Structure of prokaryotic genom, replication and gene expression in prokaryots

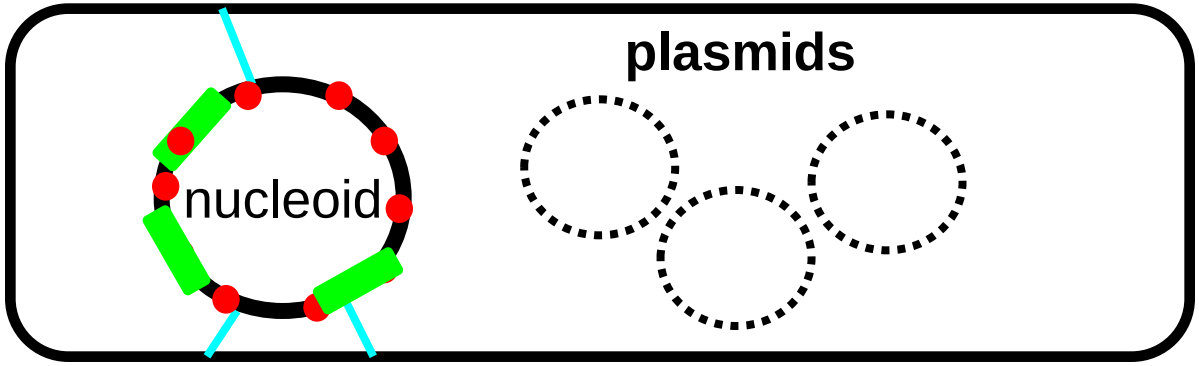
Biology

Doc. RNDr. Jan Hošek, Ph.D.
hosek@mail.muni.cz

Department of Molecular Pharmacy
FaF MU

Structure of prokaryotic genom

Genom of prokaryotic cell



- no nucleus envelope
- DNA, HLP-proteins (histon-like proteins), non-histone proteins
- nucleoid is attached to cell membrane on several places (*Inc*)

Prokaryotic chromosome

- Part of nukleoid (prokaryotic nucleus)
- Mostly circular dsDNA (linear e.g. for *Borrelia burgdorferi*)
- Superhelix divided to loops (domains)

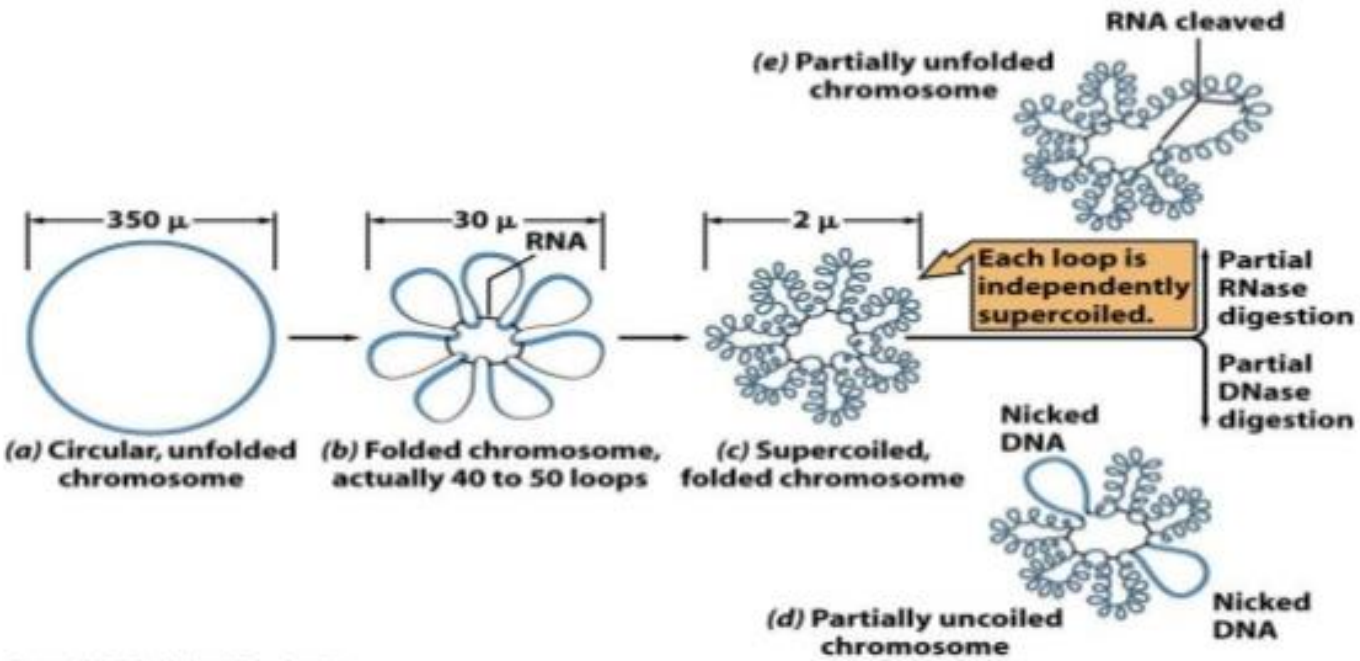
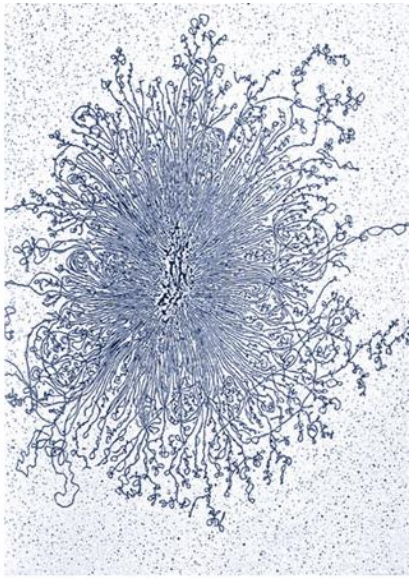


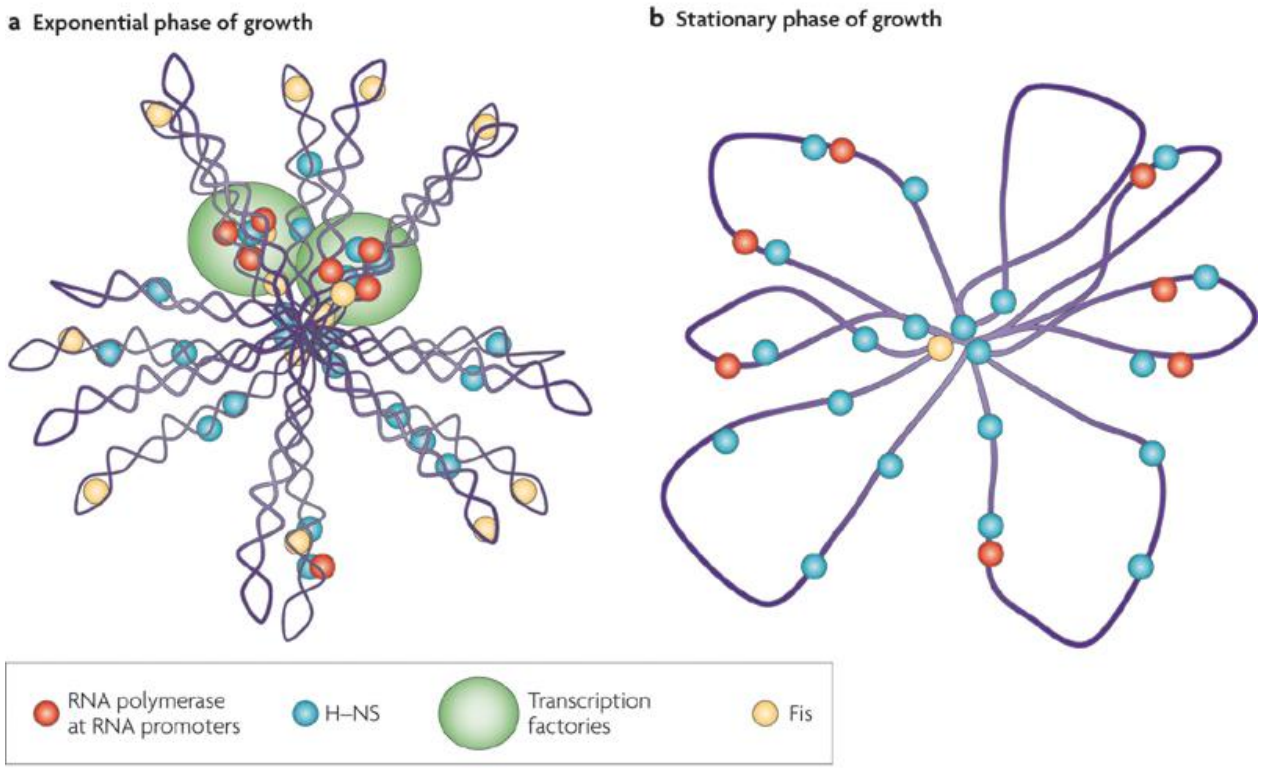
Figure 9-15 Principles of Genetics, 4/e
© 2006 John Wiley & Sons



Nature Reviews | Genetics

doi:10.1038/nrg3375

Dynamic of nucleoid



doi:10.1038/nrmicro2261

Nature Reviews | Microbiology

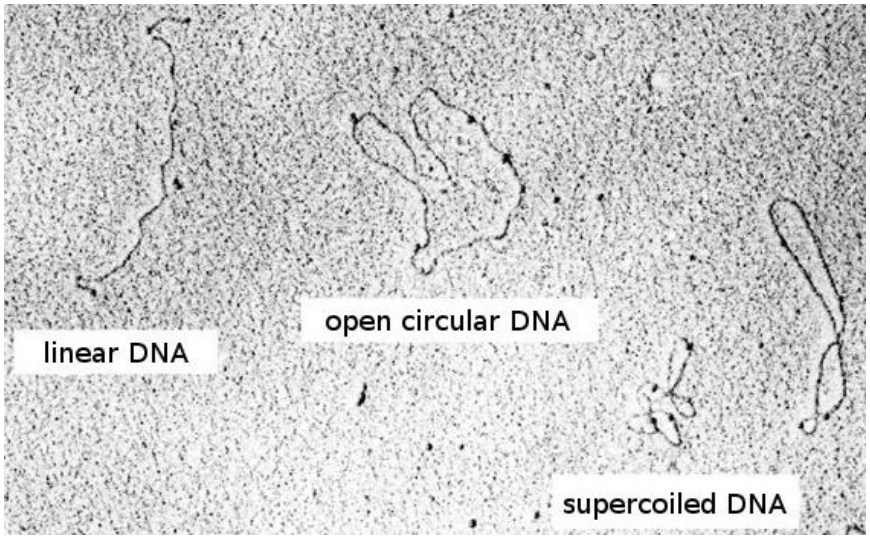
a | The folded chromosome is organized into looped domains that are negatively supercoiled during the exponential phase of growth. In this phase, the abundant nucleoid-associated proteins histone-like nucleoid-structuring protein (H-NS) and factor for inversion stimulation (Fis) bind throughout the nucleoid 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.

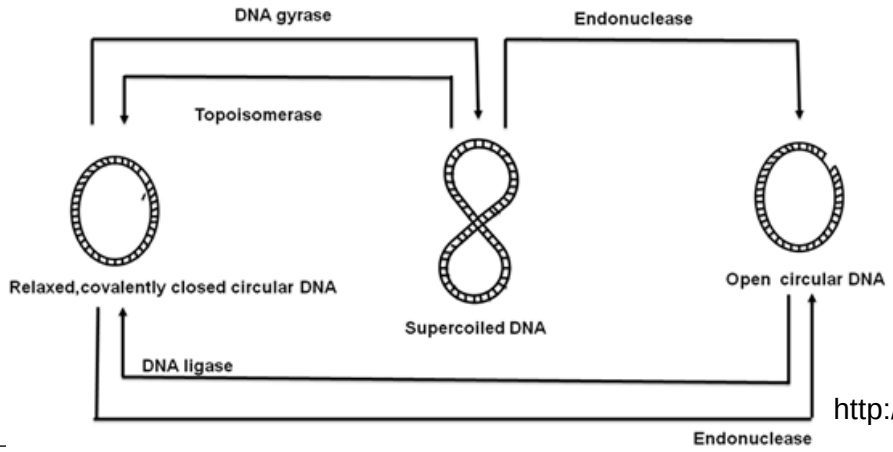
Plasmids

- bear genes which are not necessary for life (e.g., resistance to antibiotics)

http://www.wikiwand.com/

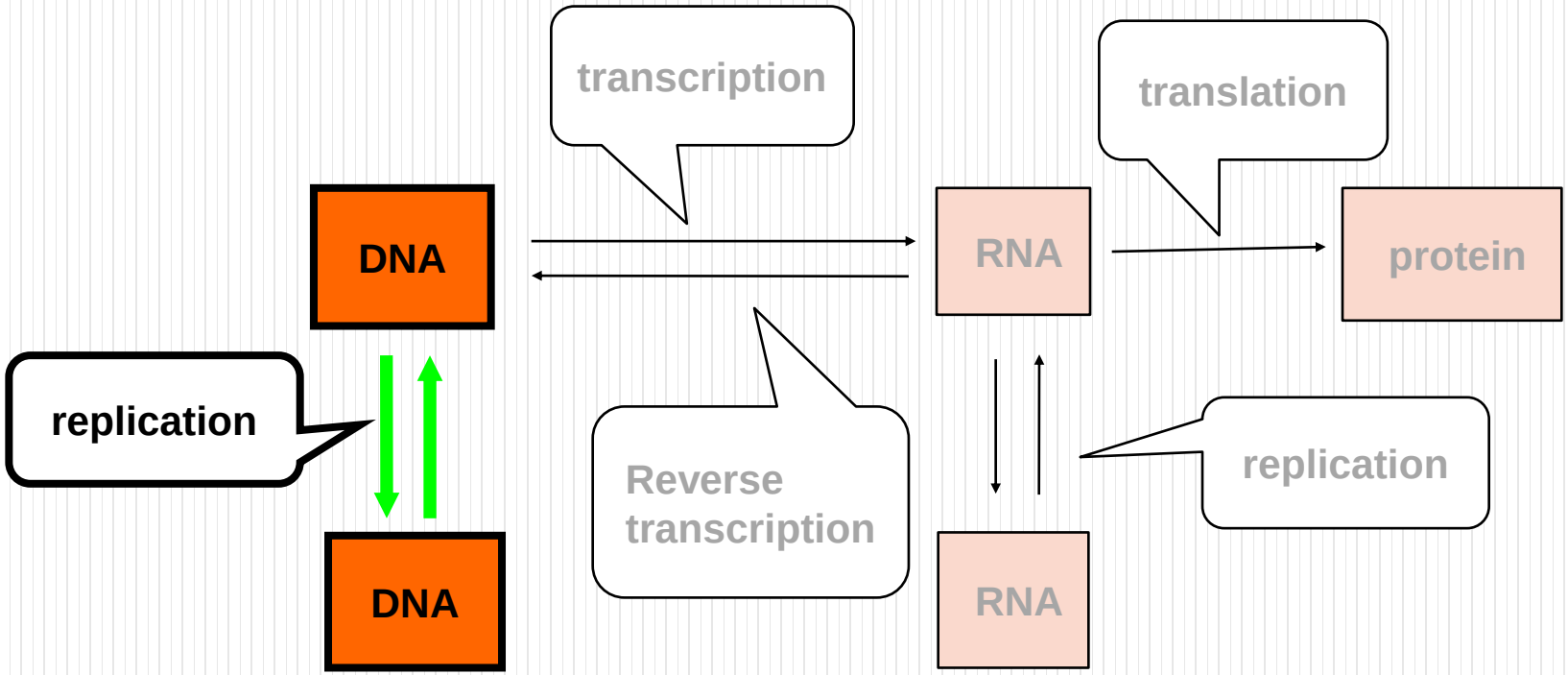
- circular dsDNA
- every is replicon = bears locus ***ori***
- Bears locus ***Inc*** for attachment to membrane





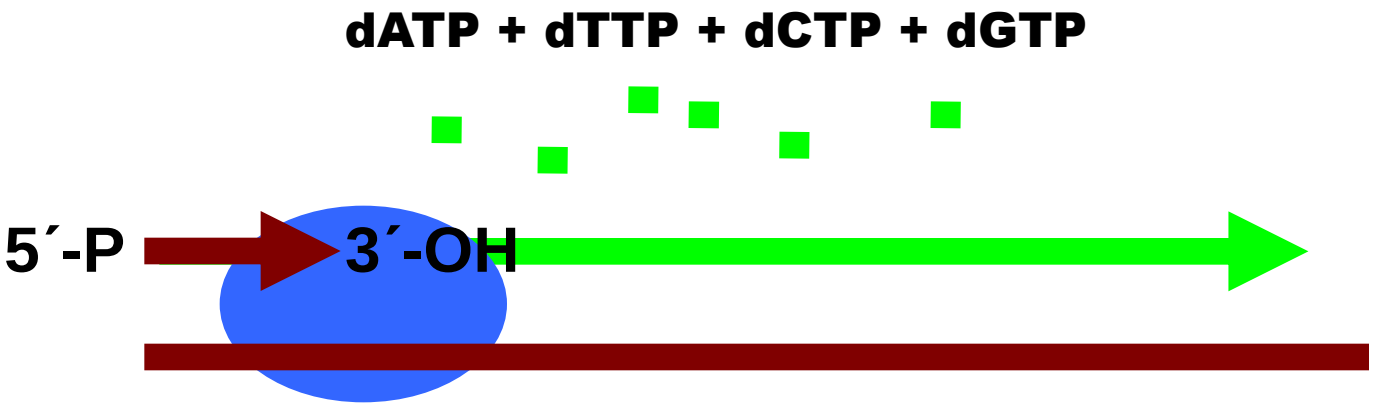
http://nptel.ac.in/courses/102103047/39

Replication of prokaryotic genom



What is needed for DNA replication ?

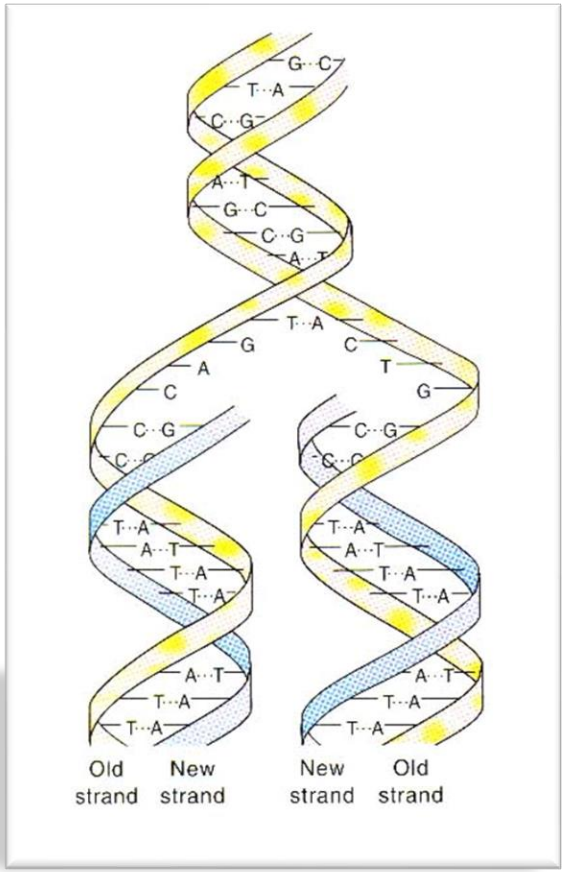
- Template strand (DNA matrix)
- Primer (free 3´-OH end)
- Polymerase + replication proteins
- dNTP



Characteristic features of replication

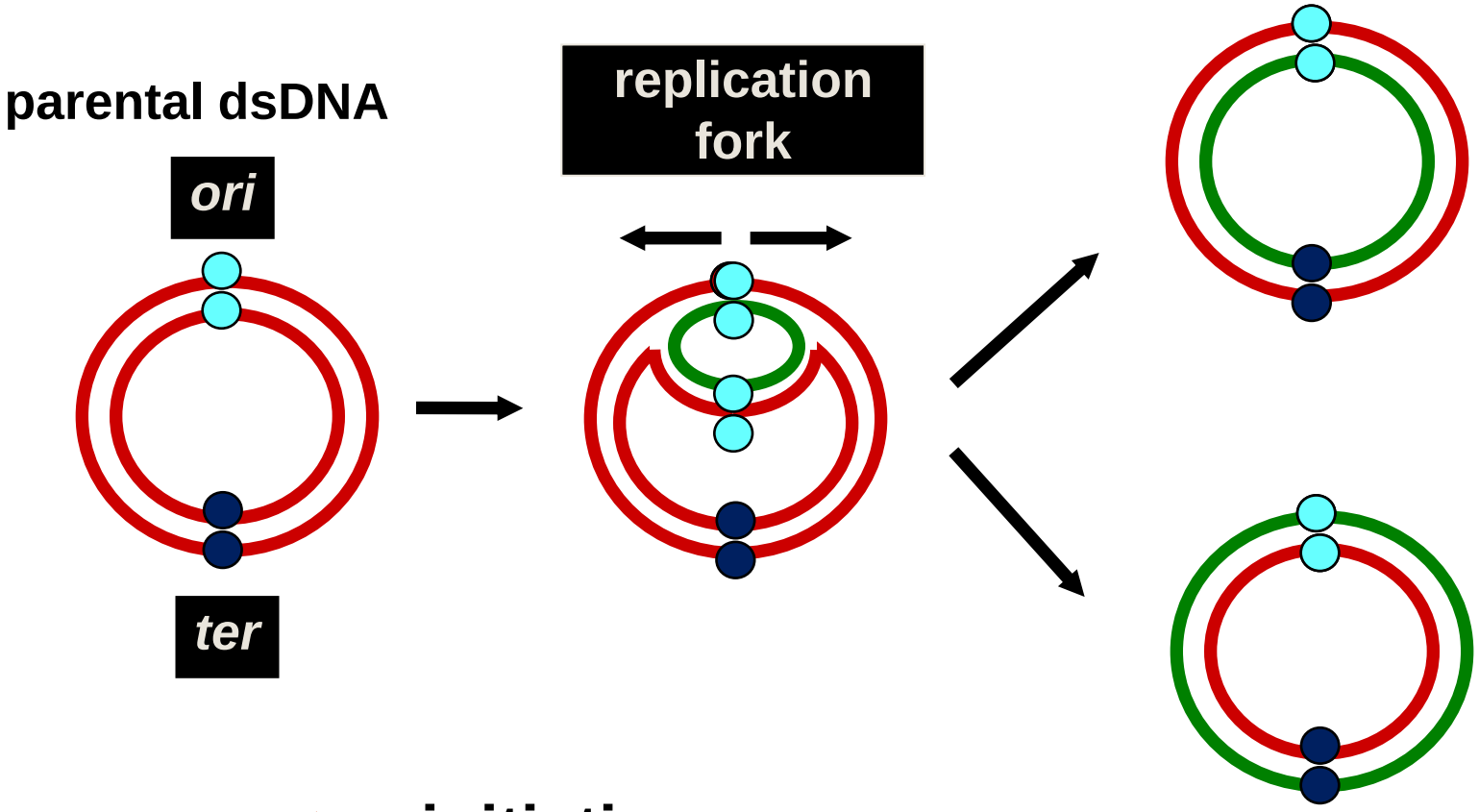
- It is **semiconservative**
  - **Meselson – Stahl experiment** → „the most beatiful experiment in biology“

https://www.youtube.com/watch?v=4gdWOWjioBE



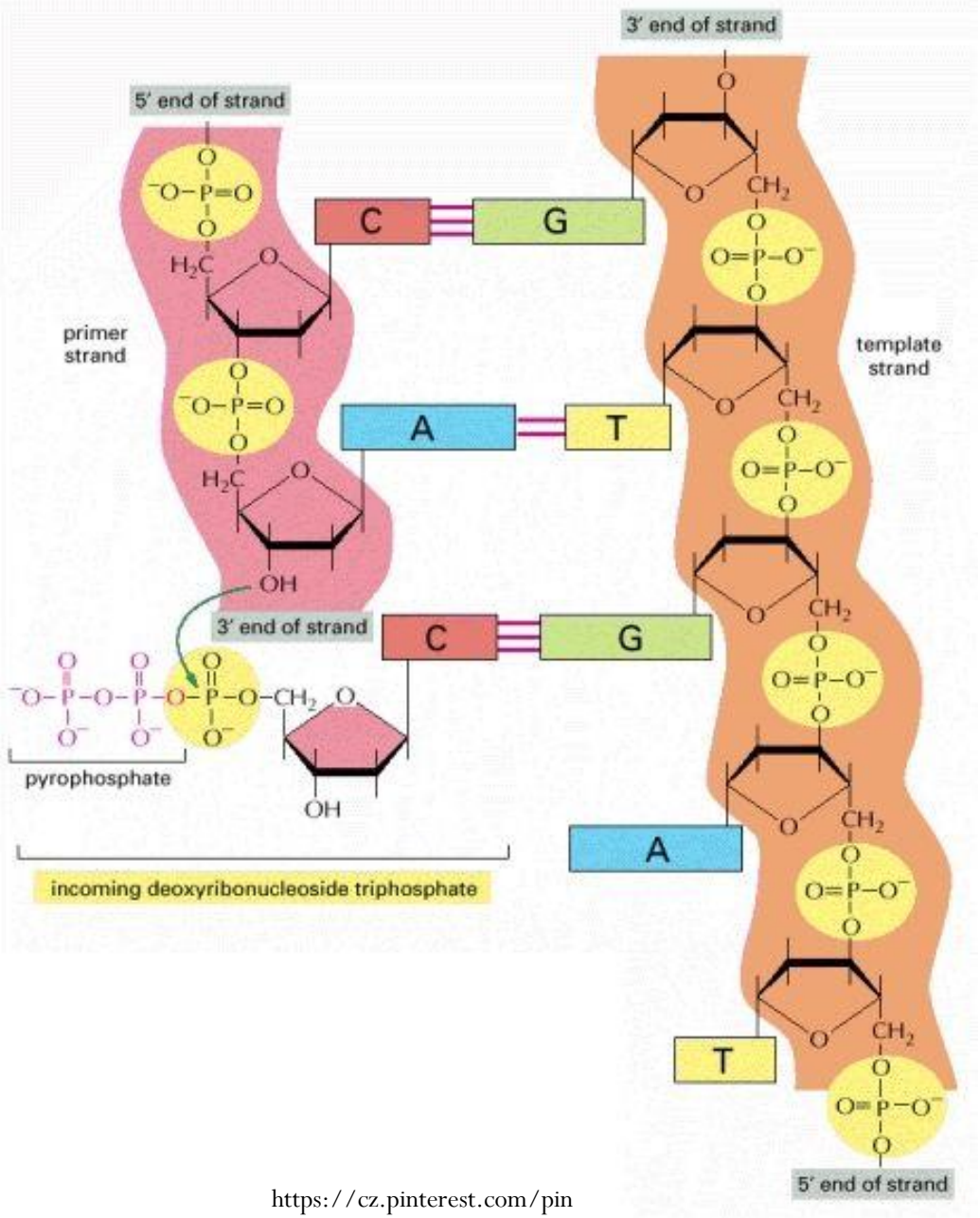
- It is **semidiscontinuous**
  - …Keep a while ☺

Replication of prokaryotic genom



- initiation
- elongation od DNA strand
- termination

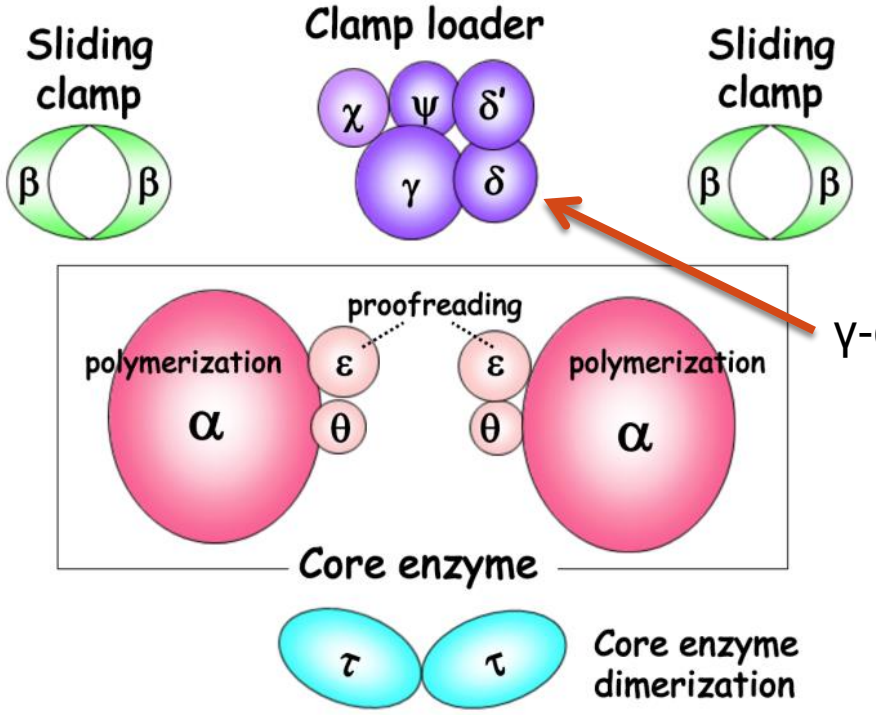
Synthesis of DNA during replication

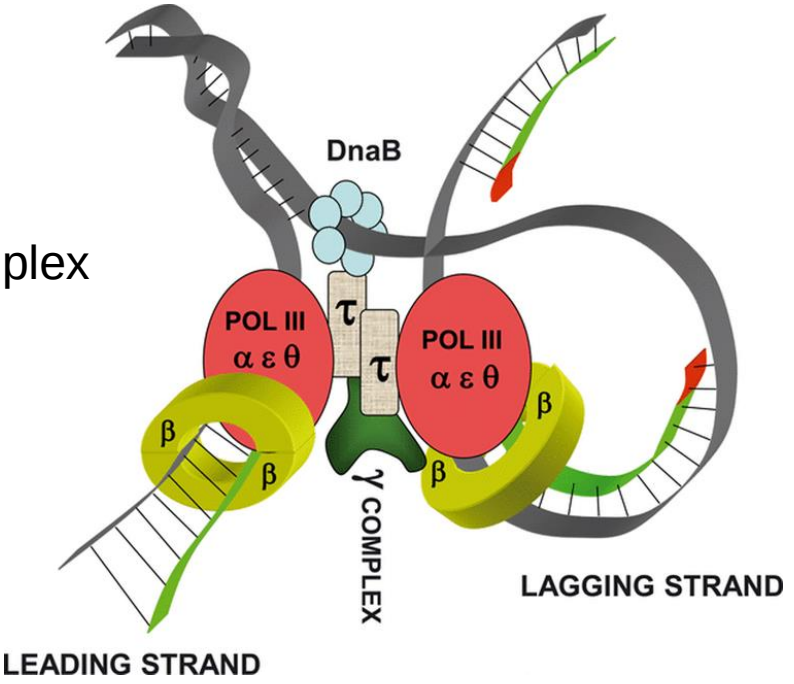


https://cz.pinterest.com/pin

Polymerase III

- Dimer of polymerase III = **PolIII***
- PolIII* - speed 20 nt/s, procesivity 11 nt
- PolIII* + β-clamp – speed 500 nt/s, procesivity „∞“



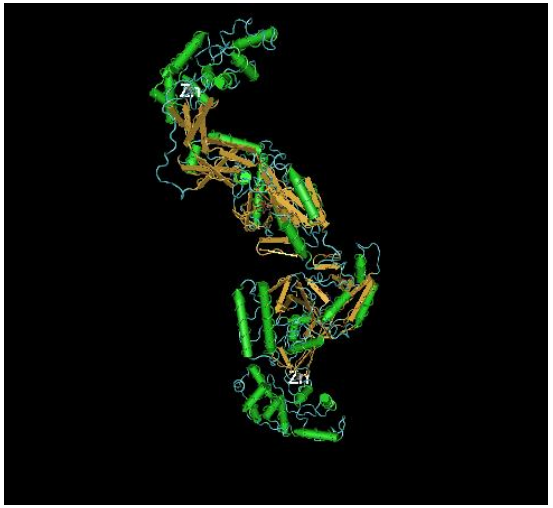


http://slideplayer.com/slide/8771653/

DOI: http://dx.doi.org/10.1111/j.1574-6976.2012.00338.x

Replication proteins - II

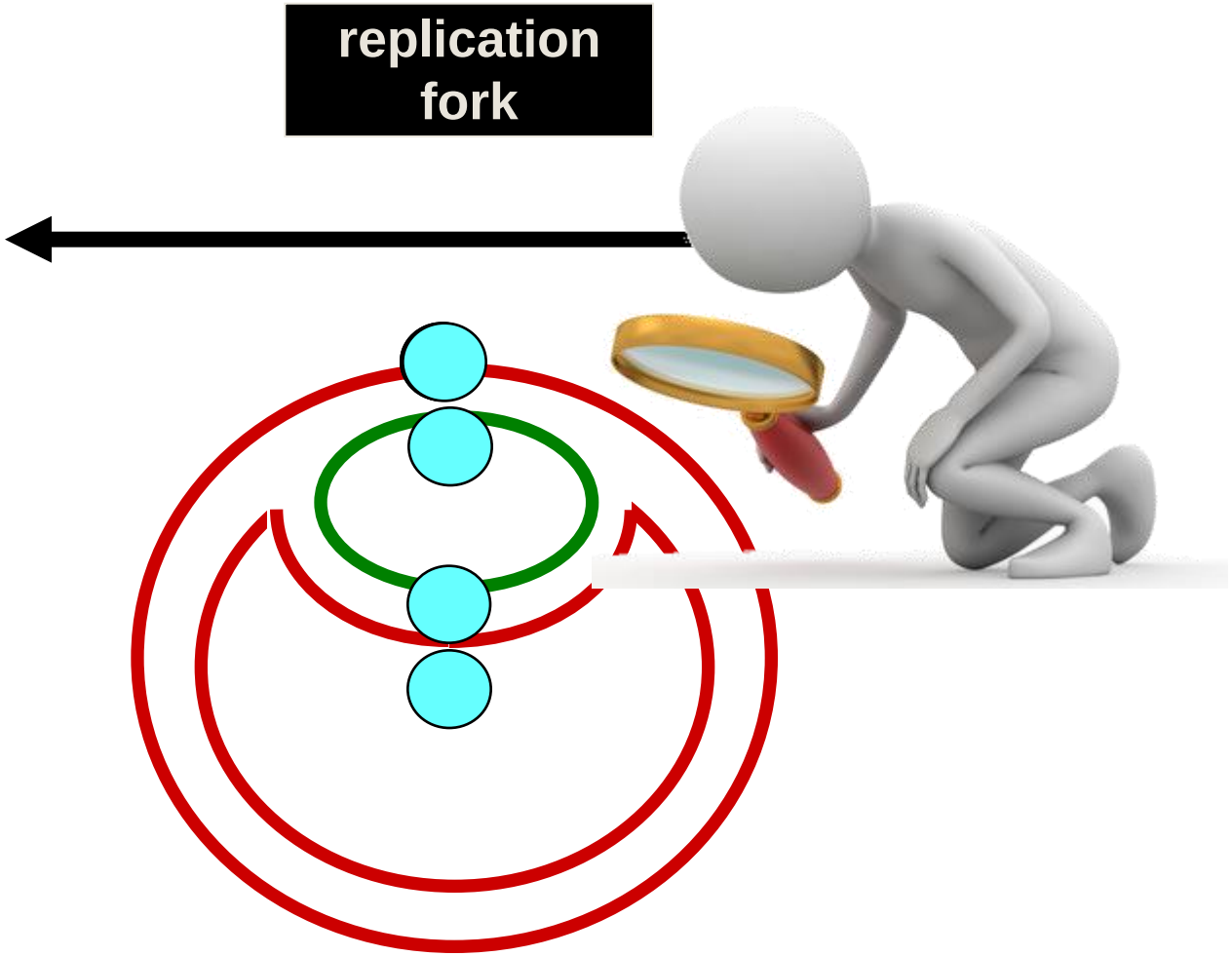
- DNA-ligase
  - It forms phosphodiester bond between 5´- and 3´- ends of two polynucleotide strands
  - It joins Okazaki fragments



Replication proteins - III

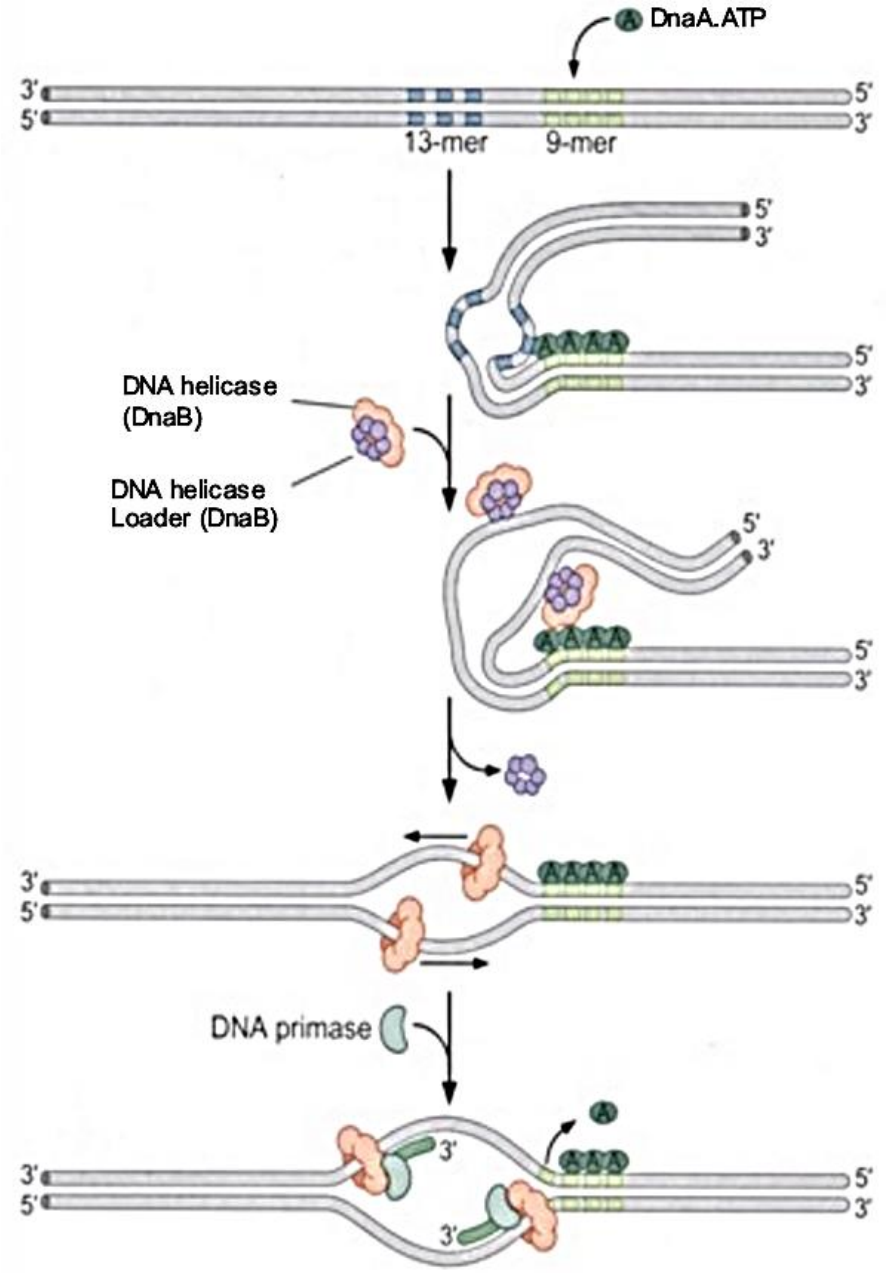
- **DNA-primase**
  - DNA-dependent RNA-polymerase
  - synthesis of RNA-primer
- **DNA-helicase**
  - untwists strands from dsDNA
  - destroyes hydrogen bonds by using the energy from NTP
- **DNA-gyrase (topoizomerase II)**
  - transform positive supercoiling to negative

Replication



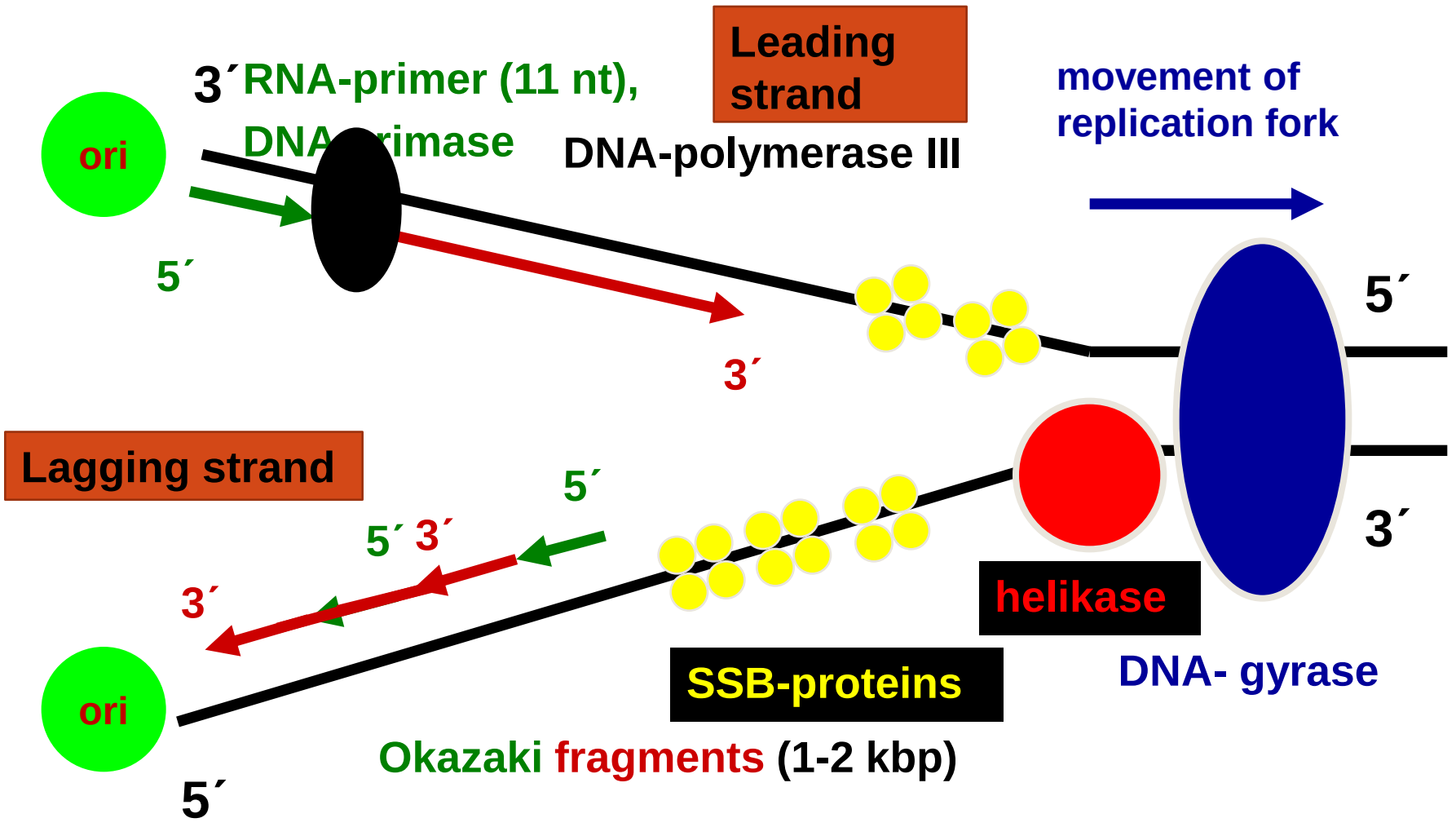
Recognition of *oriC* and initiation of replication

oriC



Watson, J. D. et al. (2004) Molecular Biology of the gene. 5th ed. CSHL Press. Fig. 8-26.

Process of replication

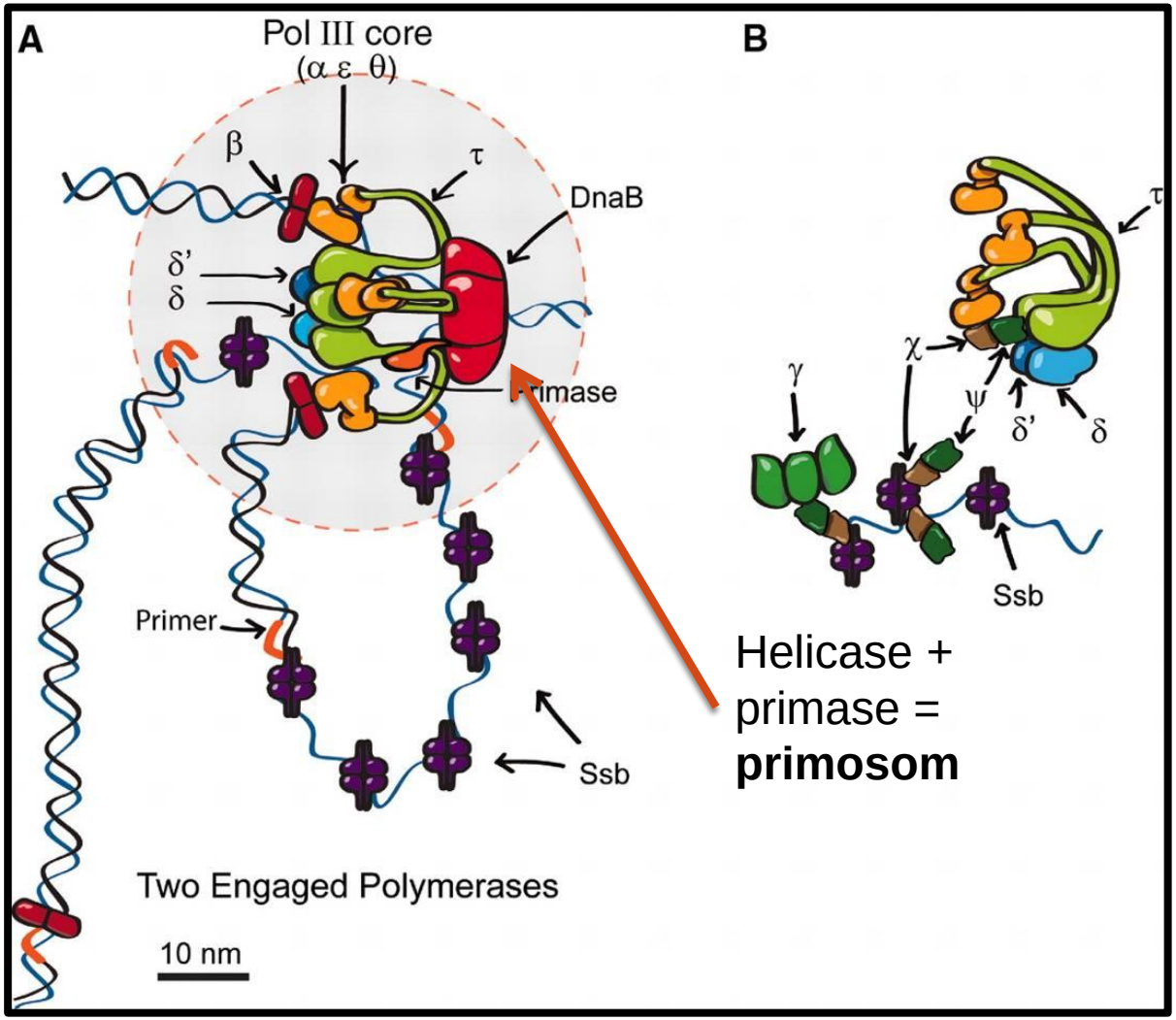


DNA polymesare I removes RNA primers and synthesize the Okazaki fragments

DNA ligase joins the Okazaki fragment

Replication is performed in replisomes

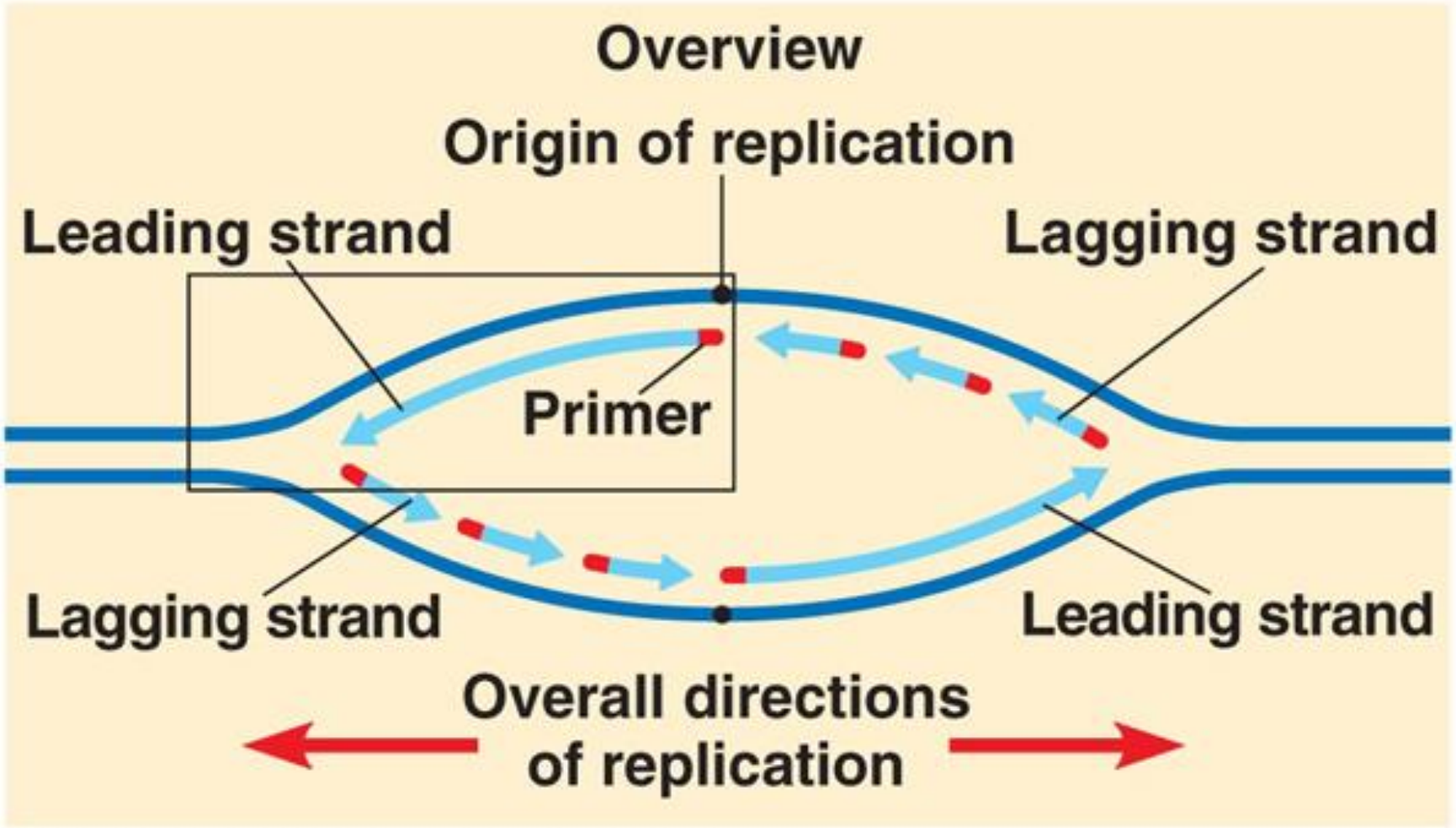
https://www.youtube.com/watch?v=TNKWgcFPHqw&ab_channel=yourgenome



Helicase + primase = **primosom**

R. Reyes-Lamothe et al., Science 328, 498-501 (2010)

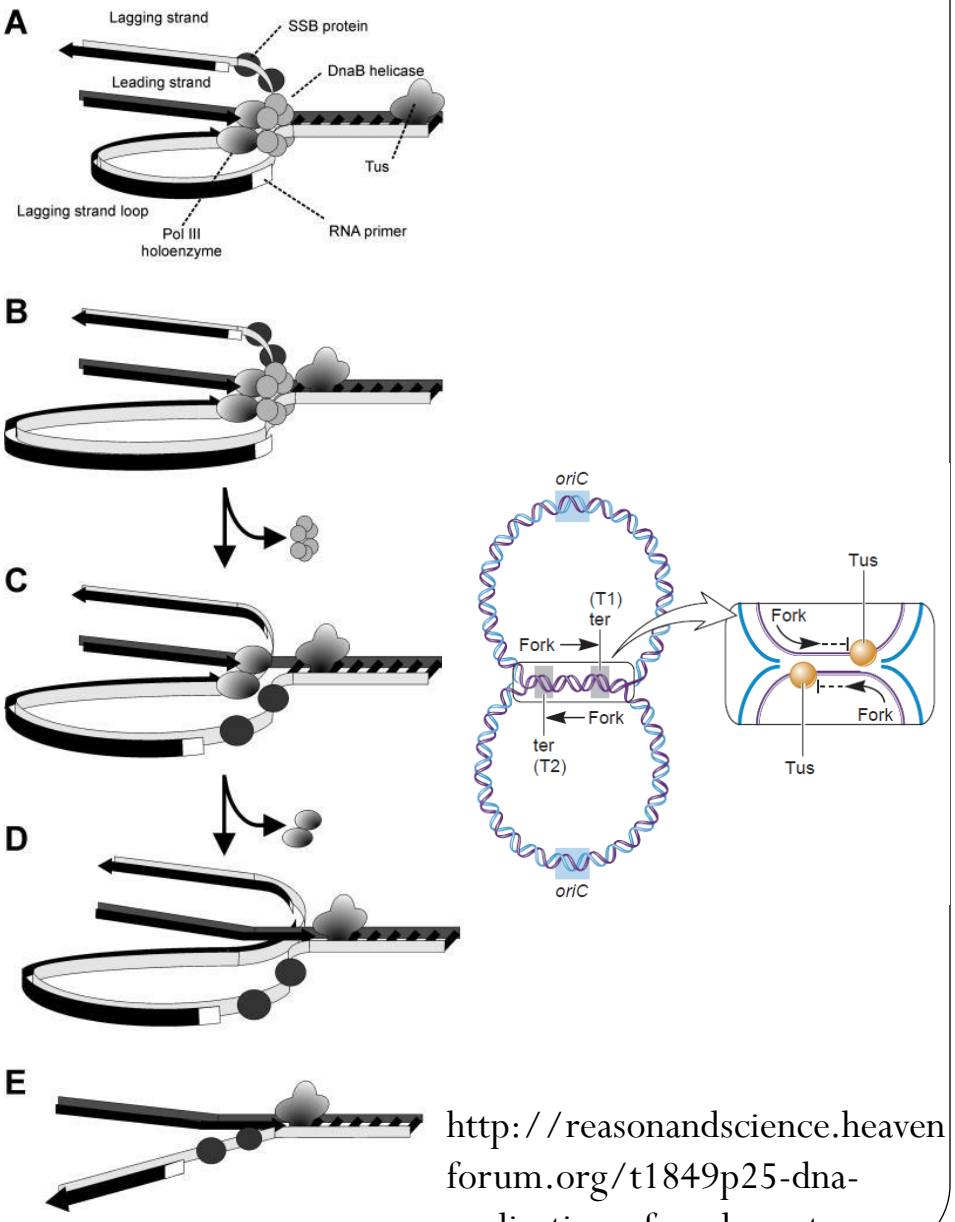
Replication is semidiscontinual and bidirectional



Copyright © 2008 Pearson Education, Inc., publishing as Pearson Benjamin Cummings.

Termination of replication

- Replication of prokaryotic chromosome ends on specific sequences named **terminators** (*ter*)
- The specific **protein Tus** binds to the terminators which inhibits activity of helicase and the formation of replication fork is stopped

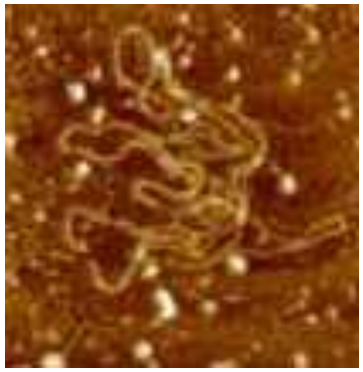


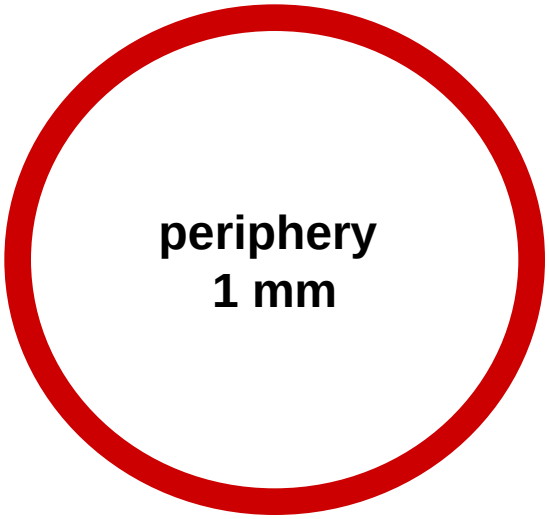
Replisome of E. coli and mechanism of replication fork arrest by a Tus-Ter complex.
(A) *The replisome moving along the DNA template approaches Tus, and the DnaB helicase assists primase to lay down the last lagging-strand primer.*
(B) *DnaB helicase action isblocked by Tus, and DnaB dissociates from the template.*
(C) *DNA polymerase III (Pol III) holoenzyme completes leading-strand synthesis up to the Tus-Ter complex and*
(D) *synthesizes the last Okazaki fragment on the lagging strand, which will eventually be ligated by DNA ligase to the penultimate fragment following removal of its RNA primer by DNA polymerase I (not shown). (E) The holoenzyme then dissociates, leaving a Y-forked structure that is single stranded on the lagging strand near the Tus-Ter complex.*

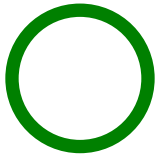
http://reasonandscience.heavenforum.org/t1849p25-dna-replication-of-prokaryotes

Replication of plasmid DNA

1) Plasmids are replicon of circle type
2) They are smaller than bacterial chromosome



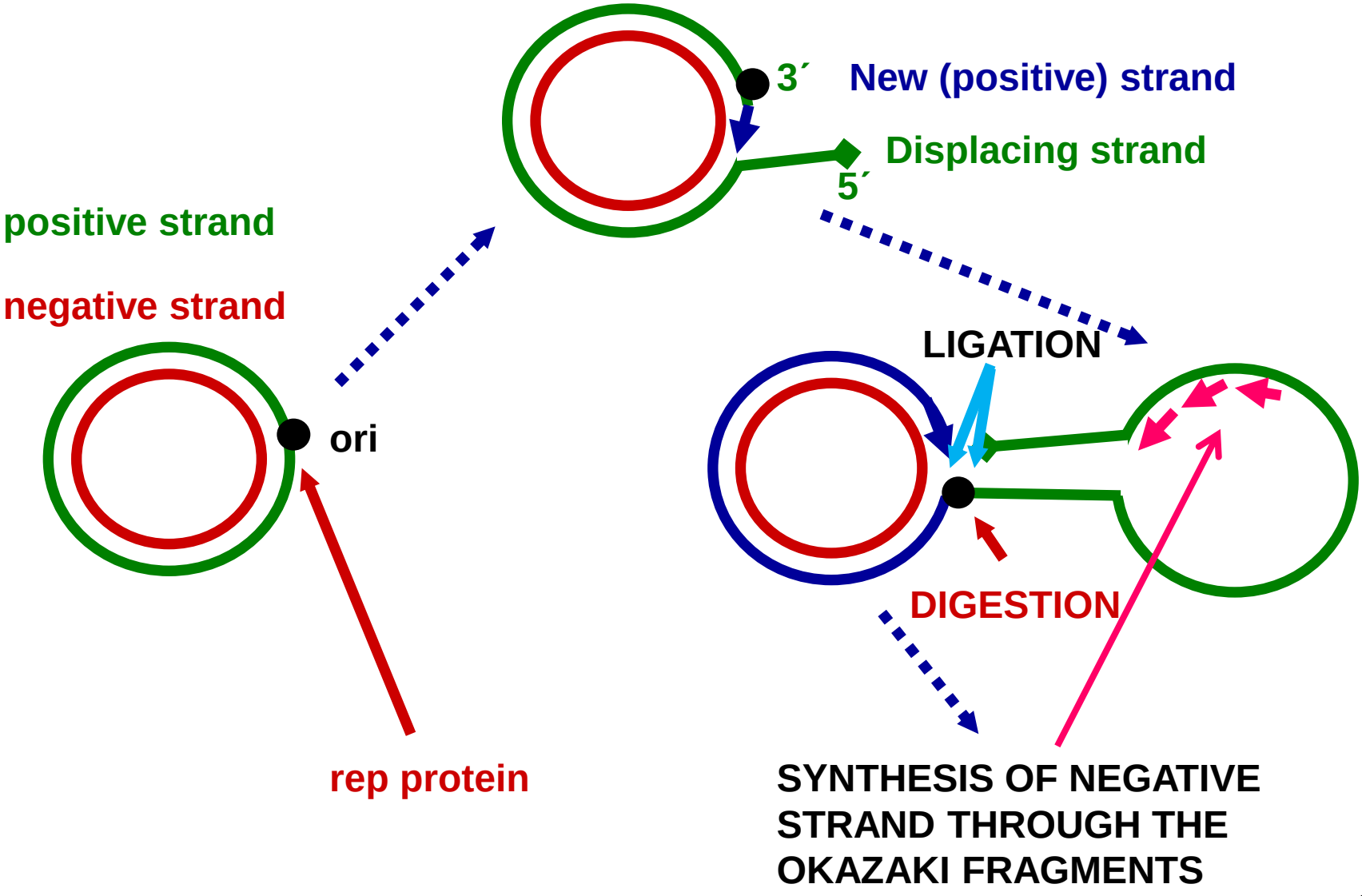




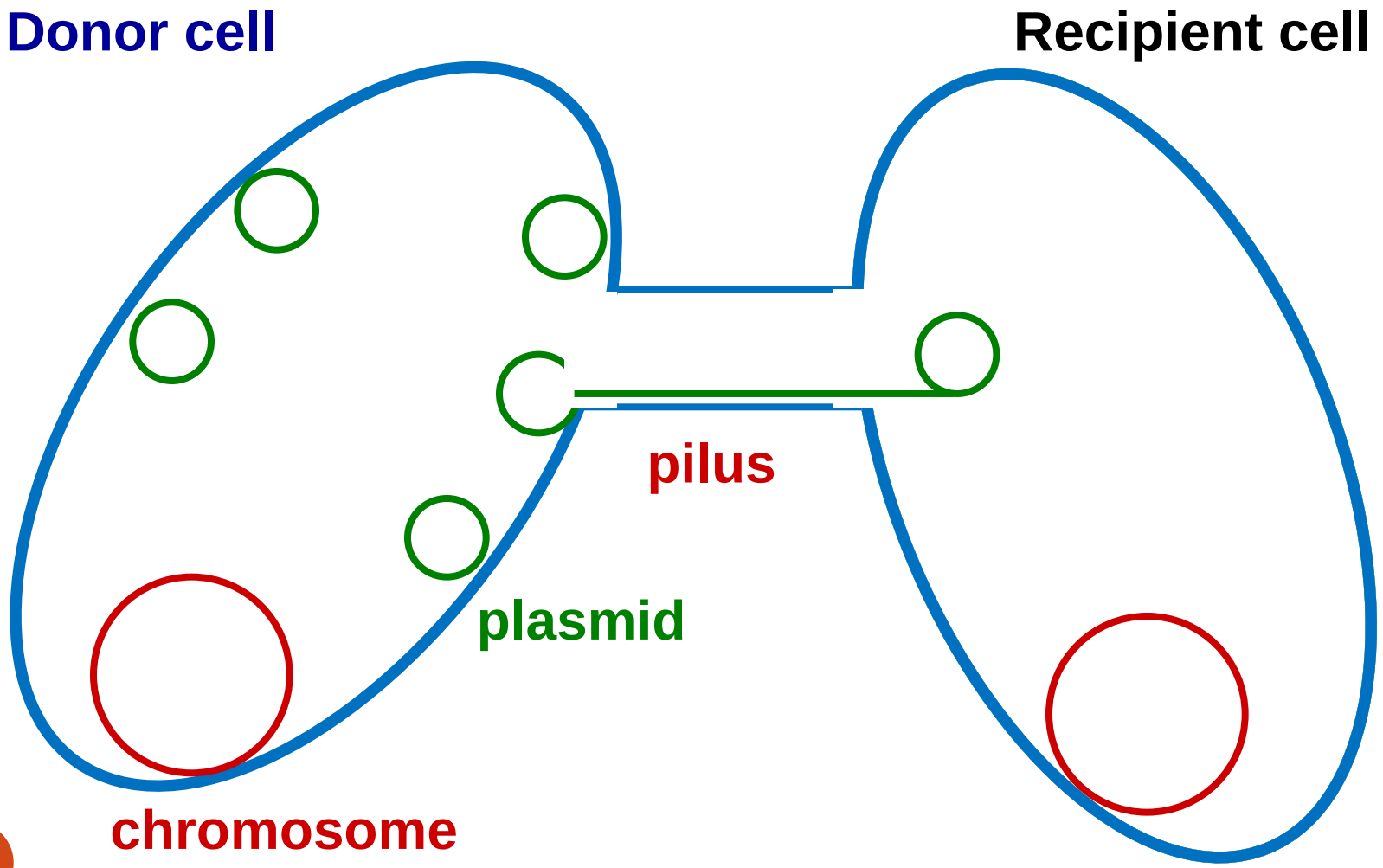
F plasmid,
periphery 31μm

- semiconservative
- semidiscontinuous
- bidirectional

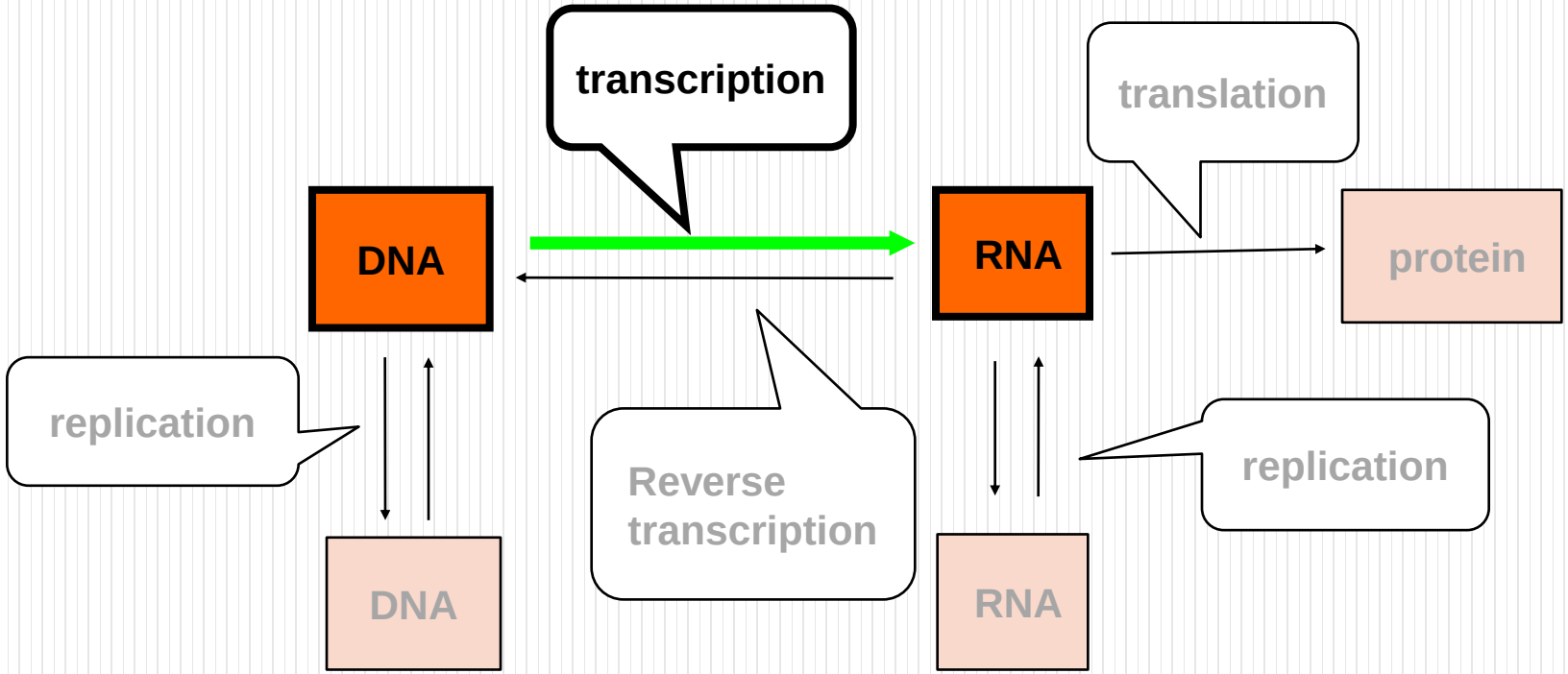
Replication by the rolling circle mechanism



Replication by the rolling circle mechanism during conjugation



The transcription of prokaryotic genome



What is the transcription?

- Process of copying genetic information in DNA into RNA = synthesis of RNA from ribonucleotides on DNA strand as a template
- DNA-dependent RNA polymerase = transcriptase = prokaryotic RNA polymerase = RNA polymerase

Functions of the RNA polymerase

- It binds to promotor sequence
- It catalyses synthesis of long primary transcripts on a template DNA strand

Which primary transcripts are created during transcription?

1) **Messenger RNA (mRNA)**

   it contains transcripts of genetic information from structural genes

2) **Precursor ribosomal RNA (pre-rRNA)**

   primary transcript of the genes for rRNA, post-transcriptionally processed to rRNA

3) **Precursor transfer RNA (pre-tRNA)**

   primary transcript of genes for tRNA post-transcriptionally processed to different types of tRNA

4) **Primary transcripts of regulatory RNAs**

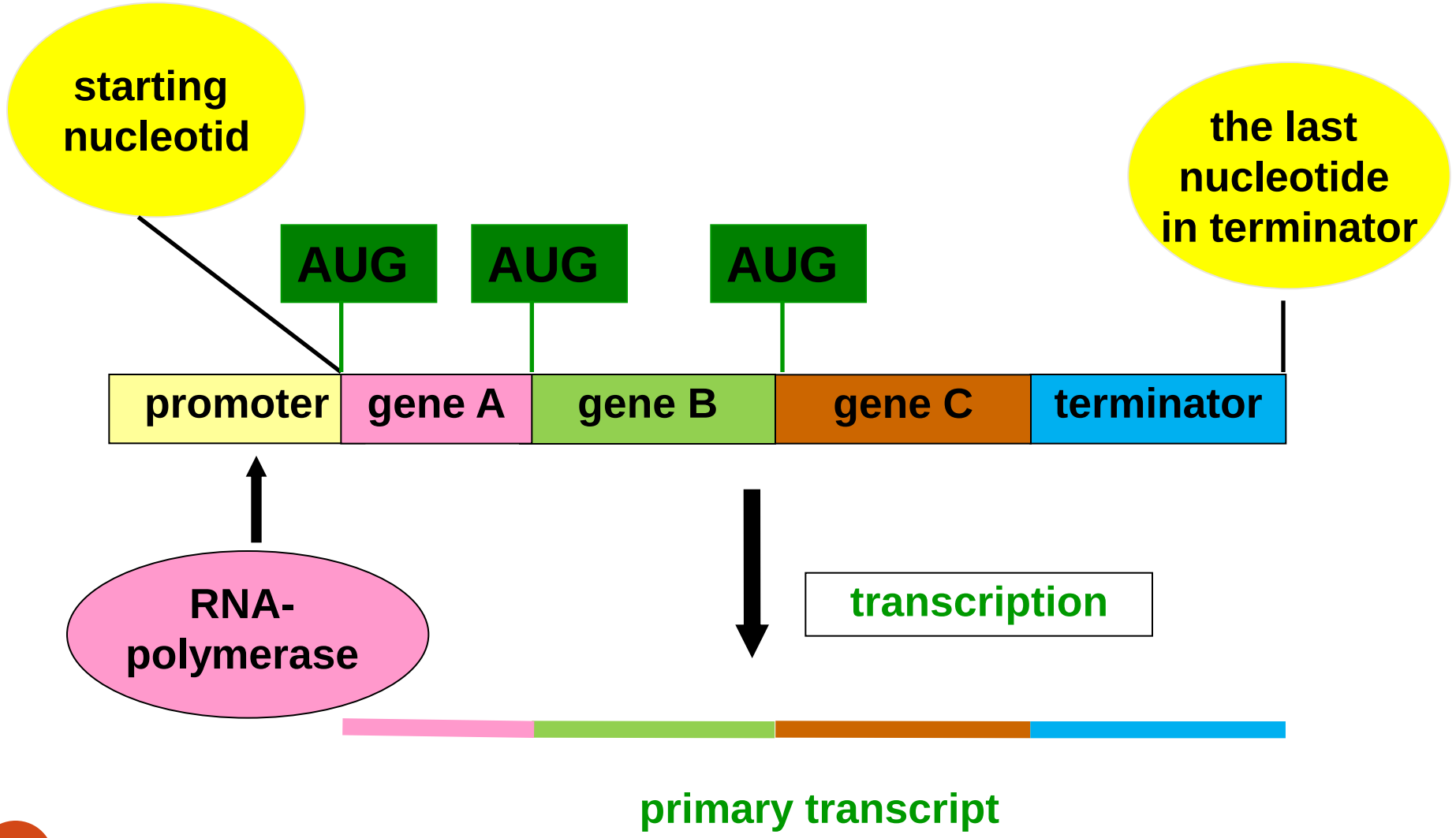
Transcription units

The transcription is performed in specific units = transcription units

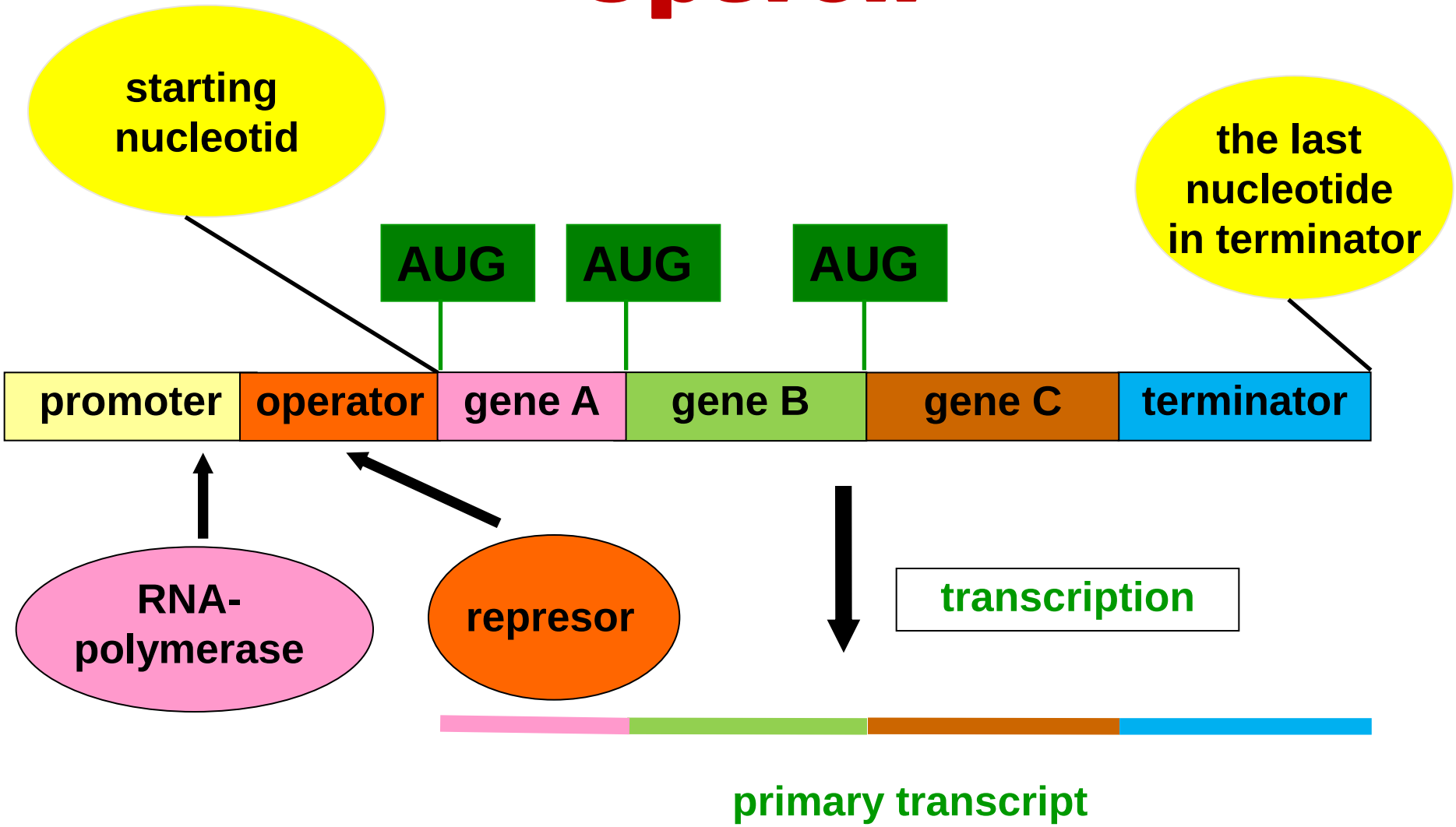
1) Transcription units of non-operon type

2) Operons

Transcription units of non-operon type



Operon



!! promotor can overlap with operator !!

Process of transcription

Initiation of transcription

Binding of RNA polymerase to promoter of the negative DNA chain and starting of RNA chain synthesis

Elongation of RNA chain

Adding of nucleoside-5´-monophosphates to 3´- end of growing RNA strand

Termination of transcription

Stop the movement of RNA polymerase → releasing the full length RNA → releasing RNA polymerase from DNA

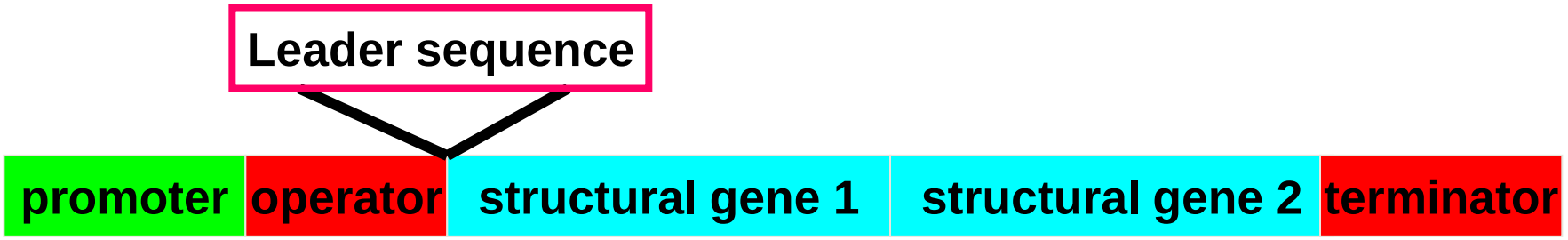
Transcription of the structural genes

1) They contain a leader sequence (only in structural genes)

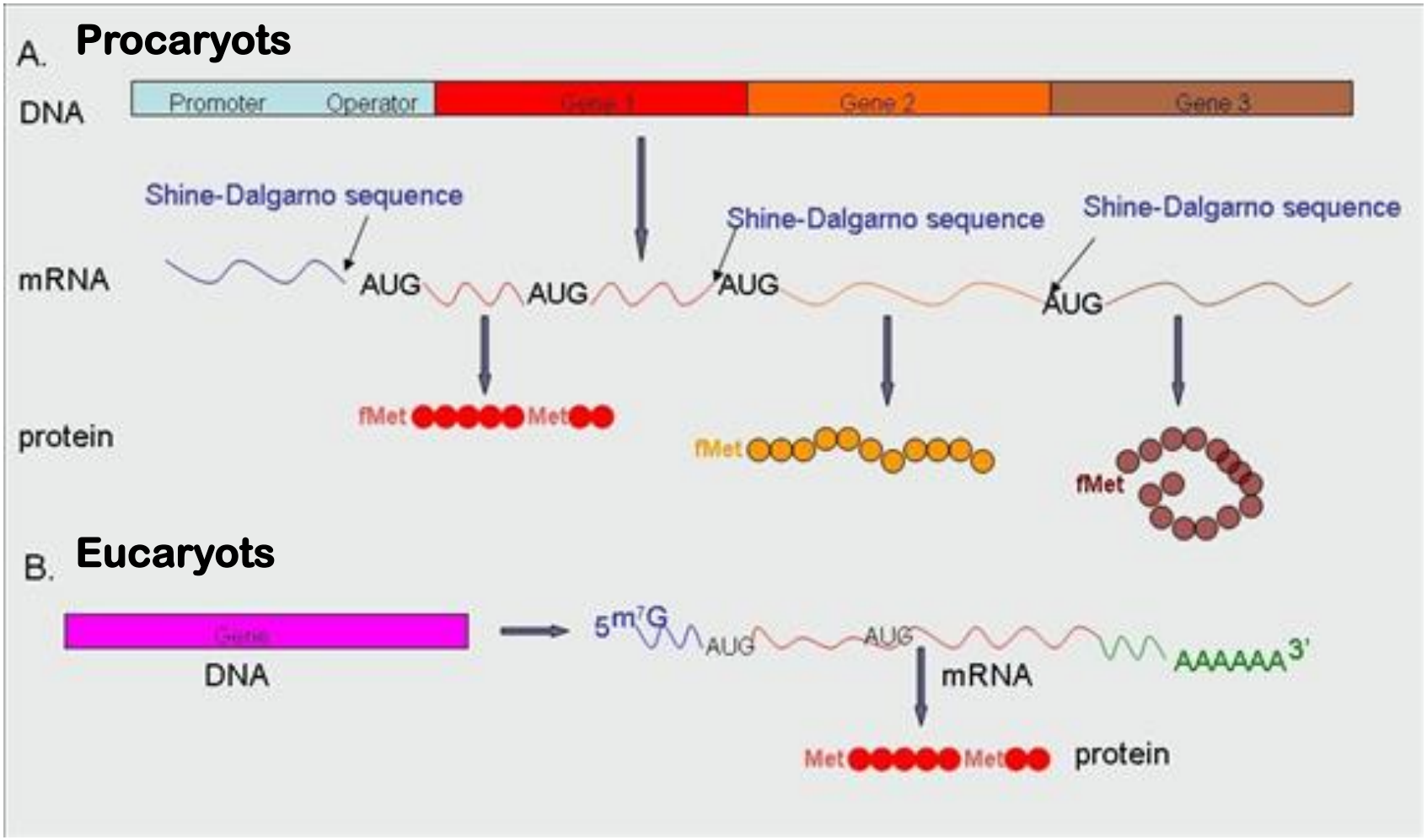
- the sequence lies between promoter and the first structural gene
- in operone it is immediately after operator

2) The leader seguence contains

- Shine-Dalgarno sequence 5´AGGA 3´
- it binds to the 3´- end of 16S-rRNA



Transcription unit of structural genes



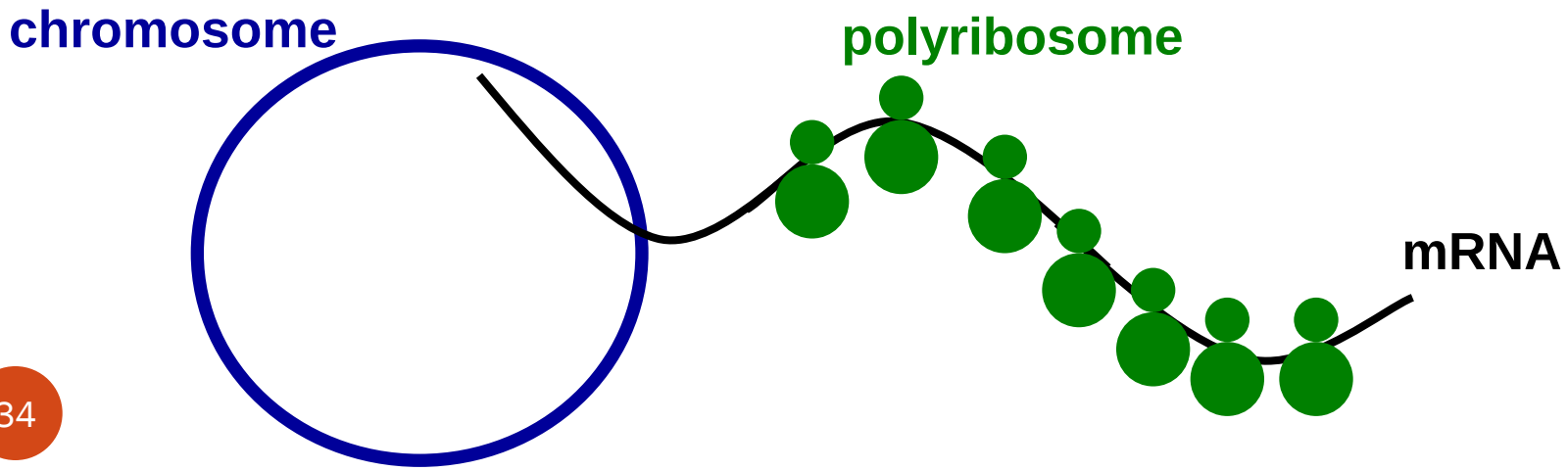
http://www.tankonyvtar.hu/en/tartalom/tamop425/0011_1A_Proteinbiotech_en_book/ch01.html

mRNA

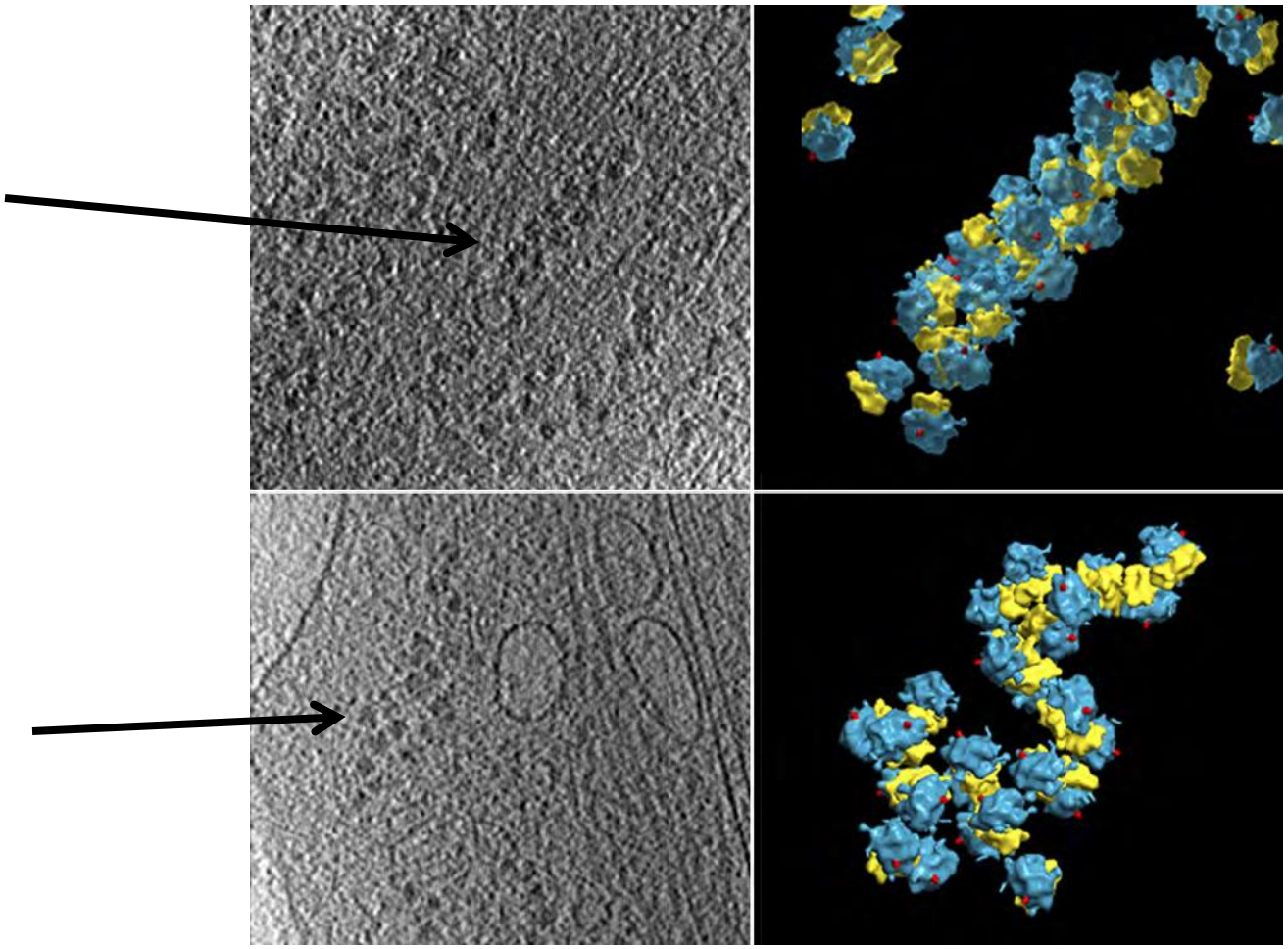
1) Primary transcript of transcription unit bearing structural genes, which are translated into polypeptide chains
2) Contains leader sequence on the 5´-end
3) Bears UUUUUUUU (ending sequence) on 3´-end
4) Contains transcripts of several genes = polycistronic (polygenic) mRNA
5) Any post-transcription processing
6) Short half-life, digestion by ribonucleases in the direction 5´- 3´
7) mRNAs represent only 3 % of total RNA in prokaryotic cell every time

Coupled of transcription with translation = coupled synthesis

1) Ribosomes bind to mRNA during transcription
2) Both process on the same mRNA (transcription + translation)
3) In some transcription units up to 15 initiations per minute = 15 new mRNA molecules
4) On each mRNA up to 30 ribosomes = 30 new polypeptide chains



Structure of polyribosome



Yellow – small subunit

Blue – large subunit

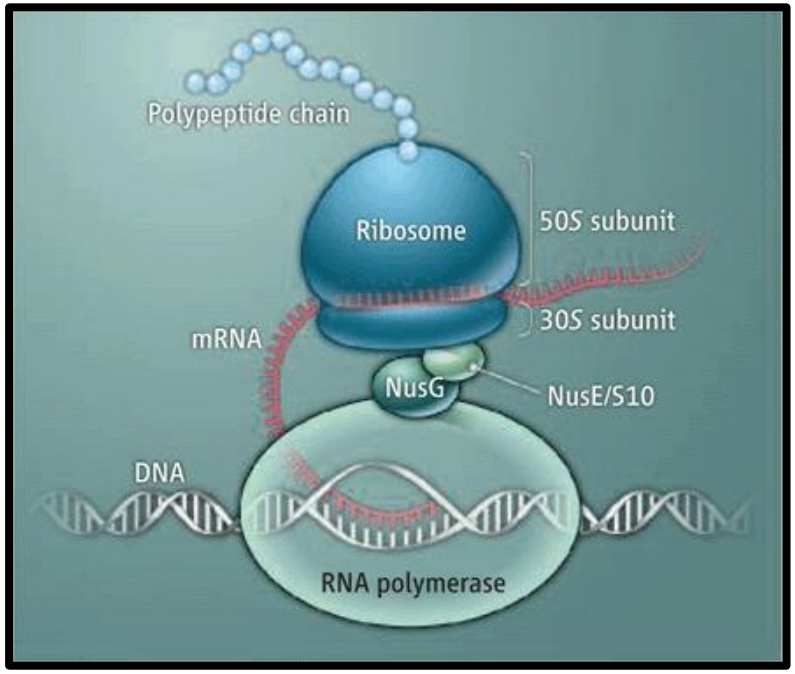
Red – polypeptide chain

Kay Grünewald and Wolfgang Baumeister, Max Planck Insitute, 2012,

Coupled syntheses

Influences speed of proteosynthesis

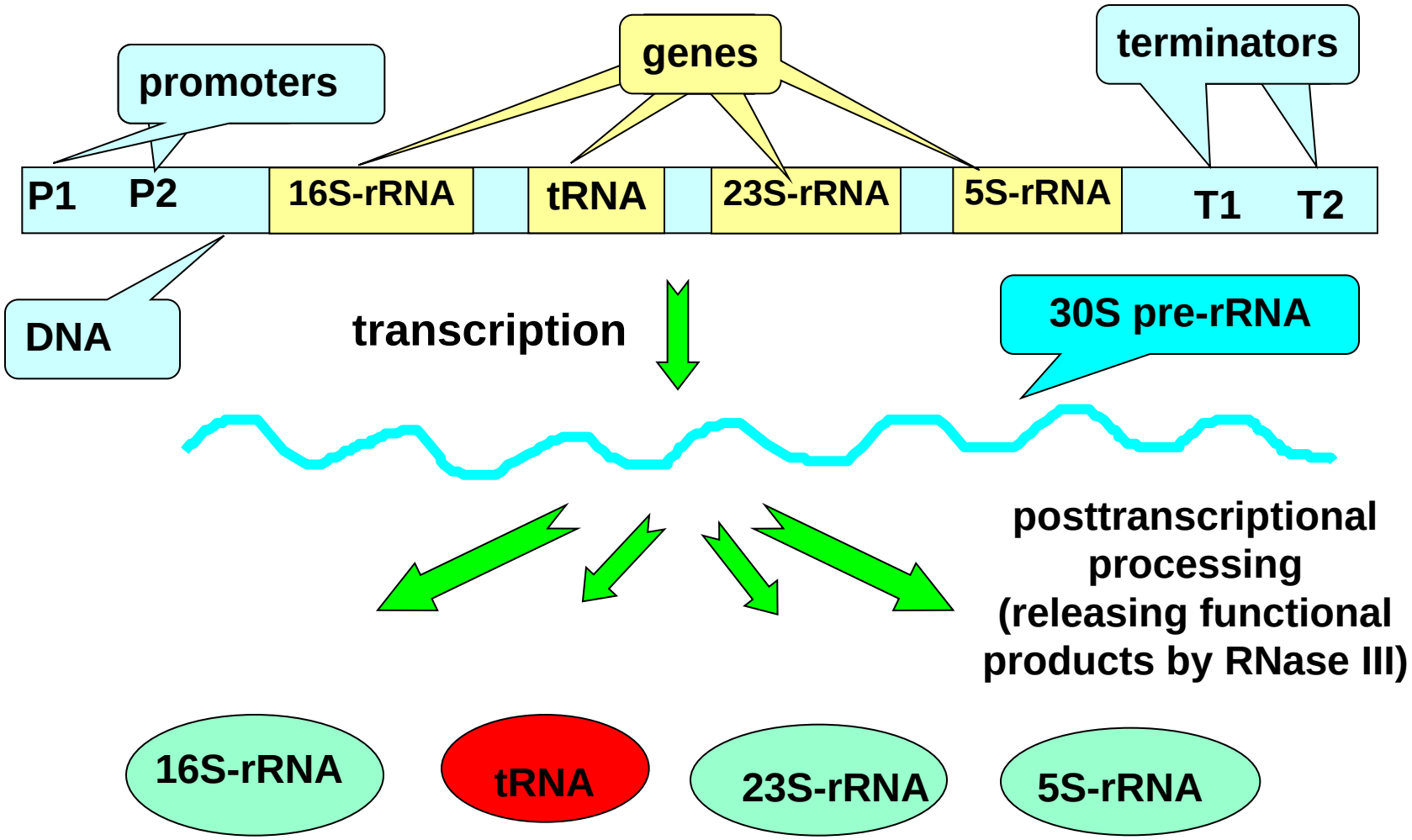
Efficient binding and progression of ribosomes along mRNA increase the speed of RNA polymerase, whereas the absence of ribosomes allows the polymerase to slow and wait for ribosomes to catch up.



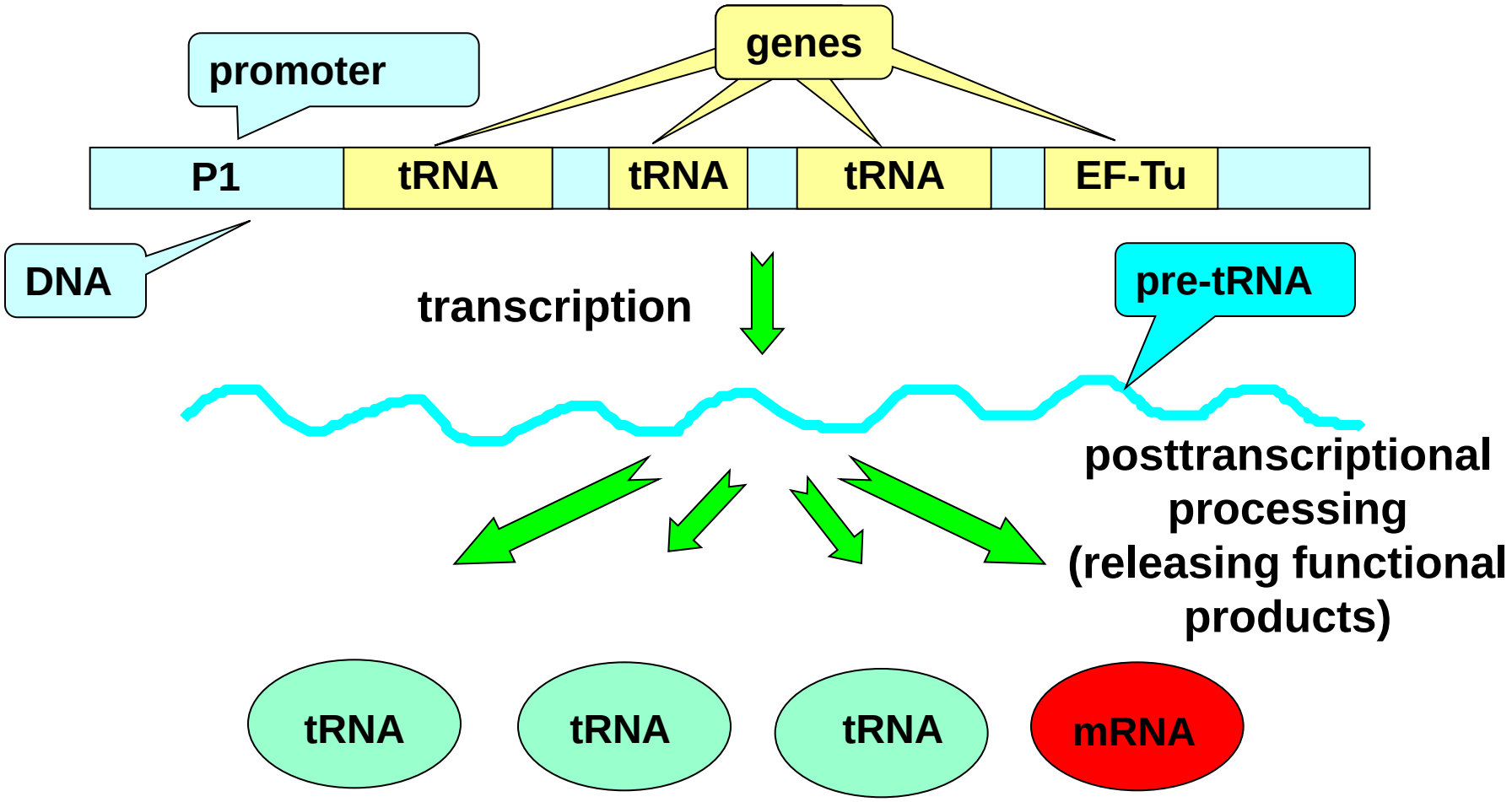
J. W. Roberts Science 328, 436-437 (2010)

Science AAAS

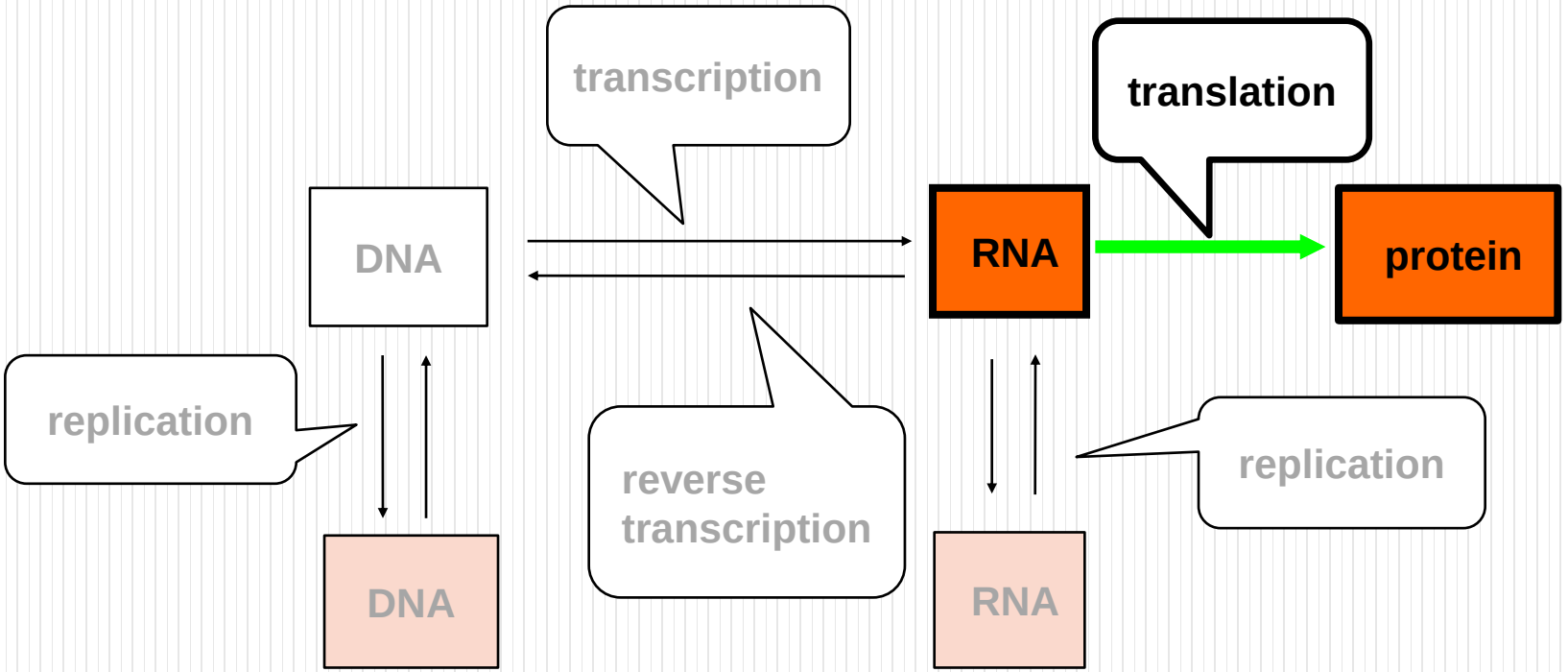
Transcription the genes for rRNA



Transcription the genes for tRNA



Translation of prokaryotic genom

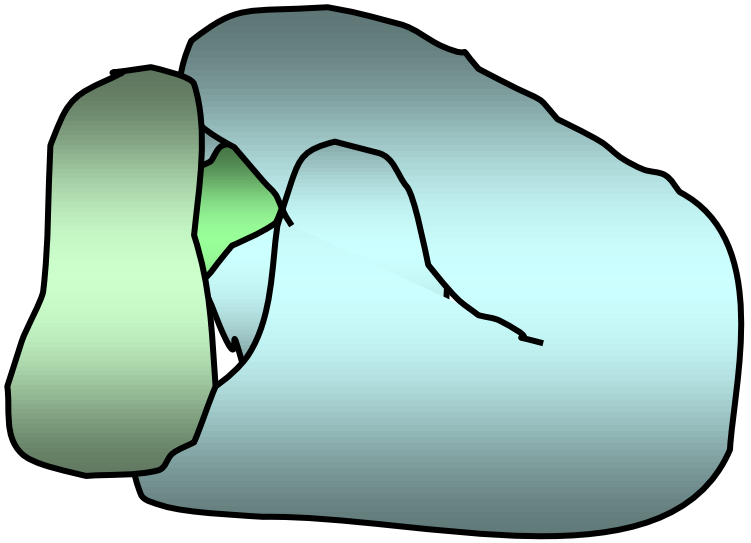


Definition of translation

- Protein synthesis
- Synthesis of polypeptide chain according the genetic code of mRNA on ribosomes
- The final process of gene (genetic information) expression

Participants of translation

- 22 activated standard amino acids
- aminoacyl-tRNA-syntetases
- tRNA
- ribosomes

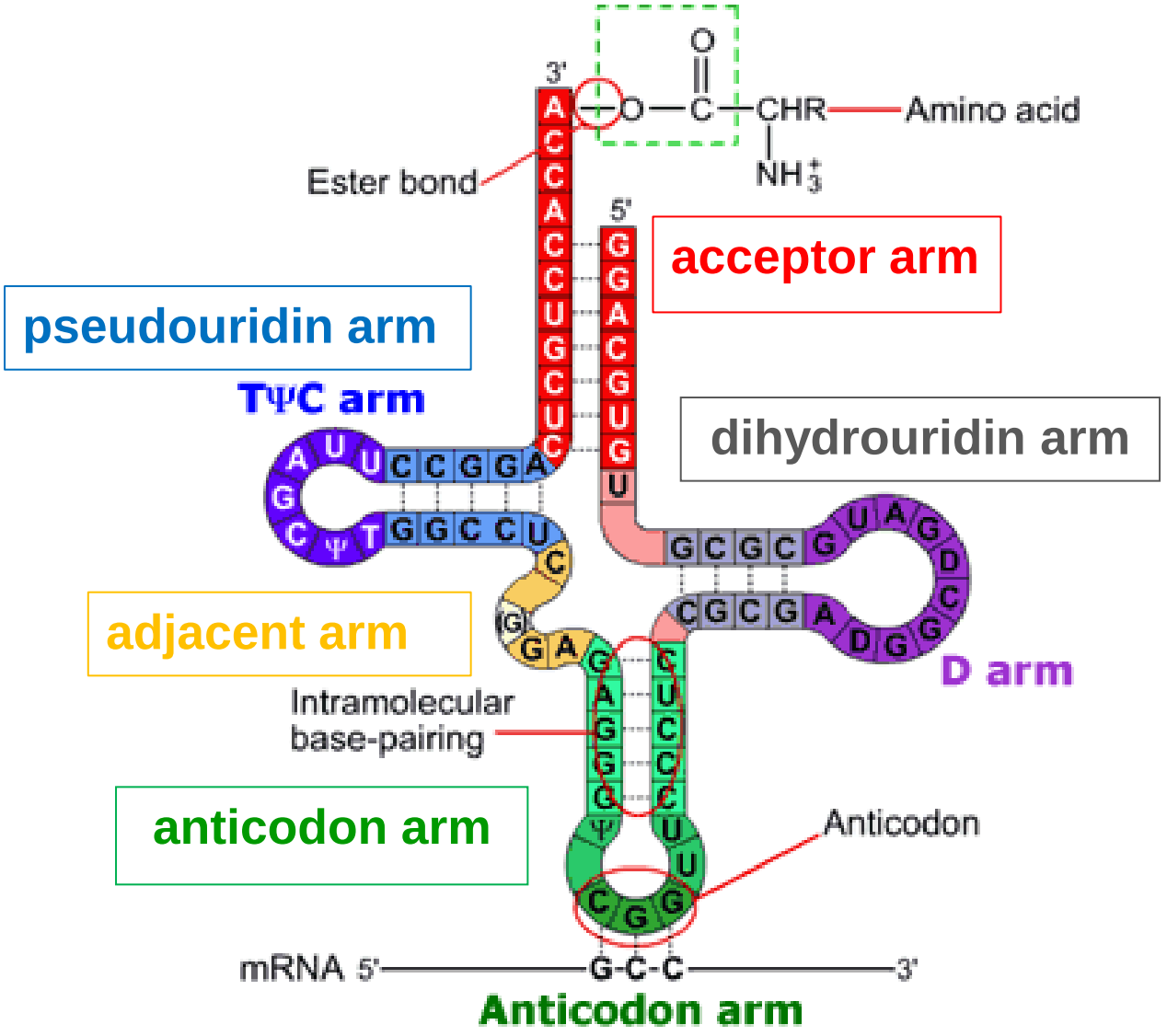


The phases of translation

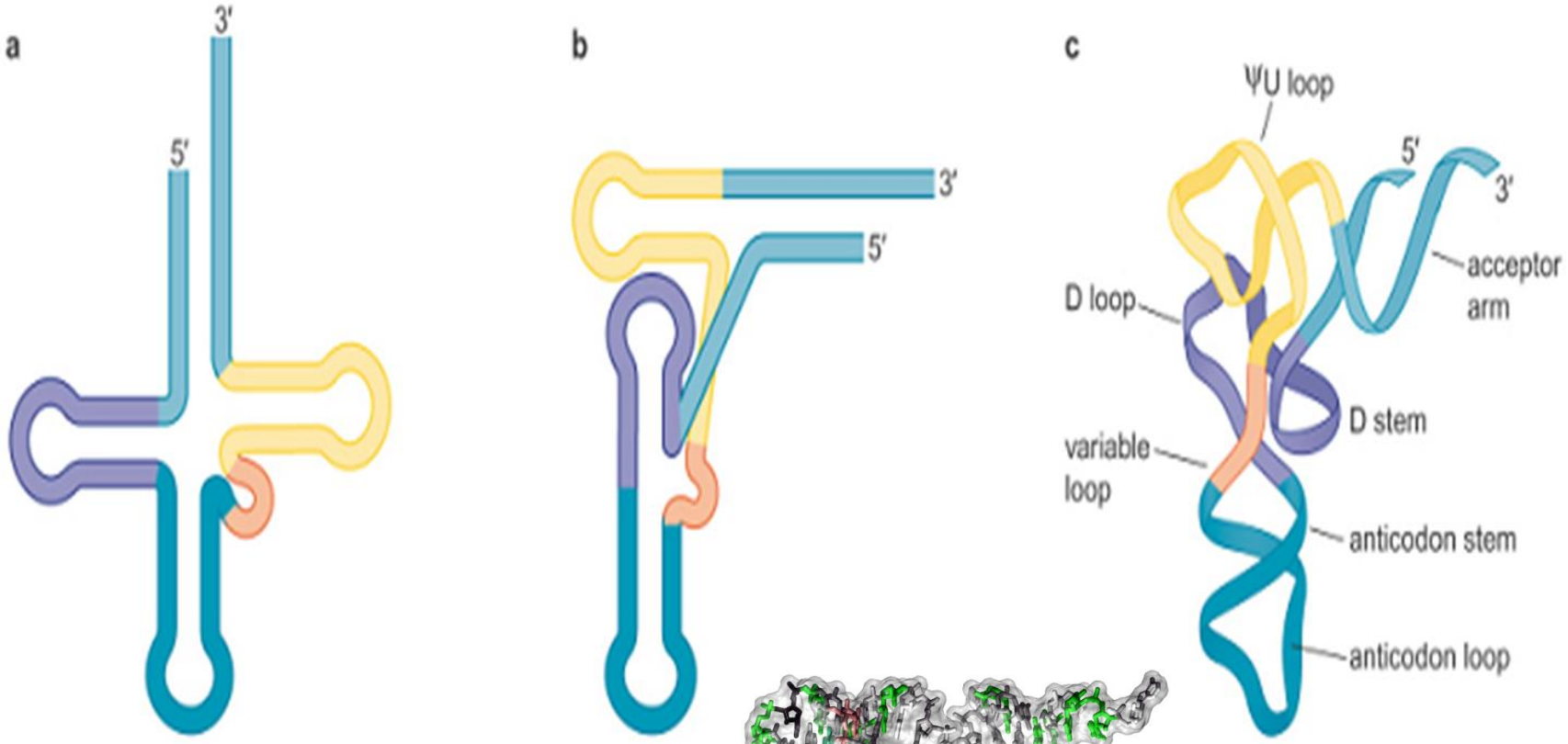
- **Activation of AA = amino acylation, charging**
  - **Process in which AA is attached to the specific tRNA**
- **Iniciation**
  - **A sequence of processes which produce initiation complex = ribosome 70S, mRNA, initiator tRNA, initiation factors**
- **Elongation**
  - **The addition of AAs to the growing polypeptide chain, elongation factors**
- **Termination**
  - **Finishing the synthesis on stop codon, releasing polypeptide from ribosome, termination factors**

The secondary structure of tRNA

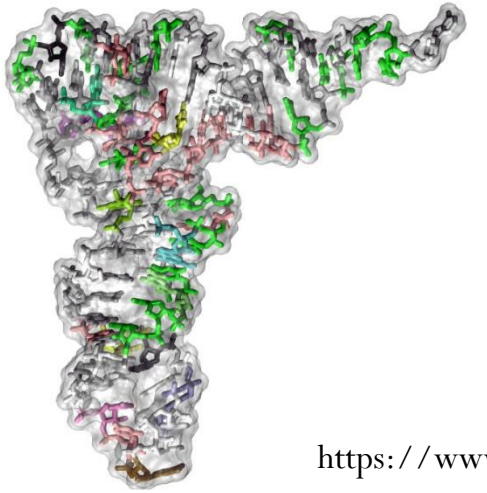
cloverleaf



The tertiary structure of tRNA

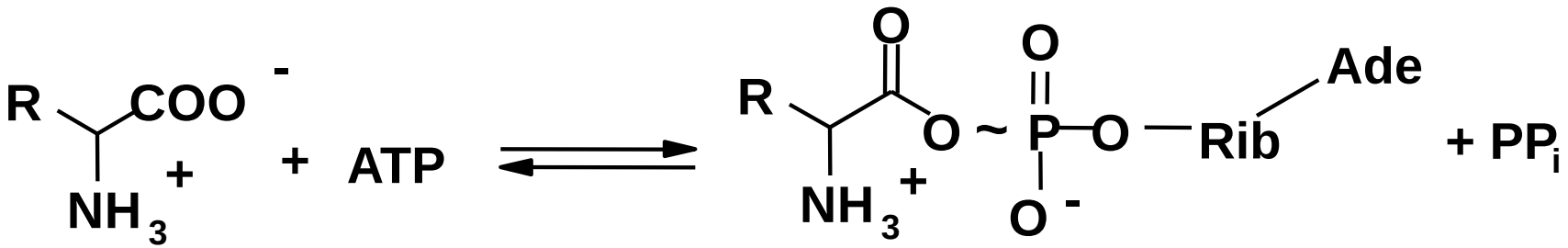


http://bioindians.org/



https://www.irbbarcelona.org

Activation of amino acids

1. 

Macroenergetic bound

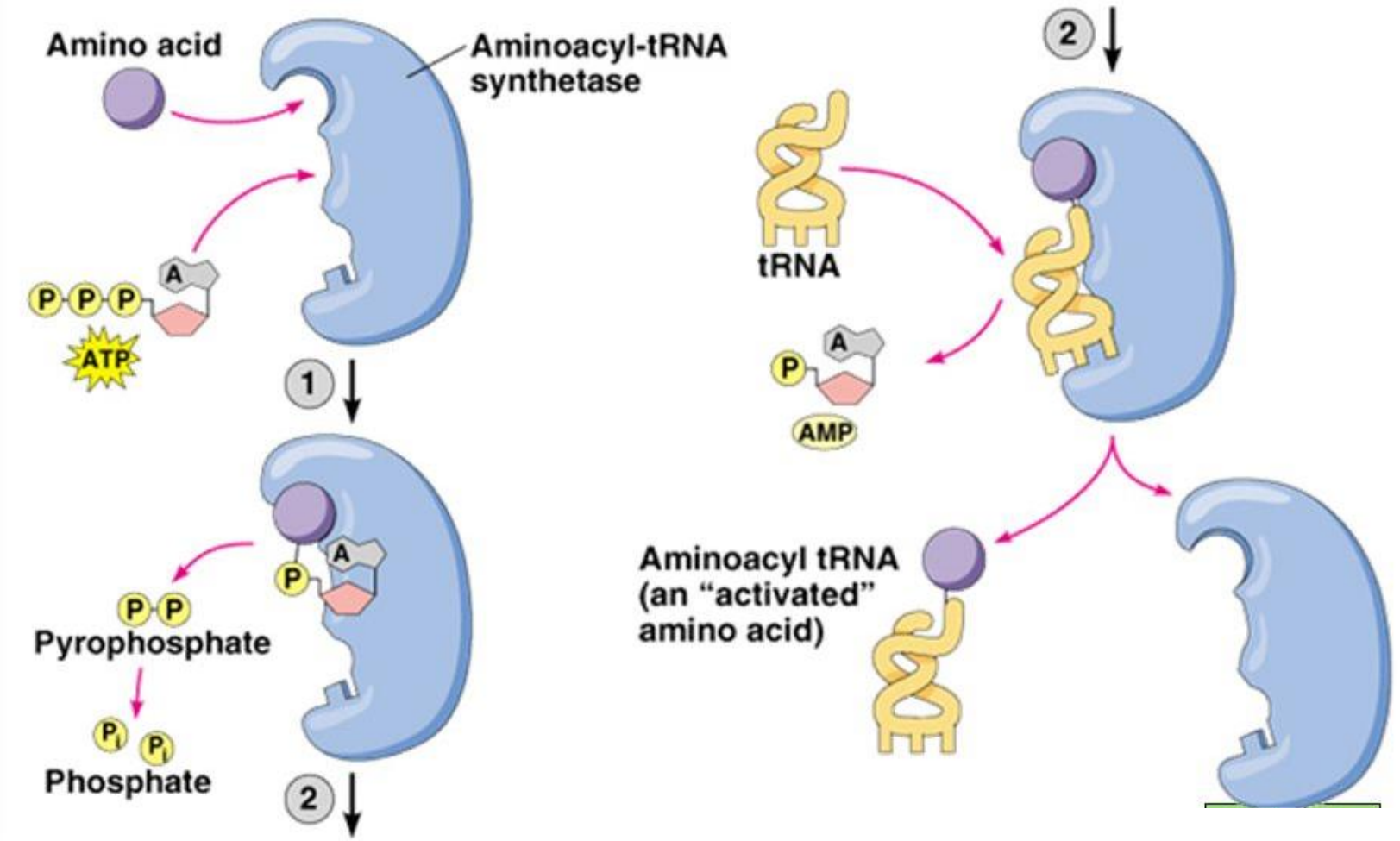
aa~AMP

aminoacyladenylate

2. aa ~ AMP + tRNA → aa ~ tRNA + AMP

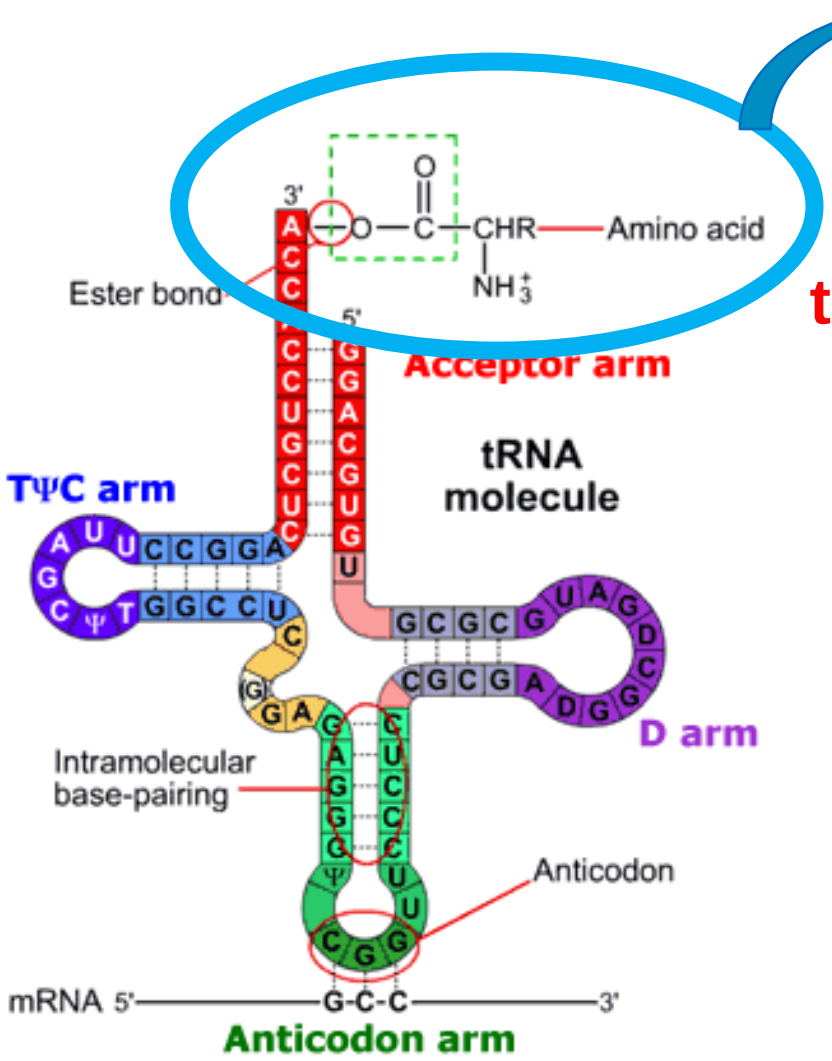
catalysed by aminoacyl-tRNA-synthetase

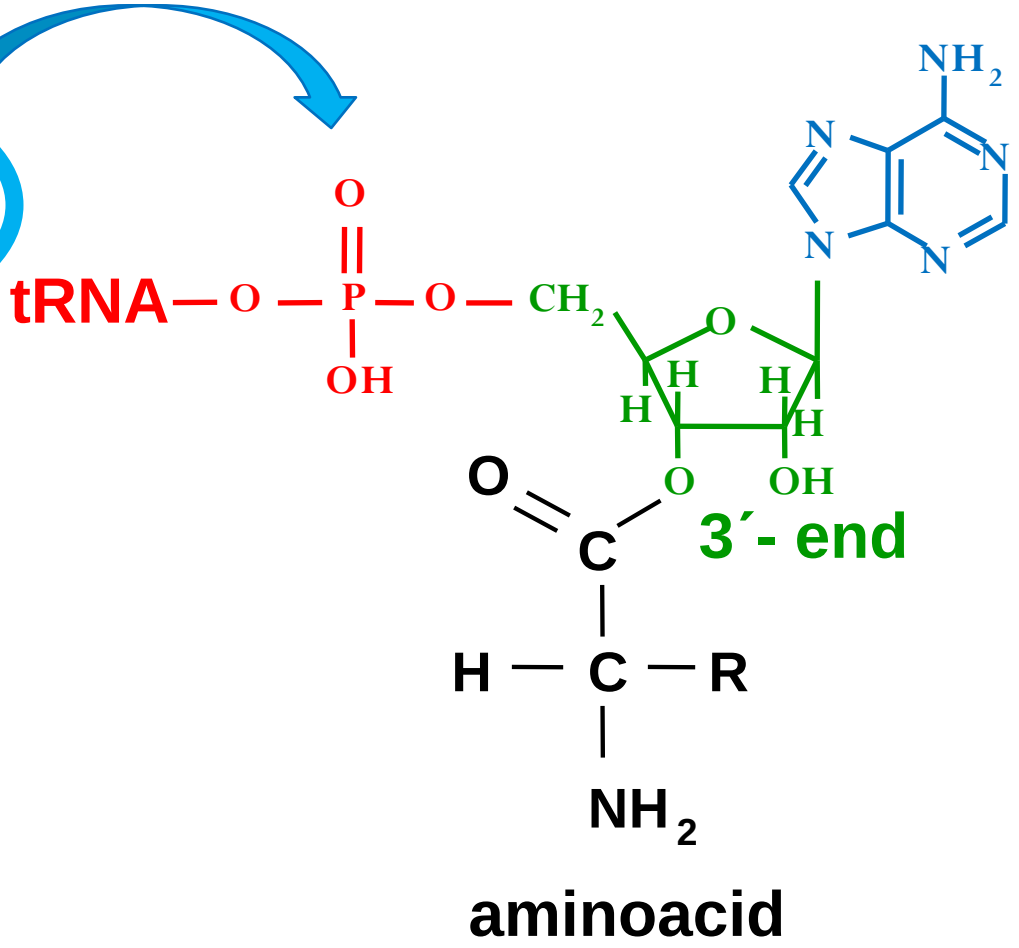
Activation of amino acids



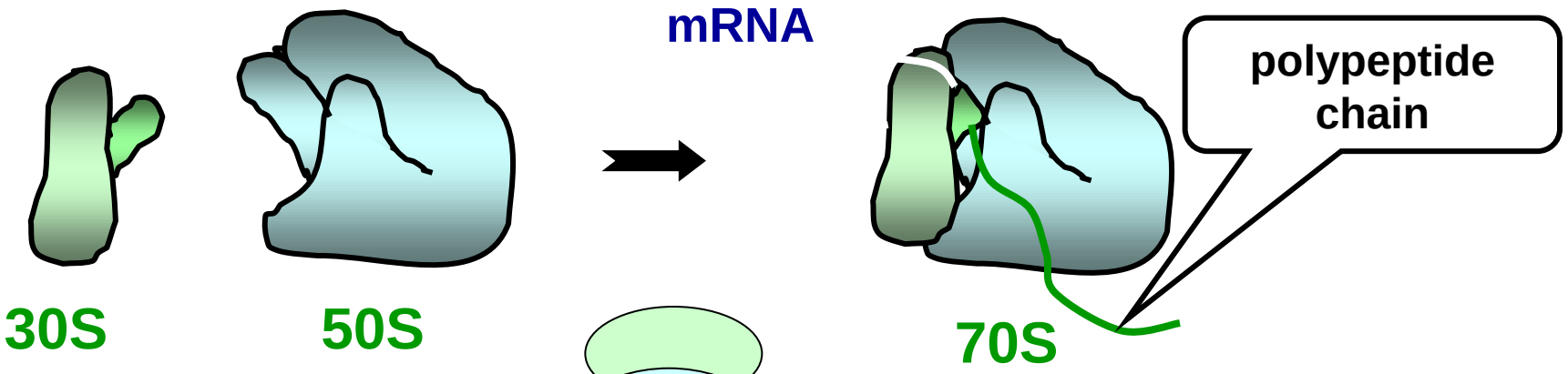
http://slideplayer.com/slide/8284821/

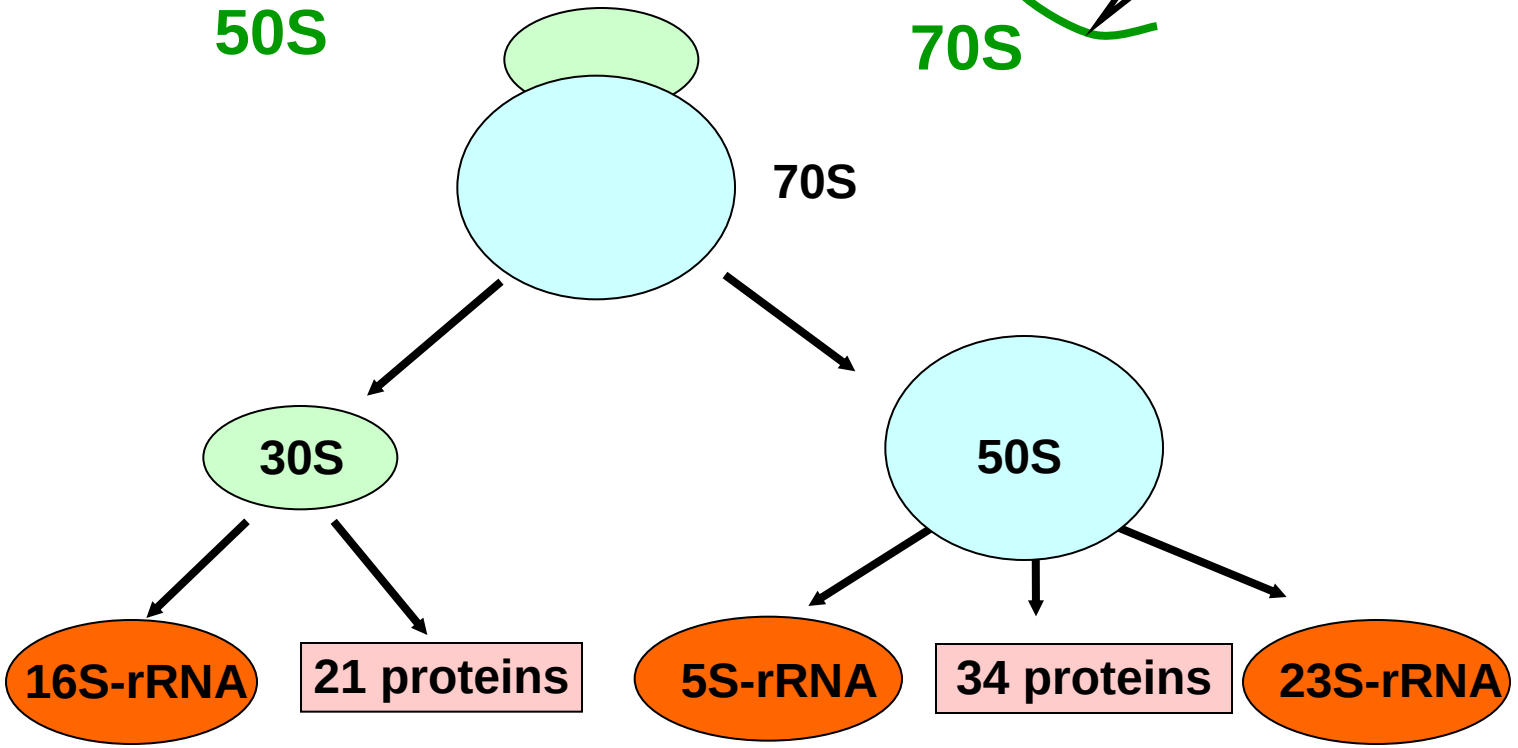
Structure of aa~tRNA



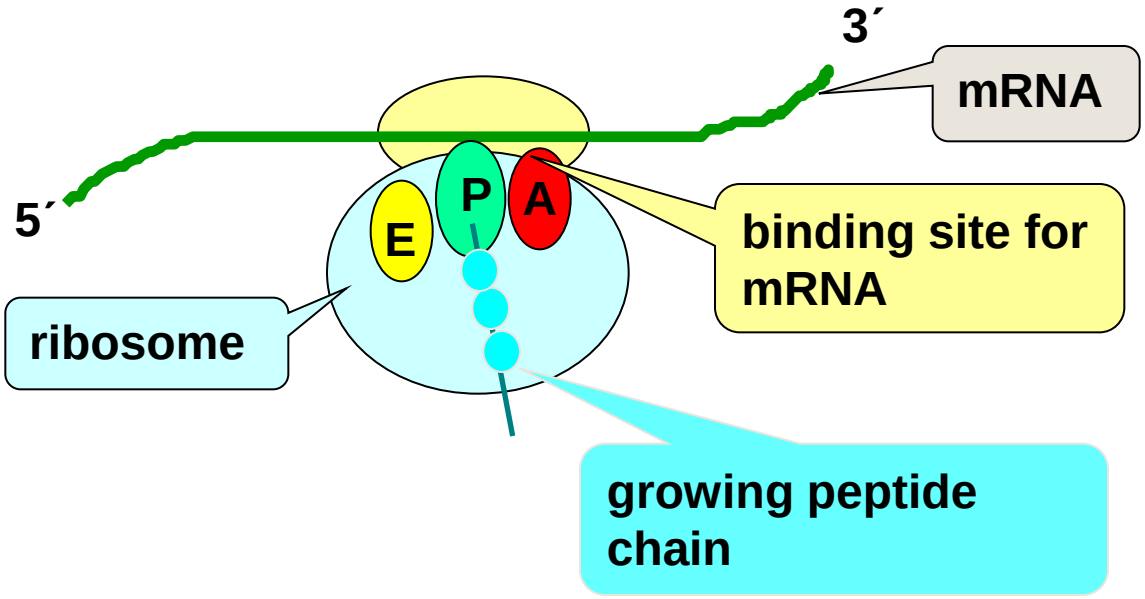


Prokaryotic ribosomes: 70S ☞ 30S a 50S





Binding sites on ribosome



- binding site for mRNA
- aminoacyl site (A site)
- peptidyl site (P site)
- exit site for deaminoacylated tRNA (E-site)
- binding sites for initiation and elongation factors

How the translation begins ?

1) **Prominence of the codon AUG**

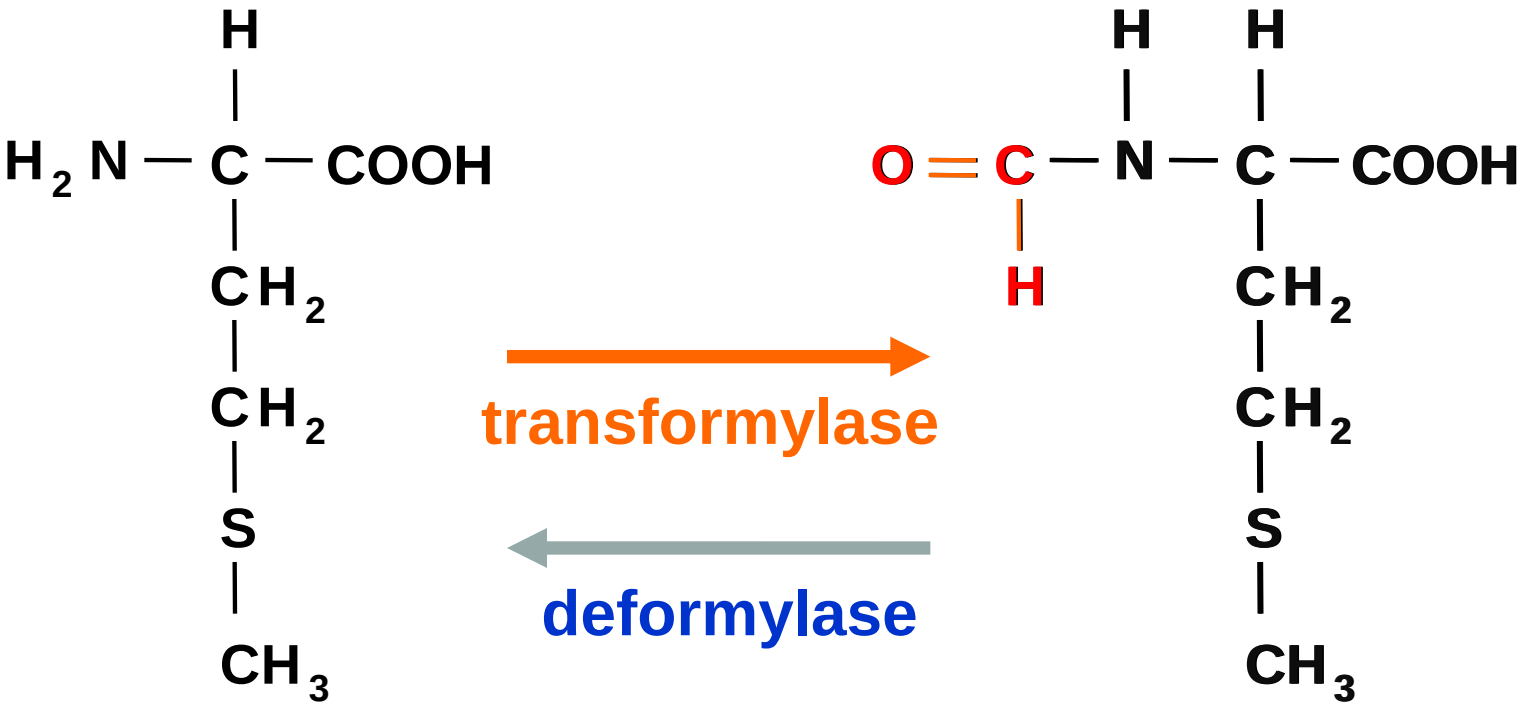
   - it codes the first AA in polypeptide chain

   - it is present also inside of polypeptide chain

2) **The codon AUG codes for methionine, nevertheless formylmethionine is at the beginning of polypeptide chain**

   - two tRNA for methionine exist – $tRNA^{Met}$ and $tRNA_i^{fMet}$

Formylmetionine

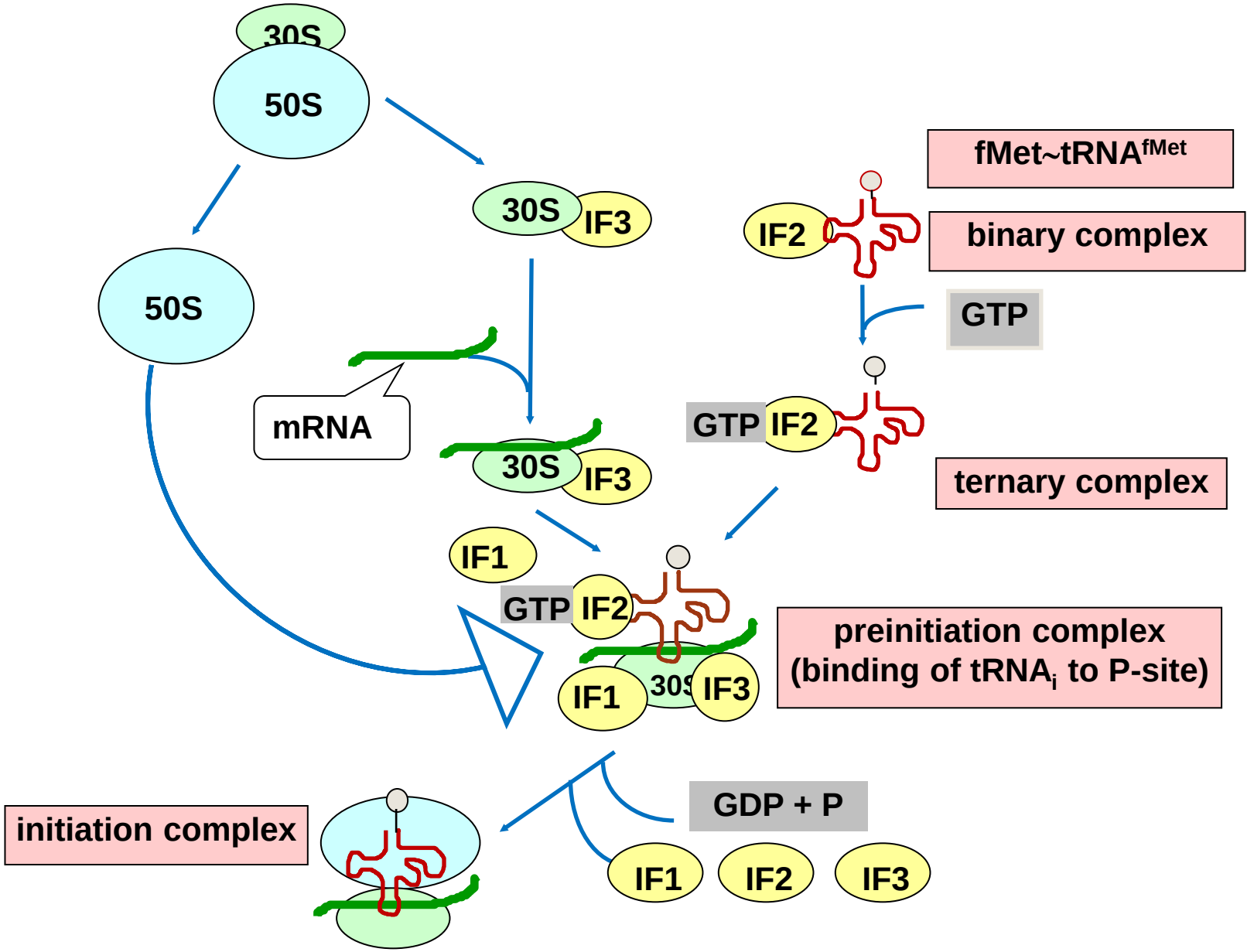


Methionin is formylated on Met ~ $tRNA^{fMet}$

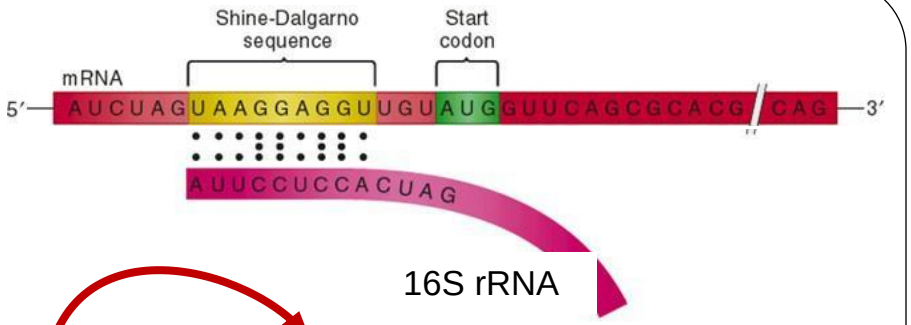
The codon 5´- AUG - 3´

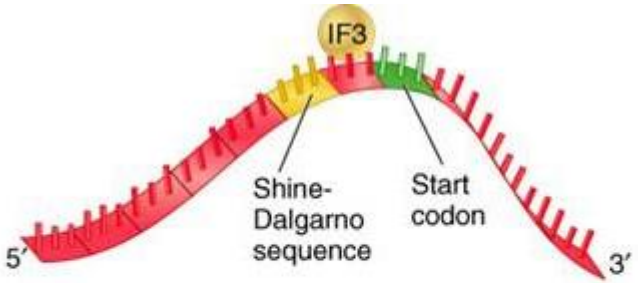
1) If it is at the beginning it binds Met~tRNA^{fMet}, which is formylated for fMet~tRNA^{fMet}, the initiation factor IF2 attends in the process
2) If it is inside, it binds Met~tRNA^{Met}, the elongation factor EF-Tu attends in the process

Initiation of translation (prokaryotic)

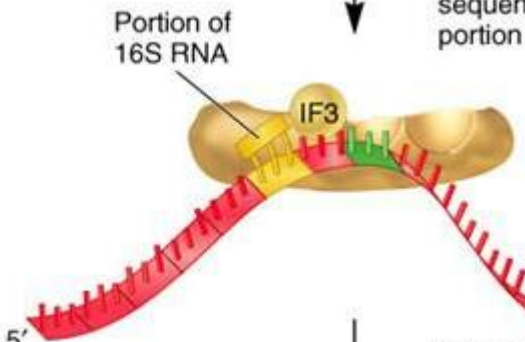


Initiation of translation

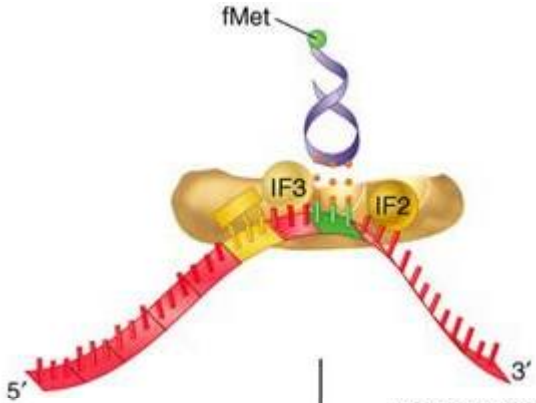




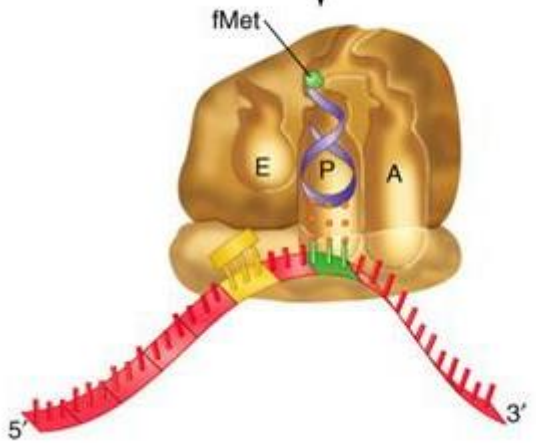
IF3 promotes the binding of mRNA to the 30S subunit. The Shine-Dalgarno sequence is complementary to a portion of the 16S rRNA.



IF2 promotes the binding of the initiator tRNA.

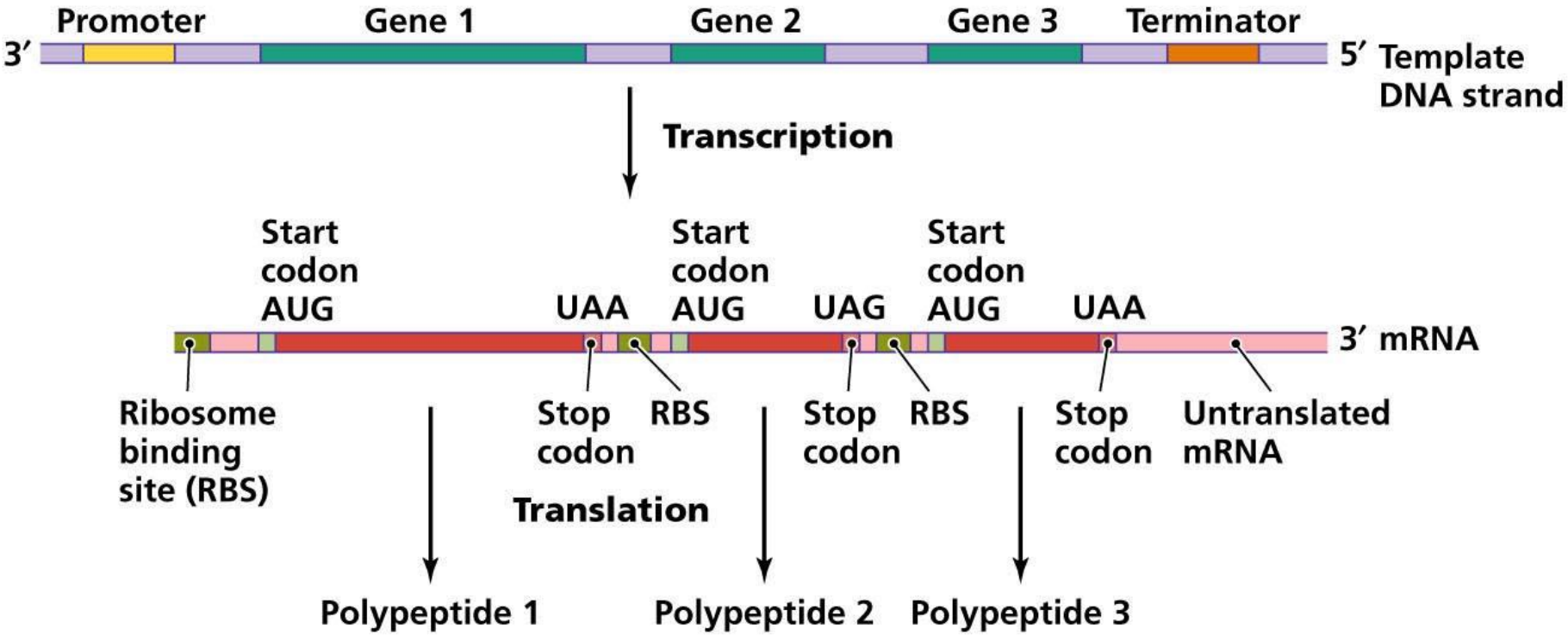


IF2 and IF3 are released. The 50S subunit associates.



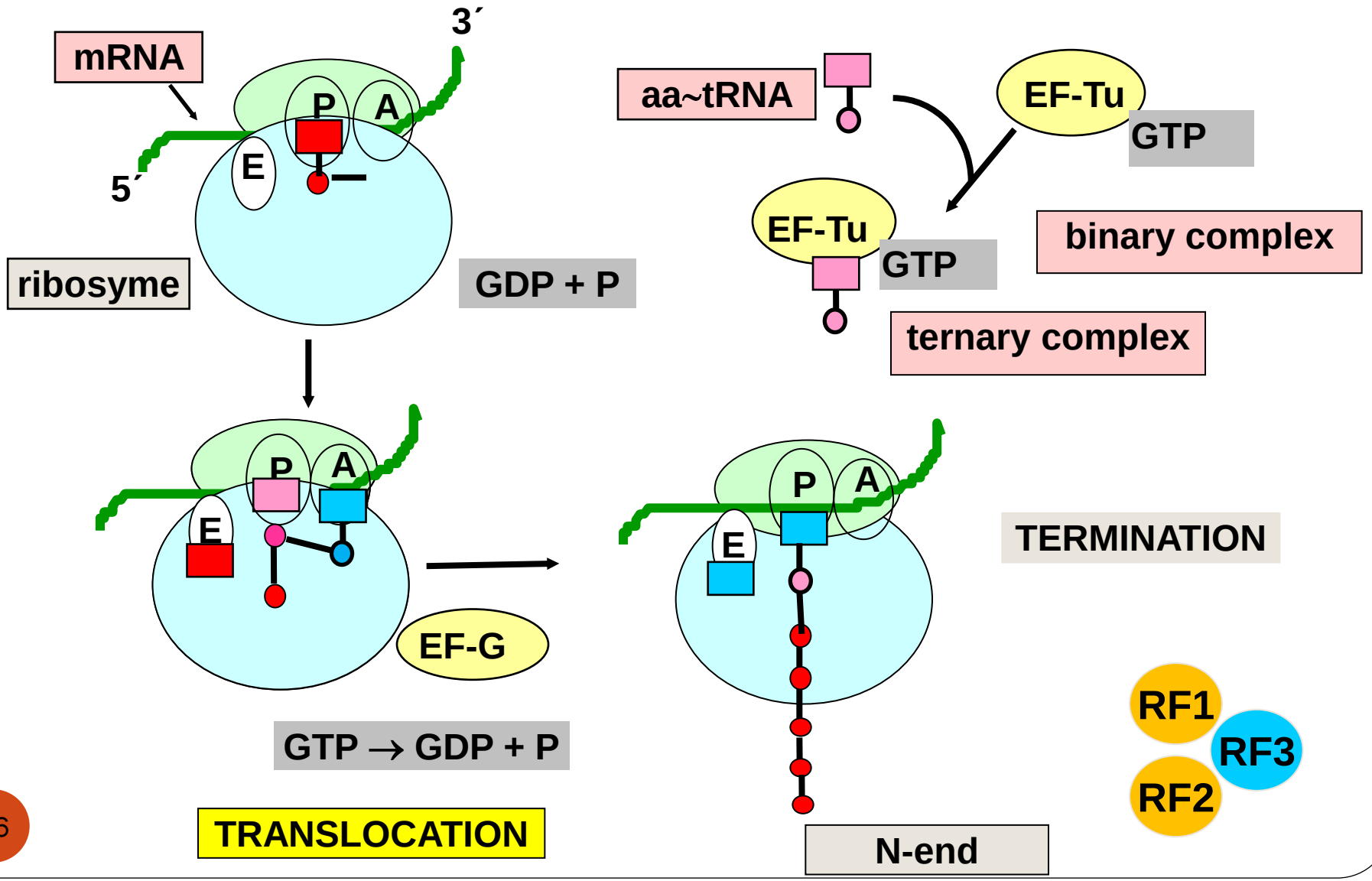
http://slideplayer.com/slide/3524858/

Ribosomes bind also on intergenes sequences

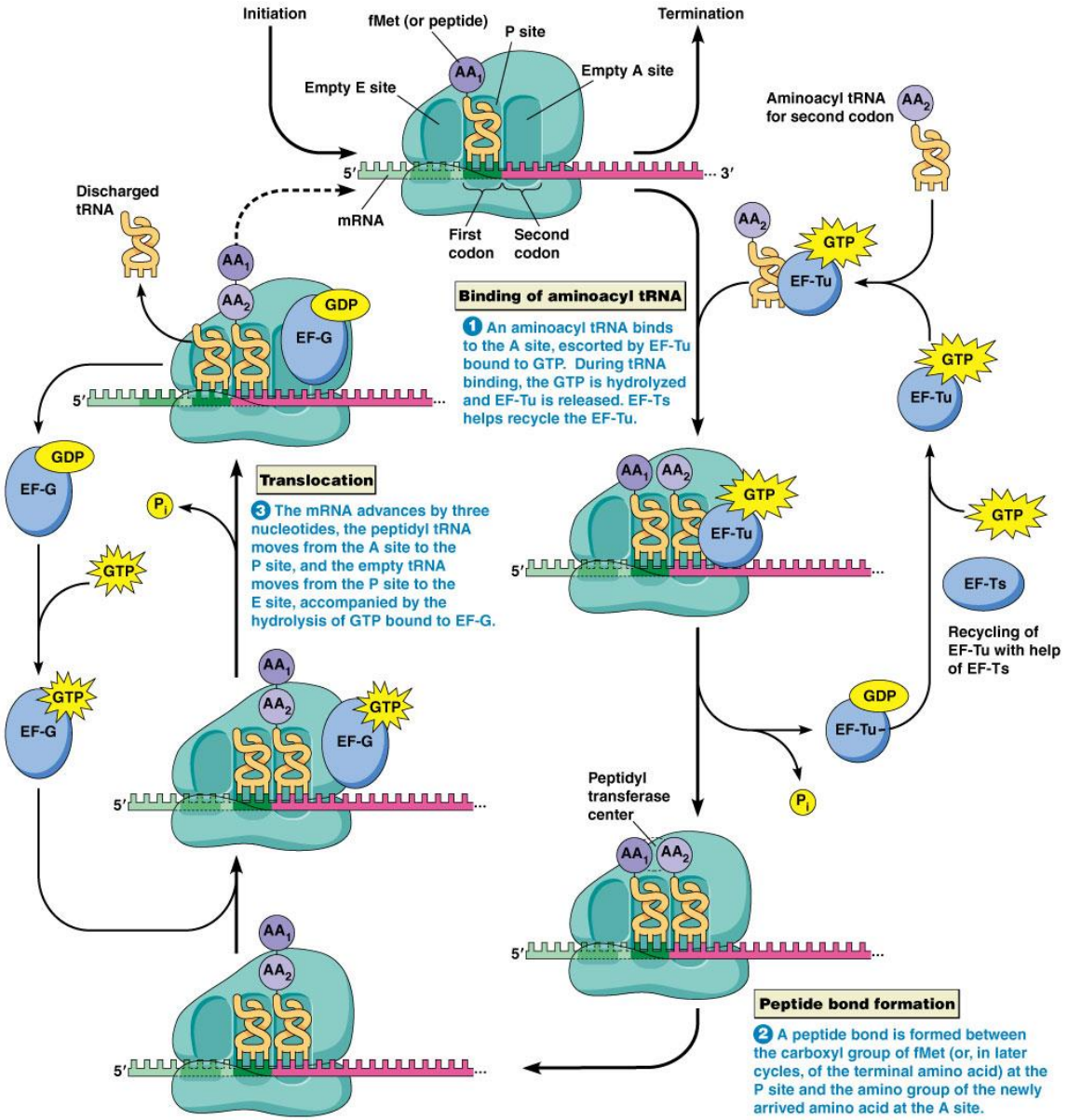


Copyright © 2006 Pearson Education, Inc., publishing as Benjamin Cummings.

Elongation of translation (prokaryotic) – Peptide growths N-end → C-end



Elongation of polypeptide chain



Speed of elongation for *E. coli* 10-20 AA/s

© 2012 Pearson Education, Inc.

Termination of translation

- the presence of nonsense codon
- the presence of releasing factors RF1 (for UAG and UAA), RF2 (for UGA and UAA) and RF3 (stimulates the effect of RF1 and RF2)
- tRNA releases from carboxy end of polypeptide chain, and growing of this chain stops
- Polypeptide chain and ribosome are released, the ribosome divides to its subunits

Posttranslation proceses

- Cotranslation modifications:
  - Deformylation
  - Cutting of AA from N-end
  - Chemical modification of AA
  - Creation of disulfidic bridges
  - Glycosilation
  - Formation of secondary and tertialy structure

- Posttranslation modifications:
  - Peptides cut off
  - Formation of quarternary structure
  - Binding of prostetic groups
  - Formation of supramolecular complexes

Translation - video

Initiation

https://www.youtube.com/watch?v=glsrY4dJzh8

Elongation

https://www.youtube.com/watch?v=PpAg2K_7ID4

Termination

https://www.youtube.com/watch?v=MNMc28EEkK0