Chapter 5

Exploring MicroRNA-mediated Binary Gene Expression

5.1 Introduction

RNAs are divided into two distinct classes: messenger RNAs (mRNAs), from which proteins are synthesized, and the non-protein-coding RNAs (ncRNAs), which are not translated into proteins. For many years it was believed that there were only a few types of ncRNAs, and they (e.g., tRNAs, rRNAs etc.) were accessories in the synthesis and functioning of proteins. In recent years, a large number of ncRNAs have been identified which function as regulatory molecules and now it is known that the cellular functions of ncRNAs, on their own or in protein complexes, are varied as well as important. On the basis of functionalities, two large classes of ncRNAs been identified so far [1, 2]:

- Small nucleolar RNA (snoRNA) family, and
- MicroRNA (miRNA) and small interfering RNA (siRNA) family

In our thesis studies, we explore the regulation of gene expression by miRNAs. MiRNAs are a class of small, noncoding RNAs which regulate gene expression in prokaryotes and eukaryotes at the post-transcriptional level. A key aspect of the regulation of gene expression is the cytoplasmic control of mRNA translation and degradation. Over the past decade, miRNAs have emerged as important regulators of translation and mRNA
decay. Usually, the regulatory pathways mediated by these miRNAs are collectively referred to as RNA silencing. They play critical roles in a number of cellular processes [3–5], such as stress response, developmental transitions, differentiation, apoptosis etc. The mechanisms of regulation by miRNAs differ in specific features in prokaryotes and eukaryotes. These are, however, based on the common principle of the regulatory RNA base-pairing with the messenger RNA (mRNA) of the target gene inhibiting translation and/or promoting mRNA degradation. Various possibilities have been suggested for the fate of the inactive complex of the regulatory RNA and the target mRNA once it is formed [6–9]: (i) the complex has a finite lifetime followed by dissociation into its free components, (ii) the regulatory RNA co-degrades with the target mRNA at the same or different rates and (iii) only the target mRNA degrades with the regulatory RNA becoming free for further activity [6–9]. The importance of miRNA-mediated regulation lies in the following facts: (i) coordination, each miRNA targets hundreds of genes and that the majority of protein-coding genes are miRNA targets [10–13], (ii) abundance, with some miRNAs expressed as highly as 50,000 copies per cell [14] and (iii) sequence conservation, with some miRNAs conserved from sea urchins to humans [15].

5.2 Some Characteristic Functional Features

Recent studies suggest that miRNA repressors are in fact distinct from transcriptional repressors at a quantitative level. A great deal of progress has been made in the past few years to explore the possible mechanisms of miRNA-mediated post-transcriptional repression. A number of functional features of miRNA-regulated gene expression have been identified so far [6, 8, 9].

Threshold Linear Response

One prominent feature is that of a threshold linear mode of action, in which the target gene protein synthesis is highly repressed below a threshold level of target mRNA production and activated in a linear fashion once the threshold is crossed. Consider the case of a miRNA with a single target in the steady state. If the rate of miRNA transcription ($\alpha_s$) is larger than that of the target mRNA, $\alpha_m$, (i.e., $\alpha_s > \alpha_m$), then the amount of miRNA present in the system will be sufficiently large to pair up rapidly with all the target mRNAs and the target will not be expressed. Conversely, if $\alpha_s < \alpha_m$ then a number of unpaired mRNAs will be free to be translated into protein molecules. In other words, the expression of a target gene is silenced as long as its transcription rate, $\alpha_m$, is smaller than that of the repressor miRNA, $\alpha_s$. This is why miRNAs can tightly repress their target genes when their synthesis rate is smaller than some threshold (set by the transcription rate), but have little or no effect at all when the synthesis rate is much
Figure 5.1: Some features of miRNA-mediated regulation. (a) Threshold-linear response associated with the threshold at $\alpha_m \approx \alpha_a$. For $\alpha_m > \alpha_a$, there is an abrupt switch-on of target gene expression. (b) The upper panels show the cellular protein levels as a function of time and individual transcription events for transcriptional regulation. The lower panels show the same graphs for miRNA-mediated regulation. In the case of transcriptional repression, rare transcription events are accompanied by large protein bursts. In the case of post-transcriptional repression by miRNAs, the transcription events are frequent but the burst size is considerably reduced.
larger than that threshold. A schematic response shown in Figure 5.1(a), where $\alpha_m$ is taken to be continuously tunable at a given $\alpha_s$. This qualitative estimation of threshold linear behaviour has been confirmed experimentally in *E. coli* using a synthetic reporter target [8].

**Suppression of Fluctuations**

Let us consider a gene, the expression of which is tightly repressed by a transcription factor, so that transcription events are rare. When mRNAs are synthesized due to a rare transcriptional event, an amplification occurs via recurring translation events resulting in a burst of proteins. The longer the lifetime of the mRNA molecule, the larger is the size of the burst. Suppressing the gene expression by miRNAs, rather than by transcription factors, makes the kinetics much more homogeneous. It reduces the fluctuations originating from protein bursts by decreasing the rate of translation as well as the lifetime of the mRNA molecules. A schematic time trace shown in Figure 5.1(b) illustrates the phenomena.

**Prioritisation of the expression of target genes**

Many of the known miRNAs have more than one mRNA target [16]. In this scenario, targets with different affinities for association with the miRNA, will compete for association with the same pool of miRNA molecules. For example, consider a case of two targets, with respective association rate constants $\mu_1$ and $\mu_2$. If $\mu_1 \gg \mu_2$, depending upon the value of $\alpha_s$, the steady state may be such that either both targets are expressed (low $\alpha_s$), only target 2 is expressed (intermediate $\alpha_s$), or none are expressed (high $\alpha_s$). An illustrative schematics is shown in the left panel of Figure 5.2. Another interesting scenario arises when we consider the example in which the transcription rate of a miRNA regulator is larger than that of either targets (suppose, $\alpha_1$ and $\alpha_2$), but smaller than their sum ($\alpha_1 + \alpha_2$). Due to the threshold-linear nature of the response (Figure 5.1(a)), either target is expected to be silenced when transcribed alone, but can be expressed if both are transcribed together (Figure 5.2, right panel). Other significant characteristics of miRNA-mediated regulation include: rapid response times and filtering of transient signals, sharpening of spatial expression patterns and stabilization of multi-stable genetic systems [9, 17]. These features should be taken into account while analyzing the responses of systems involving miRNA regulation.

### 5.3 Models & Results

Recently, a mathematical model has been proposed [6] to describe the biochemical interactions and kinetics of miRNA-mediated regulation of gene expression. The model
Figure 5.2: Coordinated response of miRNA targets in the case of discussed examples. Left panel demonstrates the scenario of two targets, with respective association rate constants $\mu_1$ and $\mu_2$. Depending upon the value of $\alpha_s$, the steady state may be such that either both targets are expressed (low $\alpha_s$), only target 2 is expressed (intermediate $\alpha_s$), or none are expressed (high $\alpha_s$). Right panel illustrates the example where transcription rate of a miRNA regulator $\alpha_s$ is larger than that of either targets ($\alpha_1$ and $\alpha_2$ respectively), but smaller than their sum ($\alpha_1 + \alpha_2$). Either target is silenced when transcribed alone but can be expressed if both are transcribed together.
5.3. Models & Results

has been experimentally validated in single cell measurements using quantitative fluorescence microscopy and flow cytometry. The experiments clearly demonstrate the high repression of target protein synthesis below a threshold level of target mRNA production, and a sensitive response above the threshold. A major finding is that the strength of the repression below the threshold has considerable cell-to-cell variation in a population of genetically-identical cells. This indicates that stochasticity may have non-trivial consequences in miRNA-mediated regulation of gene expression. Figure 5.3 shows a sketch of the different biochemical processes involved in the miRNA-mediated regulation of gene expression, which form the basis of the mathematical model proposed in [6]. The mRNA, as shown in Figure 5.3, is either free \((m)\) or is part of a miRNA-mRNA complex \((m^*)\). The target mRNA is transcribed with rate constant \(k_m\) and has a natural degradation rate constant \(\gamma_m\). The rate constants \(k_{on}\) and \(k_{off}\) are associated with the formation and dissociation of the bound complex of mRNA and miRNA. The bound miRNA becomes free either by unbinding the target mRNA (rate constant \(k_{off}\)) or by degrading the mRNA (rate constant \(\gamma_{m^*}\)). The model incorporates the important feature of molecular titration similar to the protein-protein titration analyzed earlier [18]. Protein sequestration occurs when a repressor protein binds an active protein thus forming an inactive complex. As shown in [18], the regulatory mechanism, termed molecular titration, can generate ultrasensitive input-output responses. In the case of miRNA-mediated regulation of gene expression, the inactive complex is that of mRNA and miRNA. Molecular titration is responsible for the observed sensitive dependence of protein expression on target mRNA input around a threshold level of target mRNA production.

In this chapter, we are going to analyze the dynamics of the model, the key biochemical processes of which are shown in Figure 5.3, from two different perspectives. In the first case, an additional process representing the autoactivation of the target gene expression by the target protein is included. In the second case, a simple stochastic version of the model of Figure 5.3 is considered. In this model, the only stochasticity is associated with the random transitions between the inactive and active expression states of the gene. In the inactive state, there is no transcriptional activity, i.e., mRNA synthesis. Transcription is only initiated in the active state of the gene. The degradation of the mRNA occurs in both the inactive and active states of the gene. We show that binary gene expression is a possible outcome in both of the scenarios described above. In the deterministic case, binary gene expression implies bistability, i.e., the coexistence of two stable expression states. In the stochastic case, the distribution of mRNA levels is bimodal, i.e., has two prominent peaks. In the following, we analyze the deterministic and stochastic models to investigate the origins of binary gene expression.
Figure 5.3: A schematic diagram describing the biochemical events involved in miRNA-regulated gene expression. The target mRNA is synthesized and degraded with rate constants $k_m$ and $\gamma_m$ respectively. The rate constants $k_{on}$ and $k_{off}$ describe the formation and dissociation of the bound complex $m^*$ of free mRNA $m$ and the miRNA. The bound miRNA degrades the mRNA with rate constant $\gamma_m$.

### 5.3.1 Deterministic Model

The total concentration of miRNA is assumed to be constant, since the miRNA turnover is slow compared to the timescale of gene expression [6]. The following set of differential equations describe the dynamics of the model.

\[
\frac{dm}{dt} = k_m + \frac{\beta_m p}{K + p} - k_{on} m m_i + k_{off} m^* - \gamma_m m \tag{5.1}
\]

\[
\frac{dm^*}{dt} = k_{on} m m_i - k_{off} m^* - \gamma_m m^* \tag{5.2}
\]

\[
\frac{dp}{dt} = k_p m - \gamma_p p \tag{5.3}
\]

The conservation condition for miRNA is

\[
mi_T = m_i + m^* \tag{5.4}
\]

In the equations above, $m_i$ and $mi_T$ represent the free and total miRNA concentrations. The second term on the r.h.s. of Eq. (5.1) represents the autoactivation of the target gene expression. The rate constant $\beta_m$ represents the maximum rate of mRNA synthesis due to autoactivation, with $K$ denoting the equilibrium dissociation constant for the binding of the regulatory protein at the promotor region of the target gene. The rate constants $k_p$ and $\gamma_p$ correspond to protein synthesis and degradation, respectively, with
5.3. Models & Results

Figure 5.4: (a) Steady state protein concentration $p_{st}$ versus $\alpha$. The parameter values are $k_5 = 0.01$, $K = 10$, $\phi = 0.2$ and $\lambda_\alpha = 0.1$. (b) Steady state protein concentration $p_{st}$ versus $\lambda_\alpha$. The parameter values are $k_5 = 0.01$, $K = 10$, $\phi = 0.3$ and $\alpha = 13$. (c) Phase diagram in the $\phi - \alpha$ plane showing a region of bistability between two regions of monostability. A continuous path between the two monostable regions is also possible. The parameter values are $k_5 = 0.01$, $K = 10$ and $\lambda_\alpha = 0.1$. (d) Phase diagram in the $\phi - \lambda_\alpha$ plane showing a region of bistability between two regions of monostability. The parameter values are $k_5 = 0.01$, $K = 10$ and $\alpha = 13$.

$p$ being the protein concentration. In the steady state, $\frac{dm}{dt} = 0$, $\frac{dm^*}{dt} = 0$ and $\frac{dp}{dt} = 0$ and one obtains the equations

\begin{align}
  k_m + \frac{\beta_m p}{K + p} - k_{on} m \frac{mi + k_{off} m^* - \gamma_m m}{m + \gamma_m} = 0, \quad m^* = \frac{k_{mi} m}{m + \lambda}, \quad \lambda = \frac{k_{off} + \gamma_m m^*}{k_{on}} \tag{5.5} \\
  k_m + \frac{\beta_m p}{K + p} - k_{on} m \frac{mi + k_{off} m^* - \gamma_m m}{m + \gamma_m} = 0, \quad m^* = \frac{k_{mi} m}{m + \lambda}, \quad \lambda = \frac{k_{off} + \gamma_m m^*}{k_{on}} \tag{5.6} \\
  p = \frac{\lambda p}{\gamma_p} \tag{5.7}
\end{align}

From these equations, the steady state protein concentration satisfies the equation

\begin{align}
  p_{st} = k_5 + \frac{\alpha p_{st}}{K + p_{st}} - \frac{\phi p_{st}}{p_{st} + \lambda_\alpha} \tag{5.8}
\end{align}

with $k_5 = \frac{K_m}{\lambda_\alpha}$, $\alpha = \frac{\beta_m}{\gamma_\alpha}$, $\phi = \frac{\gamma_\alpha}{\gamma_\alpha}$, $\lambda_\alpha = \frac{\lambda_p}{k_p}$ and $\gamma_\alpha = \frac{\gamma}{\gamma_p}$.

Eq. (5.8) has two stable steady state solutions, i.e., bistability in specific parameter regions. Figure 5.4(a) shows a plot of the steady state protein concentration, $p_{st}$, as a function of the parameter $\alpha$. The stable steady states are represented by solid lines.
whereas the dotted line describes the branch of unstable steady states. The plot exhibits hysteresis since the discontinuous transitions from the lower to the upper branch, and from the upper to the lower branch, occur at different values of the parameter $\alpha$, the so-called bifurcation points of the associated dynamics. Bistability constitutes a universal theme in several cell biological processes [19, 20] and signifies that a cell has a choice between two stable expression states for the same parameter values. The choice of the specific stable state depends on the previous history of the system. Figure 5.4(b) exhibits a plot of $p_{st}$ versus the parameter $\lambda_{\alpha}$. Figure 5.4(c) shows a phase diagram in the $\phi - \alpha$ plane in which a region of bistability separates two regions of monostability, corresponding to low and high expression states, respectively. Figure 5.4(d) shows a similar phase diagram in the $\phi - \lambda_{\alpha}$ plane. Bistability, in general, is an outcome of dynamics involving positive feedback and sufficient nonlinearity. So far we have mentioned possible origins of nonlinearity via the binding of the regulatory protein molecules at multiple sites of the promoter region of the gene, or when the regulatory proteins form multimers, such as dimers and tetramers, which then bind specific regions of the DNA (cooperativity in regulation) [21, 22]. We have also discussed in detail the new mechanism proposed by Tan et al. [23] by which a noncooperative positive feedback loop combined with a nonlinear protein decay term give rise to bistability in a parameter regime in the absence of cooperativity. A related study by Klumpp et al. [24] has also demonstrated the generation of a positive feedback loop due to cell growth inhibition by a protein. The nonlinear protein decay term in these cases (2nd term in the r.h.s. of Eq. (4.1)) has the same form as the last term in r.h.s. of Eq. (5.8). The origin of the nonlinearity is, however, different in the case of the post-transcriptional, i.e., miRNA-mediated regulation of gene expression. The effect of a transcriptional positive feedback loop on miRNA-regulated gene expression has been investigated in a number of earlier studies [25]. Specifically, bistability has been demonstrated in cases where the transcriptional positive feedback loop involves some form of cooperativity. Our present study establishes a new origin of bistability in miRNA-regulated gene expression, based on a non-cooperative transcriptional positive feedback loop and a nonlinear protein decay term. Since our model closely follows the experimentally-tested model proposed in Ref. [6], the inclusion of a transcriptional positive feedback loop in the original gene circuit could provide an experimental test of the new mechanism of bistability suggested by us.

5.3.2 Stochastic Model

The model is a generalization, incorporating miRNA-mediated regulation of gene expression, of an earlier stochastic model [26] in which the effect of stochasticity on unregulated/transcriptional factor-regulated gene expression was considered. More specifically, the condition for obtaining stochastic binary gene expression in terms of two gene ex-
expression parameters, \( r_1 = \frac{k_a}{\gamma_m} \) and \( r_2 = \frac{k_d}{\gamma_m} \) was derived in the previous study. The rate constants \( k_a \) and \( k_d \) are the activation and deactivation rate constants for transitions between the inactive and active states of the gene. The rate constant \( \gamma_m \) is the degradation rate constant of the gene expression product, which could be either mRNA or protein. In the present study, we determine the steady state distribution of mRNA levels. We assume that the concentration \( m^* \) of the mRNA-miRNA complex attains its steady state value at an earlier time point than the concentration \( m \) of free mRNA. As already mentioned, the only stochasticity in the model considered here is associated with the random transitions between the inactive and active states of the gene. In each state of the gene, the mRNA concentration evolves according to the equation,

\[
\frac{dm}{dt} = k_m z - \frac{\gamma^* m}{m + \lambda} - \gamma_m m
\]  

Eq. (5.9) is obtained from Eq. (5.1) by ignoring the positive feedback term and substituting the steady state concentration of \( m^* \) (Eq. (5.6)). The variable \( z = 1(0) \) when the gene is in the active (inactive) state and switches values with stochastic rate constants \( k_a (0 \rightarrow 1) \) and \( k_d (1 \rightarrow 0) \). Let \( \rho_j(m, t) \ (j = 0, 1) \) be the probability density function when \( z = j \) with the total probability density function \( \rho(m, t) = \rho_0(m, t) + \rho_1(m, t) \). The rate equations for the probability density functions are given by

\[
\frac{\partial \rho_0(m, t)}{\partial t} = -\frac{\partial}{\partial m} \left\{ \left( -\frac{\gamma^* m}{m + \lambda} - \gamma_m m \right) \rho_0(m, t) \right\} + k_d \rho_1(m, t) - k_a \rho_0(m, t) \tag{5.10}
\]

\[
\frac{\partial \rho_1(m, t)}{\partial t} = -\frac{\partial}{\partial m} \left\{ \left( k_m - \frac{\gamma^* m}{m + \lambda} - \gamma_m m \right) \rho_1(m, t) \right\} + k_a \rho_0(m, t) - k_d \rho_1(m, t) \tag{5.11}
\]

The first terms in Eqs. (5.10) and (5.11) are the so-called transport terms, representing the net flow of the probability density. The second terms represent the gain/loss in the probability density due to random transitions between the state \( j \) \((j = 1, 0)\) and the other accessible state. In the steady state, both \( \frac{\partial \rho_0}{\partial t} \) and \( \frac{\partial \rho_1}{\partial t} \) are zero and the total probability density function \( \rho \) satisfies the equation

\[
\frac{\partial \rho}{\partial m} = \frac{\left( k_a - f(m) \right) \rho}{f(m)} + \frac{\left( f(m) - k_d \right) \rho}{1 - f(m)} \tag{5.12}
\]

where

\[
f(m) = \frac{\gamma^* m}{m + \lambda} + \gamma_{new} m, \tag{5.13}
\]

\[
\gamma_{new} = \frac{\gamma_a}{k_m}, \quad \gamma_{new} = \frac{\gamma_m}{k_m}
\]

93
The steady state solution for $\rho$ is given by

$$
\rho(m) = N m^{a_1} (d_2 + m)^{a_2} (d_3 + m)^{a_3} (d_5 - m)^{a_4} (d_6 + m)^{a_4 - a_5}
$$

(5.14)

where $N$ is the normalization constant and the other terms are:

$$
\begin{align*}
a_1 &= \frac{k_a}{k_m} \left( \frac{\lambda}{\gamma_{new}^* + \gamma_{new}} \right) - 1 \\
a_2 &= \frac{k_a}{k_m} \left( \frac{\gamma_{new}^*}{\gamma_{new}^* + \gamma_{new}} \right) - 1 \\
a_3 &= 2 \\
a_4 &= \frac{k_d}{k_m} \frac{1}{2 \gamma_{new}} - 1 \\
a_5 &= \frac{k_d}{k_m} \left( \frac{1 - \gamma_{new}^* + \lambda \gamma_{new}}{2 \gamma_{new}} \right)^{-1} \frac{1}{2 \sqrt{\lambda \gamma_{new} + (\frac{\gamma_{new}^* + \lambda \gamma_{new}}{2})^2}} \\
\end{align*}
$$

(5.15)

and

$$
\begin{align*}
d_2 &= \frac{\gamma_{new}^*}{\gamma_{new}} + \lambda \\
d_3 &= \frac{\gamma_{new}^*}{\gamma_{new}} \lambda \\
d_5 &= \left( \frac{\gamma_{new}^* + \lambda \gamma_{new} - 1}{2 \gamma_{new}} \right)^2 + \frac{1}{2 \gamma_{new}} \sqrt{\left( \frac{\gamma_{new}^* + \lambda \gamma_{new} - 1}{2 \gamma_{new}} \right)^2 + 4 \lambda \gamma_{new}} \\
d_6 &= \left( \frac{\gamma_{new}^* + \lambda \gamma_{new} - 1}{2 \gamma_{new}} \right)^2 + \frac{1}{2 \gamma_{new}} \sqrt{\left( \frac{\gamma_{new}^* + \lambda \gamma_{new} - 1}{2 \gamma_{new}} \right)^2 + 4 \lambda \gamma_{new}} \\
\end{align*}
$$

(5.16)

Putting $\gamma_{new}^* = 0$ and $\lambda = 0$, i.e., considering only unregulated gene expression, one recovers from Eq. (5.14) the beta distribution [26, 27]: where $N_1$ is the normalization constant. In this case, binary gene expression is obtained in the parameter regime $r_1 = \frac{k_a}{\gamma_m}$ and $r_2 = \frac{k_d}{\gamma_m}$ both $< 1$. Two prominent peaks in the probability density function appear when $r_1$ and $r_2$ are comparable in magnitude. In the case of transcription-factor regulated gene expression, the effective activation and deactivation rate constants, $k_a'(s)$ and $k_d'(s)$, are functions of the regulatory protein (transcription factor) concentration $s$.

The gene expression response to a regulatory stimulus may be either graded or binary. The response is quantified in terms of the concentrations of mRNAs/proteins. In graded response, the average steady state concentration of the gene expression product varies continuously as the concentration $s$ of the regulatory molecules is changed, until a saturation level is reached. In the case of binary response, gene expression occurs at either of two average levels (say, low or high) and expression at other levels is minimal. The fraction of cells in the low/high expression level changes as $s$ is changed. This gives rise to a bimodal distribution in the protein/mRNA levels in an ensemble of cells. In the
case of transcription-factor regulated gene expression, binary gene expression is obtained when \( r_1 = \frac{k_a(s)}{\gamma_m} < 1 \) and \( r_2 = \frac{k_a(s)}{\gamma_m} < 1 \). One should point out that in the parameter regime in which binary gene expression occurs, unimodal distributions are obtained when \( r_1 << r_2 \) or \( r_2 << r_1 \). The full parameter regime is associated with the system exhibiting binary response to changing activation and deactivation rate constants. For example, for \( r_2 >> r_1 \), the probability distribution of mRNA levels has a single peak at a low level. As \( r_1 \) increases, a second peak appears at a high expression level with the peak becoming more prominent as \( r_1 \) approaches \( r_2 \). At the other extreme of parameter values, \( r_1 >> r_2 \), a single peak at the high expression level is obtained. As \( r_1 \) changes, the position of the peak remains the same, a characteristic of binary response. Stochastic binary gene expression refers to a bimodal distribution of mRNA/protein levels and the bifurcation from a unimodal to a bimodal distribution occurs in the parameter regime \( r_1 < 1 \) and \( r_2 < 1 \).

\[
\rho(m) = N_1 \, m^{(\frac{k_a(s)}{\gamma_m} - 1)}(\frac{k_d(s)}{\gamma_m})^{(\frac{k_d(s)}{\gamma_m} - 1)} \tag{5.17}
\]

In the case of miRNA-mediated regulation of gene expression, the most prominent contribution to binary gene expression is obtained when both \( a_1 \) and \( a_4 + a_5 \) are < 0 in Eq. (5.14), i.e., when the following inequalities are satisfied:

\[
\frac{k_a}{\gamma_m} < 1 + \frac{\gamma_{\text{new}}^*}{\lambda \gamma_{\text{new}}} \tag{5.18}
\]

\[
\frac{k_d}{\gamma_m} < 1 + \frac{1 - f}{1 + f} \quad , \quad f = \frac{\phi}{\sqrt{\phi^2 + \lambda \gamma_{\text{new}} \gamma_{\text{new}}}}
\]

with \( \phi = \frac{(1 - \gamma_{\text{new}}^* + \gamma_{\text{new}})}{2} \). One notes that the parameter region in which stochastic binary gene expression may be obtained is expanded from that (\( \frac{k_a}{\gamma_m} < 1 \) and \( \frac{k_d}{\gamma_m} < 1 \)) in the case of unregulated gene expression. Figure 5.5 shows the steady state mRNA distribution \( \rho(m) \) versus \( m \) when \( a_1 \) is < 0 and \( a_4 + a_5 < 0 \) for the parameter values (a) \( r_1 < 1, r_2 < 1 \) and (b) \( r_1 > 1, r_2 < 1 \). The other parameter values are \( \gamma_{\text{new}} = 0.1 \) and \( \gamma_{\text{new}}^* = 0.2 \). For each case, the values of \( \lambda \) are \( \lambda = 0.001, \lambda = 1 \) and \( \lambda = 20 \) respectively. We observe that increasing (decreasing) values of \( \lambda \) disfavours (favour) bimodality. For \( \gamma_{\text{new}}^* = 0.2, \lambda = 0 \), i.e., the case of unregulated gene expression, binary gene expression occurs in the parameter regime \( r_1 < 1, r_2 < 1 \) but not in the regime \( r_1 > 1, r_2 < 1 \). Figure 5.5 demonstrates the enhanced possibility of binary gene expression in the case of miRNA-mediated regulation of gene expression. One notes that if the parameter \( \lambda \) is set to zero in Eq. (5.16), the parameter regime in which binary gene expression is observed is expanded to include all values of the ratio \( r_1 = \frac{k_a}{\gamma_m} \) and \( r_2 = \frac{k_d}{\gamma_m} < 1 \). The parameter \( \lambda = \frac{k_{\text{off}} + \gamma_{\text{off}}^*}{k_{\text{on}}} \) is analogous to the dissociation constant for the formation of the bound
Figure 5.5: Plots of $\rho(m)$ versus $m$ (Eq. (5.14)) in the steady state ($a_1 < 0$ and $a_4 + a_5 < 0$). The values of $r_1$, $r_2$ and $\lambda$ are shown in individual boxes. The other parameter values are $\gamma_{new} = 0.1$, $\gamma_{new}^* = 0.2$. (a) $r_1 < 1$, $r_2 < 1$, (b) $r_1 > 1$, $r_2 < 1$. For a wide range of $\lambda$ values, binary gene expression is obtained in each case.
mRNA-miRNA complex with $\lambda = 0$ signifying infinitely strong binding. We now provide a physical understanding of the enhanced occurrence of binary gene expression when a miRNA-regulated gene expression parameter, say $\lambda$, is changed.

As we have mentioned earlier, molecular titration can generate ultrasensitive input-output responses. The origin of ultrasensitivity lies in the sequestration of an active component in an inactive complex through binding to an antagonist [6, 18, 28]. The simple mechanism involves the kinetic scheme

$$X + Y \rightleftharpoons XY$$

(5.19)

where $X$ represents the active molecule, $Y$ its antagonist and $XY$ the inactive complex. Let $K_d$ be the dissociation constant associated with the complex $XY$. The total concentrations of $X$ and $Y$ are given by $X_T = [X] + [XY]$ and $Y_T = [Y] + [XY]$. When $Y_T >> K_d$, the $X$ versus $X_T$ plot exhibits ultrasensitivity with the threshold set by $Y_T$. For $X_T < Y_T$, almost all the $X$ molecules are part of the inactive complex $XY$ so that
the concentration of free $X$ molecules is very low. At the threshold point $X_T \approx Y_T$, the sequestration of $X$ molecules is no longer dominant, so that a small increase in $X_T$ can give rise to a large increase in $X$. The sharpness of the ultrasensitive response in the simple example is controlled by the ratio $\frac{Y_T}{K_d}$ [18]. In the present study, $X$ and $Y$ represent the mRNA and the miRNA respectively. When $a_4 + a_5$ is $< 0$ in Eq. (5.14), a dominant singularity occurs at the point $m = d_5$, with the expression for $d_5$ given in Eq. (5.16). In terms of the original parameters, $d_5$ is given by

$$d_5 = \frac{1}{2} [(m_0 - \lambda - \theta_{th}) + [(m_0 - \lambda - \theta_{th})^2 + 4m_0\lambda]^{1/2}]$$  \hspace{1cm} (5.20)$$

where $m_0 = \frac{k_m}{\gamma_m}$, $\theta_{th} = \frac{\gamma^*}{\gamma_m} = \frac{\gamma^*_{miR}}{\gamma_m}$ and $\lambda$ is given by the expression in Eq. (5.6). The magnitude of $m_0$ is a measure of the total amount of mRNA. When the effective dissociation constant $\lambda \to 0$, we obtain

$$m = d_5 = \frac{1}{2} [m_0 - \theta_{th} + \sqrt{(m_0 - \theta_{th})^2}]$$ \hspace{1cm} (5.21)$$

$$= 0 \text{ if } m_0 < \theta_{th}$$

$$= m_0 \text{ if } m_0 > \theta_{th}$$

The constant $\theta_{th}$ is proportional to the total amount, $mi_T$, of miRNAs and sets the threshold of an ultrasensitive steady state response. Figure 5.6(a) shows the steady state $m$ versus $m_0$ plots (Eq. (5.20)) on a semi-logarithmic scale for the values of $\lambda = 10^{-5}$ (dash-dotted line), $\lambda = 0.001$ (dashed line), $\lambda = 0.01$ (thin solid line) and $\lambda = 0.1$ (thick solid line). One finds that the ultrasensitive behaviour is prominent only when $\lambda$ is close to zero. The value of $\theta_{th}$ is fixed at the value 2. Figures 5.6(b) - (d) show the steady state $\rho (m)$ versus $m$ curves (Eq. (5.14)) for the parameters values $\gamma^*_{new} = 0.2$, $\gamma_{new} = 0.1$ and $m_0$ greater than $\theta_{th}$ (b), almost equal to $\theta_{th}$ (c) and less than $\theta_{th}$ (d). In the last case, the second peak at $m = d_5$ ($a_4 + a_5 < 0$ in Eq. (5.14)) occurs very close to the peak at $m = 0$ ($a_1 < 0$).

The inequalities in Eq. (5.18) can be rewritten in a simpler form

$$\frac{k_a}{\gamma_{eff1}} < 1 , \quad \frac{k_d}{\gamma_{eff2}} < 1$$  \hspace{1cm} (5.22)$$

with $\gamma_{eff1} = \frac{\gamma^*}{X} + \gamma_m$ and $\gamma_{eff2} = \gamma_m(1 + \frac{1-f}{1+f})$.

The rate constants $\gamma_{eff1}$ and $\gamma_{eff2}$ can be interpreted as effective mRNA decay rate constants by noting the following. From Eq. (5.9), for $z = 0$, i.e., when the target gene is in the inactive state,

$$\frac{dm}{dt} = -\gamma^* \frac{m}{m + \lambda} - \gamma_m m = f(m)$$

On linearising $f(m)$ around the steady state value $m = 0$, one obtains
Similarly, for $z = 1$, i.e., when the target gene is in the active state, $f(m) = k_m - \frac{\gamma m}{m+\lambda} - \gamma_m m$. Again, linearising $f(m)$ around the steady state value $m = d_5$, one gets

$$\frac{dm}{dt} = k_m - \gamma_m (1 + \frac{1-f}{1+f}) m = k_m - \gamma_{eff2} m \quad (5.24)$$

Eqs. (5.23) and (5.24) are identical in forms to the equations obtained in the case of unregulated gene expression. In the latter case, however, $\gamma_{eff1} = \gamma_{eff2} = \gamma_m$. The origin of binary gene expression in the parameter regime $\frac{k_a}{\gamma_{eff1}} < 1$, $\frac{k_d}{\gamma_{eff2}} < 1$ now has a clear physical interpretation. In the inactive state of the gene, the activation rate constant has a lower value than that of the effective mRNA degradation rate constant $\gamma_{eff1}$ so that the accumulated protein level can decay to the steady state level $m = 0$ before the next transition to the active state of the gene occurs. Similarly, in the active state of the gene, the deactivation rate constant has a magnitude lower than that of the effective mRNA degradation rate constant $\gamma_{eff2}$. The gene is thus in the active state for a sufficiently long time so that the steady state mRNA level $m = d_5$ (Eq. (5.20)) is attained. The parameter regime (Eq. (5.22)) corresponds to binary response in gene expression as changing the $k_a$, $k_d$ values does not alter the peak positions but only the amplitudes. When both $\frac{k_a}{\gamma_{eff1}}$ and $\frac{k_d}{\gamma_{eff2}}$ are $> 1$, the activation/deactivation rate constant is larger than the effective mRNA degradation rate constant, so that a transition from the inactive to the active state and vice versa occurs before the mRNA level can attain the value $m = 0$ in the inactive state, and the value $m = d_5$ in the active state. As a result, the probability distribution $\rho(m)$ is unimodal, with the peak position at an intermediate mRNA level. One also obtains a graded response to changing values of the rate constants $k_a$ and $k_d$, i.e., the position of the peak shifts in a graded manner. In the steady state expression for $\rho(m)$, for $a_1 < 0$ and $a_4 + a_5 < 0$, the singularities occur at $m = 0$ and $m = d_5$. The other terms add a small background correction to the distribution. Figure 5.7 shows the plot of $\rho(m)$ versus $m$ in the case when $a_1 < 0$ and $a_4 - a_5 < 0$, $(a_4 + a_5 > 0)$. In this case, though a bimodal distribution is obtained for $r_1$, $r_2 > 1$ and $\lambda$ small, the second peak is associated with quite a broad distribution ((a) and (b)). When the magnitude of $\lambda$ is increased from $\lambda = 0.002$ (b) to $\lambda = 0.2$ (c), a unimodal distribution is obtained.

5.4 Discussions

A large number of studies, both theoretical and experimental, have been carried out in the last few years on the origins and consequences of binary gene expression, the novel feature in which the distribution of mRNA/protein levels has two prominent peaks. The
possible origins of binary gene expression are several of which three principal mechanisms have been the focus of recent studies [29]: positive feedback-based [20, 21], emergent bistability [23, 30] and purely stochastic [26, 31–36]. The first two mechanisms create the potential for the coexistence of two stable expression states and noise-induced transitions between the states give rise to the bimodal nature of the distribution of mRNA/protein levels. In the case of binary gene expression with a purely stochastic origin, there is no bistability in the deterministic limit. Experimental observations of stochastic binary gene expression have been reported in a number of studies [33, 34, 36], in agreement with theoretical results. The large number of studies carried out so far on binary gene expression consider the expression to be unregulated or transcription-factor regulated. The issue of binary gene expression in the case of post-transcriptional regulation of gene expression has been mostly confined to the investigation of models in which both transcriptional and post-transcriptional (e.g., miRNA-regulated) modes of regulation are considered and the transcriptional regulation involves a cooperative positive feedback. In this chapter, we have discussed two possible scenarios for miRNA-mediated regulation of gene expression, one deterministic and the other stochastic, and demonstrated the existence of binary gene expression in each case. In the first case, a nonlinear protein decay term, together with a noncooperative positive feedback term generate bistability in specific parameter regimes. In the second case, the conditions for obtaining stochastic binary gene expression are obtained and one finds that the parameter region in which binary gene expression occurs is more extended in comparison with the cases of unregulated and transcription factor-regulated gene expression. In the latter two cases, slow transitions between the inactive and active gene expression states are responsible for binary gene expression. The conditions \( \frac{\mu_a}{T_m} < 1 \) and \( \frac{\mu_d}{T_m} < 1 \) can be reexpressed as \( \frac{T_a}{T_m} > 1 \) and \( \frac{T_d}{T_m} > 1 \) with \( T_a, T_d \) being the average lifetimes of the active and inactive gene expression states (\( T_a \sim \frac{1}{k_a}, T_d \sim \frac{1}{k_d} \)) and \( T_m \) is the average lifetime of
the mRNAs. In the case of miRNA-mediated regulation of gene expression, ultrasensitivity generated by molecular titration plays a key role in the generation of binary gene expression. The conditions $\frac{k_a}{\tau_{eff1}} < 1$ and $\frac{k_d}{\tau_{eff2}} < 1$ (Eq. (5.22)) set new time scales in the characterization of the slowness of the transitions between the gene expression states. This results in an expanded parameter regime in which binary gene expression is observed. The theoretical predictions made in the present study could be tested in experiments involving natural and synthetic gene circuits.

Binary gene expression generates phenotypic heterogeneity in a cell population with identical genetic makeup and exposed to the same initial conditions and environment. Microorganisms adopt a number of strategies for coping with stressful situations like environmental fluctuations, nutrient depletion and application of antibiotic drugs. One such strategy is the creation of phenotypic heterogeneity so that the whole population does not suffer the same fate when subjected to stress [28, 37, 38]. Recent experiments on *E. coli* [37, 38], *B. Subtilis* [28] and *M. Smegmatis* [39, 40] have demonstrated the advantages of phenotypic heterogeneity when microorganisms are subjected to stress. The regulation of gene expression by miRNAs is known to be activated in a number of cases in which a cell population is subjected to stress [3-5]. Experimental evidence of phenotypic heterogeneity in the form of binary gene expression due to regulation by miRNAs would be of significant interest in the context of the response of cell populations to stressful conditions.

### 5.5 Summary

MicroRNA-mediated regulation of gene expression is characterised by some distinctive features that set it apart from unregulated and transcription factor-regulated gene expression. Recently, a mathematical model has been proposed to describe the dynamics of post-transcriptional regulation by microRNAs. The model explains the observations made in single cell experiments quite well. In our thesis studies, we introduce some additional features into the model and consider two specific cases. In the first case, a non-cooperative positive feedback loop is included in the transcriptional regulation of the target gene expression. In the second case, a stochastic version of the original model is considered in which there are random transitions between the inactive and active expression states of the gene. In the first case we show that bistability is possible in a parameter regime, due to the presence of a non-linear protein decay term in the gene expression dynamics. In the second case, we derive the conditions for obtaining stochastic binary gene expression. We find that this type of gene expression is more favourable in the case of regulation by microRNAs as compared to the case of unregulated gene expression. The theoretical predictions relating to binary gene expression are experimentally testable.
Bibliography


[38] NQ Balaban (2011) Persistence: mechanisms for triggering and enhancing phenotypic variability, Curr Opin Genet Dev. 21(6), 768-775.
