Chapter 7

Title: Mathematical modeling for oscillations driven by noncoding RNAs

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Abstract

In this chapter, we first survey strategies for mathematical modeling of gene regulatory networks for capturing physiologically important dynamics in cells such as oscillations. We focus on models based on ordinary differential equations with various forms of nonlinear functions that describe gene regulations. We next use a small system of a microRNA and its mRNA target to illustrate a recently discovered oscillator driven by noncoding RNAs. This oscillator has unique features that distinguish itself from conventional biological oscillators, including the absence of imposed negative feedback loop and the divergence of the periods. The latter property may serve crucial biological functions for restoring heterogeneity of cell populations on the timescale of days. We describe general requirements for obtaining the limit cycle oscillations in terms of underlying biochemical reactions and kinetic rate constants. We discuss future directions stemming from this minimal, noncoding RNA-based model for gene expression oscillation.

Keywords: Mathematical modeling. Ordinary differential equation. Limit cycle. Biochemical oscillator. microRNA.

Introduction

Biological oscillators have been studied extensively due to their functional roles in controlling cellular activities with rhythmic gene expression dynamics. These functions include metabolic cycles, circadian rhythms, developmental timing, pattern formation, and cell cycle regulation in early embryo and cancer cells [1-5]. It has been known for several decades that negative feedback loop with a delay can generate biochemical oscillations [6-8]. This concept has been helpful for designing synthetic circuits that can act as oscillators [9], in which the expression of a gene is negatively regulated by itself via an intermediate gene. The studies of negative feedback loops in gene regulatory networks also facilitated the discoveries of some natural oscillators in cells [10,11]. However, it is not always possible to relate biochemical reaction networks, including gene regulatory networks, to intuitively identifiable delayed negative feedback. For example, mathematical modeling showed that a double-phosphorylation cycle of a MAP kinase network can generate sustained oscillation without any imposed feedback loop [12,13].

Oscillatory dynamics of noncoding RNAs (ncRNAs), such as microRNAs, have been widely observed in systems such as circadian rhythms, metabolic cycles, and development [14-16]. However, until recently experimental and theoretical studies of the roles of noncoding RNAs in oscillations had been confined in the framework of the negative feedback loops of gene expression. One example of these ncRNA circuits is the Hes1-miR-9 network in which the combination of self-inhibitory loop and the cross-repression between Hes1 and miR-9 produces oscillatory dynamics [17]. While ncRNAs were not generally considered to be involved in core negative feedback loops in many systems, they were found to increase the cell-to-cell variability of their target mRNAs' expressions, and the complex correlations between the expressions of ncRNAs and their target mRNAs can be difficult to interpret in some scenarios [18-20]. A recent modeling study showed that interactions between microRNA and mRNAs alone can produce sustained oscillations even in the absence of transcription-level feedback [21], and it suggested potential biological functions of the new RNA-based oscillator. In this chapter, we will briefly survey modeling strategies for studying gene expression and ncRNA dynamics. We will then use a representative model to demonstrate key components

and assumptions for producing oscillatory dynamics from a simple ncRNA-mRNA reaction network. We will also discuss the biological implications of this and related models.

Models

To capture dynamics of gene expression, models based on ordinary differential equations (ODEs) are often used with linear or nonlinear functions that describe interactions of molecular species. There are two categories of these functions. The first type is functions that produce sigmoidal-curve-like relationship between input and output (**Figure 1A**). The most common function in this category is the Hill function. For example, an inhibitory form of the Hill function is $f(x) = 1/(1 + (x/K)^n)$, where x represents the level of a regulator for the expression of a gene (either the one coding for itself or another one), K is the inhibition threshold, and n is the Hill exponent controlling the nonlinearity or the steepness of the function. A Hill function can be used in the production term of a state variable representing a gene product. In this case, x is a transcriptional or translational regulator. If we assume gene product y is regulated by x, then the ODE for molecular species y can be written in the form

$$dy/dt = bf(x) - ky, \tag{1}$$

where b is the maximum synthesis rate and k is the degradation rate constant for this molecular species. In an ODE model for the synthetic circuit of the repressilator [9], a loop containing three genes, each expressing a protein that represses the next gene in the loop (**Figure 1B**), the level of each protein is used as the regulator of the next gene's expression in a form similar to $f(x)$. A simplified version of this repressilator model contains three ODEs in the form of Eq 1. With this model, a sustained oscillation is produced from this imposed negative feedback loop (**Figure 1C**). When there are multiple regulators for the same gene, multiplicative or additive combinations of Hill functions can be used to describe these

complex regulations [22]. In the context of ncRNAs, the Hill function can also be used to describe the influence of a regulator (ncRNA) on its target's degradation rather than production. For example, in an ODE model for the Hes1-miR-9 network, *Hes1* mRNA's half-life is regulated by miR-9 in a form similar to $f(x)$ [17]. It should be noted, however, that in this microRNA model, miR-9 is not involved in a negative feedback loop (**Figure 1D**). Instead, Hes1 regulates its own expression with a delay, and this loop is essential for producing sustained oscillations. In addition to the Hill function, a hyperbolic tangent function (sometimes referred to as a sigmoidal function) such as $g(x) = 1/(1 + e^{-\sigma(w_0+wx)})$, where σ determines the steepness of the nonlinear function, w is the weight of the activation/repression by regulator level x, and w_0 is the offset, can also be used to describe gene regulation. This form of ODE was used for modeling the circadian clock in Arabidopsis and the model was able to capture experimental data of oscillatory gene dynamics under multiple conditions [23].

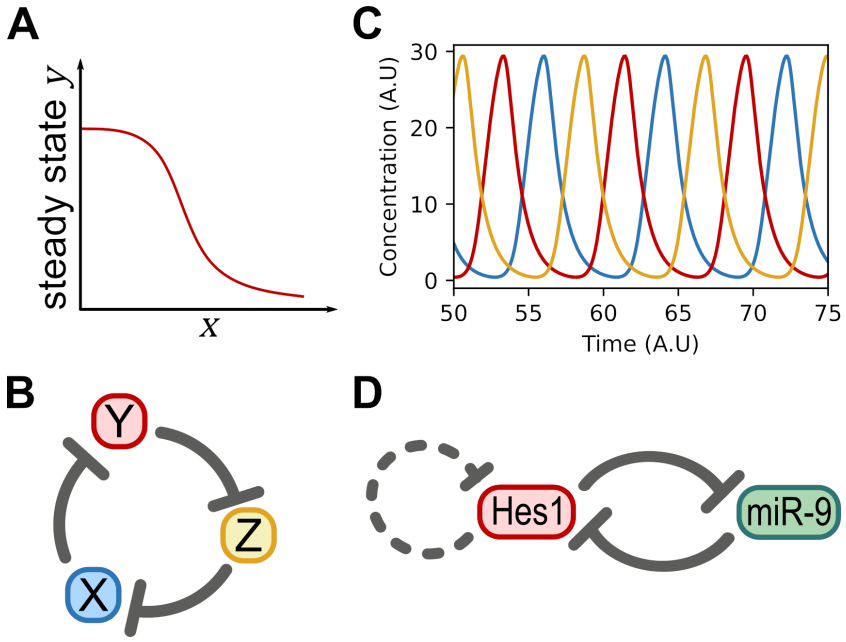

Figure 1. **Conventional approaches to model biological oscillators**. **A**. An illustration of the response curve of the sigmoidal function type. **B**. Influence diagram of the repressilator. **C**. Example simulation result for the simplified repressilator model. **D**. Influence diagram of the Hes1-miR-9 oscillator model.

While the sigmoidal function type is very popular due to its simplicity in implementation, it is phenomenological, rather than mechanistic, at the level of biochemical reactions. A requirement of using the Hill function or other similar functions is that one must abstract biochemical reactions into signed directed graphs (e.g. **Figure 1B**) before constructing a model. This abstraction is helpful in many scenarios, but it may lead to over-simplification in others. Perhaps more importantly, because of the requirement, it is not feasible to capture some interesting dynamics of gene regulation by using the sigmoidal functions or their associated signed directed graphs. For example, networks of multisite phosphorylation cycles of MAP kinase (post-translational control of protein activities) can produce multistability (a phenomenon used to be associated with a positive feedback loop in gene regulation) [24], and oscillation [12,13]. Yet, there is no intuitive way to relate the essential components for these emerging dynamics to sigmoidal functions or a positive/negative feedback loop in a graph. The strategies for describing these reaction networks involve a different category of functions based on the law of mass action. Specifically, the rate of a reaction, including binding, unbinding, production, and degradation, is proportional to concentration(s) of reactant(s) in these elementary reactions. Instead of introducing the mass-action-based ODE models in general, we will use a recent model for oscillations driven by ncRNA-mRNA interactions as an example to illustrate its structure and usefulness in studying ncRNAs [21].

In this simple model (**Figure 2A**), we describe an mRNA that contains two binding sites of a microRNA. The system contains twelve elementary biochemical reactions

$$\xrightarrow{s_R} \hat{R} \qquad \hat{R} \xrightarrow{k_R^0} \emptyset \qquad \xrightarrow{s_r} \hat{r} \qquad \hat{r} \xrightarrow{k_r^0} \emptyset \qquad \hat{R} + \hat{r} \xrightarrow{k^{\mathrm{on}}} \hat{C}_1 \qquad \hat{C}_1 \xrightarrow{k^{\mathrm{off}}} \hat{R} + \hat{r} \qquad \hat{C}_1 \xrightarrow{k_r^1} \hat{R} \qquad \hat{C}_1 \xrightarrow{k_R^1} \hat{r}$$

$$\hat{C}_1 + \hat{r} \xrightarrow{k^{\mathrm{on}}} \hat{C}_2 \qquad \hat{C}_2 \xrightarrow{k^{\mathrm{off}}} \hat{C}_1 + \hat{r} \qquad \hat{C}_2 \xrightarrow{k_r^1} \hat{C}_1 \qquad \hat{C}_2 \xrightarrow{k_R^2} 2r.$$

Here, $\hat{R}$ and $\hat{r}$ represent both the molecular species and the corresponding concentrations of unbound mRNA and unbound microRNA respectively. $\hat{C}_1$ and $\hat{C}_2$ are the concentrations of a 1:1 complex and the 2:1 complex respectively. Because the mRNA has two binding sites of the microRNA, which are assumed to be identical, $\hat{C}_1$ represents either form of the 1:1 complex and its concentration. The total concentration of 1:1 complex is $2\hat{C}_1$. s_R is the transcription rate constant of mRNA. s_r is the transcription rate constant of microRNA. k_R^0 is the degradation rate constant of the unbound mRNA. k_R^1 and k_R^2 are the degradation rate constants of the mRNA in the 1:1 complex and the 2:1 complex respectively. k_r^1 and k_r^2 are the degradation rate constants of the microRNA in the 1:1 complex and in the 2:1 complex respectively. k_r^0 is the degradation rate constant of the unbound microRNA. κ^{on} is the association rate constant. κ^{off} is the dissociation rate constant. It should be noted that this model is very general: it can be easily modified to study an mRNA with multiple binding sites for different microRNAs; and it can be used to describe other (nc)RNA species (e.g. $\hat{R}$ can represent the concentration of a long-noncoding RNA that harbors multiple binding sites for another RNA) or even proteins.

We make the following changes to relate the dimensionless variables and parameters to the original ones:

$$\hat{t} = t/k_R^0, \qquad \hat{R} = Rs_r/k_R^0, \qquad \hat{r} = rs_r/k_R^0, \qquad \hat{C}_1 = C_1 s_r/k_R^0, \qquad \hat{C}_2 = C_2 s_r/k_R^0$$

$$\sigma_R = s_R/s_r, \qquad \kappa^{\mathrm{on}} = k^{\mathrm{on}} s_r/{k_R^0}^2, \qquad \kappa^{\mathrm{off}} = k^{\mathrm{off}}/k_R^0, \qquad \gamma = k_r^0/k_R^0, \tag{2}$$

$$\alpha_1 = k_R^1/k_R^0, \qquad \alpha_2 = k_R^2/k_R^0, \qquad \beta_1 = k_r^1/k_r^0, \qquad \beta_2 = k_r^2/k_r^0.$$

Here, σ_R represents the synthesis rate constant of the second mRNA relative to that of the microRNA. γ represents the degradation rate constant of the unbound microRNA relative to that of the unbound mRNA. We define α_1, α_2, β_1 and β_2 relative degradation factors (RDFs), and they represent the degradation rate constants of the mRNA (α) and microRNA (β) in the complexes (1 and 2) relative to those of the unbound

forms of the same molecules, respectively. With the law of mass action, we then use the following ODEs to describe the dynamics of the three species

$$dR/dt = \sigma_R - 2\kappa^{\mathrm{on}}Rr + 2\kappa^{\mathrm{off}}C_1 - R + 2\beta_1\gamma C_1$$

$$dr/dt = 1 - 2\kappa^{\mathrm{on}}Rr + 2\kappa^{\mathrm{off}}C_1 - 2\kappa^{\mathrm{on}}C_1 r + 2\kappa^{\mathrm{off}}C_2 - \gamma r + 2\alpha_1 C_1 + 2\alpha_2 C_2$$

$$dC_1/dt = \kappa^{\mathrm{on}}Rr - \kappa^{\mathrm{off}}C_1 - \kappa^{\mathrm{on}}C_1 r + \kappa^{\mathrm{off}}C_2 - \alpha_1 C_1 - \beta_1\gamma C_1 + \beta_2\gamma C_2 \quad (3)$$

$$dC_2/dt = 2\kappa^{\mathrm{on}}C_1 r - 2\kappa^{\mathrm{off}}C_2 - \alpha_2 C_2 - 2\beta_2\gamma C_2.$$

Note that because of the symmetrical binding assumption, there are two identical 1:1 complexes described by the same variable C_1 (or equivalently $\hat{C}_1$). Therefore, all the reaction rates that involve the 1:1 complex and appear in variables R, r, C_2 are multiplied by 2 in Eq 3. In the model, there are four RDFs: α_1, α_2, β_1, and β_2. α_1 and α_2 represent the degradation rate constants of the mRNA in the 1:1 and 2:1 complexes, respectively, relative to its degradation rate constant in the unbound form. β_1 and β_2 are the corresponding RDFs for the microRNA. A key difference between this mass-action-based ODE model and those based on sigmoidal function type is that the nonlinearity of the mass-action-based model is only from the binding of two molecular species, for which the rate of the reaction is proportional to both concentrations.

The system shown in Eq 3 can be used to capture oscillation and other dynamics of ncRNAs. It is possible to convert Eq 3 into a two-state-variable (2D) system [21], but since it may reduce the interpretability of the model, we will use Eq 3 to describe the emergence of oscillation first. With a set of biologically plausible parameters (e.g. $\kappa^{\mathrm{on}} = 10000$, $\kappa^{\mathrm{off}} = 1$, $\gamma = 0.25$, $\alpha_1 = \beta_1 = 1$, $\alpha_2 = 12$, $\beta_2 = 12$, $\sigma_R = 3.6$), we observed sustained oscillations for all state variables in Eq 3 (**Figure 2B**). In these oscillations, the cycles of the unbound mRNA and the unbound microRNA are out of phase, whereas the 1:1 complex is in phase with the unbound mRNA. Unlike other molecular species, the 2:2 complex's amplitude is smaller, and it is not depleted even at the trough of the oscillation. With the example set of parameters, the period of the oscillation is approximately $10t_{1/2}$, where $t_{1/2}$ is the half-life of the mRNA. The half-lives of mammalian

mRNAs are typically several hours [25]. This oscillation produced form RNA-RNA interactions therefore has a slow oscillation with a period on the timescale of days. The biological functions of these slow oscillations are yet to be determined experimentally, but it was proposed that they may drive the formation of heterogeneous cell populations, a phenomenon observed in cancer and progenitor cells (see details later) [21]. An important feature of the RNA-based oscillator that further supports the proposed function is the divergence of the periods when some parameters of the model vary. For example, a slight decrease in the production rate mRNA σ_R results in a significant increase of the period (**Figure 2C**). More generally, when σ_R changes, the system undergoes Hopf bifurcations at the two points of this parameter space, which define the boundaries of the region where the system can oscillate continuously with the existence of stable limit cycles (**Figure 2D**). Importantly, when σ_R approaches the lower bound of the limit cycle region (the left Hopf bifurcation point in **Figure 2D**), the periods of the limit cycles change abruptly (**Figure 2D**, red curve). This dramatic change of periods is in stark contrast to oscillations driven by simple negative feedback loops. For example, when a similar parameter changes in the repressilator model, for example, the periods of the limit cycles do not change significantly [21]. This shows that the RNA-based oscillator free of imposed feedback loop and the negative-feedback-driven oscillator have distinct sources for limit cycle formation. In fact, the same model shown in Eq 3 can generate saddle-node bifurcations in a parameter region adjacent to the one for limit cycle oscillations. Importantly, it was shown that the lower bound of the limit cycle region is close to a saddle-node bifurcation point, and the oscillation-initiating saddle-node on the invariant circle (SNIC) bifurcation is responsible for the divergence of the periods [21]. Interestingly, even though the RNA-RNA interaction model (Eq 3) does not contain any imposed feedback, it can act as both a toggle switch and an oscillator. The toggle switch behavior of the model was previously shown to be useful for explaining experimental observations on microRNA-driven tissue boundary formation [26].

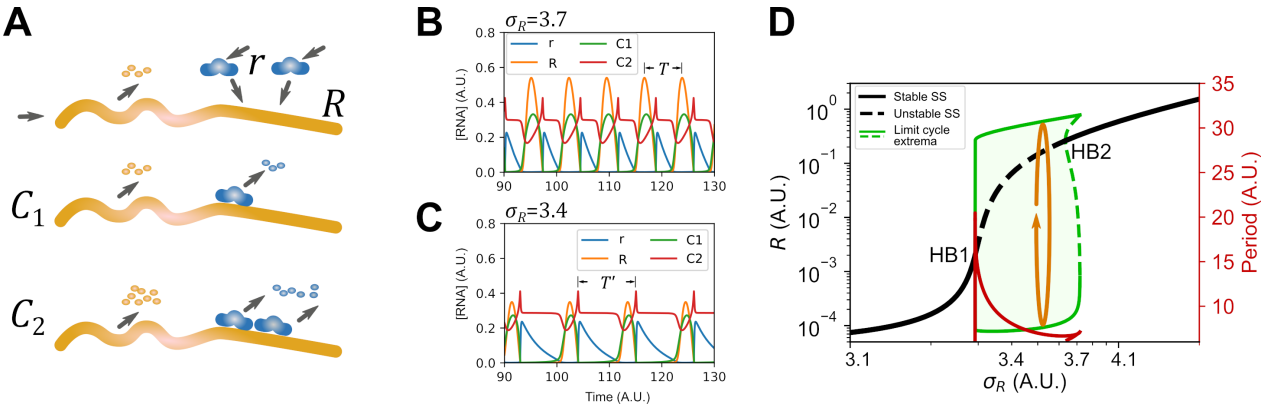

Figure 2. **Model structure and simulations of a ncRNA-based oscillator**. **A**. An illustration of biochemical reactions involving an mRNA and a microRNA. **B and C**. Simulation results for the model shown in Eq 3 (see main text for other parameter values). **D**. The left axis shows the steady state level of free mRNA with respect to the production rate constant. HB stands for Hopf bifurcation. Shaded area shows a region where only limit cycles are stable. The right axis and the red curve show the periods of the limit cycles.

Nonetheless, the divergence of the oscillations suggests that the RNA-based oscillator itself may not be a good pacemaker which one might expect from a biological oscillator. This is because slight variations of parameter values will lead to large changes of period, a key metric of a biological 'clock'. In fact, in the presence of transcriptional noise or other sources of stochasticity, the slow and divergent oscillator driven by ncRNAs can generate irregular patterns of switches between two states representing distinct concentration profiles: high-mRNA/low-microRNA, and low-RNA/high-microRNA (**Figure 3A**). Here, the changes in concentrations (high and low) are reflected in both total mRNA (microRNA) and free mRNA (microRNA). While the irregularity of state changes makes the oscillator a poor pacemaker, it can provide a strategy for a cell population to diversify themselves in a robust manner. For an initial population with homogeneous expression condition, possibility following a drug treatment or a sorting experiment, cells switch back and forth between two states asynchronously with the help of the diverging oscillator and

stochasticity, which establishes two subpopulations and maintains their proportions (**Figure 3B**). The oscillator-driven state changes generate this type of population-level, stationary-phase heterogeneity more robustly than state changes induced by stochasticity alone. The latter model had been commonly used to explain cancer and progenitor cell heterogeneity in combination with multistable systems [27], which contain point attractors rather than limit cycles.

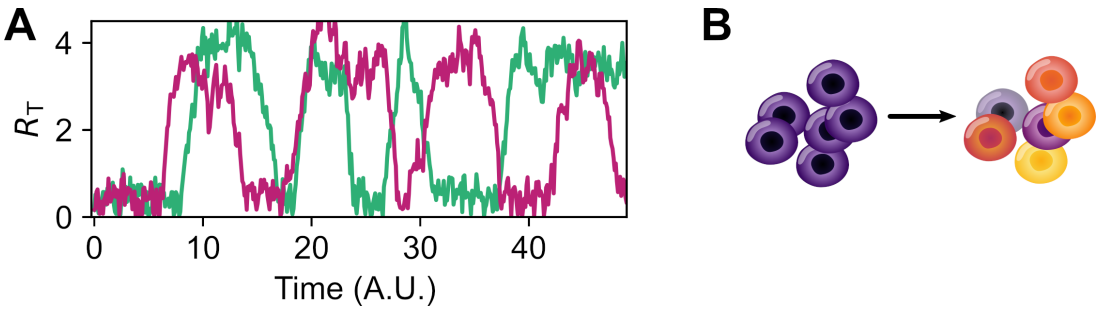

Figure 3. **Potential biological functions of the ncRNA-based oscillator**. **A**. Stochastic simulations for two representative cells under the control of the model shown in Eq 3 with the same initial condition. Transcriptional noise was included in stochastic ODEs stemming from Eq 3. **B**. An illustration of heterogeneity restoration of a cell population with a homogeneous initial condition.

There are several assumptions in the model underlying Eq 3 regarding the molecular events. It is assumed that the mRNA and microRNA can form two symmetrical 1:1 complexes each of which one of the two binding sites are occupied, respectively. It was shown that the symmetry of the 1:1 complexes is not required for the limit cycle formation: one can assume that the binding to a specific site is required and occurs before the binding to the other site without losing oscillations [21]. However, the assumption that the 1:1 complex and the 2:1 complex are formed sequentially is crucial for oscillation. In other words, state variable C_1 is necessary for Hopf bifurcation in the model shown in Eq 3. It was also shown that a similar

model with only one binding site cannot produce Hopf bifurcation and it associated oscillation [21]. The nonexistence of Hopf bifurcation for models without C_1 or C_2 with arbitrary positive rate constants can be proved with analytical methods such as the Routh-Hurwitz criterion [21]. In addition to these "structural" requirements, values of kinetic rate constants can also be important for obtaining oscillations. For example, the transcription rate of the mRNA needs to be close to a threshold of activation at which the steady state mRNA concentration starts to increase significantly. Another crucial parametric relationship for oscillation is that the 2:1 complex needs to have significantly different degradation rate constants for both mRNA and microRNA from the corresponding rate constants in free RNA forms and the 1:1 complex [21]. This requirement implies a biologically plausible cooperativity in which the formation of high-order complexes triggers the enhanced or reduced degradation of both the regulator and the target.

Conclusions and future perspectives

Mathematical modeling has been instrumental in improving the understanding of gene regulation. In particular, it connected oscillatory gene expression dynamics crucial for cellular and organismal level physiology to gene regulatory networks with negative feedback loops. The expansion of this type of connections to elementary reaction networks involving noncoding RNAs will help the discovery of new regulatory circuits in cells whose reversible state transitions are important for tissue-level functions. The absence of explicit feedback loops in the RNA-based models (e.g. Eq 3) challenges the conventional view that oscillations in gene expression requires imposed feedback loops such as negative transcriptional regulation of a gene by the products of itself. The simplicity of such RNA-based models (e.g. Eq 3) will not only facilitate the future experimental tests of the model predictions but also contribute to the understanding of complex regulatory gene networks as building blocks for larger models. Since ncRNAs and mRNAs work in systems much larger than the two-binding-site model shown in Eq 3, new emerging dynamics may arise when high-order RNA complexes are considered. For example, recent work showed

the possibility for a system of a mRNA containing four microRNA binding sites to generate three stable steady states without transcriptional feedback [28]. These systems have important functionals such as regulating cancer cell plasticity in the spectrum of epithelial-mesenchymal transition (EMT) (e.g. key EMT gene *ZEB1* mRNA harbors more than three binding sites for miR-200). It will also be interesting to investigate complex interactions of multiple limit cycles and point attractors with larger ncRNA systems in the future. Furthermore, the integration of RNA-decay regulation with translational control may lead to additional dynamical features that are not captured by the RNA-based model.

The RNA-driven oscillations predicted by the model have long and diverging periods which may render difficulties in direct observations of these oscillations experimentally. However, these unique features may allow the diverging oscillators to interact with important systems such as the cell cycle control circuit and the circadian clock to achieve useful performance. For example, diverging periods of the oscillator can help to diversify cell cycle phases [3]. The robust heterogeneity-restoring mechanism may allow cancer cells to gain survival advantages during drug treatment or enable regeneration from progenitor cells at an appropriate pace after injury. Future experimental and theoretical studies are warranted to obtain a deeper understanding of the ncRNA circuits' roles in these important physiological scenarios.

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