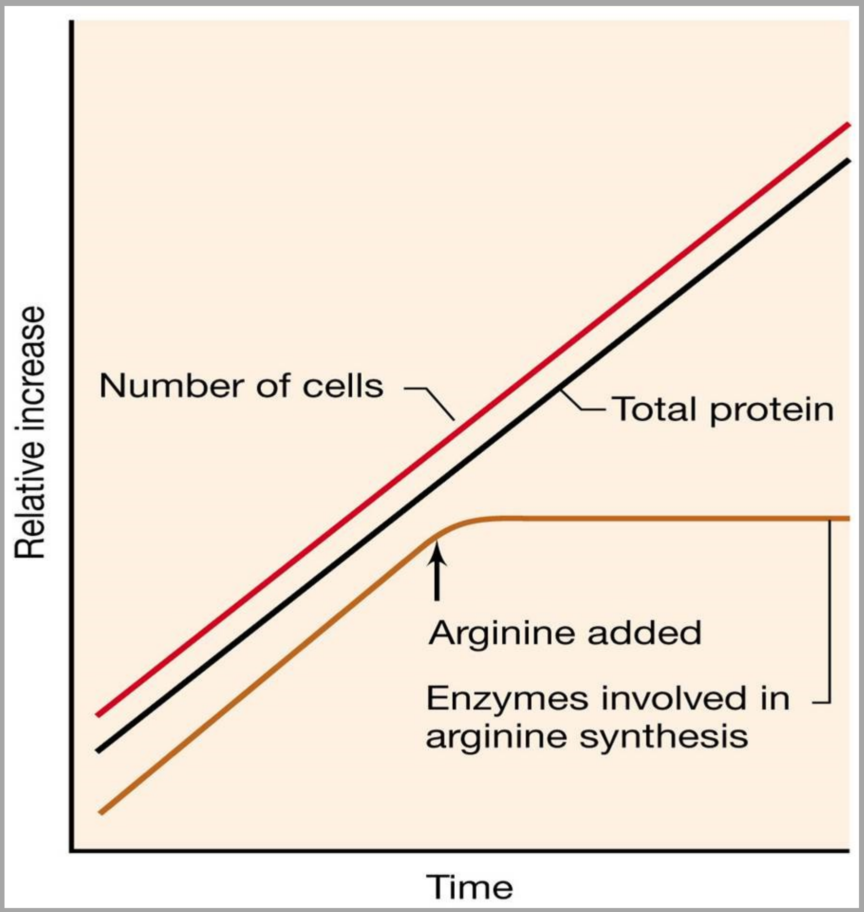
Uravnavanje izražanja genov: Prokarionti

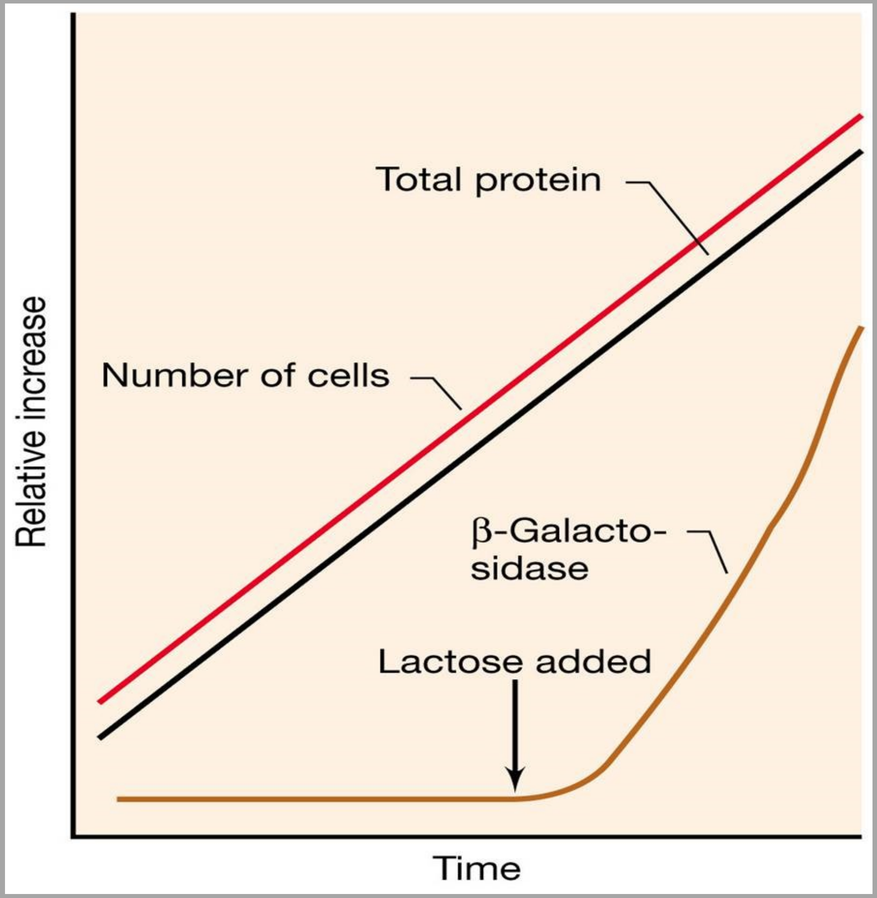
Voet 3: poglavje 31.3

Stryer 5: poglavje 31

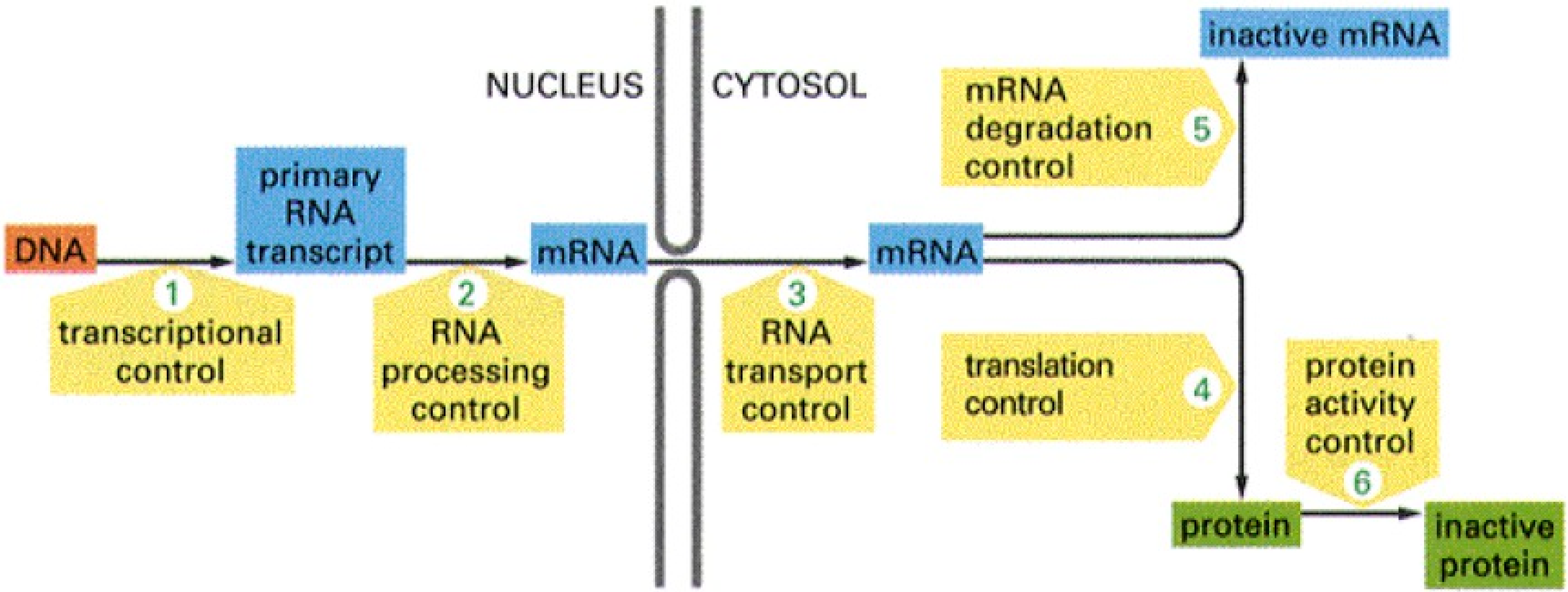
Krebs: poglavje 26



represija



indukcija

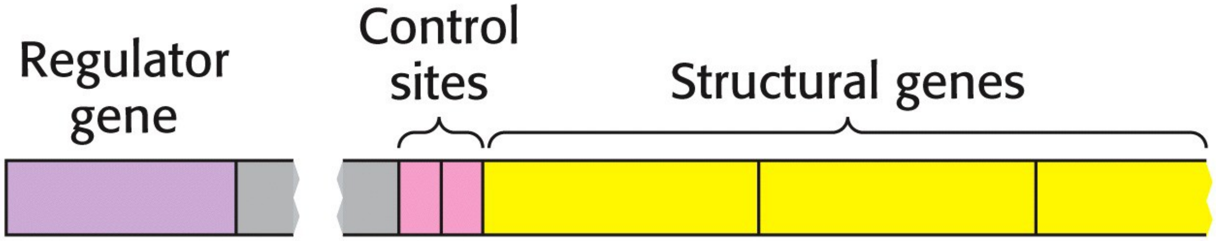

NUCLEUS
CYTOSOL
DNA
primary RNA transcript
mRNA
mRNA
inactive mRNA
protein
inactive protein
1 transcriptional control
2 RNA processing control
3 RNA transport control
4 translation control
5 mRNA degradation control
6 protein activity control

Možni načini uravnavanja koncentracije proteina v celici (splošno):

- hitrost transkripcije
- hitrost posttranskripcijskega procesiranja
- hitrost razgradnje mRNA
- hitrost sinteze proteina
- hitrost posttranslacijskega procesiranja
- hitrost razgradnje proteinov

Osnovna tipa izražanja genov:

- konstitutivno (stalna transkripcija – “hišni geni”)
- inducibilno /represibilno (aktivacija/inaktivacija genov)

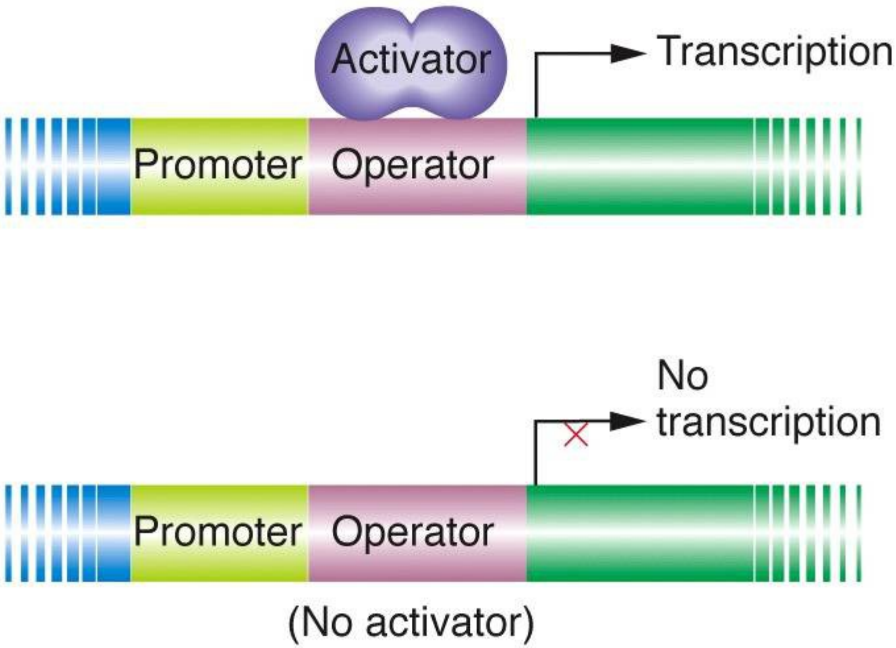


operon: niz genov z usklajeno regulacijo

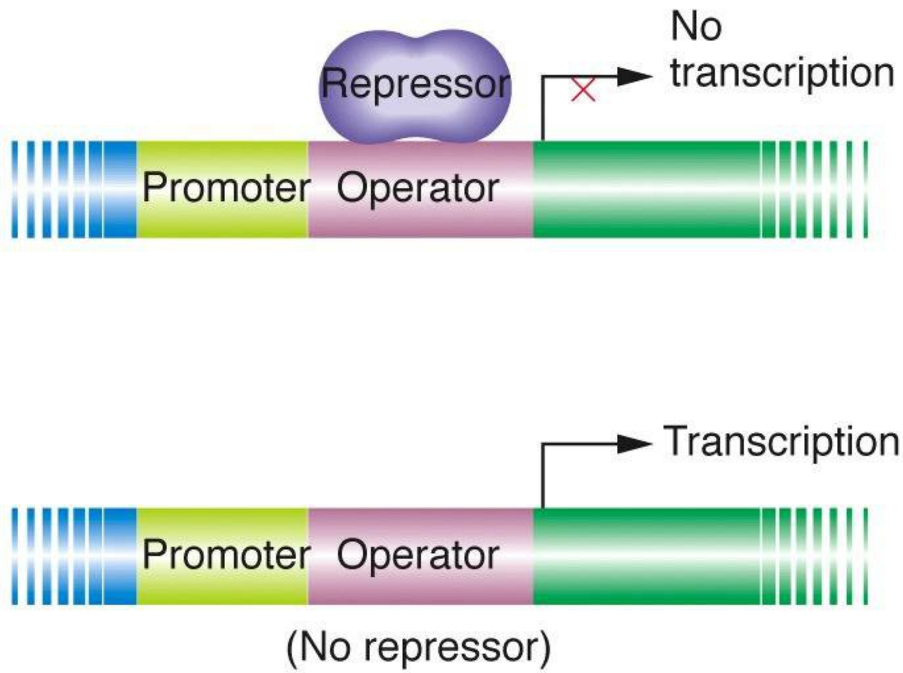
represorji / aktivatorji: regulatorji transkripcije

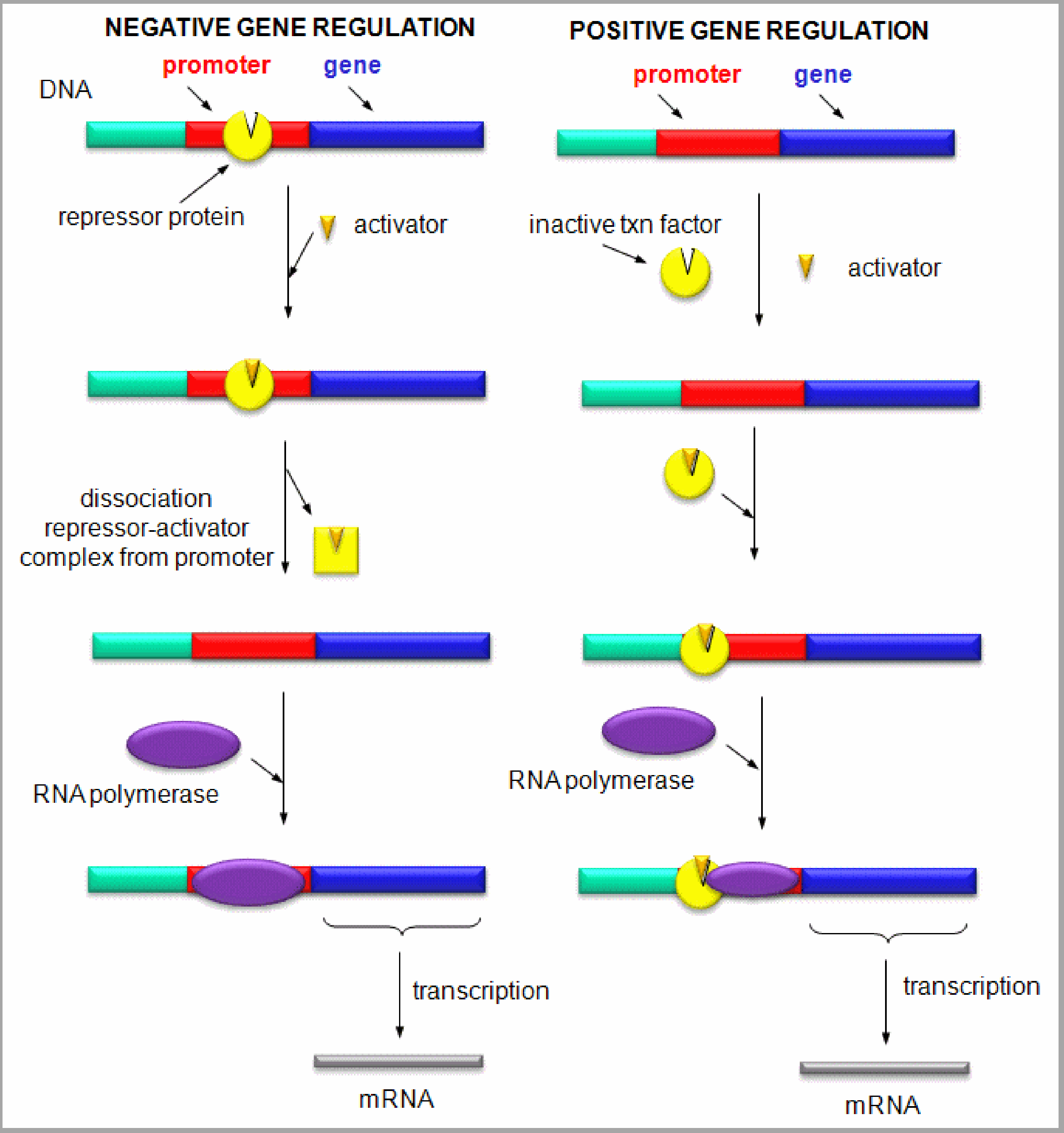
kontrola izražanja poteka najpogosteje na ravni transkripcije

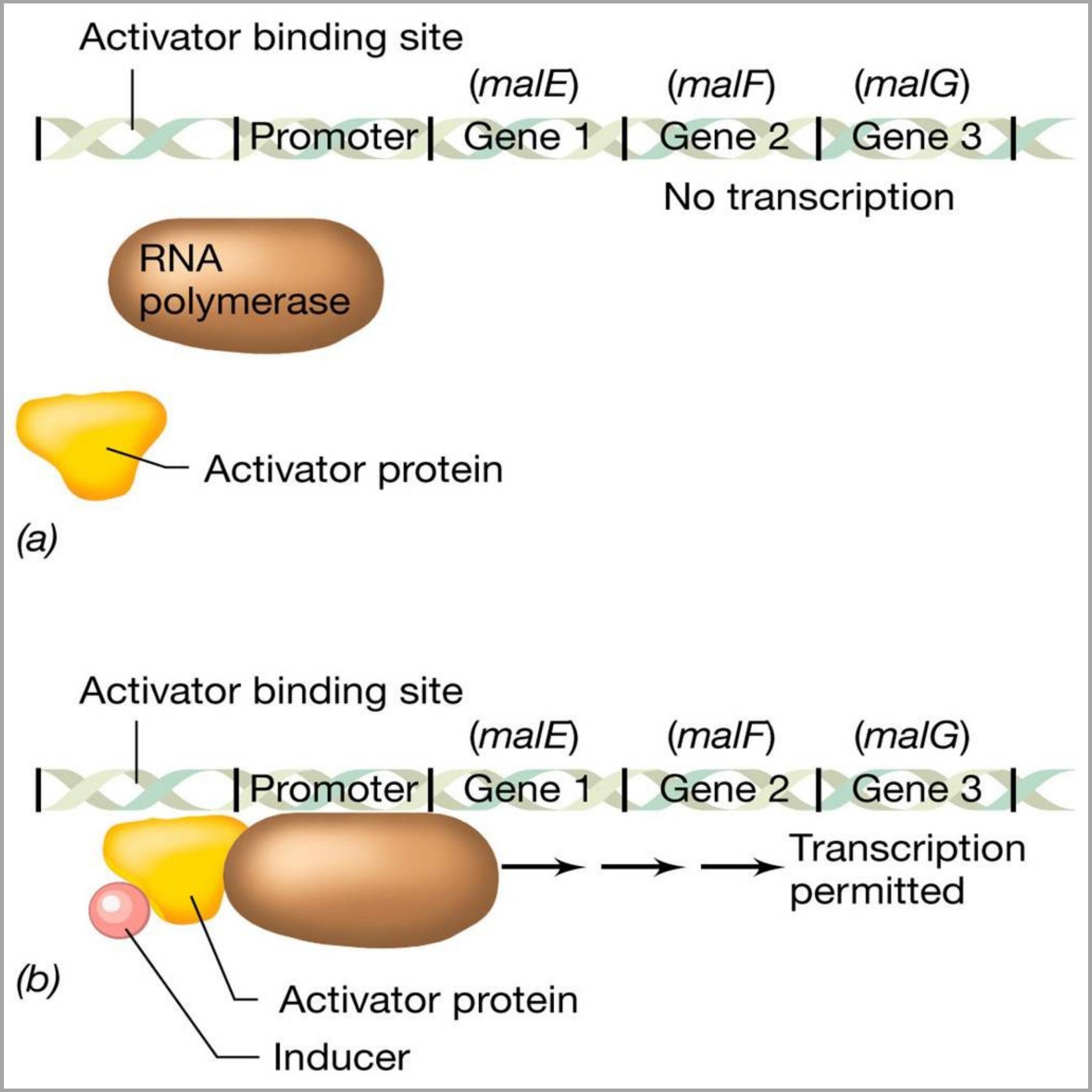
Positive regulation



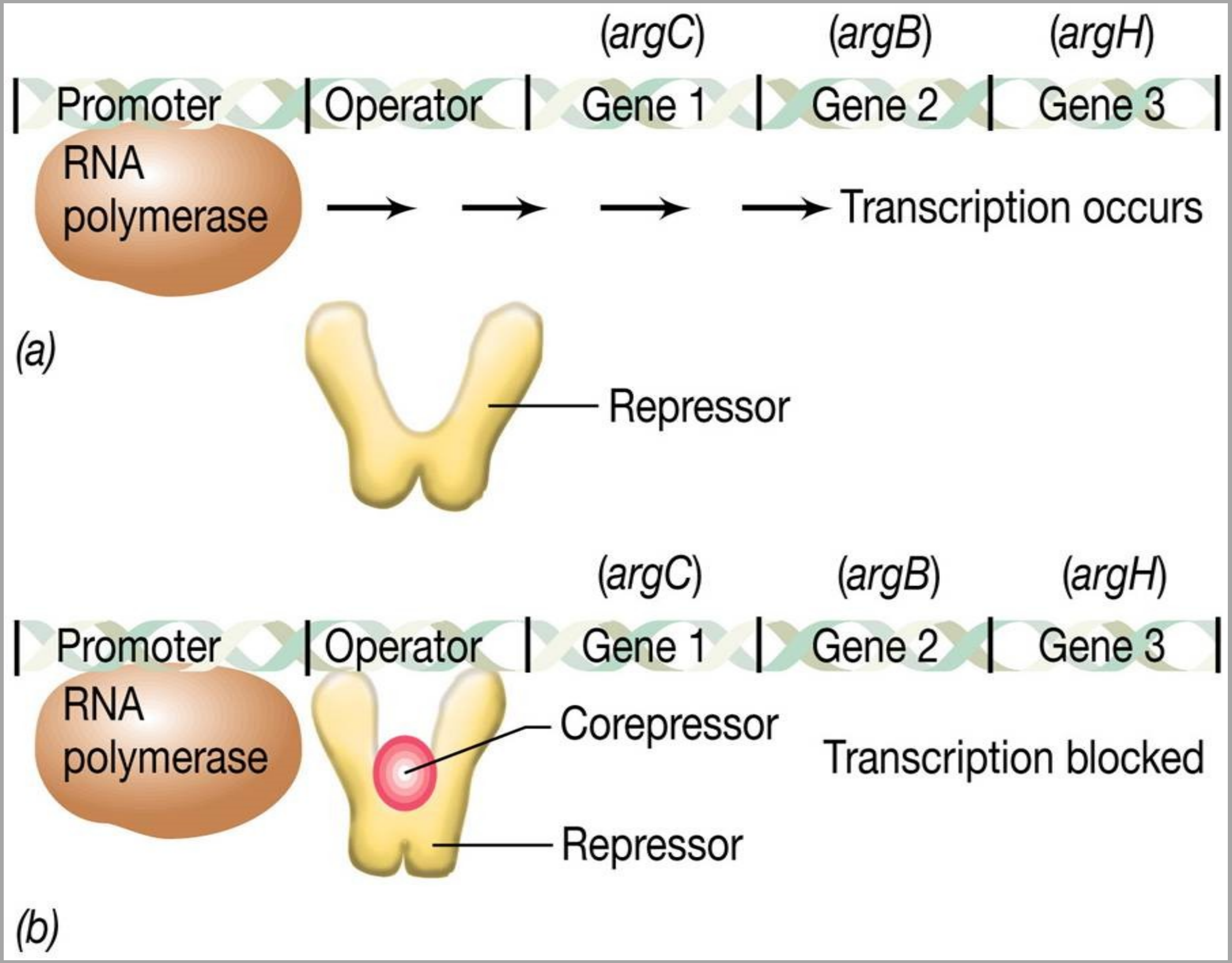
Negative regulation



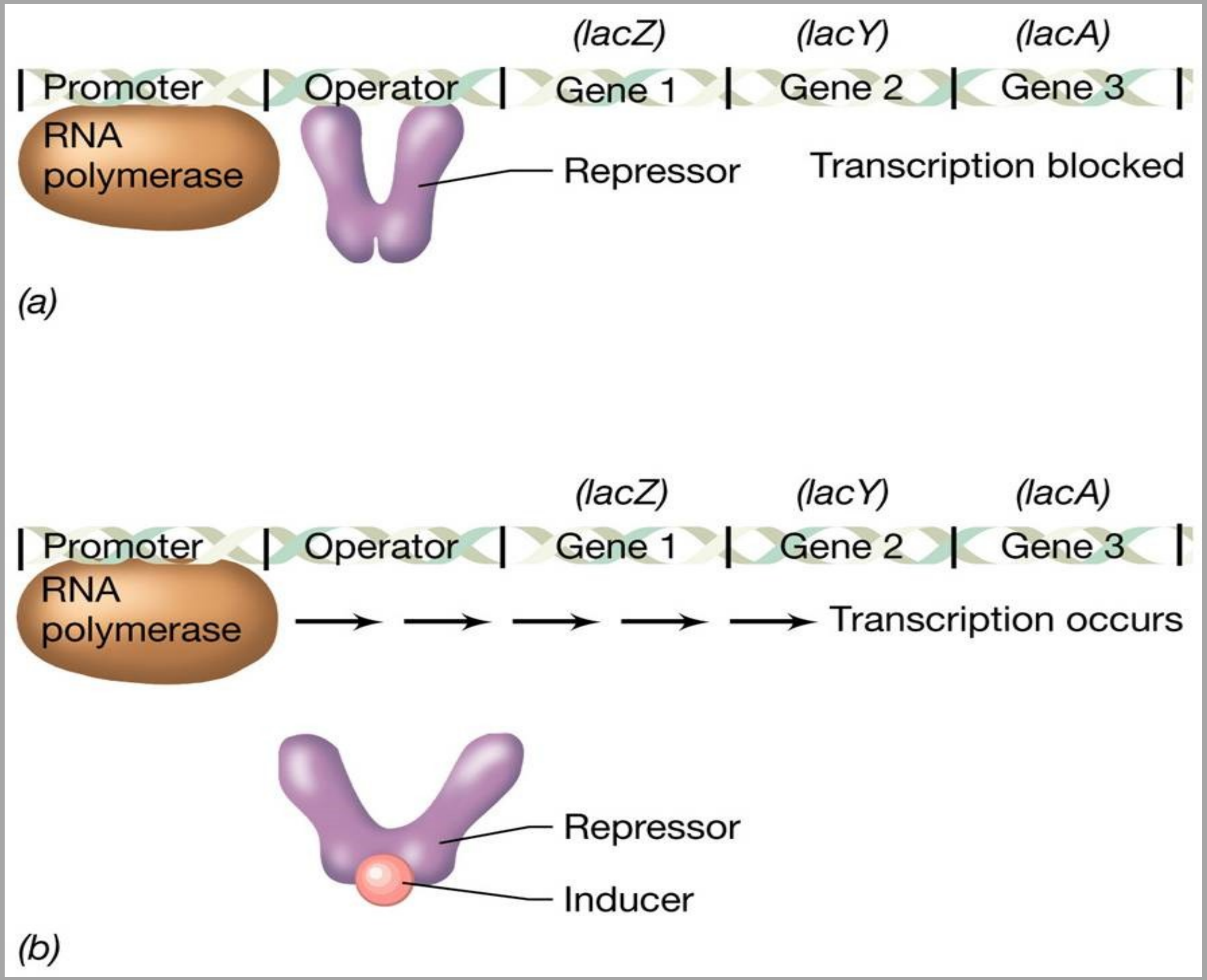

NEGATIVE GENE REGULATION
DNA
promoter
gene
repressor protein
activator
dissociation repressor-activator complex from promoter
RNA polymerase
transcription
mRNA
POSITIVE GENE REGULATION
promoter
gene
inactive txn factor
activator
RNA polymerase
transcription
mRNA



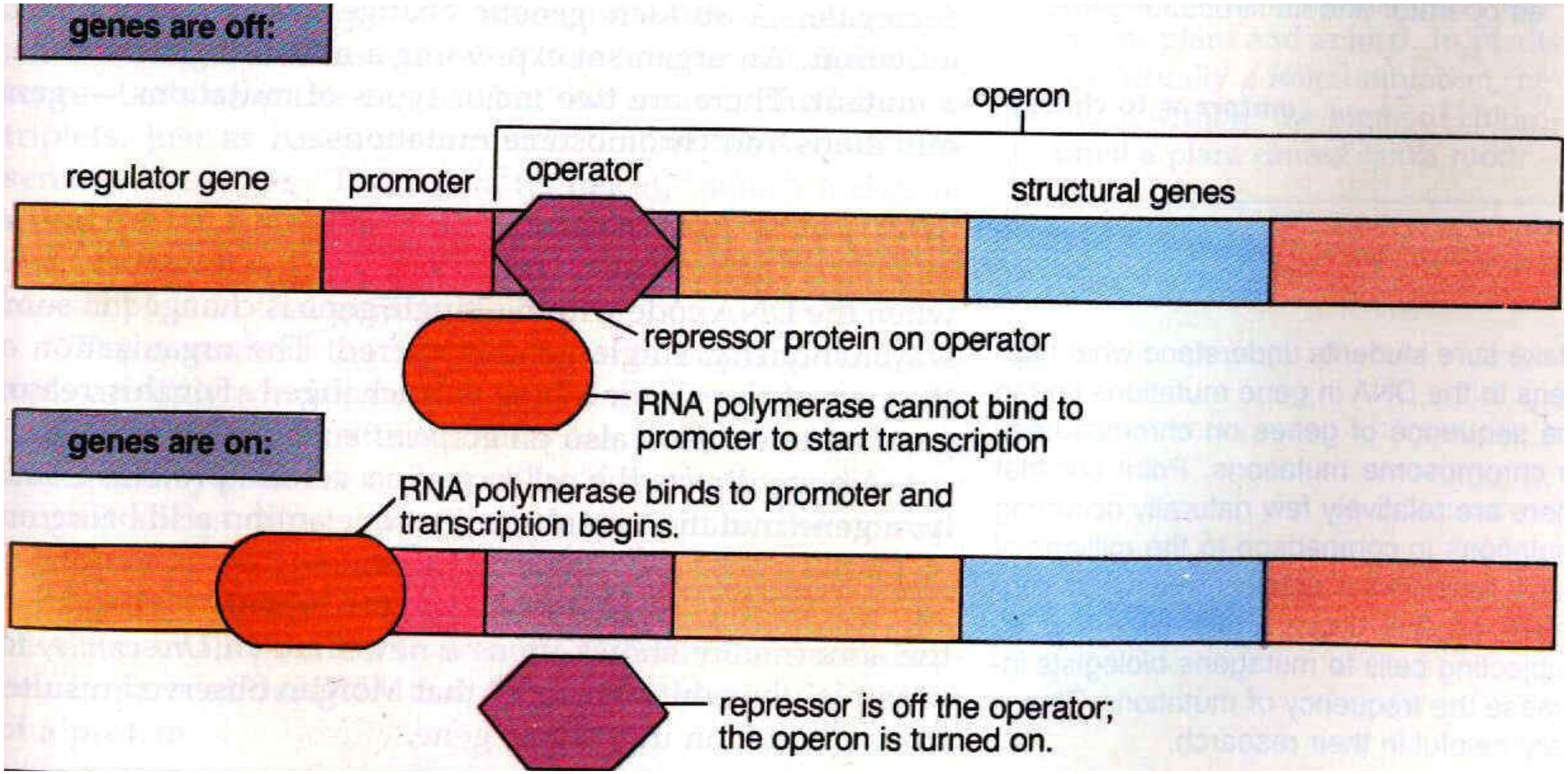
Maltozni operon: pozitivna regulacija, kjer aktivator deluje po vezavi induktorja (mal)



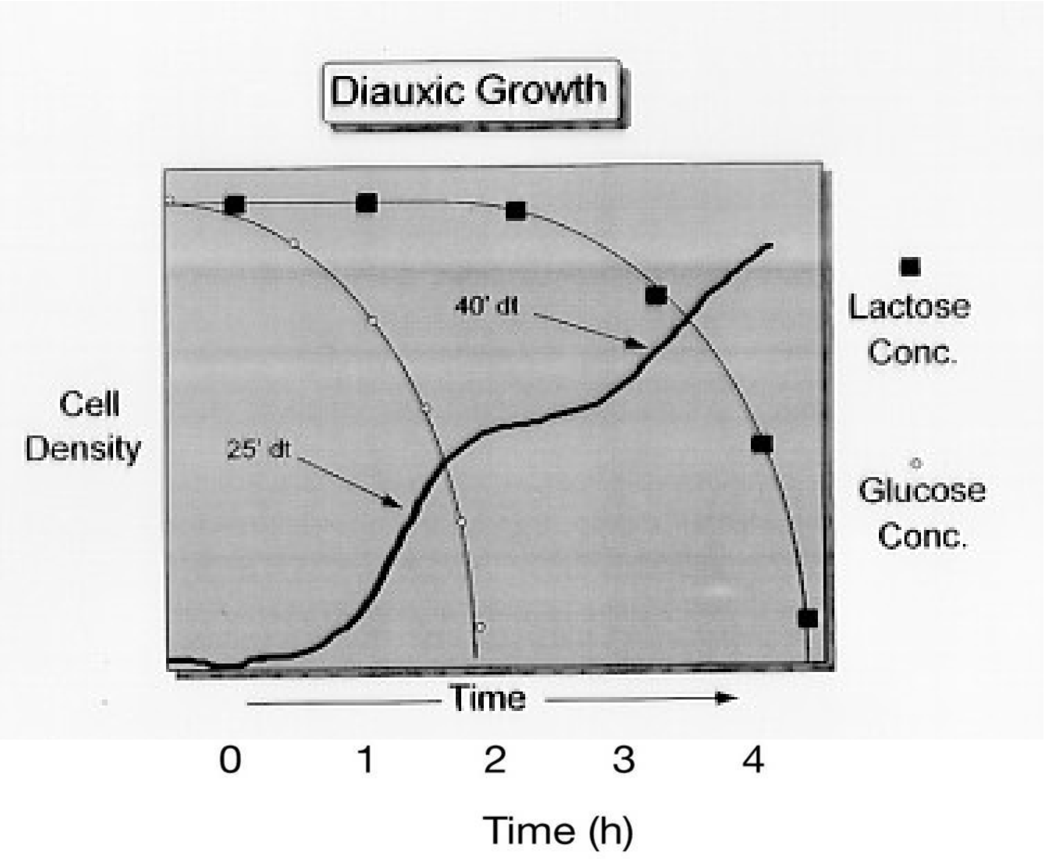
Argininski operon: represor deluje šele po vezavi korepresorja (Arg)

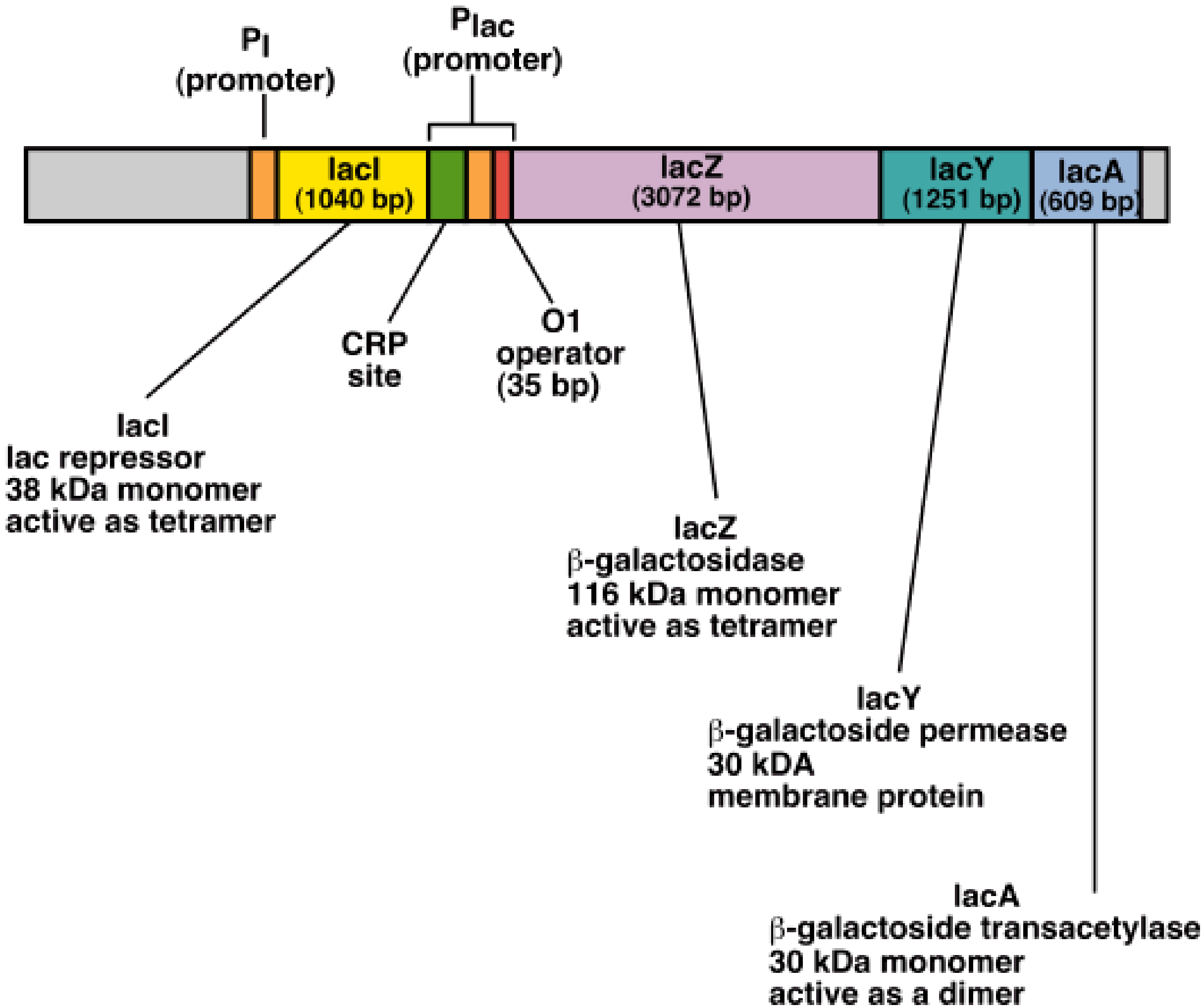


Laktozni operon: represor deluje, dokler se nanj ne veže induktor (lac)

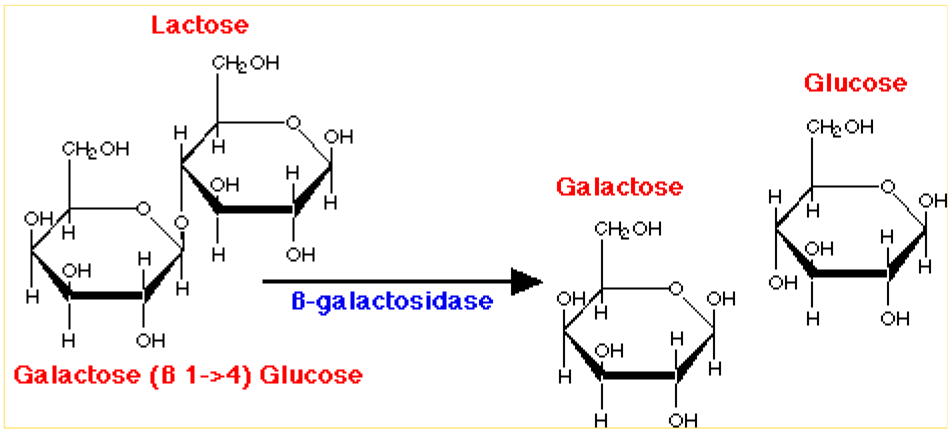


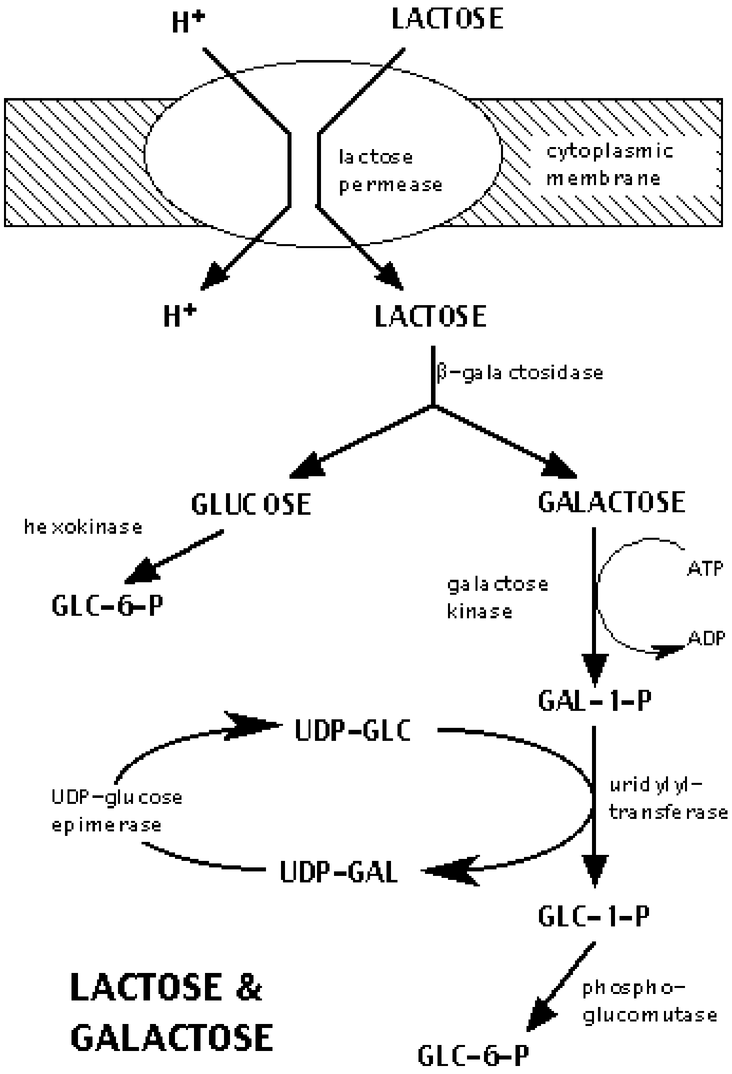
genes are off:
operon
regulator gene
promoter
operator
structural genes
repressor protein on operator
RNA polymerase cannot bind to promoter to start transcription
genes are on:
RNA polymerase binds to promoter and transcription begins.
repressor is off the operator; the operon is turned on.

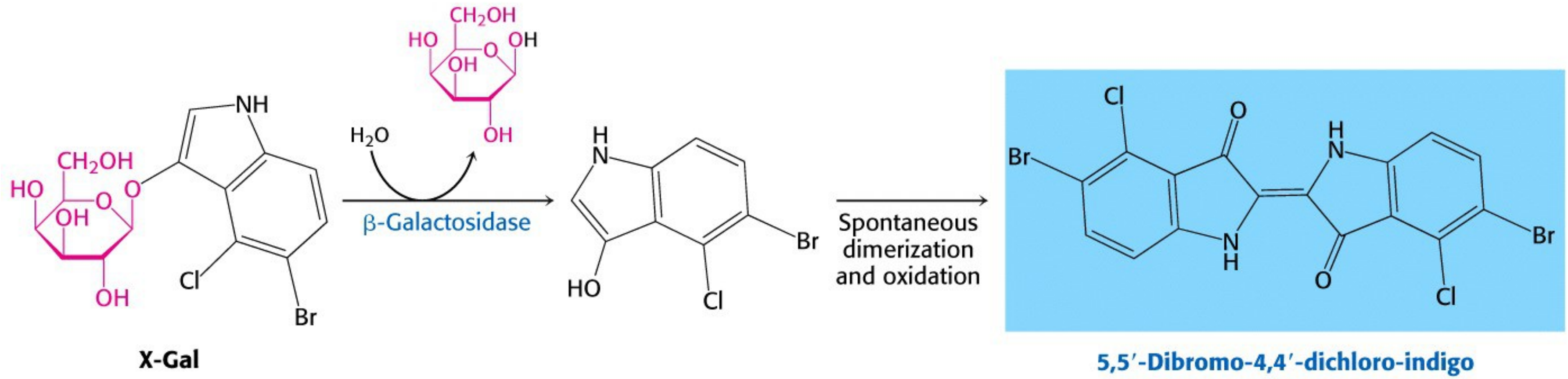

Diauxic Growth
Cell Density
25' dt
40' dt
Lactose Conc.
Glucose Conc.
Time
0
1
2
3
4
Time (h)



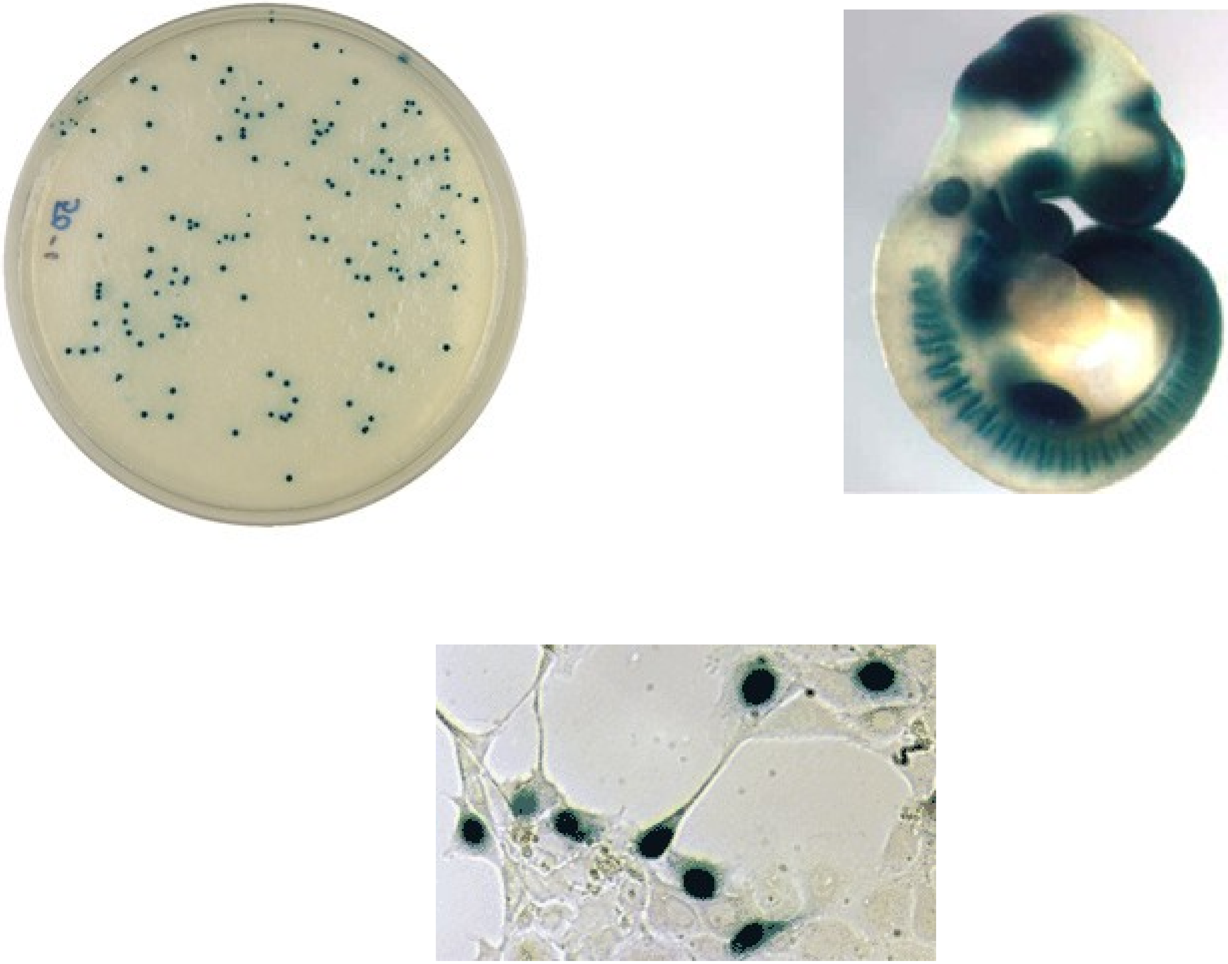
P$_I$
(promoter)
P$_{lac}$
(promoter)
lacI
(1040 bp)
lacZ
(3072 bp)
lacY
(1251 bp)
lacA
(609 bp)
CRP
site
O1
operator
(35 bp)
lacI
lac repressor
38 kDa monomer
active as tetramer
lacZ
β-galactosidase
116 kDa monomer
active as tetramer
lacY
β-galactoside permease
30 kDA
membrane protein
lacA
β-galactoside transacetylase
30 kDa monomer
active as a dimer


Lactose
Galactose (β 1->4) Glucose
β-galactosidase
Galactose
Glucose

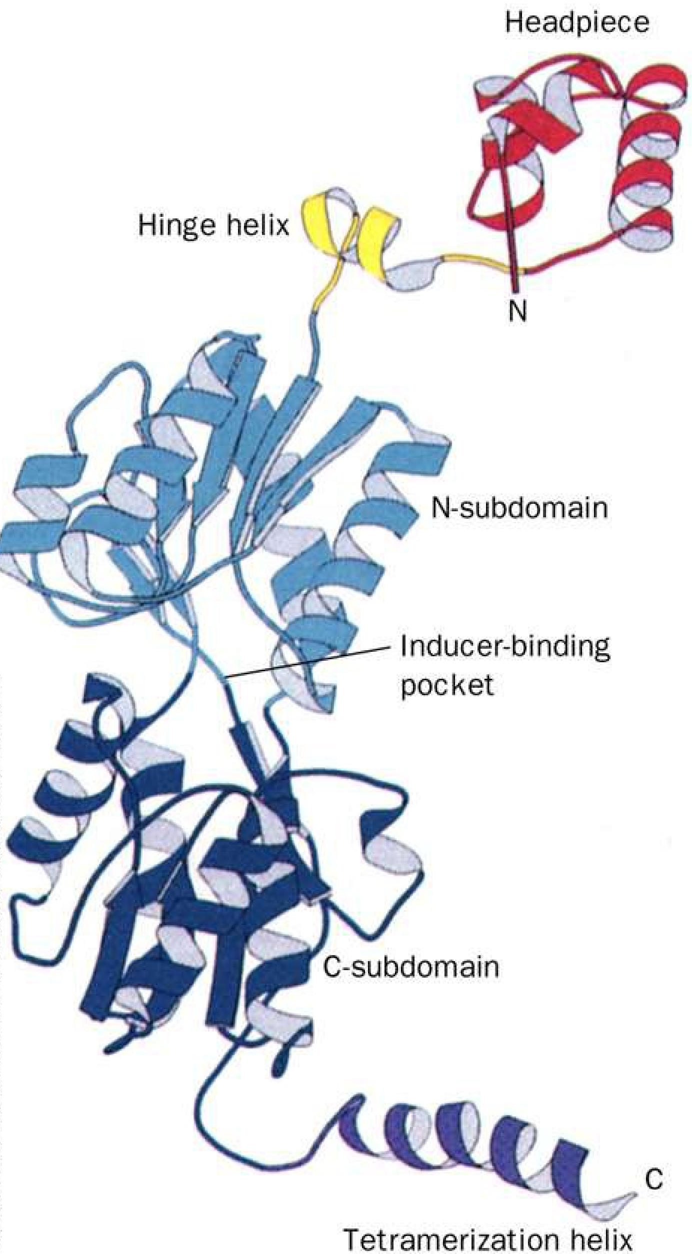

H+
LACTOSE
lactose permease
cytoplasmic membrane
H+
LACTOSE
β-galactosidase
GLUCOSE
GALACTOSE
hexokinase
GLC-6-P
galactose kinase
ATP
ADP
GAL-1-P
UDP-GLC
UDP-glucose epimerase
uridylyl-transferase
UDP-GAL
GLC-1-P
LACTOSE & GALACTOSE
phospho-glucomutase
GLC-6-P

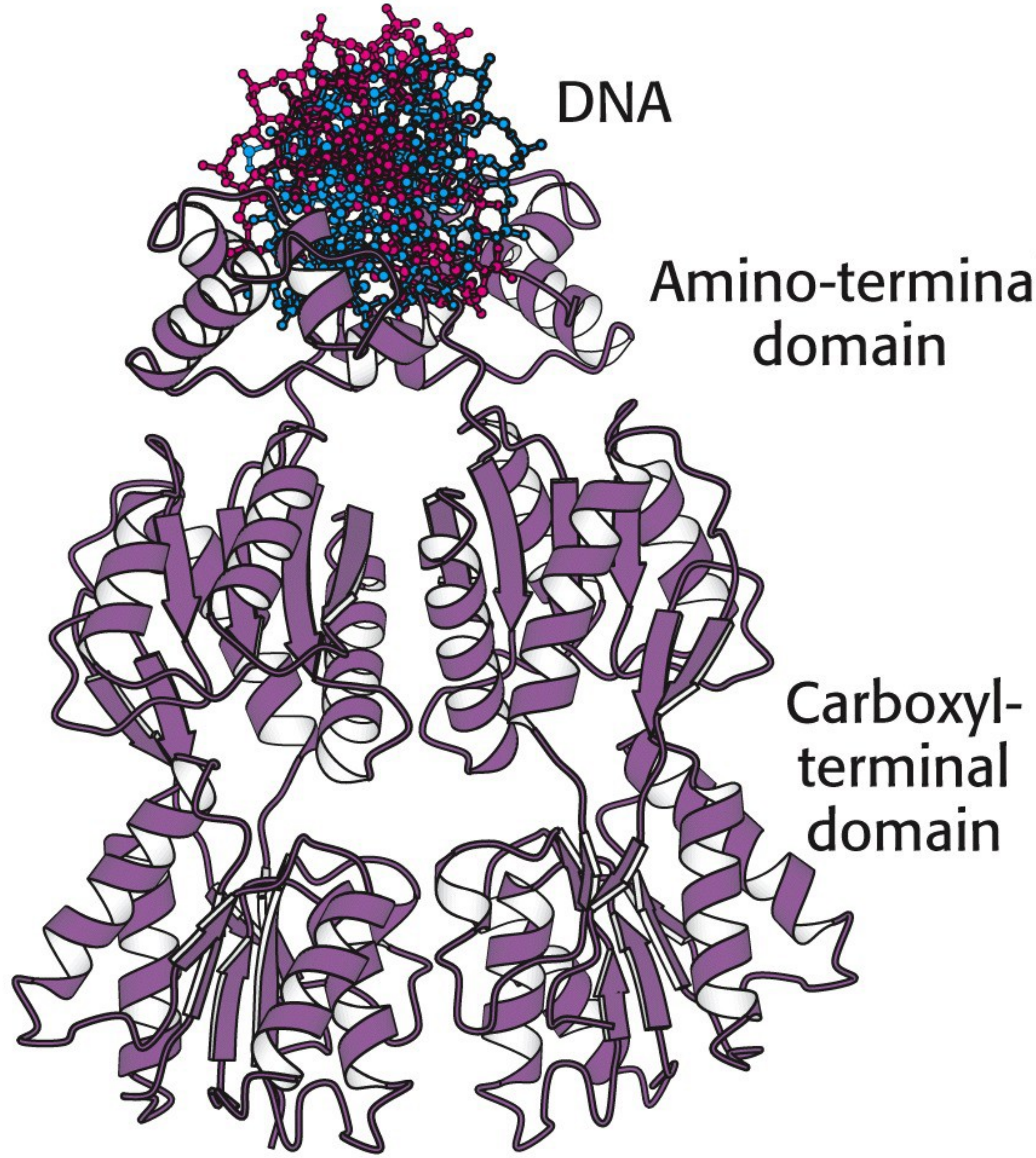


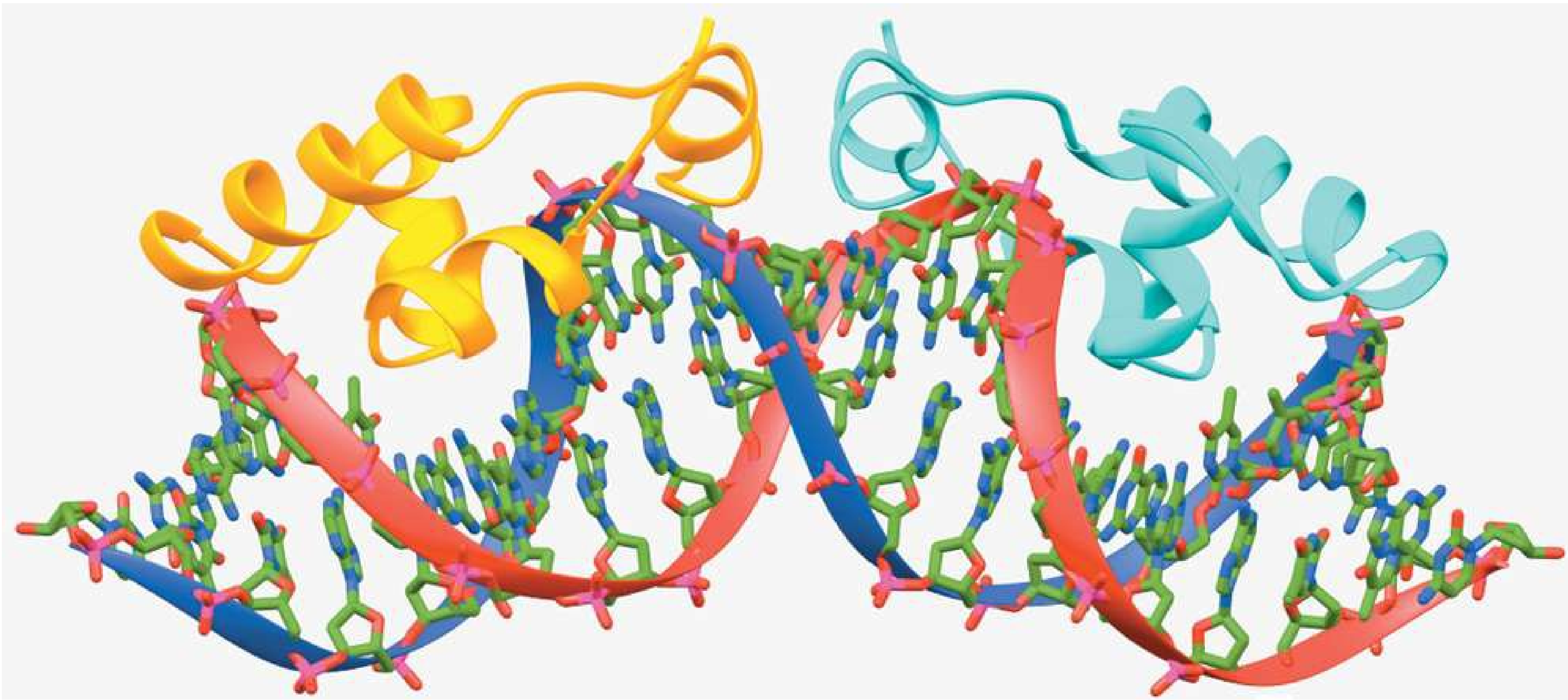
5-bromo-4-kloro-3-indolil-beta-D-galaktopiranozid



β-galaktozidaza kot reporterski encim: detekcija preko razgradnje X-gal.


Headpiece
Hinge helix
N
N-subdomain
Inducer-binding
pocket
C-subdomain
C
Tetramerization helix

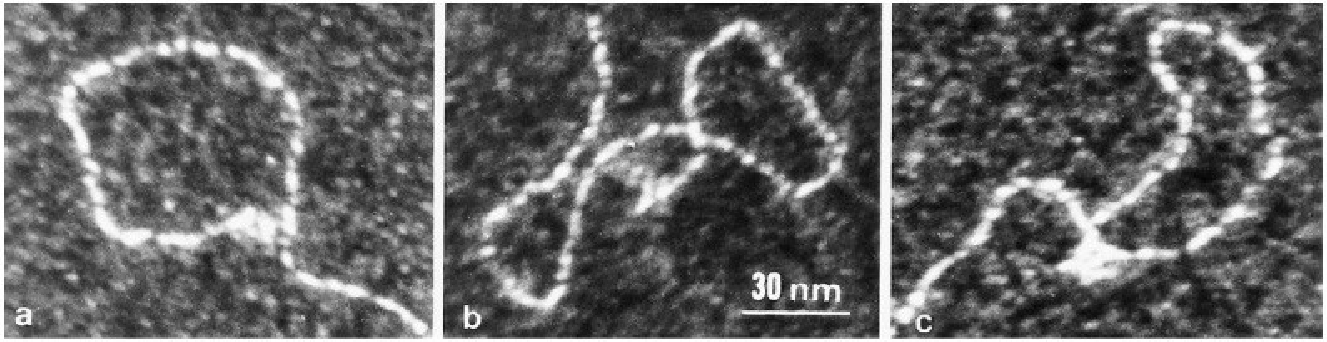

DNA
Amino-terminal
domain
Carboxyl-
terminal
domain

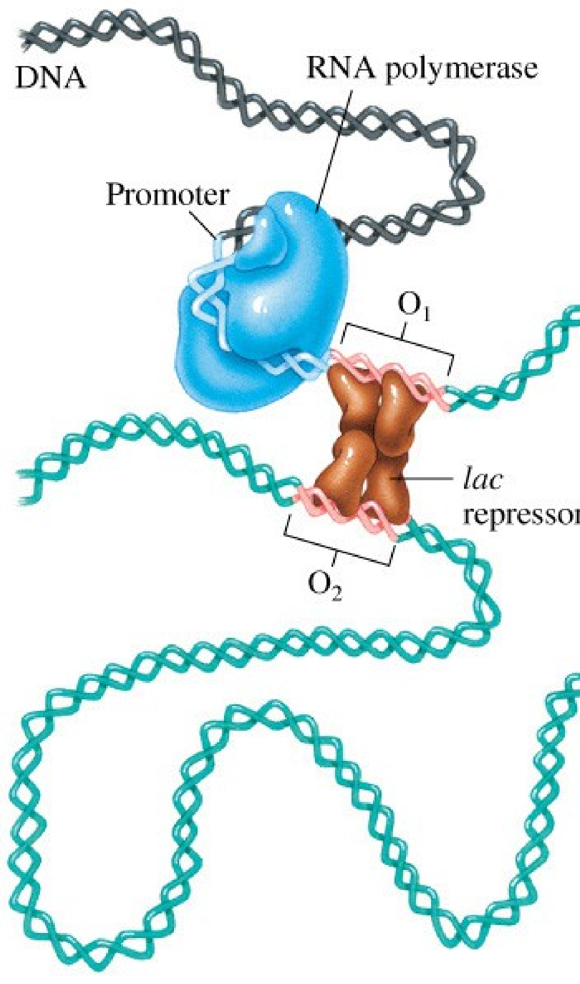


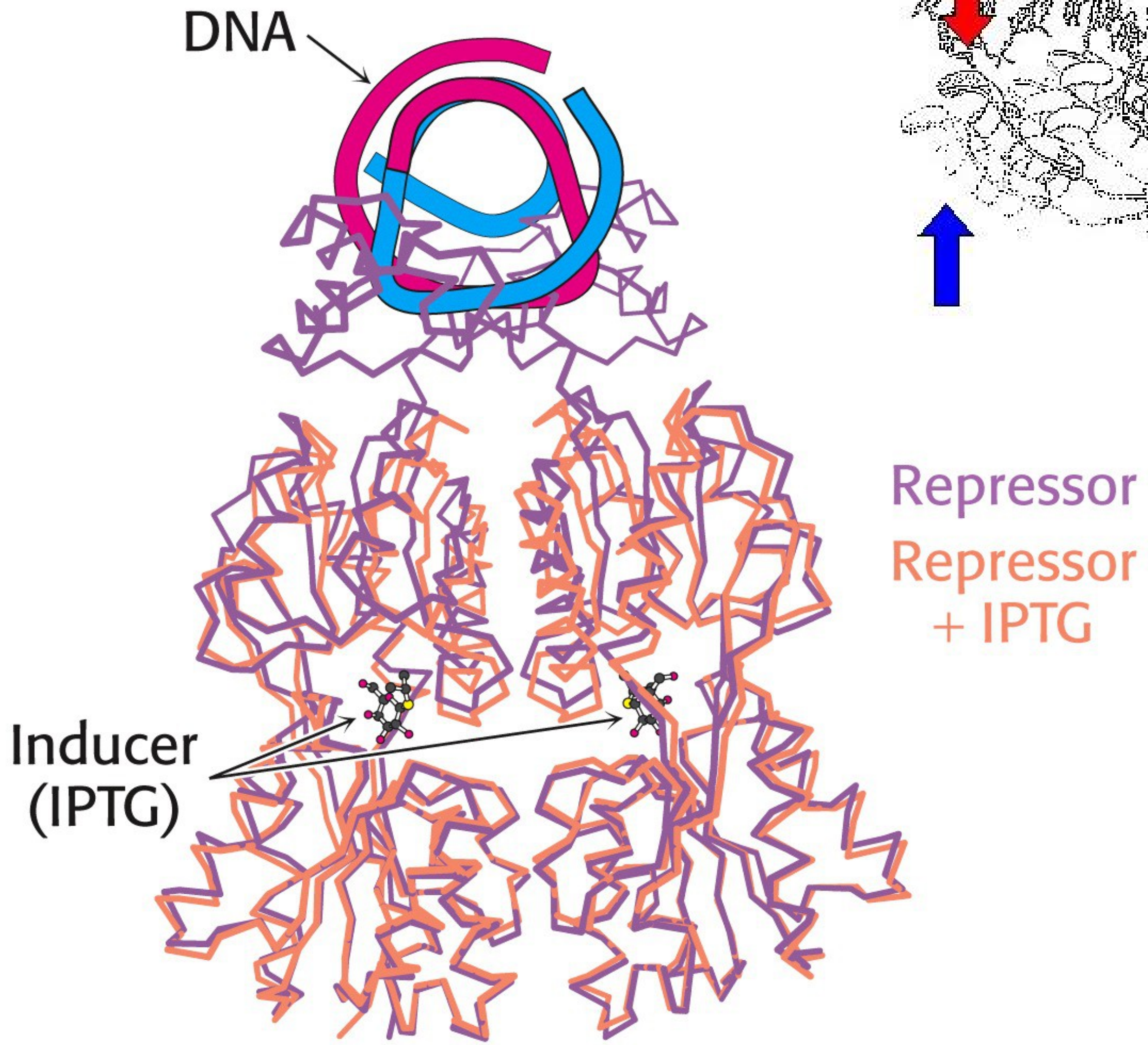
5′- . . . TGTGTGGAATTGTGAGCGGATAACAATTTCACACA . . . 3′

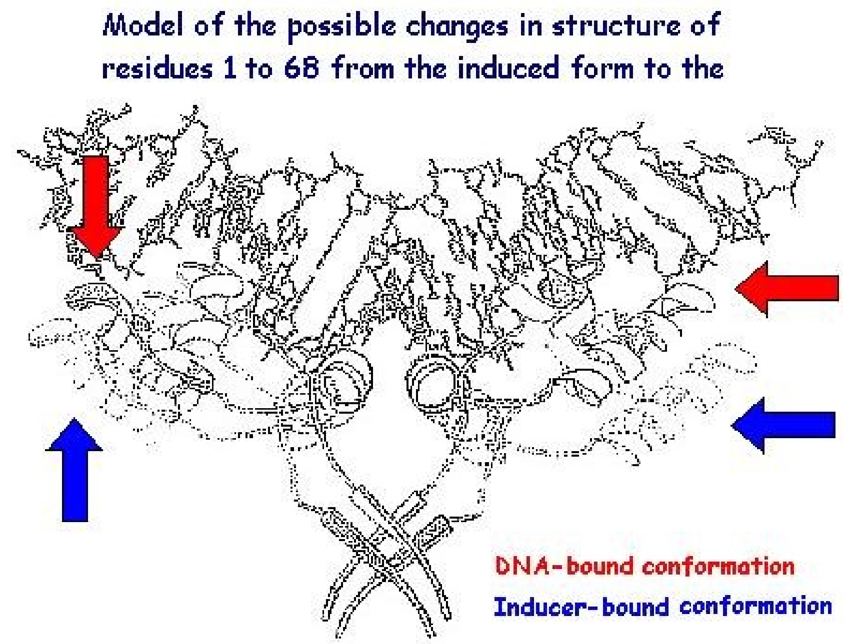
3′- . . . ACACACCTTAACACTCGCCTAATGTTAAAGTGTGT . . . 5′

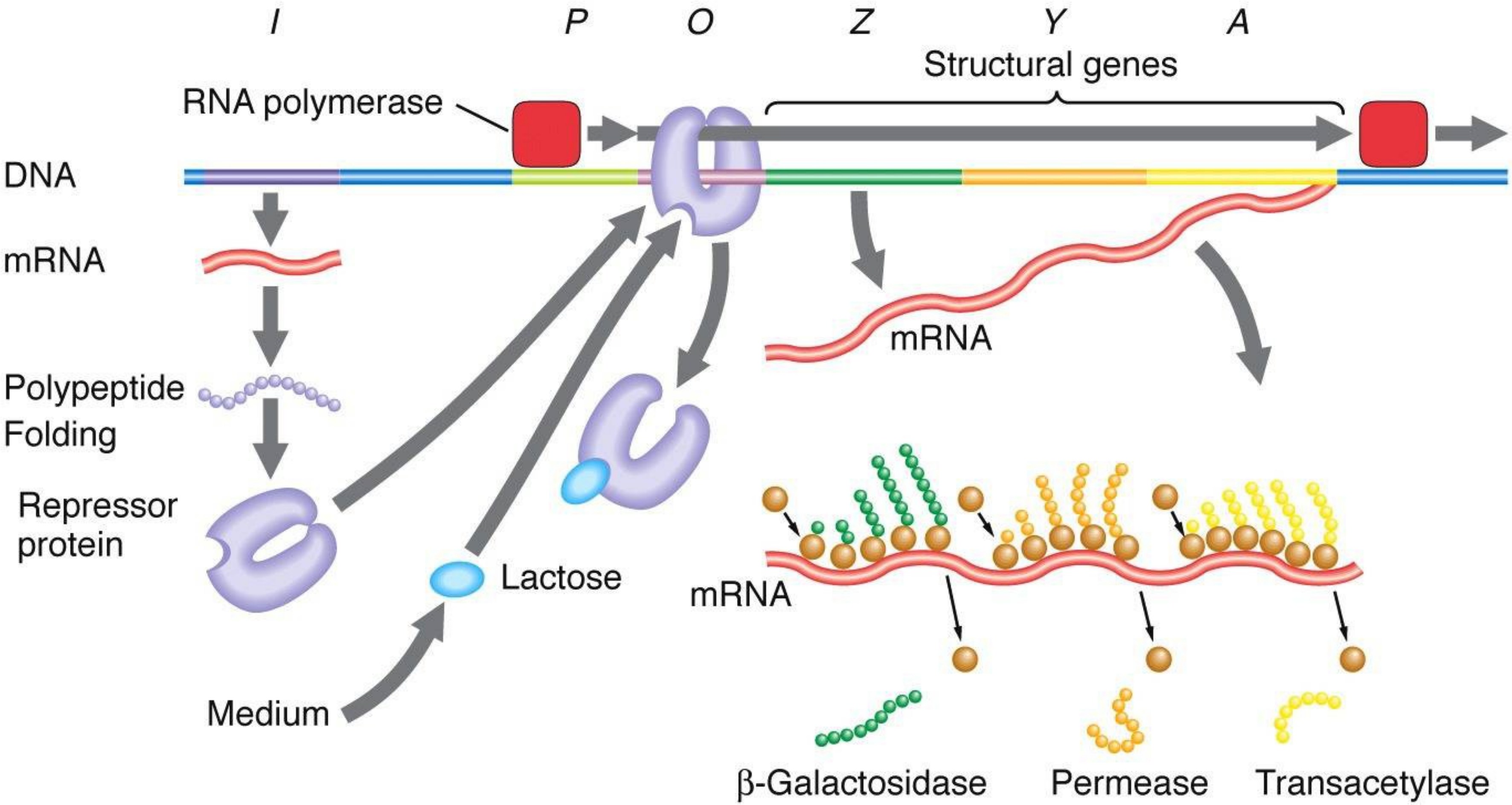
Vezavna regija lacI na operator *lac* pri *E. coli*. Struktura na osnovi NMR.

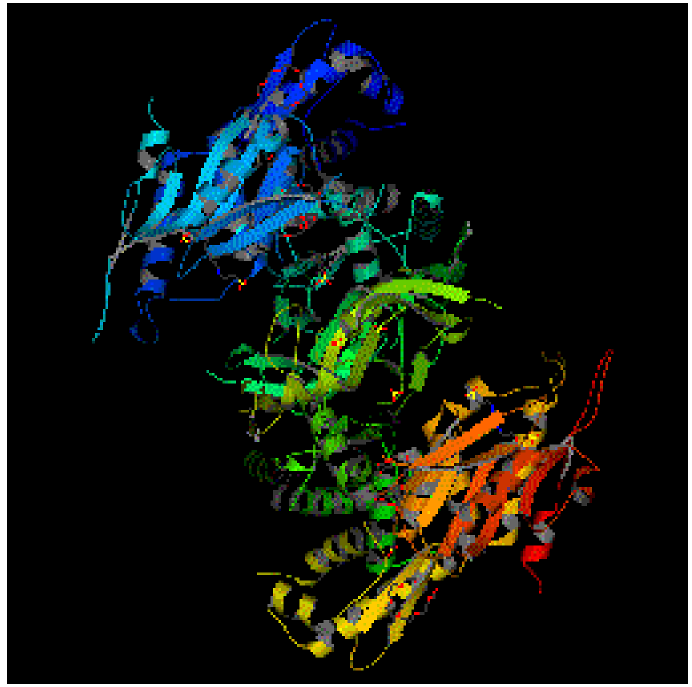

a
b
30 nm
c


DNA
RNA polymerase
Promoter
O_1
lac
repressor
O_2


DNA
Inducer
(IPTG)
Repressor
Repressor
+ IPTG

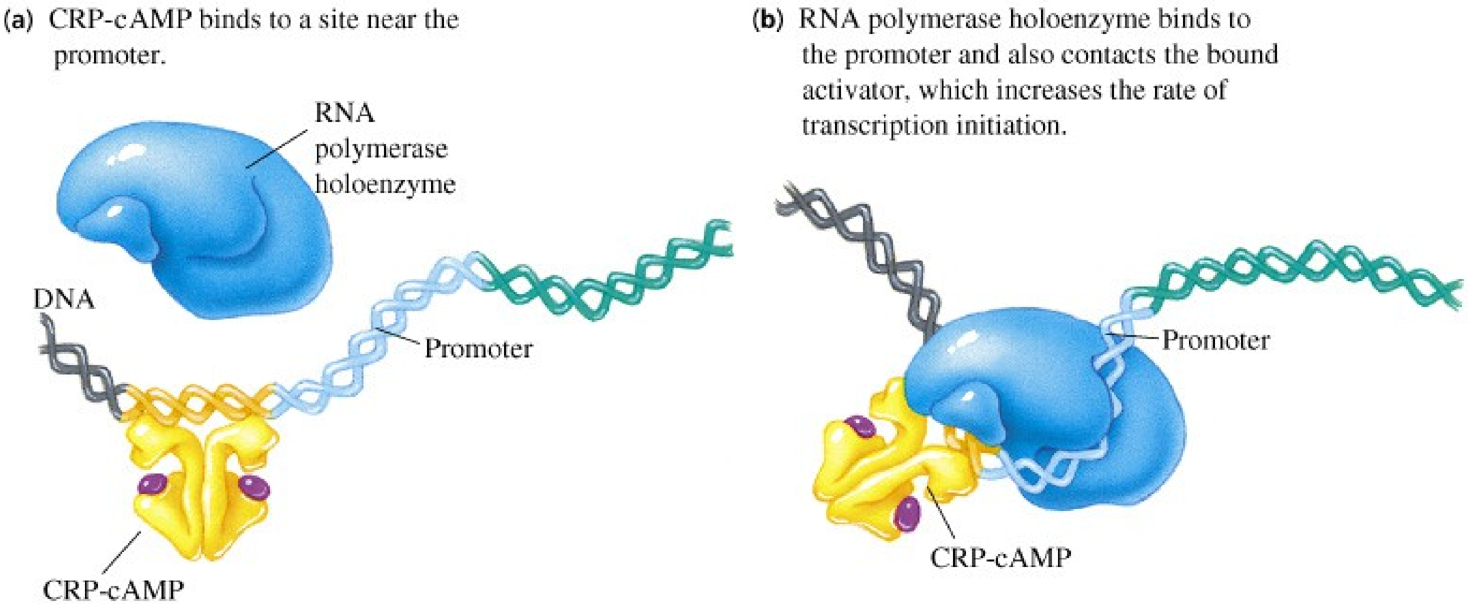

Model of the possible changes in structure of
residues 1 to 68 from the induced form to the
DNA-bound conformation
Inducer-bound conformation


I
P
O
Z
Y
A
Structural genes
RNA polymerase
DNA
mRNA
Polypeptide
Folding
Repressor
protein
Lactose
Medium
mRNA
mRNA
β-Galactosidase
Permease
Transacetylase

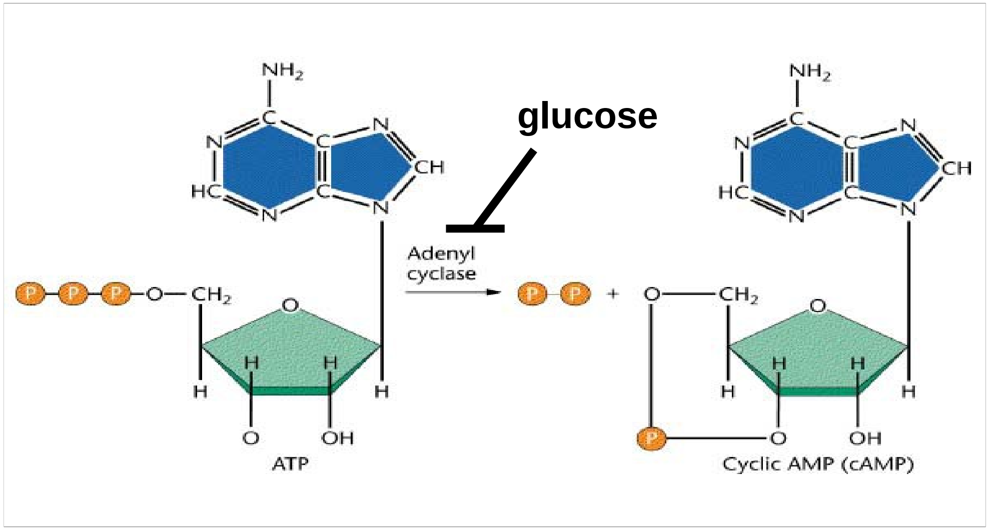


```
         1        10        20          30        40        50        60
         |         |         |           |         |         |         |
LacI     MKPVTLYDVAEYAGVSYQTVSRVVN...QASHVSAKTREKVEAAMAELNYIPNRVAQQLAGKQS
         1           10        20        30        40        50        60
         |            |         |         |         |         |         |
FruR     M...KLDEIARLAGVSRTTASYVINGKAKQYRVSDKTVEKVMAVVREHNYHPNAVAAGLRAGRT
         1          10        20           30        40        50
         |           |         |            |         |         |
PurR     MATIK..DVAKRANVSTTTVSHVIN...KTRFVAEETRNAVWAAIKELHYSPSAVARSLKVNHT
             --------     ---------         --------------     ---------
              Helix 1      Helix 2              Helix 3          Helix 4
```

	1	2	3	4	5	6	7	8	9	
Lac	N	T	T	G	T	G	A	G	C	◊
FruR	N	G	C	T	G	A	A	T	C	◊
PurR	N	A	C	G	C	A	A	A	C	◊

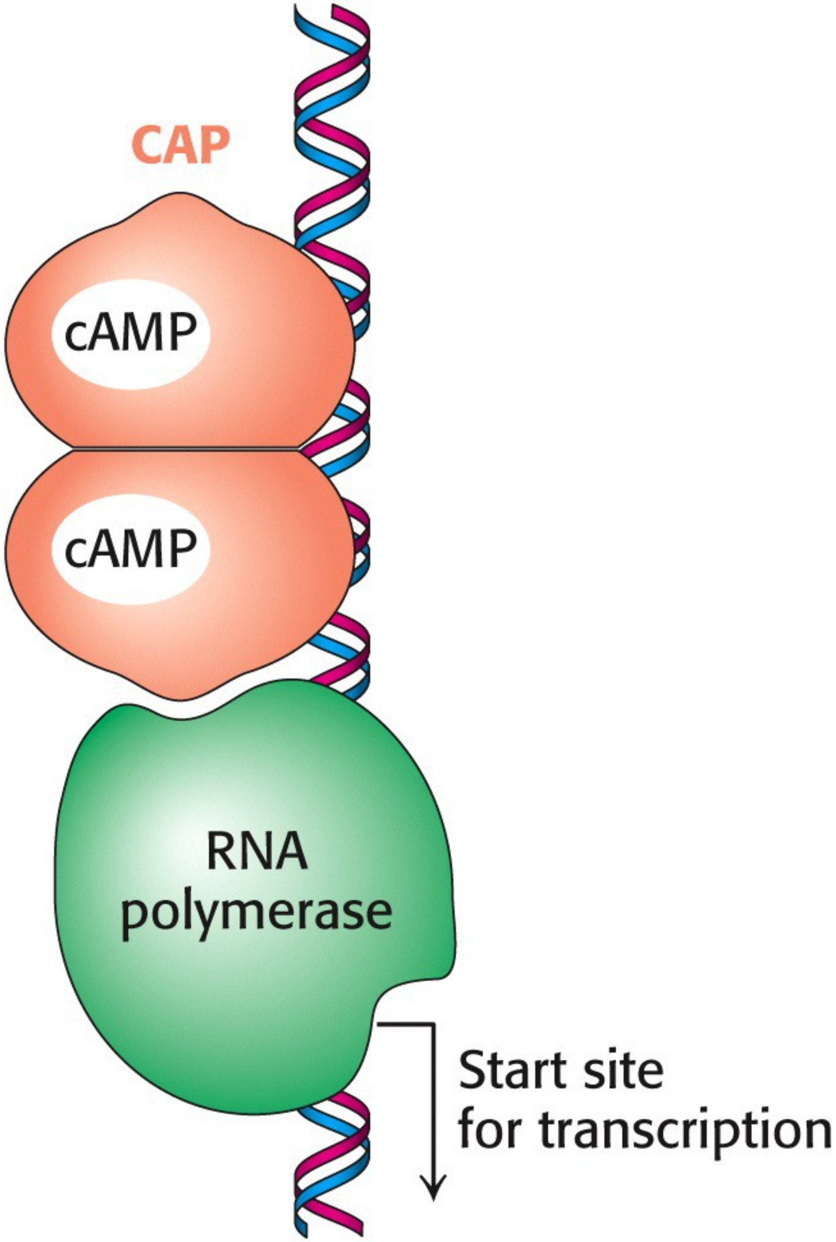


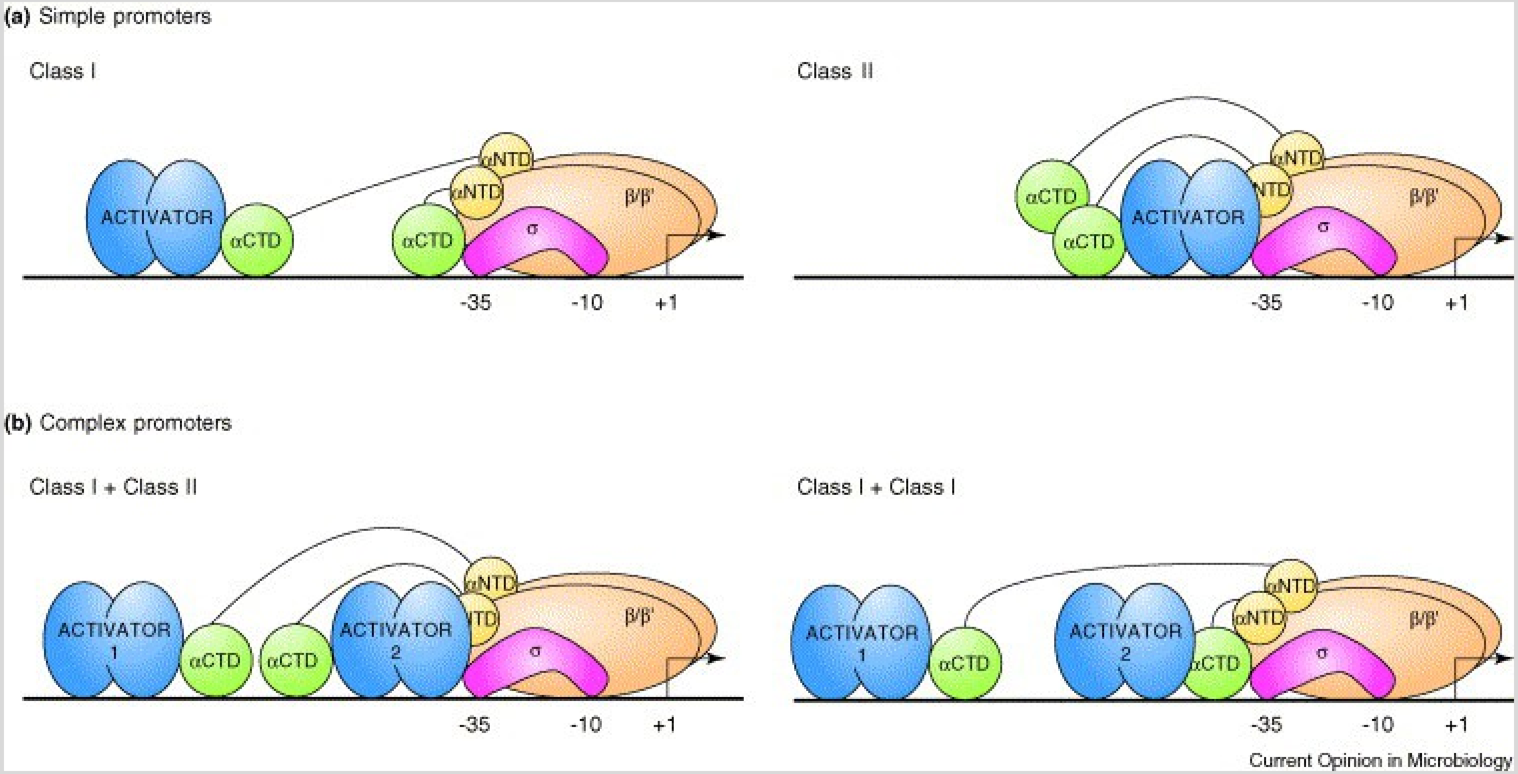
CRP: cAMP receptor protein
CAP: catabolite activator protein



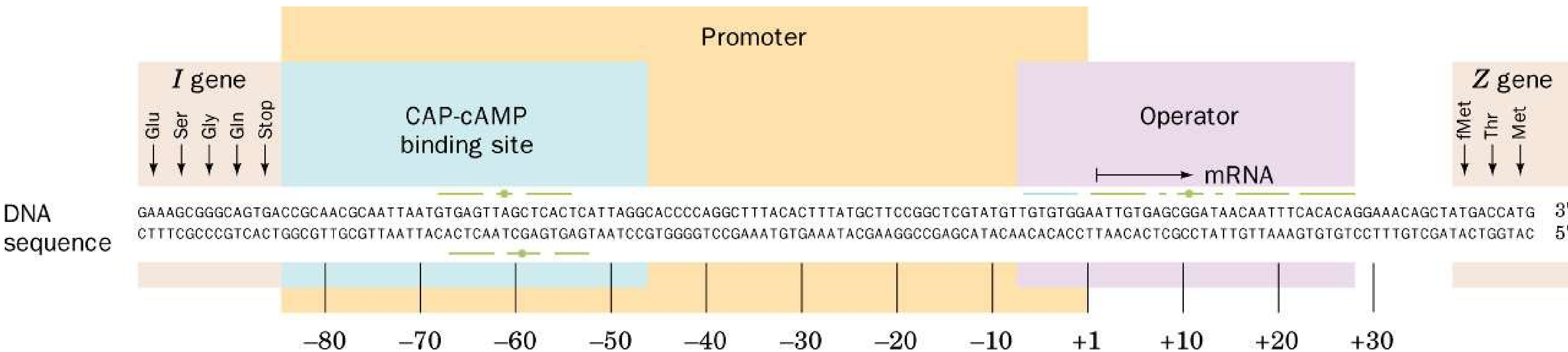
aktivacija operona *lac*
preko katabolitne represije

50x pogostejša iniciacija transkripcije

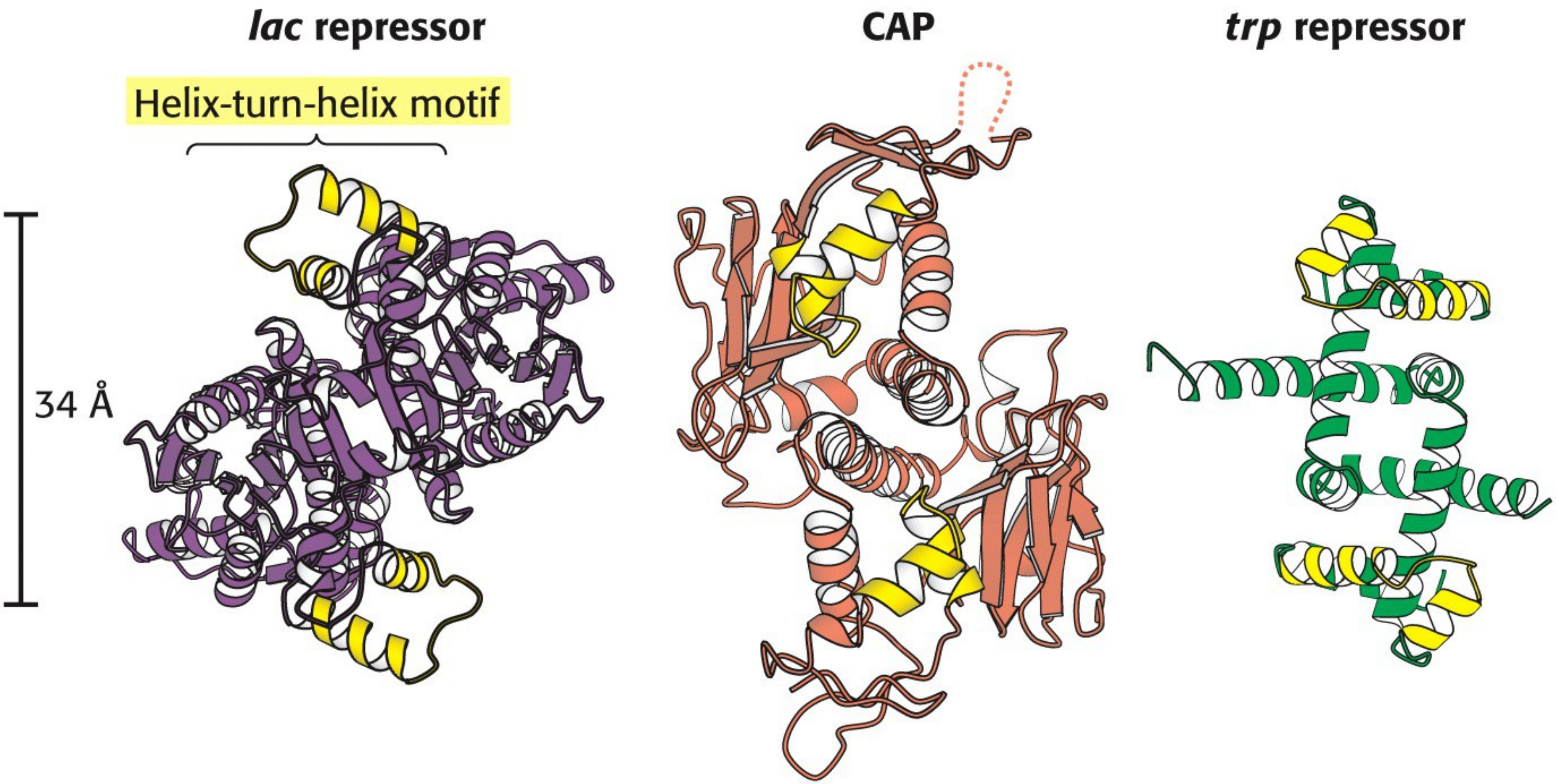




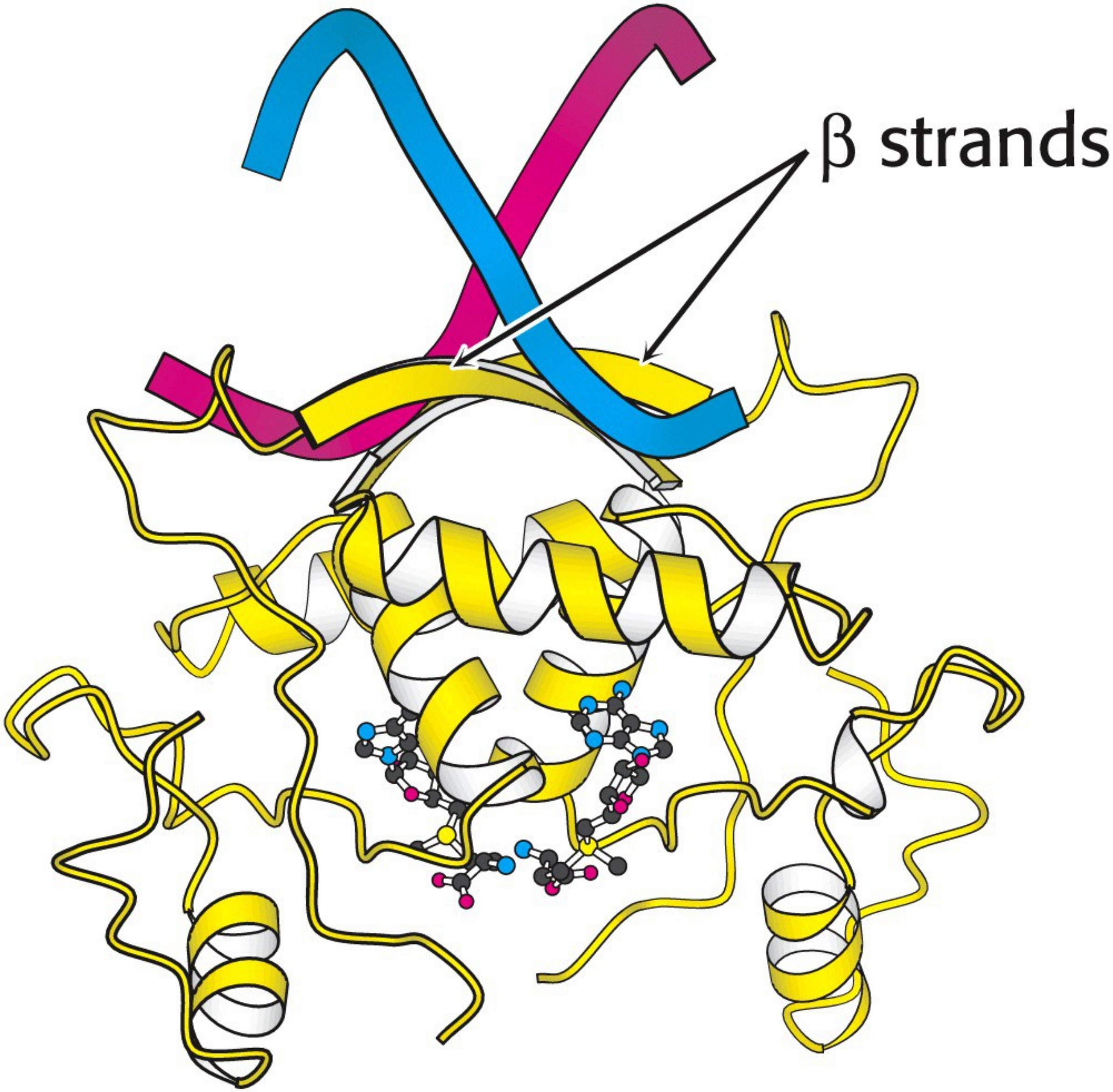
Promotorji razreda I in II se razlikujejo po oddaljenosti od promotorja in po načinu interakcije s C-končno domeno podenote alfa RNA-polimeraze in/oz. s podenoto sigma.
Razred I: npr. *lac (CAP @ -61)*; razred II: npr. *gal*P1 (@-41).



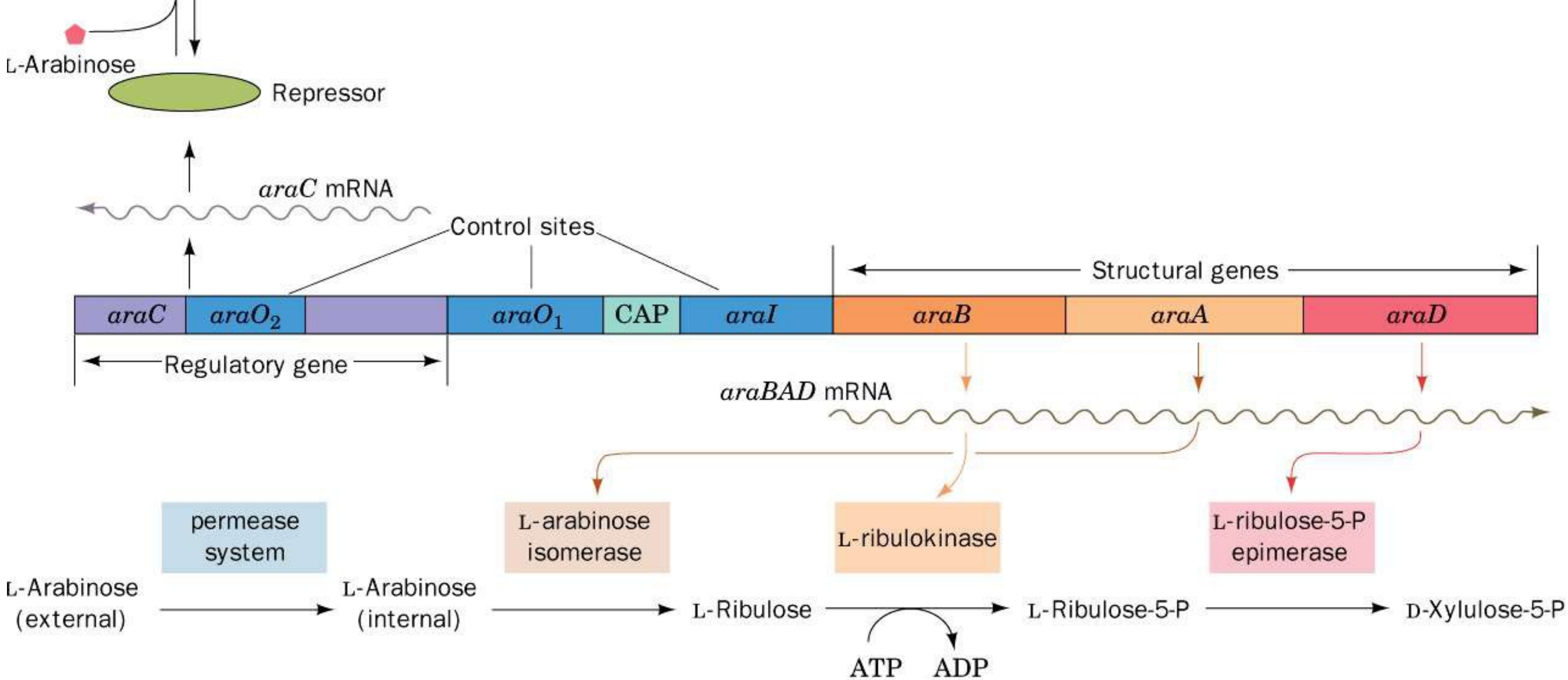
Nukleotidno zaporedje promotorsko-operatorske regije *lac*-operona *E. coli*.

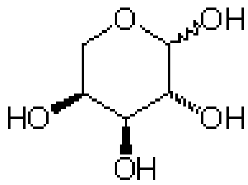


Motiv vijačnica-obrat-vijačnica (helix-turn-helix) omogoča vezavo na DNA.

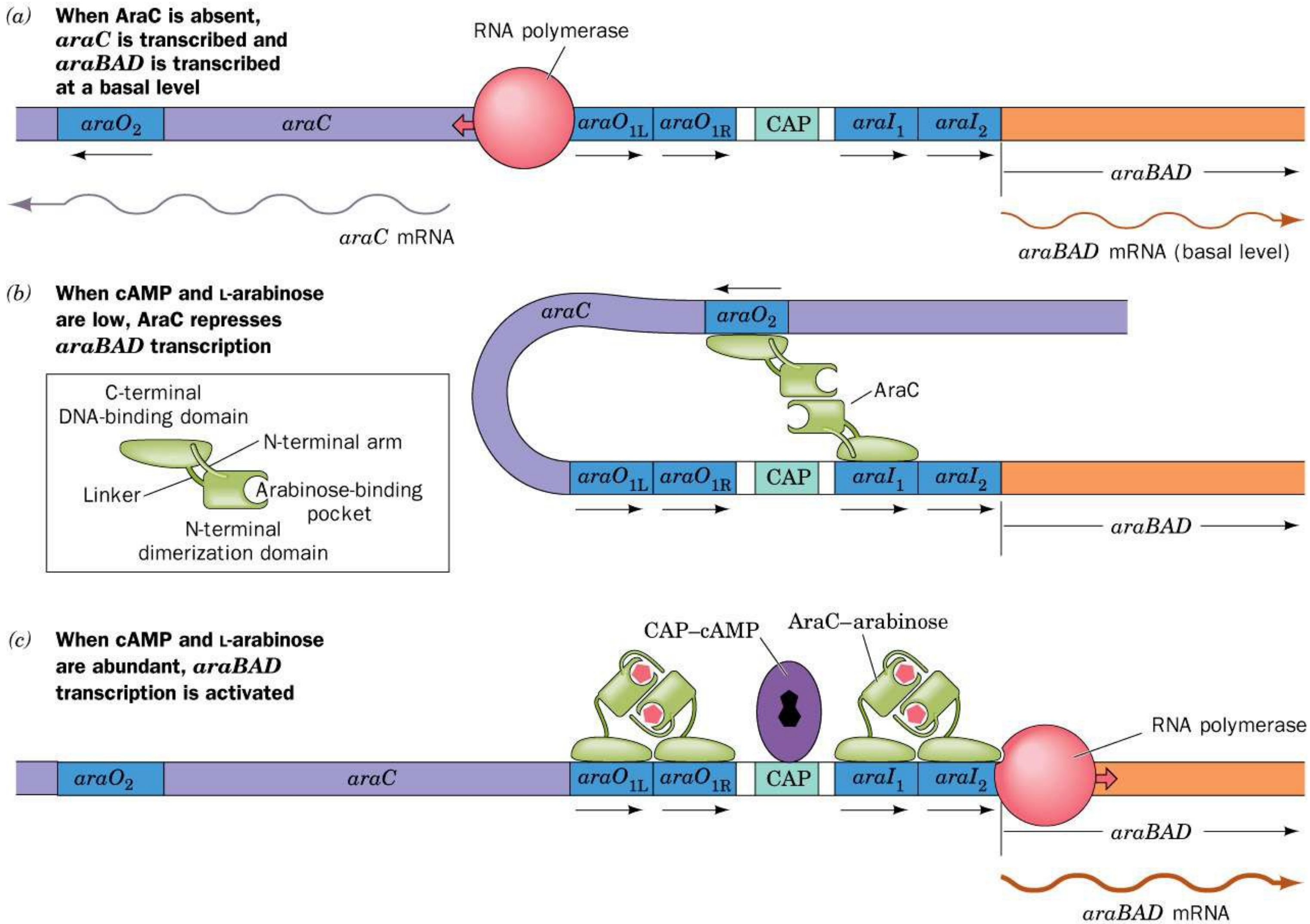


met - represor

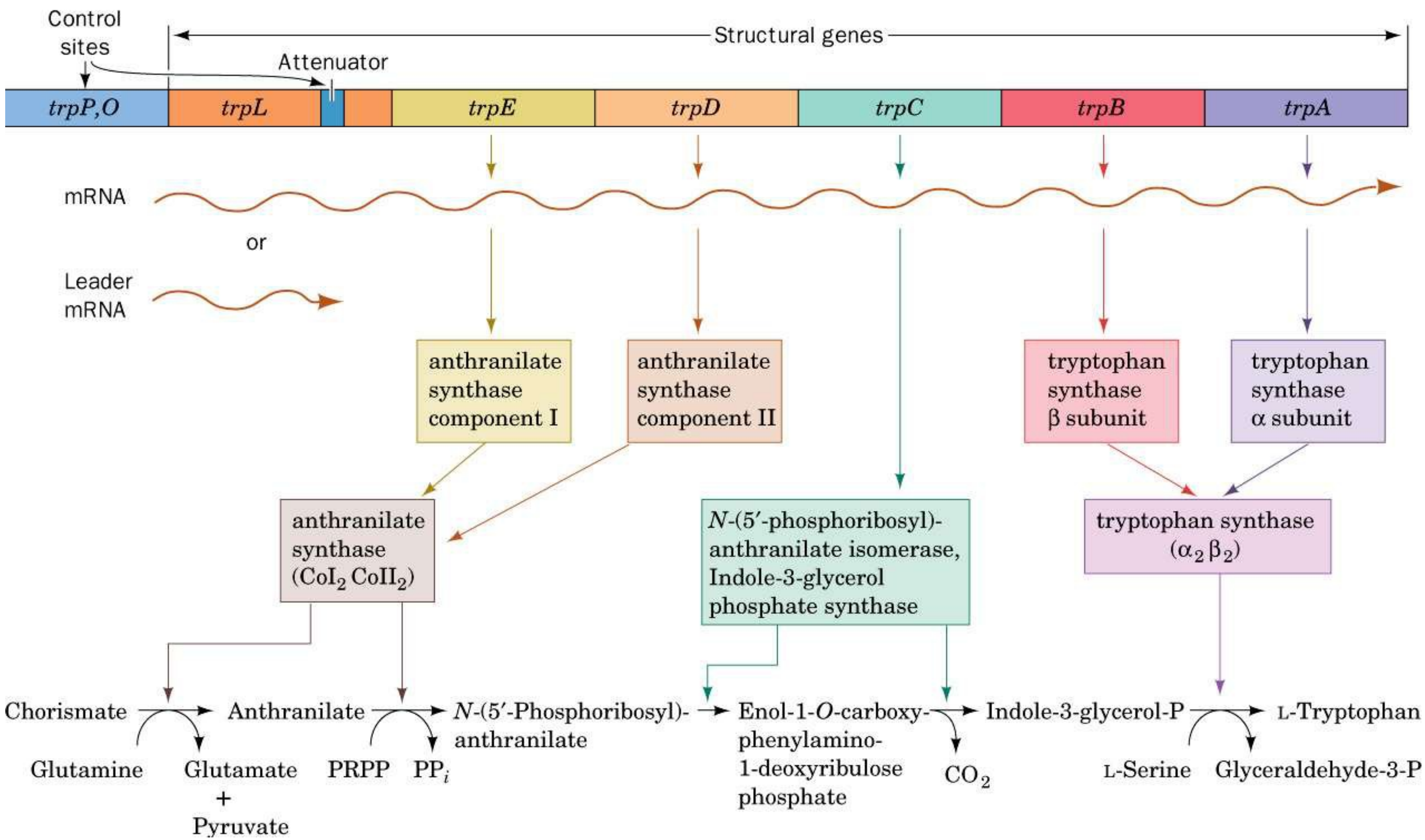




Genetska karta operonov *araC* in *araBAD E. coli*.

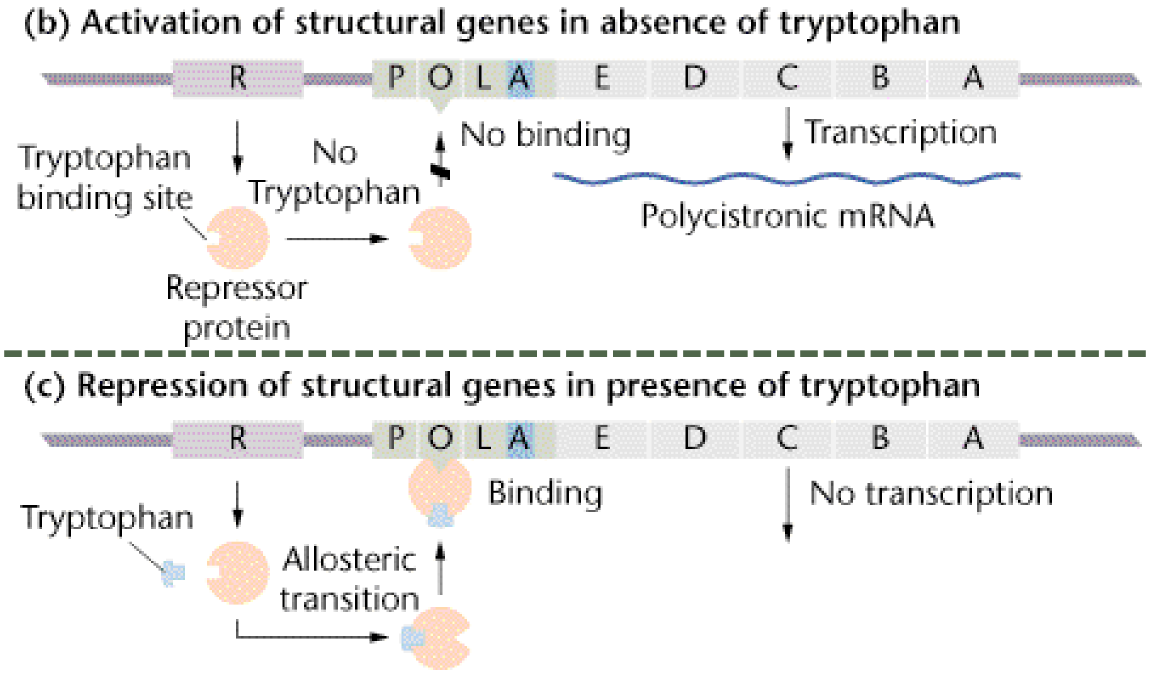


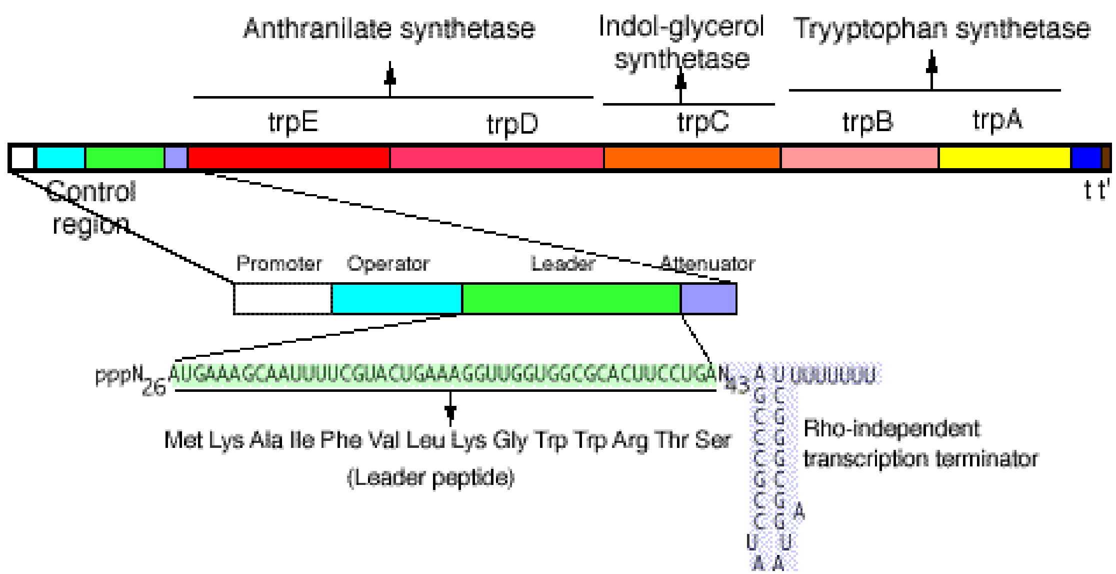
Uravnavanje *araBAD*. a) v odsotnosti araC; b) v prisotnosti araC, a brez L-arabinoze in brez cAMP; c) v prisotnosti araC, cAMP in L-arabinoze.



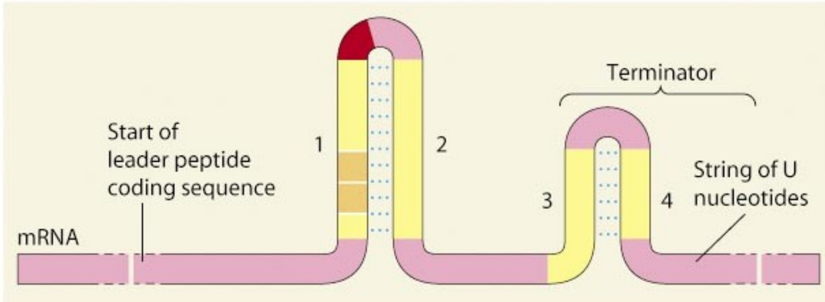
Control sites
Structural genes
Attenuator
trpP,O
trpL
trpE
trpD
trpC
trpB
trpA
mRNA
or
Leader mRNA
anthranilate synthase component I
anthranilate synthase component II
tryptophan synthase β subunit
tryptophan synthase α subunit
anthranilate synthase (CoI$_2$ CoII$_2$)
N-(5′-phosphoribosyl)-anthranilate isomerase, Indole-3-glycerol phosphate synthase
tryptophan synthase ($\alpha_2\beta_2$)
Chorismate
Anthranilate
N-(5′-Phosphoribosyl)-anthranilate
Enol-1-O-carboxy-phenylamino-1-deoxyribulose phosphate
Indole-3-glycerol-P
L-Tryptophan
Glutamine
Glutamate + Pyruvate
PRPP
PP$_i$
CO_2
L-Serine
Glyceraldehyde-3-P


trp repressor

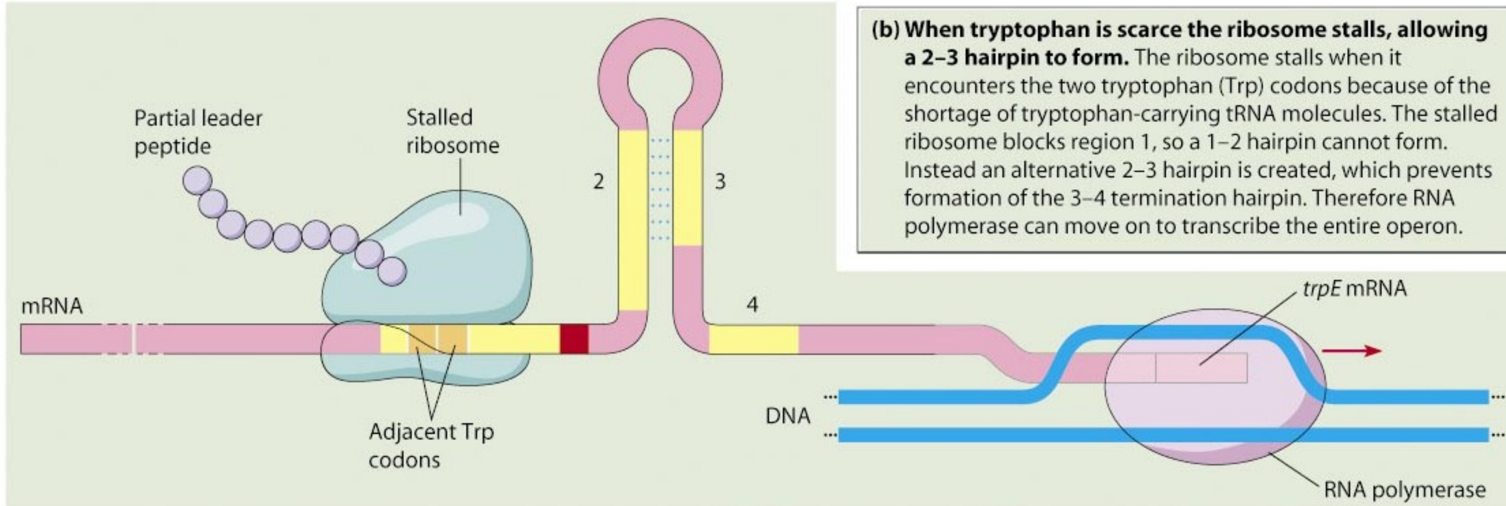

(b) Activation of structural genes in absence of tryptophan
R
P O L A E D C B A
Tryptophan binding site
No Tryptophan
No binding
Transcription
Polycistronic mRNA
Repressor protein
(c) Repression of structural genes in presence of tryptophan
R
P O L A E D C B A
Tryptophan
Binding
No transcription
Allosteric transition



atenuatorji → posttranskripcijski regulatorji

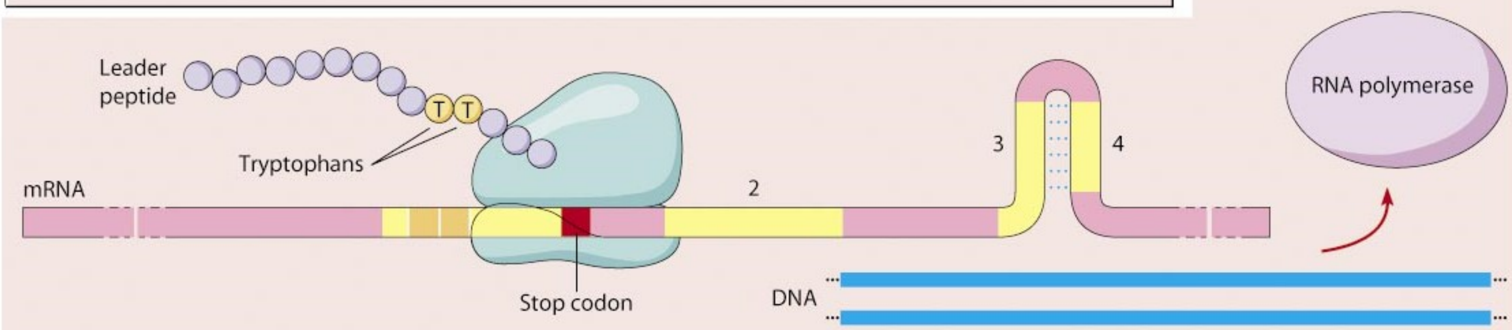


(a) The most stable secondary structure for *trp* leader mRNA. Attenuation depends on the ability of regions 1 and 2 and regions 3 and 4 of the *trp* leader sequence to base-pair, forming hairpin secondary structures. The 3–4 hairpin structure acts as a transcription termination signal.



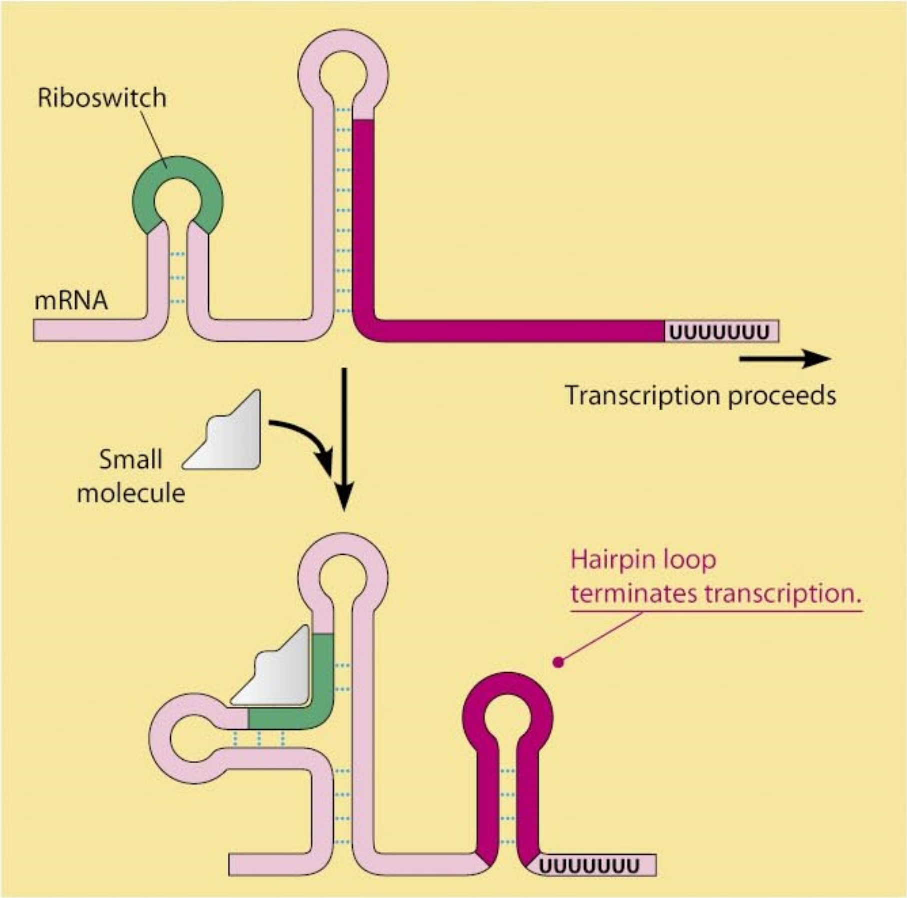
(b) When tryptophan is scarce the ribosome stalls, allowing a 2–3 hairpin to form. The ribosome stalls when it encounters the two tryptophan (Trp) codons because of the shortage of tryptophan-carrying tRNA molecules. The stalled ribosome blocks region 1, so a 1–2 hairpin cannot form. Instead an alternative 2–3 hairpin is created, which prevents formation of the 3–4 termination hairpin. Therefore RNA polymerase can move on to transcribe the entire operon.

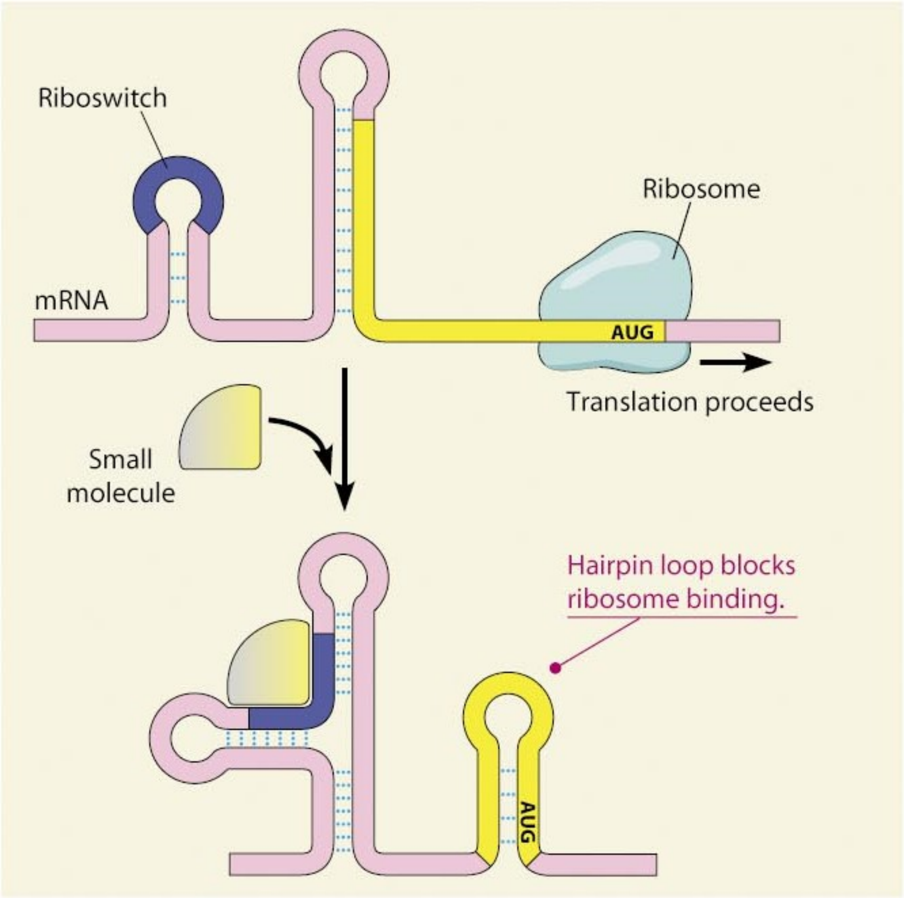
(c) When tryptophan is plentiful the ribosome continues, allowing the 3–4 transcription termination signal to form. The moving ribosome completes translation of the leader peptide and pauses at the stop codon, blocking region 2. As a result, the 3–4 structure forms and terminates transcription near the end of the leader sequence.

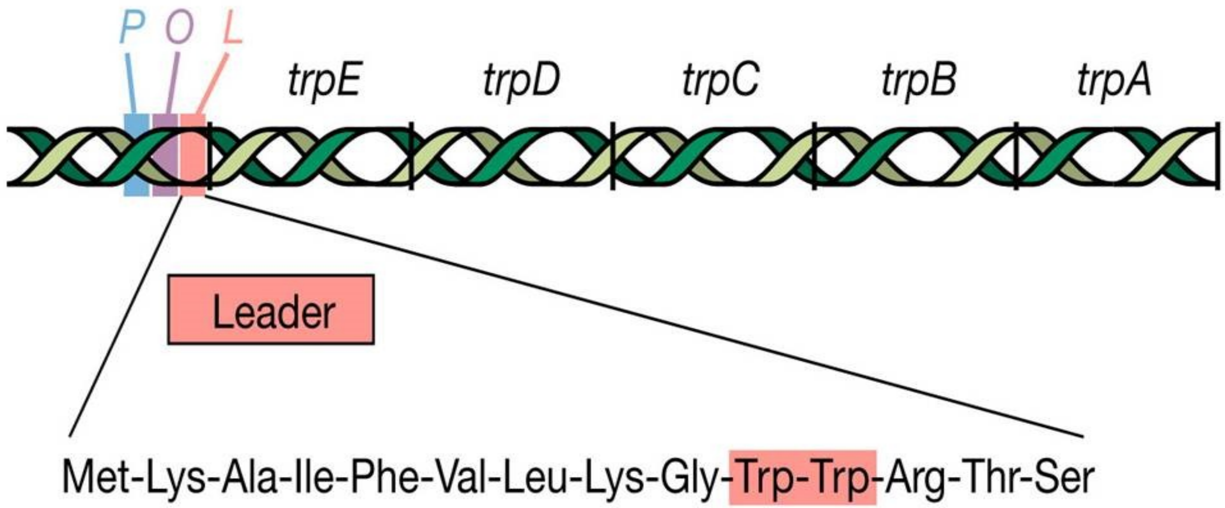


(a) Transcription termination. Binding of a small molecule to a riboswitch in the leader sequence of some mRNAs triggers the formation of a hairpin loop that terminates transcription. FMN binding to the leader sequence of the mRNA transcribed from the *rib* operon of *B. subtilis* works in this way.

(b) Translation initiation. In other mRNAs, a small molecule binding to a riboswitch triggers formation of a hairpin loop containing the site where ribosomes normally bind, thereby interfering with translation initiation. In *E. coli*, this type of control is used by FMN to inhibit translation of mRNAs coding for enzymes involved in FMN synthesis.







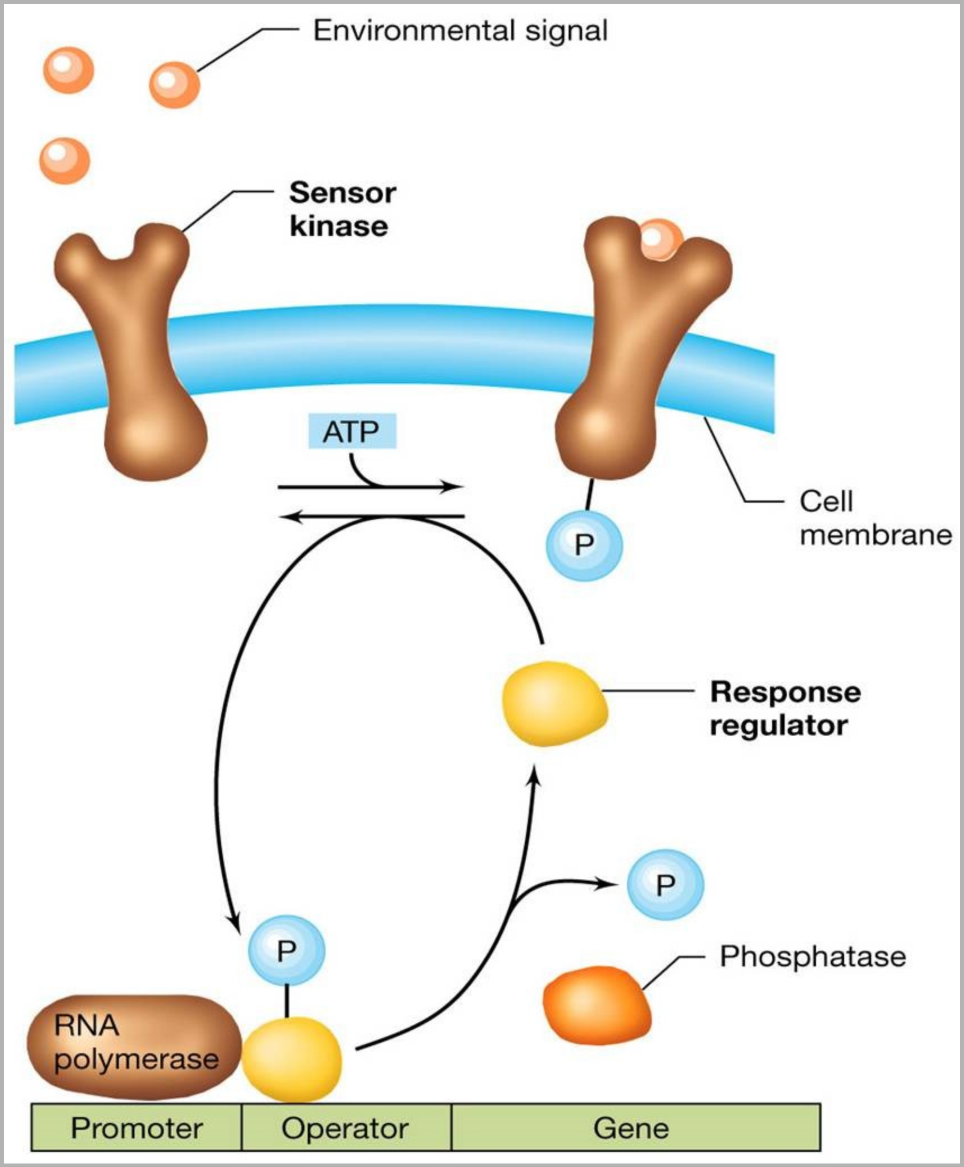
(a)

Threonine — Met-Lys-Arg-Ile-Ser-Thr-Thr-Ile-Thr-Thr-Thr-Ile-Thr-Ile-Thr-Thr-Gly-Asn-Gly-Ala-Gly

Histidine — Met-Thr-Arg-Val-Gln-Phe-Lys-His-His-His-His-His-His-His-Pro-Asp

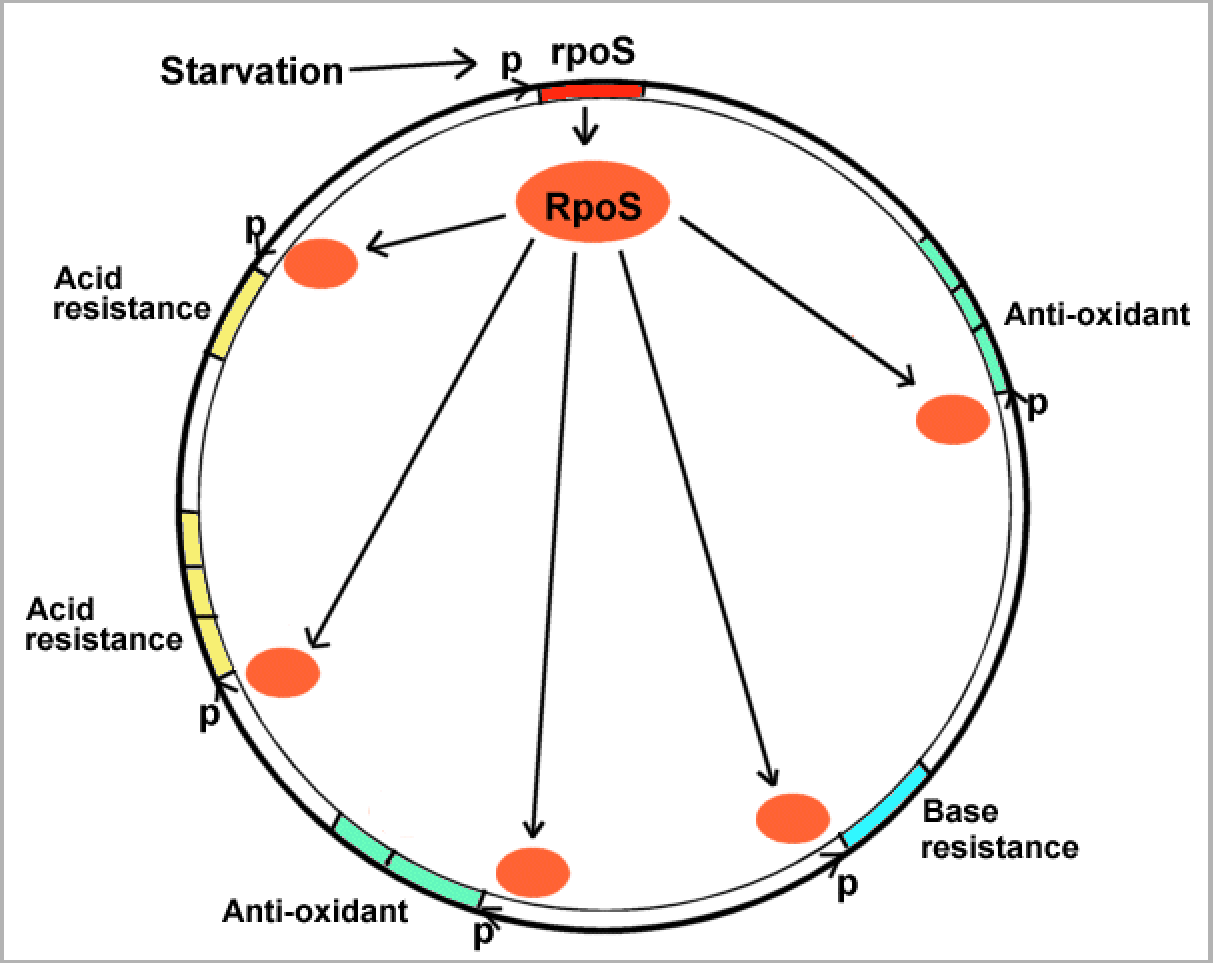
Phenylalanine — Met-Lys-His-Ile-Pro-Phe-Phe-Phe-Ala-Phe-Phe-Phe-Thr-Phe-Pro

(b)



Dvokomponentni sistem uravnavanja z receptorsko kinazo in odzivnim regulatorjem.
V opisanem primeru je fosforilirani regulator represor.

Regulon je skupina operonov, ki jih uravnava isti regulator.
To je možno, ker imajo enak promotor /operator.



RpoS je posebni faktor sigma, ki omogoča transkripcijo stresnih genov.