The Mitotic Spindle is a Protein Machine whose moving parts are made of dynamic microtubules and mitotic motors which generate pN scale forces to move chromosomes over μm scale distances with high fidelity.

This machine works in the noisy stochastic cellular nanoworld where thermal fluctuations play a dominant role in counteracting order and randomizing movements – how does it, and other protein machines, work so well in this environment?

This class aims to address this question.

The history of mitosis research also illustrates how understanding the molecular biology of subcellular processes proceeds from descriptive via molecular to physical approaches.
The Mitotic Spindle – a Protein Machine that drives Chromosome segregation.
History of Mitosis Research.

A. Discovery of Mitosis and Chromosomal Basis of Heredity (19th Century).

B. Classical Mechanics of Mitosis; the spindle as a force-generating machine (1950-1990)

C. Molecular Biology of Mitosis - molecular components and biochemical mechanism (1960-present).

(A) Discovery of Mitosis.

1. The principles of Heredity

2. The principle of Evolution by Natural Selection.

3. The Cell Doctrine.

1805: Lorenz Oken, Professor of Anatomy at University of Zurich; all organisms made of *infusoria* (cells) that multiply and differentiate.

1840: Cell Theory formalized by Zoologist Theodor Schwann and Botanist Matthias Schleiden;

-all organisms made of cells
-cells are fundamental units of all organisms
-how do they arise?

Mitosis discovered in context of origins of cells by cell division and chromosomal theory of heredity.
Discovery of Cell Division.

Leeuwenhoek; Trembley; Spallanzani (1700-1780); Cell Division.

Robert Remak, (1844-53); Nuclear division.
Chromosomes and Mitosis.

1876: Balbiani and Van Beneden saw “rods” separating into nuclei.
Mitosis and Chromosome theory of Heredity:

1. Van Beneden (1885): chromosome number and morphology is species-specific.
2. Boveri (1905): each chromosome is distinct and carries specific part of genome.
3. T.H. Morgan (1910): specific chromosomes (X) carry specific genes (Red eye).

-Mitosis segregates genes on chromosomes.
-Mechanism? Role of Spindle Fibers?
(B) The Spindle as a Machine.
Spindle fiber dynamics, sliding filaments and spindle forces.

Reality of the Spindle Fibers?

Advocates:
1. Flemming and others; spindle fibers are contractile elements.
2. Dujardin, 1835, cytoplasm full of contractile fibers made of “sarcode”.
3. Franz Unger, 1832, intracellular particle transport (blocked by EtOH) observed in pollen tubes. Driven by contractile fibers.
4. Belar, 1929 (and later Bajer) saw vigorous particle transport along spindle fibers.
5. Druner, 1894; spindle fibers contract to pull chromosomes to poles and expand to push poles apart.
6. Ris, 1949; found that 0.5% chloral hydrate blocks spindle elongation but not chromatid-to-pole motility. Defined anaphase A and anaphase B.

Critics:
However, E.B. Wilson and many others questioned the reality of the spindle fibers. E.g. could a “force field” move chromosomes and deform cytoplasm like a magnetic field acting on Fe filings. Formed passively. Fixation artifact.
1952: Mazia and Dan; mass isolation spindles SU eggs.

Spindle fibers, chromosomes and asters = coherent physical entity (But did not contribute much to spindle biochemistry).
1953: Shinya Inoue uses polarizing light microscopy

Steady state fibers polymerize at equator; depolymerize at poles.

Polymerize/push; depolymerize/pull.
E.M. analysis by McIntosh et al, 1969-80: Sliding Filaments
**Force Generation by Spindle Fibers.**

Ostegren, 1951; Force balance.

1. Antagonistic poleward forces.
2. Force proportional to distance from pole.
3. Forces balance at metaphase.
4. Poleward forces drive anaphase A.

Nicklas, 1983; 1 nN stall force.
(C) Molecular Mechanisms of Mitosis;

1. Discovery of Tubulin.
   - 1967, Borisy and Taylor purified tubulin as a $H^3$-colchicine binding protein.
   - 1972, Bob Weisenberg, tubulin assembly into MTs in vitro using 0.1M PIPES + EGTA.

2. Discovery of MT-based motors proteins.
   - 1963; Gibbons purifies dynein an ATPase from cilia.
   - 1985; Vale, Reese and Sheetz purify kinesin from bovine brain and squid axons.

Allowed study of spindle MT dynamics and mitotic motors.
**Dynamic Instability.**
(Mitchison and Kirschner, 1984).

**Microtubule Treadmilling.**
(Margolis and Wilson, 1978).

**Kinetochore motility - Pacman versus poleward flux;**
(Salmon and Mitchison et al, 1980s- present).
Purification of Mitotic Motors: Kinesins in Sea Urchin Embryos.


Clarification of the roles of motor proteins in mitosis and cell division may be complicated by their number and variety. Wright et al, 1993 suggest that some kinesin-like proteins may have essential mitotic functions, but kinesin itself does not.
Mitosis in *Drosophila* embryos.

- Biochemistry and inhibitor microinjection.
- Cytology of specific proteins in transgenic embryos.
- Mutants and sequenced genome.

- **Microtubule Polymer Ratchets:**
  - Polymerizing/pushing.
  - Depolymerizing/pulling.

<table>
<thead>
<tr>
<th>Motor Protein</th>
<th>Locus</th>
<th>Function (?)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasmic Dynein</td>
<td>64C</td>
<td>Pole/chromosome motility.</td>
</tr>
<tr>
<td>Bipolar Kinesin (KLP61F)</td>
<td>61F</td>
<td>MT crosslinking-sliding. motor.</td>
</tr>
<tr>
<td>C-terminal Kinesin (Ncd)</td>
<td>99C</td>
<td>MT crosslinking-sliding motor.</td>
</tr>
<tr>
<td>KLP67A</td>
<td>67A</td>
<td>Astral pole motor.</td>
</tr>
<tr>
<td>PAV-KLP</td>
<td>64B</td>
<td>Interzone.</td>
</tr>
<tr>
<td>KLP3A</td>
<td>3A</td>
<td>Chromokinesin/interzone.</td>
</tr>
<tr>
<td>KLP31D/E</td>
<td>31D/E</td>
<td>Chromokinesin.</td>
</tr>
<tr>
<td>KLP38B</td>
<td>38B</td>
<td>Chromokinesin.</td>
</tr>
<tr>
<td>CENP-meta</td>
<td>32E</td>
<td>Kinetochore motor.</td>
</tr>
<tr>
<td>CENP-ana</td>
<td>32E</td>
<td>Kinetochore motor.</td>
</tr>
<tr>
<td>KLP10A</td>
<td>10A</td>
<td>Spindle poles.</td>
</tr>
<tr>
<td>KLP59C</td>
<td>59C</td>
<td>Kinetochores.</td>
</tr>
</tbody>
</table>
The bipolar kinesin KLP61F drives MT-MT sliding.

The Kin-I kinesins, KLP10A and KLP59C depolymerize MTs.
Mechanism of Anaphase A

Metaphase

Anaphase A

Anaphase B
Mechanism of Anaphase B

Metaphase

Anaphase A

Anaphase B
Anaphase B Spindle Elongation

1. A motor-dependent sliding filament mechanism generates force to push the poles apart.
2. Poleward flux acts as a regulatory switch.

Model: Equations describing the dynamics of Anaphase spindle elongation.

1. \( \frac{dS}{dt} = 2 (v_{\text{speckle}} - v_{\text{flux}}) = 2 (v_{\text{sliding}} - v_{\text{flux}}) \)

2. \( \frac{dL}{dt} = 2 (v_{\text{polymerization}} - v_{\text{sliding}}) \)

3. \( \mu \cdot \frac{dS}{dt} / 2 = k \cdot N \cdot L \cdot F_m (1 - \frac{v_{\text{sliding}}}{V_m}) \)

Unknown variables:
- \( S(t) \)
- \( L(t) \)
- \( v_{\text{sliding}} \)
CONCLUSIONS.

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