TF_A Control of Ribosome Biogenesis is Conserved in *S. castellii*

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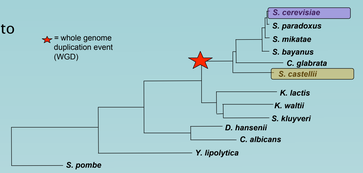
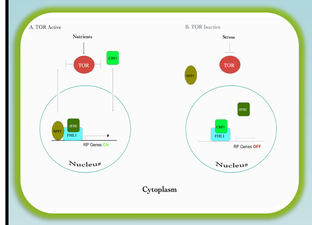
Introduction

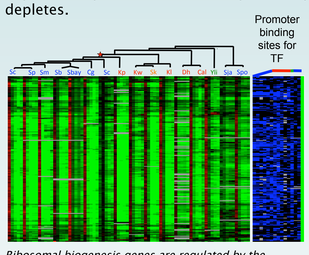
Comparative transcriptomics provides a new glimpse into the evolution of gene regulation. *Ascomycota* fungi are uniquely suited among eukaryotes for studies of regulatory evolution due to: broad phylogenetic scope, many sequenced genomes, and facility of genomic analysis.





Coordinated expression of modules of functionally related genes (such as ribosomal protein (RP) genes and ribosomal biogenesis (ribi) genes) is often conserved at great evolutionary distances. This coordinated expression is consistent with a selective pressure to conserve coordinated transcript levels to maintain functional cellular models. Genes encoding RPs are tightly coexpressed in organisms from bacteria to humans, consistent with a selective pressure to conserve coordinated transcript levels to maintain a stoichiometric balance in ribosome assembly.

In the *Ascomycete* species we study, expression of the RP module is conserved. For example, in a glucose depletion experiment conducted previously in our lab, the RP module was repressed in all species as glucose depletes.



Ribosomal biogenesis genes are regulated by the transcription factor TF_A $\log_2$ ratio: -3 3

This phenotypic conservation is not necessarily due to full conservation of the regulators. Several transcription factors controlling ribosomal gene expression have changed several times since the last common ancestor after *Ascomycota* fungi, which span *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. However, all these species possess an ortholog of the TF_A gene (see above).

Known Functions of TF_A in *S. cerevisiae*:

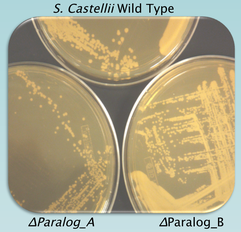
- Controls ribi genes in response to nutrients and stress
- Regulates G_2/M transitions in mitosis
- Modulates cell size
- Target of TOR pathway

Results and Data

Hypothesis: While *S. cerevisiae* only contains one *TF_A* gene, *S. castellii* has **two** homologs (*S.cas*Paralog_A and *S.cas*Paralog_B). In addition, the binding site for TF_A has been found in the promoter region of RP genes in this species. Therefore, in *S. castellii*, we will test the function of each paralog to ask if one or both are conserved for the purpose of regulating and activating RP gene expression and cell size.

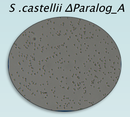
Phenotype Examination in *S. castellii*

S. Castellii Wild Type



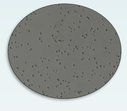
ΔParalog_A ΔParalog_B

S .castellii ΔParalog_A



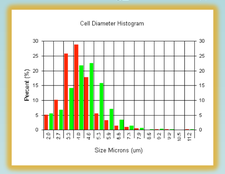
S. castellii ΔParalog_B

S. castellii Wild Type



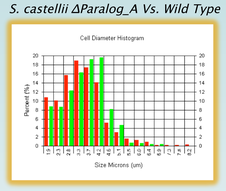
Cell Diameter Size Distribution

S. castellii ΔParalog_B Vs. Wild Type



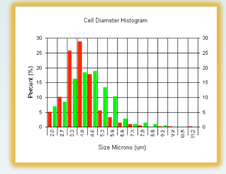
Mutant
Wild Type

S. castellii ΔParalog_A Vs. Wild Type



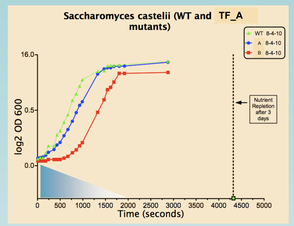
Mutant
Wild Type

S. castellii ΔParalog_B Vs. ΔParalog_A

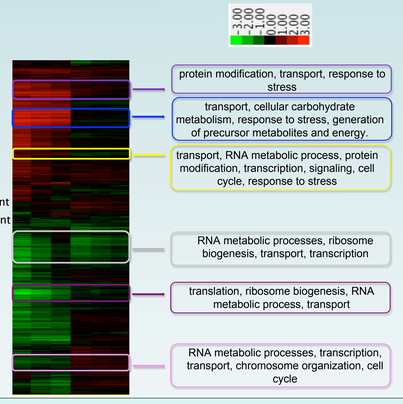


Paralog_B Mutant
Paralog_A Mutant

Growth Curves



Microarray Data Results



protein modification, transport, response to stress

transport, cellular carbohydrate metabolism, response to stress, generation of precursor metabolites and energy.

transport, RNA metabolic process, protein modification, transcription, signaling, cell cycle, response to stress

RNA metabolic processes, ribosome biogenesis, transport, transcription

translation, ribosome biogenesis, RNA metabolic process, transport

RNA metabolic processes, transcription, transport, chromosome organization, cell cycle

Conclusions

- Phenotype and Growth Evaluation
  - Observations:
    - Mutants *ΔParalog_A* and *ΔParalog_B* yield smaller colony sizes
    - Histograms reveal mutants have similar cell size, both smaller than wild type (WT)
    - Growth curve reveals *ΔParalog_A* is a much slower grower than *ΔParalog_B* and WT
    - Mutants' growth rate are similar to each other, neither mutant strain is more similar to WT
  - **Conclusion:** A slower growth rate and/or smaller cell size leads to a smaller colony size (compared to WT)
- Microarray Analysis
  - Observations:
    - Reduced ribosomal gene expression in both mutant strains
    - Many more ribosomal genes are down-regulated in *ΔParalog_A* than in *ΔParalog_B*
    - Genes involved in cell cycle are down-regulated in *ΔParalog_A*, but up-regulated in *ΔParalog_B*
  - **Conclusions:**
    - *TF_A* and its paralog (Paralog_B) regulate ribosomal gene expression
    - *ΔParalog_A* is a more global regulator of ribosomal gene expression
    - *It can be suggested this gene, TF_A Paralog_A, has conserved its function as a ribosomal protein regulator because it is syntenic with the S. cerevisiae gene.*

Future Direction

We will create a double knockout of *Paralog_A* and *Paralog_B* in *S. castellii* to determine whether the genes work independently or together. Is a double knockout viable? Further, with ~11% of genes being significantly up or down regulated in the *ΔParalog_A* mutant, we would like to resolve whether this gene is directly or indirectly responsible for this result. Does *TF_A* sit on the promoter region of all of these genes? Or is it due an indirect effect (e.g. decreased ribosomal gene expression)? In *S. cerivisiae*, TF_A is a transcriptional activator, therefore it is surprising that we saw significant up-regulation in the mutant. We plan to investigate this finding.

Acknowledgements

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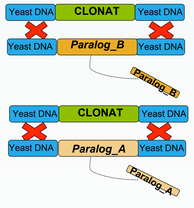
References

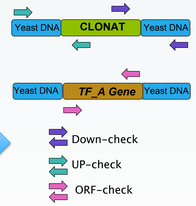
•Hassell, Christopher DR. (Photographer). (2010). Budding yeast. [Web]. Retrieved from http://greaterimmunity.com/images/Baker%27s%20yeast
•Powers, Ted. (2004). Ribosome biogenesis: giant steps for a giant problem. Cell, 119(7), Retrieved from http://www.sciencedirect.com/science?

Methods

Constructed *S. castellii* *ΔTF_A* Mutants



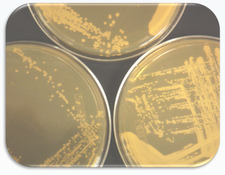
Confirmation of K.O. using PCR



Sample	Up-check	ORF-check	Down-check
gDNA (positive control)	–	+	–
mutants	+	–	+

Grew Mutant Strains Up on Agar Plates

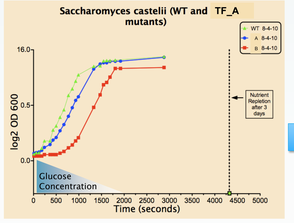
S. Castellii Wild Type



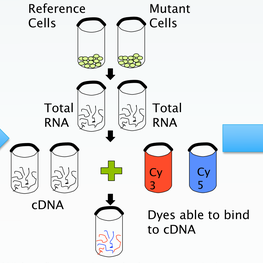
ΔParalog_A ΔParalog_B

Nutrient Repletion Experiment

Created Growth Curves During Experiment

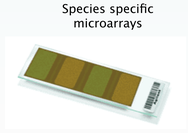


Extracted RNA and Labeled cDNA

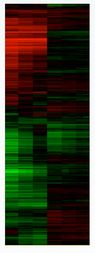


With reference in red, the mutant is in blue, the cDNAs can now put placed onto a microarray for competitive hybridization.

Placed colored cDNAs onto a Microarray



Constructed a Heat Map



Expression intensities for each gene are determined by the log ratio of mutant signal over reference signal.