The Effect of MCHM on Stress Response Pathway Regulators, Med15 and Snf1

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Abstract

Living cells respond by changing molecular and cellular pathways when they are exposed to stressful environmental conditions. Damage to intracellular molecules depends on the duration of stress exposure and the severity of the stressor. In 2014, large volumes of MCHM (4-methylcyclohexane) spilled into the Elk River of West Virginia contaminating drinking-water supplies. By studying cellular stress response through the Mediator complex in *Saccharomyces cerevisiae* (baker's yeast), the molecular effects of the industrial coal-cleaning chemical can be determined. Mediator, a highly conserved multi subunit complex relays signals from DNA binding transcription factors to RNA polymerase II. Med15, a subunit found within the tail domain of the Mediator complex works with stress-induced transcription factors and is regulated by many kinases including CDKs and the AMP kinase Snf1. Because Snf1 interacts with the Mediator complex and is required for many cellular activities such as glucose-repressed gene transcription and pH tolerance. *snf1Δ* yeasts are much more sensitive to MCHM compared to wild-type. Med15 contains a KIX domain, and two polyQ tracts separated by a polyQA tract. Genetic variation of the Med15 subunit between yeast strains (YJM789K5a and S288c) caused by the difference in the polyQ tracts leads to varying levels of MCHM tolerance. Under adverse conditions, both Med15 and Snf1 are essential for the regulation of stress response pathways. Another interesting discovery was that the 13xMyc tag that was used throughout the study, have shown to have an impact on Med15 and in turn affects its sensitivity towards MCHM and other chemical stressors. Snf1 regulates energy homeostasis and is often misregulated in cancer and other metabolic diseases, while patients DiGeorge Syndrome, who multiple developmental defects and are often sick are missing one copy of Med15. Understanding how energy homeostasis and stress response are related will improve understanding of exposure risks to MCHM and how conserved pathways interact.

Results

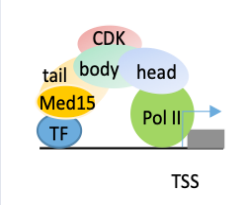
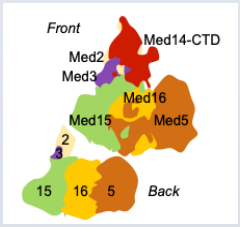


Figure 1 Interaction between the Mediator complex, transcription factors and pol II at transcription start site The head domain of the Mediator complex interacts directly with the pol II's C-terminal domain, while the middle domain associates with cyclin-dependent kinases (CDKs). The tail domain binds to regulatory factors.

Figure 2 Representation of protein components of the tail domain The tail domain of the Mediator complex is composed of Med3, Med2, Med5, Med16 and Med15 (Gal11) and the C-terminal end of Med14 connects the tail with the rest of the body of the Mediator complex.



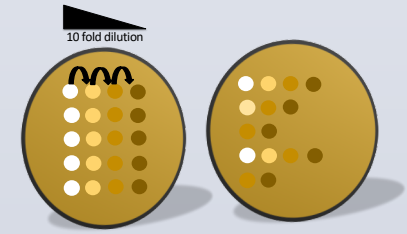


Figure 3 Growth assays used as a measure of chemical sensitivity

Yeast are grown in liquid culture and then diluted to the same appropriate concentration. Ten-fold serial dilutions are carried out and then spotted onto solid media containing chemicals or drugs of interest. YPD is rich media containing all the necessary amino acids and carbon source for yeast to grow. Plates are incubated at 30°C and then photographed over a period of 3-4 days.

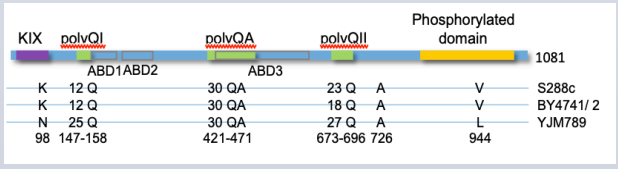


Figure 4 Schematic of Med15 subunit and differences between five genetically diverse yeast Med15 (120kDa) is made up of unique amino acids sequences that comprises the KIX domain, polyglutamine domain I (polyQI), polyglutamine/alanine domain (polyQA), polyglutamine domain II (polyQII) and the Mediator activation/ association domain (MAD). The amino acid numbers in the diagram are based on S288c. Variations in length of polyQ tracts between S288c and YJM789 could be responsible for the differences seen in MCHM tolerance.

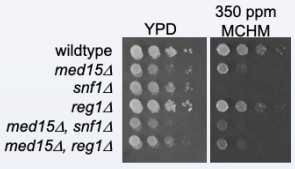


Figure 5 Impact of Med15, Snf1 and Reg1 on yeast Serial dilution of BY4741 with single and double mutants of med15Δ, *snf1Δ* and *reg1Δ* grown for two days on YPD or 350ppm of MCHM. On YPD, *snf1Δ* yeast grew a little slower than wildtype yeast on YPD and was much more sensitive in MCHM. Reg1, a negative regulator of Snf1 when deleted grew only a little slower on MCHM compared to wildtype yeast.

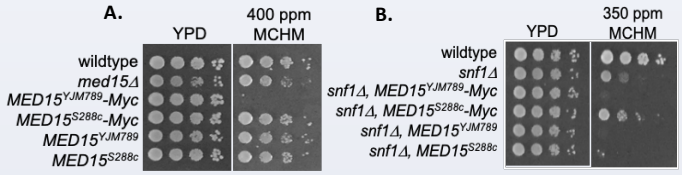
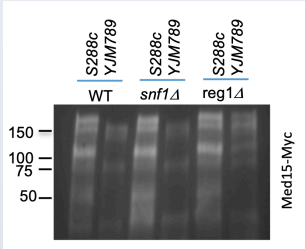


Figure 6 Effect of Myc tag on yeast expressing different alleles of Med15 (A) Serial dilution of BY4741 with different alleles of Med15 that were C-terminally tagged with 13xMyc or untagged grown on YPD or 400ppm of MCHM. The effect of Myc tag can be observed through the difference in MCHM sensitivity. (B) Serial dilution of BY4741 *snf1Δ* with different alleles of Med15 that were C-terminally tagged with 13xMyc or untagged grown on YPD or 350ppm of MCHM. Only the expression of Med15^{S288c}-Myc affected sensitivity to MCHM.

Figure 7 Western blot of different Med15-Myc alleles immunoprecipitated from BY4741 with *snf1Δ or reg1Δ* Both of the Med15 alleles were similar in the wildtype yeast and *snf1Δ*. However in the *reg1Δ*, Med15^{YJM789}-Myc shifted up and has similar banding patterns to Med15^{S288c}-Myc.



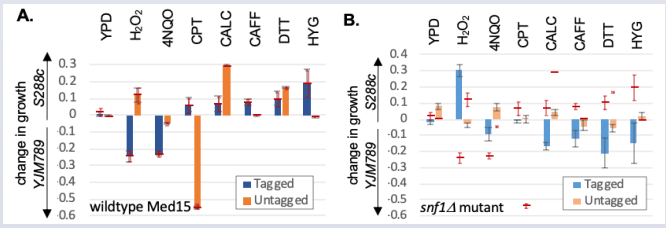


Figure 8 Quantitative growth assay of different Med15 alleles Myc-tagged and untagged with different drugs Values above the y-axis indicate increased growth of yeast with Med15^{S288c} and values below the y-axis indicate increased growth of yeast with Med15^{YJM789}. (A) Growth of yeast with different Med15 allele tagged with Myc. Med15^{YJM789}-Myc had a greater resistance against chemicals that produced free radicals such as hydrogen peroxide (H_2O_2) and 4-nitroquinoline 1-oxide (4NQO). (B) Growth of BY4741 *snf1Δ* carrying different alleles of Med15 tagged with Myc or untagged. The Myc tag flipped some preferences of the alleles and for some it exaggerated the differences. Med15^{S288c}-Myc *snf1Δ* grew better in H_2O_2 than wildtype yeast.

Conclusions and Ongoing Research

- Both Snf1 and Med15 are essential for MCHM tolerance.
- Without Snf1 and Med15 as stress response regulators, a different stress response pathway or regulators must be activated to allow for cell's survival and growth.
- Genetic variation contributes to the variation in MCHM tolerance.
- Snf1 and Med15 are part of the stress response pathway, which is necessary for cells to overcome and adapt to environmental changes.
- Myc tag is known to be a target of Snf1. Phosphorylation through Snf1 tethers to the tag and therefore can affect the function of Med15.
- We plan on determining post-translational modifications on Med15 after exposure to various chemical stressors and its interactions with other subunits using a proximity labeling technique.

Acknowledgments

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