Using "Anchor Away" to Test Mediator Presence at Pol III Genes

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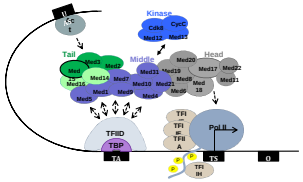
Introduction

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 (1).
•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).



Preliminary Research

•Unpublished data using chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) of temperature sensitive *med17* (*ts*) mutants suggests that Mediator subunit 17 associates with tRNA genes.
•The association between Mediator 17 and tRNA genes was lost after 1 hour incubation at 37˚C.
•However, artifactual ChIP signals have been found in highly transcribed regions of DNA that undermine the significance of the results (4).

Objective

To examine Mediator association wtih Pol II and Pol III genes before and after Mediator eviction from the nucleus using ChIP and an Anchor Away yeast strain.

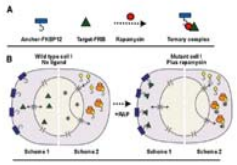
GFP (green fluorescent protein) expressed in the same strain serves as a control for false ChIP signals.

Two necessary steps for this were 1) testing the anchor away strain for Mediator eviction and 2) introducing and testing an epitope to be used in ChIP of Mediator in this strain.

Testing the Anchor Away System for Mediator Eviction

Anchor Away Strain

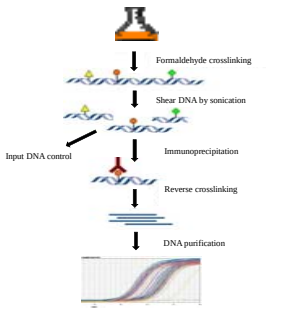
- The Anchor Away method uses rapamycin, a drug that forms a strong molecular bridge between a ribosomal anchor, RPL13A-FKBP12 and the target protein (5,6)
- In our strain, Srb4 (Med17) was fused to FRB, to allow eviction of Mediator from the nucleus upon rapamycin treatment.



Haruki (2008), *Molecular Cell* (5)

Methods

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



E. Knoll, unpublished data

Results

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

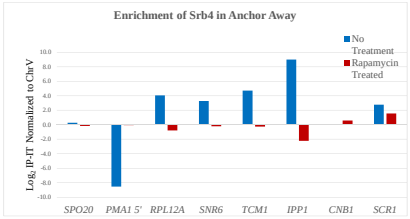


Figure 1: ChIP of Srb4 in Anchor Away Strain
SPO20, PMA1 5´, RPL12A, SNR6, TCM1, IPP1, CNB1, SCR1 molecular targets were normalized to *ChrV*. Almost all targets showed reduced association after rapamycin treatment.

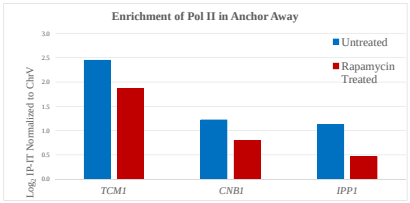
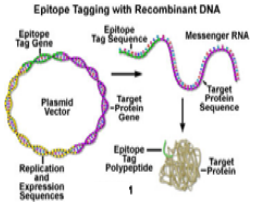


Figure 2: Enrichment of Pol II in Anchor Away Strain
Mediator is required for Pol II association with DNA. Here, Pol II association with three strongly transcribed genes is reduced after eviction of Mediator by rapamycin treatment.

Introducing a *myc* Epitope Tag to Med18/SRB5

Generating an epitope tag

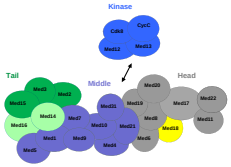
- We *myc* epitope tagged other Mediator subunits to perform ChIP because specific antibodies do not exist.
- Shown here (Fig. 3) is an example of PCR used to amplify the *SRB5-13 myc-HIS3* marked fragment from the east strain that expresses the Srb5-13myc fusion protein.



Modified from microscopy research center

Methods

- We used PCR primers SRB5 Forward: SRB5 myc to amplify the *SRB5-13 myc-HIS3* marked fragment from the yeast strain that expresses the Srb5-13myc fusion protein.
- The *HIS3* selective marker allowed integration into the *his-* (*his3-11,15*) Anchor Away strain of the *SRB5-13myc* fusion gene, with selection on CSM-leu-his plates.
- We then tested whether *SRB5-13 myc-HIS3* epitope tag was integration into the genome using ChIP.



E. Knoll, unpublished data

Results

- As expected, we observed a low signal in the absence of the epitope tag, since there is no epitope for the antibody to react with, and a strong signal in the epitope tagged strain (Fig 4).

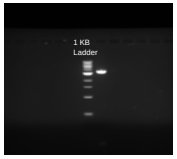


Figure 3: *Amplification of SRB5 myc-HIS3*
Gel electrophoresis of *SRB5-13 myc-HIS3* amplicon. The PCR program ran 25 cycles of denaturation at 95˚ C, annihilation 55˚ C, and extension at 72˚ C all for 1 minute.

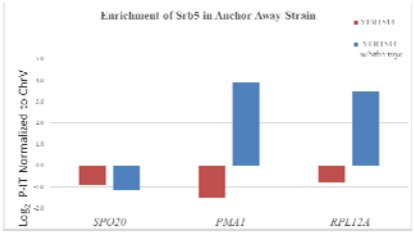


Figure 4: Enrichment of Srb5 in Anchor Away Strain.
Mediator (specifically Srb5) associates with the strongly transcribed PMA1 and RPL12A genes, but not with the very poorly transcribed SPO20 gene.

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 away strain effectively sequesters MED17 (Srb4) and Pol II from the nucleus.
•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 regulated 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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Acknowledgments

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