

Epigenetski mehanizmi regulacije ekspresije genov

Genetika 2010/11
B.Javornik

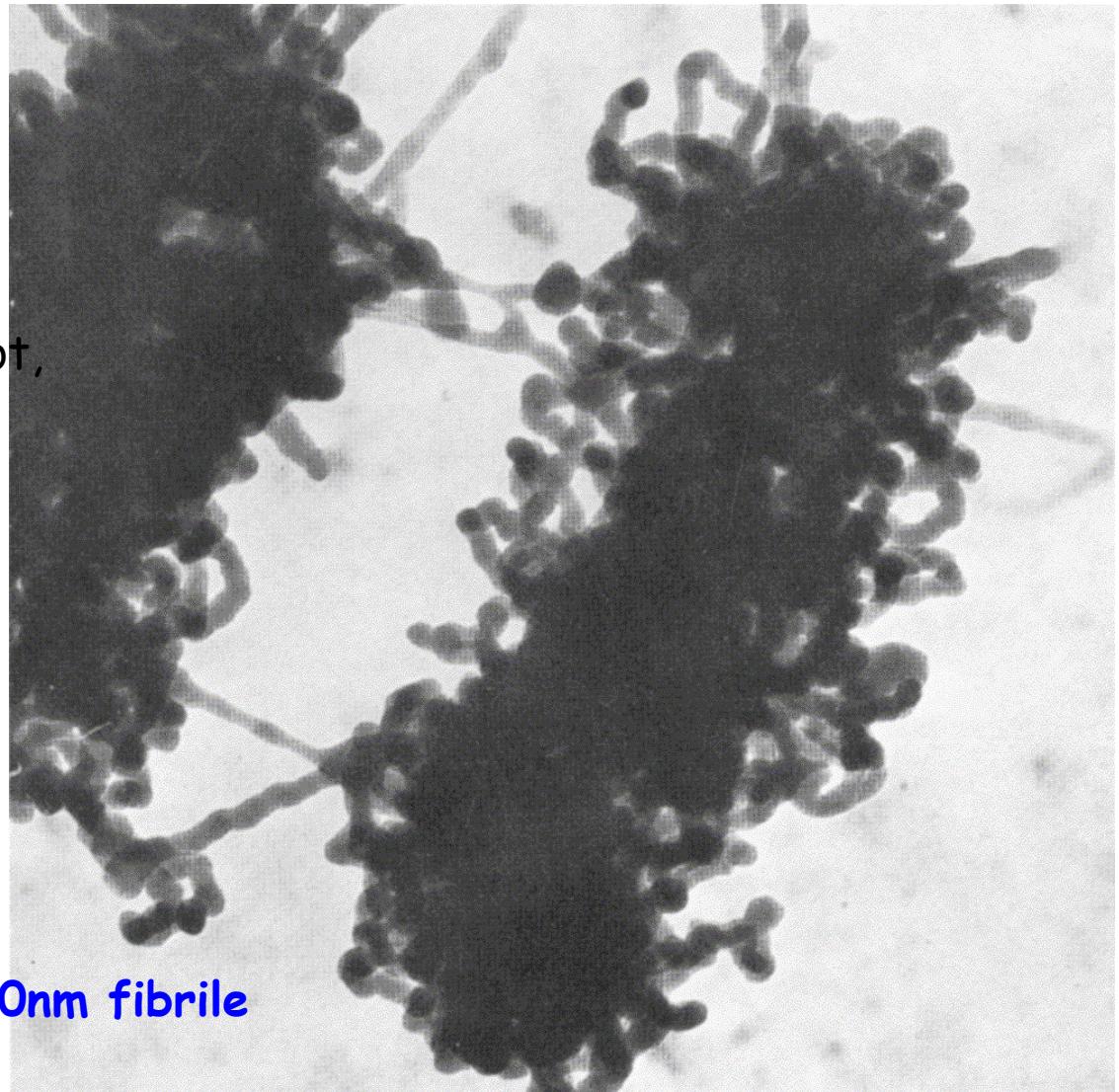
Struktura kromosomov

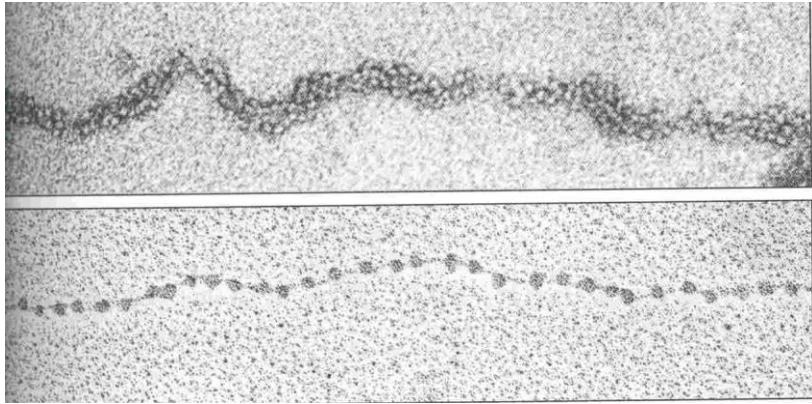
V kromosому je ena molekula DNA

Metafazna kromosoma čebele
(1968). Kromosoma izgledata kot,
da ju gradi ena neprekinjena
nitka (**30nm fibrile**)

Dolžina DNA???

30nm fibrile

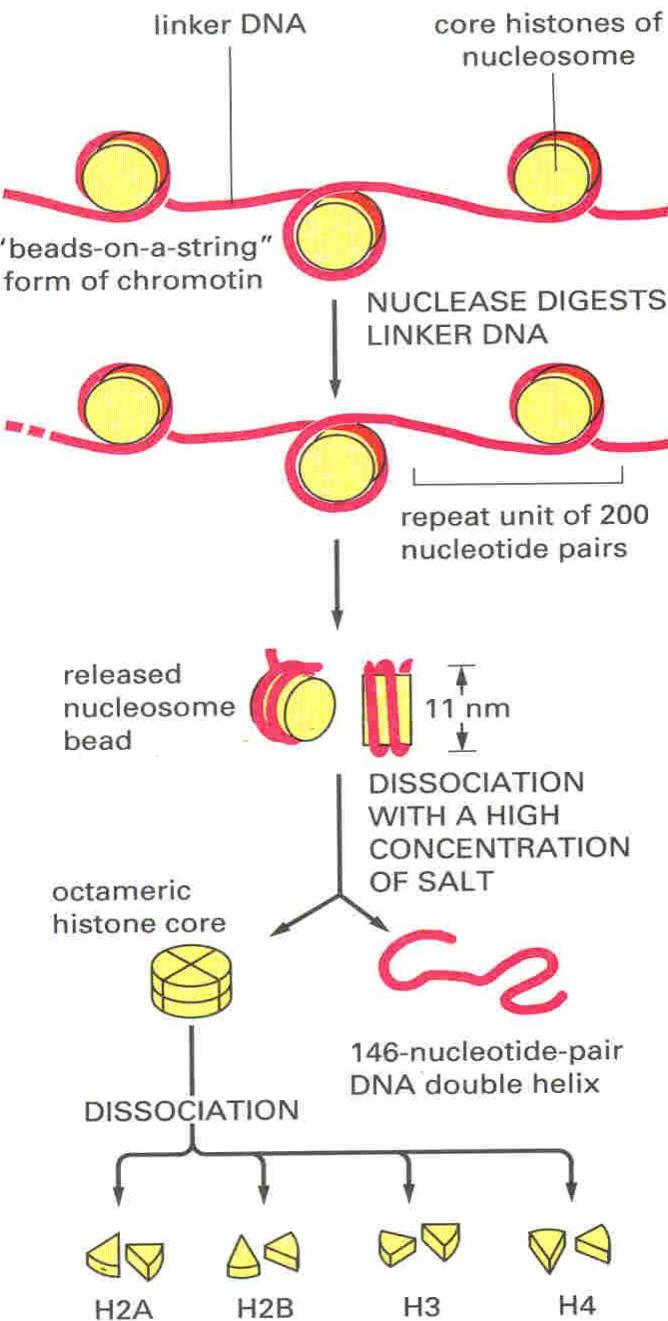




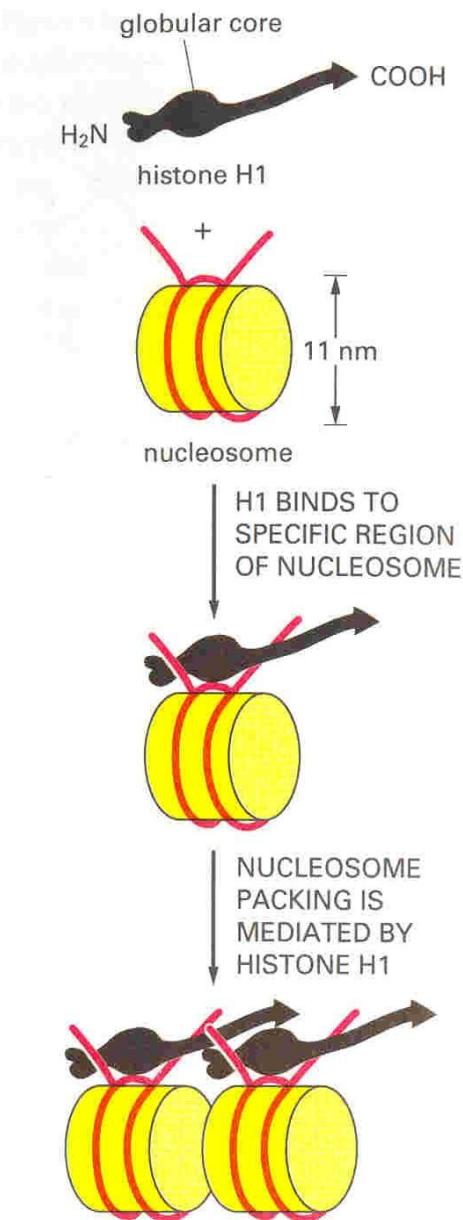
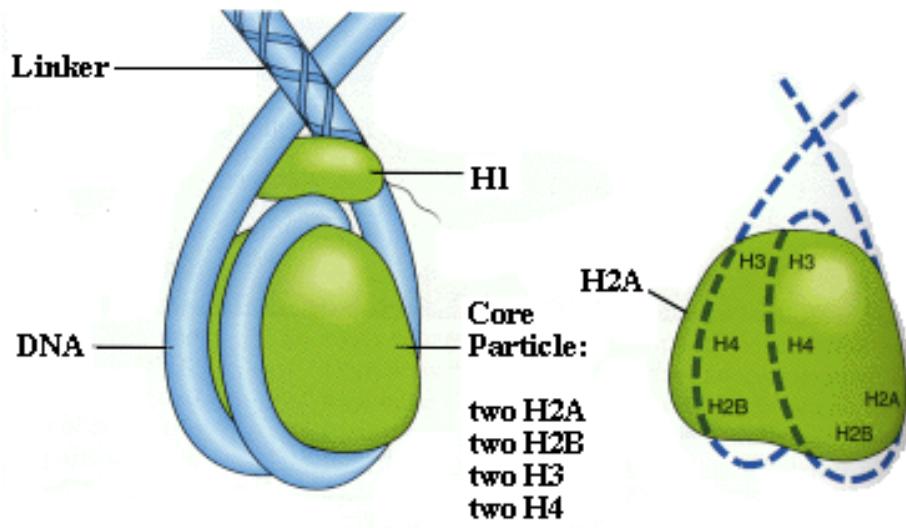
Nukleosomi

Histonska jedra

Histonske beljakovine



Nukleosomi

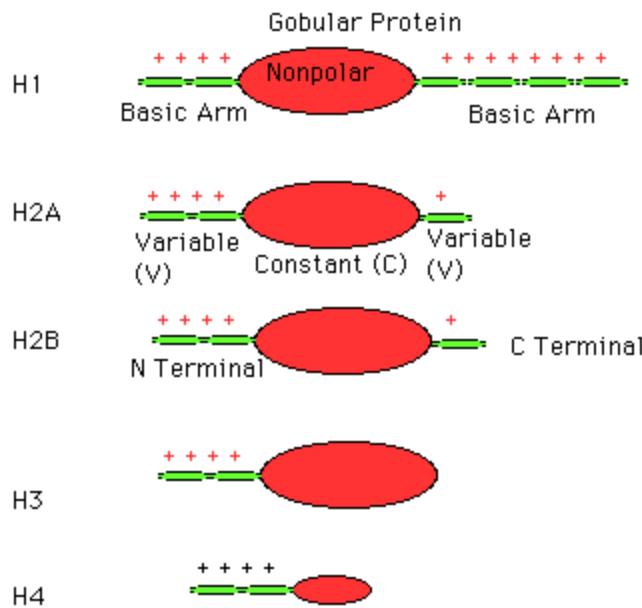


Histonske beljakovine

Histone type	Approximate Molecular Weight	Number of Amino Acids	Content (Basic amino acids)
H1	17,000 - 28,000	200-265	27% lysine, 2% arginine
H2A	13,900	129-155	11% lysine, 9% arginine
H2B	13,800	121-155	16% lysine, 6% arginine
H3	15,300	135	10%lysine, 15% arginine
H4	11,300	102	11% lysine, 4% arginine

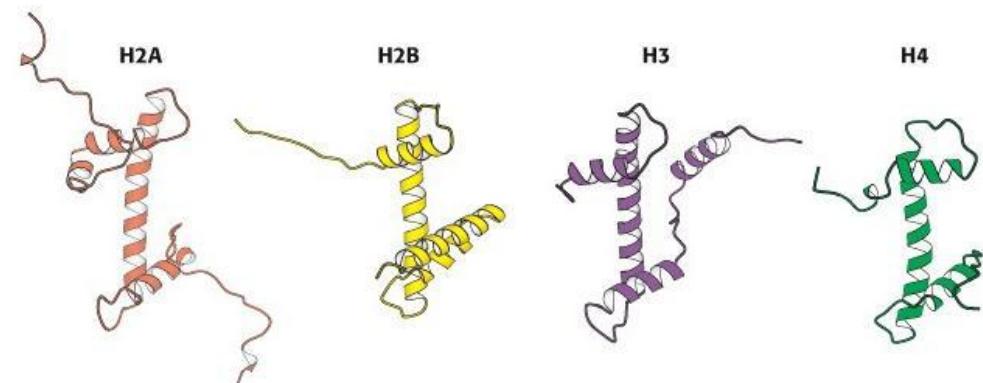
Histonske beljakovine

Structure of Histone Proteins

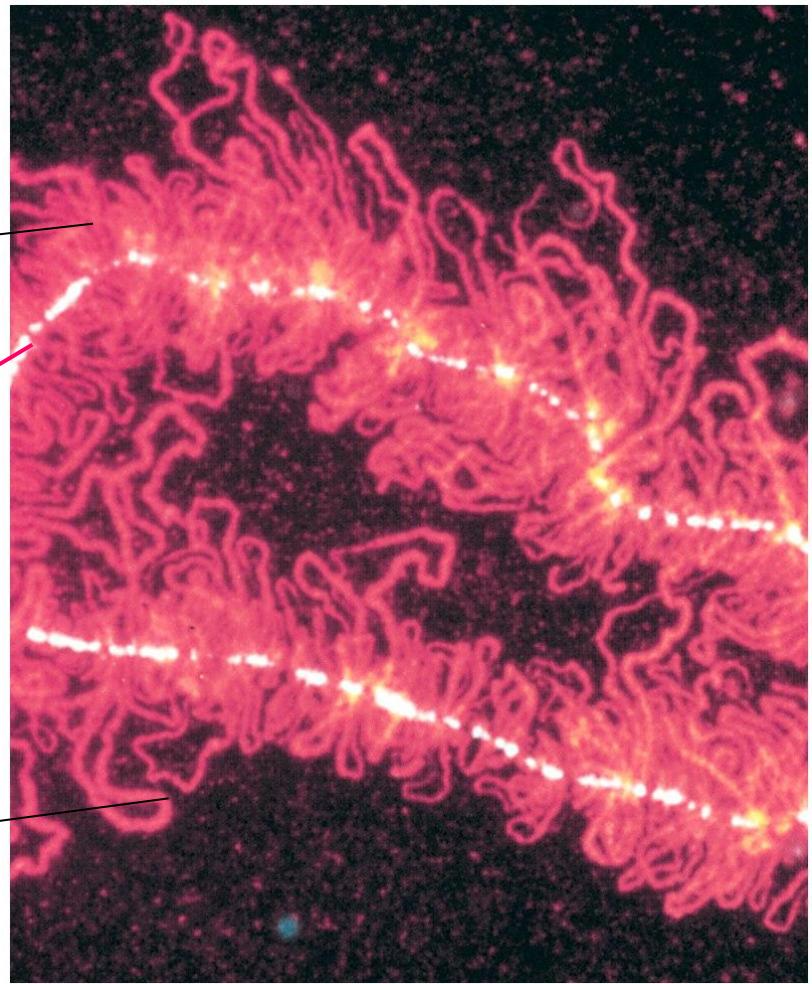
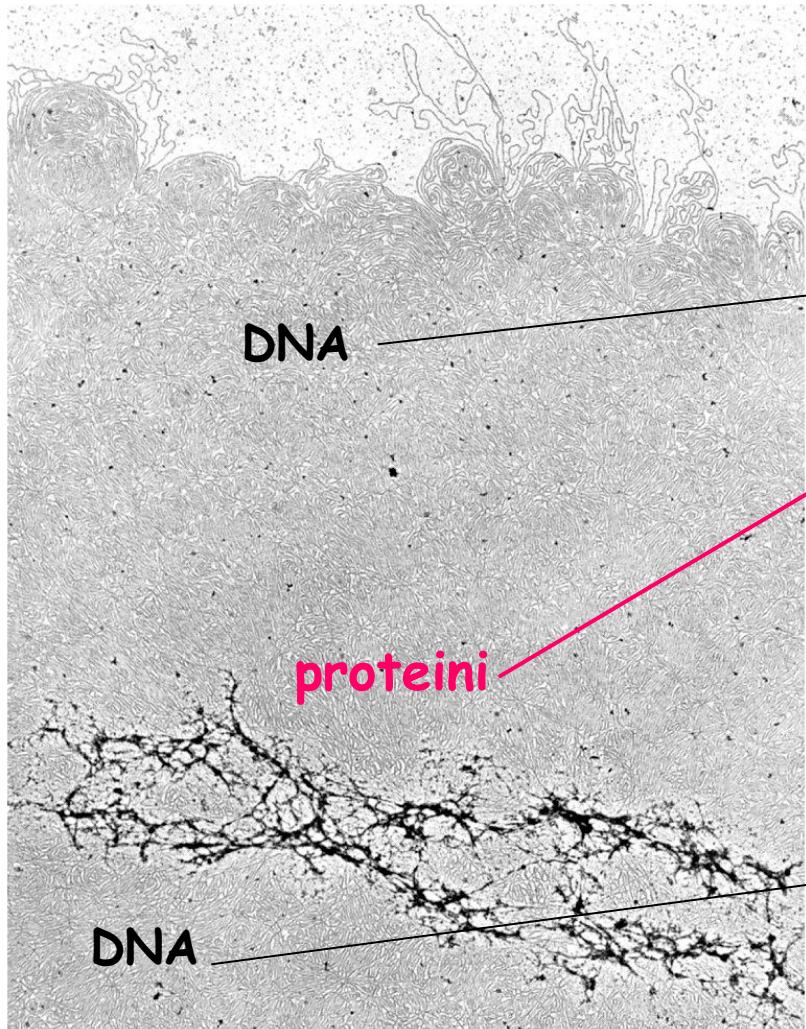


Histones 2(A,B), 3 and 4:

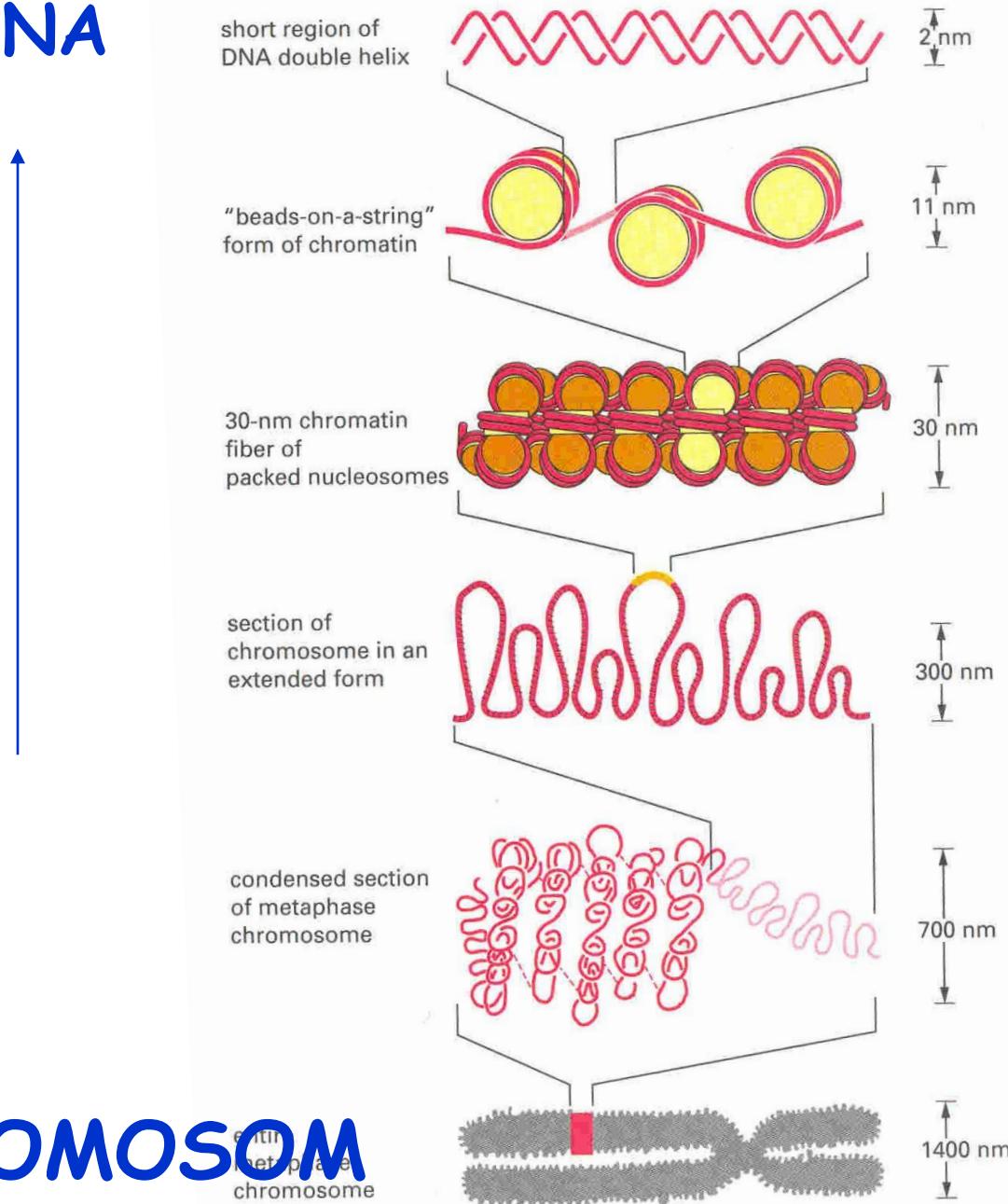
Conserved structures (and also amino acid sequences)



Nosilni proteini solenoida - proteinsko ogrodje; Ne-histonski proteini kromatina



DNA



KROMOSOM

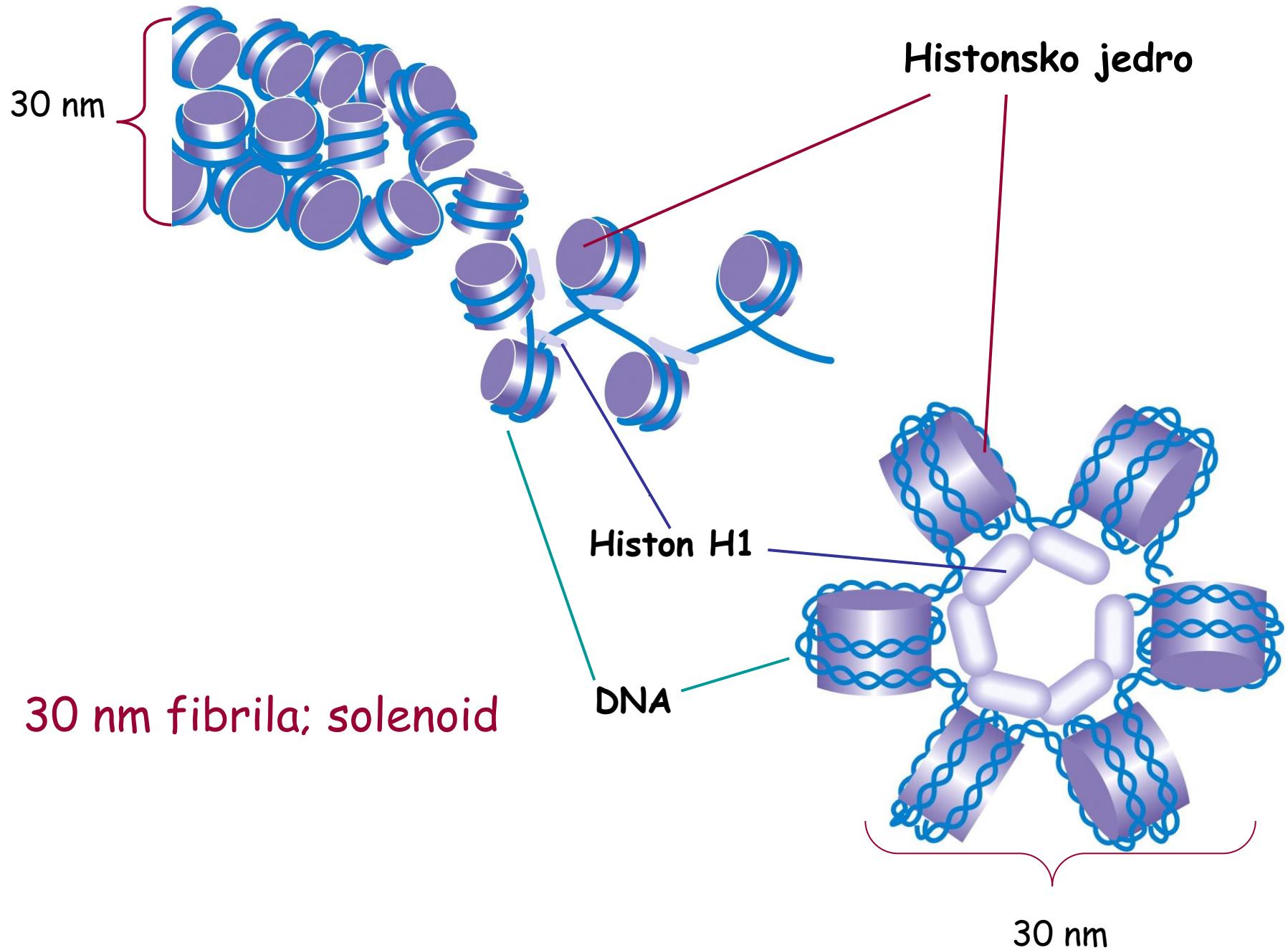
dsDNA

Nukleosomska oblika

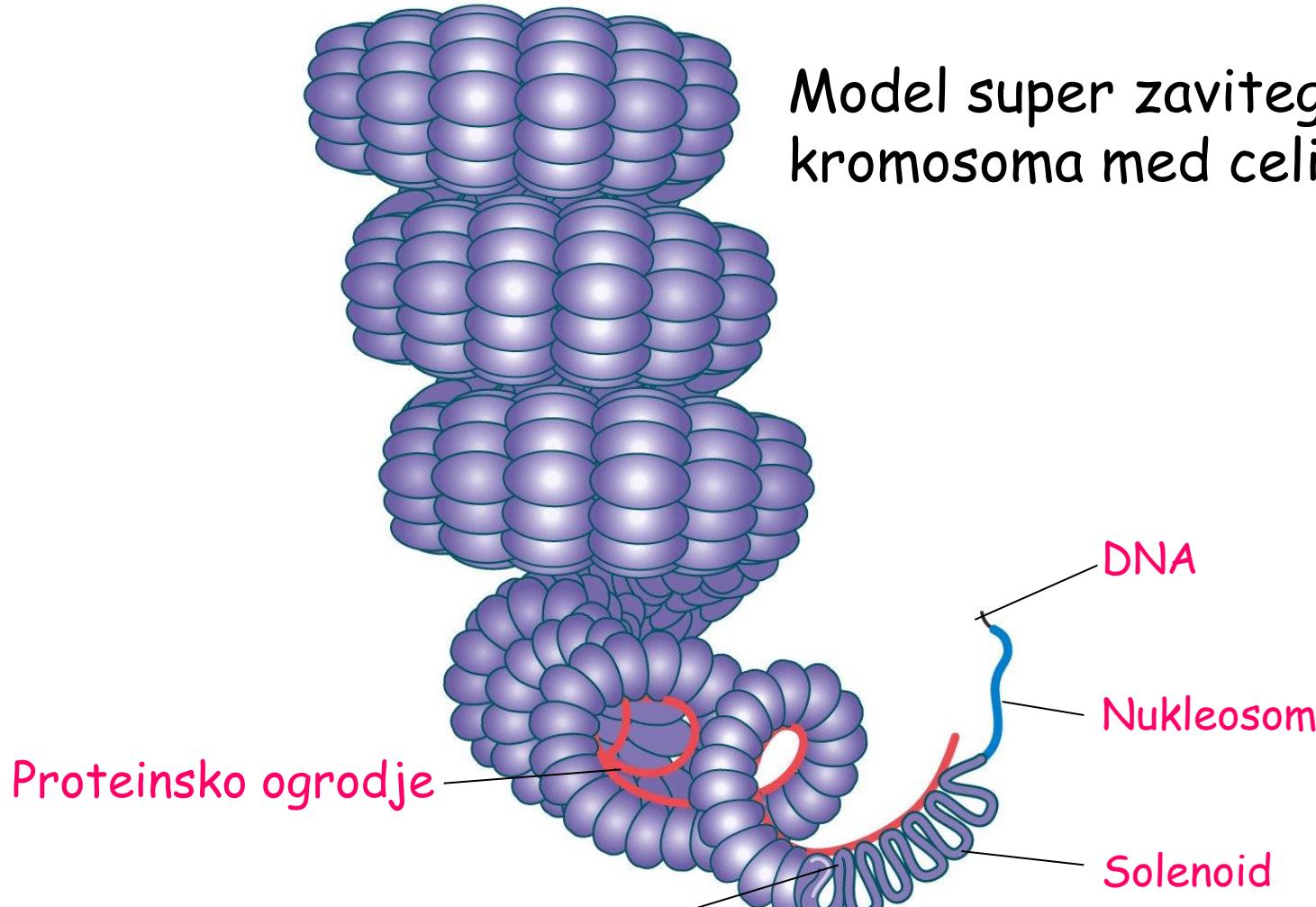
30 nm fibrila;
Solenoidna oblika

Solenoidne zanke

Kondenzirana oblika



Model super zavitega kromosoma med celično delitvijo



Specifične sekvence DNA za oprijem solenoidnih zank na proteine:
SARs: scaffold attachment regions ali
MARs: matrix attachment regions

Nivoji uravnavanja izražanja genov

TRANSKRIPCIJA

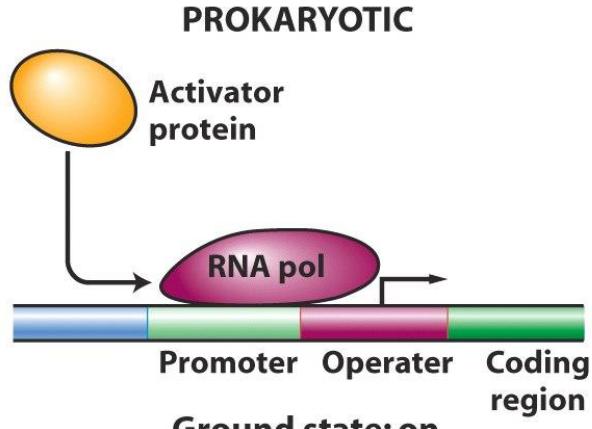
PROCESIRANJE RNA

TRANSPORT RNA

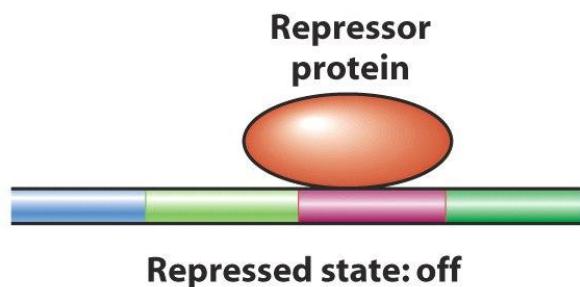
STABILNOST RNA

TRANSLACIJA

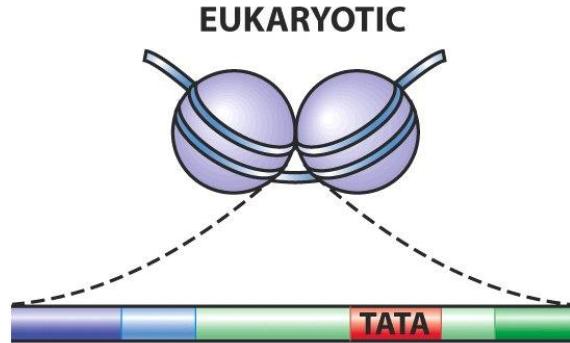
POST-TRANSLACIJSKE
MODIFIKACIJE



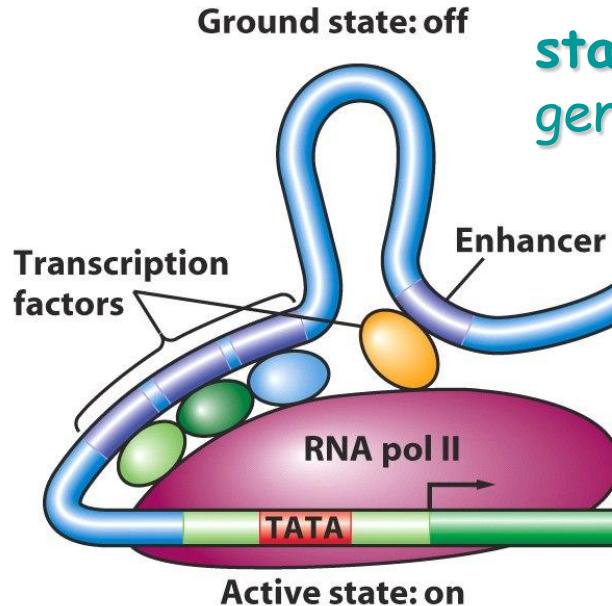
Osnovno stanje: gen se lahko prepisuje



Blokada: prepisovanje gena je onemogočeno



Osnovno stanje: nukleosomi; gen se ne prepisuje

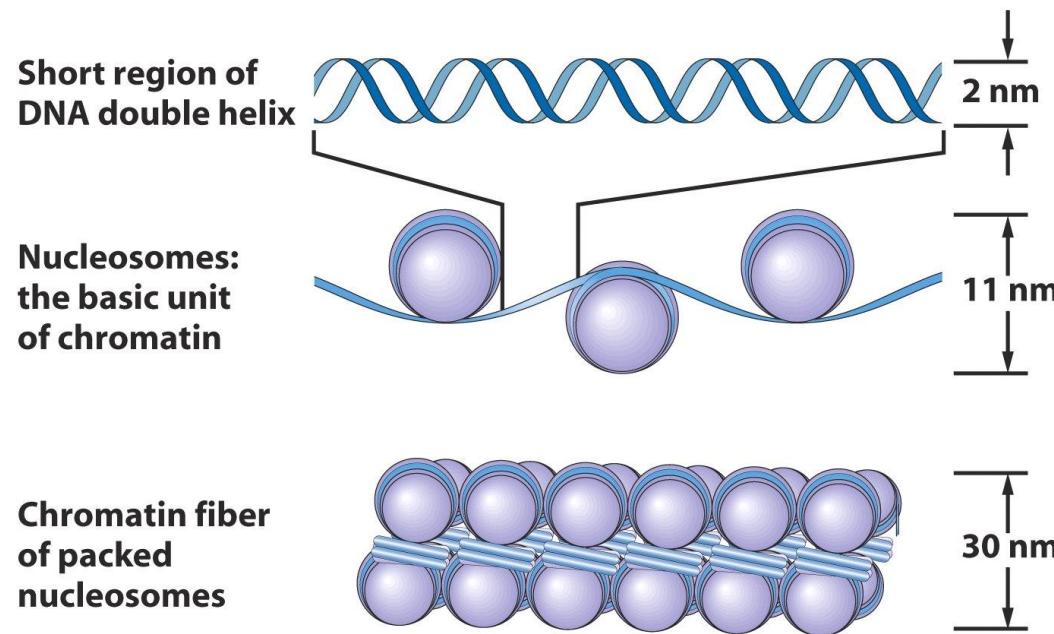


Aktivno stanje: gen se začne prepisuje

Uravanjanje transkripcije

Vloga kromatina pri uravnavanju izražanja evkariontskih genov

Kromatin ni uniform; visoko kondenzirane regije heterokromatina vsebujejo manj genov in imajo nižjo rekombinacijsko frekvenco kot evkromatinske regije. *Fenomeni inaktivacije X kr., imprintinga in pozicijskega efekta so pokazali na aktivno vlogo kromatina pri izražanju genov.*



Epigenetske spremembe

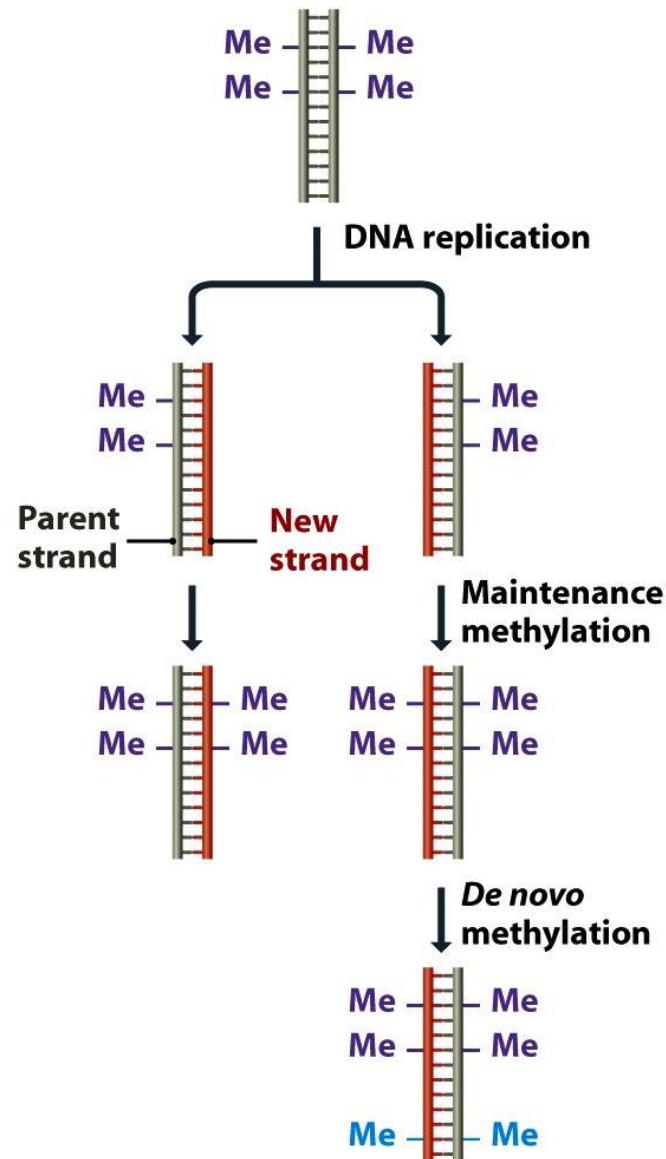
- spremembe v genski ekspresiji zaradi sprememb v kromatinski strukturi:
 - Metilacija DNA
 - Post-translacijske modifikacije histonov
 - Remodeliarnje kromatina

Metilacija DNA

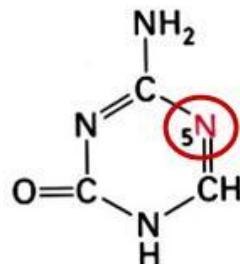
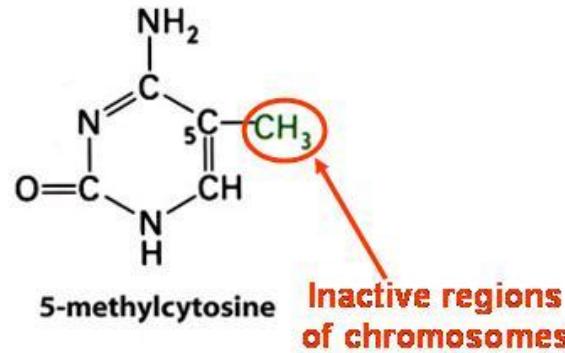
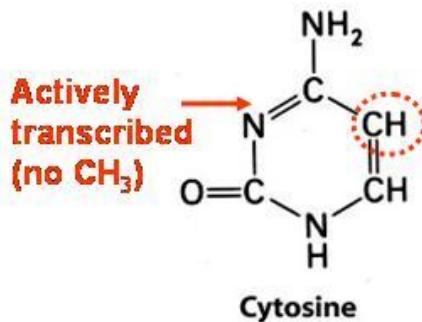
Značilna vloga pri normalnem razvoju organizma in pri razvoju bolezni

Metilacija DNA:

- Citozin - 5-metilcitozin
- DNA metiltransferaze
- 3% metiliranega C pri človeku (5'-CpG-'3),
- Vzdrževalna metilacija** - ohranjanje metilacije med replikacijo
- **de novo metilacija**, dodajanje CH_3 na nove citozine
- mCpG se nahajajo v skupinah (otoki) ali zunaj teh skupin. CpG izven otokov so večinoma metilirani.



Actively transcribed regions of chromosomes are under-methylated



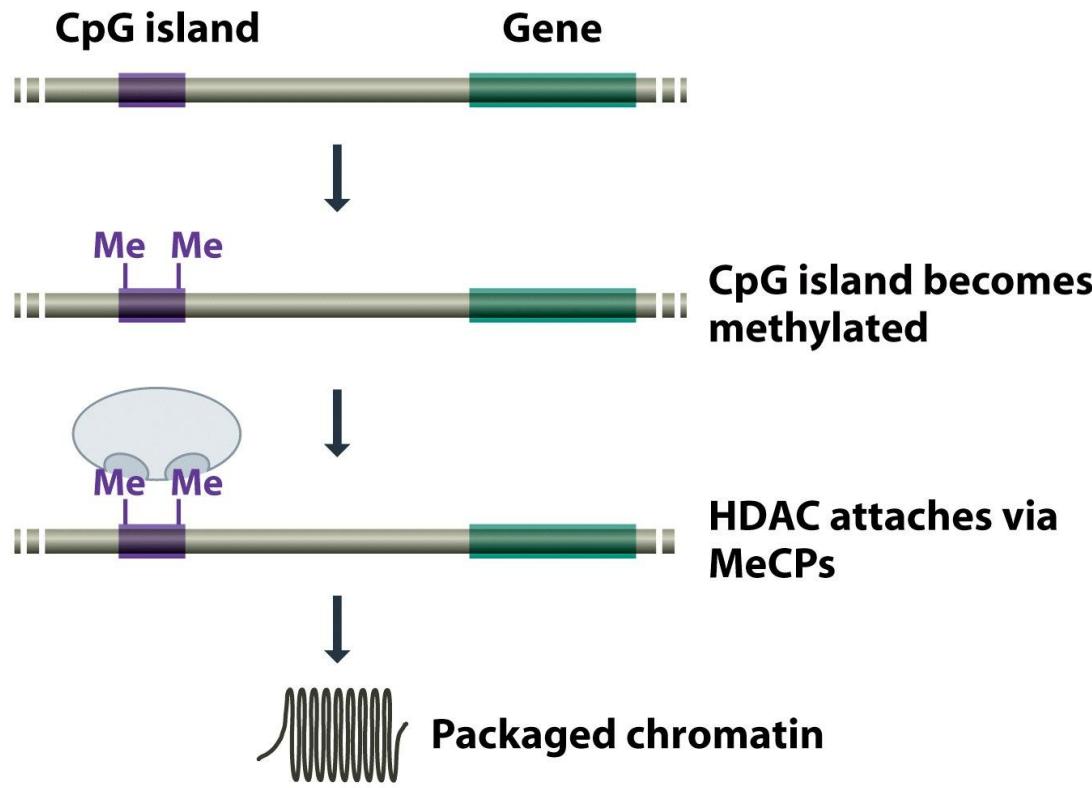
Cytosine analog:
Gets into DNA and
activates genes
(can't be methylated)

DNA metiltransferaze

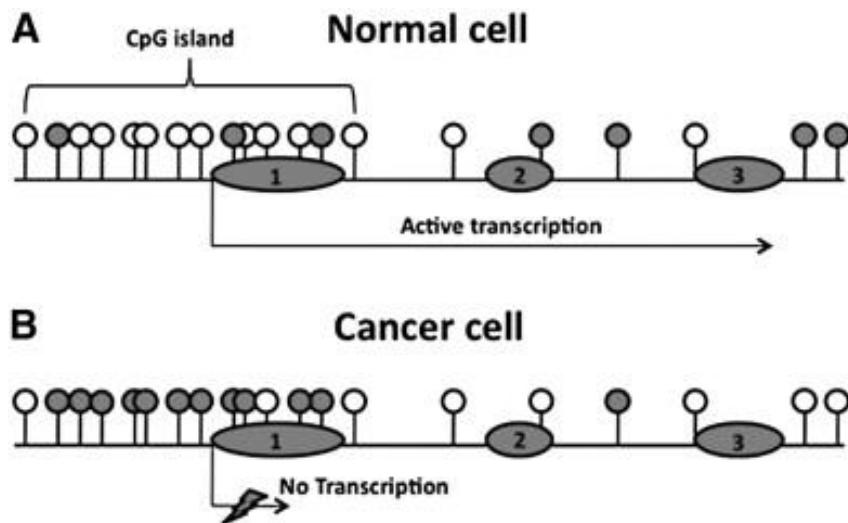
- De novo metilacija DNMT3A, DNMT3B
- vzdrževalna metilacija, DNMT1

Metil-DNA vezavni proteini

- MBD proteini (methylated DNA-binding domain)



Vzdrževalna in de novo metilacija utišata gene. CpG otoki na zgornji strani gena se metilirajo in vežejo MeCP oz. MBD (Metil-CpG-vezne proteini), ki so del histonskega deacetilacijskega kompleksa (HDAC). HDAC kompleks se aktivira, deacetilira histone in zavije kromatin. CpG otoki znotraj gena in med geni so verjetno znak za začetek transkripcije ncRNA.



[European Journal of Pharmacology](#)

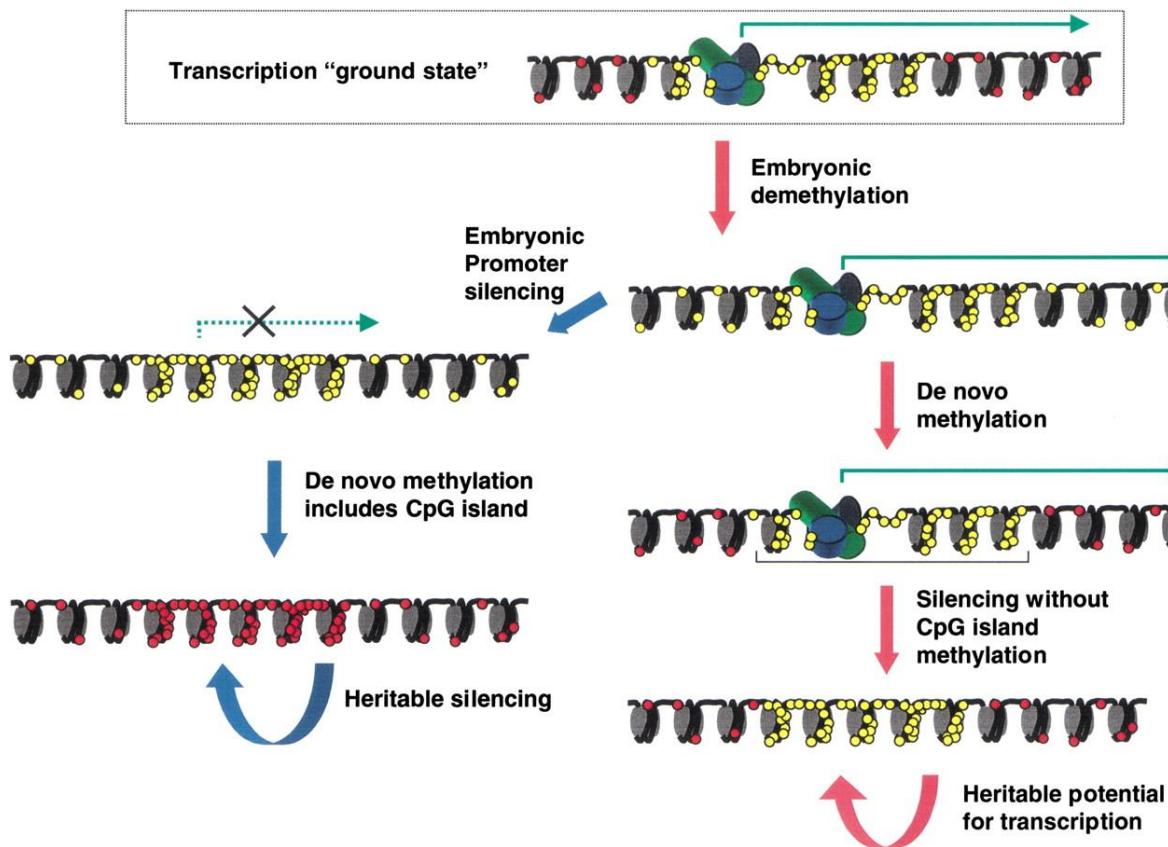
Volume 625, Issues 1-3, 25 December 2009, Pages 131-142

Epigenetics and cancer treatment

Mechanisms of Disease: neurogenetics of MeCP2 deficiency

Nature Reviews Neurology 2, 212-221 (April 2006) | doi:10.1038/ncpneuro0148

A hypothetical scenario relating embryonic transcriptional activity to DNA methylation status in mammals.

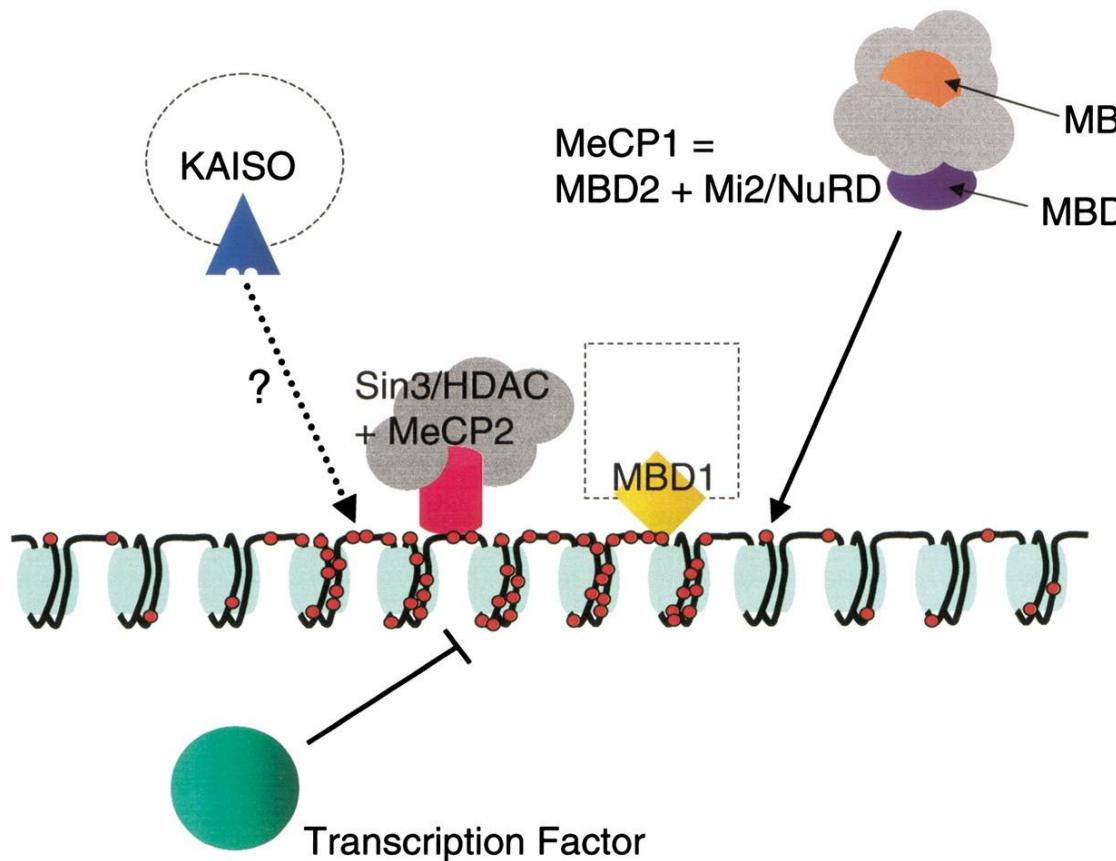


Starting from a notional transcription ground state, embryonic demethylation leads to substitution of **methylated sites (red circles)** by **nonmethylated sites (yellow circles)**. Two alternative fates are then envisaged: either transcription persists leading to restoration of the unmethylated CpG island (bracket) flanked by methylated non-island-flanking DNA (pink arrows); or transcription is extinguished by other mechanisms in the embryo and this invites de novo methylation of the CpG island and its flanks. In this way the activity of embryonic promoters is imprinted for the duration of that somatic lifetime.

Bird A Genes Dev. 2002;16:6-21



Mechanisms of transcriptional repression by DNA methylation.

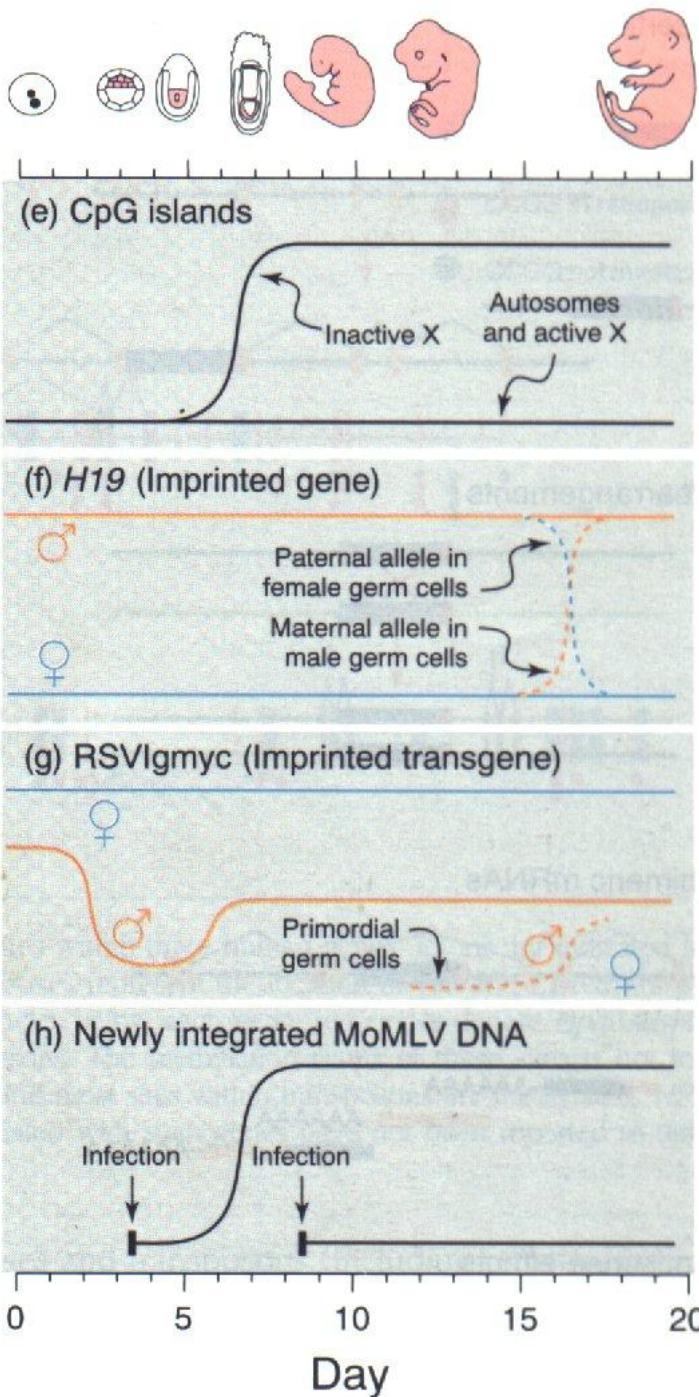
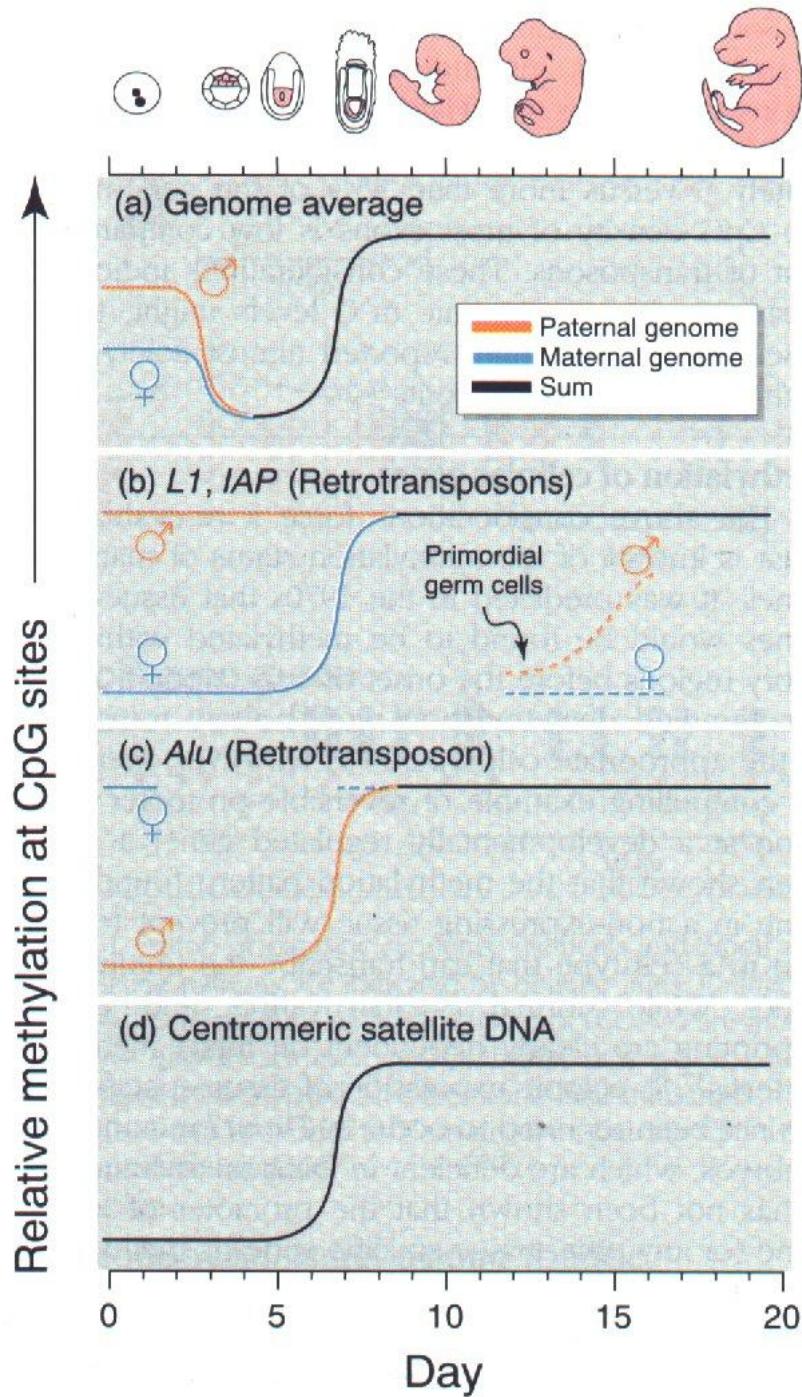


Sin3 (sesalci): HDAC1 in HDAC2, 5 pomožnih proteinov; prepoznavanje metilirane DNA z MeCP

NuRD (sesalci): HDAC1 in HDAC2, drugi set pomožnih proteinov; prepoznavanje metilirane DNA; tudi sposobnost kromatinskega remodeliranja

A stretch of nucleosomal DNA is shown with all **CpGs methylated (red circles)**. Below the diagram is a transcription factor that is unable to bind its recognition site when a methylated CpG is within it. Many transcription factors are repelled by methylation. Above the line are protein complexes that can be **attracted by methylation**, including the methyl-CpG-binding protein MeCP2 (plus the **Sin3A** histone deacetylase complex), the MeCP1 complex comprising MBD2 plus the **NuRD** corepressor complex, and the uncharacterized MBD1 and Kaiso complexes. MeCP2 and MBD1 are chromosome-bound proteins, whereas MeCP1 may be less tightly bound. Kaiso (methylated repression) has not yet been shown to associate with methylated sites *in vivo*.





Post-translacijske modifikacije histonov

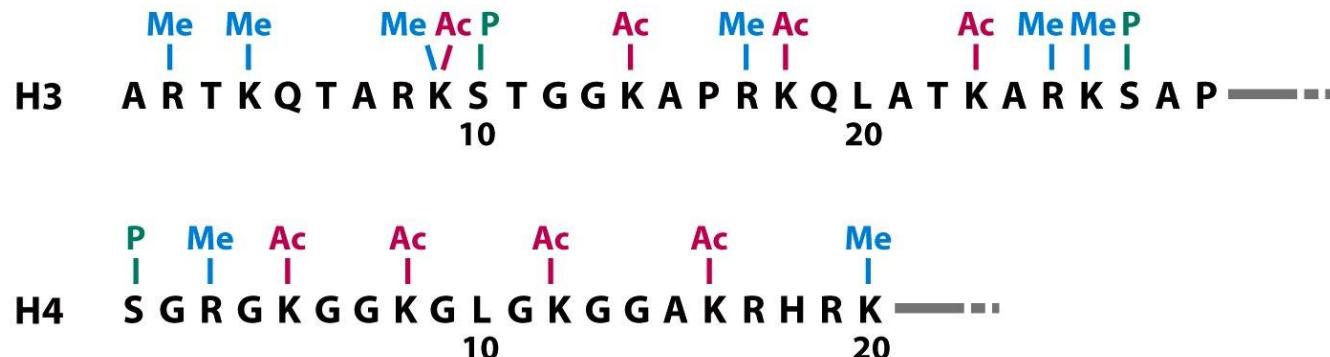
PT modifikacije histonov

- Acetilacija / deacetilacija histonov

HAT : histonska acetiltransferaza

HD : histonska deacetilaza

- Metilacija, fosforilacija, ubikvitinacija

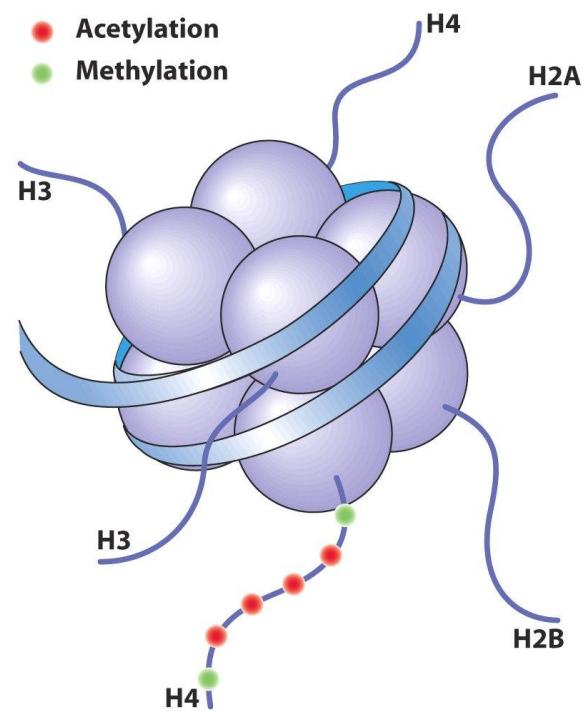
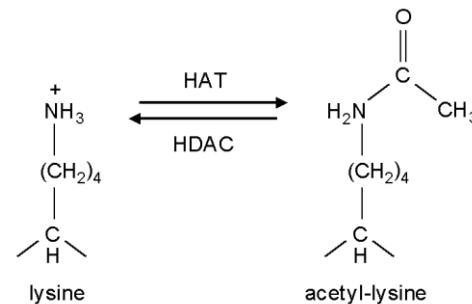


Modifikacije na histonih pritegnejo oz.
odbijajo različne proteine

Histoni:

- Univerzalni gradniki nukleosomov
 - Prosti N-terminalni konci histonskih verig (histonski repi)
 - Reverzibilne post-translacijske modifikacije AK v histonskih repih (histonski kod)
 - Acetilacija
 - HAT: acetil transferaza
 - HDAC: histonska deacetil transferaza
 - Aktivni geni: histoni acetilirani
 - Neaktivni geni: histoni ne-acetilirani (kondenziran X kr.)

Histonski kod



Acetilacija / deacetilacija histonov

Aktivni geni: acetilirani histoni

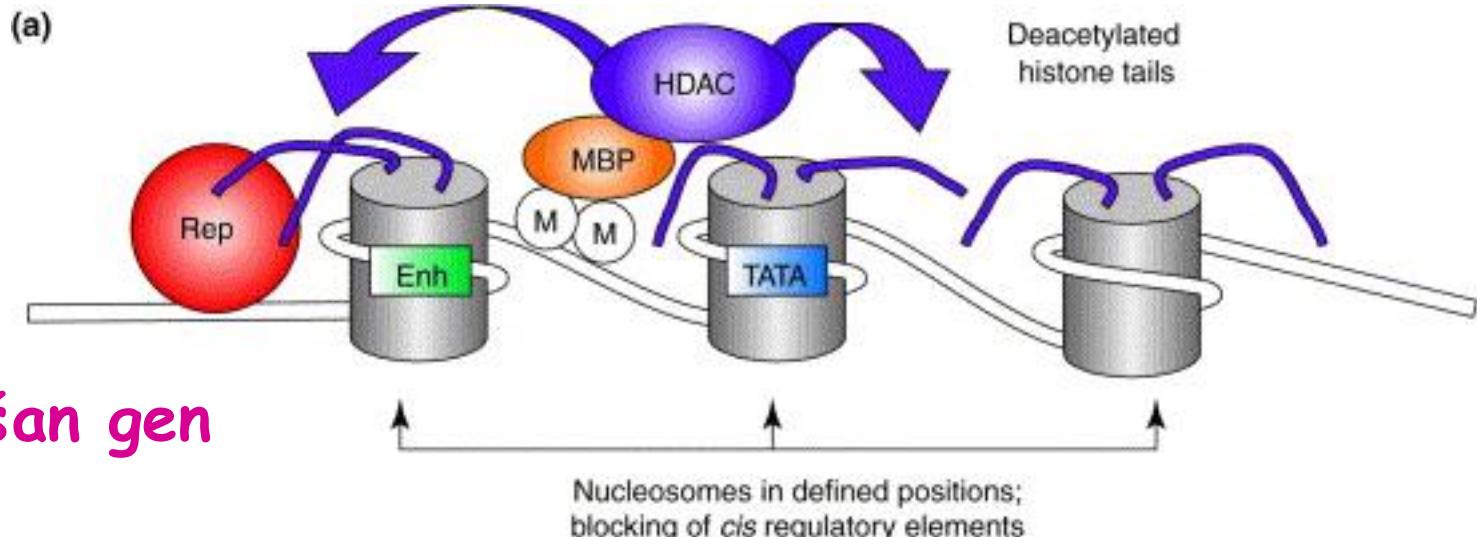
HAT : histonska acetiltransferaza,
sp. encimi za posamezne K v H4 oz. H3; npr. **GCN5**
H3(K9, K14, K18)

Utišani geni, deacetilirani histoni

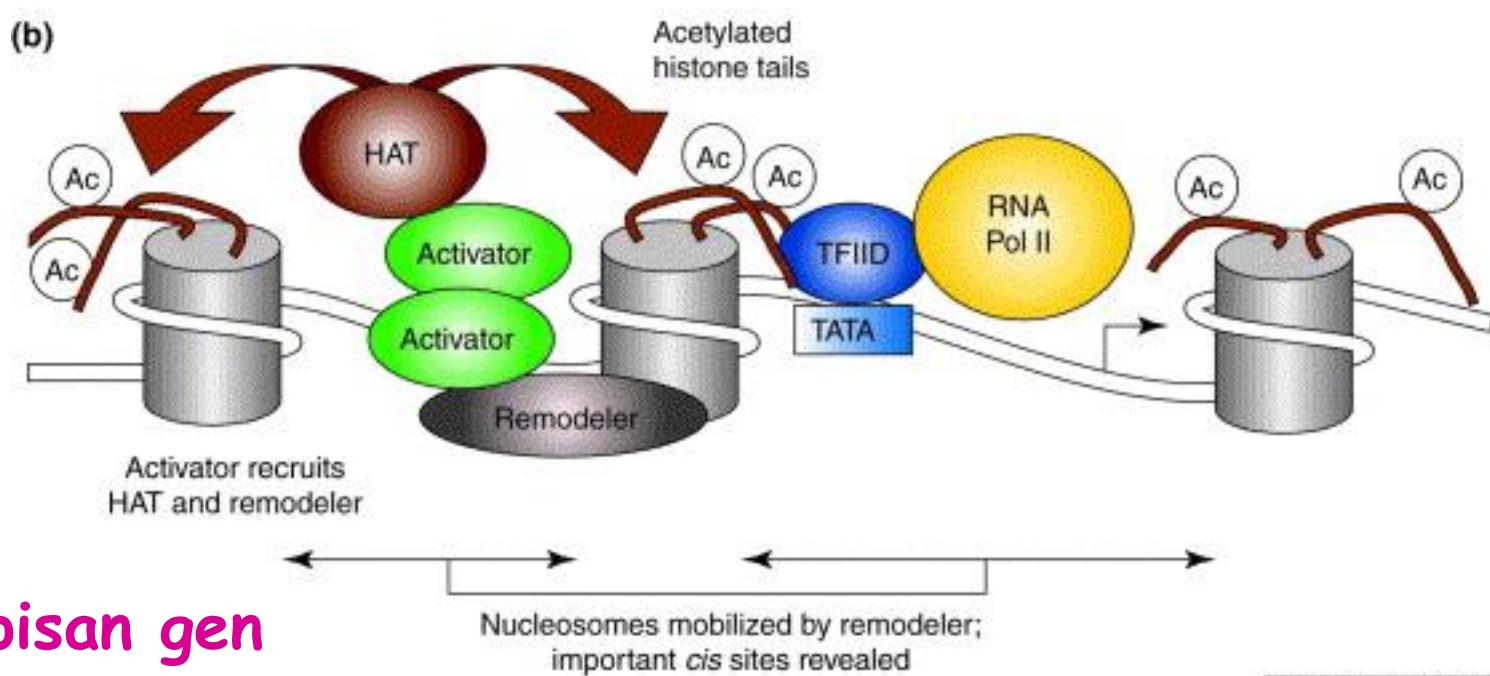
HDAC : histonska deacetilaza + proteini
Sin3 (sesalci): HDAC1 in HDAC2, 5 pomožnih proteinov;
prepoznavanje metilirane DNA z MeCP

NuRD (sesalci): HDAC1 in HDAC2, drugi set pomožnih
proteinov; prepoznavanje metilirane DNA; tudi
sposobnost kromatinskega remodeliranja

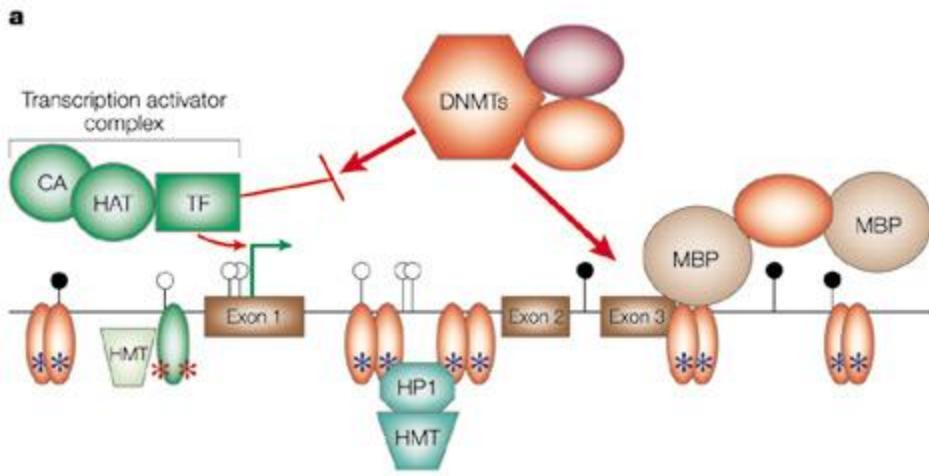
Sir2 (kvasovke): HDAC in proteini



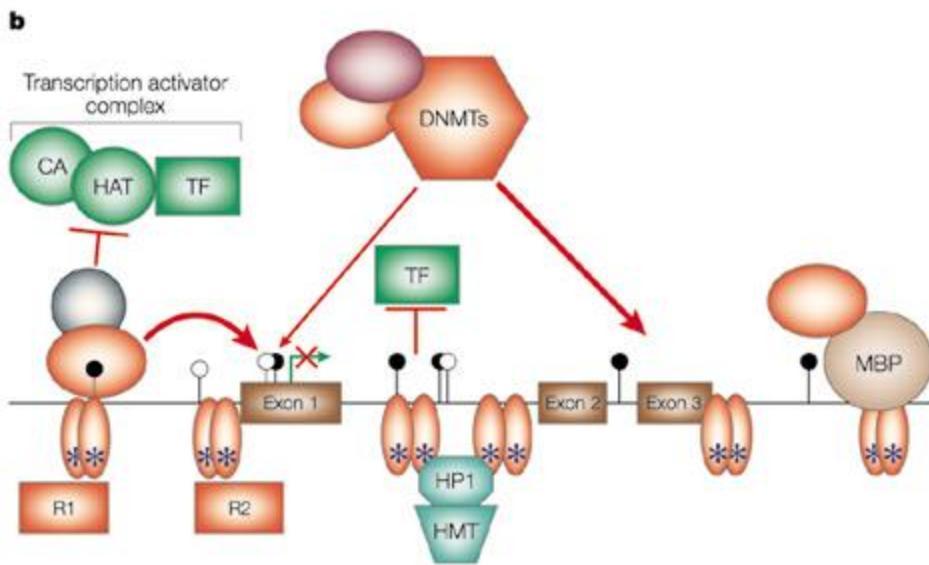
Utišan gen



Prepisani gen

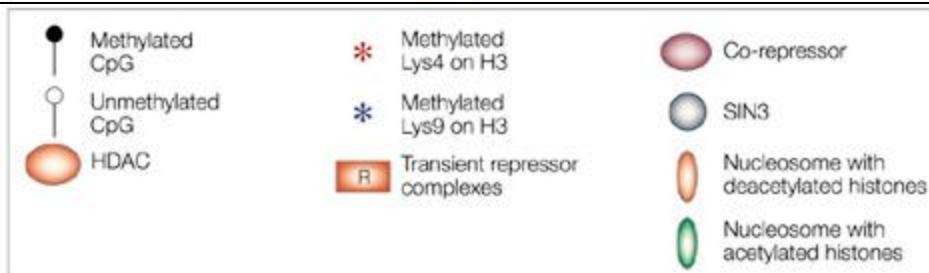


Transkripcijsko aktiven kromatin

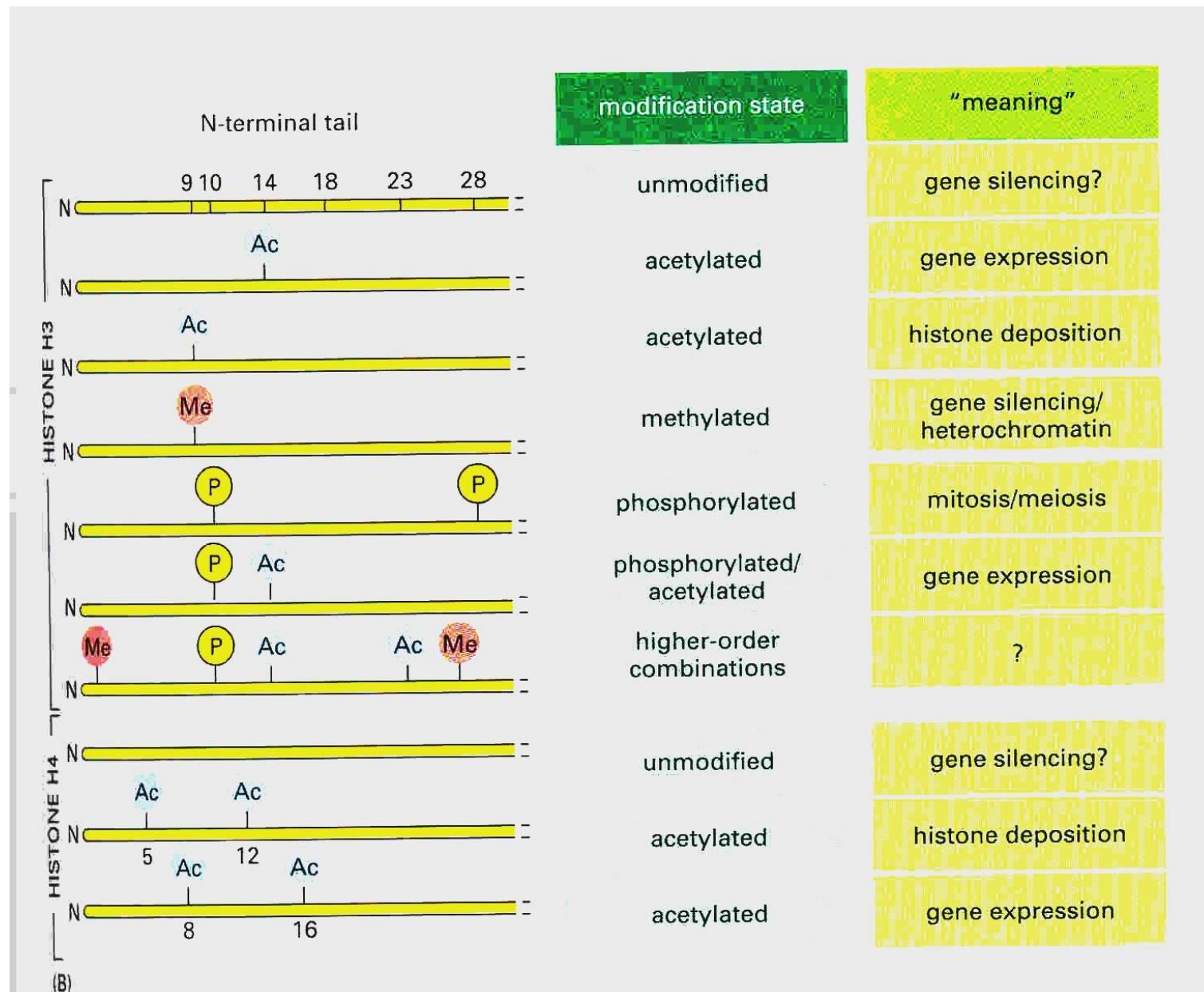


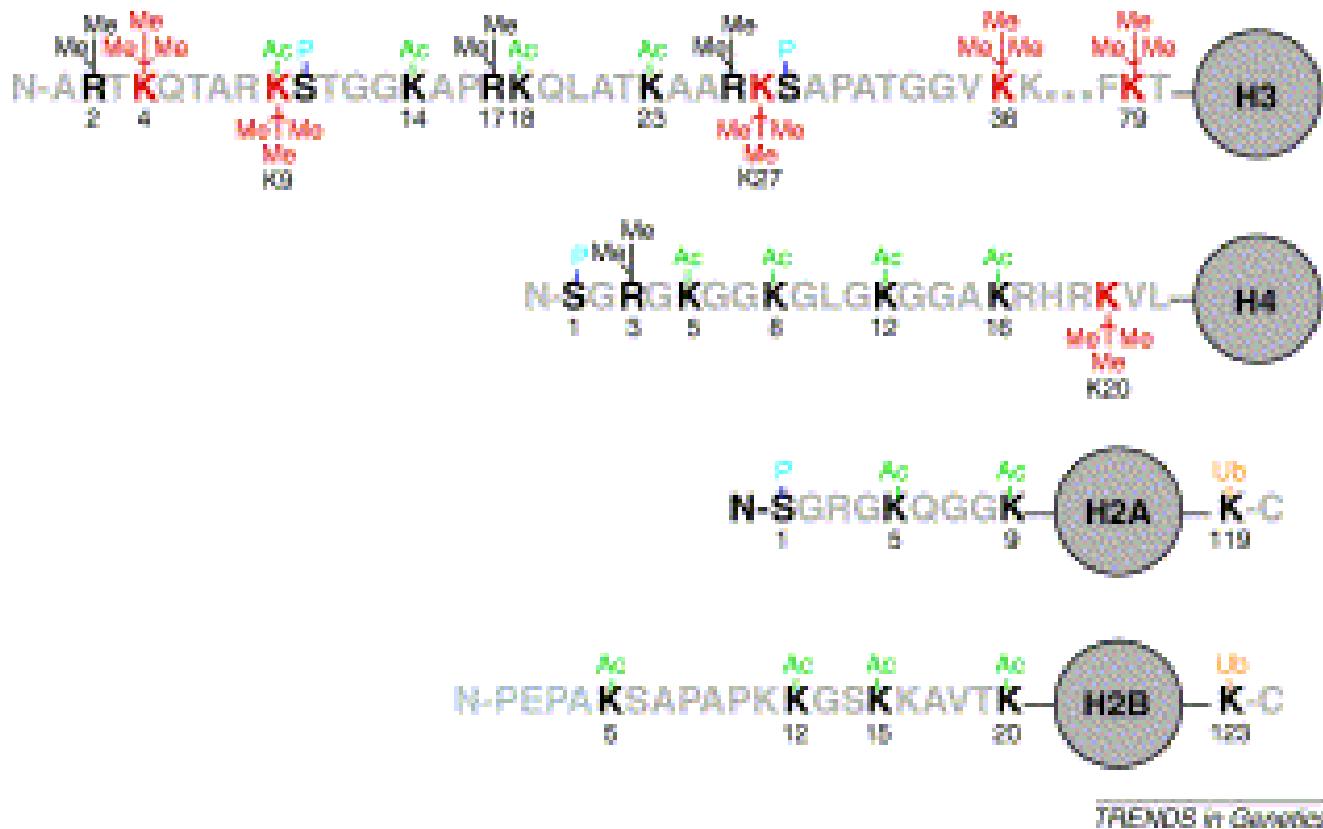
Transkripcijsko neaktiven kromatin

HAT: histonska acetilaza; **CA:** ko-aktivator;
DNMT: DNA metil transferaza; **HDAC:** histonska deacetilaza; **MBP:** metil-citozin vezni proteini;
SIN3: transkripcijski ko-represor; **HMT:** histonska metiltransferaza; **HP1:** ?protein



Modifikacije histonskih "repov" in njihov pomen





Post-translacijske kovalentne modifikacije histonov

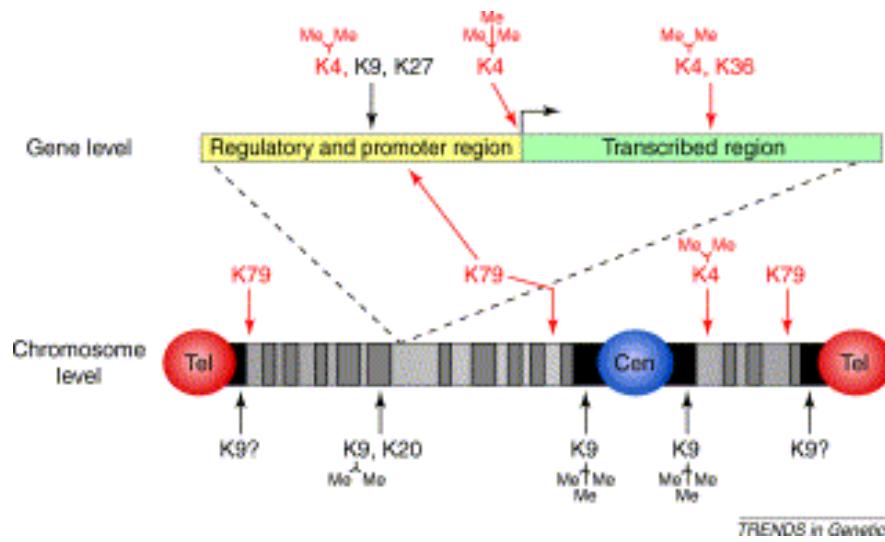
K, **K**: Lys; R: Arg; Me: metilacija; **Ac**: acetilacija; **P**: fosforilacija

HKMTs: histon lizin metiltransferaze

H3: K4, K9, K27; K36; K79

H4: K20

TiG, 2003, 19:629



TRENDS in Genetics

Metilacija lizina v histonu H3: histon lizin metiltransferaze, HKMT

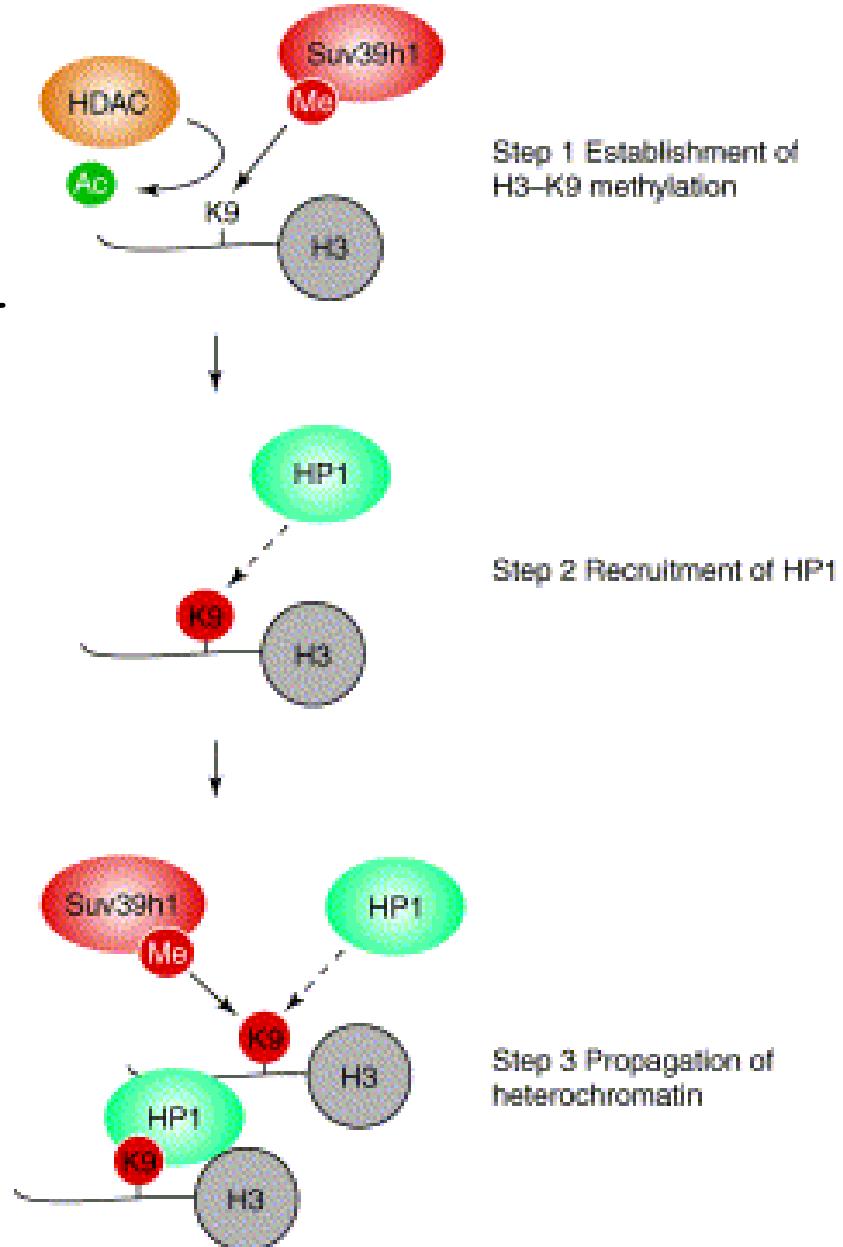
Suv39h1 : H3K9
 ASH1 : H3k4
 EZH2 (PcG) : H327

Aktivni geni: H3K4me3, H3K35me3, H3k79m3 (> acetilacija)

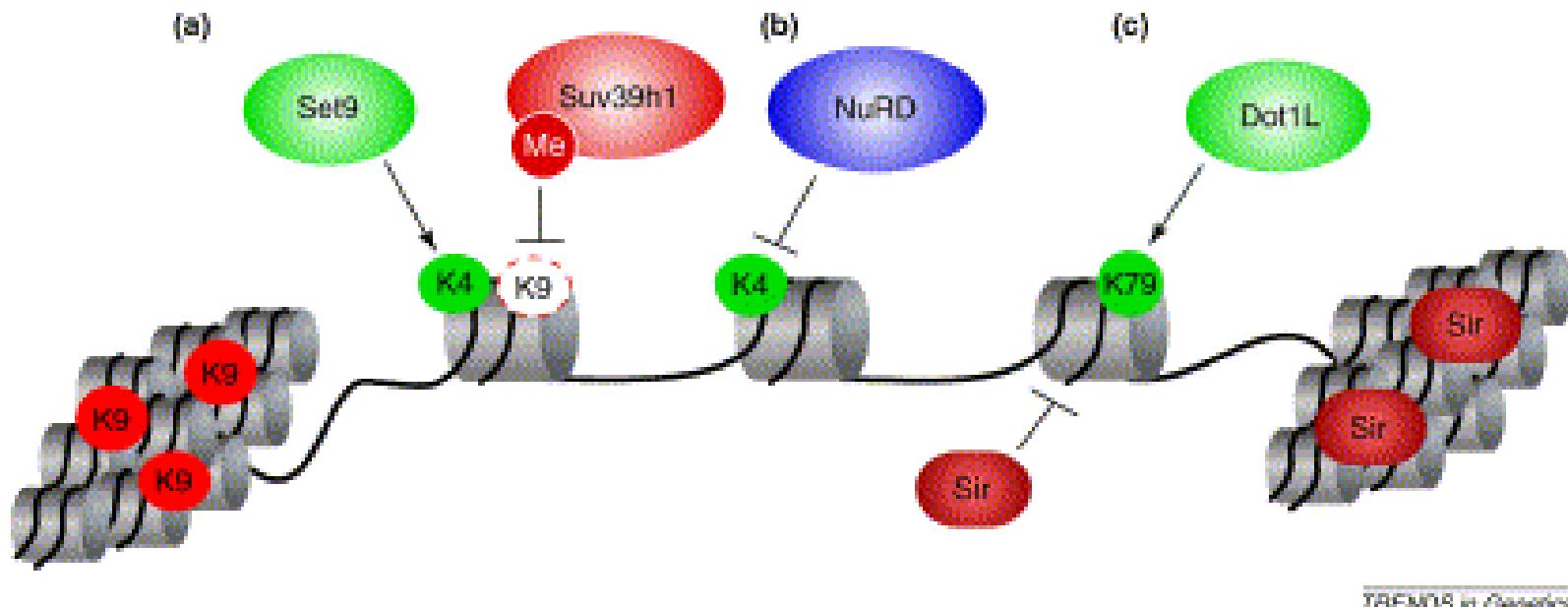
Utišani geni: H3K9me3, H3k27me3 (< acetilacija)

Vzpostavitev in vzdrževanje metilacije H3-K9

- 1) deacetilacija (HDAC) H3-K9, metilacija (HKMT - SUV39H1) H3-K9
- 2) HP1 selektivno prepozna H3-K9me.
- 3) propagacija heterokromatina: HP1 (več mol.) rekrutira Suv39H1 itd
-
-



- a) H3-K4Me prepreči Me H3-K9; b) NuRD ne veže H3
 b) c) Me z Dot1L prepreči vezavo Sir (evkromatinska meja)



TRENDS in Genetics

Suv39h1: histon lizin metiltransferaza (HKMT), H3-K9

Set9: HKMT H3-K4

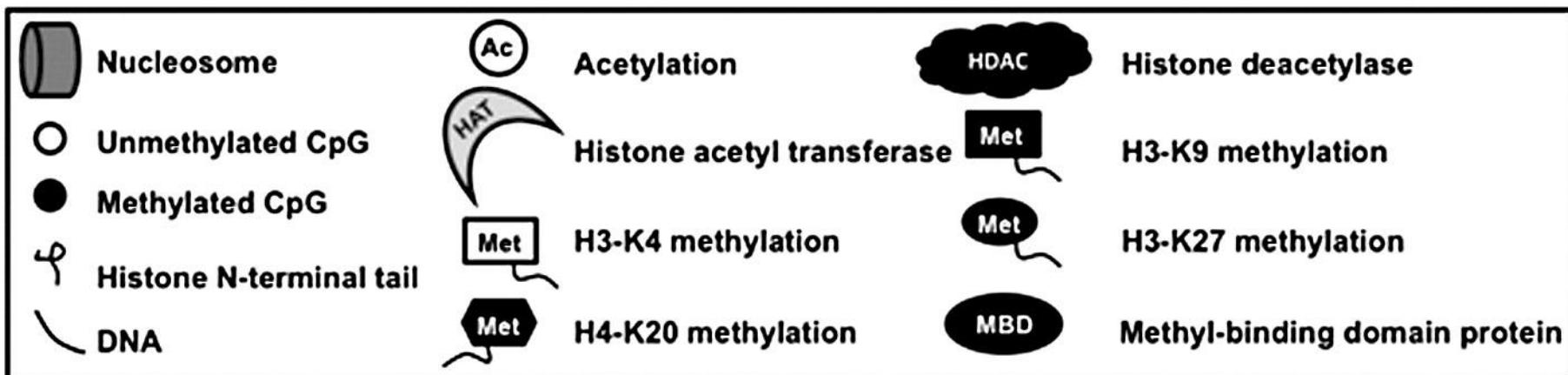
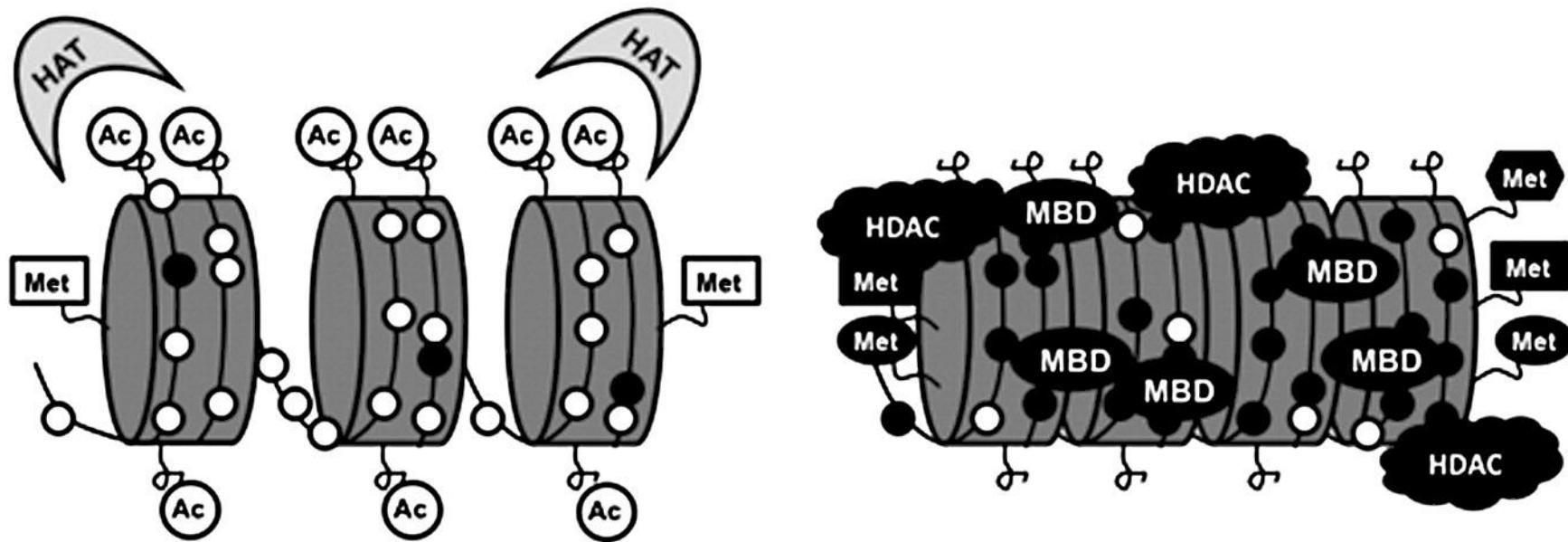
NuRD: histonski deacetilazni kompleks nukleosomskega remodeliranja (priprava Lys za Me)

Dot1L: HKMT H3-K79 (disrupter of telomeric silencing)

Sir: protein za utišanje kromatina

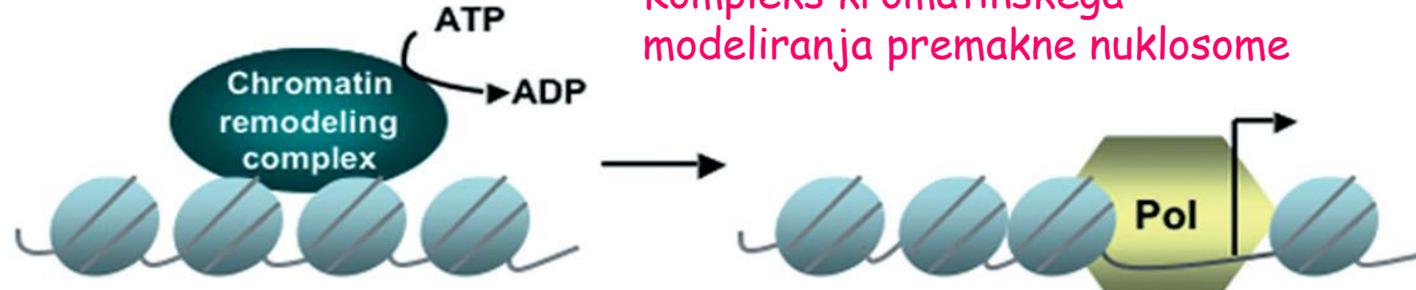
Vloga metilacije Lys 4 in Lys 79 na H3 za preprečitev utišanja kromatima z H3-K9 oz. Sir (kvas in sesalci)

Figure 5. Function of lysine 4 on histone 3 (H3-K4) and lysine 79 on histone 3 (H3-K79) methylation in the prevention of silenced chromatin. **(a)** Functional interplay between histone 3 and lysine 9 (H3-K4) and H3-K9 methylation. Methylation of H3-K4 prevents Suv39h1 from methylating H3-K9, thereby halting HP1-mediated heterochromatin spreading. Set 9 catalyzes the methylation of H3-K4 ([Table 1](#)) **(b)** The histone deacetylase nucleosome remodeling complex (NuRD) does not bind the histone H3 tail in the presence of H3-K4 methylation [\[54\]](#) and [\[55\]](#). These processes (a,b) occur in mammals and *Schizosaccharomyces pombe*. **(c)** H3-K79 methylation by the Dot1L protein blocks Sir-mediated silencing, thus participating in the creation of a euchromatic boundary. This process (c) occurs in mammals and *Saccharomyces cerevisiae*.

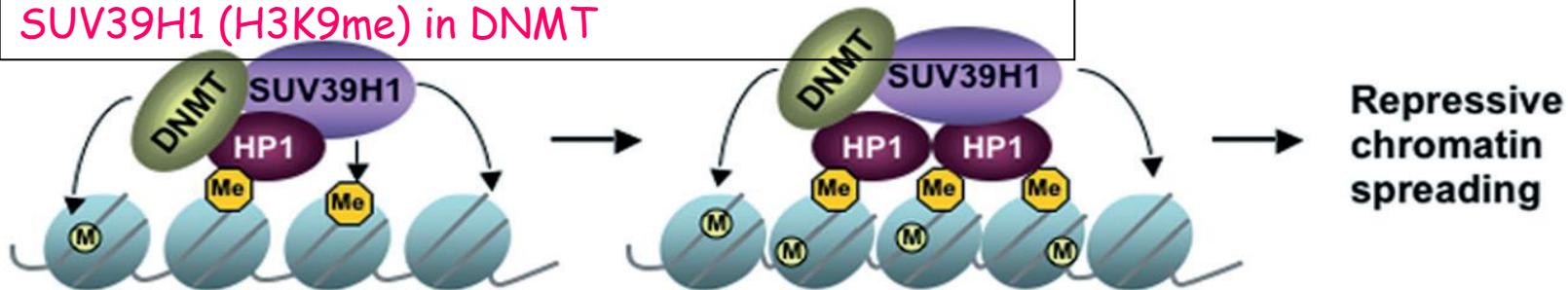


Proteini pomembni za
epigenetsko gensko regulacijo

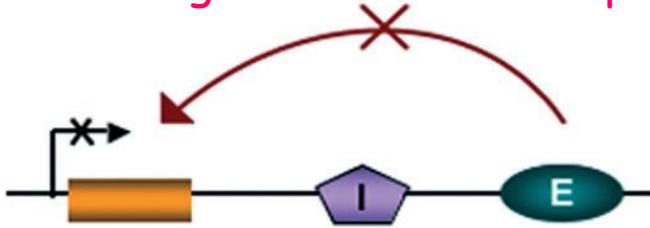
- Proteini pomembni za epigenetsko gensko regulacijo;
 - Kompleks kromatinskega remodeliranja (> energija, spreminja pozicijo nukleosomov, interakcije DNA-histoni)
 - Efektorski proteini (modifikacije histonov - vezava ef. proteina (HP1, PcG), rekrutiranje encimov - Hp1-SUV39H1..)
 - Insulatorski elementi (blokiranje ojačevalca, formiranje kromatinskih barier)

A**B**

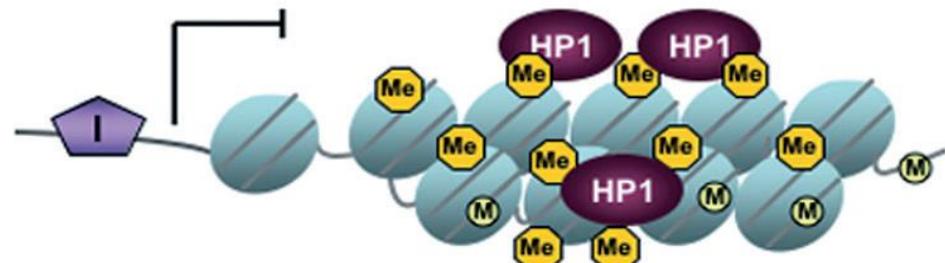
H3K9me3 pritegne HP1, na katerega se veže SUV39H1 (H3K9me) in DNMT

**C**

Vloga insulatorskih zaporedij (I)



Enhancer blocking



Proteini za remodeliranje kromatina

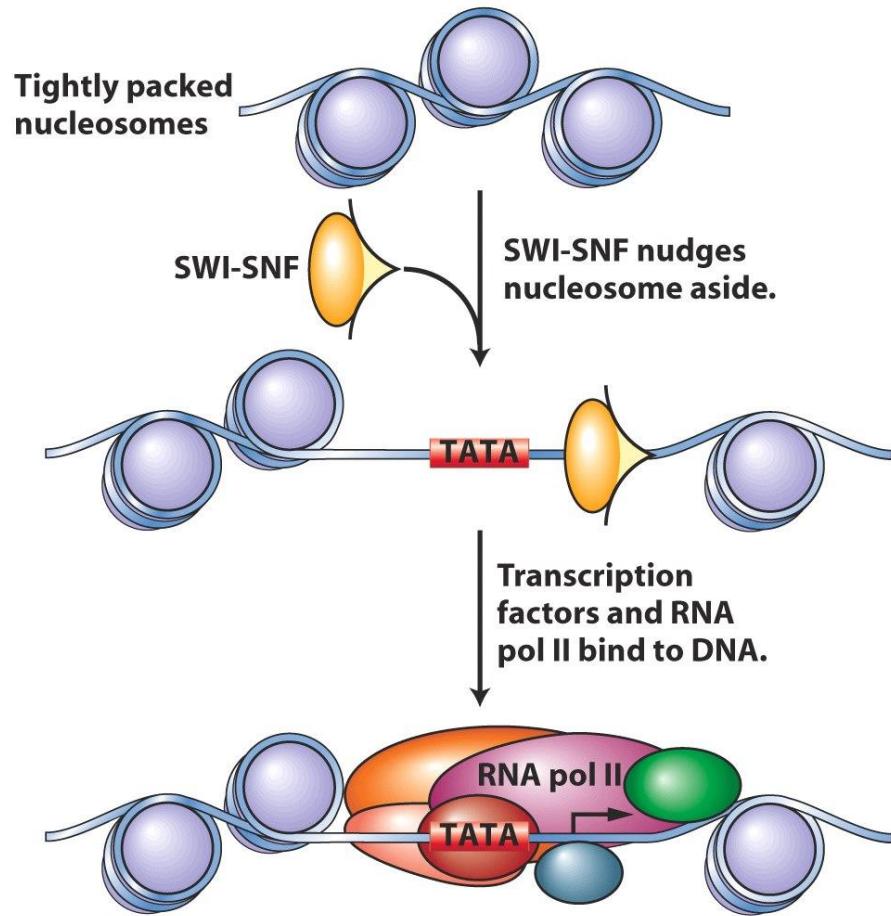
Mutacije kvasa;

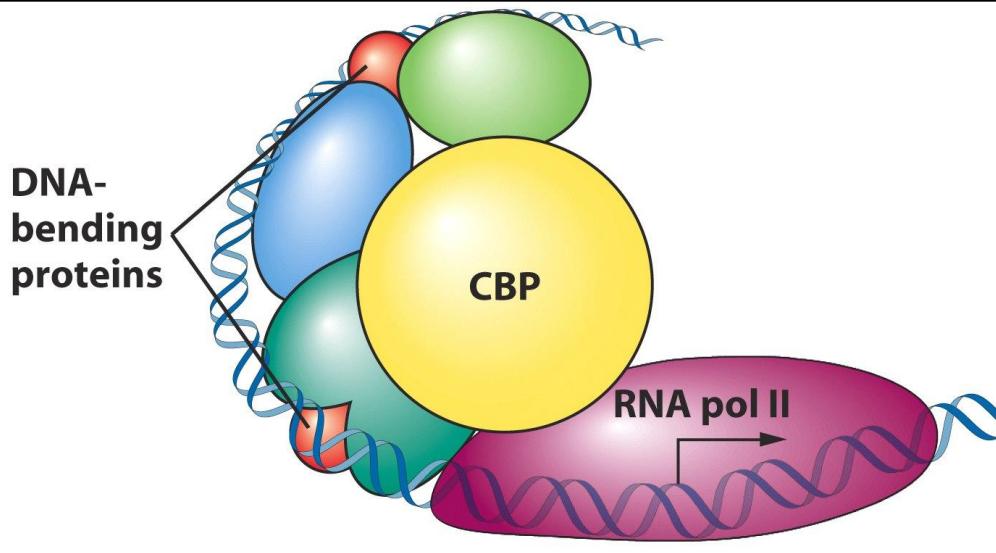
Mutant *swi-snf* (*swi*: mutacija v paritvenemu tipu; *snf*: mutacija glede fermentacije sladkorja)

SWI-SNF protein:

Del velikega proteinskega kompleksa, ki lahko spremeni položaj nukleosomov skupaj z ATP.

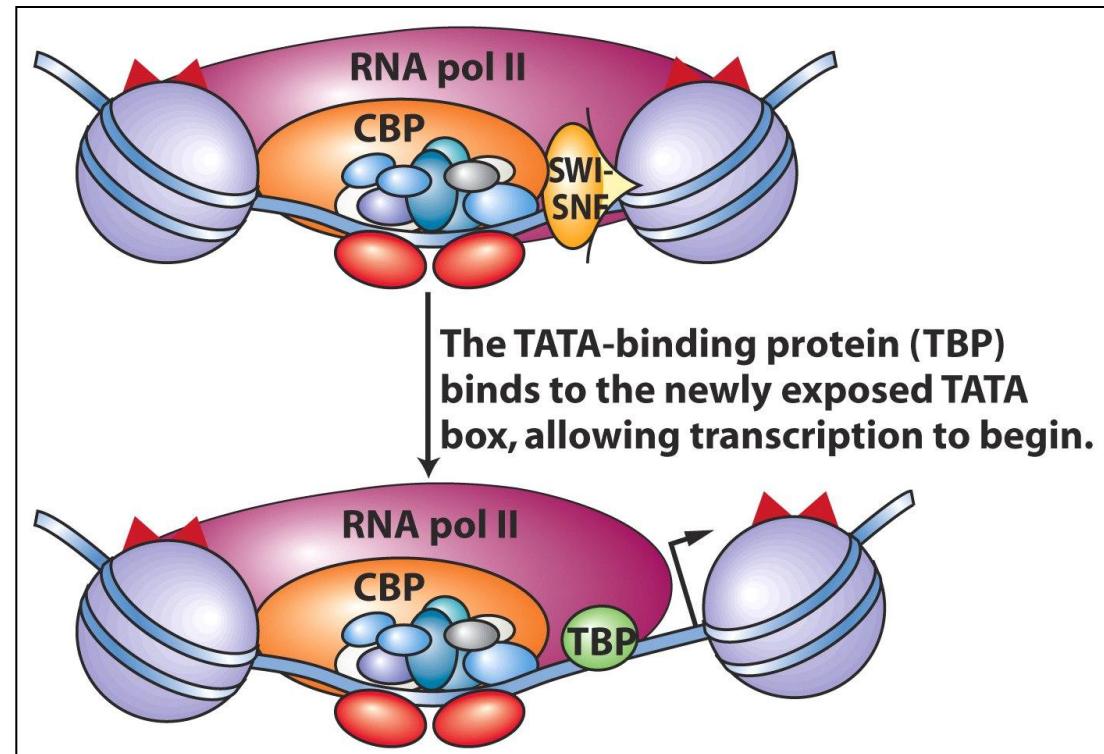
Slika: SWI-SNF premakneta nukleosom, ki zakriva TATA zaporedje; s sprostitvijo TATA se lahko veže osnovni transkripcijski kompleks.





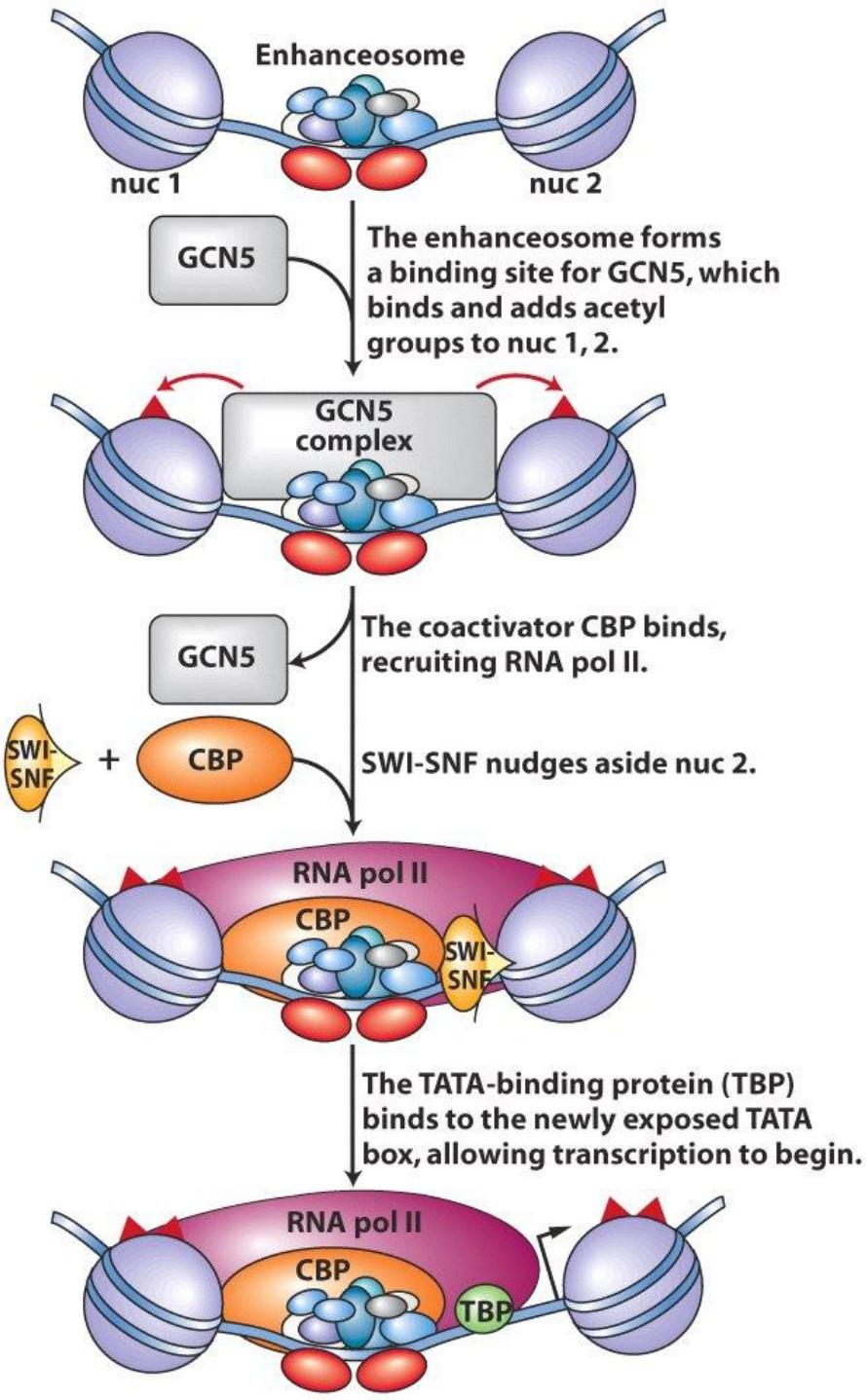
Ojačevalni kompleks gena za β -interferon brez nukleosomov

Ojačevalni kompleks gena za β -interferon z nukleosomi



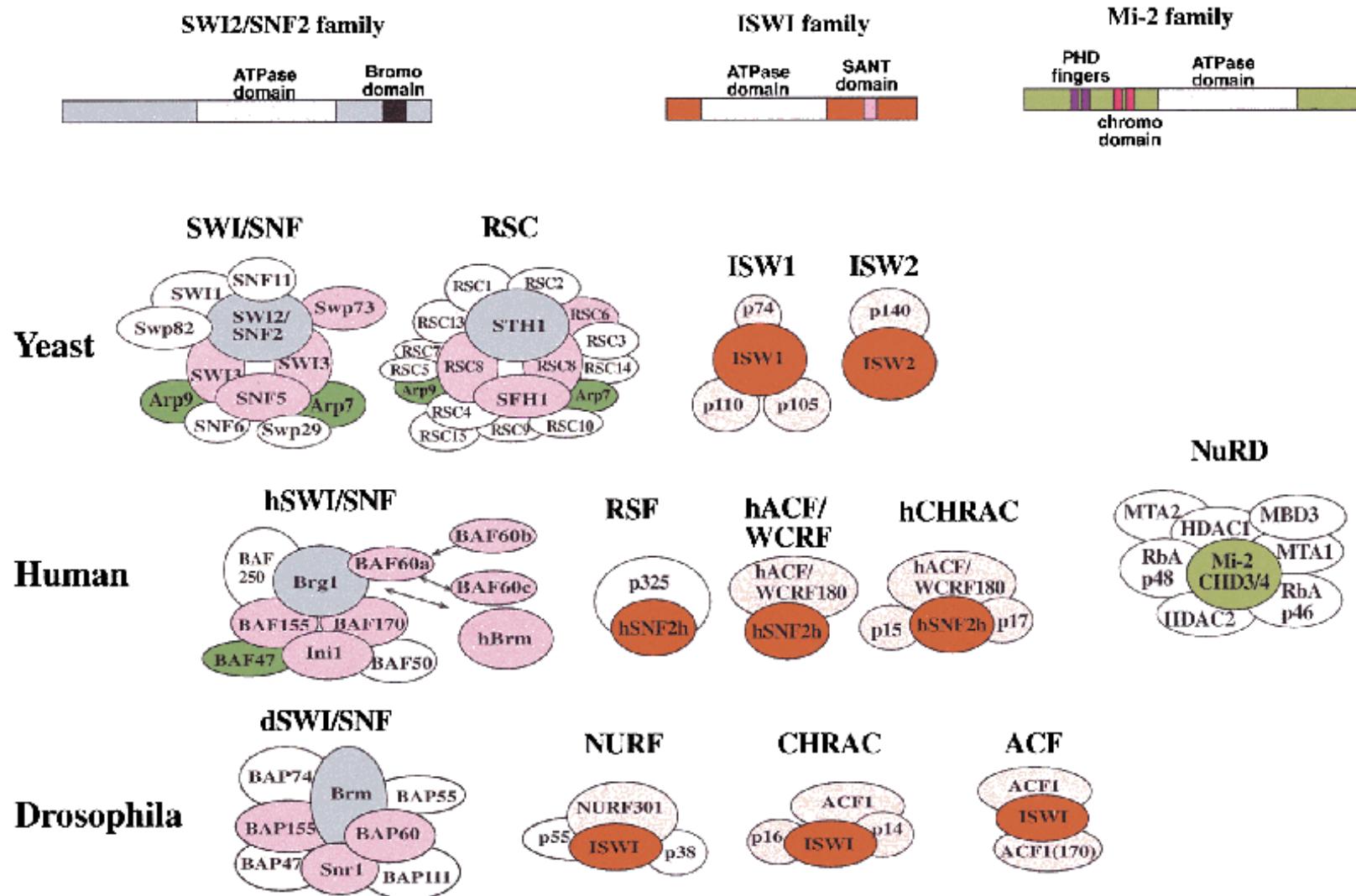
B-interferon ojačevalni kompleks premakne nukleosome s pomočjo SWI-SNF kompleksa

- ojačevalni kompleks (sinergija RP)
- acetilacija histonov (*GCN5*)
- remodeliranje kromatina (SWI-SNF)



Proteinski kompleksi za remodeliranje kromatina

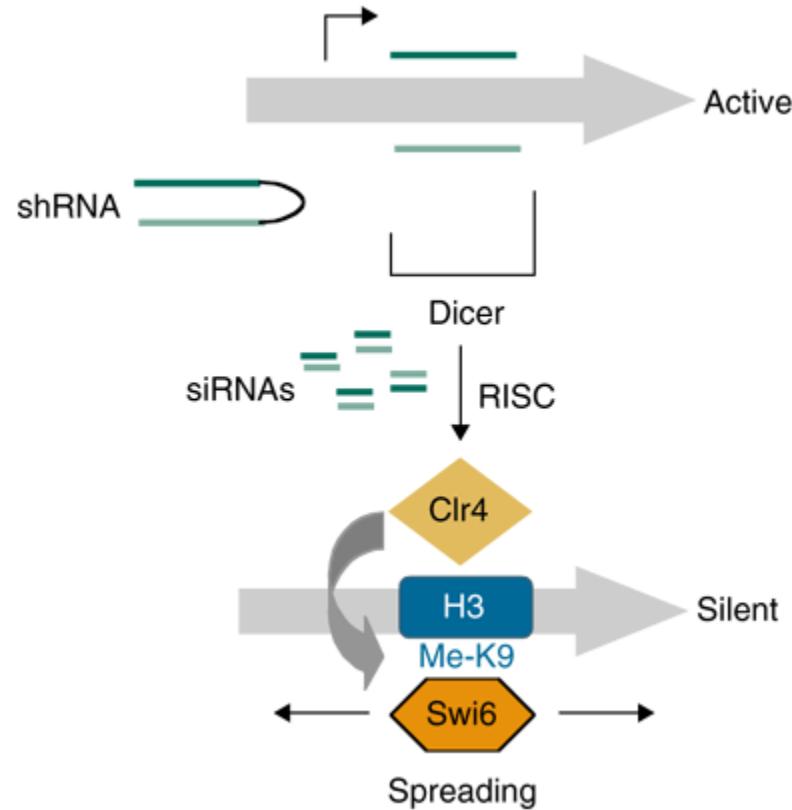
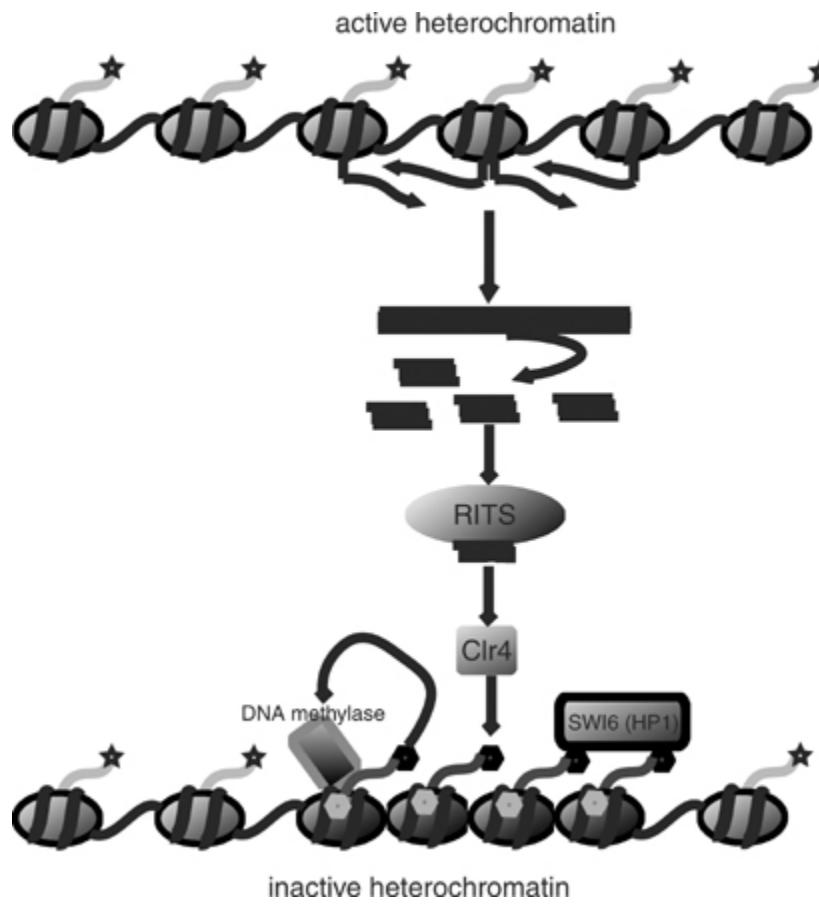
Chromatin remodeling complexes



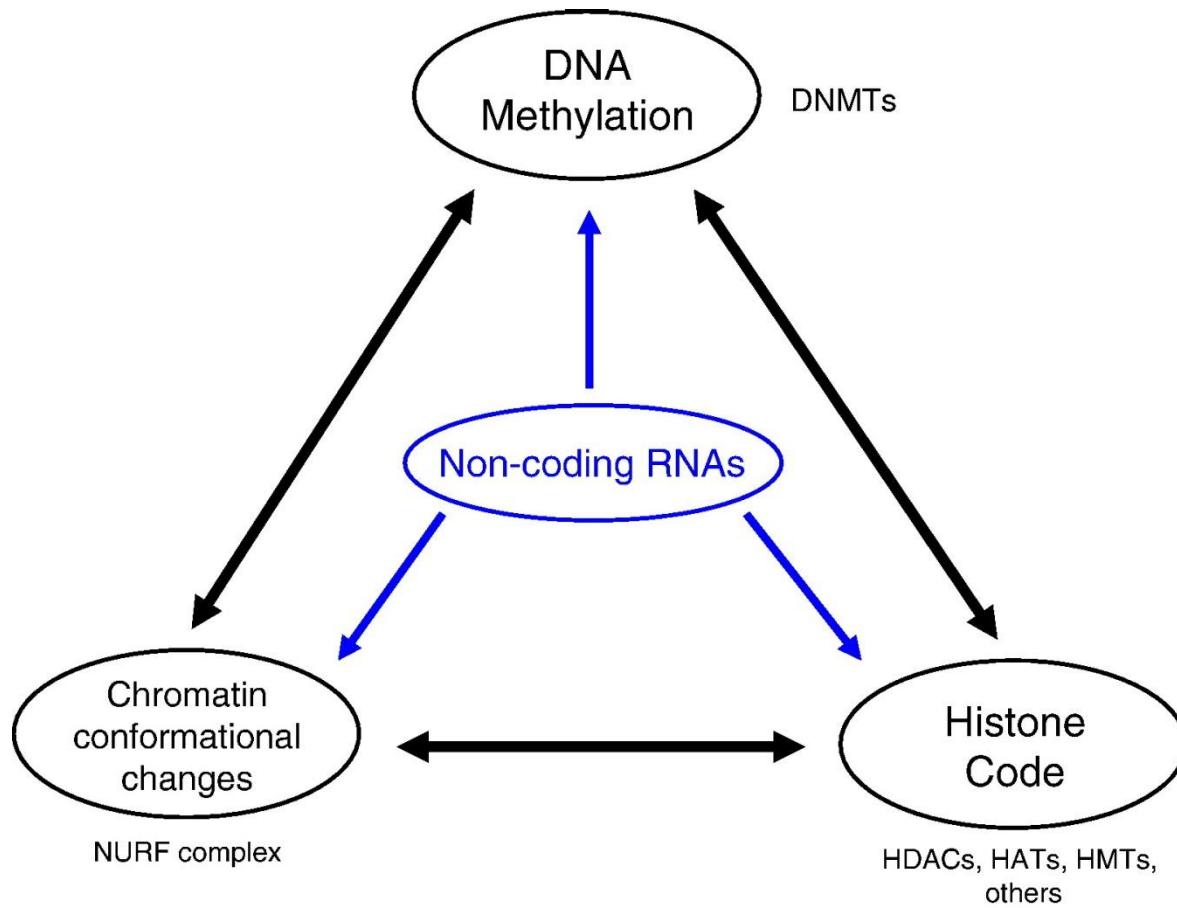
Epigenetske regulacije in RNA

Ne-kodirajoča RNA (ncRNA)

- Kratka ncRNA (miRNA - de novo metilacija, siRNA - utišanje transpozonov, piRNA)
- Dolge ncRNA (več kot 200 nt) - interakcije lncRNA s kompleksom kromatinskega remodeliranja

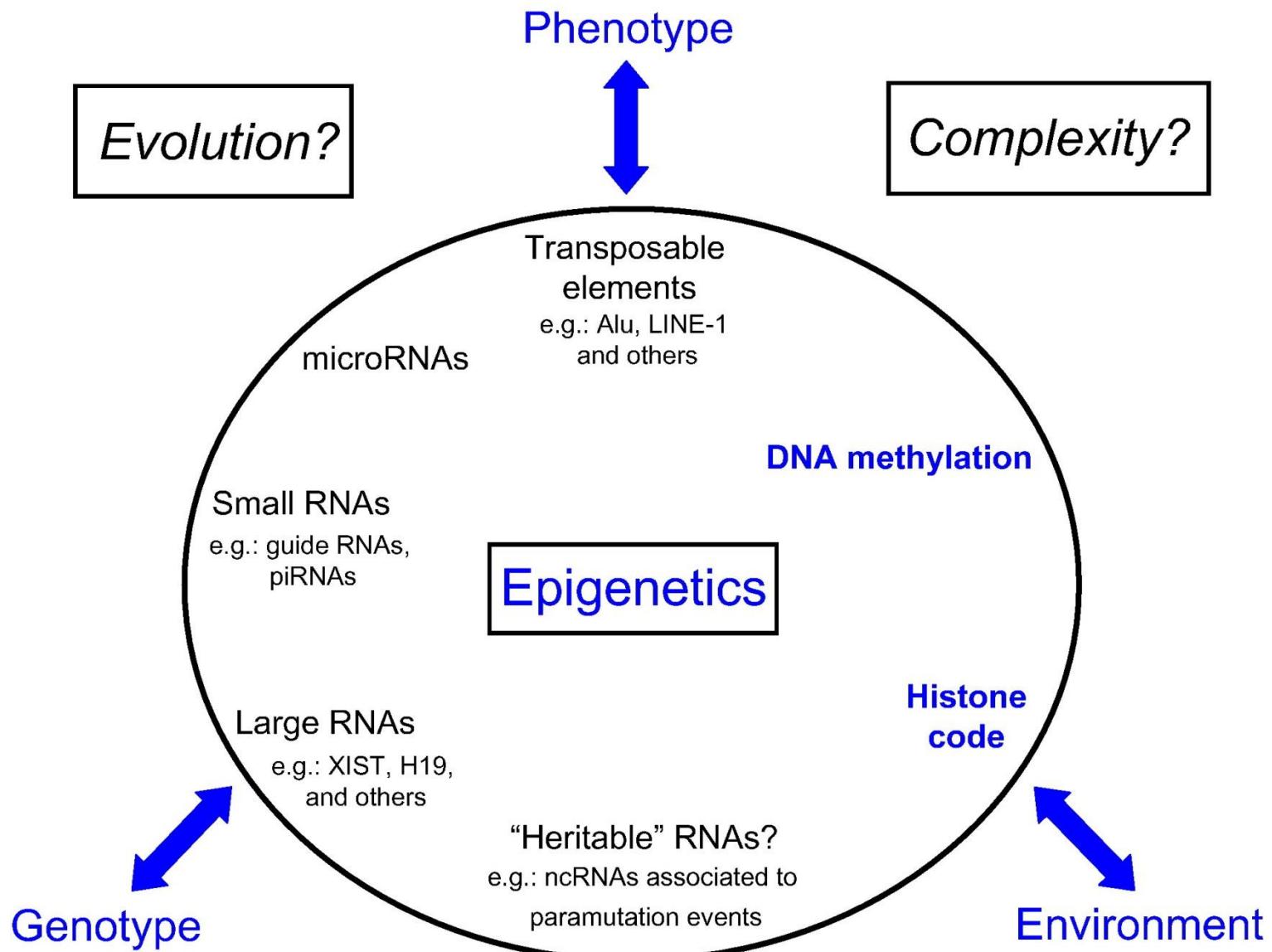


The expression of dsRNA triggers the assembly of silent heterochromatin in *Schizosaccharomyces pombe*. Overlapping transcripts hybridize and form dsRNA molecules homologous to the DNA segment. dsRNAs enter the RNAi pathway and are cleaved to siRNAs. The siRNAs are then incorporated into a new complex called **RITS** (RNAi-induced transcriptional silencing complex), which is able to target homologous DNA. The RITS recruits **Clr4** that methylates lysine 9 of histone H3, this allows binding of **Swi6**, the mammalian HP1 homolog, on the modified chromatin, and enables the formation of silent heterochromatin.



Non-coding RNAs as essential players in epigenetic mechanisms.

DNMTs, DNA methyltransferases; NURF, nucleosome remodeling factor; HDACs, histone deacetylases; HATs, histone acetyltransferases; HMTs, histone methyltransferases.



Epigenetics as a central molecular mechanism in organism complexity. In this model, the genotype (represented by the DNA molecule), the environment and all epigenetic mechanisms work in concert in order to generate specific phenotypes. Even if organisms have the same genotype, depending on environmental changes, they can have different phenotypes that are mediated by epigenetics. ncRNAs and repetitive sequences are key elements in this new concept.

Inaktivacija X kromosma

Inaktivacija X kr. pri samicah sesalcev

- Izenačenje števila kopij genov X kr. pri obeh spolih
- Naključna inaktivacija enega od X kr. v zgodnji fazi razvoja
- Inaktiviran X kr. je visoko kondenziran; Barrovo telo
- **X-inaktivacija in kromatin:**
 - Večina genov na kondenziranem X kr. je neaktivnih
 - utišanih
 - epigenetski “značke”: hipermetilirana DNA, metiliran Lys9-H3
 - Utišani geni ostanejo neaktivni v vseh celicah potomkah - dedna sprememba brez spremembe v sekvenci DNA =
EPIGENTSKA SPREMENBA

Različni mehanizmi kompenzacije doz -izenačena genska produkcija

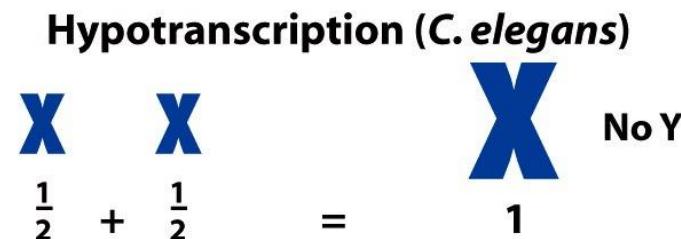
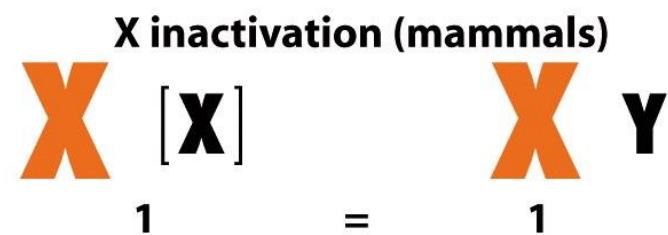
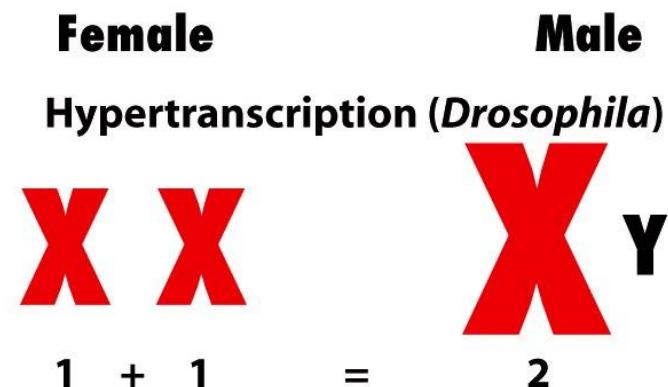
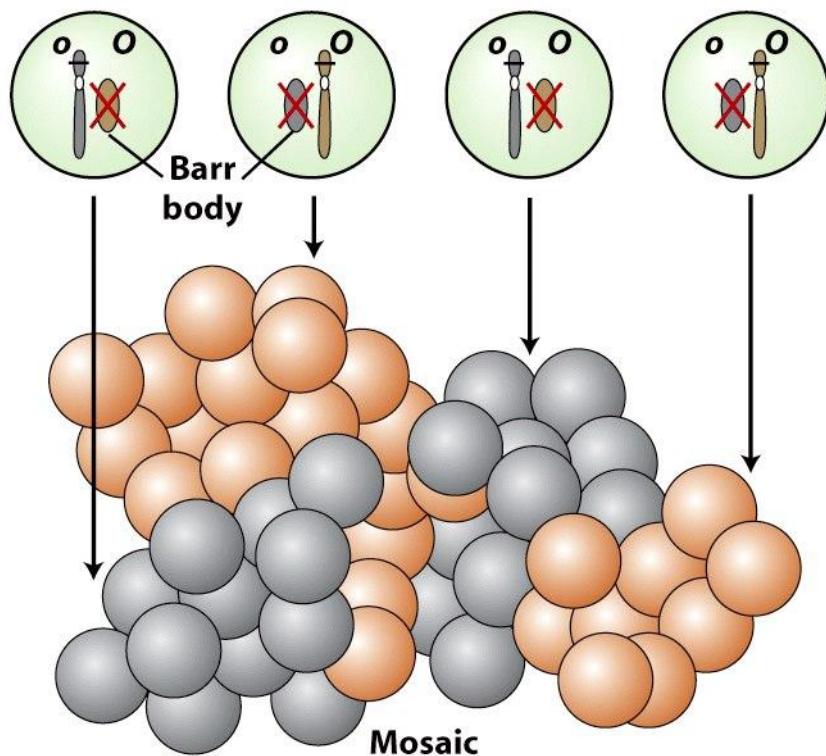
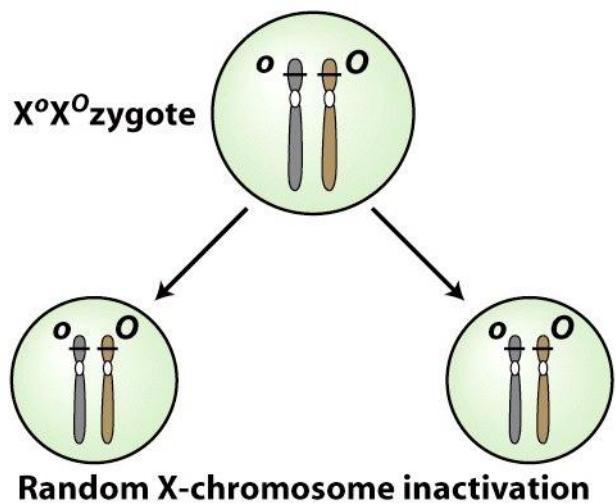


Figure 11-27
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Dedovanje dlake pri mačkah

Črna barva: X^oX^o, X^bY

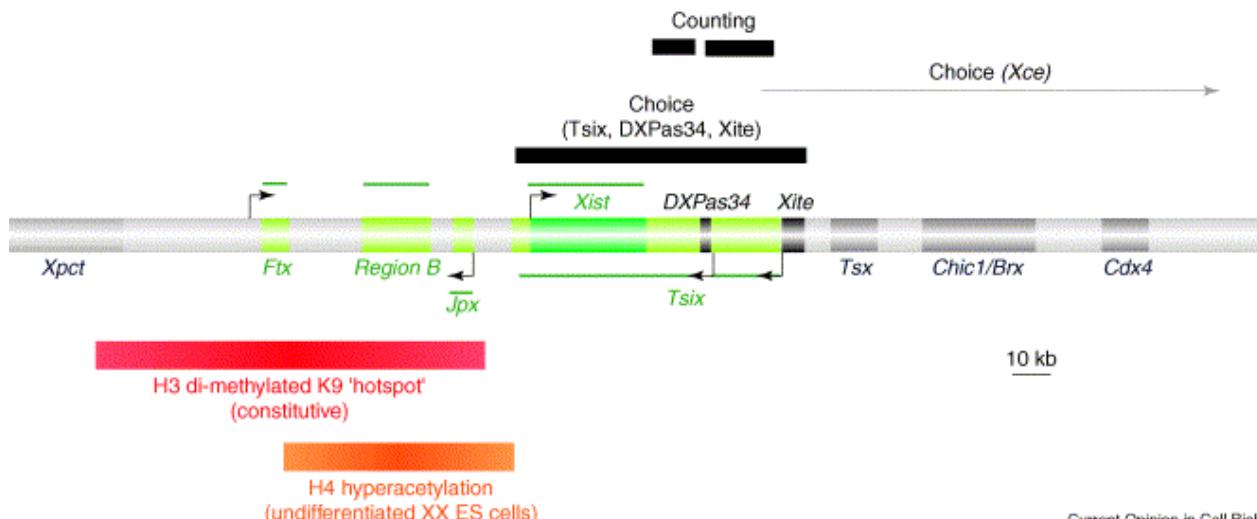
Oranžno-rjava barva:
 X^oX^o, X^oy

Lisasta: X^oX^O , naključna
inaktivacija enega od X kr.



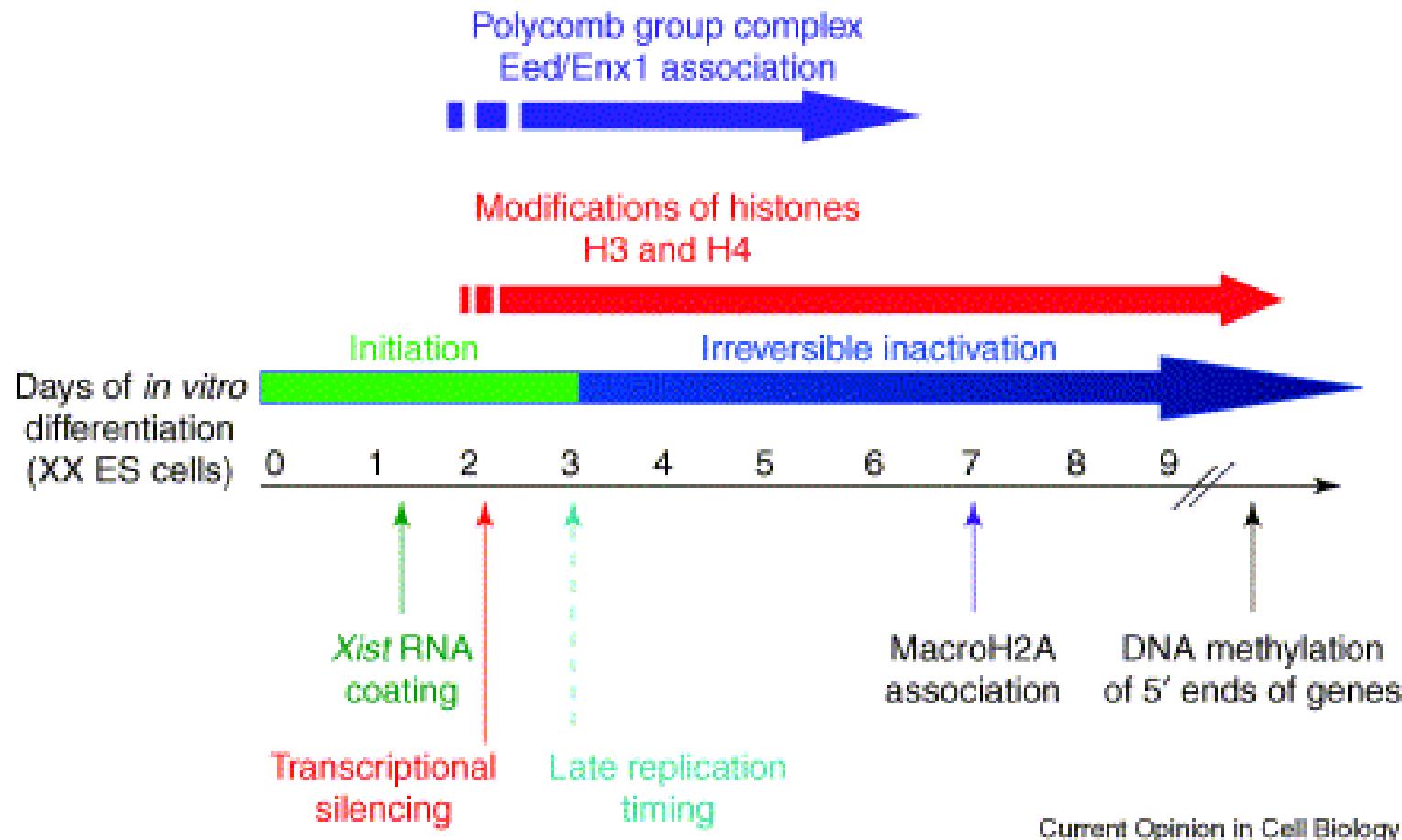
Regulacija inaktivacije X kromosma pri sesalcih

- Kompleksni lokus: X-inaktivacijski center; *Xist* (X-inactive-specific transcript) = 17 bp RNA; prekrije X-kr. in vzpodbudi inaktivacijo
- 'counting' = $1X/2n$; inaktivacija vseh, razen enega X-kr. (??avtosomni faktor se veže na X-Aktiv. in prepreči inaktivacijo)
- 'choice' = izbira enega X (??)
- Zeleno: ne-kodirajoči transkripti
- Sivo: kodirajoči transkripti; funkcije nepovezane z *Xic*
- H3-K9: stalna metilacija
- H4: hiperacetilacija samo v ženskih ES celicah pred diferencijacijo



Heard E, 2004, Recent advances in X-chromosome inactivation. Curr Opin Cell Biol, 16(3):247

Regulacija inaktivacije X kromosma pri sesalcih



Naključna X inaktivacija v diferenciranih ESC. Obdobje med Xist RNA odvisne iniciacijske faze (zeleno) in Xist RNA neodvisne irreverzibilne faze (modro) EeD/Enx1 zgodnji vzdrževalni kompleks= (histone metiltransferaze; Enx1=H3-K27 metilacija; G9a=H3-K9 metilacija)

X inactivation and the complexities of silencing a sex chromosome

Jennifer Chowa, _ and Edith Hearda,

Current Opinion in Cell Biology

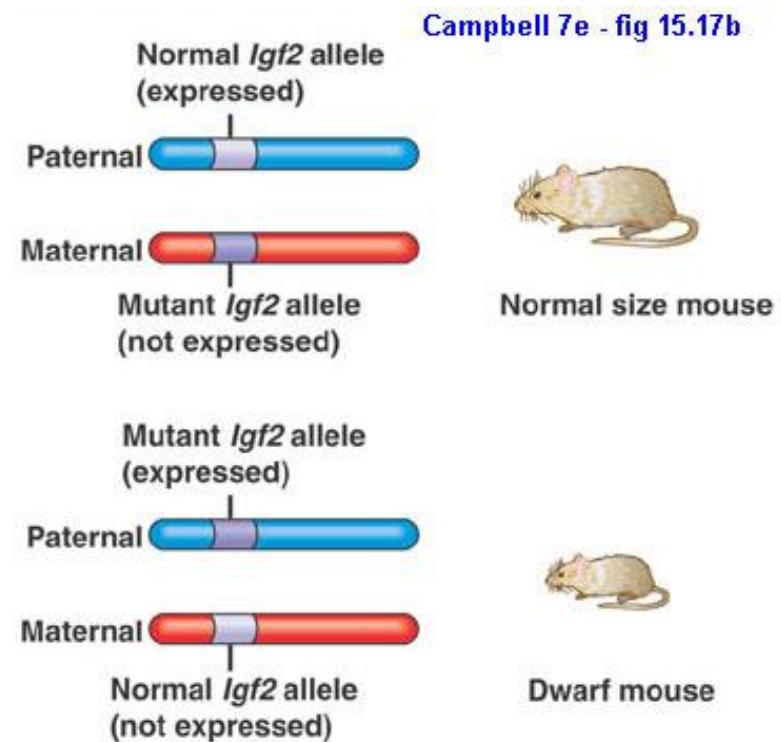
Volume 21, Issue 3, June 2009,

Pages 359-366

Imprinting - starševsko
označeni geni

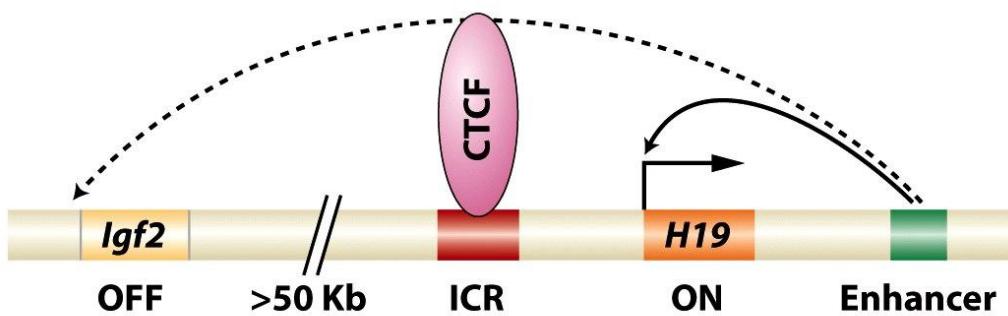
Imprinting, starševsko označeni aleli

- Epigenetska sprememba
- označeni (metilirani) aleli se ne izrazijo (materin Ifg2 alel je označen in se ne izraža)
- Imprinting geni so hemizigotni (ena kopija gena)
- imprinting - pozor - analiza rodovnikov;
- V sesalcih več sto starševsko označenih genov - pomembni za razvoj in diferencijacijo

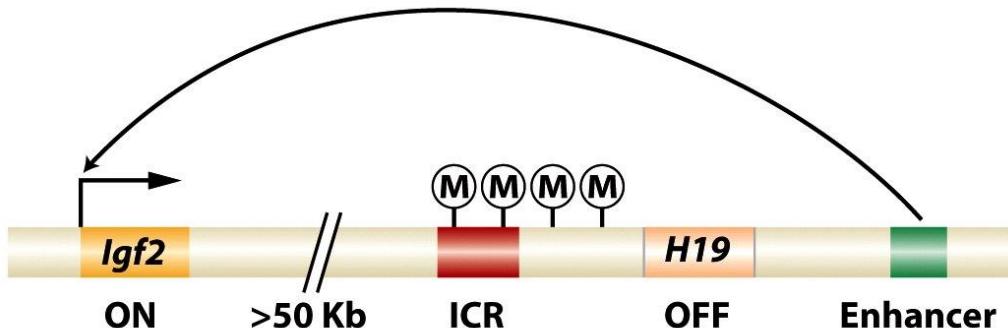


(b) When a normal *Igf2* allele is inherited from the father, heterozygous mice grow to normal size. But when a mutant allele is inherited from the father, heterozygous mice have the dwarf phenotype.

♀ Maternal allele

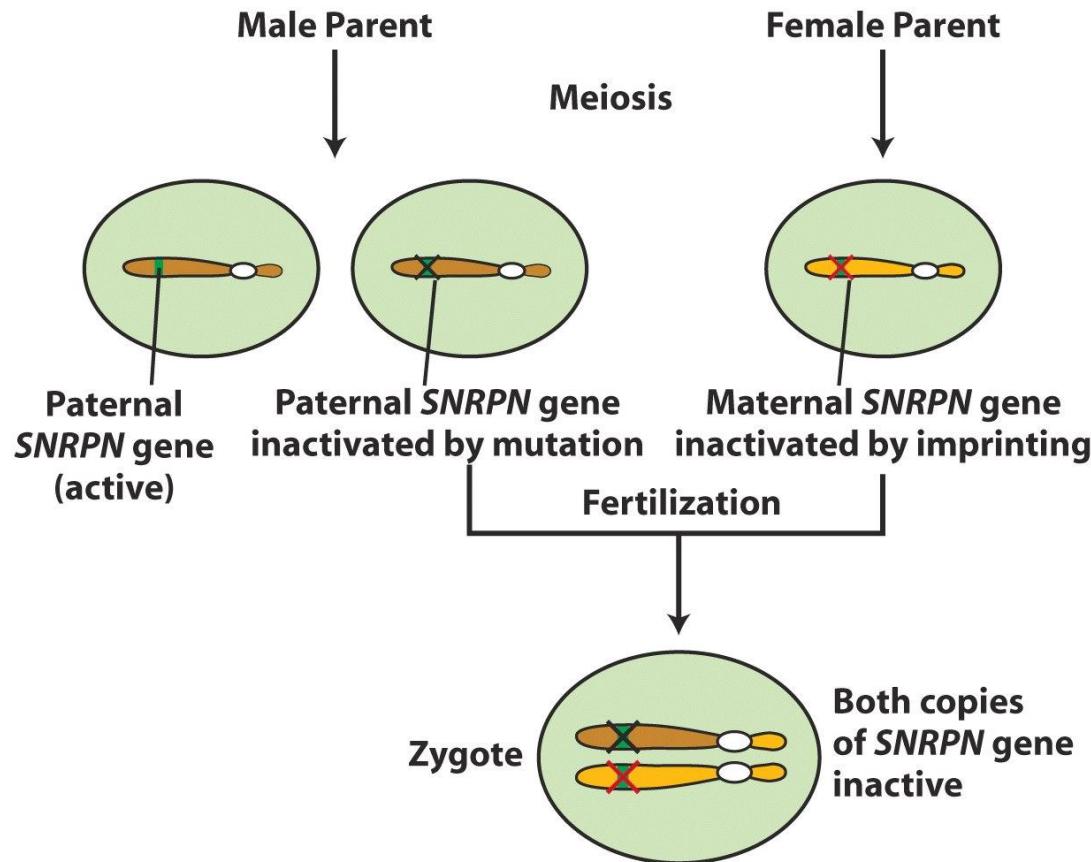


♂ Paternal allele



Starševsko označeni geni pri miški. Kontrolna regija za imprinting (ICR) je nemetilirana v gametah samic in lahko veže dimer CTCF s čimer tvori *insulator*, ki blokira delovanje ojačevalca za aktivacijo gena *Igf2*. Metilacija (M) ICR v gametah Samcev prepreči vezavo CTCF faktorja kot tudi vezavo drugih proteinov na *H19* promotor.

Prader-Willi sindrom, genetska bolezen pogojena z imprintingom materinega alela *SNRPN*, aktiven je samo očetov alel, v primeru mutacije očetovega alela se pojavi bolezen PW sindrom



These genes (*SNRPN*, *MKRN3* and *NECDIN*) have been characterized and are only expressed from the **paternally inherited allele**. Prader-Willi patients lack expression of these genes. The *SNRPN* polypeptide (SmN) is involved in splicing pre-mRNA, the *MKRN3* gene encodes a zinc finger protein and *NECDIN* is mainly expressed in post-mitotic neurons. **All these genes have a CpG island at their 5' end which is unmethylated on the expressed paternal allele and methylated on the repressed maternal allele.**

Prader-Willi Syndrome Growth Hormone Treatment



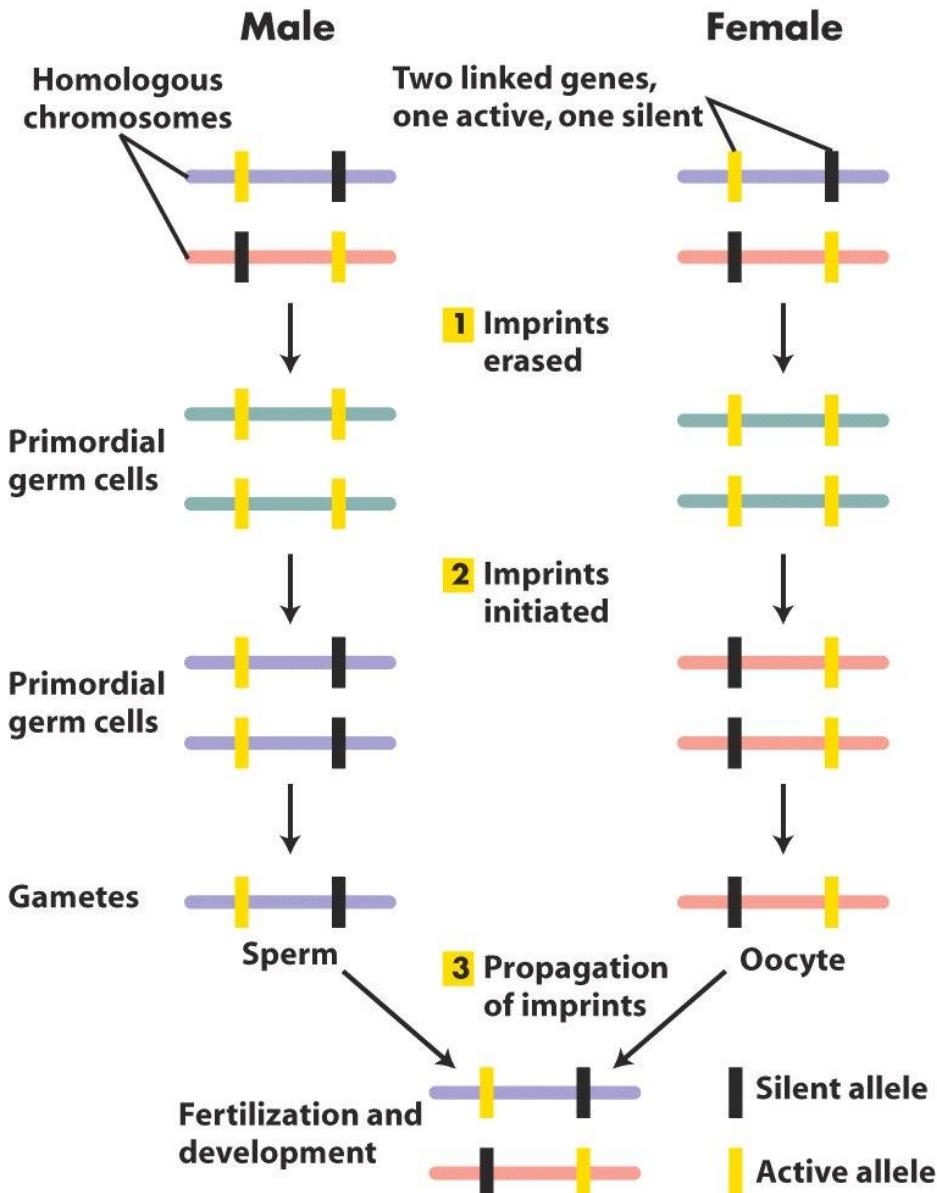
2 months of GH treatment
(14 yrs old)



13 months of GH treatment
(15 yrs old)

figure 3

Starševsko označeni aleli se v spolnih celicah na novo označijo tako, da nosijo spolno specifično oznako dajalca gamete (materni ali očetni alel)



Klonirani telički - Ali je reprogramiranje jedra vključevalo tudi reprogramiranje starševsko označenih genov????



Pozicijski efekt

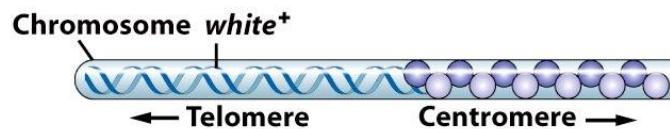
Pozicijski efekt



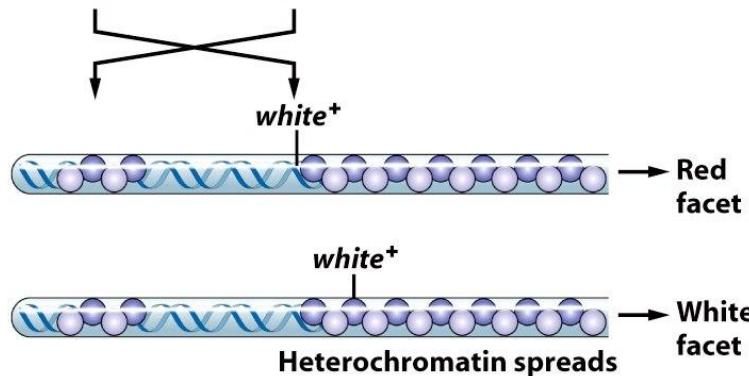
An example of Position Effect Variegation (PEV), in which the functional *white* gene is silenced in most, but not all cells, due to juxtaposition with heterochromatin. *White* is expressed in the red pigmented eye ommatidia cells, and is silenced in the white cells.

Pozicijski efekt večbarvnosti pri drozofili

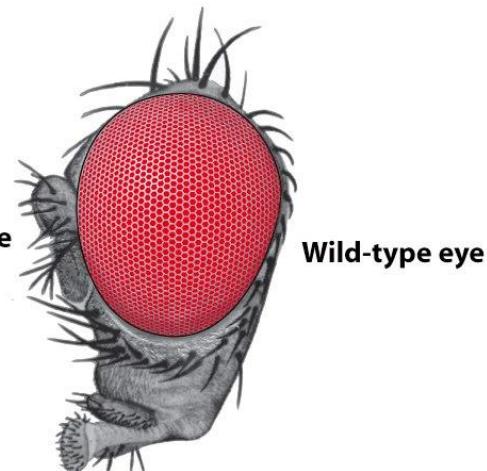
Kromosomska inverzija (mutacija) je premaknila w^+ blizu heterokromatina; heterokromatin se je širil in utišal w^+ v nekaterih celicah - **epigenetsko utišanje gena**



Inversion places $white^+$ close to heterochromatin.

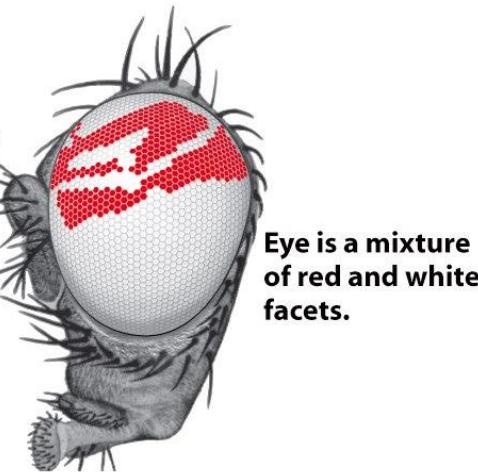


$white^+$ gene expressed



$white^+$ gene expressed

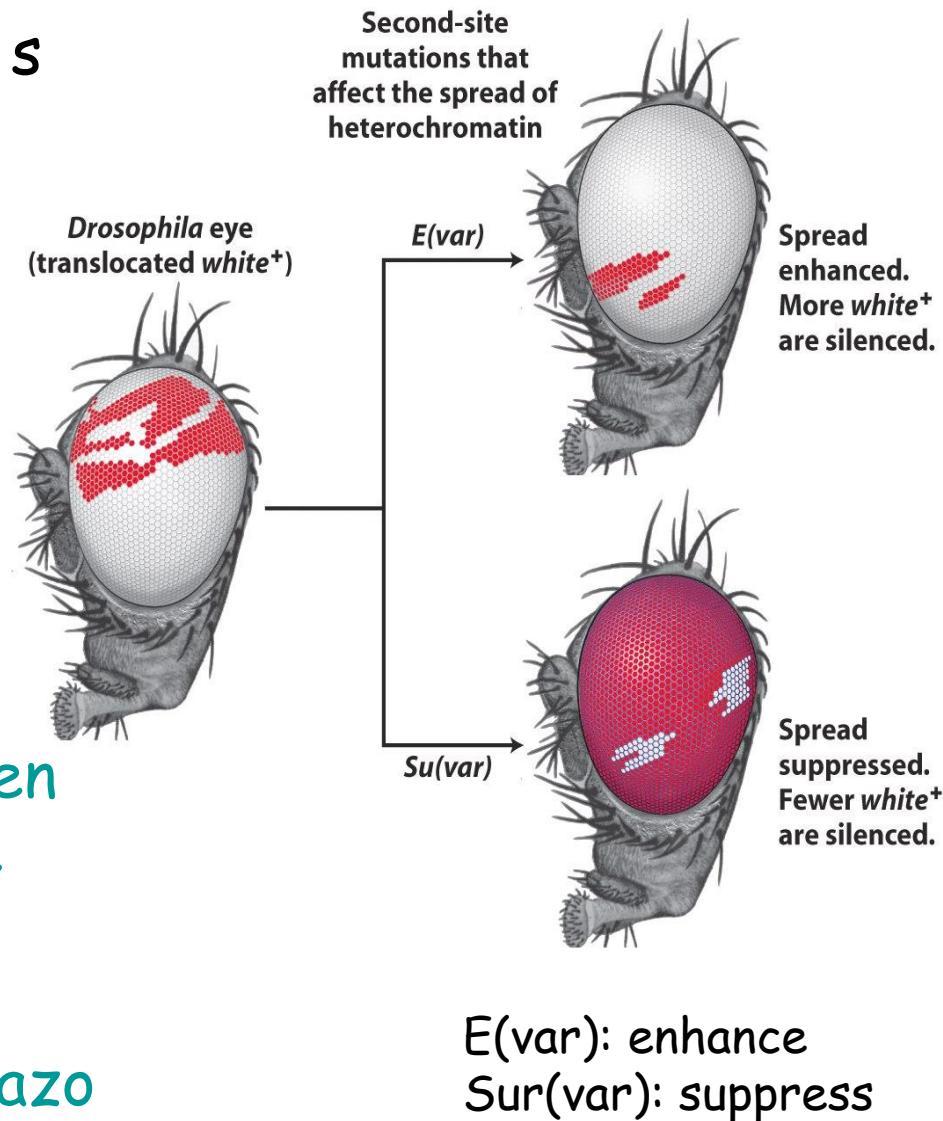
$white^+$ gene silent



Genetska analiza PEV:

Mutacija na drugem mestu, ki zavre ali poveča širjenje heterokromatina pri mutantu s translokacijo;

Pregled mutantov je pokazal povečanje (*E(var)*) oz. zmanjšanje (*Sur(var)*) širjenja heterokromatina



"Mejni" insulator rekrutira encime kot npr. histonsko acetiltransferazo (HAT) in omogoči formiranje evkromatina. M - metilacija; Ac - acetilacija; HMTase-histonska metiltransferaza HP-1- heterokromatinski protein

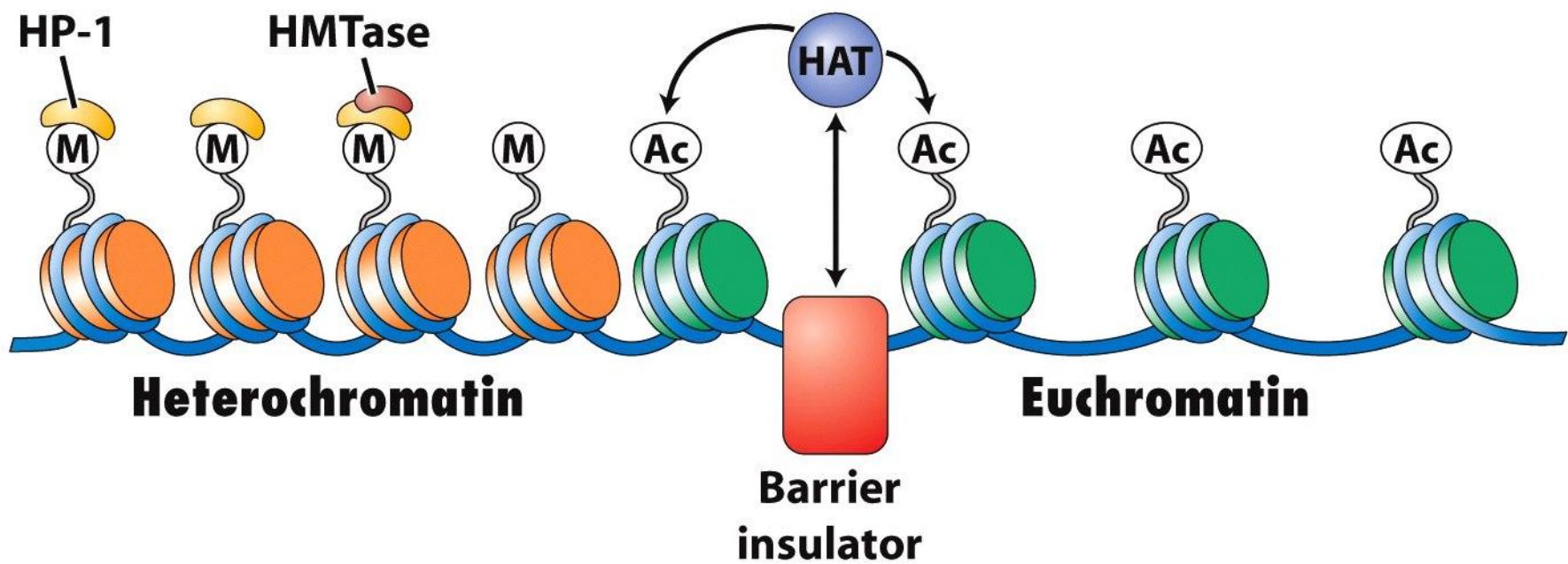


Figure 11-26

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- Gibney ER and Nolan CM (2010) Epigenetics and gene expression. *Heredity* 105:4.
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- <http://www.plosbiology.org/article/info%3Adoi%2F10.1371%2Fjournal.pbio.1000506>

The Honey Bee Epigenomes: Differential Methylation of Brain DNA in Queens and Workers

Using genome-wide methylation profiles in honey bee queen and worker brains to understand how contrasting organismal outputs are generated from the same genotype.