

# TÖRTÉNETI ÁTTEKINTÉS

1842: Nageli, kromoszómák leírása

1860-: Mendel, öröklődés szabályai

1869: Miescher, nuclein (DNS)

1882: Flemming, chromatin (sejtmag állománya)

1902: Sutton, kromoszómaelmélet

1906: Bateson, „genetika”

1946: Muller, röntgensugarak mutagén hatása

(Nobel-díj)

1933: Morgan, kromoszómák szerepe az öröklésben

(Nobel-díj)

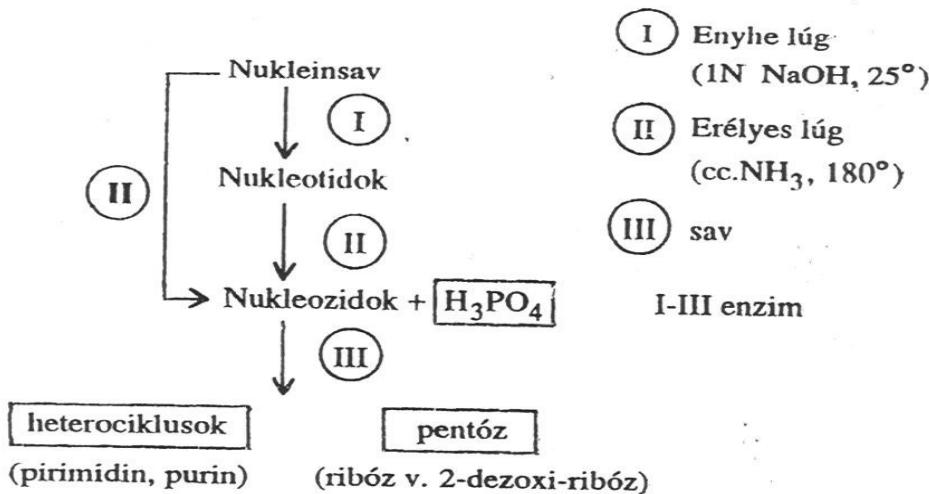
1928: Griffith, DNS genetikai szerepe

1944-től DNS-alapú genetika

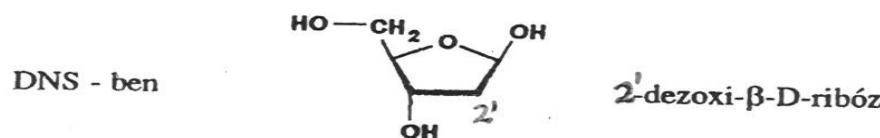
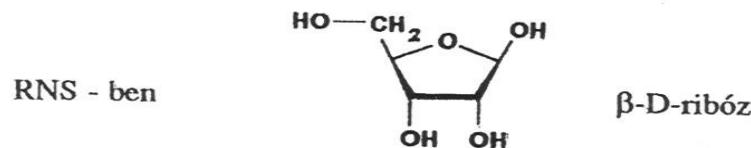
## **NUKLEINSAVAK**

Miescher (1869) gennyből

### Hidrolízis

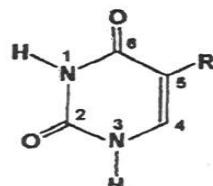


Cukorrész

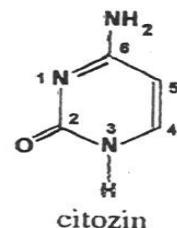


## Heterociklusok

a.) Pirimidinek:

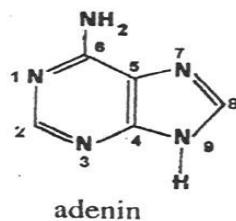


R = H uracil (RNS-ben)  
R = CH<sub>3</sub> timin (DNS-ben)

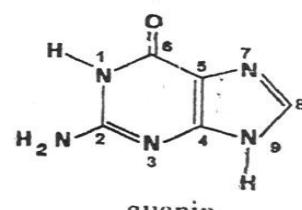


citozin

b.) Purinok:



adenin



guanin

Tautoméria

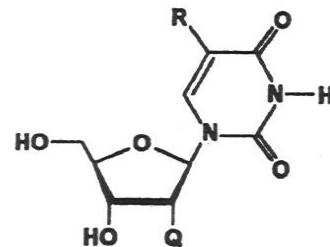


!



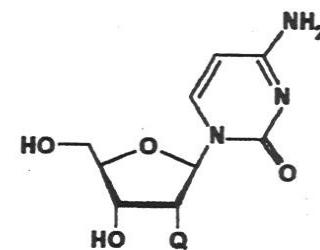
NUKLEOZIDOK

Heterociklusok („bázisok”) N-glikozidjai



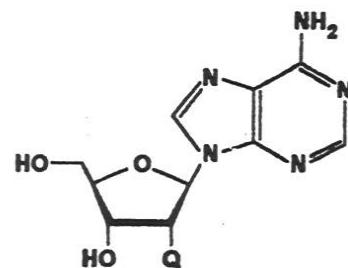
$R = H$   
 $Q = OH$

$R = CH_3$   
 $Q = H$



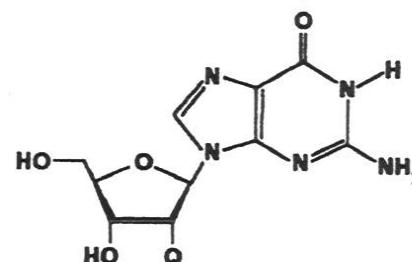
$Q = OH$  citidin

$Q = H$  dezoxi-citidin



$Q = OH$  adenozin

$Q = H$  dezoxi-adenozin



$Q = OH$  guanozin

$Q = H$  dezoxi-guanozin

Szerkezet (Levene-Todd)

1., Bázis és cukor minősége

Savas hidrolízis (glikozid!) erélyes (N-glikozid)

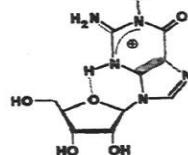
pirimidin-glik.

10%-os  $H_2SO_4$ , 125°

purin-glik.

0.01N  $H_2SO_4$ , 100

(katalízis)



Cukor károsodik (deoxi-ribóz elbomlik)

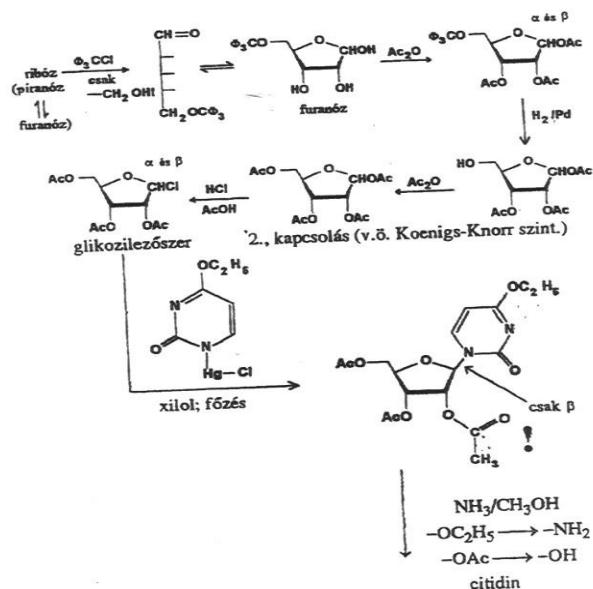
ribóz → furfurol



2., Kapcsolódási hely

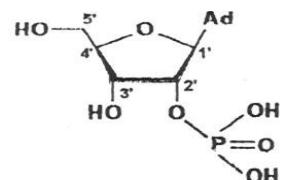
a) cukron      nem redukál      }  
nem mutarotál      } → glikozidos OH

b) bázison      bonyolult;



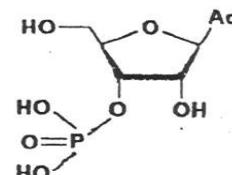
NUKLEOTIDOK

Nukleozidok foszforsav-észterei



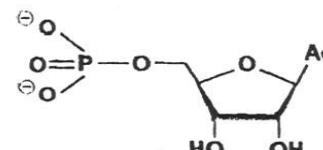
adenozin-2'-(mono)-foszfát

2'-AMP



adenozin-3'-foszfát

3'-AMP



adenozin-5'-foszfát

5'-AMP (AMP)

mindegyik  
diaron  
pH 7-en!

Ugyanígy mindegyik nukleozidból

3'-UMP

5'-CMP

2'-GMP

3'-TMP

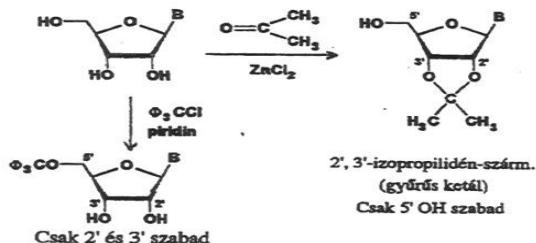
5'-dCMP = dezoxi-citidin-5'-foszfát

stb.

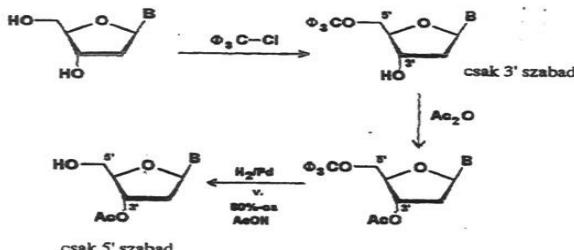
Szintézis

1.. Védett nukleozid-származék

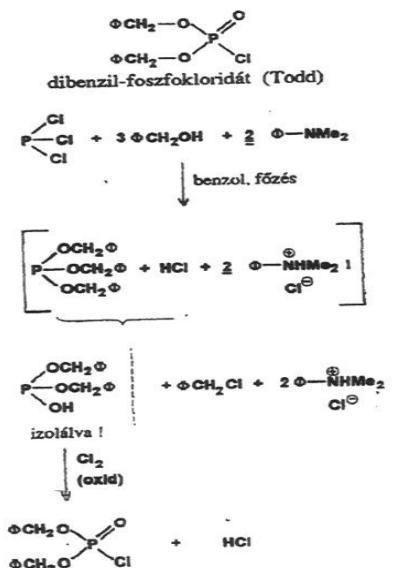
a) Ribonukleozid



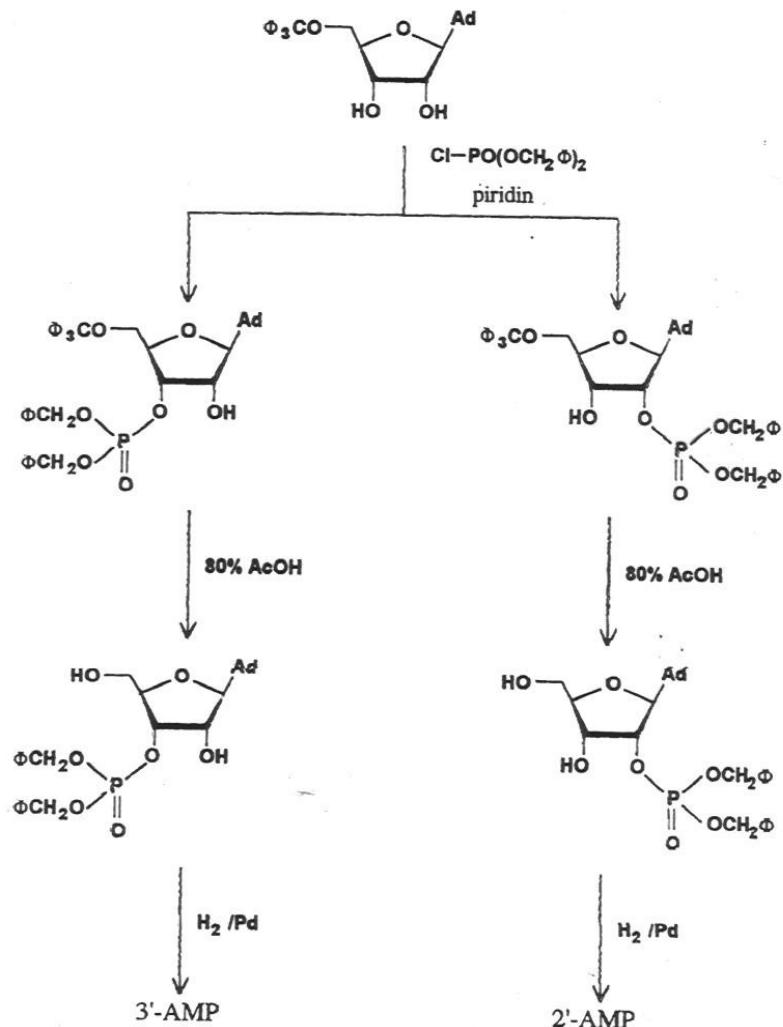
b) Dezoxi-ribonukleozidok



2.. Foszforilezőszer



Pl.



Kromatográfiásan szétválaszthatók!

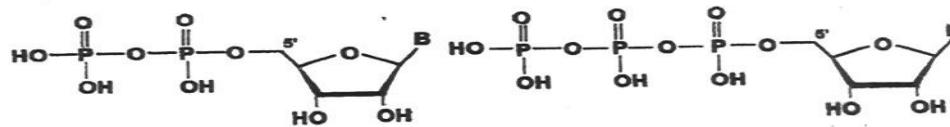
## NUKLEOTID KOENZIMEK

Koenzimek ~ az élő sejt reagensei

Enzim nemfehérje része

apoenzim + koenzim = holoenzim  
(fehérje)

Nukleozid-5'-foszfátok származéka



5'-difoszfátok

(pirofoszfátok)

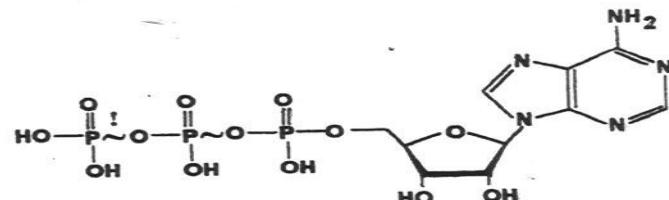
5'-trifoszfátok

Minden nukleozidnak van ilyen származéka a sejtbén  
(nukleinsav szintézis 5'-trifoszfátokból)

Legfontosabbak: adenozin-származékok

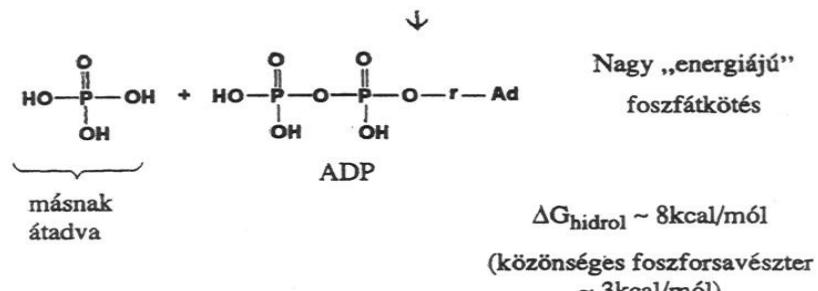
ADP és ATP

Energiaháztartás

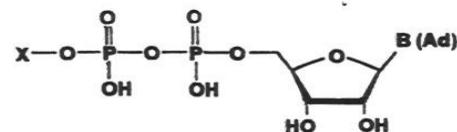
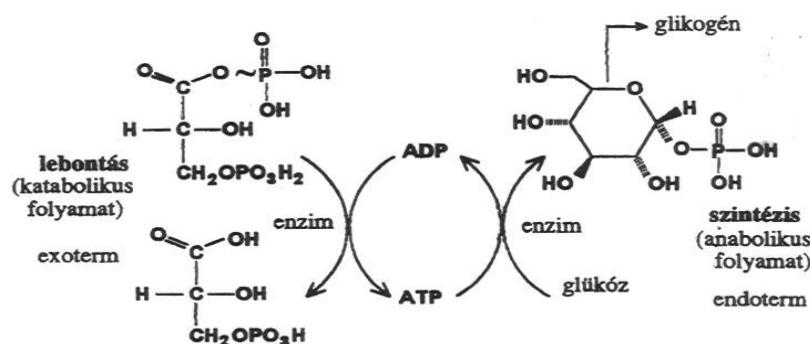


ATP (adenozin-5'-trifoszfát)



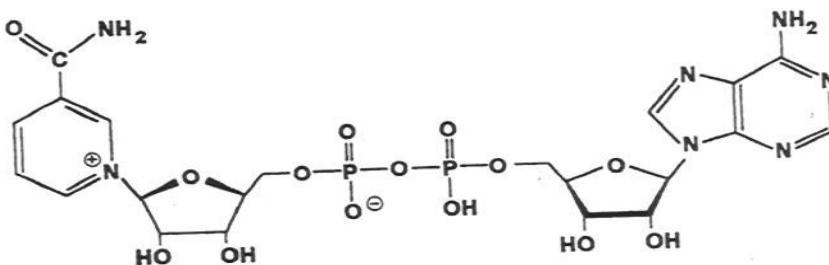


Energia  $\left\{ \begin{array}{l} \text{tárolás} \\ \text{átvitel} \end{array} \right\}$   $\text{PO}_3\text{H}_2$  - átvitelen keresztül



X ~ nukleozid      ~      „dinukleotid”

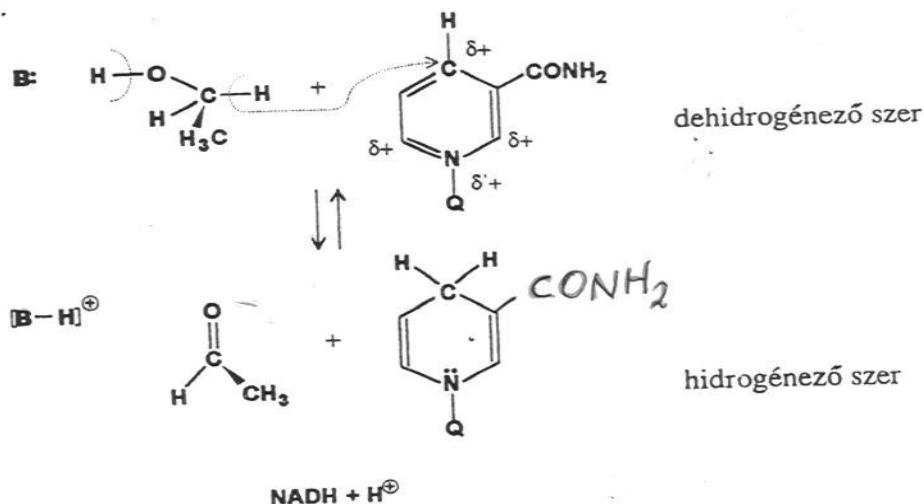
NAD<sup>+</sup>



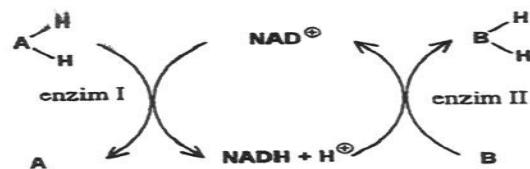
Nikotinamid-adenin-dinukleotid

Az élő sejt egyik oxidáló (dehidrogénező) szere

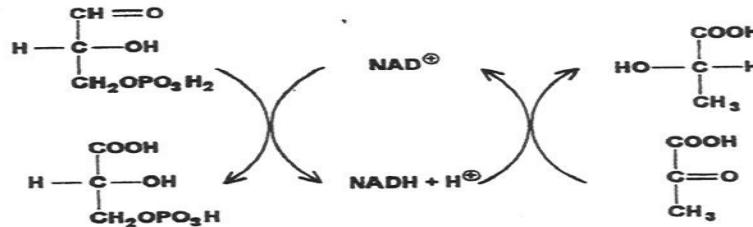
P1.



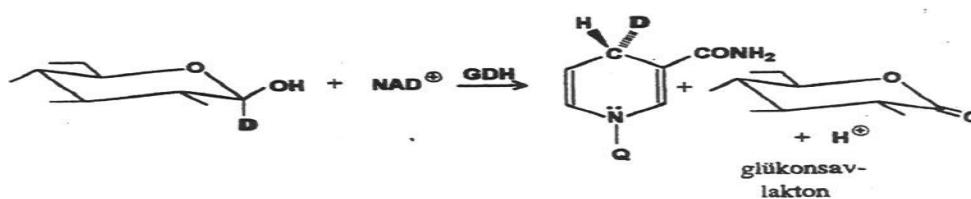
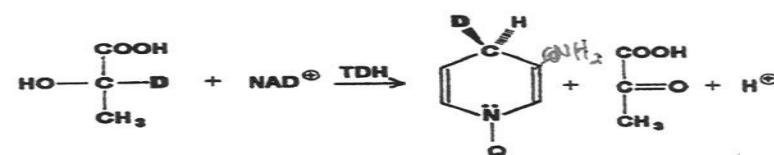
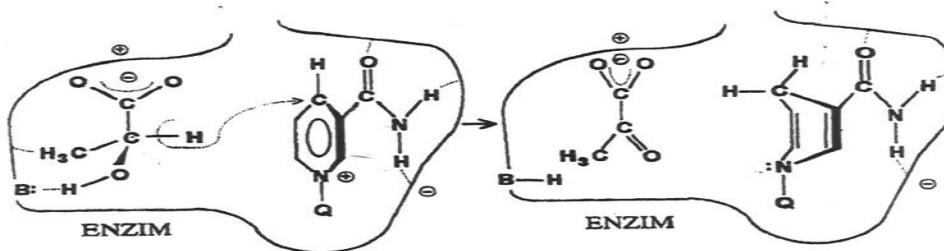
### Hidridion átvitel



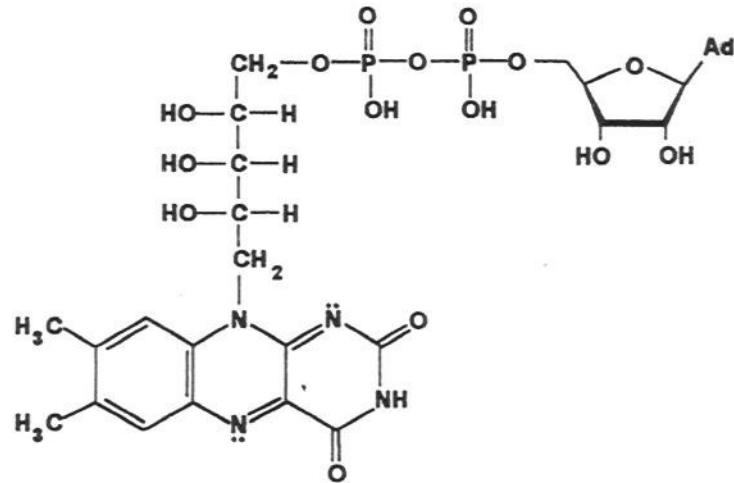
P1.



### Sztereoszelektivitás

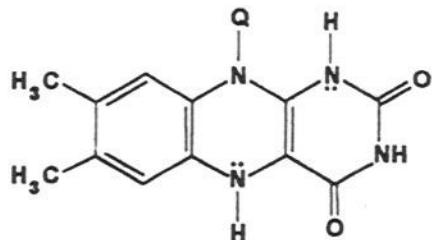


FAD

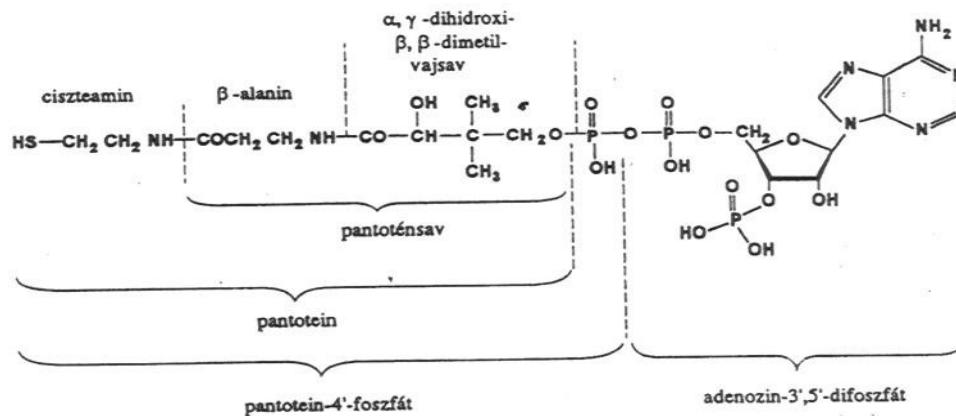


Flavin-adenin-dinukleotid

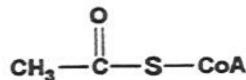
+ 2H [H: $^{\ominus}$  + H $^{\oplus}$  vagy 2H $\cdot$  ]



### Co-A

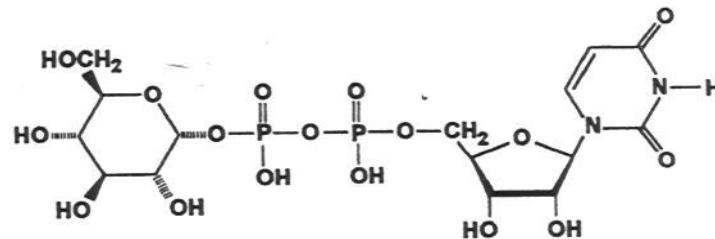


### Acil-átvitel



zsírok bioszintézise

### UDP-cukrok



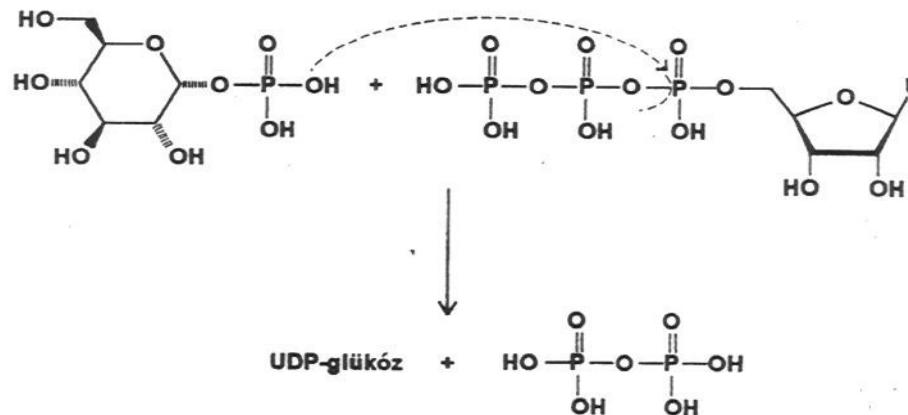
Uridin-difoszfo-glükóz

Di- és poliszacharidok szintézise

glikozilcsoport átvitel

Pl. UDP-glükóz + fruktóz  $\longrightarrow$  UDP + szacharóz

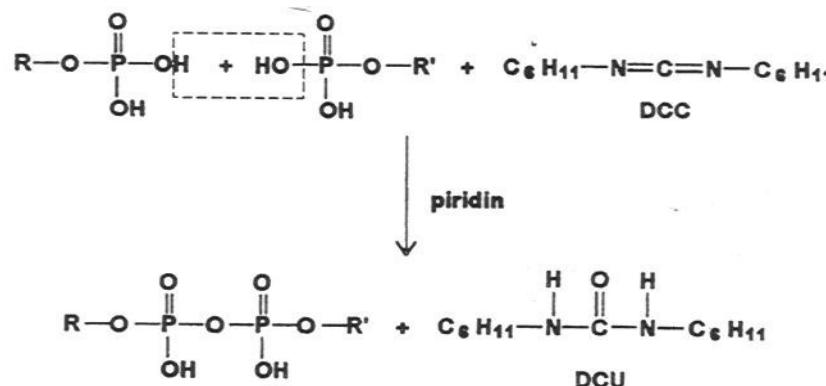
Bioszintézis:



Kémiai szintézis

Pirofoszfát-kötés kialakítása két nukleotidból

pl. DCC



## Nukleinsavak

DNS                    sejtmag (bakteriofágok)      timusz, halspermium  
                           kromoszóma      MS  $10^8\text{--}10^{12}$  !

RNS                    főleg plazma (növényi vírusok)    élesztő  
                           több típus:      MS  $10^4\text{--}10^6$

Nukleoproteid = nukleinsav + bázikus fehérje (hiszton)

### Összetétel

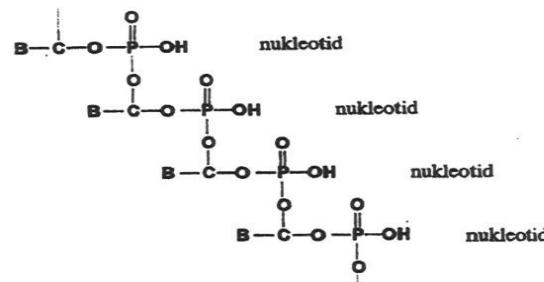
$$\begin{array}{ll} \text{DNS} & \text{Pu} = \text{Py} \\ & \boxed{\begin{array}{l} \text{A} = \text{T} \\ \text{G} = \text{C} \end{array}} \quad \frac{\text{A} + \text{G}}{\text{T} + \text{C}} = 1 \\ & 0,4 < \frac{\text{A} + \text{T}}{\text{G} + \text{C}} < 2 \end{array}$$

RNS                    Nem szabályos!

### PRIMER Szerkezet

Nukleinsav = polinukleotid

Foszforsav-diészter kötés (titrálás)



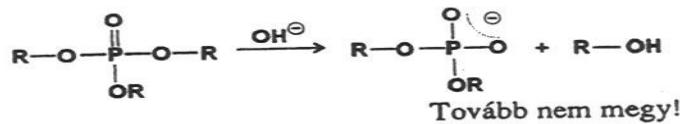
## Hidrolízis vizsgálata

### A) Lúgos hidrolízis

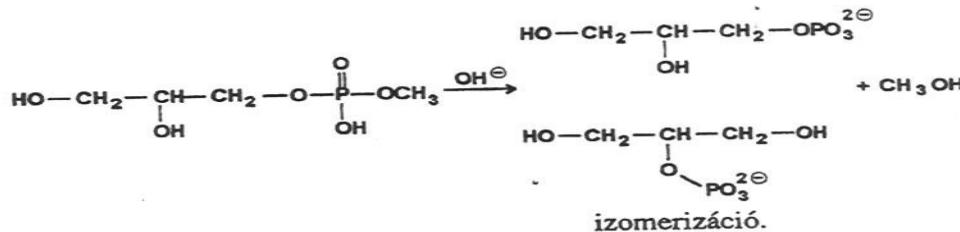
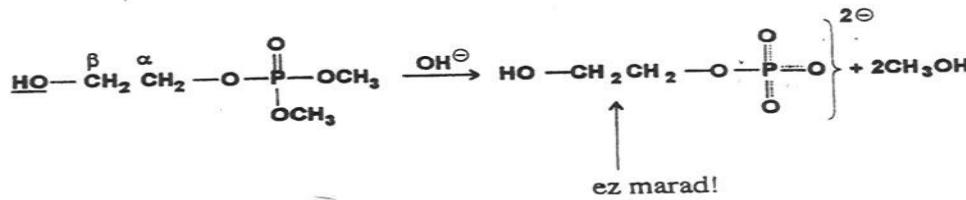
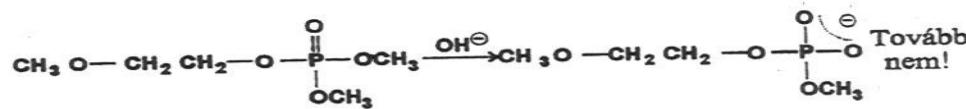
Termék: 2'- és 3'- monofoszfátok elegye  
 (Tipp: 2'-3' kötés)

Foszforsavészterek lúgos hidrolízise (Todd)

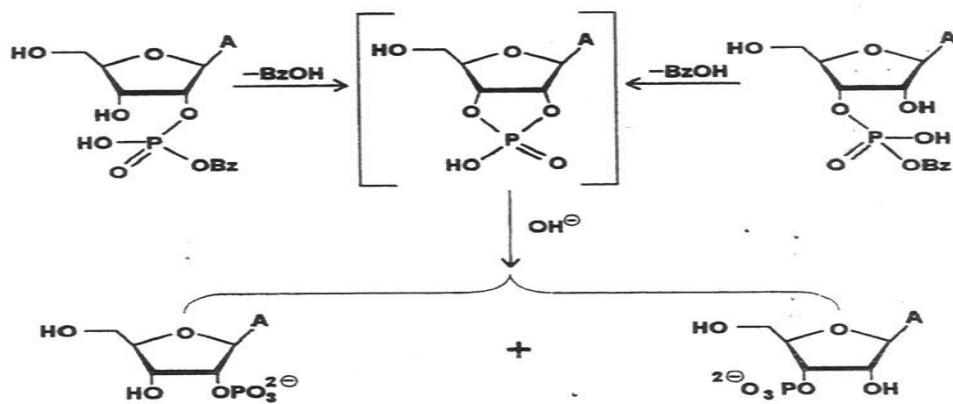
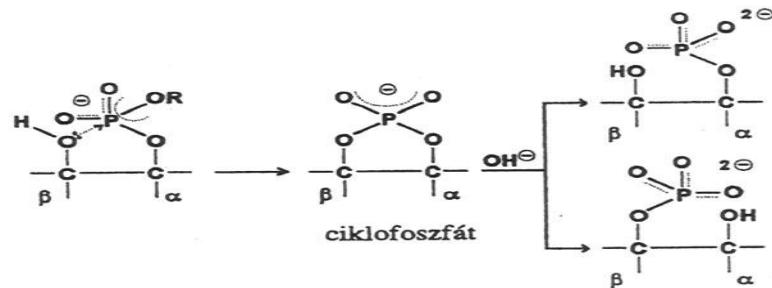
Foszforsav-tri-észter



Foszforsav-di-észter csak  $\beta$ -OH jelenlétében



Magyarázat: szomszédcsoport részvétel



A vicinális OH-ra tehát szükség van.

Nem lehet: 2'-3' vagy 2'-2' és 3'-3' (vagy 5'-5')

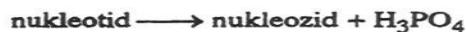
Két lehetőség: 2'-5' vagy 3'-5'

B)

#### Enzimes hidrolízis

#### Különböző fajlagosságú enzimek

##### 1.) Foszfo-monoészteráz (PME)

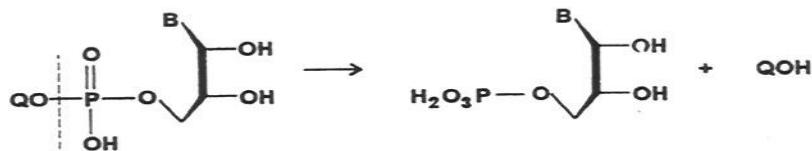


a, 3'-PME

b, 5'-PME

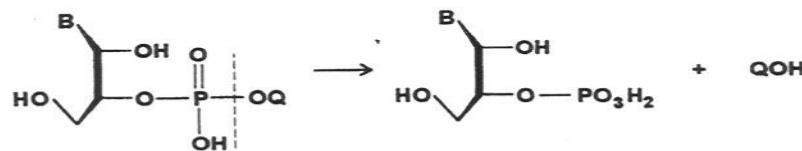
##### 2.) Foszfo-diészteráz (PDE)

a, Kígyóméreg-PDE

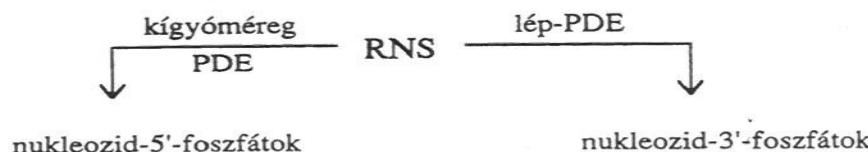


csak 5'-foszfátok észtereit bontja! (Pu, Py)

b, Lép-PDE



csak 3'-foszfátok észtereit bontja! (Pu, Py)

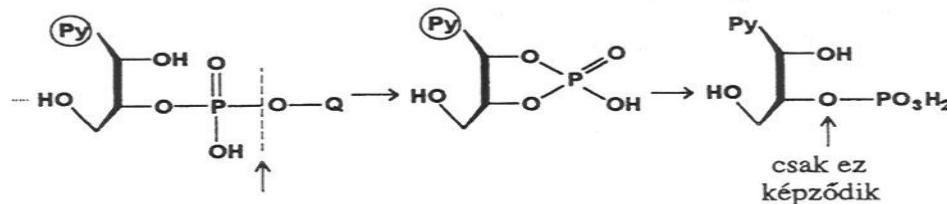


Teljes hidrolízis!

Ebből kötésmód: 3'-5'

3., Ribonukleáz

Csak pirimidin nukleozid-3'-foszfátok észterei



(Pl)

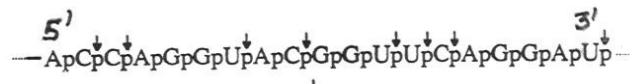
Rövidítések:

U, C, A, G ~ nukleozidok

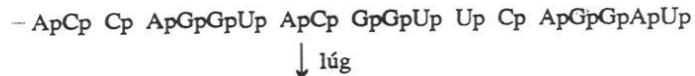


bal jobb

pC Cp  
5' 3'



↓ RNáz



pirimidin nukleozid-  
3'-foszfátok

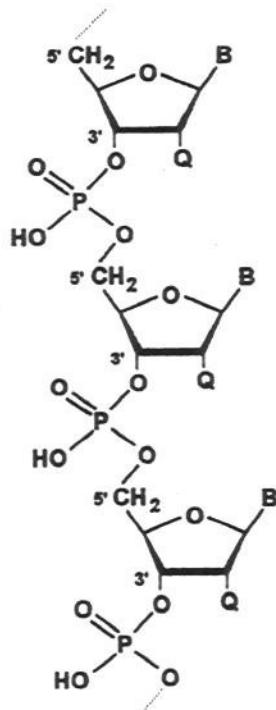
purin nukleozid-  
2'- és 3'-foszfátok elegye

DNS

kevesebb lehetőség

Enzimes hidrol. alapján: csak 3'-5' kötés!

Kapcsolódási mód:



$Q = H \sim DNS$

$Q = OH \sim RNS$

cukor-foszfát láncolat

Kapcsolódási sorrend ~ szekvencia

Megoldott kérdés

Holley (Nobel-díj ~ 1975)  
Sanger (Nobel-díj ~ 1980)

Részleges hidrolízis (pl. RNáz)

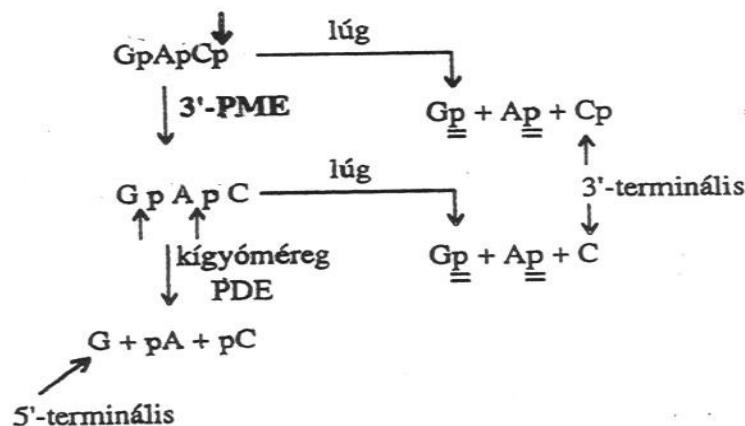
Végcsoport meghat. (pl. PME)

Lépésenkénti lebontás

Nagyon egyszerű példa

$\underline{p} = \begin{matrix} 2' \\ \text{és} \\ 3' \end{matrix}$

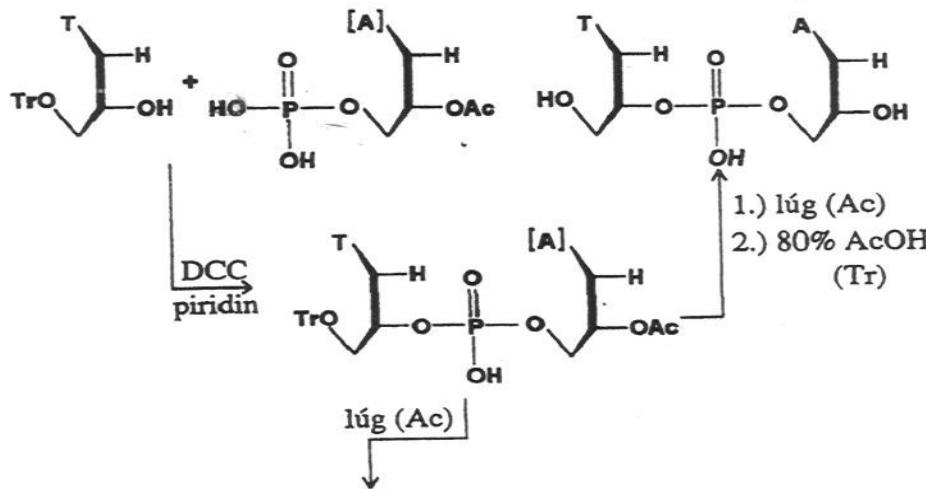
} foszfát elegye

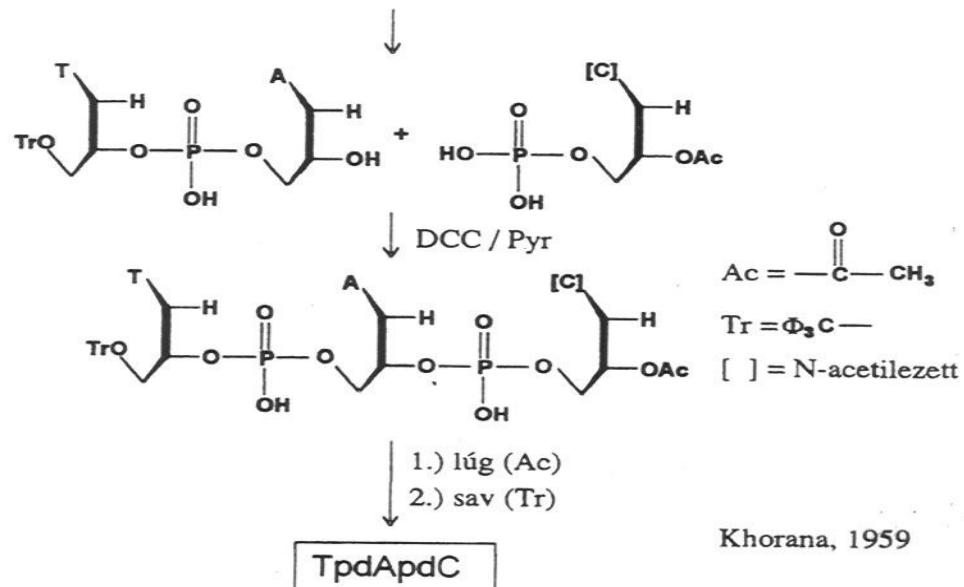


### Szintézis

Megfelelően védett nukleotidok összekapcsolása

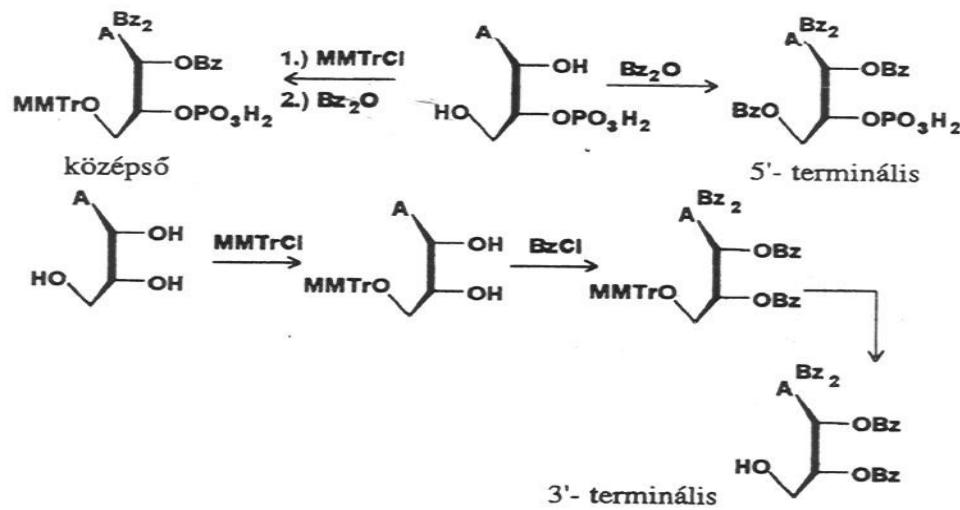
Dezoxi-sorban egyszerűbb      Todd (Nobel-díj ~ 1968)  
Khorana (Nobel-díj ~ 1975)

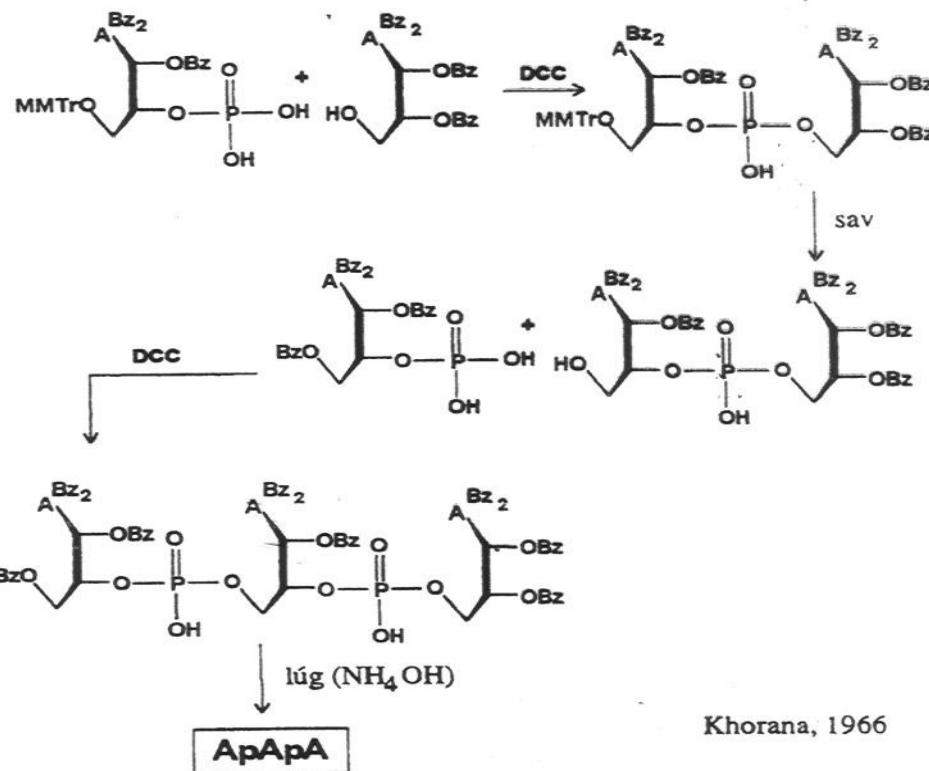
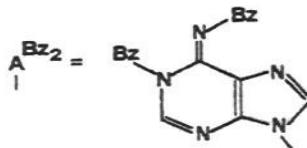
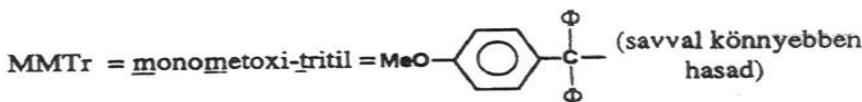
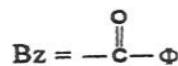




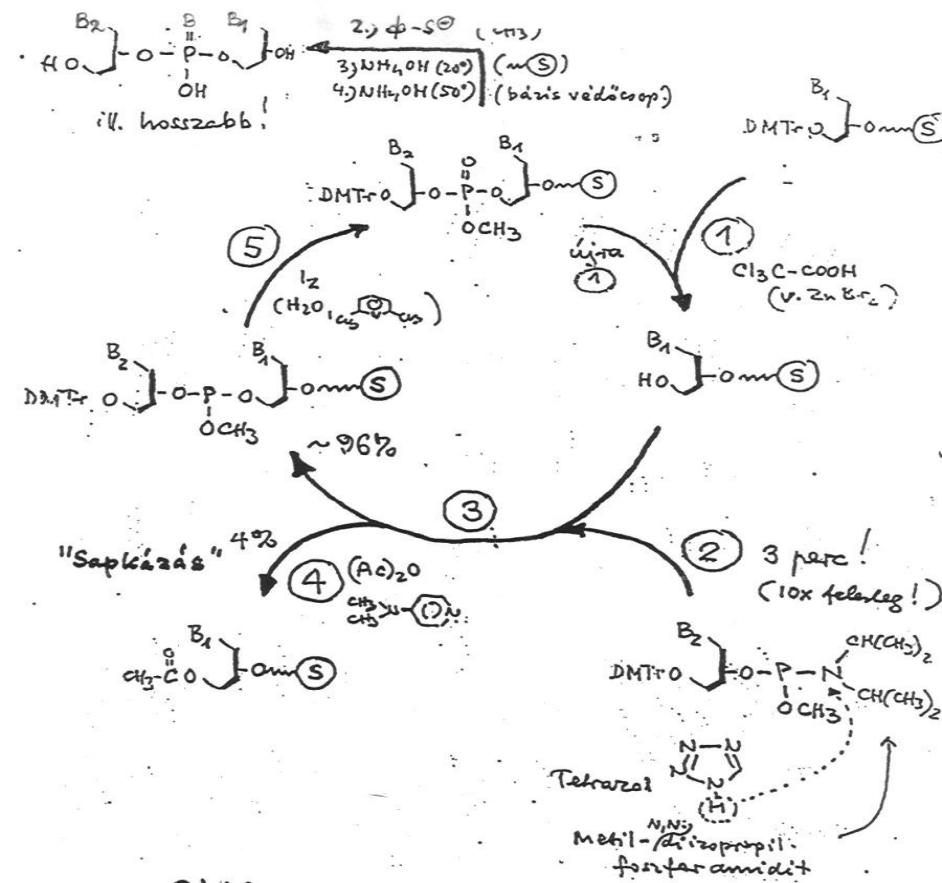
Ribo-trinukleotid szint. ApApA

Kiindulási anyagok:



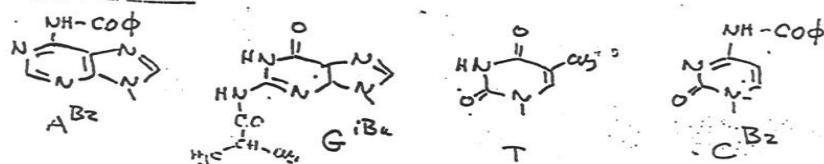


2x207 nukleotidból álló DNS (Khorana, 1979)  
speciális (enzimes) módszerrel 8-12-es darabokból

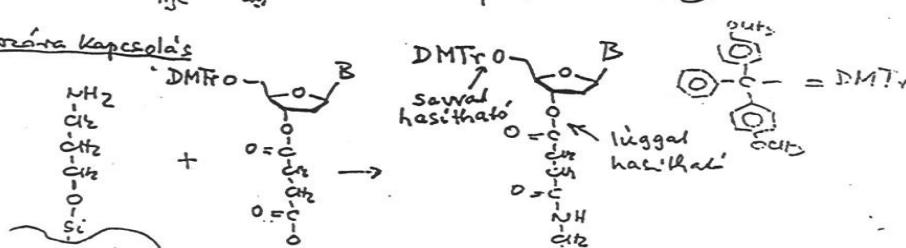


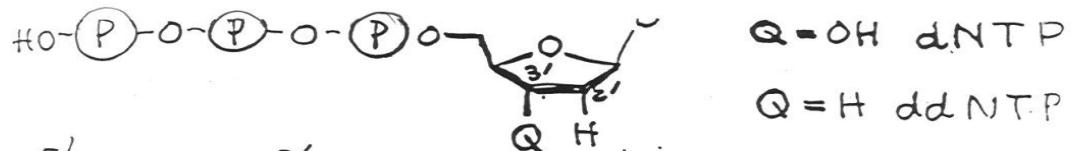
## OLIGONUKLEOTID SINTÉZIS (AUTOMATIKUS)

## Vedett bázisok

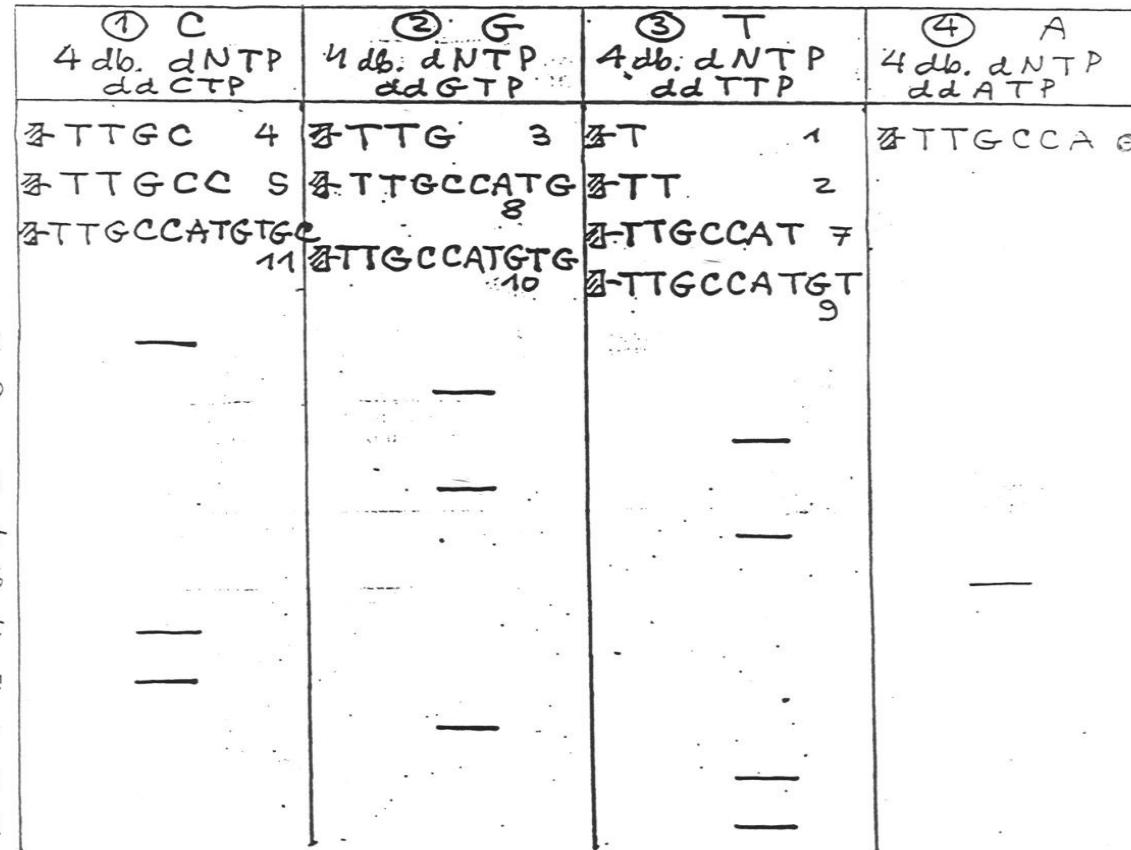


## Hordozási kapcsolat

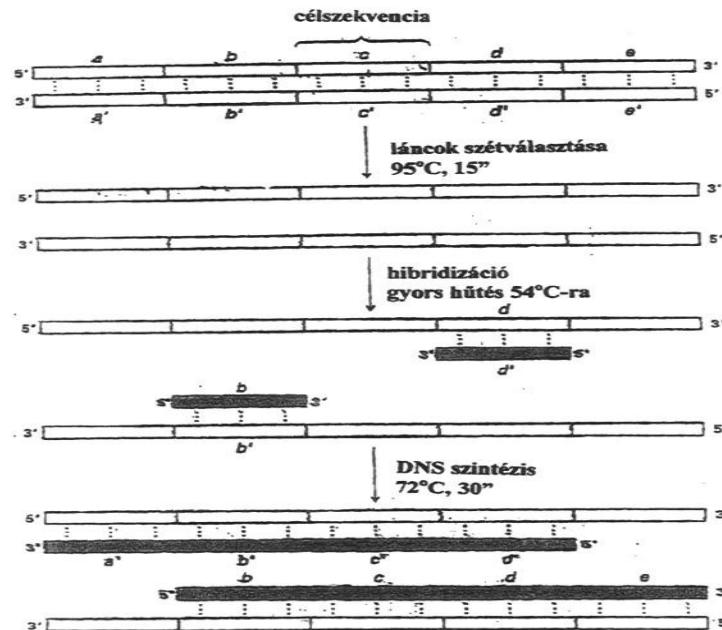




S' primer OH SANGER-FÉLÉ DIDEZOKI-MÓDSZER  
 AACGGTACACG G S' 5'



3' 5'  
 TTGCCATGTG  
 AACGGTACACG .....



**Cycle 0**

0 0  
 a a'  
 b b'  
 c c'  
 d d'  
 e e'

**Cycle 1**

0 1 1 0  
 a a' a'  
 b b' b' b'  
 c c' c c'  
 d d' d' d'  
 e e e'

**Cycle 2**

0 1 2 2 2 2 1 0  
 a a' a' a'  
 b b' b' b' b' b' b'  
 c c' c' c c' c c'  
 d d' d' d' d' d' d'  
 e e e e'

**Cycle 3**

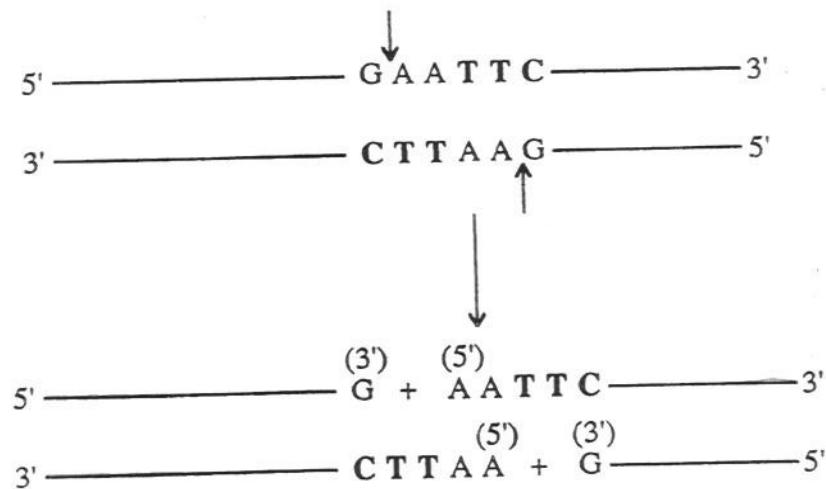
0 1 2 3 2 3 3 3 3 3 2 3 2 1 0  
 a a' a' a'  
 b b' b' b' b b b b' b' b' b b b'  
 c c' c' c' c c' c c' c' c c c c'  
 d d' d' d' d d d' d' d' d' d' d' d'  
 e e e e'

<b>Organizmus</b>	<b>Ezer bázispár</b>	<b>Hossz (μm)</b>
<b>Vírusok</b>		
Polioma (SV40)	5,1	1,7
λ fág	48,6	17
T2 fág	166	56
Vaccinia	190	65
<b>Baktériumok</b>		
Mycoplasma	760	260
E. coli	4 000	1 360
<b>Eukarióták</b>		
Élesztő	13 500	4 600
Muslica	165 000	56 000
Ember	2 900 000	990 000

## RESTRIKCIÓS ENZIMEK

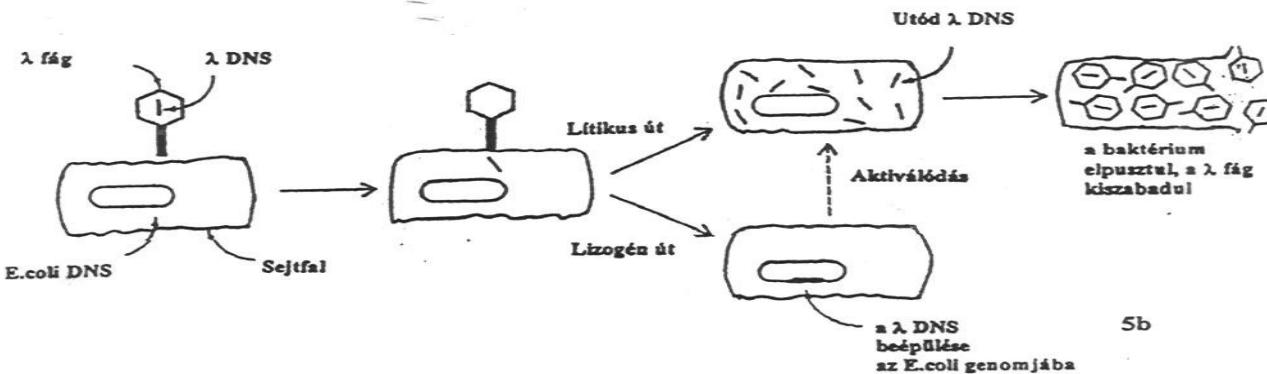
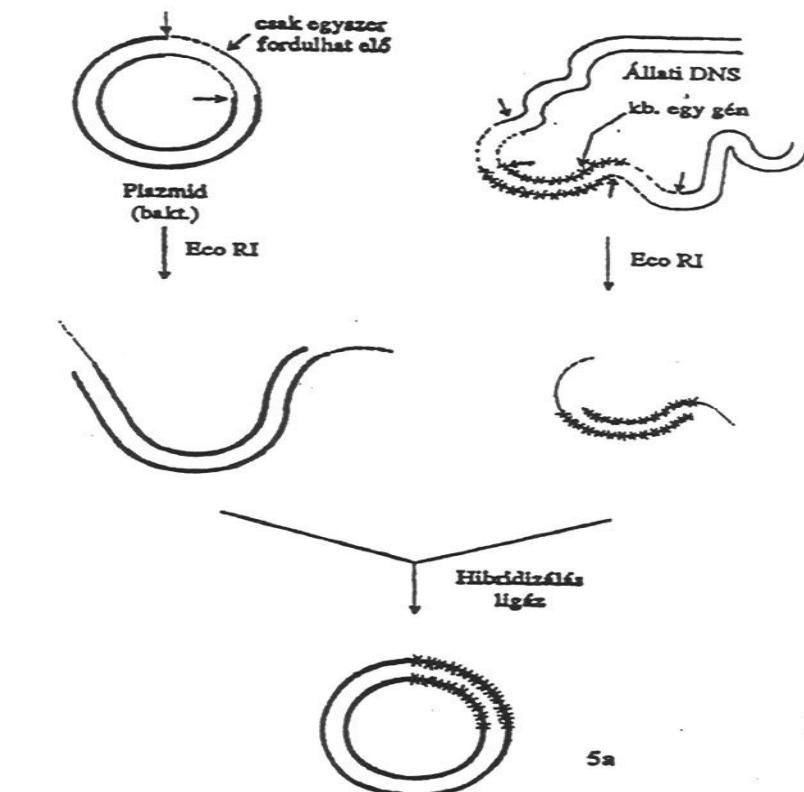
Baktériumokban pl. E. coliban Eco RI  
kb. 100 féle ismert.

Hasítási mechanizmus („palindróm” szekvenciánál)

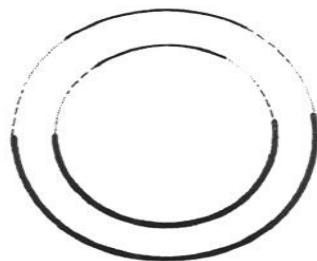


Könnyen újra összekapcsolható ligáz-zal.

Állati DNS-ből restriktiós enzimmel



5. ábra. A plazmidok és a fág vektorok működési elve

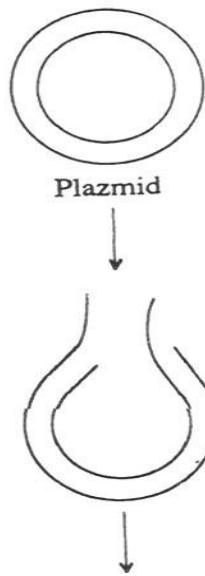
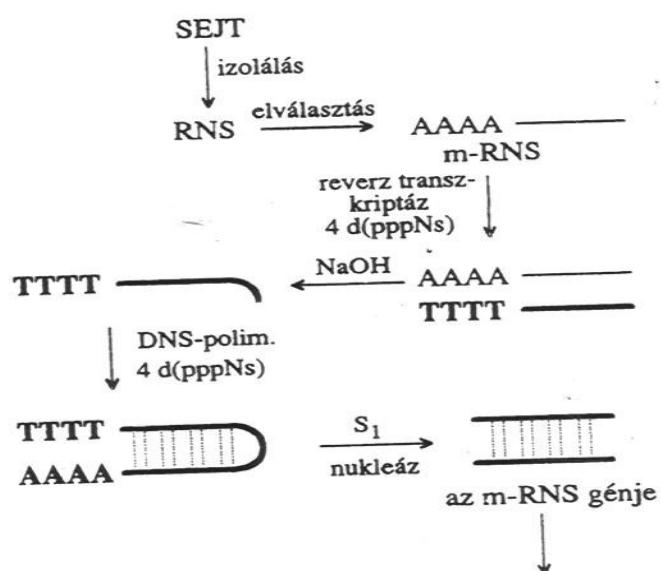


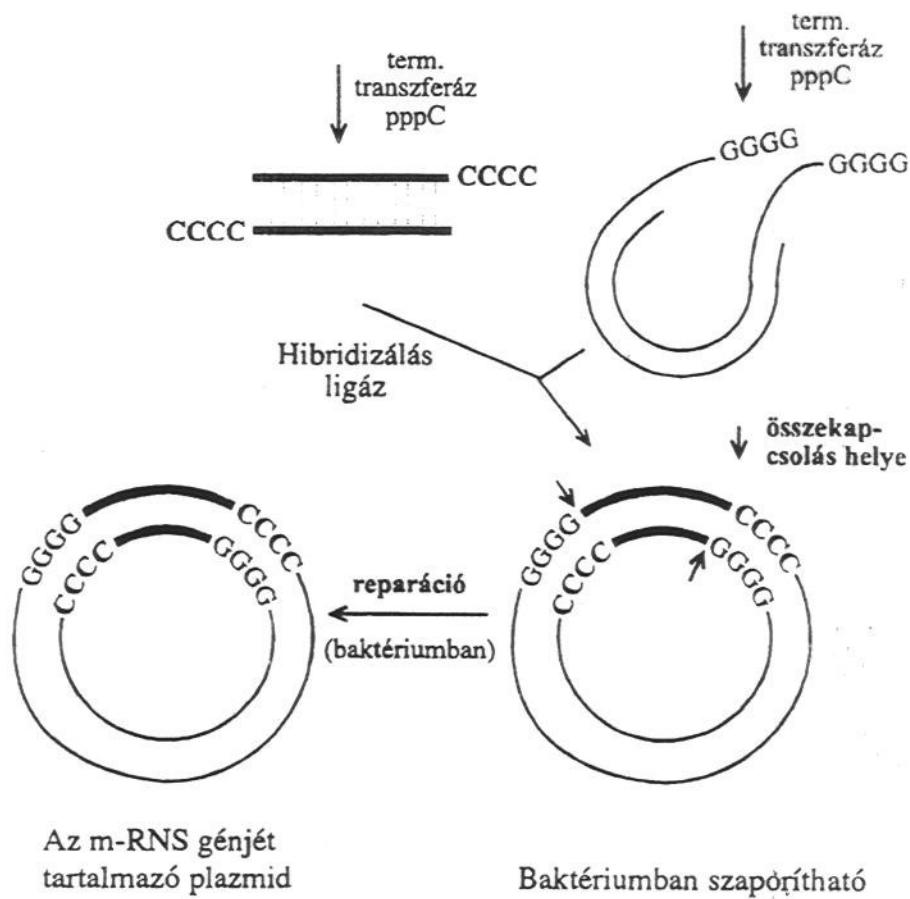
Állati gént tartalmazó plazmid  
„chiméra DNS”



Baktériumokban tovább szaporítható

B) m-RNS-ből kiindulva





Az m-RNS génjét tartalmazó plazmid

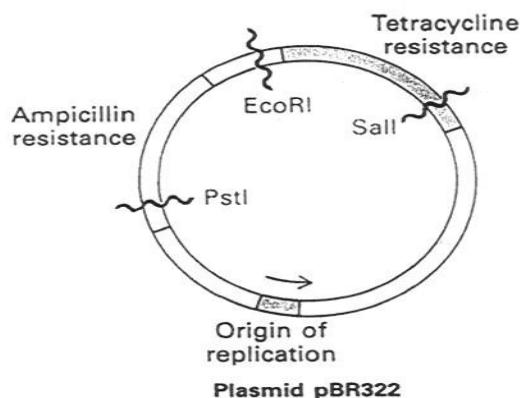
Baktériumban szaporítható

Pl.: globin génje (m-RNS retikulocitákból izolálható)

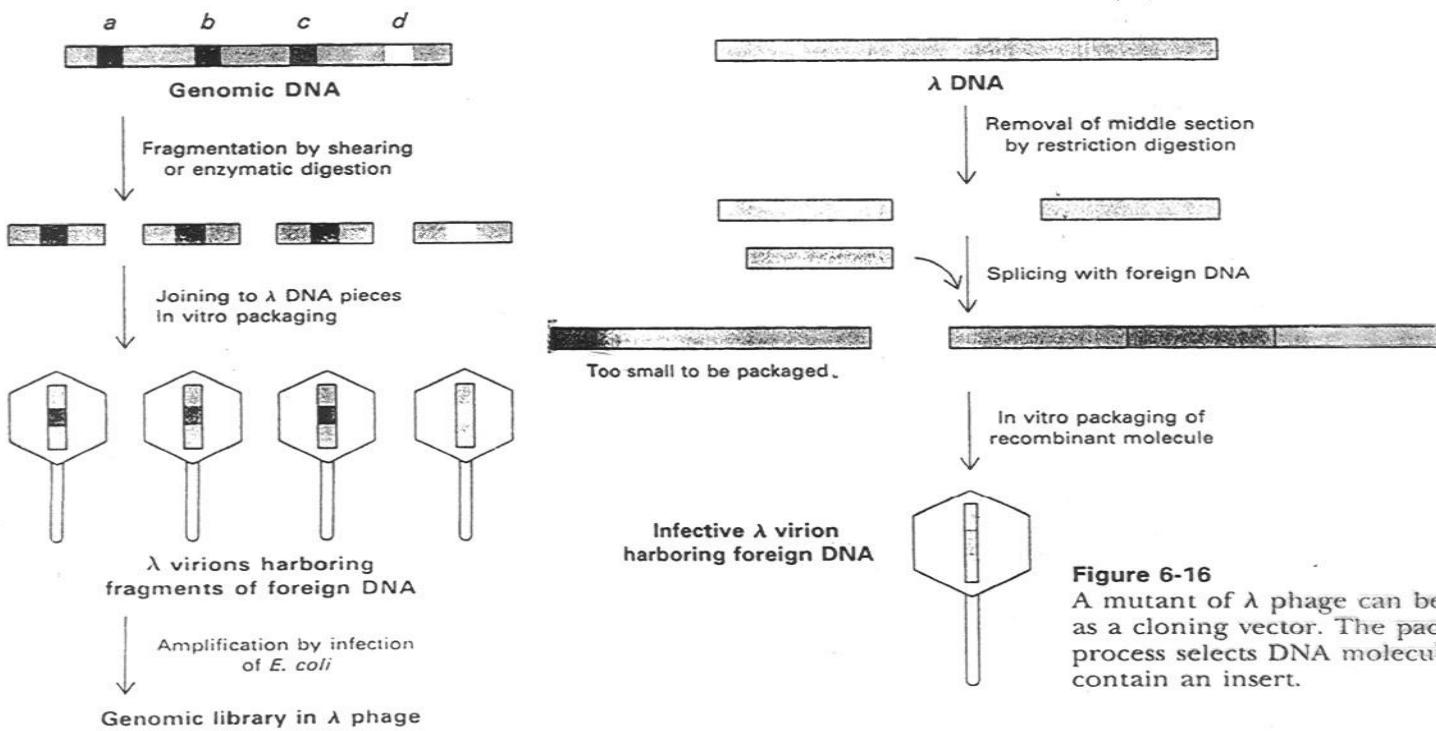
Ugyanígy „szaporíthatók” a szintetikus gének

## ÚT A HUMÁN GENOM FELDERÍTÉSÉHEZ

- 1990: - W. F. Anderson első ízben alkalmaz génterápiát egy immunrendszer betegségben szenvedő kislánynál  
- A Department of Energy (DOE) és a National Institutes of Health (NIH) beindítja a 15 évre tervezett Human Genome Project-et.  
Cél: gének lokalizálása és biológiai vizsgálata  
az emberi genom  $3 \cdot 10^9$  bázispárnyi DNS-ének szekvenálása
- 1992: - Kaliforniai kutatók felfedezik, hogy egy gén a 19. kromoszómában szívinfarktus kockázati tényező. A gén a lakosság 25-30%-ának genomjában azonosítható.  
Klinikai teszt lehetősége  
- A Wellcome Trust (Nagy-Britannia) csatlakozik a genom projekthez
- 1994: - A DOE beindítja a Microbial Genome Program-ot
- 1995: - Craig Venter és munkatársai az Institute for Genomic Research (Maryland) kutatóközpontban megfejtik az influenza vírus teljes genomját a „genome shotgun sequencing” módszer segítségével
- 1996: - Ian Wilmut és munkatársai Skóciában klónozzák a Dolly névre keresztelt bárányt egy felnőtt anyajuh sejtjéből. Ez az első nem-szexuális úton létrejött élőlény!  
- Az E. coli baktérium teljes genomjának megfejtése
- 1998: - Egy többsejtű élőlény, a C. elegans nevű féreg teljes genomjának megfejtése
- 1999: - A DOE Joint Genome Institute (genom-szekvenáló központ) létrehozása Kaliforniában  
- A muslica (Drosophila fruit fly) teljes genomjának felderítése



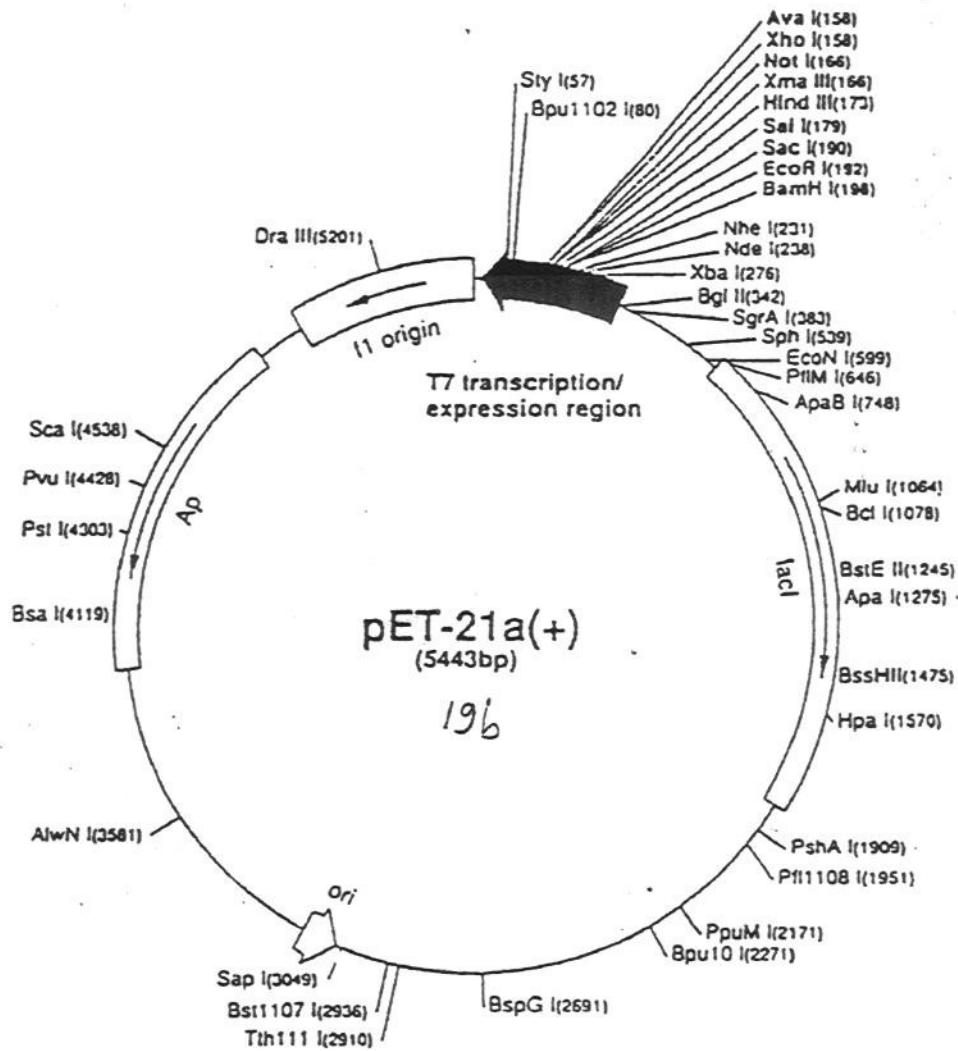
**Figure 6-14**  
Genetic map of pBR322, a plasmid with two genes for antibiotic resistance. pBR322, like all other plasmids, is a circular duplex DNA.

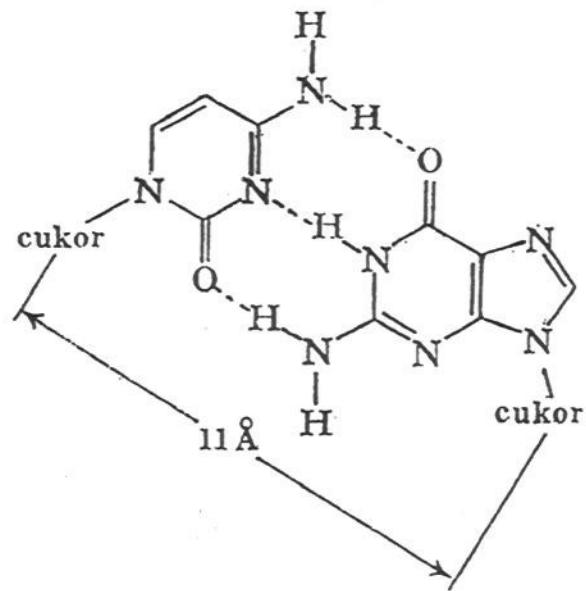
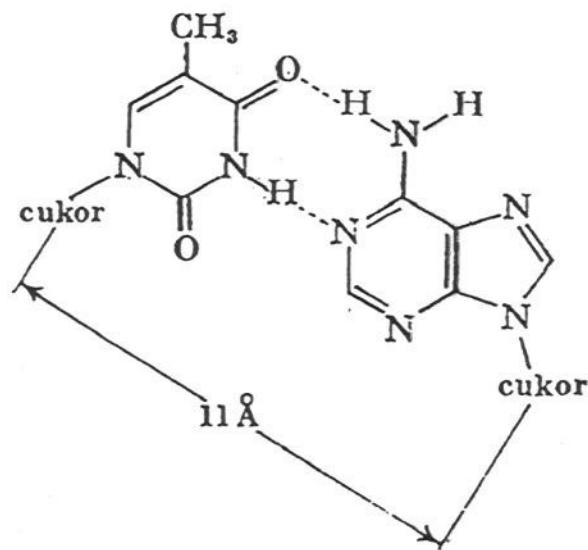


**Figure 6-16**  
A mutant of  $\lambda$  phage can be used as a cloning vector. The packaging process selects DNA molecules that contain an insert.



Novagen







Original parent molecule

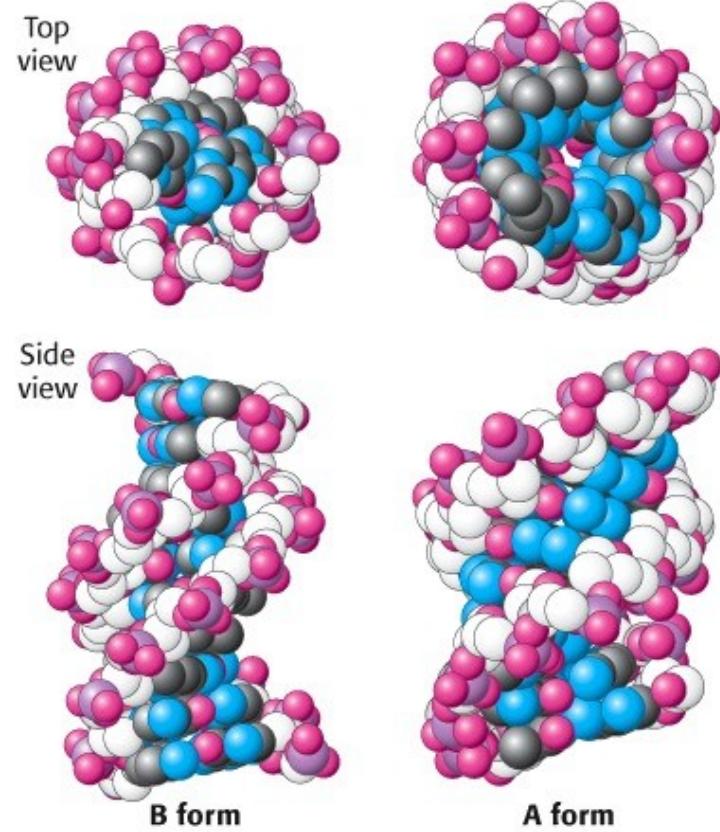
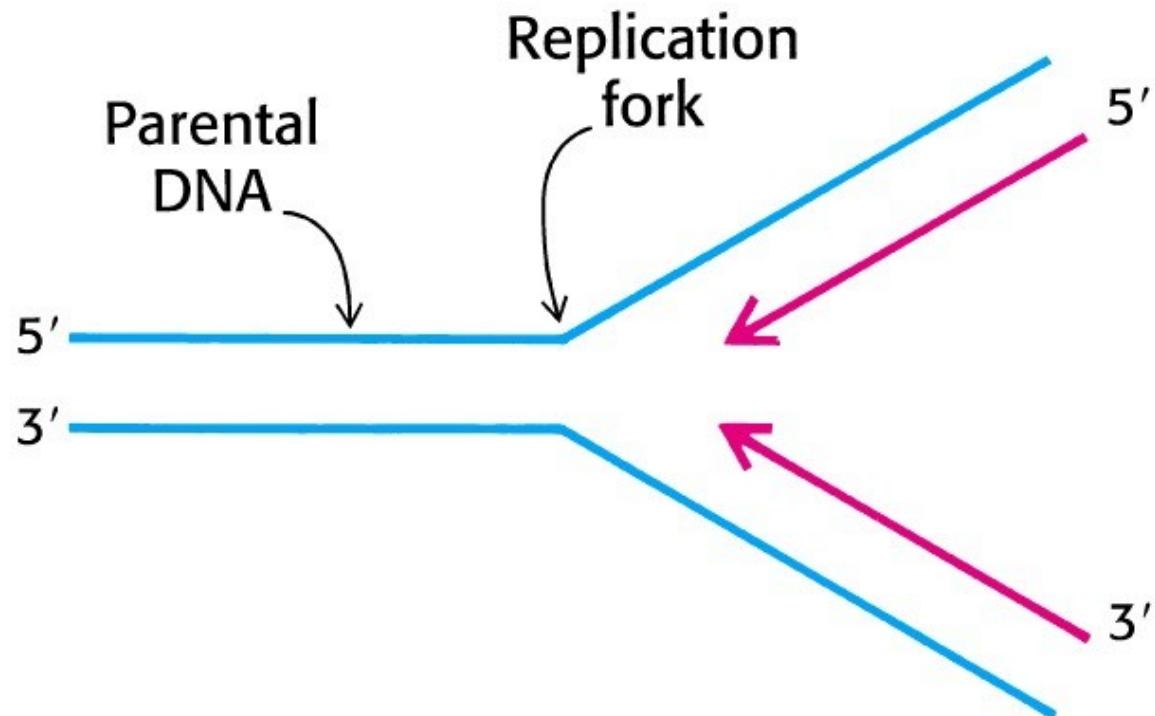


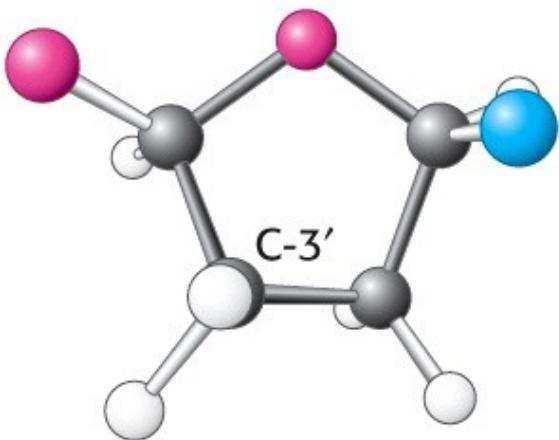
First-generation daughter molecules

Unwound

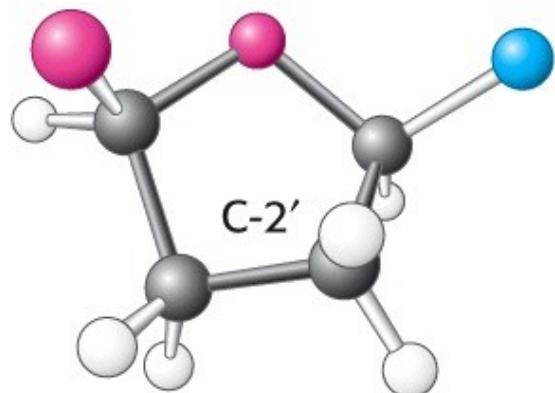
Overwound



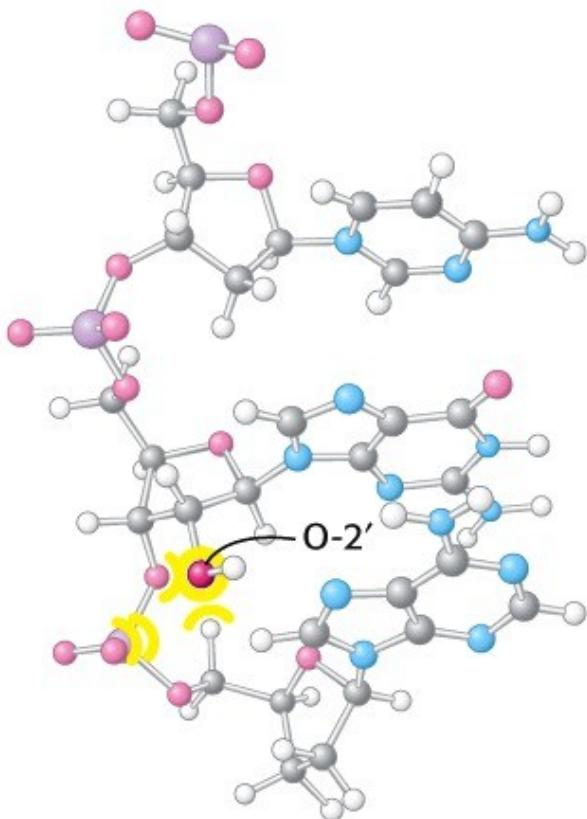


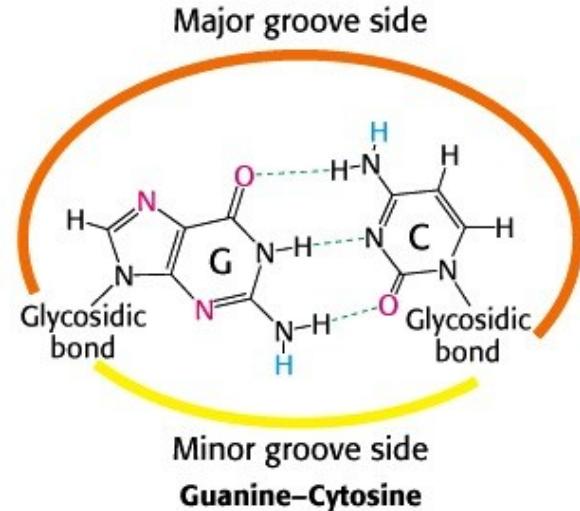
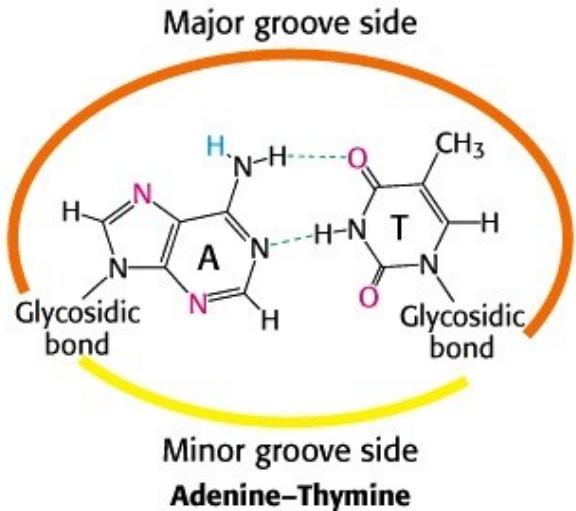
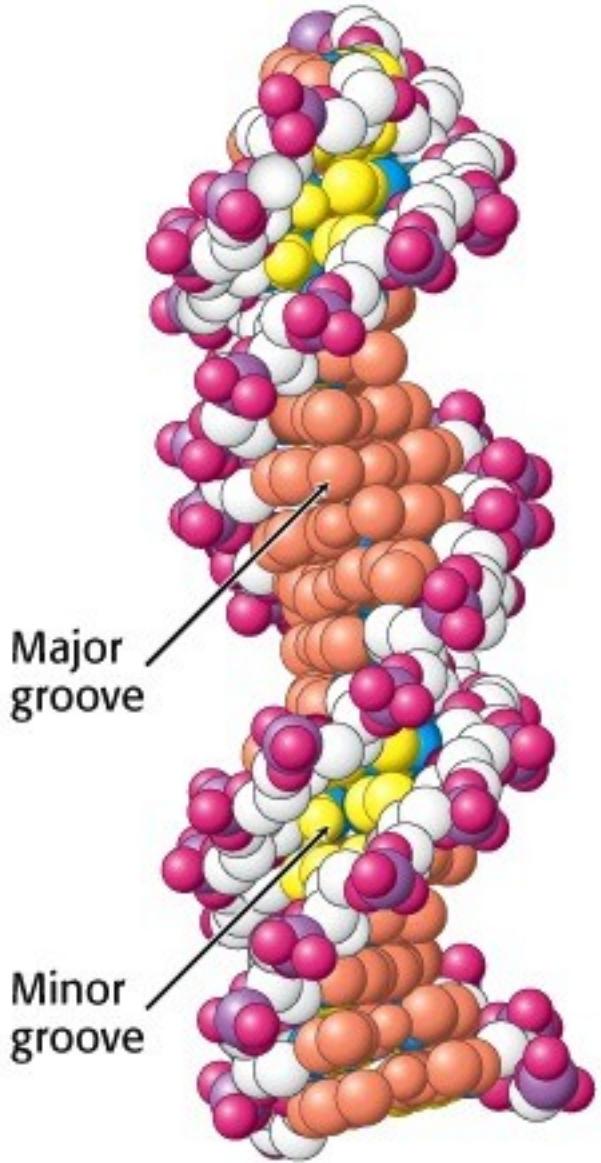


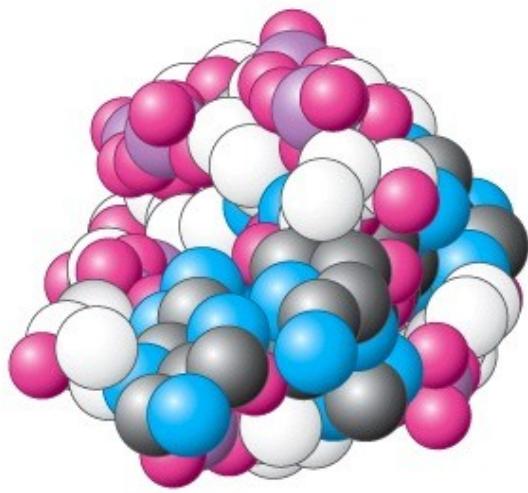
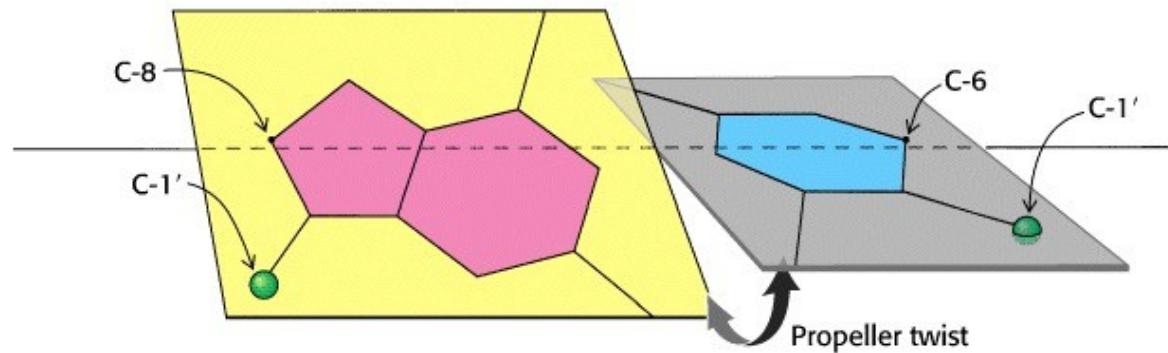
C-3' endo (A form)



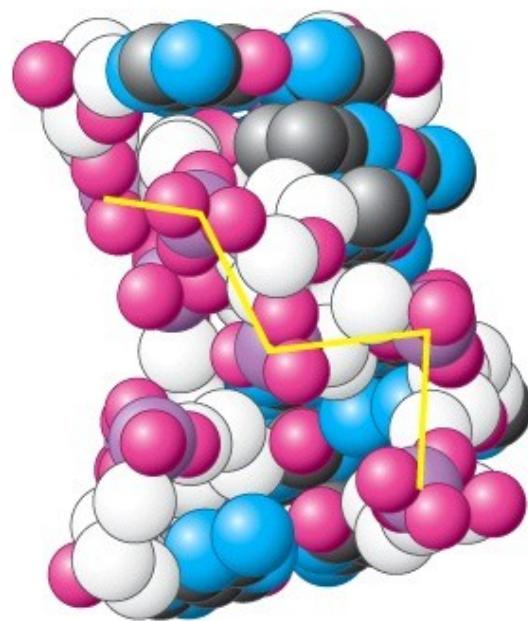
C-2' endo (B form)



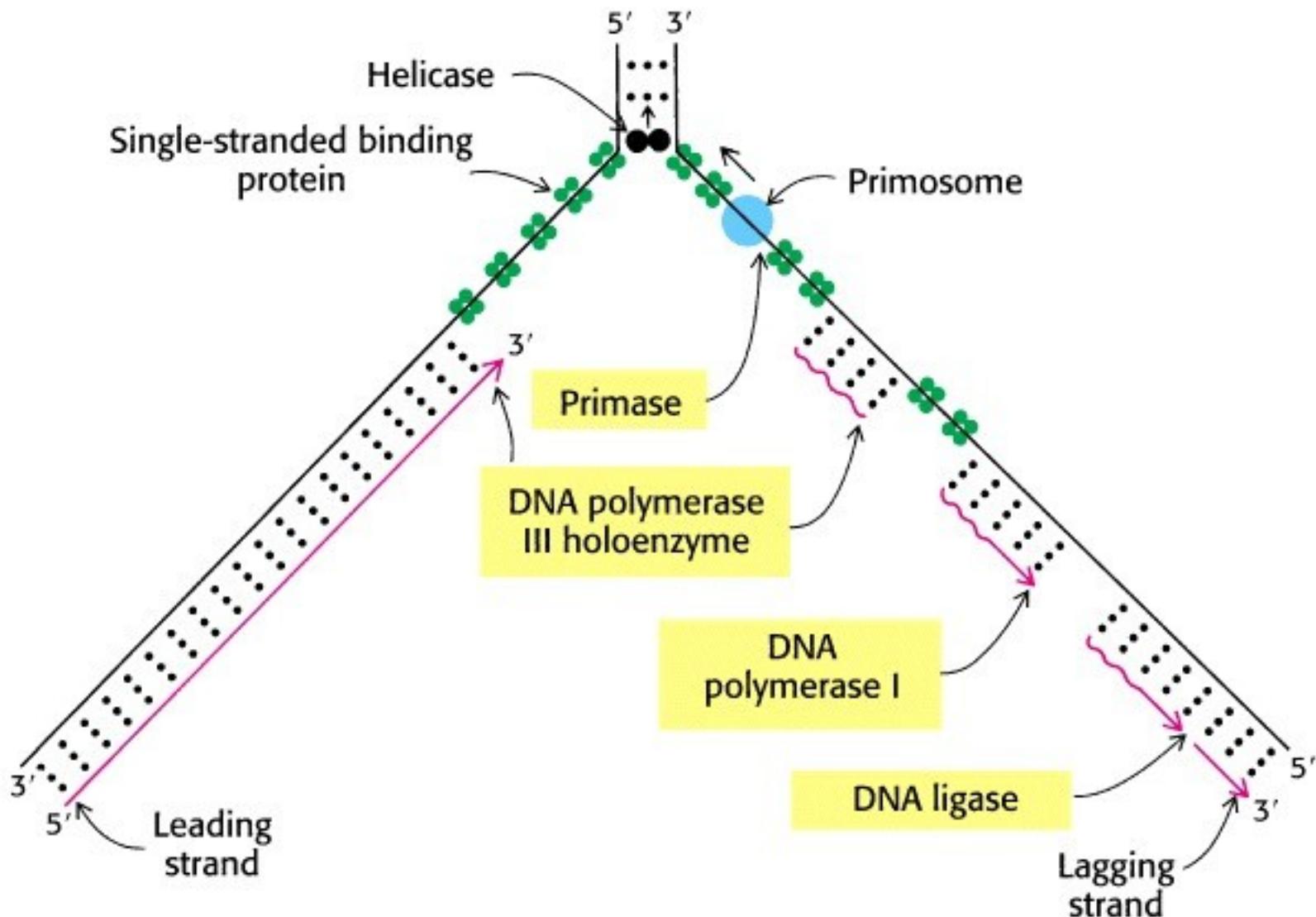


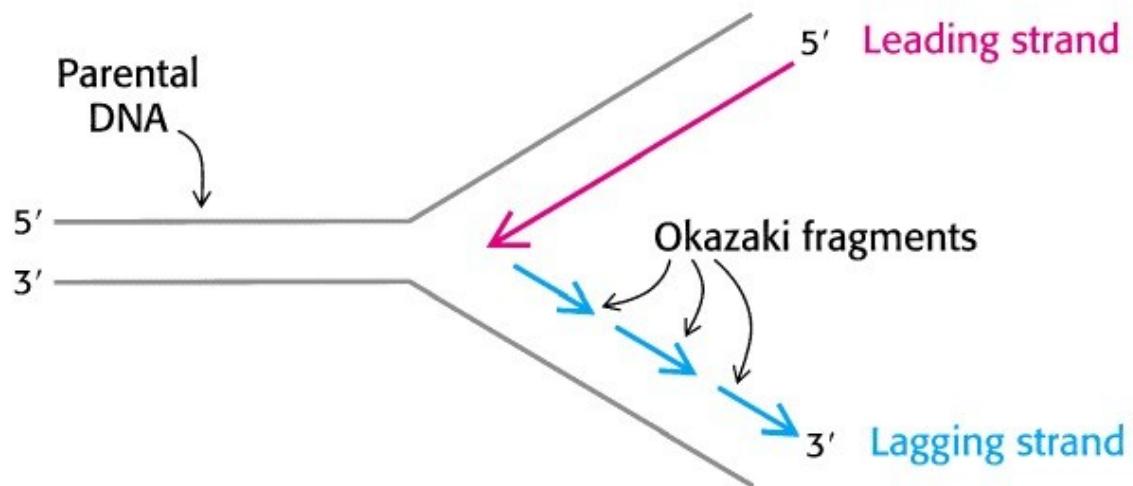
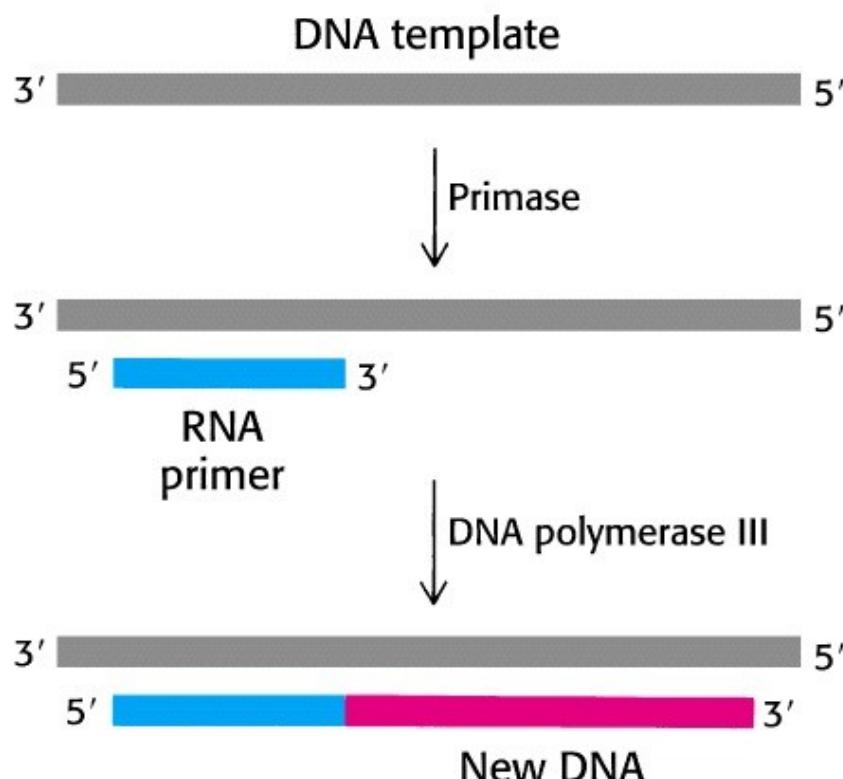


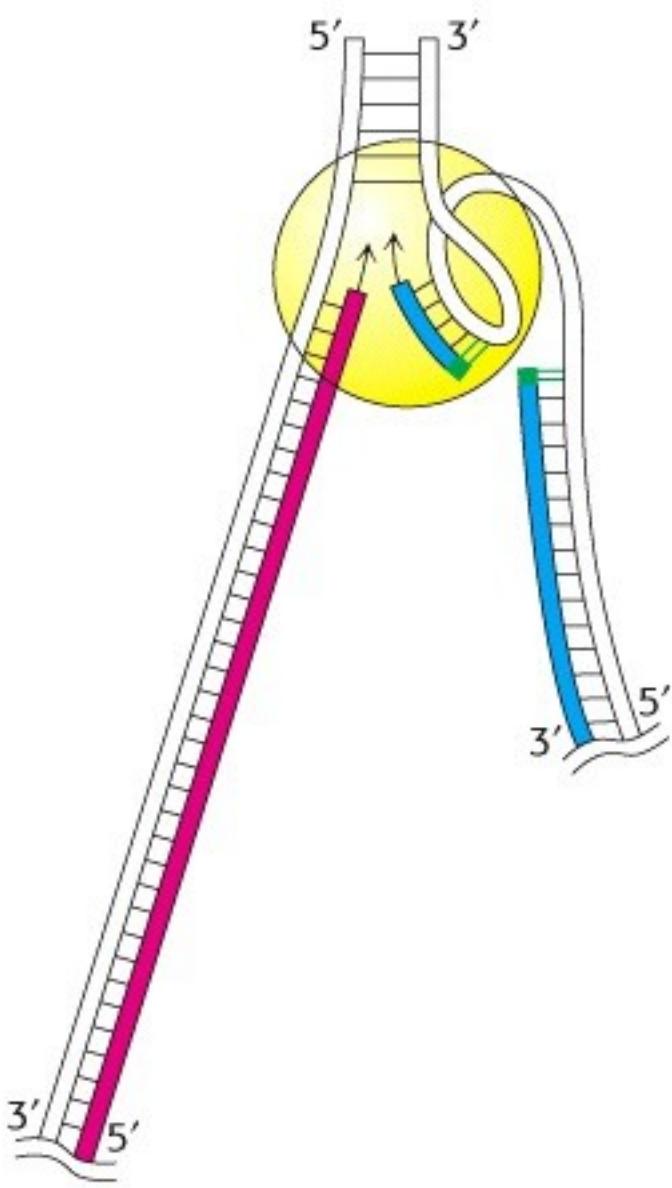
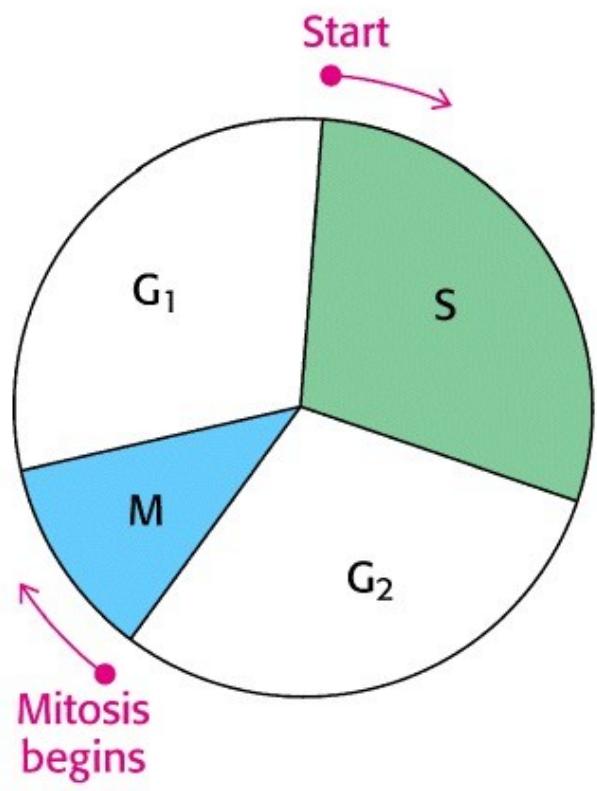
Top view



Side view

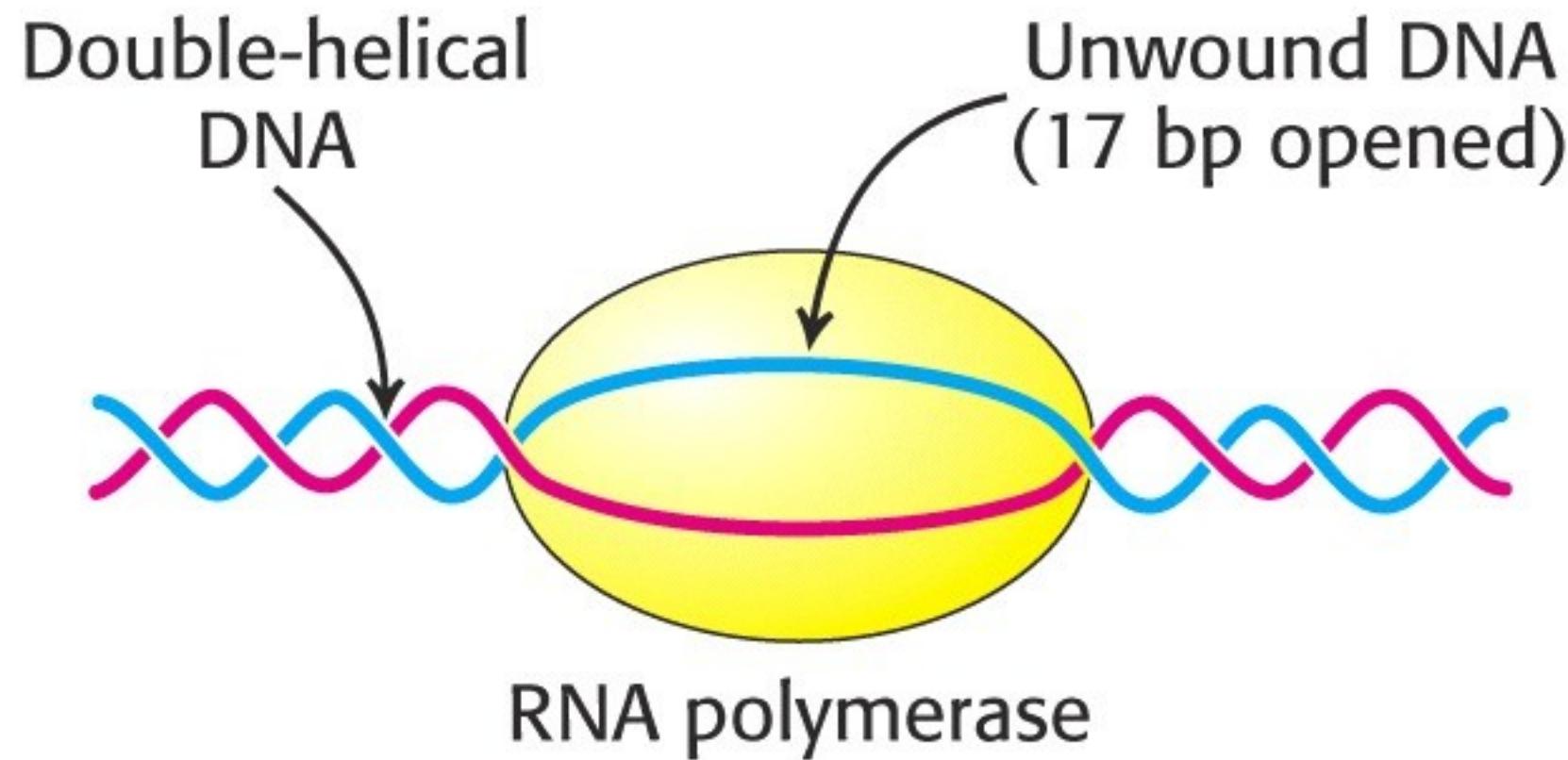
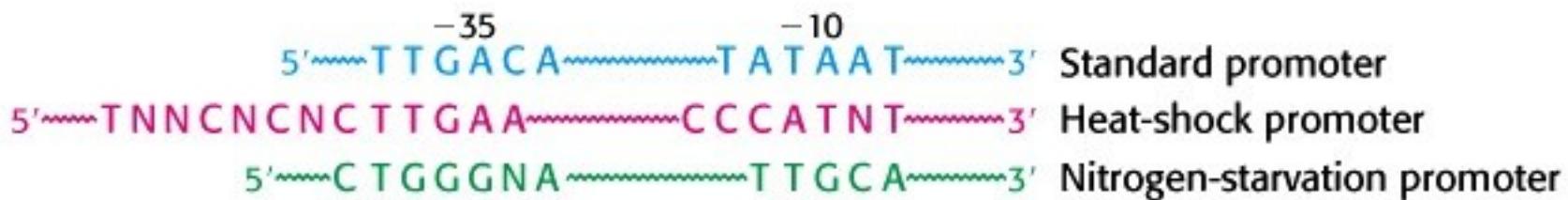


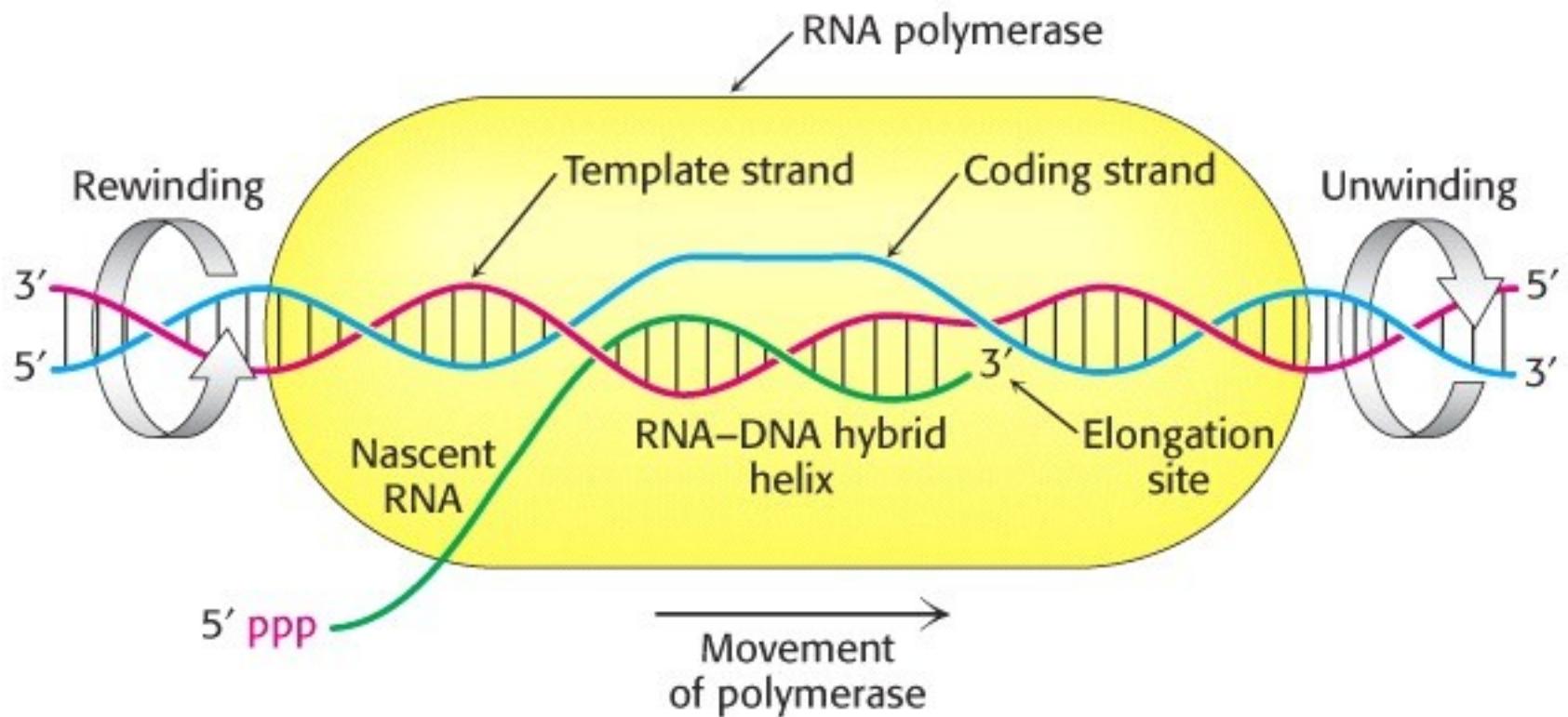


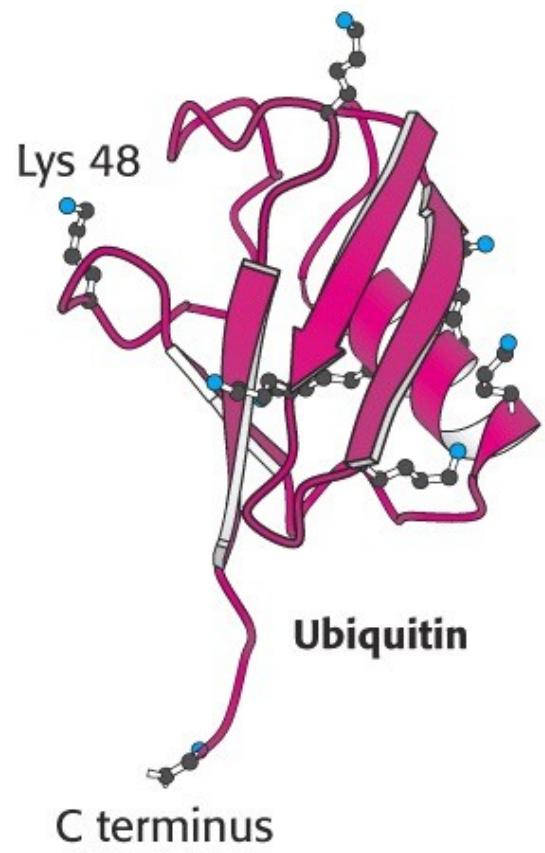
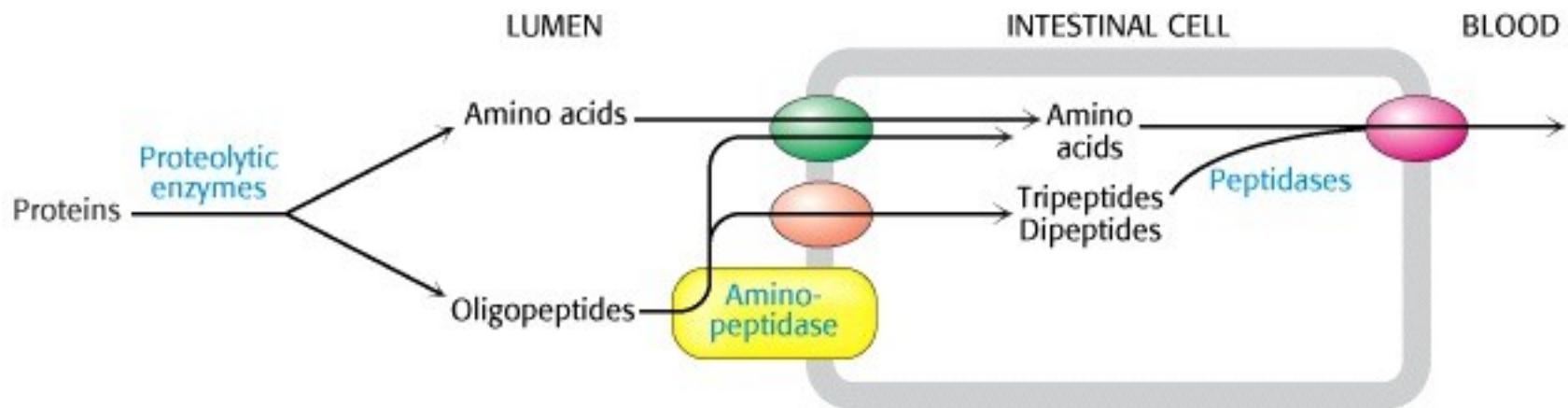


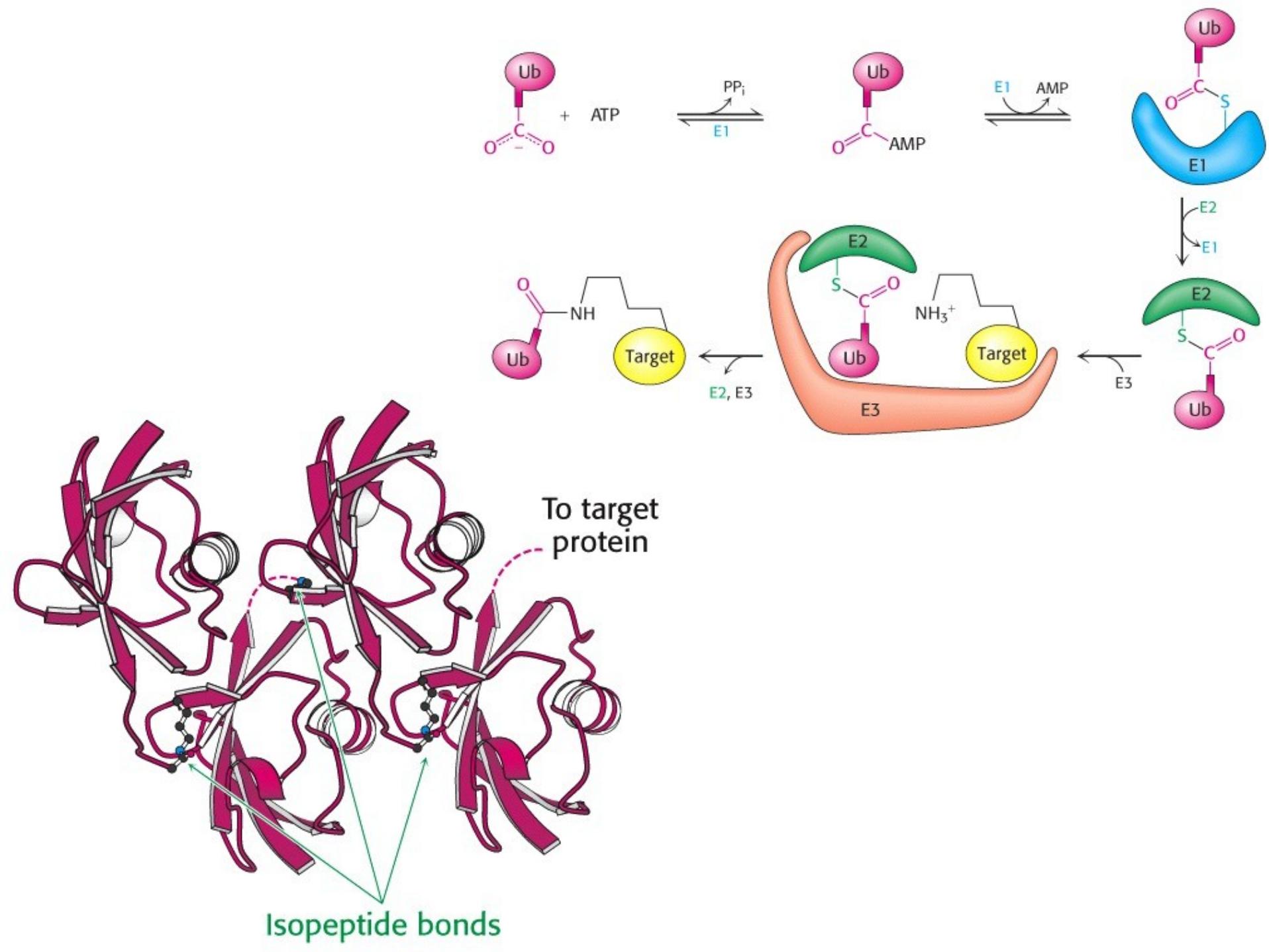
**TABLE 27.1 Comparison of A-, B-, and Z-DNA**

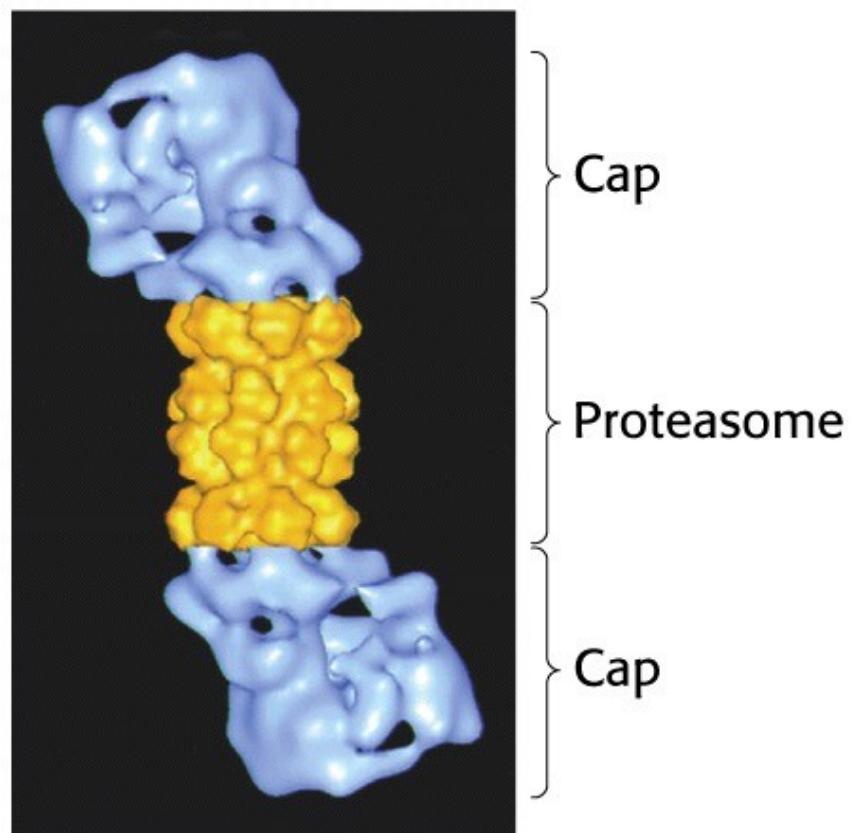
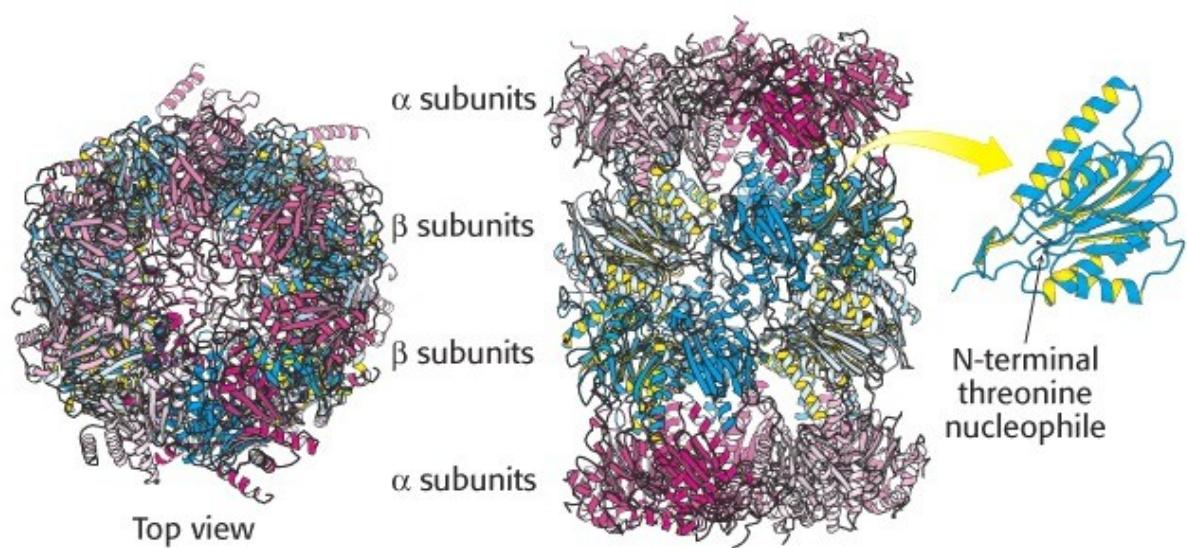
	Helix type		
	A	B	Z
Shape	Broadest	Intermediate	Narrowest
Rise per base pair	2.3 Å	3.4 Å	3.8 Å
Helix diameter	25.5 Å	23.7 Å	18.4 Å
Screw sense	Right-handed	Right-handed	Left-handed
Glycosidic bond	<i>anti</i>	<i>anti</i>	alternating <i>anti and syn</i>
Base pairs per turn of helix	11	10.4	12
Pitch per turn of helix	25.3 Å	35.4 Å	45.6 Å
Tilt of base pairs from normal to helix axis	19°	1°	9°
Major groove	Narrow and very deep	Wide and quite deep	Flat
Minor groove	Very broad and shallow	Narrow and quite deep	Very narrow and deep

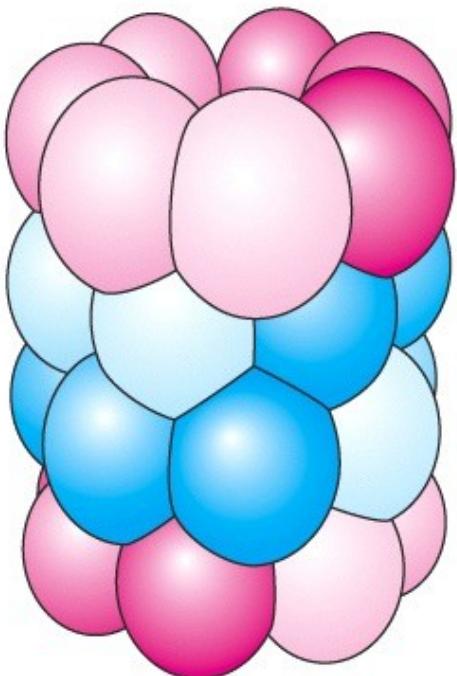




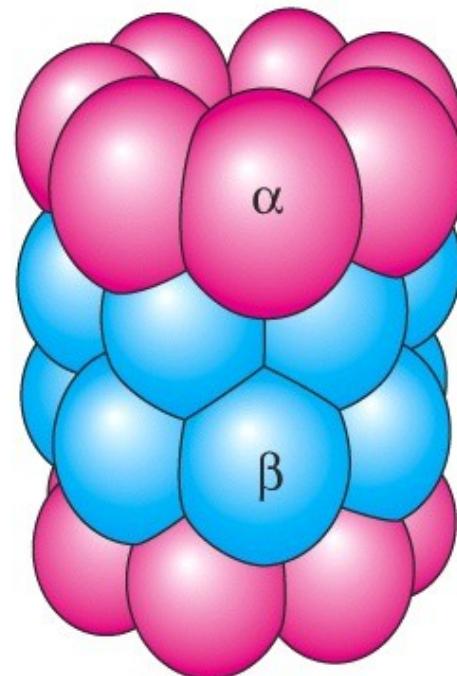




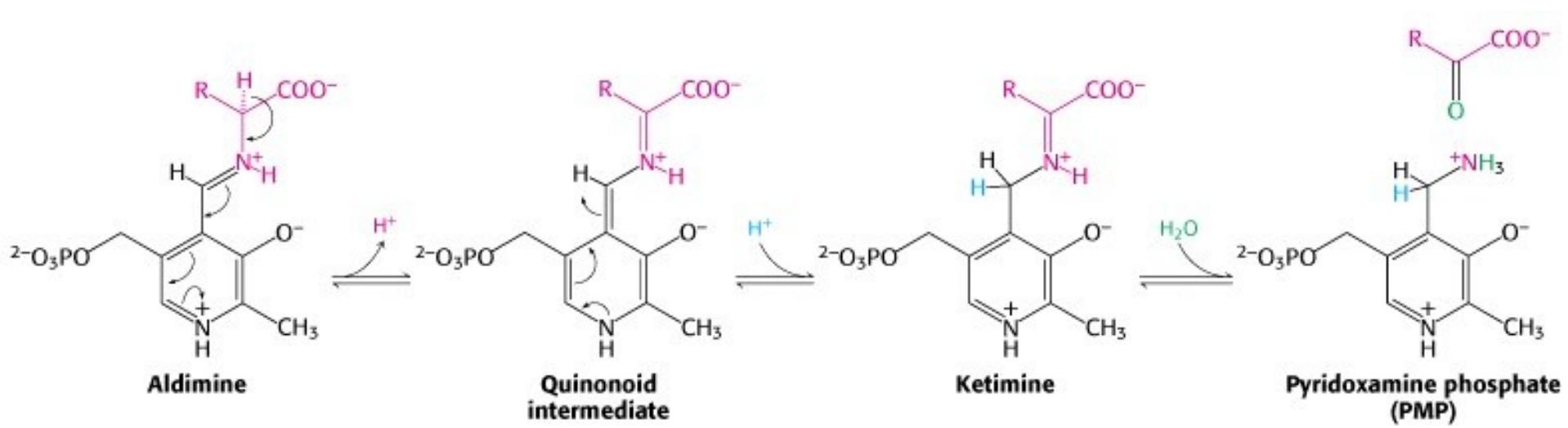


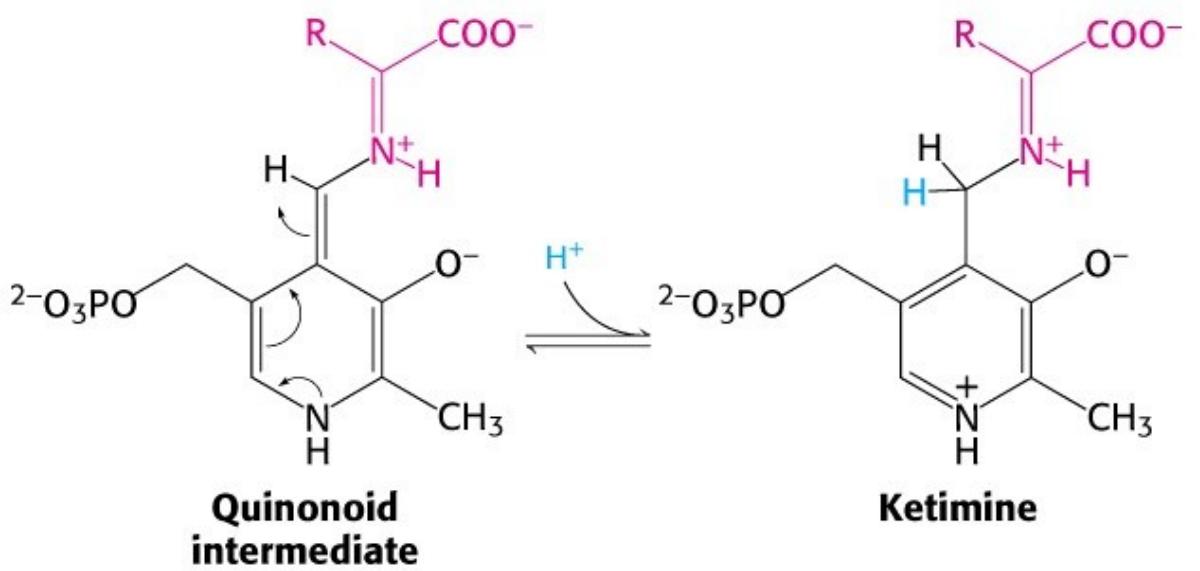
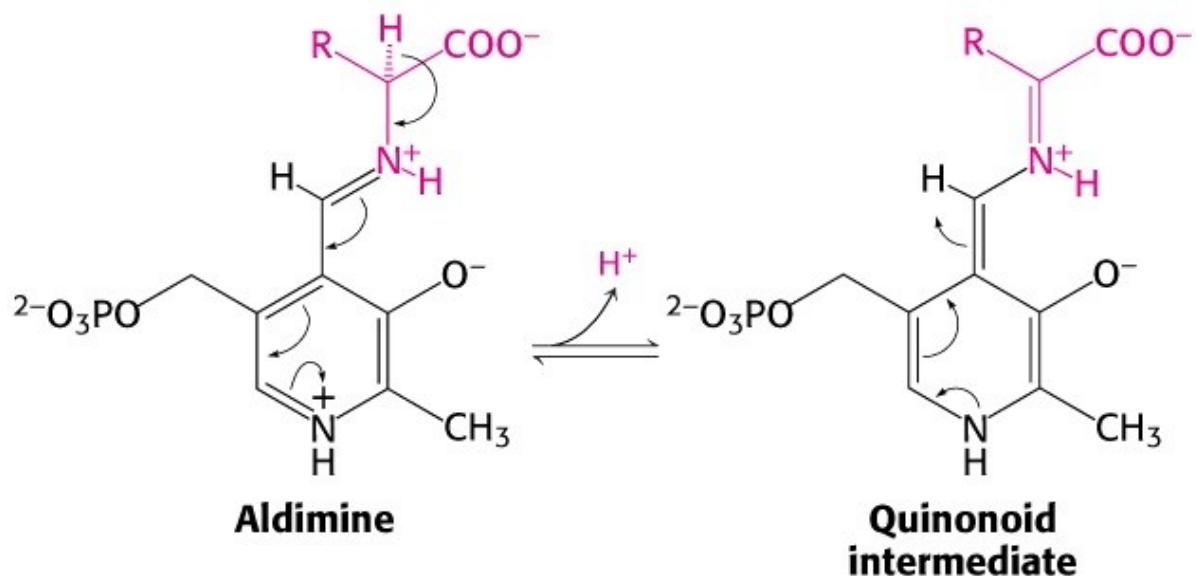


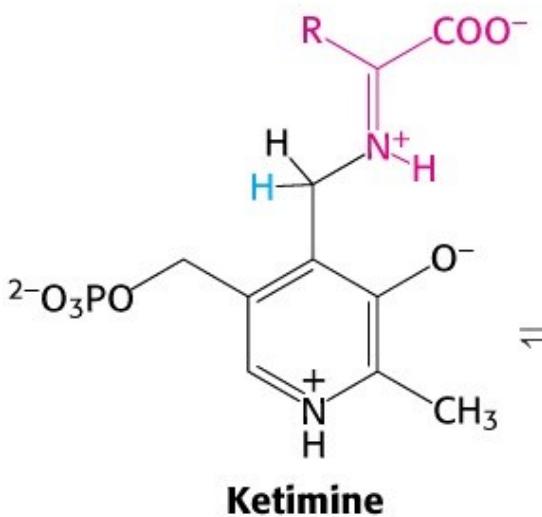
Eukaryotic proteasome



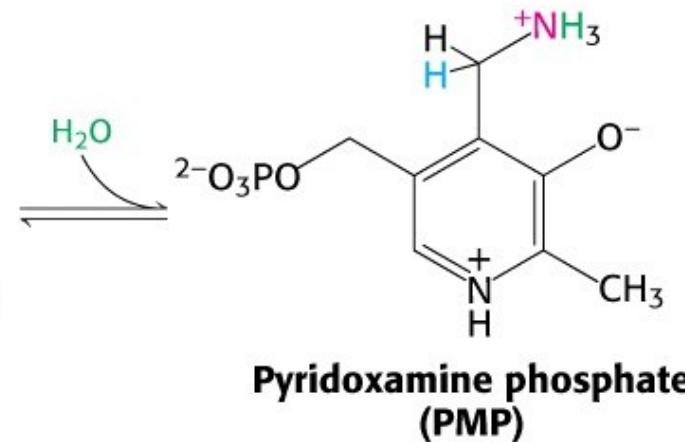
Archaeal proteasome



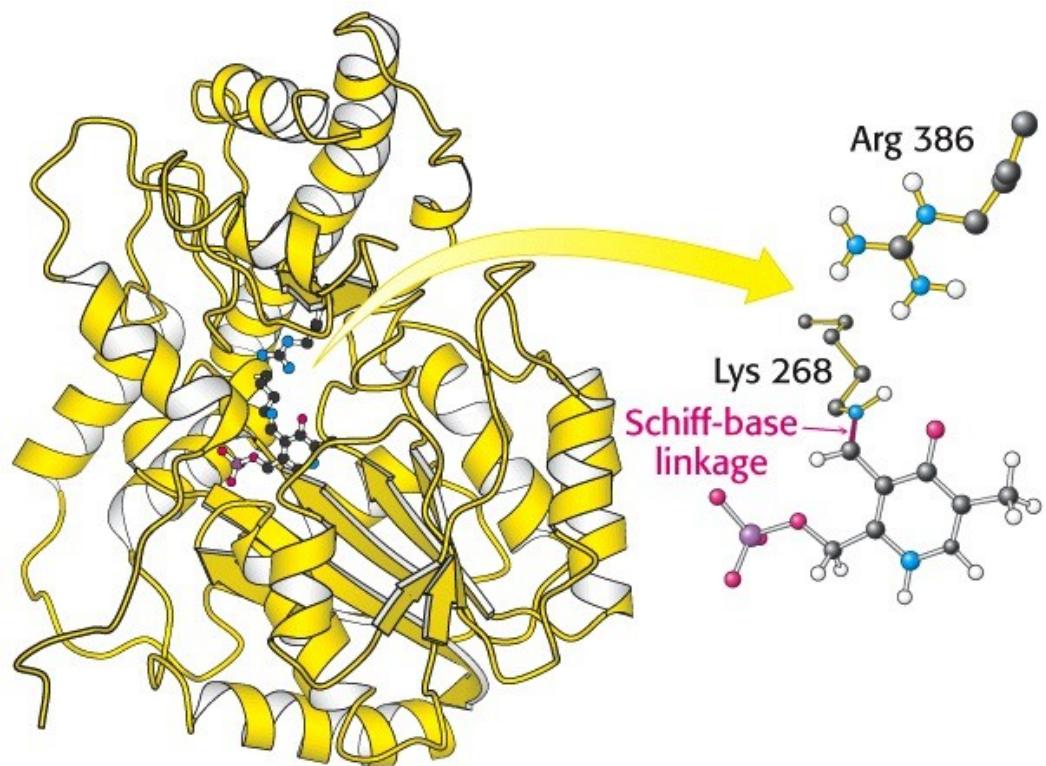


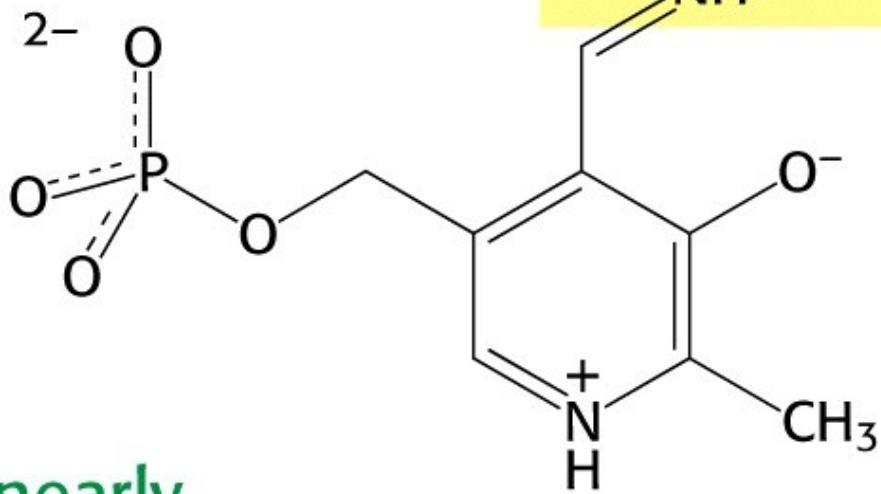
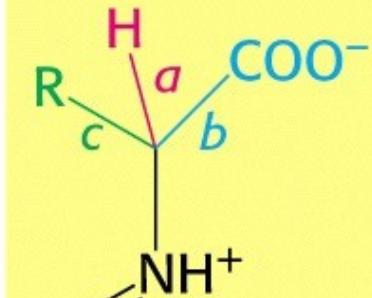


**Ketimine**

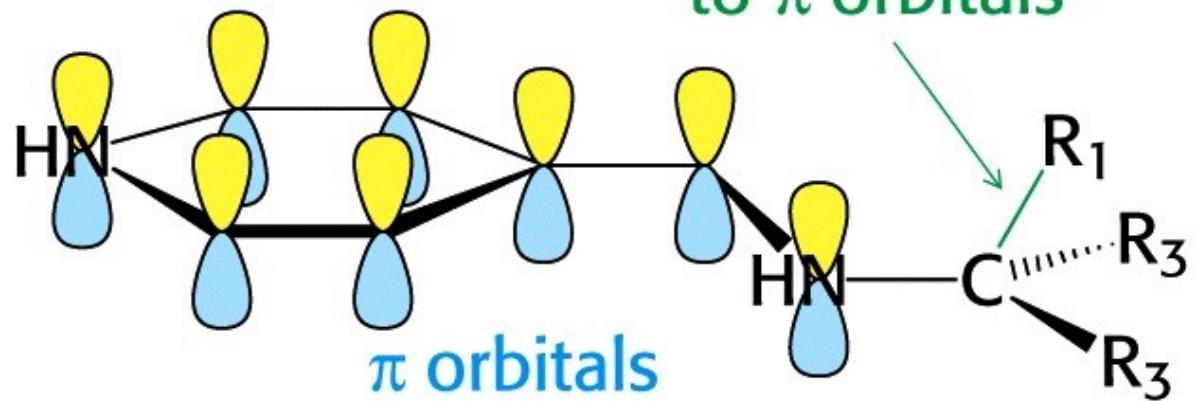


**Pyridoxamine phosphate (PMP)**

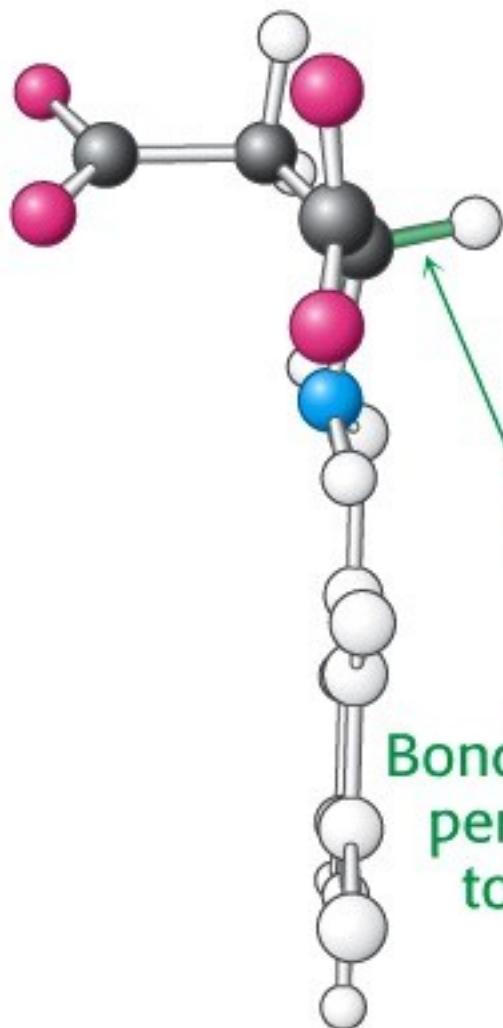




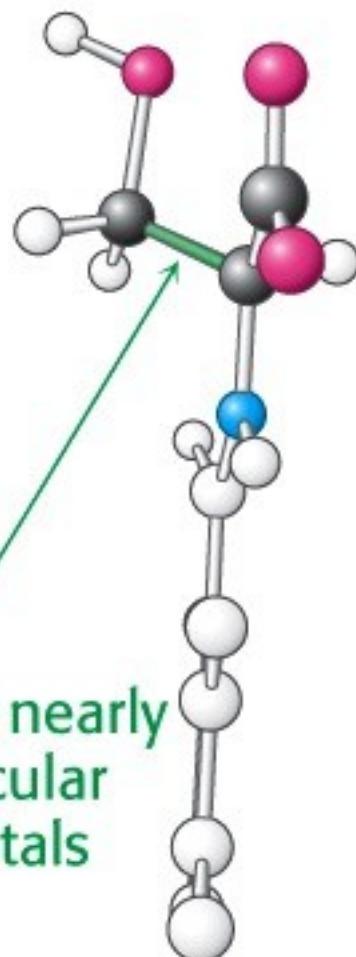
Bond most nearly  
perpendicular  
to  $\pi$  orbitals



**Aspartate  
aminotransferase**



**Serine  
hydroxymethyl-  
transferase**

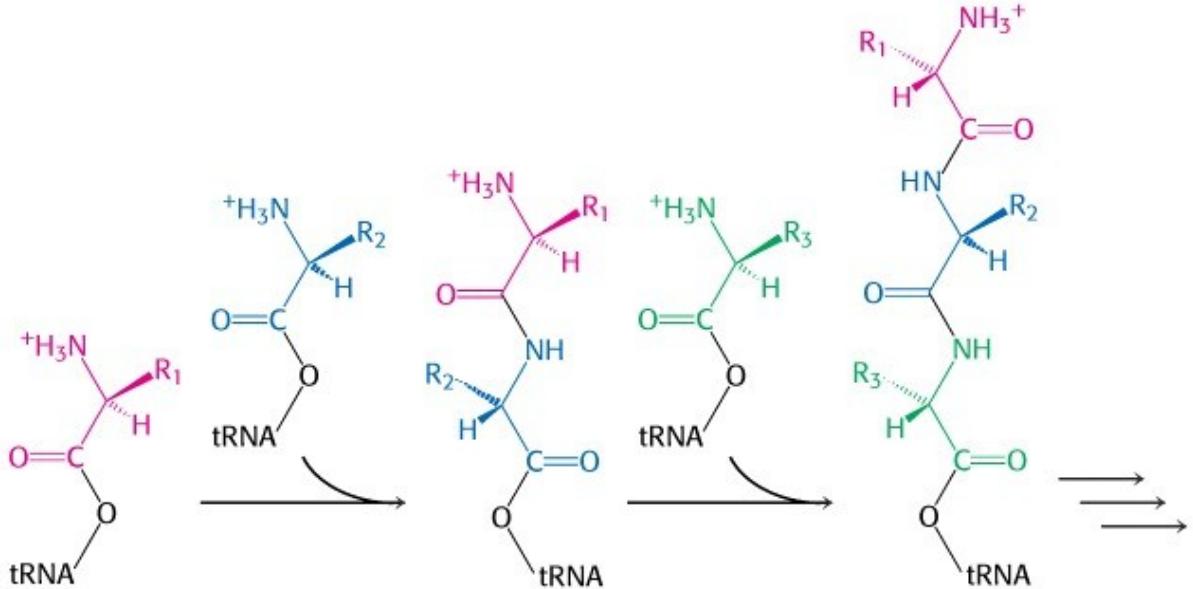
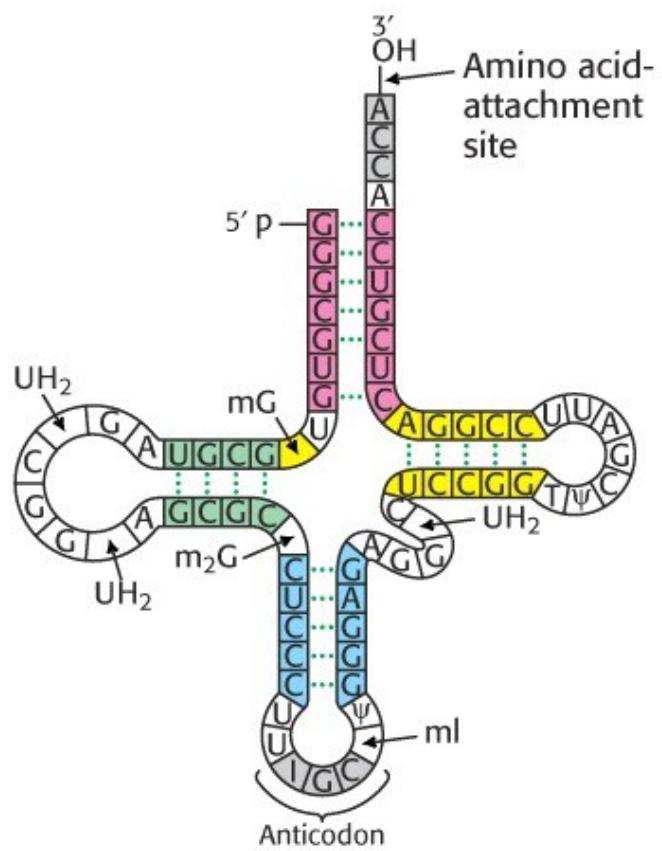


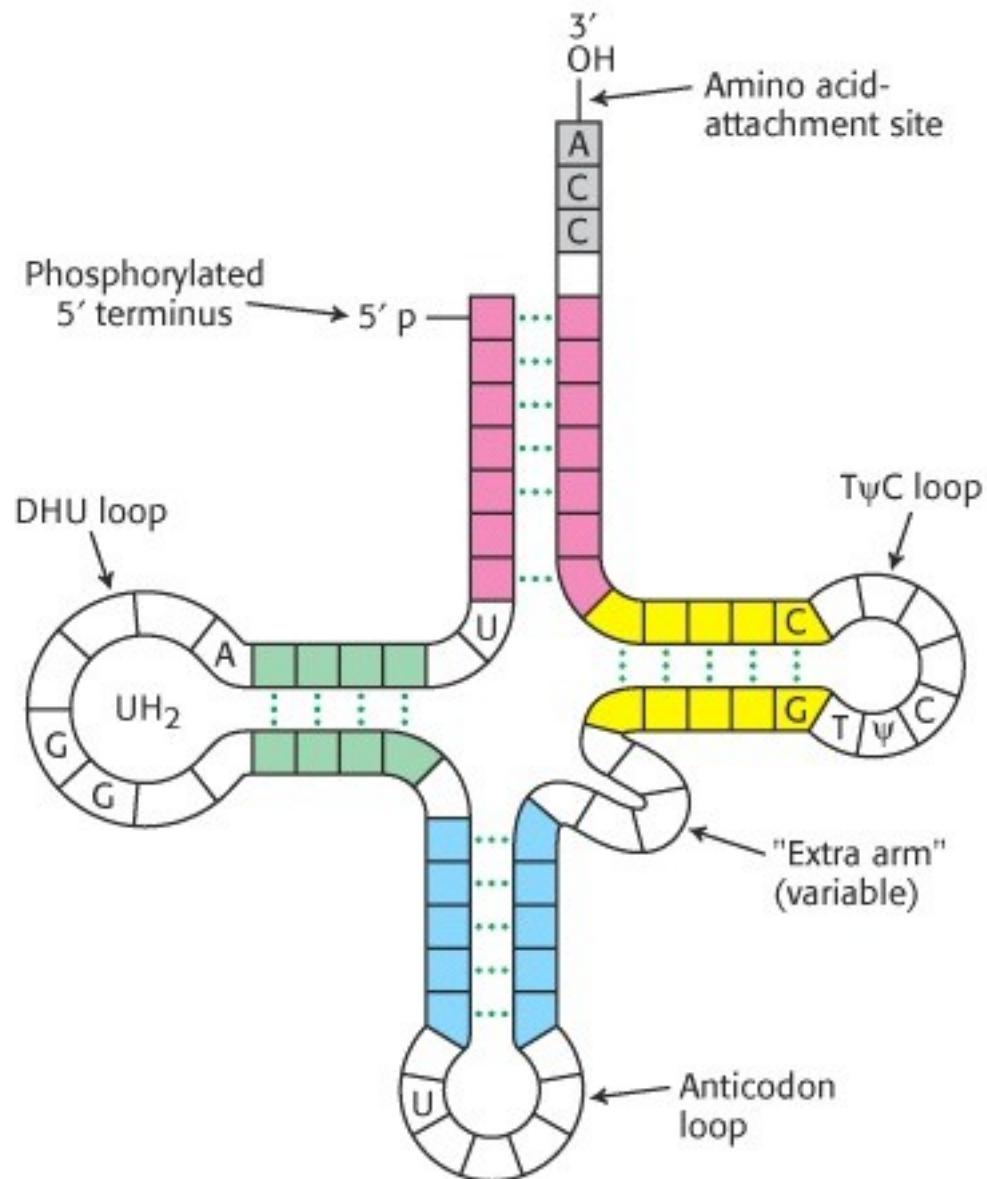
Bond most nearly  
perpendicular  
to  $\pi$  orbitals

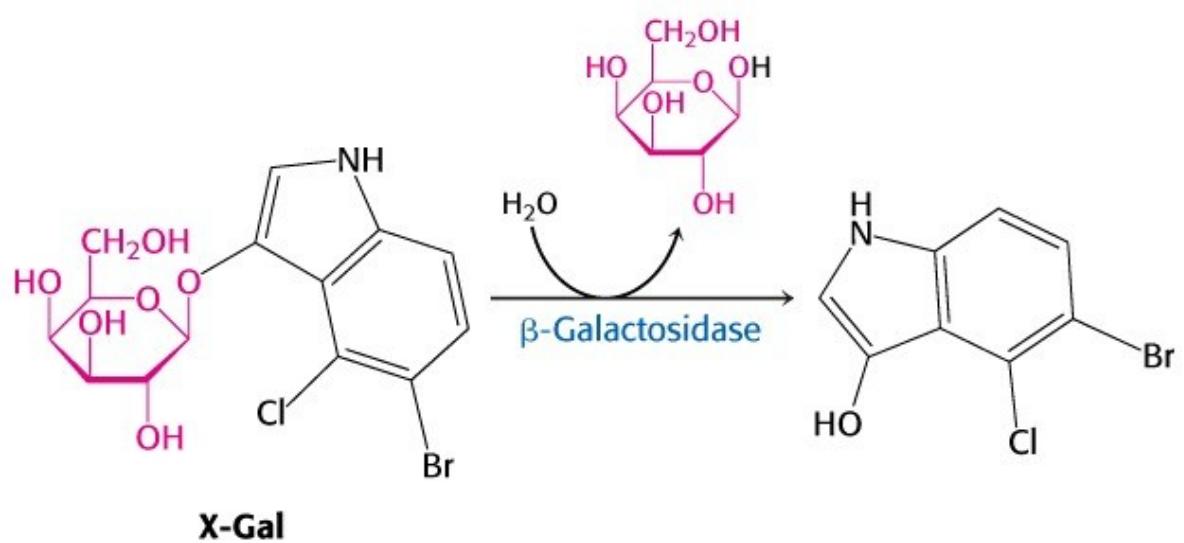
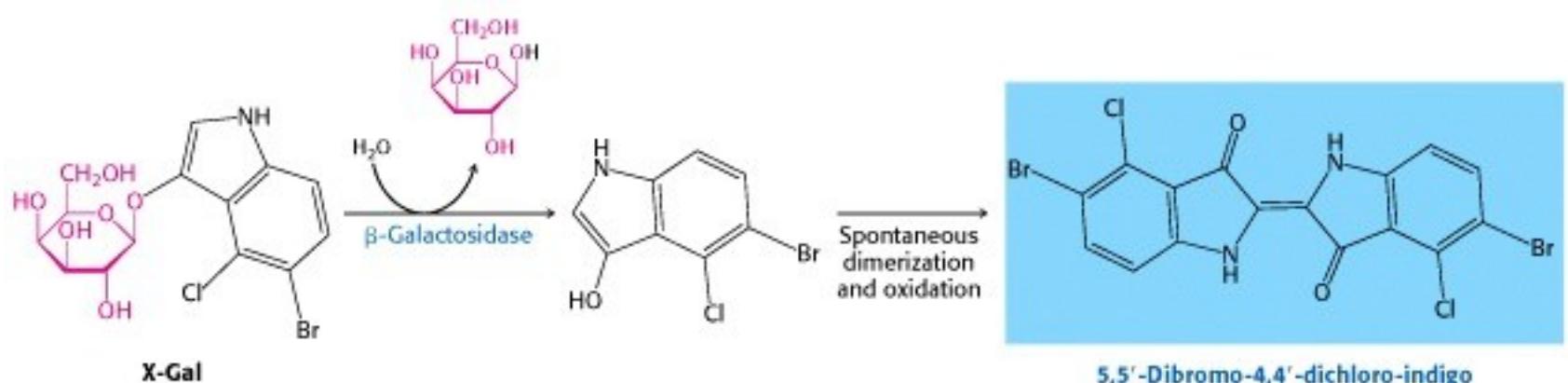
**TABLE 23.1** Dependence of the half-lives of cytosolic yeast proteins on the nature of their amino-terminal residues

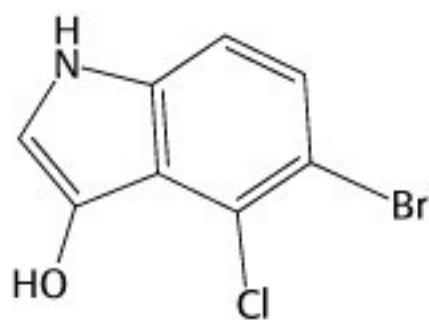
Highly stabilizing residues ( $t_{1/2} > 20$ hours)			
Ala	Cys	Gly	Met
Pro	Ser	Thr	Val
Intrinsically destabilizing residues			
$(t_{1/2} = 2$ to 30 minutes)			
Arg	His	Ile	Leu
Lys	Phe	Trp	Tyr
Destabilizing residues after chemical modification			
$(t_{1/2} = 3$ to 30 minutes)			
Asn	Asp	Gln	Glu

Source: J. W. Tobias, T. E. Schrader, G. Rocap, and A. Varshavsky. *Science* 254(1991):1374.

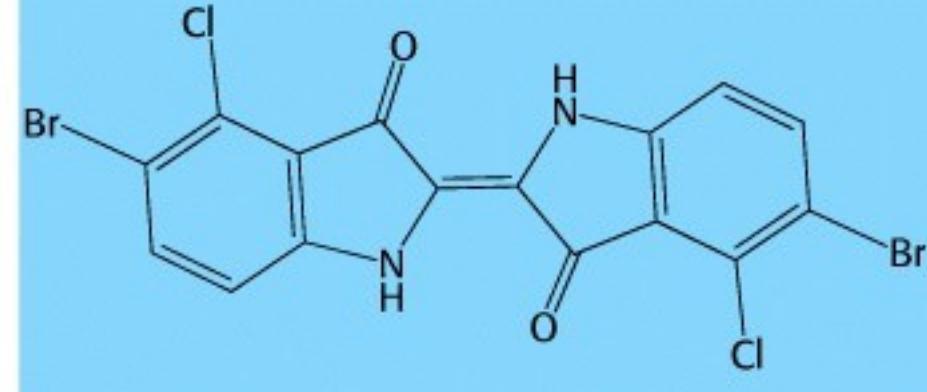




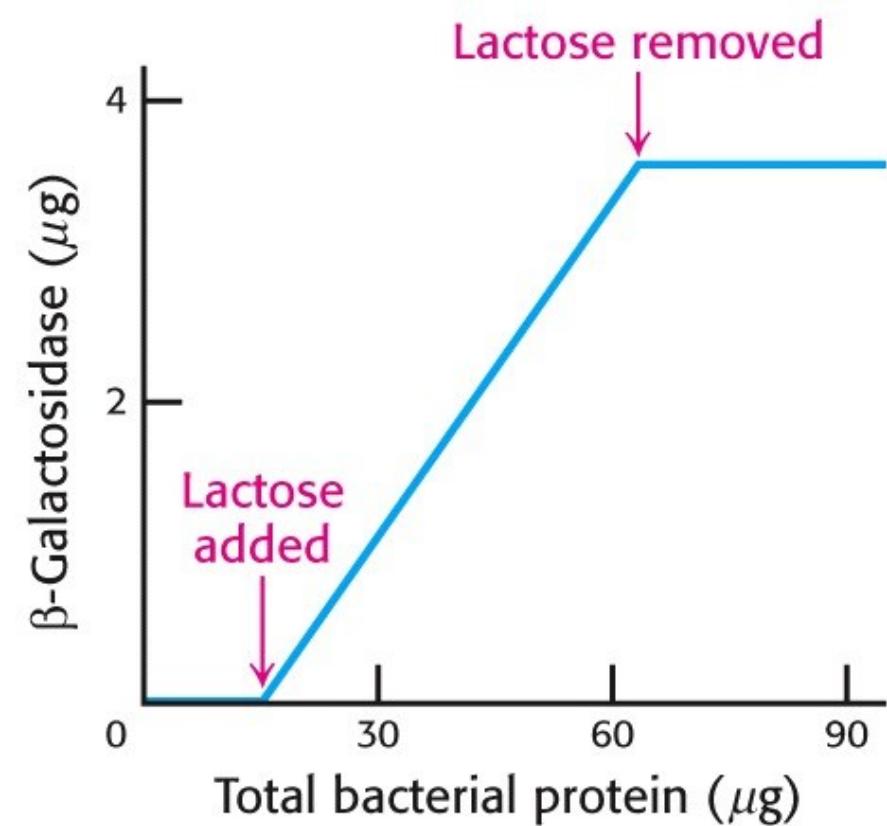


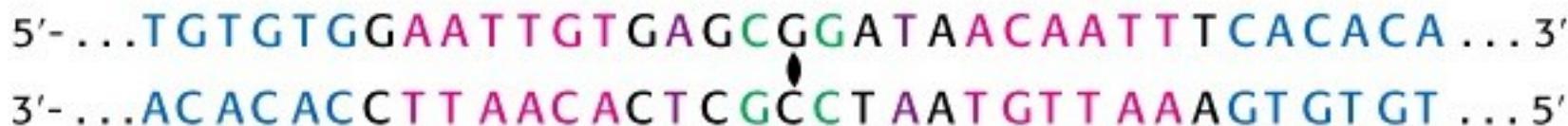
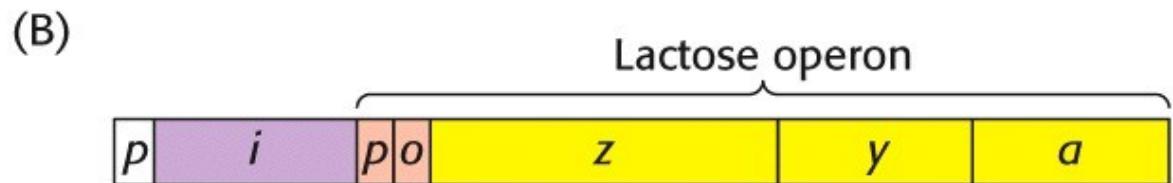
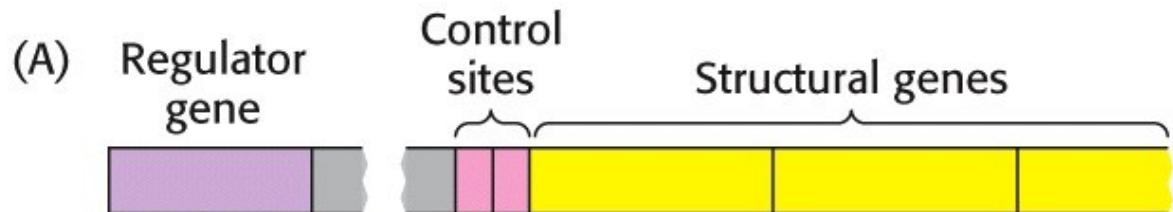


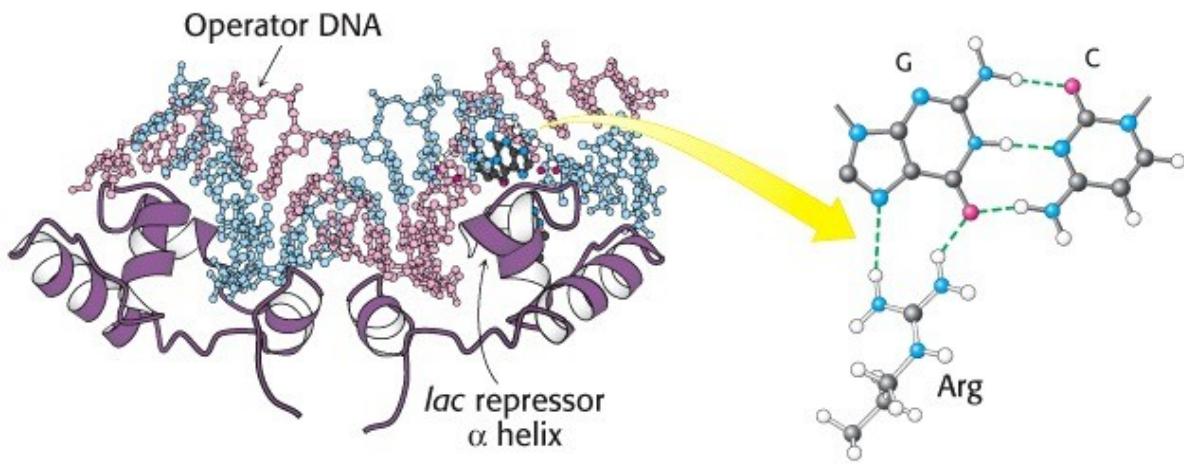
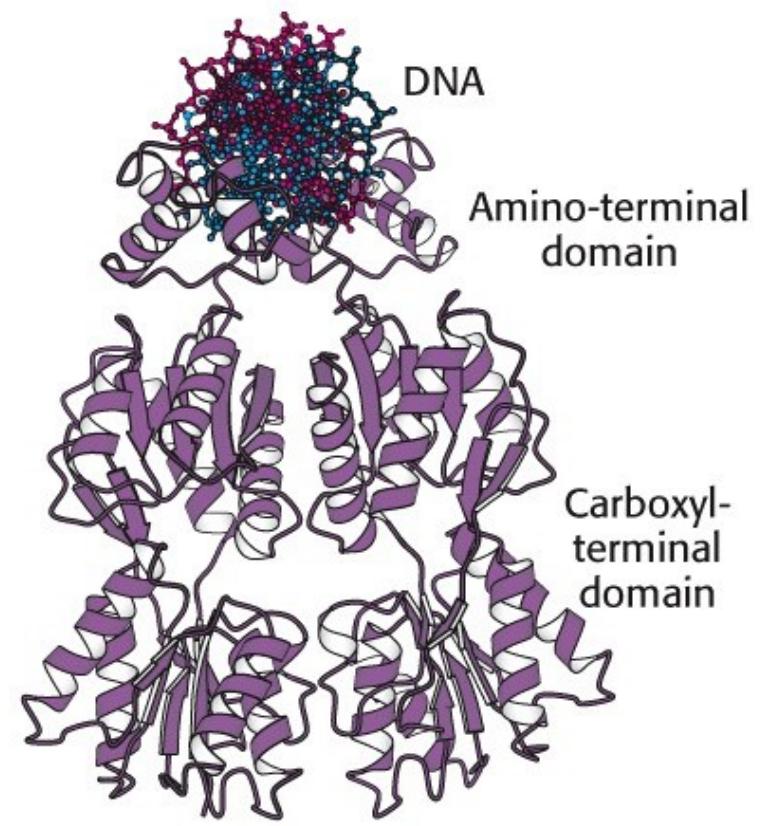
Spontaneous  
dimerization  
and oxidation

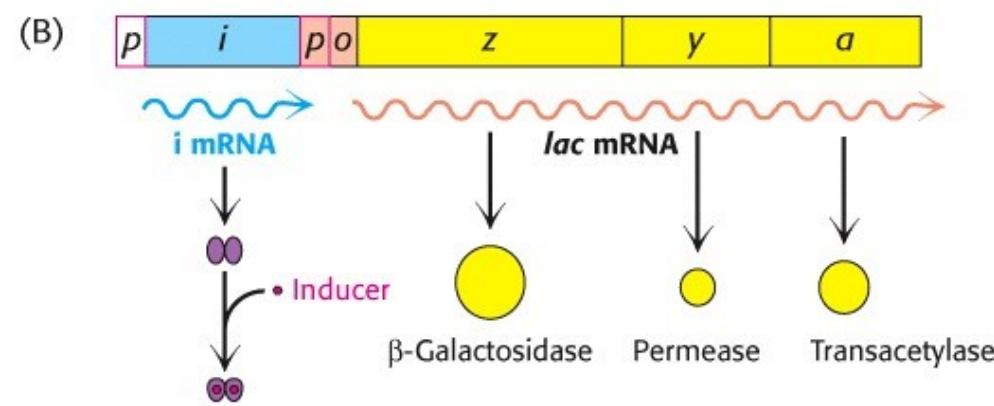
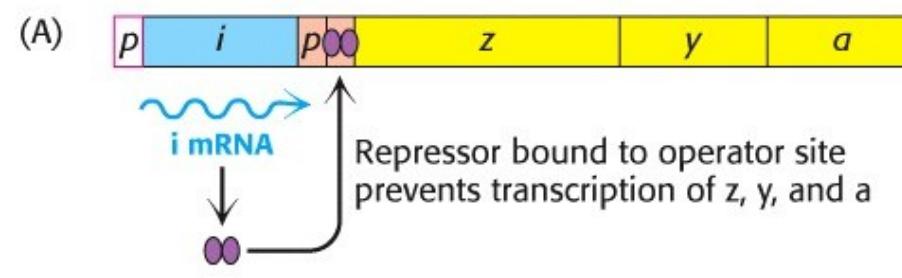
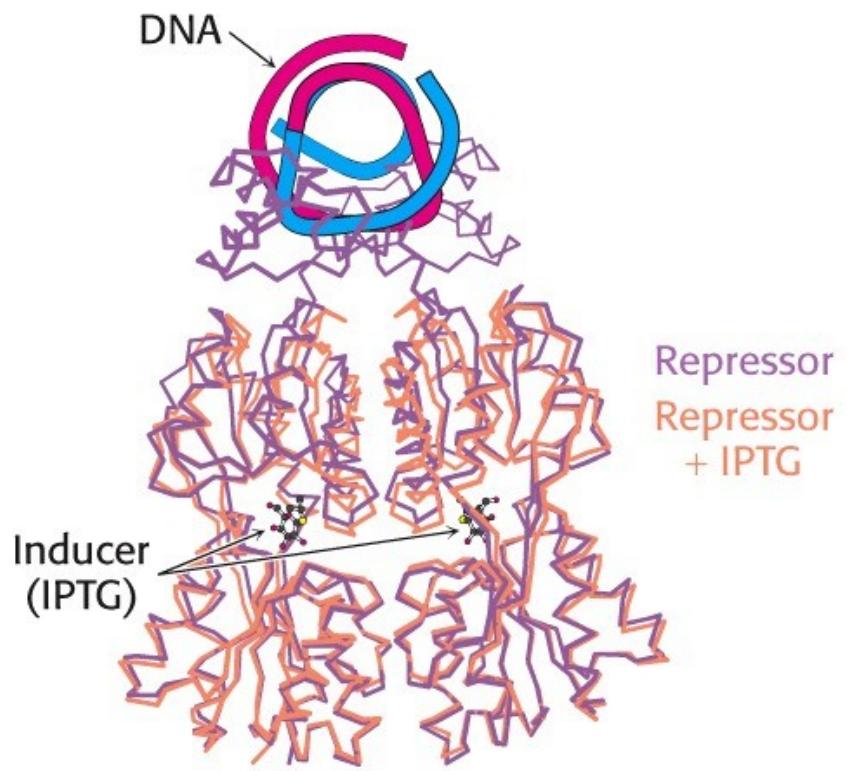


5,5'-Dibromo-4,4'-dichloro-indigo

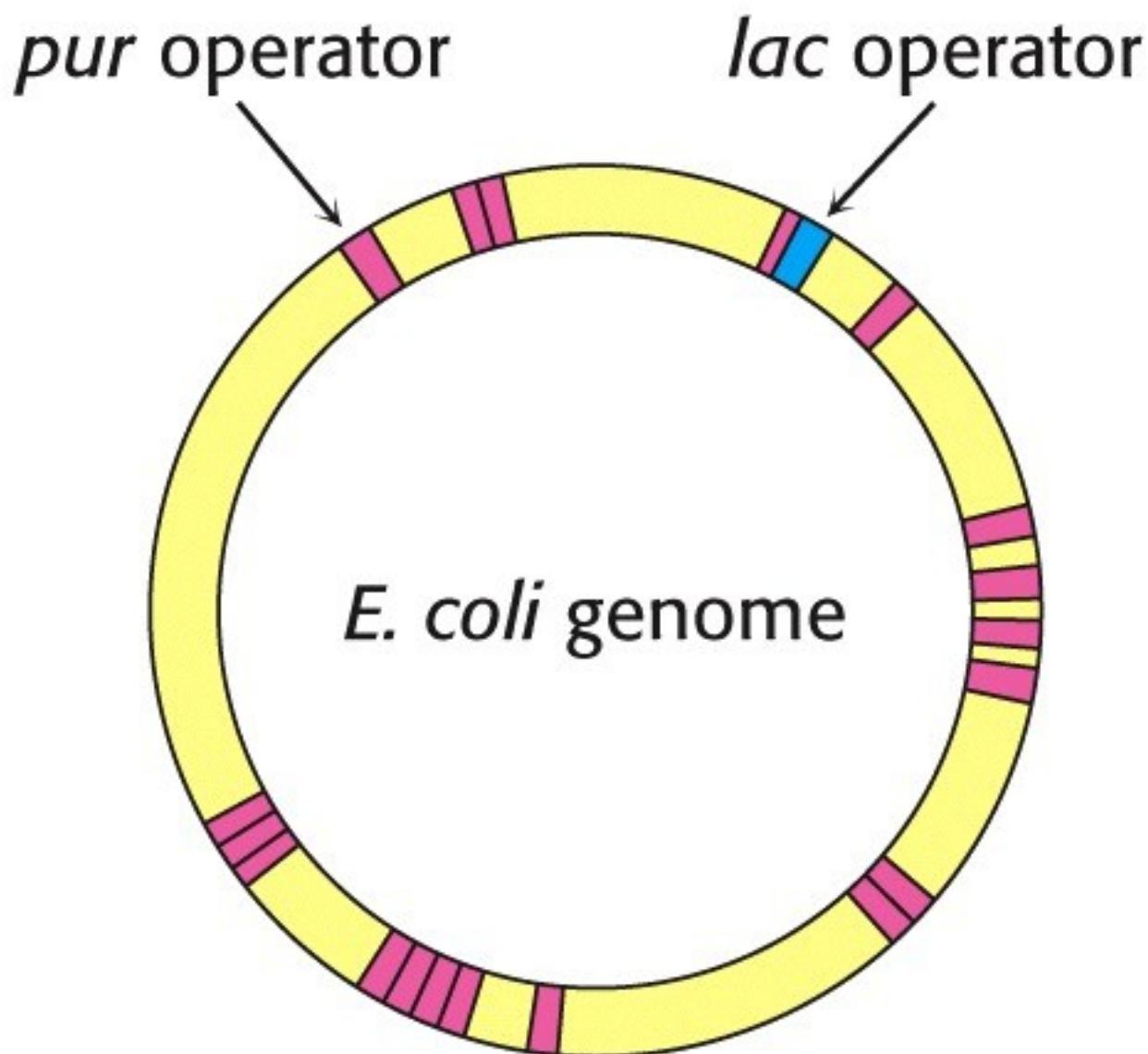


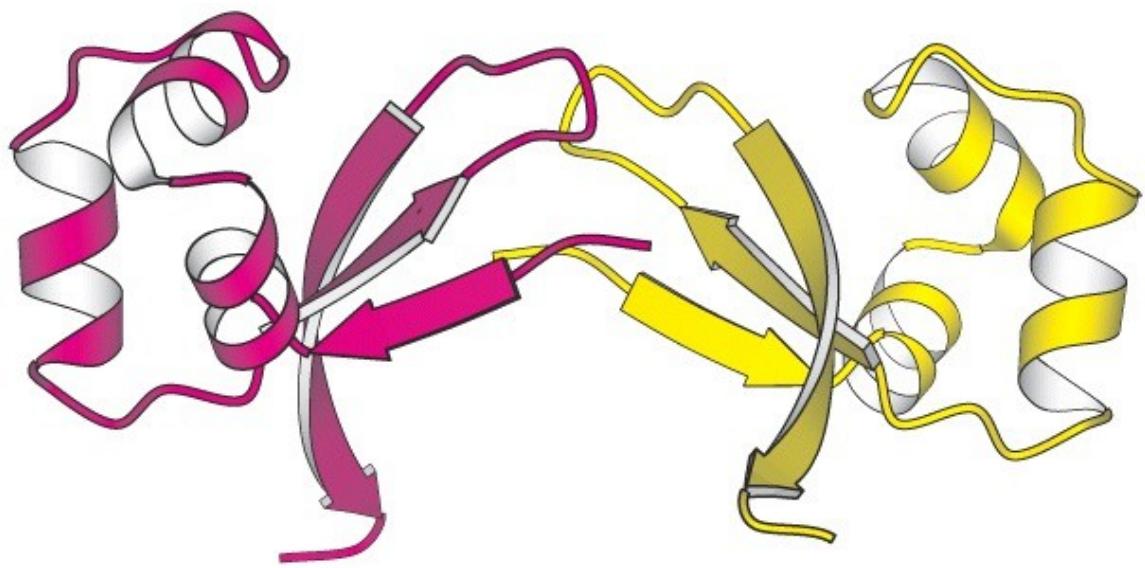
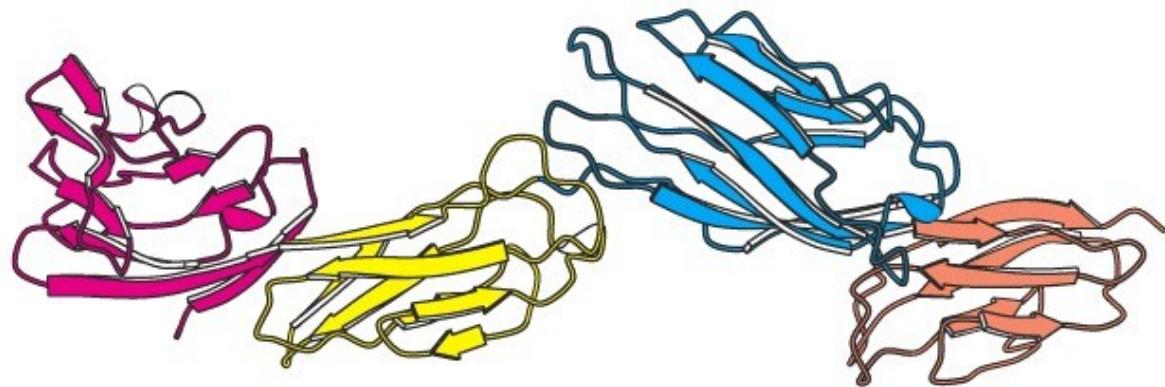




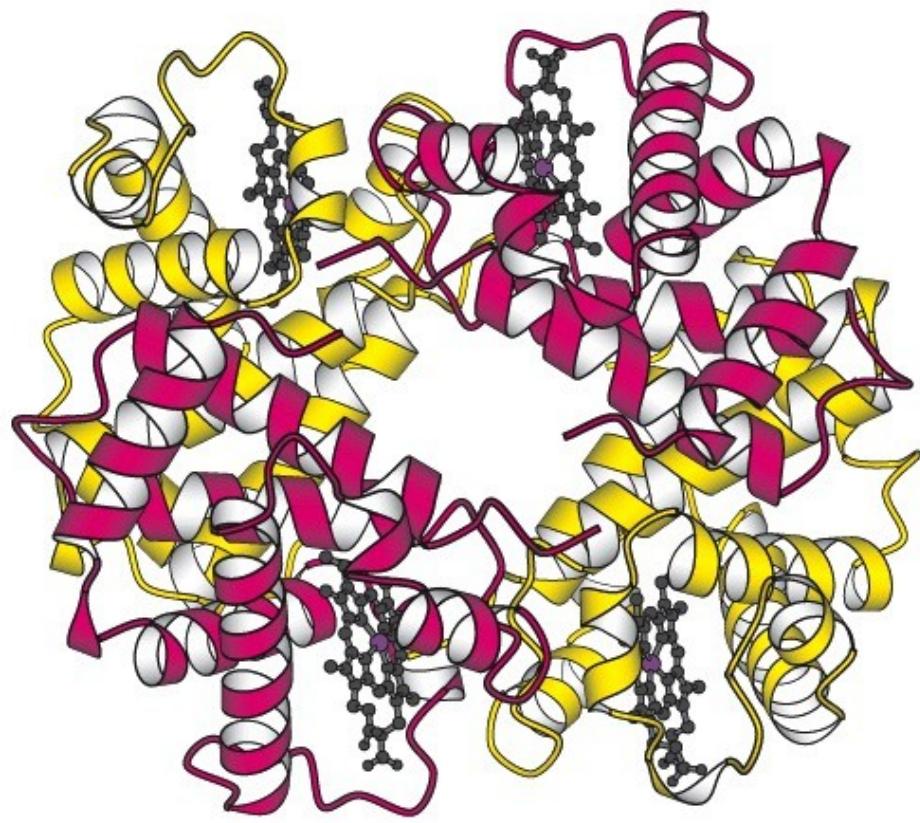
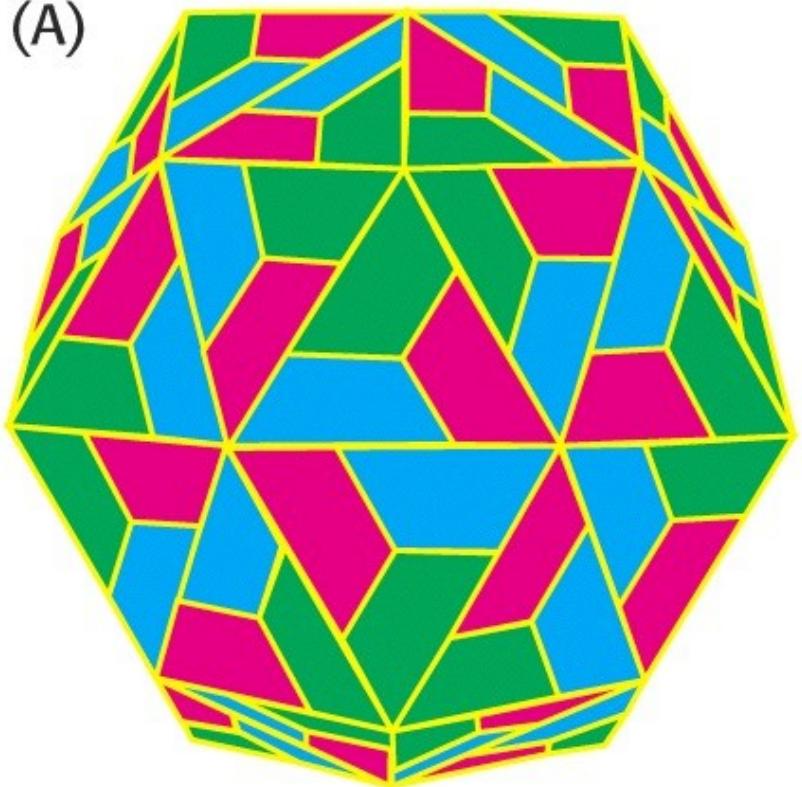


Repressor-inducer complex does not bind DNA

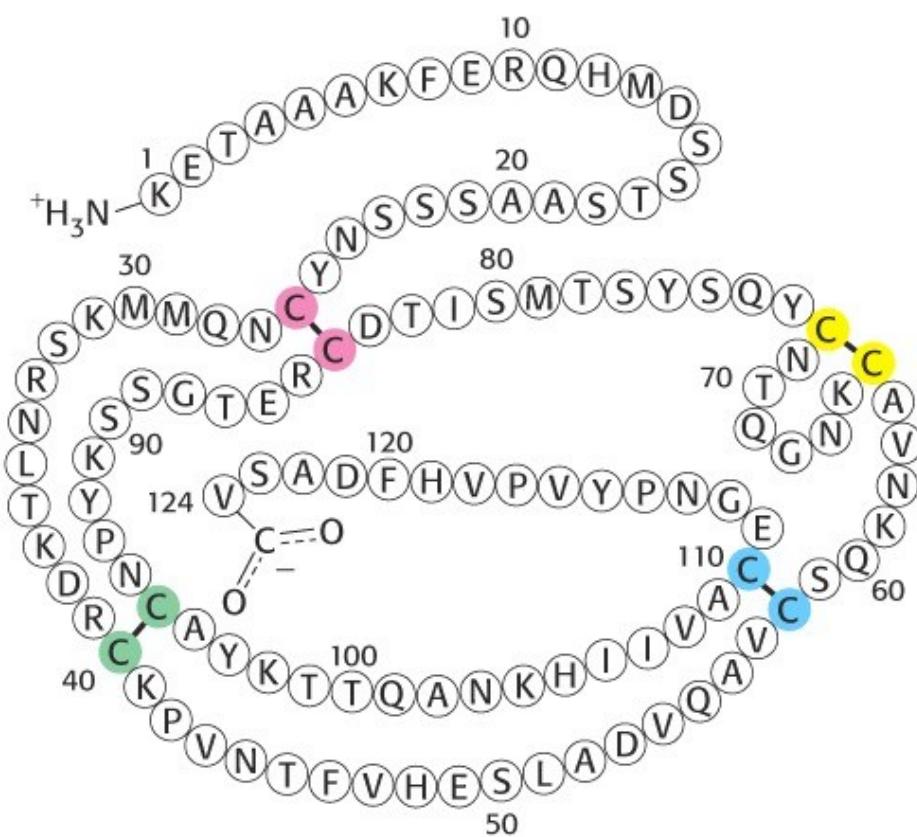
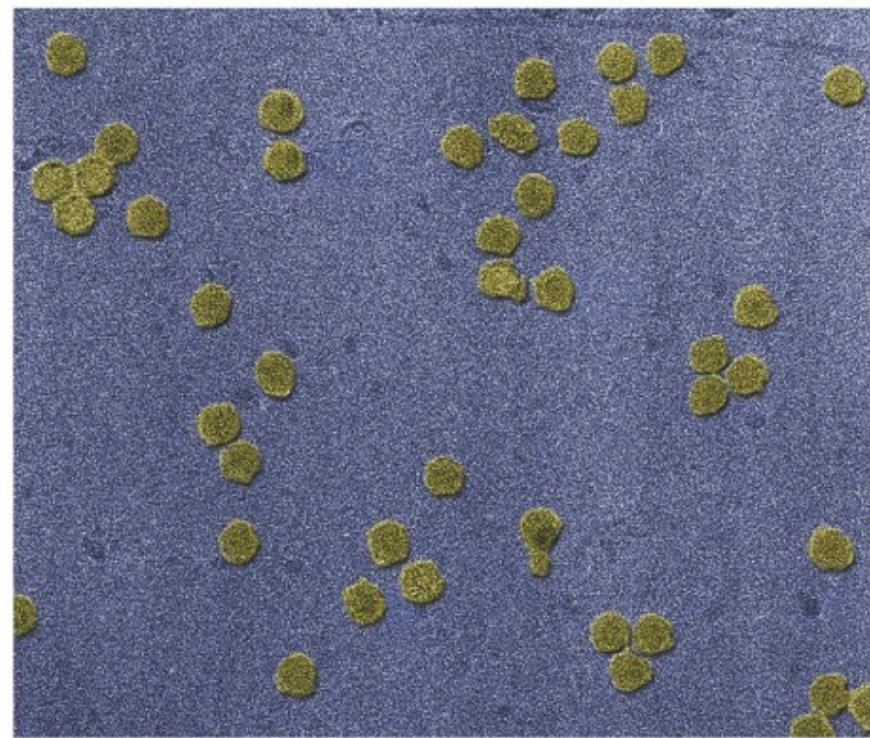


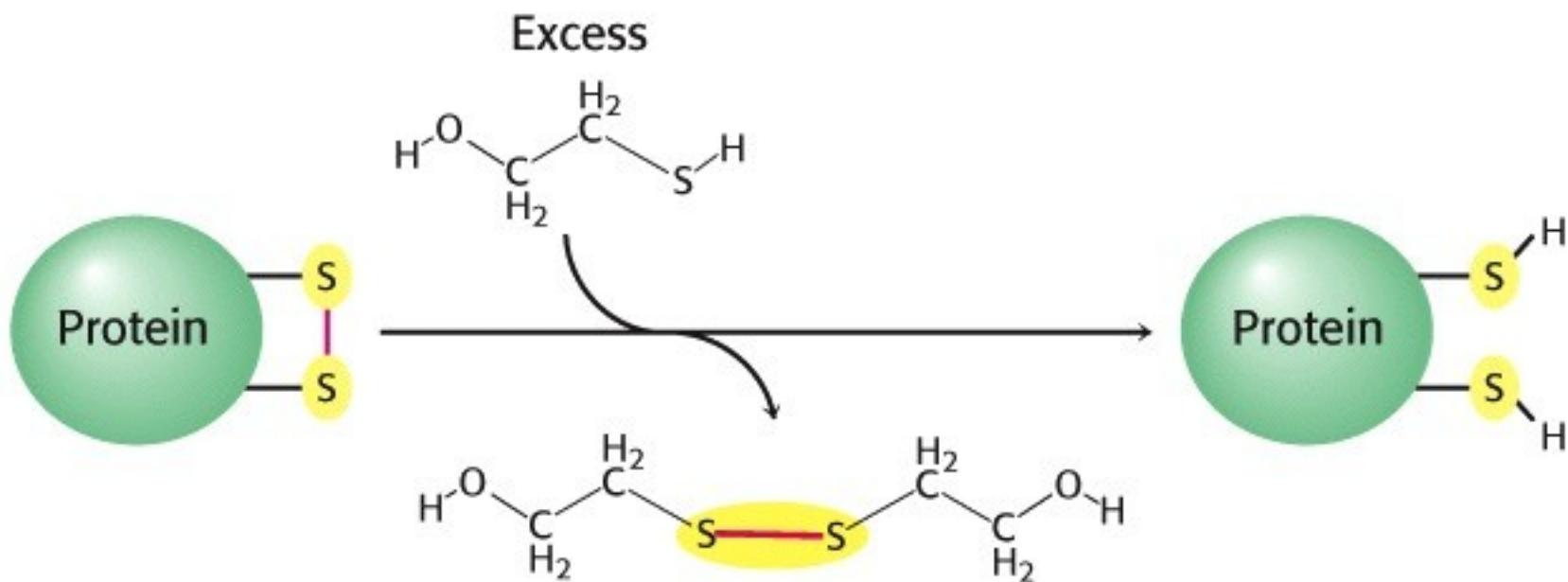


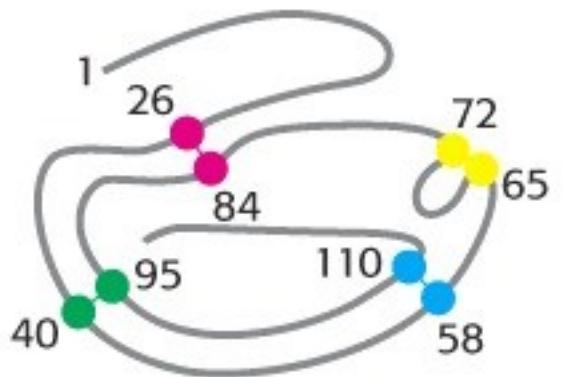
(A)



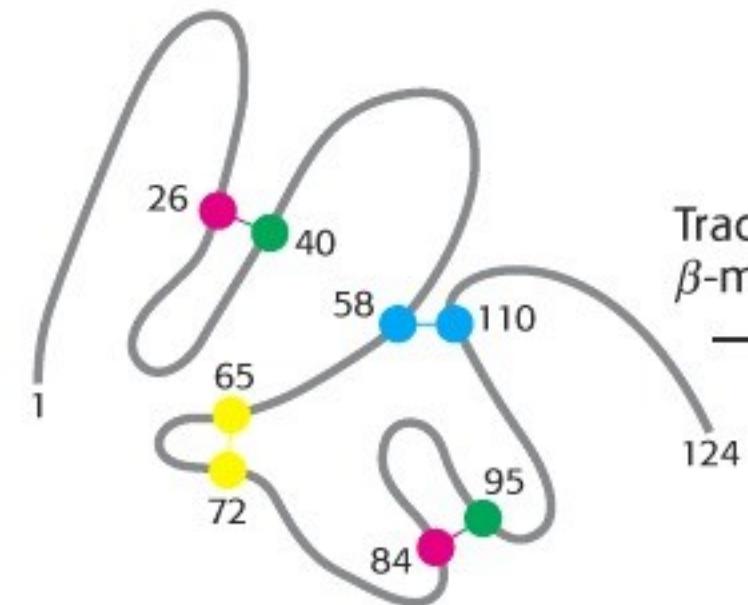
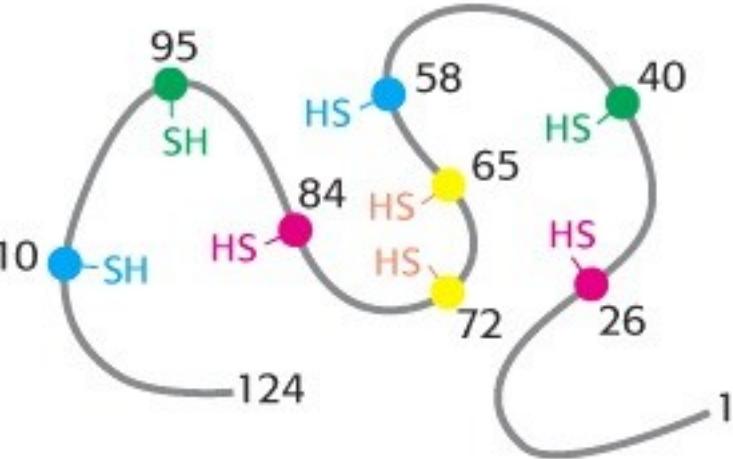
(B)



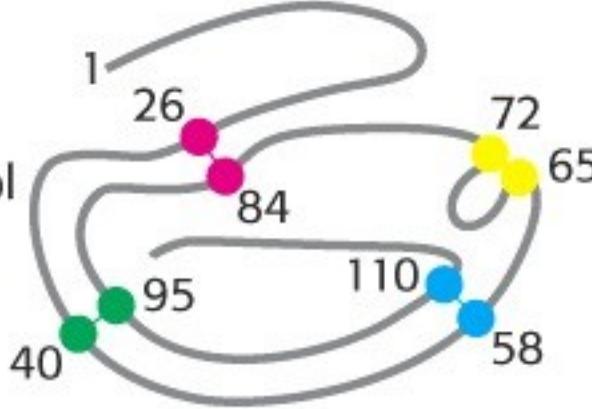




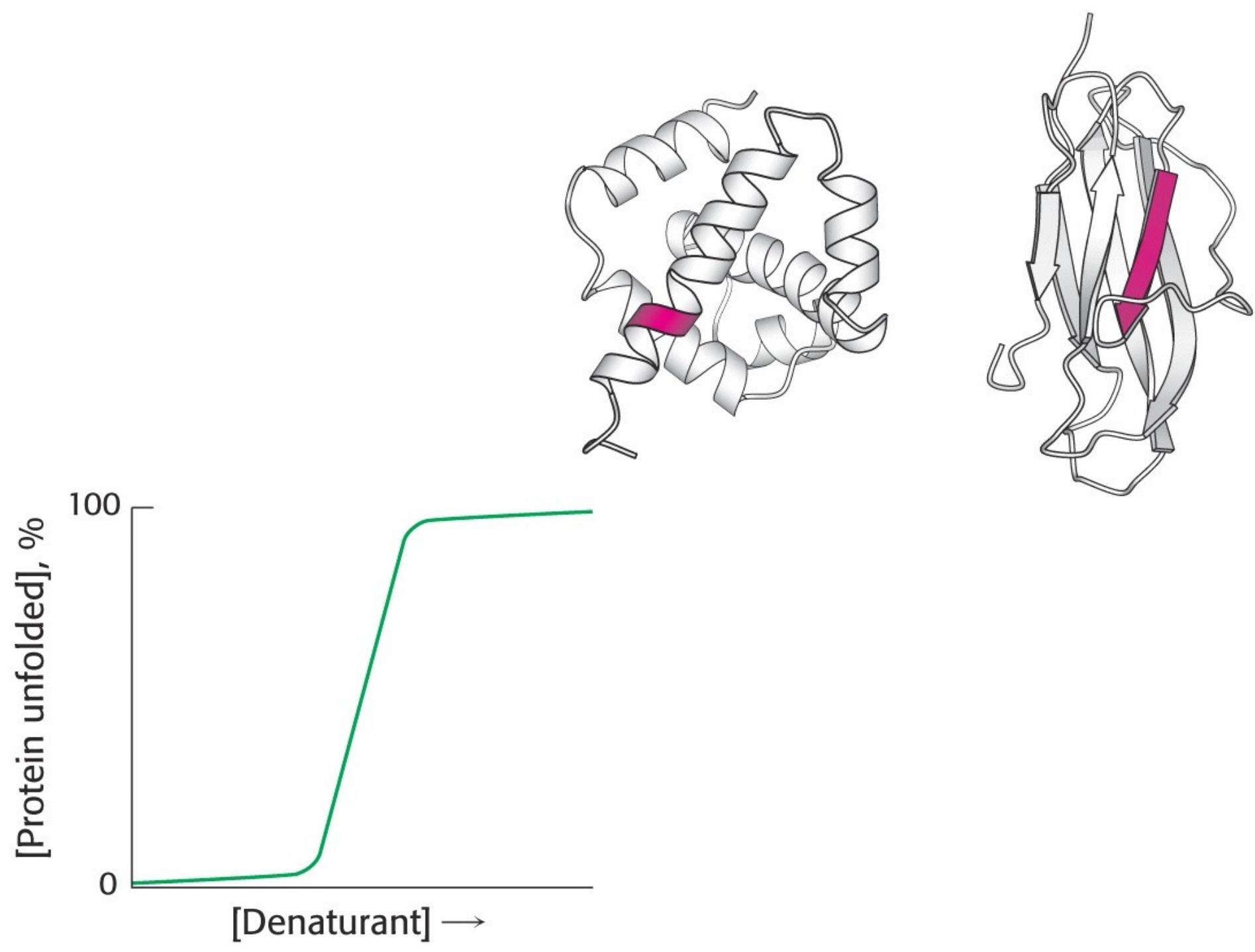
$8\text{ M}$  urea and  
 $\beta$ -mercaptoethanol

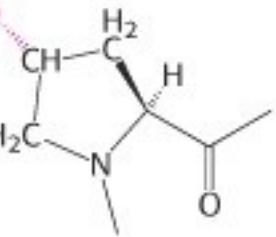
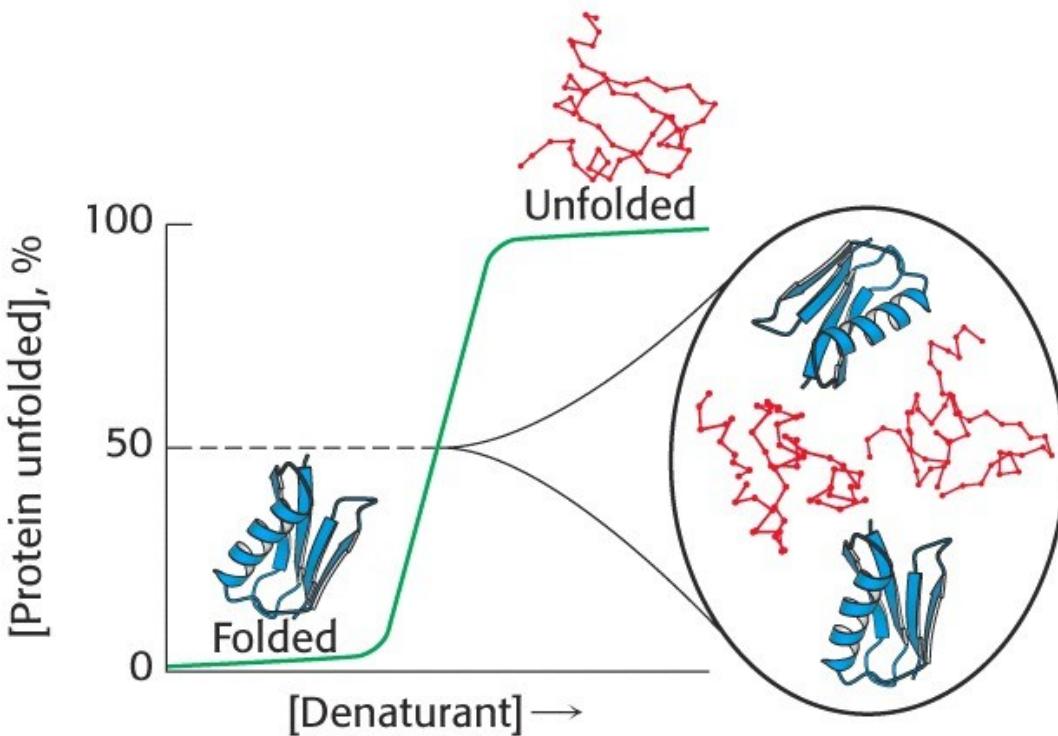


Trace of  
 $\beta$ -mercaptoethanol

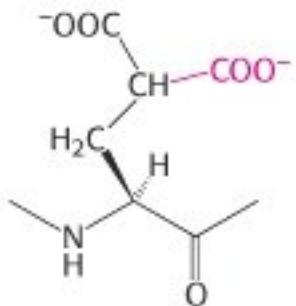


Scrambled ribonuclease

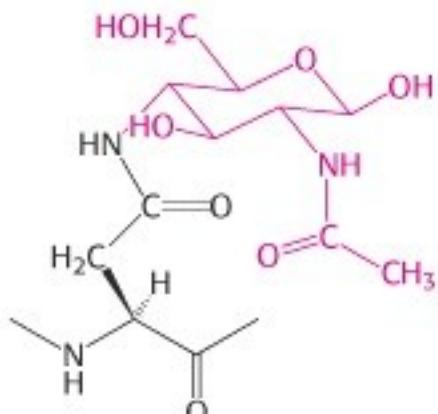




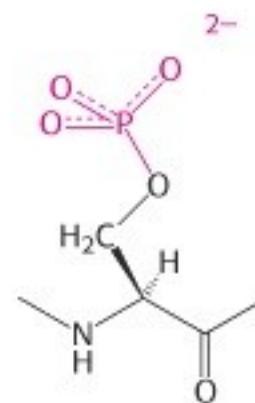
Hydroxyproline



$\gamma$ -Carboxyglutamate



Carbohydrate-asparagine adduct



Phosphoserine