Modeling coupled transcription, translation and degradation and miRNA-based regulation of this process

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Introduction

The translation-transcription process with the description of the most basic "elementary" processes consists in:

- 1) production of mRNA molecules,
- 2) initiation of these molecules by circularization with help of initiation factors,
- 3) initiation of translation, recruiting the small ribosomal subunit
- 4) assembly of full ribosomes
- 5) elongation, i.e. movement of ribosomes along mRNA with production of protein
- 6) termination of translation
- 7) degradation of mRNA molecules

A certain complexity in the mathematical formulation of this process arises when one tries to take into account the phenomenon of polysome first described in (Warner et al, 1963), when several ribosomes are producing peptides on a single mRNA at the same time. This leads to multiplicity of possible states of mRNA with various numbers of ribosomes with potentially different dynamics, interaction between ribosomes and other difficulties. The process of translation is a subject of mathematical modeling since long time ago (e.g., see (Singh, 1996)). For a recent review of existing mathematical efforts in this direction, see (von der Haar, 2012).

In the following we start with a 1) **detailed mechanistic description** of the translation process with explicit representation of every state of translating mRNA, followed by 2) deriving the **simplest and basic ODE model** of coupled transcription, translation and degradation, and 3) developing a model suitable for describing all known **mechanisms of miRNA action** on translation.

The basic model is constructed by correct lumping of the detailed model states and by separating the description of ribosomal turnover. It remains linear under assumption of that the translation is not limited by availability of ribosomal subunits or initiation factors. The only serious limitation

of this type of translation modeling is in that it does not take into account possible interactions between ribosomes. The latter might lead to more complex phenomena which can be taken into account in simulatory models of the detailed representation of translation at the cost of more difficult analytical analysis of the model.

Detailed model of translation

Let us introduce notations:

L – length of mRNA (in nucleotides)

 l_m – length occupied on mRNA by fully assembled ribosome (in nucleotides)

 k_T – rate constant of production of mRNA molecules

 k_D – rate constant of degradation of mRNA molecules

IF - initiation factors and [IF] - their concentration (that we consider constant, inexhaustible)

S40 – small ribosome component and [S40] – their concentration (that we consider constant, inexhaustible)

S60 – small ribosome component and [S60] – their concentration (that we consider constant, inexhaustible)

 k_{01} – rate constant of mRNA molecules are initiated (adding cap structure, circularization, collecting initiation factors at mRNA, etc.), we will call it **early initiation**.

 k_1 – rate constant of translation is initiated on already initiated mRNAs (molecules/sec). By this we mean rate of 40S subunit binding and shifting to the start codon.

 k_2 – rate constant of ribosome assembly, including possible transient arrest of ribosomes before starting translation (molecules/sec)

 k_r – speed at which fully assembled ribosomes moves along mRNA (nucleotides/sec); this speed include the rate of translation termination.

 k_{rd} – rate constant of spontaneous ribosome dissociation from mRNA without producing protein

 k_b – rate constant of miRNA to mRNA binding

Let us denote the total amount of mRNA molecules as MT. The simplest assumption about the production and destruction of mRNA is that the degradation process does not depend on the state of mRNA. Under this assumption the total pool of mRNAs is produced at rate k_t and destroyed with rate constant k_d , i.e. its dynamics is simple and autonomous:

$$\frac{dMT}{dt} = k_t - k_d MT \ .$$

The nearest generalization of this assumption is: the rate constants are different for some subpools of mRNA. The total pool of mRNA molecules can be separated in sub-pools of mRNA molecules in different states: R_0 – mRNA molecules in non-initiated state (not ready for translation)

 \underline{R}_0 – mRNA molecules in initiated state (ready for translation, with 40S subunit sitting at the mRNA)

 R_1 – mRNA molecules with one single ribosome assembled and moving along the mRNA

 \underline{R}_1 – mRNA molecules with one ribosome assembled and initiated for new incoming ribosome

 R_2 – mRNA molecules with two ribosomes assembled and moving along the mRNA

 \underline{R}_2 – mRNA molecules with two ribosomes assembled and initiated for new incoming ribosome

 R_{nmax} – mRNA molecules with *nmax* ribosomes assembled and moving along the mRNA

 \underline{R}_{nmax} – mRNA molecules with *nmax* ribosomes assembled and initiated for new incoming ribosome

The sum of all sub-pools of mRNA should be equal to MT:

$$MT = \sum_{i=0}^{nmax} (R_i + \underline{R}_i)$$

The number *nmax* is defined as the maximum number of ribosomes able to sit on mRNA: it may be roughly evaluated as

 $nmax = L / l_m$.

Schematically, the process of translation can be represented as in Figure 1.



Figure 1. Schematic process of detailed translation representation. It requires 2 x (nmax+1) mRNA states.

The time of passage of one ribosome along mRNA may be evaluated as

$$t_p = L/k_r ,$$

hence, the reaction rate constant of protein production and subsequent release of ribosomes from mRNA (shown in Figure 1 by backward arrows) may be evaluated as:

 $k_3 = k_r / L \; .$

The transformation of states is described by the following chemical equations:

 $R_i \rightarrow \underline{R}_i$ (with rate constant k_1), i = 0...nmax $\underline{R}_i \rightarrow R_{i+1}$ (with rate constant k_2), i = 0...nmax-1 $R_i \rightarrow R_{i-1}$ (with rate constant k_3), i = 1...nmax $\underline{R}_i \rightarrow \underline{R}_{i-1}$ (with rate constant k_3), i = 1...nmax $R_i \rightarrow R_{i-1}$ (with rate constant k_{rd}), i = 1...nmax $\underline{R}_i \rightarrow \underline{R}_{i-1}$ (with rate constant k_{rd}), i = 1...nmax

Basic model of translation, constructed by lumping the detailed model

To avoid using $2 \times (nmax+1)$ states (which potentially can be large) to represent translation, we lump the description of the detailed process in the following way.

We denote

M – amount of mRNA with translation initiation site not occupied by assembling ribosome,

F – amount of mRNA with translation initiation site occupied by assembling ribosome,

R – amount of ribosomes sitting on mRNA synthesizing proteins,

P – amount of proteins.

In terms of \underline{R}_i and R_i variables, M and F represent the lumped values:

$$M = \sum_{i=0}^{nmax} R_i$$
, $F = \sum_{i=0}^{nmax} R_i$ and $MT = M + F$.

There are two lumped reactions and two reactions representing the turnover of ribosomes (as a result of translation termination and protein synthesis or spontaneous ribosome drop-off from mRNA without protein production):

 $M \rightarrow F$ with reaction rate constant k_l ,

 $F \rightarrow M + R$ with reaction rate constant k_2 ,

 $R \rightarrow$ null with reaction rate constant k_3 .

 $R \rightarrow$ null with reaction rate constant $k_{rd}+k_d$ (ribosome drop-off and degradation without protein production).

The full reaction network describing transcription, translation and mRNA degradation is represented in Figure 2.

The corresponding list of equations is

$$\begin{cases} \mathbf{\dot{M}} = k_t - k_d M - k_1 M + k_2 F \\ \mathbf{\dot{F}} = k_1 M - k_d F - k_2 F \\ \mathbf{\dot{R}} = k_2 F - k_3 R - k_{rd} R - k_d R \\ \mathbf{\dot{P}} = k_3 R - k_p P \end{cases}$$

which has the following solution for zero initial condition M(0) = F(0) = R(0) = P(0) = 0

$$M(t) = \frac{k_t}{k_d} \frac{1}{(k_1 + k_2 + k_d)(k_1 + k_2)} \left[(k_1 + k_2)(k_2 + k_d) - k_2(k_1 + k_2 + k_d)e^{-k_d t} - k_1 k_d e^{-(k_1 + k_2 + k_d)t} \right]$$

$$F(t) = \frac{k_t}{k_d} \frac{1}{(k_1 + k_2 + k_d)(k_1 + k_2)} \Big[k_1(k_2 + k_d) - k_1(k_1 + k_2 + k_d)e^{-k_d t} + k_1k_d e^{-(k_1 + k_2 + k_d)t} \Big],$$

$$R(t) = \frac{k_t}{k_d} \frac{k_1k_2}{(k_3 + k_{rd} + k_d)(k_1 + k_2 + k_d)(k_1 + k_2)} \\ \times \Big[(k_1 + k_2) - \frac{(k_3 + k_{rd} + k_d)(k_1 + k_2 + k_d)}{(k_3 + k_{rd})} e^{-k_d t} + \frac{(k_3 + k_{rd} + k_d)k_d}{(k_3 + k_{rd} - k_1 - k_2)} e^{-(k_1 + k_2 + k_d)t} - (k_1 + k_2)e^{-(k_3 + k_{rd} + k_d)t} \Big].$$

$$P(t) = \frac{k_t}{k_d} \frac{k_1 k_2 k_3}{k_p (k_1 + k_2 + k_d) (k_3 + k_{rd} + k_d)} \left[1 - \dots e^{-k_d t} + \dots e^{-(k_1 + k_2 + k_d) t} - \dots e^{-(k_3 + k_{rd} + k_d) t} - e^{-k_p t} \right]$$



Figure 2. Basic model of translation process.

Distinguishing the initial stage of initiation in the basic model:

Specific states of mRNA such as R_0 (free mRNA) and \underline{R}_0 (initiated mRNA) can be separately represented from the total pool of mRNA. Let us denote the amount of mRNA in these states as $M_0 = R_0$ and $F_0 = \underline{R}_0$. The corresponding reaction network is shown in Figure 3. This model is able to represent specific states which will represent just produced, non-initiated mRNA.

For example, if $k_{02} \ll k_2$ then this can represent translation with membrane-bound ribosomes or SRP cycle (Singh, 1996), when there is a transient arrest in the initiated monosome state (the very beginning of the translation).

For our purposes (representing miRNA-based regulation), it is important to distinguish states M_0 and F_0 to be able to represent the initiation of mRNA and the effect of miRNA on the initiation

process. miRNA can act on k_{01} step $(M_0 \rightarrow F_0)$, thus inhibiting the early initiation process, or on k_1 step $(M \rightarrow F)$, thus, inhibiting step of 40S binding on already initiated mRNA, or on k_2 step $(F \rightarrow M+R)$, thus inhibiting ribosome assembly process. Separating M_0 and F_0 states also allows estimating the average number of ribosomes sitting on an *initiated* mRNA (the pool represented by M and F states).



Figure 3. Reaction network representing translation process with explicit representation of the fraction of initiated F_0 and non-initiated, free (more exactly, "early born") mRNA M_0 .

The corresponding system of equations is

$$\begin{cases} \frac{d[M_0]}{dt} = k_t - (k_d + k_{01})[M_0] \\ \frac{d[F_0]}{dt} = k_{01}[M_0] - (k_d + k_{02})[F_0] \\ \frac{d[M]}{dt} = k_{02}[F_0] + k_2[F] - (k_d + k_1)[M] \\ \frac{d[F]}{dt} = k_1[M] - (k_d + k_2)[F] \\ \frac{d[R]}{dt} = k_{02}[F_0] + k_2[F] - (k_d + k_{rd} + k_3)[R] \\ \frac{d[P]}{dt} = k_3[R] - k_p[P] \end{cases}$$

which has the following steady-state solution:

$$\begin{split} &[M_{0}] = \frac{k_{t}}{k_{01} + k_{d}}, [F_{0}] = \frac{k_{t}k_{01}}{(k_{01} + k_{d})(k_{02} + k_{d})}, \\ &[M] = \frac{k_{t}}{k_{d}} \frac{k_{01}k_{02}(k_{2} + k_{d})}{(k_{01} + k_{d})(k_{02} + k_{d})(k_{1} + k_{2} + k_{d})}, [F] = \frac{k_{t}}{k_{d}} \frac{k_{01}k_{02}k_{1}}{(k_{01} + k_{d})(k_{02} + k_{d})(k_{1} + k_{2} + k_{d})}, \\ &[R] = \frac{k_{t}}{k_{d}} \frac{k_{01}k_{02}(k_{1} + k_{d})(k_{2} + k_{d})}{(k_{01} + k_{d})(k_{02} + k_{d})(k_{1} + k_{2} + k_{d})(k_{3} + k_{d} + k_{rd})}, \\ &[P] = \frac{k_{3}}{k_{p}} \frac{k_{t}}{k_{d}} \frac{k_{01}k_{02}(k_{1} + k_{d})(k_{2} + k_{d})}{(k_{01} + k_{d})(k_{02} + k_{d})(k_{1} + k_{2} + k_{d})(k_{3} + k_{d} + k_{rd})}, \\ &MT = [M_{0}] + [F_{0}] + [M] + [F] = \frac{k_{t}}{k_{d}}, \end{split}$$

$$RB = \frac{R}{M+F} = \frac{(k_1 + k_d)(k_2 + k_d)}{(k_1 + k_2 + k_d)(k_3 + k_d + k_{rd})}.$$

The relaxation times are

$$\begin{aligned} rt_{M_0} &= \frac{1}{k_{01} + k_d}, \, rt_{F_0} = \frac{1}{\min(k_{01} + k_d, k_{02} + k_d)}, \\ rt_M &= rt_F = \frac{1}{k_d}, \, \, rt_R = \frac{1}{k_d}, \, \, rt_P = \frac{1}{\min(k_d, k_P)}. \end{aligned}$$

Extending the basic model of translation with miRNA-based regulation

To take into account the action of miRNA on translation, the model of translation shown in Figure 3 is supplied with mRNA states representing mRNA with a miRNA bound to it (states M'_0, F'_0, M', F', R'). The rate of miRNA binding is k_b which determines irreversible conversion of the miRNA-free states (without prime) to miRNA-bound states (primed). The corresponding rate constants which might be different from normal translation process are marked with prime symbol as well. In addition, we introduce a special *B* state which describes reversible capturing of mRNA in P-bodies, where they can be specifically degraded at a higher rate k_{bd} than during the miRNA-free translation.



Figure 4. Model of miRNA-based regulation.

Other possible model extensions

The basic lumped model can serve as a basis for other extensions by explicit splitting of particular states from the lumped states and other modifications. Let us list several possible scenarios:

1) More explicit representation of translation termination

- 2) Description of phenomena connected with uneven distribution of ribosomes along mRNA, such as described in recent literature on explicit studies of ribosome positioning on mRNAs (Ingolia et al, 2009).
- 3) Taking into account possible limitation phenomena connected to limited availability of ribosomal subuntis or initiation factors. This will, however, lead to non-linear models which can be analytically approached in the way described in (Zinovyev et al., 2010).
- 4) Mean-field models of the ribosomes' interaction: The simplest method to include the interaction of ribosomes in the lumped model is a dependence of the ribosome drop-off constant k_{rd} on the average concentration θ of the ribosomes per initiated molecule of mRNA: k_{rd}=k_{rd}(θ). For example, for the scheme presented in Fig. 3 it may be k_{rd}(θ)=a/(b-θ) and k'_{rd}(θ')=a'/(b'-θ'), where θ=R/(M+F) and θ'=R'/(M'+F').

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