THE EFFECTS OF AN INCREASED AMOUNT OF MITOCHONDRIAL DNA ON THE YEAST METABOLIC CYCLE

A Senior Honors Thesis

by

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ABSTRACT

The Effects of an Increased Amount of Mitochondrial DNA on the Yeast Metabolic Cycle (April 2008)

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When prototrophic yeast cells undergo continuous growth in nutrient-limited conditions, they experience robust metabolic oscillations. Additionally, the processes of metabolism in yeast cells have been shown to be coordinated with cell division; cell division can only occur in certain phases of the metabolic cycle. It has been hypothesized that certain mutations that influence the rate of cell division may also affect yeast metabolic oscillations. Abf2p is a mitochondrial DNA maintenance protein; overexpression of Abf2p increases the rate of cell division, however, the effects of the overexpression of Abf2p on yeast metabolism are not known. Using a chemostat apparatus, metabolic oscillations, in terms of fluctuating dissolved oxygen

concentrations, of Abf2p were obtained. Remarkably, the period of the metabolic oscillations of cells over-expressing Abf2p was more variable, but always longer than the period of wild-type cells. This was due to a clear expansion of the reductive phase. If the role of Abf2p in promoting cell division can be determined, a possible mechanism of controlling cell division may be established.

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I INTRODUCTION[1]

The Role of Cell Division and Metabolism in the Proliferation of Cancerous Cells

Despite dramatic increases in medical knowledge and technology, cancer remains a leading cause of death in the United States. In the last twenty-five years, research has revealed that cancer is a disease that entails changes in the genome sequence; specifically, cancer is the result of mutations that instigate uncontrolled cell division (Hanahan and Weinberg, 2000). For this reason, current research is focused on identifying mutations that produce the abnormal replication patterns that are associated with cancerous cells.

The cell cycle consists of a growth phase (G1) followed by DNA synthesis (S) and the second growth phase (G2), and concludes with mitosis (M). Once cells initiate division, they almost always complete it. Cell division initiation is dependent upon coordination between cellular metabolism and the division machinery as well as environmental cues. Cells decide whether or not to divide in late G1, before DNA synthesis in the S phase, at a point called START in yeast cells (Hartwell and Unger,

[1] This thesis follows the style and format of *Cell*.

1977). Overall, it is the G1 phase of growth that is variable; if the factors that increase or decrease the length of this phase can be identified, a fundamental mechanism of cell division control will be revealed.

Cancer research involves the use of various models for studies ranging from humans to mice to microscopic organisms. A commonly used model is *Saccharomyces cerevisiae*, which is also known as budding yeast. In addition to having cell division machinery similar to that of an animal cell, *S. cerevisiae* produces a bud on the cell surface when it enters the S phase, and thus can simply be examined by a microscope to see if the cells have completed START.

Several studies have been done over the last four decades showing that yeast cells experience metabolic oscillations (Benjamin P. Tu, 2006). However, it was not until approximately ten years ago that a correlation between cell division and metabolism was discovered. Recently, it was asserted that DNA replication can only occur during the reductive phase of the yeast metabolic cycle (Chen et al., 2007). Various mutations in yeast strains were tested; however, specific gain-of-function mutants were neglected. These mutations may play a pivotal role in the control of cell division.

Metabolic Control of Cell Division

Yeast oscillations were first observed in the 1960s and were reflected as varying outputs of NADH fluorescence (Chance et al., 1964). Since then, studies have shown that oscillations generally occur in continuous cultures of *S. cerevisiae* when conditions such as the flow rates of media, temperature, and rate of agitation are kept constant within a chemostat apparatus (Danilo Porro, 1988). These oscillations occur spontaneously and are related to the respiratory-fermentative metabolism of yeast; consequently, oscillations are measured in terms of varying outputs of key metabolic products such as dissolved oxygen, carbon dioxide, and ethanol (Danilo Porro, 1988). Furthermore, analysis of the oscillating cell cultures indicates that the cell population is relatively synchronized as is indicated by changes in the budding index (Fiechter, 1975; Kappeli et al., 1985; Kuenzi and Fiechter, 1972).

In 1988, a hypothesis for the origin of the spontaneous oscillations was devised by Porro et al. (1988). It was believed that yeast cells produce ethanol which changes the environment of the cell population and results in a decrease in the critical sizes needed for budding and cell division (Danilo Porro, 1988). As a result, more cells are budded, resulting in a degree of synchrony among the cell population. Thus, the period of the

oscillation must coincide with the period of the cell cycle for the cell population (Danilo Porro, 1988). However, the actual mechanism of the generation of synchrony in terms of cell division within the cell population remained to be elucidated.

Further research was conducted to determine the cellular processes that may be responsible for causing yeast oscillations. It was established that processes such as redox biochemisty and transcription occur at different times of the yeast metabolic cycle (Klevecz et al., 2004; Murray et al., 1999; Murray et al., 1998; Tu et al., 2005). It was also determined that the metabolic process of respiration is constantly occurring, but contains distinct oxidative and reductive phases (Murray et al., 2003). Recently, it was shown that a majority of the metabolites associated with the yeast metabolic cycle show oscillating behavior; most metabolite concentrations reach their highest values in the transition phase between the reductive and oxidative phases, which in turn coincides with the maximum NADPH concentration and the maximum rate of DNA synthesis (Murray et al., 2007). Furthermore, it was shown that cell division can only occur in the reductive phase of the yeast metabolic cycle (Klevecz et al., 2004; Tu et al., 2005). In addition, transcripts were studied; transcripts that are involved in biosynthesis peak during the oxidative phase, whereas transcripts involved in respiration, mitochondrial

biogenenisis, and actin arrangement peak during the reductive phase (Murray et al., 2007; Tu et al., 2005). All in all, since specific processes were attributed to particular phases of the yeast metabolic cycle, specific genes associated with these processes could be examined in order to determine mechanism of synchrony and possibly a mechanism of cell division control.

Since the correlation between cell division and metabolism had firmly been established, current research has been directed towards identifying yeast strains that have mutations affecting cell division. Oscillations of these strains are obtained and the periods of these oscillations are compared to those of wild-type yeast. Research has indicated that loss-of-function, growth-retarded mutants have shorter periods of oscillation compared to those of wild type yeast strains (Chen et al., 2007). In these mutants, DNA synthesis is not strictly confined to the reductive phase, allowing the exposed DNA to experience oxidative damage, further inducing spontaneous mutations; the process of proliferation of mutant cells may be associated with the processes that proliferate cancerous cells in humans (Chen et al., 2007). However, the effects of gain-of-function mutants on the yeast metabolic cycle has yet to be examined. Gain of function mutants are much more important that loss of function mutants in studies of cell

cycle control because gain of function mutants point to critical metabolic pathways that can actively promote cell division. It is much easier to destroy something, as is seen with loss of function mutations, than it is to improve an existing pathway, as is seen with gain of function mutations. Examination of these mutations could potentially lead to the development of a mechanism to control cell division.

Mutation of Interest

Mitochondria play an important role in the cell cycle. In addition to ATP production, mitochondria function as signaling centers that maintain the regulation of calcium, iron, and metabolites in the cytosol as well as regulate apoptosis (Friddle et al., 2004). Since mitochondria are vital for cell function, preservation of the mitochondrial DNA is especially important. Mitochondrial DNA is threatened by several processes; most significantly, mitochondrial respiration produces oxygen radicals that can attack mitochondrial DNA, which in turn leads to several disorders (Chen et al., 2007). Paradoxically, the processes that allow the cell to live also yield very dangerous byproducts. For this reason, the mitochondrial DNA must be packaged in a manner that

prevents damage, yet at the same time, does not impair its normal functions (Friddle et al., 2004).

S. cerevisiae packages mitochondrial DNA in a structure that resembles a bacterial nucleoid (Miyakawa et al., 1984; Miyakawa et al., 1987; Wintersberger et al., 1975). Mammals are also known to package mitochondrial DNA in the same manner (Satoh and Kuroiwa, 1991; Van Tuyle and McPherson, 1979), further validating the use of *S. cerevisiae* as a model for study. The mitochondrial- nucleoid structures vary significantly from the chromatin that is formed by packaging DNA in the cell nucleus (Friddle et al., 2004). Extensive research has been done on chromatin and the roles of histones in DNA compaction; however, there is not much information present on the proteins that are involved in the process of mitochondrial-nucleoid formation.

In 1988, a 20-kDa protein was discovered to be especially abundant in polypeptides that were isolated from mitochondrial DNA (Diffley and Stillman, 1988). This protein was named Abf2p (ARS binding factor 2) and is involved in DNA binding; the protein binds to nonspecific regions of DNA, but demonstrates specific binding to certain replicating sequences (Diffley and Stillman, 1988). Abf2p is a mitochondrial DNA maintenance protein; it directly binds to and causes the mitochondrial DNA to fold

and compact (Friddle et al., 2004; Stigter, 2004). In addition, Abf2p causes negative supercoiling in DNA when topioisomerase is present (Friddle et al., 2004). Abf2p is not necessary for mitochondrial DNA replication; however, changes in Abf2p affect the number of copies of mitochondrial DNA that are present (Zelenaya-Troitskaya et al., 1998). When the Abf2p gene is over-expressed double or triple the amount compared to the level of expression in wild-type, the mitochondrial DNA is increased by 50-150 percent; however, Abf2p expression that is greater than ten times the amount expressed in wild-type cells leads to the loss of mitochondrial DNA (Zelenaya-Troitskaya et al., 1998). The effects of an increase in mitochondrial DNA in metabolism and cell division have yet to be studied.

The Present Study: Examining the Effects of Overexpression of Abf2p on Yeast Metabolic Oscillations

It has been long-established that cell division and metabolism in *S. cerevisiae* are coordinated. Specific cellular processes follow a defined sequence, which in turn is reflected in varying concentrations of metabolites, thus producing metabolic oscillations that have been observed in yeast for many years. The effects of loss-of-function mutations on cell division and metabolism have been studied, but the effects of gain-of-

function mutations have yet to be examined. The present study aims to examine the effects of the overexpression of Abf2p on metabolism and cell division. Overexpression of Abf2p is known to produce an increased rate of cell division, but its effect on the yeast metabolic cycle is unknown. Metabolic oscillations of yeast cells overexpressing Abf2p will be obtained; any differences in the pattern of oscillations when compared to wild-type oscillations may be due to the enhanced rates of cell division. Once the oscillations have successfully been obtained DNA content analysis can be performed to determine the extent of coordination of cell division and metabolism in the mutant cells. Eventually this information can be used to devise a fundamental mechanism of the cell division control. This mechanism will have several implications in the treatment of diseases such as cancer, which are the direct result of uncontrolled cell division

II MATERIALS AND METHODS

Media Preparation

To make 2.5 liters of YPD media, weigh out 50 grams of peptone and 25 g of yeast extract in a large beaker. Add 1.8 liters of distilled water and stir with a magnetic stirrer until completely dissolved. Add 2.33 milliliters of anti-foam and stir. Bring up the volume to 2.5 liters. Pour 500 milliliter aliquots into glass bottles and autoclave for 30 minutes. Store at room temperature.

To make 0.5 liters of synthetic complex (SC) –ura media, weigh out 0.85 grams of yeast nitrogen base (YNB) without ammonium sulfate, 2.5 grams of ammonium sulfate, 10 grams of glucose, and 1 grams of –ura drop out powder into a beaker. Add 300 milliliters of distilled water and stir with a magnetic stirrer until compeletely dissolved. Bring up the volume to 0.5 liters and pour the media into a glass bottle. Autoclave the bottle of media for 30 minutes and then store at room temperature.

40% Glucose Preparation

To make 150 milliliters of glucose, weigh out 60 grams of glucose in a small glass beaker. Add 100 milliliters of distilled water and stir with a magnetic stirrer. Heat the solution periodically to help dissolve the glucose. Syringe filter the glucose into a small, autoclaved bottle and store at 4 °C. Add 6.25 milliliters of glucose to 500 milliliters of YPD media just prior to use.

Wild-Type Cell Culture Preparation

A bottle of 500 milliliters of YPD media is obtained. 6.25 milliliters of 40% glucose are added with a sterile pipette to the bottle of media. A colony of the wild-type cells grown on YPD plates is transferred to this media bottle. The cells are grown overnight at 30 °C.

CEN-ABF2^{+} Cell Culture Preparation

CEN-ABF2^{+} cells are transformed in the X51280 cells and grown on SC –ura plates to select for the transformed cells. For the experiment, 20.0 milliliters of the SC – ura media is placed in a 50.0 milliliters sterile plastic tube. A colony of *CEN-ABF2^{+}* is

inoculated in this media. The culture is incubated at 30 °C overnight. Afterwards, this culture is transferred to a bottle containing 500 milliliters YP with 6.25 milliliters of 40% glucose. The bottle is incubated at 30 °C for approximately 5 hours before the cell culture is loaded into the chemostat.

Chemostat Apparatus

A standard chemostat is used with a few modifications. The temperature device is removed from the apparatus. The agitation speed of the rotator is 425 revolutions per minute. 2.5 liters per minute of air are blown into the chemostat. The tube used to load the cells has a 10 milliliter pipette attached at the end of the tubing; this pipette is inserted into the cell culture in order to load the cells. A Cole-Parmer dissolved oxygen probe is inserted into the chemostat. The probe is connected to a Accumet excel XL 15 pH meter. A waster container and a media container are attached to the chemostat via tubing. The chemostat is filled with approximately 400 milliliters of distilled water and is autoclaved for 30 minutes. The media and waste container are autoclaved along with the chemostat, but are actually connected to the apparatus via tubing at a later time. The chemostat apparatus is situated in a chemical hood, but the blower of the hood is turned

off once the cell culture is present within the chemostat. Refer to Figure 1 for a visual display of the apparatus.

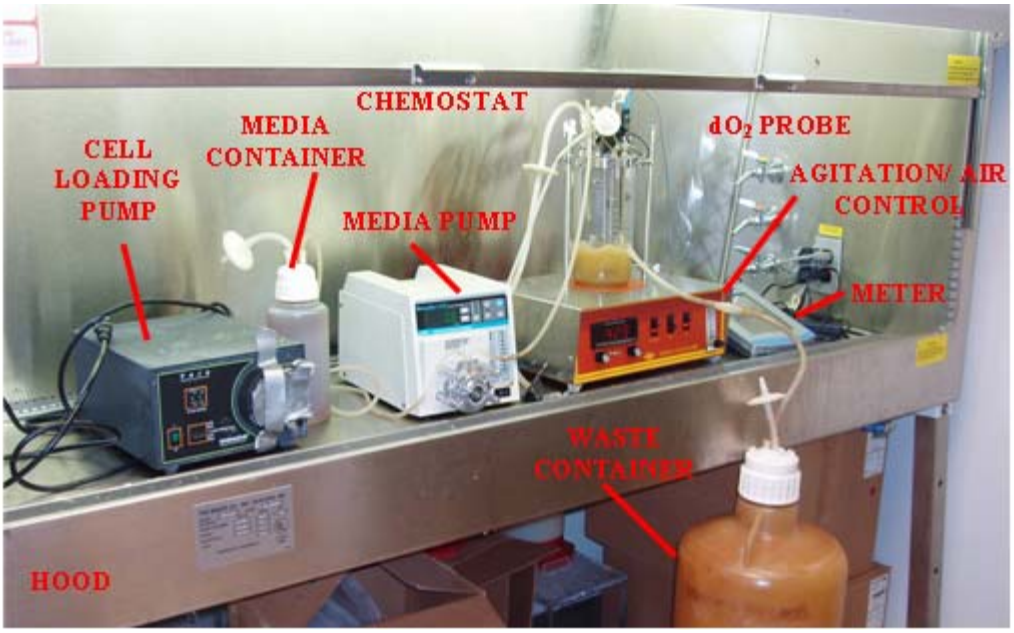

Figure 1: A picture of the actual chemostat apparatus used in the experiment. Unlike the picture, however, the hood was kept closed during the actual experiment in order to help prevent contamination.

Experimental Procedure

The cell cultures in the 500 mL YPD bottles are loaded into the chemostat. First, before the cells are loaded, the waste container is connected to the chemostat and the water used for autoclaving is emptied from the chemostat. Then, a pump is connected to the tube with the 10 milliliter pipette attached and the cells are transferred into the chemostat. Once the cells have finished loading, the pipette is removed and the tubing involved in this transfer is clamped with clips. In addition, prior to the addition of the media, the tubing that connects the chemostat vessel to the waste container is clamped with a clip as well to prevent loss of the culture. The agitation and air are started as soon as the culture has been transferred. The probe is connected to the meter that measures dissolved oxygen concentration in terms of millivolts. The meter is programmed to take readings every 15 minutes; the readings are obtained constantly as soon as the cells are loaded.

For the next portion of the procedure, wild-type cells are treated differently than the *CEN-ABF2*$^{+}$ cells. The wild-type cells are allowed to grow in the chemostat for approximately 24 hours and the millivolt values are monitored. Once the millivolt values have remained stable for several hours, media with 6.25 milliliters of 40% glucose is

added to the media container and the media container is connected to the media pump and to the chemostat. The clamp on the tubing connecting the waste container to the chemostat vessel is removed. The media pump is started at a flow rate of 0.6 milliliters/ minute. The media is allowed to flow for several days and the millivolt readings are constantly recorded at 15 minute intervals by the meter.

The *CEN-ABF2*$^{+}$ cells are also grown in the chemostat for approximately 24 hours and the millivolt values are monitored, but the millivolt values never stabilize. Once the 24 hour chemostat growth period is completed, media with 6.25 milliliters of 40% glucose is added to the media container and the media container is connected to the media pump and to the chemostat. However, the clamp on the tubing connecting the waste container to the chemostat vessel is not removed. The chemostat is allowed to fill with the media for approximately 12 hours. After approximately 12 hours have passed, the clamp preventing the flow of the chemostat culture to the waster container is removed and the culture within the chemostat is allowed to return and be maintained at a constant volume. The media is allowed to flow for several days and the millivolt readings are constantly recorded at 15 minute intervals by the meter.

III RESULTS

The majority of the work conducted for this project involved establishing the parameters for obtaining yeast metabolic oscillations of the *CEN-ABF2*$^{+}$ mutant cells. Though yeast metabolic oscillations have been obtained using a similar apparatus in the past, each strain requires slightly different parameters to oscillate. Furthermore, discrepancies exist in the apparatus components that are used; experimentation is required to determine the correct settings.

The first part of the experimental process involved obtaining the oscillations of wild-type cells. These oscillations have been obtained in the past by other research laboratories; by obtaining wild-type oscillations, it could be verified that the chemostat apparatus components were in the correct configuration and that the nutrient-limited environment created was conducive to generating yeast metabolic oscillations. These oscillations are presented in Figure 2. Based on a single trial, the period of these oscillations, as is indicated by Figure 2, was approximately 8.5 (± 0.7) hours. The average period of three oscillations obtained from two separate trials was 7.8 (± 0.6) hours. The periods of these oscillations was similar to the periods of obtained by (Danilo

Porro, 1988). These results confirmed that the apparatus in use was successfully conducive in generating yeast metabolic oscillations.

The second part of the experiment involved generating metabolic oscillations of *CEN-ABF2*$^{+}$ cells. The oscillations of these cells have never been generated. Through experimental manipulation, it was determined that these cells needed an increased amount of time to adjust to the influx of fresh media. For this reason, the chemostat vessel was allowed to fill with fresh media for approximately 12 hours. This extended amount of time allowed to *CEN-ABF2*$^{+}$ cells to become more in phase and eventually undergo metabolic oscillations. The oscillations of *CEN-ABF2*$^{+}$ cells are also presented in Figure 2. Based on a single trial, the period of these oscillations, as is indicated by Figure 2, was approximately 15.4 (± 6.1) hours. The average period of six oscillations obtained from two separate trials was 15.0 (± 4.3) hours. The oscillation pattern of the *CEN-ABF2+* cells involved a significantly longer period and was much more irregular.

There were several difficulties that occurred during the experimental process. These difficulties encompassed instrument malfunction and contamination. Several various probes and meters were used before an apparatus that consistently produced correct readings was developed. In addition, contamination remained a constant issue.

There are many stages in which the cell culture and media have to be transferred, allowing time for various contaminants to be accumulated, which cause for the experimental trial to be terminated. Consequently, only two trials of the wild-type and *CEN-ABF2*$^{+}$ oscillations were obtained; these two trials serve as the basis for the overall conclusion of this study.

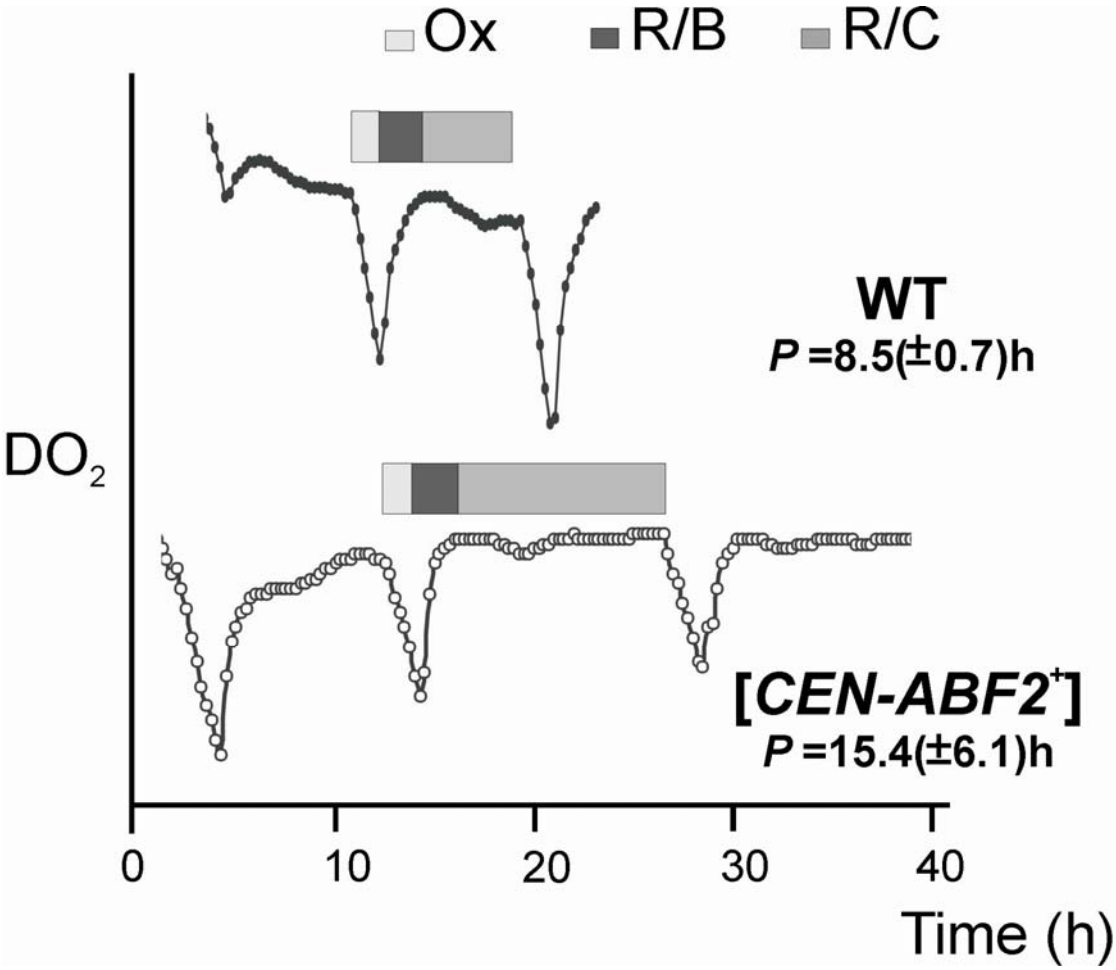

Figure 2: Yeast metabolic oscillations of wild-type (WT) and *CEN-ABF2*$^{+}$ cells. The dissolved oxygen concentration is represented in terms of millivolts. P denotes period of the oscillations in hours. The errors in the periods are in terms of the variations of the periods of the oscillations that were produced during one specific trial. Ox denotes the oxidative phase, R/B denotes reductive building phase, and R/C denotes reductive charging phase; the bars located above the oscillations are color-coded to indicate the metabolic phase that the cells are experiencing.

IV DISCUSSION AND CONCLUSION

Overexpression of Abf2p has a distinct effect on the yeast metabolic cycle by significantly expanding the period of the metabolic oscillations. Although the oscillations of the *CEN-ABF2*$^{+}$ cells are more irregular, the periods of the oscillations of these mutant cells are always much longer when compared to the periods of oscillations of wild-type cells.

The implications of the effects of Abf2p on expanding the period of metabolic oscillations are examined in terms of the lengths of the oxidative and reductive phases of metabolism. The oxidative phase is the portion of the metabolic cycle that consumes oxygen; in terms of metabolic oscillations, it is the portion of the cycle in which a decrease in the dissolved oxygen concentration occurs. The reductive phase is the portion of the metabolic cycle in which oxygen is produced; in terms of metabolic oscillations, it is the portion of the cycle in which an increase in dissolved oxygen concentration occurs. Past research has shown that cell division is only able to occur in the reductive portion of the metabolic cycle (Tu et al., 2005). Figure 2 correlates the obtained oscillations with their designated phase in the metabolic phases. It is important

to note that the reductive phase of the metabolic oscillations of *CEN-ABF2*$^{+}$ is significantly expanded. The increased length of the reductive phase may be contributing to the increased rates of cell division, which have been previously attributed to overexpression of Abf2p.

A continuation of this research project will involve specifically establishing the extent of coordination of metabolism and cell division in the *CEN-ABF2*$^{+}$ cells. DNA content analysis will be performed at distinct points of the *CEN-ABF2*$^{+}$ metabolic oscillations in order to coordinate phases of the cell cycle with specific metabolic landmarks. If a correlation is obtained, biochemical tests can then be performed to determine the role of Abf2p in the signaling pathway; this information can eventually be used to develop a mechanism of cell cycle control. A mechanism for cell cycle control may allow for many diseases, such as cancer, to be cured.

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CURRICULUM VITA

SHEFALI RAJENDRA GAJJAR

EDUCATION

Texas A&M University	Bachelor's of Science Degree
College Station, TX	Major: Biochemistry
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RESEARCH

Texas A&M Undergraduate Research Fellows Program 2007-2008

- Thesis: *The Effects of an Increased Amount of Mitochondrial DNA on the Yeast Metabolic Cycle*
- Collaborated with faculty advisors in obtaining metabolic oscillations of *Saccharomyces cerevisiae*, specifically the Abf2p overexpression mutant
- Skills: Chemostat operation, culture preparation and transfer, sterilization techniques.

Texas A&M Genetics Laboratory, College Station, TX

- Research assistant from January 2006 to present.
- Conduct lab work on research projects involving cell cycle control.

University of North Texas, Health Science Center Immunology Laboratory, Fort Worth, Texas

- Summer Multicultural Advanced Research Training (SMART) Scholar from May to August 2006.
- Established the parameters for purifying the recombinant LLTI fusion protein.

HONORS AND AWARDS

- Phi Kappa Phi Most Outstanding Junior in the College of Agriculture and Life Sciences Award (2007)
- Alpha Zeta College of Agriculture and Life Sciences Most Outstanding Sophomore Award (2006)
- Dean's List (Fall 2005- Fall 2007)
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