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Control of mRNA turnover as a mechanism of glucose repression in *Saccharomyces cerevisiae*

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Abstract

The phenomenon of glucose repression in yeast is concerned with the repression of a large number of genes when glucose is an abundant carbon source and almost all of the energy requirements of the cell can be satisfied from glycolysis. Prominent among the repressed genes are those encoding mitochondrial proteins required for respiration and oxidative phosphorylation. Past studies have characterized a pathway by which a signal generated from extracellular glucose is transmitted to the nucleus. The ultimate outcome is the repression of transcription of numerous genes, but also the induction of a limited number of others. The emphasis has been almost exclusively on transcriptional control mechanisms. A discovery made originally with the transcript of the *SDH2* gene prompted an investigation of post-transcriptional mechanisms, and more specifically a study of the turnover rate of this mRNA in the absence and presence of glucose. *SDH2* mRNA has a very short half-life in medium with glucose (YPD) and a significantly longer half-life in medium with glycerol (YPG). Experimental evidence and recent progress in understanding of (1) mRNA turnover in yeast and (2) initiation of translation on the 5' untranslated region of mRNAs, lead to a working hypothesis with the following major features: the carbon source, via a signaling pathway involving kinase/phosphatase activities, controls the rate of initiation, and thus influences a competition between eukaryotic initiation factors (prominently eIF4E, eIF4G, eIF3) binding to the capped mRNA and a decapping activity (*DCPI*) which is one of the rate limiting activities in the turnover of such mRNAs. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Many organisms, and especially microorganisms, have developed mechanisms to adapt to changing environments by modulating the expression of specific genes. The budding yeast,

Saccharomyces cerevisiae, has been a model organism for many studies in which responses to environmental factors have been investigated. Among the major variable factors one can list the following: (1) temperature, (2) osmotic stress, (3) aerobic versus anaerobic conditions, (4) availability of a nitrogen source and (5) nature and abundance of the carbon source. The only notable environmental variable to which yeast does not appear to respond dramatically is light.

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A wealth of data have been accumulated from the study of heat-shock [21]. Less common but also significant are studies on adaptations to high salt which have helped greatly in defining ion channels and various mechanisms concerned with osmoregulation in yeast [29, 79, 95]. Oxygen is an environmental factor which can be sensed to generate intracellular signals to adjust energy metabolism and gene expression [112]. Similarly, the study of the adaptation to nitrogen deprivation has been a fruitful and rewarding approach [18]. The largest body of literature has, however, been assembled on the response of yeast to the carbon source and specifically the difference between cells grown in the presence of a nonfermentable carbon source such as lactate, pyruvate or ethanol or in the presence of a fermentable carbon source, usually glucose.

In the presence of abundant glucose, yeast cells can satisfy most of their energy requirements from glycolysis/fermentation and the expression of a large number of genes is repressed. The phenomenon is therefore often referred to as 'glucose repression'. Among the gene products repressed are those required for the uptake and metabolism of other sugars such as sucrose and galactose and most dramatically, a large number of gene products required for respiration and oxidative phosphorylation in the mitochondria. In fact, the decrease in the levels of such proteins causes a very noticeable change in the morphology of mitochondria.

2. Glucose repression

The phenomenon of glucose repression was recognized two decades ago and expert reviews have appeared periodically, as insights into the mechanism of this phenomenon accumulated [44, 64, 83, 98]. Not surprisingly, the isolation and characterization of mutants and genetic studies represented approaches which could be exploited most successfully with yeast [44]. Until recently, almost all of the attention was focussed on the control of gene expression at the transcriptional level and a signal transduction pathway has emerged in outline which is schematically presented in Fig. 1. In this pathway, the following major stages can be identified:

tically presented in Fig. 1. In this pathway, the following major stages can be identified:

1. Glucose first has to enter the cell by facilitated diffusion and at least 20 potential glucose transporters exist in *S. cerevisiae*, defined by the *HXT* gene family, as well as the *SNF3* and *RGT2* genes [6, 30, 46, 50, 81]. They differ in their level of expression, their affinity for glucose, and the two proteins Snf3p and Rgt2p probably act as glucose sensors/receptors producing a transmembrane signal, rather than as true transporters [74, 75, 103]. Thus, glucose can regulate its own rate of uptake into the cell and adjust it to external concentrations as well as internal needs.
2. Glucose is phosphorylated and further metabolized. Phosphorylation is generally agreed to be necessary for the generation of an intracellular signal, but the precise nature of that signal is still hotly debated [78, 107]. It could be a metabolite such as a phosphorylated hexose, fructose 1,6 biphosphate or fructose 2,6 biphosphate [35, 41], it could be an allosteric signal from one of the three hexose kinases, or it could even be an indirect signal from changing levels in cAMP, ATP or altered ATP/ADP or AMP/ATP ratios. Experiments with 2-deoxyglucose yielded results which may have been overinterpreted before the toxic effects of this analogue were fully appreciated [39, 80]. It is likely that multiple signals in parallel and/or overlapping pathways are used for regulatory purposes. The identification of three enzymes capable of phosphorylating glucose encoded by the genes *GLK1*, *HXK1* and *HXK2* [7, 28, 62] has made it possible to study glucose repression in single, double and triple mutants and for some pathways phosphorylation of glucose is clearly a requirement (e.g. Refs. [78, 84]). For some pathways a specific hexokinase, Hxk2p, seems to be required [60, 61, 91] and allosteric mechanisms rather than production of the metabolite have to be postulated. In other signal transduction pathways glucose sensing by either Snf3p or Rgt2p may be sufficient and uptake into the cell is not necessary. In these pathways, Snf3p

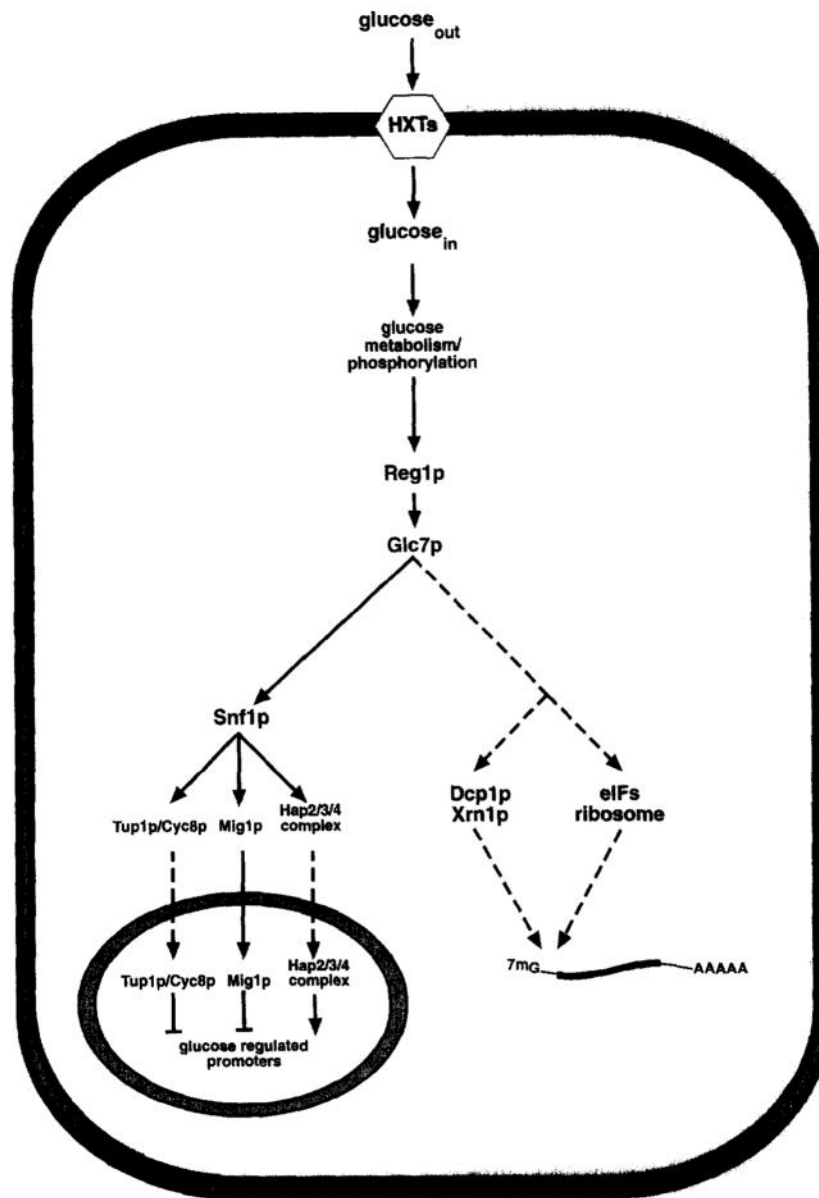


Fig. 1. Schematic representation of the signaling pathways in glucose repression. Glucose uptake into the cell is achieved by one or more glucose transporters encoded by the *HXT* genes. Phosphorylation by one of the hexokinases or glucokinase is required, but the nature of the signal immediately beyond this step is still unknown. Further downstream the gene products Reg1p, Glc7p and Snf1p are known to be involved in signal transduction to the nucleus. Glc7p is the major phosphatase in yeast which is regulated by a number of regulatory subunits, including Reg1p. A key kinase in transcriptional regulation by a variety of nuclear activators and repressors is the Snf1 kinase. Mig1p, Tup1p, Cyc8p are factors involved in transcriptional repression. The Hap2/3/4 complex is a transcriptional activator of mitochondrial and other genes. mRNA turnover takes place in the cytosol, suggesting a branch point in the signaling pathway. Experiments have shown that Reg1p (and therefore most likely Glc7p) is required for the glucose-triggered mRNA degradation. It remains to be established whether the target is the decapping enzyme (Dcp1) or one or more of the translation initiation factors.

and Rgt2p act as glucose receptors in the plasma membrane generating an intracellular signal similar to other receptor-mediated processes [75].

3. The signal is eventually conducted along the pathway by a cascade of phosphorylations and/or dephosphorylations, since mutations affecting glucose repression have been found in the major phosphatase of yeast (*GLC7*), in a regulatory subunit of this phosphatase (*REG1*) and in a serine-threonine kinase (*SNF1*) (see Refs. [44, 83, 98] for reviews).
4. Finally, nuclear factors involved in repression or transactivation of specific genes have been characterized, first by genetic means and subsequently by biochemical methods. Prominent among these factors is the transcriptional activator complex encoded by the *HAP1* or *HAP2/3/4/5* genes [66, 94, 108], the transcriptional repressor defined by the *MIG1* gene [23, 69], and the co-repressor complex composed of Cyc8p (Ssn6p) and Tup1p subunits [101].

The details of the elucidation of these transcriptional control mechanisms are beyond the scope of this review, but the major participating proteins should be kept in mind for the following discussion on post-transcriptional mechanisms. It should also be restated that glucose can cause transcriptional repression of genes as well as activation. For example, *GAL* genes are repressed in the presence of glucose by as many as three distinct mechanisms acting perhaps on a different time scale (e.g. Ref. [45]). In contrast, among the prominent genes induced by glucose one finds various *HXT* genes, and the expression of individual *HXT* genes depends on the extracellular concentration of glucose [56, 73]. In the absence of glucose a complex including Rgt1p and Ssn6p represses *HXT* gene expression. A signal generated by glucose-receptors (in the presence of glucose or by mutant proteins with a dominant mutation even in the absence of glucose) has been proposed to activate Grr1p which inhibits Rgt1p [73–75].

3. Post-transcriptional control of the expression of SDH genes

3.1. Glucose triggers *Ip* mRNA decay

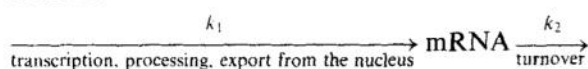
When the *SDH2* gene encoding the iron-protein (*Ip*) subunit of succinate:quinone oxidoreductase (complex II) of the mitochondrial electron transport chain was first cloned and investigated, it became quickly apparent that this protein was subject to glucose repression [58]. Like other mitochondrial activities, SDH is repressed in the presence of glucose, that is, it appears more economical for the cells to waste ethanol rather than to produce CO₂ from fully functional, respiring mitochondria. Northern blots of total RNA from yeast grown in glycerol (YPG medium) showed abundant transcripts from the *SDH2* gene, while similar blots with RNA from cells grown in glucose (YPD (dextrose)) had 6 to 12 times less *Ip* mRNA.

Initial investigations focused on the promoter of this gene and not unexpectedly, four CCAAT boxes were found within ~450 nucleotides upstream from the transcription start site [57]. Such elements had been identified in promoters such as that of cytochrome *c* (*CYC1*) as the target for the Hap2/3/4 complex [108]. Furthermore, only the proximal sites may be relevant for Hap2/3/4 complex binding (as shown by deletion analysis) [58], while the distal elements may be targets for regulation by Hap1p, which is part of a mechanism of regulation of gene expression by oxygen and heme [33, 94]. The standard approach to such studies has been to make chimeric genes with the upstream activating sequences (UAS) from the gene under study and a coding sequence for a reporter such as β -galactosidase. When such constructs were investigated, the magnitude of the response to glucose was not nearly as large as expected from the analysis of the normal, endogenous gene by Northern blots. Furthermore, the induction of the transcript in YPG was observed even when all the CCAAT boxes had been deleted from the promoter and only a minimal promoter sequence including the TATA box was present on the gene. These preliminary data suggested that transcriptional regulation was not

the entire story and that post-transcriptional regulatory mechanisms had to be seriously considered.

A confirmation of such a possibility came from an experiment in which a culture in YPG with induced levels of Ip mRNA was rapidly exposed to glucose by the addition of this fermentable substrate to the medium. Ip mRNA levels dropped precipitously with an estimated half-life of less than 5–7 min [15, 58]. Even if glucose signaled simply a transcription arrest, the half-life of the transcript must be very short to account for this observation. There was no apparent delay and no dependence on additional protein synthesis. The obvious question was whether this rapid turnover of the Ip mRNA was an intrinsic property or whether the half-life itself was subject to regulation by the carbon source. Experiments were performed with a mutant with a temperature-sensitive RNA polymerase II [71, 109]. This mutant has found applications in RNA turnover studies [4, 12, 24], because transcription by pol II is arrested quite rapidly after a shift of the cells to the nonpermissive temperature. When temperature shifts were carried out with cells in YPG with induced levels of Ip mRNA (without a change in carbon source), the mRNA appeared to be stable for up to an hour. Addition of glucose to such a culture at the non-permissive temperature caused an immediate disappearance of the transcript. On the other hand, when cells maintained in glucose at 20°C were shifted to 35°C to arrest transcription, the transcript disappeared rapidly. Unfortunately, under such conditions the level of transcript was quite low and precise measurements of the half-life proved challenging. A closely related experiment with a slightly different construct (gene) but the same transcript will be described below. It was concluded that the Ip mRNA had a differential stability depending on the carbon source.

A highly simplified scheme for understanding how steady state levels of transcripts are controlled in the cytoplasm can be formulated as follows:



From simple basic principles one can derive an expression for the steady state levels of the mRNA to be given by

$$[\text{mRNA}]_{\text{steady state}} = A \times \frac{k_1}{k_2}$$

where A is a proportionality constant; k_1 represents a composite rate constant describing the rate of transcription, processing and export of the mRNA from the nucleus, i.e. a rate of appearance of the mRNA in the cytoplasm and k_2 represents the turnover rate which itself is determined by several steps in the overall mechanism to be elaborated later. In previous studies of mRNA levels under different conditions the implicit assumption had been made that k_2 was a constant under all conditions, and the observed differences in steady state levels were simply the result of a change in k_1 (transcription, etc.). Our observations explicitly introduce k_2 (turnover) as a variable and thus observed increases in steady state levels could be the result of an increase in k_1 , a decrease in k_2 or both. A 3–4-fold increase in transcription and a 4–5-fold reduction in the turnover rate can account for a 12–20-fold increase in steady state levels of a particular mRNA. Our results suggested that a combination of the two mechanisms might be required for explaining the behaviour of the Ip mRNA in *S. cerevisiae* grown with different carbon sources. Is this the only gene and protein controlled by these mechanisms? Exhaustive investigations have not yet been performed, but the same behaviour is clearly observed with the functionally related *SDH1* transcript, encoding the flavoprotein of SDH and with *SUC2* mRNA [16]. In the earlier literature on glucose repression one can find data suggesting that levels of *CYC1* mRNA in *S. cerevisiae* [113] and *MAL6* mRNA in *S. carlsbergensis* [26] may also be regulated by turnover.

From a physiological perspective it is not obvious why the transcript should be so rapidly disposed of when a change from YPG (glycerol) to YPD (dextrose/glucose) is made. The morphological changes in the mitochondria resulting from the turnover of the inner membrane pro-

teins are not nearly as rapid [72]. However, a prompt destruction of mRNAs clearly assures that mitochondrial biogenesis is quickly shut down when glucose and glycolysis make mitochondrial functions redundant.

3.2. Is glucose-triggered mRNA turnover a transient phenomenon?

This question has been raised repeatedly by us and reviewers. There are convincing data showing that the addition of glucose to a yeast culture grown in a nonfermentable carbon source leads to a very rapid and dramatic increase in cAMP, but this elevation is temporary, and cAMP levels return to normal within minutes [31]. It is conceivable that a signal is generated that temporarily activates a mechanism of rapid mRNA turnover for specific transcripts. However, in cultures maintained continuously in glucose, the Ip mRNA may be relatively stable, its steady state level kept low by transcriptional repression alone.

In cells with a temperature-sensitive RNA polymerase II grown in glucose, and shifted to the nonpermissive temperature in the same medium, the Ip mRNA is still rapidly degraded, supporting the notion that the Ip mRNA is constitutively short-lived in glucose. Since the low steady state level in glucose makes precise measurements more difficult, a new construct was made to overcome two potential problems. The construct pGC37 combines the promoter (UAS) from the glyceraldehyde phosphate dehydrogenase gene with the TATA box and downstream portion of the *SDH2* gene. This promoter has been described as being a constitutive promoter unaffected by the carbon source [92]. Thus, it was intended that transcriptional regulation by glucose was eliminated and only post-transcriptional mechanisms would be apparent. Expression of the transcript was expected to be significant even in glucose, facilitating measurements of turnover. For reasons which will become apparent from a later discussion it was very important to obtain exactly the same transcript with a precisely conserved 5' UTR from such a chimeric gene. Transcriptional initiation

sites in yeast are variable with respect to the upstream TATA box. Therefore, it was ascertained that exactly the same transcripts were obtained from the pGC37 construct by using the upstream portion including the TATA box from the *SDH2* gene. The pGC37 construct was expressed in the ts-pol II mutant, yielding relatively high levels in both YPG and YPD. After shifting to the nonpermissive temperature there was no significant turnover in YPG, but transcript levels dropped rapidly in YPD [15]. This result again supports the conclusion that the transcript is intrinsically unstable in the presence of glucose and that its turnover is not triggered by a sudden change in the carbon source. A further discussion of the differential stability in YPG and YPD has to be deferred until the actual mechanism triggering the turnover in glucose has been further elucidated.

3.3. Elements involved in post-transcriptional control mechanisms

As introduced briefly above, genetic analyses have led to the outline of a regulatory pathway and the definition of components in the pathway that play a role in glucose repression of transcription in the nucleus. RNA turnover takes place in the cytosol, raising the question whether these two mechanisms share any regulatory metabolites or proteins. The pathway starts with glucose on the outside of the cell, but at some point two signaling pathways must diverge. By examining mutants with defects selected for their relevance to transcriptional repression it was anticipated that Ip mRNA turnover would be affected in mutants affected in proteins shared by the pathways and be unaffected in mutants with defective proteins required for transcriptional regulation.

The experiments with severely truncated promoters (UAS) of the *SDH2* gene had already been suggestive, and not surprisingly, with the transcriptional activator complex Hap2/3/4 mutated only a slightly reduced induction of the transcript in YPG was observed, i.e. a shift from YPD to YPG still caused an induction, which we attribute to mRNA stabilization; the addition of

glucose in turn triggered a rapid disappearance of the *Ip* mRNA. Mutations in the *HAP2* or *HAP3* genes had no effect on the turnover of the transcript in glucose. Clearly, such nuclear factors serve in transcriptional regulation only. Similar observations were made with a *tup1* mutant. The situation was less clear in a *cyc8/ssn6* mutant.

One of the most significant distinctions between transcriptional regulation and post-transcriptional control could be observed in a comparison of wild type cells with cells defective in the *SNF1* gene. Snf1p (a kinase) had been identified as a key element in the signaling pathway leading to the transcriptional activation of the *SUC2* gene (invertase) [14,44]. The *SUC2* gene is normally repressed in the presence of glucose, but induced in YPG. In the *snf1* mutant the *SUC2* gene is not derepressed and the cells are unable to utilize sucrose (sucrose non-fermenting) [13]. In contrast, the expression of the *SDH2* gene in *SNF1* wild type and *snf1* mutant strains shows no dramatic difference: induction of the *Ip* mRNA in YPG does not require the Snf1p, and turnover in glucose is also unaffected by the *snf1* mutation [16]. The Snf1 protein has been identified as a serine-threonine protein kinase and it is speculated that its targets are either some of the transcriptional activators or repressors themselves (e.g. Mig1p [59]) or factors just upstream from the nuclear transcription factors. The observation that Snf1p is not required for the regulation of *Ip* mRNA turnover places this element downstream from the branch-point in the glucose signaling pathways. It should also be noted that like the *SDH2* mRNA, the *SUC2* transcript is very rapidly destroyed upon addition of glucose in *SNF1* cells. The same experiment cannot be performed in *snf1* cells, because in such cells no *SUC2* mRNA is detectable.

Are phosphorylation-dephosphorylation reactions part of the signaling pathway leading to triggering of mRNA turnover? The answer appears to be yes, from observations made with another mutant strain originally isolated because it was affected in glucose repression of transcription. Our studies showed clearly that the Reg1

protein is required for the rapid turnover of the *Ip* mRNA in glucose medium [16]. In a *reg1* mutant the *SUC2* gene is constitutively expressed regardless of the carbon source, and turnover of both *SUC2* and *SDH2* mRNAs following the addition of glucose is not observed, in contrast to the wild type parent, or the mutant complemented with a normal *REG1* gene on a plasmid. Thus, the Reg1 protein appears to be a common element in the pathway(s) leading to transcriptional regulation and mRNA turnover. Its function became clear only recently when Tu and Carlson were able to demonstrate that Reg1p is a regulatory subunit of the major type 1 phosphatase (PP1) in yeast, encoded by the *GLC7 (CID1)* gene [99]. Reg1p not only activates the phosphatase, but there are indications that the specificity of the phosphatase is also influenced by Reg1p and by other regulatory subunits capable of interacting with the Glc7 catalytic subunit [2]. Thus, in the absence of Reg1p one or more targets of the *GLC7* phosphatase are likely to remain phosphorylated, preventing a further signaling to the nucleus, or a triggering of mRNA turnover. In another previous study, mutations in the *REG1 (HEX2/SRN1)* gene were found as suppressors of the *rna1-1* mutation, thus linking catabolite repression to RNA processing (pre-tRNA splicing, pre-rRNA processing and production of mature mRNA in the cytosol) [100].

Transcriptional activation of the *SUC2* gene requires a phosphorylation step by the Snf1 kinase and repression of the *SUC2* gene requires a dephosphorylation by the Reg1p-Glc7p phosphatase. It is not yet clear whether both enzymes act on the same substrate, such as Mig1p, or whether other proteins are interposed. Glucose-induced mRNA turnover requires a dephosphorylation of a target protein yet to be identified and this target protein is not a substrate for the Snf1 kinase. Another kinase for the protein regulating RNA turnover is as yet unidentified. Nevertheless, the results strongly suggest that a balance between a phosphorylated and unphosphorylated protein involved in RNA turnover is influenced by the carbon source. A *glc7* knockout is lethal in yeast and hence a direct test of the participation of the Glc7 phosphatase in the

pathway is not possible. Alleles with point mutations in the *GLC7* gene have been characterized [2, 44, 70] and some of these have an effect on transcriptional regulation of the *SUC2* gene [44]. The alleles tested by us so far have not had any significant effect on the behaviour of the Ip mRNA.

It is worth re-emphasizing that the *SUC2* gene has been used extensively but not exclusively in the development of ideas and models about glucose repression and all of these past discussions have ignored mRNA turnover. Our results show convincingly that *SUC2* mRNA turnover is triggered equally rapidly by the addition of glucose to an induced culture [16]. Therefore, post-transcriptional mechanisms in the control of the expression of invertase must be included in the considerations.

4. Mechanisms of mRNA turnover in yeast

4.1. General

Considerable progress has been made in recent years in understanding the mechanism of the turnover of mRNAs in yeast, especially the constitutively short-lived subset of mRNAs. Observations in mammalian cells may suggest that some basic features are universal and conserved [20]. The subject has been extensively reviewed [1, 4, 12, 43, 87]. The favored current model has the following aspects:

1. One of the rate-limiting steps is the shortening of the poly(A) tail of the mRNA.
2. When the tail has been shortened significantly or lost, it fails to protect the transcript from a decapping activity (*DCPI*) which removes the 7-methyl-G cap at the 5' end of the transcript.
3. The 'exposed' 5' end of the RNA now serves as a substrate for a 5' exonuclease (*XRNI*) and a rapid exonucleolytic degradation of the mRNA ensues.

Several questions can be raised immediately. Is the deadenylation of the mRNA absolutely obligatory? At least one exception has been described

(for reviews see Refs. [43, 85]). Transcripts with mutations creating premature stop codons have been shown to be inherently unstable and more so when the premature stop codon is found relatively close to the beginning of the open reading frame [43]. Such a condition, with additional dependence on downstream sequences, causes rapid turnover (NMD, nonsense-mediated decay) which also involves decapping and the 5' exonuclease, but it is independent of deadenylation [32, 77, 86, 111]. A genetic analysis has revealed the participation of a series of gene products encoded by *UPF* genes.

The roles of the decapping enzyme and the 5' exonuclease have been well defined by the use of mutants deficient in these activities. In *xrn1* mutants the turnover of various mRNAs under investigation is slowed down considerably (see below), but it is likely that other exo- and endonucleases have a back up function. *xrn1* mutants are viable, but they have abnormal phenotypes including slow growth. The diversity of phenotypes constitutes still a considerable problem to be explained in detail in terms of a missing exonuclease [25, 27, 40, 42, 53]. Similarly, a mutant with a defective decapping function (*dcp1*) has been described and it is viable [5, 51]. It appears that a single peptide/gene constitutes the active decapping enzyme [52]. In the absence of this decapping function (*DCPI*) the degradation of unstable mRNAs is also slowed down considerably. It therefore seems quite clear that the 5' cap protects the mRNA from the exonuclease. But what protects the mRNA from the decapping enzyme? And how is this protection maintained or modulated, either by the presence of a 3' poly(A) tail, or by the recognition that a premature stop codon makes an mRNA useless to the cell?

The poly(A) tail is known to be occupied by a protein referred to as poly(A) binding protein (Pab1p) and deadenylation is the result of a specific Pab1p-dependent nuclease activity [8, 9]. Pab1p is also involved in translational initiation. A case can therefore be built for an interaction between proteins binding to the poly(A) tail and proteins associated with the 5' cap and UTR of an mRNA. Included in the proteins at the 5' end

are the decapping enzyme and one or more of the numerous complexes identified as eukaryotic initiation factors (eIFs). This topic will be expanded below.

One has to ask why loss of the poly(A) tail is rapid in certain short-lived mRNAs, i.e. what distinguishes mRNAs with a short half-life from mRNAs with a long half-life? There is a general consensus that specific *cis*-acting sequences within the mRNA must play a critical role. They may be located in the 3' UTR, or further upstream. In mammalian mRNAs sequences containing the AUUUA motif in the 3' UTR have been found in certain short-lived transcripts and specific binding proteins have been isolated. How such an upstream complex activates de-adenylation is still unclear. Similar motifs have been found near the poly(A) tail in the *Ip* mRNA, but they do not seem to have such a function (see below).

The examination of the glucose-triggered turnover of the *Ip* mRNA has supported (and even anticipated) the above scheme in part [15]. It was shown that the addition of cycloheximide to a YPG-induced culture prior to the addition of glucose slowed down degradation significantly, not because of the need for protein synthesis, but because the stalled ribosomes slowed down the processive activity of a 5' exonuclease. For the first time, intermediates in mRNA turnover could be isolated which were missing the 5' UTR, but still had an intact downstream segment of the mRNA [15]. When the *xrn1* mutant became available, it was shown that in the absence of such an activity the *Ip* mRNA was considerably more stable following the shift to a glucose-containing medium [15]. Finally, in a mutant missing the decapping activity [5,51] the *Ip* mRNA remains quite stable for up to an hour after the addition of glucose (de la Cruz, unpublished observations). Thus, decapping and 5' exonuclease activity are required for the rapid turnover of this transcript in YPD. Is decapping prevented in YPG? Our working model makes this assumption, as will be elaborated below.

Is deadenylation a rate-limiting and obligatory step in glucose-triggered mRNA turnover? Our investigations suggest that there is no noticeable deadenylation of the *Ip* mRNA upon the ad-

dition of glucose to an induced culture (Prieto, unpublished observations).

4.2. Glucose-regulated mRNA turnover

The turnover of *SDH2* mRNA requires some of the same enzymes involved in general mRNA turnover, but it is worth re-emphasizing the unique features of this phenomenon. First, the lack of a preceding deadenylation is noteworthy. Second, the half-life of the transcript is dependent on the carbon source and we believe this to be a novel and particularly interesting aspect. In light of the above discussion, it follows that the decapping reaction must be controlled by the nature of the carbon source. Third, the degradation of mRNAs induced by glucose is selective: most of the studies and the discussion have focused on *SDH2* mRNA, but the functionally related *SDH1* mRNA exhibits the same behaviour and *SUC2* mRNA also appears to be subject to post-transcriptional regulatory mechanisms of the type described here. In contrast, a number of other mRNAs such as actin mRNA are not degraded when glucose is added [15] and, of course, cells continue to grow in the presence of glucose. A study on the levels of gluconeogenic mRNAs in response to environmental factors might be relevant here [67]. It shows that *FBP1* mRNA is unaffected, but *PCK1* mRNA turnover is stimulated slightly by glucose.

4.2.1. What is the relevant *cis*-acting element in *Ip* mRNA?

The discussion of mRNA turnover is usually split into two aspects. First, what *cis*-acting elements distinguish a particular mRNA and make its half-life unusually short? (There is also discussion of stabilizing sequences which may make an mRNA long-lived, but our understanding of such elements, if they exist, is very rudimentary). Second, what are the *trans*-acting factor(s) binding to the *cis*-acting element(s)? Such a factor then may represent the connection to the other step, namely the additional enzymes necessary for RNA degradation. The latter clearly include the decapping enzyme and the 5' exonuclease,

but these may represent only the ultimate reactions. For example, a prior step may involve a *cis*-acting element activating rapid deadenylation in short-lived mRNAs by interacting with the Pablp dependent poly(A) nuclease [9].

A substantial amount of effort was devoted to identifying the required *cis*-element [15]. Two experimental approaches were utilized. In the first, a large variety of altered and chimeric transcripts were expressed from non-integrating plasmids in yeast. These included large deletions from within the *SDH2* coding region towards the 3' end, substitutions of heterologous sequences between the 5' UTR and the 3' UTR, and chimeras with various lengths of the 5' portion of the *SDH2* transcript linked to downstream segments of other transcripts, or the 3' portion of the *SDH2* transcript linked to upstream portions of other mRNAs. The results appeared quite unambiguous: the 5' end of the Ip mRNA and specifically the 5' UTR, was sufficient to make the stability of any chimeric transcript tested subject to control by glucose. From primer extension analyses it was deduced that the transcription of the *SDH2* gene could start at two major and one minor sites, yielding transcripts with 5' UTRs of 59 and 45 nucleotides (major), and 50 nucleotides (minor) [57].

An extreme case tested included only the 5' UTR of Ip mRNA linked to the coding sequence, 3' UTR and poly(A) tail of the *CUP1* mRNA [15], expressed from the *SDH2* promoter. The stability in YPG and YPD of this chimeric transcript was very similar to that of the Ip mRNA, and distinctly different from the native *CUP1* mRNA. Most of the 3' UTR of the Ip mRNA could be deleted without any effect on the differential stability of the mRNA. Similarly, almost the entire open reading frame of the yeast *SDH2* mRNA was replaced with the corresponding segment from the human *SDH2* mRNA without any consequences. From these studies it was concluded that the 5' UTR was the dominant *cis*-acting determinant controlling the behaviour of this mRNA in different media.

A second approach, still incomplete, is based on making specific alterations in the 5' UTR sequence of Ip mRNA by site-directed muta-

genesis. Attempts to make additional changes in this region have been frustrated by observations that such changes can alter the transcription start site(s), making the 5' end of the transcript essentially unpredictable and highly variable. In agreement with observations by others we conclude that sequences surrounding the transcription start site are crucial for initiation of transcription in yeast. This is in contrast to mammalian promoters, where there is a fixed distance between the TATA box and the first nucleotide of the transcript.

The most revealing experiment was performed with a transcript in which a single nucleotide had been altered to obliterate the normal start codon. A second 'in frame' start codon 51 nucleotides downstream can be utilized, with the effect of doubling the length of the 5' UTR of the mRNA. The half-life of this transcript in glucose is extended by a factor 4–5 (de la Cruz et al., in preparation). There are no other changes in the entire mRNA and there are no indications that the AUG \Rightarrow AUC change would have a significant effect on any secondary structure predictable by computer. We tentatively interpret this observation to suggest that the increased length of the 5' UTR alters the interaction between the RNA and the various factors and constituents making up the translational initiation complex. As a result, access of the decapping activity to the 5' cap site is prevented or delayed and the stability of the Ip mRNA is increased.

It was mentioned above that the *SDH1*, *SDH2* and *SUC2* transcripts exhibit very similar behaviour upon being shifted from the induced state in YPG to YPD (glucose). Sequence comparisons of the 5' UTRs of these transcripts do not reveal any common consensus sequences, and there are no obvious shared secondary structures from computer predictions. Thus we do not believe that simple, short, recognizable sequence motifs constitute binding sites for as yet unidentified factors. In the absence of any detailed understanding at the molecular, structural level of the formation and activity of the translational initiation complex the interpretation of the specificity of glucose-induced turnover of a limited number of mRNAs is difficult at this time.

4.2.2. Competition between the decapping activity and translational initiation factors, a working hypothesis

Assuming that the stable Ip mRNA in glycerol retains its cap (it is known to be efficiently translated [90,93] and knowing that decapping is a prerequisite for glucose-induced turnover, it is plausible to propose that the addition of glucose causes the 5' cap of the Ip mRNA to become accessible to the decapping enzyme. It follows that the addition of glucose must alter the interaction of initiation factors with the 5' cap and the 5' UTR of the mRNA. In other words, one can envision a competition at the 5' end of the affected mRNA between the decapping enzyme and one or more factors responsible for translational initiation. The carbon source affects the outcome of this competition (Fig. 2). A strong hint of such a sequence of events comes from experiments with a temperature-sensitive mutant, *prt1-1*, in which initiation of protein synthesis stops rapidly after a shift to the nonpermissive temperature [34]. When the fate of Ip mRNA was followed after such a temperature-shift in YPG, it was found to be rapidly degraded, in contrast to other mRNAs such as the actin mRNA [15]. In other words, the arrest of a step in translation initiation caused turnover, even in the presence of glycerol. It was subsequently established in the Hershey laboratory that the *prt1-1* mutation affected a subunit of eIF3 [68].

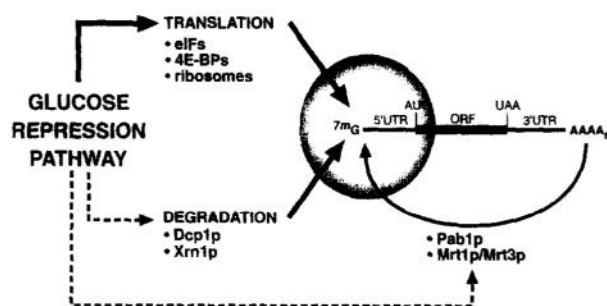


Fig. 2. Proposed scheme for glucose-triggered, specific mRNA decay. A competition between translation and degradation is influenced by a signal generated by the carbon source. It is uncertain whether an interaction between the 5' and 3' ends of the transcript via protein factors (Pab1p, Mrt1p, Mrt3p) plays a role (see text).

In order to understand the details of this working hypothesis, our present understanding of initiation factors and their role in translation must be elaborated.

5. Eukaryotic initiation factors (eIFs)

5.1. Identification of factors

Biochemical and molecular-genetic analyses have characterized six classes of initiation factors (eIF1, eIF2, eIF3, ..., eIF6). The term factor may be misleading in some cases, because some of these consist of multiple subunits forming large complexes. Most of the eIF genes in yeast are identified and cloned and the crystal structure of the 5' cap-binding protein eIF4E is available [63]. A number of recent reviews can be consulted for details for their function and participation in translational initiation and it has become abundantly clear over the past few years that control at the level of translational initiation constitutes an important mechanism in the overall control of expression of many genes [19, 37, 49, 76, 82, 88, 89]. The most intriguing aspect of the evolving picture is that many of these factors are phosphorylatable by kinase(s) and thus in principle easily incorporated into signal transduction pathways. A brief summary of the salient features and functions of some factors must suffice here.

The factor eIF2 is a GTP binding protein with three subunits (α , β , γ) which forms a ternary complex with the methionyl-tRNA. In one cycle of activity the GTP is hydrolyzed to GDP leading to the release of eIF2-GDP. Another multi-subunit factor, eIF2B, is required to promote guanine nucleotide exchange and therefore the 'recharging' of eIF2-GTP for participation in another round of initiation. Both eIF2 and eIF2B are regulated by phosphorylation, and their phosphorylation state is likely to modulate the global rate of initiation of protein synthesis. Since neither of these factors interacts directly with the mRNA, their regulation has a global effect on translation, in contrast to regulatory mechanisms which can discriminate between individual mRNAs.

The eIF4 class has had a confusing history with regard to nomenclature which has been settled only recently by an international committee [19]. The whole complex is now referred to as eIF4F, composed of individual subunits eIF4A, eIF4B, eIF4E and eIF4G (eIF4A may be absent in yeast). There is general agreement that eIF4F functions in combining the 40S ribosome with the mRNA, an interaction also requiring eIF3. There is less consensus on the order and the kinetics in which these components assemble. The eIF4E subunit binds directly to the 7-methyl-G of the 5' cap [63, 65]. The largest peptide, eIF4G, has come under close scrutiny as a potential scaffolding protein which can associate with eIF4E, with eIF3, with the factors eIF4A and B, and probably with the mRNA itself [36]. The precise function of eIF3 within this complex is still poorly understood; it may or may not interact directly with the mRNA and it has also been proposed to promote the association with the ternary met-tRNA-eIF2-GTP complex. The eIF4A protein has an ATP-dependent RNA helicase activity and eIF4B is an RNA binding protein which promotes the helicase activity without interacting directly with eIF4A. The factors eIF5 and eIF6 are required at later stages of initiation.

Several other proteins deserve mention that have no function in the initiation complex itself, but can influence the process by binding to individual factors. Prominent among those are the mammalian 4E-BPs (4E-BP1, alias PHAS-I and 4E-BP2). A functionally homologous yeast protein is encoded by the *CAF20* gene. They bind to the cap binding protein eIF4E and prevent its interaction with eIF4G. Their ability to bind eIF4E is regulated by phosphorylation of the BPs (see Ref. [10] for a recent review). Another factor is p67 which binds to eIF2 and prevents phosphorylation by eIF2 α kinases. Additionally, the poly(A) tail-binding protein Pab1p, after a long hiatus since its discovery, has also become a key player. It interacts directly with a domain of eIF4G, demonstrated so far in in vitro experiments [97] and these observations offer a more detailed explanation for numerous observations showing that the poly(A) tail acts in

synergy with the 5' cap to stimulate translation efficiency (recent references [97, 106]).

5.2. Competition between decapping activity and translational initiation factors, where does the specificity come from?

From the preceding discussion it becomes apparent that translational control at the level of initiation can be achieved a priori by multiple mechanisms. First, the amount of each factor present in an active form could be controlled. This can be achieved by controlling its synthesis, or by depleting it with specific binding proteins. Second, many of the subunits can be phosphorylated, presumably affecting the activity of the factor. The challenging question is to distinguish and explain the effect of such mechanisms on either global protein synthesis, i.e. the overall rate of translation of all mRNAs or on the efficiency of translation of specific mRNAs. If all factors were present in unlimited quantities/activities, it would be difficult to discriminate between mRNAs selected for translation. Hence, one or more factors must be limiting and the most likely candidate is eIF4E [38, 105]. But there is a second aspect. For discrimination to occur, the interaction of one or more factors with specific mRNAs must be distinguishable. The differences in the sequences of the 5' UTRs of various mRNAs provide an obvious means for discrimination, and the factor(s) playing a role in this discrimination must therefore interact directly with the mRNA. Factors eIF4E, eIF4G, eIF4A, eIF4B and eIF3 are potential candidates.

The interaction of these factors with the mRNA is not likely to be easily explained in terms of a short, contiguous nucleotide consensus sequence or secondary structure constituting a binding site for a specific factor. A unique situation is the binding of eIF4E to the 5' cap [63] found on almost all mRNAs. The remainder of the complex must cope with a large variety of sequences, secondary structures and lengths of the 5' UTR. A simplistic view of the 'scanning model' [47-49, 96] has an initiation complex slid-

ing along from the cap to first start codon, aided by hydrolysis of triphosphates and the activity of a helicase. Considering the total mass (and size) of the initiation factors eIF2, eIF3, eIF4F together with the 40S ribosome in the 45S pre-initiation complex in relation to the total length of the 5' UTR (variable, but frequently less than 100 nt), there is often not much room to 'slide', depending on the contacts made by the mRNA with these factors.

A very thoughtful review of translational regulation has recently been published by Sachs and Buratowski [88], pointing out some common themes between transcriptional and translational initiation. In this view analogies are made between the TATA box and the 5' cap, the TATA-binding protein (TBP) and eIF4E, the interaction of these sequence specific proteins with either transcriptional activators (TAFs) or eIF4G and the further stabilization of the complex by interaction with other activator proteins acting at a distance, for example, from a distant enhancer in the case of transcription, and the Pab1p bound to the 3' poly(A) tail. A basal level of activity is thus achieved by the binding of eIF4E to the cap. Interaction of eIF4E with eIF4G brings the second subunit into the complex, strengthening the complex, but allowing already for a discrimination between different mRNAs by the nature of the contacts made by eIF4G within the 5' UTR.

Another significant point raised in this review is the following: one can consider the assembly of the preinitiation complex as an orderly, sequential addition of individual soluble factors. Alternatively, several factors may pre-assemble (a holoenzyme) and the binding efficiency and affinity may be controlled at once by multiple interactions between different domains on the complex and the 5' cap + 5' UTR. Specificity and discrimination between mRNAs could be achieved by a kinetic mechanism controlling the rate of assembly of the complete initiation complex, or by a difference in affinity of the entire complex for various mRNAs. Finally, there could be a rate-limiting translocation of the complex from the 5' cap to the start codon (a version of the sliding model).

In the specific case of glucose-mediated turnover, the activity of a specific factor or the whole complex towards a subset of mRNAs must be modified by a signal initiated by glucose, and a phosphorylation or dephosphorylation reaction becomes a highly plausible suggestion.

5.3. Phosphorylation of eIFs

There is a substantial literature on this subject [38] which cannot be reviewed in detail here. The phosphorylation of 4E-BP1 has already been mentioned. The responsible kinase is sensitive to rapamycin, initially characterized as an immuno-suppressant and inhibitor of G1 cell cycle progression in mammalian cells and in yeast [10,11]. It now appears that when unphosphorylated 4E-BP1 reduces the effective concentration of eIF4E, translation of a few selected mRNAs is significantly reduced [10]. The mechanism is part of a signaling pathway for translational control by growth factors. In yeast, the p20 protein encoded by the *CAF20* gene appears to play a role analogous to mammalian 4E-BPs [3,22]. However, initial experiments in our laboratory suggest that translational control by glucose is not sensitive to rapamycin, and it is not affected in a *caf20* mutant.

eIF4E is not only stoichiometrically limiting (in mammalian cells), and subject to sequestration by the 4E-BPs, but its affinity for the cap is further boosted by phosphorylation. Overproduction of eIF4E in mammalian cells leads to a transformed phenotype [38], presumably by the imbalance of proteins produced under less stringent control. Phosphorylation of the yeast eIF4E has also been described, first with correlations to the overall rate of protein synthesis in cells [37] and second with an effect on selectivity for mRNAs [102,110].

The protein eIF4G has been investigated for functional subdomains in mammals and yeast (Pab1p, eIF3, RNA, eIF4A binding). Two genes in yeast, *TIF4631* and *TIF4632*, encode homologs that may differ in subtle ways and are referred to as eIF4G1 and eIF4G2 [97]. Phosphorylation has not been investigated in detail.

Although most of the subunits of eIF3 have now been identified in mammals and yeast, its participation in initiation of translation is still somewhat of an enigma. Little information is available about potential phosphorylation of eIF3 subunits and their role in modulating initiation.

6. Signal transduction and translational control in other organisms

A complete review of this subject is not intended here and only two examples will illustrate how post-transcriptional control mechanisms in other systems may share features of the mechanism described here for yeast.

Chan and Yu [17] have recently described how the stability of the transcript from the α -amylase gene in rice is regulated by the sugar sucrose. A four-fold decrease in the half life of α -amylase mRNA is observed when rice cells in suspension are exposed to higher levels of sucrose. These authors demonstrated that an element in the 3' UTR is responsible for the sugar-dependent stimulation of turnover of the mRNA.

Another interesting model system includes the well known stimulation of protein synthesis by insulin and other growth factors. It is not possible to do justice to this subject in a short paragraph or two. Expert recent reviews are available [54, 55]. Reference has already been made in Section 5 to the control of the activities of 4E-BP1 and 4E-BP2, proteins which in their unphosphorylated state bind to eIF4E. The activity of eIF4E can itself be modulated by phosphorylation. Signal transduction pathways from the insulin receptor to the phosphorylation reactions involving 4E-BPs and eIF4E have been partially elucidated [55]. One of the pathways includes the following components: mSOS \Rightarrow Ras \Rightarrow Raf-1 \Rightarrow MEK \Rightarrow MAP kinase. Another, independent pathway includes phosphatidylinositol 3-kinase (PI3-kinase) and at least one hypothetical kinase further downstream. The PI3-kinase pathway also appears to have another target. The factor eIF2B is activated by insulin. A requirement for PI3-kinase but not MAP

kinase has been demonstrated for this activation of the factor which promotes GDP/GTP exchange on eIF2 [104].

7. Summary and future directions

Glucose repression of the expression of a select number of genes is a mechanism that can now be seen to operate at two levels. First, regulation of transcription is achieved by the modulation of the affinity of transcriptional repressors for elements in the promoter of such genes. Second, the half-life of the transcripts can be strongly influenced by the carbon source. Thus, in the presence of a nonfermentable carbon source like glycerol, the steady state level of *SDH1* and *SDH2* mRNAs (and others) is elevated by a combination of induced transcription and stabilization of the mRNAs.

The outline of a signaling pathway for transcriptional regulation has been established. Key components are glucose transporters and sensors in the plasma membrane, enzymes needed for glucose metabolism such as hexokinase 2 (*HXK2*), the major type 1 phosphatase (*GLC7*) and some of its regulatory subunits (*REG1*), a serine-threonine kinase (*SNF1*) and a combination of transcriptional activators or repressors (*HAP2/3/4/5*, *MIG1*, *SSN6*, *TUP1*). Additional intermediates, regulators and/or substrates for the phosphatase and kinase may exist. The expression of some of these regulatory components is subject to glucose repression, creating feedback loops which permit a wide range of responses to varying carbon sources.

The signaling pathway for the regulation of specific mRNA turnover is less well defined. Not unexpectedly, some upstream components appear shared, and the pathways branch with one branch leading to nuclear targets (transcriptional regulation), while the other targets cytoplasmic factors (mRNA turnover). The branch point is upstream of the Snf1 kinase whose function is not required for triggering mRNA turnover. The Reg1/Glc7 phosphatase complex plays a role in both mechanisms, suggesting that another kinase is involved in the regulation of mRNA

stability. This kinase and its target(s) remain to be defined; one or more of the eIFs are plausible targets.

Starting from a consideration of the mechanism of mRNA turnover, our studies have established that the glucose signal exposes the 5' 7-methyl G cap of select mRNAs to a decapping activity and once decapped, the RNA is rapidly degraded by a 5'-3' exonuclease (*XRN1*). It takes a small leap of imagination to recognize that decapping and degradation are in direct competition with the initiation of translation of the mRNA, involving the 5' cap and a combination of initiation factors, specifically the cap binding factor eIF4E, the other components of eIF4F (eIF4G and B) and eIF3. Modulation of the activity of these eIFs (and to some extent their preferential interaction with subsets of mRNAs) by protein phosphorylation is now being recognized as a very general and significant mechanism of translational control. From such considerations a working hypothesis has emerged postulating that the glucose-induced signal is ultimately targeted at one or more of these eIFs. As a result of the modulation of eIF activity/specificity the balance between translational initiation and decapping is tilted towards decapping in the presence of glucose, resulting in rapid turnover of the mRNA. A proof of this hypothesis requires the identification of a specific kinase (casein kinase II?) in competition with a phosphatase (Glc7p or other), as well as the identification of the specific eIF and the site at which it is phosphorylated.

Prior deadenylation of the mRNAs is not required. The specificity of the reaction appears to be determined by the 5' UTR of the mRNA. The existence and participation of a specific RNA binding protein analogous to the iron-responsive factor binding to the iron-responsive element of ferritin mRNA cannot be excluded at this time, but the absence of a consensus sequence in several affected mRNAs and the results observed after small alterations in the 5' UTR make this possibility less likely. In fact, understanding the precise characteristics of the *cis*-acting element in the 5' UTR in combination with the various RNA binding domains of sev-

eral of the eIFs remains a major challenge for the future.

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