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A QUANTITATIVE MODEL FOR THE TRANSLATIONAL CONTROL OF *GCN4* IN YEAST

Tao You[1,2], George M. Coghill[1*] and Alistair J.P. Brown[2]
[1] School of Physical Sciences, University of Aberdeen, Computing Science Department, Aberdeen, UK AB24 3UE
[2] School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, UK AB25 3ZD

Abstract

Cells are capable of adapting to environmental changes by reprogramming their gene expression. The yeast *Saccharomyces cerevisiae* responds to amino acid starvation by inducing the expression of enzymes on nearly all amino acid biosynthetic pathways (Natarajan *et al*., 2001; Hinnebusch, 2005). The transcription factor Gcn4 plays a key role in this general amino acid control (GCN) response. The *GCN4* mRNA is translationally repressed under amino acid replete conditions, and this repression is alleviated to enhance the production of Gcn4p during amino acid starvation. This *GCN4* translational regulation lies at the heart of GCN regulation. We report the mathematical formulation that describes the translational control of the *GCN4* mRNA. Our modeling of previously published data on *GCN4* translational regulation suggests differential scanning rates for the 40S ribosomal subunit on the 5' leader sequence of the *GCN4* mRNA under amino acid replete and starvation conditions. This discovery could be attributable to changes in relative helicase activities under the two conditions and requires further investigation.

Keywords

GCN4 translational control, Kinetic model, 40S subunit scanning rate

INTRODUCTION

The yeast *GCN4* gene encodes a transcription factor that plays a central role in the GCN response to amino acid starvation (Natarajan *et al*., 2001; Hinnebusch, 2005). *GCN4* expression is regulated through a variety of control mechanisms at different levels, and can be induced or suppressed via different regulatory molecules under diverse conditions. These include the tight regulation of *GCN4* mRNA translation. Our model of this process, which is one of the best characterized examples of translational regulation in eukaryotic cells, exploits Gillespie's Stochastic Chemical Kinetics. These topics are reviewed briefly herein.

* To whom all correspondence should be addressed

GCN4 Translational Control

The GCN response is essential for yeast cells to grow under amino acid starvation conditions (Hinnebusch, 2005). The regulation of *GCN4* expression is central to the kinetic behaviour of this response (Hinnebusch, 2005). *GCN4* expression is regulated primarily at the translational level through an unusually long 5'-leader region (about 500 nucleotides in length) which contains four short upstream open reading frames (uORFs) (Figure 1a). Under amino acid replete conditions, these uORFs essentially function as barriers to the translation of the main Gcn4-encoding open reading frame (ORF). The translation of these uORFs induces the detachment of scanning ribosomes from the *GCN4* mRNA (Figure 1b). uORF1 and uORF2 are relatively weak barriers that allow nearly half of the scanning ribosomes to remain on the mRNA. In contrast, uORF3 and uORF4 are strong barriers, causing nearly all of the ribosomes to disassociate from the *GCN4* mRNA after their translation (Abastado, 1991).

During translation initiation in eukaryotic cells, a scanning 40S ribosomal subunit must bind the ternary complex (TC) in order to recognize an initiation codon (AUG triplet). This critical ternary complex comprises eIF2·GTP and Met-tRNA$_i^{Met}$. The resultant preinitiation 43S complex (40S + TC) can then recognize the next start codon it encounters and translate the corresponding ORF. For the main *GCN4* ORF to be translated, scanning 40S subunits must bypass the inhibitory uORF3 and uORF4 in the 5'-leader of the *GCN4* mRNA. This can happen if the concentration of TC is relatively low, thereby reducing the probability of forming an active preinitiation 43S complex before the scanning 40S subunit bypasses uORF3 and uORF4 (Hinnebusch, 2005). *GCN4* mutants that lack uORF2 and uORF3 display essentially intact translational behaviour (Hinnebusch, 2005). Therefore, for the sake of simplicity, only uORF1 and uORF4 are considered in our model.

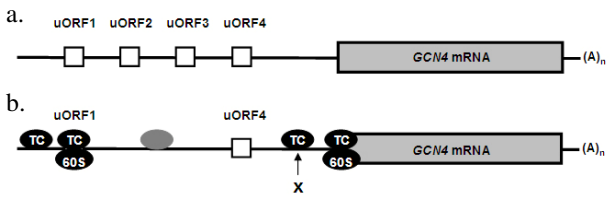

Figure 1. GCN4 Translational Control. (a) Cartoon of GCN4 mRNA structure. (b) Cartoon of the GCN4 main ORF translation.

When amino acids are replete, the concentration of active TC is relatively high. Therefore, 40S ribosomal subunits re-associate with TC efficiently after uORF1 translation, forming active preinitiation complexes. Hence these 43S complexes reinitiate at uORF3 or uORF4, thereby aborting translation of the *GCN4* mRNA before the main open reading frame. Hence, rates of translation of Gcn4 protein are low. However, when yeast cells are starved of amino acids, the abundance of eIF2·GTP declines as a result of Gcn2-mediated signaling events (Hinnebusch, 2005). Consequently the concentration of active TC declines, thereby leading to reduced rates of TC recruitment by 40S subunits. This leads to an overall reduction in yeast mRNA translation, but an increase in *GCN4* mRNA translation because more scanning 40S subunits bypass the inhibitory uORF3 and uORF4 and reach the main *GCN4* ORF (Hinnebusch, 2005). Gcn4 synthesis is derepressed about 25-fold under amino acid starvation conditions, leading to the activation of amino acid biosynthetic genes (Hinnebusch, 2005).

More recent work from the Hinnebusch laboratory on the relationship between the intercistronic distances between the upstream and main ORFs and the rate of reinitiation at the main *GCN4* ORF has implicated an additional unidentified factor (factor X) in the recognition of the *GCN4* start site (Figure 2b) (Grant *et al*, 1994). In fact, modulating the rate of each step in the translation initiation process affects the behaviour of the scanning 40S ribosomal subunits downstream of uORF1 on the *GCN4* mRNA, and changes the outcome of the *GCN4* translation. Those mutations that impair TC recruitment by the 40S subunit increases the amount of time needed for these subunits to regain their ability to reinitiate translation. This favours the bypassing of uORF3 and uORF4, and increases the likelihood of translating the main *GCN4* ORF (Hinnebusch, 2005). On the other hand, mutations that slow down the rate of 40S scanning downstream of uORF1 increase the chances of TC assimilation before the downstream uORFs. These mutations reduce translation at the main *GCN4* ORF thereby preventing proper activation of the GCN System (Hinnebusch, 2005). In summary, the efficiency of TC recruitment by 40S subunits, and the 40S scanning rate are two critical factors that determine the rate of *GCN4* translation. The *GCN4* translational control model should reflect the effects of both of these factors.

Gillespie's Stochastic Chemical Kinetics

The Gillespie's Stochastic Chemical Kinetics deals with thermally equilibrated chemical reaction systems. For such systems, reaction parameter a_μ is introduced to denote how likely a reaction happens (Gillespie, 1977). It is the counterpart of reaction rate in regular kinetics (which is customarily expressed in moles per second per unit volume), and is measured in moles per second (Gillespie, 1977). For a reaction system comprising α reactions under given initial conditions (chemical concentrations, known temperature, etc.) at time t, denote the probability that the next reaction will be an R_μ reaction and will happen during the next infinitesimal time interval $(t+\tau,\ t+\tau+d\tau)$ as $P(\tau,\mu)\cdot d\tau$, which is given by

$$P(\tau,\mu)\cdot d\tau = \exp[-\tau\cdot(\sum_{\alpha} a_\mu)]\cdot a_\mu \cdot d\tau \qquad (1)$$

Equation (1) explicitly shows that $P(\tau,\mu)\cdot d\tau$ conforms to an exponential distribution with regard to the relative time τ given the initial time t. This system has some very desirable properties. Integrating this joint probability function over all the time and all the reactions respectively, it is possible to derive the marginal probabilities with respect to reaction and time.

$$P(\mu) = \int_0^{+\infty} P(\tau,\mu)d\tau = \frac{a_\mu}{\sum_{\alpha} a_\mu} \qquad (2)$$

$$P(\tau) = \sum_{\alpha} P(\tau,\mu) = (\sum_{\alpha} a_\mu)\cdot\exp[-\tau\cdot(\sum_{\alpha} a_\mu)] \qquad (3)$$

Equation (2) demonstrates that $P(\mu)$ is independent of time (Gillespie, 1977). Rather, the probability of which reaction will fire in the next time interval $(t,\ t+\tau)$ is only determined by the reaction parameters. Similarly, Eq. (3) manifests that the probability distribution for when the next reaction will happen is only determined by the total rate of reactions (Gillespie, 1977).

MATHEMATICAL MODEL

In a recent report, the expression of a series of *GCN4-lacZ* reporters, each containing different 5'-leader lengths, was examined to study the relationship between intercistronic distance and the proportion of 40S ribosomal subunits competent to reinitiate translation downstream of uORF1 under steady state conditions (Grant *et al*., 1994). Our model, which mathematically formulates this work, includes assumptions are that are stated below:

1. Random disassociation of scanning 40S ribosomal subunits, and mistranslation by translating ribosomes were neglected.
2. Reverse 3'-to-5' scanning was ignored.
3. 40S ribosomal subunits that reacquire a TC translate the next 3'-proximal reading frame.
4. 40S ribosomal subunits that translate uORF4 dissociate from the *GCN4* mRNA immediately after uORF4 translation, and were thus prevented from reaching *GCN4*. Hence, only those 40S ribosomal subunits that reacquire TC after they have scanned past uORF4 can translate the main *GCN4* open reading frame.

Formulation of the reinitation at uORF4

Now we consider the physical picture necessary to describe the 40S reinitiation at uORF4. This process comprises only two reactions: the translocation reaction in which the 40S move forward by a constant number of nucleotides per unit time, and the binding reaction in which the 40S reacquires TC. Denote the translocation reaction parameter by a_s, and the binding reaction parameter by a_{re} (that is $k{\cdot}TC{\cdot}(40S{\cdot}mRNA_{leader})$ in quantity). Using Eq. (3), the expected time for the next reaction to happen is calculated to be

$$\int_0^{+\infty} \tau \cdot P(\tau) d\tau = \frac{1}{a_{re} + a_s} \tag{4}$$

If the next reaction is scanning, the 40S subunit will move forward by $\frac{a_s}{a_{re} + a_s}$ nucleotides. For an mRNA 5' leader sequence of n nucleotides, the 40S subunit would need to move as many as $\frac{n \cdot (a_{re} + a_s)}{a_s}$ steps to finish scanning. Given by Eq. (2), the probability for the next reaction to be scanning is $\frac{a_s}{a_{re} + a_s}$.

Therefore, the probability for a 40S to scan past the entire stretch of the 5' leader without re-associating with a TC is given by expression (5). And the proportion of the ribosomes that have reacquired TC among all the ribosomes that scanned n nucleotides is given by expression (6). This conclusion formed the basis for the parameter estimation that follows.

$$\left(\frac{a_s}{a_{re} + a_s}\right)^{\frac{n \cdot (a_{re} + a_s)}{a_s}} \tag{5}$$

$$1 - \left(\frac{a_s}{a_{re} + a_s}\right)^{\frac{n \cdot (a_{re} + a_s)}{a_s}} \tag{6}$$

Estimating a_{re} and a_s for uORF4 translation

In the work by Grant *et al.* (1994), the percentages of competent ribosomes that reinitiate at uORF4 were calculated by comparing the lacZ activities of mutants with only uORF1 and *GCN4* (denoted as A_1) and the corresponding mutants that also include uORF4 (denoted as A_2) by the following formula. The calculated probability values (Table 1) were used to estimate a_{re} and a_s.

$$\frac{A_1 - A_2}{A_1} \tag{7}$$

Table 1. The percentages of ribosomes reinitiated at uORF4 for different intercistronic distances between uORF1 and uORF4 under the two conditions.

Intercistronic distances (nt)	32	200	230	273	346
Repressing condition (%)	76	97	96	96	96
Derepressing condition (%)	22	72	73	88	91

To estimate the best fit of the model, a Euclidian distance was introduced. It was defined as the distance between the predicted values of competent ribosome percentages for various 5'-leader lengths and the experimental results, and was calculated with respect to each condition. Suppose the predicted values for amino acid replete cells are P_n ($n=1, 5$), and P_n' ($n=1, 5$) for amino acid starved cells, with the experimental results P_n^{obs} ($n=1,5$) and $P_n^{obs'}$ ($n=1,5$) under the two conditions respectively, the Euclidian distance used to optimize the parameters are defined as the following.

$$\sqrt{\sum_{n=1}^{5} \left(P_n - P_n^{obs}\right)^2} \tag{8a}$$

$$\sqrt{\sum_{n=1}^{5} \left(P_n^{'} - P_n^{obs'}\right)^2} \tag{8b}$$

In the present case, it was assumed that a_s has a value of 10 nt/sec when amino acid is replete (Berthelot, 2005). Using expression (8a), the value of a_{re} under the repressing condition was searched in the range (0, 1], by employing an evolutionary optimization algorithm that has a stochastic ranking procedure to efficiently walk through the user-defined parametric space to find the minimum (Runarsson, 2000). Minimizing the distance given by

expression (8b), the a_{re} and a_s values under the derepressing condition were searched in a separate optimization using the same algorithm, in which a_s was allowed to assume a different value in the range (0, 100] nt/sec other than the fixed value 10 nt/sec, and the value of a_{re} was searched in the range of (0, 1]. Each search evolved 1000 generations to find the best possible fit. The computation was done in MATLAB® R2006b on a Pentium 4 PC with 2Gb of RAM.

Formulation of the translation reinitiation at GCN4

It was assumed that the assembly of factor X to 40S is independent to the binding of the TC to 40S, both spatially and temporally (Grant *et al.*, 1994). Therefore, for an mRNA with only uORF1 and the main *GCN4* ORF, the probability for the 40S to reinitiate at the main *GCN4* ORF is equal to the product of the probabilities that it assembles both factors before reaching there. Denoting the binding rate for TC as a_{re1}, and introducing an additional binding rate for factor X, a_{re2}, the above statement is formulated as the following.

$$\left[1-\left(\frac{a_s}{a_{re1}+a_s}\right)^{\frac{n\cdot(a_{re1}+a_s)}{a_s}}\right]\cdot\left[1-\left(\frac{a_s}{a_{re2}+a_s}\right)^{\frac{n\cdot(a_{re2}+a_s)}{a_s}}\right] \quad (9)$$

Estimating a_{re} and a_s for uORF4 translation

The probabilities of *GCN4* recognition at different intercistronic distances between uORF1 and the main *GCN4* ORF under the two conditions were calculated by comparing the lacZ activities of mutants with uORF1 and *GCN4* (denoted as A_1) to the corresponding ones lacking uORF1 (denoted as A_2). These results were subsequently normalized by the percentage of ribosomes that reinitiated at *GCN4* when uORF1 was separated by 350 nucleotides from *GCN4*, as expression (10). The calculated probability values (Table 2) were used to estimate a_{re} and a_s.

$$\frac{A_1}{A_2}\Big/\frac{680}{1600} \quad (10)$$

Table 2. The dependency of ribosome reinitiation at GCN4 *with intercistronic distances under the two conditions.*

Intercistronic distances (nt)	32	50	140	172	350
Repressing condition (%)	11	31	62	52	89
Derepressing condition (%)	11	26	74	50	100

The values of a_s and a_{re1} under the two conditions were taken from the previous optimization results. To estimate the corresponding a_{re2} values under the two conditions, a new Euclidian distance expression (11) was introduced to indicate the goodness of fit. Parameter estimation was carried out using the same evolutionary optimization algorithm.

$$\sqrt{\sum_{n=1}^{5}\left(P_n-P_n^{obs}\right)^2+\sum_{n=1}^{5}\left(P_n^{'}-P_n^{obs'}\right)^2} \quad (11)$$

RESULTS AND DISCUSSION

Reinitiation at uORF4

As described above, reinitiation at uORF4 is central to the translational repression of GCN4 under amino acid replete conditions. Figure 2 shows that the reinitiation competence is highly sensitive to the TC concentration. Under the repressing conditions, 40S subunits quickly assimilate TC and regain their ability to recognize the next start codon. In fact, 76% of the 40S ribosomal subunits have reacquired TC after scanning only 32 nucleotides. Under the derepressing conditions, however, the ribosomes reacquire the TC at a slower rate. Only 22% of the ribosomes regain their translational competence after scanning an equivalent number of nucleotides, in sharp contrast with repressing conditions.

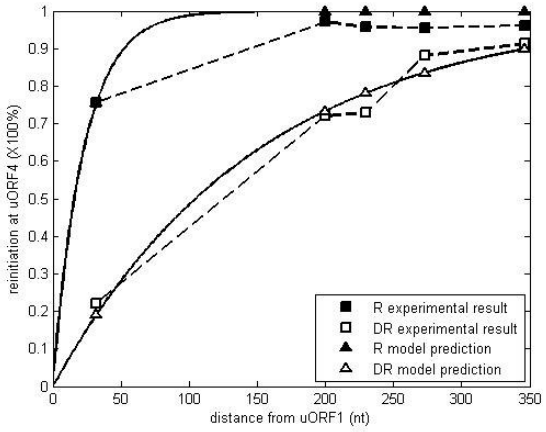

Figure 2. Plot of reinitiation at uORF4 as a function of the intercistronic distance from uORF1 to uORF4. The parameters of the two conditions were searched in two separate optimizations. Under the repressing condition, a_{re} = 0.43, with a Euclidian distance of 0.078. Under the derepressing condition, a_{re} = 0.19, and a_s = 29, with a Euclidian distance of 0.079.

The parameter estimation results show that the optimization algorithm successfully located an excellent fit to the experimental data. Since the original experiments were repeated 3 times and results were claimed to have less than 23% standard errors (Grant *et al.*, 1994), the model predictions are virtually indistinguishable from the wet data. In line with what was observed experimentally, the model is also sensitive to TC concentration, allowing 76% of ribosomes to re-associate with TC after scanning

32 nucleotides under the repressing condition, while only 19% under the derepressing condition.

The parameter estimation results also suggest a 3-fold increase in the scanning rate under derepressing conditions. To investigate whether this change is essential for the model to optimally account for the experimental results, the Euclidian distance given by expression (8b) was plotted as a function of TC binding rate and 40S scanning rate (Figure 4). A steep groove of the saddle-like surface is conspicuous on the perspective projections. The side views further demonstrate that the surface has a flat bottom with many possible combinations of the two parameters producing small Euclidian distances near the optimum value 0.079.

In fact, the formation of the linear steep grove is a direct result of expression (6). $\frac{a_s}{a_{re}+a_s}$ can be taken as a single variable, scanning rate ratio (*SRR*). Therefore, expression 5 becomes

$$1-(SRR)^{\frac{n}{SRR}} \qquad (12)$$

An optimization using expression (12) as the estimator of the probabilities gives the *SRR* values under the repressing and derepressing conditions are 0.955 and 0.993, respectively. Therefore, the model can best fit the experimental data for the derepressing condition as long as $\frac{a_s}{a_{re}+a_s}=0.993$, or simply, $\frac{a_s}{a_{re}}=\frac{0.993}{0.007}$. This explains the observed linearity on Figure 3, and quantitatively reiterated the previous point that the *GCN4* translational control relies on both parameters and neither of them should be neglected when studying this system.

Therefore, the 3-fold change in 40S scanning rate is dispensable. However, this does not violate the conclusion that the scanning rate has to change in order to fit the experimental observations. Since the global translation rate of H98 (the *GCN4-lacZ* reporter used to mimic amino acid starvation) is only decreased by less than 3-fold (Moehle, 1991) (0.43/3 ≈ 0.14, corresponds to 22 nt/sec along the scanning rate axis in Figure 3), we shall reasonably expect that the 40S scanning rate is enhanced by around 2-fold (22/10 ≈ 2). These results were used to estimate a_{re2} in the next subsection.

Reinitiation at GCN4

The model predictions are consistent with the observation that the probability of reinitiation at main *GCN4* ORF is much less sensitive to the TC level (Figure 4). Under the derepressing condition, the 2-fold increase in 40S scanning rate is suppressed by the large elevation in factor X binding efficiency (Grant *et al.*, 1994). When amino acids are plentiful, the *GCN4* reinitiation is also modulated by the factor X binding rate to maintain a nearly constant recognition probability. Similar to the case for uORF4, the 20-fold change is unnecessary (Figure 5). In fact, a roughly 10-fold change is already adequate for the model to accommodate the data (see (0.55, 0.06) in Figure 5 inset a). Again, this does not violate the conclusion that the recognition of *GCN4* is modulated by factor X binding to maintain a nearly constant dependency for the translational competence on the intercistronic distance for the two conditions.

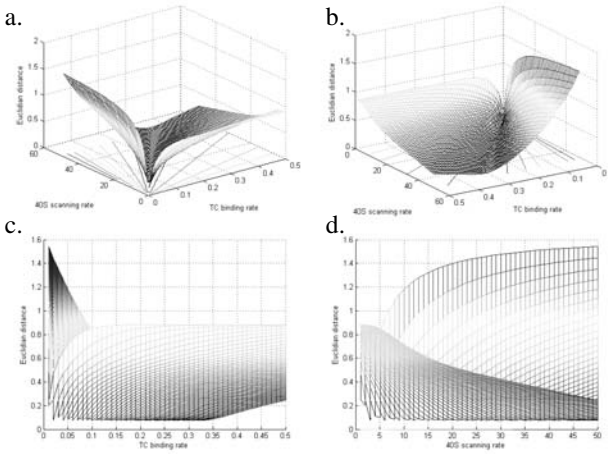

Figure 3. The Euclidian distance given by formula (8b) (z axis in inset a) was plotted as a function of the TC binding rate (x axis in inset a, 0.01 to 0.5, step length: 0.01) and the 40S scanning rate (y axis in inset a, 1 to 50 nt/sec, step length: 1nt/sec). Both perspective projections (a, b) and side views (c, d) are illustrated.

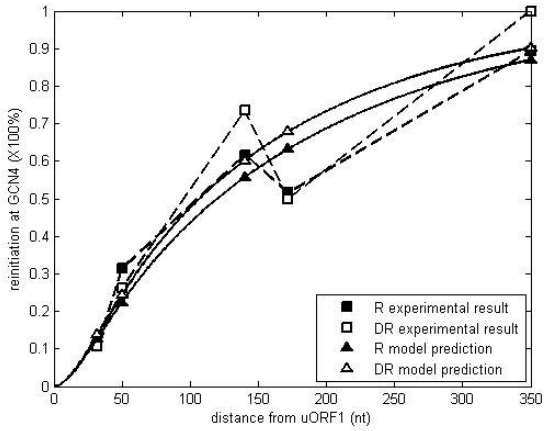

Figure 4. Plot of reinitiation at GCN4 as a function of the intercistronic distance from uORF1 to GCN4. The parameter estimation predicted that the best a_{re2} values for the model are 0.058 and 1.2 under the repressing and derepressing conditions, respectively, giving an optimal Euclidian distance of 0.296. In other words, the binding of factor X is 20 times more efficient when amino acid is starved.

A possible mechanism for the 40S scanning rate change

The previous discussion suggests that the 40S scanning rate changes from 10nt/sec to 22nt/sec during amino acid starvation. This paradoxical increase under the

derepressing condition can possibly be explained by a change in the relative helicase activities under these conditions.

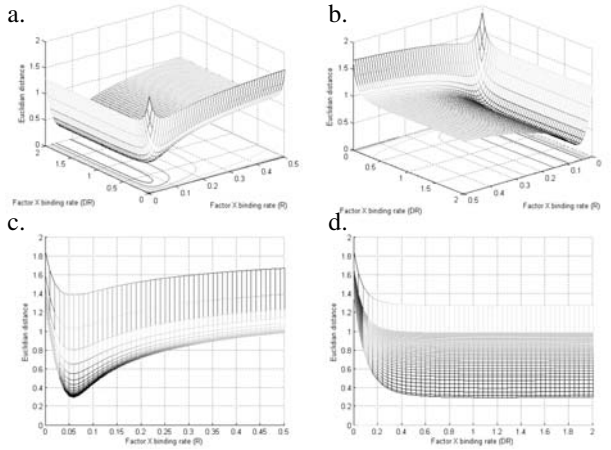

Figure 5. The Euclidian distance of GCN4 translation (z axis in inset a) was plotted as a function of the factor X binding rates under the two conditions (repressing condition, x axis in inset a, 0.01 to 0.5, step length: 0.01; derepressing condition, y axis in inset a, 1 to 50 nt/sec, step length: 1nt/sec). Both perspective projections (a, b) and side views (c, d) are illustrated.

There are two major ATP-dependent helicases in yeast, eIF4A/B and Ded1. eIF4A and eIF4B are found to be very abundant in the cytoplasm, having saturating numbers of 106000 and 24000 molecules per cell on average under the regular growth conditions, respectively (Huh *et al.*, 2003). However, eIF4A has a low helicase activity in RNA unwinding and annealing, even when bound to its stimulator eIF4B. On the other hand, Ded1 is less abundant, but has a much higher RNA unwinding efficiency. Ded1 has been reported to have a 40-fold higher ATPase activity compared to eIF4A, and 25-fold higher activity than eIF4A·eIF4B in an 11 base-pair RNA duplex unwinding assay (Marsden *et al.*, 2006). Ded1 loads onto the duplex in a manner dependent on the single-stranded region (Yang, 2006). This suggests the hypothesis that eIF4A·eIF4B and Ded1 might load competitively onto mRNA. When amino acids are replete, eIF4A·eIF4B may bind more favorably than Ded1 due to its higher abundance in the cell. According to this model, this would limit the scanning rate.

Under the amino acid starvation conditions there is a 75% reduction in the transcription of eIF4A and eIF4B genes (Natarajan, 2001). In contrast, the *DED1* gene is transcribed constitutively under these conditions (Natarajan, 2001). Therefore, Ded1 levels probably increase under derepressing conditions, relative to eIF4A·eIF4B. According to this hypothesis, relatively more Ded1 may become loaded onto mRNA during amino acid starvation, thereby enhancing the unwinding of the *GCN4* 5'-leader region and enhancing the scanning rate. In brief, this hypothetical mechanism, which is based on the differential regulation of the two helicases Ded1 and eIF4A·eIF4B during amino acid starvation, can provide an explanation for the change in scanning rate under these conditions.

CONCLUSIONS

A kinetic model was rigorously formed based on Gillespie's Stochastic Chemical Kinetics. Exploiting the experimental data for *GCN4-lacZ* reporters with mutant 5' leaders, this model was reliably parameterized. The model strongly suggests the existence of differential scanning and factor X binding rates under the amino acid replete and starvation conditions, which deserve further investigation in the future.

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