Quantifying mRNA synthesis and decay rates using small RNAs

Vlad Elgart, Tao Jia, Rahul Kulkarni,
Department of Physics, Virginia Tech,
E-mail: elgart@vt.edu, kulkarni@vt.edu.

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Abstract

Regulation of mRNA decay is a critical component of global cellular adaptation to changing environments. The corresponding changes in mRNA lifetimes can be coordinated with changes in mRNA transcription rates to fine-tune gene expression. Current approaches for measuring mRNA lifetimes can give rise to secondary effects due to transcription inhibition and require separate experiments to estimate changes in mRNA transcription rates. Here, we propose an approach for simultaneous determination of changes in mRNA transcription rate and lifetime using regulatory small RNAs to control mRNA decay. We analyze a stochastic model for coupled degradation of mRNAs and sRNAs and derive exact results connecting RNA lifetimes and transcription rates to mean abundances. The results obtained show how steady-state measurements of RNA levels can be used to analyze factors and processes regulating changes in mRNA transcription and decay.

Cellular adaptation to changing conditions is critically dependent on processes that enable rapid responses to environmental fluctuations. While considerable research has focused on changes in transcription, research over the past several years has demonstrated that control of mRNA decay plays an increasingly important role in cellular responses [1, 2, 3]. Correspondingly, there is significant interest in understanding factors and processes which govern regulation of mRNA decay.

The traditional approach for measuring mRNA lifetimes involves quantification of mRNAs remaining at different times following inhibition of transcription, e.g., by the addition of rifampicin [2]. This procedure requires multiple measurements during time intervals of the order of the mRNA lifetime, hence high temporal resolution is required for short-lived mRNAs. More significantly, the procedure for inhibition of transcription can give rise to secondary effects which influence mRNA decay [2], hence it is of interest to consider alternative approaches. In the following, we outline a novel proposal for quantifying mRNA decay.
A naturally occurring process which regulates turnover for many bacterial mRNAs involves interactions with regulatory small RNAs (sRNAs). Experiments have shown that post-transcriptional regulation of gene expression can occur via binding and subsequent coupled degradation of the mRNA and regulatory sRNA which occurs rapidly [10, 17]. The corresponding coarse-grained model of gene expression has been analyzed by several groups [9, 8, 11, 12, 13] and shown to be consistent with experimental observations [9]. We will show that the kinetic scheme for this mode of regulation leads to an exact mathematical result relating RNA decay rates. The obtained result is valid even in the presence of large fluctuations that are typical for low abundance mRNAs.

Our analysis considers a generalized stochastic model for regulation by sRNAs as illustrated in Fig. 1. The complexity of processes leading to transcription suggests that RNA synthesis in many instances is not adequately modeled as a Poisson process [7, 14]. Hence, we model mRNA and sRNA production by arbitrary stochastic processes with mean arrival rates $k_m$ and $k_s$ respectively. Degradation of a mRNA and a sRNA can occur either independently (with constant probability per unit time $\frac{1}{\tau_m}$ and $\frac{1}{\tau_s}$ respectively) or through the coupled degradation process.

The experimental setup in our proposal for quantifying mRNA decay is as follows. Consider three different strains as shown in Fig. 1B: two unregulated strains (i.e., with either sRNA or mRNA deleted) and the wild type (WT) strain. In the WT strain, both mRNA and sRNA are present and regulate each other. In steady state, we derive the following exact relations (Appendix) connecting
mRNA/sRNA lifetimes and transcription rates to the mean abundances

\[ k_m \tau_m = \langle m \rangle \]
\[ k_s \tau_s = \langle s \rangle \]

for the unregulated strains, and

\[ \frac{\tau_m}{\tau_s} = \frac{\langle m \rangle - \langle \tilde{m} \rangle}{\langle s \rangle - \langle \tilde{s} \rangle}. \]

Here \( \langle m \rangle \) and \( \langle s \rangle \) are the mean mRNA(sRNA) abundances in strains lacking the sRNA(mRNA), and \( \langle \tilde{m} \rangle \) and \( \langle \tilde{s} \rangle \) are the mean mRNA and sRNA levels in WT strain where both are present.

The above relations suggest an alternative approach (Fig. 1B) for quantifying decay times for mRNAs that either have a naturally occurring small RNA regulator or for which an antisense RNA regulator can be designed. Consider an experimental setup expressing the sRNA from an inducible promoter such that its transcription rate is primarily controlled by inducer concentration. The mean transcription rate \( k_s \) can, in principle, be determined using single-molecule methods [18]. Now the basic parameters for the coupled system are \( k_m, k_s, \tau_m \) and \( \tau_s \). If \( k_s \) is known, then the values of the other parameters can be determined using experimental measurements of \( \langle m \rangle, \langle s \rangle, \langle \tilde{m} \rangle \) and \( \langle \tilde{s} \rangle \) in combination with equations Eqns. 1-3 above. Alternatively, experiments can be designed to keep \( k_s \) fixed while factors regulating mRNA decay are changed e.g., by deletion of a protein known to play a role in mRNA decay. The above equations can be used to simultaneously determine the corresponding fold-changes of the mRNA/sRNA lifetimes and the mean mRNA transcription rate.

The proposed approach can be used to address several important questions of current interest, some of which are highlighted in the following. By targeted mutagenesis of specific mRNA sequence elements, the induced fold-change in mRNA lifetime, as well as the corresponding change in the transcription rate \( k_m \), can be determined using the same experimental setup. This is an important feature, given that recent experiments have observed coordination between changes in transcription and changes in mRNA degradation [10]. Quantifying the change in mRNA lifetimes induced by mutations to different components of cellular degradation pathways can address such issues as the role of polyadenylation in mRNA decay [6]. It would also be of interest to design high-throughput experiments for different mRNAs which are regulated by corresponding antisense RNAs, all of which are expressed from identical inducible promoters and thus have the same \( k_s \). The proposed procedure can then be used for genome-wide determination of relative transcription rates and lifetimes of mRNAs. These effective parameters, in turn, serve as critical inputs to systems-level models of cellular processes [15].

In summary, we have proved an exact relation for a nonlinear stochastic model of cellular post-transcriptional regulation. The derived results suggest a novel procedure for simultaneous determination of mRNA production rates and mRNA lifetimes. While the focus was on bacterial mRNAs, the procedure
outlined can also be applied to higher organisms and used to systematically explore the sequence determinants and processes involved in regulation of mRNA decay.

Appendix

The proposed experimental setup involves measurements of mRNA/sRNA abundances for three strains, the sRNA deleted \( \Delta(sRNA) \) strain, mRNA deleted \( \Delta(mRNA) \) strain, and the wild type (WT) strain (both species are present and regulate each other.) The regulation model (with constant creation rates for mRNA and sRNA) has been analyzed by several groups [9, 11, 13] and is summarized in the reaction scheme described in Fig.1.

Recent experiments which provide evidence for transcriptional bursting [14] point towards the need to go beyond the Poisson process as a model for RNA synthesis. Accordingly, we take both mRNA and sRNA creation events as arbitrary stochastic processes. Degradation of RNA is assumed to be a Poisson process with rate \( \tau_x^{-1} \), where \( \tau_x \) is the mean lifetime of \( x \)RNA , \( x = \{ m, s \} \). In the WT strain, mRNA and sRNA undergo a coupled degradation process. We assume that this process is symmetric with respect to the number of mRNA and sRNA molecules involved, e.g., \( M + S \rightarrow \emptyset \).

Let us choose a particular realization of the system evolution during time interval \( t = [0, T] \). For large values of \( T \), we derive

\[
x(T) - x(0) = C_x(T) - Y(T) - \tau_x^{-1} \int_0^T dt x(t),
\]

where \( x(t) \) is the number of molecules of the species \( x = \{ m, s \} \) at the time \( t \).

In Eq.3 \( C_x(t) \) is the total number of molecules of the species \( x \) created during system evolution until time \( T \), and \( Y(T) \) is the total number of molecules of either species that is mutually degraded within the time interval \([0, T]\). Finally, using the law of large numbers, the number of molecules degraded naturally in \([0, T]\) is given by the last term in the Eq.3.

Dividing both sides of Eq.3 by \( T \) and taking a limit \( T \rightarrow \infty \) we obtain

\[
\lim_{T \rightarrow \infty} \frac{x(T) - x(0)}{T} = k_x - \tau_x^{-1} \langle \bar{x} \rangle - \lim_{T \rightarrow \infty} \frac{Y(T)}{T},
\]

where \( \langle \bar{x} \rangle \) is average number of molecules in the system. We also defined \( k_x \) as a mean arrival rate of the species \( x = \{ m, s \} \). The limit on the left hand side of Eq.4 vanishes in the case of finite degradation rates \( \tau_x^{-1} \) (number of molecules at any time is finite.) Note that \( Y(T) \) is an extensive quantity (it is monotonic increasing function of \( T \)) and therefore, the limit on the right hand side of Eq.4 is finite.
Hence, we derive

\[ k_m - \tau_m^{-1}\langle \tilde{m} \rangle - \lim_{T \to \infty} \frac{Y(T)}{T} = 0, \]

\[ k_s - \tau_s^{-1}\langle \tilde{s} \rangle - \lim_{T \to \infty} \frac{Y(T)}{T} = 0, \quad (5) \]

which immediately yields the following expression

\[ k_m - \tau_m^{-1}\langle \tilde{m} \rangle = k_s - \tau_s^{-1}\langle \tilde{s} \rangle. \quad (6) \]

In the unregulated case \( Y(T) \equiv 0 \) for any \( T \), since one of the RNA species is deleted and there is no coupled degradation. In this situation one gets

\[ 0 = k_m - \tau_m^{-1}\langle m \rangle, \quad 0 = k_s - \tau_s^{-1}\langle s \rangle, \quad (7) \]

where \( \langle x \rangle \), \( x = \{m, s\} \) are the average number of molecules during unregulated system evolution. Combining the set of equations above with Eq. (6), we derive the results in the main text Eqs 1-3. We note that the derived results are valid even if the binding of mRNA and sRNA is taken to be reversible and the lifetime of the mRNA-sRNA complex is finite. Finally, the time average can be replaced by the ensemble average in the steady state.

We have validated the derived results using stochastic simulations based on the Gillespie algorithm [4]. Production of RNA molecules was taken to occur in transcriptional bursts [14] i.e. each burst corresponds to the arrival of a random number of RNAs drawn from a geometric distribution, conditional on the production of at least 1 RNA molecule [5]. The waiting-time between bursts was a random variable drawn from exponential or Gamma distributions. As expected, the results from the simulations were in excellent agreement with the derived analytical results.

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