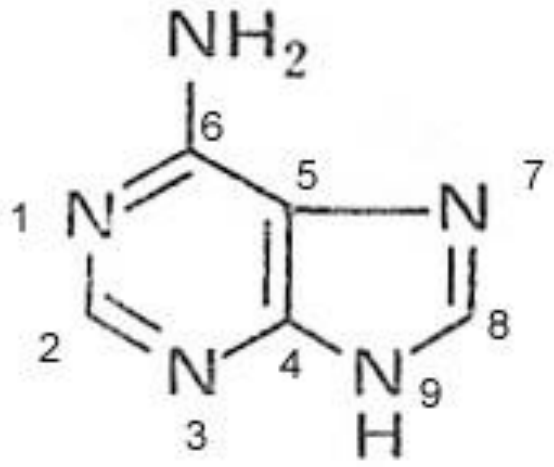
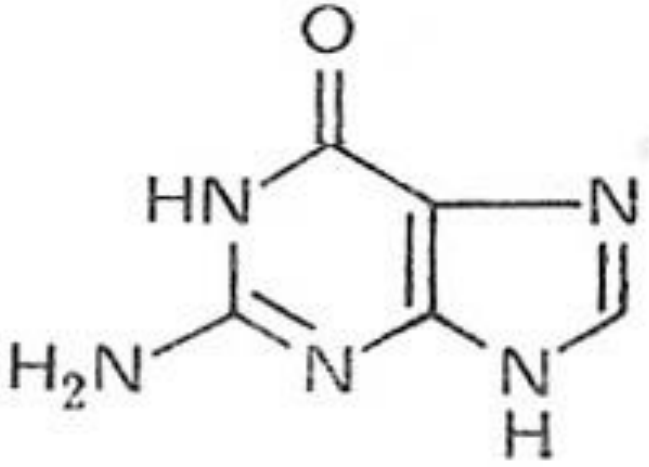
Struktura, vlastnosti a význam nukleových kyselin, DNA a RNA, DNA v jádře, histony, chromatin.

Interakce DNA-protein, transkripční faktory

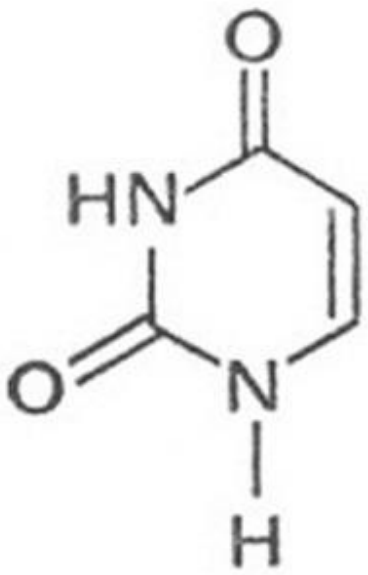


adenin

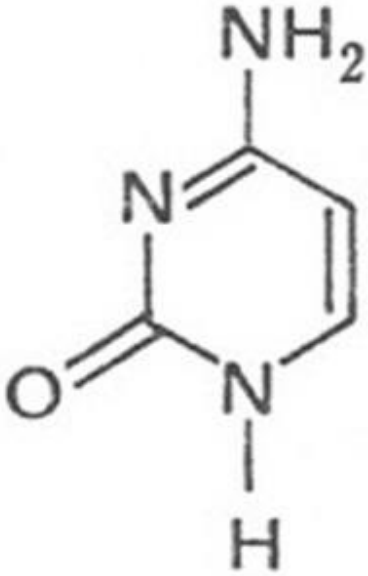


guanin

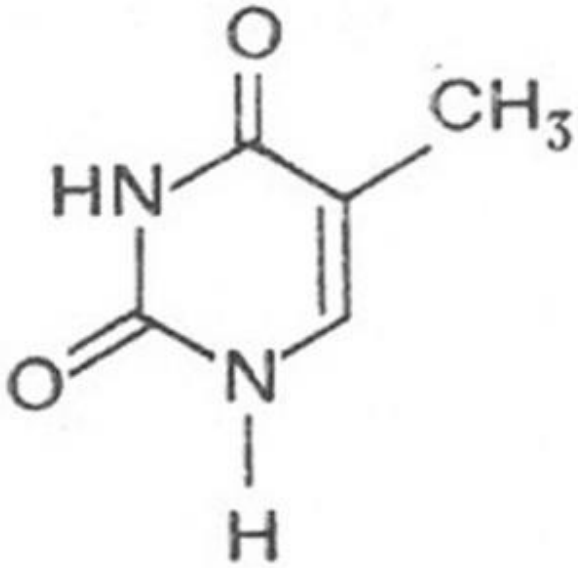
purinové base



uracil

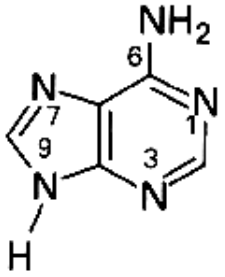


cytosin

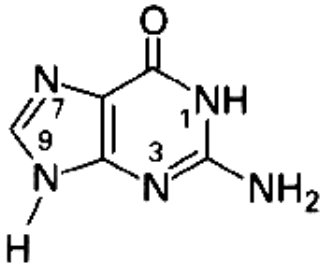


thymin

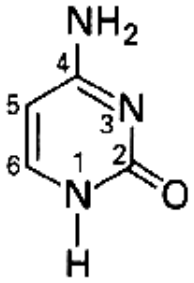
pyrimidinové base



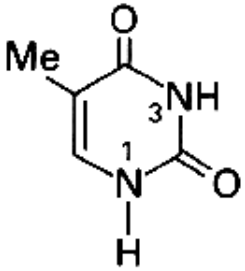
adenine



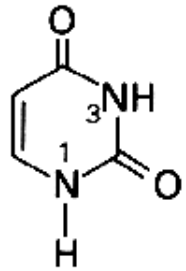
guanine



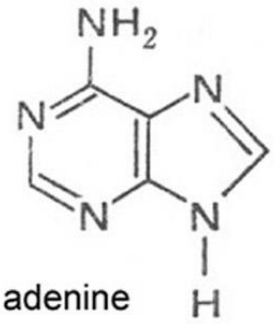
cytosine



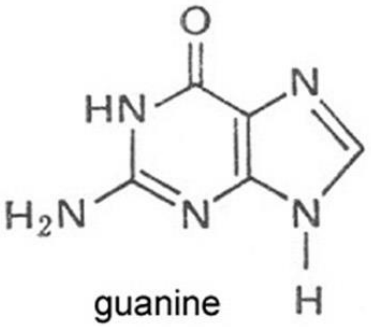
thymine



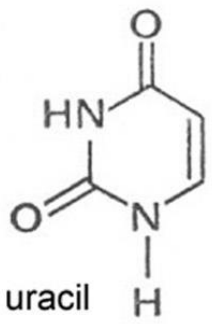
uracil



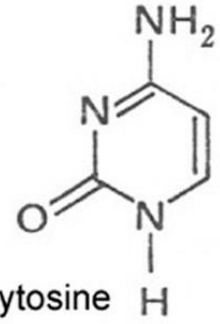
adenine



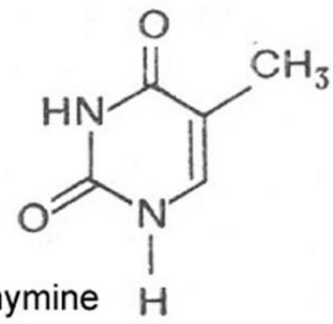
guanine



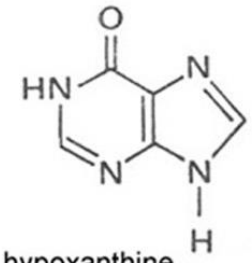
uracil



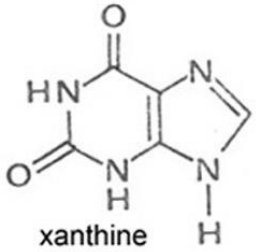
cytosine



thymine



hypoxanthine



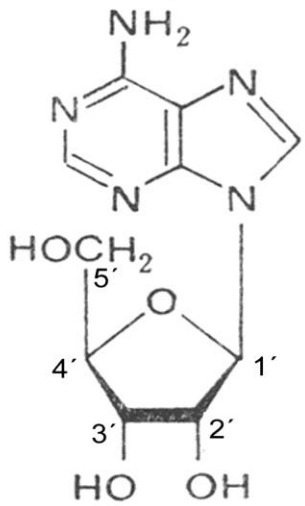
xanthine

in DNA: A, G, C, T	(with 2'-deoxyribose)	dA, dG, dC, dT
in RNA: A, G, C, U	(with ribose)	

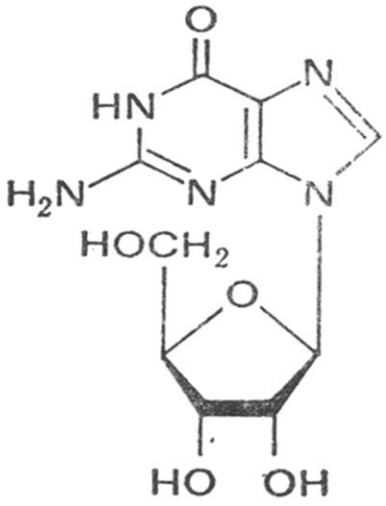
Nukleové base - purinové a pyrimidinové

Ribonukleosidy - base + ribosa
Deoxyribonukleosidy base + 2´- deoxyribosa

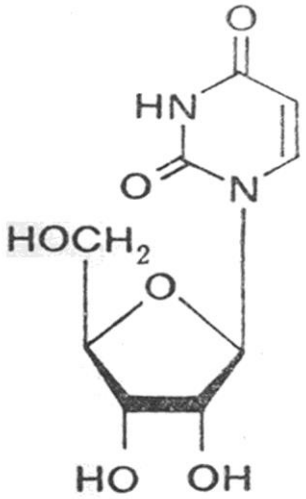
Nukleotidy, deoxynukleotidy – nukleosidy s fosfát. skupinou



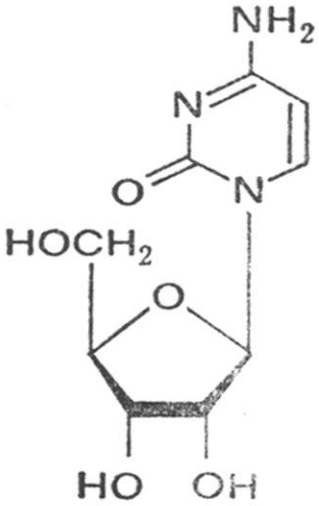
adenosin



guanosin

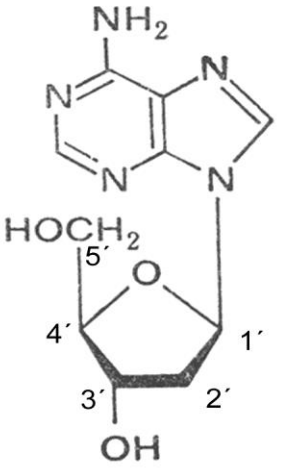


uridin

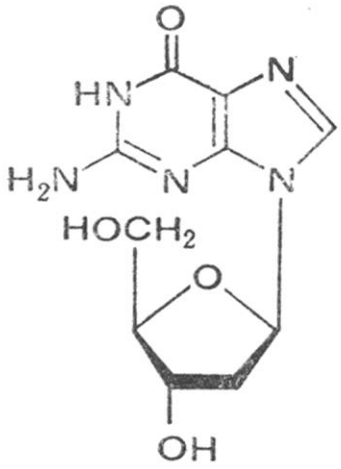


cytidin

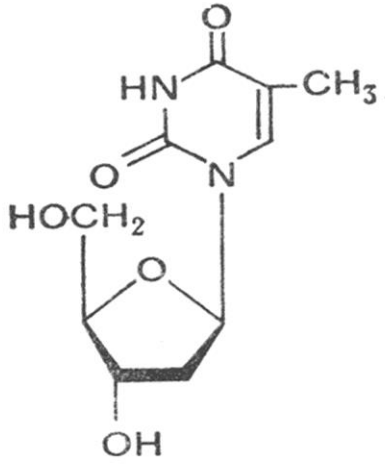
Ribonukleosidy



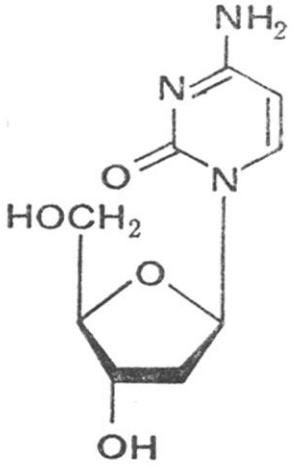
2'-deoxyadenosin



2'-deoxyguanosin



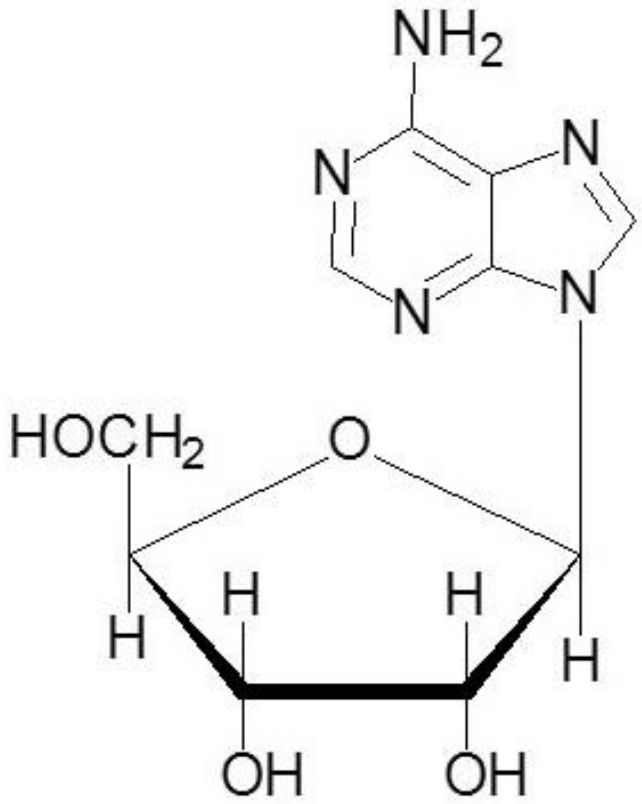
2'-deoxythymidin

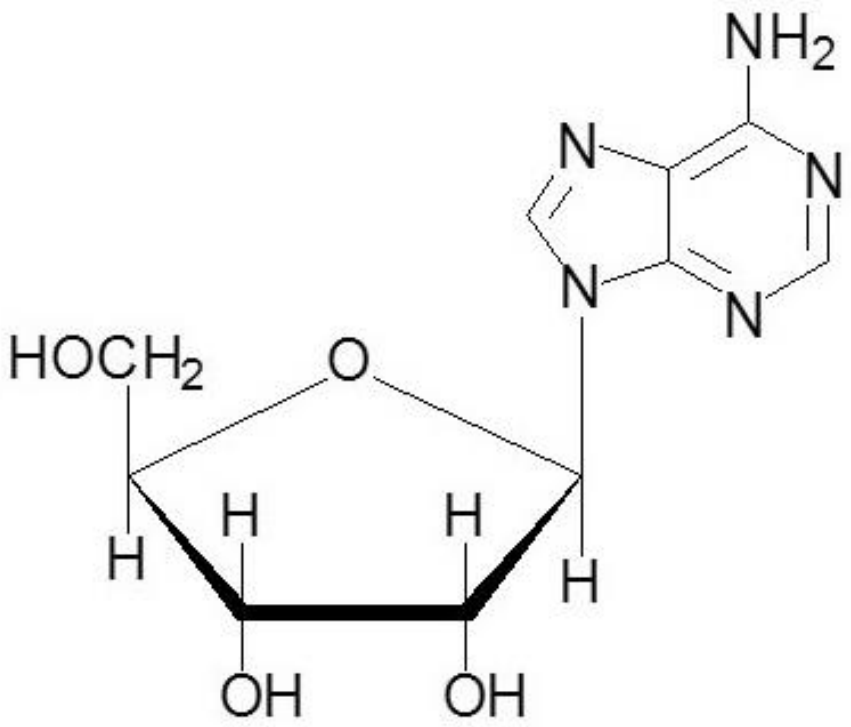


2'-deoxycytidin

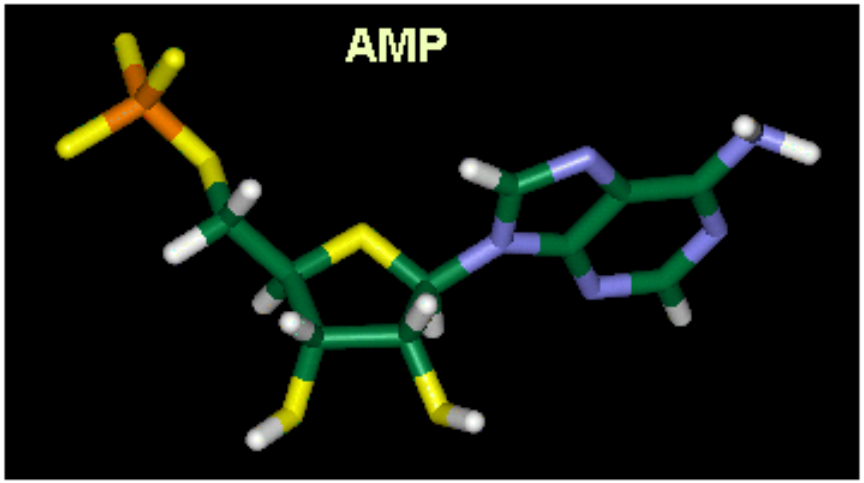
Deoxyribonukleosidy

Syn- a anti- adenosin

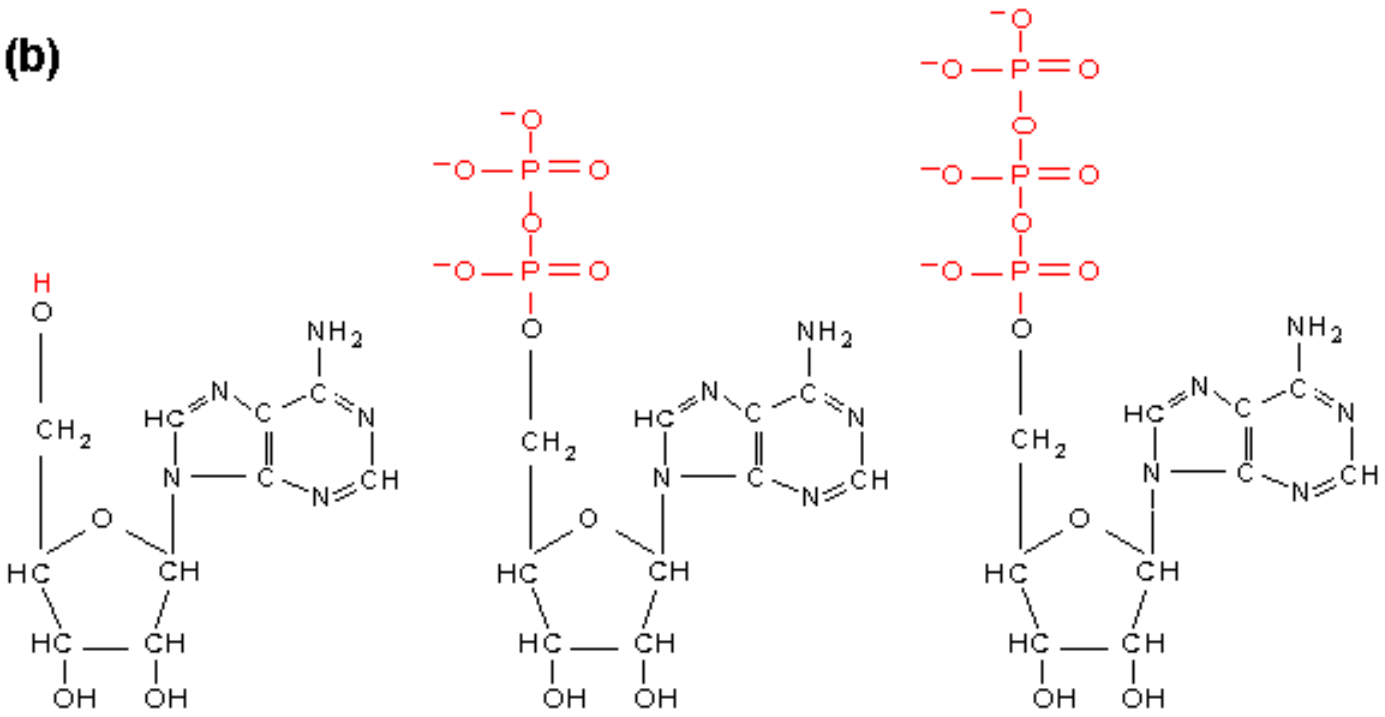


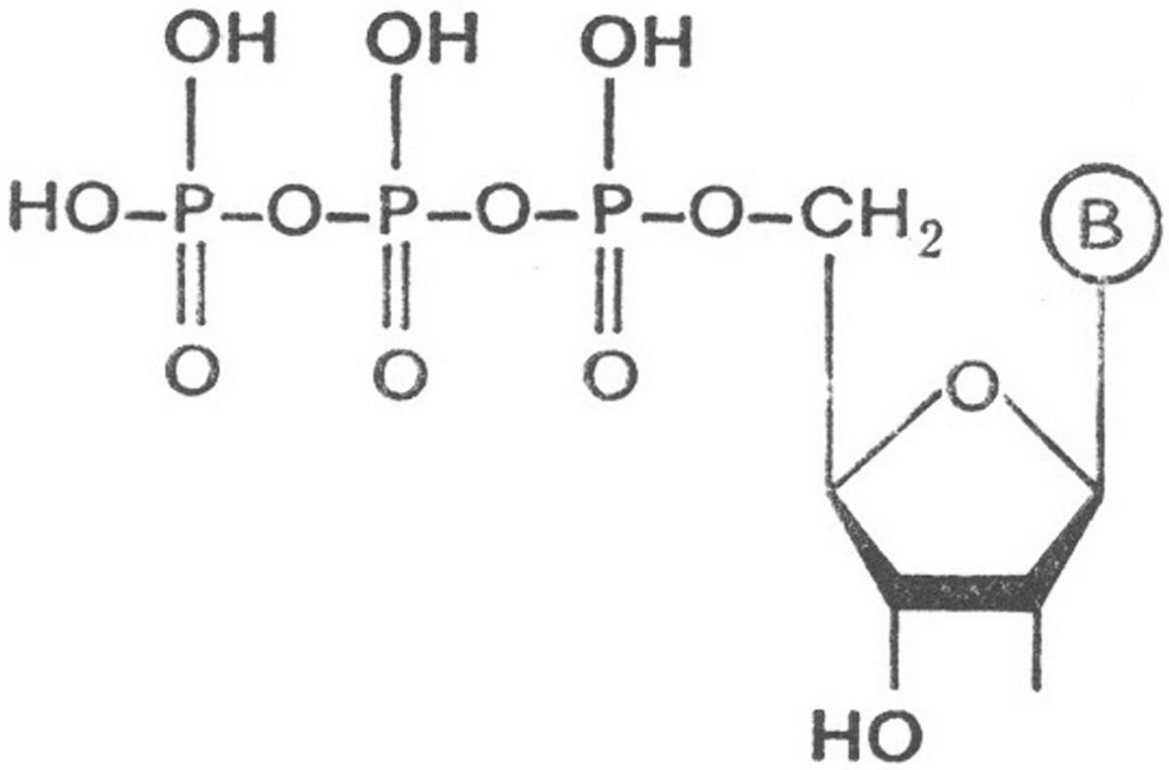


(a)



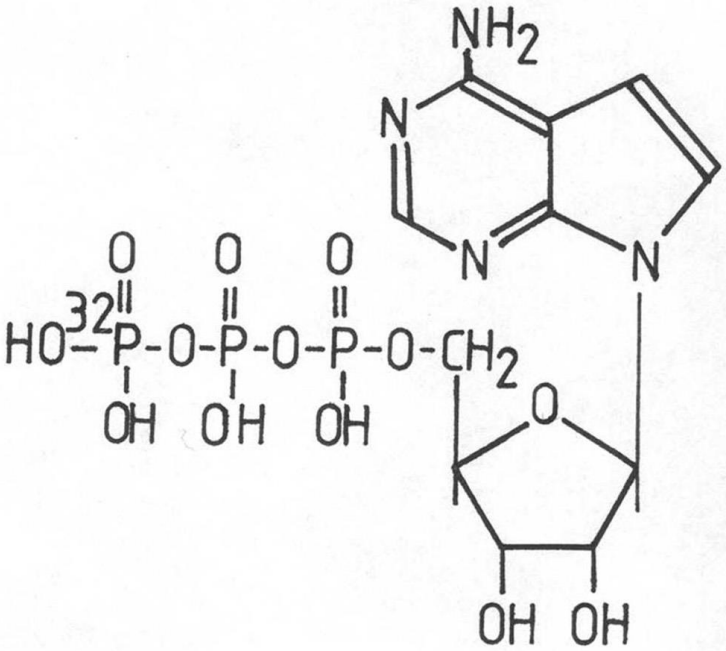
(b)



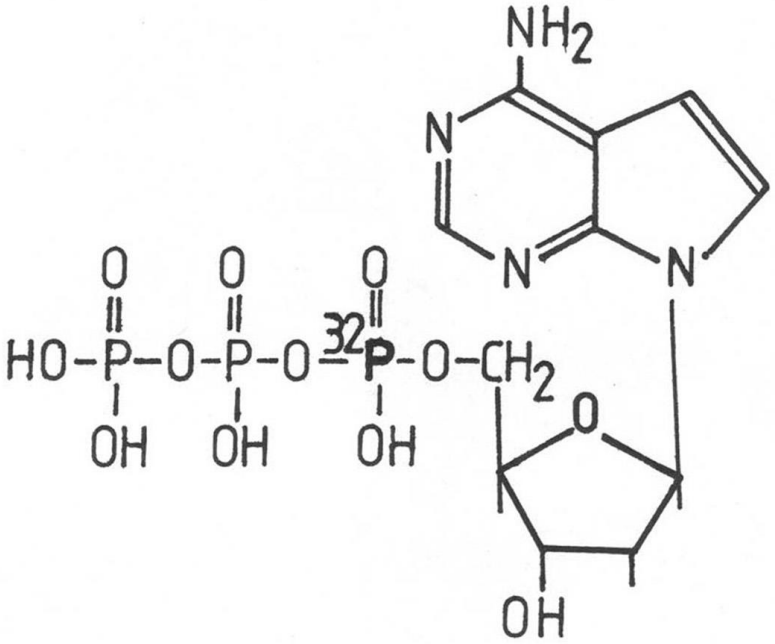


2'-deoxynucleoside 5'-triphosphate

Radioaktivní fosfát v ATP/dATP

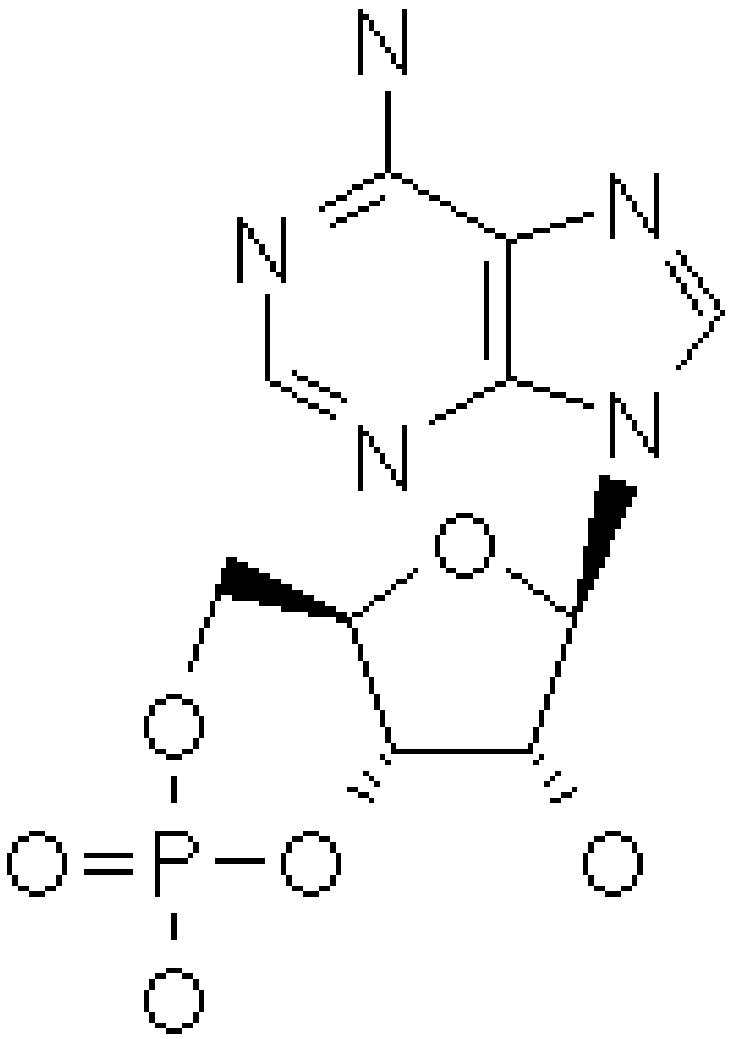


γ-^{32}P-ATP



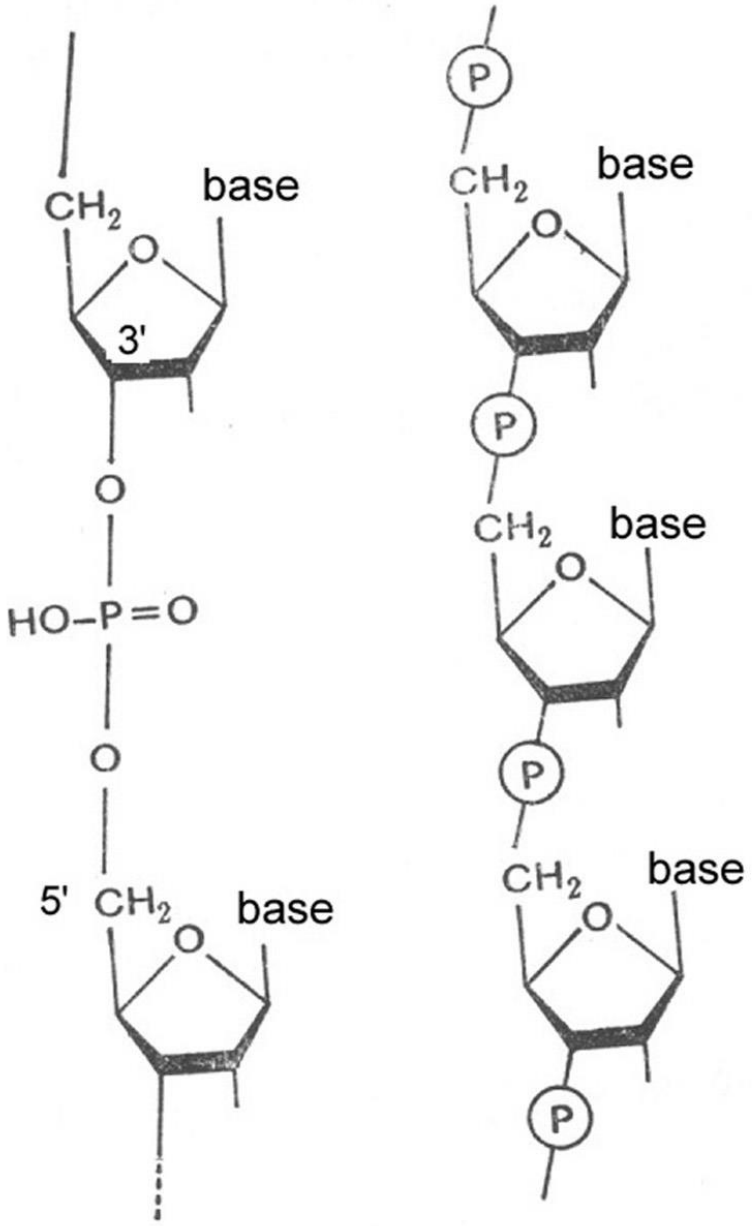
α-^{32}P-dATP

Cyklický 3´,5´AMP



C00575

Vlákno DNA: fosfodiesterové vazby



Typy NK:
DNA (jaderná a mitochondriální), rRNA, mRNA, tRNA, malé molekuly RNA s různou funkcí

TRANSKRIPCE = přepis informace z DNA do RNA

DNA duplex: 5′ ... TACGACGAGCTACG ... 3′
3′ ... ATGCTGCTCGATGC ... 5′

RNA: 5′ ... UACGACGAGCUACG ... 3′

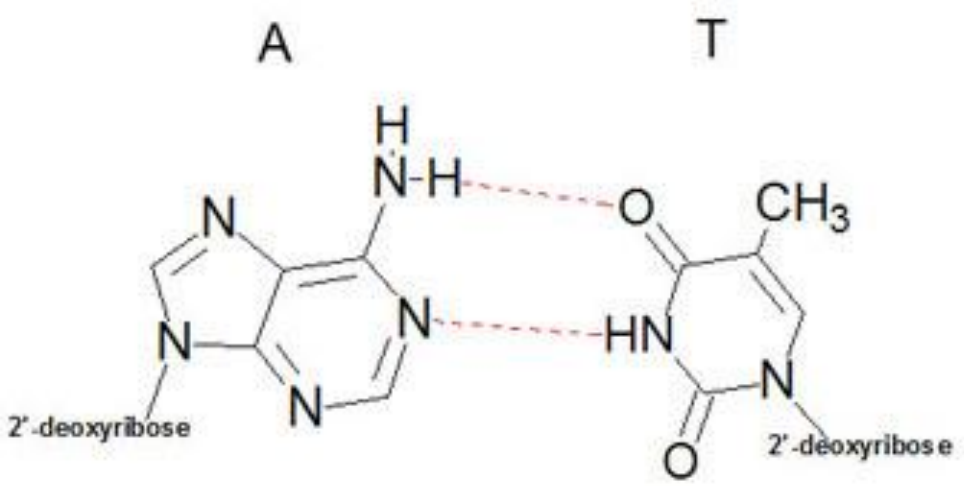
(reverzní transkripce: RNA > DNA, např. mRNA > cDNA)

DNA	RNAs
STRUKTURA:	
2´-deoxyribosa	**ribosa**
- thymin	**- uracil**
- dvoušroubovice+vyšší struktury v jádře	**- jeden řetězec, sekundární struktura**
FUNKCE:	
- uchovávání genetické informace	**- úloha v expresi genetické informace**
Základní procesy kterých se účastní:	
- replikace, transkripce (ssDNA as template)	**- transkripce, translace**
Localizace v buňce:	
- jádro, (mitochondrie)	**- jádro,cytoplasma (mitochondria)**
Tvorba hybridů:	
DNA x DNA	
DNA x RNA	
RNA x RNA	

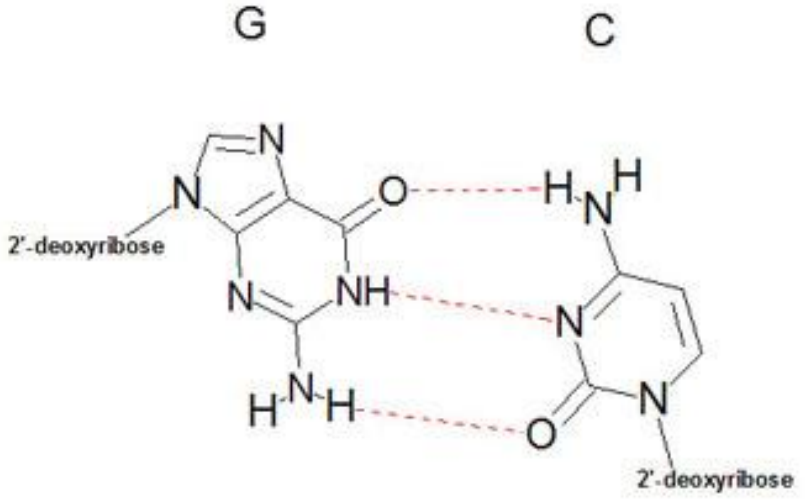
DNA, chromatin, histony

DNA je v jádře organizována v chromatinu.

Párování bazí

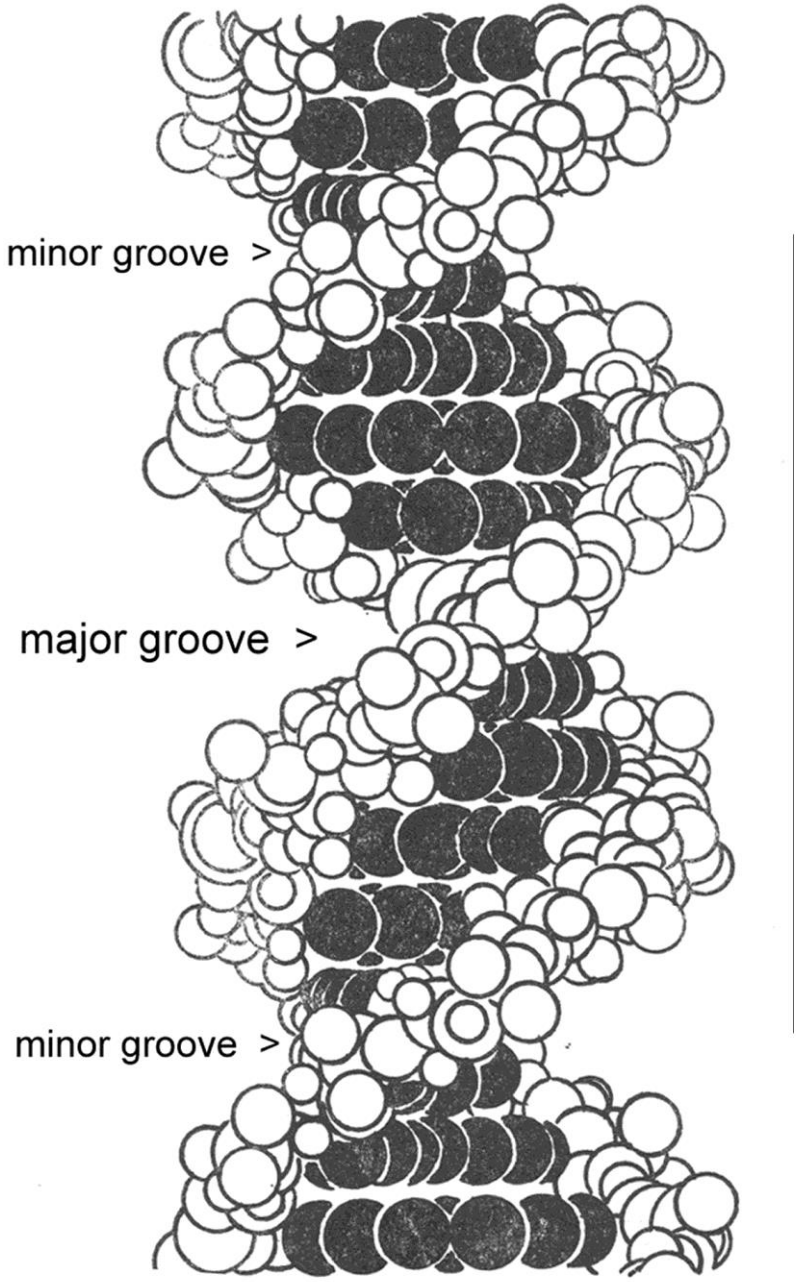


A-T

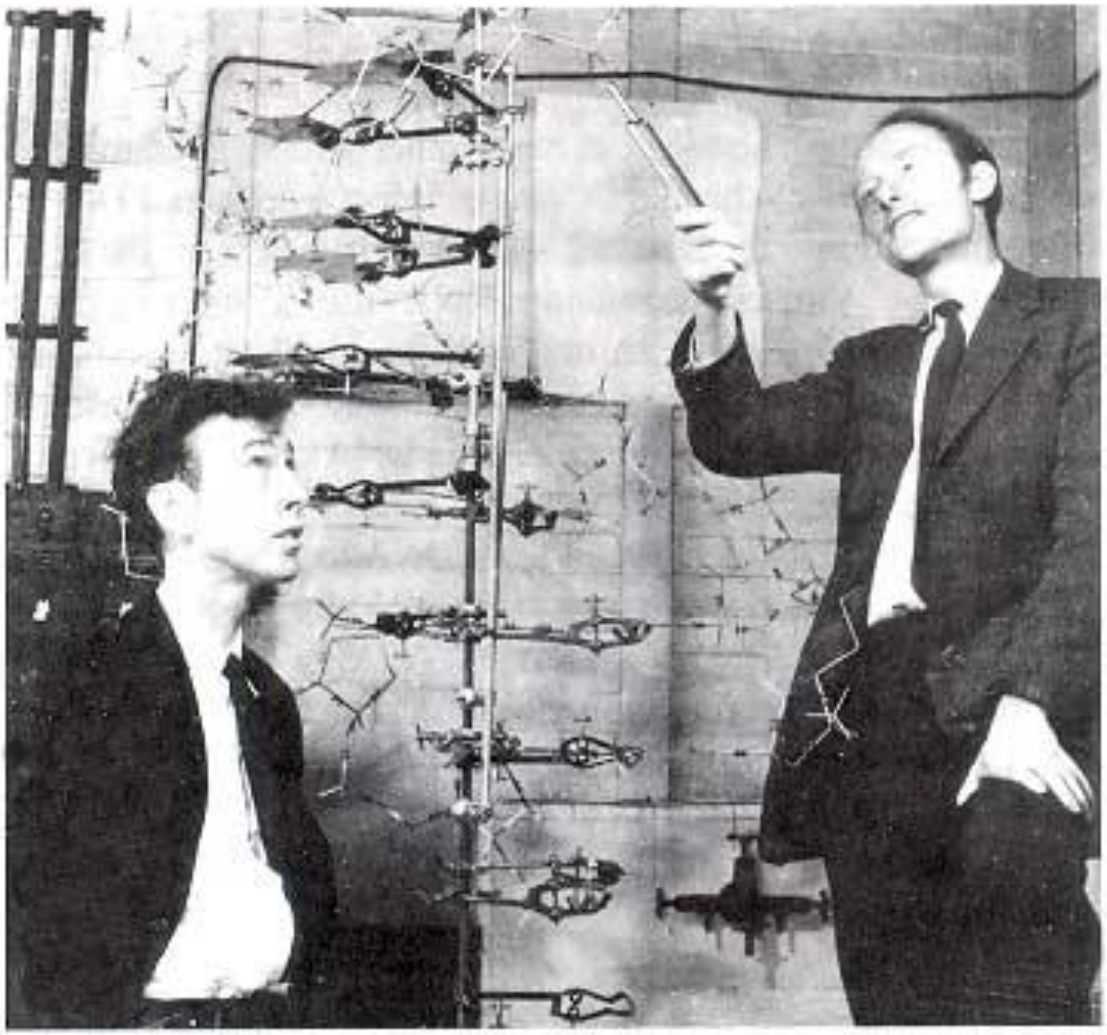


G-C

Double helix of DNA



~ 10 base pairs
(3,5 nm)



Watson and Crick.

In 1953 Watson and Crick deduced the structure of DNA. James Watson (peering up at their homemade model of the DNA molecule) was a young American postdoctoral student, and Francis Crick (pointing) was an English scientist.

Konformace dvoušroubovice DNA:

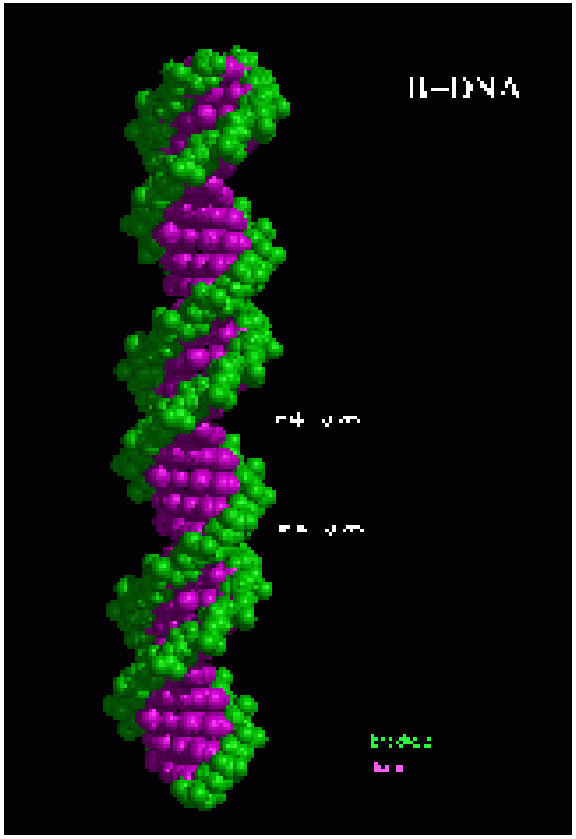
B-DNA: pravotočivý helix, DNA v chromatinu, při nižších koncentracích solí

A-DNA: pravotočivý helix, při vyšších konc. solí nebo v přítomnosti alkoholu

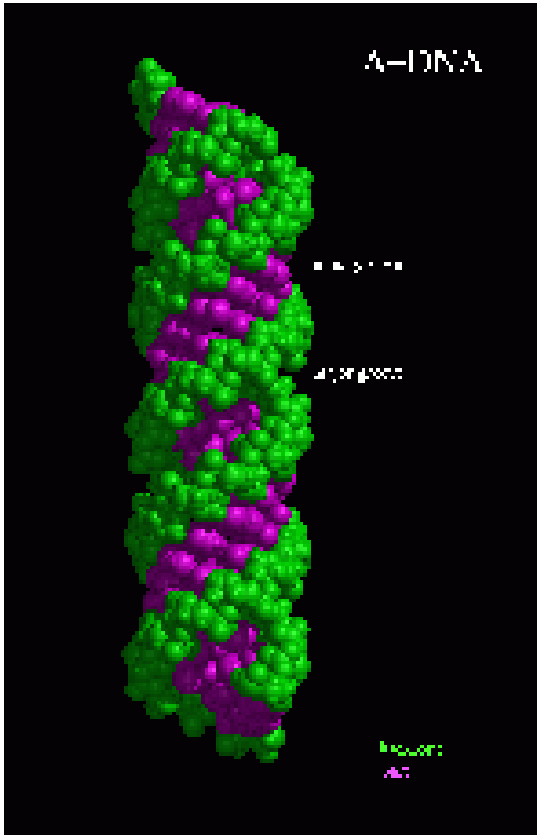
Z-DNA: levotočivý helix GC oblastí, při vyšších konc. solí nebo v přítomnosti alkoholu

RNA je jednovláknová, (pokud je lokálně dvoušroubovicová, konformace je podobná A-formě DNA

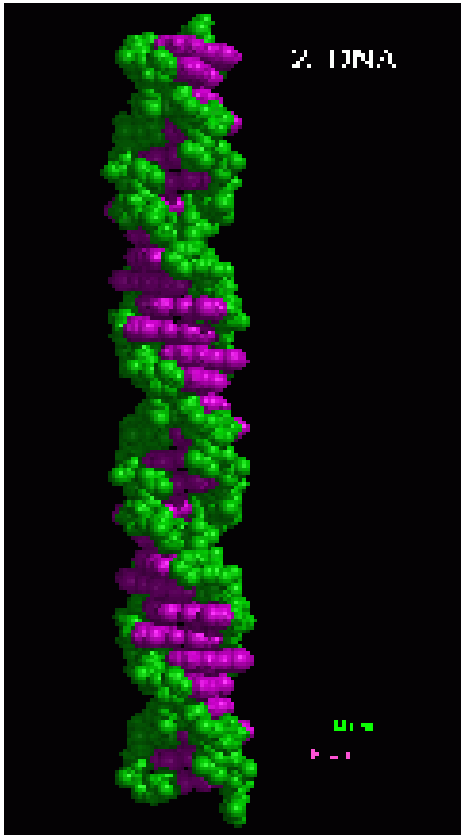
B-DNA



A-DNA



Z-DNA



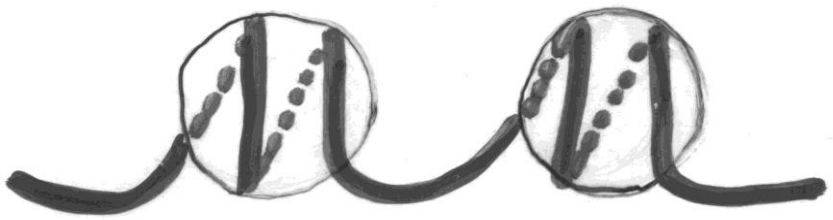
Uspořádání DNA v chromatinu:

Nukleosom > chromatinové vlákno > kondensovaný chromosom.

Chromatin je dynamická struktura, hraje aktivní roli při transkripci.

Euchromatin, heterochromatin.

Nukleosomy

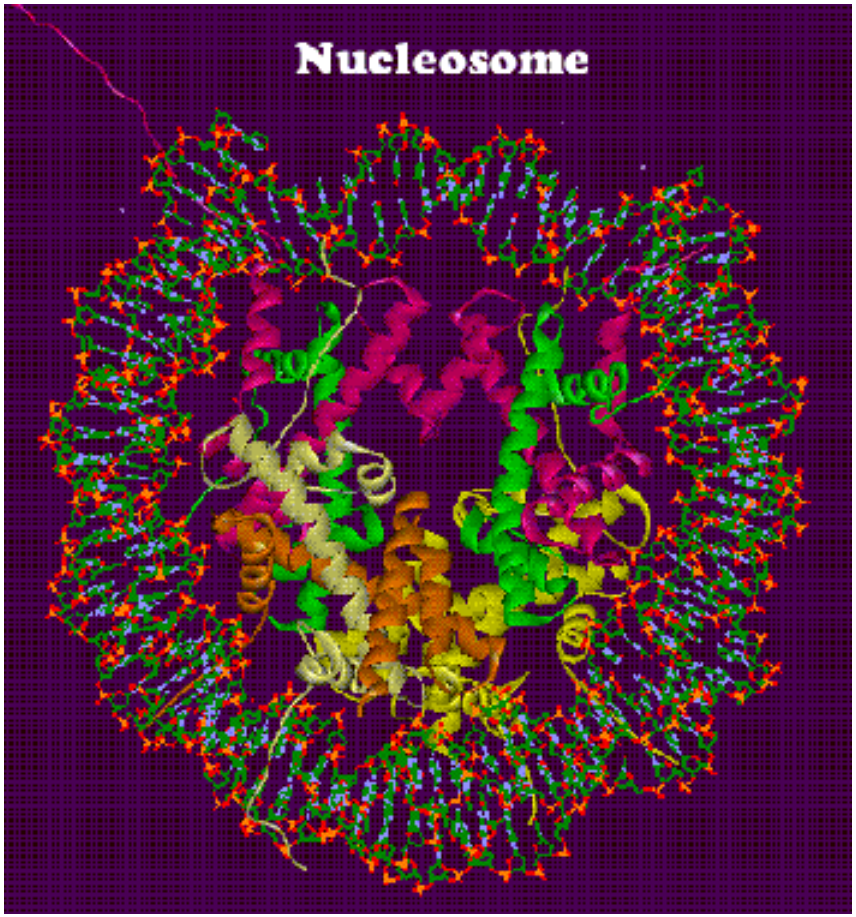


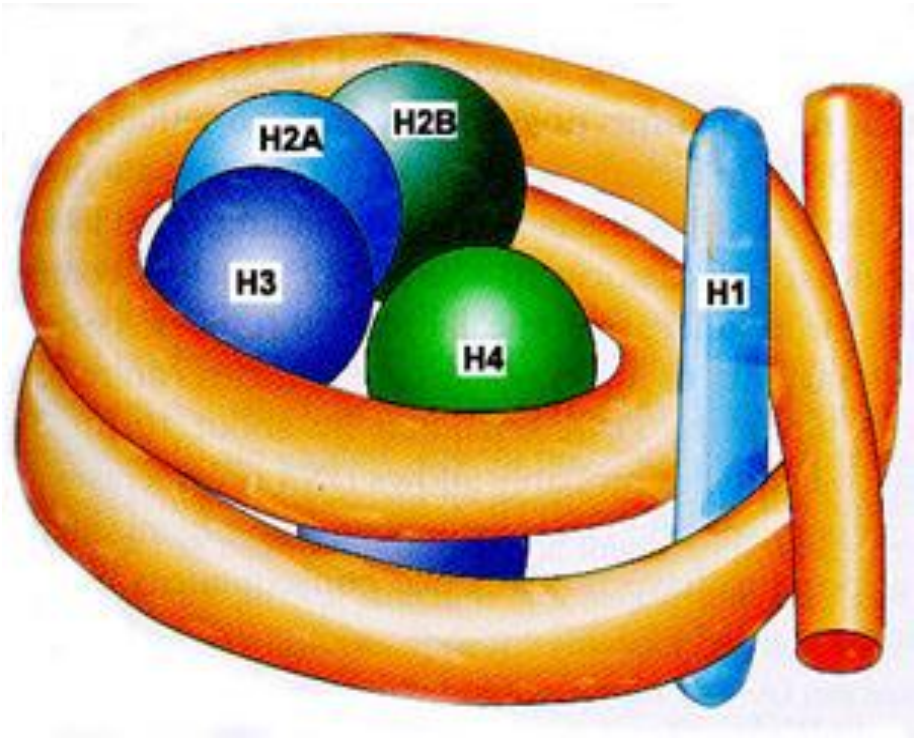
Histony: 2A, 2B, 3 a 4 (celkem 8 molekul histonů)

DNA: 146 bp., necelé 2 otáčky kolem histonového jádra + cca 40 bp. spacer

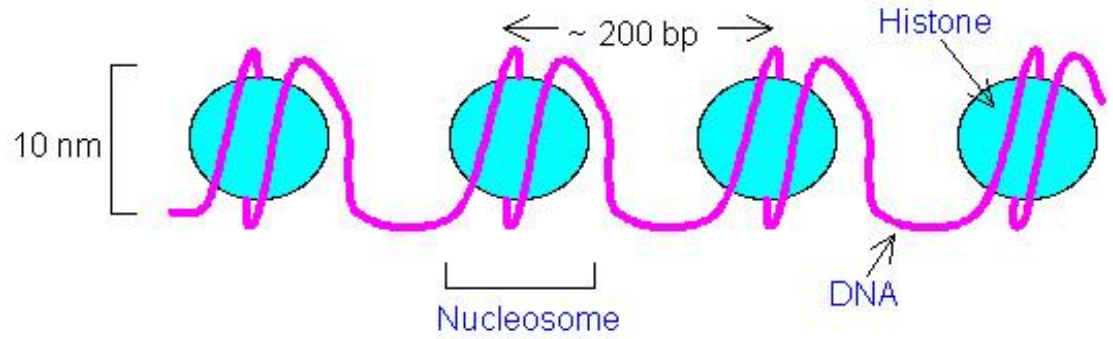
změny ve struktuře histonů: acetylace/deacetylace histonů, "remodelace" multiproteinovými komplexy - význam při transkripci

Nukleosom

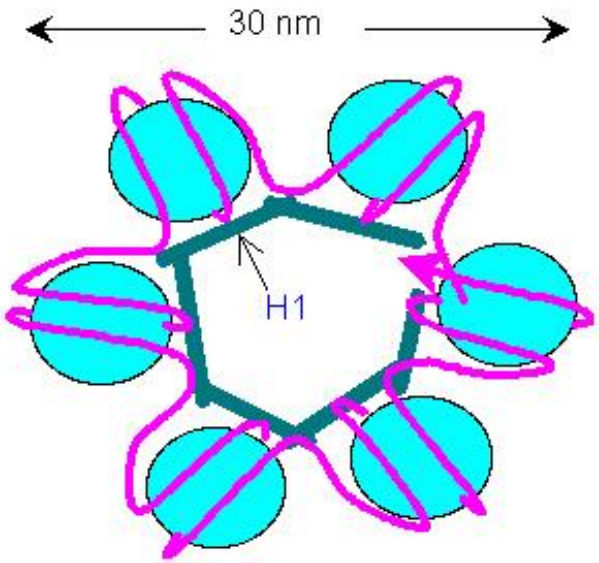




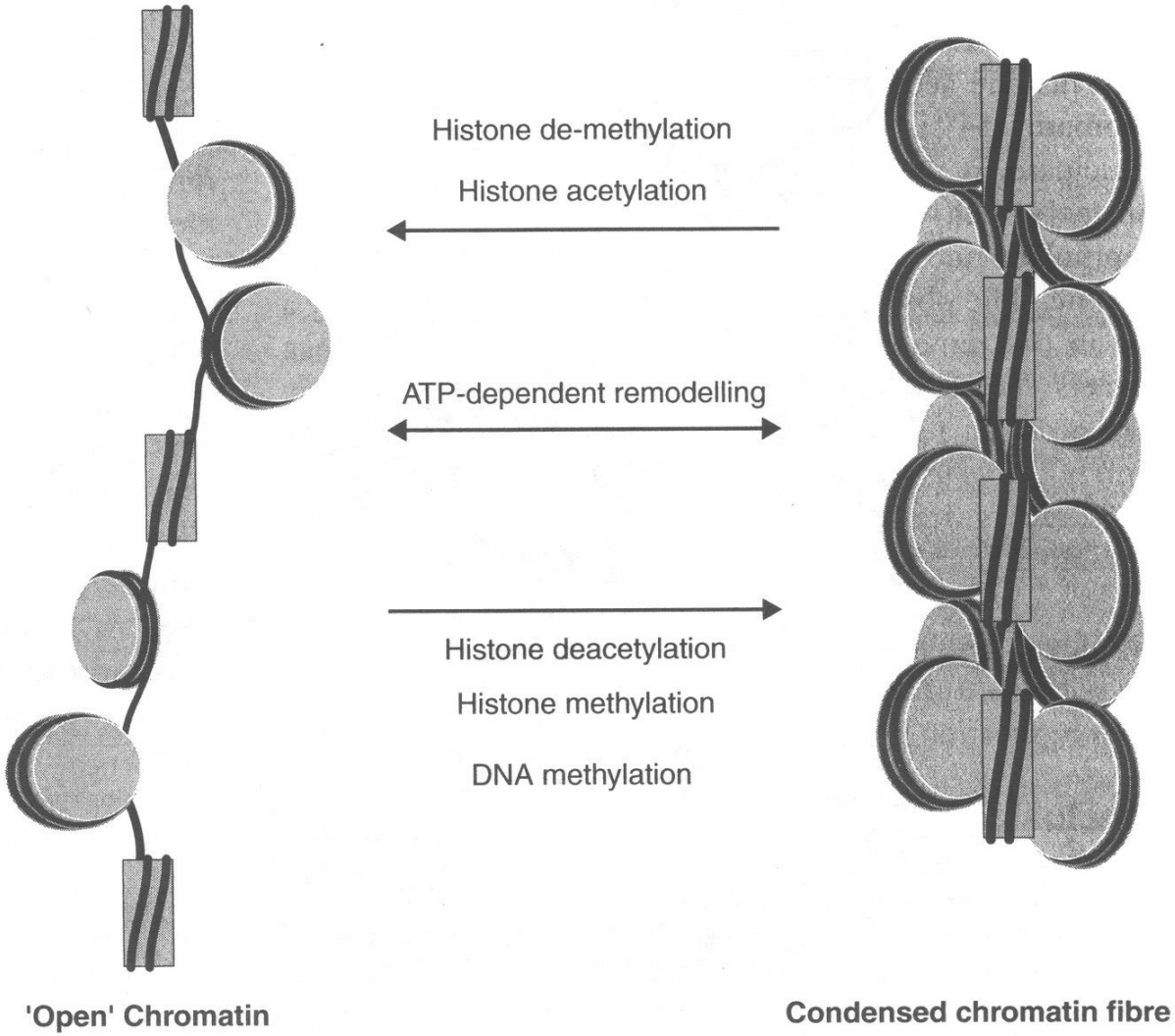
(a) Beads-on-a-String Conformation



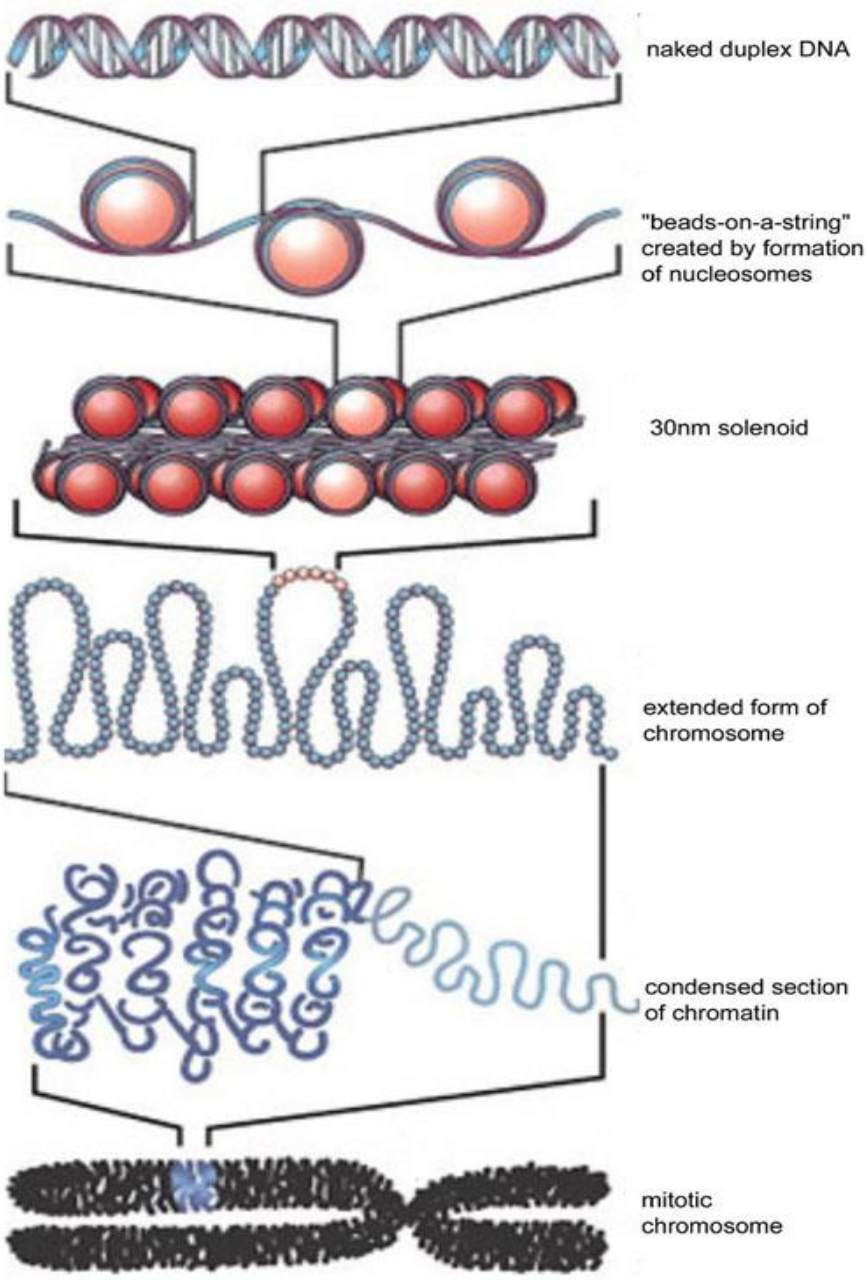
(b) One Turn of Solenoid



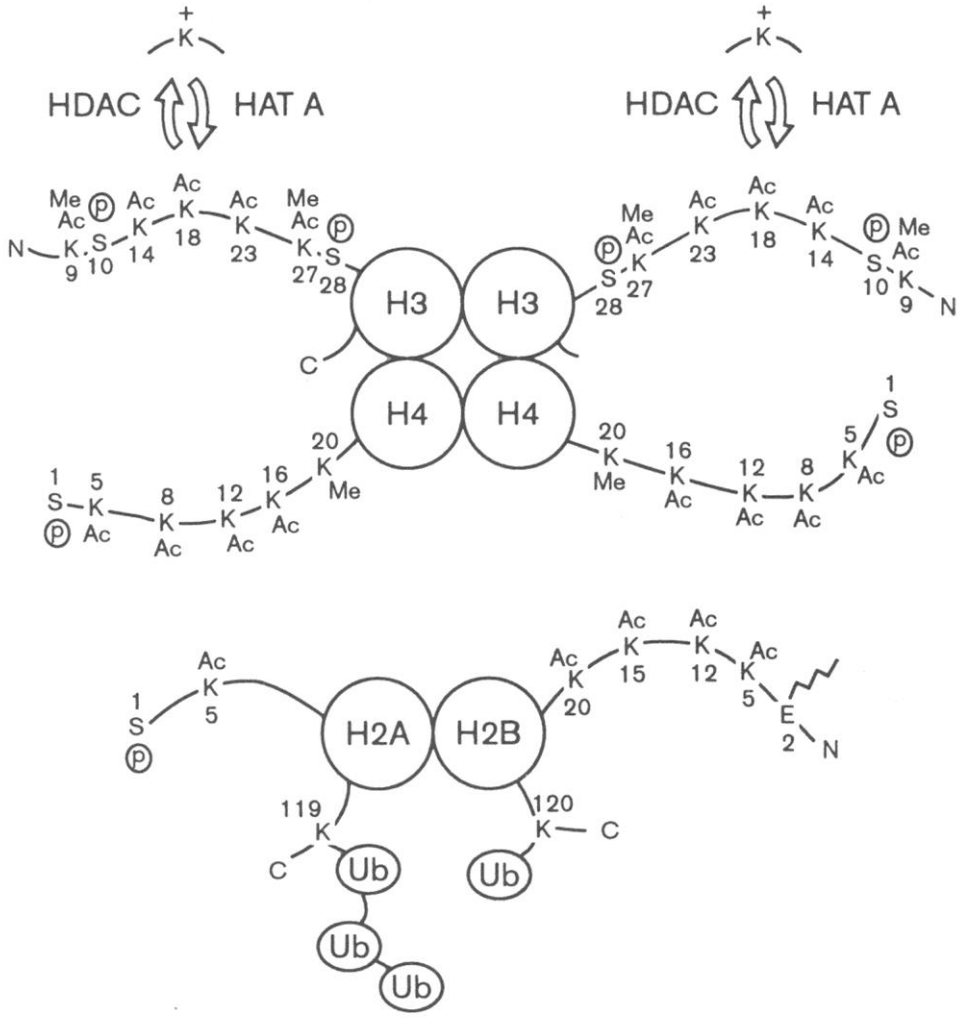
DNA v jádře je organizována v nukleosomy a vyšší struktury (nukleosomové řady a chromatinová vlákna)



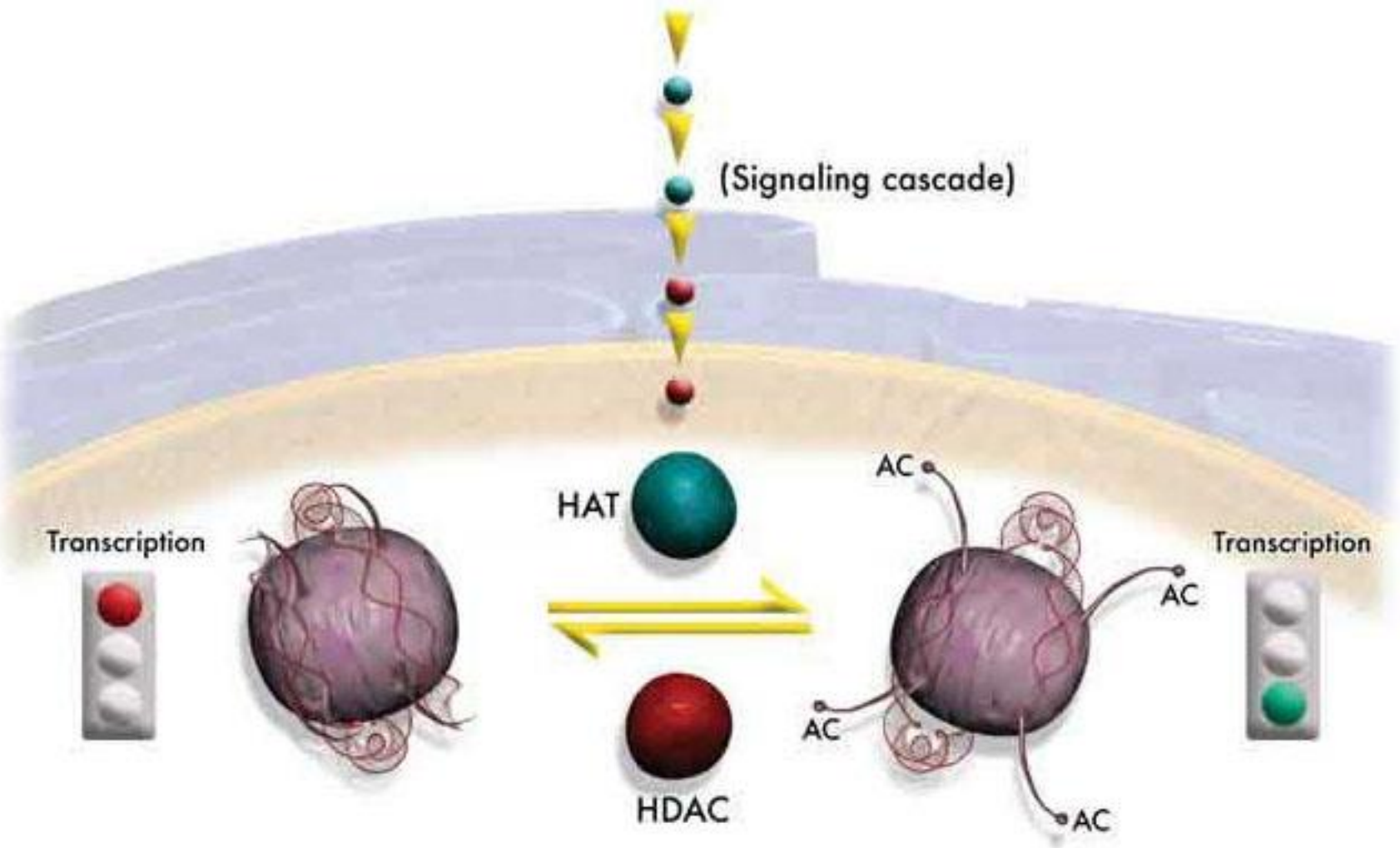
DNA v jádře je organizována v nukleosomy a vyšší struktury (nukleosomové řady a chromatinová vlákna)



Acetylace a deacetylace histonů



HAT = histon acetyltransferasa HDAC = histone deacetylasa

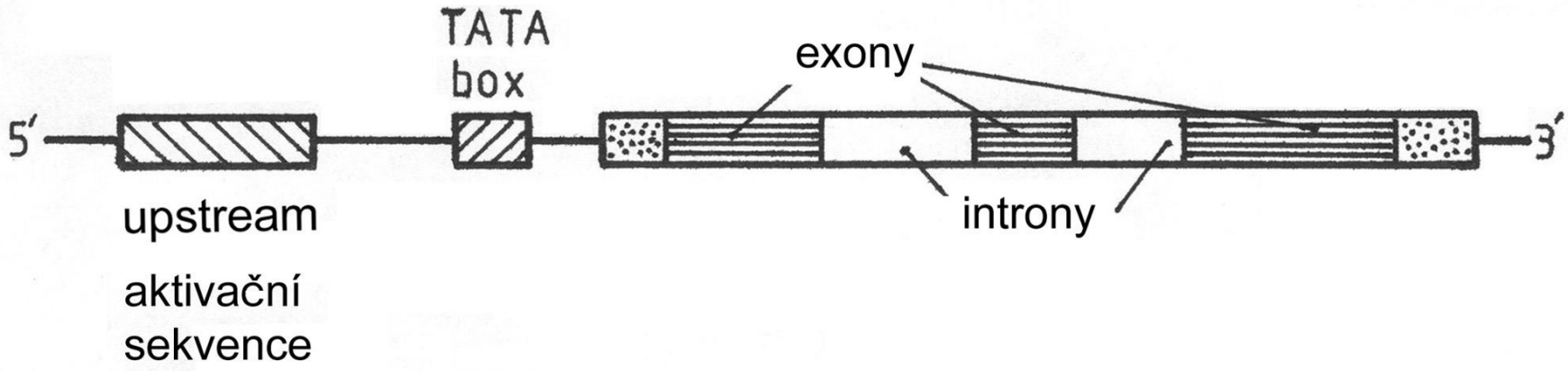


Signaling to Chromatin

Genomová DNA:

Gény,
Satelitní DNA

Repetitivní DNA – LINEs, SINEs, ...



Typický eukaryotický gen

Lidská genomová DNA (~ 3,3 x 10^9 nukleotidů)

Gény:

- protein-kódující DNA *
  - solitární gény (včetně intronů) do cca 15%
  - duplikované gény, génové rodiny do cca 15%
- tandemové gény kódující rRNA, tRNA, snRNA a histony (20-300 tandemových kopií) 0,3 %

* Sekvence skutečně kódující proteiny (exony) jsou jen cca max. 1,5%.

„Mimogénová“ DNA (=repetitivní sekvence a blíže necharakterizovaná DNA).

Lidská genomová DNA (~ 3,3 x 10^9 nukleotidů)

Repetitivní sekvence:

- **Satelitní DNA** 3% genomu

a) opakování 14-500 nt. o délce 20-100 kb (častá v oblasti centromér)
- minisatelity (15-100 nt v délce 1-5 kd), její rozdíly slouží k identifikaci jedince (DNA fingerprinting).

b) mikrosatelity (1-13 nukleotidů, nejčastěji jen 1-4 nt, o délce cca 150 nt)
- telomerová DNA (cca 15 kb) – opakující se sekvence GGGTTA

- **Mobilní DNA** (= transposony, „roztroušená DNA, sobecká DNA“) celkem cca 45%
  - DNA transposony 3%
  - Retrotransposony 8%
    - LTR-retrotransposony
    - non-LTR-retrotransposony
      - LINEs (long interspersed elements) **L1**, L2, L3 21%
      - SINEs (short interspersed elements) (Alu sekvence) 13 %

Blíže necharakterizovaná DNA cca 25%

Genom retroviru:

2 identické molekuly RNA (každá 35S),
„cap“ na 3´-konci, polyA na 5-´konci

sekvence RNA kóduje:

gag - strukturní proteiny virové kapsidy

pol - reverzní transkriptasu (+ proteasu)

env - virový obal

Typy molekul RNA

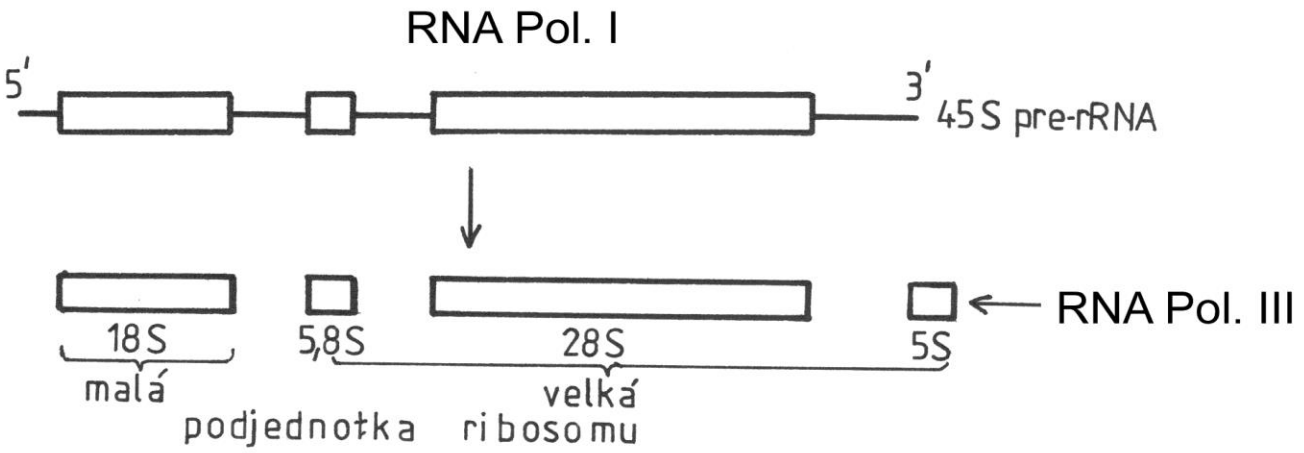
rRNA - ribozomální RNA (28S, 18S, 5,6S, 5S)

mRNA - messengerová RNA

tRNA - transferová RNA

malé jaderné RNA - účast při sestřihu mRNA, úpravách rRNA v jadérku, ...

Ribosomální RNA

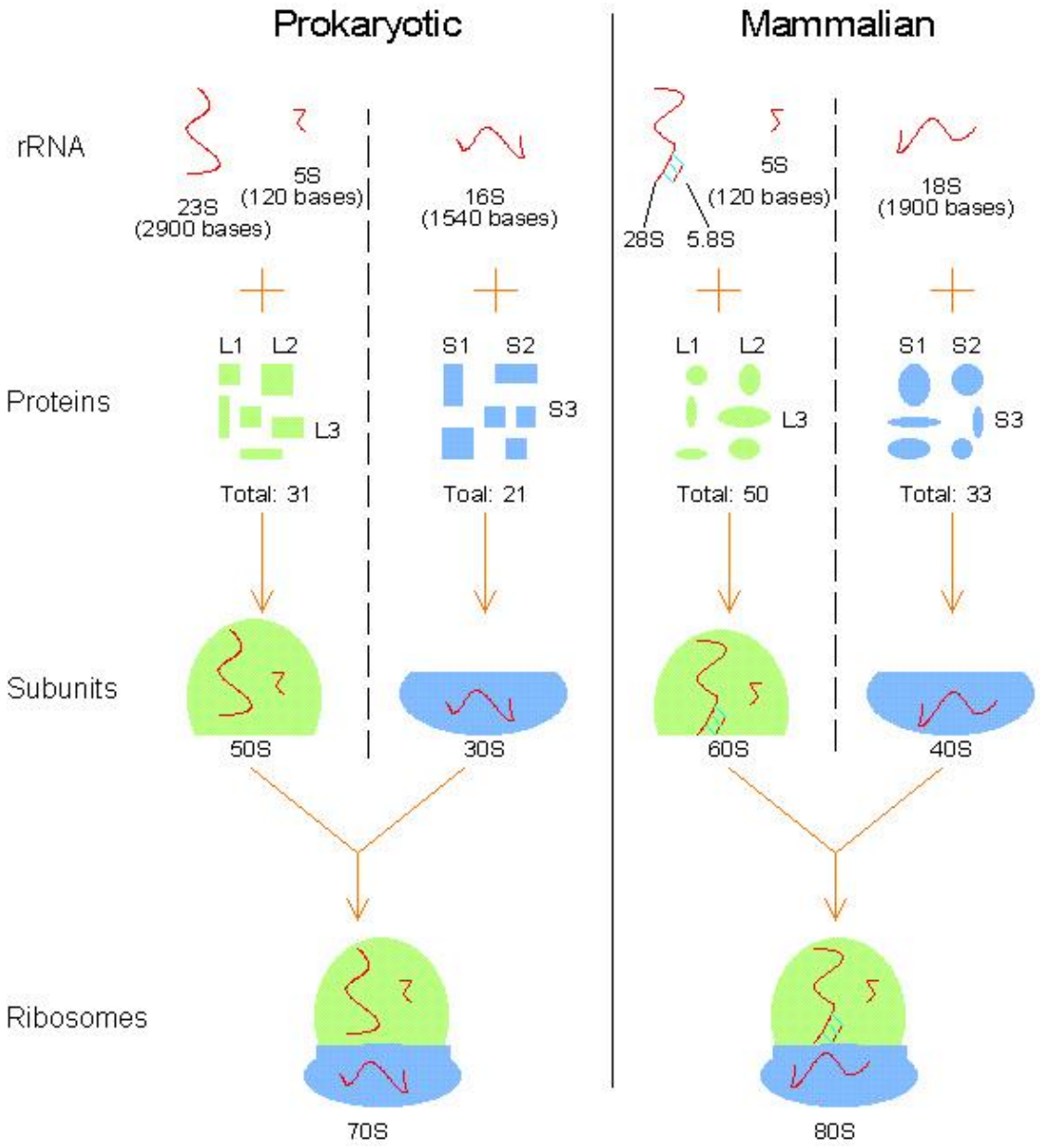


~200 kopií genu pro pre- rRNA

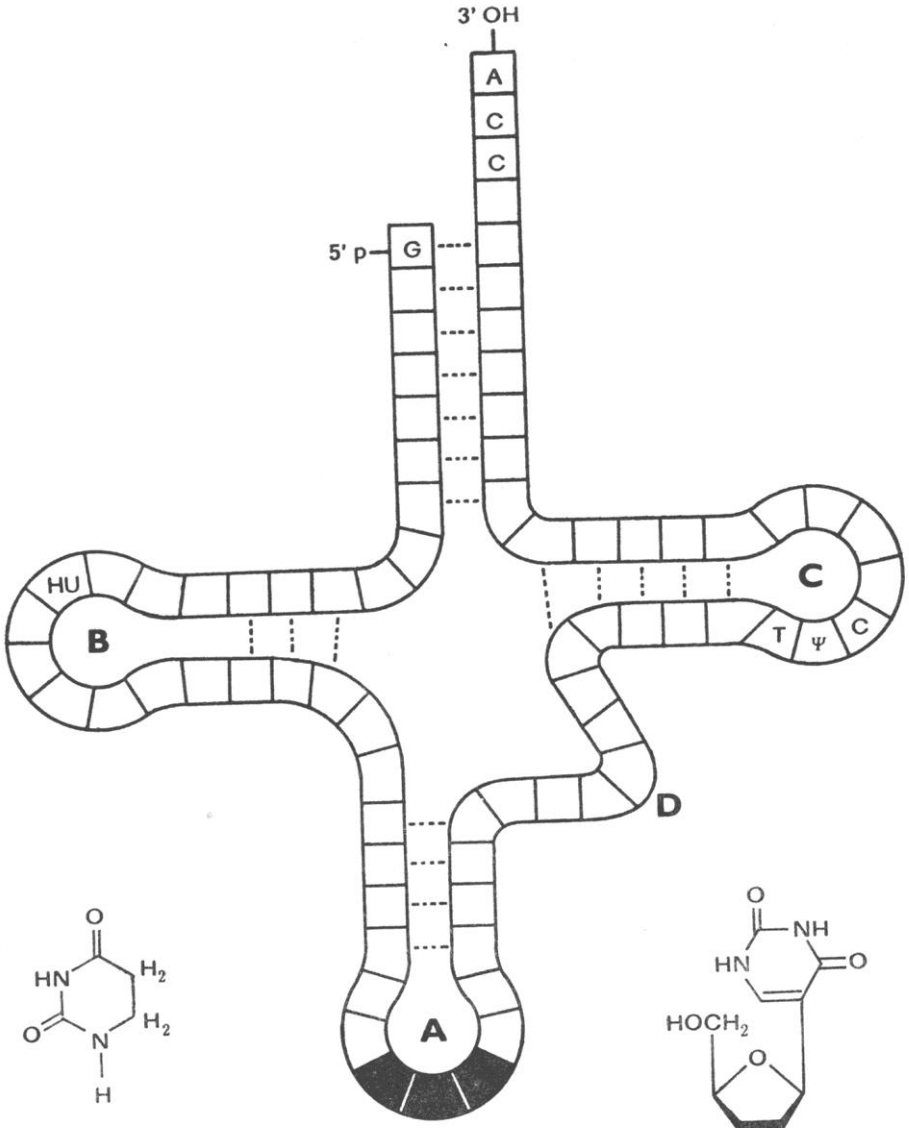
transkripce RNA pol. I., vyštěpení
jednotlivých rRNA a složení ribozomálních podjednotek
probíhá v nukleolu

nukleové baze v 18S, 28S a 5,8S jsou
často modifikovány pomocí snoRNP komplexů

Struktura ribosomu.



Transferová RNA má typickou sekundární strukturu

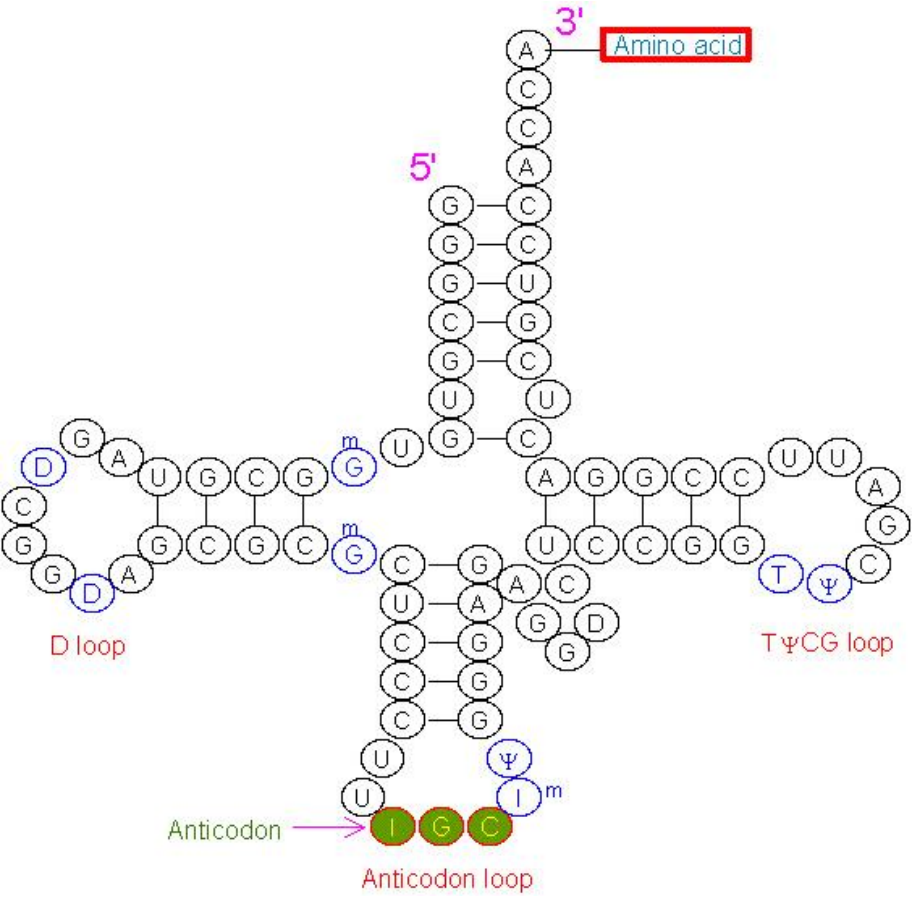


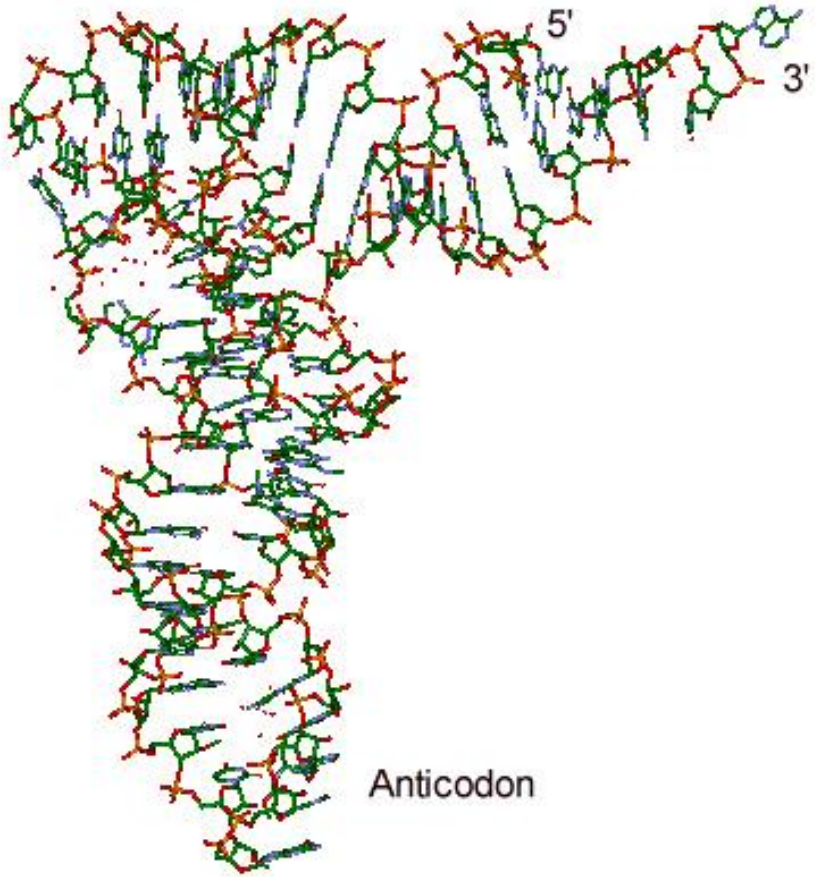
dihydrouracil

(HU)

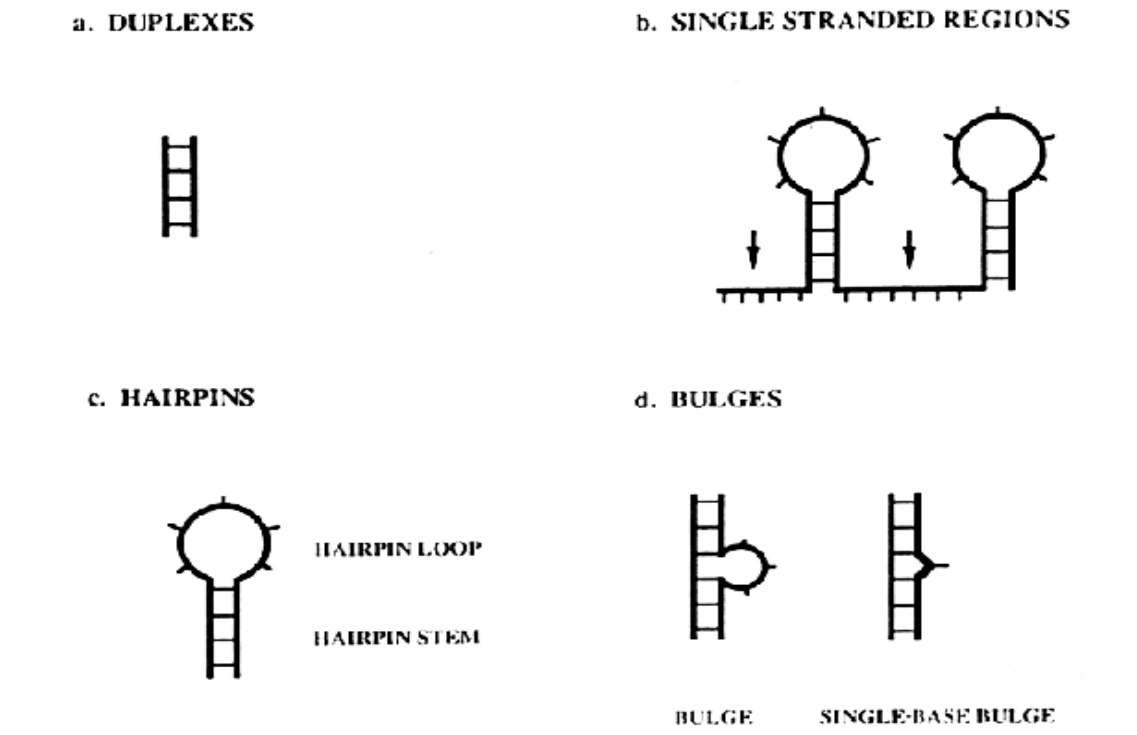
pseudouridine

Sekundární a terciární struktura transferové RNA





Sekundární struktury RNA

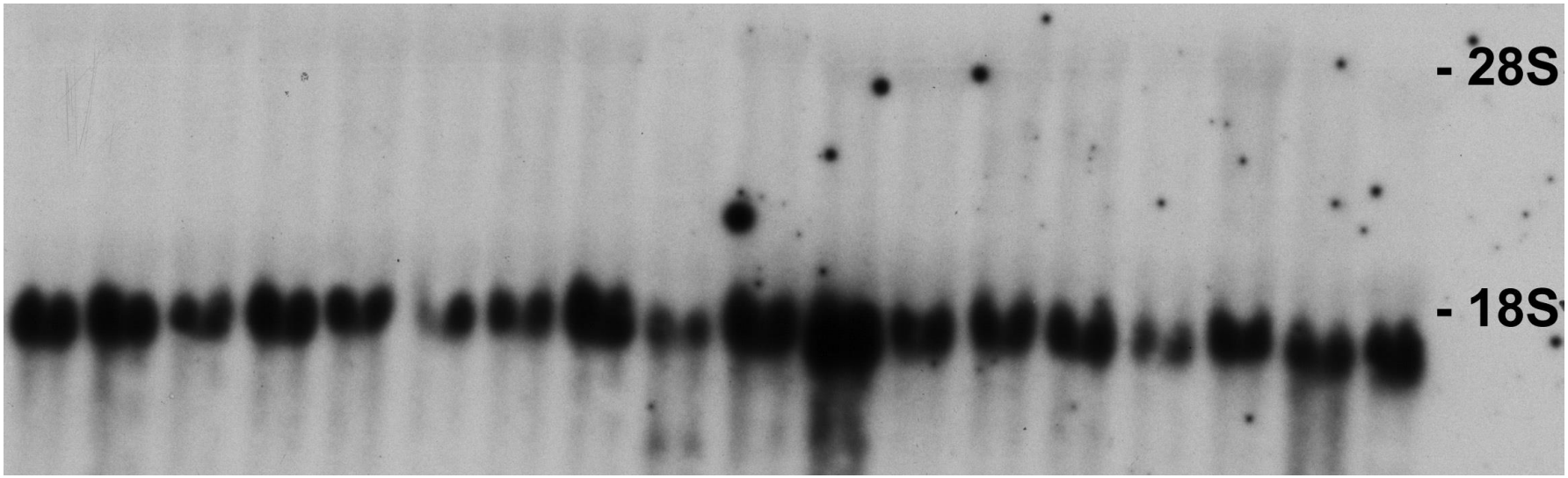


REGULACE TRANSKRIPCE

VAZBA DNA-PROTEIN

TRANSKRIPČNÍ FAKTORY

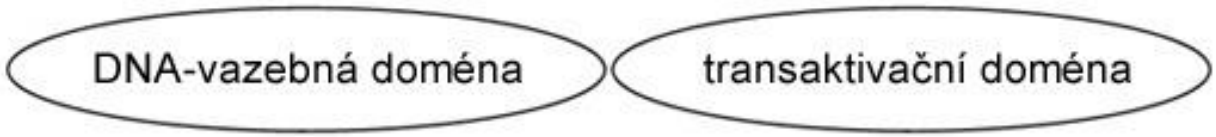
Ukázka N-blotu (GAPDH)



Transkripční faktory

MODULY:

DNA-vazebná doména Oligomerizační oblast Transaktivační doména



Obecné transkripční faktory:
(pro RNA polymerasu II)

TFIIA, B, D, E, F, H

TFIID komplex: TBP a TAFs

SPECIFICKÉ TRANSKRIPČNÍ FAKTORY

KOAKTIVÁTORY, KOREPRESORY

Příklady vazebných motivů pro transkripční faktory v DNA:

Sp1:	GGGCGG
CREB:	TGACGTCA
bHLH TFs:	CANNTG (E-box)
E2F:	TTCGCGC

Transkripční faktory

DNA-vazebné domény:

1. basicka doména
2. „zinc-finger“
3. „helix-turn-helix“

Dimerizační domény:

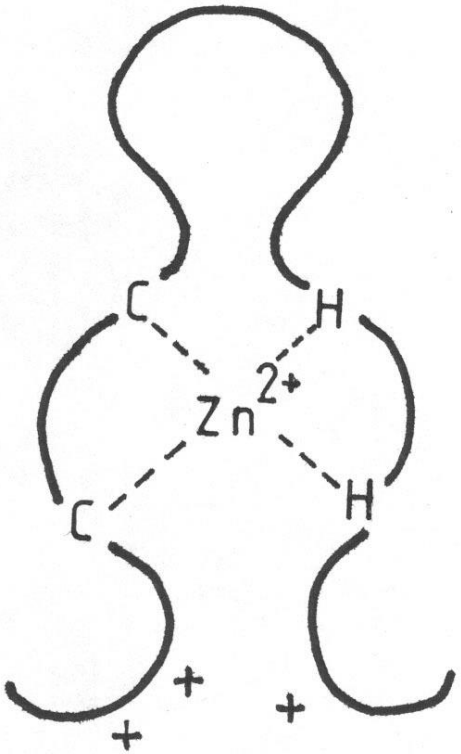
1. HLH („helix-loop-helix“)
2. LZ („leucine zipper“)

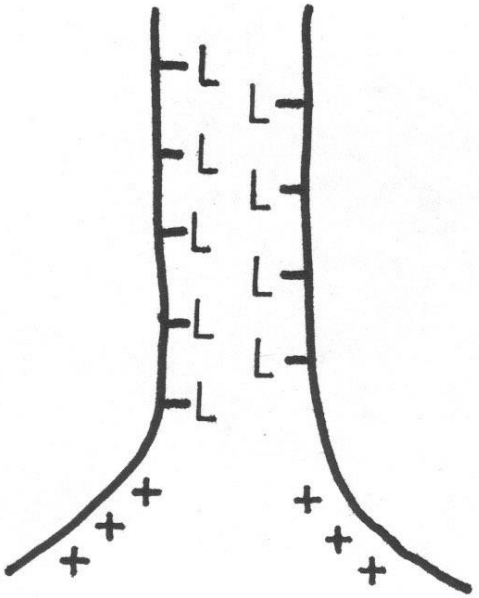
V transkripčních faktorech jsou v kombinaci, např. basic-HLH nebo basic-HLH-LZ (bHLH-LZ)

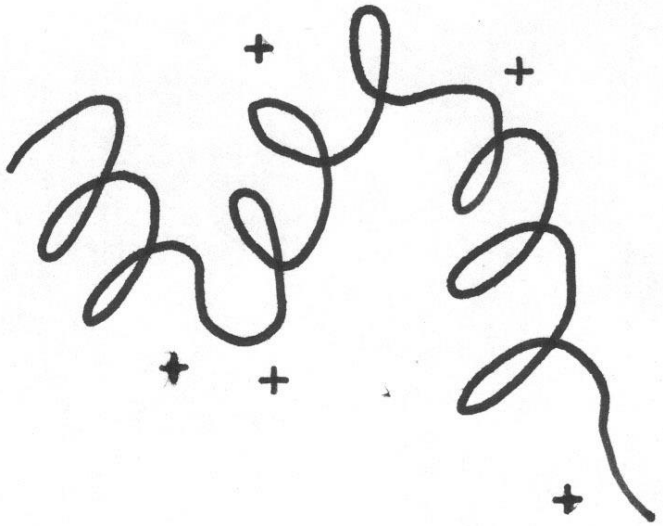
Transkripčně-aktivační domény:

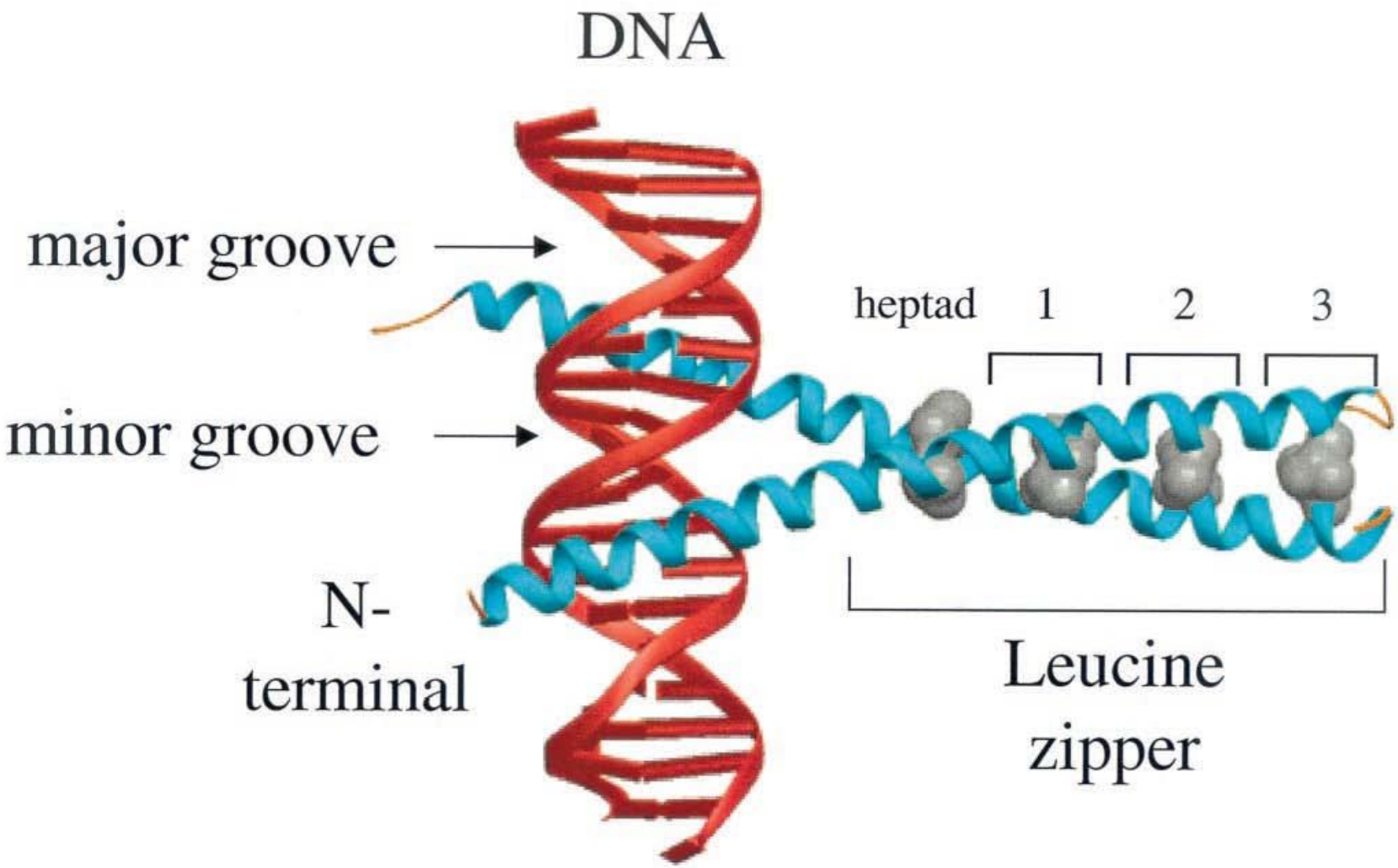
1. z kyselých aminokys. zbytků („acidic domains“)
2. bohaté na glutamin
3. bohaté na prolin

zinc-finger leucine zipper homeodoména

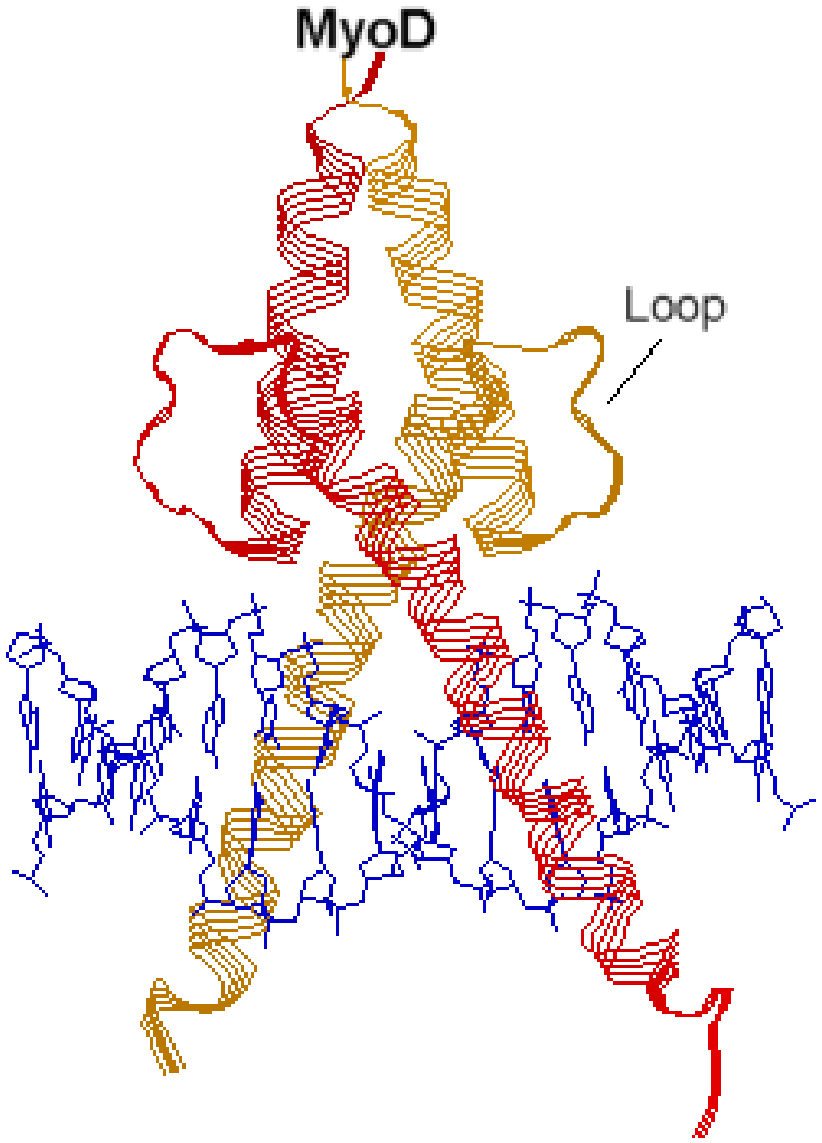







DNA
major groove
minor groove
N-
terminal
heptad
1
2
3
Leucine
zipper

Binding of transcription factor (HLH) to DNA



1. TF s basickou doménou

1.1 Leucine zipper (bZIP)

c-Jun
c-Fos
CREB

1.2 Helix-loop-helix (bHLH)

E12
E47
MyoD, Myogenin, Myf-5
ASH
NeuroD

1.3 Helix-loop-helix / leucine zipper (bHLH/ZIP)

USF
TFEB, TFEC, TFE3
c-Myc (L-myc, N-myc, B-myc)
Max, Mad, Mxi1
E2F (1-5)

2. TF s zinc-finger doménou

Receptory pro steroidní hormony (kortikoidy, progesteron, estrogen, androgeny)
Receptory pro thyroidní hormony
Receptory pro retinoidy

3. TF s Helix-turn-helix doménou

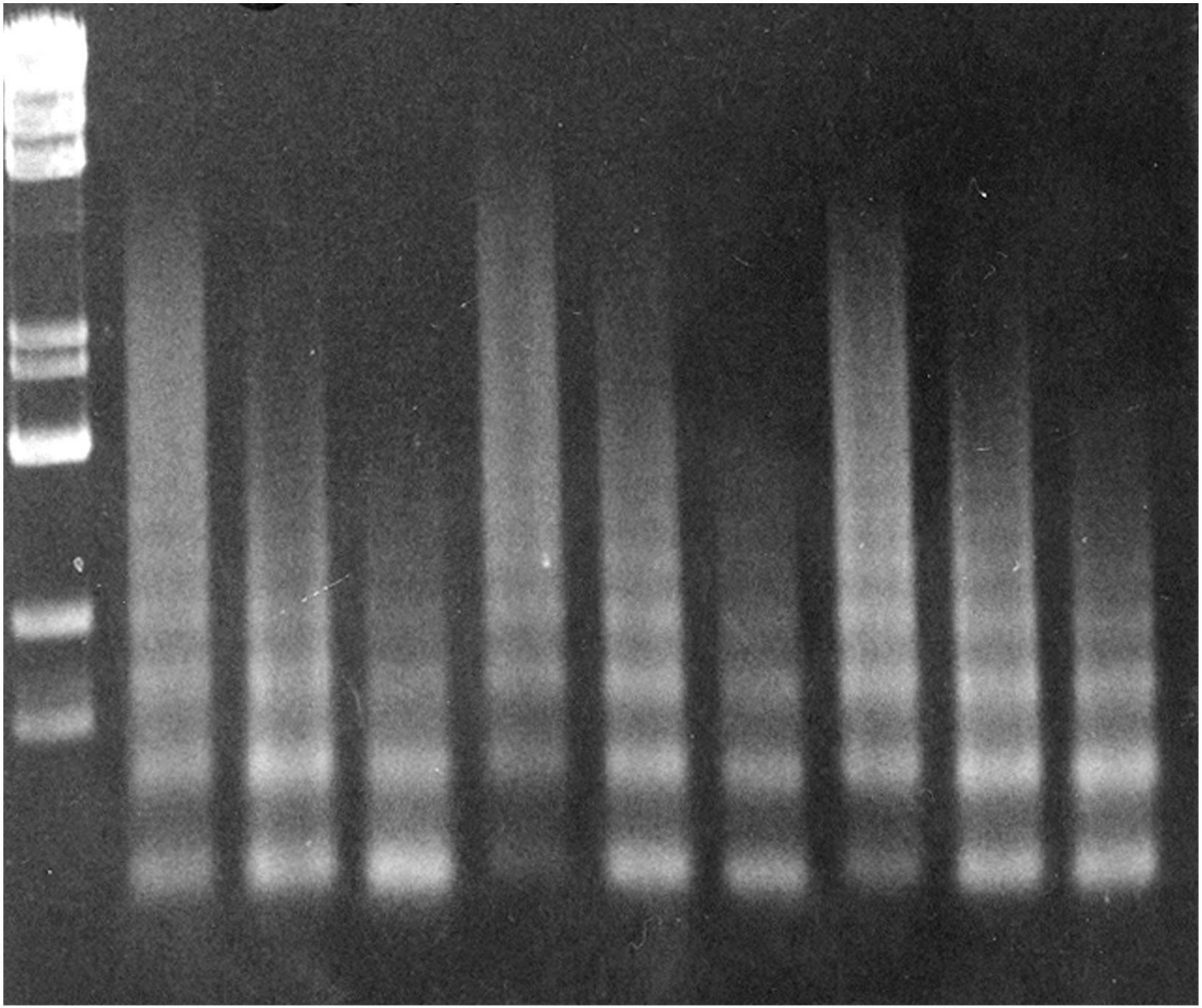
Homeo doména (Hox proteiny)
POU doména (homeo + POU-specifická doména)
Paired domain = párová doména (samotná nebo v kombinaci s homeo)

4. TF vážící se v obl. minor groove

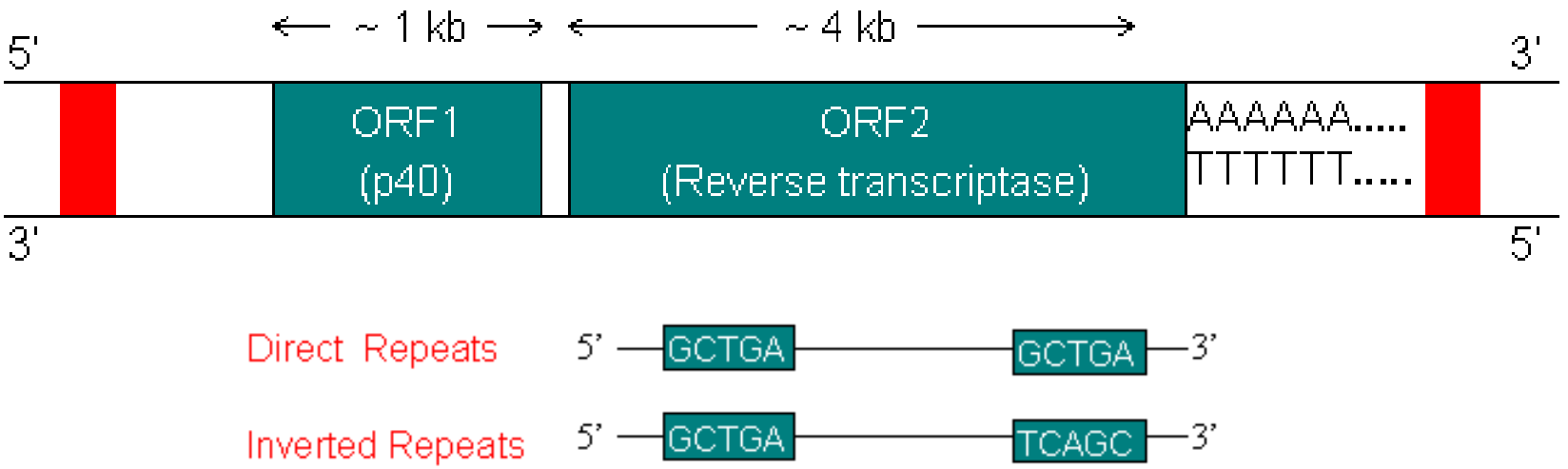
(HMG skupina, TBP)

Děkuji za pozornsot

Štěpení DNA mikrokokovou nukleasou na mono-, di, tri-,...nukleosomy



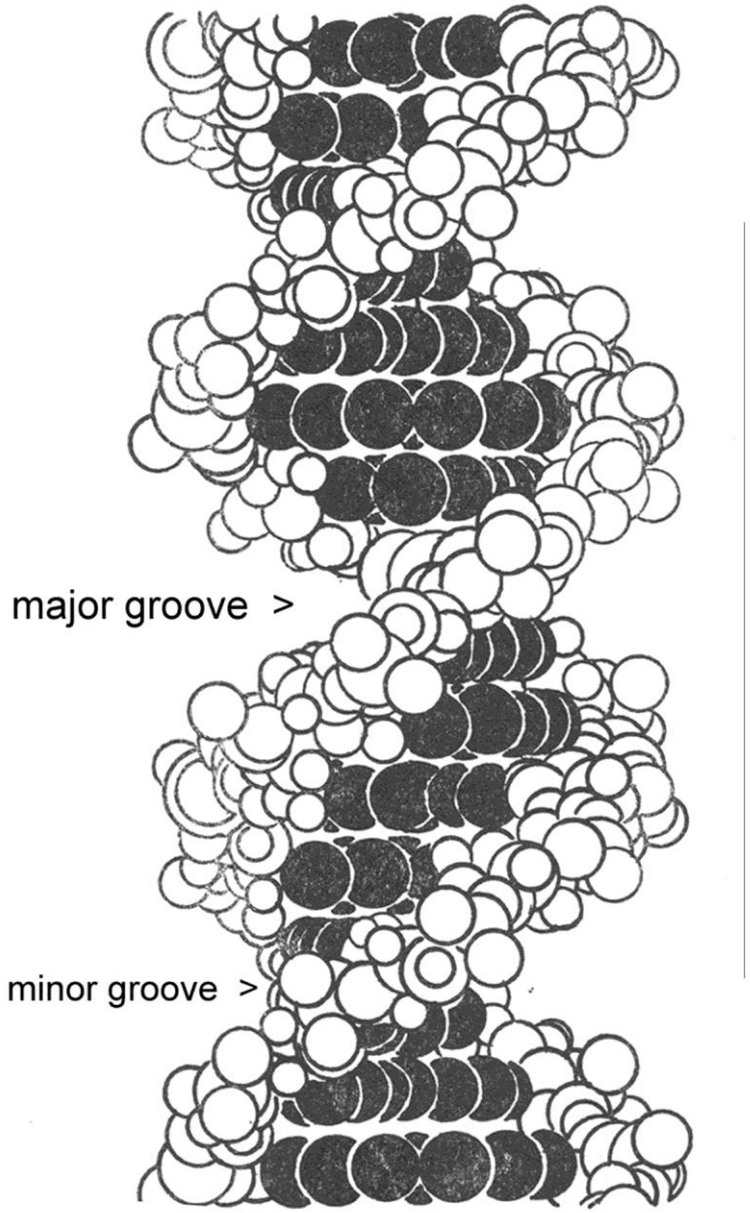
LINEs (délka 6-8 kb, cca 900 000x v genomu):

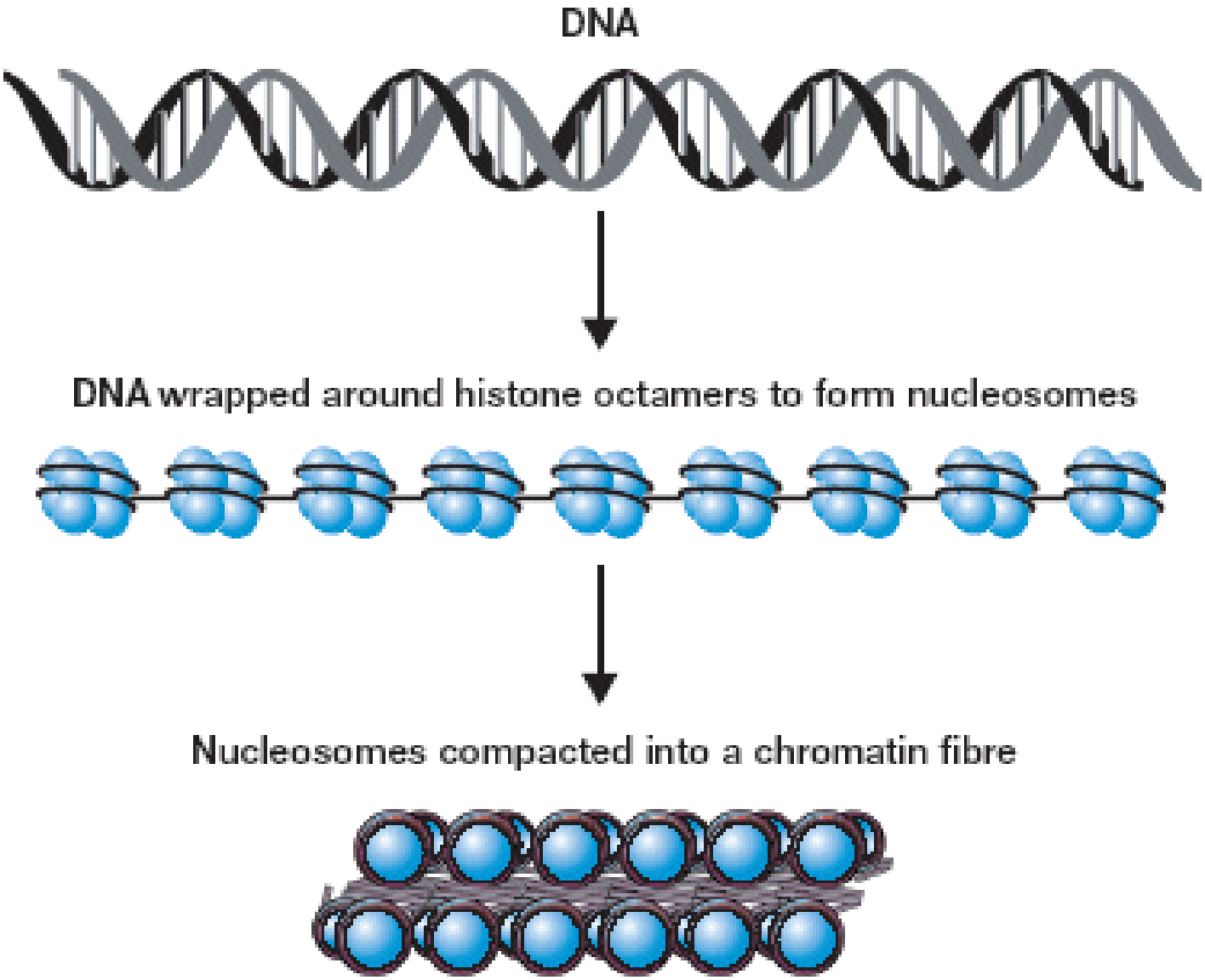


SINEs (délka cca 300 bp.)

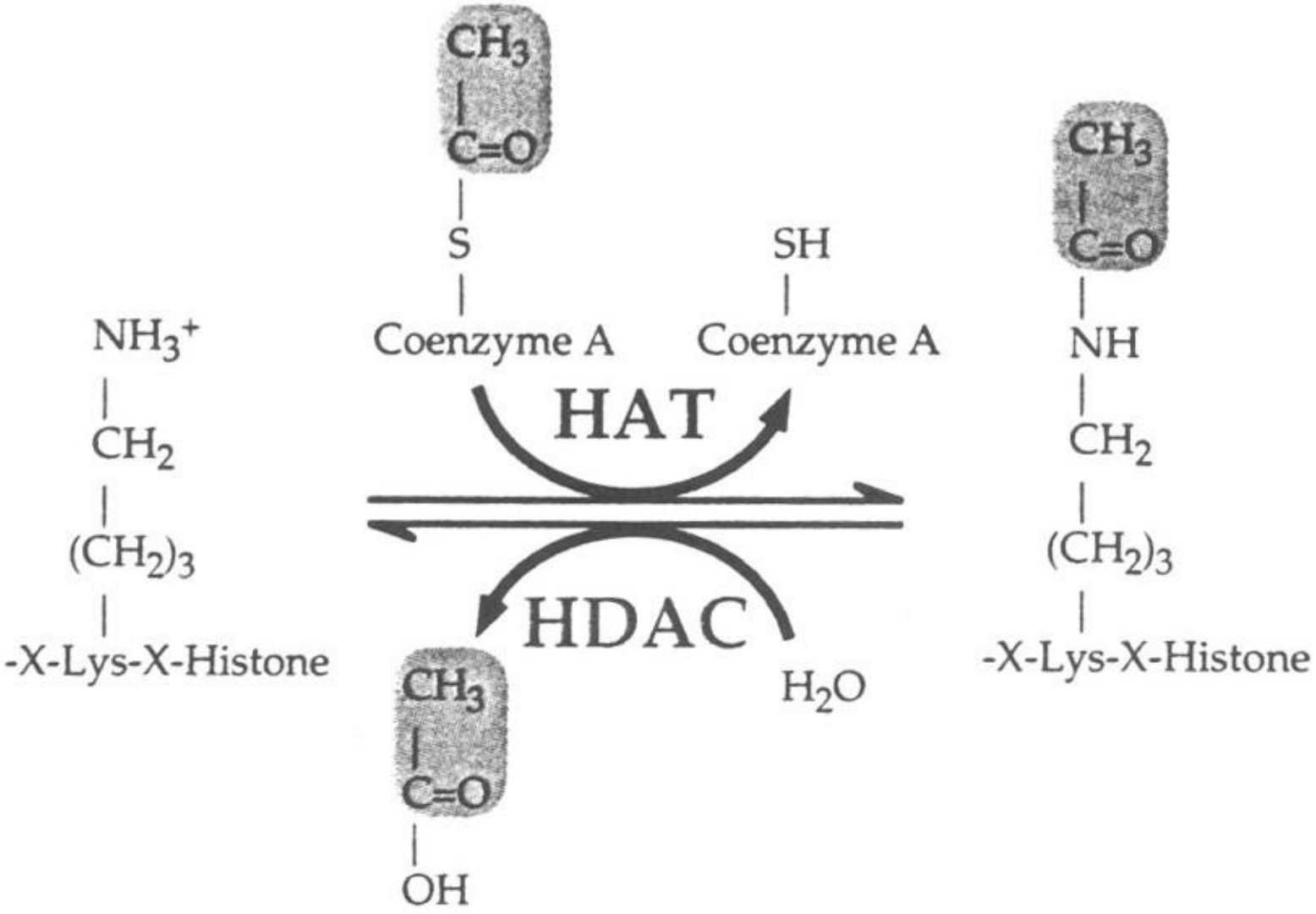
Nejčastější jsou Alu sekvence (v počtu asi 700 tis - 1 mil. v genomu)

Double helix of DNA

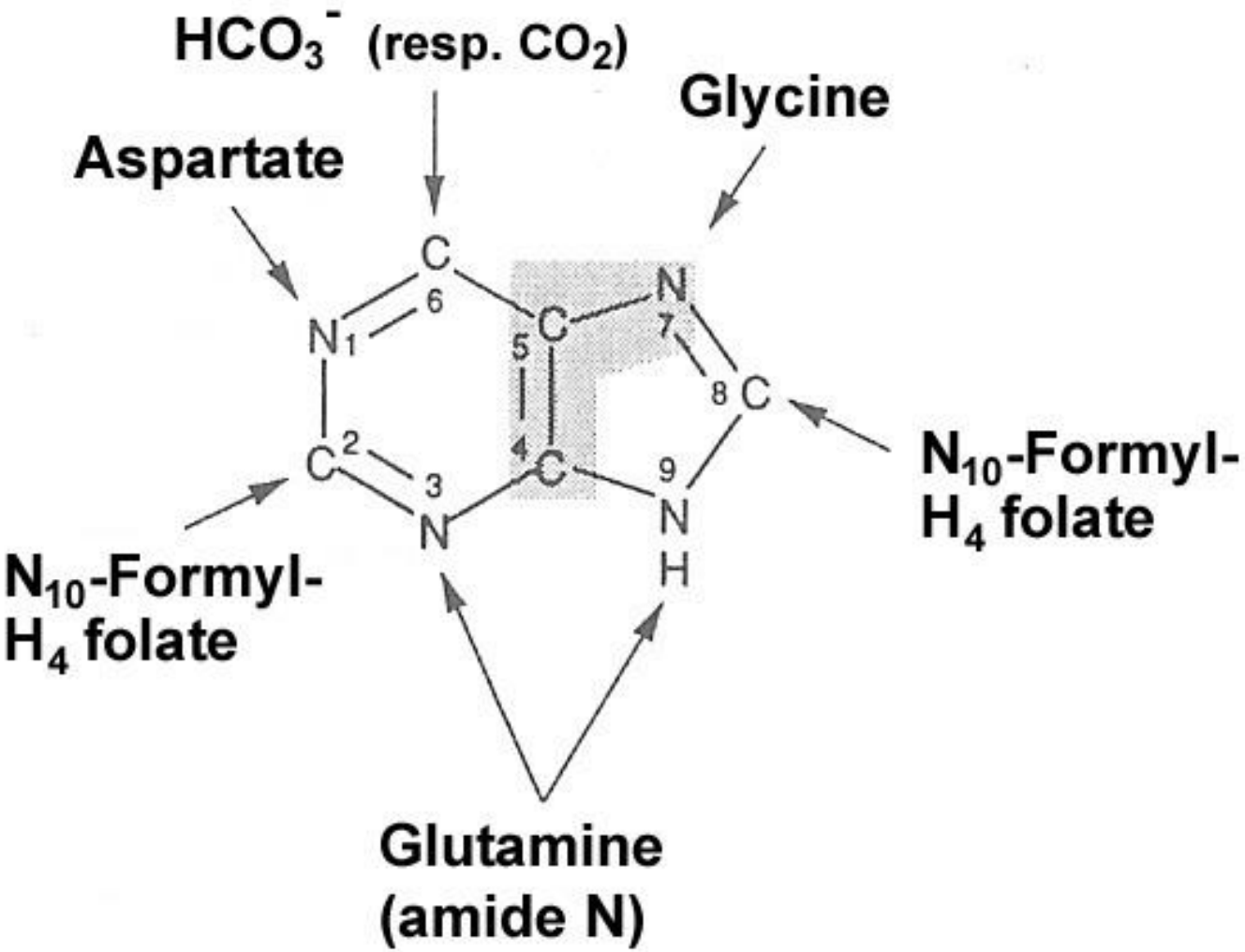



DNA
DNA wrapped around histone octamers to form nucleosomes
Nucleosomes compacted into a chromatin fibre

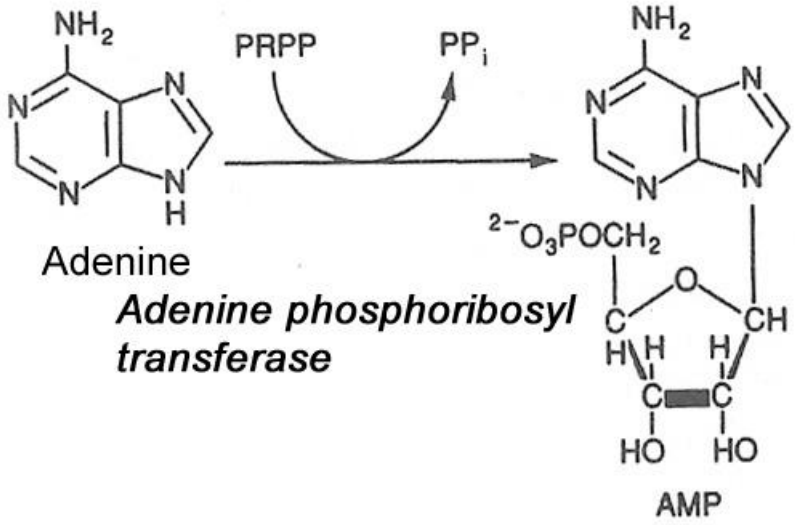
Acetylace a deacetylace lysinového zbytku v histonech

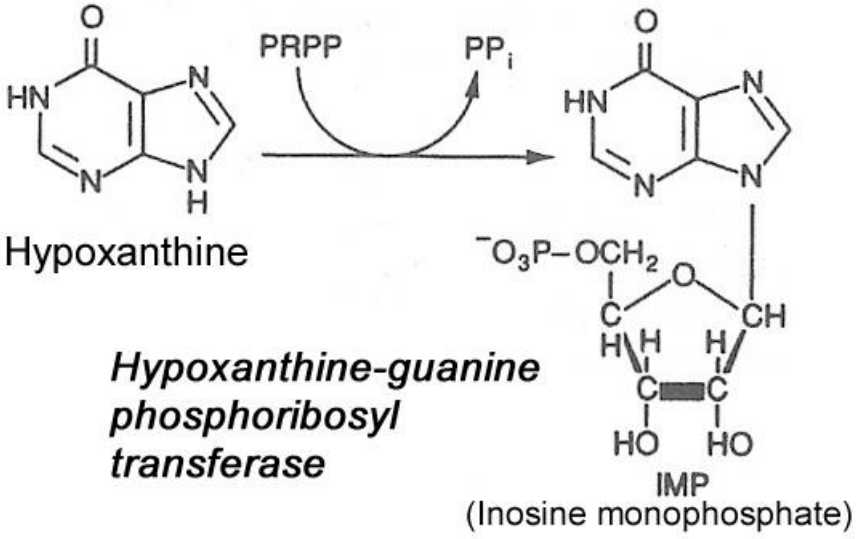


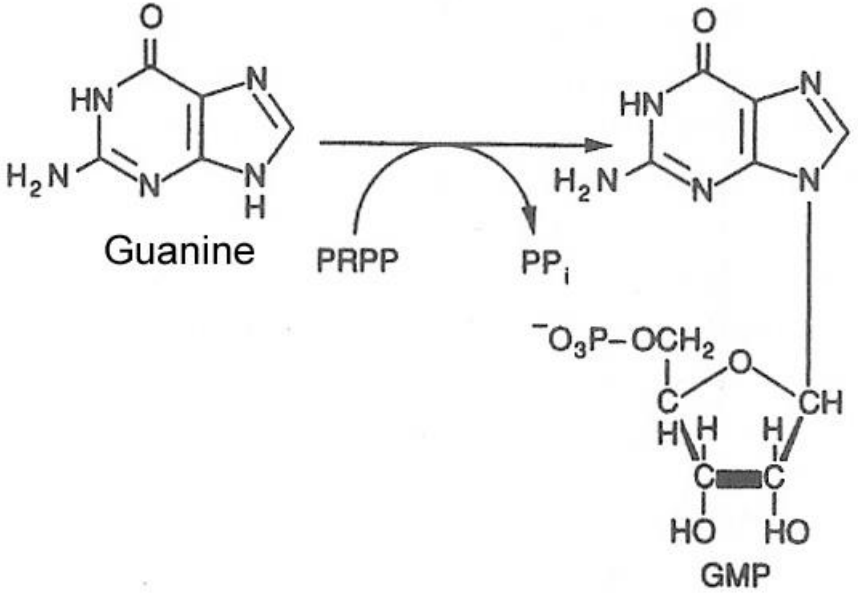
Overview of the sources of atoms in the newly synthesized purine ring



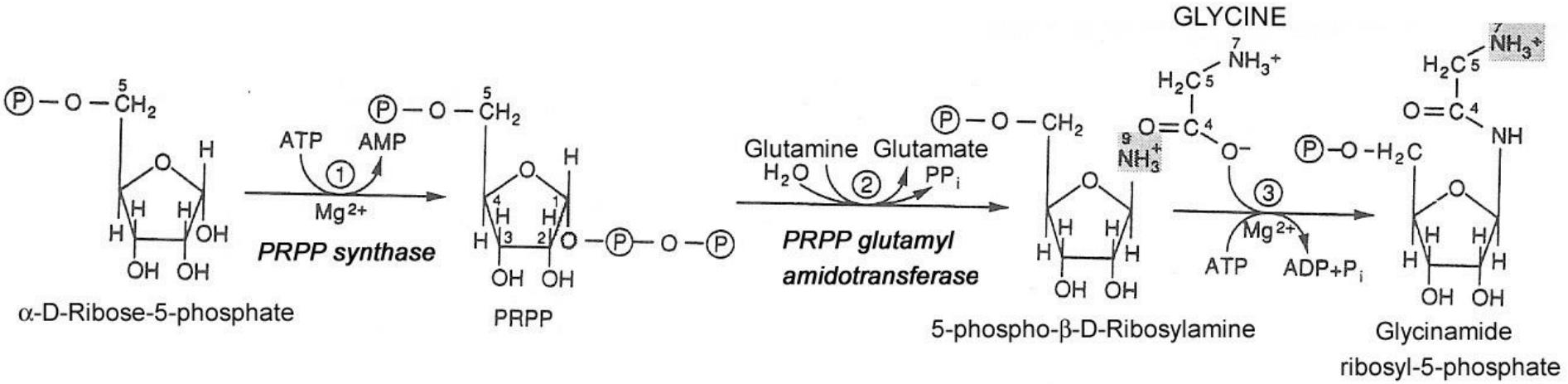
Enzymes in "salvage reactions" couple PRPP directly to purine bases

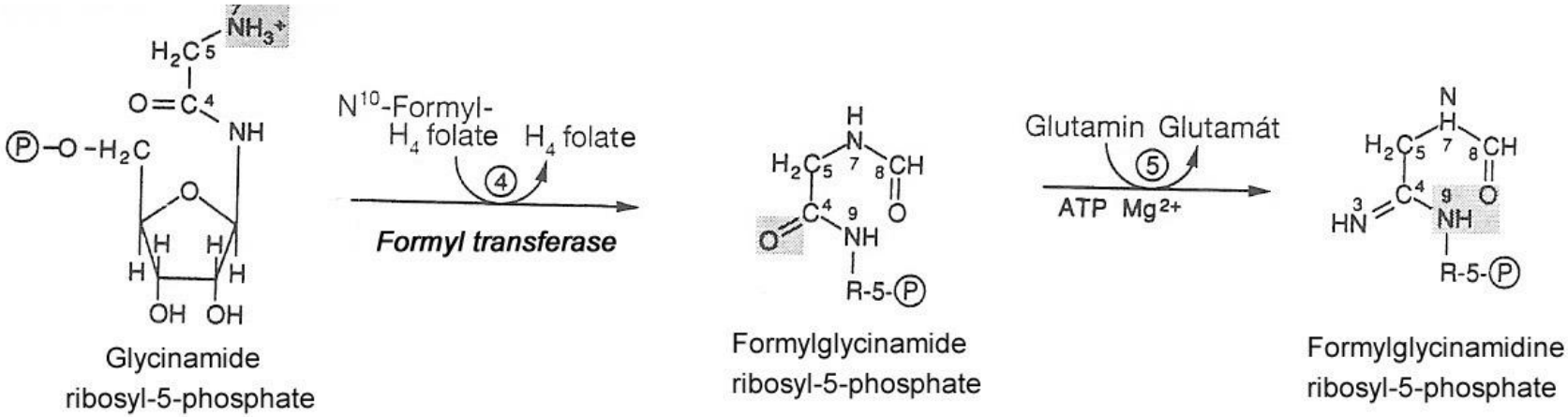




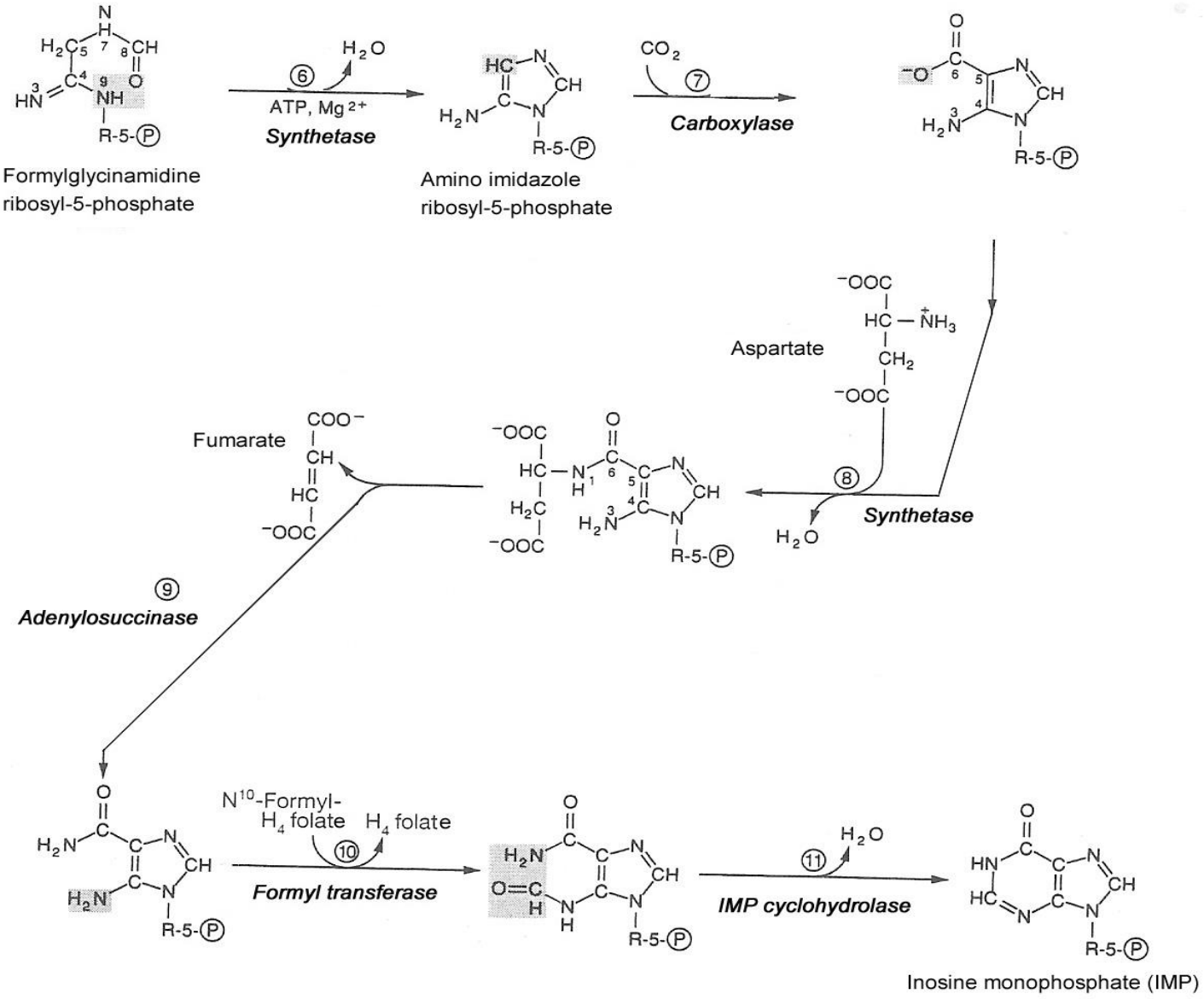


Purine biosynthesis





Purine biosynthesis - cont.



AMP and GMP are formed from IMP