Identification and characterization of supressors for *rng15* deletion in fission yeast cytokinesis

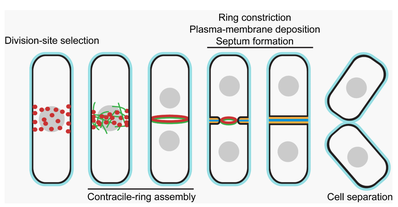
Michaelanne Sauer, Kenneth Gerien, Jian-Qiu Wu
Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210, USA

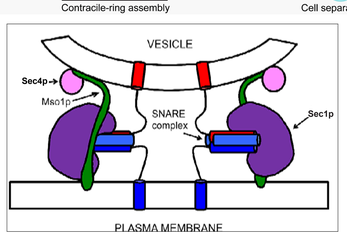
Abstract

While much is known about cytokinesis, the final stage of cell-division cycle, our knowledge on roles of vesicle trafficking during cytokinesis is limited. To further our understanding of exocytosis during cytokinesis, we investigate Rng15 in fission yeast, a popular model organism for cytokinesis. Little is known about Rng15, though our preliminary studies and its homology with budding yeast Mso1 suggest that it participates in exocytosis. Rng15 also shares homology to Mint1, a mammalian adaptor protein involved in exocytosis. Cells with *rng15* gene deleted (*rng15*Δ) cannot grow at 36°C due to defective cytokinesis and accumulate secretory vesicles at the division site.

To further investigate how Rng15 functions in cytokinesis, we screened for high-copy suppressors of rng15Δ at 36°C by transforming rng15Δ cells with plasmid DNA from a fission yeast genomic DNA library. We reasoned that if the proteins being produced from the plasmids rescue the growth of *rng15*Δ cells at 36°C, these proteins are involved in similar pathways as Rng15. So far we have isolated that Rng15 and Gmh5 from the screen. Gmh5 is a membrane protein of the Golgi mannosyltransferase complex, which is predicted to be involved in elongation of the polysaccharide mannan backbone and cell wall biogenesis. We will study Gmh5 functions by testing its localization, deletion phenotype, and genetic interactions with *rng15*Δ and other mutations in cytokinesis. In addition, we plan to identify more suppressors with additional screen. Through this investigation, we will have a better understanding of the role of exocytosis in the delivery of materials during fission yeast cytokinesis. Furthermore, we hope to provide others in the field with potential insights on how Mso1/Rng15 family proteins work in cytokinesis in other model systems, such as mammals.

Introduction



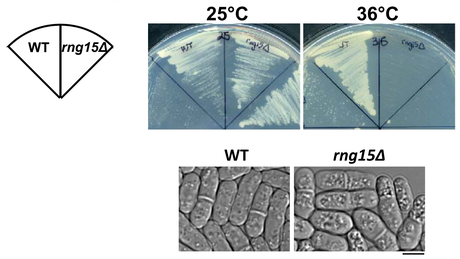


Lee et al., 2012, Cytoskeleton

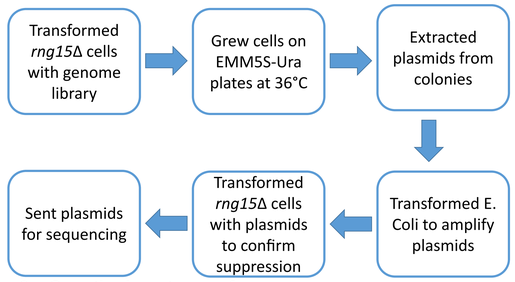
Weber-Boyvata M. et.al. 2013, Mol Biol Cell. 24:331-341.

Methods and Results

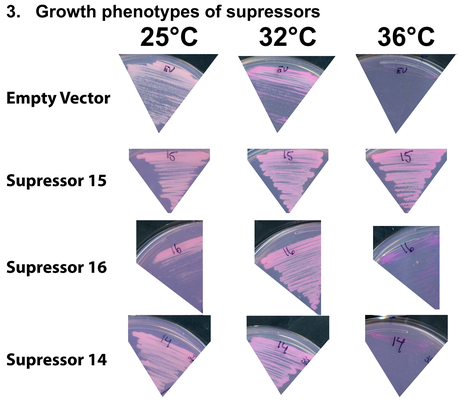
1. At 36°C *rng15Δ* is lethal, with accumulation of vesicles



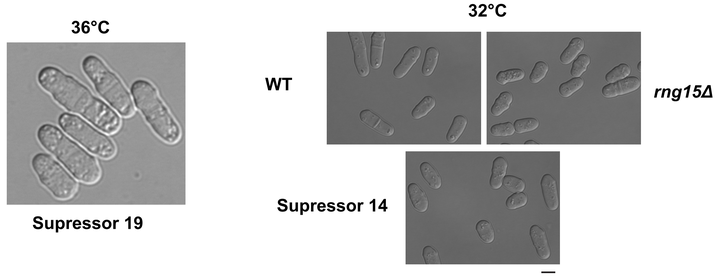
2. Supressor Screen Approach



3. Growth phenotypes of supressors



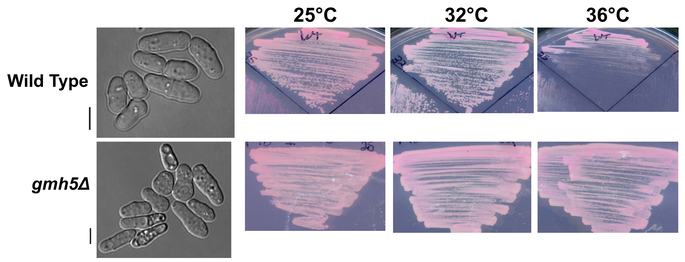
4. Supressors are able to partially rescue the phenotype of *rng15Δ*



5. Gmh5 Rescues Rng15 deletion

Suppressor	Gene	Function
9,10,15,19,22,24	Rng15	Unknown
16	Cob1	Electron Transport
14	Gmh5	Cell Wall Biogenesis

6. *gmh5Δ* cells are viable, but sick



7. Cross of *rng15Δ* with *gmh5Δ*

1. *gmh5Δ*
2. Double Mutant
3. *rng15Δ*
4. WT

1. WT
2. Double Mutant
3. *rng15Δ*
4. *gmh5Δ*

Conclusions

1. *rng15Δ* does not grow at 36°C
2. Gmh5 is a possible supressor for *rng15Δ* phenotype
3. There appears to be no synthetic lethality for Rng15 and Gmh5

Future Directions

1. Overexpression of Gmh5 in *rng15*Δ
2. Further screens to identify other supressors
3. Analyze localizations and functions of Gmh5 and other potential supressors

Acknowledgements

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