Cell Nucleus
- Structure, Dynamics, and Regulation -

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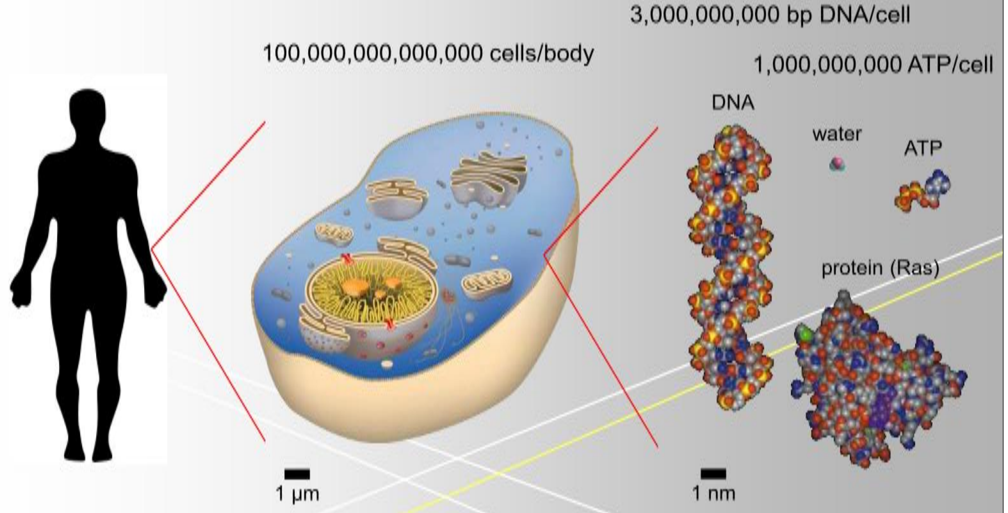
Organism, Cell, and Molecule

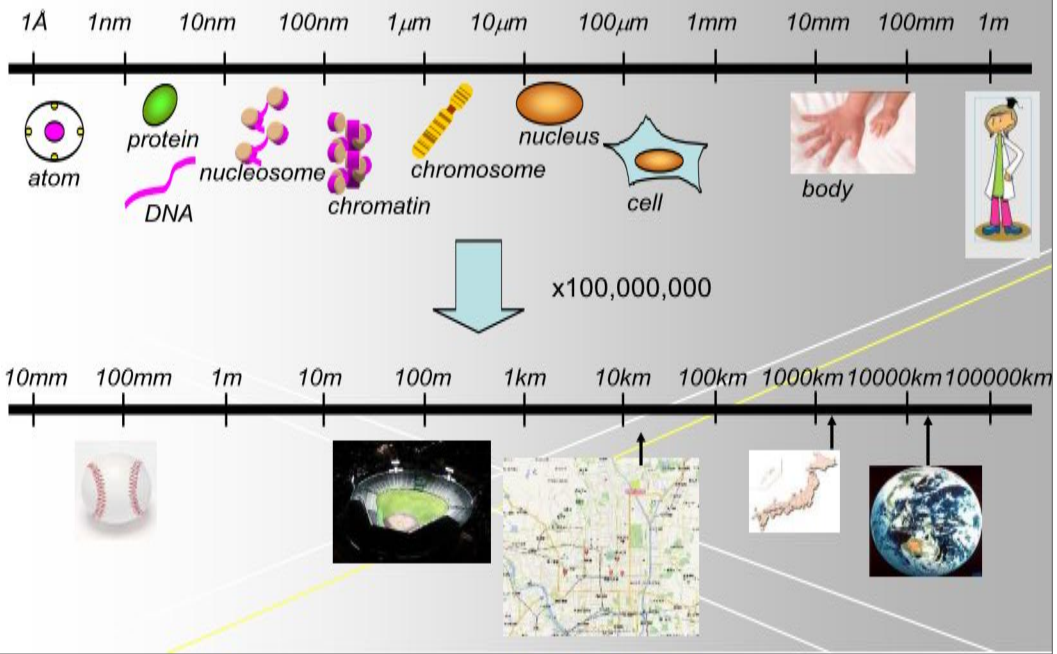


Molecular function → Cellular behavior → Property of the organism

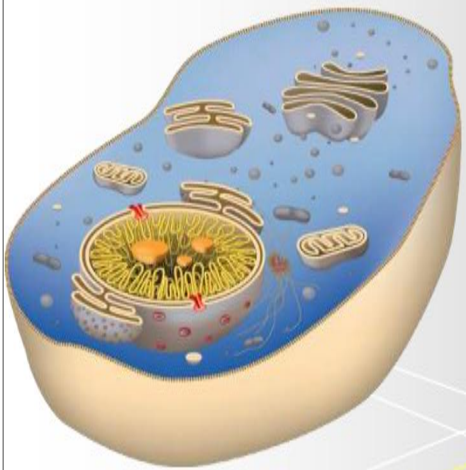
Mi-ke (三毛猫) - Tricolor or Calico Cat

- Cat with three colored fur
- Typically white, black, brown
- Often found in Japan, already very popular in the Edo-period
- Becoming popular in other countries, called "Mi-ke"
- **Almost 100% female**

Worlds of Cells and Molecules



Subcellular Compartmentation



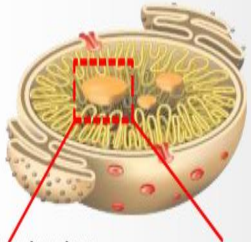
Organelle

- Membrane-enclosed:
- Non-membranous:

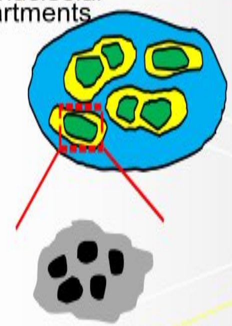
Benefits of subcellular compartmentation

-
-
-

More Magnification, More Structures



3 sub-nucleolar compartments



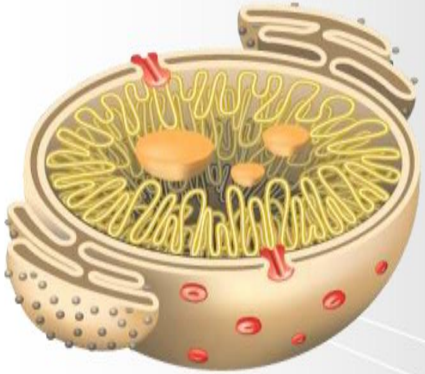
"core and clouds"

Sub-nucleolar Structures

- Fibrillar Center (FC):
  center for rRNA transcription
  markers: RNA polymerase I, UBF
- Dens Fibrillar Compartment (DFC):
  rRNA early processing
  markers: fibrillarin
- Granular Compartment (GC):
  rRNA late processing, ribosomal assembly
  markers: nucleolin, B23

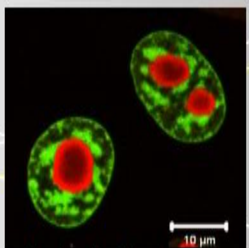
TCOF-1 (FC)

Nuclear Structure

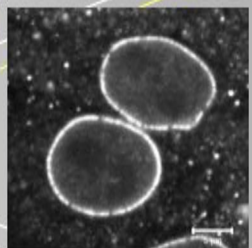


Sub-nuclear Structures

- Nuclear envelope:
- Nucleoplasmic:



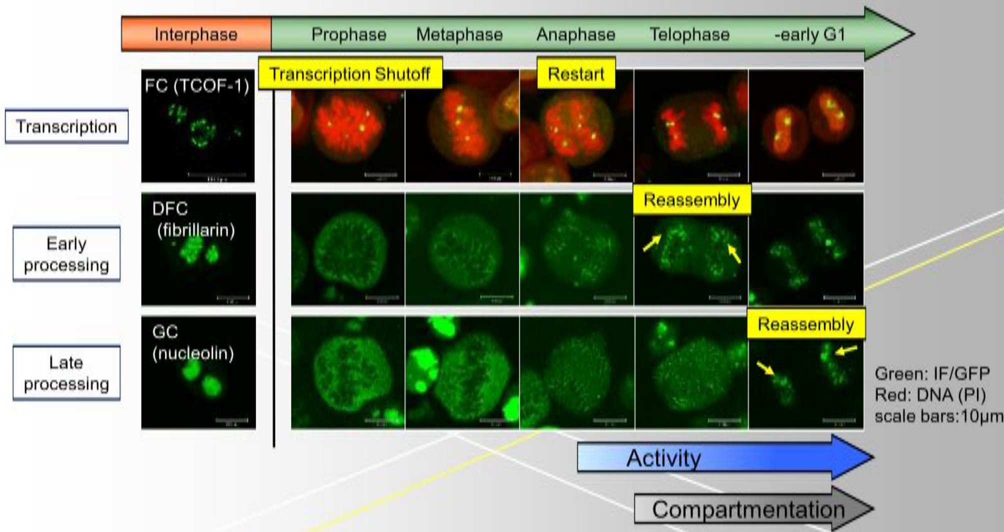
Nucleolin (Nucleolus)
NuMA (Nuclear speckle)



Nucleoporin (Nuclear pore)

Activity and Compartment

Nucleolar organization: Activity ensures the Compartment



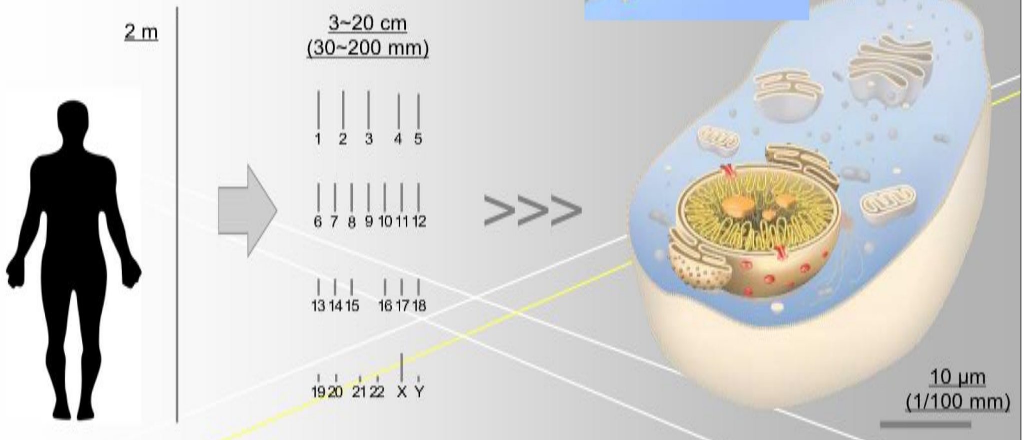
Compartmentation is closely related to its activity

DNA Packing in the Nucleus

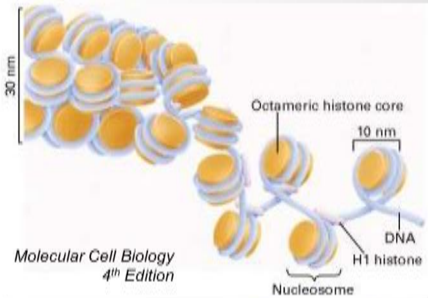
Human genome DNA
Length: 2 m
Diameter: 2 nm
Nucleus: 10 µm

x1,000,000

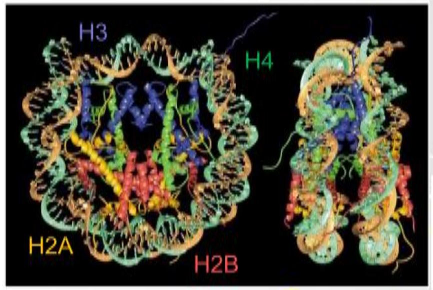
2000 km
2 mm
10 m

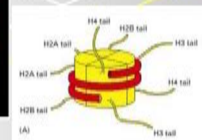


Nucleosome

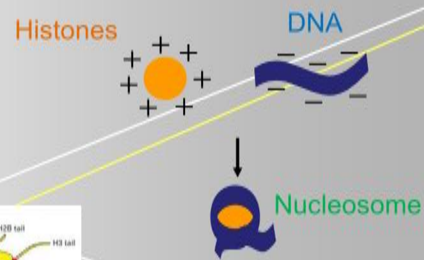


Molecular Cell Biology
4th Edition





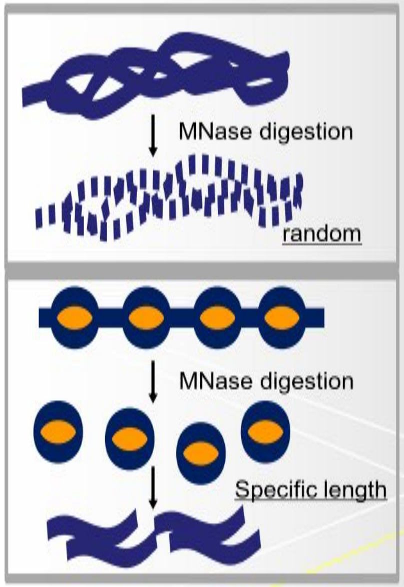
		MW	K/R contents
Core histones	H2A	14,000	20%
	H2B	13,900	22%
	H3	15,400	23%
	H4	11,400	24%
Linker histone	H1	20,800	32%

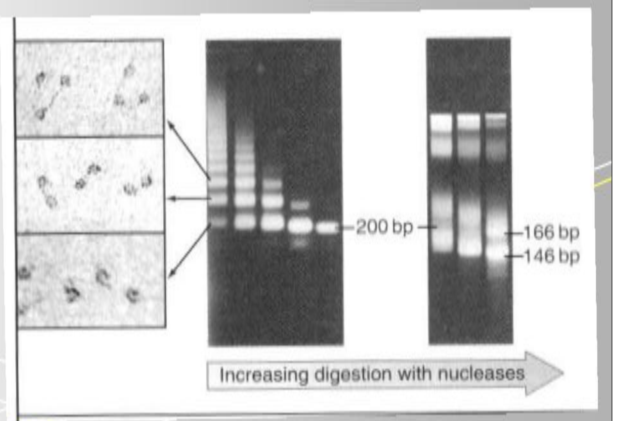


Molecular Biology of the Cell
4th Edition

Nucleosome is made of histone octamer rapping ~146bp DNA

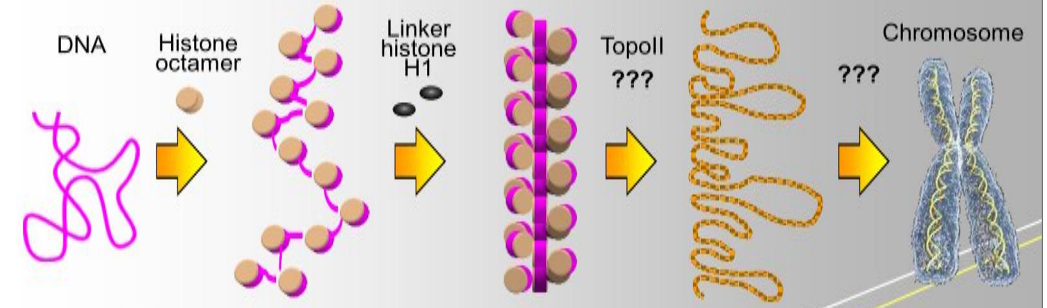
Structural "Unit" of Genomic DNA

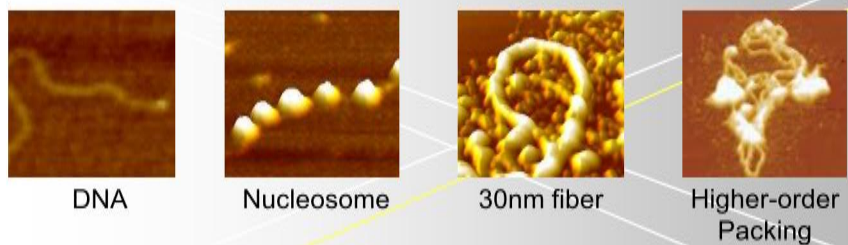




Chromosomal DNA contains fundamental unit consist of 146 base pairs

Higher-Order Structure of the Genome

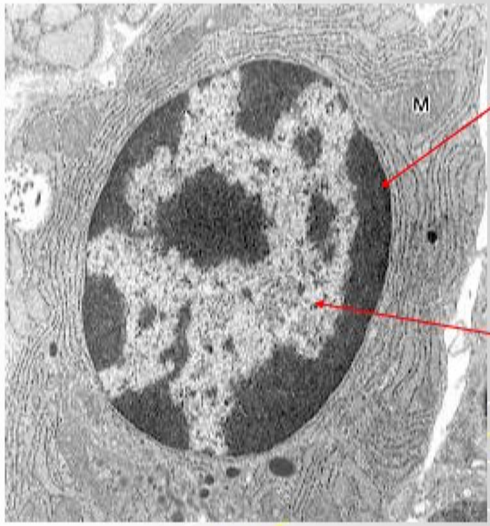




How these structures are related to the genome function?

Heterogenous Packing

EM image of leucocyte cell nucleus



Heterochromatin

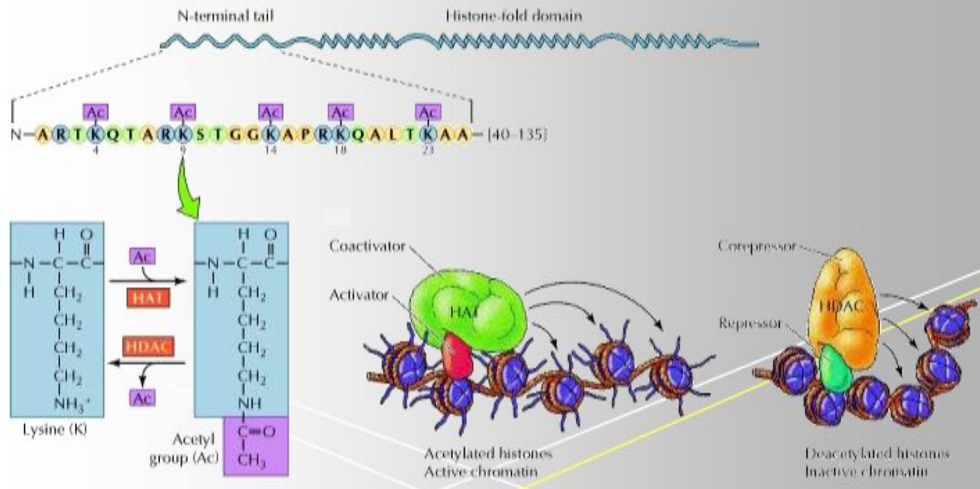
Highly packed
Functionally inactive

Euchromatin

Loosely packed
Functionally active

How these different structures are organized?

Histone Acetylation



The Cell: A Molecular Approach. 2nd edition

Histone acetyltransferase (HAT)

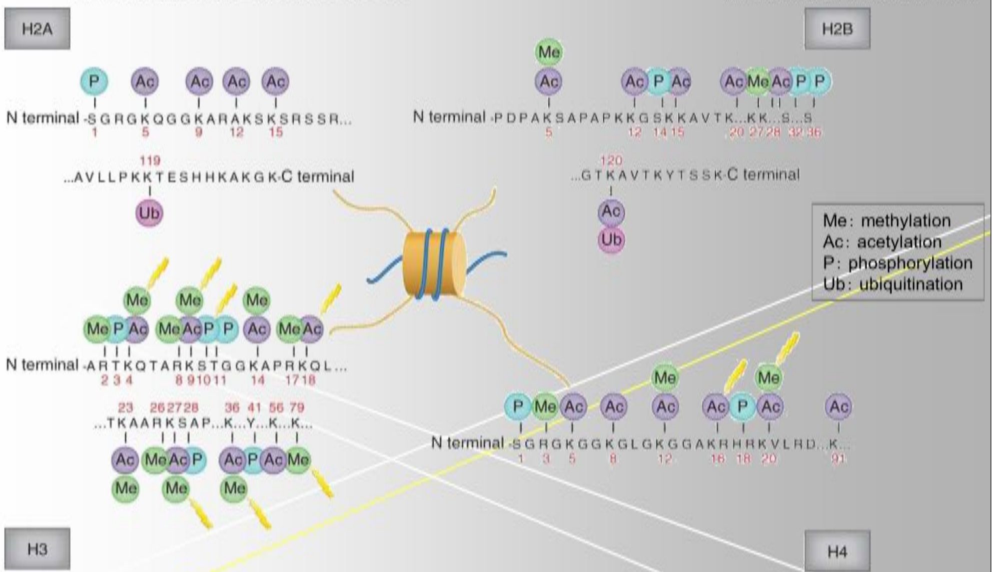
Histone deacetylase (HDAC)

Acetylation neutralize Lysine charge and attenuate histone-DNA interaction

Histone Modification

Known histone modifications

M. Paredas (2011) Nature Medicine

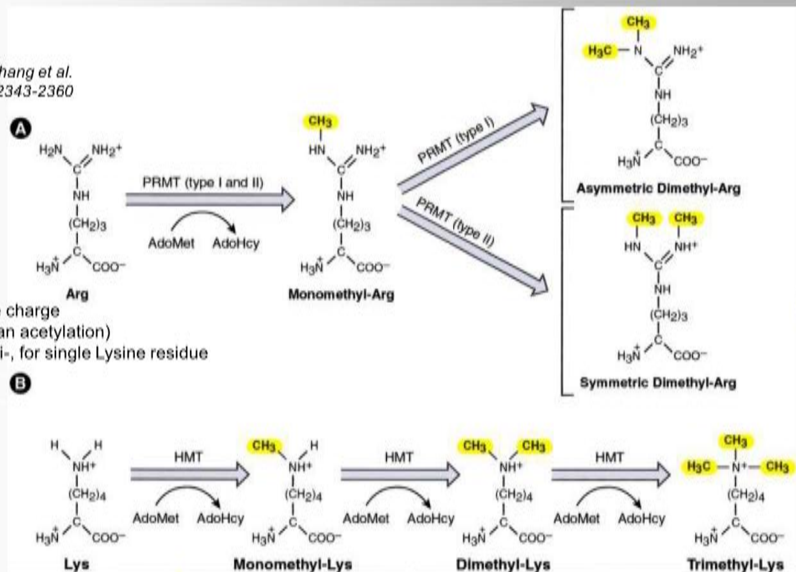


Histone tails contain >50 modification sites

Histone Methylation

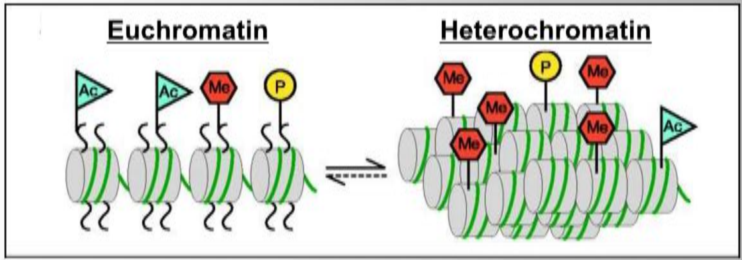
Yi Zhang et al.
Genes & Dev. 2001. 15: 2343-2360

Histone methylation
1) does not change the charge
2) has long half-life (than acetylation)
3) can be mono-, di-, tri-, for single Lysine residue



Histone methylation is often function as a flag to DNA-binding proteins

Histone Code



T. Jenuwein and CD. Allis (2001) Science

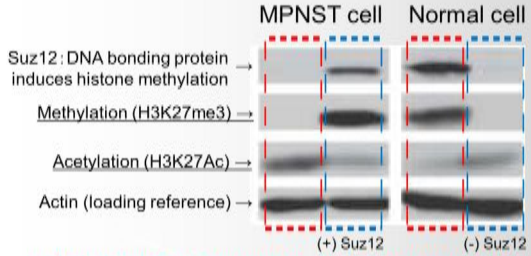
Histone	H3					H4
Site	K4	K9	K27	K36	K79	K20
mono-Me	Transcriptional activation	Transcriptional activation	Transcriptional activation	Transcriptional activation	Transcriptional activation	Transcriptional silencing
di-Me	DNA repair	Transcriptional repression	Transcriptional repression			
tri-Me	Transcriptional activation	Transcriptional repression Heterochromatin formation	Transcriptional silencing X inactivation			Heterochromatin formation

1. Histone modification affects DNA-histone interaction, leading to the genome regulation
2. Histone modification recruits specific DNA-binding proteins, leading to the genome regulation

Histone modification functions as a “second code” of the genome

Dynamic Balancing of Epigenetic Status

MPNST (malignant peripheral nerve sheath tumor)
(悪性末梢神経鞘腫瘍、神経細胞のガン)



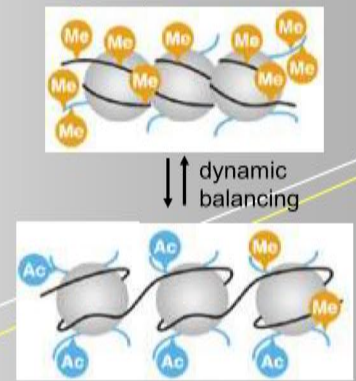
Normal: Suz12 expression, histone methylation at H3K27, no acetylation at the site
MPNST: Loss of Suz12, lack of histone methylation, facilitated acetylation at H3K27

Histone modification status is altered by both knockdown of Suz12 from normal cells and introduction of Suz12 in MPNST cells.

TD. Raedt (2014) Nature

Balancing in epigenetic status

Suppressive modification
inactivate gene expression

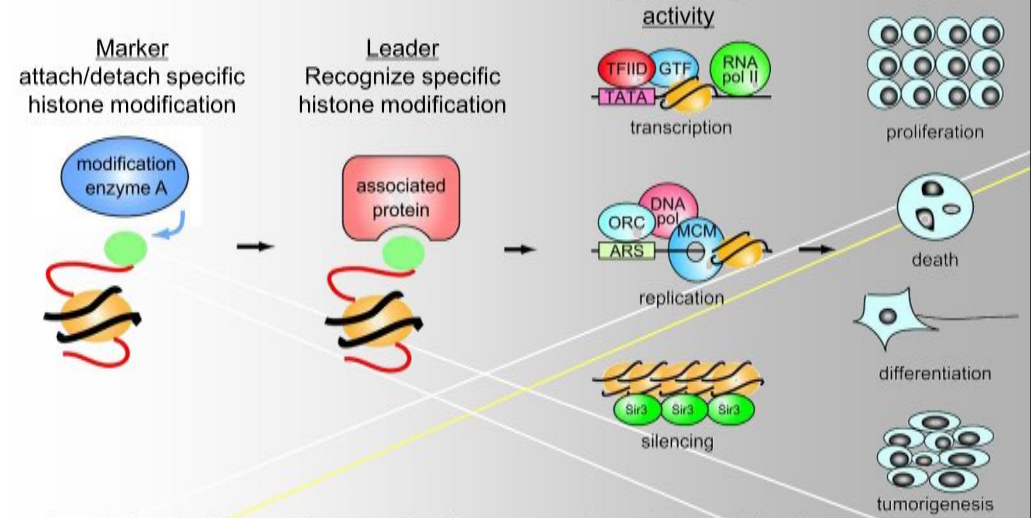


Promotive modification
activate gene expressions (→tumor)

A variety of different types of cells are produced, from identical genome information

Epigenetics

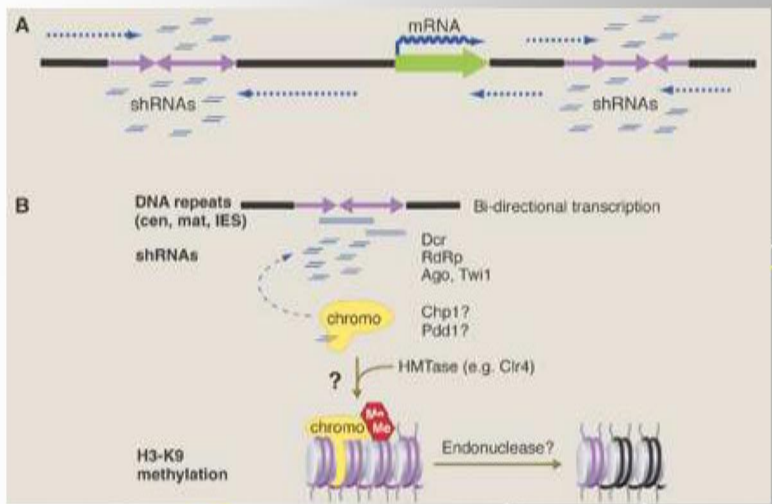
Gene regulation resulting from changes in chromosomal status, without altering the DNA sequence



A variety of different types of cells are produced, from identical genome

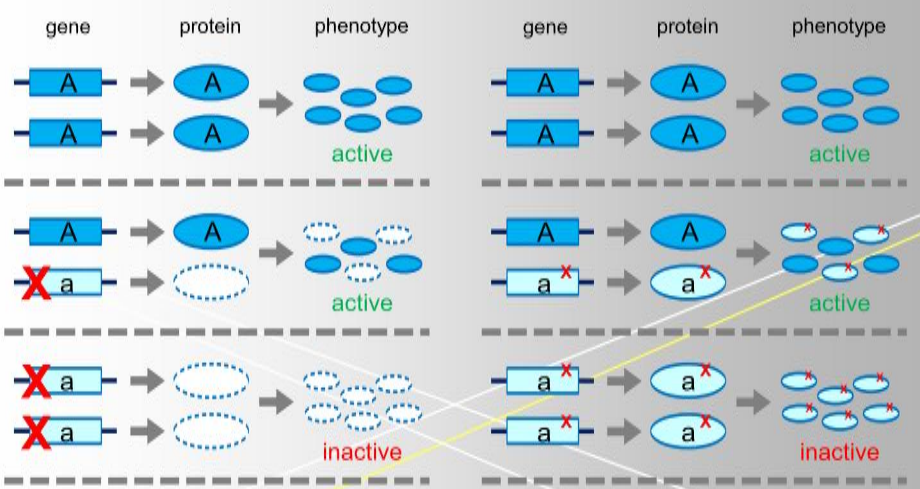
RNA-based Regulation

An RNA-Guided Pathway for the Epigenome



Jenuwein T. (2002) Science

Molecular Basis for Dominant/Recessive



Chromatin structure is related to the regulation at transcriptional level

Genes Controlling Cats' Color

Gene					
All white	W / w	W-	ww	ww	ww
White spot	S / s	--	ss	S-	S-
Orange/Black	O / o	--	oo	oo	OO

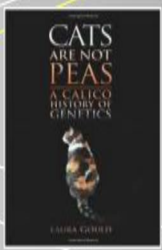
How can a cat grow both black and brown furs?

Genes Controlling Cats' Color

Gene		Phenotype
White (all white)	W	white fur grows in entire body (*1)
	w	fur can be other colors
(white) Spot	S	white spots
	s	no spot
Orange/Black (*2)	O	brown (orange) fur
	o	black fur

(*1) dominant white gene dominates all other genes
(*2) brown/black are allelic phenotype

[reference]
Cats Are Not Peas: A Calico History of Genetics
Laura Gould (2008), ISBN: 1568813201
「三毛猫の遺伝学」(日本語訳)

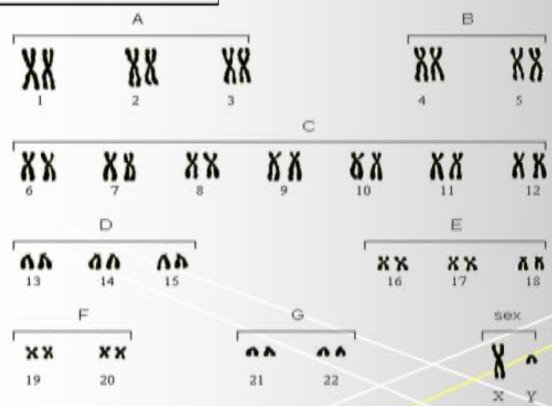


*There are at least 9 genes know to affect fur color

Sex Chromosomes

Karyotype

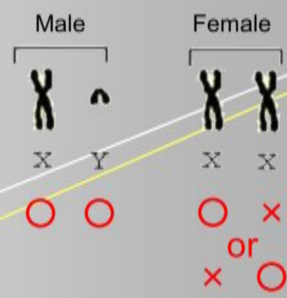
Autosomal chromosomes



Sex chromosomes

Sex chromosomes

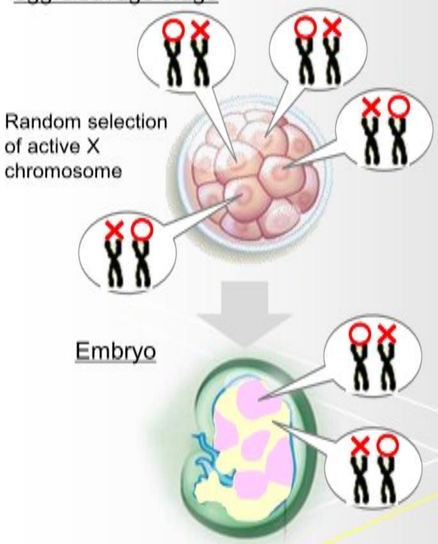
X: 163 million bp, 1100 genes
Y: 51 million bp, 80 genes

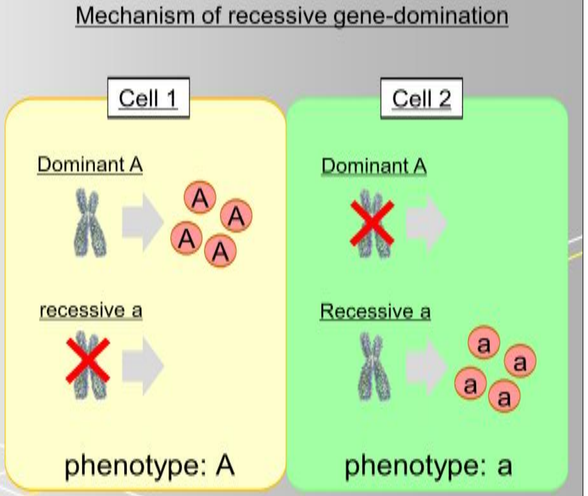


Functional chromosome

* Cat contains 19 pairs 38 chromosomes

X-Chromosome Inactivation





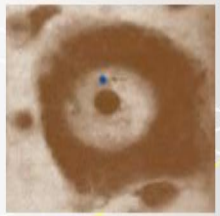
Mosaic pattern of one X-working area is made in XX genotype body

Discovery of X-Chromosome Inactivation

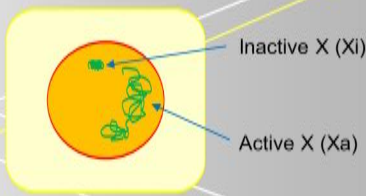
X chromosome inactivation: Lyonization

Murray Barr (1949)
Observe characteristic chromosomal aggregation in cat's neuronal cell, named "barr body".
This was found only in female cells.

Susumu Ohno (1960)
Barr body contains one of the X chromosomes.



Barr and Bertram (1949) Nature

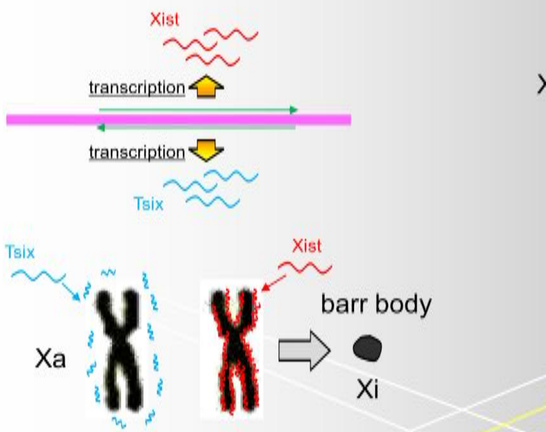


Genotype of Mi-ke

Gene		White	Black	W/Bl	W/Br	W/Br/Bl Mi-ke!!
All white	W / w	W-	ww	ww	ww	
White spot	S / s	--	ss	S-	S-	
Orange/Black (X chromosome)	O / o	--	oo (F) o (M)	oo (F) o (M)	OO (F) O (M)	

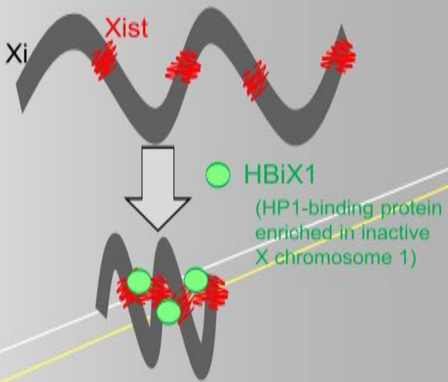
Molecular Mechanisms

Non-coding RNA "Xist" and "Tsix"



Xist binds and inactivate X (heterochromatin formation)
Tsix prevents Xist function

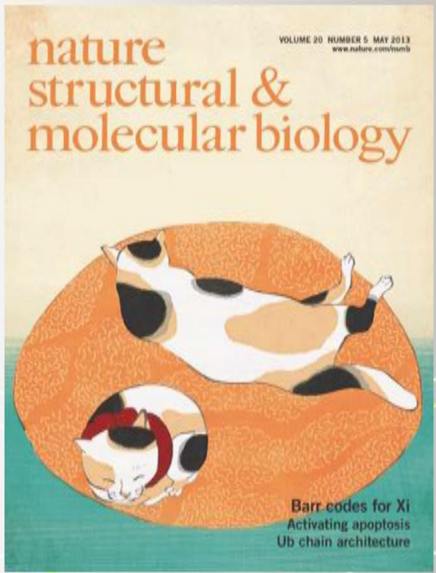
"HBiX1" mediates Xist-dependent heterochromatin formation



RS Nozawa, C Obuse, et al. (2013) Nature Struct. Mol. Biol.

Xist and HBiX1 cooperates to inactivate Xi by forming heterochromatin

Mi-ke Must be Female

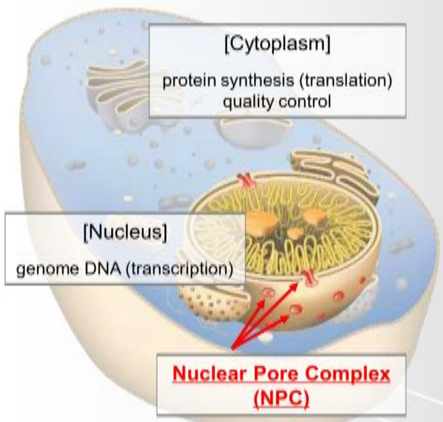


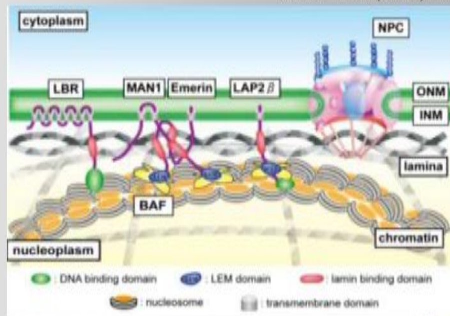
RS Nozawa, C Obuse, et al. (2013) Nature Struct. Mol. Biol.

Mi-ke should contain two X chromosomes, that means, must be female

Nucleocytoplasmic Communication

Hirano et al. (2008) EJP (Review)





What should go across the nuclear pore?

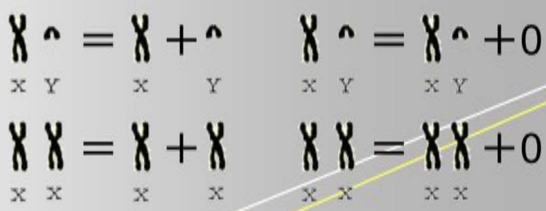
- ☐ Basal need/
  - Import: transcription-related proteins, nuclear structural proteins, nucleolar proteins
  - Export: mRNA complex, ribosomes, other cytosolic RNAs
- ☐ Adaptive response/
  - Import: replication factors, cell cycle-related nuclear factors, signaling molecules

However…

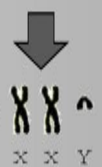
There are some male Mi-ke…

Klinefelter's syndrome

Normal gametogenesis | Abnormal division



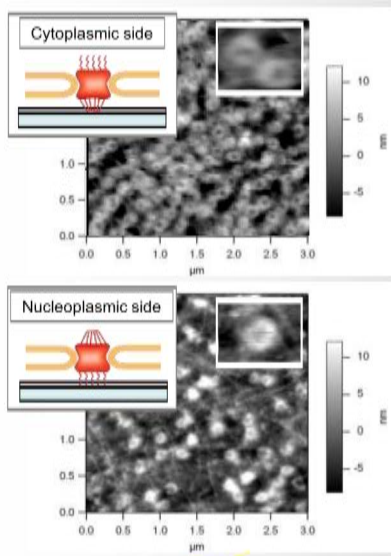
male with:



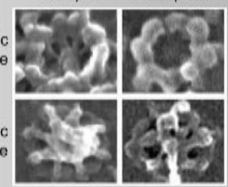
- probability: 1/30,000 or less (?)
- sometimes appear in (Japanese) newspapers when it is born
- traded at several million yen (?)

Nuclear Pore Complex (NPC)

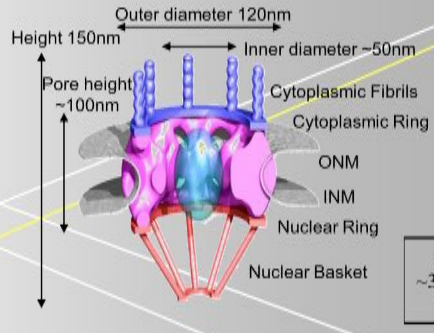
AFM observation of NPC (Xenopus egg)



EM observation of NPC



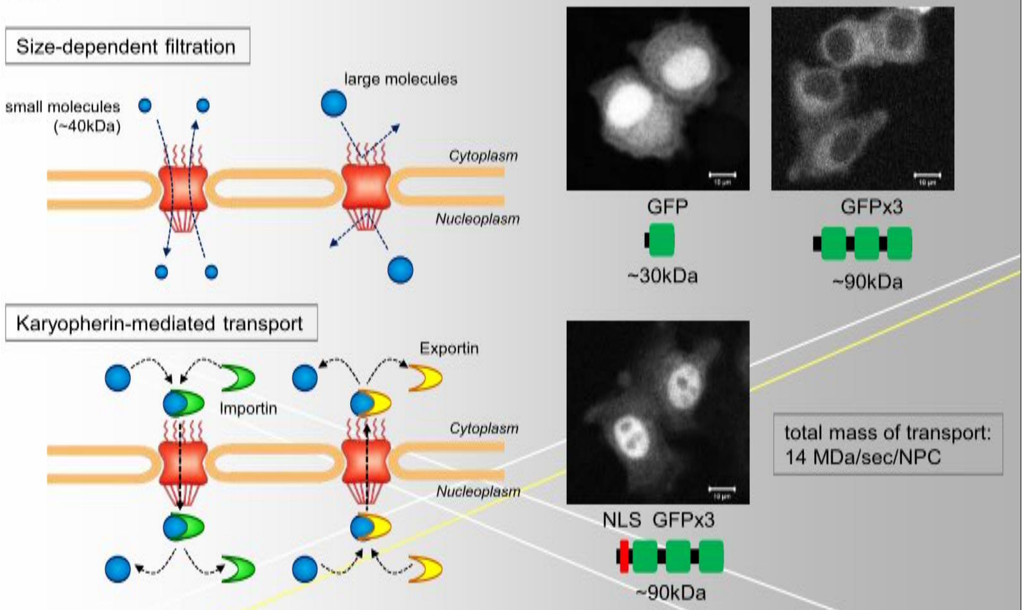
Brohawn SG et al. Structure (2009)



Octamer
~30 subunits
120MDa

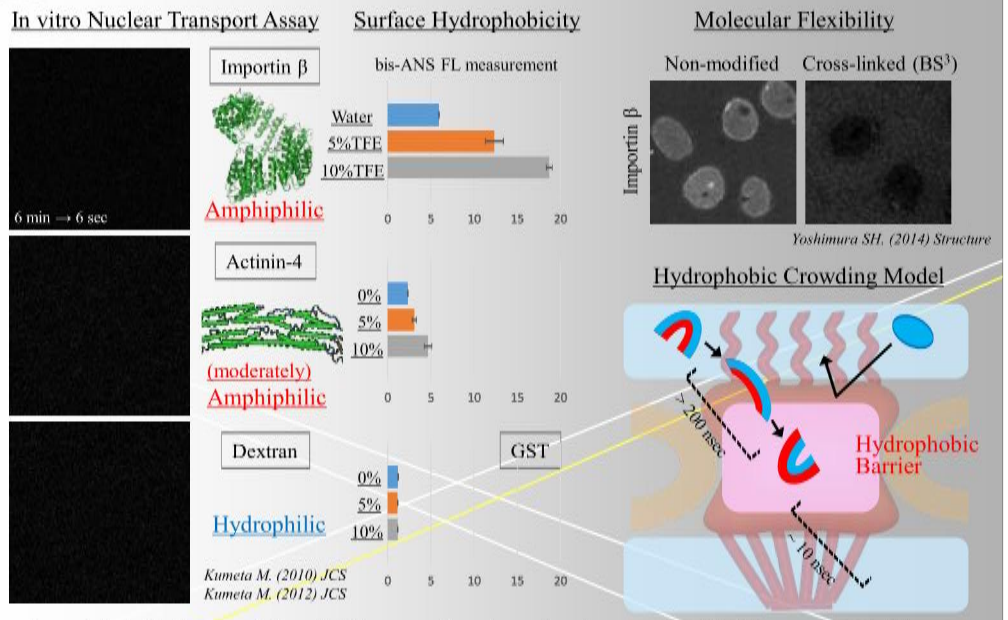
Standard cells contain 3,000~5,000 NPCs, ~5 NPC/μm^2, ~400nm intervals

Selectivity of the NPC



Why Karyopherin can pass through the pore?

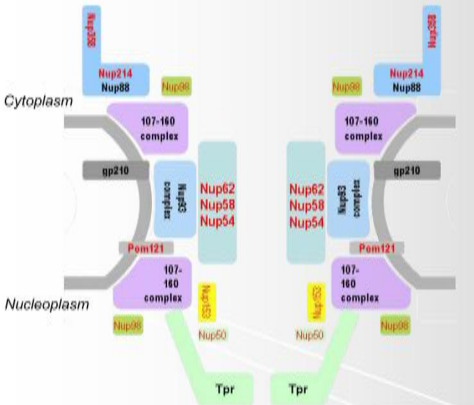
Property of the NPC-Permeable Cargoes



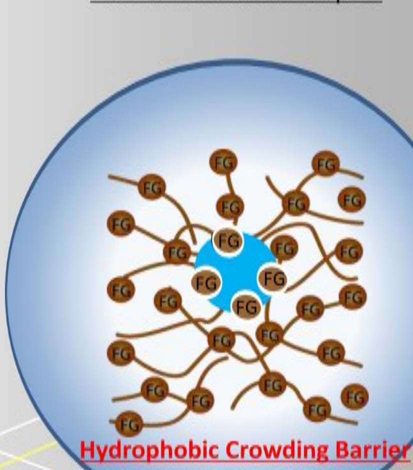
Amphiphilicity and flexibility are the key features for NPC permeability

Property of the NPC Barrier

Subunit composition and FG-repeats



Environment inside the pore

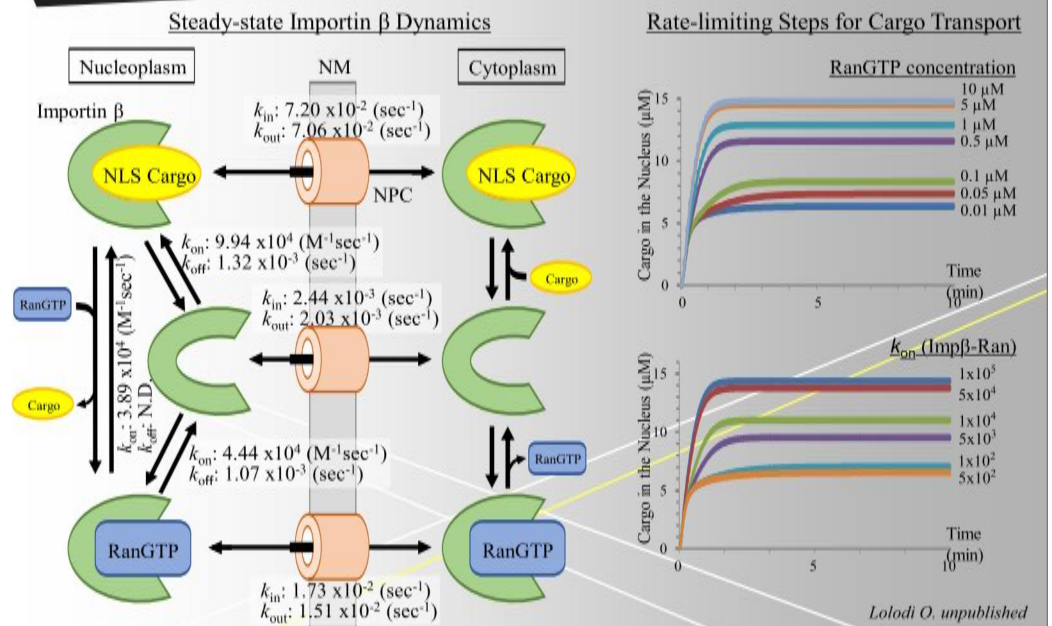


Nup153 (1200-1435)

-- FXFG, FG --

SSTPATSAGGGIFGSSTSSSNPPVATFVFGQSSNPVSSS
AFGNTAESSTSQSLLFSQDSKLATTSSTGTAVTPFVFGP
GASSNNTTTSGFGFGATTTSSSAGSSFVFGTGPSAPSAS
PAFGANQTPTFGQSQGASQPNPPGFGSISSSTALFPTGS
QPAPPTFGTVSSSSQPPVFGQQPSQSAFGSGTTPNSSS
AFQFGSSTTNFNFTNNSPSGVFTFGANSSTPAASAQP

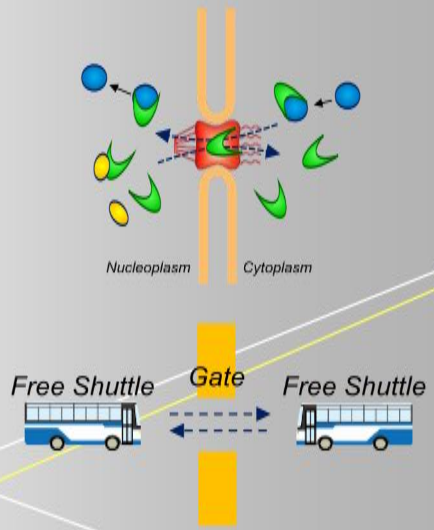
Transport Mediator: Catch-and-Release



Biological Benefits of the NPC Machinery

Cellular strategy for nuclear transport

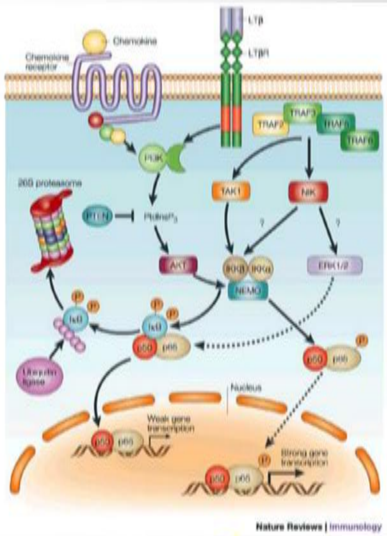
- Directed transport is achieved by non-directed Karyopherins
- Catch-and-release mechanism enables gradient localization of the cargoes
- Passage itself does not require energy consumption. (If it requires one ATP/passage, roughly 3,000,000 ATP/sec is required)



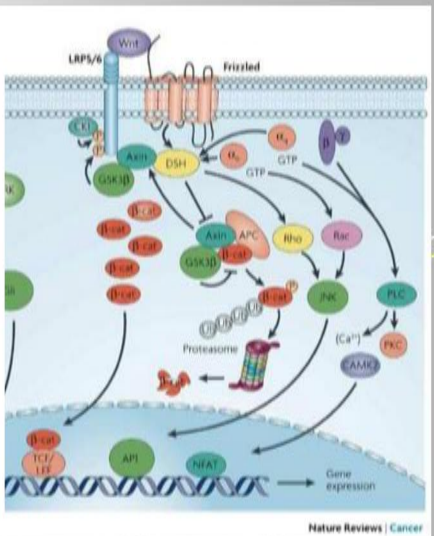
NPC mechanism is so elegant and sophisticated !!

Nuclear Transport in Cell Signaling

NFκB signaling

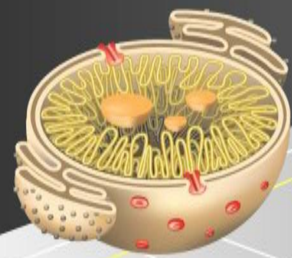


Wnt signaling



Shh, Notch, and others, related to development, differentiation, cancer

Cell Nucleus
- Structure, Dynamics, and Regulation -



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