Osnovne karakteristike 3-D strukture molekula DNK i RNK

- Rendgenska strukturna analiza (vlakana) DNK
- Watson-Crickov model (B) DNK
- Zašto dvostruki heliks?
- Polimorfizam DNK:
  - kanonske (standardne/prosečne) strukture A i B heliksa
  - Z heliks
- Struktura molekula RNK
- Zašto polimorfizam DNK i konzervatizam RNK?
- Život je započeo sa RNK! Zašto RNK?

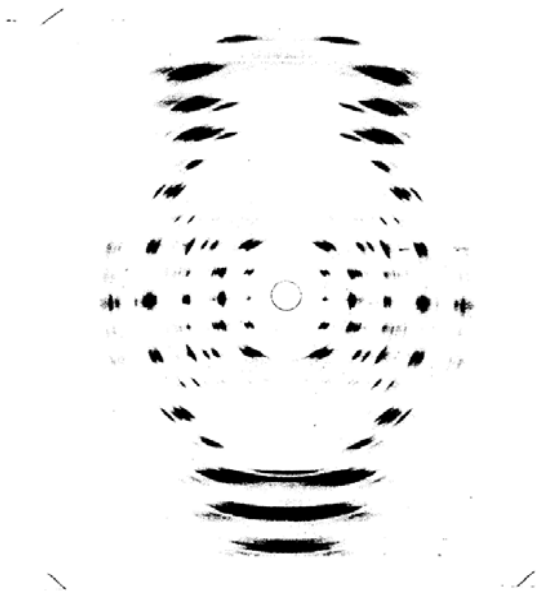
Rendgenska strukturna analiza (vlakana) DNK

1920-1958

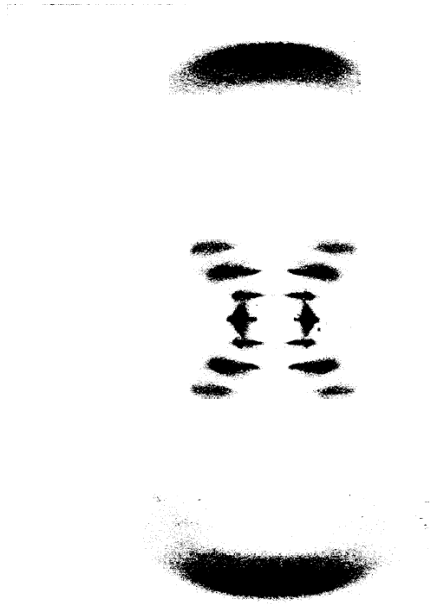
1916-2003

U periodu 1940-1952.g. su fizičar, M.Wilkins i hemičarka, R.Franklin (uglavnom) radili na određivanju 3-D strukture DNK metodom difrakcije x-zraka!!!

Došli su do važnih saznanja o strukturi, ali nisu rešili kompletnu strukturu!



A-DNK



B-DNK

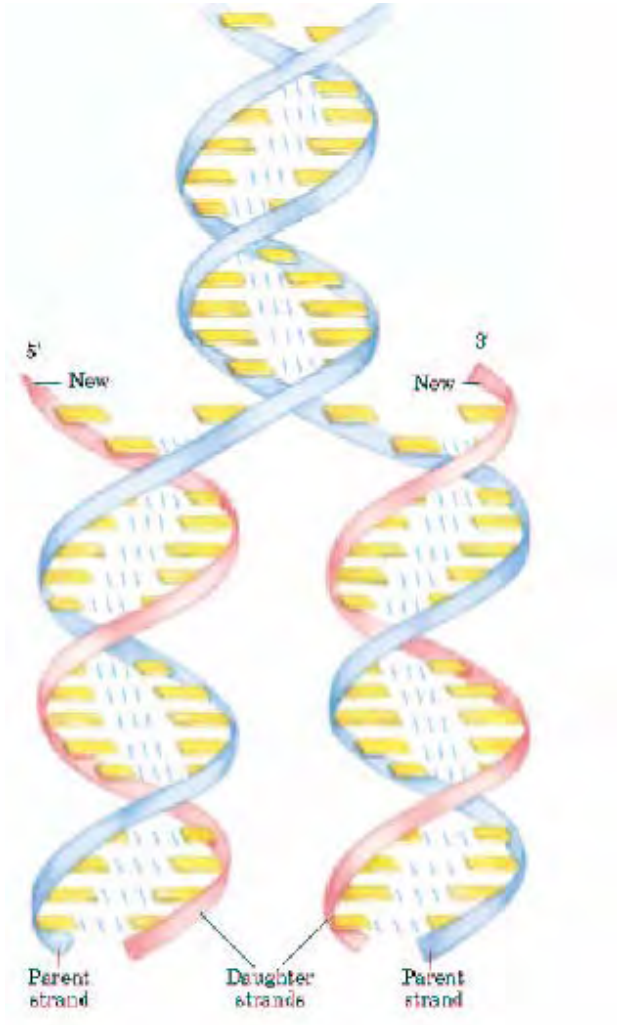
Difrakcione slike A i B DNK

NB: preparati VLAKANA DNK se nalaze u rastvoru u kojem su taloženi, preparat B sa više, i preparat A sa manje vlage!

A Structure for Deoxyribose Nucleic Acid

J. D. Watson and F. H. C. Crick , *Nature* **(3),** 171, 737-738 (1953)

"It has not escaped our notice (12) that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" **……..**


5'
New
3'
New
Parent strand
Daughter strands
Parent strand

Kako su Watson i Crick dešifrovali 3-D strukturu DNK?

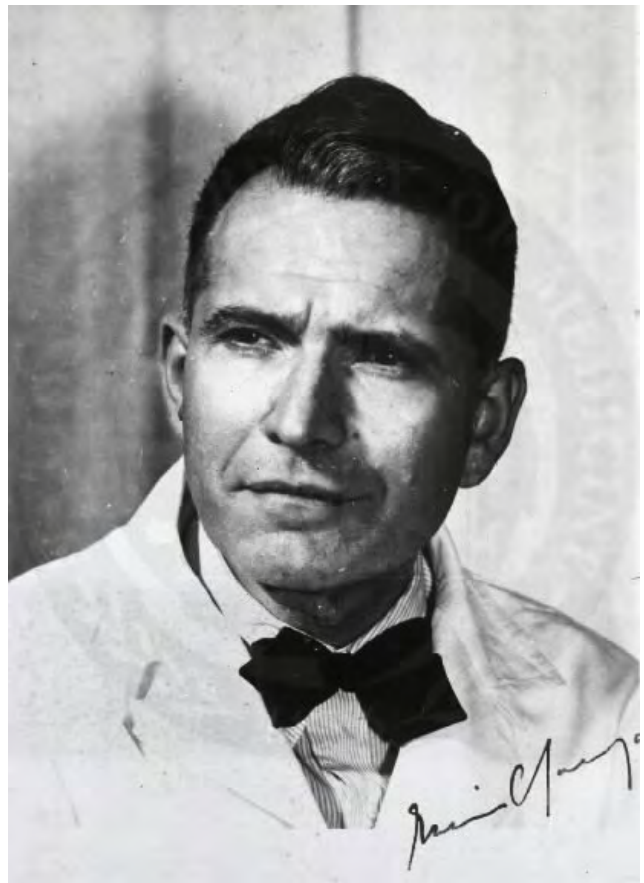
Oblast	Podaci	Izvor
Kristalografija (difrakcija x-zraka)	Slepljeni slojevi subjedinica u spiralama; dugi lanac, ne isključuju se dva lanca; šećer-fosfat okrenut spolja.	M.Wilkins i R. Franklin (uglavnom Franklin)
Organska hemija	4 nukleotida	P. Levene
Biohemija	α-helix, modeli	L. Pauling
Hromatografija na hartiji	Odnos baza %A = %T %C = %G	E. Chargaff
Hemijske veze	Ispravan (tautomerni) oblik baza	J. Donahue
Matematika	Privlačne sile među bazama	J. Griffith

Odnos baza u preparatima DNK

Edwin Chagraff (1905-2002) je primenom hromatografije na hartiji primetio “pravilnosti” u sastavu baza svih preparata DNK koje je analizirao:

$$\%A = \%T$$

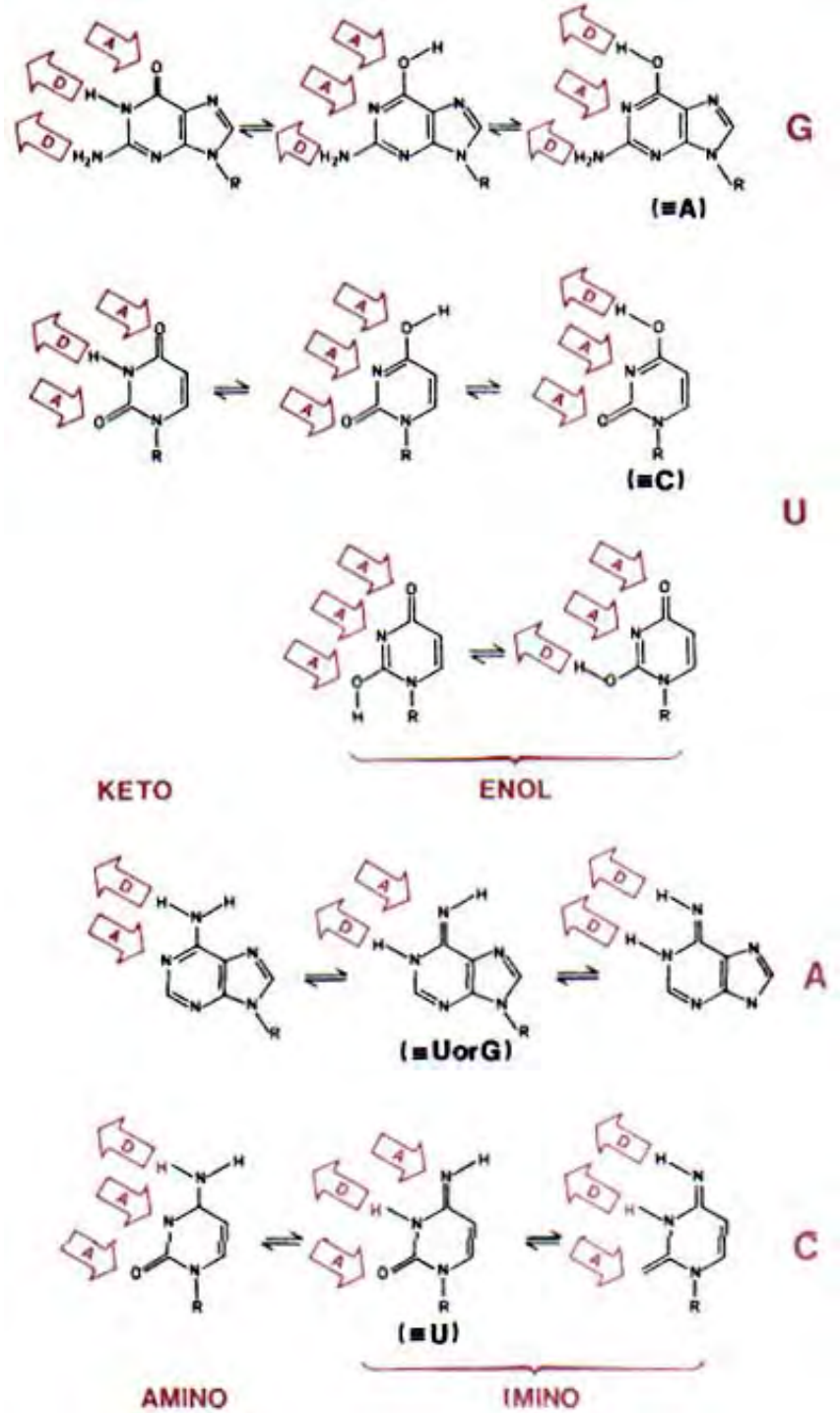
$$\%C = \%G$$



Tautomerne strukture baza

- Keto vs. enol
- Amino vs imino

- Do ~1950 smatralo se da su baze u enolnom obliku. Zašto?



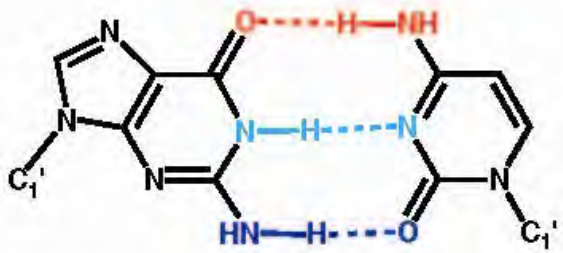
Šta je doprinos Watsona i Cricka?

- W & C su zapazili na osnovu modela da parovi baza A-T i C-G imaju isti prečnik i oblik.

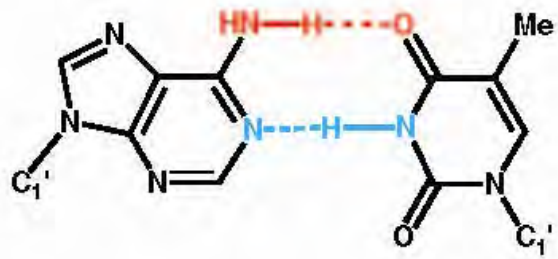
- To im je omogućilo da:
  - (1) razumeju konstantni prečnik molekula DNK (što je proizilazilo iz rendgenske analize!)
  - (2) primene Chargaffova pravila.

- Napravili su model 3-D strukture molekula DNK!

W-C sparivanje baza



dG dC



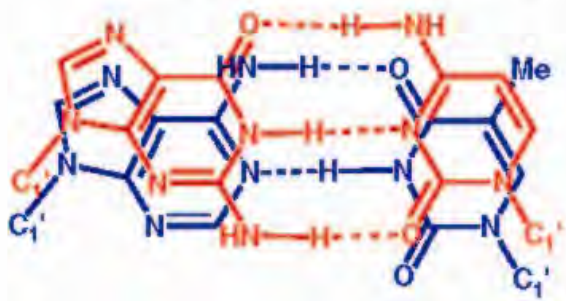
dA dT

H bond 2.8 Å

H bond 2.9 Å

$C_{1'}$ to $C_{1'}$ 10.85Å

Isomorphous base pairing



W-C sparivanje baza u molekulu DNK

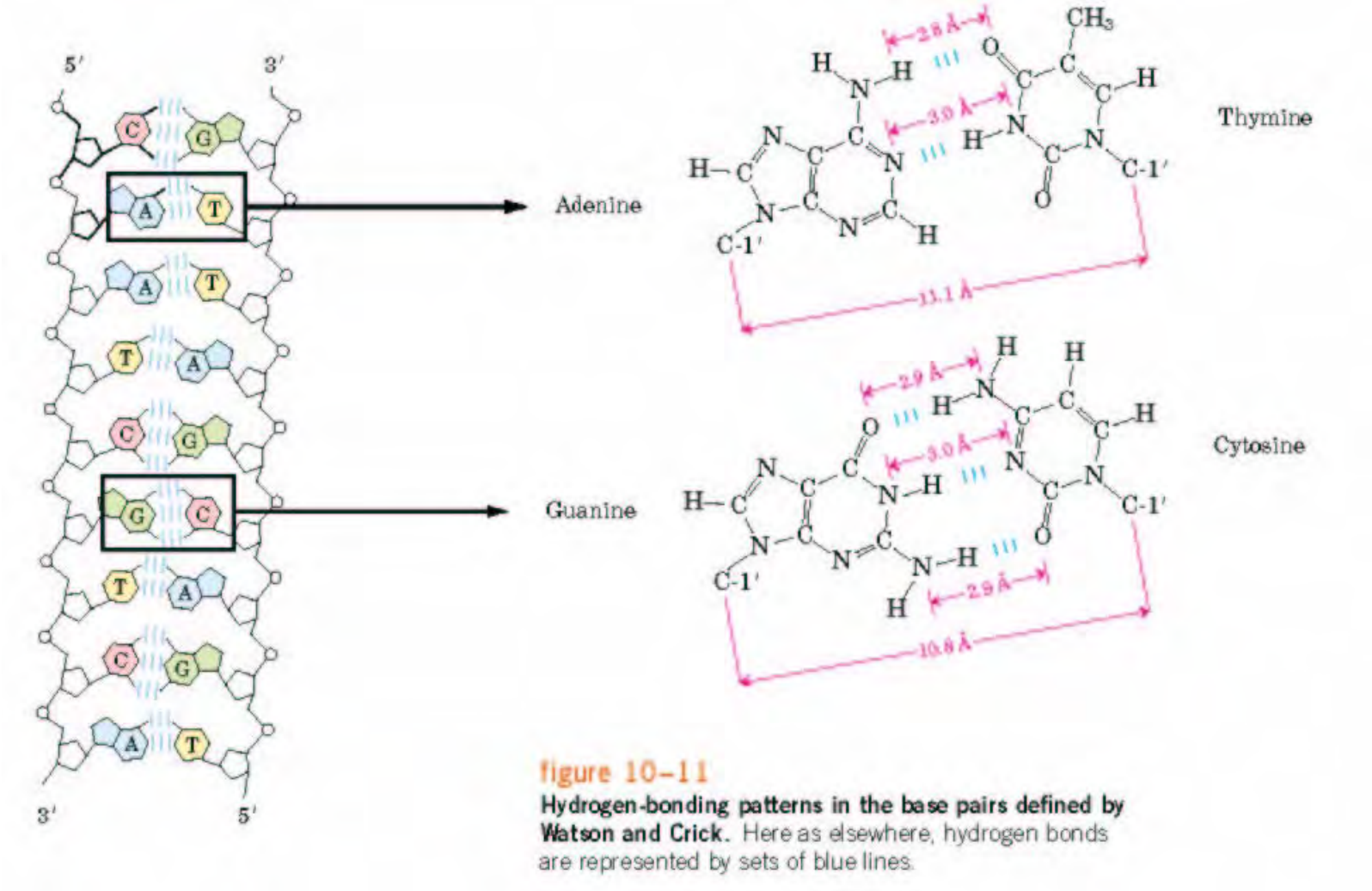
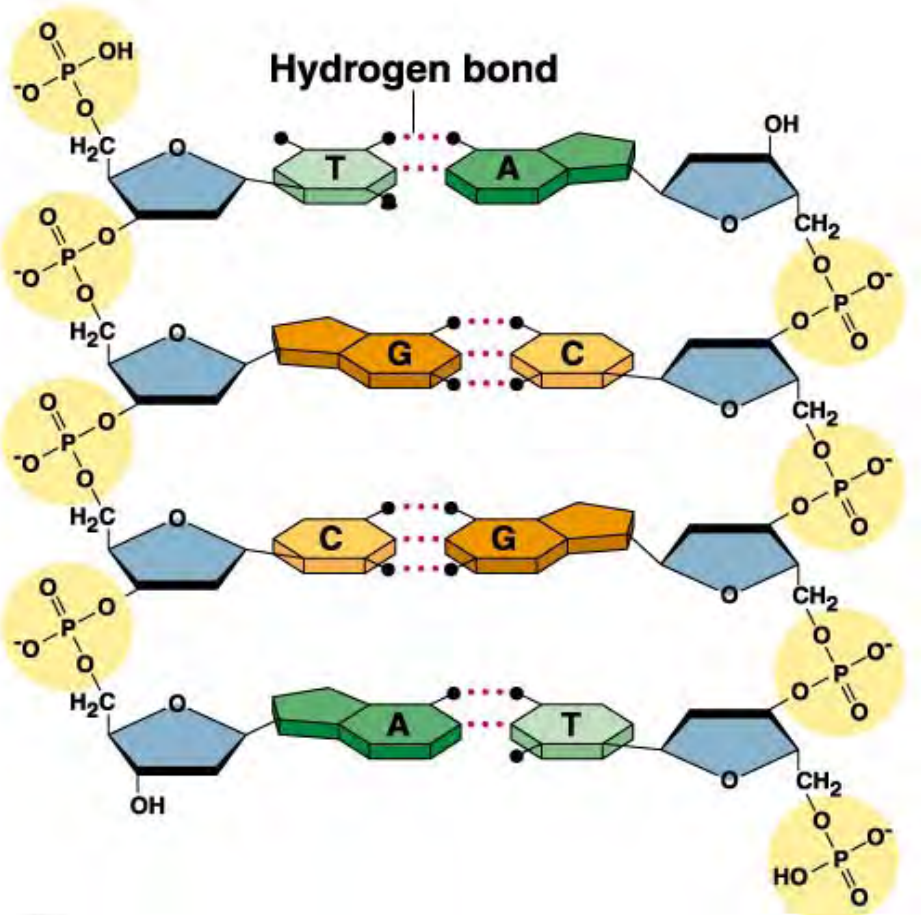
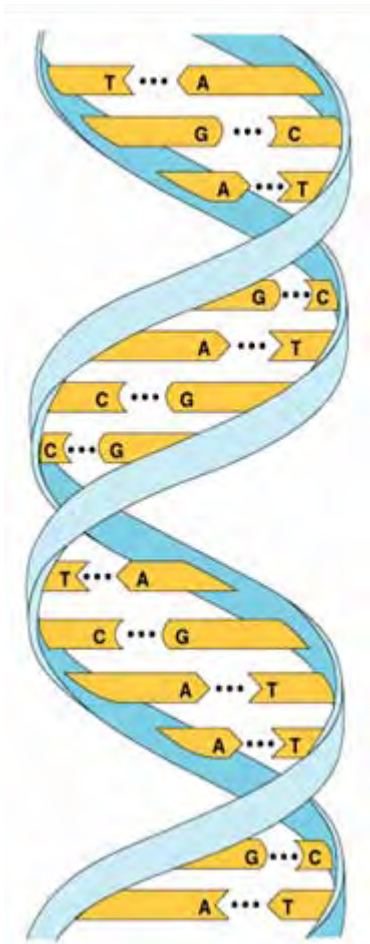


figure 10–11

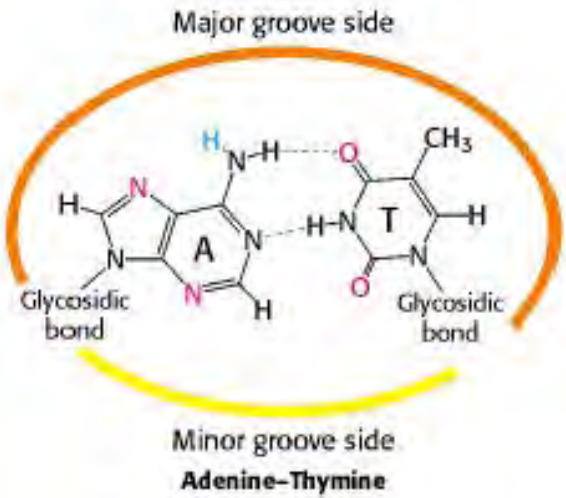
Hydrogen-bonding patterns in the base pairs defined by Watson and Crick. Here as elsewhere, hydrogen bonds are represented by sets of blue lines.

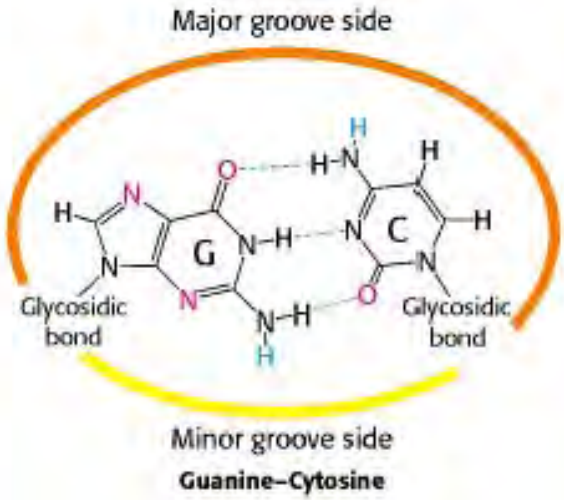
Watson-Crickov model (B) DNK

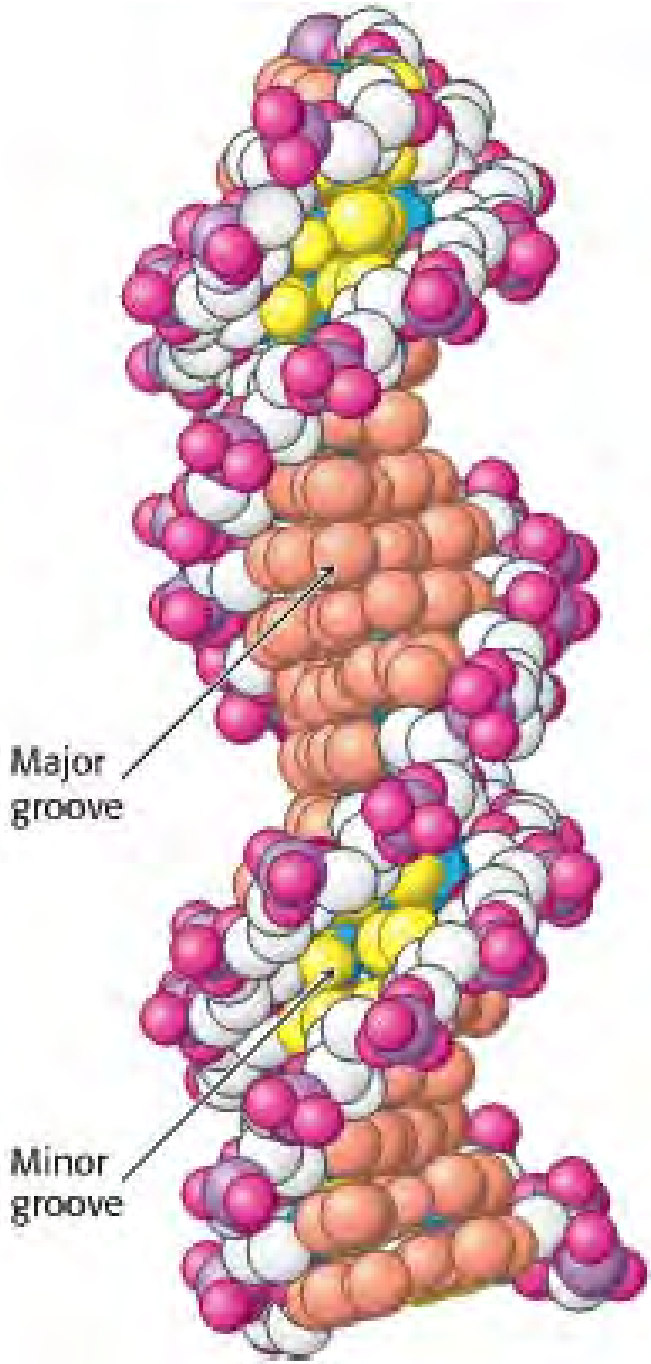




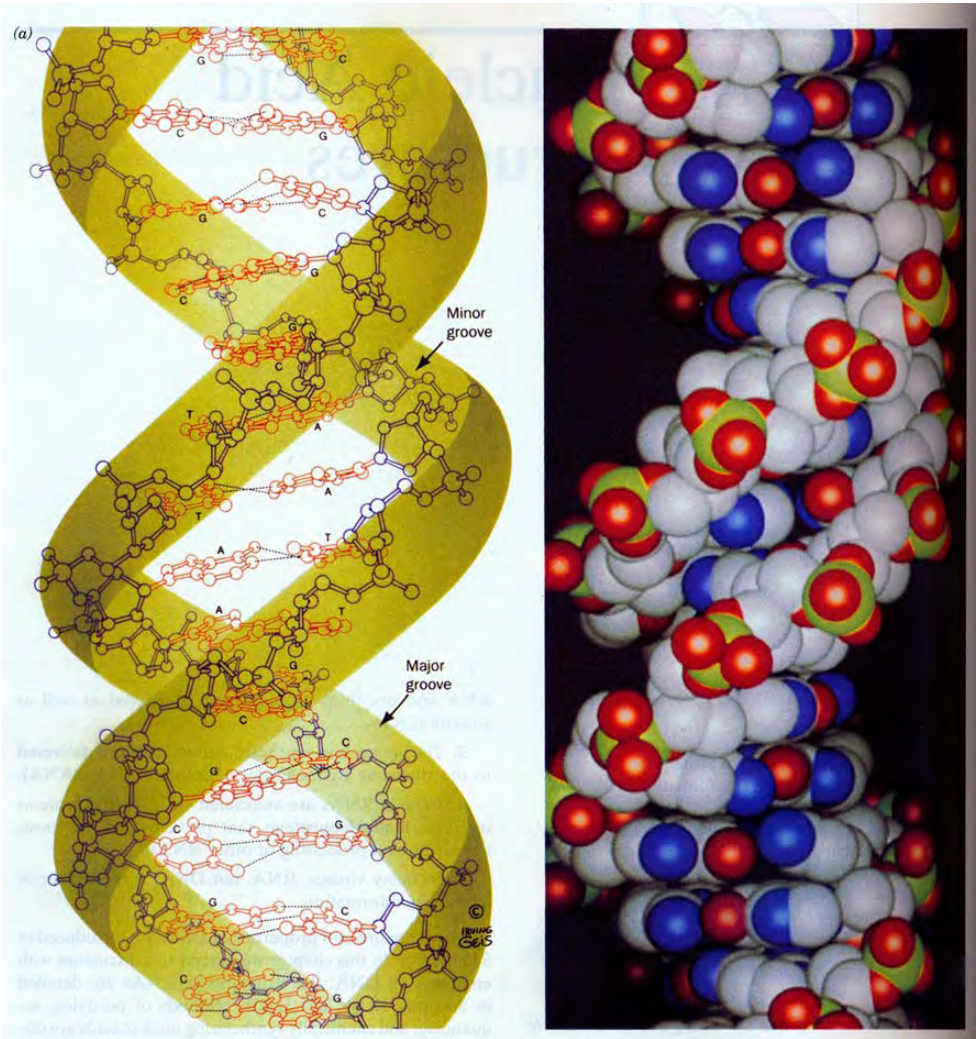
B-DNK


Major groove side
A
T
Glycosidic bond
Glycosidic bond
Minor groove side
Adenine–Thymine


Major groove side
G
C
Glycosidic bond
Glycosidic bond
Minor groove side
Guanine–Cytosine

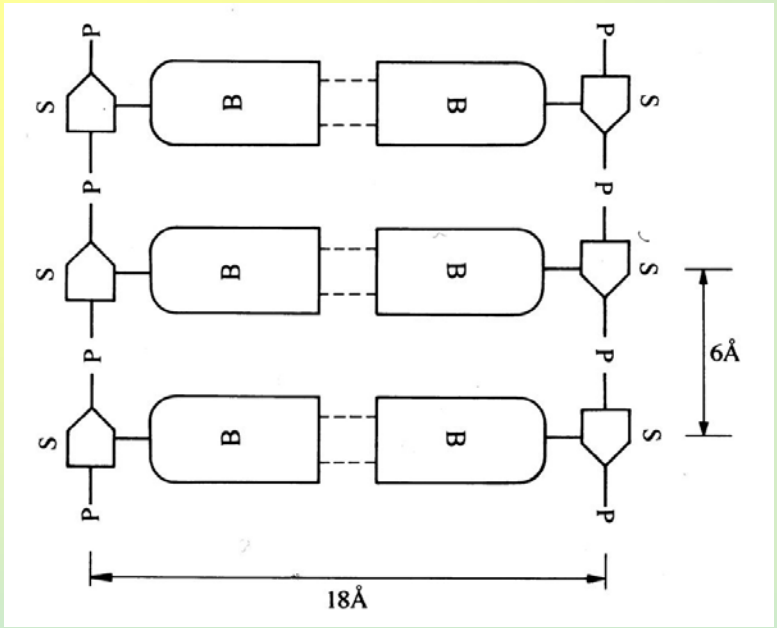

Major groove
Minor groove

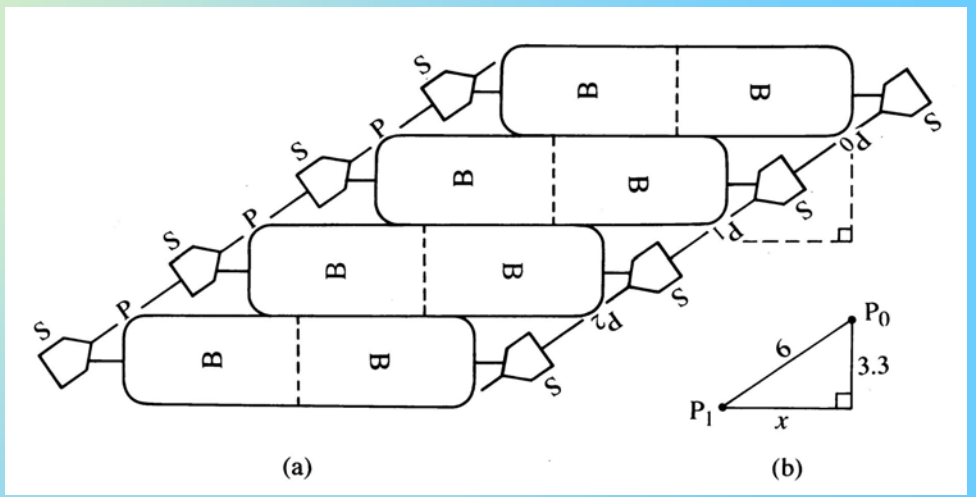
Velika vs mala brazda - različita okruženja - važno za prepoznavanje i vezivanje!

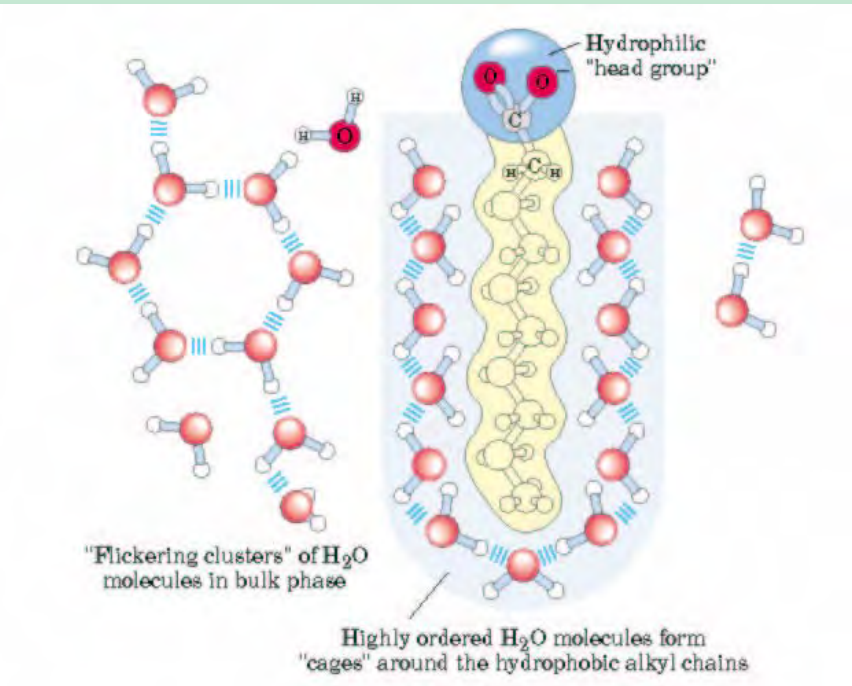


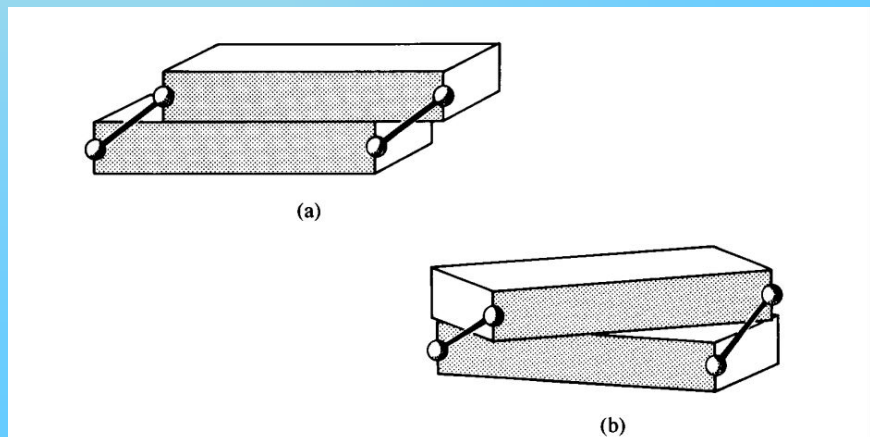
- Mala brazda
  - hidrofobni H atomi na dezoksiribozi
- Velika brazda
  - bogatija u bazama

Zašto dvostruki heliks?

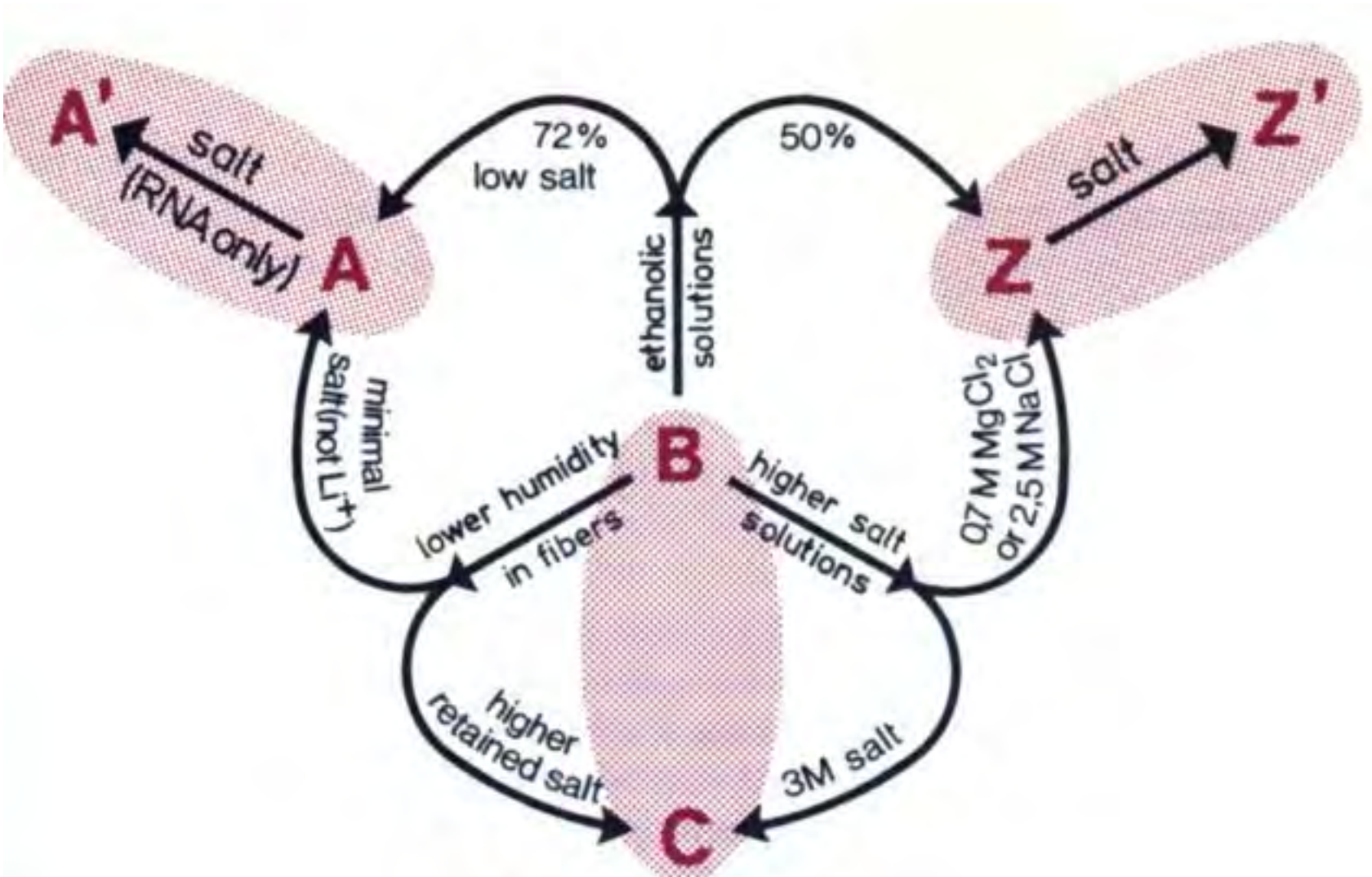






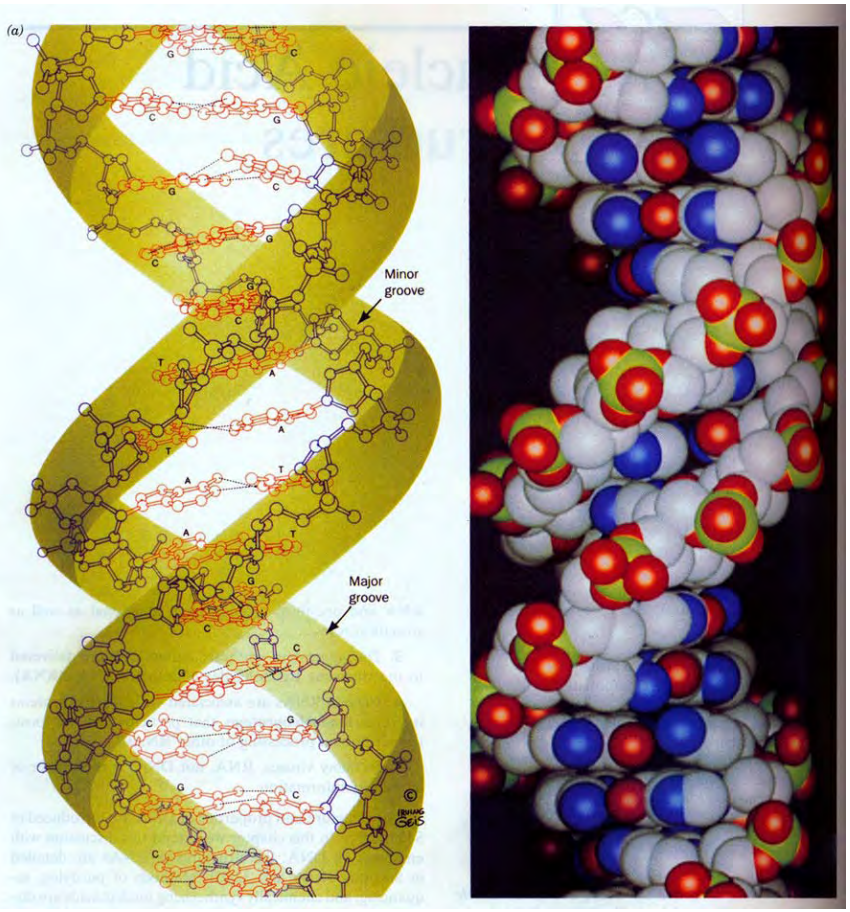


Polimorfizam DNK vs konzervatizam RNK

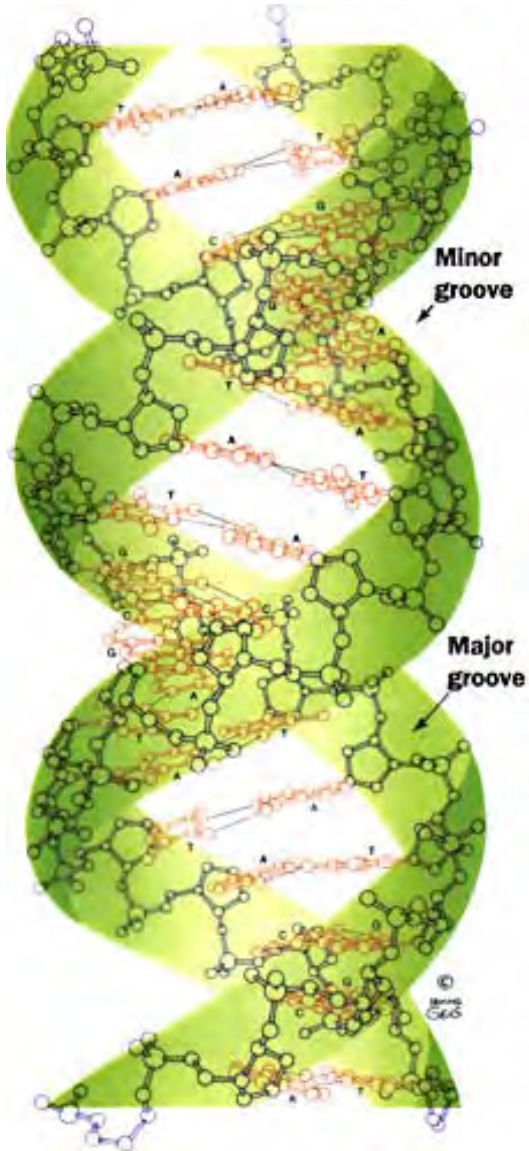


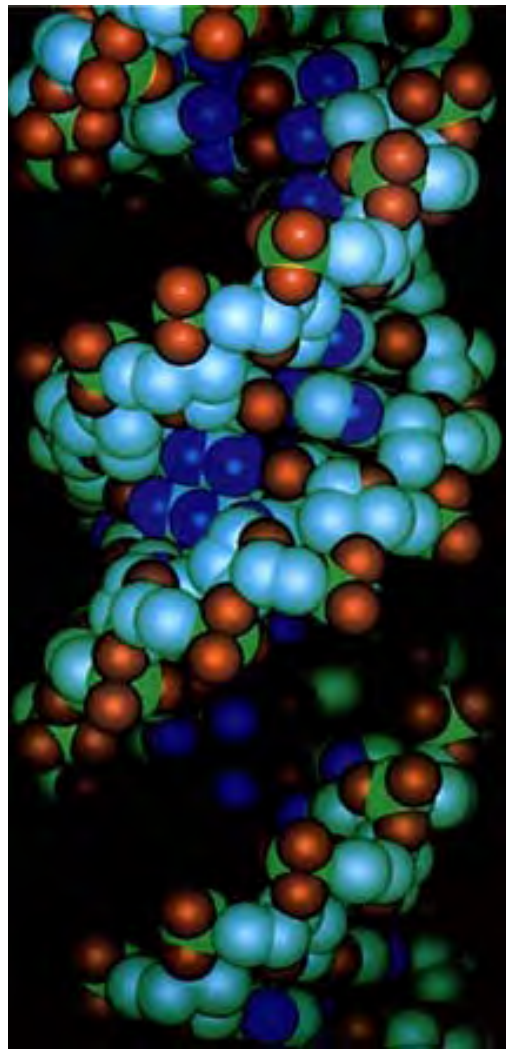
Kanonska (standardna/prosečna) struktura

B-DNK

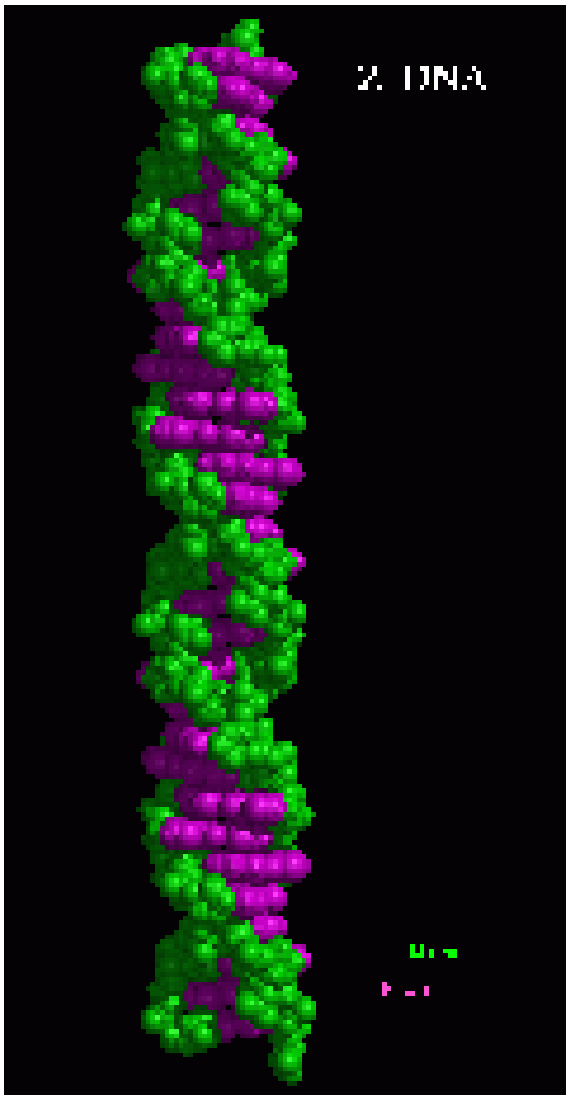


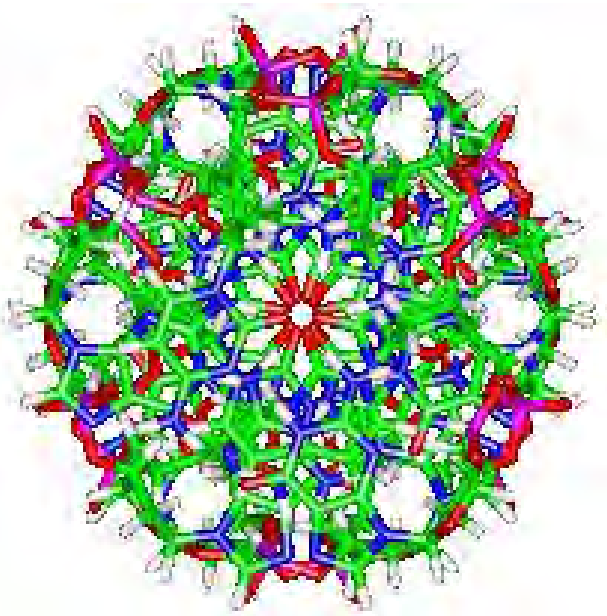
A DNK



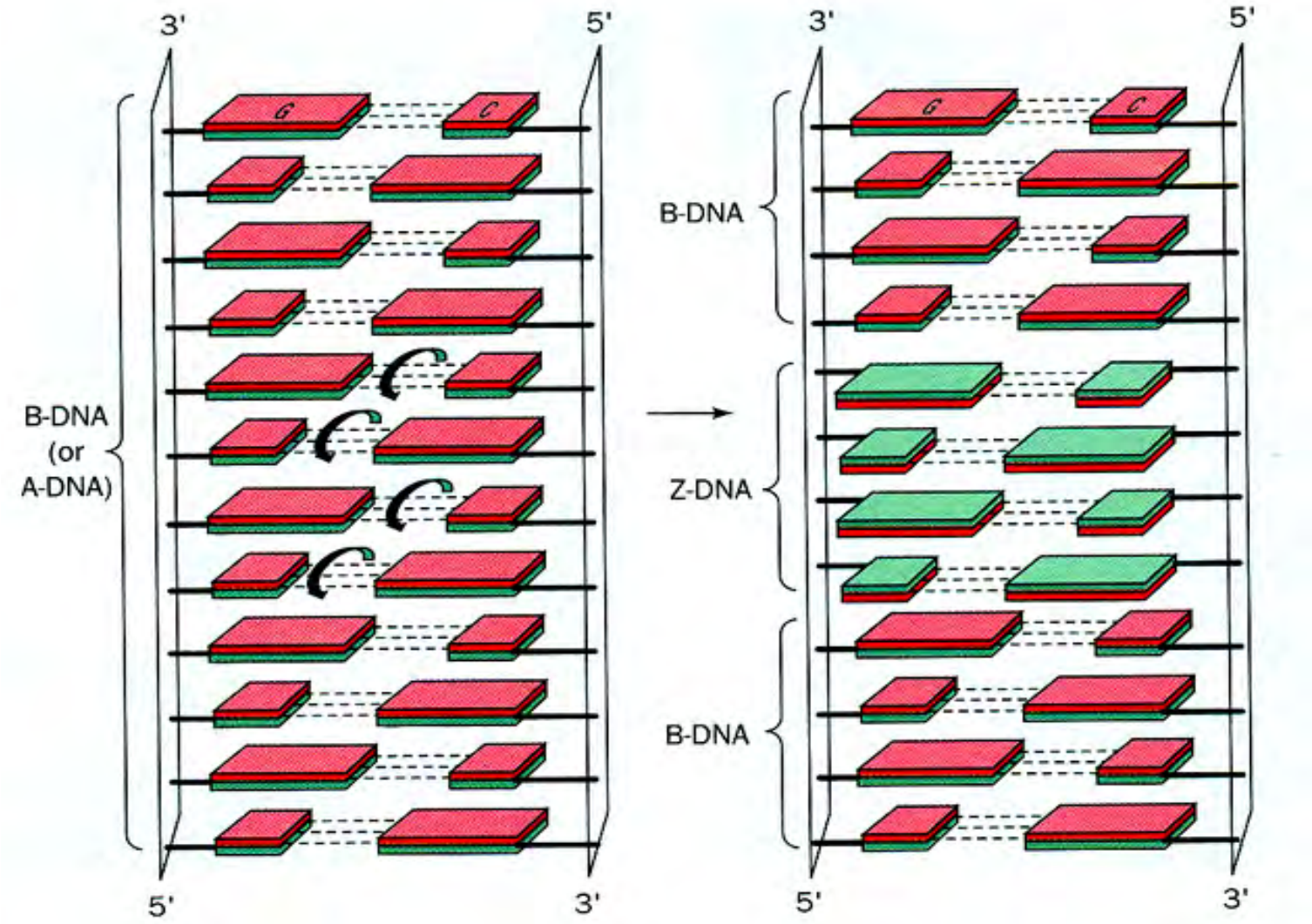


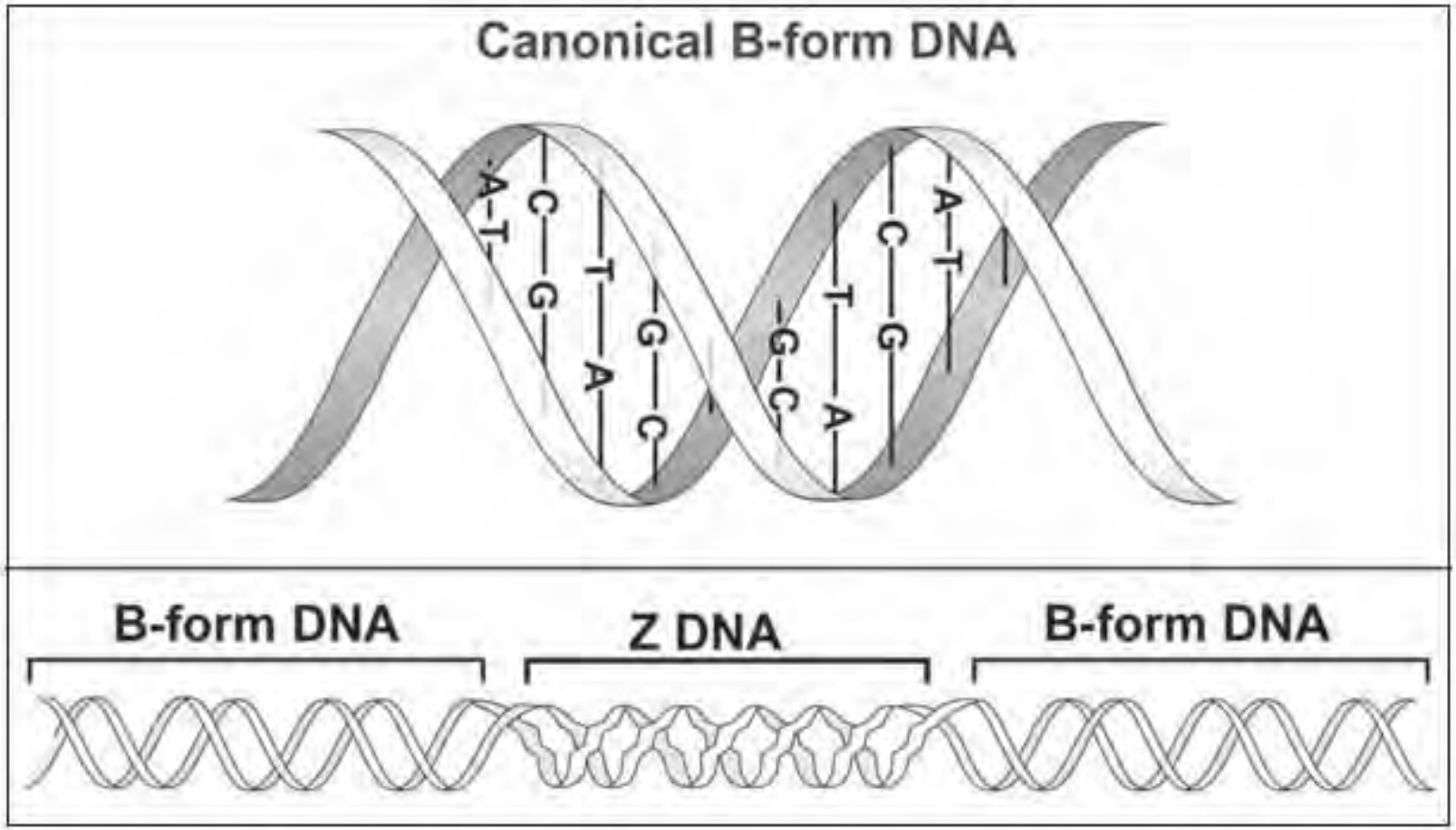
Z-DNK



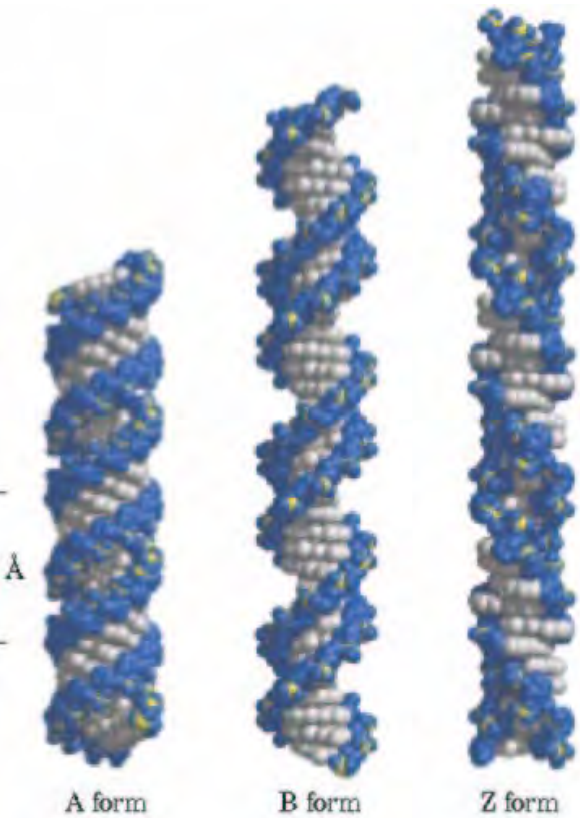


Z helix: purin-pirimidin




Canonical B-form DNA
A–T
C—G
T—A
G—C
G—C
T—A
C—G
A—T
B-form DNA
Z DNA
B-form DNA

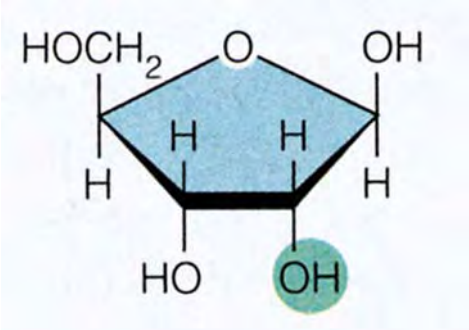
Standardne (prosečne) konformacije DNK



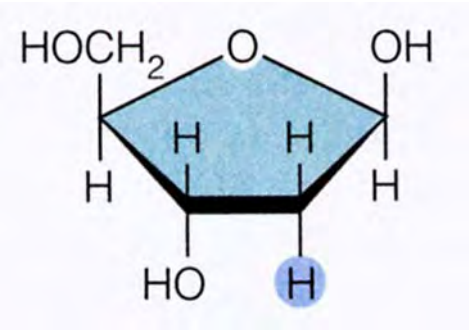
	A form	B form	Z form
Helical sense	Right handed	Right handed	Left handed
Diameter	~26 Å	~20 Å	~18 Å
Base pairs per helical turn	11	10.5	12
Helix rise per base pair	2.6 Å	3.4 Å	3.7 Å
Base tilt normal to the helix axis	20°	6°	7°
Sugar pucker conformation	C-3′ endo	C-2′ endo	C-2′ endo for pyrimidines; C-3′ endo for purines
Glycosyl bond conformation	Anti	Anti	Anti for pyrmidines; syn for purines

figure 10–19
Comparison of A, B, and Z forms of DNA. Each struc-

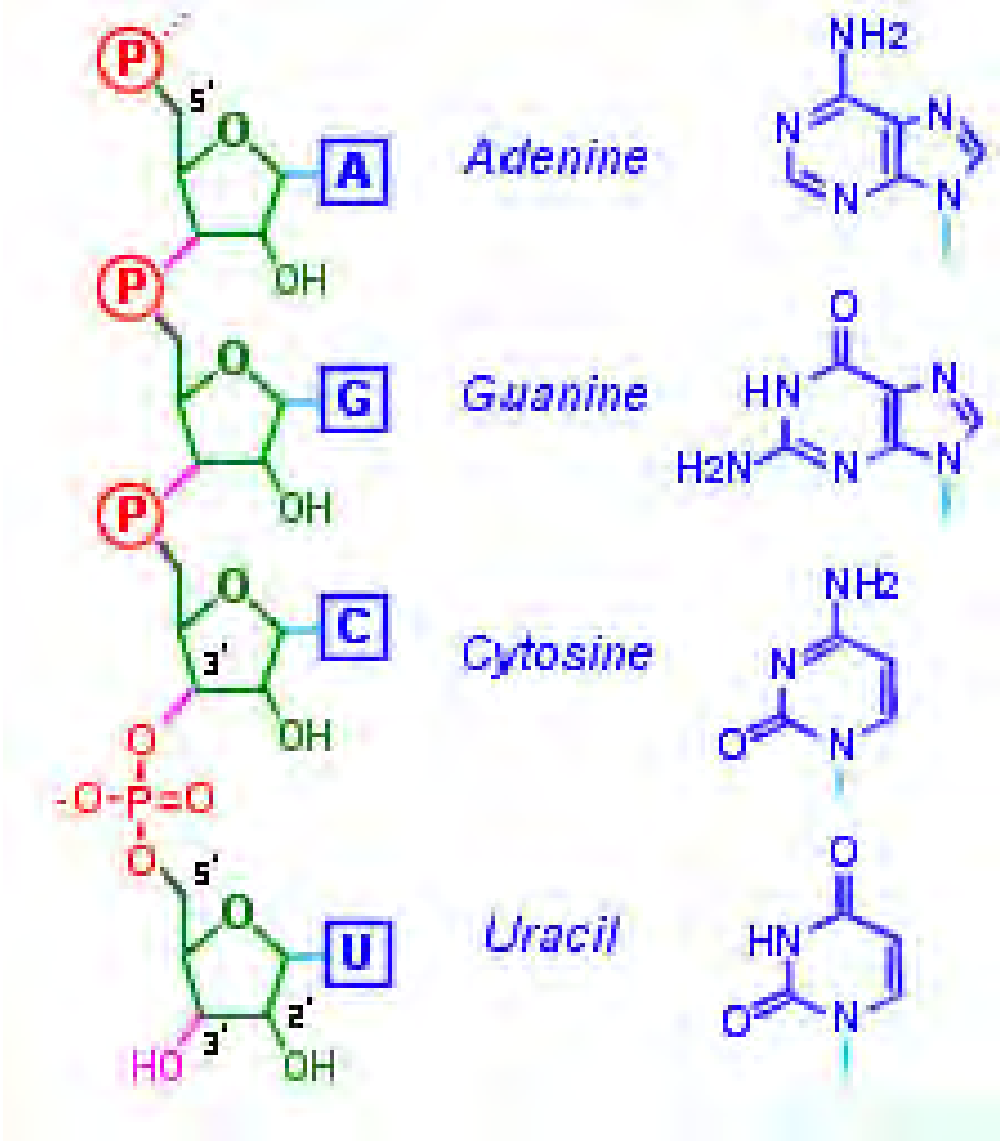
RNK vs. DNK



Riboza



Dezoksiriboza



RNK: informaciona, transportna, ribozomalna

► RNK linearan polimer:

adenin (A), citozin (C), guanin (G) i uracil (U).

► RNK može da se uvija dajući:

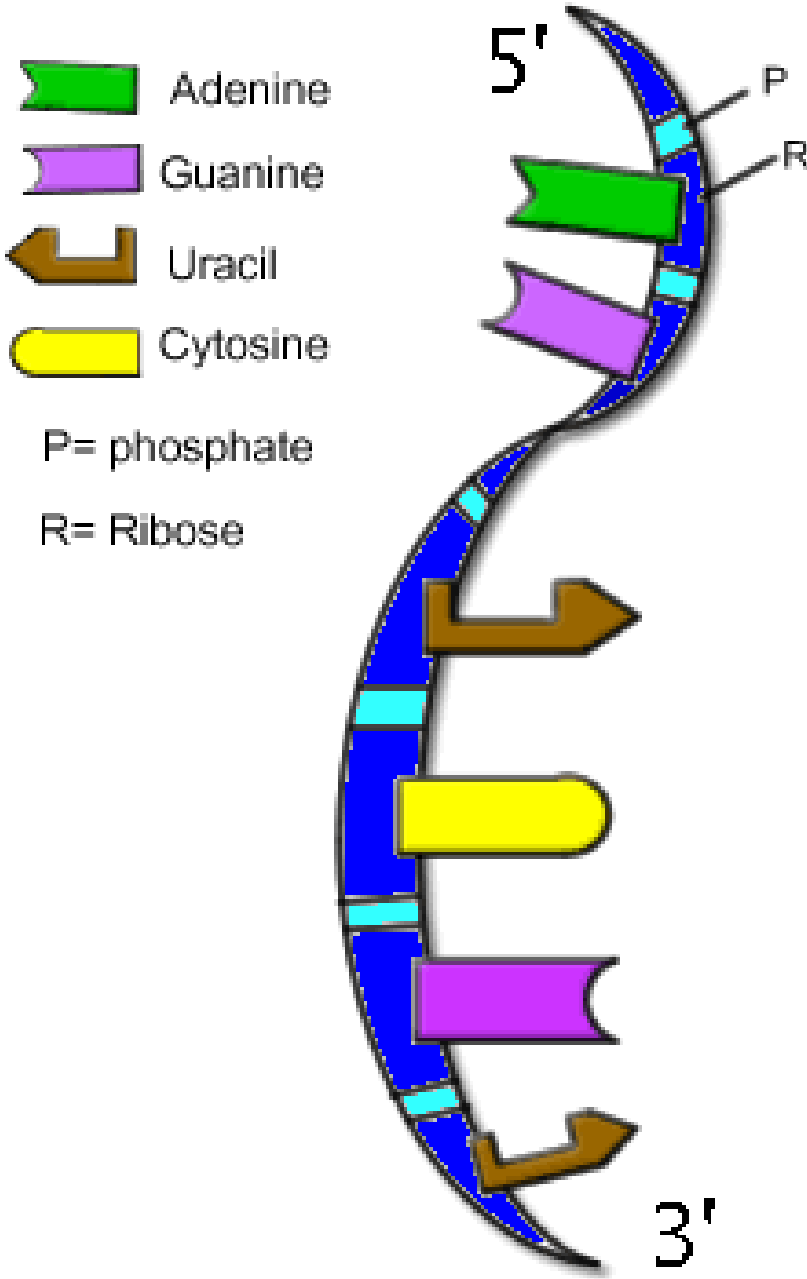
-sekundarne strukture

-tercijernu strukturu

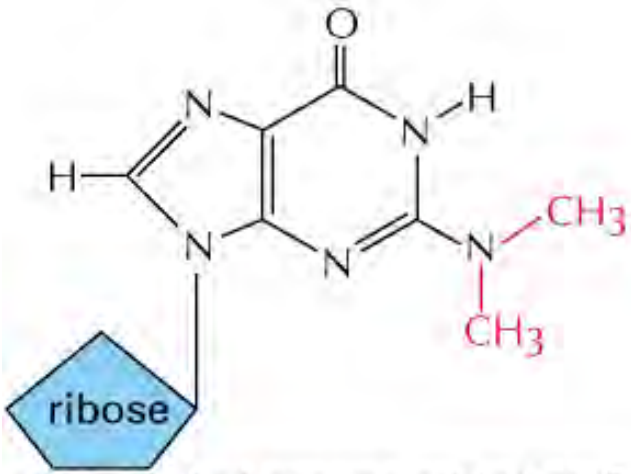
► Sparivanje baza u RNK:

AU i GC (W-C)

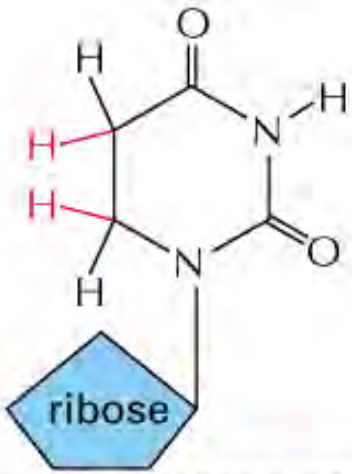
GU “wobble”



Modifikovane baze nađene u tRNK

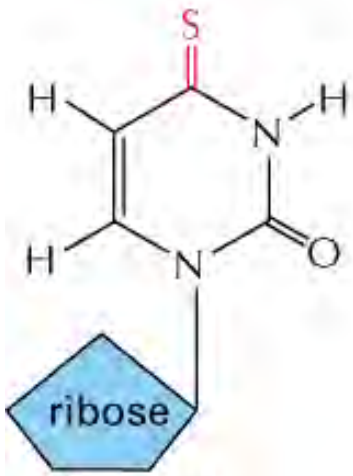


two methyl groups added to G
(*N,N*-dimethyl G)

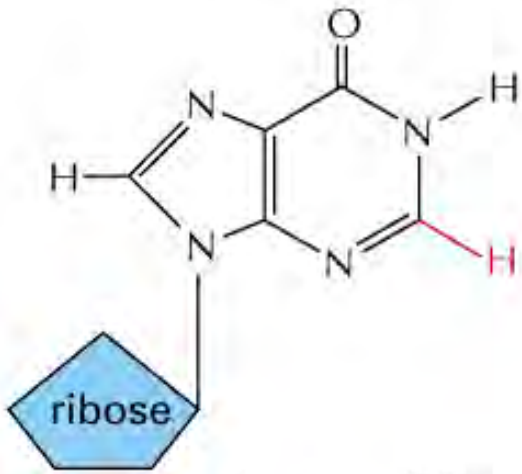


two hydrogens added
to U (dihydro U)

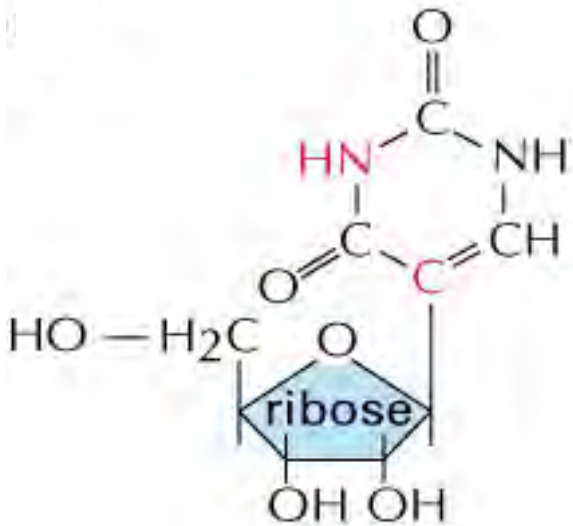
$= UH_2$



sulfur replaces oxygen in U
(4-thiouridine)



deamination of G
(inosine)



pseudouridine

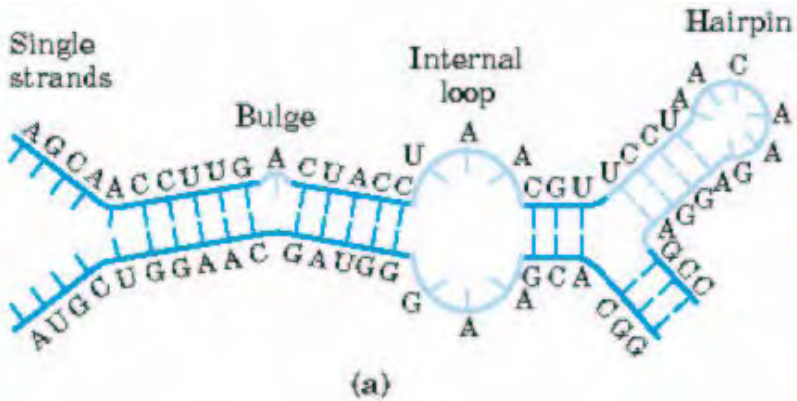
Figure 6–55. Molecular Biology of the Cell, 4th Edition.

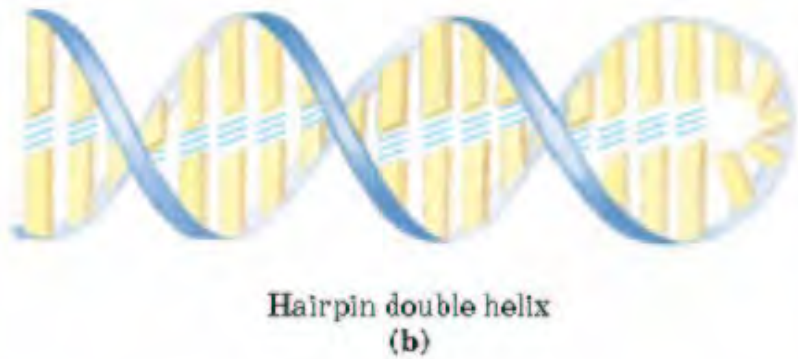
Informaciona RNK (iRNK)

figure 10–25

Typical right-handed stacking pattern of single-stranded RNA. The bases are shown in white, the phosphate atoms in yellow, and the riboses and phosphate oxygens in green. Green is used to represent RNA strands in succeeding chapters, just as blue is used for DNA.

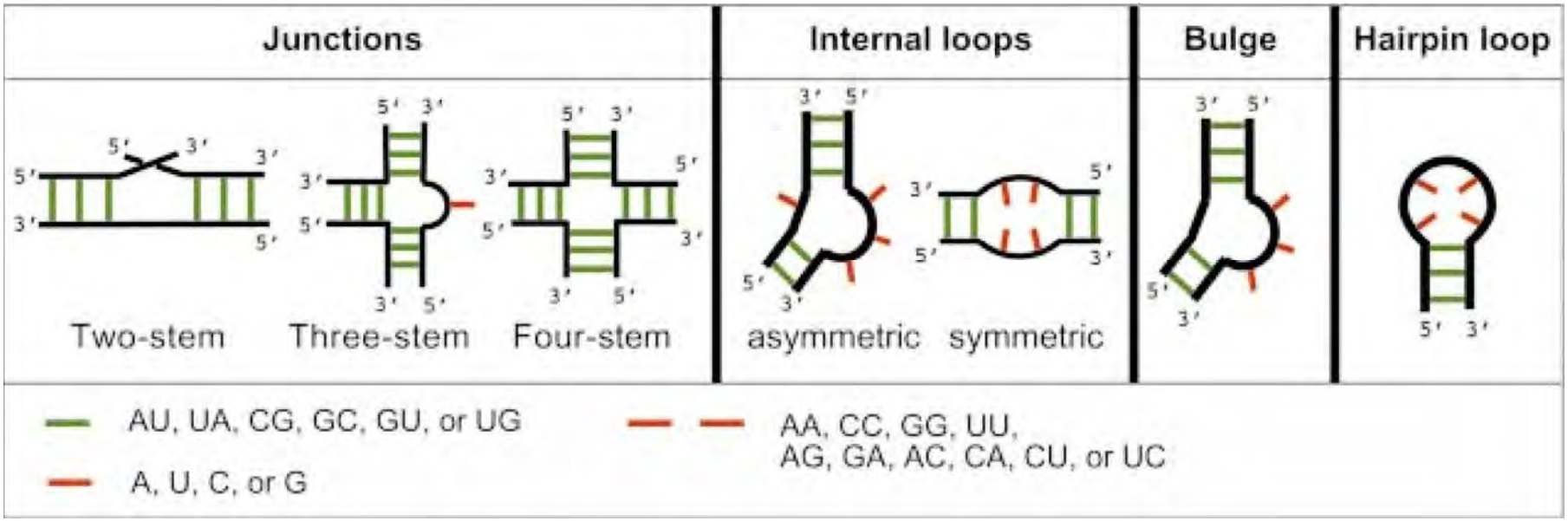
Sekundarne strukture tRNK, rRNK





A heliks!!!

RNK: sekundarne strukture



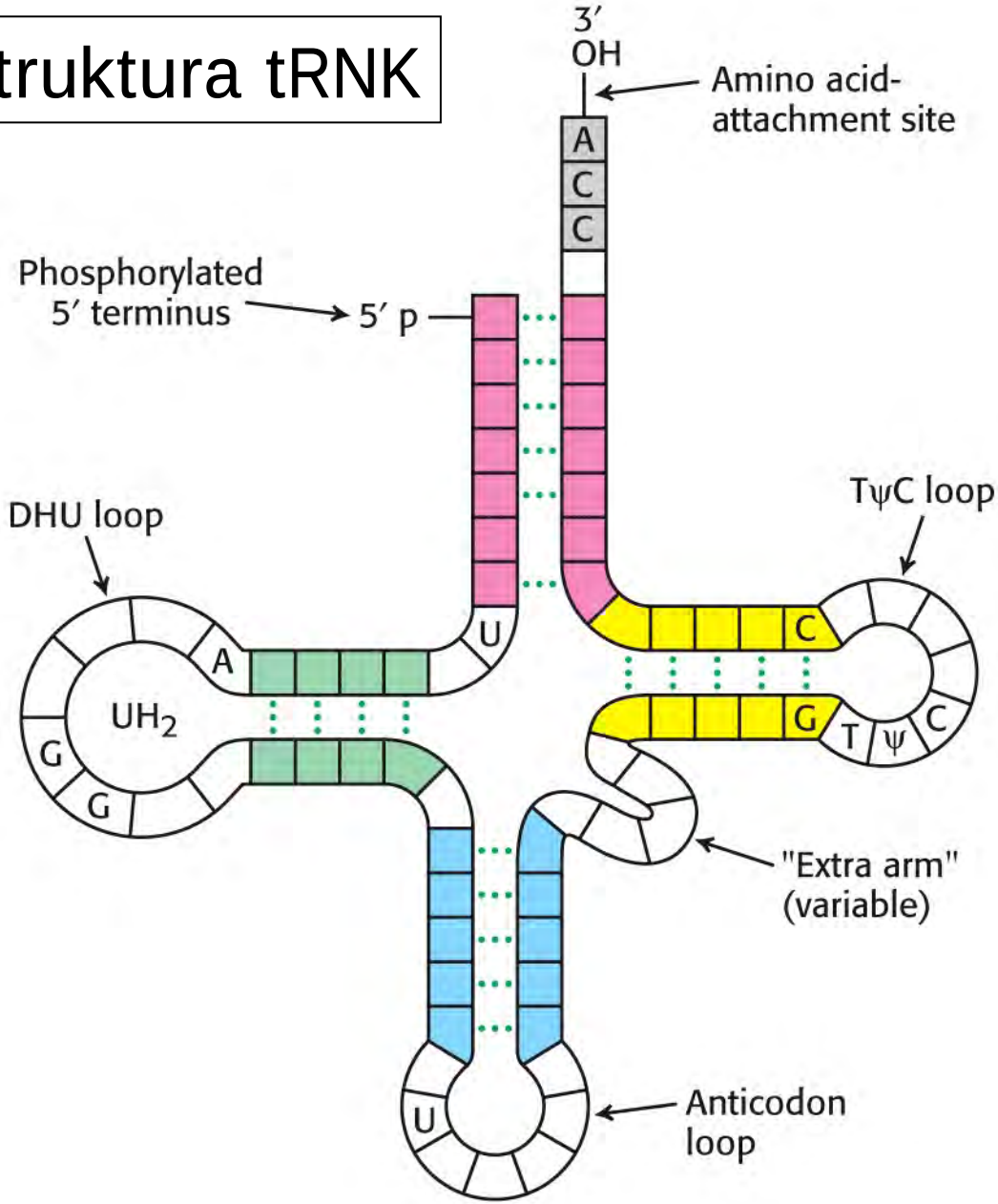
Helikoidne peteljke (ili regioni) – komplementarni delovi niza

Spojevi (“junctions”) – gde se spajaju 2 ili više peteljki

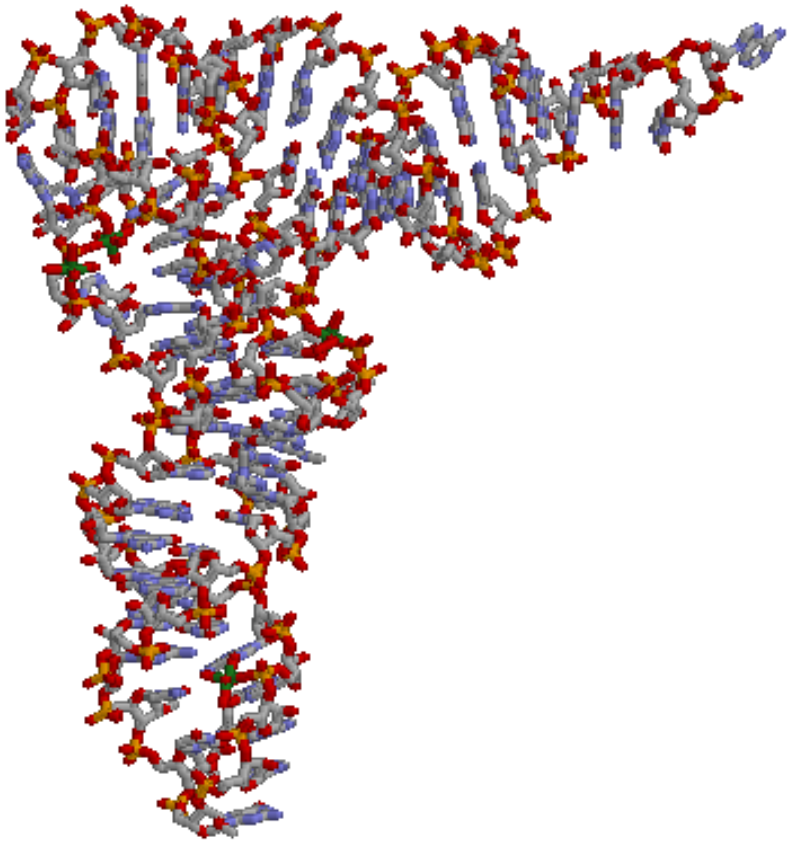
Petlje (“Loops”)

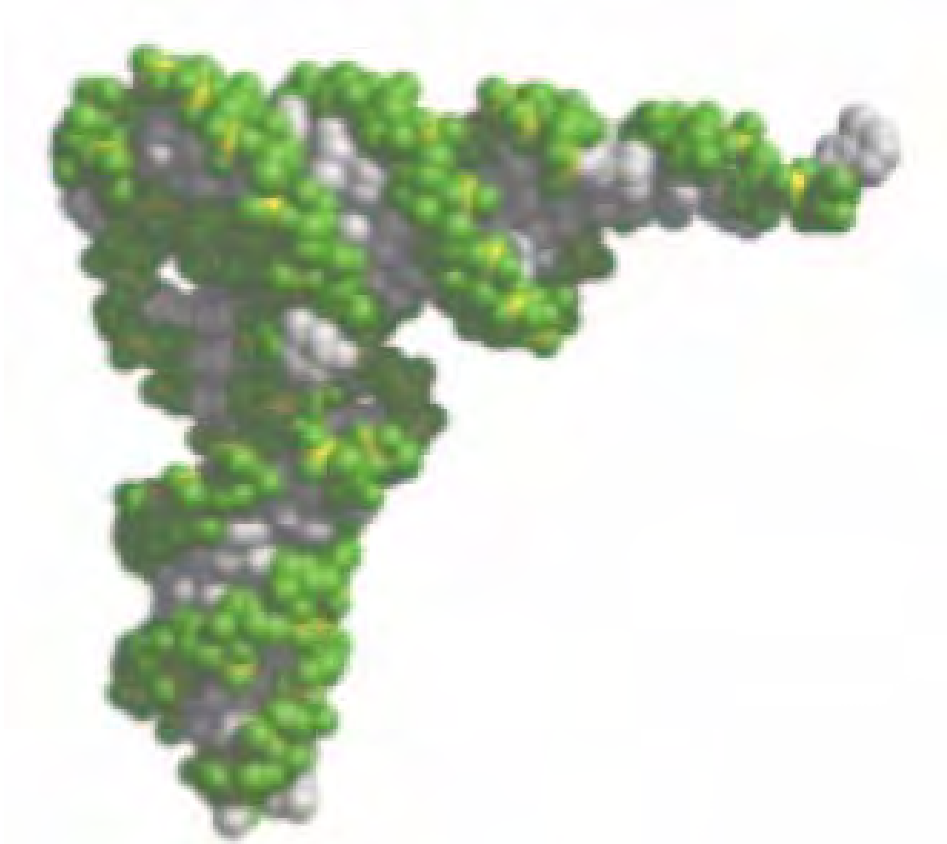
- **Interne petlje – imaju 2 ili više nesparenih ili pogrešno spareniha baza na obe strane**
- **Izbočine (“bulge”)– imaju 1 ili više nesparenih ili pogrešno sparenih baza na jednoj, ali ne i na drugoj strani**
- **Ukosnice (“hairpin”) – imaju 4 ili više nesparenih baza**

Opšta struktura tRNK

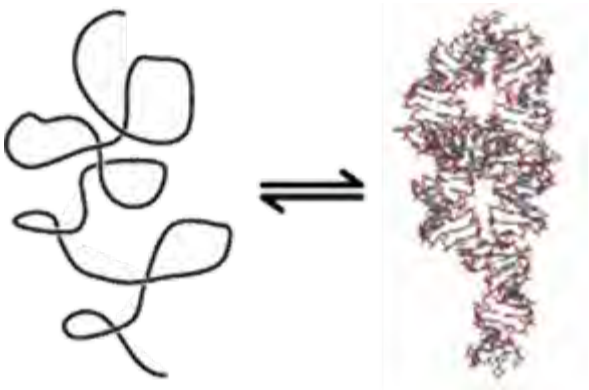


t-RNK: tercijarna struktura

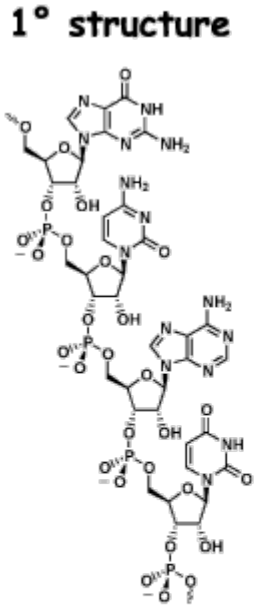




Problem uvijanja RNK

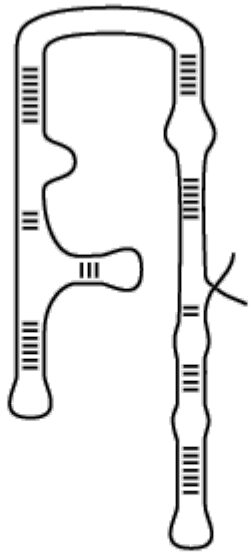


Uvijanje RNK je slično uvijanju proteina!



1° structure

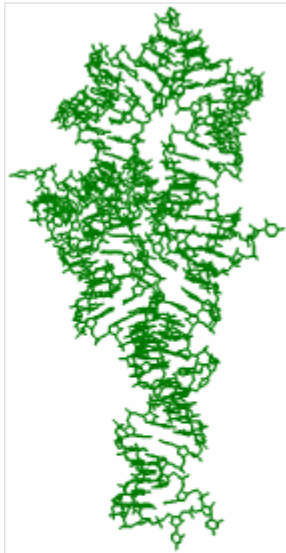
form 2° structure



2° structure

Mg^{2+}

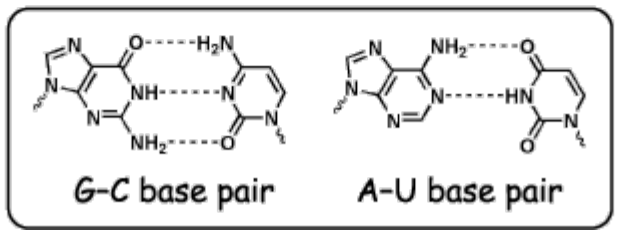
form 3° structure



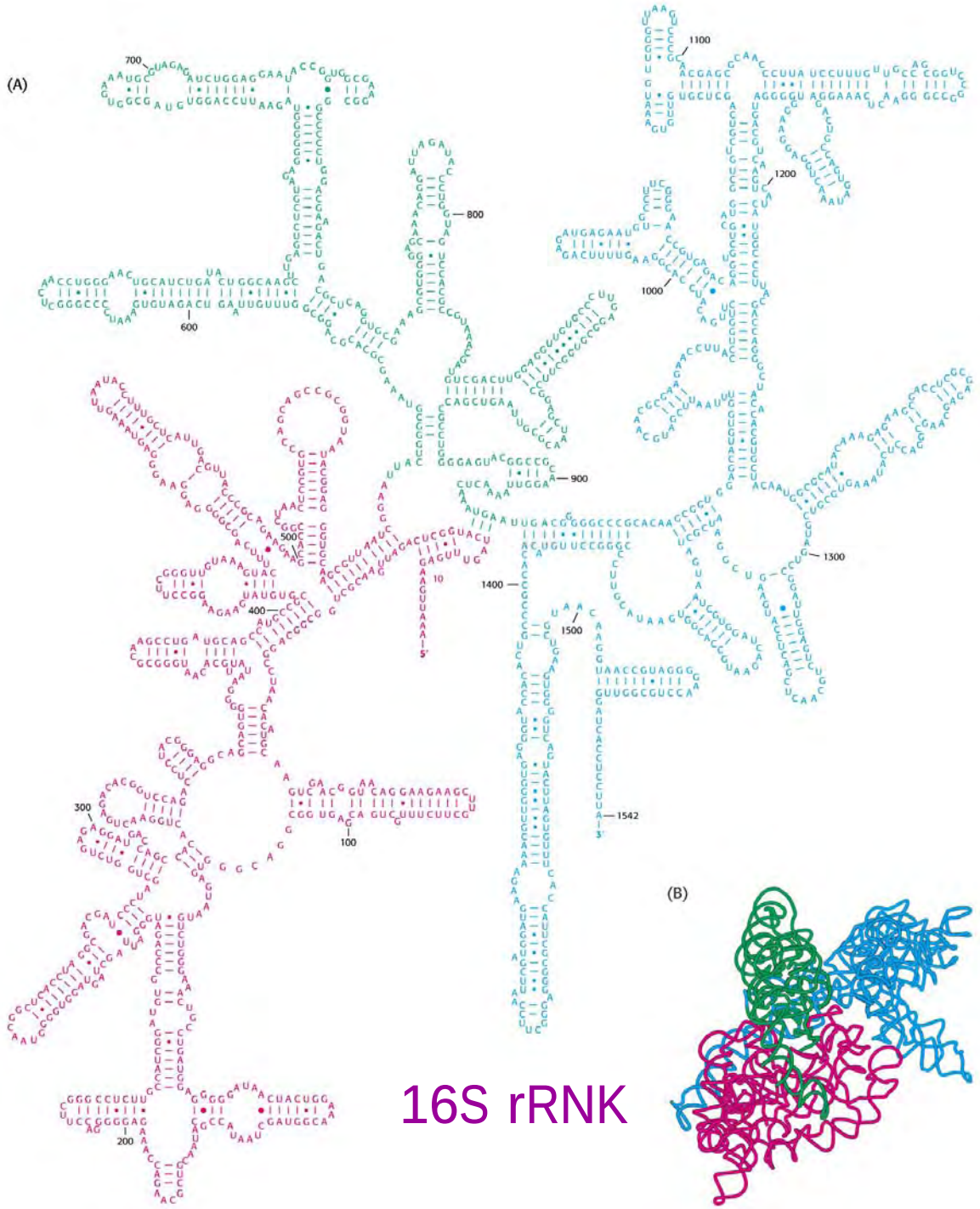
3° structure

Razlika (u odnosu na protein):

- uloga metalnih jona (Mg^{2+});
- stabilnost izolovanih sekundarnih struktura



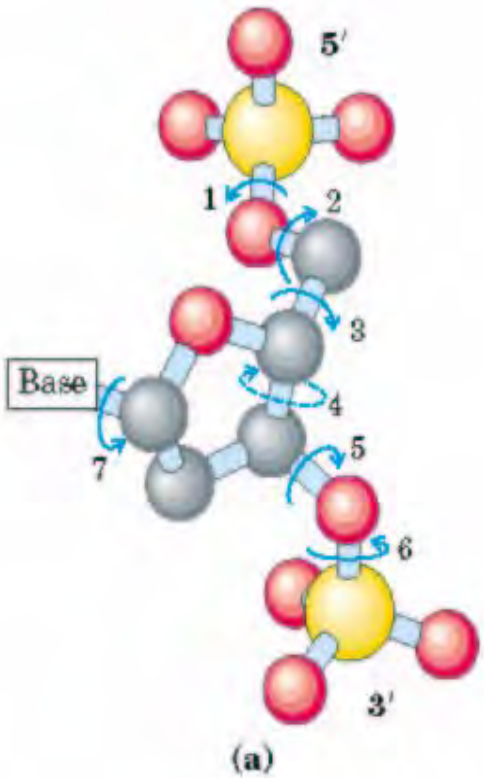
Ribozomalna RNK

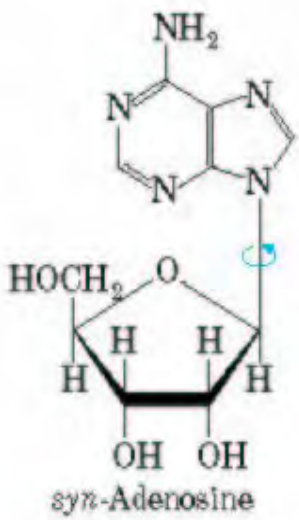


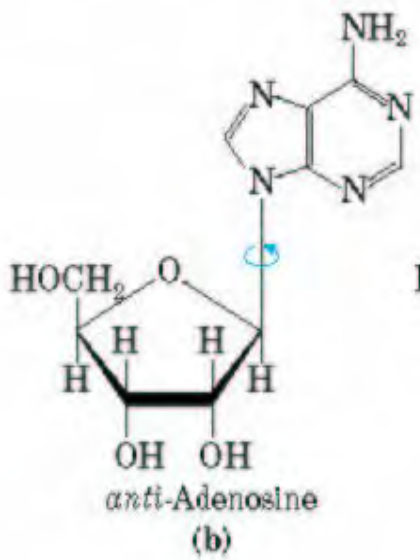
Zašto polimorfizam u DNK
i konzervatizam u RNK???

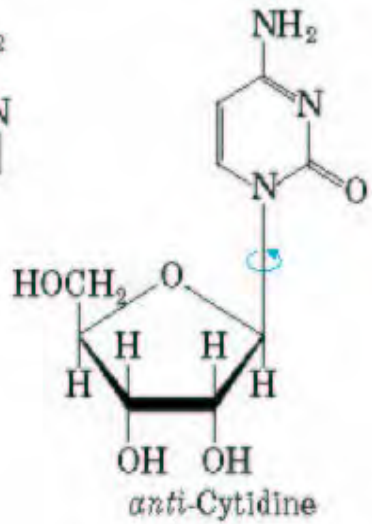
– Efekat fosfodiestarke kičme (šećera!)
– Efekat sekvence baza

Konformaciona analiza nukleotida

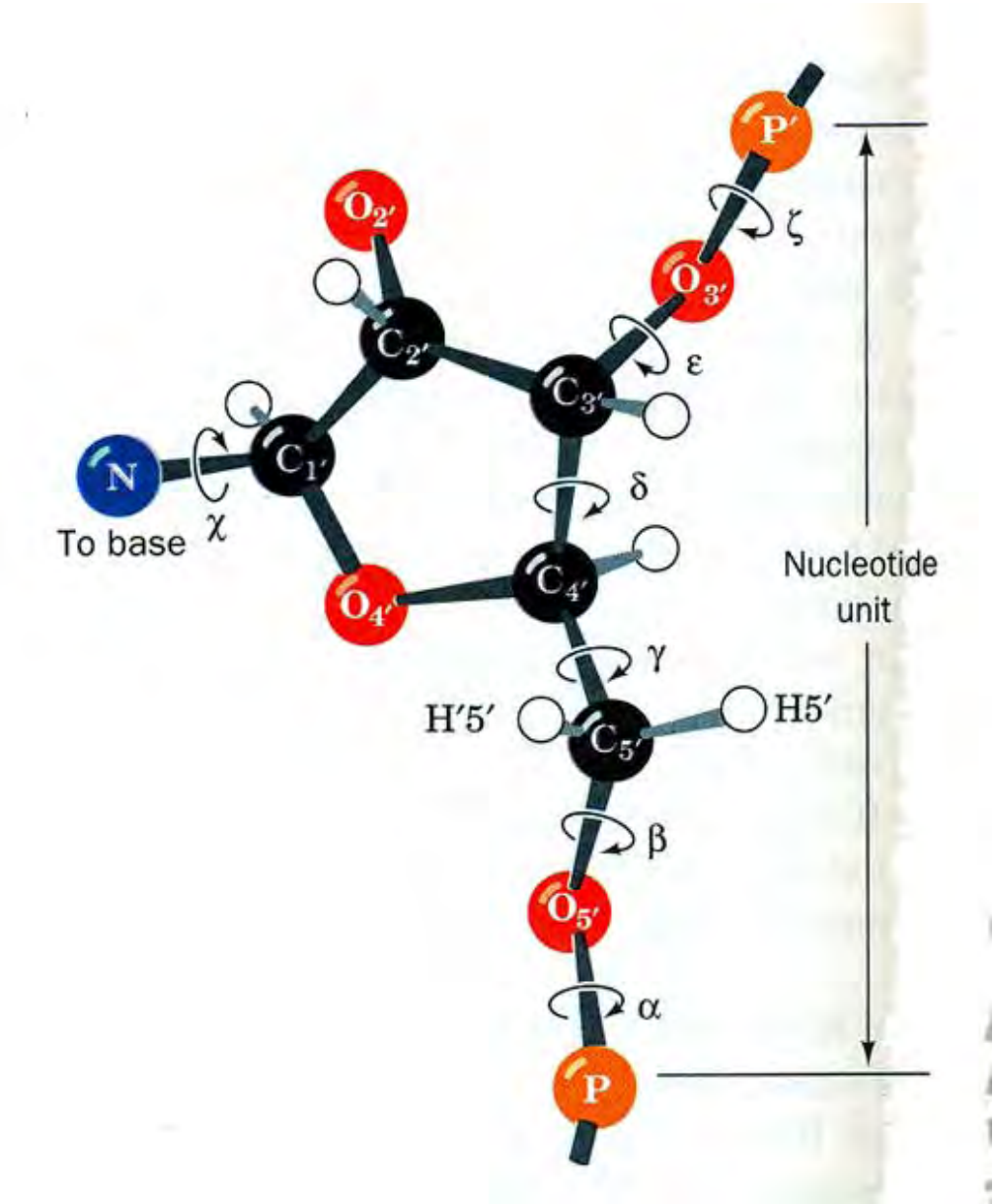




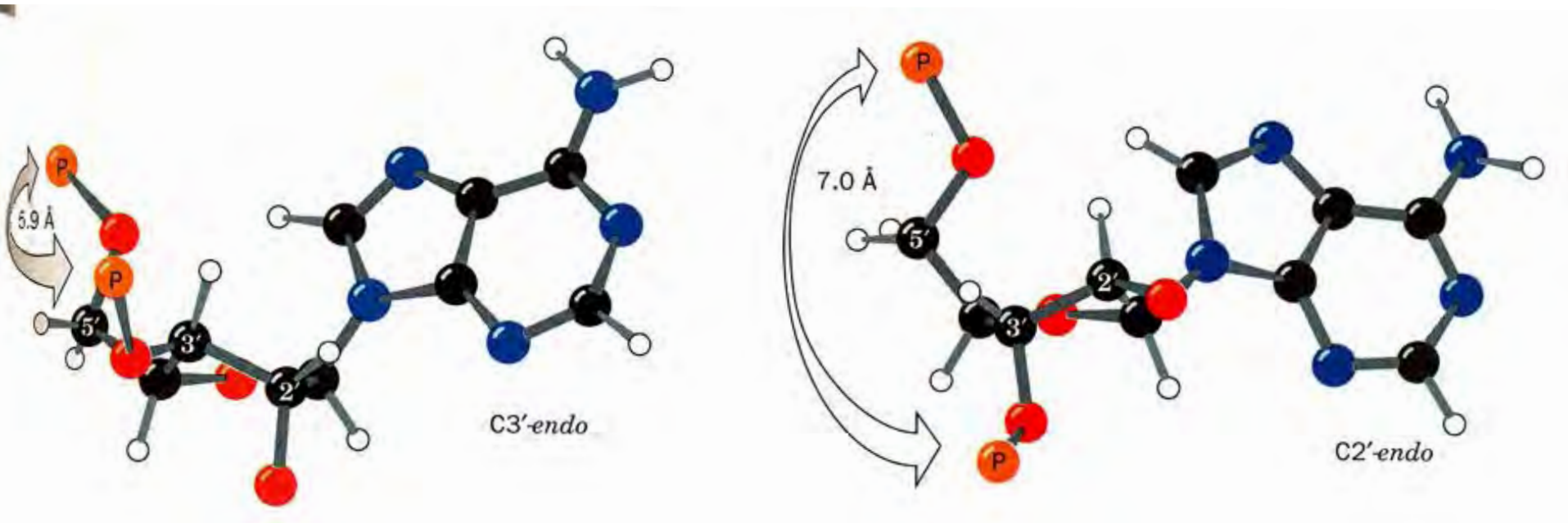




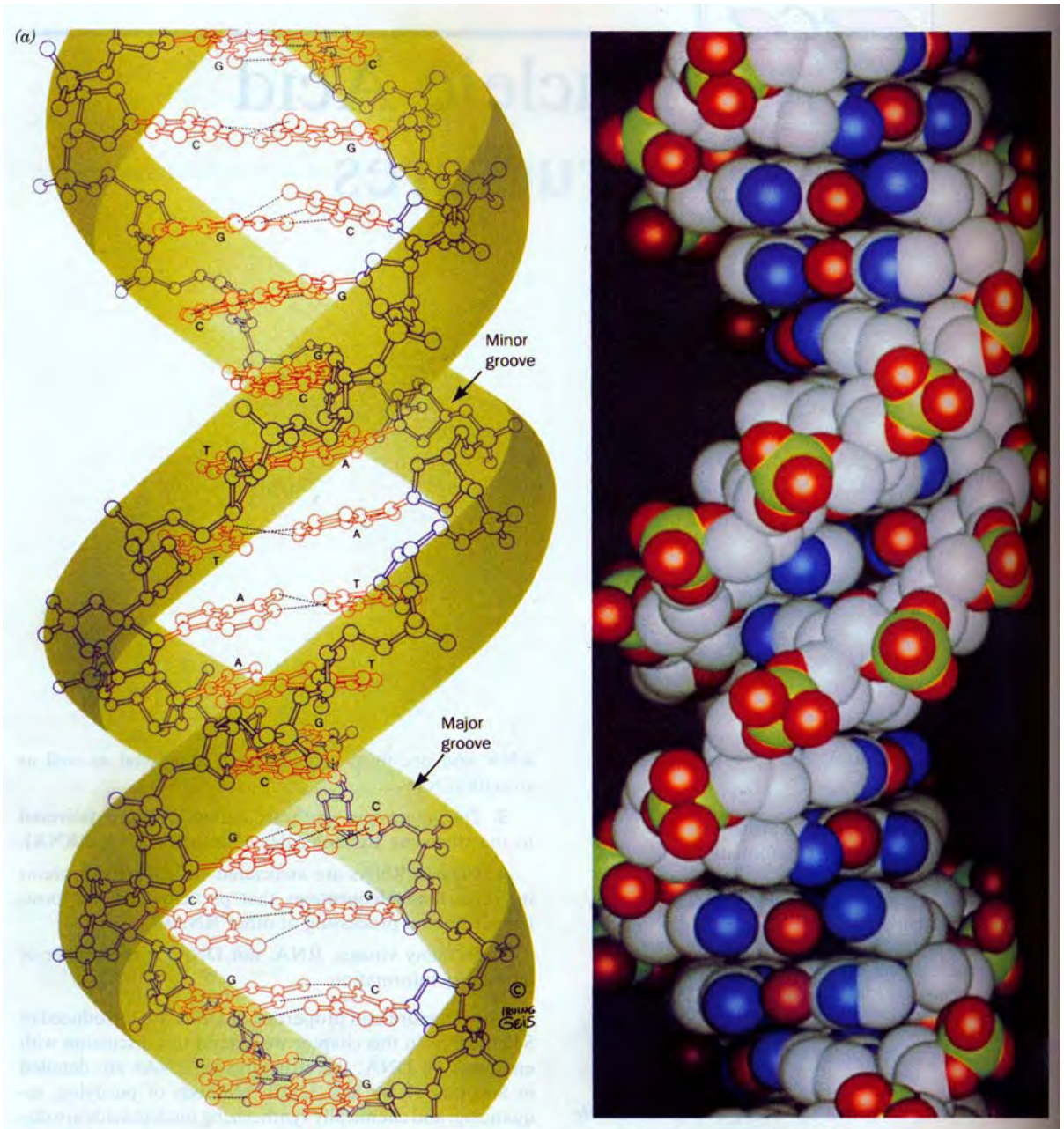
Konformaciona analiza nukleotida



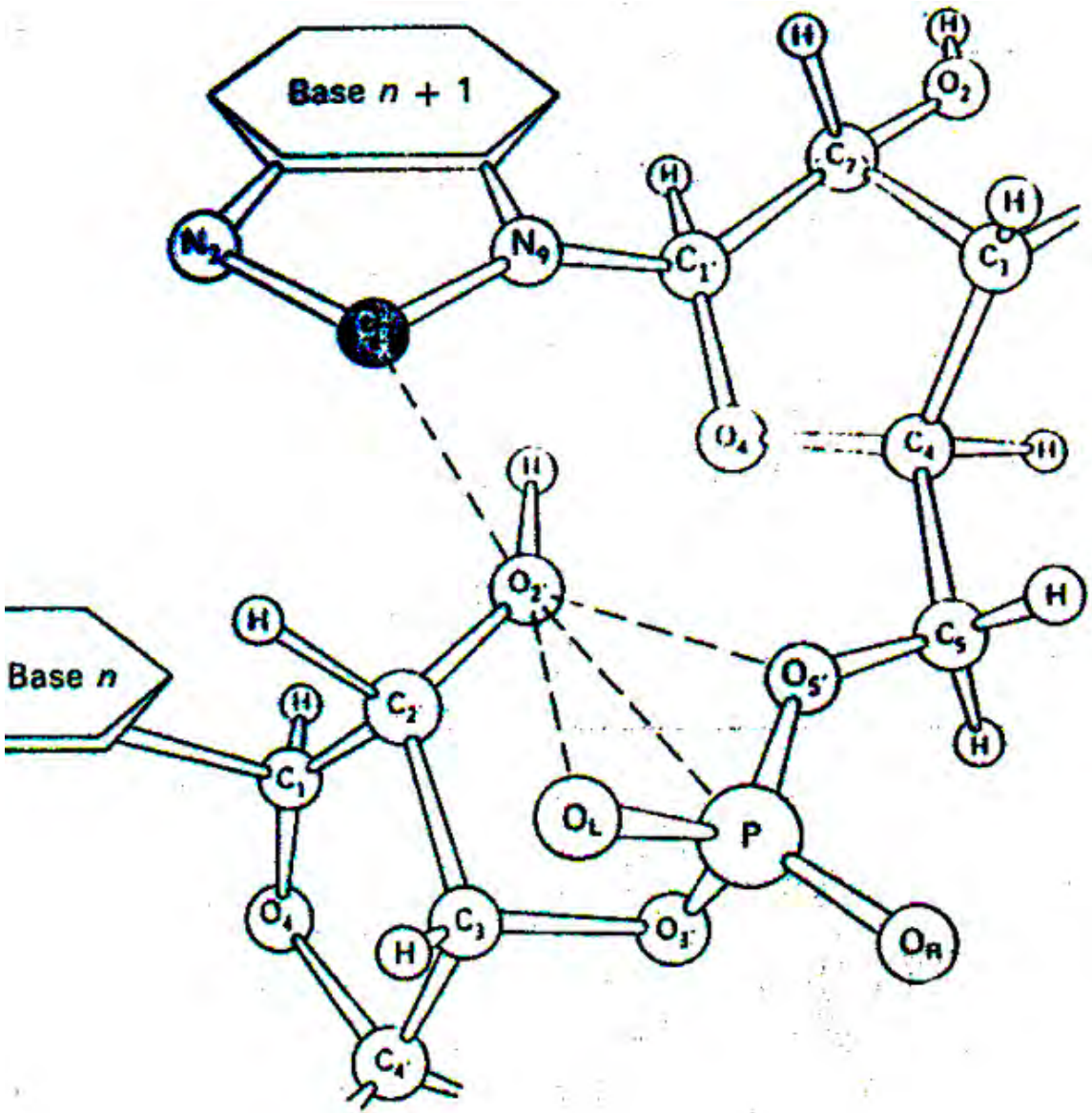
Konformaciona analiza šećera



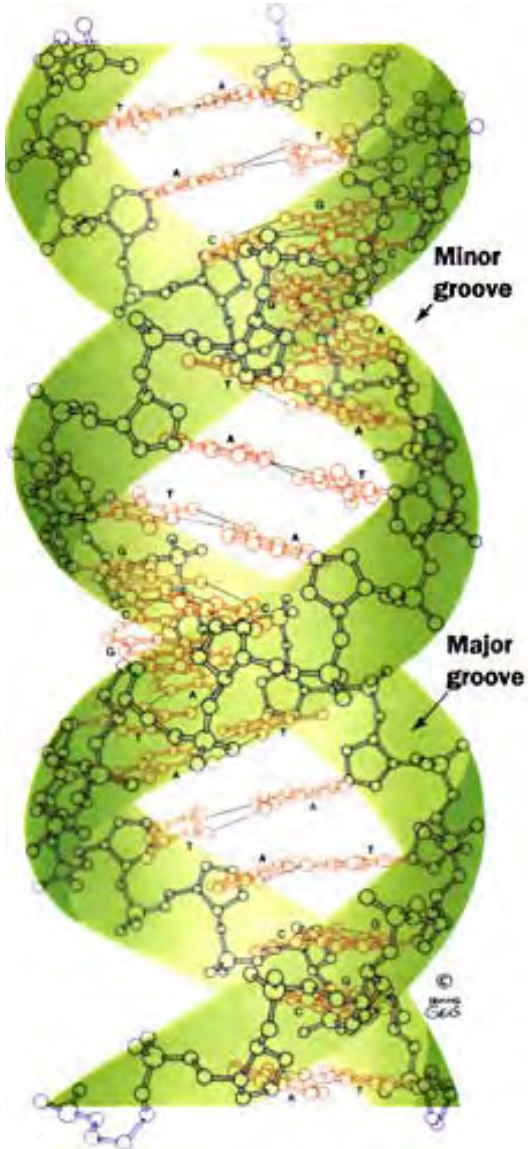
Zašto riboza ne može u B heliks?

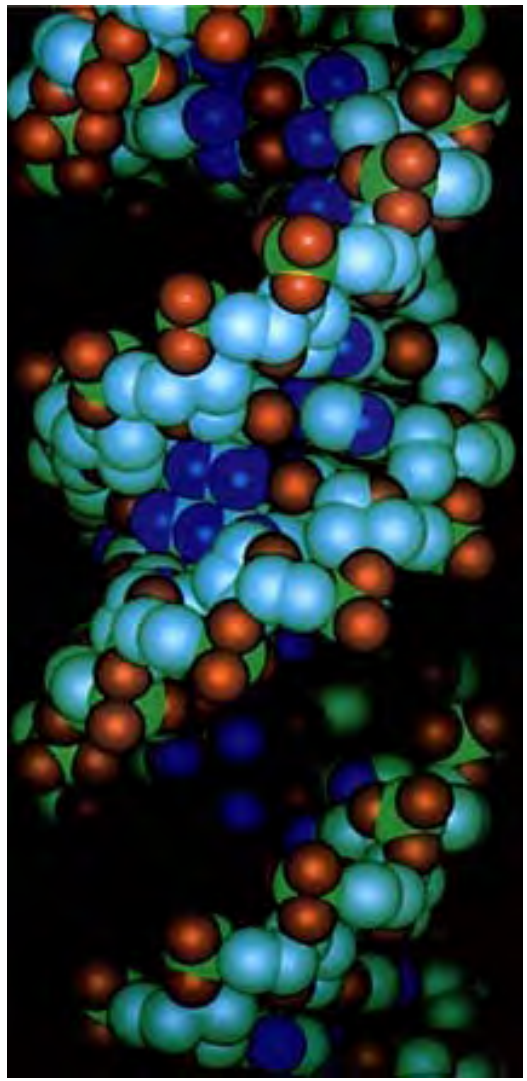


Zašto riboza ne može u B-heliks?

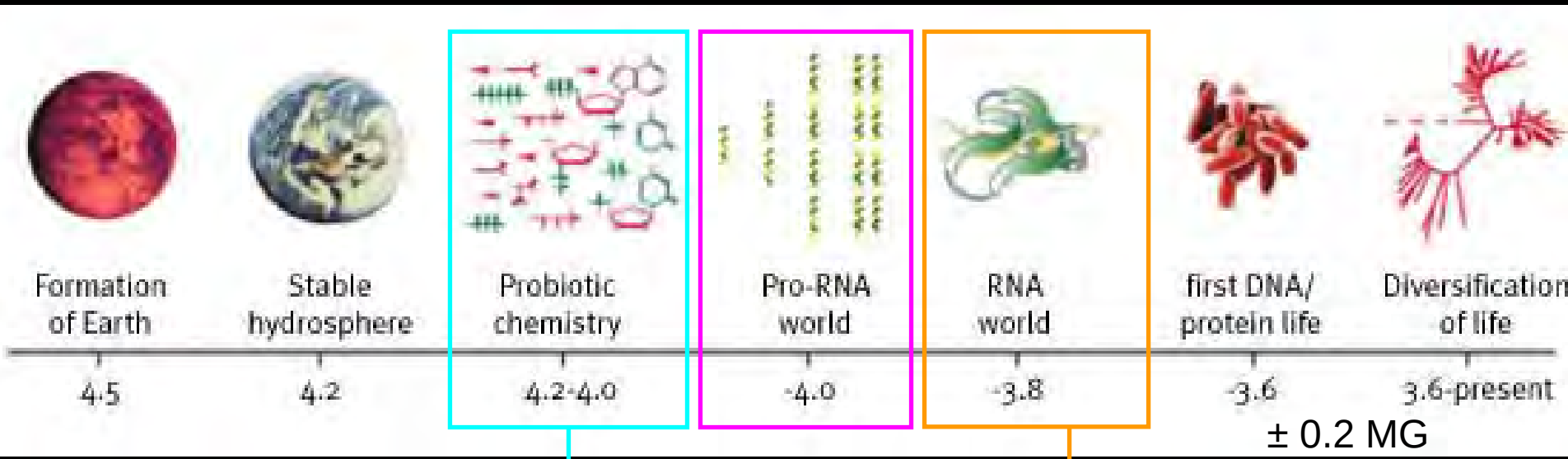


.....a može u A-heliks?



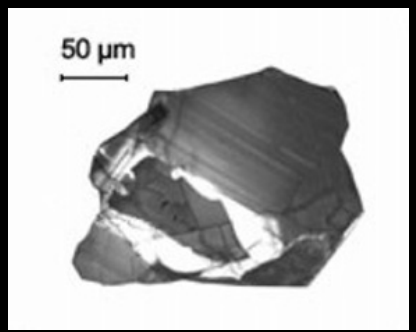


Evolucija života



Nastanak života!

Prebiološka evolucija



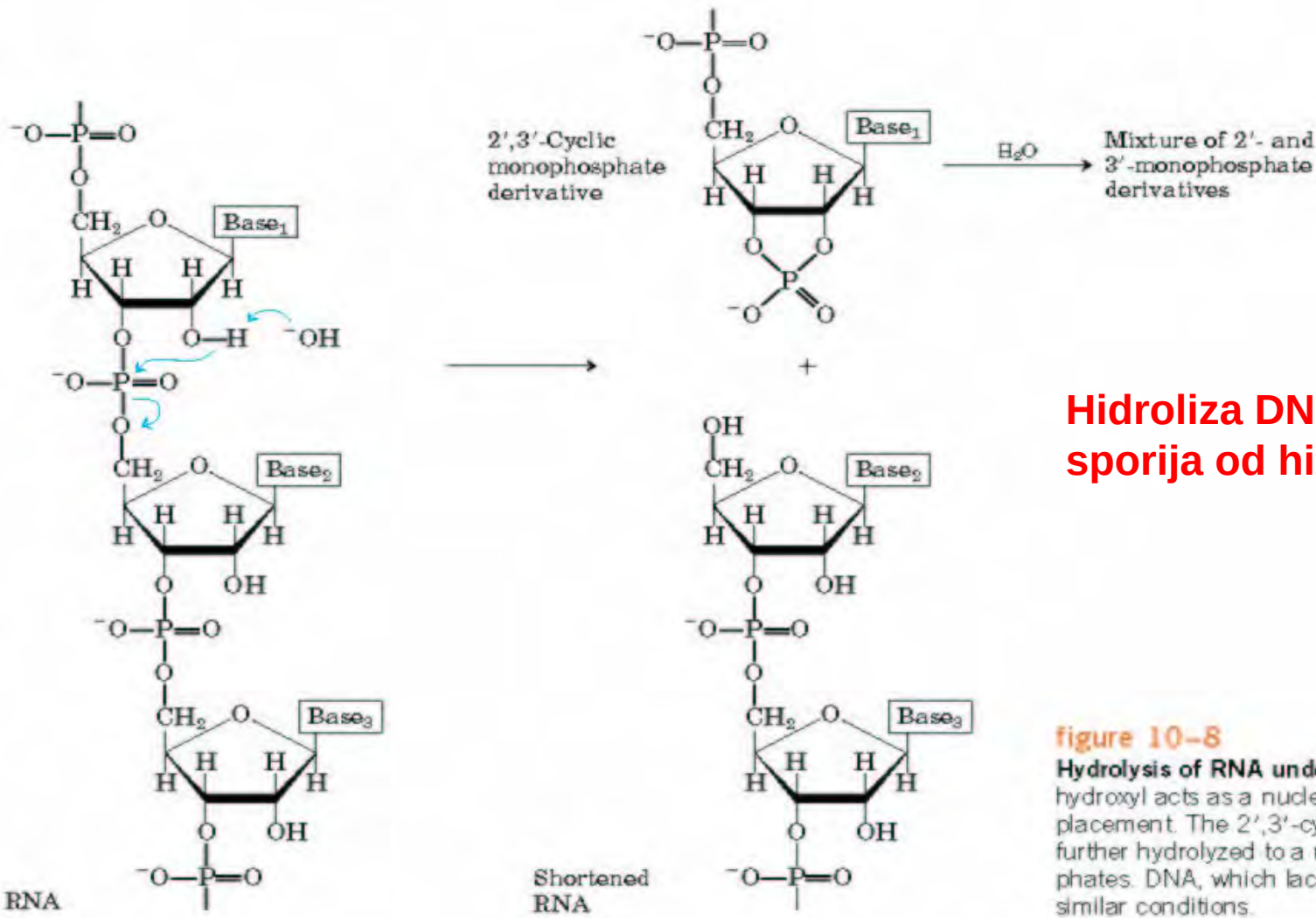
Hemijski dokazi:
prebiotička sinteza (hemijska evolucija): biomolekula

-Biohemijski dokazi

-RNK može da bude i katalizator

-DNK je stabilnija u vodenoj sredini od RNK!!!!

Zašto je RNK podložnija hidrolizi od DNK?



Hidroliza DNK je 100x sporija od hidrolize RNK!!!!

figure 10–8
Hydrolysis of RNA under alkaline conditions. The 2′ hydroxyl acts as a nucleophile in an intramolecular displacement. The 2′,3′-cyclic monophosphate derivative is further hydrolyzed to a mixture of 2′- and 3′-monophosphates. DNA, which lacks 2′ hydroxyls, is stable under similar conditions.