Regulation of gene expression

I. A DNA

1953. DNA structure (James Watson and Francis Crick)

Discovering transcription, translation

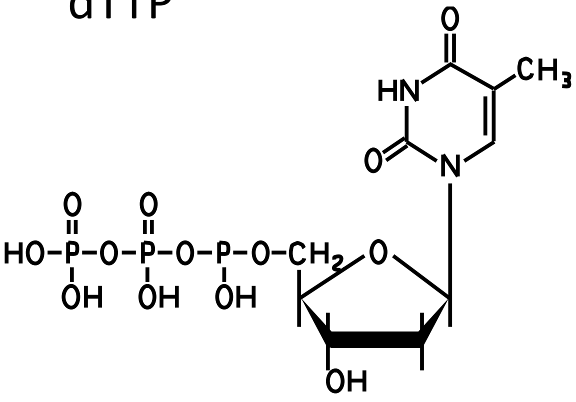
↓

Genetic information coded in DNA

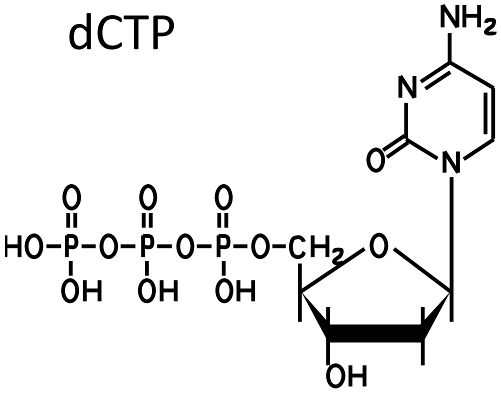
Nucleotides of the DNA

Containing pirimidine base:

dTTP

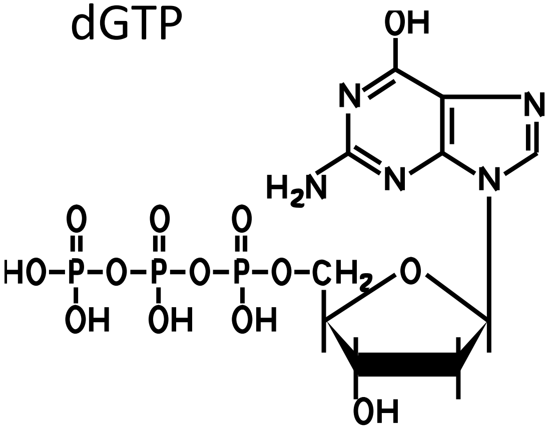


dCTP

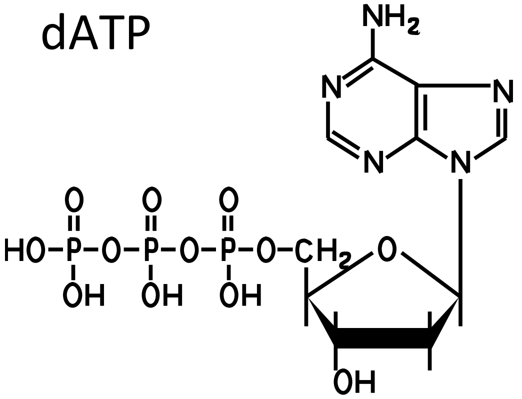


Containing purine base:

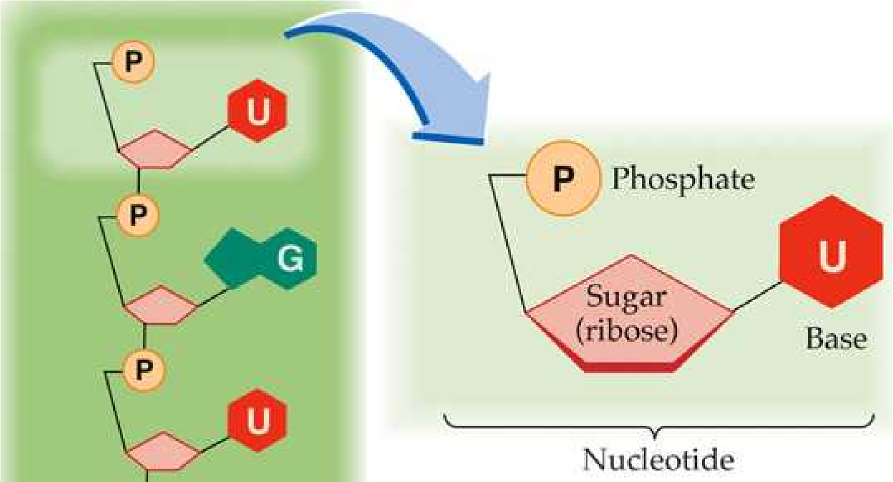
dGTP



dATP



Structure of DNA



Primary structure:
sequence of nucleotides

✓ Covalent bonds: desoxyribose and phosphate group (phosphodiester bond)

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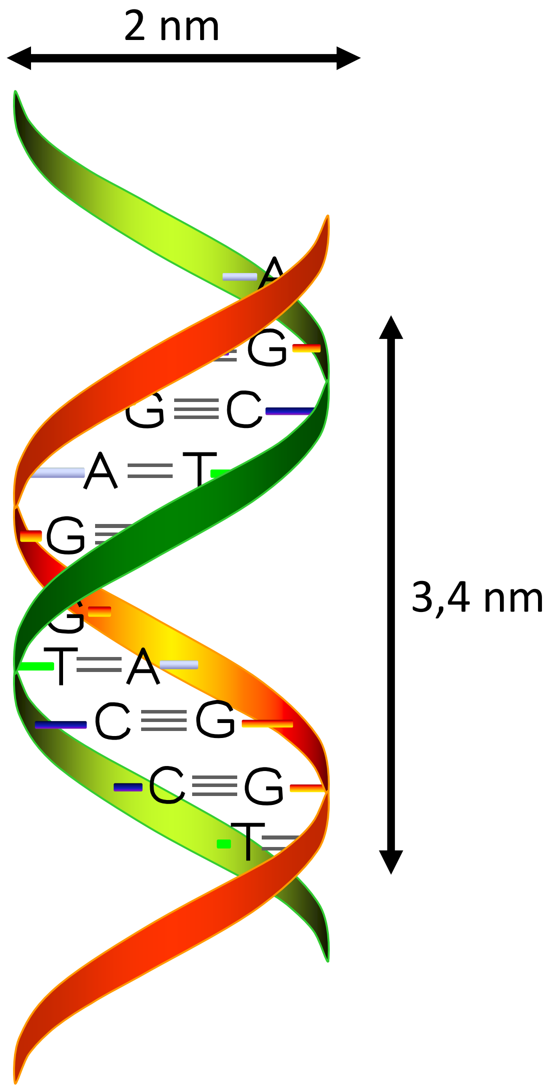
Sugar-phosphate
backbone

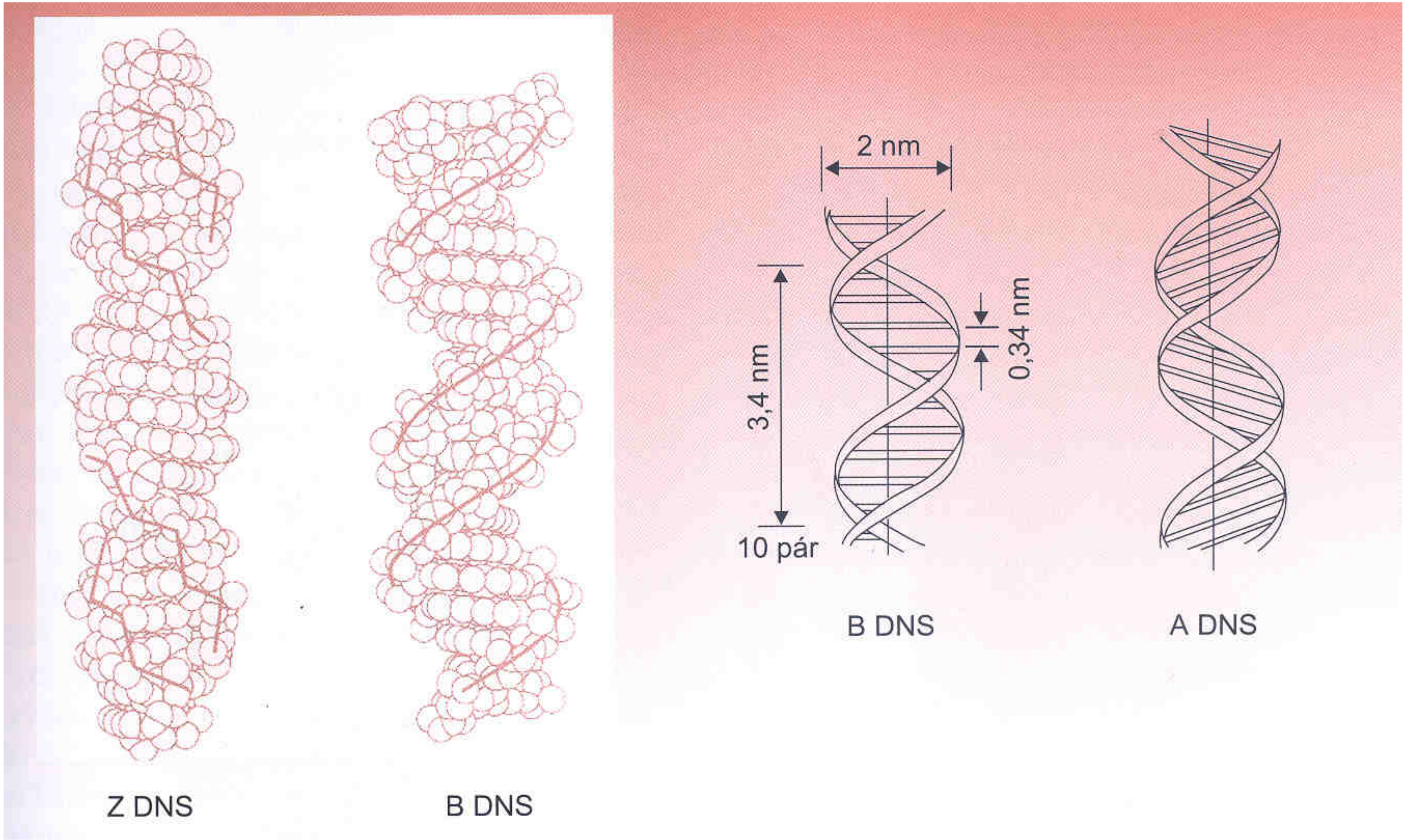
✓ genetic information is coded by the sequence of the nucleotides

The double helix model



- ✓ two polynucleotide strands (antiparallel)
- ✓ Hydrogen bonds stabilise the base pairs
- ✓ major groove and minor groove – binding sites of transcription factors

The double helix



Z: 12 bp/round, winds to the left

B: 10 bp/round, winds to the right

A: 10-11 bp/round, winds to the right

Chromatin structure

Complex of DNA and proteins that make up the contents of the nucleus of a cell

Histones:

- ✓ alcaline proteins → structure units
- ✓ most conserved proteins
- ✓ H1, H2A, H2B, H3, H4
- ✓ no histones in prokaryotes

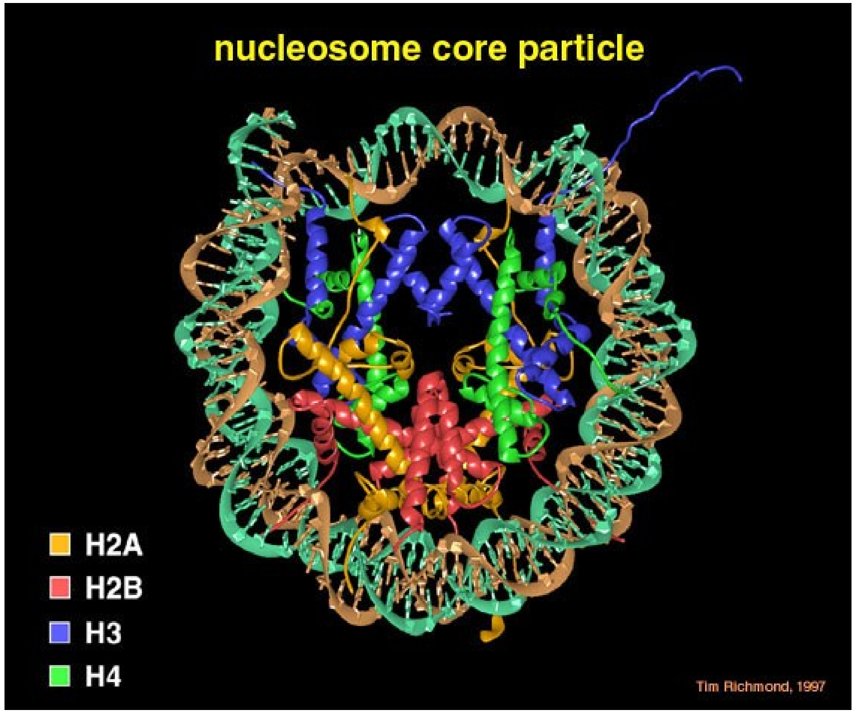
Non-histone proteins:

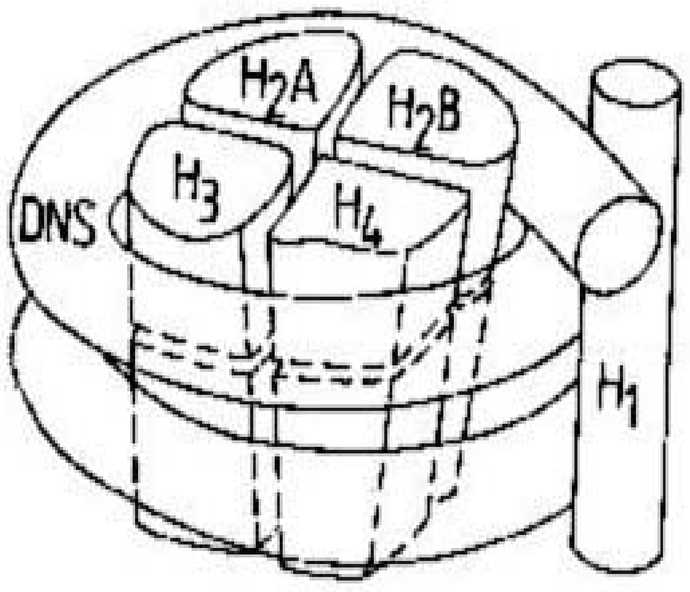
- ✓ DNA replication, RNA synthesis, gene expression regulation
- ✓ acidic or neutral proteins
- ✓ high level of heterogenity

Chromatin structure

Nucleosome= DNA + histone octamers

Histone octamer: H2A, H2B, H3 and H4 histones



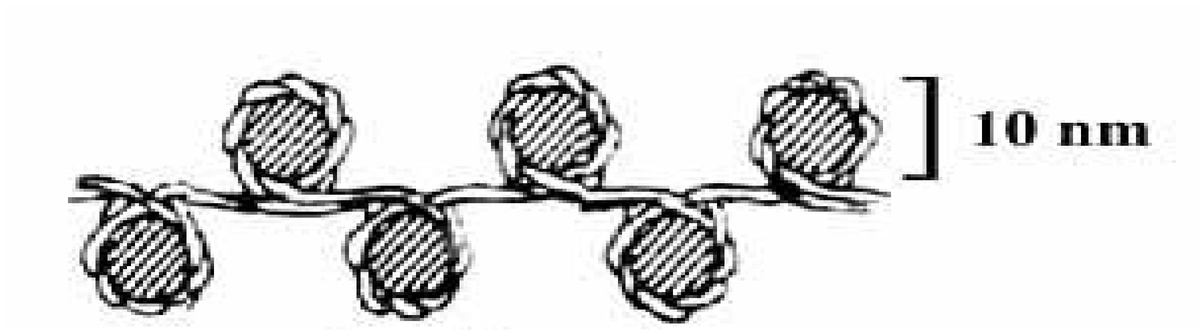


Kromatin structure

Around a histone octamer: 146 bp DNA

Between the nucleosomes: linker DNA

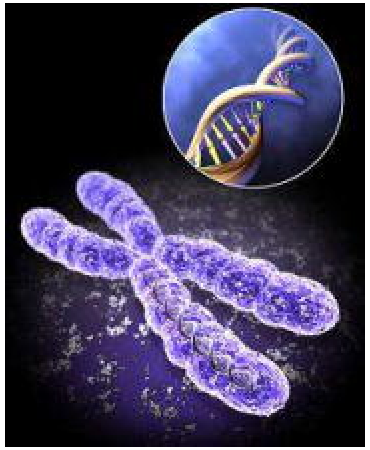
→ The structure resembles a pearl necklace



Between 2 nucleosome: H1 histones → more tight package of DNA

Modification of histones (acethyl-, methyl-, phosphate groups)

Chromatin structure



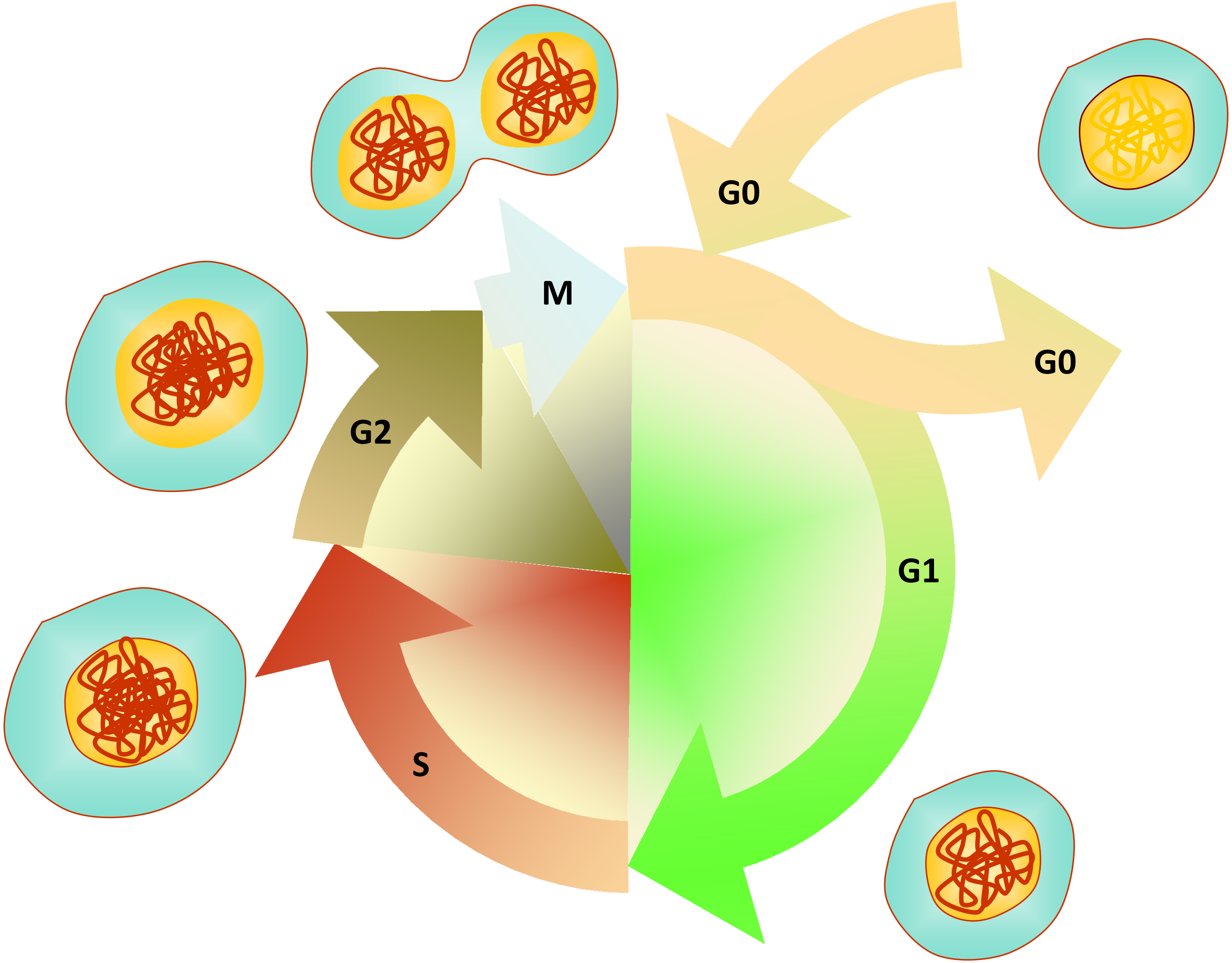
Chromosomes

Single units of the chromatin, highly condensed form of DNA

- ✓ Mitosis – most compact structure
- ✓ Interphase – more loose

- ✓ euchromatin: active transcription
- ✓ heterochromatin: highly condensed (inactive)

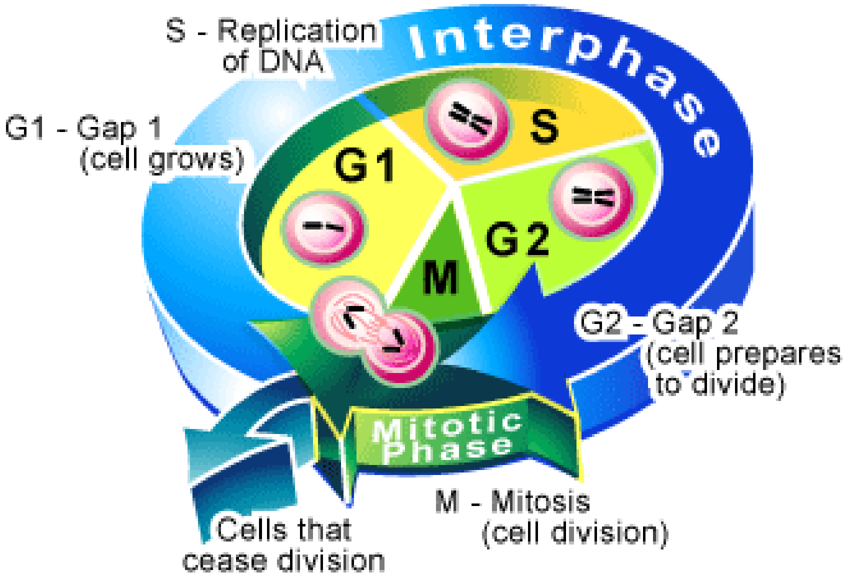
The cell cycle



The cell cycle

S phase: DNA duplication

G1 phase: synthesis of macromolecules needed in the S phase



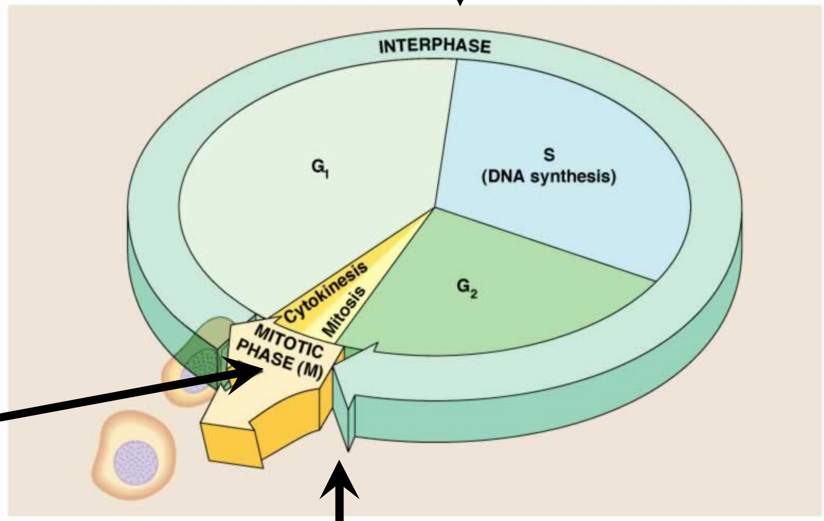
G2 phase: synthesis of cell organelles and macromolecules needed for the Mitosis

G_0 phase: resting phase

M phase: duplication of the cell

Check points of the cell cycle

G1/S checkpoint – Is the cell big enough? Are the conditions appropriate?



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M checkpoint –Are all the chromosomes bound to the kinetochores?

G2/M checkpoint – Did all the DNA molecules divide?

Regulation of the cell cycle

- cyclines ad cyclin-dependent kinases (CDK)
- the amount of cyclines and activity of Cdk-s show a characteristic oscillation during the cell cycle
- Cdk-s phosphorylate numerous proteins
- 3 groups:

  ✓ G1/S cyclins

  ✓ S cyclins

  ✓ M cyclins

Regulation of the cell cycle

Retinoblastoma protein (Rb):

- G1/S checkpoint
- non-phosphorilated Rb: binds E2F transcription factor → keeps the cell in G1
- cyclin-Cdk complex phosphorylate Rb → S phase
- tumor suppressor

Rb protein has been discovered in a tumor that appears during childhood and affects the eye.

Regulation of the cell cycle

p53:

- transcription factor - DNA repair genes
- only a small amount is the cells but e.g. UV raises its level
- DNA damage → activation of protein kinases – phosphorylation of p53 → transcription of repair proteins
- inhibition of the cell cycle until the damage is repaired
- important in promoting apoptosis
- tumor suppressor

Regulation of the cell cycle

Gettin out of G_0 phase

- Chemical signals → G1 phase
- Stimulating molecules: growth factors, cytokines

Mutation of the signal transduction pathways can cause tumors (protooncogene → oncogene)

Receptor proteins (EGFR), G-proteins (e.g. ras), protein kinases (e.g. src), transcription factors (e.g. jun, myc)

Regulation of the cell cycle

Protooncogenes:

- growth and cell division stimulators
- mutation (protooncogene → oncogene) → stronger effect → tumor formation (in heterozygotes too)

Tumor suppressors

- inhibition of cell proliferation
- only in homozygotes
- loss of function → tumor formation

DNA repair

- numerous mutagenes → DNA damage e.g. UV, radiation
- if corrected before replication: the mutation disappears

What kind of damages have to be corrected?

- mismatch
- timine dimers
- depurination
- deamination
- breaks of strands
- point mutations

DNA repair

Repair enzymes

- endonucleases
- glycosidases
- ß-DNA-polymerase (prokaryotes: DNA-polymerase I.)
- DNA-ligase

DNA repair regulation

Most important regulator: p53

- transcription factor - DNA repair genes
- only a small amount is the cells but e.g. UV raises its level
- DNA damage → activation of protein kinases – phosphorylation of p53 → transcription of repair proteins
- inhibition of the cell cycle until the damage is repaired
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- tumor suppressor

RNA types

rRNA

- ribosome
- **5S**; **5,8S**; **28S** rRNA-ek: large subunit rRNA
- **18S** rRNA: small subunit rRNA
- 70-80% of all RNA

tRNA

- amino acid transport
- 12% of all RNA

mRNA

- genetic information (DNA→ribosomes)
- 3-5% of all RNA

RNA types

hnRNA

- heteronuclear RNA
- → mature mRNA

snRNA

- small nuclear RNA
- mRNA maturation (splicing)

scRNA

- small cytoplazmic RNA
- ribosomes → ER

Structure and expression of prokaryotic genes

- genes → operons
- polycistronic mRNA
- 1 enzyme: RNA polymerase
- no splicing
- transcription and translation are connected in time and space (no cell compartments)

Eukaryotic genome

- Only a small % of DNA encodes genes
- 1990. A Human Genom Project
- Results (2000)

    - ✓ 20000-25000 gene
    - ✓ function is known: 6000 genes

- non-coding control regions, repetitive sequences, pseudogenes

Az eukarióta genom

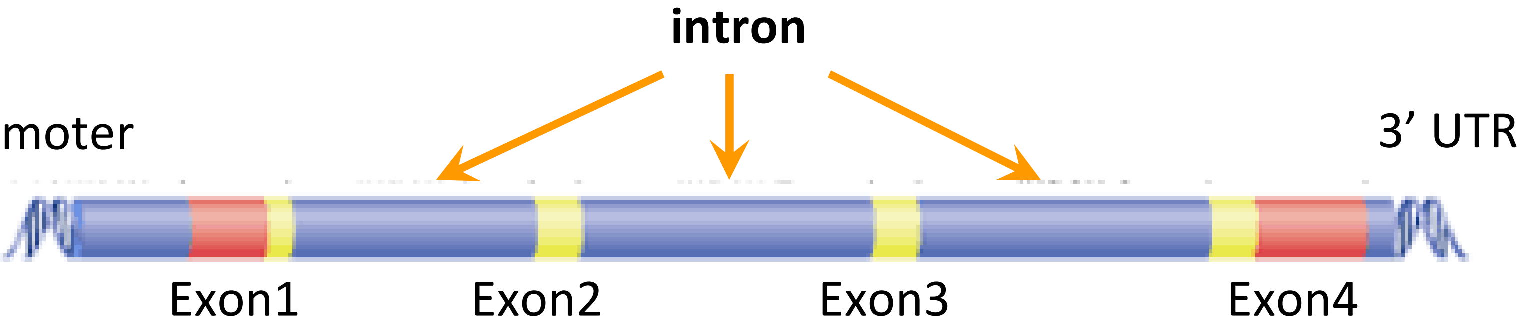
Based on their repetition → 3 groups

- a few times e.g. rRNA, histone genes
- a few hundred times e.g. transcription regulator proteins
- a few thousand times e.g. telomere, centromere

UTR:

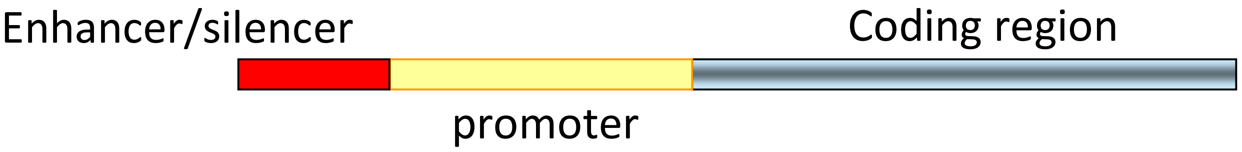
- untranslated region
- mRNA 5’, 3’ ends
- stability of mRNA (3’), ribosome binding (5’)

Structure and expression of eukaryotic genes



- Monocistronic
- length of introns: 80-1000 bp
- modification of primary transcription product
- 3 different RNA polymerase enzymes
- transcription and translation are separated
    - ✓ mRNA synthesis (transcription): nucleus
    - ✓ Protein synthesis (translation): cytoplasm

Transcription regulation



✓ enhancer: cis-regulatory module, binds activating transcription factors (5’, 3’, intron)

✓silencer: inhibition of transcription

Response element

- specific DNA sequences
- not longer than 10 nucleotides
- reacts to specific chemical signals
- e.g. increase of cAMP-level → CRE (cAMP response element)

↓

Binding of specific transcription factors
cAMP response element binding protein (CREB)

↓

transcription activation

Transcription regulation

Transcription activation

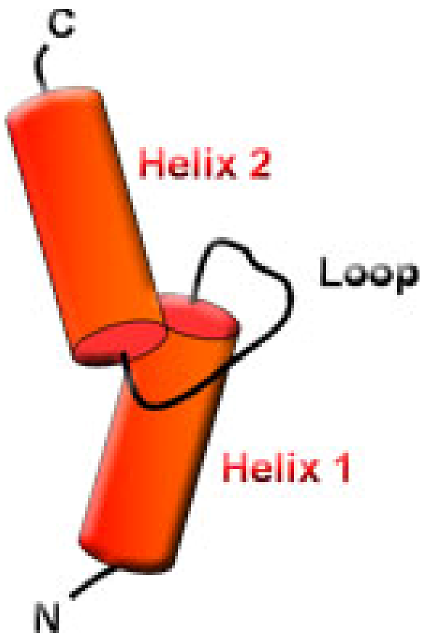
Transcription inhibition

Characteristic motifs

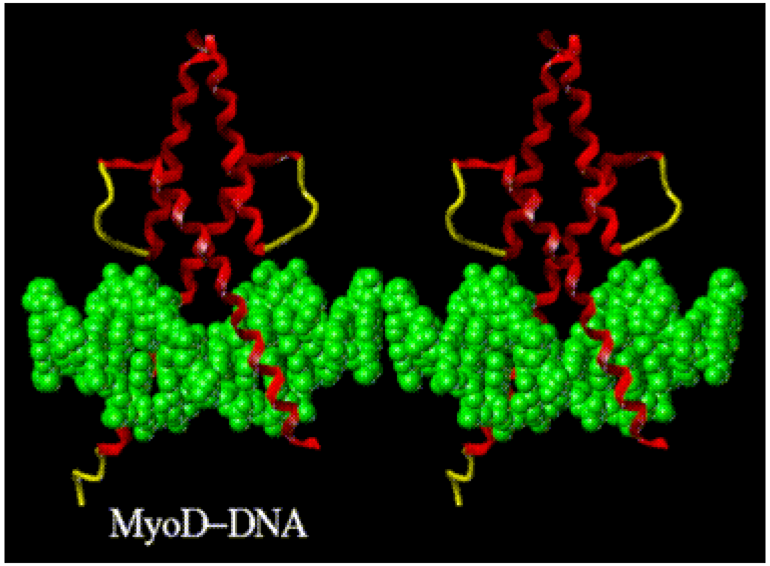
- ✓ helix-loop-helix (HLH proteins)
- ✓ ZN-finger
- ✓ Leucin zipper

A lot of transcription factors are hormone receptors (intracellular receptors of steroid hormons) or tumor suppressors

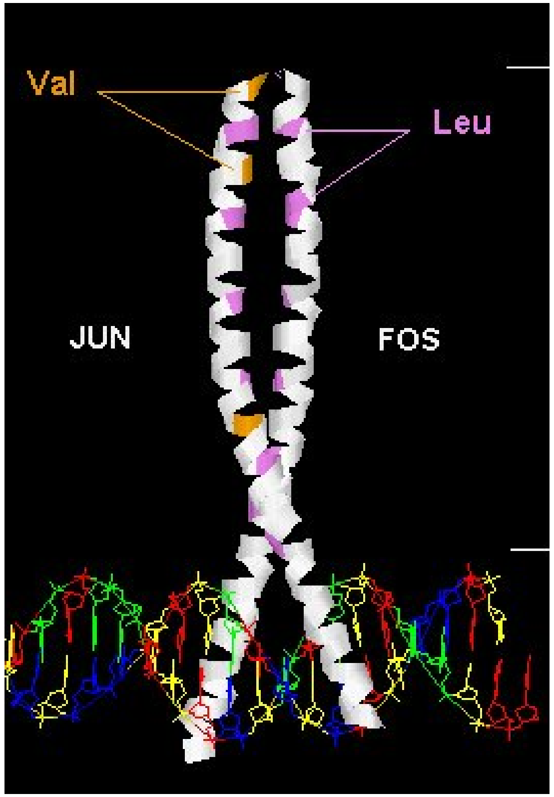
HLH proteins



- binds into the major groove
- dimers
- e.g. MyoD, Myogenin (muscle differentiation)
- Achete-scute complex (Drosophila)

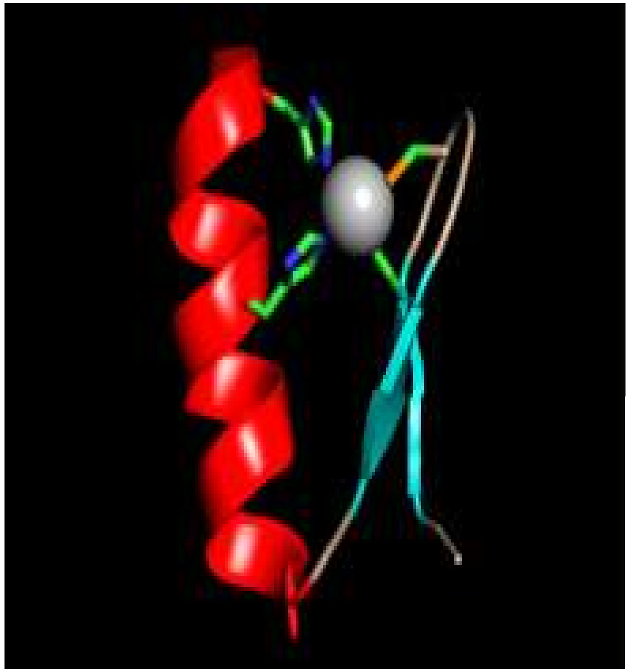


Leucine zipper proteins

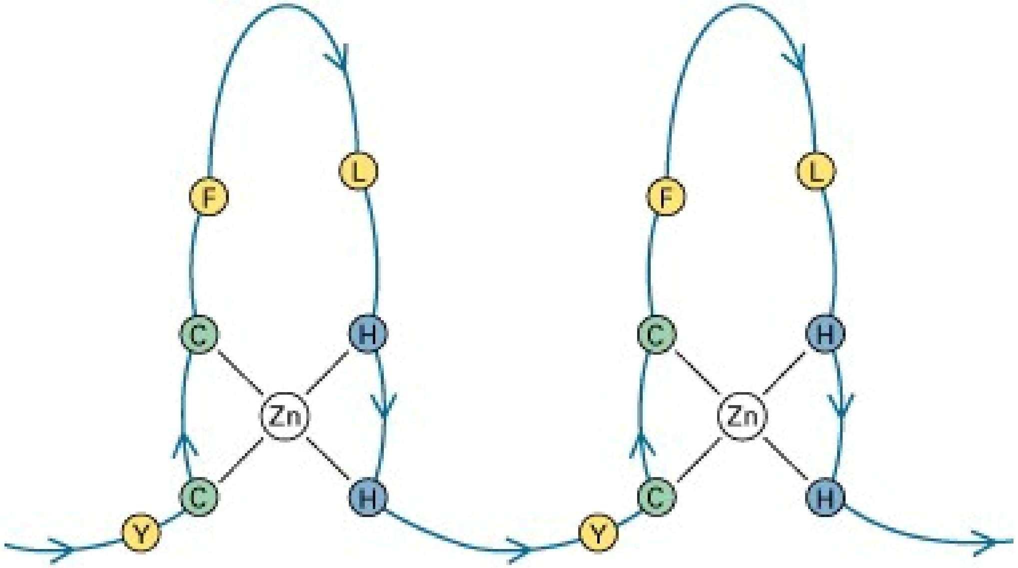


- not in DNA binding
- dimerisation
- at least 4 leucines in the helical part(leu-6as.-leu-6as.-leu…)
- e.g. fos, jun, myc proteins (protooncogenes)

Zn-finger proteins



- 2 His and 2 Cys or 4 Cys bind Zn
- e.g. steroid receptors, TFIIIA protein (regulation of 5S rRNA transcription)



Other transcription factors

A) p53 protein

- regulation of cell cycle
- activation of transcription of DNA repair enzymes
- Promotes apoptosis

B) Hox proteins

- a group of related genes that control the body plan of an embryo along the anterior-posterior (head-tail) axis

RNA-polymerases

RNA-polymerase I.

- 5,8S + 28S + 18S rRNA
- in the nucleus
- resistant to α-amanitin

RNA-polymerase II.

- transcription of mRNAs and snRNAs
- in the nucleoplasm
- α -amanitin inhibits

RNA-polymerase III.

- transcription 5S rRNA, tRNA, snRNA
- nucleoplasm

Transcription

A transcription szakaszai:

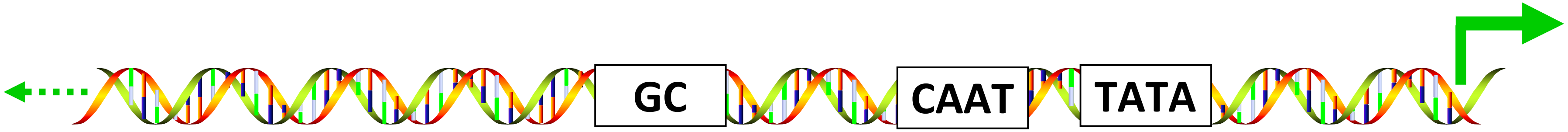
initiation → elongation → termination

RNA-polymerase cannot bind DNA by itself

↓

Transcription factors are needed

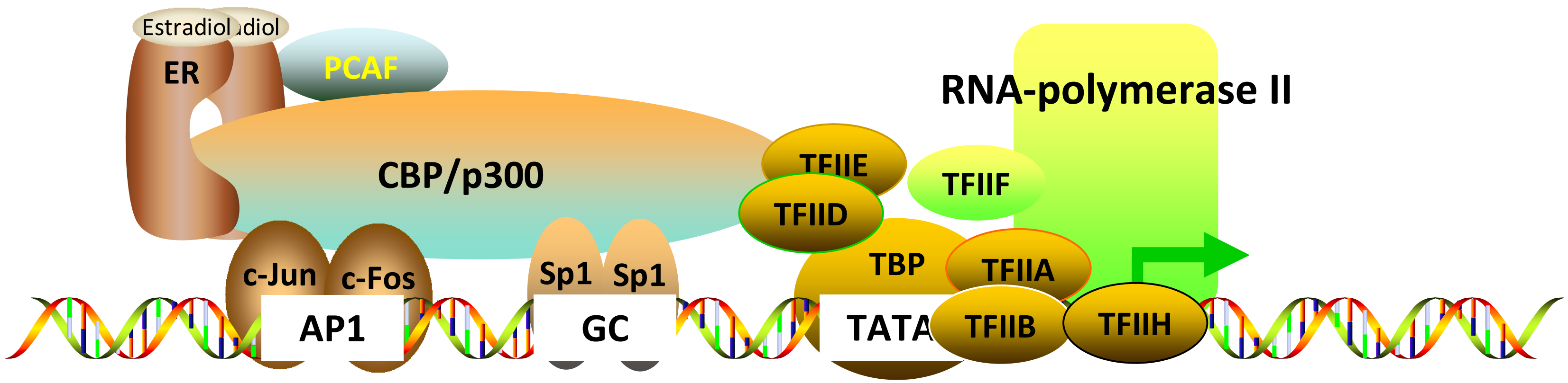
Eukaryotic promoter structure



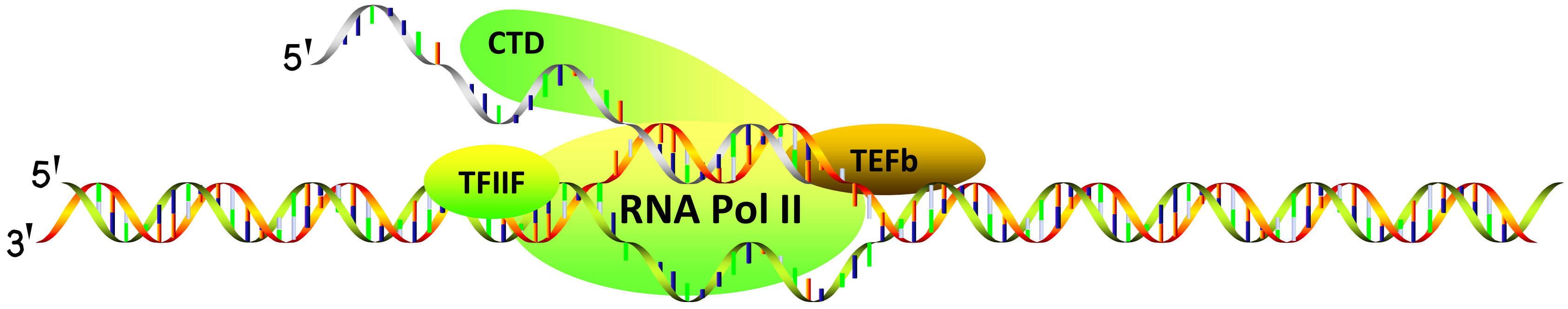
- not always 5’, it can be in an intron also
- TATA-box: TBP (TATA Binding Protein) – minor groove

Transcription

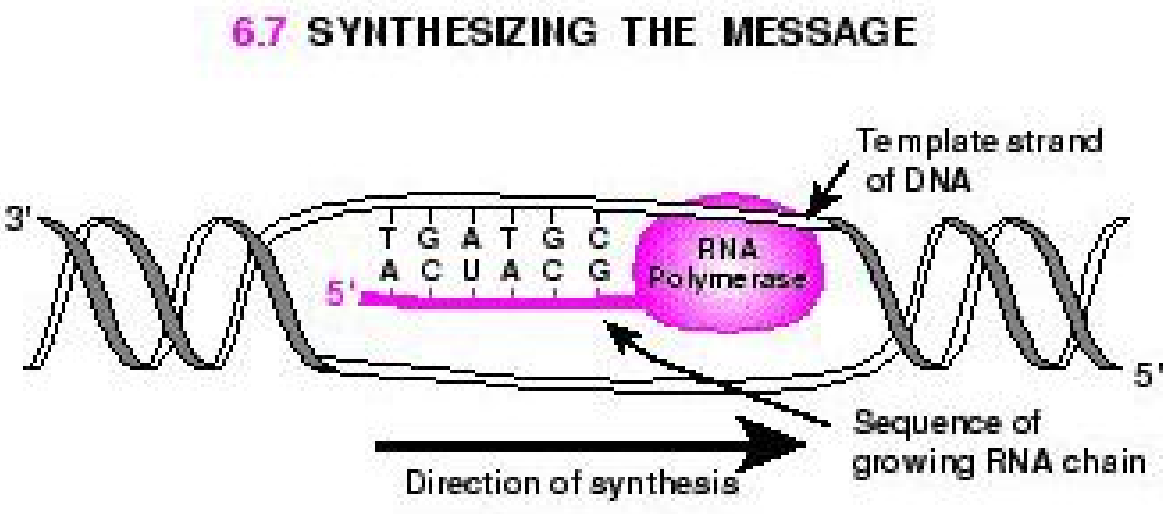
Initiation complex



Elongation complex



Elongation

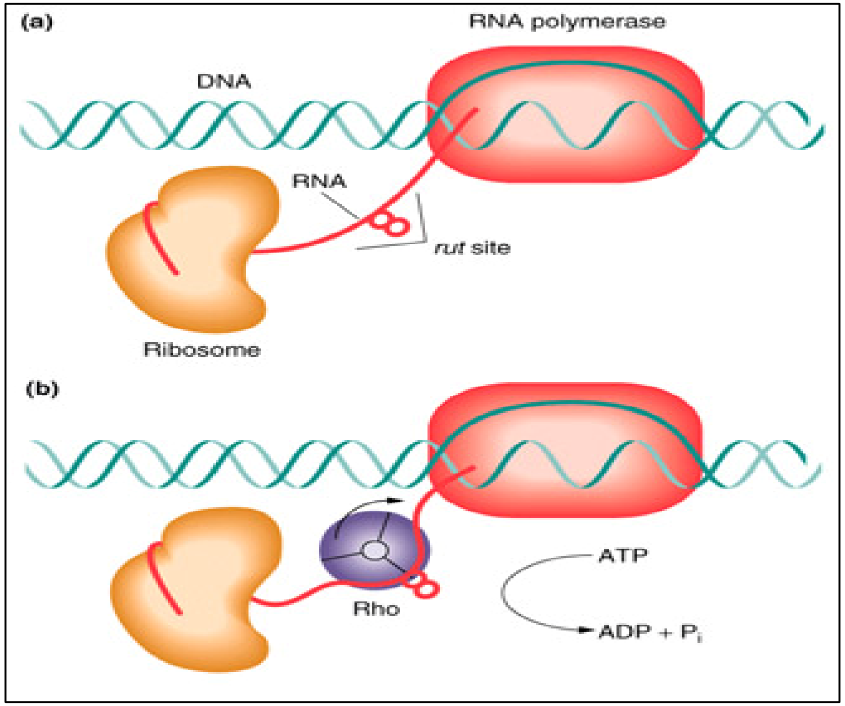


Insertion of nucleotides

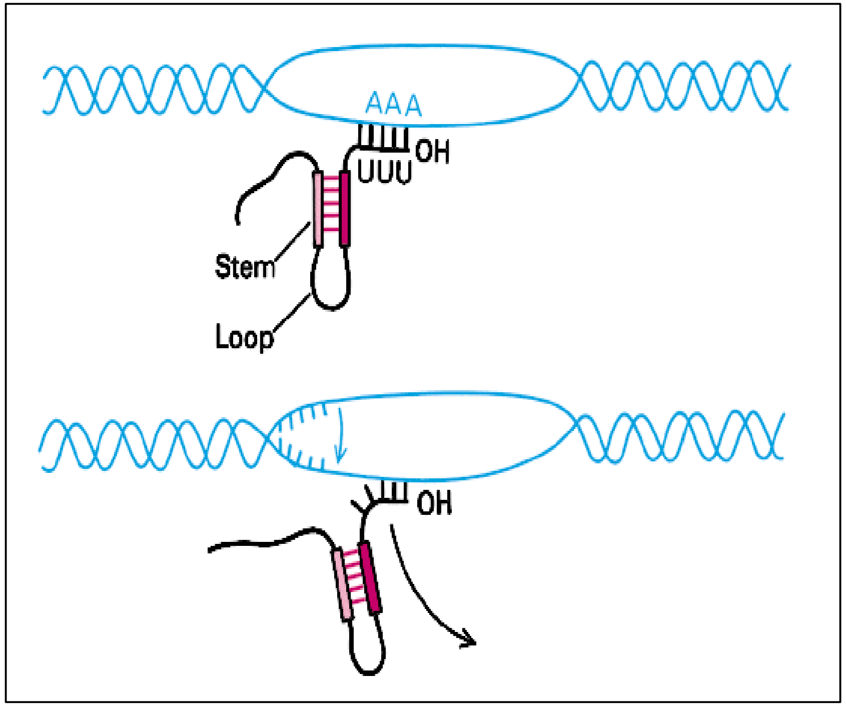
- nucleotide-triphosphates → hnRNA

Termination

Rho factor dependant



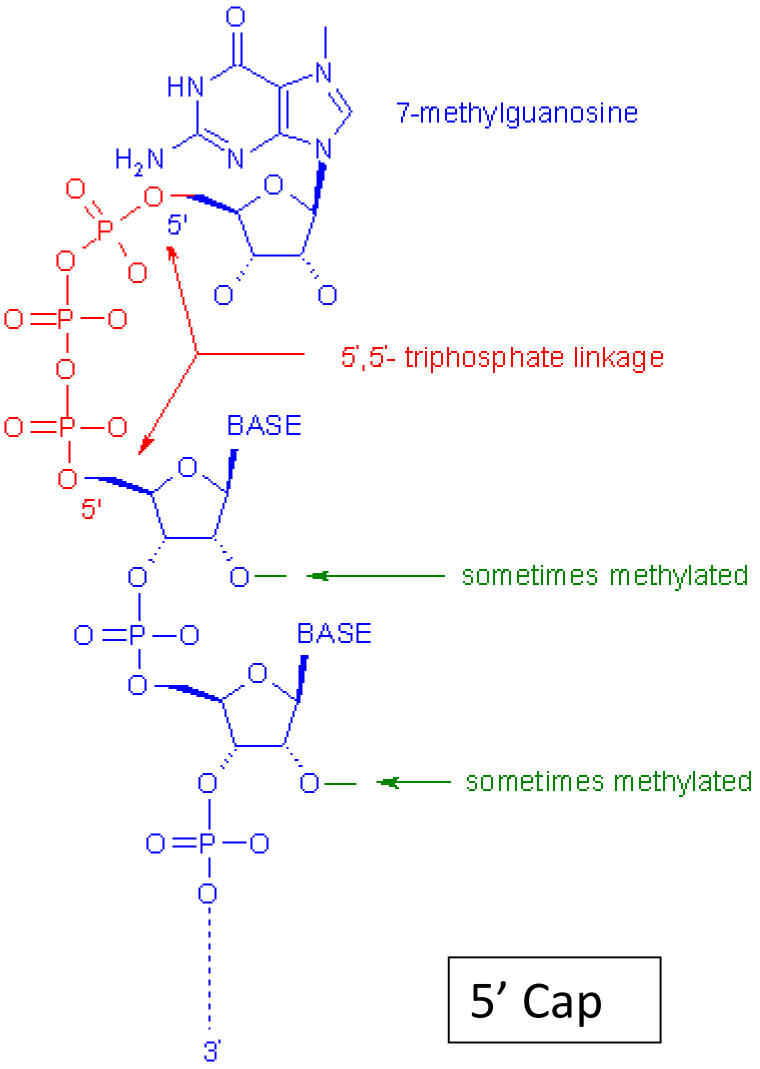
Rho factor independant



Oligomer factor ’pulls RNA out’

Loop in the forming RNA → dissociates from DNA

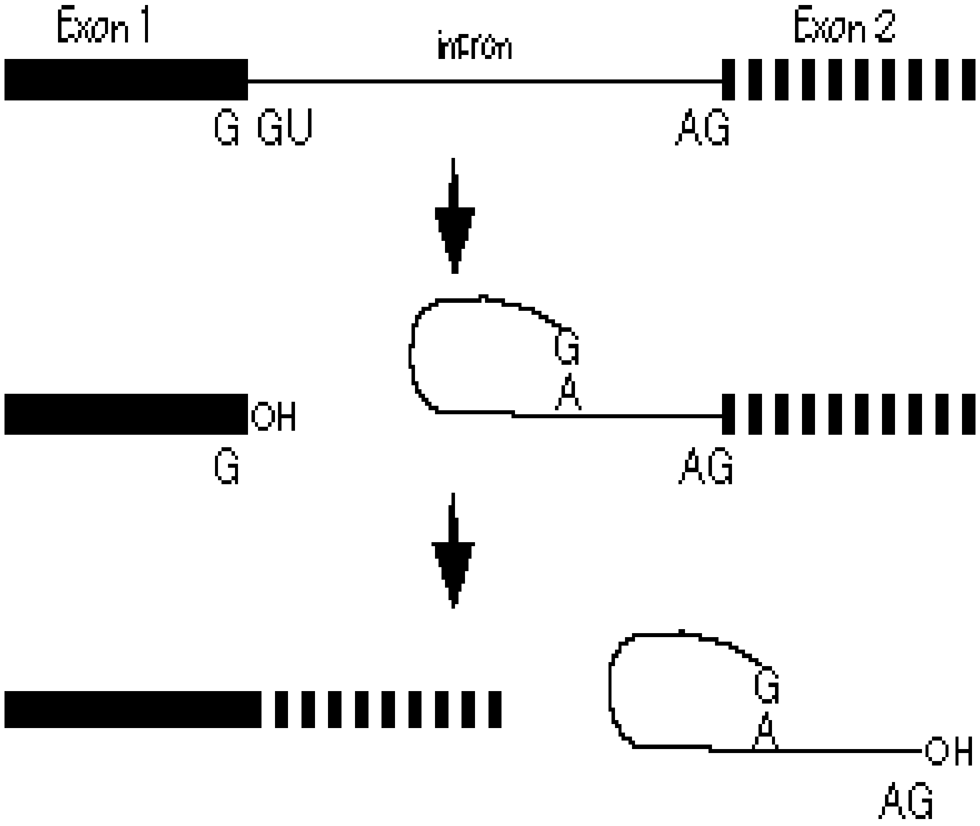
hnRNA maturation



5’ Cap

- modification of 5’-end = Cap-formation
- modification of 3’-end = poliA tail
- splicing

Splicing



Alternative splicing, splice errors, antisense RNA

- splicing: specific – tissue, stade of development – different exons are expressed = alternative splicing
- Examples:
    - Synthesis of Ig heavy chains
    - synthesis of rat tropomyosin
- damage of splicing is known in several diaseases e.g. thalassemias
- ***antisense RNA***: a single-stranded RNA that is complementary to a messenger RNA (mRNA) strand transcribed within a cell → inhibiton of the translation of a specific protein

Translation

- amino acids are determined by nucleotide triplets = **codon**s (mRNA)
- 64 codons
  - 3 **stop codons**
  - 60 codons encoding amino acids

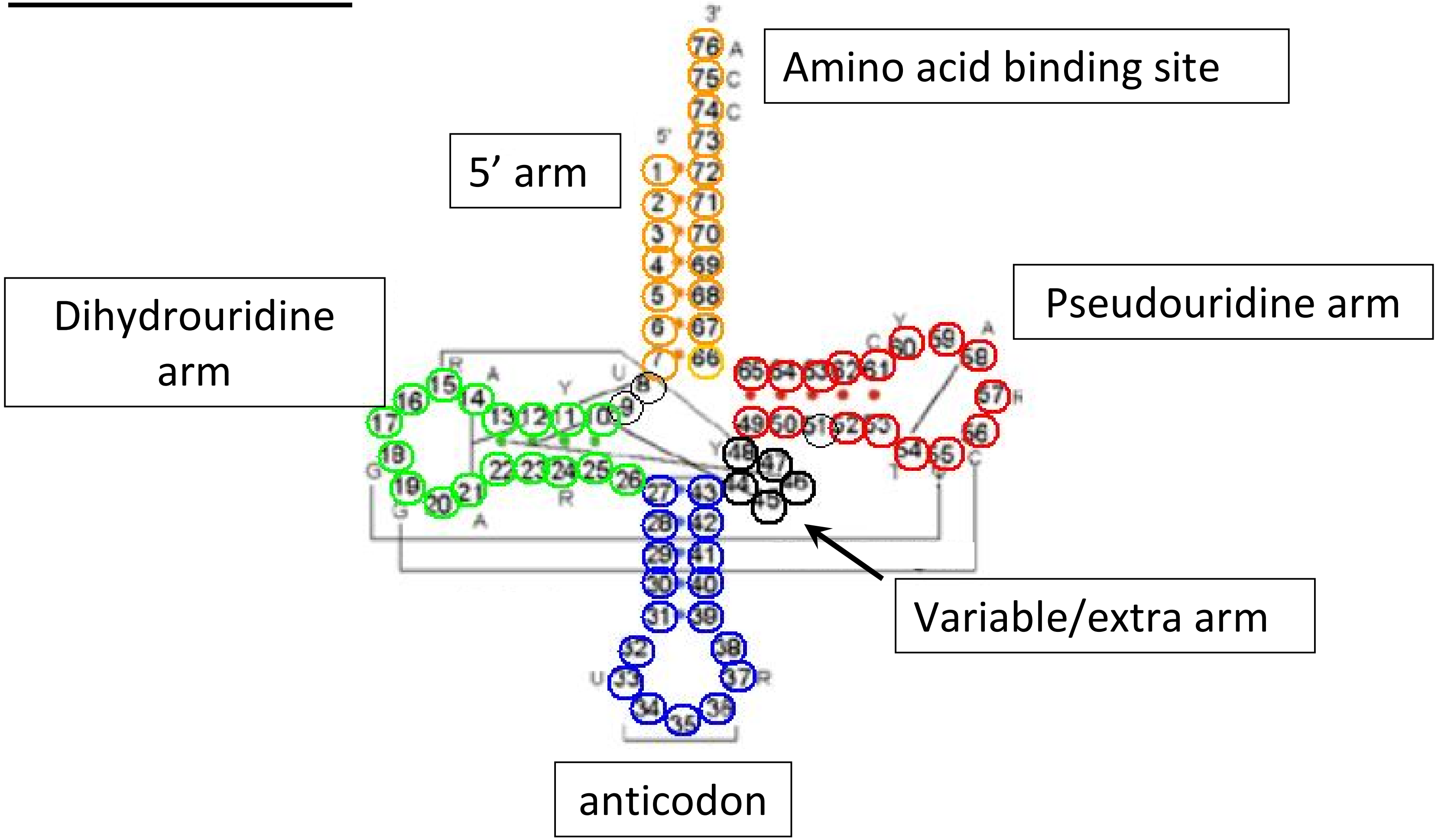
↓

1 amino acid – more codons

- initial codon: AUG → methionine (eukaryotes) (prokaryotes: formyl-methionine)
- tranfer of amino acids: **tRNA** (46)

Transfer of amino acids

tRNA structure



Amino acid binding site

5' arm

Dihydrouridine arm

Pseudouridine arm

Variable/extra arm

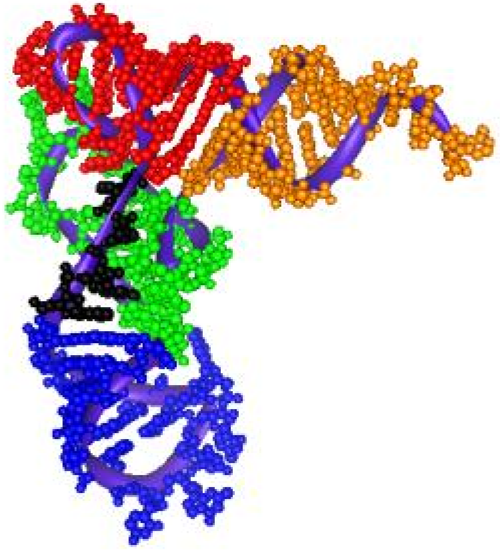
anticodon

Activation of amino acids

$$\text{Amino acid} + \text{tRNA} + \text{ATP} \xrightarrow{\textbf{E}} \text{aminoacyl-tRNA} + \text{AMP} + \text{PP}_i$$

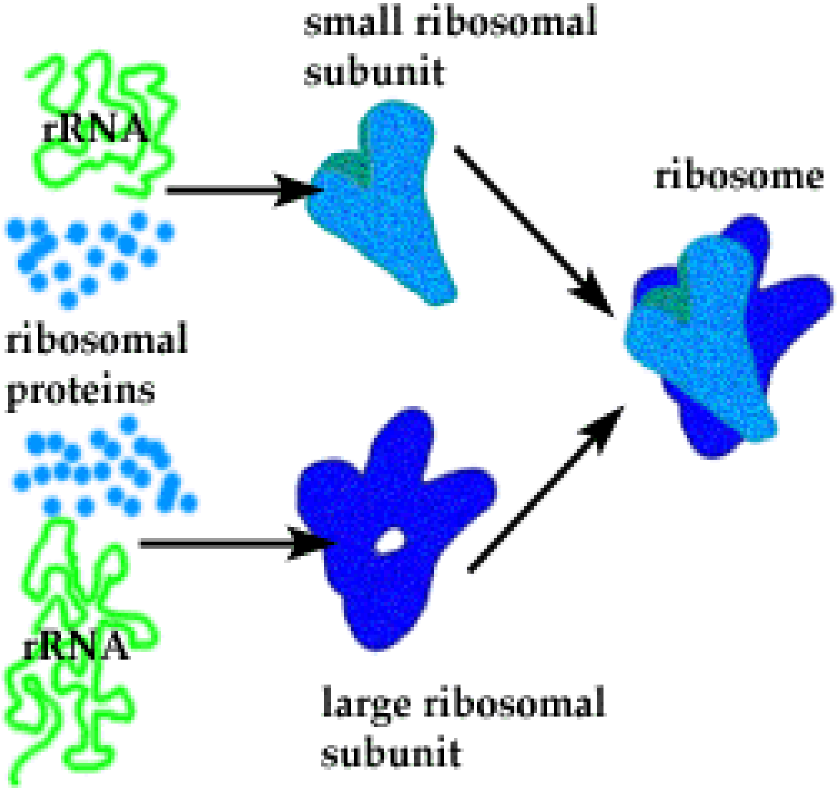
E = aminoacyl-tRNA-synthetase

- 2 steps
- macroergic bond between the tRNA and the amino acid
- aminoacyl-tRNA-synthetase: highly specific enzymes



tRNA structure

Ribosome



Prokaryotes: 70S ribosome

- large subunit: 50S
- small subunit: 30S

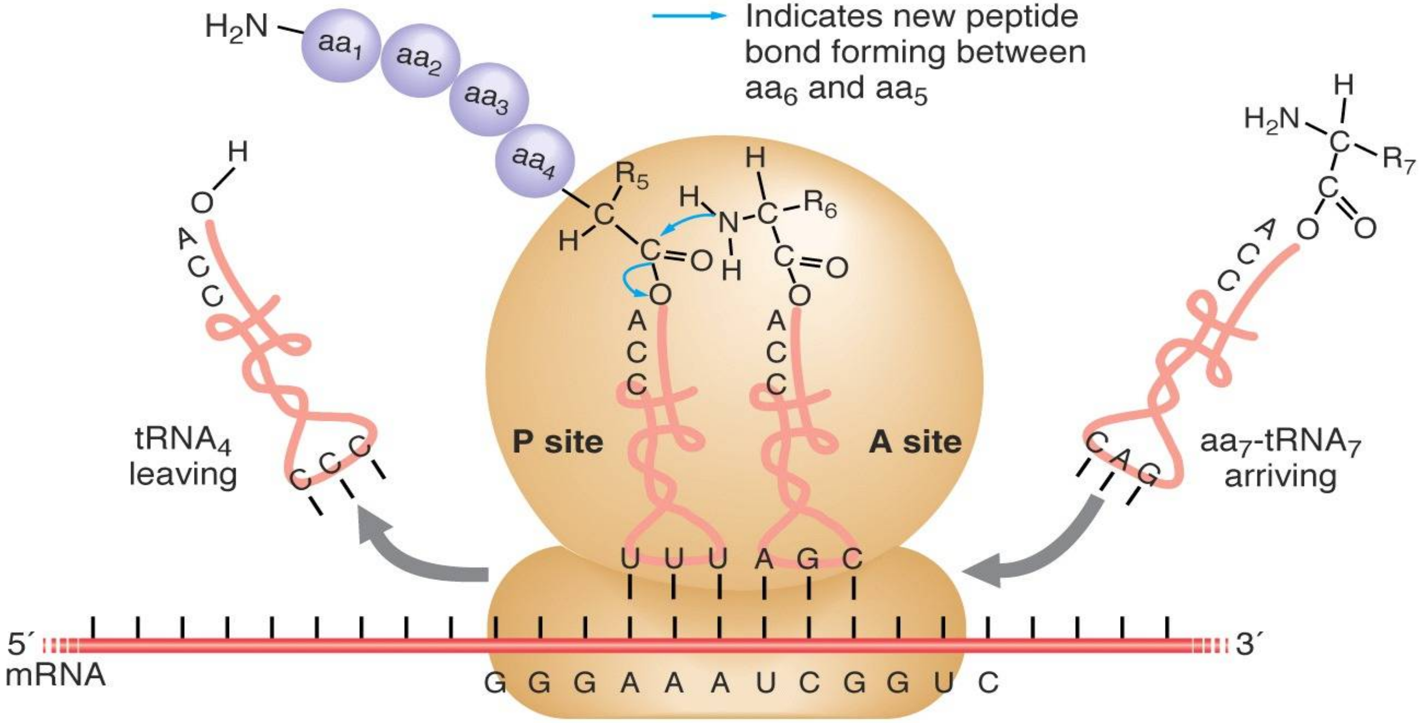
Eukaryotes: 80S ribosome

- large subunit: 60S
- small subunit: 40S

Ribosome

Binding sites:

- P = peptidyl-tRNA binding site
- A = aminoacyl-tRNA binding site



Protein synthesis

Steps of translation (prokaryotes):

1) Initiation: formation of initiation complex
   - mRNA bind small subunit of ribosome
   - Initiator tRNA binds ribosome (formyl-methionine-tRNA)
   - initiation factors (IF1, IF2, IF3)

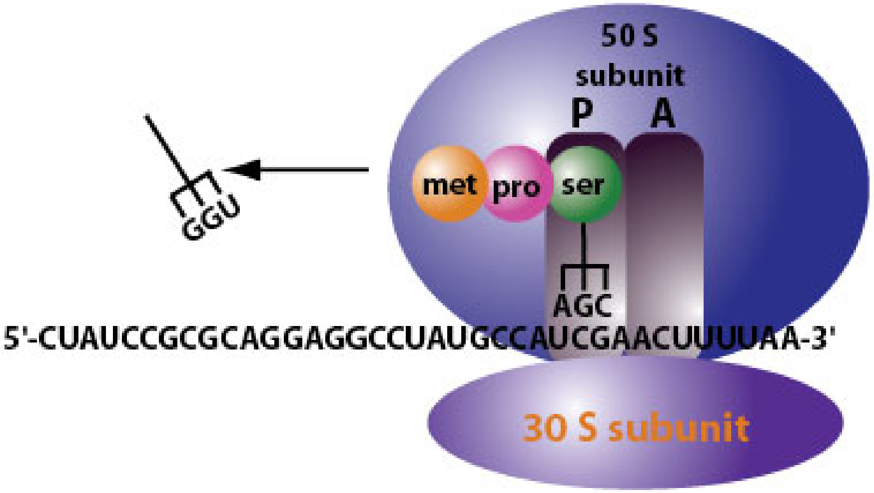
Function of initiation factors

- IF1: stabilisation of the complexet
- IF2: GTP binding protein
- IF3: prevents the conncetion of empty 30S and 50S subunits

Protein synthesis

2) Elongation: 3 steps

- aminoacyl-tRNA binds to ‘A’ site
- peptide bonds between ‘P’ and ‘A’ tRNA’s amino acids
- translocation (tRNA dissociates from ‘P‘ site, A site→P site)

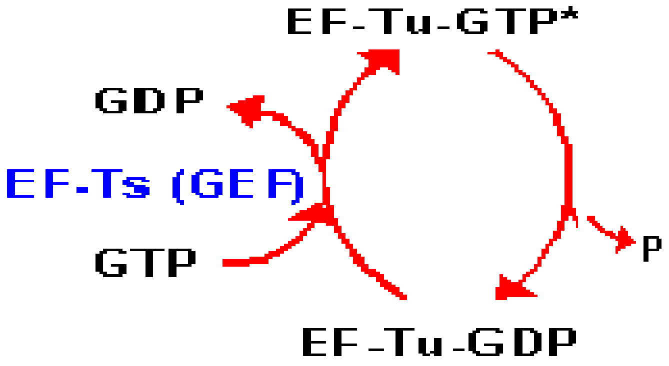


Protein synthesis

Specific factors which help elongation = elongation factors

- EF-Tu: helps aminoacyl-tRNA to bind ribosome (GTP hydrolysis)
- EF-Ts: exchange protein, helps GDP to dissociate from EF-Tu
- ET-G: helps translocation (GTP hydrolysis)

Peptidyl-tranferase caralyzes the formation of the peptide bond



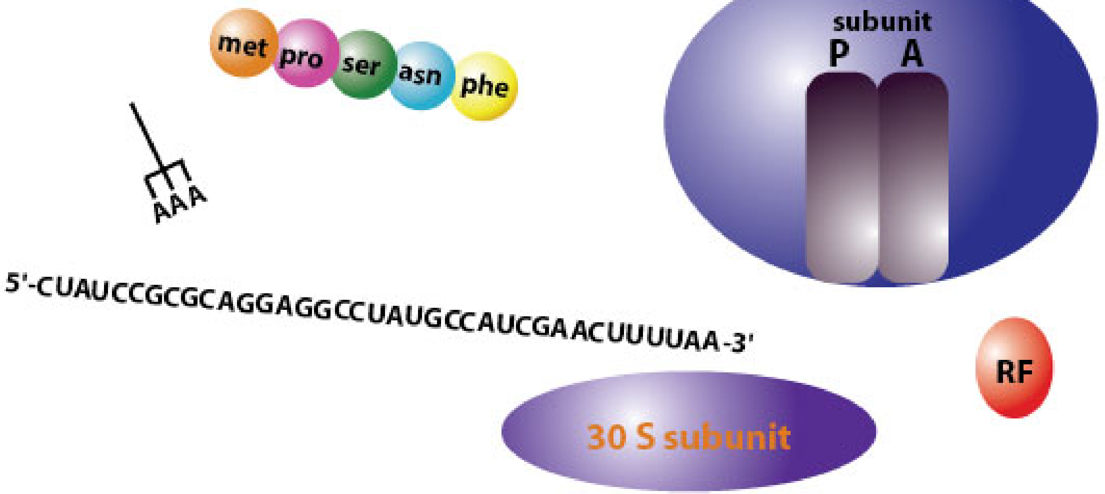
Protein synthesis

3) Termination: ‘A’ site – stop codon → no tRNA

↓

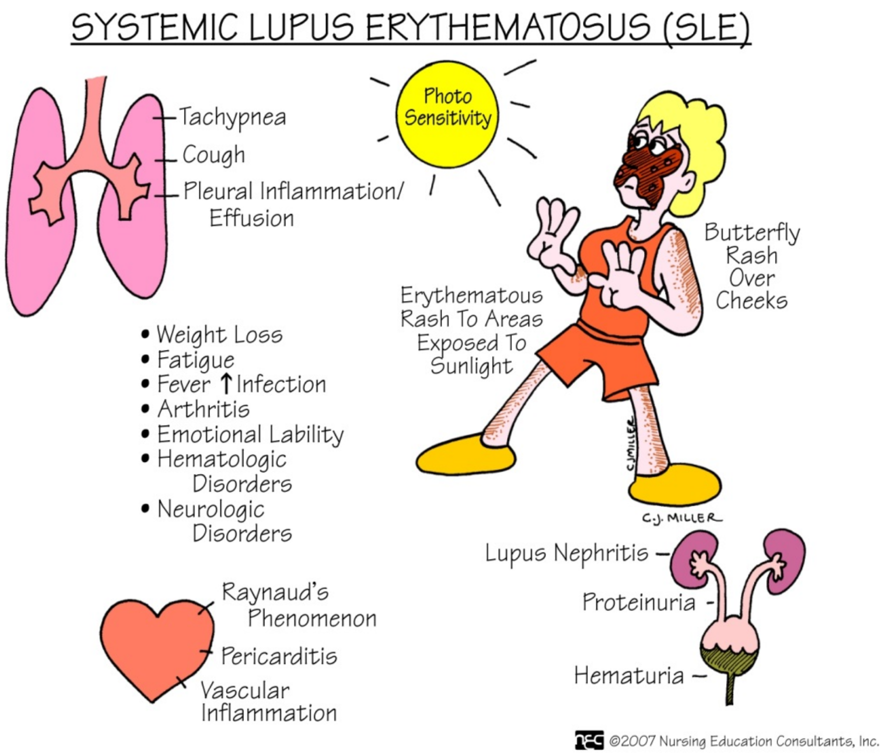
Termination factors = release factors → peptidyl-transzferase → polypeptide is released from the last tRNA

Ribosome dissociates



Systemic lupus erythematosus (SLE)

- chronic, systemic, autoimmune disease – hiigh level of antibodies → immunocomplex precipitation in the tissues → widespread destruction
- mostly women (20-40 yrs)
- Complement factor defficiency (C1q, C2, C3) (elimination of immunocomplexes is inhibited)
- Autoantibodies against C1q
- Symptoms e.g.
    - Butterfly rash over cheeks
    - fever, arthritis
    - Nephritis
    - Hemolytic anaemia



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Thalassemias

- Genetic defect → the synthesis of normal globin chains is distracted
- Single chains: accumulation in the erythropoetic stem cells → toxic effect → ineffective erythropoesis
- Types:
  1. β THALASSAEMIA
     - β globin chain defect
     - β chain can be replaced by δ and γ chains → Hb A2($\alpha 2\delta 2$) and Hb F ($\alpha 2\gamma 2$)
     - Aggregation of α-chains → mutant red blood cells
     - Variable symptoms
  2. α THALASSAEMIA
     - α globin chain defect
     - Can be asymptomatic (depends on the genetic status)