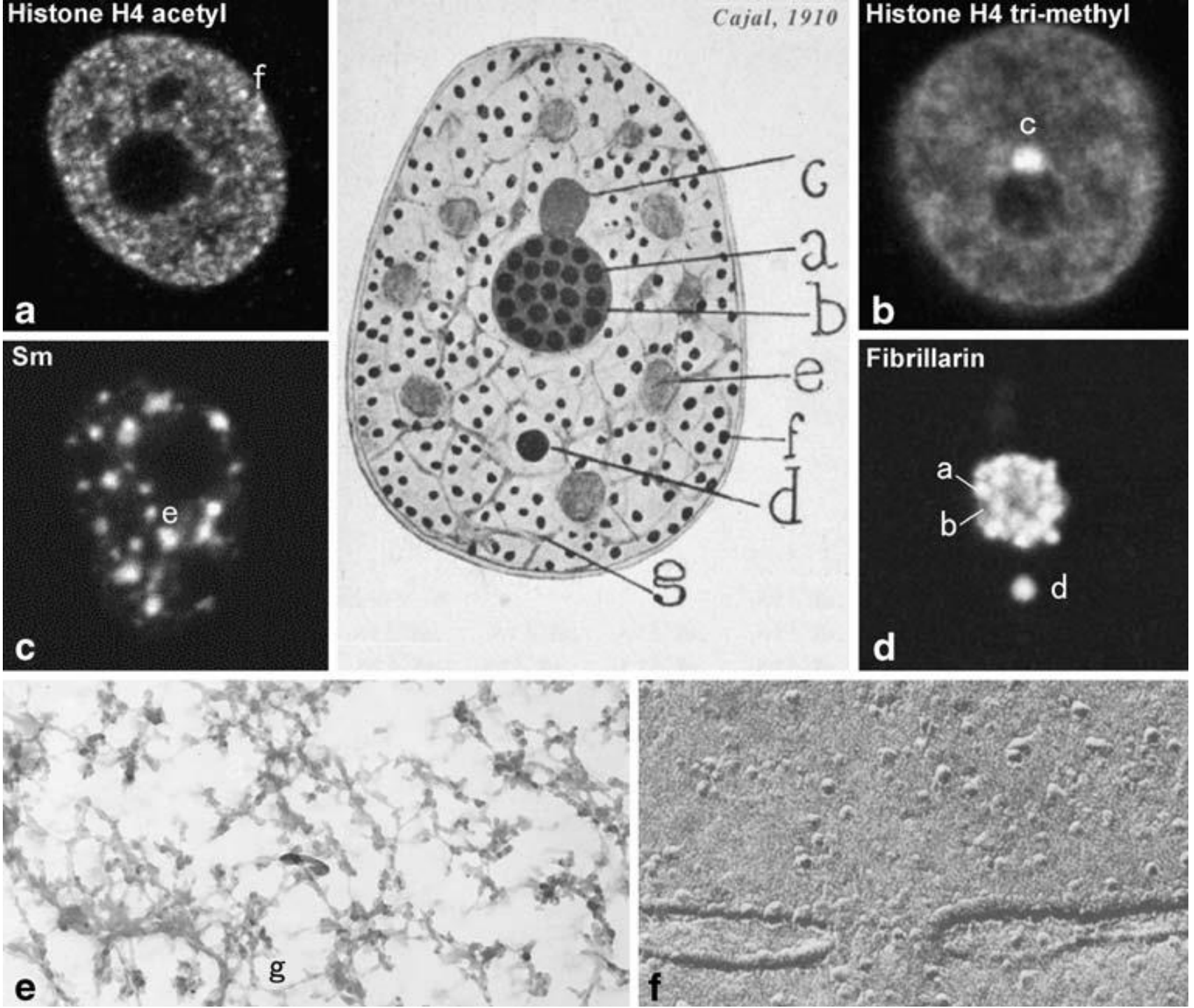
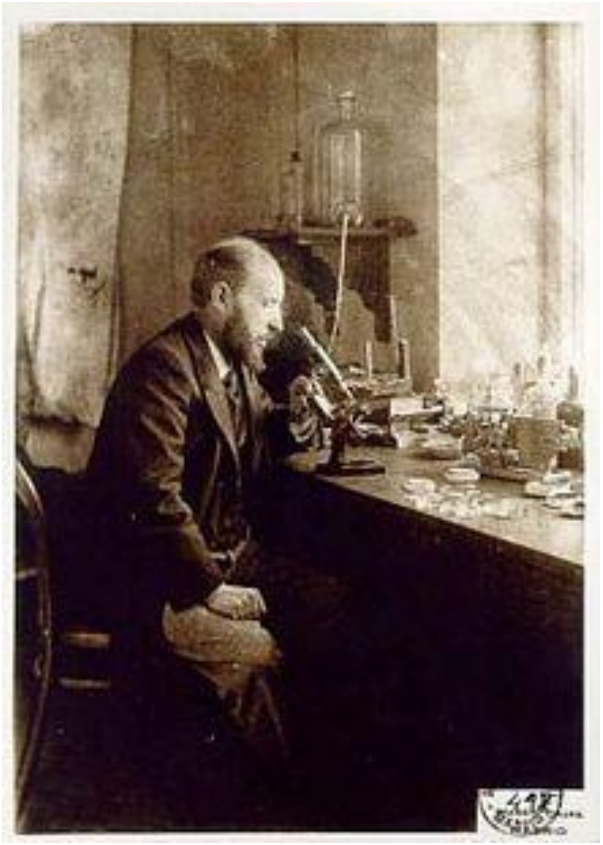
Regulacija transkripcije in jedrni transport

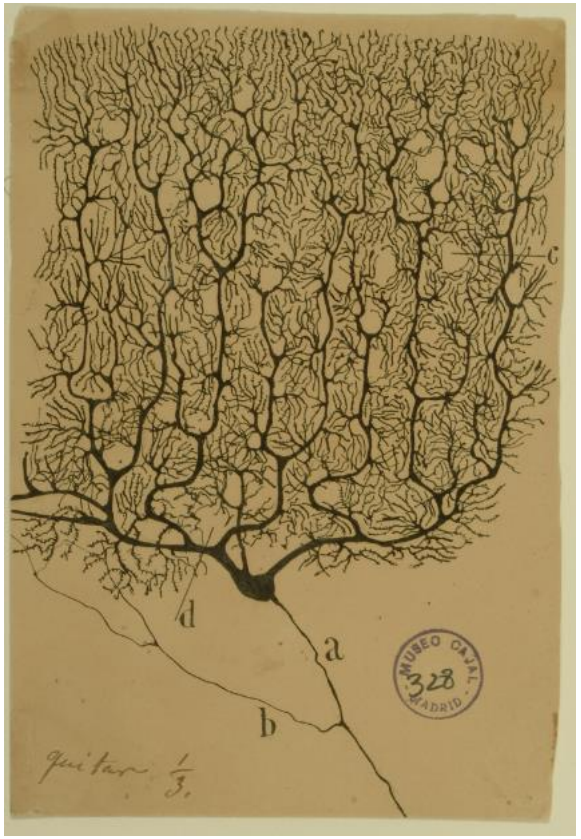
12. marec 2014


Histone H4 acetyl
f
a
Cajal, 1910
c
a
b
e
f
d
g
Histone H4 tri-methyl
c
b
Sm
e
c
Fibrillarin
a
b
d
d
g
e
f

1906 Nobelova nagrada za fiziologijo ali medicino - za strukturo živčnega sistema

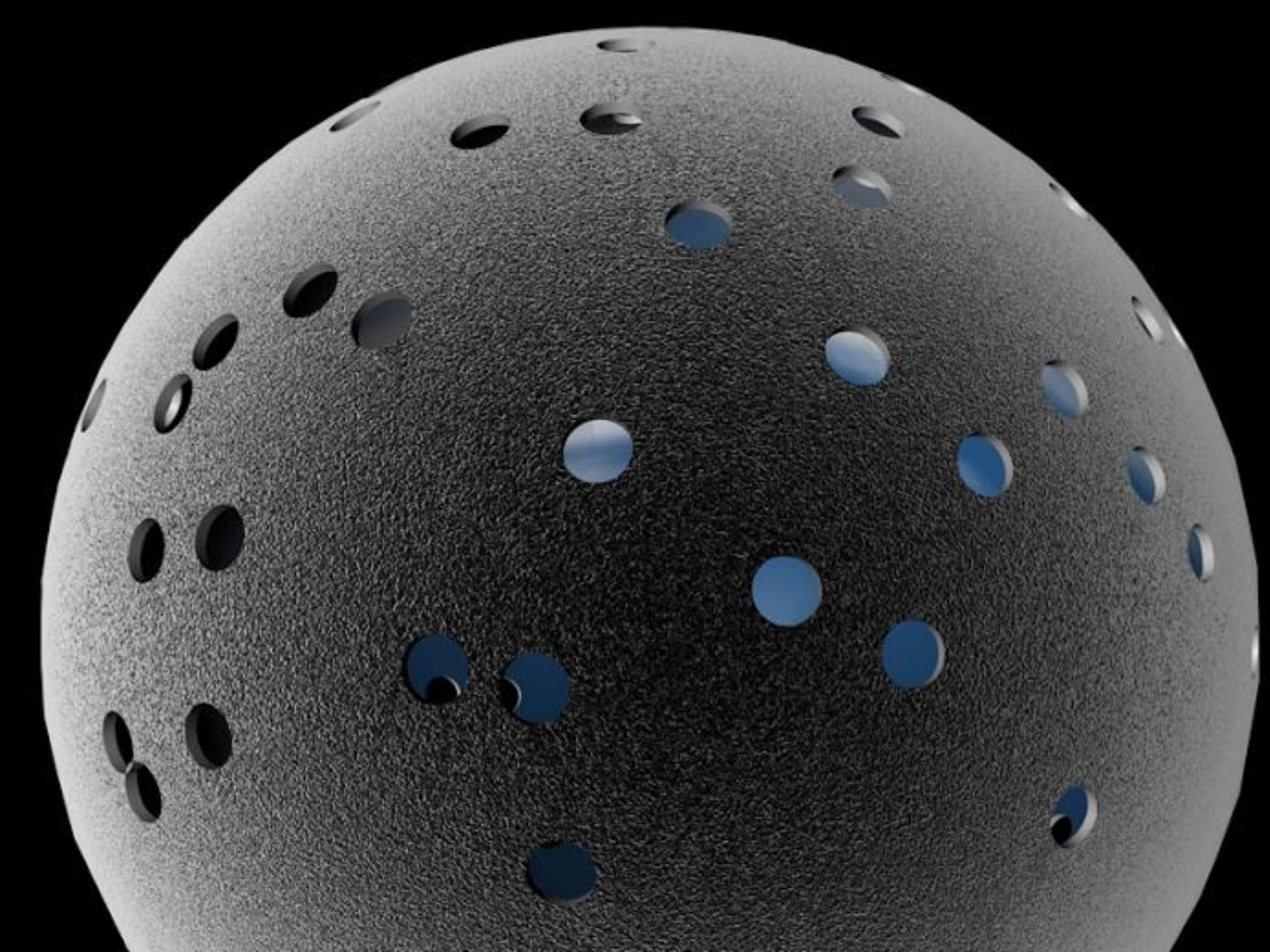
Santiago Ramón y Cajal



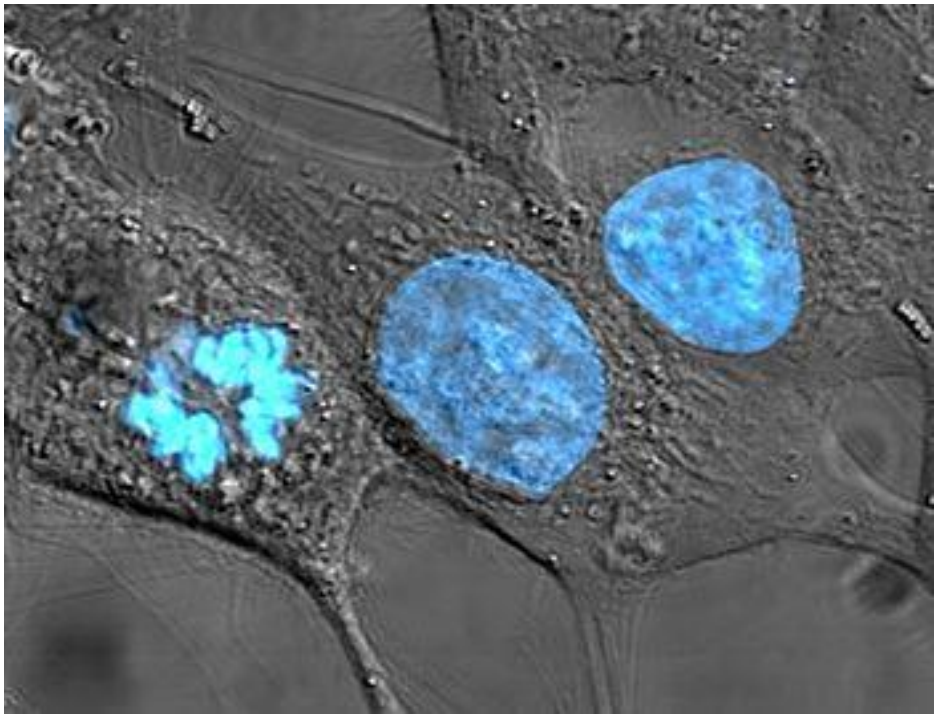


Camillo Golgi

JEDRA SO MOŽGANI CELIC

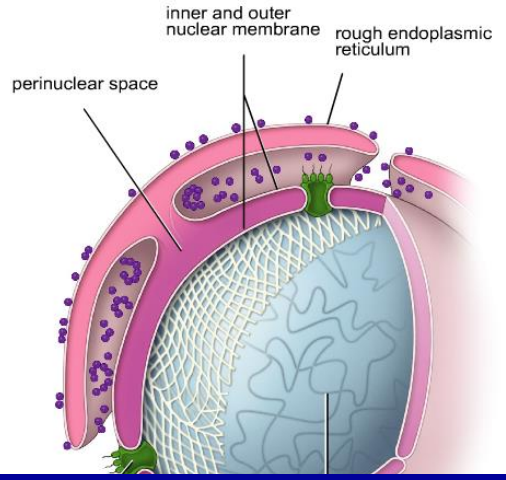


Jedro v HeLa celicah

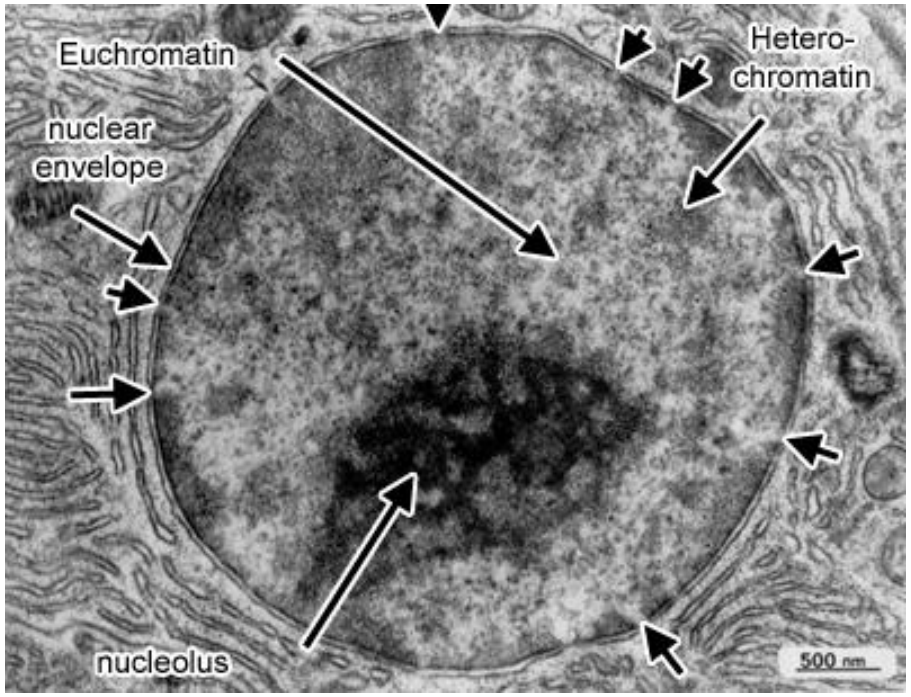


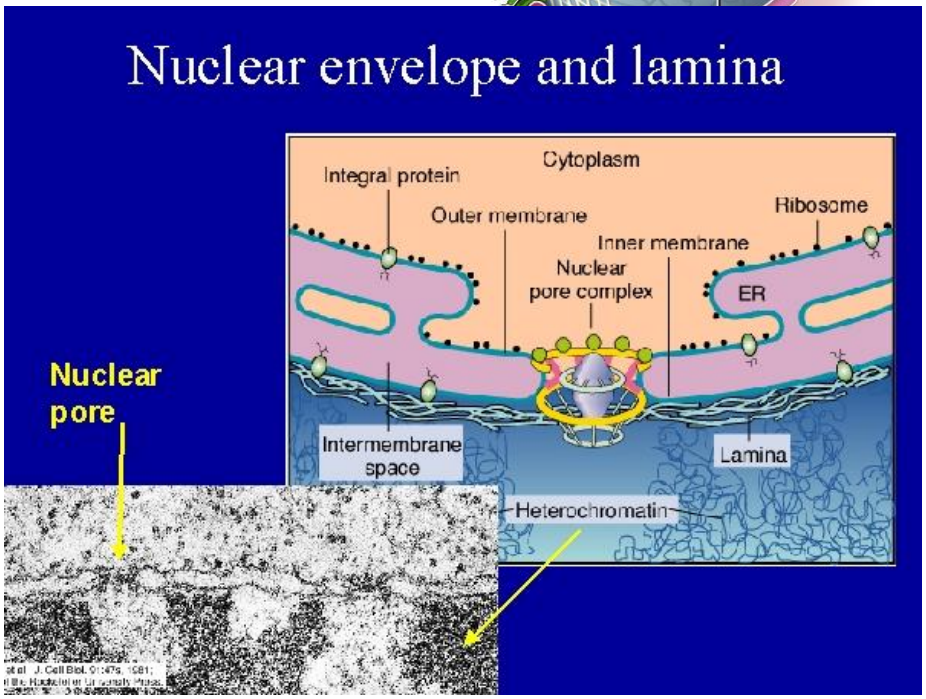
Desni dve celici sta v interfazi, leva pa v procesu mitoze, kjer je DNA kondenzirana.

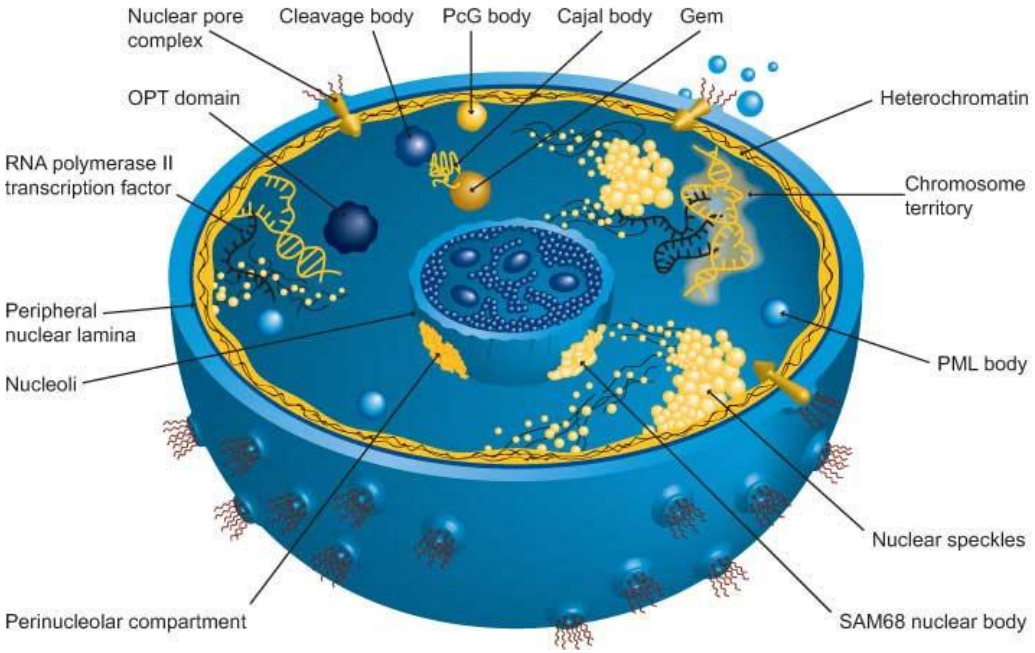
Jedro

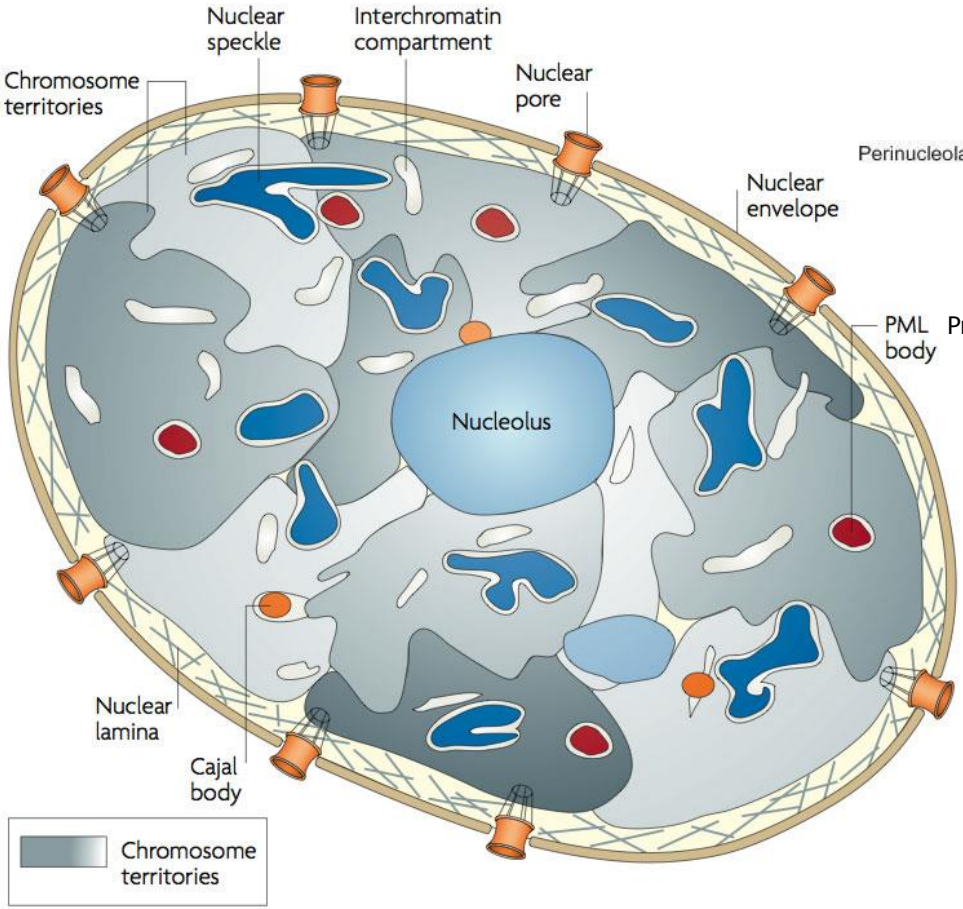


1-10 µm
Jedero je zelo organizirana struktura.







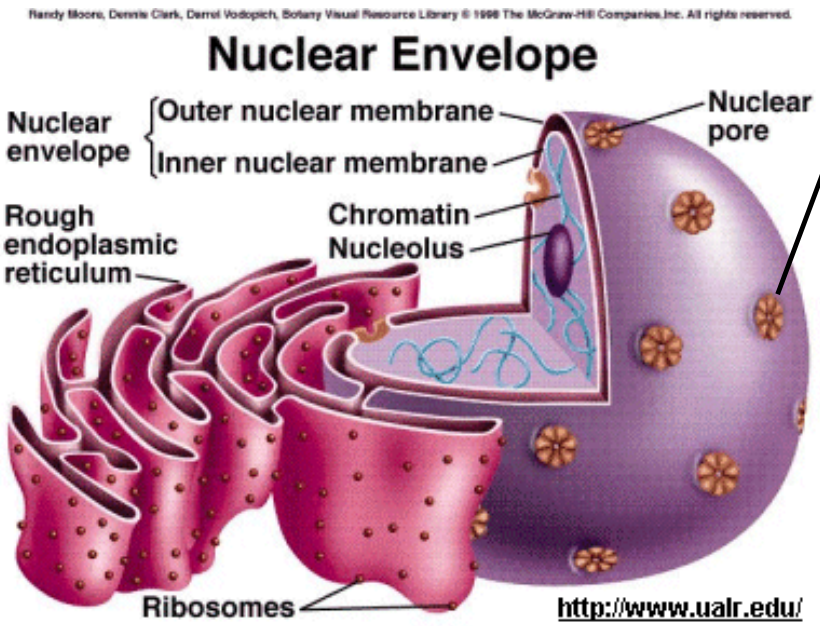


Promyelocytic leukaemia bodies

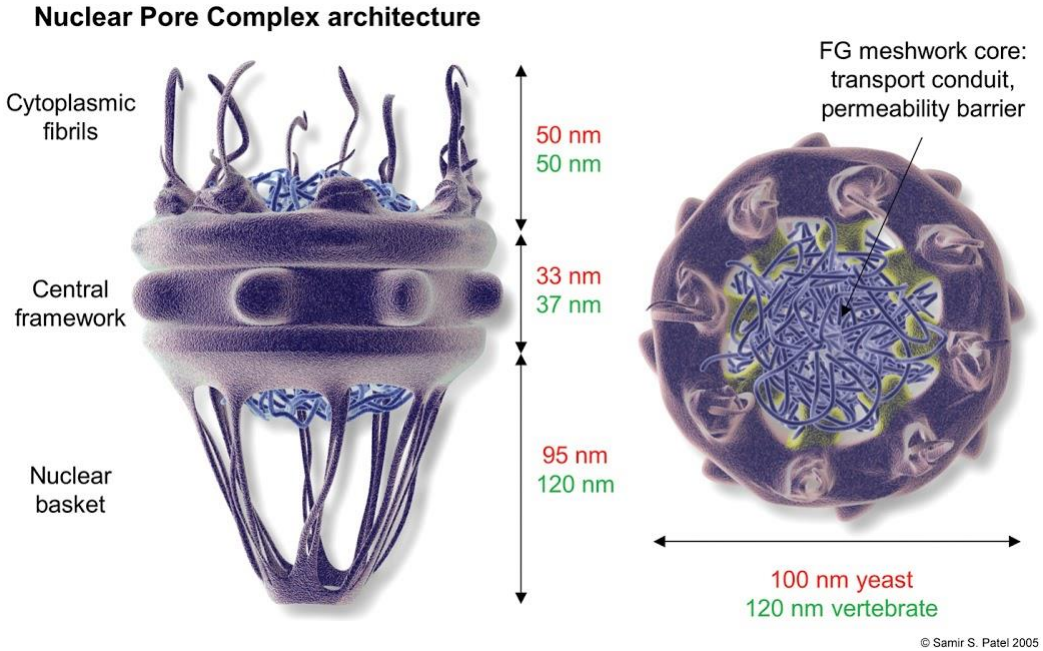
Komunikacija med citoplazmo in nukleoplazmo poteka skozi jedrno poro

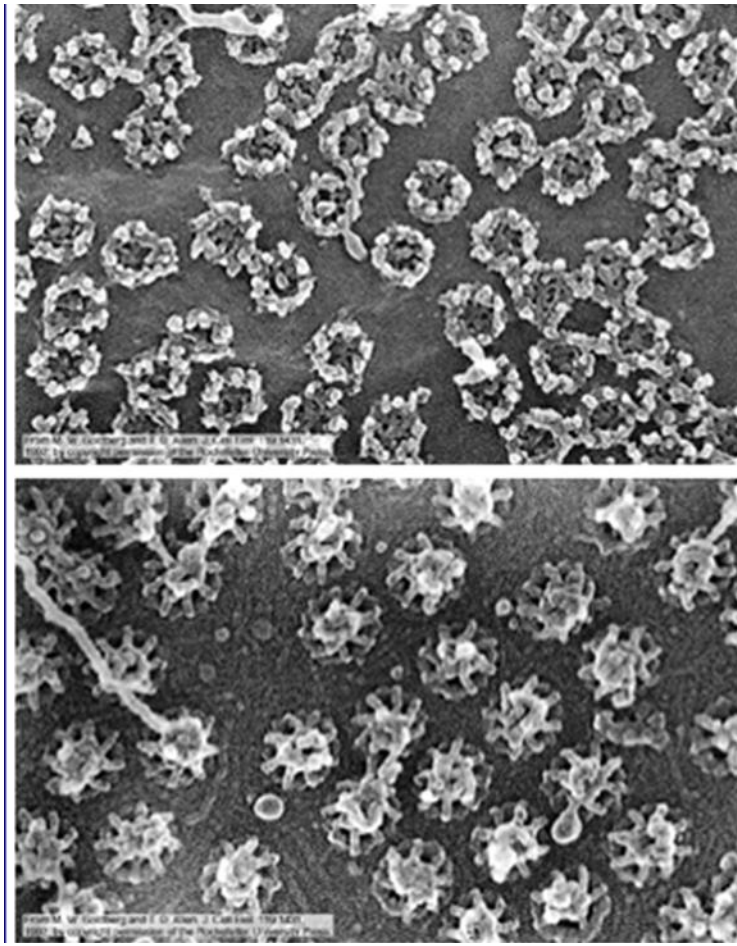
Jedrna pora je velik kompleks proteinov.

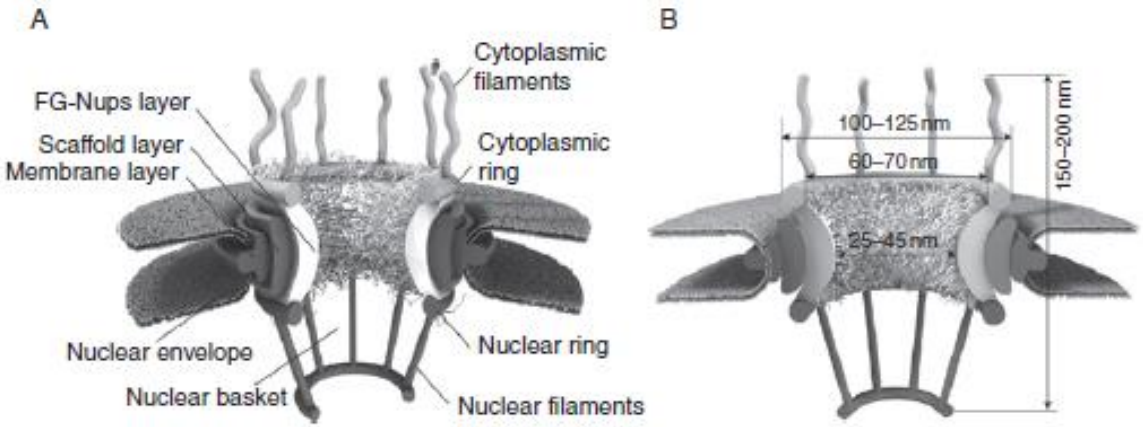
- Število por v jedru:
  - 200 pri kvasovkah
  - 2000 pri človeku
  - 20.000 pri dvoživkah
- Velikost:
  - 60 MDa pri kvasovkah
  - 120 MDa pri človeku
  - 30x večja od ribosomov
- Poro sestavljajo strukturni proteini:
  - 30 (*S. cerevisiae*)
  - 50-100 (Vertebrates)



Jedrna pora


Nuclear Pore Complex architecture
Cytoplasmic fibrils
Central framework
Nuclear basket
50 nm
50 nm
33 nm
37 nm
95 nm
120 nm
FG meshwork core: transport conduit, permeability barrier
100 nm yeast
120 nm vertebrate
© Samir S. Patel 2005

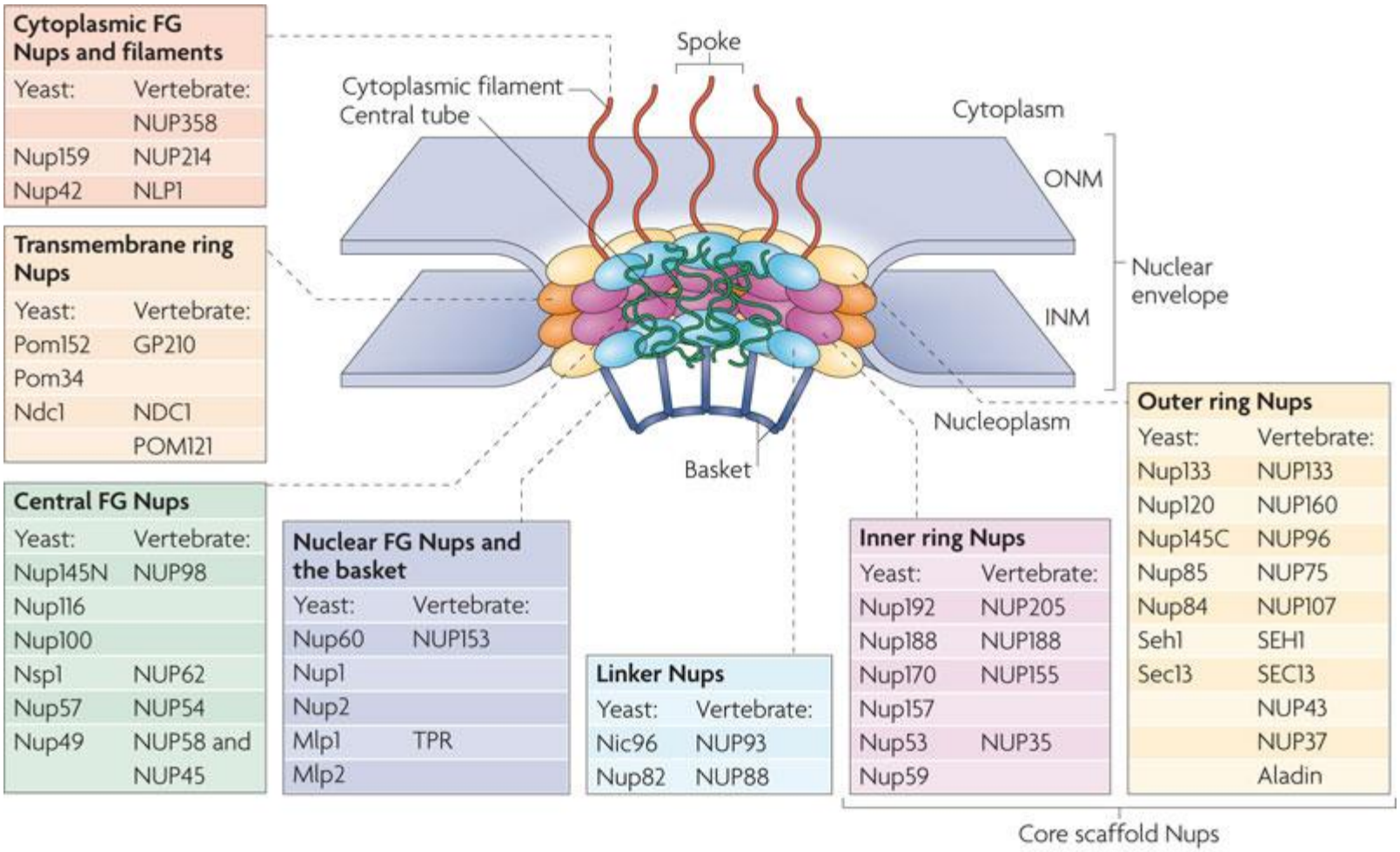



A
FG-Nups layer
Scaffold layer
Membrane layer
Cytoplasmic filaments
Cytoplasmic ring
Nuclear envelope
Nuclear basket
Nuclear ring
Nuclear filaments
B
100–125 nm
60–70 nm
25–45 nm
150–200 nm

Nukleoporini

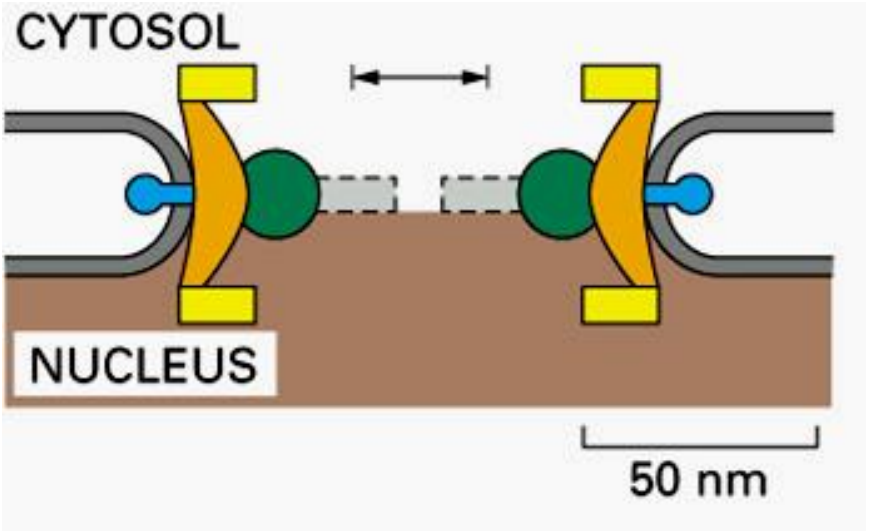
- So proteini v kompleksu jedrne pore.
- Nekateri so transmembranski, da zasidrajo poro v membrano.
- NPC se med mitozo razgradi in ponovno sestavi (odprta mitoza).
- Nekateri nukleoporini so vezani, drugi so dinamični: hitro lahko asoiciirajo z membrano ali oddisociirajo stran.

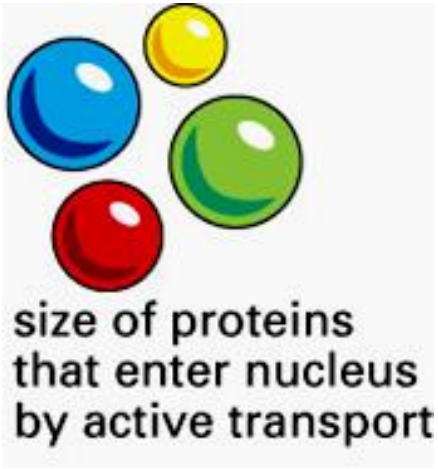
Struktura jedrne pore



Nature Reviews | Molecular Cell Biology

Transport skozi jedrno poro

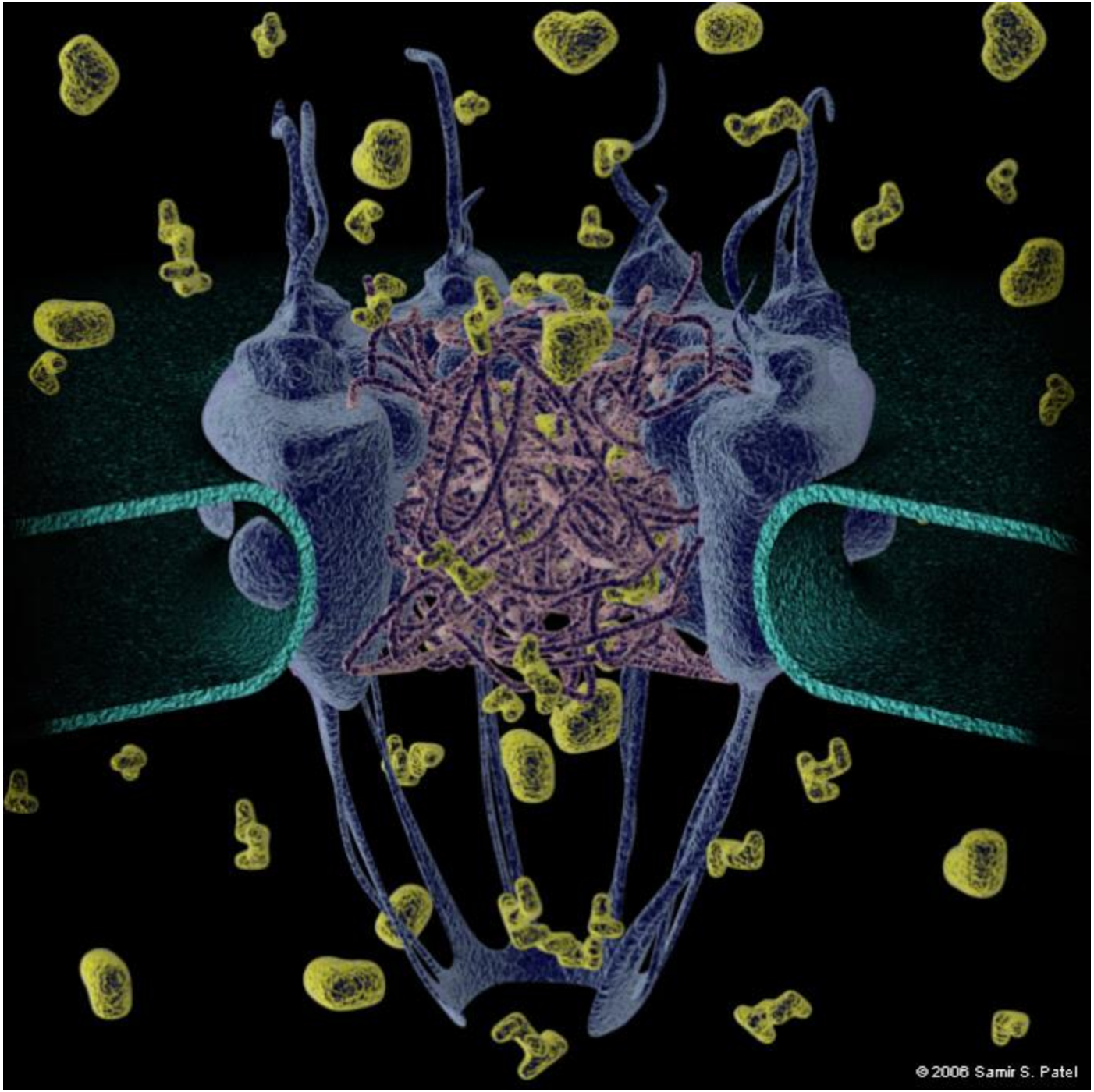


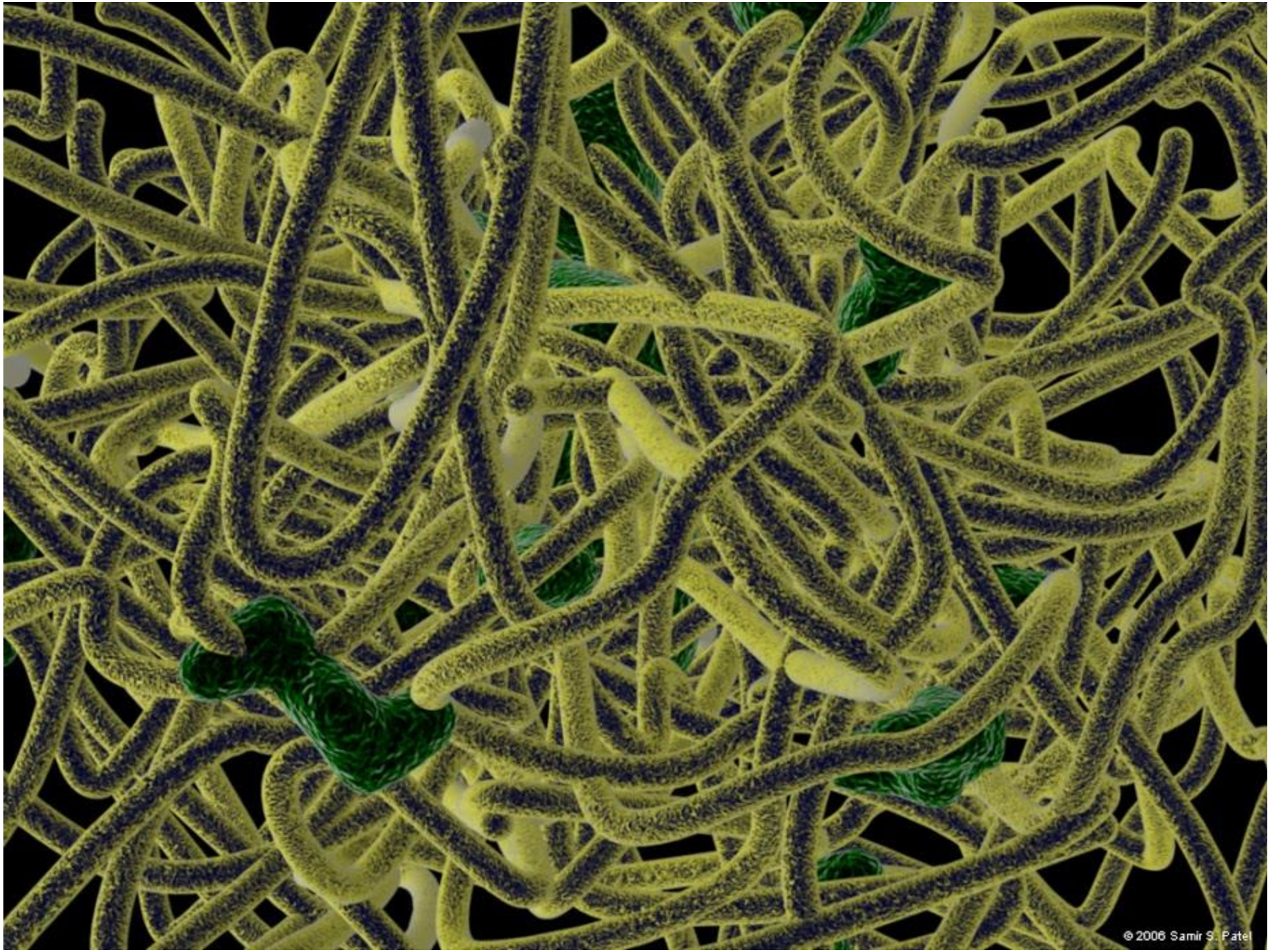


- Molekule, manjše od 100 Da prehajajo skozi membrano.
- Skozi poro potuje 500-1000 (1 milijon/s, Cardarelli, 2012) molekul na sekundo v obe smeri.
- Pasivni prehod – difuzija:

  do 5000 Da hitro

  do 60.000 Da možno, počasi (premer ~ 9 nm)
- Aktivni prehod z receptorji **karioferini** (importini, eksportini) (do 39 nm)


© 2006 Samir S. Patel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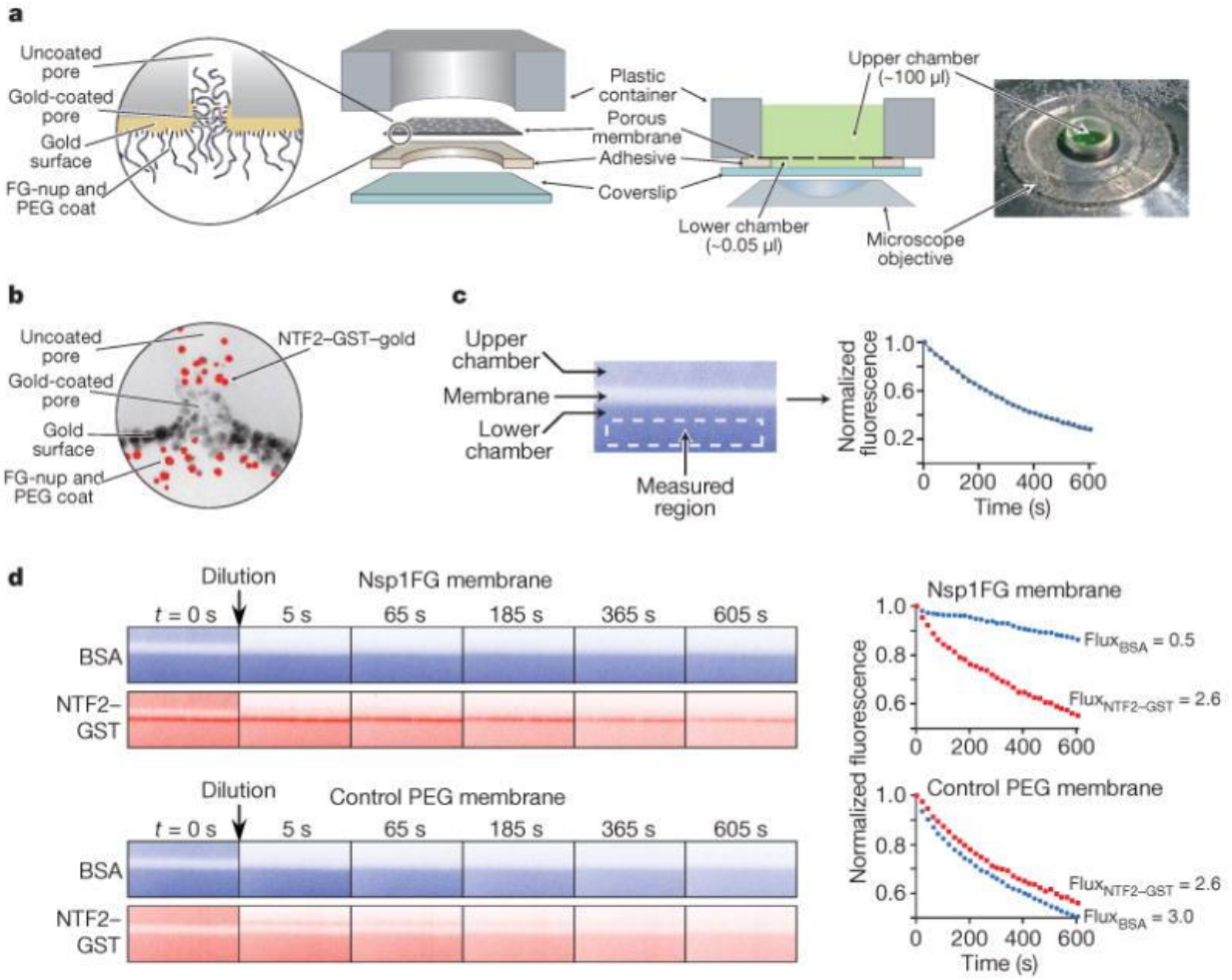
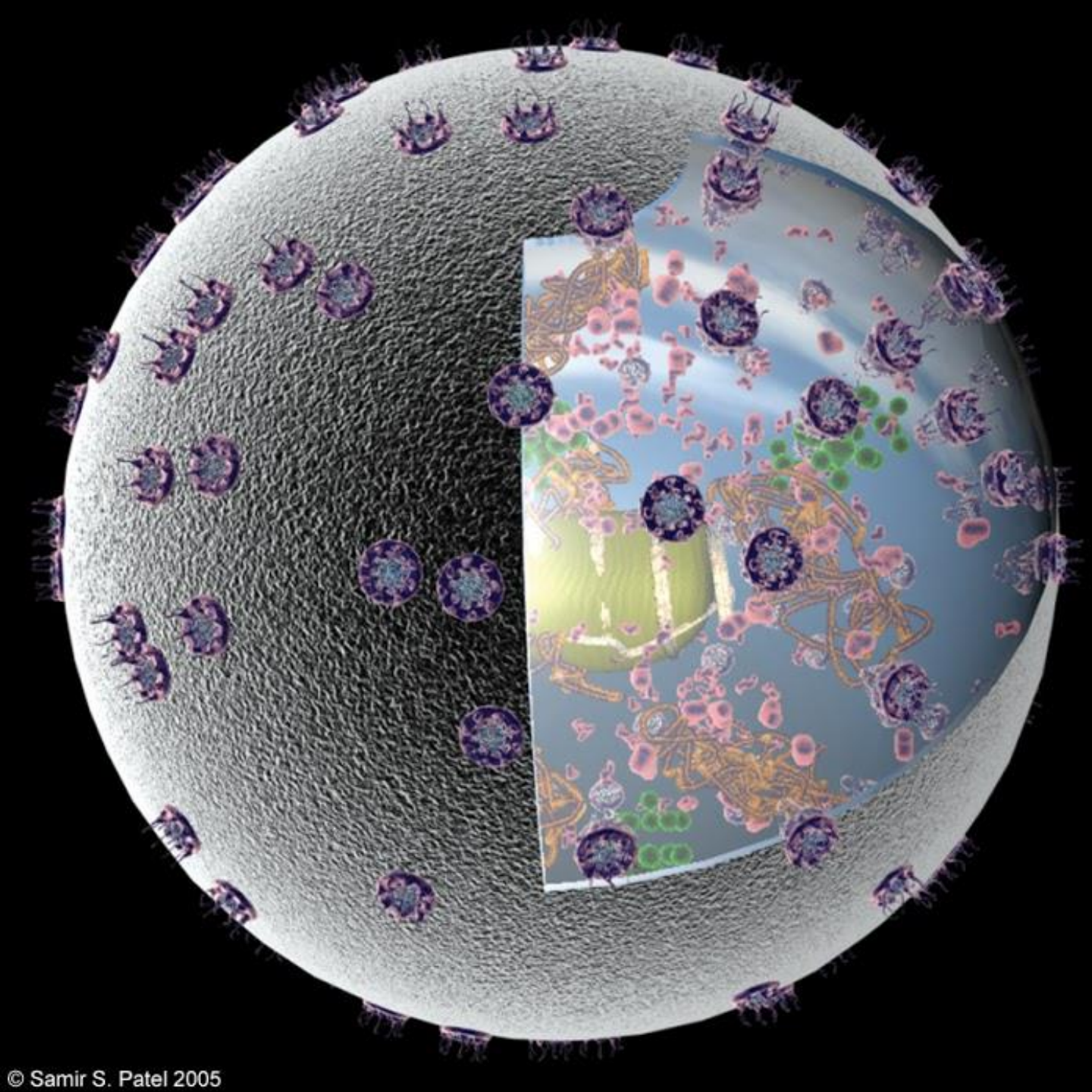
Nanopore z FG ponovitvami posnemajo transport skozi NPC



Jovanovic-Talisman, Nature 2009



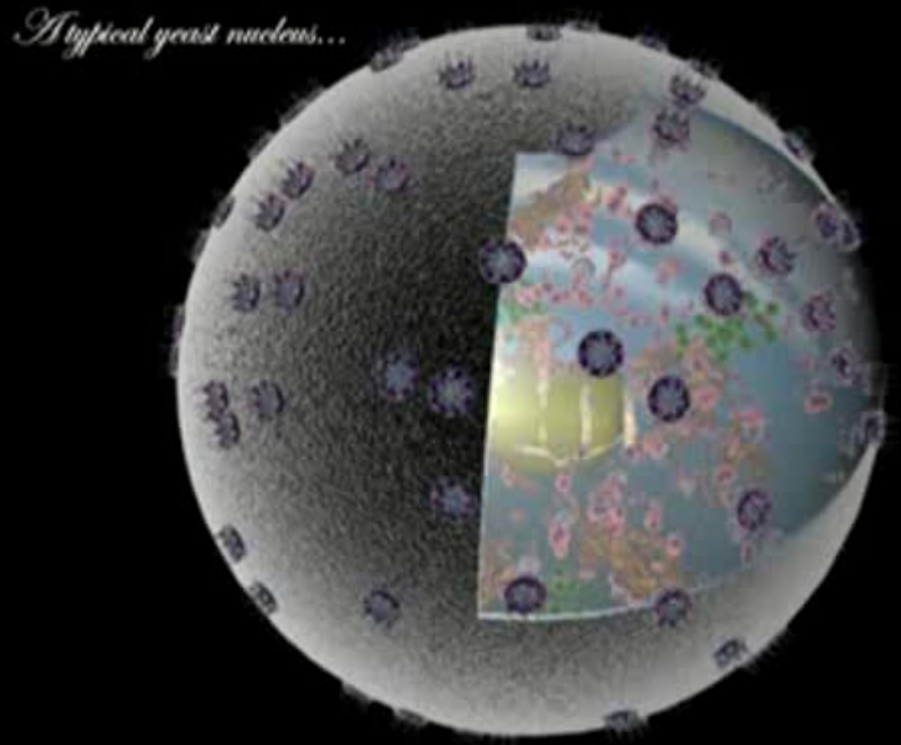
Nuclear Import

ribosomal proteins
nucleolar proteins
histones
transcription factors
snRNPs
rRNPs
replication factors
viral genomes

Nuclear Export

proteins
ribosome subunits
mRNA
tRNA
snRNA
rRNA
viral RNP's

Transport proteinov v jedro



Značilna signalna zaporedja za transport proteinov

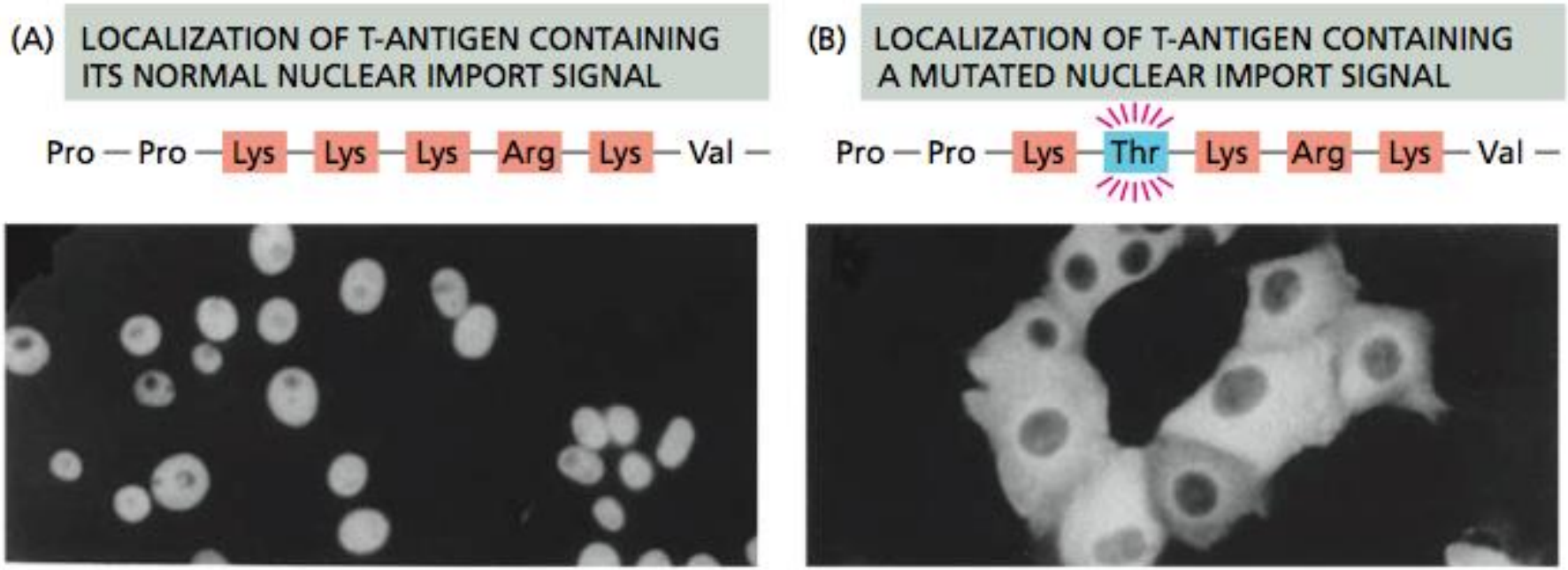
Table 12–3 Some Typical Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

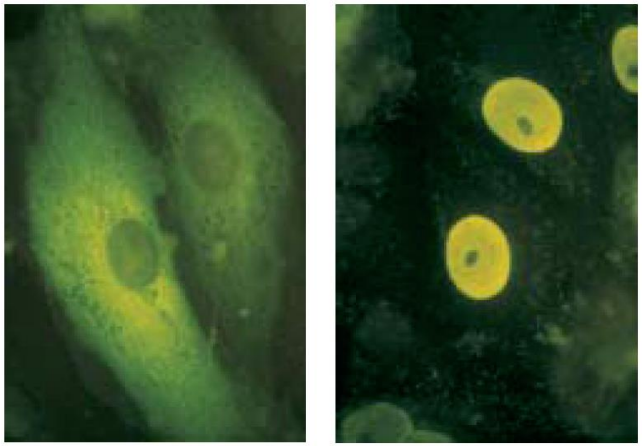
- Signal za prenos v jedro - NLS (nuclear localisation signal) je večinoma bazičen, lahko tudi hidrofoben.
- Protein ima lahko 1 ali več NLS.

Signal za lokalizacijo v jedru - NLS

I.



II. Citosolnemu proteinu so dodali NLS, zato se lokalizira v jedru.

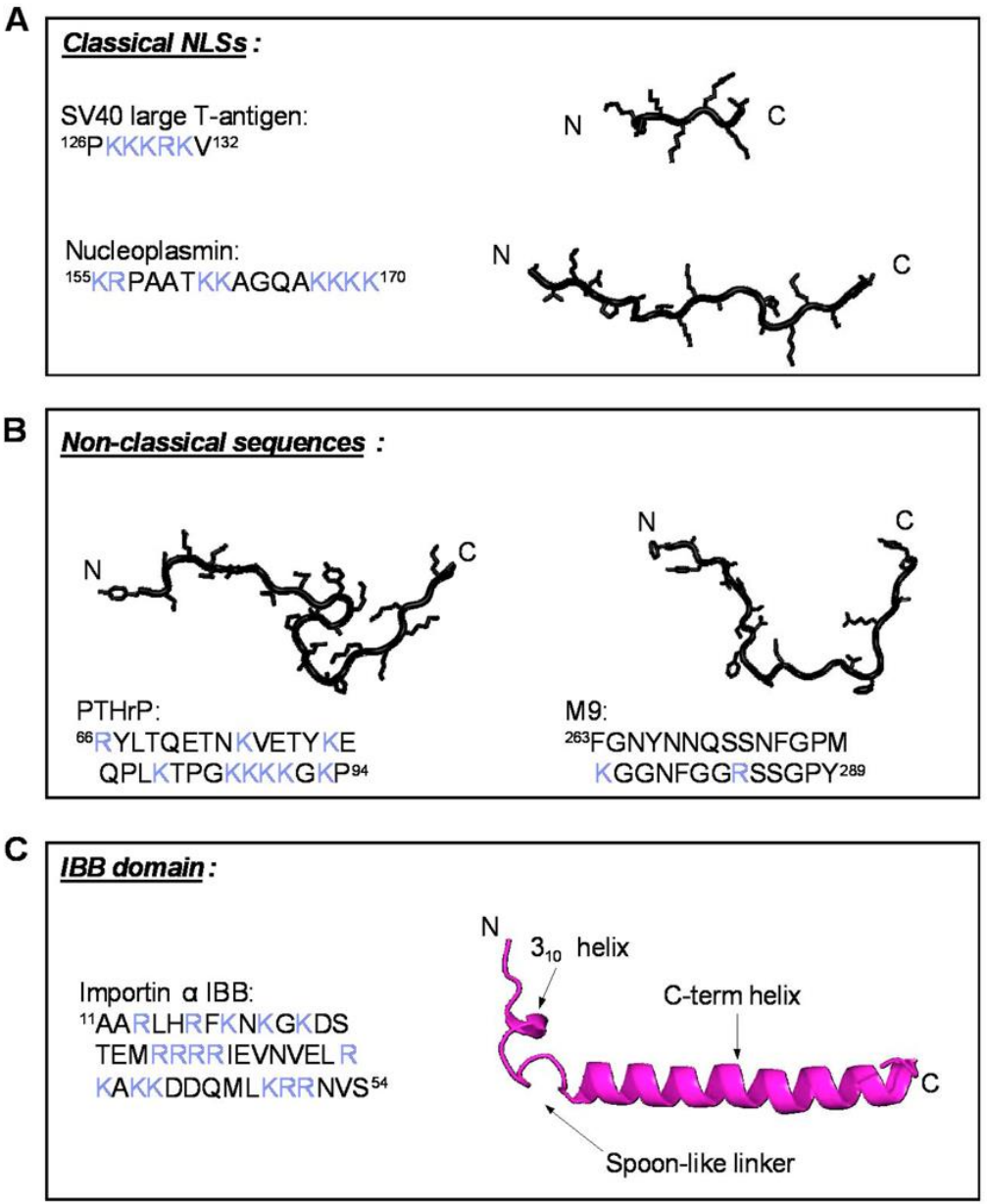


Signal za lokalizacijo v jedru - NLS

- KLASIČEN (tovor se veže na Impβ)
  - Monopariten: 5-7 bazičnih ak, bogat z Lys ali Arg, Impα-Impβ
  - Bipartiten

- NEKLASIČNI – različni

  Konformacijski – PY-NLS (prolin, tirozin)
  - 9-12/20 ak
  - N-konec je hidrofoben ali bazičen, C-konec $RX_{2-5}PY$



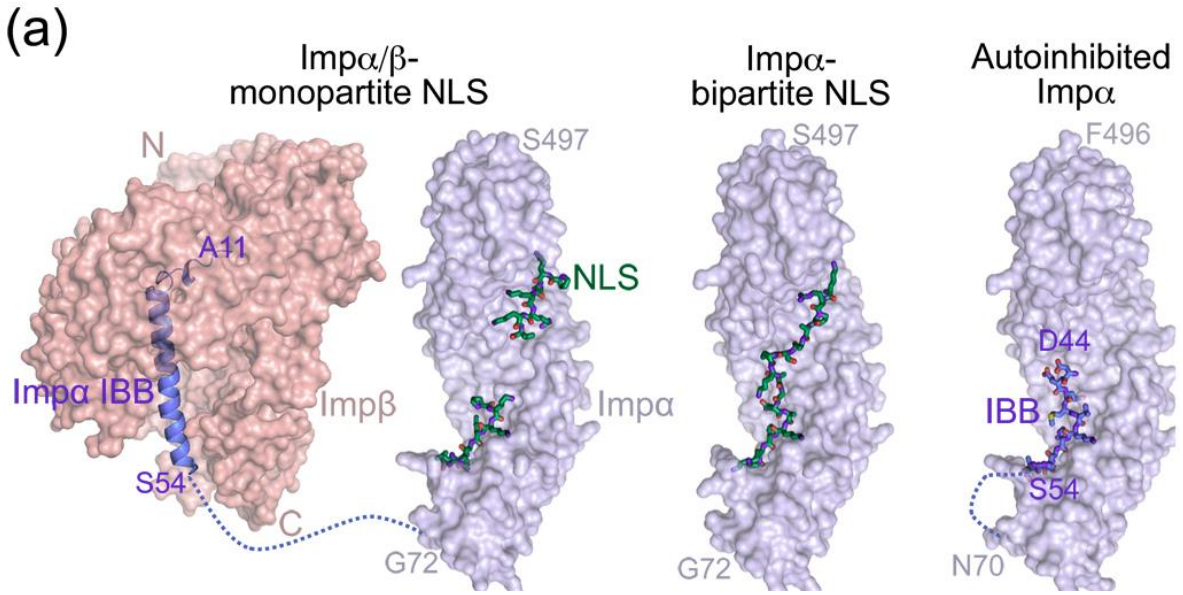
Karioferini (Kapβ) – receptorji jedrnega transporta

- > 20 človeških karioferinov (11 importinov)
- 90 – 150 kDa
- 10 -20 % sekvenčno ohranjeni
- 20 HEAT ponovitev (**H**untingtin, elongation factor 3 (**E**F3), protein phosphatase 2A (PP2**A**), and the yeast kinase **T**OR1)
- Vloga:
  - jedrni transport
  - genska ekspresija
  - prenos signala
  - imunski odgovor
  - širjenje virusov

Proteini s klasičnim NLS se v jedro prenesejo z Impβ (Kapβ1)

Impβ se veže na Impα (50% vseh tovorov, ki gredo v jedra),

Impα-β kompleks je najbolj robusten, direktna vezava molekul na Impβ je hitrejša in porabi manj energije.

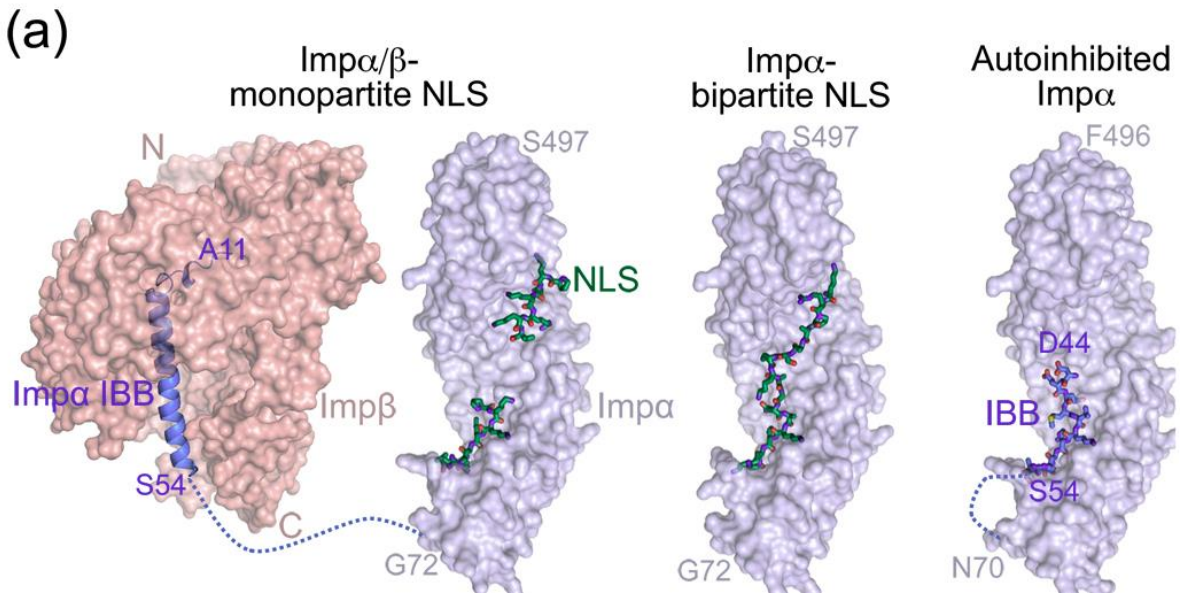


Prenos v jedro z Impβ

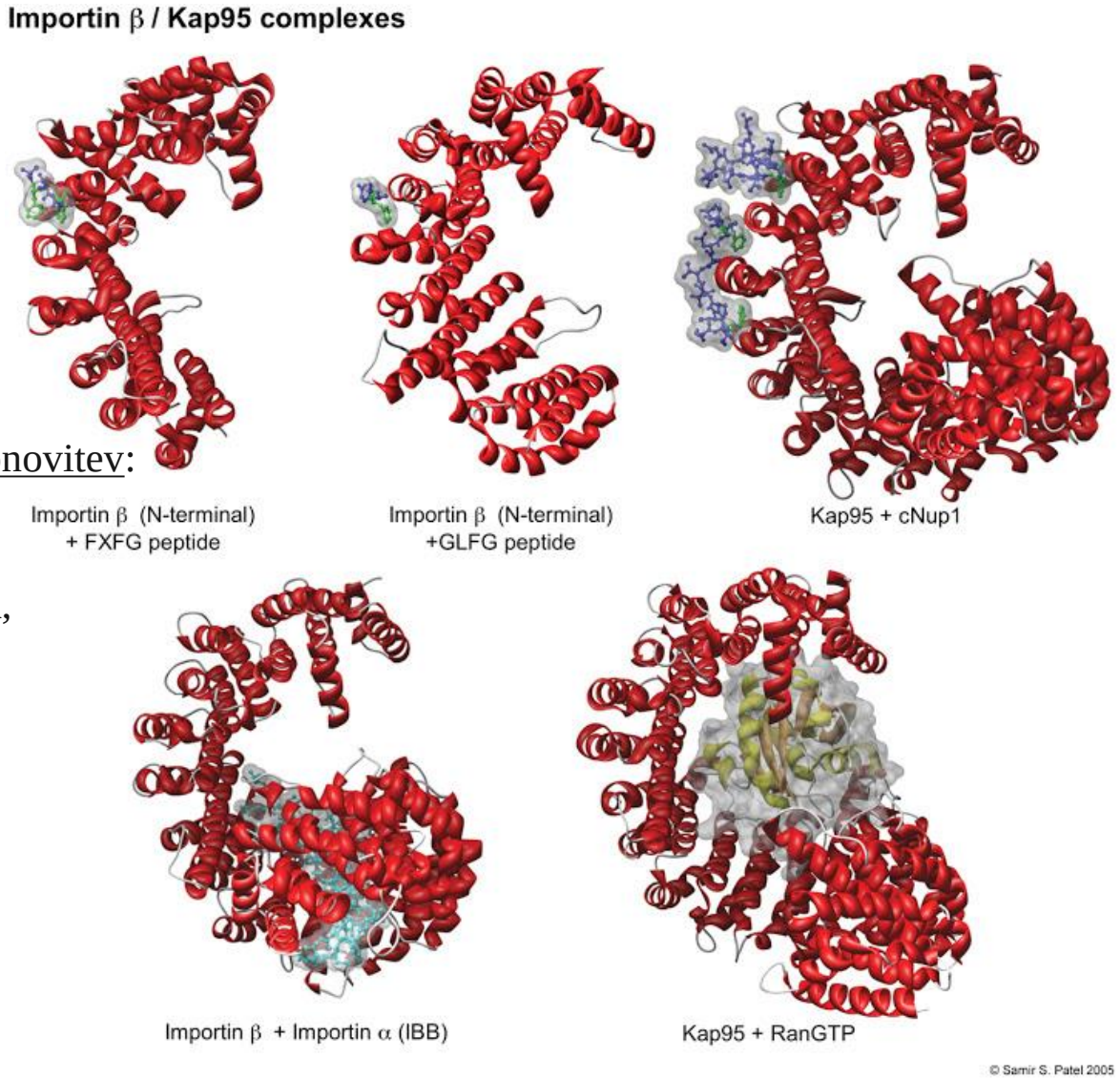
Impβ se veže na Impα, na snuportin1 ali direktno na tovor. Te molekule imajo različna vezavna mesta na Impβ.

Impα: 10 armadilo ponovitev (ARM), vsaka ima 3 helikse, skupaj tvorijo cilindrični superheliks.

Z IBB domeno se veže na Impβ (kot snuportin).



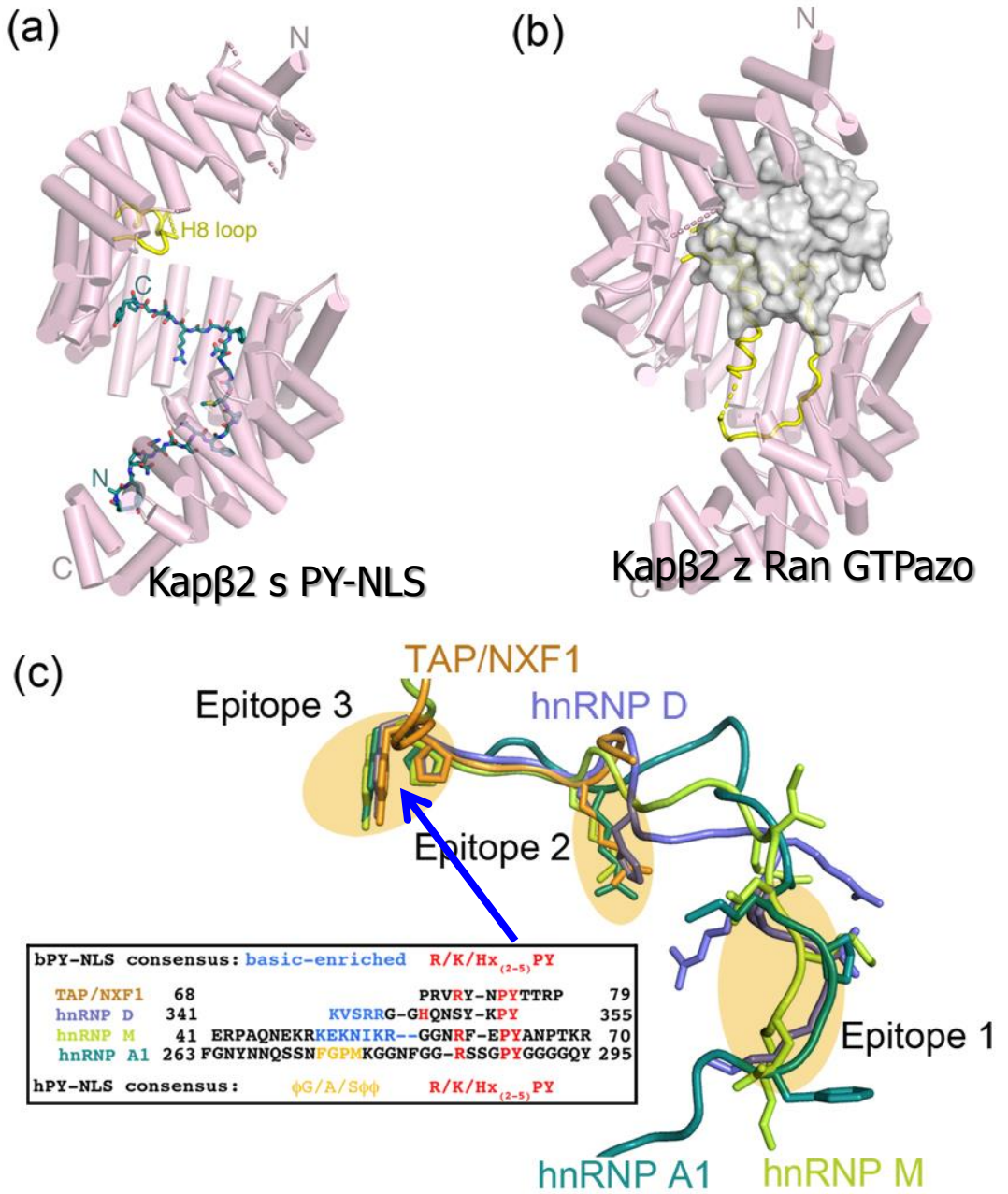
Strukture Importina β glede na vezavnega partnerja



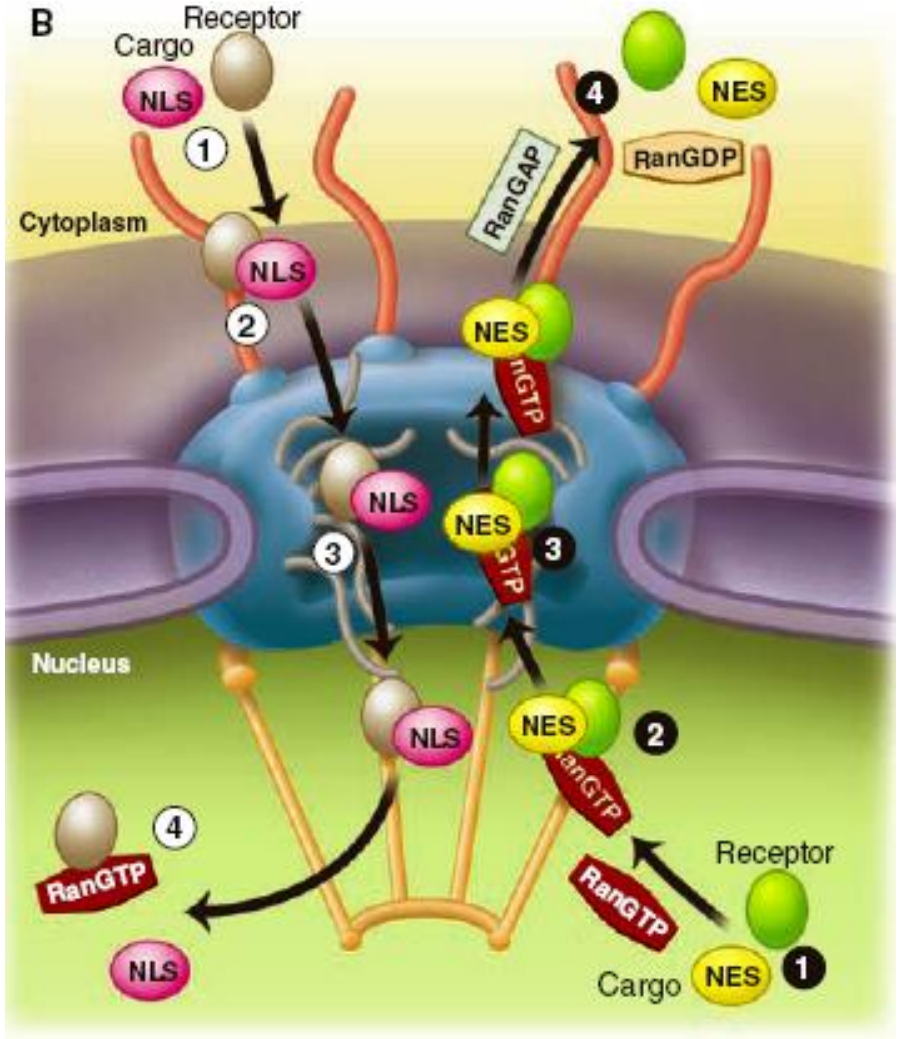
<u>19 tandemnih HEAT ponovitev</u>:
Huntingtin,
Elongation factor 3,
Protein phosphatase 2A,
TOR1 – PI3 kinase

Vnos v jedro z importinom Kapβ2

- Manj znanih tovorov – 20 RNA-BP, 100 kandidatov, znani hnRNP A1, HuR, NXF1, hnRNO D, JKTB
- NLS je raznolik – PY-NLS
- 15-30 ak, epitopi
- N-del hidrofoben ali bazičen, C-del ima $RX_{2-5}PY$
- Različni PY-NLS se z vsakim epitopom veže drugače



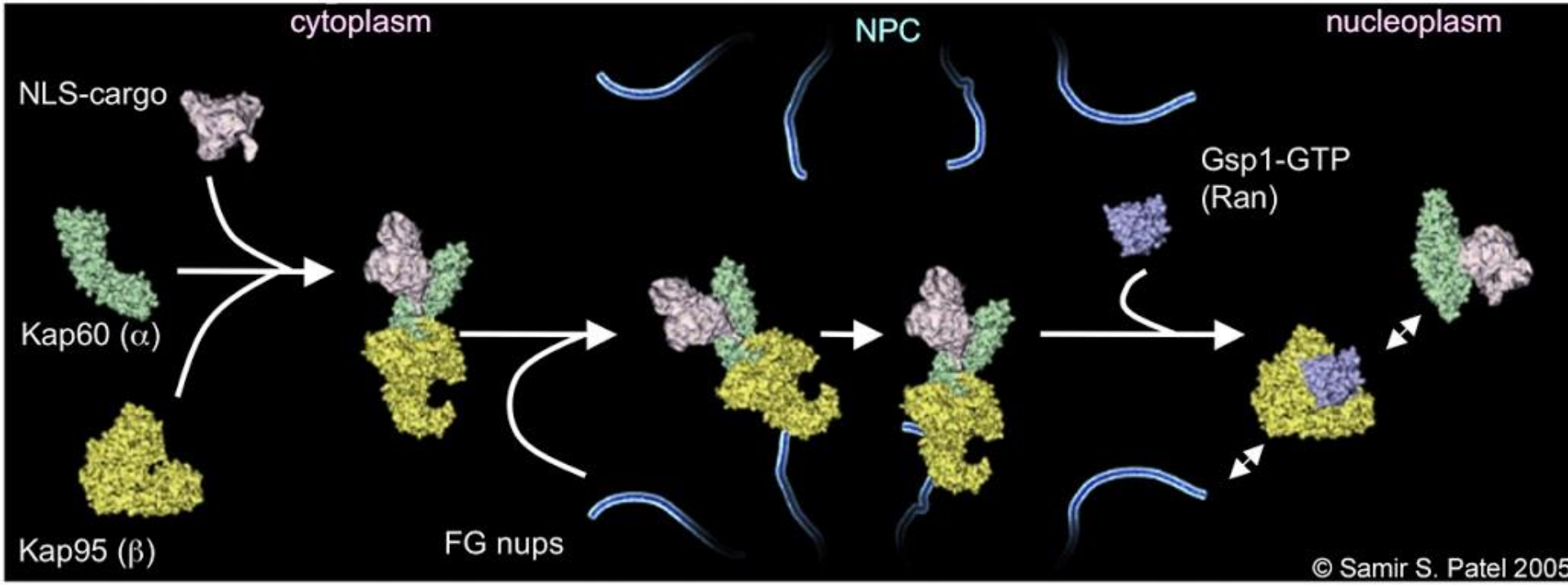
Vnos v jedro



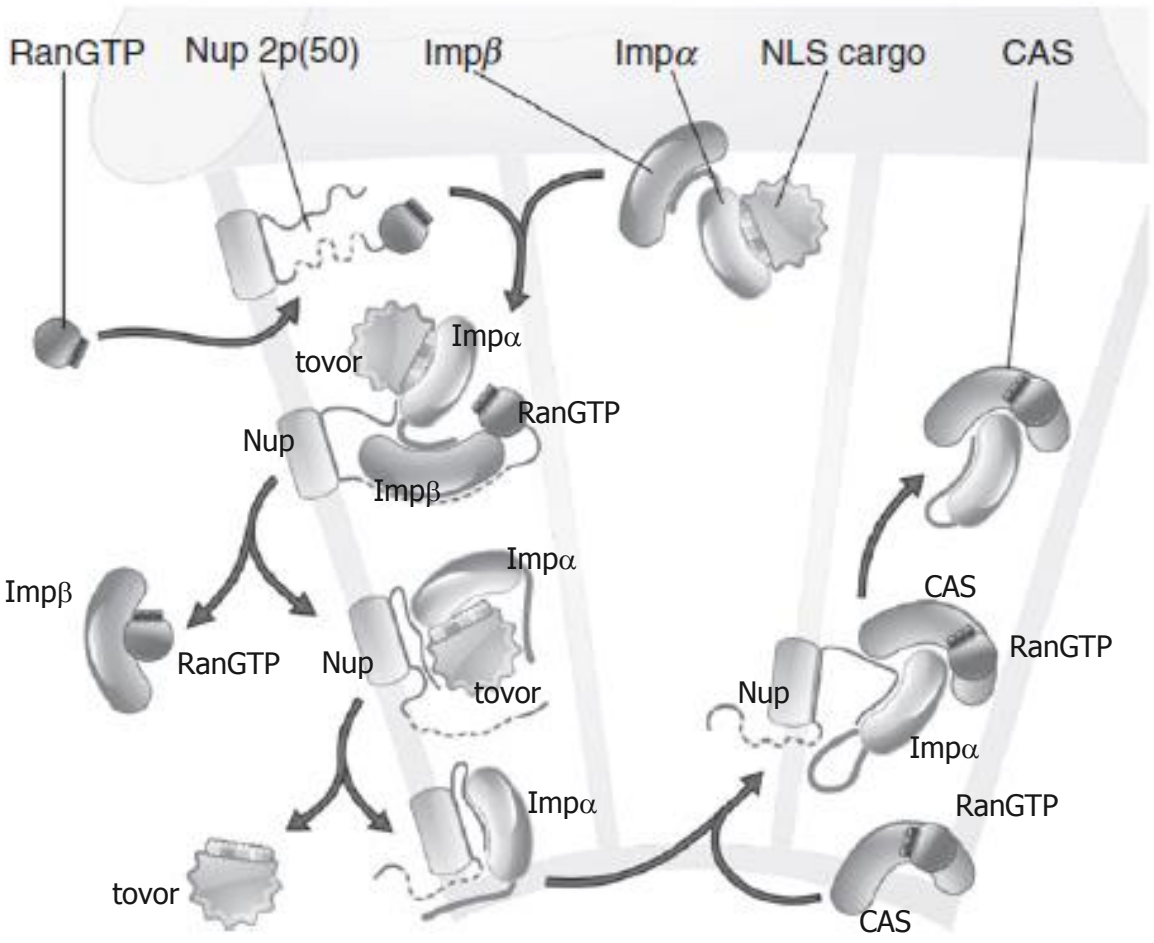
- Receptorji (karioferini) se vežejo na tovor (NLS) neposredno ali preko adapterjev (sorodni karioferini α).
- različni karioferini pomagajo vnesti enak tovor
- različni tovori tekmujejo za en karioferin
- Reguliran z Ran GTPazo.

Science, 318, 1412

Vnos v jedro z Importinom β

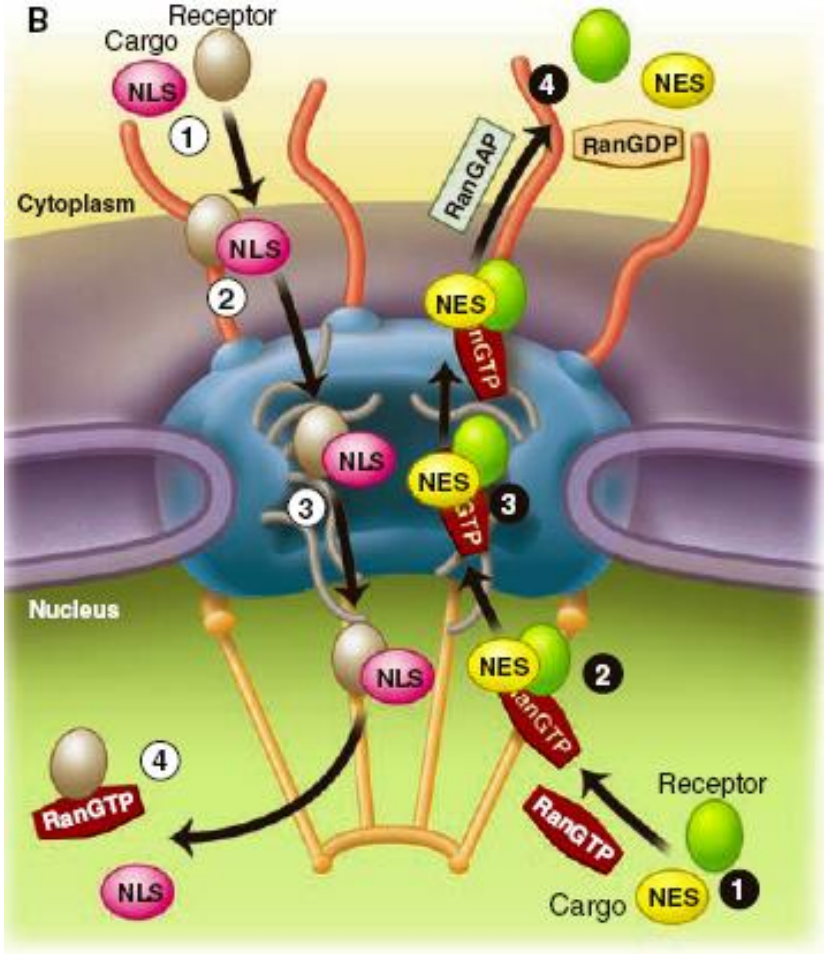


Jedrni transport v košari

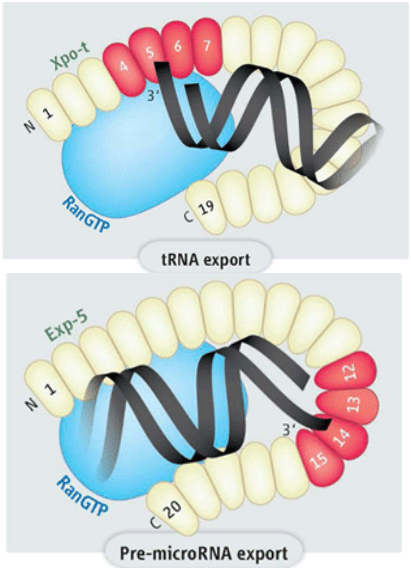


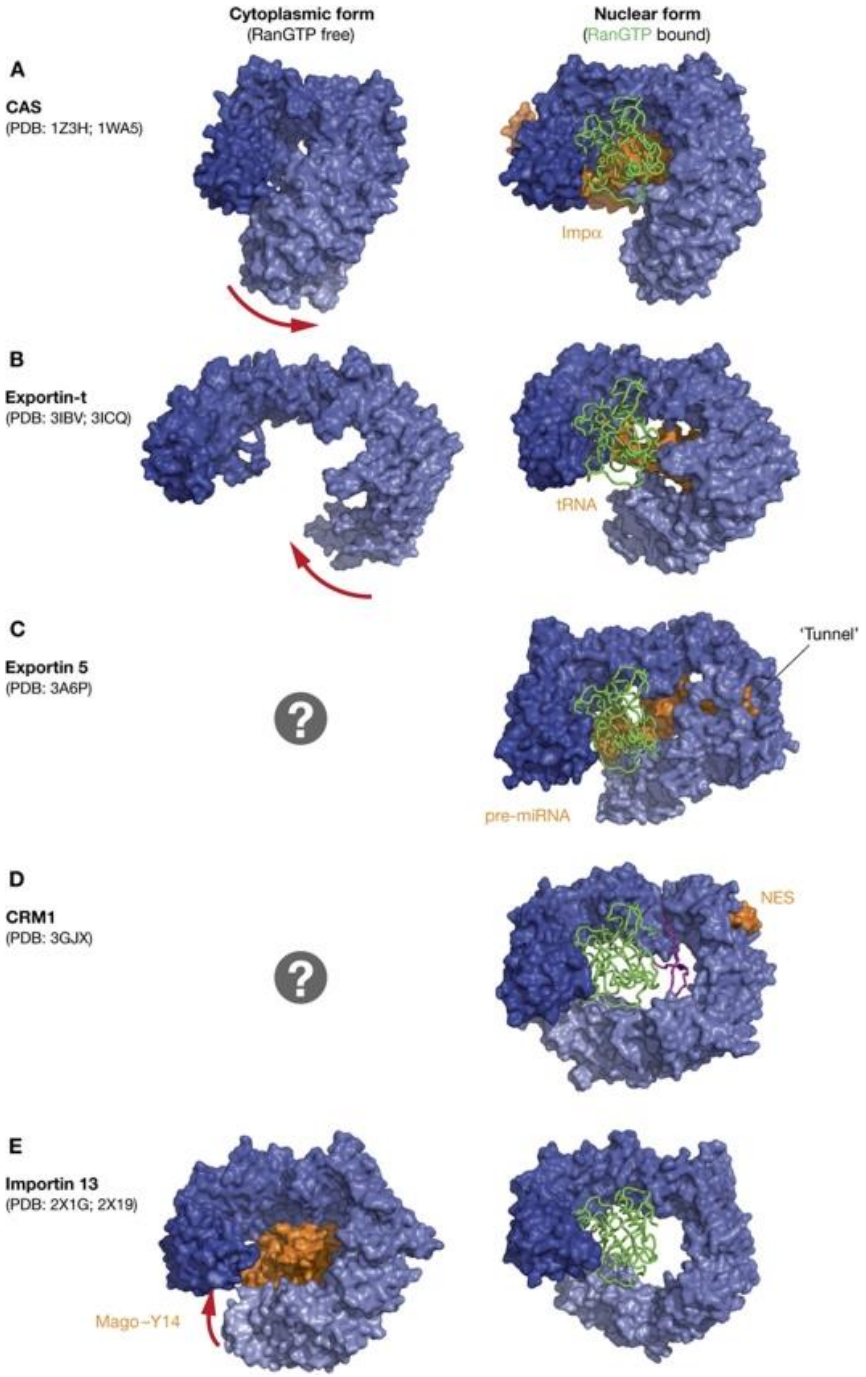
Prenos iz jedra

- Signal za prehod iz jedra (NES) – bogat z Leu (10-15 ak, > 200 znanih tovorov, inhibitor je leptomicin B).
- mRNA (hnRNP), snRNA, tRNA, ribosomalne podenote, miRNA, proteini
- Receptorji so iz družine karioferinov (eksportini): CRM1 za proteine, NXF1/NXT1 za mRNA



Strukture eksportinov





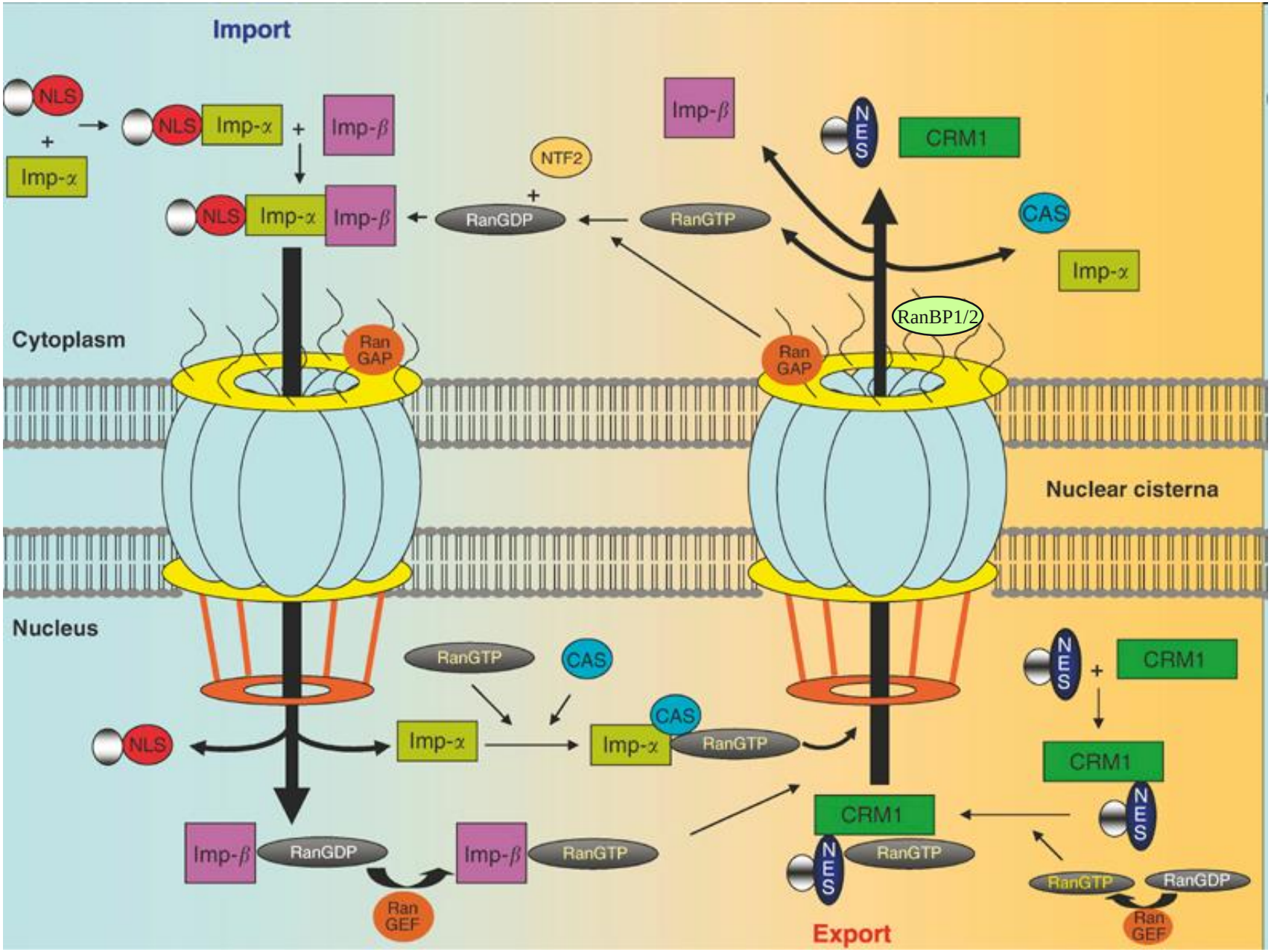
Eksportini

NTR	Cargoes (selection)	Adapter	Selected references
Exportins			
CRM1 (Exportin 1) Xpo1p/Kap124p	Leu-rich NES cargoes		Fischer *et al* (1995); Wen *et al* (1995); Fukuda *et al* (1997); Stade *et al* (1997); Fornerod *et al* (1997a)
	HIV genomic RNA	HIV Rev	Fischer *et al* (1995)
	m^7G-capped UsnRNAs	PHAX + CBC	Izaurralde *et al* (1995); Ohno *et al* (2000)
	60S pre-ribosomal subunits	NMD3	Ho *et al* (2000); Thomas and Kutay (2003)
	Snurportin 1 (SPN1)		Paraskeva *et al* (1999)
CAS (Exportin 2) Cse1p/Kap109p	Importin αs Srp1p/Kap60p		Kutay *et al* (1997); Solsbacher *et al* (1998)
Exp-t (Xpot) Los1p/Kap127p	tRNA		Kutay *et al* (1998); Arts *et al* (1998a); Hellmuth *et al* (1998)
Exportin 5 (Xpo5)	tRNA, eEF1A (via aa-tRNA)		Bohnsack *et al* (2002); Calado *et al* (2002)
	dsRNA-binding proteins (via dsRNA)		Brownawell and Macara (2002)
	Pre-miRNAs		Yi *et al* (2003); Bohnsack *et al* (2004); Lund *et al* (2004)
	60S pre-ribosomal subunits		Wild *et al* (2010)
Exportin 6	Actin–profilin complexes		Stüven *et al* (2003)
Exportin 7	p50RhoGAP, 14-3-3σ		Mingot *et al* (2004)
Bidirectional NTRs			
Importin 13	*Import:* Mago-Y14, Ubc9, histone fold heterodimers		Mingot *et al* (2001); Kahle *et al* (2005); Walker *et al* (2009)
	Export: eIF1A		Mingot *et al* (2001)
Msn5p/Kap142p (orthologous to Exp5)	*Import:* RPA		Yoshida and Blobel (2001)
	Export: tRNA, dsRNA, phosphorylated Pho4p		Kaffman *et al* (1998); Shibata *et al* (2006)
Exportin 4	*Import:* Sox2, SRY		Gontan *et al* (2009)
	Export: eIF5A, Smad3		Lipowsky *et al* (2000); Kurisaki *et al* (2006)

S. cerevisiae NTRs that are functional orthologues of the vertebrate export receptors are named in blue. Only a selection of cargoes and references is listed. Adapters are proteins that link a cargo to an exportin and that are rapidly recycled back to the nucleus following export. See main text for more information.

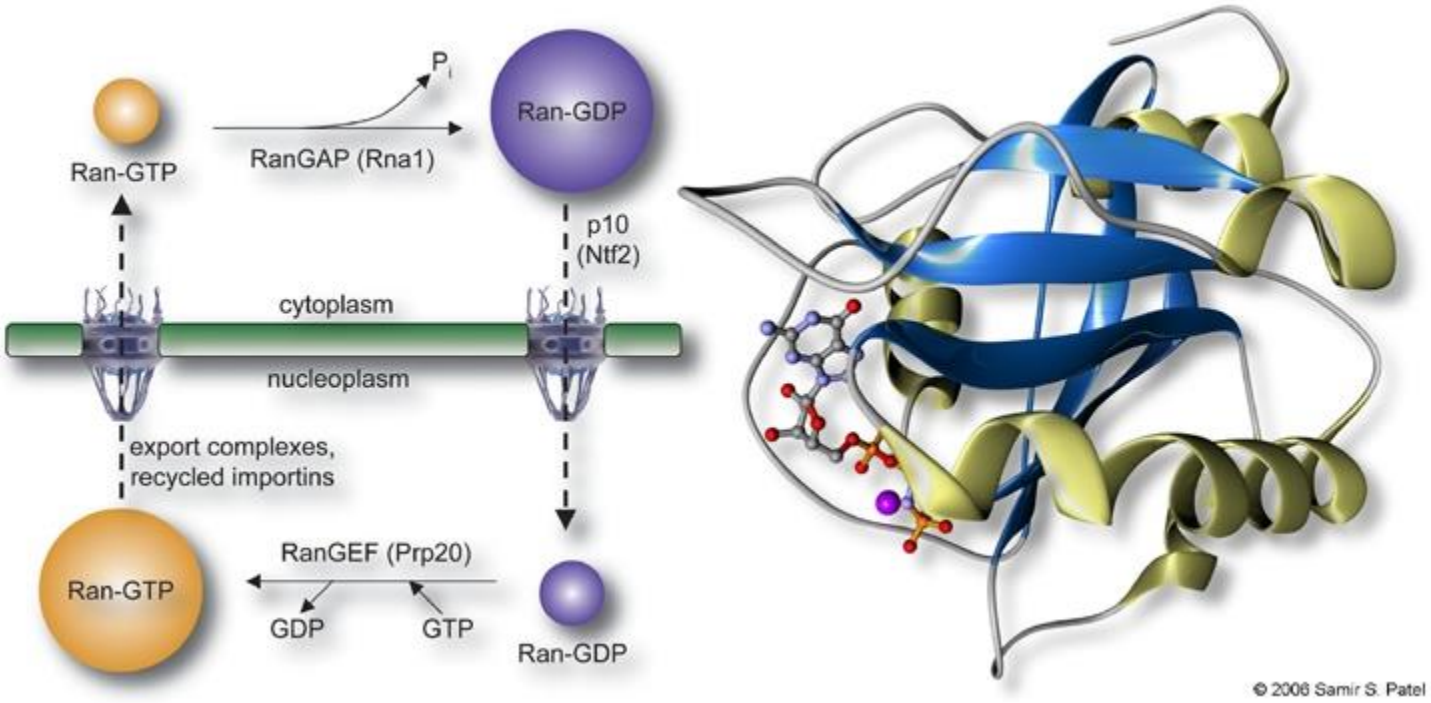
Guttler in Gorlich, 2011

Transport proteinov med jedrom in citosolom

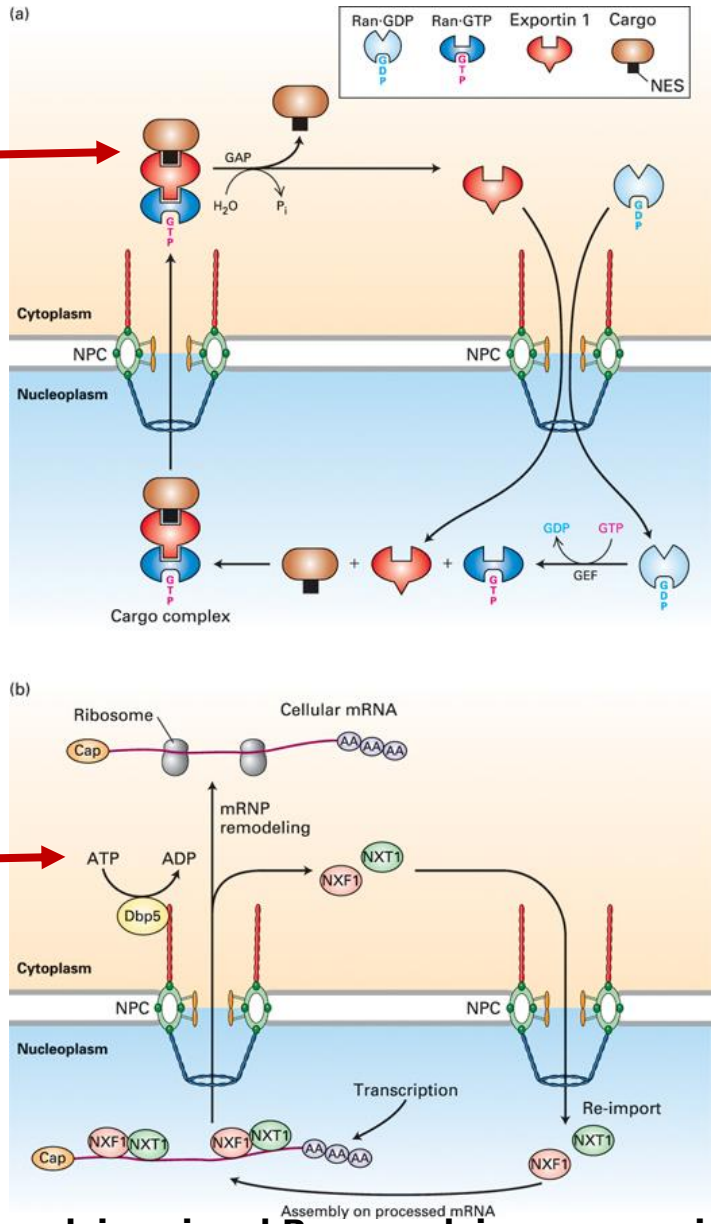


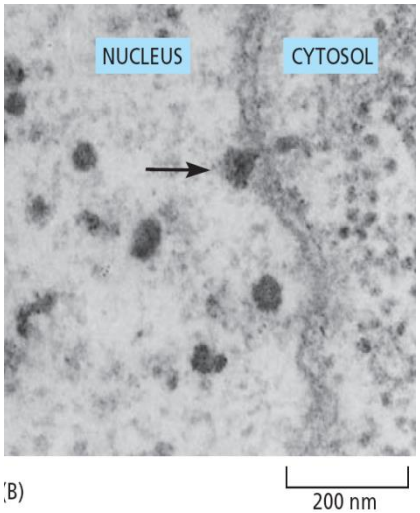
Faustino et al., Clinical Pharmacology & Therapeutics 2007

Ran-GTP / Ran-GDP



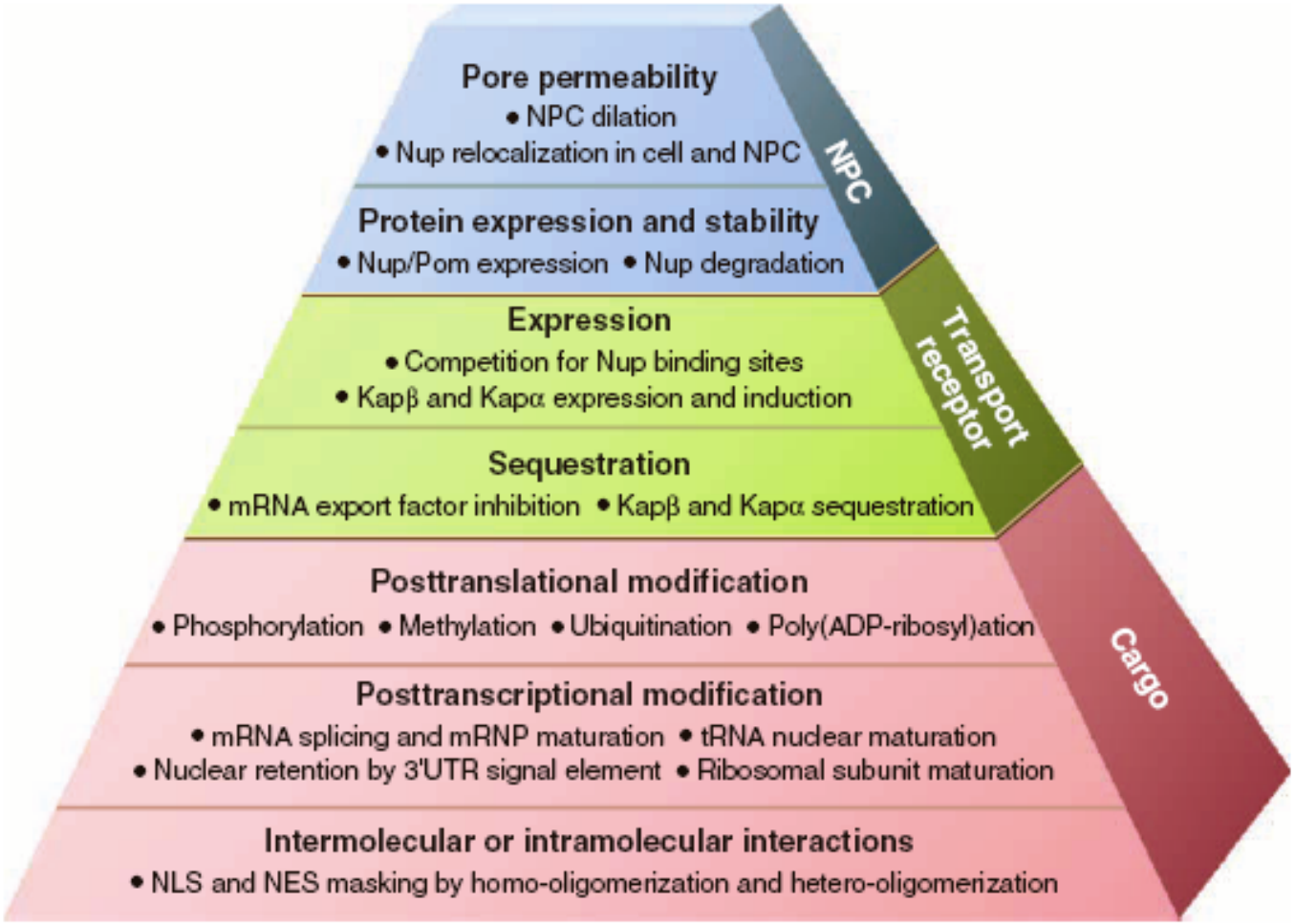
Dve glavni poti prehoda iz jedra





Od Ran-odvisen in od Ran-neodvisen prenos iz jedra.

Regulacija prenosa proteinov med jedrom in citoplazmo



Mutacije komponent jedrnega transporta in bolezni

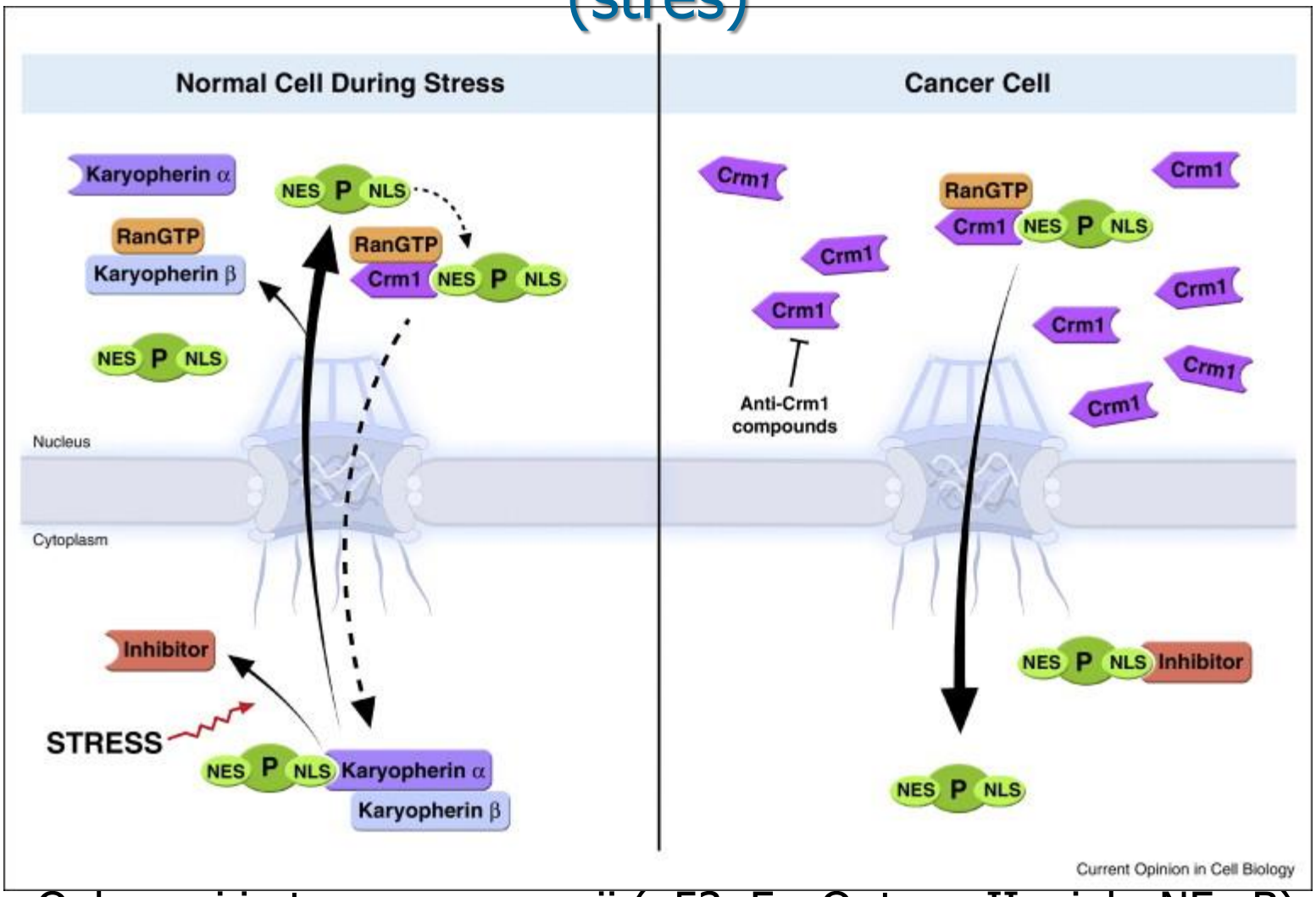
Table 1 | Tissue-specific roles of metazoan nucleoporins

Metazoan nucleoporins (*Saccharomyces cerevisiae* homologue)	Tissue-specific disorder or developmental aspect	Nature of nucleoporin mutation	References
Human Nup155 (Nup157, Nup170)	AF, which is a cardiac disorder characterized by clinical arrhythmia	Homozygous missense R391H in humans; large truncation (leaving 271/1,391 amino acids) in mouse model	Zhang *et al*, 2008
Drosophila Nup154 (Nup157, Nup170)	Male and female gametogenesis, various steps of oogenesis and spermatogenesis	P-element insertion in the 5' region of the gene, resulting in reduced expression (hypomorph)	Gigliotti *et al*, 1998; Grimaldi *et al*, 2007
Human Nup98, mouse Nup98 (Nup145N, Nup116)	Acute myeloid leukaemia (and other haematological malignancies); haematopoietic stem-cell proliferation	Multiple genomic translocations, which result in fusion of Nup98 fragment, including FG repeats, to another gene	Nakamura *et al*, 1996; Slape & Aplan, 2004; Takeda *et al*, 2006
Mouse Nup96 (Nup145C)	Immune-system function, interferon response, B-cell and T-cell proliferation	Genetic knockout by an inserted early stop codon	Faria *et al*, 2006
Human Nup214 (Nup159)	T-cell acute lymphoblastic leukaemia	Genomic translocations, which fuse Nup214/CAN fragment to another gene	Graux *et al*, 2004; Saito *et al*, 2004
Drosophila Nup88/mbo (Nup82)	Trachea, central nervous system, imaginal discs of larvae; immune response	Genetic null by removal of 5'-coding sequences	Uv *et al*, 2000
Human Nup62 (Nsp1)	Primary biliary cirrhosis (autoimmune liver degeneration)	Autoimmune antigen	Wesierska-Gadek *et al*, 2007
Human Nup62 (Nsp1)	Autosomal recessive infantile bilateral striatal necrosis (degeneration of the basal ganglia)	Homozygous missense Q391P mutation	Basel-Vanagaite *et al*, 2006
Human Gp210 (–)	Primary biliary cirrhosis (autoimmune liver degeneration)	Autoimmune antigen	Tartakovsky & Worman, 1995
Human Nup358/RanBP2 (–)	Familial cases of infection-triggered acute necrotizing encephalopathy	Heterozygous missense mutation T585M	Neilson *et al*, 2009
Mouse Nup133 (Nup133)	Neural stem/progenitor cell differentiation	Functional null mutation owing to truncation/stop codon	Lupu *et al*, 2008
Human Aladin (–)	Triple A syndrome (adrenal insufficiency, absence of tear secretion and achalasia)	Various nonsense, frameshift and missense mutations	Cronshaw & Matunis, 2003
Zebrafish ELYS/Flo (–)	Proliferating progenitor cells in developing retina and intestine	Truncated protein owing to early stop codon and lower expression levels	Davuluri *et al*, 2008; de Jong-Curtain *et al*, 2008

AF, atrial fibrillation; CAN, Nup214/CAN; ELYS, embryonic large molecule derived from yolk sac; Flo, flotte lotte; Gp210, glycoprotein of 210 kDa; mbo, members only; Nup, nucleoporin; RanBP2, Ran-binding protein 2.

Capelson and Hetzer, EMBO R, 2009

Nenormalen izvoz proteinov v rakastih celicah (stres)



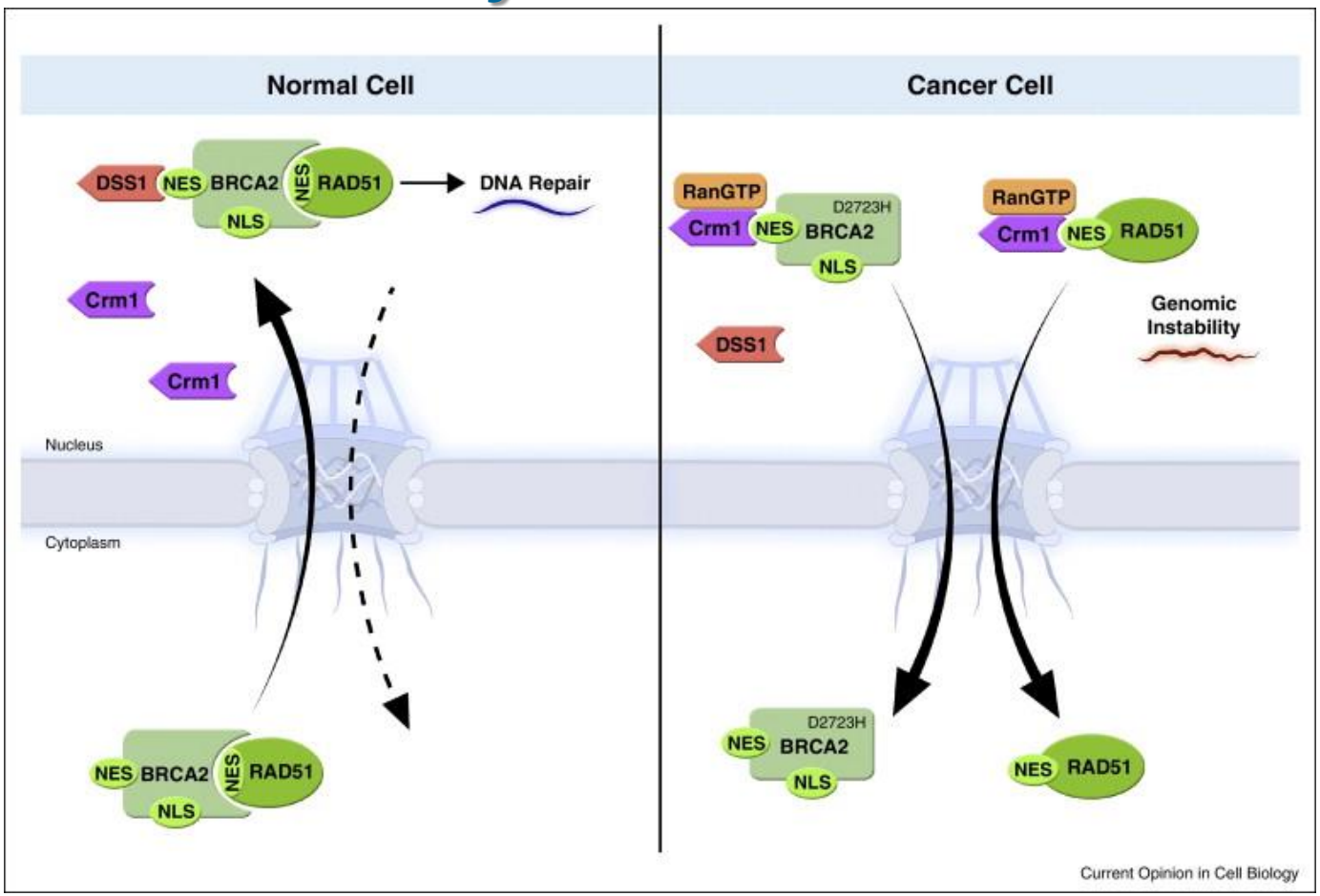
Onkogeni in tumor supresorji (p53, FoxO, topo-IIa, inh. NF-κB) potujejo iz jedra, ker je prekomerno izražen CRM1 (glioma, osteosarkoma, levkemija), inducirana proliferacija.

Izražanje CRM1 pri človeških rakih

- Prekomerno izražanje sovpada s povečanim metastaziranjem, povečnimi tumorji, znižano stopnjo preživetja.
- Rak na jajčniki, pankreatični rak, osteosarkom, glioma, rak maternični vratu.
- Inhibitorji: LMB, SINE (selektivni inh. jedrnega izvoza)

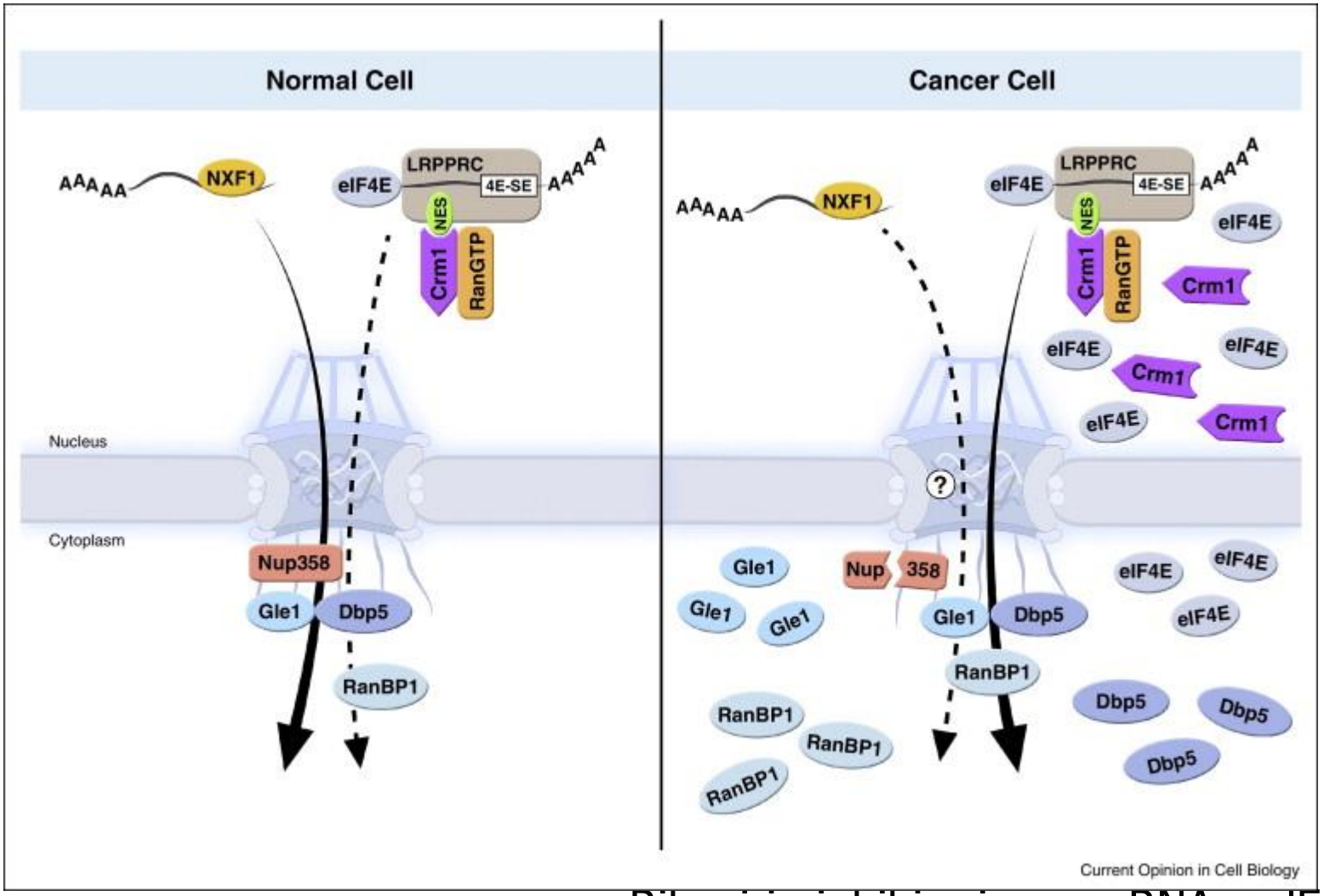
Turner et al., Bioch Pharm, 2012

Mutacije v BRCA2 namesto popravljanja DNA vodijo v nastanek raka



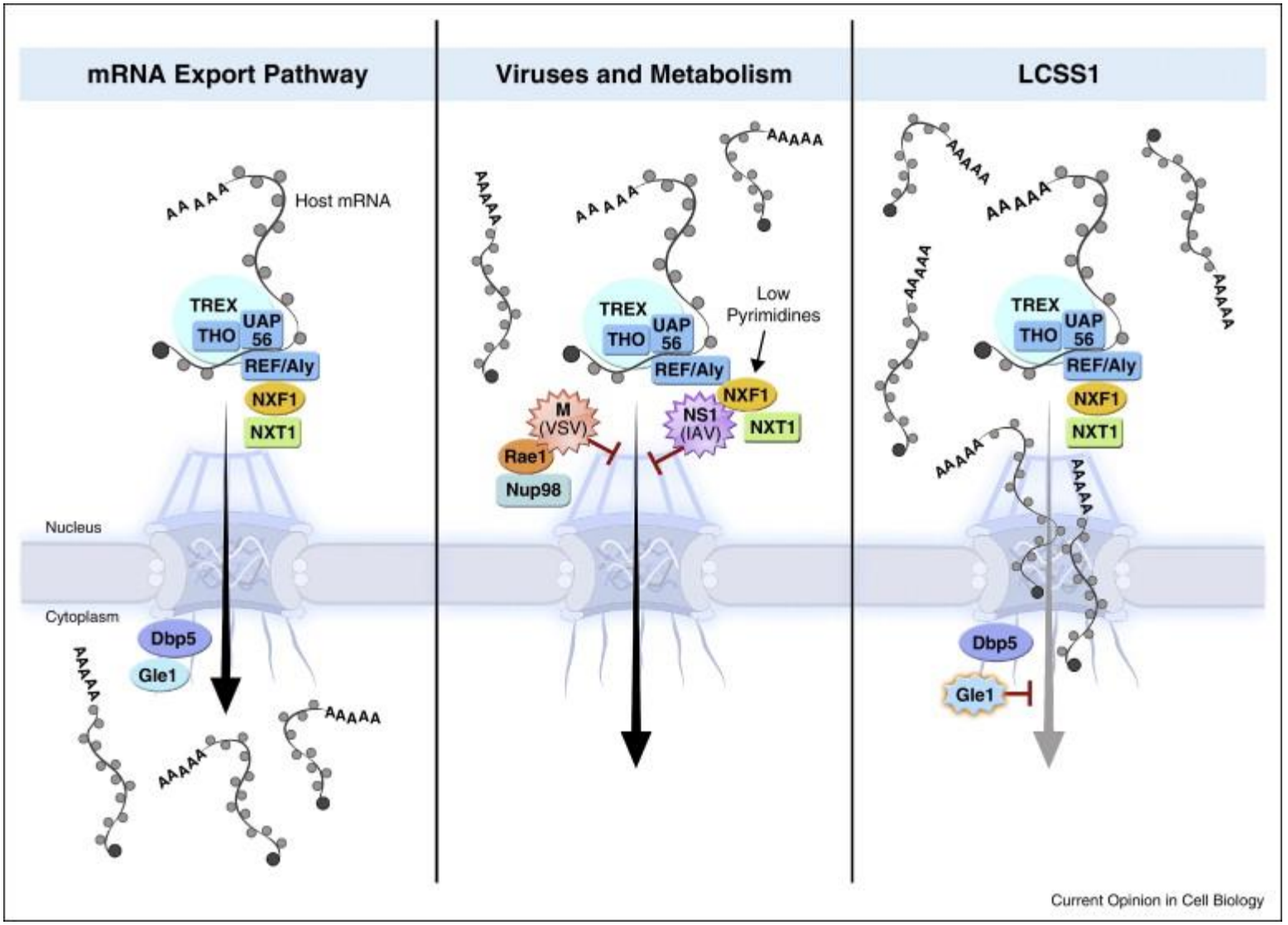
Mutiran BRCA2 ni vezan na DSS1, zato je NES izpostavljen in protein potuje v citoplazmo, enako RAD51. Oseba z mutiranim BRCA2 ima predispozicijo za raka na prsih, jajčnikih in pankreasu.

Tumorigeneza zaradi povečanega prenosa mRNA za proliferacijo celic v citoplazmo



Ribavirin inhibira izvoz mRNA z eIF4E.

Vpliv virusov na jedni transport



Nepravilna lokalizacija proteinov

Napačna lokalizacija proteinov povzroči nastanek bolezni. Prevladujejo raki in nevrodegenerativne bolezni.

Table 1. Mislocalized proteins that have been associated with human diseases

Protein	Disease	Mechanism	Mislocalization	Reference
SRY	Swyer syndrome	Mutation of NLS	Loss of nuclear localization	(McLane and Corbett, 2009)
SHOX	Léri–Weill dyschondrosteosis	Mutation of NLS	Cytoplasmic retention	(Sabherwal et al., 2004)
TRPS1	TRPS	Mutation of NLS	Loss of nuclear localization	(Kaiser et al., 2004)
ARX	XLAG	Mutation of NLS	Loss of nuclear localization	(Shoubridge et al., 2010)
FOXP2	Speech–language disorder	Mutation of NLS	Loss of nuclear localization	(Mizutani et al., 2007)
AIRE	APECED	Mutation of ZFD	Cytoplasmic retention	(Bjorses et al., 2000)
RPS19	Diamond–Blackfan anemia	Mutation of NoS	Loss of nucleolar localization	(Da Costa et al., 2003b)
AGT	Primary hyperoxaluria type 1	Polymorphism and/or mutation	Mitochondrial mislocalization	(Djordjevic et al., 2010)
hsMOK2	Laminopathy	Mutation of lamin A/C	Formation of nuclear aggregates	(Dreuillet et al., 2008)
SHOC2	Noonan-like syndrome	Acquired N-myristoylation	Mislocalization to the plasma membrane	(Cordeddu et al., 2009)
Rhodopsin	Retinitis pigmentosa	Mutations	ER retention	(Mendes et al., 2005)
AVPR2	Nephrogenic diabetes insipidus	Mutations	ER retention	(Robben et al., 2006)
ATP7B	Wilson disease	H1069Q mutation	ER retention	(Payne et al., 1998)
ABCA1	Tangier disease	Mutations	Loss of plasma membrane localization	(Tanaka et al., 2003)
Tau	Neurodegenerative diseases	Hyperphosphorylation	Mislocalization to dendritic spines	(Hoover et al., 2010)
TARDBP	ALS and FTLD	Unknown	Cytoplasmic mislocalization	(Winton et al., 2008)
FUS	FTLD	Mutations	Cytoplasmic mislocalization	(Vance et al., 2009)
FOXO	Various types of cancer	Post-translational modifications	Cytoplasmic mislocalization	(Dansen and Burgering, 2008)
p53	Various types of cancer	Mutations, post-translational modifications	Cytoplasm	(Fabbro and Henderson, 2003)

APECED, autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy; ALS, amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; TRPS, trichorhinophalangeal syndrome; XLAG, X-linked lissencephaly with absent corpus callosum and ambiguous genitalia.

Hung and Link, J Cell Sci, 2011

Terapevtske tarče

Terapevtske tarče pri nuklearnem transportu so deli NPC, receptorji ali tudi molekule tovora.

Table 2. Examples of agents that can interfere with protein trafficking

Agent	Direct target	Localization effect	Potential application	Reference
IN3	Gonadotropin-releasing hormone receptor (GNRHR)	Cell surface expression of GNRHR	Reproductive disorders	(Finch et al., 2010)
Rapamycin	MTOR	Restoration of nuclear TARDBP	Various neurodegenerative diseases	(Caccamo et al., 2009)
CF35Es	Mutant rhodopsin	Proper rhodopsin trafficking	Retinitis pigmentosa	(Ohgane et al., 2010)
SMIP001/004	Unknown	Nuclear p27KIP localization	Prostate cancer	(Rico-Bautista et al., 2010)
ETP-45648	PI3K	Nuclear FOXO localization	Various types of cancer	(Link et al., 2009)
CHS828	IKK	Cytoplasmic NF-κB	Various types of cancer	(Olsen et al., 2004)
PITs	Pleckstrin homology domain	Loss of AKT at the plasma membrane	Various types of cancer	(Miao et al., 2010)
Palmostatin B	APT1	Loss of precise RAS localization	Lung cancer	(Dekker et al., 2010)
Tipifarnib	Farnesylated proteins	Mistargeting of farnesylated proteins	Hematologic malignancies	(Martinelli et al., 2008)
Poloxin	PLK1	PLK1 mislocalization	Various types of cancer	(Reindl et al., 2008)
Resveratrol	SIRT1	Nuclear FOXO1	Various types of cancer	(Frescas et al., 2005)
GSIs	NOTCH1	Loss of ICN1	T cell acute lymphoblastic leukemia	(Real et al., 2009)
Elliticine	Unknown	Increased nuclear p53 localization	Various types of cancer	(Xu et al., 2008)
INCAs	Calcineurin and NFAT	Cytoplasmic NFAT	Inflammatory and autoimmune diseases	(Roehrl et al., 2004)
WGA	N-Acetyl-D-Glucosamine (GlcNac)	Unspecific nuclear exclusion	ND	(Gasiorowski and Dean, 2003)
bimax1/2	Importin-α	Resistance to nuclear cargo release	Viral infection, Atherosclerosis	(Kosugi et al., 2008)
LMB analogues	CRM1	Unspecific nuclear trapping	Various types of cancer	(Mutka et al., 2009)

ND, not determined.

Hung and Link, J Cell Sci, 2011

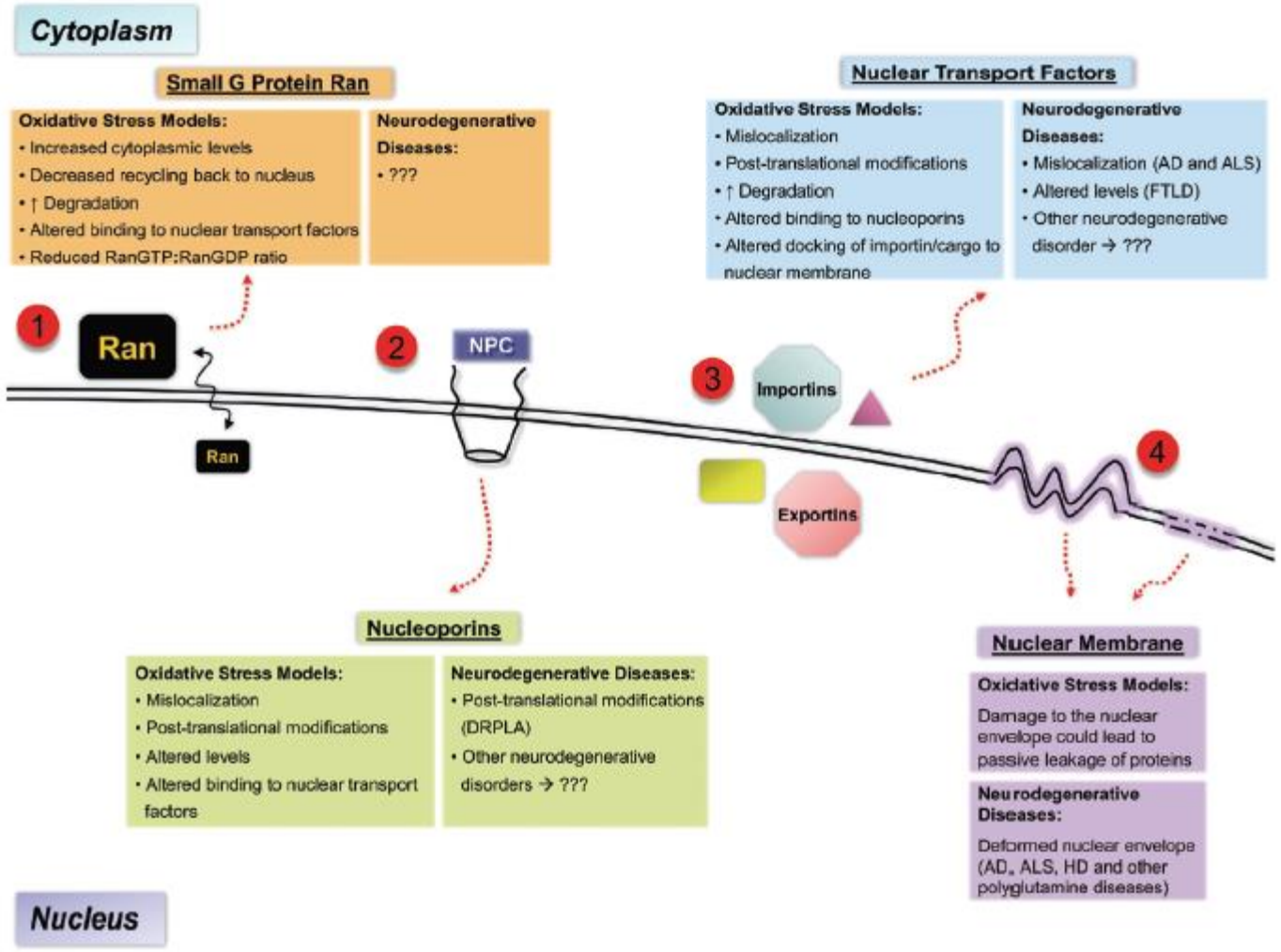
Nepravilna lokalizacija proteinov

- Nevrodegenerativne bolezni, raki, shizofrenija, diabetes isipidus, cistična fibroza

Terapije: inhibitorji komponent jedrnega transporta.

Turner et al., Bioch Pharm, 2012

Spremembe v jedrnem transportu v modelih oksidativega stresa in pri nevrodegenerativnih boleznih



Patel in Chu, Int J Clin Exp Pathol, 2011

Jedrna pora in bolezni

- Rak
- Avtoimune bolezni
- Nevrodegenerativen bolezni
- Infekcije z virusi
- Stres…

Vnos virusov v jedro