The Cell Nucleus

1. The cell nucleus – structural components:
   - nuclear envelope
   - chromatin – sex chromatin (Barr body)
   - nucleolus
   - nuclear matrix (nucleoplasm)

2. Chromosomes – structure and composition

3. Karyotype, chromosomal alterations

4. Structure and replication of DNA, genome
Membranous organelles

- Endoplasmic reticulum
- Annulate lamellae
- Mitochondria
- Golgi apparatus
- Lysosomes
- Proteasomes
- Secretory vesicles
- Transport vesicles
- Peroxisomes
- Coated vesicles
- Nucleus
Membrane-Limited Compartment

- **nucleus**
- genetic material container
- cell division
- cell differentiation

- Golgi apparatus
- Secretory vesicles
- Centrioles
- Rough endoplasmic reticulum
- Fat droplets
- Nucleolus
- Mitochondria
- Nuclear envelope
- Lysosomes
- Smooth endoplasmic reticulum

Prof. Dr. Nikolai Lazarov
- R. Brown, 1831 - (Lat. *nucleus*, kernel; Gr. *karyon*, nut)
- in all eukaryotic cells – with exception of Er
- number – uninuclear, binuclear, multinuclear cells
- number of chromosomes – haploid, diploid, polyploid (tetra-, octa- etc.), aneuploid
- localization – centrally or peripherally
- **external morphology:**
  - shape – species-diversified – spherical, egg-shaped, rod-like, kidney-shaped, segmented etc.
  - size – 10% of the cell volume; 5 µm (spermatozoon), 40 µm (oogonium)
Nucleus – structure

- Structural components:
  - nuclear envelope
  - chromatin
  - nucleolus
  - nuclear matrix (nucleoplasm)

The Cell Nucleus

- Outer membrane
- Inner membrane
- Nucleoplasm
- Nucleolus
- Chromatin
- Nuclear envelope
- Pore in nuclear envelope
Nuclear envelope

- First description: M. Watson, 1955
- Ultrastructure:
  - outer nuclear membrane – 6 nm
    - ribosomes ⇄ rER
    - vimentin filaments
  - inner nuclear membrane – 6 nm
    - nuclear lamina – 100-300 nm
      - lamins A, B, C ⇄ chromatin
  - perinuclear space – 10-40 nm
  - nuclear pores
Prof. Dr. Nikolai Lazarov

Nuclear envelopopathies

- **Nuclear envelopopathies** (2000):
  - diseases associated with defects of the nuclear envelope

- **Laminopathies**: first reports in the late 1990s
  - a group of rare genetic disorders caused by mutations in genes encoding proteins of the nuclear lamina

- **Emery-Dreifuss muscular dystrophy**
  - named after Alan Emery (1928-) and Fritz Dreifuss (1926-1997)
  - mutations in the gene EMD for the protein emerin and gene LMNA for two similar proteins lamin A and lamin C
# Nuclear envelopathies

<table>
<thead>
<tr>
<th>Disease</th>
<th>Form of inheritance</th>
<th>Clinical phenotype</th>
<th>Mutated NE protein</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Emery-Dreifuss muscular dystrophy</td>
<td>X-linked</td>
<td>Slowly progressing contractures and muscle weakness, wasting of skeletal muscle and cardiomyopathy</td>
<td>Emerin</td>
<td>22, 24, 25</td>
</tr>
<tr>
<td>Emery-Dreifuss muscular dystrophy</td>
<td>Autosomal dominant</td>
<td>Slowly progressing contractures and muscle weakness, wasting of skeletal muscle and cardiomyopathy</td>
<td>Lamin A/C</td>
<td>29</td>
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<tr>
<td>Limb girdle muscular dystrophy</td>
<td>Autosomal dominant</td>
<td>Slowly progressive pelvic girdle weakness, later development of contractures and cardiac disturbances</td>
<td>Lamin A/C</td>
<td>32, 34</td>
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<tr>
<td>Dilated cardiomyopathy with conduction system disease</td>
<td>Autosomal dominant</td>
<td>Dilated cardiomyopathy</td>
<td>Lamin A/C</td>
<td>33, 36</td>
</tr>
<tr>
<td>Charcot-Marie-Tooth</td>
<td>Autosomal recessive</td>
<td>Motor and sensory neuropathy, diabetes</td>
<td>Lamin A/C</td>
<td>37, 38</td>
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<tr>
<td>Mandibuloacral dysplasia</td>
<td>Autosomal dominant</td>
<td>Postnatal growth retardation, craniofacial anomalies, skeletal malformations, mottled cutaneous pigmentation</td>
<td>Lamin A/C</td>
<td>41</td>
</tr>
<tr>
<td>Dunnigan-type familial partial lipodystrophy</td>
<td>Autosomal dominant</td>
<td>Subcutaneous fat loss, adipose tissue accumulates (face and neck), insulin resistance and diabetes</td>
<td>Lamin A/C</td>
<td>42, 43</td>
</tr>
<tr>
<td>Hutchinson-Gilford progeria syndrome</td>
<td>De novo mutation</td>
<td>Premature aging, dwarfism, alopecia, craniofacial disproportion, delayed tooth formation, aged-looking skin, osteoporosis and joint problems. Early death due to atherosclerosis and cardiovascular disease.</td>
<td>Lamin A/C</td>
<td>47, 52</td>
</tr>
<tr>
<td>Atypical Werner's syndrome</td>
<td>Autosomal recessive</td>
<td>Scleroderma-like skin, cataract, subcutaneous calcification, premature arteriosclerosis, diabetes mellitus, prematurely aged facies</td>
<td>Lamin A/C</td>
<td>55</td>
</tr>
<tr>
<td>Pelger-Huët anomaly</td>
<td>Autosomal dominant</td>
<td>Hypolobulated neutrophil nuclei, coarse chromatin, varying degrees of developmental delay, epilepsy and skeletal abnormalities</td>
<td>Lamin B receptor</td>
<td>58</td>
</tr>
<tr>
<td>Greenberg skeletal dysplasia</td>
<td>Autosomal recessive</td>
<td>Lethal course, fetal hydrops, short limbs, abnormal chondro-osseous calcification</td>
<td>Lamin B receptor</td>
<td>60</td>
</tr>
<tr>
<td>Restrictive dermopathy</td>
<td>Autosomal recessive</td>
<td>Early neonatal lethal course. Intrauterine growth retardation, tight and rigid skin with erosions, mineralization defects of the skull, pulmonary hypoplasia.</td>
<td>Lamin A/C</td>
<td>61</td>
</tr>
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<td>Restrictive dermopathy (RD)</td>
<td>Autosomal recessive</td>
<td>Early neonatal lethal course. Intrauterine growth retardation, tight and rigid skin with erosions, mineralization defects of the skull, pulmonary hypoplasia.</td>
<td>ZMPSTE24 (FACE-1)</td>
<td>61</td>
</tr>
</tbody>
</table>

Nuclear envelopathies represent a group of inherited diseases that arise through mutations in genes encoding proteins of the nuclear lamina. In each disease, the form of inheritance, clinical phenotype, the mutated NE protein, and citations are presented.
Nuclear pore complex

- **Denomination:** M. Watson, 1959
- **Nuclear pore:**
  - total number – 3000-5000
  - length – 70-80 nm
- **Nuclear pore complex:**
  - average diameter – 130 nm
  - 100 proteins (nucleoporins)
  - 3 rings x 8 subunits:
    - cytoplasmic ring ⇒ filaments
    - luminal – internal and external
    - intranuclear ⇒ “nuclear cage”
  - diaphragm – 9 nm
  - central granule
- **First description**: W. Flemming, 1882

- **Heterochromatin** (condensed) – 90%
  - Gr. *heteros*, other + *chroma*, color:
    - marginal chromatin – nuclear membrane
    - chromocenters – nucleoplasm
    - nucleolar-associated chromatin:
      - perinucleolar
      - intranucleolar
  - constitutive heterochromatin – inactive, around the chromosome centromere and near telomeres
  - facultative heterochromatin ⇒ euchromatin and sex chromatin

- **Euchromatin** (extended) – 10%
  - Gr. *eu*, good, true:
    - a lightly packed form of chromatin (DNA, RNA and protein)
    - comprises the most active portion of the genome within the cell nucleus – replication and transcription
synonym: **Barr body** – only in females
Barr, Bertram, 1949
✓ tightly packed inactive X chromosome
✓ localization:
  ➢ adhering to the nuclear envelope
  ➢ “drumstick-like” appendage to the nuclei

**Medical application:**
✓ diagnostics in endocrinology
✓ forensic medicine practice
✓ study of inherited chromosome anomalies
  – Klinefelter and Turner syndromes etc.
✓ disclosure of the genetic sex
  – in hermaphroditism and pseudohermaphroditism
Chromatin composition

- **Composition:**
  - coiled strands of DNA
  - proteins:
    - non-histone proteins (20%) – acid proteins ⇒ nuclear protein matrix
    - histones (80%) – basic proteins only in the nucleus
      - H1 – keeping in place the wrapped DNA, binds to the "linker DNA"
      - H2A, H2B, H3, H4 – nucleosome core

- **Nucleosome (U-body):**
  - basic structural unit of chromatin
  - core of histone octamers (10-11 nm)
  - two loops of DNA (2 nm) – 146 base pairs
seven levels of chromatin organization:

- DNA double helix – 2 nm
- nucleosome
- “beads-on-a-string” form:
  - nucleosome particles – 11 nm
  - stretches of linker DNA
- solenoid model – 30 nm fiber:
  - condensed chromatin threads
- extended chromatin fibers – 300 nm
- condensed chromosome – 700 nm
- metaphase chromosome – 1400 nm
**Nucleolus**

- **First description:** Fontana, 1774

- **Number:** 1- max. 10:
  - metabolically active cells
  - embryonic cells
  - during proliferation
  - rapidly growing malignant tumors

- **External morphology:**
  - non-membrane bound structure
  - shape – spherical; compact, reticular, annular etc.
  - diameter – 1-3 µm

- **Formation:**
  - nucleolar organizers – short arms of chromosomes 13, 14, 15, 21 and 22 (the acrocentric chromosomes)
Nucleolus – structure

Electron microscopically: three distinct components

- **Fibrillar center** – pale-stained region:
  - fine filaments
  - RNA polymerase I $\Rightarrow$ transcription of rRNA

- **Fibrillar part, pars fibrosa:**
  - nucleolonema – 5-10 nm fibers
  - newly synthesizes rRNA

- **Granular part, pars granulosa:**
  - ribonucleoprotein particles – 15-20 nm granules
Nuclear matrix (nucleoskeleton)

- **synonyms**: nucleoplasm, karyoplasm
- **a highly dynamic structure**
- **Composition – amorphous**:
  - proteins + RNA – nucleoskeleton
  - nuclear lamina – lamins A, B, C, actin, emerin, nesprin-1 and -2
  - numerous enzymes
  - metabolites
  - ions
  - crystalline inclusions
  - viruses and
  - other inclusions
Chromosomes

- Denomination: W. von Waldeyer, 1888

- Definition:
  - a very long DNA molecule (that contains many genes) and associated proteins, carrying portions of the hereditary information of an organism

- Total number in humans – 46 (2n) (23 homologous pairs):
  - 22 pairs of autosomes (1-22)
  - 1 pair of gonosomes (sex chromosomes) – X and Y

- Size:
  - length – 0.1 to 30 µm (average 3-8 µm) (51 million to 245 million base pairs)
  - thickness – 0.5-2 µm
  - single DNA molecule – 1.7-8.5 cm
  - total length – 1.7 m
Structure:

- **arms** – long and short
- **primary constriction**, **centromere** – microtubule organizing center (Gr. *kentron*, center + *meros*, part)
- **kinetochore** (Gr. *kinetos*, moving + *chora*, central region)
  - single = S-chromosomes (chromatids)
  - double = D-chromosomes
- **telomeres**
- **secondary constriction**, **satellites** (nucleolar organizers)

Types of chromosomes – according to centromere position:

- **metacentric**
- **submetacentric**
- **acrocentric – satellites**
  - 5 pairs (13, 14, 15, 21, 22)
- **telocentric**
Cytogenetics – chromosome (genetic) diseases

Human karyotype:
- number of chromosomes
  - polyploidy
  - aneuploidy
- size
- structure

Metaphase chromosomes:
- staining – banding technique; A-T (G bands) and G-C (R bands)

Denver classification, 1960:
- 7 groups autosomes (A-G)
- sex chromosomes or gonosomes (X and Y)
Chromosomal anomalies

- Definition: any change in the normal structure or number of chromosomes

- Numerical abnormalities:
  - aneuploidy (an abnormal number of chromosomes)
    - monosomy
      - Turner syndrome
    - trisomy, tetrasomy etc.
      - trisomy 21 (Down syndrome)
      - trisomy 18 (Edward syndrome)
  - polyploidy

- Structural abnormalities:
  - deletions (loss of chromosomal material)
  - duplications (gain of chromosomal material)
  - translocations (transfer of a portion from one to another chromosome)
  - inversions and rings (re-arrangement of the genetic material)

- Mosaicism:
  - the presence of two populations of cells with different genotypes in one individual
  - chromosomal change is only in some cells of the body
DNA – The Matrix of Life
Structure of DNA

- **DNA**: a nucleic acid that contains the genetic information (genes)

- **Chemical composition:**
  - long polymers of nucleotides:
    - phosphate group
    - deoxyribose - five-carbon sugar
    - nitrogenous bases
      - purine – adenine and guanine
      - pyrimidine – thymine and cytosine

- **Structure:**
  - arranged in the form of a double helix
  - two anti-parallel strands
    - it looks like a twisted ladder:
      - the sides of the ladder are composed of nucleotides
      - the rungs of the ladder are bonds between the bases where adenine only forms a bond with thymine, and guanine with cytosine

"for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material"

The Nobel Prize in Physiology or Medicine 1962
The Largest Human DNA Helix
DNA replication: a fundamental process occurring in all living organisms to copy their DNA. DNA replication is the basis for biological inheritance.

A SUMMARY OF DNA REPLICATION:

1. Helicases unwind the parental double helix.
2. Single-strand binding proteins stabilize the unwound parental DNA.
3. The leading strand is synthesized continuously in the 5’ → 3’ direction by DNA polymerase.
4. The lagging strand is synthesized discontinuously. Primase synthesizes a short RNA primer, which is extended by DNA polymerase to form an Okazaki fragment.
5. After the RNA primer is replaced by DNA (by another DNA polymerase, not shown), DNA ligase joins the Okazaki fragment to the growing strand.

Overall direction of replication.
Mutations in DNA

- changes in the DNA sequence of a cell's genome

- Mutation causes:
  - radiation, viruses, transpoisons and mutagenic chemicals
  - errors that occur during meiosis or DNA replication

- Mutation types:
  - lethal mutations
  - loss-of-function mutations
  - silent mutations etc.

![Diagram of DNA mutations](image-url)
The Nobel Prize in Chemistry 2015
Tomas Lindahl, Paul Modrich, Aziz Sancar

The Nobel Prize in Chemistry 2015 was awarded jointly to Tomas Lindahl, Paul Modrich and Aziz Sancar "for mechanistic studies of DNA repair".
Human genome

- Human genome: the genome of *Homo sapiens*, which is stored on 23 chromosome pairs

- Genome:
  - the entirety of an organism's hereditary information
  - includes both the genes and the non-coding sequences of the DNA

- Genes:
  - the basic units of heredity in a living organism
  - a portion of DNA that contains both "coding" sequences that determine what the gene does, and "non-coding" sequences that determine when the gene is active (expressed)

- Genetic code:
  - the set of rules by which a gene is translated into a functional protein
Ask your genome

Thank you...

Prof. Dr. Nikolai Lazarov