Cell Separation Delay and Membrane Trafficking Defects in Cdc42 GAP Mutants

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Abstract

Cytokinesis is the final step in cell division, where a cell separates into two daughter cells. Cytokinesis involves many steps that must be organized in a spatiotemporal manner. In most eukaryotes, this involves the assembly and constriction of an actomyosin ring. The fission yeast *Schizosaccharomyces pombe* serves as a good model system to study cytokinesis because they divide via actomyosin-dependent-cytokinesis. The Rho-family of small GTPases are molecules involved in the regulation of cell growth and division. The GTPase Cdc42 helps promote timely onset of ring constriction and septum formation in fission yeast. Studies with many other organisms show that Cdc42 must also be inactivated at certain points during cell division for proper cytokinesis. Cells lacking *rga4* and *rga6*, the GAPs that inactive Cdc42, exhibit delayed cell separation, due to overactive Cdc42.
We find that the GAP mutants display membrane remodeling defects during cell abscission. Cdc42 is likely involved in the regulation of membrane trafficking. Indeed, fimbrin, an endocytosis marker displays abnormal localization in the GAP mutant. This suggests that there is an endocytic defect in cells lacking both *rga4* and *rga6*. Future directions will investigate how membrane remodeling defects impair cytokinesis.

Introduction and Background

Fission yeast divide by actomyosin-ring-dependent cytokinesis. Cytokinesis involves multiple steps that are spatiotemporally organized for successful cell separation. Ring constriction happens concurrently with septum ingression. The septum is formed from the synthesis of a primary and secondary septum. Simultaneous to septum formation, the membrane ingresses to form a barrier. The cell separates when certain enzymes digest the primary septum, leaving the secondary septum to form the cell wall of the new daughter cells. All of this must occur in the correct order for successful cell division. We have discovered that the spatiotemporal activation pattern of the GTPase Cdc42 promotes these events during cytokinesis. Cdc42 is spatiotemporally activated by two distinct GEFs, Gef1 and Scd1, to promote different events during cytokinesis. Gef1 localizes to the actomyosin ring and activates Cdc42 to promote timely onset of ring constriction and septum ingression. The activator Scd1 localizes to the membrane barrier behind the ring and facilitates proper septum formation.[1] We have seen that constitutively overactive Cdc42 leads to cytokinetic defects. These defects can be seen in many organism such as *Xenopus, Drosophila, and S.pombe.* We know that Cdc42 is antagonistic for cell separartion. It must be turned off at certain points for proper cell abscission. This information lead us to ask what happens when this inactivation does not happen? This Is the question that lead us to look at the negative regulators of Cdc42, the GAPs *rga4* and *rga6*.

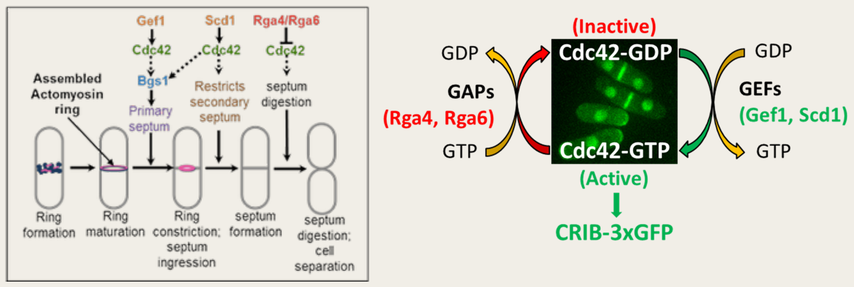

Figure 1: Cdc42 spatiotemporally organizes different cytokinetic events and is activated and inactivated by GEFs and GAPs.

1. Delayed Cell Separation

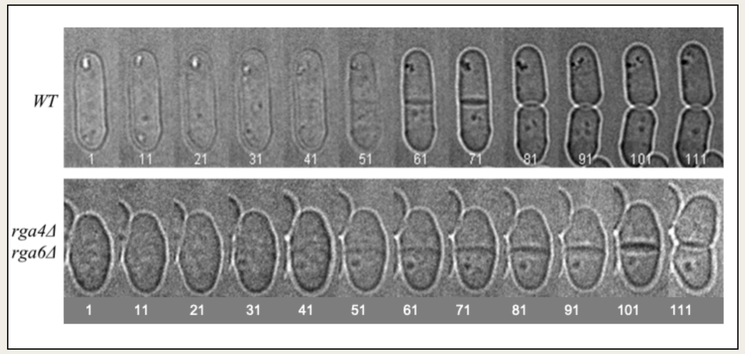

Figure 2:The Cdc42 double GAP mutant, rga4Δrga6Δ, shows a delay in cell separation when compared to wild type

2. Is Cell Separation Delay Cdc42 or Rho2 Dependent

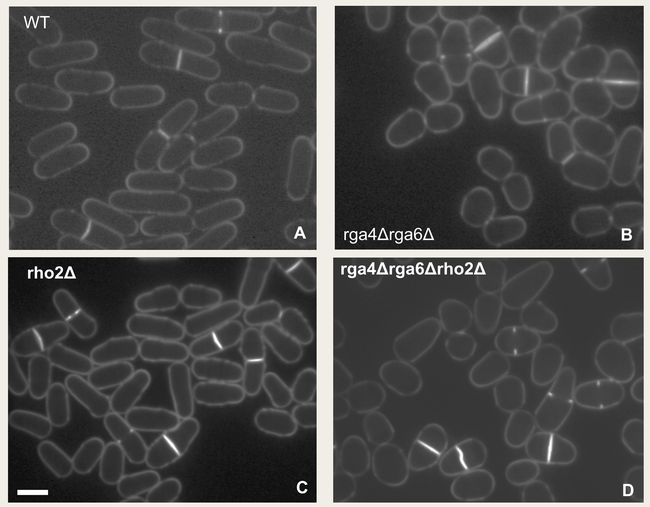

Figure 3: Rga4 and Rga6 are also Rho2 GAPs, so calcofluor images were taken to determine if the delay was Cdc42 or Rho2 dependent. All four strains were imaged using a confocal microscope and analyzed to produce septation indices (counting fraction of cells with complete septum). Figure 3 above shows calcofluor images of each strain. Scale bar is 5 microns.

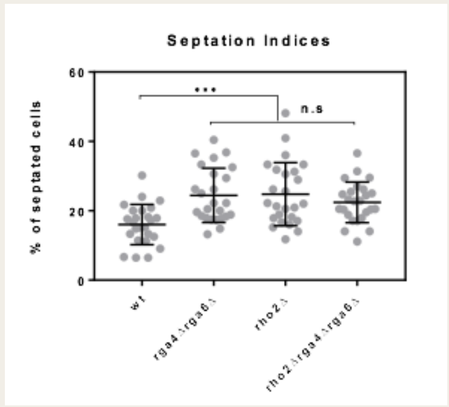

Figure 4: Septation Index Quantification

If the delay seen was Rho2 dependent, then deleting Rho2 should bring septation index back to wild type level. As seen in the quantification in Figure 4, deleting Rho2 did not have this effect. This told us the defect was not Rho2 dependent.

3. Membrane Remodeling Defects

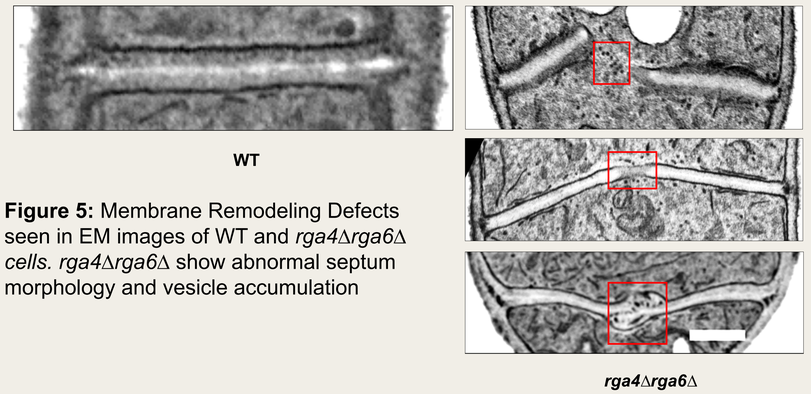

Figure 5: Membrane Remodeling Defects seen in EM images of WT and *rga4Δrga6Δ cells. rga4Δrga6Δ* show abnormal septum morphology and vesicle accumulation

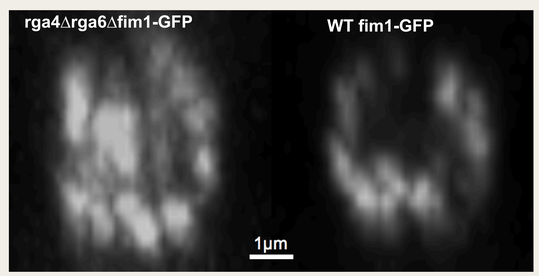

Figure 6: Because of Cdc42's likely role in membrane remodeling, we first looked at the localization of fimbrin, an endocytic marker.

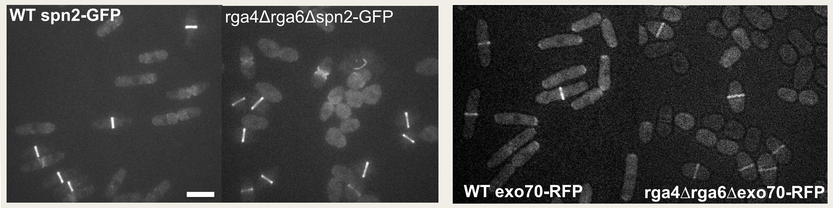

Figure 7: Localization of exocytic proteins *spn2* and *exo70.* Scale bar is 5 microns.

Conclusion and Future Directions

The unique activation patterns of the GTPase Cdc42 by GEFs and GAPs play a pivotal role in proper cytokinesis. Deleting the GAPs, *rga4 and* rga6, that inactivate Cdc42 leads to a delay in cell separation and membrane remodeling defects during cell abscission. The abnormal localization of fimbrin, an endocytic marker, in the GAP mutant suggest that the likely cause of the defects seen is related to endocytosis. In the future we will focus on the role GAPs play in endocytosis, and how exactly these endocytic membrane remodeling defects impair cytokinesis.

References

1. Wei, B., B.S. Hercyk, N. Mattson, A. Mohammadi, J. Rich, E. DeBruyne, M.M. Clark, and M. Das. 2016a. Unique Spatiotemporal Activation Pattern of Cdc42 by Gef1 and Scd1 Promotes Different Events during Cytokinesis. Molecular biology of the cell.

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