Structure of prokaryotic genom, replication and gene expression in prokaryots

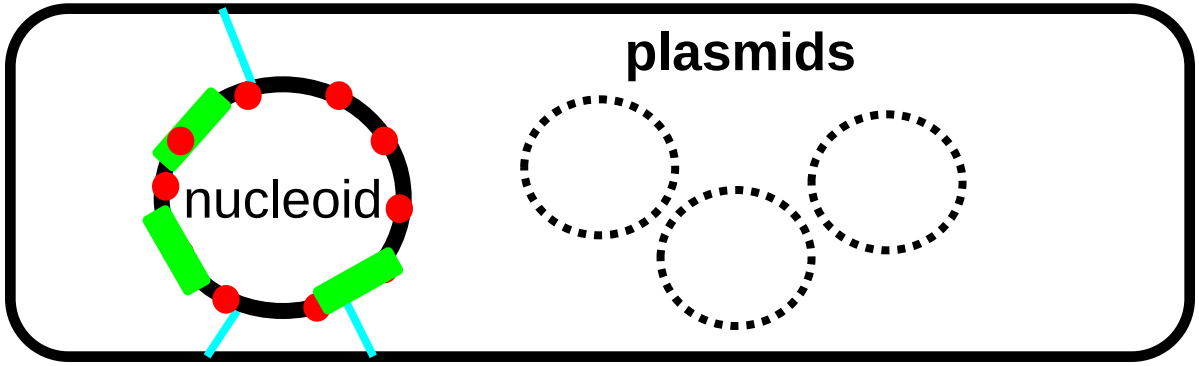
Molecular Biology

RNDr. Jan Hošek, Ph.D.

hosekj@vfu.cz

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)

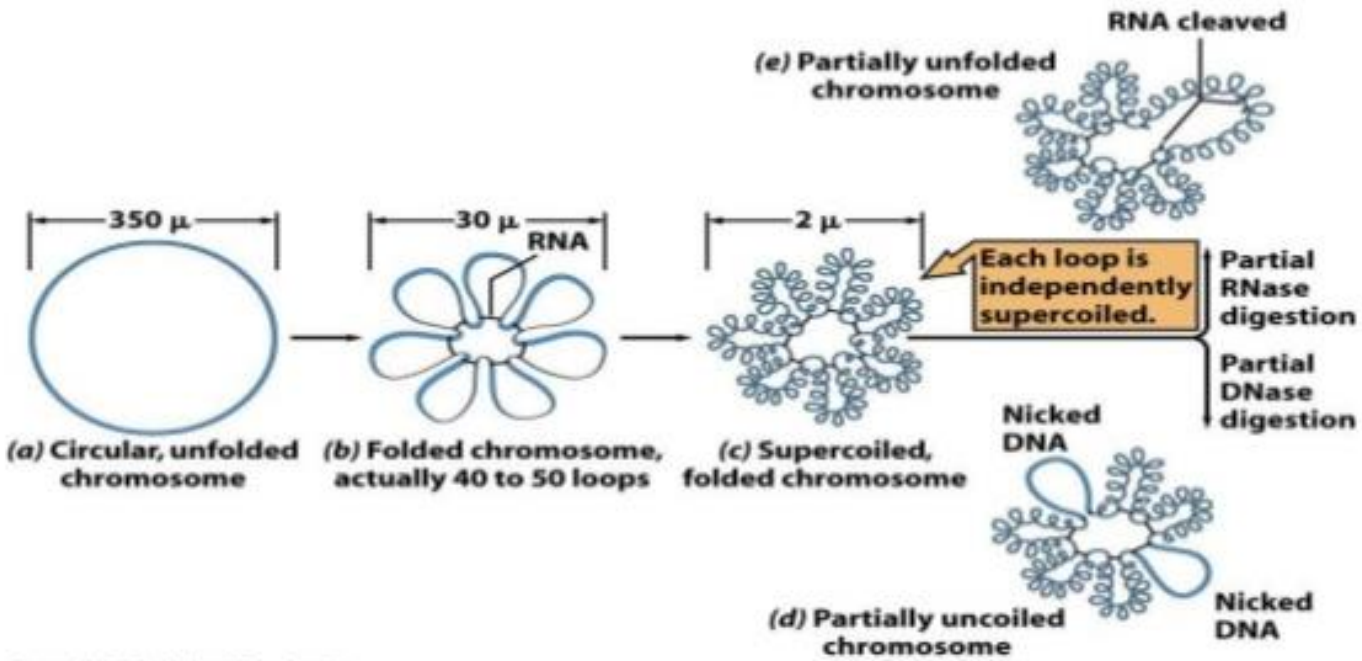


Figure 9-15 Principles of Genetics, 4/e
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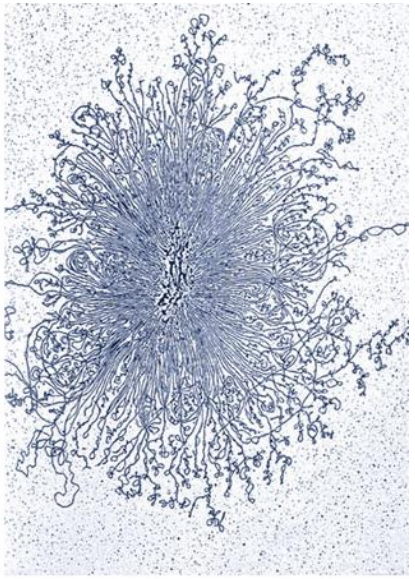
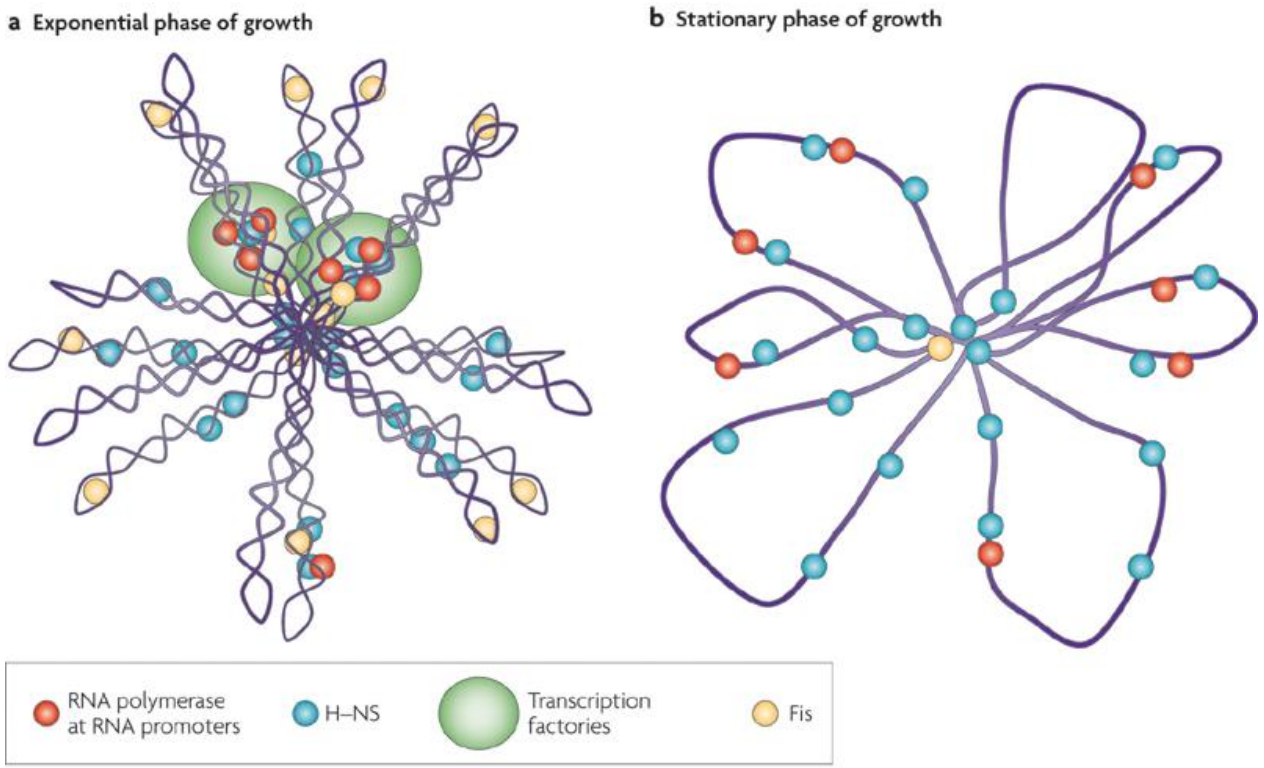


Nature Reviews | Genetics

doi:10.1038/nrg3375

Dynamic of nucleoid



doi:10.1038/nrmicro2261

Nature Reviews | Microbiology

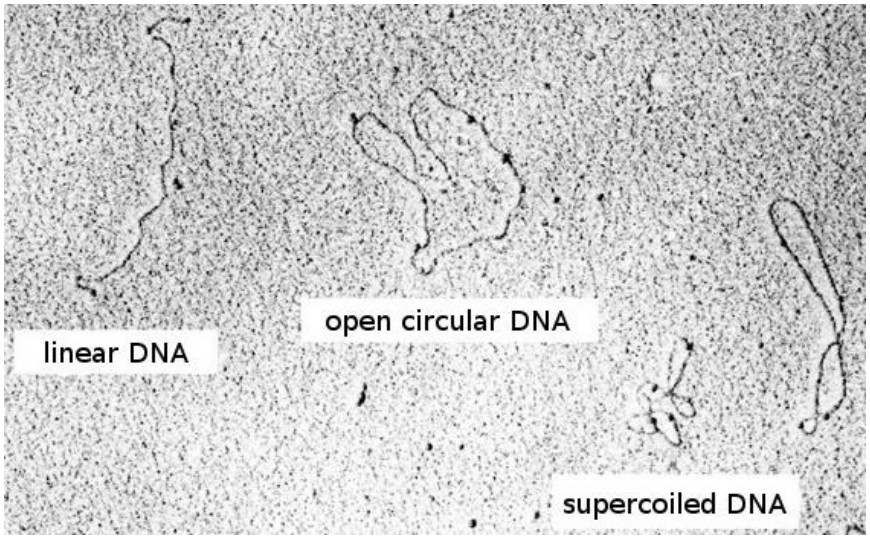
a | The folded chromosome is organized into looped domains that are negatively supercoiled during the exponential phase of growth. In this phase, the abundant nucleoid-associated proteins histone-like nucleoid-structuring protein (H-NS) and factor for inversion stimulation (Fis) bind throughout the nucleoid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

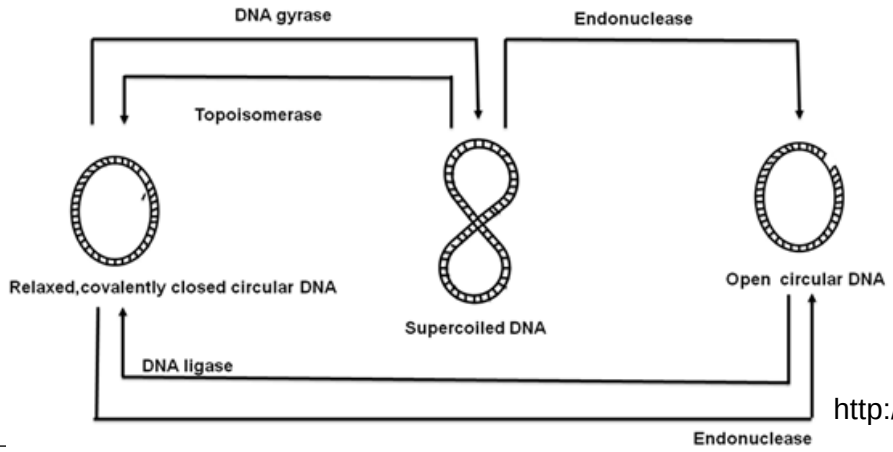
Plasmids

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

http://www.wikiwand.com/

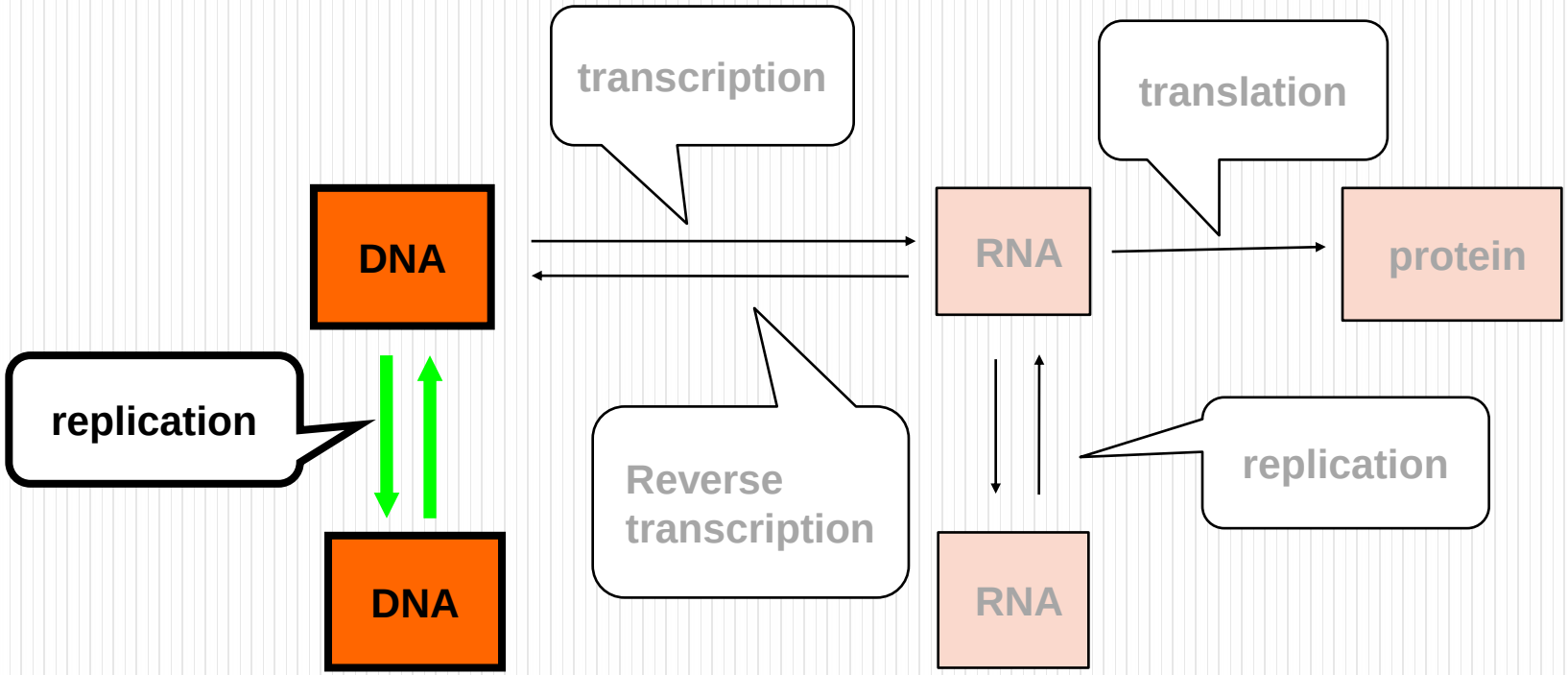
- circular dsDNA
- every is replicon = bears locus ***ori***
- Bears locus ***Inc*** for attachment to membrane





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

Replication of prokaryotic genom



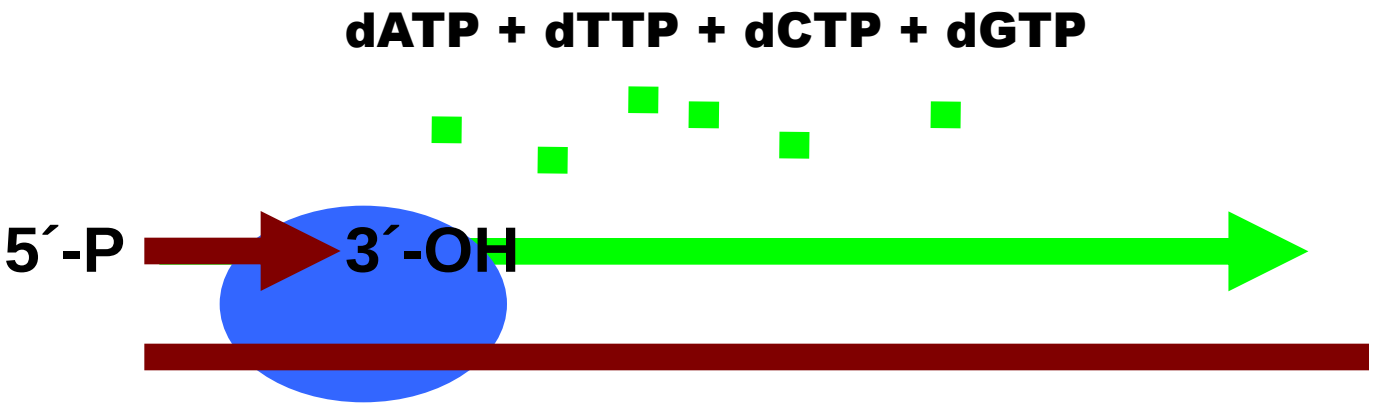
Replication

DNA replication is a process in which the two strands of a DNA double helix are separated and a new complementary strand of DNA is synthesized on each of the two parental template strands.

This mechanism ensures that genetic information will be copied faithfully at each cell division.

What is needed for DNA replication ?

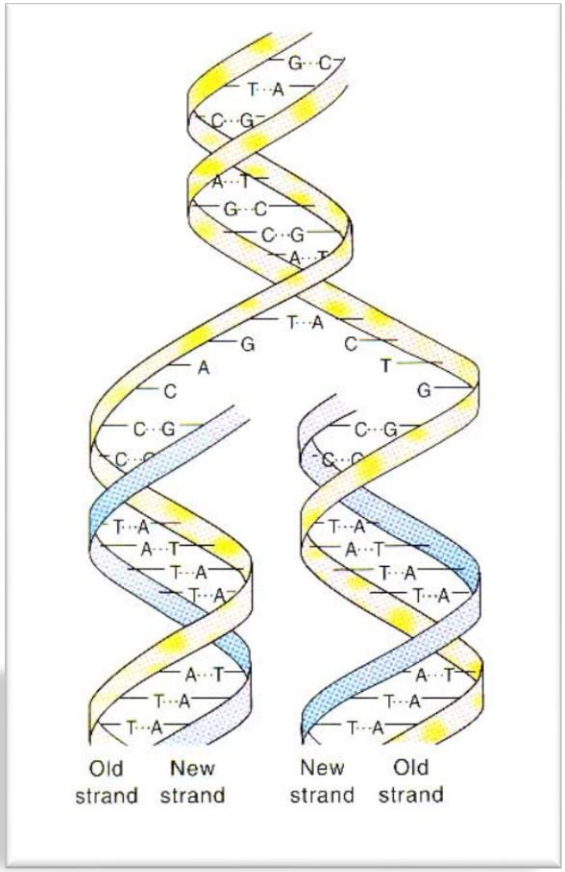
- Template strand (DNA matrix)
- Primer (free 3´-OH end)
- Polymerase + replication proteins
- dNTP



Characteristic features of replication

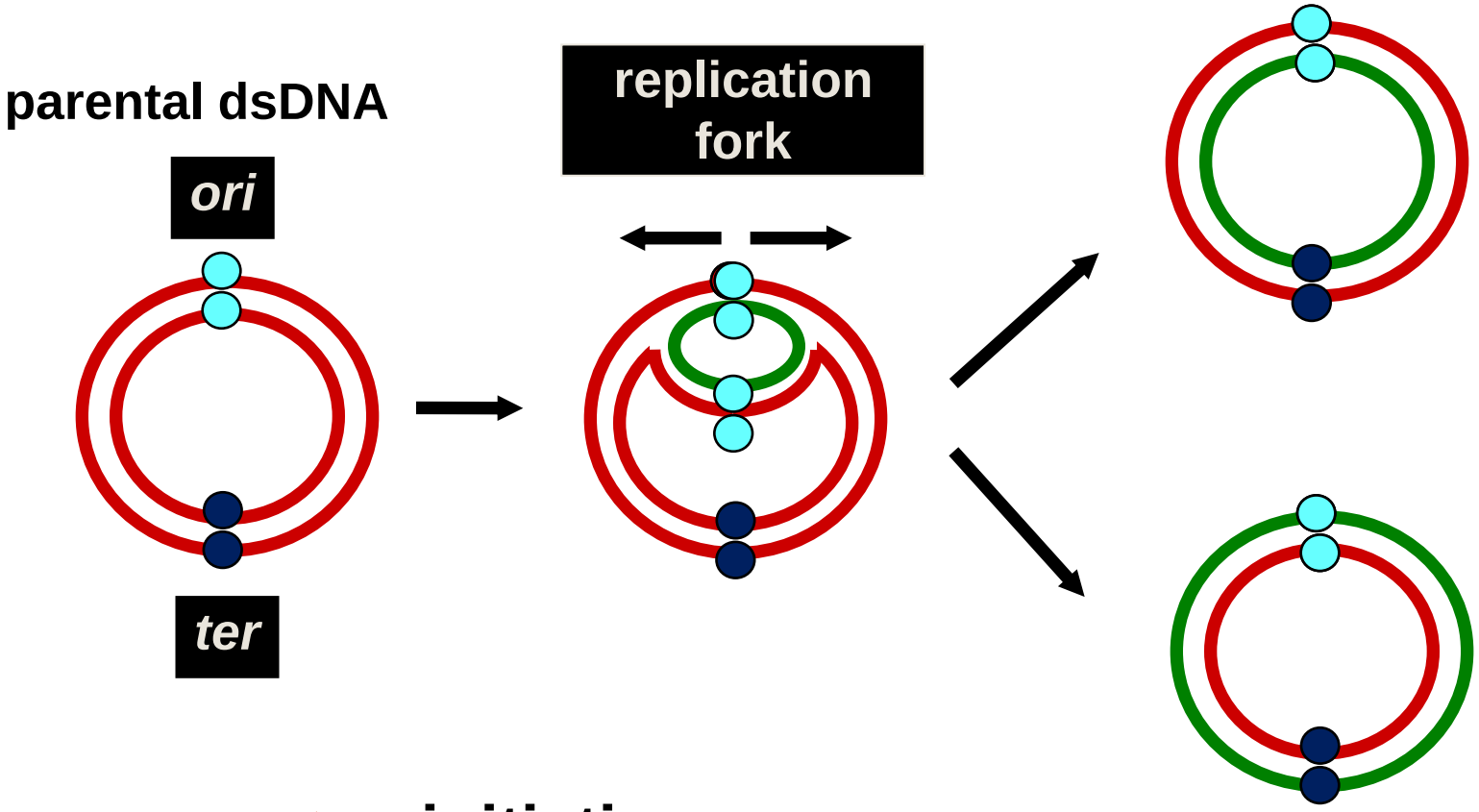
- It is **semiconservative**
  - **Meselson – Stahl experiment** → „the most beatiful experiment in biology“

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



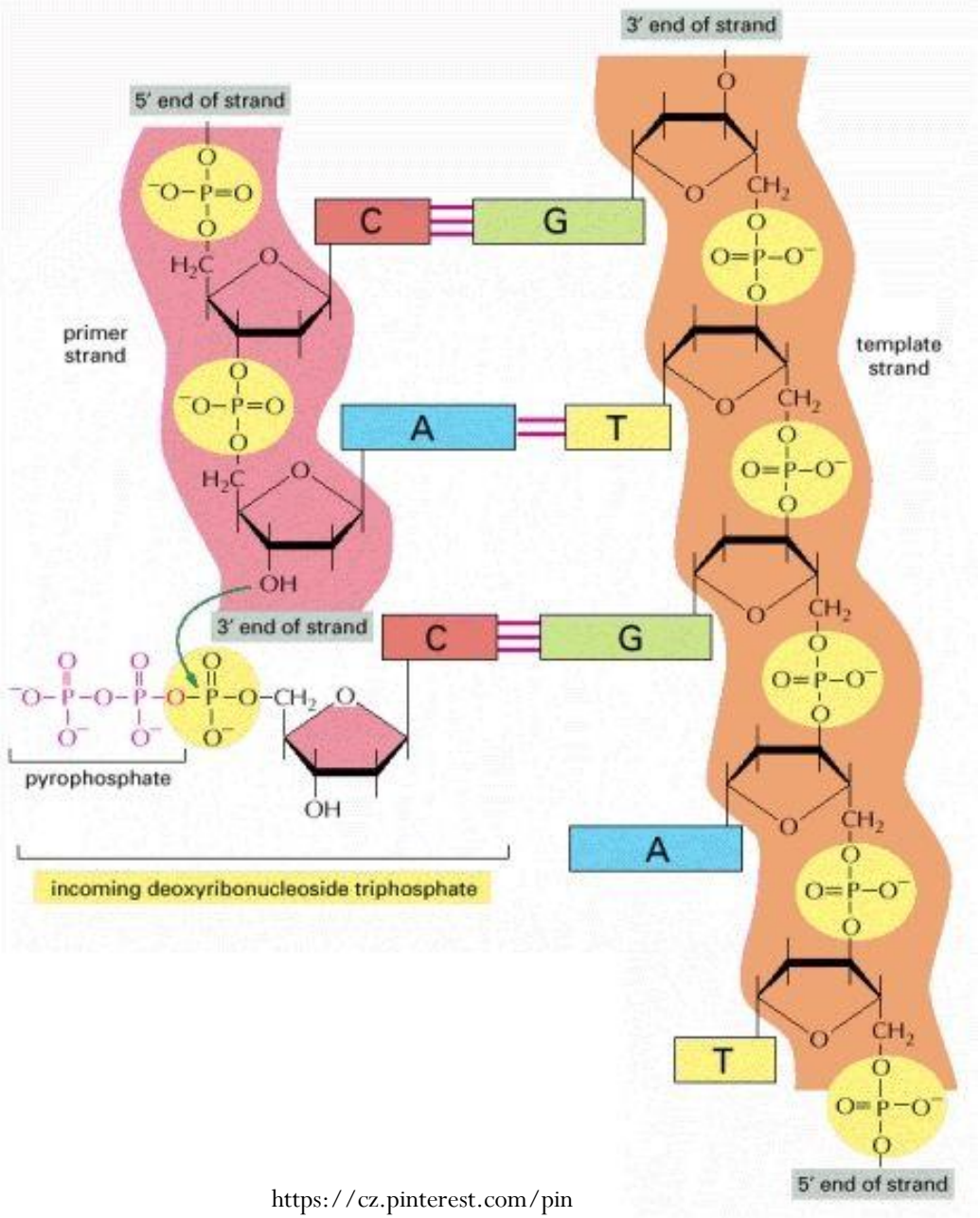
- It is **semidiscontinuous**
  - …Keep a while ☺

Replication of prokaryotic genom



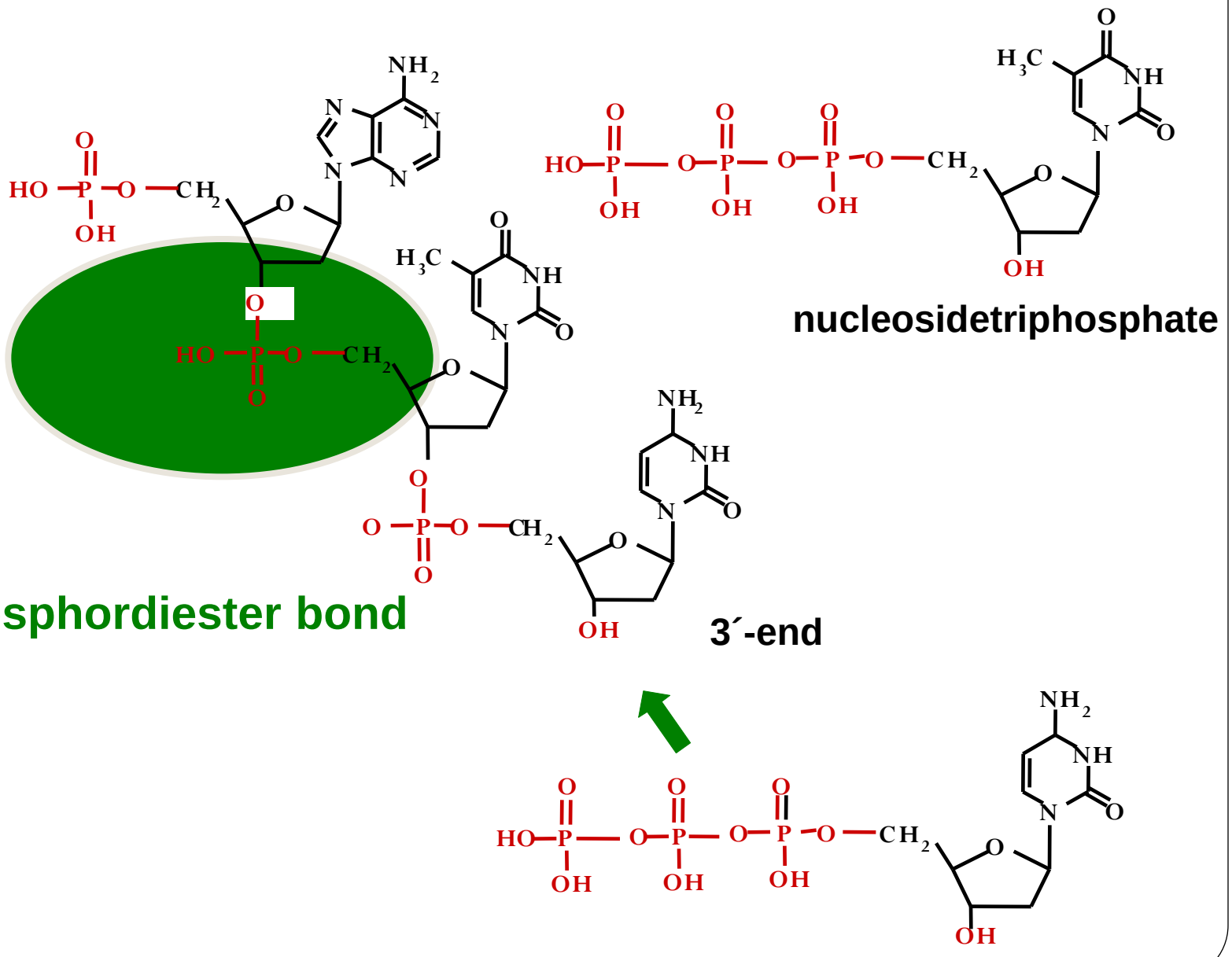
- initiation
- elongation od DNA strand
- termination

Synthesis of DNA during replication



https://cz.pinterest.com/pin

Growing of nucleotide chain



Replication proteins - I

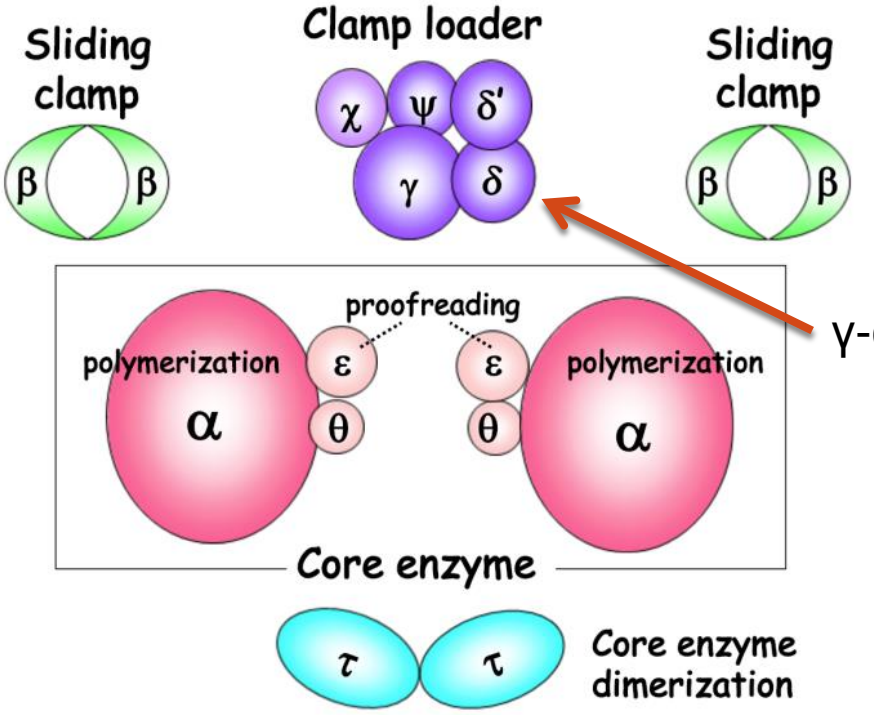
- **DNA polymerase I**
  - **one globular polypeptide, M = 109 000**
  - **polymerase, and 5´- 3´, 3´- 5´ exonuclease activities**
  - **It catalyses replication in space between Okazaki fragments**
  - **It removes RNA-primers by its 5´- exonuclease activity**

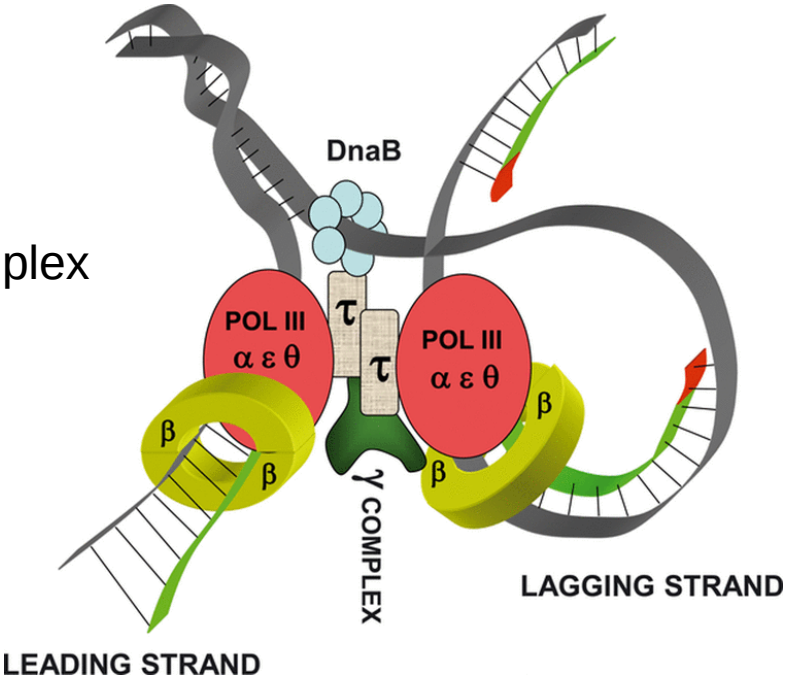
- **DNA polymerase II**
  - **monomeric, M = 90 000**
  - **Polymerase activity**
  - **3´- 5´, 5´- 3´exonuclease activity**

- **DNA polymerase III**
  - **M = 900 000, oligomeric protein which consists of from several units**
  - **It catalyse synthesis of leading strand and Okazaki fragments during replication**
  - **It polymerize by speed 500 nt/min**
  - **It is processive for the whole DNA molecule**

Polymerase III

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

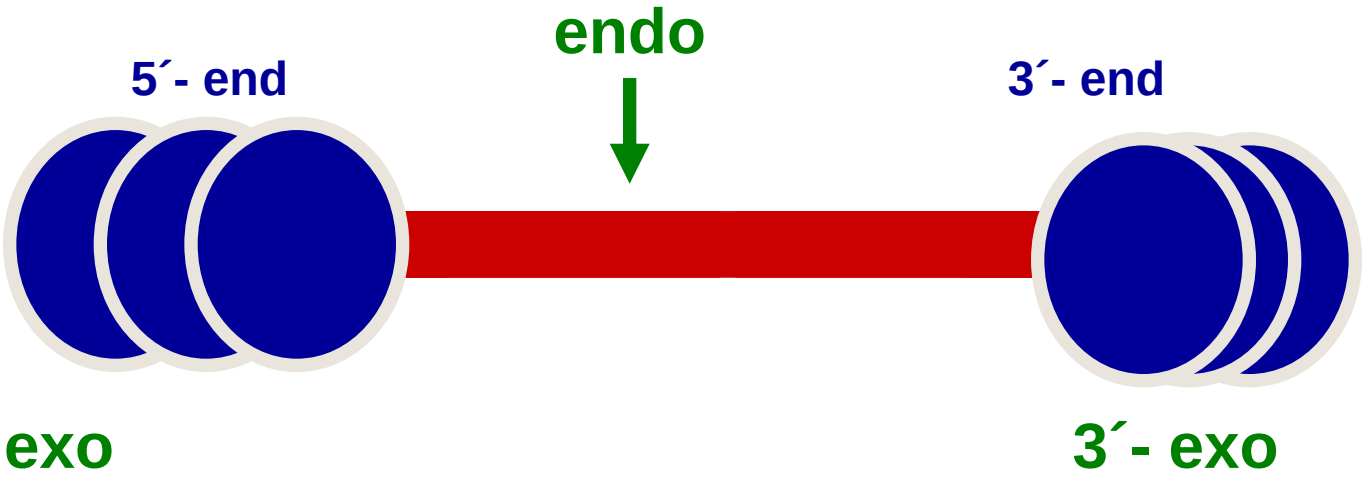




http://slideplayer.com/slide/8771653/

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

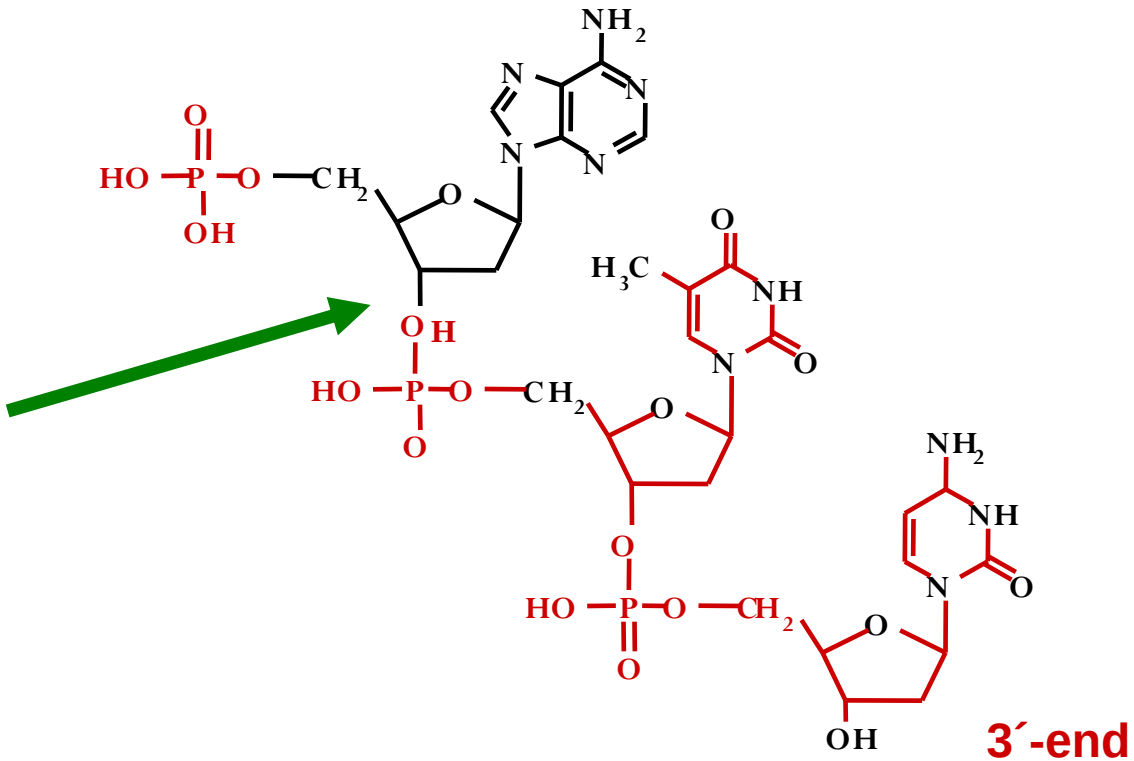
Endo- a exo-nuklease activities



-P a -OH ends !!!

5´- exonuklease activity

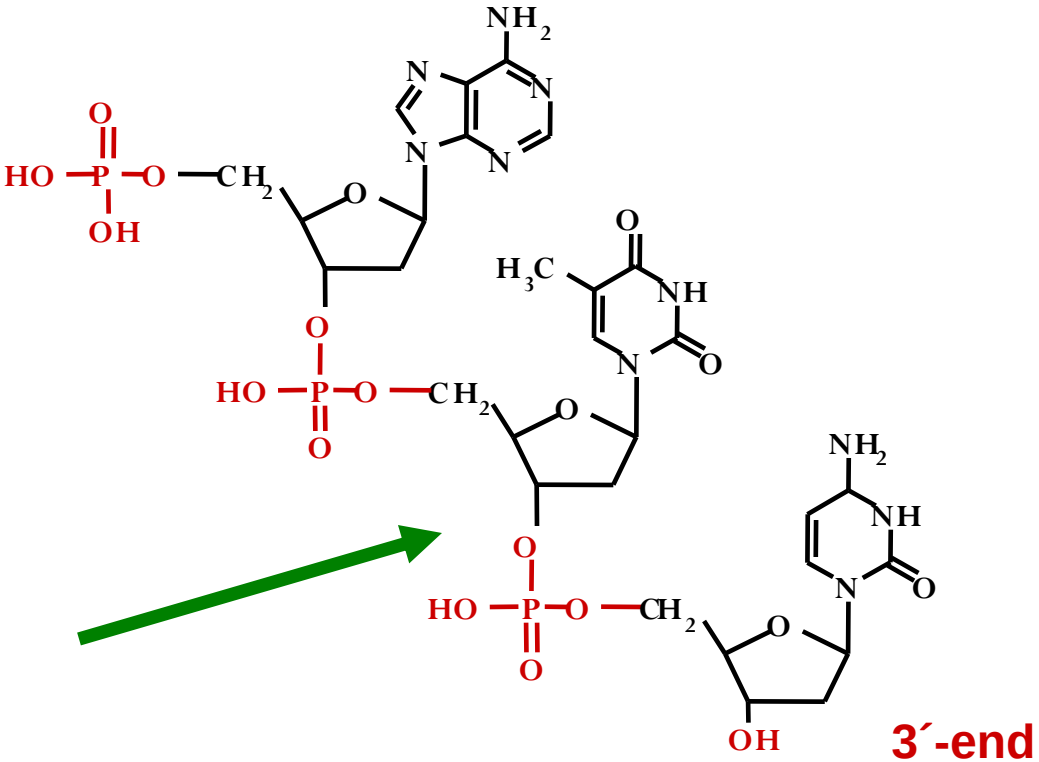
5´-end



3´-end

3´- exonuklease activity

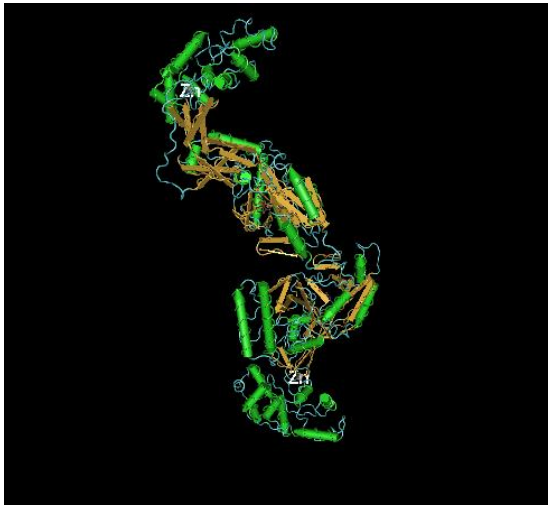
5´-end



3´-end

Replication proteins - II

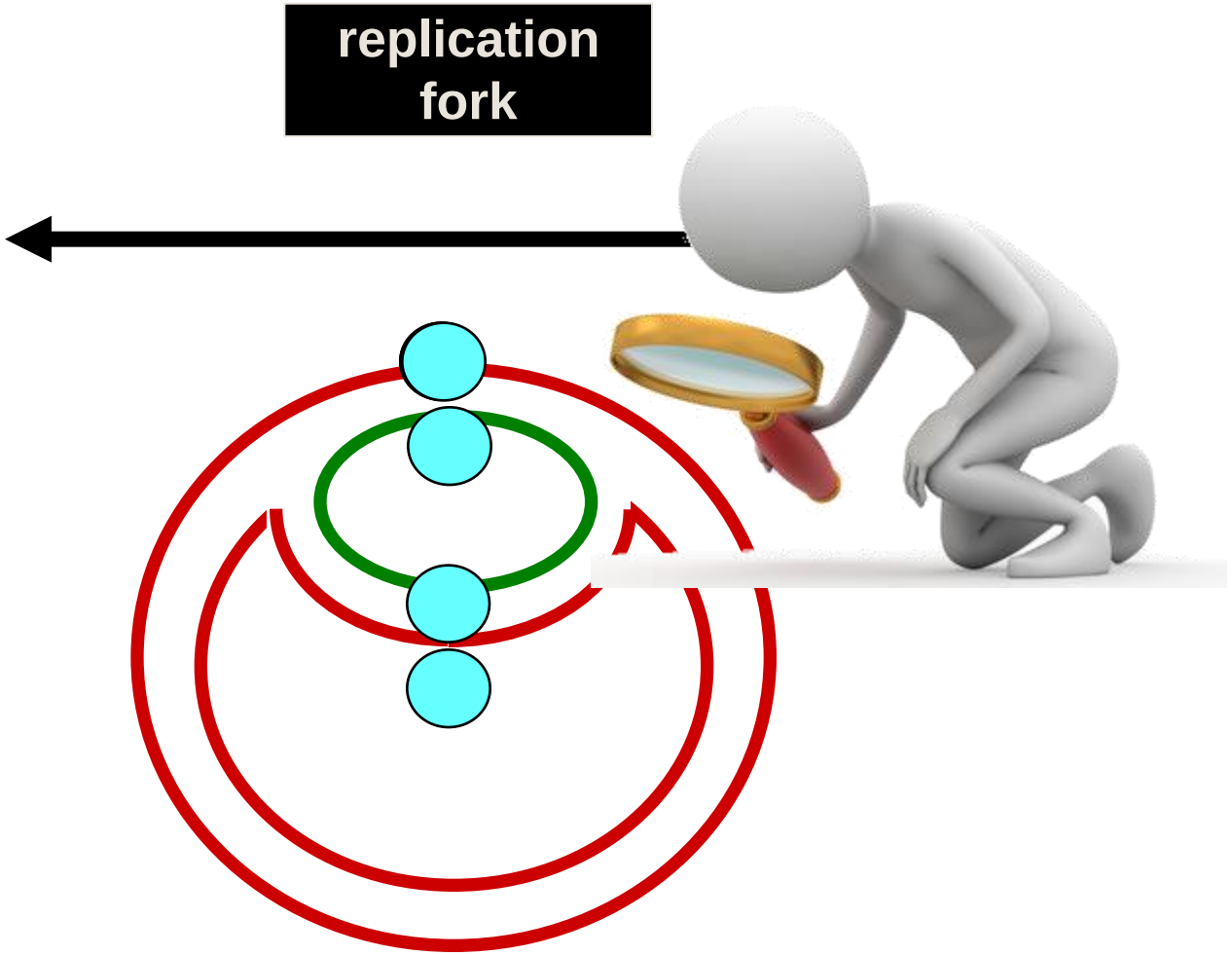
- DNA-ligase
  - It forms phosphodiester bond between 5´- and 3´- ends of two polynucleotide strands
  - It joins Okazaki fragments



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

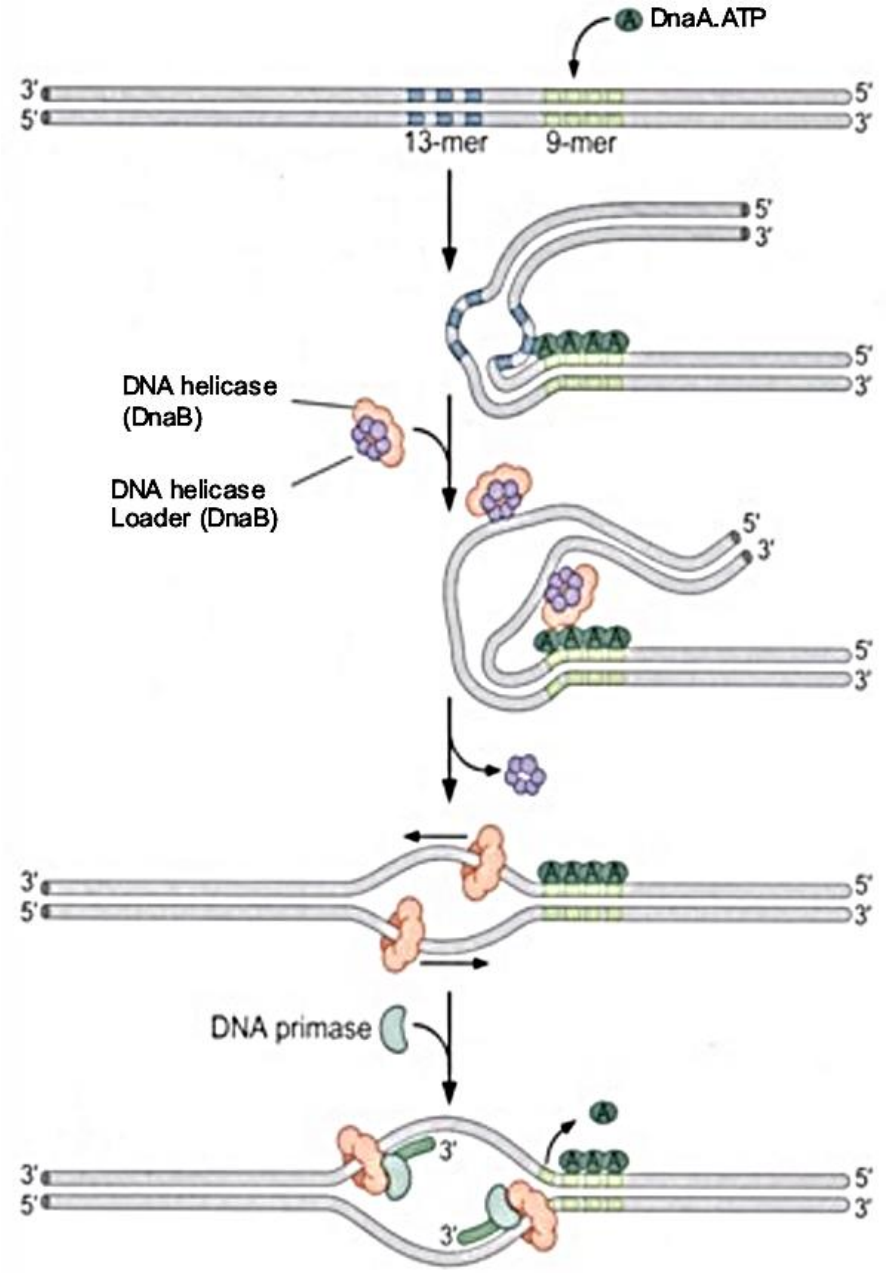


Initiation of replication

- **DnaA proteins recognise the origin of replication *oriC* (245 nt) → disintegration of H-bonds → opening of *oriC***
- **Helicases bind on released free DNA strands → unwinding of dsDNA in the direction 5´- 3´ → creation of replication fork**
- **SSB-proteins bind to ssDNA parts, the proteins keep the strands in outstretched conditions; it protects reforming of dsDNA**

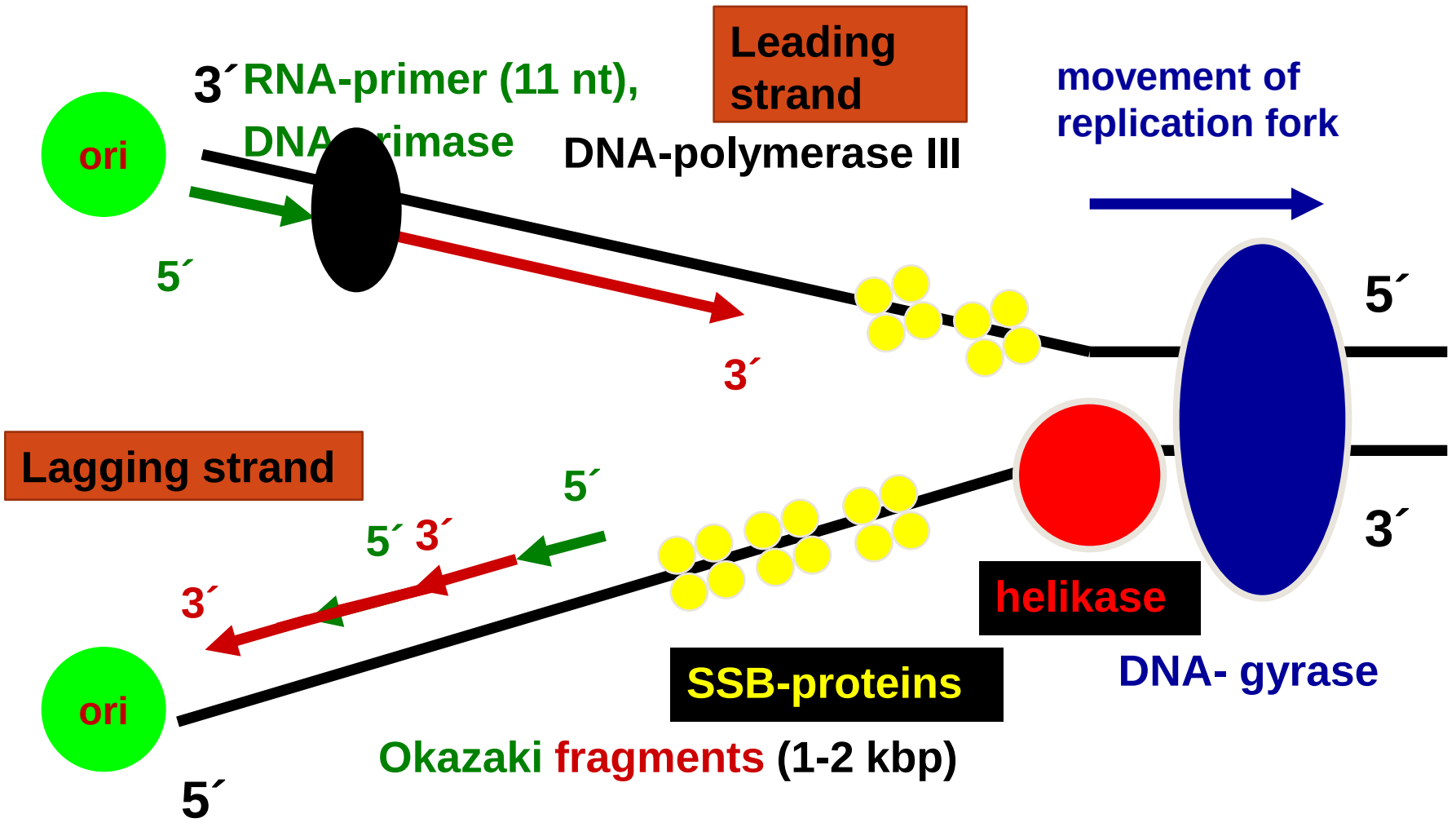
Recognition of *oriC* and initiation of replication

oriC



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

Process of replication

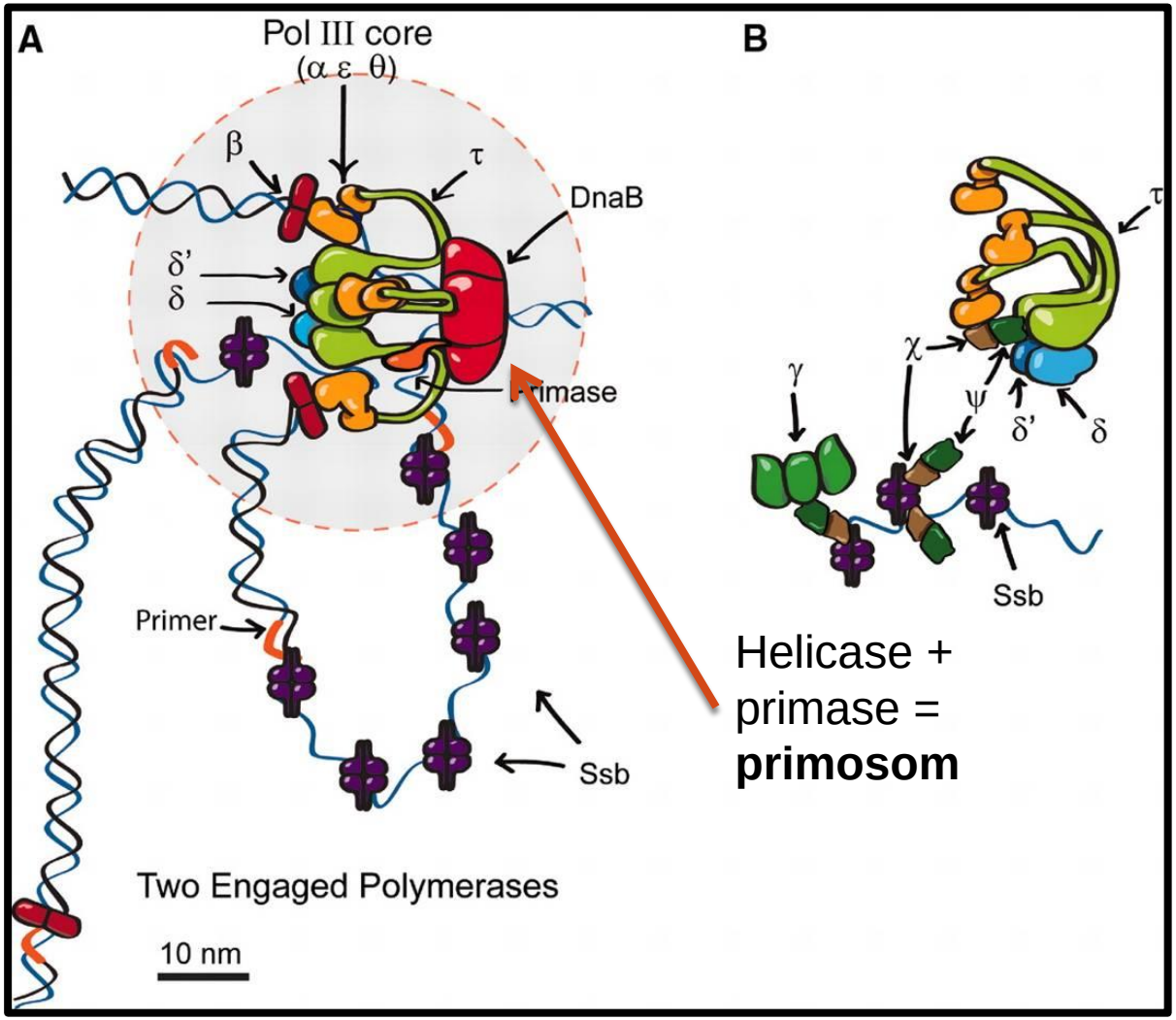


DNA polymesare I removes RNA primers and synthesize the Okazaki fragments

DNA ligase joins the Okazaki fragment

Replication is performed in replisomes

https://www.youtube.com/watch?v=G1AoVF3k9Hg



Helicase + primase = **primosom**

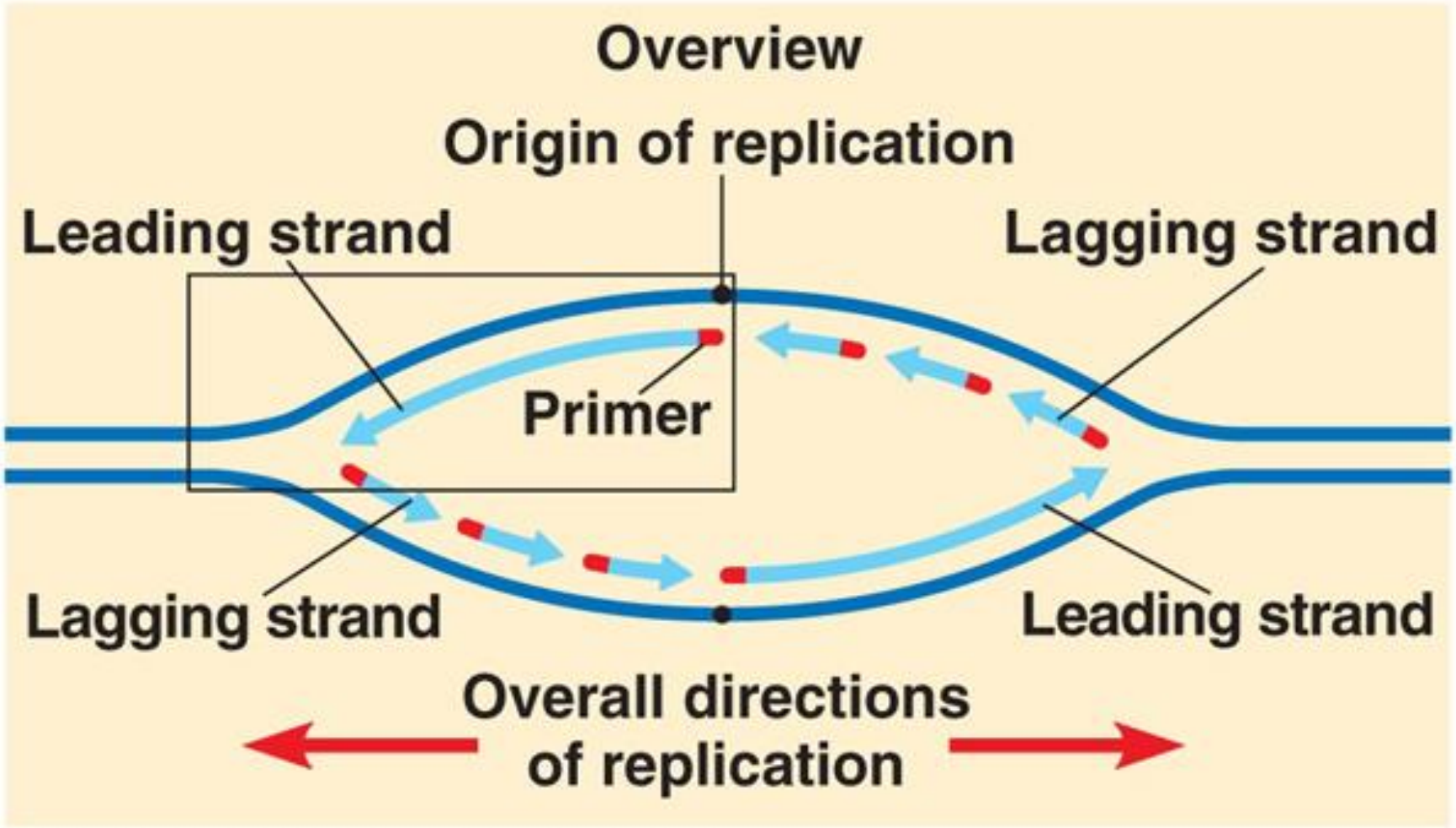
R. Reyes-Lamothe et al., Science 328, 498-501 (2010)

Text to the previous picture

Fig.: Schematic model for replisome components.

- **(A) Two engaged polymerases and one of the three β-clamps at a distance from the core replisome (circle of diameter 50 nm shown in gray). The data indicate that ~75% of replisomes have this organization, whereas ~25% have all three β clamps associated with the core replisome and potentially associated with active Pol III.**
- **(B) Expanded view of clamp loader (3') and three additional molecules of interacting with Ssb tails. The heterodimer bound to the clamp loader may also contact Ssb (*14*); (shown as a trimer, but the stoichiometry is unknown) then interacts with Ssb-associated.**

Replication is semidiscontinual and bidirectional



Replication - elongation

Synthesis of new DNA strands is <u>semidiscontinuous</u>

- **leading strand**
- **lagging strand**
- **Okazaki fragments**

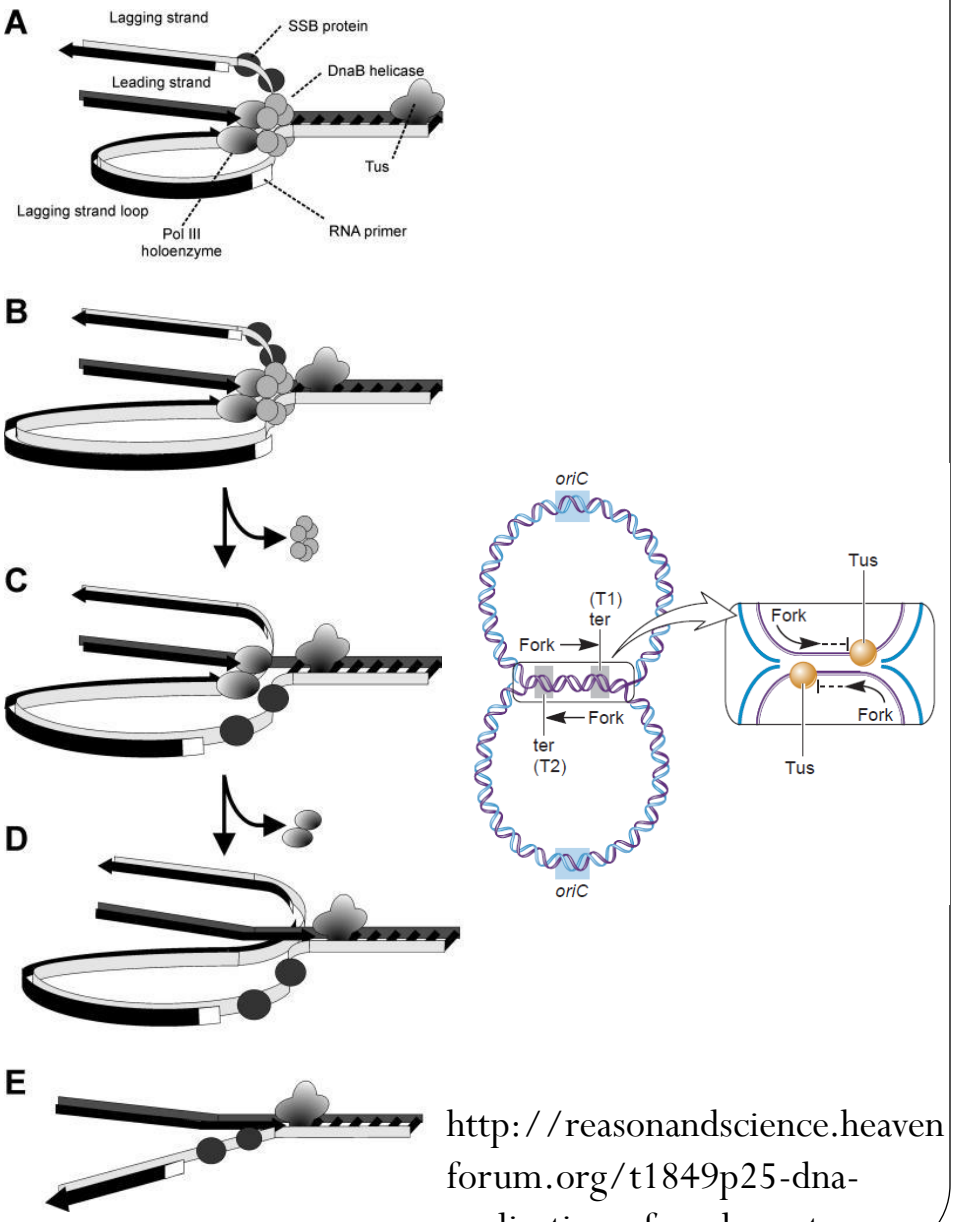
Tsuneko Okazaki

Reiji Okazaki

In 1968, Okazaki discovered the way in which the lagging strand of DNA is replicated via fragments, now called Okazaki fragments.

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

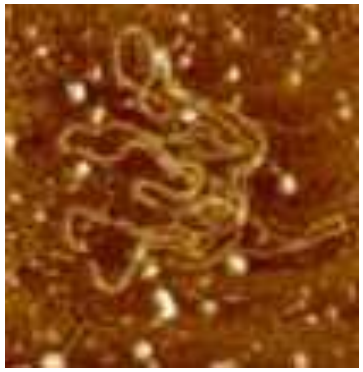


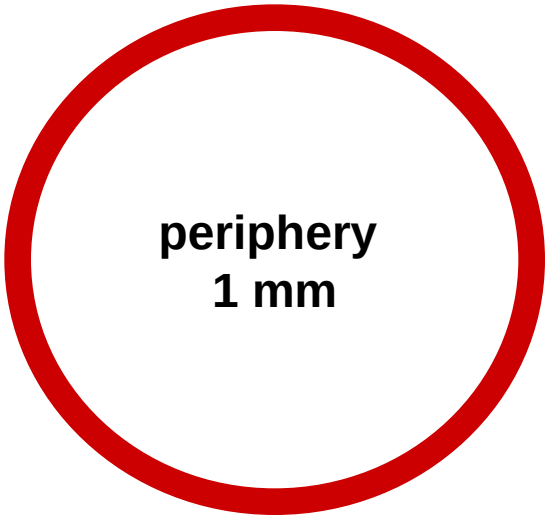
Replisome of E. coli and mechanism of replication fork arrest by a Tus-Ter complex.
(A) *The replisome moving along the DNA template approaches Tus, and the DnaB helicase assists primase to lay down the last lagging-strand primer.*
(B) *DnaB helicase action isblocked by Tus, and DnaB dissociates from 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.*

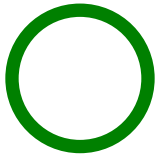
http://reasonandscience.heavenforum.org/t1849p25-dna-replication-of-prokaryotes

Replication of plasmid DNA

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



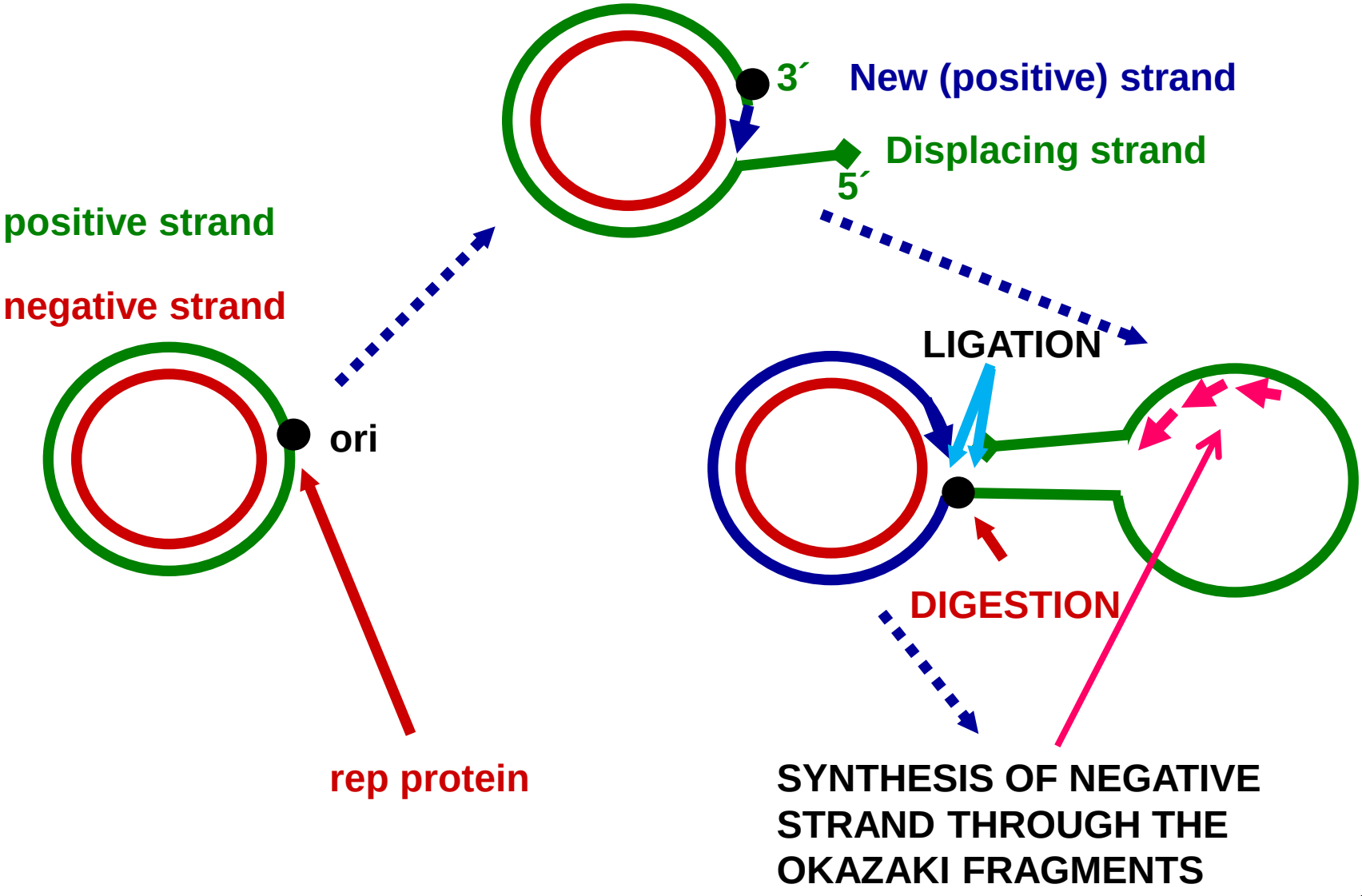




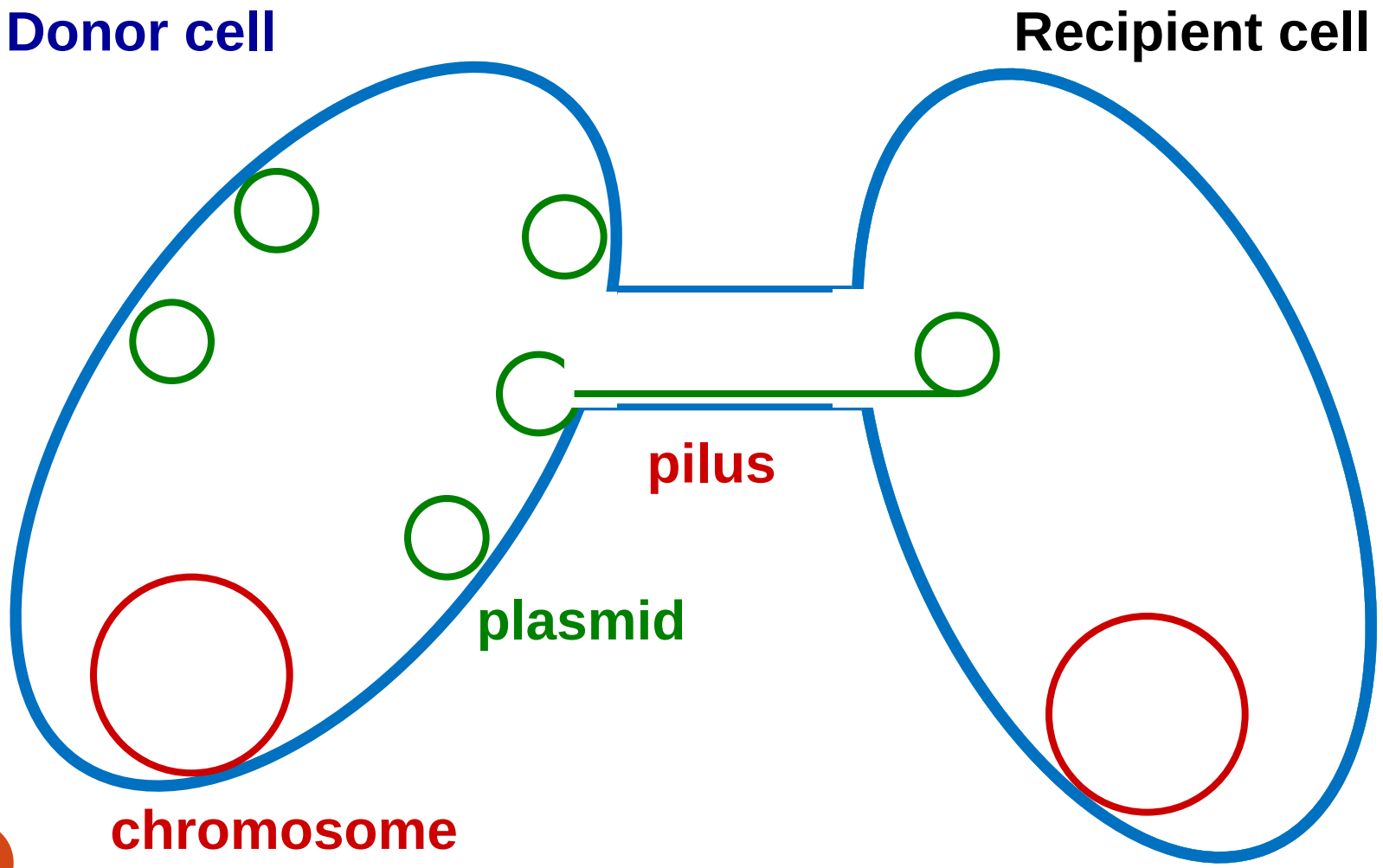
F plasmid,
periphery 31μm

- semiconservative
- semidiscontinuous
- bidirectional

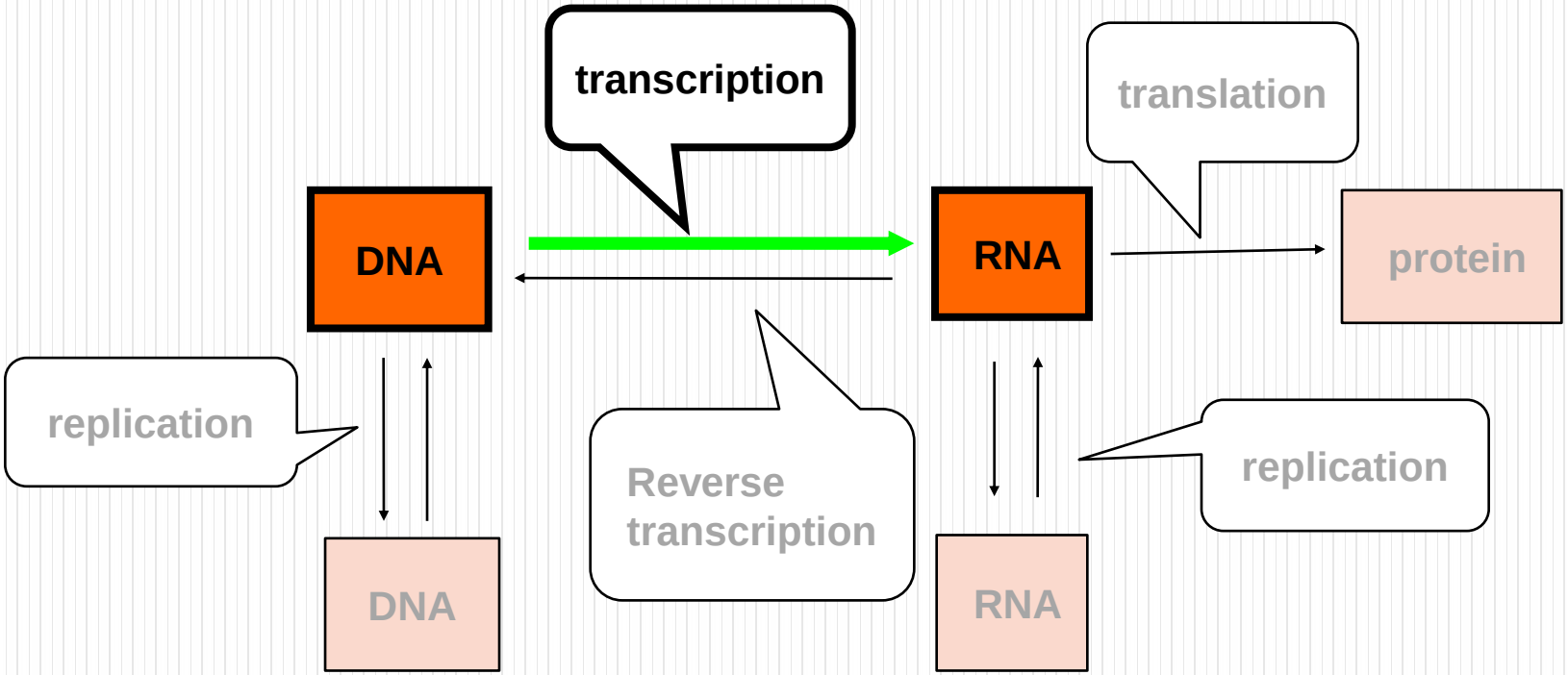
Replication by the rolling circle mechanism



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**

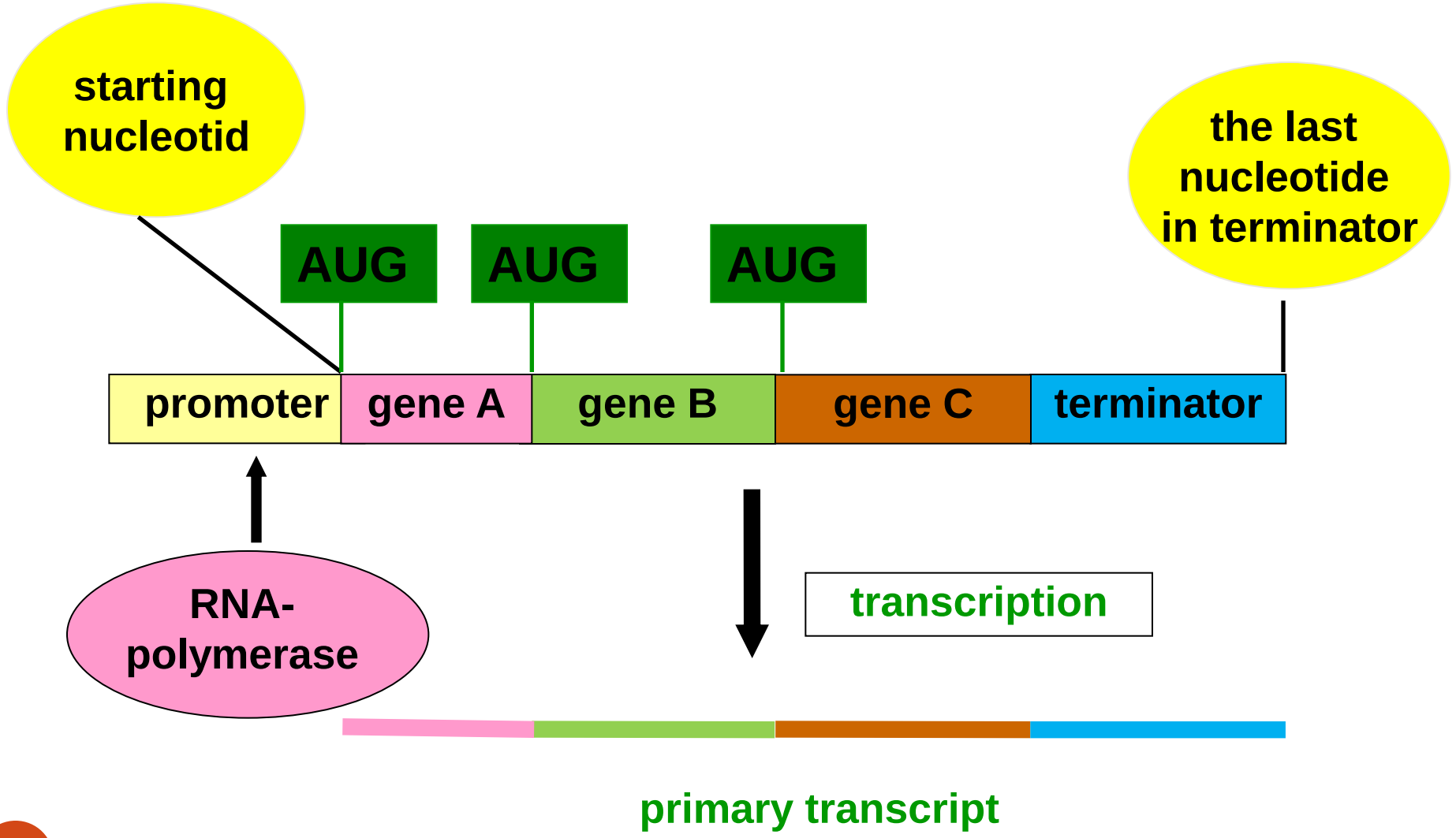
Transcription units

The transcription is performed in specific units = transcription units

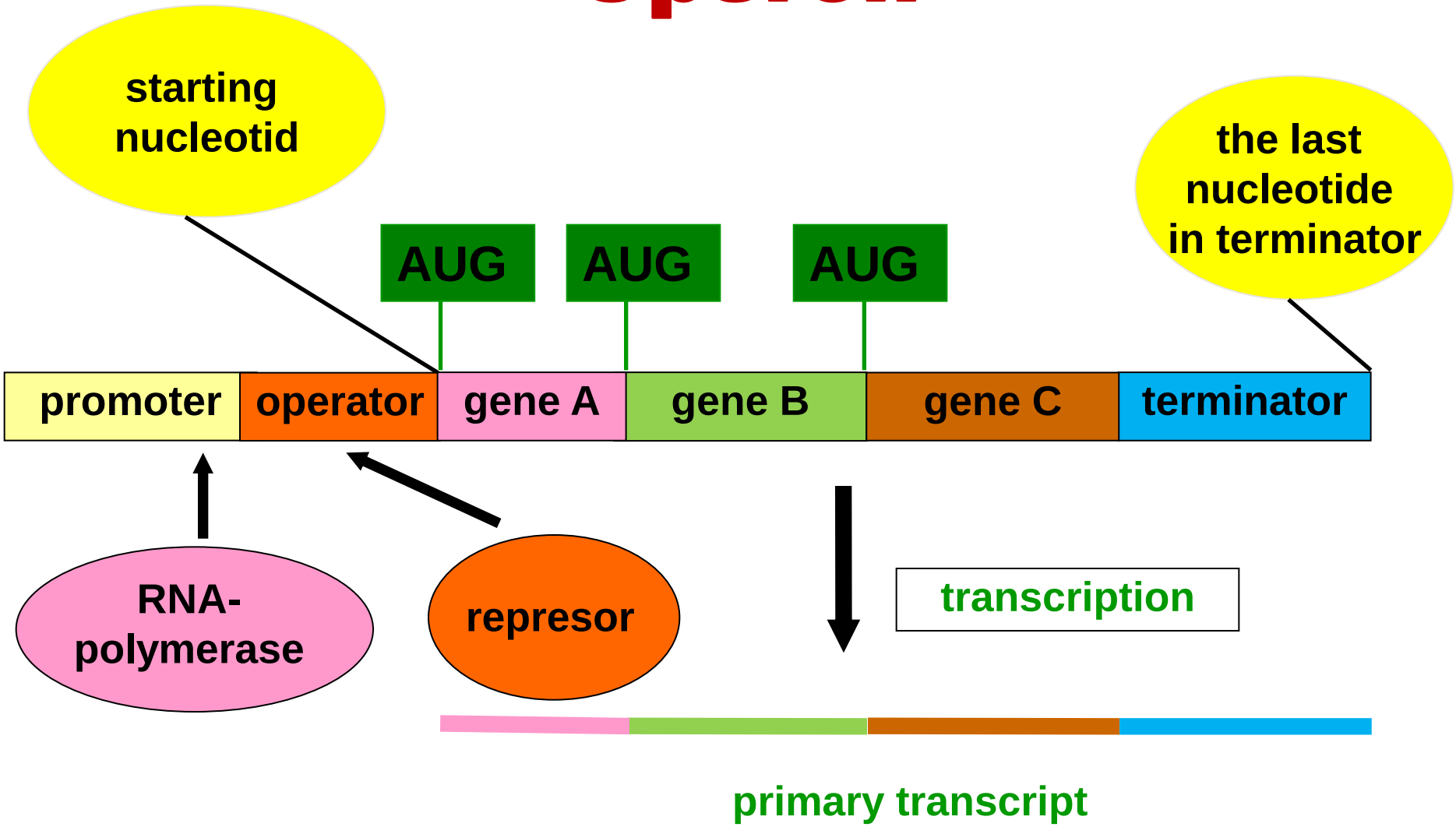
1) Transcription units of non-operon type

2) Operons

Transcription units of non-operon type



Operon



!! promotor can overlap with operator !!

Types of transcription units

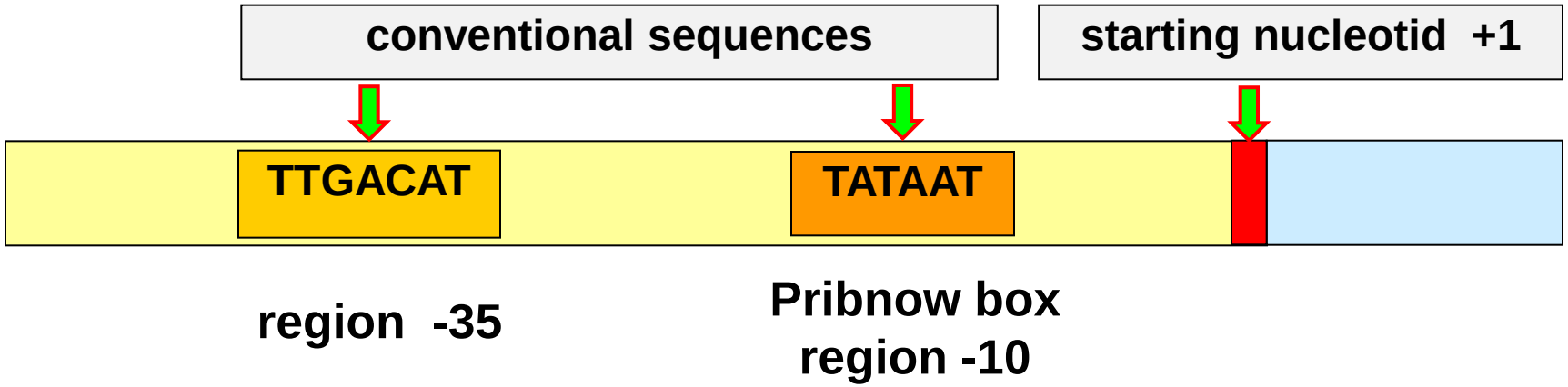
1) The transcription units which contain structural genes

2) The transcription units which contain genes for rRNA

3) The transcription units which contain genes for tRNA

4) The transcription units for regulatory RNA

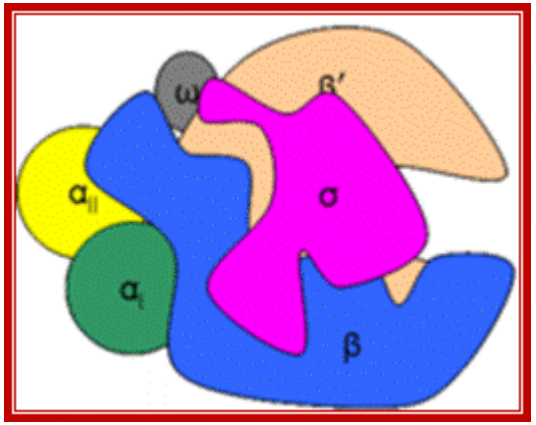
Prokaryotic promoter



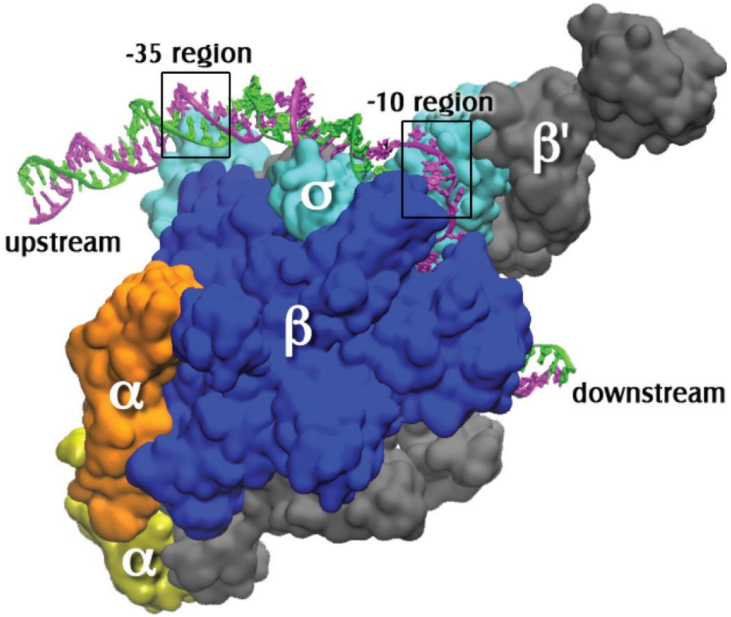
- Similarity of promoters enable their affinity to single RNA polymerase
- Differences are responsible for extent of this affinity
  - strong bacterial promoter – more similar to conventional sequences
  - weak bacterial promoter – less similar to conventional sequences

Bacterial RNA polymerase

1) It is able to recognize the promoters of all transcription units
2) It consists of 5 subunits



- 2x α (M = 40 000), responsible for stability
- 1x β (155 000), used for binding ribonucleotides to the enzyme
- 1x β´ (160 000), responsible for binding RNA polymerase to template DNA strand
- 1x ω, regulation
- 1 x σ (85 000), σ-factor, responsible for binding RNA polymerase to promoter



doi:10.3390/biom5020668

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

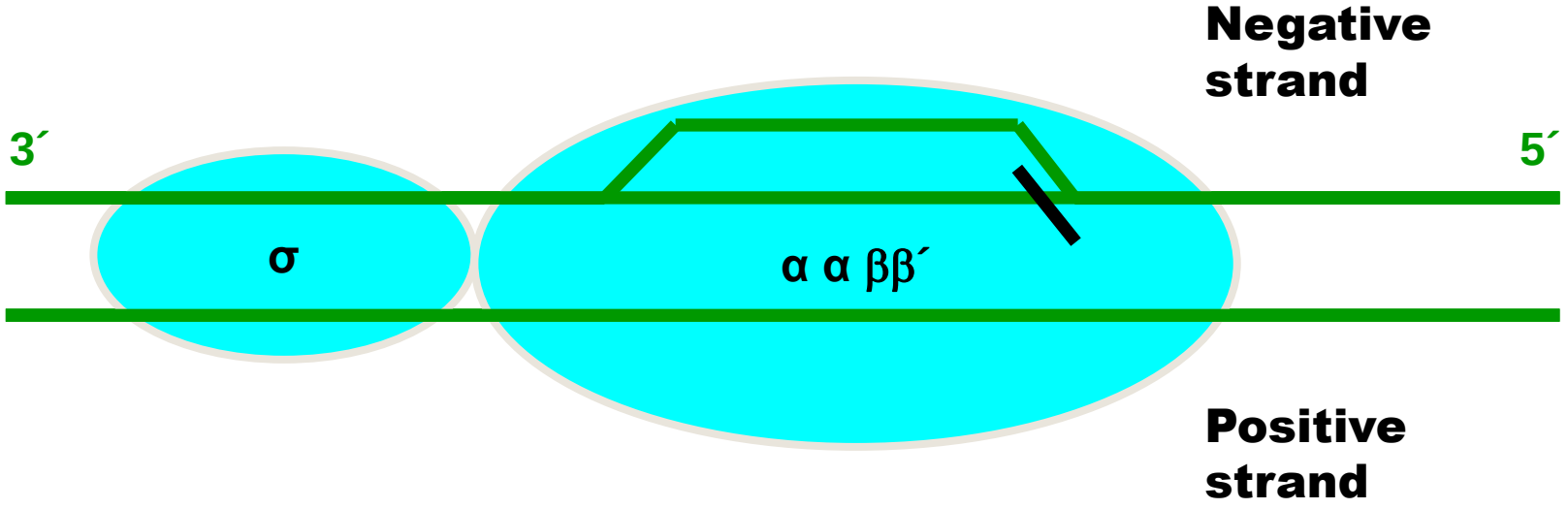
Stop the movement of RNA polymerase → releasing the full length RNA → releasing RNA polymerase from DNA

Initiation of transcription

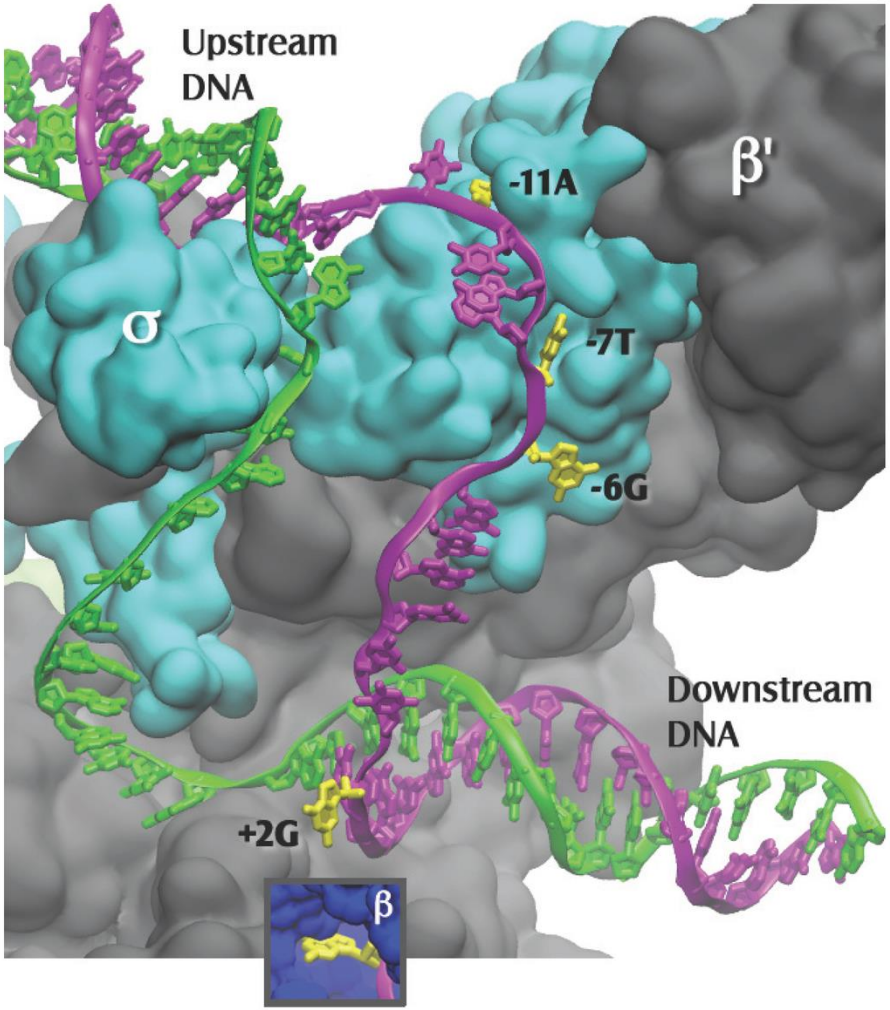
1) Binding of RNA polymerase to the sequence -35 and to the Pribnow box (closed transcription binary complex)

2) Releasing hydrogen bonds between two DNA strands in the Pribnow box (open transcription binary complex)

3) Transcription of the first two nucleotides (open transcription ternary complex)



Open transcription binar complex

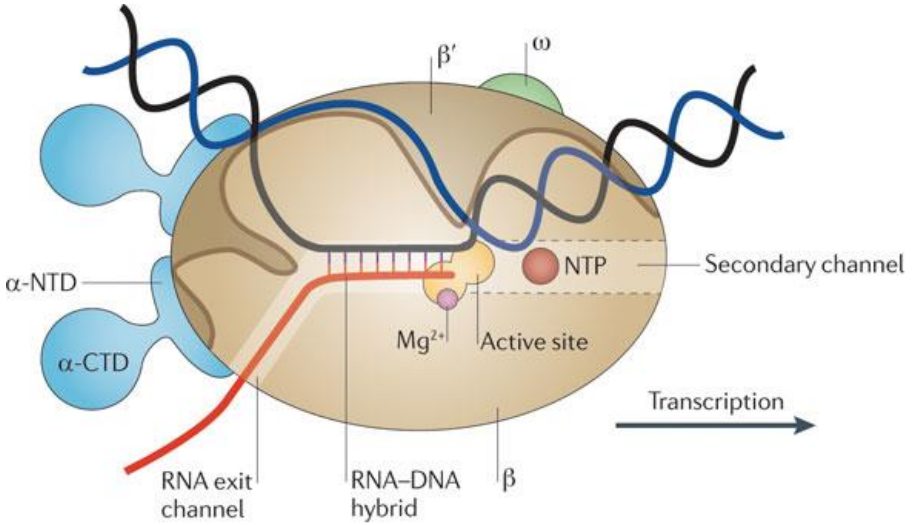


The β subunit was removed to reveal the transcription bubble and the flipped bases in their pockets. Template DNA is in green and nontemplate DNA is in magenta, with the flipped-out bases in yellow. Bases −11A and −7T interact solely with the σ subunit. Base −6G is at the σ-β subunit interface. Base +2G interacts solely with the β subunit (insert). The −12T nontemplate base is shown in the figure as unpaired, as it is in the 4G7O coordinate set; it is likely base paired in the native promoter.

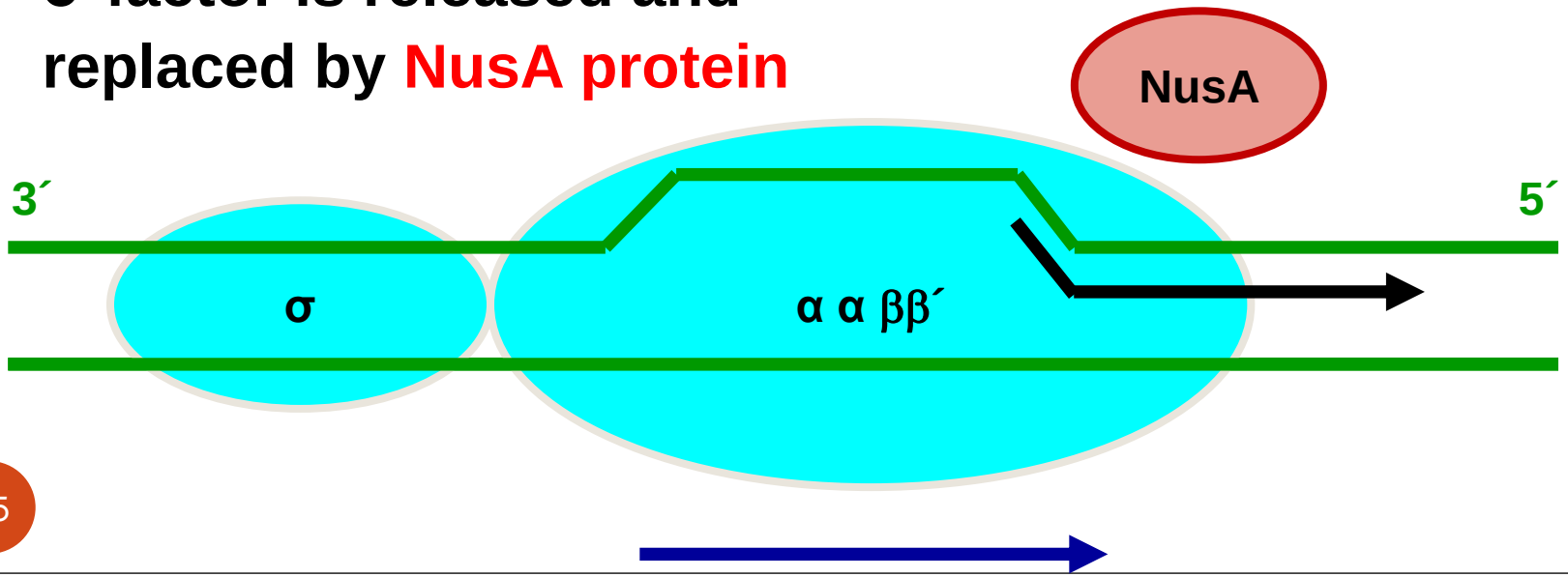
Biomolecules **2015**, *5*(2), 668-678;
doi:10.3390/biom5020668

Elongation of transcription

1) RNA synthesis continues in 5´-3´ direction and RNA polynucleotide grows
2) The speed of synthesis is about 40 nt/s
3) Once elongation starts, σ-factor is released and replaced by NusA protein

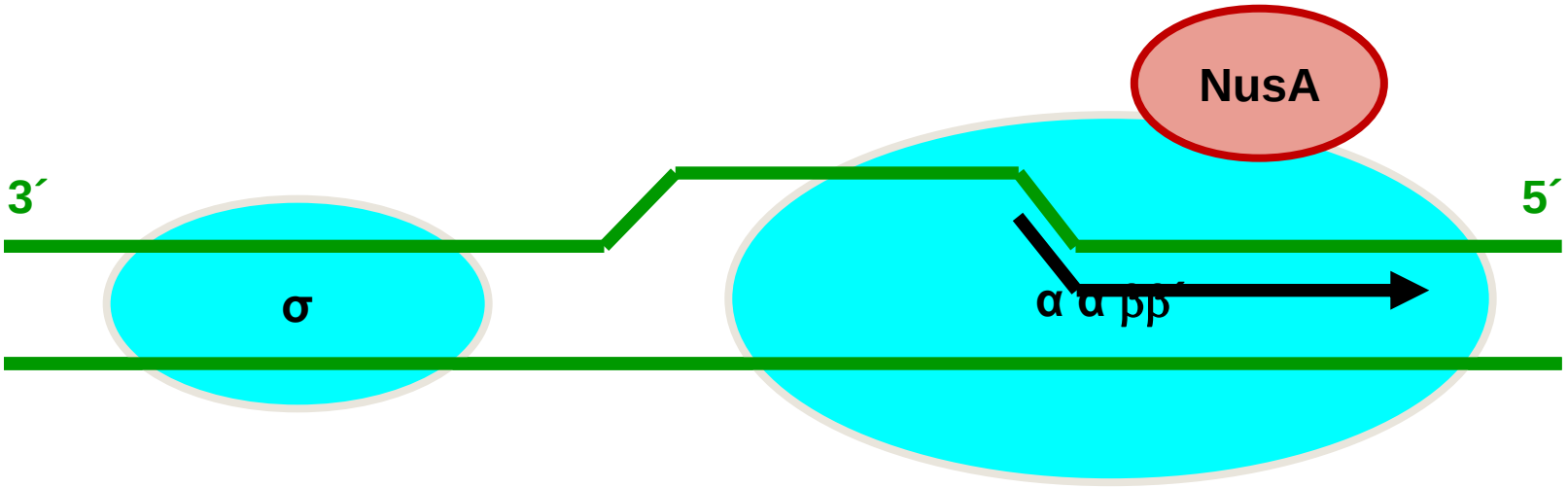


Nature Reviews | Microbiology
DOI: 10.1038/nrmicro2560



Termination of transcription

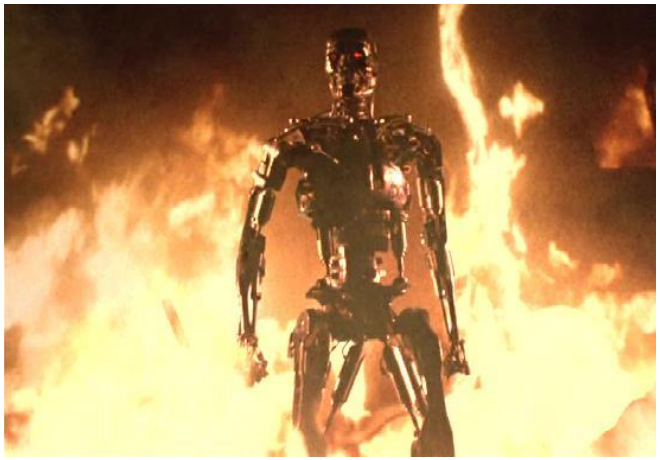
1) The RNA polymerase stops its movement
2) The full length RNA is released
3) The RNA polymerase is released from DNA
4) Disociation of NusA protein from RNAP



Bacterial terminators of transcription

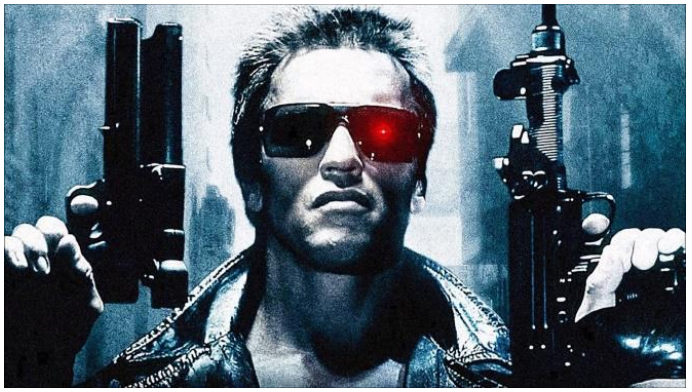
1. **Rho-independent terminators**

   transcription is terminated without the presence of specific ρ-factor

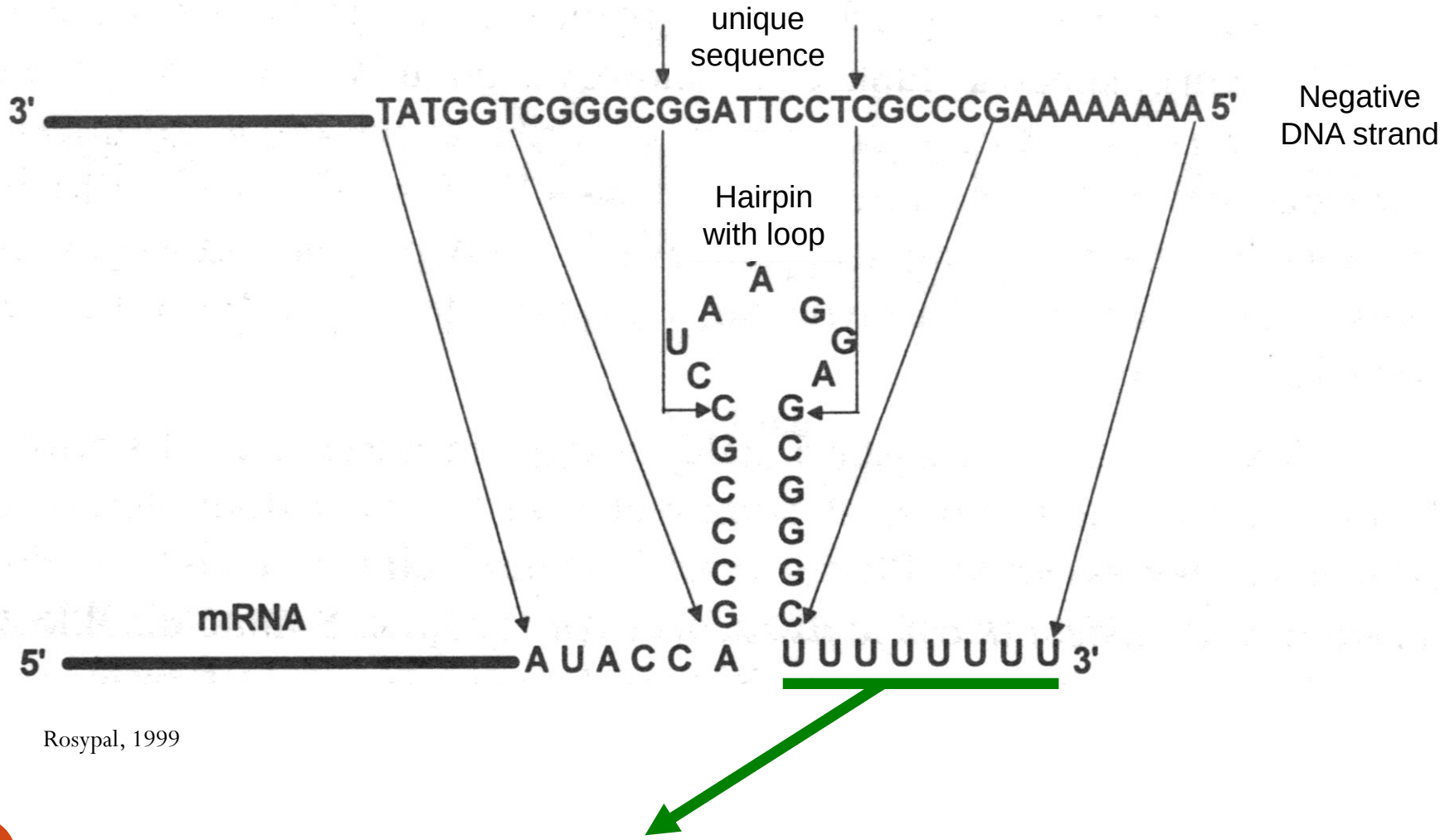


2. **Rho-dependent terminators**

   transcription is terminated in the presence of specific ρ-factor



Rho-independent terminators

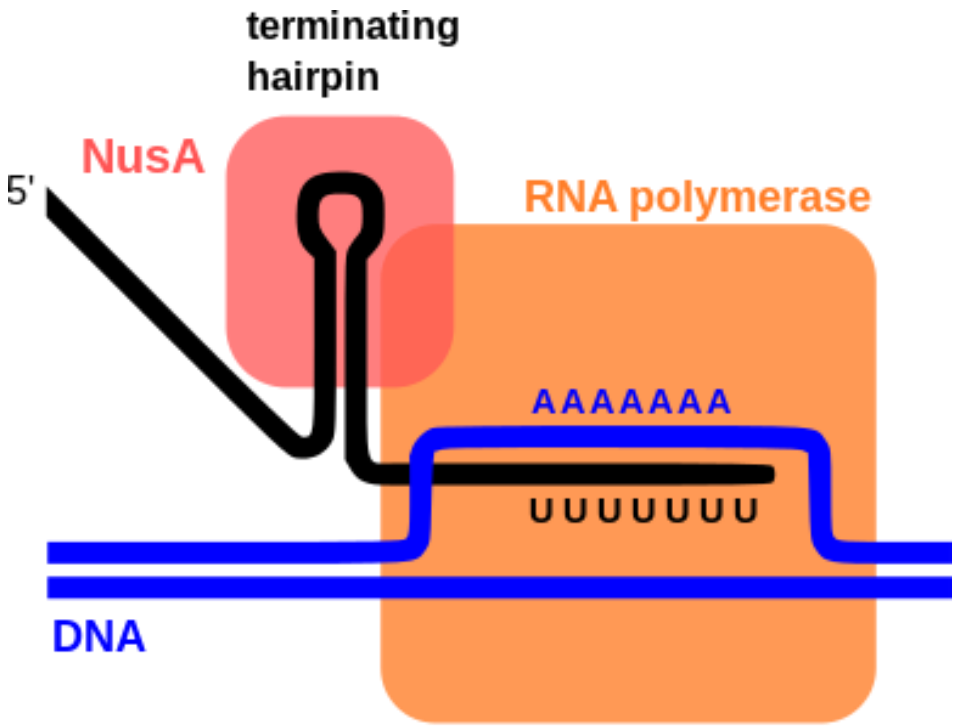


Rosypal, 1999

Sequence necessary for RNA release

Termination of transcription ρ-factor independent

- Hairpin binds to NusA protein
- Stop of RNAP movement
- Finishing of 8U sequence transcription
- RNAP releases from DNA and again associate with σ-factor



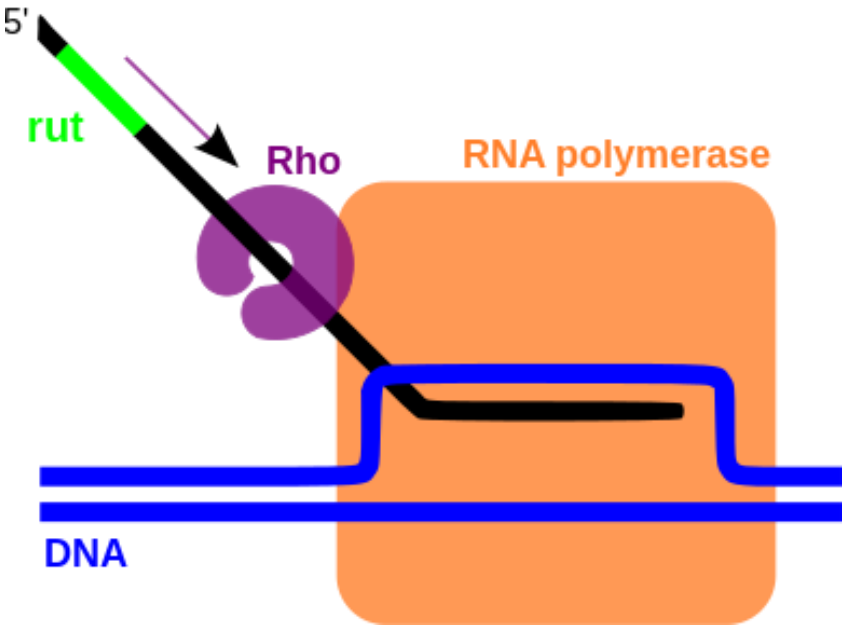
Wiki commons; © Oalnefo1

Rho-dependent terminators

1) Similar structure
2) The sequence AAAAAAA is replaced by another, for example GTTAGAA
3) This sequence is transcribed to CAAUCUU
4) No sequence UUUUUUUU → no signal for RNA polymerase releasing → dependency on ρ-factor

Termination of transcription ρ-factor dependent

- ρ-factor binds to *rut* locus of nascent RNA
- Subsequently moves towards RNAP
- Once ρ-factor reaches a RNAP, RNAP disociates from DNA



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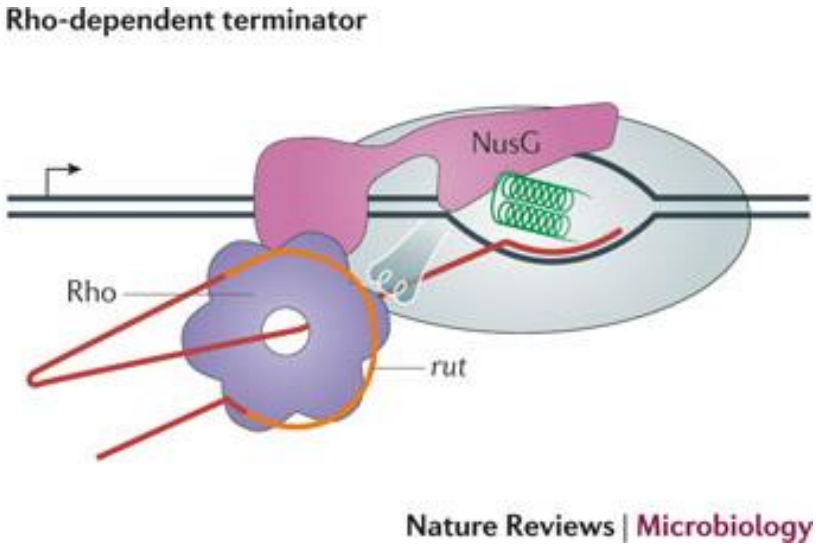


DOI: 10.1038/nrmicro2560

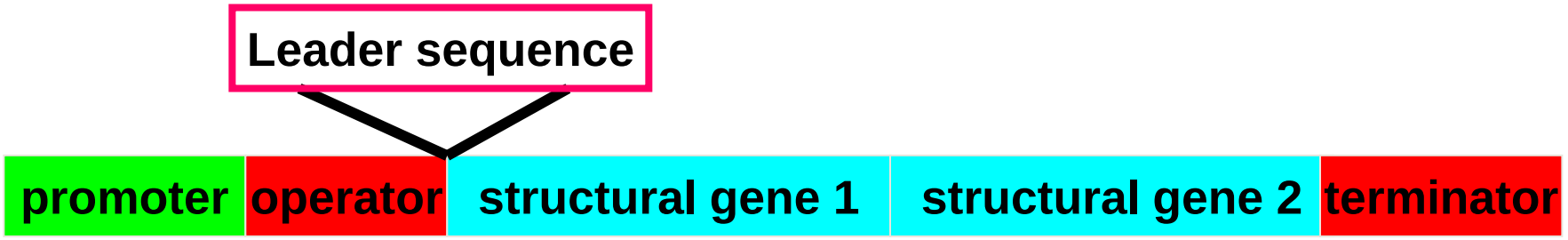
Transcription of the structural genes

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

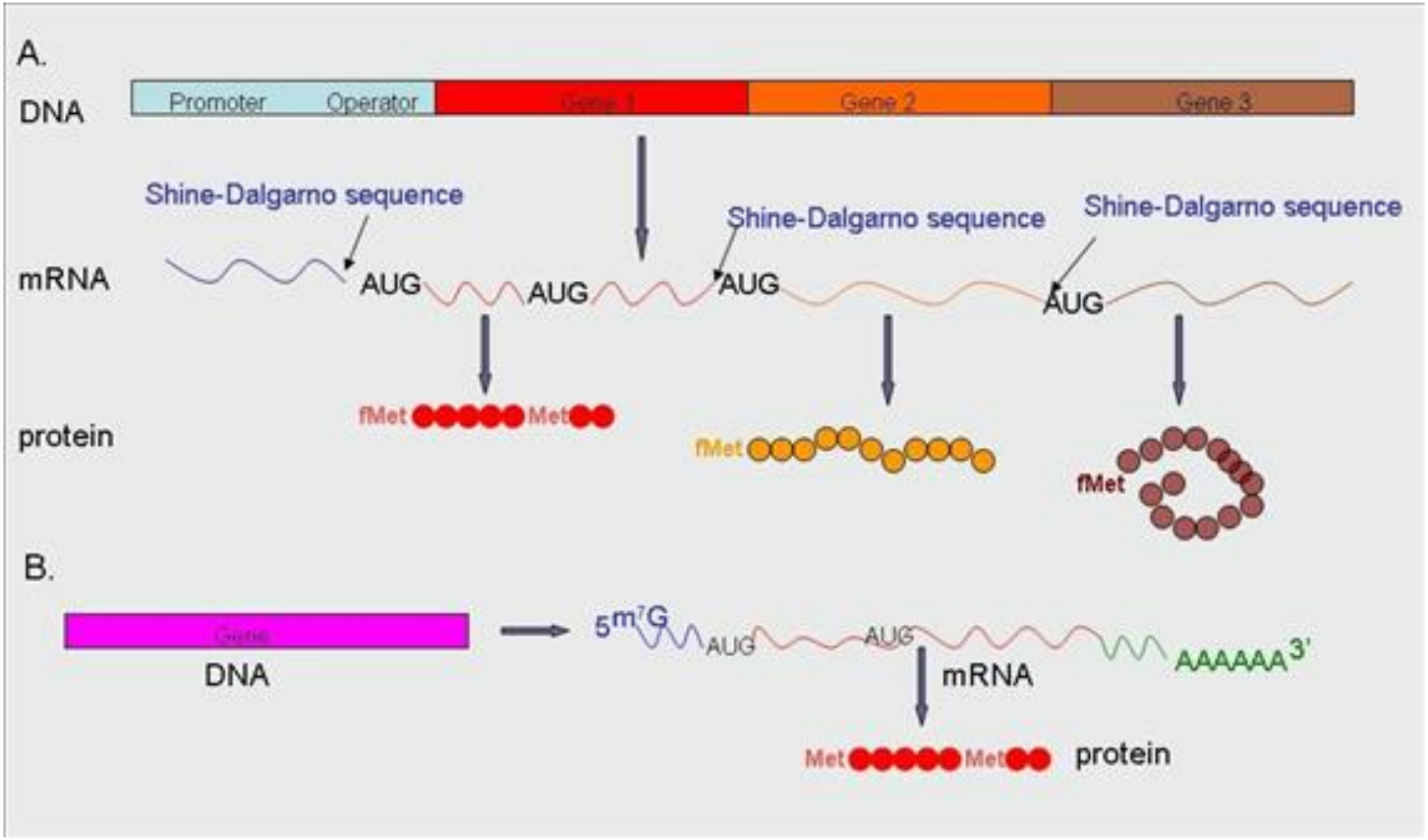
- the sequence lies between promoter and the first structural gene
- in operone it is immediately after operator

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



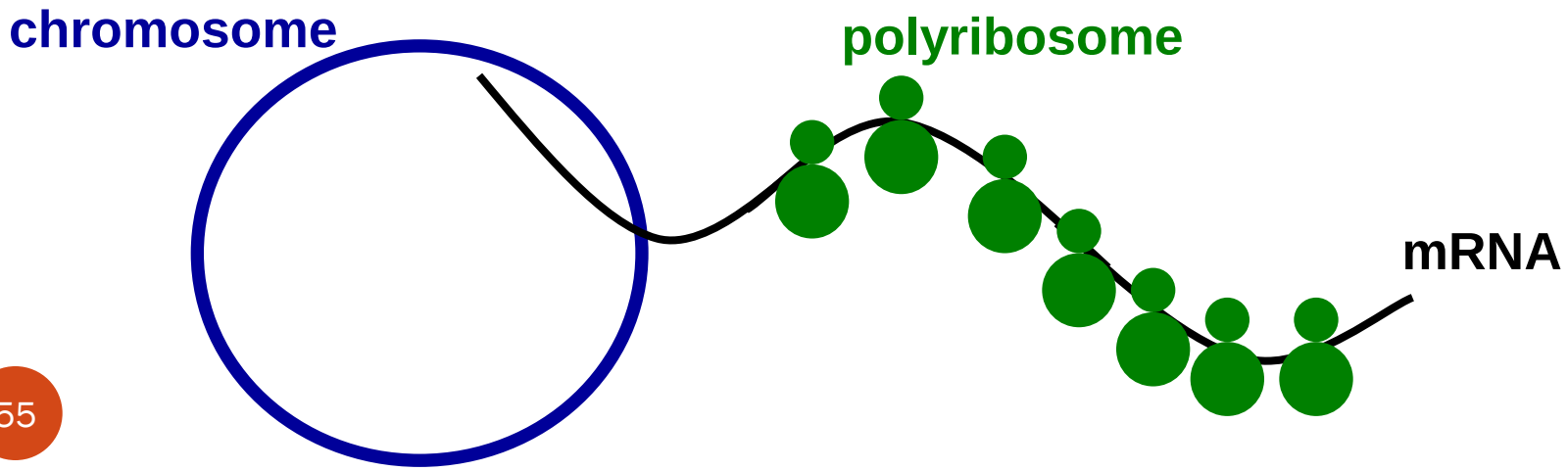
http://www.tankonyvtar.hu/en/tartalom/tamop425/0011_1A_Proteinbiotech_en_book/ch01.html

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



Coupled syntheses

Influences speed of proteosynthesis

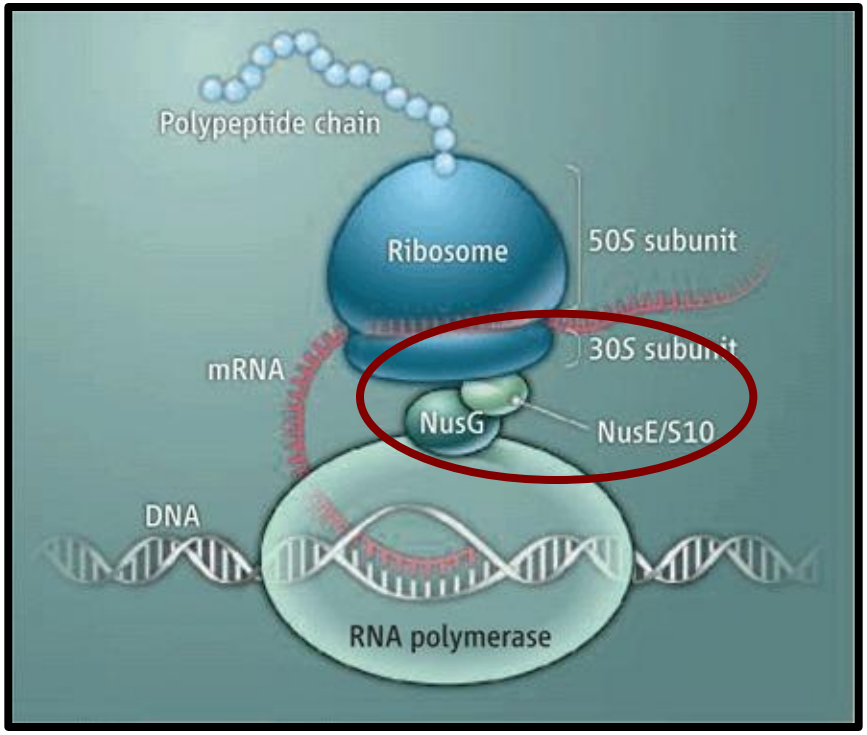
Efficient binding and progression of ribosomes along mRNA increase the speed of RNA polymerase, whereas the absence of ribosomes allows the polymerase to slow and wait for ribosomes to catch up.

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

Coupled syntheses

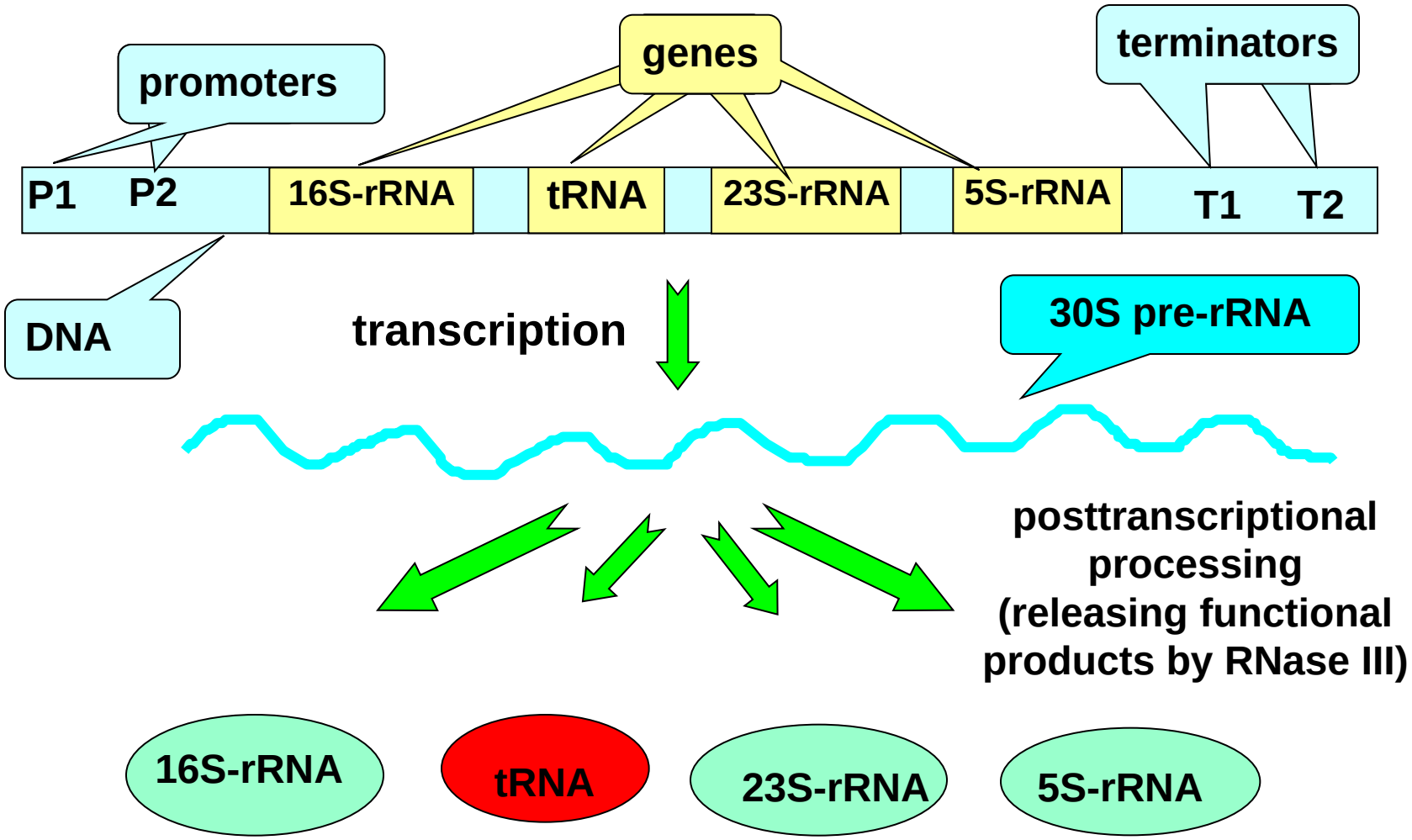
1) The first ribosome translating a mRNA associates with RNA polymerase through the NusE-NusG-polymerase interaction

2) This prevents retraction of the emerging mRNA into RNA polymerase, and thus inhibits backtracking-associated pauses that slow RNA polymerase in the absence of the ribosome.

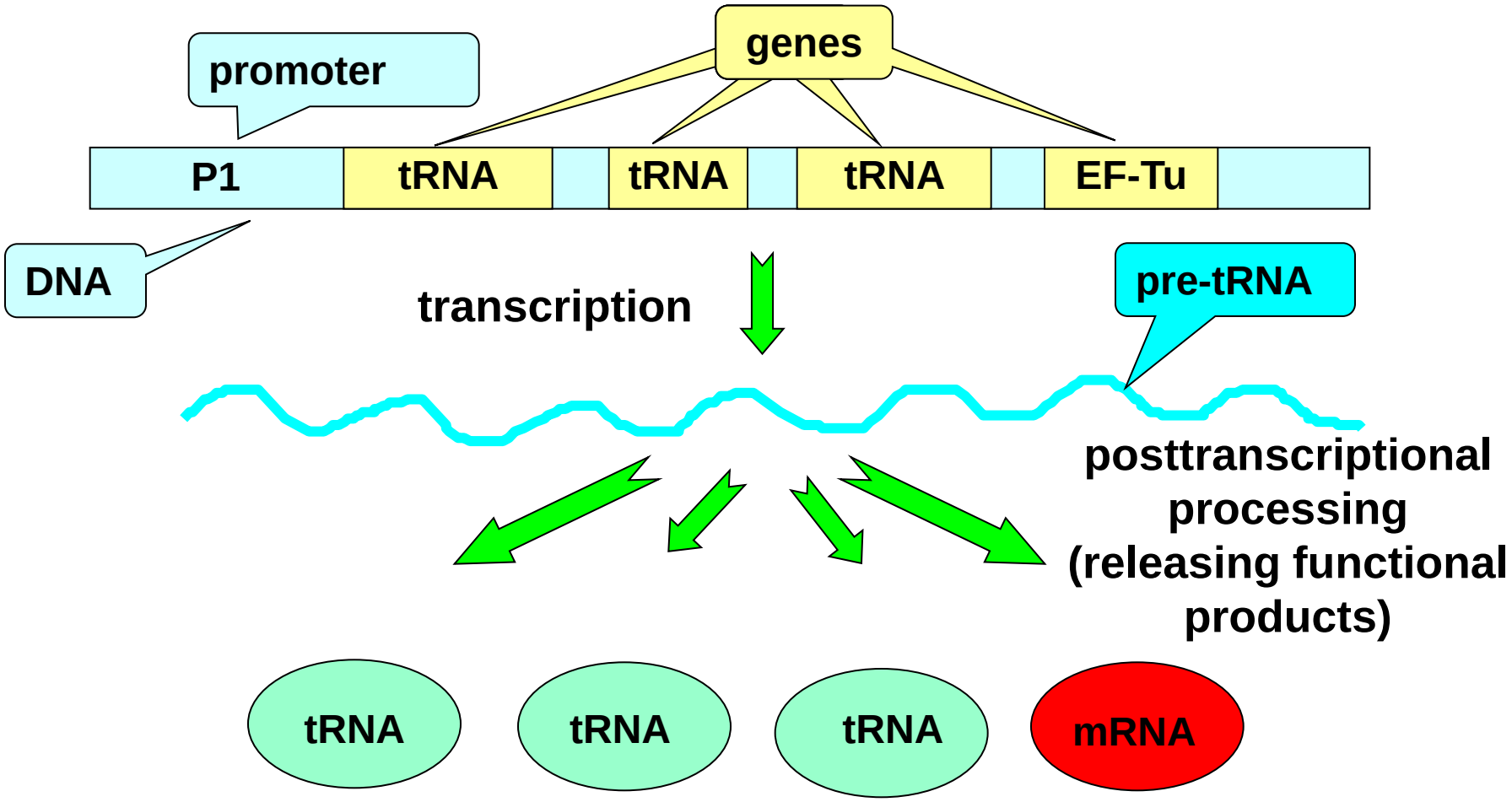


J. W. Roberts Science 328, 436-437 (2010)

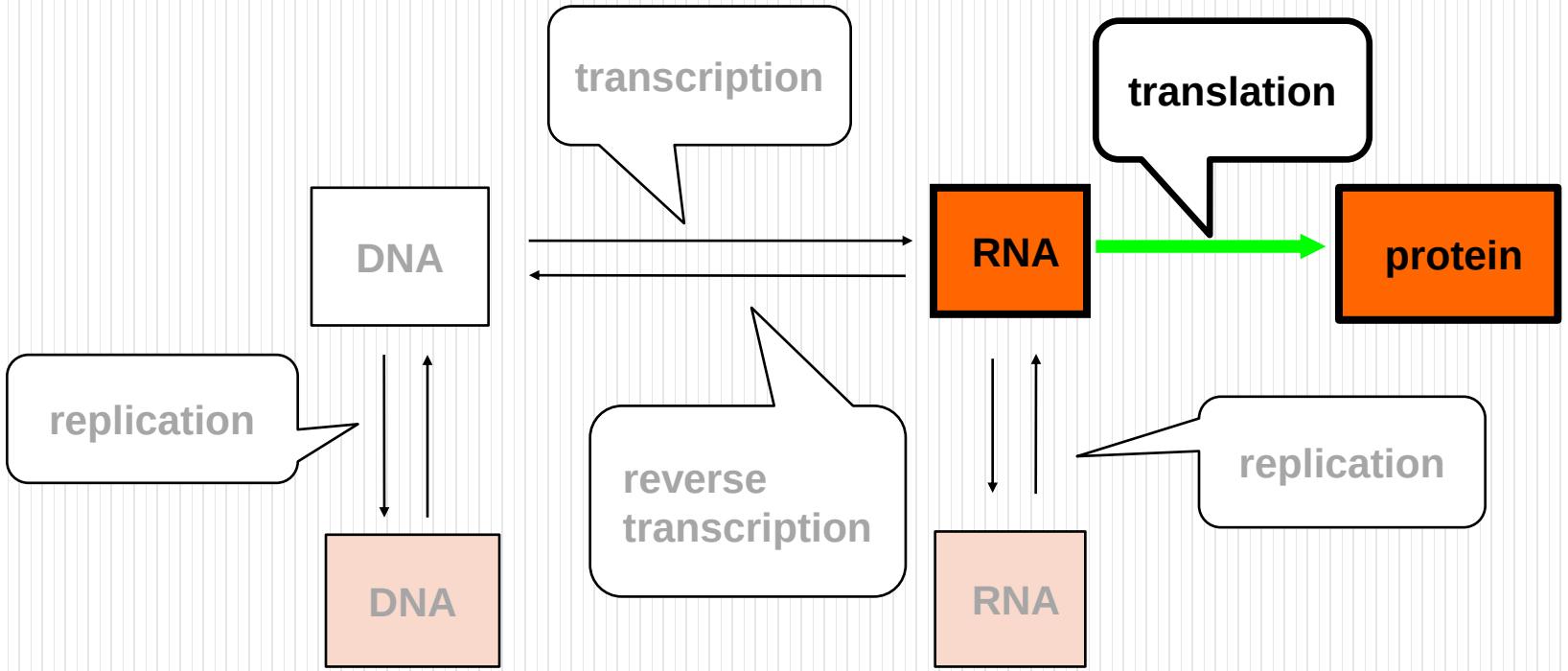
Transcription the genes for rRNA



Transkripce genů pro tRNA



Translation of prokaryotic genom

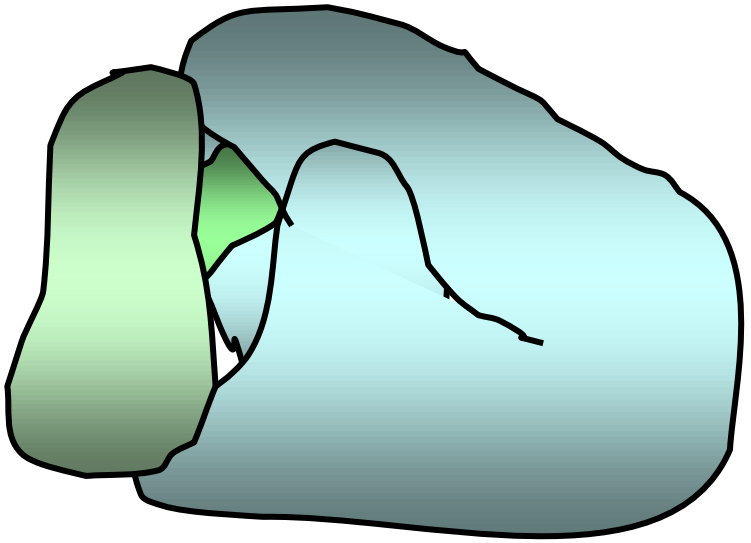


Definition of translation

- 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

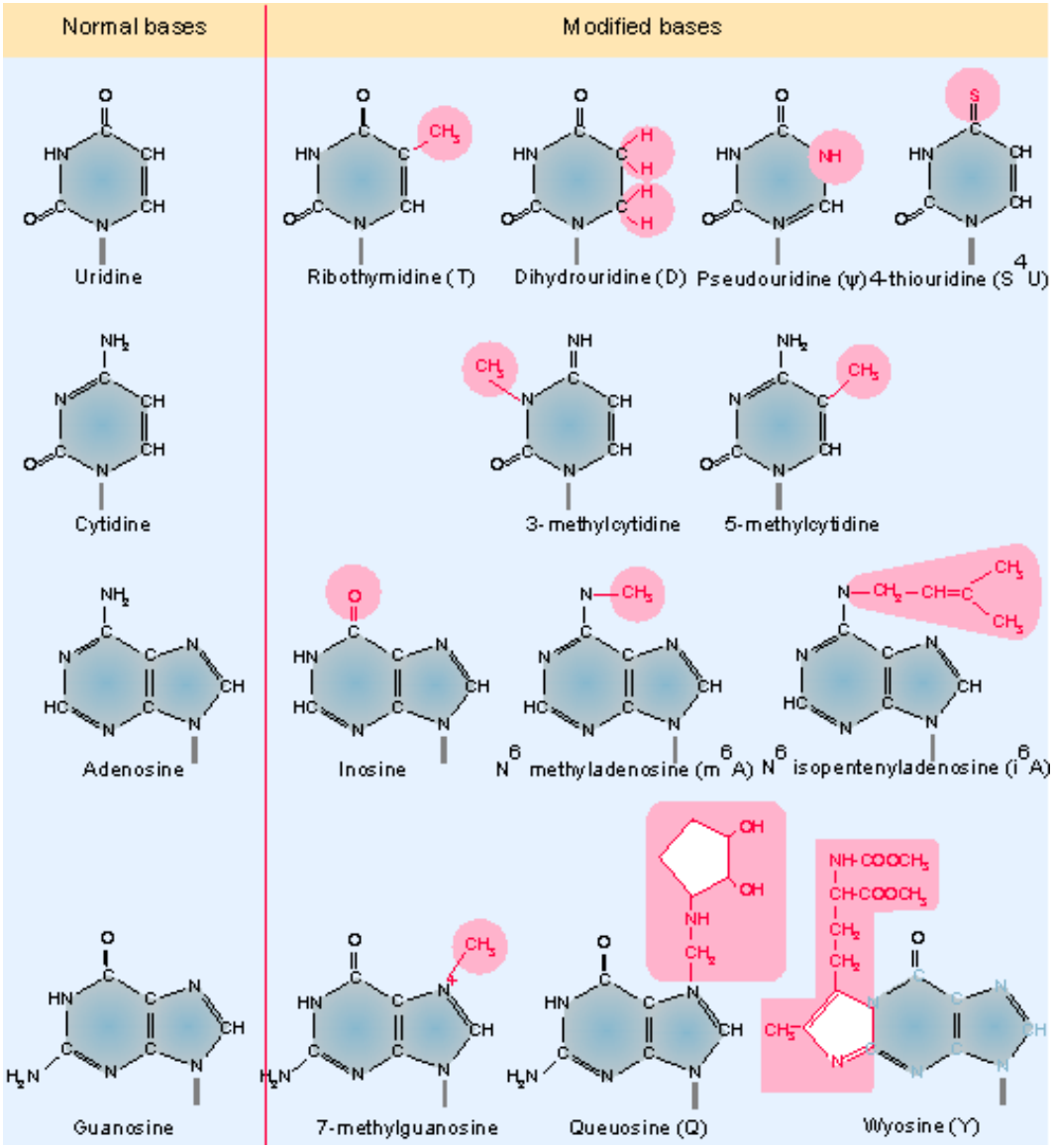
- **Activation of AA = amino acylation, charging**
  - **Process in which AA is attached to the specific tRNA**
- **Iniciation**
  - **A sequence of processes which produce initiation complex = ribosome 70S, mRNA, initiator tRNA, initiation factors**
- **Elongation**
  - **The addition of AAs to the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

The primary structure of tRNA

- length about 74 - 95 nucleotides
- molecular mass 80 000
- 3´- end = 5´- CCA - 3´
- integral part of the sequence are modified bases
  - they originate by enzyme modification after transcription

- marking $tRNA^{Ala}$, $tRNA^{Leu}$, ...
- Ala ~ $tRNA^{Ala}$, Leu ~ $tRNA^{Leu}$

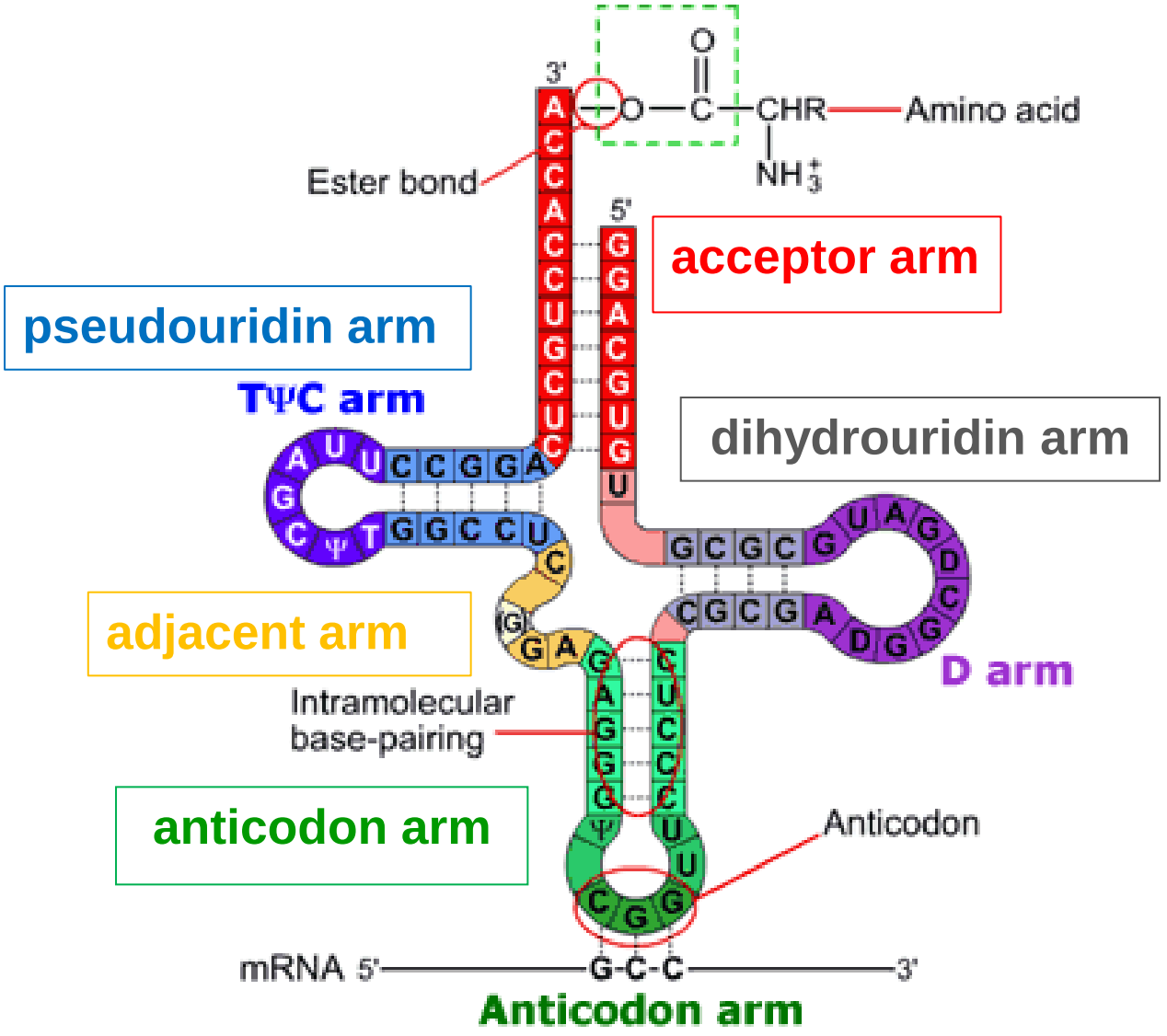
The modified bases in the primary structure of tRNA



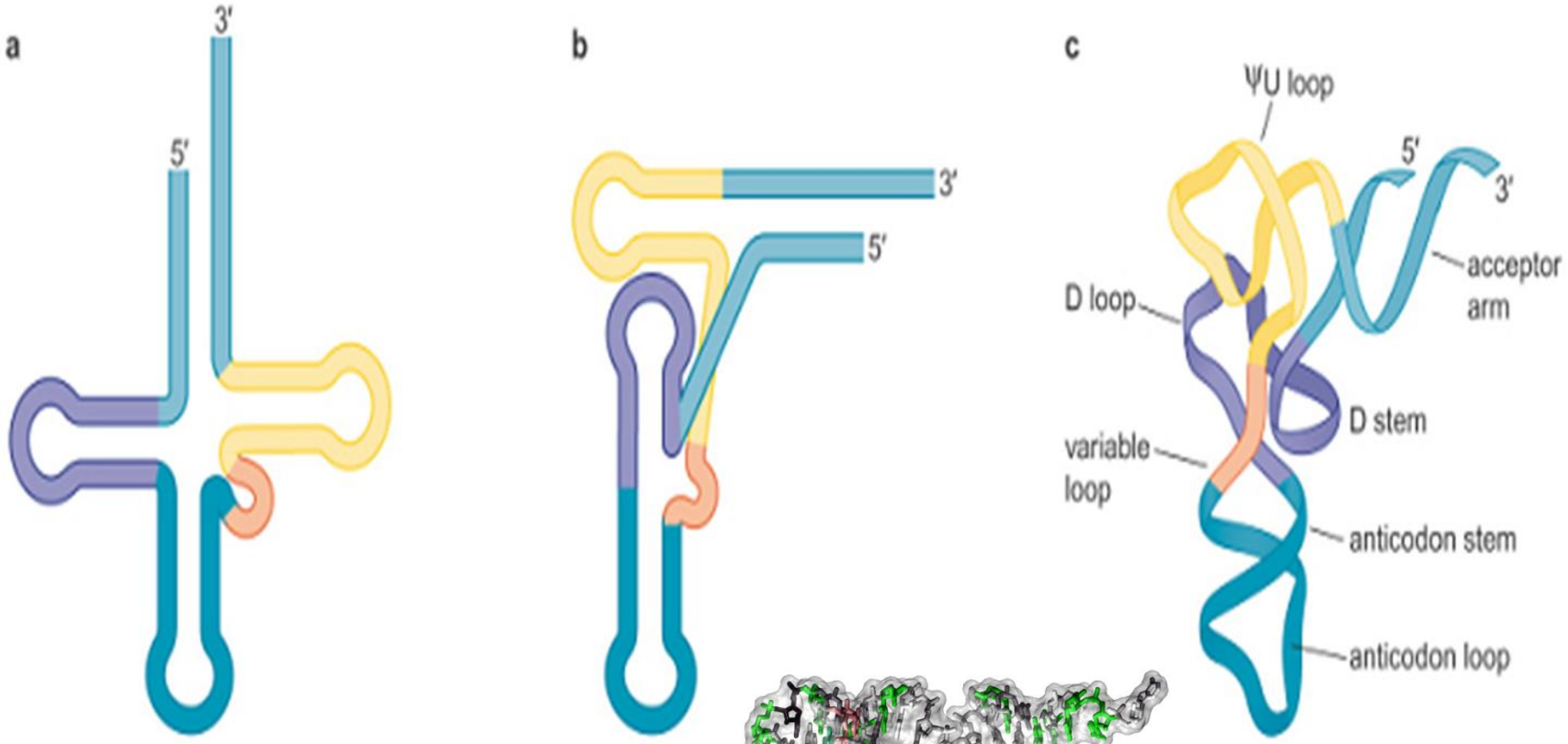
http://genes.atspace.org/7.3.html

The secondary structure of tRNA

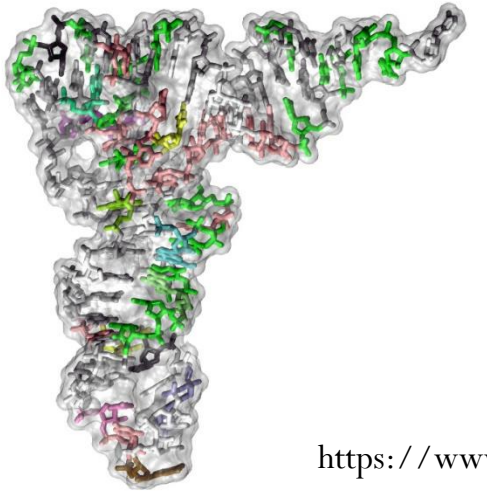
cloverleaf



The tertiary structure of tRNA

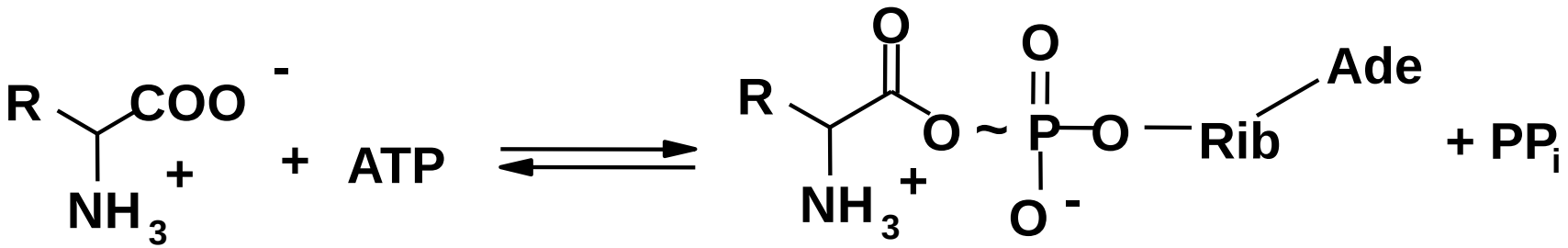


http://bioindians.org/



https://www.irbbarcelona.org

Activation of amino acids

1. 

Macroenergetic bound

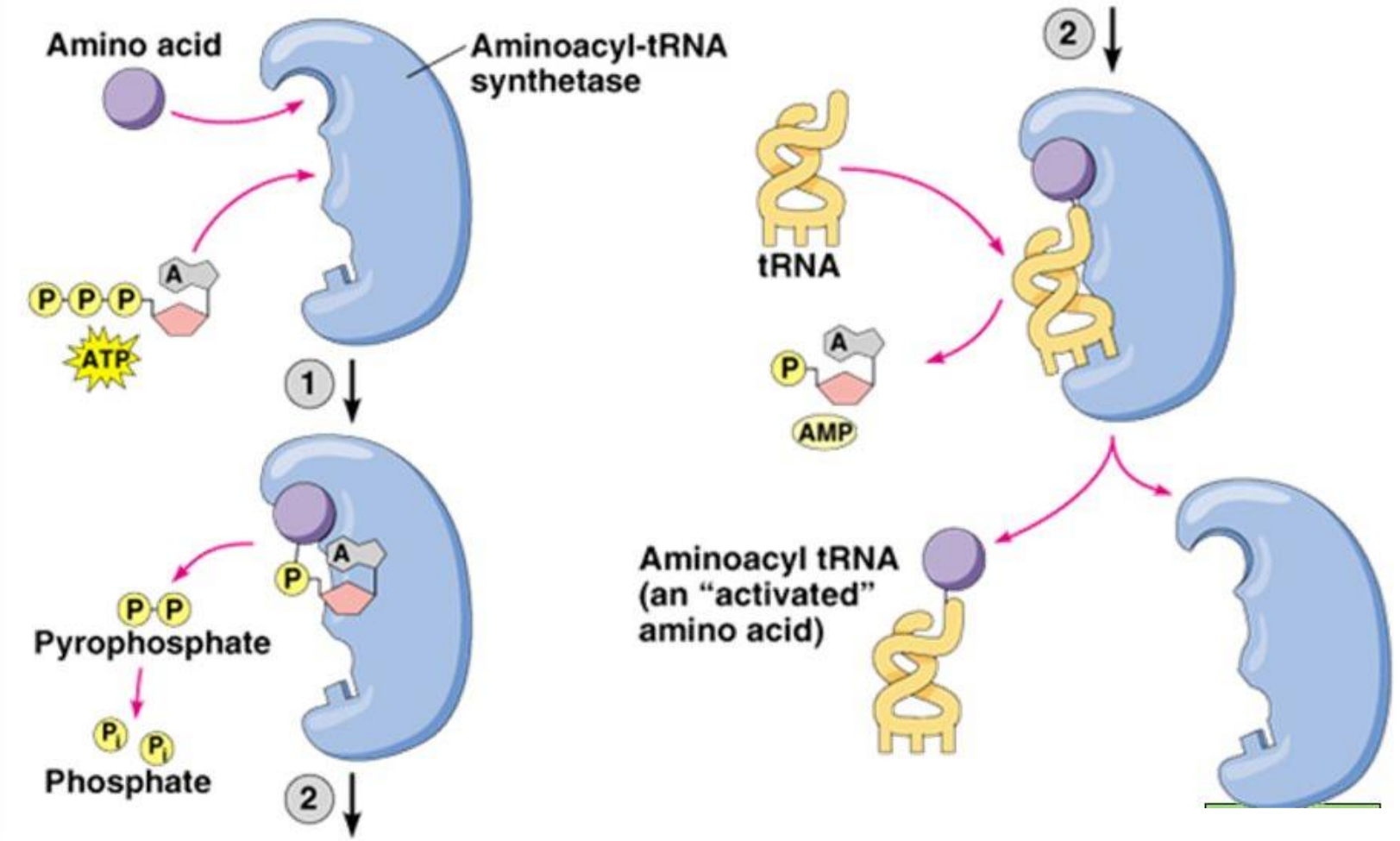
aa~AMP

aminoacyladenylate

2. **aa ~ AMP + tRNA → aa ~ tRNA + AMP**

catalysed by aminoacyl-tRNA-synthetase

Activation of amino acids



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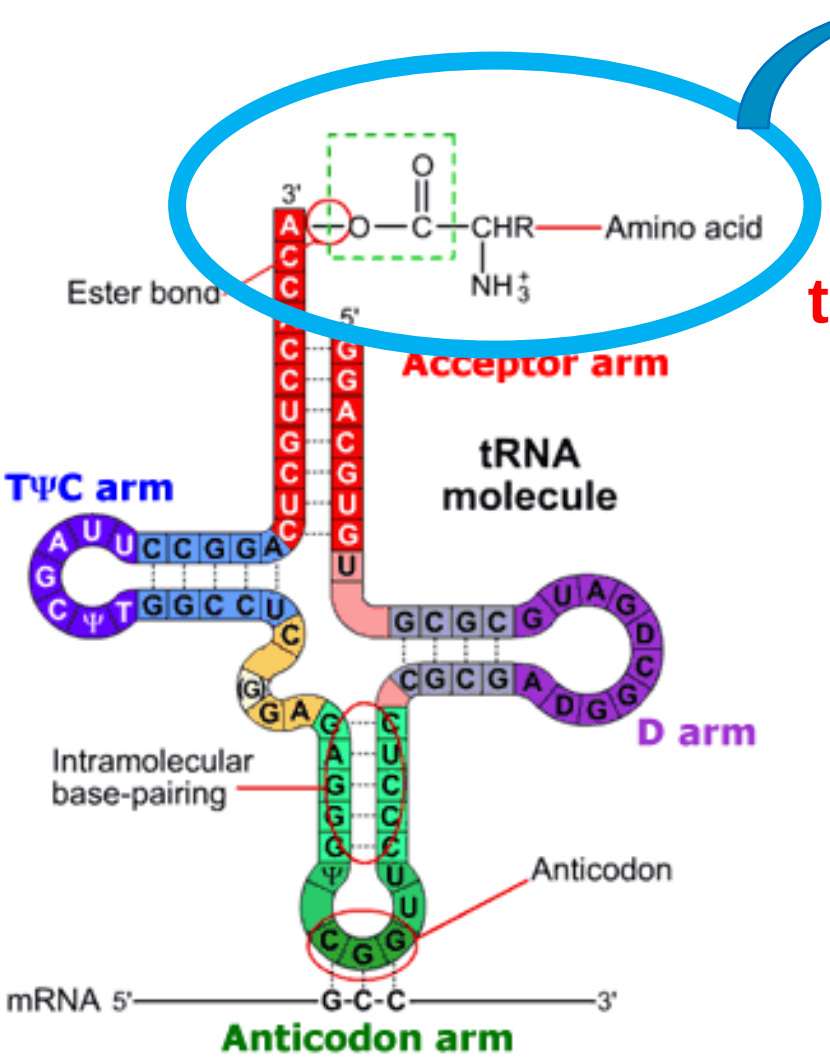
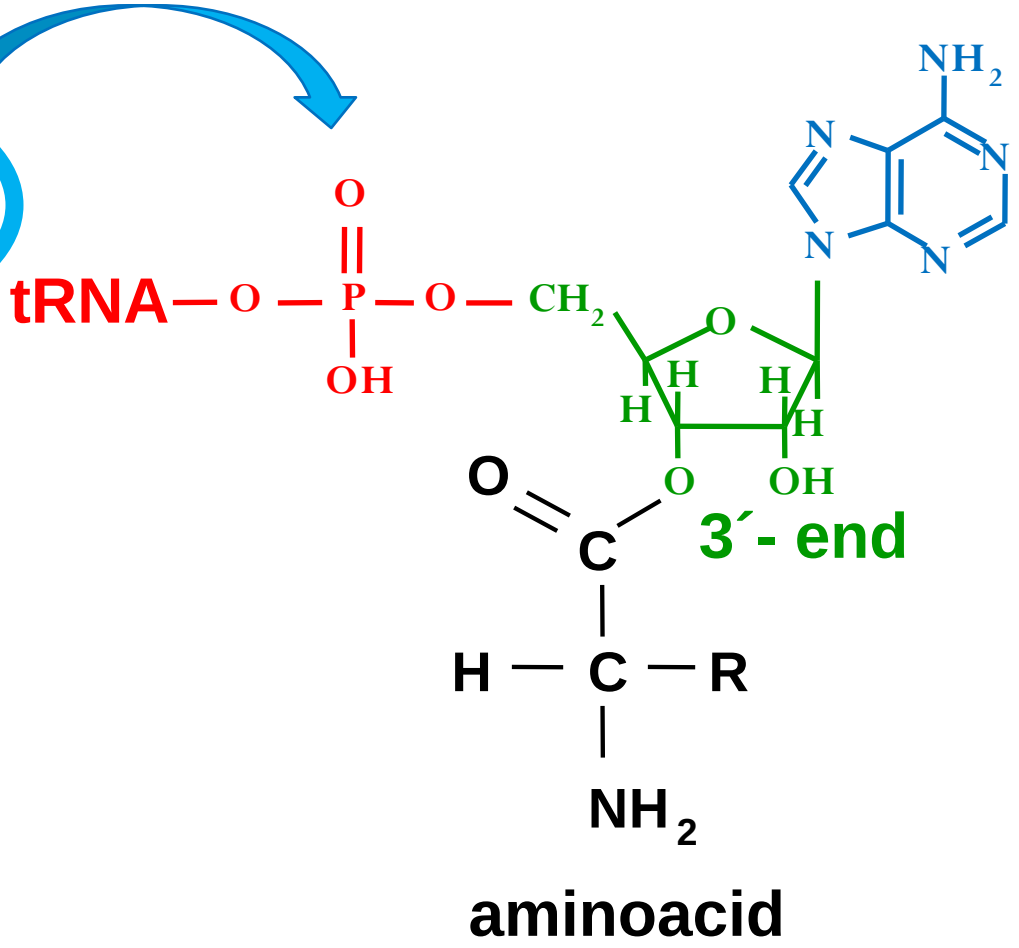
Structure of aa~tRNA





Aminoacyl-tRNA-syntetases

- molecular mass = 40 000 - 100 000
- low homology in primary structure
- several conservative sequences
- each tRNA is specific for only one AA
- binding site for AA
- binding site for tRNA
  - binding site for tRNA is able to bind similar tRNA !
- binding site for ATP

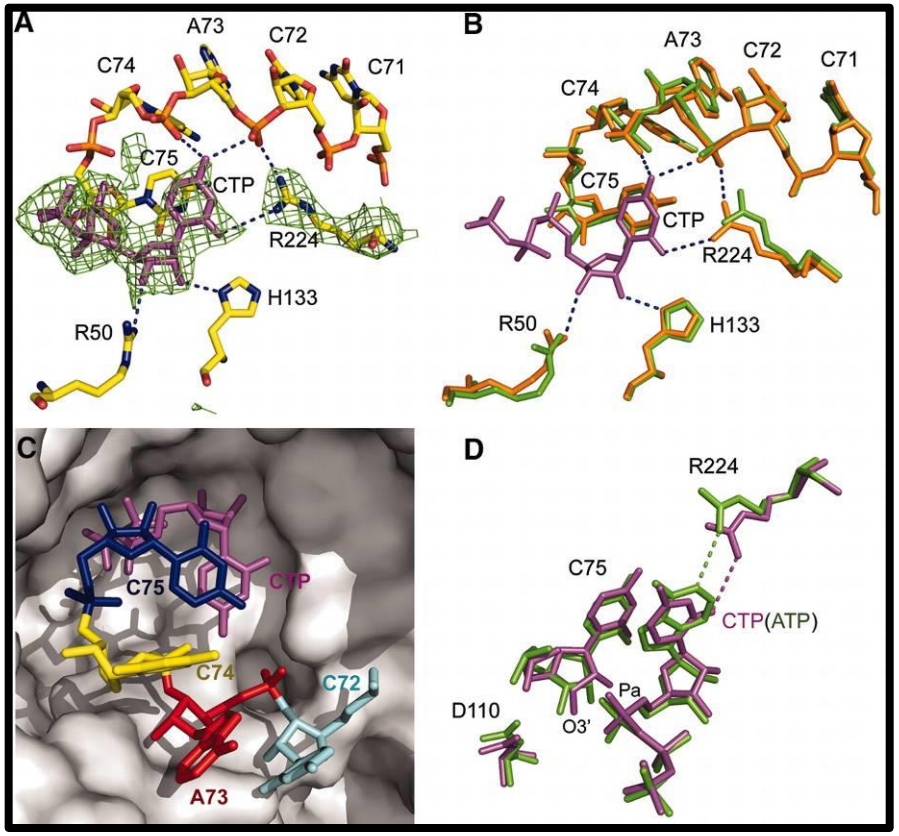
From what coming triplex CCA ?

- This triplex of nucleotides is present at 3´- end of each tRNA
- It is added by nucleotidyltransferase during posttranscriptional processing of theprimary transcript (without any template!)
- Two groups of nucleotidyltransferase exist
  - group I = Archae
  - group II = prokaryotes and eukaryotes
- How the C is added is known for many years
- How the A is added was described in 2010 – crystalography studies by Pan et al.

B. Pan et al., Science 330, 937-940 (2010)

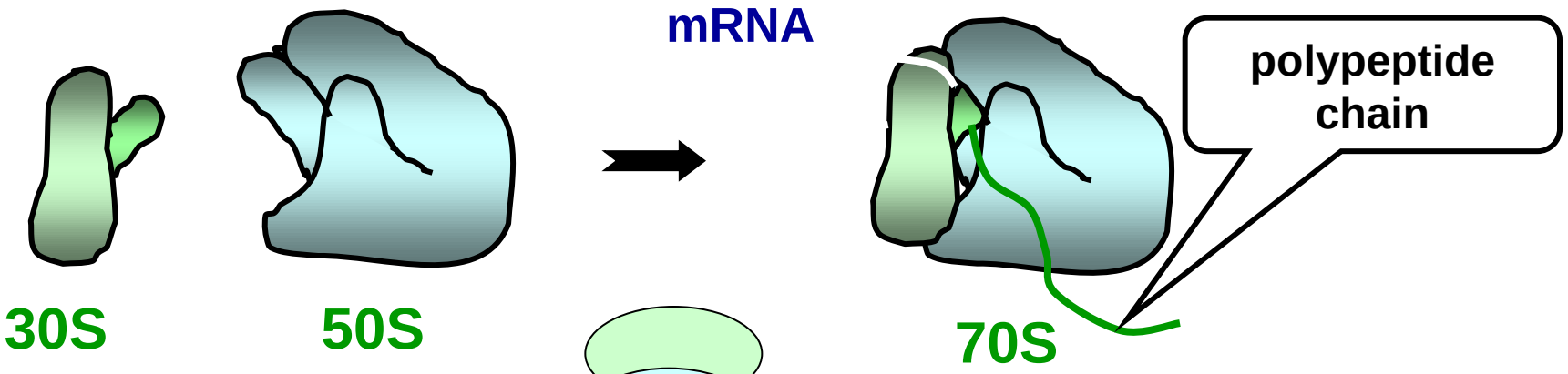
From what coming triplex CCA ?

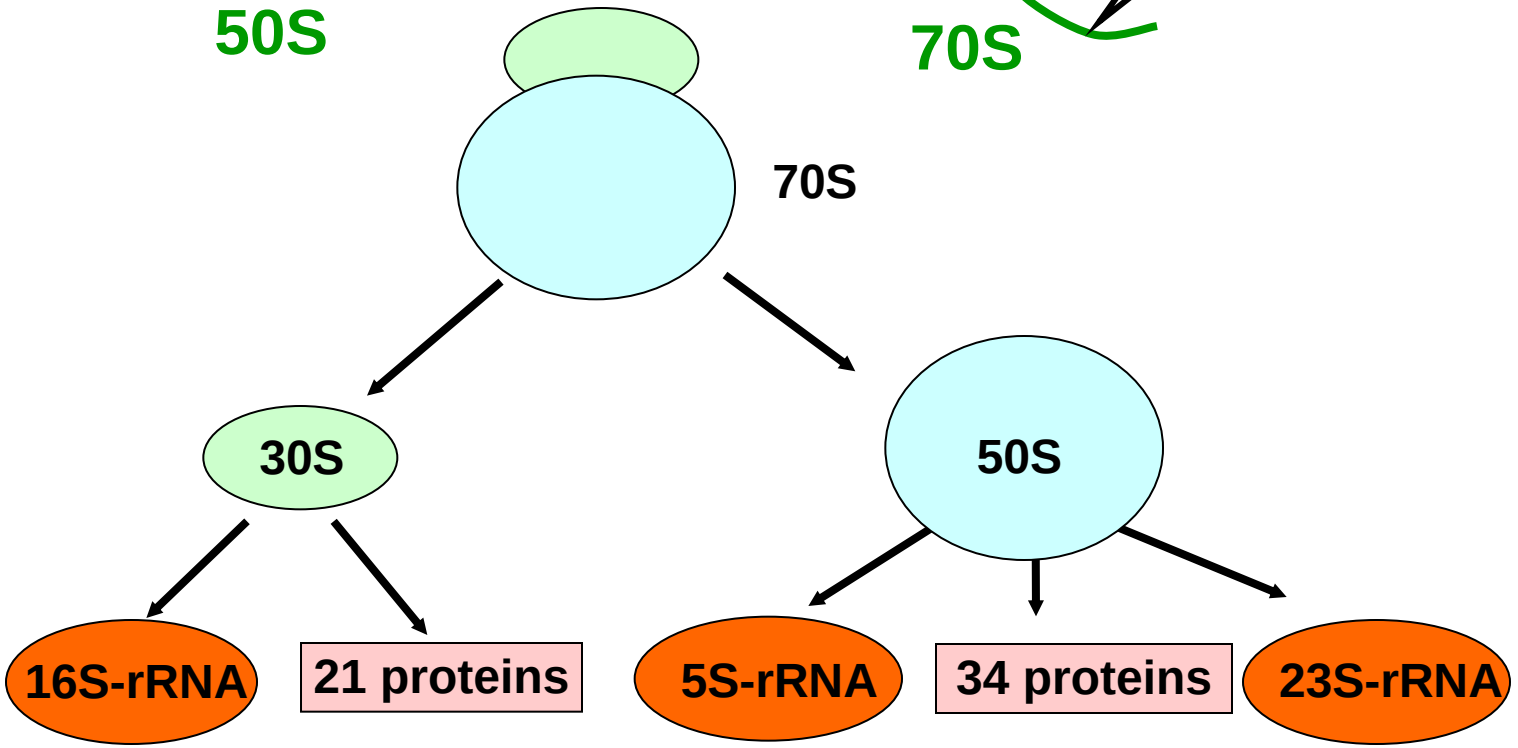
The COOH group from the asparagine acid in position 110 is used as a common base



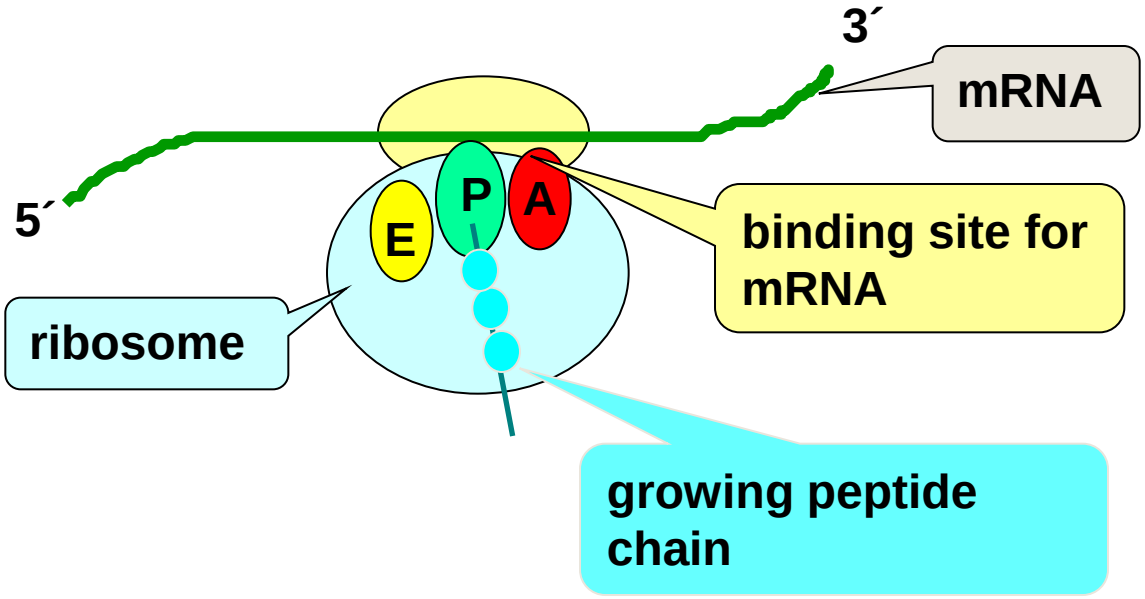
More information in paper B. Pan et al., Science 330, 937-940 (2010)

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

The Nobel prize for chemistry 2009

For research of structure and function of ribosomes
1950 - 1999

Venkatraman Ramakrishnan

MRC Laboratory of Molecular Biology, Cambridge, UK

Ada Yonath

Weizmann Institute, Israel

Thomas A. Steitz

Howard Hughes,

Yale University, USA

How the translation begins ?

1) **Prominence of the codon AUG**

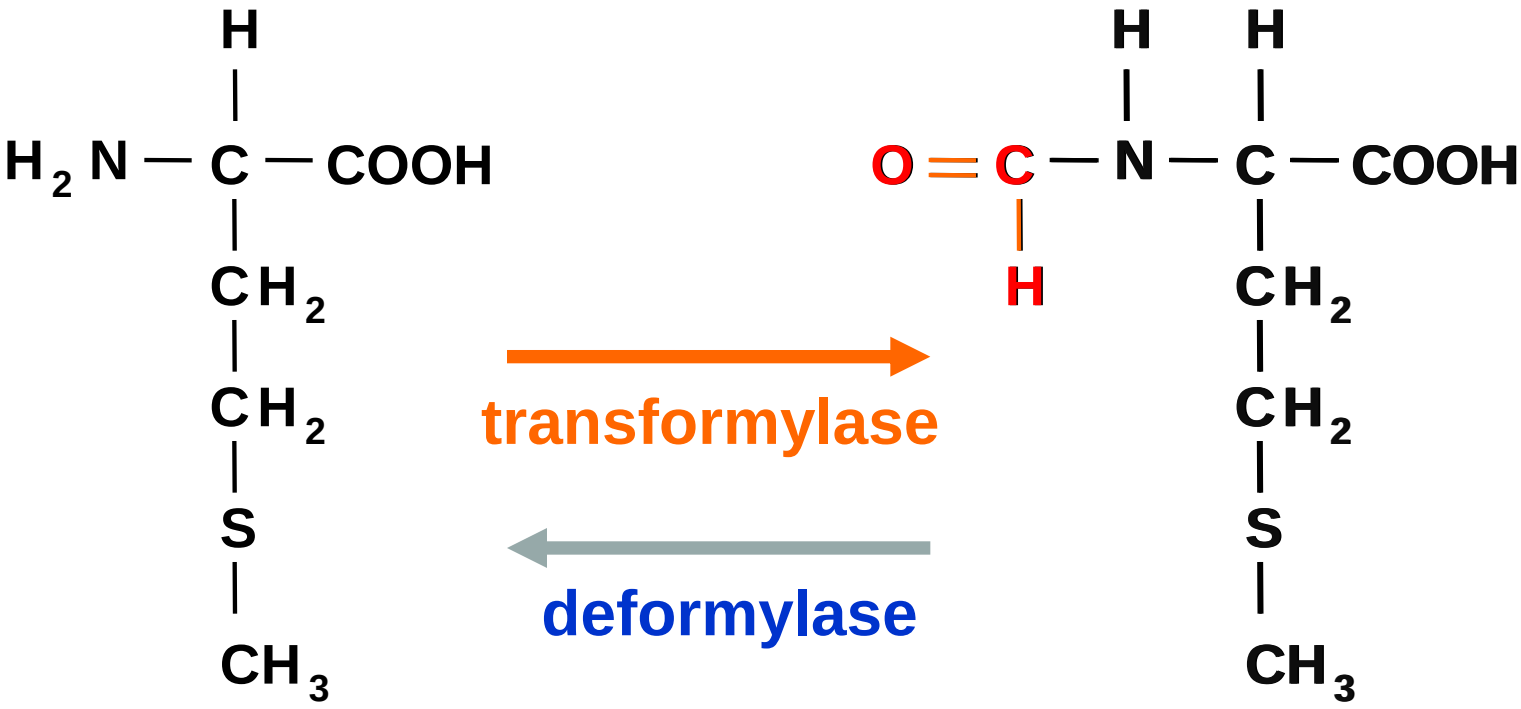
   - it codes the first AA in polypeptide chain

   - 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

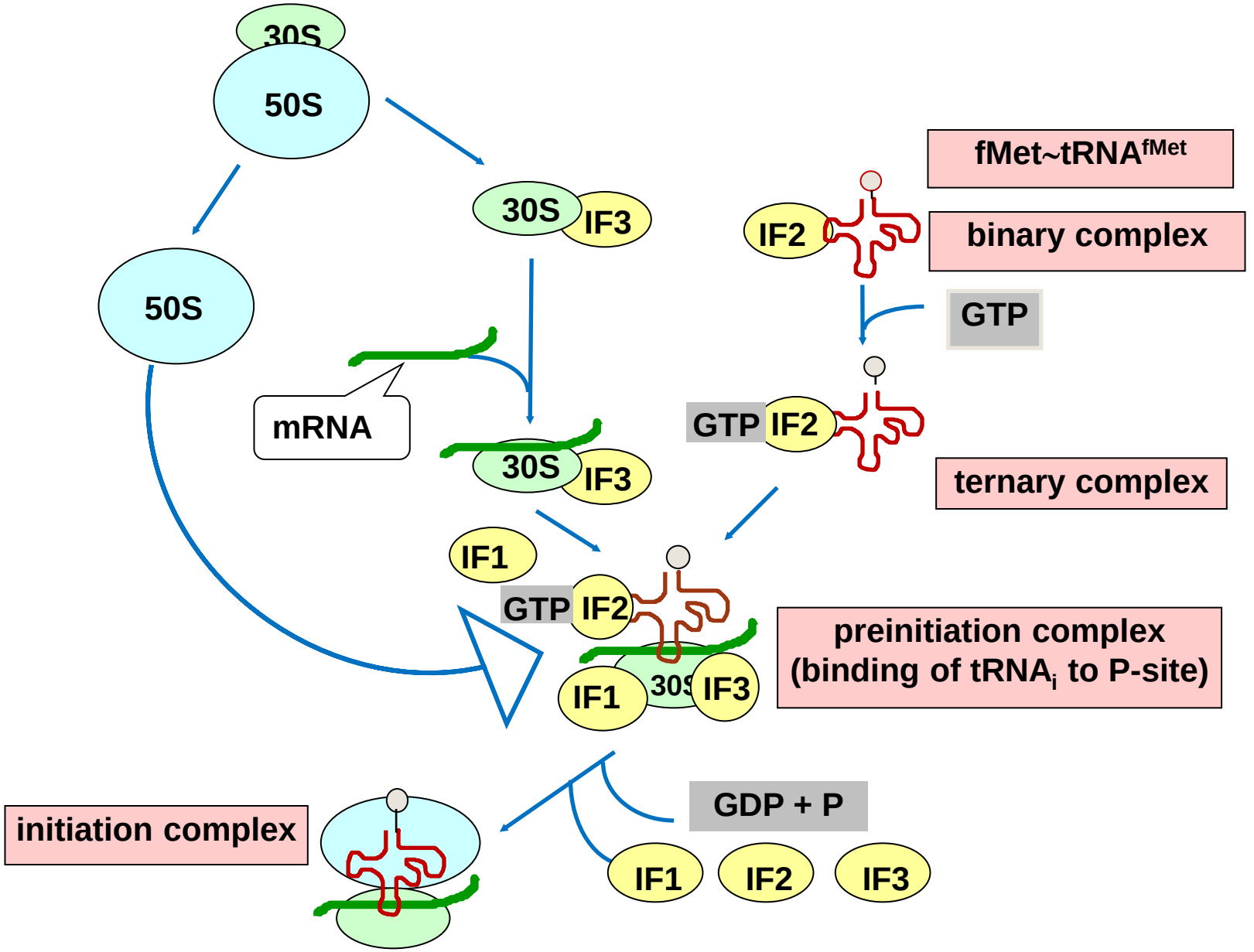


Methionin is formylated on Met ~ $tRNA^{fMet}$

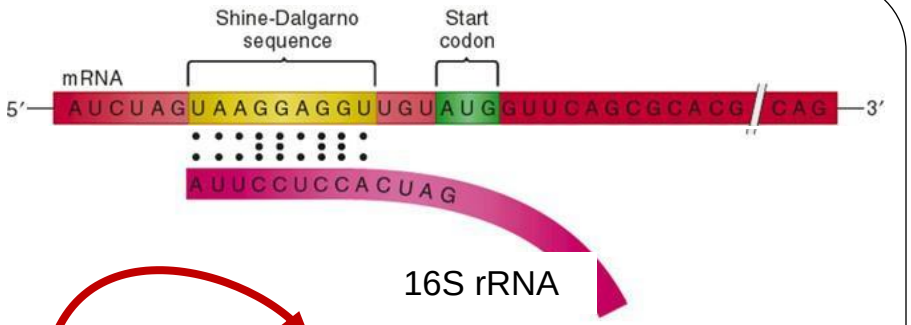
The codon 5´- AUG - 3´

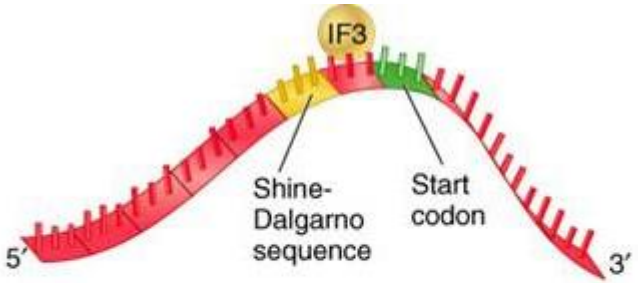
1) If it is at the beginning it binds Met~tRNA^{fMet}, which is formylated for fMet~tRNA^{fMet}, the initiation factor IF2 attends in the process
2) If it is inside, it binds Met~tRNA^{Met}, the elongation factor EF-Tu attends in the process

Initiation of translation (prokaryotic)

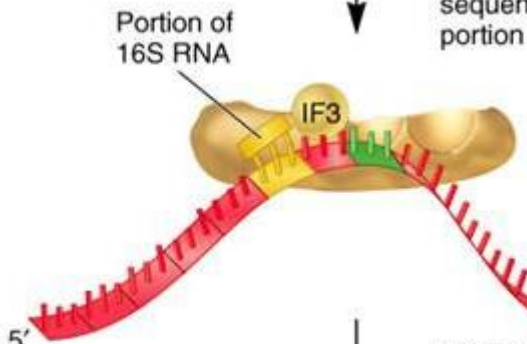


Initiation of translation

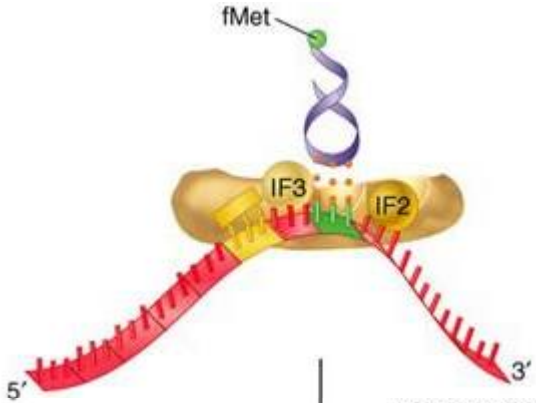




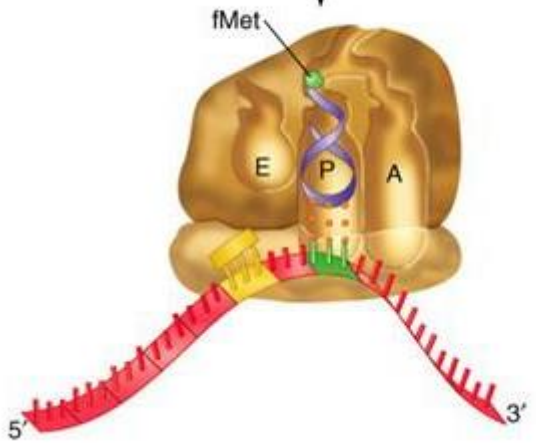
IF3 promotes the binding of mRNA to the 30S subunit. The Shine-Dalgarno sequence is complementary to a portion of the 16S rRNA.



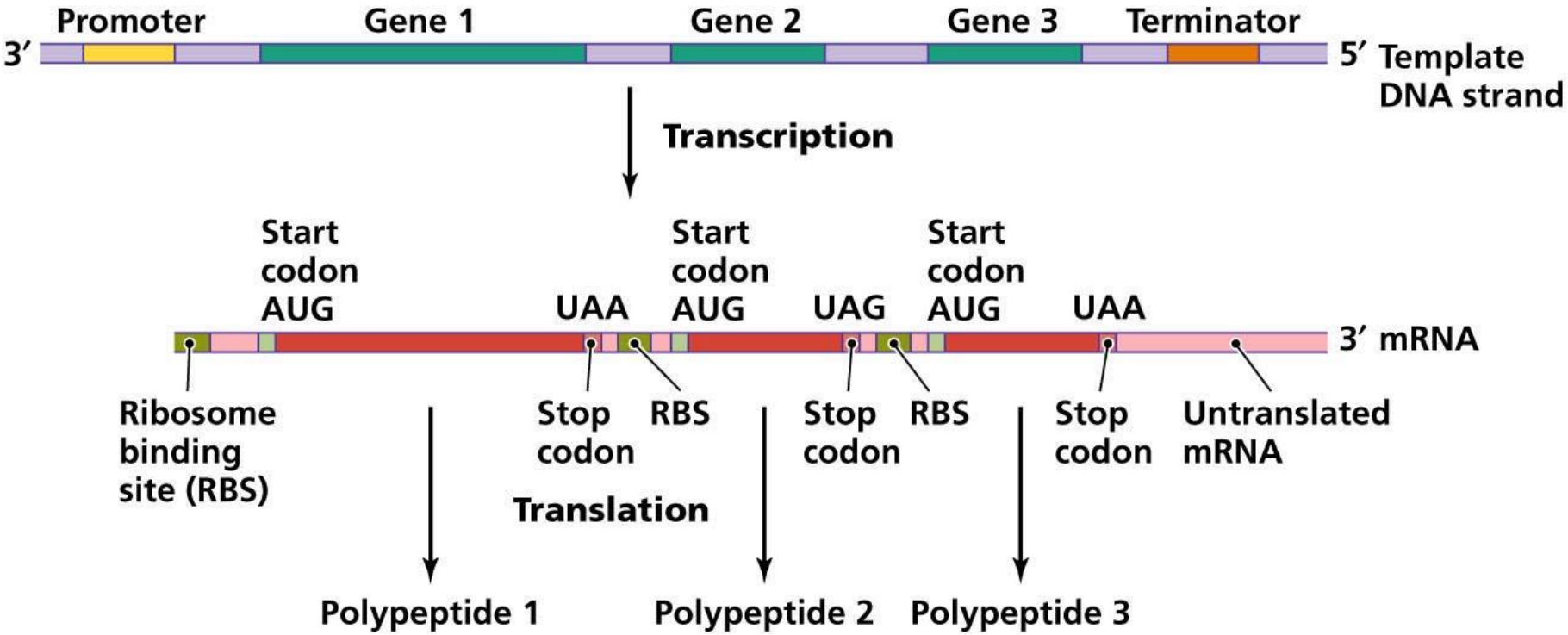
IF2 promotes the binding of the initiator tRNA.



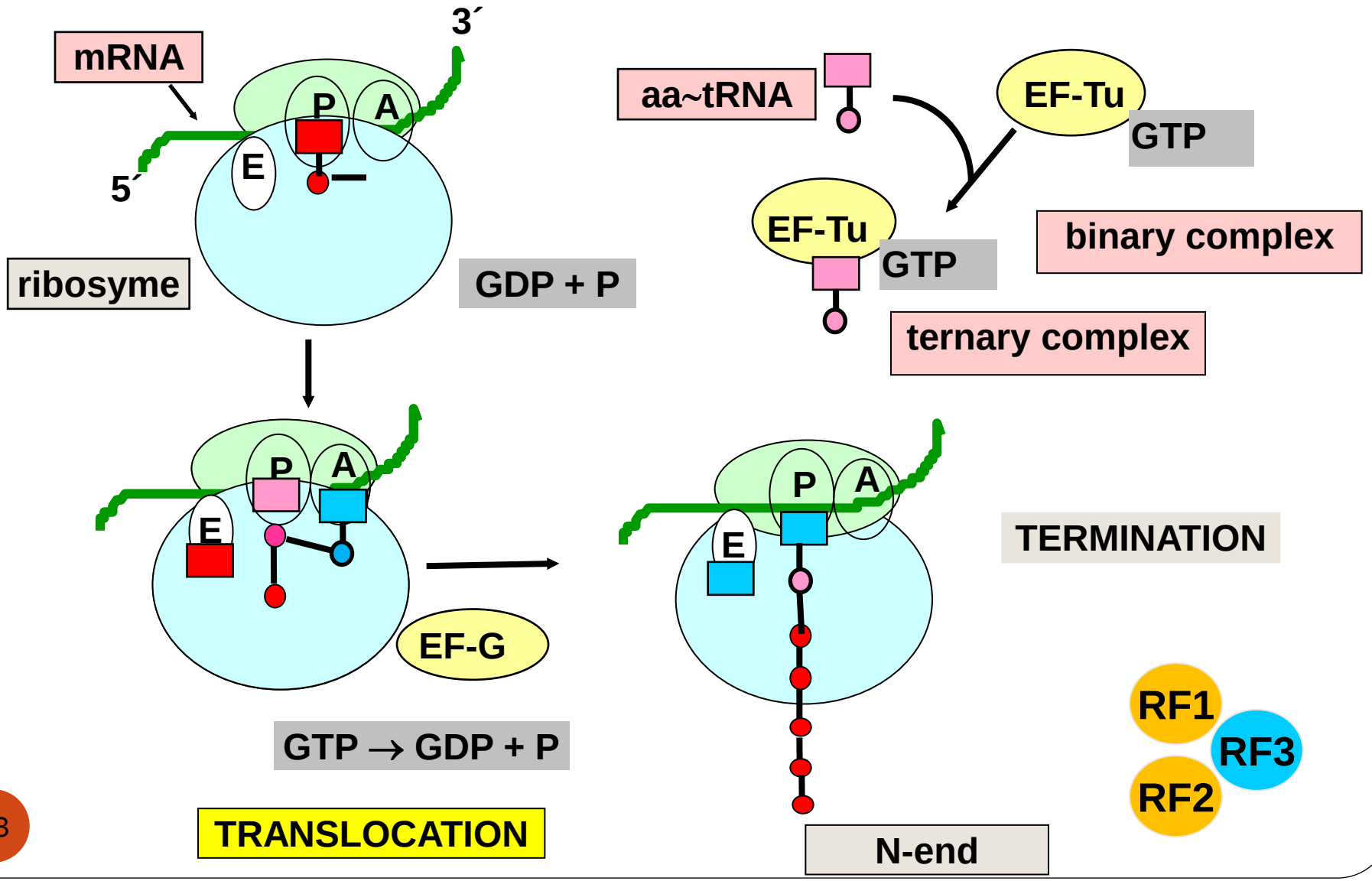
IF2 and IF3 are released. The 50S subunit associates.



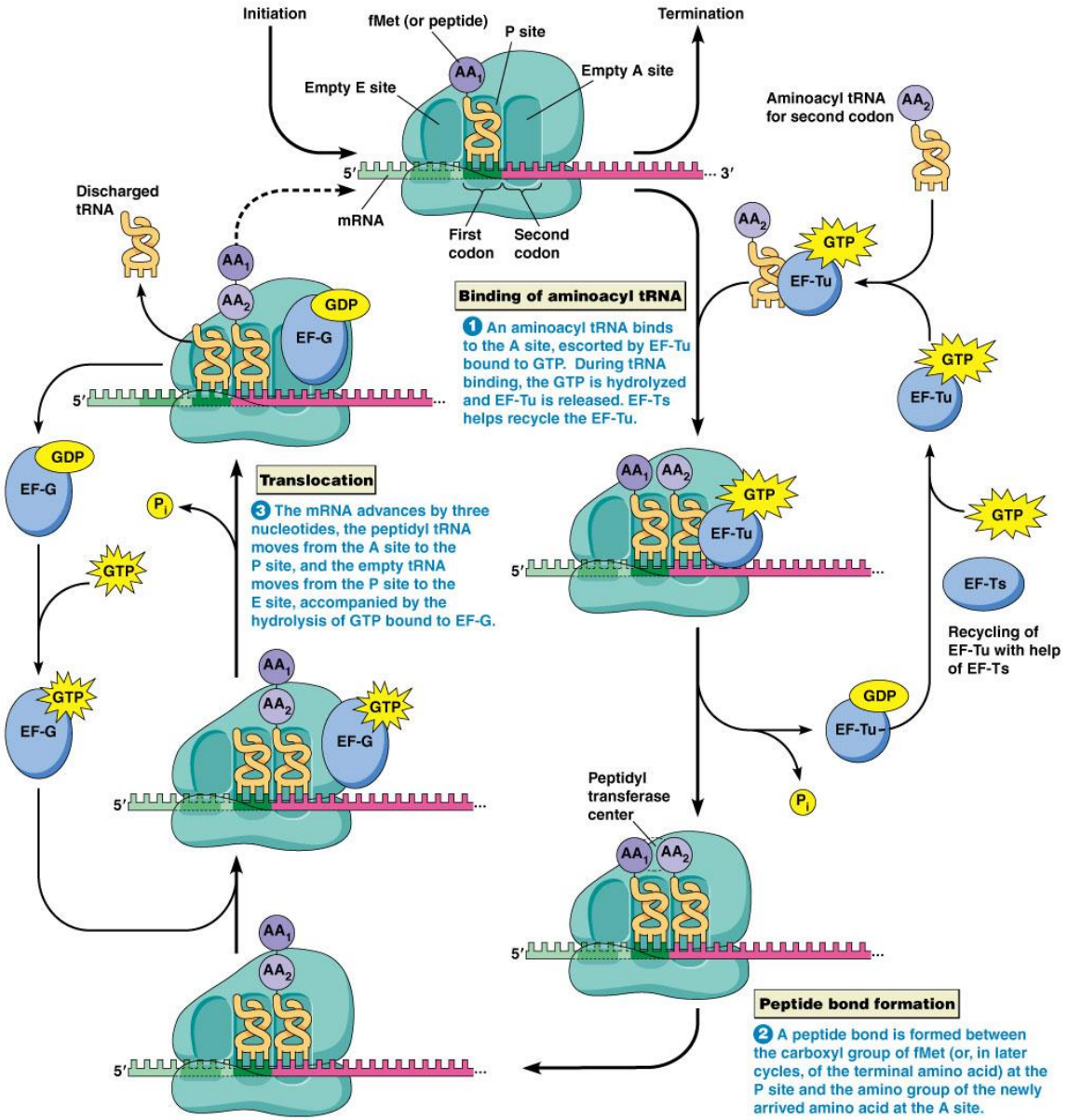
Ribosomes bind also on intergenes sequences



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



Elongation of polypeptide chain



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

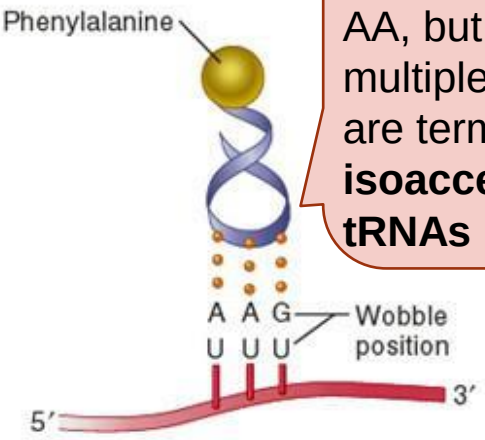
Wobble base paring in tRNA

- 22 AA – 64 triplets – 40 tRNAs

????

- Some tRNAs recognise more codons
- F. Crick proposed the wobble hypothesis in 1966 to explain the pattern of degeneracy
- 1st two bases of the codon-anticodon (5´-XXo-3´) pair strictly by watson-Crick rules
- The 3rd (5´-ooX-3´) can wobble and this movement allows alternative H-bonding between bases to form non-Watson-Crick base paring

tRNAs charged with the same AA, but recognise multiple codons are termed **isoacceptor tRNAs**



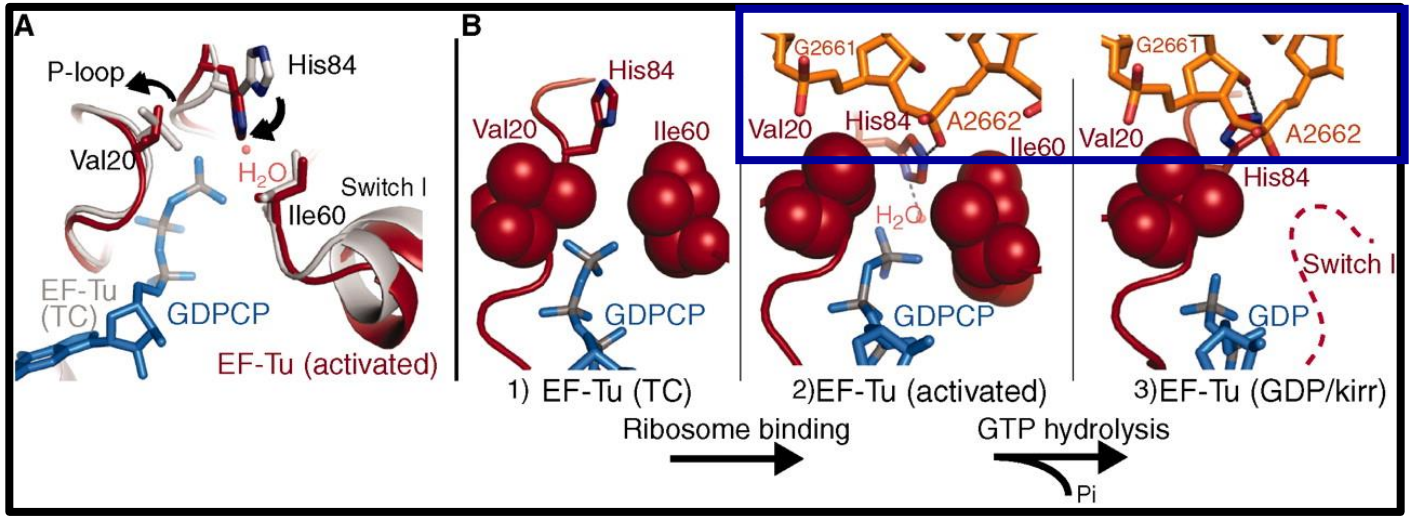
http://slideplayer.com/slide/3524858/

Revised wobble rules

Nucleotide of anticodon	Third nucleotide of codon
G	C, U
C	G
A	U, C, (A), G
U	A, U, G, (C)
I	U, C, A
xm^5s^2U xm^5U^5 Um xm^5U	A, (G)
xo^5U	U, A, G
k^2C	A

The central role of GTPase

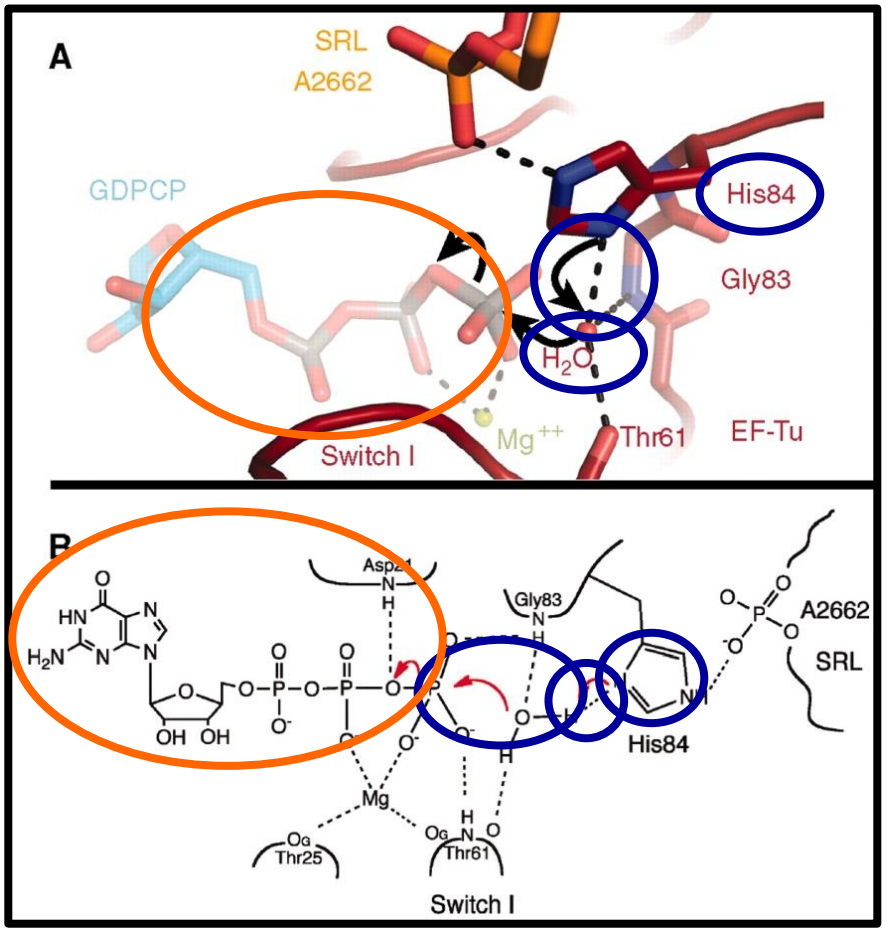
- GTPase is involved in the process
- EF-Tu is a part of GTPase
- A domain which binds GTP is evolutionary highly conservative and is present from bacteria to higher eukaryotes
- Hydrolysis of GTP is associated with conformation change, in which A2662 from 23S-rRNA is involved



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

Chemism of GTP hydrolysis?

- His84 acts as a general base
- which activate the catalytic water molecule by removing a proton
- the proton attacks γ-phosphate of GTP
- GDP is released



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

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

Translation - video

Initiation

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

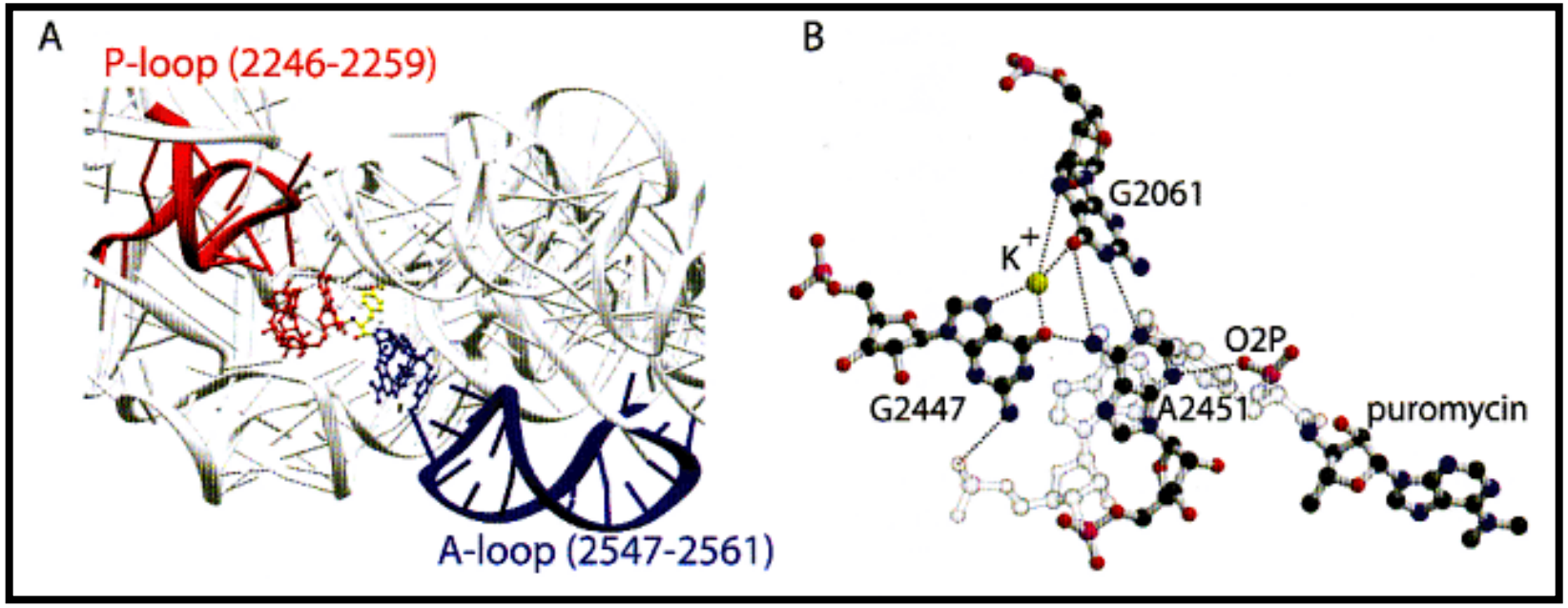
Elongation

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

Termination

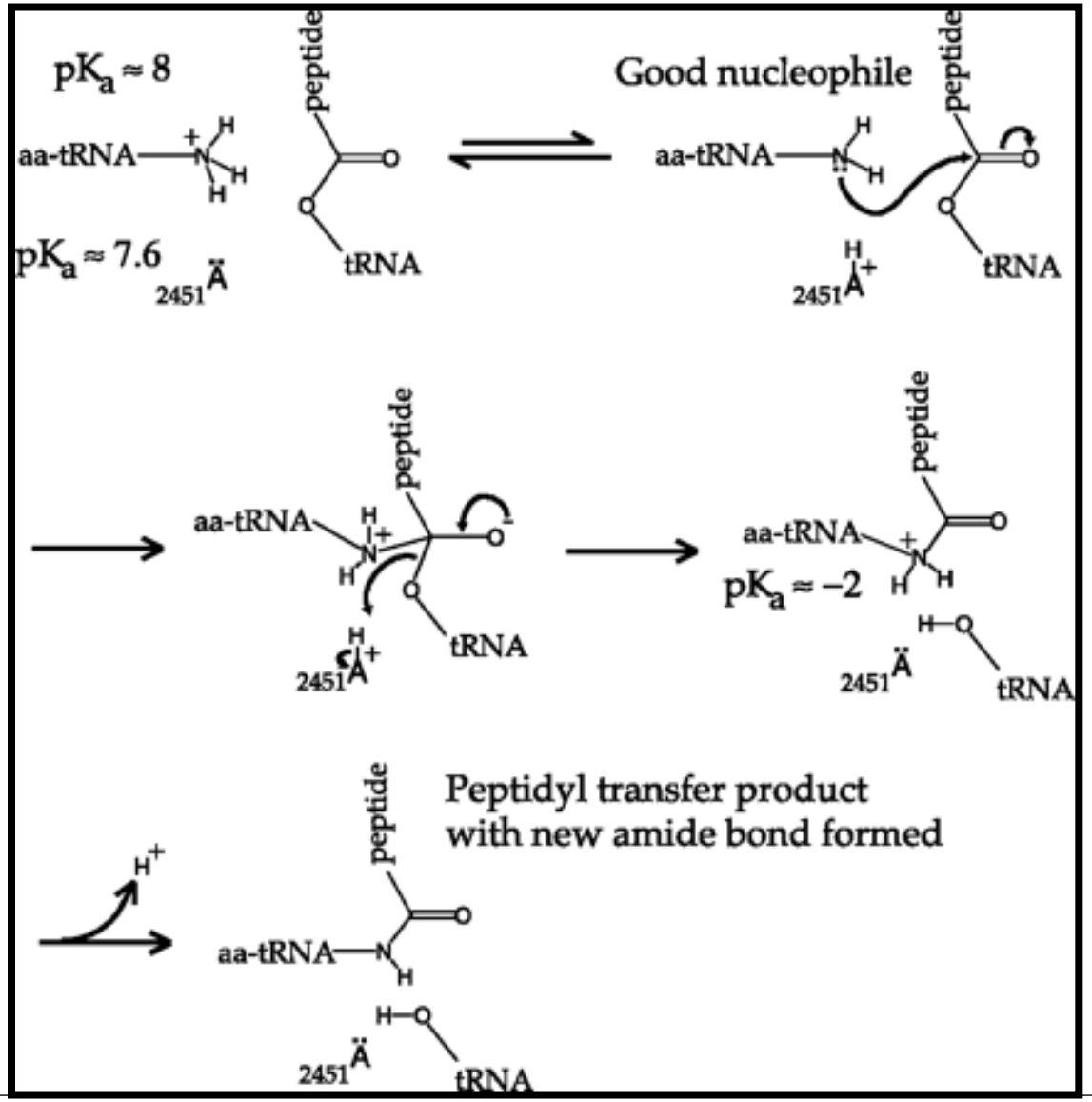
https://www.youtube.com/watch?v=MNMc28EEkK0

The catalytic site for peptide bond formation

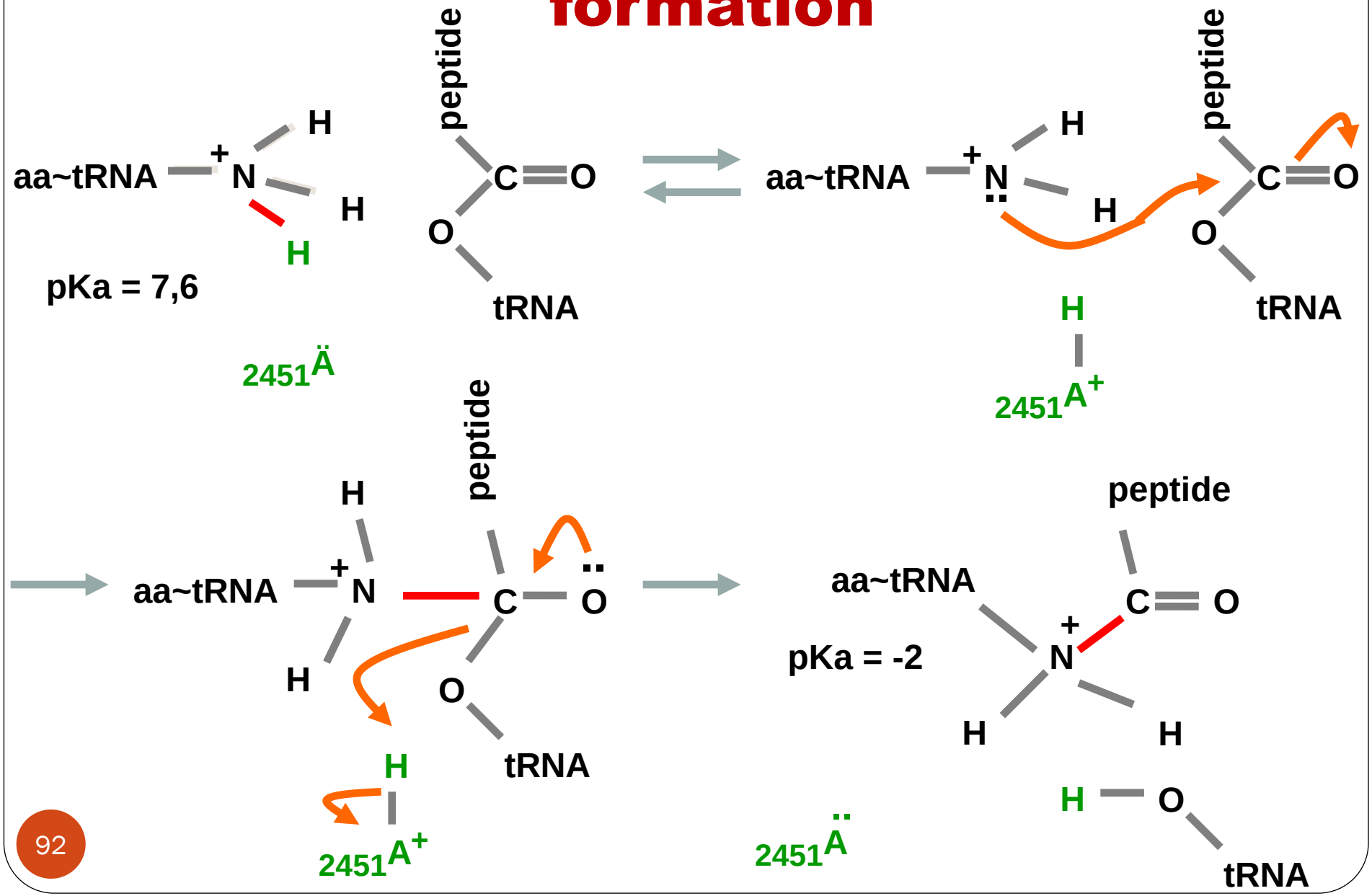


- P-loop and A-loop are the parts of 23S-rRNA
- No protein in the distance 18A from the catalytic site
- crucial is nitrogen N3 on adenine A2451

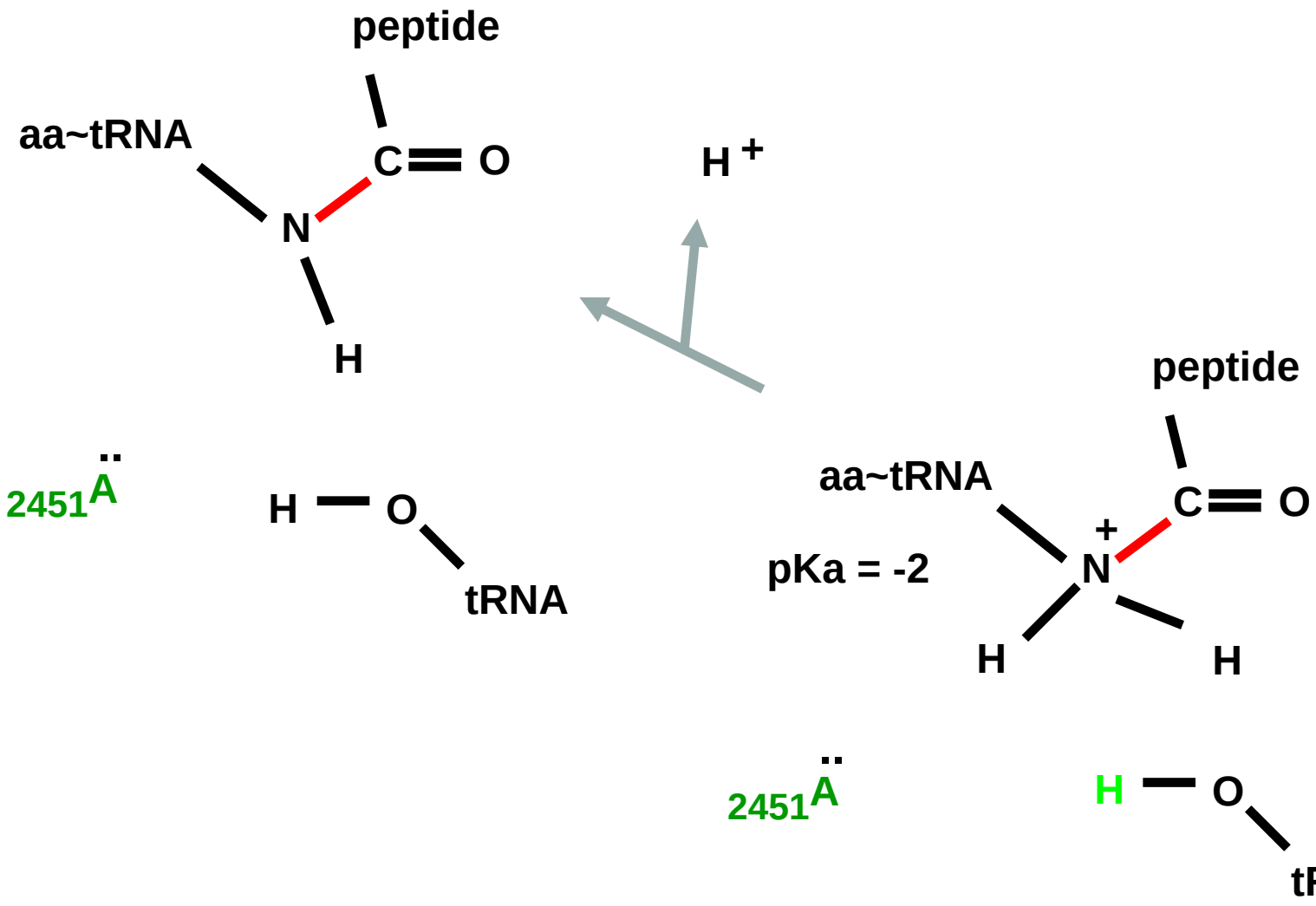
The mechanism of peptide bond formation



The mechanism of peptide bond formation



The mechanism of peptide bond formation



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