

N-terminally Truncated Spastin Causes Long Intercellular Bridges in PTK1 Cells

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Introduction

Abstract:

PTK1 cells are commonly used to study mitosis however, PTK1 genes are poorly characterized. Spastin is a microtubule-severing protein involved in microtubule regulation in mitosis, as well as post-mitotic cells such as neurons. Spastin was cloned in PTK1 cells.² An N-terminal deletion of spastin generated in PTK1 cells resulted in a loss of 180 amino acids, including the microtubule-interacting and endosomal trafficking (MIT) domain. The loss of the MIT domain generated a mutant apparently incapable of proper recruitment to the midbody. This resulted in a loss of microtubule severing capabilities during abscission, as well as lengthy intracellular bridges and microtubule protrusions.

Introduction:

Abscission refers to the severing of microtubules during cytokinesis. This process is important for the separation of daughter cells, resulting in the completion of the mitotic process.⁴ Curious about this process, microtubule-severing proteins were investigated for their role during abscission, specifically Katanin and spastin. These known microtubule severing proteins were both cloned and transfected into PTK1 cells. Following transfection, an N-terminal deletion in the spastin gene arose, piquing interest in the function of this truncated mutant in PTK1 cells. The loss of 180 amino acids, meant the loss of the microtubule-interacting and endosomal trafficking domain (MIT). The microtubule binding domain (MTBD) and the AAA ATPase (required for microtubule severing) domain remained intact.

Previous studies have suggested that the MIT domain of spastin is required for abscission and promotes spastin localization to the midbody. The loss of the MIT binding domain hinders midbody localization of spastin and microtubule severing functions during abscission.²

Methods:

We made a plasmid containing a truncated spastin fused to RFP. The truncated mutant was transfected into PTK1 cells. siRNA primers were designed against endogenous spastin, resulting in knockdown of endogenous spastin. We utilized both live and fixed imaging methods, including confocal microscopy.

Sequence Analysis:

PTK1 cells come from the *Potorous tridactylus*, a species of rat kangaroo. They are commonly used to study mitosis however, their genome is poorly understood and very few genes have been successfully cloned. Sequence analysis was performed on the spastin gene in PTK1 cells. Sequence analysis revealed that the MIT domain and the MTBD are evolutionarily conserved between humans and marsupials. However, the AAA ATPase region contained 2 amino acid variations between the two species.

Taxonomy	Animal	Scientific name	Query Cover	Percent Identity
Marsupial	Common Wombat	<i>Vombatus ursinus</i>	100	97.33
Marsupial	Tasmanian devil	<i>Sarcophilus harrisii</i>	100	96.11
Marsupial	Koala	<i>Phascolarctos cinereus</i>	100	97.33
Placental	Alpaca	<i>Vicugna pacos</i>	100	90.24
Placental	House cat	<i>Felis catus</i>	100	89.86
Placental	Tiger	<i>Panthera tigris</i>	100	89.86

Figure 1. Sequence Analysis of spastin

Results

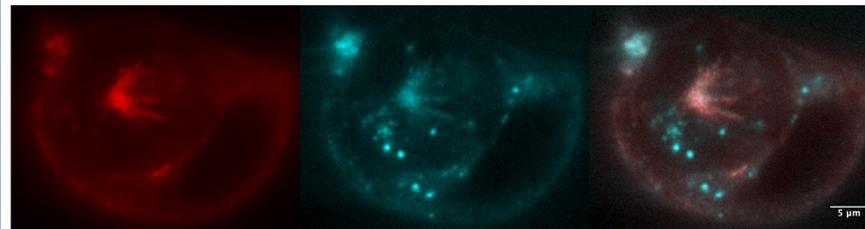


Figure 2. Ptk1 half-spindle in metaphase. Imaged microtubules (red, left), spastin (cyan, middle). Merged on right.

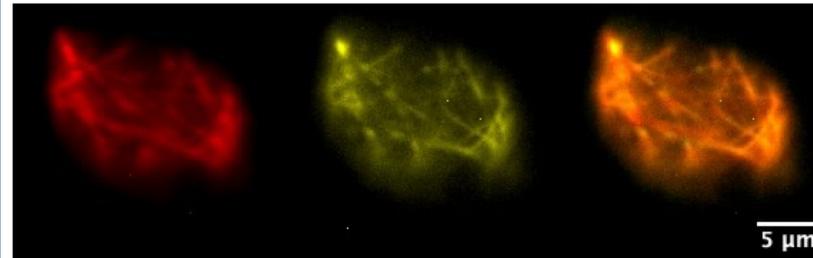


Figure 3. Ptk1 cortical microtubules at interphase. Imaged microtubules (red, left), spastin (yellow, middle). Merged on right.

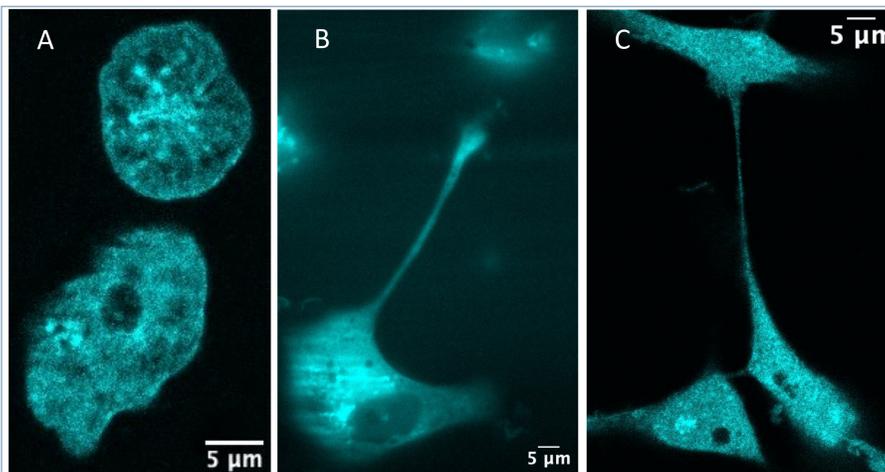


Figure 4. (A) Wild-type PTK1 cells containing endogenous spastin stained with DAPI. (B,C) PTK1 cells containing truncated mutant of spastin, displaying intracellular bridge phenotype.

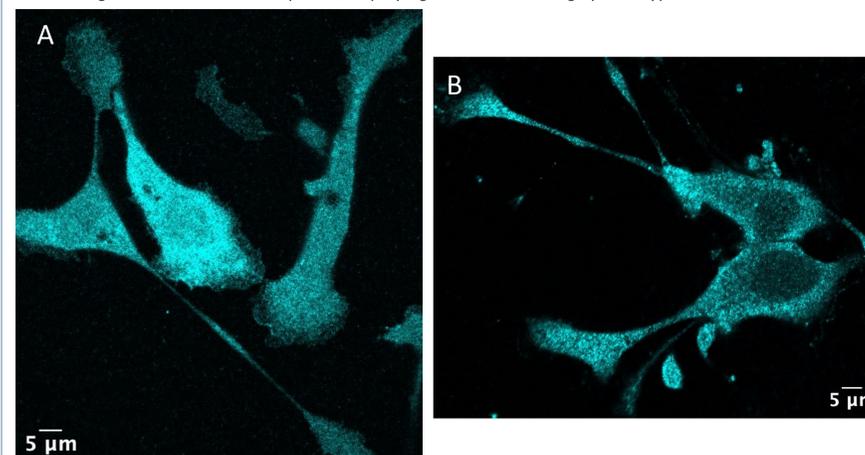


Figure 5. (A) PTK1 cells containing truncated mutant of spastin, showing intracellular bridge phenotype. (B) PTK1 cells containing truncated mutant of spastin, displaying microtubule protrusions.

Conclusion

Following deletion of the N-terminal region of the spastin gene, proper localization to microtubules during the formation of a mitotic spindle remained intact. This suggests that the MTBD and the AAA ATPase domain are sufficient for proper cellular localization of microtubules.

Deletion of the MIT domain resulted in intracellular bridges and microtubule projections. Spastin failed to localize to the midbody during mitosis. The loss of the MIT domain was responsible for failed abscission in PTK1 cells.

Future Directions:

Our experimental conditions lack a sample of PTK1 cells containing full-length exogenous RFP-tagged spastin. This sample would have been used as a control for reference against our truncated spastin samples. In future studies, further care would have been taken to maintain a control sample of exogenous spastin expressed in PTK1 cells.

This project represents one trial therefore, the sample size should be further expanded to see if the observed phenotypes could be replicated.

Overall, additional imaging experiments of truncated spastin should be performed and proper transfection of full length spastin should be obtained.

References:

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