

Research Article

Ribosome reinitiation can explain length-dependent translation of messenger RNA

David W. Rogers^{1,¶*}, Marvin A. Böttcher^{2,¶}, Arne Traulsen², Duncan Greig^{1,3}

¹Experimental Evolution Research Group and ²Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Plön, Germany

³Department of Genetics, Evolution, and Environment, University College London, London, WC1E 6BT, United Kingdom

¶These authors contributed equally to this work.

*Corresponding author

E-mail: rogers@evolbio.mpg.de (DWR)

Short title: Ribosome reinitiation on circular transcripts

1 **Abstract**

2 Models of mRNA translation usually presume that transcripts are linear; upon reaching the
3 end of a transcript each terminating ribosome returns to the cytoplasmic pool before initiating
4 anew on a different transcript. A consequence of linear models is that faster translation of a
5 given mRNA is unlikely to generate more of the encoded protein, particularly at low ribosome
6 availability. Recent evidence indicates that eukaryotic mRNAs are circularized, potentially
7 allowing terminating ribosomes to preferentially reinitiate on the same transcript. Here we
8 model the effect of ribosome reinitiation on translation and show that, at high levels of
9 reinitiation, protein synthesis rates are dominated by the time required to translate a given
10 transcript. Our model provides a simple mechanistic explanation for many previously
11 enigmatic features of eukaryotic translation, including the negative correlation of both
12 ribosome densities and protein abundance on transcript length, the importance of codon
13 usage in determining protein synthesis rates, and the negative correlation between transcript
14 length and both codon adaptation and 5' mRNA folding energies. In contrast to linear models
15 where translation is largely limited by initiation rates, our model reveals that all three stages
16 of translation - initiation, elongation, and termination/reinitiation - determine protein synthesis
17 rates even at low ribosome availability.

18 19 **Introduction**

20 The physiological state of a cell is largely determined by the identity and abundance of the
21 proteins encoded by its genome. Understanding how genetic information is first transcribed
22 into messenger RNA and then translated into protein is therefore fundamental to our
23 understanding of biological systems. A wide variety of technologies has allowed detailed
24 investigations of transcription, but - until very recently - a lack of similar tools for empirical
25 research on translation has meant that the study of post-transcriptional regulation has been
26 largely restricted to mathematical models with little opportunity for parameterization or
27 evaluation. Recent advances in both sequencing technology and mass spectrometry have

1 now produced large amounts of data on the translation of eukaryotic mRNA, revealing how
2 transcript features, RNA-binding proteins, and non-coding RNAs influence translation [1,2].
3 While many of the determinants of translation rates revealed by these empirical studies were
4 predicted by existing models, some remain difficult to explain. Perhaps the most striking
5 correlate of translation rate is the length of the transcript itself. Multiple experimental studies,
6 across a wide range of eukaryotic organisms, have demonstrated a steep negative
7 correlation between the length of a given coding sequence (CDS) and three different
8 measures of translation: translation initiation rates [3-5], the density of ribosomes on a
9 transcript [5-15], and the abundance of the encoded protein [16-19].

10 Ribosome and polysome profiling experiments have shown a positive relationship between
11 ribosome density and protein abundance, leading to the conclusion that transcripts with
12 higher ribosome densities have higher translation rates [9,11,20]. A positive relationship
13 between ribosome density and translation rate can occur when translation is limited by low
14 initiation rates. In traditional models of translation, initiation can be limiting when other steps
15 in translation, such as elongation, occur quickly enough to prevent collisions between
16 ribosomes [20]. Consistent with this key role of initiation rates in determining translation
17 rates, Arava et al [6] found that the higher densities of ribosomes on shorter transcripts was
18 most consistent with shorter transcripts having exponentially higher initiation rates than
19 longer transcripts, estimating a halving of the initiation rate with every 400-codon-increase in
20 CDS length. More recent analyses [3,4] have revealed that the relationship between CDS
21 length and initiation rates is better described by a power law: the initiation rate is roughly
22 halved for every doubling of CDS length (i.e. a log-log slope of approximately -1). However,
23 the assumption of initiation-limitation leaves little room for variation in elongation rates to
24 influence translation rates, which is at odds with recent work demonstrating that codon usage
25 can be an important determinant of protein yields [21,22].

1 If translation is limited by the ability of transcripts to capture ribosomes from the cytoplasmic
2 pool (the *de novo* initiation rate), mechanisms that allow transcripts to retain terminating
3 ribosomes for subsequent rounds of translation should improve translation rates. The closed-
4 loop model of translation was first proposed as a hypothetical mechanism to improve
5 translation efficiency through intrapolyosomal ribosome reinitiation [23,24]. By bringing the
6 sites of termination and initiation into close proximity through circularization of the mRNA, the
7 closed-loop complex allows ribosomes that have finished translating to reinitiate translation
8 on the same mRNA molecule rather than returning to the cytoplasmic pool. The closed-loop
9 model was initially based on the appearance of many polysomes in electron micrographs as
10 circular, rather than linear, structures (detailed high resolution tomographic analyses of
11 circular polysomes are now available [25]). Recent theoretical and experimental studies have
12 shown that secondary structures in single stranded RNAs bring the 5' and 3' ends close
13 together (equivalent to the distance spanned by 9-16 nucleotides) meaning that mRNAs are
14 effectively circularized [26,27]. Interactions between initiation factors bound to the 5' end, and
15 proteins associated with the 3' end including release and recycling factors, and the poly(A)
16 binding proteins, are thought to facilitate translation, possibly by stabilizing the closed-loop
17 structure or by actively promoting reinitiation [28,29].

18 The importance of reinitiation of ribosomes on circular transcripts in determining protein yield
19 is well established *in vitro* [23,24,30-32]. Measuring translation of the luminescent protein
20 luciferase in a eukaryotic cell-free system, Kopeina et al [31] showed that circular polysomes
21 rarely exchanged ribosomes with the free pool or lost ribosomes to other transcripts, but
22 linear polysomes did so frequently. On circular polysomes, most terminating ribosomes
23 immediately reinitiated on the same mRNA molecule (see also [30]). Alekhina et al [32] found
24 that protein production in a similar cell-free system does not rapidly reach a steady state, as
25 would be expected under a linear model of translation, but rather accelerates over the
26 lifetime of the transcript, consistent with reinitiation on the same transcript. They proposed

1 that the translation rate initially depends on slow *de novo* initiation of ribosomes from the free
2 pool but soon becomes dominated by the much faster process of reinitiation.

3 Here, we use a minimal computational model to investigate the consequences of ribosome
4 reinitiation on translation, with particular focus on transcript length and codon usage. We find
5 that reinitiation causes ribosome densities, overall initiation rates, and protein yields to
6 decrease with increasing transcript length. Furthermore, higher levels of reinitiation increase
7 the importance of codon usage in determining translation rates in a length-dependent
8 manner, even at low ribosome densities or low *de novo* initiation rates. Reinitiation therefore
9 provides a potential mechanistic explanation for multiple previously-enigmatic patterns
10 observed in empirical studies of translation.

11 **Model Description**

12 We use a totally asymmetric simple exclusion process (TASEP, reviewed in [33]) to
13 investigate the closed loop model of translation. The TASEP (Fig. 1) models each transcript
14 as a one-dimensional lattice consisting of a number of sites equal to the number of codons in
15 the CDS: each site represents a single codon. Each site can be either free or occupied by a
16 ribosome. Ribosomes move along the transcript in the 5' to 3' direction and cannot occupy
17 the same codon(s) as any other ribosome. In our model, the transcript is circularized,
18 meaning that terminating ribosomes can not only be released into the cytoplasmic pool (as in
19 a linear TASEP) but can also move to the initiation site of the same transcript (reinitiation).

20 Four different types of reactions can take place in the TASEP: (i) *de novo* initiation: a free
21 ribosome can be placed onto the 5' end of the transcript (the initiation site) at the *de novo*
22 initiation rate; (ii) elongation: ribosomes at any codon on the transcript (except the
23 termination site) can move forward one codon in the 3' direction at the elongation rate; if a
24 ribosome occupies the termination site, it can either (iii) leave the transcript at the release
25 rate or (iv) it can move to the initiation site at the reinitiation rate.

1 We model ribosomes as extended particles that occupy ten codons each: the A-sites (where
2 each codon is translated) of adjacent ribosomes must be spaced apart by at least 10 codons.
3 Thus, the elongation reaction is only possible when the A-site of the next ribosome in the 3'
4 direction is > 10 codons downstream. Similarly, neither *de novo* initiation nor reinitiation is
5 possible if any of the first 10 codons is occupied by an A-site.

6 Analytical solutions of the TASEP are possible, but currently can only be applied to the
7 steady state. Consequently, most TASEP models, including a recent study of reinitiation [34],
8 investigate translation at the steady state, where the rate at which ribosomes join a transcript
9 equals the rate at which they leave, and the translation rate is constant. However, every real
10 transcript spends some proportion of its lifetime outside of the steady state, where these
11 solutions do not apply; the assumption of a perpetual steady state is therefore an
12 approximation. A new transcript does not instantly acquire ribosomes distributed along its
13 length. Instead, ribosomes join at the 5' end and gradually progress towards the stop codon,
14 where they can be released. In the absence of reinitiation, the steady state can be reached
15 once the first ribosome to join a transcript is released. The duration of this "pioneer round"
16 [35] increases with transcript length, but generally represents a small proportion of eukaryotic
17 transcript lifetimes. In the absence of reinitiation the steady state is therefore a good
18 approximation (although it can be inappropriate for prokaryotes with short-lived transcripts
19 [36,37]). However under reinitiation, ribosomes do not necessarily leave the transcript upon
20 termination, which causes the effective initiation rate (and the translation rate) to increase
21 over time [32]. The time to reach the steady state therefore increases with both transcript
22 length and reinitiation probability, and the time spent outside of the steady state thus
23 represents a greater proportion of transcript lifetimes (Fig. S1A). The steady state
24 assumption consequently becomes a much worse approximation of translation at higher
25 levels of reinitiation, overestimating translation rates on long transcripts and underestimating
26 translation on short transcripts (Fig. S1B). It is therefore impossible to make a fair

1 comparison of translation at different reinitiation levels using the steady state approximation,
2 particularly for transcripts of different lengths.

3 Since we do not assume that translation on any given transcript is always at the steady state,
4 we cannot use the steady state analytical solutions of the TASEP. Instead, we perform
5 stochastic simulations using the Gillespie algorithm [38], which capture both the steady state
6 and the non-steady state. In models that assume the steady state, all translation that occurs
7 in simulations prior to the steady state is ignored. For example, in a recent reinitiation-based
8 model of translation in yeast, the first 10^5 s of simulations was discarded [34]. Given that the
9 average lifetime of yeast transcripts is on the scale of 10^3 - 10^4 s [39], this means that all
10 translation occurring over biologically plausible lifetimes was excluded from the analysis.
11 Here, we make no assumptions about the steady state; we simply account for all translation
12 that occurs during the lifetime of a transcript (both before and after the steady state is
13 achieved). We simulated translation on each transcript independently. Each run generated a
14 time evolution of the ribosome occupancy at each codon on a given transcript. We computed
15 three measures of translation: ribosome density (the average number of ribosomes on a
16 transcript over its lifetime divided by one tenth of CDS length, because each ribosome
17 occupies 10 codons), effective initiation rate (the total number of initiations occurring through
18 either *de novo* initiation or reinitiation divided by transcript lifetime) and protein yield (the total
19 number of ribosomes reaching the stop codon of a transcript). We averaged the results of
20 1000 runs to produce results that are not subject to large stochastic fluctuations. We do not
21 consider untranslated regions and our transcripts therefore represent only the CDS. The
22 code for our TASEP is available at: <https://github.com/marvinboe/reTASEP>

23 Changing any transcriptome-wide parameter can dramatically alter global ribosome usage.
24 For instance, at a given *de novo* initiation rate, increasing the reinitiation probability increases
25 the total number of actively translating ribosomes. While this effect may be true, given that
26 reinitiation is expected to allow more efficient use of ribosomes (see Discussion), it makes

1 parameterizing the model difficult because the actual level of reinitiation is unknown. To keep
2 all simulations consistent with empirical values, we have adjusted the *de novo* initiation rate
3 to maintain the empirically observed average ribosome density. For simplicity, we have kept
4 the number of ribosomes on a 400-codon-long transcript constant (at 6 ribosomes) for all
5 transcriptome-wide reinitiation probabilities and elongation rates.

6 **Results**

7 **High levels of reinitiation generate length-dependent translation**

8 Our model captures the negative correlation between ribosome density and CDS length
9 observed in empirical studies, but only if the probability of reinitiation is high (Fig. 2). This
10 result is intuitive; if reinitiation were perfect, all ribosomes that initiate would continue to
11 reinitiate and translate, never leaving a transcript until it degrades. The density of ribosomes
12 on a transcript of a given length and age would therefore be determined exclusively by the *de*
13 *nov*o initiation rate. If the *de novo* initiation rate is the same for all transcripts, then all
14 transcripts of a given age should carry the same number of ribosomes and ribosome density
15 will be the inverse of CDS length (with a log-log slope of -1). At a given elongation rate, the
16 time required for a ribosome to complete one cycle (travel from the start codon to the stop
17 codon) is less for short transcripts than for long transcripts. This means that, prior to the
18 steady state, reinitiation occurs more frequently on shorter transcripts resulting in higher
19 protein yields for short transcripts than long transcripts. When all or nearly all terminating
20 ribosomes reinitiate, the effective initiation rate is much higher for shorter transcripts -
21 providing a simple mechanism that could explain the length-dependence of initiation rates
22 predicted by recent studies of translation [3-5,8]. The higher ribosome densities on shorter
23 transcripts, and corresponding higher levels of reinitiation, result in higher occupancies of the
24 initiation site on shorter transcripts, occasionally blocking new ribosomes from initiating on
25 short transcripts. This initiation interference [21] slightly reduces the length-dependence of

1 ribosome density, effective initiation rate, and protein yield (Fig. 2), resulting in a log-log
2 slope less steep than -1.

3 When reinitiation is not perfect, ribosomes can return to the cytoplasmic pool after
4 termination, and the effect of CDS length on ribosome density, effective initiation rate, and
5 protein yield is diminished. Even small reductions in reinitiation probability greatly weaken
6 length-dependence (Fig. 2). This is because short transcripts have more opportunities to lose
7 ribosomes than do long transcripts. While a successful reinitiation event only guarantees that
8 a ribosome remains associated with the transcript until the next termination event, ribosome
9 loss is permanent. In the complete absence of reinitiation, length-dependence is therefore
10 abolished.

11 **Reinitiation, but not *de novo* initiation, has a larger effect on short transcripts** 12 **than long transcripts**

13 While changing transcriptome-wide parameters can dramatically affect global ribosome
14 usage (see Model Description), altering parameters of transcripts encoded by a single gene
15 will have little effect on global ribosome usage. This is because nearly all endogenous genes
16 are expressed at low levels, so changing the translation parameters of the transcripts
17 produced by a single gene will have a negligible effect on global ribosome availability
18 [4,20,41]. By studying transcripts of individual genes, we can therefore investigate the
19 consequences of changing a single parameter while holding all other values constant. We
20 first tested the effects of altering the reinitiation rate of transcripts encoded by a single gene
21 (Fig. 3 A, B). Doubling the reinitiation rate results in an extremely similar increase in all three
22 measures of translation (ribosome density, effective initiation rate, and protein yield; results
23 are therefore only shown for ribosome density), but the effects are greater for short
24 transcripts than long transcripts. These effects are mirrored by a length-dependent decrease
25 in translation when the reinitiation rate is halved (Fig. 3B). Furthermore, the length-
26 dependent effects of changing the reinitiation rate of a single transcript species are generally

1 stronger at higher transcriptome-wide reinitiation probabilities, except when reinitiation is so
2 high that ribosomes rarely leave the transcript (e.g. 99.9%).

3 We next tested the effects of altering the *de novo* initiation rate of a single transcript species
4 (Fig 3 C, D). In the absence of reinitiation, doubling the *de novo* initiation rate had an equal
5 effect on ribosome density for transcripts of all lengths. However, at higher levels of
6 reinitiation, doubling the *de novo* initiation rate resulted in a smaller increase in ribosome
7 density on short transcripts than on long transcripts, caused by increased initiation
8 interference; the higher density of ribosomes on short transcripts under reinitiation increases
9 the probability that the initiation site is blocked, preventing successful *de novo* initiation. The
10 effects of altering the *de novo* initiation rate on the effective initiation rate and protein yield
11 are very similar to the effects on ribosome density.

12 **High levels of reinitiation couple effective initiation rates and protein yields to** 13 **the elongation rate**

14 So far, we have assumed that all transcripts have identical elongation rates, but in reality the
15 elongation rate varies between transcripts encoded by different genes [42]. We therefore
16 investigated the consequences of changing the elongation rate of a single CDS from 10s^{-1} to
17 either 20s^{-1} or 5s^{-1} (Fig. 4). Increasing the elongation rate reduces the amount of time
18 between initiation and termination. In the absence of reinitiation, this causes ribosomes to
19 spend less time on the altered transcript resulting in decreased ribosome density, but has
20 little effect on the initiation rate or protein yield since these elongation rates are generally not
21 limiting. Altered elongation rates do affect how long it takes to clear the initiation site and
22 therefore the amount of initiation interference, explaining the relatively small differences in
23 initiation rates and protein yields seen at 0% reinitiation [21].

24 Under perfect reinitiation, terminating ribosomes explicitly reinitiate on the same transcript.
25 Changing the elongation rate of a single gene therefore has no effect on the density of

1 ribosomes on the altered transcript. However, by altering the time between reinitiation
2 events, changing the elongation rate results in an equal change in the effective initiation rate
3 of the altered transcript (Fig. 4). The protein yield of any endogenous gene is therefore
4 exquisitely sensitive to changes in elongation rate under perfect reinitiation. Under perfect
5 reinitiation, this effect is seen at all CDS lengths. The importance of the elongation rate
6 decreases dramatically when reinitiation levels are reduced: faster elongation results in more
7 opportunities to lose ribosomes, particularly on short transcripts.

8 **Length-dependent consequences of a single slow step on translation**

9 So far, we have only considered the effects of changing the average elongation rate of a
10 transcript. However, it is difficult to imagine a mechanism that could simultaneously alter the
11 elongation rate of all codons in a single transcript species without affecting the global
12 elongation rate. Instead, transcripts are likely altered by mutations affecting a single codon at
13 a time. Codon usage can affect elongation by determining the stability of secondary
14 structures in the mRNA, but different codons are also decoded at different rates depending
15 on the cellular availability of the appropriate tRNA. Most amino acids are encoded by multiple
16 codons, and some codons (including synonymous codons that code for the same amino
17 acid) are decoded faster than others [42,43]. We therefore investigated the consequences of
18 a single slow step on translation of transcript species of different lengths (Fig. 5). Here, we
19 only examined translation at 99.9% reinitiation; similar results would be expected for other
20 models of length-dependent translation. Introducing a single slow step into any transcript
21 reduces its effective initiation rate and protein yield, but the effects are much larger for short
22 transcripts than for long transcripts (Fig. 5). The length-dependence of a single slow step
23 arises from two sources. First, a single site represents a larger proportion of a short transcript
24 than a long transcript and consequently results in a greater decrease in the average
25 elongation rate [46]. Second, short transcripts have higher ribosome densities and are
26 therefore more prone to collisions or "traffic jams" than are long transcripts. Effective initiation

1 rates and protein yields are particularly sensitive to single slow steps near the start codon,
2 with larger effects on shorter transcripts: slow clearance of the initiation site delays
3 reinitiation and blocks *de novo* initiation resulting in lower ribosome densities on affected
4 transcripts.

5 **A yeast-specific model of translation with reinitiation**

6 Given the importance of variation in elongation rates to translation under reinitiation, we used
7 our model to simulate translation in *S. cerevisiae* using codon-specific decoding rates. We
8 used decoding rates (see Table S1) estimated by Gilchrist & Wagner [47] which are based
9 on tRNA availability and wobble pairing rules and scaled so that the average decoding rate is
10 10s^{-1} ; they are related to measures of codon occupancy ($r = 0.494$, $n = 61$, $P < 0.0001$ [11]).
11 Since efficient reinitiation couples protein production to elongation rates, synonymous codon
12 usage should have detectable consequences for protein yield at high levels of reinitiation.
13 We tested the effects of synonymous codon usage at 99.9% reinitiation by predicting the
14 yields of nine different synthetic GFP constructs [48] that differ only in their synonymous
15 codon usage (Fig. 6A). We compared these predictions to observed protein abundances
16 measured in *S. cerevisiae* expressing each construct, and found a strong positive correlation
17 between predicted yields and observed abundances ($r = 0.750$, $n = 9$, $P = 0.020$); our model
18 predicted approximately half of the observed effect of using different synonymous codons
19 (relative expression of highest vs. lowest construct, model = 2.4-fold, observed = 5.4-fold).
20 Thus, efficient reinitiation correctly predicts a role for synonymous codon usage in
21 determining yield.

22 Having established that Gilchrist & Wagner's [47] codon-specific elongation rates are
23 realistic, we used them to simulate the entire budding yeast translome. The results of our
24 simulations at 99.9% reinitiation are strongly correlated (Fig. 6B) with experimental measures
25 of ribosome densities ($r = 0.932$, $n = 5542$, using data from [6]) and calculated initiation rates
26 ($r = 0.742$, $n = 5348$, using estimates from [3]; $r = 0.618$, $n = 3728$, using estimates from [4]).

1 Our yield predictions are less strongly correlated with measured protein abundances ($r =$
2 0.478, $n = 4686$, data from Peptide Atlas 2013). This weaker correlation is unsurprising as
3 our predictions of yield omit many important determinants of protein abundance including
4 transcript abundance and protein stability. Results of simulations at other reinitiation levels
5 are included in Fig. S3 (fixed transcript lifetime) and Fig. S4 (experimentally measured
6 transcript lifetimes).

7 **Discussion**

8 We have shown that a fixed transcriptome-wide level of ribosome reinitiation can generate
9 both length-dependent translation and a powerful transcript-specific role for codon usage, but
10 only when reinitiation is extremely efficient. The level of reinitiation in live cells is unknown,
11 but multiple studies have established that reinitiation is much more frequent than *de novo*
12 initiation in cell-free systems. Furthermore, if reinitiation benefits the cell, we would expect it
13 to evolve to become highly efficient. Maintaining a large pool of ribosomes consumes a
14 substantial part of a cell's energy budget and selection will favor mechanisms that allow
15 ribosomes to be used efficiently [49]. If, as reported by Nelson & Winkler [30] and Kopeina et
16 al [31], reinitiation of post-termination ribosomes is faster than *de novo* initiation from the free
17 ribosome pool, then efficient reinitiation reduces the amount of "dead time" ribosomes spend
18 in the pool waiting to be recruited [34,50]. Each ribosome in a cell is therefore able to
19 complete more rounds of translation in a given time interval under high levels of reinitiation
20 compared to low levels of reinitiation. Reinitiation levels should be very closely associated
21 with fitness: the translation initiation rate is thought to be the principal determinant of the rate
22 of cell division [51,52]. Consequently, if reinitiation does occur in living cells, it is hard to
23 imagine why it would not work very efficiently. Direct measurement of the level of reinitiation
24 *in vivo* may soon be possible thanks to recent technological advancements enabling
25 selective labeling of ribosomes [53] and the visualization of translation on individual mRNAs
26 [54,55].

1 A single fixed level of reinitiation is not necessary to explain length-dependent translation;
2 efficient reinitiation is only required on short transcripts (Fig. S5). Studies in living cells have
3 shown that some transcripts are more likely to be associated with translation factors required
4 to form the closed-loop complex than others [56]. If the closed-loop complex is required for
5 efficient reinitiation, then reinitiation levels likely vary between transcripts. More specifically,
6 shorter transcripts likely experience higher levels of reinitiation since they are both more
7 likely to be enriched with closed-loop factors [15,57) and to form more stable closed-loop
8 complexes [58]. Additionally, cellular depletion of both the closed loop factor eIF4G and the
9 translational regulator Asc1/RACK1 has also been shown to have a greater effect on the
10 translation of short transcripts than on long transcripts [13,15]. Using length-dependent
11 reinitiation levels in our simulations allows the empirical relationship between CDS length
12 and ribosome density, effective initiation rate, and protein yield to be captured at an average
13 reinitiation level orders of magnitude smaller (~90%; Fig. S5) than does a fixed reinitiation
14 level (99.9%; Fig. S5).

15 Beyond acting on global mechanisms, natural selection also operates to maximize the
16 protein yield of transcripts encoded by individual genes (translational efficiency [46]).
17 Selection for increased translational efficiency can not only increase the abundance of a
18 given protein in a cell, but can also maintain protein levels while minimizing the cost of
19 transcription, which has been shown to be an important determinant of fitness in yeast [59].
20 The strength of selection depends on the magnitude of the effect of a given mutation on
21 translational efficiency; mutations with larger effects are subject to stronger selection. We
22 have shown that the magnitude of the effect on translational efficiency of altering a given
23 parameter by an equal amount can vary with the length of the altered transcript species.
24 Thus, the strength of selection on mutations that affect a given parameter can be length-
25 dependent [46]. For instance, doubling the reinitiation rate of a single transcript species
26 results in a bigger increase in translational efficiency for shorter transcripts (Fig. 3). Mutations
27 affecting the reinitiation rate of short transcripts are therefore more likely to be selected than

1 are those than occur on long transcripts, potentially contributing to higher levels of reinitiation
2 on shorter transcripts as discussed above (Fig. S5). In contrast, doubling the *de novo*
3 initiation rate does not result in higher translational efficiency on shorter transcripts and,
4 under reinitiation, can actually have smaller effects on shorter transcripts due to increased
5 initiation interference (Fig. 3). Selection for increased translational efficiency on individual
6 transcript species is therefore not predicted to result in higher *de novo* initiation rates on
7 shorter transcripts. Instead, selection under reinitiation will be more effective at reducing
8 initiation interference on shorter transcripts.

9 At high levels of reinitiation, we have shown that a single slow step in translation causes a
10 greater reduction in the translational efficiency of short transcripts than that of long
11 transcripts (Fig. 5). Eliminating slow steps has larger effects on the translation of short
12 transcripts compared to long transcripts and therefore selection to eliminate slow steps will
13 be most effective in genes encoding short transcripts. Length-dependent selection against
14 slow steps under reinitiation therefore offers an explanation for the negative correlation
15 between codon adaptation and CDS length observed across eukaryotes ([4, 46, 60-64] but
16 see also [65]). Translational efficiency is particularly sensitive to slow sites near the start
17 codon (Fig. 5, see also [21]): slow clearance of the initiation site delays reinitiation (promoting
18 ribosome loss) and blocks *de novo* initiation resulting in lower ribosome densities on affected
19 transcripts. Multiple mechanisms can determine how slowly ribosomes vacate the initiation
20 site including the presence of one or more slow codons [21] or the presence of stable 5'
21 secondary structures in the transcript [66]. As both features reduce yield to a greater extent
22 on short transcripts compared to long transcripts (Fig. 5), selection should be more efficient
23 at eliminating them on shorter transcripts, consistent with the negative correlations between
24 CDS length and both 5' mRNA folding energy and 5' codon adaptation [60]. Thus, length-
25 dependent translation generated by high levels of reinitiation will generate length-dependent
26 selection against slow steps [46], which will in turn reinforce patterns of length-dependent
27 translation.

1 Reinitiation provides a simple mechanistic explanation for empirically observed patterns of
2 length-dependent translation including negative correlations between CDS length and
3 ribosome density, effective initiation rate, protein yield, transcript codon adaptation, 5' codon
4 adaptation, 5' folding energy, and association with closed-loop factors. Under reinitiation,
5 these patterns are expected to emerge through selection for efficient ribosome usage,
6 maximizing protein yield, and translational efficiency on individual transcript species. This is
7 in sharp contrast to linear models in which, at low ribosome availability, length-dependence
8 arises through direct selection for higher *de novo* initiation rates on shorter transcripts [3,4].
9 Our model is consistent with the emerging view that translation is controlled not only by
10 initiation, but also by elongation and termination/reinitiation [21,22,67]. This conceptual shift
11 makes clear that manipulating any these stages can have profound consequences on
12 translation, and presents factors associated with elongation, release, and recycling as new
13 targets for therapeutic intervention (cf. [68]).

14

1 **Parameter Estimates and Justification**

2 **Transcript lifetime, age distribution and mode of decay**

3 The decay of eukaryotic transcripts is initiated by the stepwise removal of adenine residues
4 from the poly(A) tail [69]. Deadenylation is the slowest step in mRNA decay, and during this
5 process there is no apparent decay of the transcribed portion of the mRNA [70,71].

6 Enzymatic digestion of the poly(A) tail is distributive: the deadenylase binds to a poly(A) tail,
7 cleaves a small number of residues, then dissociates from the transcript before binding to a
8 different poly(A) tail [72]. Thus deadenylation consists of a series of sequential first-order
9 reactions, resulting in a hypo-exponential distribution of full-length transcript lifetimes [69,73].

10 Hypo-exponential distributions, by definition, have lower variances than an exponential
11 function with the same mean lifetime and age-matched populations of eukaryotic transcripts
12 are expected to show very little degradation for long periods then decay rapidly. When the
13 number of sequential deadenylation steps is high (~30), the distribution of transcript lifetimes
14 becomes symmetrical and approximates the normal distribution [74]. We have therefore, for
15 computational simplicity, assumed that all transcripts in a given simulation have the same
16 fixed lifetime.

17 In our model, all translation ceases as soon as the transcript lifetime is reached; ribosomes
18 that have a new round of translation are not allowed to finish and therefore do not contribute
19 to the protein yield. Consequently, protein yield in our model does not exactly match the
20 effective initiation rate. There is evidence that eukaryotic mRNA decay is co-translational:
21 transcript degradation in the 5'-3' direction follows the last translating ribosome allowing all
22 ribosomes to complete their final round [75]. If this is the sole mechanism of eukaryotic
23 transcript decay, then the true yield becomes the effective initiation rate multiplied by the
24 transcript lifetime (a constant in our model).

25

1 ***De novo* initiation rates**

2 The average number of ribosomes per transcript is remarkably constant across eukaryotes.
3 Polysome profiling studies estimate that the median *S. cerevisiae* transcript contains 6.0
4 ribosomes (based on weighted averages for all measured transcripts [6]; an identical
5 average was calculated in [7]) while the average human embryonic kidney HEK293T cell
6 contains 5.6-6.1 ribosomes [11]. Direct counts of the numbers of ribosomes in polysomes
7 [14] using atomic force microscopy provide similar values (*S. cerevisiae* = 6.5 ribosomes per
8 transcript, HEK293T = 8.7 ribosomes per transcript, human MCF-7 cells = 8.3 ribosomes per
9 transcript). Direct polysome counts are almost certainly over-estimates of the average
10 number of ribosomes per transcript as they ignore unoccupied transcripts (approximately 15-
11 30% of mRNAs [6,11]) and mRNAs occupied by a single ribosome. To be consistent with
12 these counts, in all of our models, for any transcript lifetime and reinitiation probability, we
13 have adjusted the *de novo* initiation rate such that the average transcript (CDS length = 400
14 codons) carries 6 ribosomes (white line on each heatmap in Fig. S2). This causes the *de*
15 *nov*o initiation rate to decrease with increasing reinitiation level and decreasing transcript
16 lifetimes.

17 Maintaining six ribosomes on a 400-codon-long transcript for transcripts with different
18 lifetimes requires much larger changes to the *de novo* initiation rate at high reinitiation
19 probabilities compared to low reinitiation levels (Fig. S2). Under perfect reinitiation, the
20 lifetime protein yields of transcripts of a given CDS length will be similar, but the rate of
21 protein production over time (proteins per mRNA per unit time) will be higher for short-lived
22 transcripts compared to long-lived transcripts. Thus, organisms with short-lived transcripts,
23 such as yeast, will exhibit much higher protein synthesis rates than organisms with long-lived
24 transcripts, such as mammals. Current estimates suggest that protein production rates in
25 mammals are considerably lower than in yeast, consistent with the much higher doubling
26 rates and much lower protein stabilities in yeast compared to mammals [76-78]. In contrast,

1 assuming similar elongation rates across species, linear models predict that yeast and
2 mammals should have similar protein production rates since they have similar average
3 ribosome densities.

4 **Model parameters**

5 **Full model.** We explored the consequences of different reinitiation levels on the average
6 ribosome density, effective initiation rate and protein yield for transcripts with different CDS
7 lengths using a wide range of transcript lifetimes and *de novo* initiation rates (Fig. S2). For
8 each combination of transcript lifetime and *de novo* initiation rate, we simulated translation
9 for transcripts of the following log-uniform distributed CDS lengths (in codons): 50, 63, 79,
10 100, 126, 158, 200, 251, 316, 399, 502, 632, 796, 1002, 1263, 1590, 2002, 2522, 3176, and
11 4000, and then calculated the slope of the resulting estimates of ribosome density, effective
12 initiation rate, and protein yield over CDS length. All codons were decoded at a rate of 10s^{-1}
13 based on the average level in yeast [79] and similar to the average rate observed in a mouse
14 embryonic cell line (5.6s^{-1} [80]). Termination rates (the sum of the release rate and the
15 reinitiation rate) are set equal to the elongation rate at 10s^{-1} . Different reinitiation levels are
16 achieved by setting the reinitiation rate as the corresponding proportion of the termination
17 rate. Translation of each transcript was averaged over 1000 runs.

18 **General model.** To explore the consequences of different reinitiation levels in more detail,
19 we present a model using an arbitrary lifetime of 3000s (50 minutes) for all transcripts (Figs.
20 2-5). This value is intermediate between estimates of median transcript half-lives in yeast
21 (10-30 minutes [81]) and mammalian cell lines (300-600 minutes [81]). Simulations were
22 performed with a constant elongation rate of 10s^{-1} (except for Fig. S3 where we perform the
23 same simulation at 5s^{-1} and 20s^{-1}). The model is otherwise the same as the full model,
24 except that a single *de novo* initiation rate is used at each reinitiation level. *De novo* initiation
25 rates are adjusted for each reinitiation level such that a 400-codon-long transcript carries an
26 average of 6 ribosomes. The *de novo* initiation rates used with a transcriptome-wide

1 elongation rate of 10s^{-1} were: 100% = 0.00438s^{-1} , 99.9% = 0.00458s^{-1} , 99% = 0.00586s^{-1} ,
2 95% = 0.01289s^{-1} , 90% = 0.02285s^{-1} , 80% = 0.04199s^{-1} , 50% = 0.09570s^{-1} , 0% = 0.17578s^{-1} .

3 **Yeast-specific model.** We computed the average ribosome density, effective initiation rate,
4 and protein yield (Fig. 6) for 5888 *S. cerevisiae* transcripts ranging in CDS length from 16 to
5 4910 codons (median length = 405 codons) in our model. We used the codon-specific
6 elongation rates calculated by Gilchrist & Wagner [47]; these rates are scaled such that the
7 average elongation rate is 10s^{-1} . As above, we adjusted *de novo* initiation rates for each
8 reinitiation level such that a 400-codon-long transcript (ignoring variation in decoding rates)
9 contained an average of 6 ribosomes. The exact *de novo* initiation rates used were: 100% =
10 0.00859s^{-1} , 99.9% = 0.00869s^{-1} , 99% = 0.01016s^{-1} , 95% = 0.01641s^{-1} , 90% = 0.02568s^{-1} ,
11 80% = 0.04492s^{-1} , 50% = 0.09766s^{-1} , 0% = 0.17578s^{-1} .

12 Most studies of mRNA stability report transcript half-lives. If eukaryotic transcripts decay with
13 biphasic (slow-then-fast) kinetics, then transcript lifetimes cannot be calculated from
14 observed half-lives by assuming first-order kinetics [71]. We have therefore based our
15 estimate of transcript lifetime on a study of nascent transcription rates in *S. cerevisiae* which
16 estimated that the entire set of mRNAs in a cell turns over more than four times per 6780s
17 (113 minute) cell cycle [39], resulting in an average transcript lifetime of 1553s (26 minutes).
18 Although most yeast studies predict fairly similar median transcript half-lives, gene-specific
19 estimates show little correlation across studies [81]. Consequently, we have made the
20 simplifying assumption that all transcripts have the same 1553s lifetime.

1 **References**

- 2 **1.** Lackner DH, Bähler J. Translational control of gene expression: from transcripts to transcriptomes. *Int Rev Cell*
3 *Mol Biol.* 2008;271: 199-251.
- 4 **2.** Kuersten S, Radek A, Vogel C, Penalva LOF. Translation regulation gets its 'omics' moment. *Wiley Interdiscip*
5 *Rev RNA.* 2013;4: 617-630.
- 6 **3.** Ciandrini L, Stansfield I, Romano MC. Ribosome traffic on mRNAs maps to gene ontology: genome-wide
7 quantification of translation initiation rates and polysome size regulation. *PLoS Comp Biol.* 2013;9: e1002866.
- 8 **4.** Shah P, Ding Y, Niemczyk M, Kudla G, Plotkin JB. Rate-limiting steps in yeast protein translation. *Cell.*
9 2013;153: 1589-1601.
- 10 **5.** Weinberg DE, Shah P, Eichhorn SW, Hussmann JA, Plotkin JB, Bartel DP. Improved ribosome-footprint and
11 mRNA measurements provide insights into dynamics and regulation of yeast translation. *Cell Rep.* 2016;14: 1-13.
- 12 **6.** Arava Y, Wang Y, Storey JD, Brown PO, Herschlag D. Genome-wide analysis of mRNA translation profiles in
13 *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA.* 2003;100: 3889-3894.
- 14 **7.** MacKay VL, Li X, Flory MR, Turcott E, Law GL, Serikawa KA, et al. Gene expression analyzed by high-
15 resolution state array analysis and quantitative proteomics. *Mol Cell Proteomics.* 2004;3: 478-489.
- 16 **8.** Arava Y, Boas FE, Brown PO, Herschlag D. Dissecting eukaryotic translation and its control by ribosome
17 density mapping. *Nucl Acids Res.* 2005;33: 2421-2432.
- 18 **9.** Lackner DH, Beilharz TH, Marguerat S, Mata J, Watt S, Schubert F, et al. A network of multiple regulatory
19 layers shapes gene expression in fission yeast. *Mol Cell.* 2007;26: 145-155.
- 20 **10.** Qin X, Ahn S, Speed TP, Rubin GM. Global analyses of mRNA translational control during early *Drosophila*
21 embryogenesis. *Genome Biol.* 2007;8: R63.
- 22 **11.** Hendrickson DG, Hogan DJ, McCullough HL, Myers JW, Herschlag D, Ferrell JE, et al. Concordant regulation
23 of translation and mRNA abundance for hundreds of targets of a human microRNA. *PLoS Biol.* 2009;7:
24 e1000238.

- 1 **12.** Ingolia NT, Ghaemmaghami S, Newman JRS, Weissman JS. Genome-wide analysis in vivo of translation with
2 nucleotide resolution using ribosome profiling. *Science*. 2009;324: 218-223.
- 3 **13.** Park EH, Zhang F, Warringer J, Sunnerhagen P, Hinnebusch AG. Depletion of eIF4G from yeast cells
4 narrows the range of translational efficiencies genome-wide. *BMC Genomics*. 2011;12: 68.
- 5 **14.** Lauria F, Tebaldi T, Lunelli L, Struffi P, Gatto P, Pugliese A, et al. RiboAbacus: a model trained on
6 polyribosome images predicts ribosome density and translational efficiency from mammalian transcriptomes. *Nucl*
7 *Acids Res*. 2015;43: e153.
- 8 **15.** Thompson MK, Rojas-Duran MF, Gangaramani P, Gilbert WV. The ribosomal protein Asc1/RACK1 is required
9 for efficient translation of short mRNAs. *eLife* 2016;5: e11154.
- 10 **16.** Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, et al. Global analysis of protein
11 expression in yeast. *Nature*. 2003;425: 737-741.
- 12 **17.** Gunaratne J, Schmidt A, Quandt A, Neo SP, Saraç OS, Gracia T, et al. Extensive mass spectrometry-based
13 analysis of the fission yeast proteome. *Mol Cell Proteomics*. 2013;12: 1741-1751.
- 14 **18.** Vogel C, de Sousa Abreu R, Ko D, Le S-Y, Shapiro BA, Burns SC, et al. Sequence signatures and mRNA
15 concentration can explain two-thirds of protein abundance variation in a human cell line. *Mol Syst Biol*. 2010;6:
16 400.
- 17 **19.** Wang T, Cui Y, Jin J, Guo J, Wang G, Yin X, et al. Translating mRNAs strongly correlate to proteins in a
18 multivariate manner and their translation ratios are phenotype specific. *Nucl Acids Res*. 2013;41: 4743-4754.
- 19 **20.** Plotkin JB, Kudla G. Synonymous but not the same: the causes and consequences of codon bias. *Nat Rev*
20 *Genet*. 2011;12: 32-42.
- 21 **21.** Chu D, Kazana E, Bellanger N, Singh T, Tuite MF, von der Haar T. Translation elongation can control
22 translation initiation on eukaryotic mRNAs. *EMBO J*. 2014;33: 21-34.
- 23 **22.** Tarrant D, von der Haar T. Synonymous codons, ribosome speed, and eukaryotic gene expression regulation.
24 *Cell Mol Life Sci*. 2014;71: 4195-4206.
- 25 **23.** Phillips GR. Haemoglobin synthesis and polysomes in intact reticulocytes. *Nature*. 1965;205: 567-570.

- 1 **24.** Baglioni C, Vesco C, Jacobs-Lorena M. The role of ribosomal subunits in mammalian cells. Cold Spring Harb
2 Symp Quant Biol. 1969;34: 555-565.
- 3 **25.** Afonina ZA, Myasnikov AG, Shirokov VA, Klaholz BP, Spirin AS. Conformation transitions of eukaryotic
4 polyribosomes during multi-round translation. Nucl Acids Res. 2015;43: 618-628.
- 5 **26.** Yoffe AM, Prinsen P, Gelbart WM, Ben-Shaul A. The ends of a large RNA molecule are necessarily close.
6 Nucl Acids Res. 2011;39: 292-299.
- 7 **27.** Leija-Martínez N, Casas-Flores S, Cadena-Nava R, Roca JA, Mendez-Cabañas JA, Gomez E, Ruiz-Garcia J.
8 The separation between the 5'-3' ends in long RNA molecules is short and nearly constant. Nucl Acids Res.
9 2014;42: 13963-13968.
- 10 **28.** Mazumder B, Seshadri S, Fox PL. Translational control by the 3'-UTR: the ends specify the means. Trends
11 Biochem Sci. 2003;28: 91-98.
- 12 **29.** Wilkie GS, Dickson KS, Gray NK. Regulation of mRNA translation by 5'- and 3'-UTR-binding factors. Trends
13 Biochem Sci. 2003;28: 182-188.
- 14 **30.** Nelson EM, Winkler MM. Regulation of mRNA entry into polysomes. J Biol Chem. 1987;262: 11501-11506.
- 15 **31.** Kopeina GS, Afonina ZA, Gromova KV, Shirokov VA, Vasiliev VD, Spirin AS. Step-wise formation of
16 eukaryotic double-row polyribosomes and circular translation of polysomal mRNA. Nucl Acids Res. 2008;36:
17 2476-2488.
- 18 **32.** Alekhina OM, Vassilenko KS, Spirin AS. Translation of non-capped mRNAs in a eukaryotic cell-free system:
19 acceleration of initiation rate in the course of polysome formation. Nucl Acids Res. 2007;35: 6547-6559.
- 20 **33.** Chou T, Mallick K, Zia RKP. Non-equilibrium statistical mechanics: from a paradigmatic model to biological
21 transport. Rep Prog Phys. 2011;74: 116601.
- 22 **34.** Marshall E, Stansfield I, Romano MC. Ribosome recycling induces optimal translation rate at low ribosomal
23 availability. J R Soc Interface. 2014;11: 20140589.
- 24 **35.** Maquat LE, Tarn WY, Isken O. The pioneer round of translation: features and functions. Cell. 2010;142: 368-
25 374.

- 1 **36.** Nagar A, Valleriani A, Lipowsky R. Translation by ribosomes with mRNA degradation: exclusion processes on
2 aging tracks. *J Stat Phys.* 2011;145: 1385-1404.
- 3 **37.** Gorissen M, Vanderzande C. Ribosome dwell times and the protein copy number distribution. *J Stat Phys.*
4 2012;148: 628-636.
- 5 **38.** Gillespie DT. Exact stochastic simulation of coupled chemical reactions. *J Phys Chem.* 1977;81: 2340-2361.
- 6 **39.** Pelechano V, Chávez S, Pérez-Ortín JE. A complete set of nascent transcription rates for yeast genes. *PLoS*
7 **ONE.** 2010;5: e15442.
- 8 **40.** Wang M, Weiss M, Simonovic M, Haertinger G, Schimpf SP, Hengartner MO, et al. PaxDb, a database of
9 protein abundance averages across all three domains of life. *Mol Cell Proteomics.* 2012;11: 492-500.
- 10 **41.** Charneski CA, Hurst LD. Positively charged residues are the major determinants of ribosomal velocity. *PLoS*
11 **Biol.** 2013;11: e1001508.
- 12 **42.** Yu CH, Dang Y, Zhou Z, Wu C, Zhao F, Sachs MS, et al. Codon usage influences the local rate of translation
13 elongation to regulate co-translation protein folding. *Mol Cell.* 2015;59: 744-754.
- 14 **43.** Presnyak V, Alhusaini N, Chen YH, Martin S, Morris N, Kline N, et al. Codon optimality is a major determinant
15 of mRNA stability. *Cell.* 2015;160: 1111-1124.
- 16 **46.** Comeron JM, Kreitman M, Aguadé M. Natural selection on synonymous sites is correlated with gene length
17 and recombination in *Drosophila*. *Genetics.* 1999;151: 239-249.
- 18 **47.** Gilchrist MA, Wagner A. A model of protein translation including codon bias, nonsense errors, and ribosome
19 recycling. *J Theor Biol.* 2006;239: 417-434.
- 20 **48.** Lanza AM, Curran KA, Rey LG, Alper HS. A condition-specific codon optimization approach for improved
21 heterologous gene expression in *Saccharomyces cerevisiae*. *BMC Syst Biol.* 2014;8: 33.
- 22 **49.** Warner JR. The economics of ribosome biosynthesis in yeast. *Trends Biochem Sci.* 1999;11: 437-440.
- 23 **50.** Chu D, Thompson J, von der Haar T. Charting the dynamics of translation. *BioSystems.* 2014;119: 1-9.
- 24 **51.** Soifer I, Barkai N. Systematic identification of cell size regulators in budding yeast. *Mol Syst Biol.* 2014;10:
25 761.

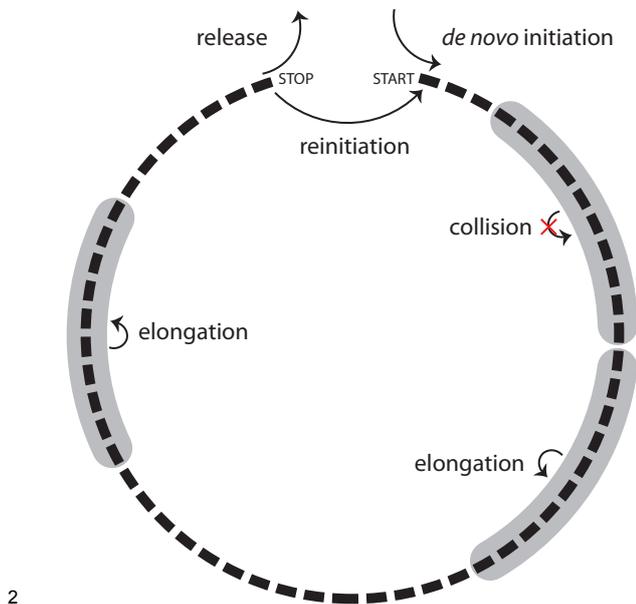
- 1 **52.** Polymenis M, Aramayo R. Translate to divide: control of the cell cycle by protein synthesis. *Microbial Cell*.
2 2015;2: 94-104.
- 3 **53.** Jan CH, Williams CC, Weissman JS. Principles of ER cotranslational translocation revealed by proximity-
4 specific ribosome profiling. *Science*. 2014;346: 1257521.
- 5 **54.** Wang C, Han B, Zhou R, Zhuang X. Real-time imaging of translation on single mRNA transcripts in live cells.
6 *Cell*. 2016;165: 990-1001.
- 7 **55.** Yan X, Hoek TA, Vale RD, Tanenbaum ME. Dynamics of translation of single mRNA molecules in vivo. *Cell*.
8 2016;165: 976-989.
- 9 **56.** Archer SK, Shirokikh NE, Hallwirth CV, Beilharz TH, Preiss T. Probing the closed-loop model of mRNA
10 translation in living cells. *RNA Biol*. 2015;12: 248-254.
- 11 **57.** Costello J, Castelli LM, Rowe W, Kershaw CJ, Talavera D, Mohammed-Qureshi SS, et al. Global mRNA
12 selection mechanisms for translation initiation. *Genome Biol*. 2015;16: 10.
- 13 **58.** Amrani N, Ghosh S, Mangus DA, Jacobson A. Translation factors promote the formation of two states of the
14 closed-loop mRNP. *Nature*. 2008;453: 1276-1280.
- 15 **59.** Kafri M, Metzl-Raz E, Jona G, Barkai N. The cost of protein production. *Cell Rep*. 2016;14: 22-31.
- 16 **60.** Ding Y, Shah P, Plotkin JB. Weak 5'-mRNA secondary structures in short eukaryotic genes. *Genome Biol*
17 *Evol*. 2012;4: 1046-1053.
- 18 **61.** Duret L, Mouchiroud D. Expression pattern and, surprisingly, gene length shape codon usage in
19 *Caenorhabditis*, *Drosophila*, and *Arabidopsis*. *Proc Natl Acad Sci USA*. 1999;96: 4482-4487.
- 20 **62.** Marais G, Duret L. Synonymous codon usage, accuracy of translation, and gene length in *Caenorhabditis*
21 *elegans*. *J Mol Evol*. 2001;52: 275-280.
- 22 **63.** Kliman RM, Irving N, Santiago M. Selection conflicts, gene expression, and codon usage trends in yeast. *J*
23 *Mol Evol*. 2003;57: 98-109.
- 24 **64.** Waldman YY, Tuller T, Shlomi T, Sharan R, Ruppin E. Translation efficiency in humans: tissue specificity,
25 global optimization and differences between developmental stages. *Nucl Acids Res*. 2010;38: 2964-2974.

- 1 **65.** Coghlan A, Wolfe KH. Relationship of codon bias to mRNA concentration and protein length in
2 *Saccharomyces cerevisiae*. *Yeast*. 2000;16: 1131-1145.
- 3 **66.** Gu W, Zhou T, Wilke CO. A universal trend of reduced mRNA stability near the translation-initiation site in
4 prokaryotes and eukaryotes. *PLoS Comput Biol*. 2010;6: e1000664.
- 5 **67.** Merrick WC, Harris ME. Control not at initiation? Bah, humbug! *EMBO J*. 2014;33: 3-4.
- 6 **68.** Richter JD, Collier J. Pausing on polyribosomes: make way for elongation in translational control. *Cell*.
7 2015;163: 292-300.
- 8 **69.** Cao D, Parker R. Computational modeling of eukaryotic mRNA turnover. *RNA*. 2001;7: 1192-1212.
- 9 **70.** Dreyfus M, Régnier P. The poly(A) tail of mRNAs: bodyguards in eukaryotes, scavenger in bacteria. *Cell*.
10 2002;111: 611-613.
- 11 **71.** Chen CYA, Ezzeddine N, Shyu AB. Messenger RNA half-life measurements in mammalian cells. *Methods*
12 *Enzymol*. 2008;448: 335-357.
- 13 **72.** Chen CYA, Shyu AB. Mechanisms of deadenylation-dependent decay. *Wiley Interdiscip Rev RNA*. 2010;2:
14 167-183.
- 15 **73.** Decker CJ, Parker R. A turnover pathway for both stable and unstable mRNAs in yeast: evidence for a
16 requirement for deadenylation. *Genes & Dev*. 1993;7: 1632-1643.
- 17 **74.** Kuo TC, Huang WC, Wu SC, Cheng PL. A case study of inter-arrival time distributions of container ships. *J*
18 *Mar Sci Technol*. 2006;14: 155-164.
- 19 **75.** Pelechano V, Wei W, Steinmetz LM. Widespread co-translational RNA decay reveals ribosome dynamics.
20 *Cell*. 2015;161: 1400-1412.
- 21 **76.** Jackson DA, Pombo A, Iborra F. The balance sheet for transcription: an analysis of nuclear RNA metabolism
22 in mammalian cells. *FASEB J*. 2000;14: 242-254.
- 23 **77.** von der Haar T. A quantitative estimation of the global translational activity in logarithmically growing yeast
24 cells. *BMC Systems Biol*. 2008;2: 87.

- 1 **78.** Schwanhäusser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, et al. Global quantification of mammalian
2 gene expression control. *Nature*. 2011;473: 337-342.
- 3 **79.** Waldron C, Jund R, Lacroute F. The elongation rate of proteins of different molecular weight classes in yeast.
4 *FEBS Lett*. 1974;46: 11-16.
- 5 **80.** Ingolia NT, Lareau LF, Weissman JS. Ribosome profiling of mouse embryonic stem cells reveals the
6 complexity and dynamics of mammalian proteomes. *Cell*. 2011;147: 789-802.
- 7 **81.** Geisberg JV, Moqtaderi Z, Fan X, Ozsolak F, Struhl K. Global analysis of mRNA isoform half-lives reveals
8 stabilizing and destabilizing elements in yeast. *Cell*. 2014;156: 812-824.
- 9 **82.** Lipson D, Raz T, Kieu A, Jones DR, Giladi E, Thayer E, Thompson JF, Letovsky S, Milos P, Causey M.
10 Quantification of the yeast transcriptome by single-molecule sequencing. *Nat Biotech*. 2009;27: 652-658.
- 11 **83.** Miura F, Kawaguchi N, Yoshida M, Uematsu C, Kito K, Sakaki Y, Ito T. Absolute quantification of the budding
12 yeast transcriptome by means of competitive PCR between genomic and complementary DNAs. *BMC Genomics*
13 2008;9: 574.

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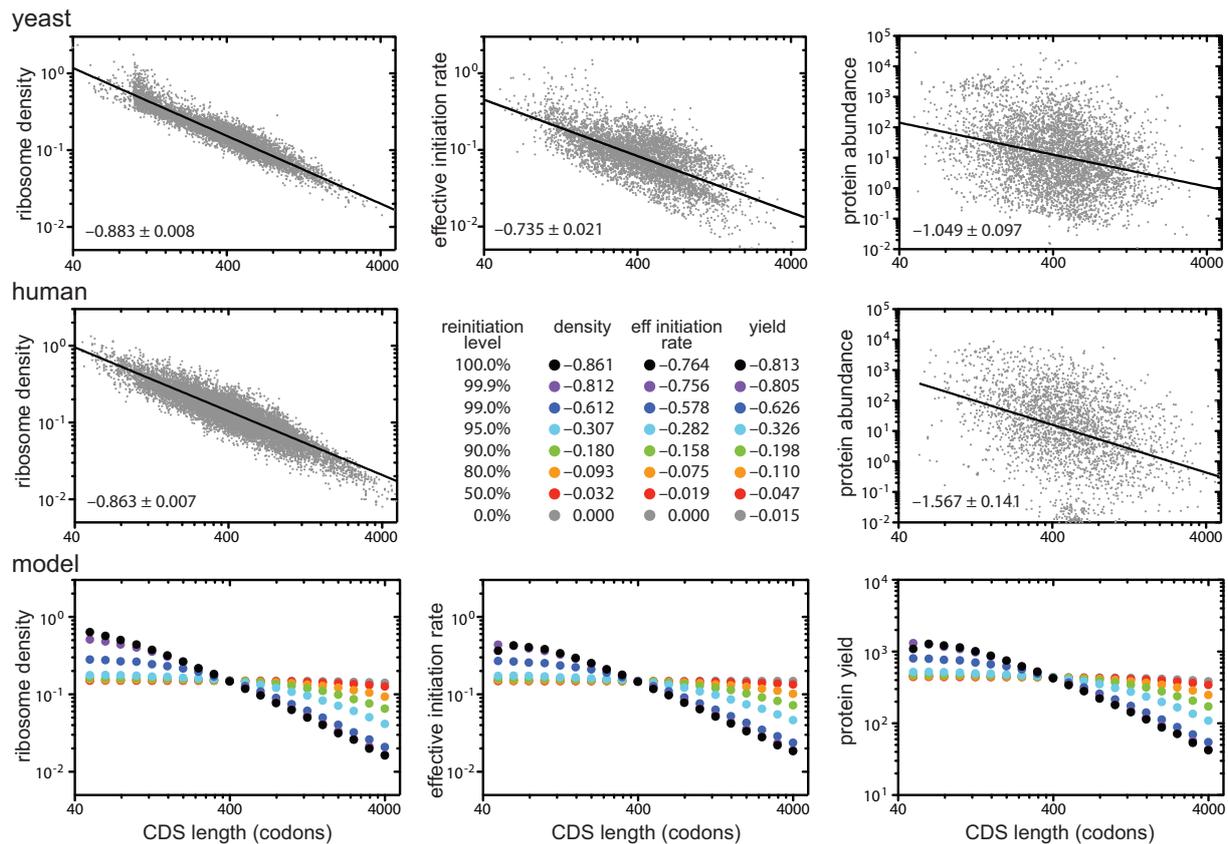
1 Figures



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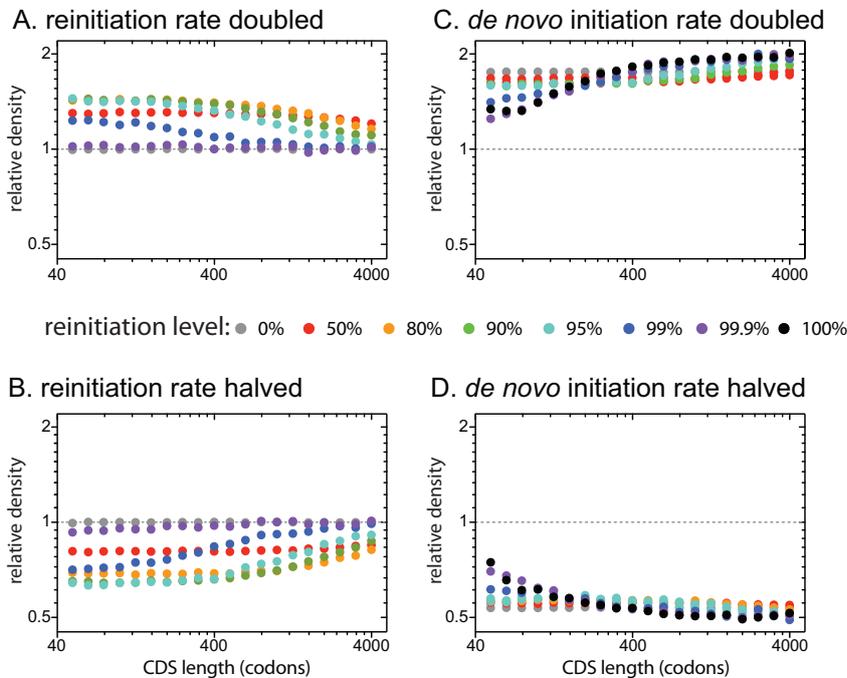
3 **Figure 1. The closed-loop model of translation.** Ribosomes (shaded in grey), modeled as extended particles
4 that occupy 10 codons (black boxes), join the transcript at the start codon from the cytoplasmic pool at the *de*
5 *novovo* initiation rate, and hop to the next codon (in the 5' to 3' direction) at the elongation rate. Upon reaching the
6 stop codon, ribosomes either return to the cytoplasmic pool at the release rate or return to the start codon at the
7 reinitiation rate. The reinitiation level is determined by the reinitiation rate divided by the sum of the reinitiation and
8 release rates. If the initiation site is occupied (i.e. any of the first 10 codons is being decoded), new ribosomes fail
9 to join the transcript and reinitiating ribosomes either remain at the termination site or return to the cytoplasmic
10 pool at the release rate. An elongating ribosome fails to step forward if the distance between its center and that of
11 the ribosome in front is ≤ 10 codons (collision). Stochastic simulations were performed using the Gillespie
12 algorithm. The Gillespie algorithm consists of multiple steps: (1) Initialization: the simulation time is set to zero; at
13 this point in our simulations, all transcripts are empty and the only possible reaction is *de novo* initiation; (2) List
14 all possible reactions: all possible reactions are determined and their rates are used to calculate the total rate of
15 possible reactions; (3) Monte Carlo step: two random numbers are generated, the first determines the waiting
16 time until the next reaction based on the total rate of possible reactions, and the second determines which
17 reaction occurs using each reaction rate as a probabilistic weight; (4) Update: the time is increased by the
18 randomly generated waiting time from step 3 and the chosen reaction is performed; (5) Iteration: repeat from step
19 2 unless the transcript lifetime has been reached.

20



1
2 **Figure 2. Reinitiation of post-termination ribosomes causes length-dependent translation.** Ribosome
3 density is the average number of ribosomes occupying a transcript during its lifetime divided by one tenth of the
4 CDS length (since each ribosome occupies 10 codons). The effective initiation rate is the total number of initiation
5 events (*de novo* initiation and reinitiation) divided by transcript lifetime. Protein yield is the total number of
6 ribosomes reaching the stop codon during the lifetime of a transcript. Slopes (95% confidence intervals) are
7 indicated in the bottom left corner of each panel. *De novo* initiation rates were adjusted at each reinitiation level
8 so that a 400-codon long transcript carried 6 ribosomes. **Top:** experimentally observed relationships between
9 CDS length and ribosome density (left), initiation rate (center) or protein abundance (right) in the budding yeast
10 *Saccharomyces cerevisiae*. **Middle:** experimentally observed relationship between CDS length and ribosome
11 density (left) and protein abundance in the human HEK293T cell line. Estimates of the initiation rate are not
12 currently available for this cell line, so we have used this space to list the slopes from our simulations. **Bottom:**
13 predicted relationships between ribosome density (left), the effective initiation rate (center), and protein yield
14 (right) and CDS length at different reinitiation levels (different colours) from our simulations. Simulations were
15 performed using a fixed elongation rate of 10s^{-1} (see Fig. S3 for simulations at other fixed elongation rates). **Data**
16 **sources:** Yeast densities are weighted averages of the signals in polysomal fractions for 6071 transcripts from
17 [6]; initiation rates for 5348 transcripts were calculated by Ciandrini et al [3] based on ribosome density data from
18 [7]; protein abundances of 4694 proteins included in the Peptide Atlas 2013 dataset from PaxDb [40] normalized

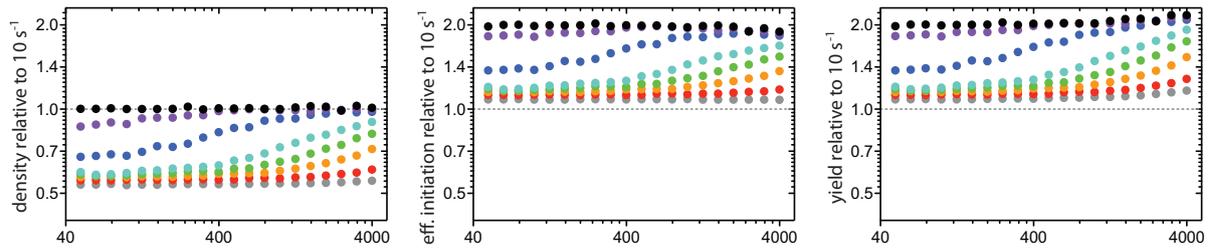
1 against the total number of proteins (expressed as parts per million). HEK293T densities were calculated from
2 mean ribosome numbers (across 3 replicates) reported by Hendrickson et al [11]; protein abundances of 2636
3 proteins with identified CDS lengths included in the Geiger MCP 2012 data set (based on spectral counting) from
4 PaxDb [40].



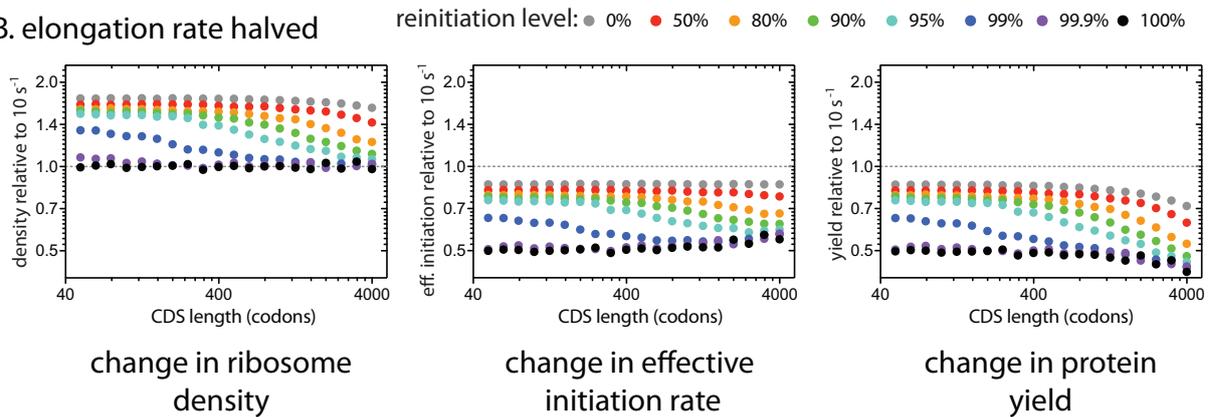
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6 **Figure 3. Transcript-specific change of the reinitiation rate, but not the *de novo* initiation rate, has larger**
7 **effects on short transcripts than long transcripts.** We simulated the effects of changing the reinitiation rate (A,
8 B) or the *de novo* initiation rate (C, D) of a single transcript species by 2-fold or 0.5-fold at different transcriptome-
9 wide reinitiation levels. For each transcriptome-wide reinitiation level (different colours), doubling (or halving) the
10 reinitiation rate shifted the reinitiation level to: 99% to 99.5% (98.0%); 95% to 97.5% (90.5%); 90% to 94.7%
11 (81.8%); 80% to 88.9% (66.7%); 50% to 66.7% (33.3%); 0% to 0% (0%). Doubling or halving the reinitiation rate
12 at very high transcriptome-wide reinitiation levels (e.g. 99.9%) has little effect on translation since ribosomes
13 rarely leave transcripts. Y-axes (log 2 scaled) show the ribosome density of altered transcripts relative to an
14 equivalent transcript at the transcriptome wide reinitiation level. The effects of changing either the reinitiation rate
15 or the *de novo* initiation rate on the effective initiation rate and protein yield were nearly identical to the effects on
16 ribosome density. Transcriptome-wide *de novo* initiation rates were adjusted at each reinitiation level so that a
17 400-codon long CDS at the transcriptome-wide reinitiation level carried 6 ribosomes.

A. elongation rate doubled



B. elongation rate halved



1

2

Figure 4. Transcript-specific change in translation caused by altering the average elongation rate of a

3

single coding sequence. We simulated the effects of changing the average elongation rate of a single transcript

4

species from 10s^{-1} to either (A) 20s^{-1} or (B) 5s^{-1} at different reinitiation levels (different colours). CDS length refers

5

to the length of the altered coding sequence. Y-axes (log 2 scaled) show the effect of altering the elongation rate

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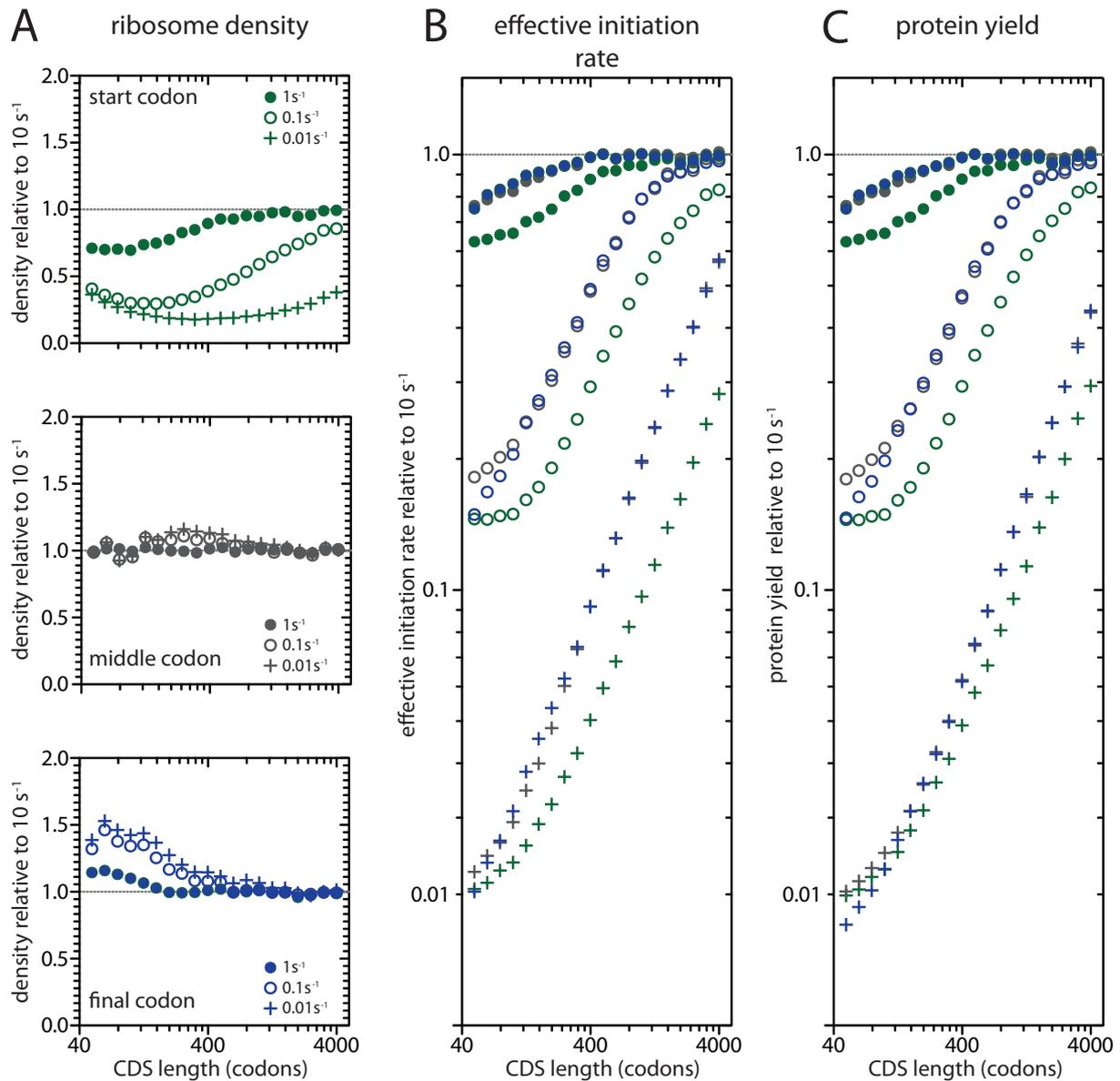
on the ribosome density (left), effective initiation rate (center), and protein yield (left) of the altered transcript at the

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higher or lower elongation rate relative to 10s^{-1} (dotted line). *De novo* initiation rates were adjusted at each

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reinitiation level so that a 400-codon long transcript with a fixed elongation rate of 10s^{-1} carried 6 ribosomes.



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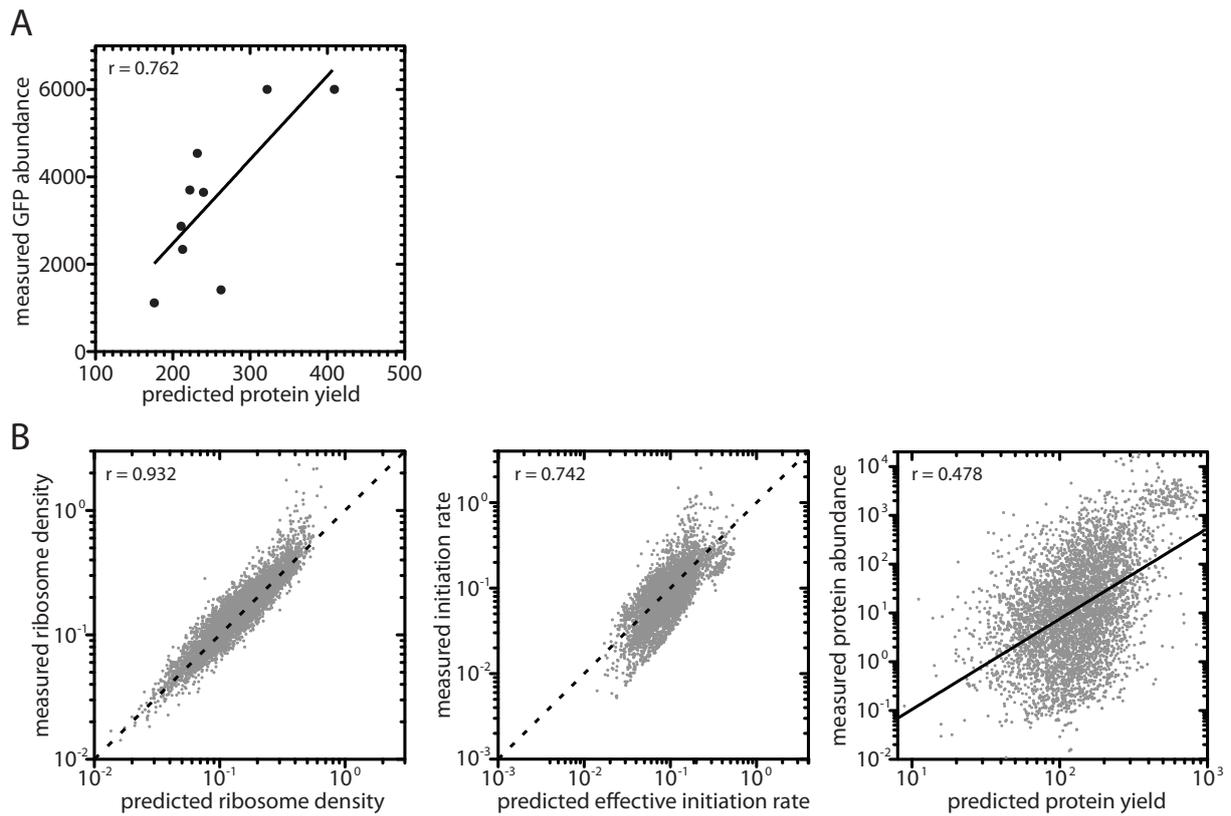
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Figure 5. The consequences of a single slow step under length-dependent translation. We investigated the consequences of introducing a slow elongation step at either the start codon (green), the middle codon (grey), or the final codon (immediately before the stop codon, blue) under 99.9% reinitiation. CDS length refers to the length of the altered coding sequence. Slow codons were translated at 1 s^{-1} (filled circles), 0.1 s^{-1} (open circles), or 0.01 s^{-1} (plus signs). Y-axes show the effect of a single slow step on the ribosome density (right), effective initiation rate (center), and protein yield (right) at the lower elongation rate relative to 10 s^{-1} (dotted line).



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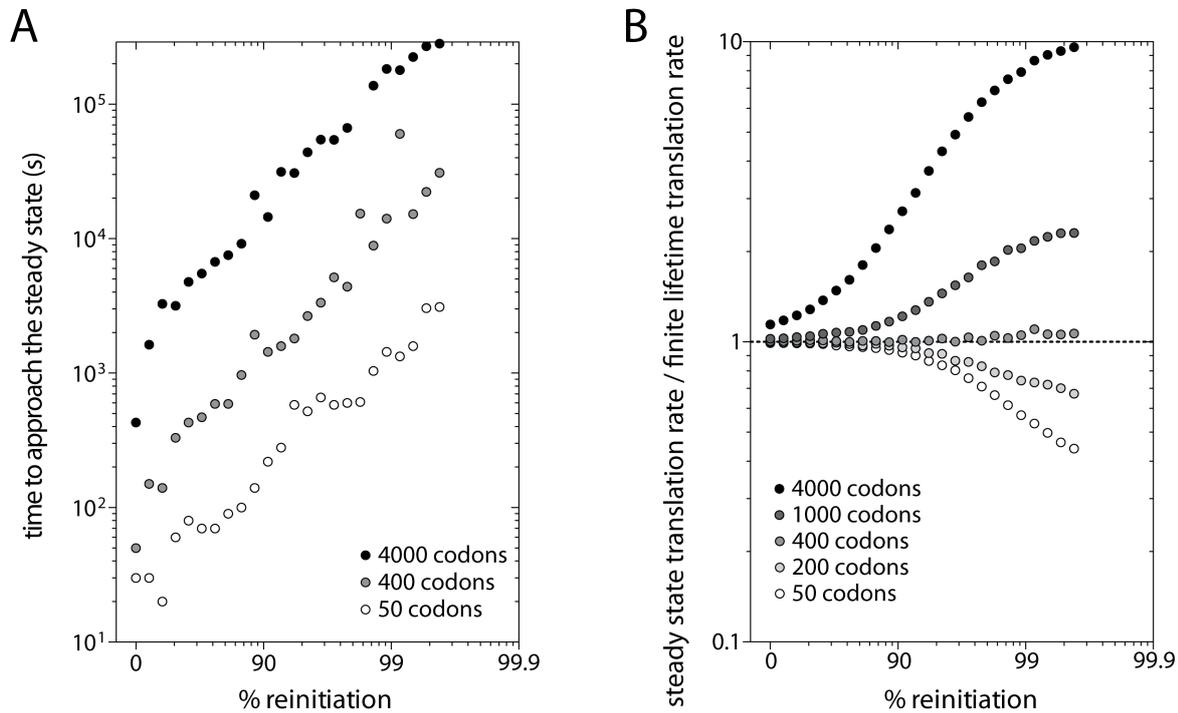
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Figure 6. Simulating translation in the budding yeast *S. cerevisiae*. Our simulations were run using yeast-specific decoding rates [47] and a transcript lifetime of 1553s [39]. Results are shown for 99.9% reinitiation (see Figs. S3 & S4 for simulations at other reinitiation levels). (A) Correlation of model-predicted protein yields (proteins per mRNA per lifetime) for 9 differently codon-optimized GFP coding sequences with experimentally measured abundances (arbitrary units) from [48]. (B) Correlation of model-predicted ribosome density, effective initiation rate, and protein yield with experimentally measured values. Sources of experimental data are the same as in Fig. 2. When X and Y-axis scales are equivalent, the 1:1 line is represented by a dotted line. When X and Y-axis scales are not equivalent, the solid line is the regression line.

1 Supporting information



2

3 **Figure S1. The steady state is a poor approximation of translation at high reinitiation levels for transcripts**

4 **with finite lifetimes.** (A) Time to approach the steady state ribosome density at different reinitiation levels for

5 transcripts of different lengths. Simulations were run for 3×10^5 s using the parameter values described for our

6 general model. *De novo* initiation rates were adjusted at each reinitiation level so that a 400-codon transcript

7 carried an average of 6 ribosomes at the steady state. The steady state ribosome density was calculated as the

8 average density over the final 10^4 s of 1000 iterations (if this value showed no directional change over time). The

9 first passage time represents the earliest time point that the average density of 1000 iterations equaled or

10 exceeded the steady state density. Long transcripts (4000 codons) failed to reach the steady state during the

11 3×10^5 s run time at reinitiation levels above 99.6%. Data points at higher reinitiation levels were therefore

12 excluded. (B) Steady state translation rate (yield s^{-1}) relative to the average translation rate (yield s^{-1}) on

13 equivalent transcripts with a finite 3000 s lifetime. Deviations from a ratio of 1 represent the magnitude of the

14 misestimation of the translation rate caused by assuming a perpetual steady state on transcripts with finite

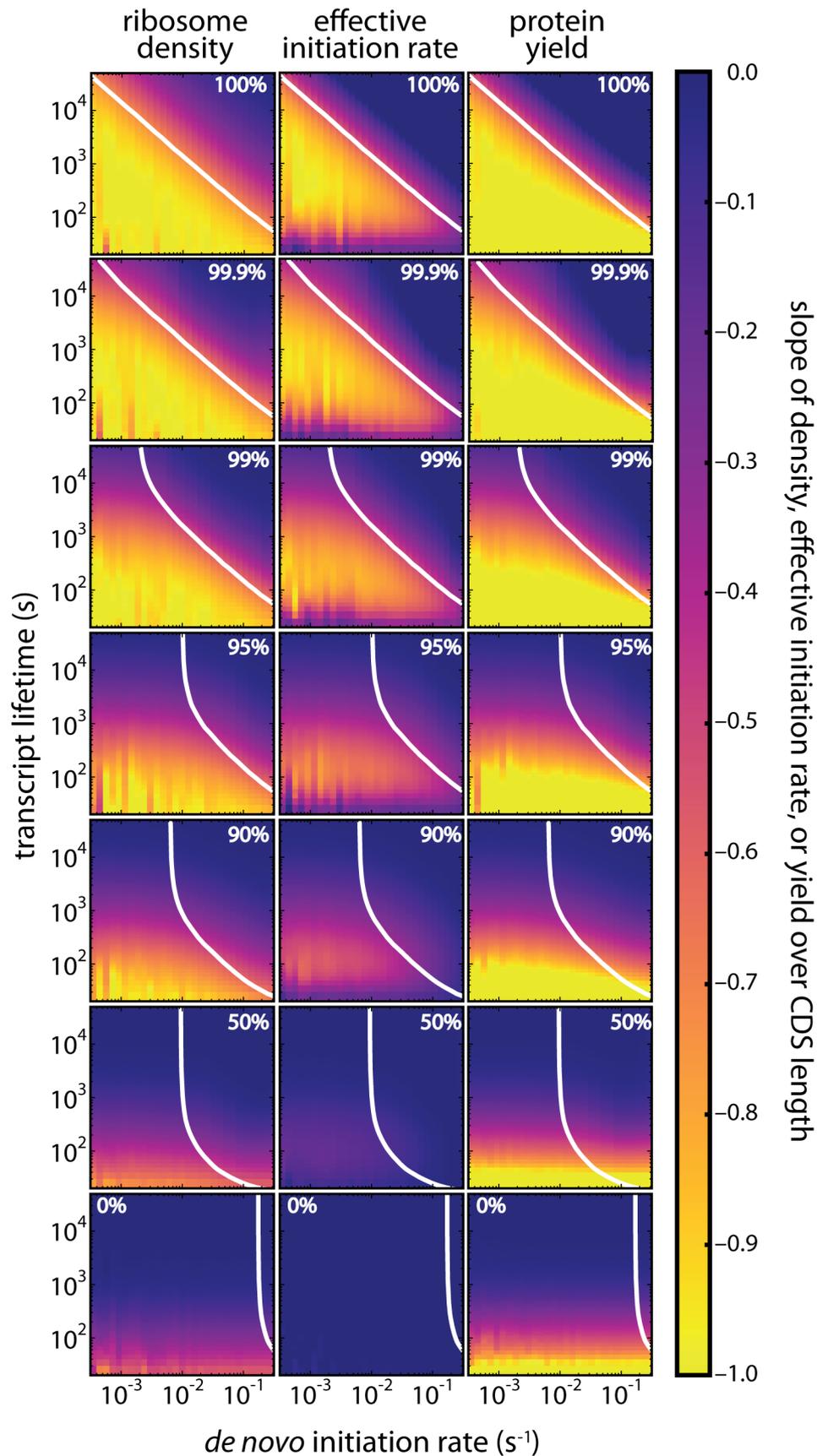
15 lifetimes. *De novo* initiation rates were adjusted at each reinitiation level such that the average 400 codon-

16 transcript carried 6 ribosomes either at the steady state or on average over its 3000 s lifetime. Consequently, all

17 400 codon transcripts have the same average ribosome density allowing fair comparison. At high reinitiation

18 levels, the steady state approximation overestimates ribosome density on long transcripts and underestimates

19 ribosome density on short transcripts.



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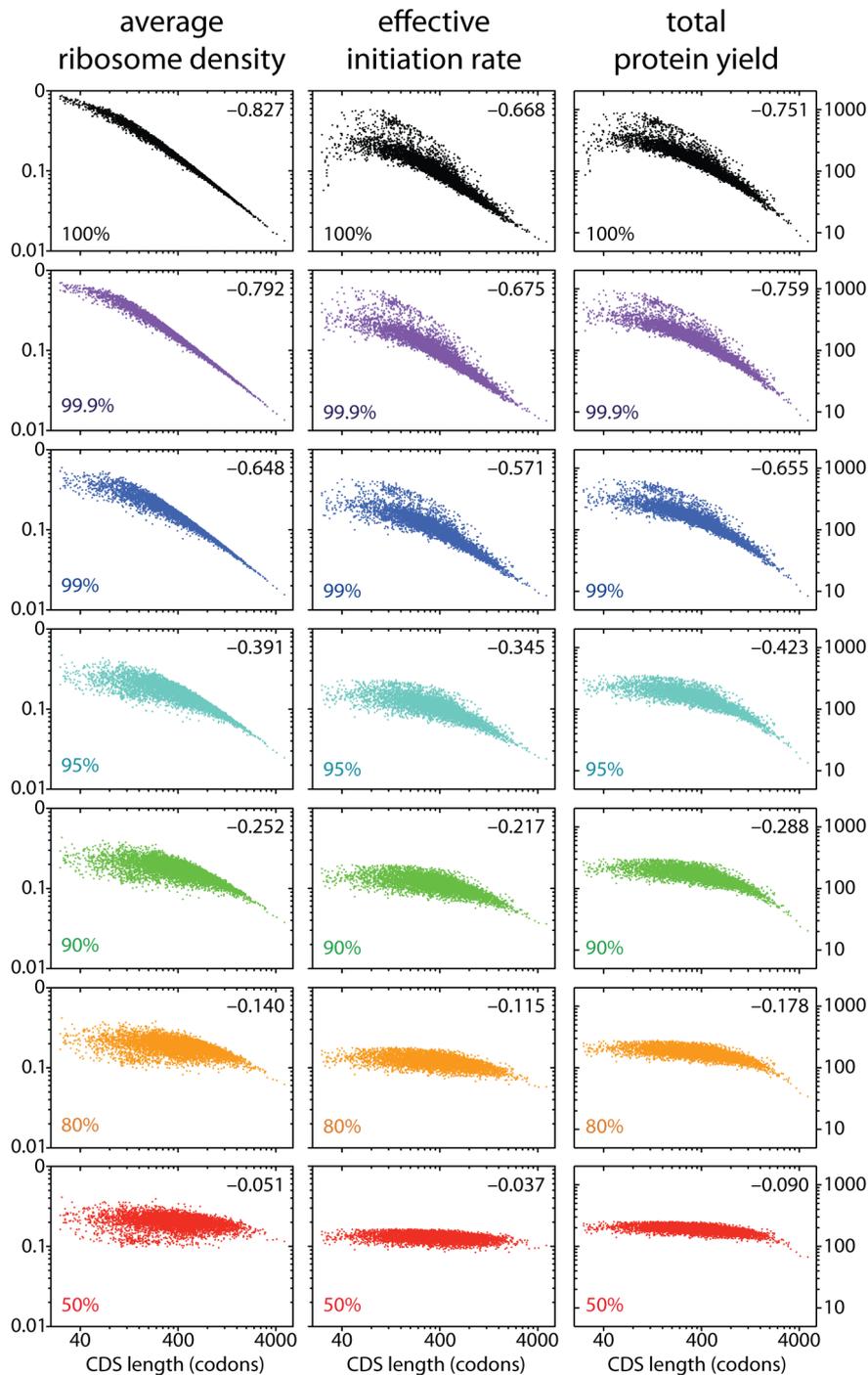
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Figure S2. Full model predictions of ribosome density, effective initiation rate, and protein yield at

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different reinitiation levels. We simulated translation for a wide range of transcript lifetimes and *de novo*

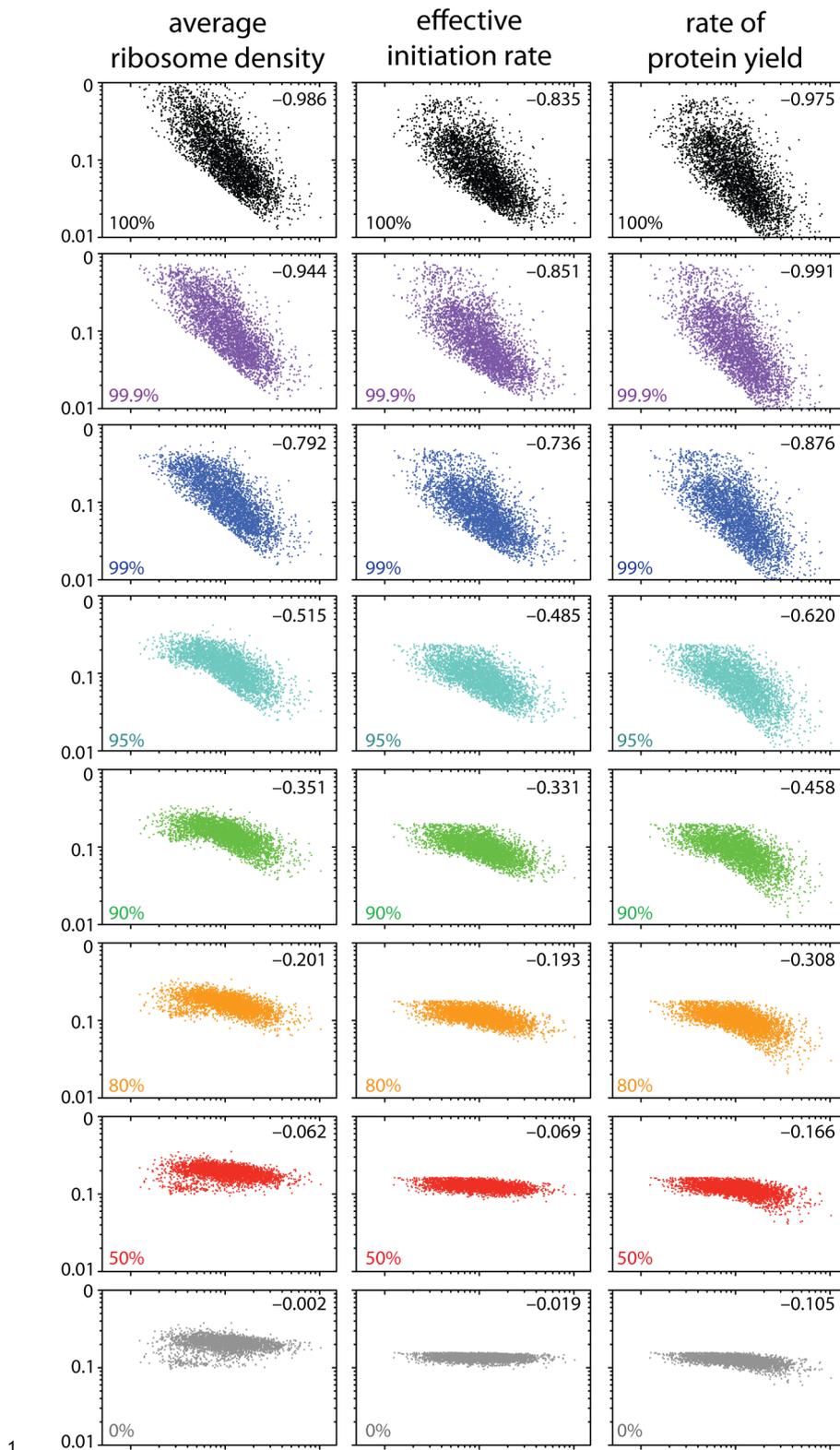
1 initiation rates. For any given combination of transcript lifetime and *de novo* initiation rate, we simulated
2 translation for transcripts with different CDS lengths and then calculated the slope of ribosome density, effective
3 initiation rate, and protein yield over CDS length. Slopes are indicated in different colours (see colour bar),
4 reflecting the degree of length-dependence. The white line in each panel shows the *de novo* initiation rate
5 required at each lifetime such that a 400-codon long transcript carries 6 ribosomes. Our model predicts that high
6 levels of reinitiation can cause length-dependent translation across a wide range of transcript lifetimes and *de*
7 *nov*o initiation rates. At high levels of reinitiation, length-dependence is strongest for short transcript lifetimes and
8 low *de novo* initiation rates; since ribosomes are unlikely to leave the transcript, at long lifetimes or high *de novo*
9 initiation rates, all transcripts eventually become saturated with ribosomes. As a result, the number of ribosomes
10 per transcript becomes proportional to CDS length (since longer transcripts can carry more ribosomes) and
11 ribosome density, the effective initiation rate, and protein yield become similar for all CDS lengths. As the
12 reinitiation level falls, the effective initiation rate becomes dominated by the *de novo* initiation rate, which is the
13 same for all CDS lengths, and ribosome density, the effective initiation rate, and protein yield all become
14 independent of CDS length. In a linear model of translation (0% reinitiation), negative slopes for density and yield
15 are only seen for extremely short transcript lifetimes simply because the first initiating ribosome fails to reach the
16 stop codon on long transcripts before the maximum lifetime is reached. This effect disappears when transcript
17 lifetimes exceed the time required for the first ribosome to reach the stop codon of the longest transcript (~400
18 seconds).



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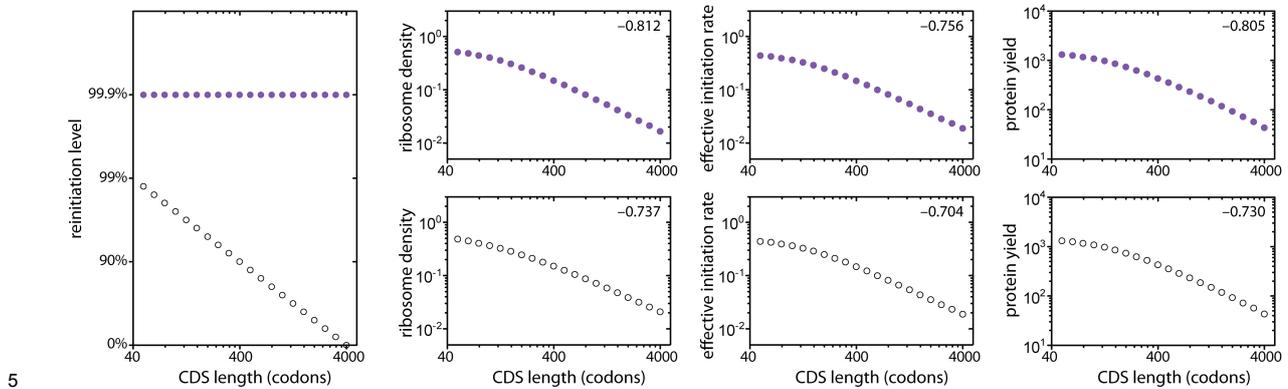
2 **Figure S3. Simulating translation in the budding yeast *S. cerevisiae* at different reinitiation levels.**

3 Predicted ribosome density, effective initiation rate, and protein yield for all 5888 budding yeast transcripts
4 simulated at different reinitiation levels using codon-specific decoding rates from [47]. The left y-axis scale applies
5 to ribosome density (left) and effective initiation rates (center); the right y-axis scale applies to protein yield (right).
6 Slopes are indicated in the top-right corner. As in Fig. 6, all transcripts had a fixed lifetime of 1553 seconds. *De*
7 *novo* initiation rates were adjusted at each reinitiation level so that a 400-codon long transcript with a fixed
8 decoding rate of 10s^{-1} carried an average of 6 ribosomes.



1
2 **Figure S4. Simulating translation in the budding yeast *S. cerevisiae* at different reinitiation levels using**
3 **empirical estimates of transcript lifetime.** The simulations shown in Fig. S3 were repeated using empirical
4 estimates of transcript lifetimes. Transcript lifetimes were calculated using relative abundances from [82]
5 (measured using single-molecule sequencing digital gene expression which eliminates the length-bias associated

1 with RNA-Seq), a total of 36,000 transcripts per cell [83], and nascent transcription rates from [39]. For each
2 transcript, absolute abundance was divided by the transcription rate to obtain the average lifetime. Transcripts
3 with average lifetimes below 400s were excluded to prevent bias towards increased length-dependence (see Fig.
4 S2).



5
6 **Figure S5. Length-dependent translation only requires high levels of reinitiation on short transcripts.**

7 Decreasing reinitiation levels with transcript length (open circles) produces very similar relationships between
8 CDS length and ribosome density, effective initiation rate, and protein yield as does a fixed reinitiation level of
9 99.9% (purple circles). Slopes of each relationship are shown in the upper right corner of each panel. Length-
10 dependent reinitiation levels capture length dependent translation at much lower reinitiation levels than that
11 required using a fixed reinitiation level. We have arbitrarily chosen to decrease the reinitiation level as a function
12 of CDS length according to the formula $100\% \cdot (1 - \text{CDS length}/4000)$. The *de novo* initiation rate was set such that
13 a 400-codon transcript carried an average of 6 ribosomes (99.9% reinitiation = 0.00458s^{-1} , length-dependent
14 reinitiation = 0.02285s^{-1}).

Amino acid	Codon	Decoding rate	Amino acid	Codon	Decoding rate	Amino acid	Codon	Decoding rate
ALA	GCU	18.2	GLY	GGC	27.0	PRO	CCA	15.6
ALA	GCC	11.7	GLY	GGA	4.69	PRO	CCG	15.6
ALA	GCA	7.82	GLY	GGG	3.13	SER	UCU	22.4
ALA	GCG	7.82	HIS	CAU	8.43	SER	UCC	14.4
ARG	CGU	4.21	HIS	CAC	13.8	SER	UCA	7.28
ARG	CGC	2.70	ILE	AUU	20.3	SER	UCG	1.56
ARG	CGA	2.70	ILE	AUC	13.0	SER	AGU	3.82
ARG	CGG	1.56	ILE	AUA	3.13	SER	AGC	6.26
ARG	AGA	17.2	LEU	UUA	10.9	THR	ACU	17.4
ARG	AGG	1.72	LEU	UUG	19.2	THR	ACC	11.2
ASN	AAU	9.56	LEU	CUU	0.956	THR	ACA	6.26
ASN	AAC	15.6	LEU	CUC	1.56	THR	ACG	1.56
ASP	GAU	15.5	LEU	CUA	9.00	TRP	UGG	11.7
ASP	GAC	25.3	LEU	CUG	9.00	TYR	UAU	10.4
CYS	UGU	4.56	LYS	AAA	6.70	TYR	UAC	17.0
CYS	UGC	7.47	LYS	AAG	15.1	VAL	GUU	19.3
GLN	CAA	14.1	MET	AUG	7.82	VAL	GUC	12.4
GLN	CAG	1.56	PHE	UUU	8.9	VAL	GUA	3.13
GLN	GAA	18.4	PHE	UUC	14.6	VAL	GUG	2.87
GLN	GAG	3.13	PRO	CCU	3.13			
GLY	GGU	16.5	PRO	CCC	2.01			

1
2 **Table S1.** *S. cerevisiae* codon-specific elongation rates from [47].