The Role of the Human Translationally Controlled Tumor Protein (TCTP) in Mitosis

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Abstract

Mitosis is the complex process that results in division of DNA and other cellular components into two daughter cells. Successful cell division requires that microtubules of the mitotic spindle attach to the kinetochores of mitotic chromosomes. These attachments are used for both aligning chromosomes at the spindle equator and for the physical separation of sister chromosomes during anaphase. In the study of how microtubules and kinetochores work together during mitosis, researchers are searching for new proteins that may help to piece together this complex puzzle. The Translationally Controlled Tumor Protein (TCTP) has been suggested to play a role in mitotic cell division, as it has been immuno-localized to the mitotic spindle, however, its precise function in mitosis remains unknown. The goal of my project is to determine the role of human TCTP in mitosis. Thus far, my research has shown that TCTP is located at the microtubules during mitosis, and when knocked down by siRNA treatment, human HeLa H2B-GFP cells (GFP-tagged chromosomes) are either unable to complete mitosis or take an extended time to do so. Currently, I am undergoing procedures to pick the most suitable human cell line in which to study the roles of TCTP during mitosis (HeLa-S3, HeLa-Kyoto, or HeLa H2B-GFP), and a mitotic index and characterization of this cell line. In the future, I plan to look in to the association between F-actin and TCTP, recently brought to light by Bazile et al, 2009.

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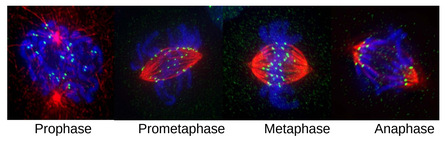
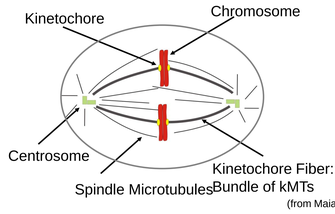
Background: Mitosis

- The process by which eukaryotic cells divide their genetic material and cellular components
- Mitosis takes place in the following steps:
  - Prophase: chromosomes condense
  - Prometaphase: begins with nuclear envelope breakdown; centrosomes begin to separate to opposite sides of the cell; chromosomes begin to line up in the middle of the cell
  - Metaphase: chromosomes are lined up in the center of the cell with microtubule attachments by each sister chromatid to one spindle pole
  - Anaphase: sister chromatids are pulled apart, one to each spindle pole, destined for separate daughter cells



Prophase Prometaphase Metaphase Anaphase

Mitotic Spindle



(from Maiato et al., 2005)

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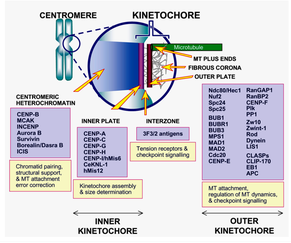
Background: Kinetochore

- Kinetochore links a sister chromatid to its corresponding microtubule from a single spindle pole
- Critical for robust microtubule attachments to chromosomes
- The inner kinetochore is composed of chromatin, while the outer kinetochore is a dynamic, proteinacious network
- Microtubules bind to the outer kinetochore, however the mechanism is still unknown

The Kinetochore



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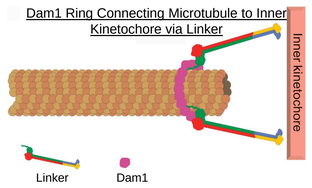
Background: Dam1 Complex

- The Dam1 complex is essential for accurate chromosomal segregation in yeast (Wang et al., 2007)
- It is a ring-like structure that self-assembles around microtubules and binds to both kinetochores and microtubules (Wang et al., 2007)
- Dam1 plays an active role in chromosome segregation and may serve as the link between kinetochore and spindle microtubules (Cheeseman et al., 2000)
- A Dam1 homolog has not yet been found in higher eukaryotes
- Dam1 has been found to be methylated during mitosis



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Objectives and Approach

TCTP was picked up by an antibody screen to identify methylated proteins

TCTP is methylated during mitosis so is being investigated in hopes of finding the higher eukaryote homolog of Dam1.

My objective is to determine the role of TCTP in mitosis by answering the specific questions:
- What is the mitotic phenotype when hTCTP is knocked down?
- What is the mitotic phenotype when hTCTP is overexpressed?

Methods Used:
- Live cell and fixed cell imaging of HeLa cells (Kyoto and H2B-GFP)
  - Measure mitotic timing
- Knock down accomplished with hTCTP siRNA (confirmed with Western Blot)
- Overexpression accomplished with GFP clone of hTCTP sequence

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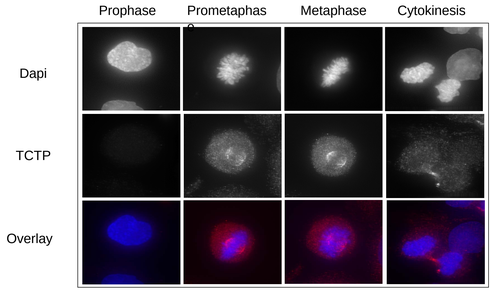
TCTP Localization During Mitosis

HeLa-Kyoto cells stained with TCTP at 1:50 in 4% paraformeldehyde fixation



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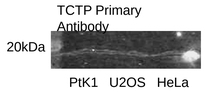
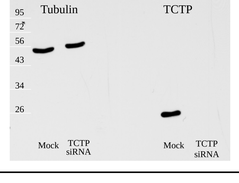
TCTP siRNA Knockdown

Western Blot analysis shows that the TCTP primary antibody works in PtK1 (Rat Kangaroo), U2OS (Human), and HeLa-Kyoto (Human) cell lines (below).



Western Blot protocols also show that TCTP is successfully knocked down in HeLa H2B-GFP cells by the TCTP siRNA (below).



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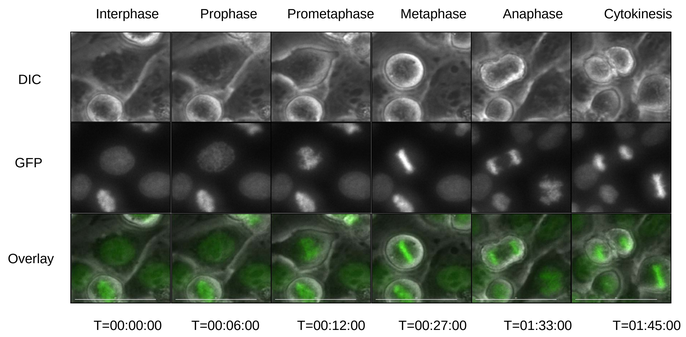
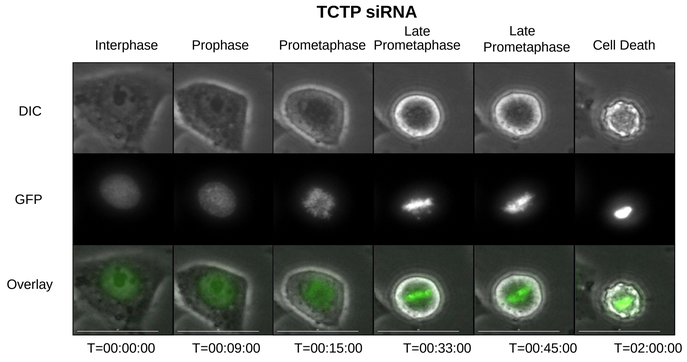
HeLa H2B-GFP: Live-Cell Imaging

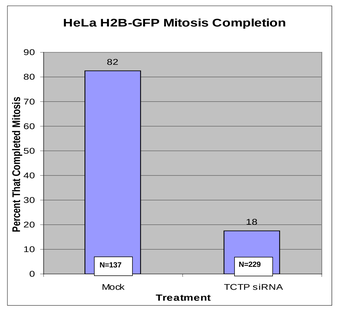
HeLa H2B-GFP cells were treated with TCTP siRNA for 72 hr and then filmed overnight. Timing through mitosis was measured for control and siRNA-treated cells.

Mock



TCTP siRNA

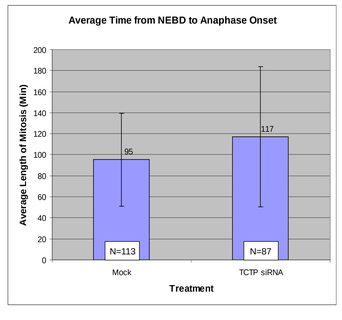




(Left) In the **mock** transfection condition, 82% of cells were able to complete mitosis

In the TCTP **siRNA** knockdown condition, only 18% of cells were able to complete mitosis

(Right) Of the cells in the mock and knockdown conditions that were able to complete mitosis, the average length of time (minutes) between nuclear envelope breakdown (NEBD) and anaphase onset was:
- 95 minutes for the Mock condition
- 117 minutes for the TCTP siRNA condition



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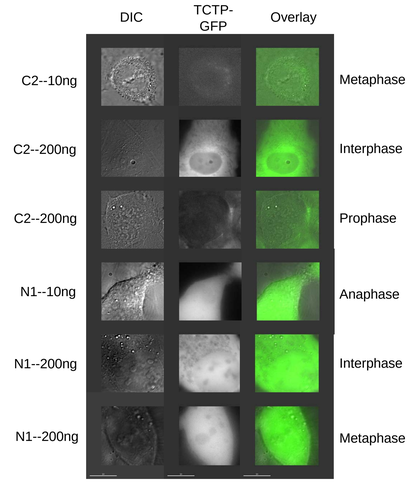
TCTP-GFP Overexpression



- N-terminal and C-terminal TCTP-GFP fusion constructs were generated
- The constructs were transfected into U20S cells (200ng and 10ng)
- There was no microtubule localization of TCTP-GFP found in any phase of the cell cycle, regardless of the concentration used for transfection
- TCTP may not localize to microtubules, or the high levels of cytoplasmic TCTP may obscure visualization at microtubules

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Bazile, et al. (2009): TCTP Publication

- TCTP colocalizes with tubulin to the spindle pole during mitosis
- TCTP is not a MAP (microtubule associated protein):
  - Microtubules and TCTP can be localized independently of each other
  - TCTP does not co-precipitate with tubulin like other typical MAPs
- TCTP associates with F-actin, particularly at spreading cell boarders
  - TCTP shows preference for actin-rich foci when actin depolymerization is induced by cytochalasin D
- TCTP knockdown by siRNA causes drastic changes in cell shape:
  - Elongation of the cell, accompanied by cytoplasmic protrusions
  - These cytoplasmic protrusions can lead to unequal division into daughter cells

Conclusions:
- TCTP is not a direct tubulin or actin-binding protein
- TCTP plays a significant role in cytoskeleton, but these roles have not yet been elucidated by researchers

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Summary, Conclusions, and Future Directions

- TCTP was initially thought to be a MAP (microtubule associated protein), but has been shown by Bazile et al., that this is not the case.
- Immunofluorescence by the TCTP primary antibody shows localization to the spindle pole during mitosis
- Western blot analysis shows that TCTP can be detected in HeLa (human), U2OS (human), and PtK1 (rat kangaroo) cell extracts. Additionally, siRNA knock down of TCTP in HeLa-H2B GFP cells was confirmed by this method.
- Live cell microscopy of HeLa H2B-GFP cells treated with the TCTP siRNA shows a mitotic phenotype of a large percentage of cell death, especially between metaphase and anaphase
- A cloned TCTP-GFP construct over-expressed in human U2OS cells does not show punctate localization at either 200ng or 10ng concentrations for either construct (N-terminus or C-terminus) in either interphase or mitotic cells.

Future Directions:
- Perform TCTP siRNA Western blot of HeLa H2B-GFP cells in comparison to HeLa-Kyoto and HeLa-S3 cells to determine the best cell line in which to repeat the live cell mitotic phenotype index
- Repeat live cell analysis and begin fixed cell analysis in comparison to tubulin and actin
- Determine TCTP's relationship with actin during mitosis and interphase

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