Cell Nucleus  
- Structure, Dynamics, and Regulation -

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Organism, Cell, and Molecule

100,000,000,000,000 cells/body
3,000,000,000 bp DNA/cell
1,000,000,000 ATP/cell

Molecular function → Cellular behavior → Property of the organism

 Worlds of Cells and Molecules

1Å 1nm 10nm 100nm 1μm 10μm 100μm 1mm 10mm 100mm 1m

atom protein nucleosome chromatin chromosome nucleus cell body

 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
**Subcellular Compartmentation**

- **Organelle**
  - Membrane-enclosed:
  - Non-membranous:

**Benefits of subcellular compartmentation**

**Nuclear Structure**

- **Sub-nuclear Structures**
  - Nuclear envelope:
  - Nucleoplasmic:

**Activity and Compartment**

**Nucleolar organization: Activity ensures the Compartment**

- **Transcription**
  - FG (TGF1)
  - Restart
  - Reassembly

- **Early processing**
  - DFC (fibrillarin)

- **Late processing**
  - GC (nucleolin)

**Compartmentation is closely related to its activity**
**DNA Packing in the Nucleus**

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

  2000 km
  2 mm
  10 m

**Structural “Unit” of Genomic DNA**

- MNase digestion
  - Random
  - Specific length

Chromosomal DNA contains fundamental unit consist of 146 base pairs

**Nucleosome**

- Nucleosome is made of histone octamer rapping ~146bp DNA

**Higher-Order Structure of the Genome**

- DNA → Histone octamer → Linker histone
- Topol? → Chromosome

How these structures are related to the genome function?
**Heterogenous Packing**

How these different structures are organized?

**Histone Acetylation**

Acetylation neutralize Lysine charge and attenuate histone-DNA interaction

**Histone Modification**

Known histone modifications

Histone tails contain >50 modification sites

**Histone Methylation**

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

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 Code**

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)
(Malignant peripheral nerve sheath tumor)

<table>
<thead>
<tr>
<th>MPNST cell</th>
<th>Normal cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suz12: DNA binding protein induces histone methylation</td>
<td>Methylation (H3K27me3)</td>
</tr>
<tr>
<td>Acetylation (H3K27Ac)</td>
<td>Actin (loading reference)</td>
</tr>
<tr>
<td>(+)Suz12</td>
<td>(+)Suz12</td>
</tr>
</tbody>
</table>

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. Roessler (2014) Nature

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

- **Marker**: attach/detach specific histone modification
- **Leader**: recognize specific histone modification

**RNA-based Regulation**

An RNA-Guided Pathway for the Epigenome

A variety of different types of cells are produced, from identical genome information
Molecular Basis for Dominant/Recessive

Transcriptional level: 
- Gene (A) leads to active protein (A), which results in a phenotype.
- Gene (A) leads to active protein (A), which results in a different phenotype.

Protein level:
- Gene (A) leads to active protein (A), which results in a phenotype.
- Gene (a) leads to active protein (a), which results in a different phenotype.

Chromatin structure is related to the regulation at transcriptional level.

Genes Controlling Cats’ Color

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenytoype</th>
</tr>
</thead>
<tbody>
<tr>
<td>White (all white)</td>
<td>W: white fur grows in entire body (*1) w: fur can be other colors</td>
</tr>
<tr>
<td>(white) Spot</td>
<td>S: white spots s: no spot</td>
</tr>
<tr>
<td>Orange/Black</td>
<td>O: brown (orange) fur o: black fur</td>
</tr>
</tbody>
</table>

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

How can a cat grow both black and brown furs?

Genes Controlling Cats’ Color

<table>
<thead>
<tr>
<th>Gene</th>
<th>W</th>
<th>W-</th>
<th>ww</th>
<th>nw</th>
</tr>
</thead>
<tbody>
<tr>
<td>All white</td>
<td>W</td>
<td>W-</td>
<td>ww</td>
<td>nw</td>
</tr>
<tr>
<td>White spot</td>
<td>S</td>
<td>ss</td>
<td>S-</td>
<td>S-</td>
</tr>
<tr>
<td>Orange/Black</td>
<td>O</td>
<td>oo</td>
<td>oo</td>
<td>OO</td>
</tr>
</tbody>
</table>

Sex Chromosomes

Karyotype

Autosomal chromosomes

Sex chromosomes

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

Male: Sex chromosomes
Female: Functional chromosome

* Cat contains 19 pairs 38 chromosomes

[Reference]
Cats Are Not Peas: A Calico History of Genetics
「三毛猫の遺伝学」(日本語訳)
**X-Chromosome Inactivation**

**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

**Molecular Mechanisms**

Non-coding RNA “Xist” and “Tsx”

“HBiX1” mediates Xist-dependent heterochromatin formation

Xist binds and inactivate X (heterochromatin formation)

Tsx prevents Xist function

Xist and HBiX1 cooperates to inactivate Xi by forming heterochromatin
Mi-ke Must be Female

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

However...

There are some male Mi-ke...

Klinefelter's syndrome

<table>
<thead>
<tr>
<th>Normal gametogenesis</th>
<th>Abnormal division</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X^+) (X^+)</td>
<td>(X^+) (X^+) (+0)</td>
</tr>
<tr>
<td>(XX) (X^+)</td>
<td>(XX) (X^+) (+0)</td>
</tr>
<tr>
<td>(XX) (XX)</td>
<td>(XX) (XX)</td>
</tr>
</tbody>
</table>

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

Nucleocytoplasmic Communication

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

Nuclear Pore Complex (NPC)

AFM observation of NPC (Xenopus egg)

- Cytoplasmic side
- Nucleoplasmic side

EM observation of NPC

- Xenopus
- Drosophila

Brooks SG et al. Structure (2009)

Standard cells contain 3,000~5,000 NPCs, \(\sim5\) NPC/\(\mu\)m\(^2\), \(\sim400\) nm intervals
Selectivity of the NPC

Size-dependent filtration

- Small molecules (~40kDa)
  - Cytoplasm
  - Nucleoplasm

- Large molecules
  - Cytoplasm
  - Nucleoplasm

Karyopherin-mediated transport

- Importin
  - Cytoplasm
  - Nucleoplasm

- Exportin
  - Cytoplasm
  - Nucleoplasm

GFP

- ~30kDa
- Cytoplasm

GFPx3

- ~90kDa
- Cytoplasm

Why Karyopherin can pass through the pore?

Property of the NPC-Permeable Cargos

In vitro Nuclear Transport Assay

Surface Hydrophobicity

Molecular Flexibility

Importin β

bi-ANS FL measurement

Water

55 TFE

10 TFE

Actin-4

45%

55%

100%

Amphiphilic

Dextran

0%

55%

100%

Hydrophilic

Kameoka M. (2019) JCS
Kameoka M. (2012) JCS

Hydrophobic Crowding Model

Amphiphilicity and flexibility are the key features for NPC permeability

Property of the NPC Barrier

Subunit composition and FG-repeats

Nup153 (1200-1435)

SSPFLSAGGQGSSSNNPPFFYFVYQGSSNPVVSS
AFN13ETSSQSLFQSGDLKASIISGTAATFPPQGQP
CGSASSNITTSQGQGATTTTSASGSPVQGTPSAAS
PAGQANTQFTGQGQGQAGQCNPPQFGGQSSTALFTGQGC
QPAPPFTGGVSSSSQPPFQGQGQPQSGAFSSTTNSSS
AFPQGSSSTNNFNTNNSPGQFVPQQANSTTPAQSAQP

Environment inside the pore

- Cytoplasm
  - 101-193
  - Nup

- Nucleoplasm
  - 101-193
  - Nup

Transport Mediator: Catch-and-Release

Steady-state Importin β Dynamics

- Nucleoplasm
- NM
- Cytoplasm

Rate-limiting Steps for Cargo Transport

- RanGTP concentration
- Cargo in the Nucleus (µM)
- Time (min)

kcat (Import-B-Ran)

10 µM
5 µM
1 µM
0.5 µM
0.1 µM
0.05 µM
0.01 µM

kcat (Import-B-Ran)

10 µM
5 µM
1 µM
0.5 µM
0.1 µM
0.05 µM
0.01 µM

kcat (Import-B-Ran)

10 µM
5 µM
1 µM
0.5 µM
0.1 µM
0.05 µM
0.01 µM

Loladó O unpublished
**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!!

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**Nuclear Transport in Cell Signaling**

- **NFκB signaling**
- **Wnt signaling**

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