

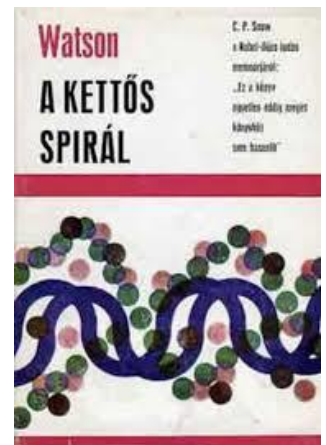
4. Előadás

Nukleozidok, nukleotidok, nukleinsavak

Történeti háttér

Savas karakterű anyagok a sejtmagból

- 1869-71 DNS a sejtmag fő komponense - "nuclein"
Friedrich Miescher (Svájc, 1844-1895), izolálás
1970: FM Institute for Biomedical Research, Basel
Western blotting (protein)
- 1882 Walther Flemming: „Chromatin” elnevezés
W. Waldeyer: CHROMO → (szín) SZOMA
(„coloured body”, festék láthatóvá tétel)
- 1934 - DNS polimer és molekulatömege kb. 500 000
E. Hammersten, T. Caspersson
- tisztított növényi vírusban (tobacco mosaic virus) is van
W. M. Stanley (USA), Nobel díj, kémia, 1946
- 1940 első elektronmikroszkópos kép
→ DNS
→ pozitívan töltött fehérjék
- 1947-50 nukleotid összetétel - E. Chargaff (USA)
- 1953 kettős hélix - J. Watson, F. Crick (UK) (N.d. 1962)
- 1958 DNS polimeráz I enzim (első DNS „készítő” enzim)
- 1960 RNS polimeráz; mRNS felfedezése
- 1964 $^3\text{H-RNS}_{\text{Ala}}$ szekvenálása - R. Holley (USA)
- 1966 genetikai kód megfejtése
- 1968 védett nukleotidok összekapcsolása
(A. Todd, H. G. Khorana)



- 1970 DNS hasító enzim (restriktációs) felfedezése
- 1973 DNS fragmensek beépítése plazmidba E. coli
„Androméda-törzs” (1971)
<https://videa.hu/videok/film-animacio/az-andromeda-torzsz-1971-x0MQSDagaZGVtI0v>
- 1975 RNS kromoszóma szekvenálása; MS2 fág (3 fehérje)
- 1977 GENENTECH
- 1978 somatostatatin: az első emberi hormon,
rekombináns technológiával
(W. Arber, D. Nathans, H. O. Smith: Nobel-díj: restriktációs enzimek)
- 1979 malignus sejtből származó DNS-sel „fertőzni” lehet
egészséges sejtvonalat
- 1980 Nobel-díj: DNS szekvenálás; rekombináns DNS szintézis
F. Sanger; W. Gilbert
- 1982 - humán inzulin (DNS technológia) a piacon (humulin)
- első onkogének izolálása, expresszálása, szekvenálása
(egy aminosav különbség)
- 1983 DNS a λ baktérium fágból (48 502 bázispár)
- 1984-03 HUMAN GENOM projekt elfogadása (USA Kongresszus)
kb. 3×10^9 bázispár 3 milliárd USD 1 USD/ bázispár
- 1992 The Sanger Centre, Cambridge (DNA szekvenálás, HUGO)
- 2000 Wellcome Sanger Institute
„the role of genetics in health and disease”
- 2009 Nobel-díj: Telomer/telomeráz védi a kromoszómát
E. H. Blackburn, C. W. Greider, J. W. Szostak
- 2015 The Francis Crick Institute, London
(1500, inc. 1250 kutató, GBP 100 millió)

Felosztás

Nukleotid koenzimek

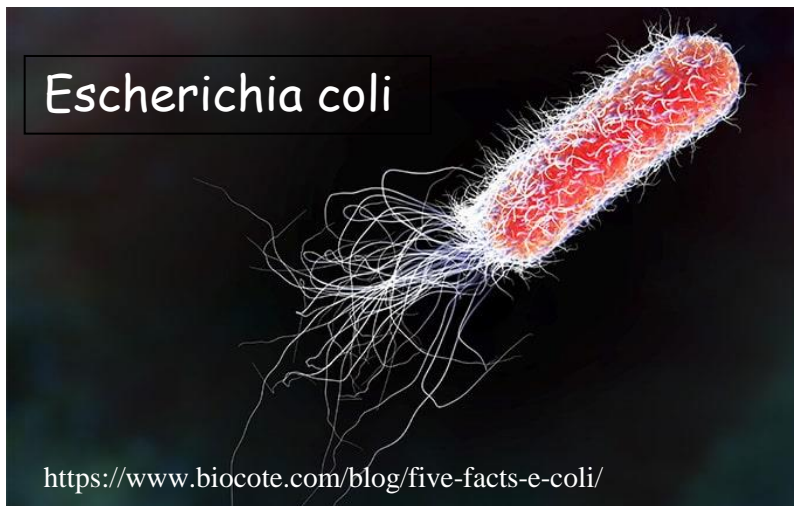
ADP, ATP
NAD
FAD
Koenzim-A (CoA)
uridin-difoszfát
(UDP)

RNS

Mt: 10^4 - 10^6
plazma
vírusok
láncossz: $> 3 \times 10^3$

DNS

Mt: 10^8 - 10^{12}
sejtmag
vírusok
láncossz: $> 10^7$



Felfedezés:
Theodore Eschrich,
1884

Gram-negative

O157:H7 törzs:
E. coli sejt

0,4 %

db/sejt $1,2 \times 10^7$

6 %

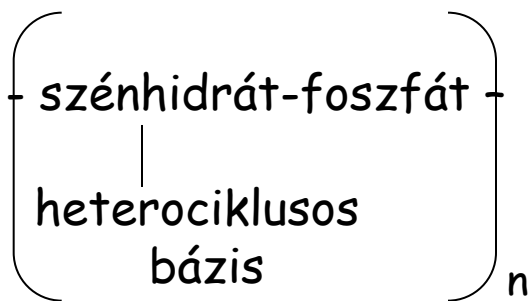
rRNS: 6×10^4
tRNS: 4×10^5
mRNS: 10^3

1 %

4

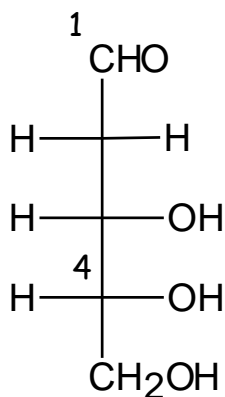
A nukleinsavak primer szerkezete

Alapkomponensek

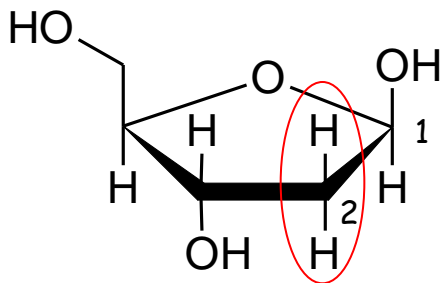


1. Szénhidrát: monoszacharid, aldopentóz

β -D-2-dezoxiribóz (DNS)

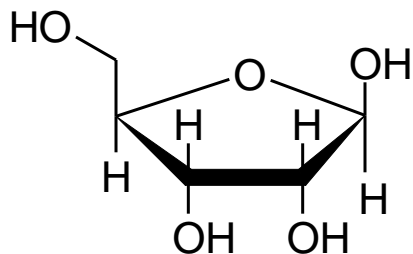
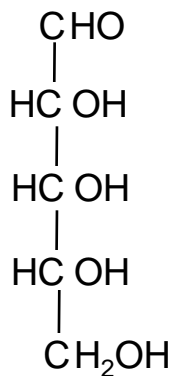


D-2-dezoxiribóz



β -D-2-dezoxiribóz

β -D-ribóz (RNS)

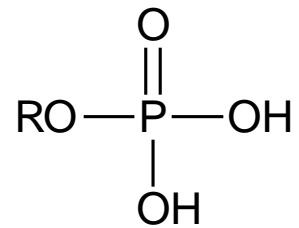
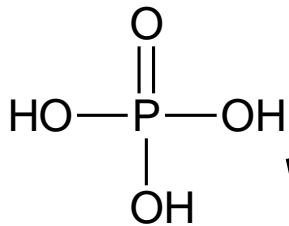


β -D-ribóz

2. Foszforsav(ak)

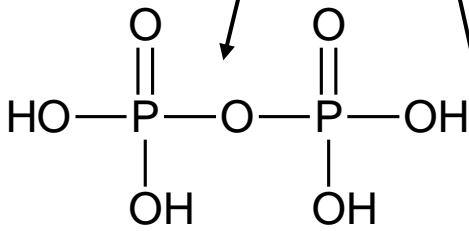
sav	konjugált bázis	K_a	pK_a
H_3PO_4	$H_2PO_4^-$	$7,1 \cdot 10^{-3}$	2,15
$H_2PO_4^-$	HPO_4^{2-}	$6,3 \cdot 10^{-8}$	7,20
HPO_4^{2-}	PO_4^{3-}	$4,2 \cdot 10^{-13}$	12,38

foszforsav
(ortofoszforsav)



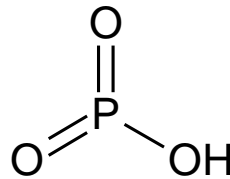
monoészter

160 °C

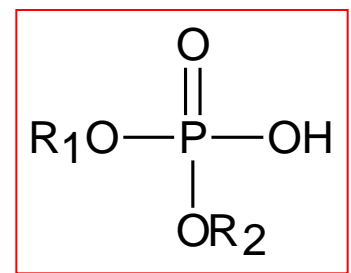


difoszforsav

>300 °C

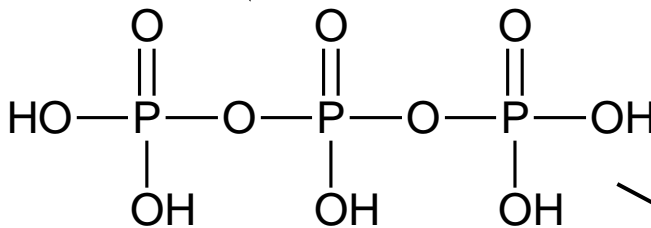


metafoszforsav

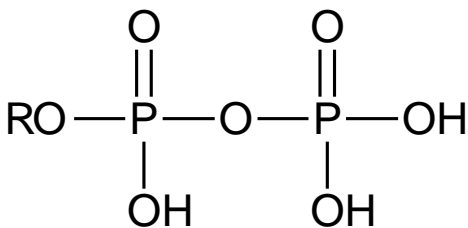


foszfodiészter

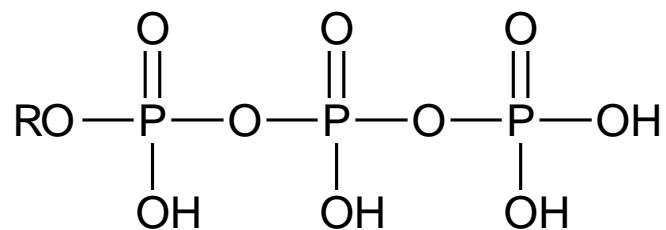
- lineáris
- ciklusos



trifoszforsav

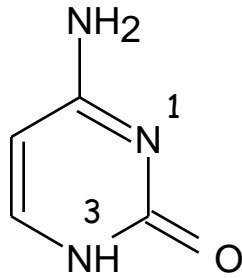


difoszforsav-észter

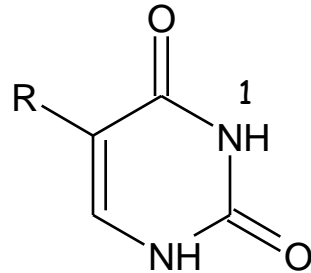


trifoszforsav-észter

3. Heterociklusos bázis

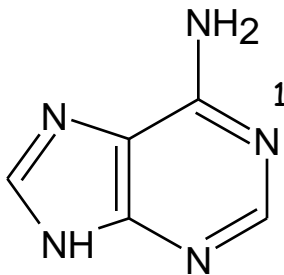


citozin
(C)

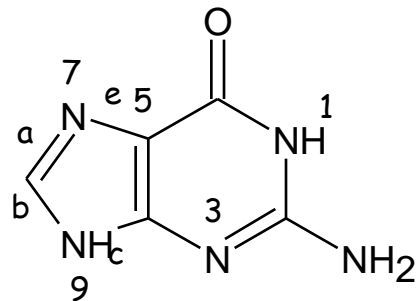


timin R = -CH₃ (T)
R = -H (U) (C)

pirimidin



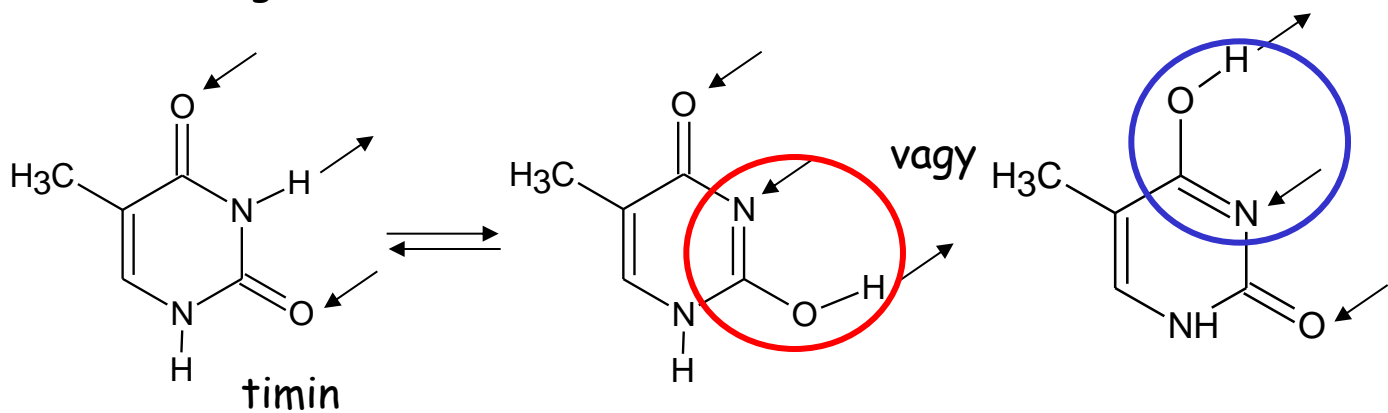
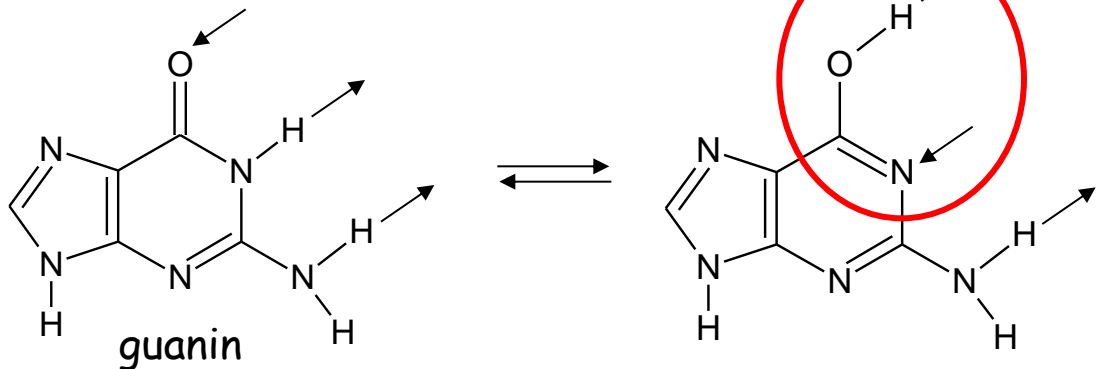
adenin
(A)



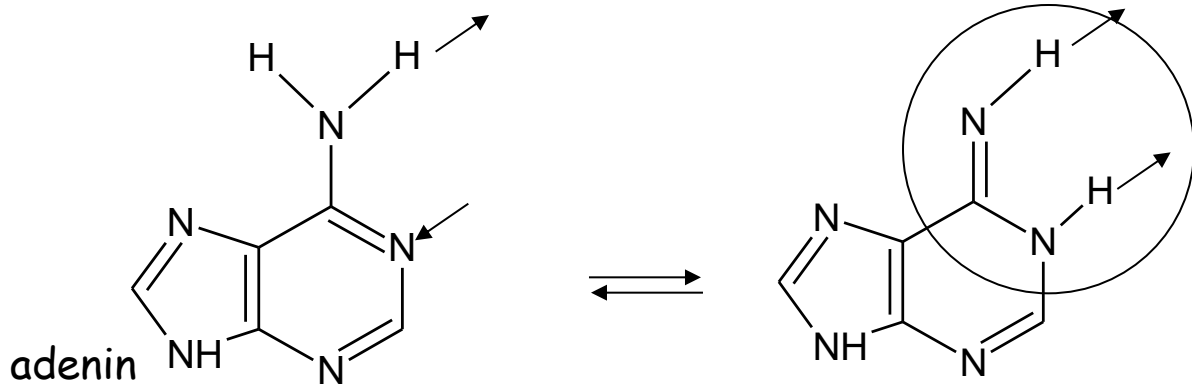
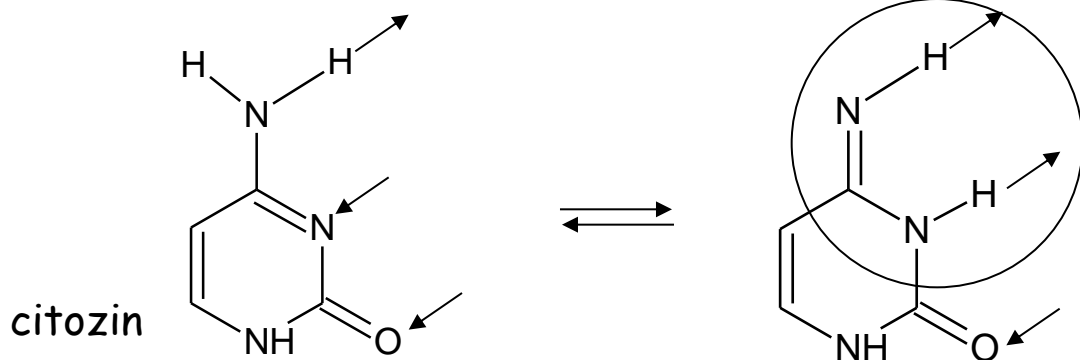
guanin,
(G)

purin (pirimidin(d)imidazol)

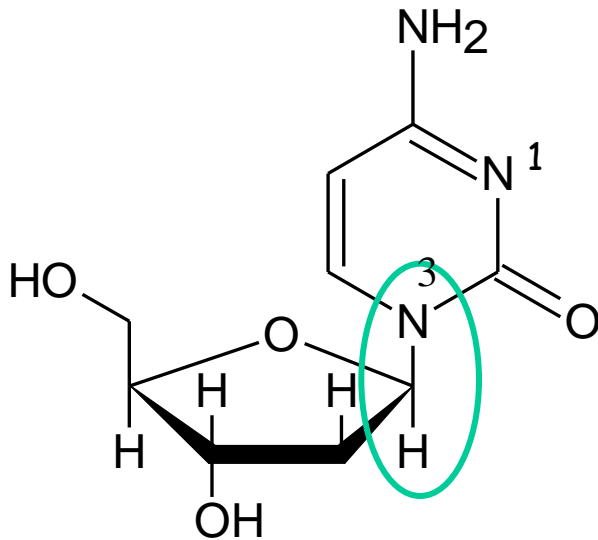
Keto-enol tautoméria



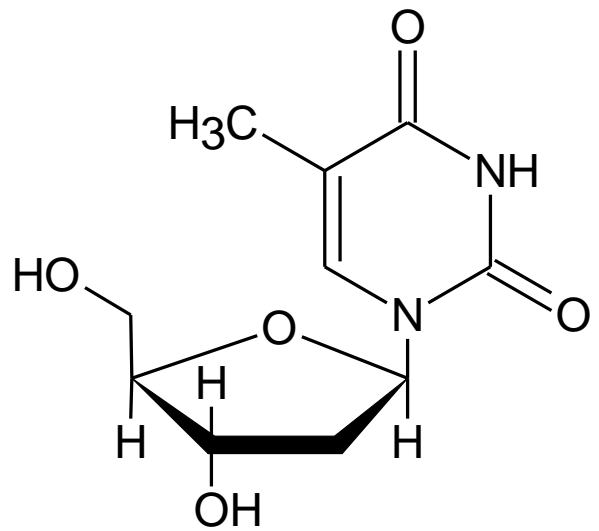
Amino-imino tautoméria



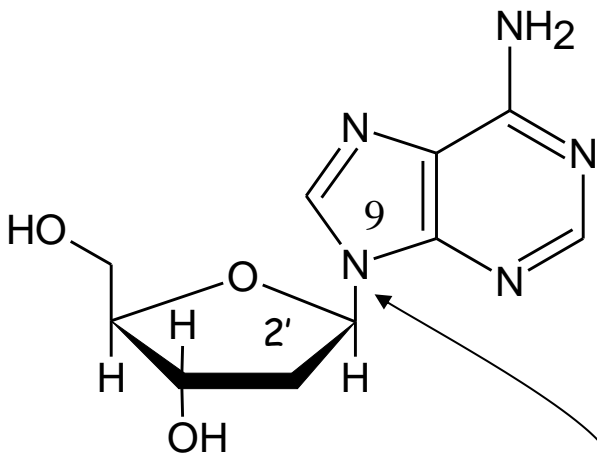
Nukleozidok szénhidrát és heterobázis (*N*- glikozid kötés)



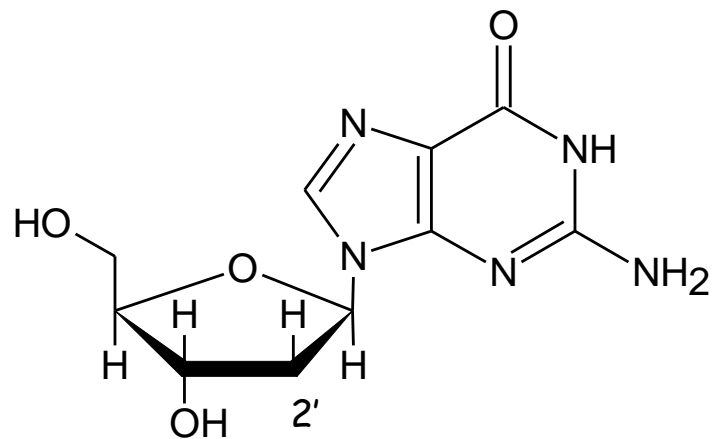
2'-dezoxicitidin



2'-dezoxitimidin



2'-dezoxiadenozin

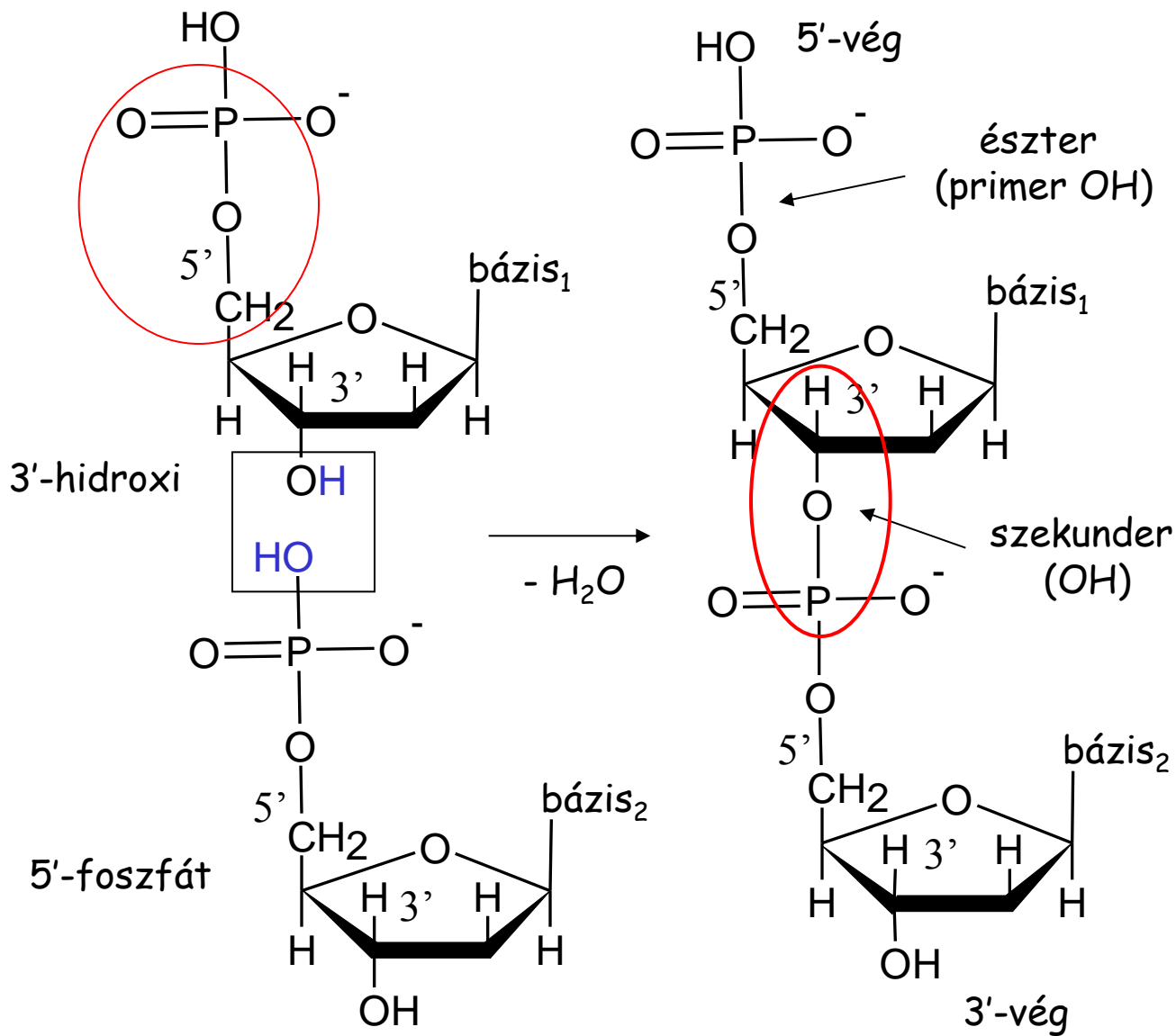


2'- dezoxiguanozin

β -*N*-glikozid

Nukleotidok

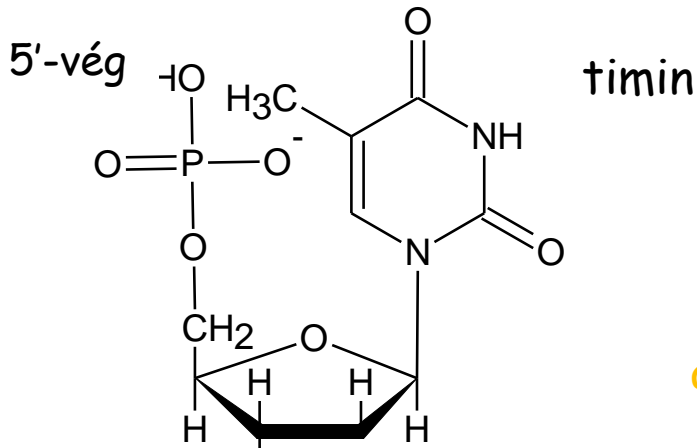
szénhidrát és foszforsav (3',5'- diészter kötés)



Ribonukleotidok ionizációs állandói (pK)

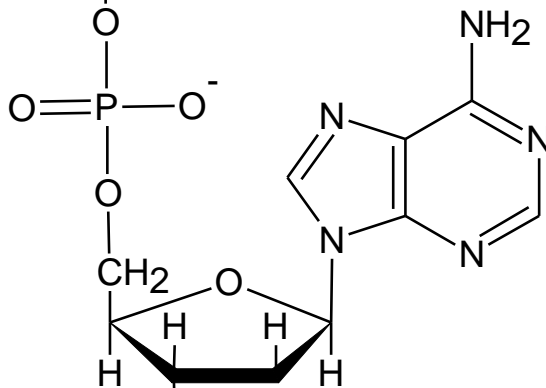
	Bázis	Szekunder foszfát	Primer foszfát
Adenozin-5'-foszfát (5'-AMP)	3,8	6,1	0,9
Uridin -5'-foszfát (5'-UMP)	9,5	6,4	1,0
Citidin-5'-foszfát (5'-CMP)	4,5	6,3	0,8
Guanin-5'-foszfát (5'-GMP)	2,4, 9,4	6,1	0,7

A polinukleotid lánc primer szerkezete



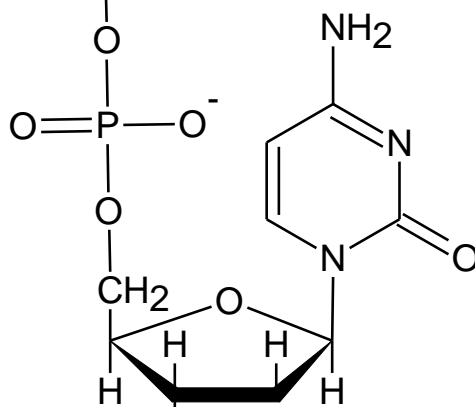
adenin

pK = 3,8



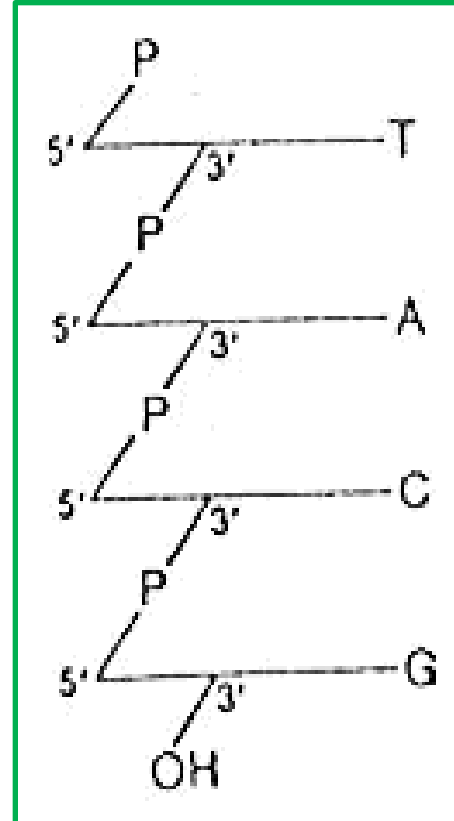
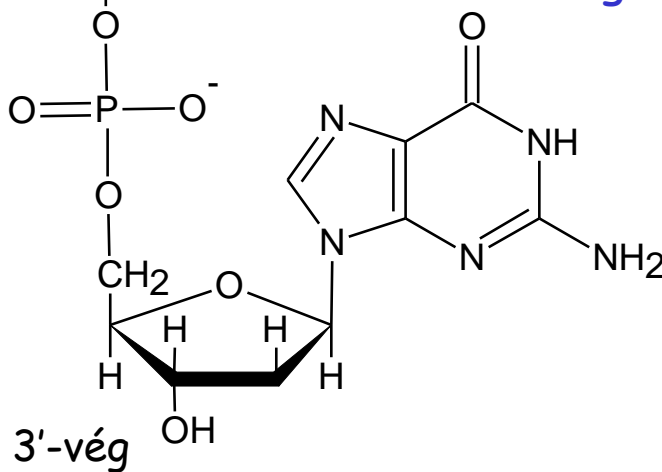
pK = 4,5

citozin

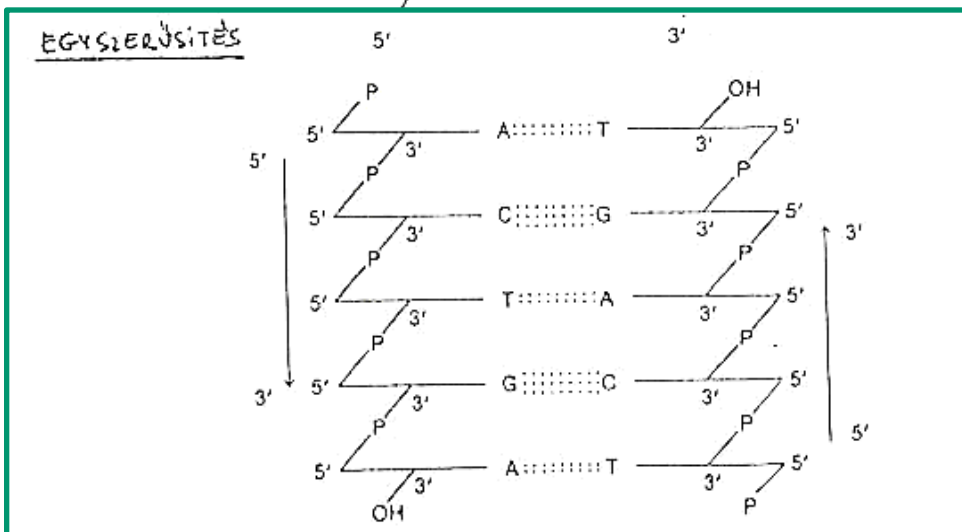
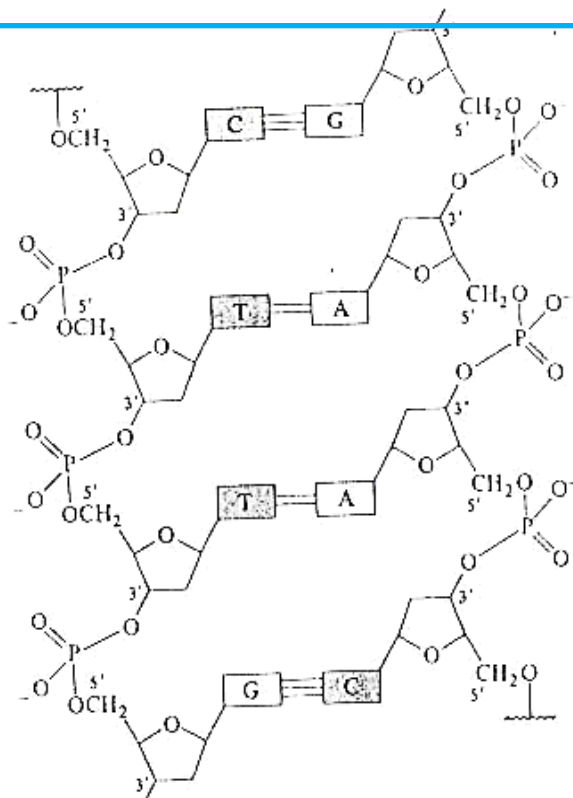
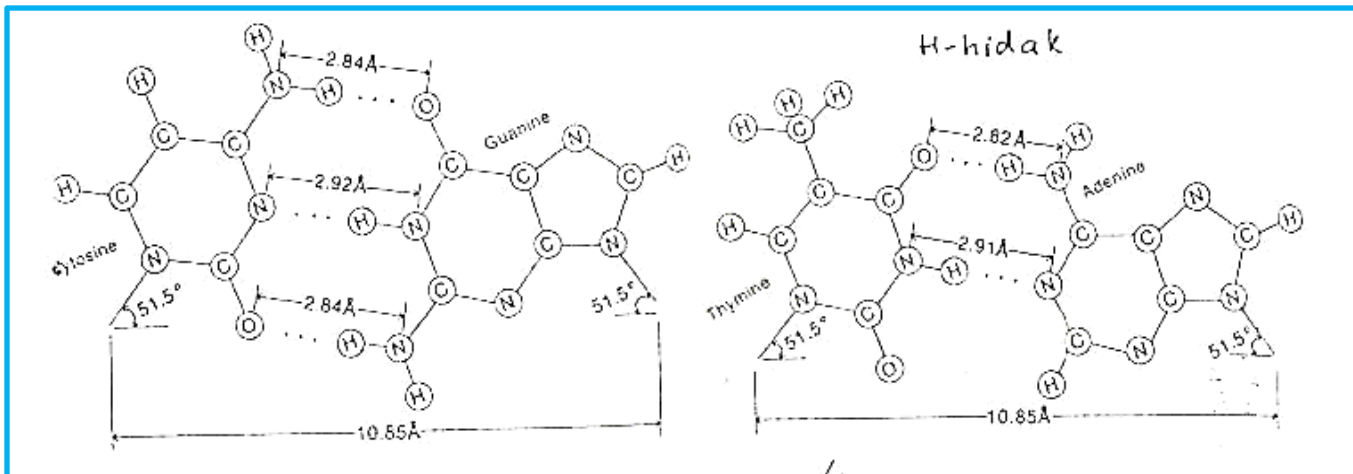


guanin

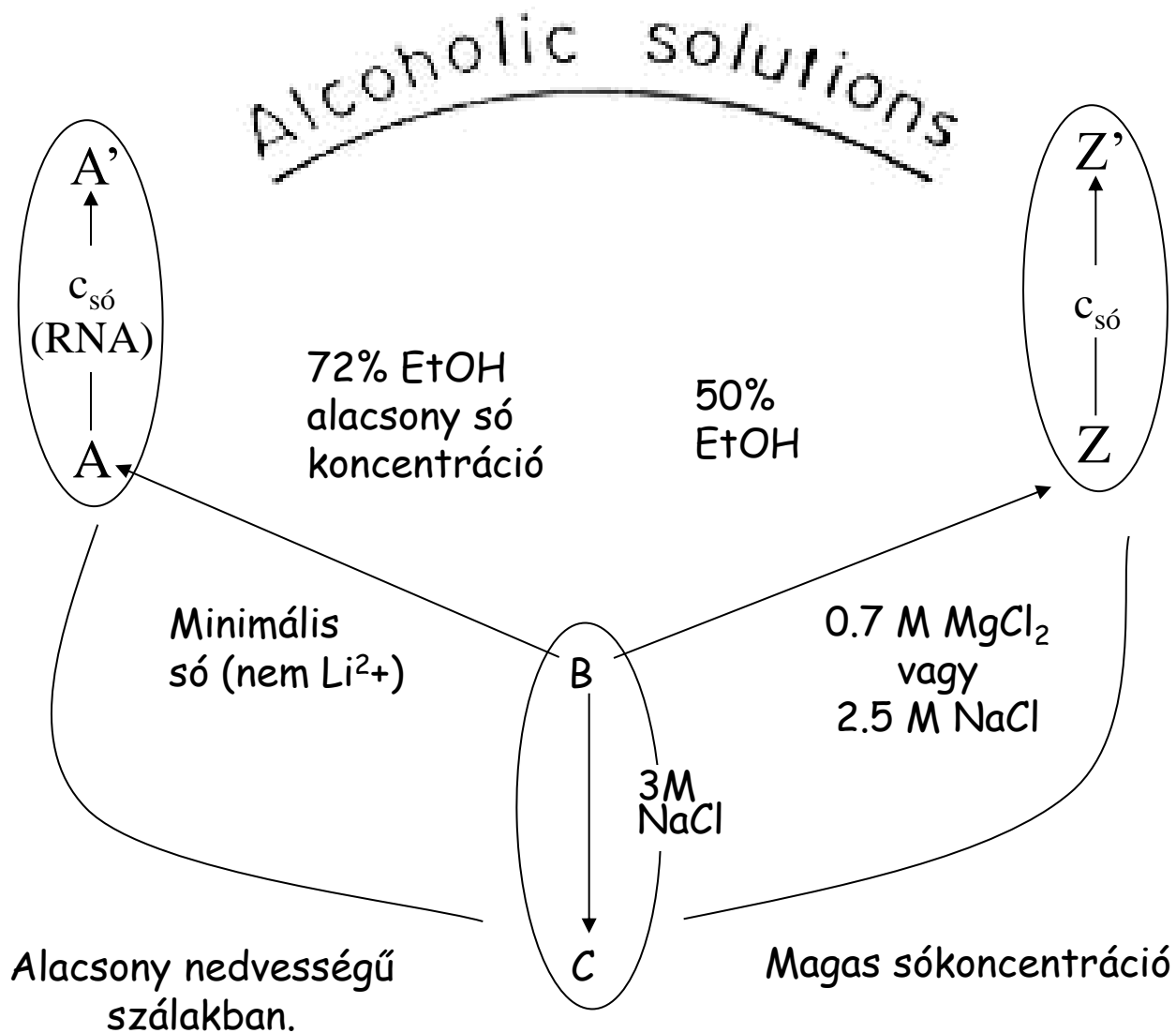
pK= 2,4
9,4



A kettős polinukleotid lánc primer szerkezete



Nukleinsav „társzerkezetek”

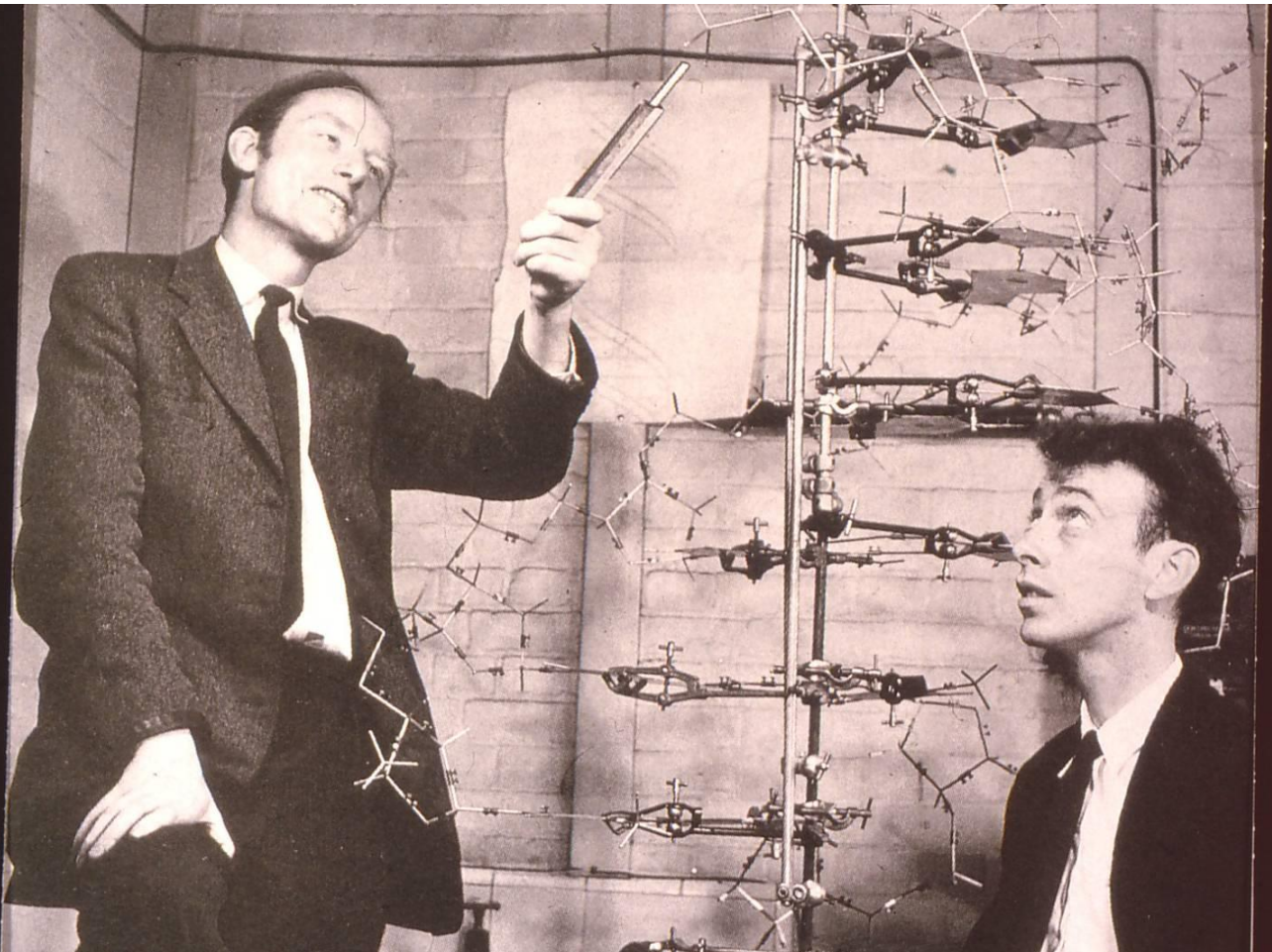




Francis Crick, 1916-2004



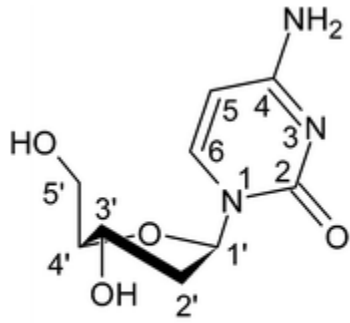
James D. Watson, 1928-



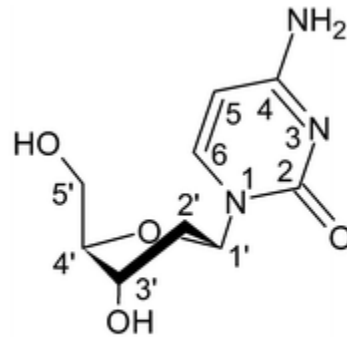
James D. Watson: *A kettős spirál*, Gondolat, 1970, 1972

A nukleinsavak térszerkezete

A 2-dezoxi-D-ribóz téralkata
(a gyűrű síkja fölötti C atom szerint)

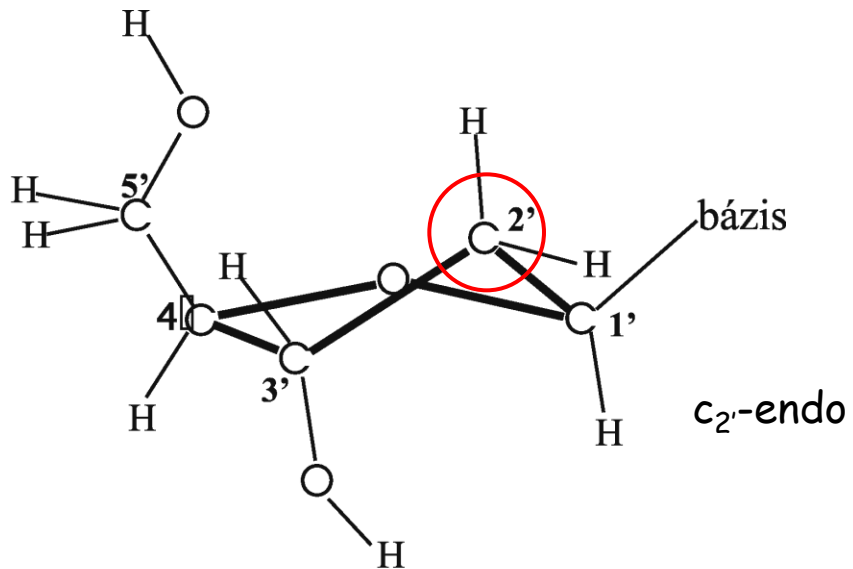


C3'-endo (N)

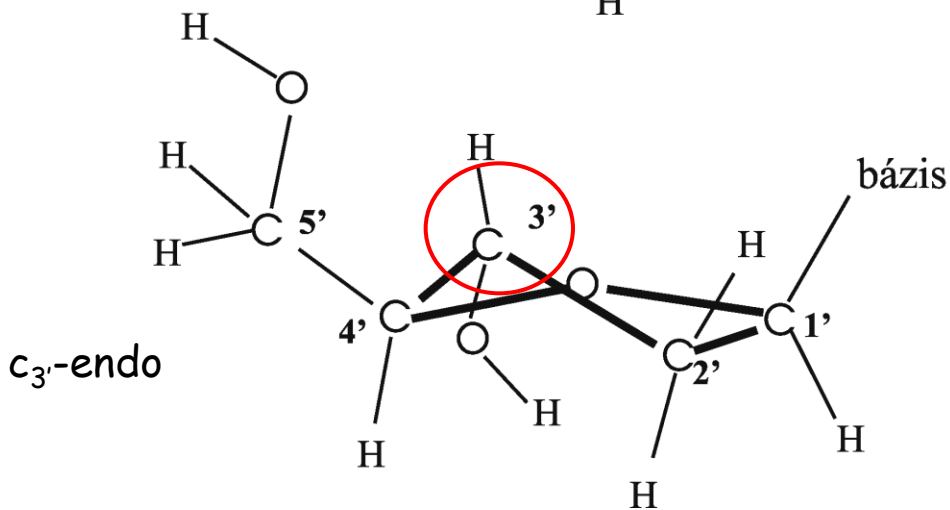


C2'-endo (S)

0,5 Å

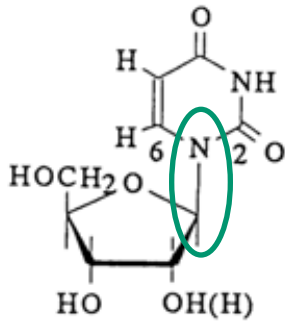


c₂'-endo

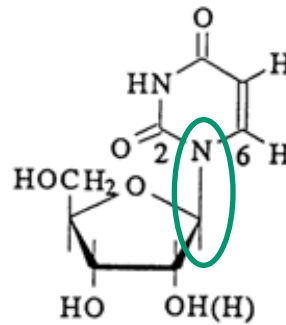


c₃'-endo

A β -glikozidkötéshez kapcsolódó konformerek

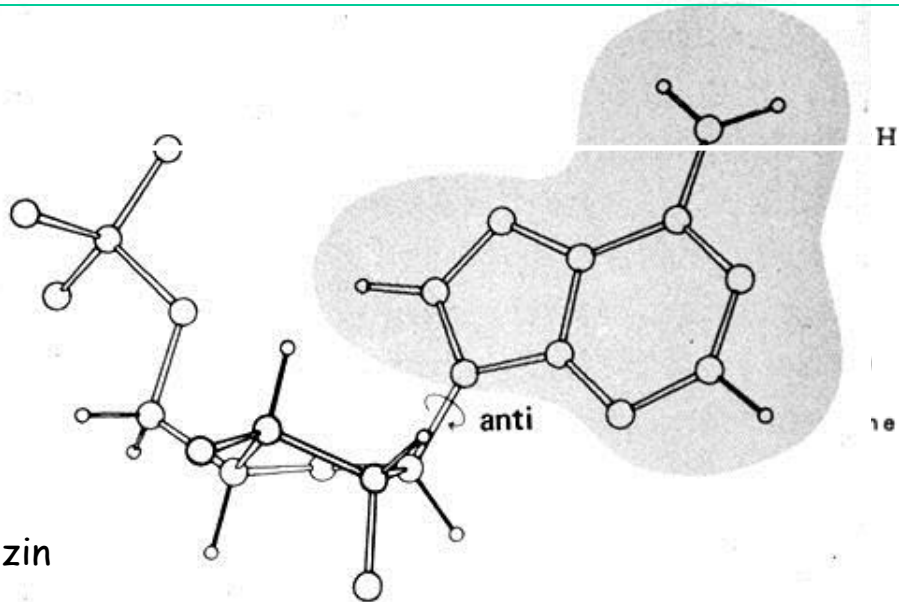


anti-uridin

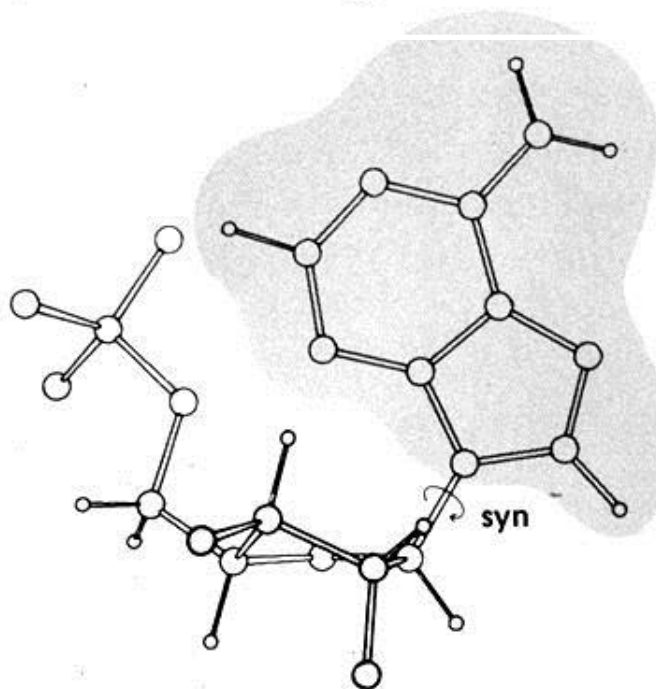


syn-uridin

anti-adenozin



anti-adenozin



A, B és Z típusú DNS jellemzői

	A	B	Z
Hélix irány	Jobbmenetű	Jobbmenetű	Balmenetű
Bázispár per kanyar	11	10,4	12 (6 dimers)
Bázispárok távolsága	2.3 Å	3.4 Å	3.8 Å
Menetemelkedés	25,3 Å	35,4 Å	45,6 Å
Bázispár dőlésszög	19 °	1 °	9 °

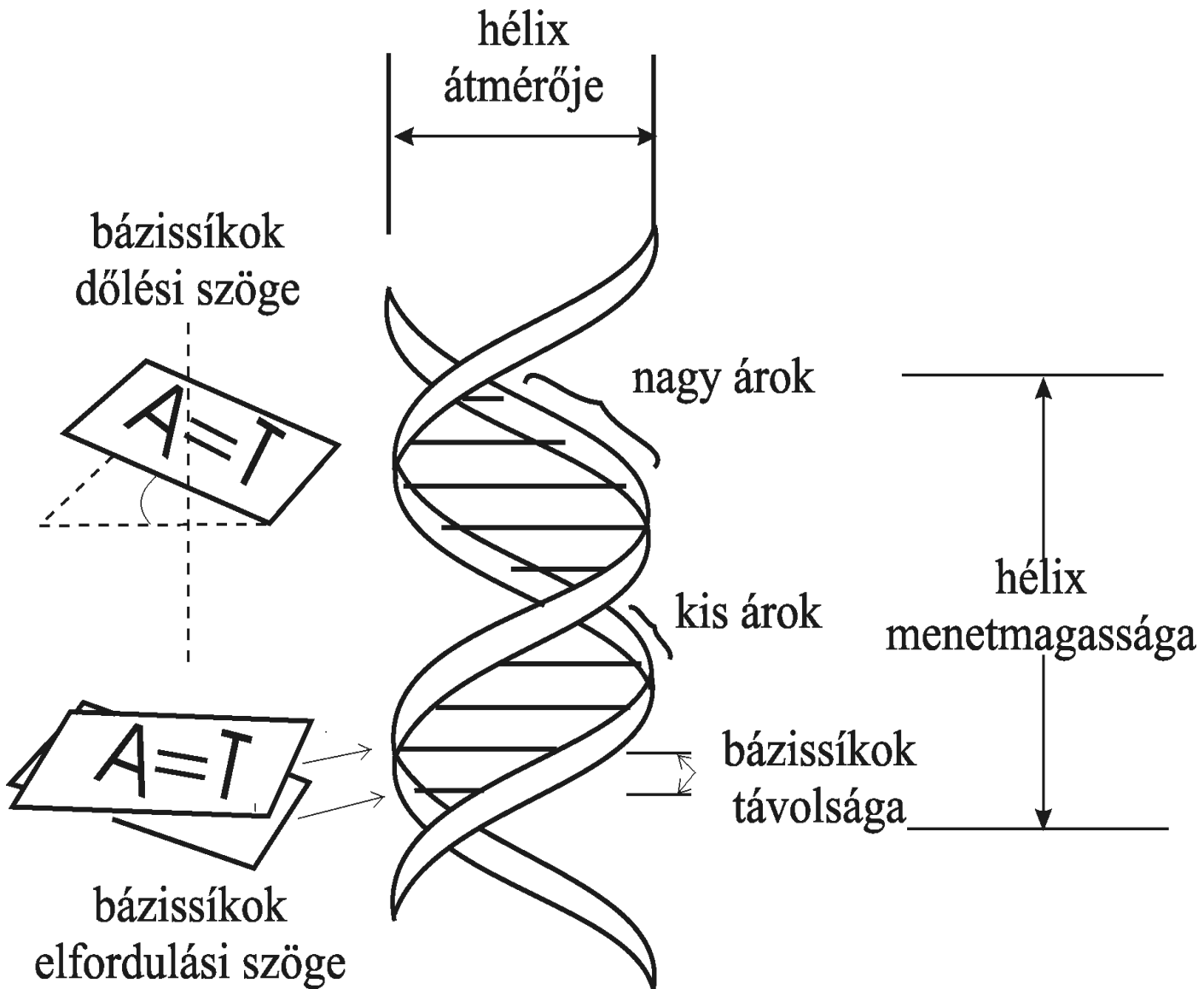
Glikozid konformáció

Dezoxicitidin	<i>Anti</i>	<i>Anti</i>	<i>Anti</i>
Dezoxiguanozin	<i>Anti</i>	<i>Anti</i>	<i>Syn</i>

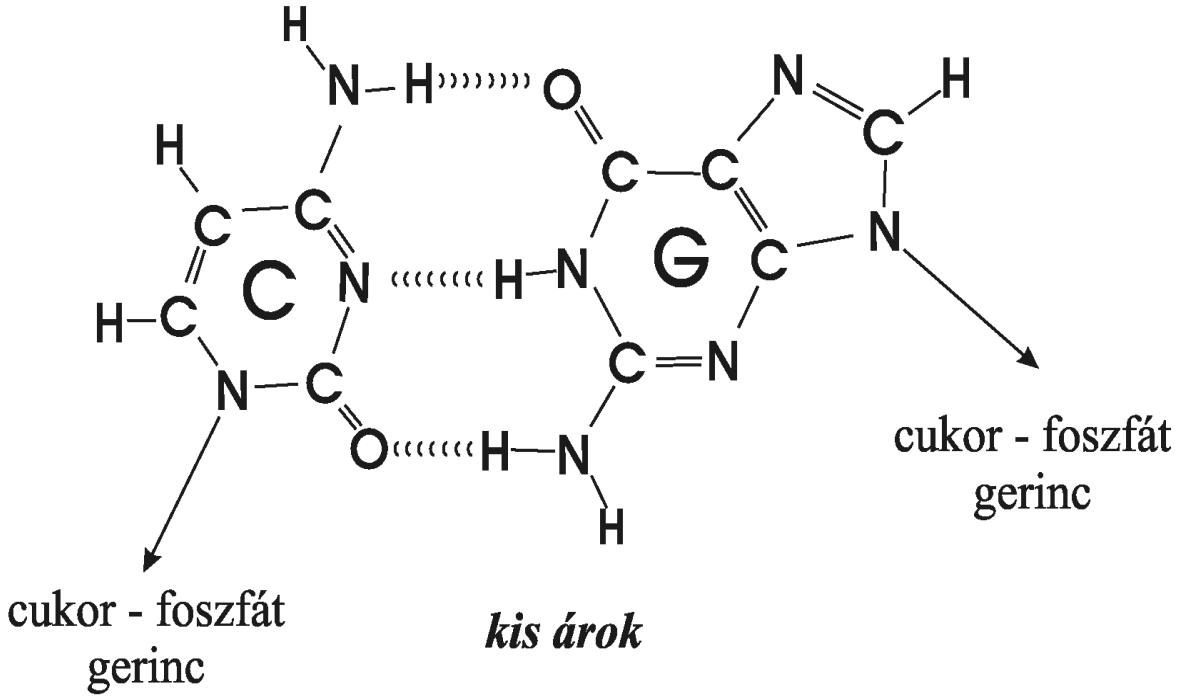
Szénhidrát konformáció

Dezoxicitidin	C-3'-endo	C-2'-endo	C-2'-endo
Dezoxiguanozin	C-3'-endo	C-2'-endo	C-3'-endo

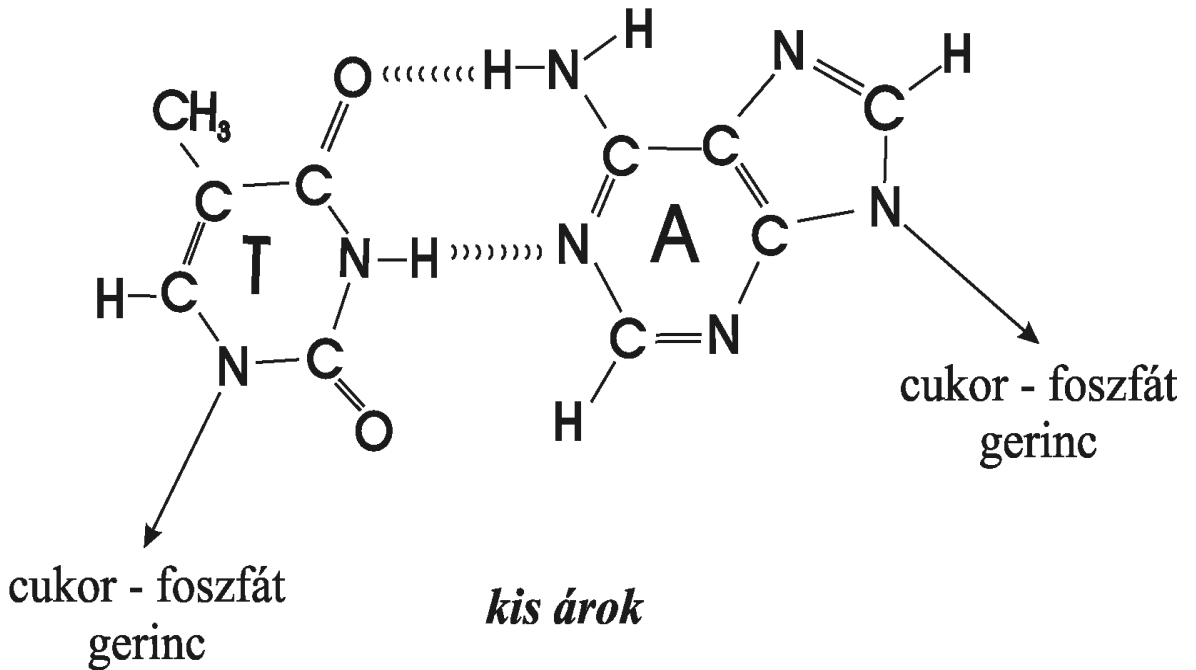
Értelmezés



nagy árok

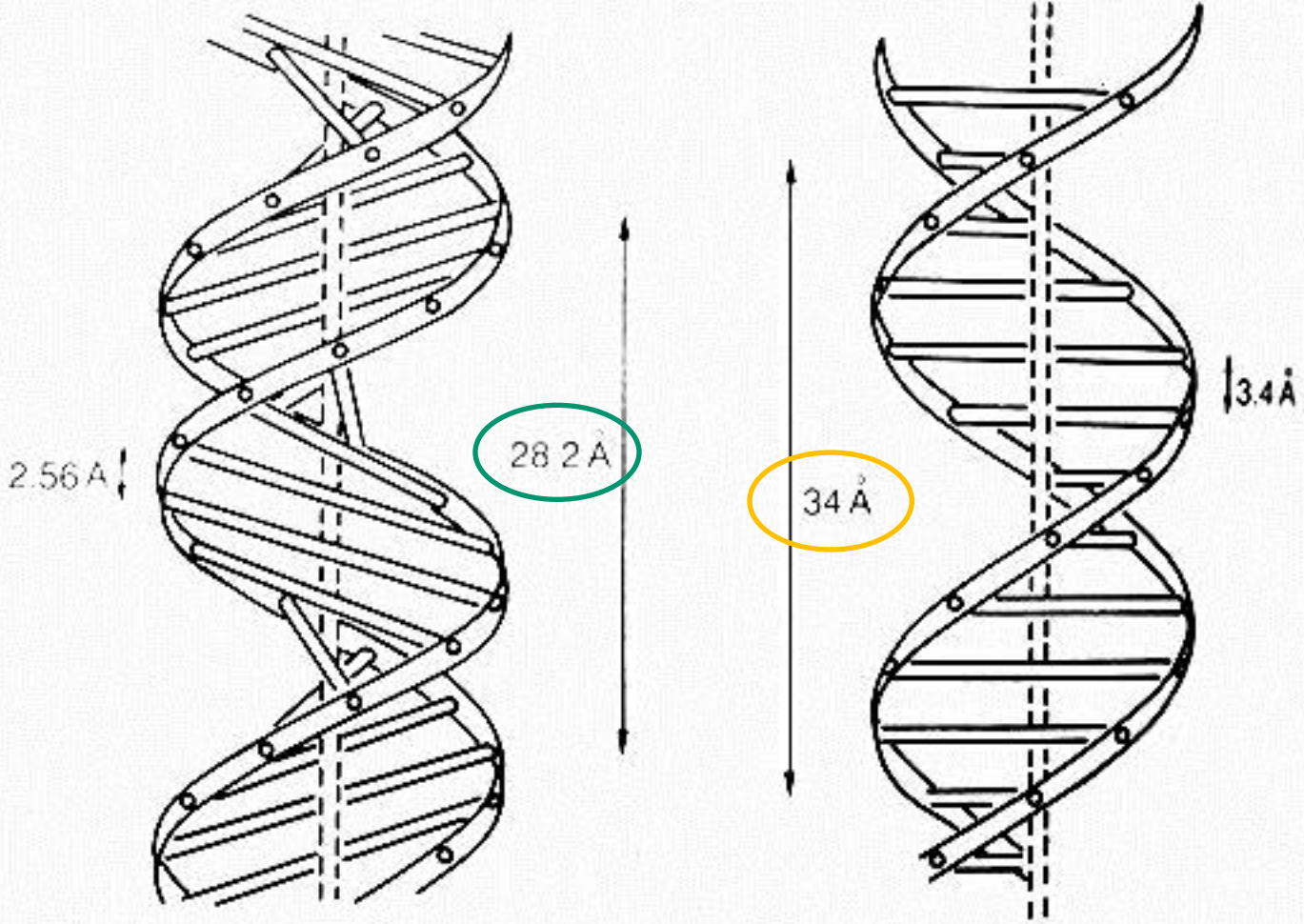


nagy árok



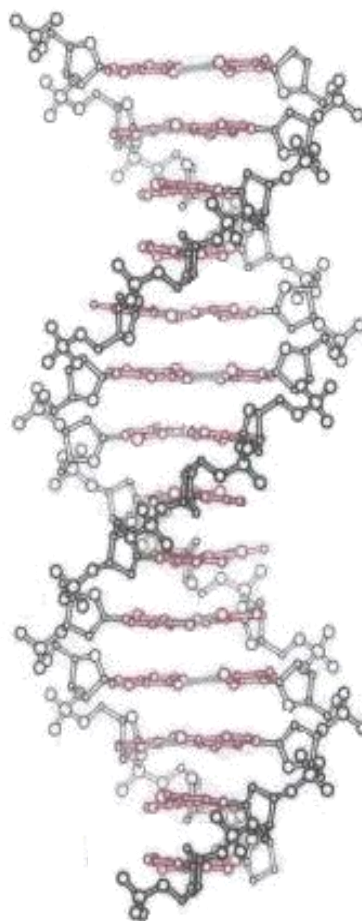
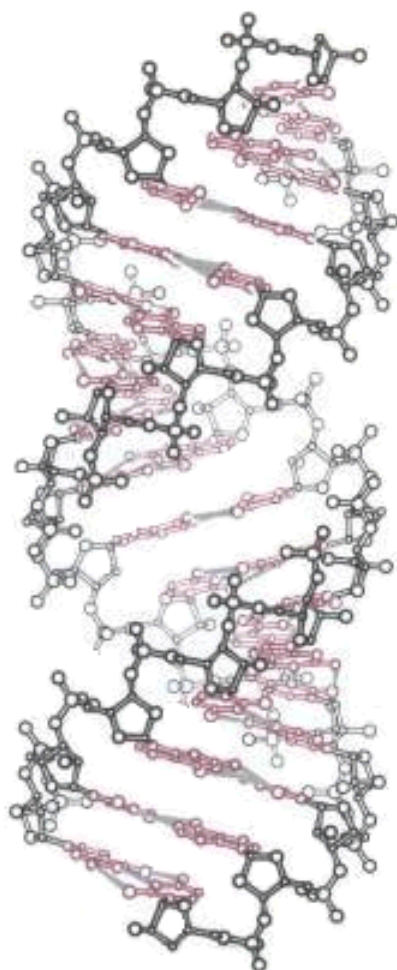
A-DNS

B-DNS

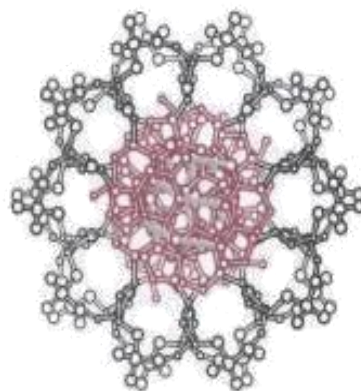
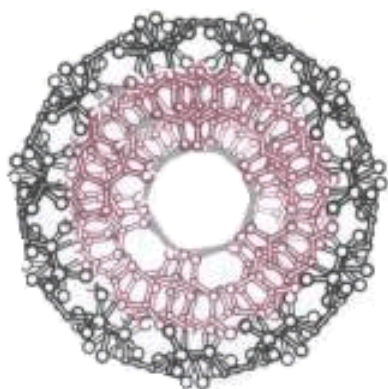


A-DNS

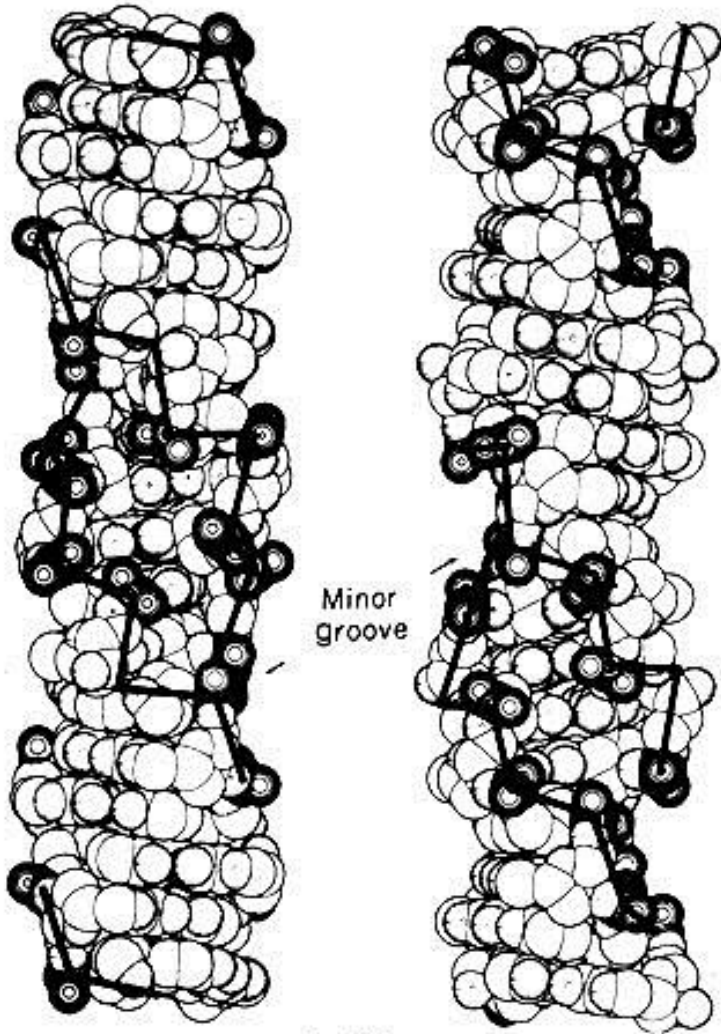
B-DNS



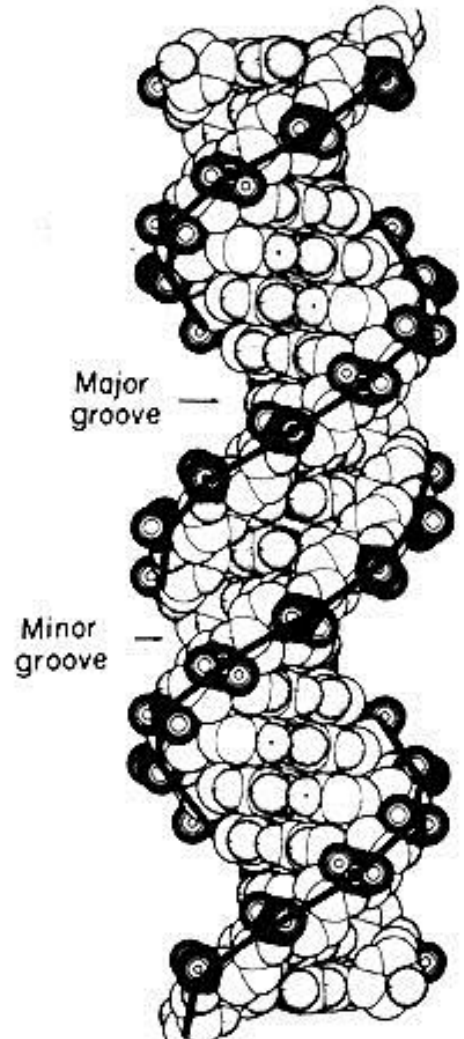
(a)



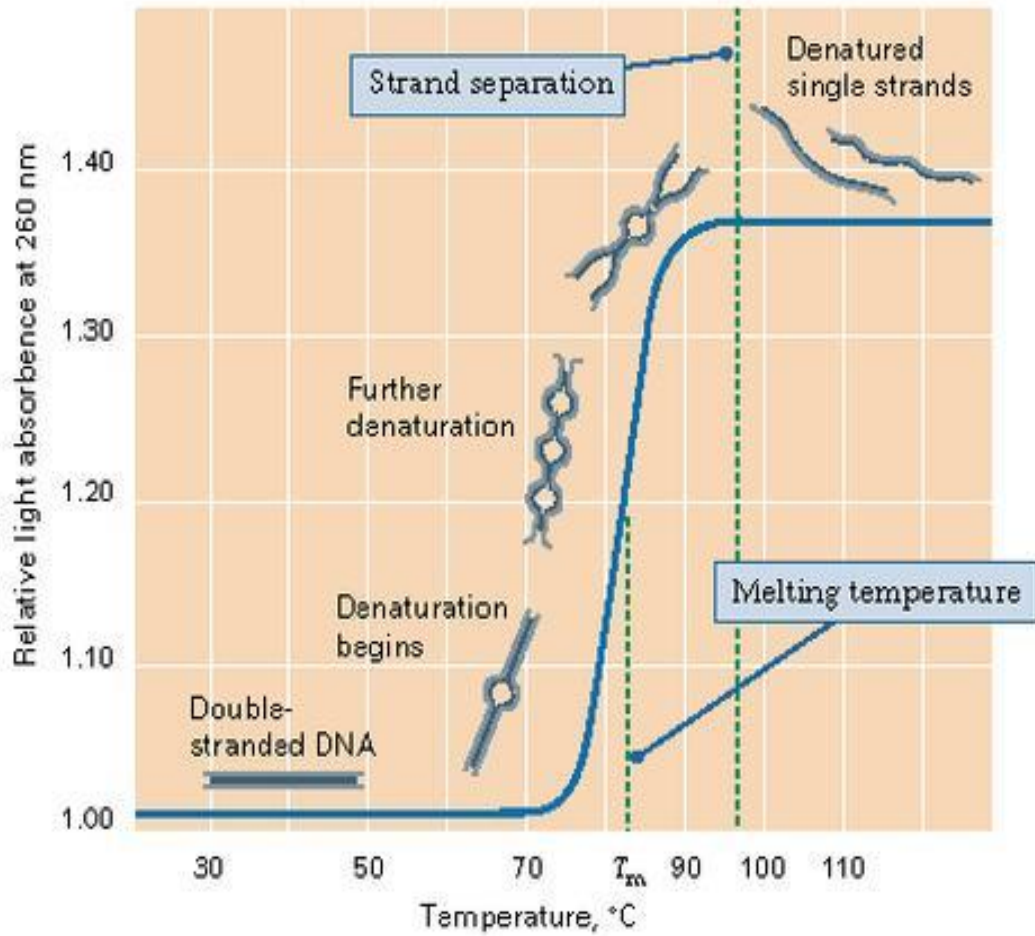
Z-DNS



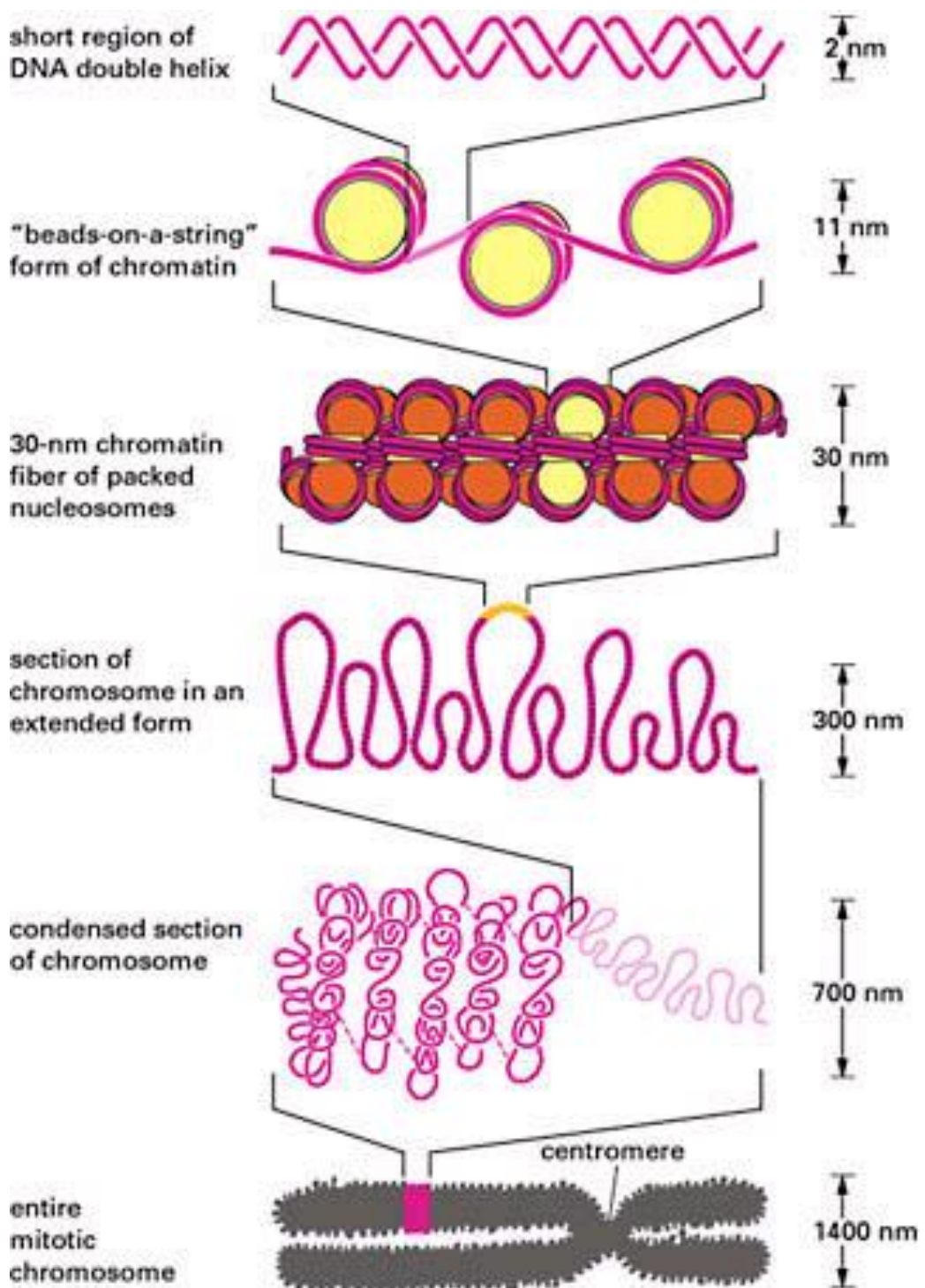
B-DNS



DNS hő-denaturáció

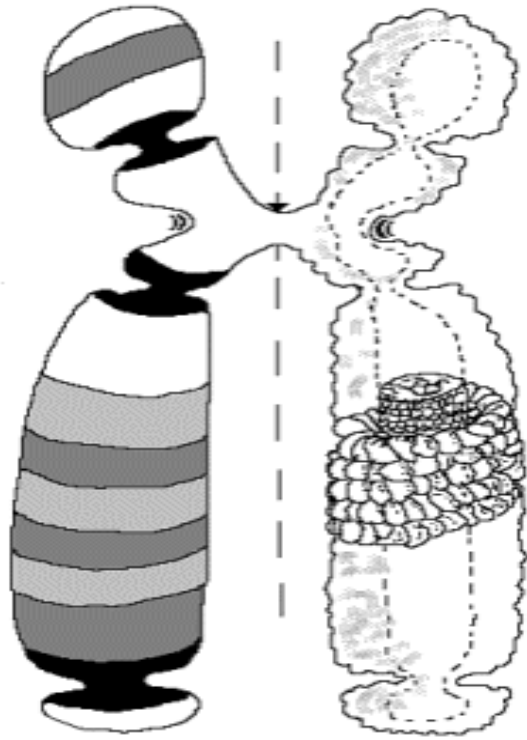


A DNS-től a kromoszómáig

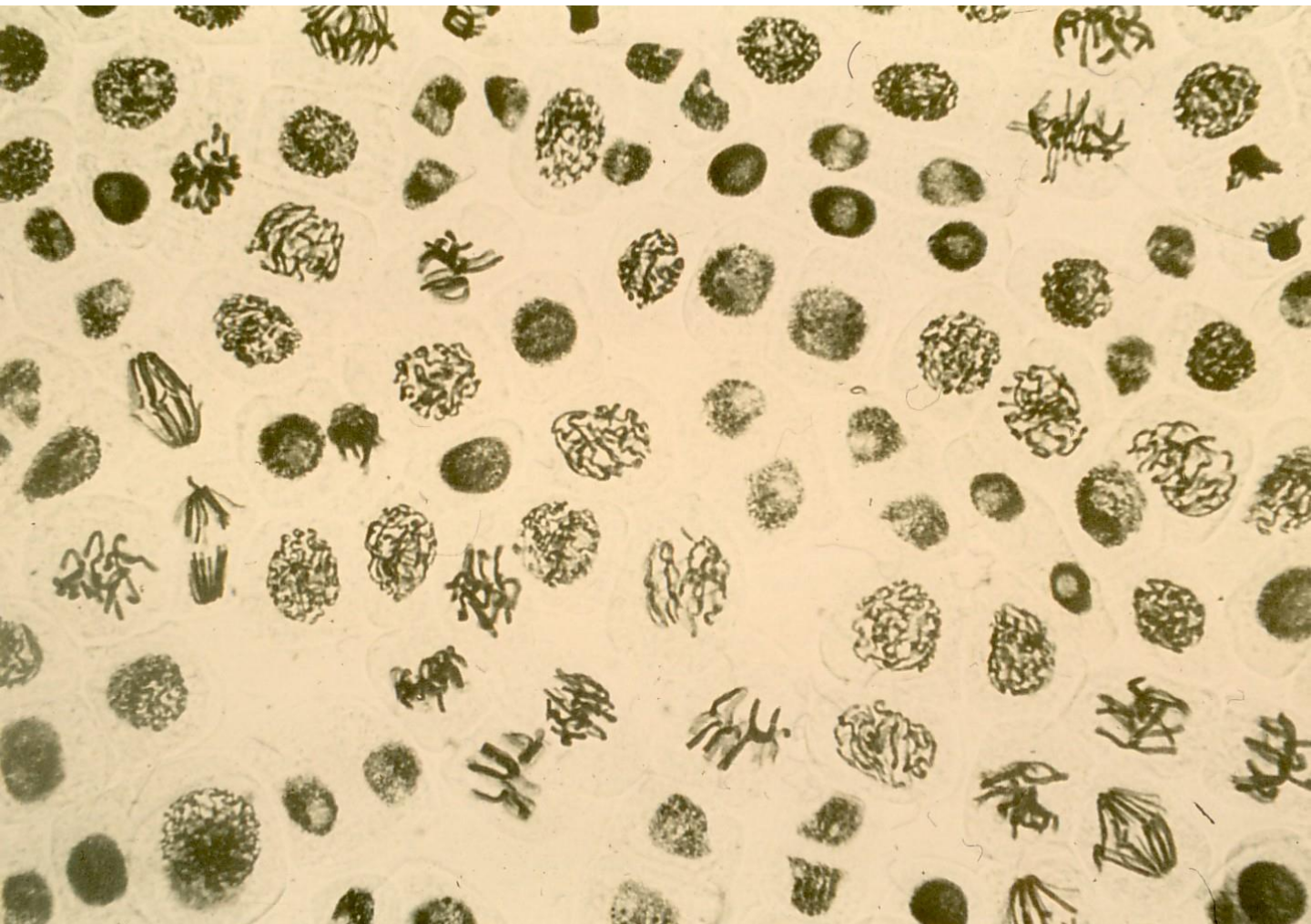


NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 50,000x SHORTER THAN ITS EXTENDED LENGTH

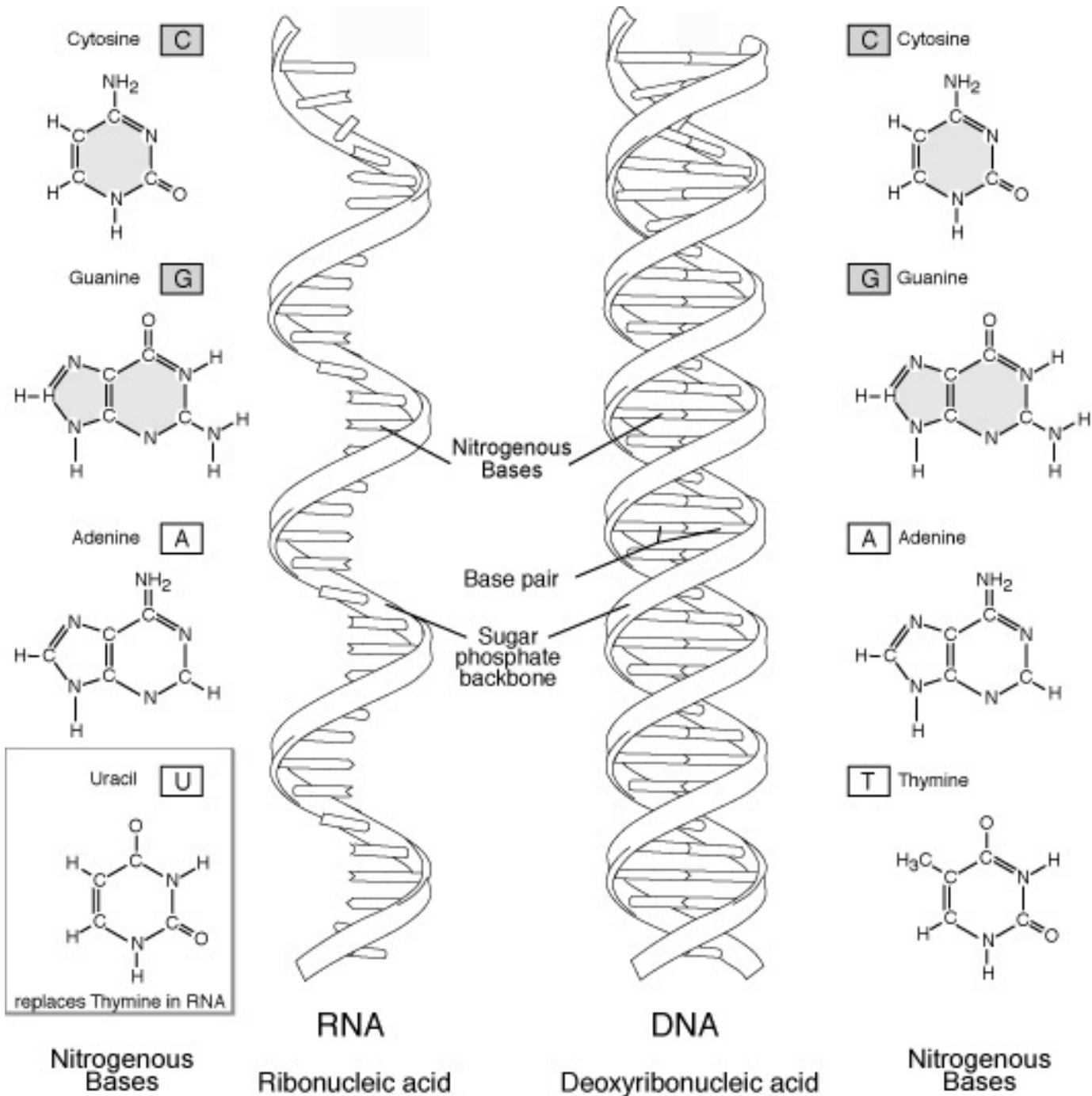
Egy kromoszóma
és...



Metaphasic Chromosome

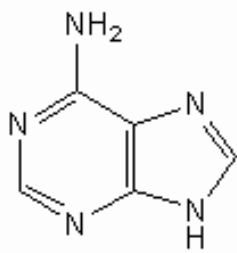


Összevetés: DNS és RNS

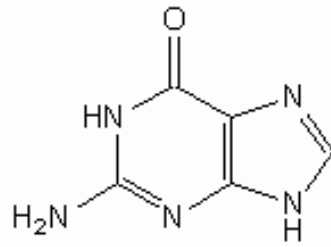


Az RNS

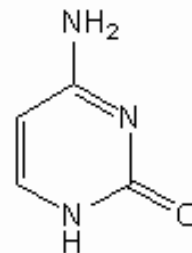
Nucleoside bases found in RNA:



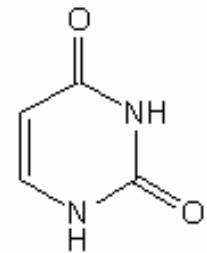
adenine (A)



guanine (G)

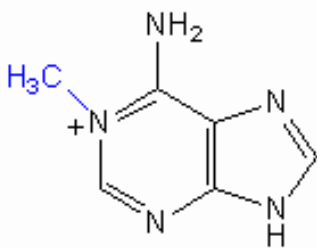


cytosine (C)

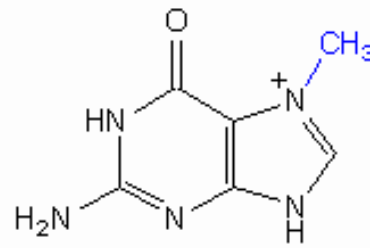


uracil (U)

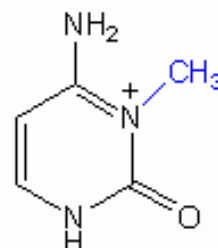
Examples of modified bases found in tRNA:



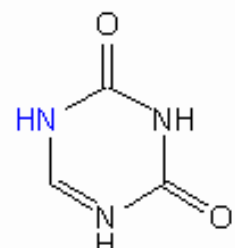
1-methyladenine (m^1A)



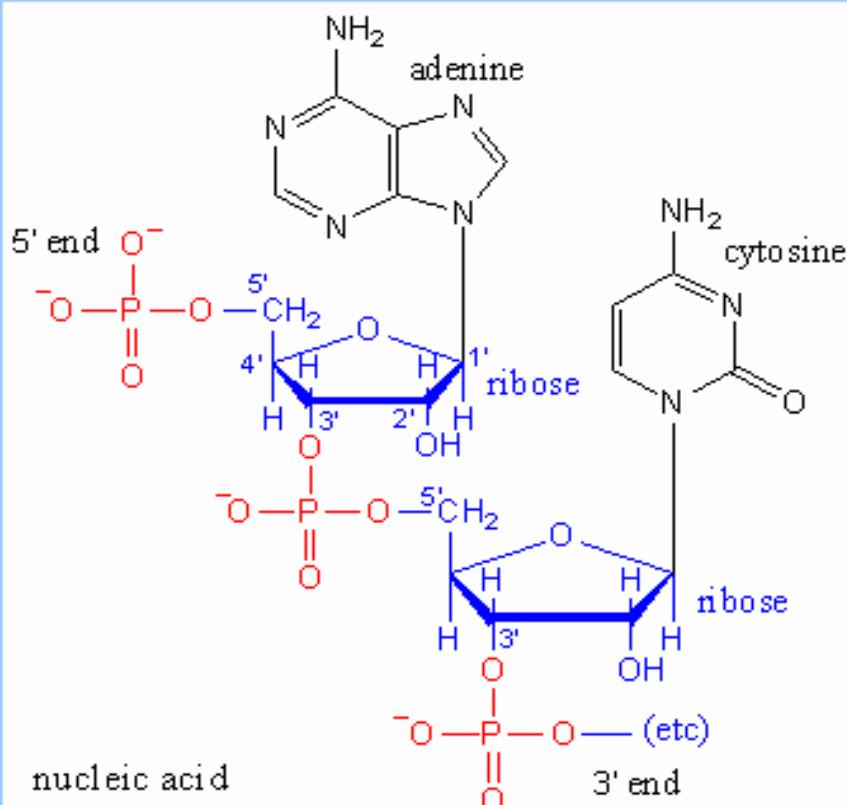
7-methylguanine (m^7G)



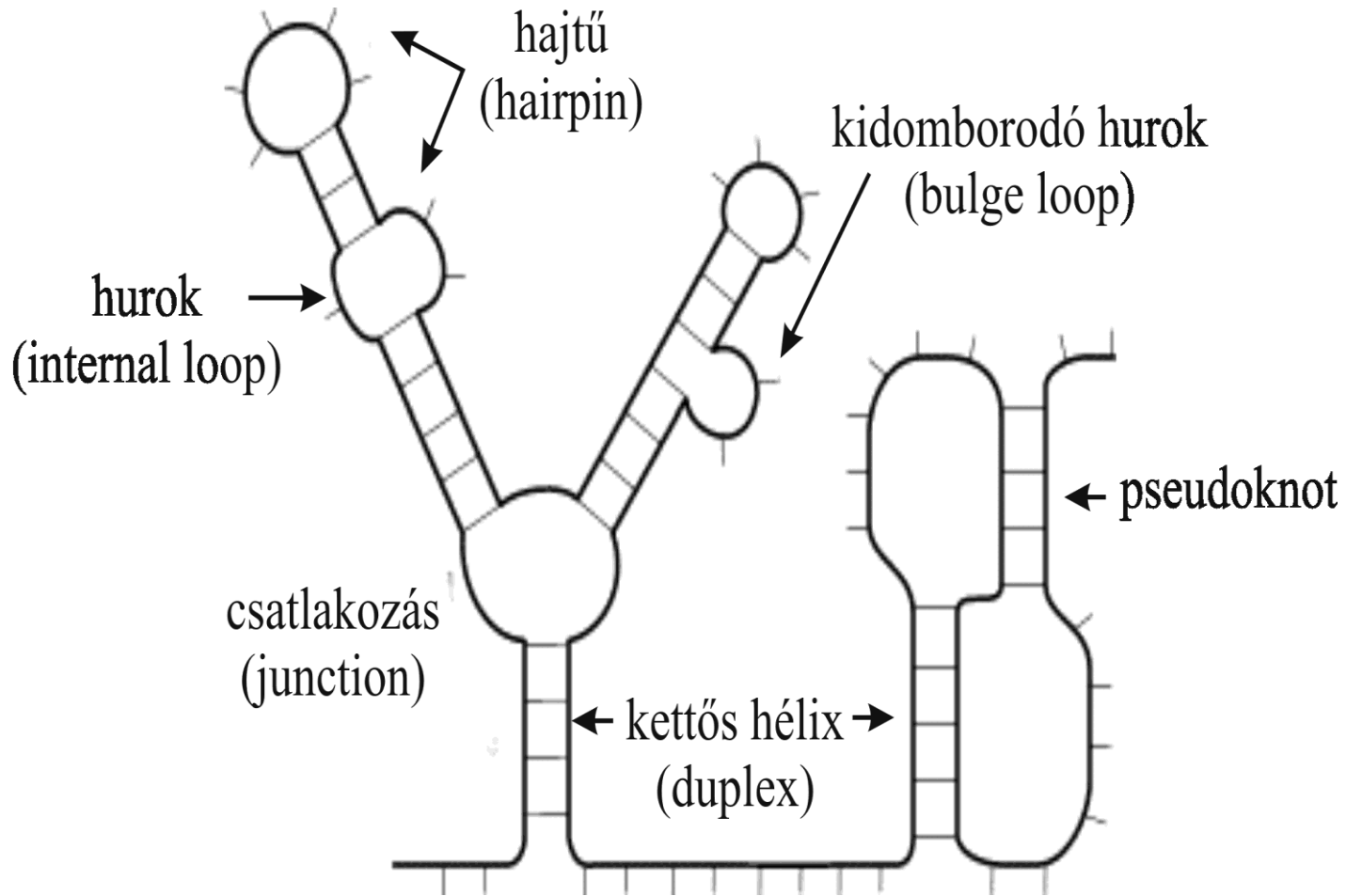
3-methylcytosine (m^3C)



pseudouracil (Ψ)



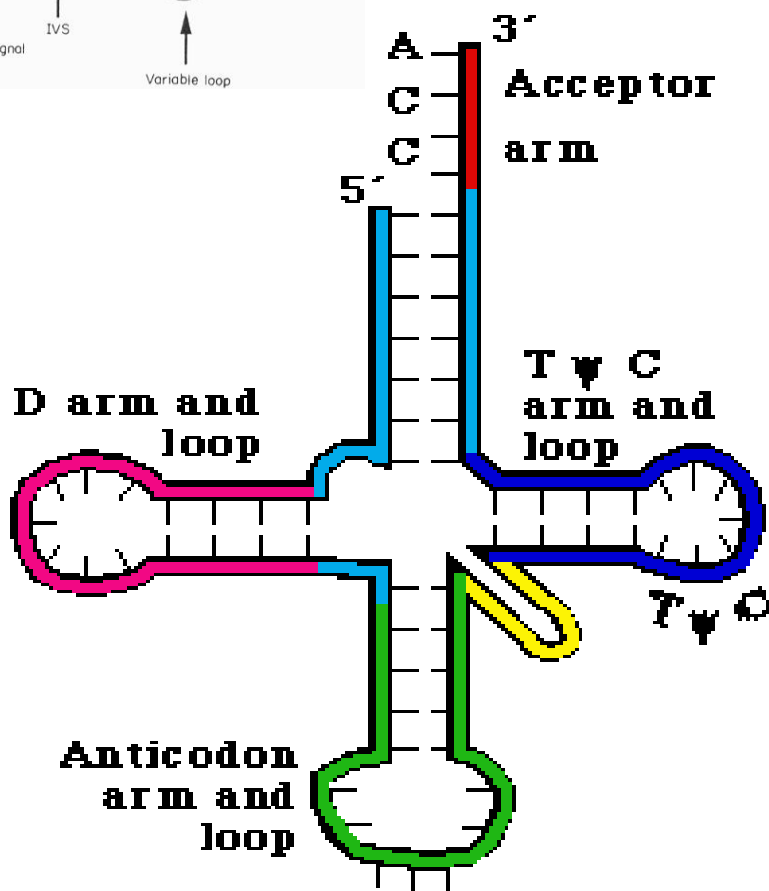
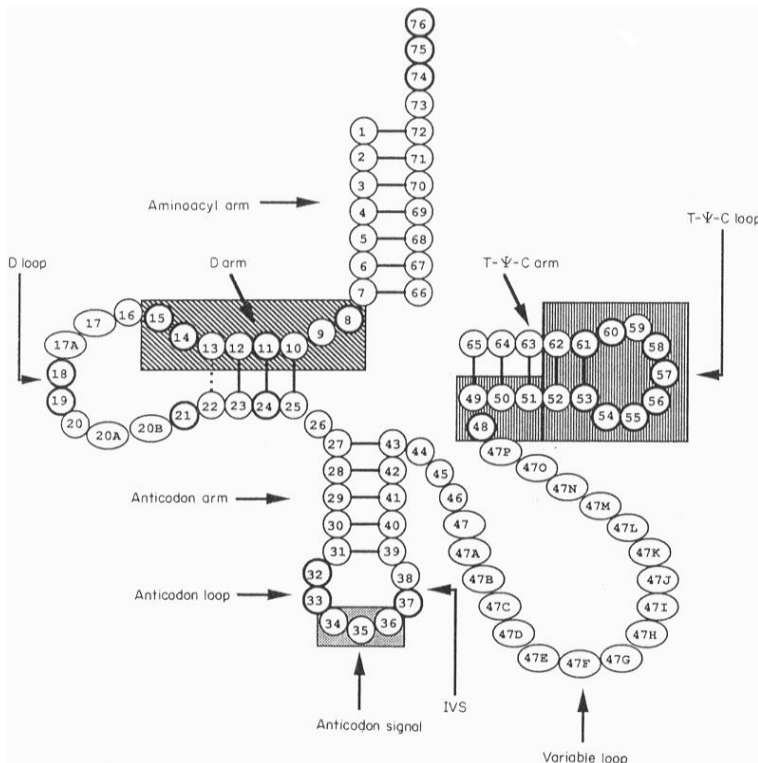
mRNS másodlagos szerkezete



tRNA másodlagos szerkezete

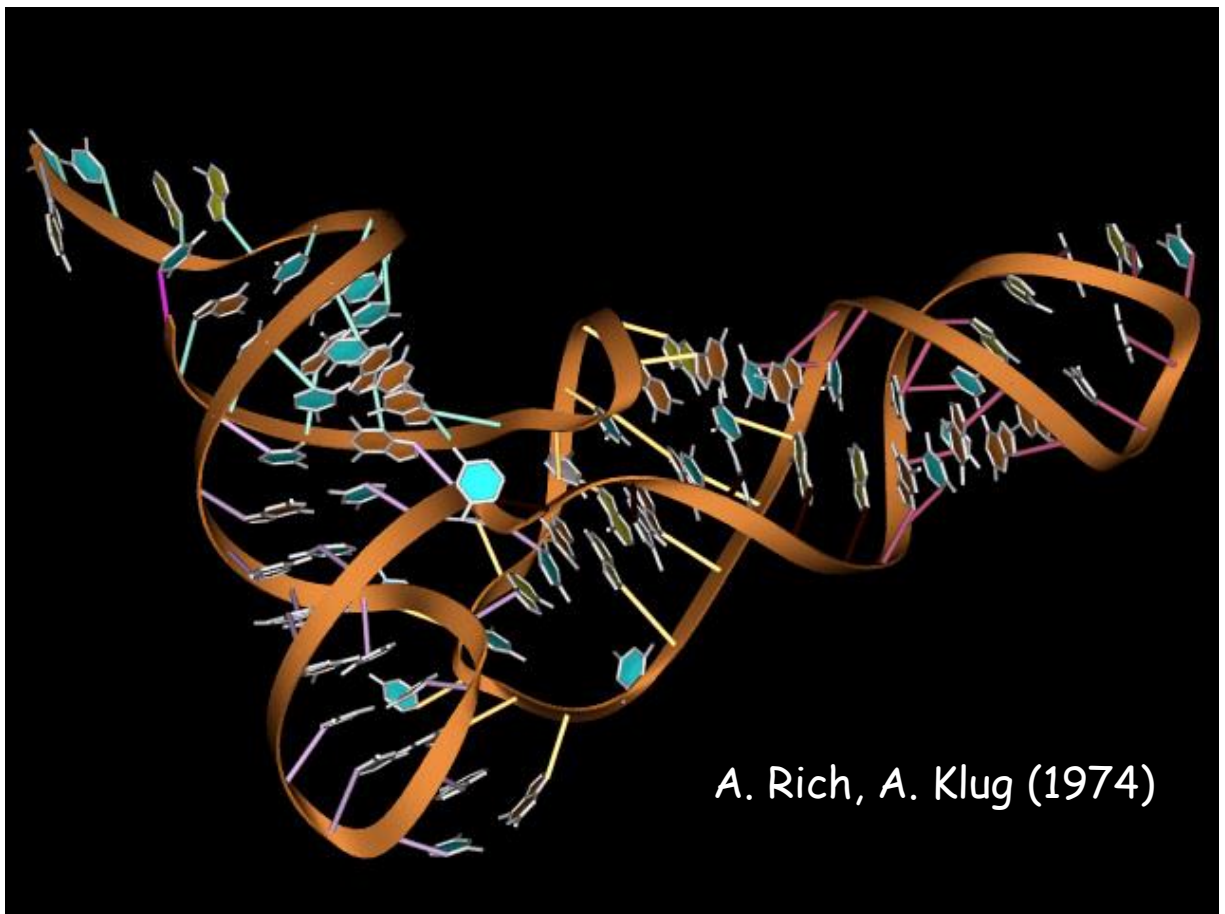
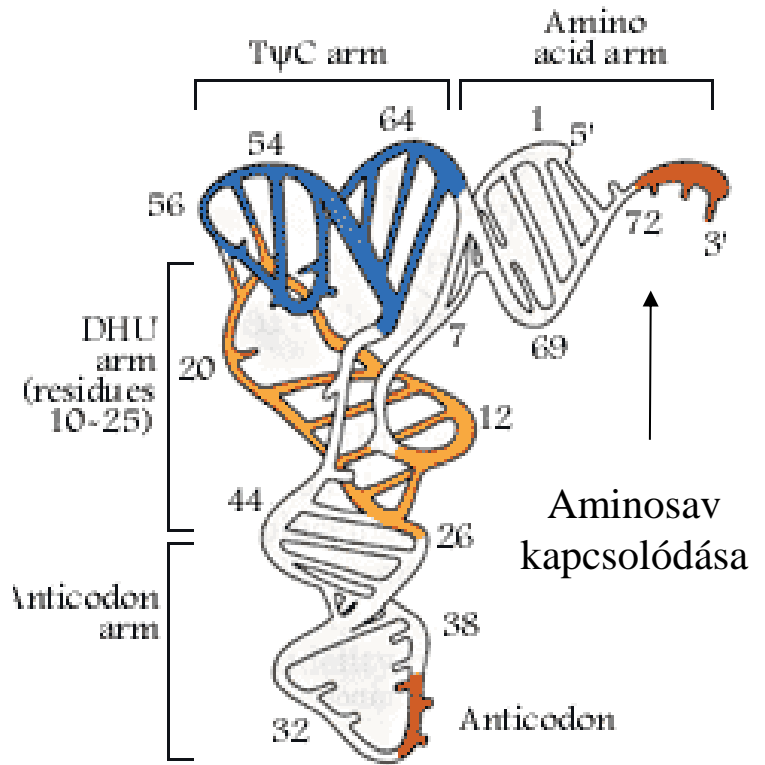
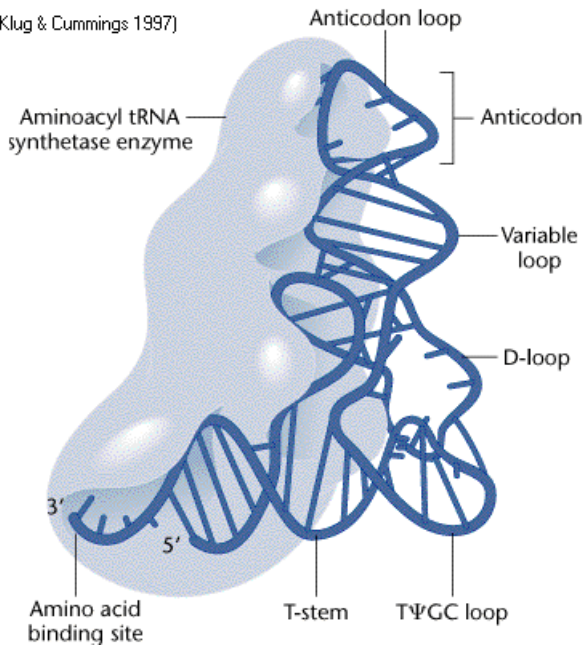
(75-95 nukleotid)

tRNA^{Ala}
(Holley, N.d. 1968)



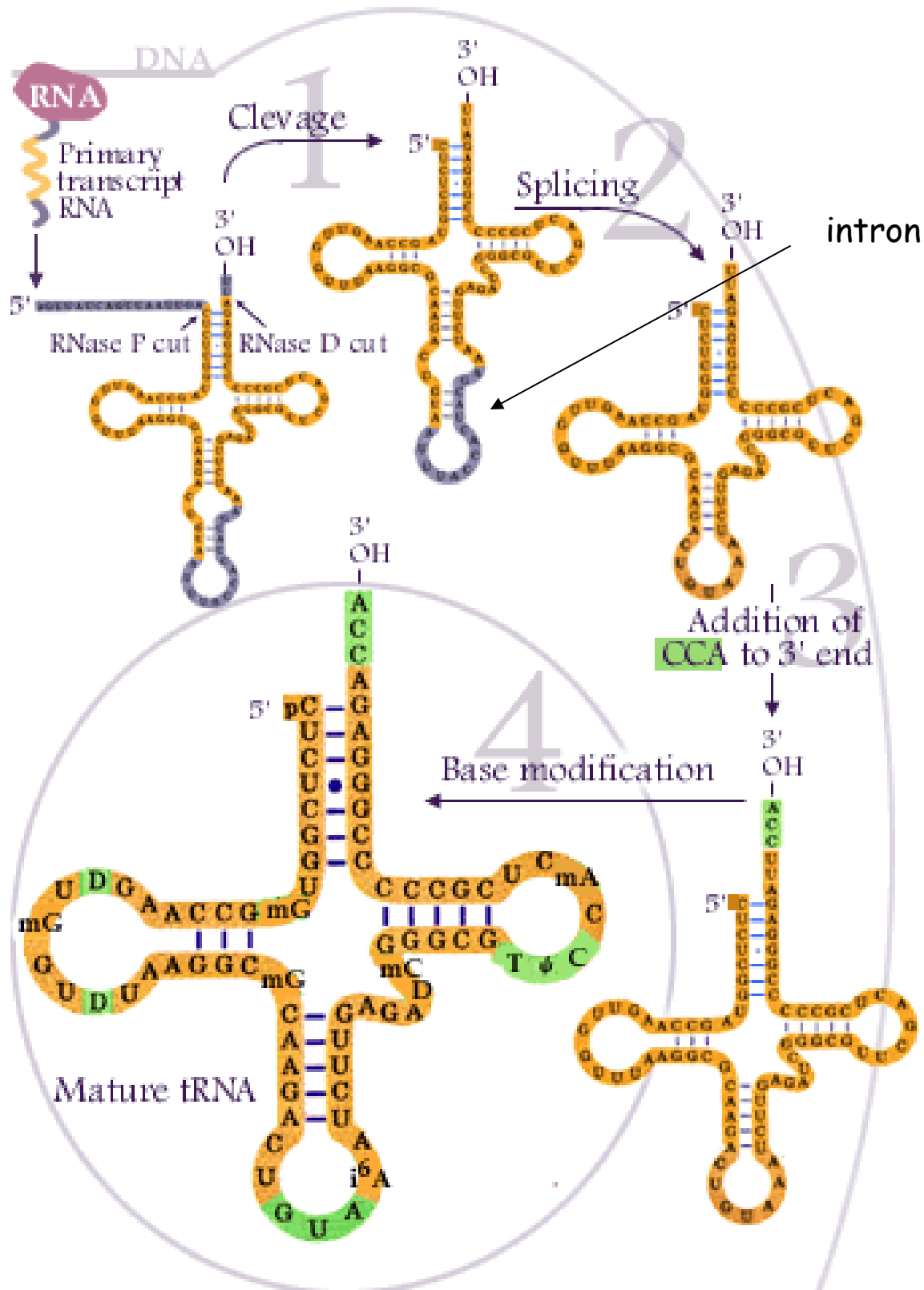
tRNS térszerkezete

(Klug & Cummings 1997)



A. Rich, A. Klug (1974)

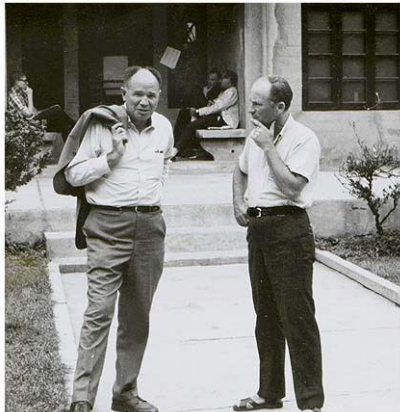
tRNS bioszintézise



10 % módosított bázis

†RNS - aminosav szintézise

P. Zamencik,
M. Hoogland,
1957

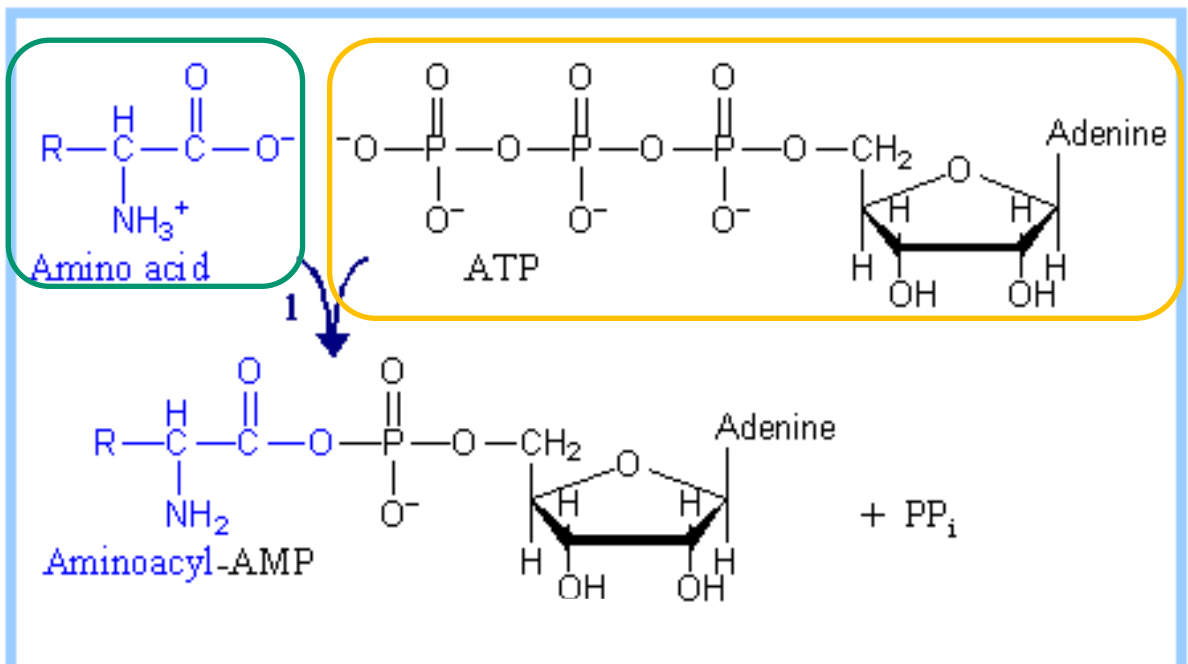


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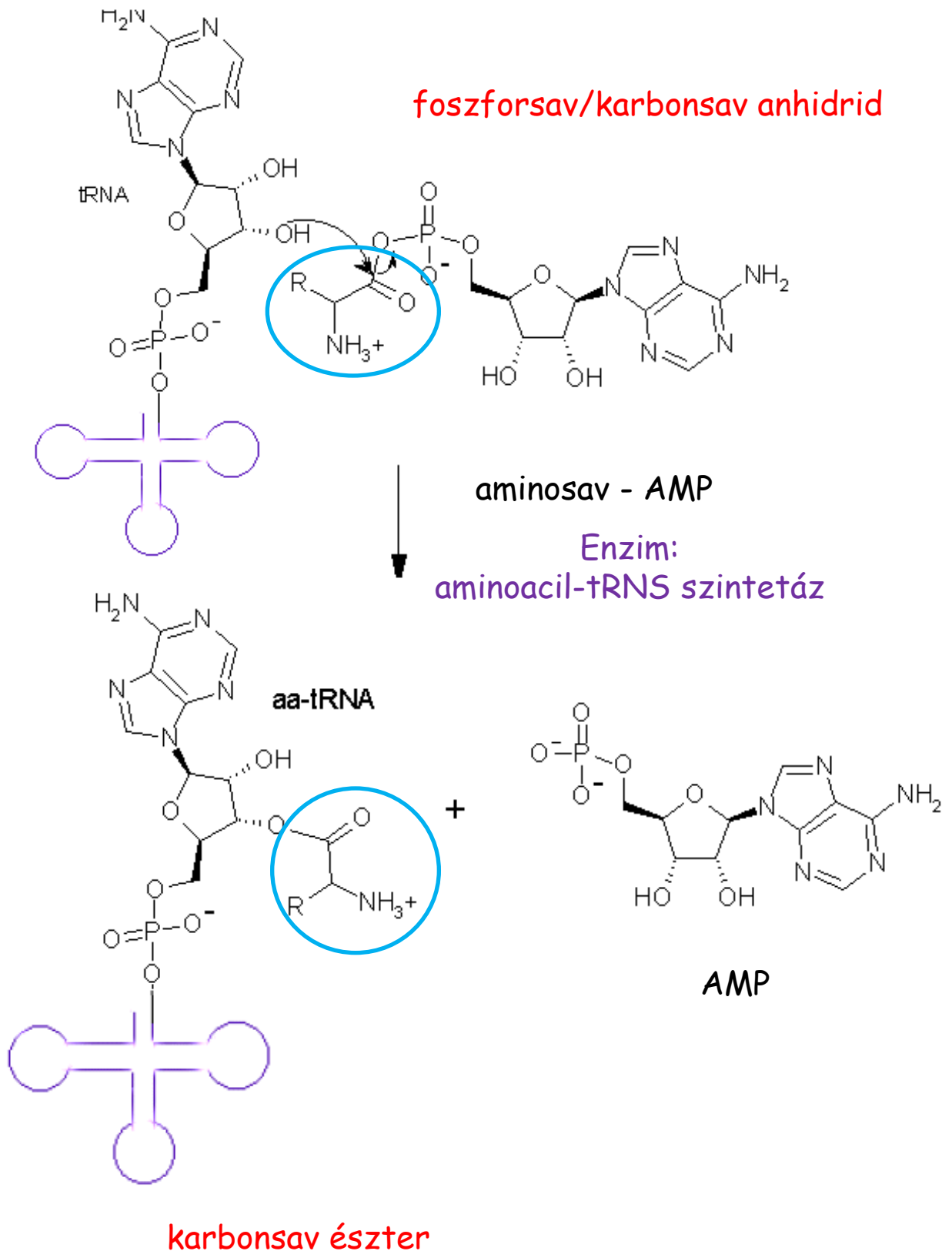
Paul Charles Zamecnik
(November 1912 – October 2009)

Mahlon Bush Hoagland
(October, 1921 – September 2009)

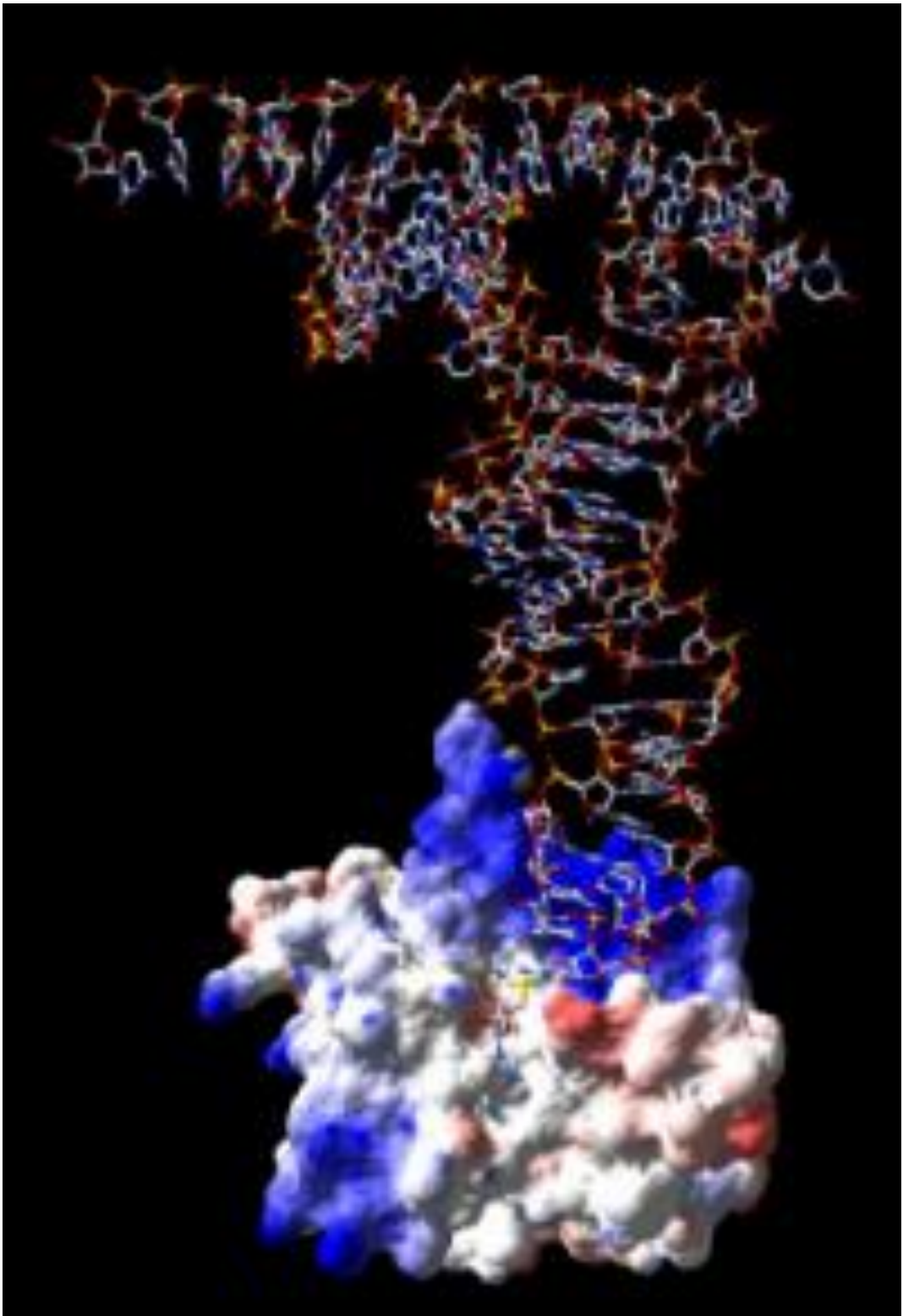
1. Aminoacyl-adenilát (AMP) keletkezése



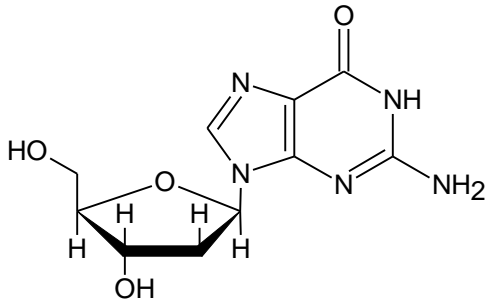
2. Aminoacil-tRNS keletkezése



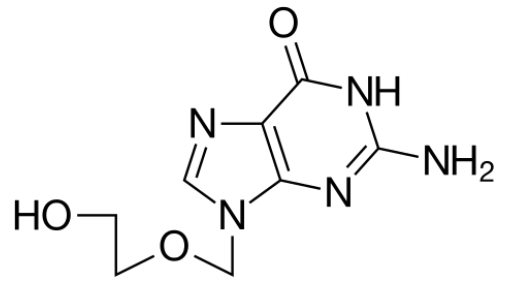
tRNS - aminosav



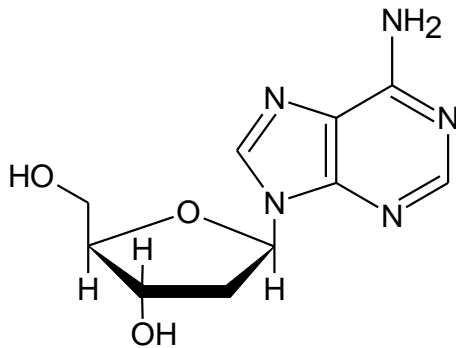
Nukleozid hatóanyagok



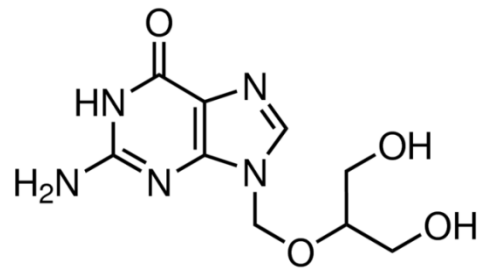
2-dezoxiguanozin



„Aciclovir“
(acicloguanozin)
HSV, VZV, 1977



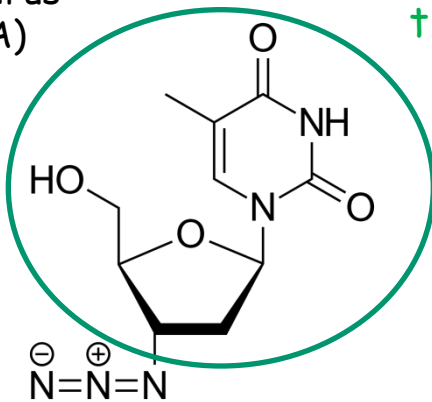
2-dezoadenozin



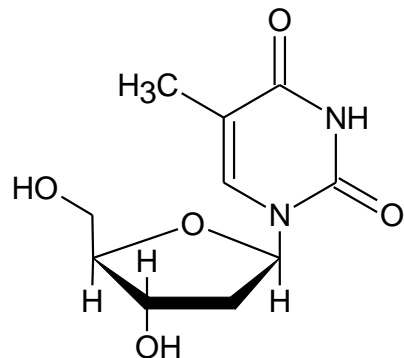
ganciclovir
HCMV, 1989

HIV, retrovirus
1986 (USA)

thymidine



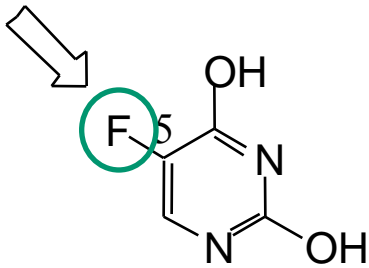
zidovudin
azidothymidine (AZT)



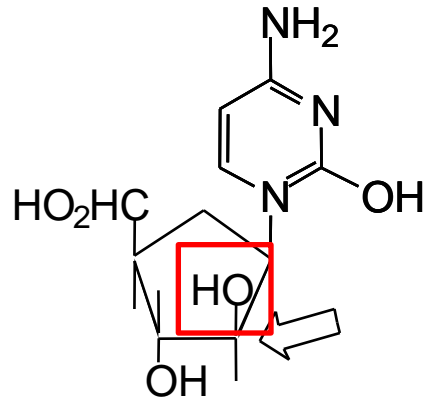
2-dezoxitimidin

Nukleotid származékok - tumorellenes hatóanyagok

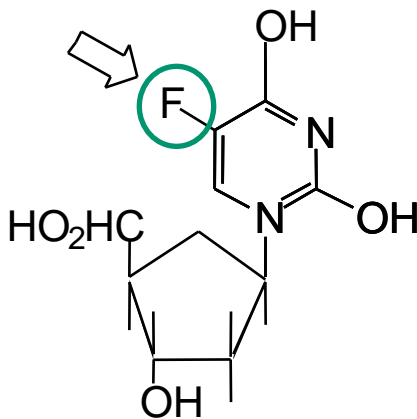
Pirimidin analógok



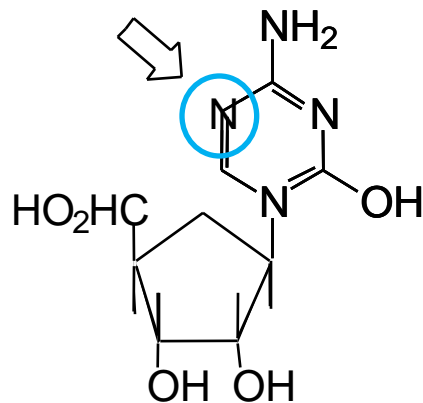
5 - fluoro - uracil
(Cl, Br, I)



citozin arabinozid (Ara-C)

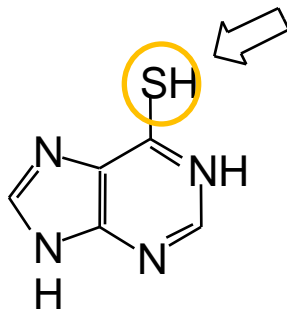


5 -fluoro - 2' dezoxiuridin

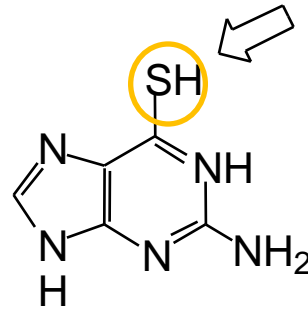


5 - aza - citidin

Purin analógok



6-merkpto-adenin



6-merkpto-guanin