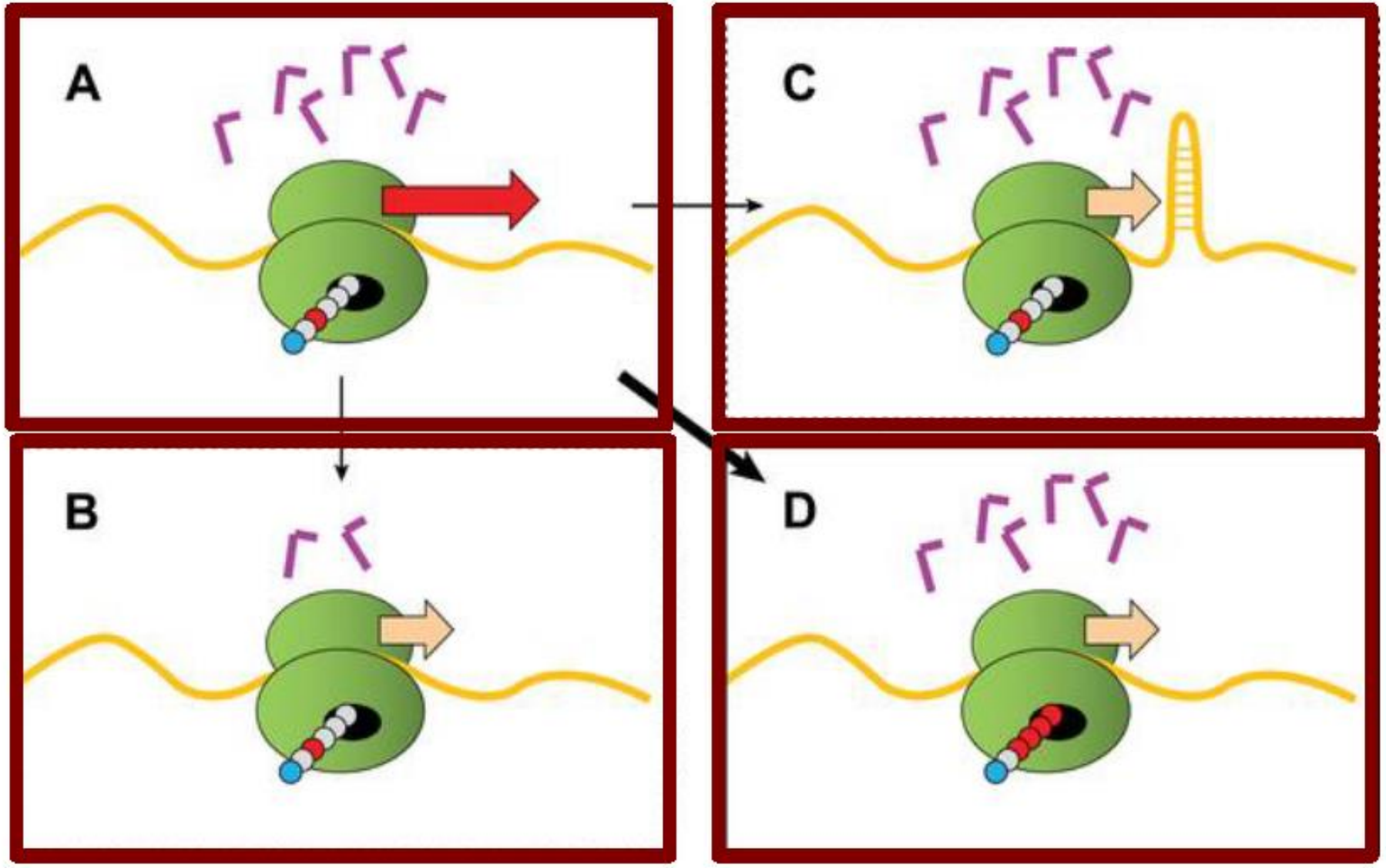


Termination of translation

- the presence of an nonsense codon where AA does not bind, but the termination factors do
- presence of termination factors RF1 (for UAG and UAA), RF2 (for UGA and UAA) and RF3 (stimulates the effect of RF1 and RF2)
- From the carboxyl terminus of the polypeptide chain, the tRNA is released to stop its elongation
- This frees both the polypeptide chain and the ribosome, which then breaks down into its subunits



Regulation of translation speed



A – standard ribosome movement

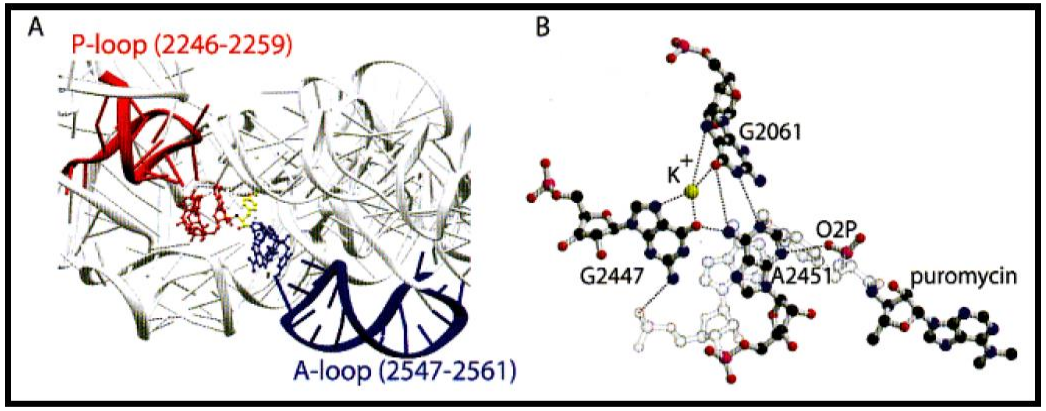
B – slowing of ribosome due to codons for less frequent AA

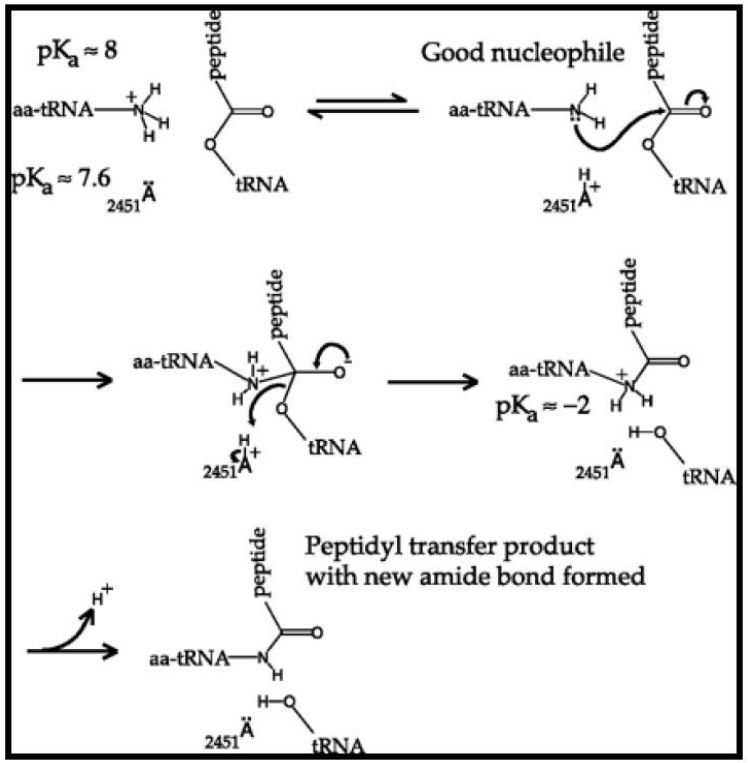
C – slowing of the ribosome due to secondary mRNA structure

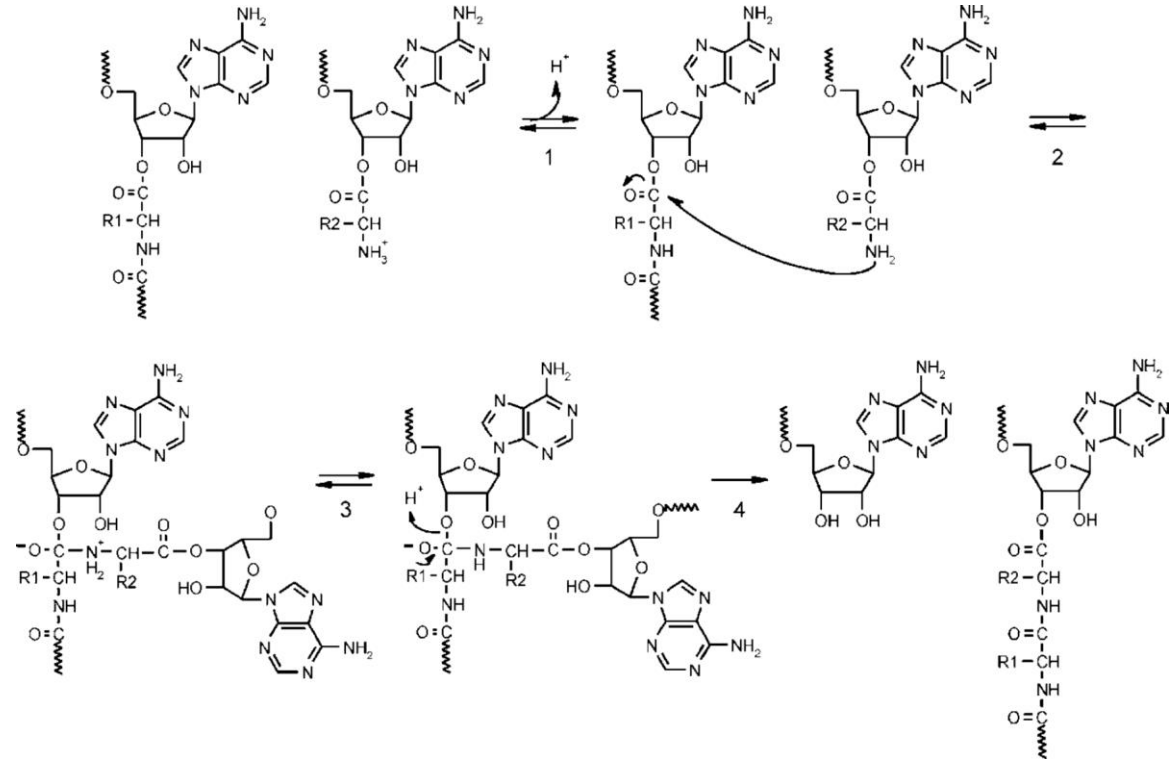
D – slowing of the ribosome due to positively charged AA - electrostatic interactions reduce the speed of movement in the tunnel

Mechanism of peptide bond formation

- P-loop and the A-loop are part of 23S-rRNA
- There is no protein in the vicinity of 18A from the catalytic site
- N3 nitrogen of adenine A2451 is decisive







Genes are expressed differently

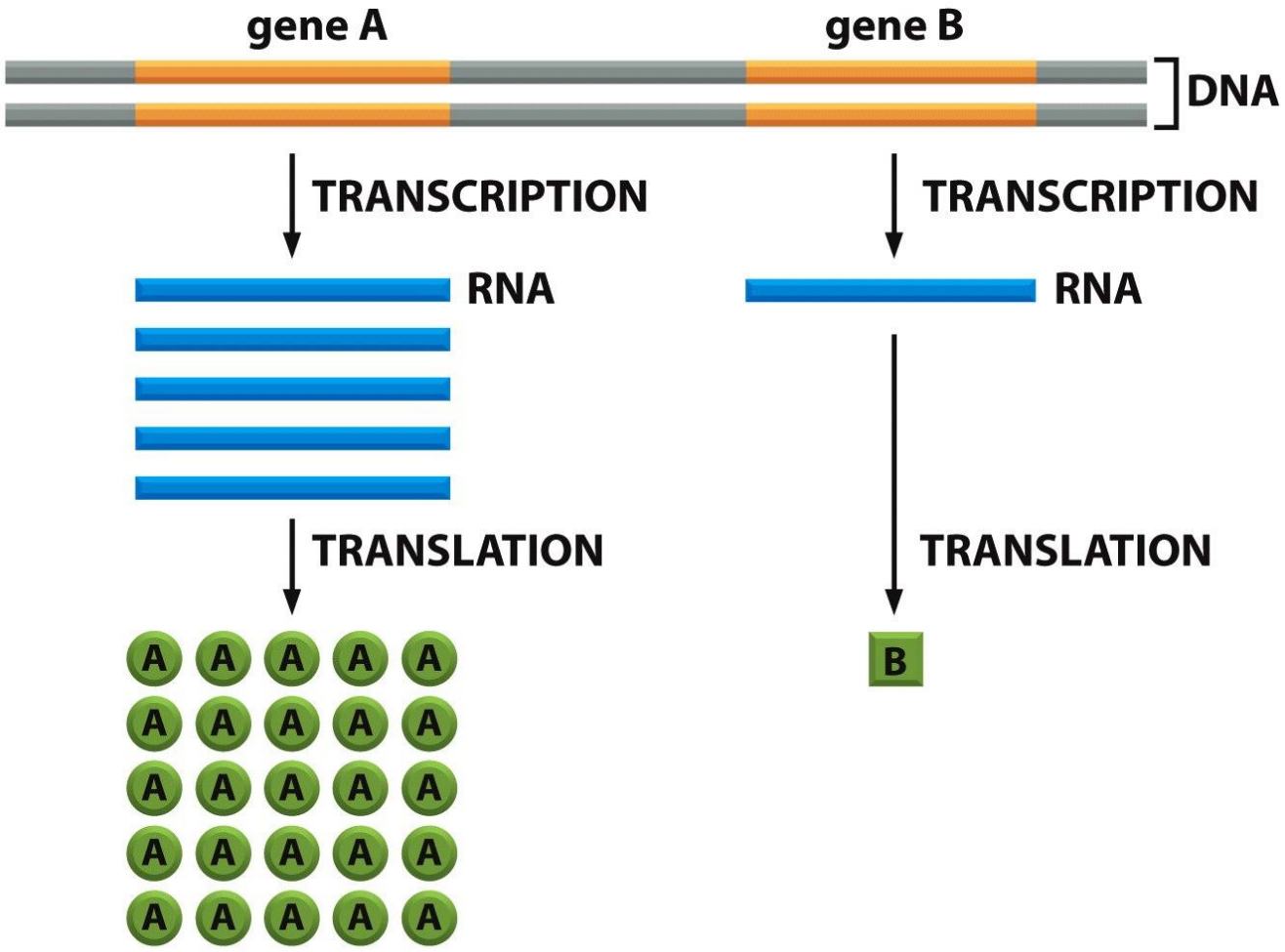


Figure 6-3 Molecular Biology of the Cell 5/e (© Garland Science 2008)