Forces in the mitotic spindle

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products of cellular metabolism. In addition, he was interested in the involution of adipose tissue, and studied the fine structure of the fibres of connective tissue and their swelling during treatment with acids.

At a time when the focus of Flemming's interest was still the behaviour of individual cells, research into the process of cell division had already begun. In 1873, Schneider sketched the important steps of cell division. He saw the transformation of the nucleus into rod-like structures (Stäbchen), which assembled in the centre of the cell (at what we now know as the metaphase plate). At a stage that we now call anaphase, two groups of Stäbchen could be seen in the elongated cell.

Between 1874 and 1876, Flemming described these steps in more detail. Whereas Schneider had postulated that the nucleus undergoes deformation during cell division, Flemming provided a more detailed account of the process. Flemming's work laid the foundation for the understanding of the mitotic process, which was later expanded upon by other scientists.

Although Flemming was initially interested in the sensory organs of molluscs, his work on adipose tissue contributed to the understanding of connective tissue. He also studied lipid droplets, which were defined as connective tissue components.

Flemming's contributions to cell biology were significant, and his work on cell division and adipose tissue provided important insights into the processes of cellular metabolism and the structure of connective tissue. Flemming's legacy continues to influence the field of cell biology and has paved the way for further research in this area.
RESPECT the SPINDLE
Spin Infinite Yarns with One Amazing Tool

ABBY Franquemont
The mitotic spindle

Alberts et al., Molecular Biology of the Cell
Kinetochores

Functions of the kinetochore

• attachment of chromosomes to spindle MTs
• activation of the spindle checkpoint (tension sensing)
• force generation for chromosome movement
The principal task of the spindle is to segregate the chromosomes without errors.

Chromosome loss: 1 in 10,000 cell divisions in yeast (Hartwell & Smith, 1985).

Aneuploidy: genetic disorders, cancer
The challenge

1. How does the spindle self-assemble?

2. What forces act on chromosomes?
How does the spindle self-assemble?

The time required for microtubules to capture all kinetochores exceeds the duration of prometaphase other mechanisms.

Kirschner and Mitchison, 1986
Mechanisms that accelerate kinetochore capture

Bias in MT dynamics towards the chromosomes

Nucleation of MTs at spindle MTs

Carazo-Salas et al. 2001
Wilde et al. 2001

Goshima et al. 2008
Mechanisms that accelerate kinetochore capture

Nucleation of MTs at the kinetochore

Nucleation of MTs at the chromosome

Witt et al. 1980
De Brabander et al. 1981

Karsenti et al. 1984
Carazo-Salas et al. 1999
Mechanisms that accelerate kinetochore capture

Chromosome movements

Paul et al. 2009
Observation of individual capture events

Fission yeast
*S. pombe*

GFP-tubulin
ndc80-tdTomato

Iana Kalinina
Pivoting of microtubules is random

\[ \Delta t \text{ (s)} \]

MSAD (degree^2)

\[ \alpha \]

spindle pole

KC

spindle pole

\[ \text{MT} \]

\[ 1 \text{ pixel}^2 \]

\[ 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \quad 30 \]

\[ 0 \quad 50 \quad 100 \quad 150 \quad 200 \quad 250 \]

\[ 0 \quad 5 \quad 10 \quad 15 \quad 20 \quad 25 \quad 30 \]
• Microtubule pivots around the spindle pole.

• Kinetochore diffuses.

Amitabha Nandi, Benjamin Lindner, Nenad Pavin
Test of the model

Pivoting model — 3 minutes
“Search-and-capture” model — 100 minutes
Pivoting of microtubules changes with temperature

\[ \Delta t \text{ (s)} \]

\[ \text{MSAD (degree}^2\text{)} \]

- 32°C, n=101
- 24°C, n=106
- 14°C, n=101
Tests of the model

![Graph showing the fraction of lost KCs over time at 14°C and 32°C. The graph at 14°C shows a downward trend with error bars, while the graph at 32°C also shows a downward trend.](image)
MT pivoting around the spindle pole accelerates capture

The spindle consists of parallel and antiparallel MT bundles

Grishchuk & McIntosh, 2006
MT pivoting accelerates spindle assembly

03:02 min:s

1µm

GFP-tubulin

Lora Winters
MTs growing from kinetochores pivot

Pivoting motion of kinetochore-bound MTs helps spindle assembly in higher cells.

Drosophila S2 cells
Maiato et al. 2004
What forces act on chromosomes?

Force generation is a key task of the spindle.
Forces on kinetochores are generated only by k-fibers.
Our hypothesis

Kinetochore (KC)  Chromatin

K-fiber  Cross-linkers

Bridging MTs

Spindle pole  Spindle pole

Sister k-fibers are connected by MTs in addition to the chromatin spring.
Non-kinetochore MTs bridge sister k-fibers

HeLa cell
Tubulin-GFP
mRFP-CENP-B

bridging fiber
Non-kMTs have been seen near kinetochores in EM

African blood lily

Jensen et al., J Cell Biol 1982 (lily)
McDonald et al., J Cell Biol 1992 (PtK1)
Ohi et al., Dev Cell 2003 (Xenopus)

Function of these MTs?
Laser-cutting assay for the study of bridging MTs

Cut the outermost k-fiber

Look at the fast response

Similar laser-cutting of k-fibers:
Maiato et al., *J Cell Biol* 2004
Sheykhani et al., *Cytoskeleton* 2013
Sikirzhytski et al., *J Cell Biol* 2014
Elting et al., *J Cell Biol* 2014
Bridging MTs are connected with k-fibers in HeLa cells

Tubulin-GFP
mRFP-CENP-B

Janko Kajtez, Anastasia Solomatina
Do bridging fibers contain antiparallel overlaps?
PRC1 is found in the bridging fiber

PRC1-GFP (overlap regions of MTs)    mRFP-CENP-B
Bridging fiber contains dynamic MTs

2xGFP-EB3
mCherry-CENP-A

1 µm

time

0

kinetochores
EB3

12s

1.9±0.4 EB3 spots/min

Bruno Polak
Shape of the k-fiber

\[ \Theta_C \] did not change after the cut.

\[ \Theta_K \] increased after the cut.
In:
- Measured geometry of the spindle:
  - spindle length and width
  - angles at the spindle pole and at the kinetochore
- Bending rigidity of the k-fiber and of the bridging fiber:
  - bending rigidity of a single MT
  - number of MTs in the k-fiber and in the bridging fiber

Out:
- position of the junction, \( x_j \) \( \sim 1 \mu m \) away from the KC
- force at the spindle pole, \( F_C \) \( \sim 50 \) pN
- force at the kinetochore, \( F_K \) \( \sim 300 \) pN
How important is the bridging fiber for the force balance?

Bridging fiber with more MTs $\Rightarrow$ larger force at the pole
$\Rightarrow$ larger force in the bridging fiber
$\Rightarrow$ faster straightening after the force is released
Cells with thicker bridging fibers

PRC1-GFP
Tubulin-mCherry
mRFP-CENP-B
n = 23 ± 5 MTs
What happens with the tension on KCs after cutting?

Prediction: Cutting closer to the KC should result in a greater release of tension at KCs.
The distance between KCs decreases after cutting

Results

and the stored elastic potential energy of the bent microtubule fiber is converted into kinetic energy in an attempt of the fiber to straighten out. Our experiments support this claim as the straightening of both the ablate stub and the intact k-fiber was observed as the response to k-fiber severing. Force that drives the motion after ablation is balanced in the intact fiber through the fixed anchoring at the spindle pole.

3.2.2 Kinetochores are under tension

Just as the motion of the spindle element away from the spindle center indicates the presence of compressive forces along the k-fiber, movement of sister kinetochores relative to each other gives us information about the forces acting in the centromeric region. According to our predictions, we observed that following k-fiber ablation sister kinetochores move towards each other (Figure 12a). In order to follow the response of the kinetochore pair to the force disruption in all the ablated cells, the position of each outermost sister kinetochore was tracked using tracking software developed in-house [53]. Analysis of this data revealed that in 84% (43/51) of ablated cells the distance between sister kinetochores decreased within the first frame after the accompanying k-fiber was ablated (Figure 12b). This confirms that, prior to

Figure 12: a) Time laps images of HeLa metaphase spindle following the ablation with the focus on interkinetochore distance. White circles mark the position of the two outermost sister kinetochores. The interkinetochore distance clearly decreases after laser ablation (marked by white triangle) implying the centromeric region was stretched prior to ablation. Maximum projection of two planes. Bars, 1 µm.

b) Interkinetochore distance normalized to the value at the moment of the cut as a function of time. Distance between sister kinetochores decreases in majority of cells. Grey line and light green area mark mean and standard deviation respectively.

Table 1

<table>
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<tr>
<th>Time (s)</th>
<th>KC-KC distance (µm)</th>
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<tbody>
<tr>
<td>0</td>
<td>2.0</td>
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<tr>
<td>10</td>
<td>1.6</td>
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<td>20</td>
<td>1.2</td>
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n=43
Bridging microtubules link sister k-fibers and balance the tension on kinetochores.
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