A cell cycle cue triggers cell growth resumption after division

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Abstract

The decoupling of the fundamental processes of cell division and growth is important for maintaining cell integrity. Through a chemical approach, we delayed the clock that controls when cells separate, uncoupling cell division and cell growth. This led to polarized cell growth before the cells completed separation. Using fluorescent markers to denote the cell's stage in the cell cycle, we observed that only cells that were in mitosis exhibited this uncoupling. Previously it was thought that growth resumption occurred after completion of cell division, but this observation suggests that growth is triggered earlier, from a mitotic cue. This mitotic cue allows the tips to become better equipped to compete with the septum, causing the septum not to separate. Cdc42, a conserved GTPase that is a master regulator of cell division and polarity in eukaryotes, appears at the septum and remains there until the cells separate then oscillates between the cell tips. However, in the uncoupled cells, Cdc42 leaves during septum formation, before the cells separate and is found at the tips. Further investigations into the specific cues could lead to a better understanding of what this cell cycle trigger is and how this cue affects Cdc42 at the tips.

Introduction and Background

Before mitosis, wild type *S. pombe* cells must stop their growth in order to ensure proper cell division. Then growth is restored once the cells divide. This is fundamental in ensuring that cells maintain proper size. The cue that causes cell growth to resume is largely not understood.

How does growth resume after cell division?

Cdc42, a highly conserved GTPase, is a master regulator of cell division and polarity. Cdc42 is controlled by GEFs that activate Cdc42 and GAPs that inactivate Cdc42. Active Cdc42 has been found to oscillate between the ends, demonstrating a competition between the two ends [1].

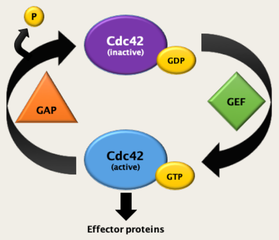


Figure 1: Shows the regulation cycle of Cdc42 through activation by the GEFs and inactivation by the GAPs.

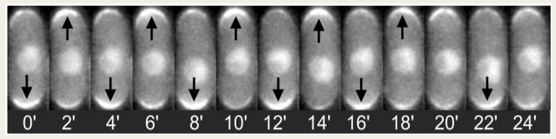
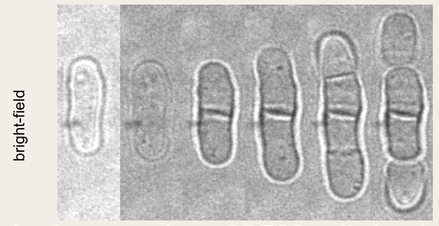


Figure 2: Active cdc42 is tagged with CRIB-GFP, showing the oscillation of active cdc42 between the cell tips [2].

Press (**Pr**ematurely **E**longating **S**ans **S**eperation) Phenotype

Figure 3: When cells are treated with LatA a portion of the cells resume growth either during or after septum formation, but these cells fail to separate. The LatA disrupts the actomyosin ring and therefore delays cell separation, but the cells continue to grow. Therefore they become long and end up becoming multi-septated.



What cell cycle phase are these PRESS cells in when they are treated with LatA ?

Methods

Latrunculin A (LatA)
The cells are treated with 10μM of LatA for 30 minutes and then rinsed, and then the cells are allowed to recover. The LatA depolymerizes all of the actin in the cell. Leading to the disruption of both actin cables, the actomyosin ring and actin patches [2].

Lectin
Lectin was used to stick the cells to the plate, this allows the same cells to be imaged before during and after the treatment of the cells. This technique was adapted to our lab in order to observe the effects of LatA on the cells [3].

Results

Mitotic cue triggers growth

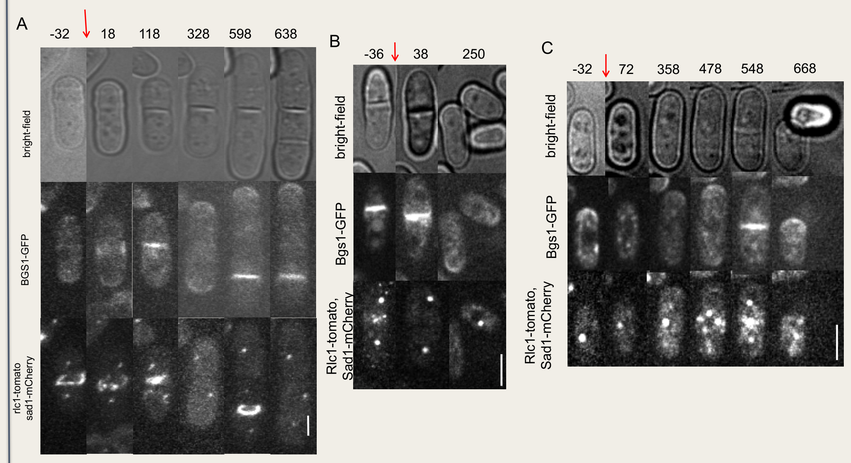


Figure 4: Wild type cells tagged with Bgs1-GFP, Rlc1-tomato and Sad1-mCherry and cells stuck to plate by lectin. The tagged spindle pole bodies were used to see what cell cycle the cells were in. (A) Cells treated with LatA during mitosis exibit the PRESS phenotype (B) Cells treated with LatA after septum formation no not exhibit the PRESS phenotype (C) Cells treated with LatA while in interphase do not exhibit the PRESS phenotype

Cdc42 leaves during septum formation

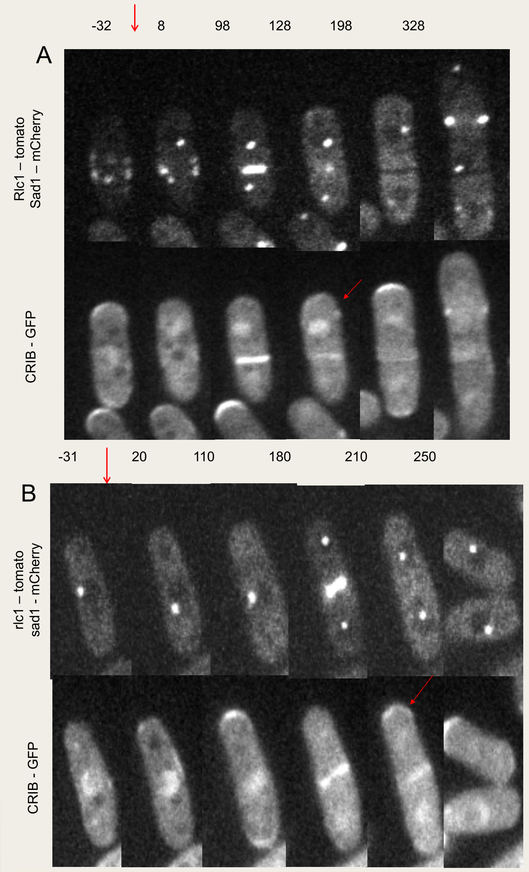


Figure 5: Wild type cells tagged with CRIB-GFP, Rlc1-tomato and Sad1-mCherry and cells stuck to plate by lectin. The tagged spindle pole bodies were used to see what cell cycle the cells were in. The CRIB-GFP associates with active Cdc42.The cells were treated for 30 minutes with 10μM LatA. (A) The cells treated with LatA during mitosis the active Cdc42 leaves the cell middle and begins to accumulate at the tips before the ring has completely constricted and therefore before the septum has completely formed (B) When cells are treated with LatA before the onset of mitosis the Cdc42 does not begin to be found at the tips until the the ring has completely constricted and the septum has completely formed.

Conclusion

Only the cells that were in mitosis when the actin was disrupted, showed the PRESS phenotype leading to the conclusion that there must be a mitotic cue that triggers the resumption of growth after cell division. The cells must pass this certain cue before the cells can resume growth, and this is not dependent on the physical separation of the cells. Also, it was shown that in the uncoupled cells, Cdc42 leaves the middle of the cells while septum was still forming and went to the tips. This could show that there is a link between this mitotic cue and the localization of Cdc42 at the tips of the cells following cell separation. This mitotic cue allows the tips to become better equipped to compete for resources with the middle. Future work in finding this cell cycle trigger could lead to a better understanding of the cell cycle and a better understanding of how this cue affects the localization of Cdc42 at the tips.

References

[1] Das M, Drake T, & Verde F (2012). Oscillatory dynamics of Cdc42 GTPase in the control of polarized growth. *Science* 337(6091), 239-243.
[2] Spector I, Schochet NR, Kashman Y, & Groweiss A (1983). Latrunculins: Novel marine toxins that disrupt microfilament organization in cultured cells. *Science* 214, 493-495.
[3] Ye Dee Tay, Marcin Leda, Andrew B. Goryachev, & Kenneth E. Sawin (2018) Local and global Cdc42 guanine nucleotide exchange factors for fission yeast cell polalrty are coordinated by microtubules and the Tea1-Tea4-Pom1 axis. *Journal of Cell Science* (131).