BME 42-620 Engineering Molecular Cell Biology

Lecture 16:
DNA Packaging
Structure of Cell Nucleus

Chapter 4
Course Administration Notes

• Reading assignment 4
  - Presentation format
  - Presentation scheduled on Nov-10, 2011
    Group 5: Jackie Chen, Jaclyn Brackett, Pitirat Pholpabu
    Group 6: Simone Costa, Christine Bronikowski, James Rockwell

• Lecture on Nov-08 cancelled. A make-up lecture will be scheduled.
Outline

• DNA packaging and structural organization
• Overview of kinetochore structure and functions
• Overview of cell nucleus structure
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The Problem: DNA Storage and Access

- Spacing between base pairs $\approx 3.4\text{Å}$
- For human genome, approximately 3.2 billion base pairs
- Total length $\approx 3.4\times10^{-10}\times3.2\times10^{9}\times2 \approx 2.2\text{m}$
- Diameter of a nucleus: $5\sim10\times10^{-6}\text{m}$
- Access to genetic information must be provided and regulated.
Chromosome Organization in the Cell Cycle

- DNA are packaged with associated proteins into chromosomes.
- Chromosomes undergo dramatic reorganization when cells divide.
Organization of Human Chromosomes (III)

- Much (40~50%) of non-protein-coding DNA in the human genome is transcribed into RNA.

- Coding regions are usually unique.

- Eukaryotic genomes often contain large numbers of repetitive DNA sequences that are present in many copies.
  - Transposons
The Problem: DNA Storage

- Long sequence of DNA must be stored within the geometry of a nucleus
  - Example: human chromosome 22, 48 million bp
  - Extends to length of ~1.5 cm
  - Measures 2 μm in mitosis
  - Packaging ratio on the level of $10^4$ in mitosis
  - Packaging ratio ~500 in interphase

- Packaged DNA must provide controlled access to regions required for gene expression.
The Solution: Multiple Levels of DNA Packaging

![Diagram of DNA packaging levels](image)

**NET RESULT:** EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH
Packaging of DNA into Nucleosomes

- DNA is coiled around a protein core to form nucleosomes: 142 hydrogen bonds.
- ~7 folds in packaging.
- Histone H2A, H2B, H3, H4 with 147 bp DNA.
- Nucleosomes repeat at every 200 bp.
  So ~30 million nucleosomes in a human cell.
- Total mass of histones approximately equal to that of DNA.
Histone Organization

- N-terminal tail is subject to different forms of modification.

- H2A & H2B form a dimer through handshaking.

- H3 & H4 form a dimer in a similar fashion and then a tetramer.

- Extensive interactions between histones and DNA
Dynamics of the Nucleosome

• Nucleosomes are dynamic.

• Eukaryotic cells have a large variety of ATP-dependent chromatin remodeling complexes.
Second level of DNA Packaging: 30-nm Fibre

- Chromatin structure beyond nucleosomes is generally less well understood.
- Nucleosomes are further packaged into 30-nm fibers.
- The precise structure of the 30-nm fiber is not yet known.
- ~40 folds in packaging.
Regulation of Chromatin Structure (I)

- Certain types of chromatin structures can be inherited.
- This is one form of epigenetic inheritance.
- Epigenetic information is usually but not always erased during the formation of eggs and sperms.
Regulation of Chromatin Structure (II)

- **Heterochromatin vs euchromatin**
  - Heterochromatin refers to a highly condensed region of a chromosome that is generally inactivated for transcription.
  - Euchromatin refers to a uncondense region of a chromosome that is active for transcription.
Regulation of Chromatin Structure (III)

- Core histones can be covalently modified at different sites.
- All these modifications are reversible.
- Such modifications enable the recruitment of specific regulatory proteins.

![Diagram of Lysine Acetylation and Methylation](image)

![Diagram of Serine Phosphorylation](image)

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The Histone Code Hypothesis

• The hypothesis: Covalent modifications and histone variants of histones encode specific controls of gene expression.
Higher Order Structure of Chromosomes (I)

- Two different cases:
  - interphase chromosomes
  - and mitotic chromosomes.

- Different organization configurations:
  - loops and band
  - Condensation

- Higher order structures are also actively regulated for control of gene expression.
Higher Order Structure of Chromosomes (II)

- Organization of mitotic chromosome involves condensation.
Mitotic Chromosome Scaffold Protein: SMC

- Condensation of mitotic chromosomes is mediated by condensin, a large protein complexes built from SMC dimers.

- SMC protein: structural maintenance of chromosome.

- Cohesin: holds sister chromatids together; cleaved by separase.

- Hundreds of other chromosome scaffold proteins with unknown functions.

Hirano T, Genes Dev. 13:11-19, 1999
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Kinetochore (I)

- Kinetochore becomes visible in mitosis.

Kinetochore (II)

• Embedded in the surface of centromere.
• Kinetochores assemble in prophase; dissemble after mitosis.
• Primary functions
  - Directs chromosome movement
  - Regulating microtubule dynamics
  - Form signaling pathways to regulate cell cycle
• Fibrous corona is detected on unattached kinetochores.

**Kinetochore (III)**

- Kinetochore is a very large protein assembly.

- At least 70 kinetochore associated proteins have been identified in budding yeast.

- Its structure is likely to be fully solved in the near future.

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The Cell Nucleus

• The cell nucleus is the largest organelle in a eukaryotic cell.

• This compartmentation serves many purposes.
Structure of the Cell Nucleus

- The cell nucleus is organized into multiple subdomains:
  - nuclear membrane
  - subnuclear organelles
The nuclear envelope breaks down during mitosis and reassembles upon exit.
Traffic Between Nucleus and Cytoplasm

- Traffic between nucleus and cytoplasm goes through the nuclear pore complex and is controlled by different signal sequences.

- Proteins imported into the nucleus bear a nuclear localization sequence (NLS: PKKRRKV).

- Proteins exported from the nucleus bear a nuclear export sequence (NES: LQLPPLERLTL).

- Immature RNAs bear a nuclear retention signal (RNS).
Structural Compartmentation of the Nucleus

• Individual chromosomes tend to concentrate within discrete territories with limited intermingling.

• Chromosomes active in transcription (euchromatin) tend to concentrate to the middle of the nucleus.

• Chromosomes inactive (heterochromatin) in transcription tend to concentrate at the periphery.
Questions ?