Izražanje genov

Izražanje genov je kombinacija procesov, ki vključuje prepisovanje gena v mRNA, njeno procesirjanje in prevajanje v protein.

Vsi geni se ne izražajo v enaki meri v vseh celicah in pod vsemi pogoji.

Dve vrsti genov

- **vzdrževalni geni** – se **konstitutivno** izražajo v približno enakih količinah

- **regulirani geni** – se izražajo kot odziv na določen signal

  inducibilni geni – izražanje se **inducira** (sproži/poveča) kot odziv na signal

  represibilni geni – izražanje se reprimira (zmanjša/prekine) kot odziv na signal

Izražanje genov

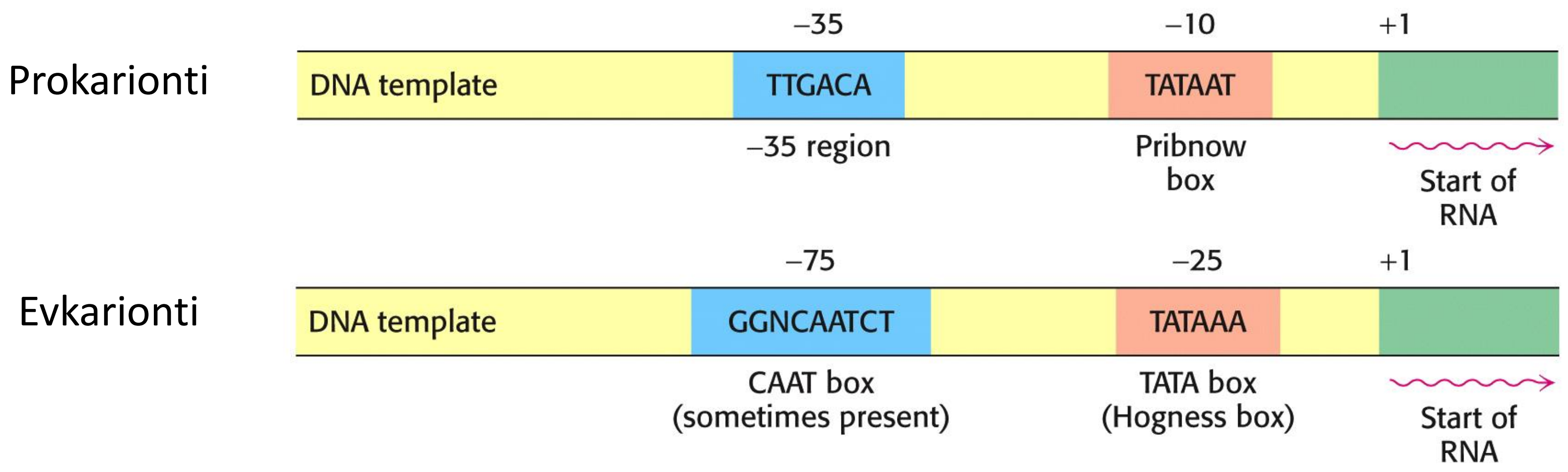
TABLE 31.1 Highly expressed protein-encoding genes of the pancreas and liver (as percentage of total mRNA pool)

Rank	Pancreas	%	Liver	%
1	Procarboxypeptidase A1	7.6	Albumin	3.5
2	Pancreatic trypsinogen 2	5.5	Apolipoprotein A-I	2.8
3	Chymotrypsinogen	4.4	Apolipoprotein C-I	2.5
4	Pancreatic trypsin 1	3.7	Apolipoprotein C-III	2.1
5	Elastase IIIB	2.4	ATPase 6/8	1.5
6	Protease E	1.9	Cytochrome oxidase 3	1.1
7	Pancreatic lipase	1.9	Cytochrome oxidase 2	1.1
8	Procarboxypeptidase B	1.7	α-1-Antitrypsin	1.0
9	Pancreatic amylase	1.7	Cytochrome oxidase 1	0.9
10	Bile salt-stimulated lipase	1.4	Apolipoprotein E	0.9

Sources: Data for pancreas from V. E. Velculescu, L. Zhang, B. Vogelstein, and K. W. Kinzler, Serial analysis of gene expression, *Science* 270(1995):484–487. Data for liver from T. Yamashita, S. Hashimoto, S. Kaneko, S. Nagai, N. Toyoda, T. Suzuki, K. Kobayashi, and K. Matsushima, Comprehensive gene expression profile of a normal human liver, *Biochem. Biophys. Res. Commun.* 269(2000):110–116.

Regulacija izražanja genov

Regulatorni elementi so proteini, hormoni in metaboliti, ki ponavadi interagirajo s promotersko regijo in regulirajo delovanje RNA polimeraze.



Pri *E. coli* poznamo tri glavne tipe regulatornih elementov:

- Specifičnostni faktorji – σ podenote RNA polimeraze
- Aktivatorji – pospešijo vezavo RNA-polimeraze na promoter
- Represorji – preprečujejo vezavo RNA-polimeraze na promoter

Specifičnostni faktorji

σ podenota RNA polimeraze je tista, ki prepozna promoter in omogoča vezavoholoencima na DNA. V E. coli poznamo sedem različnih σ podenot, ki prepoznajo različne promoterje. Večino promoterjev *E. coli* prepozna podenota σ^{70}:

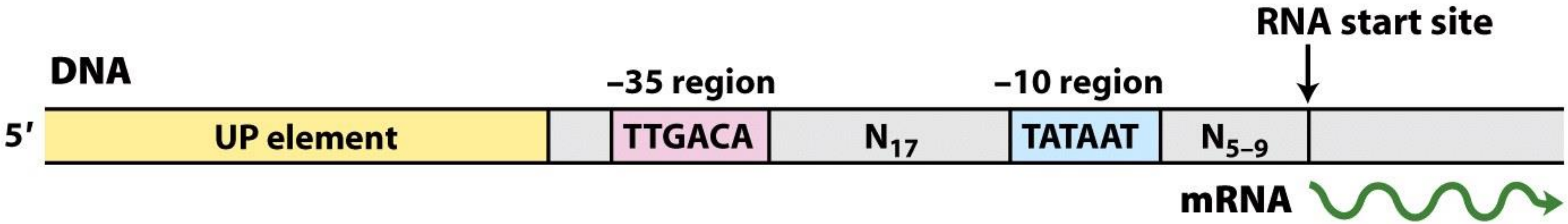


Figure 28-2
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Pri toplotnem šoku σ^{70} podenoto zamenja σ^{32} podenota, ki sproži prepisovanje genov za proteine toplotnega šoka, ki imajo drugačen promoter:

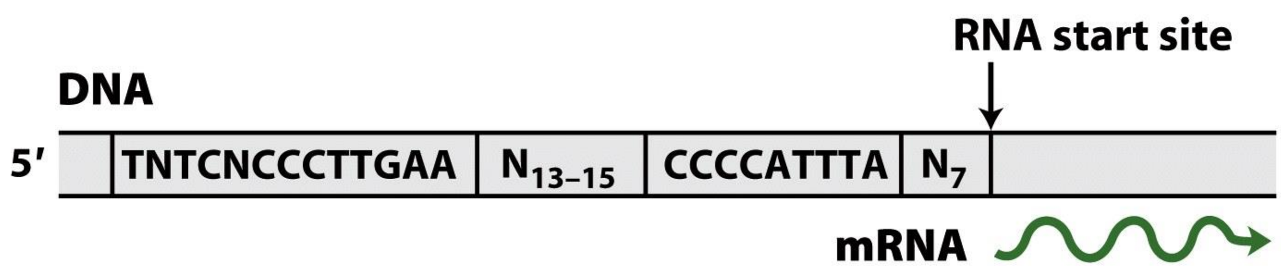

Figure 28-3
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Specifičnostni faktorji

TABLE 26–1	The Seven Subunits of *Escherichia coli*			
σ subunit	K_d (nM)	Molecules/cell*	Holoenzyme ratio (%)*	Function
s^{70}	0.26	700	78	Housekeeping
s^{54}	0.30	110	8	Modulation of cellular nitrogen levels
s^{38}	4.26	<1	0	Stationary phase genes
s^{32}	1.24	<10	0	Heat shock genes
s^{28}	0.74	370	14	Flagella and chemotaxis genes
s^{24}	2.43	<10	0	Extracytoplasmic functions; some heat shock functions
s^{18}	1.73	<1	0	Extracytoplasmic functions, including ferric citrate transport

Source: Adapted from Maeda, H., Fujita, N., & Ishihama, A. (2000) *Nucleic. Acids Res.* **28**, 3500.

Note: σ factors are widely distributed in bacteria; the number varies from a single σ factor in *Mycoplasma genitalium* to 63 distinct σ factors in *Streptomyces coelicolor.*

*Approximate number of each σ subunit per cell and the fraction of RNA polymerase holoenzyme complexed with each σ subunit during exponential growth. The numbers change as growth conditions change. The fraction of RNA polymerase complexed with each σ subunit reflects both the amount of the particular subunit and its affinity for the enzyme.

Table 26-1
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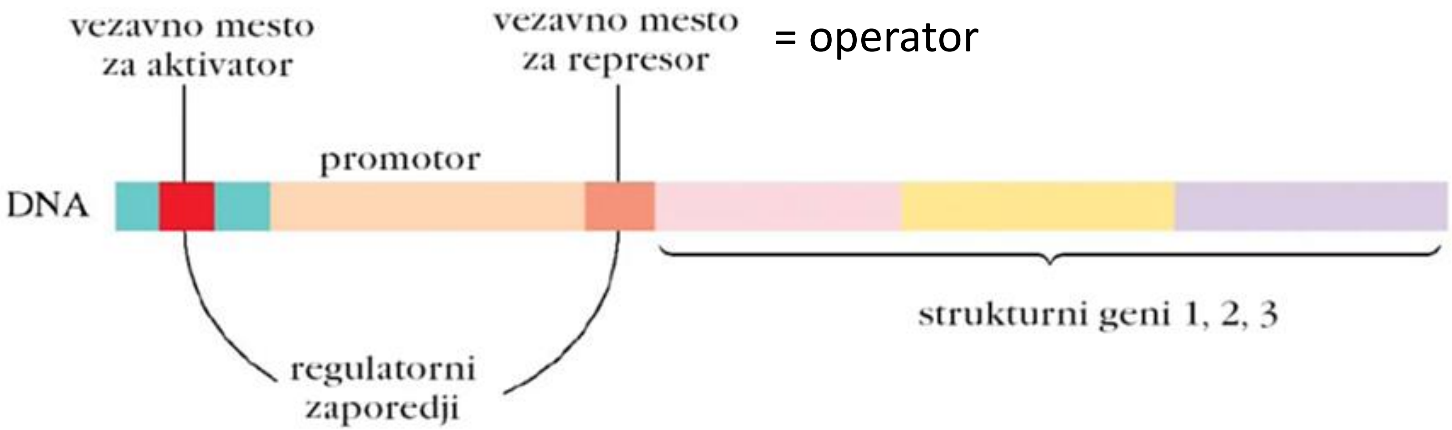
Operonski model regulacije

Regulacija izražanja genov z aktivatorji in represorji v *E. coli* poteka po t.i. operonskem modelu. Splošna shema bakterijskega operona:



Operonski model regulacije

Možni so štirje različni mehanizmi uravnavanja izražanja kot odziv na signal iz okolja:

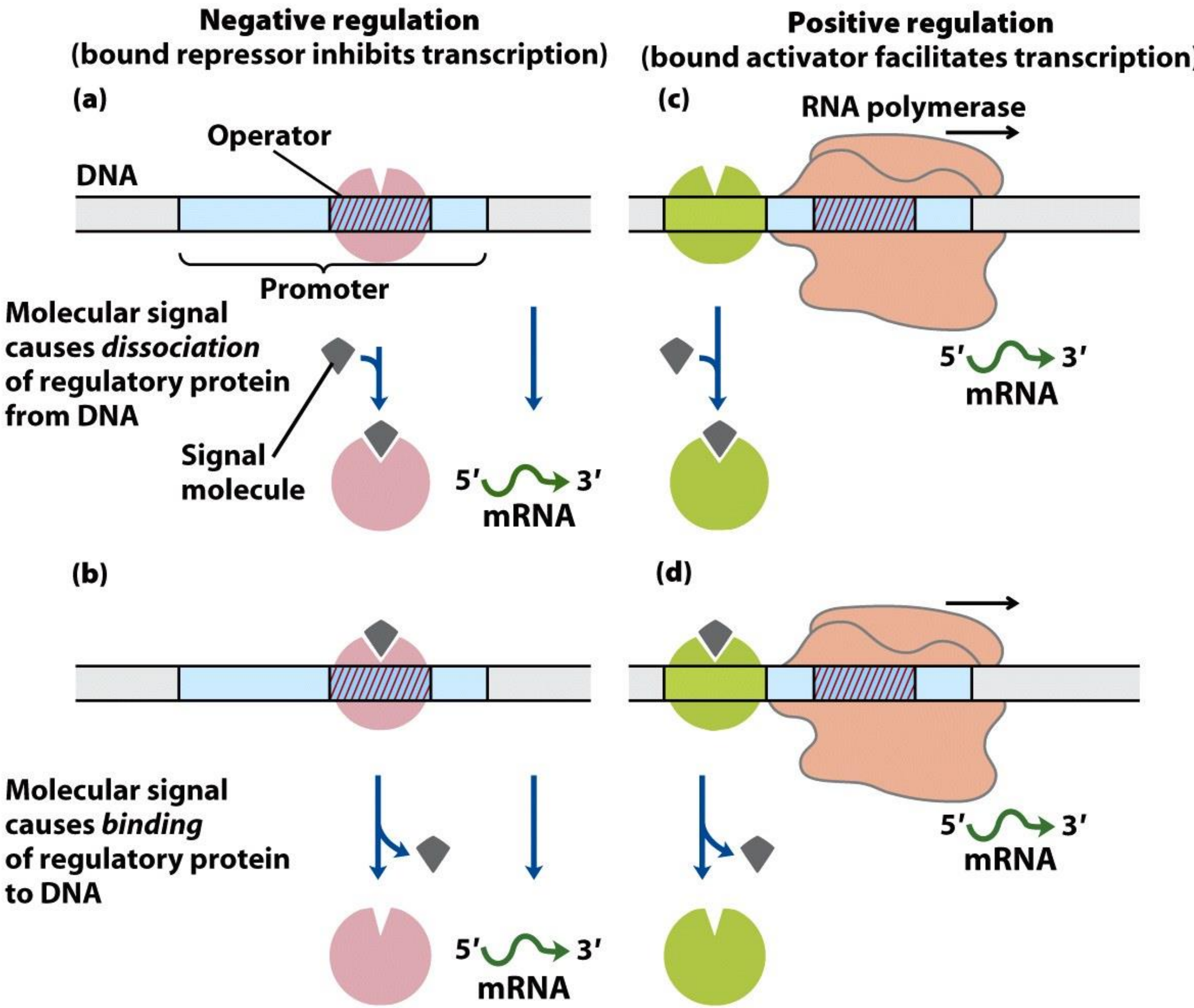


Figure 28-4
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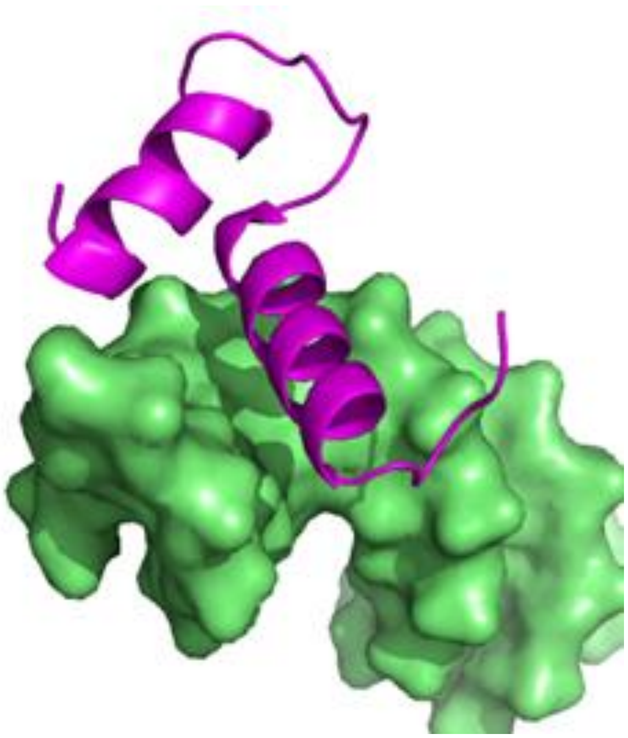
Motivi vezave regulatorjev na DNA

Regulatorni proteini se na DNA vežejo preko enega izmed DNA-vezavnih motivov. Ponavadi se vežejo kot dimeri ali v obliki tandemnih ponovitev.

1. Motiv vijačnica-zavoj-vijačnica (HTH)

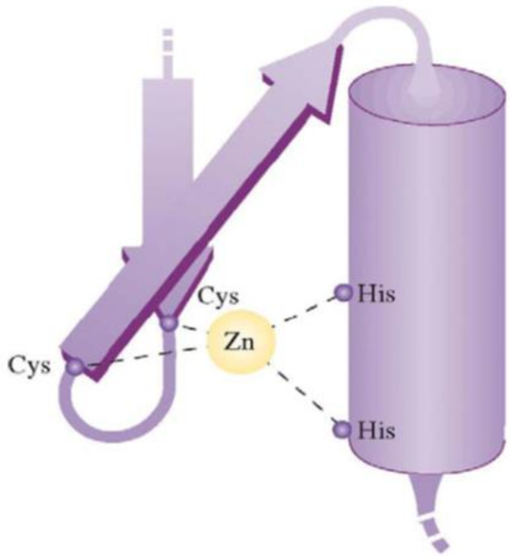
vezava v veliki žleb

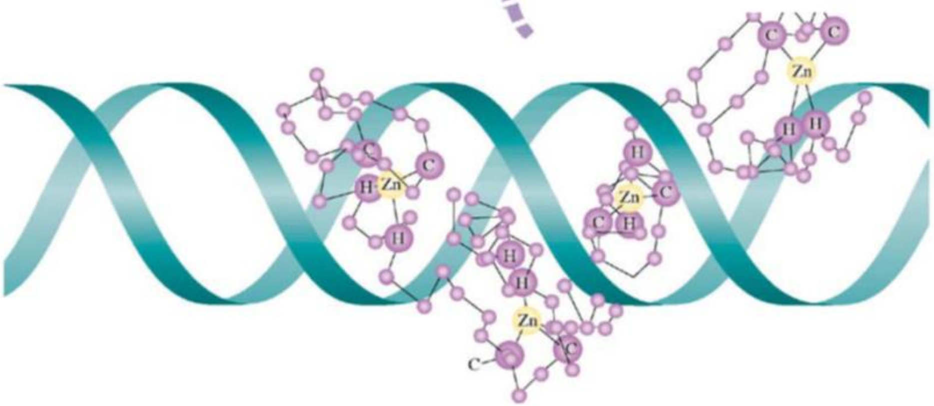


Motivi vezave regulatorjev na DNA

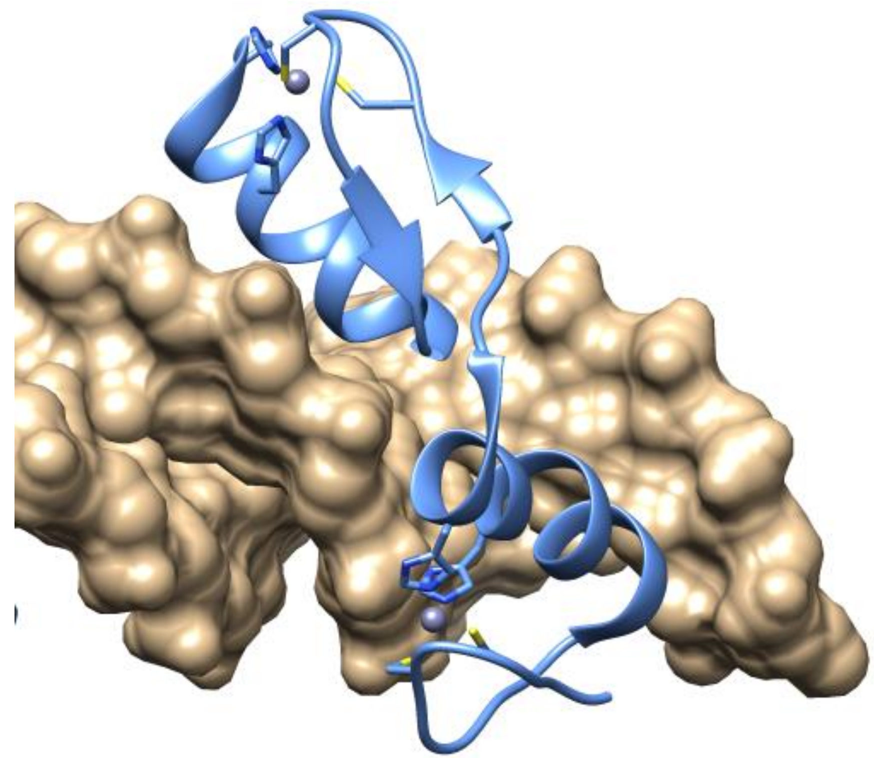
Regulatorni proteini se na DNA vežejo preko enega izmed DNA-vezavnih motivov. Ponavadi se vežejo kot dimeri ali v obliki tandemnih ponovitev.

1. Motiv cinkovega prsta





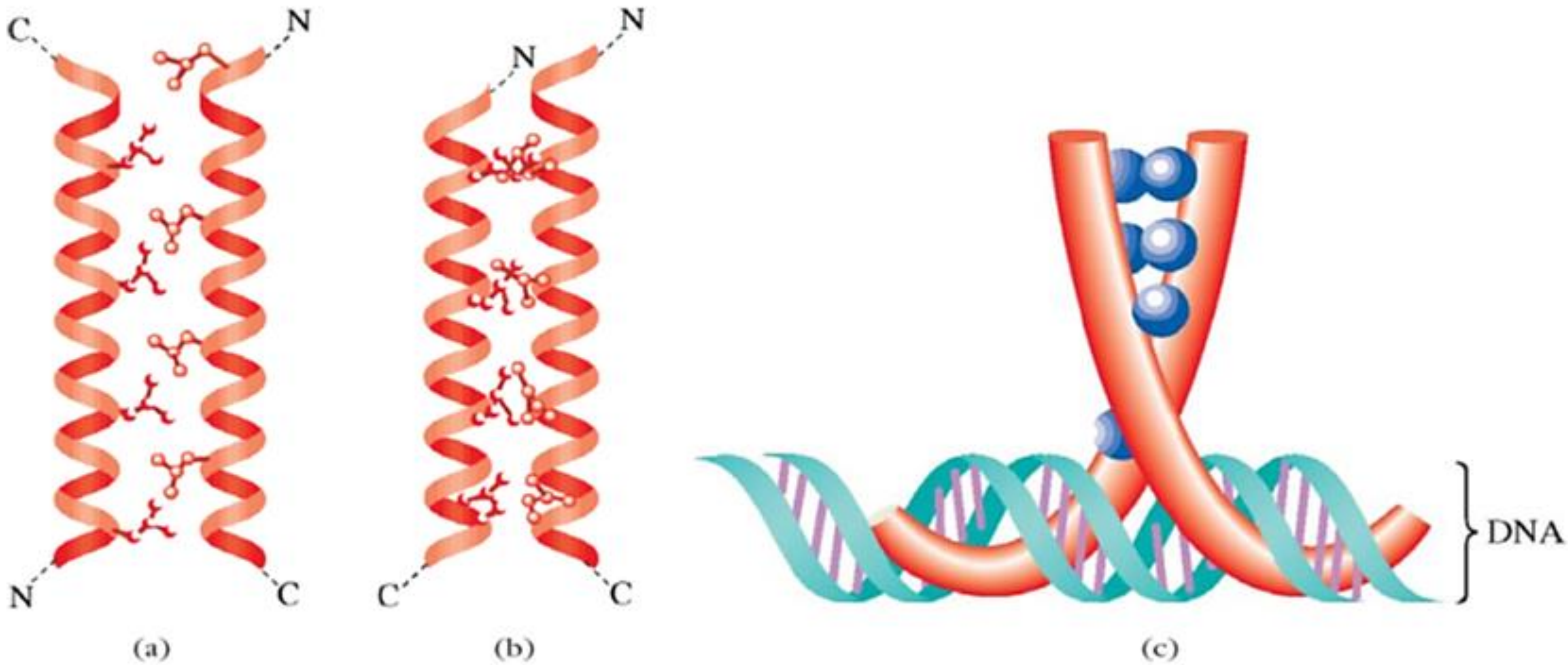
vezava v veliki žleb



Motivi vezave regulatorjev na DNA

Regulatorni proteini se na DNA vežejo preko enega izmed DNA-vezavnih motivov. Ponavadi se vežejo kot dimeri ali v obliki tandemnih ponovitev.

1. Motiv levcinske zadrge

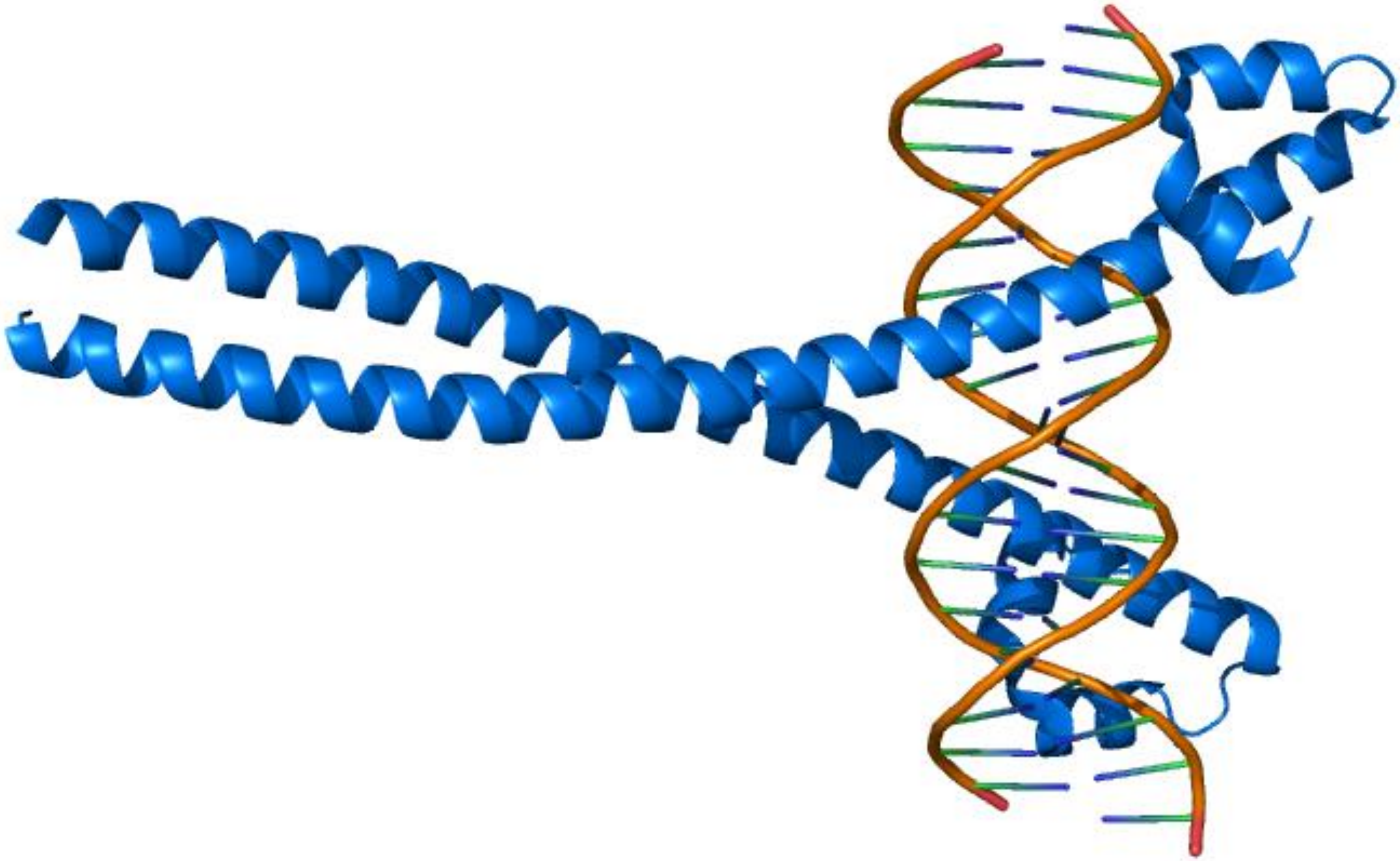


vezava v veliki žleb

Motivi vezave regulatorjev na DNA

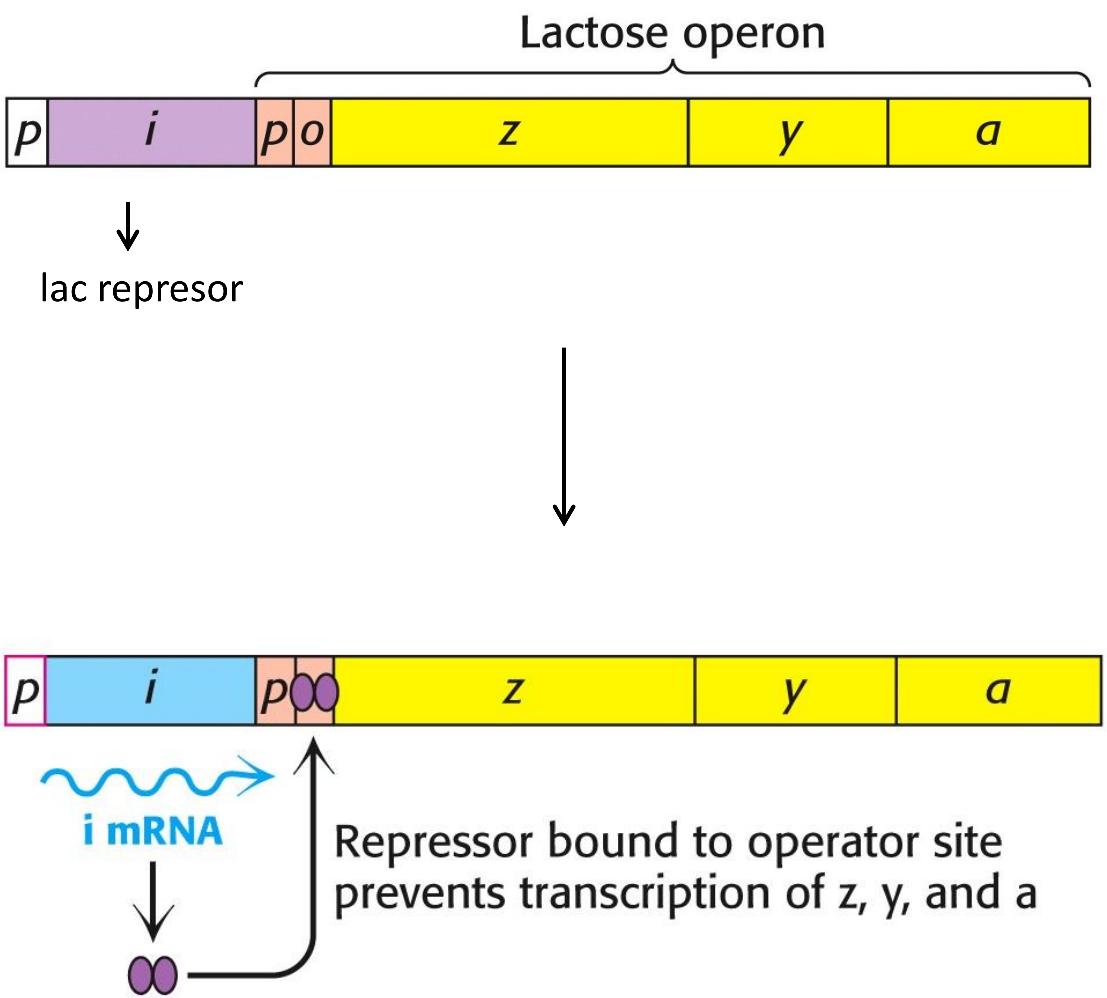
Regulatorni proteini se na DNA vežejo preko enega izmed DNA-vezavnih motivov. Ponavadi se vežejo kot dimeri ali v obliki tandemnih ponovitev.

1. Motiv levcinske zadrge



Uravnavanje *lac* operona

lac operon *E. coli* kodira za tri proteine, potrebne za vnos in razgradnjo glukoze v celici.



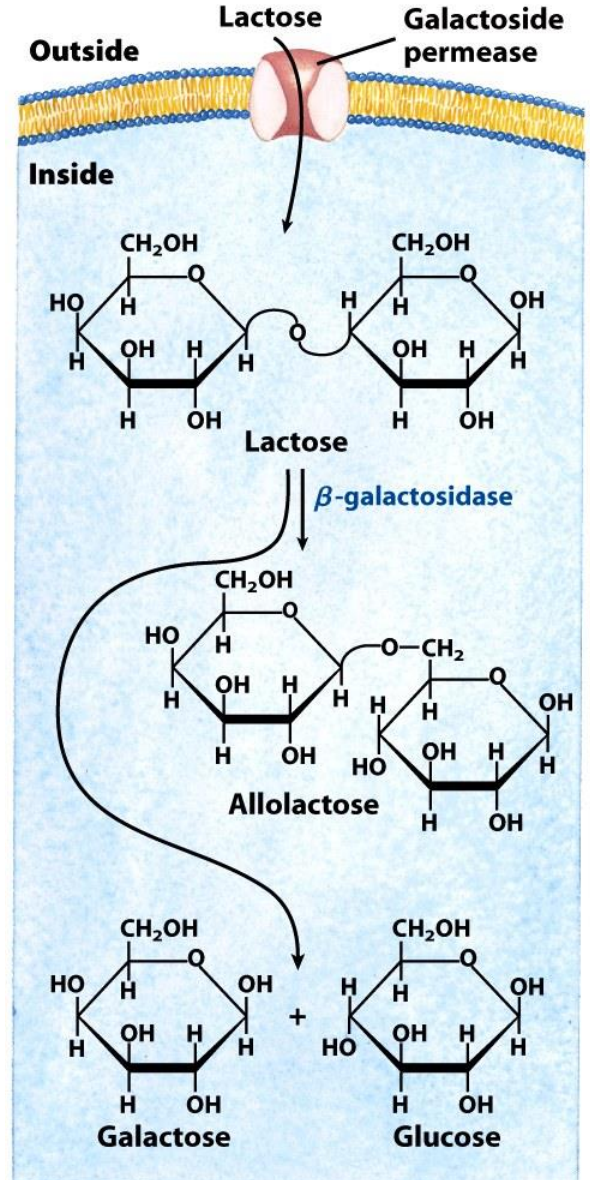


Figure 28-6
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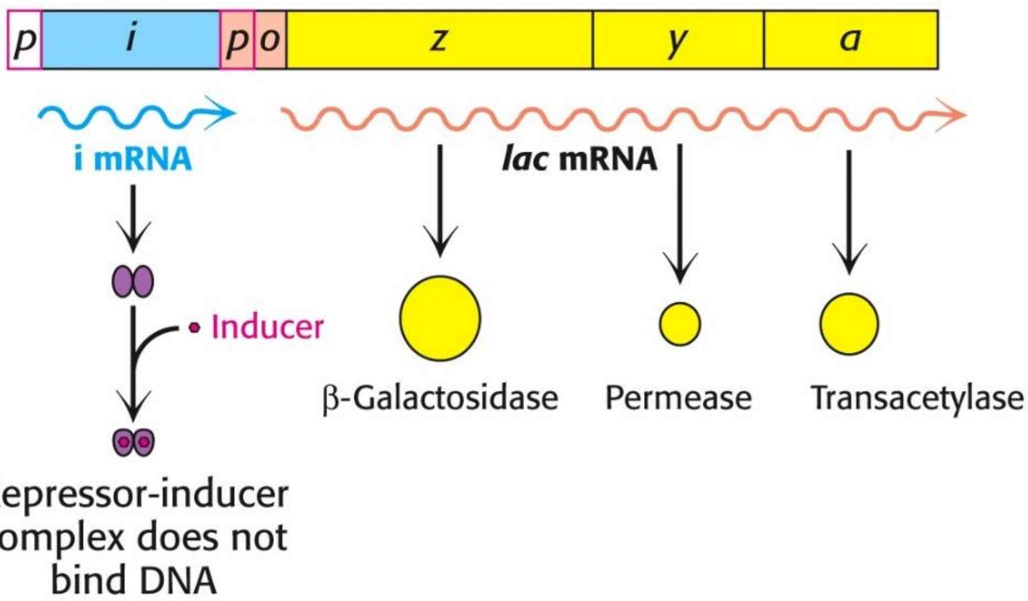
Uravnavanje *lac* operona

lac operon *E. coli* kodira za tri proteine, potrebne za vnos in razgradnjo glukoze v celici.

Ob prisotnosti induktorja, le-ta inaktivira *lac* represor in RNApol začne prepisovati *lac* operon.



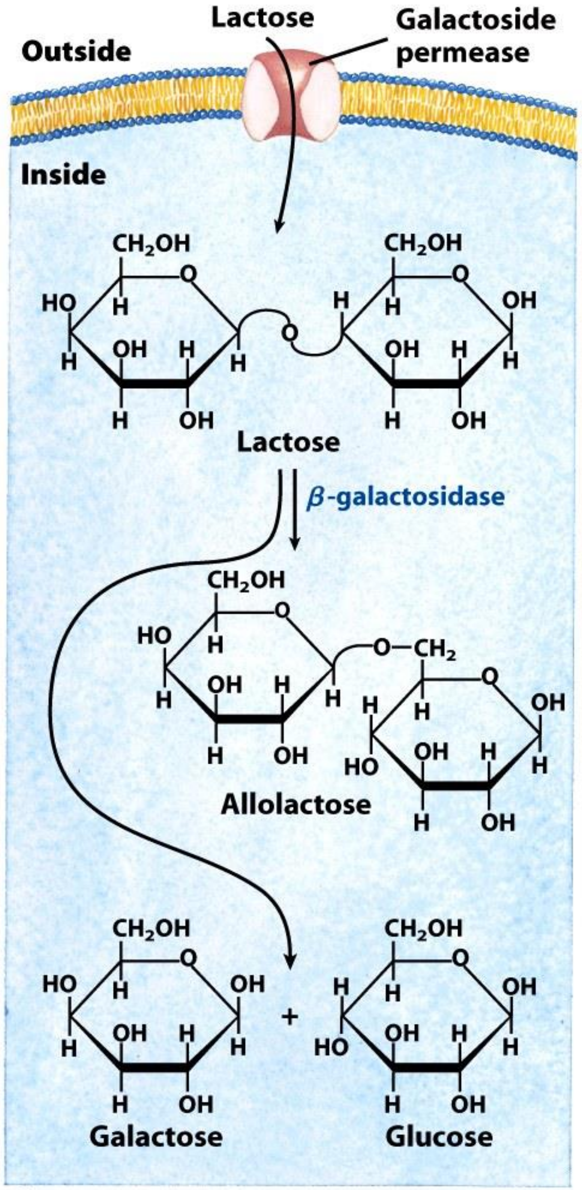


Figure 28-6
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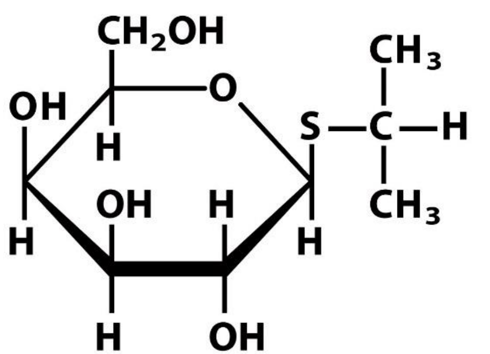
Uravnavanje *lac* operona

lac operon *E. coli* kodira za tri proteine, potrebne za vnos in razgradnjo glukoze v celici.

<u>Induktorji</u>:

- alolaktoza
- IPTG



Isopropyl-β-D-thiogalactoside (IPTG)

Unnumbered 28 p1121
Lehninger Principles of Biochemistry, Fifth Edition

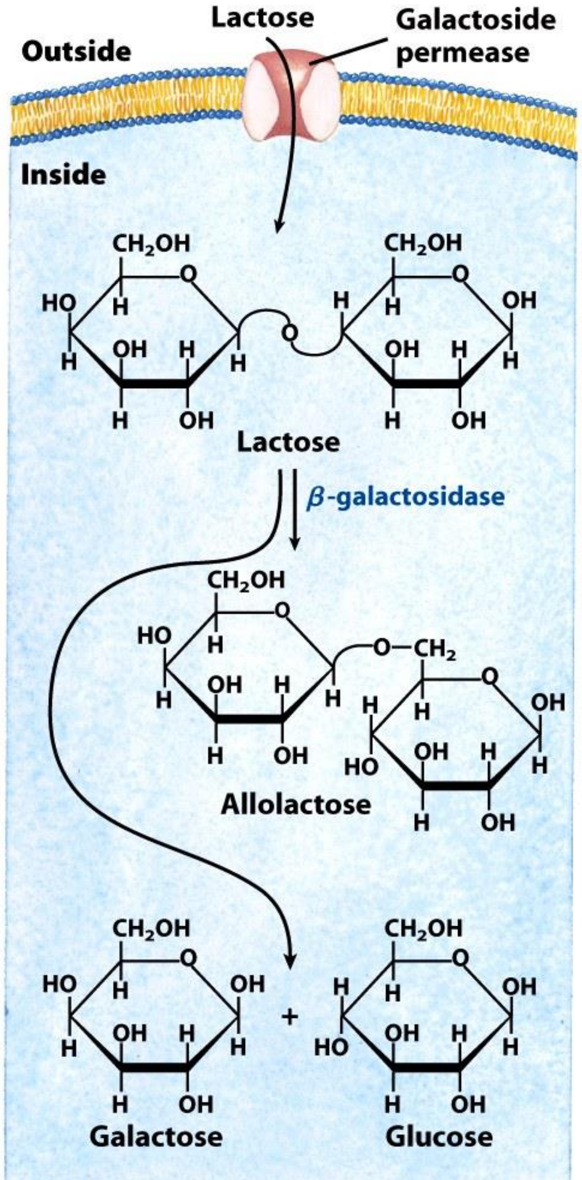


Figure 28-6
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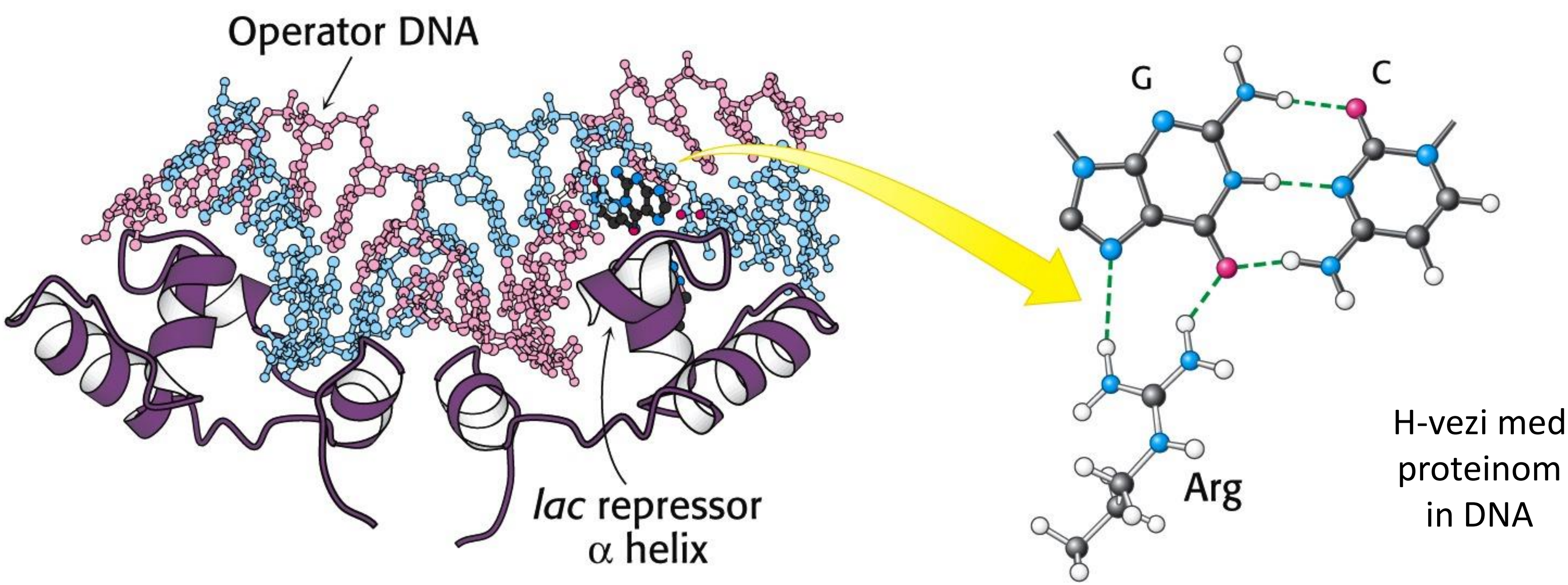
Struktura *lac* represorja

Operatorska regija skoraj popolnoma palindromna:

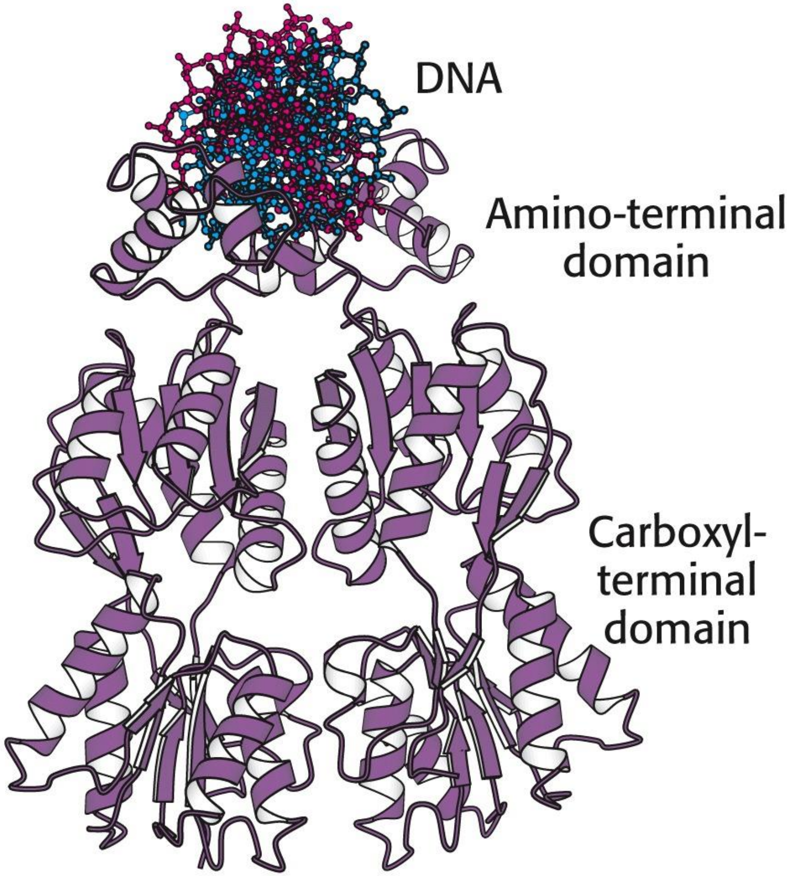
5′-...TGTGTGGAATTGTGAGCGGATAACAATTTCACACA...3′
3′-...ACACACCTTAACACTCGCCTAATGTTAAAGTGTGT...5′

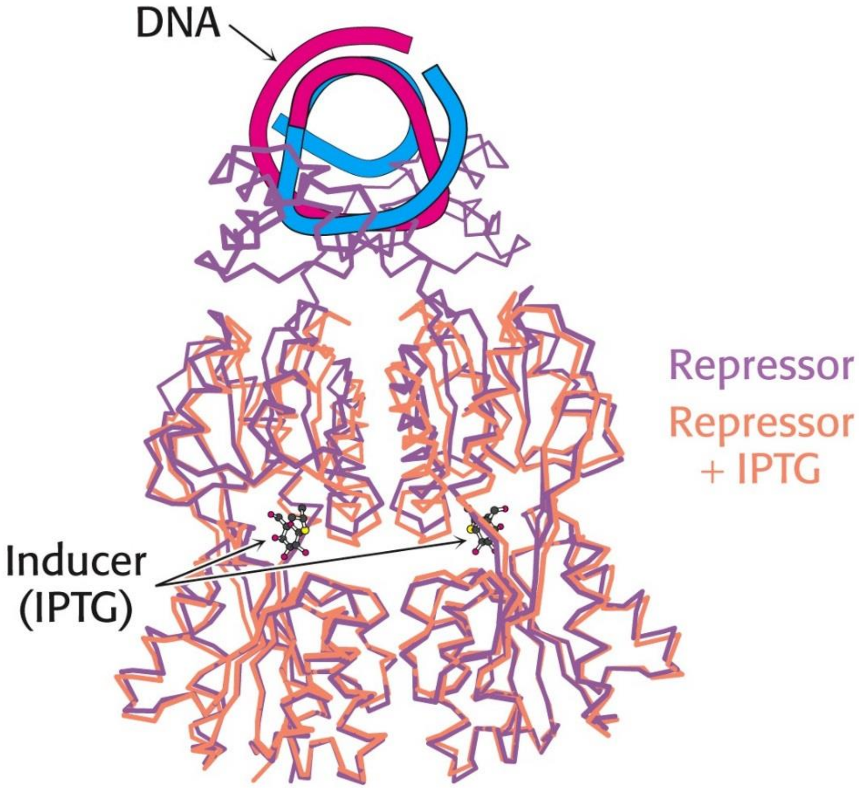
Dimer *lac* represorja z operatorsko regijo interagira preko dveh motivov HTH.



Struktura *lac* represorja

lac represor je večdomenski protein. Induktor se veže med N- in C-končno domeno in povzroči konformacijsko spremembo, ki onemogoči vezavo na DNA.





Struktura *lac* represorja

Dejanska oblika lac represorja je tetramer (dimer dimerov).. Kot tak lahko interagira z dvema regijama DNA hkrati. Dejansko so na *lac* operonu prisotne tri kopije *lac* operatorja. Represor interagira hkrati z glavnim operatorjem O1 in enim od pomožnih operatorjev O2 ali O3. Afiniteta do slednjih je nižja kot do O1.

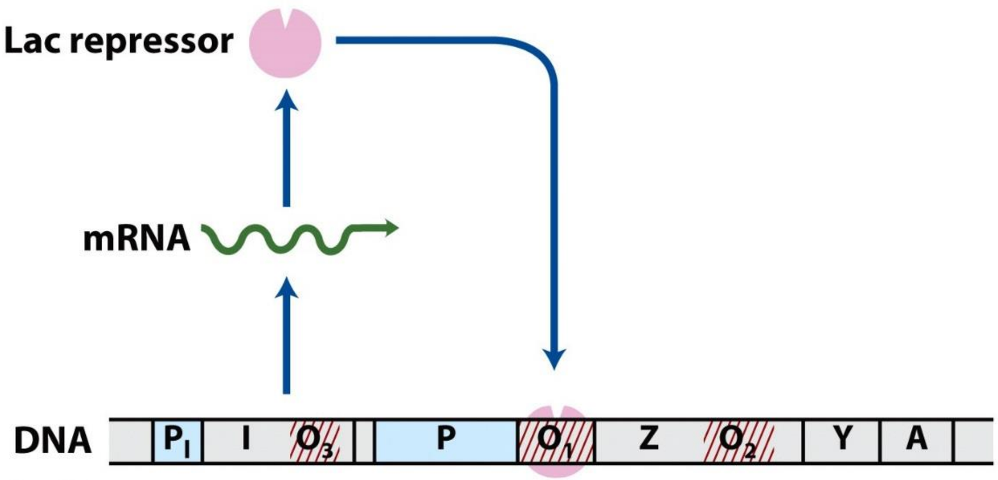


Figure 28-7a
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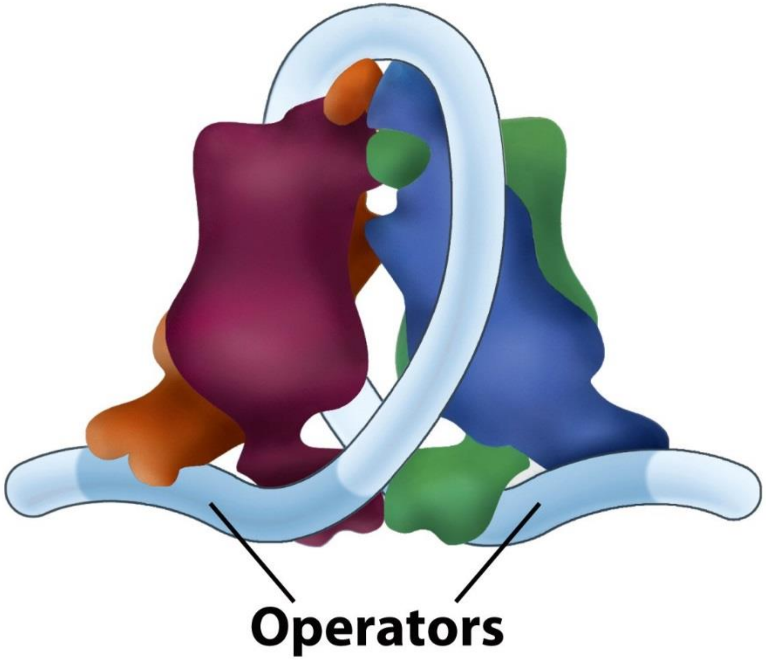

Figure 28-7b
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Struktura *lac* represorja

Tetramer *lac* represorja

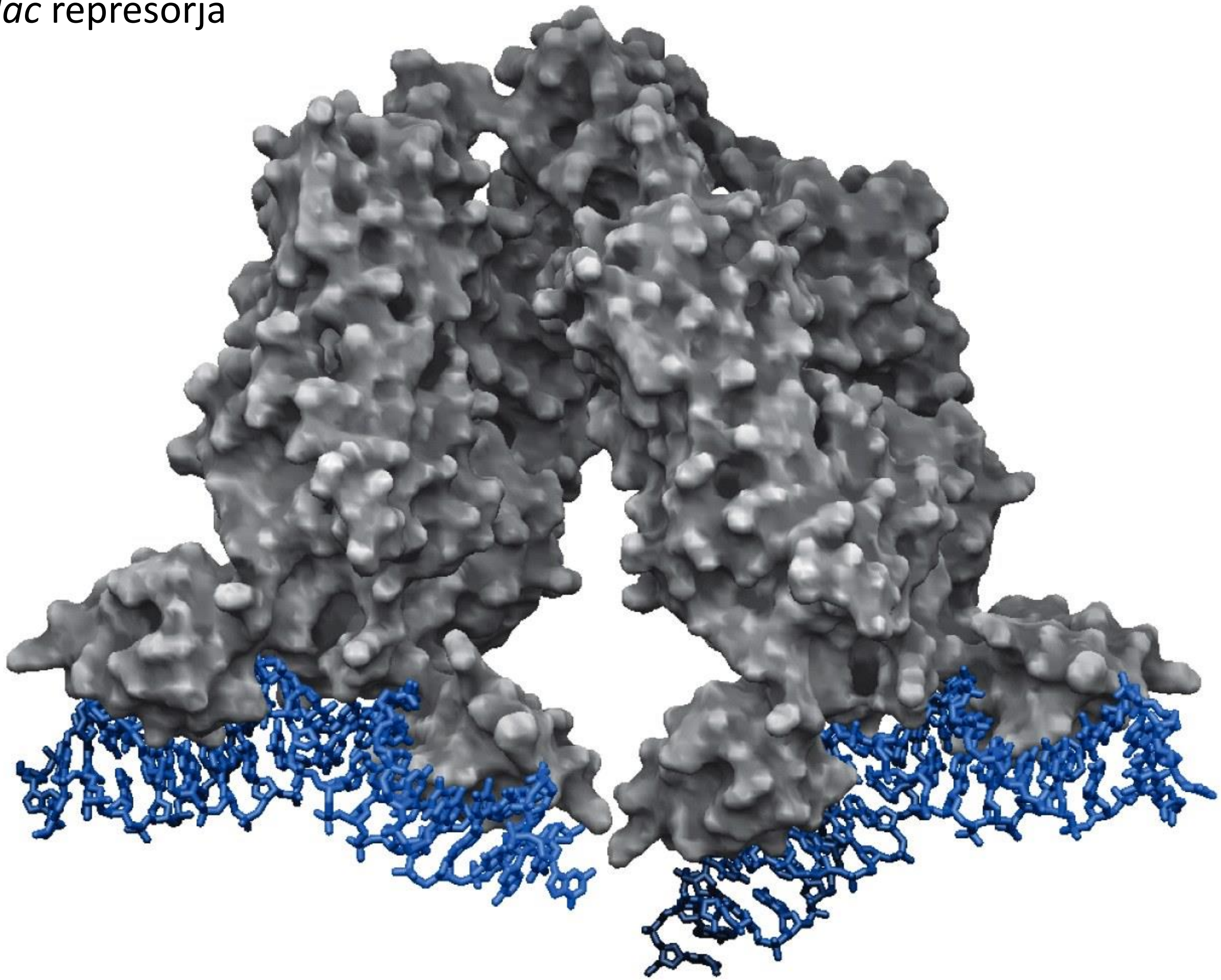

Figure 28-7c
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Katabolna represija

Poleg *lac* represorja je *lac* operon reguliran še z enim regulatorjem – CRP, ki se odziva na koncentracijo glukoze v celici. V prisotnosti glukoze je neaktiven, ko pa se konc. glukoze zmanjša, to povzroči tvorbo cAMP, ki se veže na CRP. Kompleks CRP-cAMP se veže na lac operon in sproži njegovo prepisovanje. Mehanizmu odzivanja celice na koncentracijo glukoze pravimo katabolna represija, ker je sistem v prisotnosti glukoze inhibiran.

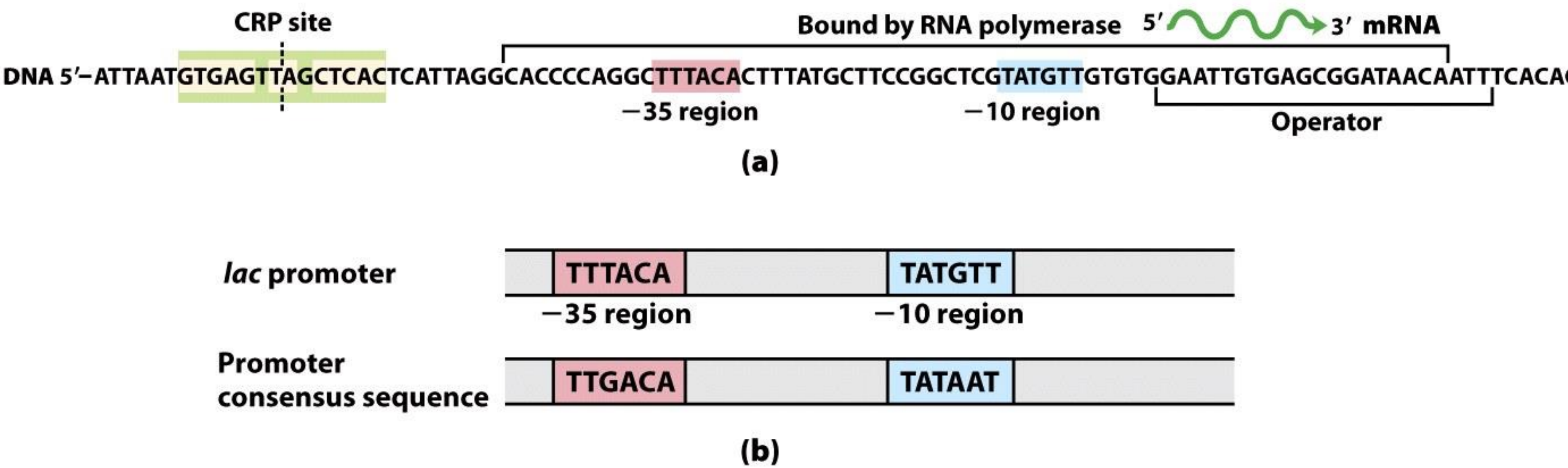

Figure 28-17
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CRP ... cAMP receptorski protein

Katabolna represija

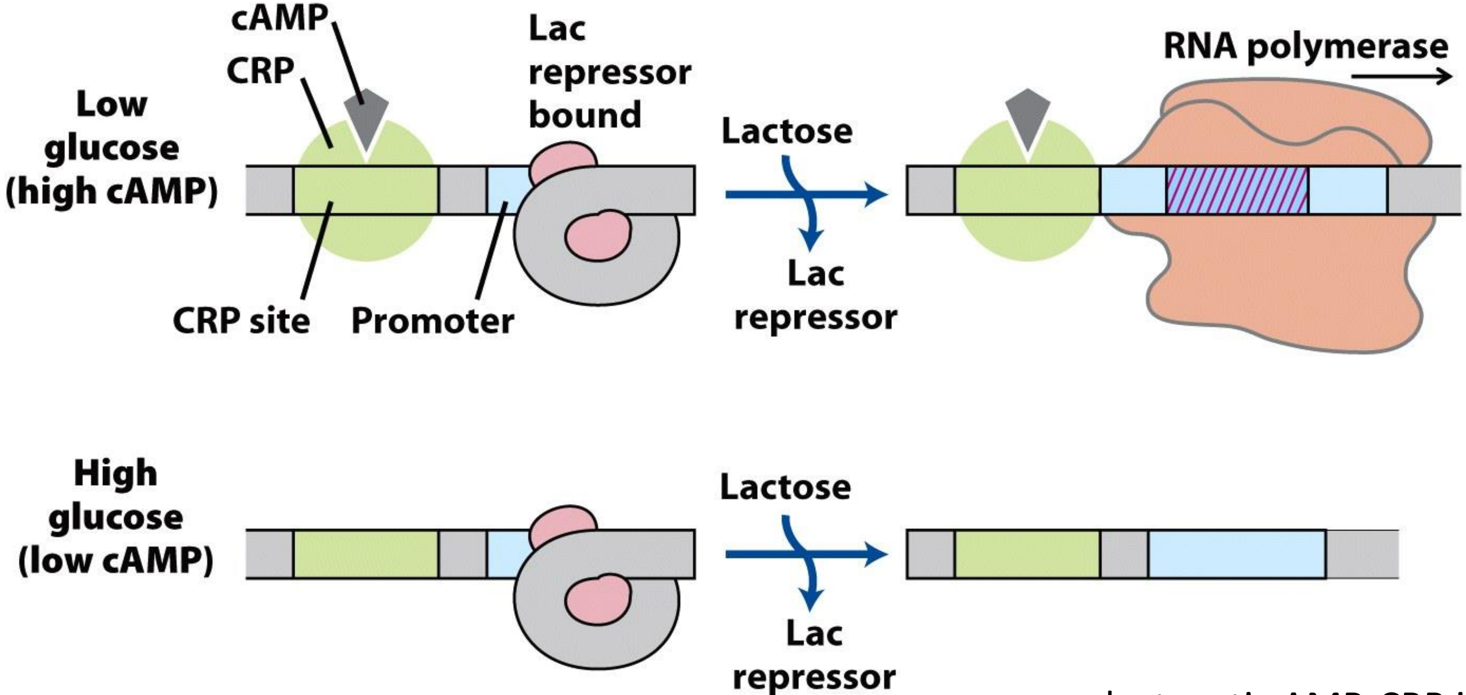

Figure 28-18
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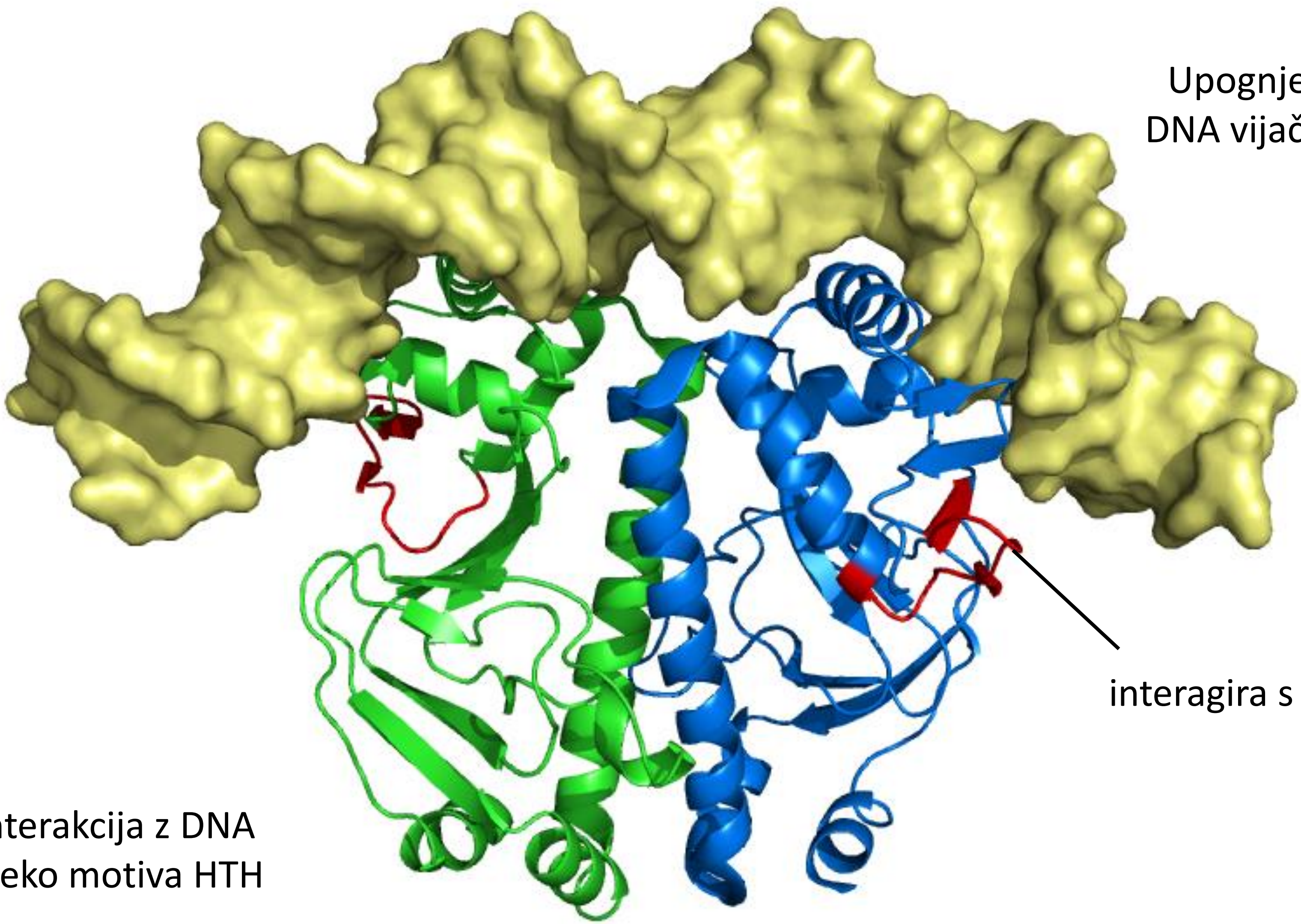
v odsotnosti cAMP-CRP je *lac* operon slabo aktiven.

Poleg lac operona CRP-cAMP regulira še druge operone, ko kodirajo za encime za prebavo sekundarnih sladkorjev. Vsem operonom pod kontrolo enega regulatorja rečemo **regulon**.

Struktura CRP



trp operon

Dva mehanizma regulacije:

1. trp represor
2. atenuator v vodilnem peptidu

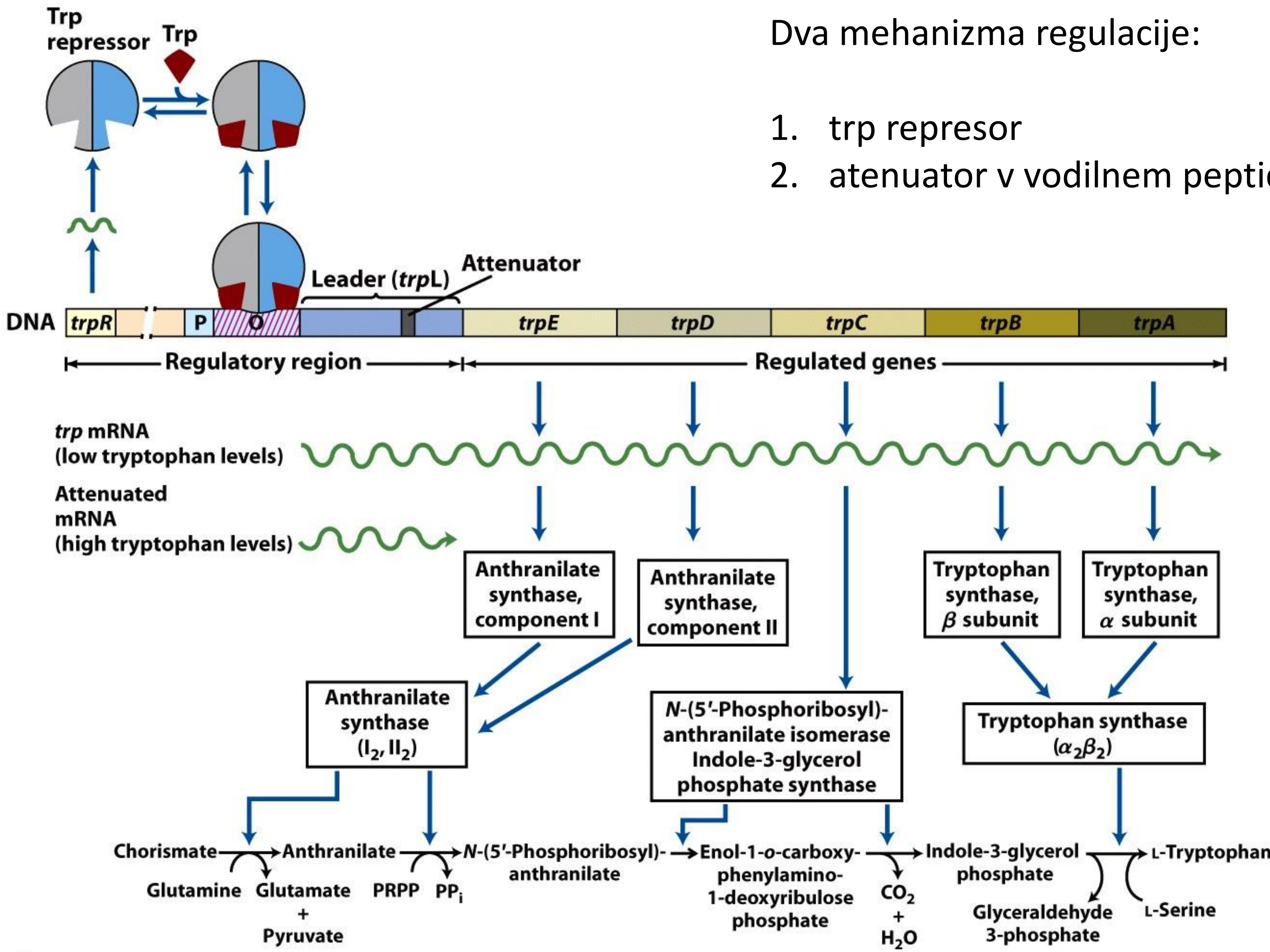


Figure 28-19
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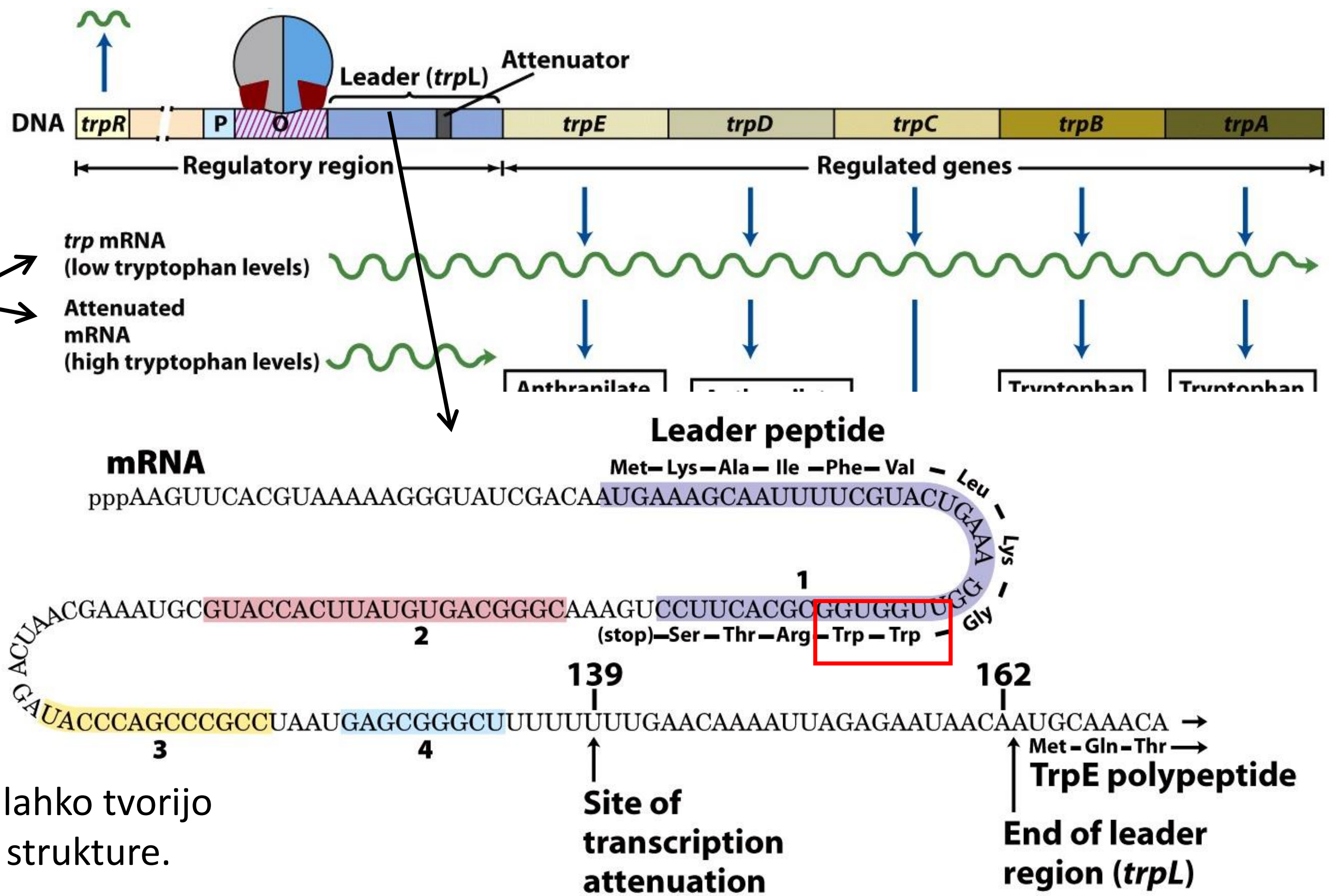
Mehanizem atenuacije

Atenuacija temelji na kotranskripcijski translaciji – takojšnjem prevajanju mRNA v protein še med prepisovanjem DNA v RNA. Gre za mehanizem, s katerim se hitrost sinteze proteinov regulira s konc. Trp v celici (razlika v hitrosti do 700x).

atenuacija



Regije 2,3 in 4 lahko tvorijo sekundarne strukture.

Figure 28-21a
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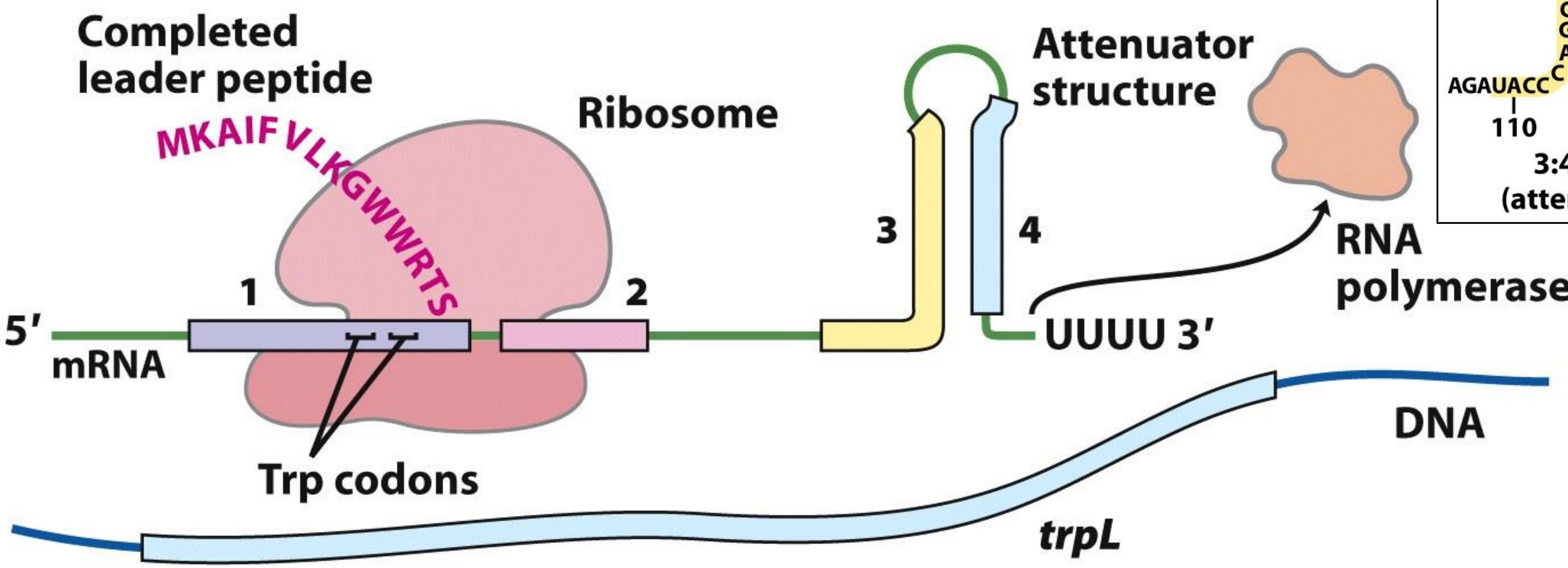
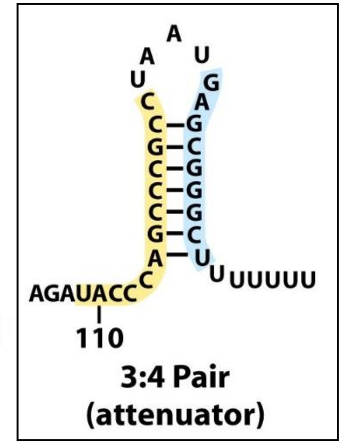
Mehanizem atenuacije

Če je triptofana dovolj, se vodilni peptid hitro prepiše, nastane atenuacijska zanka 3,4 in transkripcija se konča.





When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like attenuator structure formed by sequences 3 and 4.

Figure 28-21b part 1
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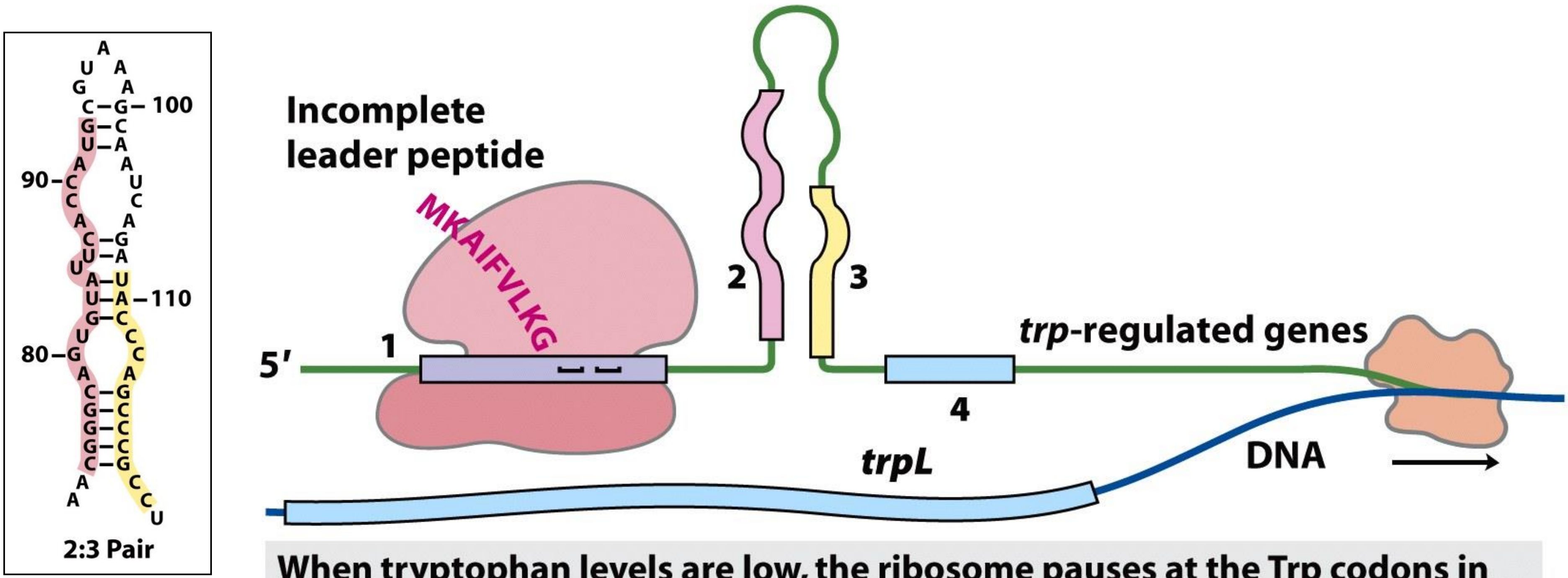
Mehanizem atenuacije

Če triptofana ni dovolj, se ribosom zaustavi na dveh zaporednih kodonih za Trp. Vzpostavi se zanka 2,3 (ki ni atenuator) in RNA polimeraza nadaljuje s prepisovanjem genov za sintezo Trp.



When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation, because sequence 3 is no longer available to form the attenuator structure with sequence 4. The 2:3 structure, unlike the 3:4 attenuator, does not prevent transcription.

Figure 28-21b part 2
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