Self-organization of microtubule assemblies

Intro: self-organization of molecules into “living” systems
Case-study: self-organization of meiotic spindles

Tim Mitchison
Harvard Medical School
7_2011

Self-organization of molecules into living systems

Outline & key concepts

- *Self-organization* allows molecules to from living systems
- *Polymerization* allows protein molecules to assemble into cell-spanning filaments
- Microtubules and other cytoskeleton polymers hydrolyze GTP to promote polymerization dynamics
- Mitotic spindles self-organize and have life-like properties such as the ability to adapt in size, and repair damage
- These properties depend on spindles being steady state assemblies that continuously re-build themselves

Genomes encode proteins directly….

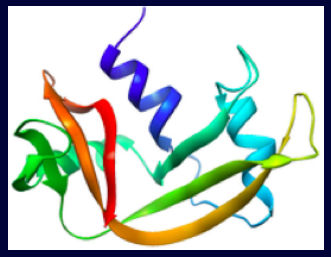
Genes

GGCAGAAACTGCCTTCT
CTCTCTCAGACATCAAAC
TAGAGACCCAGG……
.

Polypeptides

PSLGKETAAAKFERQH
MDSSTSAASSSNYCNQ
MMKSRNLTK……

Protein machines



…..but how proteins self-organize into living systems is less clear

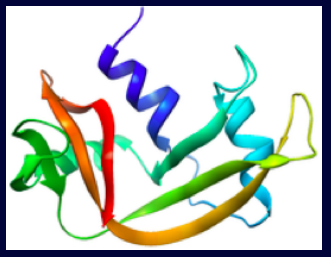
Genes

GGCAGAAACTGCCTTCT
CTCTCTCAGACATCAAAC
TAGAGACCCAGG……
.

Polypeptides

PSLGKETAAAKFERQH
MDSSTSAASSSNYCNQ
MMKSRNLTK……

Protein machines

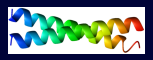


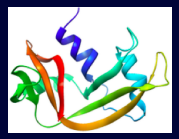
Life!

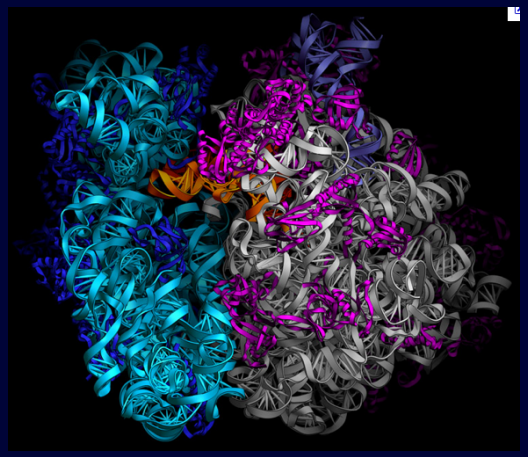
Dynamic self-organization

Life processes presents a scaling problem:

How do *molecules*, which are nm long and typically operate in msec regimes, organize *cells* which are μm and operate in min regimes?

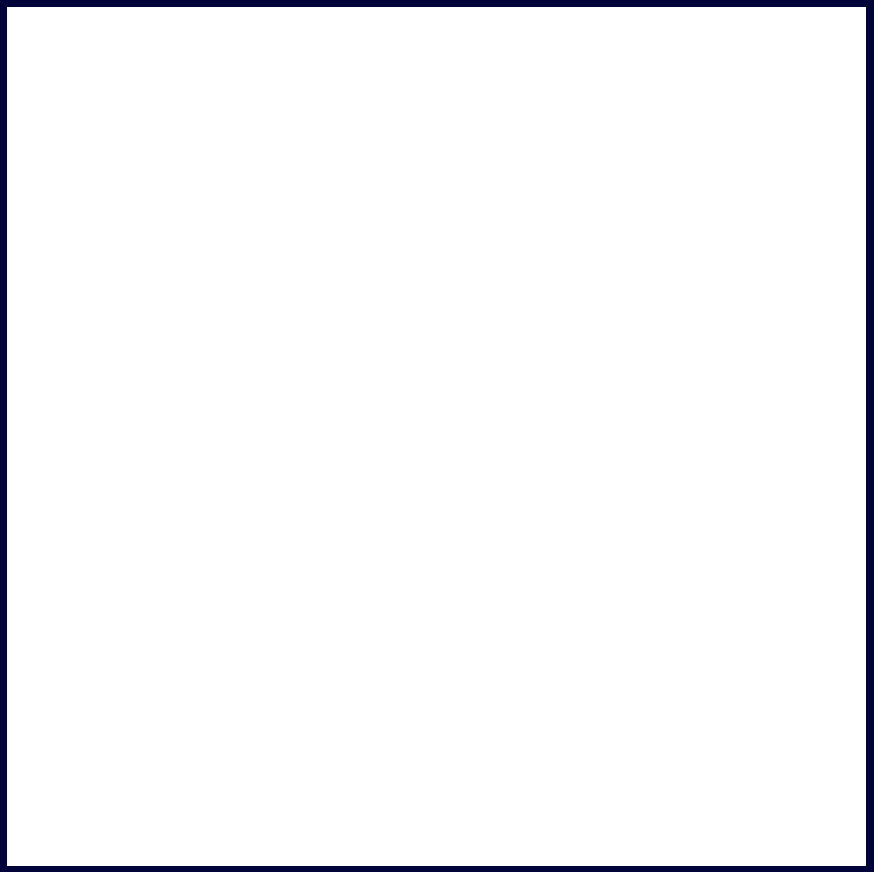






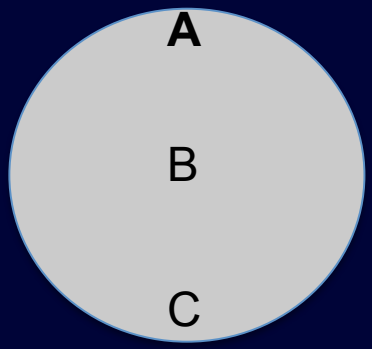
Proteins and protein machines ~3-30nm

?



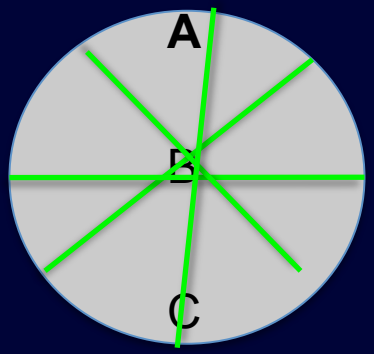
Frog egg dividing
~1 mm in diameter, divides every 30 min

Principles for organizing whole cells

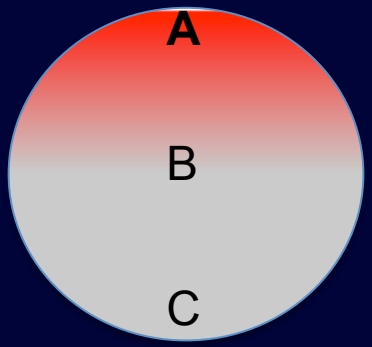


How could different parts of a cell communicate over many μm?

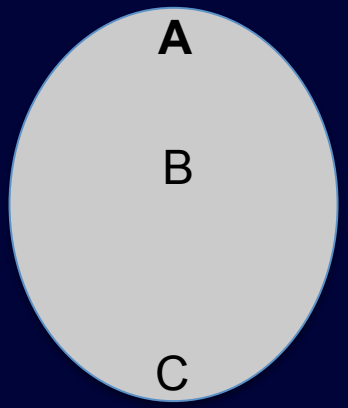
Principles for organizing whole cells



Long protein polymers

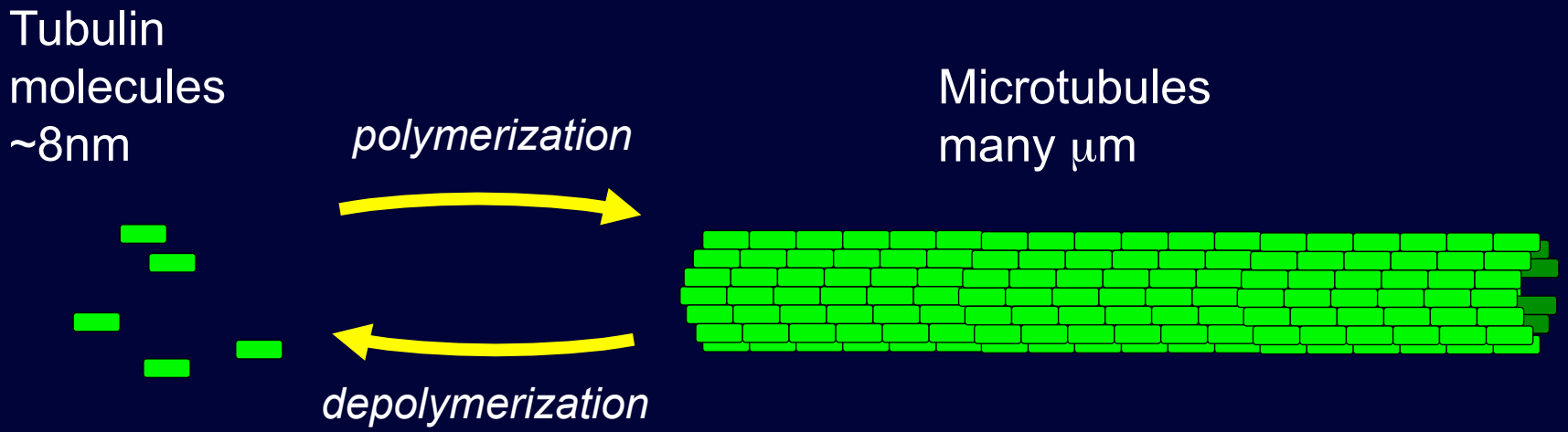


Chemical gradient



Mechanical forces

Long proteins polymers help integrate cells



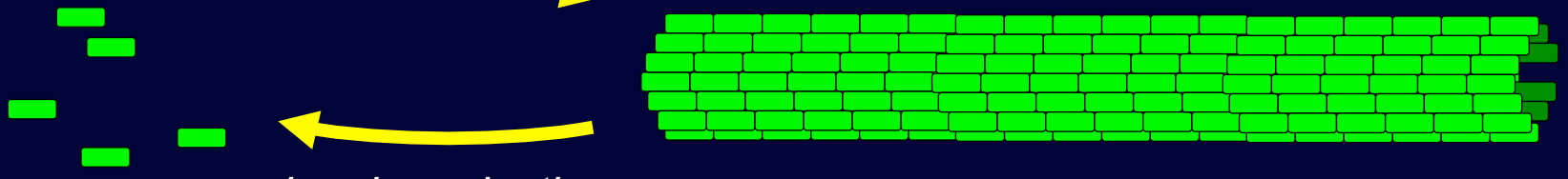
Long proteins polymers help integrate cells

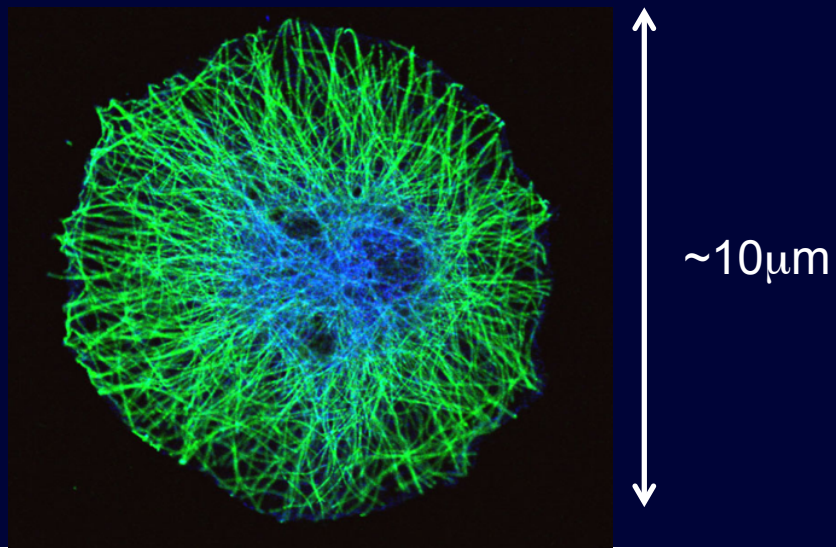
Tubulin molecules ~8nm long

polymerization

depolymerization

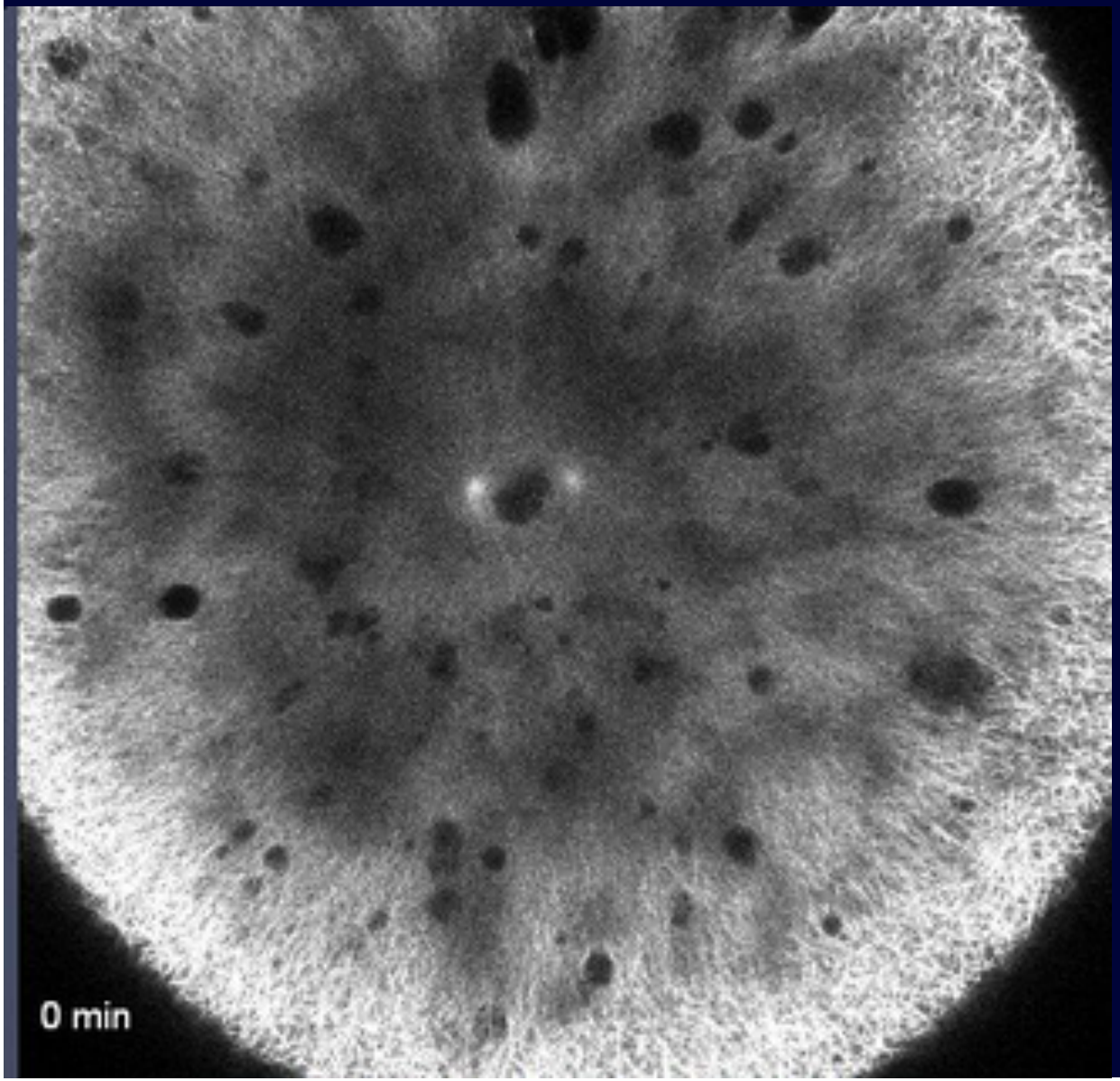
Microtubules ~10s of μm long





Approximately radial array of microtubules in a small cell (Drosophila tissue culture cell)

Radial microtubule arrays called “asters” organize much larger cells in early embryos



Microtubules in a Zebrafish embryo

This movie starts at prophase of 1st mitosis and continues to cytokinesis

The field of view is ~500μm

Wühr et al (2011) Meth Cell Biol

Microtubule assemblies provide a great example of self-organization in cells

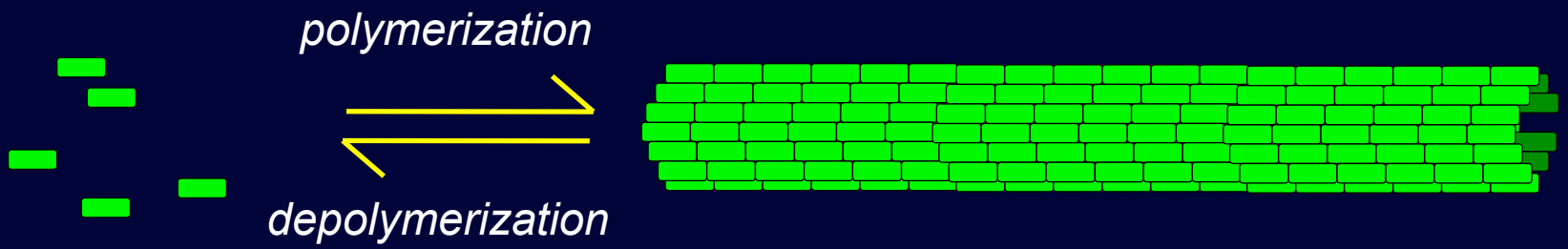
One of the great students of self-organization is Shinya Inoue

He used polarization microscopy of living cells to observe mitotic spindles appearing and disappearing in egg cells for the first time

Jellyfish embryo
Polarization microscopy
Shinya Inoue

Self-assembly vs. self-organization

the role of chemical energy



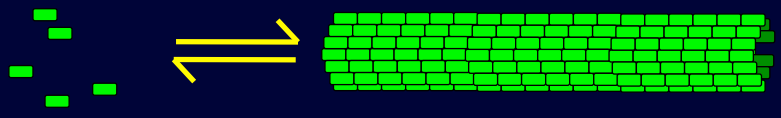
Imagine a protein polymer where polymerization and depolymerization are simply forward and back steps of the same reaction, with no other changes

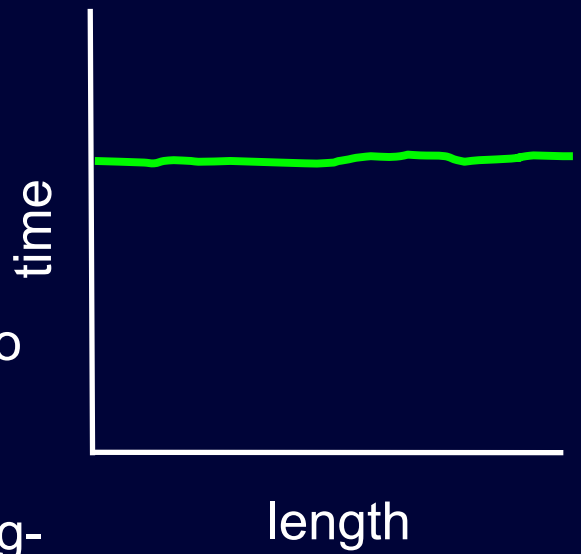
This would be an example of self-assembly (the “lego block” principle)

Self-assembly is the way most protein machines and assemblies are built, with no need for ATP hydrolysis or other energy-dissipating steps

Self-assembly vs. self-organization

the role of chemical energy



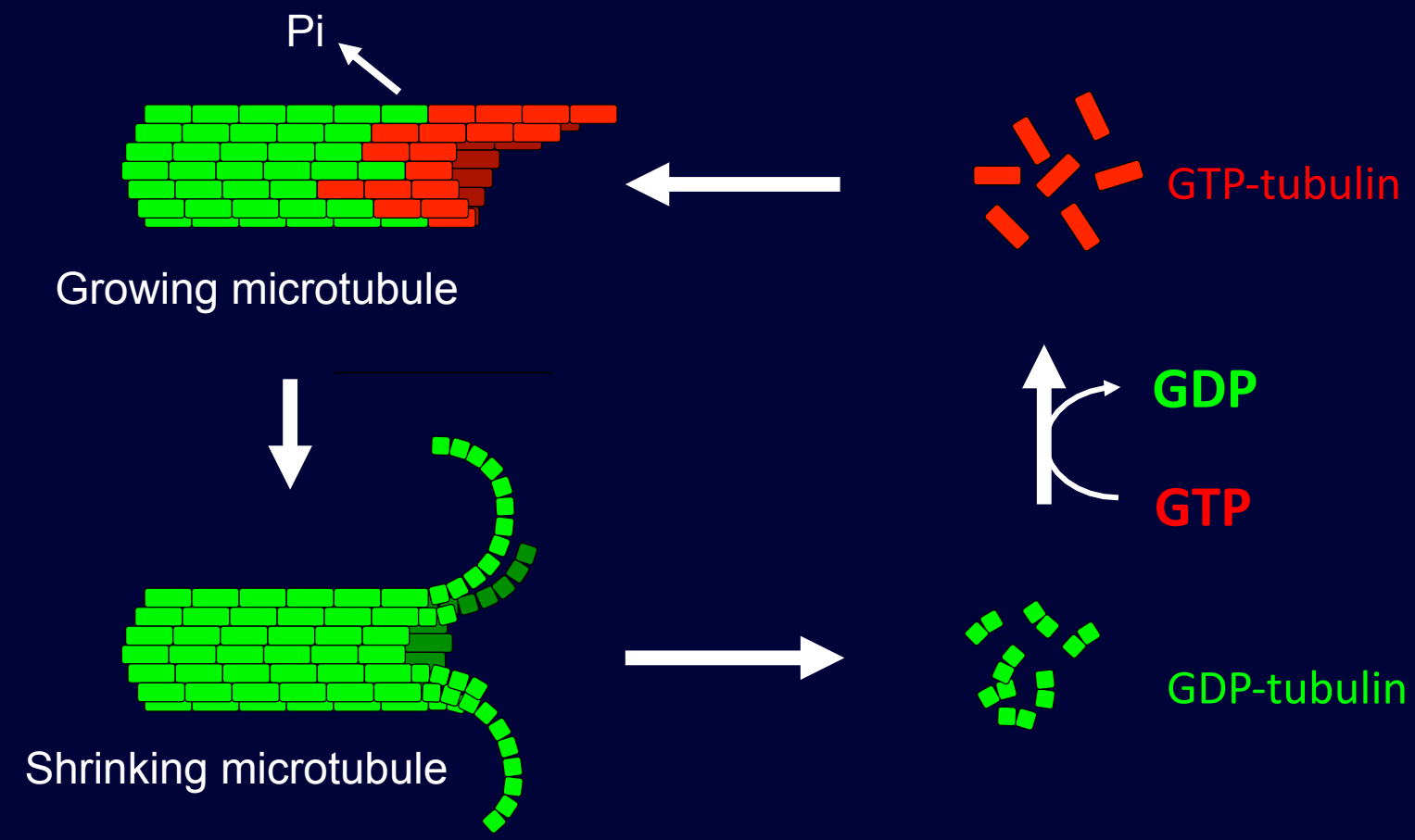


A simple self-assembled polymer would tend to keep the same length over time

This would make ideal rod component in a long-lived structure – like a virus particle, or a bacterial flagellum

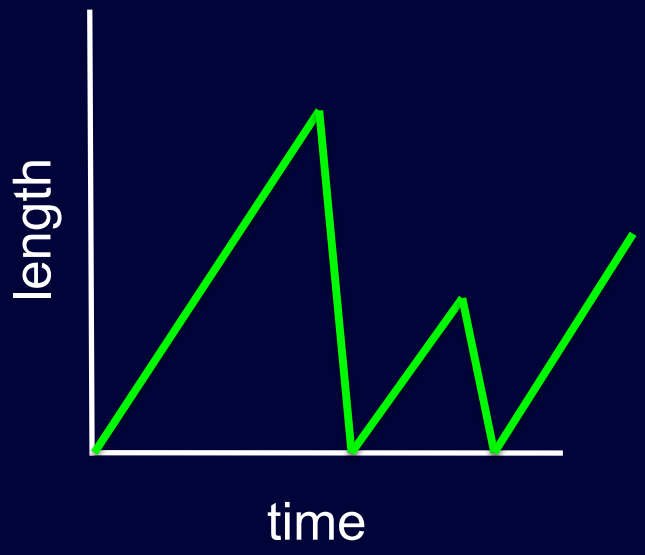
But is would not be suitable for building a mitotic spindle or interphase microtuble aster which has to assemble and disassemble in minutes

Microtubule polymerization is coupled to GTP hydrolysis



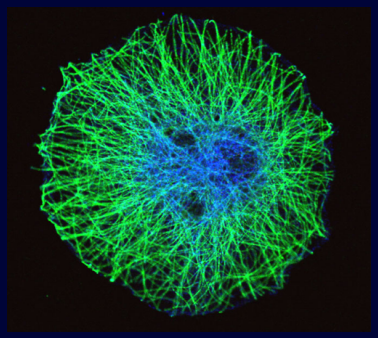
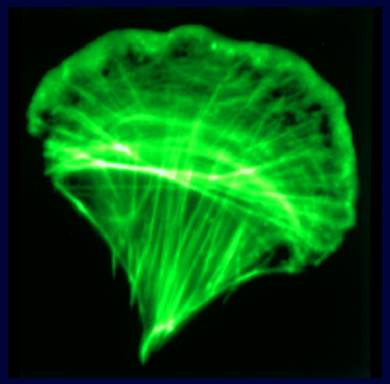
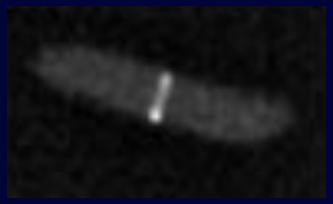
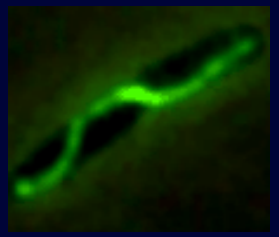
- Polymerization and depolymerization involve different chemical species
- For every tubulin that goes through this cycle, ~12 kcal/mol of chemical energy is dissipated through hydrolysis of one molecule of GTP

GTP hydrolysis powers large fluctuations in microtubule length called “dynamic instability”

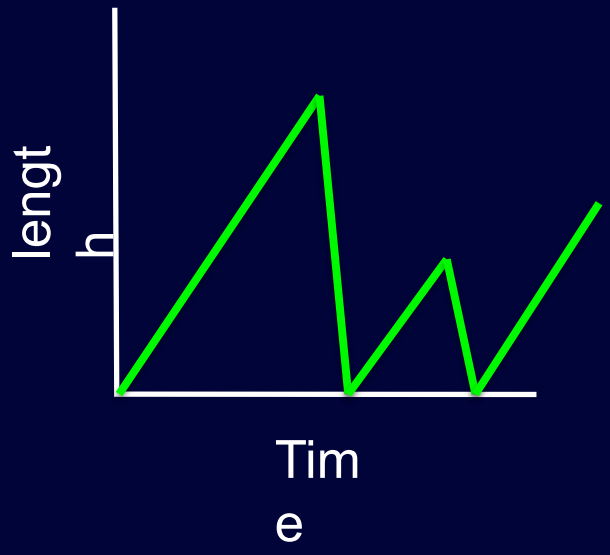


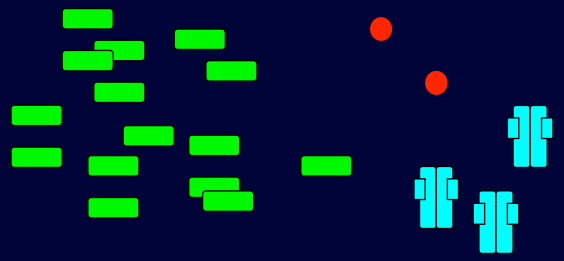
Microtubules growing from centrosomes in mitotic egg extract
Tournebize, Hyman et al 1997

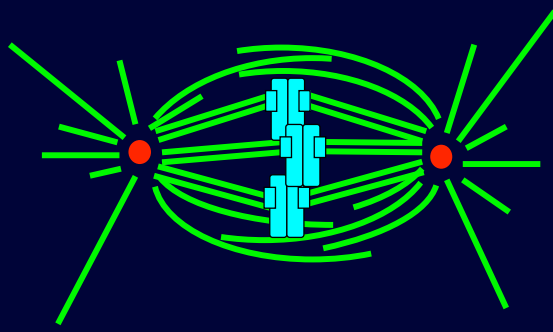
Diverse cytoskeleton polymers hydrolyze GTP or ATP to promote polymerization dynamics

	Tubulin family Hydrolyze GTP	Actin family Hydrolyze ATP
Animal and plant cells (Eukaryotes)	 Microtubules	
Bacteria (Prokaryotes)	 FtsZ	 ParM

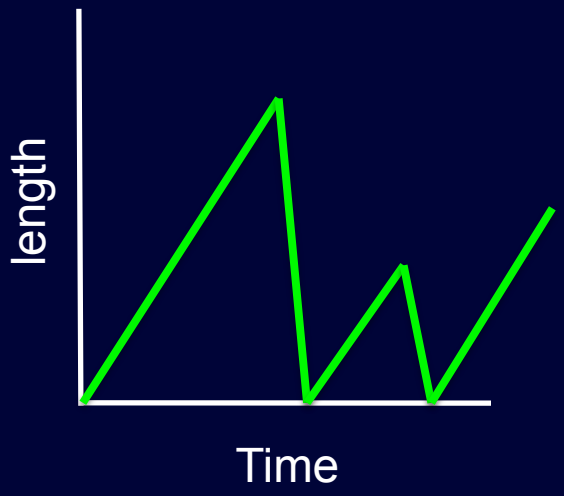
How might GTP-promoted microtubule length fluctuations promote self-organization?



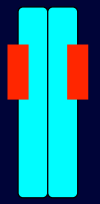




Length fluctuations promote *spatial exploration*

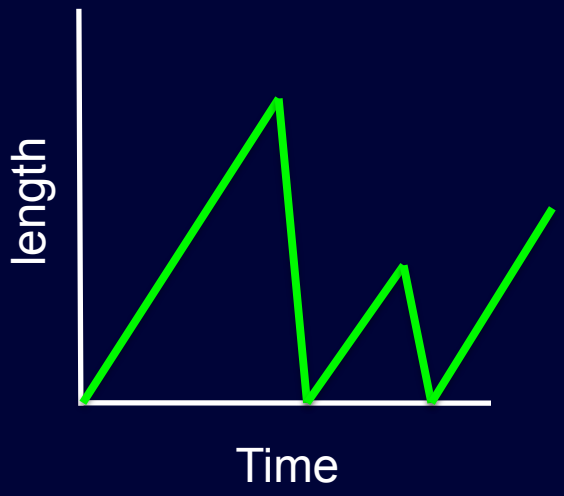


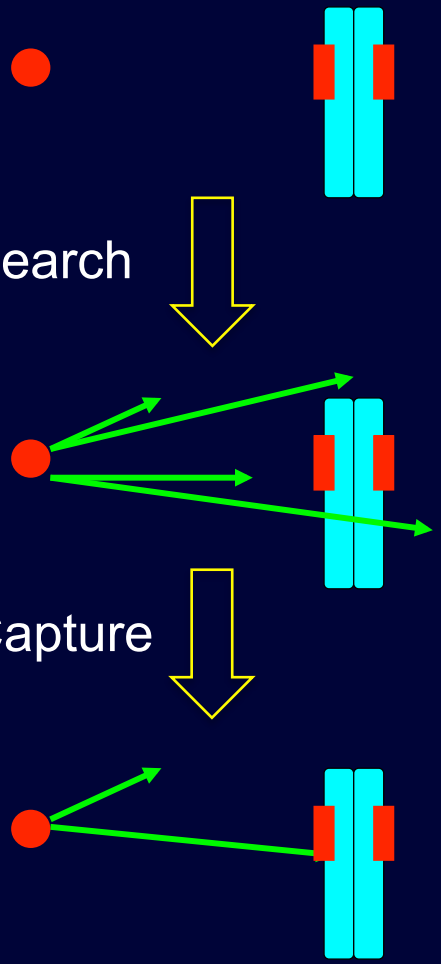
Centrosome
Nucleates
microtubules



Kinetochore
Binds
microtubules

Length fluctuations promote *spatial exploration*





Kirschner and Mitchison (1986) Cell

Microtubules assemblies have “life-like” properties

- Spontaneous assembly from soluble precursors
  (at a controlled time and place in the cell)
- Characteristic, but adaptable, shape and size
- Rapid recovery from damage

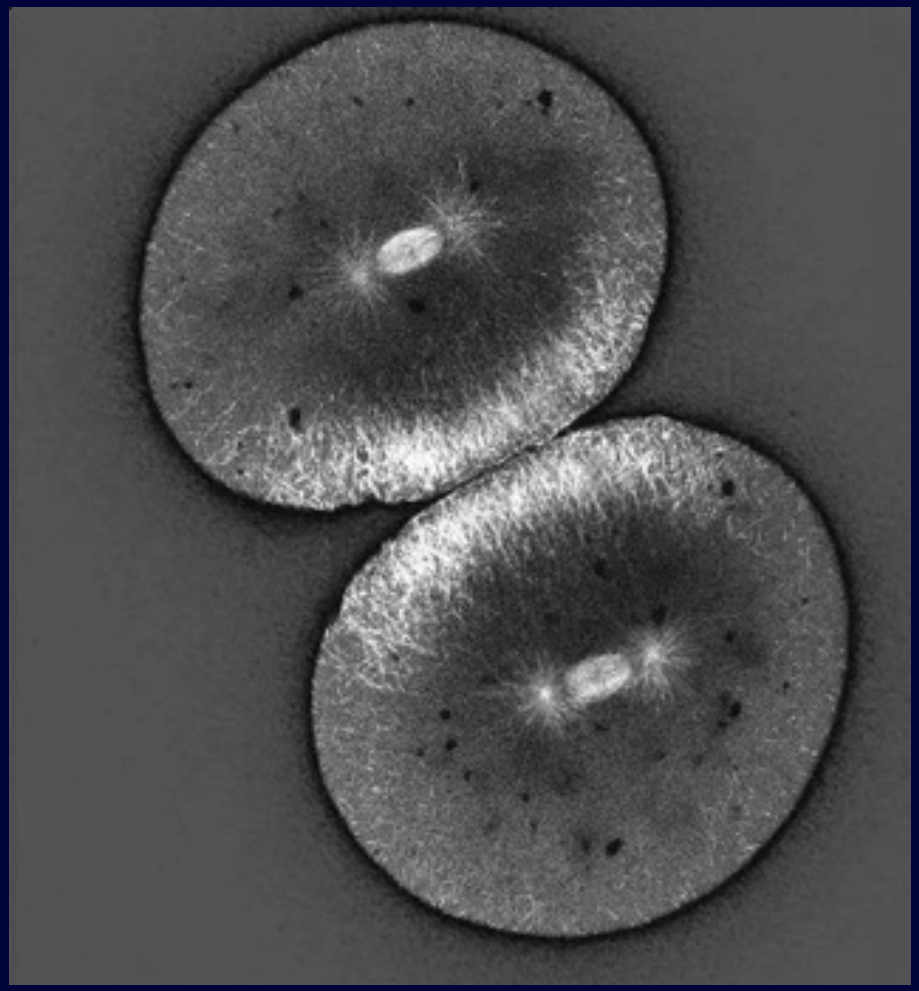
These properties are common to self-organizing systems

Microtubule arrays adapt to the size of the cell

Microtubules in an early Zebrafish embryo

The size of the mitotic spindle scales with the size of the cell, while its shape remains relatively constant

Movie by Martin Wühr
(2011) Meth Cell Biol

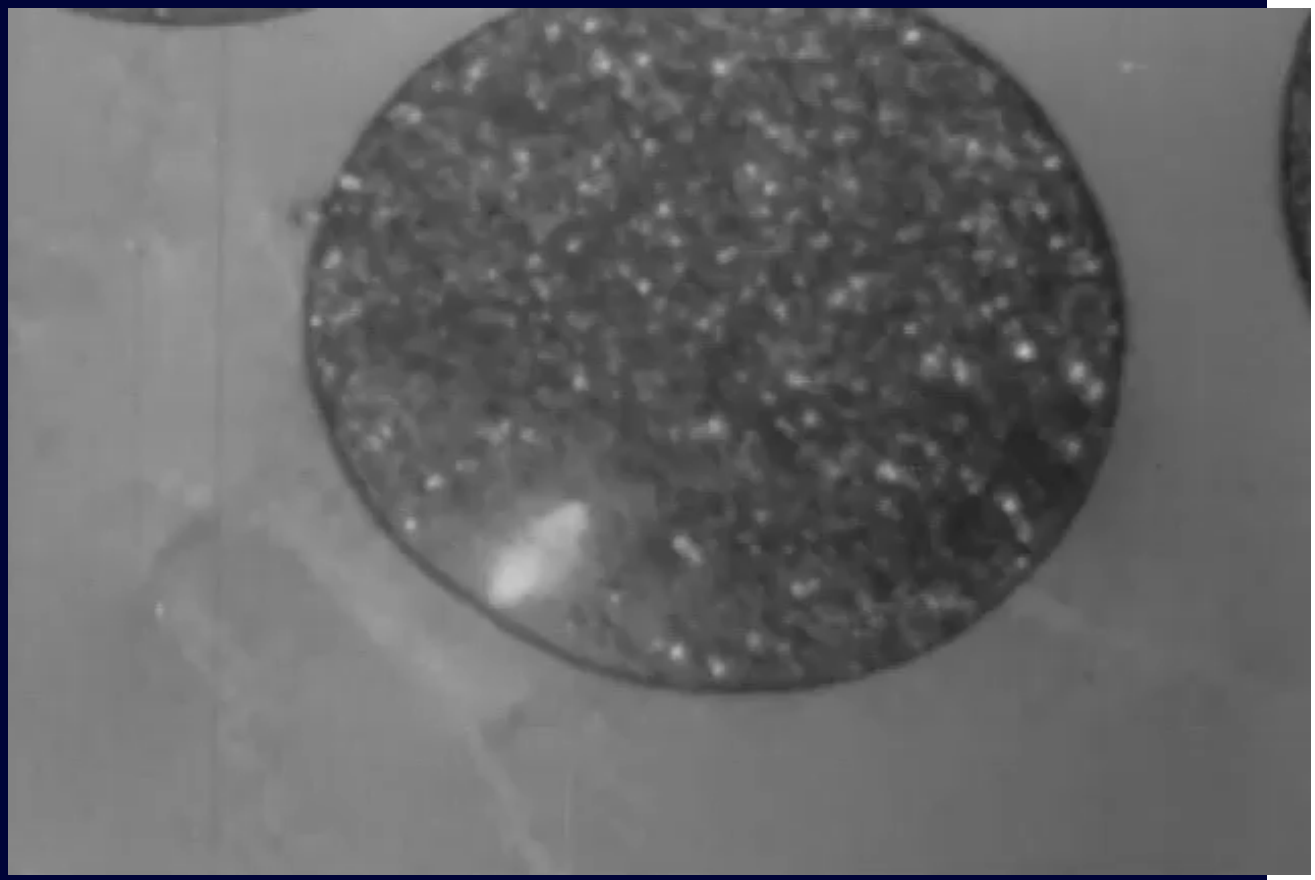


Microtubule assemblies recover rapidly from damage

Meiotic spindle in an egg subjected to repeated cycles of high pressure which depolymerizes microtubules, followed by recovery at low pressure

Note rapid recovery of the spindle to its original size and shape

Chaetopterus (parchment worm)
Polarization microcopy
Salmon and Inoue (1975)



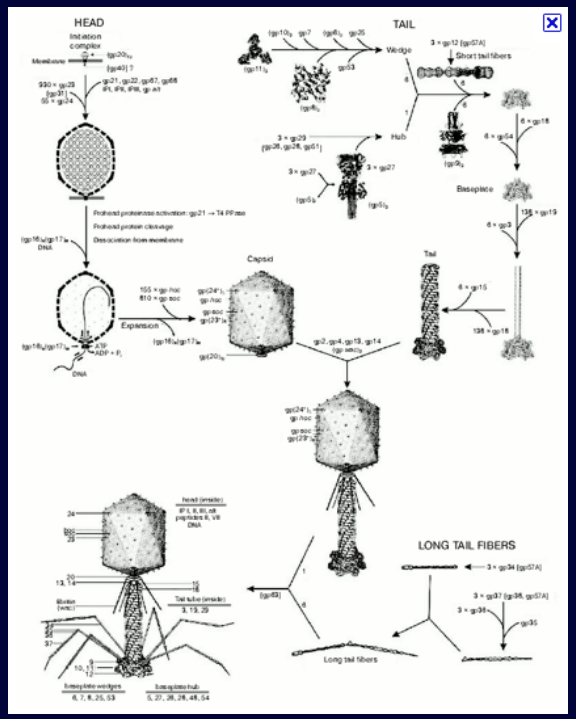
Microtubule assemblies recover rapidly from damage

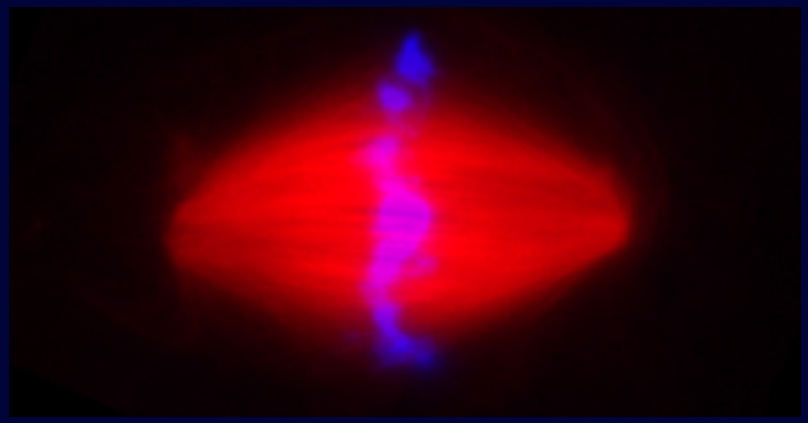
Two meiotic spindles brought into contact using microneedles

The two fuse into a single spindle over ~30min

Xenopus egg extract
Polarization microscopy
Gatlin et al Curr Biol (2009)

Self-assembly vs. self-organization





Bacteriophage

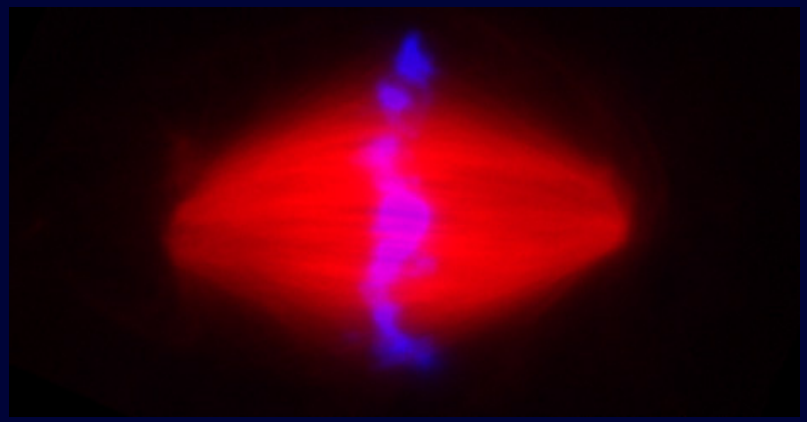
Complex assembly pathway leads to a low-energy structure with invariant size and shape

Spindle

Self-organization leads to a dynamic, energy-dissipating assembly that can adapt in size and repair damage

Self-organization generates *steady-state* assemblies

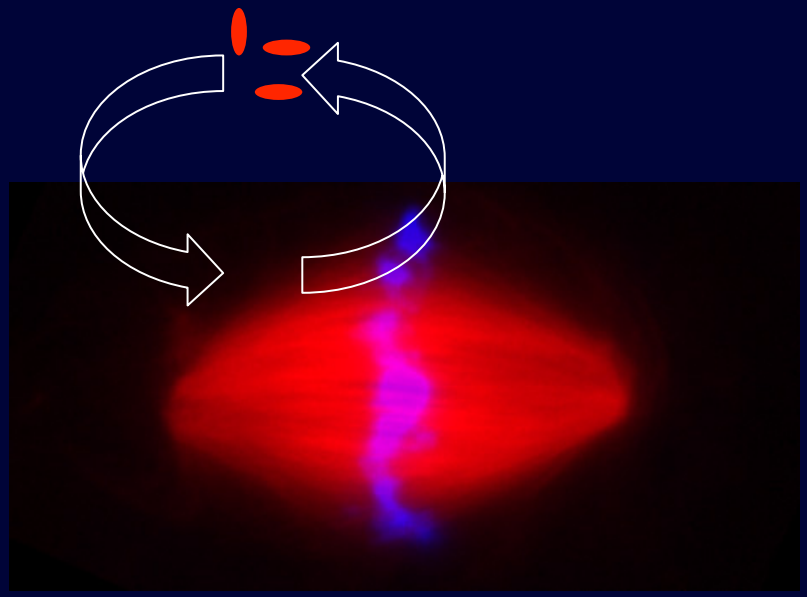
Meiosis-II spindle self-assembled in Xenopus egg extract



Metaphase meiotic spindles retain the same size and shape for hours, yet their internal contents are highly dynamic. They represent a *stable steady state* of a complex dynamical system

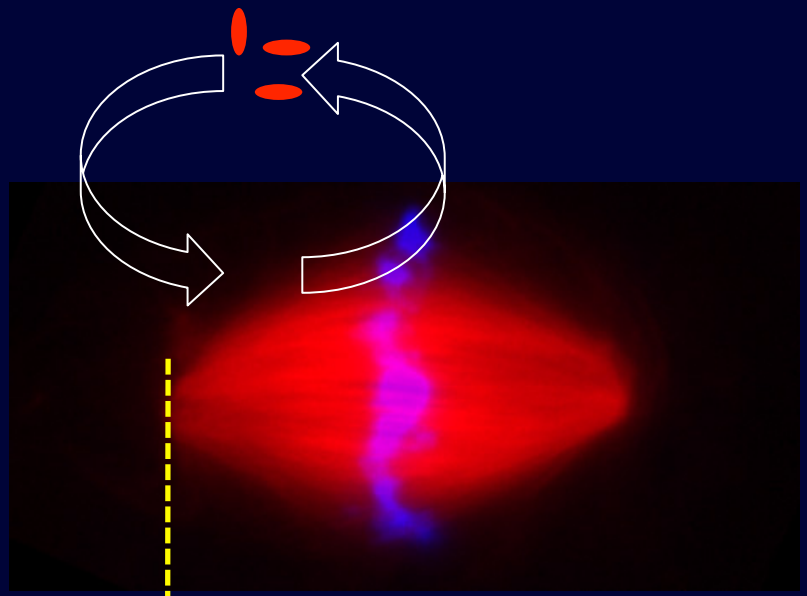
Spindles are steady-state assemblies that continuously re-build themselves

1) Spindle proteins exchange rapidly with soluble subunits in the bulk cytoplasm



Spindles are steady-state assemblies that continuously re-build themselves

1) Spindle proteins exchange rapidly with soluble subunits in the bulk cytoplasm

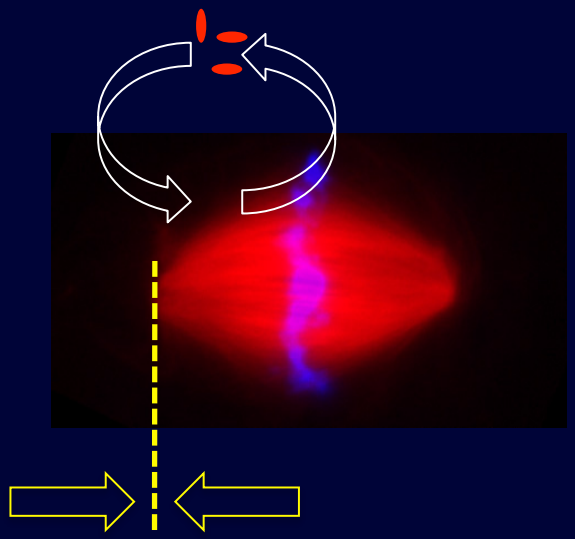


Pulling poles in

Pushing poles out

2) Forces within the spindle are balanced

Spindles are steady-state assemblies that continuously re-build themselves



Rapid turnover of subunits and continuous re-balancing of forces generate a steady state in mass and shape

These processes require substantial energy dissipation (ATP and GTP hydrolysis)

The net effect is an assembly that continuously renews itself, and can thus adapt and repair damage

Self-organization is a general organizing principle for cells

Cells are built from multiple assemblies that self-organize and continuously re-build themselves

Indeed, the cell itself can be viewed as such an assembly

Microtubule cytoskeleton

Actin cytoskeleton

ER, Golgi, secretory and endocytic pathways

Plasma membrane