Krajowa Konferencja Zastosowań Matematyki w Biologii i Medycynie

Jugowice, 11th–15th September 2017

A SIMPLE MATHEMATICAL MODEL OF RIBOSOME PROFILE

Daria Kogut[1,2], Dorota Hudy[1,3] and Jarosław Śmieja[1,4]

[1]Silesian University od Technology
ul. Akademicka 16, 44-100 Gliwice
[2]daria.kogut@polsl.pl, [3]dorota.hudy@polsl.pl,
[4]jaroslaw.smieja@polsl.pl

ABSTRACT

MicroRNA (miRNA) is involved in many intracellular processes (for example embriogenesis, apoptosis, immunity or virial infections) and regulates gene expression at post-transcriptional level. About 30 % of genes are regulated by miRNA. There is a lot of research on pathways and gene expression regulated by miRNA aimed at unveiling new mechanisms employed by these molecules that are unknown so far. This paper is concerned modeling of translation processes leading to a polysomal distribution transcript-ribosome complexes that might be regulated by miRNA and is observed in experiments. We propose a simple mathematical model, describing probabilities of the number of ribosomes on an RNA matrix. This model should constitute a base for further research focused on regulatory mechanisms involving miRNA and taking into account stochastic effects in such regulation.

INTRODUCTION

Systems biology is a field of study, which describes biological processes, transduction pathways or intracellular processes (where as a system we understand for example a single cell) with mathematical models. In order to create a good mathematical model of biological system it is essential to employ wide knowledge about regulatory structure and dynamics of intracellular processes.

Differential equations are one of possible ways to describe such systems. Usually, molecules concentrations are the variables. However, in this paper we present a less commonly used approach, modelling the processes under consideration as a finite state automaton.

One of the regulatory molecules affecting gene expression is microRNA – a small (21-25 nt), single-stranded, non-coding RNA molecule. Its main function is post-transcriptional regulation of gene expression and it plays an important role in gene silencing. its actions involve either inhibition of translation through binding to its target mRNA and thus inhibiting the access for the ribosome or labeling the target for degradation [5]. This is achieved in several ways, through inhibition of attaching the 60S ribosomal subunit, premature ribosome drop-off or inhibition of protein elongation process, cleavage of mRNA or destabilization of mRNA [3, 4]. However, the exact mechanisms, through which miRNAs exert their actions are still not fully explained. The model developed in this work is meant to be the first step in trying to elucidate them.

BIOLOGICAL BACKGROUND AND EXPERIMENTAL RESULTS

Ribosomes are super organized machineries which participate in the protein synthesis. The process starts with a small subunit attaching to the 5' end of mRNA and scanning for the "start" codon AUG. Then, a large subunit is recruited and the synthesis of a protein may start [2]. Each transcript can be processed by several ribosomes at the same time. When there is more than one ribosome on a mRNA, such construct is called a polysome[7]. Polysomes may have different structures, e.g. circular, spiral, rosette, staggered line or caterpillar-like double-rowed [5]. The existence of polysomes can be measured, thus allowing to discern highly processed transcripts from those less processed or not processed at all. Theoretically, the more ribosomes attached to a transcript, the faster new proteins are obtained in the translation process. Therefore, the rate of processing of mRNA can be indirectly measured by isolating the polysomes and determining the actual ribosome number on a transcript of interest.

The plasmid which was used in our experiments includes two reporter genes sequences (Renilla and Firefly luciferases). One of them had eight motifs to which microRNA could bind and the other one served as the transfection control, without miRNA binding sequences. Activity of luciferases was assessed and assumed to be proportional to the amount of molecules.[10]

For the HCT 116 cell line we measured several polysome profile for reporter genes. Aforementioned Renilla and Firefly luciferases were introduced to cells by PEI transfection 48 hours before collecting the material. Cells were incubated with the medium containing cycloheximide needed to prevent the ribosomes from dropping off the mRNA during cell processing necessary to take the measurements. Then they were collected and flash froze in liquid nitrogen. Next day they were lysed and centrifuged on the sucrose gradient. The final result of the procedure was obtaining polysomal fractions distributed along the sucrose gradient. They were subsequently collected. Finally, the whole probe was divided for 12 parts containing subunits or a different number of ribosomes attached to the mRNAs. Because of the imperfect collecting technique, we could not distinguish the accurate number of ribosomes attached to the mRNA of interest. Therefore we divided the measured profiles into heavy, light and monosome fractions, with respect to the number of ribosomes (Fig.1).

The experimental results were subsequently used in model development.

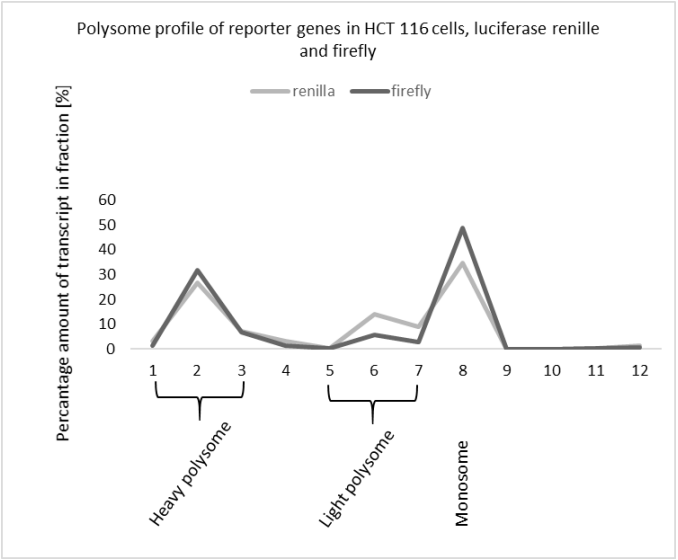

Figure 1. Percentage amount of reporter transcripts through polysomal fractions in HCT 116 cells.

MATHEMATICAL MODEL OF THE TRANSLATION PROCESS INCLUDING MIRNA-BASED REGULATION

Despite the relative short history of miRNA research, there is a rich literature devoted to its modeling, dealing with specific pathways[11], involving various feedback loops, cell cycle regulation[6] or simply in translation process [10]. The latter describes this process by the simplest linear model, which contains four species: 40S subunit of ribosome, F, A and P (where F is the initiation site for ribosome, A (Aminoacyl) is the A- binding site of the ribosome in translation, P (peptidyl) is the P- binding site of ribosome), and three chemical reactions. The reactions describe the initiation process, late and cap-independent initiation, and elongation with termination. Another, nonlinear model has been proposed in [9]. It takes into account recycling of initiation factors and ribosomal subunits (40S and 60S). In addition to species introduced in [10], this model includes the 60S subunit, eIF4F (translation initiation factor) and R (number of ribosomes fully assembled on miRNA-free mRNA) instead of P. Similarly to the simplest linear model, there are four reactions – assembly of the initiation complex, cap-independent initiation steps, protein translation and recycling of ribosome subunits. All of these reactions are assumed irreversible.

This model inspired Zinovyev et al to write a general model of miRNA - mediated translation regulation[3]. This model treats miRNAs as a translation regulator that can act through nine different modes of operation and incorporates all of them. Furthermore, that model takes into account important processes, which were not included in previous models, such as synthesis and degradation. Additionally, various initial conditions have been tested.

As the examples cited above prove, microRNA is a very interesting molecule. The model proposed in this paper is aimed at finding differences in regulation of gene expression depending on the number of ribosomes on mRNA. None of the existing models deals with that phenomenon and, in fact, this has not been the subject of experimental research either. The mathematical model is used to check the level of miRNA-mediated gene silencing on two mRNAs- Renilla and Firefly luciferases, based on experimental results.

ASSUMPTIONS OF THE MODEL

The model is in the form of a finite state automata, in which the state of the system is related to the number of ribosomes attached to the transcript. The variables P_i denote probabilities of the system being in the state x_i. Four states are taken into account in the model (Fig. 2).The first one, x_0, represents mRNA without any ribosomes attached, while x_1-x_3 represent mRNA with 1-3 ribosomes, respectively. It is assumed that in a given short time interval the system may either stay in the same state (no new ribosome complex has attached to the transcript), one additional ribosome may have attached or one ribosome may have detached. The latter case may be due to either completion of a single protein molecule synthesis or rare random event in which the ribosome has detached without producing a functional protein. Binding of a new ribosome is a Poisson process, in which the probability of binding a new one increases with the number of ribosomes occupying the transcript[2].

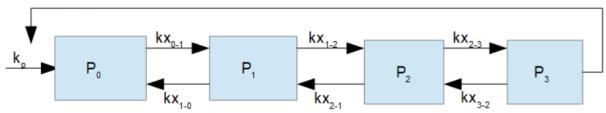

Figure 2. A block diagram representing the model.

$$P_0 = k_p(1 - P_3) - k_{x0-1}F_0 + k_{1-0}F_1, \tag{1}$$

$$P_1 = k_{x0-1}P_0 - k_{x1-2}P_1 + k_{x2-1}P_2, \tag{2}$$

$$P_2 = k_{x1-2}P_1 - k_{x2-3}P_2 + k_{x3-2}P_3 - x_{x2-1}P_2, \tag{3}$$

$$P_3 = k_{x2-3}P_2 - k_{x3-2}P_3. \tag{4}$$

SIMULATION VS EXPERIMENTAL RESULTS

Sample transient system responses, shown in Fig. 3 (parameters are given in Table 1) may represent activation of some regulatory mechanisms following e.g. stress conditions. However, in the experiments no such stress has been used, nor is it included in the mathematical model. As the experimental results are single time point measurements (repeated several times), the underlying assumption is that the cells in the experiment are in homeostasis. Therefore, steady state of the model is compared to experimental data As we can see in (Fig. 4), the highest intensity of transcript level both in simulation and biological plots we can see for monosomes, next for mRNA with light ribosomes, then for heavy polysomes. Unoccupied transcripts are negligible. This reflects experimental observations, though the quality of the fit is not very high.

Table 1. Values of coefficients used in model.

Coefficient	Value
k_p	0.03
k_{x0-1}	0.13
k_{x1-2}	0.025
k_{x2-1}	0.08
k_{x2-3}	0.15
k_{x3-2}	0.067

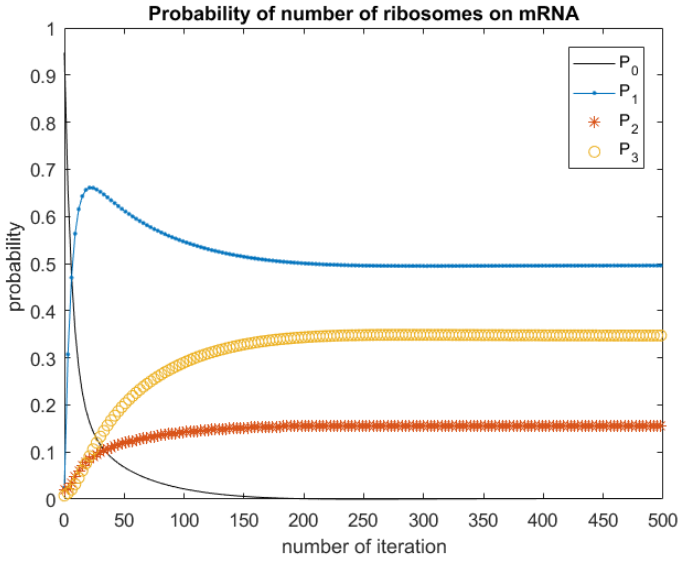

Figure 3. Simulation of probabilities of number of ribosomes on mRNA.

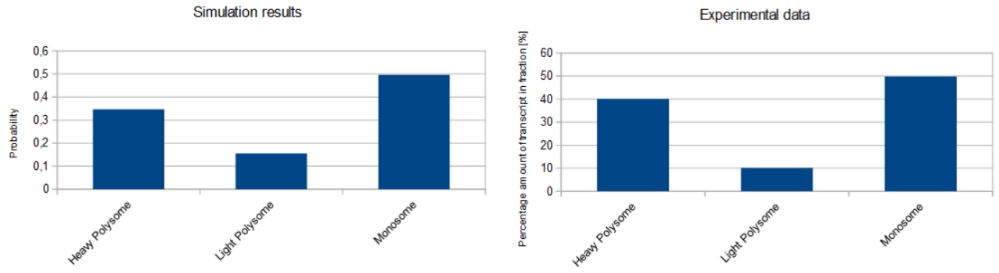

Figure 4. Comparison of simulation in stationary state (left) and experimental data (right). The bars in the right panel represent the maximum values for each fraction depicted in Fig. 1

CONCLUSIONS

The simplistic model presented in this paper captures some of the biological system properties, observed experimentally. However, it needs to be modified and expanded to be a useful tool supporting analysis of a biological system. First, the number of states in the automata should be adjusted to cover all possible cases, taking into account the size of the ribosome complex, minimum distance between two ribosomes occupying the same transcript. In fact, it is estimated that up to 7-8 ribosomes may be attached to the renilla transcript. Parameter fitting should be performed for the full model. The next step should involve modeling of miRNA-mediated control actions. These might be represented by setting the parameters to be functions of miRNA concentration. These functions should be chosen depending on the hypothetical type of miRNA action.

ACKNOWLEDGEMENTS

The work has been partialy supported by the NCN grant DEC-2013/11/B/ST7/01713 .

REFERENCES

[1] A.E. Borujeni and H.M. Salis: *Translation Initiation is Controlled by RNA Folding Kinetics via a Ribosome Drafting Mechanism*, Journal of the American Chemical Society (2016).

[2] A.G. Hinnebush: *Molecular Mechanism of Scanning and Start Codon Selection in Eukaryotes*, Microbiol Mol Biol Rev. **75** (2011), 434–467.

[3] A. Zinovyev, N. Morozova, A.N. Gorban, and A. Harel-Belan: *Mathematical Modeling of microRNA-Mediated Mechanisms of Translation Repression*, Advances in Experimental Medicine and Biology (2013), 189–224.

[4] D.P. Bartel: *MicroRNAs: Genomics, Biogenesis, Mechanism, and Function*, Cell (2004), 281–297.

[5] G. et al. Viero: *Three distinct ribosome assemblies modulated by translation are the building blocks of polysomes*, JCB **208** (2015), 581–596.

[6] H.W. Kang, M. Crawford, M. Fabbri, G. Nuovo, M. Garofalo, S.P. Nana–Sinkam, and A. Friedman: *A Mathematical Model for MicroRNA in Lung Cancer*, PLoS One **8** (2013).

[7] J.R. Warner, A. Rich, and C. E. Hall: *Electron Microscope Studies of Ribosomal Clusters Synthesizing Hemoglobin*, Science **138** (1962), 1399–1403.

[8] M.D. Faye, D.E. Graber, and M. Holcik: *Assessment of Selective mRNA Translation in Mammalian Cells by Polysome Profiling*, J Vis Exp. (2014).

[9] T. Nissan and R. Parker: *Computational analysis of miRNA repression of translation: implication for models of translation initiation inhibition*, RNA (2008), 1480–1491.

[10] T. Schagat and J. Vidugiriene: *MicroRNA Biosensors: Application for the psiCHECKtm-2 Vector*, Promega Notes (2008), 16–18.

[11] X. Lai, O. Wolkenhauer, and J. Vera: *Understanding microRNA-mediated gene regulatory networks through mathematical modelling*, Nucleic Acid Research (2016), 6019–6035.