Introduction

This lecture introduces the nucleus and how information is transferred from stable stored information (DNA) converted to an intermediate (mRNA, rRNA, tRNA) of variable stability, exported from the nucleus to the cytoplasm where mRNA is then translated into Protein. This is gene expression, the products of this process are used either within the cell, exported (exocytosis) or used to replace worn out components.

We will study this topic looking at the key organelle in this process, the nucleus.

Nucleolar Assembly Movie | Movie - nuclear pore complexes (http://jcb.rupress.org/cgi/content/full/154/6/1147/FIG6/DC1)
Objectives

- Understand the concept of the cell nucleus
- Understand the overall structure and components within the nucleus
- Understand the functions of the nucleus
- Brief understanding of chromosomal structure

Eukaryotes

Difference between Prokaryotes and Eukaryotes

- Cytoskeleton
- DNA structure
  - circular, linear
  - Packing (histones)
  - RNA processing (splicing)

Eukaryote Gene Expression

DNA -> mRNA -> Protein

DNA (transcription) -> mRNA (translation) -> Protein (function)

Nucleus

DNA (transcription) -> mRNA Nuclear processing (export)

- DNA -> mRNA splicing (introns removed, exons joined) -> mRNA

Cytoplasm

mRNA (translation by ribosomes) -> Protein (processing)

- Protein Processing cytoplasm (free ribosomes), rough endoplasmic reticulum (bound ribosomes)

Some proteins are returned to the nucleus by a Nuclear Localization Sequence (NLS)

- SV40 Large T-antigen (http://www.plosone.org/article/fetchObject.action?uri=info:doi/10.1371/journal.pone.0010475.g001&representation=PNG_M) - PKKRRKV (single part)
- nucleoplasmin - KR[PAATKKAGQA]KKKK (two part) two clusters of basic amino acids separated by 10 amino acids.

Membrane Evolution

Postulated that an early "coating" structure lead to the infolding of the primitive plasma membrane to form the many membrane covered organelles in the cytoplasm.

These modules may also be the evolutionary precursor to the nuclear pore structures and account for the double membrane that coats the nucleus.

Nuclear Compartment

- Nuclear envelope
- Nuclear cytoskeleton
- Nucleolus
- Chromosome territories
Nucleus Membrane Evolution

- Nuclear envelope and endoplasmic reticulum system
- Euchromatin is a lightly packed form of chromatin (DNA, RNA and protein)
- Heterochromatin is a tightly packed form of DNA
- Interchromatin compartment
- "speckles" interchromatin granule clusters
  - Splicing speckles or SC 35 domains
  - Thought to be sites of storage of mRNA splicing factors
- Nuclear bodies - Cajal and PML

Links: MBOC - A cross-sectional view of a typical cell nucleus (http://www.ncbi.nlm.nih.gov/books/NBK26821/?rendertype=figure&id=A606)

Nucleus Size

- Cell "karyoplasmic ratio" relatively constant (ratio of nuclear volume to cell volume)
  - Most other cellular organelles (ER and mitochondria) can vary greatly in amounts
- Multinucleated fission yeast cells
  - Relative amount of cytoplasm surrounding each nucleus controls the size of individual nuclei


Nuclear Envelope

- Forms structural compartment
- Nuclear envelope two concentric membranes
- Breaks down each mitosis (recycled)
- Outer membrane continuous with Endoplasmic Reticulum (Endoplasmic Reticulum is covered in Lecture 5)
- Contains holes "nuclear pores"
Nuclear architecture
The nuclear cytoskeleton has 2 layers

- **outer** - less organised surrounds nuclear envelope
- **inner** - **nuclear lamina** - thin shell (20 nm) underlying the nuclear envelope
  - associates with both the inner nuclear membrane and underlying chromatin
  - can regulate gene expression
  - provides anchor sites for nuclear pore complexes
  - broken down each cell division
Nuclear Lamins

Intermediate filaments covered in Cytoskeleton Lecture – Intermediate Filaments

- Lamins - Class V intermediate filaments
- Vertebrate lamins are classified into 2 types - A and B
- 10 nm in diameter, forms rope-like networks
- polypeptide form dimers - central alpha-helical regions of two polypeptide chains are wound around each other
- assembly - head-to-tail association of dimers form linear polymers, side-by-side association of polymers form filaments
- B-type - B1 and B2 (586 aa protein, Mr 66,334 Da) lamins are ubiquitously expressed throughout development
- A-type - lamins A and C (Mr 74 kD and 65 kD) lamins in many organisms expression does not appear until midway through embryogenesis (possible role in differentiation)
- lamins phosphorylation state affects nuclear envelope assembly state (dephosphorylation nuclear envelope assembly, phosphorylation nuclear envelope disassembly)
- Lamins also link DNA to nuclear envelope (Lamin B1 interacts with chromatin)

Nuclear lamina and lamina-interacting proteins

Nucleoskeleton to the cytoskeleton complexes

Lamin Abnormalities - (laminopathies) mutations in lamins can lead to human disease (Hutchinson-Gilford Progeria Syndrome)

Nuclear Transport History

1999 Nobel Prize Medicine - Günter Blobel for the discovery that "proteins have intrinsic signals that govern their transport and localization in the cell"


Nuclear Pores
- Protein complex
- External diameter of about 120 nm (30 times the size of a ribosome)
- Channel diameter 25 nm
- Channels between nucleus and cytoplasm (import/export)
- Passive passage of small polar molecules, ions, (<40-60 kDa)
- Active (selective/regulated) passage of larger macromolecules, proteins, and RNAs
- Importin - cytosolic protein
Nuclear Bodies

- Functional compartments

Nucleus Movie 1

Cajal Bodies

![Image of Cajal Bodies](image)

- also called - nucleolar accessory bodies, coiled body, gems
- 0.1 - 2.0 microns, 1-10/ nucleus
- Gems and Cajal bodies two forms of same structure
- GEMS (Gemini of coiled bodies)

- proposed sites where small nuclear ribonucleoproteins (snRNPs) and small nucleolar RNAs (snoRNPs) are modified.
  - snRNPs are particles that combine with pre-mRNA and various proteins to form spliceosomes
  - snoRNAs are a class of small RNA molecules that are involved with modifications of ribosomal RNAs (rRNAs) and other RNA genes

Cajal bodies were first reported in 1903 by the Spanish cytologist/histologist Ramón y Cajal, who christened them "nucleolar accessory bodies".


See also Nature Reviews - The centennial of the Cajal body

PML Bodies

- promyelocytic leukaemia nuclear bodies
- also called PODs, ND10 or Kremer bodies
- Function unknown
- regulation of diverse cellular functions?
- viral infection, cellular transformation, innate immunity, growth control, apoptosis
dynamic hubs sensing stress and DNA damage

**Chromosomes**

- not “visible” at interphase, condense for mitosis (1,000 fold)
- condensation allows chromosomes to move along mitotic spindle without breaking or tangling
- eukaryotes have separate chromosomes
  - Human 23 pairs, 22 autosome pairs, 2 sex chromosomes
- diploid 2 copies of each chromosome (inherited one male/one female)
  - except male sex chromosomes X from mother Y from father
- DNA and protein
- packing of DNA
- DNA structure
- encodes genome (humans 30,000 genes, draft sequence published in 2001)
- DNA genes encode RNA and proteins
- DNA can also encodes nothing

**Chromosome Territories**
Space within the nucleus occupied by individual chromosomes
  ■ Several different models as to how these territories interact
  ■ Intrachromosomal domains possibly RNA processing and transport


Nucleolus

Appearance
  ■ Fibrillar center, dense fibrillar component, and granular component
  ■ Nucleolus changes during the cell cycle:
    ■ during mitosis - nucleolus breaks up as chromosomes condense
    ■ after mitosis - nucleolus reforms from coalesce of tips of 10 chromosomes

Function
  ■ Sites of ribosomal (rRNA) gene transcription, processing, and ribosome assembly
  ■ Nucleolus size depends on cell metabolic activity
  ■ Sites of ribosomal (rRNA) gene transcription, processing, and ribosome assembly
  ■ All cells contain multiple copies of rRNA genes

Links: Movie - Dynamics of nucleolus-derived foci throughout the cytoplasm and the disappearance of NDF during telophase (http://jcb.rupress.org/content/suppl/2000/08/03/150.3.433.F2.DC1) | Movie - Dynamics of nucleolar reassembly in telophase were analyzed by the visualization of fibrillarin-GFP (http://jcb.rupress.org/content/suppl/2000/08/03/150.3.433.F3.DC1)

Chromosome Features

■ 2 telomeres, centromere, replication origins
■ Telomere- at ends of chromosome (bacterial DNA circular)
■ Centromere- holds duplicated DNA together
■ Kinetochore - protein complex forms around the centromere forms during mitosis
■ Chromatin - DNA packed by DNA binding proteins (histones and non-histones) form 30nm DNA fibre
■ 2 types of chromatin in interphase nuclei (based on cytology)
  ■ heterochromatin - highly condensed (restricted gene transcription)
  ■ euchromatin - less condensed (gene transcription)

Telomere
- at ends of all chromosomes (not bacterial DNA circular)
- roles in chromosome replication and maintenance
- replication
  - for replicating the ends of linear chromosomes
- maintenance
  - proposed to provide each cell with a replication counting mechanism that helps prevent unlimited proliferation
- each cell division shortens telomere 50–100 nucleotides
- DNA 100s to 1,000s repeats of a simple-sequence containing clusters of G residues (humans AGGGTT)
- Telomerase enzyme maintains length

**Centromere**

- directs movement of each chromosome into daughter cells every time a cell divides
- centromere embedded in heterochromatin
- satellite DNA sequences (AT-rich) repeated many thousands of times
- proteins assemble on this to form Kinetochore
  - attachment site for spindle microtubules

**Links:** MBOC - Centromere (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?&rid=mboc4.figgrp.672)

**Replication Origins**
DNA replication initiates at multiple origins (ori)
- in both prokaryotic and eukaryotic DNA
- multiple origins in eukaryotes (human genome about 30,000 origins)
- each origin produces two replication forks (moving in opposite directions)

Chromosome DNA Packing

Euchromatin ("good chromatin") - light, transcriptionally active, about 10% of all chromatin.

Heterochromatin - condensed, transcriptionally inactive, about 90% of all chromatin.

Nucleosomes
- formed by DNA wrapped around histones
- unit particle of chromatin (nucleosomal histones) (discovered 1974)
- EM unfolded DNA has "beads on a string" appearance
- second order folding forms 300 nm fibre
- condensed DNA for mitosis 700 nm fibre

Histones
- only in eukaryotes
- small proteins positively charged (binds negatively charged DNA)
- not sequence specific binding (as in transcription factors)
- 4 core histones (H2A, H2B, H3, and H4)
- 2 linker histones (H1/H5)

Abnormalities

Hutchinson-Gilford Progeria Syndrome
- In more than 80% of cases the gene defect responsible for HGPS is a single spontaneous mutation in codon 608 of the LMNA gene, which encodes both lamin A and lamin C
- single-base substitution in exon 11 that reveals a cryptic splice site in the LMNA gene thus producing a truncated protein
- progerin is a mutant form of the nuclear architectural protein lamin A

**Emery-Dreifuss Muscular Dystrophy**


Loss of a-Type Lamin Expression Compromises Nuclear Envelope Integrity Leading to Muscular Dystrophy (http://jcb.rupress.org/cgi/content/abstract/147/5/913)

**Eukaryote Gene Expression**

DNA -> mRNA -> Protein
- DNA (transcription) -> mRNA (translation) -> Protein (function)
- DNA -> mRNA splicing (introns removed, exons joined) -> mRNA
- DNA -> rRNA, tRNA, siRNA (RNA interference (RNAi) pathway)

**Nucleus** DNA (transcription) -> mRNA Nuclear processing (export) **Cytoplasm** mRNA (translation) -> Protein (cytoplasm, rough endoplasmic reticulum)

**Protein Modification**

Protein - cytoplasmic (free ribosomes), rough endoplasmic reticulum (bound ribosomes)

**Exocytosis**

Rough Endoplasmic Reticulum -> transport vesicle -> Golgi apparatus -> secretory vesicle

**History**

Below are some example historical research finding related to cell junctions from the JCB Archive (http://jcb.rupress.org/misc/fromthearchive.shtml) and other sources.

1961 The nucleolar origin of rRNA (http://jcb.rupress.org/cgi/content/full/168/4/524-a)
Base compositions and half-lives suggest to Jan-Erik Edström that the nucleolus is the source of rRNA.

**References**


**Textbooks**

Search Online Textbooks


Books

- The Cytoskeleton - cellular architecture and choreography (http://books.google.com/books?id=JNHuxHzTm7IC)

Reviews

Selma Osmanagic-Myers, Thomas Dechat, Roland Foisner Lamins at the crossroads of mechanosignaling. Genes Dev.: 2015, 29(3);225-37 PubMed 25644599


Roderick Y H Lim, Ueli Aebi, Birthe Fahrenkrog Towards reconciling structure and function in the nuclear pore complex. Histochem. Cell Biol.: 2008, 129(2);105-16 PubMed 18228033

Articles


L Yang, T Guan, L Gerace Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis. J. Cell Biol.: 1997, 137(6);1199-210 PubMed 9182656

Acronyms

- AA - amino acid
- DNA - deoxyribonucleic acid
- EM - electron microscopy
- FL - fluorescent
- GEMS - Gemini of coiled bodies
- INM - inner nuclear membrane
- KASH - Klarsicht/ANC-1/Syne-1 homology domain–containing protein
- LEM domain - fold identified in LAP2, emerin, and MAN1 confers direct binding to dsDNA
- LINC - nucleoskeleton to the cytoskeleton complexes
- mRNA - messenger RNA
- NE - nuclear envelope
Nuclear pore complexes are fixed in place Daigle et al. (http://jcb.rupress.org/cgi/content/abstract/154/1/71) report that nuclear pore complexes (NPCs) undergo limited movements (http://jcb.rupress.org/cgi/content/full/200101089/F4/DC1) that match the deformations of the nuclear envelope as tracked using a grid (http://jcb.rupress.org/cgi/content/full/200101089/F4/DC2) of bleached nuclear lamins. NPCs are therefore remarkably stable complexes, and are probably anchored to a protein network in the nuclear envelope.

Nucleoporins reassemble around post-mitotic chromatin A conserved nuclear pore subcomplex was characterized and tracked by Belgareh et al. (http://jcb.rupress.org/cgi/content/abstract/154/6/1147), who found that the proteins were recruited (http://jcb.rupress.org/cgi/content/full/154/6/1147/FIG6/DC1) during telophase in a rim pattern surrounding the chromosomes. A low level of staining was also apparent on the kinetochores throughout mitosis.

Nucleolar re-formation after mitosis Savino et al. (http://jcb.rupress.org/cgi/content/abstract/153/5/1097) follow the re-formation (http://jcb.rupress.org/cgi/content/full/153/5/1097/F3/DC1) of nucleoli after mitosis. Prenucleolar bodies (PNB) form on the chromosome surface and nucleolar material flows along links between PNBs (http://jcb.rupress.org/cgi/content/full/153/5/1097/F4/DC2) and towards (http://jcb.rupress.org/cgi/content/full/153/5/1097/F6/DC1) a developing nucleolar organizer region (http://jcb.rupress.org/cgi/content/full/153/5/1097/F4/DC1) (NOR). Eventually this leads to the fusion (http://jcb.rupress.org/cgi/content/full/153/5/1097/F9/DC1) of nucleoli to form a single entity.

Processing complexes may help reassemble nucleoli Nucleolar reassembly (http://jcb.rupress.org/cgi/content/full/150/3/433/F3/DC2) during telophase is shown by Dundr et al. (http://jcb.rupress.org/cgi/content/abstract/150/3/433) to require mitotically preserved processing complexes.

Speckles - A splicing factor has limited mobility Based on the limited mobility (http://jcb.rupress.org/cgi/content/full/150/1/41/F1/DC1) of a splicing factor, Kruhlak et al. (http://jcb.rupress.org/cgi/content/abstract/150/1/41) determine that the factor undergoes frequent but transient interactions with relatively immobile nuclear binding sites, both when associated with speckles and when dispersed in the nucleoplasm. This a 3-D video that should be viewed using red/green 3-D glasses.

2016 Course Content


Laboratories: Introduction to Lab | Microscopy Methods | Preparation/Fixation | Cell Knockout Methods | Cytoskeleton Exercise | Immunohistochemistry | Project Work | Confocal Microscopy | Tissue Culture | Stem Cells Lab | Microarray Visit

2016 Projects:

Dr Mark Hill 2015, UNSW Cell Biology - UNSW CRICOS Provider Code No. 00098G


Categories: Nucleus | Organelle | Science-Undergraduate | 2016ANAT3231
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