ABSTRACT OF THE DISSERTATION

Selective translation and regulated decay of yeast mRNAs in response to carbon source: *cis* elements and *trans* factors

by

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Doctor of Philosophy in Biology

University of California, San Diego, 2000

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The yeast *Saccharomyces cerevisiae* is adapted to respond rapidly to changes in their environment. One example of rapid response is glucose repression. *Saccharomyces* preferentially uses glucose and consequently shuts-off many genes involved in metabolism of non-fermentable substrates, mitochondrial biogenesis, and oxidative phosphorylation. However, when fermentable substrate is absent, these genes are derepressed as yeast switches to cellular respiration. The gene *SDH2*, which encodes the iron protein of the succinate dehydrogenase enzyme, and is part of mitochondrial complex II, is glucose repressed. It had been found that stability of the *SDH2* message is affected by carbon source. When yeast are grown on YPD, *SDH2* mRNA is present at low level (repressed). When yeast are grown on YPG (derepressed), the *SDH2* mRNA is stabilized and is present at much higher level. When glucose is added to a YPG culture, the *SDH2* mRNA is rapidly degraded. Prior work showed that the 5'UTR of the *SDH2* mRNA was necessary and sufficient to confer glucose-dependent stability to yeast mRNAs. It is now shown that the 5'UTRs of the *SDH1* and *SUC2* mRNAs also

confer instability in glucose. Furthermore, it is demonstrated that alterations of the 5'UTR can block glucose-triggered degradation of the *SDH2* mRNA. Finally, it is shown that stabilization of mRNA in glucose correlates to changes in translation initiation efficiency. A working model is described that proposes changes in *SDH2* mRNA stability are due to a competition between translation initiation and mRNA degradation depending on carbon source.

CHAPTER 1

INTRODUCTION

1.1 HISTORY

The Scheffler lab has been investigating cellular respiration and mitochondrial function for many years. Studies of mitochondrial complex II, the succinate dehydrogenase, led to the development of the yeast *Saccharomyces cerevisiae* as a model system (Lombardo et al., 1990; Lombardo and Scheffler, 1989). The SDH complex is composed to two peripheral membrane proteins, the iron protein encoded by the *SDH2* gene, and the flavoprotein encoded by the *SDH1* gene. Two additional genes, *SDH3* and *SDH4*, encode the membrane-bound component of complex II that couple SDH into the mitochondrial electron transport chain.

It had been observed that the *SDH2* gene is glucose-repressed. Specifically, when yeast was grown on glucose (YPD), there was a low level of the *SDH2* mRNA. On the other hand, when yeast was grown without glucose (YPG), a 6-12 fold increase in the *SDH2* mRNA level was seen (Lombardo et al., 1992). Most interestingly, when glucose was added to a derepressed culture, the level of *SDH2* mRNA was found to fall precipitously without delay.

1.2 GLUCOSE REPRESSION

1.2.1 Overview

Many microorganisms have evolved to rapidly alter their gene expression patterns in response to changes in their environment. Examples of environmental changes include temperature, osmolarity, and nutritional requirements, particularly nitrogen and carbon source. One such change in gene expression is glucose repression. Certain microorganisms, including *Saccharomyces*, prefer to generate energy (ATP) by glycolysis (fermentation) rather than respiration. Thus when glucose is present, genes involved in functions such as mitochondrial biogenesis, oxidative phosphorylation, and metabolism of non-fermentable substrates are shut-off. It is only when fermentable substrate is limiting that these genes are turned on. When fermentable substrate becomes available, these genes are again shut-off.

The model gene for studying glucose repression has been *SUC2*, which encodes the enzyme invertase (e.g., (Carlson et al., 1984; Neigeborn and Carlson, 1984)). *SUC2* is very tightly repressed in glucose with essentially no detectable transcript. When glucose is limiting, *SUC2* mRNA is present at extremely high level.

1.2.2 Glucose signaling pathway and transcriptional regulation

Research into how cells detect glucose and regulate genes in response to glucose is quite extensive (Carlson, 1988; Carlson, 1998; Carlson, 1999; Entian, 1986; Gancedo, 1998; Johnston, 1999; Ronne, 1995; Trumbly, 1992; Ullmann, 1996). The key steps of

the glucose-signaling pathway from external glucose to transcriptional repression are as follows (Figure 1.1):

1) Detection and uptake of external glucose. Several hexose transporters have been identified (e.g., (Reifenberger et al., 1997)). In addition, two proteins, Rgt2p and Snf3p, while structurally similar to transporters, may act as sensors and not transport glucose *per se* (Ozcan et al., 1996).
2) Phosphorylation of glucose. Once glucose is taken up into the cell, it is phosphorylated by hexokinases including Hxk1p, Hxk2p, and Glk1p (Bisson et al., 1993) to glucose-6-phosphate. Further conversions to fructose-6-phosphate, fructose-1,6-bisphosphate, and fructose-2,6-bisphosphate also occur.
3) Phosphorylation of glucose leads to a signal to the Reg1p, a regulatory subunit of Glc7p, the major protein phosphatase I (Tu and Carlson, 1994; Tu and Carlson, 1995). The precise nature of the signal from phosphorylated glucose to Reg1p is as yet unknown.
4) Reg1p targets Glc7p to dephosphorylate Snf1p, a protein kinase. Snf1 kinase then acts via additional kinases (e.g., Snf4p (Jiang and Carlson, 1997; Ludin et al., 1998)) eventually impinging upon transcription factors including Tup1p and Mig1p to repress some genes, and the Hap2/3/4/5 complex to derepress others (reviewed (Johnston and Carlson, 1992)).

1.2.3 Glucose signaling pathway and post-transcriptional regulation

While much of the focus of glucose repression has focused on transcriptional regulation, it was found that in the case of glucose-triggered decay of the *SDH2* mRNA

a post-transcriptional mechanism was also involved. However, it was found that there is a fork in the glucose-signaling pathway, one branch leading toward transcriptional regulation and the other toward regulated decay of the *SDH2* mRNA (Cereghino and Scheffler, 1996). Specifically, Snf1p is not involved in the regulated decay of *SDH2* mRNA but is necessary for transcriptional depression (Figure 1.1).

1.3 POST-TRANSCRIPTIONAL REGULATION

Post-transcriptional gene regulation can operate at several levels (McCarthy, 1998) involving mRNA and/or protein. One mode involves regulating the level of mRNA by turnover. mRNA "activity", i.e. translation efficiency, can also be regulated. Also, level of protein can be regulated by turnover, and the activity of a protein can be modulated by post-transcriptional modifications such as phosphorylation. However, the focus here will be on mechanisms involving mRNA.

The steady-state level of an mRNA is determined both by the rate of synthesis (transcription, processing, and nuclear export) and the rate of decay of the mRNA (Hargrove et al., 1991). An example where mRNA level is regulated by changes in its decay rate involves the iron response element (IRE) and the mammalian transferrin receptor mRNA (Harford and Klausner, 1990; Theil, 1993). Transferrin receptor imports iron when cellular levels are low. The IRE is a hairpin-loop structure present in the 3'UTR of the transferrin receptor mRNA. When iron levels are low, the IRE binds a specific protein (IRE binding protein, or IRE-BP). Binding of the IRE-BP to the

transferrin receptor mRNA 3'UTR prevents its degradation and allows the mRNA to accumulate without interfering with its translation.

Another level of regulation involves modulating the rate/efficiency of translation. One example again involves an IRE located in the 5'UTR of the ferritin mRNA (Theil, 1990; Theil, 1993); when cellular iron is low, the IRE-BP binds the ferritin mRNA and blocks translation. Another example is the *GCN4* mRNA in yeast, which contains upstream open reading frames (uORFs) and is translated only during nitrogen starvation (Hinnebusch, 1993). In rice, translation and stability of the alpha amylase mRNA is affected by carbohydrate starvation (Sheu et al., 1994; Sheu et al., 1996). Translation of the mRNA of the mammalian glucose transporters GLUT1/4 also depends on cellular glucose levels (Qi and Pekala, 1999; Taha et al., 1999). Even translation of translation initiation factor (eIF) and ribosomal protein mRNAs depends on the TOR (target of rapamycin) pathway, which is impinged in part by insulin (Pause et al., 1994).

An intriguing example where both decay and translation are linked is the case of β-tubulin mRNA (Cleveland, 1988; Coulson and Cleveland, 1993; Theodorakis and Cleveland, 1996). Increase in the intracellular concentration of tubulin subunits leads to specific degradation of β-tubulin mRNA. Decay depends on translation of the first thirteen codons. This partial peptide is thought to interact via a "co-factor" with free tubulin subunits and this causes the decay of the translated β-tubulin mRNA. Decay occurs even if the first four codons are altered by silent mutations. Decay does not occur if translation is abrogated or the peptide sequence is changed. It may be that the co-factor mediating the interaction of peptide and tubulin subunits is in fact the ribosome.

1.4 MECHANISMS OF mRNA DECAY

mRNA decay has been extensively studied and frequently reviewed (Beelman and Parker, 1995; Decker and Parker, 1993; Peltz and Jacobson, 1992; Ross, 1995; Ross, 1996; Sachs, 1993). Two models of mRNA decay are broadly sketched for consideration here: a general constitutive decay of short-lived mRNAs and specific decay of selected mRNAs.

1.4.1 General decay of constitutively short-lived mRNAs

Parker and colleagues have described a general mechanism of mRNA degradation also called deadenylation-dependent decay, or constitutive mRNA decay (Beelman and Parker, 1995) (Figure 1.2). There are three main sequential steps for the decay of constitutively short-lived mRNAs:

1) Deadenylation. The poly(A) tail is added to mRNA post-transcriptionally in the nucleus. In yeast, the initial poly(A) tail length averages about 40-60 nucleotides. After the mRNA leaves the nucleus, a progressive shortening of the tail occurs until only an oligo(A) tail remains (<<20 nucleotides). This is thought to be accomplished by poly(A)nuclease (Brown and Sachs, 1998). The rate of deadenylation is a function of sequence elements present in the 3'end of the mRNA including AUREs (AU-rich elements) (Asson-Batres et al., 1994; Chen and Shyu, 1995; Shaw and Kamen, 1986). In some cases it is thought there may be signals that trigger catastrophic shortening of the poly(A) tail of specific mRNAs.

2) Decapping. Once the poly(A) tail has been sufficiently shortened, decapping by the Dcp1p enzyme occurs (Beelman et al., 1996). The 5'cap is a 7-methyl-G residue added post-transcriptionally to the 5'end of mRNAs. This cap serves at least two functions. First, it protects the mRNA from 5'-3'exonucleolytic decay. Second, the cap localizes the translation initiation complex through recruitment of the eIF4F cap-binding complex.
3) 5'-3' decay. Finally, there is rapid 5'-3' exonucleolytic degradation of the decapped mRNAs by various ribonucleases including Xrn1p (Larimer et al., 1992). This step is thought to be constitutive and unregulated. Note, while Xrn1p represents the major exonucleolytic enzyme in yeast (~30% of total activity), there are a host of other exonucleases with redundant function.

The first two steps—deadenylation and decapping—are considered the main regulatory points and are thought to be sequential, although recent work has indicated these steps may be decoupled in certain mutant yeast strains (Morrissey et al., 1999).

1.4.2 Specific decay of select mRNAs

Specific degradation of mRNA may be best illustrated by work on NMD, or nonsense-mediated decay. A cellular machinery, which is encoded by the UPF genes, is capable of identifying mRNAs containing premature stop codons and directing the aberrant mRNA for immediate decapping and 5'-3' degradation (Culbertson, 1999; Czaplinski et al., 1998). In other cases of specific degradation, the target mRNA is internally cleaved and cleavage products are then removed by general decay mechanisms (e.g. (Decker and Parker, 1993)). NMD and similar pathways are

sometimes referred to as deadenylation-independent decay, because the degradation is independent of the state of the poly(A) tail.

It should be noted that multiple modes of mRNA stabilization and degradation may be functioning at the same time in a single transcript if multiple *cis*-elements and/or *trans*-factors are acting in concert. Overall stability or degradation of a particular mRNA then depends on the multiple mechanisms that are in play.

1.5 FOCUS OF THESIS: REGULATED DEGRADATION AND SELECTIVE STABILITY OF THE *SDH2* AND OTHER mRNAs

The research herein is focussed on three aspects of the selective stability and regulated decay of the *SDH2* mRNA. The first aspect involves elucidating the *trans*-factors involved in glucose-triggered decay of the *SDH2* and other glucose-sensitive mRNAs, i.e. the mechanism of glucose-triggered decay. The second aspect focuses on analysis of the nature of the *cis*-element directing glucose-triggered decay of selected mRNAs, specifically, what is contained within the 5'UTR that confers glucose-sensitive decay? The third aspect examines how changes in translation correlate with changes in mRNA stability depending on the carbon source.

1.5.1 Mechanism of glucose-triggered decay

Initial studies on the *SDH2* mRNA to determine the *cis*-element conferring selective stability found that the 5'UTR was necessary and sufficient. This immediately suggested that the *SDH2* mRNA is degraded from the 5' end. The last decay step—5'-3' exonucleolytic degradation by Xrn1p—was tested by Cereghino et al. by looking at

glucose-triggered decay in an *xrn1* yeast mutant. It was found that lack of Xrn1p activity blocked glucose-triggered decay of the *SDH2* mRNA (Cereghino et al., 1995).

It remained to be shown, however, whether decapping and/or deadenylation were obligate steps for glucose-triggered degradation. These questions are addressed in Chapter 3.

1.5.2 Analysis of the 5'UTR of the *SDH2* and other mRNAs

It had been observed in YPD that there was a low-but-detectable level of the *SDH2* mRNA. On the other hand, when yeast was grown on glycerol (YPG), a 6-12 fold increase in the *SDH2* mRNA level was seen (Lombardo et al., 1992). An analysis of the yeast promoter (or upstream activating sequence, UAS), found several regulatory elements involved in glucose repression. However, even when the *SDH2* coding sequence, including 5'UTR, ORF, and 3'UTR, was fused to glucose-insensitive UAS, a distinct difference in *SDH2* mRNA level was still seen between YPD and YPG. However, the difference was not as dramatic (3-4 fold vs. 6-12 fold). Additional work using a yeast strain with a temperature-sensitive RNA polymerase II (*rbp1-1*) to shut off mRNA transcription confirmed that a post-transcriptional mechanism, specifically change in mRNA stability, was involved in determining the level of mRNA (Cereghino et al., 1995).

Studies swapping the 5'UTR, ORF, and 3'UTR of the *SDH2* mRNA with those from other heterologous mRNAs demonstrated that the 5'UTR was the key element conferring glucose-sensitive behavior (Cereghino et al., 1995). However, what specifically about the *SDH2* 5'UTR specifies it as a target for glucose-sensitive

degradation remained unknown. Is there a specific consensus sequence or secondary structure? Or is it a general property of the entire 5'UTR such as nucleotide composition or length? It's known that non-specific secondary structures can block translation, particularly since unwinding of the 5'UTR is not particularly efficient in yeast (Koloteva et al., 1997; Oliveira et al., 1993; Vega Laso et al., 1993). Also, length of the 5'UTR can play a role, i.e. longer leader sequences, irrespective of actual sequence, are generally capable of binding 40S ribosomes more readily (Kozak, 1991). Finally, much work by Kozak has examined how the sequence around the translation start site (AUG context) affects translation efficiency in both mammals and yeast (Kozak, 1987; Kozak, 1992; Kozak, 1999).

It was decided to focus on the following four elements: (1) consensus sequence elements in the 5'UTR, (2) secondary structure in the 5'UTR, (3) length of the 5'UTR, and (4) AUG context. To address points 1 and 2, first, the repertoire of 5'UTRs that confer glucose-sensitive instability was expanded by examining behavior of the *SDH1* and *SUC2* mRNAs. In addition, the 5'UTRs of the *SDH2*, *SDH1*, and *SUC2* mRNAs were fused to a heterologous mRNA, and stability of the fusion constructs were examined. The results of these studies are described in Chapter 4. To address points 3 and 4, small sequence alterations in the 5'UTR of the *SDH2* mRNA were made, and constructs were examined for alterations in glucose-sensitive decay. The results of these studies are described in Chapter 5.

1.5.3 Role of translation on selective stability of the *SDH2* mRNA

Elucidation of the mechanism of glucose-triggered decay of the *SDH2* mRNA suggested that the degradation machinery itself was not the target of glucose signaling. However, several lines of evidence pointed at translation as being the key to the selective stability of the *SDH2* mRNA (Cereghino et al., 1995). For example, cycloheximide may be administered to halt translation by locking ribosomes on mRNA. When cycloheximide was added prior to glucose, it was found that decay started at the 5'end, but was blocked by ribosomes. *SDH2* mRNA decay was also examined in a temperature-sensitive mutant of the eIF3 complex, *prt1-1*. At the nonpermissive temperature, translation is halted due to failure of complexing the 40S and 60S ribosomal subunits together. It was found that the *SDH2* mRNA decayed even in YPG when the *prt1-1* strain was shifted to a nonpermissive temperature. This again suggested that translation is important to stability of the *SDH2* mRNA.

It was proposed that stability of the *SDH2* mRNA depended on a competition for the 5'cap and 5'UTR between the translation initiation and the enzymatic machinery of degradation, and that this competition was affected by the carbon source (Figure 1.3). The model is tested by investigating the changes in the distribution of mRNAs on a polysome gradient depending on carbon source. The results of these studies are described in Chapter 6.