Synchronization of Genetic Oscillaton Induced by mi-RNA

Submitted in partial fulfillment of the requirements for the award of the degree of

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by
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DECLARATION

I hereby declare that the dissertation tilted **"Synchronization of Genetic Oscillators Induced by mi-RNA"** submitted to School of Computational & Integrative Sciences (SCIS). Jawaharlal Nehru University (JNU). New Delhi in partial fulfillment of the requirement for the award of the Degree of M.Tech in Computational and System Biology is an authentic record of the work carried out by me under the guidance of **Dr. R. K. Brojen Singh**. faculty. School of Computational and Integrative Science, JNU and that this dissertation has not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or similar title to any candidate of any other university. I will not publish the work without permission of **Dr. R. K. Brojen Singh** as the intellectual property rights belong to JNU. New Delhi.

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CERTIFICATE

This is to certify that the dissertation entitled **"Synchronization of genetic oscillators induced by miRNA"** is an authentic record and this dissertation is carried out by **Saurabh Kumar Sharma** at School of Computational and Integrative Sciences, Jawaharlal Nehru University, under my supervision and guidance. The contents of the project work, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. The work fulfills the requirements for the award of the M.Tech. degree in Computational and Systems Biology.

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SAURABH KUMAR SHARMA

Abstract

Background: We have studied the properties of miRNA within the cell and outside the cell and verified the impact of miRNA on the genetic oscillator in one cell in terms of both by control of amplitude and frequency. mi-RNAs are a class of small RNA molecules (18–24 nucleotides) that are known to regulate gene expression at the post-transcriptional level by reducing the amount of proteins produced by translation. Oscillation is the repetitive variation, typically in time, of some measure about a central value (often a point of equilibrium) or between two or more different states. Biological oscillator is an inhibitory feedback loop, which includes one or more oscillating variables. The miRNA of the outside the cell is stable more than the inside the cell so we would like the examine that can it be treat as a information agent. So we have taken two identical cells and diffused by miRNA from one cell to another cell as a communication channel. We went for it and want to find that can both cells be synchronized or not after a certain time.

Result: We have studied simple biological cell. We synchronized the two cell with diffusion constant and come to know that the information from one cell to another cell is reached. We also see with different different parameters of diffusion constant including diffusion constant with random numbers and comparison it. We also see the relation between the diffusion constant and rate constant of miRNA.

Conclusion: The extracellular miRNA are more stable than the intracellular miRNA. miRNA can act as a coupling constant from one cell to another cell for communication between two cells.

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Chapter 1
INTRODUCTION

One of a new inter- disciplinary field of study is system biology which focuses on very large complex interactions within the biological systems. using a better approach to biological and biomedical research. For understanding biology at the system level. we have to firstly understand the structure and dynamics of cellular and organismal function. not the characteristics of various parts of a cell or organism. The system's properties like robustness emerge as an important issue. and understanding these properties would have a help on the future of medicine [1].

Presumption motivated research in systems biology. So there will be a proposed system where we planned for that assumption. Systems can be both its created either naturally or manually [2]. The system represents a computable set of assumptions that need to be tested or supported experimentally. Computational "dry" experiments belongs as a simulation. on systems reveal computational correctness of the assumptions that manually in each systems. Any lacking systems would disclose inconsistencies with experimental facts, and so some changes are necessary [3]. Systems that pass this test become subjects of a thorough system analysis where a number of predictions may be made. A group of assumptions that can differentiate a correct system among other competing systems is chosen for "wet" experiments. Successful experiments are those that eliminate inadequate systems. Systems that handle this process are believed to be consistent with current experimental data [2]. Although it is an glorify process in the research of systems biology. the prospect is that improvement of research in computational science transform biological research to meet this cycle for a more systematic and assumption-driven science., scientific methods. technical knowledge for measurements, and genomics will deliberately [1].

Since biological systems are very complex. For understanding it requires the mainly two important ways the integration of experimental and computational research means we can say also in other words a systems biology approach [1]. Computational biology, through pragmatic modeling and theoretical exploration, provides a powerful foundation from which to address critical scientific questions head-on. Computational systems biology addresses questions fundamental to our understanding of life, yet progress here will lead to practical innovations in medicine, drug discovery and engineering [2].

1.1 Modeling Biological systems

Consider a well stirred molecular system of size V having N molecular species given by $X = [X_1, X_2, \ldots\ldots\ldots\ldots, X_N]^T$ undergoing M reaction channels given by,

$$aX_1 + bX_2 + \ldots\ldots\ldots \xrightarrow{k_m} pX_1 + bX_2 + \ldots\ldots\ldots$$

Where, a, b, ... are the number of reactant molecules and p, q, ... are number of product molecules respectively. $\{k_m\}$ is the set of rate constants with, m=1,2.....M.
The state change vector v is given by,

$$v_m = [p - a, q - b, \ldots]^T$$

Most of the biological systems are complex in nature with complicated reactions where molecular species could be multi-functional (involved in various functional modules). However, the complicated reactions can be reduced to simpler elementary reactions where in each reaction, at most one or two or three molecular interaction is involved [3]. Such molecular interaction picture can be modeled with two approaches described below.

1.1.1 Deterministic Approach

The time evolution of a system of chemically reacting molecules, the classical way of describing such interaction is to do a molecular dynamics simulation, exactly capturing all the molecule's locations and velocities, and in the variation of the species' populations accordingly whenever a chemical reaction occurs. However, if the number of molecules

(molecular population) is large ($\sim 10^{12\text{-}23}$) describing such interaction picture by probing each and every molecule's dynamics could be almost impossible. One way of looking at the dynamics of such system is to use classical chemical kinetics ie by interpreting the complicated reaction channels to a set of ordinary differential equations of all the variables using mass action law;

$$\frac{dX_1}{dt} = f_1\,[X_1\,,\ X_2\,, \ldots\ldots\ldots\ldots, X_N]$$

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$$\frac{dX_N}{dt} = f_n\,[X_1\,,\ X_2\,, \ldots\ldots\ldots\ldots, X_N]$$

where, f_1, ... f_n are functions whose forms depends on types of the reaction channels. Chemical reactions in this approach are observed as different, importantly rapid physical events, and there are categories of two types of elementary reactions: uni molecular, having as a result of development of internal fluctuations to a single molecule, and bimolecular, happening as a result of a interaction between two molecules. From a classical mechanics prospective, any one might pretend that this type of a system is deterministic, such that the state of the system at some later time can be described by tracing back the history of the system [4]. If the system derived deterministically with regards to the locations, velocities, and the specie's molecular populations, it will not emerge deterministically with regards to the species populations only. The indeterminate quantum inevitably gets in like in a uni-molecular reaction; indeed we are not able to know when a molecule will convert itself into a different isomeric pattern. Further, chemical systems are normally not mechanically confined; rather they are constantly in touch with a external heat bath, whose random disorder preserves the system in thermal equilibrium at some temperature [5]. The time evolution of the trajectory of the molecular species can be traced by solving the set of differential equations given by (3). However, the deterministic description could not able to pick up the noise fluctuations as the time evolution of the variables are average dynamics.

1.1.2 Stochastic Approach

Stochastic chemical kinetics describes the time evolution of a well-stirred chemically reacting system in a way that takes into account the fact that molecules come in whole numbers and exhibit some degree of randomness in their dynamical behavior due to various random interactions of internal molecules and external fluctuations [4]. In the stochastic approach, the trajectories of molecules are some kind of random-walk process which is driven through a single differential difference equation that is master equation where all possible interactions are included. Fairly simple kinetic theory arguments show that the stochastic formulation of chemical kinetics of the interacting molecules has a firmer physical basis than the deterministic formulation, but unfortunately the stochastic master equation is often mathematically intractable and difficult to solve for complex systems [5]. There is, however, a way to make exact numerical calculations within the framework of the stochastic formulation without having to deal with the master equation directly [6]. It is a relatively simple digital computer algorithm which uses a rigorously derived Monte Carlo procedure to numerically simulate the time evolution of the given chemical system. Like the master equation, this "stochastic simulation algorithm" (SSA) correctly accounts for the inherent fluctuations and correlations that are necessarily ignored in the deterministic formulation [5].

Even though quantum mechanical effects are avoided, still the prediction of the events in the trajectories of the stochastic system has indeterminacy, with unpredictable state of the system along the trajectories [4]. The design of the biological system is possibly looking for successful qualitative behavior of the system when it may go for the deterministic approach. However, it is very far away from situation where one might predict a good system behavior of pragmatic size and complexity by attractive time steps in the time evolution of the system at the expense of huge computational cost. We have to use such type of model that find out all details about its state of a system, like location, direction and energy of every single molecule under study. For these points of prospects, when we go for prospects of above level, the system's dynamics are not deterministic, but essentially stochastic [6]. To expose the actual description of stochastic mechanism directing the system dynamics, the study of statistical physics is mandatory. Stochastic

simulation will permit us to analyze the distribution related with the time to elimination. which is not easily possible using deterministic approach. We cannot easily implement a deterministic model which is not applicable to experimental data with the use of implied rate constant, because the deterministic approach cannot capture quantitative behavior of the system. However, stochastic approach can able to capture this realistic behavior and patterns in a systematic way [4].

1.1.3 The Master Equation formalism

Master equation is a classic method for determining the stochastic time evolution of chemical reaction channels which involve decay and creation of molecules in the process [5]. McQuarrie has given a nice report of the master equation approach to chemical kinetics [6], where the main features of master equation formalisms and the concept for stochastic simulation approaches can be extracted. Even though the concept of "grand probability function" is the key feature in master equation formalism which involves transition probabilities of decay and creation of molecules in the process, it helps giving key concepts to formulate the stochastic simulation algorithm [5].

$P(X_1, X_2, \ldots\ldots\ldots\ldots, X_N ; t)$ = probability that there will be in V at time t X_1 molecules of species S_1, and X_2 molecules of species S_2 and X_N molecules of species S_N

The calculation of above function gives us a totally characteristics of the "Stochastic state" of the system at time t [5].

1.1.4 Outline of the Gillespie algorithm:

The Gillespie algorithm which is known as SSA borrowed the key idea from Master equation formalism, but the algorithm is not directly derived from this formalism [5]. The basic concept of this algorithm is based on the two questions arising from the well stirred interacting molecular system: 1. at what time 2. Which reaction takes place? The reaction time and selection of reaction which occur during that time interval are in fact random process which can be extracted from the probabilities of reaction time and selection of reaction reflected from the master equation of the whole system [5]. Then SSA can be

developed by generating two uniform random numbers, one used for reaction time and the other for selecting reaction which will fire during the time interval. Then starting from an initial state defined by a vector of populations of molecular species, one can trace the trajectories all molecular species by simulating repeatedly with a huge time steps updated by reaction time. It is Monte Carlo like simulation algorithm that can be used for slow reactions.

The reactions in nature have different types, namely, very slow, slow, fast, very fast and delay reactions. Gillespie algorithm cannot able to identify these types of reactions except slow one from the mixture of all these reactions. Therefore there have been various modified Gillespie algorithm which can be used to identify different types of reactions, for example, delay stochastic simulation algorithm which can be used to identify delay reactions, hybrid model to identify slow and fast reactions etc. In this work we will be using SSA only because even though it needs huge computational cost, it gives exact molecular trajectories [5].

Life is a rhythm. Rhythm shows oscillation, which is the repetitive variation, typically in time, of source measure about a central value generally called a point of equilibrium of between two or more different states [9]. In general biological oscillations are generated by various processes, such as, by an inhibitory feedback loop, which includes one or more oscillating variables; by a source of delay in this feedback loop, which allow an oscillating variable to overshoot a steady – state values before the feedback inhibition is fully effective.

We then study specifically two models, chemical oscillator (Brusselator model) and Genetic oscillator (circadian oscillator) which are described below and implement SSA in order to study important behaviors of these systems.

1.2 The Brusselator model

Lotka noticed that set of coupled. autocatalytic reactions in 1920 which is following

$Y_1 + X \xrightarrow{k_1} 2Y_1$

$Y_1 + Y_2 \xrightarrow{k_2} 2Y_2$

$Y_2 \xrightarrow{k_3} Z$

These reactions consumed some important dynamic properties. Volterra examined reactions independently and make a mathematically model of predator– prey ecosystem [7]. Thus, reaction 2 explained that a predator species Y_2 regenerate by feeding on a prey species Y_1. Reaction 1 explained that Y_1 regenerate by feeding with a certain foodstuff X, which is supposed to be here to be only marginally reduced by that: and the isomerization reaction 3 explained the eventual decrease of Y_2 by natural disaster [5, 7]. The brusselator is related to the Lotka Volterra model which is the naturally stable character of the oscillation. At that time there was a question that is there any model possible of chemically reacting system with two intermediates species Y_1 and Y_2 which has an importance that no matter what is initial condition. the system will finally completely orbiting around a well manner. closed. stable path in Y_1 Y_2 plane.

Then Brussels's workers modeled based on the above shortcomings [8]. with a design following example of such a "limit cycle" chemical oscillator [5], which is known as Brusselator model.

Brusselator 4 reactions

1. $X_1 \xrightarrow{k_1} Y_1$
2. $Y_1 + X_2 \xrightarrow{k_2} Y_2 + Z_1$
3. $2Y_1 + Y_2 \xrightarrow{k_3} 3Y_1$
4. $Y_1 \xrightarrow{k_4} Z_2$

The above set of reactions has been designate the "Brusselator" by Tyson[9] and so for many study have been done by a number of worker [8,9]. A stochastic simulation computer program was written for the Brusselator (putting M, number of reactions= 4, N, number of species = 2,

$X_1 = Y_1$, $X_2 = Y_2$, $h_1 = X_1$, $h_2 = X_2 Y_1$,

$h_3 = \frac{(Y_2 * Y_1 * (Y_1 - 1))}{2}$ and $h_4 = Y_1$

1.3 Circadian rhythms model

The environment changes in an extremely periodic way. There are also many other changes like daily cycles of light and dark and as well as every year cycles of dynamic climates and physical conditions. This type of environmental periodicity might be constructing the requirement for organisms to develop internal time-keeping mechanisms to exactly forecast these external changes and modify their State respectively [12].

The change in environment and fluctuations allow the organisms to keep internal sense of daily time and regulates their behavior. accordingly a broad range of organisms use circadian clocks modulated by this change. Mostly these clocks use intracellular genetic networks established on negative and positive regulatory elements. The integration of these negative and positive circuits at the cellular level introduces strong constraints on their functioning and design [23]. Circadian rhythms have been determining in many different organisms from a cell to man as a large range of behavioral, physiological and biochemical parameters. [10, 11, 12, 13, 14].

An internal period of around 24 hours is being characterized these rhythms [11] and the phase of the oscillation is shifted by the strength of physical or chemical pulses [15]. Else more, temperature is also play an important role in these rhythms [16, 17]. The phase-shifts are time dependent, the pulse is given in the phase of the circadian cycle [12, 15]. It is also acquired that such persevering phase-shifts of free-running rhythms should be explained as conformation for effects of the perturbing agent on the clock itself [18][19] [20]. Protein synthesis has been also important related to its desirable involvement in the molecular mechanism of circadian rhythms [21, 18, 20, 22]. With the help of repressor.

the dynamics of an activator protein and the concentration of a repressor protein construct an inactive complex. So the clock does not necessary to depend on mRNA dynamics to oscillate, which makes it principally resistant to variations [12,23].

An importance property of circadian clocks is the capability to manage a constant period over a broad range of internal and external fluctuations [12]. The clock runs correctly and generates the expression of clock-dependent genes at the desired time of the day is assured by such type of robustness. There is also role of temperature which is disturbing chemical reaction rates when temperature fluctuates and might perturb the oscillatory behavior. There is also another source of fluctuations in circadian clock due to the presence of internal noise generated by the random interaction of chemical reactions [24]. The oscillatory behavior of the biochemical network may be disturbed by the less numbers of molecules which responsible for random fluctuations [25].

1.4 Introduction to miRNA and its function

Ambros and his colleagues discovered miRNAs. miRNAs are a class of non-coding small RNA molecules, whose size is 18-24 nucleotides, playing a crucial role in a biological process. It regulates gene expression at post-transcription level by reducing the amount of proteins produced by translation [26.29] and as well as resolving targeted hydrolysis and translation inhibition of mRNAs [27]. Actually miRNAs have been shown too many places to engage in the regulation of various cellular functions and also involved in many diseases like cancer [30-32]. Chim and his collegues, firstly reported that miRNAs are present in biological fluids and also found in placental miRNAs in maternal blood plasma in freely noticeable concentrations [28].

1.4.1 How are miRNAs produced

Actually in nature miRNAs are single stranded RNA molecules transcribed from noncoding genomic regions [37,38]. This development is achieved by RNA polymerase II via long pri-miRNA precursors that encode one or more miRNAs, which is standardized in a 60–70 nucleotide hairpin structure which is separated by a single-stranded RNA (Figure 1) [37,39]. With multi-protein complex the pri-miRNA is

processed in the cell nucleus that consist RNAse III called Drosha and the double – stranded RNA binding protein called Pasha [40,41] generating stem loop pre-miRNA sequences. From the cytoplasm Exportin-5 transported this pre-miRNA where the RNase III Dicer cuts out the single stranded loop producing a miRNA duplex [42, 43]. Dicer also participates in the assembly of mi-RISC (miRNA RNA-induced silencing complex) where one of the two strands of miRNA duplex is depraved. [44].

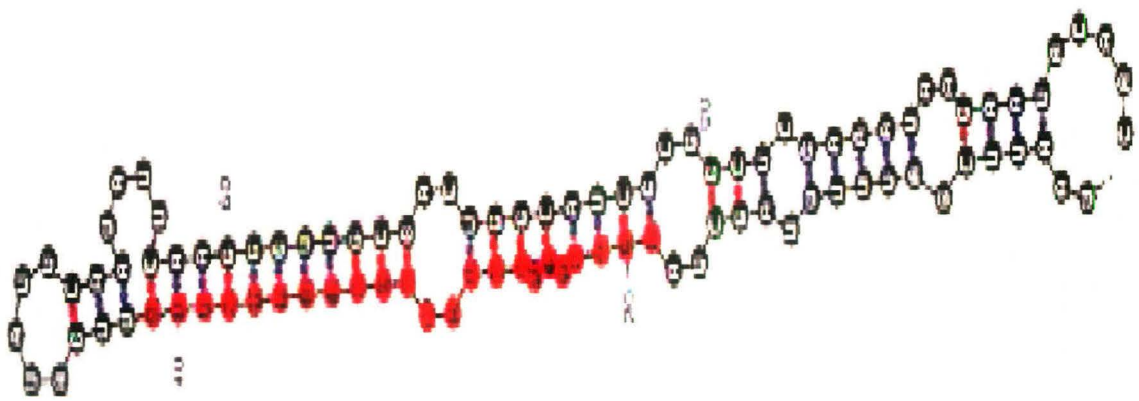

Figure 1: pre-miRNA's secondary structure. The anticipated stem loop structure of pre-mir-219-1, represent circadian miRNA in the mouse. The red one is the mature sequence in the figure. 30.2 kcal/mole is the free energy ΔG assumed for this structure [45].

1.4.2 The functions of miRNA

miRNAs regulate gene expression in various context-dependent ways by different miRNA processing complexes [46]. The communication of miRNA with destination transcripts could be largly grouped into two different pathways (Figure 2). The base pairing between a miRNA and mRNA-target triggers mRNA cleavage by the 'silencing' mechanism. Incomplete pairing of miRNA with 3 UTR of target mRNA leads to binding of miRISC to the target, which results to repression of translation. The mode of action of

miRNAs is not completely understood while it is engaged in base pairing between the 5 end of the miRNA (which is 7–8 nucleotides, called 'seed'), and the 3 UTR of the target mRNA [46,47]. Such pairing may induce (1) degradation of mRNA, or (2) change in the efficiency of translation by either influencing the ribosomal drop-off from the mRNA or by inhibiting 80S ribosome synthesis at the start of the translation [48, 49]. There was also shown that miRNA may affect the stability of mRNA through de-adenylation process and de-capping process to the target mRNA. It was shown that miRNAs can also affect the stability of mRNA by de-adenylation and de-capping of the target mRNA [50].

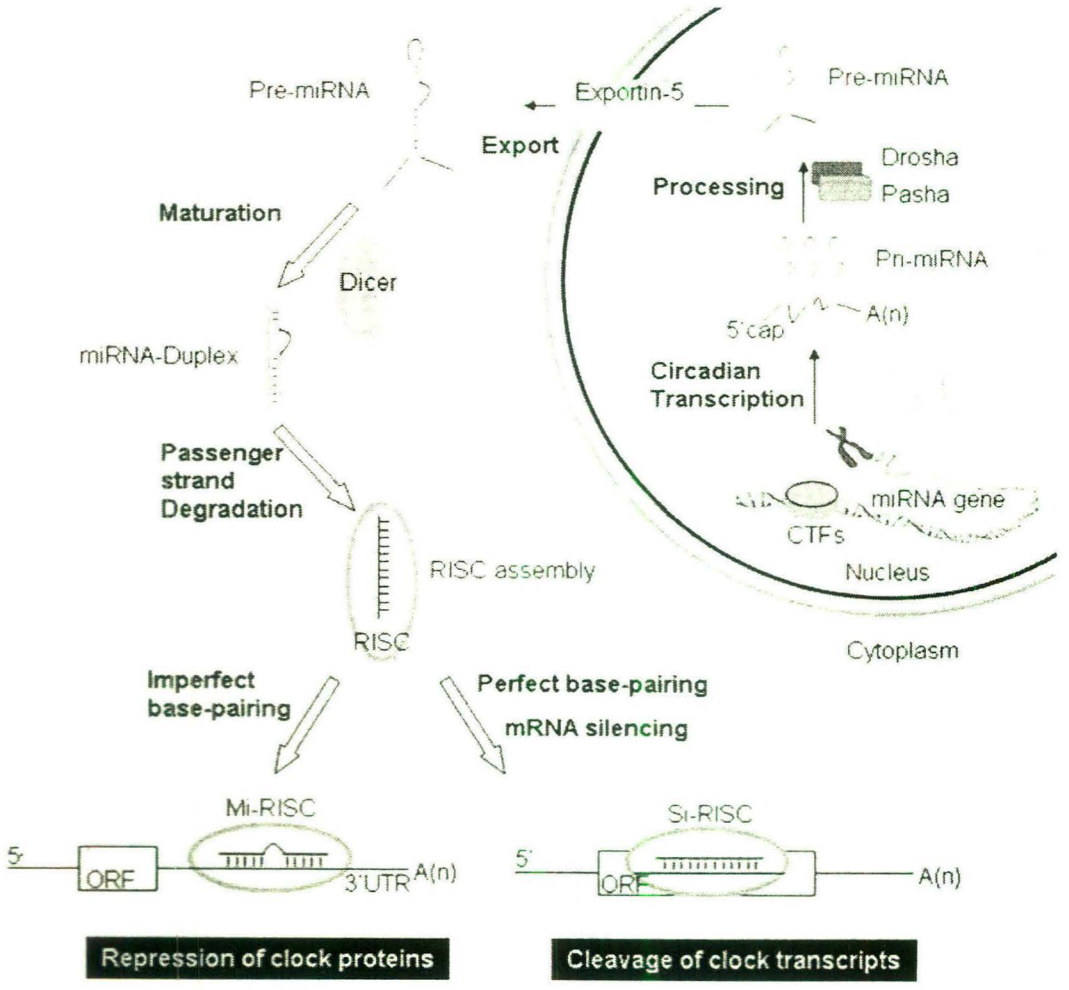

Figure 2: Function of microRNA: Transcription factors (CTFs) induces the transcription of miRNA genes [50].

Further Drosha–Pasha multi-protein complex interact with the pri-miRNA leads to the synthesis of 60–70 nt pre-miRNA. Exportin-5 translocates the pre-miRNA from the nucleus to the cytoplasm. The role of Dicer to cut the pre-miRNA single stranded loop and generates miRNA duplex. The degradation of microRNA passenger strand takes place at the RISC. This modified form of single strand miRNA further loaded at the RISC. Base pairing between miRNA and target mRNA leads to the mRNA cleavage (si-RISC dependent silencing), whereas imperfect base-pairing between 3 UTR of target mRNA and miRNA leads to mi-RISC-dependent inhibition of translation [50].

1.4.3 Molecular mechanisms in Circadian clocks

The earth's rotation plays an important role in daily routine of light and temperature and drives the forces in the evolution of circadian clock, permitting organisms to forecast and prepare for their daily and seasonally changing environment [51]. The molecular description of the circadian clock may differ in different sense: the principle of a system of self continuous transcriptional–translational assessment loops is conserved [52]. Transcription factors of mammalian CLOCK and BMAL1, bind to clock-specific motifs and triggers the transcription of negative regulators, which are transported to the cytoplasm and then forward to be translated. These type of negative factors, which physically interact with each other, giving heterodimers (in mouse PER-CRY, or in fly PER-TIM), are come back to the nucleus after some time and then repress the positive circadian transcription factors, through down regulating their own transcription there [53,54]. After some time the negative factors decreased in plenty of amount in the circadian cycle progressively leads to their own depression which permit the positive transcription factor for trigger a new circadian cycle. A transcription rhythm of downstream clock-controlled genes (ccgs) is dependent upon the everyday oscillation of the core clock proteins. The several reports of the Genomewide expression analysis from several microarray in different organisms suggests, the expression of as much as (5-10) % of transcripts in a particular tissue oscillates in a circadian manner [55, 56].

There is analysis of the mammalian cycling proteome suggest that cytosolic proteins is rather higher than found in the case of microarray studies and there are found a constant

accumulation at the level of the mature transcript in many case of cycling proteins. This variation suggests that post-transcriptional and post-translational mechanisms are important components of circadian rhythmicity. There is also found evidence (reviewed in Zheng and Sehgal 2008) suggests that cycling of clock transcripts and proteins is not totally important for clock function and pointing that the 'central dogma' of the transcriptional negative feedback loop is probably enough but not necessary and post-transcriptional/translational process are an important part of the circadian clock [45].

1.4.4 Role of miRNA in the circadian clock

The report about microarray of Drosophila heads verified the expression of 78 miRNAs from flies entrained to light–dark cycles and analyze to the correlated expression in the clock mutant cyc01 [57]. There are two miRNAs dme-miR-263a and 263b, which have significant cycling that were suppressed in the mutant. It has been validated expression of such type of miRNA by qPCR tool, which also declared that both these products cycled in continuous darkness [56]. The fold-change of miR-263a was little small than miR-263b which is 1.7-fold and 2-fold oscillations, respectively. On comparing with the fold-change of circadian clock transcripts (e.g. 4–5 [56], the quite change of miRNA has been seen which conclude that they are not biologically relevant. Yang and his colleagues [57] profiled the expression in whole heads and seen that expression of these specific miRNAs change a little in individual neurons at a higher level. And one condition is also possible that the fold change in moderate in inherent property of miRNAs, reflects the regulators in fine tuning of expression of miRNA. It has been reported that in the rat's brain the miRNA levels have quite large magnitude of fold-change. For example, miRNA levels after sleep deprivation in the rat's brain also show modest magnitude of fold-change (1.5–2.5) [58].

It is also seen that some clock miRNA may not show oscillation everyday but also play an important role in circadian regulation [57]. If the miRNA level is constant then it may present as a threshold which 'gates' circadian oscillations. If there is change in the gates of oscillation of others clock protein than it may reflect the responds of miRNA towards the various cues. It is seen by Yang and his colleagues about six miRNA which did not

cycle while had a quite different profile compared than the cyc0 mutant. There are many prediction algorithms. Yang and his colleague had used and found that a number of clock genes were identified which possibly provide a target for circadian miRNAs. with per. Clock, tim, dbt, cwo and tws [57].

1.4.5 Extracellular miRNA:

There are many reports which give the evidence of the presence of miRNA in all other parts of body fluids like saliva, urine, breast milk, seminal plasma, tears, amniotic fluid, colostrum, bronchial lavage, cerebrospinal fluid, peritoneal fluid and pleural fluid after the exploration of extracellular miRNA in blood plasma and serum [33-36]. By numerous pathological conditions it has been seen that the changes in miRNA spectra in certain fluids, implying that extracellular miRNAs may serve as informative biomarkers to determine the pathological status of the body [33-35].

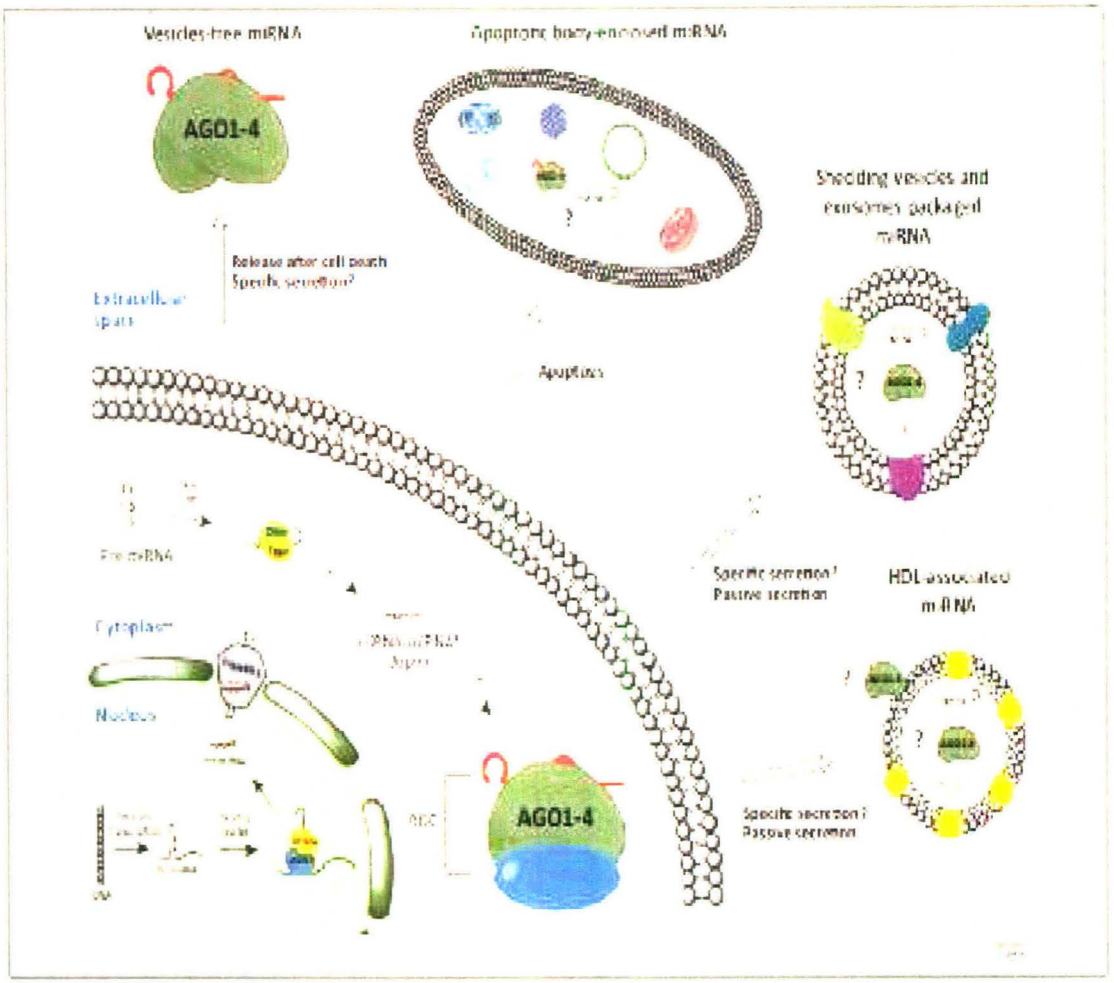

Figure 3 Biogenesis of miRNA in the cell and the modes of extracellular miRNA packaging [27].

The important fact of forming of all miRNAs is RNA polymerase II which is greater than 200 nucleotide primary miRNA transcripts. Maturation of primary miRNA starts in the nucleus with cleavage by the endonuclease complex Drosha-DGCR8 into the amount of 60-70 nucleotide hairpin intermediates which is called miRNA precursors. Then pri-miRNA transport from the nucleus into the cytoplasm by exportin-5 and then it cut into 22-24 nucleotides miRNA/miRNA* duplexes by the endonuclease Dicer. After that the miRNA strand aggregate into Argonaute family which has four proteins (AG01, AG02, AG03, and AG04). These four proteins are the important components of RNA-induced silencing complex (RISC) and miRNA* strand which is decayed afterwards. There are

three types of membranous vesicles which contain extracellular miRNA are (1) apoptosis bodies. (2) shedding vesicles and (3) exosomes. There are also another extracellular miRNA which is vesicles free and integrated with two proteins either too AGO proteins alone or aggregated into HDL particles. Remaining two vesicles shedding and exosomes fall in the class of microvesicles which are restricted by lipid bilayer [27].

1.5 Synchronization: a means of cellular communication

Dutch researcher Christiaan Huygens observed for the first time and explained the synchronization of two pendulum clocks coupled by weak signal by putting close together in the seventeenth century [60]. He took a couple of pendulum clocks and hanged from a common support and discovered that both was synchronized after sometime which means that the oscillation of these two clocks coincided fully and pendula moved regularly in the same direction [64]. Consider two oscillating systems $X = [X_1, X_2, \ldots\ldots\ldots, X_N]^T$ and $Y = [Y_1, Y_2, \ldots\ldots\ldots, Y_N]^T$ and if the two systems are allowed to couple through a coupling mechanism (direct, diffusive, time delay etc) with coupling parameter C via a certain variable say (X_i, Y_i), then at sufficiently large value of C, the other variables other than X_i, Y_i are synchronized. If we consider the instantaneous phases of any corresponding variable from the two systems to be ϕ_k^1 and ϕ_k^2 , then the two systems will synchronized if the following condition satisfy.

$m\phi_k^1 - n\phi_k^2 = constant$

where, m and n are constants. This condition is known as phase locking condition which is true for phase synchronization. There are different types of synchronization, namely, complete synchronization (the two systems are synchronized completely), delay synchronization (delay induced synchronization), generalized synchronization (a system drives two other systems to synchronize) etc.

1.5.1 Coupling Mechanism

There are different types of coupling mechanisms, namely, direct coupling (corresponding one variable in the two systems are made exactly equal and the two systems are synchronized), delay coupling (time delay is considered as coupling

parameter and the two systems are synchronized). mean-field coupling (in a group of systems a fraction of information is allowed to diffused in each system and the systems become synchronized). We use diffusive coupling mechanism in this work to induce synchronization between two systems. Consider two systems defined by $X = [X_1, X_2, \ldots\ldots\ldots, X_N]^T$ and $Y = [Y_1, Y_2, \ldots\ldots\ldots, Y_N]^T$. then if X_i and Y_i are corresponding variables that can diffuse from one system to another by

$$X_i \xrightarrow{C} Y_i$$

$$Y_i \xrightarrow{C''} X_i$$

where C and C'' are diffusive rates. Then for sufficiently large value of $C = C''$ the two systems will achieve synchronization.

Molecular communication is a very ubiquitous property of the living organism. It leads to the synchronization between two or more system e.g., biological cell. which is due to adjustment of their behavior or motion to a common path due to coupling or forcing. In synchronization the path of one of the system will obey to same value as the other side. They will remain in step with each other. The synchronization in biological system is also refers to communication between the cells with almost information [59]. A synchronization phenomenon is found to be a very common phenomenon in biology. due to collective behavior of communication between neurons, cells, or animals. There are several means of communication takes place among the biological systems, such as direct coupling, mean field coupling and diffusive coupling. In the synchronization, the systems jointly create and then come with a signal with a common manner, like electrical field which shows that each system connected to each other directly [64].

There are various open questions which will be main focus of this work. They are 1. several studies have been done so far to study the impact of miRNA on genetic oscillator. 2. But still there is a scope to find how the dynamics of genetic oscillator behave due to the impact of miRNA at molecular level?, 3. and also how miRNA act as a synchronizing agent is still an open question? and some extracellular miRNA species might also carry cell- cell signaling function?

Chapter 2

Stochastic kinetics of molecular interaction

The well stirred molecular system can be described via two formalisms for mathematically describing the dynamics of the spatially homogeneous chemical system: the first one is the deterministic approach which concern with the time evolution of the system as a continuous, in which one can predict the process provided initial histories and calculated by constructing simple differential equation using "the rate reaction law". The second one is the stochastic approach, which concerns with the time evolution of the system as a type of random-walk process, calculated by the master equation formalism. The stochastic approach to the chemically reacting system within the master equation is in general cannot be solved easily. Gillespie argued systematic computational algorithm to solve the dynamics of such system without solving master equation based on the framework of two key questions: at what time which reaction occurs. In this paper he had taken a fixed volume V which contains N chemical species which undergo M reaction channels with initial concentration of molecules of each species at the initial time and want to find the status of molecular population after any instant of time. He further explained that for simple reaction occurrence there should be collision of two or more molecules in an appropriate way and the molecular collision occurs in random manner in thermal equilibrium. Therefore, he had taken a system which is in thermal equilibrium which has two molecules S_1 and S_2 inside some volume V. Then the probability for colliding the two molecules in small time interval by the below equation.

$\delta \frac{V_{Coll}}{V} = V^{-1} \pi r_{12}^2 v_{12} \delta t$ = average probability that a particular 1-2 molecular pair will be collide in the next vanishingly small time interval δt.

..... (1)

Where v_{12} = the relative velocity of two molecules.

V_{Coll} = is the collision volume of where two molecules may collide.

V = total volume of system.

r_{12} = 1-2 collision will occur whenever the center-to-center distance between an S_1 molecule and an S_2 molecule decreases to $r_{12} = r_1 + r_2$.

t= time interval

Then he had taken another case in which X_1 of S_1 and X_2 of S_2 molecules in V at given time t and calculate the distinct X_1- X_2 molecular pairs. which was done by below equation (2)

$X_1 X_2 V^{-1} \pi r_{12}^2 v_{12} \delta t$ = probability that a 1-2 collision will occur somewhere inside V in the next infinitesimal time interval (t. t+ dt) ..... (2)

Therefore for a thermally equilibrated system, the molecular event described by above equation is known as "collision probability per unit time". and the coefficient of dt in it is called as "collision rate". This is reason why these collisions constitute a stochastic Markov process instead of a deterministic rate process.

Since the well stirred molecular system is homogeneous in nature. one can inter relate stochastic rate constant and classical rate constant. The stochastic rate constant c_μ and classical rate constant k_μ are connected by

$c_\mu = k_\mu V^{1-\nu}$

Further there is also an important role of system's temperature and the physical properties of the molecules such that

$c_\mu\, dt$ = average probability that a particular combination of R_μ. reactant molecules will react accordingly in the next infinitesimal time interval dt. ..... (3)

The "average" in the above equation means that, if we multiply $c_\mu dt$ by the total number of distinct combinations of reactant molecules in R_μ in the system at time t, the probability that an R_μ reaction will occur somewhere inside V in the next infinitesimal time interval will be obtained. For such system, the molecular mechanisms in the reaction picture comprises of two notions one for reaction time and the other for which reaction will fire during that time interval. The probability of occurring μth reaction during τ reaction time is given by,

$P(\tau, \mu)\, d\tau$ = probability, if the given state $[X_1, X_2, \ldots\ldots\ldots, X_N]$ at time t , then the next reaction will be fired in volume V in the very small time interval $(t+\tau, t+\tau+d\tau)$ and that reaction will be R_μ ..... (4)

The $P(\tau, \mu)$ is generally defined as "reaction probability density function" which is a joint probability. Since the probabilities of a reaction will fire at time τ, $P(\tau)$ and the probability of μth reaction will occur are independent of each other, one can write

$P(\tau, \mu)=P(\tau)P(\mu)$

Then propensity function (a_μ) for finding the probability that a μth reaction will occur in V in $(t, t+\tau)$, within a given system in the state $[X_1, X_2, \ldots\ldots\ldots, X_N]$ at time t can be described by,

$$a_\mu\, dt \equiv h_\mu c_\mu\, dt \qquad \text{---------- (5)}$$

Where a_μ = Propensity function.

h_μ= Number of distinct R_μ molecular reactant combinations available in the state.

c_μ = Stochastic rate constant.

Then defining a parameter

$$a_o = \sum_{i=1}^{M} a_i S$$

and solving equation (5) one can get the expression for τ as

$\tau = \frac{1}{a_0}\left(ln\left(\frac{1}{P_\tau}\right)\right)$ and condition for selecting μ_{th} reaction is given by,

$$\sum_{i=1}^{\mu-1} a_i < a_o P_\mu \leq \sum_{i=1}^{\mu} a_i$$

The P_τ and P_μ can be replaced by a set of two independent uniform random numbers. This is the backbone of Gillespie algorithm.

M.G Jose et. al described about the genetic oscillator which considers interaction of repressor and activator generating 24 hours oscillating cycle. They explained that as environment changes in a periodic manner like day night cycle and cycle of climate changes etc may create the necessity for organisms to develop internal time-keeping mechanisms to accurately predict these types of external changes and modify their state accordingly. All organisms have this 24 hours period biological clock which is called circadian rhythms. The availability of intracellular transcription regulation networks with a set of clock elements gives rise to stable oscillations in gene expression. The clock mechanism has two elements, one positive which activate genes coupled to the circadian clock and also at simultaneously the other one is negative which in turn the positive element. So due to the degradation of negative element and re-expression of the positive element, the cycle complete itself.

One of the important properties of circadian clock is the ability to control a constant period of internal and external fluctuation, and this is responsible for that which protein is necessary to be triggered at the appropriate time of the day. Further, there may be fluctuation due to the molecular interactions which is stochastic in nature giving rise to intrinsic noise which is responsible for random movement which can affect the oscillatory behavior of biochemical network dynamics. The model consists of two genes one is an Activator (A) and the second one is Repressor (R) which are responsible for producing corresponding proteins via their respective mRNAs [23]. The interaction network consists of the positive and the negative feedback loops.

A. Nandi et al studied the role of miRNA in circadian oscillator to regulate the dynamics of the clock. They explained how miRNA affect the amplitude and as well as frequency of circadian oscillator by introducing general reaction mechanism which involve miRNA [26]. The miRNA control temporal behavior of the biochemical network of the extended circadian oscillator with miRNA regulation at the post-transcriptional level was studied. The regulation of circadian rhythm via various rates of miRNA related reactions showed the activity of miRNA on the rhythmic scenario. However, it is not still clear that how suppression of genes has effected in biological decision- making after many study of the effect of miRNA based suppression on gene expression on various biological processes. Further the intercellular and intracellular signal transduction process need to be investigated in a wider range to understand the role of miRNA in regulating cellular processes.

K. Wang et al reported that miRNA can be consider as a new class of regulators of gene expression given a new direction of research activities but not the answer of many questions like about the process of gene regulation function and other as well as how it is integrated with other molecules in the network. It had been seen that sufficient amount of miRNAs are present in plasma and as well as in other body fluids in human. So this fact suggested that the sufficient amount of miRNAs found outside the cell seem to be stable outside the cell. So a new idea came from it that the biological function of miRNAs might be come outside the cell and helped for the cell to cell communication [62].

Chapter 3

Modeling chemical and miRNA induced genetic oscillators

The chemical oscillator (Brusselator) and genetic oscillator are modeled and studied using stochastic simulation algorithm due to Gillespie. We have developed SSA program code which is written in python language. The study has been described below.

3.1 Brusselator Oscillator

This chemical oscillator consists of two molecular species Y_1 and Y_2 and four reaction channels described in the Table 3.2. The prophensity functions as well as the values of the rate constants are also provided in Table 3.2. The parameters, their descriptions and initial values taken in our simulation are listed in Table 3.1.

Sr. No.	Molecule Species	Description	Initial Value
1.	X_1	Foodstuff	1000
2.	X_2	Foodstuff	2000
3.	Y_1	Predator Species	1000
4.	Y_2	Prey Species	2000
5.	Z_1	Constant	-
6.	Z_2	Constant	-

Table: 3.1 Brusselator

Sr. No.	Reaction Channel	Propensity Function	Value of Rate Constant
1.	$X_1 \xrightarrow{k_1} Y_1$	$a_1 = k_1 * X_1$	$k_1 = 5$
2	$Y_1 + X_2 \xrightarrow{k_2} Y_2 + Z_1$	$a_2 = k_2 * (Y_1 * X_2)$	$k_2 = 0.025$
3.	$2Y_1 + Y_2 \xrightarrow{k_3} 3Y_1$	$a_3 = k_3 (Y_2 * Y_1 * (Y_1 - 1))$ /2	$k_3 = 0.00005$
4.	$Y_1 \xrightarrow{k_4} Z_2$	$a_4 = k_4 * (Y_1$	$k_4 = 5$

Table: 3.2 Reaction channels of Brusselator

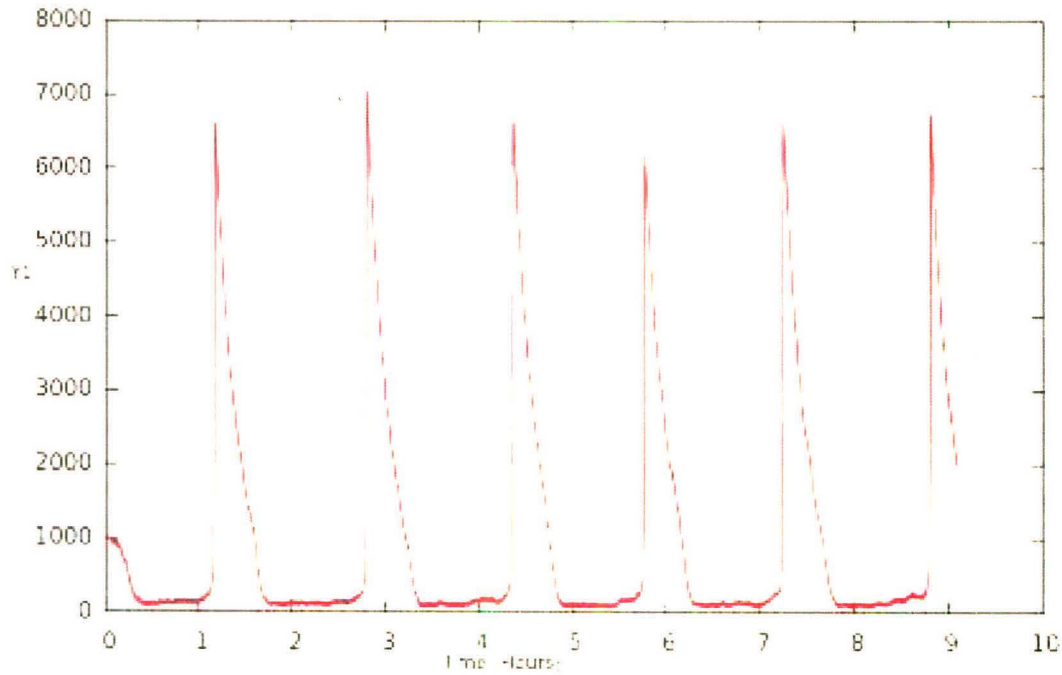

Figure-3.1- Brusselator oscillator Y_1 Vs. Time

Figure-3.2 - Brusselator oscillator Y_2 Vs. Time

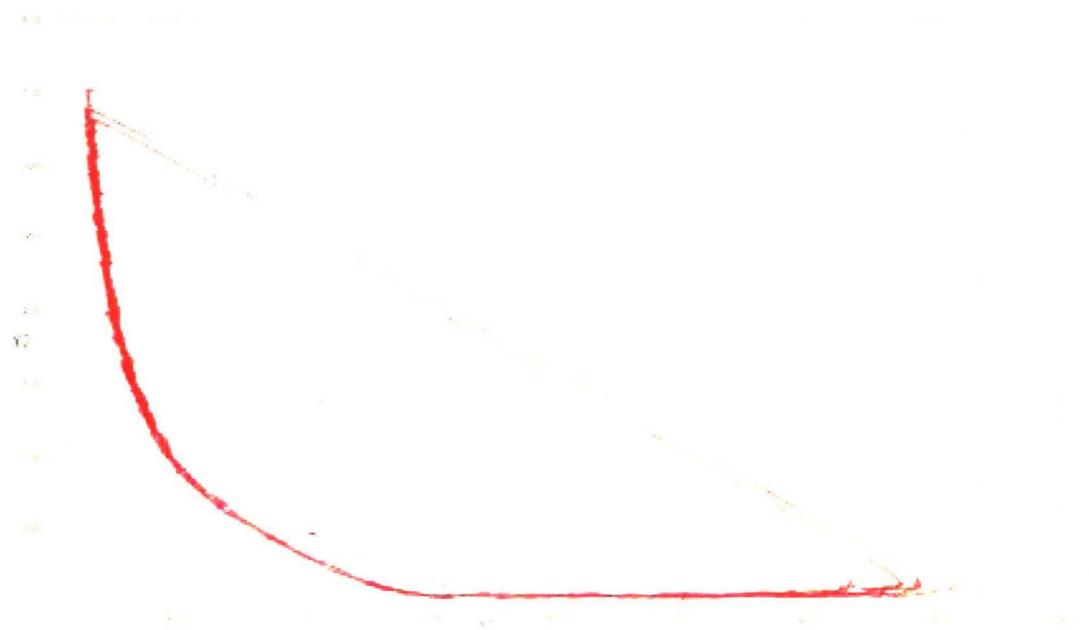

Figure-3.3 Brusselator oscillator Y_2 Vs. Y_1

Figures 3.1 and 3.2 describe how Y_1 and Y_2 evolve with time and the dynamics show fluctuations due to stochastic nature of the time evolution of the system. The time periods of Y_1 and Y_2 are 0.7 and 0.68 units of time respectively. The average amplitudes of the Y_1 and Y_2 are 6579 and 6985 respectively. Due to fluctuation in amplitudes of oscillation of the molecular species, one can see stable limit cycle of broaden thickness (Figure 3.3). It is also shown that the system consistently retrace its previous path on the diagonal of the limits cycle and this diagonal traces is directly related to the maximum variation in the height of vertical axis means Y axis. There is also fluctuation due to random process which can be seen at the microscopic level [5].

3.2 The miRNA induced genetic oscillator model:

Nomenclature of first oscillator:

D_A the number of activator genes with activator protein (A) bound to its promoter and D_A the number of activator genes without activator protein (A) bound to its promoter. D_R' the number of repressor gene with activator protein (A) bound to its promoter and D_R the number of repressor genes without activator protein (A) bound to its promoter. M_A is the number of mRNA of the activator protein (A) and M_R is the number of mRNA of the repressor protein (R). Synthesis of activator-repressor complex is C. m denotes the number of miRNA. C_{RISC} is the complex of miRNA with mRNA.

Second oscillator: D''_A the number of activator genes with activator protein (A') bound to its promoter and D^1_A the number of activator genes without activator protein (A') bound to its promoter. D''_R the number of repressor gene with activator protein (A') bound to its promoter and D^1_R the number of repressor genes without activator protein (A') bound to its promoter. M'_A is the number of mRNA to the activator protein (A') and M'_R is the number of mRNA to the repressor protein(R'). Synthesis of activator-repressor complex is C'. m' denotes the number of miRNA. C'_{RISC} is the complex of miRNA with mRNA.

Formation of D'_A occurs with rate constant k_2 and again dissociation of D'_A into D_A takes place with rate constant k_1.Formation of D'_R occurs with rate constant k_4 and dissociation of D'_R ' into D_R with rate constant k_3 and also dissociation into A occurs with rate constant k_{16} and also dissociation into M_R occurs with rate constant k_5 . Dissociation of D_R into M_R takes place with rate constant k_6. Dissociation of M_R into R takes place with rate constant k_{11} and degradation of M_R takes place with rate constant k_7. Formation of C_{RISC} takes place with rate constant k_{21}.Formation of C occurs with rate constant k_{18} and again dissociation of C into R takes place with rate constant k_{13}. Degradation of R takes place with rate constant k_{12}. Degradation of C_{RISC} takes place with rate constant k_{22}. Formation of m occurs with rate constant k_{19} and again degradation takes place with rate constant k_{20}. Formation of A occurs with rate constant k_{15}. Formation of M_A occurs with rate constant k_8 and again dissociation of M_A into A takes place with rate constant k_{14}. Degradation of A takes place with rate constant k_{17}.Dissociation of D_A into M_A takes place with rate constant k_9. Degradation of M_A takes place with rate constant k_{10}.

Now consider the two systems are coupled via diffusive coupling. This coupling can be done by allowing the diffusion of m of one cell into other cell takes place with rate constant k45 and also the diffusion of m' of other cell into cell takes place with rate constant k_{46}. This can be expressed by introducing two extra reaction channels in the system.

This can be expressed by introducing two extra reaction channels in the system.

$$m \xrightarrow{k_{45}} m'$$

$$m' \xrightarrow{k_{46}} m$$

Then we look for synchronization in other corresponding variables in the two coupled systems. We consider various possibilities of coupling constants are taken which can induce cellular communication via miRNA. For the sake of convenience we take $k_{45}=k_{46}$. Since the interaction among the systems are random in nature depending on the availability of the miRNA diffused in and out of the systems, we introduce random coupling between the two cells to understand how they interact.

The genetic oscillator described by Vilar et al has been used by incorporating miRNA regulatory reaction channels. The model is shown in Figure 3.5. The description of the molecular species involved in the model is given in the Table 3.3. The set of reaction channels, their prophensity functions and values of the rate constants used in the simulation are listed in Table 3.3. The simulation results of the variables R (repressor) and A (activator) are shown in Figure 3.3. The dynamics of R and A show fluctuations due to stochastic interaction of molecular species. The value of system size V is taken to be zero.

List of the molecular species:

Sr. No.	Molecule Species	Description	Molecule
1.	D_A	Activator genes without activator protein bound to its promoter	Y_1
2.	D'_A	Activator genes with activator protein bound to its promoter	Y_2
3.	A	Activator Protein	Y_3
4.	D_R	Repressor gene without activator protein bound to its promoter	Y_4
5.	D'_R	Repressor gene with activator protein bound to its promoter	Y_5
6.	M_R	mRNA to the repressor protein	Y_6
7.	M_A	mRNA to the activator protein	Y_7
8.	R	Repressor Protein	Y_8
9.	C	Synthesis of activator-repressor complex	Y_9
10.	m	miRNA	Y_{10}
11.	C_{RISC}	Complex of miRNA with mRNA	Y_{11}

Table: 3.3 Notation of miRNA role in genetic oscillator

List of the reactions channels, propensity function and value of rate constant:

Sr. No.	Reaction Channel	Propensity Function	Value of Rate Constant
1.	$Y_2 \xrightarrow{k_1} Y_1$	$a_1 = k_1 * Y_2$	$k_1 = 50$
2	$Y_1 + Y_3 \xrightarrow{k_2} Y_2$	$a_2 = k_2 * (Y_1 * Y_3)$	$k_2 = 1$
3.	$Y_5 \xrightarrow{k_3} Y_4$	$a_3 = k_3 * Y_5$	$k_3 = 100$
4.	$Y_4 + Y_3 \xrightarrow{k_4} Y_5$	$a_4 = k_4 * (Y_4 * Y_3)$	$k_4 = 1$
5.	$Y_5 \xrightarrow{k_5} Y_6 + Y_5$	$a_5 = k_5 * Y_5$	$k_5 = 50$
6.	$Y_4 \xrightarrow{k_6} Y_6 + Y_4$	$a_6 = k_6 * Y_4$	$k_6 = 0.01$
7.	$Y_6 \xrightarrow{k_7} \phi$	$a_7 = k_7 * Y_6$	$k_7 = 0.5$
8.	$Y_2 \xrightarrow{k_8} Y_7 + Y_2$	$a_8 = k_8 * Y_2$	$k_8 = 500$
9.	$Y_1 \xrightarrow{k_9} Y_7 + Y_1$	$a_9 = k_9 * Y_1$	$k_9 = 50$
10.	$Y_7 \xrightarrow{k_{10}} \phi$	$a_{10} = k_{10} * Y_7$	$k_{10} = 10$
11.	$Y_6 \xrightarrow{k_{11}} Y_6 + Y_8$	$a_{11} = k_{11} * Y_6$	$k_{11} = 5$
12.	$Y_8 \xrightarrow{k_{12}} \phi$	$a_{12} = k_{12} * Y_8$	$k_{12} = 0.2$
13.	$Y_9 \xrightarrow{k_{13}} Y_8$	$a_{13} = k_{13} * Y_9$	$k_{13} = 1$
14.	$Y_7 \xrightarrow{k_{14}} Y_7 + Y_3$	$a_{14} = k_{14} * Y_7$	$k_{14} = 50$
15.	$Y_2 \xrightarrow{k_{15}} Y_2 + Y_3$	$a_{15} = k_{15} * Y_2$	$k_{15} = 50$
16.	$Y_5 \xrightarrow{k_{16}} Y_5 + Y_3$	$a_{16} = k_{16} * Y_5$	$k_{16} = 100$
17.	$Y_3 \xrightarrow{k_{17}} \phi$	$a_{17} = k_{17} * Y_3$	$k_{17} = 1$
18.	$Y_8 + Y_3 \xrightarrow{k_{18}} Y_9$	$a_{18} = k_{18} * (Y_8 * Y_3)$	$k_{18} = 2$
19.	$\phi \xrightarrow{k_{19}} Y_{10}$	$a_{19} = k_{19} * 1$	$k_{19} = 20$

20.	$Y_{10} \xrightarrow{k_{20}} \phi$	$a_{20} = k_{20} * Y_{10}$	$k_{20} = 0.029$
21.	$Y_{10} + Y_6 \xrightarrow{k_{21}} Y_{11}$	$a_{21} = k_{21} * (Y_{10} * Y_6)$	$k_{21} = 6.0$
22.	$Y_{11} \xrightarrow{k_{22}} \phi$	$a_{22} = k_{22} * Y_{11}$	$k_{22} = 0.6$

Table: 3.4

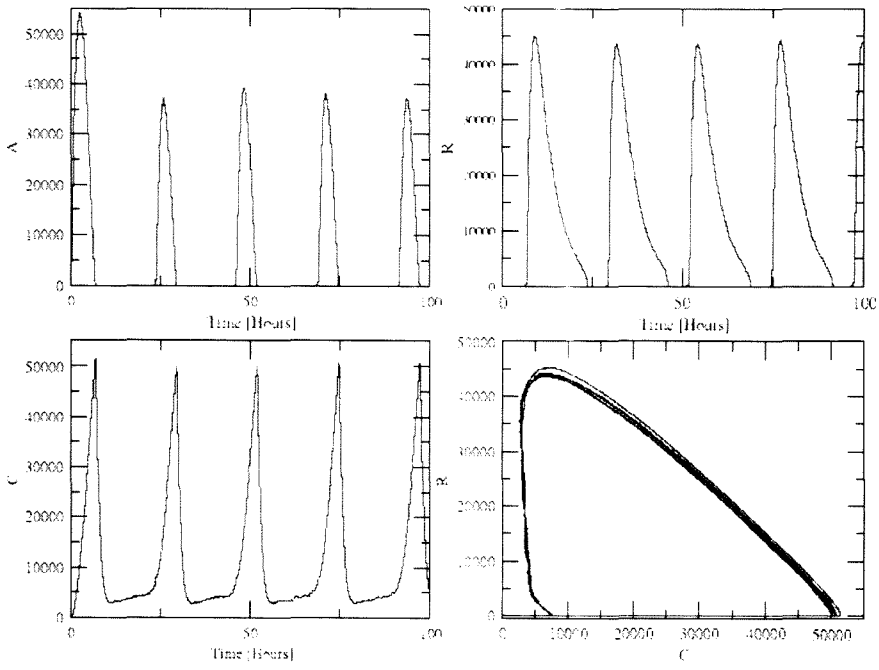

Figure- 3.4 Genetic oscillator

Oscillator-1

Sr. No.	Molecule Species	Description	Molecule
1.	D_A	Activator genes without activator protein bound to its promoter	Y_1
2.	D^*_A	Activator genes with activator protein bound to its promoter	Y_2
3.	A	Activator Protein	Y_3
4.	D_R	Repressor gene without activator protein bound to its promoter	Y_4
5.	D^*_R	Repressor gene with activator protein bound to its promoter	Y_5
6.	M_R	mRNA to the repressor protein	Y_6
7.	M_A	mRNA to the activator protein	Y_7
8.	R	Repressor Protein	Y_8
9.	C	Synthesis of activator-repressor complex	Y_9
10.	m	miRNA	Y_{10}
11.	C_{RISC}	RISC of miRNA with mRNA	Y_{11}

Table: 3.5

Oscillator -2

Sr. No.	Molecule Species	Description	Molecule
12.	D^1_A	Activator genes without activator protein bound to its promoter	Y_{12}
13.	D^{**}_A	Activator genes with activator protein bound to its promoter	Y_{13}
14.	A'	Activator Protein	Y_{14}
15.	D^1_R	Repressor gene without activator protein bound to its promoter	Y_{15}
16.	D^{**}_R	Repressor gene with activator protein bound to its promoter	Y_{16}
17.	M^*_R	mRNA to the repressor protein	Y_{17}
18.	M^*_A	mRNA to the activator protein	Y_{18}
19.	R^*	Repressor Protein	Y_{19}

20.	C'	Synthesis of activator-repressor complex	Y_{20}
21.	m'	miRNA	Y_{21}
22.	C_{RISC}	RISC of miRNA with mRNA	Y_{22}

Table: 3.6

All Reactions Channels

Sr. No.	Reaction Channel	Propensity Function	Value of Rate Constant
1.	$Y_2 \xrightarrow{k_1} Y_1$	$a_1 = k_1 * Y_2$	$k_1 = 50$
2	$Y_1 + Y_3 \xrightarrow{k_2} Y_2$	$a_2 = k_2 * (Y_1 * Y_3)$	$k_2 = 1$
3.	$Y_5 \xrightarrow{k_3} Y_4$	$a_3 = k_3 * Y_5$	$k_3 = 100$
4.	$Y_4 + Y_3 \xrightarrow{k_4} Y_5$	$a_4 = k_4 * (Y_4 * Y_3)$	$k_4 = 1$
5.	$Y_5 \xrightarrow{k_5} Y_6 + Y_5$	$a_5 = k_5 * Y_5$	$k_5 = 50$
6.	$Y_4 \xrightarrow{k_6} Y_6 + Y_4$	$a_6 = k_6 * Y_4$	$k_6 = 0.01$
7.	$Y_6 \xrightarrow{k_7} \phi$	$a_7 = k_7 * Y_6$	$k_7 = 0.5$
8.	$Y_2 \xrightarrow{k_8} Y_7 + Y_2$	$a_8 = k_8 * Y_2$	$k_8 = 500$
9.	$Y_1 \xrightarrow{k_9} Y_7 + Y_1$	$a_9 = k_9 * Y_1$	$k_9 = 50$
10.	$Y_7 \xrightarrow{k_{10}} \phi$	$a_{10} = k_{10} * Y_7$	$k_{10} = 10$
11.	$Y_6 \xrightarrow{k_{11}} Y_6 + Y_8$	$a_{11} = k_{11} * Y_6$	$k_{11} = 5$
12.	$Y_8 \xrightarrow{k_{12}} \phi$	$a_{12} = k_{12} * Y_8$	$k_{12} = 0.2$
13.	$Y_9 \xrightarrow{k_{13}} Y_8$	$a_{13} = k_{13} * Y_9$	$k_{13} = 1$

14.	$Y_7 \xrightarrow{k_{14}} Y_7 + Y_3$	$a_{14} = k_{14} * Y_7$	$k_{14} = 50$
15.	$Y_2 \xrightarrow{k_{15}} Y_2 + Y_3$	$a_{15} = k_{15} * Y_2$	$k_{15} = 50$
16.	$Y_5 \xrightarrow{k_{16}} Y_5 + Y_3$	$a_{16} = k_{16} * Y_5$	$k_{16} = 100$
17.	$Y_3 \xrightarrow{k_{17}} \phi$	$a_{17} = k_{17} * Y_3$	$k_{17} = 1$
18.	$Y_8 + Y_3 \xrightarrow{k_{18}} Y_9$	$a_{18} = k_{18} * (Y_8 * Y_3)$	$k_{18} = 2$
19.	$\phi \xrightarrow{k_{19}} Y_{10}$	$a_{19} = k_{19} * 1$	$k_{19} = 20$
20.	$Y_{10} \xrightarrow{k_{20}} \phi$	$a_{20} = k_{20} * Y_{10}$	$k_{20} = 0.029$
21.	$Y_{10} + Y_6 \xrightarrow{k_{21}} Y_{11}$	$a_{21} = k_{21} * (Y_{10} * Y_6)$	$k_{21} = 6.0$
22.	$Y_{11} \xrightarrow{k_{22}} \phi$	$a_{22} = k_{22} * Y_{11}$	$k_{22} = 0.6$
23.	$Y_{13} \xrightarrow{k_{23}} Y_{12}$	$a_{23} = k_{23} * Y_{13}$	$k_{23} = 50$
24.	$Y_{12} + Y_{14} \xrightarrow{k_{24}} Y_{13}$	$a_{24} = k_{24} * (Y_{12} * Y_{14})$	$k_{24} = 1$
25.	$Y_{16} \xrightarrow{k_{25}} Y_{15}$	$a_{25} = k_{25} * Y_{16}$	$k_{25} = 100$
26.	$Y_{15} + Y_{14} \xrightarrow{k_{26}} Y_{16}$	$a_{26} = k_{26} * (Y_{15} * Y_{14})$	$k_{26} = 1$
27.	$Y_{16} \xrightarrow{k_{27}} Y_{17} + Y_{16}$	$a_{27} = k_{27} * Y_{16}$	$k_{27} = 50$
28.	$Y_{15} \xrightarrow{k_{28}} Y_{17} + Y_{15}$	$a_{28} = k_{28} * Y_{15}$	$k_{28} = 0.01$
29.	$Y_{17} \xrightarrow{k_{29}} \phi$	$a_{29} = k_{29} * Y_{17}$	$k_{29} = 0.5$
30.	$Y_{13} \xrightarrow{k_{30}} Y_{18} + Y_{13}$	$a_{30} = k_{30} * Y_{13}$	$k_{30} = 500$

31.	$Y_{12} \xrightarrow{k_{31}} Y_{18} + Y_{12}$	$a_{31} = k_{31} * Y_{12}$	$k_{31} = 50$
32.	$Y_{18} \xrightarrow{k_{32}} \phi$	$a_{32} = k_{32} * Y_{18}$	$k_{32} = 10$
33.	$Y_{17} \xrightarrow{k_{33}} Y_{17} + Y_{19}$	$a_{33} = k_{33} * Y_{17}$	$k_{33} = 5$
34.	$Y_{19} \xrightarrow{k_{34}} \phi$	$a_{34} = k_{34} * Y_{19}$	$k_{34} = 0.2$
35.	$Y_{20} \xrightarrow{k_{35}} Y_{19}$	$a_{35} = k_{35} * Y_{20}$	$k_{35} = 1$
36.	$Y_{18} \xrightarrow{k_{36}} Y_{18} + Y_{14}$	$a_{36} = k_{36} * Y_{18}$	$k_{36} = 50$
37.	$Y_{13} \xrightarrow{k_{37}} Y_{13} + Y_{14}$	$a_{37} = k_{37} * Y_{13}$	$k_{37} = 50$
38.	$Y_{16} \xrightarrow{k_{38}} Y_{16} + Y_{14}$	$a_{38} = k_{38} * Y_{16}$	$k_{38} = 100$
39.	$Y_{14} \xrightarrow{k_{39}} \phi$	$a_{39} = k_{39} * Y_{14}$	$k_{39} = 1$
40.	$Y_{19} + Y_{14} \xrightarrow{k_{40}} Y_{20}$	$a_{40} = k_{40} * (Y_{19} * Y_{14})$	$k_{40} = 2$
41.	$\Phi \xrightarrow{k_{41}} Y_{21}$	$a_{41} = k_{41} * 1$	$k_{41} = 20$
42.	$Y_{21} \xrightarrow{k_{42}} \phi$	$a_{42} = k_{42} * Y_{21}$	$k_{42} = 0.029$
43.	$Y_{21} + Y_{17} \xrightarrow{k_{43}} Y_{22}$	$a_{43} = k_{43} * (Y_{21} * Y_{17})$	$k_{43} = 6.0$
44.	$Y_{22} \xrightarrow{k_{44}} \phi$	$a_{44} = k_{44} * Y_{22}$	$k_{44} = 0.6$
45.	$Y_{10} \xrightarrow{k_{45}} Y_{21}$	$a_{45} = k_{45} * Y_{10}$	$k_{45} = 3.0$
46.	$Y_{21} \xrightarrow{k_{46}} Y_{10}$	$a_{46} = k_{46} * Y_{21}$	$k_{46} = 3.0$

Table: 3.7 Reaction channels synchronization between two oscillator

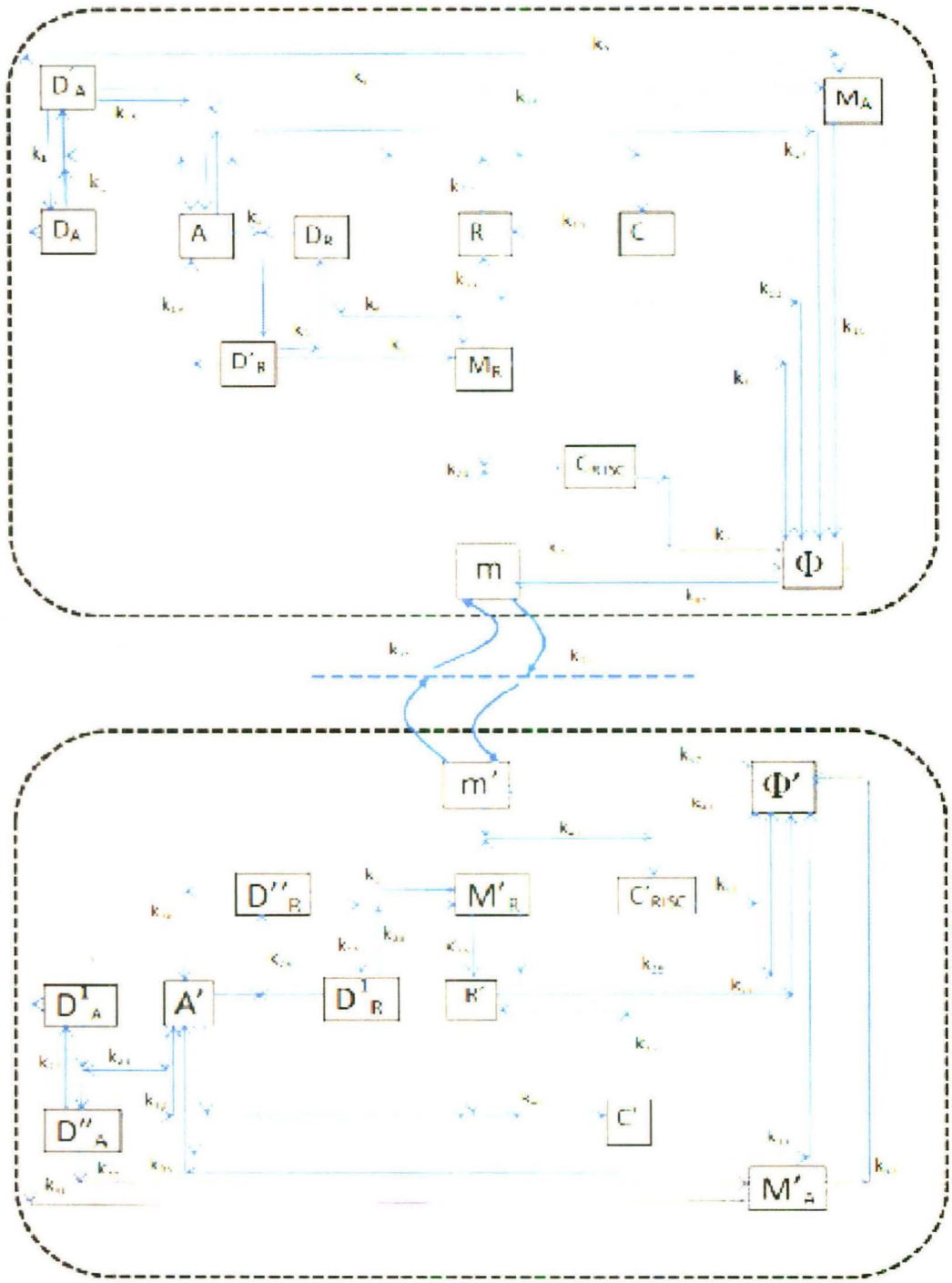

Figure- 3.5 Coupled two genetic oscillator model induced by miRNA

The role of miRNA in regulating genetic oscillator is very important to study with in order to understand its activities in wider cellular networks. Further, its role in cellular communication and how it helps in signal processing are still open questions to be investigated. We will address these problems in this work and try to understand some of the fundamental working principle of miRNA in genetic regulation.

Chapter 4

miRNA induced regulation of Genetic oscillator and synchronization

We have our proposed the model of interacting two identical genetic oscillators via diffusive coupling and checked for synchronization at various values of coupling constant. At zero value of coupling constant we got the dynamics of the variable in the two oscillators evolve independently (Fig. 4.1) showing the oscillators are uncoupled and desynchronized. Therefore, the dynamics of Activator protein (A) of one oscillator uncorrelates with the corresponding dynamics of Activator protein (A') of another oscillator. Therefore, there is no information flow from one oscillator to the other. Same is shown for dynamics of repressor proteins R and R' of the two uncoupled oscillators.

We then changed the value of coupling constant (ε) and as the value of coupling strength increases the two oscillators start process signal each other via diffusing miRNA molecules. Then at ε=3 we found strong synchronization between corresponding variables (A,A') and (R,R') as shown in Figure 4.2. The synchronization is not a complete synchronization because of noise induced due to stochastic nature of the system. The synchronization of the set of variables (A,A') and (R,R') is supported by two dimensional recurrence plots (Fig. 4.4) where points in the plots concentrate towards the diagonal due to correlation between the corresponding variables when the two variables are synchronized. If the two oscillators are not synchronized then the points will scatter away from the diagonal. Therefore, the rate of concentration of the points towards the diagonal in fact indicates the degree of strong synchrony the two oscillators will have.

We found that miRNA affect the frequency and amplitude of the genetic oscillator and regulate the network. The change in the value of coupling constant (reaction 45-46) and miRNA rate constant (reaction 19-22) in Table 7, affects the dynamics of A and R of the oscillator. Biologically the increase in the value of coupling constant will induce stress in system and the excess value of it may cause apoptosis which is programmed cell death or it may lead to cancer.

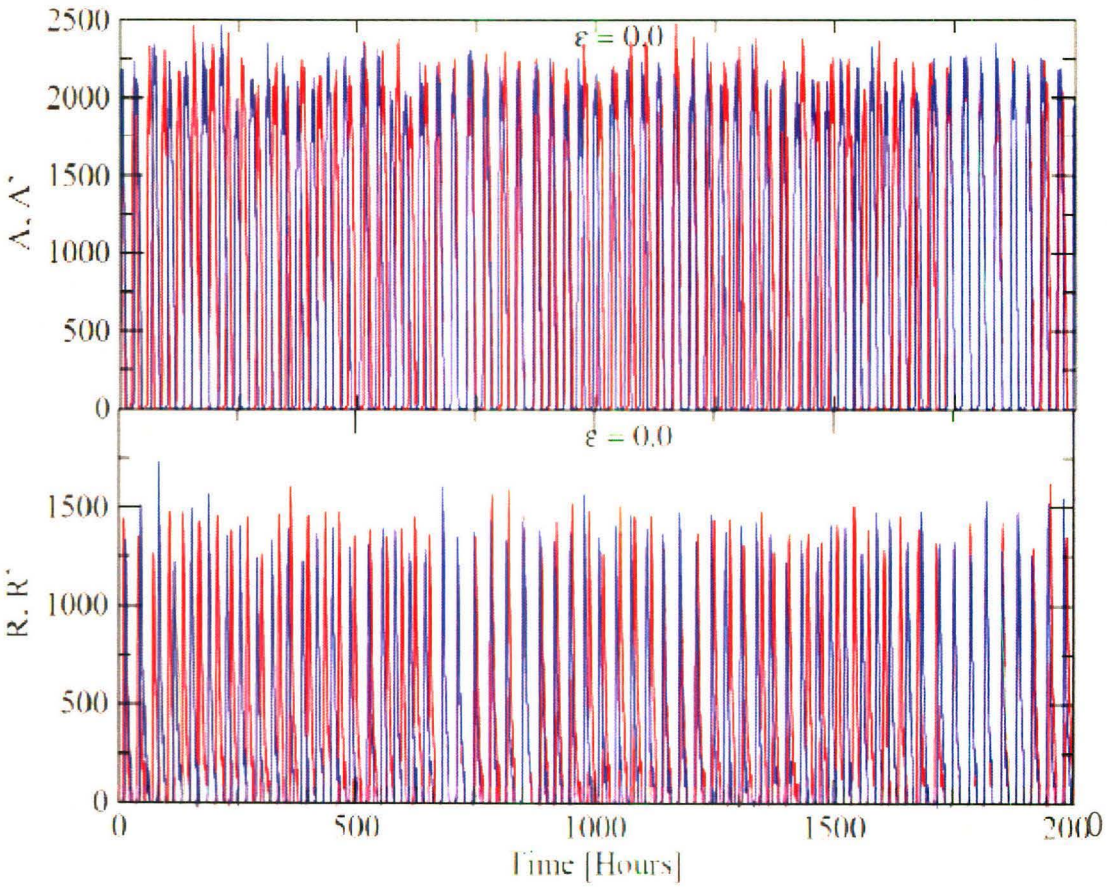

Figure- 4.1 the desynchronization of stochastic genetic oscillators.

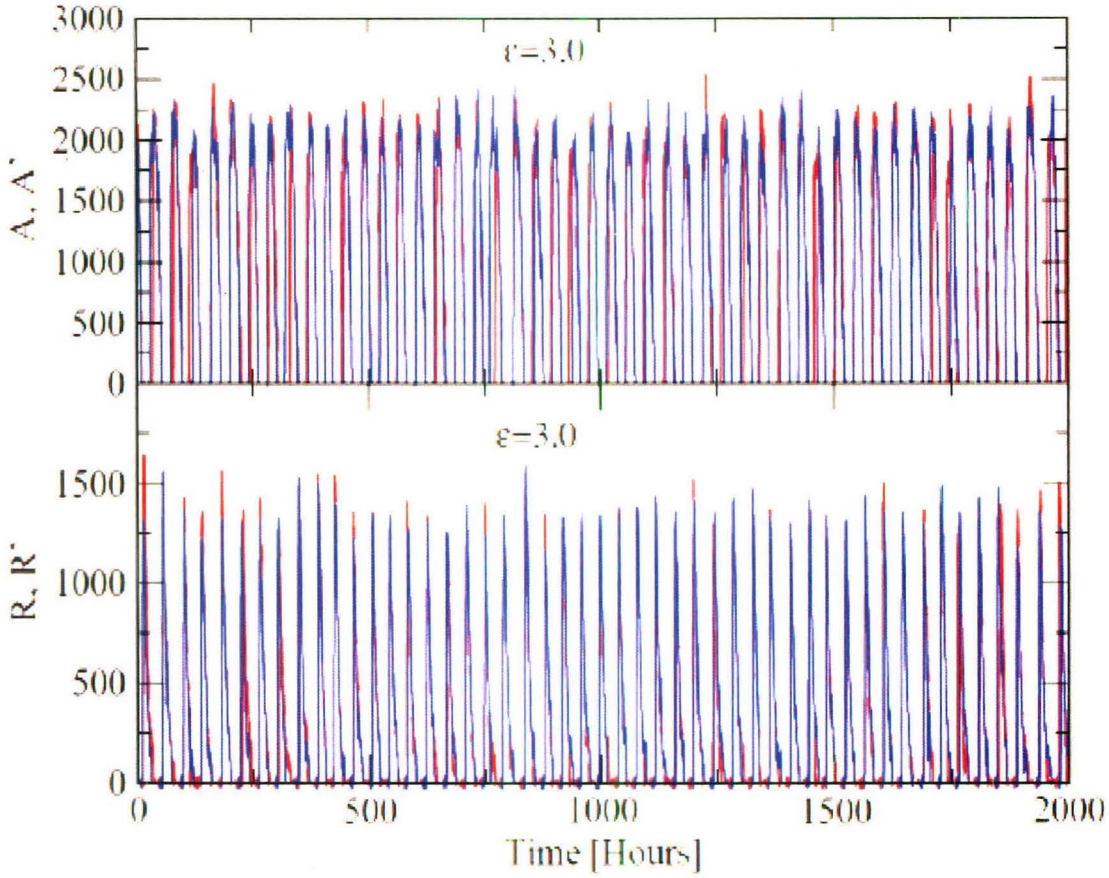

Figure- 4.2 synchronization of stochastic genetic oscillators.

If the switching of coupling is done at a particular time in the dynamics, the coupled genetic oscillators do not show synchrony instantaneously but take some time to get synchronization (Fig. 4.3). The coupling is switched on with value ε=3 at 250 hours in the dynamics of two coupled oscillators, and synchronization between A and A', and R and R' takes place after 70 hours later (320 hours onwards). We could able to see uncoupled or desynchronized regime (>250hours), transition regime ([250-320] hours) where the oscillators start interacting each other and strongly synchronized regime. These phase transitions are unique and independent of initial conditions. However, if the oscillators are far away at initial condition, the range of the regimes slightly alter but the three regimes are distinct and always there in the dynamics.

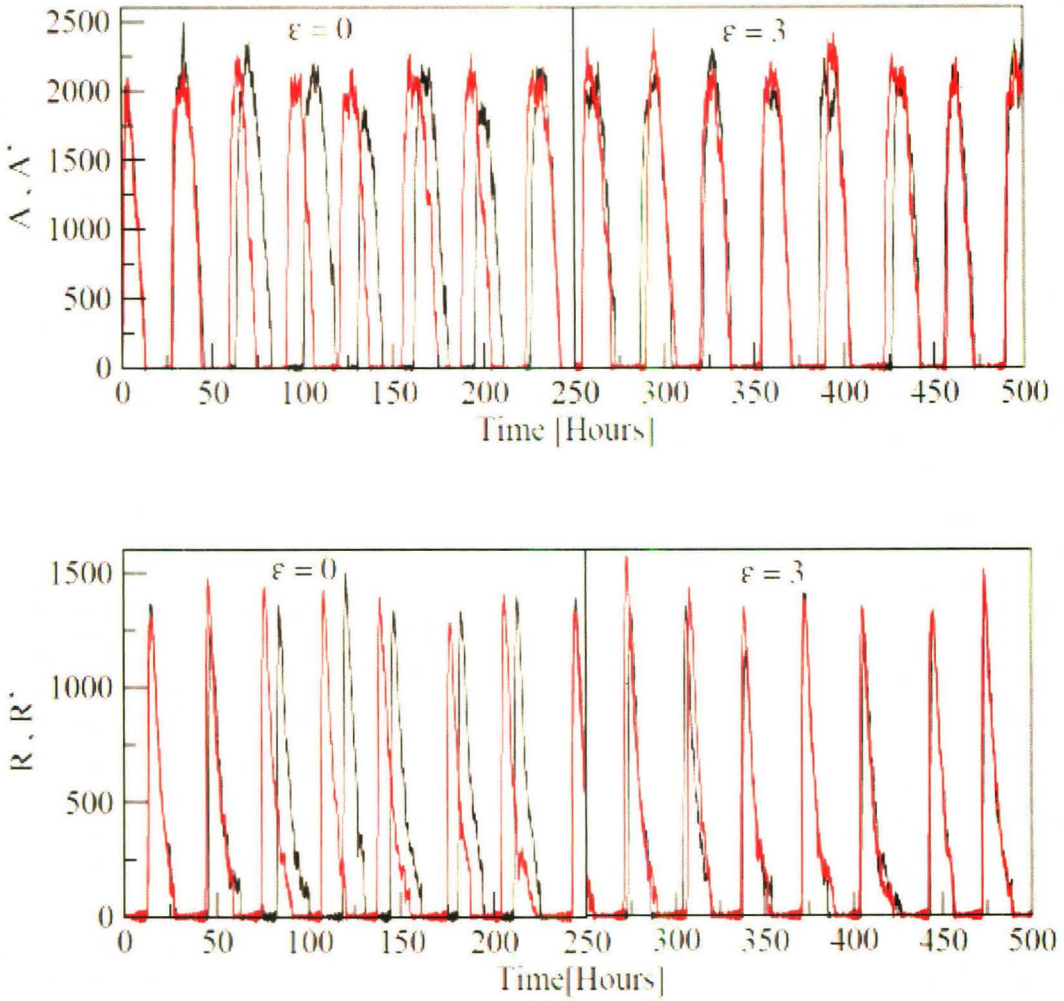

Figure 4.3 Generalized synchronization and Desynchronization stochastic genetic oscillators.

The recurrence plots between A and A', and R and R' for ε =0 show random distribution of points throughout the entire plain indicating the two oscillators are uncoupled and behave independent of each other (Fig. 4.4). However, at coupling constant ε =3, the points in plain of recurrence plot start concentrating towards the diagonal showing the correlation of the points indicating the synchronization of the two oscillators induced by miRNA. The broadening of the concentrating points towards diagonal is due to stochastic nature of the systems where intrinsic noise is the main player which resists from complete synchronization.

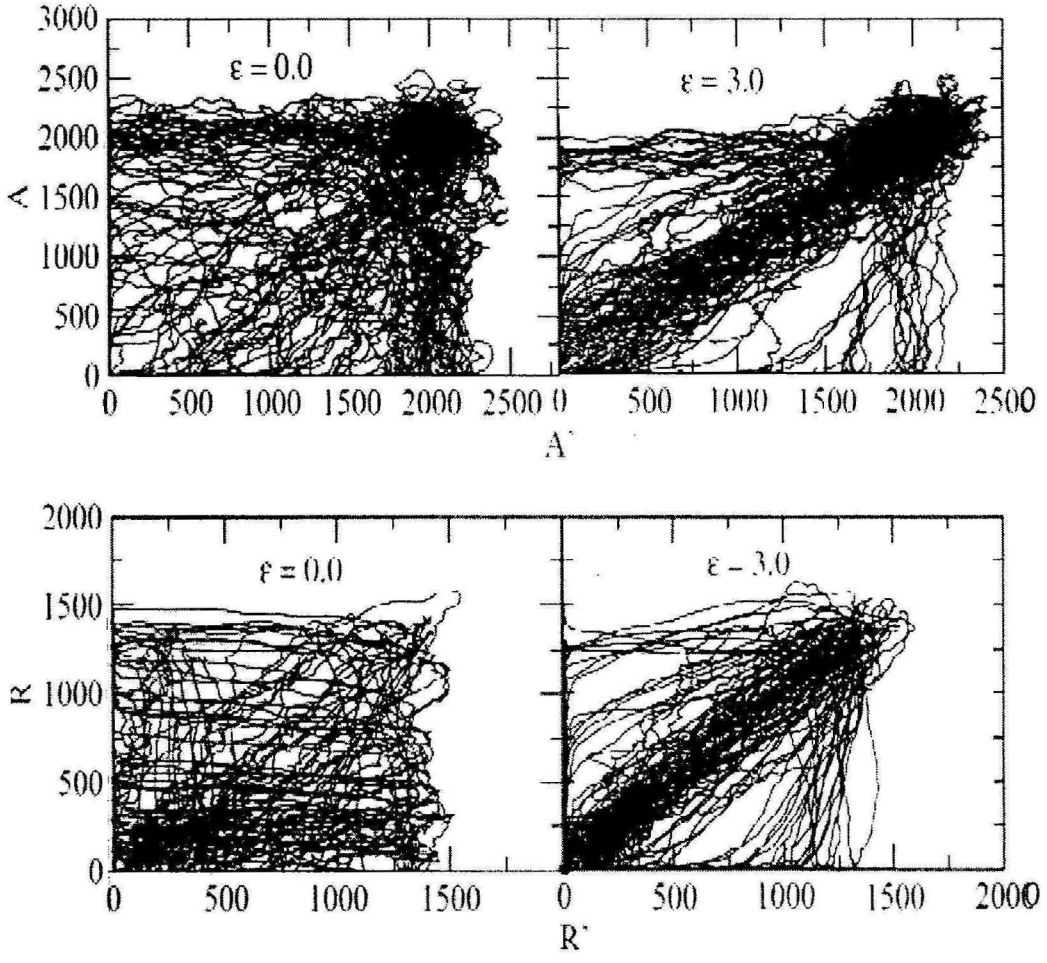

Figure-4.4 Recurrence plot

4.1 The impact of miRNA rate constant on coupling constant

The genetic oscillator is regulated by miRNA consists of four extra reactions in the usual reaction network of genetic oscillator. Since the decay and synthesis rates of miRNA and related complexes induce impact in the overall behavior of the network and signal processing, we study how different rate constants of the four reactions regulate the overall signal processing of the network. This study is done by varying the rate constants and see how they influence the coupling constant.

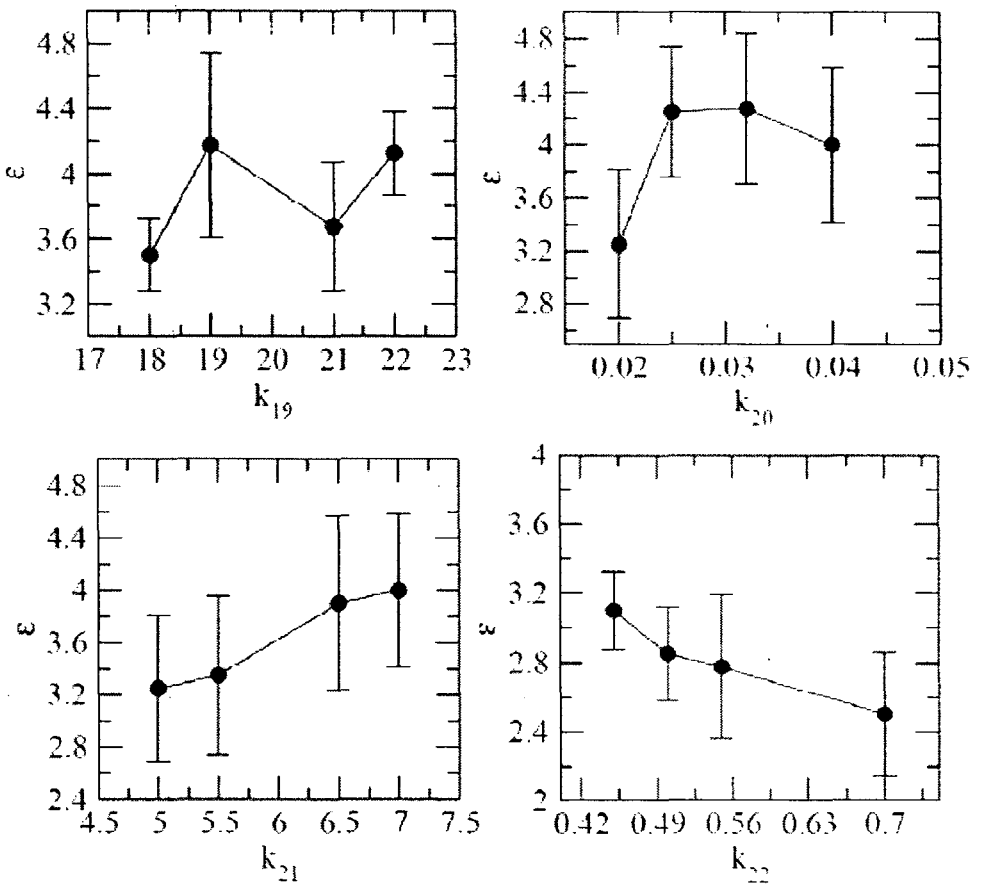

Figure-4.5 Phase Diagram

The variation in k_{19} shows fluctuations in the coupling constant and the error bars in each data point indicates the range in which the point can fluctuate. Since the coupling constant on an average remains constant as a function of k_{19} it shows that there is no interference of k_{19} to the signal processing or synchronization phenomenon. If the range of k_{19} is in between [18-22] the ε becomes 3.8. This means that changing k_{19} the the genetic oscillators need higher value of ε for achieving synchronization.

We then look for the impact k_{20} on the synchronization efficiency. We varied k_{20} in the range [0.02-0.04] to understand the impact on ε. The result show that initially ε (3.2) increases as k_{20} increases to reach a maximum value of ε (4.6), then stays stationary for some range of k_{20} [0.025-0.035] then decreases to a value 4 as k_{20}. This indicates that the

response of ε due to k_{20} has three fold impact scenario: first, for small values of k_{20} the oscillators do synchronize quickly, secondly for larger values of k_{20} oscillators need higher values of ε to get synchrony due to resistance of miRNA regulation, and thirdly for very large values of k_{20} the oscillators achieve synchrony quickly again. Therefore, k_{20} has interesting impact on genetic oscillator regulation.

We now study the impact of k_{21} which is the formation rate of C_{RISC} on coupling constant in Fig. 4.5. The results show that the value of ε increases (3.2 to 4.1) as k_{21} increases (5-7). This means that the increase in the population of C_{RISC} resist the signal processing between the oscillators.

The degradation rate k_{22} of C_{RISC} has also strong impact on the coupling constant. The results show that as k_{22} increases ε decreases almost exponentially. This means that the decrease in the population of C_{RISC} the synchronization between the oscillators enhances and they can achieve synchrony at a small value of ε.

4.2 Competition between synchronizing ability and stress induced by miRNA

miRNA induce stress to the system by regulating and exploiting amplitude and time period of the genetic oscillator which is toxic to the system. At the same time it also induce synchrony among the identical systems by acting as synchronizing agent which plays constructive role in the signal processing. We study the the working range of miRNA in terms of coupling constant ε which is a measurable parameter. As we have shown in previous sections that the diffusively coupled two genetic oscillators show synchronization at ε=3.0. At this situation, the synchronization is found between the A and A'; as well as between R and R' of the two systems as well (Fig. 4.6). We can see this behavior in both temporal behavior and as well as in recurrence plot where points in the plain are more towards diagonal.

We then increase the value of ε upto a certain interval and found that there is lack of synchronization between two oscillators as ε increases (both between A, A' and also between R, R'). When the coupling constant is 15 and above, we found that the two

oscillators become desynchronized, which can be seen both in temporal and recurrence plots of both variables (A,A') and (R,R'). The results show that in moderate values of ε the role of miRNA is constructive which is actively taking part in signal transduction and processing. However, if the population of diffusing miRNA is large (ε is large) then giving stress or toxic to the system is much more than the constructive role by violating the oscillating behavior of the system.

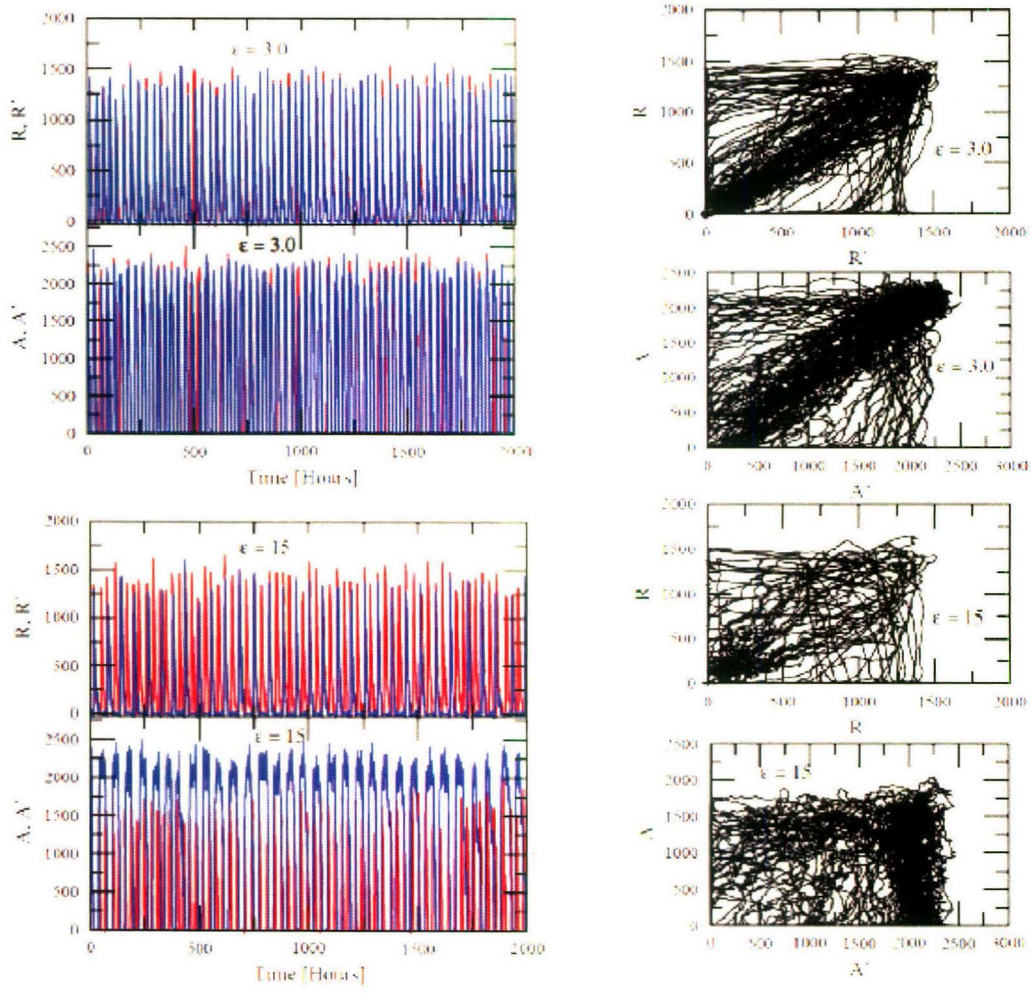

Figure- 4.6 Limitation of coupling constant

4.3 Synchronization due to random interaction

Generally natural systems specially biological systems interact among them randomly with random coupling strengths and are dependent on various factors such as how random the distribution of the systems is, environmental fluctuations and many other situations. We study this problem by allowing the two systems to couple with randomly. This is done by introducing a random number r to randomize the coupling constant as ε in bidirectional diffusive coupling. Other parameters and their values remains the same.

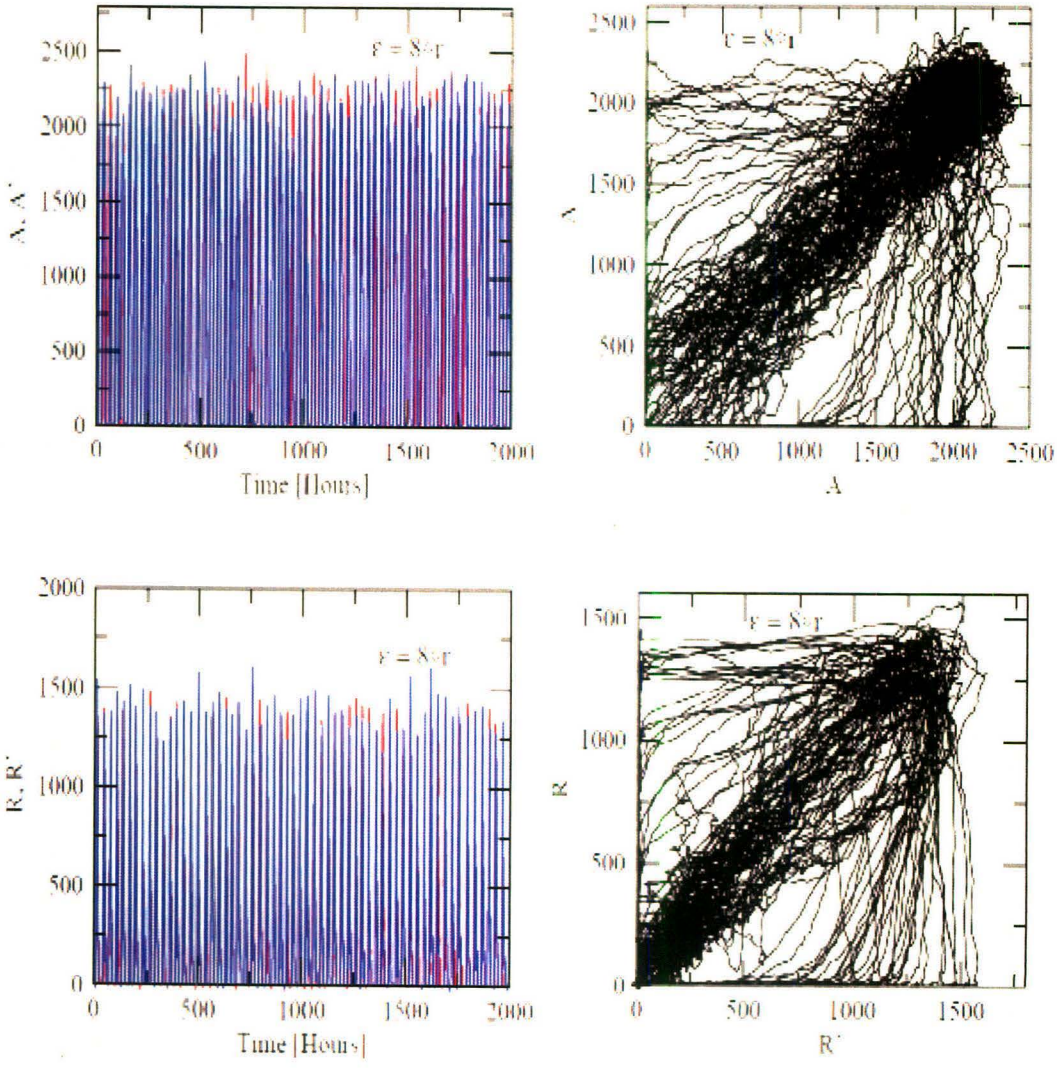

Figure 4.7 Synchronization with random coupling constant

The results show that the two oscillators show synchronization at $\varepsilon = 8r$ as different from the earlier value $\varepsilon=3$ (Fig. 4.7). The temporal behavior and recurrence plot of (A, A') and (R,R') support this claim of synchronization. The coupling constant becomes as a function of random number r. The deviation of this value of ε could be due to random interaction of the two systems which depends on the situation of random interactions arising out of systems and environment.

The study clearly shows that miRNA has strong regulating impact on genetic regulatory network. The moderate population of miRNA in the network has constructive role to regulate the network in terms signal processing, stabilizing the system. However, if the population of miRNA is large then it may induce toxic (stress) to the system and may lead to violate the rhythmic behavior of the system and destroy signal processing among them.

Chapter 5

Conclusion:

miRNA play a precious role in biological functions as it is in the transcription-translation feedback model and gives us an information about molecular clock [65]. Organisms need to adopt the sense of internal time in day today life and therefore biological clock arise through the evolutionary process of internal time kipping mechanisms that generate different rhythms. It has seen that miRNA induce change in the amplitude and frequency in circadian or genetic oscillator and also the effects on the genetic regulatory networks which is a controlling fundamental biological process [26]. Molecular and genetic studies suggest that a 24 hours time period creates a interconnected feedback cycles that is regulate the transcription of a few number of clock genes[63].

miRNA inside the cell is less stable than the outside the cell [27]. So there is some question raised about extracellular miRNA's function. It is also suggested and showed by our study that the extracellular miRNA may act as signaling molecule in cell- cell communication [62]. There is an important role of coupling constant of miRNA in regulating as well as signal processing of the cellular systems because at moderate values of this coupling constant the cells do process signal actively and quickly. However, at comparatively large values of coupling constant the oscillatory behavior is destroyed as well as signal processing is destroyed. Therefore miRNA acts two distinct and contrast roles depending on the available population of miRNA in the system in regulating the regulatory network.

There are challenging future works along this direction because the role of miRNA in inducing excess stress that may lead to either apoptosis or cancer is not well studied

problem. There could many types of miRNAs are involved in regulating circadian rhythm and these miRNAs need to be incorporated in modeling the system in order to get realistic results those may verify experimentally. The incorporation of time delay parameter in the system dynamics as well as in long range or relay signal processing of the cellular systems could be interesting problem to investigate which may give deep insight how miRNA works.

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