



The role of the 5k untranslated region (UTR) in glucose-dependent mRNA decay

Bernard J de La Cruz, Susana Prieto, Immo E Scheffler

► To cite this version:

Bernard J de La Cruz, Susana Prieto, Immo E Scheffler. The role of the 5k untranslated region (UTR) in glucose-dependent mRNA decay. *Yeast*, 2002, 19 (10), 10.1002/yea.884 . hal-03848483

HAL Id: hal-03848483

<https://hal.science/hal-03848483>

Submitted on 10 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The role of the 5' untranslated region (UTR) in glucose-dependent mRNA decay

Bernard J. de la Cruz, Susana Prieto and Immo E. Scheffler*

Department of Biology, University of California, San Diego, La Jolla, CA 92093–0322, USA

*Correspondence to:

I. E. Scheffler, Department of Biology, University of California, San Diego, La Jolla, CA 92093–0322, USA.

E-mail: ischeffler@ucsd.edu

Abstract

When *S. cerevisiae* are grown with glucose, *SDH2* mRNA encoding the iron protein of the succinate dehydrogenase complex is unstable and present at low level. In yeast grown without glucose, *SDH2* mRNA is stable and its level rises. Addition of glucose to a glucose-limited culture causes the *SDH2* mRNA level to fall rapidly with a half-life of ~5–7 min. Previously the 5'UTR of the mRNA of *SDH2* was shown to be necessary and sufficient to destabilize it in glucose (Lombardo *et al.*, 1992). We now show that the *SDH1* and *SUC2* 5'UTRs are capable of conferring glucose-sensitive mRNA instability. We also examine how changes in the *SDH2* 5'UTR affect glucose-triggered degradation. Finally, we show that changes in mRNA stability are correlated with changes in translational efficiency for these transcripts. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: glucose repression; mRNA turnover; translation initiation; eukaryotic initiation factors; 5' UTR; *Saccharomyces cerevisiae*

Introduction

Many microorganisms have evolved the ability to rapidly alter their metabolism and gene expression patterns depending on changes in environmental and nutritional conditions. The bakers' yeast *Saccharomyces cerevisiae* has been studied extensively with respect to the phenomenon of glucose repression (Johnston and Carlson, 1993; Gancedo, 1998; Carlson, 1998, 1999). Yeast preferentially use glucose and other fermentable carbon sources during exponential growth and rely primarily on glycolysis (fermentation) to meet energy needs, shuttling other carbon and nitrogen into cell mass. Consequently, genes involved in mitochondrial biogenesis and oxidative phosphorylation as well as genes involved in metabolism of non-fermentable carbon sources are shut off, while genes involved in glycolysis/fermentation are active.

Explorations of the mechanism of glucose repression have used *SUC2*, encoding invertase, which is encoded by the *SUC2* locus, as a model gene for study (Neigeborn and Carlson, 1987; Tamai *et al.*, 1994; Celenza and Carlson, 1989; Sarokin and Carlson, 1985). Most of these studies have sought to explain glucose repression in terms of transcriptional regulation (Jiang and Carlson, 1997; Ozcan

et al., 1997). *SDH2*, another gene that is glucose-repressed, encodes the iron protein subunit of the succinate dehydrogenase (complex II) in mitochondria (Lombardo and Scheffler, 1989). Previous work has shown that while *SDH2* transcription is repressed in glucose, there is also significant post-transcriptional regulation of its mRNA (Cereghino *et al.*, 1995). *SDH2* mRNA is unstable and present at low levels when yeast are grown on glucose (YPD); *SDH2* mRNA is stabilized (half-life > 20 min) and present at a level that is 6–12-fold greater in cells grown in a non-fermentable carbon source. When glucose is added to a YPG-grown culture, the *SDH2* transcript is degraded rapidly, with a half-life of ~5–7 min. The 5'UTR of the *SDH2* mRNA is the key element conferring glucose-sensitive instability (Cereghino *et al.*, 1995). The mRNA of the *SDH1* gene, which encodes the flavoprotein of the succinate dehydrogenase, and of the *SUC2* gene are also subject to glucose-triggered decay (Cereghino, 1995).

Here we measure and compare the half lives of these three mRNAs and show that all have relatively short half-lives in glucose and long half-lives in glycerol, and, when glucose is added, *SDH1* and *SUC2* mRNA levels, like *SDH2* mRNA, rapidly fall to low levels. We show that their

5'UTRs are capable of destabilizing the transcript from a reporter construct combining the respective 5'UTRs with a GFP reading frame and the *PGK1* 3'UTR and termination site. We also analyse a series of alterations in the 5'UTR of the *SDH2* mRNA and their effect on glucose-triggered degradation. Finally, we correlate changes in mRNA stability with changes in the distribution of these transcripts on polysome gradients, depending on the carbon source. The polysome results suggest differences in the translatability of these mRNAs in YPD and YPG media. Our working hypothesis is that a competition between formation of the translation initiation complex and the degradation machinery at the 5'cap and 5'UTR determine the stability of the *SDH2*, *SDH1* and *SUC2* mRNAs, and that this competition is affected by glucose (Figure 10).

Materials and methods

Plasmids and yeast strains

GFP fusion constructs (pME01, pME02 and pME04) were created by PCR amplification of the UAS and 5'UTR of the *SDH2*, *SDH1* and *SUC2* genes, respectively, with flanking *SacII* and *BamHI* sites. The UAS–5'UTR was fused in-frame to a PCR-amplified fragment containing the GFP coding sequence and *PGK1* terminator from pRH475 (kindly provided by Dr R. Hampton, Department of Biology, UCSD) with engineered *BamHI* and *ApaI* sites. The entire fusion was placed in a *SacII*–*ApaI*-digested pRS315 shuttle vector (Sikorski and Hieter, 1989) (Figure 1).

The *SDH2* constructs with alterations in the 5'UTR are diagrammed in Figure 2. pRSIp7-2b,

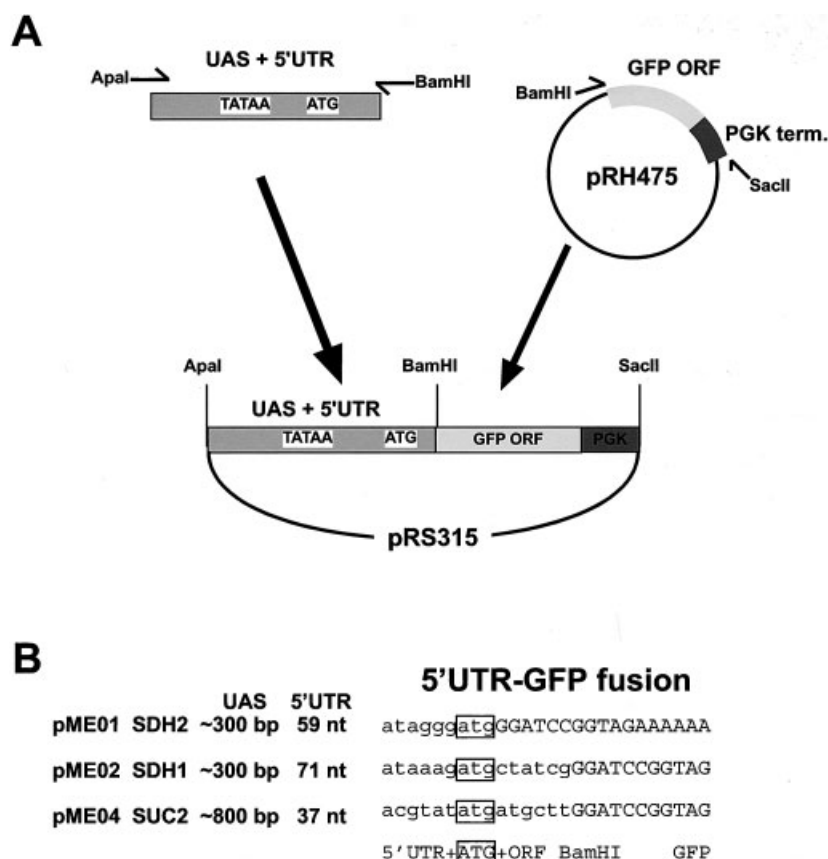


Figure 1. Construction scheme of pME01, pME02 and pME04. (A) Diagram of fusion of each UAS–5'UTR and the GFP ORF–*PGK1* terminator at the engineered *BamHI* site into the *ApaI*/*SacI* sites of the pRS315 shuttle vector. (B) Table of approximate UAS size, 5'UTR lengths and sequence of the 5'UTR–GFP fusion site

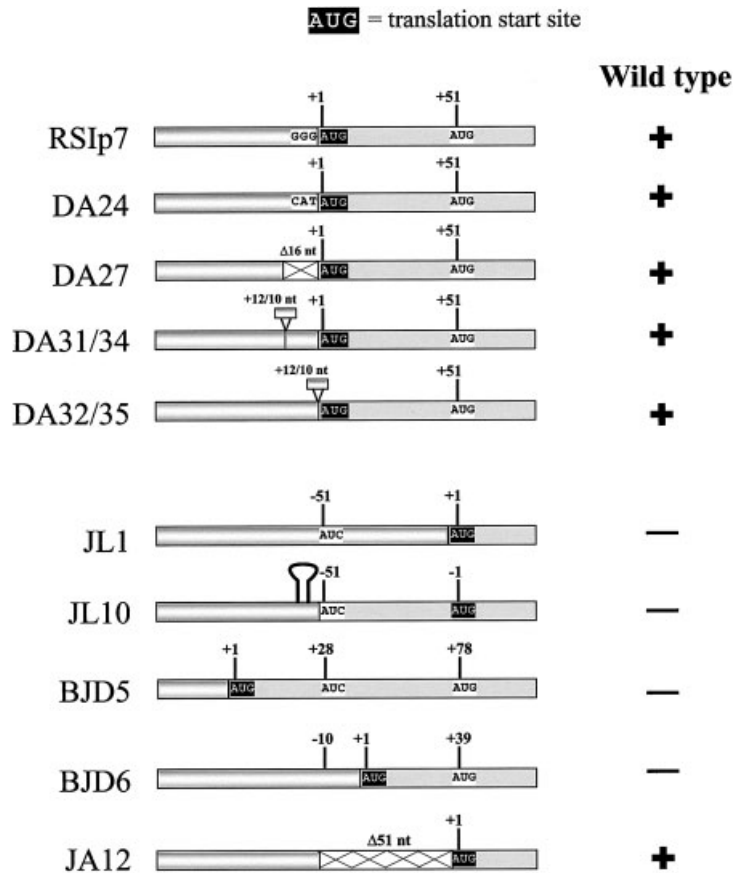


Figure 2. Constructs with altered *SDH2* 5'UTRs. Left, schematic diagrams of 5'UTR alterations. Right-hand column indicates wild-type (unstable) behaviour (+) or altered (stable) behaviour (—) in glucose

also in a pRS315-based vector (Sikorski and Hieter, 1989), is derived from the pRSIp7 (Lombardo and Scheffler, 1989), with a 467 bp deletion of the *SDH2* UAS from an internal *NdeI* site to a *SmaI* site in the pRS315. pJL1 was created by site-directed mutagenesis, converting the first AUG of pRSIp7-2b to an AUC which lengthens the 5'UTR from 59 to 110 nucleotides, since a second in-frame AUG exists at position +51. Starting with the pJL1 construct, pBJD5 and pBJD6 were made by creating in-frame start codons at positions -27 and +10, respectively, by site-directed mutagenesis. In the pJL10 construct, a 26 nucleotide insert containing a hairpin loop ($\Delta G = -20.3$ kcal/mol) has been added to the pJL1 construct at position -2 of the original AUG (Cereghino *et al.*, 1995). pRSIp7-2b is the parent plasmid for the pDA series. A *NdeI* site was created in front of the first ATG of the pRSIp7-2b by site-directed mutagenesis (GGGATG → CATATG) to generate pDA24. In pDA27, 16

nucleotides are deleted between the *NdeI* site and an upstream *BstI*1107 site in pDA24. pDA31 and pDA32 contain a *BamHI* linker (12 nucleotides) at the *BstI*1107 and *NdeI* sites, respectively, while pDA34 and pDA35 contain a *SacI* linker (10 nucleotides).

Yeast strains used in these experiments are the wild-type DL1 (*MAT α ura3 leu2-3,112 his3*) and YJL1, a Δ *sdh2* strain derived from DL1. All plasmids were introduced into either the wild-type DL1 yeast or YJL1 by the lithium acetate method (Ito *et al.*, 1983).

Yeast derepression and glucose-triggered decay

Yeast precultures were grown to stationary state on selective media. 200 ml YPD were inoculated with 1 ml preculture, then shaken (~250 rpm) at 30°C to an OD₆₀₀ ~ 0.5–0.8. 20 ml YPD culture (steady-state in YPD) were collected immediately by freezing in a

dry ice/ethanol bath. For subsequent samples, 20 ml of yeast culture were transferred to 50 ml conical tubes, pelleted and resuspended in 20 ml YPG, and grown for another 3 h at 30°C with shaking. After 3 h, one 20 ml culture was collected by freezing in a dry ice/ethanol bath (steady-state in YPG). To each of the remaining samples (G + D), 1 ml 50% glucose (dextrose) solution was added and the samples shaken at room temperature. At the various times after glucose addition, additional aliquots were frozen in a dry ice/ethanol bath. Frozen samples were centrifuged at 2000 rpm until the cells were pelleted. Pellets were resuspended in ~1 ml RNA extraction buffer (0.5 M NaCl, 0.2 M Tris-HCl, pH 7.0, 10 mM EDTA, in DEPC-treated water), quick-frozen again in dry ice/ethanol, then stored at -70°C until further use.

RNA extractions

Initial RNA extractions were by the phenol/glass bead method as described previously (Cereghino *et al.*, 1995). Later experiments used the RNeasy reagent (Ambion) in conjunction with glass bead beating as follows: frozen cells were thawed by centrifugation for ~3 min at room temperature. RNA extraction buffer was removed and 0.5 ml RNeasy and 0.5 ml glass beads (0.4–0.6 µm acid-washed, Sigma) were added. Cells were broken in a Retsch bead beater (Glen Mills) by shaking samples for 1.5 min, then placed on ice for 2 min; this cycle was repeated three times. The samples were then incubated at room temperature for 5 min. Next, 0.1 ml chloroform was added and samples vortexed for 20 s. The samples were again incubated for 10 min at room temperature. The samples were then centrifuged for >15 min at 4°C. The aqueous phase was transferred to a fresh tube and mixed with 1/2 volume (0.25 ml) DEPC-treated water plus 1 volume (0.5 ml) 100% isopropanol, vortexed, and set at room temperature for 5 min. The samples were again centrifuged for >15 min at 4°C. The supernatant was then removed and the RNA pellets washed with 1 volume (0.5 ml) 75% ethanol. The samples were centrifuged for 5 min at 4°C, then the supernatant was aspirated. RNA pellets were air-dried for >10 min and redissolved in 0.5% SDS.

Northern analysis

RNA samples were fractionated on formaldehyde-agarose gels, then vacuum-transferred as previously

described (Cereghino *et al.*, 1995). Blots were pre-hybridized for >4 h in 20 ml hybridization solution (50% formamide, 5 × Denhart's reagent, 2 × SSC, 0.1% SDS, and 20 µg/ml sheared salmon sperm DNA). After prehybridization, the hybridization solution was removed and replaced with a fresh 20 ml. Probe was added to a specific activity of 0.5–1.0 × 10⁶ cpm/ml and hybridized overnight (12–16 h) at 42°C with rocking. Probes were labelled by random priming as described previously (Cereghino *et al.*, 1995). To account for variations in loading, mRNA signals were normalized to 25S rRNA by incubating the filter for 1 h with ~100 000 cpm of an oligonucleotide probe specific for the large ribosomal RNA (5'-ATCCGCTAAGGAGTGTGTAACAACCTCACC-3'), end-labelled with [γ -³²P] ATP by T4 polynucleotide kinase (Promega). These conditions had been determined to give a linear response and accurate measurement of the RNA loading, while yielding a signal comparable to that of the derepressed *SDH2* mRNA signal.

Blots were washed once for ~10 min at room temperature and twice for ~10 min at the appropriate temperature in 2 × SSC and 0.1% SDS. Blots were visualized by mounting on a phosphorimager screen overnight and analysed using the Bio-Rad GS-525 phosphorimager and Molecular Analyst software (Bio-Rad).

Polysome analysis

The polysome extraction method was adapted from protocols by Brown and others (Dickson and Brown, 1998). YPD (~400 ml) was inoculated from pre-cultures and grown overnight at 30°C to OD₆₀₀ ~ 0.8–1.0. Once cultures reached the appropriate OD, cycloheximide was added to a final concentration of 50 µg/ml, and the culture was shaken for an additional 10 min. For derepressed (YPG) cultures, the YPD culture was pelleted in a GSA rotor at 5000 rpm for 10 min, the medium was decanted, and the pellet resuspended in 400 ml YPG; the YPG culture was then shaken for an additional 3 h at 30°C prior to cycloheximide addition.

The culture was then harvested by pelleting at 4°C in a GSA rotor at 5000 rpm for 10 min. The medium was removed, and the pellet was resuspended in 10 ml polysome extraction buffer (10 mM NaCl, 30 mM MgCl₂, 10 mM Tris-HCl, pH 7.5, 50 µg/ml cycloheximide, 1 mM DTT, 1 mM PMSF, 0.5 µg/ml leupeptin and 0.4 µg/ml pepstatin), then centrifuged again at 4°C at 5000 rpm for 5 min. The

final yeast pellet was resuspended in 1 ml polysome extraction buffer and stored at -70°C prior to polysome extraction.

To extract polysomes, cell suspensions were distributed into microfuge tubes containing ~ 0.5 ml cell suspension and ~ 0.5 ml glass beads. The tubes were shaken for 1.5 min in a Retch bead beater (Glen Mills) then placed on ice for 2 min. This cycle was repeated three times. Tubes were centrifuged for 20 min at 4°C , 10 000 rpm, then supernatants pooled (~ 0.8 – 1.0 ml) and transferred to a fresh microfuge tube. OD_{260} was measured for 2 μl sample. 150–300 OD_{260} units (~ 200 – 900 μl) polysome extracts were loaded onto 7–47% (w/w) sucrose gradients. Gradients were spun in an ultracentrifuge SW-41 rotor at 35 000 rpm for 165 min and allowed to come to a halt without braking (total spin time ~ 3 h 15 min). Gradients were collected from bottom to top using a needle and peristaltic pump. OD_{254} was read continuously with a UV monitor (Pharmacia) and plotted on a chart recorder; 14–18 fractions of ~ 0.6 ml each were collected into tubes containing 70 μl 10% SDS and 70 μl 1 M Tris-HCl, pH 7.0, then immediately frozen in dry ice/ethanol and stored at -70°C .

To extract RNA from the polysome fractions, samples were thawed quickly in a 65°C water bath and 0.6 ml phenol:chloroform:IAA (25:24:1) was added. The samples were vortexed for 20 s then centrifuged at room temperature for 4 min to separate the phases. The aqueous phase was transferred to a fresh tube and extracted with 0.6 ml 100% chloroform. After chloroform extraction, LiCl was added to the aqueous phase to a final concentration of 1 M and the tube topped off with 100% isopropanol. Samples were incubated overnight at -20°C , then centrifuged for >20 min at 4°C . The supernatant was completely removed and the pellets air-dried for >10 min. The RNA pellet was resuspended in 20 μl 0.5% SDS plus 3 μl with loading dye (Ausubel *et al.*, 1988) and loaded onto formaldehyde/agarose gels, size-fractionated and probed as described above.

Results

SDH1 and *SUC2* mRNAs share the glucose-sensitive instability of the *SDH2* mRNA

The decay rates in YPD of all *SDH1*, *SDH2* and *SUC2* mRNAs were quantified by Northern analysis. An increase in the levels of all three mRNAs

was seen as yeast are shifted from YPD to YPG. However, the relative increase of each message level is not the same: the increase is most dramatic for the tightly repressed *SUC2* mRNA, then *SDH2* and *SDH1* (results not shown). When glucose is added to the YPG-derepressed cultures, the levels of the *SDH2*, *SDH1* and *SUC2* decay rapidly but at slightly different rates (Figure 3). The half-life was determined to be of the order of 5–7 min for *SDH2* mRNA, ~ 10 min for *SDH1* mRNA, and 3–5 min for *SUC2* mRNA (Prieto *et al.*, 2000). Thus, the stability of all three mRNAs appears to be affected by glucose.

The 5'UTRs of the *SDH1*, *SDH2* and *SUC2* mRNAs confer glucose-sensitive decay to a heterologous construct

The *SDH2* 5'UTR destabilizes the *CUP1* mRNA in a glucose-dependent manner (Cereghino *et al.*,

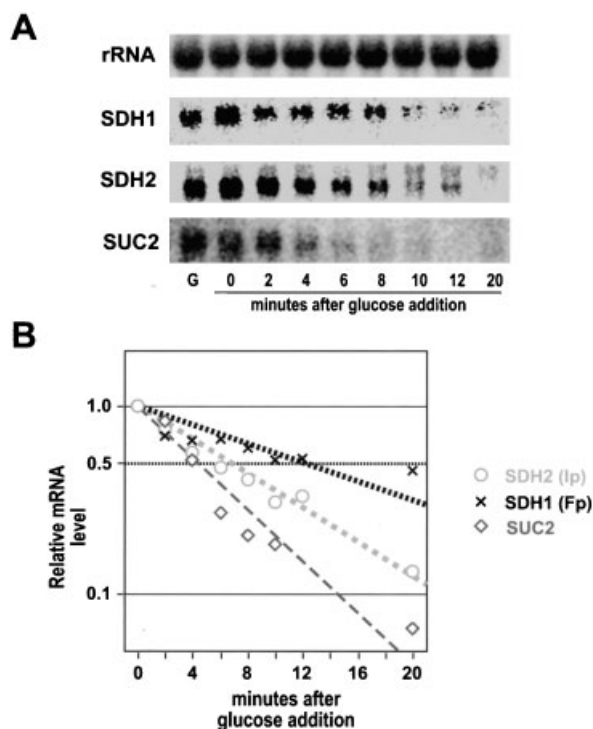


Figure 3. Glucose-triggered decay of endogenous mRNAs. (A) Northern blots probed for the *SDH1*, *SDH2* and *SUC2* mRNAs. Lane G, YPG-derepressed culture; lanes 0–20, min after addition of glucose to YPG-derepressed culture. (B) Decay kinetics of *SDH1*, *SDH2* and *SUC2* mRNAs. Levels of each mRNA in each lane are normalized to the 25S rRNA signal. Initial mRNA level is set to 1.0 at 0 min after glucose addition

1995). We extended this earlier experiment by fusing the UAS and 5'UTRs of the *SDH2*, *SDH1* and *SUC2* mRNAs to the GFP coding sequence and the *PGK1* 3'UTR and polyadenylation site. The UAS–5'UTR combination was retained to assure that the resulting transcripts would have exactly the same start site as the original mRNAs; with heterologous promoters, transcription start sites are difficult to predict in yeast. The glucose-sensitive stability of these chimeric mRNAs was examined and compared to that of the endogenous *SDH2* mRNA. Note that each of these 5'UTRs have different lengths and different nucleotide sequences (Table 1). GFP is not endogenous to yeast and its transcript would not *a priori* be expected to show sensitivity to the carbon source. *PGK1* mRNA is highly stable, with its 3'UTR neutral with respect to decay (LaGrandeur and Parker, 1999).

The results for the 5'UTR–GFP heterologous transcripts are shown in Figure 4. As a control for glucose-triggered decay, the endogenous *SDH2* mRNAs are probed on the same Northern blots. Like the endogenous *SDH2* mRNA, the transcripts from the fusion constructs are derepressed in YPG. The derepression of these chimeric genes can also be clearly observed by comparing the relative fluorescence of whole cells grown in either YPD or YPG (results not shown). Glucose-triggered decay is clearly seen. The *SDH2* 5'UTR–GFP (pME01) transcript decays at a rate only slightly lower than that of the endogenous *SDH2* mRNA (Figure 4A, left). The *SDH1* 5'UTR–GFP (pME02) transcript decays at approximately the same rate as *SDH2* mRNA (Figure 4B, left). The *SUC2* 5'UTR–GFP transcript (pME04) has a shorter half-life compared to that of the *SDH2* mRNA (Figure 4C). In the latter

case there is a correlation with the very fast decay of the *SUC2* mRNA (Figure 3B).

Does the ORF of GFP contribute to the control of the stability of these chimeric transcripts? Experiments (results not shown) with transcripts containing the GFP ORF in different contexts (5'UTR, 3'UTR) have shown that such transcripts are not degraded rapidly in YPD. In one experiment such a transcript was induced from a GAL promoter, and transcription was repressed by the addition of glucose. Nevertheless the half-life of this transcript was significantly longer than the half-life of the *SDH2* transcript under the same conditions.

Alterations to the *SDH2* 5'UTR that block glucose-triggered decay

Next, attention was focused on how small alterations of the *SDH2* 5'UTR might affect glucose-sensitive degradation. One set of constructs was generated by small sequence insertion/deletions in the 5'UTR of *SDH2* mRNA. In a second set of constructs the position of the first AUG was altered, i.e. the length of the 5'UTR was changed with a minimal perturbation to the surrounding sequence, especially regarding the transcriptional start sites. It should be noted that the *SDH2* mRNA has two major (–59 and –44) and one minor (–50) transcription start sites. However, all three transcripts exhibit the same glucose instability (Lombardo *et al.*, 1992). The lengths of 5'UTRs are stated with reference to wild-type transcripts having the longest 5'UTR (59 nt). Other more major alterations further upstream caused unpredictable changes in transcription start site(s) and were not included in the analysis.

When the decay kinetics of these constructs were analysed, it was observed that they fall into two categories: those that show wild-type behaviour, i.e. decay upon addition of glucose, and those that do not decay upon the addition of glucose (Figure 2). Within the short-lived group, variations in half-life were relatively insignificant. Northern blots and decay kinetics for three examples of altered 5'UTRs that retain normal or near normal behaviour are shown in Figure 5. The pDA27 transcript has a deletion of 16 nucleotides immediately upstream of the first AUG shortening the 5'UTR to 43 nucleotides. As seen in the Northern blot (Figure 5A, left) and its quantitative analysis (Figure 5A, right), pDA27 mRNA is degraded like the wild-type *SDH2* mRNA. The pDA31 transcript contains a 12 nt

Table 1. *SDH1*, *SDH2*, and *SUC2* 5'UTR sequences

Name	Sequence
<i>SDH1</i> (Fp) Accession No. M86909	gaagauaaaaaagaagaagaagaagaaga aauccaaauuacauaguacgaagaagaacga gaauaaag AUG –71
<i>SDH2</i> (Ip) Accession No. V01311	gaaaggaauauaguugagcuauacuucuuuga aaucuggagauuacauuuuuuaggg AUG –59
<i>SUC2</i> (Inv) Accession No. J05487	caagcaaaacaaaagcuuuuuuuuuacuaa cguau AUG –37

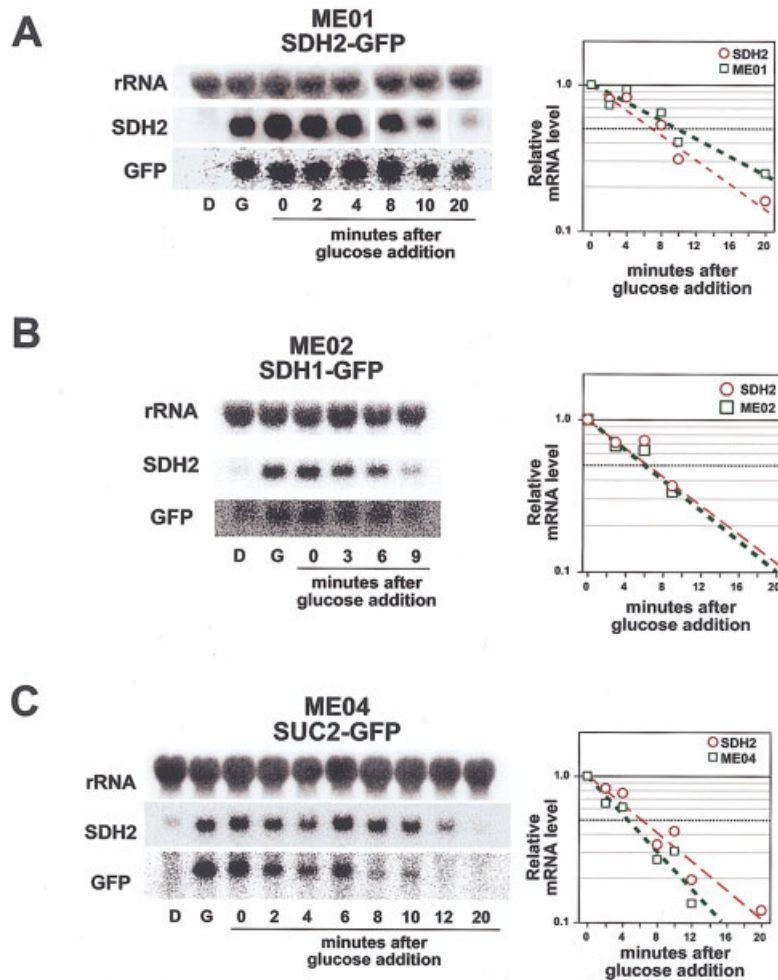


Figure 4. Glucose-triggered decay of chimeric, heterologous mRNAs. Left, Northern blots probed for 25S rRNA, endogenous *SDH2* mRNA, and the chimeric 5'UTR-GFP mRNA. Right, decay kinetics of the endogenous *SDH2* mRNA and the chimeric 5'UTR-GFP mRNA. (A) Results for pME01 (*SDH2*-GFP fusion). (B) Results for pME02 (*SDH1*-GFP fusion). (C) Results for pME04 (*SUC2*-GFP fusion)

*Bam*HI linker placed immediately upstream of the first AUG, thus lengthening the 5'UTR to 71 nucleotides. In this case it can be seen that the stability of the message may be increased slightly to ~7–8 min (Figure 5B). This holds true for the other linker insertion constructs pDA32, pDA34 and pDA35 (data not shown).

To further show the importance of the 5'UTR vs. downstream sequences in the open reading frame, pJA12 has the 51 nucleotide region deleted between the first AUG through the second in-frame AUG, yet its transcript shows the same decay kinetics as that from the endogenous gene (Figure 5C).

For the second group, half-lives of >20 min are

found. The most striking result is seen with the construct pJL1, where the first AUG is converted to an AUC (Figure 6). There is evidence (see below) suggesting that the second in-frame AUG at position +51 is used as the new translation start site, thus doubling of the 5'UTR length to 110 nt. That this single nucleotide change so radically alters the stability of the JL1 (AUG +51) mRNA is intriguing. Even at steady-state in glucose, JL1 (AUG +51) mRNA level is relatively higher compared with the wild-type mRNA. This is consistent with the observation that the JL1 mRNA is more stable in glucose. In the construct pJL10, an insert capable of forming a short hairpin is added at a position –2

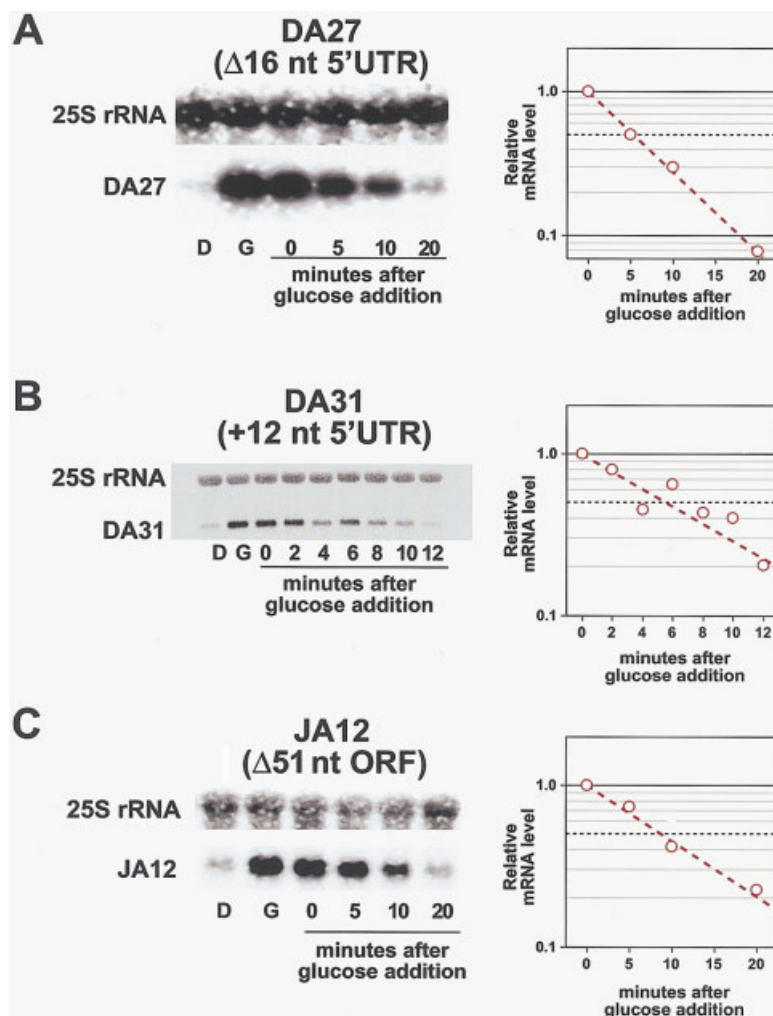


Figure 5. Glucose-triggered decay of constructs with altered *SDH2* 5'UTRs exhibiting wild-type behaviour. Left, Northern blots probed for 25S rRNA and *SDH2* mRNA. Right, decay kinetics of the altered mRNA. (A) Results for pDA27 (-16 nt deletion). (B) Results for pDA31 ($+12$ nt insertion). (C) Results for pJA12 (-51 nt ORF)

from the original AUG (Figure 2), and its mRNA was also found to be stable. However, the same hairpin when present in front of the original AUG (construct pGC35; Cereghino, 1995) blocks translation as determined by Western blot and the absence of growth on YPG (data not shown).

It is also of interest that the pJL1 and pJL10 constructs will complement $\Delta sdh2$ yeast cells when grown on glycerol, albeit at much slower growth rates compared to yeast complemented with a wild-type construct. In addition, assays for SDH activity show pJL1 and pJL10 to restore SDH activity to low levels (unpublished observations). The observation that pJL1 does not complement

the $\Delta sdh2$ mutant as well as the wild-type construct (pRSIp7) can be rationalized by noting that a translation start at the second AUG would produce a protein missing 17 amino acids and hence a large part of the signalling sequence for mitochondrial import. However, the fate of the truncated protein represents an aspect of the pJL1-complemented mutant that is not relevant to the problem of mRNA stability.

To determine the importance of the position of the first AUG with respect to glucose-triggered decay, pJL1 (AUG $+51$) construct was used as a starting point to introduce new in-frame AUGs at positions -27 (pBJD5) and $+10$ (pBJD6) relative

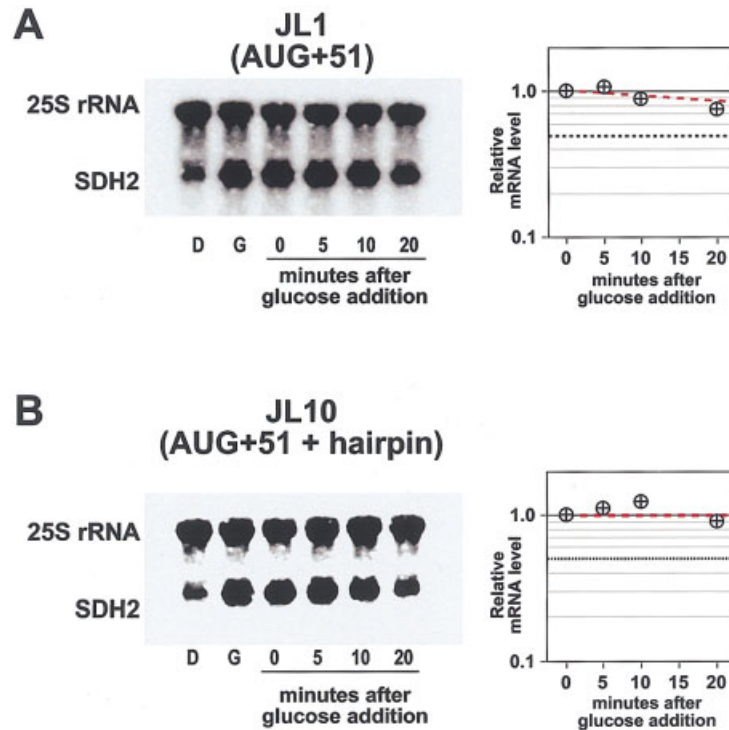


Figure 6. Northern analysis and decay kinetics of mRNAs from stable constructs. (A) Results for pJL1 (AUG +51). (B) Results for pJL10 (AUG +51 plus hairpin)

to the original AUG (Figure 2). These would generate new transcripts with 5'UTRs 32 nt and 69 nt in length, while keeping the rest of the surrounding sequences unchanged (Table 2). The new in-frame

Table 2. AUG contexts of wild-type genes and SDH2 5'UTR alterations

Gene (Genbank)	5'UTR length (nt)	Context
<i>Endogenous genes</i>		
SDH2	59	Aua G ggAUGuugaa
SDH1	71	Gag A caAUGagaga
SUC2	39	ucuau A UGaugcc
ACT1	141	uua A caAUGGauuc
Kozak consensus	(-3/+4)	Rnn R ccAUGR
<i>Stable alterations</i>		
JL1/JL10	110/136	Agg G guAUGGcuac
BJD5 (AUG -27)	37	ucuuga A UGacugg
BJD6 (AUG +10)	69	uug G acAUGcuauu
<i>Unstable alterations</i>		
DA24	59	Auacau A UGuugaa
DA27 (Δ 16 nt)	43	Gaguau A UGuugaa
DA32 (+12 nt)	71	Auacau A UGuugaa
DA35 (+10 nt)	71	uccgu A UGuugaa

AUGs would generate proteins having an additional seven amino acids (pBJD5) or missing three amino acids (pBJD6). We find in both cases that the transcripts remain as stable as the JL1 (AUG +51) mRNA (Figure 7). Incidentally, all of these transcripts retain the A-rich runs at the beginning of the 5'UTR, and yet their behaviour is changed. These A-rich runs are therefore not part of the *cis*-element specifying glucose-triggered instability.

Resistance to glucose-triggered decay corresponds to more efficient translation initiation

A working hypothesis has proposed that glucose-sensitive stability is a consequence of a competition for the 5'UTR of the *SDH2* mRNA between the translation initiation complex and the degradation machinery, and that this competition is affected by the presense or absense of glucose (Scheffler *et al.*, 1998). Specifically, the decapping enzyme Dcp1p and translation initiation factors (including the cap-binding eIF4F complex and eIF3) compete directly for the 5' cap. Highly efficient translation initiation is expected to help in stabilizing the mRNA, while

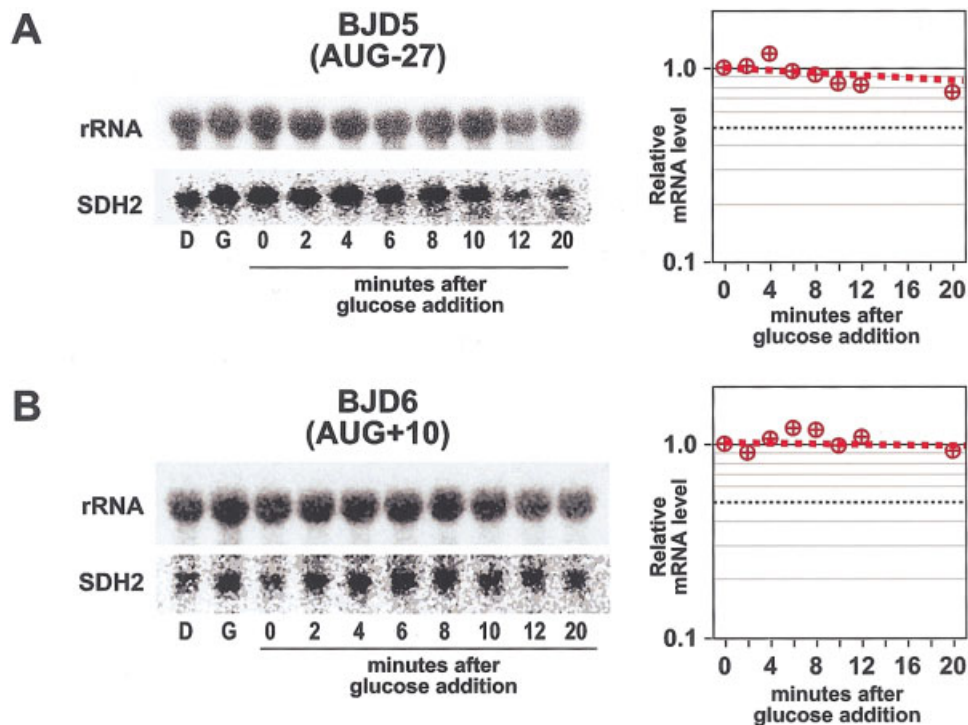


Figure 7. Stable transcripts from constructs with altered first AUG position. (A) Results for pBJD5 (AUG -27). (B) Results for pBJD6 (AUG +10)

inefficient translation may allow the decapping enzyme access to the mRNA.

Preliminary data were suggestive of such a scenario for the wild-type *SDH2* mRNA. Previously we demonstrated that while $\geq 90\%$ of *SDH2* mRNA is present on polysomes when yeast are grown in YPG, this fraction drops to $\sim 70\%$ found in polysomes when yeast are grown in YPD. In contrast, $\geq 90\%$ of the *ACT1* mRNA is found on polysomes regardless of whether yeast are grown in YPD or YPG (Prieto *et al.*, 2000).

The investigation was expanded to include polysome profile analysis from yeast containing constructs having different stabilities. To recapitulate the earlier observations, polysome analysis was done on wild-type DL1 yeast. The results are shown in Figure 8. Focusing on the endogenous *SDH2* mRNA, it is seen that the majority of the transcript ($\geq 90\%$) is in polysomes when yeast are grown on YPG. In contrast, the endogenous *SDH2* mRNA from cells grown in glucose exhibits a broader and multiphasic distribution on the gradient, with a significant portion found outside of the polysome region.

Similar results are seen for the *SDH2* 5'UTR-GFP

fusion (pME01) from the same cells (Figure 8). In the case of the *SUC2* 5'UTR-GFP fusion (pME04), there is essentially no detectable GFP mRNA in YPD, due to extremely tight repression by the *SUC2* UAS. However, in YPG most of the *SUC2* 5'UTR-GFP mRNA, like the *SUC2* mRNA (Figure 8), is present in polysomes.

It was predicted for transcripts resistant to glucose-triggered decay that most of the mRNA would be present in polysomes when yeast are grown either on YPD or on YPG. This is in fact observed for the JL1 (AUG +51) transcript (Figure 9). Similar results were obtained for the pBJD5 (AUG -27) and pBJD6 (AUG +10) transcripts (data not shown).

Discussion

Although glucose repression has been studied extensively from the perspective of the signaling pathway and its target at the transcriptional level (Trumbly, 1992), a post-transcriptional mechanism is also important (Cereghino *et al.*, 1995; Cereghino and Scheffler, 1996; Prieto *et al.*, 2000). In particular, it was noted that the steady-state levels of

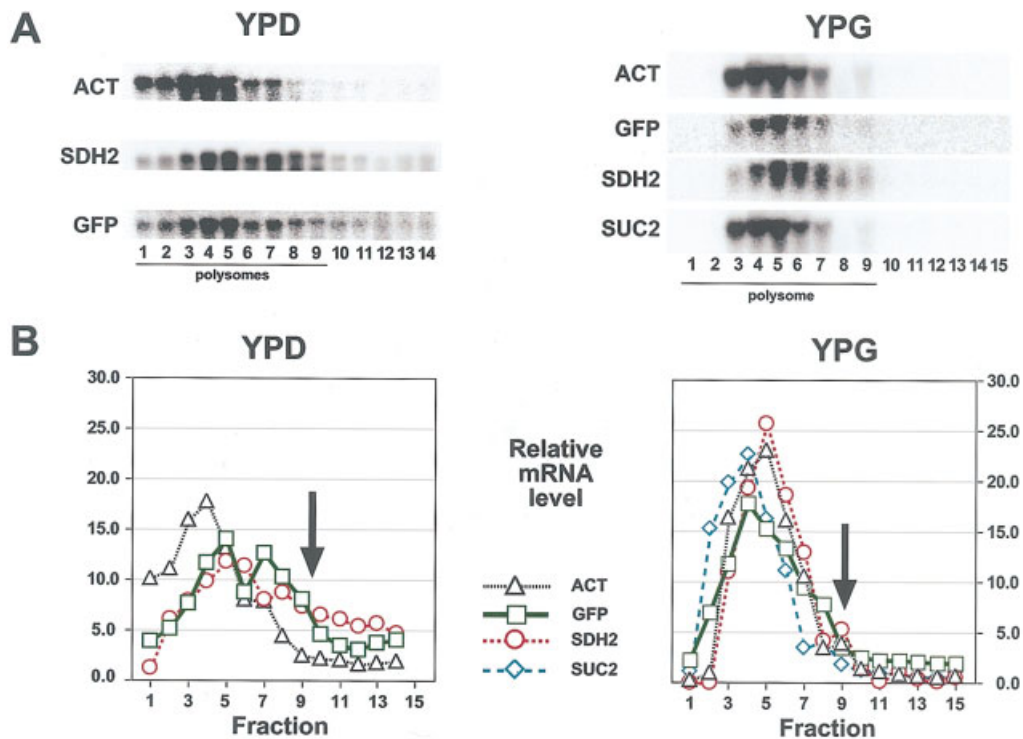


Figure 8. Polysome profiles from DLI yeast containing pME01 (*SDH2*–GFP fusion). Left, results for yeast grown in YPD. Right, results for yeast grown in YPG. (A) Northern blot from YPD and YPG grown yeast. Probed for *ACT1*, *SDH2*, ME01 (*SDH2*–GFP fusion) and *SUC2* (YPG blot only) mRNAs. Lanes represent fractions of the sucrose gradient from bottom (heavy) to top (light). Fractions containing polysomes are indicated by the bar. (B) Distribution of mRNAs in the YPD vs. YPG polysome profiles. Fraction of each mRNA present plotted for each lane. Arrow indicates break between polysomes and the 80S monosome

SDH2 mRNA are controlled by the differential stability of the *SDH2* mRNA. The 5'UTR was found to be the major *cis*-acting element regulating stability and selective degradation of the *SDH2* mRNA (Cereghino, 1995). Our working hypothesis is that the degradation and translation machinery compete for the 5' cap, and that the outcome of the competition can depend on a signal from the carbon source (Figure 10).

An obvious challenge is to identify the feature of the 5'UTR that is responsible for the selective turnover of these mRNAs. Four major properties come to mind: (a) shared consensus sequences for protein binding; (b) characteristic secondary structures; (c) overall length of the 5'UTR; (d) Kozak context of the start codon. The *SDH1* and *SUC2* mRNAs exhibit similar behaviour to the *SDH2* mRNA. Each property was examined by comparing these 5'UTRs, and by making small alterations in the 5'UTR of the *SDH2* mRNA. Our results clearly suggest that none of these, alone or in combination,

can provide a simple predictive model for the observed behaviour of the transcripts.

Multiple alignment and searches for common sequence motifs failed to reveal shared recognizable sequence elements in these 5'UTRs, e.g. uORFs (Oliveira and McCarthy, 1995), 5'TOPs (Levy *et al.*, 1991; Avni *et al.*, 1994, 1997; Jefferies *et al.*, 1997; Hornstein *et al.*, 1999), or IREs (Casey *et al.*, 1988; Hentze *et al.*, 1988; Haile *et al.*, 1989; Klausner and Harford, 1989; Theil, 1990a, 1990b; Klausner *et al.*, 1993). The 5' end of each of these leaders is rich in As, but no significance for them was found. A calculation of potential secondary structures did not reveal stable hairpins or loops in the *SUC2* and *SDH1* 5'UTRs, while the *SDH2* 5'UTR had the potential for forming such structures with a free energy of formation of approximately -8 kcal (Jaeger *et al.*, 1989; Zuker, 1989; Mathews *et al.*, 1999). Otherwise, no similarities in predicted secondary structures between the three transcripts stand out. The above 5'UTRs differ significantly in

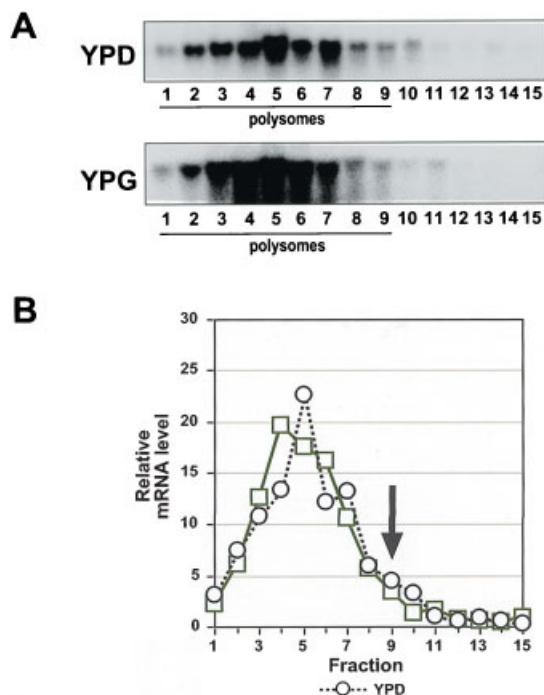


Figure 9. Polysome profile from YJL1 ($\Delta sdh2$) yeast containing pJL1 (AUG +51) construct. (A) Northern blots probed for *SDH2* mRNA. Lanes represent fractions of the sucrose gradient from bottom (heavy) to top (light). Fractions containing polysomes indicated by the bar. (B) Distribution of JL1 (AUG +51) mRNA in YPD and YPG polysome gradients. Arrow indicates break between polysomes and the 80S monosome

length, ranging from 37 to 71 nt (Table 1). It should be pointed out that one frequently finds multiple transcription start sites in yeast, and for the *SDH2* gene two major and one minor start sites have been established (Lombardo and Scheffler, 1989), yielding transcripts with 5'UTRs from 44 to 59 nt, all of which appear to behave in the same way in response to glucose. Finally, with regard to the canonical Kozak sequence at the -3 and $+4$ positions (Kozak, 1986), none have the optimal sequence for efficient translation (Table 2). The lack of optimal Kozak sequence suggests that translation efficiency may be a factor in the turnover of these transcripts. However, comparison of these 5'UTRs, two of which are functionally related while the third is not, failed to reveal shared primary or secondary structures.

Next, the focus was on introducing alterations in the *SDH2* 5'UTR that would alter the behaviour of the transcript in YPD or YPG. Surprisingly, many changes including point mutations and deletion or

insertion of short oligonucleotides caused no deviation from the behaviour of the wild-type (Figure 2). Some of the alterations result in changes in the length of the 5'UTR, and in some both the length of the 5'UTR and the context of the AUG are altered. Although the AUG context alterations are not exhaustive, changes to either the -3 or $+4$ position suggest that neither alone is sufficient to stabilize the mRNA. Also, a construct (pJA12) that preserved the *SDH2* 5'UTR but deleted the first 50 nucleotides in the ORF was also unstable in glucose. This result reinforces the idea that the ORF does not contain significant *cis*-acting elements or binding sites for proteins that affect mRNA stability.

The most dramatic effect was seen in the JL1 (AUG +51) transcript, where a single nucleotide was altered to convert the first AUG to AUC, and it was found that the JL1 mRNA is highly stable in glucose. The change effectively doubles the length of the 5'UTR. The JL1 mRNA is translated as determined by pulse labelling (unpublished results) but provides only partial SDH activity ($\sim 10\%$). It is believed to be due to loss of 17 amino acids crucial for mitochondrial import of Sdh2p, thus also confirming that the first AUG is the normal translation start. It is considered unlikely that this single nucleotide substitution would destroy a consensus site for an RNA binding regulatory protein, or have a dramatic effect on secondary structure. In other words, if a destabilizing sequence was present in the 5'UTR of *SDH2* mRNA, it should still be present in the JL1 mRNA (unless it happened to include the original start codon).

The 5'UTR of the pJL1 construct was further modified by introducing new in-frame start codons either upstream in pBJD5 (AUG -27) or downstream in pBJD6 (AUG $+10$) from the original start codon, without any other changes in the JL1 sequence. Regardless of the increase or decrease in 5'UTR length, these mRNAs remained stable in glucose. Adding a hairpin loop in the middle of the 5'UTR of the pJL1 transcript (pJL10) did not block translation or destabilize it as might be expected. However, since the hairpin is relatively far from the start codon, it may not be sufficient to block translational initiation (Oliveira *et al.*, 1993; Sagliocco *et al.*, 1993; Vega Laso *et al.*, 1993).

At this point it is interesting to contrast the behaviour of two constructs, pDA34 and pDA35, which have short oligonucleotide insertions to lengthen the 5'UTR and are both unstable in

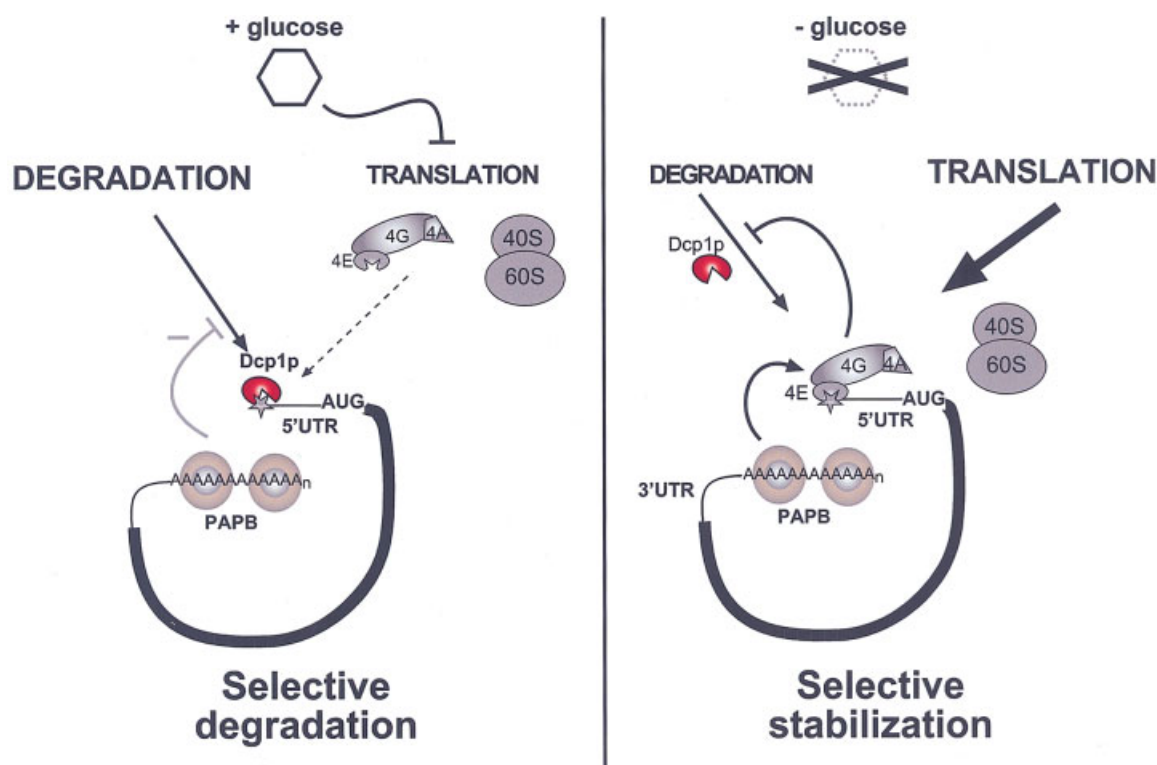


Figure 10. The working model of selective translation and degradation of glucose-repressed mRNAs

glucose, with that of the pBJD6 (AUG +10) construct, which lengths the 5'UTR by nucleotide substitution. All three transcripts have a 5'UTR of 69 nt. A comparison of each Kozak context finds no obvious improvement to make pBJD6 more efficiently translated. It has not been investigated whether the new translation start sites in pBJD5 and pBJD6 are used *in vivo*. Both proteins would have abnormal mitochondrial targeting sequences and thus are unlikely to restore 100% SDH activity. Biochemical assays for SDH activity show only ~10% SDH activity (unpublished results). However, an analysis of the actual proteins made, and of their fate (including import, turnover, incorporation into complex II), is a peripheral issue.

An analysis of the distribution of these mRNAs on polysome gradients was used to examine the role of carbon source on the competition between degradation and translation initiation (Figure 10). Two comparisons were made. First, polysome profiles from cells grown on YPG were compared with those from cells grown on YPD. Polysome profiles from YPG-grown cells found both stable (*ACT1*) and unstable (*SDH2*, *SUC2* and 5'UTR-GFP fusion) mRNAs located under a single prominent

peak on polysomes (Figures 8 and 9). The region corresponding to the monosomes and lighter RNA-protein complexes is free of detectable transcripts. In contrast, polysome profiles from YPD-grown cells found unstable transcripts had a broader distribution with multiple peaks and with a significant fraction found in the region with the lighter RNA-protein complexes (Figure 8). The presence of transcript above the polysome region appear to correlate with the observed rapid turnover in YPD. Furthermore, since the lighter complex is subject to the degradative mechanism, one would not expect significant accumulation.

Second, profiles of transcripts that are unstable in YPD were compared with those of transcripts that are stable. The stable JL1 transcript is found almost exclusively in the polysome region in both YPD and YPG (Figure 9). Thus, it appears that initiation of translation of the JL1 mRNA is efficient in YPD; this coincides with the observation that the JL1 transcript is more resistant to degradation in glucose. Similar measurements with the *SUC2* mRNA and with the *SUC2* 5'UTR-GFP (pME04) transcript have confirmed that these mRNAs are exclusively found in the polysome region from cells

grown in YPG (results not shown). Unfortunately, cells in YPD express so little of *SUC2* or *SUC2* 5'UTR–GFP mRNA that measurements of their distribution were technically impossible.

Wild-type yeast cells do grow in YPG, and even better in YPD. Therefore, global protein synthesis and translation must be efficient in both media. However, a subset of specific mRNAs, including the *SDH2*, *SDH1* and *SUC2* mRNAs, are rapidly degraded when glucose is added to a derepressed (glycerol) culture. Previous work showed that glucose-triggered decay does not trigger deadenylation (Prieto *et al.*, 2000), and only requires decapping by the enzyme Dcp1p followed by degradation by the 5'–3' exonuclease Xrn1p (reviewed by Decker and Parker, 1994; Beelman and Parker, 1995; Tucker and Parker, 2000). It is noted that the 5' cap also has a role in translation initiation. The 'scanning model' has the eIF4G complex and 40S ribosomal subunit recruited to the 5' cap (reviewed by Day and Tuite, 1998; Tucker and Parker, 2000). The 40S subunit then scans the 5'UTR until it reaches a translation start site. This model seems insufficient to explain the change in stability of specific mRNAs, especially considering the length of the 5'UTR relative to the size and mass of the eIFs and ribosomal subunits. Instead it is likely that the 5'UTR is contacted by one or more eIFs at multiple points. Various translation initiation factors including eIF4G, eIF4A and eIF3 have non-specific RNA binding motifs. Also, various eIFs can be differentially phosphorylated, likely altering their activities (Frederickson and Sonenberg, 1992). Our laboratory has shown that glucose-triggered decay of *SDH2* mRNA is abolished in mutants of *reg1*, which is a regulatory subunit of the Glc7 phosphatase and part of the glucose-signalling pathway (Cereghino and Scheffler, 1996). It is then possible that carbon source may modulate activity of each eIF by differential phosphorylation.

Another connection between translation and degradation is seen in the interaction between Dcp1p and the eIF4G–eIF4E translation initiation complex and the poly(A) binding protein (Pab1p) (Schwartz and Parker, 2000; Tucker and Parker, 2000). Dcp1p also interacts with Pat1p, a gene product involved in formation and stabilization of preinitiation translation complexes (Wyers *et al.*, 2000); the *PAT1* gene was previously identified as *MRT1*, a factor stimulating decapping. Hence, cap binding protein (eIF4E) and the decapping enzyme compete for the same substrate and interact with

each other indirectly through other proteins associated with translation. Any change in the activity of one or the other can affect the half-life of a transcript. Mutations of eIFs have been shown to modulate translation efficiency of specific transcripts (Schwartz and Parker, 1999). As described here, a signal dependent on carbon source can do the same. Specificity arises from particular features of the mRNA including 5'UTR length and sequence. Such a mechanism would be distinct from that affecting global protein synthesis under stress or during amino acid or glucose starvation (Ross, 1996).

Acknowledgements

This project was supported by a grant from the American Cancer Society to IES. B.J. de la Cruz was supported by a training grant from the United States Public Health Service. S. Prieto was a postdoctoral fellow supported by the Spanish Ministerio de Educacion y Cultura. The authors would like to thank Drs R. Hampton, S. Emr and T. Hope for fruitful discussions and for making constructs available (R.H.). Special thanks are owed to Drs G. Cereghino, D. Atencio, M. S. Englehart and J. Lee for making some of the constructs used in this study.

Abbreviations

3'UTR/5'UTR, 3'/5' untranslated region; DEPC, diethylpyrocarbonate; GFP, green fluorescent protein; IAA, isoamyl alcohol; ORF, open reading frame; UAS, upstream activating sequence; YPD, 1% yeast extract, 2% peptone, 2% dextrose; YPG, 1% yeast extract, 2% peptone, 3% glycerol.

References

- Ausubel FM, Brent R, Kingston RE, *et al.* 1988. *Current Protocols in Molecular Biology*. Wiley: Media, PA.
- Avni D, Biberman Y, Meyuhas O. 1997. The 5' terminal oligopyrimidine tract confers translational control on TOP mRNAs in a cell type- and sequence context-dependent manner. *Nucleic Acids Res* **25**: 995–1001.
- Avni D, Shama S, Loreni F, Meyuhas O. 1994. Vertebrate mRNAs with a 5'-terminal pyrimidine tract are candidates for translational repression in quiescent cells: characterization of the translational *cis*-regulatory element. *Mol Cell Biol* **14**: 3822–3833.
- Beelman CA, Parker R. 1995. Degradation of mRNA in eukaryotes. *Cell* **81**: 179–183.
- Carlson M. 1998. Regulation of glucose utilization in yeast. *Curr Opin Genet Dev* **8**: 560–564.

- Carlson M. 1999. Glucose repression in yeast. *Curr Opin Microbiol* **2**: 202–207.
- Casey JL, Hentze MW, Koeller DM, *et al.* 1988. Iron-responsive elements: regulatory RNA sequences that control mRNA levels and translation. *Science* **240**: 924–928.
- Celenza JL, Carlson M. 1989. Mutational analysis of the *Saccharomyces cerevisiae* SNF1 protein kinase and evidence for functional interaction with the SNF4 protein. *Mol Cell Biol* **9**: 5034–5044.
- Cereghino GP. 1995. The Control of mRNA Turnover as a Mechanism of Glucose Repression in *Saccharomyces cerevisiae*. PhD Thesis, University of California at San Diego.
- Cereghino GP, Atencio DP, Saghbini M, Beiner J, Scheffler IE. 1995. Glucose-dependent turnover of the mRNAs encoding succinate dehydrogenase peptides in *Saccharomyces cerevisiae*: sequence elements in the 5' untranslated region of IP mRNA play a dominant role. *Mol Biol Cell* **6**: 1125–1143.
- Cereghino GP, Scheffler IE. 1996. Genetic analysis of glucose regulation in *Saccharomyces cerevisiae*: control of transcription versus mRNA turnover. *EMBO J* **15**: 363–374.
- Day DA, Tuite MF. 1998. Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. *J Endocrinol* **157**: 361–371.
- Decker CJ, Parker R. 1994. Mechanisms of mRNA degradation in eukaryotes. *TIBS* **19**: 336–340.
- Dickson LM, Brown AJP. 1998. mRNA translation in yeast during entry into stationary phase. *Mol Gen Genet* **259**: 282–293.
- Frederickson RM, Sonenberg N. 1992. Signal transduction and regulation of translation initiation. *Sem Cell Biol* **3**: 107–115.
- Gancedo JM. 1998. Yeast carbon catabolite repression. *Microbiol Mol Biol Rev* **62**: 334–361.
- Haile DJ, Hentze MW, Rouault TA, Harford JB, Klausner RD. 1989. Regulation of interaction of the iron-responsive element binding protein with iron-responsive RNA elements. *Mol Cell Biol* **9**: 5055–5061.
- Hentze MW, Caughman SW, Casey JL, *et al.* 1988. A model for the structure and functions of iron-responsive elements. *Gene* **72**: 201–208.
- Hornstein E, Git A, Braunstein I, Avni D, Meyuhos O. 1999. The expression of poly(A) binding protein gene is translationally regulated in a growth-dependent fashion through a 5'-terminal oligopyrimidine tract motif. *J Biol Chem* **274**: 1708–1714.
- Ito H, Fukuda Y, Murata K, Kimura A. 1983. Transformation of intact yeast cells treated with alkali cations. *J Bacteriol* **153**: 163–168.
- Jaeger JA, Turner DH, Zuker M. 1989. Improved predictions of secondary structures for RNA. *Proc Natl Acad Sci U S A* **86**: 7706–7710.
- Jefferies HBJ, Fumagalli S, Dennis PB, Reinhard C, Pearson RB, Thomas G. 1997. Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70^{S6k}. *EMBO J* **16**: 3693–3704.
- Jiang R, Carlson M. 1997. The Snf1 protein kinase and its activating subunit, Snf4, interact with distinct domains of the Sip1/Sip2/Gal183 component in the kinase complex. *Mol Cell Biol* **17**: 2099–2106.
- Johnston M, Carlson M. 1993. Regulation of carbon and phosphate utilization. In *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*, Broach J, Jones EW, Pringle J (eds). 1993. Cold Spring Harbor Laboratory Press: New York; 193–281.
- Klausner RD, Harford JB. 1989. *cis-trans* Models for post-transcriptional gene regulation. *Science* **246**: 870–872.
- Klausner RD, Rouault TA, Harford JB. 1993. Regulating the fate of mRNA: the control of cellular iron metabolism. *Cell* **72**: 19–28.
- Kozak M. 1986. Influences of mRNA secondary structure on initiation by eukaryotic ribosomes. *Proc Natl Acad Sci U S A* **83**: 2850–2854.
- Kozak M. 1992. A consideration of alternative models for the initiation of translation in eukaryotes. *CRC Crit Rev Biochem* **27**: 385–402.
- LaGrandeur T, Parker R. 1999. The *cis*-acting sequences responsible for the differential decay of the unstable MFA2 and stable PGK1 transcripts in yeast include the context of the translational start codon. *RNA* **5**: 420–433.
- Levy S, Avni D, Hariharan N, Perry RP, Meyuhos O. 1991. Oligopyrimidine tract at the 5' end of mammalian ribosomal protein mRNAs is required for their translational control. *Proc Natl Acad Sci U S A* **88**: 3319–3323.
- Lombardo A, Cereghino GP, Scheffler IE. 1992. Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*. *Mol Cell Biol* **12**: 2941–2948.
- Lombardo A, Scheffler IE. 1989. Isolation and characterization of a *Saccharomyces cerevisiae* mutant with a disrupted gene for the IP subunit of succinate dehydrogenase. *J Biol Chem* **264**: 18874–18877.
- Mathews DH, Sabina J, Zuker M, Turner DH. 1999. Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J Mol Biol* **288**: 911–940.
- Neigeborn L, Carlson M. 1987. Mutations causing constitutive invertase synthesis in yeast: genetic interactions with snf mutations. *Genetics* **115**: 247–253.
- Oliveira CC, McCarthy JEG. 1995. The relationship between eukaryotic translation and mRNA stability. A short upstream open reading frame strongly inhibits translational initiation and greatly accelerates mRNA degradation in the yeast *Saccharomyces cerevisiae*. *J Biol Chem* **270**: 8936–8943.
- Oliveira CC, Van den Heuvel JJ, McCarthy JEG. 1993. Inhibition of translational initiation in *Saccharomyces cerevisiae* by secondary structure: roles of the stability and position of stem loops in the mRNA leader. *Mol Microbiol* **9**: 521–532.
- Ozcan S, Vallier LG, Flick JS, Carlson M, Johnston M. 1997. Expression of the SUC2 gene of *Saccharomyces cerevisiae* is induced by low levels of glucose. *Yeast* **13**: 127–137.
- Prieto S, de la Cruz BJ, Scheffler IE. 2000. Glucose-regulated turnover of mRNA and the influence of poly(A) tail length on half-life. *J Biol Chem* **275**: 14155–14166.
- Ross J. 1996. Control of messenger RNA stability in higher eukaryotes. *Trends Genet* **12**: 171–175.
- Sagliocco FA, Vega Laso MR, Zhu D, Tuite MF, McCarthy JEG, Brown AJP. 1993. The influence of 5' secondary structures upon ribosome binding to mRNA during translation in yeast. *J Biol Chem* **268**: 26522–26530.
- Sarokin L, Carlson M. 1985. Upstream region of the SUC2 gene confers regulated expression to a heterologous gene in *Saccharomyces cerevisiae*. *Mol Cell Biol* **5**: 2521–2526.
- Scheffler IE, de la Cruz BJ, Prieto S. 1998. Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*. *Int J Biochem Cell Biol* **30**: 1175–1193.
- Schwartz DC, Parker R. 1999. Mutations in translation initiation

- factors lead to increased rates of deadenylation and decapping of mRNAs in *Saccharomyces cerevisiae*. *Mol Cell Biol* **19**: 5247–5256.
- Schwartz DC, Parker R. 2000. mRNA decapping in yeast requires dissociation of the cap binding protein, eukaryotic translation initiation factor 4E. *Mol Cell Biol* **21**: 7933–7942.
- Sikorski RS, Hieter P. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19–27.
- Tamai KT, Liu X, Silar P, Sosinowski T, Thiele DJ. 1994. Heat shock transcription factor activates yeast metallothionein gene expression in response to heat and glucose starvation via distinct signalling pathways. *Mol Cell Biol* **14**: 8155–8165.
- Theil EC. 1990a. Ferritin mRNA translation, structure, and gene transcription during development of animals and plants. *Enzyme* **44**: 68–82.
- Theil EC. 1990b. Regulation of ferritin and transferrin receptor mRNAs. *J Biol Chem* **265**: 4771–4774.
- Trumbly RJ. 1992. Glucose repression in the yeast *Saccharomyces cerevisiae* (MicroReview). *Mol Microbiol* **6**: 15–21.
- Tucker M, Parker R. 2000. Mechanisms and control of mRNA decapping in *Saccharomyces cerevisiae*. *Ann Rev Biochem* **69**: 571–595.
- Vega Laso MR, Zhu D, Sagliocco FA, Brown AJP, Tuite MF, McCarthy JEG. 1993. Inhibition of translational initiation in the yeast *Saccharomyces cerevisiae* as a function of the stability and position of hairpin structures in the mRNA leader. *J Biol Chem* **268**: 6453–6462.
- Vilela C, Velasco C, Ptushkina M, McCarthy JEG. 2000. The eukaryotic mRNA decapping protein Dcp1 interacts physically and functionally with the eIF4F translation initiation complex. *EMBO J* **19**: 4372–4382.
- Wyers F, Minet M, Dufour E, Vo LTA, Lacroute F. 2000. Deletion of the PAT1 gene affects translation initiation and suppresses a PAB1 gene deletion in yeast. *Mol Cell Biol* **20**: 3538–3549.
- Zuker M. 1989. On finding all suboptimal foldings of an RNA molecule. *Science* **244**: 48–52.