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Glucose-regulated Turnover of mRNA and the Influence of Poly(A) Tail Length on Half-life*

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Glucose repression in *Saccharomyces cerevisiae* can now be seen to operate at two levels: regulation of transcription of certain genes and control of the half-life of the corresponding mRNAs (Scheffler, I. E., de la Cruz, B. J., and Prieto, S. (1998) *Int. J. Biochem Cell Biol.* 30, 1175–1193). For example, the steady state levels of *SDH2* mRNA and *SUC2* mRNA are significantly determined by their differential rates of turnover. A current model for the mechanism of mRNA turnover includes three distinct steps: a rate-limiting deadenylation, removal of the 5'-7-methyl-G (decapping), and 5'-3' exonuclease digestion. We have investigated the same three reactions during glucose-induced degradation of these transcripts. Our results indicate that while decapping (by Dcp1p) and 5'-3' exonuclease digestion (by Xrn1p) are obligatory steps for the rapid degradation of these mRNAs, the dependence on deadenylation is more complicated. At steady state in glycerol these transcripts have very short poly(A) tails but are nevertheless very stable; the addition of glucose causes immediate decapping and degradation without further deadenylation; in contrast, newly made *SUC2* mRNA (after a shift from glucose to glycerol) has significantly longer poly(A) tails, and such transcripts are not rapidly degraded upon addition of glucose. A constitutive deadenylation reaction that is independent of the carbon source eventually makes the stability of these transcripts very sensitive to glucose. These results are interpreted in terms of a working hypothesis proposing a competition between translational initiation and decapping influenced by the carbon source. The presence of a long poly(A) tail may also affect this competition in favor of translational initiation and mRNA stabilization.

In the presence of abundant glucose yeast cells can satisfy most of their energy requirements from glycolysis/fermentation, and the expression of a large number of genes is repressed. The phenomenon is therefore often referred to as "glucose repression." Among the gene products repressed are those required for the uptake and metabolism of other sugars such as sucrose and galactose, as well as a large number of gene products required for respiration and oxidative phosphorylation in the mitochondria. Until recently, almost all of the at-

tention was focused on the control of gene expression at the transcriptional level (2–6).

Our laboratory has investigated how glucose regulates the expression of the *SDH2* gene encoding the iron-protein subunit of succinate:quinone oxidoreductase (complex II) of the mitochondrial electron transport chain in *Saccharomyces cerevisiae* (7–9). When a culture in YPG with de-repressed levels of *SDH2* mRNA is rapidly exposed to glucose by the addition of this fermentable substrate to the medium, *SDH2* mRNA levels drop precipitously with an estimated half-life of 5–7 min (8, 9). Experiments performed with a mutant with a temperature-sensitive RNA polymerase II (10) revealed that these differences in mRNA levels depending on the carbon source are due to differential stability of *SDH2* mRNA. Studies to identify what distinguishes mRNAs with a short half-life from mRNAs with a long half-life (*i.e.* what are the required *cis*-acting elements within the *SDH2* mRNA?) concluded that the 5'-UTR¹ was the dominant *cis*-acting determinant controlling the behavior of this mRNA in different media (9). Exhaustive investigations have not yet been performed, but the same kind of behavior is clearly observed with the functionally related *SDH1* transcript, encoding the flavoprotein subunit (Fp) of mitochondrial complex II, and with the *SUC2* mRNA, encoding invertase, and previously believed to be controlled exclusively at the level of transcription (11). The phenomenon of glucose repression, therefore, appears to involve at least two independent mechanisms, one acting at the level of transcription, and another controlling mRNA stability.

Considerable progress has been made in recent years in understanding the mechanism of mRNA turnover in yeast, especially with reference to the subset of constitutively short-lived mRNAs. The subject has been extensively reviewed (12–16). A favored current model has the following three steps: 1) shortening of the poly(A) tail, 2) decapping, and 3) 5'-3' exonucleolytic degradation. Poly(A) tail shortening is thought to be a key rate-limiting and obligate step in the decay of constitutively short-lived mRNAs. Factors including Pab1p, the poly(A)-binding protein, and a nuclease associated with Pab1p as well as *cis*-elements within the mRNA (primarily at the 3' end) are thought to regulate the rate of deadenylation. However, Pab1p also interacts with the 5' end of the mRNA via translation initiation factor 4G (eIF4G), giving Pab1p a potential role in regulating translation efficiency (17).

After the tail is sufficiently shortened, removal of the cap by the decapping enzyme follows. However, deadenylation can also be uncoupled from decapping (18). Finally, the rapid 5'-3' exonucleolytic degradation is due primarily to the constitutive Xrn1 nuclease, accounting for a large fraction (~30%) of such an activity in yeast.

The 5'-exonuclease encoded by the *XRN1* gene was found to

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¹ The abbreviations used are: UTR, untranslated region(s); nt, nucleotides(s); eIF, eukaryotic initiation factor.

TABLE I
Strains used in experiments

Strain	Genotype	Origin	Ref
DL1	<i>MATa leu 2-3 ura3 his3</i>	M. Yaffe	Van Loon <i>et al.</i> 1983 (58)
Y260	<i>MATa ura3-52 rpb1-1</i>	T. Young	Nonet <i>et al.</i> (10)
YNN27	<i>MATa ura3 trp1</i>	A. Stevens	Larimer <i>et al.</i> (31)
YNN27 xrn	<i>MATa ura3 trp1 xrn1::URA3</i>	A. Stevens	Larimer and <i>et al.</i> (31)
YRP840	<i>MATa his4-534 leu2-3, 112 trp-1 ura3-52 cup1::LEU/PGK1pG/MFA2pG</i>	R. Parker	Beelman <i>et al.</i> (28)
YRP1069	<i>MATa his4-539 leu2-3, 112 trp-1 ura3-52 cup1::LEU/PGK1pG/MFA2pG dcp1::URA3</i>	R. Parker	Beelman <i>et al.</i> (28)

be required for the glucose-induced degradation of the *SDH2* and *SDH1* mRNAs (9). The required role of Dcp1p is documented in the present studies, and it seems clear that the 5' cap protects these mRNAs from the exonuclease. It was also unresolved whether the glucose signal was targeted at the potentially rate-limiting deadenylation reaction. In other words, does glucose trigger a rapid shortening of the poly(A) tails, resulting in rapid degradation?

To address these questions the present study examined the length and size distribution of the poly(A) tails on two different mRNAs, *SDH2* and *SUC2* mRNA, during derepression, at high steady state levels during growth in YPG, and immediately after the addition of glucose. The glucose signal did not induce any further changes that could have accounted for the rapid initiation of turnover. Our working hypothesis therefore proposes that the glucose signal is directed at events at the 5' ends of the specific mRNAs.

EXPERIMENTAL PROCEDURES

Yeast Strains and Vectors—The strains used in this work were generously provided by other investigators and have been previously described (Table I). Yeast cells were grown on YPD (1% Bacto Yeast Extract, 2% Bacto Peptone, 2% dextrose), or YPG (1% Bacto Yeast Extract, 2% Bacto Peptone, 3% glycerol) (19).

Northern Analysis—Total RNA was extracted from yeast as described (20), followed by two additional 2 M lithium chloride precipitations (21) to increase the purity of the RNA. The total RNA concentration of each sample were determined spectrophotometrically. Northern analyses were carried out by standard protocols (20). Filters were UV cross-linked for 75 s in a Fotodyne DNA transfer lamp. Prehybridization and hybridization solutions consisted of 50% formamide, 5 × Denhardt's solution, 0.5% SDS, and 200 µg/ml denatured salmon sperm DNA, for random primer hybridization. For oligonucleotide hybridizations 15% formamide was used instead of 50%.

The signal on Northern blots was quantitated with a Bio-Rad (Hercules, CA) Molecular Imager System GS-525. The amount of specific mRNA in each lane was normalized using 25 S rRNA as a constitutive control. In order to obtain an rRNA signal that is precisely proportional to total RNA loading, different hybridization conditions (time of incubation with the probe, amounts of probe) were optimized for the oligomeric 25 S rRNA probe. The probe was an oligonucleotide of the following sequence: 5'-ATCCGCTAAGGAGTGTGTAACAACTCACC-3' (22) end-labeled with [γ -³²P]ATP by polynucleotide kinase. A 1-h incubation with 100,000 cpm of rRNA radioactive probe for each blot was chosen as the condition yielding a linear response and a signal comparable to the signal from a *SDH2* random primed probe.

Analysis of Poly(A) Tail Length—To measure the length of the poly(A) tails of the *SDH2* mRNA and *SUC2* mRNAs, these mRNAs were first cleaved by RNase H in the presence of oligonucleotides complementary to a selected region of the 3'-UTR of the respective mRNA (as described in the figure legends). Ten micrograms of total RNA were hybridized with 300 ng of mRNA-specific oligonucleotide (OL1, INVER-1) in the presence or absence of 2 µg of oligo(dT) (Life Technologies, Inc.) and incubated with 0.025 units/µl of RNase H in 50 mM Tris-HCl (pH 7.9), 10 mM MgCl₂, 100 mM NaCl, 1 mM dithiothreitol, and 0.27 units/µl RNasin for 20 min at 37 °C (23). RNA fragments were resolved on 12% polyacrylamide, 7.7 M urea gels, transferred by an

electroblotter to a Hybond-N membrane (Amersham Pharmacia Biotech) and hybridized with specific ³²P-labeled oligonucleotides (OL2, INVER-2). Size standards in flanking lanes were visualized by ethidium bromide staining and used to estimate the length of the undigested transcripts, and of the single and double-digested 3' fragments. Details for the quantitative analysis are given in the figure legends.

Probes—The probe for the 5' region of the *SDH2* transcript was derived from a 568-base pair *PstI-XbaI* fragment of the coding sequence (7) and labeled by random priming. The oligonucleotides used for probing the 3' fragments are described in Table II and were end-labeled with [γ -³²P]ATP using the T4 polynucleotide kinase reaction as described (20).

Standard Assay of mRNA Levels—Yeast cells were grown overnight in YPD to an A₆₀₀ of approximately 0.5. Aliquots of the glucose-grown cells (20 ml) were placed into 50-ml tubes and centrifuged for 4 min at 2000 rpm. The supernatant was removed, and the cell pellets were resuspended in YPG, or again in YPD for some controls, and grown with vigorous shaking at 30 °C. After some time (see figures), one of the YPG tubes, as well as the one with YPD, were processed. One ml of 50% glucose was added to the remaining tubes containing YPG (to obtain 2.5% glucose, YPG + D), and these cultures were harvested after the desired incubation times and immediately processed for RNA extraction.

Polyribosome Analysis—Polyribosomes were prepared and fractionated basically as described by Mangus and Jacobson (24). Extracts (DL1 strain) were obtained from 400-ml cultures (A₆₀₀ = 0.5–0.8) after growth under three different conditions: (i) glucose-repression conditions in YPD; (ii) 30 min after derepression in YPG; (iii) 3 h after derepression in YPG. Cultures were centrifuged and quickly resuspended in 10 ml of lysis buffer. Lysis buffer contained: 10 mM Tris-HCl (pH 7.4), 100 mM NaCl, 30 mM MgCl₂, 50 µg/ml cycloheximide, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0.5 µg/ml leupeptin, and 0.4 µg/ml pepstatin. After a brief centrifugation (until the rotor has reached 3,000 × g), the supernatant was discarded. The cell suspension was transferred to a new tube and the cells were suspended in 1 ml of lysis buffer. The suspension was divided into two Eppendorf tubes containing 400 µl of glass beads and "retched" for 3 times for 90 on a Glens Mills Retch bead-beater, cooling down in ice for 2 min between agitations. After lysis, the mixture of beads and broken cells was centrifuged at 12,000 × g for 10 min. The supernatant was transferred to a new Eppendorf tube and centrifuged in a microcentrifuge at top speed for 10 min. The supernatant was removed and gently laid on top of an 11-ml 7–47% (w/w) sucrose gradient. The gradient buffer contained: 50 mM Tris-HCl (pH 7.0), 50 mM NH₄Cl, 12 mM MgCl₂, 1 mM dithiothreitol and appropriate amount of sucrose. The gradients were centrifuged in a Beckman SW41 rotor at 35,000 rpm for 2 h and 45 min and then pumped through an UV monitor (A₂₅₄ filter) with a peristaltic pump. The profile of the gradients was monitored on a chart recorder and 0.6-ml samples were collected using a fraction collector. The samples were frozen at -70 °C until used for RNA extraction.

Total RNA was isolated from each fraction by addition of SDS and Tris-HCl (pH 7.5) to 1.2% and 120 mM, respectively, and then by three phenol/chloroform extractions followed by one chloroform extraction. The samples were then ethanol precipitated (in the presence of 0.2 M sodium acetate) and resuspended into 20 µl of diethyl pyrocarbonate-treated water with RNasin. Electrophoresis of RNA, blotting, and hybridization with oligonucleotides were performed as described above.

Miscellaneous Methods and Reagents—[α -³²P]dCTP and [γ -³²P]dATP

TABLE II
Oligonucleotides used for RNase H digestion and for probing at the 3'-UTR region of the mRNAs

Name	Sequence	Application
ACT1-REV	5'-CGATAGATGGGAAGACAGCACGAGGAGCG-3'	Hybridization with actin mRNA on polysome gradients
OL1	5'-AGTCATAGAGAGTAAACATA-3'	<i>SDH2</i> 3'-UTR RNase H digestion
OL2	5'-GAACACCTTGTGCGCTATG-3'	<i>SDH2</i> 3'-UTR hybridization after RNase H digestion with OL1
INVER-1	5'-CATTTCATATGGTTTGGAGG-3'	SUC2 3'-UTR RNase H digestion
INVER-2	5'-GGTAACTGGGGTCTGGGAGAA-3'	SUC2 3'-UTR hybridization after digestion with INVER-1

were purchased from ICN (Cleveland, OH). All other chemicals used were of the highest grade available. Restriction enzymes were purchased from New England Biolabs and used according to the manufacturer's specifications. Bacterial transformations were performed using the heat shock protocol (25). Yeast transformations were performed by the lithium acetate method (26). Invertase was assayed following a protocol from the laboratory of Dr. S. Emr (University of California, San Diego) adapted from Goldstein and Lampen (27).

RESULTS

The 5'-3' Exonuclease (Xrn1p), as Well as the Decapping Enzyme (Dcp1p), Are Involved in the Degradation of the SDH2 mRNA—It has previously been described how the glucose-induced turnover of *SDH2* mRNA in yeast is slowed down in an *xrn1* mutant strain ($t_{1/2} > 20$ min versus $t_{1/2} = 5$ min for the wild type) after glucose addition to a glycerol-grown culture (9). Others have described that in the absence of the decapping function (*Dcp1p*) the degradation of several yeast mRNAs, including transcripts with rapid, intermediate, and slow decay rates, is slowed down considerably (28, 29).

When the gene (*DCP1*) for the decapping enzyme was identified, we examined the kinetics of glucose-induced turnover of *SDH2* mRNA in a knock-out compared with its wild type (Fig. 1B). The *xrn1* strain and its wild type parent (Fig. 1A) were also included in this quantitative analysis. The half-life of the message was calculated (Fig. 1, bottom plots) using an improved method for quantitation (see "Experimental Procedures") where the signal is normalized to the amount of 25 S rRNA loaded per lane. The rate of *SDH2* mRNA turnover in both mutants is strongly affected, especially in the *dcp1* strain, where the *SDH2* mRNA levels persist for more than 15 min after addition of glucose to the YPG medium. Although it is the major 5'-3' exonuclease, *Xrn1p* accounts only for approximately 30–40% of the total RNase activity of crude yeast extracts (30, 31); therefore it is not surprising that we can still observe some degradation of the *SDH2* mRNA in the *xrn1* mutant.

These results prove that the degradation of the *SDH2* mRNA induced by glucose shares at least the last two steps of a general mechanism of mRNA turnover in yeast. It seems quite plausible that the 7-methyl-G cap at the 5' of the transcript protects the mRNA from the exonuclease in the absence of glucose.

The Poly(A) Tail of SDH2 mRNA during Glucose-induced Degradation of the Message—The first step, frequently considered as a rate-limiting step of the mRNA degradation process, is the shortening of the poly(A) tail (32, 33). However, at least one exception has been described: nonsense-mediated mRNA decay also involves decapping and the 5'-exonuclease, but it is independent of deadenylation (16, 34–39). Morissey *et al.* (18) also described how decapping and 5'-3' degradation can be uncoupled from deadenylation in yeast *pab1* mutants (18). Glucose-regulated mRNA stability constitutes a novel phenomenon in mRNA turnover (9, 11) in which deadenylation can be explored.

First, the poly(A) tail distribution of *SDH2* mRNA from a wild type strain, DL1, was studied (Fig. 2). Poly(A) tail length was measured using RNA from de-repressed cells in YPG, and following the addition of glucose to these cultures. To enhance

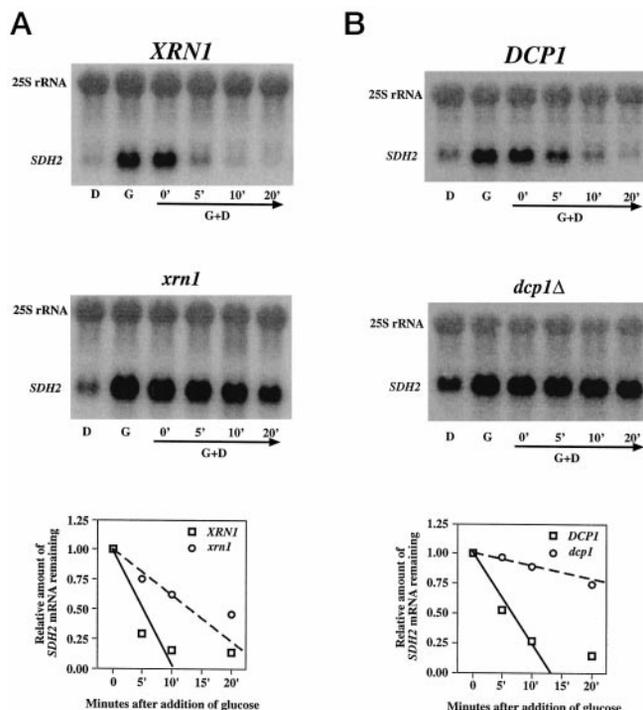


FIG. 1. Effect of 5'-exonuclease and decapping mutants on *SDH2* mRNA decay. A, Northern blots from *XRN1* and *xrn1* yeast strains. Blots were probed with a 5' *SDH2* fragment and with a 25 S rRNA oligo probe ("Experimental Procedures"). The upper bands represent the 25 S rRNA signal used for normalization (RNA loading). The lower bands represent the *SDH2* mRNA. Lanes D, steady-state YPD cultures; lanes G, YPG-de-repressed cultures; lanes G + D, YPG-de-repressed cultures harvested at 0, 5, 10, and 20 min after addition of glucose. The plot at the bottom shows the decay kinetics of *SDH2* mRNA after addition of glucose. Lines represent a linear best fit to the 0, 5-, and 10-min time points. *SDH2* mRNA levels in each lane were normalized to 25 S rRNA, and the relative amount at the time of glucose addition was set to 1.0. B, Northern blots from *DCP1* and *dcp1* yeast strains and decay kinetics of *SDH2* mRNA after addition of glucose.

the resolution, the body of the transcript was shortened by cleavage with RNase H at a downstream site defined by an oligonucleotide complementary to sequences near its 3' end. The distribution of the lengths of the poly(A) tails at each time point, before and after glucose addition to the YPG culture, was obtained from a comparison of the 3' fragments obtained after RNase H digestion with a single oligonucleotide, and a sample where the poly(A) tail had also been removed in the presence of oligo(dT). The details are described under "Experimental Procedures" and in the figure legends.

The results were the following. 1) The uncut RNA and the digested samples migrated to the expected positions (uncut mRNA, 1.2 kilobases, and the double-digested fragment, 170 bases, results not shown), in agreement with the position of the poly(A) site determined independently (9). 2) There is always a difference between the samples subjected to single (3' specific oligonucleotide) and double digestion (3' specific oligo + oligo(dT)), proof that the achieved resolution is sufficient to distinguish between RNA fragments containing even a relatively

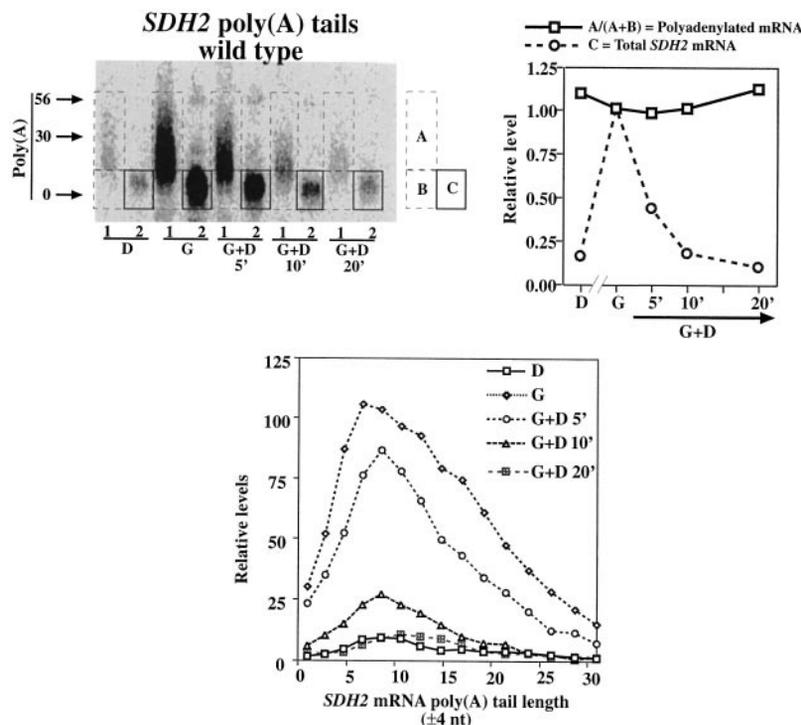


FIG. 2. ***SDH2* mRNA poly(A) tail length in wild type strain.** RNA was prepared from cultures grown in YPD, (*D* time points), de-repressed in YPG for 3 h (*G* time points), or with glucose added to YPG cultures (*G+D* time points). Digested RNA samples were resolved on 12% acrylamide, 7.7 M urea gel either before or after removal of the poly(A) tail, transferred onto a membrane, and probed with OL2. The *left lanes* of each pair (1) represent RNA samples digested in the presence of OL1; the *right lanes* (2) represent samples digested with both OL1 and oligo(dT). Size estimates were made by comparison with markers and a calibration of the gel mobilities. Signal quantitation was performed using boxes A, B, and C (see scheme on the *right* of the blot). Counts within C boxes (○), corresponding to deadenylated fragments, were used to calculate the total relative amounts of *SDH2* mRNA present (normalized to 25 S rRNA) at each time point (D, G, or G+D). Counts in A boxes represent *SDH2* mRNA with poly(A) tails, while counts in B boxes represent *SDH2* mRNA with very short or no poly(A) tails. The ratio $A/(A+B)$ (□) is a measure of the fraction of polyadenylated molecules. Counts in box C as well as the $A/(A+B)$ ratio were set to 1.0 for the sample extracted from the YPG culture (G). *Bottom*, distribution of poly(A) tail lengths in *SDH2* mRNA molecules during the glucose-induced decay. The region with the signal in lanes 1 was subdivided into 15 boxes, and the signal in each box was measured and plotted against the estimated poly(A) tail length.

short poly(A) tail, and those without one. The signal in the C boxes corresponding to double-digested, deadenylated 3' fragments (Fig. 2) reflects the derepression of *SDH2* mRNA from YPD to YPG, and the turnover after addition of glucose to the YPG culture (*dotted line* in the inset graph), as expected.

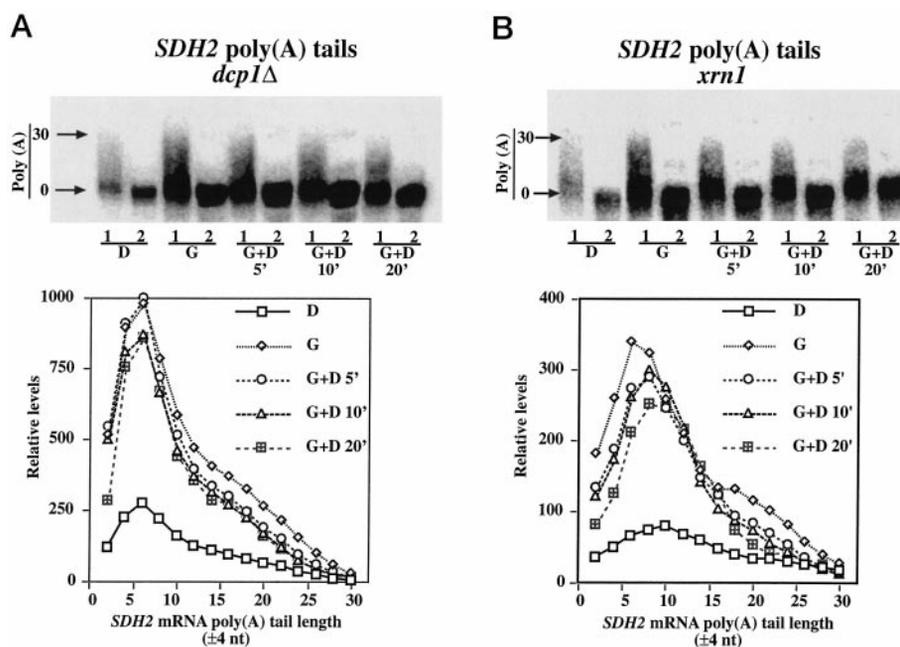
From a visual inspection of the blot, the signal corresponding to *SDH2* poly(A) tails also appears to diminish with time after glucose addition to the YPG culture, but a plot of the $A/(A+B)$ ratios (*solid lane* in the *inset graph*) suggests that there is no significant change with time for *SDH2* mRNAs extracted from YPG or from YPG + D cultures. This ratio estimates the fraction of molecules with a poly(A) tail. Most of the mRNAs have poly(A) tails less than 30 nt long. A more detailed quantitative analysis of the data is shown at the bottom of Fig. 2. By subdividing the entire range (A + B) into smaller boxes and quantitating their content, a complete distribution of poly(A) tail lengths can be obtained. The distributions at various times after addition of glucose exhibit a change in peak height, reflecting the decay, but no significant change in the position of the peak or the overall shape of the curve, reflecting length distributions. The tail is already quite short at steady state in YPG. Therefore, a quantitative analysis of the signal distribution on the gel reveals that there is global *SDH2* mRNA turnover but no glucose-induced deadenylation. A repeat experiment with wild type cells focused on a higher time resolution of the behavior during the first 10 min (results not shown). Again, the distribution of poly(A) tail lengths did not change appreciably in this time frame, in contrast to what would be expected if glucose-induced deadenylation was obligatory to allow the rapid degradation.

The analysis of poly(A) tail lengths in the wild type strain is complicated by the rapid turnover of the *SDH2* mRNA. The use of the *dcp1* and *xrn1* mutants permitted the study of the *SDH2* poly(A) tail in a time frame when most of the *SDH2* mRNA population would be degraded in a wild type strain.

The experiment performed with the *dcp1* mutant is shown in Fig. 3A. After 10 min, more than 50% of the *SDH2* mRNA present in glycerol would have been degraded in the wild type strain (see Fig. 1), and an obligatory, rate-limiting deadenylation triggered by glucose would have been expected to be detectable at that time. The distribution of the signal in the samples from a single digestion reveals a high proportion of the fragments concentrated at a position not much different from that of the double-digested samples. This observation suggests that most of the *SDH2* mRNA population appears to have extremely short poly(A) tails in YPG medium even prior to the addition of glucose. This observation is not entirely unexpected. Because in the *dcp1* mutant RNA turnover is slow, the average age of all RNA molecules is greater than in wild type cells under similar conditions, and a constitutive poly(A) shortening is expected to occur (see "Discussion"). Nevertheless, in YPG even molecules with very short or apparently no tails are completely stable. The signals corresponding to double digested samples show how the mRNA is de-repressed in glycerol (due to transcriptional activation) but is relatively stable following glucose addition due to the *dcp1* mutation. Due to the slow turnover there is even a stronger signal in glucose compared with the wild type.

A quantitative analysis similar to that performed for the wild type cells (Fig. 2, *bottom*) shows the distributions of tail lengths

FIG. 3. Poly(A) tail length in *dcp1* and *xrn1* strains during glucose-induced *SDH2* mRNA turnover. RNA was prepared from cultures grown in YPD (*D* time points), de-repressed on YPG for 3 h (*G* time points), or after glucose addition to YPG-de-repressed cultures (*G+D* time points). RNase H digestion, gel fractionation, and probing of blots were performed as described in the legend to Fig. 2. *A*, experiment with the *dcp1* strain. *Top panel*, the *left lanes* of each pair (1) represent RNA samples digested in the presence of OL1; the *right lanes* (2) represent samples digested with both OL1 and oligo(dT). *Bottom panel*, the region with the signal in *lanes 1* was subdivided into 15 boxes, and the signal in each box was measured and plotted against the estimated poly(A) tail length. Size estimates are derived from RNA standards ("Experimental Procedures"). *B*, same experiment with the *xrn1* strain.



at various times before and after the addition of glucose (Fig. 3*A*, *bottom*). Peak heights and peak positions do not change appreciably with time. These data suggest strongly that glucose does not promote deadenylation, or at least not rapidly enough to be rate-limiting in the turnover of this mRNA. Deadenylation does not appear to be part of the signaling mechanism in glucose-induced degradation for this transcript.

The same kind of experiment was performed with the *xrn1* mutant (Fig. 3*B*). As before, the *SDH2* mRNA is de-repressed in glycerol, but is more stable than in the wild type strain after glucose addition due to the *xrn1* mutation. Again a large proportion of the *SDH2* mRNA population appears to be already deadenylated under all conditions due to the fact that the average age of these mRNAs is greater in these mutants compared with wild type cells, and therefore the relatively short poly(A) tails could be the result of a slow constitutive deadenylation. 10–20 min after glucose addition, when most of the *SDH2* message would have been degraded in the wild type strain, the poly(A) tail length distributions are not changed significantly.

The Poly(A) Tail of Newly Made and Steady State *SDH2* mRNA—Average lengths of poly(A) tails in newly made yeast mRNAs have been reported to be in the range of 40–93 nt (32, 33, 40). The *SDH2* mRNA at steady state in YPG (Fig. 2) shows a disperse poly(A) length distribution where the average number of adenine nucleotides appears quite small, but nevertheless the transcript is very stable under these conditions (9). In an effort to elucidate if the newly synthesized transcripts from this gene contain a longer poly(A) tail (at least in the range of those previously described for *S. cerevisiae*), we studied the poly(A) tail length during the de-repression in YPG media. Samples were taken at several time points after a transfer from YPD to YPG. In Fig. 4, the levels of *SDH2* transcript are seen increasing with time in glycerol media (see plot on the right for quantitation) as expected. The distribution of poly(A) tail lengths in YPD and at various times in YPG is shown in Fig. 4*B*. The position of the peak and the shape of the curve do not change appreciably, indicating that molecules with very short poly(A) tails arise at the earliest times. This is in distinct contrast to the observations with the *SUC2* transcript (see below).

The relative stability of the poly(A) tails of *SDH2* mRNAs at steady state in YPG was investigated in a temperature-sensi-

tive RNA pol II mutant (10). After an arrest of transcription at the non-permissive temperature the *SDH2* mRNA level remains relatively constant for up to 120 min. This result was obtained previously (8), and re-confirmed here (Fig. 5*A*). When the distributions of poly(A) tail lengths of such populations of *SDH2* mRNA were examined over this period, we observed no appreciable change (Fig. 5*B*). At steady state in YPG (G_{3h}), and at the time of arrest of transcription by a temperature shift, the population included many molecules with relatively short tails (10–12 nt), and others with long tails similar to those made shortly after de-repression. Over the next 120 min there was neither significant turnover of either population, nor a change in the position of the peak. No further trimming of the poly(A) tails of *SDH2* mRNAs in YPG is observed.

Other mRNAs Subject to Glucose-induced Turnover—The *SUC2* gene is another example of a glucose-repressed gene. Initially it was considered to be influenced solely at the level of transcription (see Ref. 4 for a review), but it has been shown that differential stability on different carbon sources also plays a role in determining the steady-state level of the *SUC2* mRNA (11). The poly(A) tail of this message was therefore analyzed to see if the glucose-induced *SUC2* mRNA turnover was independent of deadenylation. The results of this study (Fig. 6*A*) reveal the dramatic de-repression of *SUC2* mRNA from YPD to YPG, and the rapid turnover of this message upon glucose addition to YPG-grown yeast cells at steady state (*dotted line* in the *lower graph*). As in Fig. 2, the global *SUC2* mRNA turnover makes the interpretation of the blot less apparent. The signal corresponding to the *SUC2* mRNA fragments with poly(A) tails becomes smaller and smaller with time after glucose addition to a YPG culture. However, this is not due to poly(A) shortening, because no significant or rapid change in the proportion of polyadenylated molecules in the whole population is detectable (the $A/(A+B)$ ratio (*solid line* in the *lower graph*)). It remains constant during the entire interval when most of the mRNA degradation occurs and increases slightly at the end. As in the case for *SDH2* mRNA, there is no obligatory deadenylation preceding *SUC2* mRNA degradation induced by glucose.

Studies on *SUC2* mRNA poly(A) tails were also performed using an *xrn1* strain. In this mutant the message was detectable even in steady-state glucose cultures (Fig. 6*B*, *lane D₁*), in contrast to the wild type strain, where no transcript is observed in YPD-grown cells (Fig. 6*A*, *lane D₁*). Minimal decay is de-

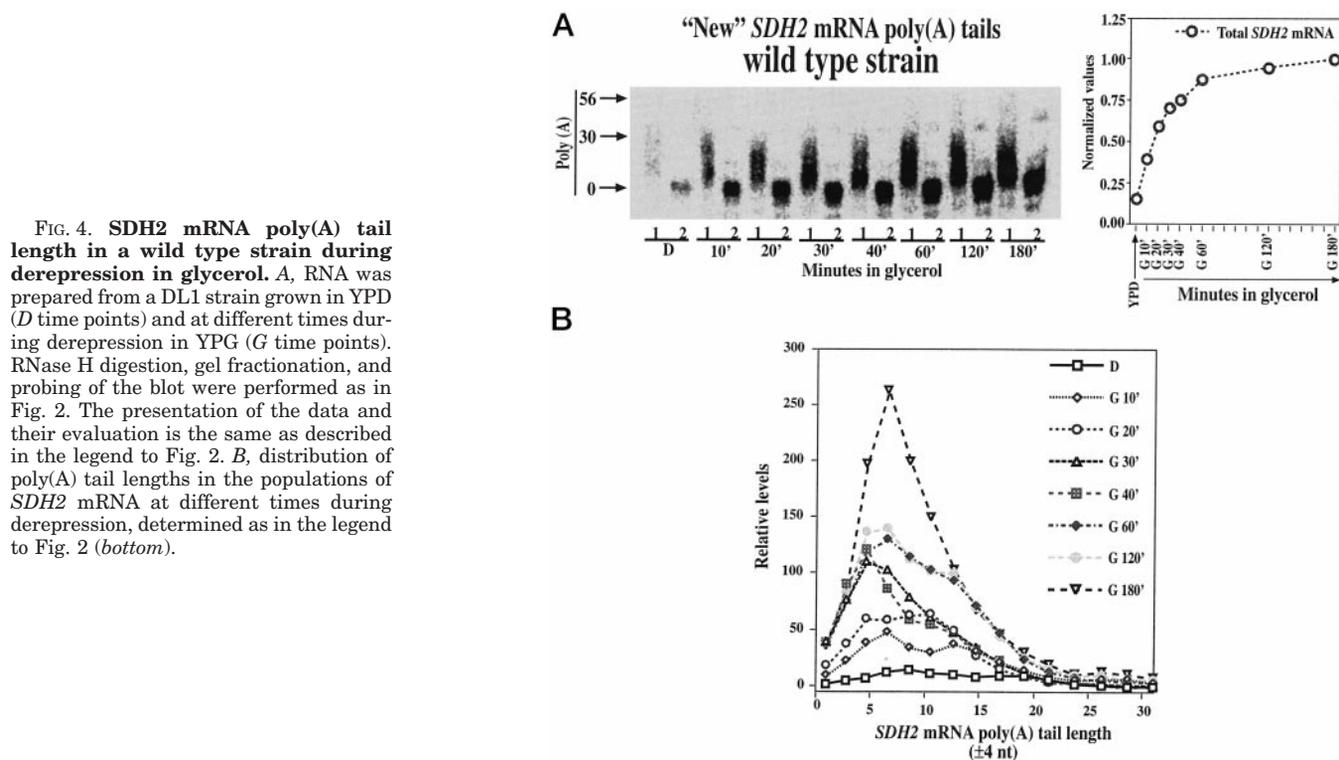


FIG. 4. *SDH2* mRNA poly(A) tail length in a wild type strain during derepression in glycerol. **A**, RNA was prepared from a DL1 strain grown in YPD (*D* time points) and at different times during derepression in YPG (*G* time points). RNase H digestion, gel fractionation, and probing of the blot were performed as in Fig. 2. The presentation of the data and their evaluation is the same as described in the legend to Fig. 2. **B**, distribution of poly(A) tail lengths in the populations of *SDH2* mRNA at different times during derepression, determined as in the legend to Fig. 2 (bottom).

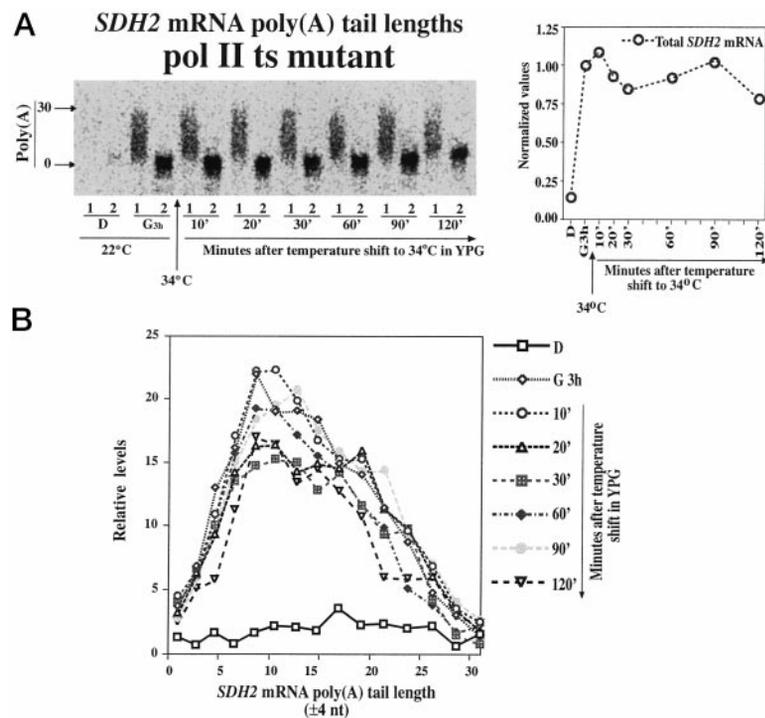


FIG. 5. **Stability of the poly(A) tail in YPG.** **A**, a culture of the strain Y260 with a temperature-sensitive RNA polymerase was transferred from YPD (*D*) to YPG (*G*) for 3 h at the permissive temperature, and the temperature was then raised to the non-permissive temperature. Aliquots were analyzed as described before. There is no appreciable decay of the *SDH2* mRNA in glycerol over 2 h. **B**, distributions of poly(A) tail lengths of samples after various times at the non-permissive temperature, determined as described in the legend to Fig. 2. The signal in YPD is too small to yield a meaningful distribution.

tected after glucose addition, confirming that the Xrn1 nuclease is also involved in glucose-induced degradation of the *SUC2* mRNA. As in the experiment performed with *SDH2* mRNA (Fig. 3B), no difference was observed in the signal corresponding to poly(A) tails of mRNAs extracted from glycerol culture, compared with those after glucose addition. During the same time interval all the *SUC2* transcripts would have been degraded in the wild type strain. The constant A/(A+B) ratio reveals no alterations in the fraction of polyadenylated molecules. Therefore, no further deadenylation is required for glucose-induced *SUC2* mRNA degradation.

The Poly(A) Tail in Newly Made and Steady State SUC2

mRNA—Other groups examining mRNA degradation have used a distinct approach placing the gene encoding the transcript of interest under the control of an inducible promoter, for example, the *GALI* upstream activating sequence (32). The same combination of promoter and carbon sources was not suitable for the study of glucose-induced mRNA turnover. However, as pointed out above, no *SUC2* mRNA is detectable in a wild type strain grown in YPD, and the de-repression in YPG is rapid and dramatic (11). Thus, total RNA was extracted at various times after a change from YPD to YPG, and an analysis of poly(A) tail lengths was performed as in previous experiments (Fig. 7). 45 min after the switch from YPD to YPG

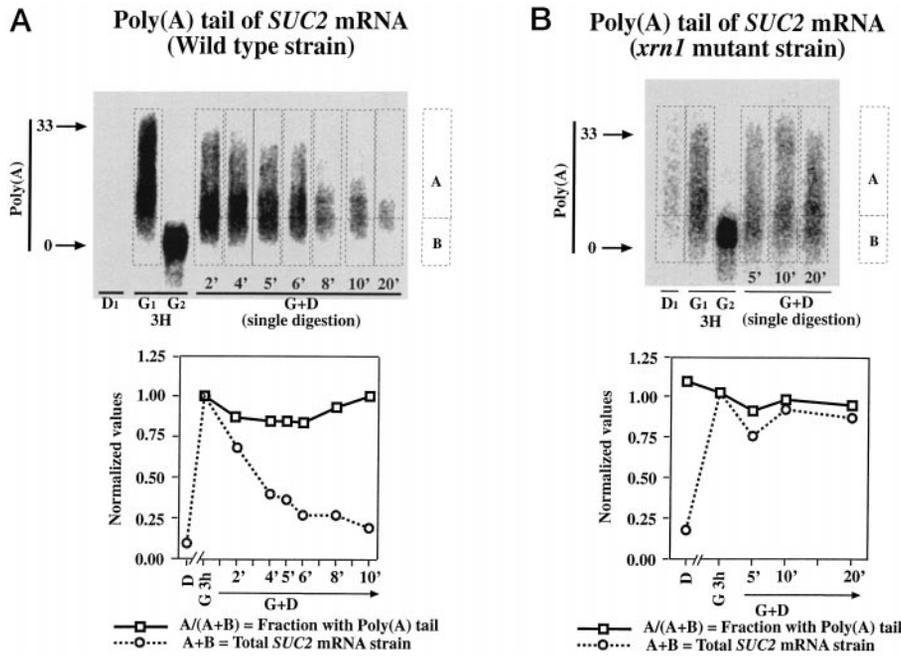


FIG. 6. *SUC2* mRNA poly(A) tail length during glucose-induced mRNA turnover. *A*, wild type strain. RNA was prepared from cultures grown in YPD (*D* time points), de-repressed in YPG for 3 h (*G* time points), and with glucose added to YPG (*G+D* time points). Total RNA from each sample was used to perform RNase H digestion in the same manner as for the *SDH2* mRNA. The oligonucleotide used to digest in the 3'-UTR of the *SUC2* mRNA (INVER-1) generates a fragment of ~120 nucleotides + the length of the poly(A) tail (*D1*, *G1*, and *G+D* single digestion, even though *D1* shows no signal). Only the sample extracted from glycerol culture was used in this case to show the presence and length of the poly(A) tail. *G1* and *G2* represent the difference between single digestion, with INVER-1, and double digestion, with INVER-1 + oligo(dT), respectively. Fractionation by gel electrophoresis, blotting, and probing were performed as above, except that an oligo probe specific for the remaining *SUC2* 3' fragment (INVER-2) was used. Quantitative analysis was carried out using schemes similar to those described in earlier figures. Due to the absence of double-digested samples for the different time points, counts in *A+B* were used in this case to calculate the total relative amount of *SUC2* mRNA at each time point. Counts in *A+B* as well as in *A/(A+B)* were set to 1.0 for the sample extracted from the YPG culture (*G1*). *B*, the same experiment with the *xrn1* strain. Counts in *box A+B* and the ratio *A/(A+B)* were set to 1.0 for the sample extracted from the YPG culture (*G*).

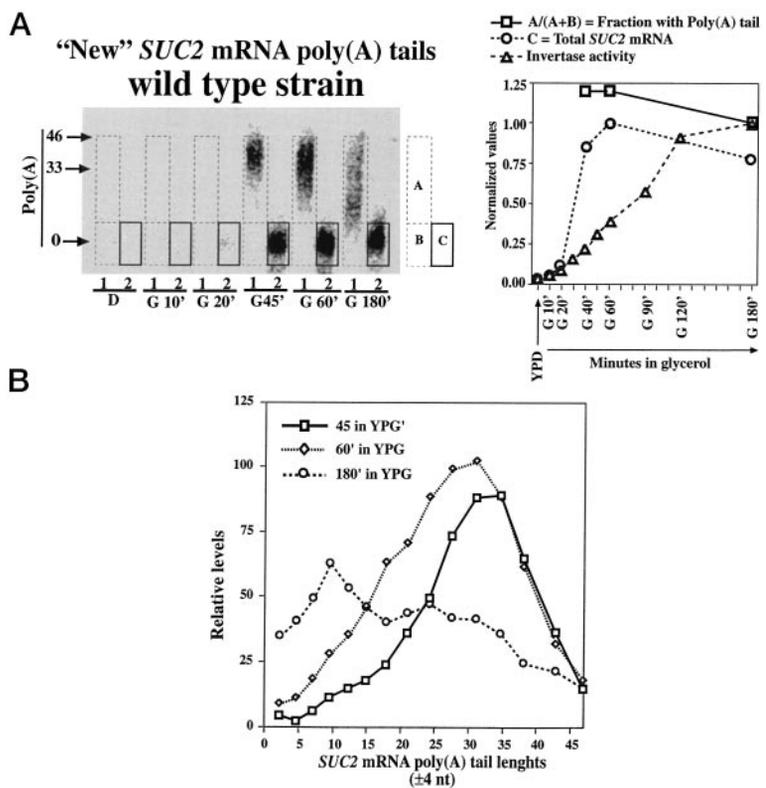


FIG. 7. *SUC2* mRNA poly(A) tail length in a wild type strain during derepression in glycerol. *A*, RNA was prepared from cultures grown in YPD (*D* time points), and at different times during derepression in YPG (*G* time points). RNase H digestion, gel fractionation, probing of the blot, and quantitative analysis were performed as described in the legend to Fig. 2, but using *SUC2*-specific oligos for RNaseH digestion and probing. \square , fragments from the double digestion (*box C*) representing the total relative amounts of *SUC2* mRNA present (normalized to 25 S rRNA and to the sample extracted after 1 h in glycerol (*G 60'*)); \square , a plot of the ratio *A/A+B* representing the fraction of polyadenylated molecules; it was set to 1.0 for the sample extracted after 3 h in glycerol (*G 180'*). The figure also shows the appearance of total invertase activity in cells collected at the indicated times (Δ). *B*, distribution of poly(A) tail lengths in populations of *SUC2* mRNA at various times after derepression, determined as described in the legend to Fig. 2.

polyadenylated fragments were detectable with a tail of ~35–50 adenine nucleotides (Fig. 7A). In this case, the fragment population was concentrated in a narrow band, suggest-

ing the presence of a relatively homogeneous population of molecules with very similar tail lengths. However, when this culture was maintained in YPG for longer periods, the *SUC2*

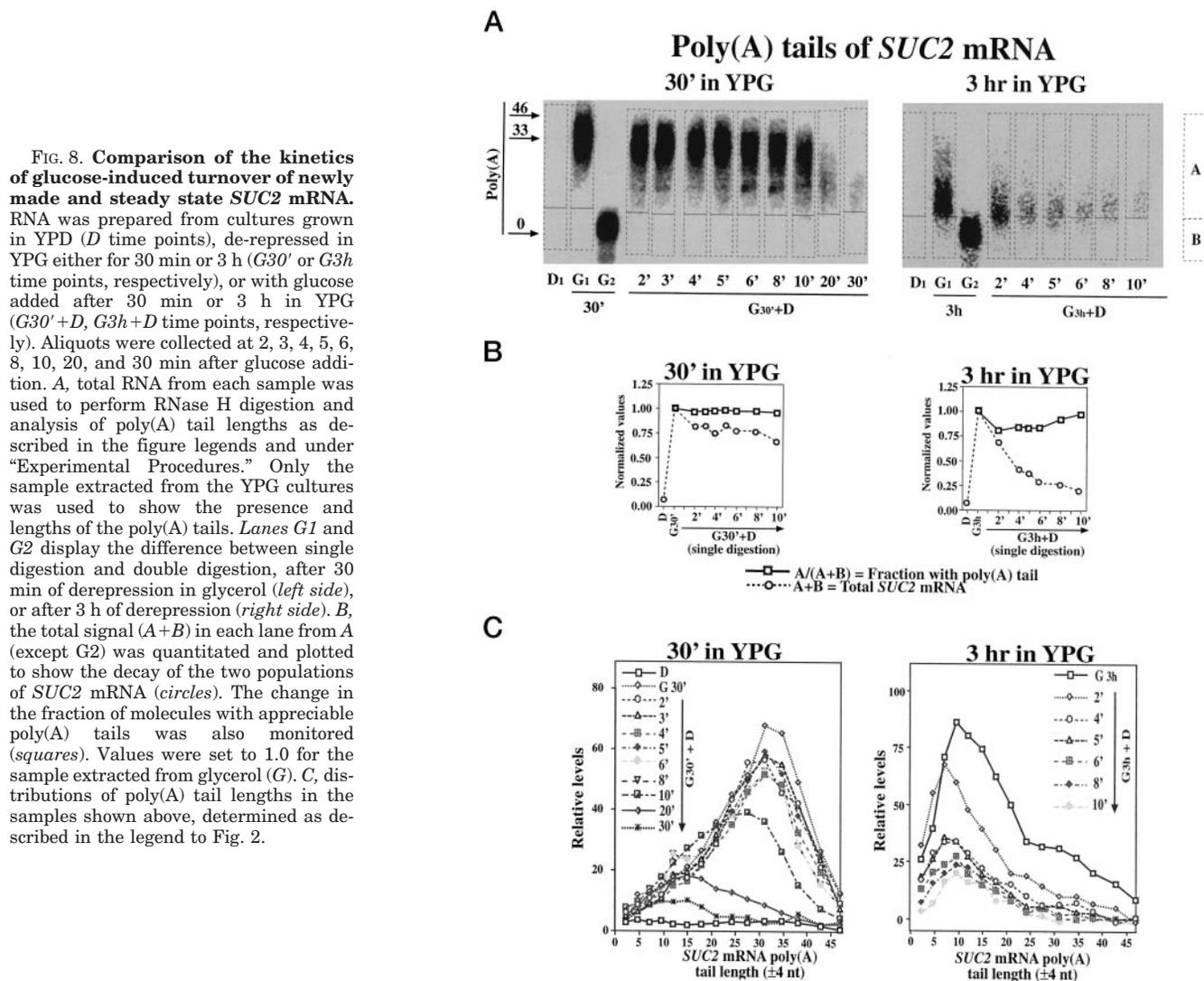


FIG. 8. Comparison of the kinetics of glucose-induced turnover of newly made and steady state *SUC2* mRNA. RNA was prepared from cultures grown in YPD (*D* time points), de-repressed in YPG either for 30 min or 3 h (*G30'* or *G3h* time points, respectively), or with glucose added after 30 min or 3 h in YPG (*G30'+D*, *G3h+D* time points, respectively). Aliquots were collected at 2, 3, 4, 5, 6, 8, 10, 20, and 30 min after glucose addition. **A**, total RNA from each sample was used to perform RNase H digestion and analysis of poly(A) tail lengths as described in the figure legends and under “Experimental Procedures.” Only the sample extracted from the YPG cultures was used to show the presence and lengths of the poly(A) tails. *Lanes G1* and *G2* display the difference between single digestion and double digestion, after 30 min of derepression in glycerol (*left side*), or after 3 h of derepression (*right side*). **B**, the total signal (*A+B*) in each lane from **A** (except *G2*) was quantitated and plotted to show the decay of the two populations of *SUC2* mRNA (*circles*). The change in the fraction of molecules with appreciable poly(A) tails was also monitored (*squares*). Values were set to 1.0 for the sample extracted from glycerol (*G*). **C**, distributions of poly(A) tail lengths in the samples shown above, determined as described in the legend to Fig. 2.

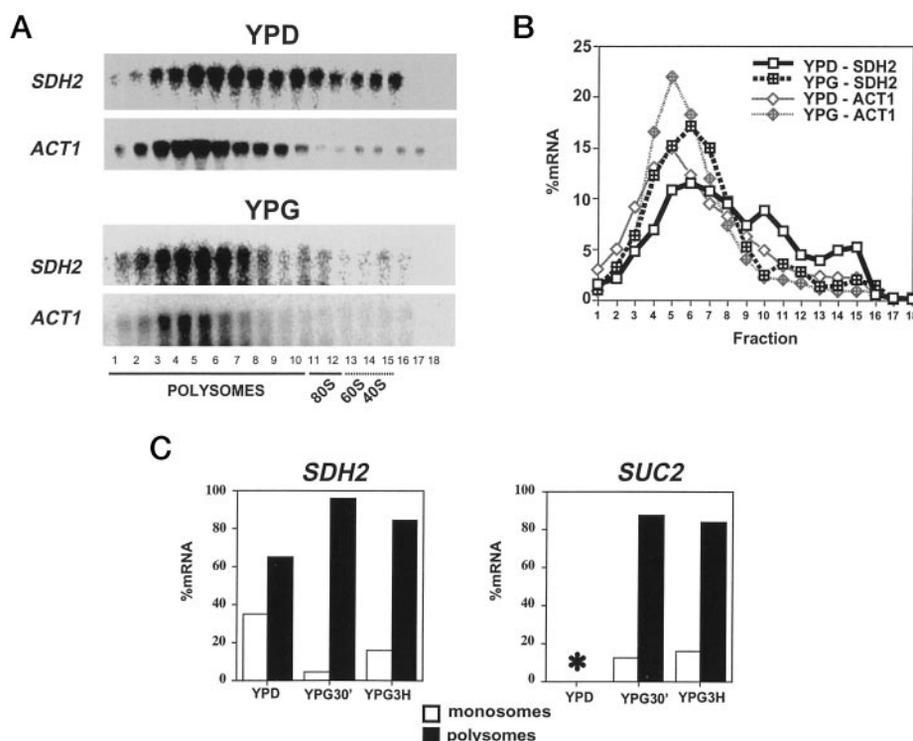
mRNA population evolved to a steady state level with a significantly more disperse distribution of poly(A) tail lengths. A substantial fraction of the molecules had a relatively short poly(A) tail, as previously noticed for *SUC2* mRNA at steady state in YPG (Fig. 6A). The kinetics of *SUC2* mRNA derepression are also represented in Fig. 7A (*right panel*). The *A/(A+B)* ratio (*solid line* in the *inset graph*) cannot be calculated at the beginning of the derepression because no signal is detected, but a small decrease is detected between 60 and 180 min of derepression due to the accumulation of molecules containing very short poly(A) tails at steady state. The complete distributions of poly(A) tail lengths during derepression are shown in Fig. 7B. It is clear that 45 min after derepression the population at the peak has a poly(A) tail in the 35-nucleotide range, but at later times the curve broadens and short-tailed *SUC2* mRNAs accumulate as seen at steady state.

The above observations opened up the possibility of obtaining within the same strain two different populations of *SUC2* mRNA: a population of “young” transcripts with a relatively long poly(A) tail (>35 nt) in cells shortly after an de-repression in YPG, or a population of “old” transcripts with a substantial subpopulation having a short tail (<20 nt) at steady state after prolonged culture in YPG. The addition of glucose is expected to have two effects: it inhibits transcription (the experiment becomes equivalent to a “chase”), and it initiates a second signal that can trigger degradation. Would there be a difference in the rates of turnover, depending on the age of the *SUC2* mRNA

population present at the moment of glucose addition? To test this, a YPD culture was transferred to YPG and then divided into two halves. Glucose was added to one-half after 30 min in YPG and to the other half after 3 h in YPG. Total RNA was then extracted from each at different times after glucose addition. Northern blot analysis of full length *SUC2* mRNA (normalized to 25 S rRNA) showed the glucose-induced decay kinetics of the two *SUC2* mRNA populations to differ markedly (results not shown). Measurements of the 3' fragments after RNase H digestion of aliquots from the same samples (Fig. 8A) also show this difference in decay rate. The “younger” transcripts decay after some delay (~20 min). An experiment with the same time resolution as that shown in Fig. 8A, but without glucose addition was also performed (results not shown). It shows that the same slow constitutive shortening of *SUC2* poly(A) tails occurs in glycerol and in the absence of turnover. 50 min after the medium change (corresponding to the 20 min time point after glucose addition), a majority of the poly(A) tails are short. At that point the transcripts become susceptible to glucose-induced decay.

The difference in poly(A) tail lengths in the two cultures is again evident in this experiment. The kinetics of decay for the two populations of 3' fragments are shown in Fig. 8B, and a complete analysis of the time-dependent distributions of poly(A) tail lengths is shown in Fig. 8C. The difference in half-lives is quite obvious and significant. This reinforces the idea that the glucose-induced turnover rate of this transcript

FIG. 9. Polysome profiles and analysis of mRNA distributions. A, comparisons of *SDH2* and *ACT1* mRNAs in YPD and YPG. Cells were grown in YPD or in YPG at steady state. Extracts were made and fractionated on sucrose gradients as described under "Experimental Procedures." Fractions were collected after passage through a UV monitor, and from the profile the position of the polysomes, 80 S ribosome and the ribosome subunits could be established. Individual fractions were analyzed by Northern blots and quantitated by PhosphorImager. B, the graph displays the distributions of the actin mRNA (thin lines), and the *SDH2* mRNA (thick lines) in YPD and YPG. C, comparison of *SDH2* and *SUC2* mRNA distributions early (30 min) and late (180 min) after derepression. The bar graphs represent the fraction of the total signal in the polysome region of the gradients (tubes 1–10) and the fraction on monosomes or smaller (tubes 11–18). For the *SDH2* transcript these measurements were also made in YPD, but the *SUC2* transcript was undetectable in YPD (*).



depends on its age at the time of glucose addition. Young, long-tailed *SUC2* mRNAs decay slowly (note the superposition of the curves from the 2 to 8-min samples). When the *SUC2* mRNA population has short poly(A) tails in a large proportion of molecules, glucose-induced turnover is very fast. Significantly, after 3 h in YPG no appreciable change in the distribution of poly(A) lengths is seen during the glucose-induced decay (Fig. 8C, right side).

It could be argued that the very young *SUC2* mRNA molecules seen after 30 min of de-repression are stable because they have not yet reached a state or "compartment" in the cytosol where they would be subject to glucose-triggered deadenylation or degradation. For example, they could still be largely in the nucleus. We investigated such a possibility first by measuring the accumulation of the *SUC2* mRNA in parallel with measurements of invertase activity in total cell extracts (Fig. 7A, right side). Invertase is active immediately after its co-translational import into the endoplasmic reticulum (41), and thus only a short delay (a few minutes) between appearance of *SUC2* mRNA in the cytosol and appearance of enzyme activity would be expected. On the time scale of interest here (0–30 min), the increase in mRNA levels is accompanied by an increase in invertase activity. In other words, we see no significant delay in the appearance of invertase activity after derepression compared with the appearance of mRNA, and hence we see no evidence for a temporary arrest of the mRNA in a compartment where it would be protected from degradation. Since each mRNA is translated multiple times, the mRNA levels and invertase activity are not necessarily proportional until steady state is reached.

A more stringent test is to follow the mRNA distributions on polysome gradients. Polysomes were obtained from cells in YPD, and from cells 30 and 180 min after derepression in YPG. They were fractionated on sucrose gradients as described under "Experimental Procedures," followed by Northern analysis of individual fractions. Results are shown in Fig. 9. The top left of the figure shows the signals obtained with an *SDH2* probe, and an actin probe (*ACT1*) from cells at steady state in YPD

and YPG. Their quantitation and distribution is shown on the right panel, and a further distinction between polysome-associated transcripts and those in the monosome region is shown in Fig. 9C. Most of the *ACT1* mRNA is found in the polysome region at all times and in both media. An analysis similar to that in Fig. 9C reveals $\sim 10 \pm 5\%$ of *ACT1* transcripts in the monosome region in YPG and YPD (results not shown).

A very intriguing result is observed for the *SDH2* mRNA. In YPG most of the transcripts are found in the polysome fractions suggestive of efficient translation. This transcript can also be detected in YPD, and, in contrast, here we reproducibly detect multiple peaks in the distribution of this mRNA on the gradients (Fig. 9B). A substantial fraction of the *SDH2* mRNA is found in the region of the monosomes, the 80 S ribosome, or in a fraction with an even lower sedimentation coefficient. This result may be indicative of a relatively inefficient initiation of translation in YPD. *SUC2* mRNA is absent in YPD, but in YPG the majority of *SUC2* transcripts are also found in the polysome region of the gradient (Fig. 9C, right). This is true 30 min after de-repression and after 180 min in YPG. Thus, the young population with long poly(A) tails (Figs. 7, 8) is not found in some protected compartment, but is fully engaged in translation. Their relative stability following glucose addition can therefore not be attributed to their failure to be engaged on polysomes.

DISCUSSION

A common decay mechanism of many mRNAs in *S. cerevisiae*, particularly constitutively short-lived mRNAs, involves a pathway comprised of three sequential steps: deadenylation, decapping, and degradation by a 5'-3' exonuclease (13, 29). The turnover of other subpopulations of mRNA in yeast appears to be dependent on a different set of proteins and *cis*-acting elements (42, 43).

The case of glucose-induced mRNA turnover presents a novel aspect of mRNA degradation. This regulated mRNA turnover requires a signaling pathway with input from external glucose, and as targets *trans*-acting effector molecules regulating or

participating in the hydrolysis reaction. The nature of the *cis*-acting sequences distinguishing a select subgroup of transcripts is also of considerable interest. Previous work had demonstrated that the 5'-UTR is a major *cis*-acting determinant, directing our attention to the activities at the 5'-UTR (9).

The behavior of an *xrn1* mutant strain was re-investigated in this study, in conjunction with the study of a novel *dcp1* mutant (29), by an improved quantitative analysis ("Experimental Procedures"). We conclude that the removal of the 7-methyl-G cap by the Dcp1 protein is essential, and that the Xrn1 protein represents the major but not exclusive nuclease activity in glucose-induced mRNA degradation. What prevents or slows the attack of these degradative enzymes in YPG where these mRNAs are significantly more stable (8)? One formal possibility is that the decapping activity is activated by glucose, and down-regulated in glycerol, but such a mechanism is not likely to be able to account for the specificity in this process for a subpopulation of mRNAs. *SDH2* mRNA has also been shown to be unstable in YPG at the nonpermissive temperature in a *prt1* mutant in which initiation is arrested at the nonpermissive temperature (9). On the other hand, the Dcp1 protein can be phosphorylated,² and it remains to establish 1) how its phosphorylated state is controlled, and 2) what the physiological significance of such a modification might be.

The occupation of the 5' cap and 5'-UTR by initiation factors, eIFs, constitutes an alternate protective mechanism, and hence an explicit link to the initiation of translation. A role for the poly(A) tail in this process can now be rationalized by numerous observations suggesting that the 3' and 5' ends of an mRNA may interact in a functionally significant way by means of proteins associated with these segments of RNA (16, 44–47). Such a model would also explain how the loss of the poly(A) tails could contribute to a weakening of the interaction of the eIFs with the 5'-UTR and hence expose the cap to the decapping enzyme (48), although Morrissey *et al.* (18) have also described the independence of decapping from deadenylation in a *pab1* mutant.

Two plausible targets emerge for the signaling mechanism initiated by glucose: 1) the deadenylation reaction(s) could be accelerated significantly, or 2) the affinity of the eIFs for select mRNAs (specifically their 5'-UTRs) could be subject to modulation by phosphorylation/de-phosphorylation reactions induced by glucose. The results in this study suggest strongly that the mechanism of glucose-triggered turnover of specific mRNAs does not include deadenylation as an obligatory, rate-determining step. In this respect this regulated turnover resembles the rapid decay of mRNAs mediated by premature stop codons (nonsense-mediated decay) (16, 34, 39), but, as described previously, the *UPF1* function is not required (9).

Not unexpectedly, the poly(A) tails of *SDH2* mRNA at steady state in YPG exhibited a broad distribution with respect to lengths, up to a maximum of ~50 adenylate residues. Molecules with long tails represented a small percentage of the total population, while the vast majority had very short tails (<<20 adenylate residues, see Figs. 3–5). For comparison, deadenylated 3' fragments were produced by RNase H digestion with two oligonucleotides, one specific for a sequence in the 3'-UTR, the other an oligo(dT). Since the cleavage is not necessarily precisely at the junction between the 3'-UTR and the poly(A) tail, and since even the polyadenylation site is somewhat variable in yeast (9), the deadenylated fragments themselves have a range of sizes with an uncertainty of ~5–10 nucleotides. Nevertheless, the distributions of double-digested fragments with no poly(A) tail, and the very prominent lower end of the

distribution of poly(A)-containing fragments (single digest) overlap very significantly (Figs. 2 and 4) and make it appear that a substantial fraction of the *SDH2* transcripts has almost no poly(A) tail.

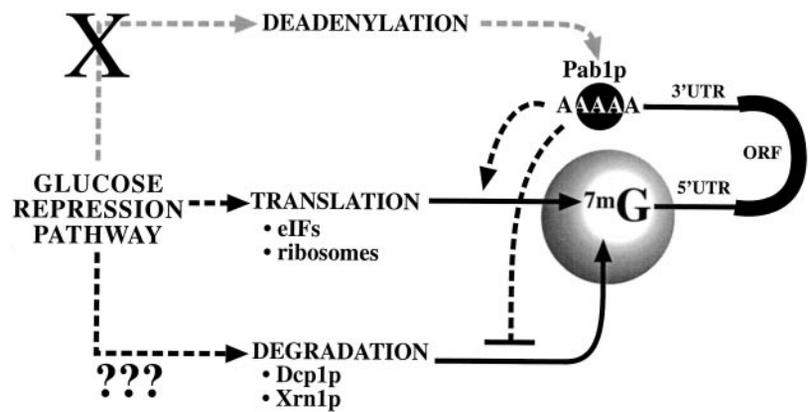
A quantitative analysis before and after addition of glucose to wild type cells shows that the rapid disappearance of transcripts is not preceded by or associated with any measurable change in the distribution (and peak position) of tail lengths (Fig. 2, *bottom*). A similar observation was made with the *xrn1* and *dcp1* mutants. When the degradation of the transcripts is prevented, there is no significant change in the fraction of poly(A) containing molecules over a period of 5–20 min, and specifically no induced, rapid disappearance of the longer poly(A) tails (Fig. 3). Since glucose-triggered mRNA degradation is quite rapid (half-lives <10 min), one would have expected to see deadenylation within a 10-min time frame, if it was obligatory and rate-limiting. It should be noted that in the two mutants the average age of mRNA molecules is greater due to a reduced overall turnover rate, and thus one might expect such molecules to have shorter poly(A) tails at steady state due to constitutive poly(A) shortening. This is not particularly evident for *SDH2* mRNAs, since the tails are already very short in a large fraction of wild type cells. It is not a central issue here, and our results (Fig. 4) suggest either that new *SDH2* mRNAs are made with very short tails, or that shortening occurs very rapidly in the course of derepression and mRNA maturation in YPG. The distribution of short tail lengths observable during derepression and at steady state in YPG is not altered with further aging of the mRNAs (Fig. 5). A second point is that even though the *SDH2* mRNAs have very short tails, short poly(A) tails are not sufficient under these conditions to promote degradation of these transcripts (Fig. 5 and Ref. 8). One may view the transcripts in YPG to be at a threshold, where exposure of the cells to glucose rapidly tips the balance in favor of decapping and turnover.

Can such mRNAs be protected in glucose by longer poly(A) tails? Previous studies on poly(A) tail length by other groups have been performed using an inducible promoter to produce exclusively newly synthesized transcripts (young transcripts) with uniformly long poly(A) tails (32). We considered the need for a system that would allow us to distinguish newly synthesized transcripts with long tails after derepression in glycerol, and to measure the rate of turnover of this exclusive class of molecules following glucose addition. With that goal, we investigated the kinetics of derepression of the *SDH2* mRNA in YPG and studied the poly(A) tail length at different times during the derepression (Fig. 4). Molecules with very short poly(A) tails predominate at the earliest times. It should be noted that the study of newly synthesized *SDH2* mRNA molecules at the earliest times (<10 min) is complicated by the continued presence of "older" molecules made at steady state in YPD with a distribution of relatively short poly(A) tails. This small amount masks the equally small amount of newly synthesized molecules at early time points (<10 min) after derepression. It is very difficult to distinguish between the poly(A) tail of new transcripts and that of the steady state population existing in YPD. Nevertheless, it is clear that there is no appreciable transient population of long-tailed *SDH2* mRNAs at any time during derepression (Fig. 4).

SUC2 mRNA is another transcript subject to glucose-induced turnover (11). When glucose was added to a steady state culture in YPG, the rapid degradation of the *SUC2* message was also found to take place without prior changes in the distribution of poly(A) lengths (see Fig. 6A). Again, glucose-induced turnover appears independent of deadenylation. In the *xrn1* mutant strain where degradation was prevented, poly(A)

² R. Parker, personal communication.

FIG. 10. Model for the proposed competition between initiation and turnover of specific mRNAs controlled by the carbon source.



tail lengths also did not shift appreciably upon addition of glucose. No *SUC2* mRNA was detected in the presence of glucose (11), but this transcript is rapidly accumulated in YPG, making it possible to detect newly synthesized *SUC2* mRNA molecules containing relatively homogeneous poly(A) tails with >40 adenylate residues within 30–45 min (Figs. 7 and 8). Glucose-induced turnover of these young transcripts was found to be very slow when compared with the glucose-induced turnover of such mRNAs in YPG at steady state (3 h) (Fig. 8). One difference between these two populations of mRNAs is clearly in the average length of the poly(A) tails. A long poly(A) tail appears to stabilize this mRNA even in the presence of glucose. Changes in the size distribution of poly(A) tails have been previously correlated with alterations in mRNA decay rates (33) (for review, see Refs. 49 and 50), hence the stabilizing effect of a long poly(A) tail may not be a surprise. What needs to be re-emphasized, and is well illustrated by the example of the *SUC2* transcript, is that the shortening of the poly(A) tail alone is not sufficient to make this transcript unstable in YPG. From a different perspective, an accelerated deadenylation in young *SUC2* mRNA molecules is not triggered by glucose (Fig. 8, left side)

It could be argued that the young *SUC2* mRNA is not subject to the normal mechanisms of glucose-induced mRNA decay because of a delay in mRNA biogenesis or maturation of cytoplasmic ribonucleoprotein. This explanation was forwarded (33) to explain the observation that newly synthesized mRNAs with long poly(A) tails (even those with premature stop codons) were not degraded at the expected rapid rate in a mutant in which the *PAB1* gene had been deleted. While we cannot exclude this possibility, several points can be made. First, our studies were performed in a wild type yeast strain. Second, the appearance of invertase activity was not delayed following the derepression of the *SUC2* gene (Fig. 7). Finally, the majority of both young and old *SUC2* transcripts are found on polysomes, indicative of their participation in translation (Fig. 9C). Therefore, the stability of the young transcripts cannot be ascribed to their localization in some protected state.

In summary, our experiments demonstrate that the signaling pathway initiated by glucose does not target the deadenylation mechanism. In that sense, glucose-regulated mRNA turnover is independent of deadenylation. On the other hand, the stable mRNAs investigated here at steady state in YPG already have very short poly(A) tails that require no further reduction before degradation by the Dcp1p and Xrn1p activities can be triggered. When young *SUC2* mRNAs were investigated their stability was enhanced, presumably due to their long tails, but other mechanisms cannot be completely excluded.

The experiments involving polysome gradients provide some direct evidence for the view that the carbon source can influence the efficiency of translational initiation of specific mRNAs

(Fig. 9). In YPG the vast majority of *SDH2* mRNAs is found on polysomes, but in YPD a very significant fraction of these transcripts is found at distinct peaks in the region of the gradient containing monosomes (80 S) and even smaller complexes (60 S to 40 S). In contrast, this difference is not observed for *ACT1* mRNA, a transcript that is not destabilized by glucose. The complete repression of *SUC2* mRNA in YPD unfortunately precludes a similar comparison for this transcript, but as expected, most of it is found on polysomes in YPG (Fig. 9C).

These results can be fitted readily into a working hypothesis proposed in previous publications (1, 11). In this model (Fig. 10), the carbon source controls the outcome of a competition between two events at the 5'-UTR of the relevant mRNAs: initiation of translation and decapping/exonuclease activity. In YPG the initiation complex including eIFs, 40 S ribosome, and Met-tRNA is formed rapidly, and the cap-binding protein, eIF4E, protects the transcript against Dcp1p. The poly(A) tail with bound Pab1p may contribute to the stabilization, but the shortness of the tails at steady state suggests that this mechanism is relatively insignificant. The addition of glucose alters the affinity of the initiation complex for the 5'-UTR. The results of this study eliminate a signaling mechanism that includes deadenylation and the Pab1p interaction with eIF4G. Instead, it is proposed that the phenomenon of glucose repression involves a signaling pathway changing the activity of one or more eIFs. The failure to induce turnover by glucose in a *reg1* mutant is highly suggestive of a signaling pathway altering phosphorylation states (9). The experiments with the temperature-sensitive mutant, *prt1-1*, with a defective subunit of the translation initiation factor eIF3 are also relevant here (51). It is notable, however, that the modulation of the activity of the eIF(s) by glucose is insufficient when young transcripts with long poly(A) tails are involved. Binding of multiple Pab1p molecules and stabilization of the initiation complex by the type of interaction proposed by Sachs and colleagues (45–47) may account for this observation.

The phosphorylation of specific eIFs in yeast and in mammalian cells has been abundantly demonstrated (52–57). A major distinction needs to be made between mechanisms that affect protein synthesis globally, and mechanisms that may play a role in determining the translation efficiency of specific mRNAs. The final target of the glucose signaling pathway remains to be established: which eIF is modulated, which site on the protein is phosphorylated, and which specific kinase/phosphatase is responsible for the modification?

A final comment will address the 5'-UTR, the major *cis*-acting determinant in glucose-regulated mRNA turnover. In our view there appears to be no short consensus sequence for binding a specific RNA-binding protein involved in this mechanism. Instead, a large portion of the 5'-UTR (~50–60 nt) is probably associated with a very large complex including the

40 S ribosome, eIF4E, eIF4G, eIF3, met-tRNA-eIF2E-GTP, and possible eIF4A and eIF4B. The mass of proteins involved may cover almost the entire length from the 5'-cap (binding eIF4E) to the start codon. Specific interactions with the 5'-UTR can be expected to depend on the entire sequence and its length.³ The efficiency of formation of this complex and its stability are key to translational regulation and indirectly the control of the half-life of an mRNA.

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³S. Prieto, B. J. de la Cruz, and I. E. Scheffler, unpublished observations.

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