Crosstalk between Rho-family GTPases at the division site during cytokinesis

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Abstract

Cytokinesis is the final step of cell division, when a parent cell separates into two daughter cells. All cells divide, and cells with cytokinetic defects can become cancerous, which is why studying cytokinesis is of interest to many labs. We are interested in researching the mechanisms that regulate cytokinesis, using *Schizosaccharomyces pombe,* or fission yeast, as a model organism. Two Rho-family GTPases Rho1 and Cdc42 in *S. pombe*, participate in and have distinct roles in cytokinesis. Both Rho1 and Cdc42 are activated by specific guanine nucleotide exchange factors (GEFs), Rgf3 and Gef1, respectively. The mechanisms of Rho1 activation and regulation at the division site are poorly understood. In both WT and *gef1*Δ cells, the Rho1 GEF Rgf3 localizes to the division site at approximately the same time, in early anaphase A which coincides with the completion of actomyosin ring assembly. Interestingly, Rho1 is activated immediately after ring assembly in *gef1*Δ cells, but later at the onset of ring constriction in WT cells. Our results suggest that Gef likely via Cdc42 activation, inhibits Rho1 activation until the time of ring constriction by an unknown mechanism. Current research is focused on how Gef1 inhibits Rho1 activation and the significance of this inhibition.

Keywords: GTPase, Rho1, Cdc42, cytokinesis, Gef1, Rgf3, actomyosin ring constriction, septum ingression

Introduction

Cytokinesis in Fission Yeast

Here, we use the model organism *S. pombe*, or fission yeast, to study cell division. Fission yeast is a rod-shaped unicellular eukaryotic organism that is a great model system in which to study cytokinetic processes in space and time. Fission yeast reproduces rapidly via actomyosin ring-dependent binary fission with a generation time of 2-4h in supplemented (yeast extract) and minimal media (Hayles and Nurse, 2017). About 70% of fission yeast genes identified have human orthologs, many of which are implicated in human diseases (Wixon, 2002). In mammalian cells, cytokinesis occurs via the constriction of a medially assembled actomyosin ring. Fission yeast cells also assemble a contractile actomyosin ring, however the initiation of constriction is more complex, due to the fact that they have a cell wall. The actomyosin ring constriction is tightly coupled to synthesis of new cell wall material called 'septum' to divide into two daughter cells (Proctor et al., 2012). Unlike in mammalian cells that constrict as soon as the actomyosin ring forms, fission yeast actomyosin rings undergo a maturation phase lasting roughly 15-20 minutes, and this is when the proteins required to synthesize the septum are recruited (Lee et al., 2012). It is easy to visualize how the cell combines a pulling force (ring constriction) and a pushing force (septum ingression) to successfully complete cytokinesis (Figure 1). We are interested in how the cell regulates the simultaneous processes of ring constriction and septum ingression in space and time to ensure proper cell separation.

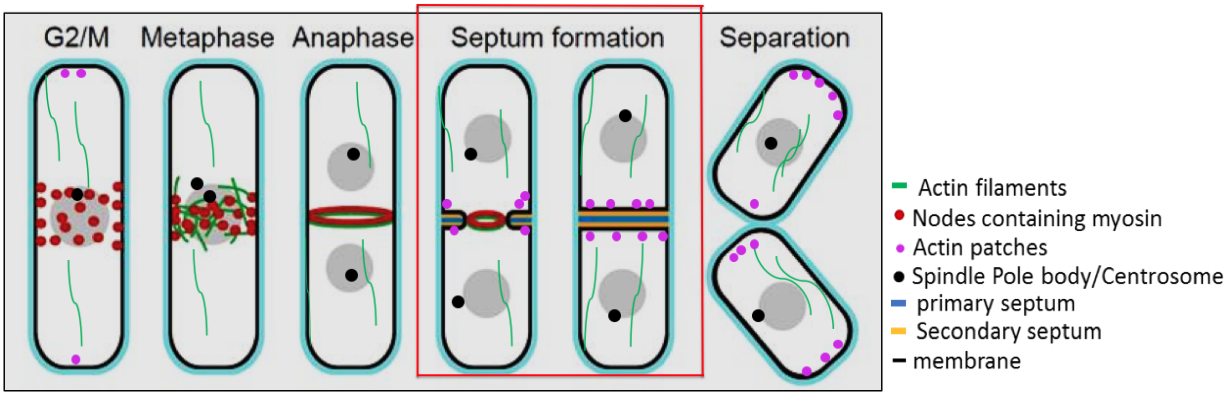


Figure 1. Cytokinesis in fission yeast Diagram illustrating fission yeast cytokinesis. Red box indicates the temporal focus of this research, when the actomyosin ring starts to constrict and the septum is synthesized following the contracting ring. *Modified from Wu et al., 2012*

Rho1 and Cdc42

Two Rho-family GTPases, Rho1 and Cdc42, are known to coordinate cytokinetic events. GTPases are proteins that act as molecular on-and-off switches by cycling between an active, guanine triphosphate (GTP) bound state, and an inactive, guanine diphosphate (GDP) bound state. GTPases are positively regulated, or activated, by guanine nucleotide exchange factors, or GEFs, and negatively regulated, or inactivated, by GTPase activating proteins, or GAPs (Figure 2). For this research, we worked exclusively with the GEF of Rho1, Rgf3, and the GEF of Cdc42, Gef1 (Figure 2). Though there are many Rho1 GEFs, we chose to focus on Rgf3 for the purposes of this research, as it is the only Rho1 GEF that is shown to be essential for cell viability.

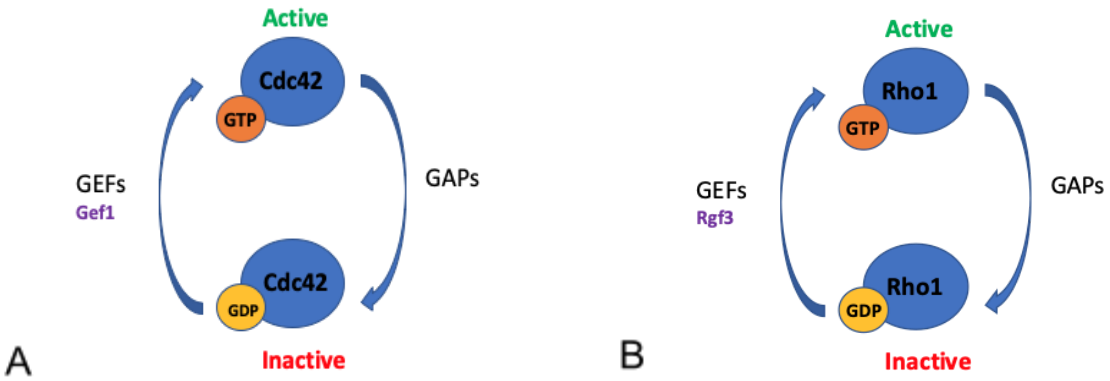
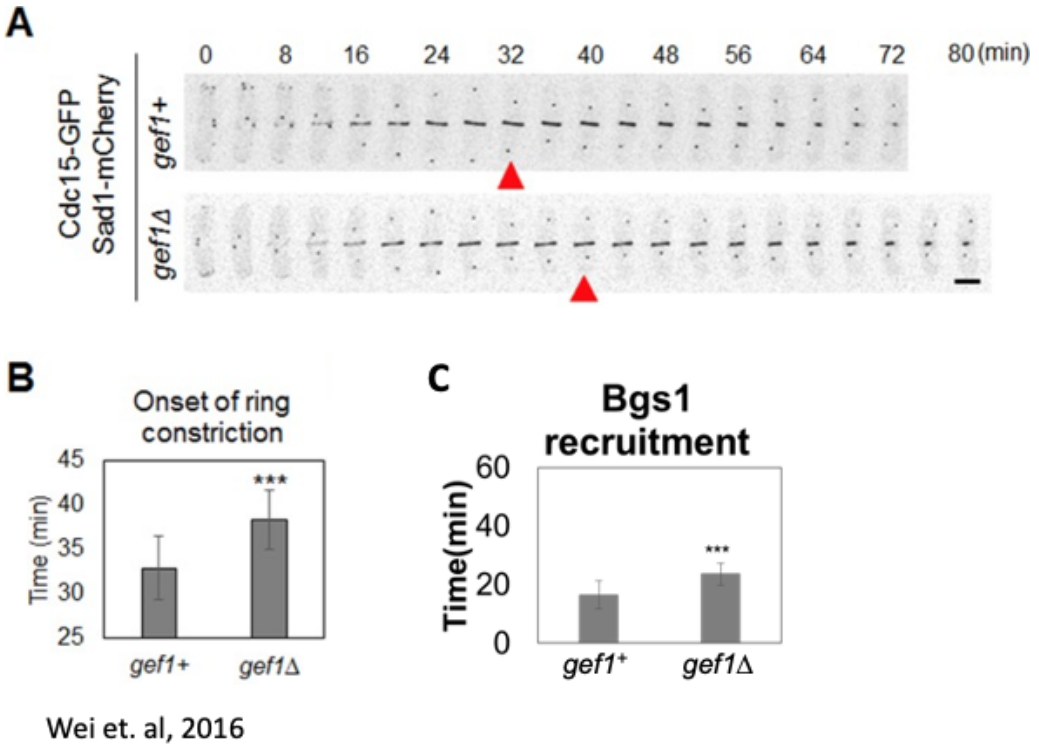


Figure 2. GTPase activity (A) Diagram representing the activation pattern of Cdc42 as it pertains to this research. (B) Diagram representing the activation pattern of Rho1 as it pertains to this research.

Both Rho1 and Cdc42 have important, yet distinct roles in the promotion of fission yeast cytokinesis. They are both required for cell viability and are highly conserved across all different kinds of organisms, through to humans (Hercyk and Das, 2019). In other organisms, Rho1 and Cdc42 have been shown to have interactions in the regulation of various processes, for example the formation of hyphae in *Candida albicans* (Corvest et al., 2013). However, interactions and mechanisms of crosstalk between Cdc42 and Rho1 have not been well studied nor well characterized in fission yeast (Hercyk and Das, 2019). Previous work done in our lab has shown that Cdc42 localizes to the division site in a unique spatiotemporal manner to promote the onset of ring constriction (Wei et al., 2016). As evidence to this, upon deletion of Gef1, there is a delay in the onset of ring constriction (Wei et al., 2016). Another role of Cdc42 during cytokinesis is the recruitment of a β-glucan synthase that synthesizes the primary septum, Bgs1, to the division site (Hercyk and Das, 2019). A delay in Bgs1 recruitment to the division site is experienced in *gef1*Δ mutants (Wei et al., 2016). In turn, active Rho1 is shown to be required for the formation of the primary septum and thus the timely onset of septum ingression, as Rho1 serves as an

activator of not only Bgs1, but other β-glucan synthases as well, including the synthases that synthesize the secondary septum. Because of this novel interplay, we hypothesized that Cdc42 and Rho1 have routes of crosstalk during cytokinesis (Figure 3).



<u>Figure 3. Cdc42 promotes the timely onset of ring constriction and recruitment of Bgs1</u> (A) Time lapse images showing progression through mitosis. The actomyosin ring is visible via a fluorescently tagged ring component, Cdc15. Spindle pole bodies are visible via a fluorescently tagged spindle pole body protein, Sad1. Red arrows indicate the onset of ring constriction, showing a delay in *gef1*Δ mutants. (B) Quantification of data shown in (A). (C) Quantification of data showing a delay in Bgs1 recruitment to the division site in *gef1*Δ mutants. *Figures from Wei et al., 2016.*

Results and Discussion

We sought out to test our hypothesis that there is crosstalk between Cdc42 and Rho1 during cytokinesis by deleting *gef1* and observing the phenotypic effects with fluorescent microscopy. First, we looked at Rgf3 localization to the division site, and we found that there is

no difference in the timing of Rgf3 localization to the division site in both wild-type cells and *gef1*Δ mutants, as shown by Figure 4. We used spindle pole body separation distance as a molecular timer and we plotted this against fluorescence frequency. Spindle pole body distance is commonly used in fission yeast as a mitotic timer. In fission yeast, the spindle pole bodies are analogous to the microtubule organizing center in mammalian cells. As a result, as the spindle forms and mitosis progresses, the spindle pole bodies separate. As Figure 4 indicates, Rgf3 localizes to the division site at the same time in our WT and our *gef1*Δ mutants. It localizes in early Anaphase A, when the contractile ring has just finished assembly.

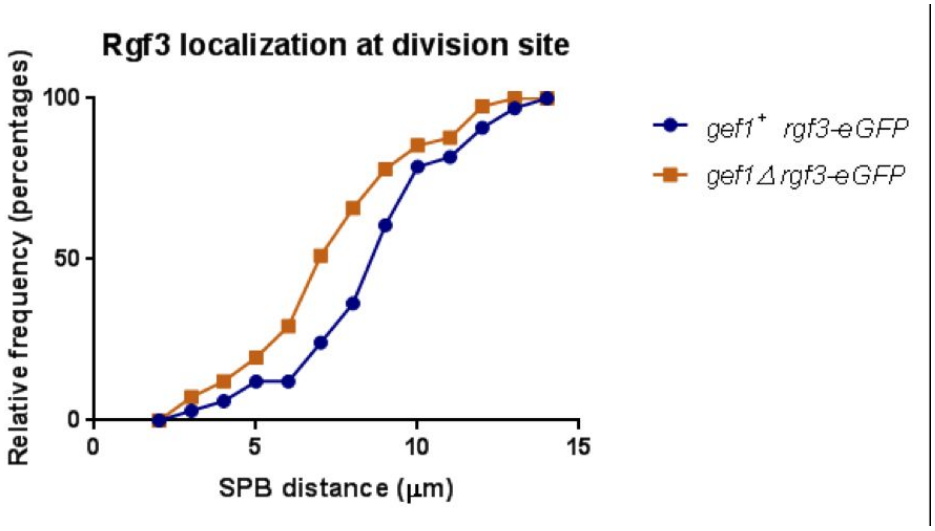


Figure 4. Timing of Rgf3 localization to the division site Spindle pole body (SPB) separation distance (μm) can be used to time cytokinetic events. In both *gef1*$^{+}$ and *gef1Δ* cells, Rgf3 localizes to the division site at the same time, when the spindle pole bodies have separated 2.8 μm, in early anaphase A.

Next, we looked at Rho1 activation in both WT and *gef1*Δ cells. To do this, we constructed a fluorescent probe which selectively binds active Rho1. Because Rgf3 localizes to the division site at the same time in both the WT and *gef1*Δ mutants, we expected to see that Rho1 is activated at the same time in both WT and *gef1*Δ. However, this is not what was

observed. To visualize the timing of Rho1 activation, we took time lapse images, and we found that Rho1 is activated almost 15 minutes earlier in *gef1*Δ cells (Figure 5). Quantification of the data from the time lapse imaging can also be seen in Figure 5.

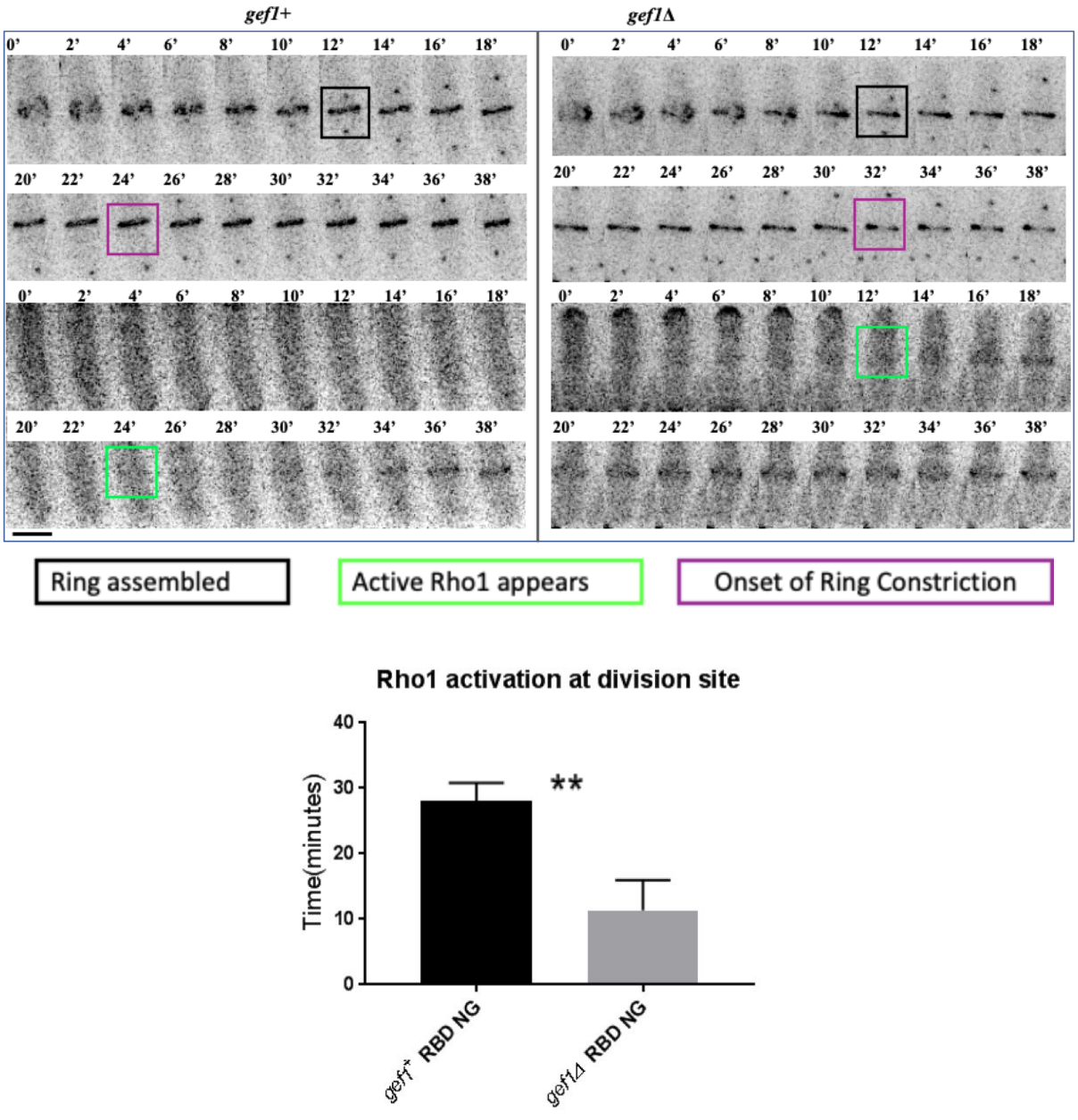


Figure 5. Time lapse images indicating timing of Rho1 activation To visualize Rho1 activity, we constructed a bio-probe that specifically binds to the active GTP bound form of Rho1, thus creating a marker that reports its spatiotemporal activation pattern in live cells. We have found

that in *gef1*Δ cells, Rho1 is activated much earlier than in WT cells. Intervals are in minutes. In *gef1*Δ cells, Rho1 is active about 15 minutes earlier than WT. p=0.0019

Conclusion

The final step in cell division, cytokinesis, is a highly spatiotemporally regulated process (Wei et al., 2016). It requires the cooperation of many proteins, including the Rho-family GTPases Cdc42 and Rho1, to ensure that the cell divides properly (Wei et al., 2016; Hercyk and Das, 2019). We observe that when we delete the GEF of Cdc42, Gef1, Rho1 is activated about 15 minutes earlier than in wild-type cells even though the GEF of Rho1, Rgf3, localizes in both strains at the same time. Theoretically, this could be significant to the survival of the cell because early Rho1 activation could lead to premature septum formation even before the chromosomes successfully segregate leading to ploidy defects. This interplay leads us to conclude that there is a mechanism of crosstalk between Cdc42 and Rho1 at the division site during cytokinesis.

We have found that Rgf3 localizes to the division site at the same time in both *gef1*$^{+}$ and *gef1*Δ mutants, immediately after actomyosin ring formation (Figure 4). However, Rho1 activation pattern differs greatly. This is interesting because it is rarely documented in the literature for a GEF to localize and not activate its GTPase. In *gef1*$^{+}$ cells, Rho1 is not activated until the onset of ring constriction. In *gef1*Δ mutants, Rho1 is activated much earlier, soon after the ring has formed and about the same time when Rgf3 is recruited (Figures 5, 6). Based on these findings we propose that Cdc42, through the activity of Gef1, inhibits Rgf3-mediated Rho1 activation at the division site. This gives us a possible mechanism of crosstalk between Rho1 and

Cdc42 at the division site during cytokinesis and future research are focused on testing this hypothesis.

Methods and Materials

Strains and Cell Culture

The *S. pombe* strains utilized in this study are all isogenic to the original strain isolated by Paul Nurse, PN972. All strains were cultured and grown exponentially in supplemented minimal media at 25℃. Supplemented minimal media was used as a method for increasing fluorescence intensity for better protein visualization.

Microscopy

For cell imaging, A VT-Hawk two-dimensional array laser scanning confocal microscopy system with an Olympus IX-83 inverted microscope with a 100x numerical aperture was used. Images were acquired at room temperature. Data shown from this study was collected through time-lapse image acquisition using Mattek coverslipped culture dishes, exactly as reported in Onwubiko et al., 2019.

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