Influence of a prion protein on the TOR pathway in Saccharomyces Cerevisiae

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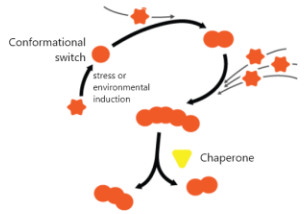
Abstract

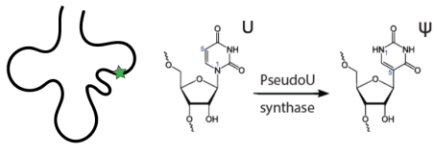
Pseudouridine synthases are critical RNA modifiers in eukaryotes. One member of this family of enzymes, encoded by the Pus4 gene in the budding yeast Saccharomyces cerevisiae, forms a prion protein, named [BIG+]. Rather than resulting in cell death, as for known mammalian prion diseases, [*BIG*$^+$] promotes increased cell proliferation and cell size. These observations raise the question of how the prion promotes cell growth. One possible mechanism is an alteration to a fundamental eukaryotic growth control pathway, mediated by the TOR complex ("target of rapamycin"). One target of TOR, a protein kinase, is Sch9, an AGC kinase, which is activated via phosphorylation by the TOR complex. Sch9 activity promotes multiple processes essential for growth such as ribosome biogenesis, translation control, and cAPK activity. To better understand the relationship between [*BIG*$^+$] and the TOR pathway, we have introduced hyperactive mutants of TOR or Sch9 into [*BIG*$^+$] and naïve (non-prion) cells. By monitoring growth rate in media with varying levels of arginine, we can monitor [*BIG*$^+$] response to different nutrient conditions. We found that [*BIG*$^+$] cells are resistant to perturbations that would normally alter the growth of cells through the TOR complex. This contributes to our understanding of how the prion and TOR complex are interacting to influence cell growth epigenetically.

Introduction

Prions are alternative protein conformations that catalyze their own propagation.

- Prion formation occurs purely by chance during translation. The frequency of formation can be increased through exposure to stress.
- Prions are the cause of diseases such as Creutzfeldt-Jakob, Kuru, and Scrapie in sheep.
- Chaperone proteins assist in propagation to cell progeny by breaking up protein aggregates.



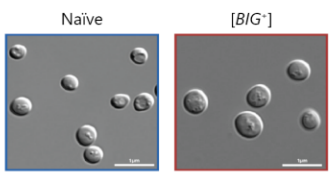


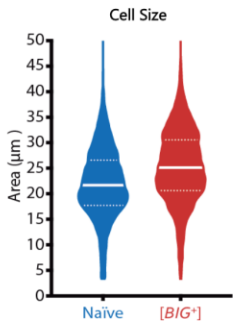
Pus4 is a pseudouridine synthase that converts uridine 55 on tRNAs to pseudouridine.

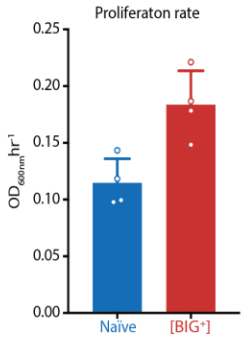
- This process is conserved throughout life. In humans, this gene is known as TruB.
- The prion form of this protein results in a gain-of-function phenotype deemed [*BIG*$^+$] (better in growth).

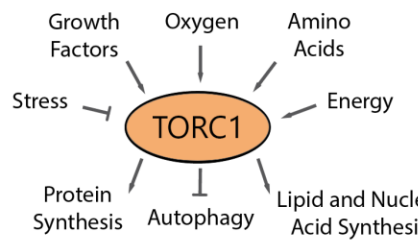
[*BIG*$^+$] cells are larger and proliferate faster than naïve cells.

- These cells perform better in rich growth media, but die faster than naïve cells.
- Larger cell size and growth rate suggests an interaction with the TOR complex.



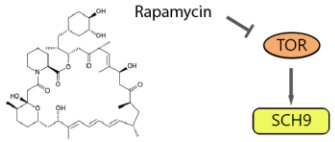






The TOR complex controls the use of available nutrients in cells.

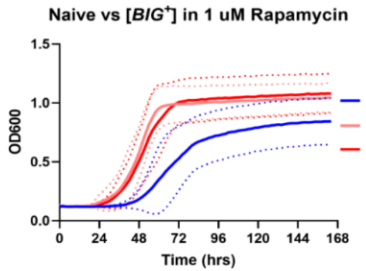
- The target of rapamycin (TOR) was discovered in yeast and is highly conserved.
- Rapamycin strongly inhibits this complex, slowing cell growth.

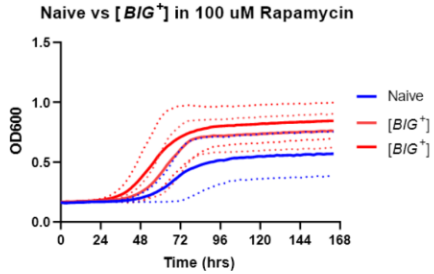


Results

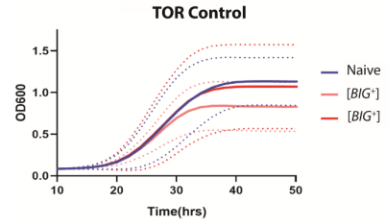
Monitoring TOR activity in yeast populations with and without the prion.

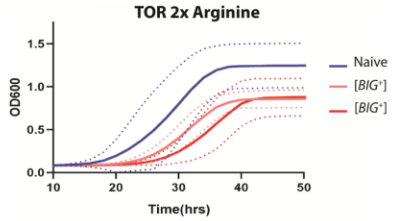
- Viewing the responses of both naïve and [*BIG*$^+$] cells gives insight into how the prion is affecting the cell.
- Eight replicates of each strain were grown to produce this figure. Cells containing the prion were slowed in their growth, but naïve cells did considerably worse.





[*BIG*$^+$] cells grow differently than naïve cells in excess arginine, further suggesting and interaction with the TOR complex.

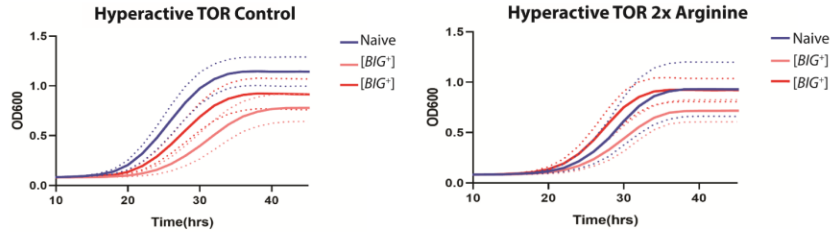




Doubling the concentration of Arginine strongly increases the growth of naïve cells while [*BIG*$^+$] cells maintain a consistent growth pattern.

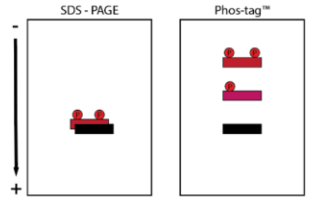
This suggests that the interaction between the prion and TOR results in a nutrient independent pathway that is resistant to growth perturbation.

Introducing a hyperactive mutant of TOR should provide a similar result to doubling the concentration of arginine. We can view this parallel growth pattern for naïve cells.



Combining hyperactive mutants and doubling the concentration of arginine results in a decrease in naïve growth due to overactivation. Growth rate of [*BIG*$^+$] remains relatively constant in all four conditions.

On Going Research



Sch9 is a useful target for determining TOR activity rather than growth rate.

- Sch9 is a kinase controls essential growth functions such as ribosome biogenesis and translation initiation.
- This protein has six phosphorylation sites that are regulated by the TOR complex. Determining the degree of phosphorylation of this protein will show us the activity of TOR.

/ / / SuperSep™ Phos-Tag™ gels incorporate zinc cations into the polyacrylamide structure to slow the mobility of phosphorylated proteins. These gels proved difficult to use so we found a new assay that utilizes 2-nitro-5-thiocyanobenzoic acid (NTCB) to cleave Sch9, shortening the protein. This allows us to view the degree of phosphorylation with regular SDS-PAGE. By growing cells in the presence of both rapamycin and cycloheximide, an activator of TOR, we can view how TOR is controlled differently in cells harboring the prion. Once we determine the activity of Sch9, we plan to view other pathways that TOR controls.

Conclusions

Resistance to rapamycin with increased cell size and proliferation rate suggests [*BIG*$^+$] interacts with TOR.

[*BIG*$^+$] grow slower in minimal media but are resistant to common signaling mechanisms of the TOR pathway which results in consistent growth patterns.

Future Experiments

Repeat growth assay with hyperactive mutants of TOR with different amino acids to confirm results.

Cleavage of n-terminal portion of Sch9 will improve western blot resolution and allow us to determine the activity of TOR.

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