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Control of mRNA Turnover as a Mechanism of Glucose Repression in *Saccharomyces cerevisiae*

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We have examined the expression of the gene encoding the iron-protein subunit (Ip) of succinate dehydrogenase in *Saccharomyces cerevisiae*. The gene had been cloned by us and shown to be subject to glucose regulation (A. Lombardo, K. Carine, and I. E. Scheffler, J. Biol. Chem. 265:10419–10423, 1990). We discovered that a significant part of the regulation of the Ip mRNA levels by glucose involves the regulation of the turnover rate of this mRNA. In the presence of glucose, the half-life appears to be less than 5 min, while in glycerol medium, the half-life is >60 min. The gene is also regulated transcriptionally by glucose. The upstream promoter sequence appeared to have four regulatory elements with consensus sequences shown to be responsible for the interaction with the HAP2/3/4 regulatory complex. A deletion analysis has shown that the two distal elements are redundant. These measurements were carried out by Northern (RNA) analyses of Ip mRNA transcripts as well as by assays of β-galactosidase activity in cells carrying constructs of the Ip promoter linked to the *lacZ* coding sequence. These observations on the regulation of mRNA stability were also extended to the mRNA of the flavoprotein subunit of succinate dehydrogenase and in some experiments of iso-1-cytochrome *c*.

Our laboratory has described the isolation and sequencing of the gene for the iron-protein (Ip) subunit of succinate dehydrogenase (SDH) in *Saccharomyces cerevisiae* (15). The subunit is one of four peptides making up complex II of the mitochondrial electron transport chain (for reviews, see references 12, 29, and 31).

In the course of our initial characterization and expression of this gene, we introduced it on a vector into a yeast strain with a disrupted endogenous gene. We observed, not unexpectedly, that expression of this gene was markedly repressed by glucose (15). The phenomenon of glucose repression in yeast cells has been known for some time (7). In the presence of an abundant supply of glucose in the medium, yeast cells use glycolysis as their primary pathway of energy metabolism, and expression of the genes of the oxidative phosphorylation pathway is repressed. When the glucose concentration is reduced, or in the presence of a nonfermentable carbon source, the expression of mitochondrial functions is increased.

Studies of this phenomenon have addressed different aspects of this problem. A number of different genes and gene products are involved in sensing of the glucose concentration or of some secondary metabolite and in the signal transduction pathway which ultimately leads to factors associated with transcriptional activation or suppression (1, 3, 6, 17, 19, 25, 30, 33, 34). At the transcriptional level, the HAP2/3/4 protein complex plays a prominent role (4, 6, 10, 11, 22, 23). In a few cases, it has been shown to interact with a specific sequence motif (TNATTGGT) found in the upstream activation sequences (UASs) of genes such as iso-1-cytochrome *c* (21), α-ketoglutarate dehydrogenase subunit of the α-ketoglutarate dehydrogenase complex (24), and other mitochondrial genes (18). These studies have emphasized transcriptional control as the primary mechanism of control of gene expression by glucose, with the focus on the promoter and on specific and nonspecific transcription factors.

The analysis of the promoter region of the SDH Ip gene revealed the presence of four potential HAP2/3/4 binding consensus sites, and one of our initial goals was to define the smallest promoter segment which still permitted the normal operation of the glucose repression mechanism. Thus, we constructed a series of deletion mutants with the original coding sequence of the Ip gene, as well as a similar series in which the truncated Ip promoters were cloned in front of the coding sequence for β-galactosidase. A large apparent discrepancy between the results observed with the Ip gene and those obtained with the chimeric gene (different coding sequences and 3′ untranslated regions) suggested strongly that a posttranscriptional mechanism (mRNA stability) plays an important role in regulating the steady-state level of Ip and functionally related mRNAs. In this report, we demonstrate that the half-life of these transcripts is vastly shorter in glucose-containing media than in media with a nonfermentable carbon source.

MATERIALS AND METHODS

Yeast strains and vectors. The parental yeast strain DL1 (*leu2 ura3 his3*) (32) was obtained from M. Yaffe, Department of Biology, University of California, San Diego. SDH-deficient mutant derived from this strain has been described by us (16). It was used as the host for the shuttle vector pRS315 (27) and for various constructs made with this vector. L. Guarente's laboratory at the Massachusetts Institute of Technology kindly provided strains BWG1-7a (*MATa leu2-3,112 his4-519 ade1-100 ura3-52*) (8), JO1-1a (*leu2-3,112 his4-519 ade1-100 ura3-53 hap2Δ*), and SHY40 (*leu2-3,112 his4-519 ade1-100 ura3-52 hap3:HIS4*) (28), and we used strain BWG1-7a as host for the vector pSEYC 102 (5), its derivatives with the Ip promoter-*lacZ* fusions, and plasmid

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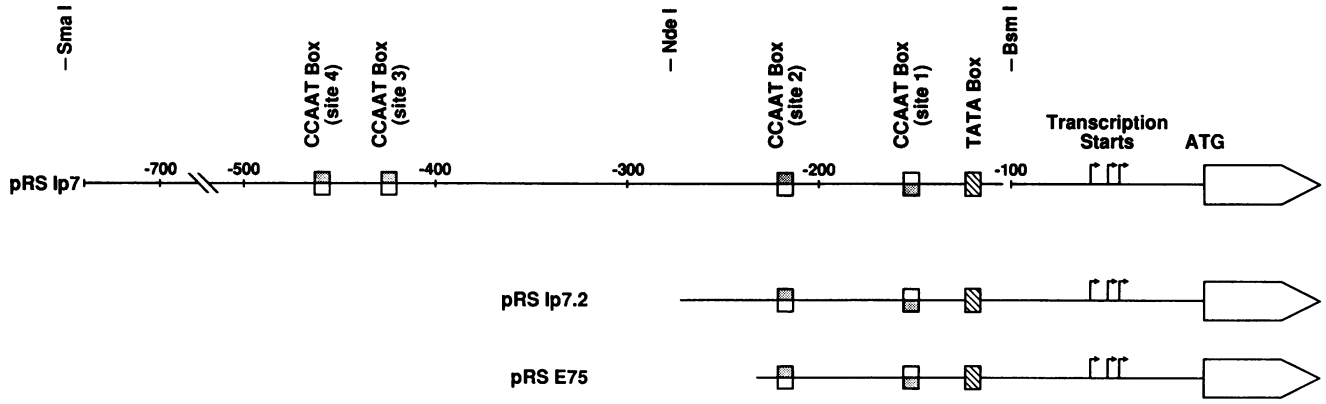

FIG. 1. Key features of the promoter region of the SDH Ip gene in *S. cerevisiae*. There are four consensus sites ATTGG (or CCAAT) for binding of the HAP2/3/4 complex. The various deletions used in our analysis are shown below. Ip7.2 starts at nt −272 relative to the start codon, and E75 starts at nt −234. Their construction is described in Materials and Methods.

pLG 669z, containing the *CYC1* promoter fused to *lacZ* (9) (also made available by L. Guarente).

Yeast mutant strains Y260 (*MAT***a** *ura3-52 rpb1-1*) and Y136 (DB1033) (*Mat*α *ura3-52*) were generously provided by Richard Young's laboratory at the Massachusetts Institute of Technology (20). Strain DB1033 is the parental strain for the mutant strain with a temperature-sensitive RNA polymerase II.

Yeast cells were grown on YPD (1% Bacto Yeast Extract, 2% Bacto Peptone, 2% dextrose), on YPG (1% Bacto Yeast Extract, 2% Bacto Peptone, 3% glycerol), or on SD or SG synthetic medium (0.67% Difco yeast nitrogen base without amino acids, with 2% dextrose or 3% glycerol, respectively) (26) with the appropriate supplements.

All yeast transformations were performed by the lithium acetate method (14). At the end of the experiments, the glucose concentrations in the media were routinely monitored by Chemstrip bG (Boehringer Mannheim) to confirm that the glucose concentration had not been significantly depleted.

The Ip gene and construction of the deletion mutants. The SDH Ip gene from *S. cerevisiae* had been cloned by us into the shuttle vector pRS315 to yield the new vector, pRS Ip7 (15). It contains 740 bp of promoter sequence and complements an SDH-deficient mutant in which the chromosomal Ip gene had been disrupted by targeted recombination (16).

Deletions from the 5′ end of the Ip promoter were constructed by cleavage at the *Nde*I site (position −273 relative to the translation start site) and further digestion with exonuclease III and S1 nuclease (Nested Deletion Kit; Pharmacia). The fragment upstream of the original *Nde*I site was removed by *Sma*I digestion, and the remaining plasmid was recircularized. A series of different deletions was obtained by varying the length of the exonuclease treatment. All deletions were further characterized by DNA sequencing (Sequenase Kit; United States Biochemical Corp.).

The SDH Ip promoter-*lacZ* fusion genes were constructed as follows. A *Sac*I-*Hin*dIII fragment from pRS Ip7 or *Kpn*I fragments from the series of pRS deletions were cut with *Fok*I, blunt ended with S1 nuclease, and subcloned into the *Sma*I site of the vector pGEM3z f(−) (Promega). The orientation of the fragment was checked by sequencing, and the *Eco*RI-*Bam*HI fragments from the appropriate pGEM constructs were cloned into the pSEYC 102 vector cut with the same enzymes. As a result, the various Ip promoters and four codons of the Ip coding sequence are fused in frame with the *lacZ* coding sequence. The desired fusion was verified by sequencing, using a specific oligonucleotide primer from the β-galactosidase coding region. The primer was synthesized by Operon Technologies (Alameda, Calif.).

Other genes. The gene for the flavoprotein (Fp) subunit of SDH in *S. cerevisiae* was isolated by K. B. Chapman, S. D. Solomon, and J. D. Boeke (Johns Hopkins University) and kindly made available to us prior to publication. L. Guarente's laboratory provided clone pYeCYC1 containing the iso-cytochrome 1 gene.

Other methods. Standard protocols were used both for Northern (RNA) analysis (2, 16) and for the assay of β-galactosidase activity (2). Radioactive probes were made by the random primer DNA labeling system from Bethesda Research Laboratories. The Ip probe was a 568-bp *Pst*I-*Xba*I fragment of the coding sequence; the Fp probe was a 1,030-bp *Bgl*II-*Bgl*II fragment from the pKC59 plasmid of Chapman et al. (see above), and the *CYC1* probe was derived as an *Eco*RI-*Hin*dIII fragment from plasmid pYeCYC1 provided by L. Guarente.

Restriction enzymes, isotopes, and other reagents. Restriction enzymes were purchased from Bethesda Research Laboratories or from New England Biolabs. They were used according to the instructions provided by the suppliers. [α-^{32}P]dCTP (specific activity, 3,000 Ci/mmol) was purchased from Amersham Corp. All other chemicals were of the highest grade available.

RESULTS

Promoter deletions and vector constructs. Figure 1 shows the promoter region of the yeast Ip gene as well as significant landmarks such as transcription start sites, the TATA box, and several segments containing a consensus sequence for the HAP2/3/4 complex involved in glucose repression (CCAAT box) (4, 22, 24). The region shown had been demonstrated to be sufficient for maximum regulated expression compared with the wild-type gene on chromosome VII (15). 5′ deletions of the promoter region were constructed as described in Materials and Methods. A summary of the deletion mutants used in this study is also shown in Fig. 1.

Alternatively, the same series of truncated promoters was cloned in front of the β-galactosidase coding sequence in the vector pSEYC 102 (5) as described in Materials and Meth-

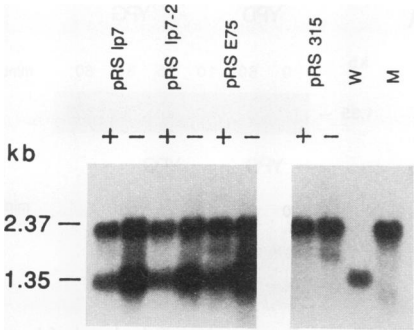

FIG. 2. Northern analysis of total RNA from wild-type strain DL1 (W), its derivative with the disrupted SDH Ip gene (M), the same mutant transfected with the shuttle vector pRS 315, and a series of strains with the disrupted chromosomal gene and various promoter deletions in the Ip gene carried in the vector pRS315. Cells were grown in SD medium (+) and transferred to SG medium (−) for 30 min before isolation of the RNA. The 2.4-kb transcript is derived from transcription from the disrupted gene extending into the insert; the 1.35-kb transcript corresponds to normal Ip mRNA.

ods. The resulting chimeric gene contains four codons from the signal sequence of the Ip subunit (amino acids MLNV).

Both plasmids are nonintegrating plasmids whose copy number after transfection into yeast cells is similar and close to one (results not shown). It should be pointed out, however, that the Ip promoter sequences in the two series are identical from the 5′ endpoints of the various deletions to the translation start site, while vector sequences immediately upstream are different.

Northern analysis of Ip gene expression. The Ip gene with partial promoter deletions on plasmid pRS315 was introduced into yeast strain DL1 (phenotype His$^-$ Leu$^-$ Sdh$^-$) in which the Ip gene on chromosome VII had been disrupted by insertion of a *URA3* gene (16). Selection in the absence of leucine yielded plasmid-containing cells which were routinely maintained in SD medium.

Northern analysis of Ip transcripts in wild-type, Sdh$^-$ mutant, and Sdh$^-$ mutant cells carrying different plasmids is shown in Fig. 2. Total RNA was isolated from cells grown in SD medium (high glucose) or after a shift to SG medium (low glucose) for 30 min.

In wild-type cells, the normal Ip transcript (1.35 kb) was induced very significantly when the cells were grown in low-glucose medium (not shown), and a similar increase was observed when the same 1.35-kb mRNA was transcribed from the complete gene on the nonintegrating plasmid pRS Ip7. It is noteworthy that the chimeric transcript (~2.4 kb) from the disrupted chromosomal gene appeared slightly elevated relative to the normal transcript in cells grown in high-glucose medium (see also Fig. 2 in reference 15 and Fig. 4). At low glucose concentrations, the increase in the level of the 2.4-kb transcript was relatively modest by comparison. A further explanation of this discrepancy will be provided below. The results in Fig. 2 (lanes pRS Ip7-2 and pRS E75) indicate that upstream sequences can be deleted from the promoter to position −234 (which leaves the HAP2/3/4 site [site 2] at ~−220 intact) without any significant effect on the inducibility of the normal 1.3-kb transcript at low glucose concentrations.

Chimeric genes with the Ip promoter controlling the expression of β-galactosidase activity. We also constructed a series of chimeric genes in the pSEYC 102 vector. These genes have the same promoters as tested in the experiments described above but contain only four codons of the Ip coding sequence, followed by the coding sequence of the enzyme β-galactosidase (Materials and Methods). The host was strain BWG1-7a.

TABLE 1. β-Galactosidase activities

Construct	β-Galactosidase activity[a]	
	+Dextrose	−Dextrose
pSEYC Ip7	2.7 ± 0.23	5.6 ± 1.2
pSEYC Ip7-2	9.6 ± 0.41	30.0 ± 1.2

[a] Values represent averages of independent determinations from three different yeast cultures (normalized with respect to total protein in the extracts).

β-Galactosidase assays were performed as described in Materials and Methods, with extracts from cells grown in high- or low-glucose medium. With an Ip promoter segment of 750 nucleotides (nt) (pSEYC Ip7) or as few as 280 nt (pSEYC Ip7.2), the expression of β-galactosidase was measurable in glucose-repressed cells, and it increased two- to fourfold after 30 min in the absence of glucose (Table 1). The observed derepression is considerably smaller than that expected on the basis of the Northern analysis with the Ip coding sequence (see Fig. 2 and 4), but it is in the same range as the observed induction of the 2.4-kb chimeric mRNA, which is transcribed from the same promoter. We also measured β-galactosidase activity and induction under our conditions with plasmid pLG 669z, provided by the laboratory of L. Guarente (Materials and Methods). It has a β-galactosidase gene with the *CYC1* promoter. The induction of activity under derepressed conditions was also of the order of two- to fourfold when this plasmid was introduced into BWG1-7a.

Induction in *hap* mutants. To test the role of the *HAP* genes in the induction of β-galactosidase activity, we introduced plasmid pLG 669z as well as selected plasmids with the Ip promoter linked to the β-galactosidase gene into mutants JO1-1a (*hap2*Δ) and SHY40 (*hap3:HIS4*) (Materials and Methods). As expected, in the absence of the *HAP2* or *HAP3* gene product, no derepression of enzyme activity in YPG medium was observed. We concluded that the derepression of the β-galactosidase activity in glucose-deficient medium was entirely due to the HAP2/3/4-dependent transcriptional activation, in complete agreement with previous studies (21).

In contrast, the observed increase in the Ip mRNA level as assayed by Northern analysis under the same conditions could not be accounted for entirely by the fourfold increase in the rate of transcription suggested by our *lacZ* data. If a posttranscriptional mechanism was involved, one might predict accumulation of Ip mRNA following glucose deprivation even under conditions in which transcriptional activation is eliminated by mutation in the *HAP2* or *HAP3* gene. In such mutants, the Ip promoter is expected to be a weak constitutive promoter. In fact, the same experiments monitoring Ip, Fp, and *CYC1* mRNA levels in such mutants demonstrate a very significant elevation 30 min after removal of glucose (Fig. 3). The HAP2/3/4 complex appears to play no role in a posttranscriptional mechanism regulating the accumulation of the Ip and Fp mRNAs in YPG medium. A return to YPD medium caused a rapid decrease of the signals for all of the mRNAs (see below). There may be some quantitative differences in the rates for the individual mRNAs. It should be

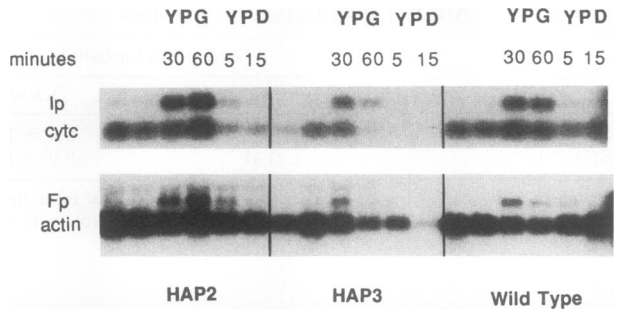

FIG. 3. Induction of various mRNAs in wild-type and *hap* mutants. Cells were grown in YPD (left two lanes of each panel), switched to YPG, from which aliquots were taken at 30 and 60 min, and transferred back to YPD, from which aliquots were taken at 5 and 15 min. After being probed with a mixture of Ip and *CYC1* probes, the filters were washed and reprobed with a mixture of actin and Fp probes.

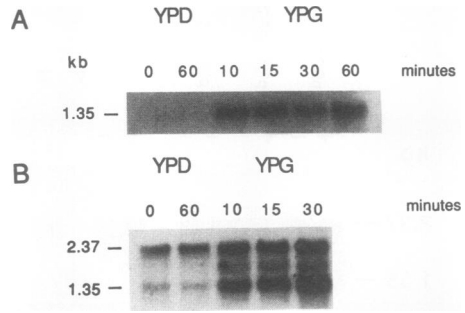

FIG. 4. Kinetics of derepression of the level of Ip mRNA upon a shift to glucose-poor medium YPG. Wild-type DL1 cells (A) or cells with a chromosomal disruption of the Ip gene and carrying the vector pRS Ip7 with 750 nt of promoter region (B) were grown in SD medium (repressed state), collected by centrifugation, and resuspended in YPG medium or in YPD medium. Aliquots of cells were harvested at the indicated times, and total RNA was prepared for Northern analysis.

noted that none of these data reflect true steady-state conditions, and the behavior of *CYC1* may in fact be less dramatic. These results prompted us to consider mRNA turnover under different conditions.

The kinetics of induction and repression of Ip mRNA under different conditions. A series of experiments was conducted to define the rate at which mRNA levels changed following a shift to glucose-poor medium, YPG (induction), or conversely, from a nonfermentable carbon source to glucose-rich medium, YPD (repression). Northern analyses were performed with total RNA from cells harvested at various times after the change in medium. Experiments were carried out with cells having a normal allele of the Ip gene on chromosome VII or with cells carrying the Ip gene with the complete promoter (780 nt) on a nonintegrating, single-copy plasmid, pRS Ip7.

In cells shifted from glucose to a nonfermentable carbon source, the level of Ip mRNA increased rapidly, and the new steady-state level was approached in 30 to 60 min. It reached a maximum within this time range, regardless of the location of the normal Ip gene (chromosome or plasmid). There were some variations from experiment to experiment due to loading (only one experiment is shown), but the rapid increase within 10 to 30 min was consistently observed (Fig. 4).

The blots in Fig. 4 also show the corresponding changes in the 2.4-kb chimeric transcript from the disrupted gene of the SDH-deficient mutant cells. As shown before, the level of this transcript was higher initially, and it increased much less dramatically than did the 1.35-kb Ip mRNA.

The return to glucose-repressed conditions was followed by a rapid decline of the 1.35-kb Ip mRNA level, regardless of whether the transcript had been from the chromosome or from the plasmid vector (Fig. 5). The decrease to the new steady state was observed to occur within 5 to 10 min, suggesting a relatively rapid turnover of the existing Ip mRNA. However, in distinct contrast, the chimeric 2.4-kb transcript remained at the slightly elevated level throughout the 60-min period over which these observations were made.

The same blots were stripped and hybridized with either a mixture of probes for the Fp and actin mRNAs or a probe for the iso-1-cytochrome *c* mRNA. It is clear that the actin mRNA was quite stable over the 1-h period investigated, even when the cells had been transferred to glucose. In YPG, the signals for each of the mRNAs vary in parallel with the variation in the actin mRNA signal; i.e., the variation is due to loading. Like the Ip mRNA, the Fp and *CYC1* mRNAs are rapidly degraded after the addition of glucose; less than 5 min appears to be sufficient for an almost complete disappearance of the signals.

A similar series of experiments was performed with the temperature-sensitive strain Y260 and its wild-type parent, DB1033 (20). A mutation in the RNA polymerase II permits a rapid arrest (5 to 15 min) in mRNA synthesis following a

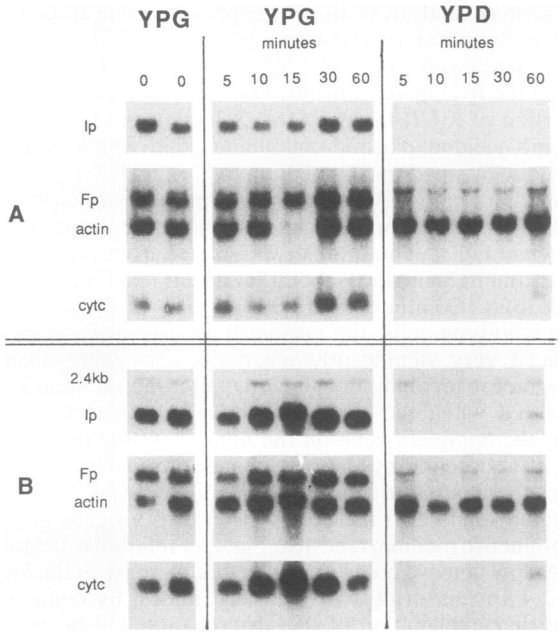

FIG. 5. Northern analysis. Parental cells (A) or cells carrying a gene on the vector to complement the disrupted chromosomal gene (B) were grown in YPG medium (induced state), collected by centrifugation, and resuspended in either YPG or YPD medium. Aliquots were collected at the indicated times, and RNA was prepared for Northern analysis. After being probed with the Ip probe, the filters were washed and reprobed with a mixture of Fp and actin probes. After another wash, the filters were probed with the *CYC1* probe. The small signal above the Fp signal was due to an artifactual hybridization with the actin probe.

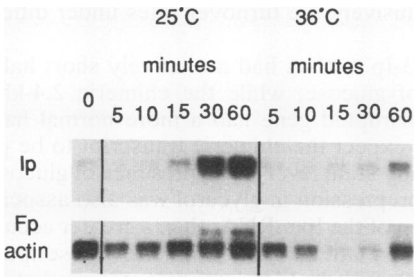

FIG. 6. Test for leakiness of the temperature-sensitive polymerase mutation. Cells were grown in glucose-rich medium to repress the Ip and Fp mRNAs (lane 0). Cultures were then transferred to YPG medium, and prewarmed medium (36°C) was added to half of each culture. Aliquots were collected at either 25 or 36°C at the indicated times for preparation of total RNA. After being probed with the Ip probe, the filters were washed and reprobed with a mixture of Fp and actin probes.

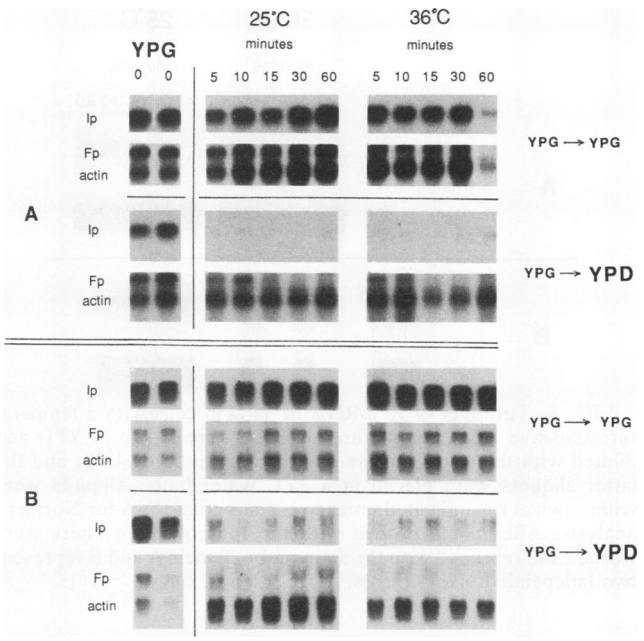

FIG. 7. Measurement of the turnover of Ip mRNA in yeast cells with a temperature-sensitive RNA polymerase II (A) and in the corresponding parental cells (B). Cells were grown at the permissive temperature in YPG medium to induce high levels of the mRNAs of interest (lanes 0). Aliquots were diluted with an equal volume of YPG medium at 25 or at 48°C, and the latter was shifted to a 36°C water bath (YPG→YPG); similar aliquots were diluted with equal volumes of glucose-containing medium to raise the final glucose concentration to that of YPD. Again, one set was diluted with prewarmed medium and placed in the 36°C water bath (YPG→YPD). Aliquots were withdrawn at the indicated times for RNA isolation. Blots were first probed with the Ip probe and, after the filters were washed, with a mixture of Fp and actin probes. There is a small artifactual signal due to the actin probe just above the Fp band which persists in all samples in either medium or at either temperature.

temperature shift from 25 to 36°C. As shown by Herrick et al. (13), the temperature-sensitive mutants can be used reliably for measuring the half-life of mRNAs at 36°C without significant complications from the effects of heat shock proteins.

A preliminary experiment was used to assess the completeness of the arrest in transcription (Fig. 6). Cells were induced by a shift from glucose to glycerol, but aliquots of cells were transferred to the nonpermissive temperature at the same time. While a large and rapid induction occurred in these cells at the permissive temperature, comparable to results shown in Fig. 4, the increase at 36°C was relatively small, even after 60 min. The observation that there was an increase at all could be due to a very low level of residual transcription at the nonpermissive temperature, coupled with the stabilization of the Ip mRNA in YPG medium. Repeat experiments confirmed the apparent small leakiness of the temperature-sensitive mutation.

Cells maintained at 25°C and fully induced in YPG medium to have maximum levels of the Ip and Fp transcripts were shifted to 36°C or left at 25°C; half of the aliquots received glucose, while the other half was kept continuously in glycerol. The addition of prewarmed medium to raise the temperature (and the glucose concentration in half of the samples) permitted the first sampling at 5 min after the change in conditions. A very dramatic decrease in the level of Ip mRNA is already observable after such a short time in glucose, in contrast to the observations with cultures maintained in glycerol (Fig. 7A). The addition of glucose caused an extremely rapid turnover of the Ip and Fp mRNAs.

Regardless of whether transcription is allowed to continue after the addition of glucose, the approach to the new steady state is dominated by the degradative mechanism, since the observed kinetics are very similar at this level of resolution and precision at either temperature in mutant and wild-type cells. The degradation of the Fp mRNA may be slightly slower. Comparison of the results at the permissive and nonpermissive temperatures also suggests that there is no significant influence of the heat shock response on the turnover of the Ip mRNA.

The possibility has to be considered that the rapid turnover observed in the experiments described above was a transient phenomenon caused by the shift in media. A temperature shift of the temperature-sensitive mutant cells while they remain in YPD permits a direct measurement of the turnover of Ip mRNA in otherwise unperturbed cultures. It should be noted that the Ip mRNA level under these conditions is low to start with. The results shown in Fig. 8 show that the rapid turnover of the mRNA occurs at all times in glucose-rich medium. When transcription is arrested at 36°C, the mRNA levels drop dramatically within 5 min.

DISCUSSION

The experiments described in this report were aimed at obtaining a clearer understanding of the mechanism by which glucose regulates the expression of genes involved in mitochondrial electron transport in *S. cerevisiae*. The gene for the (Ip) subunit of SDH was the initial focus of our investigation (15). Subsequently, the gene for the Fp subunit of SDH was made available to us (Materials and Methods) to be included in the analysis.

Our investigation began with an analysis of the nature and function of UASs, but we were soon led to a consideration of posttranscriptional mechanisms, which we believe to play a significant role in regulating the steady-state levels of Ip and Fp mRNAs and other mRNAs.

Northern analysis of transcripts from the disrupted gene (but with an intact promoter) on the chromosome in the presence of the gene with promoter deletions on the nonin-

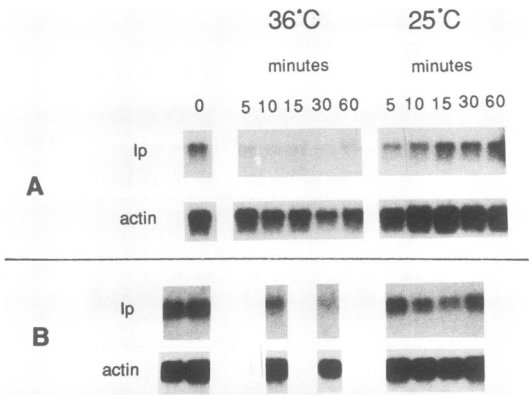

FIG. 8. Turnover of Ip mRNA in YPD in cells with a temperature-sensitive RNA polymerase II. Cells were grown in YPD and diluted with the same glucose-rich medium at 25 or 48°C, and the latter aliquots were placed in a 36°C water bath. Aliquots were withdrawn at the indicated times to prepare total RNA for Northern analysis. After being probed with the Ip probe, the filters were washed and reprobed with the actin probe. Panels A and B represent two independent experiments.

tegrating plasmid in principle constituted a system in which the effect of the deletion could be compared directly with an internal control, since the disrupted gene was still expressed from the wild-type promoter. Although the two genes were present at comparable copy numbers (results not shown), we observed a large difference in the steady-state amounts of mRNA even when the promoter on the plasmid was essentially wild type. More significantly, upon a shift to glucose-deficient medium, induction of the 1.3-kb Ip mRNA was always far greater than induction of the 2.4-kb chimeric transcript. Comparable results were obtained when the nonfermentable carbon source was either glycerol, raffinose, lactate, or ethanol (results not shown).

On the other hand, while Northern analysis of the Ip transcript suggested an up to 20-fold derepression in the presence of a nonfermentable carbon source, the same promoters in front of the β-galactosidase coding sequence yielded a consistently smaller induction of enzyme activity.

These results suggested to us that the promoter alone could not account for this difference. Instead, it should be recognized that both the transcript from the disrupted resident Ip gene and the transcript from the β-galactosidase chimeric gene have 3′ coding and noncoding sequences completely different from those of the normal Ip mRNA. Therefore, one might expect the corresponding mRNAs to have completely different half-lives, and this factor could contribute to the differences in steady-state mRNA levels (and enzyme activity) under the two conditions.

Another revealing experiment was the induction of β-galactosidase activity or the Northern analysis of the Ip transcript in either a *hap2* or a *hap3* mutant after a shift to glucose-deficient medium. In agreement with expectations, β-galactosidase activity was not induced in these *hap* mutants when *lacZ* transcription was directed by the Ip promoter (results not shown). In contrast, there was a very significant induction and accumulation of Ip mRNA in the absence of a functional HAP2/3/4 complex when cells were transferred from YPD to YPG medium (Fig. 3). In the absence of the HAP complex, the Ip promoter can be considered a constitutive promoter in either medium (11, 21). Hence, the steady-state levels of Ip and Fp mRNAs will reflect exclusively the turnover rates under different conditions.

If the 1.3-Ip mRNA had a relatively short half-life in the presence of glucose, while the chimeric 2.4-kb transcript from the disrupted gene had a more normal half-life, then one would expect the chimeric transcript to be present at a higher steady-state level in the presence of glucose. Furthermore, if derepression in glycerol was also associated with a stabilization of the Ip mRNA, then a greater accumulation of this mRNA relative to the chimeric transcripts would be expected. Results in Fig. 4 confirm this prediction.

The kinetics experiments measuring the return to the steady-state levels of the glucose-repressed condition supported this interpretation. It was apparent from the experiments shown in Fig. 5 that the Ip mRNA must have a very short half-life in the presence of glucose, since the repressed steady-state level appeared to be reached after 5 min or less, even when transcription was continuing. In the more elaborate series of experiments shown in Fig. 7 and 8, we attempted to measure the half-life more precisely by shutting off transcription in the temperature-sensitive mutant by a shift to the nonpermissive temperature. Upon addition of glucose, the observed half-life was less than 5 min, while the Ip mRNA level in the presence of glycerol remained almost the same over a period of 1 h in the absence of further synthesis. Turnover of the Ip mRNA was so rapid that it was irrelevant whether polymerase II transcription was turned off or not.

We also tested the stability of the Ip mRNA after blocking transcription in a temperature-sensitive polymerase II mutant under conditions in which no change in carbon source occurred. When cells are maintained in glycerol, there is virtually no turnover of Ip mRNA at the nonpermissive temperature over a period of 60 min; a similar experiment in YPD led to a rapid disappearance of the transcript when further synthesis was prevented (Fig. 8).

The availability of a probe for the functionally related Fp gene transcript (Materials and Methods) made it possible to extend these studies to a second mRNA. In all experiments, we observed a similar behavior: a very short half-life (<10 min) in the presence of glucose and a much longer half-life (≥60 min) in glycerol. A more detailed kinetics analysis may confirm some small differences in the corresponding rate constants for synthesis and turnover, reflecting the size difference between the two mRNAs.

The behavior of the *CYC1* mRNA deserves some additional comments. The data in Fig. 5 suggest that its half-life is also strongly influenced by the nature of the carbon source, but the results shown in Fig. 3 indicate that in comparison with the Ip and Fp mRNAs, the behavior may be less dramatic. Further experiments comparing different strains will be required to confirm these preliminary data.

Two reports published some time ago (35, 36) have provided somewhat contradictory results. The first report described that glucose rapidly repressed cytochrome *c* synthesis, and the results were explained by postulating either a very fast decay of the corresponding mRNA or the existence of a translational block. In a follow-up of this work, the authors measured the rate of in vivo *CYC1* mRNA transcription by DNA excess hybridization of pulse-labeled RNA. They concluded that the half-life of *CYC1* mRNA was unchanged under repressed and derepressed conditions, but to account for the previous results, they had to invoke a difference in the functional and hybridizable half-life of the mRNA: a modification induced by glucose rendered the mRNA untranslatable without destroying it. Clearly, the

discrepancy between our measurements and those obtained by pulse-chase experiments (35) requires further attention.

As far as we are aware, most of the studies on gene expression and glucose repression in *S. cerevisiae* have emphasized regulation at the transcriptional level and the nature of the interaction of transcription factors such as the HAP2/3/4 complex with UASs, for example, the UAS1 or UAS1UP1 of the *CYC1* gene (11, 22, 23). In fact, the use of LacZ fusions to study yeast promoters (24) ignores the possibility of posttranscriptional mechanisms of regulation.

The experiments described here add another dimension to the study of the phenomenon of glucose repression. They raise the exciting prospect of studying a system in which the half-life of an mRNA can easily be controlled by manipulations of media. Furthermore, the experiments with the chimeric mRNA from the disrupted gene exclude for the moment the 5′ portion of the Ip mRNA as the relevant part of the transcript. It seems more likely that the 3′ half of the Ip mRNA, and possibly the 3′ untranslated region, has sequence elements which respond to *trans*-acting factors whose activity is controlled indirectly by glucose. A future task will be to identify more precisely the domain of the mRNA which is responsible for the interaction with the hypothesized *trans*-acting factor. It may be helpful that several mRNAs investigated in this study have exhibited the same behavior, and the relevance of certain sequences in the 3′ untranslated region is under investigation.

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