Glucose-dependent Turnover of the mRNAs Encoding Succinate Dehydrogenase Peptides in *Saccharomyces cerevisiae*: Sequence Elements in the 5' Untranslated Region of the Ip mRNA Play a Dominant Role

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We have demonstrated previously that glucose repression of mitochondrial biogenesis in *Saccharomyces cerevisiae* involves the control of the turnover of mRNAs for the iron protein (Ip) and flavoprotein (Fp) subunits of succinate dehydrogenase (SDH). Their half-lives are >60 min in the presence of a nonfermentable carbon source (YPG medium) and <5 min in glucose (YPD medium). This is a rare example in yeast in which the half-life of an mRNA can be controlled by manipulating external conditions. In our current studies, a series of Ip transcripts with internal deletions as well as chimeric transcripts with heterologous sequences (internally or at the ends) have been examined, and we established that the 5'-untranslated region (5' UTR) of the Ip mRNA contains a major determinant controlling its differential turnover in YPG and YPD. Furthermore, the 5' exonuclease encoded by the *XRN1* gene is required for the rapid degradation of the Ip and Fp mRNAs upon the addition of glucose. In the presence of cycloheximide the nucleolytic degradation of the Ip mRNA can be slowed down by stalled ribosomes to allow the identification of intermediates. Such intermediates have lost their 5' ends but still retain their 3' UTRs. If protein synthesis is inhibited at an early initiation step by the use of a *prt1* mutation (affecting the initiation factor eIF3), the Ip and Fp mRNAs are very rapidly degraded even in YPG. Significantly, the arrest of translation by the introduction of a stable hairpin loop just upstream of the initiation codon does not alter the differential stability of the transcript in YPG and YPD.

These observations suggest that a signaling pathway exists in which the external carbon source can control the turnover of mRNAs of specific mitochondrial proteins. Factors must be present that control either the activity or more likely the access of a nuclease to the select mRNAs. As a result, we propose that a competition between initiation of translation and nuclease action at the 5' end of the transcript determines the half-life of the Ip mRNA.

INTRODUCTION

The phenomenon of "glucose repression" in *Saccharomyces cerevisiae* has received considerable attention,

because it represents an excellent model system to study gene expression in response to a changing environment (Fraenkel, 1982; Szekely and Montgomery, 1984; Celenza and Carlson, 1986; Johnston and Carlson, 1992; Trumbly, 1992). In the absence of a fermentable carbon source, the cell optimizes its energy metabolism by inducing the large number of genes required for the biosynthesis of active mitochondria.

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In the presence of glucose in the medium, the assembly of the mitochondrial electron transport chain is suppressed. The expression of other genes encoding nonmitochondrial proteins, involved in carbohydrate transport and metabolism, is also being regulated in such a carbon source-dependent manner (Ronne, 1995).

A fruitful approach to identify components involved in glucose repression has been to isolate mutants in this regulatory mechanism. Mutations have been identified that cause constitutive repression or derepression regardless of carbon source, suggesting the involvement of both positive and negative factors in this regulatory system (Carlson *et al.*, 1981; Celenza and Carlson, 1986; Neigeborn and Carlson, 1987; Trumbly, 1988; Williams and Trumbly, 1990; Williams *et al.*, 1991; Laurent and Carlson, 1992; Trumbly, 1992; Winston and Carlson, 1992). Many of the factors identified are involved in the regulation of gene expression at the transcriptional level. Past work has demonstrated the HAP2/3/4 complex of peptides to be a transcriptional activator that binds sequence elements in the promoter regions of relevant target genes (Forsburg and Guarente, 1989). A role in transcriptional regulation has also been proposed for the multifunctional DNA-binding proteins ABF1 and CPF1 (De Winder and Grivell, 1992). Certain other gene products, such as the SNF1 protein kinase and the REG1 protein, are part of a signal transduction network by which a signal from extra-cellular glucose is transmitted through the cytoplasm to the nucleus (Johnston and Carlson, 1992; Trumbly, 1992). Thus, several types of proteins with different functions must interact for the presence of glucose to cause transcriptional changes at selected promoters (Levitzi, 1988; Gross *et al.*, 1992; Trumbly, 1992).

Our own studies have focused on the expression of the genes for succinate dehydrogenase (SDH), an enzyme of the Krebs cycle and a constituent of complex II on the inner mitochondrial membrane that couples electron flow from the Krebs cycle to the electron transport chain. The SDH complex consists of an iron protein subunit (Ip; *SDH2* gene) and a flavoprotein subunit (Fp; *SDH1* gene) (Lombardo *et al.*, 1990; Chapman *et al.*, 1992). In our initial study of the regulation of the Ip gene, we obtained evidence for transcriptional control involving the HAP2/3/4 complex binding to CCAAT boxes in upstream activator elements of the promoter, but by far the most dominant response to the absence or presence of glucose appeared to be the change in the half-life of the Ip and Fp mRNAs (Lombardo *et al.*, 1992). In *hap2* or *hap3* mutants the accumulation of these mRNAs in glycerol was still observed. From experiments with temperature-sensitive RNA polymerase II mutants we concluded that in medium with glycerol as the carbon source (YPG, G = glycerol), the half-life was >60 min; in contrast, the

half-life of Ip mRNA in glucose-containing medium (YPD, D = dextrose) was <5 min. A relatively high steady state level of Ip and Fp mRNAs in YPG could be reduced to a new and low steady state within 5–10 min after the addition of glucose (Lombardo *et al.*, 1992). The results suggested that the addition of glucose to induced yeast cells caused a very rapid activation of a specific mechanism for the turnover of these two mRNAs. Our observations are similar to those reported by Federoff *et al.* (1983), who demonstrated that the loss of maltase mRNA due to catabolite repression was also caused in part by a change in the half-life of this transcript.

Three types of mRNA turnover have been distinguished in yeast as follows: 1) There are constitutively short-lived transcripts such as the *MAT α 1* mRNA (Parker and Jacobson, 1990; Caponigro *et al.*, 1993) and the *MFA2* mRNA (Lowell *et al.*, 1992; Muhrad and Parker, 1992). 2) There is a pathway for degrading mRNAs containing nonsense mutations, which is dependent on the UPF1 gene product (Leeds *et al.*, 1991, 1992). 3) There are regulated pathways revealed by our studies (Lombardo *et al.*, 1992) and the analysis of certain meiotic transcripts (Surosky *et al.*, 1994). Expert recent reviews by Sachs (1993) and Decker and Parker (1993 and 1994) have summarized the salient features of the first two pathways, what is known about destabilizing sequences in those mRNAs, and the coupling of mRNA turnover to translation and deadenylation. It is unclear whether the first two classes share any common features with glucose-sensitive turnover.

Along with comparing the mechanism of regulated mRNA turnover to constitutive and nonsense codon-mediated mRNA decay, our initial goal was to understand how an element within the Ip mRNA could confer differential stability to the transcript. In these studies, we have localized the *cis*-acting sequences responsible for the selective destabilization to the 5' end of the transcript. We present data suggesting that the Ip mRNA is degraded in a 5' $\rightarrow$ 3' direction, and we identify a gene product primarily responsible for the nucleolytic attack. Finally, these observations lead us to investigate the roles of translational initiation and elongation in this regulated turnover. Taken together, our results suggest a mechanism by which glucose controls the access of nuclease to the 5' end of the transcript.

MATERIALS AND METHODS

Yeast Strains and Vectors

The SDH-deficient yeast strain (YSC16) derived by disruption of the Ip gene has been described by us (Lombardo *et al.*, 1992). It was used as a host for the shuttle vectors pRS313, pRS314, and pRS315 (Sikorski and Hieter, 1989), carrying various gene constructs. The other strains used in this work were generously provided by other investigators and have been previously described (Table 1) (van Loon *et al.*, 1983; Lombardo and Scheffler, 1989; Larimer and Stevens, 1990).

Table 1. Strains used in experiments

Strain	Genotype	Origin	Reference
DL1	<i>MATα ura3 his3 leu2</i>	M. Yaffe	van Loon <i>et al.</i> 1983
YSC16	<i>MATα ura3 his3 leu2 sdh2::URA3</i>	A. Lombardo and Scheffler	Lombardo <i>et al.</i> 1989
PLY22	<i>MATα ura3 his4 met14 SUF1-1</i>	M. Culbertson and A. Atkin	Leeds <i>et al.</i> 1992
PLY22a	<i>MATα ura3 his4 met14 SUF1-1 upf1-Δ2::URA3</i>	M. Culbertson and A. Atkin	Leeds <i>et al.</i> 1992
YNN27	<i>MATα ura3 trp1</i>	A. Stevens	Larimer and Stevens, 1992
YNN27xrn	<i>MATα ura3 trp1 xrn1::URA3</i>	A. Stevens	Larimer and Stevens, 1992
MCY1845	<i>MATα ade2-10 ura3 snf1-Δ10</i>	M. Carlson	Celenza and Carlson, 1986
Δ cup1-51	<i>MATα cup1Δ::URA3 leu2 trp1 lys2 ade his3 ura3 GAL⁺</i>	C. Guthrie	Lesser and Guthrie, 1993
YAS504	<i>MATα prt1-1 trp1 ura3 leu2 his3</i>	A. Sachs	Personal communication
YDPA1	<i>MATα ura3 leu2 rpb1-1 sdh2::ura3</i>	D. Atencio	This work
Y262	<i>MATα ura3-52 his4-539 rpb1-1</i>	T. Young	Nonet <i>et al.</i>
H952	<i>MATα gcd2-1 ura3-52 leu2-3 leu2-112</i>	A. Hinnebusch	Cigan <i>et al.</i>
F232	<i>MATα ura3-52 his4-303 sui2-1</i>	A. Hinnebusch	Cigan <i>et al.</i>
PHY1086	<i>MATα prt1-1 ade2 his3 leu2 trp1 ura3</i>	P. Herman	Personal communication
JRRY2334	<i>MATα PRT1 ade2 his3 leu2 trp1 ura3</i>	P. Herman	Personal communication

We made the double mutant carrying the disrupted *Ip* gene and the *upf1* mutation by mating and sporulating the resulting diploids. Haploid cells unable to grow on SG medium were tested by Southern analysis for the disrupted *UPF1* gene. In later studies we also made use of a double mutant (YDPA1) with the temperature-sensitive RNA polymerase II (Nonet *et al.*, 1987) and a new, nonreversible disruption of the endogenous *Ip* gene (Lee, unpublished data).

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 media (0.67% Difco yeast nitrogen base without amino acids, with 2% dextrose or 3% glycerol, respectively) (Sherman *et al.*, 1982), with the appropriate supplements.

The plasmid pYactI (Ng and Abelson, 1980) was kindly provided by D. Atencio and M. Yaffe (University of California, San Diego, CA). pYac4 (Burke *et al.*, 1987) was a gift of R. Ketudat-Cairns (UCSD), and pKC59 was generously contributed by K. Chapman and J. Boeke (Johns Hopkins University, Baltimore, MD) (Chapman *et al.*, 1992).

Northern Analysis

Total RNA was extracted from yeast as described (Ausubel *et al.*, 1988), followed by two additional 2 M lithium chloride precipitations (Sambrook *et al.*, 1989) to increase the purity of the RNA. RNA concentrations of each sample were determined spectrophotometrically. Northern analyses were carried out by standard protocols (Ausubel *et al.*, 1988). Filters were UV cross-linked for 75 s in a Fotodyne DNA transfer lamp. Prehybridization and hybridization solutions consisted of 50% formamide, 5 $\times$ SSPE (pH 7.4), 5 $\times$ Denhardt's solution, 0.5% SDS, and 200 μ g/ml denatured salmon sperm DNA.

In general, at least two independent clones were analyzed in repeat experiments. In the later stages of this project, the signal on Northern blots was quantitated with the FUJIX BAS1000 imager system or the Bio-Rad Molecular Imager System (Richmond, CA).

Probes

Radioactive probes were made with the Random Primer DNA Labelling System (Life Technologies, Gaithersburg, MD). The 5' *Ip* probe was derived from a 568-bp *PstI*-*XbaI* fragment of the coding

sequence (49). The 3' *Ip* probe consisted of an 832-bp *XbaI*-*HindIII* fragment that included the last 150 bp of open reading frame and the entire 3'-untranslated region (UTR) extending beyond the poly(A) addition site (Lombardo *et al.*, 1990). The 5' *Ip* oligo probe was an end-labeled 20 mer derived from bases +111 to +136 of the *Ip* coding sequence. The *Fp* probe was a 1030-bp *BglIII*-*BglIII* fragment from pKC59 (Chapman *et al.*, 1992). The 5' *ACT1* probe was derived from a 2.2-kb *PstI*-*KpnI* fragment, while the 3' *ACT1* probe was an 870-bp *KpnI*-*EcoRV* fragment of pYactI. The *HIS3* probe was derived from a 493-bp *NdeI*-*PstI* fragment of pYac4. The *URA3* probe was an 845-bp *NsiI*-*PstI* fragment from pRS316. The *LEU2* probe corresponded to a 486-bp *EcoRI*-*Clal* fragment of pRS315. A 335-bp *KpnI*-*PstI* fragment from the pG24 plasmid (Lesser and Guthrie, 1993) was used as the *CUP1* probe.

Standard Assay of mRNA Levels

SDH-deficient yeast cells (YSC16) harboring the plasmids of interest were grown overnight in YPD to an OD₆₀₀ of approximately 0.5. An aliquot of these glucose-grown cells (approximately 20 ml) was removed and processed at this point. The remaining culture was centrifuged for 3 min at high speed (2000 rpm), the supernatant was removed, and the cell pellet was resuspended in prewarmed YPG and grown with vigorous shaking at 30°C for 45 min. After removing and processing an aliquot of the glycerol-induced culture, sufficient glucose was added to the remaining culture to bring the glucose concentration to 2% (YPG + D). These cultures were harvested after the desired incubation times and immediately processed for RNA extraction.

Plasmid Constructions

A short summary and description of the various plasmids constructed for this study is presented in Table 2. The correct construction of chimeric genes was confirmed by extensive enzyme restriction analysis and/or sequencing.

The pGCΔA and pGCΔB 3' deletion series were produced in the following manner: pRSIp7, a pRS315 based plasmid with the 2.2-kb SDH *Ip* gene inserted at the *SacI* and *HindIII* sites (Lombardo *et al.*, 1990), was linearized at the *XbaI* site (+650) located in the coding sequence. The DNA was treated for increasing periods with *ExoIII* exonuclease, blunt-ended with S1 nuclease as prescribed (Nested

Deletion Kit, Pharmacia, Piscataway, NJ), and then ligated to an excess amount of *Xba*I linkers (New England Biolabs, Beverly, MA; pTGCTCTAGAGCA). The series was then digested with *Xba*I and *Hind*III, and the resulting deletion fragments representing the 3' portion of the Ip gene were cloned into the corresponding sites of pGem 3zf(–) (Promega, Madison, WI). After being sequenced, the cloned fragments were excised with *Xba*I and *Hind*III and used to replace the corresponding wild-type *Xba*I–*Hind*III fragment of the Ip gene in the pRSIp7 vector. Hence, each member of the deletion series pGCΔA and pGCΔB contains the intact Ip promoter and the noncoding and coding sequence upstream of the *Xba*I site.

Table 2. Plasmids made for this study

Plasmid	Description
pRSIP7	pRS315 containing the wild-type Ip gene
pAL-E55	Complete Ip transcript under the control of a deleted Ip promoter that contains only 41 bp upstream of its TATA box
pAL-E56	Complete Ip transcript under the control of a deleted Ip promoter that contains only 4 bp upstream of its TATA box
pGCΔA and ΔB	Series of pRSIp7 derivatives possessing the Ip promoter, 5' UTR, and majority of its coding sequence with increasing deletions starting at the <i>Xba</i> I site and extending toward the polyA site
pGC2	Fusion of the Ip promoter, 5' UTR, and 5' coding sequence (+1 to +402) ligated to the actin 3' coding sequence, 3' UTR, and polyA site
pGC3	Fusion of the Ip promoter, 5' UTR, and 5' coding sequence (+1 to +311) ligated to the HIS3 3' coding sequence, 3' UTR, and polyA site
pGC4	Fusion of the Ip promoter, 5' UTR, and 5' coding sequence (+1 to +642) ligated to the HIS3 3' coding sequence, 3' UTR, and polyA site
pGC7	Fusion of the actin promoter, 5' UTR, and coding sequence ligated to the Ip 3' coding sequence, 3' UTR, and polyA site
pGC9	Fusion of the actin promoter, 5' UTR, and coding sequence ligated to a minimal region of the Ip 3' UTR and polyA site
pGC15	Deletion in the Ip coding sequence of pRSIp7 resulting in a premature stop codon 15 aa after the start codon
pGC19	In frame deletion of 70% of the Ip coding sequence of pRSIp7
pGC40	Internal substitution of >90% of the coding sequence and 3' UTR of pRSIp7 with 300 bp of the HIS3 gene
pGC43	Fusion of the Ip promoter and "elongated" 5' UTR (up to position +28) with complete coding sequence, 3' UTR, and polyA site of the CUP1 gene
pGC62	Fusion of the LEU2 promoter, 5' UTR, and coding sequence ligated to the Ip 3' coding sequence, 3' UTR, and polyA site
pJB1	Complete Ip transcript with a premature stop codon introduced 35 codons after the initiation codon
pMS8	Internal substitution of 75% of the Ip coding sequence of pRSIp7 with the respective region of the human Ip coding sequence
pMS13	Fusion of the Ip promoter and 5' UTR (up to position –2) with complete coding sequence, 3' UTR, and polyA site of the CUP1 gene

The plasmid pGC2 was constructed by combining the *Kpn*I–*Eco*RV *ACT1* fragment (870 bp) of pYact1 with the 1.15-kb *Kpn*I–*Kpn*I fragment including the Ip promoter and 5' portion of Ip coding sequence. This chimeric 5' Ip-3' *ACT1* gene was reinserted into pRS315. pGC3 was derived by excising the 1.16-kb *Nhe*I–*Hind*III (filled in) fragment from pRSIp7 and replacing it with the 458-bp *Nhe*I–*Ssp*I fragment of pRS313 that contains the 3' end of the *HIS3* gene. pGC4 was constructed by exchanging the *Xba*I–*Hind*III (filled in) fragment of pGCΔB3, which lacks a polyA addition signal, with the same *Nhe*I–*Ssp*I *HIS3* fragment used for pGC3. pGC7 was constructed by combining the *Eco*RI–*Kpn*I fragment of pYact1 with the *Xba*I–*Hind*III fragments from the 3' end of the Ip gene. This 5' *ACT1*-3' Ip chimeric construct was then cloned into the *Pst*I site of pRS315.

The internal substitutions and deletions were made as follows. pGC14 and pGC19 were constructed by removing various internal restriction fragments from the Ip coding sequence, filling in the overhangs and blunt end ligation. The 2.0-kb *Sac*I–*Xho*I insert was excised from pGC14 and cloned into the polylinker of pRS314 to yield pGC15. pGC40 was created by replacing the *Stu*I–*Xba*I fragment of pGCΔA9 with the 298-bp *Bal*I–*Nhe*I fragment of the *HIS3* coding sequence. The construction of the chimeric human/yeast Ip genes has been described elsewhere (Saghebini *et al.*, 1994).

pMS13 was constructed by first amplifying a 428-bp fragment containing the entire *CUP1* coding sequence and 3' UTR by polymerase chain reaction (PCR) and the use of the following primers: pCCGGATCCATGTTTCAGCGAATTAA and pCCCTGCAGCTATACAGAGTTGTAAG. The PCR product was digested at the *Bam*HI and *Pst*I sites located in the primers and cloned into the *Gsu*I site (blunt-ended and modified with *Bam*HI linker) and the *Pst*I site of pRSIp7. The resulting construct possesses the Ip promoter, the 5' UTR to the –2 position, an inserted *Bam*HI site, and the entire *CUP1* ORF and 3' UTR.

pGC21 was made by first amplifying a 213-bp fragment containing the *ACT1* 5' upstream region and its N-terminal coding sequence with PCR and the following primers: pTTTGAATGCGCTCTCTTTTATCTTCC and pTTCCATGGCAGCAATCCATTGTTAATTC. The product was digested at the *Bsm*I and *Nco*I sites in the primers and cloned into the corresponding sites of pRSIp7, thereby replacing the Ip 5' UTR and a portion of its coding sequence. Secondly, the *Bsp*HI–*Xho*I (1.6 kbp) fragment of pYact1 was used as a substitute for the *Nco*I–*Xho*I fragment in this construct. The resulting construct possesses the Ip promoter and TATA box fused to the *ACT1* 5' UTR, an *ACT1* coding sequence that has an in-frame internal deletion (*Nco*I–*Bsp*HI), and the entire *ACT1* 3' UTR.

Site-directed mutagenesis was employed to insert the hairpin loop 5' GGTACCCGCGGTTCCGCCGCGGTACC 3' ($\Delta G = -20.3$ kcal/mol) at the –2 position with respect to the Ip initiation codon in pRSIp7 to yield pGC35.

pGC36 resulted from first fusing the *Sst*I–*Pst*I fragment containing the glyceraldehyde phosphate dehydrogenase (*GPD*) promoter, including its own TATA element, of pG3 (Schena *et al.*, 1991) to the *Pst*I–*Xho*I fragment of an altered Ip gene extending from the –40 position to the transcriptional termination region. This fusion product was cloned into the *Pst*I–*Xho*I sites of pRS315. The resulting transcript contains approximately 30 nucleotides of *GPD* 5' UTR, 40 nucleotides of Ip 5' UTR, and the Ip coding region and 3' UTR. pGC37 was constructed by fusing the *Pst*I–*Xmn*I portion of the *GPD* promoter, which lacks its TATA element, to the *Sst*I (filled in)–*Xho*I fragment of pAL-E56 (see below). The resulting construct contains the upstream *GPD* promoter sequence, the Ip TATA element, and the entire Ip transcribed region.

pGC43 was constructed by first using site-directed mutagenesis to alter the initiation codon in pRSIp7 to ATC. The 418-bp blunt-ended *Bam*HI–*Pst*I *CUP1* fragment from pMS13 was then used to replace the 1.4-kb *Stu*I–*Pst*I fragment of the mutated pRSIp7 clone. The new construct thus contained the Ip promoter, the Ip 5' UTR elongated by 28 nucleotides, a destroyed *Bam*HI site, and the entire *CUP1* ORF and 3' UTR.

In the pGC62 construct the *EcoRI*–*EcoRV* fragment of the *LEU2* coding sequence was replaced with a modified *XbaI*–*HindIII* fragment of the *Ip* gene yielding a 5' *LEU2*–3' *Ip* chimeric gene/transcript. For selecting this vector in the YSC16 host, we inserted a *HIS3* gene into the *BglIII* site of pRSIp7.2 (Lombardo *et al.*, 1992).

The construction of pAL-E55 and pAL-E56 have been described previously along with the mapping of the features of the *Ip* promoter (Lombardo *et al.*, 1992). pAL-E55 contains 41 bp of promoter sequence upstream of its TATA box, while pAL-E56 contains only 4 bp upstream of its TATA box along with the *Ip* coding sequence and downstream elements.

The 2.2-kb *SacI*–*HindIII* fragment of pRSIp7 was excised and cloned into pRS314 to produce pRSIp8. Similarly, the same fragment was moved from pJB1 (see site-directed mutagenesis) into pRS314 to yield pJB2.

Mapping of the PolyA Addition Site

The polyA addition sites of the wild-type *Ip*, pGCΔA9, and pGCΔB2 transcripts were mapped by using a modification of the 3' RACE protocol (Frohman *et al.*, 1988). Briefly, 5 μg of total RNA, either intact or RNase-treated (control), was processed according to the protocol and the cDNA products were diluted to a total volume of 500 μl. Five microliters were used in a PCR to amplify the 3' end of the *Ip* gene using the following oligonucleotides: pTTGGATCCTG-GTTTGGCTATTGCTG or pTTGGATCCAACAGCTGGCAGCTA-CG, and pGACTCGAGTCGACATGGATT₁₄. The GeneAmp PCR Kit (Perkin-Elmer, Norwalk, CT) was utilized with the following 30-cycle regimen: 94°C, 1 min; 52°C, 2 min; and 72°C, 2 min. The reaction mixture was purified using a G-50 Sepharose column (Sambrook *et al.*, 1989) and 2.5 M ammonium acetate precipitation (Sambrook *et al.*, 1989). The PCR products were then digested with *Sall* and *Bam*HI, purified by agarose gel electrophoresis, and cloned into pGem 3zf(–). The authenticity of the products was verified by Southern analysis, and positives were subsequently sequenced.

Mapping of the Transcription Start Site

The transcriptional start sites and capping state of various messages were determined by using the 5' RACE method of Bahring *et al.* (1994).

Site-directed Mutagenesis

Mutagenesis was executed by using the Altered Sites Mutagenesis System (Promega) or the Transformer Mutagenesis Kit (Clontech, Cambridge, UK). The early stop mutation in pJB1, the mutated initiation codon in pGC43, and the inserted hairpin structure of pGC35 were confirmed by sequencing and then cloned into pRS315.

Miscellaneous Methods and Reagents

[α-³²P]dCTP, [γ-³²P]dATP, and [³⁵S]methionine were purchased from ICN (Cleveland, OH) and Amersham (Arlington Heights, IL). Cycloheximide and 2-deoxyglucose were purchased from Sigma (St. Louis, MO). All chemicals used were of the highest grade available.

Restriction enzymes were purchased from Life Technologies or New England Biolabs. They were used according to supplier specifications. DNA sequencing was performed using the Sequenase Kit from U.S. Biochemical (Cleveland, OH). Custom oligonucleotides were purchased from Operon. Bacterial transformations were performed using the BTX 600 electroporator with standard procedures of the manufacturer. All yeast transformations were performed by the lithium acetate method (Ito *et al.*, 1983) or the PLATE method (Elble, 1992). At the end of the experiments, the glucose concentration in the media was routinely monitored by Chemstrip bG (Boehringer Mannheim, Indianapolis, IN) to confirm that the glucose concentration had not been significantly altered.

RESULTS

Ip Transcripts from Altered or Heterologous Promoters Show Glucose-sensitive, Differential Turnover

After disrupting the chromosomal copy of the *Ip* gene in the DL1 strain, we supplied this mutant with a CEN plasmid harboring the *Ip* gene with a wild-type or truncated promoter (Table 2). When these cells carrying the complete promoter (pRSIp7) were grown in glucose (YPD media), the *Ip* mRNA level was relatively low (Figure 1A). Within 45 min after the removal of the glucose media and a shift to glycerol as a carbon source (YPG), this level was elevated approximately 8- to 12-fold. The addition of glucose (or specifically dextrose) to such an induced culture caused the rapid disappearance of the *Ip* transcript in less than 10 min (YPG+D). The message for the SDH *Fp* gene displayed similar induction and turnover.

The *Ip* promoter and its four CCAAT boxes (HAP2/3/4 binding sites) have been characterized (Lombardo *et al.*, 1992), and a series of promoter deletions were made and described in part previously (Lombardo *et al.*, 1992). The plasmids pAL-E55 and pAL-E56 represent two such examples with deleted promoters: the pAL-E55 contains 41 nucleotides (nt) upstream from the TATA box and retains the most proximal CCAAT box; pAL-E56 has no CCAAT box and retains only 4 nt upstream of the TATA box. Transcripts arising from both deleted promoters accumulated in glycerol and were degraded rapidly upon addition of dextrose (Figure 1A). The induction of the pAL-E56 transcript in YPG is slightly reduced in comparison to those of the wild-type promoter; however, it is reminiscent of the induction of the *Ip* transcript in *hap2* or *hap3* mutants (Lombardo *et al.*, 1992). In these mutant backgrounds, the *Ip* promoter behaved as a minimal (constitutive) promoter lacking glucose-responsive UAS sequences (enhancers). We previously observed that the *Ip* transcript accumulated in glycerol and degraded in glucose in the absence of transcriptional activating proteins (HAP2/3/4) just as it did when it originated from the truncated promoter (pAL-E56). Thus, our current findings using truncated promoters support our postulate that *Ip* transcript behavior results primarily from message stabilization rather than transcriptional activation.

In a second attempt to negate the influence of the *Ip* promoter and concentrate on changes in message stability, we replaced the *Ip* promoter with a heterologous and constitutive *GPD* promoter described previously (Schena *et al.*, 1991). The pGC36 construct contains the *GPD* promoter including its TATA box and ~30 nt of *GPD* 5' UTR fused to the *Ip* gene at position –40 relative to the start codon. The expected transcript is essentially the shorter of two major *Ip* mRNAs, with 4 nt at the 5' end replaced by 30 nt from

Figure 1. (A) Analysis of the constructs with minimal Ip promoters. Total RNA was isolated from SDH-deficient yeast cells (YSC16) carrying various plasmids with deletions in the Ip promoter. Cells were harvested after overnight growth in 2% glucose (YPD), after shift to 3% glycerol (YPG) for 45 min, and 10 min after addition of glucose to the glycerol-induced cultures (YPG+D). Subsequent Northern blots were probed with Ip, Fp, and ACT1 probes. ACT1 is used as an internal standard to monitor loading. pRSIp7 contains the complete wild-type Ip promoter including CCAAT boxes (HAP2/3/4 binding sites; only the proximal three sites are shown). pAL-E55 has a truncated Ip promoter containing the TATA box and one of the upstream CCAAT boxes whereas pAL-E56 contains only the TATA box and four nucleotides upstream. (B) Analysis of constructs with heterologous promoters. SDH-deficient yeast cells carrying the illustrated constructs were harvested after induction on 3% glycerol (YPG) for 90 min, and at the indicated times after addition of glucose to the glycerol-induced cultures (YPG+D). The resulting Northern blot was probed with Ip, Fp, and ACT1 probes. The upstream portion of the *GPD* promoter was used to substitute for the por-

tion of the Ip (*SDH2*) promoter that contains the upstream activating sequences (UAS) and hence the presumed glucose-sensitive promoter elements. pGC36 contains the *GPD* upstream promoter elements, the *GPD* TATA box, and ~30 nucleotides of its 5' UTR fused to the Ip gene at position -40 relative to the start codon. pGC37 contains the *GPD* promoter upstream of its TATA box fused to the Ip TATA box and its downstream transcribed region. 5' RACE analysis verified that pGC36 transcripts contain chimeric 5' UTRs with *GPD* and Ip sequences while pGC37 produces wild-type Ip transcripts.

the *GPD* 5' UTR. In the pGC37 construct, the *GPD* promoter was cut ~20 nt upstream from its TATA box, and the truncated Ip gene from the pAL-E56 construct was added, i.e., the basal Ip promoter with 4 nt upstream of its TATA box (Figure 1B). Wild-type Ip transcripts were made from this heterologous constitutive promoter, as verified by the 5' RACE protocol (see MATERIALS AND METHODS).

Expression of these transcripts in cells with a disrupted endogenous gene revealed a very interesting difference (Figure 1B). The levels of pGC36 and pGC37 transcript were comparable in YPG medium. However, when glucose was added to such cultures, the pGC36 transcript level was only slightly diminished after 7.5 min, while the pGC37 transcript was reduced

to less than half of its level (Figure 1B). At later times we reproducibly observed some additional decrease in the pGC36 transcript, while the pGC37 transcript appeared to have almost reached its steady state level after ~7.5 min.

The pGC36 and pGC37 transcripts were also expressed in the *sdh2* ts-*PolIII* double mutant. Cells were grown in either YPG or YPD at the permissive temperature, and aliquots of cells were analyzed by Northern blots at various times after a shift to the nonpermissive temperature to arrest transcription (Figure 2). Both transcripts were very stable in YPG, but unstable in YPD. The pGC37 transcript disappeared noticeably faster than the pGC36 transcript. The only significant difference between these two tran-

Figure 2. Both pGC36 and pGC37 constructs were transformed into a strain (YDPA1) that contains a deletion of the chromosomal *Ip* gene and a temperature-sensitive allele of an RNA polymerase II subunit (*rpb1-1*). Cells harboring either transcript were grown on either YPD or YPG media at the permissive temperature (21°C) and then shifted to the nonpermissive temperature (37°C) to shut off transcription and allow the measurement of each message's rate of decay in the two media. Total RNA was harvested from cells at the indicated times after the temperature shift, probed with *Ip* and *ACT1* probes, and the normalized results are presented in the accompanying graphs. *Ip/ACT1* transcript ratios were quantitated with the FUJIX phosphorimager system.

scripts is at the 5' end: in the pGC36, mRNA between 4 and 19 nt from the normal *Ip* mRNA have been removed (multiple start sites), and replaced with 30 nt from the *GPD* 5' UTR. This point will be addressed again below. The pGC37 transcript, identical to the *Ip* transcript, but made from a different promoter, and thus shows the same differential stability in YPG and YPD. This behavior must therefore reflect a property of the mRNA and not of the promoter. A post-transcriptional mechanism is the most likely explanation.

The pGC37 composite promoter was made late in the course of these studies. All the chimeric constructs used to investigate *cis*-acting elements were made from the *Ip* promoter to maintain the same *Ip* transcription start sites and 5' UTR. However, we do not believe that the *Ip* promoter influences the post-transcriptional behavior of the *Ip* mRNA, based on previous (Lombardo *et al.*, 1992) and present results.

3' RACE Analysis of the *Ip* Transcript Reveals a Long 3' UTR with AU-rich Elements

The results described above clearly emphasize the importance of the 5' end in determining the differential stability of the transcript. The 5' UTR had been defined previously by primer extension and nuclease protection assays (Lombardo *et al.*, 1990), and we have confirmed that three major transcription start sites at -44, -50, and -59 are used in both YPG and YPD. However, to identify the *cis*-acting sequences responsible for making the half-life of this transcript regulated by glucose, we were required to exactly define the entire transcript, and in particular, the 3'-UTR. Its precise size was established by the 3' RACE protocol (Frohman *et al.*, 1988). As shown in Figure 3, in 9 of 10 independent clones derived by reverse transcription PCR from *Ip* mRNA, we found the polyadenylation to start at positions clustered around +1155 to 1165, or

~360 nucleotides downstream from the end of the coding sequence. A closer inspection of the untranslated downstream sequences revealed a stretch of nucleotides (+1108 → +1143) that was extremely rich in A and U. This portion of the Ip 3' UTR also contained three pentanucleotide sequences of the type AUUUA.

Such AU-rich elements (ARE) in the 3' UTR had also been found in transcripts in mammalian cells that were known to have a short half-life, for example, *c-fos*, *c-myc*, and granulocyte macrophage-colony-stimulating factor. Their significance in rapid mRNA turnover has been demonstrated *in vivo* and *in vitro* (Cleveland and Yen, 1989; Bohjanen *et al.*, 1991, 1992; Brewer, 1991; Peltz *et al.*, 1991; Shyu *et al.*, 1991; Roy *et al.*, 1992; Savant-Bhonsale and Cleveland, 1992; You *et al.*, 1992). We also thought it

noteworthy that the Fp mRNA and the *CYC1* mRNA, which degrade rapidly in the presence of glucose, have similar sequence elements in their 3' UTRs (Zaret and Sherman, 1984; Russo *et al.*, 1991; Robinson and Lemire, 1992). Our attention thus became focused on this portion of the Ip mRNA, suspecting that it may be the *cis*-acting element responsible for rapid degradation in glucose.

The Long 3' UTR of the Ip mRNA Is Not Necessary or Sufficient for Rapid Degradation in Glucose

To investigate a possible role for the ARE's in the differential stability of Ip mRNA, a series of internal deletions were constructed by ExoIII exonuclease digestion from the *Xba*I site near the end of the coding sequence toward the 3' end of the gene (Figure 3). If

Figure 3. Deletion series of the 3' end of the Ip gene. The figure shows the entire Ip transcript (top), and the three most relevant deletions ($\Delta A9$, $\Delta B2$, and $\Delta B3$); their endpoints (from sequencing) are indicated above the sequence at the bottom. The polyadenylation sites of the wild-type transcripts are indicated by the filled triangles above the sequence. The transcripts from $\Delta A9$ are polyadenylated at positions indicated by an asterisk (*) below the sequence; the transcripts from $\Delta B2$ are polyadenylated at positions indicated by filled squares above the sequence. The AU-rich elements (ARE) and the AUUUA motif within it are underlined. No transcripts are observed from the $\Delta B3$ deletion, whose deletion extends past the wild-type polyadenylation sites (nt 1182–1189). The right side of the figure shows Northern analyses of transcripts from wild-type and deleted Ip genes ($\Delta A9$, $\Delta B2$, and $\Delta B3$) expressed in the YSC16 strain. Ip, Fp, and ACT1 represent the probes used for detecting the relevant transcripts. The three lanes (+, -, and +) refer to cells grown overnight in YPD, shifted for 45 min to YPG, and 10 min after the addition of glucose to cells in YPG, respectively. The lower part of the figure shows the results with a portion of the Ip coding sequence and the Ip 3' UTR attached to heterologous upstream sequences. pGC7, transcript consisting of the ACT1 5' UTR, and coding sequence, a portion of Ip coding sequence, the Ip 3' UTR and polyadenylation site; pGC62, transcript consisting of the LEU2 5' UTR and coding sequence, a portion of Ip coding sequence, the Ip 3' UTR, and polyadenylation site. Ip, Fp, and ACT1 probes were used to detect the relevant chimeric transcripts. The blots were probed with an ACT1 fragment to confirm equal loading of all samples.

these AU-rich sequences conferred glucose-sensitive degradation, we reasoned that their removal from the Ip transcript should stabilize the resulting mRNA in dextrose. The most significant representatives of this deletion series and the behavior of the corresponding transcripts are described in Figure 3. The $\Delta A9$ deletion stops just upstream of the putative ARE region, and the transcript behaved normally in YPD and YPG. The clone $\Delta B2$ has the ARE deleted, but to our surprise the transcript still exhibited the regulated behavior in YPD and YPG. An examination of the end of this transcript revealed that the polyadenylation site had shifted further downstream, and several clones obtained by 3' RACE (MATERIALS AND METHODS) had variable polyadenylation sites (Figure 3). This shift caused the new transcripts to include other AU-rich elements and one AUUUA sequence within one of them. Similarly, we found that the transcripts from the gene with the $\Delta A9$ deletion also did not always get polyadenylated at the normal position, but at sites further downstream (Figure 3).

The design of transcripts excluding the AU-rich regions might be difficult, because in yeast the definition of a polyadenylation signal is much less precise than in mammalian cells (Proudfoot, 1991; Wahle and Keller, 1992); the examination of this 3' UTR region revealed one potential transcription termination signal (1182–1189) (Irniger *et al.*, 1991). We found quite unequivocally that a deletion, $\Delta B3$, extending to position +1198 led to the failure of the appearance of a measurable transcript, most likely because of the destruction of the polyadenylation signal (Figure 3). In summary, our attempts to delete all the AUUUA pentamers from the 3' UTR were stymied by the requirement for a polyadenylation signal; however, removal of >95% of the 3' UTR did not significantly affect the Ip mRNA turnover rate.

Because some transcripts possess two independently acting destabilization domains (Shyu *et al.*, 1991), there existed the possibility that a second sequence element in the Ip 5' UTR could compensate for the loss of the 3' UTR and maintain normal message behavior. To examine the possibility that the Ip 3' UTR was sufficient to confer differential stability to a heterologous mRNA, we replaced the 3' UTRs of stable transcripts with the 3' UTR of the Ip mRNA. Chimeric transcripts containing the 5' UTR and part of the coding sequence of the *ACT1* ($t_{1/2} = 45$ min) or *LEU2* ($t_{1/2} = 14$ min) message were fused to a short portion of Ip coding sequence together with the entire Ip 3' UTR (Table 2). These hybrid mRNAs were not downregulated in YPD and not rapidly degraded when glucose was added to a culture grown in YPG (Figure 3, pGC7, pGC62). Similar transcripts with the same *ACT1* or *LEU2* segments joined to the Ip terminal region from pGC $\Delta A9$ (Figure 3) were also constitutively expressed in YPG and YPD. Thus, the Ip 3' UTR, and specifically

the AU-rich region (including the AUUUA elements) just upstream from the poly(A), have no significant effect on the regulated instability in glucose, although they may contribute to the formation of a mature, polyadenylated transcript.

The Coding Sequence of the Ip Gene Is Not Responsible for Regulated Turnover

To determine whether the coding region played a role in the transcript's rapid degradation in glucose, several deletions were made in this portion of the Ip mRNA. Although some complications arise from the inadvertent introduction of premature stop codons in some of the coding sequences (see below), the results appear to be quite clear. A deletion of ~70% of the coding sequence from the *StuI* site near the beginning (10 codons left) to the *XbaI* site (from which previous deletions were made in a downstream direction) still allowed the abundant accumulation of a shorter transcript in YPG, which is rapidly degraded upon the addition of glucose (pGC19 in Figure 4). In this case the joining of the modified restriction sites preserved the reading frame, so that the normal start and stop codons of Ip mRNA would be utilized.

Two kinds of internal substitutions were also investigated (Table 2). The first (pGC40.2) involved a 298-bp *BalI-NheI* fragment from the *HIS3* gene replacing the *StuI-XbaI* fragment of pGC $\Delta A9$, which represents an in-frame substitution. Only the 5' UTR, the first 10 codons, and the last 60 nt of the 3' UTR and polyadenylation site from the Ip transcript are left. The resulting transcript was regulated normally in YPD and YPG (pGC40.2 in Figure 4). In addition, a series of chimeric genes were constructed by swapping coding segments of the yeast Ip with the corresponding human cDNA sequences (Saghebini *et al.*, 1994). In every case the open reading frame is preserved and some of these chimeric proteins are functionally active. A member of this series, the pMS8 transcript, was expressed and regulated normally in YPG and YPD, even with more than 70% of yeast coding sequence replaced by human sequence (Figure 4). Therefore, sequences within the coding region can be substituted without significantly disrupting the wild-type behavior of the transcript.

Chimeric mRNAs Starting with the Ip 5' UTR Display Glucose-sensitive Turnover

The analysis of the coding sequence and 3' UTR suggested that the relevant *cis*-acting sequences in Ip mRNA that control the differential stability in YPD and YPG are located in the 5' end of the transcript. To test the hypothesis that this region was sufficient for the rapid turnover of the Ip transcript in glucose, a series of constructs were made by fusing the 5' end of the Ip gene, including a

Figure 4. Northern analyses of transcripts from *Ip* genes modified in the coding region. The three lanes (+, −, and +) refer to YSC16 cells carrying these constructs grown in YPD, after 45 min in YPG, and 10 min after the addition of glucose to cells induced in YPG, respectively. The blots were probed with the *Ip* or *HIS3* fragments to detect the relevant chimeric mRNAs. (See Table 2 or MATERIALS AND METHODS for more detailed description of each construct). WT, the wild-type transcript with 5' UTR, coding sequence, and 3' UTR; pGC19, transcript with a large portion of coding sequence deleted in frame; pMS8, transcript with a large portion of yeast coding sequence replaced by the corresponding human coding sequence; pGC40.2, transcript with most of the *Ip* coding sequence and the proximal portion of the 3' UTR replaced with a segment of coding sequence from the *HIS3* gene. The AU-rich region of the *Ip* 3' UTR and the polyadenylation site are retained.

portion of the coding sequence, to the 3' portions of the *ACT1* (pGC2) or *HIS3* genes (pGC3, pGC4); neither the *ACT1* ($t_{1/2} = 45$ min) nor the *HIS3* mRNA turnover ($t_{1/2} = 7$ min) is glucose sensitive. These chimeric messages accumulated in glycerol and decayed rapidly when glucose was added back to the derepressed cells (Figure 5A). All of the corresponding transcripts, which contain the *Ip* 5' end, appeared to have regulated stabilities in YPD and YPG similar to the authentic *Ip* mRNA.

Finally, to examine the sufficiency of the 5' UTR specifically, this 44–59 nucleotide domain was fused to a region of the *CUP1* transcript in two different constructs. The *CUP1* gene product confers copper resistance to yeast. pMS13 contains the intact *CUP1* coding sequence, 3' UTR, and polyadenylation site linked to the *Ip* 5' UTR at position −2, and pGC43 represents a similar construct in which 28 nucleotides of *Ip* coding sequence are also included, but the *Ip* start codon has been eliminated by site-directed mutagenesis. The plasmids were introduced into a $\Delta cup1$ mutant (Lesser and Guthrie, 1993). The chimeric *CUP1* transcripts were induced in YPG and rapidly disappeared upon the addition of glucose (Figure 5B), paralleling the behavior of the endogenous *Ip* mRNA. It should be noted that a functional protein is produced, and the transfected strains have been shown to be more resistant to copper in SG compared with SD minimal medium. When the *CUP1* mRNA ($t_{1/2} = 18$ min) (Belasco and Brawerman, 1993) is transcribed from its endogenous promoter in the isogenic parent

strain, it accumulates to a significantly higher steady state level in YPD relative to YPG (Figure 5B, top lanes). However, the addition of glucose to YPG does not cause a rapid change in the level of this normal *CUP1* transcript.

All the above constructs possess the *Ip* promoter and the *Ip* 5' UTR. To eliminate the possibility that the *Ip* promoter influenced the fate of the transcript stability (Enssle *et al.*, 1993), we constructed pGC21. In this clone, the *Ip* promoter is connected to the *ACT1* 5' UTR, coding sequence with an internal deletion, 3' UTR, and polyadenylation site. As shown in Figure 5B (bottom lanes), the resulting transcript level was not significantly affected by the carbon source, and in particular, it was not degraded immediately upon the addition of glucose to YPG. This behavior of the mutated *ACT1* message, which contains no *Ip* sequence, indicates that the *Ip* promoter does not influence its nascent transcript's rate of turnover.

In our original publication we had interpreted the detection of a 2.4-kb transcript with the *Ip* probe to represent a transcript from the chromosomal *Ip* promoter, with a 5' segment of *Ip* mRNA, followed by a sequence from the insert that disrupts the gene. Further analysis has now shown that this transcript arises from a cryptic promoter in the insert and contains a 3' portion of coding sequence and 3' UTR. It is stable in glucose. Another transcript from the chromosomal *Ip* promoter is induced in glycerol (albeit at a reduced level) and rapidly degraded after the addition of dextrose. It contains only a 5' segment of the *Ip* mRNA

Figure 5. (A) Northern analysis of chimeric transcripts with the 5' end of the *Ip* mRNA and 3' portions and polyadenylation sites of *ACT1* (pGC2) or *HIS3* (pGC3 and pGC4) mRNAs. All transcripts were expressed from the *Ip* promoter. YSC16 cells harboring the constructs were assayed as described in the previous figures. YPD, overnight growth in glucose; YPG, after 45 min in glycerol medium; YPG+D, 10 min after the addition of dextrose to the induced cultures. Restriction sites in the *Ip* gene are indicated at the top (WT), and the corresponding breakpoints in the *Ip* gene in the chimeric constructs are indicated for pGC2, pGC3, and pGC4, respectively. The chimeric *Ip* transcripts were detected by a probe derived from the 5' region of the *Ip* gene while an *ACT1* probe was used to standardize for equal loading and to detect the pGC21 transcript. (B) Top: Steady state levels and degradation of *Ip* and *CUP1* mRNAs in YPD, YPG, and after the addition of glucose to YPG. Wild-type (WT) cells were grown in YPD, shifted for 45 min to YPG, and harvested 10 and 30 min after the addition of glucose to cells in YPG, respectively. The mRNAs are expressed from the wild-type, chromosomal genes. Middle: Steady state levels and degradation of *Ip* and *Ip-CUP1* chimeric mRNAs expressed from the *Ip* promoter. $\Delta cup1$ cells expressing chimeric *Ip-CUP1* mRNAs were assayed as described in the section above. The transcript detected by the *Ip* probe is produced by the endogenous, normal *Ip* gene. The pMS13 and pGC43 transcripts, detected by the *CUP1* probe, are from chimeric genes with *Ip* promoters introduced into a $\Delta cup1$ strain. pMS13 mRNA has the *Ip* 5' UTR (from -59, -50, or -44 to -2) linked to the *CUP1* coding sequence and 3' UTR; pGC43 mRNA contains the complete *Ip* 5' UTR and 28 nucleotides of coding sequence, but with the start codon removed by mutagenesis, fused to the *CUP1* coding sequence and 3' UTR. The blots were probed with an *ACT1* fragment to confirm equal loading of all samples. Bottom: The pGC21 transcript was transcribed from the *Ip* promoter and contains the *ACT1* 5' UTR, an internally deleted (*NcoI-BspHI*), in-frame *ACT1* coding sequence, and the *ACT1* 3' UTR (Table 2).

and ends with a sequence from the insert. These results agree with our new interpretation that the most significant domain for the regulated turnover of Ip mRNA is in the 5' UTR.

The 5' Exonuclease XRN1 Is Involved in the Degradation of the Ip mRNA

The XRN1 gene product is a protein of 170 kDa that is involved in a variety of functions (Kearsey and Kipling, 1991), but a major activity appears to be a 5' → 3' exonuclease that accounts for 30–40% of the RNase activity of crude yeast extracts (Stevens, 1980; Larimer *et al.*, 1992). The half-lives of two short-lived mRNAs increase two- to fourfold in yeast mutants in which this gene had been disrupted, while stable mRNAs were not affected significantly (Larimer *et al.*, 1992). Recent experiments by Parker's group have also implicated the XRN1 nuclease in the degradation of short-lived mRNAs after they are decapped (Muhlrad *et al.*, 1994). We therefore tested the behavior of the endogenous Ip mRNA in a strain with the *xrn1* mutation. In distinct contrast to the observations in the parental cells, the high level of Ip and Fp transcripts induced in YPG persisted for at least 15 min after the addition of glucose in the *xrn1* strain (Figure 6). These results suggest that the 5' → 3' exonuclease encoded by the XRN1 gene plays a central role in this regulated process. After 30–60 min the transcript is slowly degraded, suggesting that other nucleases may eventually become involved.

Cycloheximide Slows Down the 5' → 3' Degradation of the Ip Message

A number of studies on mRNA turnover in yeast and other organisms have indicated a coupling of mRNA degradation to protein synthesis and the association of nucleolytic activity with polysomes (Santiago *et al.*, 1987; Brawerman, 1989; Brown, 1989; Atwater *et al.*, 1990; Herrick *et al.*, 1990; Kruys *et al.*, 1990; Parker and Jacobson, 1990; Koeller *et al.*, 1991; Leeds *et al.*, 1991; Peltz *et al.*, 1991; Wisdom and Lee, 1991; Knutsen *et al.*, 1992; Savant-Bhonsale and Cleveland, 1992; Theodorakis and Cleveland, 1992; Yarchuk *et al.*, 1992). A question raised by our observations was whether the glucose-triggered degradation of Ip mRNA required the synthesis of new proteins. In view of the extremely rapid triggering of turnover by the addition of glucose, it seems unlikely that new proteins have to be synthesized, but rather that pre-existing proteins are altered or activated (see DISCUSSION).

We investigated whether an arrest in protein synthesis just before the change in medium could affect Ip mRNA degradation by using the translation inhibitor cycloheximide (Figure 7A). The addition of cycloheximide delayed the dextrose-sensitive degradation for

Figure 6. Northern analysis of Ip and Fp transcripts from the *xrn1* mutant and its wild-type parent. Wild-type (YNN27) and two independent clones of *xrn1* yeast (YNN27*xrn1*) were grown in YPD, shifted for 45 min to YPG, and harvested 5 and 15 min after the addition of glucose to cells in YPG. Only the 15 min timepoint is shown for the second clone. The indicated probes were used to monitor the behavior of the endogenous, chromosomally derived transcripts.

approximately 30 min. We also observed the transcripts remaining after 60 min to be not only fewer in quantity, but reduced in size (Figure 7A). The same RNA samples were re-run at higher resolution and hybridized with either an oligonucleotide from within the first 150 nucleotides of the coding region (5' oligo probe), or a probe corresponding to the 3' UTR (3' Ip probe). Figure 7B shows that the signal obtained with the 5' oligo probe disappeared after less than 30 min following the addition of glucose (much of it after 10 min), while the signal from the 3' Ip probe disappeared more slowly and again reflected a shortening transcript. These results are consistent with a degradation process starting at the 5' end and proceeding toward the 3' end. In this case it may have been slowed down by the stalled ribosomes paused on the message, making it possible to identify intermediates of reduced length. Separate control experiments indicated that the addition of cycloheximide to induced cells remaining in YPG did not cause any degradation of Ip mRNA for up to 90 min.

The Ip Message Is Destabilized in Glycerol If Translation Initiation Is Disrupted

We desired to examine the relationship between translation and the regulated transcript turnover without having to consider the pleiotropic side effects of a drug. An alternate means of inhibiting translation was to use a *ptf1* mutant, in which an essential factor (eIF-3) for initiation of protein synthesis is temperature sensitive (Hanic-Joyce *et al.*, 1987). When such mutant cells were grown in YPG at the permissive temperature (21°C), Ip mRNA was induced as expected. The addition of glucose caused rapid degradation, but most significantly, the rapid degradation of the Ip mRNA was also initiated in YPG simply by switching the cells to the nonpermissive temperature

(Figure 8). It should also be noted that *ACT1* and *CUP1* mRNA were not degraded under these conditions. Furthermore, the Ip transcript was stable in YPG at 36°C in the isogenic wild-type parent under the same experimental conditions. A similar behavior was observed for the Fp transcript, and with another strain carrying the *prt1-1* allele (YAS504, Table 1) (results not shown).

Two other temperature-sensitive mutants in initiation of protein synthesis were examined. In *ts sui2-1* and *ts gcd2-1* strains (Cigan *et al.*, 1991; Foiani *et al.*, 1991) the behavior of the Ip mRNA at the nonpermissive temperature was distinctly different (Figure 8): the Ip mRNA was completely stable in YPG. Significantly, the *prt1* mutation causes a complete failure to form the ternary preinitiation complex, including the 40 S subunit, at the 5' CAP of an mRNA (Feinberg *et al.*, 1982), whereas in the other two mutants this complex is formed, but later steps in initiation such as scanning or joining with the 60S subunit at the AUG codon are impaired (Keireleber *et al.*, 1986; Bushman *et al.*, 1993).

The Differential Turnover of the Ip Transcript Does Not Depend on the Upf1 Protein or Translational Elongation

Several studies have indicated that the introduction of premature stop codons into mRNAs can have a dramatic effect on the stability of such mRNAs. If the stop codon is near the beginning of the coding sequence, most mRNAs are rapidly degraded by a mechanism that involves the UPF proteins (Leeds *et al.*, 1991, 1992). In a possibly unique case, if a stop codon is introduced upstream from a destabilizing region in

the coding sequence of the *MAT α 1* mRNA, it increases the half-life of the transcript. This result suggests that the passage of ribosomes through this region helps determine the short half-life of this transcript (Parker and Jacobson, 1990).

To determine whether UPF1 function was involved in the glucose-regulated turnover, we examined the behavior of Ip and Fp mRNAs in a mutant lacking this gene product. The regulated turnover of these messages was completely normal, indicating that the UPF1 protein plays no role in the glucose-induced degradation of these transcripts (Figure 9). Two altered Ip transcripts were also investigated to determine whether the dextrose-sensitive turnover depends on the presence of ribosomes on the downstream region of the mRNA. The pGC15 transcript has an internal deletion from the Ip coding sequence, causing a frame shift and hence a premature stop codon 16 codons downstream from the start codon. The pJB1 transcript has a stop codon at codon #36 introduced by site-directed mutagenesis (MATERIALS AND METHODS). Both of these transcripts were quite unstable in YPG in a *UPF1* (wild type) background (Figure 9). We then introduced these plasmids into a double mutant carrying the disrupted Ip (*SDH2*) gene and the *upf1* mutation. The corresponding transcripts were then induced to the expected levels in YPG, and rapidly decayed following the addition of glucose (Figure 9). There is thus no need for the translation of the greater part of the Ip mRNA to exhibit the regulated stability described in this study. Translational elongation is required for the general stability but not the differential turnover of the transcript.

Figure 7. (A) Effect of cycloheximide on the turnover of Ip mRNA. YSC16 yeast expressing the wild-type Ip gene from pRSIp7 were induced in YPG. Cycloheximide (final concentration = 100 μ g/ml) or water was then added 5 min before the addition of glucose to the induced cultures. Aliquots of cells were harvested at the indicated times after the addition of glucose, and the resulting RNA was probed with a fragment from the 3' portion of the Ip gene. The control without cycloheximide is shown on the right. Complete inhibition of protein synthesis was verified by monitoring the incorporation of [35 S]methionine into trichloroacetic acid-precipitable material (The elevated signal at 60 min is a loading artifact).

(B) Northern analysis of the transcripts remaining in the presence of cycloheximide with 5' and 3'-specific Ip probes. The RNA samples from the cycloheximide-treated cultures of Section A were rerun for improved resolution. The samples were hybridized first with an oligonucleotide probe from the 5' portion of the Ip gene, stripped, and then reprobbed with a random-primed fragment from the 3' portion of this gene. Five to ten minutes after the addition of glucose, the transcript is barely detectable with the 5' oligo probe, but its smaller derivative is detectable with the 3' probe up to 90 min.

Figure 8. Effect of the arrest of initiation of protein synthesis on the stability of Ip mRNA. (A) Northern analysis of RNA from temperature-sensitive *prt1* mutant and isogenic parent cells grown in YPG at 21°C and at indicated times after a shift to the nonpermissive temperature (36°C). Cells possessing temperature-sensitive alleles of *SUI2* and *GCD2* were assayed in the same manner. RNA samples were harvested at the indicated times and hybridized with the indicated probes. [³⁵S]methionine incorporation into trichloroacetic acid precipitable material was monitored to confirm a block in translation.

Another approach to investigating the relationship between mRNA structure, involvement in translation, and turnover was to place a relatively stable secondary structure just upstream from the start codon, to prevent the initiation complex (including the 40S ribosome) from scanning to the first AUG codon (Beelman and Parker, 1994; Sagliocco *et al.*, 1994). Our goal was to determine if this step participates in stabilizing the Ip transcript in the absence of glucose. The transcript from the pGC35 construct, which includes a hairpin loop at the -2 position, was unable to complement the SDH-deficient mutant cells, although an mRNA of the expected size was expressed. The cells were unable to grow in YPG, did not have any measurable SDH activity (< 1%), and contained no detectable Ip peptide in a Western blot (results not shown). Although this structure blocked translation of the first AUG codon,

this mRNA turned over just as the wild-type transcript (Figure 10). Therefore, the later stages of translational initiation and elongation appear to be unnecessary for modulating the stability of the Ip transcript.

DISCUSSION

Our observation, that the half-lives of mRNAs for certain mitochondrial proteins are regulated by the carbon source in the medium, has added a new dimension to the phenomenon of "glucose repression." Specifically, the mRNAs for the SDH peptides Ip and Fp have long half-lives in media containing a nonfermentable carbon source such as glycerol ($t_{1/2} > 60$ min), while their turnover in glucose-containing media is extremely rapid ($t_{1/2} < 5$ min) (Lombardo *et al.*, 1992). An earlier study of carbon catabolite repression of maltase synthesis in *Saccharomyces carlsbergensis* had hinted at a similar phenomenon (Federoff *et al.*, 1983).

Although transcriptional regulation played a minor role, regulation of the Ip message level by a post-transcriptional mechanism was shown by our studies with glucose-insensitive, minimal promoters (Figure 1), and in our experiments with *hap2* and *hap3* mutants in which essential transcriptional activation factors are absent (Lombardo *et al.*, 1992).

The major emphasis in the studies described in this paper is on the extremely rapid degradation of the transcripts when glucose is added to an already induced culture grown in YPG. An understanding of the mechanisms that govern the instability of such mRNAs must include the identification of *cis*-acting sequences and the characterization of *trans*-acting factors. Our initial efforts were directed toward the identification of sequence elements in the Ip mRNA that might dictate the differential stability in YPD and YPG.

Figure 9. Northern analysis of selected mRNAs in a UPF1 or upf1 background. ipUPF1 (YSC16) and ipupf1 (YGC2) yeast expressing the indicated constructs were assayed in the usual manner: + - + refers to cells in YPD, YPG (45 min), and YPG+glucose (10 min). pRSIP7 expresses the wild-type transcript. The pGC15 transcript contains a premature stop codon after 15 codons, and has a long deletion in the coding sequence; the pJB1 transcript consists of the entire Ip mRNA sequence but has a premature stop codon after 35 codons. Samples were probed as indicated.

Figure 10. Effect of the arrest of elongation of protein synthesis by a stable secondary structure 2 nt in front of the start codon of the Ip transcript. A stable hairpin loop was inserted at the -2 position with respect to the first AUG in the Ip-transcribed region by using the Transformer Mutagenesis Kit (Clontech) to yield pGC35. YSC16 cells expressing either the wild-type Ip mRNA (pRSIp7) or the translationally impaired Ip mRNA (pGC35) were assayed by the standard procedure, and the resultant Northern blots are shown. Blockage of Ip peptide synthesis was verified by Western blot and SDH activity assay. Ip/*ACT1* transcript ratios for each time point were quantitated as described previously and are indicated on the bar graph.

From the study of chimeric transcripts and a deletion series, we could conclude that the Ip 3' UTR is not necessary or sufficient to cause the Ip message to be differentially stable depending on the carbon source. Next, several constructs were tested in which large portions of the coding sequence of the Ip mRNA were either deleted or substituted by other sequences. The resulting transcripts exhibited a behavior that was indistinguishable from the normal Ip mRNA. Because the inherent stability of the heterologous sequence (i.e., *ACT1*, *HIS3*, and *LEU2*) contributes to the overall stability of the chimeric transcript, we joined the Ip mRNA to messages with different half-lives. Regardless of the heterologous sequence used, the 5' UTR emerged as the major sequence element with a potential regulatory function.

All chimeric transcripts that start with the 5' UTR of Ip were differentially stable in YPG and YPD. In the extreme case of the pMS13 construct the chimeric mRNA contains only the entire Ip 5' UTR (from the transcription start site(s) to the -2 position), followed by a complete and functional *CUP1* coding sequence, 3' UTR, and poly(A) tail. The 5' UTR is also notably AU-rich and includes an AUUUA sequence, but the detailed exploration of sequence elements in the 5' UTR by site-directed mutagenesis remains to be carried out. Preliminary experiments with Fp-*CUP1* fusions suggest that the 5' end of Fp mRNA is also responsible for the destabilization of such transcripts in glucose.

Because most of the transcripts with the Ip 5' UTR were derived from the Ip promoter, it could be argued

that the addition of glucose causes rapid shutoff of transcription and a constitutively unstable Ip mRNA decays in the same short time interval. Several observations argue against such an interpretation. 1) When transcription is arrested in a temperature-sensitive RNA polymerase II mutant, the Ip transcripts are very stable in YPG but rapidly degraded in YPD. 2) The minimal promoter in the pAL-E56 construct would also have to be sensitive to the carbon source and respond within minutes to repression by glucose. 3) The heterologous *GPD* promoter in the pGC36 construct would have to be less sensitive to the carbon source, while the almost identical promoter in the pGC37 construct would have to be repressible by glucose. If this were true, the significant promoter element conferring glucose-repression would be centered around and downstream of the TATA box of the Ip promoter. 4) The results with the pGC21 construct also demonstrate that the Ip promoter including its TATA box and 28 nt downstream from it do not cause instability in a stable, truncated *ACT1* mRNA.

The identification of important *cis*-acting sequence elements at the 5' end of the transcript is consistent with the finding that the 5' → 3' exonuclease (XRN1) plays a role in the glucose-regulated turnover of Ip and Fp mRNAs. More definitive evidence for the involvement of a 5' → 3' exonuclease such as XRN1 was derived from the experiment where cycloheximide was added before dextrose. Under these conditions the 5' → 3' exonuclease appears to be slowed down, allowing the detection of intermediates in the degradation. These intermediates have lost their 5' ends but

are still intact at the 3' end, confirming the direction of degradation and consistent with observations by Decker and Parker (1994 and 1993).

In some other respects, the data shown in Figure 6 remain puzzling: the rapid degradation of accumulated Ip mRNA upon addition of glucose to induced cells is delayed, but cells grown overnight (16 h) in YPD have low levels of Ip and Fp transcripts. If the XRN1 protein was the only nuclease involved in the control of turnover, and if the rate of turnover was the only determinant of steady state levels, one would have expected the transcript levels to remain high in YPD. However, the contribution of transcriptional regulation is probably not negligible over a longer time period (16 h), and it is conceivable that other, slower-acting XRN1-like nucleases are also involved in maintaining the steady state level in glucose (Hsu and Stevens, 1993; Kenna *et al.*, 1993). Because different responses occur over different time periods in glucose repression, caution must be used when comparing changes that occur within an hour to changes that occur overnight.

It is tempting to speculate on the mechanism of this differential mRNA stability. Suggestions have been made that the XRN1 exonuclease targets uncapped RNA (A. Stevens, personal communication), and therefore regulation of this degradation can be controlled at the level of decapping. An mRNA-decapping enzyme from *Saccharomyces cerevisiae* has been reported (Stevens, 1988), raising the intriguing possibility that its access or activity may be controlled by the carbon source. These considerations must be related to the fact that the 5' end of the Ip mRNA is bound by factors (e.g. eIF-3 and eIF4F) involved in the initiation of protein synthesis (Hershey, 1989, 1990, 1991), and the small ribosomal subunit. It is therefore possible to imagine that a competition between initiation factors and a 5' exonuclease for the 5' end of the Ip is influenced by the carbon source.

Data in support of this hypothesis were obtained with the *prt1* ts mutant strain. A complete arrest in initiation of protein synthesis at the nonpermissive temperature (Hanic-Joyce *et al.*, 1987) resulted in a very rapid disappearance of Ip and Fp mRNAs even when the cells were maintained in glycerol. This result suggests that the failure to form the complex with eIF-2, GTP, tRNA^{met}, 40S ribosomes, and the 5' end of the mRNA left the 5' end susceptible to nucleolytic attack. Others had shown that a subset of mRNAs of the *HSP70* heat shock genes was also specifically degraded when the PRT1 gene product was inactivated (Barnes *et al.*, 1993). In contrast, in mutants (*sui2-1* and *gcd2-1*) impaired in later stages of initiation (eIF2) the protective effect of the complex is still observed. Similarly, the experiments with the hairpin just upstream from the start codon also suggest that it is not necessary to complete initiation of translation by the addi-

tion of the 60S ribosomal subunit upon reaching the start codon. The protection of the 5' end by early initiation factors alone may be sufficient to stabilize the transcript in YPG.

On the other hand, when protein chain elongation was prematurely terminated by the creation of an early stop codon, the behavior of the Ip and Fp mRNAs was normal, provided the UPF1 protein was eliminated by mutation. With the UPF1 protein present, such mRNAs are degraded by an independent pathway designed to eliminate transcripts with premature stop codons (Leeds *et al.*, 1991, 1992).

At this time it is still difficult to relate the regulated turnover of the Ip and Fp mRNAs to the general mechanisms proposed by Sachs (1993) and Decker and Parker (1993) for the turnover of constitutively unstable mRNAs. These models emphasize that destabilizing sequences in an mRNA act directly or indirectly with a nuclease to deadenylate its poly(A) tail. Deadenylation is hypothesized to be an obligatory and rate-determining step preceding the complete nucleolytic degradation. Our future studies will investigate whether the removal of the poly(A) is affected by the presence of glucose. To fully understand how the turnover of a subset of mRNAs is regulated by the carbon source, one needs to elucidate the signaling pathway from the extracellular glucose to the ultimate rate-determining nucleolytic attack on the transcript. The behavior of the Ip message is currently being studied in yeast mutants that are affected in glucose repression. For instance, we have found that SNF1 (a protein kinase involved in transcriptional induction of glucose-repressed genes) (Celenza and Carlson, 1986; Estruch *et al.*, 1992) does not appear to play a role in the regulated Ip mRNA turnover. Our goal is to link regulators of glucose repression to factors involved in RNA degradation.

In this context, another result is noteworthy. Contrary to expectations, we found that 2-deoxyglucose does not cause the rapid degradation of Ip mRNA when added to an induced culture. This analogue, which cannot be metabolized further after its initial phosphorylation, causes the transcriptional repression of glucose-repressible genes (Neugeborn and Carlson, 1987). Transcriptional repression by glucose is believed to be triggered when it enters the cell and is phosphorylated (Trumbly, 1992). The failure of 2-deoxyglucose to trigger Ip mRNA degradation suggests that a phosphorylated glucose intermediate may need to be further metabolized before it can activate the signaling pathway controlling nucleolytic activity. Thus, the novel phenomenon described here and in our previous communication (Lombardo *et al.*, 1992) may differ quite significantly from classical glucose repression with respect to the signaling pathway.

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REFERENCES

- Atwater, J.A., Wisdom, R., and Verma, I.M. (1990). Regulated mRNA stability. *Annu. Rev. Genet.* 24, 519–541.
- Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G., and Struhl, K. (1988). *Current Protocols in Molecular Biology*, Media, PA: John Wiley and Sons.
- Bahring, S., Sandig, V., Lieber, A., and Strauss, M. (1994). Mapping of transcriptional start and capping points by a modified 5' RACE technique. *BioTechniques* 16, 807–808.
- Barnes, C.A., Singer, R.A., and Johnston, G.C. (1993). Yeast prt1 mutations alter heat-shock gene expression through transcript fragmentation. *EMBO J.* 12, 3323–3332.
- Beelman, C.A., and Parker, R. (1994). Differential effects of translational inhibition in *cis* and in *trans* on the decay of the unstable yeast MFA2 mRNA. *J. Biol. Chem.* 269, 9687–9692.
- Belasco, J., and Brawerman, G. (1993). *Control of Messenger RNA Stability*, San Diego, CA: Academic Press.
- Bohjanen, P.R., Petryniak, B., June, C.H., Thompson, C.B., and Lindsten, T. (1991). An inducible cytoplasmic factor (AU-B) binds selectively to AUUUA multimers in the 3'-untranslated region of lymphokine mRNA. *Mol. Cell. Biol.* 11, 3288–3295.
- Bohjanen, P.R., Petryniak, B., June, C.H., Thompson, C.B., and Lindsten, T. (1992). AU RNA-binding factors differ in their binding specificities and affinities. *J. Biol. Chem.* 267, 6302–6309.
- Brawerman, G. (1989). mRNA decay: finding the right targets. *Cell* 57, 9–10.
- Brewer, G. (1991). An A+U-rich element RNA-binding factor regulates c-myc mRNA stability in vitro. *Mol. Cell. Biol.* 11, 2460–2466.
- Brown, A.J.P. (1989). Messenger RNA stability in yeast. *Yeast* 5, 239–257.
- Burke, D.T., Carle, G.F., and Olson, M.V. (1987). Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. *Science* 236, 806–812.
- Bushman, J.L., Asuru, A.I., Matts, R.L., and Hinnebusch, A.G. (1993). Evidence that GCD6 and GCD7, translational regulators of GCN4, are subunits of the guanine nucleotide exchange factor for eIF-2 in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 13, 1920–1932.
- Caponigro, G., Muhlrad, D., and Parker, R. (1993). A small segment of the *MATa1* transcript promotes mRNA decay in *Saccharomyces cerevisiae*: a stimulatory role for rare codons. *Mol. Cell. Biol.* 13, 5141–5148.
- Carlson, M., Osmond, B.C., and Botstein, D. (1981). Mutants of yeast defective in sucrose utilization. *Genetics* 98, 25–40.
- Celenza, J.L., and Carlson, M. (1986). A yeast gene that is essential for release from glucose repression encodes a protein kinase. *Science* 233, 1175–1180.
- Chapman, K.B., Solomon, S.D., and Boeke, J.D. (1992). SDH1, the gene encoding the succinate dehydrogenase flavoprotein subunit from *Saccharomyces cerevisiae*. *Gene* 118, 131–136.
- Cigan, A.M., Foiani, M., Hannig, E.M., and Hinnebusch, A.G. (1991). Complex formation by positive and negative translational regulators of GCN4. *Mol. Cell. Biol.* 11, 3217–3228.
- Cleveland, D.W., and Yen, T.J. (1989). Multiple determinants of eukaryotic mRNA stability. *New Biol.* 1, 121–126.
- De Winder, J.H., and Grivell, L.A. (1992). Global regulation of mitochondrial biogenesis in *Saccharomyces cerevisiae*: ABF1 and CPF1 play opposite roles in regulating expression of the *QCR8* gene, which encodes subunit VIII of the mitochondrial ubiquinol-cytochrome *c* oxidoreductase. *Mol. Cell. Biol.* 12, 2872–2883.
- Decker, C.J., and Parker, R. (1993). A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a requirement for deadenylation. *Genes Dev.* 7, 1632–1643.
- Decker, C.J., and Parker, R. (1994). Mechanisms of mRNA degradation in eukaryotes. *Trends Biochem. Sci.* 19, 336–340.
- Elble, R. (1992). A simple and efficient procedure for transformation of yeasts. *BioTechniques* 13, 18–20.
- Enssle, J., Kugler, W., Hentze, M.W., and Kulozik, A.E. (1993). Determination of mRNA fate by different RNA polymerase II promoters. *Proc. Natl. Acad. Sci. USA* 90, 10091–10095.
- Estruch, F., Treitel, M.A., Yang, X., and Carlson, M. (1992). N-Terminal mutations modulate yeast SNF1 protein kinase function. *Genetics* 132, 639–650.
- Federoff, H.J., Eccleshall, T.R., and Marmur, J. (1983). Carbon catabolite repression of maltase synthesis in *Saccharomyces carlsbergensis*. *J. Bacteriol.* 156, 301–307.
- Feinberg, B., McLaughlin, C.S., and Moldave, K. (1982). Analysis of temperature-sensitive mutant ts 187 of *Saccharomyces cerevisiae* altered in a component required for the initiation of protein synthesis. *J. Biol. Chem.* 257, 10846–10851.
- Foiani, M., Cigan, A.M., Paddon, C.J., Harashima, S., and Hinnebusch, A.G. (1991). GCD2, a translational repressor of the GCN4 gene, has a general function in the initiation of protein synthesis. *Mol. Cell. Biol.* 11, 3203–3216.
- Forsburg, S.L., and Guarente, L. (1989). Communication between mitochondria and the nucleus in regulation of cytochrome genes in the yeast *Saccharomyces cerevisiae*. *Annu. Rev. Cell Biol.* 5, 153–180.
- Fraenkel, D.G. (1982). Carbohydrate metabolism. In: *The Molecular Biology of the Yeast Saccharomyces cerevisiae: Metabolism and Gene Expression*, ed. J.N. Strathern, E.W. Jones, and J.R. Broach, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1–38.
- Frohman, M.A., Dush, M.K., and Martin, G.R. (1988). Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA* 85, 8998–9002.
- Gross, E., Goldberg, D., and Levitzki, A. (1992). Phosphorylation of the *S. cerevisiae* Cdc25 in response to glucose results in its dissociation from Ras. *Nature* 360, 762–765.
- Hanic-Joyce, P.J., Singer, R.A., and Johnston, G.C. (1987). Molecular characterization of the yeast PRT1 gene in which mutations affect translation initiation and regulation of cell proliferation. *J. Biol. Chem.* 262, 2845–2851.
- Herrick, D., Parker, R., and Jacobson, A. (1990). Identification and comparison of stable and unstable mRNAs in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 10, 2269–2284.
- Hershey, J.W.B. (1989). Protein phosphorylation controls translation rates. *J. Biol. Chem.* 264, 20823–20826.
- Hershey, J.W.B. (1990). Overview: phosphorylation and translation control. *Enzyme* 44, 17–27.
- Hershey, J.W.B. (1991). Translational control in mammalian cells. *Annu. Rev. Biochem.* 60, 717–755.

- Hsu, C.L., and Stevens, A. (1993). Yeast cells lacking 5'-3' exoribonuclease 1 contain mRNA species that are poly(A) deficient and partially lack the 5' cap structure. *Mol. Cell. Biol.* 13, 4826-4835.
- Irniger, S., Egli, C.M., and Braus, G.H. (1991). Different classes of polyadenylation sites in the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 11, 3060-3069.
- Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* 153, 163-168.
- Johnston, M., and Carlson, M. (1992). Regulation of carbon and phosphate utilization. In: *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*, vol. 2, ed. J. Broach, E.W. Jones, and J. Pringle, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 193-281.
- Kearsey, S., and Kipling, D. (1991). Recombination and RNA processing: a common strand? *Trends Cell Biol.* 1, 110-113.
- Keireleber, C., Wittekind, M., Qin, S., and McLaughlin, C.S. (1986). Isolation and characterization of PRT1, a gene required for the initiation of protein biosynthesis in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 6, 4419-4424.
- Kenna, M., Stevens, A., McCammon, M., and Douglas, M.G. (1993). An essential yeast gene with homology to the exonuclease-encoding *XRN1/KEM1* gene also encodes a protein with exoribonuclease activity. *Mol. Cell. Biol.* 13, 341-350.
- Knutsen, H.K., Austlid Taskén, K., Eskild, W., Jahnsen, T., and Hansson, V. (1992). Half-lives of different sized mRNAs for the PKA subunit RI are regulated differently in response to inhibition of transcription and translation. *Biochem. Biophys. Res. Commun.* 184, 454-460.
- Koeller, D.M., Horowitz, J.A., Casey, J.L., Klausner, R.D., and Harford, J.B. (1991). Translation and the stability of mRNAs encoding the transferrin receptor and *c-fos*. *Proc. Natl. Acad. Sci. USA* 88, 7778-7782.
- Kruys, V., Beutler, B., and Huez, G. (1990). Translational control mediated by UA-rich sequences. *Enzyme* 44, 193-202.
- Larimer, F.W., Hsu, C.L., Maupin, M.K., and Stevens, A. (1992). Characterization of the *XRN1* gene encoding a 5' → 3' exoribonuclease: sequence data and analysis of disparate protein and mRNA levels of gene-disrupted yeast cells. *Gene* 120, 51-57.
- Larimer, F.W., and Stevens, A. (1990). Disruption of the gene *XRN1*, coding for a 5' → 3' exoribonuclease, restricts yeast cell growth. *Gene* 95, 85-90.
- Laurent, B.C., and Carlson, M. (1992). Yeast SNF2/SWI2, SNF5, and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. *Genes Dev.* 6, 1707-1715.
- Leeds, P., Peltz, S.W., Jacobson, A., and Culbertson, M.R. (1991). The product of the yeast *UPF1* gene is required for rapid turnover of mRNAs containing a premature translational termination codon. *Genes Dev.* 5, 2303-2314.
- Leeds, P., Wood, J.M., Lee, B.-S., and Culbertson, M.R. (1992). Gene products that promote mRNA turnover in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12, 2165-2177.
- Lesser, C.F., and Guthrie, C. (1993). Mutational analysis of pre-mRNA splicing in *Saccharomyces cerevisiae* using a sensitive new reporter gene, *CUP1*. *Genetics* 133, 851-863.
- Levitzki, A. (1988). Transmembrane signalling to adenylate cyclase in mammalian cells and in *Saccharomyces cerevisiae*. *Trends Biochem. Sci.* 13, 298-303.
- Lombardo, A., Carine, K., and Scheffler, I.E. (1990). Cloning and characterization of the iron-sulfur subunit gene of succinate dehydrogenase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 265, 10419-10423.
- Lombardo, A., Cereghino, G.P., and Scheffler, I.E. (1992). Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 12, 2941-2948.
- Lombardo, A., and Scheffler, I.E. (1989). Isolation and characterization of a *Saccharomyces cerevisiae* mutant with a disrupted gene for the Ip subunit of succinate dehydrogenase. *J. Biol. Chem.* 264, 18874-18877.
- Lowell, J.E., Rudner, D.Z., and Sachs, A.B. (1992). 3'-UTR-dependent deadenylation by the yeast poly(A) nuclease. *Genes Dev.* 6, 2088-2099.
- Muhlrad, D., Decker, C.J., and Parker, R. (1994). Deadenylation of the unstable mRNA encoded by the yeast *MFA2* gene leads to decapping followed by 5' → 3' digestion of the transcript. *Genes Dev.* 8, 855-866.
- Muhlrad, D., and Parker, R. (1992). Mutations affecting stability and deadenylation of the yeast *MFA2* transcript. *Genes Dev.* 6, 2100-2111.
- Neigeborn, L., and Carlson, M. (1987). Mutations causing constitutive invertase synthesis in yeast: genetic interactions with *snf* mutations. *Genetics* 115, 247-253.
- Ng, R., and Abelson, J. (1980). Isolation and sequence of the gene for actin in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 77, 3912-3916.
- Nonet, M., Scafe, C., Sexton, J., and Young, R. (1987). Eucaryotic RNA polymerase conditional mutant that rapidly ceases mRNA synthesis. *Mol. Cell. Biol.* 7, 1602-1611.
- Parker, R., and Jacobson, A. (1990). Translation and a 42-nucleotide segment within the coding region of the mRNA encoded by the *MATa1* gene are involved in promoting rapid mRNA decay in yeast. *Proc. Natl. Acad. Sci. USA* 87, 2780-2784.
- Peltz, S.W., Brewer, G., Bernstein, P., Hart, P.A., and Ross, J. (1991). Regulation of mRNA turnover in eukaryotic cells. *Crit. Rev. Eukaryotic Gene Exp.* 1, 99-126.
- Proudfoot, N. (1991). Poly(A) signals. *Cell* 64, 671-674.
- Robinson, K.M., and Lemire, B.D. (1992). Isolation and nucleotide sequence of the *Saccharomyces cerevisiae* gene for the succinate dehydrogenase flavoprotein subunit. *J. Biol. Chem.* 267, 10101-10107.
- Ronne, H. (1995). Glucose repression in fungi. *Trends Genet.* 11, 12-17.
- Roy, N., Laflamme, G., and Raymond, V. (1992). 5'-untranslated sequences modulate rapid mRNA degradation mediated by 3' AU-rich element in *v-c-fos* recombinants. *Nucleic Acids Res.* 20, 5753-5762.
- Russo, P., Li, W.-Z., Hampsey, D.M., Zaret, K.S., and Sherman, F. (1991). Distinct *cis*-acting signals enhance 3' endpoint formation of *CYC1* mRNA in the yeast *Saccharomyces cerevisiae*. *EMBO J.* 10, 563-571.
- Sachs, A.B. (1993). Messenger RNA degradation in eukaryotes. *Cell* 74, 413-421.
- Saghibini, M., Broomfield, P.L.E., and Scheffler, I.E. (1994). Studies on the assembly of complex II in yeast mitochondria using chimeric human/yeast genes for the iron-sulfur protein subunit. *Biochemistry* 33, 159-165 (Abstract).
- Sagliocco, F.A., Zhu, D., Vega Laso, M.R., McCarthy, J.E.G., Tuite, M.F., and Brown, A.J.P. (1994). Rapid mRNA degradation in yeast can proceed independently of translational elongation. *J. Biol. Chem.* 269, 18630-18637.
- Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

- Santiago, T.C., Bettany, A.J.E., Purvis, I.J., and Brown, A.J.P. (1987). Messenger RNA stability in *Saccharomyces cerevisiae*: the influence of translation and poly(A) tail length. *Nucleic Acids Res.* 15, 2417–2429.
- Savant-Bhonsale, S., and Cleveland, D.W. (1992). Evidence for instability of mRNAs containing AUUUA motifs mediated through translation-dependent assembly of a >20S degradation complex. *Genes Dev.* 6, 1927–1939.
- Schena, M., Picard, D., and Yamamoto, K.R. (1991). Vectors for constitutive and inducible gene expression in yeast. *Methods Enzymol.* 194, 389–398. (Abstract).
- Sherman, F., Fink, G.R., and Hicks, J.B. (1982). *Methods in Yeast Genetics*, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
- Shyu, A.-B., Belasco, J.G., and Greenberg, M.E. (1991). Two distinct destabilizing elements in the *c-fos* message trigger deadenylation as a first step in rapid mRNA decay. *Genes Dev.* 5, 221–231.
- Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* 122, 19–27.
- Stevens, A. (1980). Purification and characterization of a *Saccharomyces cerevisiae* exoribonuclease which yields 5' mononucleotides by a 5' → 3' mode of hydrolysis. *J. Biol. Chem.* 255, 3080–3085.
- Stevens, A. (1988). mRNA-decapping enzyme from *Saccharomyces cerevisiae*: purification and unique specificity for long RNA chains. *Mol. Cell. Biol.* 8, 2005–2010.
- Surosky, R.T., Strich, R., and Esposito, R.E. (1994). The yeast *UME5* gene regulates the stability of meiotic mRNAs in response to glucose. *Mol. Cell. Biol.* 14, 3446–3458.
- Szekely, E., and Montgomery, D.L. (1984). Glucose represses transcription of *Saccharomyces cerevisiae* nuclear genes that encode mitochondrial components. *Mol. Cell. Biol.* 4, 939–946.
- Theodorakis, N.G., and Cleveland, D.W. (1992). Physical evidence for cotranslational regulation of β -tubulin mRNA degradation. *Mol. Cell. Biol.* 12, 791–799.
- Trumbly, R.J. (1988). Cloning and characterization of the *CYC8* gene mediating glucose repression in yeast. *Gene* 73, 97–111.
- Trumbly, R.J. (1992). Glucose repression in the yeast *Saccharomyces cerevisiae* (MicroReview). *Mol. Microbiol.* 6, 15–21.
- van Loon, A.P.G.M., van Eijk, E., and Grivell, L.A. (1983). Biosynthesis of the ubiquinol-cytochrome c reductase complex in yeast: discoordinate synthesis of the 11 kd subunit in response to increased gene copy number. *EMBO J.* 2, 1765–1770.
- Wahle, E., and Keller, W. (1992). The biochemistry of 3'-end cleavage and polyadenylation of messenger RNA precursors. *Annu. Rev. Biochem.* 61, 419–440.
- Williams, F.E., Varanasi, U., and Trumbly, R.J. (1991). The *CYC8* and *TUP1* proteins involved in glucose repression in *Saccharomyces cerevisiae* are associated in a protein complex. *Mol. Cell. Biol.* 11, 3307–3316.
- Williams, F.E., and Trumbly, R.J. (1990). Characterization of *TUP1*, a mediator of glucose repression in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 10, 6500–6511.
- Winston, F., and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.* 8, 387–391.
- Wisdom, R., and Lee, W. (1991). The protein-coding region of *c-myc* mRNA contains a sequence that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. *Genes Dev.* 5, 232–243.
- Yarchuk, O., Jacques, N., Guillerez, J., and Dreyfus, M. (1992). Interdependence of translation, transcription and mRNA degradation in the *lacZ* gene. *J. Mol. Biol.* 226, 581–596.
- You, Y., Chen, C.-Y.A., and Shyu, A.-B. (1992). U-rich sequence-binding proteins (URBPs) interacting with a 20-nucleotide U-rich sequence in the 3'-untranslated region of *c-fos* mRNA may be involved in the first step of *c-fos* mRNA degradation. *Mol. Cell. Biol.* 12, 2931–2940.
- Zaret, K.S., and Sherman, F. (1984). Mutationally altered 3' ends of yeast *CYC1* mRNA affect transcript stability and translational efficiency. *J. Mol. Biol.* 176, 107–135.