RNA synthesis, regulation of gene expression

(E.T.)

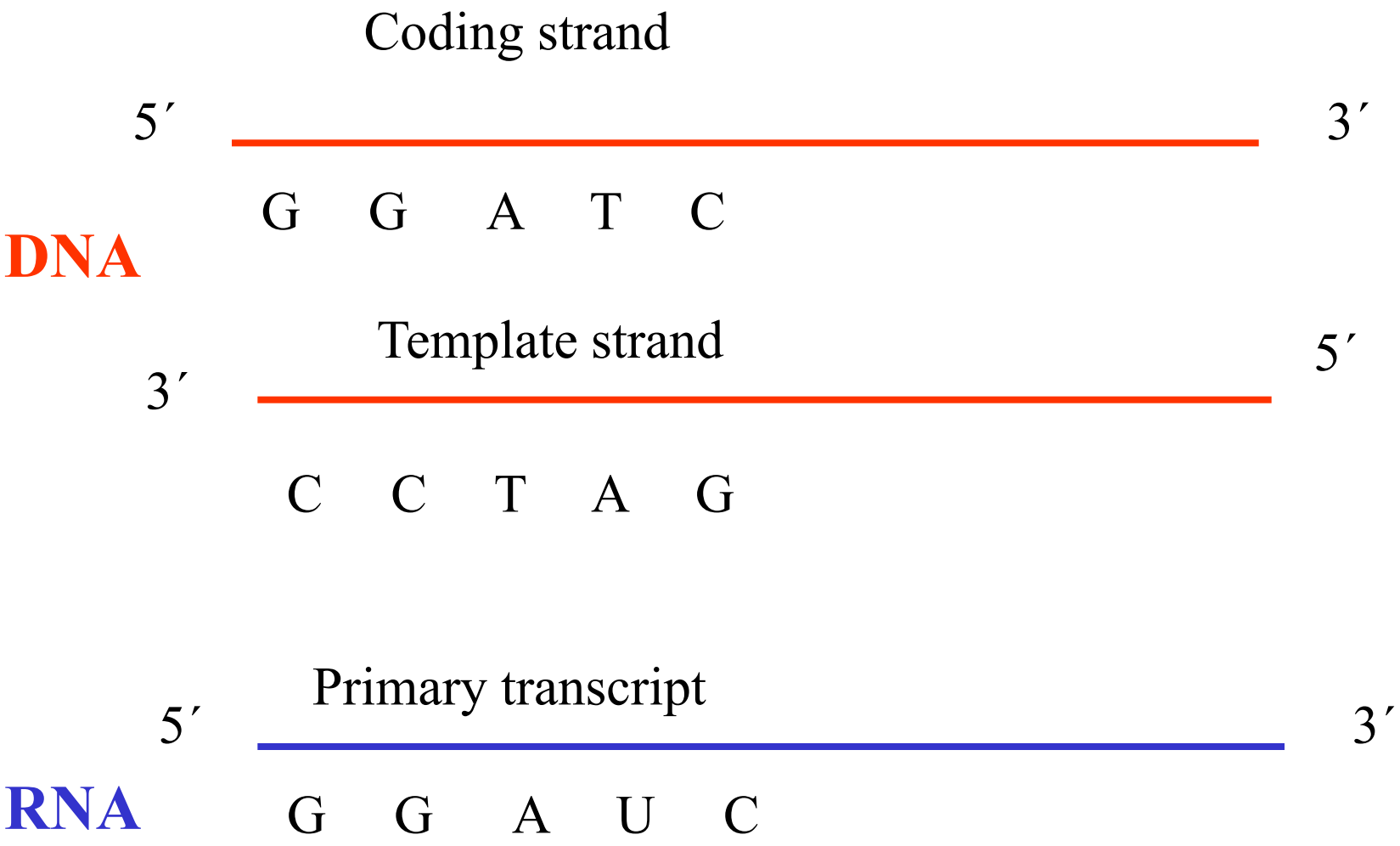
Transcription

Synthesis of RNA from a DNA template

Only one strand of double helix DNA is transcribed – template strand.

The second strand is coding strand (its sequence is identical with primary transcript only U is replaced by T)

Terminology



RNA transcript is synthetized in the 5′→ 3′ direction

For transcription are necessary:

dsDNA

RNA-polymerase

ATP, GTP, CTP, UTP

Mg^{2+} ions

Replication x Transcription

Characteristic	**replication**	**transcription**
Enzymes:	DNA-polymerases	RNA-polymerases
Location:	On chromosome at S phase	Selected fragment of DNA
Initiation:	RNA primer is necesssary	no primer is necessary
Procedure:	Both strands are copied	Only one strand is copied
Control:	Proofreading and DNA repair	polymerase do not include proofreading
nucleotides:	dATP, dGTP, dCTP, dTTP	ATP, GTP, UTP, CTP

Transcription is carried out by DNA-dependent RNA polymerases (transcriptases)

Prokaryotes:

•only one polymerase composed of 5 subunits plus sigma factor.

• it transcripts all forms of RNA

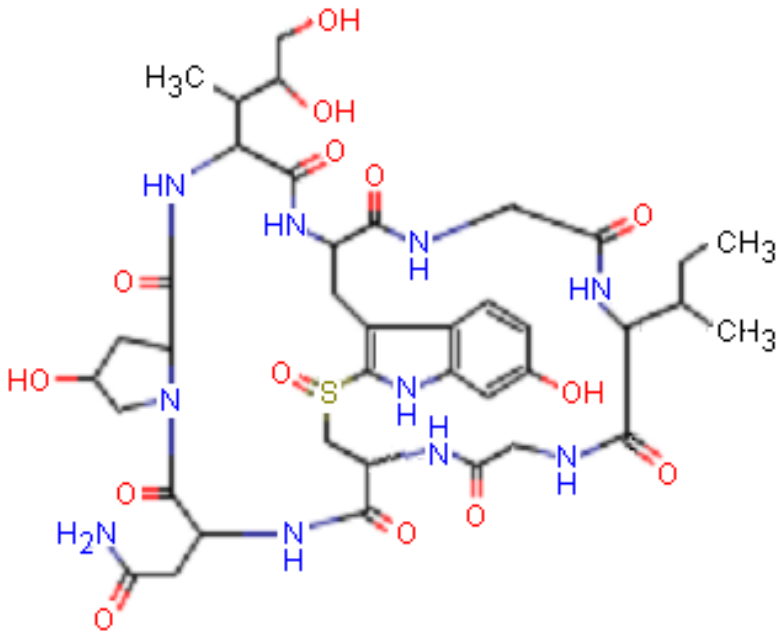
Eukaryotes

4 different RNA polymerases
RNA pol I – synthesis of r RNA (in nucleolus)
RNA pol II – synthesis of mRNA (nucleus)
RNA pol III – synthesis of tRNA, 5S RNA (nucleus)
RNA pol IV - synthesis of mitochondrial RNA

The mechanism of the action is the same, they recognize various promoters

Amanitine (bacterial toxine from Amanita phalloides) - cyclic octapeptide with unussual amino acids

Inhibitor of eukaryotic RNA polymerase (mainly of II-type)



Three phases of transcription

- initiation
- elongation
- termination

Mechanism of RNA polymerase function

•Synthesis of RNA occurs in 5´→3 direction

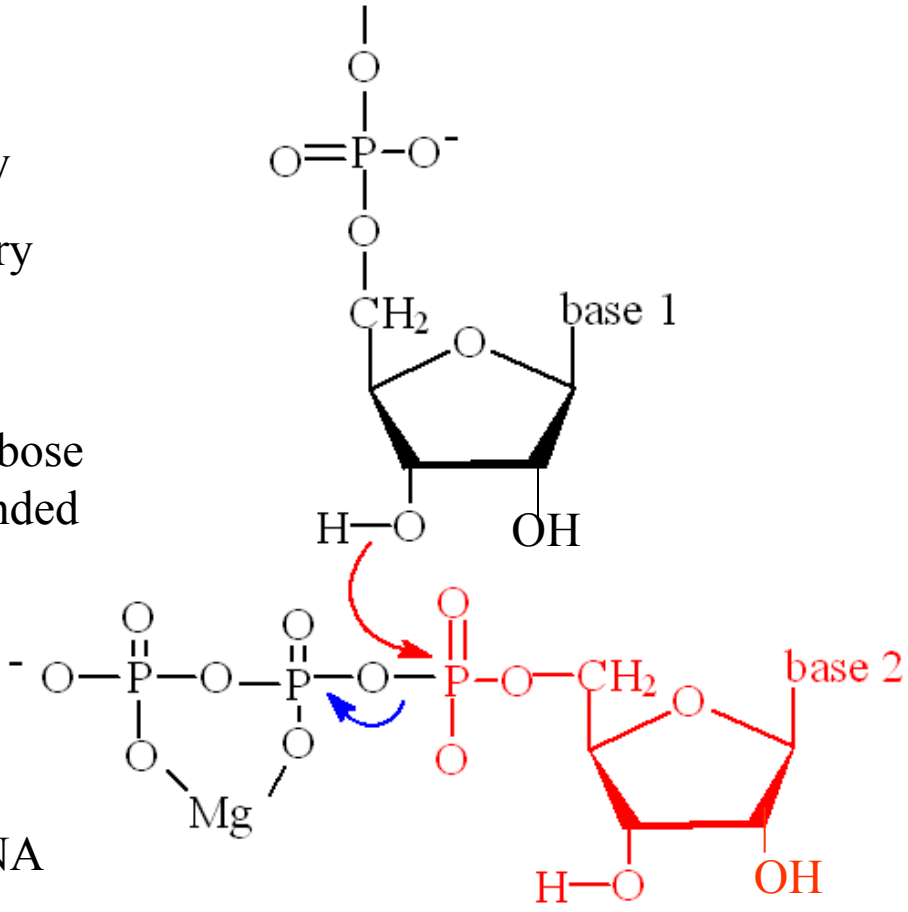
•Nucleotides ATP,GTP,CTP,UTP are necessary

• Each nucleotide pairs with the complementary nucleotide on the DNA template

• Polymerase catalyzes formation of phosphodiester bond between 3 -OH end of ribose on growing RNA-strand and α-phosphate bonded to 5 OH of ribose new nucleotide

• the energy for polymeration is provided by cleavage of NTP and release of diphosphate

•In contradistinction to DNA polymerases, RNA polymerases don´t exhibit any nuclease (proof-reading) activity so that they cannot correct mismatches.



+ PPi

Terminology of transcription

Promoter – specific sequence on DNA template about 40 nucleotides long lying in upstream position to the initiation site

Transcription unit - sequence of nucleotides in DNA that codes for a single RNA molecule, along with the sequences necessary for its transcription; normally contains a promoter, an RNA-coding sequence, and a terminator

Boxes (elements): small sequences in the promoter region

Cis-acting sequences: lying on the same molecule of DNA that is transscribed, near the gene

Trans-acting factors : proteins that bind to these DNA sequences and facilitate or prevent binding of DNA polymerase (genes for their synthesis are lying on different chromozomes)

Primary transcript - RNA product synthesized in direction u 5′→3′

Initiation of transcription

•RNA polymerase (RNAP) binds to specific nucleotide sequences – promoters
• stable complexes with template DNA strand and RNA at the promoter region are formed

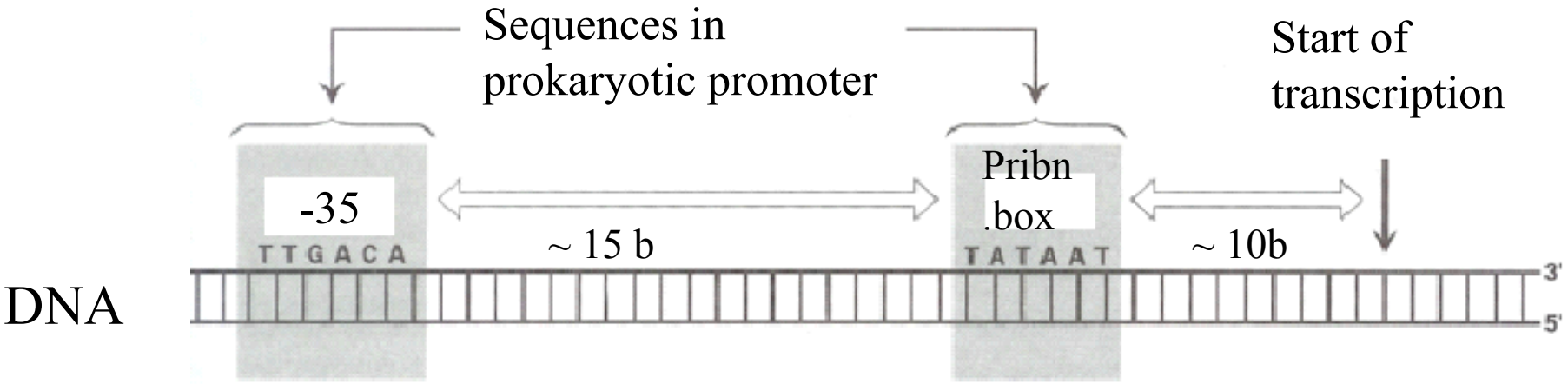
The promoter contains characteristic consensus sequences (common conserved regions that are found in certain area of all genes)

Promoter in DNA of prokaryotes

In position ~ -10 TATAAT box (Pribnow box)

In position ~ -35 another sequence TTGACA

These sequences are recognized by σ -factor of prokaryotic RNA polymerase



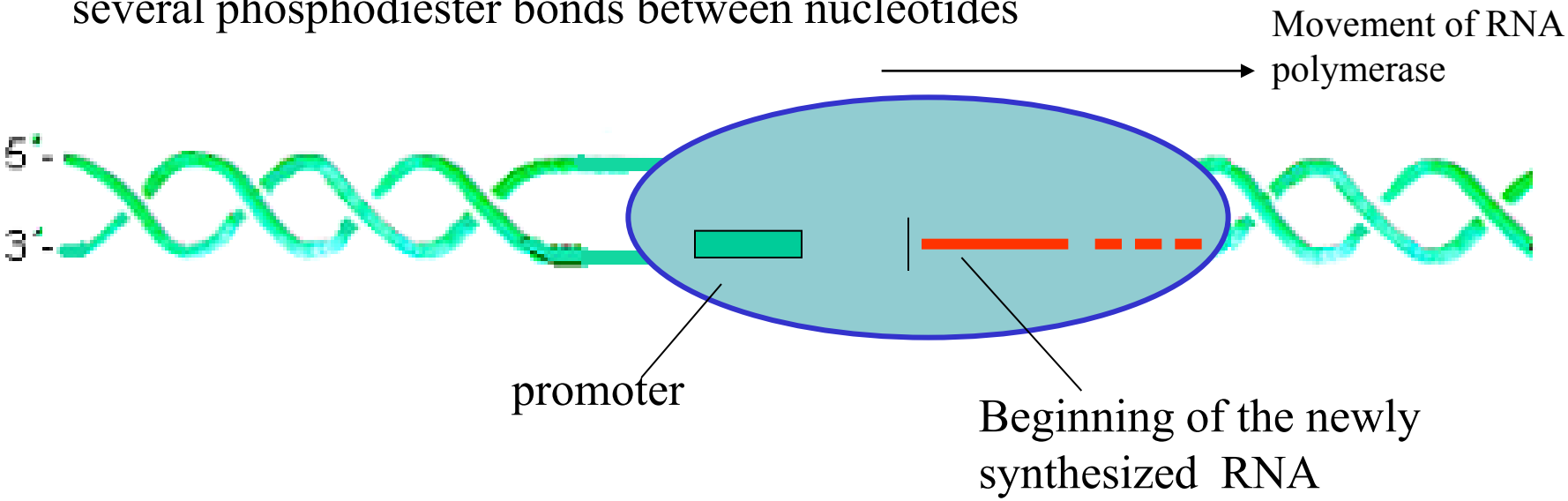
Transcription in prokaryotes

Iniciation:

Binding of RNA-polymerase to promotor region of DNA by sigma subunit

Local unwinding of DNA caused by RNA polymerase

Pairing of ribonucleotide bases with template strand and formation of several phosphodiester bonds between nucleotides



RNA polymerase is inhibited by antibiotics rifampicine

Transcription in prokaryotes

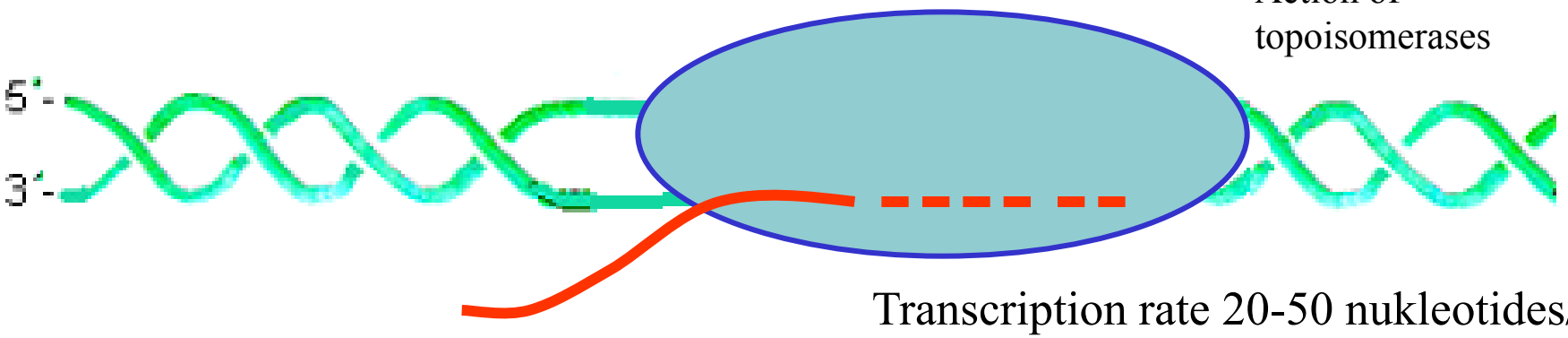
Elongation:

Release of sigma subunit from RNA polymerase

RNA polymerase moves along the template strand, unwinding of double helix

Formation of covalent ester bonds among the nucleotides

Newly formed RNA is released

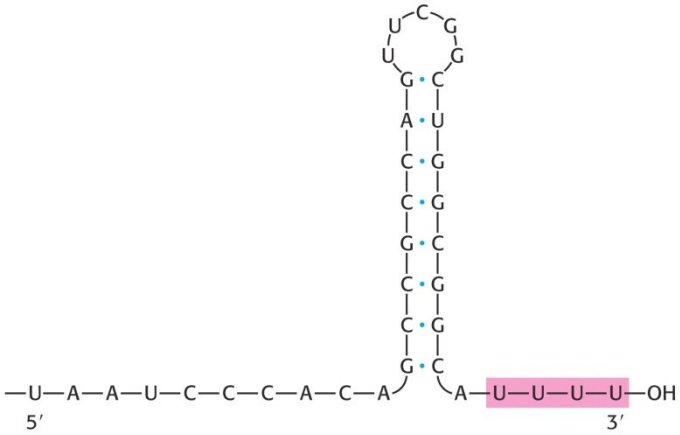


Transcription rate 20-50 nukleotides/s

Termination signal – transcription is finished. Newly synthesized RNA separates from DNA template.

Termination

Termination signals usually contain a palindromic (selfcomplementary) GC-rich region and an AT-rich region.
Thus the mRNA transcripts of this DNA palindrome can pair to form a hairpin structure with a stem and loop followed by a sequence of more uracil base – RNA transcripts end within or just after them



Transcription in eukaryotes

It is far more complicated than transcription in prokaryotes

DNA is temporary released from chromatine structure

Most active are relaxed parts of chromatin – euchromatin

Relaxation of chromatin is mediated by acetylation of lysine residue at the amino terminus of histone proteins by the action of histone acetyltransferase

Promoter u eukaryotes (RNA polymerase II)

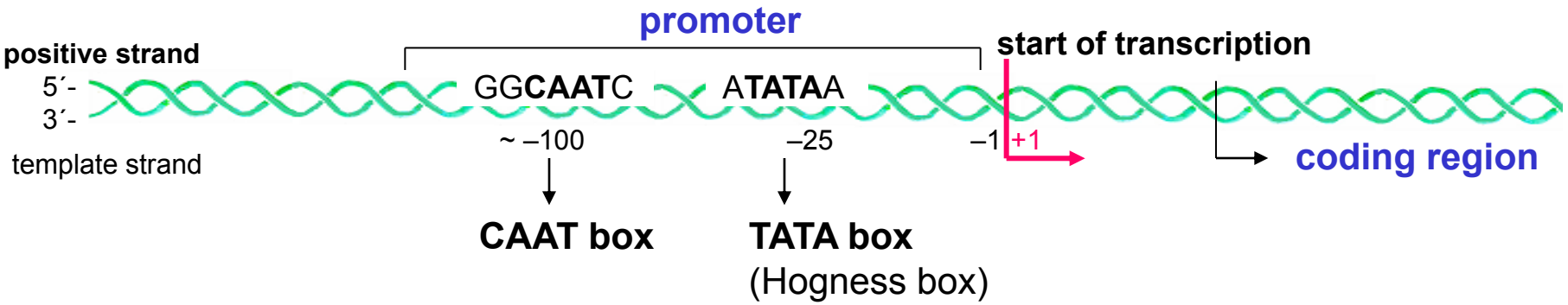
Transcription factors mediate the binding of RNA polymerase and the initiation of transcription.

Many transcription factors are involved that bind to different regions of DNA

Boxes in the promotor of eukaryotes:

TATA (analogic to Pribnow sequence)

Sometimes present CAAT



in basal gene expression specifies the frequency of initiation

directs TF II D and RNA pol II to the correct site

Transcription factors in eukaryotes

Basal transcription factors

transcription factors bound onto the promoter

Are necessary for transcription of all genes

Gene specific transcription factors

bind to regulatory DNA sequences distant from promoters.

Basal transcription factors in eukaryotes

- They must be attached to RNA polymerase before the transcription starts and are at the same time associated with promoter sequences
- They are necessary for recognition of promoter and facilitate the binding of RNA polymerase
- Polymerase II and transcription factors bound onto the promoter form a complex called the **basal transcription apparatus**. It regulates basal gene expression
- At least six basal transcription factors in eukaryotes

Basal TF = are necesary for transcription of all genes

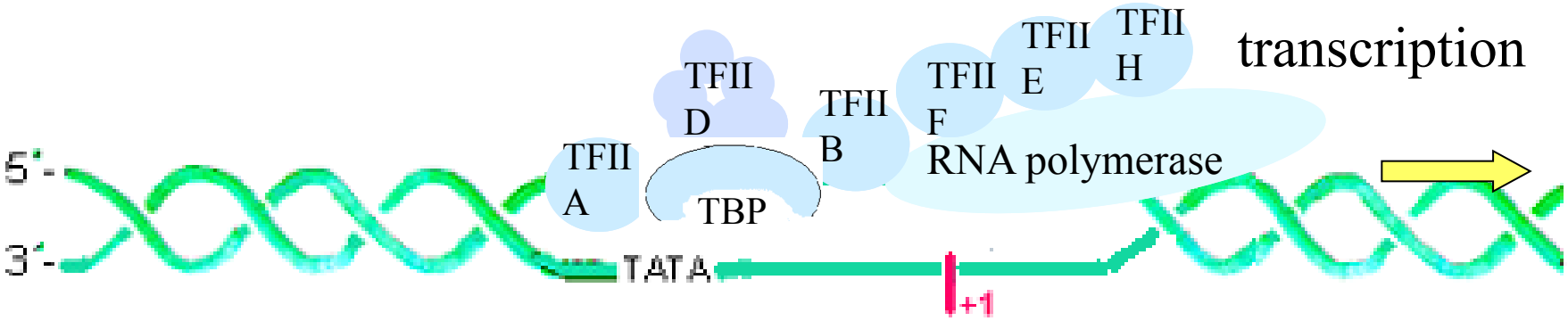
Basal transcription factors

TFIID – the biggest of basal factors of transcription - 11 subunits

One of the subunits is TBP (TATA box binding protein).

TBP binds to TATA box, the other subunits reacts also with TBP and RNA polymerase

One of factors is an ATP-dependent **helicase** that separates the DNA duplex for the **polymerase II**



Polymerase II then slides to the start of transcription and initiate transcription

http://www.youtube.com/watch?v=WsofH466lqk

Gene specific regulatory proteins (factors)

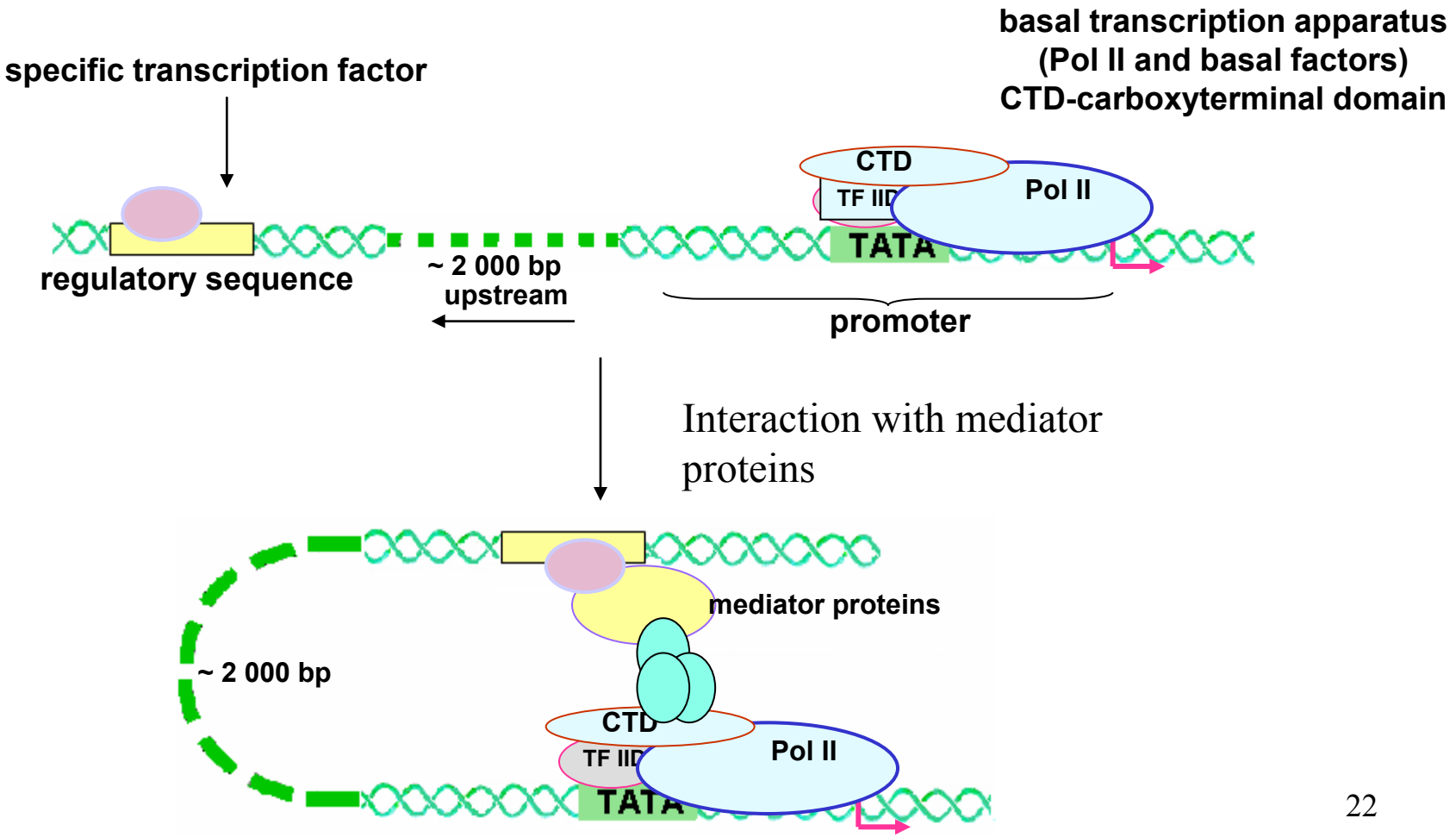
Specific transcription factors - proteins that bind to **specific regulatory DNA sequences (enhancers, silencers, HRE)** lying on the same chromosome, distant from promoters (very often in large distance).

They act as activators or repressors of the given gene transcription.

Specific transcription factors interact with mediator proteins (coactivators, corepressors) that are in contact with basal transcription factors.

A typical gene coding synthesis of a protein in eukaryotes has many binding sites for specific transcription factors in the template DNA strand

Regulation of a typical eukaryotic gene by a specific transcription factor



Examples of specific transcription factors

Specific transcription factors are proteins whose specific effect is often activated by cellular signal pathways:

Examples of specific transcription factors and their activation

a) Intracelular receptors activated by binding of hydrophobic hormones

b) Membrane receptors of hormons producing second messenger after the binding of hormon. One of the effects of second messenger is activation of proteinkinase that activates transcription factor by phosphorylation

c) Ras signal pathway activated by binding various growth factors on membrane receptor is terminated by phosphorylation of transcription factors (proteins Fos, Jun, Myc a dalších)

d) SREBP (sterol regulatory element binding protein) activated at low sterol concentration in the cell

e) Binding of cytokine to membrane receptors activates JAK-STAT signal pathway. Phosphorylated STAT protein functions as transcription factor.

a) Intracellular receptors of hormons are specific transcription factors

• receptors of steroidal (thyroidal) hormones are present in cytoplasma or nucleus in inactive form. They bind inhibitory protein in inactive form (e.g. heat shock protein).

• hormon permeates across the plasmatic membrane to the cell and is specifically bonded to the receptor in cytoplasma or nucleus

• inhibitory protein is separated, the complex hormon-receptor is formed, the conformation of receptor protein is changed

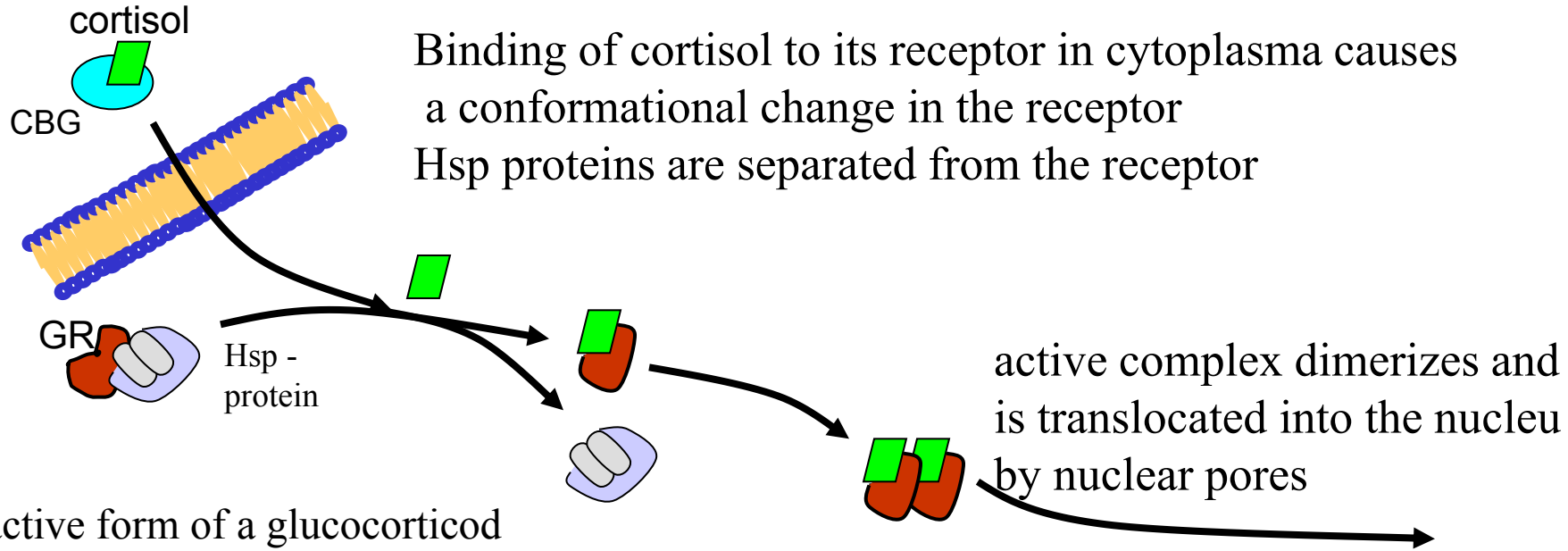
•the complex hormon receptor is translocated to the nucleus

• the complex hormon-receptor acts as the specifis transcription factor in the nucleus and binds to DNA at hormon response element (HRE)

• the complex hormon-receptor attached to DNA reacts at the same time with coaktivatr (mediator protein) that is in contact with basal transcription complex. Thereby is transcription of a gene stimulated or inhibited.

Example: Initiation of transcription by cortisol

- cortisol in plasma is transfered by CBG (corticosteroid-binding globulin)
- hydrophobic molecule difuses into a cell



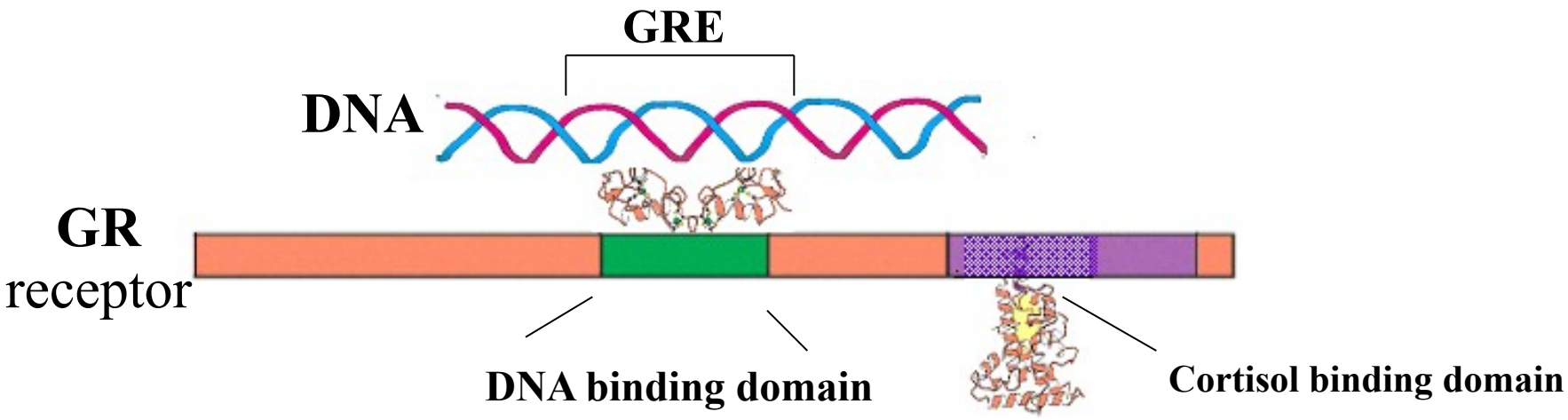
Binding of cortisol to its receptor in cytoplasma causes
a conformational change in the receptor
Hsp proteins are separated from the receptor

active complex dimerizes and
is translocated into the nucleu
by nuclear pores

Inactive form of a glucocorticod receptor is present in cytoplasma and is associated with dimer of hsp 90 protein (chaperon) and other proteins

Binding sites of the glucocorticoid receptor

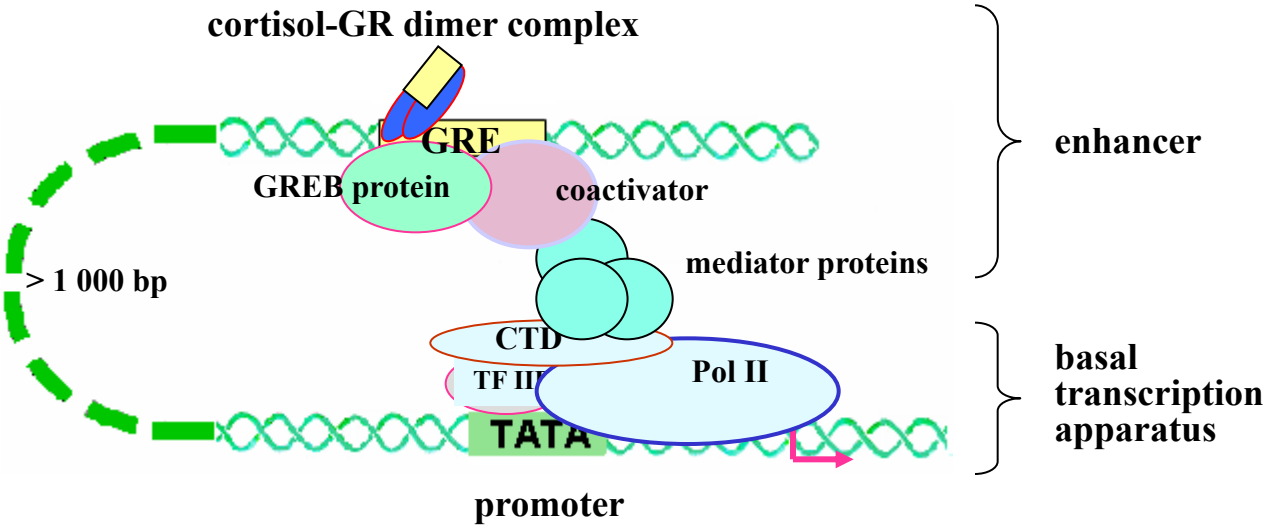
Dimeric complex cortisol-receptor binds to dsDNA at the specific GRE sequence (glucocorticoid response element), it means on HRE (hormone response element) specific for glucokortikoids.



Initiation of transcription by cortisol - continuation

Active complex cortisol-receptor **binds onto DNA** at the specific sequence **GRE** (glucocorticoid response element, one of the HRE – hormone response elements).

The **coactivator** and specific **hormone response element-binding proteins** (HREB-proteins) are also attached. This complex supports initiation of transcription on the promoter by means of **mediator proteins**.



GR dimer – intracellular glucocorticoid receptor (dimer)

GRE – glucocorticoid response element

GREB protein – GRE binding protein (a specific transcription factor)

Intracelular receptors for hydrophobic hormons

•Members ofthe nuclear receptor superfamily (there is known more than 150 proteins).

•Present in cytoplasma or nucleus

•Main group are steroidal-thyreoidal receptors

Examples:

Androstane receptor AR

Estrogene receptor ER

Progesterone receptor PR

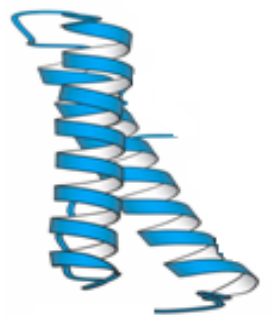
Glucocorticoid receptor GR

Mineralocorticoid receptor MR

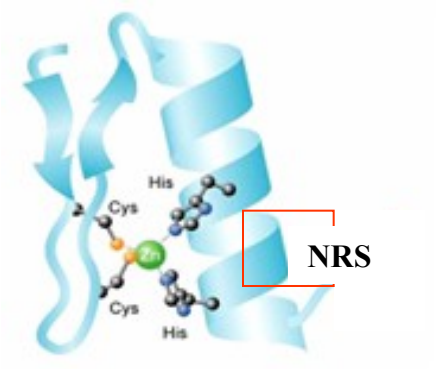
Transcription factors that bind onto regulatory DNA sequences

comprise mostly one of the **typical structural motifs:**
helix-turn (or loop)-**helix, zinc-finger,** and **leucine zipper.**

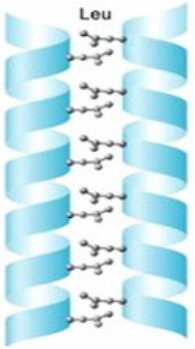
Only the small part of protein molecule (called DNA-binding domain) is responsible for the interaction with DNA. It is usually represented by two adjacent α-helical segments.



helix-turn-helix



zinc finger

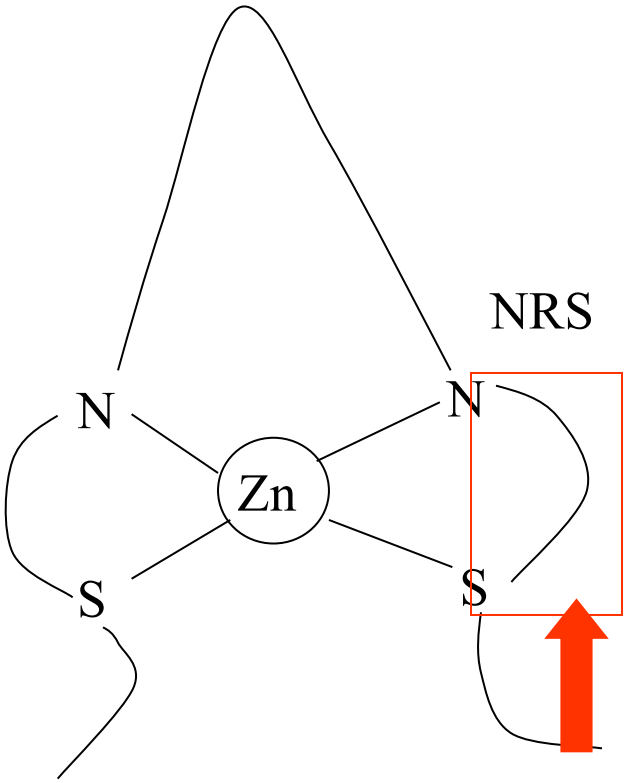


leucine zipper

Zinc finger, e.g., occurs in DNA binding domains of steroid-hormone receptors. **NRS** (nucleotide recognition signal) is a part of α-helix containing amino acid sequence that is able to recognize specific regulatory sequence of nucleotides in the major groove DNA.

Transcription factors are attached to DNA usually in the major groove.

Zinc finger



E.g.binding domains of steroidal hormone receptors.

Zn^{2+} is chelated by four ligands ether by histidine(N) or by cysteine (S)

Zn^{2+} maintains the tertiary structure of the domain

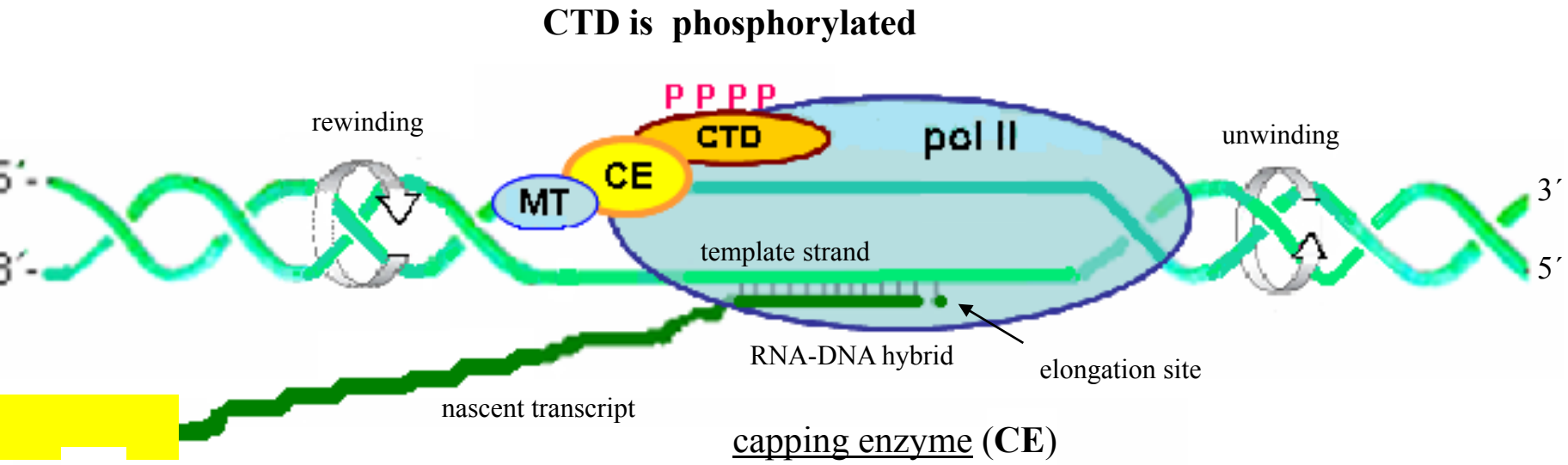
NRS (nucleotide recognition signal) is a part of α-helix containing the sequence of amino acids that serves for recognition of specific sequence in major groove DNA

Initiation of transcription - summarization

- Transcription is initiated only after all transcription factors are attached
- The completed assembly of transcription factors and RNA polymerase bind to the promoter, forming a transcription initiation complex.
- RNA polymerase is attached to the transcription factors and DNA in promoter region
- It melts 10-15 nucleotide base pairs around the transcription start site, allowing for ribonucleotides to bind to the template strand.
- After the first bond is synthesized, the RNA polymerase must clear the promoter, most of transcription factors are separated

Elongation phase

As transcription proceeds, RNA polymerase traverses the template strand and uses base pairing complementarity with the DNA template to create an RNA copy.

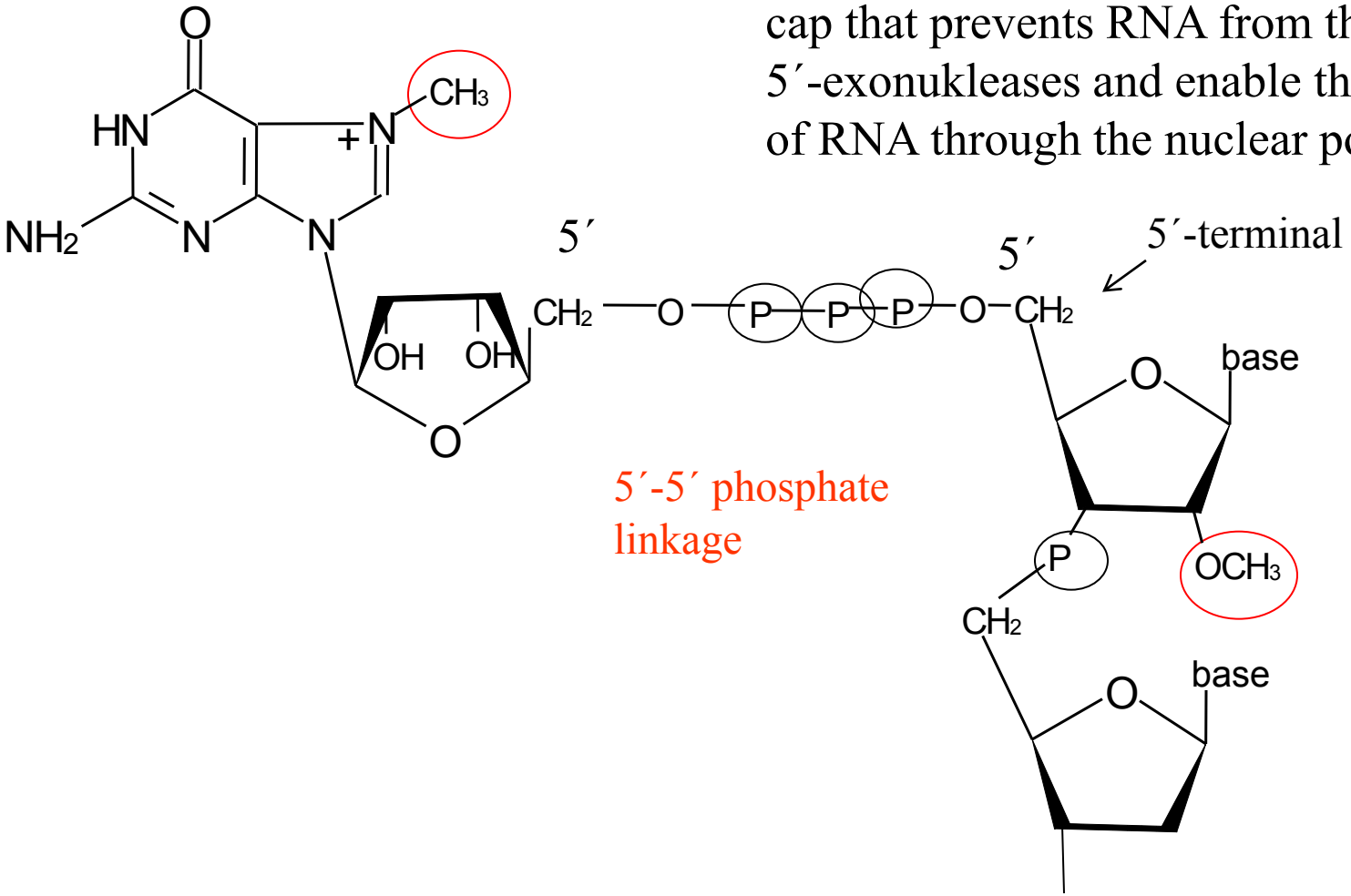


capping enzyme (**CE**)
methyltransferase (**MT**)
Both those enzymes modify the 5′-end of the nascent transcript to **5′-m^{7}Gppp-cap**

Capping of mRNA

7-methylguanosine is attached by 5´-5´phosphate bond to the 5´-terminal end of the mRNA

Complex of proteins is then bonded to the cap that prevents RNA from the action of 5´-exonukleases and enable the transport of RNA through the nuclear pores



Čapka je také nutná pro zahájení translace

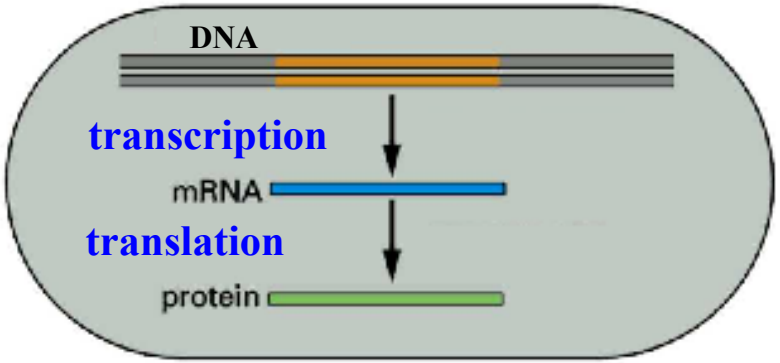
Termination in eukaryotes

no perspicuous termination signal has been found.

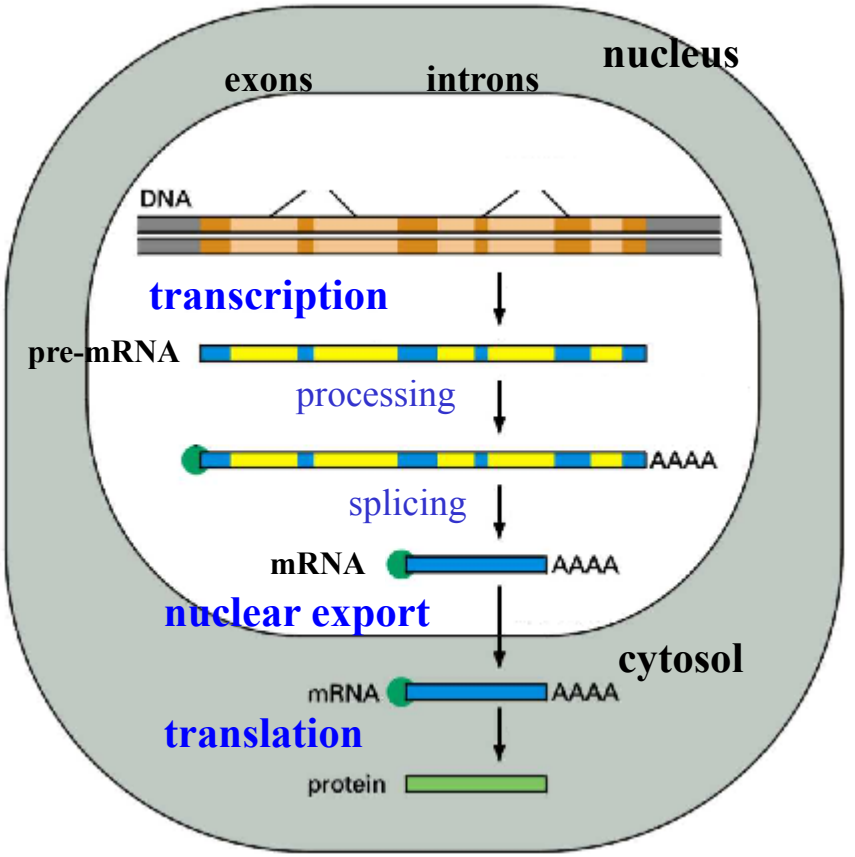
Transcripts produced by DNA polymerase II are released from the transcription apparatus after the polyadenylation signal AAUAAA and the GU- or U-rich sequence that is able to bind cleavage stimulation factor (CStF) had been transcribed.

The terminal sequences of the transcripts are decomposed in the course of 3´-polyadenylation (not encoded by template DNA).

Eukaryotic transcription and translation are separated in space and time



Prokaryotes



Eukaryotes

Differences between prokaryotes and eukaryotes

Prokaryotes - transcription occurs in the cytoplasm. Translation of the mRNA into proteins also occurs in the cytoplasm.
DNA is much more accessible to RNA polymerase than DNA in eukaryotes.
RNA polymerase interacts directly with prokaryotic DNA.
mRNA produced as a result of transcription is not modified in prokaryotic cells.

Eukaryotes - transcription occurs in the cell's nucleus. mRNA then moves to the cytoplasm for translation.
Eukaryotic DNA is wrapped around histones to form nucleosomes.
Eukaryotic DNA is packed to form chromatin.
Other proteins mediate the interation between RNA polymerase and DNA in eukaryotes.
Eukaryotic cells modify mRNA by RNA splicing, 5' end capping, and addition of a polyA tail.

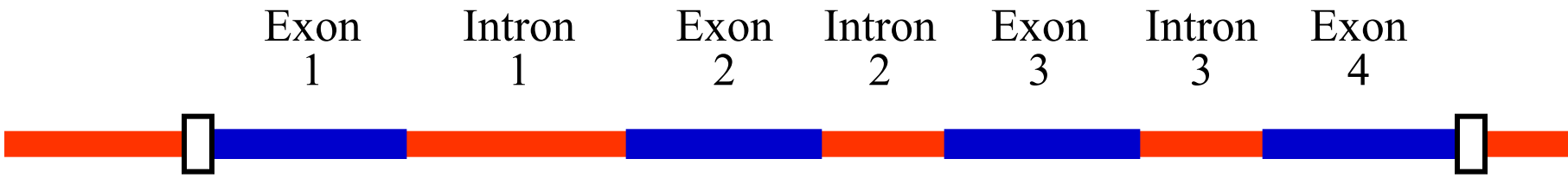
Processing of primary transcripts

- Primary transcript is precise copy of precursor template DNA with exception of T→U
- Primary transcripts of tRNA and rRNA are posttranscriptionaly modified by nucleases in both prokaryotes and eukaryotes
- Prokaryotic mRNA is practically identical with primary transcript (is used for translation before the synthesis finishes)
- Eukaryotic mRNA is significantly modified

Processing of eukaryotic mRNA

The primary transcript is hnRNA

It is a transcript of the structural gene at which the coding sequences (exons) alter with non-coding sequences (introns or interventing sequences).



Non-coding sequences must be removed during processing

Procesing of hnRNA in nucleus

- Chemical modification (capping at 5′ terminal) – prevents mRNA against 5′-endonucleases and it is also the marker recognized in proteosynthesis.

- Splicing (removal of introns)

- Polyadenylation (addition of polyA on 3′ terminal) – it prevents against the 3′exonucleases

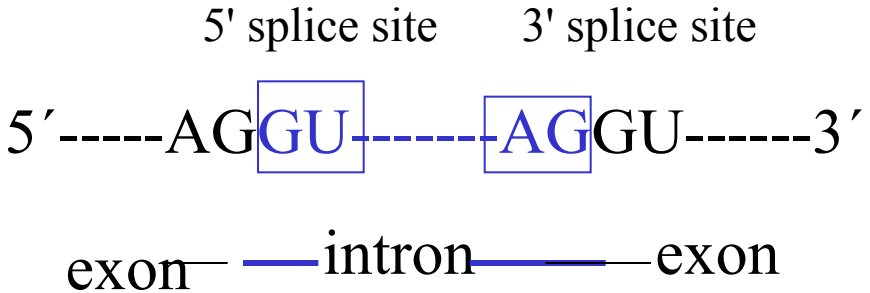
Splicing of hnRNA

Splicing is ensured by the action of small nuclear complexes – splicesomes

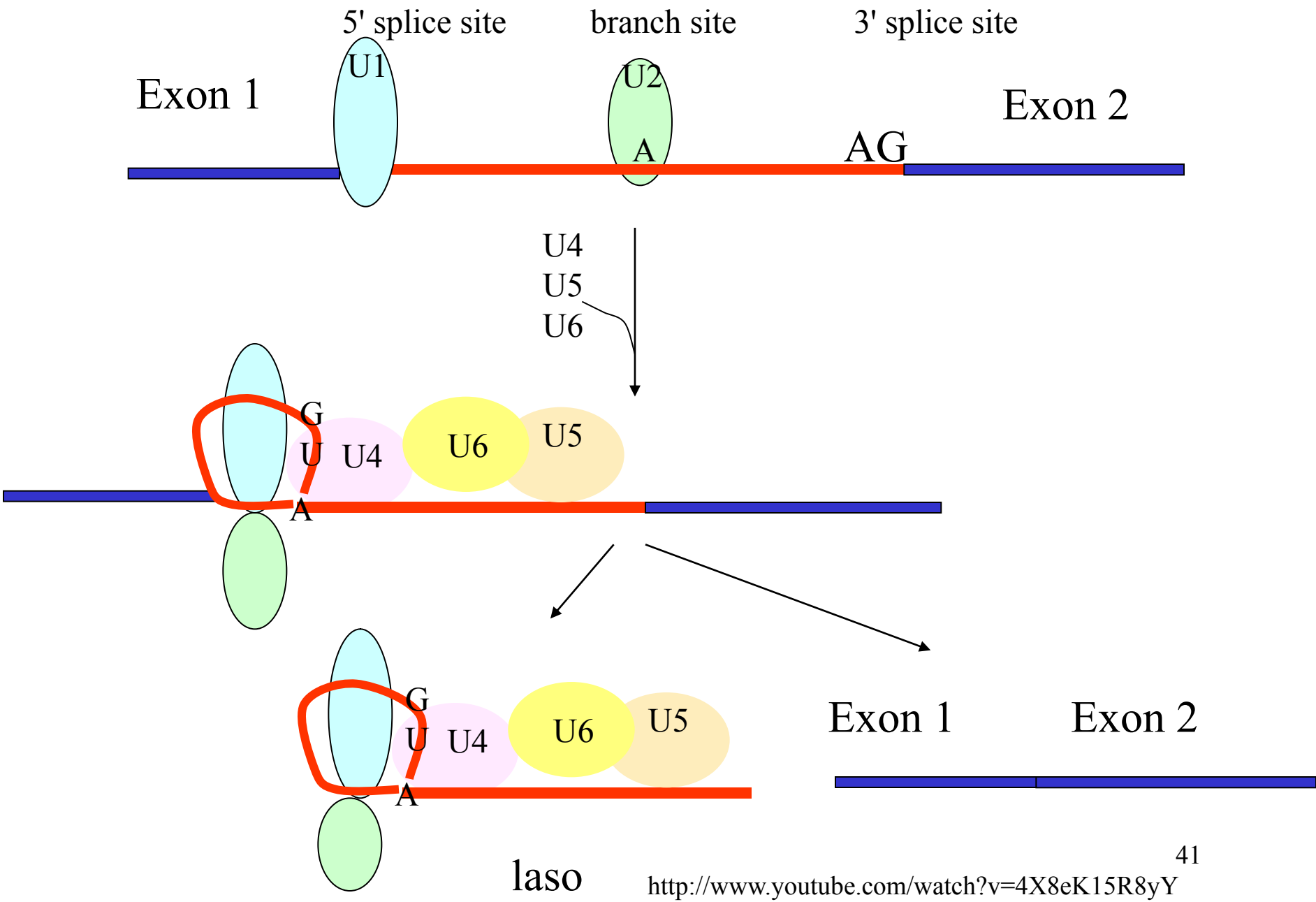
Splicesomes contain five small RNA rich in uracil (U1, U2,U4,U5 and U6)

Small RNAs are associated with proteins and form snRNPs (small nuclear ribonucleoprotein particles).

Nucleotide sequence AGGU determine the splice sites.These sequentions are recognized by snRNPs.

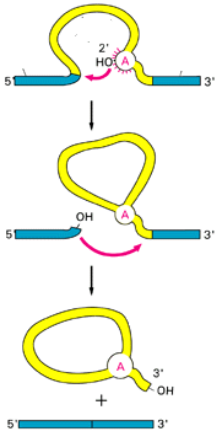


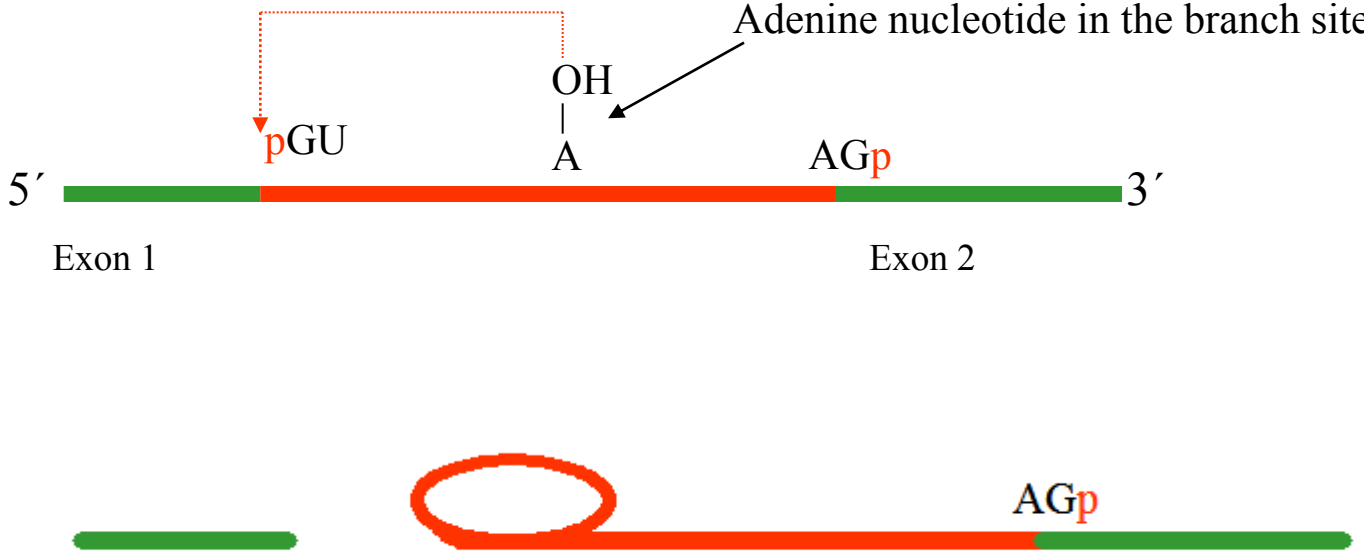
Splicing



http://www.youtube.com/watch?v=4X8eK15R8yY

Mechanisms of RNA-splicing





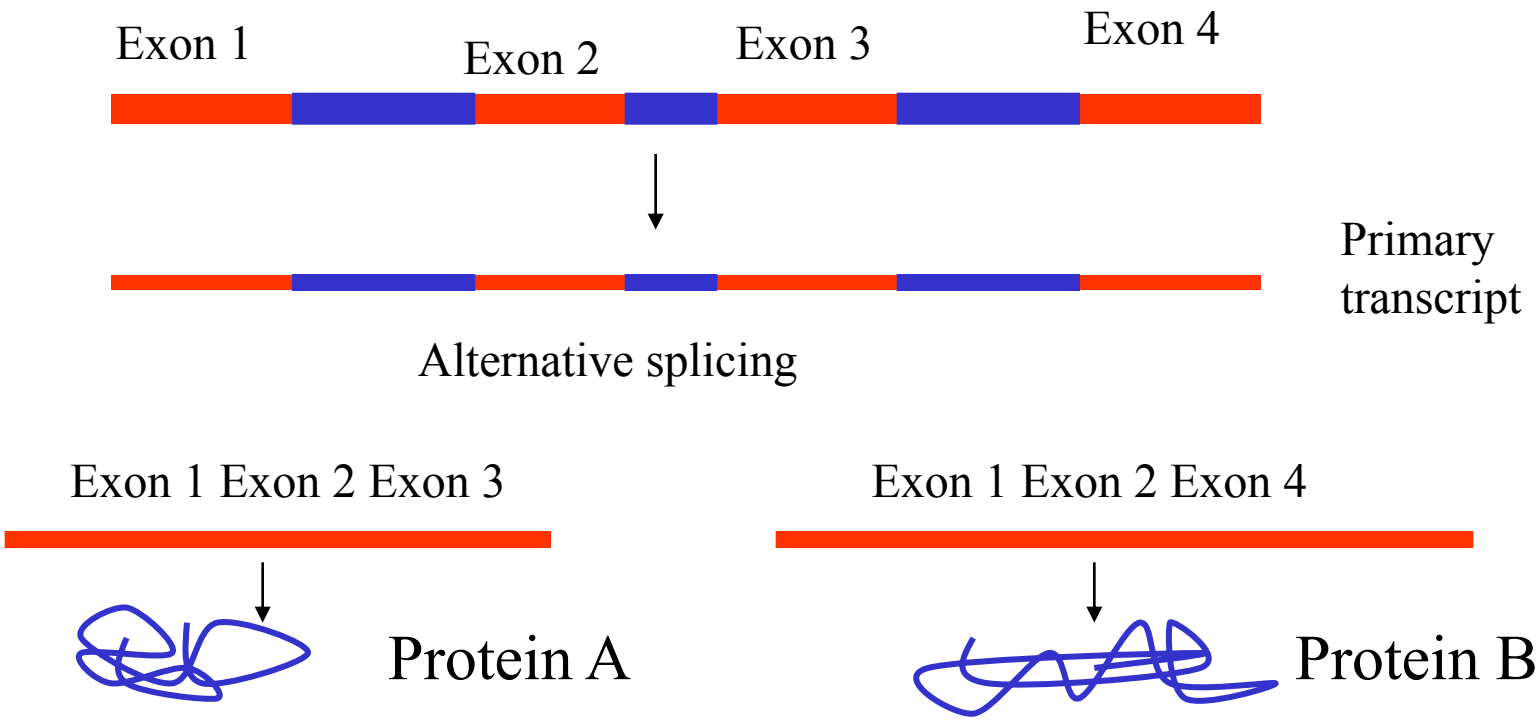
2′-OH group of adenine nucleotide attacches phosphate in the position 5′of guanine nucleotide at the place of splicing and forms a lariat. The chain on the 3′end of exone 1 is interrupted, 3′-OH become free and attaches the 5′-end of exon 2

mRNA

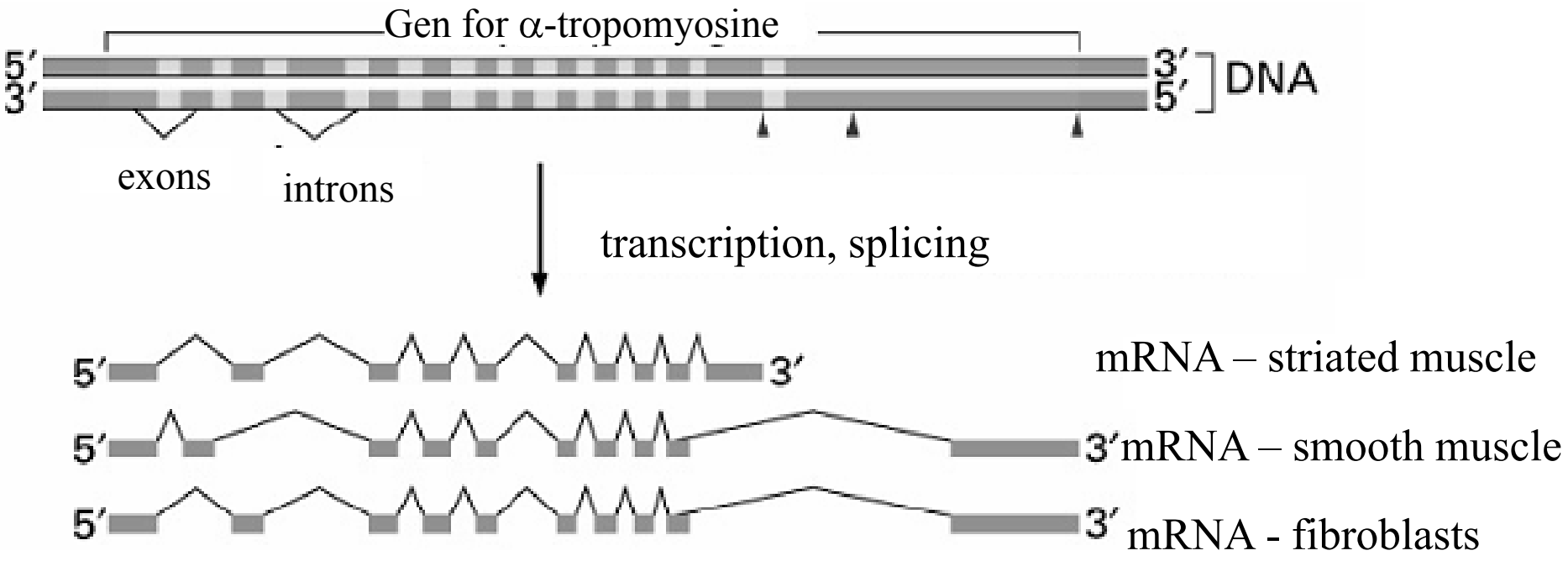
spliced intron

Alternative splicing

Alternative splicing – various groups of exon originating from one gene form various molecules of mRNA that provide various proteins



Alternative splicing of m RNA



In many cases, the splicing process can create a range of unique proteins by varying the exon composition of the same messenger RNA. Alternative splicing can occur in many ways. Exons can be extended or skipped, or introns can be retained.

Splice site mutations

Mutation at splice sites can lead to improper splicing and production of abberant proteins

E.g. β - thalasemia:

β-subunit of hemoglobin is not formed in sufficient amount

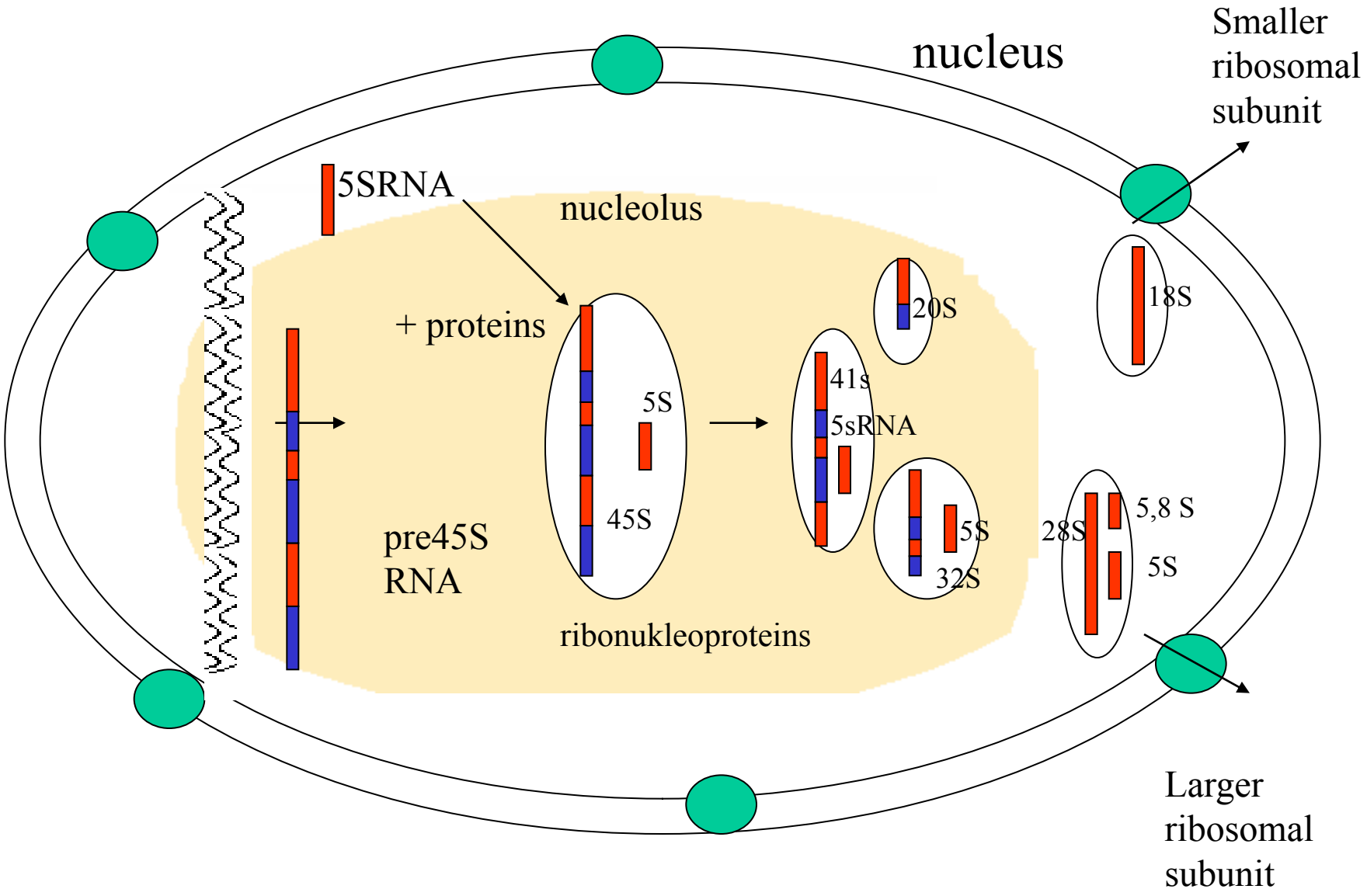
It results from point mutation in β-globin gene where the

G→A mutation occurs

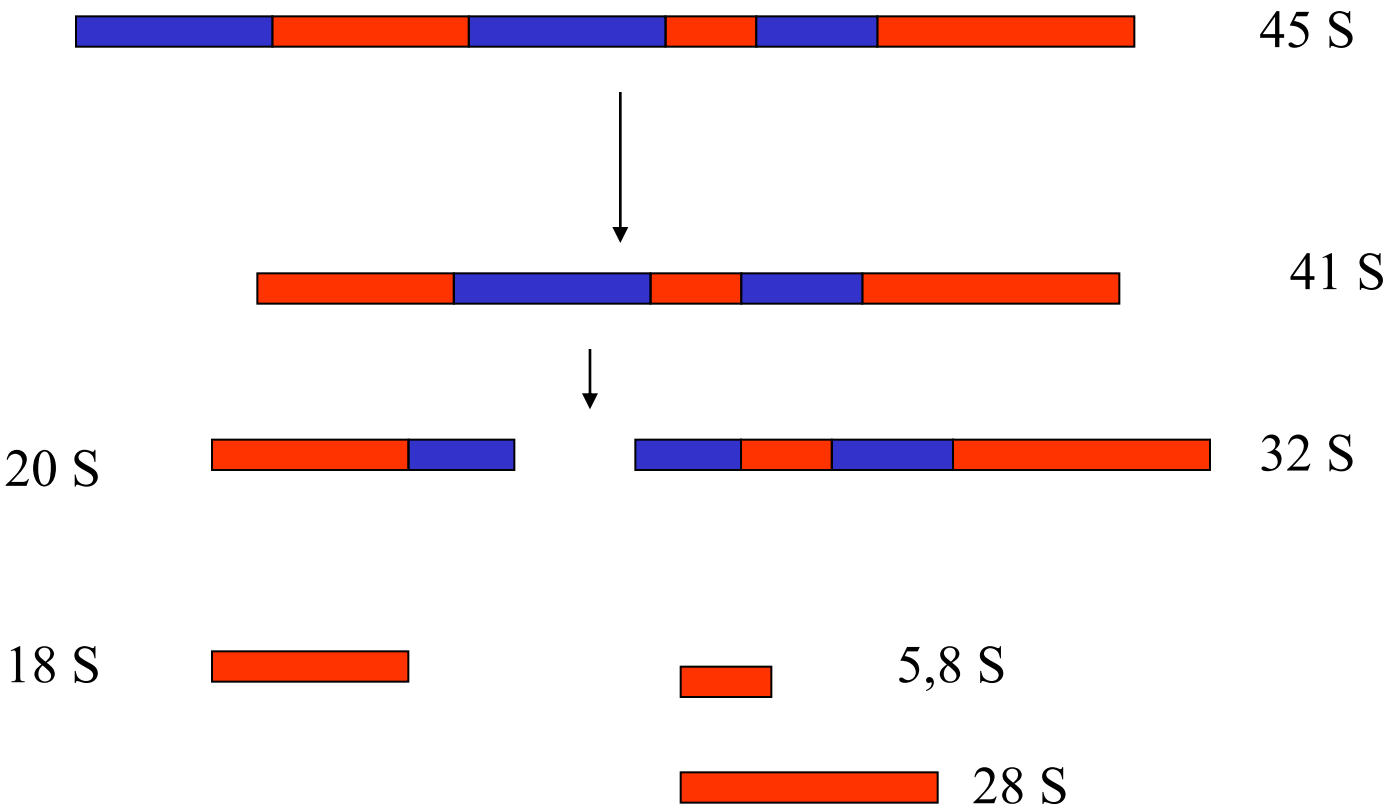
This creates a new splice acceptor site nineteen nucleotides upstream from the normal splice acceptor

A faulty beta-globin protein is made, leading to severe anemia.

Syntéza eukarytic rRNA



Processing of 45 S eukaryotic rRNA



Synthesis of eukaryotic rRNA

Nucleolus:

45S RNA is synthetized in form of preRNA

Complexation with proteins – formation of ribonucleoproteins

Methylation and shortening

5S RNA is synthetized in nucleoplasma, it migrates into the nucleolus and is attached to ribonucleoproteins

Transport of shortened RNAs to nucleoplasma and through nuclear pores to cytoplasma. Formation of ribosomes.

Synthesis of eukaryotic tRNA

Synthesis in a form of pre t-RNA in the nucleus

Removal of nucleotide sequences on 5´, 3´terminal and nucleotide intron in the anticodon loop

Modification of bases: methylation of uracil to thymin

dehydrogenation of uracil

formation of pseudouridine (C-C bond between uracil and ribose)

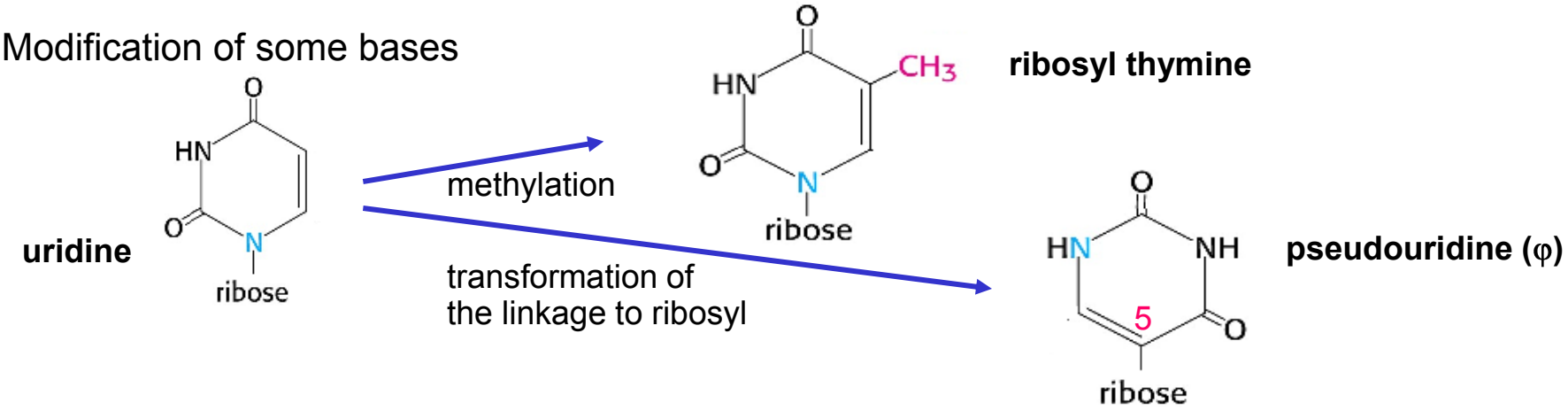
deamination of adenosine to inosine

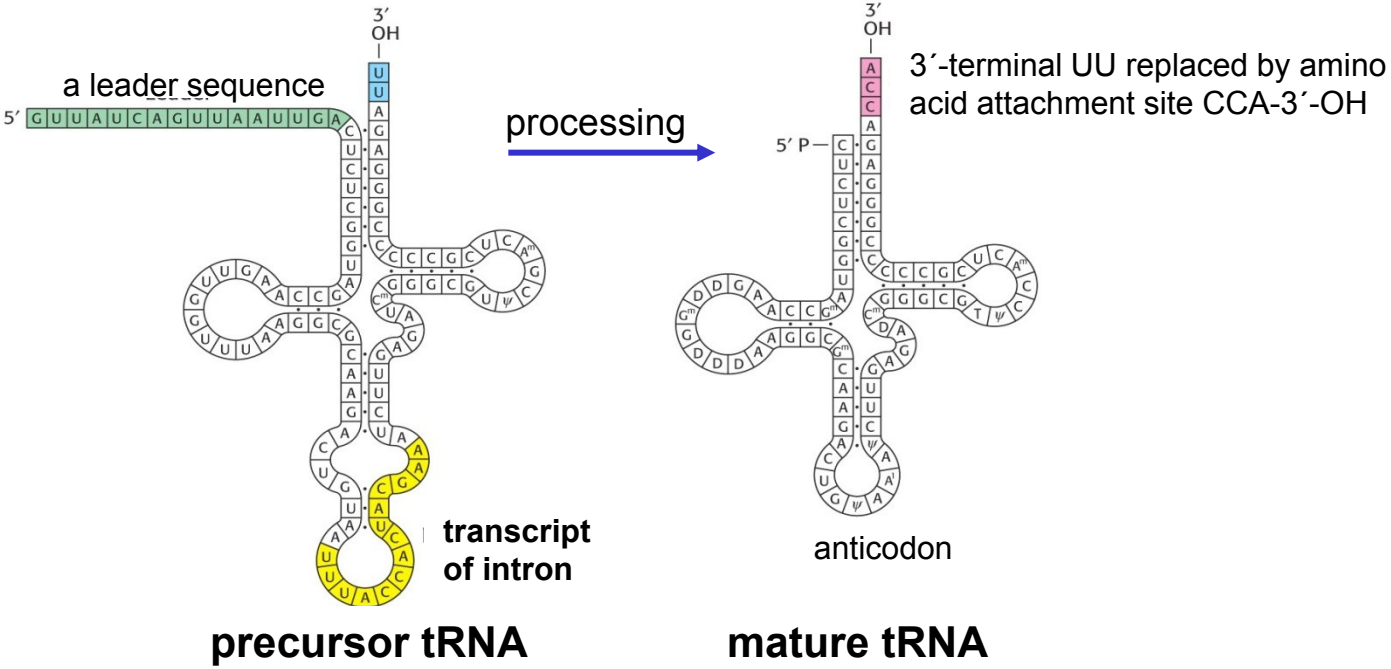
Addition of CCA sequence on 3´end

Migration to cytoplasma

Examples of tRNA processing:

Modification of some bases





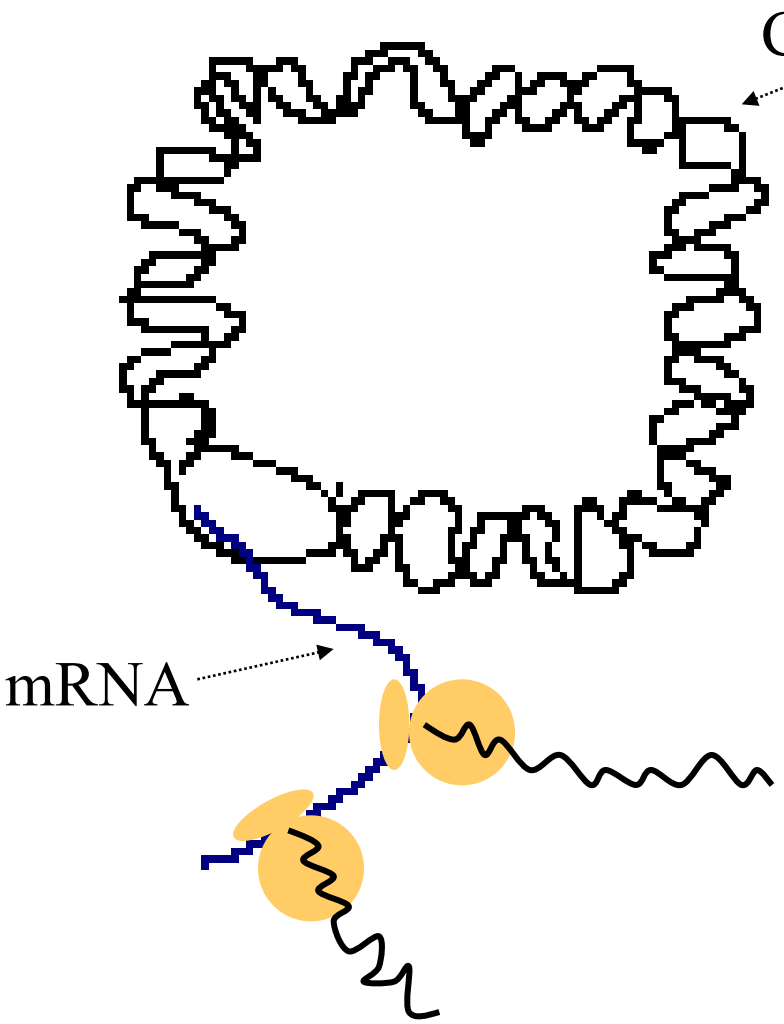
Regulation of gene expression

Gene expression – formation of proteins or RNA products

Generally only small fraction of genes in a cell are expressed at any time

Gene expression is regulated differently in prokaryotes and eukaryotes

The main features of gene expression in prokaryotes



- only one DNA in the cell
- DNA is not complexed with histones
- nucleus is not separated from cytoplasma
- transkripts of genes do not include introns
- translation and transcription occur simultaneously

Regulation of gene expression in prokaryotes

Regulation is less complex than in the multicellular eukaryotes

Gene expression is regulated mainly by controlling the initiation of gene transcription.

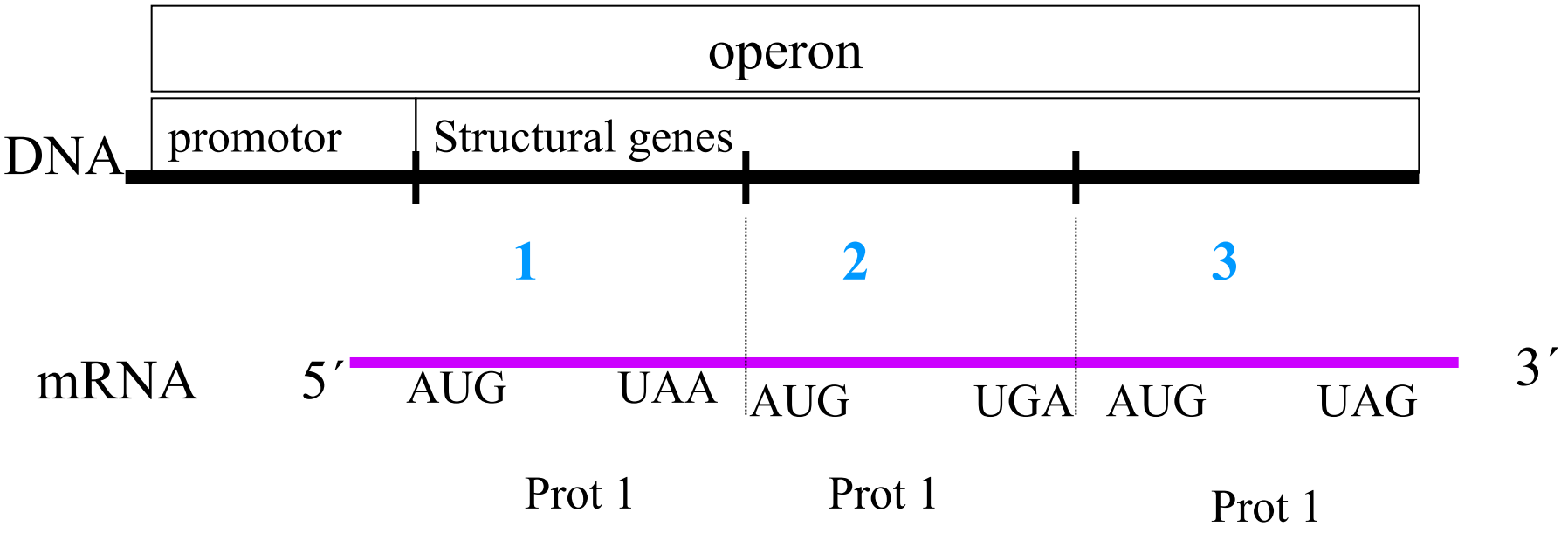
The most extensive studied is bacterium E.coli. Its genom includes $4x10^6$ base pairs $\rightarrow$E.coli should be able of making several thousands of proteins

Under normal conditions they synthesize only about 600-800 different proteins.

Many genes are inactive and only those genes are expressed that generate the proteins required for growth in that particular enviroment

Operons theory

Structural gens of bacterias are grouped into units called operons



Operon

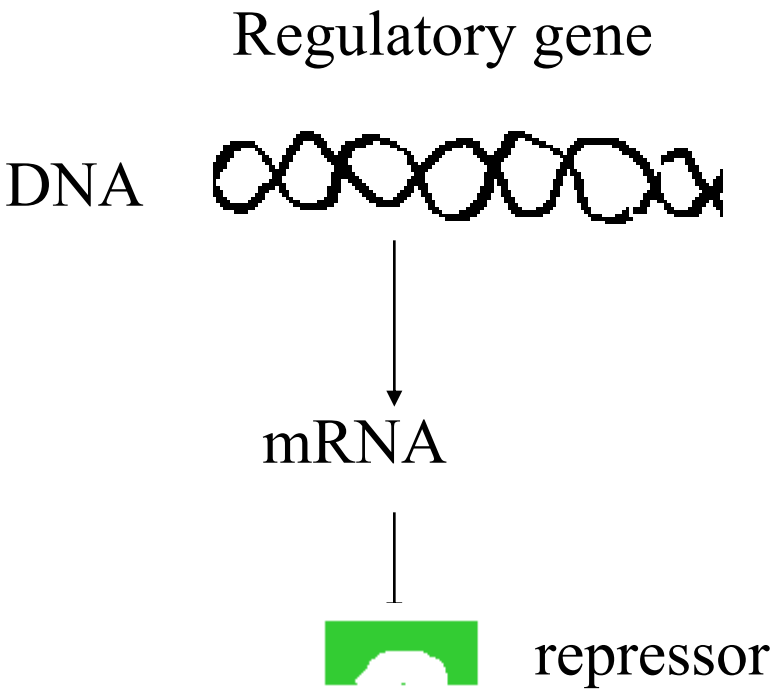
• It includes structural genes for proteins that are metabolically related

•Genes in operon are ussually coordinately expressed (they are either all „turned on“, or all „turned off“)

•The product of transcription is single polycistronic mRNA

•Transcription is regulated by single promotor which is located in the operon at the 5´-end, upstream from the structural genes

Regulation of RNA polymerase binding by repressors – negative control



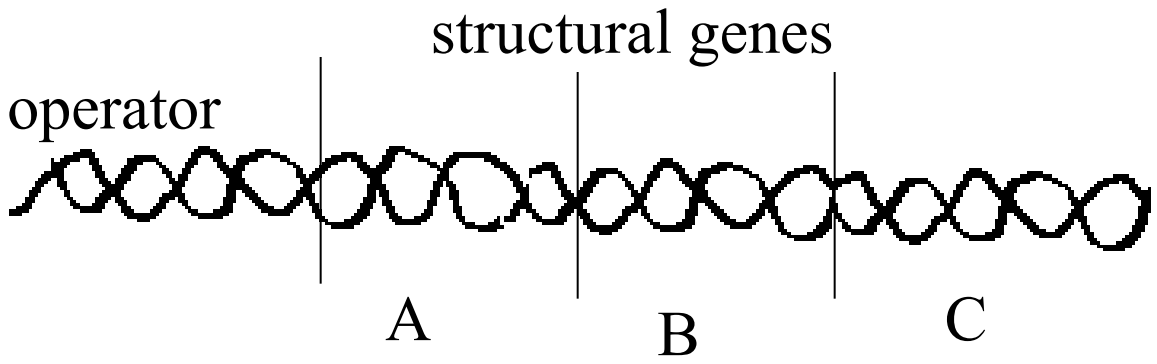
repressor je encoded by regulatory gene

Its product, the repressor protein, difuses to the promoter and binds in the region of the operon called operator

Operator is located within the promoter or near of its 3´-end

Repressor blocks the binding of RNA-polymerase to the promotor

Synhesis of mRNA does not occur



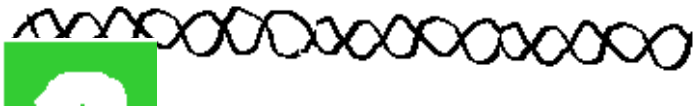
Repressor is controlled by two mechanisms

Induction

Inductor is a small molecul that binds to repressor, changes its conformation and triggers its release from the operator

transcription can start

Inductors: small molecules of nutrients or their metabolites



Corepression

repressor is not active until corepressor is bonded to it. The complex repressor-corepressor binds to operator preventing binding of RNA polymerase

transcription stops

Corepressors: small molecules of nutrients or their metabolites

Example of induction

Induction of *lac* operon in E.coli by lactose

Enzymes for metabolizing glucose by glycolysis are produced constitutively

If the milk sugar lactose is available, the cell adapt and begin to produce three additional enzymes required for lactose metabolism

These enzymes are encoded by *lac* operon

A metabolite of lactose (**allolactose -**isomer of lactose that is formed spontaneously) serves as an inducer, binds to the repressor and inactivates it.

RNA polymerase can bind to promotor and transcribe the structural genes of the *lac* operon (β-galactosidase, permease and transacetylase)

Glucose can prevent activation of the *lac* operon (see fig.80)

Example of corepression

Corepression of *trp* operon (synthesis of tryptophane at E.coli)

genes for enzymes of tryptophane synthesis (5 enzymes) are located in *trp* operon

Tryptophan ise corepresssor, it binds toinactive repressor, binds its conformation.

The complex tryptophan-repressor inhibits transcription of operon.

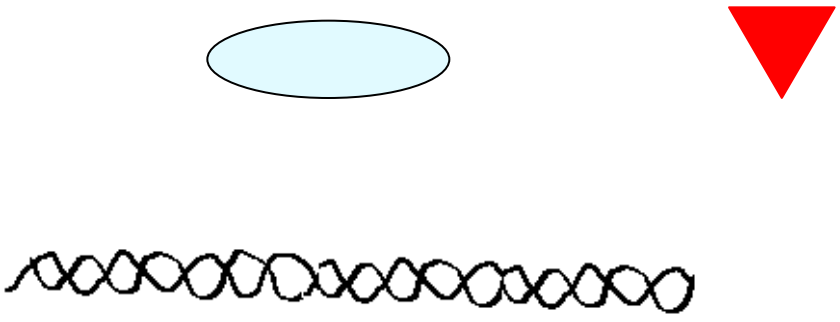
http://www.biology.ualberta.ca/facilities/multimedia/uploads/genetics/trpoperon2.swf

Stimulation of RNA polymerase binding - positive control

Regulatory proteins binds to promoter and stimulate the binding of RNA-polymerase

Regulatory protein is activated on the base of presence/or absence of small molecule of nutrient of its metabolite in the cell

Catabolite repression



Example of positive control

Transcription of *lac* operon at E.coli

transcription of *lac* operon is affected by allolactose only when the glucose is absent

Decrease of glucose level results in increase of cAMP (it is not known why)

cAMP binds to its receptor in the cell (cAMP-receptor protein → CRP)

Complex cAMP-CRP binds to the regulatory site of *lac* operon, stimulates the binding of RNA polymerase to promotor and transcription of genes for metabolism of lactose

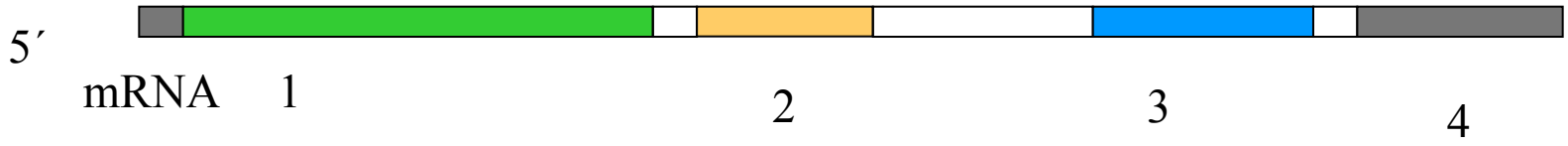
→ cells metabolize lactose, only when it does not have adequate supply of glucose

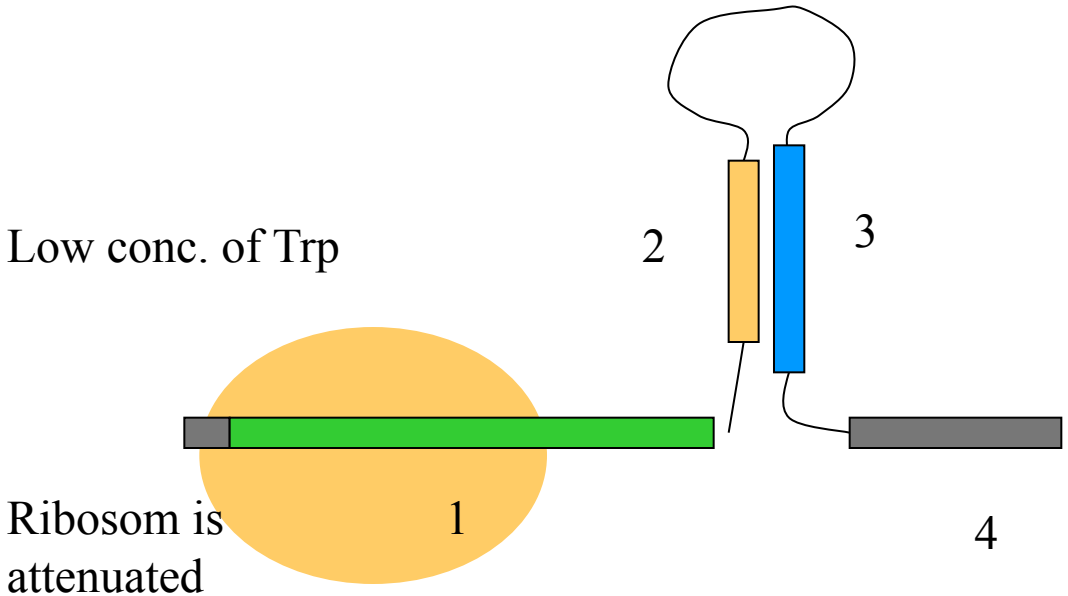
Atenuation of transcription

- Some operons are regulated by process that interrups (attenuates) transcription after it has been initiated
- The cause is the change of secondary structure of mRNA
- Attenuator („retarder“) is a sequence included in operon
- Processes of translation occurs at the same time with transcription
- The rate of transcription affects the formation of hair-pin loops on mRNA

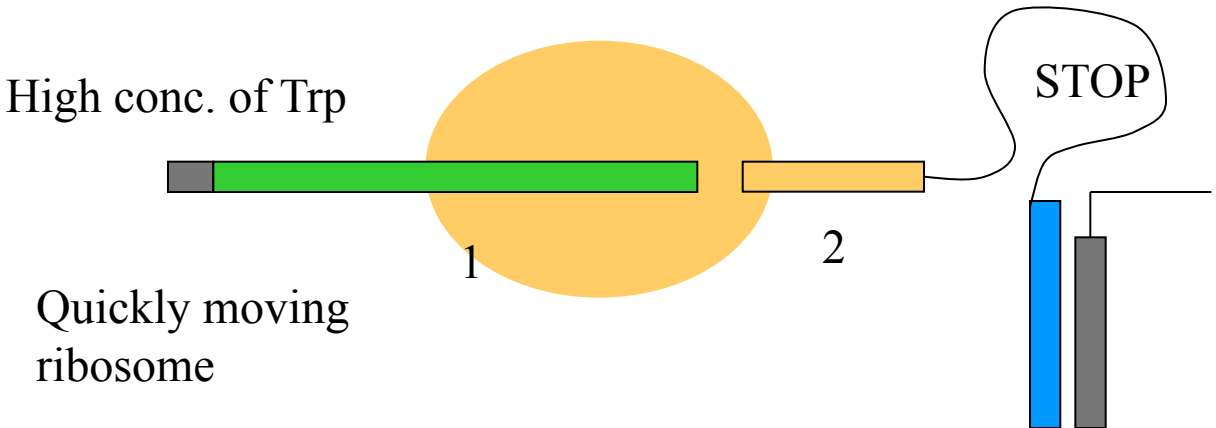
http://www.biology.ualberta.ca/facilities/multimedia/uploads/genetics/trpoperon2.swf

Atenuation of transcription – trp operon E.coli





Sequence 1 contains codones for Trp



Atenuation

• mRNA is transcripted from the trp operon, ribosomes bind to it and rapidly begin to translate the transcript.

• RNA polymerase is followed by moving ribosome

• near the 5´end of the transcript there are number of codons for trp.

• initially, high levels of trp in the cell result in high levels of trp-$tRNA^{trp}$ and rapid translocation of the transcript

• rapid translocation generates a hairpin loop in the mRNAthat serves as a termination signal for RNA polymerase and transcription terminates.

• when trp levels are low, levels of trp-$tRNA^{trp}$ are low and ribosome stall at codons for trp. A different hairpin loop forms in mRNA that does not terminate transcription

•RNA polymerase can complete the transcription

•The level of transcription is regulated by the amount of trp in the cell

Regulation of protein synthesis in eukaryotes

The main features of gene expression in eukaryotes (differences from prokaryote):

- DNA is organized in nucleosomes of chromatin
- gene must be in an active structure to be exxpressed in a cell
- operons are not present
- genes encoding metabolically related proteins are located on different chromosomes
- each gen has its promotor
- transcription and translation are separated

Regulation of eukaryotic cells gene expression occurs at multiple level

- A) DNA and the chromosome including chromosome remodeling and gene rearrangement
- B) transcription, primarily through transcription factors affecting binding of RNA polymerase
- C) processing of transcripts
- D) initiation of translation and stability of mRNA

Regulation of availability of genes for transcription

In cells of differentiated tissues only the genes that have some role in the cell are active

Chromatin in the nucleus:

- Condensed (heterochromatin) – genes are inactive
- Euchromatin- genes produce mRNA

Long-term changes in the activity of genes occur during development as chromatin goes from a diffuse to a condensed state and vice versa.

A) Examples of regulation on the chromosom structure level

- Chromatin remodeling
- DNA methylation
- gene rearangement
- gene amplification
- gene deletion

➢Chromatin remodeling

- change of chromatin state that results in activation of transcription

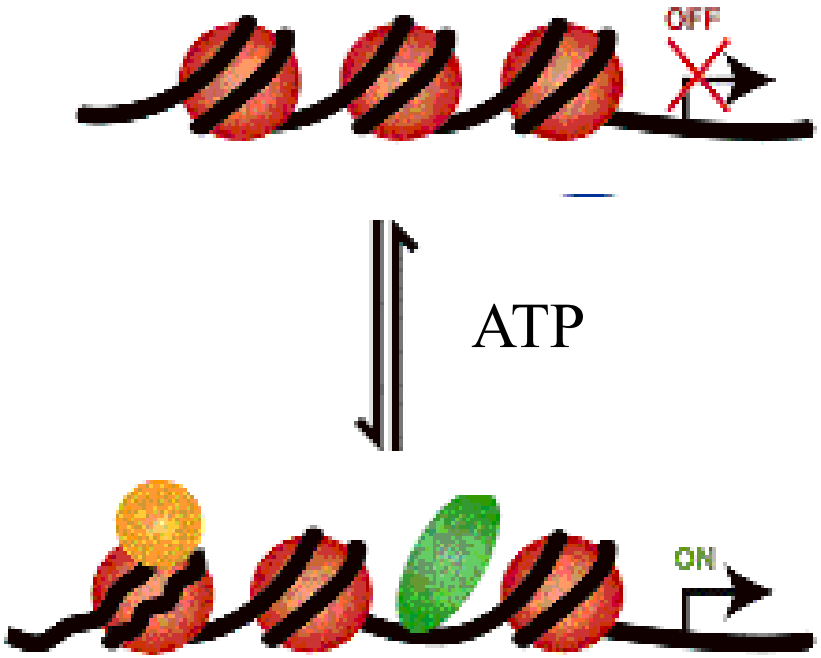
→ displacement of of nucleosome from chromatin so that the transcription can start

Remodeling mechanisms:

• an ATP driven unwinding of certain section of DNA from nuclosome core

• covalent modification of the histone tails through acetylation or deacetylation (acetylation of ε -amino group in side chain of lysin on N-terminals of histones H2A,H2B,H3 a H4).

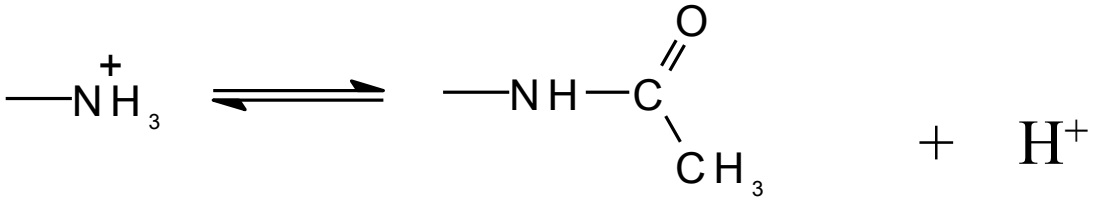
ATP driven unwinding of certain section of DNA from nuclosome core



Significance of histon-acetyltransferase and histon deacetylase

Histon-acetyltransferase catalyzes acetylation of histones.

This reaction removes a positive charge from the ε--amino group of the lysine, thereby reducing the electrostatic interactions between the histones and the negatively charged DNA→ this results in an easier unwinding. This makes RNA polymerase and transcription factors easier to access the promoter region. Therefore, in most cases, histone acetylation enhances transcription while histone deacetylation represses transcription.



➢DNA methylation

Methylation of cytosine residues in DNA by SAM → 5-methylcytosine

SAM + DNA → DNA-CH_3 + S-adenosylhomocystein

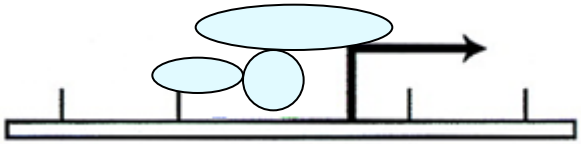
Methylations are located in GC-rich sequences (GC-islands) that are often near or in the promoter region of gene (postsyntetic modifiation of DNA – enzym methylase)

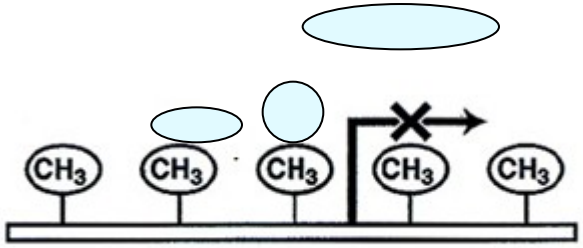
Genes that are methylated are less readily transcribed

Example: globin genes are more extensively methylated in nonerythroid cells than in cells in which these genes are expressed (erytroblasts and retikulocytes)

DNA methylation

Initiation of transcription





Transcription is inhibited by methylation

➢Gene rearrangement

Segments of DNA can move from one location to another in the genome, associating with each other in various ways

Example : rearrangement of genes in cells that produce antibodies (imunoglobulins)
(see Imunology)

➢Gene amplification

Certain region of a chromosome undergo repeated cycles of DNA replication
The newly synthesized DNA is excised and forms small, unstable chromosomes called double minutes.
They intgrate into other chromosomes thereby amplifying the gene in the process.
It is not usual physiological means of regulating of gene expression in normal cells, it occurs in response to certain stimuli
Normally gene amplification occurs through errors during DNA replication and cell division – then cells containing amplified genes may have a grow advantage, if the enviromental conditions are appropriate.

Example: patients treated by methotrexate (nhibitor of dihydrofolate reductase) can develop drug resistance.
The cause is that some rapidly deviding cancer cells amplify the gene for dihydrofolate reductase, producing hundreds of copies in the genome. These cells generate large amount of dihydrofolate reductase and normal doses of methotrexate are not longer adequate.

B) Regulation at the level of transcription

Basal regulation of transcription (common for all genes)

Regulation by the components of „basal transcription complex“ (RNA polymerase binding the TATA box, TATA binding proteins and further basal transcription factors binding on promoter or RNA-polymerase)

Genes regulated only in this way:

constitutively expressed genes

Specific regulation of gene expression:

Gene specific transcription factors bind to the specific regulatory sequences.

Regulation of transcription factors

Down(up)-regulation of transcription factors

formation (see SRBP in synthesis of cholesterol)

Modulation by binding of stimulatory and inhibitory ligands (CREBP)

Mutual cooperation of transcription factors

Phosphorylation/ dephosphorylation of transcription factors regulated growth actors, cytokines, peptide hormones atd.

C) Postranscriptional regulation

Alternative splicing

Alternative splicing and variation of the site of polyadenylation cause that one gene can produce various proteins (see the lecture 13)

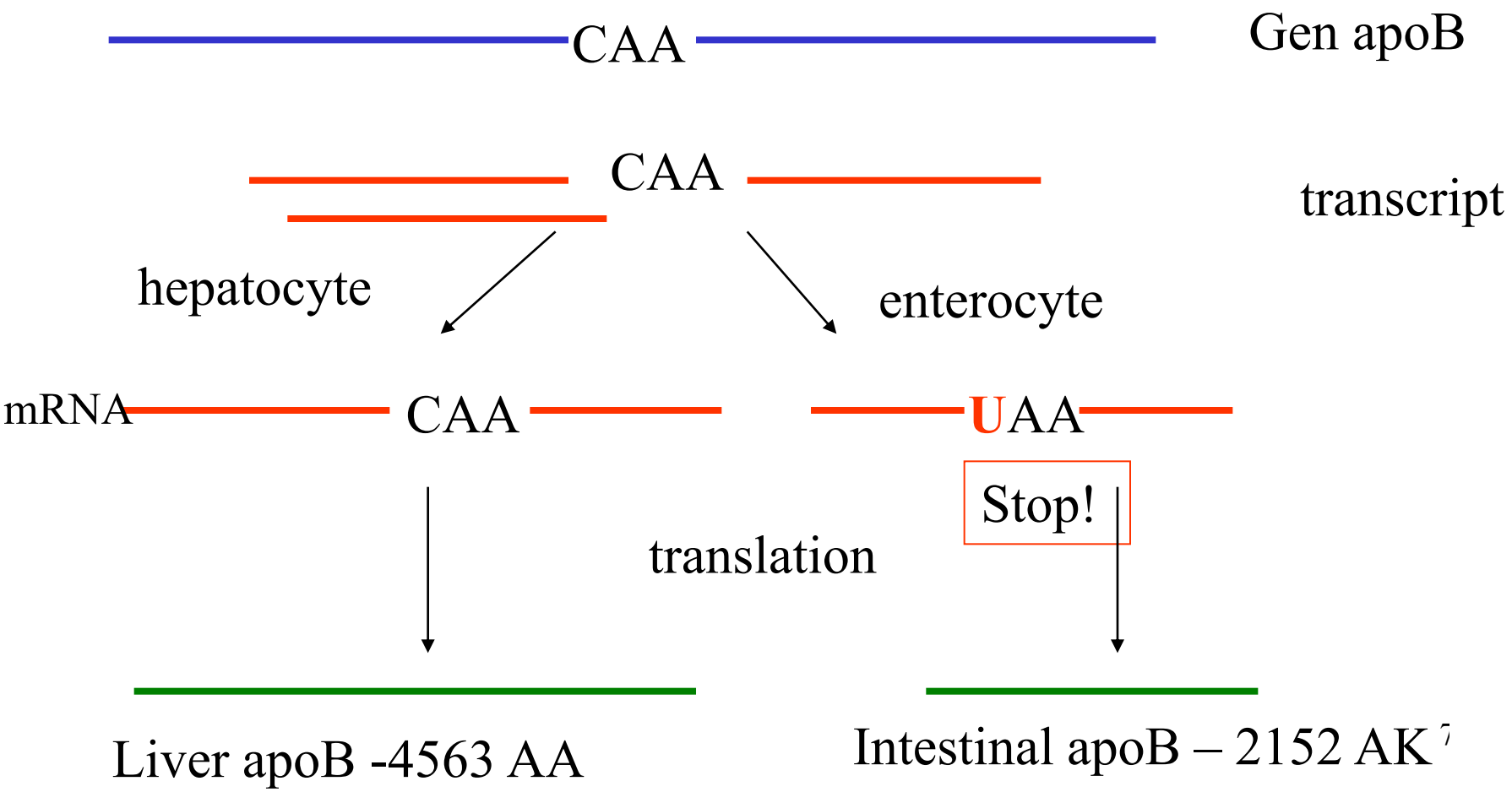
RNA editing

In some instances, RNA is „edited“ after transcription.

Primary transcript and the sequence of gene are the same, but bases are altered or nucleotides are added or deleted after the transcript is synthesized.

RNA editing

synthesis of apoB in hepatocytes and enterocytes



Synthesis apoB in hepatocytes and enterocytes (apo B is a component of chylomicrons and VLDL)

Gen apoB produces protein containing 4563 amino acids in liver

The same gene produces apoB containing only 2152 amino acids in enterocytes

Conversion of C(cytosin) to U (uracil) by deamination of mRNA transcript generates the stop-codon in intestinal mRNA. Thus the protein formed in the enterocyte has only 48% of lenght in comparision with apoB in liver

D) Regulation at the level of translation

Regulation ussualy involves the initiation of protein synthesis by eIFs (eukaryotic initiation factors)