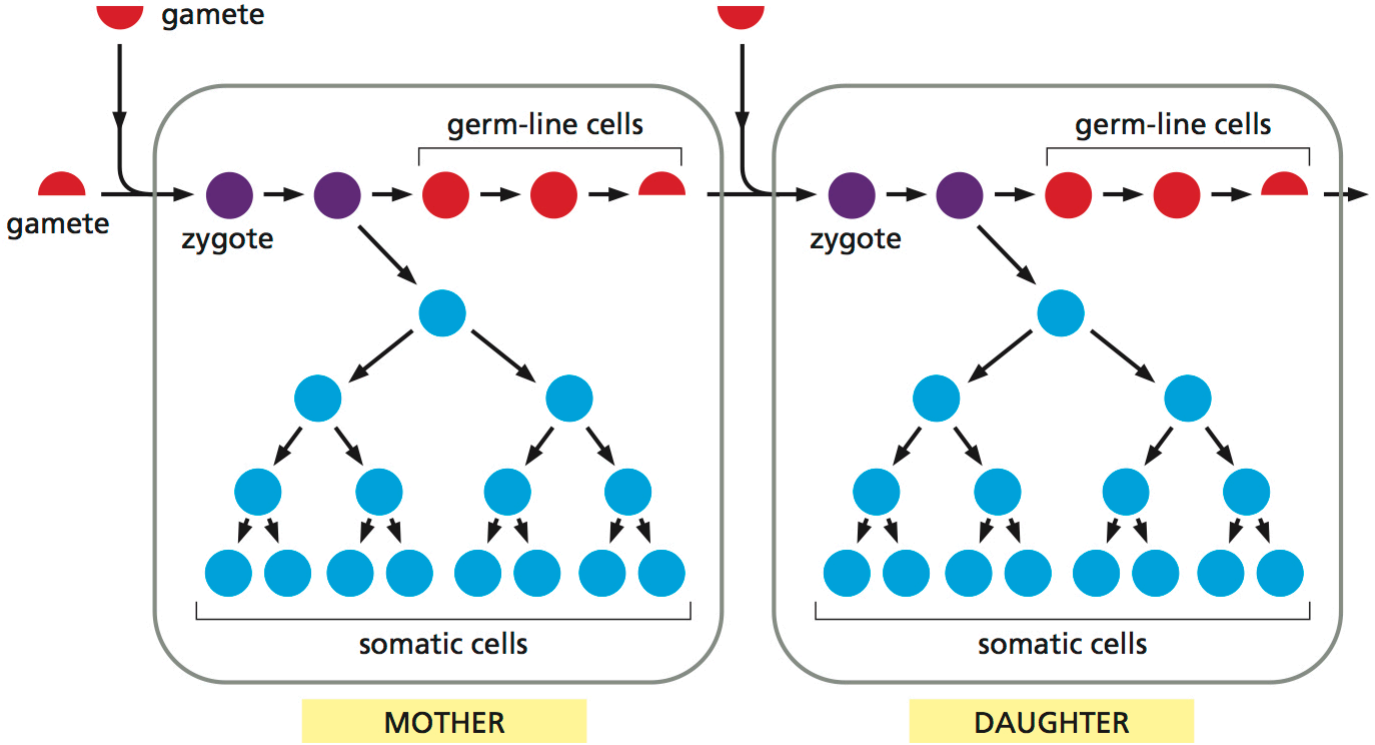
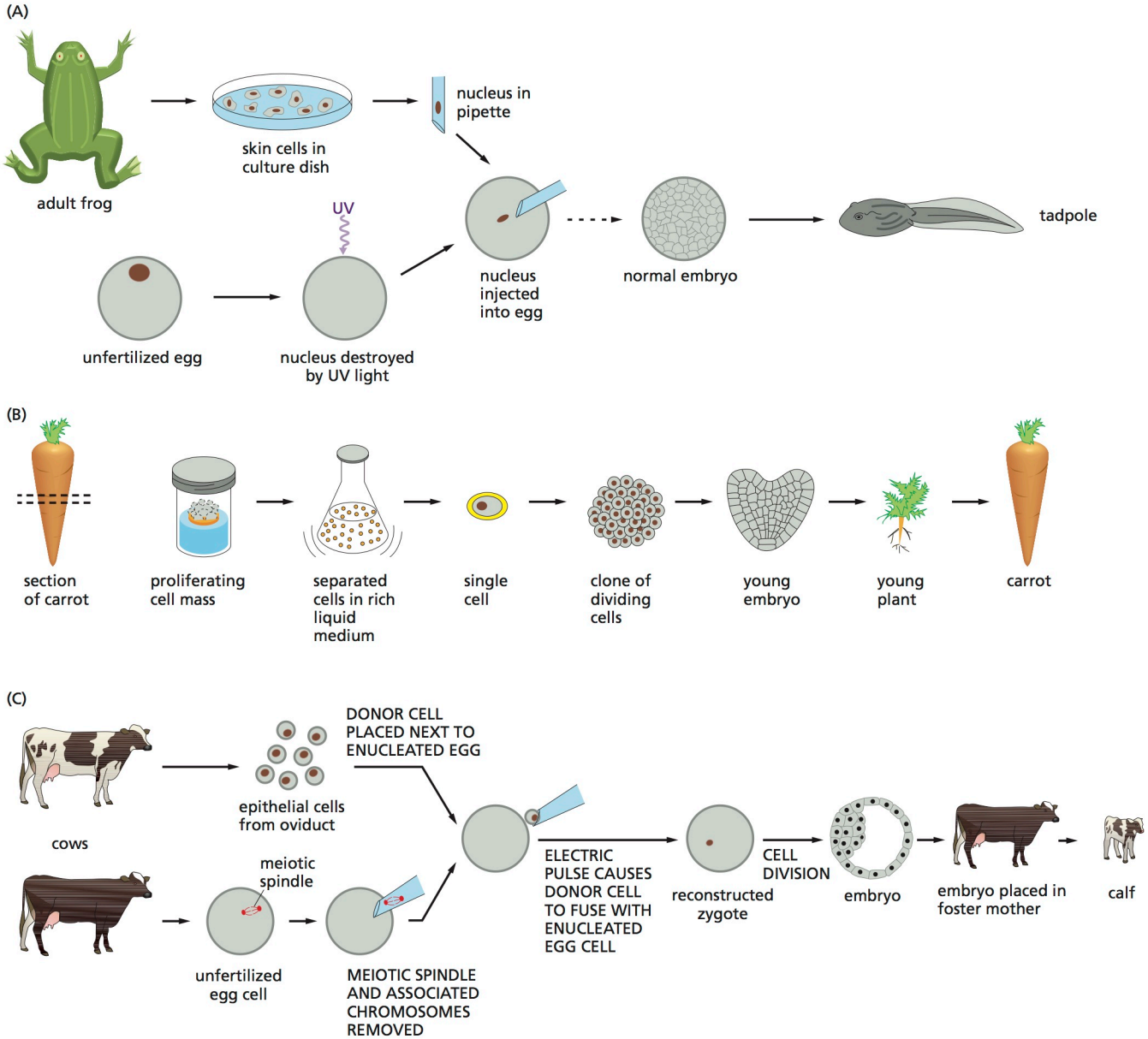
6. Princípy kontroly expresie génov

- Úrovne kontroly expresie génov.
- Operónový model. Pokusy Jacoba a Monoda.
- Negatívna a pozitívna kontrola expresie.
- Katabolická represia.
- Atenuácia.
- Regulácia životného cyklu fága lambda.
- Porovnanie kontroly génovej expresie v prokaryotických a eukaryotických bunkách.
- Kontrola na úrovni transkripcie a posttranskripčné úpravy RNA.
- Kontrola na úrovni translácie a posttranslačné úpravy proteínov.

Zárodočné a somatické bunkové línie majú odlišné funkcie

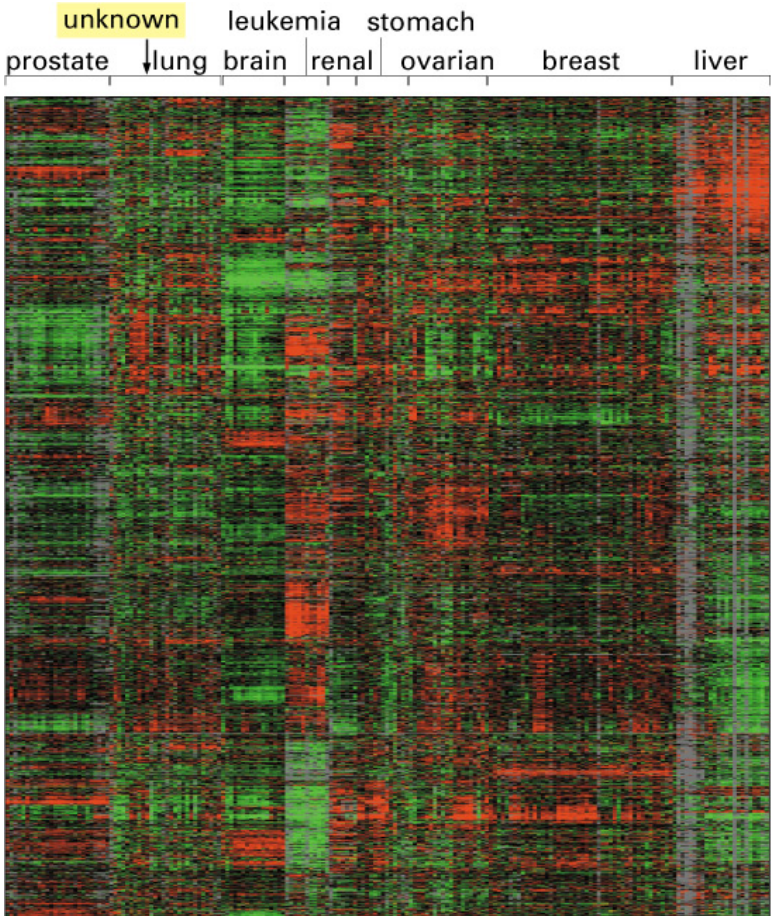


Diferencované (somatické) bunky obsahujú kompletnú genetickú informáciu pre tvorbu celého organizmu

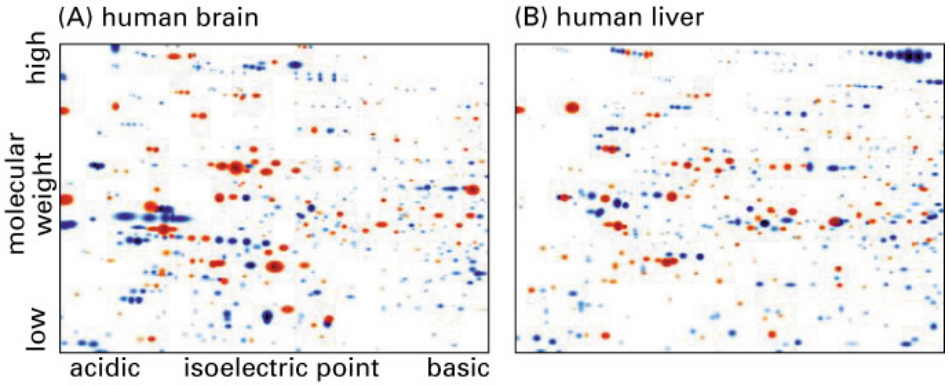


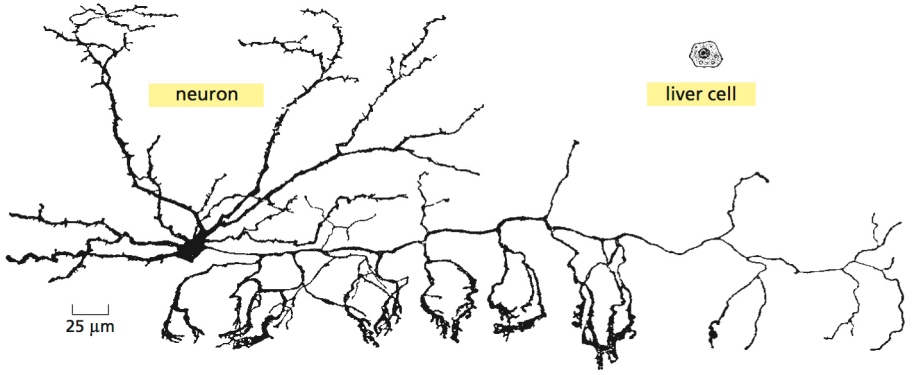
Bunky rôznych tkanív exprimujú rozdielne súbory génov

mRNA
(DNA chip/microarray)

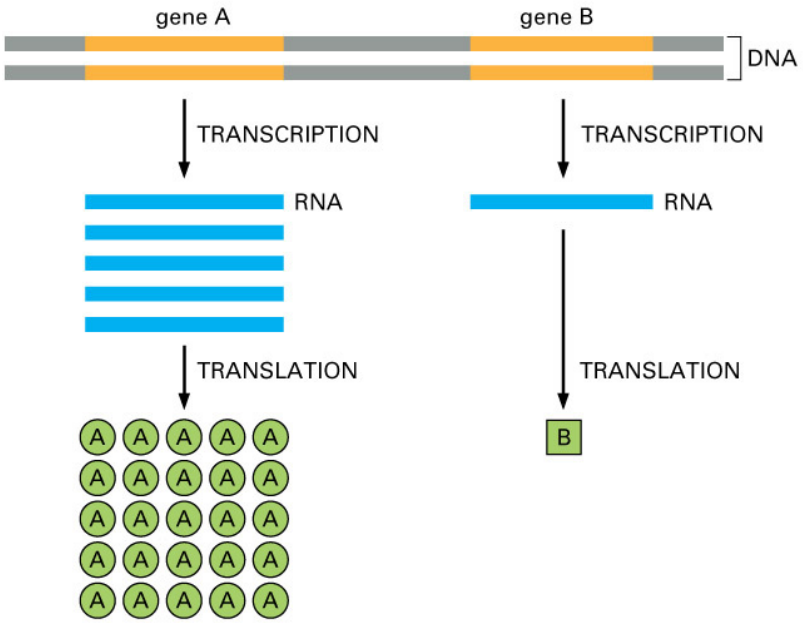


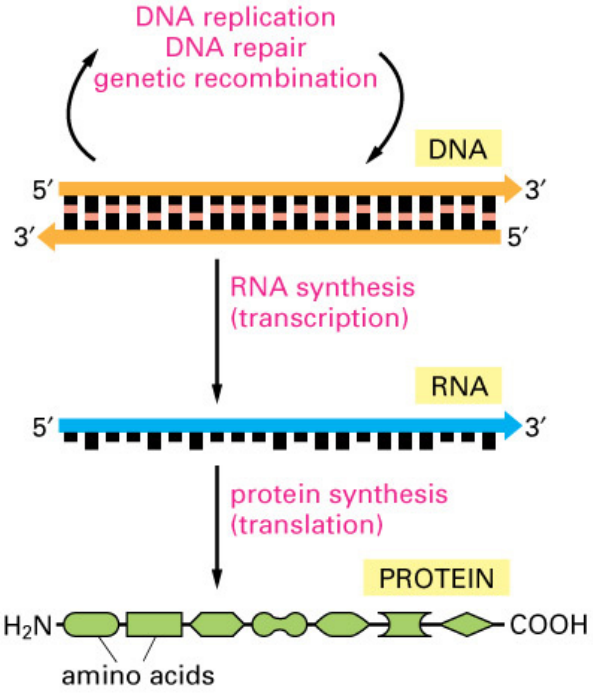
proteíny
(2D elektroforéza/mass-spec)





Kontrola expresie génov





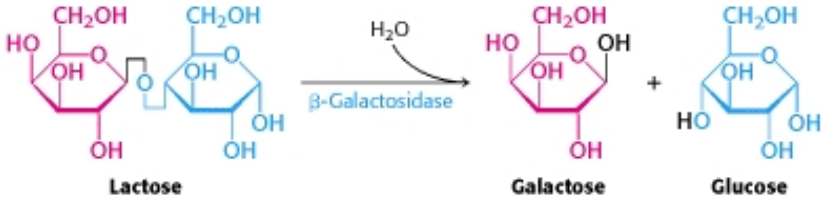
The Nobel Prize in Physiology or Medicine 1965 was awarded jointly to François Jacob, André Lwoff and Jacques Monod ***"for their discoveries concerning genetic control of enzyme and virus synthesis"***

François Jacob
(1920-2013)
France

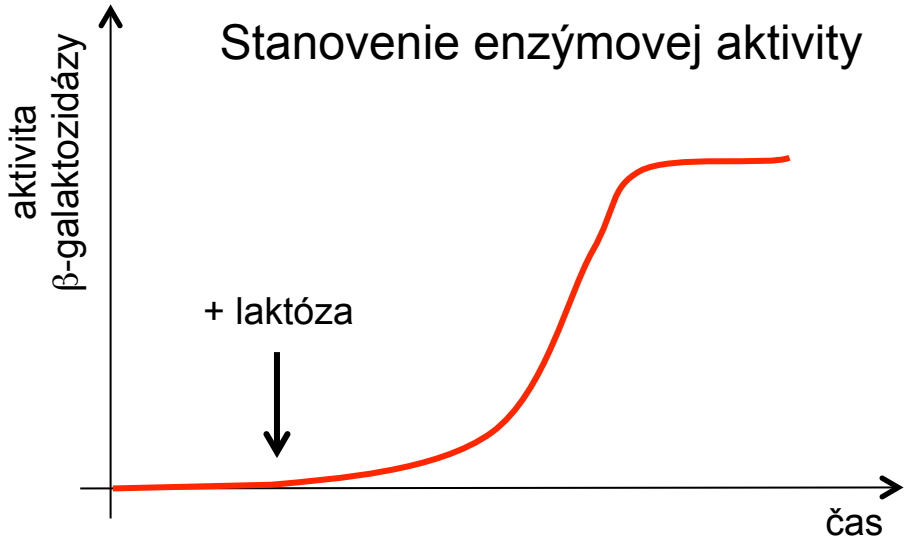
André Lwoff
(1902-1994)
France

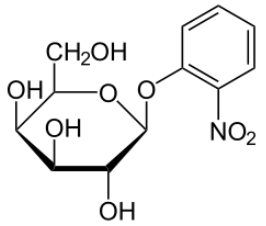
Jacques Monod
(1910-1976)
France

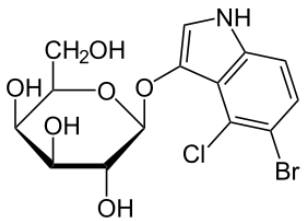
Modelový systém: Regulácia metabolizmu laktózy v baktériách *E.coli*



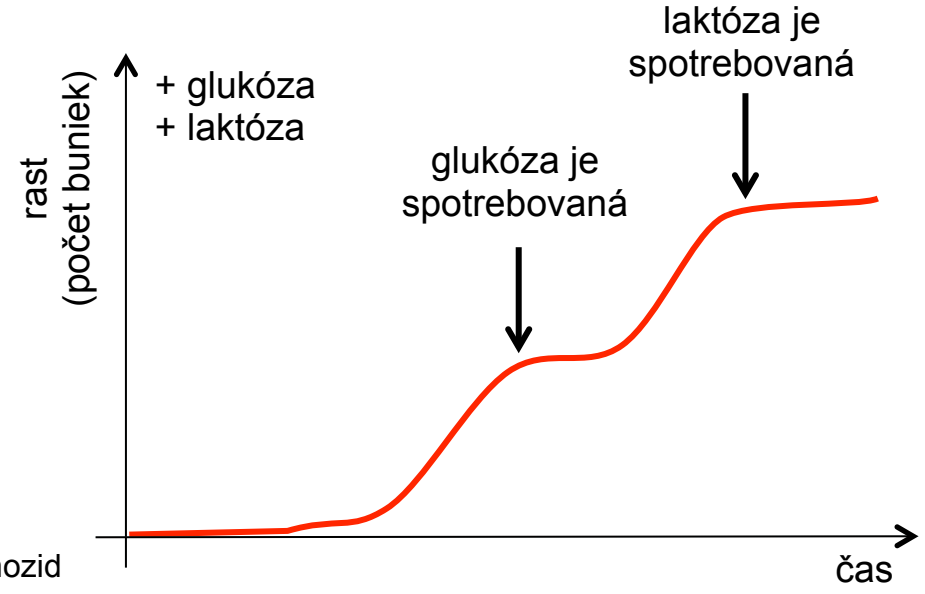
Stanovenie enzýmovej aktivity

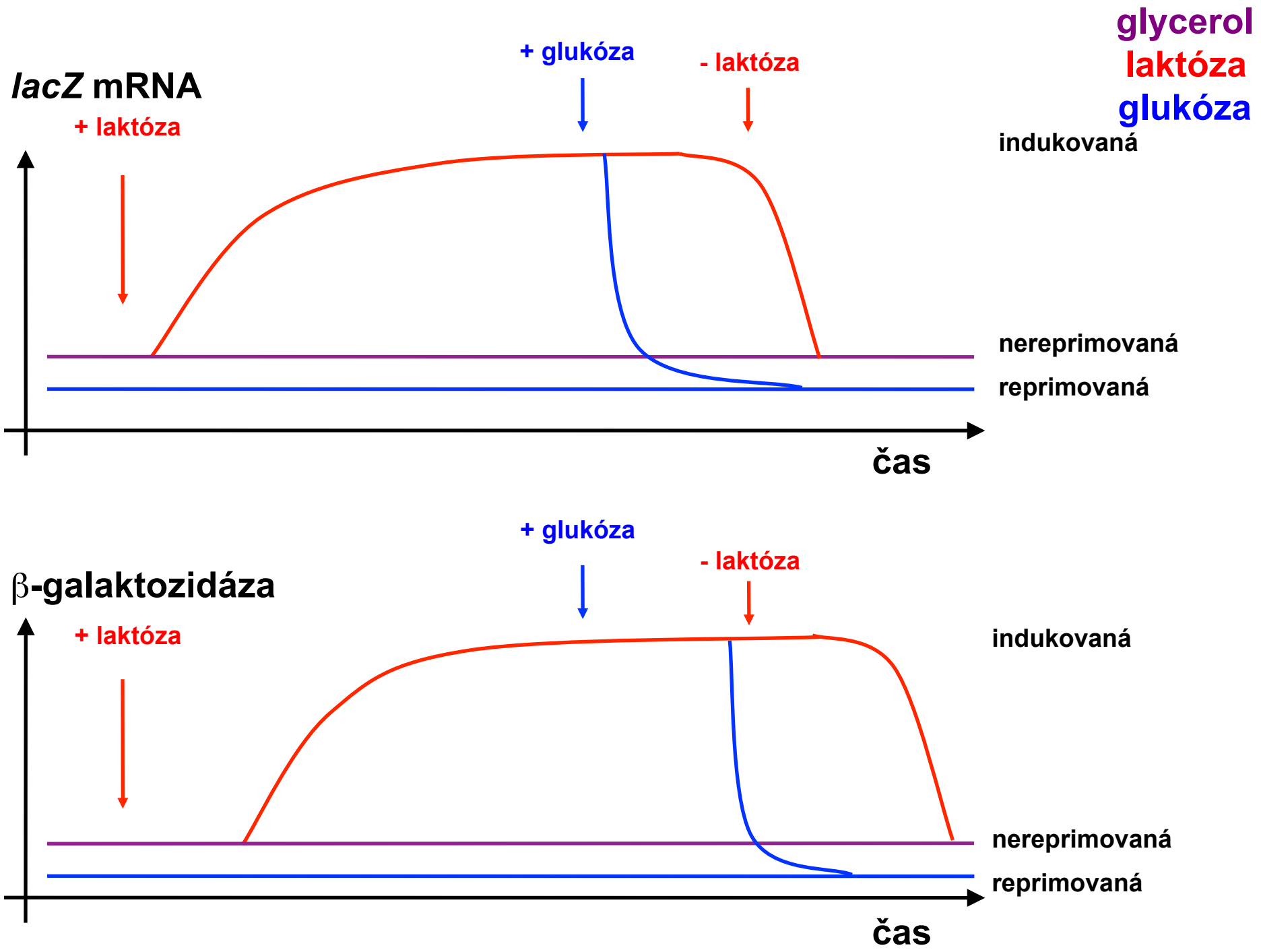




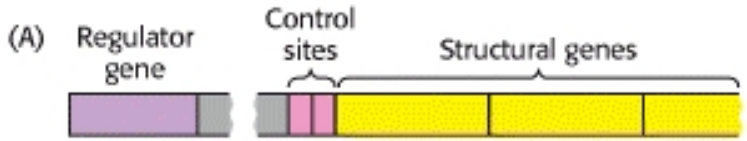


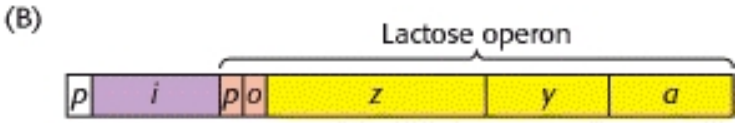
Rastové krivky (diauxický rast)

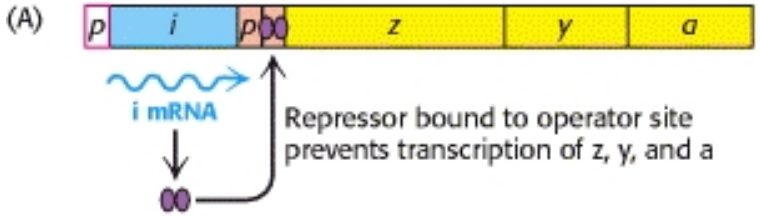


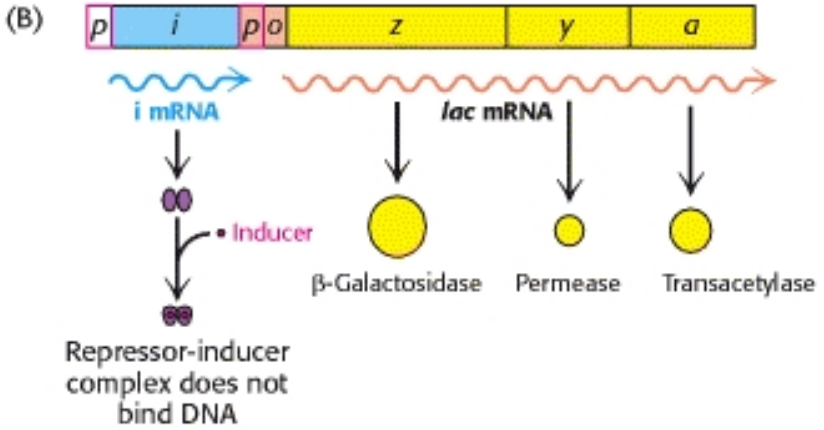

glycerol
laktóza
glukóza
lacZ mRNA
+ laktóza
+ glukóza
- laktóza
indukovaná
nereprimovaná
reprimovaná
čas
β-galaktozidáza
+ laktóza
+ glukóza
- laktóza
indukovaná
nereprimovaná
reprimovaná
čas

Operónový model: *lac* operón

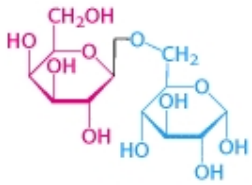




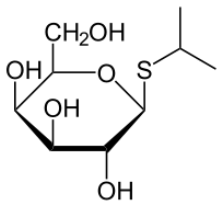




LacI → represia
laktóza (alolaktóza, IPTG) → induktor



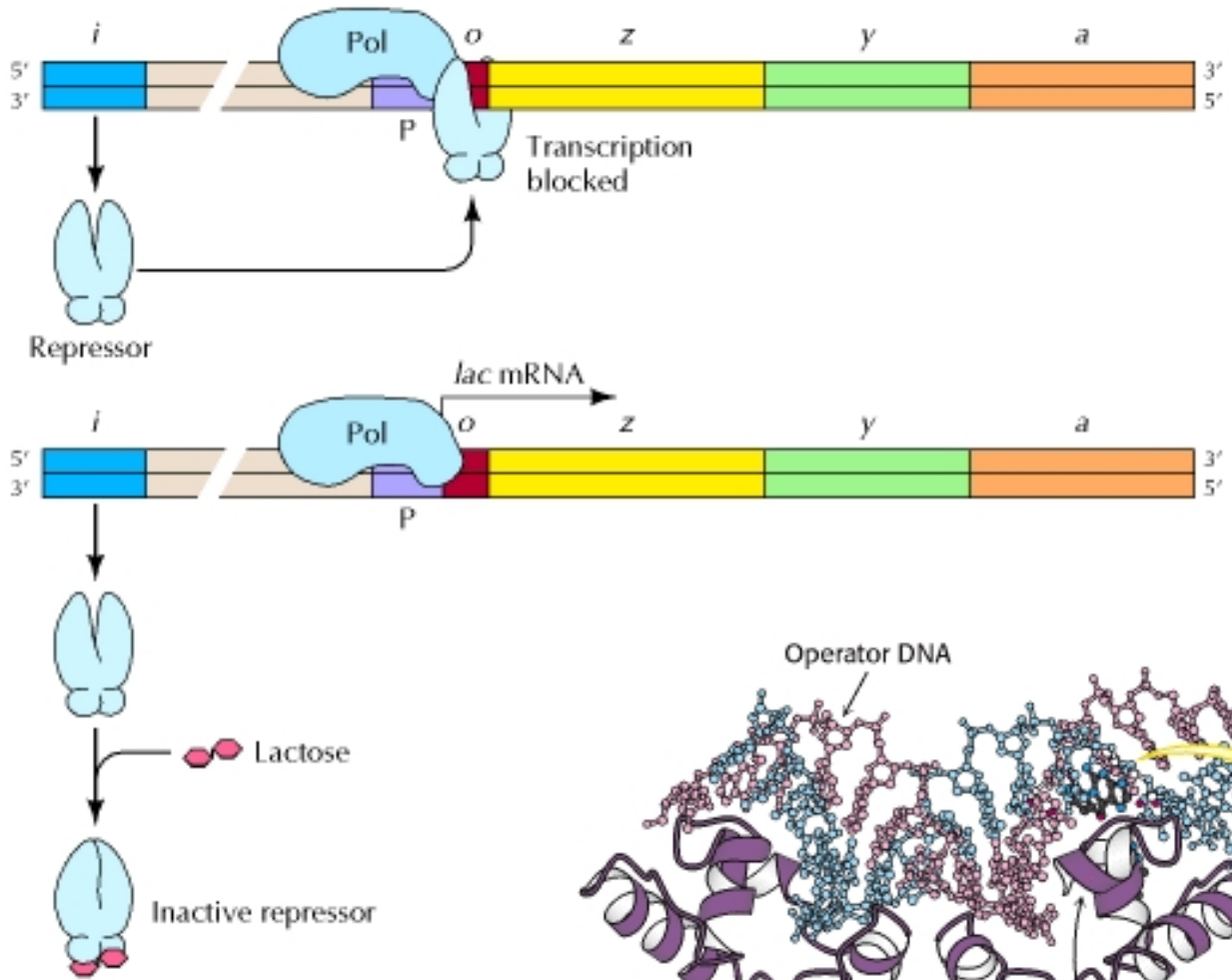
1,6-Allolactose

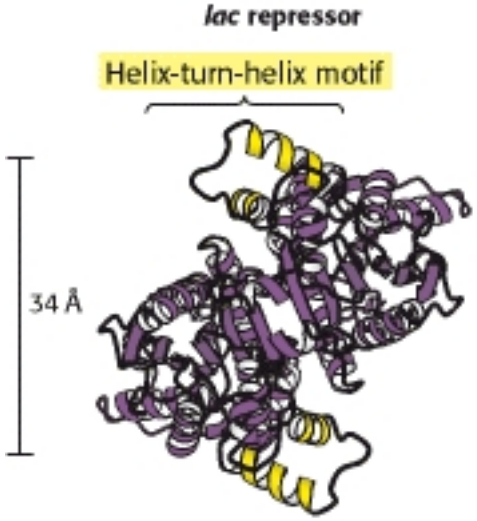


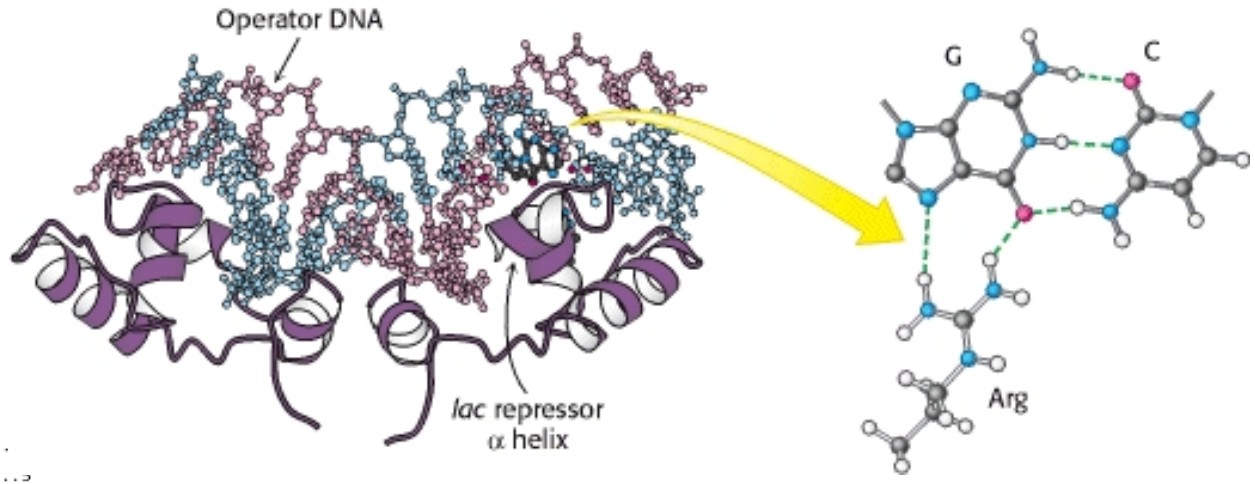
IPTG
izopropyl-β-D-tiogalaktopyranozid

Proteín LacI reprimuje expresiu *lac* operónu

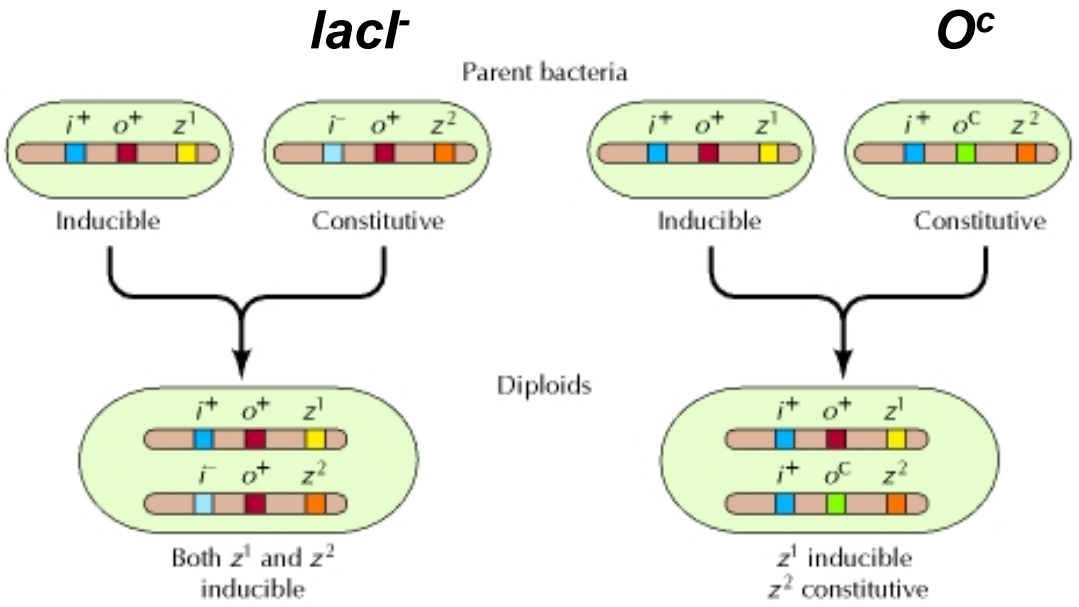
Represor LacI viaže sekvenciu DNA operátora a blokuje transkripciu







Experimenty s regulačnými mutantmi

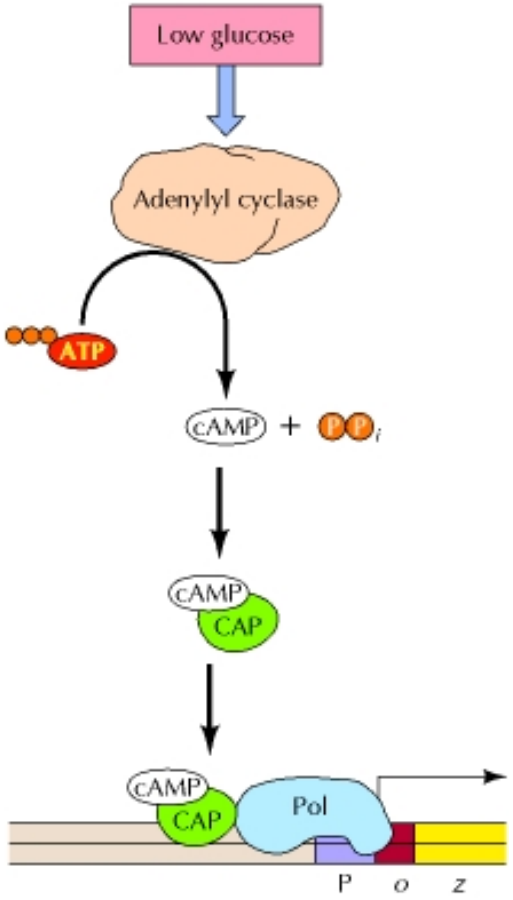


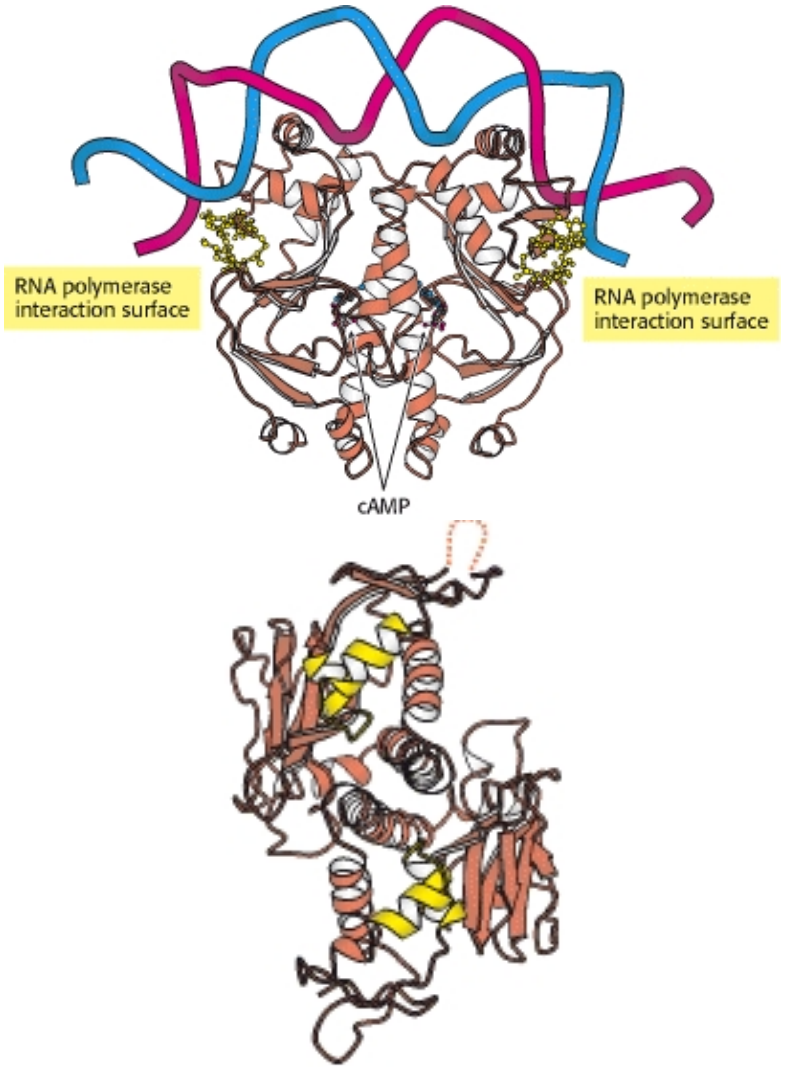
represor (LacI) je *trans*-regulačný faktor

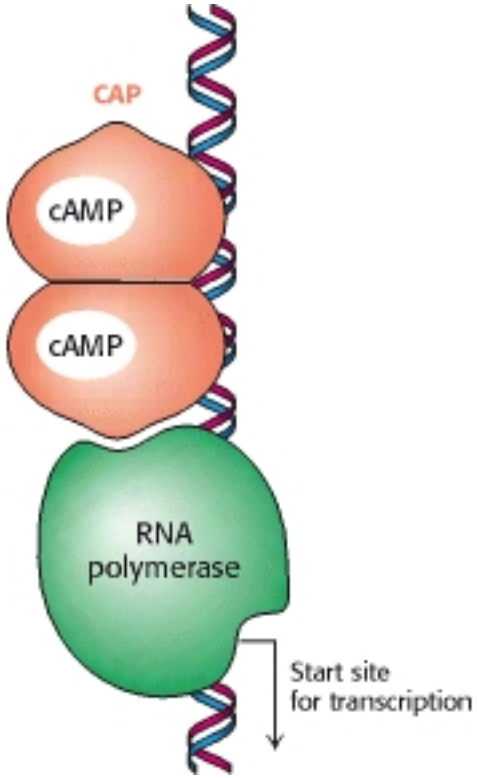
operátor je *cis*-regulačný element

Proteín CAP (*catabolite activator protein*) v komplexe s cAMP aktivuje expresiu *lac* operónu

Komplex CAP-cAMP viaže regulačnú sekvenciu DNA a interaguje s RNA polymerázou

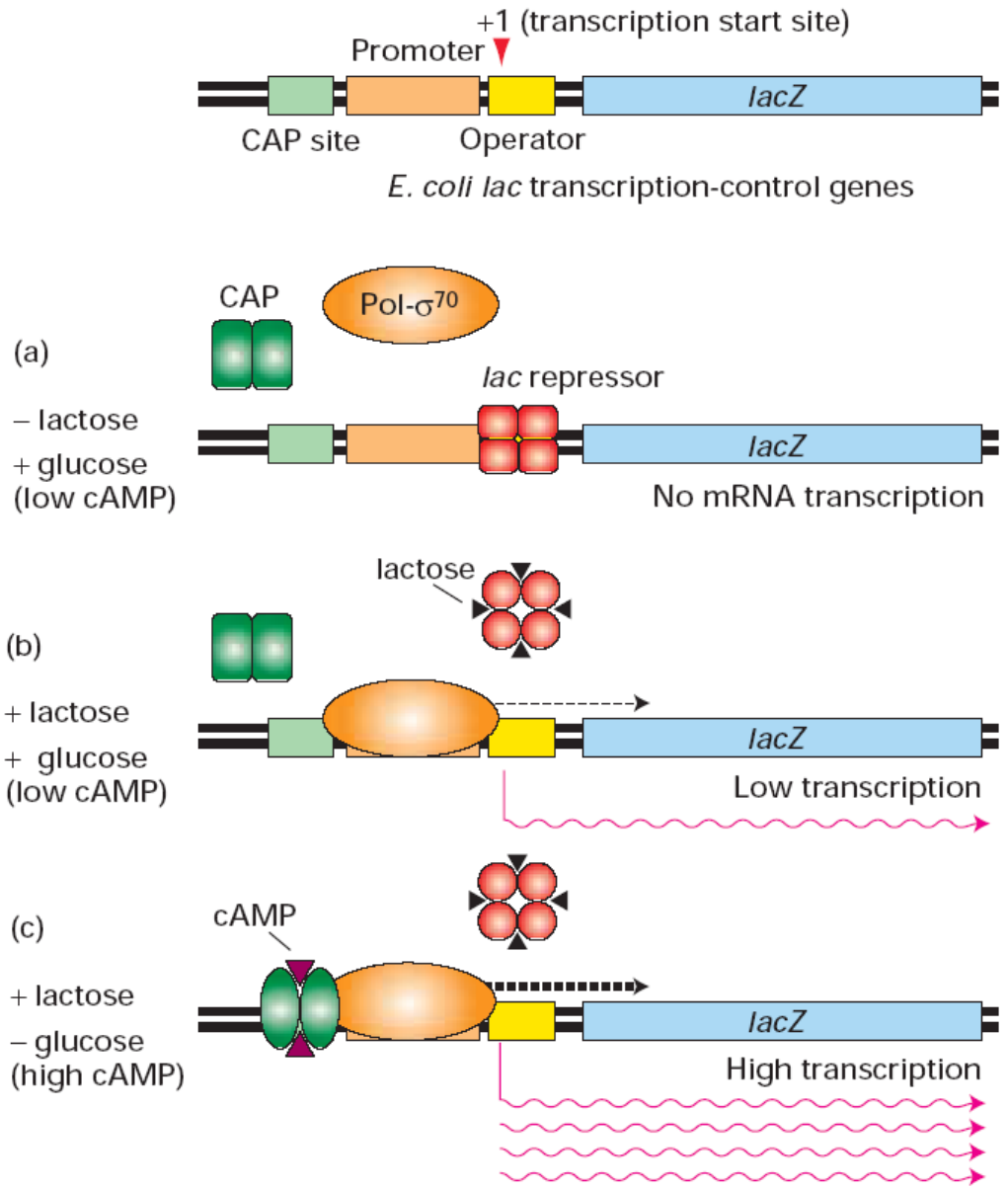




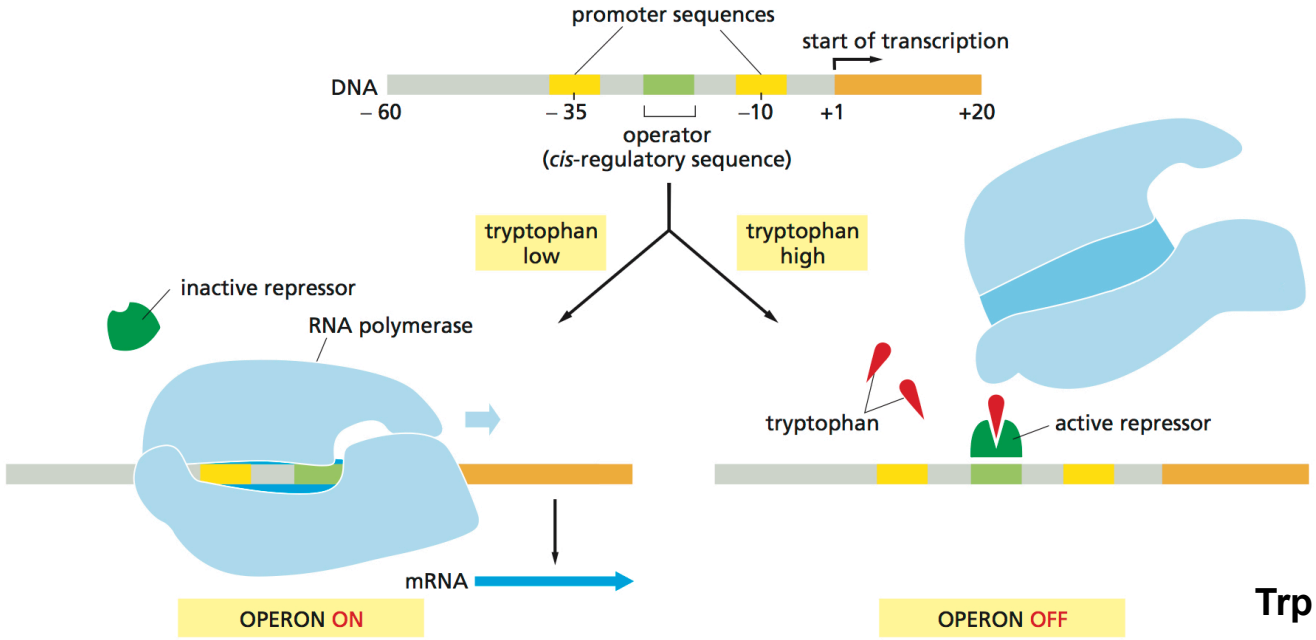


väzba na DNA vyžaduje cAMP

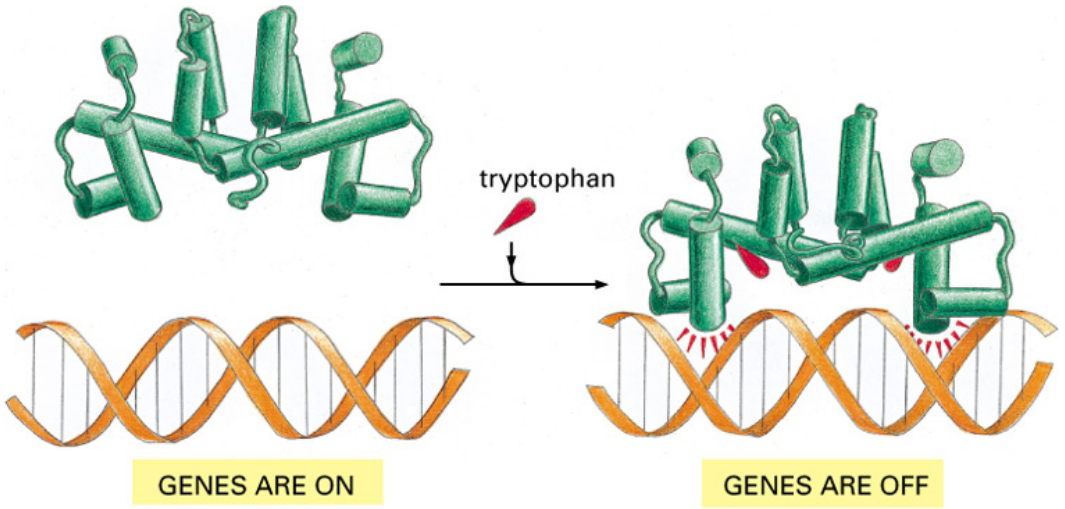
Pozitívna a negatívna kontrola *lac* operónu



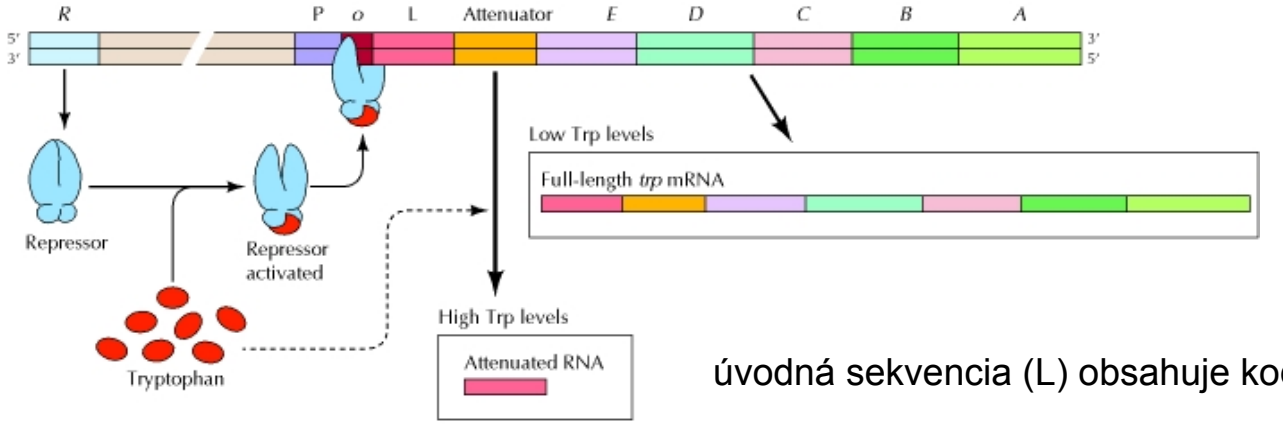
trp operón: negatívna kontrola represorom a korepresorom



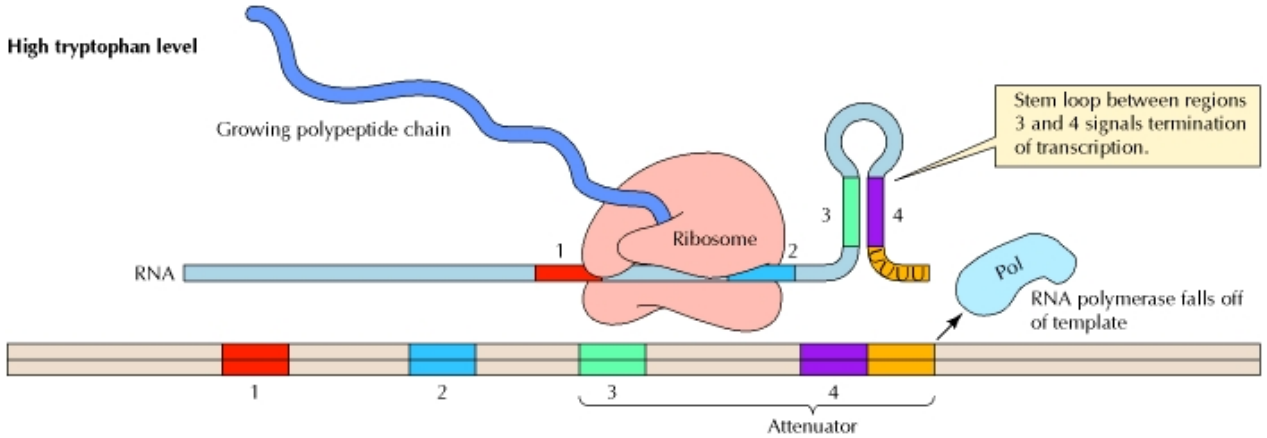
Trp → korepresor

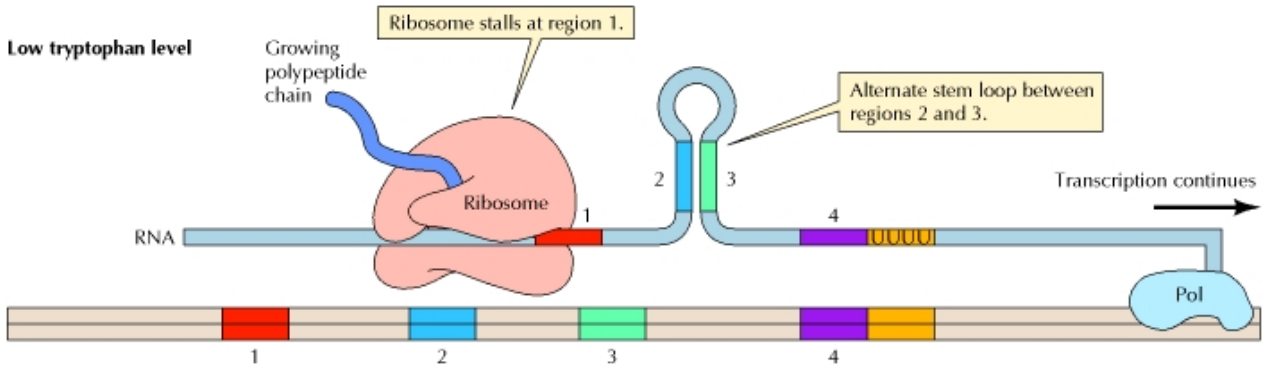


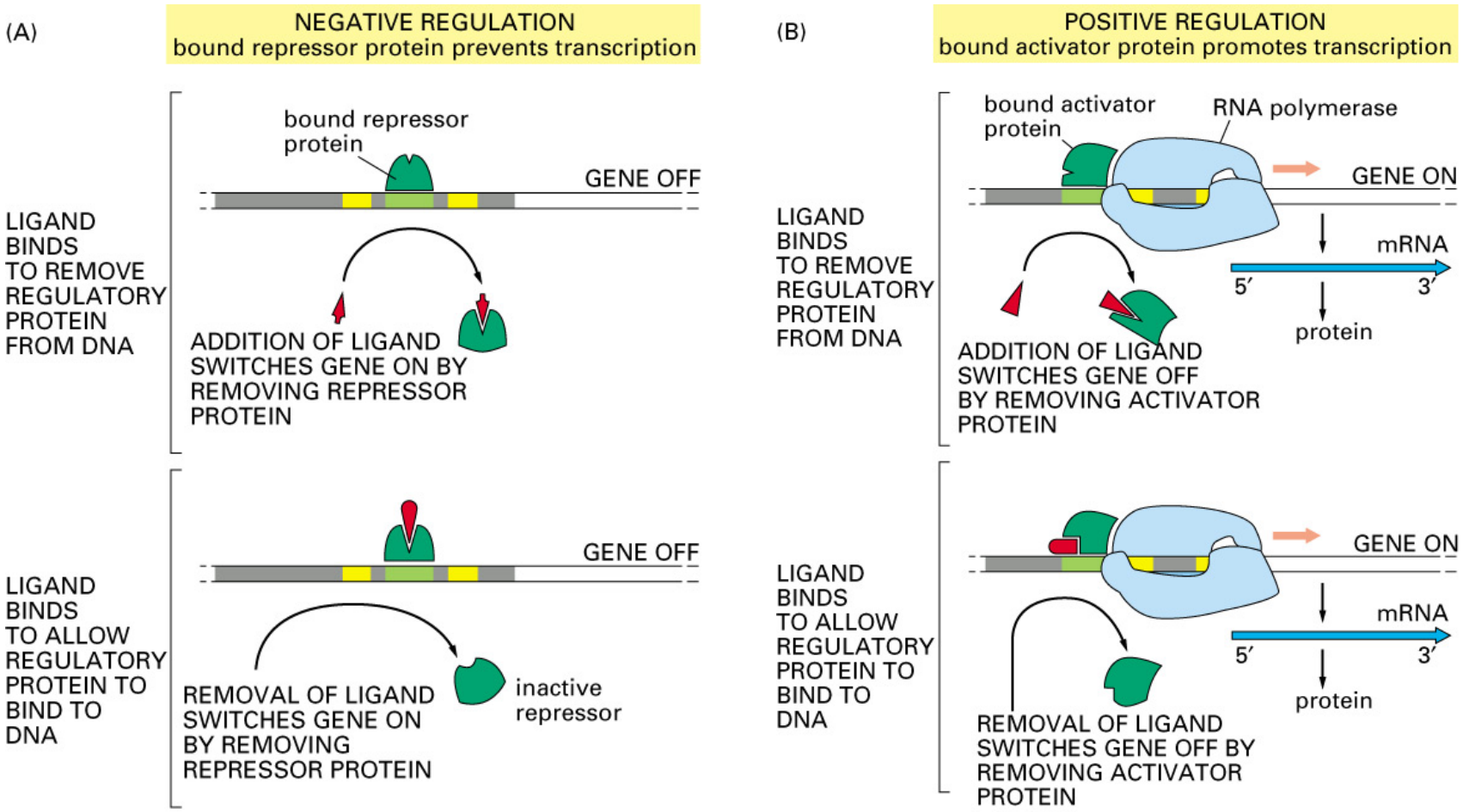
trp operón – negatívna kontrola atenuáciou



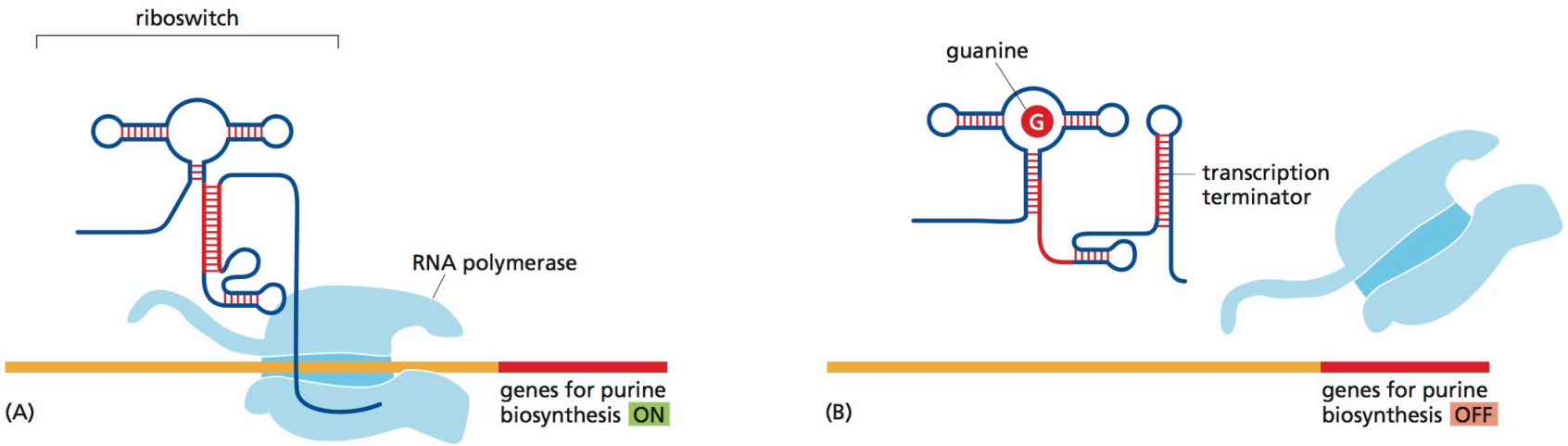
úvodná sekvencia (L) obsahuje kodóny pre tryptofán



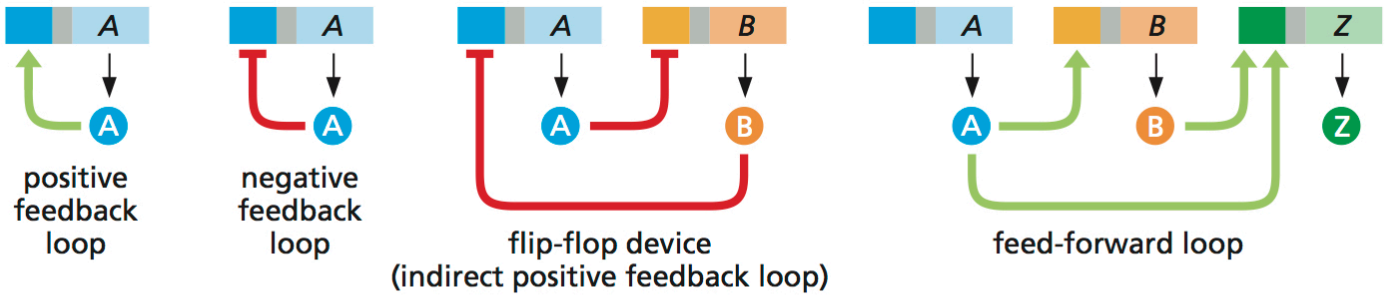



(A)
NEGATIVE REGULATION
bound repressor protein prevents transcription
LIGAND BINDS TO REMOVE REGULATORY PROTEIN FROM DNA
bound repressor protein
GENE OFF
ADDITION OF LIGAND SWITCHES GENE ON BY REMOVING REPRESSOR PROTEIN
LIGAND BINDS TO ALLOW REGULATORY PROTEIN TO BIND TO DNA
GENE OFF
REMOVAL OF LIGAND SWITCHES GENE ON BY REMOVING REPRESSOR PROTEIN
inactive repressor
(B)
POSITIVE REGULATION
bound activator protein promotes transcription
LIGAND BINDS TO REMOVE REGULATORY PROTEIN FROM DNA
bound activator protein
RNA polymerase
GENE ON
mRNA
5′
3′
protein
ADDITION OF LIGAND SWITCHES GENE OFF BY REMOVING ACTIVATOR PROTEIN
LIGAND BINDS TO ALLOW REGULATORY PROTEIN TO BIND TO DNA
GENE ON
mRNA
5′
3′
protein
REMOVAL OF LIGAND SWITCHES GENE OFF BY REMOVING ACTIVATOR PROTEIN

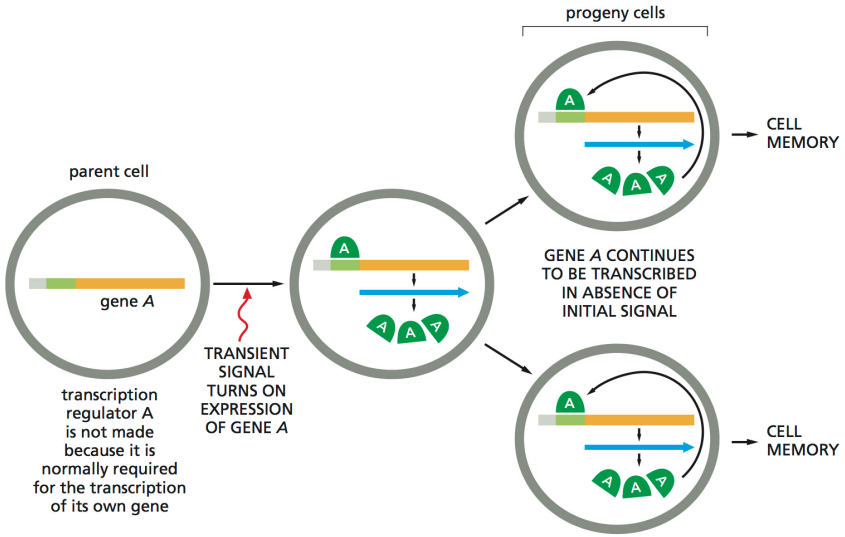
Negatívna kontrola transkripcie pomocou ribo-prepínačov (*riboswitch*)



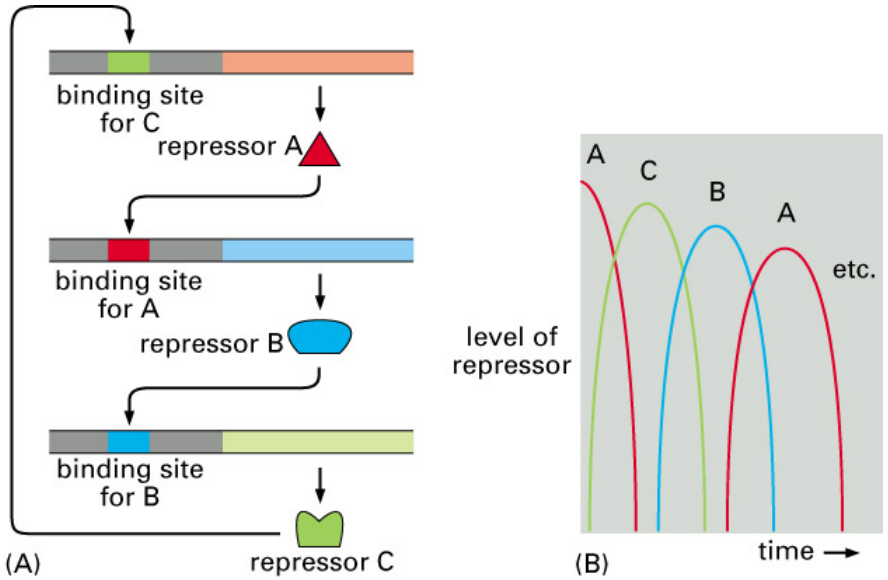
Regulačné siete v transkripcii génov



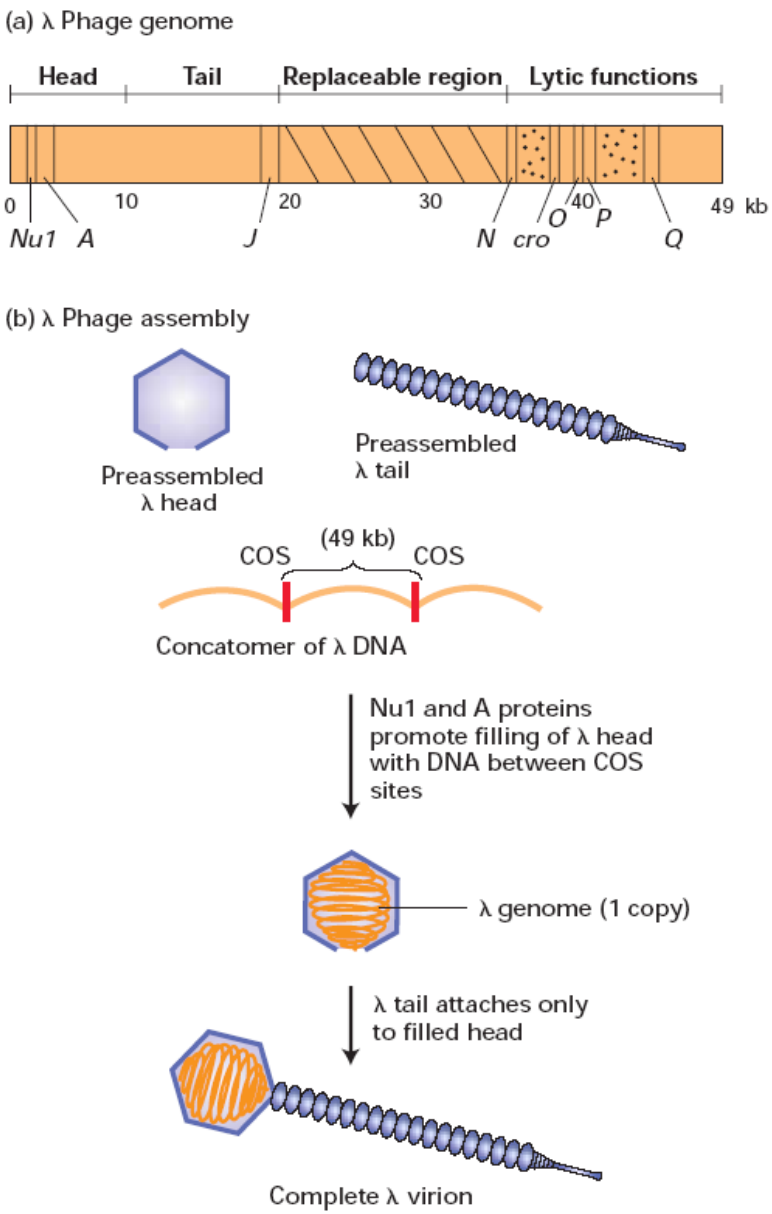
Pozitívna spätná väzba



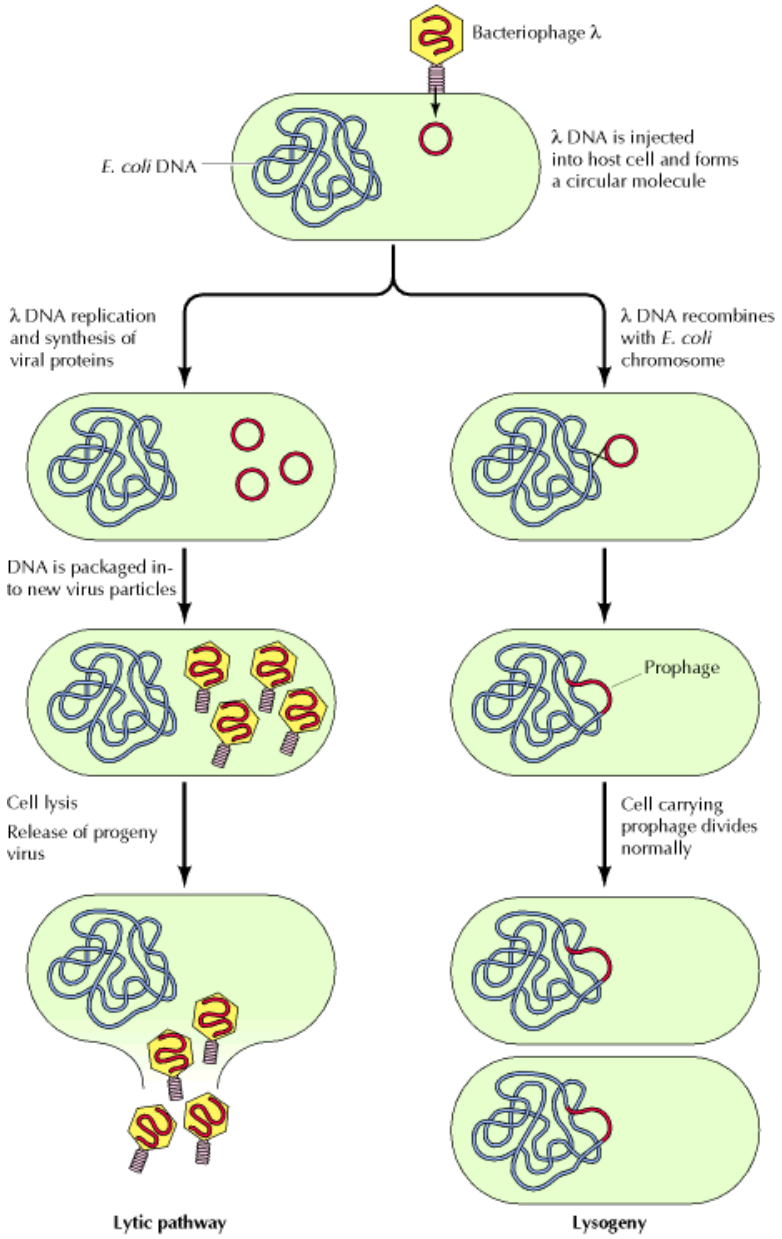
Negatívna spätná väzba



Bakteriofág λ ako modelový systém kontroly expresie génov

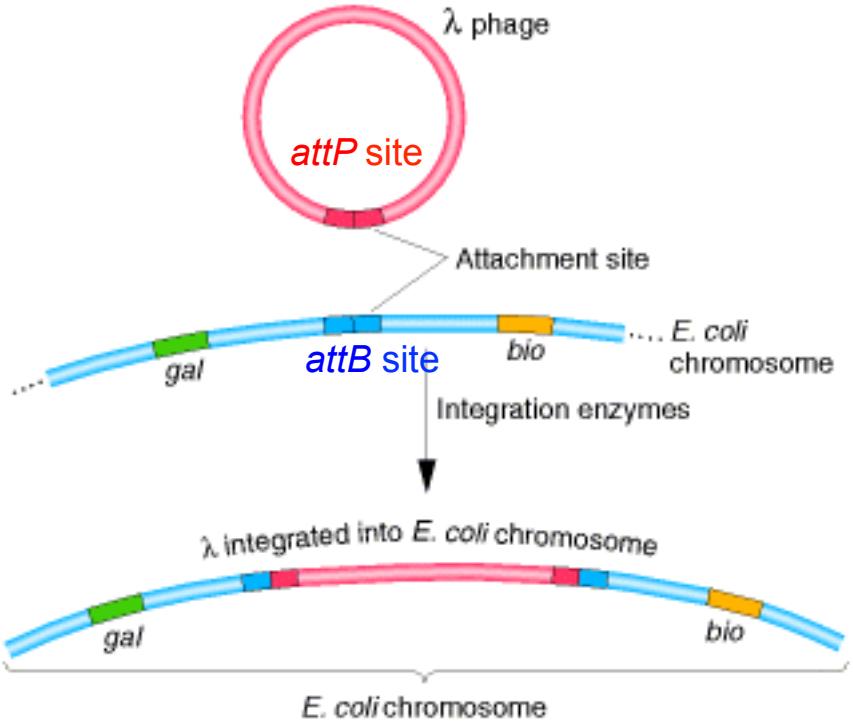


Dve dráhy životného cyklu bakteriofága λ

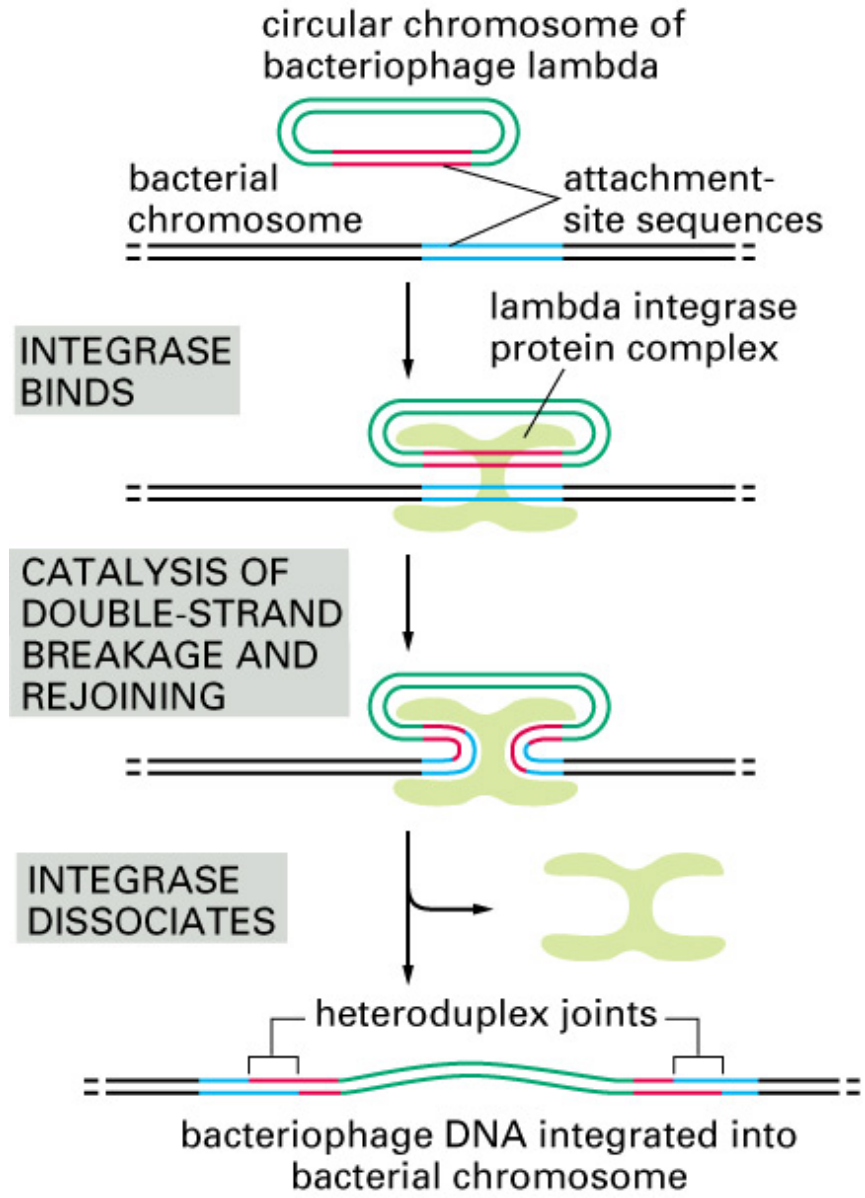


- Kapsid obsahuje lineárnu DNA bakteriofága
- Po vstupe do buniek DNA cirkularizuje – *cos* sekvencie

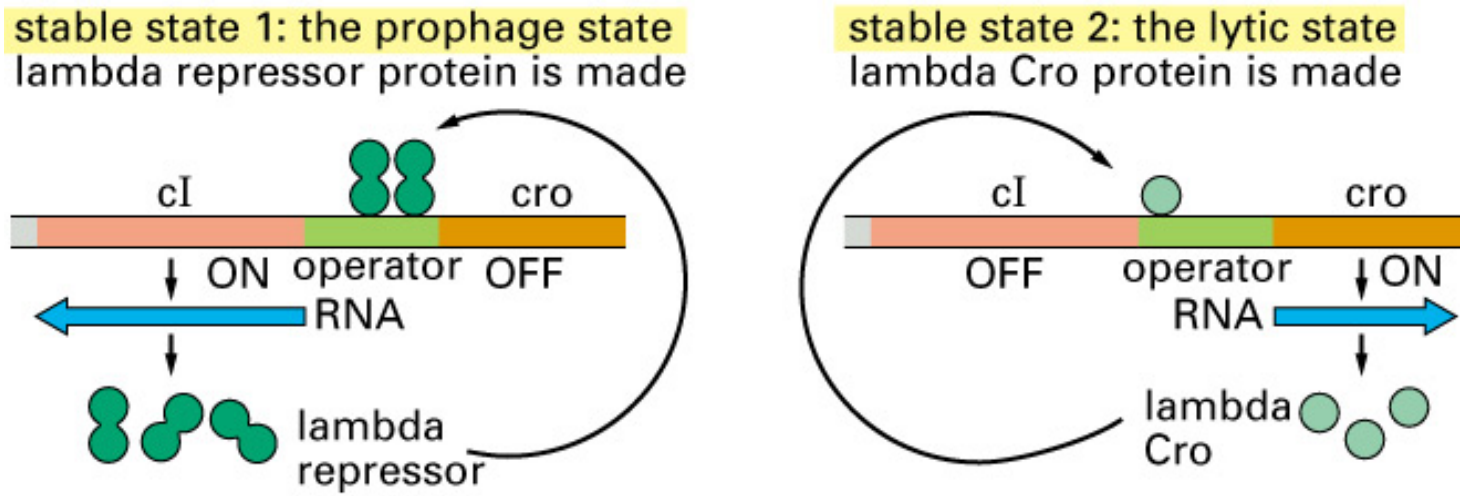
- V lytickej dráhe sa amplifikuje rolling-circle mechanizmom
- V lyzogénnej dráhe sa λ DNA integruje do DNA hostiteľa



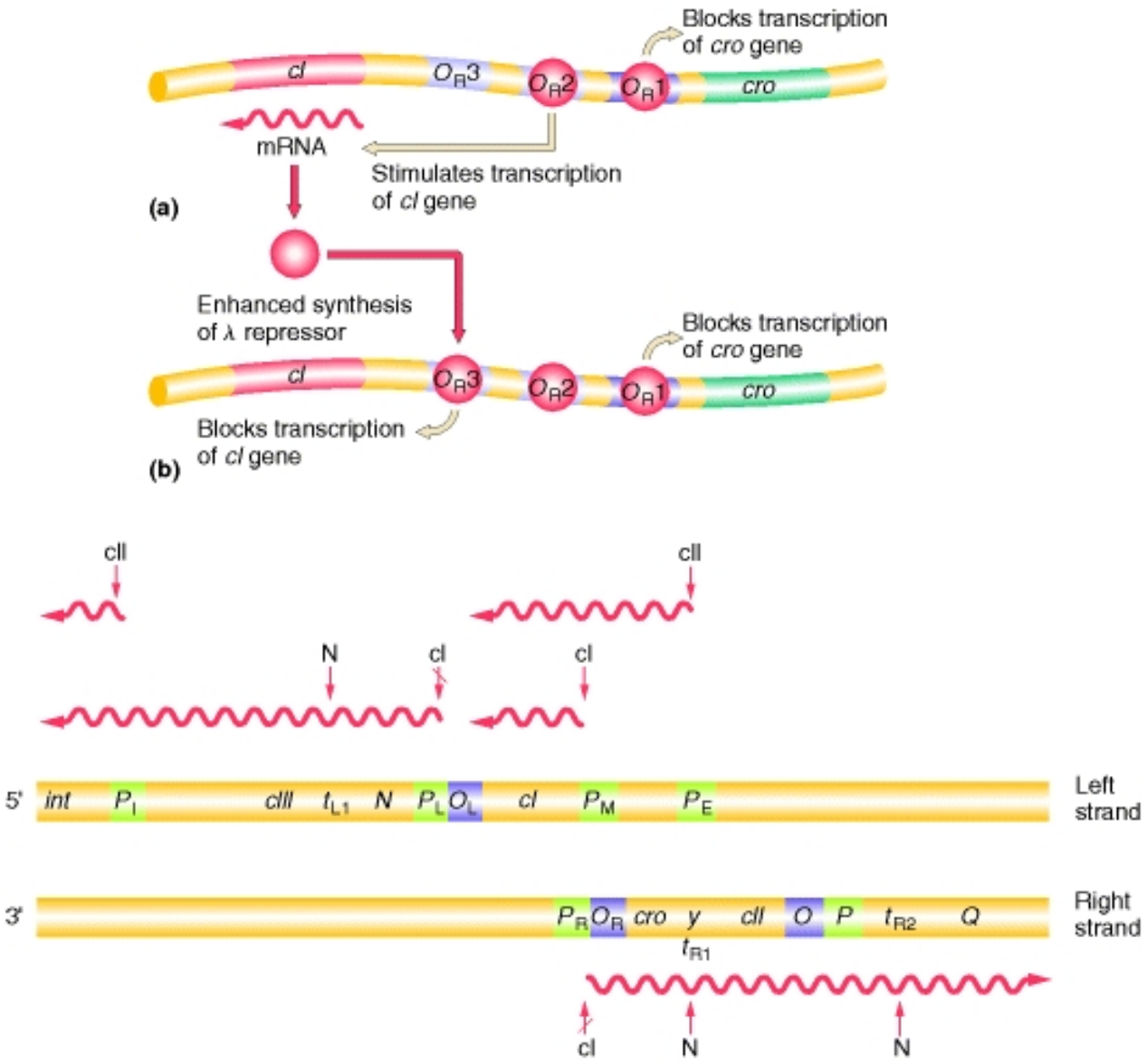
Integráciu do DNA hostiteľa katalyzuje λ integráza



Proteíny cI a cro rozhodujú medzi lytickou a lyzogénnou dráhou životného cyklu bakteriofága λ

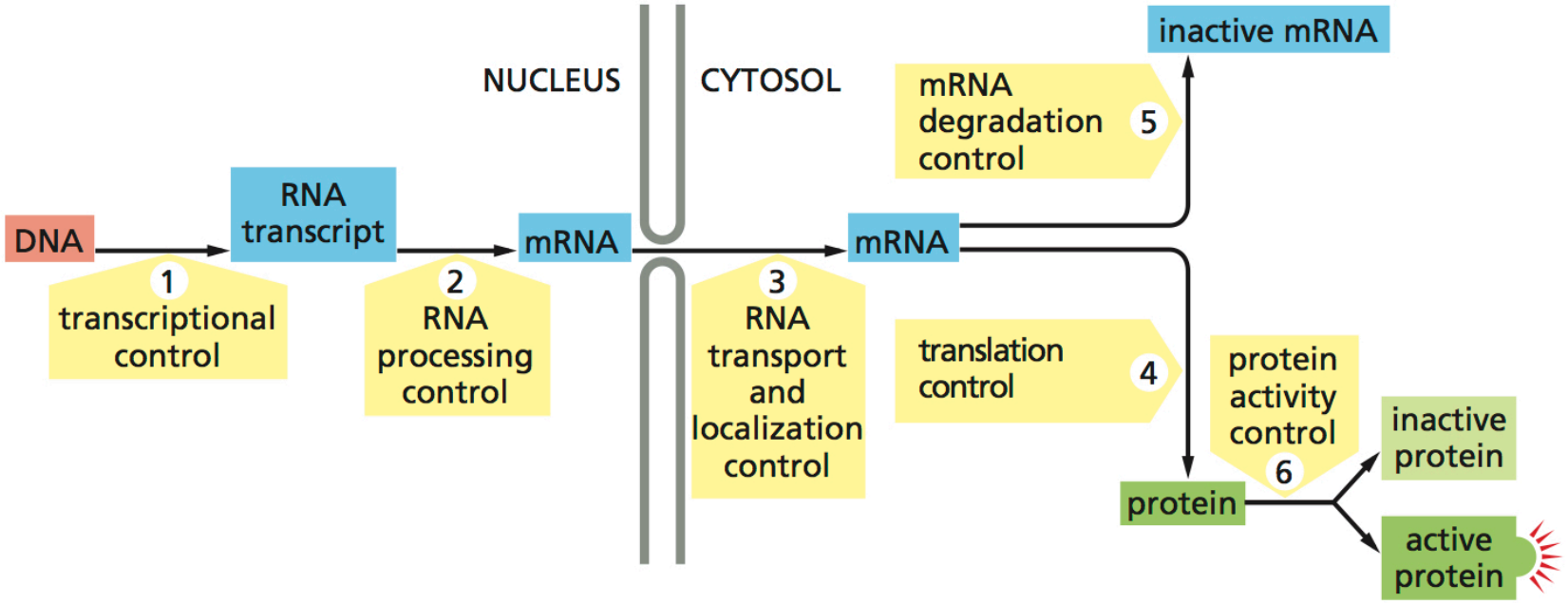


*c*I – (*clear* I) represor lytickej dráhy
cro – (*control of repressor's operator*) represor lyzogénnej dráhy

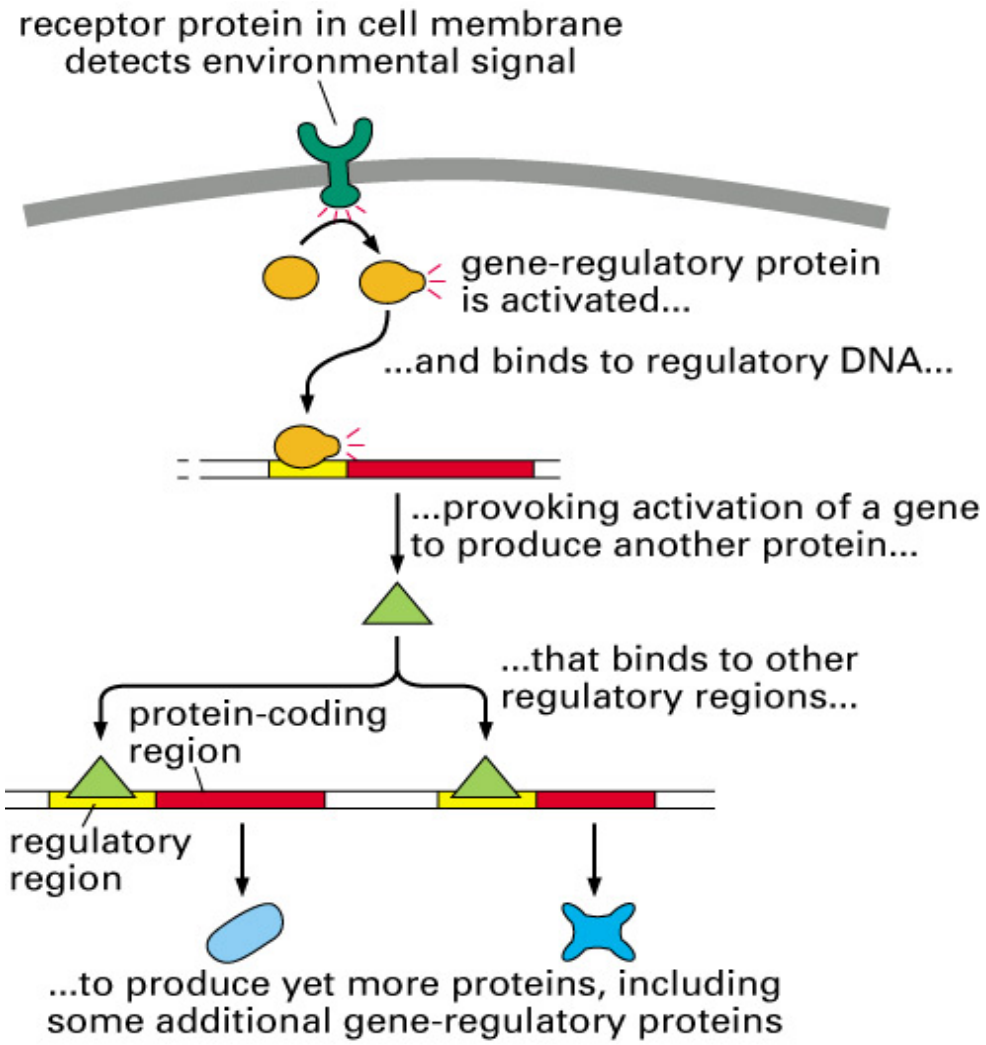


cI – (*clear* I) represor lytickej dráhy
cII – (*clear* II) aktivátor lyzogénnej dráhy
N – a<u>N</u>titerminátor

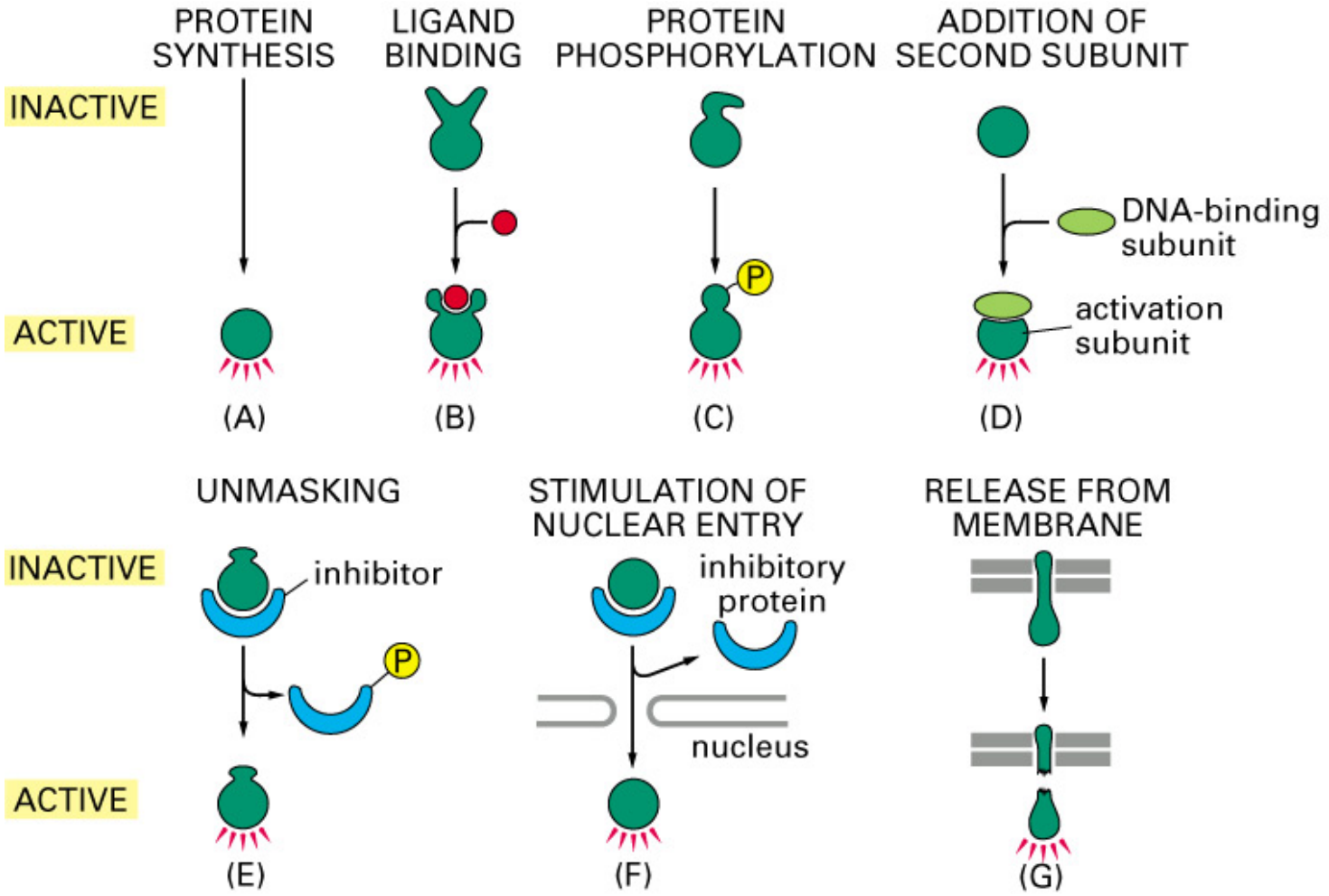
Úrovne kontroly expresie génov v eukaryotickej bunke



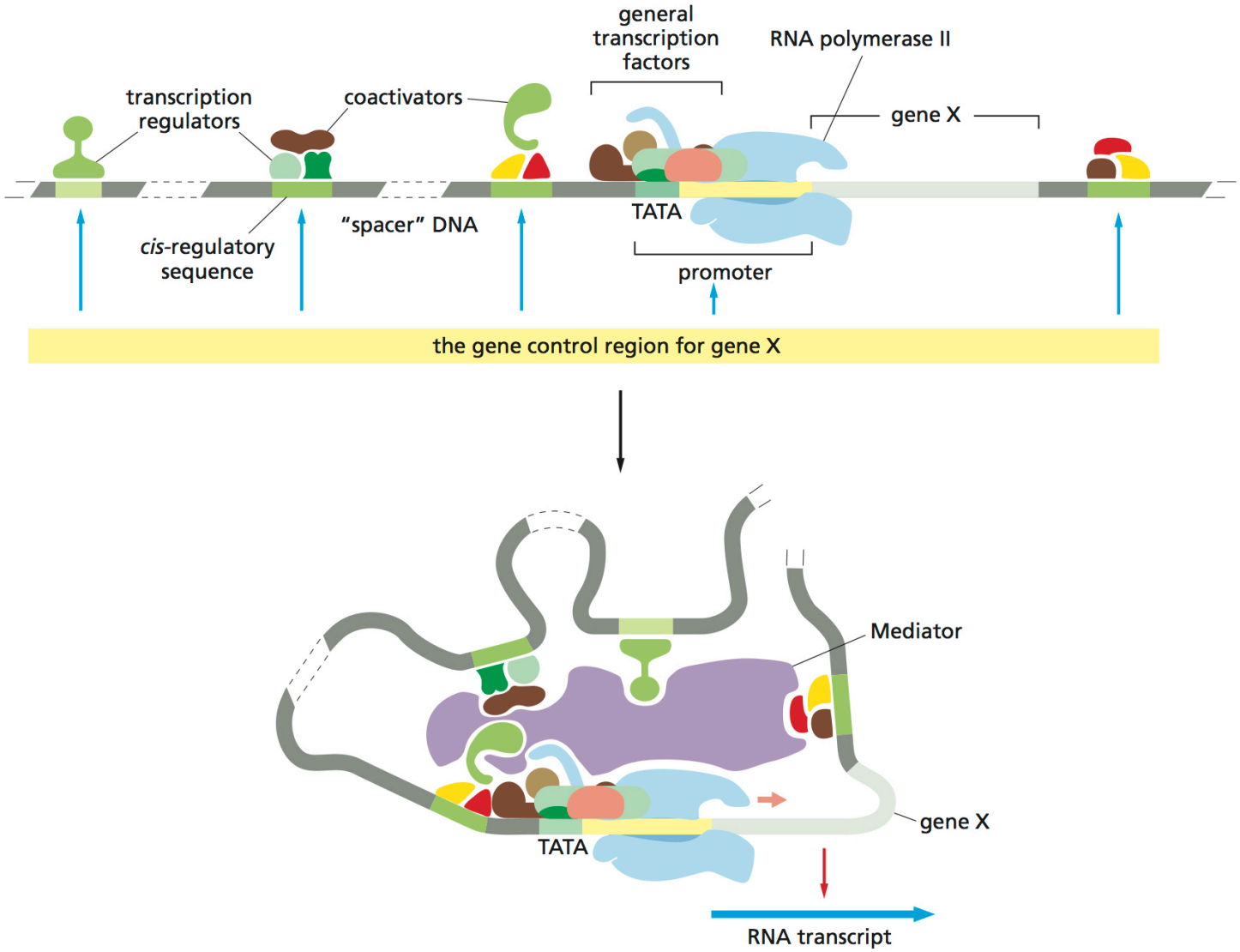
Transkripčný faktor kontroluje viacero transkripčných jednotiek regulónový model



Možnosti aktivácie regulačných proteínov v eukaryotickej bunke



Transkripcia eukaryotických génov je kontrolovaná viacerými proteínovými faktormi



Významnú úlohu v regulácii expresie má chromatín

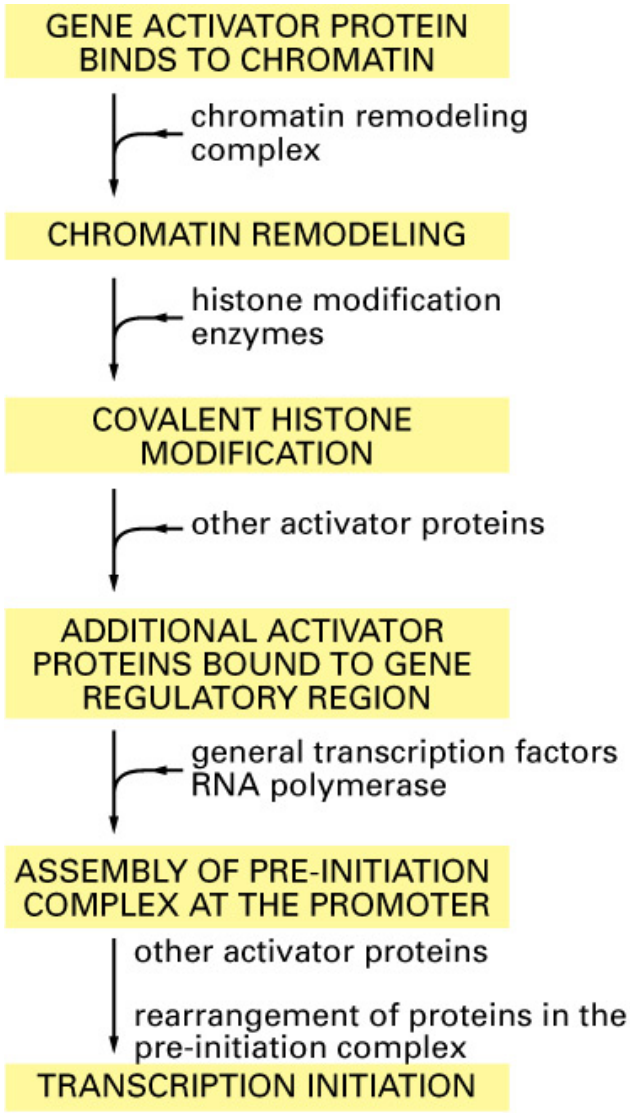
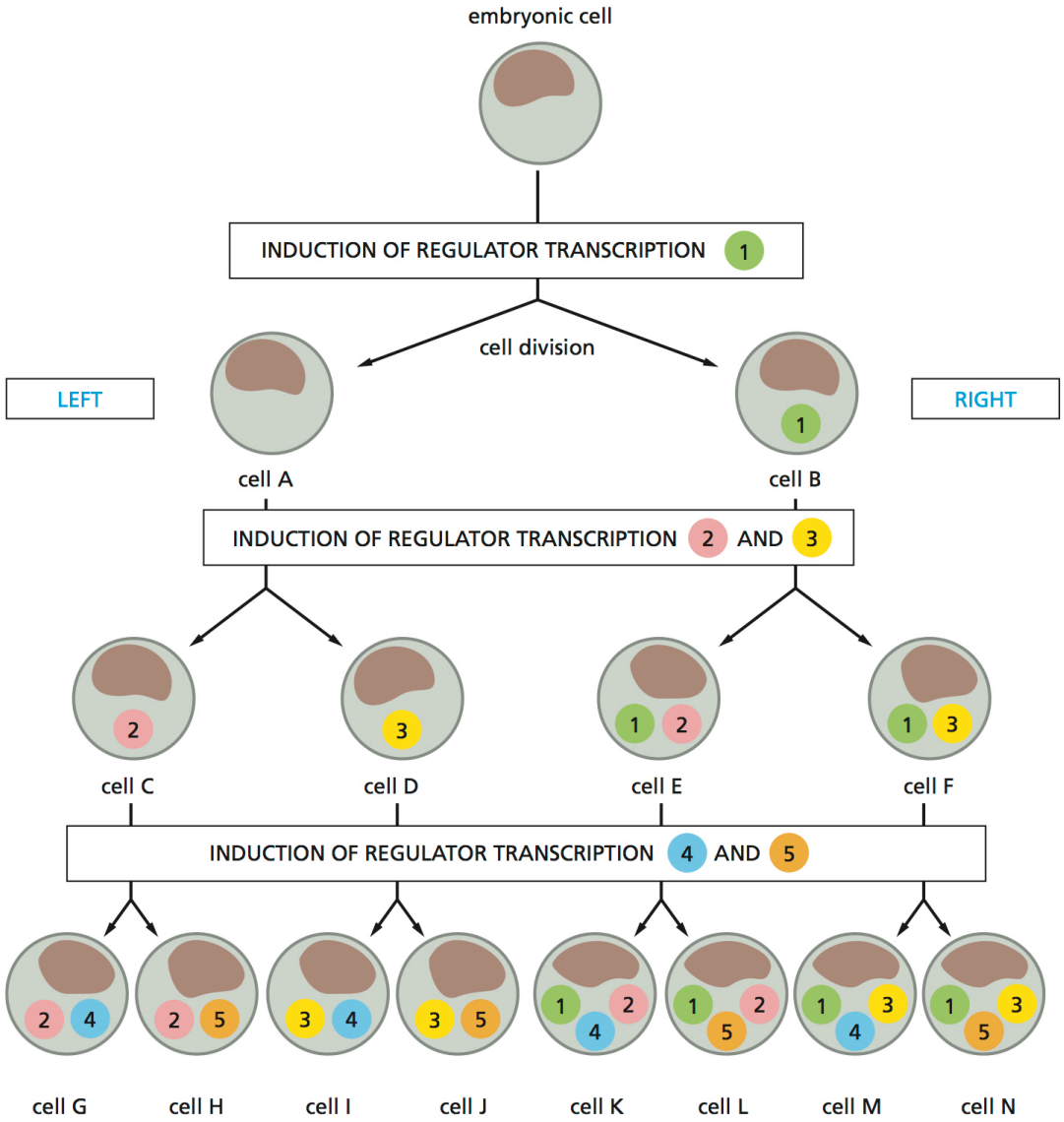


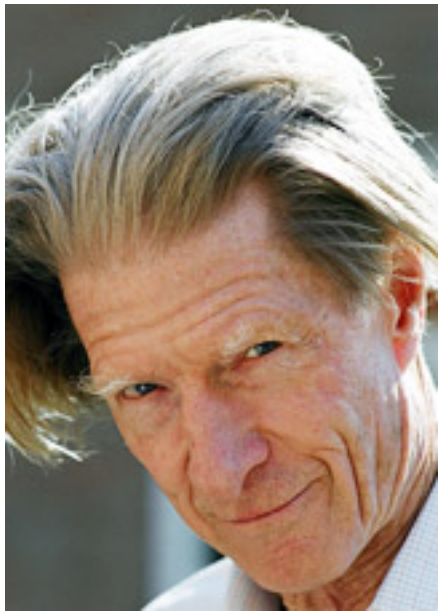
Figure 7–48. Molecular Biology of the Cell, 4th Edition.

Kombinatorická kontrola diferenciácie buniek

kombinácie malého počtu regulačných proteínov



The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka "*for the discovery that mature cells can be reprogrammed to become pluripotent*"



Sir John B.Gurdon
(1933-)
UK

Shinya Yamanaka
(1962-)
Japan / USA

Nabudúce:

7. Úloha biologických membrán v eukaryotickej bunke

- Kompartmentalizácia bunky.
- Štruktúra a funkcie membrán.
- Transport cez membrány.
- Vektorové procesy viazané na membrány.
- Úloha membrán v prenose nervového signálu.