# Cellular Division and TANGLED in Maize

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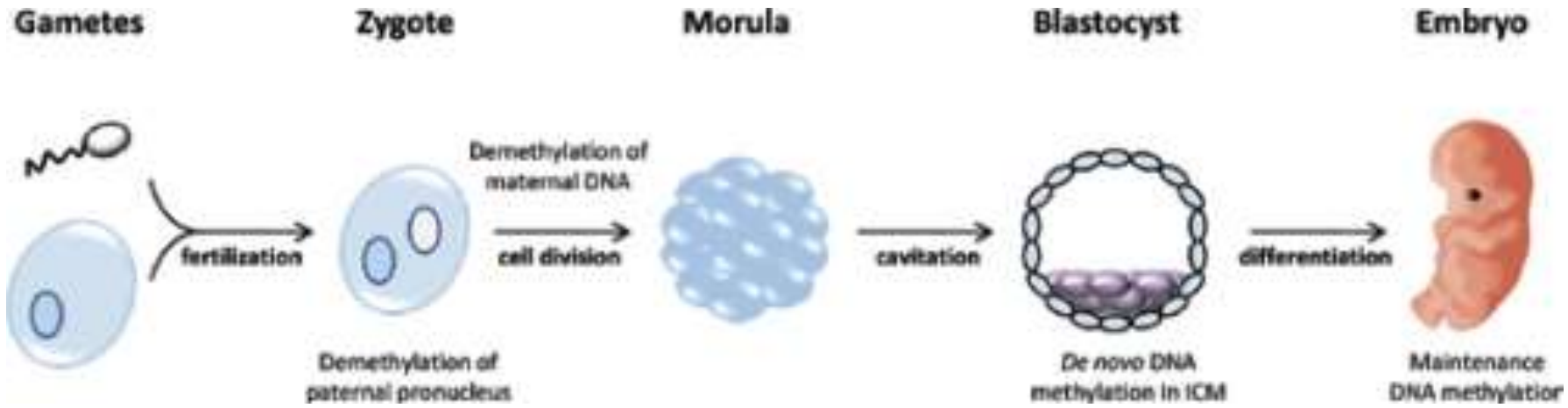
# Cellular Division is essential for life

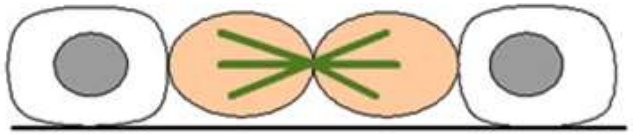
Healthy cellular division ensures the proper growth and development of many organisms.



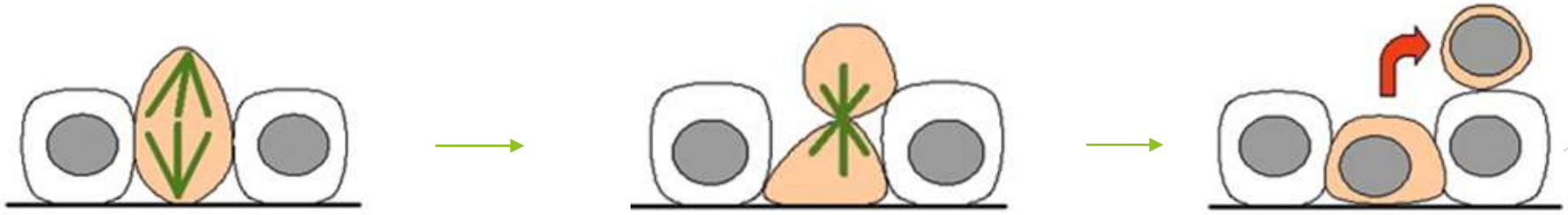

http://epigenie.com/epigenetics/epigenetic-regulation/

# Orientation of cellular division plays a key role in the metastasis of cancers

Normal Cell Orientation



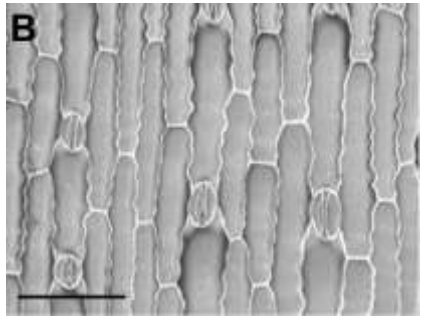
Abnormal Cell Orientation



Vasiliev J. M. et.al. PNAS 2004;101:12526-12530

# This mechanism can be explored with maize

Plants are a great model system to explore the orientation of cellular division because their cells are fixed, and have rigid shape.

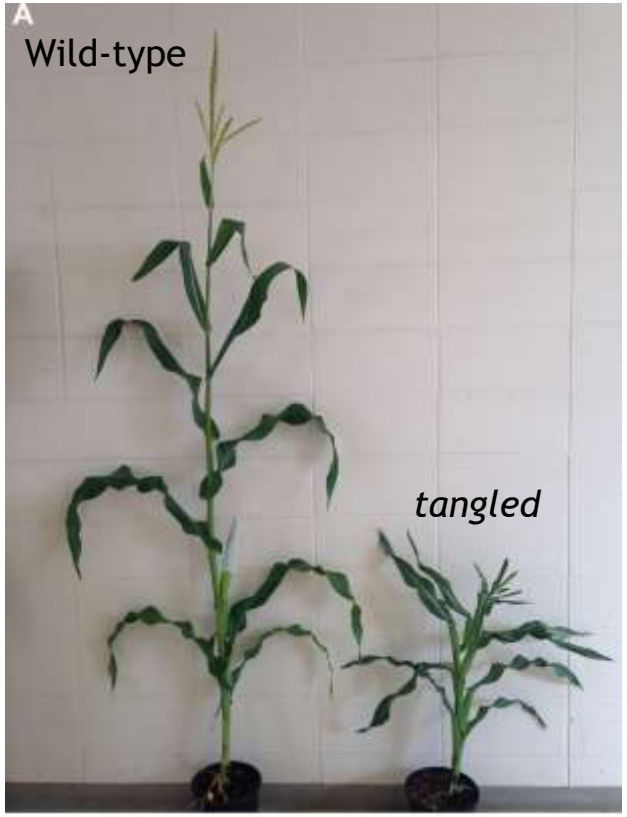




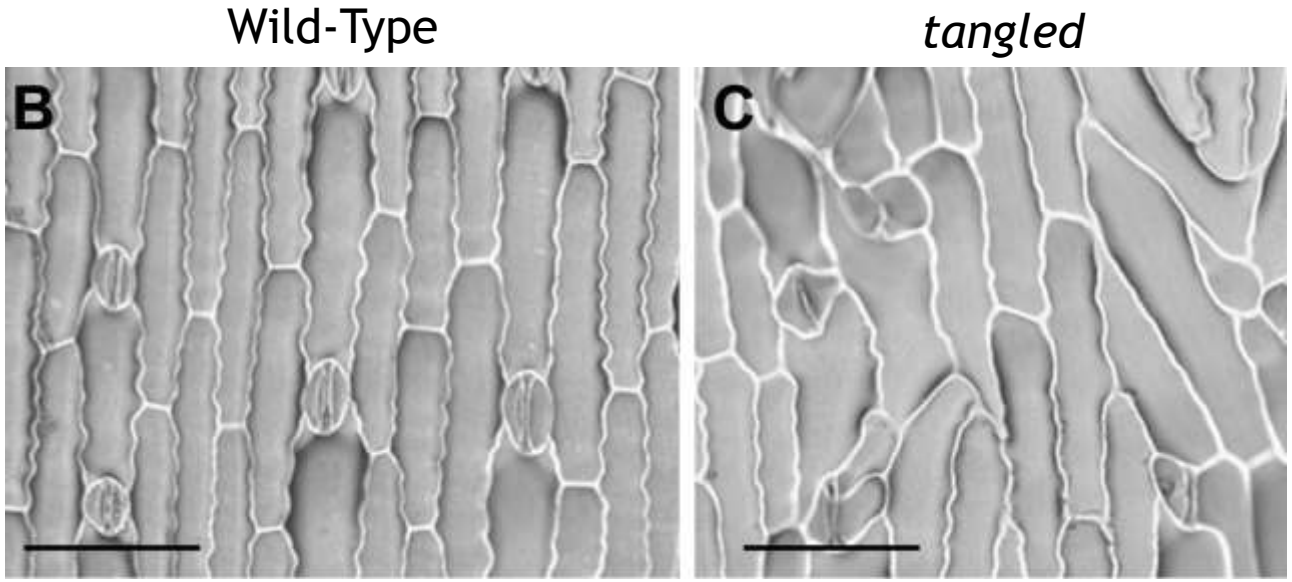
However, the knowledge we can gain from plant cells can be applied to animal cells due to these shared properties:

- Anti-parallel array of microtubules
- Extensive vesicle trafficking toward the site of division

# In maize, a mutant is present called ‘tangled’



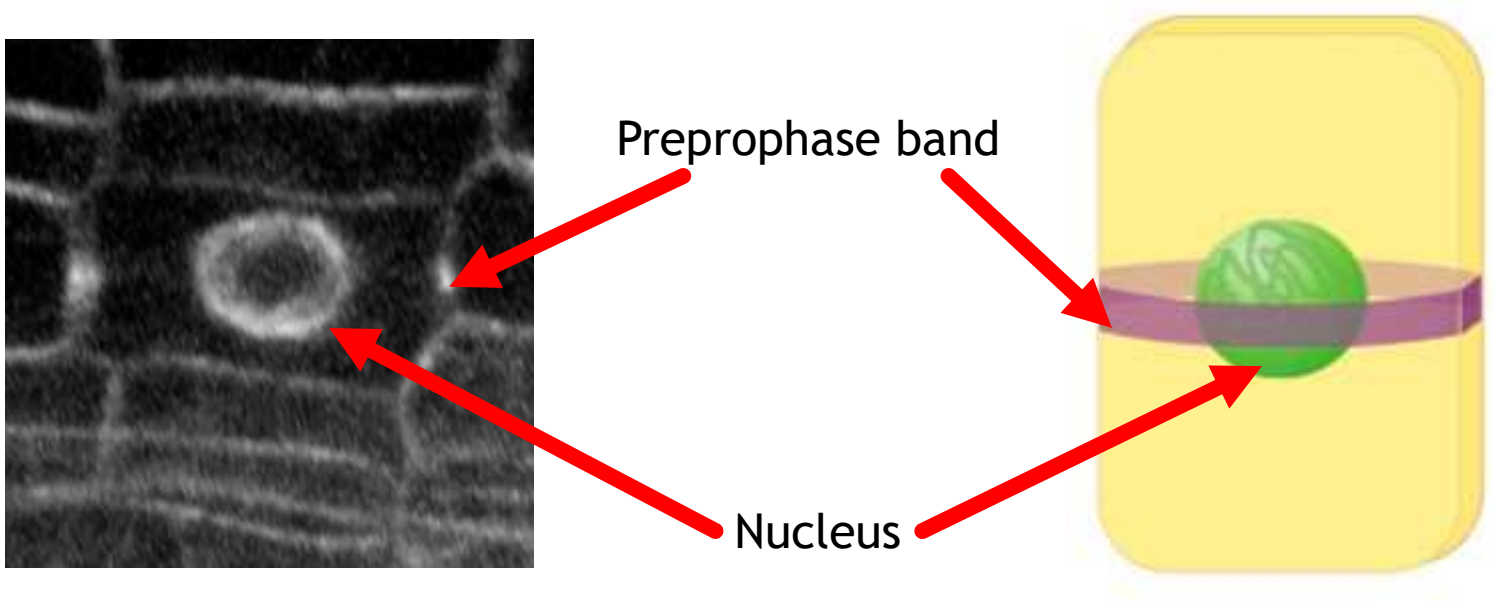

The *tangled* mutant is much shorter than the wild-type plant.




The cells of *tangled* mutants are much more disorganized.

# Division begins with prophase

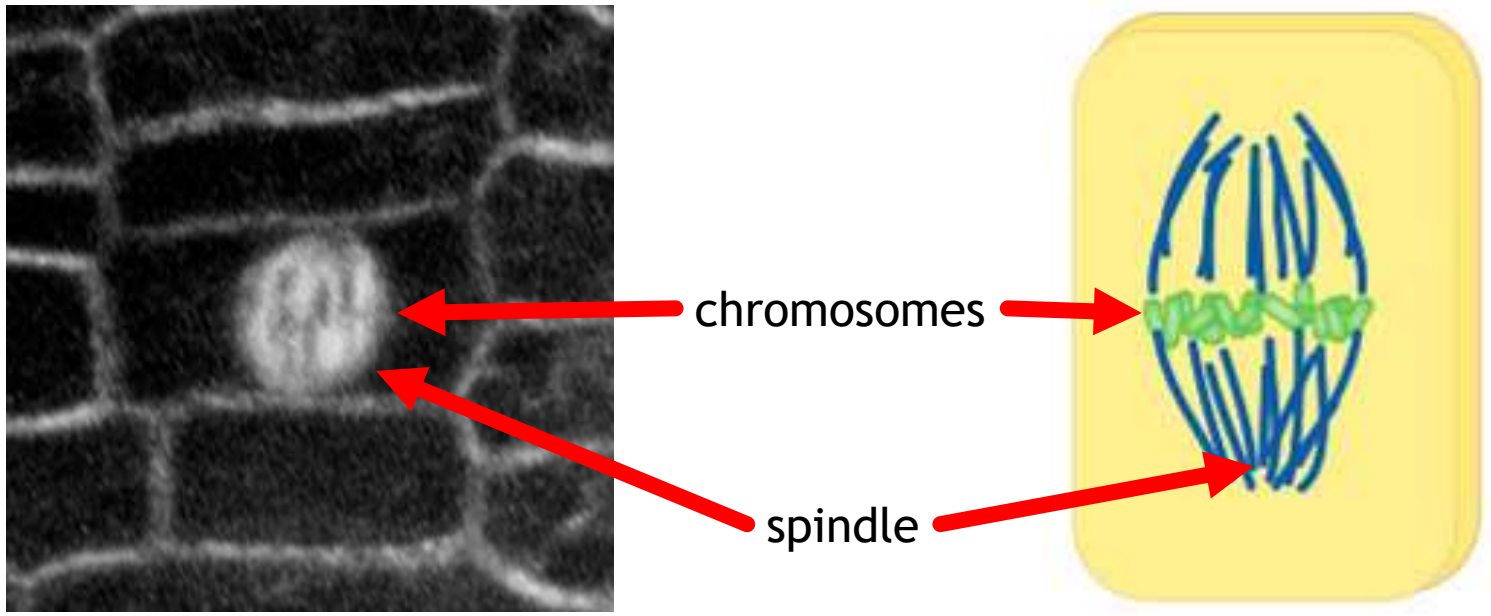
- First, a preprophase band forms around the cell, and acts as a marker for where the new cell wall will form.
- The DNA begins to condense inside the nucleus to form chromosomes




Modified from: Rasmussen, CG et al Annual Reviews Plant Biology 2011

# Next, the cell progresses through metaphase

- The spindle forms and chromosomes align.

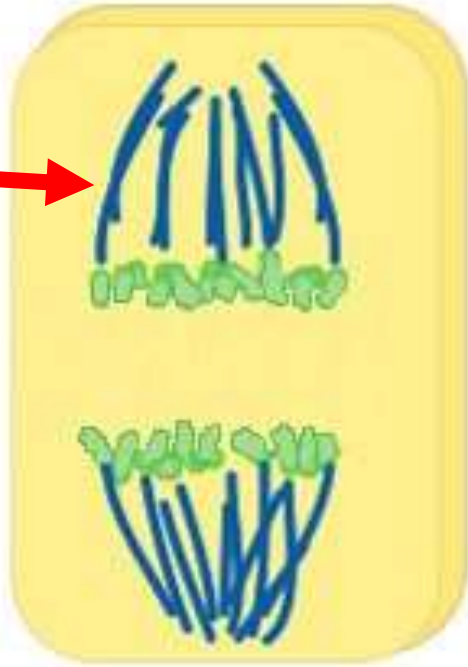



Modified from: Rasmussen, CG et al Annual Reviews Plant Biology 2011

# In anaphase, the chromosomes are pulled to either side of the cell

- The spindle pulls the two sets of chromosomes to opposite sides of the cell.

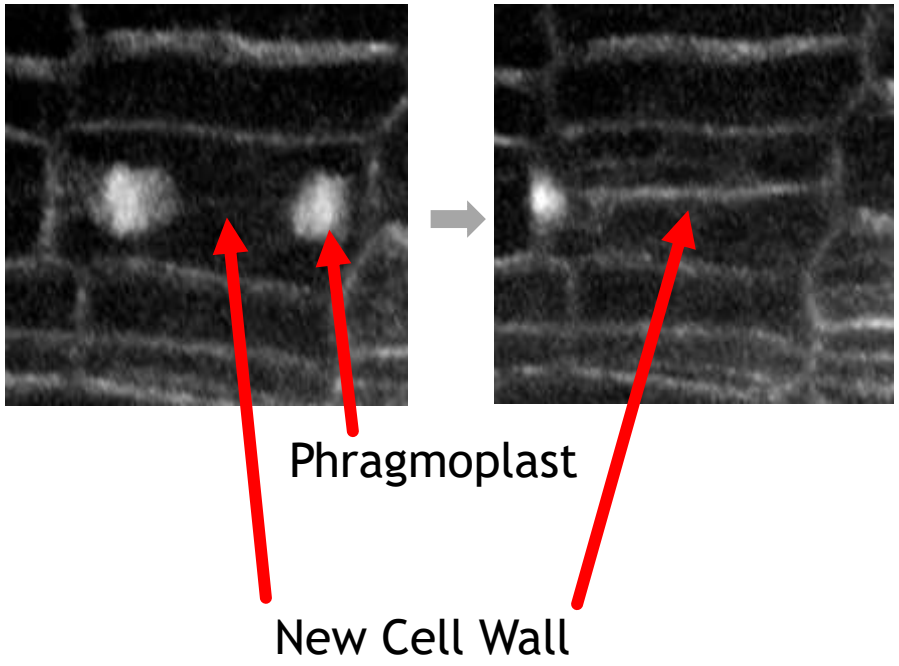
spindle

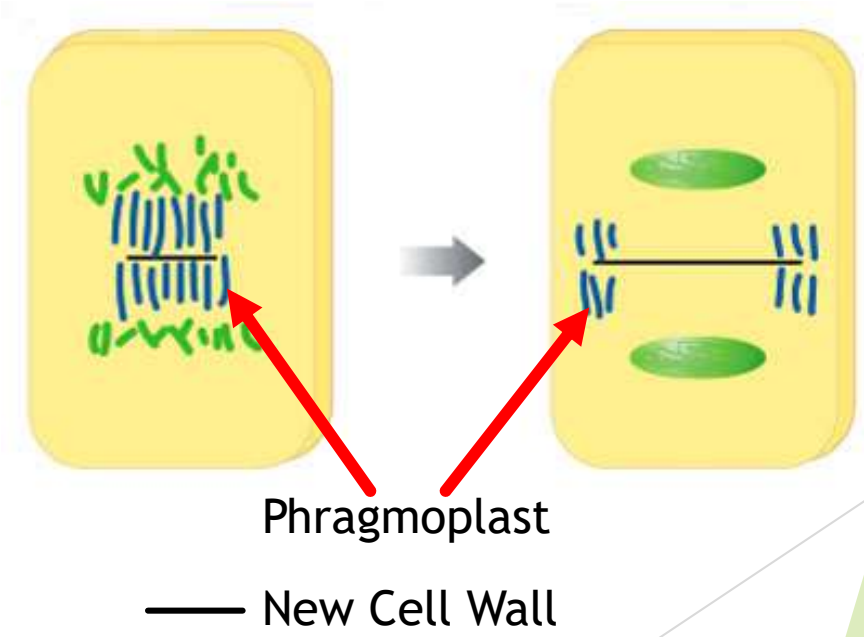


Modified from: Rasmussen, CG et al Annual Reviews Plant Biology 2011

# Finally, the cell completes division with telophase.

- The new cell wall begins to form with support from the phragmoplast, and chromosomes uncondense into new nuclei.




Modified from: Rasmussen, CG et al Annual Reviews Plant Biology 2011

# Why do *tangled* cells display disorganization and misorientation?

Hypothesis: Wild-type and *tangled* plants show differences during cellular division

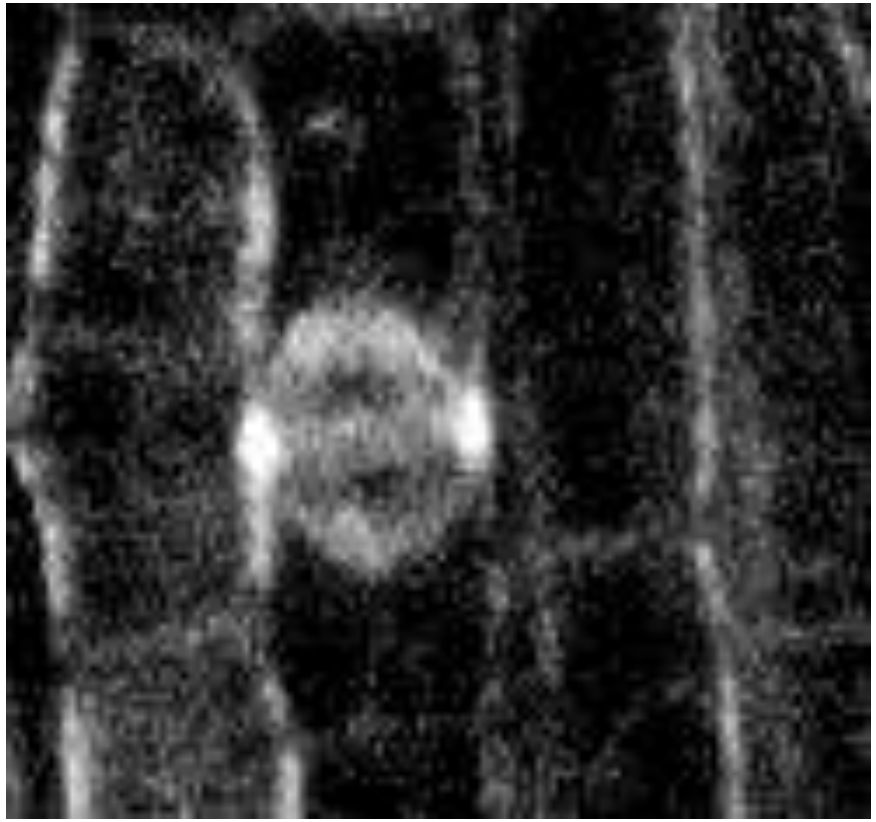
# Observing cellular division

- Both wild type and *tangled* plants were tagged with a fluorescent marker, CFP-Tubulin
- Fluorescence is only visible under 443nm laser applied to the sample during division

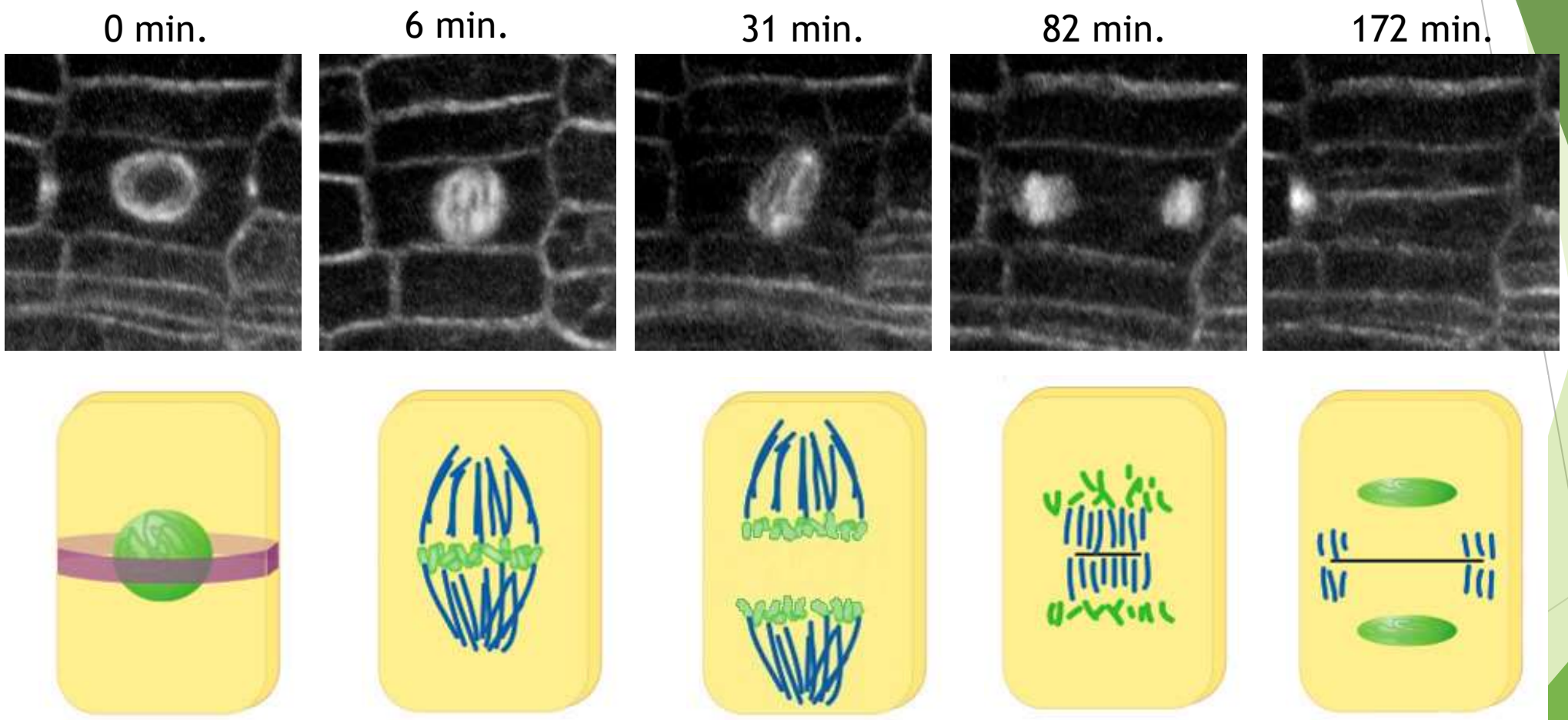
# Materials and Methods

- Samples were taken from 3-5 week old plants of each phenotype
- Samples were observed on a confocal microscope using 20x and 40x objectives
- Cellular structures were illuminated with a 443 nm laser
- Live cell imaging was performed on the cells from prophase until telophase.
- Data were collected, and completion times for each mitotic stage was recorded.

# Timelapse progression of a wild-type cell

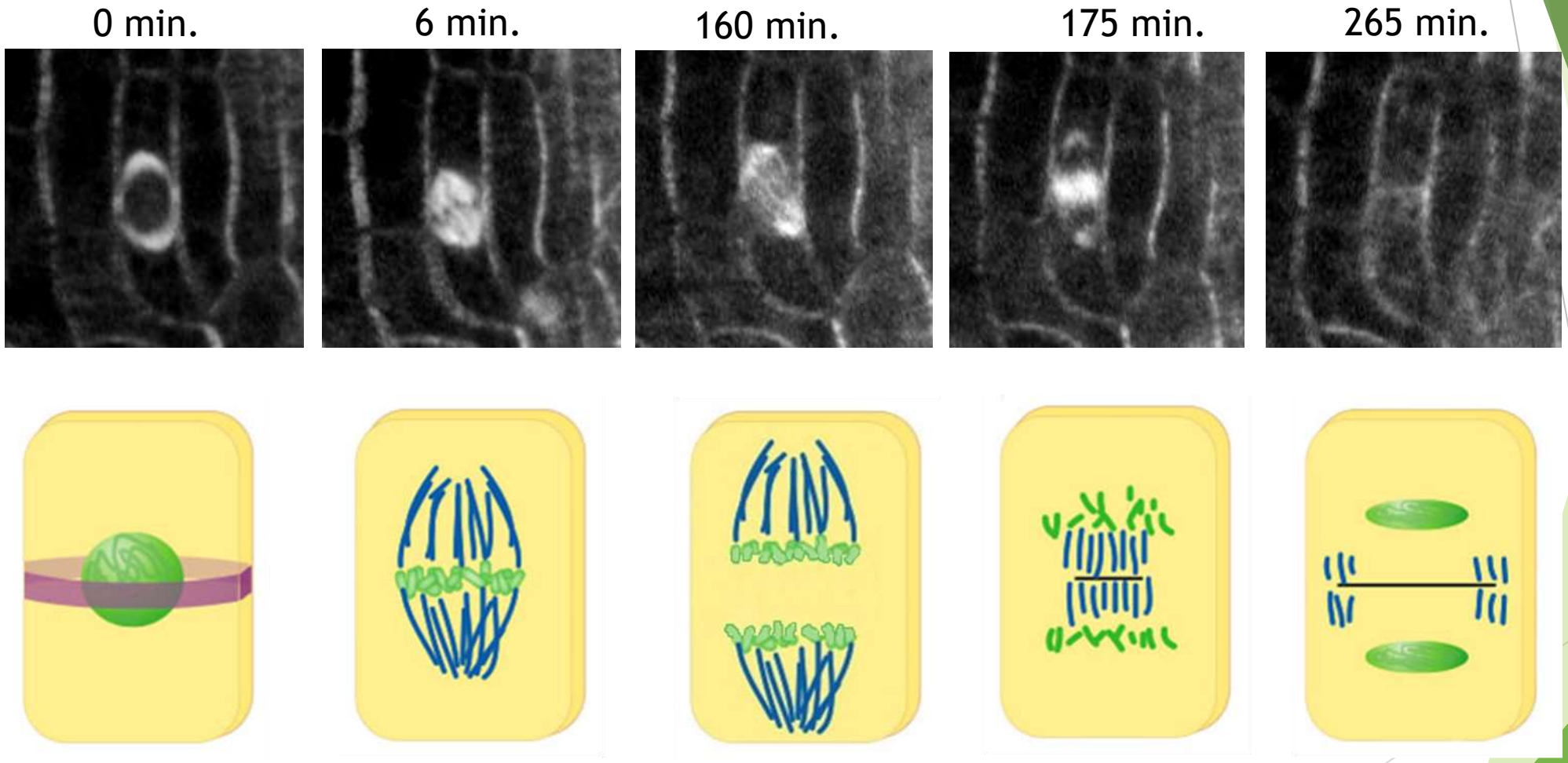


# Representative wild-type cells




Modified from: Rasmussen, CG et al Annual Reviews Plant Biology 2011

# Representative *tangled* cells

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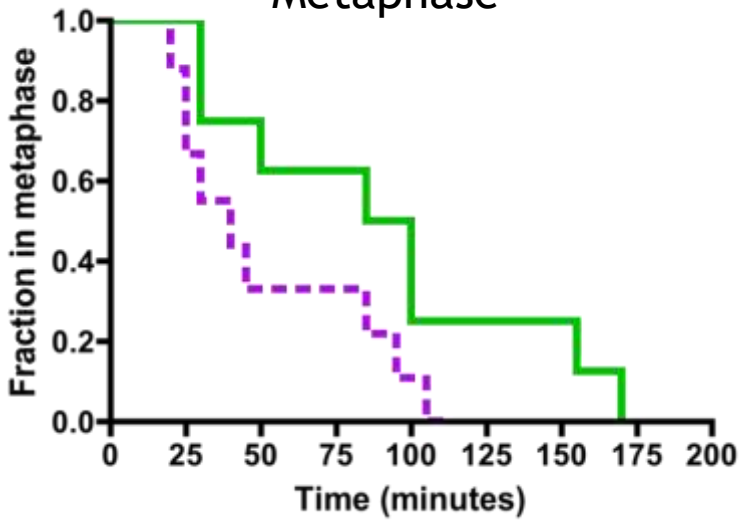
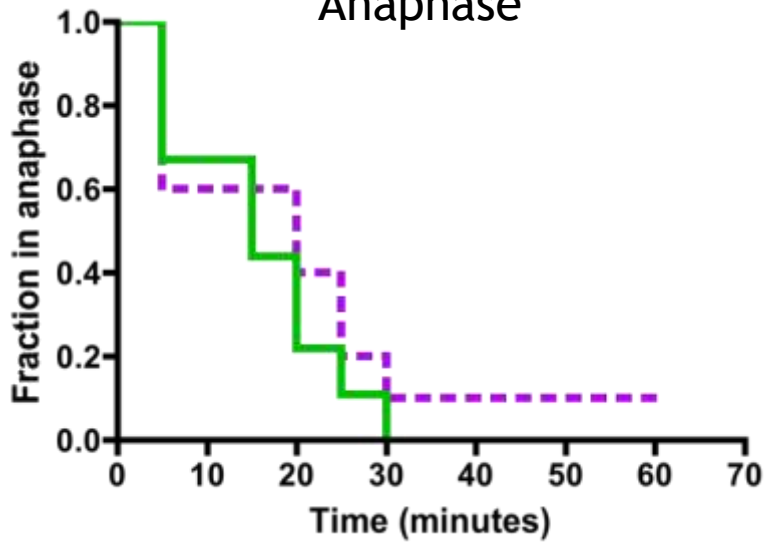
# Stage duration in wild-type and *tangled* cells

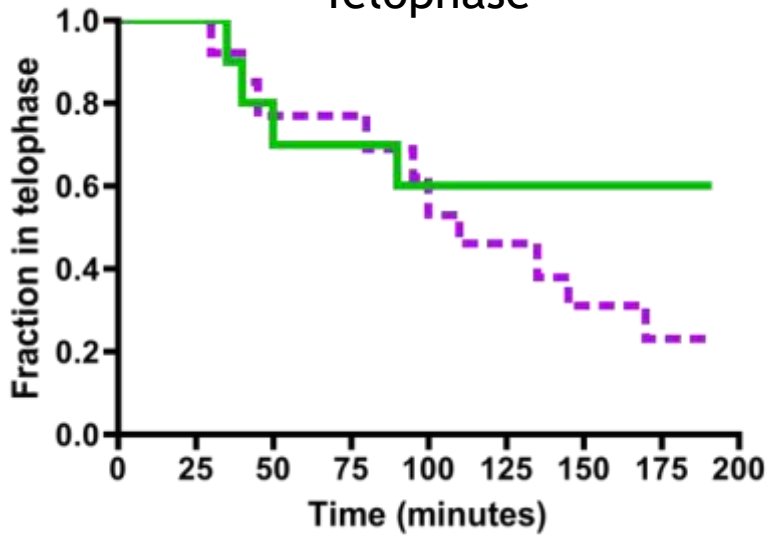
Metaphase




Anaphase




Telophase



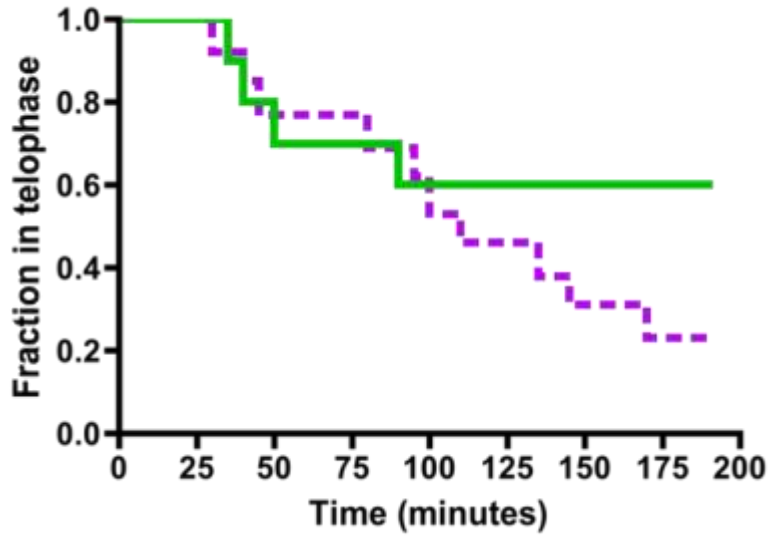

Legend

tangled

wild-type

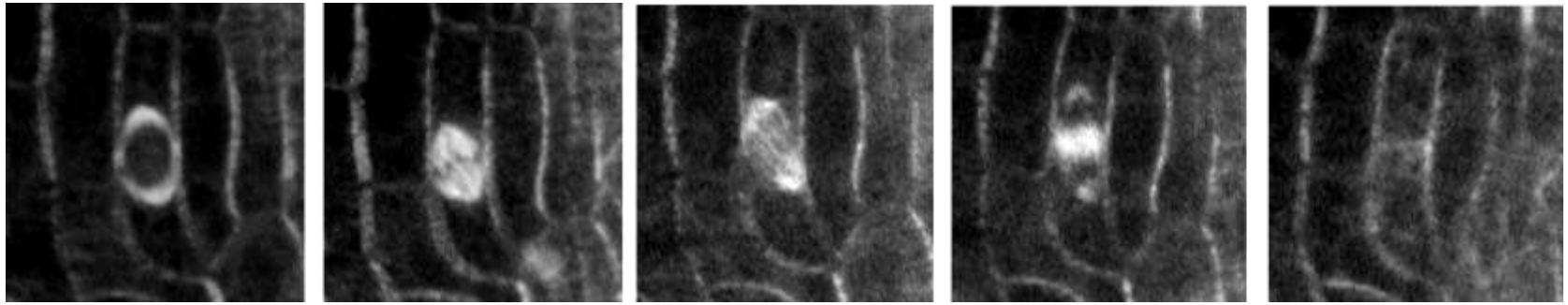
# Discussion of Results

- The abnormally high rates of stalling for tangled cells in telophase suggest that the protein TANGLED is critical during this stage.



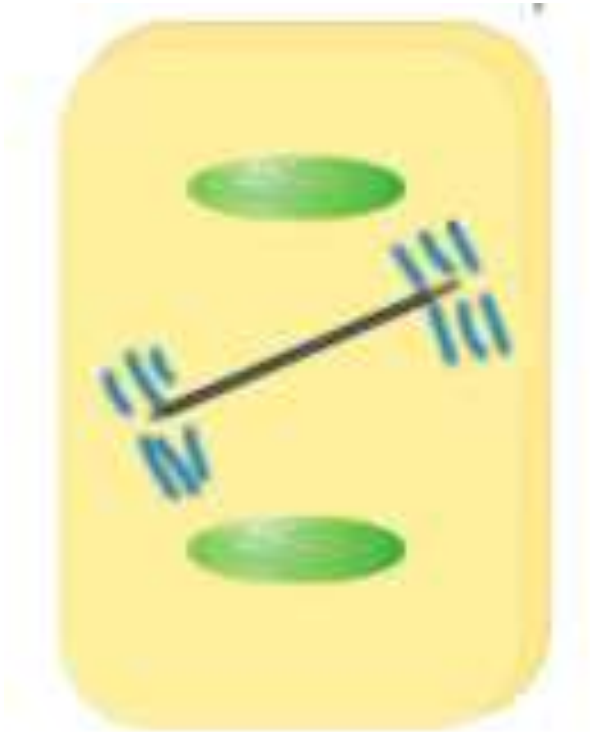
# Discussion of Results

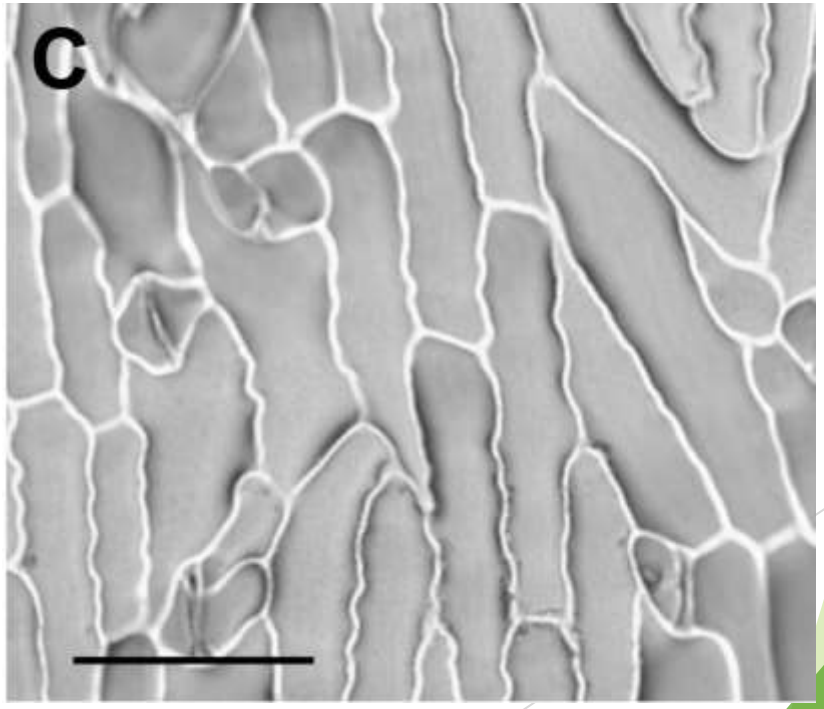
- The cytokintetic machinery was not observed becoming misoriented.



# Future Directions

- In order to clarify the mechanism by which the division site is maintained, it would help to run perform longer time-lapse imaging of tangled cells.



# Conclusions

- Tangled mutants show slowed division, largely due to delays in telophase.
- Long delays in telophase combined with the absence of misoriented pre-telophase cytokinetic machinery suggests high TANGLED activity in telophase
- Observation of telophase completion will be crucial in illuminating the true role of tangled in the cellular division orientation.

# Acknowledgements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# References


- Cleary AL, Smith LG. (1998). The Tangled1 gene is required for spatial control of cytoskeletal arrays associated with cell division during maize leaf development. Plant Cell 10: 1875-88
- Mohanty A, Luo A, DeBlasio S, Ling X, Yang Y, et al. (2009). Advancing cell biology and functional genomics in maize using fluorescent protein-tagged lines. Plant Physiol 149: 601-5
- Smith, L.G., Hake, S, and Sylvester, A.W. (1996). The tangled-1 mutation alters cell division orientations throughout maize leaf development without altering leaf shape. Development, 122, 481-489.
- Rasmussen, C. G., Humphries, J. A., & Smith, L. G. (2011). Determination of symmetric and asymmetric division planes in plant cells. Annu Rev Plant Biol, 62, 387-409.
- Walker, K. L., Muller, S., Moss, D., Ehrhardt, D. W., & Smith, L. G. (2007). Arabidopsis TANGLED identifies the division plane throughout mitosis and cytokinesis. Curr Biol, 17(21), 1827-1836.
- http://epigenie.com/epigenetics/epigenetic-regulation/
- http://openi.nlm.nih.gov/detailedresult.php?img=2842294_pone.0009654.g003&req=4