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Mathematical Modelling of Post-Transcriptional Regulation of Gene Expression

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Abstract

This report aims to understand the role of microRNA and Argonaute, combined to RISC, during post-transcription on the production of protein. It modifies the Klironomos and Berg model (Klironomos & Berg, 2013) as well as Nyayanit and Gadgil model (Nyayanit & Gadgil, 2015).

In the Nyayanit and Gadgil model, a counter-intuitive result is obtained, where increasing the microRNA concentration increases the concentration of protein produced. It is investigated further whether the result is due to the translation of the mRNA - RISC complex that is taken into account in the model.

1 Introduction

1.1 Biological background

Protein is known as the building block of life. It is an essential component of various parts of our body, from head to toe. To obtain it, our body has a protein factory inside our cells. Like any other items that we make, we need a recipe to produce protein. The recipe is stored in a molecule called DNA. DNA is two strands twisted around each other in the form of double helix. It is located in the nucleus of the cell. Sometimes, the two strands unwind. When it does, the process of replication and transcription may take place.

Replication is the process whereby DNA makes copies of itself, whereas transcription is when DNA produces mRNA, also known as messenger RNA. mRNA brings the information from the DNA out of the nucleus to the ribosome, where protein is made. Therefore, mRNA is needed to transport the information. In the ribosomes, the information in mRNA is translated into protein in the process of translation. One of the factors that determine the rate of protein production is the rate of translation. When there is more mRNA available, there is more translation taking place hence more protein produced.

However, protein production is also regulated at the post-transcription stage. Post-transcription is the time in between transcription and translation. Following transcription, mRNA may bind to RISC, RNA Induced Silencing Complex. RISC is made of Argonaute and microRNA. Argonaute is a protein itself and is the active part of the RISC. microRNA is a class of regulators in post-transcriptional regulation (Nyayanit & Gadgil, 2015). The combination of mRNA and RISC is not unique so that one mRNA can bind to several different RISCs and vice versa (Nyayanit & Gadgil, 2015). When mRNA binds to RISC, there will be less mRNA to produce protein. Hence, the conventional picture is that if there is more microRNA, there will be more RISC and less mRNA, and as a result, there will be less

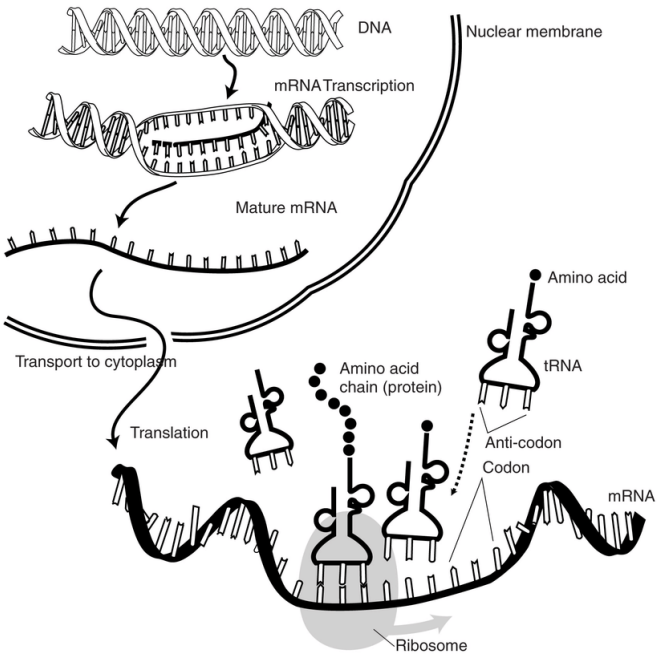

Figure 1: Diagram of protein production (Mesuere, 2016)

protein. This report aims to understand further whether more microRNA always means less protein produced, when the various binding steps and competition between microRNAs is taken into account.

1.2 Mathematical background

In this report, I am concerned with reactions between various species. Hence, I begin by introducing the theory underpinning the mathematical models of these processes, based on my supervisor's lecture notes (Green, 2012).

1.2.1 Law of Mass Action

The Law of Mass Action is a mathematical model which describes the rate of change of concentration of the reactants and products in a chemical reaction. It states that the change in concentration of product is proportional to the product of the concentrations of the reactants. If we have the reaction

$$A + B \xrightarrow{k} C,$$

the rate of change of the concentration of product C is given by

$$\frac{dc}{dt} = kab,$$

where k is a reaction constant and a, b, c are the concentrations of A, B, C respectively. If we have a reversible reaction

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C,$$

the rate of change of each substance is given by

$$\begin{aligned}\frac{dc}{dt} &= k_1 ab - k_2 c,\\ \frac{da}{dt} &= k_2 c - k_1 ab,\\ \frac{db}{dt} &= k_2 c - k_1 ab,\end{aligned}$$

where k_1 and k_2 are reaction constants.

1.2.2 Steady States

Let $\frac{dx}{dt} = f(x)$. A steady state, fixed point, or equilibrium of a differential equation are the values of x such that

$$\frac{dx}{dt} = 0 \Leftrightarrow f(x) = 0.$$

If we start close to a steady state x^*, we might want to know if we will move towards or away from it. It is explained by the stability of each steady state. To be able to determine the stability of steady states, there are three possible scenarios. In these scenarios, δx means that we move the x value by a little and see what happens to the value of x.

- If $\frac{df}{dx} < 0$, then x is decreasing for $\delta x > 0$ and increasing for $\delta x < 0$ so the system moves back towards x^*. Therefore, x^* is stable.
- If $\frac{df}{dx} > 0$, then x is increasing for $\delta x > 0$ and decreasing for $\delta x < 0$ so the system moves further away from x^*. Therefore, x^* is unstable.
- If $\frac{df}{dx} = 0$, then x^* is stable from one direction and unstable from another. It is also known as semi-stable fixed point.

1.2.3 Systems of Ordinary Differential Equations

If we have more than one Ordinary Differential Equations (ODEs), we need to set all of the equations to be zero to obtain a steady state. For example, if we have x_1 and x_2, we need

$$\frac{dx_1}{dt} = f_1(x_1, x_2) = 0 \text{ and } \frac{dx_2}{dt} = f_2(x_1, x_2) = 0.$$

If we have linear system

$$\frac{dx_1}{dt} = \lambda_1 x_1 \text{ and } \frac{dx_2}{dt} = \lambda_2 x_2,$$

we can write the ODEs in the form of a matrix,

$$\frac{d}{dt}\begin{pmatrix} x_1 \\ x_2 \end{pmatrix} = \begin{pmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{pmatrix}\begin{pmatrix} x_1 \\ x_2 \end{pmatrix}$$

or equivalently

$$\frac{d\boldsymbol{x}}{dt} = \begin{pmatrix} \lambda_1 & 0 \\ 0 & \lambda_2 \end{pmatrix}\boldsymbol{x}.$$

In this case, λ_1 and λ_2 are eigenvalues of the 2x2 matrix. The eigenvalues of the matrix can determine the stability of the steady states:

- $\lambda_1 > 0$, $\lambda_2 > 0$ is unstable node,
- $\lambda_1 > 0$, $\lambda_2 < 0$ is a saddle,
- $\lambda_1 < 0$, $\lambda_2 < 0$ is stable node.

This is a simple method to calculate the stability of steady state of a small system ($n = 2$ in this case). However, for larger values of n, it is a complicated process. Instead, we can investigate the stability of steady states numerically.

1.2.4 Nonlinear Systems

If the system is nonlinear, we can linearise the system about the steady state. Consider the system

$$\frac{dx_1}{dt} = f_1(x_1, x_2) \text{ and } \frac{dx_2}{dt} = f_2(x_1, x_2),$$

where f_1 and f_2 are continuously differentiable functions.

Let $(\bar{x}_1, \bar{x}_2)$ be a steady state of the system such that

$$f_1(\bar{x}_1, \bar{x}_2) = f_2(\bar{x}_1, \bar{x}_2) = 0.$$

Let $\epsilon \ll 1$ and have

$$x_1(t) = \bar{x}_1 + \epsilon z_1(t), x_2(t) = \bar{x}_2 + \epsilon z_2(t),$$

where $z_1(t)$ and $z_2(t)$ are small deviations from the system. The Taylor expansions of f_1 and f_2 around $(\bar{x}_1, \bar{x}_2)$ is

$$f_1(\bar{x}_1 + z_1, \bar{x}_2 + z_2) = f_1(\bar{x}_1, \bar{x}_2) + \epsilon z_1 \frac{\partial f_1}{\partial x_1}\Big|_{\bar{x}_1,\bar{x}_2} + \epsilon z_2 \frac{\partial f_1}{\partial x_2}\Big|_{\bar{x}_1,\bar{x}_2} + O(\epsilon^2),$$
$$f_2(\bar{x}_1 + z_1, \bar{x}_2 + z_2) = f_2(\bar{x}_1, \bar{x}_2) + \epsilon z_1 \frac{\partial f_2}{\partial x_1}\Big|_{\bar{x}_1,\bar{x}_2} + \epsilon z_2 \frac{\partial f_2}{\partial x_2}\Big|_{\bar{x}_1,\bar{x}_2} + O(\epsilon^2),$$

or

$$\boldsymbol{f}(\bar{\boldsymbol{x}} + \epsilon \boldsymbol{z}) = \boldsymbol{f}(\bar{\boldsymbol{x}}) + \epsilon J(\bar{\boldsymbol{x}})\boldsymbol{z} + O(\epsilon^2),$$

where J is the Jacobian matrix of $\boldsymbol{f}$ at the steady state $(\bar{x}_1, \bar{x}_2)$. Since $\bar{\boldsymbol{x}}$ is a steady state, $\boldsymbol{f}(\bar{\boldsymbol{x}}) = 0$. Since

$$\frac{dx_1}{dt} = \epsilon \frac{dz_1}{dt}, \ \frac{dx_2}{dt} = \epsilon \frac{dz_2}{dt},$$

at leading order, the system becomes

$$\frac{d}{dt}\begin{pmatrix} z_1 \\ z_2 \end{pmatrix} = \begin{pmatrix} f_{1_{x_1}} & f_{1_{x_2}} \\ f_{2_{x_1}} & f_{2_{x_2}} \end{pmatrix} \begin{pmatrix} z_1 \\ z_2 \end{pmatrix} \Leftrightarrow \frac{d\boldsymbol{z}}{dt} = J(\bar{\boldsymbol{x}})\boldsymbol{z},$$

where $\boldsymbol{z} = (z_1(t), z_2(t))^T$ and J is the Jacobian matrix of $\boldsymbol{f} = (f_1, f_2)^T$.

Now the nonlinear system has been reduced to linear system about the steady state. We can use the same technique of looking at the eigenvalues of $J(\bar{\boldsymbol{x}})$ to determine the stability of the steady state. This conditions where the stability of nonlinear system can be determined by linearisation is described in the following definition and theorem.

Definition: The steady state $(\bar{x}_1, \bar{x}_2)$ is called **hyperbolic** if all eigenvalues of the Jacobian $J(\bar{x}_1, \bar{x}_2)$ have nonzero real part.

Theorem (Hartman-Grobman): Assume that $(\bar{x}_1, \bar{x}_2)$ is a hyperbolic equilibrium. Then, in a small neighbourhood of $(\bar{x}_1, \bar{x}_2)$ the phase portrait of the non-linear system

$$\frac{dx_1}{dt} = f_1(x_1, x_2) \text{ and } \frac{dx_2}{dt} = f_2(x_1, x_2)$$

is equivalent to that of the linearised system.

However, as mentioned above, we can investigate the stability of steady states of large system numerically since the process gets complicated with large values of n.

2 Statement of Authorship

I discussed and developed the mathematical models with my supervisor. I modified the MATLAB code provided by my supervisor to solve the differential equation models and find steady states. I wrote the MATLAB code to plot the steady states when changing the values of the parameters. I explored different values of parameters of the model and discussed the appropriate values with my supervisor. I interpreted the graphs and wrote this report. This report is then proofread by my supervisor.

3 Mathematical model for a single microRNA

I now present our mathematical model for post-transcriptional gene regulation. I begin by considering one microRNA. The following notation is in number of species per cell unit volume, used to represent the concentration of substances.

- m represents concentration of microRNA,
- A represents concentration of Argonaute,
- A_m represents concentration of Argonaute-microRNA (RISC),
- R represents concentration of mRNA (messengerRNA),
- P represents concentration of protein,
- c represents concentration of complex formed by RISC and mRNA.

3.1 Klironomos and Berg Model, 2013

The Klironomos and Berg model (Klironomos & Berg, 2013) has equations representing the reactions with single microRNA, as shown in black below. It takes into account microRNA, Argonaute, RISC, mRNA, and protein. The rate of change of concentration of RISC is the negative of Argonaute since the total amount of A and A_m is constant. The model does not consider the complex formed by RISC and mRNA, which I added to the model as shown in red.

As seen in the model, I have included decomposition of the complex, $d_c c$, as well as the decay of the complex, $l_c c$. The decomposition of the complex is when the complex dissociates to RISC and mRNA whereas the decay of the complex is degradation of the complex. In the model I include a term representing the translation of the complex (the $b_P c$ term) though biologically it is not clear if this happens in practice.

$$\frac{dm}{dt} = \underbrace{k_m}_{\text{production of m}} - \underbrace{d_m m}_{\text{degredation of m}} - \underbrace{k_A m A}_{\text{production of RISC}} + \underbrace{d_A A_m}_{\text{decay of RISC}} \tag{1}$$

$$\frac{dA_m}{dt} = -\frac{dA}{dt} = \underbrace{k_A m A}_{\text{production of RISC}} + \underbrace{d_A A_m}_{\text{decay of RISC}} - \underbrace{d_{Rm} A_m R}_{\text{binding RISC to R}} + \underbrace{d_c c}_{\text{decomposition of complex}} \tag{2}$$

$$\frac{dR}{dt} = \underbrace{k_R}_{\text{production of R}} - \underbrace{d_R R}_{\text{decay of R}} - \underbrace{d_{Rm} A_m R}_{\text{decay due to binding to RISC}} + \underbrace{d_c c}_{\text{decomposition of complex}} \tag{3}$$

$$\frac{dP}{dt} = \underbrace{k_P R}_{\text{translation of P}} - \underbrace{d_P P}_{\text{decay of P}} + \underbrace{b_P c}_{\text{translation of complex}} \tag{4}$$

$$\frac{dc}{dt} = \underbrace{d_{Rm} A_m R}_{\text{production of complex}} - \underbrace{d_c c}_{\text{decomposition of complex}} - \underbrace{l_c c}_{\text{decay of complex}} \tag{5}$$

3.2 Method

Equations (1) to (5) are non-dimensionalised to reduce the number of parameters, giving Equations (6) to (10). The scale for A and A_m is the constant A_0, where $A_0 = A + A_m$, the total amount of Argonaute and RISC. For the other variables, I nondimensionalise them as follows, with tilde indicating dimensionless equations:

$$T = \frac{1}{k_A A_0}\tilde{t}\ ,\ m = \frac{d_A}{k_A}\tilde{m}\ ,\ (A, A_m) = A_0(\tilde{A}, \tilde{A_m})\ ,\ R = \frac{k_R}{d_R}\tilde{R}\ ,\ P = \frac{k_P k_R}{k_A A_0 d_R}\tilde{P}\ ,\ c = \frac{d_{Rm} k_R}{k_A d_R}\tilde{c}.$$

This gives the following model equations (dropping tildes):

$$\frac{dm}{dt} = \delta_1 - \delta_2 m - m(1 - A_m) + A_m \tag{6}$$

$$\frac{dA_m}{dt} = -\frac{dA}{dt} = \alpha_1[m(1 - A_m) - A_m] - \alpha_2 A_m R + \alpha_3 c \tag{7}$$

$$\frac{dR}{dt} = \beta_1(1 - R) - \beta_2 A_m R + \beta_3 c \tag{8}$$

$$\frac{dP}{dt} = R - \psi_1 P + \psi_2 c \tag{9}$$

$$\frac{dc}{dt} = A_m R - \gamma_1 c - \gamma_2 c \tag{10}$$

where

$$\delta_1 = \frac{k_m}{d_A A_0}\ ,\ \delta_2 = \frac{d_m}{k_A A_0}\ ,\ \alpha_1 = \frac{d_A}{k_A A_0}\ ,\ \alpha_2 = \frac{d_{Rm} k_R}{k_A A_0 d_R}\ ,\ \alpha_3 = \frac{d_c d_{Rm} k_R}{k_A^2 A_0^2 d_R}\ ,$$
$$\beta_1 = \frac{d_R}{k_A A_0}\ ,\ \beta_2 = \frac{d_{Rm}}{k_A}\ ,\ \beta_3 = \frac{d_c d_{Rm}}{k_A^2 A_0}\ ,\ \psi_1 = \frac{d_P}{k_A A_0}\ ,\ \psi_2 = \frac{b_c d_{Rm}}{k_A k_P}\ ,\ \gamma_1 = \frac{d_c}{k_A A_0}\ ,\ \gamma_2 = \frac{l_c}{k_A A_0}.$$

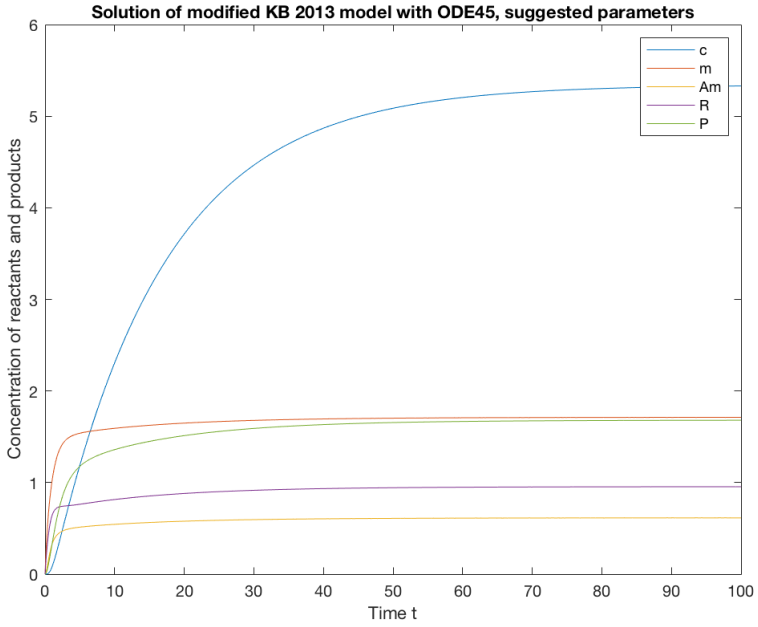

Figure 2: Solution of modified KB 2013 model

As it is not possible to solve the system above analytically, I used MATLAB to plot numerical solutions to the differential equations. Using the *ode45* solver as a built-in function of MATLAB, I plotted the results into a graph as shown in Figure 2 using the parameter values in Table A. The graph shows that the concentration of each substances reach a steady state at some point. Hence, I wrote another code to find all of the steady states of the model. This is done by solving the equations when set to zero, using the built-in function *solve*. Then I varied the values of δ_1 to be 0.1, 2.1 and 4.1. The values of $\alpha_2 = \beta_2$ is varied between 1 and 2 with interval of 0.5. The values of $\psi_2 = 0.005$ and $\gamma_2 = 0.1$ was set and other parameter values are shown in Table A. The steady states were plotted in Figure 3.

3.3 Results

As time increases, the concentration of each of the substances reaches a steady state as shown in Figure 2. Hence, the steady states were explored further. As I changed the values of the formation of the complex, the steady states changes. This can be seen in Figure 3. There are two steady states visible for each rate of formation of microRNA. One of each pair of steady states has negative protein concentration. Exploring on those steady states, I found that they are unstable steady states so that it will not be observed in real life. This aligns with the fact that negative concentration is impossible to achieve in experiments.

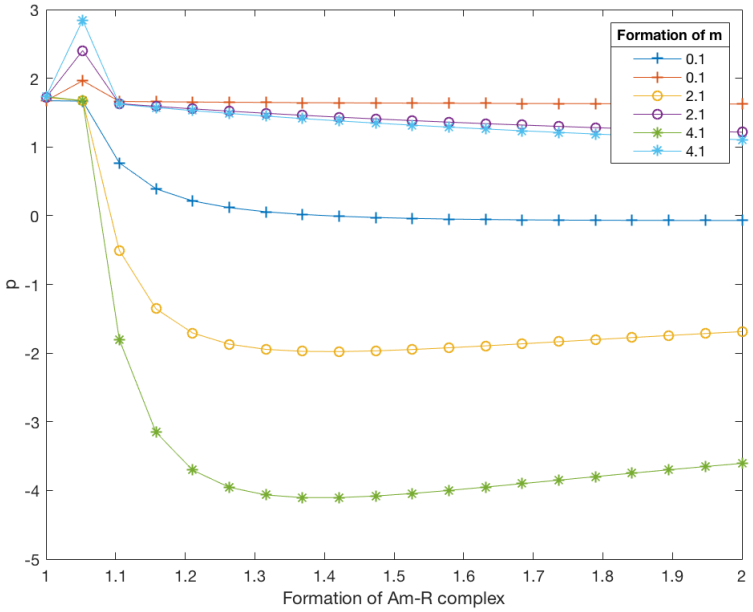

Figure 3: Steady states of modified KB model

As the formation of the complex increases, the general trend is that the concentration of protein decreases. However, there is a spike visible when the formation rate is around 1 to 1.2. This shows that the increase in the formation of complex actually increases the protein produced. However, these steady states turns out to be unstable. Therefore, it cannot be observed in experiments.

4 Mathematical model for two microRNAs

After exploring the model for one microRNA, now I will explore the model when there are two microRNAs in the system.

4.1 Nyayanit and Gadgil Model, 2015

The Nyayanit and Gadgil model (Nyayanit & Gadgil, 2015) considers the possibility of having two microRNAs in the system, as shown in Equations (11) to (16). However, it does not take into account Argonaute and RISC. It assumes that the microRNA combines directly with the mRNA, producing the complexes c_1 and c_2. The Nyayanit and Gadgil model also assumes that there still can be translation occuring even though the messenger RNA has combined with the RISC, expressed by b_1 and b_2. However, these values are set to be very low, since, as described earlier, it is not clear biologically if translation of the complex can occur in practice.

$$\frac{dm_1}{dt} = \underbrace{k_{m_1}}_{\text{production of } m_1} - \underbrace{d_{m_1}m_1}_{\text{degradation of } m_1} - \underbrace{k_1Rm_1}_{\text{binding } m_1 \text{ to R}} + \underbrace{d_{c_1}c_1}_{\text{decomposition of complex 1}} \tag{11}$$

$$\frac{dm_2}{dt} = \underbrace{k_{m_2}}_{\text{production of } m_2} - \underbrace{d_{m_2}m_2}_{\text{degradation of } m_2} - \underbrace{k_2Rm_2}_{\text{binding } m_2 \text{ to R}} + \underbrace{d_{c_2}c_2}_{\text{decomposition of complex 2}} \tag{12}$$

$$\frac{dR}{dt} = \underbrace{k_R}_{\text{production of R}} - \underbrace{d_RR}_{\text{decay of R}} - \underbrace{k_1Rm_1}_{\text{binding } m_1 \text{ to R}} - \underbrace{k_2Rm_2}_{\text{binding } m_2 \text{ to R}} + \underbrace{d_{c_1}c_1}_{\text{decomposition of complex 1}} + \underbrace{d_{c_2}c_2}_{\text{decomposition of complex 2}} \tag{13}$$

$$\frac{dP}{dt} = \underbrace{k_PR}_{\text{translation of P}} - \underbrace{d_PP}_{\text{decay of P}} + \underbrace{b_1c_1}_{\text{translation of complex 1}} + \underbrace{b_2c_2}_{\text{translation of complex 2}} \tag{14}$$

$$\frac{dc_1}{dt} = \underbrace{k_1Rm_1}_{\text{production of complex 1}} - \underbrace{d_{c_1}c_1}_{\text{decomposition of complex 1}} - \underbrace{l_{c_1}c_1}_{\text{decay of complex 1}} \tag{15}$$

$$\frac{dc_2}{dt} = \underbrace{k_2Rm_2}_{\text{production of complex 2}} - \underbrace{d_{c_2}c_2}_{\text{decomposition of complex 2}} - \underbrace{l_{c_2}c_2}_{\text{decay of complex 2}} \tag{16}$$

Modifiying the code used for Klironomos and Berg model, I recreated Figure 4 as shown in the Nyayanit and Gadgil paper, Figure 4B (Nyayanit & Gadgil, 2015). The Nyayanit and Gadgil model has a counter intuitive result where increasing the concentration of microRNA actually increases the concentration of protein produced for certain parameter values as shown in Table B (Nyayanit & Gadgil, 2015). This is not expected since we expect more microRNA will give less mRNA for translation and hence less protein produced.

Now I consider the Nyayanit and Gadgil model without the translation of the complex by setting b_1 and b_2 to zero. The values of the other parameters are the same as Figure 4. This produces Figure 5. Similar trend to Figure 4 is observed, where increasing the rate of formation of microRNA increases the protein production. However, the values of $p/pref$ has increased drastically from around 5 to around 2000. This is due to the fact that the value of $pref$ decreases significantly.

4.2 Modified Nyayanit and Gadgil Model, 2015

The Nyayanit and Gadgil model is modified to include the Argonaute and RISC, with the modifications from the original model highlighted in red, as shown in Equation (17) to (24).

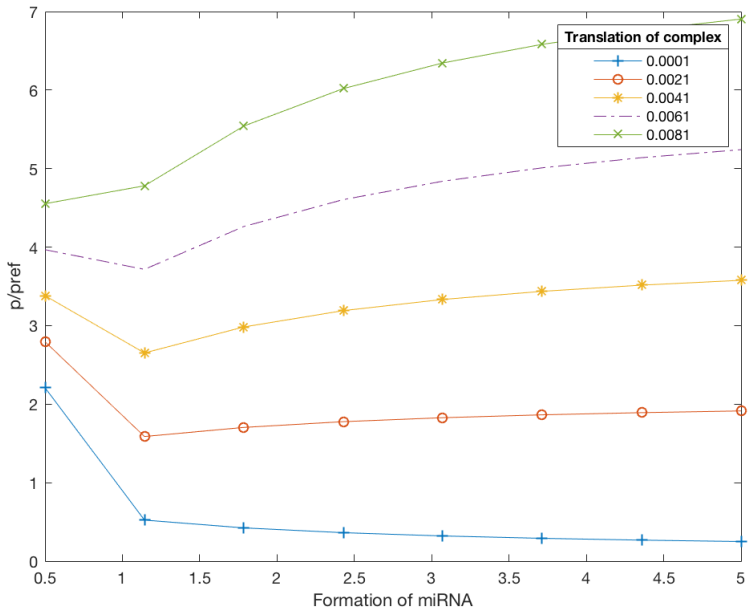

Figure 4: Steady states of NG model

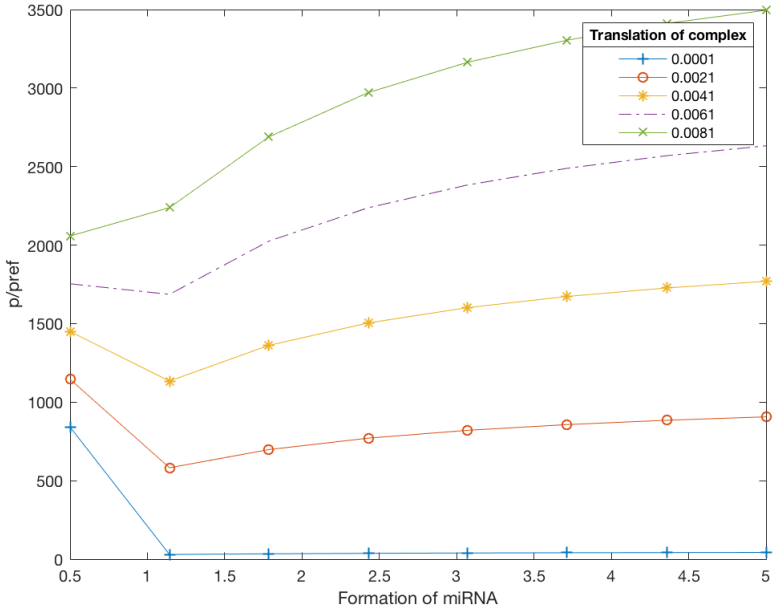

Figure 5: Steady states of NG model, no translation of complex

$$\frac{dm_1}{dt} = \underbrace{k_{m_1}}_{\text{production of } m_1} - \underbrace{d_{m_1}m_1}_{\text{degradation of } m_1} - \underbrace{k_{A_1}m_1A}_{\text{binding } m_1 \text{ to A}} + \underbrace{d_{A_1}A_{m_1}}_{\text{decomposition of } A_{m_1}} \tag{17}$$

$$\frac{dm_2}{dt} = \underbrace{k_{m_2}}_{\text{production of } m_2} - \underbrace{d_{m_2}m_2}_{\text{degradation of } m_2} - \underbrace{k_{A_2}m_2A}_{\text{binding } m_2 \text{ to A}} + \underbrace{d_{A_2}A_{m_2}}_{\text{decomposition of } A_{m_2}} \tag{18}$$

$$\frac{dA_{m_1}}{dt} = \underbrace{k_{A_1}m_1A}_{\text{binding } m_1 \text{ to A}} - \underbrace{d_{A_1}A_{m_1}}_{\text{decomposition of } A_{m_1}} - \underbrace{d_{Rm_1}A_{m_1}R}_{\text{binding } A_{m_1} \text{ to R}} + \underbrace{d_{c_1}c_1}_{\text{decomposition of complex 1}} \tag{19}$$

$$\frac{dA_{m_2}}{dt} = \underbrace{k_{A_2}m_2A}_{\text{binding } m_2 \text{ to A}} - \underbrace{d_{A_2}A_{m_2}}_{\text{decomposition of } A_{m_2}} - \underbrace{d_{Rm_2}A_{m_2}R}_{\text{binding } A_{m_2} \text{ to R}} + \underbrace{d_{c_2}c_2}_{\text{decomposition of complex 2}} \tag{20}$$

$$\begin{aligned}\frac{dR}{dt} = {} & \underbrace{k_R}_{\text{production of R}} - \underbrace{d_RR}_{\text{decay of R}} + \underbrace{d_{c_1}c_1}_{\text{decomposition of complex 1}} + \underbrace{d_{c_2}c_2}_{\text{decomposition of complex 2}} \\ & - \underbrace{d_{Rm_1}A_{m_1}R}_{\text{binding } A_{m_1} \text{ to R}} - \underbrace{d_{Rm_2}A_{m_2}R}_{\text{binding } A_{m_2} \text{ to R}}\end{aligned} \tag{21}$$

$$\frac{dP}{dt} = \underbrace{k_PR}_{\text{translation of P}} - \underbrace{d_PP}_{\text{decay of P}} + \underbrace{b_1c_1}_{\text{translation of complex 1}} + \underbrace{b_2c_2}_{\text{translation of complex 2}} \tag{22}$$

$$\frac{dc_1}{dt} = \underbrace{d_{Rm_1}A_{m_1}R}_{\text{production of complex 1}} - \underbrace{d_{c_1}c_1}_{\text{decomposition of complex 1}} - \underbrace{l_{c_1}c_1}_{\text{decay of complex 1}} \tag{23}$$

$$\frac{dc_2}{dt} = \underbrace{d_{Rm_2}A_{m_2}R}_{\text{production of complex 2}} - \underbrace{d_{c_2}c_2}_{\text{decomposition of complex 2}} - \underbrace{l_{c_2}c_2}_{\text{decay of complex 2}} \tag{24}$$

Equations (17) to (24) are nondimensionalised as follows, with tilde indicating dimensionless equations:

$$T = \frac{1}{k_{A_1}A_0}\tilde{t}\ ,\ m_1 = \frac{d_{A_1}}{k_{A_1}}\tilde{m_1}\ ,\ m_2 = \frac{d_{A_2}}{k_{A_2}}\tilde{m_2}\ ,\ (A, A_{m_1}, A_{m_2}) = A_0(\tilde{A}, \tilde{A_{m_1}}, \tilde{A_{m_2}})\ ,\ R = \frac{k_R}{d_R}\tilde{R}\ ,$$
$$P = \frac{k_Pk_R}{k_{A_1}A_0d_R}\tilde{P}\ ,\ c_1 = \frac{d_{Rm_1}k_R}{k_{A_1}d_R}\tilde{c_1}\ ,\ c_2 = \frac{d_{Rm_2}k_R}{k_{A_2}d_R}\tilde{c_2}.$$

This gives the following model equations (dropping tildes):

$$\frac{dm_1}{dt} = \delta_{11} - \delta_{12}m_1 - m_1(1 - A_{m_1} - A_{m_2}) + A_{m_1} \tag{25}$$

$$\frac{dm_2}{dt} = \frac{k_{A_2}}{k_{A_1}}\Big(\delta_{21} - \delta_{22}m_2 - m_2(1 - A_{m_1} - A_{m_2}) + A_{m_2}\Big) \tag{26}$$

$$\frac{dA_{m_1}}{dt} = -\frac{dA_1}{dt} = \alpha_{11}[m_1(1 - A_{m_1} - A_{m_2}) - A_{m_1}] - \alpha_{12}A_{m_1}R + \alpha_{13}c_1 \tag{27}$$

$$\frac{dA_{m_2}}{dt} = -\frac{dA_2}{dt} = \frac{k_{A_2}}{k_{A_1}}\Big(\alpha_{21}[m_2(1 - A_{m_1} - A_{m_2}) - A_{m_2}] - \alpha_{22}A_{m_2}R + \alpha_{23}c_2\Big) \tag{28}$$

$$\frac{dR}{dt} = \beta_1(1 - R) - \beta_{12}A_{m_1}R - \beta_{22}A_{m_2}R + \beta_{13}c_1 + \beta_{23}c_2 \tag{29}$$

$$\frac{dP}{dt} = R - \psi_1 P + \psi_{12}c_1 + \psi_{22}c_2 \tag{30}$$

$$\frac{dc_1}{dt} = A_{m_1}R - \gamma_{11}c_1 - \gamma_{12}c_1 \tag{31}$$

$$\frac{dc_2}{dt} = \frac{k_{A_2}}{k_{A_1}}\Big(A_{m_2}R - \gamma_{21}c_2 - \gamma_{22}c_2\Big) \tag{32}$$

where

$$\delta_{11} = \frac{k_{m_1}}{d_{A_1}A_0}\ ,\ \delta_{12} = \frac{d_{m_1}}{k_{A_1}A_0}\ ,\ \delta_{21} = \frac{k_{m_2}}{d_{A_2}A_0}\ ,\ \delta_{22} = \frac{d_{m_2}}{k_{A_2}A_0}\ ,$$

$$\alpha_{11} = \frac{d_{A_1}}{k_{A_1}A_0}\ ,\ \alpha_{12} = \frac{d_{Rm_1}k_R}{k_{A_1}A_0d_R}\ ,\ \alpha_{13} = \frac{d_{c_1}d_{Rm_1}k_R}{k_{A_1}^2A_0^2d_R}\ ,\ \alpha_{21} = \frac{d_{A_2}}{k_{A_2}A_0}\ ,\ \alpha_{22} = \frac{d_{Rm_2}k_R}{k_{A_2}A_0d_R}\ ,\ \alpha_{23} = \frac{d_{c_2}d_{Rm_2}k_R}{k_{A_2}^2A_0^2d_R}\ ,$$

$$\beta_1 = \frac{d_R}{k_{A_1}A_0}\ ,\ \beta_{12} = \frac{d_{Rm_1}}{k_{A_1}}\ ,\ \beta_{22} = \frac{d_{Rm_2}}{k_{A_1}}\ ,\ \beta_{13} = \frac{d_{c_1}d_{Rm_1}}{k_{A_1}^2A_0}\ ,\ \beta_{23} = \frac{d_{c_2}d_{Rm_2}}{k_{A_1}k_{A_2}A_0}\ ,$$

$$\psi_1 = \frac{d_P}{k_{A_1}A_0}\ ,\ \psi_{12} = \frac{b_1d_{Rm_1}}{k_{A_1}k_P}\ ,\ \psi_{22} = \frac{b_2d_{Rm_2}}{k_{A_2}k_P}\ ,\ \gamma_{11} = \frac{d_{c_1}}{k_{A_1}A_0}\ ,\ \gamma_{12} = \frac{l_{c_1}}{k_{A_1}A_0}\ ,\ \gamma_{21} = \frac{d_{c_2}}{k_{A_2}A_0}\ ,\ \gamma_{22} = \frac{l_{c_2}}{k_{A_2}A_0}.$$

Equations (25) to (32) are solved using *ode45* in MATLAB and the steady states are plotted in Figure 6. The values of the parameters are given in Table C, whereas $\alpha_{12} = \beta_{12} = \alpha_{22} = \beta_{22}$ are varied between 1 to 2 with step size 0.05. There are four steady states visible in the graph. Increasing the formation of microRNA complex rate decreases the concentration of protein as expected. This does not reflect the results from the Nyayanit and Gadgil model.

There are negative values for the steady state of the protein in Figure 6. The stability of these steady states has been examined and they are unstable. Hence, these steady states will not be observed in experiments, which aligns with the fact that negative concentration is not physically feasible.

To explore the modified Nyayanit and Gadgil model, the rate of formation of microRNA complex of the second microRNA is doubled so that $\delta_{21} = 4.2$. The other parameter values are set to be the same as Figure 6, giving Figure 7. There are five steady states visible on the graph with same trend as Figure 6. However, it can be seen that the values are more spread out.

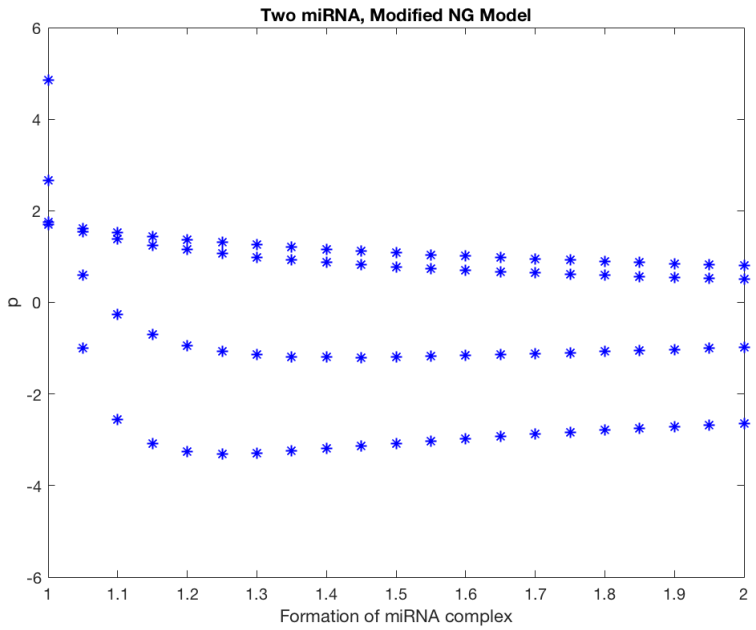

Figure 6: Steady states of modified NG model

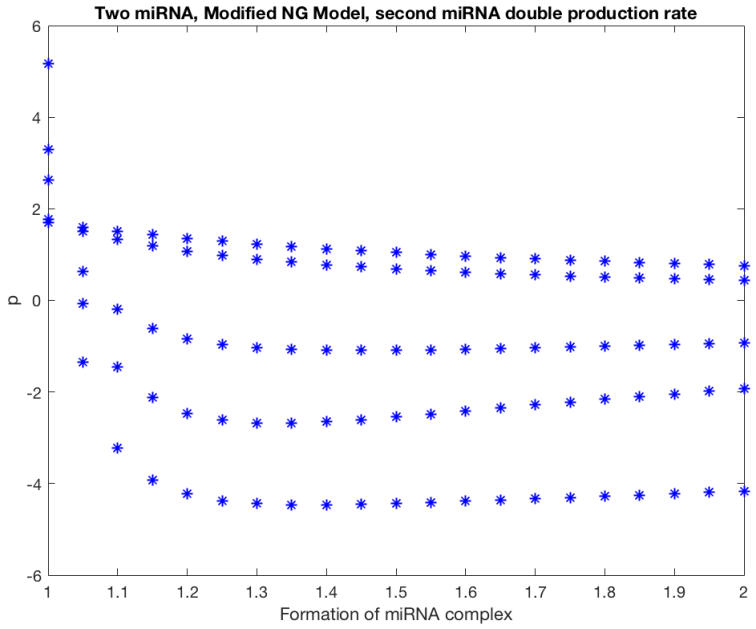

Figure 7: Steady states of modified NG model, second miRNA complex formation rate doubled

5 Discussion and Conclusion

In this project I have learned about modelling chemical reactions, specifically using the Law of Mass Action. It models the rate of change of concentration of product proportional to the concentration of reactants. Also, I have learned about the stability of Ordinary Differential Equations (ODEs) and simulating them in MATLAB.

I have looked through the Klironomos and Berg model (Klironomos & Berg, 2013), as well as the Nyayanit and Gadgil model (Nyayanit & Gadgil, 2015). The Klironomos and Berg model was extended to have two microRNAs in the system whereas the Nyayanit and Gadgil model was extended to include Argonaute and RISC. When simulating the extended Klironomos and Berg model, I found the counter-intuitive result, where increasing the microRNA concentration increases the protein production. However, when explored further, the steady states are unstable. Hence, it cannot be observed in experiments. Looking through the Nyayanit and Gadgil model, similar results occured in the original model. However, the steady states are stable in this case, meaning that it can be observed when doing experiments. Yet, when the Nyayanit and Gadgil model was modified to include the Argonaute and RISC, the result was not observed for the parameter values we considered.

In the original Nyayanit and Gadgil model, it is questionable whether or not the translation of the complex is possible. However, when the translation of the complex is set to be zero, the counter-intuitive result was still observed. The next step of this project would be to investigate thoroughly the parameter values that causes the counter-intuitive result to occur. This will enrich our understanding on the behaviour of microRNA, Argonaute and RISC, as well as expand possible utilizations of them.

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Appendices

A Parameter value for Figure 2

Parameter	Value
δ_1	2.1
δ_2	1.2
α_1	1.2
α_2	1
α_3	0.1
β_1	1.2
β_2	1
β_3	0.1
ψ_1	0.6
ψ_2	0.01
γ_1	0.1
γ_2	0.01

B Parameter value for Figure 5

Parameter	Value
k_{m_1}, k_{m_2}	1
d_{m_1}, d_{m_2}	0.0025
k_1, k_2	0.2943
d_{c_1}, d_{c_2}	0.0208
k_R	2
d_R	0.002
k_P	0.01
d_P	0.001
b_1, b_2	0.001
l_{c_1}, l_{c_2}	0.00228

C Parameter value for Figure 6

Parameter	Value
δ_{11}	2.1
δ_{21}	2.1
δ_{21}	1.2
δ_{21}	1.2
α_{11}	1.2
α_{21}	1.2
α_{13}	0.1
α_{23}	0.1
β_{11}	1.2
β_{21}	1.2
β_{13}	0.1
β_{23}	0.1
ψ_1	0.6
ψ_{12}	0.01
ψ_{22}	0.01
γ_{11}	0.1
γ_{21}	0.1
γ_{21}	0.01
γ_{22}	0.01