ROLE OF THE MEDIATOR COMPLEX IN ETHANOL TOLERANCE IN YEAST

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ABSTRACT

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Mediator is a protein complex in eukaryotes which integrates signals from a diverse range of transcription factors and relays them to RNA polymerase. Much of mediator is highly conserved, but it is expected that some sequences would become specialized according to the regulatory needs of particular organisms. Since the ability to tolerate high concentrations of ethanol is a characteristic feature of *Saccharomyces cerevisiae*, we studied mediator structure/function relationships through growth analysis of mutants on 2% glucose ± 6% ethanol. From this work, we identified a 35 amino acid sequence within Med8 in the "neck" region of the head module that plays a key role in ethanol tolerance under certain conditions, but which has little impact on growth on 2% glucose alone. Surprisingly, this region was only required for ethanol tolerance when the sequences that tether Med18 & Med20 to the head module were deleted or when the nearby Med31 subunit was deleted. Based on cryo-EM, these are both conditions which induce flexibility of the head module. Our working model is that this region of the mediator "neck" stabilizes a conformation of the mediator complex which is necessary for appropriate expression of key genes that confer ethanol tolerance, but which is not necessary for growth on glucose without ethanol. This is likely due to gene and context-specific changes in the structure and activity of the adjacent RNA polymerase CTD binding region.

ACKNOWLEDGEMENTS

I would like to thank my research advisor, Dr. Park, for his guidance and wisdom which he has so graciously shared with me. He is a man of thought and of science, and I hope that I have learned some of these qualities from him.

Thanks also to Miles Nicholson, another undergraduate in Dr. Park's lab, who has worked hard in performing many of these experiments and who I value as a colleague and companion.

NOMENCLATURE

bp	base pairs
cryo-EM	cryo-electron microscopy
DDT	Dithiothreitol
PCR	Polymerase Chain Reaction
SBP	Streptavidin binding protein
YCp	Yeast centromeric plasmid

CHAPTER I

INTRODUCTION

Mediator is 25–30 subunit protein complex in the nuclei of eukaryotes which acts as an intermediary between a wide range of transcription factors and RNA polymerase (Poss *et al.*, 2013). Many parts of mediator are highly conserved across all eukaryotic groups (Poss *et al.*, 2013). However, as might be expected due to the varying metabolic and regulatory needs of the various species, some sequences diverge rapidly even among closely related groups.

Structural studies using X-ray crystallography and cryo-electron microscopy (cryo-EM) have elucidated the structure of mediator at near-atomic resolution (Plaschka *et al.*, 2015; Robinson *et al.*, 2016; Tsai *et al.*, 2017). The structures resulting from these studies demonstrate that the three-dimensional structure of mediator is also highly similar across groups, even from those as far apart as mammals, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*.

Mediator is involved in a variety of aspects of gene expression, including recruitment of RNA polymerase, facilitation and inhibition of pre-initiation complex formation, elongation, and mRNA transport (Kornberg, 2005; Conaway and Conaway, 2013; Poss *et al.*, 2013; Schneider *et al.*, 2015). Due to mediator's intermediary role between transcription factors and RNA polymerase, it is sometimes described as a "processor" of genetic information (Allen and Taatjes, 2015). However, the mechanisms of this processing, or integration, of signals from the various transcription factors and signal transduction pathways to regulate the expression of individual genes are not well understood.

Prior to my arrival in the Park lab, preliminary results indicated that Med8 was important for ethanol tolerance. Specifically, a series of C-terminal deletions suggested that amino acids 138-189 (i.e. $Med8_{138\text{-}189}$) played a key role.

Med8 is an essential subunit located in the "head" region of mediator. It is known to contain two regions of interest: a portion of the binding region for the carboxy-terminal domain (CTD) of RNA polymerase II ($Med8_{107-121}$) and a conserved C-terminal domain ($Med8_{190-223}$) reported to tether Med18 and Med20 to the rest of the complex (Lariviere *et al.*, 2006; Plaschka *et al.*, 2015; Robinson *et al.*, 2016). Med8 also contains a helical region adjacent to the CTD binding region that is not required for viability ($Med8_{138-172}$), and divergent sequences at both ends and between amino acids 173 and 189 which are not visible in the crystal structure (Plaschka *et al.*, 2015).

The present study examines the effect of a series modifications to Med8 on the ability of *Saccharomyces cerevisiae* to grow on rich media containing 2% glucose ± 6% ethanol.

CHAPTER II

MATERIALS AND METHODS

Materials

G block 1778	Synthetic partial Med8 containing 40 bp of 5' flanking sequence, Med8 coding sequence for amino acids 1-138, a stop codon, and the first 40 amino acids of the promoter for His3MX6
pRSII415	Yeast centromeric plasmid with full length Med8 and Leu2 selectable marker
PB30	Yeast centromeric plasmid pRSII415 containing full length wild type Med8 and the Leu2 gene [YCp Med8 Leu2]
PB37	Yeast centromeric plasmid pRSII415 containing the full length 223 amino acid Med8 sequence fused in frame to the TAP tag and also His3MX6 [YCp $Med8_{1-223}$-TAP His3 Leu2]
PB38	Yeast centromeric plasmid pRSII415 containing the $Med8_{1-138}$ fused in frame to TAP and also His3MX6 [YCp $Med8_{1-138}$-TAP His3 Leu2]
PB45	The same as PB37, except that $Med8_{138-176}$ was replace with SBP, an unrelated sequence (Streptavidin Binding Peptide) [YCp $Med8_{SBP-223}$-TAP His3 Leu2]
PB53	Yeast centromeric plasmid containing $Med8_{1-189}$-TAP, but with Med8 $_{132-176}$ replaced by SBP [YCp $Med8_{SBP-189}$-TAP His3 Leu2]

PY25	BY4741 MATa Med8-TAP His3Mx6 S. cerevisiae with C-terminal-TAP tagged Med8 purchased from Dharmacon
PY36	BY4742 med8Δ0::KANMX6 [YCp Med8 Ura3] BY4742 derivative in which chromosomal Med8 is deleted and Med8 function is provided by centromeric plasmid pRSII416 containing full length Med8 and Ura3
PY68	BY4742α med8Δ0 [YCp $Med8_{1-138}$ His3 Leu2]
PY69	BY4742α med8Δ0 [YCp $Med8_{1-138}$-TAP His3 Leu2]
PY84	BY4742 med8Δ0::KANMX6 [YCp Med8 Ura3] med31Δ0::hph Same as PY36, but with Med31 coding region deleted

Methods

All work on this project was conducted using BL-1 procedures in accordance with Texas A&M Institutional Biosafety Guidelines under permit IBC2015-073. Experiments were designed by Dr. William Park, and were performed by Dr. Park, Miles Nicholson, or myself.

General approach for preparation of yeast strains

Most of the yeast strains used in these experiments were derived from BY4742. The genotype of this strain is: MATα, his3Δ0, leu2Δ0, med15Δ0, ura3Δ0. Unless otherwise indicated, yeast and bacterial manipulations were performed as described in Methods in Yeast Genetics and Genomics (Dunham *et al.*, 2015) or Molecular Cloning (Sambrook *et al.*, 1989).

Preparation of Med8$_{1-138}$ without TAP tag

Med8Δ0 Leu2 was isolated by amplification of all of PB30 [YCp Med8 Leu2], except the Med8 coding region, using primers 1724_R and 1725_F (shown in figure 1 below) and the high fidelity "Phusion" polymerase from New England BioLabs. The mobility of the PCR product on a 0.7% agarose gel was consistent with the expected size of 6489 base pairs.

His3MX6 fused to Med8 3' flanking sequences was isolated by amplification of genomic DNA isolated from the yeast strain PY25 (containing C-terminal-TAP tagged Med8 fused to His3MX6) using primers 1777_F and 1738_R and "Phusion" polymerase. As shown on Figure 1 below, this selectively excludes the TAP tag. Mobility of the PCR fragment on a 1% agarose gel was consistent with the expected size of 1578 base pairs.

Med8Δ0 Leu2, His3MX6, and G block 1778 (containing Med8$_{1-138}$) were fused using Gibson Assembly (Gibson, 2011). The recombinant plasmid was then transformed into frozen competent DH5α *E. coli*. Transformants were analyzed via PCR using primers 1737_F and 1738_R, and agarose gel electrophoresis. The absence of TAP was confirmed using amplification with primers 1780_F, which binds to Med8 coding sequence, and 1781_F, which binds to His3MX6 promoter, followed by agarose gel electrophoresis. This resulted in a 509 bp fragment, indicating the absence of TAP. The resulting bacterial strain was named PB46 [YCp Med8$_{1-138}$ His3 Leu2] and a frozen glycerol stock was prepared.

The plasmid YCp Med8$_{1-138}$ His3 Leu2 was purified with the ZR Plasmid Miniprep kit from Zymo Research. The resulting plasmid was quantified using a NanoDrop spectrophotometer.

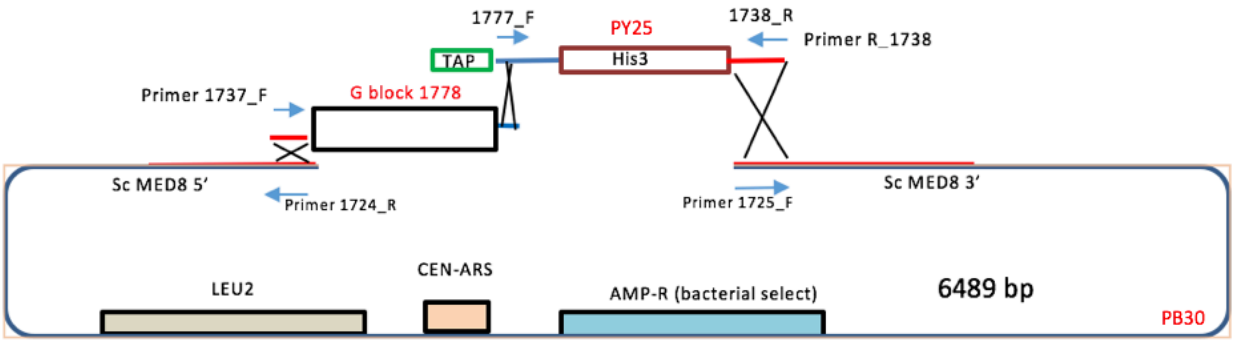

Figure 1. Preparation of Med8$_{1\text{-}138}$ without TAP tag

Transformation of Med8$_{1\text{-}138}$ ±TAP into yeast

A Med8Δ0 haploid yeast strain cannot be directly prepared because Med8 is an essential gene. Consequently, the Med8±TAP strains were prepared indirectly from cells containing [YCp Med8 Ura3] plasmid to complement the deletion of the chromosomal copy of Med8 using the following plasmid shuffle process.

Yeast strain PY36 BY4742 med8Δ0 [YCp Med8 Ura3]) was transformed with purified PB46 [YCp Med8$_{1\text{-}138}$ His3 Leu2] DNA. In parallel, PY36 med8Δ0 was also transformed with PB38 [YCp Med8$_{1\text{-}138}$-TAP His3 Leu2] to allow direct, side-by-side comparison of yeast strains with and without the TAP tag.

Yeast transformation was performed using 1 ml of an overnight culture of PY36 resuspended in 0.8 ml of a solution consisting of 1.0 mL 0.2M lithium acetate/40% polyethylene glycol 3350, 120 µL 0.1M DDT, and 30 µL 2 mg/mL single-stranded salmon sperm DNA. For each transformation, 0.1 ml of the cell suspension-transformation solution was incubated with 1 µg of plasmid DNA at 45°C for 30 minutes before plating on synthetic complete media, containing 2% glucose and all essential amino acids except histidine (SC-His). Streaking for single colonies

yielded strains which contained [YCp Med8$_{1-138}$±TAP His3 Leu2]. Most of these also contained the wild type complementing plasmid [YCp Med8 Ura3] present in PY36.

To remove the complementing [YCp Med8$_{1-223}$ Ura3] plasmid, cells were gridded on YPD (rich media with 2% glucose) and incubated at 30 °C for 24 hours. PY36 was also included as a negative control. This plate was replicated onto SC-Leu 5-FOA (synthetic complete media without leucine, and containing 5-FOA, a compound which is metabolized into a toxin by yeast strains containing Ura3) and incubated at 30 °C for 48 hours. This resulted in no growth from the negative PY36 control, but robust growth from the ±TAP strains. Streaking for single colonies yielded PY68 (BY4742α med8Δ0 [YCp Med8$_{1-138}$ His3 Leu2]), the strain without TAP, and PY69 (BY4742α med8Δ0 [YCp Med8$_{1-138}$-TAP His3 Leu2]), the strain with TAP.

Preparation of YCp Med8$_{SBP-189}$-TAP His3 Leu2 Plasmid

From previous experiments, we had yeast strain PB45A, which has full length Med8 with 138-176 replaced by the streptavidin binding peptide (SBP), an unrelated sequence containing a helical segment. Note, SBP is two amino acids longer than the Med8$_{138-172}$ segment it replaced, but has a similar helical structure. To remove Med8$_{190-223}$-TAP, we purified the plasmid from PB45A, and amplified it with primers 1737_F and 1779_R (shown in figure 2 below) flanking Med8$_{SBP-189}$, resulting in a 649 base pair fragment. TAP was isolated with a TAP-His3 cassette and primers 1740_F and 1738_R, resulting in a 2136 base pair fragment. Each fragment was confirmed via gel electrophoresis. The modified Med8 and Tap-His3 fragments were assembled via Gibson assembly and the resulting plasmids inserted into bacteria and screened by PCR and gel electrophoresis as described above.

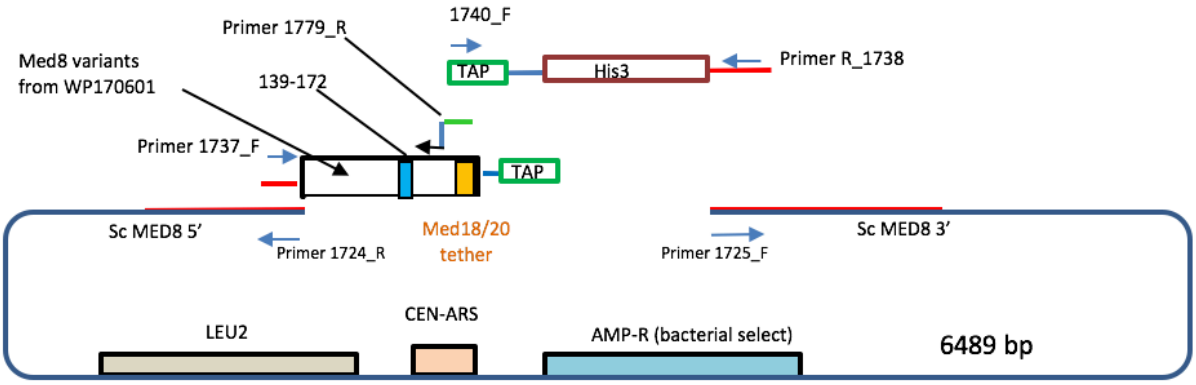

Figure 2: Diagram of construction of Med8$_{SBP-189}$-TAP plasmids.

Transformation of yeast and isolation of PY72

The plasmid YCp Med8$_{SBP-189}$-TAP His3 Leu2 (a.k.a. Med8$_{SBP-189}$-TAP was introduced into BY36 med8Δ0 [YCp Sc Med8 Ura3] via the one-step lithium acetate method and the complementing wild type Ura3 plasmid evicted by the "shuffle" technique described above.

As expected, a transformation control with no added plasmid DNA produced no colonies, and the new yeast strain PY72 (BY4742α med8Δ0 [YCp Med8$_{SBP-189}$-TAP His3 LEU2]) grew robustly.

Source of Med31Δ0

For this experiment, Dr. Park prepared PY84, a med31Δ0 version of BY36, and inserted Med8$_{1-138}$-TAP, Med8$_{SBP-189}$-TAP and Med8$_{SBP-223}$-TAP. I was involved in the growth analyses of this strain.

Measurement of growth rates

Because the absorbance of a liquid yeast culture at 600 nm increases as the number of yeast suspended in the culture increases, absorbance at 600 nm can be used as a measure for the number of cells. A basic problem, however, is that ethanol evaporates at a significant rate under the conditions normally used to grow yeast – e.g. shaking 25 ml of media in 250 ml flask with vented caps at 30C. Fortunately, prior research in the Park lab has shown that in the presence of glucose, yeast grow exponentially without shaking in 13 x 100 mm tubes sealed with gas permeable tape. This limits ethanol evaporation, but allows very reproducible growth measurements.

To obtain saturated cultures for inoculation, 5 ml cultures were grown in YPD (2% glucose) for 48 hours on a rotating wheel at 30 °C. Absorbance at 600 nm of the 48-hour culture was then measured using a 100-fold dilution of the same culture. Duplicate 4 ml cultures in appropriate media (frequently YPD, i.e. 2% glucose ± 6% ethanol) in 13 x 100 mm tubes were then inoculated with 0.1 optical density (OD) of cells, where 1 OD is defined as 1 absorbance value. This corresponds to approximately 10^6 cells. The 13 x 100 mm tube screw caps were opened ½ turn and sealed with one round of 3M Micropore tape to allow some gas exchange while minimizing ethanol loss. All experimental cultures were grown at 30 °C without shaking other than being mixed gently before measurement of A600 at intervals of 3-12 hours.

CHAPTER III

RESULTS

Ethanol tolerance in $Med8_{138}$ ± TAP

Prior to my arrival in the Park lab, a series of C-terminal, TAP-tagged deletions of Med8 had been prepared and tested for growth 2% glucose ± 6% ethanol. $Med8_{1\text{-}189}$-TAP, which deletes the region of Med8 reported to tether Med18 and Med20 to the rest of the mediator complex (Lariviere *et al.*, 2006; Lariviere *et al.*, 2008) had only a modest effect on ethanol tolerance and was comparable to $Med8_{1\text{-}172}$-TAP. $Med8_{1\text{-}138}$-TAP still grew vigorously on 2% glucose alone, but growth was almost completely blocked in 2% glucose + 6% ethanol.

The question that I first addressed was whether Med8 amino acids 138-172 actually play a key role in ethanol tolerance or whether the lack of ethanol tolerance in $Med8_{138}$-TAP, compared to longer clones such as $Med8_{172}$-TAP, was due to proximity of the large TAP tag to the CTD binding region of the mediator complex. This was done a growth assay of PY68 and PY69 ($Med8_{138}$ ± TAP tag) and directly comparing their growth in glucose-containing media ± 6% ethanol.

PY68 and PY69 ($Med8_{138}$ ± TAP) both had very similar growth patterns. On YPD (2% glucose) both strains grew from an initial A600 value of ≈ 0.025 to A600 >1 in less than 24 hrs. After a short lag, the growth curves were linear when plotted as $\log_2$ of A600 – as would be expected for logarithmic growth. PY68 and PY69 also had very similar growth curves in ethanol (YPDE 2% glucose + 6% ethanol). However, growth was much slower and essentially stopped after 20 hours.

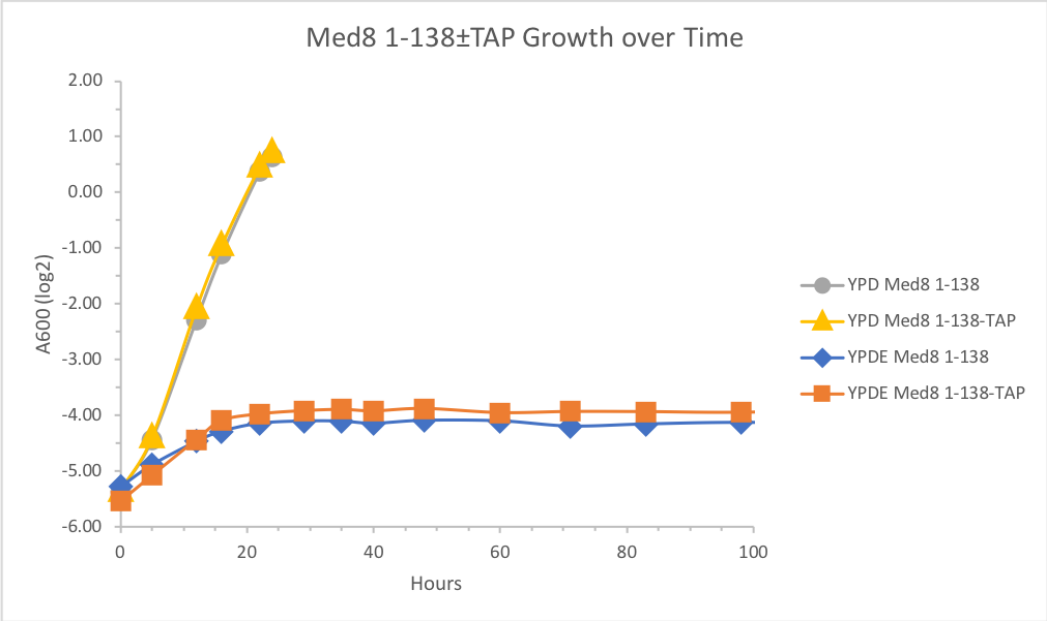

Figure 3: Growth of $Med8_{1\text{-}138}$ ±TAP variants on YPD (2% glucose) and YPDE (2% glucose + 6% ethanol).

Growth of constructs in which $Med8_{138\text{-}176}$ was replaced by SBP

Despite the importance of $Med8_{138\text{-}172}$ in C-terminal deletions, it was observed prior to my arrival in the Park lab that replacement of $Med8_{138\text{-}176}$ with the unrelated sequence streptavidin binding peptide (SBP) in full length Med8 ($Med8_{SBP\text{-}223}$-TAP) does not reduce ethanol tolerance. To further examine this apparent paradox, we prepared $Med8_{SBP\text{-}189}$-TAP and grew the variants summarized in Figure 4 in 2% glucose + 6% ethanol.

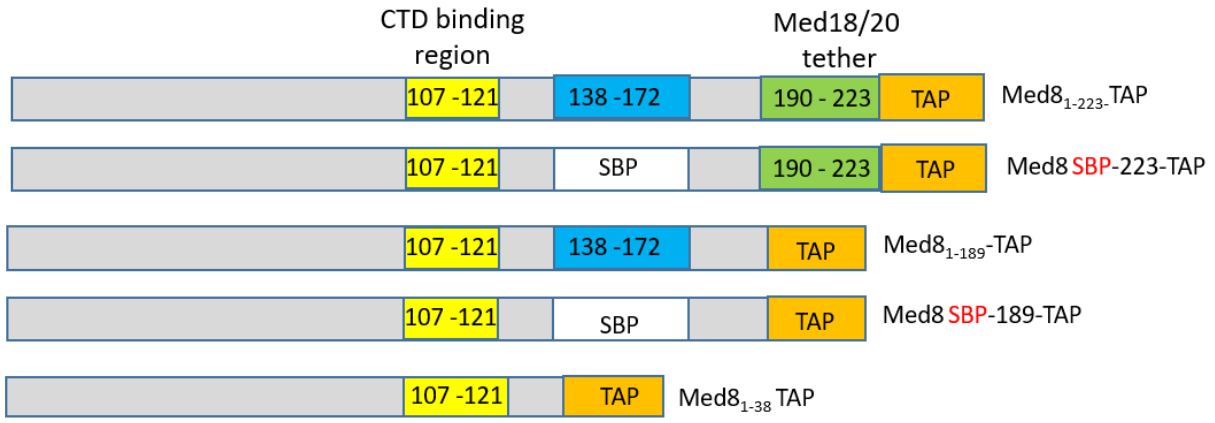

Figure 4: Summary of Med8 length and content variants

As shown in figure 5 below, yeast containing full length Med8$_{223}$-TAP grow relatively well on 6% ethanol. When the Med18/20 tether is absent, replacing Med8 $_{138-172}$ with SBP (Med8 $_{SBP-189}$-TAP) resulted in an almost complete loss of ethanol tolerance; similar to that seen with Med8$_{1-138}$-TAP. However, when the Med18/20 tether was present (Med8$_{SBP-223}$-TAP), replacing Med8$_{132-176}$ had no effect on growth on 2% glucose + 6% ethanol.

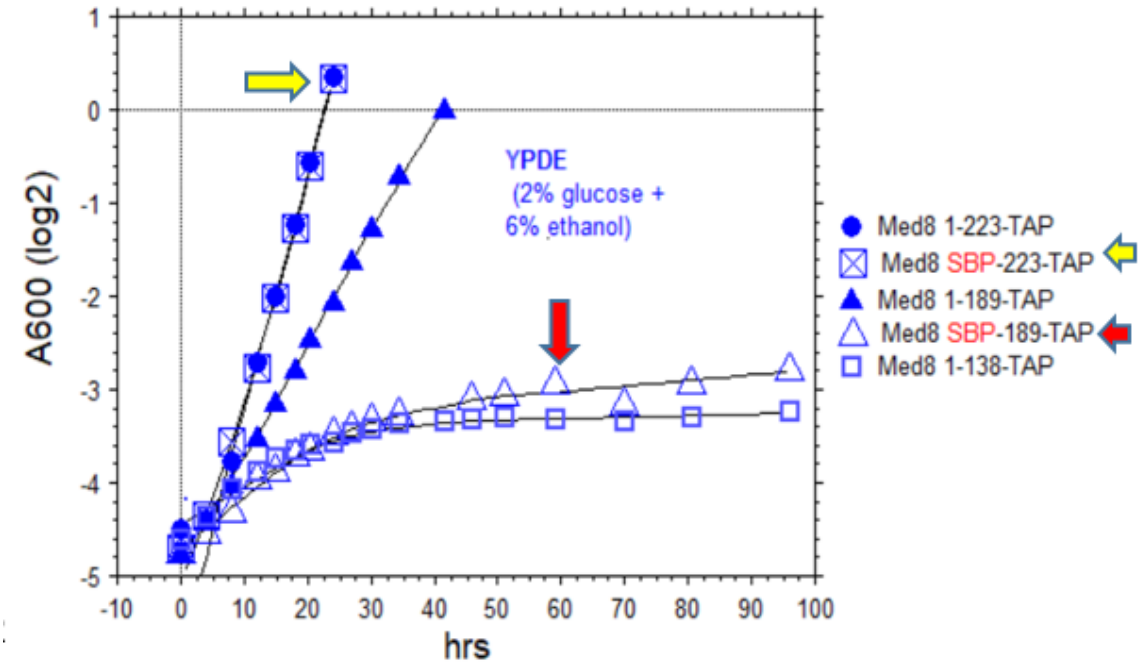

Figure 5: Growth of Med8 length and content variants on 2% glucose + 6% ethanol.

Interaction between Med31 and Med8$_{138\text{-}176}$

Med31 is located directly across the carboxy-terminal domain (CTD) binding gap from Med8 and near the critical Med8$_{139\text{-}172}$ region. Thus it is possible that the normal structure in the head module is due in part to an interaction between Med31 and Med8 across the CTD-binding gap that involves Med8$_{138\text{-}176}$. In this hypothetical case, one might expect synergistic interactions between Med31Δ0 and Med$_{138\text{-}176}$ mutations.

Dr. Park prepared PY84, a med31Δ0 derivative of PY36 (BY4742 med8Δ0 [YCp Med8 Ura3] med31Δ0) and I helped transform it with Med8$_{1\text{-}138}$-TAP, Med8$_{SBP\text{-}189}$-TAP and Med8$_{SBP\text{-}223}$-TAP and to assay relative growth on 2% glucose ± 6% ethanol.

Interestingly, med31Δ0 was synthetically lethal when the Med18/20 tether region of Med8 was absent. [YCp Med8$_{138}$-TAP His3 Leu] and [YCp Med8$_{SBP\text{-}189}$-TAP His3 Leu] were both successfully introduced into the PY84, but no cells survived after the complementing [YCp Med8 Ura3] plasmid was evicted with 5-FOA. Note that deletion of both Med18/20 tether and Med31 was lethal even on rich media containing glucose without ethanol. In contrast, BY4742 med8Δ0 [YCp Med8 SBP-223] med31Δ0) transformants grew normally on media with 2% glucose.

Consistent with the results shown above, cells containing Med8$_{SBP\text{-}223}$-TAP had the same relative growth rate in 2% glucose + 6% ethanol as the corresponding wild type (Figure 6). In both cases, the specific growth rate on 2% glucose + 6% ethanol was approximately 55% of that on 2% glucose alone. Cells containing med31Δ0 alone were somewhat more less ethanol tolerant, with a relative growth rate of approximately 0.3. The double mutant, however had a much lower growth rate than either med31Δ0 or Med8 SBP-223-TAP alone (relative growth rate of approximately 0.01).

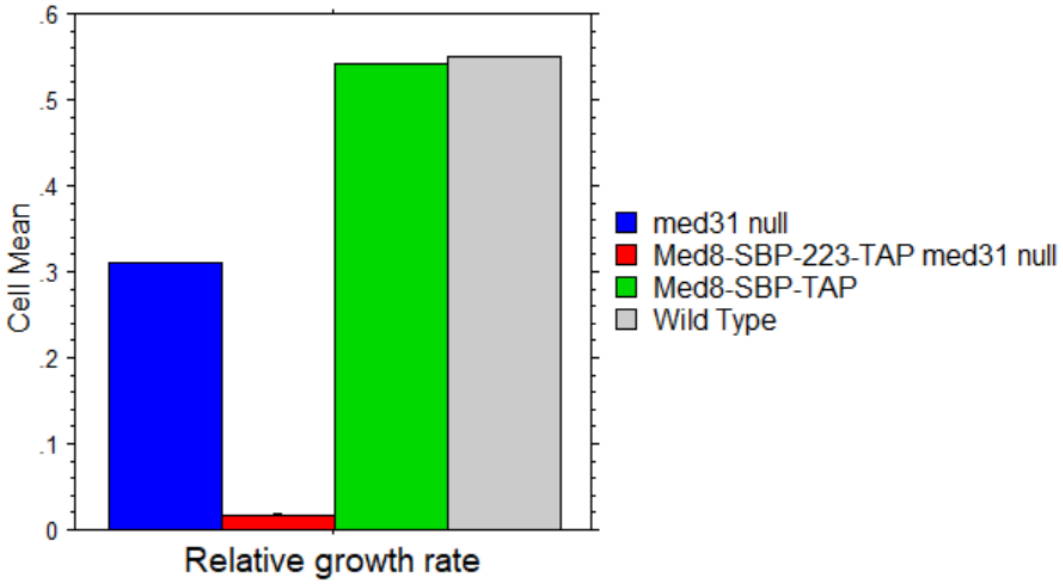

Figure 6. Relative growth rate (Growth rate on 2% glucose ± 6% ethanol) of yeast containing a Med31 deletion, in which $Med8_{138\text{-}176}$ was replaced with the SBP, the double mutant and the corresponding wild type strain, BY4742.

CHAPTER IV

DISCUSSION

$Med8_{1-138}$ ethanol susceptibility cannot be explained by TAP steric effects

The initial observation that $Med8_{138}$-TAP grew poorly in 6% ethanol did not prove that Med8 amino acids 139-172 were essential for ethanol tolerance. Since $Med8_{138-172}$ is near the mediator CTD binding region, a reasonable alternate explanation was that the bulky TAP tag could have sterically hindered the conformational rearrangement of mediator associated with pre-initiation complex formation (Tsai et al. 2017) in a fashion that particularly effected one or more critical genes required for ethanol tolerance. To test this possibility, we prepared a $Med8_{1-138}$ variant without the TAP tag and found that growth in the presence or absence of 6% ethanol was essentially identical. This result strongly suggests that proximity of the TAP tag to critical regions of the mediator complex was not responsible for the reduced ethanol tolerance of $Med8_{138}$-TAP variants, but rather that the actual $Med8_{138-223}$ sequence was directly involved.

The role of $Med8_{138-172}$ is context dependent

Despite the dramatic effect of removing $Med8_{138-172}$ in C-terminal deletions, replacing it with the unrelated SBP sequence in full length Med8 had no effect on growth rate ± 6% ethanol However, like $Med8_{138-176}$, SBP contains a short helical region. Thus it was possible that, despite lack of sequence homology, SBP was able to functionally substitute for $Med8_{138-176}$. To test this possibility, we replaced $Med8_{138-176}$ with SBP when the Med18/20 tether was absent ($Med8_{SBP-189}$-TAP). This resulted in a loss of ethanol tolerance with growth patterns similar to $Med8_{1-138}$-TAP. Therefore, under normal conditions, the presence of the Med18/20 tether in the mediator complex is epistatic to the ethanol-resistance function of $Med8_{139-172}$.

The role of $Med8_{138\text{-}176}$ was also clearly evident in med31Δ0 cells. Deleting Med31 alone had only a modest effect in cells with wild type Med8, reducing the relative growth rate from 0.55 to 0.3. Substituting SBP for $Med8_{138\text{-}176}$ in $Med31^{+}$ cells had no effect on growth ± 6% ethanol. However, the $Med8_{SBP\text{-}223}$-TAP, med31Δ0 double mutant almost completely blocked growth on 6% ethanol. This synergistic effect suggests that Med31 and $Med8_{138\text{-}176}$ make independent contributions to ethanol tolerance.

In summary, $Med8_{138\text{-}176}$, $Med8_{189\text{-}223}$ (the Med18/20 tether), and Med31 each function to enable ethanol-resistance. The Med18/20 tether is epistatic to $Med8_{138\text{-}176}$. $Med8_{138\text{-}176}$ and Med31 act synergistically to support ethanol tolerance.

Working model

During formation of the transcription preinitiation complex, mediator undergoes a conformational shift which optimizes the gap between mediator "neck" and "knob" domains that bind the CTD of RNA Polymerase II (RNA Pol II) (Figures 7 and 8 below) (Tsai *et al.*, 2017). Med8 is part of the "neck" domain and contains a portion of the CTD binding domain (Robinson *et al.*, 2016). Med31 (yellow in Figure 7 below) forms the majority of the "knob" domain and also directly interacts with the CTD-binding domain (Tsai *et al.*, 2017). The conformational shift also optimizes interactions with the foot domain of RNA Pol II and Med18 and Med20 (orange and red in Figure 7), which collectively form the "moveable jaw" of mediator.

Deletion of Med31 and $Med8_{189\text{-}223}$ may contribute to flexibility in the mediator head module. Tsai *et al.* (2014) observed by cryo-EM that the head module of the mediator complex becomes more flexible in both med18Δ0 and med31Δ0 cells. While Med18 was still active in all of the strains used in these experiments, Med18 and Med20 are no longer tightly bound to the

mediator complex when Med8$_{190-223}$ are deleted (Lariviere *et al.*, 2006). Thus, many of the constructs used in these growth assays contain modifications which cause flexibility in the head module under the conditions used for cryo-EM. It is possible that this same flexibility occurs *in vivo*.

Med31, Med8$_{138-172}$ and the Med18/20 tether region of Med8 may all play key roles in stabilizing a specific conformation of the mediator complex that is required for expression of ethanol-stress related genes, but which is not critical for growth on glucose alone. Under conditions that favor enhanced mediator head module flexibility such as deletion of Med31or the Med18/20 tether, interactions between Med8$_{138-172}$ and other subunits or interacting proteins may be critical for expressing key genes required ethanol tolerance. However, when Med31, and the Med18/20 tether region are intact (and thus Med18, Med20, and the C-terminal end of Med8 are tightly bound), the role of Med8$_{138-172}$ may be less critical. The observed synthetic lethality of Med31 and Med18/20 tether deletions, and the synergy between med31Δ0 and Med8$_{SBP-223}$-TAP are all consistent with Med31, Med8$_{138-172}$ and the Med18/20 tether playing mutually reinforcing roles in gene- and contextdependent changes in the structure or activity of the nearby CTD binding region.

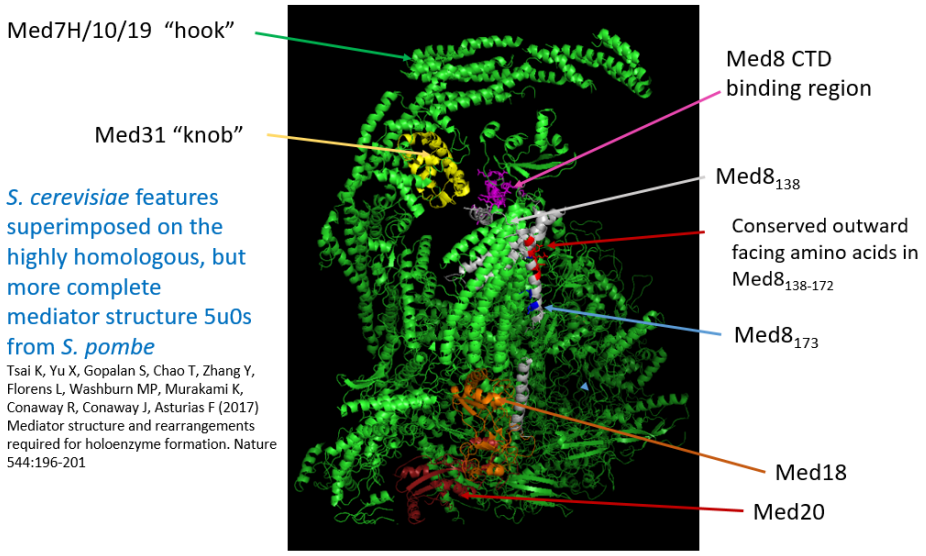

Figure 7: *S. cerevisiae* features superimposed on the highly homologous, but more complete mediator structure 5u0s from *S. pombe.*

The proposed role of Med8$_{138\text{-}172}$, as illustrated in figure 8, can be envisioned, in the cartoon of mediator function from Tsai *et al*., 2017, as stabilizing conformations of the mediator "neck" during CTD, binding, holoenzyme formation and subsequent CTD phosphorylation and initiation of transcription that are critical for expression of key genes involve in ethanol tolerance, but which are less important for growth under normal conditions without ethanol.

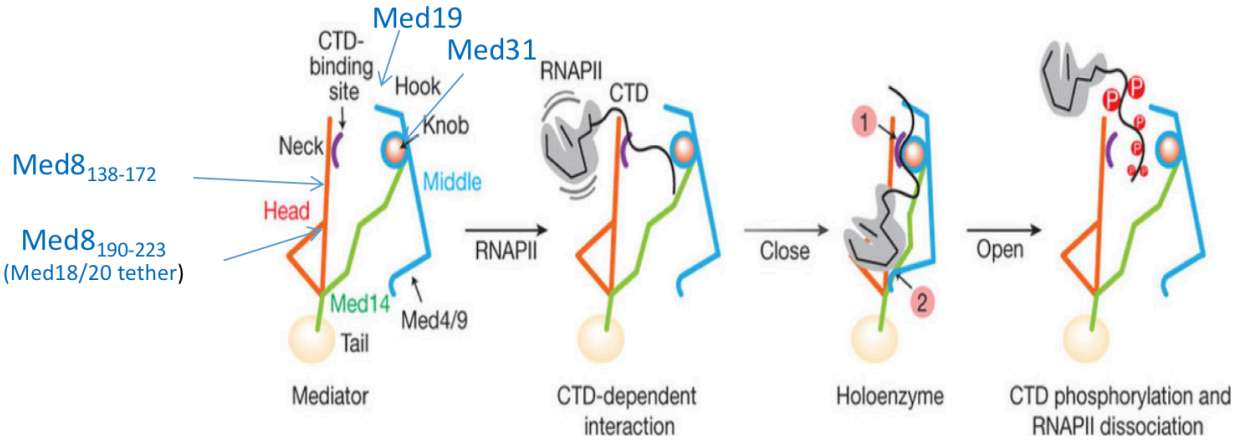

Figure 8: Cartoon from Tsai *et al*., 2017 illustrated to show the critical locations of Med8$_{138\text{-}172}$, the Med18/20 tether, Med19 and Med31.

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