Modeling mRNA Populations

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Abstract

This paper presents a model to describe the dynamics of protein translation. A system of ordinary differential equations is derived to describe the number of ribosomes bound to a strand of mRNA at a given time. The number of ribosomes bound to an mRNA at a given time is referred to its ribosome load. The mRNA is classified based on its ribosome load and whether or not it's decapped for future degradation. Distribution of ribosome counts is assumed to be related to the translation initiation rate, translation completion rate, degredation marking rate, and length of the mRNA. The length of the mRNA's coding region plays the role of controlling the number of ribosome counts which, in turn, determines the number of ODEs in the system. A goal of this work is to see how the equilibrium distribution between classes as changes with coding region length. A closed form solution to the density in the i^{th} ribosomal class in a system with $i_{\max}$ states is presented for the equilibrium distribution of the decapped classes in terms of the capped classes. The equilibrium solutions in the capped classes are shown to be related to the full determinant of the tri-diagonal matrix used to describe the system, as well as all the determinants of the minors associated to it. In general, there is no closed form for the determinant of a tri-diagonal matrix, only a recurrence relation that can be used to find determinants. However, in this model a closed form exists for the full determinant as it changes with changing values of $i_{\max}$ and its formula is presented . This closed form for the determinant provides a method to efficiently find equilibrium solutions for the entire system. Additionally, a continuous approximation using PDE is derived and also used to find equilibrium solutions to the system. Both of these methods for determining equilibrium solutions are utilized in an effort to find the set of parameters that maximizes the likelihood of a given data set. A process for mapping the equilibrium model results to data is also presented and used to begin preliminary estimation of model parameters

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and to verify model function.

alternate abstract: Modeling Ribosomal Loading of mRNA

A model is presented to describe the dynamics of protein translation related to the ribosomal load of an mRNA. The number of ribosomes bound at a given time is referred to as ribosome load, and using this value a population of mRNA are classified. A system of ordinary differential equations (ODEs) is derived and solved for the equilibrium distribution of a population of mRNA. Distribution of ribosome counts is assumed to be related to the translation initiation rate, translation completion rate, degradation marking rate, and length of the mRNA. Methods are developed to find analytical equilibrium solutions to the system of ODEs and a system of partial differential equations (PDEs) are derived to find numerical approximations to the ODE system at equilibrium as well. Both the PDE continuous approximation and the analytical solutions to the ODE system agree offering two different methods for finding solutions at equilibrium within optimization routines. Additionally, a tool is developed and presented that is used to compare the model results to empirical microarray data measures of ribosome load.

Keywords: bioinformatics, mRNA population, protein translation, ribosome loading, ribosome count, polysome, mathematical model

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Paper Outline

1. Motivation - **(Mike)**
   (a) Why is this process important?
   (b) What will this model enable researchers to do?
   (c) Other modeling efforts?
2. Derivation and Assumptions
   (a) Physical processes captured (Ideally, have a quick discussion of process and inline definitions of variables used to represent process, followed by a total recap in a table) - **(Nate)**
      i. System described as population model: Dichotomy of decapped and capped mRNA. State variables based on an mRNA's ribosome load.
      ii. Process of mRNA production
      iii. Process of Marking mRNA for degradation supposed
      iv. Three processes of : Initiation, translation, and completion
   (b) Definition/Discussion of system boundaries - (**MIKE**)
      i. Physical boundaries as a cell and relation to parameters
      ii. Discussion of perceived upper and lower limits to state variables and parameters
      iii. Temporal boundaries and relation to steady state
   (c) Assumptions: Such as initial assumptions of specific functional forms, i.e. marking rate constant among classes - **(Nate)**
   (d) Justify consideration of system as two subsystems, decapped and capped. - **(Nate)**
3. Model Formulation: Total model presented and then analysis of capped and decapped systems - **(Nate)**
   (a) ODE/Discrete system
      i. Present system of ODEs (Total, capped, and decapped)
      ii. Matrix Representation of ODE model (Total, capped, and decapped)
      iii. Steady state formulations
   (b) PDE/Continuous system

i. Explain motivation for deriving PDE

ii. Explain framing as 'non-linear birth and death process'

iii. Explain derivation using Taylor expansion

iv. Present PDE for capped class

v. Present non-dimensionalized system

vi. Present 2nd order ODE to be solved for non-dimensionalized PDE at Equilibrium

vii. Motivate and present equation for decapped class at equilibrium

viii. (Make decision to present results for steady state values for PDE here or in a separate section to follow)

4. Results - **(Nate)**

   (a) Present solution strategies/methods

      i. ODE/Discrete system: Matrix inversion technique

      ii. PDE/Continuous system: Numerical solver of 2nd order ODE that arises at equilibrium

      iii. Discussion of alternative solution approaches

   (b) Present actual solutions for a couple sets of parameters: Highlight agreement of ODE and PDE system

   (c) Present solutions for discrete system under further simplifications for translation and initiation

5. Opportunities for Future Research - **(Nate and Mike)**

   (a) Application of model to real data. Can highlight sources of data.

   (b) Alternate functional forms and relaxed assumptions

   (c) Further establish connection (in simplified system) to potential probability distributions

   (d) How to move forward with analytical solutions, specifically connection to solving 2nd order partial difference equation arising from tri-diagonal form of matrix, note here that boundary conditions exist that may be utilized which are not normally present.

1. Introduction

This section addresses such topics as why modeling this process important, what this model will enable researchers to do, and what other modeling efforts exist that seek to achieve the same goals.

1.1. mRNA and Translation

1. Gene expression short overview
   (a) Gene expression is often stated as the central dogma in which genetic information encoded in the DNA is transcribed into mRNA which is subsequently translated into protein.
   (b) Often, a greater amount of attention is focused on explaining gene expression at the transcriptional level and prevailing changes of mRNA transcript levels.
   (c) However, multiple studies across all kingdoms of life have shown that transcript expression level is only moderately predictive of the final protein expression.
   (d) Gene expression at the post transcriptional level is controlled by mRNA transcript stability and degradation, translation and protein maturation/degradation.
   (e) The model presented in this paper encompasses gene expression regulation occuring at the translational and the mature mRNA population level.
2. Biology controlling mRNA stability and translation
   (a) Mature mRNAs in the cytosol are called the free mRNA pool, and are in one of three states.
   (b) They are actively being translated by ribosomes and will continue to initiate new rounds of translation until the transcript is degraded.
   (c) Transcripts are degraded directly from the free mRNA pool.
   (d) Transcripts are protected from degradation by RNA binding protein chaperones or are found in processesing bodies awaiting translation initiation or degradation.
   (e) Degradation of mature mRNAs is controlled by numerous processes depending on whether they are bound to ribosome, in processing bodies or in the free mRNA pool.
   (f) Free mRNAs can be decapped or deadenylated followed by exonuclease digestion.
   (g) Ribosomes can destine transcripts to degradation under multiple conditions.
   (h) The first ribosome to bind to a freshly exported transcript performs the "pioneer round of translation", which is charged with assesing the mRNA's quality.

(i) There are 3 processes which occur in the pioneer round of translation, all of which detect different mRNA defects.

(j) No Go Decay (NGD) detects a stalled ribosome, either due to mRNA structural features, slowly translating sequence or inteference of translation elongation.

(k) No stop decay (NSD) detects a missing stop codon and nonsense mediated decay (NMD) detects potential mis splicing or nonsense mutations.

(l) All three decay mechanisms, NMD, NSD and NGD lead to the eventual degradation of their bound transcripts.

(m) While NSD and NMD are a restricted to the pioneering round of translation, NGD can also uccur during the following rounds of translation.

(n) As transcripts are cleared by the pioneering round of translations more ribosomes can attach to the transcript, once more than one ribosome is on a transcript this ribosome mRNA complex is called a polysome.

(o) Transcripts associated to ribosomes are generally assumed to be protected from degradation and only degraded once ribosomes are off the transcript, however both NGD, (sRNA silencing) and a process called cotranslational decay can degrade actively translated transcripts.

(p) Cotranslational decay involved the decapping of actively translating mRNA transcripts and subsequent 5' to 3' mRNA degradation which follows a 3 nucleotide periodic pattern in step with the Ribosome.

3. Current Models/Research and how our model fits in the current field

1. The basic representation of the central dogma dictates that epression of protein coding genes starts from genes encoded in DNA that are transcribed to mRNA and subsequently translated to Protein.

2. A more careful representation considers that the final protein production is dependent on both the maintenance of an actively translating mRNA population, the association of ribosomes on the population and finally the degradation of the protein itself.

3. The maintenance of mRNA populations relies on the balance of mRNA transcription rates, the translation status of transcripts and numerous mRNA decay pathways.

4. mRNA degradation relies on removing protective and translation enhancing componenets of the mRNA. These include the 5' mG cap and the 3' polyadenosine tail.

5. Additionally mRNA degradation can be promoted through endonucleolytic cleavage by RISC (and siRNAs).

6. mRNA degradation can occur in both a ribosomal associated or a ribosome free manner.

7. Ribosomal association of transcripts can lead to both protection of viable transcripts as well as quality control degradation of faulty transcripts.

8. When a viable transcript is bound by the ribosomal and translational machinery, the 5' cap is bound by translational initation factors and the 3' tail is bound by poly A binding proteins. This protects transcripts from exonucleic attack and degradation.

9. Endonucleic degradation is still possible, but reduced due to a reduced accessibility of the siRNA binding sequence on the transcript through competition with ribosomes.

10. However multiple mechanisms of mRNA decay are carried out in association with the ribosome. Nonsense mediated decay, no go decay and no stop decay all rely on ribosomes detecting faults in the transcript and susbsequently interacting with degradation machinery to remove the faulty transcript.

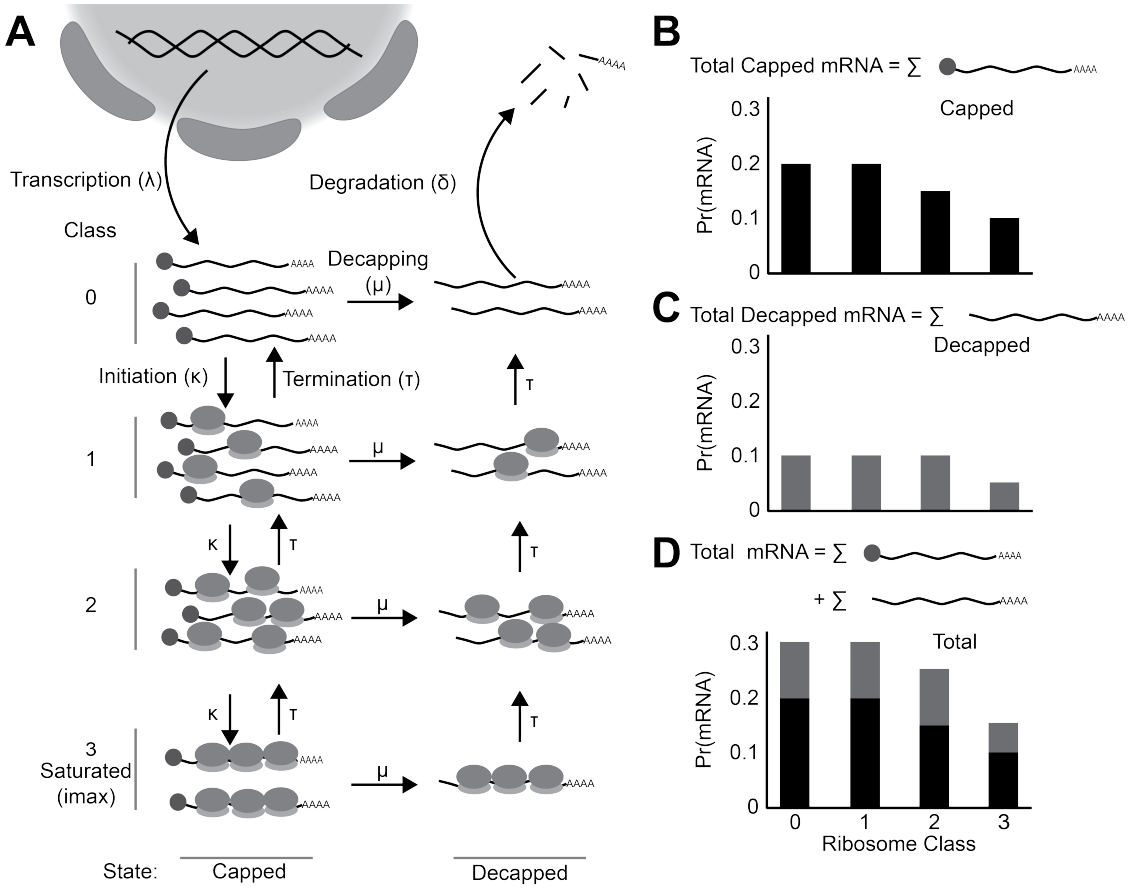
11. With some mechanisms of mRNA decay, decay can occur co-translationally. This is mainly seen in 5' decapping. When a translating transcript is decapped the 5' to 3' exonucleic degradation machinery trails the most upstream ribosome. As the ribosome translates the mRNA is degraded.

12.

2. Methods

2.1. Model Overview

Figure 1: Cartoon Representation of model in biological context. A) Model overview. Transcripts enter the sytem into the capped state at class 0 (no ribosomes bound). They enter the state at rate λ through transcription. Transcripts are free to move up and down ribosomal classes at rates κ for translation initiation and τ for elongation/termination. Transcripts can also be decapped and enter the decapped state at rate μ. Finally, upon reaching class 0 in the decapped state transcripts are fully degraded at rate δ. B) Probability of finding an mRNA in each class in the capped state. C) probability of finding an mRNA in each class in the decapped state. D) Joint probabilty of finding an mRNA in each class across each state. This reflect the total protein production potential.



The model captures some of the basic processes governing mRNA populations: transcript production, degradation and the process of translation (Figure 1A). Transcripts can exist in one of two states: capped and decapped which captures the the role of the 5' cap in mRNA protection and translation initiation. Capped transcripts are translationally competent, meaning that new ribosome can be loaded onto the transcript. Individual transcripts in the cell will be found with a set number of ribosomes (none, 1, 2, etc). The number of ribosomes on a transcript determines that transcripts ribosomal class. The model seeks to determine how the population of transcipts of a single gene are distributed between ribosomal classes and capped and decapped states. Transcripts enter into the model as defined by the transcription rate λ into the capped state with no ribosomes (class 0) From capped class 0 a transcript can have two fates. The transcript can be decapped for degradation at rate μ and move into the decapped class 0. Alternatively, a ribosome can initiate translation at rate $\kappa * (1 - i/imax)$ and be loaded onto the transcript and move it into capped class 1.Where i is the current transcript class and imax is the maximal ribosomal occupancy on the transcript A ribosome on transcript can then elongate and terminate at a rate of $\tau * i$. After a ribosome fully elongates and terminates it leaves the transcript and the transcript falls to a lower class. Capped transcripts move through rounds of translation initiation and elongationtermination and distribute along the different ribosomal classes. From any ribosomal class in the capped state the transcript can be decapped at rate μ and move into the decapped state while maintaining the same ribosomal class. decapped transcripts can no longer initate new rounds of translation, but allow for currently loaded ribosomes to complete translation. This process represent co-translational decay, a common method of mRNA decay in eukaryots (Citation) After all ribosome complete translation, the mRNA is in decapped class 0 and completely degraded at a rate δ. The saturated state of a transcript is denoted as *imax*, meaning that that transcript can no longer accept any more ribosomes. The model produces two outputs. First, the total mRNA in each state and therefore the system (Figure 1B-D). Second, The distribution of the mRNAs in each mRNA in each ribosomal class. (Figure 1 B-D). The total protein output at steady state from our model can be obtrained by calculating the average ribosomal class in the system by the total mRNA in the system (Figure 1D).

2.2. Model Definition and Analysis

For simplicity, we begin by defining our model equations using generic functions to describe the transition of mRNAs between different classes or states. We then constrain the model by assuming specific functions to describe the transition of mRNAs between classes.

General model equations of the density independent initation model

Our model consists of two sets of time dependent and coupled ODEs. Each set of ODEs describes the abundance of mRNAs that are either capped and decapped for degradation. The ODEs within each sets equations are structured by the ribosome load of the mRNA. The coupled ODEs within a set of equations describe how mRNAs are introduced to the set, the transitions in ribosome load via initiation or completion of protein translation, and the transition between sets either via the marking of capped mRNAs or the degradation of decapped mRNAs with a ribosome load of 0.

Specifically, new mRNA enter the 0^{th} capped class $m_0(t)$ at a rate.. Ribosomal bind mRNAs in the i^{th} capped class at a rate $\kappa(i)$, increasing the mRNA's ribsome load to the $i+1^{th}$ class. By definition, $\kappa(i_{\max}) = 0$, i.e. mRNAs with a ribosome load of $i_{\max}$ cannot accommodate any addtional mRNAs. An average ribosomal footprint covers 9 codons (27 nucleotides). Therefore for a protein of 270 amino acids in length, the maximal ribsomal load, $i_{\max} = 10$. Capped mRNAs with ribosome load i are decapped at a rate of $\mu(i)$. Accordingly, the ribosome load of decapped mRNAs remains unchanged, but they are transitioned from the capped class $m_i(t)$ to the decapped class $m_i^*(t)$. Ribosome movement along an mRNA is assumed to occur independent of whether or not its capped or decapped for degradation. Thus, ribosomes complete translation of both decapped and capped mRNAs with ribosome load i at rate $\tau(i)$, decreasing the mmRNA's ribosome load to the $i-1^{th}$ class. It is important to note that $\tau(i)$ is not the same as the average elongation rate. $\tau(1) = \textit{average elongation rate}/(9 \cdot i_{\max})$ Since mRNA's with a ribosome load of 0 have no ribosomes which can complete translation, by definition $\tau(0) = 0$. Only mRNAs decapped for degradation and with a ribosome load of 0 are removed from the system. Specifically, decapped mRNAs are removed from the 0^{th} class at a rate of $\delta\, m_0^*(t)$.

The functional form of the capped subsystem is:

$$
\begin{aligned}
\frac{dm_0}{dt} &= \lambda + \tau(1)m_1 - (\kappa(0) + \mu(0))\, m_0 \\
\frac{dm_1}{dt} &= \kappa(0)m_0 + \tau(2)m_2 - (\tau(1) + \kappa(1) + \mu(1))\, m_1 \\
&\vdots \\
\frac{dm_i}{dt} &= \kappa(i-1)m_{i-1} + \tau(i+1)m_{i+1} - (\tau(i) + \kappa(i) + \mu(i))\, m_i \\
&\vdots \\
\frac{dm_{i_{\max}}}{dt} &= \kappa(i_{\max}-1)m_{i_{\max}-1} - (\tau(i_{\max}) + \mu(i_{\max}))\, m_{i_{\max}}
\end{aligned}
$$

Similarly, the functional form of the decapped subsystem is:

$$\begin{aligned}
\frac{dm_0^*}{dt} &= \mu(0)m_0 + \tau(1)m_1^* - \delta m_0^* \\
\frac{dm_1^*}{dt} &= \mu(1)m_1 + \tau(2)m_2^* - \tau(1)m_1^* \\
&\vdots \\
\frac{dm_i^*}{dt} &= \mu(i)m_i + \tau(i+1)m_{i+1}^* - \tau(i)m_i^* \\
&\vdots \\
\frac{dm_{i_{\max}}^*}{dt} &= \mu(i_{\max})m_{i_{\max}}^* - \tau(i_{\max})m_{i_{\max}}
\end{aligned}$$

2.3. The density dependent initiation model

As stated above, we assume mRNAs marked for degradation cannot initiate translation. Thus the initiation function for the decapped set of mRNAs is $\kappa(i) = 0$. In contrast, ribosomes can initiate translation on a capped mRNA. For simplicity, we assume ribosome binding of an mRNA is independent of their ribosome load, but that successful initiation of translation is affected by ribosome load. Specifically, we assume the start codon must be unoccupied by a ribosome in order for translation initiation to be successful. Because we not modeling the explicit movement of ribosomes along an mRNA, we assume that at steady state probability of finding a ribosome at any given codon position within the coding sequence follows a uniform distribution. As a consequence of this assumption, the probability of a ribosome occupying a given position on an mRNA with a ribosome load of i is simply $i/i_{\max}$. Thus, the probability the start codon is unoccupied is $1 - i/i_{\max}$ and, in turn, our translation initiation rate function can be defined as,

$$\kappa(i) = \kappa_0 \left(1 - \frac{i}{i_{\max}}\right), \tag{1}$$

where κ_0 is a gene specific parameter that describes the rate at which capped mRNAs encounter and are bound by ribosomes within the cytosol (i.e. it is an implicit function of the abundance of free ribosomes which we assume is constant).

Similarly, the probability a ribosome is at the penultimate codon (i.e. the codon immediately upstream from the stop codon) is $i/i_{\max}$. Assuming that the rate of translation termination is greater than the average elongation rate (i.e. translation termination is not a rate limiting step), we can define

our translation completion rate function as,

$$\tau(i) = \tau_0 i / i_{\max} \tag{2}$$

where τ_0 describes the average elongation rate of a ribosome along the protein coding sequence of that gene and, thus, could vary between genes. Finally, we assume that capped mRNAs are decapped for degradation rate independent of their ribosome load, i.e. $\mu(i) = \mu_0$. As with τ_0, μ_0 is assumed to fixed for a given gene, but may vary between genes.

Incorporating these explicit functional forms leads to representation of capped subsystem as:

$$\begin{aligned}
\frac{dm_0}{dt} &= \lambda + \tau_0 \frac{1}{i_{\max}} m_1 - (\kappa_0 + \mu_0)\, m_0 \\
\frac{dm_1}{dt} &= \kappa_0 m_0 + \tau_0 \frac{2}{i_{\max}} m_2 - \left(\tau_0 \frac{1}{i_{\max}} + \kappa_0 \left(1 - \frac{1}{i_{\max}} \right) + \mu_0 \right) m_1 \\
&\vdots \\
\frac{dm_i}{dt} &= \kappa_0 \left(1 - \frac{i-1}{i_{\max}} \right) m_{i-1} + \tau_0 \frac{i+1}{i_{\max}} m_{i+1} - \left(\tau_0 \frac{i}{i_{\max}} + \kappa_0 \left(1 - \frac{i}{i_{\max}} \right) + \mu_0 \right) m_i \\
&\vdots \\
\frac{dm_{i_{\max}}}{dt} &= \kappa_0 \left(1 - \frac{i_{\max}-1}{i_{\max}} \right) m_{i_{\max}-1} - \left(\tau_0 \frac{i_{\max}}{i_{\max}} - \mu_0 \right) m_{i_{\max}}
\end{aligned}$$

and the decapped subsystem as,

$$\begin{aligned}
\frac{dm_0^*}{dt} &= \mu_0 m_0 + \tau_0 \frac{1}{i_{\max}} m_1^* - \delta m_0^* \\
\frac{dm_1^*}{dt} &= \mu_0 m_1 + \tau_0 \frac{2}{i_{\max}} m_2^* - \tau_0 \frac{1}{i_{\max}} m_1^* \\
&\vdots \\
\frac{dm_i^*}{dt} &= \mu_0 m_i + \tau_0 \frac{i+1}{i_{\max}} m_{i+1}^* - \tau_0 \frac{i}{i_{\max}} m_i^* \\
&\vdots \\
\frac{dm_{i_{\max}}^*}{dt} &= \mu_0 m_{i_{\max}}^* - \tau_0 \frac{i_{\max}}{i_{\max}} m_{i_{\max}}.
\end{aligned}$$

2.4. Matrix-vector Formulation of ODE System

It is frequently useful to work with the matrix-vector formulation for a system of ODE. In this model, the dynamics of the decapped and capped mRNAs can be represented as,

$$\vec{M}' = \boldsymbol{F}\vec{M} + \vec{B}, \tag{3}$$

where $\vec{M} \in \mathbb{R}^{2(i_{\max}+1)}$ is a vector of all state variables, ordered here as m_0, m_1, ..., $m_{i_{\max}}$, m_0^*, m_1^*, ..., $m_{i_{\max}}^*$, $\vec{M}'$ is the vector containing the first derivatives of $\vec{M}$ with respect to time, $\boldsymbol{F} \in \mathbb{R}^{2(i_{\max}+1)\times 2(i_{\max}+1)}$ is the matrix representing the full system (Equation 4), and $\vec{B} \in \mathbb{R}^{2(i_{\max}+1)}$ is the vector of λ as the first component and 0s else. Using the functional forms presented above, matrix formulations are provided next.

As opposed to explicitly listing elements of the full system matrix-vector representation we found that it is more convenient to utilize the block structure that emerges in this system and explicitly provide the block components. The matrix $\boldsymbol{F}$ is block lower-diagonal and is given in Equation 4.

$$\boldsymbol{F} = \begin{pmatrix} \boldsymbol{U} & \boldsymbol{0} \\ \\ \boldsymbol{\mu} & \boldsymbol{R} \end{pmatrix}. \tag{4}$$

The upper-left block, $\boldsymbol{U}$, corresponds to the capped state variables, where $\boldsymbol{U}$'s general form is provided in Equation 5. The upper-right block is a matrix of all zeros, $\boldsymbol{0} \in \mathbb{R}^{i_{\max}+1\times i_{\max}+1}$. Using $\boldsymbol{I}$ to represent the $i_{\max}+1 \times i_{\max}+1$ identity matrix, the lower-left block is $\boldsymbol{\mu} = \mu_0\boldsymbol{I}$, a diagonal matrix with the constant μ_0 on the diagonal and 0s else. The lower-right block, $\boldsymbol{R}$, corresponds to the decapped state variables and its form is provided in Equation 6.

The matrix $\boldsymbol{U}$ is $(i_{\max}+1 \times i_{\max}+1)$ dimensional and is tri-diagonal with non-zero entries on the

diagonal, super-, and sub-diagonals,

$$\boldsymbol{U} = \begin{pmatrix} -(\kappa_0+\mu_0) & \tau_0\frac{1}{i_{\max}} & & & & & & \\ \kappa_0 & \left(1-\frac{1}{i_{\max}}\kappa_0+\mu_0+\tau_0\frac{1}{i_{\max}}\right) & \tau_0\frac{2}{i_{\max}} & & & & & \\ & \ddots & \ddots & \ddots & & & & \\ & & & 1-\frac{(i-1)}{i_{\max}}\kappa_0 & -\left(1-\frac{i}{i_{\max}}\kappa_0+\mu_0+\tau_0\frac{i}{i_{\max}}\right) & \tau_0\frac{i+1}{i_{\max}} & & \\ & & & & \ddots & \ddots & \ddots & \\ & & & & & \frac{1}{i_{\max}}\kappa_0 & -\left(\mu_0+\tau_0\frac{i_{\max}}{i_{\max}}\right) & \end{pmatrix} \tag{5}$$

In the representation given in Equation 5, all blank entries are 0. The $(i_{\max}-1)^{\text{th}}$ row has been suppressed in Equation 5, but it can be generated using the formula included for the i^{th} row.

The matrix $\boldsymbol{R}$ is the lower-right block in the block lower-diagonal matrix $\boldsymbol{F}$ (Equation 4),

$$\boldsymbol{R} = \begin{pmatrix} -\delta & \tau_0\frac{1}{i_{\max}} & & & & & & \\ & -\tau_0\frac{1}{i_{\max}} & \tau_0\frac{2}{i_{\max}} & & & & & \\ & & \ddots & \ddots & & & & \\ & & & -\tau_0\frac{i-1}{i_{\max}} & \tau_0\frac{(i+1)}{i_{\max}} & & & \\ & & & & \ddots & \ddots & & \\ & & & & & -\tau_0\frac{(i_{\max}-2)}{i_{\max}} & \tau_0\frac{i_{\max}}{i_{\max}} & \\ & & & & & & -\tau_0\frac{i_{\max}}{i_{\max}} & \end{pmatrix}, \tag{6}$$

$\boldsymbol{R}$ is upper-diagonal with only non-zero entries on the diagonal and the super-diagonal.

2.4.1. Capped Subsystem Matrix-vector Representation

As a group the capped subsystem decouples from the decapped subsystem, as such the capped subsystem can be solved independently of the decapped subsystem. The matrix-vector formula representing the capped subsystem is

$$\vec{m}' = \boldsymbol{U}\vec{m} + \vec{b}, \tag{7}$$

where $\vec{m} \in \mathbb{R}^{i_{\max}+1}$ is the vector of capped state variables ordered m_0, ..., $m_{i_{\max}}$, $\vec{m}'$ is the vector containing the first derivatives of $\vec{m}$ with respect to time, $\boldsymbol{U} \in \mathbb{R}^{i_{\max}+1 \times i_{\max}+1}$ is the matrix representing the capped subsystem (Figure 5), and $\vec{b} \in \mathbb{R}^{i_{\max}+1}$ is the vector of λ as the first component and 0s else. With all equations defined for the full ODE system, include matrix-vector representations, the next section outlines methods for finding steady-state solutions to the system.

3. Determining steady-state solutions

With a closed-form expression for the determinant of the capped class matrix $\boldsymbol{U}$ we are now able to formulate another method to determine equilibrium solutions in the capped class by utilizing the matrix-vector formulation of the capped subsystem. At equilibrium $\vec{m}' = 0$, and so the matrix-vector equation (Eq. 7), when solved for the vector of state variables, $\vec{m}$, becomes,

$$\vec{m} = -\boldsymbol{U}^{-1}\vec{b}. \tag{8}$$

and making determination of equilibrium solutions come down to deriving an efficient technique for inverting $\boldsymbol{U}$.

The closed-form of the determinant enables the identification of parameters in which $\boldsymbol{U}$ is invertible. Using the fact that $\boldsymbol{U}$ is invertible iff $\det[\boldsymbol{U}] \neq 0$. Equation **??** shows that the only possible scenario where $\boldsymbol{U}$ is non-invertible is when $\mu_0 = 0$. Given that μ_0 controls the rate at which capped mRNA become decapped for deletion, the physical interpretation of $\mu_0 = 0$ is no degradation of capped mRNA and thus no loss in the system, leading to linear, unbounded growth in capped mRNA populations equal to the mRNA transcription rate λ.

With a classification of when $\boldsymbol{U}$ is invertible we now consider a particular method for matrix inversion that can make use of the closed-form of the determinant. Namely, the method considered here is the determinant-adjoint approach given by,

$$\vec{m} = -\frac{1}{\det[\boldsymbol{U}]} Adj[\boldsymbol{U}]\vec{b}.$$

Thus inverting $\boldsymbol{U}$ now falls onto the task of simplifying the product of $Adj[\boldsymbol{U}]\vec{b}$. Owing in part to the tri-diagonal form of $\boldsymbol{U}$ and to the fact that $\vec{b}$ is only non-zero in the first component, an expression for $\vec{m}$ was able to be determined as follows,

4. Results

1. The capped system can be split into two components: Total transcript count and the distribution of transcripts.

   (a) The capped class transcript number is determined by λ, μ

   (b) In the capped class the total transcript abundance is determined by a similar relationship as Equation 21.

   $$\frac{\lambda}{\mu} = \sum_{i=0}^{i_{\max}} m_i, \tag{9}$$

   (c) Where λ is only a scaling factor for the system as a whole. I.e. the distribution of transcripts across all classes is determined by κ, τ, μ and δ.

   (d) μ affects both the total transcript abundance and the distribution of ribosomal classes across a particular species of transcript.

   (e) First μ controls the rate of outflow from capped unto decapped, and second it shifts mRNAs to lower ribosomal classes.

   (f) The solution to the system, as presented previously, can be expressed in the determinant-adjoint form:

   $$\vec{m} = -\frac{1}{\det[\boldsymbol{U}]} Adj[\boldsymbol{U}]\vec{b}.$$

   (g) As $\vec{b}$ is [λ 0 0 0 ... 0]. Only the first column of the adjoint matrix contributes to the result.

   $$Adj[\boldsymbol{U}]\vec{b} = \lambda\vec{a}$$

   and

   $$\sum_{j=0}^{i_{\max}} \vec{a}_j = a_{tot}$$

   (h) With this we can factor our solution into two parts: 1) the total transcript abundance and 2) The distribution of transcript across the ribosomal classes.

   $$\vec{m} = -\frac{\lambda a_{tot}}{\det[\boldsymbol{U}]} \frac{\vec{a}}{a_{tot}} = \frac{\lambda}{\mu}\vec{p}_m$$

   (i) The terms on the left hand side of the equation represent the total transcript population. The right hand side is the vector of probabilities, one entry for each class and is a function of κ, τ, and μ.

(j) This formulation has three interesting properties

i. First it gives a determinant free solution to our system. Now, to obatin a full solution of the capped solution to our model we only need the first column of the Adjugate matrix.

ii. Second it splits the two funtions of μ; Its effect on transcript number and its effect on transcript distribution. And allows for their separate analysis.

iii. Third, it permits analysis of the underlying transcript distribution even under conditions where the model has no solution. For example, when $\mu = 0$, both solutions are indeterminate. However, the determinant free solution allows for us to explore what the transcript distribution would be when μ=0.

2. Ribosomal distribution on the decapped class is determined by the ratio of κ/τ and μ

(a) Figure 4 shows a representative contour plot of model solutions for a broad range of κ values.

(b) For $\kappa < 0.1$ there is no ribosome loading, while loading maxes out beyond 10^4, with a smooth transition between boundaries.

(c) In order to better interpret the model we will focus on parameter ranges dervied from literature.

i. To approximate μ transcript half-lives calculated by Sorenson (2018) in cordycepin treated *Arabidopsis thaliana* seedlings. where μ is:

$$\mu = \frac{ln(2)}{t_{1/2}} \tag{10}$$

Where $t_{1/2}$ is the half-life. Model solutions were produced for a range of half-lives: 1, 5, 25, 50, 75, 95 and 99 percentile values of the half-lives found in Sorenson (2018)

ii. The resulting range of μ is from 10^-3to10^-6.

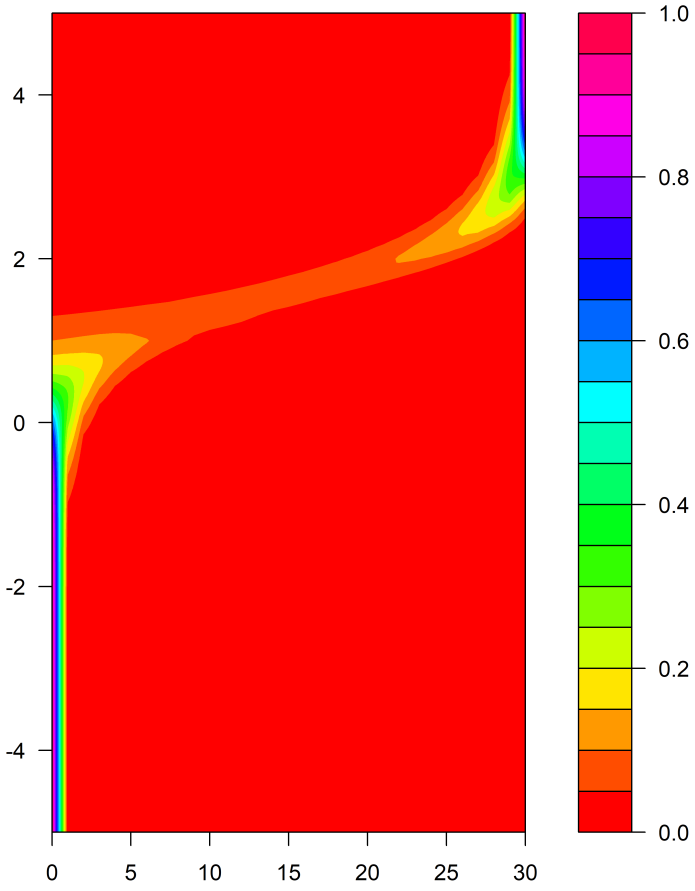
iii. The range for τ is based from (insert citations here) and ranges from 4-20 aa/s depending on the organism. A plausible biological range of 0.1-20 aa/s was explored.

iv. The

v. As λ is only a scaling factor and does not affect the distribution of the ribosomes it was set to 1.

3. The decapped class depends on the distribution of the capped class, τ, μ and δ

Figure 2: Contour plot of capped class over a range of kappa values: $\kappa = 10^{-}5 - 10^{5}.i_{\max} = 30, \lambda = 1, \tau 0 = 1, \mu 0 = 0.1, \delta = 100,000$. Color scale is percent ribomose load for each kappa value across all 31 ribosome classes.



(a) The decapped subsystem can be further divided into the m_0^* class and the m_i^* classes for $i > 0$.

(b) the m_0^* class in the current model design represents only two forms of degradation, free mRNA degradation and the degradation of the remanents of XRN1 mediated degradation.

(c) δ only determines the accumulation of transcripts in the m_0^* class. It is reasonable to explore the model with large δ since decapped transcripts are translationally incompetent.

Data Sources

1. Protein Length data

(a) Saccharomyces cerevisiae

(b) Arabidopsis thaliana

2. mRNA half life

(a) Saccharomyces cerevisiae

(b) Arabidopsis thaliana

3. Defining parameter space

(a) Saccharomyces cerevisiae

(b) note on At and Hs

Main results:

Supplemental Figure 1

1. The model solution at equilibrium has three outputs.

2. First the total number of mRNA transcripts. Second split between the capped and decapped states. And third the distribution of the transcripts across ribosomal classes for each of the states.

3. s

4. After exploring the model solutions it was determined that the transcription rate λ acts simply as a scalar for the whole system.

Text for discussion of figure 1

1. The model presented most closely resembles the co-translational decay pathway from eukaryotes.

2. There are a few current limitations of the model. First and foremost it doesn't model endonucleolytic decay pathways such as gene silencing by siRNA and the RISC complex or the NMD, NGD and NSD pathways or degradation initiated through ribosomal collisions.

3. Implementation of endonucleolytic decay would require an ability to track ribosomal position, which is not possible in our model.

4. Alternatively a probabilistic model of ribsomal position given a particular load could allow for redistribution of capped transcripts into decapped states of lower ribosomal class.

5. Currently there is a debate whether mRNA stability is regulated primarily through the protective effects of ribosomal association or through the suboptimal codons causing ribosomal stalling and the subsequect ribosome associated decay pathways (Chan elife).

6. We explored the interaction of ribosomal loading and marking rate, noting that increased marking leads to a lower expected ribosomal load. This particularly noticable for genes with short half lives and is less sensitive with transcripts with halflives above 2 hours.

7. In the current implementation of the model we did not directly explore the protective effects of ribosomal loading. This could be first implemented by including a similar weighting term analogous to the wieght for the intiation rate of (1-i/imax).

8. However, biology suggests a more complex behavior.

9. The protective effects of translation could increase per ribosome, but eventually at high loads could trigger ribosome associated decay pathways through ribosomal collisions.

10. Our model does not model elongation at a single codon level like TASEP, nor at a course grained approximation of groups of codons like the ribo flow models. This means that it is not capable of analyzing the effect of individual non optimal codons, or stretches of mRNA which are non-optimal

11. Our model reflects the average elongation rate over a transcript. However it can be set to reflect the slowest bottle neck across a transcript.

12. Another alternative to modeling bottlenecks is increasing granularity to a very coarse grained ribo-flow model comprising of only two or three sectors, depending whether the transcript has one or two bottlenecks.

13. This splits transcripts into an intial sections where the bottleneck effectively "shortens" the transcript length to the first bottleneck site. Increasing the effect of ribosome loading on iinitiation interference.

14. However, the transcript physically has more length and can bear a larger ribosomal load on the section section. This results in a transcript having and "effective" imax between the actual coding sequence length and the lenght of the transcript to the first bottleneck.

Model predicts mRNA distribution across ribosomal classes

1. The model

2. Density independent initiation and density independent initiation models

3. The model predicts the steady state total mRNA quantity in both decapped and capped states as well as the mRNA's distribution across different ribosomal classes in each state.

4. Figure X Left,

The model provides an upper bound on translational efficiency

1. The behavior of both DDI and DII models were explored at equilibrium. At equilibrium, the rate of new initiation events must be equal to the rate of ribosomes leaving the transcript.

2. The two key parameters that control this process are initiation (κ) and elongation (τ). Changes in either parameter alter the predicted distribution of transcripts across ribosomal classes.

3. To summarize their joint behavior, we chose to focus on the elongation scaled initiation rate (κ τ) as this provides a single unique parameter for each model response.

4. Exploration of the steady state solution showed that the transcription rate only acts to scale the steady state solution. Meaning that the distribution of transcripts across the ribosomal classes is independent of the number of transcripts being produced. All model solutions presented here have the same transcription rate to allow for comparison.

5. The degradation rate (δ) only controls the transcript population in the decapped zeroth class. To reduce interference from this we set a very high degradation rate.

6. To interpret model behavior within a biologically meaningful context, empirically determined rates for translation initiation, elongation, and half-life (as a proxy for marking rate) were obtained from the literature.

7. Introduce scaled initiation rate range. Discuss upper and lower limits vis a vis the model output.

8. *** This is a very important question. How do we show in a convincing manner that the model is recapitulating actual biological results? I have previously used the arguement that both polysome profiling and single molecule imaging of in-vivo ribosomes all agree with the approximate range predicted by the model, especially within the scaled initiation values. That is the model predicts that the ribosome load will be between 10-50%0, with median loads of 2

- 13 ribosomes for a transcript of median length. This is reasonable and the median behavior aligns with what polysome profiles show. However, Polysome profiles contain all of the mRNAs in a cell or organism at once. This means that the fact that the model predic

(-3 to -0.6 in log ten). The marking rate was determined from Arabidopsis mRNA half-lives with a median half-life of 115 min (short 1st percentile: 11.5 min, long 99th percentile: 1,500 min) [32] To establish the base model behavior, DII and DDI models were run with a long half-life across a broad range of scaled initiation on a transcript encoding a protein of median length (369aa) (Figure 1). In the DII model, all ribosomal classes are populated. At the high end of the biological scaled initiation transcripts reach full ribosomal occupancy (Figure 1A). The DII model ignores initiation interference caused by ribosomes already present on the transcript. The DDI model accounts for initiation interference, and as expected, a decrease in ribosomal load is observed compared to DII. Even with interference, access to most ribosomal classes is attainable in the DDI model. Ribosomal profiling experiments suggest that most transcripts have one to ten ribosomes loaded with a strong preference for smaller polysomes. Live single molecule fluorescence experiments do show higher polysome numbers rising into the 50s [33]. For the long reporters used in these experiments that equates to a 12 to 30 percent ribosomal load. Thus the DDI model probably overestimates the loading for a particular scaled initiation rate.

Marking rates have only a mild effect on ribosomal load, but a large effect on protein production.

1. From the solution of the capped system we can define two separate effects of transcript marking.
   (a) First, marking determines how many transcripts can accummulate in the capped class, thus determining the total transcript population
   (b) Second, marking rate shifts the distribution of transcripts in the capped class towards lower ribosomal classes.
2. Marking only has a mild effect on the distribution of transcripts on the capped class

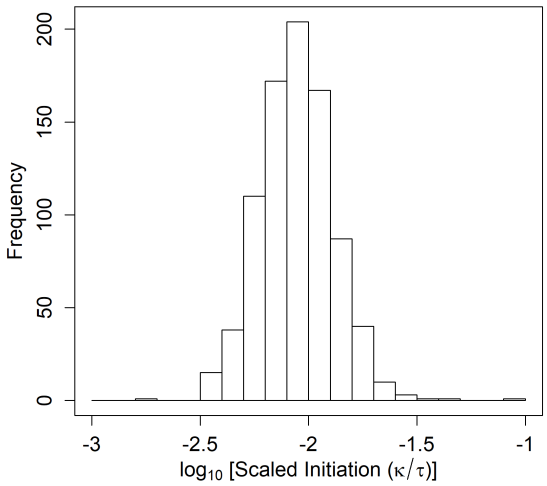
Under equilibrium protein length affects ribosomal load but not ribosomal density

1. stuff

Figure 2 points for discussion

1.

Figure 3: Comparison of density independent initiation (DII) and density dependent initiation (DDI)



1. As more ribosomes are loaded onto a transcript there is reduced space available for new ribosomes to occupy.

2. This in turn would reduce the ability to initiate new rounds of translation.

3. To investigate the effect of ribosomal density on initiation we created two versions of the model. A density dependent initiation (DDI) model which scales the initiation rate by the number of ribosomes on the transcript. And a density intedpendent initiation (DII) version of the model which has a fixed initiation rate.

4. In the DII model the ribosome load increases linearly with the scaled initiation rate ($\kappa \ \tau$) until it reaches

5. Our model does not explicitly capture the mechanism of translation, it just keeps track of the available spaces left on a transcript. As such the model would underestimate the actual e ffect of ribosomal interference

Figure 4: Comparison of density independent initiation (DII) and density dependent initiation (DDI)

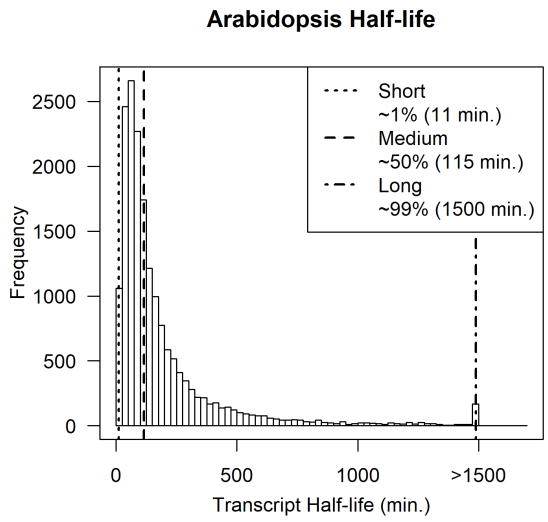


Figure 5: Comparison of density independent initiation (DII) and density dependent initiation (DDI)

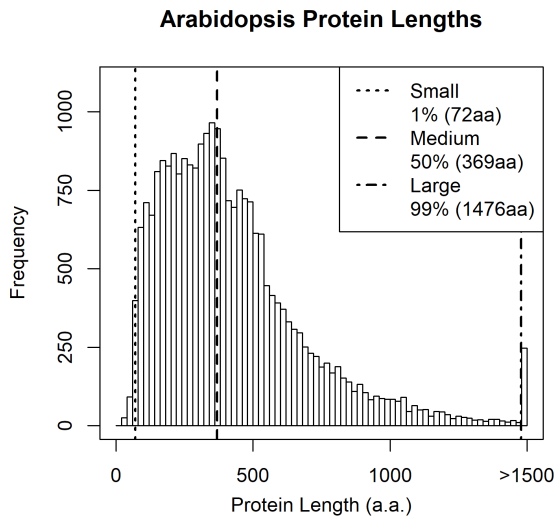


Figure 6: Comparison of density independent initiation (DII) and density dependent initiation (DDI)

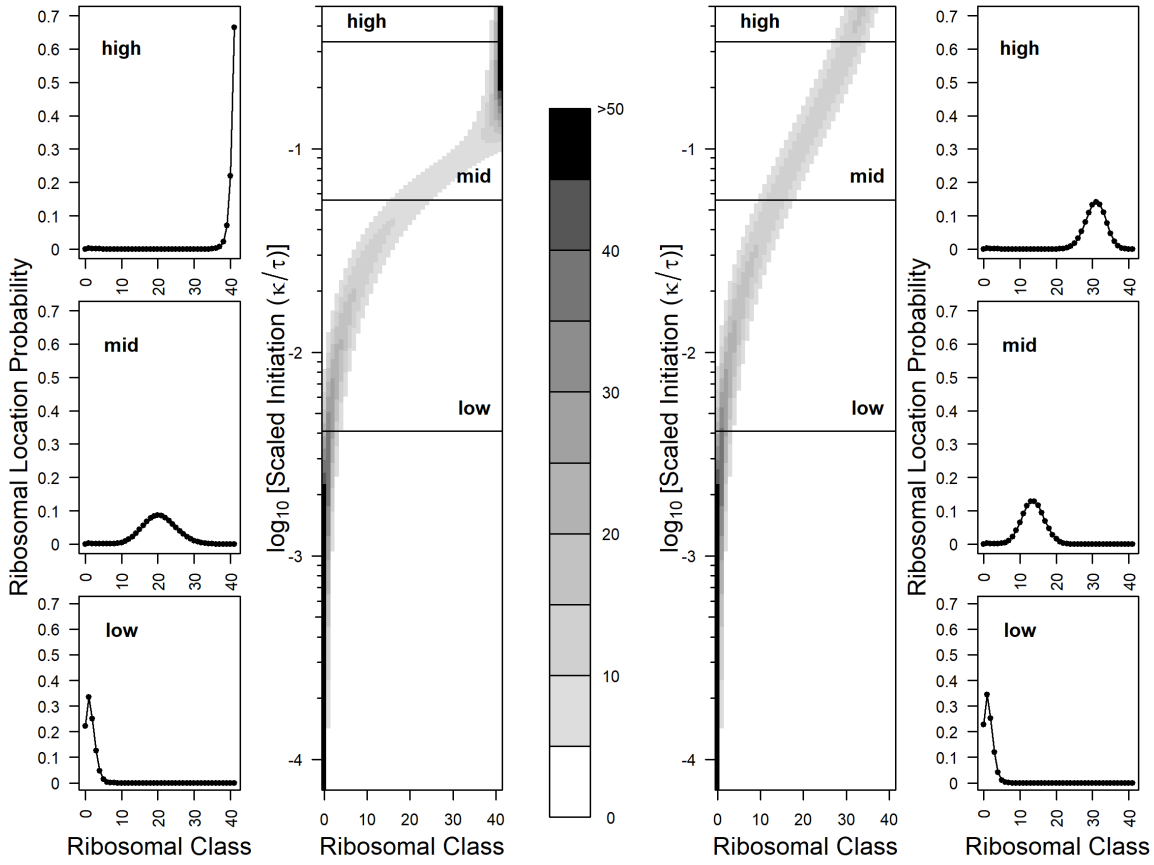


Figure 7: Comparison of density independent initiation (DII) and density dependent initiation (DDI)

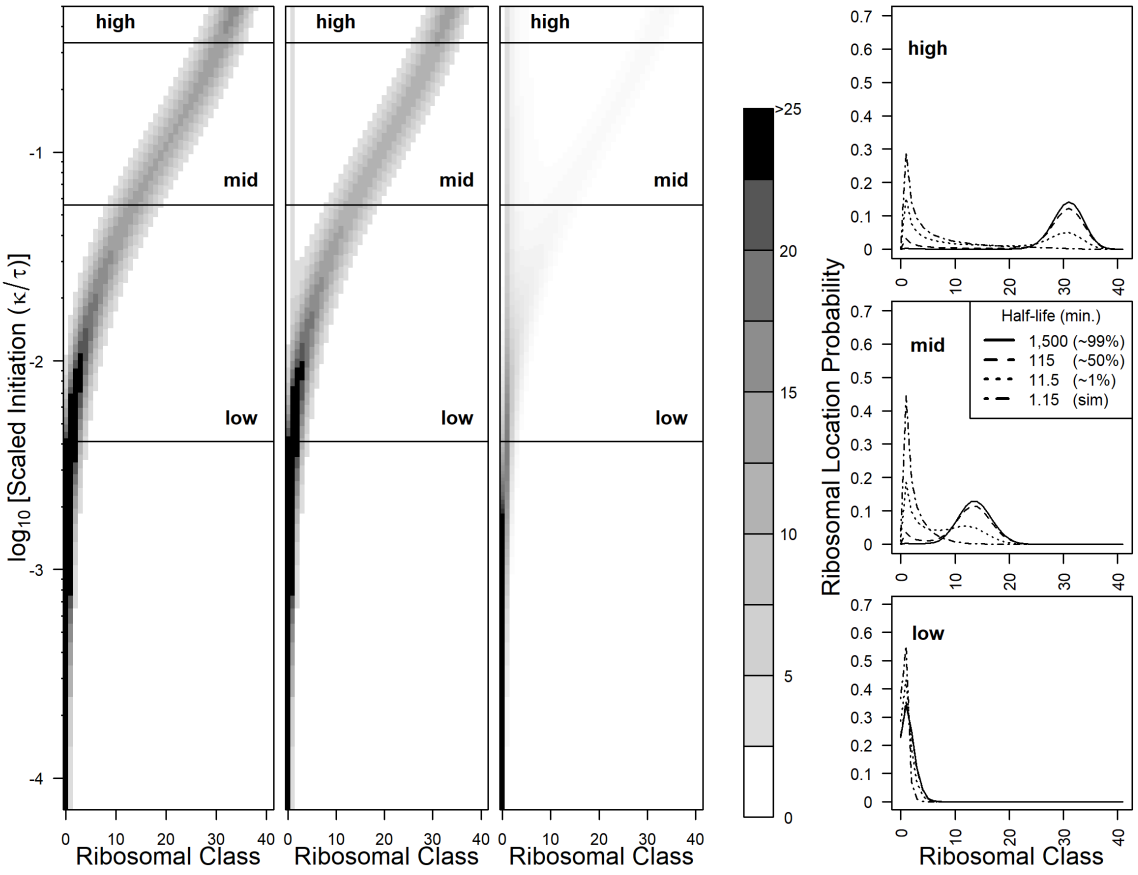


Figure 8: Comparison of density independent initiation (DII) and density dependent initiation (DDI)

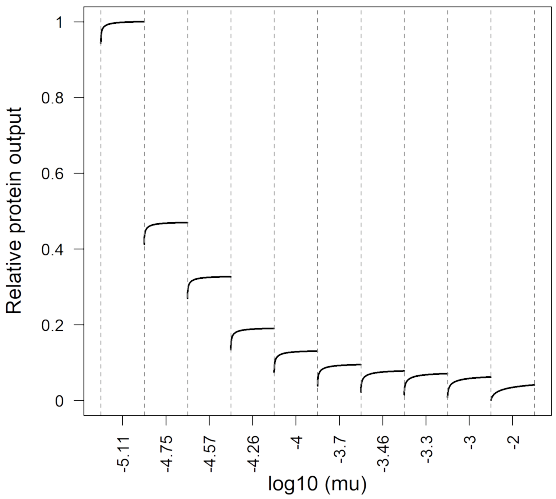


Figure 9: Comparison of density independent initiation (DII) and density dependent initiation (DDI)

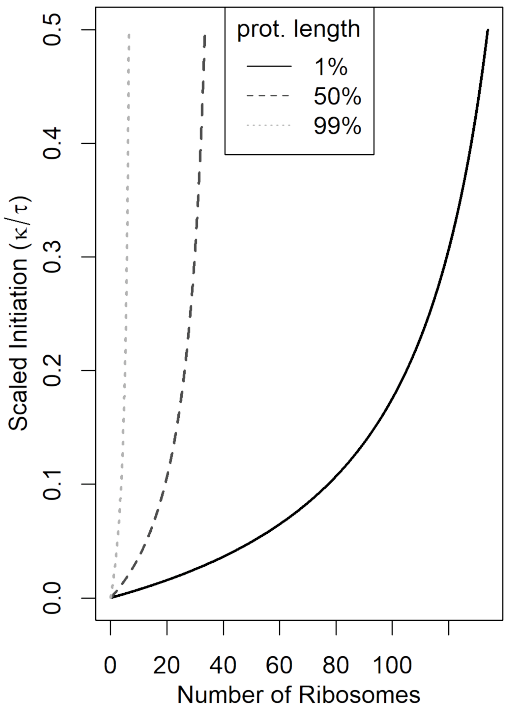


Figure 10: Comparison of density independent initiation (DII) and density dependent initiation (DDI)