Ribosome recycling, diffusion, and mRNA loop formation in translational regulation

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ABSTRACT We explore and quantify the physical and biochemical mechanisms that may be relevant in the regulation of translation. After elongation and detachment from the 3' termination site of mRNA, parts of the ribosome machinery can diffuse back to the initiation site, especially if it is held nearby, enhancing overall translation rates. The elongation steps of the mRNA-bound ribosomes are modeled using exact and asymptotic results of the totally asymmetric exclusion process (TASEP) [Derrida & Evans 1997]. Since the ribosome injection rates of the TASEP depend on the local concentrations at the initiation site, a source of ribosomes emanating from the termination end can feed back to the initiation site, leading to a self-consistent set of equations for the steady-state ribosome throughput. Additional mRNA binding factors can also promote loop formation, or cyclization, bringing the initiation and termination sites into close proximity. The probability distribution of the distance between the initiation and termination sites is described using simple noninteracting polymer models. We find that the initiation, or initial ribosome adsorption binding required for maximal throughput can vary dramatically depending on certain values of the bulk ribosome concentration and diffusion constant. If cooperative interactions among the loop-promoting proteins and the initiation/termination sites are considered, the throughput can be further regulated in a nonmonotonic manner. Potential experiments to test the hypothesized physical mechanisms are discussed.

Keywords: polymers, asymmetric exclusion process, protein production

INTRODUCTION

The rate of protein production needs to be constantly regulated for all life processes. Genetic expression, protein production, and post-translational modification, as well as transport and activation, are all processes that can regulate the amount of active protein/enzymes in a cell. Although much recent research has focused on the biochemical steps regulating the switching of genes and rates of transcription, translational control mechanisms, post-translational processing, and macromolecular transport are also important. For example, during embryogenesis, nuclear material is highly condensed, transcriptional regulation is inactive, and translational control is important [Browder 1991, Wickens et al. 1996]. In other instances, transcriptional regulation is accompanied by long lag times, particularly with long genes. Translational regulation is also the only means by which RNA viruses express themselves.

Protein production, as with other cellular processes, requires the assembly of numerous specific enzymes and cofactors for initiation. This assembly occurs in free solution and on the 5' initiation site of mRNA. Translation involves unidirectional motion of the ribosome complex along the mRNA strand as amino-acid-carrying tRNA successively transfer amino acids to the growing polypeptide chain. Images of mRNA caught in the act of translation often show numerous ribosome complexes attached to the single-stranded nucleotide (Fig. 1A). The multiple occupancy is presumably a consequence of very active translation, when many copies of protein are desired.

Under certain conditions, the local concentration of tRNA, ribosomes, initiation factors, etc., will control protein production. One possible physical feedback mechanism underlying all the other biochemical regulation processes utilizes local concentration variations of the components of translation machinery. Moreover, there is ample biochemical evidence that the 5' and 3' ends of eukaryotic mRNA interact with each other, aided by proteins that bind to the poly(A) tail and/or regions near the initiation site [Sachs 1990], particularly if the 5' initiation terminus is capped. The presence of both a poly(A) tail and a 5' cap have been found to synergistically enhance translation rates in a number of eukaryotic systems [Gallie 1991]. Numerous proteins that initiate translation, such as eukaryotic initiation factor eIF4, have been identified to bind to the cap and initiate ribosomal binding [Mathews et al. 1996, Munroe & Jacobson 1990, Preiss & Heutze 1999, Sachs 2000]. A different set of proteins, poly(A) binding proteins (PAB) such as Pab1p, are found to bind to the poly(A) tail. The proteins on the 5' cap and the poly(A) tail are also known to form a complex (cap-eIF4E-eIF4G-Pab1p-poly(A) tail) which can increase translation rates [Jackson 1996, Munroe & Jacobson 1990, Sachs 1997, Sachs 2000]. In vitro solutions of capped, poly(A)-tailed mRNA, tRNA, and ribosomes fail to display synergy [Gallie 1991], indicating that additional factors are required for cooperative interactions between the cap and the poly(A) tail. However, in vitro systems that include caps, poly(A) tails, eIF's, and PAB's reveal circularized mRNA structures in electron micrograph (EM) (Fig. 1A) and atomic force microscopy (AFM) (Fig. 1B) images. In this way, it is thought that various components of the translation machinery can be recycled after termination without
completely reentering the enzyme pool in the cytoplasm.

Even in uncapped mRNA, there is evidence that certain sequences in the terminal 3' untranslated region (UTR) can enhance translation to levels comparable to those seen in capped mRNAs [Wang et al. 1997, Jackson 1996]. Additionally, there are indications that proteins near the termination end can, upon contact, directly activate [Gallie 1991] or inactivate [Curtis et al. 1995, Dubnau & Struhl 1996] ribosome entry at the 5' initiation site. Loops also appear to be a common motif in DNA structures [Goddard et al. 2000, Martin & Hagerman 1996] and appear to take part in transcriptional regulation [Martin & Hagerman 1996, Dunn et al. 1984, Wyman et al. 1997]. Double stranded DNA has a much longer persistence length than single-stranded nucleic acids (such as mRNA) and is much less likely to form loops without accompanying binding proteins or specific sequences. Direct evidence for RNA “circularization” is shown in Figure 1B, which shows loop formation of relatively short double-stranded mRNA in the presence of loop-binding factors at their ends [Hagerman 1985]. It is reasonable to expect that the more flexible single-stranded mRNA decorated with ribosomes can form similar loops. Besides the AFM-imaged loop of double stranded RNA shown in Fig. 1B, there is also substantial evidence, particularly in viral mRNAs, that base pairing between uncapped 5' regions and non-polyadenylated 3' regions forms closed loops of many kilobases [Wang et al. 1997]. This loop formation by direct base pairing, or “kissing,” is a very plausible mechanism by which the 3' UTR recruits ribosomes and delivers them to the 5' initiation site [Guo et al. 2001].

In this paper, we model the proposed cyclization, or “circularization” [Sachs 1997] and ribosome recycling mechanisms. Cooperative interactions of the initiation and termination sites with eIF’s and PAB proteins will also be considered within a number of reasonable assumptions. Since translation employs an immense diversity of mechanisms and proteins that vary greatly across organisms [Mathews et al. 1996], we will only develop an initial, qualitative physical picture of cytoplasmic mRNA translation consistent with the ingredients mentioned above. Three different coupled effects are considered in turn: (i) a totally asymmetric exclusion process (TASEP) describing the unidirectional stochastic motion of the ribosome along the mRNA, (ii) the diffusion and adsorption/desorption kinetics from the mRNA initiation/termination sites, and (iii) the polymer physics associated with how the termination and initiation sites are spatially distributed relative to each other. The ribosome density along the mRNA, as well as the time-averaged throughput of ribosomes, the ribosome “current,” are described by solutions of the TASEP. The parameters in the TASEP are the internal hopping rates and the injection and extraction rates at the initiation and termination sites, respectively. Since ribosome components that diffuse in bulk must adsorb on the initiation site, the injection rate used in the TASEP will be proportional to the local concentration of the rate-limiting ribosome. Ribosomes that reach the termination site desorb and reenter the pool of diffusing ribosomes. The distance between the termination end and the initiation site, when ribosomes are released, can thus influence the absorption rate and hence the overall translation rate. The initiation-termination end-to-end distance distribution can be estimated with basic polymer physics. The end-to-end distance distribution can include effects such as specific binding of poly(A) associated proteins with the 5' cap, thereby forming a loop, bringing the initiation and termination sites into close proximity. Although our

FIG. 1: (A) An electron micrograph of polysomes on mRNA. (B) An AFM micrograph of circularization of mRNA mediated by loop forming proteins. From Wells et al., (1998). These images are of double stranded RNA of approximate length 2-4× the dsRNA persistence length. Single stranded end segments with loop binding factors comprise the ends.
model applies only to cytoplasmic mRNA translation, many of its components can also be adapted to treat mRNA adsorption on endoplasmic reticulum (ER) and ER-assisted translation.

**PHYSICAL MODELS**

We now consider the physical processes necessary to describe the above-mentioned translation processes. At the relevant time scales, we will see that fluctuations in these physical mechanisms are uncorrelated with each other. This allows us to consider simple steady-states where time or ensemble averages of the TASEP, ribosome diffusion in the cytoplasm, and the mRNA chain conformations are uncorrelated and can be taken independently of each other. A simplifying schematic of the basic ingredients of mRNA translation is given in Fig. 2.

**The Asymmetric Exclusion Process**

The TASEP is one of a very small number of interacting nonequilibrium models with known exact solutions. Asymmetric exclusion models have been used to effectively model qualitative features of diverse phenomena including ion transport [Hahn et al. 1996, Chou 1999, Chou & Lohse 1999], traffic flow [Schreckenberg et al. 1995], and the kinetics of biopolymerization [MacDonald et al. 1968, MacDonald & Gibbs 1969]. Briefly, the model consists of a 1D lattice of N sites, each of approximately the molecular size of a ribosome unit. Each variable $\hat{\sigma}_i = \{0, 1\}$ represents the ribosome occupation at site $i$ of the coding region of mRNA. Each site can be occupied by at most one ribosome and the mean occupation $\sigma_i \equiv \langle \hat{\sigma}_i \rangle$ at each site $1 \geq \sigma_i \geq 0$. The probability in time $dt$ that an individual ribosome moves forward to the next site (toward the 3' end) is pdf, provided the adjacent site immediately in front is unoccupied. Backward moves are not allowed, since ribosomes are strongly driven motors that move unidirectionally from 5' to 3'. The entrance and exit rates at the initiation ($i = 1$) and termination ($i = N$) sites are denoted $\alpha$ and $\beta$, respectively (cf. Fig 2C). The exact steady state solutions to this kinetic model, including the average density $\sigma_i$, and the mean particle (ribosome) current have been found by Derrida and Evans [Derrida et al. 1993], using a matrix product ansatz, and by Schütz and Domany [Schütz & Domany 1993], using an iteration method. An exact representation for the steady-state current across an $N$-site chain is [Derrida et al. 1993]

$$J_N \equiv J(\alpha, \beta, p) = p S_{N-1}(p/\beta) - S_{N-1}(p/\alpha) / S_N(p/\beta) - S_N(p/\alpha),$$

where

$$S_N(x) = \sum_{k=0}^{N-1} \frac{(N-k)(N+k-1)!}{N!k!} x^{N-k+1}$$

In the $N \to \infty$ limit, the 1D TASEP (Eq. 1) admits three nonequilibrium steady-state phases, representing different regimes of the steady state current $J$:

(I) $\alpha < \frac{p}{2}$, $\alpha < \beta$ \hspace{1cm} $J \equiv J_L = \alpha(1 - \frac{\alpha}{p}) \hspace{1cm} \sigma \equiv \frac{\alpha}{p}$

(II) $\beta < \frac{p}{2}$, $\beta < \alpha$ \hspace{1cm} $J \equiv J_R = \beta(1 - \frac{\beta}{p}) \hspace{1cm} \sigma \equiv 1 - \frac{\beta}{p}$

(III) $\alpha, \beta \geq \frac{p}{2}$ \hspace{1cm} $J \equiv J_{\text{max}} = \frac{p}{4} \hspace{1cm} \sigma \equiv \frac{1}{2}$

FIG. 2: A cartoon of mRNA translation in eukaryotes. The intermediary proteins and cofactors are not depicted. (A) An mRNA chain loaded with ribosomes (green), in various stages of protein (black) production. Ribosomal components as well as other components such as tRNA exist at a uniform background concentration. The initiation and termination sites are additional sinks ($i = 1$) and sources ($i = N$), respectively, of ribosomes. (B) Binding factors (yellow and dark grey) can increase the probability of loop formation or “circularization,” which brings the poly(A) tail (red) in better proximity to the initiation site, enhancing ribosome recycling. (C) Schematic of the associated TASEP with injection ($\alpha$), internal hop ($p$), and desorption ($\beta$) rates labelled.
increases in \( \alpha \) valid only in the Eqs. 3 and the associated phase diagram in Fig. 3 are the constant current \( J \), the rate limiting step \( J_\text{empty} \), and has a small current expected at large but finite phase diagram is qualitatively accurate for the currents \( i \) the opposite limit of fast desorption at low current which is a function of only the slow step \( \beta \). \( \beta = \alpha \) \( \beta > \alpha \) \( \beta = 1 \). Therefore, the high occupancy phase (II) has a low current which is a function of only the slow step \( \beta \). In the opposite limit of fast desorption at \( i = N \), the system attains maximal current \( J = p/4 \) where the effective rate-limiting steps are internal hopping rates \( p \). In this phase, the constant current \( J = p/4 \) is independent of further increases in \( \alpha \) or \( \beta \). The ribosomal currents given by Eqs. 3 and the associated phase diagram in Fig. 3 are valid only in the \( N \to \infty \) limit. Nonetheless, the \( N = \infty \) phase diagram is qualitatively accurate for the currents expected at large but finite \( N \).

There may appear to be a microphysical inaccuracy: The TASEP defined above corresponds to individual movements with step length equal to the ribosome size. However, ribosomes typically occlude \( \sim 10 \) codons, so that it takes \( \sim 10 \) microscopic steps for the ribosome to move the distance of its own size [Lakatos & Chou 2003, Shaw et al. 2003]. An accurate approximation for the throughput \( J \) (Eq. 1) is to assume that each step between two sites defined in our model consists of \( \sim 10 \) actual tRNA transfers. The effective rate \( p \) is thus the average tRNA transfer rate reduced by a factor of \( \sim 10 \). With this consideration, the TASEP completely determines the steady state ribosome throughput as long as the effective rate \( p \) is appropriately defined. Therefore, we will treat the mRNA translation problem using steps sizes equal to the ribosome size, with the understanding that for appropriately rescaled transition rates, our results will be qualitatively correct. The exact currents of a TASEP, where the particle diameters are \( q \) times the step size, is given in Appendix B [MacDonald et al. 1968]. Explicit Monte-Carlo simulations have also been performed on large particle/ small step size dynamics to confirm the accuracy of the results [Lakatos & Chou 2003, Shaw et al. 2003].

What remains is to determine the self-consistent dependence of the model parameters, in particular \( \alpha \) and \( \beta \), on the local ribosome concentration (which in turn depends on the mean current \( J \)), diffusion rates, circularization, etc. For example, the injection rate \( \alpha \) at the initiation site will be proportional to a microscopic binding rate \( k \) times the local ribosome concentration.

**Steady-State Release, Diffusion, and Capture**

The complete mRNA translation machinery is extremely complicated, since it is comprised of many auxiliary RNA and protein cofactors, as well as a collection of active mRNA chains. Since there are many active mRNA chains in the cytoplasm, each mRNA chain feels the sinks (initiation sites) and sources (termination sites) of all the other mRNA chains. However, these other randomly distributed chains, each with their own initiation and termination sites, contribute an averaged background ribosome concentration. Thus, it is only the termination site (ribosome source) associated with the initiation site on the same mRNA chain that resupplies the initiation site in a correlated manner. We thus consider a single “isolated” mRNA chain and for the sake of simplicity, assume that a single component, say phosphorylated elongation initiation factor eIF4F or eIF2, say [Clemens 1996, Sachs 2000], is key to a rate limiting step.

We will generically call this component the “ribosome.” Consider a source of newly-detached ribosomes (emanating from the 3’ termination site) at position \( r \) away from the 5’ initiation site. The probability of finding this particle within the volume element \( dr \) about \( r \) obeys the linear diffusion equation with the termination site acting as a source,

\[
\partial_t P(r, t) - D \nabla^2 P(r, t) = J(t) W_{\text{eff}}(r, t), \tag{4}
\]

where \( D \) is the bulk ribosome diffusion constant, \( J(t) \) is the instantaneous rate of ribosome release from the termination end, and \( W_{\text{eff}}(r) \, dr \) is the probability that the termination site is within the positions \( r \) and \( r + dr \) from the initiation site. Although Eq. 4 can be solved exactly...
for all times, the TASEP result (Eq. 1) is appropriate only in the steady-state, so we must consider that limit for all processes.

The typical mRNA passage time of a single ribosome is on the order of one minute. The bulk diffusion constant of the 10-15mm radius (a ~ 15nm) ribosome unit is $D \sim 10^{-8} - 10^{-7} \text{cm}^2/\text{s}$. A ribosome molecule will diffuse the length of a 1kB pair mRNA strand in ~ 0.1s. Therefore, with each release of a ribosome from the termination site, the probability density appears as a pulse which passes through the initiation site over a time scale shorter than it takes for a ribosome to stochastically hop a few lengths of its size along the mRNA chain. Therefore, an upper bound on the amount of correlation between concentration fluctuations and $\hat{\sigma}_1$ can be found by considering the equal time two-point correlation in the maximal current phase ($\hat{\sigma}_1\hat{\sigma}_N - \sigma_1\sigma_N \sim N^{-3/2}/8$ [Derrida & Evans 1993]. Two-point correlations in other current regimes are smaller, and decay exponentially with $N$ [Essler & Rittenberg 1996]. Therefore, we can neglect the correlation of the current $J(t)$ with the occupancy $\hat{\sigma}_1$ at the initiation site. Moreover, the end-to-end distribution $W_{\text{eff}}$ arises from the statistics of the mRNA polymer configurations and is also assumed independent of both $J(t)$ and $\hat{\sigma}_1$. The steady state ribosome distribution can thus be found by setting $\partial_t P(r, t) = 0$ on the left-hand-side of Eq. 4 and taking the time, or ensemble, average of the remaining Poisson equation to obtain

$$\langle \nabla^2 P(r, t) \rangle = \nabla^2 C(r) = -\frac{J}{D} W_{\text{eff}}(r),$$

(5)

where $J \equiv \langle J(t) \rangle$ is the steady-state current of ribosomes emanating from the termination end of the mRNA re-entering the bulk ribosome pool, and $C(r) = \langle P(r, t) \rangle$ is the ensemble average of $P(r)$.

The boundary condition for $C(r)$ at the initiation site will depend on the occupancy of that site. When it is empty, there is a flux due to the microscopic adsorption step onto the first site. When $\hat{\sigma}_1 = 1$, the bulk ribosome probability distribution will obey perfectly reflecting boundary conditions. Since the probability at $r = a$, $P(r = a, t)$ depends on the occupation $\hat{\sigma}_1$, $\langle P(a, t)\hat{\sigma}_1 \rangle \neq C(a)\sigma_1$. The mean concentration at $r = a$ must be found by averaging the currents in the two states, $\hat{\sigma}_1 = 1$, and $\hat{\sigma}_1 = 0$. When the initiation site is empty,

$$J(\hat{\sigma}_1 = 0) \equiv J_0 = 4\pi a^2 D \partial_r C(r = a) = kC(r = a).$$

(6)

Since the steady state current $J(\hat{\sigma}_1 = 1) = 0$ when the initiation site is full, the averaged steady-state current is

$$J = (1 - \sigma_1)J(\hat{\sigma}_1 = 0) + \sigma_1 J(\hat{\sigma}_1 = 1) = (1 - \sigma_1)J_0,$$

(7)

where $(1 - \sigma_1)$ is the fraction of time that the initiation site is unoccupied, ready to absorb a ribosome from the bulk. This probability is not directly dependent on the distribution $W_{\text{eff}}(r)$, but will depend on the time-averaged local concentration $C(r)$, which in turn depends on $W_{\text{eff}}$ only through the distance of the source site at $i = N$.

The solution to Eq. 5, obeying the boundary conditions Eq. 6 and $C(r \to \infty) = C_\infty$, is

$$C(r) = C_\infty - C_\infty \left( \frac{ka}{4\pi Da + k} \right) + \frac{J}{D} \int dr' G(r - r') W_{\text{eff}}(r'),$$

(8)

where $r$ is distance measured from the initiation site, and

$$G(r, r') = \frac{1}{4\pi |r - r'|} - \sum_{m = -\ell}^{\infty} \left( \frac{ka}{4\pi a^2 D(\ell + 1)a^{-\ell - 2}} \right) \times Y^\ell_{\ell m}(\Omega) Y_{\ell m}(\Omega')$$

(9)

is the associated Green function. In Eq. 9, $r_\leq(r_>)$ is the smaller(larger) of $|r|, |r'|$ and $Y_{\ell m}(\Omega)$ are the spherical harmonic functions of the solid angle $\Omega$ defined by the vector $r$ [Arfken 1985]. The first two terms in Eq. 8 arise from the uniform concentration $C_\infty$ at infinity and the effects of a sink of radius $a$ at the initiation site. The sink decreases the effective concentration to a level below that of $C_\infty$. The last term proportional to $J$ increases the local concentration and is the result of the source (termination site) some finite distance away from the initiation site. If $k \to 0$, and ribosomes do not bind even when the initiation site is empty, the current $J$ must vanish, and $C(r) \to C_\infty$, as expected. However, one cannot simply consider the limit $k \to 0$ in Eq. (8) because $k$ and $\sigma_1$ are related through $J$, the current determined by the TASEP in the rest of the chain. This can be seen by considering the limit $k \to \infty$. If the rest of the TASEP contains the rate-limiting step to ribosome throughput, making $J$ very small, it will effectively block clearance of the initiation site, since all sites of the chain will be nearly occupied. In this case, $\sigma_1 \approx 1$ and $k(1 - \sigma_1)$ is small (despite a large $k$), and $C(r) \approx C_\infty$, as expected. However, if the rest of the chain is not rate-limiting, and if clearance of the initiation site can occur fast enough, $\sigma_1 < 1$ and $k(1 - \sigma_1)$ can be large. In this case, $C(r) \approx C_\infty(1 - a/r) + JD^{-1} \int dr' G(r - r') W_{\text{eff}}(r')$. The TASEP current $J$ will eventually be balanced with $J = (1 - \sigma_1)J_0$. Note that $J$ is determined by Eq. 1 which in turn depends on the entry rate $\alpha$ (in other words, $kC(a)$). Thus, steady-state currents need to be self-consistently determined, since $C(a)$ and $\sigma_1$ are not parameters, but dynamical variables that will in turn be determined by setting $J = (1 - \sigma_1)J_0$. The analysis which uses Eq. 1 to find self-consistent explicit expressions for $J$ will be presented in the Results and Discussion.
Since the averaged bulk concentration profile is spherically symmetric about the initiation site, only the $\ell = 0$ terms in the expression for $G(\mathbf{r} - \mathbf{r'})$ survive and

$$J_a = kC(a)$$

$$= \frac{4\pi a^2 D kC_{\infty}}{ka + 4\pi a^2 D} + \frac{4\pi a^2 D k J}{4\pi D(ka + 4\pi a^2 D)} \int_{r' > a} dr' \frac{W_{\text{eff}}(r')}{r'}$$

$$= \frac{ka}{k + 4\pi a D} \left[ 4\pi DC_{\infty} + \frac{J}{R} \right],$$

where

$$\frac{1}{R} \equiv \left( \frac{1}{r} \right) = \int dr \frac{W_{\text{eff}}(r)}{r}. \quad (10)$$

The surface concentration at the sink surface $a$ is reduced from the “bulk” value by a factor of $1 + 4\pi a D/k$, due to adsorption and diffusional depletion. However, part of this initiation site concentration is also replenished at a rate proportional to the flux $J$, due to the presence of a nearby termination (source) site. The effects of this replenishment are measured by the mean inverse separation $1/R$. The “harmonic distance” $R$ defines the effective distance felt by diffusing ribosomes as they make their way from the termination end back to the initiation site. This particular $r^{-1}$ scaling is a consequence of the solution to Poisson’s equation (Eq. 5) in three dimensions, and is related to the capture probability of diffusing ligands, as analyzed by Berg and Purcell [Berg & Purcell 1977]. Equation 10 contains two unknowns, $C(a)$ and $\sigma_1$. We can use the explicit solution Eq. 1 if we identify the injection rate $\alpha$ of the TASEP with the unoccupied initiation site current $J_0 = kC(a) \equiv \alpha$. Equation 1 then relates $kC(a)$ to $\sigma_1$. A second equation can be used by noticing that the flux itself must be balanced. Upon using $J = kC(a) (1 - \sigma_1)$ in Eq. 10, a second relationship between $kC(a)$ and $\sigma_1$ can be found. Substitution of the solution for $kC(a)$ (in terms of experimentally known or controlled parameters $k, C_{\infty}, a, R, D$) into Eq. 1 determines the self-consistent, steady-state ribosome current. This analysis, using the three different explicit forms of Eq. 1 (in the long chain limit) is presented in the Results and Discussion.

End-to-End Distribution $W_{\text{eff}}$

We now find $W_{\text{eff}}(r)$ in order to compute $R$ and obtain $C(a)$. In some cases, the mRNA chain may be anchored to cellular scaffolding or ER membranes such that the initiation-termination separation is fixed. If one is interested in steady-state protein production over a period which allows little change in initiation-termination distance, $W_{\text{eff}}(r) = \delta(r - R)$, and $R = |R|$. In other cases, the mRNA may be free to explore numerous conformations on the protein production time scale. Although it is possible that long mRNA strands may contain secondary structure, we will assume that ribosomes, as they move along the mRNA, melt out these structures. Although there is evidence that mRNA can contain small, local loops [Hagerman 1985, Wang et al. 1997], it is less likely that they have larger-scale tertiary structure. Thus, we will estimate $W_{\text{eff}}$ and $R$ with simple polymer models.

FIG. 4: A schematic of the effects of loop forming factors. The coding region of the mRNA is blue (the ribosomes and the poly-A tail are not shown), the noncoding spacers of $m$ and $n$ persistence lengths $\varepsilon$ are solid black, while the the neglected short ends are dashed curves. The loop binding factors are of typical size $d$. (A) Nonlooped conformations in which the initiation-termination site distribution function is governed by $W(r|\text{open})$. (B) The initiation-termination distribution function in looped configurations is denoted $W(r|\text{loop})$. $W(r|\text{loop})$ is weighted more strongly at small $r$ relative to $W(r|\text{open})$. For stronger attraction between loop binding factors the probability of loop formation increases, decreasing the effective distance $R$ that ribosomes must diffuse to be recycled back to the initiation site.

As shown in Fig. 2, the mRNA is comprised of three segments divided between two qualitatively distinct regions. Typical coding regions are $\sim 10^3$ base pairs, corresponding to $N \sim 300$. At low ribosome densities, the uncovered mRNA base pairs will be rather flexible, and the effective persistence length $\ell$ will be a local average between $a$ and the 2-4 nucleotide persistence length $\varepsilon$ of uncovered mRNA. Large reductions in the persistence length of dsDNA containing segments of single-stranded regions have also been observed by Mills et al. [Mills et al. 1994]. More sophisticated theories for variable persistence lengths can be straightforwardly incorporated; however, for simplicity, we approximate the persistence length in the coding region to be a uniform con-
stant on the order of $\ell = a$, the individual ribosome exclusion size. The contour length of the coding region is thus $L_N = Na$ with $N \sim 50 - 500$. The untranslated regions, or UTRs between the initiation site and the binding factor (dark gray), and between the termination site and the loop-binding factor (yellow), with persistence lengths $\epsilon$, have contour lengths of $L_m = m \epsilon$ and $L_n = n \epsilon$, respectively. Typical $L_m, L_n$ are on the order of 100 bases so that $n, m \sim 20 - 50$. However, extremely long noncoding segments of order 1kbp can exist [Mathews et al. 1996] where $m, n \sim 300$. In what follows we will also neglect all the excluded volume effects of the remaining short ends of the mRNA chain.

As demonstrated by Wells et al. [Wells et al. 1998] in Figure 1B, mRNA can form loops in the presence of binding proteins. Therefore, we expect that $W_{\text{eff}}(r)$ (and hence $1/R$) will be a linear combination of $W(r|\text{open})$ and $W(r|\text{loop})$, the initiation-termination probability distributions in open and looped mRNA configurations, respectively. These configurations are shown in Figs. 4A, B. For simplicity, we will use probability distributions associated with noninteracting (phantom) chains and approximate the distributions $W(r)$ with both a freely jointed chain (FJC) and worm like chain (WLC) models with appropriate persistence lengths $\ell$. The finite-sized, short distance behavior of the $W(r|\text{open},\text{loop})$ will be important for accurately computing $(1/R)$. As we will see, $W(r|\text{loop})$ can be constructed from the more fundamental quantity $W(r|\text{open})$ [Liverpool & Edwards 1995, Sokolov 2002]. Since we are eventually interested in either ribosome transport from termination to initiation or in activation/deactivation of initiation or release sites due to direct contact with the end proteins, we compute in Appendix C the distance distribution $W(r|\text{open})$ in the state where site $i = N$ is occupied and site $i = 1$ is unoccupied.

Using the $W(r|\text{open})$ computed in Appendix C, we can thus consider the contributions of looped configurations to the effective end-to-end distance distribution. The binding energy between the 5’-cap and poly(A) tail proteins, $-U_0$ (in units of $k_B T$), determines the probability that the chain is looped:

$$P_{\text{loop}}(n, m; N; U_0) = \frac{\exp(-G_{\text{loop}})}{\exp(-G_{\text{loop}}) + \exp(-G_{\text{open}})} = \frac{e^{-U_0}}{e^{-U_0} + \Omega_0(\text{open})/\Omega_0(\text{loop})},$$

where the free energies of a closed and open mRNA chain are $G_{\text{loop}} = -U_0 - S_{\text{loop}}$ and $G_{\text{open}} = -S_{\text{open}}$, respectively. Since the ratio of the number of configurations under looped and open chain conditions is the ratio of probabilities of loop formation in the absence of head-tail interactions ($U_0 = 0$), $\Omega_0(\text{open})/\Omega_0(\text{loop}) = (1 - P_{\text{loop}}(0))/P_{\text{loop}}(0)$, and

$$P_{\text{loop}}(0) = \frac{e^{U_0} P_{\text{loop}}(0)}{e^{U_0} P_{\text{loop}}(0) + (1 - P_{\text{loop}}(0)).}$$

The probability that the ends of a noninteracting (in the absence of loop binding proteins) chain intersects itself within the interaction volume defined by a thin spherical shell of thickness $\delta$ (the binding interaction range) is approximately

$$P_{\text{loop}}(0) \approx 4\pi d^2 \delta \int_{r_m, r_m + N > d} W_{\text{r}}(r_m|\text{open}) W_{\text{r}}(r_{m+N} - r_m|\text{open}) \times W_{\text{e}}(r_{m+N}|\text{open}) \, dr_1 \, dr_2 \approx \sqrt{\frac{6}{\pi}} \left( \frac{d}{L_T} \right)^2 \left( \frac{\delta}{L_T} \right) [1 + O(d/L_T)],$$

where $d$ is the typical size of the loop binding factors and $L_T = \sqrt{L_N^2 + L_m^2 + L_n^2} = \sqrt{N a^2 + (m + n)\epsilon^2}$. We have assumed the total radius of gyration $L_T \gg a$, and used a Gaussian chain as a qualitative approximation for the distributions used in the calculation of $P_{\text{loop}}(0)$. The conditional probability distribution $W(r|\text{loop})$ for a looped chain is

$$W(r|\text{loop}) = \frac{W_{\text{a}}(r|\text{open}) W_{\text{e}}(r|\text{open})}{\int_{r > a} W_{\text{a}}(r'|\text{open}) W_{\text{e}}(r'|\text{open}) \, dr'},$$

where $W_{\text{a}}(r|\text{open})$ denotes the single segment, open chain probability distributions in the two segments with persistence lengths $\ell = a, \epsilon$. For $a \sqrt{N} \gg \epsilon (m + n)$, the loop distribution given by Eq. 15 is qualitatively similar to the distribution function $W_{\text{e}}(r|\text{open})$ of the short segment of persistence length $\epsilon$.

Using Eqs. 13, 14, 15, and C5 we construct the effective initiation-termination distance distribution

$$W_{\text{eff}}(r) = (1 - P_{\text{loop}}(0)) W(r|\text{open}) + P_{\text{loop}}(0) W(r|\text{loop}).$$

$W_{\text{eff}}(r)$ is plotted in Appendix C (Fig. 11) for various $U_0$. Qualitatively similar loop probability distributions have also been computed by Liverpool and Edwards [Liverpool & Edwards 1995] within the WLC model but without finite-sized molecules at the ends. Here and in all subsequent analyses, we use the typical parameters $\epsilon/a = 0.2, d = a$, and $\delta/a = 0.1$. As $U_0$ is increased, the distance distribution function switches over from $W(r|\text{open})$ to $W(r|\text{loop})$. The statistics of $W(r|\text{open})$ and $W(r|\text{loop})$ are governed by $L_N = Na$ and $L_m = (m + n)\epsilon$, respectively. The loop forming factors, since they are close to the initiation and termination sites ($L_m \ll L_N$), enhance the probability that the ends are close to each other, particularly when the binding energy $U_0$ is large.
in Eqs. 13 and 16 qualitatively describes a crossover in \( U_A, B \) \( R/a \) dependence of \( U \) self primarily in the low \( U \) lengths (ratio of noncoding persistence lengths to coding persistence predominantly increases the typical distance decreasing the length of the short noncoding ends of the mRNA of a free chain to that of a loop occurs near \( U \) The crossover between the end-to-end distribution function initiation and termination sites are brought closer together.

The crossover between the end-to-end distribution function of a free chain to that of a loop occurs near \( U_0^\ast \) \( \sim 8 \). Increasing the length of the short noncoding ends of the mRNA predominantly increases the typical distance \( R \) in the large \( U_0 \), looped regime. (B) The \( N \)-dependence of \( R/a \) with the ratio of noncoding persistence lengths to coding persistence lengths \( (m + n)/N = 1/2 \). The \( N \)-dependence manifests itself primarily in the low \( U_0 \), open chain regime. (C) The \( N \) dependence of \( R/a \) for various \( U_0 \).

The harmonic distance, \( R \), determined using \( W_{\text{eff}} \) is shown in Figs. 5A, B as functions of loop binding energy \( U_0 \). The result given by the last line in Eq. 14, when used in Eqs. 13 and 16 qualitatively describes a crossover in \( W_{\text{eff}} \) from \( W(\mathbf{r}|\text{open}) \) to \( W(\mathbf{r}|\text{loop}) \) behavior at

\[
U_0^\ast \approx \ln \left[ \sqrt{\frac{\pi}{6}} \left( \frac{L_T}{d} \right)^2 \frac{L_T}{\delta} \right] + O(d^2\delta/L_T^3). \tag{17}
\]

In Fig. 5A, \( R/a \) is shown with \( N = 100 \), but at various noncoding lengths \( m + n \). In the large binding strength limit, \( R/a \) depends only on the short distance \( (m + n)\varepsilon \). When loops rarely form, the typical separation between initiation and termination sites can only depend on \( L_N \) which is the only quantity varied in Fig. 5B. Notice that the exact FJC solution (Appendix C), or truncated WLC solution for \( W(r \leq a|\text{open}) = 0 \) ensures that \( R/a > 1 \) for all values of \( m, n, N \), and \( U_0 \). The dependence of \( R/a \) on \( N \) is shown in Fig. 5C for various \( U_0 \). When \( U_0 \) is small, the initiation-termination harmonic distance \( R \) is controlled by \( L_N \) and increases as \( \sqrt{N} \). For larger \( U_0 \), the chain is partially bound into a loop where the distance is controlled by the much shorter \( L_{m+n} \). The harmonic distance \( R \) remains small unless \( N \) becomes extremely large so that entropy can dominate and the loop ends can unbind.

We now couple our mathematical models by incorporating the \( W_{\text{eff}} \)-weighted inverse harmonic distance \( a/R \) into the local, effective concentration \( C(a; R) \) given by Eq. 10. The effective injection rates \( \alpha = kC(a) \) that control the translation rate within the steady-state TASEP are then self-consistently determined.

**RESULTS AND DISCUSSION**

Here, we compute the possible currents \( J \) and the parameter space in which each are valid. We will use the exact solution Eq. 1, or its three asymptotic forms (Eqs. 3), as well as \( J = kC(a)(1 - \sigma_1) \) in Eq. 10, to find all relevant quantities and parameter phase boundaries.

Substitution of \( J = kC(a)(1 - \sigma_1) \) into Eq. 10 and solving for \( \sigma_1 \), we find

\[
1 - \sigma_1 = \frac{4\pi DR}{k} \left( 1 - \frac{C_{\infty}}{C(a)} \right) + \frac{R}{a}. \tag{18}
\]

Upon multiplying Eq. 18 by \( kC(a) \), we find

\[
kC(a)(1 - \sigma_1) = 4\pi DR(C(a) - C_{\infty}) + \frac{R}{a} kC(a) \equiv J(kC(a), \beta, p) = \begin{cases} 
  kC(a)(1 - kC(a)/p) \\
  \beta(1 - \beta/p) \\
p/4
\end{cases} \tag{19}
\]

To find \( C(a) \) in terms of known parameters, we use the explicit solutions of the TASEP for the current \( J(kC(a), \beta, p) \) (Eq. 1 or 3) as indicated on the right-hand-side of Eq. 19. The exact solution Eq. 1 yields an
$N + 2$ order equation in $kC(a)$ which we solve numerically. Only one of the $N + 2$ roots of Eq. 19 is real, yielding occupations between zero and one, and is the physically relevant. The self-consistent solutions for $kC(a)$ are used to evaluate $J(kC(a), \beta, p)$, which are plotted in Figures 6A, B. As expected, shorter chains yield slightly higher current. Larger $D$ also increases the current and makes the approximate maximal current phase obtainable at smaller $kC_\infty/p$. Asymptotic limits for the current near phase boundaries and at large $N$ are given in Appendix D.

\begin{equation}
C(a) = \frac{p/4 + 4\pi DRC_\infty}{4\pi DR + Rk/a}.
\end{equation}

The criterion for maximal current, $k > p/(2C(a))$, is thus

\begin{equation}
k > \frac{p(4\pi DR + k(R/a))}{p/2 + 8\pi DRC_\infty}.
\end{equation}

Upon solving Eq. 21 for $k$, we find the minimum $k = k^*$ required to achieve maximal current $J = p/4$:

\begin{equation}
\frac{kC_\infty}{p} > \frac{k^*C_\infty}{p} = 2 - \frac{p}{4\pi aDC_\infty} (1 - \frac{a}{2R}).
\end{equation}

Note that for large enough $p/(4\pi aDC_\infty)$ the critical value $k^*$ can diverge. The divergence is more likely or larger $R$ and occurs when there is simply not enough ribosome nearby to provide a large enough “on” rate $\alpha$ to achieve maximal current. Even when the source (termination end) is held at the initiation site ($R = a$), there is the possibility that $k^*$, and maximal current, are never attained. This behavior arises because even for ribosomes released at an infinitely absorbing spherical initiation surface, there is a probability of escape [Berg & Purcell 1977].

Next, let us consider small $\beta$ and large $\alpha = kC(a)$. The mRNA has a high ribosome occupancy and a steady-state current $J = \beta(1 - \beta/p)$. This regime (phase II) is termination rate-limited and occurs for $\beta < p/2$ and $\beta < \alpha = kC(a)$. Upon using $J = \beta(1 - \beta/p)$ in Eq. 19,

\begin{equation}
\beta < kC(a) = k\frac{\beta(1 - \beta/p) + 4\pi DRC_\infty}{4\pi DR + kR/a}.
\end{equation}

The only physical range of $\beta$ that satisfies inequality 23 is

\begin{equation}
\beta < \beta^*(k) = \frac{p}{2} \left( \frac{R}{a} (\bar{D} + 1) - 1 \right)
\times \left[ 1 + \frac{4(R/a)\bar{D}kC_\infty}{p((\bar{D} + 1)R/a - 1)^2} - 1 \right],
\end{equation}

where $\bar{D} \equiv 4\pi aD/k$. Equation 24 defines the phase boundary between the high-density, exit rate-limited phase (II) and the low-density, initiation rate-limited phase (I). This phase boundary is plotted as a function of $kC_\infty/p$ for fixed $4\pi aDC_\infty/p = 0.5$ in Figs. 7B. In the

FIG. 6: The numerically determined, steady state currents at finite $N$. The self-consistent currents were found by numerically finding the roots to the polynomial in $J$ obtained by substituting the last line of expression 19 into the exact equation 1. (A) Steady-state currents as a function of the injection rate $kC_\infty/p$ for $R/a = 3$ and various $D = 4\pi aD/k = 0.25, 1.0, 10$. For $D = 10$, $N = 10$ and $N = 50$ are compared. (B) $J$ as a function of length $N$ for $D = 1, 10$ and $kC_\infty/p = 0.3, 1$.

The numerical solutions depicted in Fig. 1 show, that for even modest $N \geq 10$, the currents are accurately described by their asymptotic expressions Eq. 3. Therefore, we can very accurately solve for $kC(a)$ and steady-state ribosome currents by separately considering each phase and its associated asymptotic form of $J$.

First assume that the detachment rate $\beta \geq p/2$ and consider the maximal current (phase III in the TASEP) where $J = p/4$. This occurs when both $\alpha, \beta > p/2$. To determine the parameter regime in which $J = p/4$ holds, we solve for $C(a)$ and determine for what range of parameters $\alpha = kC(a) > p/2$. Using $J = p/4$ in Eq. 19, we find

\begin{equation}
\end{equation}
limit $kC_\infty/p \to 0$, the phase boundary straightens as in the standard TASEP and is approximately

$$\frac{\beta^*/p}{C_\infty} = \frac{kC_\infty}{p} \left[ 1 - \frac{(1 - \alpha/R)k}{4\pi aDR} + O(k^2) \right].$$  \quad (25)

Finally, when $\beta > \beta^*(k)$, but the entrance rate $kC(a)$ is low ($< p/2$), a low density phase with $J = \alpha(1 - \alpha/p) = kC(a)(1 - kC(a)/p)$ exists. The phase boundary delineating the low density phase (I) is defined by $k < k^*$ and $\beta = \beta^*(k)$. Upon using the current $J = kC(a)(1 - kC(a)/p)$ in Eq. 19, we find $kC(a) = \beta^*$, and the current in the initiation rate-limited phase (I):

$$J_L = \frac{p}{2\pi aD} (\beta + 1) \left( \frac{R}{a} (\beta + 1) - 1 \right) \times \left[ \sqrt{1 + \frac{4(R/a)DkC_\infty}{p((\beta + 1)R/a - 1)^2}} - 1 \right] - 4\pi aDkC_\infty.$$  \quad (26)

In the limit $p/(kC_\infty) \to \infty$,

$$J_L(p \to \infty) = \frac{4\pi aDkC_\infty}{4\pi aDkC_\infty(k(1 - a/R) + k(1 - a/R) + 4\pi aD)^3} \left( \frac{kC_\infty}{p} \right) + O(p^{-2}),$$  \quad (27)

which reduces to the result one would expect from infinitely fast initiation site clearance.

Summarizing, the large-$N$ steady-state ribosome currents (given by Eq. 1) in terms of ribosome concentrations and kinetic “on” rates are

(I) $k < k^*, \beta > kC(a)$ \quad $J \equiv J_L = kC(a)(1 - kC(a)/p)$

(II) $\beta < \frac{p}{2}, \beta < kC(a)$ \quad $J \equiv J_R = \beta(1 - \frac{\beta}{p})$

(III) $k > k^*, \beta \geq \frac{p}{2}$ \quad $J \equiv J_{\max} = \frac{p}{4}$,  \quad (28)

where $kC(a)$ in phase (I) is expressed in terms of known parameters according to Eq. 19. The mean occupations of the initiation and termination sites, in each regime, can now be readily found. At the first site, $\sigma_1 = 1 - J/(kC(a))$, where we use $J = J_L, J_R, \beta(1 - \beta/p)$, or $p/4$ (currents associated with each phase), and $kC(a)$ found from Eqs. 19, 23, or 20. Similarly, the occupation at the last site is $\sigma_N = J/\beta$. All of our results can be expressed in terms of three of the four nondimensional parameters: $\tilde{D} = 4\pi aD/k$, $kC_\infty/p$, $4\pi aDkC_\infty/p$, and $R/a$. We shall present our results in terms of the relevant nondimensional parameters appropriate for the discussion at hand.

Figure 7A shows the critical value $k^*$, above which an $N \to \infty$ TASEP is in the maximal current phase (provided $\beta/p > 1/2$). When $C_\infty$ is small and $p$ is large, there is not enough ribosome in the cytoplasm to feed the initiation fast enough compared to the clearance rate $p$. Therefore the maximal current ($J = p/4$) arises only when the binding is efficient and $k > k^*$ is large. For smaller $R$ (termination site close to the initiation site), smaller values of $4\pi aDkC_\infty/p$ can still support maximal current. From Eq. 22, we see that when $4\pi aDkC_\infty/p \leq (1 - \alpha/(2R))/2$, the critical value $k^*$ diverges and the maximal current can never be reached. There is simply not enough ribosomes or the diffusion is too slow for there to be sufficient concentration at the initiation site to support the maximal current phase.

If the diffusion constant $D$ and $C_\infty$ are chosen such
that, for example, $4\pi a DC_{\infty}/p$ is small, the critical values $k^*$ vary considerably with $R/a$, as shown by the green points ($4\pi a DC_{\infty}/p = 1/2$) in Fig. 7A. The effects of depletion arise suddenly, with onset only at values of $4\pi a DC_{\infty}/p \lesssim 0.6$. For large $R/a$, values of $4\pi a DC_{\infty}/p \sim 0.5$ will render the critical $k^*$ values very sensitive to $R$. If the initiation site has an interaction size of $a \sim 10$nm, and $p \sim 2 - 3/s$ (20-30 codons/s) [Kruger et al. 1998], a diffusion constant of $D = 10^{-8}$ cm$^2$/s requires an effective concentration of $C_{\infty} \sim 0.01 - 0.02$µM for the phase diagram to be sensitive to diffusional depletion and $R$. Although typical total cytoplasmic ribosome concentrations are $C_{\infty} \sim 1$µM, many components must assemble in order to activate a translation-viable ribosome. For example, eIF4F exists at 0.01-0.2 times the total ribosome concentration [Duncan et al. 1987]. Furthermore, this already low abundance of eIF often needs to be further phosphorylated to be active. Thus, the effective concentrations $C_{\infty}$ (and even diffusion constants) appropriate for our model may very well be low enough to fall within the range for the phase boundaries to be extremely sensitive to diffusional effects.

Figures 7B, C show the steady-state phase diagrams as functions of $\beta/p$ and effective binding rate $kC_{\infty}/p$. In these phase diagrams, as in the unperturbed ones defined by Eq. 3, the upper left region corresponds to a low density phase, the lower right region corresponds to a high density phase, and the upper right region describes a half-occupied (except near the ends $i = 1, N$) maximal current phase. The current $J$ is constant throughout the maximal current phase and is not changed if $kC_{\infty}/p$ or $\beta$ is increased beyond $k^* C_{\infty}/p$ and 1/2, respectively. The phase diagram is modified by ribosome diffusion and depletion near the initiation site. The unmodified phase boundary between phases (I) and (II) of the TASEP (Eq. 3) would simply be defined by the straight line segment $\beta/p = kC_{\infty}/p$. The main effects of diffusional depletion (by the initiation sink) and replenishment (by the termination source) on the standard phase diagram Fig. 3 is to shift the low density-maximal current phase boundary to larger effective injection rates $kC_{\infty}/p$ and bend the low density-high density phase boundaries accordingly. Figure 7B depicts the phase boundaries defined by Eqs. 22 and 24 for fixed $R/a = 3/2, 4, 10, \infty$, and fixed $4\pi a DC_{\infty}/p = 1/2$ as indicated by the green points in Fig. 7A. In this example $k^* C_{\infty}/p = 3/2, 4, 10$ for $R/a = 3/2, 4, 10$, respectively. Note that for $R/a \to \infty$ that $k^*$ diverges and the maximal current phase is never attained. If $4\pi a DC_{\infty}/p < 1/2$, then there will be a finite value of $R/a$ such that $k^*$ diverges.

If, instead, $\bar{D} = 4\pi a D/k$ is held fixed, the phase boundaries are nearly straight, as shown in Fig. 7C. Here, we fixed $R/a = 10$, and plotted the phase diagrams for $\bar{D} = 0.05, 0.1, 0.5, 3$. The corresponding values of $kC_{\infty}/p$ above which the maximal current phase is attained are $k^* C_{\infty}/p = (1/2)(1 + (1 - a/(2R))/D = 10, 21/4, 29/20, and 79/120, respectively.

Our results up to this point are contingent on the fact that measurements are averaged over time scales such that the TASEP and the diffusion processes have reached steady-state, and the mRNA chain distribution has thermally equilibrated. The possibility exists that the chain conformations are not in thermodynamic equilibrium while the TASEP and the bulk ribosome diffusion has reached steady-state for a given chain conformation. Thus, although not relevant within each of the three well-defined physical processes, the issue of kinetic versus thermodynamic control of ribosome throughput arises when one considers measurements over time scales that are insufficient to allow equilibration of the mRNA chain. The consequences of this are discussed in the following section.

**EXPERIMENTAL CONSEQUENCES AND PROPOSED MEASUREMENTS**

The basic physical mechanisms described in our model for mRNA translation suggest a number of experimental tests. However, it must be emphasized that the model is meant to provide qualitative guidelines most useful for studying trends and how they depend on physical parameters. Translation occurring in vivo involve too many molecular species and biochemical processes to be quantitatively modeled, especially in the absence of significantly more detailed experimental findings. Nonetheless, our proposed mechanisms can be probed with carefully designed, simplified, in vitro experiments. Here, we discuss in detail the basic expected phenomena and their regimes of validity.

First note from Figure 6 and from Appendix D that the exact currents for a finite number of codons $N$ very rapidly approach the asymptotic values given by Eq. 3 as $N$ increases. Even when $N$ is only $\sim 10 - 50$, the steady-state ribosome currents are only a few percent off the exact $N = \infty$ results. In other words, the exact solution Eq. 1 is a very good approximation to Eq. 3 for $N \gtrsim 10$. Therefore, as a mental guide, it is typically sufficient to consider the currents $J$ corresponding to an infinite chain ($N = \infty$) given by Eq. 3, but nonetheless consider a finite initiation-termination separation (measured by the harmonic distance $R$).

**Polysomal Density Variations**

Although we have focused on the steady-state current, the particle (ribosome) densities in each of the three current regimes are different and may be detected. In the TASEP model, the ribosome density profiles along the mRNA chain vary only near the initiation and termination ends. In the interior of the mRNA, the density is relatively uniform and are given by the last column in Eqs. 3. In the exit-rate limited phase (small $\beta/p$), where $J = \beta (1 - \beta/p)$, the midpoint density $\sigma_{N/2} \sim 1 - \beta/p$ is high, while in the low injection rate case, $J = \alpha(1 - \alpha/p)$, and $\sigma_{N/2} \sim \alpha/p$ is low. The typical density in the maximal current regime is $\sigma \sim 1/2$. These densities are also
approximately correct when one explicitly treats large ribosomes that occlude many codon “lattice sites.” Therefore, we might expect that one may be able to predict in which current regime translating mRNA exists if ribosome densities can be estimated from images taken with e.g. AFM or EM techniques. For example, in Figure 1A, the high density of ribosomes suggests that the system is in phase (II) where the steady-state current $J = \beta(1 - \beta/p)$ is a function only of the detachment rate $\beta$.

Kinetic Binding Rate and Ribosome Concentration Dependences

Figure 7C shows the minimum effective attachment rate $k^* C_{\infty}/p$ necessary for a large system to be in the maximal current regime (where the ribosome current $J \approx p/4$) as a function of the effective ribosome diffusion constant. An additional requirement is that the effective detachment rate $\beta/p > 1/2$. The value of $k^*$ can be tuned perhaps by substitution of the codons comprising the initiation sites, or by other physical means. Although ribosome diffusion constants are difficult to vary over a wide range (by modifying the solution viscosity), the critical $k^*$ is a very sensitive function of $D$, particularly for small $D$. It is thus possible that slightly increasing the ribosome diffusivity can dramatically decrease the $k^*$ necessary for the system to be in the maximal current regime.

As mentioned, changing the mRNA length $N$ does not significantly affect the overall steady-state current along the chain (beyond about $N \approx 10 - 20$) but it can change the statistics of the initiation-termination separation by changing $R$. Increasing the harmonic separation $R$ has qualitatively the same effect as decreasing the ribosome diffusivity, since terminated ribosomes now have further to diffuse back to the initiation site. For

$$D < \frac{p(1 - a/(2R))}{8\pi a C_{\infty}},$$

(29)

the maximal current regime is never reached. This can be easily seen from equation 22. Thus, rather than tuning the ribosome diffusivity, decreasing $C_{\infty}$ may preclude the system from entering the maximal current phase if Eq. 29 is satisfied. There is simply not enough ribosome available for sufficient initiation to be achieved so that the maximal current phase arises.

When inequality 29 is not satisfied, the maximal current phase can exist. In Figure 8A, we replotted the phase diagram corresponding to $R/a = 10$ shown in Fig. 7. Fixing the parameter $4\pi a D C_{\infty}/p = 0.6$ allows $k$ to be the only free parameter. This kinetic “on” rate $k$ can be tuned by varying ribosome recruitment proteins such as eIF4E. If $\beta/p > 1/2$, $C_{\infty}, D$, and $p$ are held constant, increasing $k$ from a sufficiently small value allows one to traverse the trajectory $S_1$. The steady-state ribosome current starts in the low density phase (I) with current given by Eq. 26. As $k$ is increased, the steady-state current increases until it continuously crosses over into the maximal current regime (III), where the ribosome throughput is given by $J = p/4$. Further increasing $k$ when inside the maximal current phase (III) will no longer affect the steady-state ribosome current. If, however, $\beta/p < 1/2$, the current behavior abruptly crosses over (along trajectory $S_2$) from that given by Eq. 26 to $J = \beta(1 - \beta/p)$ corresponding to the high ribosome density phase (II). In this phase the detachment step is rate-limiting, and further increases in $k$ will no longer affect the throughput.

If $k$ is held fixed and the ribosome concentration is independently varied instead, it is more instructive to plot the phase diagram for fixed $D = 4\pi a D/k$ and $R/a$, as shown in Fig. 8B. Here, we choose the representative values $R/a = 10$ and $D = 4\pi a D/k = 0.25$ and motivate parameter trajectories obtained by varying only $C_{\infty}$. For $\beta/p > 1/2$, increasing the bulk ribosome concentration traces out the trajectory $S_3$ continuously from the low density phase (I) (Eq. 26) to the maximal current ($J = p/4$) phase. Further increasing the concentration well into the maximal current phase will no longer affect the throughput. Similarly, if $\beta/p < 1/2$, increasing $C_{\infty}$ can shift the behavior from that of the low density phase to that of the high density, exit rate-limited phase. Alternatively, one may vary $p$, the mean elongation rate of individual ribosomes, by controlling the tRNA concentration in solution. For example, decreasing available tRNA will move the system from the lower left to upper right in Fig. 8B, eventually reaching a steady-state current $J = p/4$.

Despite the apparent fundamental importance of the
kinetic binding, or “on” rate in translation, there are no systematic and independent measurements of \( k \) in the literature. The required independent estimates of \( k \) may be achieved by perhaps combined kinetic and affinity measurements of the association of a minimal set of components, including only the ribosomes and a portion of the 5' initiation codons and cofactors. For the off rate \( \beta \), similar ideas can be employed. The tRNA or ribosome release factor concentrations for the last codon can also be adjusted to tune the off rate \( \beta \).

**Codon and UTR Length Dependences**

In experiments where it is possible to vary the number of codons \( N \), the typical harmonic distance \( R \) can also be tuned. The phase diagrams in Figs. 3, 7, and 8 all correspond to different regimes of Eq. 1 in the large \( N \) limit. In practice, Eq. 1, is no longer sensitive to \( N \) for \( N \gg 10 \); however, the harmonic distance \( R \) between initiation and termination sites continues to increase as \( \sqrt{N} \), affecting the local concentration \( C(a) \), and thus the effective parameter \( \alpha = kC(a) \) in Eq. 1. As shown in Fig. 7C, increasing \( R/a \) shifts the phase boundaries to the right, making the maximal current phase (III) harder to attain unless \( k \) or \( C_{\infty} \) is concomitantly increased. However, due to the \( \sqrt{N} \) dependence, this effect would be relatively weak for all but enormous values of \( N \). Hence we have chosen the qualitatively reasonable value \( R/a = 10 \) in Figs. 8A, B.

Although there may be a weak increase in \( R/a \) as one increases the mRNA length, the effects of increasing the coded sections (\( N \)) or the noncoded sections (the untranslated regions \( m,n \)), can be different depending on \( U_0 \). For large \( U_0 \), looped configurations dominate and the distance between initiation and termination sites will be more sensitive to \( m+n \), the shortest distance between them (cf. Fig. 4B). The effect of lengthening \( m+n \) on \( R/a \) in the high \( U_0 \) regime is clearly shown in Fig. 5A. For small \( U_0 \), open configurations dominate and the short segments \( m \) and \( n \) at the two ends do very little to affect \( R/a \) relative to \( N \). Thus, although length dependences are expected to be weak, increasing the codon length \( N \) would more likely increase \( R/a \) (and hence decrease throughput \( J \)) in the small \( U_0 \), or repulsive limit. Conversely, increasing \( m,n \) would more likely increase \( R/a \) when \( U_0 \) is large and loops dominate the mRNA conformations.

**Initiation-Termination Cooperative Effects**

We have so far considered only the effects of the binding energy \( U_0 \) on loop formation, \( 1/R \), and the resulting local ribosome concentration at the initiation site. However, evidence suggest that contact between elongation factor proteins and/or poly(A) tail proteins can enhance or suppress the kinetic binding rates \( k \) through direct molecular contact and cooperativity [Jackson 1996, Munroe & Jacobson 1990, Sachs 1997, Sachs 2000]. There is the possibility that in looped states, PAB’s can interact with \textit{initiation} machinery and modify \( k \), and/or elongation factors can assist or hinder detachment of ribosomes at \textit{termination}. Modification of \( k \) and/or \( \beta \) through direct contact between proteins associated near the initiation and termination sites may be an additional mechanism by which translation rates can span the regimes shown in Figs. 7B, C and 8. Qualitatively, the experimental finding that contact between the mRNA ends affect the initiation or possibly termination processes can be modeled by assuming effective “on” or “off” rates

\[
k_{\text{eff}}[U_0] = k_0(1 - P_{\text{loop}}) + k_1P_{\text{loop}}
\]

\[
\beta_{\text{eff}}[U_0] = \beta_0(1 - P_{\text{loop}}) + \beta_1P_{\text{loop}},
\]

where \( k_0, \beta_0 \) and \( \beta_1, k_1 \) are the binding and “off” rates when the mRNA is open and looped, respectively. As \( U_0 \) is varied, both the intrinsic rates as well as the sink-source separation \( R \) are modified. Using expression 30 for \( k \) and \( \beta \) in equations 22 and 24, the dependence of \( J \) on the binding energy \( U_0 \) can be mapped. A number of qualitatively different scenarios are possible. If \( \beta_0 = \beta_1 \) but \( k_1 > k_0 \), the current is a monotonically increasing function of \( U_0 \) because the binding rate increases and the ribosome source (3’ terminus) is brought closer. Both of these effects monotonically increase the steady-state current. However, if for fixed \( \beta \), \( k_1 < k_0 \), then these two effects partially balance each other and there is the possibility of a maximum in \( J(U_0) \). A maximum occurs when initially, as \( U_0 \) is increased, the decrement in \( k_{\text{eff}} \) cannot keep up with the enhancement in local ribosome concentration due to the increasing likelihood of loop formation (i.e., the shifting of the high current phase boundary to lower \( k_{\text{eff}} \)). However, if \( k_1 \) is sufficiently small, \( k_{\text{eff}} \) eventually diminishes, such that one arrives at the low density, low current regime. These effects are illustrated in the sequence of figures 9A–C. The steady-state current, self-consistently calculated from Eqs. 1, 19, and 30, has a possible maximum and is shown as a function of \( U_0 \) in figure 9D. Here, we have chosen \( k_0C_{\infty}/p = 50, k_1C_{\infty}/p = 0.3, \beta = 0.75, N = 100, m = m = 30, \varepsilon = 0.2, a = 1, \) and \( \delta = 0.1 \). Only certain sets of parameters permit a maximum. Small values of \( 4\pi aD_{\infty}/p \) and large \( N \) result in the largest maxima. For large values of \( 4\pi aD_{\infty}/p \), diffusion is fast, local ribosome concentrations are not significantly depleted by the initiation site, and the high current regime is already pushed to low values of \( kC_{\infty}/p \). Therefore, increasing \( U_0 \) and decreasing \( R \) does not further drive the high current regime towards significantly lower \( kC_{\infty}/p \). For essentially the same reason, smaller \( N \) enhance ribosome recycling, increasing the current at low \( U_0 \), thereby rendering the maximum in \( J \) to lower values of \( U_0 \). As illustrated in the exampled given in figure 9D, increases of \( 50 – 60\% \) above the background current are possible as \( U_0 \) is varied. Thus, we see that the two processes, direct molecular catalysis of initiation and termination, and ribosome diffusional depletion, balance each other and may provide delicate control mechanisms during later stages of gene regulation.
a changing phase diagram. As $U$ show hypothetical, qualitative trajectories in the presence of $k$dition, if approximation is used with persistence length $\pi a DC_{\infty}$ decreases with increasing $k$. FIG. 9: The current (Eq. 1) as a function of $U_0$ only for small $4\pi a DC_{\infty}$ equilibrium (in the presence of loop-forming proteins), a state before the mRNA chain reaches conformational polymer statistics). Since it is possible for diffusion and thermodynamic equilibrium (for the statistics of the individual ribosome movement along the mRNA) can be used to independently determine the distribution of times the mRNA chain is looped or unlooped [Goddard et al. 2000]. Only when $U_0$ or $U^*$ are large does ribosome recycling get significantly enhanced by loop formation. Transient measurements, as well as fluctuations of the measured throughput, is beyond the scope of the paper.

**SUMMARY**

We have constructed a simple model and road map for the possible physical effects at play during translation. The model incorporates driven diffusive motion which obeys exclusion statistics for ribosomes along mRNA. The initiation and termination sites are considered as sinks and sources of ribosome concentration, described by the steady-state diffusion equation (Laplace’s equation). The average conformations of the mRNA chain define the typical initiation-termination distance which determines the how terminated ribosomes directly diffuse back to the initiation site and affect the local concentration there. This local concentration is a parameter (the injection rate) in the exclusion process, but also depends on the overall ribosome throughput (the strength of the sink and source). Thus, the current $J$ needs to be solved self-consistently. Direct cooperative enhancement of kinetic binding and “off” rates were also incorporated. Although it is thought that the rate-limiting step is binding and initiation of ribosomes at the initiation site [Clemens 1996, Mathews et al. 1996], the fact that polysomes have been found to exist in both high and low ribosome occupancy states suggests that under physiological conditions, steady-state ribosome fluxes can span the regimes defined by the phase diagrams depicted Figs. 3 and 7B, C. At high occupancy, the rate limit-
ing step is the off rate $\beta$ which controls the steady-state flux (cf. Phase (II) in Fig. 3). Ribosome depletion by the sink and replenishment by the source can drastically affect the constant $k_2$ phase diagram, as shown in Figures 7. The critical values of $k_2 C'_\infty/p$ that define the the left boundary of the maximal current phase (in the $N \to \infty$ limit) is most sensitive to the dimensionless parameter $4\pi a DC'_\infty/p$ when $4\pi a DC'_\infty/p \approx 0.15 - 0.3$. For sufficiently small $4\pi a DC'_\infty/p$, the effective injection rate cannot reach 1/2 and the maximal current phase cannot be attained. When $N \neq \infty$, the explicit currents were computed from Eq. 1 and plotted in Figure 6. Given the possibility of cooperative interactions in looped mRNA configurations, we have also found a maximum in ribosome throughput as a function of loop-binding energy $U_0$.

Many molecular and chemical details have been neglected. As mentioned, we have ignored the fact that numerous components must assemble before initiation and have modelled only an “effective” rate-limiting component. The surface concentration parameter $C(a)$ in our model would be an effective concentration reflecting the total density of ribosomes capable of initiation. Proposed mechanisms of ribosome scanning [Jackson 1996], whereby ribosomes attach to segments of mRNA and undergo one-dimensional diffusion before encountering the initiation site, can be adequately modeled with the present approach if one assumes that the rate-limiting step is initial adsorption onto an mRNA segment. Furthermore, we have assumed that the ribosomes do not detach from the mRNA until they reach the termination site and that their forward hopping rates are uniform across the whole coding region. Finally, in our simple polymer model, we have neglected both self-avoidance (of both chain-chain and chain-ribosome exclusion) and the fact that the effective persistence length may vary along the mRNA, depending on the local ribosome density.

Despite these simplifying assumptions, we find that qualitatively, subtle control mechanisms can come into play, depending on biologically reasonable physical parameters. Although there are numerous experiments probing translation, both in vivo and in vitro, many different systems and physical conditions are employed, rendering quantitative comparison with measurements difficult. Nonetheless, our model suggests new measurements that can be used to qualitatively probe the various physical hypotheses and exhibit our predicted physical trends. For example, the effective $C'_\infty$ can be varied in a number of ways to test with the predicted current regimes. Occupancy along the mRNA can also be correlated with the high, low, and intermediate density phases. Additionally, the noncoding regions between the elongation factors and the initiation site, and the termination site and the poly(A) tail-bound PAB can be varied to test possible cooperative interactions defined by Eq. 30. Since the loop formation probability $P_{\text{loop}}$ depends on the total statistical length $L_T$, which is dominated by the length of the coding region ($L_N a^2 \gg (m + n)\varepsilon^2$), varying $m$ and $n$ would affect, through the likelihood of molecular contact in the looped states, only $k_{\text{eff}}$ and $\beta_{\text{eff}}$, respectively. The actual probability of loop formation $P_{\text{loop}}$, and hence $R$, would not be significantly affected. Chemical modification of the elongation factors or the PAB’s would affect $U_0$, and hence $k_{\text{eff}}$, $\beta_{\text{eff}}$, and $R$ through $P_{\text{loop}}$. Using micromanipulation techniques [Bustamante et al. 2000], it might also be possible to fix the initiation-termination distance in vitro.

Numerous extensions to the presented models can be straightforwardly incorporated to more precisely model the chemical and microphysical processes. Codon and tRNA concentration-dependent variations in the internal transition rates $p$ [Kruger et al. 1998], as well as random detachment processes, can be implemented using simple lattice simulations. Sites along the mRNA chain at which ribosomes pause can be treated as “defects” in a TASEP and the whole process can be treated with mean-field theory [Kolomeisky 1988]. Multiple coding regions in prokaryotic translation (Shine-Dalgarno sequences) can be modeled as a sequence of initiation (sinks) and termination (sources) sites. Similarly, cap-independent initiation at internal ribosome entry sites [Jackson 1996, Martínez-Salas et al. 2001, Sachs 1997] (IRES) can also be treated as sinks within our basic model. Translation of ER-associated mRNA further involve ribosomes that attach the mRNA at certain points on the ER membrane. In this case, one expects that density of cytoplasmic and ER-bound ribosomes to have a strong effect on localization of mRNA to ER and overall translation rates. One can also consider cases where the protein product itself is a ribosome product necessary for its self-translation; these processes would result in initially autocatalytic protein production. Although these more complicated and interesting extensions have not been considered here, the simple models we have presented represent a first step towards the rich problem of identifying and quantifying the physical and biological mechanisms that control late stages of expression.

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APPENDIX A: PHYSICAL ASSUMPTIONS AND MATHEMATICAL APPROXIMATIONS

Although our model arrives at a number of conclusions that are developed by combining three different physical theories, the assumptions and approximations used in each are well developed in the condensed matter physics and biophysics literature. Here, we summarize the main
physical assumptions and review the mathematical approximations used:

- **Steady-state and equilibrium assumptions**: Ribosome diffusion and motion along the mRNA are treated within steady state, while the configurational distribution of the mRNA polymer is not directly coupled to ribosome diffusion or motion, and is considered in thermodynamic equilibrium. The inverse “harmonic distance” $1/R$ is determined from equilibrium mRNA configurational distributions, but parametrically influence the nonequilibrium steady-state processes of diffusion and the TASEP. Equilibration times of unentangled polymers and diffusion times over the length of the mRNA are on the order of milliseconds to seconds, while the relaxation to steady-states in the TASEP occur over seconds to on the order of a couple minutes. Thus, on experimental time scales longer than these, transients in the ribosome throughput have dissipated, and the steady-state and equilibrium assumptions are appropriate.

One might be tempted to formulate the specific mechanisms in terms of the common notions of reactions being kinetically or thermodynamically controlled. In this biochemical terminology, the TASEP is kinetically controlled, since the ribosomes take irreversible steps as each amino acid is added during elongation. The mRNA configurations, computed under equilibrium conditions, are by definition thermodynamically controlled. However, each of the proposed mechanisms is a simple, single, independent process, the notion of kinetic control versus thermodynamic control is irrelevant. Within each mechanism, there are no alternate “reaction paths” or outcomes for kinetic or thermodynamic control to apply. However, it is possible that the mRNA conformations and the binding protein-mediated loop formation does not reach equilibrium on the time scale of measurements of ribosome throughput. This possibility is also discussed in the Experimental Consequences and Proposed Measurements section.

- **Gaussian chain polymer model for mRNA**: Unlike tRNA, the coding regions of mRNA is relatively devoid of secondary structure. The single-stranded mRNA is treated using standard statistical physics of polymers that assumes nonintersecting random walks of step size defined by the polymer persistence length. For single-stranded mRNA without adsorbed proteins, the persistence length $\approx 2 - 3$ bases. When loaded with large ribosomes, we assume that the persistence length is on the order of the ribosome size and that it is approximately uniform along the chain. Although the ribosome loading might varying slightly along the chain, this variation occurs only near the ends and does not appreciably affect the equilibrium end-to-end distributions. Although we treat only phantom (nonintersecting) polymers, effects due to the binding of finite-sized PABs and cap proteins are explicitly treated when computing the end-to-end distribution functions in the small distance regime where steric exclusion of the end proteins are important.

- **Single component “ribosomes”**: The assembly of ribosomes before or during adsorption onto the initiation site can be modeled as an effectively single, rate-limiting component that undergoes standard diffusion in the bulk solution. Including more chemical details will not qualitatively alter our results, since in diffusive steady-state, all species’ concentrations would be spatially distributed as $1/R$ and parametrically affect the TASEP in the same qualitative manner.

- **Equal particle and step sizes**: Ribosomes moving along mRNA are treated with a discrete TASEP where the step size is exactly equal to the particle diameter. However, ribosomes are large and occlude $\sim 10$ codons so that they move one particle diameter only after about $q = 10$ steps (amino acid transfers). Nonetheless, the qualitative behavior of the currents for different $q$ remain unchanged. For the sake of simplicity and clear analytic expressions (Eqs. 22, 24, and 26), we have restricted our analysis to $q = 1$. Exact large $N$ asymptotic expressions for the steady state current for general $q$ are given in Appendix D.

- **Uniform elongation step rates in the TASEP**: The analytic solutions represented by Eqs. 1, 2, and 3 are based on uniform elongation rates $p$ along the mRNA. It is known that $p$ can vary by factors of 2-10 [Kruger et al. 1998], depending on the codon in question and the availability of the associated tRNA. As a first step, we have simply assumed a scenario in which the elongation rates do not vary appreciably over the coding region. More elaborate models that include specified elongation rates $p_q$ across the mRNA chain would require extensive simulations for each realization of $\{p_i\}$.

- **Bulk diffusion limited adsorption**: Ribosomes, or the relevant rate-limiting component of a ribosome, diffuses in bulk and directly attaches to the initiation site. Capture of the ribosome by the initiation end of the mRNA may occur in a two-step process of non-specific adsorption from bulk, followed by linear diffusion along a segment of the mRNA, before ultimately interacting specifically with the initiation site [von Hippel & Berg, Stanford et al. 2000]. Although studied in the context of linear diffusion and search along DNA [Berg & Purcell 1977], direct evidence for such scanning mechanisms in the initiation of mRNA translation has been hard to obtain [Jackson 1996]. For example, secondary structure in the form of small mRNA knots near the 5’ region must be melted before efficient ribosome scanning can occur [Kozak 1989]. Nevertheless, one-dimensional diffusion of ribosomes along the mRNA near the initiation sight is implicitly included in our model. The conjectured scanning mechanisms suggest that ribosomes scan locally near the start codon [Jackson 1996, Wang et al. 1997]. Thus, if ribosome recycling via diffusion through the bulk is rate-limiting, the scanning region near the initiation where the linear diffusion occurs can be considered as binding region of larger effective capture radius $a$. 
APPENDIX B: MEAN FIELD ANALYSIS FOR LARGE PARTICLES

Consider identical particles that are driven through a long one-dimensional lattice of $L$ sites. The lattice is discretized into steps of unit length (a step size corresponding to a codon step), while the particles are of integer size $q \geq 1$. For each particle to move a distance roughly equal to its diameter, $q$ consecutive steps must be taken. Thus, we expect that effectively, the mean current would be approximately described by equations 1 or 3 but with $p$ replaced by $p/q$. A mean field model for the asymmetric exclusion process containing particles that occupy $q$ substrate lattice sites (mRNA codons) has been solved. The analysis is beyond the scope of this paper, but the resulting steady-state currents follow the same qualitative “phase diagram” (Fig. 3) as the TASEP with particles of size $q = 1$. That is, for large entrance and exit rates, there is a maximal current phase (III), bounded by low (I) and high (II) density phases. The effects of increasing the particle size to $q > 1$ only quantitatively changes the values of the currents in each of these phases, and can be straightforwardly integrated into the present study.

The general (for all particle sizes $q$) result for the steady-state currents in the infinite chain length limit are

(I) $\alpha < \frac{p}{2}, \beta < \alpha \quad J \equiv J_L = \frac{\alpha(1 - \alpha/p)}{1 + (q - 1)\alpha/p}$

(II) $\beta < \frac{p}{2}, \beta < \alpha \quad J \equiv J_R = \frac{\beta(1 - \beta/p)}{1 + (q - 1)\beta/p}$ (B1)

(III) $\alpha, \beta \geq \frac{p}{2} \quad J \equiv J_{\text{max}} = \frac{p}{(\sqrt{q} + 1)^2}$

These results have been verified to be exact (to within numerical precision) by extensive Monte-Carlo simulations. Note that for large $q$, the maximal current $J_{\text{max}}$ is that given by Eq. 3 but with $p \rightarrow p/q$. These results only serve to quantitatively shift the phase boundaries between the different current regimes and decrease the magnitude of the currents. For example, if $q = 2, 3$, the phase boundary between the low density and the maximal current regime occurs at $\alpha/p = 0.41, 0.37$, respectively, rather than at 0.5. For the sake of simplicity and manageable algebraic expressions, we have in this study only considered the $q = 1$ case. Our analysis should be applied to the mRNA translation problem with the understanding that $p$ in Eq. 3 and subsequent equations is roughly the rate for a ribosome to move its molecular size, not the rate for an individual tRNA transfer. If, however, the above expressions were used, then $p$ in expressions B1 would be identified with the typical single amino acid transfer rate.

FIG. 10: Schematic of the geometry near the initiation-termination end of a looped mRNA. The mRNA loop binding factors are shown in yellow and black, while a ribosome of radius $a$ is situated at the initiation site (not drawn to scale). $m$ and $n$ correspond to the number of bases of the UTR’s which are assumed to be relatively protein-free and have short persistence length $\varepsilon$. Here, the persistence lengths in the coding regions (thick curve, described by the TASEP) is $\ell \sim a$.

APPENDIX C: OPEN CHAIN PROBABILITY DISTRIBUTIONS

Consider the probability distribution $W(r|\text{open})$ of the initiation-termination separation in the absence of loop formation. Since the ribosome can be much larger than the typical persistence length in the noncoding region of single-stranded mRNA, $a \gg \varepsilon$. For $a \sim 10\varepsilon$, $a \ll L_{mn}$, unless the noncoding regions are very long, with $m + n \gg 100$. For shorter noncoding regions, the expression for $W(r; L_{mn}|\text{open})$ must be evaluated more carefully, particularly for small $r$, in order to compute $\int dr W(r)/r$ correctly. Assume the termination site starts a random walk from any position on the sphere. Details of the different segments of mRNA are shown in figure 10. The problem maps to that of heat diffusion from a sphere of size $a$ with reflecting boundary conditions and an instantaneous uniform temperature source on the surface. The probability that the initiation site (that is linked to the termination site via $m + n$ persistence lengths) is within $r$ of the sphere can also be described by the temperature near a sphere with an exterior instantaneous source of temperature. The diffusion equation for the probability distribution $W(r; L_{mn}|\text{open}) \equiv \hat{W}$ obeys

$$\hat{W}(r, t) = \kappa \Delta \hat{W}(r, t)$$ (C1)

where the thermal conductivity is associated with the squared persistence length, $\kappa \equiv \varepsilon^2/6$, and time corresponds to the length $t \equiv m + n$. The initial and boundary conditions corresponding to a chain that originates
from the surface of the otherwise impenetrable ribosome particle are

\[ \partial_r W(r = a) = 0, \quad W(r, t = 0) = \frac{\delta(r - a)}{4\pi a^2}, \quad (C2) \]

where we have assumed spherical symmetry. Following Carslaw and Jaeger (1959), we define \( W(r, t) = f(r, t)/r \) to reduce (C1) to \( \dot{f}(r, t) = \partial_r^2 f(r, t) \), with boundary conditions

\[ \partial_r f(r = a) = \frac{1}{a} f(a), \quad f(r, t = 0) = \frac{r\delta(r - a)}{4\pi a^2}. \quad (C3) \]

The solution for \( f(r, t) \) is found using Laplace transforms, and is

\[ f(r, t) = \frac{1}{8\pi a \sqrt{\pi nt}} e^{-(r-a)^2/(4\kappa t)} - \frac{e^{r/a-1} e^{\kappa t/a^2}}{4\pi a^2} \text{Erfc} \left[ \frac{r-a}{2\sqrt{\kappa t}} + \frac{\sqrt{\kappa t}}{a} \right]. \quad (C4) \]

The probability distribution is thus

\[ W(r, L|\text{open}) = \frac{\sqrt{3} e^{-3(r-a)^2/(2N\ell^2)}}{2(2\pi)^{3/2} \sqrt{\pi Na\ell}} - \frac{e^{r/a-1} e^{\kappa t/a^2}}{4\pi a^2} \text{Erfc} \left[ \frac{3}{2N} \left( \frac{r-a}{\ell} + \frac{\sqrt{N\ell}}{6a} \right) \right]. \quad (C5) \]

Note that if \( a/L \ll 1 \), as it is for \( L = L_N \), equation (C5) would be approximately

\[ W(r, L|\text{open}) \approx \left( \frac{3}{2\pi L^2} \right)^{3/2} e^{-3r^2/(2L^2)} \times \left[ 1 - 36\pi^2 \left( \frac{a^2}{L^2} \right) + O \left( \frac{a^4}{L^4} \right) \right], \quad (C6) \]

which reduces to end-to-end probability distribution for a Gaussian random chain. However, since \( a/L \ll 1 \), we need to use the full expression Eq. C5 for the loop contribution (Eq. 15) in the calculation of \( W_{\text{eff}}(r) \) and \( 1/R \).

For the WLC, an approximate probability distribution function can be reconstructed from commonly used phenomenological force-extension relationships. If the force-extension interpolation given by Marko and Siggia [Marko & Siggia 1995] is shifted to take into account the finite-sized origin,

\[ f(z) = \ell^{-1} \left[ \frac{1}{4 \left( 1 - \frac{z-a}{N\ell} \right)^2} + \frac{(z-a)}{N\ell} - \frac{1}{4} \right]. \quad (C7) \]

The initiation-termination distance distributions can be estimated using

\[ W_{WLC}(\text{open}|r) \approx \frac{\exp \left[ -\int_{a/N}^{a/N} f(z)dz \right]}{\int_a^{a/N} dr \exp \left[ -\int_{a/N}^r f(z)dz \right]}. \quad (C8) \]

This end-to-end probability distribution from both FJC and WLC models are plotted in Figs. 11A, B. The WLC model gives qualitatively similar distributions to those of the FJC model, provided the contour length is appropriately reduced. Furthermore, the WLC and FJC models provide qualitatively similar averages \( \langle a/r \rangle \) if the \( N \) used in the WLC is sufficiently reduced. Upon using Eqs. 15 and 16, one can compute the effective end-to-end distribution of a chain with segments of different persistence length and with attached loop binding proteins, as shown in Fig. 11C.

**APPENDIX D: ASYMPOTICS FOR \( J_N \)**

Asymptotic expressions for the steady-state current given by Derrida et al. [Derrida et al. 1993] are valid only far from the phase boundaries. However, in our present model, we are interested in how a change in the mRNA length \( N \) allows the system to cross over from one behavior to another. For the sake of completeness, we derive limiting forms for the current \( J_N \) near phase boundaries. An asymptotic expansion in the rates about \( \alpha = 1/2 \) is taken first, with \( N \) fixed. From the exact expression Eq. 2 given by [Derrida et al. 1993], we find the following asymptotic expansion

\[ S_N(x = 2) = \frac{4N}{\sqrt{\pi}} \frac{\Gamma(N+1/2)}{N!} \]

\[ \sim 4^N \frac{2}{\sqrt{N\pi}} \left[ 1 - \frac{1}{8N} + \frac{1}{128N^2} + O(N^{-3}) \right] \quad (D1) \]

For \( \beta > 1/2 \), and \( \alpha = 1/2 + \varepsilon \), we take the large \( N \) limit, but with \( \varepsilon \sqrt{N} \rightarrow 0 \). The resulting current across the maximal current-low density phase boundary is

\[ J \sim \frac{1}{4} \left[ 1 + \frac{1}{N} + \frac{\beta(\beta - 1)}{(2\beta - 1)^2 N^2} + O(N^{-3}) \right] + \frac{3\sqrt{\pi}}{32} \left[ \frac{52\beta^2 - 52\beta + 17}{8(2\beta - 1)^2 \sqrt{N}} + O(N^{-3/2}) \right] \varepsilon + O(\varepsilon^2). \quad (D2) \]
FIG. 11: (A) FJC and WLC models for $W(r|\text{open})$ for $\ell/a = 0.2$. The WLC distribution approximates that of the FJC if the effective number of persistence lengths $N$ is reduced. This reduction compensates for the stiffness of the chain that tends to give more weight at larger distances. (B) FJC and WLC distributions for $\ell/a = 1$. Note the heuristic cutoff applied to the WLC model at $r = a$. As expected, for equal $N$, the WLC model gives a typically larger separation and hence smaller $a/R$; however, $a/R \propto N^{-1/2}$ for $N \to \infty$ in all cases. (C) The effective end-to-end distance distribution $W_{\text{eff}}$ constructed from $W(r|\text{open})$ via equations 14 and 15.

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