EFFECT OF *TDA1* DELETION ON CELL CYCLE STATE IN *S. CEREVISIAE* DURING NUTRIENT LIMITATION

An Honors Fellow Thesis

by

EVELYN MARIA HOOVER

Submitted to Honors and Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

HONORS UNDERGRADUATE RESEARCH FELLOW

May 2012

Major: Microbiology

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Approved by:

Research Advisor: Michael Polymenis
Associate Director, Honors and Undergraduate Research: Duncan MacKenzie

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ABSTRACT

Effect of *TDA1* Deletion on Cell Cycle State in *S. cerevisiae* During Nutrient Limitation.
(May 2012)

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Protein kinases are enzymes which phosphorylate other proteins and often play critical regulatory roles in cellular metabolism. We studied the effects of deletion of the *TDA1* gene in *Saccharomyces cerevisiae*. *TDA1* encodes a protein kinase of largely unknown function in yeast, but its human ortholog, *NUAK1*, has been implicated in oncogenesis. We initially examined the growth rate or the mean rate of increase in size of *tda1* mutants. These preliminary studies showed that homozygous diploid *tda1* mutants have an approximately 15-20% reduced growth rate in galactose and glycerol media, compared to the wild-type. These results suggest loss of *TDA1* leads to a nutrient-specific metabolic deficiency.

In order to elucidate the effect of the *tda1* deletion on metabolism, asynchronous yeast cultures were grown in chemostats in glycerol and ammonium nutrient-limited media. Individual cells from both *tda1*Δ and WT samples taken at different dilution rates were analyzed for budding index and DNA content using flow cytometry. This analysis

examines the cell cycle state of a yeast population under specific nutrient limitation at a given dilution rate. Glycerol and nitrogen limitations were tested. We found that the mutant seems to have a reduced ability to produce glycerol. These experiments provide valuable data on the metabolic function of Tda1p and its role in the relationship between cell division and metabolism.

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NOMENCLATURE

BI	Budding index
S. cerevisiae	*Saccharomyces cerevisiae*
WT	Wild type

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CHAPTER I

INTRODUCTION

Protein kinases are enzymes which phosphorylate other proteins and often play critical regulatory roles in cellular metabolism and division [1,2]. The *TDA1* gene in *Saccharomyces cerevisiae* encodes a protein kinase of largely unknown function in yeast. However, its human ortholog, *NUAK1*, has been implicated in oncogenesis, and is thought to suppress apoptosis induced by starvation [3,4]. Therefore, we examined homozygous diploid *tda1* mutants in nutrient limitations, to examine the effect of *TDA1* on cell metabolism and division. Our initial studies examined the growth rate or the mean rate of increase in size of *tda1* mutants. These preliminary studies showed that homozygous diploid *tda1* mutants have an approximately 15-20% reduced growth rate in galactose and glycerol media, compared to the wild-type. This suggested that loss of *TDA1* leads to a nutrient-specific metabolic deficiency.

We used chemostats and flow cytometry to analyze the effect on cell cycle of limiting glycerol and ammonium on *tda1* mutants. Glycerol is involved in carbon metabolism in a variety of ways. Under aerobic conditions it can be used as a sole carbon source [5]. Glycerol is also thought to be important in recycling inorganic phosphate after glycolysis

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This thesis follows the style of PLOS Genetics.

[6,7]. Also, glycerol-3-phosphate is a key intermediate in the synthesis of lipids [8-10]. Ammonium is involved in nitrogen metabolism. It is considered a good source of nitrogen and supports better growth than a poor nitrogen source such as urea. Ammonium may also be involved in inhibiting poorer nitrogen sources like urea [11-13].

CHAPTER II

METHODS

Strains

The two strains of *S. cerevisiae* used in this study were BY4743 (WT) and YMR291W (*tda1*Δ).

Media

We tested the two strains with a chemostat using two types of nutrient limitations: glycerol and nitrogen. Both contained 1.7 g/L yeast nitrogen base without ammonium sulfate or amino acids, 20 mL/L uracil [1g/L], 60 mL/L leucine [1g/L], and 20 mL/L histidine [1 g/L]. The glycerol limited media contained by weight 0.08% dextrose, 0.4% ammonium chloride, 0.05% magnesium sulfate heptahydrate. The nitrogen limited media contained by weight 2% dextrose, 0.0016% ammonium chloride, 0.06% magnesium sulfate heptahydrate.

Chemostat

For each chemostat experiment we started at a dilution rate of 0.1 h^{-1}. We raised the dilution rate 0.05 h^{-1} after at least 5 generations. At every dilution rate we took two samples, a couple hours apart and examined the budding index as well as DNA content. Budding index was calculated by counting the number of budded versus total cells [14]. The DNA content was measured using flow cytometry.

Flow cytometry

Steven B. Haase's protocols [15] were followed with minor modifications. A 25µL aliquot of a1mL chemostat sample was centrifuged for 30 seconds at 30,000 RPM using a table microcentrifuge, fixed in 1mL of 70% Ethanol, sonicated and stored overnight at 4ºC. The sample was then stained overnight with 500µL of SYTOX Green solution containing 50m citrate buffer (pH 7.0), 0.25 mg/mL RNase A, and 1µM SYTOX. The flow cytometer was set to analyze 20,000 cells.

CHAPTER III

RESULTS

Glycerol limitation

Table 1 shows the results from the chemostat with glycerol limited media. The average percent of cells in G2 increased from 34.8 to 47.67 in WT. In YMR291W the average percent of cells in G2 increased from 25.9 to 45.3. The difference between WT and *tda1*Δ in percent G2 cells was greatest at low dilution rates, and decreased at higher dilution rates as shown by Figure 1. The budding indexes obtained were not as reliable as the flow cytometry data due to a smaller throughput. When calculating budding index between 100-200 cells were counted. Average percentage of cells in G1 and G2 were found using flow cytometry data, which included DNA content measurements for 20,000 cells.

Table 1. Chemostat data from glycerol limited media. The average budding indexes, percent of G1 cells, and percent of G2 cells are shown above for both BY4743 and YMR291W in glycerol limited media.

	BY4743			YMR291W		
Dilution Rate (h^{-1})	Average BI	Average %G1	Average %G2	Average BI	Average %G1	Average %G2
0.1	0.51835737	65.195	34.795	0.59246	74.09	25.9
0.15	0.62982768	59.225	40.765	0.66714	68.445	31.545
0.2	0.67579908	54.565	45.425	0.68522	60.64	39.35
0.25	0.69211610	52.32	47.67	0.69447	55.17	46.4
0.3				0.66236	54.725	45.265

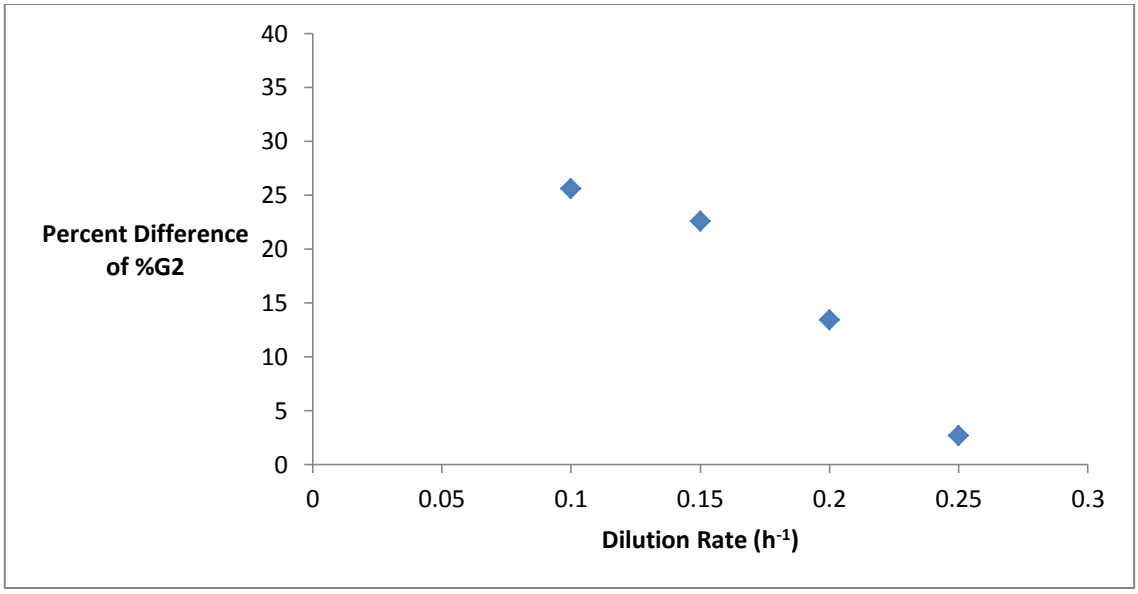

Figure 1. Difference of average G2 between WT and *tda1*Δ in glycerol chemostat. This figure shows that at lower dilution rates the percent difference between WT and YMR291W is greater than at higher dilution rates. YMR291W seems to be able to overcome its deficiencies if given more nutrients (still lacking glycerol).

Ammonium limitation

Table 2 shows the results for the ammonium limited media. According to the obtained results, the average percent G2 of BY4743 was between 48-52% lower than YMR291W. Figure 2 illustrates the trends of the average percent G2 in each strain, emphasizing the growing difference between the two strains. However, the flow cytometry data obtained for the BY4743 ammonium limitation may be inaccurate, as 500µL of the original sample was used instead of the 25µL that were used in the other experiments. Due to the concentration of cells being about twenty times greater, the amount of SYTOX green dye used was too low. Also, the flow cytometer could not distinguish between cells.

After this first trail, 25 µL of the original sample was discovered to give a better concentration of cells.

Table 2. Chemostat data from ammonium limited media. The average budding indexes, percent of G1 cells, and percent of G2 cells are shown above for both BY4743 and YMR291W in nitrogen limited media.

	BY4743			YMR291W		
Dilution Rate (h-1)	Average BI	Average %G1	Average %G2	Average BI	Average %G1	Average %G2
0.1	0.475496	82.92	17.07	0.610224	78.525	21.465
0.15	0.532856	75.025	24.965	0.574713	67.91	32.08
0.2	0.544996	76.54	23.45	0.645403	56.52	43.47
0.25	0.53987	76.94	23.05	0.68506	51.83	48.16
0.3	0.478382	79.275	20.715			

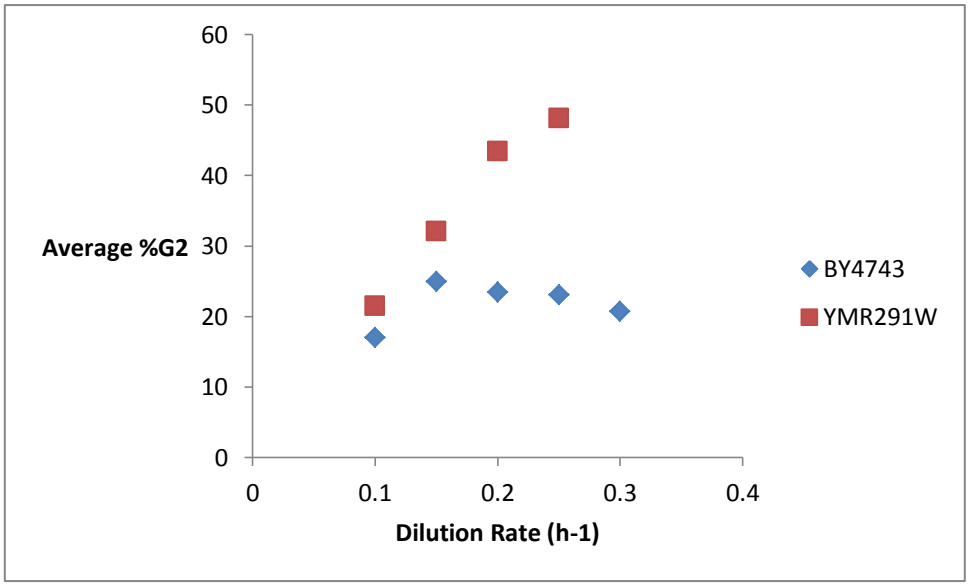

Figure 2. Percent G2 in BY4743 versus YMR291W in ammonium limitation. This data shows that YMR291W had a greater average percent of G2 cells. However, the data for the WT may be unreliable due to an error in flow cytometry analysis.

CHAPTER IV

SUMMARY AND CONCLUSIONS

After analyzing the results, we focused on DNA content measured by flow cytometry. This data analyzed thousands of cells, as opposed to the budding index which only examined hundreds. Therefore, the resolution of the flow cytometry data is much higher.

Based on our results from the glycerol limitation, *Tda1* does seem to have a role in glycerol metabolism. *Tda1* mutants seem to be unable to utilize glycerol efficiently. However, if given a greater amount of media with glucose, they seem to be able to produce enough to make up for their deficiency. These results suggest that the Tda1p kinase may be involved in a signaling pathway linking metabolism with initiation of cell division.

A recent study determined that *TDA1* was critical for the function of Hxk2, and may either encode for the enzyme or a supporting protein [16]. Hxk2 is one of the three hexose kinases in *S. cerevisiae*, and is involved in regulation of glycolysis [17]. The effect of the glycerol limitation we found is consistent with *TDA1* being involved with glycolysis. Further studies should be done to determine if *TDA1* leads to other nutrient-specific metabolic deficiencies. Limitations, which may lead to a greater understanding of the functions of *TDA1*, include galactose and glucose nutrient-limitations. Finally,

obtaining reliable ammonium results with a significant difference between the WT and *tda1*Δ could indicate that *TDA1* was involved with more than just glycolysis.

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