Proposed Mechanisms of Function of Taf14-Containing Nuclear Complexes Involved in Transcriptional Regulation of *Saccharomyces cerevisiae*

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Aakanksha Gundu[1], Ashley Aragon[1], Dr. Taylor A. Lundy, Ph.D[1], Dr. Brian D. Strahl, Ph.D[1,2]

[1]Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC, [2]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC

UNC SCHOOL OF MEDICINE

Abstract

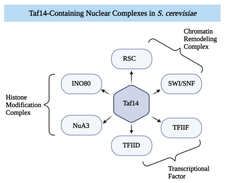
Understanding transcriptional regulation in *Saccharomyces cerevisiae* has key implications for cancer treatment because cancer is caused by abnormalities in transcription and the DNA–repair response. YEATS–domain proteins play an essential role in transcriptional regulation of the yeast genome by reading histone post–translational modifications. Taf14 is a nuclear hub that plays a fundamental role in the control of DNA processes. This protein recognizes transcriptional coactivator proteins of 6 nuclear complexes: RSC, SWI/SNF, TFIIF, TFIID, NuA3, and INO80. Recent research has discovered a conserved binding motif on each of these complexes that is recognized by Taf14 to induce protein–protein interactions.

The purpose of this project is to propose mechanisms of function of the TFIIF and TFIID complexes and how they may be involved in the DNA–repair pathway. After mutating the conserved binding sequences recognized by Taf14 on the complexes, the mutant strains, such as Taf2, Tfg1–1M4, and Tfg1–2M4, were grown in the presence of DNA–damaging agents, including hydroxyurea, camptothecin, methyl methanesulfonate, and a caffeine stressor. The pathways by which the DNA–damaging agents act sheds light on the role of proteins involved in these complexes.

Introduction

The yeast metabolic cycle acts as a transcriptional regulator through periodic expression of the yeast genome. During the high oxygen consumption phase, yeast cells undergo aerobic respiration, experience rapid growth, have high levels of acetylated histones, and greater acetyl–CoA usage for cell activities. During the low oxygen consumption phase, yeast cells repair mitochondria, increase crotonylation, undergo fermentation, and begin the cell cycle.

YEATS domain proteins, such as Taf14, recognize histone post–translational modifications by engaging chromatin associated proteins to express specific genes. Taf14 is a nuclear hub involved in the DNA–damage response, the heat response pathway, cytoskeleton organization, and more cellular processes. Taf14 associates with 6 complexes through a conserved binding motif. Mutating the essential residues on the binding motif of a complex prevents its protein–protein interaction with Taf14 and inhibits the normal pathway.



DNA–damaging agents have unique mechanisms of actions to prevent cell proliferation. Hydroxyurea inhibits ribonucleotide reductase, which decreases the concentration of available deoxyribonucleotides for DNA synthesis. Camptothecin inhibits DNA topoisomerase I activity, which leads to DNA strand breaks by blocking ligation. Methyl Methanesulfonate induces mutagenesis and interferes with the base excision repair (BER) pathway. Caffeine is a stressor that inhibits the growth rate of yeast cells.

Research Goal

To postulate possible mechanisms of function of TFFID and TFIIF nuclear complexes and their purpose in the cell after altering their common binding motif with the YEATS domain protein Taf14

Results

Tested 8 Strains:

1. WT
2. Taf14 W81A
3. Taf14 Δ
4. Taf14 YEATS Δ
5. Taf14 YEATS Δ with intron
6. Taf2M4
7. Tfg1–1M4
8. Tfg1–2M4

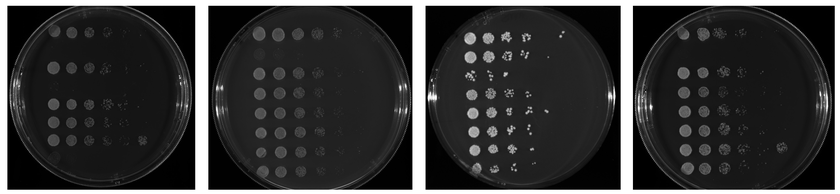
Mutations for Conserved Motif Recognized by Taf14

Mutations made by Dr. Taylor A. Lundy

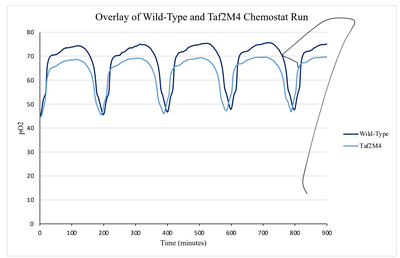
General Transcriptional Factor				
TFIID	Taf2	1392	SRSFMVKIRTKNDAKN	1407
TFIIF	Tfg1-1	613	IKNCVIILKGDKKILK	628
	Tfg1-2	685	EAIGDGKVNIKEFGKF	700

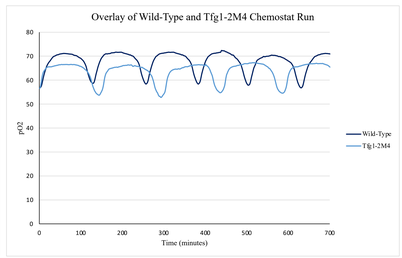
**Amino acids colored in blue were mutated to alanine*

Spotting Assay

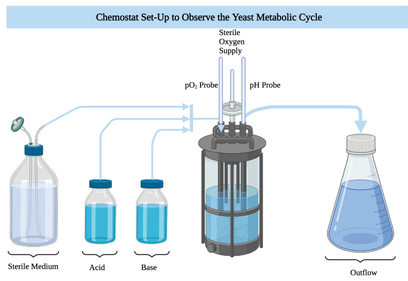


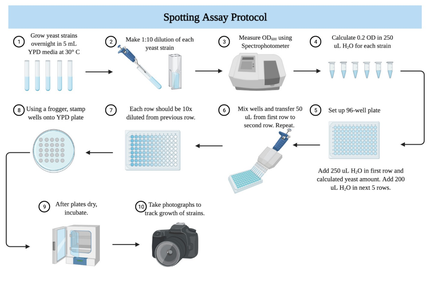
Chemostat





Methods





Conclusions

- Although TFIIF and TFIID are both complexes involved in transcriptional regulation and the DNA–repair response, the mechanisms by which they act are different.
- Based on the results, we can postulate that TFIIF may be playing a role in DNA–repair by increasing the concentration of available deoxyribonucleotides for DNA synthesis because the Tfg1–2M4 mutation prevents the yeast strain from growing in hydroxyurea.
- The Taf2, Tfg1–1M4, and Tfg1–2M4 strains show differing growth in the presence of various DNA–damaging agents, which may be due to unique mechanisms of DNA–repair, even though all three strains represent mutations in two types of transcriptional factor complexes.
- Caffeine's effect on Taf14 suggests it may be involved in the stress response.
- A mutation in Taf14, such as Taf14W81A, seems to be detrimental to yeast growth in the presence of various DNA–damaging agents.
- Even though Tfg1–2M4 did not grow in hydroxyurea, the strain's ability to grow in the presence of camptothecin suggests that there is some mechanism that offset the effect of camptothecin inhibiting DNA topoisomerase I activity.

Future Directions/Implications

- Test more DNA–damaging agents with similar mechanisms
- Mutate the common binding motif on the other complexes that interact with Taf14 to observe how this affects yeast cell proliferation
- Research the specific genes that are upregulated and downregulated by each complex
- Study the exact mechanism by which Taf14 interacts with each complex

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Strahl Lab, UNC School of Medicine, Department of Biochemistry and Biophysics