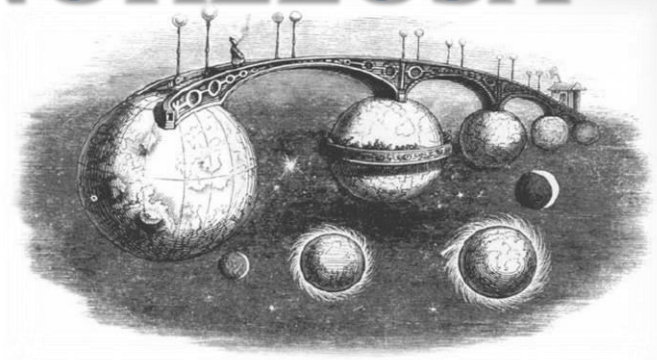
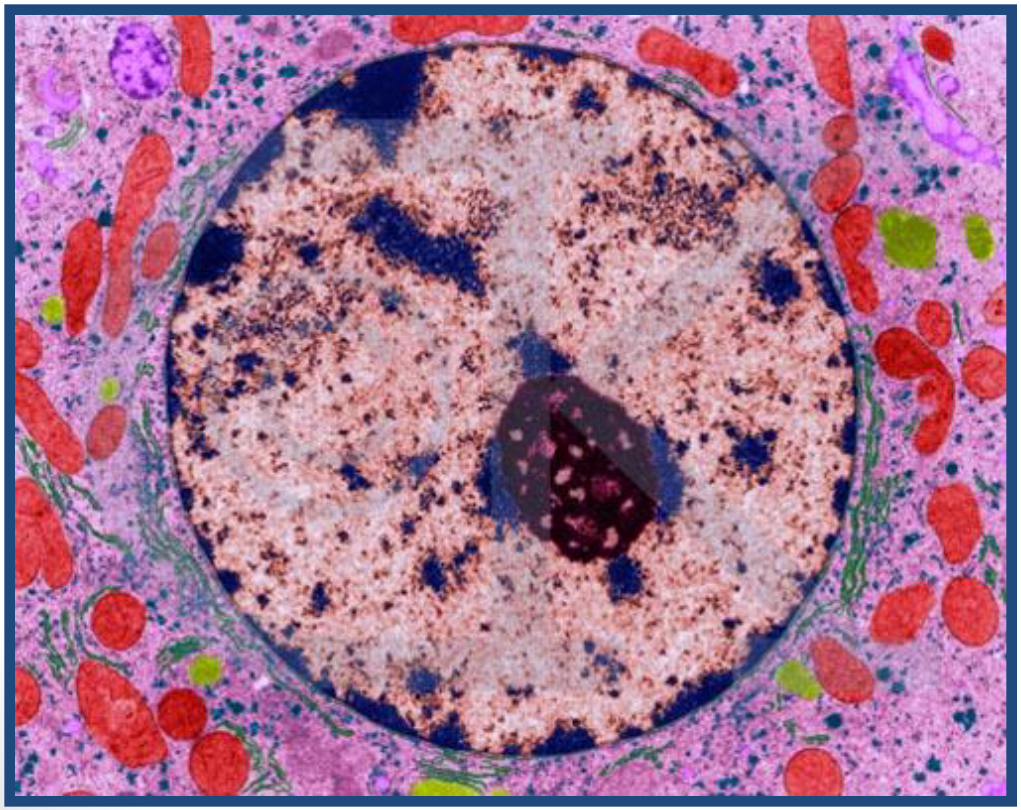
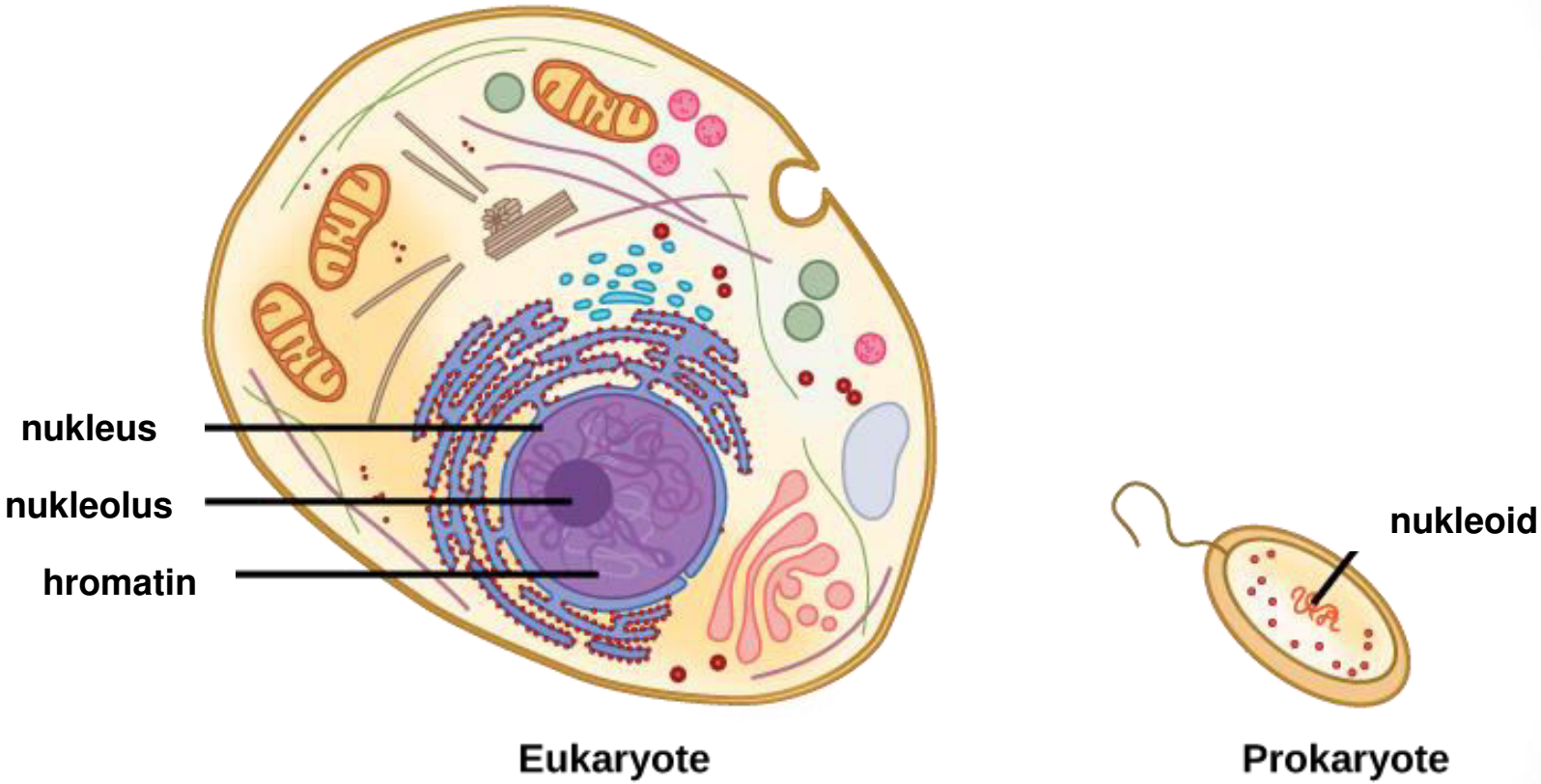
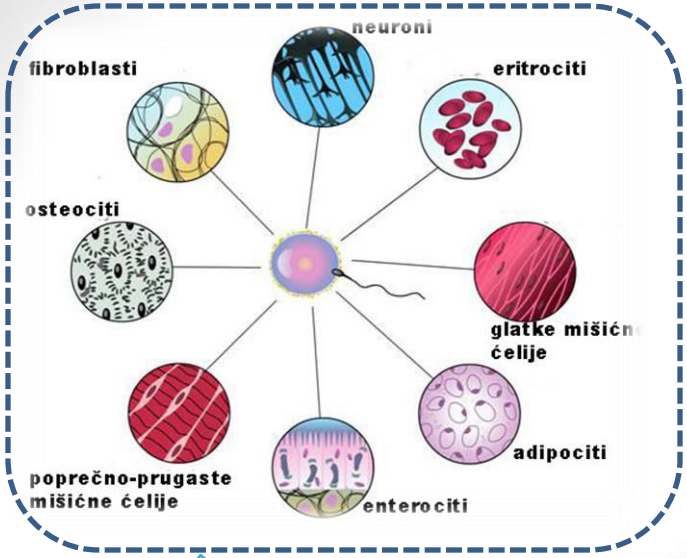
ORGANIZACIJA NUKLEUSA






nukleus
nukleolus
hromatin
Eukaryote
nukleoid
Prokaryote

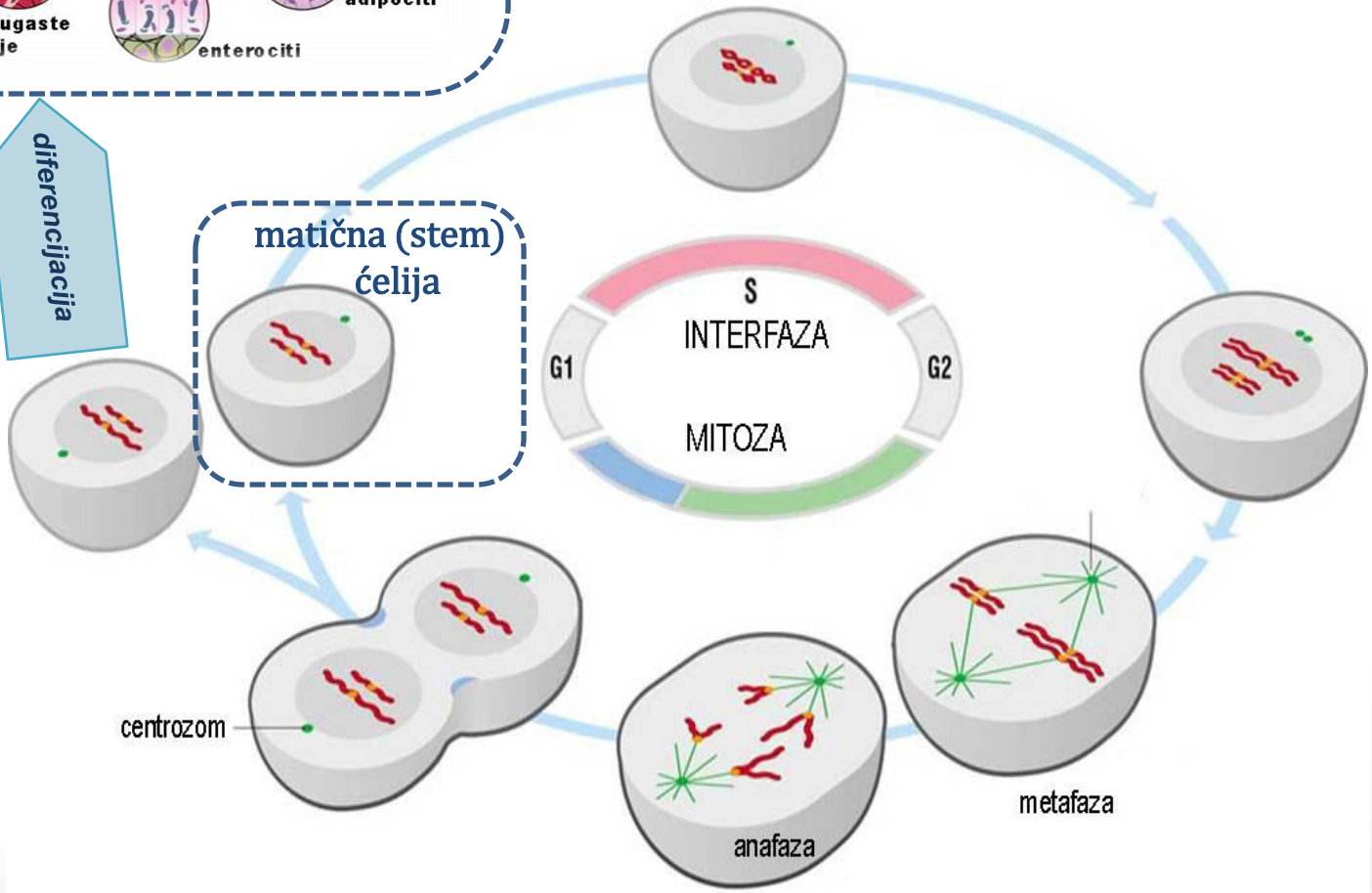


„Omnis cellula a cellula“

Rudolf Virchow, 1858.

diferencijacija

matična (stem) ćelija

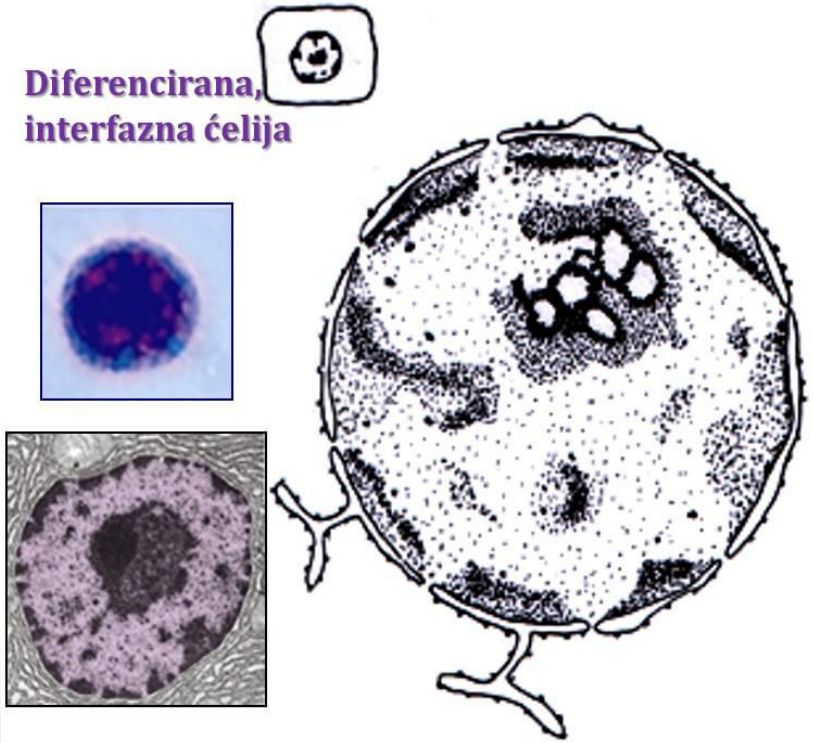


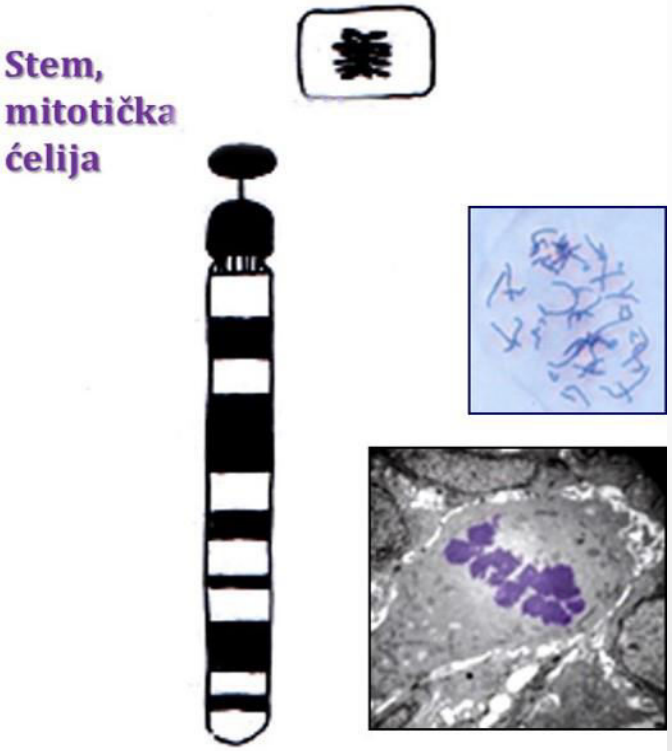
NUKLEUS DIFERENCIRANE ĆELIJE

TRANSKRIPCIJA, OBRADA I TRANSPORT RNK

NUKLEUS ĆELIJE U DEOBI

PRAVILNA REPLIKACIJA I PODELA GENOMA



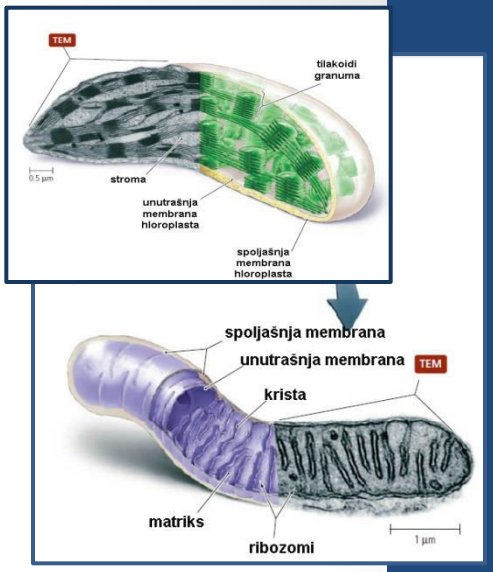


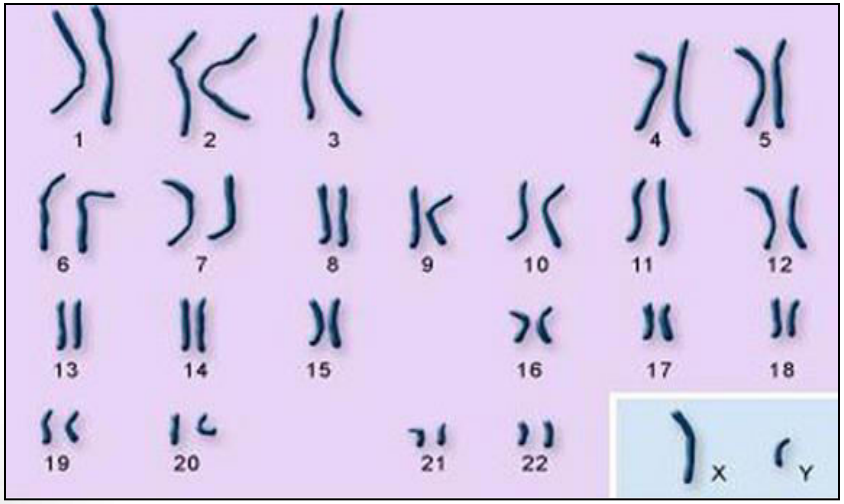
cirkularan molekul DNK

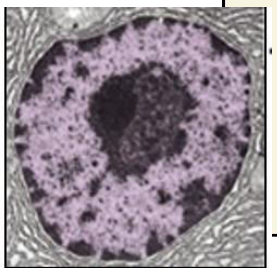
- **STRUKTURA HROMOZOMA**
- Eukarioti – linearan molekul DNK, dva seta hromozoma (od oca i od majke).

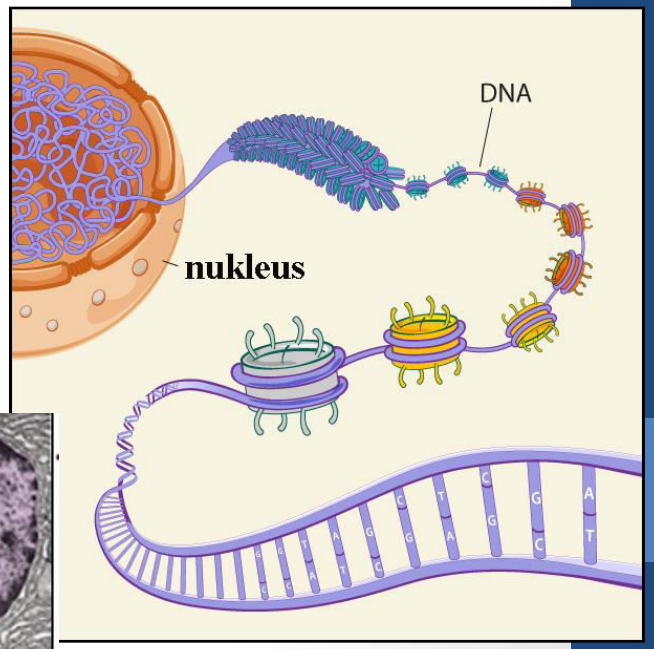
Broj hromozoma. Homologi hromozomi. Čovek – 23 para homologih hromozoma (23 par determiniše pol). Diploidne i haploidne ćelije.

- Pojam hromatina (molekul DNK i histonski i nehistonski proteini)
- Dužina molekula DNK – pakovanje (spiralizacija) - dinamičnost - u zavisnosti od transkripcione aktivnosti ćelije i ćelijskog ciklusa. Maksimum kondenzacije (pakovanja) hromatina je u deobi (maksimum u metafazi)
- Porces transkripcije zahteva nekondenzovan DNK molekul
- Hromatinska slika
- Pre deobe, ćelija replicira DNK – sestre hromatide povezane su u regionu hromozoma koji se označava centromera









Nukleus ćelije u deobi

telomera

centromera

krak

hromatida

maksimum kondenzacije

difuzan

jedna kopija

dve kopije

metacentričan

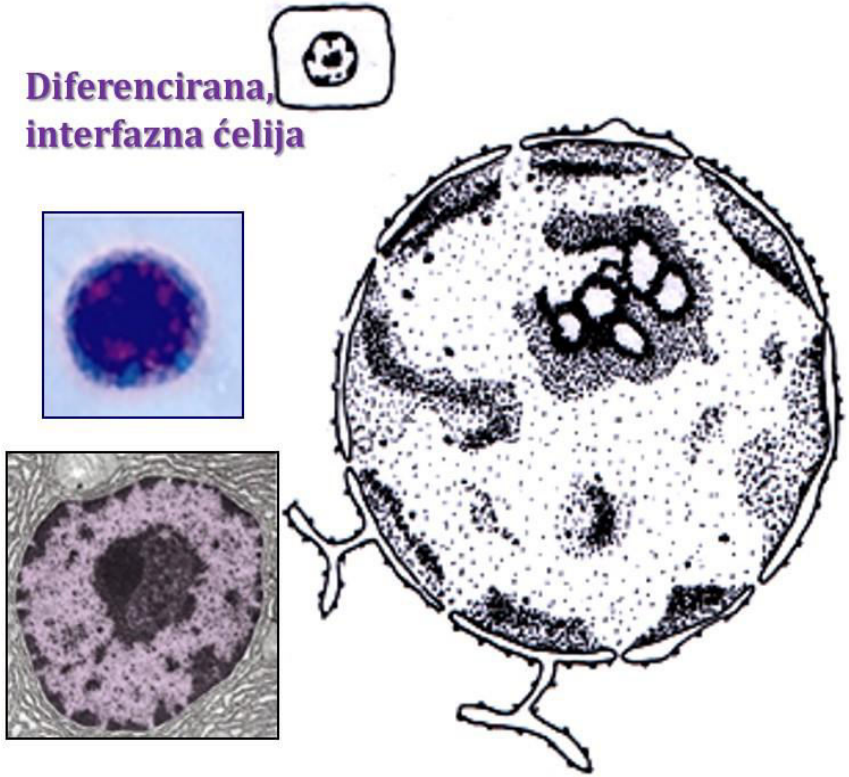
submetacentričan

akrocentričan

telocentričan

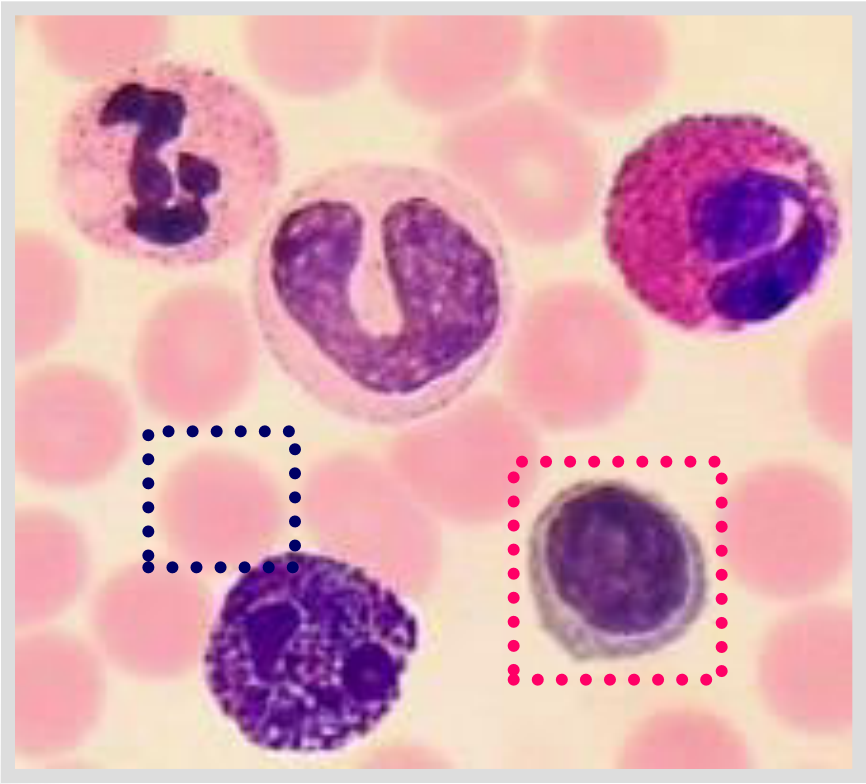
Nukleus diferencirane ćelije

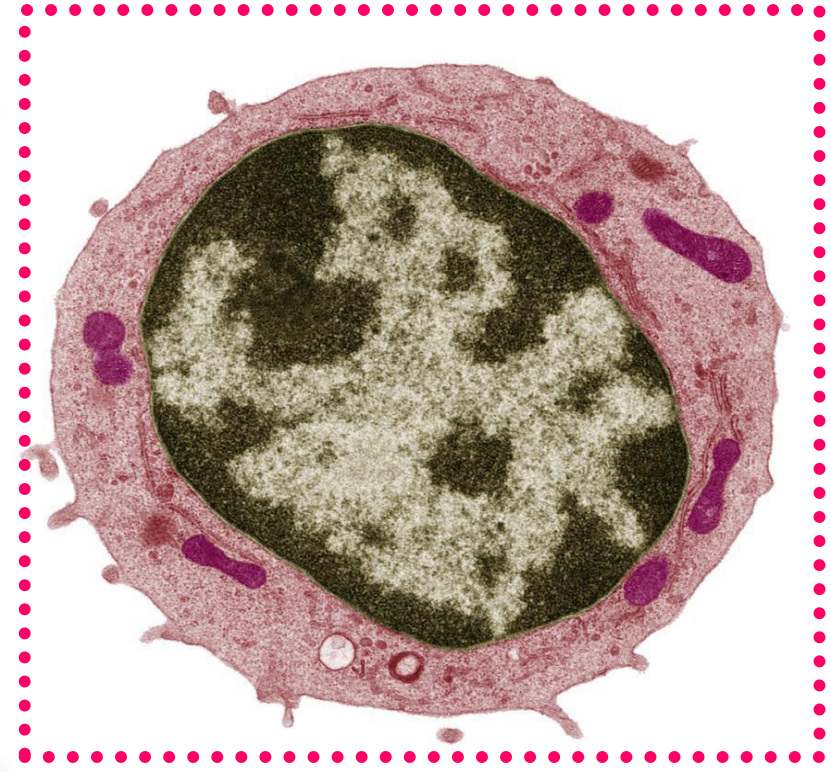
TRANSKRIPCIJA, OBRADA I TRANSPORT RNK



BROJ, OBLIK, POZICIJA I VELIČINA NUKLEUSA

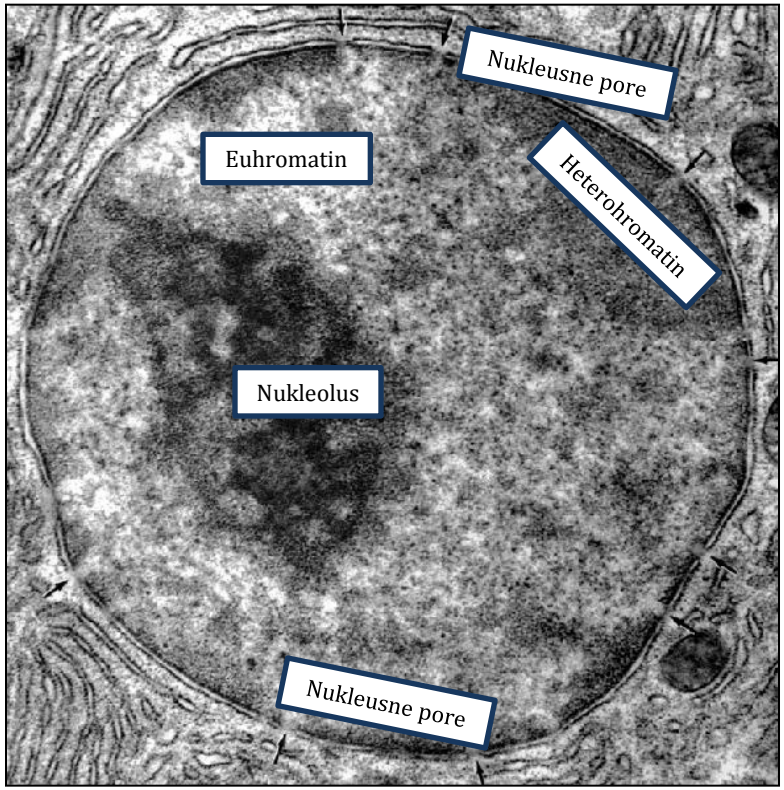
otkriće i naziv - **Robert Brown**, 1931. god.

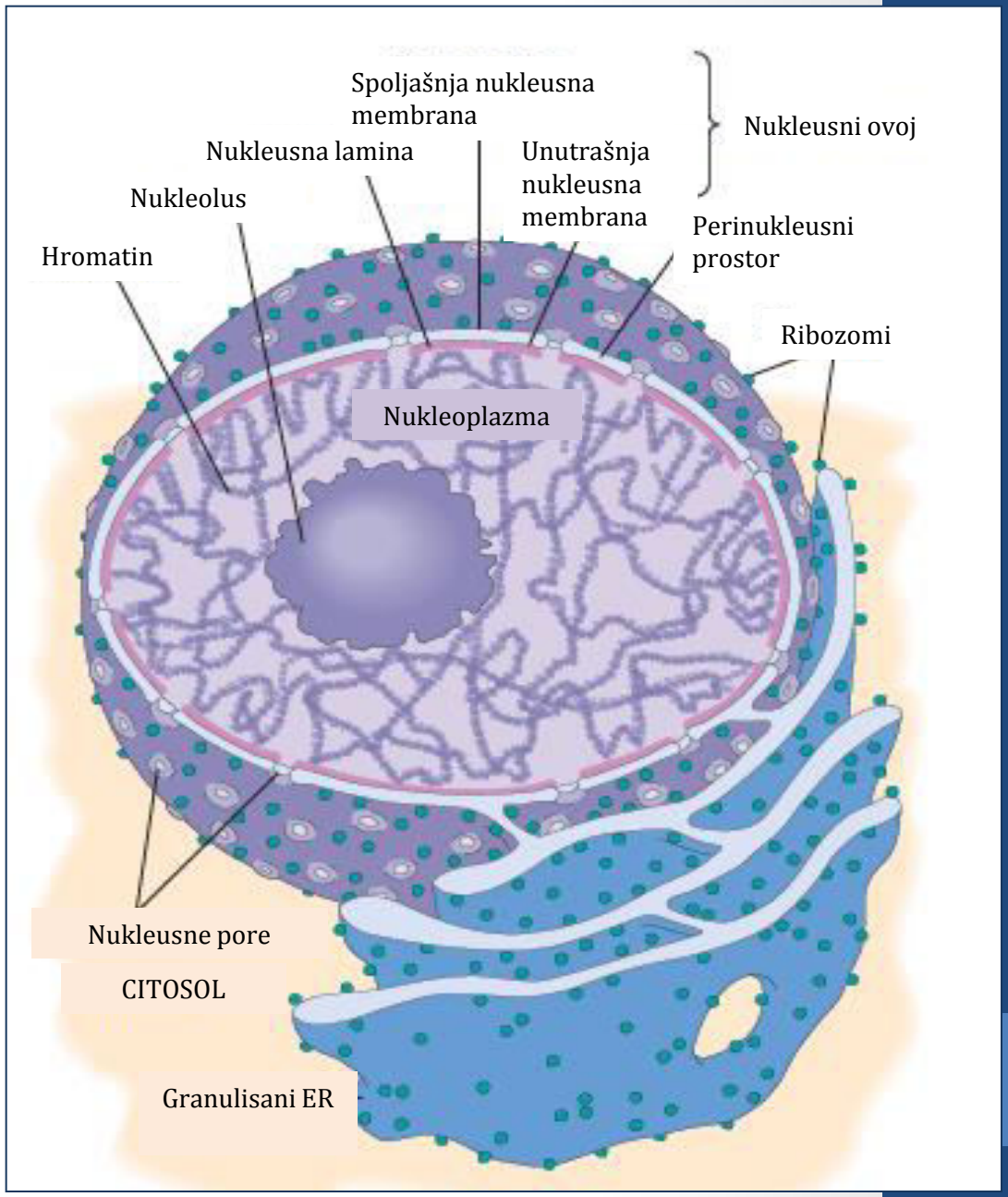




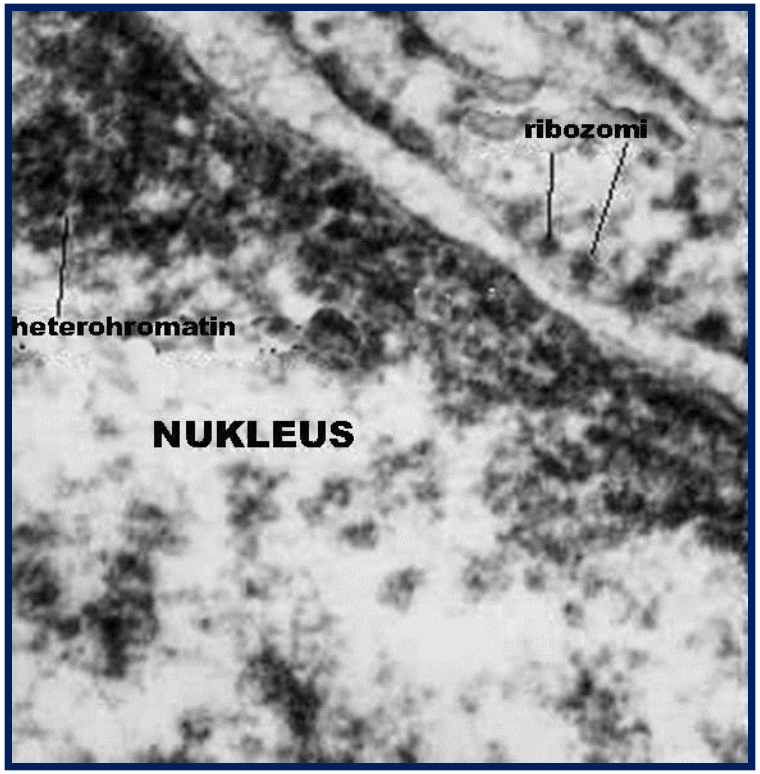
SM **TEM**

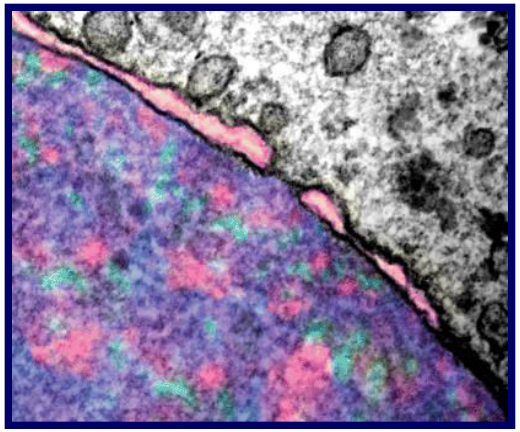
GRAĐA NUKLEUSA

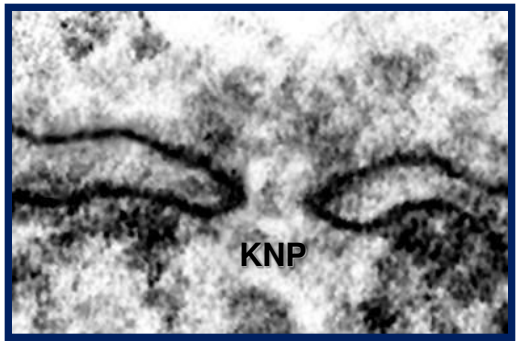




GRAĐA NUKLEUSA – nukleusni ovoj



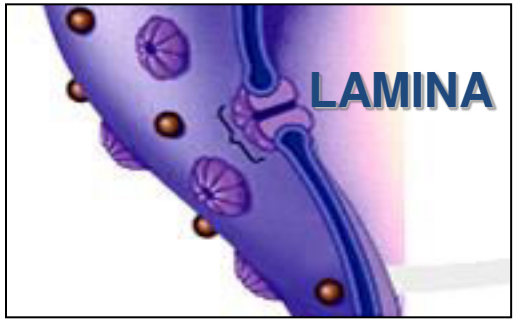


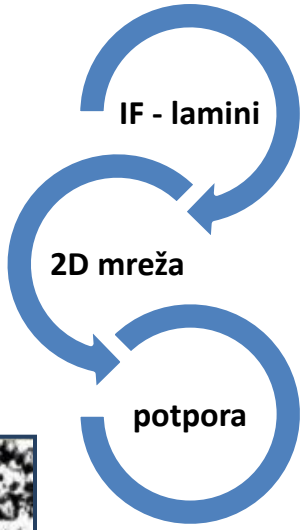


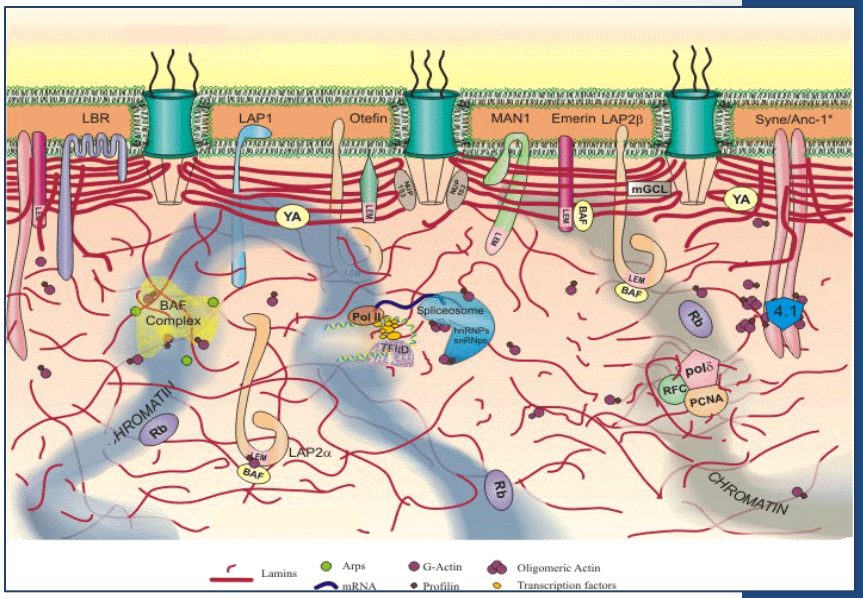
NUKLEUSNI OVOJ

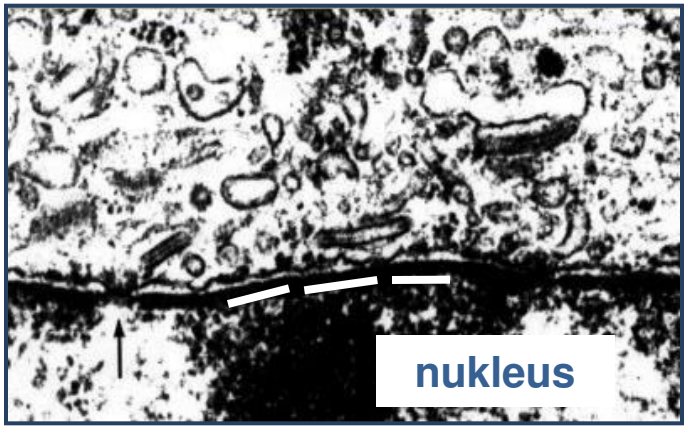
- debljina membrana nukleusnog ovoja (NO) 6.5 nm
- sastav SNM i UNM se razlikuje
- perinukleusna cisterna - dijametar 20-30 nm
- kontinuitet NO je prekinut - nukleusne pore
- UNM – integralni i periferni proteini - receptori za lamine
- SNM - u kontinuitetu sa ER

GRAĐA NUKLEUSA – nukleusna lamina

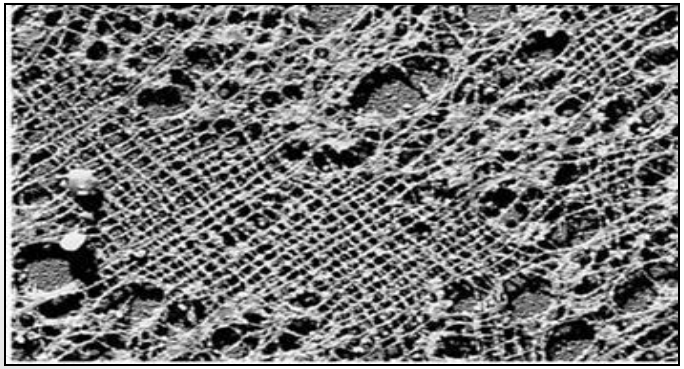


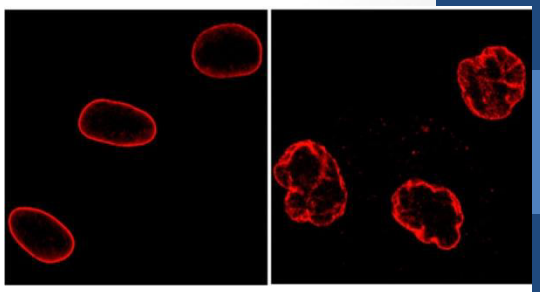






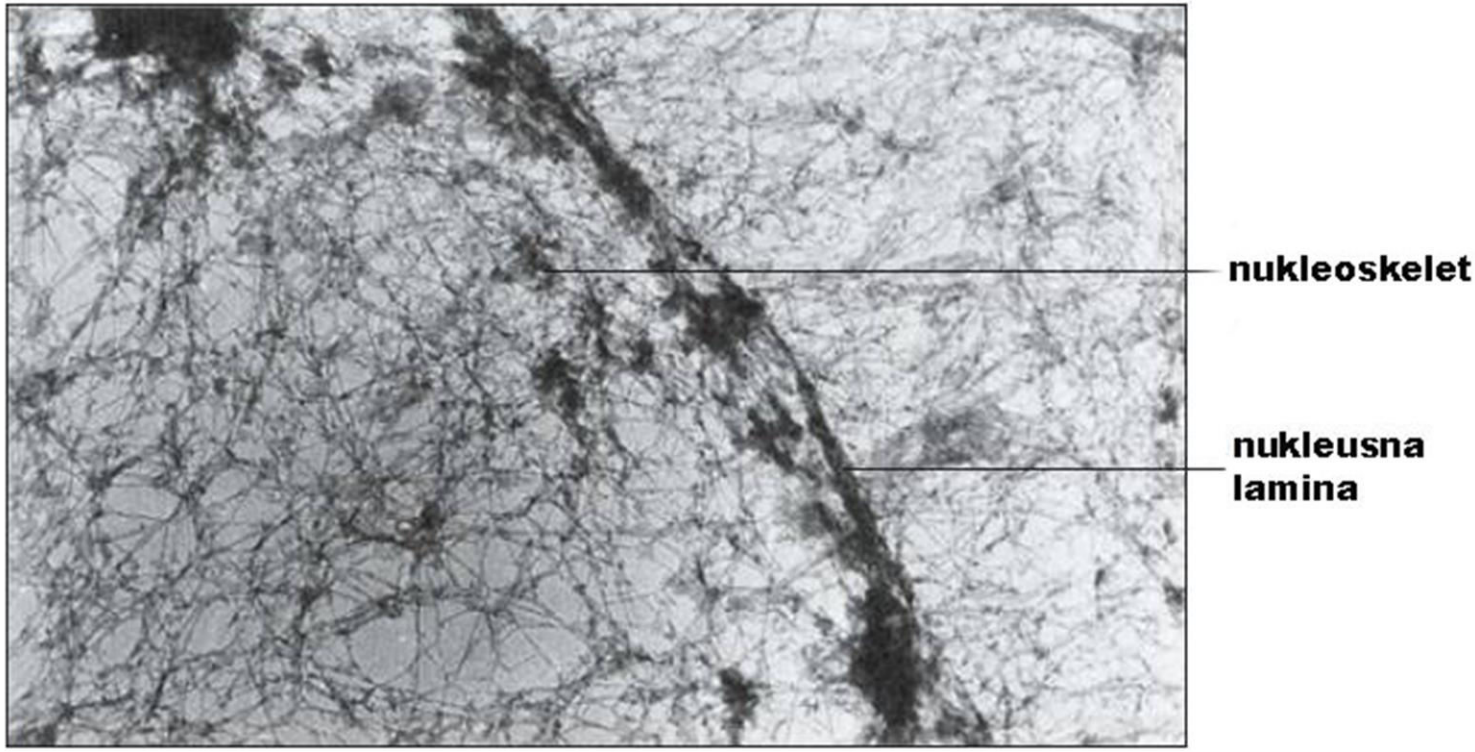
- Lamina se povezuje sa specifičnim proteinima UMN i sa hromatinskim vlaknima
- Pored potporne uloge, lamina učestvuje u organizaciji hromatina i kompleksa nukleusnih pora
- regulacija DNK replikacije i deobe





9 god. 86 god.

GRAĐA NUKLEUSA – nukleoskelet

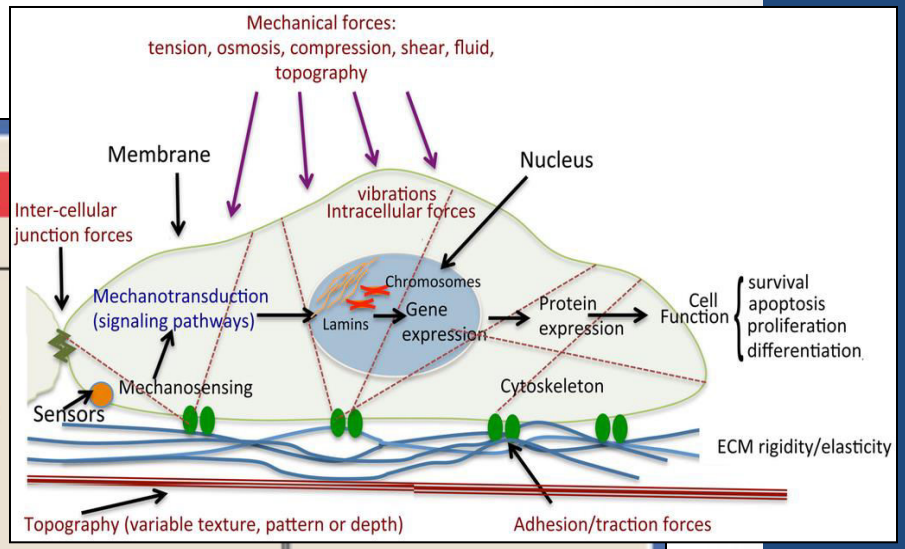


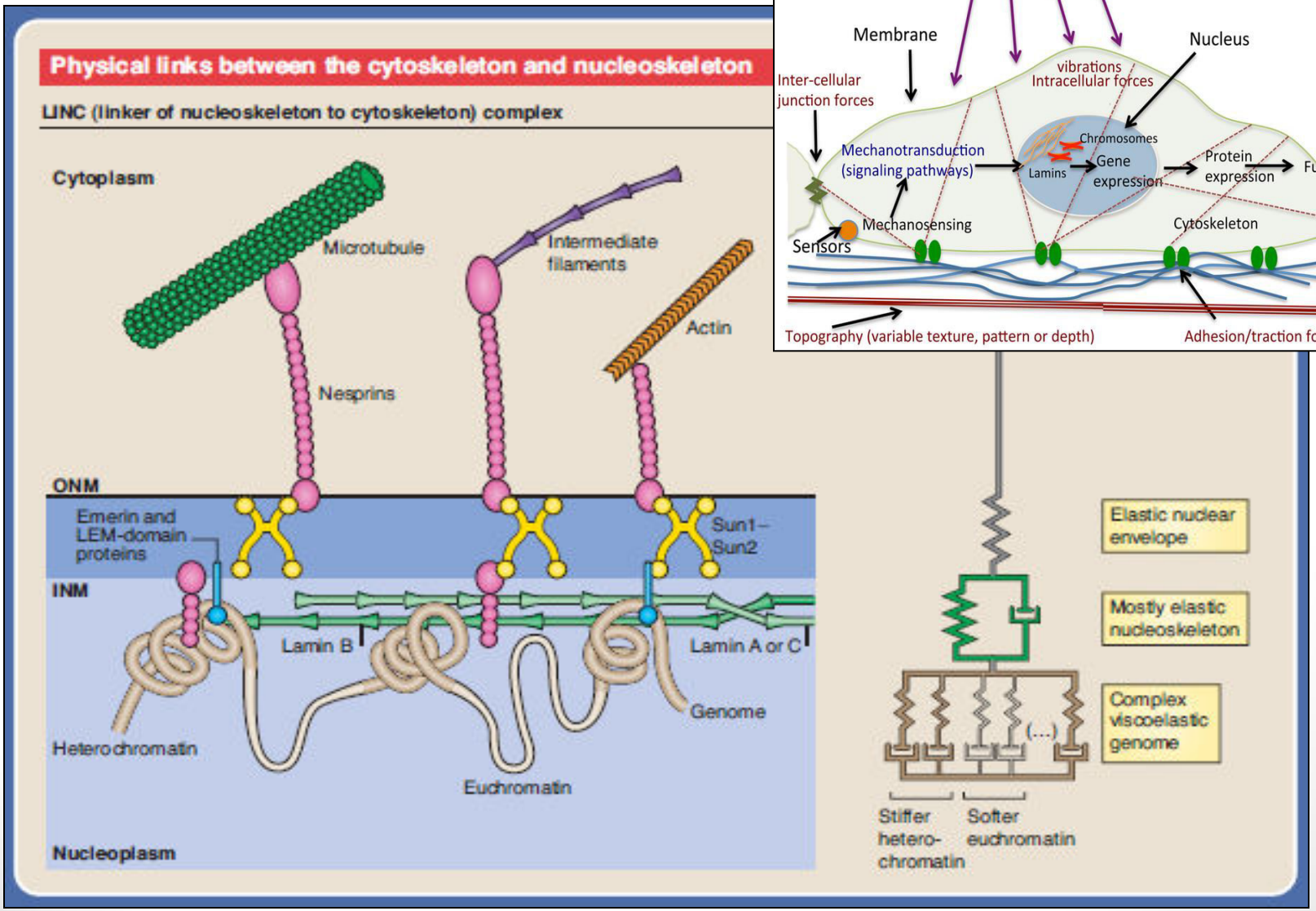
povezivanje nukleoskeleta za laminu

Nukleoskelet - proteinska mreža koju najvećim delom čine IF - lamini.
Aktinski filamenti su kratki (nekoliko monomera), uloga - strkutruna podrška, RNK obrada i eksport , prisustvo miozina i spektrina....?)
Potporna uloga nukleoskeleta
Uloga u RNK sintezi, regulaciji aktivnosti RNK polimeraze

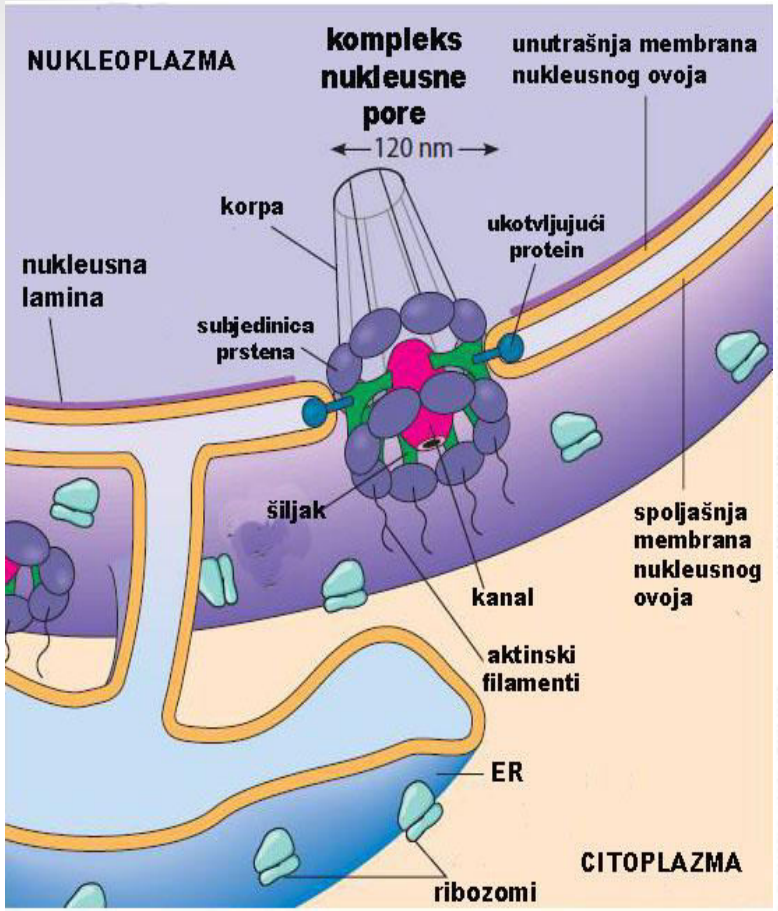
VEZA – nukleoskelet i citoskelet

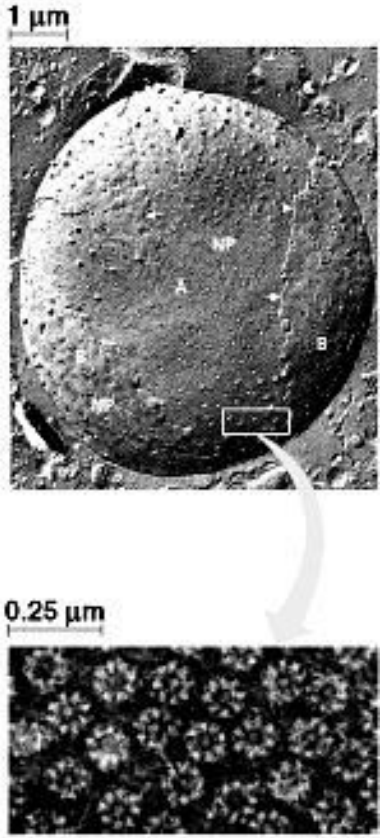
Integrini – citoskelet - nukleoskelet –remodeliranje DNK molekula – dostupnost za TF i trackripciju





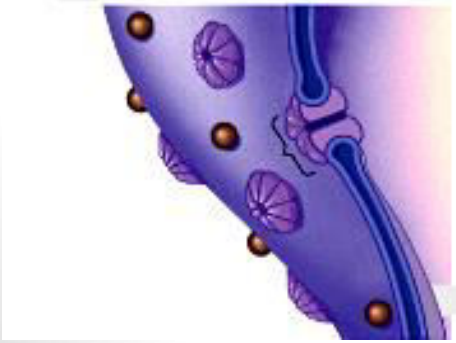
GRAĐA NUKLEUSA – kompleks nukleusne pore

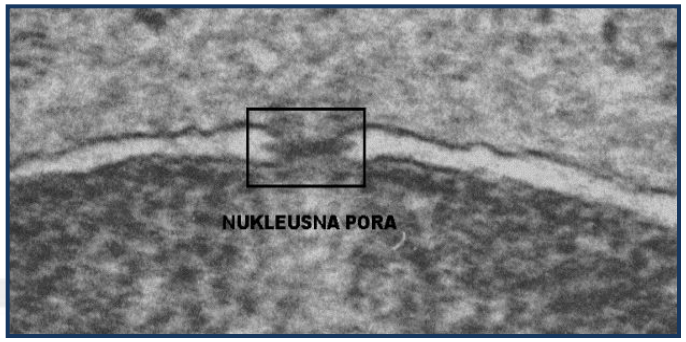


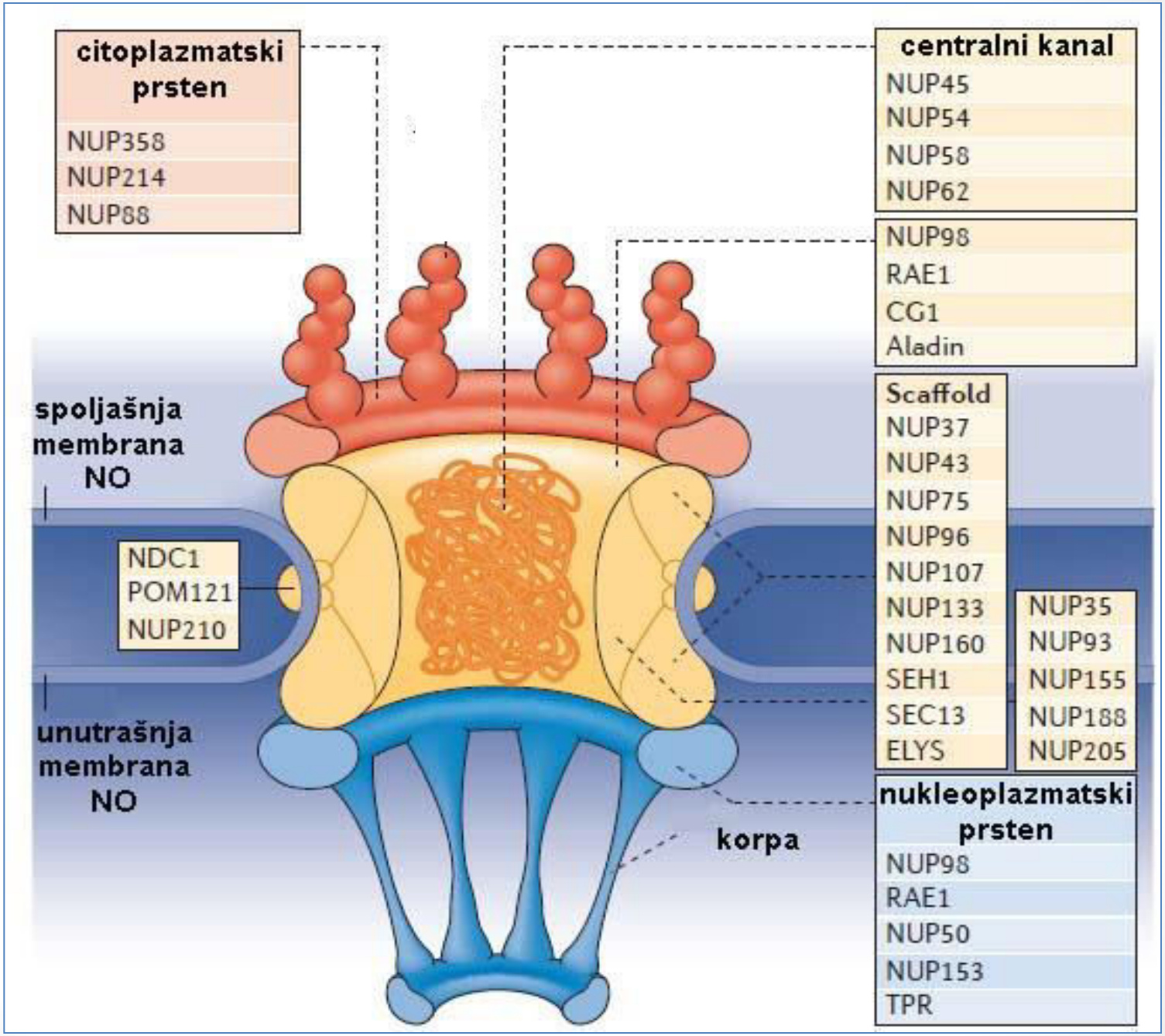


- ukotvljen u nukleusni ovoj;
- dijametar kompleksa 120 nm;
- dijametar kanala 9 nm
- nukleoporini (Nups)
- 3000-4000 pora po ćeliji u proseku
- transport 100 molek/min/ knp

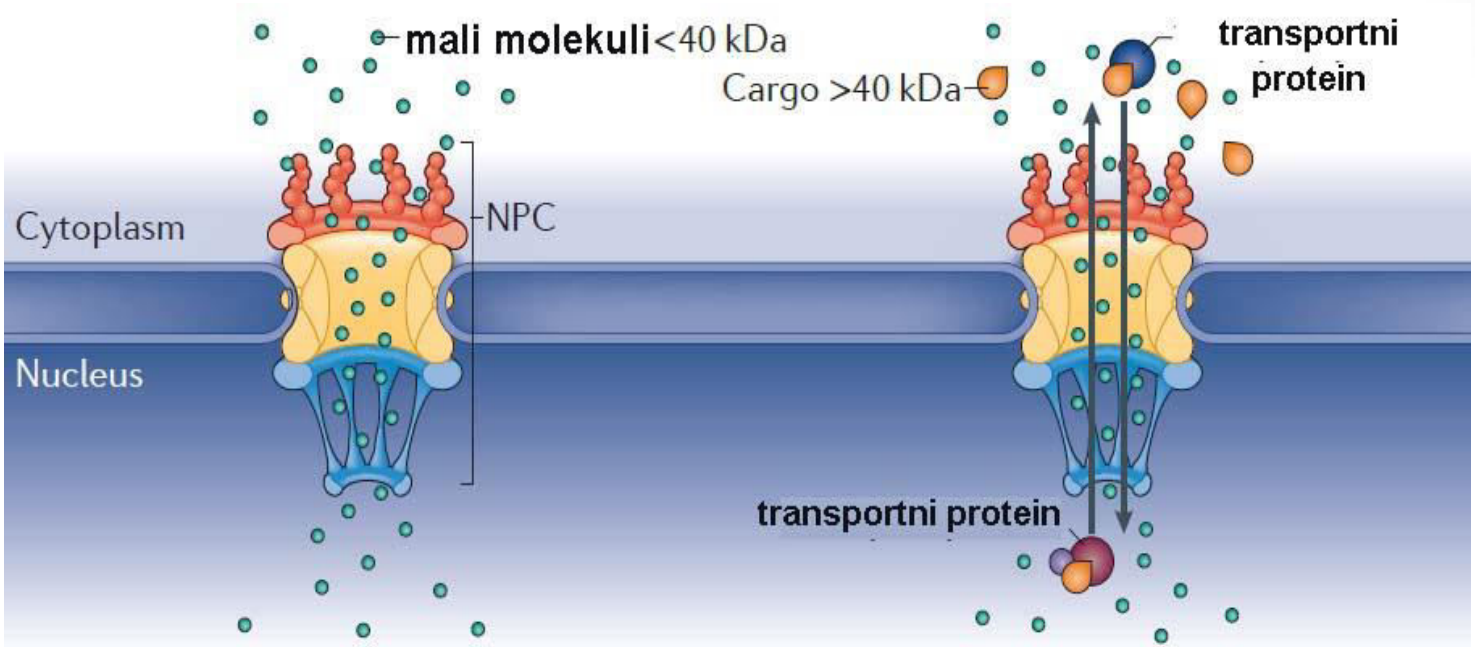
= osmougaona simetrija






citoplazmatski prsten
NUP358
NUP214
NUP88
centralni kanal
NUP45
NUP54
NUP58
NUP62
NUP98
RAE1
CG1
Aladin
spoljašnja membrana NO
NDC1
POM121
NUP210
Scaffold
NUP37
NUP43
NUP75
NUP96
NUP107
NUP133
NUP160
SEH1
SEC13
ELYS
NUP35
NUP93
NUP155
NUP188
NUP205
unutrašnja membrana NO
korpa
nukleoplazmatski prsten
NUP98
RAE1
NUP50
NUP153
TPR

difuzija *aktivan transport*

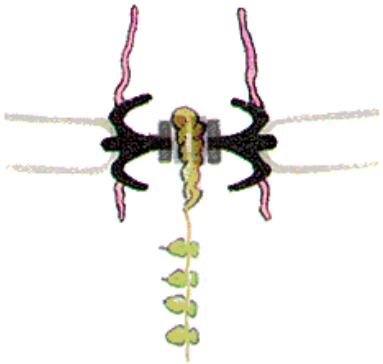


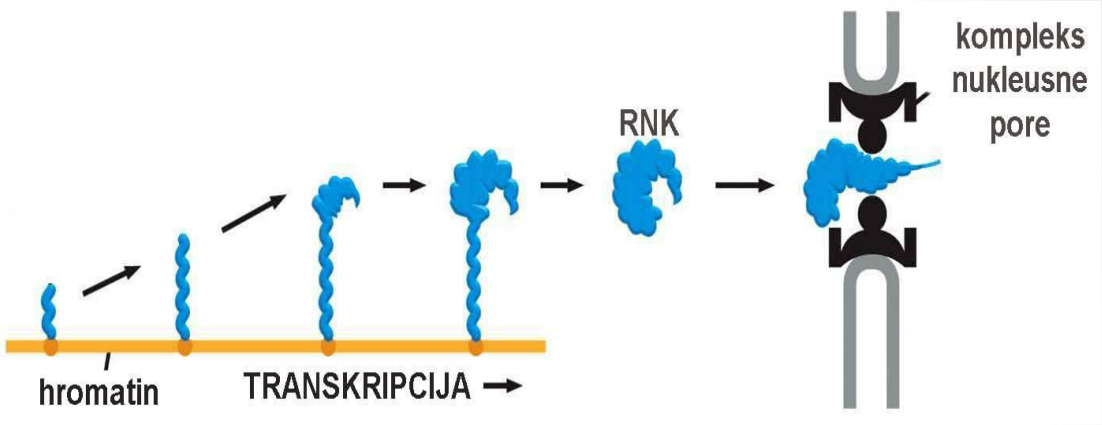
- preribozomi, RNK, joni
- proteini (histoni, transkripcioni faktori, enzimi...)
- <u>TRANSPORT U OBA SMERA</u>

NUKLEUSNI RECEPTORI – *importini* i *eksportini*

Specifične sekvence kojima se vrši prepoznavanje nukleusnih receptora i karga – NLS (nukleus lokalizujuća sekvenca) i NES (nukleus eksportna sekvenca)

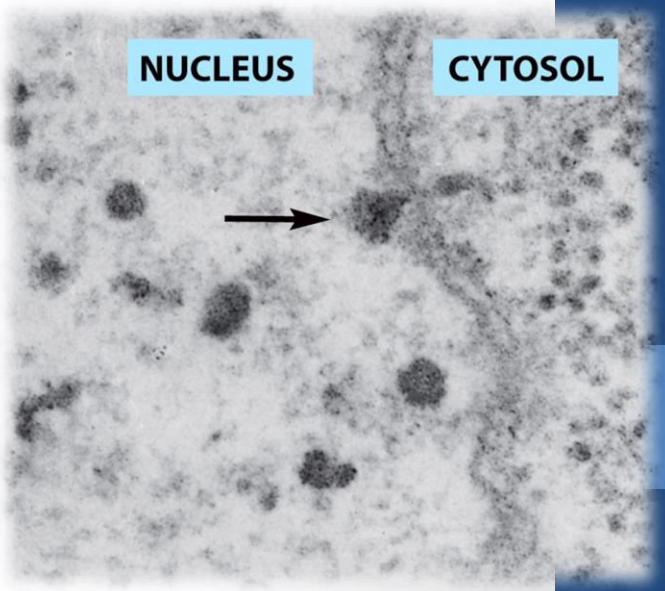
Model tansporta kroz kompleks nukleusne pore



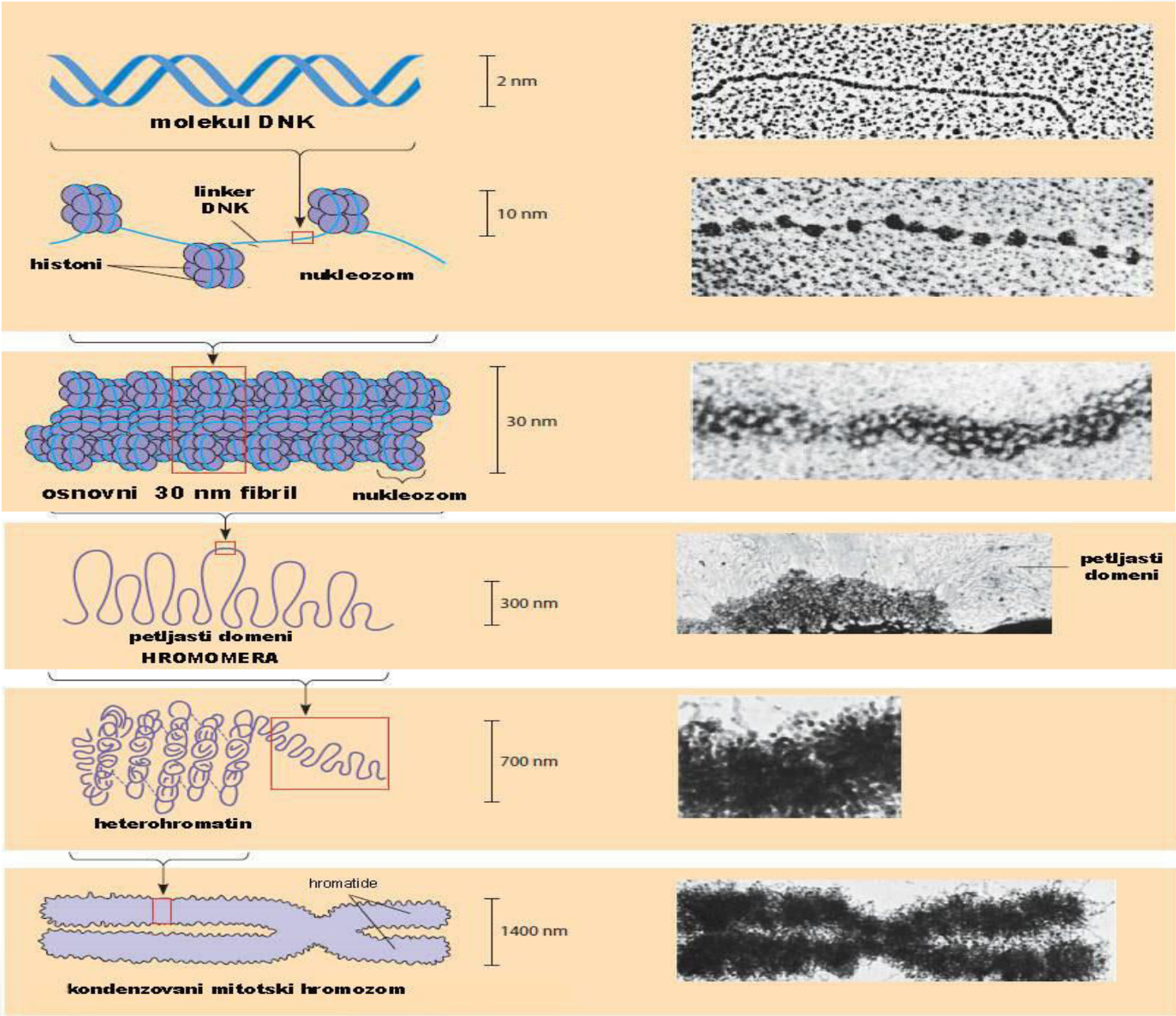


1. SVE ZAJEDNO - iRNK se savija u prostoru, njoj se pridružiju preribozomi i tRNK; takav kompleks pristiže do komleksa nukleusne pore. Odmah po ulasku u citoplazmu formira se polizom i počinje sinteza proteina.

1. KOMPONENTE SE TRANSPORTUJU ODVOJENO

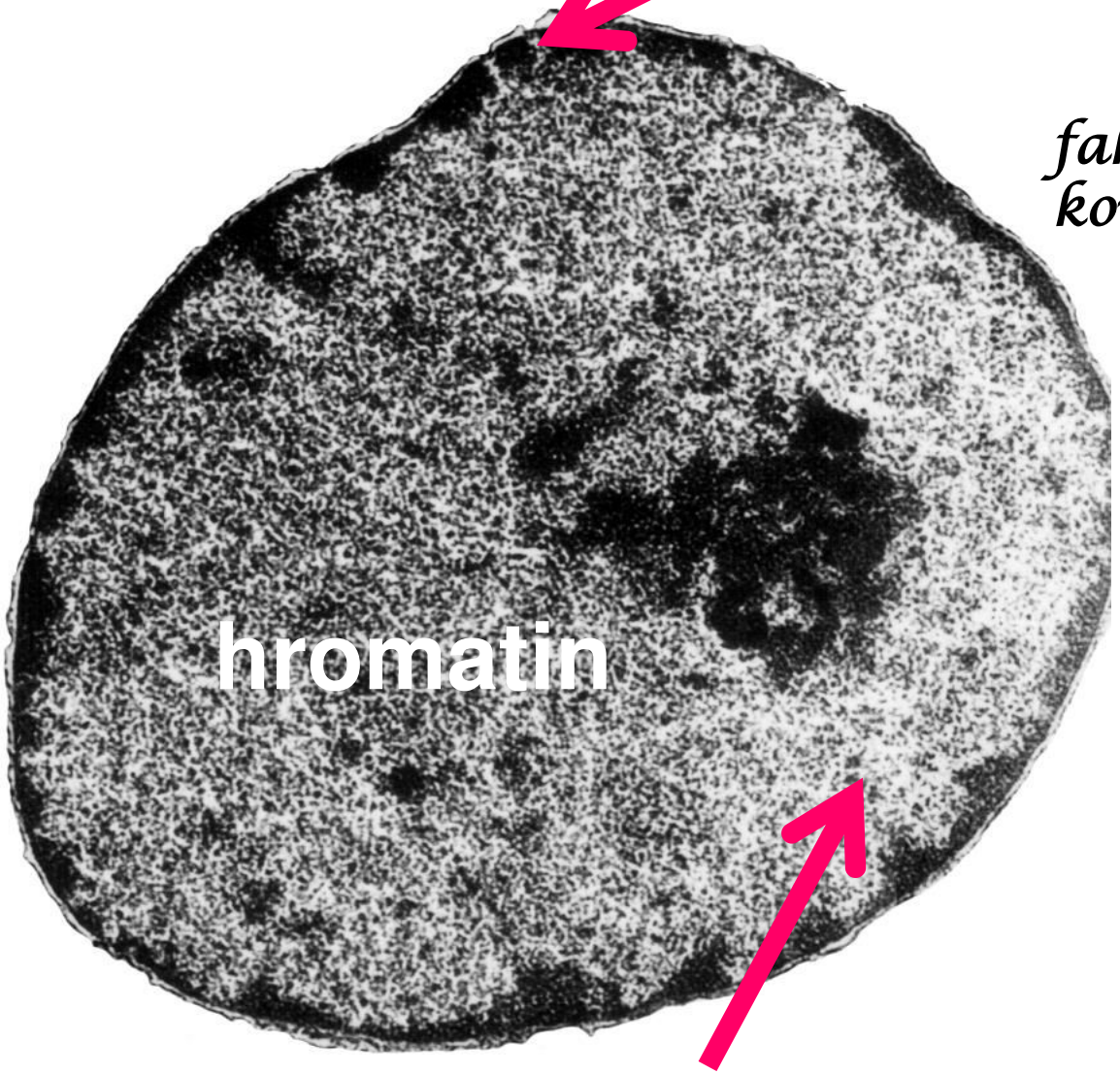


ORGANIZACIJA INTERFAZNIH HROMOZOMA



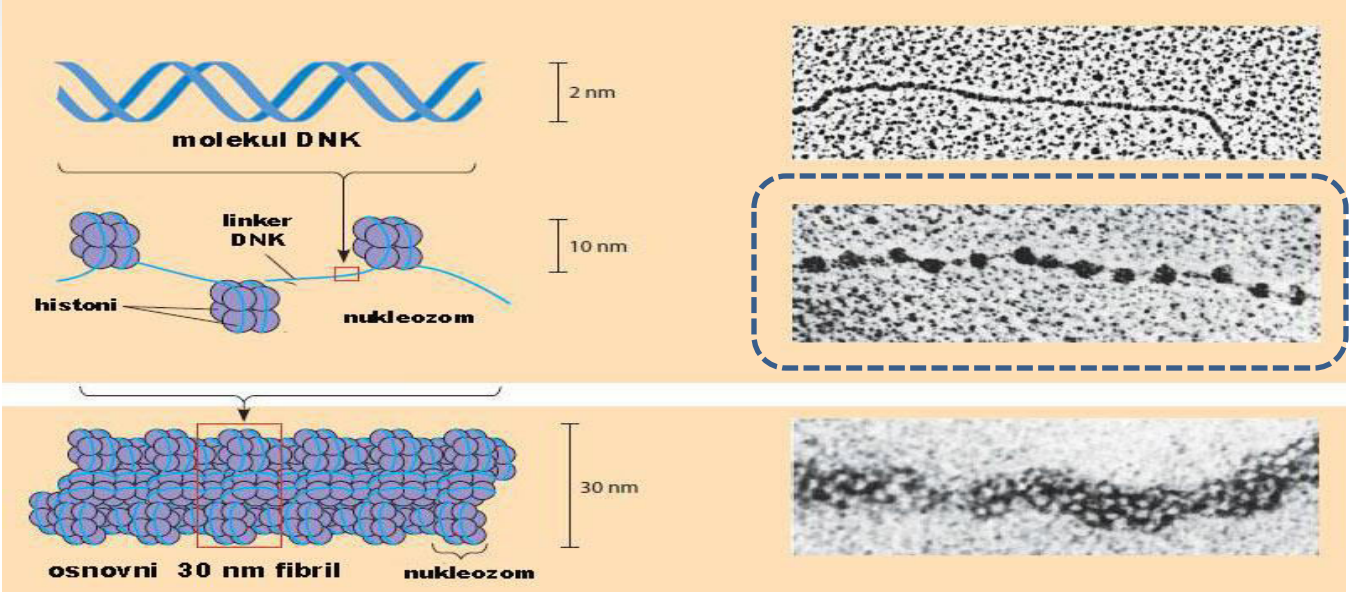
HETEROhromatin

fakultativní
konstitutivní

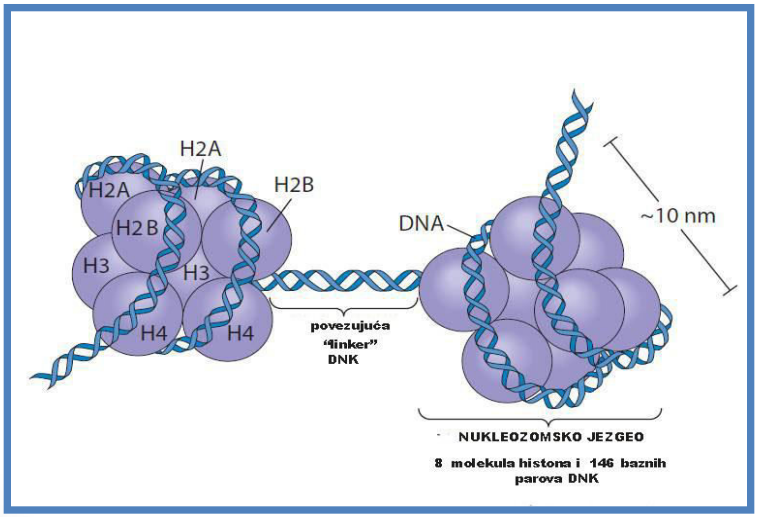


EUhromatin

ORGANIZACIJA INTERFAZNIH HROMOZOMA

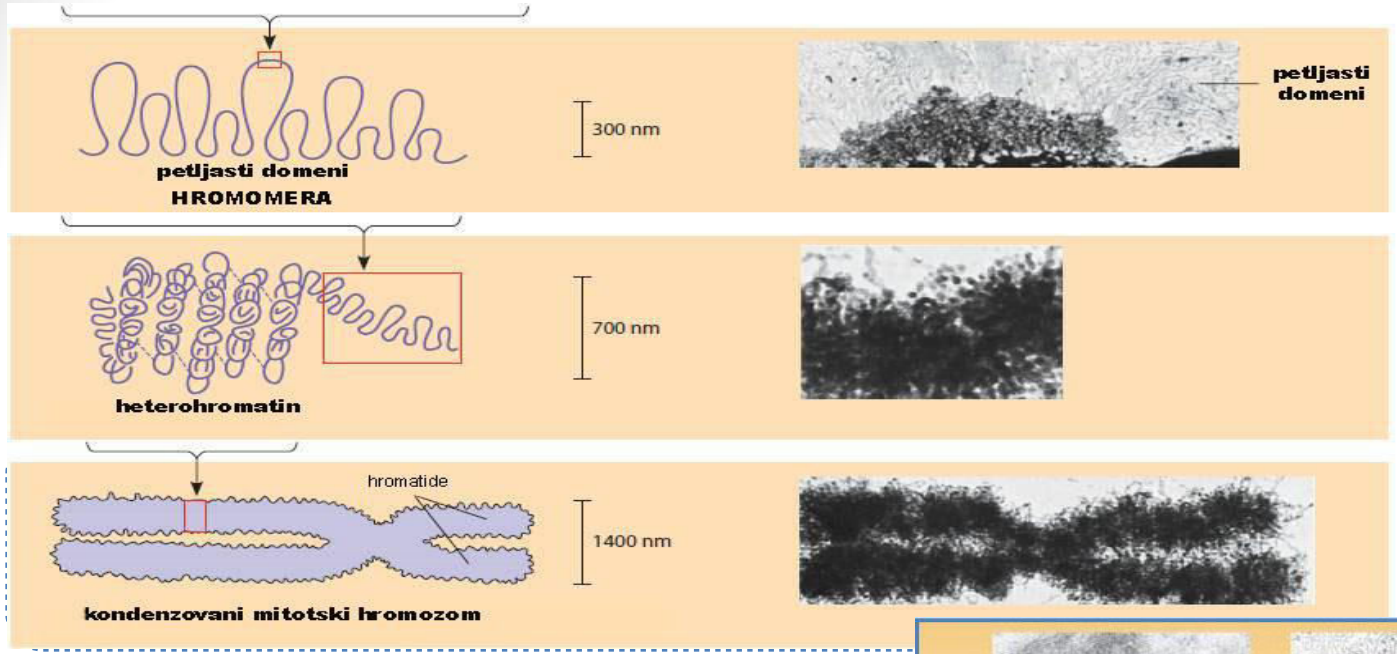


Kondezacija molekula DNK počinje molekulima histona, a zatim se nastavlja proteinima nukleoskeleta. Molekul DNK se uvija oko loptaste strukture (**nukleozomsko jezgro**) i to na takav način da se susedne strukture nalaze sa suprotnih strana. Na taj način nastaje prvi stupanj kondenzacije koji se naziva **nukleozom** i debljine je 11 nm.



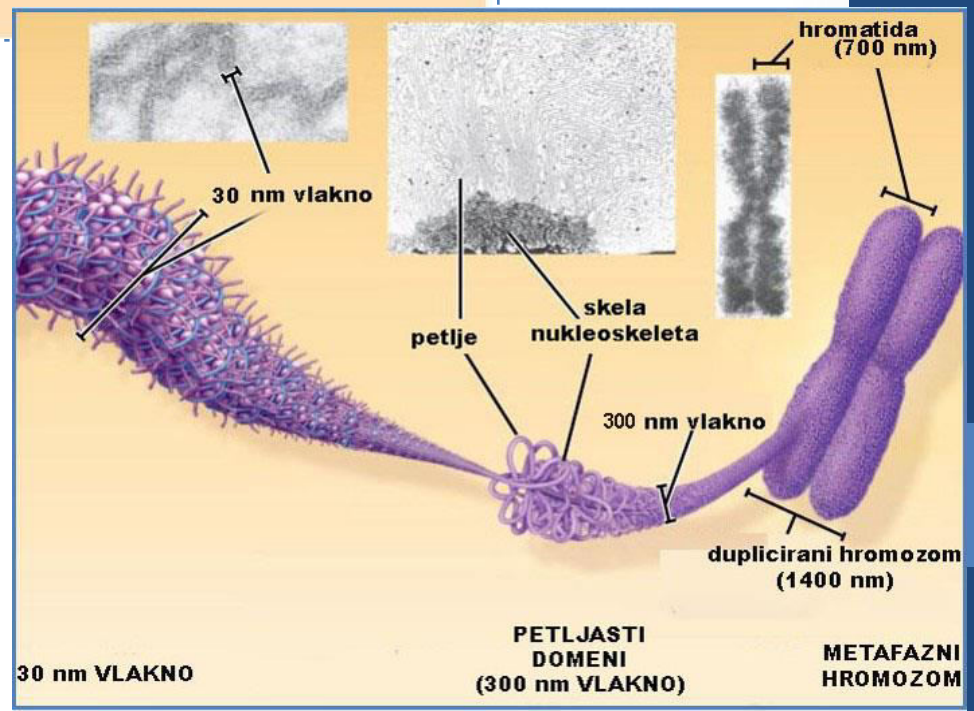
U sledećem stupnju histon H1 će uhvatiti linker DNK poput ukosnice, približiti dva nukleozoma i nastaće jedna nestabilna struktura koja će početi da se uvija u prostoru i nastaje **OSNOVNI 30 nm FIBRIL**

ORGANIZACIJA INTERFAZNIH HROMOZOMA

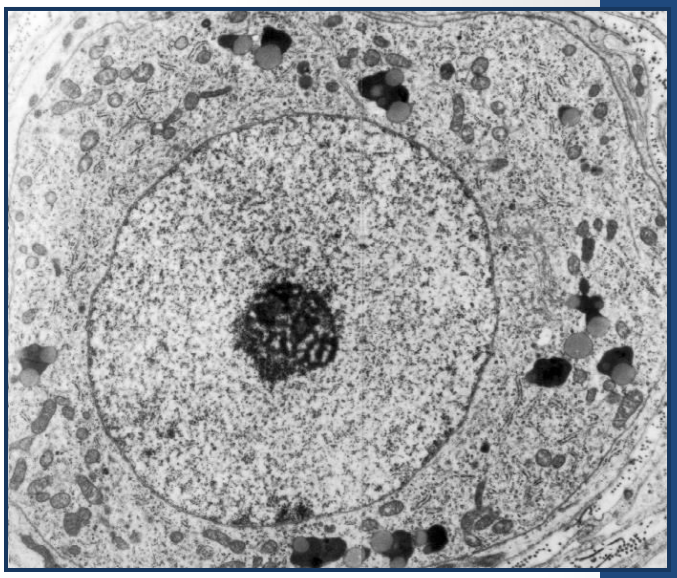


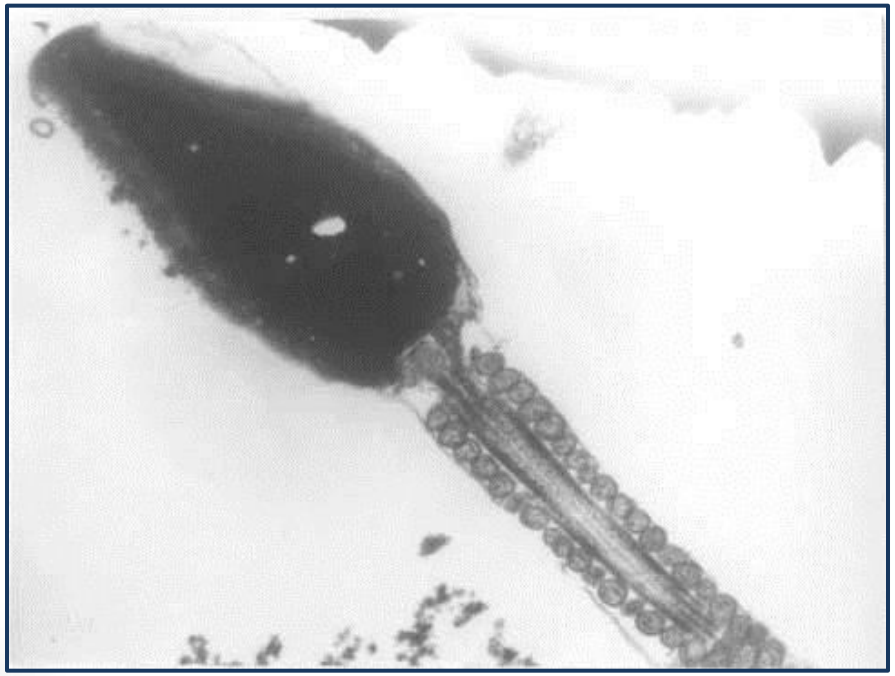
Petlje se dodatno kondenzuju proteinima – kondenzinima. Ovi proteini se zakače za petlje i „vuku“ ih ka centru hromozoma. Na taj način dobija se na stotinak malih petljica.

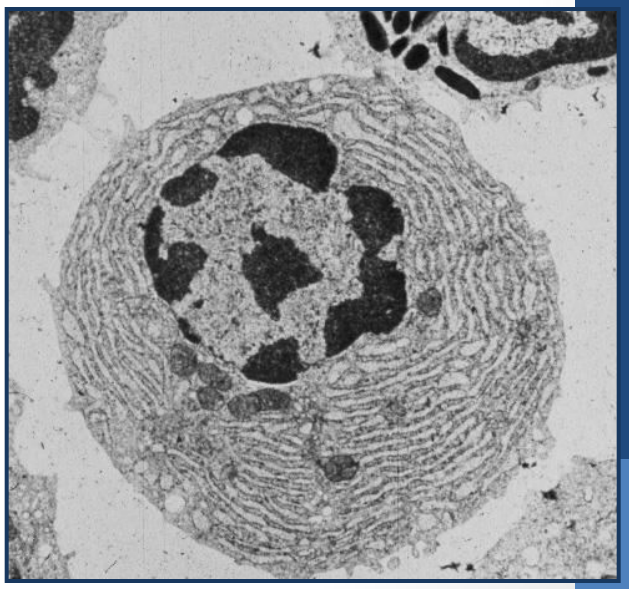
Nakon formiranja osnovnog 30 nm fibrila prestaje kondenzacija pomoću histona. Proteini koji učestvuju u daljem kondenzovanju su proteini nukleoskeleta. Osnovni 30 nm fibril poseduje sekvence za vezivanje komponenti nukleoskeleta. Na taj način će se „zakačiti“ dva regiona a između će ostati delovi DNK koji vire u vidu petlji. Dalja kondenzacija se odvija na isti način, „upetljavanjem“ petlji, čime sa skraćuje hromozom i nastaje fibril debljine 300 nm.



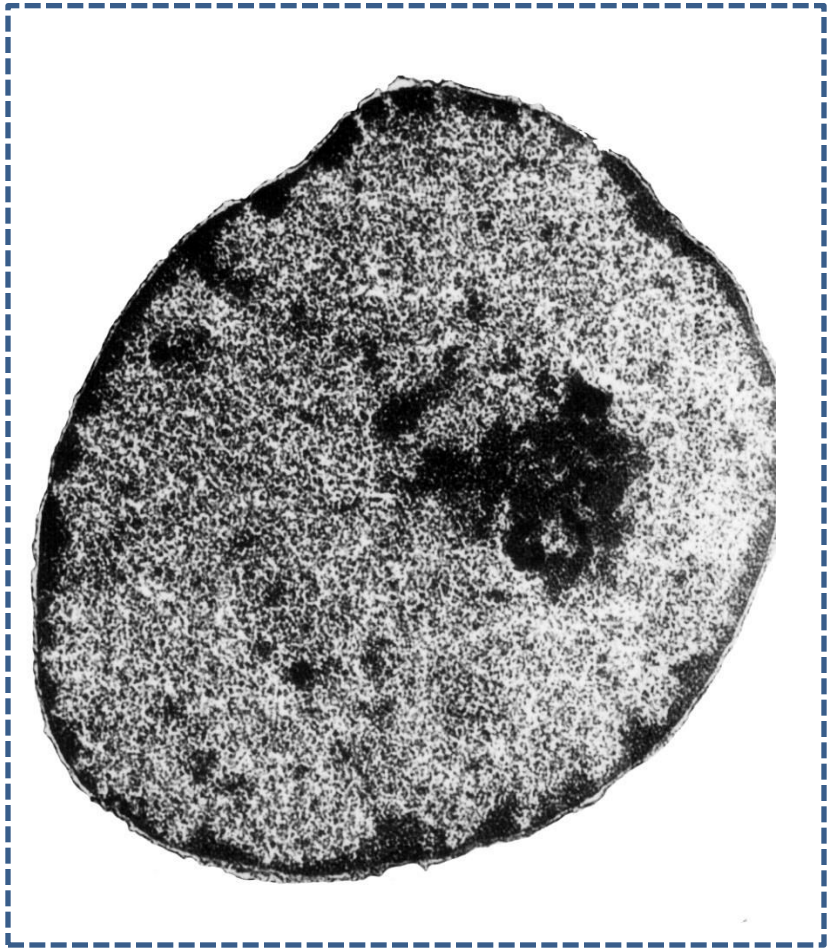
- Hromatin i hromatinska slika
- Nivoi organizacije hromatina

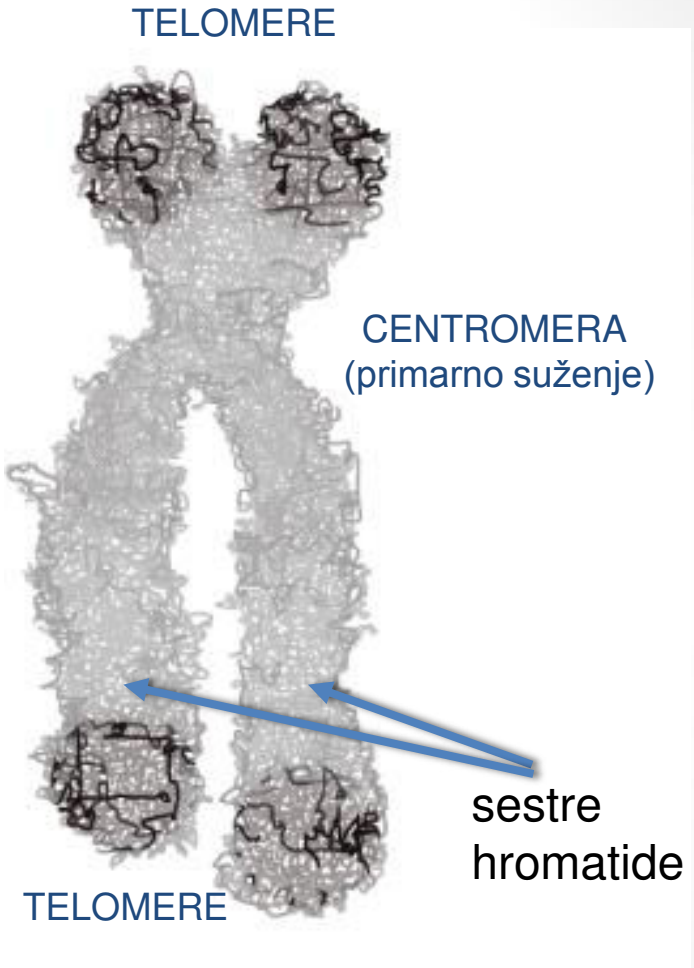






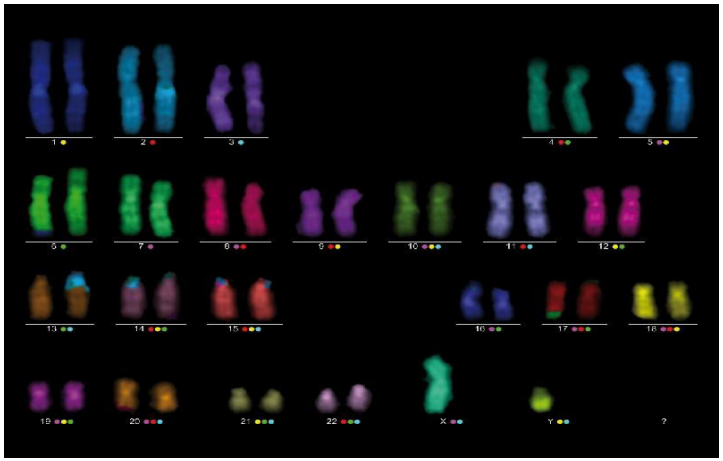
HROMATIN I HROMOZOMI





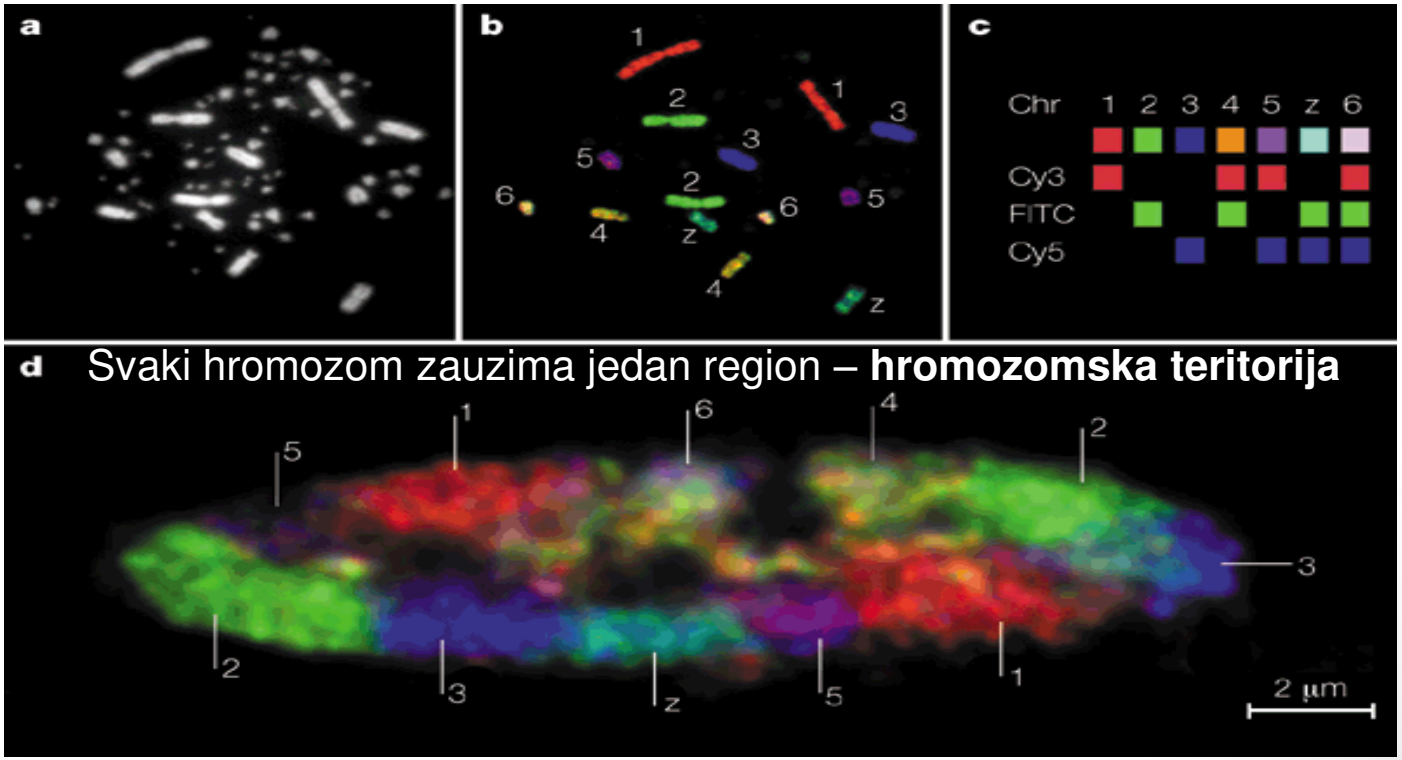
Da li ovakva organizacija hromozoma postoji i u interfaznom nukleusu?

Fluorescent *In-Situ* Hybridization (FISH)
"Chromosome Painting"



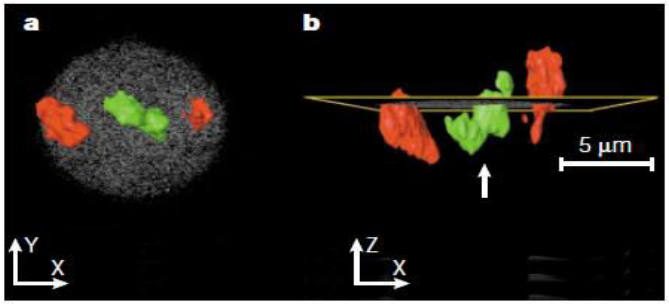
KARIOGRAM

Fluorescentna *in situ* hibridizacija (FISH) je metoda kojom možemo da obeležimo hromozome korišćenjem proba, odnosno specifinih sekvenci nukleotida koje su obeležene fluorescentnim bojama.
Na taj način, denaturacijom DNK i hibridizacijom, uz pomoć proba obeleži se željeni hromozom (hromozome).

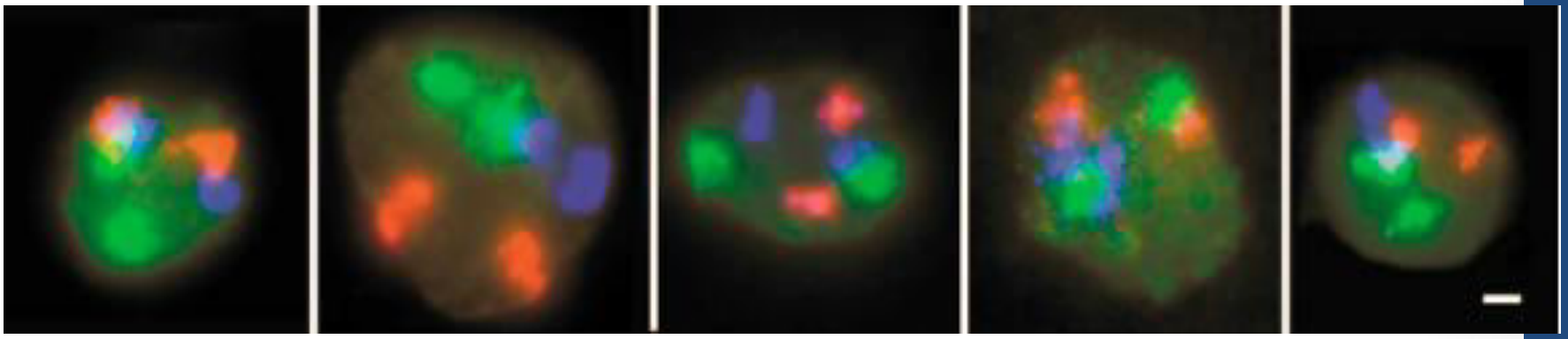


ĆELIJSKA - TKIVNA SPECIFIČNOST

U INTERFAZNOM HROMOZOMU – 2n



12 14 16

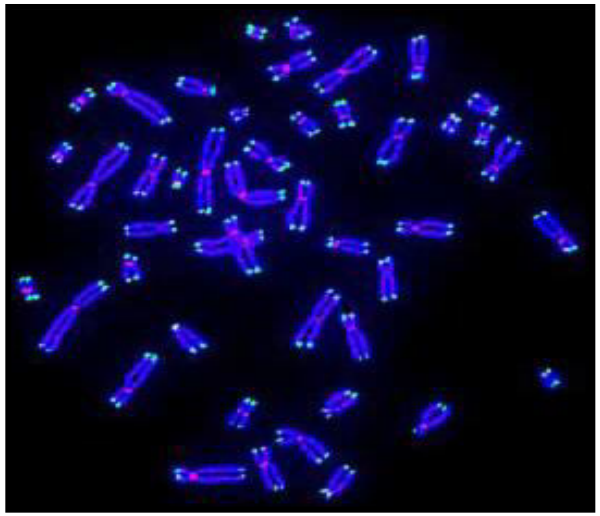


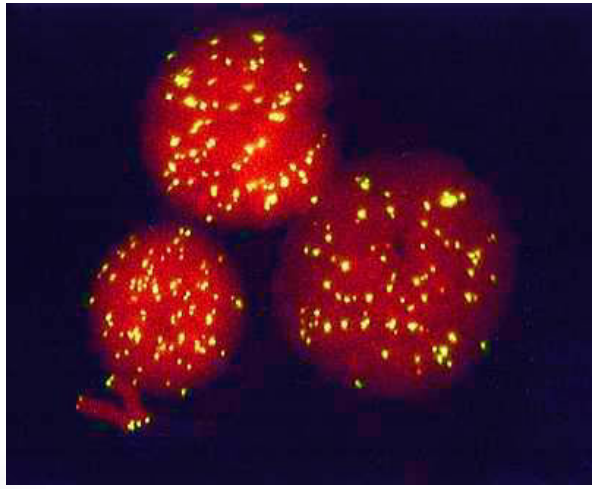
limfocit | veliki pneumocit | mali pneumocit | bubrežna ćelija | hepatocit

Pozicija i položaj hromozoma je ćelijski i tkivno specifična – posledica funkcije (ekspresija)

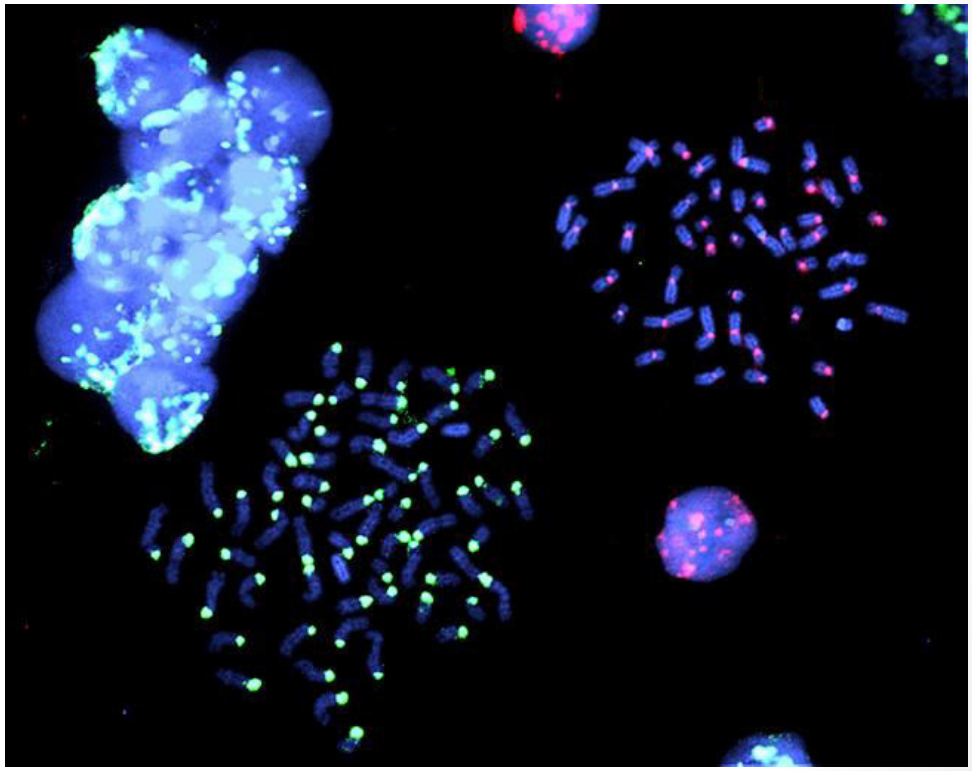
POZICIJA U HROMOZOMSKOJ TERITORIJI

TELOMERE



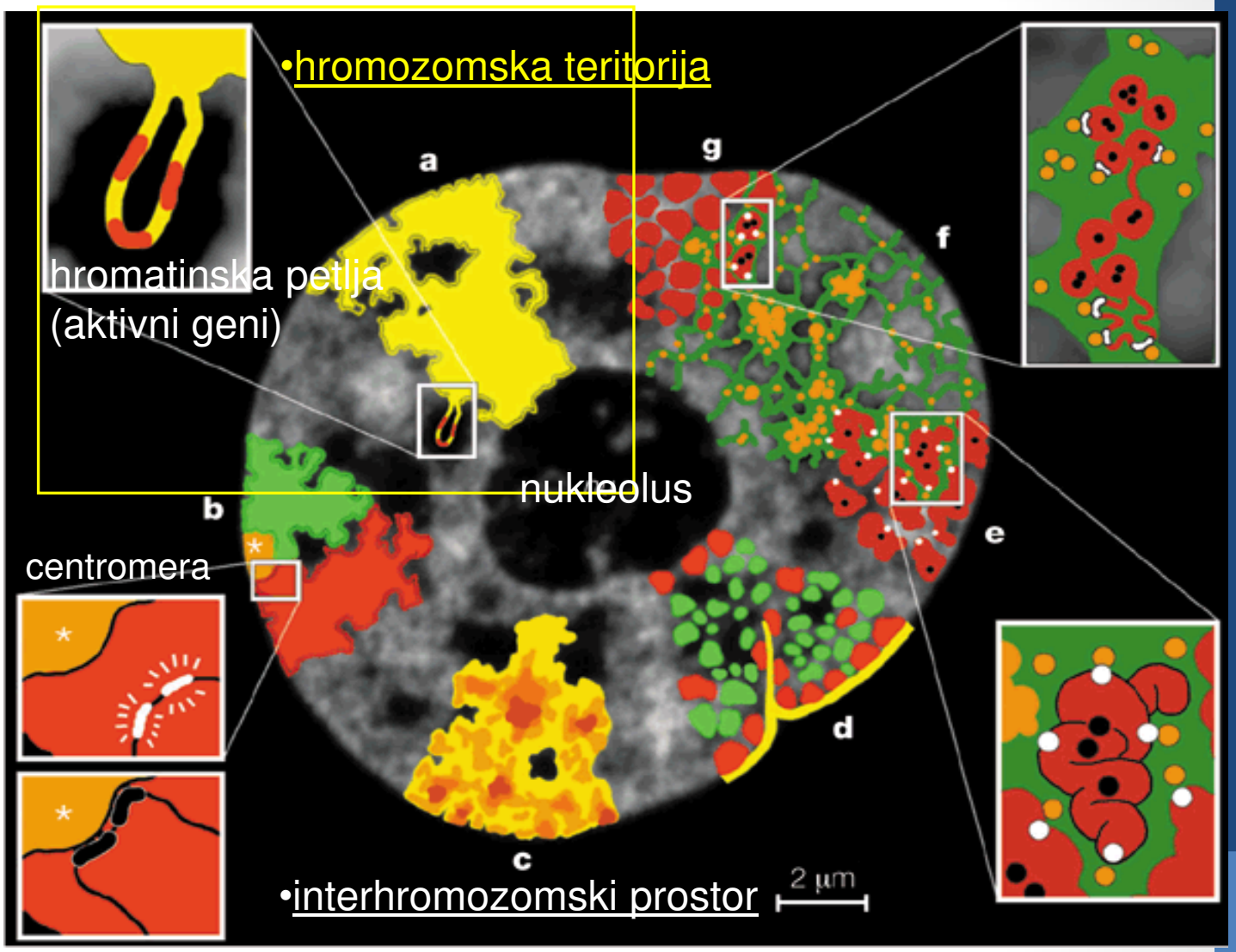


CENTROMERE



a) Hromozomska teritorija (HT) – sunđerasta struktura; brojne šupljine – **interhromatinski prostori**. Na površini HT petlje - delovi sa kojih se vrši transkripcija.

F) Narandžasta boja prikazuje poziciju kompleksa koji učestvuje u procesu transkripcije i obrade.



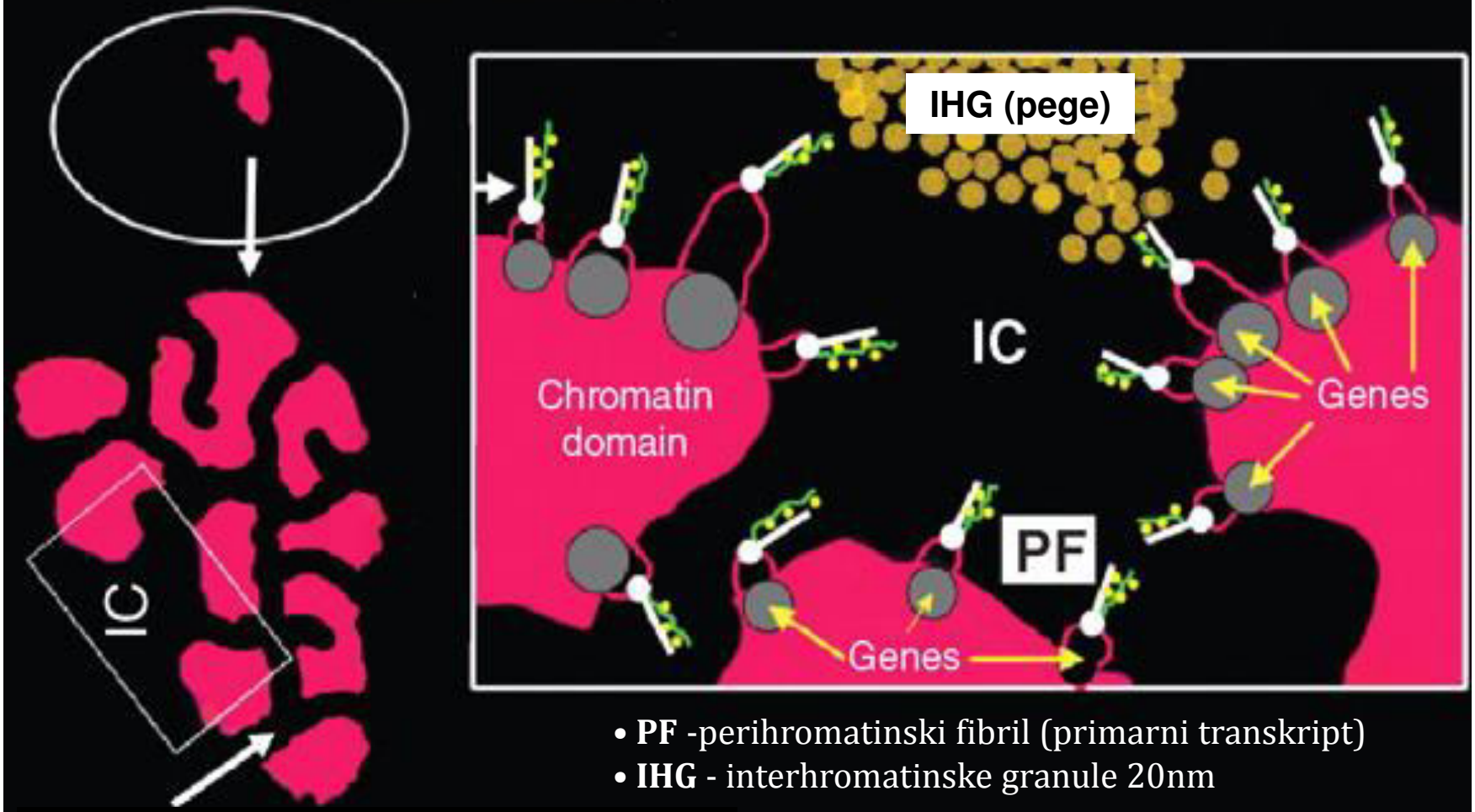
b) Pozicija **centromere** unutar HT.

c) Unutar HT postoji **razlika u kondenzaciji hromatina**. Tamni delovi HT su oblasti veće kondenzacije, svetli delovi su delovi manje kondenzacije.

e) **Aktivni geni** (bele tačke) su na površini HT, **neaktivni geni** (crne tačke) su u unutrašnjosti HT.

SINTEZA, OBRADA I TRANSPORT RNK

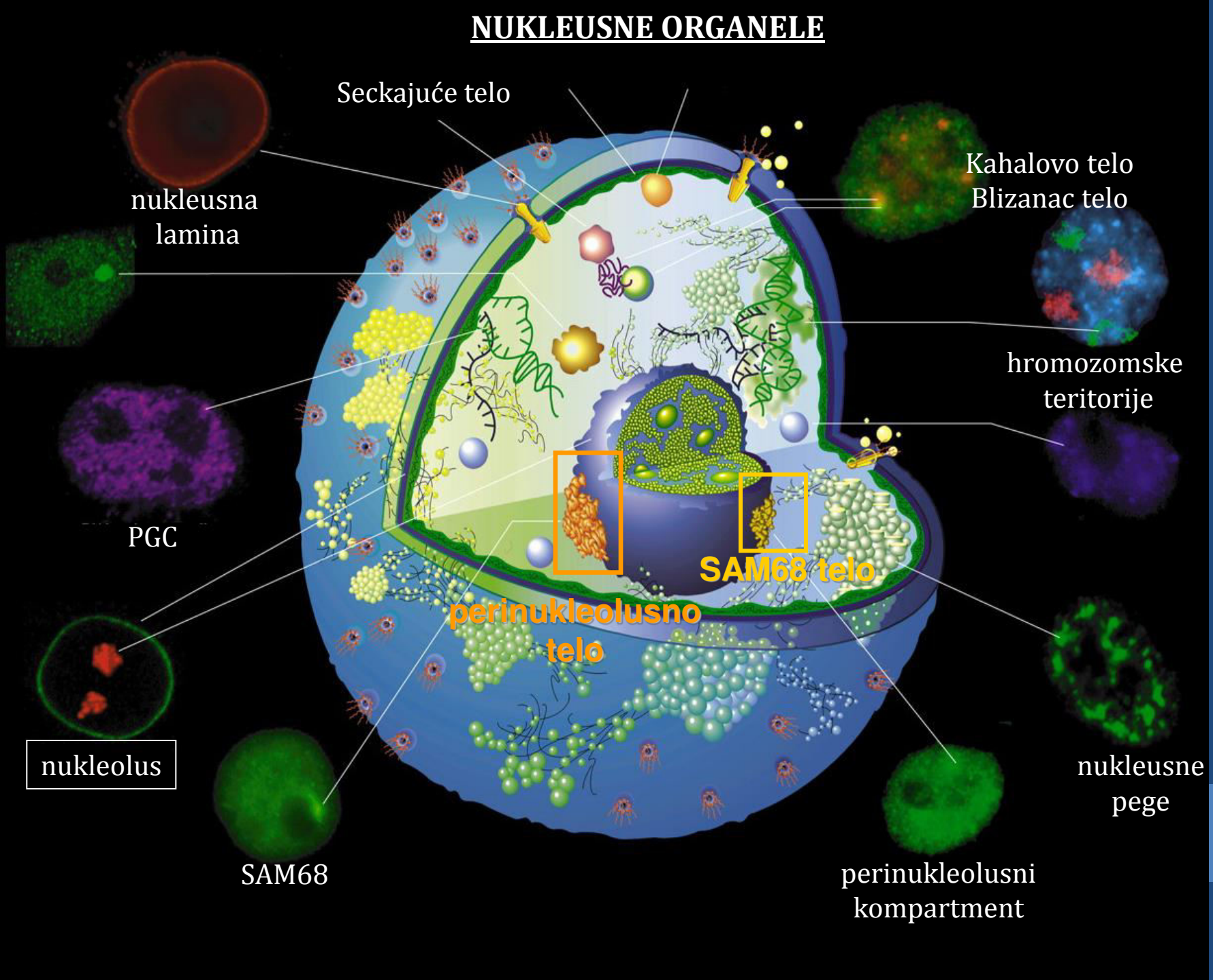
Hromozomska teritorija



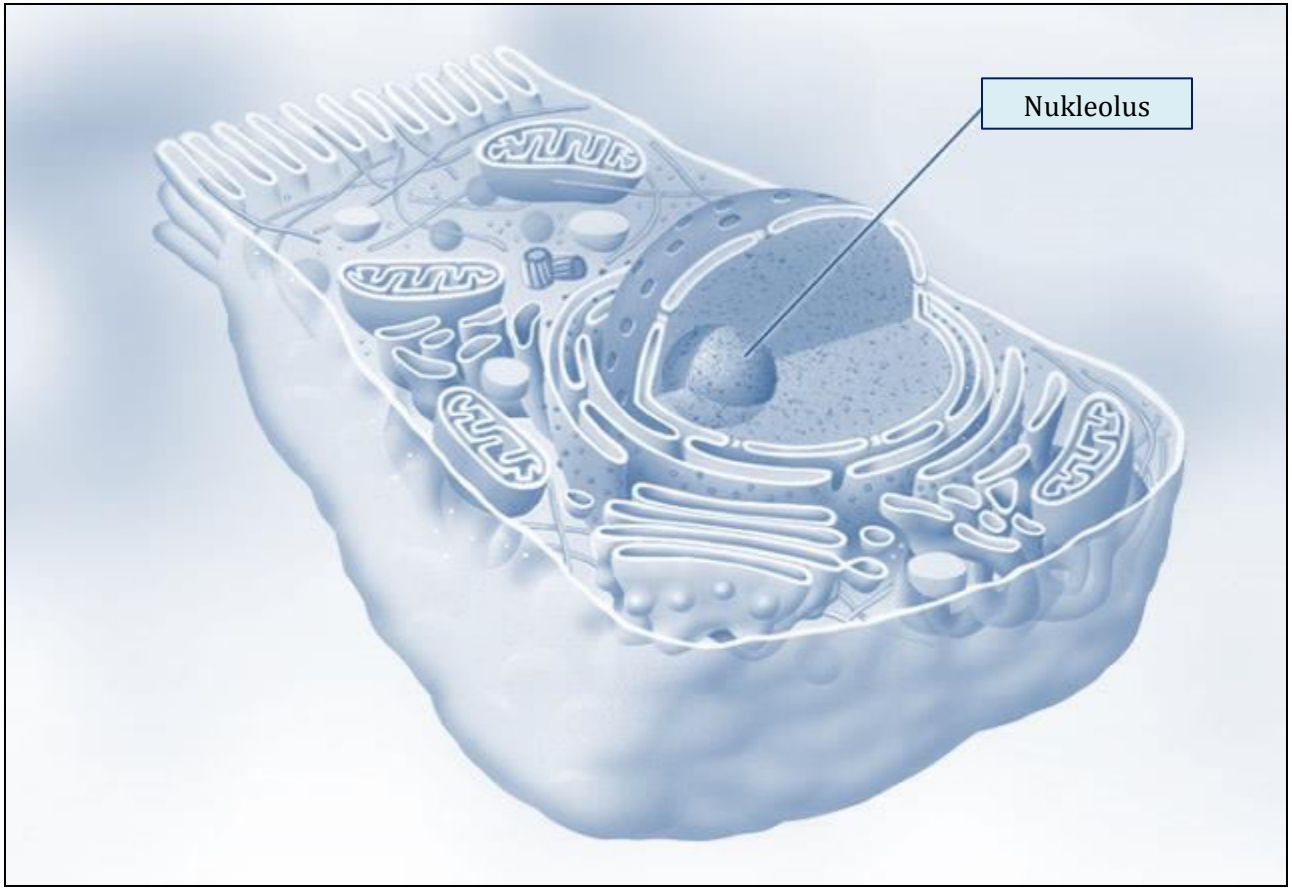
- **PF** -perihromatinski fibril (primarni transkript)
- **IHG** - interhromatinske granule 20nm

Interhromozomski kanal

NUKLEUSNE ORGANELE

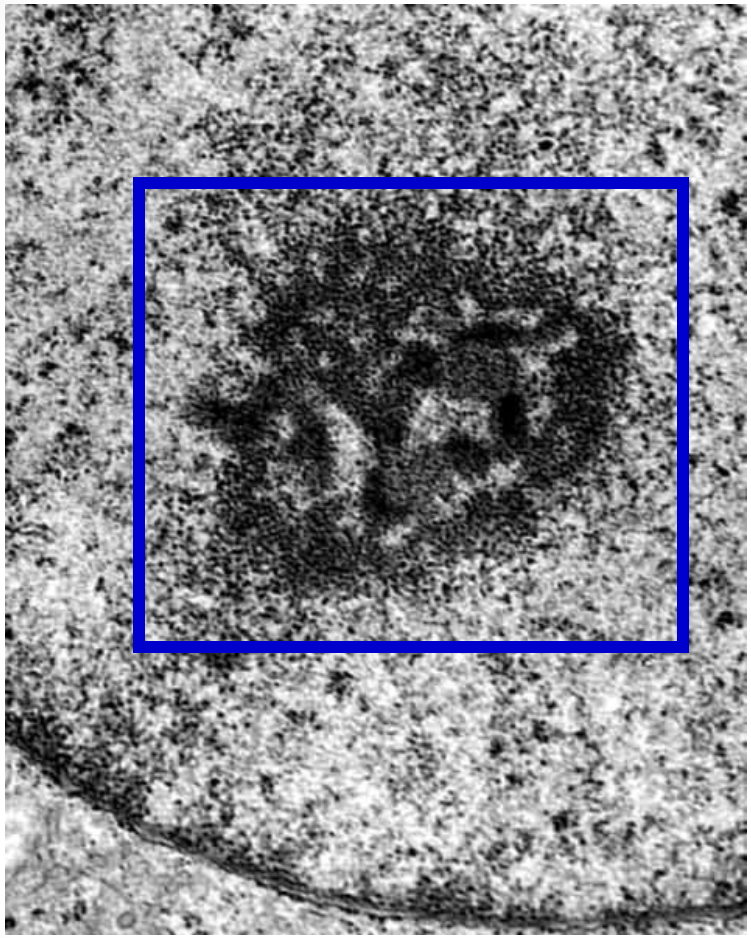


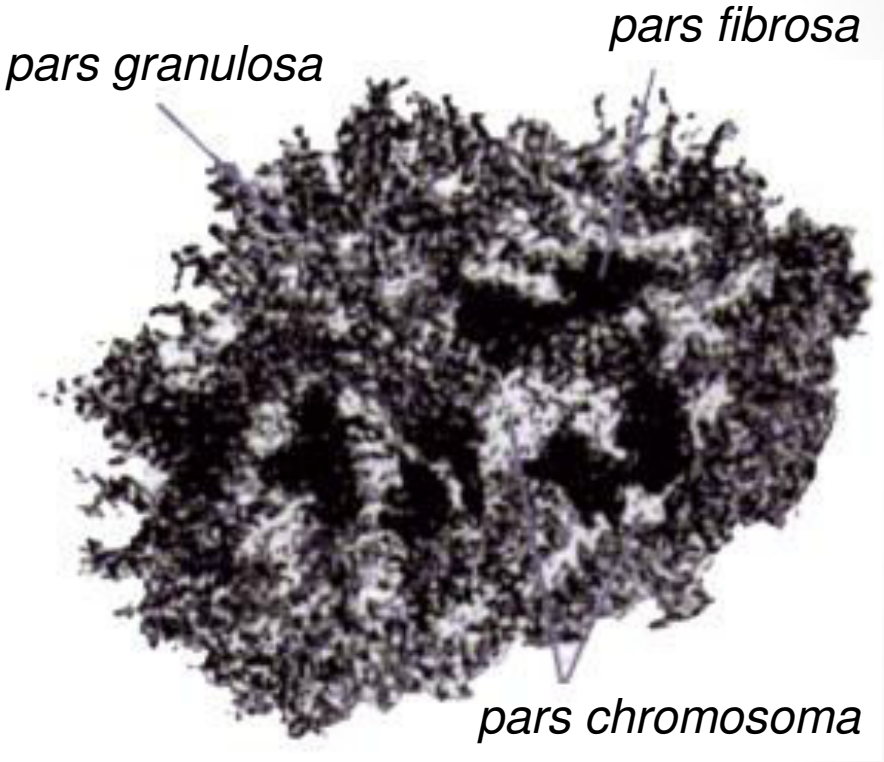
NUKLEOLUS



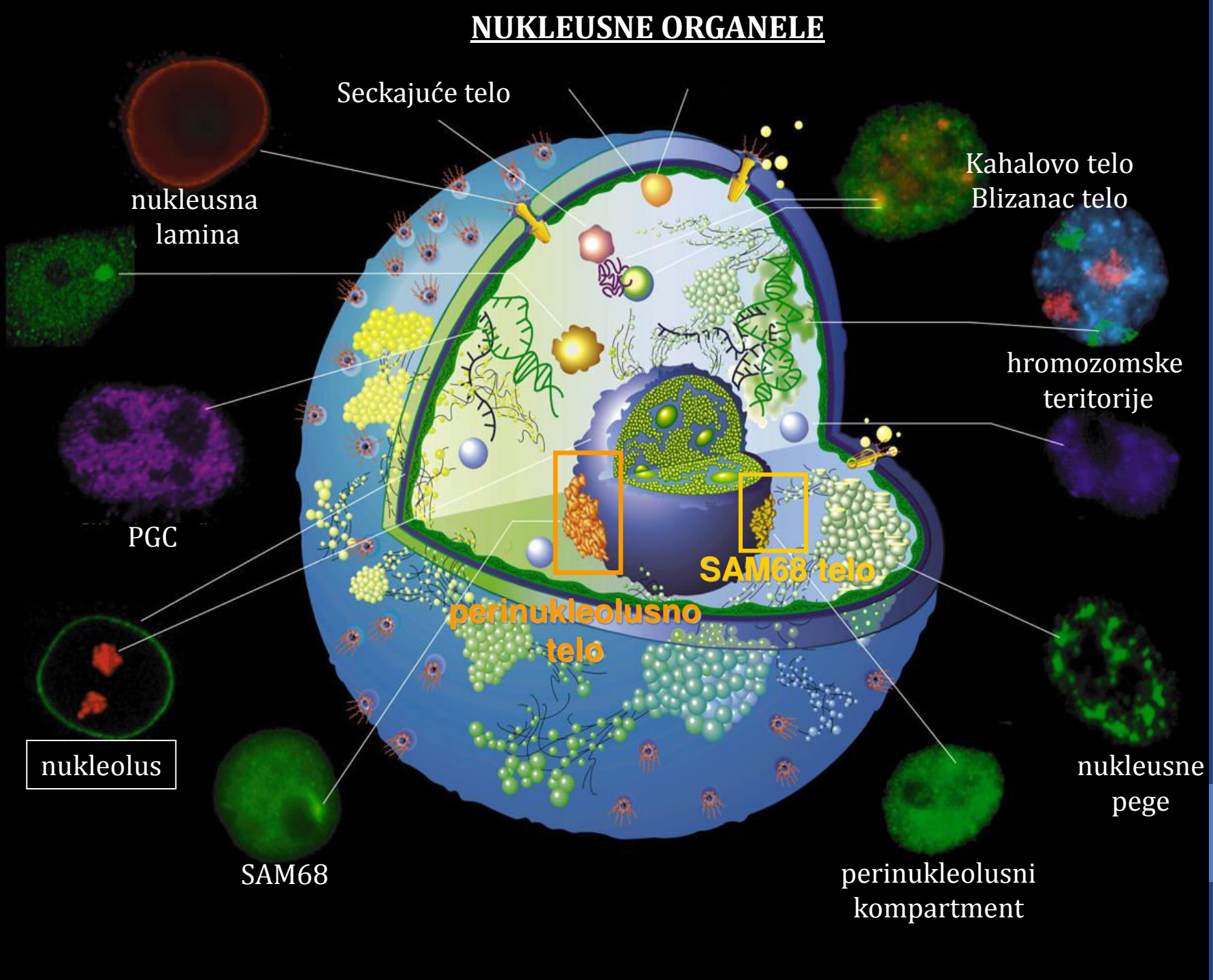
- nastaje iz oblasti sekundarnog suženja – organizator nukleolusa
- višestruko ponovljeni geni za ribozomalnu RNK
- broj sekundarnih suženja određuje broj nukleolusa (u našim ćelijama 1-5)
- broj nukleolusa u ćeliji je obrnuto proporcionalan njihovoj veličini

NUKLEOLUS – proces biogeneze ribozoma





NUKLEUSNE ORGANELE



SnapShot: Cellular Bodies

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Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA

Cell

	Body Name	Number/ Cell	Typical Size and Shape	Marker Protein	Description	Image
Nuclear Bodies	Cajal Body	0–6	0.1–2.0 μm; round	Coilin	Involved in snRNP and snoRNP biogenesis and posttranscriptional modification of newly assembled spliceosomal snRNAs.	
	Clastosome	0–3	0.2–1.2 μm; irregular	20S core catalytic component of proteasome	Contains ubiquitin conjugates, the proteolytically active 20S core and 19S regulatory complexes of the 26S proteasome, and protein substrates of the proteasome.	
	Cleavage Body	1–4	0.2–1.0 μm; round	CstF 64 kDa	Contains several factors involved in 3′ cleavage of mRNAs. ~20% contain newly synthesized RNA. Some cleavage bodies localize adjacent to Cajal and PML bodies.	
	Nuclear Speckle or Interchromatin Granule Cluster	25–50	0.8–1.8 μm; irregular	SC35, SF2/ASF	Contains proteins for pre-mRNA processing. Involved in the storage, assembly, and/or modification of pre-mRNA splicing factors.	
	Nuclear Stress Body	2–10	0.3–3.0 μm; irregular	HSF1	Induced by heat shock response. Associates with satellite III repeats on human chromosome 9q12 and other pericentromeric regions; recruits various RNA-binding proteins.	
	OPT Domain	1–3	1.0–1.5 μm; round	PTF	Contains several transcription factors (Oct1/PTF) and RNA transcripts; predominant in late G1 cells. Often localizes close to nucleolus.	
	Paraspeckle	10–20	0.5 μm; round	p54nrb, PSP1	Contains several RNA-binding proteins and nuclear-retained CTN-RNA.	
	Perinucleolar Compartment	1–4	0.3–1.0 μm; cap	hnRNPI (PTB)	Cap on surface of nucleolus; found mainly in transformed cells. Contains RNA pol III transcripts and several RNA-binding proteins.	
	PML Body	10–30	0.3–1.0 μm; round	PML	Suggested to play a role in aspects of transcriptional regulation and/or nuclear protein sequestration.	
	Polycomb Body	12–15	0.3–1.0 μm; round/ irregular	Bmi1, Pc2	Contains silencing proteins associated with Polycomb repressive complex 1; associates with heterochromatin.	
Cytoplasmic Bodies	Aggresome	1	2.0–10.0 μm; irregular	CFTR	Forms when proteasome's degradative capacity is exceeded. May sequester aggregated proteins/substrates for lysosomal degradation via autophagy. Associated with microtubule organizing center, encaged by vimentin.	
	Processing Body (P Body)	0–30	0.1–1.0 μm; round	Ago1/2, GW182	Contains decapping enzymes, a 5′-to-3′ exoribonuclease, LSm proteins, and RNAi machinery components. Also involved in storage of miRNA-repressed mRNAs.	
	Stress Granule	5–30	0.4–5.0 μm; irregular	eIF3	Formed upon stress. Contains "stalled" mRNAs, mRNA-binding proteins, translation initiation factors, and 40S ribosomal subunits. mRNAs can shuttle between stress granules and P bodies.	

See online version for references and acknowledgments.