Using “Anchor Away” to Test Mediator Presence at Pol III Genes

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Introduction

Mediator Complex

- Mediator is a large multi-subunit complex that is critical for transcription of RNA Polymerase II (Pol II) regulated genes and is conserved across eukaryotic organisms.
- Mediator consists of head, middle, and tail modules, as well as a cyclin dependent kinase (CDK) domain.
- The head module, specifically Med17, associates directly with Pol II, the tail module associates with activators and repressors that are bound to DNA elements in the upstream activating sequence (2).
- Mediator serves to channel regulatory signals from activator and repressor proteins to affect changes in gene expression (2).
- Diseases spanning congenital malformations to cancer are associated with mutations in Mediator subunits (3).

Methods

- Schema of standard chromatin immunoprecipitation (ChIP) analysis to test whether Mediator and Pol II are evicted after rapamycin treatment in our strain.
- Chromatin was immunoprecipitated from whole cell extracts using antibodies against untagged Pol II and Srb4(Med17).

Results

- Findings depict that the Anchor Away system applied to Mediator was an effective way to evict Mediator from the nucleus.

Discussion

- The Anchor Away method is a technique developed to address the limitations of using temperature sensitive mutants (5).
- Our findings provide a good indication that the anchor strain effectively sequesters Mediator from the nucleus upon rapamycin treatment.
- Thus, the anchor away system is viable to test whether ChIP signals reflect genuine association of Mediator with Pol III genes.

Future Directions

- Our results are preliminary and lack sufficient biological replicates to conclude association of Mediator at Pol III genes.
- Future analysis of the yeast strain with Srb5myc epitope tag will be more informative about Mediator enrichment at Pol III.
- Artifactual ChIP signals observed in highly transcribed regions of DNA (such as PMA1) need to be addressed with the analysis of Anchor Away yeast strains transformed with the RPL255 NLS-GFP-LEU2 plasmid.

Literature cited


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