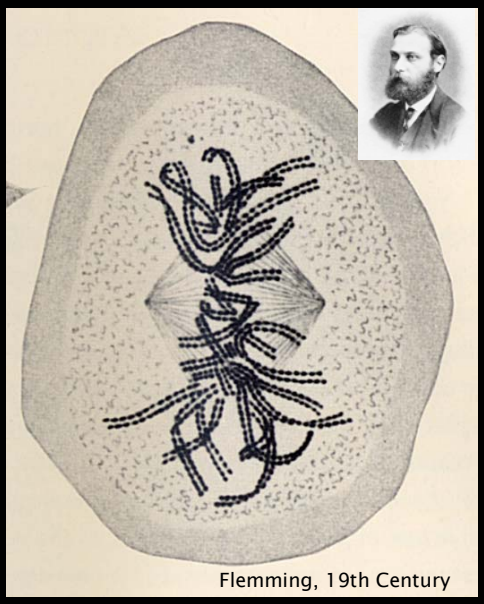
Part III: Mining the Genome for Mitotic Treasures

An RNAi Screen for Mitotic Spindle Assembly

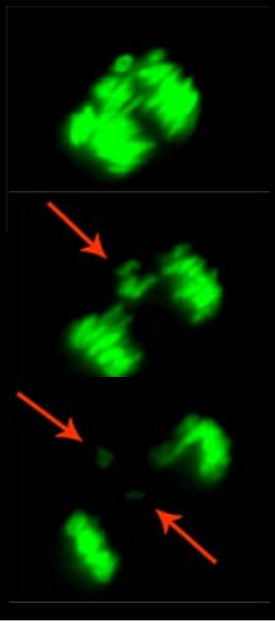
Ron Vale
UCSF
HHMI

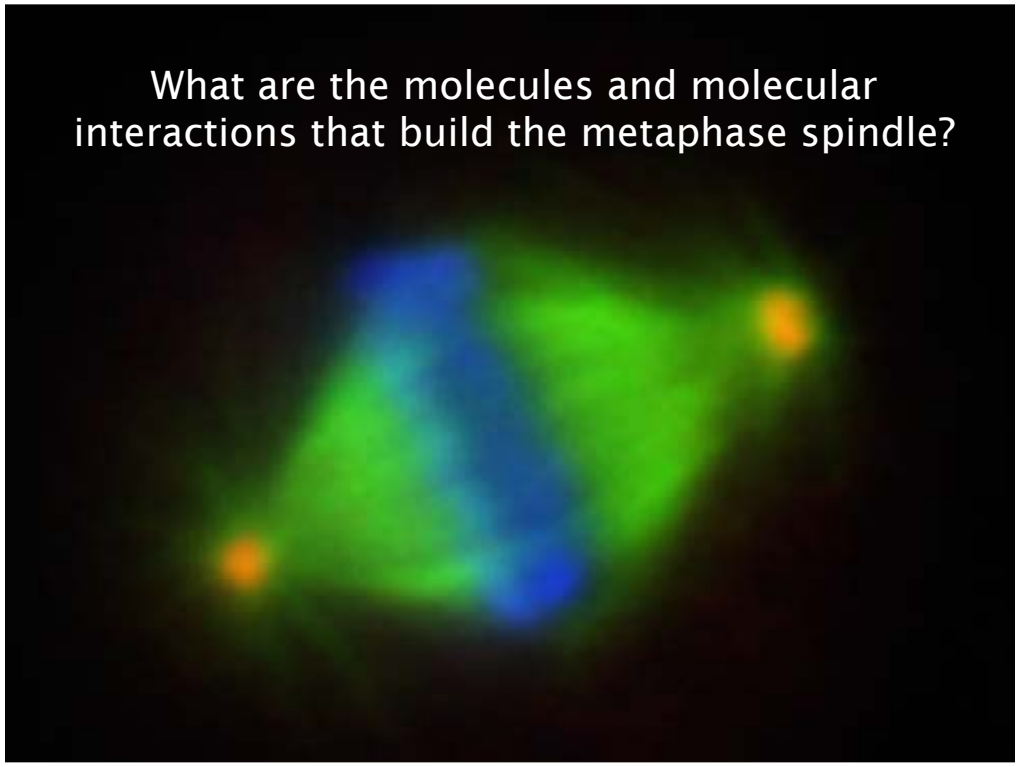
Movie by Sarah Goodwin, UCSF

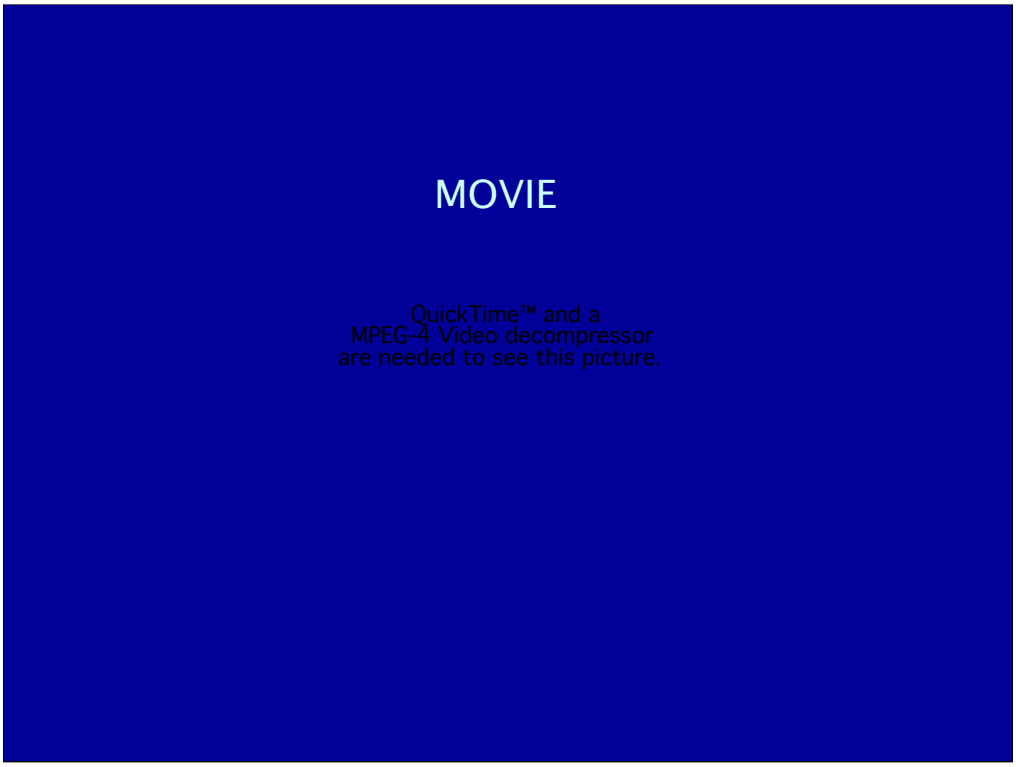
Mitosis is an old problem

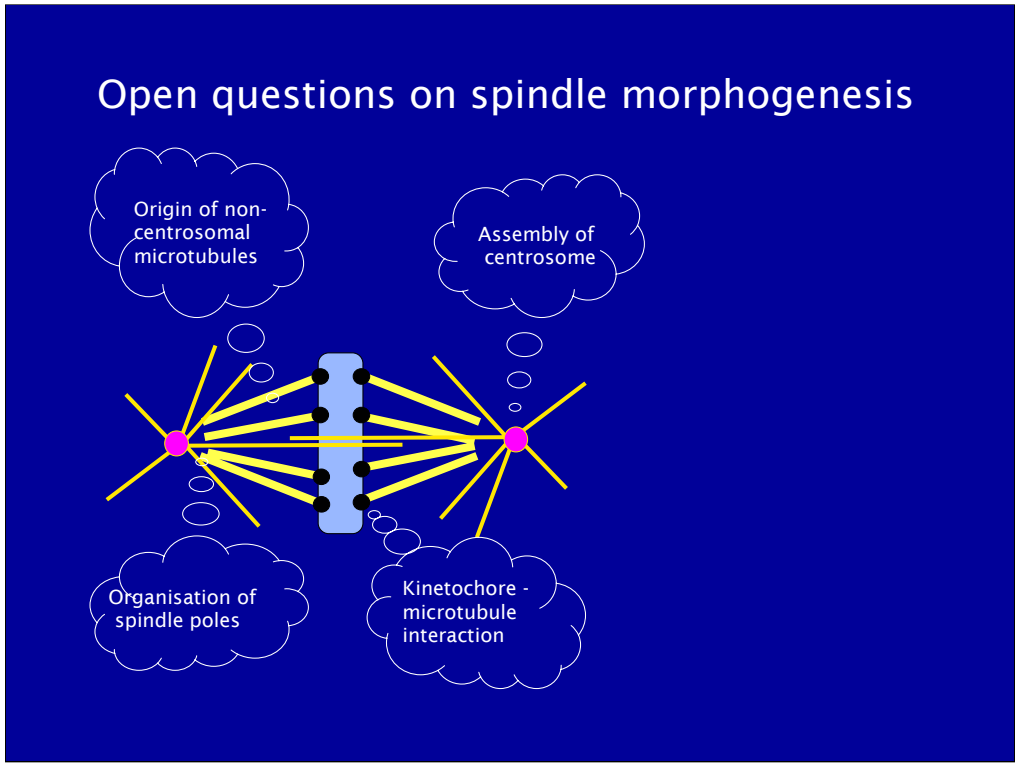


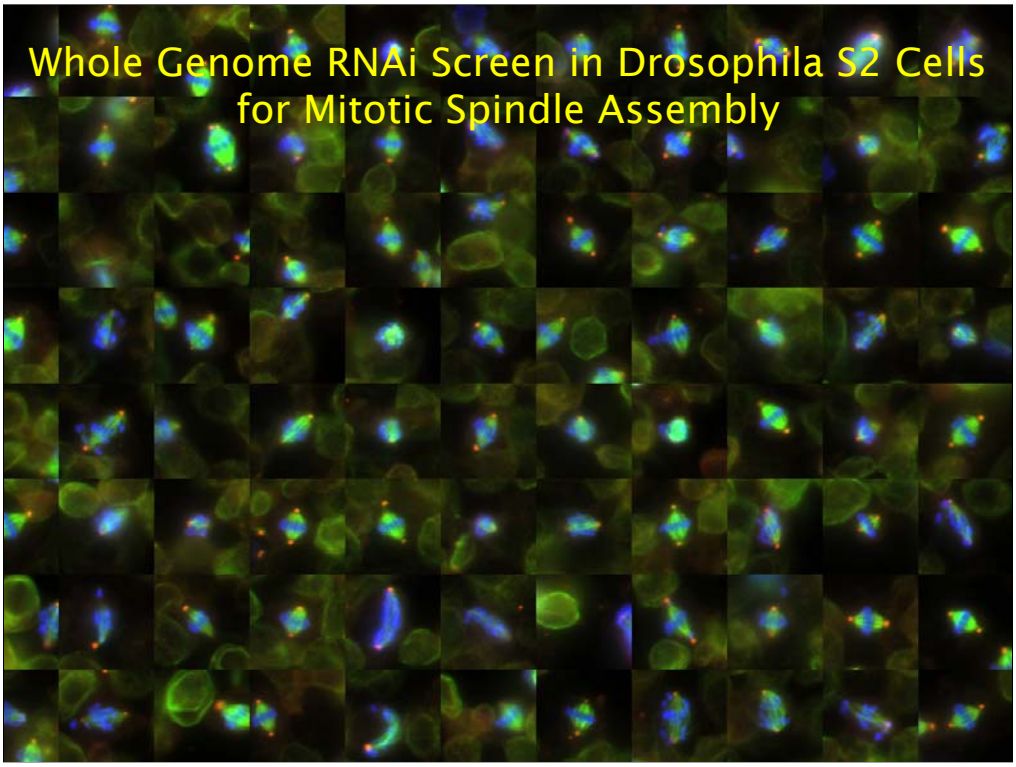
and a medically important…
(aneuploidy in cancer, cancer chemotherapy)




What are the molecules and molecular interactions that build the metaphase spindle?


MOVIE
QuickTime™ and a
MPEG-4 Video decompressor
are needed to see this picture.

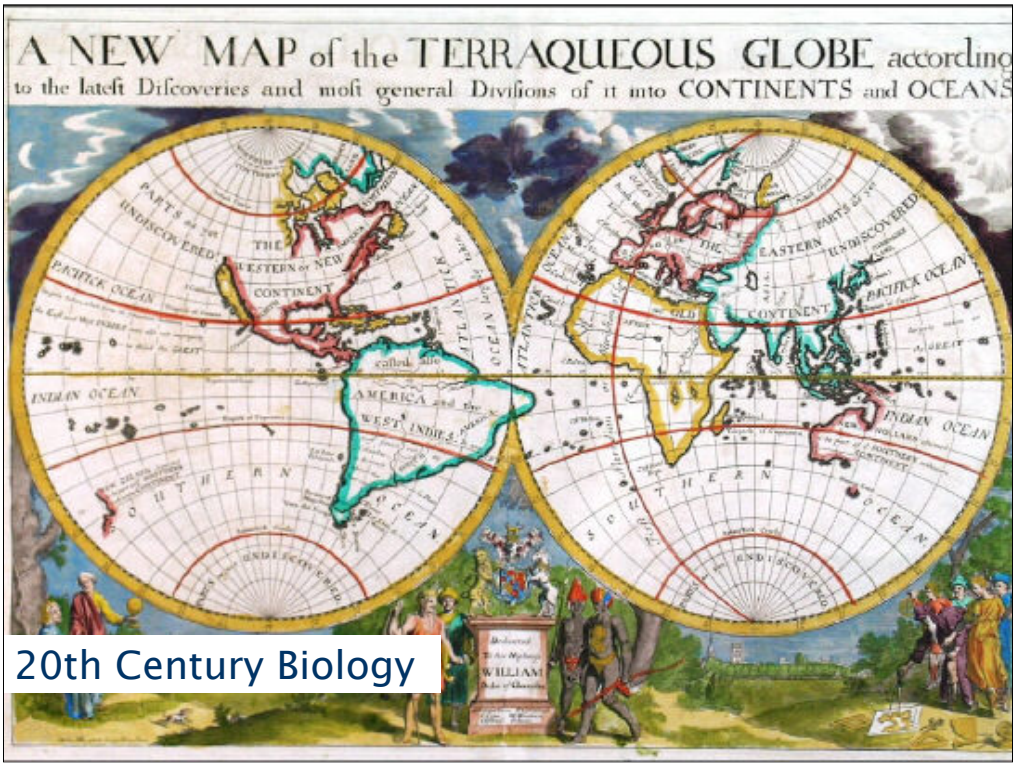

Open questions on spindle morphogenesis
Origin of non-centrosomal microtubules
Assembly of centrosome
Organisation of spindle poles
Kinetochore - microtubule interaction


Whole Genome RNAi Screen in Drosophila S2 Cells
for Mitotic Spindle Assembly

Whole Genome RNAi Screen in Drosophila S2 Cells for Mitotic Spindle Assembly

Gohta Goshima, Nico Stuurman,
Nan Zhang, Sarah Goodwin (UCSF)

Roy Wollman,
Jon Scholey (UC Davis)

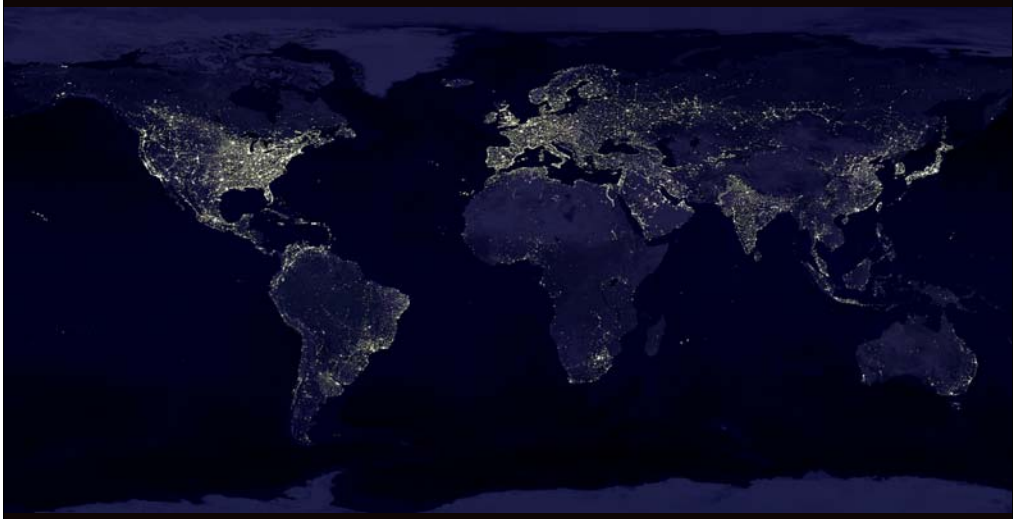


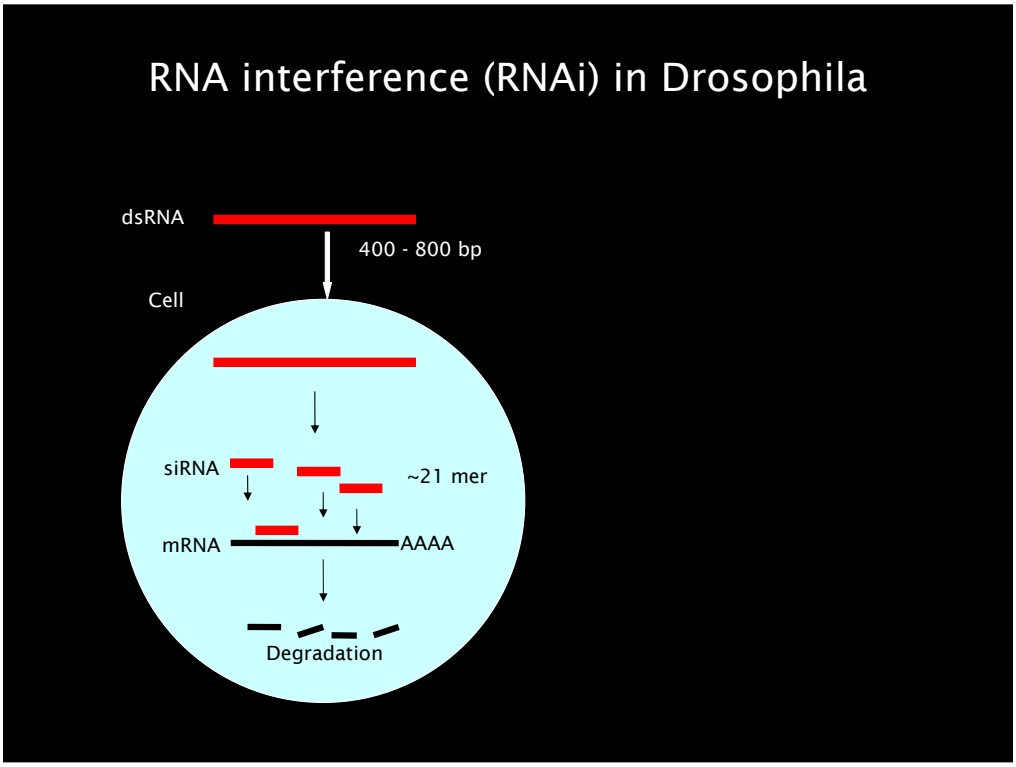
20th Century Biology

2007

Genomes have become finite, but

"undiscovered" genes still remain




RNA interference (RNAi) in Drosophila
dsRNA
400 - 800 bp
Cell
siRNA
~21 mer
mRNA
AAAA
Degradation

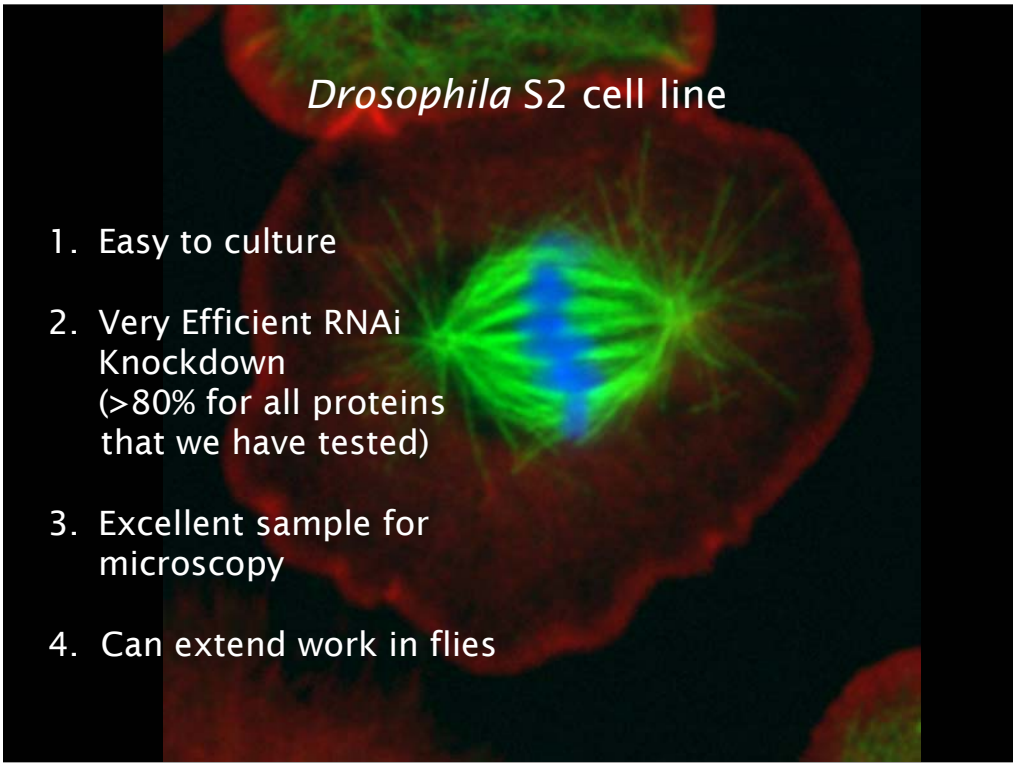
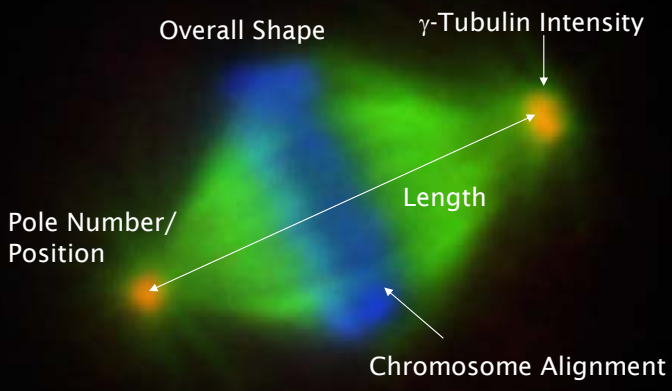

Drosophila S2 cell line
1. Easy to culture
2. Very Efficient RNAi Knockdown (>80% for all proteins that we have tested)
3. Excellent sample for microscopy
4. Can extend work in flies

Image-Based Approach for Identifying Spindle Defects Generated by RNAi

(Goshima et al. Science, April 23, 2007))



14,400 Genes and
4,000,000 Spindles Analyzed in this Screen

High-throughput RNAi Screen

1. Full Fly Genome dsRNA Library:

Designed by Nico Stuurman (UCSF) (available at Open Biosystems, Inc.)

High-throughput RNAi Screen

2. Treat S2 Cells with dsRNA for 4 days

96-well, plastic dish x 146
(each well has dsRNA for one gene)

+ APC dsRNA to induce metaphase arrest

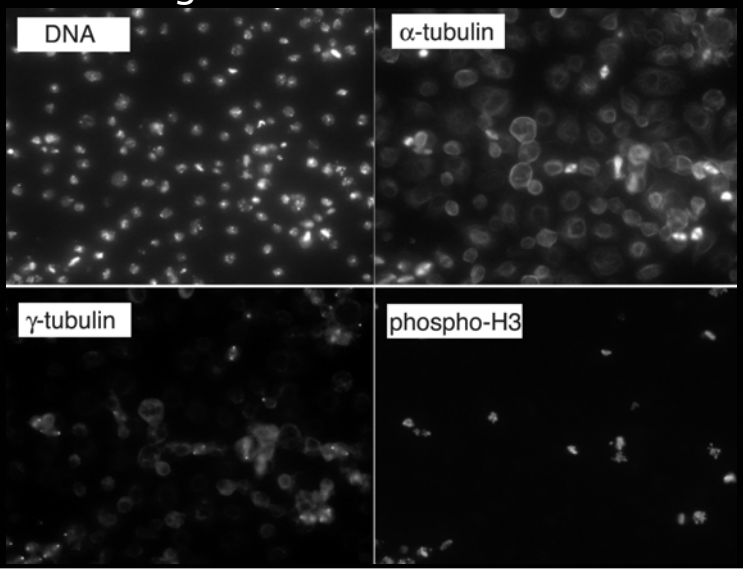
High-throughput RNAi Screen

2. High-throughput Microscopy

96-well, glass-bottom dish
for 40X, 0.95 NA imaging

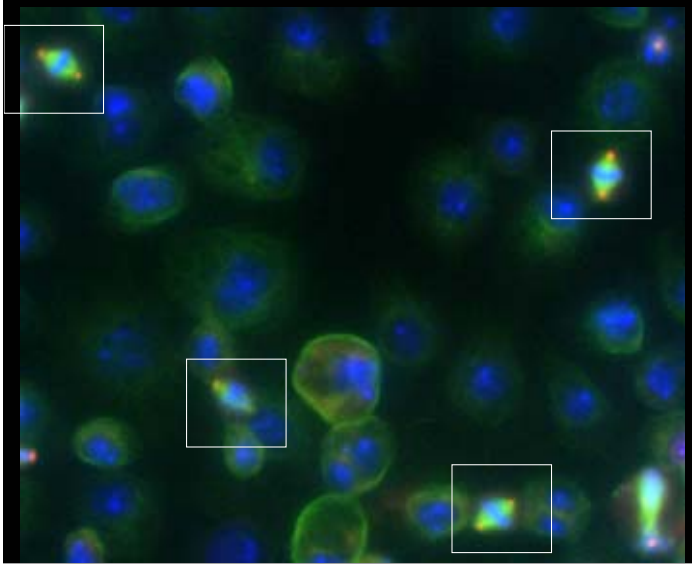
High-throughput RNAi Screen

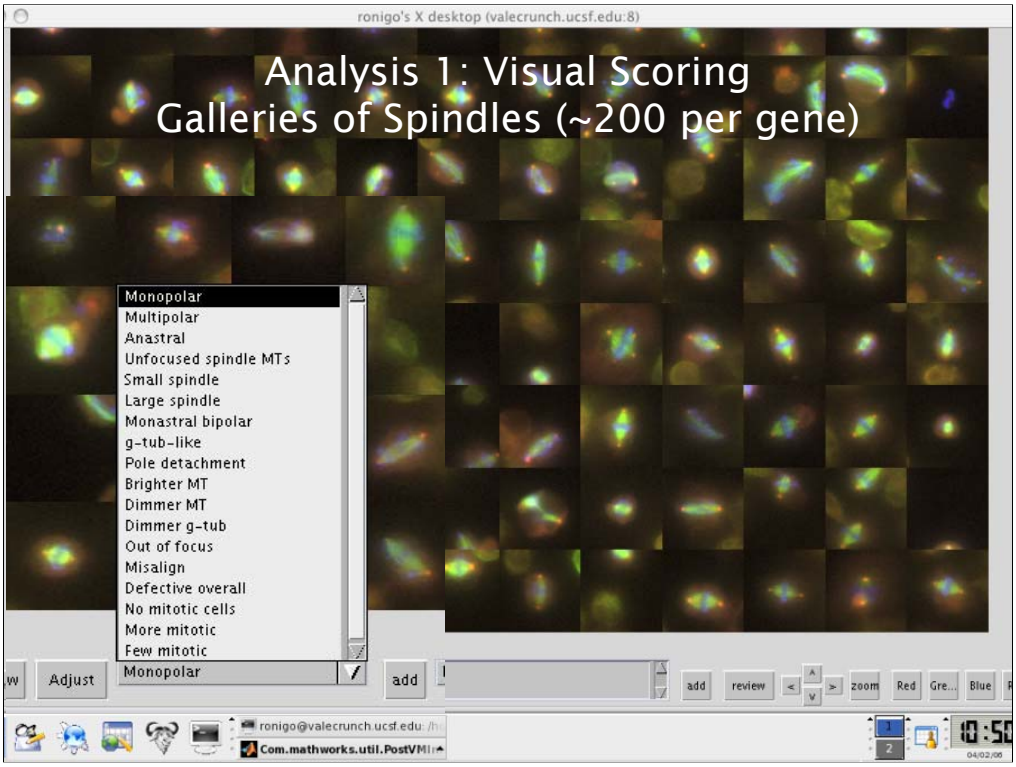
3. Image Data



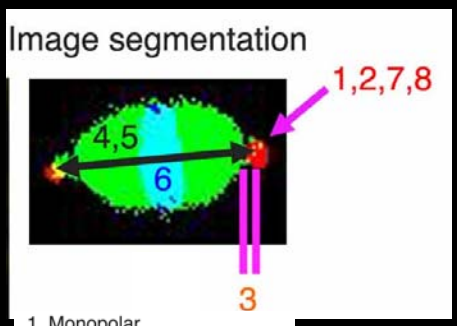
High-throughput RNAi Screen

4. Automated Mitotic Cell Identification




ronigo's X desktop (valecrunch.ucsf.edu:8)
Analysis 1: Visual Scoring
Galleries of Spindles (~200 per gene)
Monopolar
Multipolar
Anastral
Unfocused spindle MTs
Small spindle
Large spindle
Monastral bipolar
g-tub-like
Pole detachment
Brighter MT
Dimmer MT
Dimmer g-tub
Out of focus
Misalign
Defective overall
No mitotic cells
More mitotic
Few mitotic
Adjust
Monopolar
add
add
review
zoom
Red
Blue

Analysis 2:
Image Segmentation and Computational Analyses

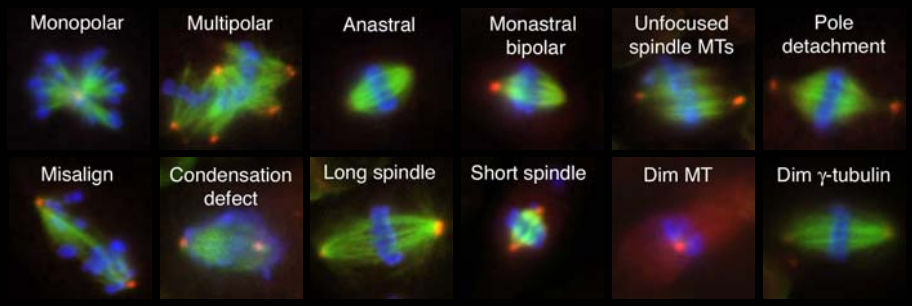


1. Monopolar
2. Multipolar
3. Pole detachment
   - frequency
   - mean distance
4. Long spindle
5. Short spindle
6. Misalignment
   - Chromatin area size
   - # of chromatin masses
   - Circumference/area ratio
7. Large γ-tubulin area
8. Dim γ-tubulin

MatLab Code by Roy Wollman (UC Davis) (available at http://rnai.ucsf.edu/mitospindlecreen)

Results: RNAi of ~200 genes produced metaphase spindle defects

Phenotypes



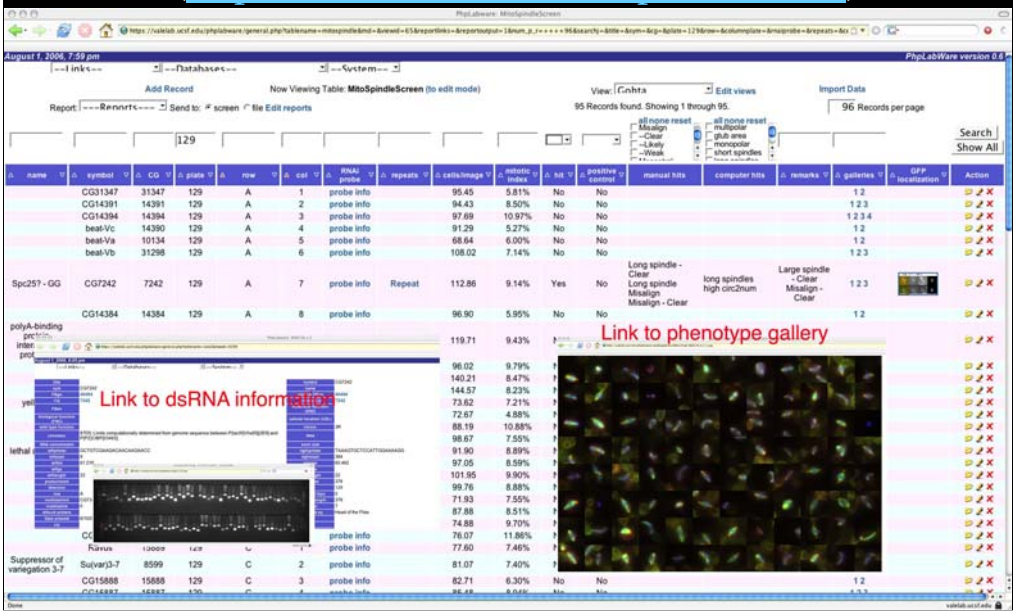
45 of 49 known mitotic genes in S2 cells identified

~90 Subunits of multi-protein complexes: Ribosome, Spliceosome, Proteosome, RNA Polymerase II

~60 Novel or Unexpected Genes

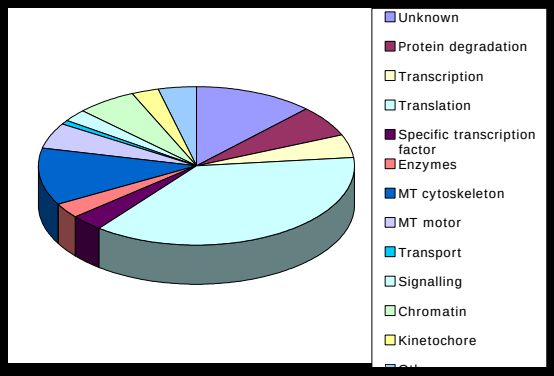
Vale Lab Screen Data Base

(http://rnai.ucsf.edu/mitospindlecreen)



What can one learn from an RNAi sceen?

Gene (CG#)	Phenotype
1453	Long Spindle
4654	Long Spindle
5785	Long Spindle
11006	Long Spindle
13628	Long Spindle
3265	Short Spindle
3992	Short Spindle
5000	Short Spindle
5650	Short Spindle
8222	Short Spindle
9028	Short Spindle
10648	Short Spindle
12396	Short Spindle
14354	Short Spindle
14735	Short Spindle
14781	Short Spindle
17077	Short Spindle
18397	Short Spindle
31763	Short Spindle
32435	Short Spindle
33130	Short Spindle



An overview of the most important proteins involved in spindle assembly

What can one learn from an RNAi sceen?

An unexpected result….
a mystery that might
lead to a new insight
into spindle assembly.

To Track Down These Unexpected Results, a Broad Range of Secondary Assays is Essential!

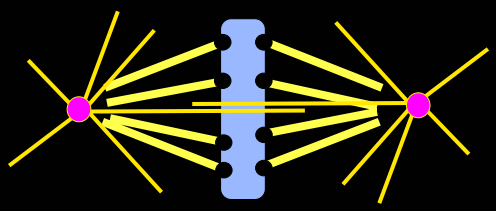
GFP tagging (often N and C termini to be certain of localization results)

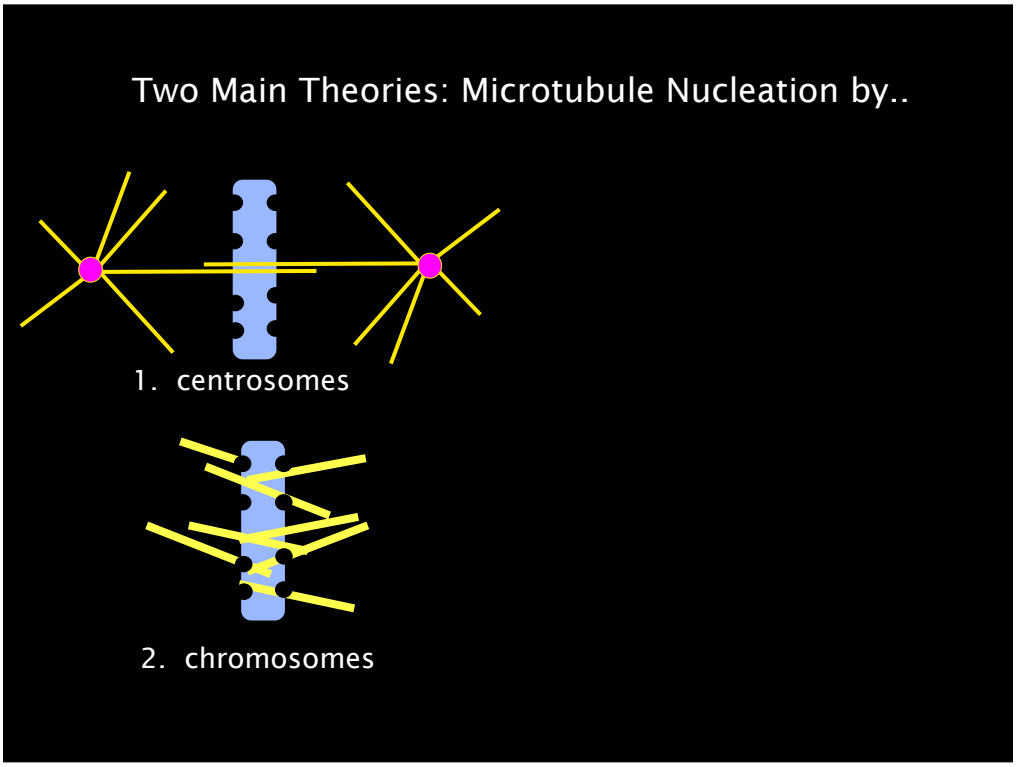
Time-lapse imaging of phenotypes

Additional RNAi/drug/localization experiments to understand mechanism

One story from the screen

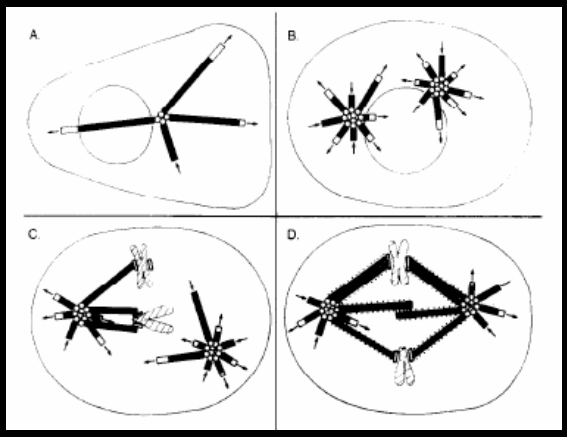
Making Microtubules
to Build the Spindle




Two Main Theories: Microtubule Nucleation by..
1. centrosomes
2. chromosomes

How are Spindle Microtubules Made?

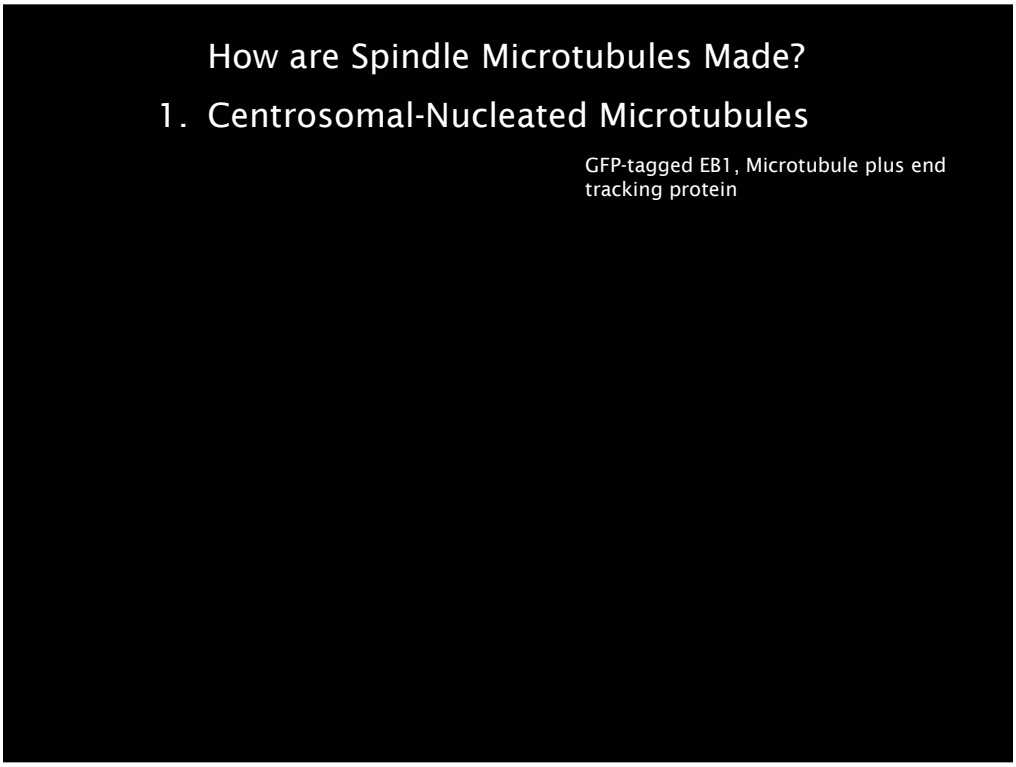
1. Centrosomal-Nucleated Microtubules



"Search and capture" from the centrosome
Kirschner and Mitchison, Cell 1986

How are Spindle Microtubules Made?

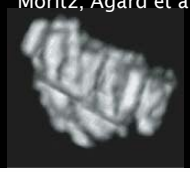
1. Centrosomal-Nucleated Microtubules

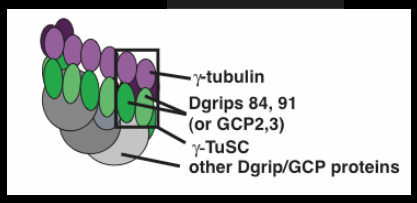


GFP-tagged EB1, Microtubule plus end tracking protein

The γ-Tubulin Ring Complex

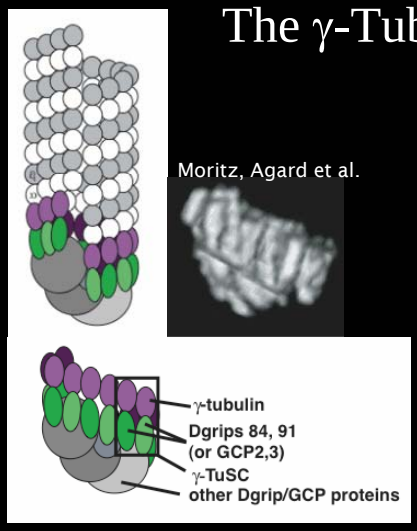
Moritz, Agard et al.





γ-TuSC (2 proteins + γ-Tubulin) is a stable subcomplex of γ-TuRC

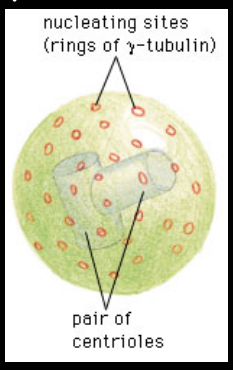
The γ-Tubulin Ring Complex



γ-TuSC (2 proteins + γ-Tubulin) is a stable subcomplex of γ-TuRC

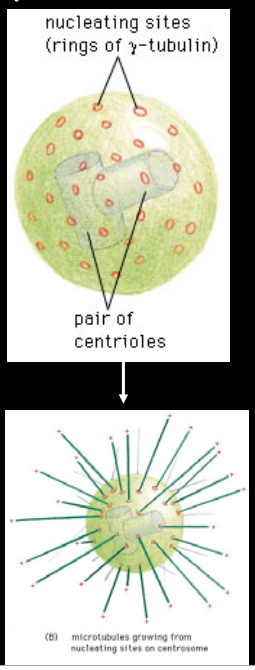
The γ-Tubulin Ring Complex

Centrosome Docking of γ-Tubulin is mediated by the "Centrosomin" Protein (Kaufman et al.)

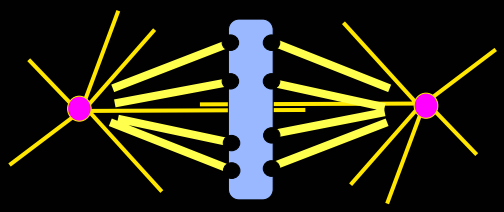


The γ-Tubulin Ring Complex

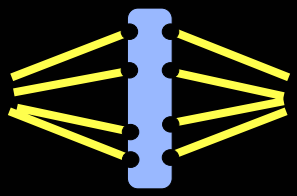
Centrosome Docking of γ-Tubulin is mediated by the "Centrosomin" Protein (Kaufman et al.)



However spindles can form without centrosomes!



However spindles can form without centrosomes!



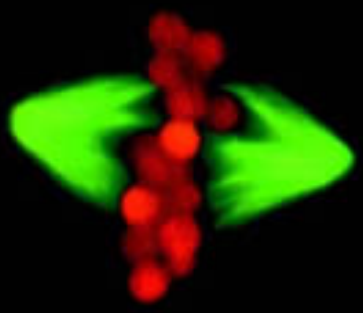
Plant cells and animal germ cell meiosis

Khodjakov, Reider et al. have shown that somatic cells also make mitotic spindles when their centrosomes are ablated.

How are Spindle Microtubules Made?

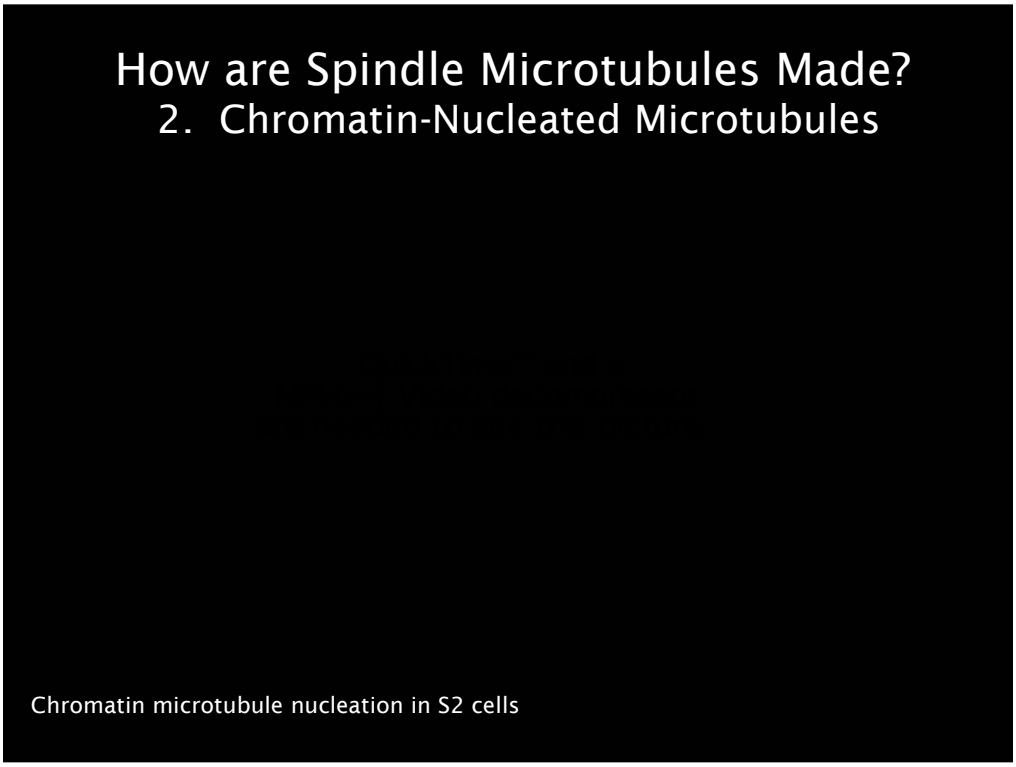
2. Chromatin-Nucleated Microtubules

Microtubule Nucleation Around DNA Beads in Xenopus Meiotic Extracts



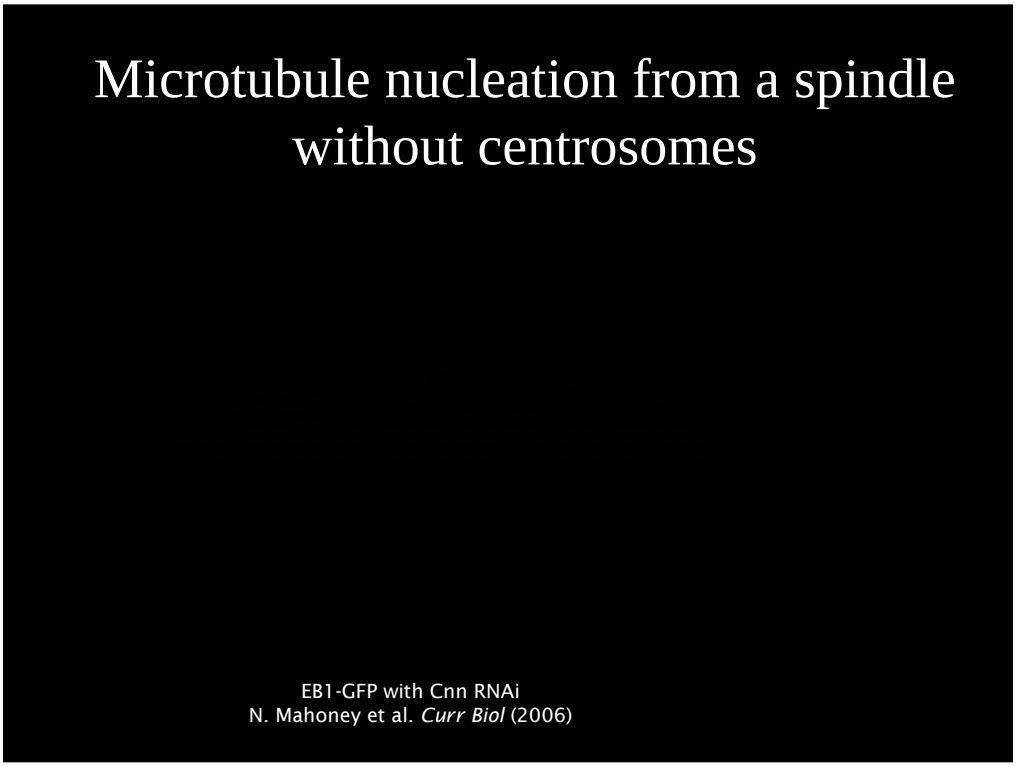
Heald and Karsenti, Nature 1996

How are Spindle Microtubules Made?
2. Chromatin-Nucleated Microtubules



Chromatin microtubule nucleation in S2 cells

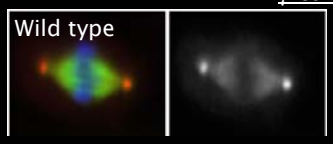
Microtubule nucleation from a spindle without centrosomes



EB1-GFP with Cnn RNAi
N. Mahoney et al. *Curr Biol* (2006)

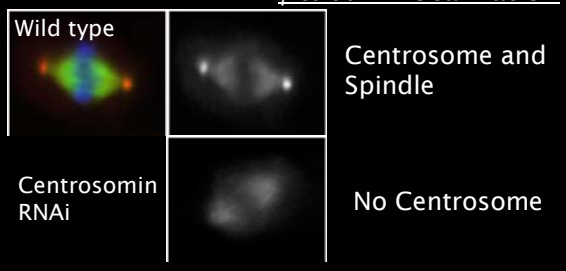
Dgt (dim γ-tubulin) RNAi phenotype

γ-tubulin localization

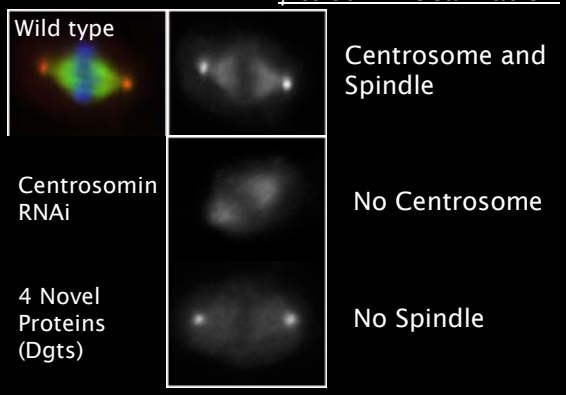


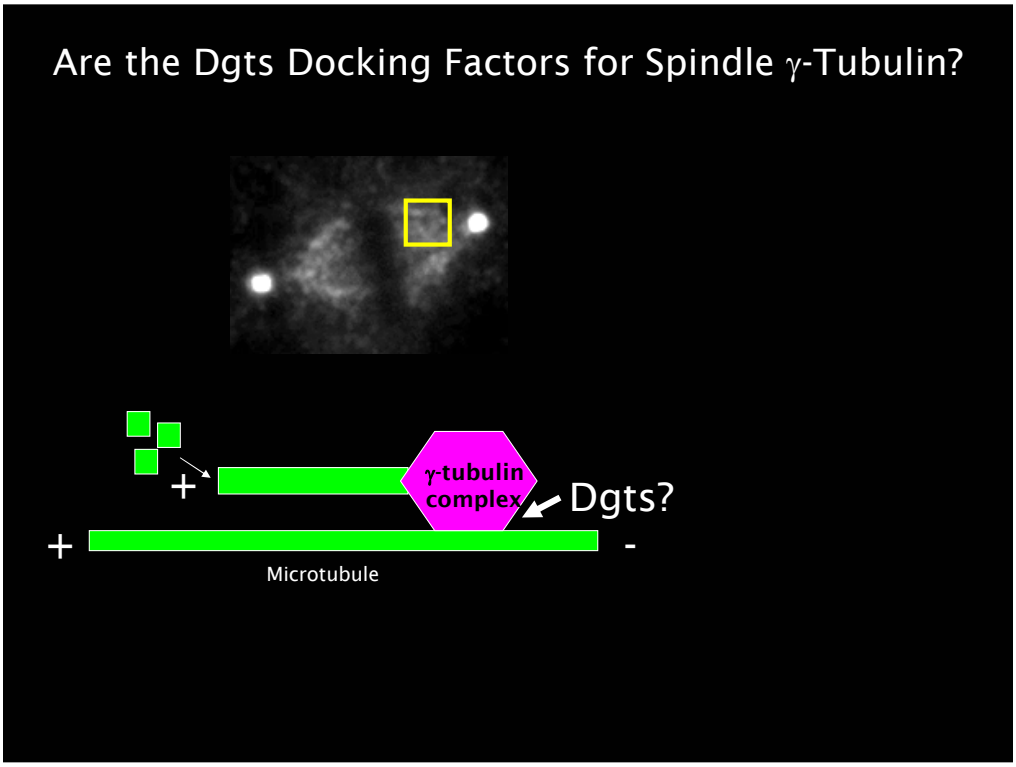
Centrosome and Spindle

Dgt (dim γ-tubulin) RNAi phenotype



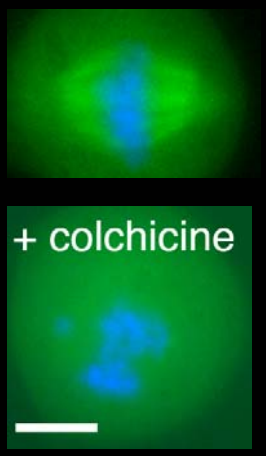
Dgt (dim γ-tubulin) RNAi phenotype




Are the Dgts Docking Factors for Spindle γ-Tubulin?
γ-tubulin complex
Dgts?
+
+
-
Microtubule

Are the Dgts Docking Factors for Spindle γ-Tubulin?

Dgt3-GFP

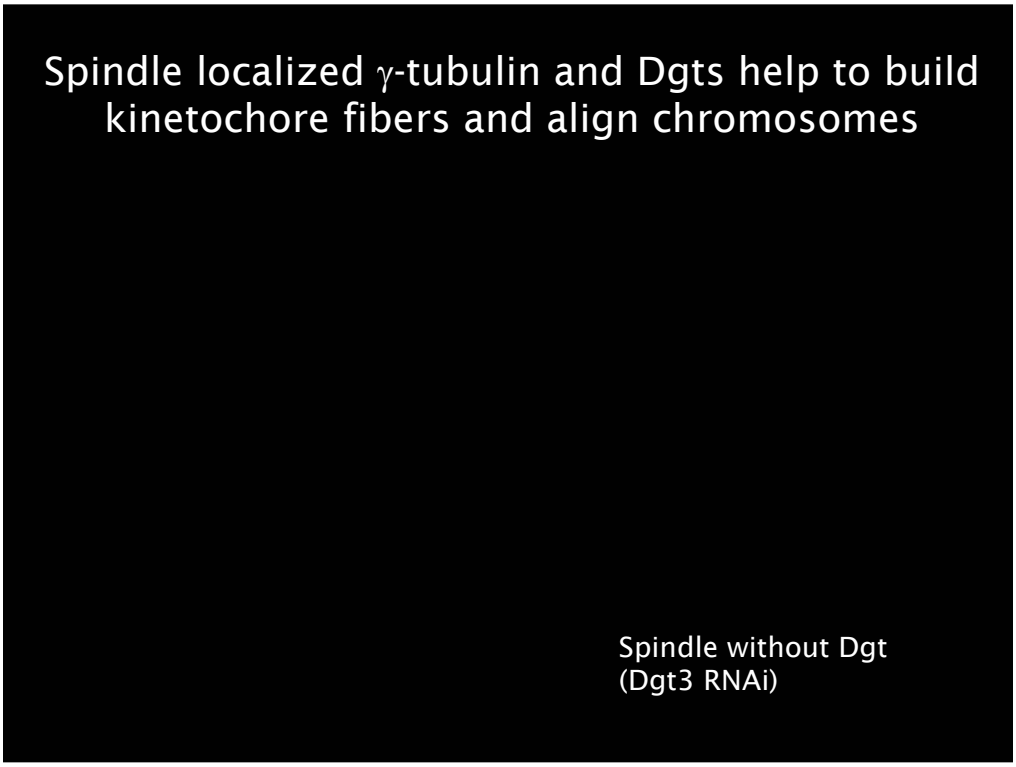


Dgts are in the right spot to be spindle-specific γ-tubulin docking factors

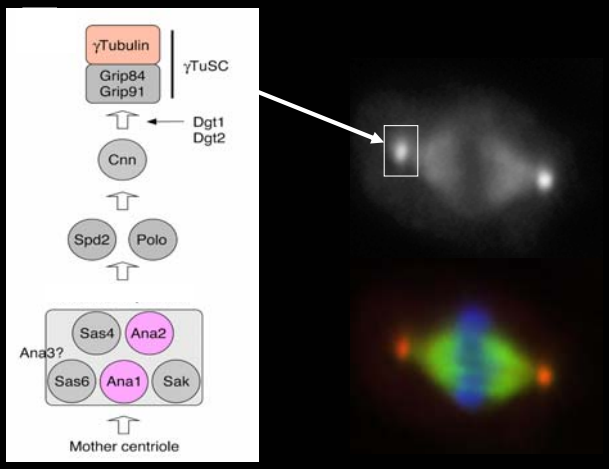
Is spindle localization of γ-tubulin by Dgts important?

High Throughput Live Cell Imaging to Examine RNAi Phenotypes

Spindle localized γ-tubulin and Dgts help to build kinetochore fibers and align chromosomes

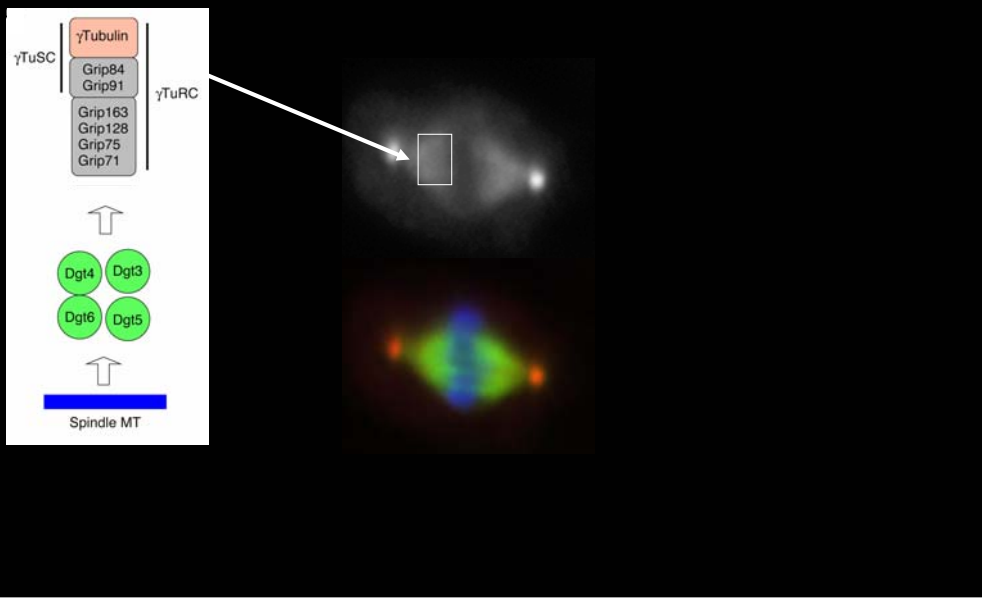


Molecular Model for γ-Tubulin Localization (From the RNAi screen and follow-up)



J. Luders, U.K. Patel, T. Stearns *Nature Cell Biol.* (2006)
Verollet et al. *J. Cell Biol.* (2006)

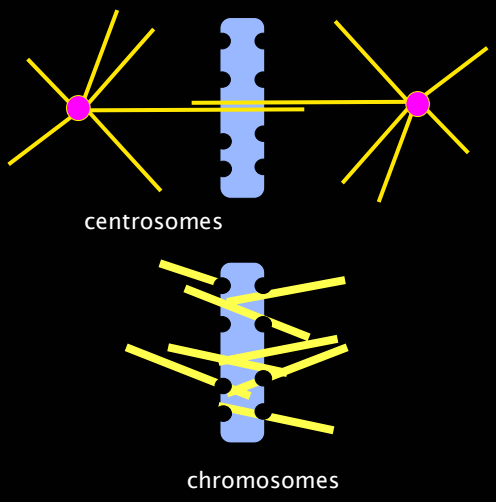
Molecular Model for γ-Tubulin Localization (From the RNAi screen and follow-up)

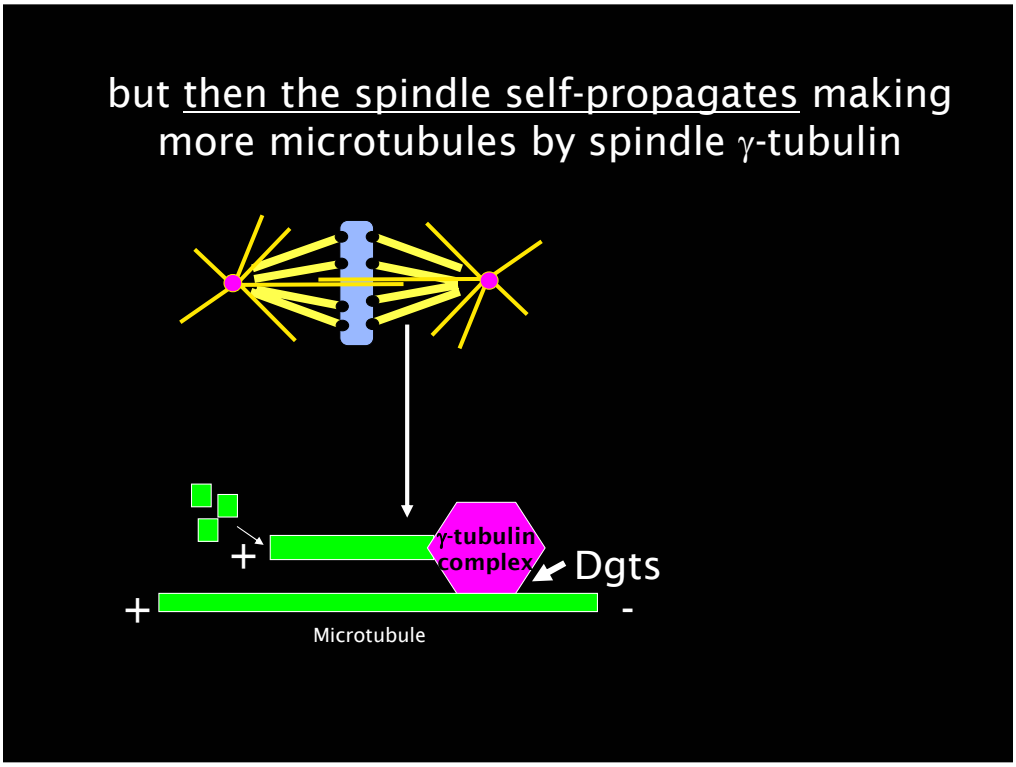


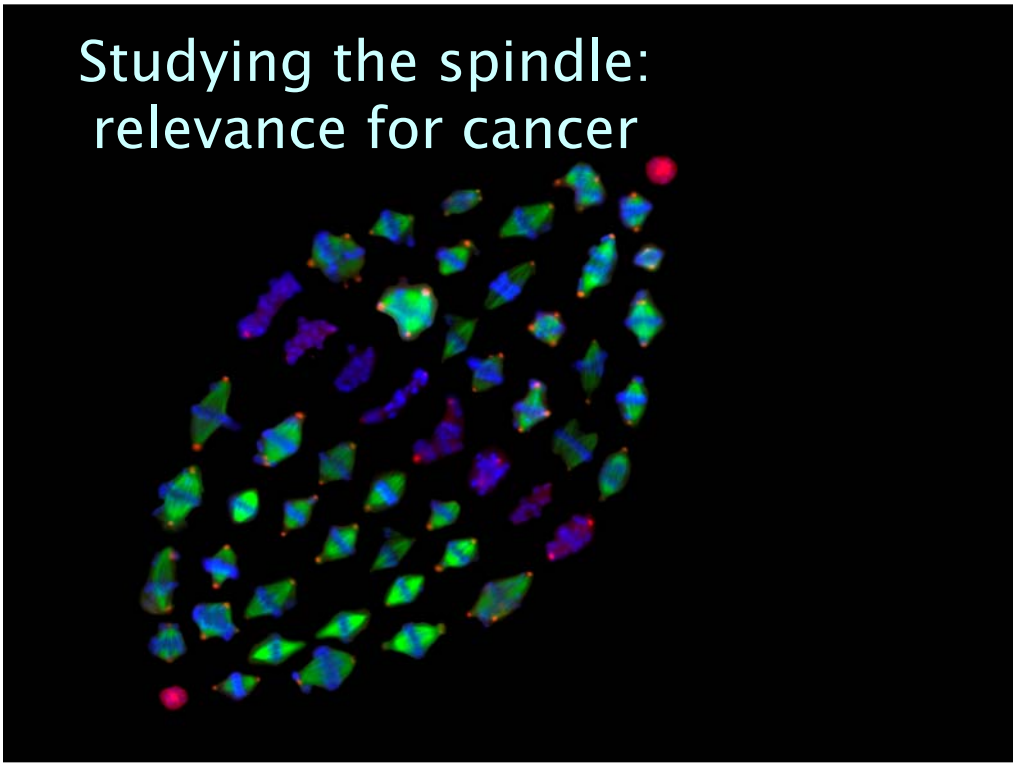
Spindle localized γ-tubulin appears to be more important than the centrosome in Drosophila!

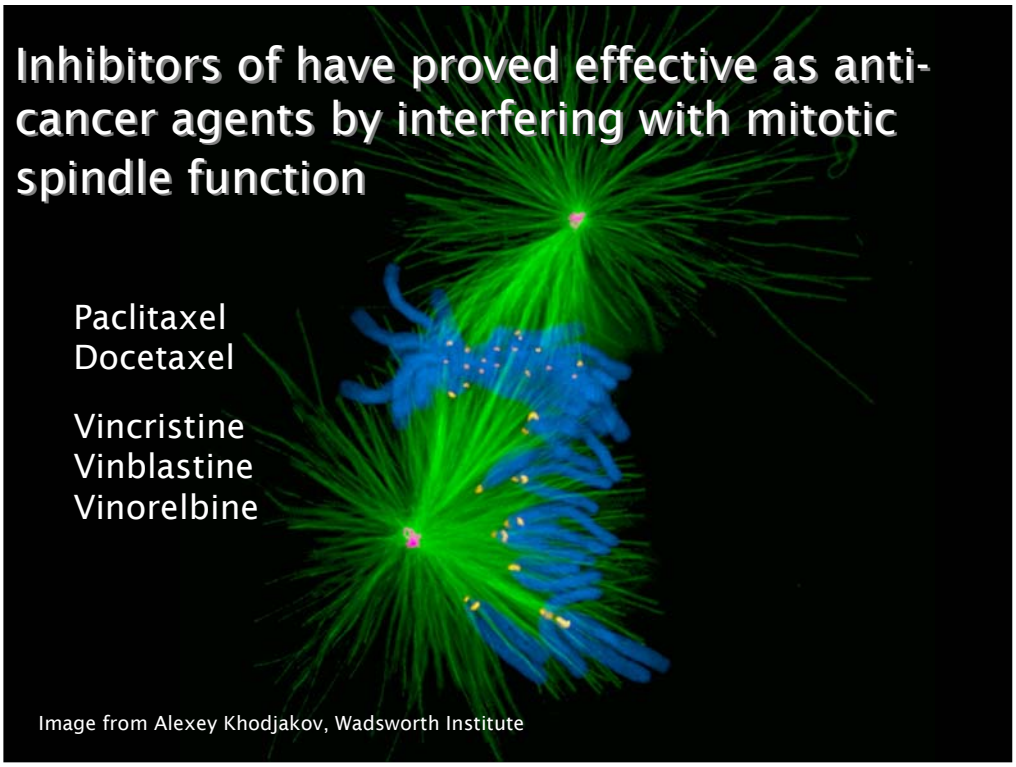
(Loss leads to spindle defects/chromosome misalignment)

Hypothesis:
Centrosomes and/or chromatin get the spindle started by nucleating the initial microtubules,



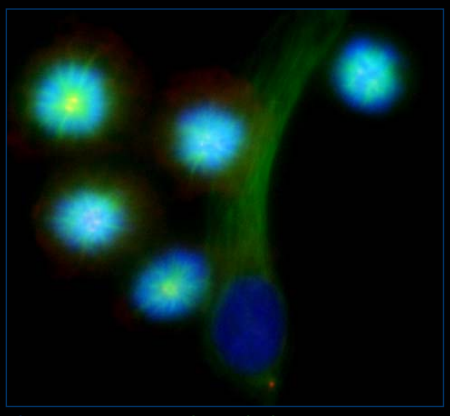

but then the spindle self-propagates making more microtubules by spindle γ-tubulin
γ-tubulin complex
Dgts
+
+
-
Microtubule


Studying the spindle:
relevance for cancer


Inhibitors of have proved effective as anti-cancer agents by interfering with mitotic spindle function
Paclitaxel
Docetaxel
Vincristine
Vinblastine
Vinorelbine
Image from Alexey Khodjakov, Wadsworth Institute

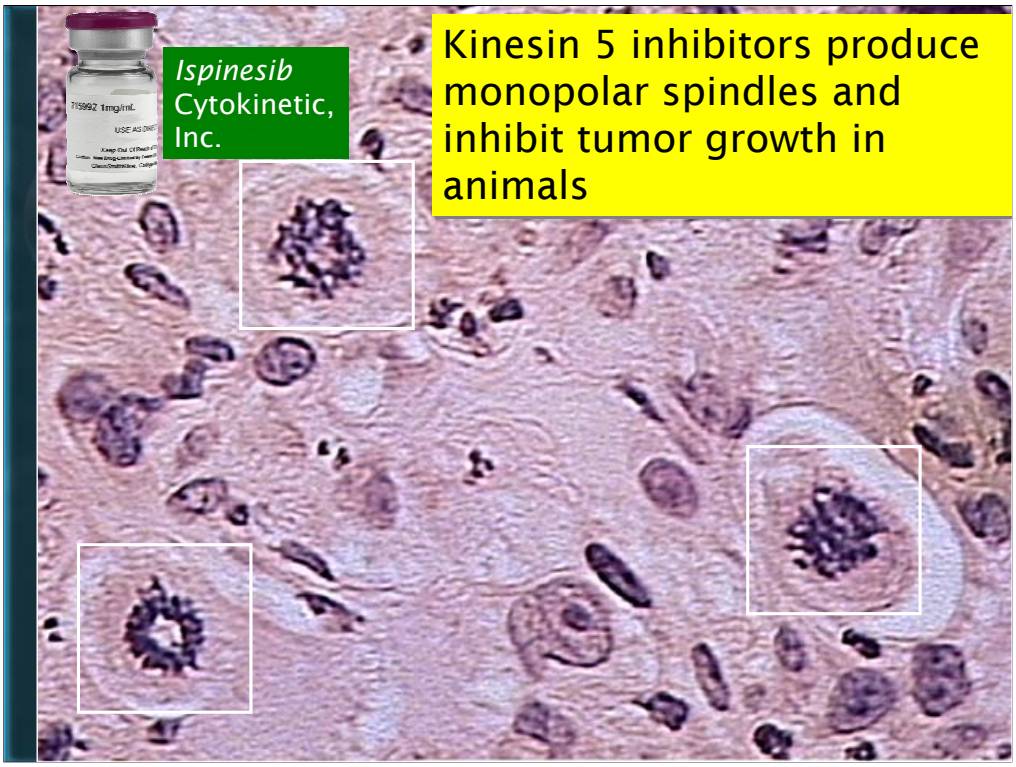
Kinesin 5 (Mitosis Specific) is Essential for Bipolar Spindle Formation

Without Kinesin 5 function- monopolar spindles

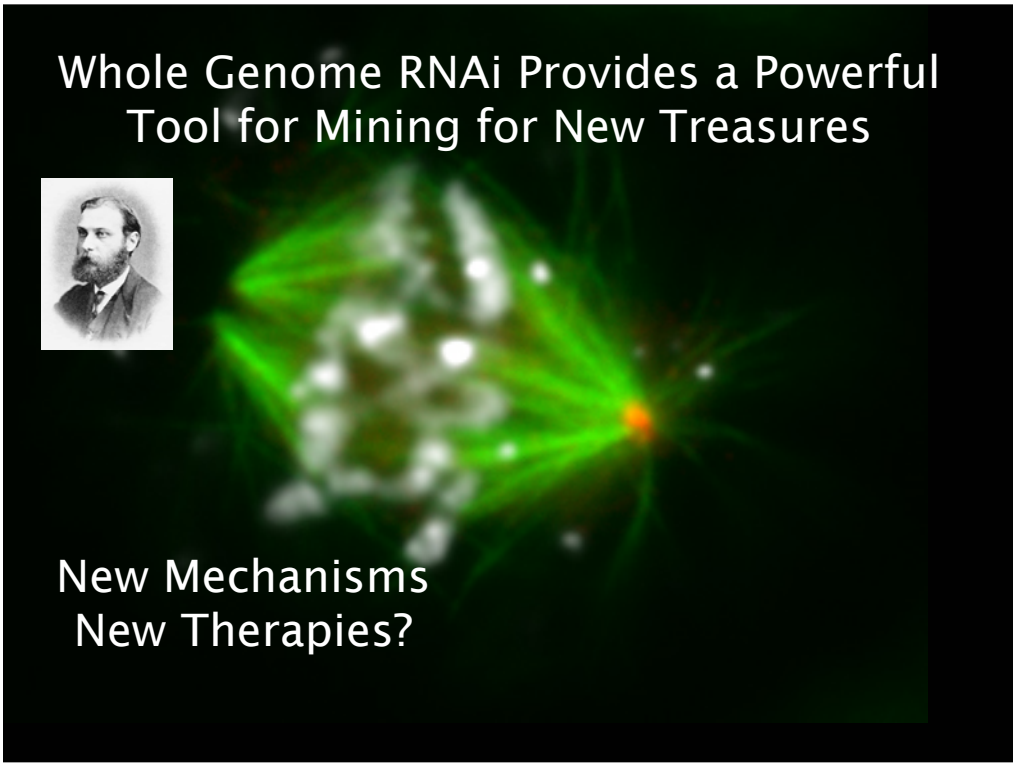


Chromosomes – Microtubules – Centrosomes

(SKOV3)


Ispinesib
Cytokinetic, Inc.
Kinesin 5 inhibitors produce monopolar spindles and inhibit tumor growth in animals


Ispinesib
Cytokinetic, Inc.
Inhibitors of Mitotic Kinesins are Now in Phase II Clinical Trials


Whole Genome RNAi Provides a Powerful Tool for Mining for New Treasures
New Mechanisms
New Therapies?