Mathematical Modeling of Circadian Clock

by

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This is to certify that I have examined this copy of a master's thesis by

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Anneme, Babama ve Abime…

ABSTRACT

Circadian rhythms are endogenously driven periodic oscillations of physiological, biochemical and behavioral processes within approximately 24 hours periodicity in diverse species range of orgaisms. In this thesis, we developed a detailed, predictive mathematical model for mammalian circadian clock. The concentrations of proteins are represented by ordinary differential equations, and first order mass action kinetic is assumed for every biological rate. These differential equations are solved by GAMS solver.

In the first part of this thesis, we try to optimize the equations by orthogonal collocation method, which is performed by GAMS, Those equations represent mRNA concentrations of oscilatting clock genes (Pers and Crys) and they are optimized to show a circadian behavior. After obtaining the results, the profiles are compared with real time data, and it is seen that the model is accordant with empirical results.

In the second part of the thesis, the aim is to knock out the genes that play role in the circadian clock mechanism. Equations, which are related to the knocked out gene, are removed from the model. Then the kinetic data set, which is obtained from the optimization, is fixed, and finally, simulation is performed. This procedure is applied for all the genes in the circadian clock. After obtaining the concentration profiles, those profiles are compared with the RT-PCR results which are found from the literature. It is seen that the model is capable of simulating the knock-out procedure.

In the last part, gene dosing is performed on core clock genes. Transcriptional rate that is obtained from the optimization part is multiplied by a certain number, and the rest of the system is left with the original kinetic data set. Per1, Per2, Cry1, Cry2 genes are subjected to gene dosing and it is seen that changing the transcription rates affects the circadian clock dramatically.

We built a predictive, detailed circadian model which is capable of simulating the RT-PCR concentration profiles of clock mRNAs. The most important advantage of this model is that it uses real time data, which makes the simulation biologically meaningful. Also including the post-translational modifications makes the system more realistic. The most important contribution of this study is the usage of optimization in order to find the kinetic parameters.

OZET

Biyolojik saat, belirli protein ve bu proteinlerin mRNA'larinin ortalama 24 saat suren periyodik salınımlar yapmasıdır. PERIOD 1-2 (PER 1-2) ve CRYPTOCHROME 1-2 (CRY1-2) proteinleri memelilerde bulunan sistemin ana proteinleridir. Biyolojik saat tek hücrelilerden memelilere kadar cok ceşitli canlı gruplarında bulunur. Bu çalışmada, biyolojik saat mekanizmasini detaylı bir şekilde modelledik. Ayrica bu model, biyolojik saat mekanizması üzerinde yapılan genetik değişiklikleri tahmin etme kapasitesine sahiptir. Modelde protein ve mRNA konsantrasyonları, birinci dereceden denklemlerle tanımlanmaktadır. Bu denklemler GAMS adı verilen, ve ozellikle endüstri mühendisliği alanında yayginca kullanilan bir program tarafindan çözülmüştür.

Tezin ilk kısmında, yukarıda bahsedilen denklemleri, dikey düzenleme adı verilen ozel bir metodla çözdük. Bu denklemler literatürde bulunan gerçek zamanlı PCR bilgilerine gore optimize edildi. Bu sekilde, mRNA ve protein konsantrasyonlarını temsil eden bu denklemlerin konsantrasyon profilleri, gerçek değerlere yaklaştırıldı.

Tezin ikinci kısmında, belirli genleri devre dışı bırakarak, o genlerin üretiminden sorumlu oldugu proteinlerin sistem uzerindeki tekil etkilerini inceledik. Bunu sağlayabilmek icin, bu genleri, ve uretikleri mRNA ve proteinleri sembolize eden denklemleri sistemden silip, geride kalan butun sistemi, optimizasyon kısmında elde ettigimiz verilerle beraber simüle ettik. Sistemdeki her gen icin bu prosedür yapıldı, ve model sonucları gercek zamanlı PCR datalarıyla karşılatırıldığında, modelin büyük ölcüde genetik degisiklikleri tahmin edebildiği görüldü.

Son olarak sistem uzerinde gen dozajı denen, ve genlerin çalışma hızını degiştirerek, bu degisimin sistem üzerindeki etkisini inceleyen bir metod uygulandı. Per1, Per2, Cry1 ve Cry2 genlerine uygulanan bu metodla, bu genlerin calışma hızının sistem uzerinde büyük etkileri olduğu görüldü.

Sonuç olarak bu çalismada, gercek zamanlı PCR verilerini kullanarak, biyolojik olarak tutarlı, tahmin gücü yüksek bir biyolojik saat modellemesi yaptık. Biyolojik saat mekanizmasiyla ilgili olan butun genleri sisteme ekledik. Bu çalışmanın alana yaptığı en onemli katkı, optimizasyon yonteminin biyolojik saat modeline uygulanmasıdır.

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NOMENCLATURE

Per1	Period 1
Per2	Period 2
Per3	Period 3
Cry1	Cryptochrome 1
Cry2	Cryptochrome2
KO	Knock-out
RT-PCR	Real-time Polymerase Chain Reaction
WT	Wild Type
BCC	Bmal1-Clock Complex
Q_{10}	Gauge of temperature dependency of a process
WC-1	White Collar 1
WC-2	White Collar 2
SCN	Suprachiasmatic Nucleus
Frq	Frequency
LD	Light-Dark
DD	Dark-Dark
CK	Casein Kinase
BVP	Boundary Value Problem
Po	Period1
Pt	Period2
Ro	Cryptochrome1
Rt	Cryptochrome2
D1	Dec1
D2	Dec2

Chapter 1

INTRODUCTION

Circadian rhythms are endogenously driven periodic oscillations of physiological, biochemical and behavioral processes within approximately 24 hours periodicity in diverse species ranging from cyanobacteria to mammals. The term "circadian" comes from the Latin circa, meaning "around", and diem or dies, meaning "day". Circadian rhythmicity is present in the sleeping and feeding patterns of animals, including human beings.
The disruption of circadian clock leads to disorders. A disrupted clock can lead to jetlag, sleeping disorders and cancer.

The circadian clock mechanisms are similar for many species that shows response to day light cycle. There are three key characteristics that are common in different species, which are persistence, temperature compensation and entrainment.

Drosophila and Neurospora are the first organisms in which circadian clock mechanism is discovered. With the identification of PERIOD protein in Drosophila, the molecular genetic studies on circadian clock have started.

After determining the clock mechanism for simpler organisms [1] [2], research has been extended to diverse biological systems. With the identification of new proteins in the mechanism, the negative and positive feedback loops of mammal's biological clock are observed. Those feedback mechanisms form the basis of circadian rhythm.

The circadian clock in mammals is located in the suprachiasmatic nucleus (SCN), a pair of distinct groups of cells located in the hypothalamus. There are about 20000 cells that create the circadian clock signal by acting simultaneously. Destruction of the SCN

results in the complete absence of a regular sleep–wake rhythm and rythm. The SCN receives information about light through the eyes.

At the molecular level circadian clock is composed of negative and positive feedback loops. Those loops are based on transcription and translation, and they employ a heterodimer of basic helix-loop-helix protein-containing transcription factors CLOCK and BMAL1 as activator, and also a family of PERIOD proteins and CRYPTOCHROME proteins as negative elements [3].

At the beginning of the circadian day, high levels of BMAL1 protein lead to formation of CLOCK:BMAL1 heterodimer (BCC). BCC promote the transcription of Per1, Per2, Cry1, Cry2 and Rev-Erbα genes by binding to a sequence called E-Box. Then mRNAs of those proteins are transferred to cytoplasm for translation. Afterwards, Rev-Erbα protein is located into nucleus, in where it represses the BMAL1 expression. In cytoplasm PER-CRY complex forms and it is located back to nucleus in where it inhibits BCC activity. So, all mRNA concentrations start to decrease, which triggers a decline in protein concentrations with a time lag. The output of the circadian clock mechanism is the oscillating concentration profiles of mCry, mPer, mDec and their proteins, mBmal1 and its protein. Light is responsible for starting the new circadian day.

Biological clock is a very large and complex system which needs to be explained in details. Empirical results are not enough to explain the overall system briefly, so building models enables scientists to understand the mechanism in details.

The most detailed circadian clock model is proposed by Forger and Peskin in 2003 [4]. In this model, every protein, which are found to be relevant to circadian clock by that time are included, also post transcriptional modifications are shown in details. Mass action kinetics is used to represent the biological reactions. The reaction rate constants represent biological rates. In this study, we extend the Forger-Peskin model by adding DEC proteins to the overall clock mechanism. After creating new equations which are representing the

role of DEC proteins in the system, the reaction rate constants are found. Those constants are found by trial and error in Forger-Peskin model like every previous model, however in our model we find those constant by optimization. We used optimization software called GAMS, which is capable of finding kinetic parameters that produce oscillating concentrations as observed in RT-PCR results. This is achieved by entering RT-PCR data, which are found from literature [5] [6] [7], to the software. Orthogonal collocation method, which is a polynomial approximation to the differential equations, is used to solve equations. This method gives the most realistic results since the optimization is done based on real data. Hence, the model is biologically meaningful.

Finally, knock-out procedure of certain genes is performed on model. Our main target is to knock-out Dec genes. After completing the procedure, we found that our model perfectly works in terms of knock-out, since we obtained similar results with experimental data.

Chapter 2 provides necessary background and literature review on circadian rhythm. The overall biological clock mechanism is explained in details, which includes details on every protein in the system, and their individual effect on the clock.

In Chapter 3 and 4, orthogonal collocation, the method used to solve the model, is discussed in details. After, the concentration results obtained from the software are given graphically. Their meanings and the effects of each protein on the overall system are discussed in details. Also the kinetic data that are optimized by the GAMS software are tabulated, by changing those parameters one by one; the robustness of the system is tested. These results are given in graphical representation.

The thesis is concluded with a short summary of the performed study.

Chapter 2

LITERATURE REVIEW

2.1 Overview

2.1.1 Chronobiology

Time can be defined as the measured or measurable period during which an action, process, or condition exists or continues. Since most of biological processes are time dependent, the area that investigates the relation with timing and biology is very crucial to understand the characteristics of organisms.Chronobiology (Chronos means time, bios means life and logos means to study), a word derived from Greek, is the area that comprises the scientific study that is related to living timing processes in plants and animals. In early times, timing ability of living organisms was known to be as the response of organisms to environmental stimuli. But throughout the time, the understanding of time effect on organism has changed. With the studies of two very famous scientists, who are also known as the two pioneers of chronobiology, Colin S. Pittendrigh and Jurgen Aschoff, the timing of organisms started to be seen as an internal mechanism, but not only a response to external environmental stimuli. With the study on daily locomotor activity rhythm of mice by Pittendrigh, chronobiology studies have started.

Throughout time, chronobiology has become one of the most important fields of biology. And also it is one of the most interdisciplinary fields. Chronobiology collaborates with different areas, which can be seen on Figure 2.1.

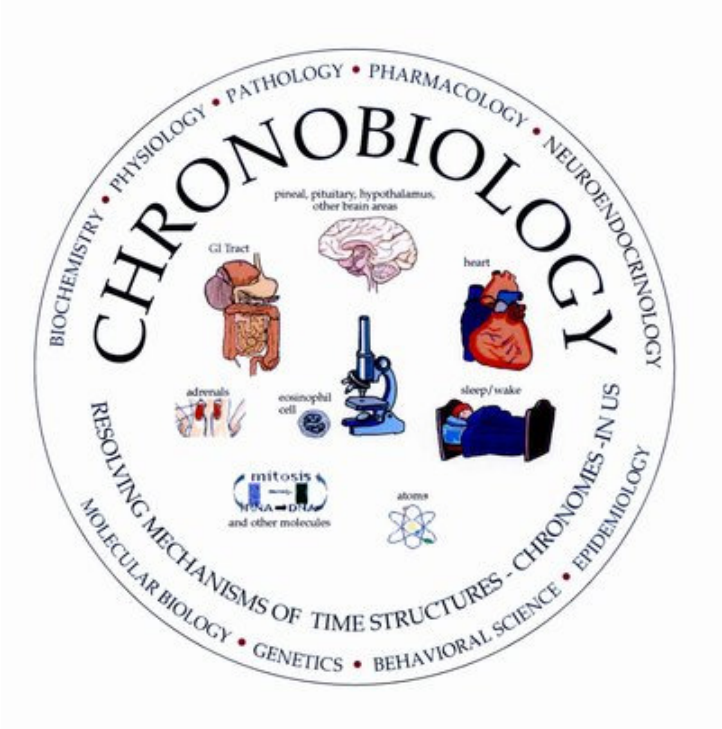

Figure 2-1 Interdisciplinary nature of chronobiology [8]

The timing process is achieved by organisms with the help of a biological mechanism, which is called the circadian clock mechanism.

2.1.2 What is Circadian Clock?

Circadian rhythms are endogenously driven periodic oscillations of physiological, biochemical and behavioral processes within approximately 24 hours periodicity in diverse species ranging from cyanobacteria [9]to mammals. The term "circadian" comes from the Latin circa, meaning "around", and diem or dies, meaning "day". Circadian rhythmicity

is present in the sleeping and feeding patterns of animals, including human beings. There are also clear patterns of core body temperature, brain wave activity, hormone production, cell regeneration and other biological activities. In addition, photoperiodism, the physiological reaction of organisms to the length of day or night, is vital to both plants and animals, and the circadian system plays a role in the measurement and interpretation of day length.

The disruption of circadian clock leads to disorders. A disrupted clock can lead to jetlag, sleeping disorders, Alzheimer's and even cancer.

2.1.2.1 Characteristics of Circadian Clock Mechanism

The core circadian clock mechanisms are similar for many species; biochemical mechanisms have evolved a common path for diverse organisms. There are three main key characteristics that are common in different species, namely persistence, temperature compensation and entrainment.

Circadian rhythm stays persistent in constant temperature, constant light or constant dark conditions with a period of approximately 24 hours. And the same biological behavior can be observed on every consecutive day.

The free running circadian period lengths are not affected from different ambient temperatures. In most cases, the reaction rates of biochemical reactions double or triple with a change of 10 ^{0}C. $Q_{10,}$ which is a gauge of temperature dependency of a process, is 2-3 for such processes. However for free runs of circadian rhythms, Q_{10} values range from 0.8 to 1.4 [3].

Thirdly, the circadian rhythm is entrained by 24 hour environmental cues. Those cues can be light-dark cycle, temperature cycles or other stimuli. Those stimuli are referred

as zeitgebers, which means time givers. For circadian clock, light and dark signals are the most important environmental entraining agents.

With the studies of Pittendrigh and Aschoff, chronobiology research has started. The chronobiology studies can be divided into two main eras, pre-molecular genetics era and molecular genetics era. In the former era, a variety of suggestions were made to explain how the clock might work. Those models are incapable of showing the circadian clock mechanism correctly, however, with the accompany of empiric studies, they helped to have a better understanding of the system.

Chronon model is one of the best known pre-molecular genetics era models for circadian clock. In this model there are hundreds of genes which are responsible for time keeping. With the transcription of the first gene, the circadian day starts. After translation of the mRNA, protein is responsible for the initiation of the transcription of the next gene. This mechanism is applied for every consecutive gene, and finally the last gene is responsible for initiating the transcription of the first gene [8].

Membrane models are important models in pre-genetic era. Like the Chronon model, Membrane models are also incapable of creating a correct understanding of the circadian clock mechanism [9].

With the rise of the molecular biology, the understanding of circadian mechanism has changed. The first gene that plays an important role in the circadian system is found in Drosophila, which is an organism that is under observation for a long time. This gene, and its protein is called as PER, which represents period, and it is found to be synthesized for 24 hours with an oscillating behavior [10]. After a while another gene is also found in Neurospora, which is called FRQ [11]. With these identifications of genes, the chronobiology entered a new era, called molecular genetics era.

2.1.2.1.1 Circadian Oscillators in Eukaryotes

Circadian rhythms are important for the daily and seasonal adaptations of all higher (eukaryotic) organisms, but are also found in lightsensing prokaryotes such as cyanobacteria [12]. In this review eukaryotic circadian clock will be discussed.

Early genetic and molecular biology studies on Drosophila and Neurospora indicated a common mechanism involving a transcriptional–translational negative feedback loop. However new discoveries suggest the presence of multiple loops and oscillators. In Chronon model, there was only a positive feedback mechanism loop, in where a protein is responsible for initiating the transcription of its successive gene.

With the progress in molecular biology, new proteins are found to be related with circadian clock mechanism in Drosophila and Neurospora. In Drosophila, PER and TIM (timeless) proteins are the most important proteins, in Neurospora, WHITE COLLAR-1 (WC-1) and WHITE COLLAR-2 (WC-2) are the other proteins taking role in circadian clock mechanism [13] [14].

Circadian clock mechanism of mammalians is controlled from suprachiasmatic nucleus (SCN). In 1990 Ralph et al. restored circadian wheel-running activities in Syrian hamsters that had their suprachiasmatic nucleus (SCN) removed, by transplanting back intact SCN tissue, indicating that the mammalian circadian clock is located in the SCN [15]. The SCN are small paired structures in the anterior hypothalamus, just above the optic chiasm. Each nucleus contains about 10,000 neurons. The nuclei are strategically positioned for receiving visual input for light-dark entrainment through both direct and indirect retina-to-SCN pathways.

There is now increasing evidence that clock genes are expressed in the oocyte and during early embryonic development [16]. The mammalian circadian clock is a complex auto-regulatory transcriptional and translational feedback program, which is composed of positive and negative regulators. Those loops are based on transcription and translation, and they employ a heterodimer of basic helix-loop-helix (bHLH)–PER-ARNT-SIM (PAS) protein-containing transcription factors CLOCK and BMAL1 as activator, and also a family of PERIOD proteins and CRYPTOCHROME proteins as negative elements [3].

At the molecular level, the clockwork of the SCN involves several proteins that participate in positive and negative transcriptional feedback loops. BMAL1 (for brain and musclearyl hydrocarbon receptor nuclear translocator (A RNT)-like protein 1) and CLOCK are transcription factors that contain two basic helix-loop-helix domains and bind E-box elements (CACGTG) in the Period and Cryptochrome clock genes and thereby effect a positive feedback loop of circadian rhythm regulation [17] [18]. The mammalian PERIOD proteins (PER1 and PER2; collectively designated PER) and CRYPTOCHROME proteins (CRY1 and CRY2; collectively designated CRY) act as negative regulators of transcription driven by the BMAL1/CLOCK heterodimer [19] [20]. PER and CRY form heterodimers that interact with casein kinase Iε (CKIε) and then translocate into nucleus where CRY acts as a negative regulator of BMAL1/CLOCK–driven transcription.

Then mRNAs of those proteins are transferred to cytoplasm for translation in ribosome. Afterwards, REV-ERBα protein is located into nucleus, in where it represses the BMAL1 expression. In cytoplasm, PER proteins are phosphorylated by casein kinase (CK1) enzyme which leads to the formation of PER-CRY complexes. A second phosphorylation step is responsible for localization of those complexes into the nucleus. Eventually PER-CRY-CK1 complex blocks BCC activity. This leads to a decline in the mRNA concentrations of Cry, Per and Rev- Erbα mRNA. After a time lag, their protein

concentrations shows a similar behavior, too. It is also known that PER2 protein is an activator for Bmal1 transcription rate [21].

The output of the circadian clock mechanism is the rhythmic expression of mCry, mPer, mDec, mBmal1 [9]. A similiar concentration profile is also observed for their protein concentrations. There is a time lag between the peaks of mRNA and their protein levels [23]. All concentration levels are at their lowest value at the beginning of the circadian day. With the triggering of light, the new circadian day begins by transcription of those mRNAs [3].

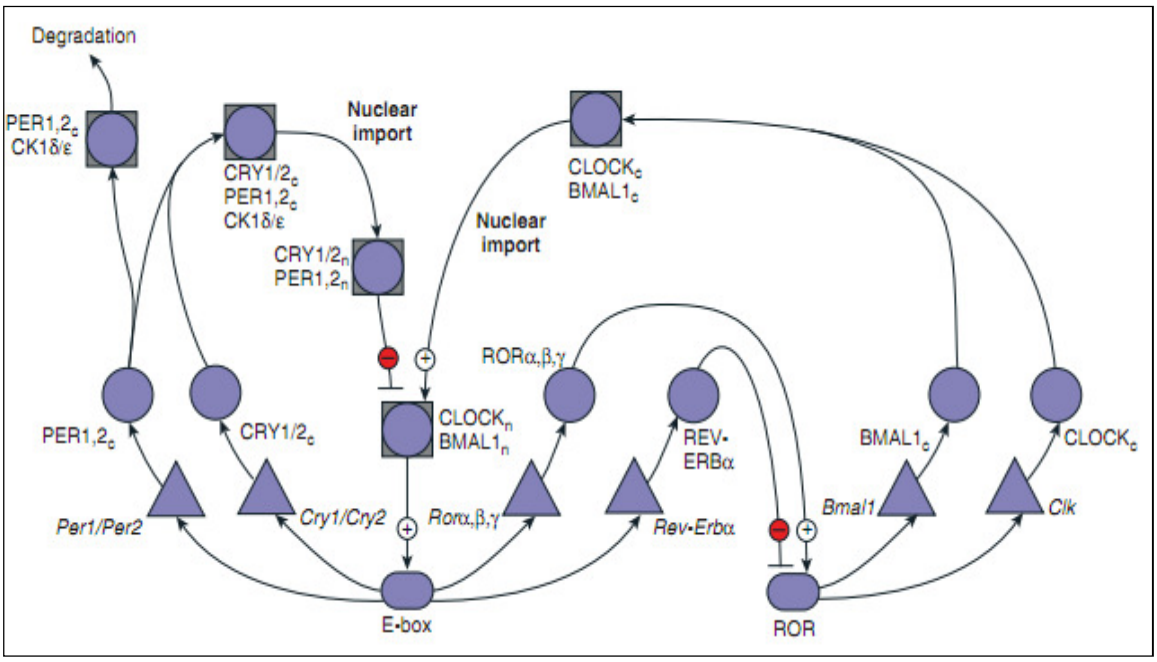

Figure 2-2 The mammalian circadian clock mechanism [64]

In addition to the biochemical feedback loop that regulates cycling at the E-box, circadian gene expression is mediated by the transcription at the ROR/REV-ERB and the DBP-

E4BP4 (D-box) binding elements NR1Ds and RORs (subfamilies of nuclear hormone receptors) are either activates or repress gene transcription form the ROR components in several clock gene [22]. There are also other transcription factors like bZIP, DBP, TEF and HLF carry out a similar function on the D-box element [23]. There are number of studies in the literature regarding the roles of these genes on circadian rhythm. For example, analysis mice knockout (Rev-erbα, Rorα, or Rorβ deficient mice) indicated that their circadian rhythm is greatly affected in terms of lower amplitude rhythm, abnormal period lengths [24] [25]. Further analysis show that a coactivator of ROR proteins called PGC1α is in fact modulating Bmal1 expression and Pgc1α knockout mice exhibit long-periods locomotors activity behavior [26]. All these data indicate that the important role of the clock gene network in regulating circadian amplitude, resistance perturbation and modulation of period length.

2.1.2.1.2 DEC1 and DEC2 Proteins

Recently, a new set of proteins found to have a regulative role on circadian clock mechanism. DEC1 and DEC2, basic helix–loop–helix transcription factors, repress CLOCK-BMAL1 induced transactivation of the Per1 promoter through direct protein–protein interactions with BMAL1 or competition for E-box elements [54]. Dec1 and Dec2 are expressed in the suprachiasmic nucleus in a circadian fashion, with a peak in the subjective day. A brief light pulse induced Dec1 but not Dec2 expression in the suprachiasmic nucleus in a phase-dependent manner. DEC1 and DEC2 are regulators of the mammalian molecular clock, and form a fifth clock-gene family [27]. Also DEC1 represses the transcription of DEC2. In other words, there is another negative feedback loop between these proteins [27] [28].

2.1.2.1.3 Light Effect on Circadian Clock

The circadian clock mechanism is controlled by the SCN which is located in the hypothalamus part of the brain. SCN generates endogenous circadian rhytms, however this does not mean that the clock mechanism is not affected by external cues. Light is the most important signal that regulates the circadian clock mechanism, in other words light is the zeitgeber for the system, it is responsible for the trigerring the starting of the circadian day. By the stimulation of melanopsin containing ganglion cells, light information reaches the SCN through the retinohypothalamic tract [29]. SCN is synchronized to a 24 hour cycle, with day and light periods. If this cycle changes, the system required days to readjust the clock mechanism. And during those days, different disorders in body can be observed, such as jet-lag.

Light up-regulates the transcriptions of mPer1 and mDec1 in mammals, light has a similar effect on mammalians when compared to other organisms, like Neurospora [30]. Also, it is known that exposure to bright light at night has important effects on the circadian clock such as altering hormone production and enhancing alertness [31].

Since light triggers the transcription of Per1 and Per2 genes, constant darkness or constant light has significant effects on their transcription rates, too. The phases of the RNA cycles of the period genes Per1 and Per2 shifts with changing the light effect [32].

2.2 Modeling of Circadian Clock

2.2.1 The Aim of Modeling

With the progress in molecular biology, many proteins have been identified which act as biological clock components, but scientists have only begun to understand how the components interact functionally to generate the circadian oscillation. For instance, it is known that the phosphorylation steps of PERIOD proteins has a very important role in the circadian clock mechanism, but the behavior of a system with a reduced or non-existent phosphorylation is not known experimentally. Only by gene-knock out, the effect of a clock gene can be observed individually.

The enormous and growing amount of detailed information available about biological systems demands an integrative modeling approach. Modeling enables the scientist to comment on the biological data easier. In addition to this, modeling leads to a better understanding on the overall functioning of the biological systems.

Biological clock is a very large, complex system which needs to be explained in more details beyond empirical results. That is why from an early time, models have been built to understand the circadian system. Since the research has started with simple organisms, the early models were built for those living entities.

2.2.2 Models for Circadian Clock

First, the models for Drosophila and Neurospora are developed [33] [34] [35] [36]. Those models and all their followers use ordinary differential equations to represent the biological systems. Although those models give idea about the system, they are incapable of examining the overall circadian mechanism since the models are too simplified.

Models are capable of showing the oscillations of the core proteins; however they do not include post translational modifications like phosphorylation [37] [38] [39]. Post-translational modifications play a very important role in the circadian clock mechanism.

Few models use Michaelis Menten kinetics in order to represent the circadian clock reaction kinetic rates [40] [41] [42]. But, since all the reactions are not enzyme substrate reactions, Michaelis Menten kinetics are not capable of producing biologically meaningful results. And, like previous models, they are too simplified.

2.2.3 Detailed Models for Circadian Clock

The most detailed circadian clock model is proposed by Forger and Peskin in 2003 [4]. Another study, which refines the kinetic parameter set obtained in Forger's work is also published [43]. In this model, every protein, which are found to be relevant to circadian clock by that time are included, also post transcriptional modifications are shown in details. Mass action kinetics is used to represent the biological reactions. The reaction rate constants represent biological rates, like transcription, translation or phosphorylation. The major drawback of the model is that those constants are found by trial and error method, which is time consuming. The mRNA concentrations show oscillations, however the concentration profiles do not match with RT-PCR results. A stochastic model of circadian clock is also proposed by Forger [44].

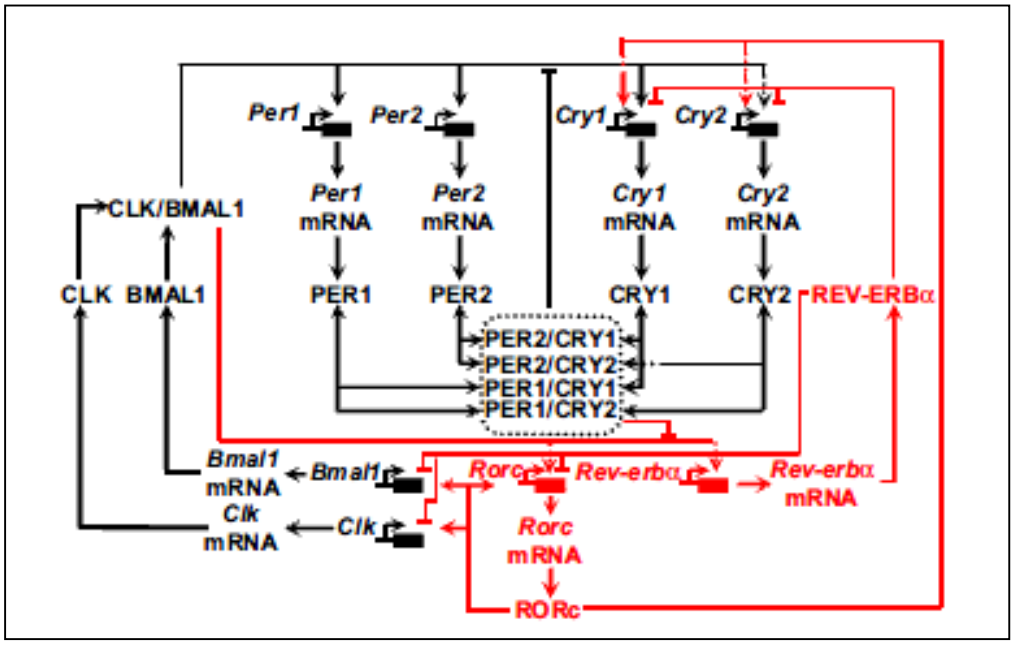

Figure 2-3The schematic representation of Mirsky circadian model [45]

Another important model of the cell-autonomous mammalian circadian clock is proposed by Mirsky et al [45]. In this model, all the important proteins are included in the system, but simplifications of post-translational modifications makes the system unrealistic, and the model is not biologically meaningful.

2.3 Perturbations on Circadian Clock Mechanism

2.3.1 Gene Knock Out

Simply, making a gene inoperative is called gene knock-out. Organisms, known as knockouts, are used for learning about a gene that has been sequenced, but which has an

unknown or incompletely known function. Researchers draw inferences from the difference between the knockout organism and normal individuals, in other words, wild types.

The main proteins that are related to circadian clock mechanism are PER1, PER2, PER3, CRY1, CRY2, CLOCK, BMAL1. By gene knock out procedure, the effect of each protein can be observed.

One of the most important characteristic of circadian clock mechanism is the periodicity. Periodicity is not affected by external effects, such as temperature. But knock-out of certain genes, changing their activity level can be effective on disturbing the periodicity of circadian clock mechanism. Knock-out can result in a longer or shorter circadian day length. Absence of certain genes can even result in total disturbance of the periodicity of circadian clock.

2.3.2 Knock-out of Cry1 and Cry2 Genes

The mammalian proteins CRY1 and CRY2, which are members of the family of plant blue-light receptors (cryptochromes) and photolyases, are two of core proteins that plays an important role in the circadian clock mechanism. Inhibiting the gene activity of both CRY proteins has a significant effect on the system. Individual knocking has a slight effect; it changes the length of the circadian day, whereas knocking out both genes results in arrhythmic.

To observe the biological function of each gene in cell, Cry1 and Cry2 mutant genes are created by gene targeting [46]. By using the reverse transcription-long-range polymerase chain reaction, it is seen that there is no Cry gene action. So null-mutant mice are created.

In order to see the effect of each protein, mice are subjected light-dark (LD) and dark-dark (DD) cycle. Those two cycles gives different results:

In DD cycle, Cry1 mutant mice show a faster behavior and the circadian day shortens. However, Cry2 mutant mice show exactly the opposite behavior, and the circadian day is longer that the wild type.

In LD cycle, the response of mice to knock-out one gene is totally different. The circadian day length shows no difference with compared to wild type animals. This is surprising, but can be explained with the replacement of the non-existent gene by its homolog pair, with a higher transcription rate.

In LD cycle, the individual effect of cry proteins cannot be seen clearly because of the replacement. So, double mutant mice should be observed. However, double mutant mice are again rhythmic when subjected to LD cycle.

The response of mice to double mutation of Cry genes change when they are subjected to DD cycle. The mice clock become arrhythmic, this shows that there is no internal circadian clock running.

In LD cycle, knockout of Cryptochrome genes has a smaller effect on other circadian clock mRNA concentration profiles. In order to see the individual effects of both CRY proteins, experiments are carried on DD cycle.

Knock-out of both Cry genes results in arrhythmicity of Period mRNA concentration profiles. In Figure 2.4, the relative mRNA abundance of Period mRNA's when Cry1, Cry2 or both are knocked out can be seen.

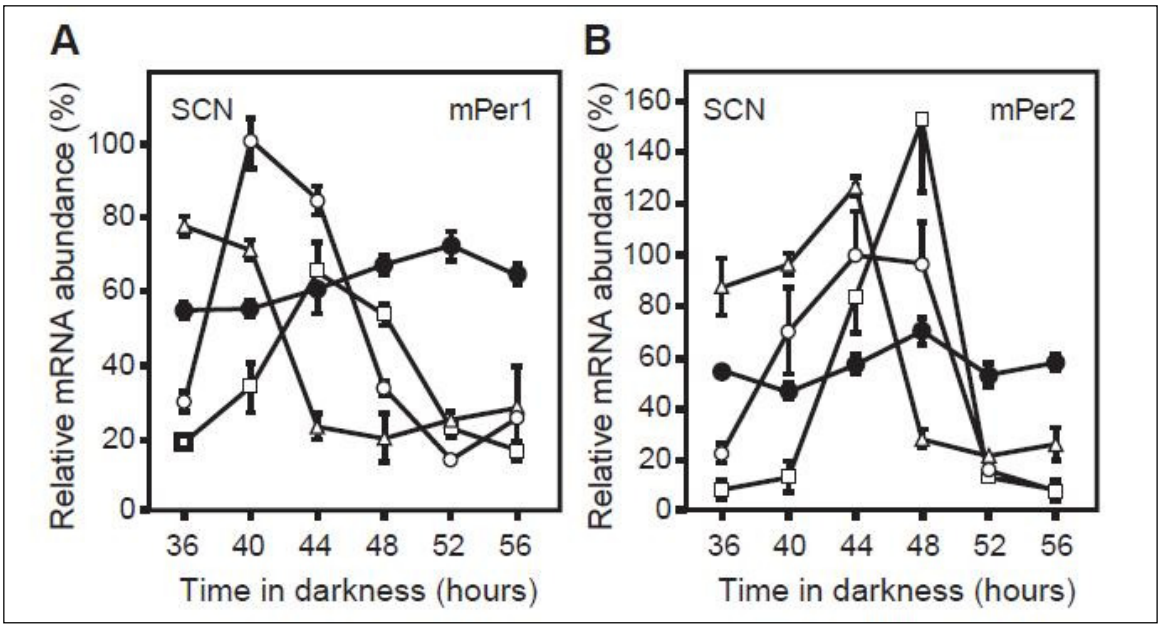

Figure 2-4 The effect of knocked out Cry genes: Phase shifts and relative concentration level changes are observed [47].

In Figure 2.4, wild type results are plotted with open circles, $Cry1^{-/-}$ with open triangles, $mCry2^{-/-}$ with open squares and duble mutant with closed circles. Rhythmic expression of mPer1 is not disturbed when Cry1 is knocked out. However, knocked out Cry2 gene results in a lowered level of mPer1 with a rhythmic expression. A phase shift is also seen. Knocking out both genes results in almost linear (statistically) concentration level.

A different result is seen on mPer2 when Cry genes are knocked out. In individual knocking out procedure, still a rhythmic expression is seen, but with different concentration levels and different peak points. When both genes are knocked out, a very slight peak and then a decrease in the expression level can be seen, but still it cannot be accepted as a rhythmic expression. It should be noted that these observations are done on a DD cycle.

2.3.3 Knock-out of Per1 and Per2 Genes

PERIOD1 and 2 proteins are two of the core elements of the circadian clock mechanism. They are known to play a very important role in the biological rhythm. By disrupting the activity of Period genes individually or together, changes in the circadian clock mechanism are observed. Like CRYPTOCHROMES, individual knocking out is less effective than a double deficiency.

An mPer1 deficient mouse is created by replacing genomic DNA containing exons of mPer1 with a neomycin resistance cassette. A same procedure is also followed for targeting an mPer2 deficient mouse [48].

To see a free-running behavior, mice are first subjected to LD cycle, and after a while DD cycle.

mPer1 deficient mice show a shorter circadian day length. The difference between the wild type circadian day length and mutated mice circadian day length varies between 0.69 hour and 0.77 hour, which is statistically significant [49]. Despite the shorter circadian day, rhythmic circadian day is still observed.

Like mPer1 deficient mice, mPer2 deficient animals also show a shorter circadian period length with respect to wild type animals. It is reported that the difference between the wild type and mutated mice circadian day length is 1.6 hour, which is statistically meaningful. If the mice are kept in constant darkness longer, the circadian rhythmicity is lost [50].

The behavior of double Per mutant mice is similar to double Cry mutant animals. Arrhythmicity is observed when both Per1 and Per2 genes are knocked out [48]. PER3, which is another protein of period family, does not affect the circadian clock mechanism

significantly in SCN. PER3 appears to participate in timekeeping in the pituitary and lung [51]. Per3 knock out shows no significant change in the concentrations of other circadian clock proteins [50].

Knock-out mPer1 gene did not affect the concentration profiles of Cry1, Bmal1, Per2 with respect to wild type animals. There is no significant difference between mutant and normal mice [50]. However, the protein concentrations show difference.

It is reported that by the knock-out Per2 gene, mPer1 concentration level decreases, also Bmal1 and Cry1 mRNA levels are lower than the wild type mice. Like their mRNA levels, concentrations of all 3 proteins are lower than the wild type animals.

2.3.4 Knock-out of Bmal1 Gene

Bmal1 is one of the most important proteins for circadian clock mechanism. By knock-out of Bmal1 gene, the individual effect of this protein is seen. As expected, Bmal1 protein is crucial for biological rhythm.

A wild type mice group and a Bmal1 deficient mice group are subjected to LD and DD cycles respectively [52]. In DD cycle, wild type animals' circadian clocks show rhythmicity, however Bmal1 deficient mice show no sign of rhythmic behavior. No protein, except BMAL1, is responsible for creating an arrhythmic circadian clock individually. This makes Bmal1 a unique protein for the system. The knock-out of BMAL1 protein has a significant effect on the rhythmic expression of mPer1 and mPer2. In wild type animals, Per1 and Per2 mRNAs show a rhythmic behavior, however deleting Bmal1 drops the concentration levels dramatically.

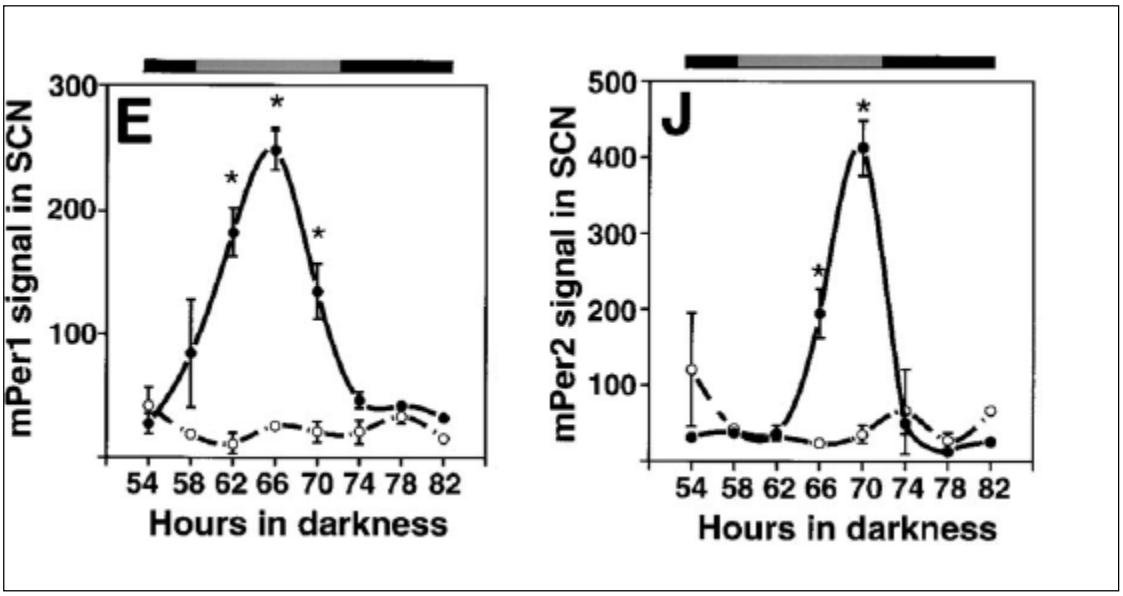

Figure 2-5The effect of knocking out of Bmal1 on Per1 and Per2 mRNA concentrations [52]

In mammalian circadian system, each core protein exists as pairs (PER1 and PER2, CRY1 and CRY2). To create arrhythmicity, it is essential to knock down both genes, but as stated before, the only exception is BMAL1. However, BMAL1 also exists with it pair BMAL2. It is known that BMAL2 has a very little role in clock mechanism. When Bmal1 is knocked-out, it is reported that transcription of Bmal2 also decreases and vanishes with time. So a substitution of BMAL2 with BMAL1 cannot be observed [52]. However, it is also observed that, with a specific technique (constitutively expressed promoter), the expression of Bmal2 is conserved, and even by knocking out Bmal1 gene, the rhythmicity is still conserved [53].

2.3.5 Knock-out of Dec1 and Dec2 Genes

DEC1 and DEC2 proteins are newly found to be important with respect to other circadian clock proteins; however their role is significant in setting the circadian mechanism. Unlike PER and CRY proteins, the knocking out both Dec genes do not result in loss of rhythmicity, this shows that DEC proteins are essential for fine tuning of the circadian functions such as period control, clockwork resetting and entrainment in the SCN [52]. For instance, knocking out both Dec genes result in a longer circadian day (0.5h longer) [54]. DEC1 protein is crucial for the tuning of the expression level of Per1. As seen on Figure 2.6, when Dec1 gene is knocked out, a phase shift in the concentration profile of Per1 mRNA is observed. Also, since there is a small feedback mechanism between DEC1 and DEC2 proteins, absence of Dec1 gene results in a similar manner with Per1 concentration profile. In both cases, the concentration levels are also increased significantly [55].

DEC proteins have no effect on transcriptions of Per2, Cry1, and Cry2 mRNAs. This fact can be observed on Figure 2.6. The mRNA concentrations are not affected with knocked out Dec1 gene.

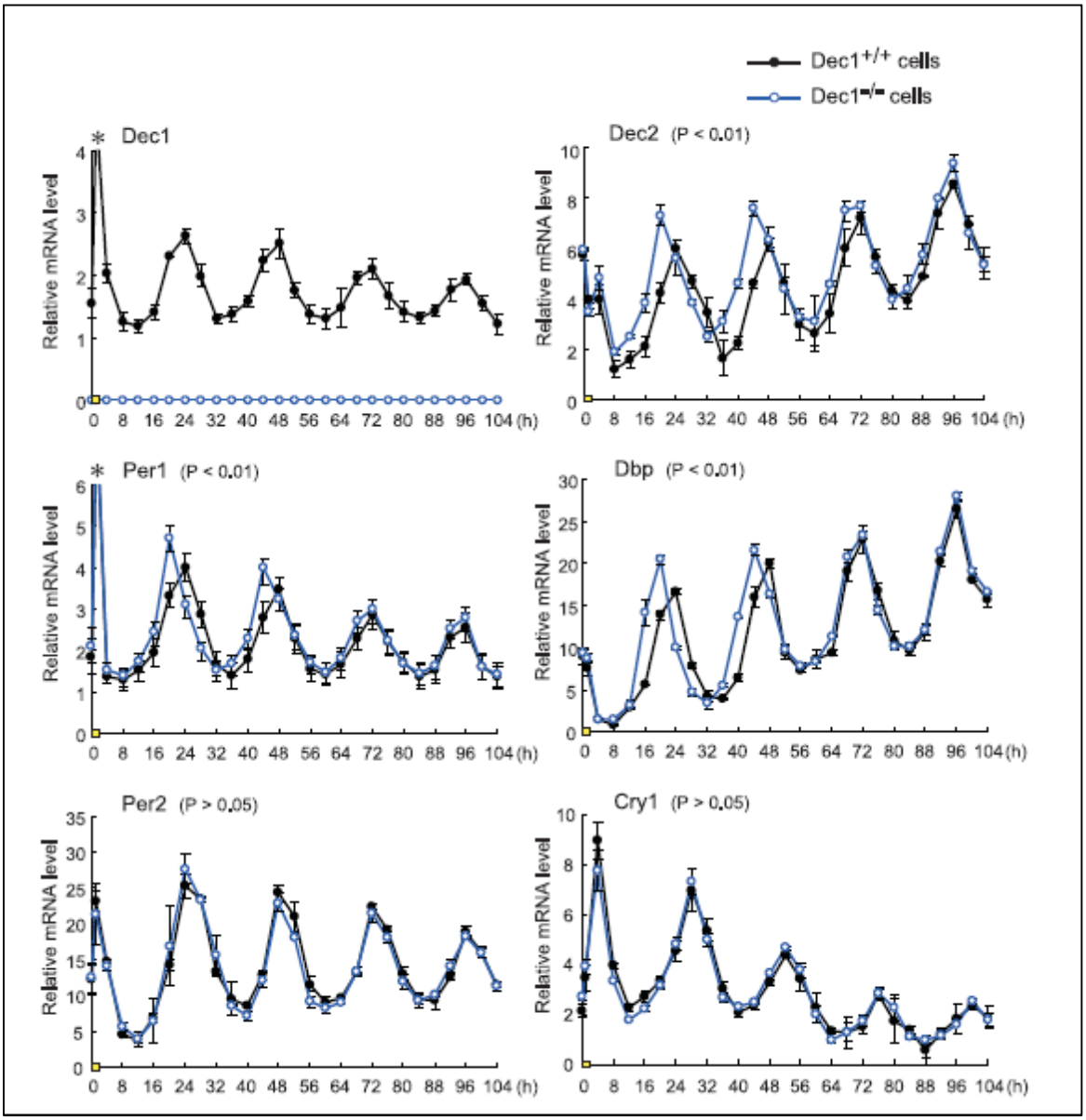

Figure 2-6 The effect of Dec1 Gene on overall circadian clock mechanism [55]

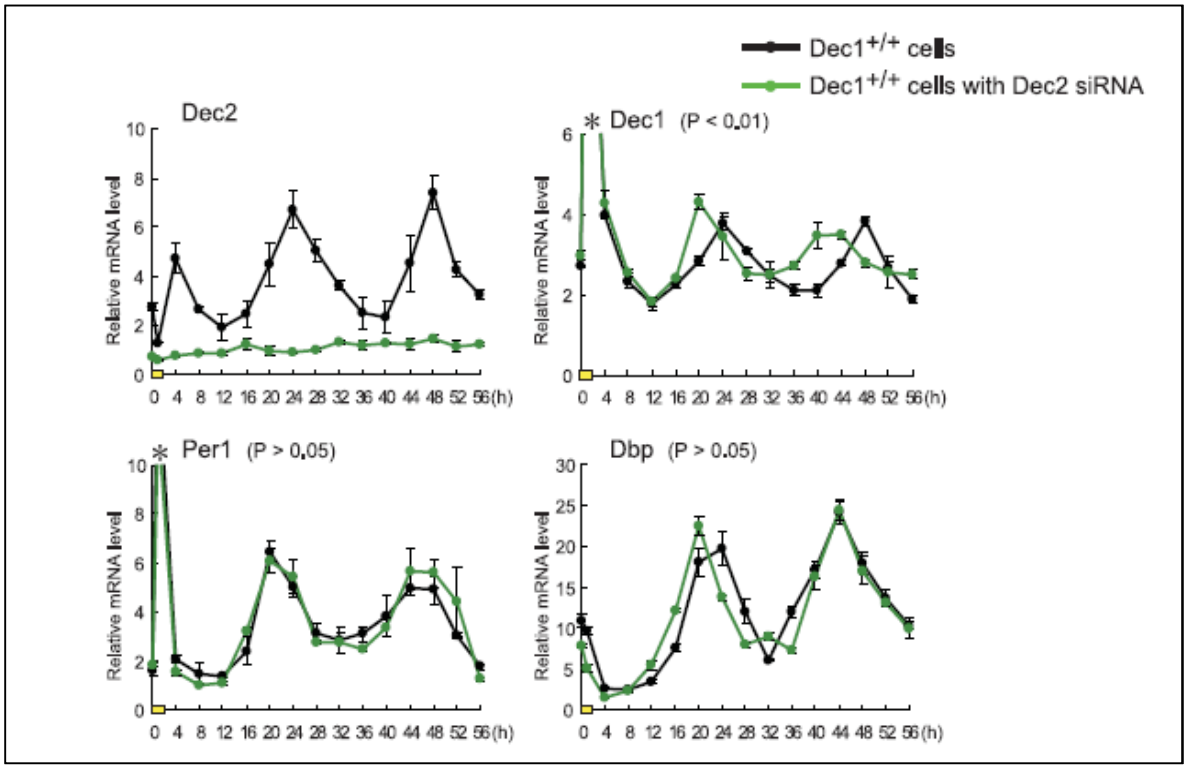

Figure 2-7 Knock-out Results of Dec2 gene: Slight changes are observed on oscillations of clock genes [55]

In Figure 2.7, the effect of knock-out of Dec2 gene is observed. Dec2 gene do not have an effect on Per1 transcription, however it increases the concentration level of Dec1, and also a phase shift is seen.

The overexpression of Dec1 gene shows the opposite behavior with respect to knock-out of Dec1 gene. The concentration levels of Per1 and Dec2 mRNAs decrease, however like the knock out results, over expression do not have significant effect on transcription of Per2 and Cry genes.

Lastly, the expression of Bmal1 and Clock genes are also affected by Dec proteins, their concentration are down-regulated when Dec1 and Dec2 genes are knocked out from the circadian system. Ror expression is also decreased with the deficiency of Dec genes, since Ror elements are the activators of Bmal1 and Clock genes, decline in Bmal1 and Clock concentration with the absence of Dec genes can be explained [55].

2.3.6 Knock-out of Clock Gene

CLOCK protein is known to be an important element for the mammalian circadian clock. It forms a heterodimer with BMAL1, and then this heterodimer binds to E-Box sequence of Period and Cryptochromes promoter and activates their transcription. However, a mutation of CLOCK protein showed that the rhythmicity of circadian clock mechanism is not altered by the disruption of Clock gene. To observe a free-running circadian clock behavior, mice are kept at LD cycle for 2 weeks, and then the animals are subjected to DD cycle for 4 weeks [56].

It is seen that deletion of Clock gene does not disrupt the rhythmicity of circadian clock mechanism. However, the circadian day is 20 minutes shorter when compared to wild type animals. The rhythmicity is not disrupted even after 4 weeks. This proves that CLOCK protein is not required to have robust circadian rhythms [56].

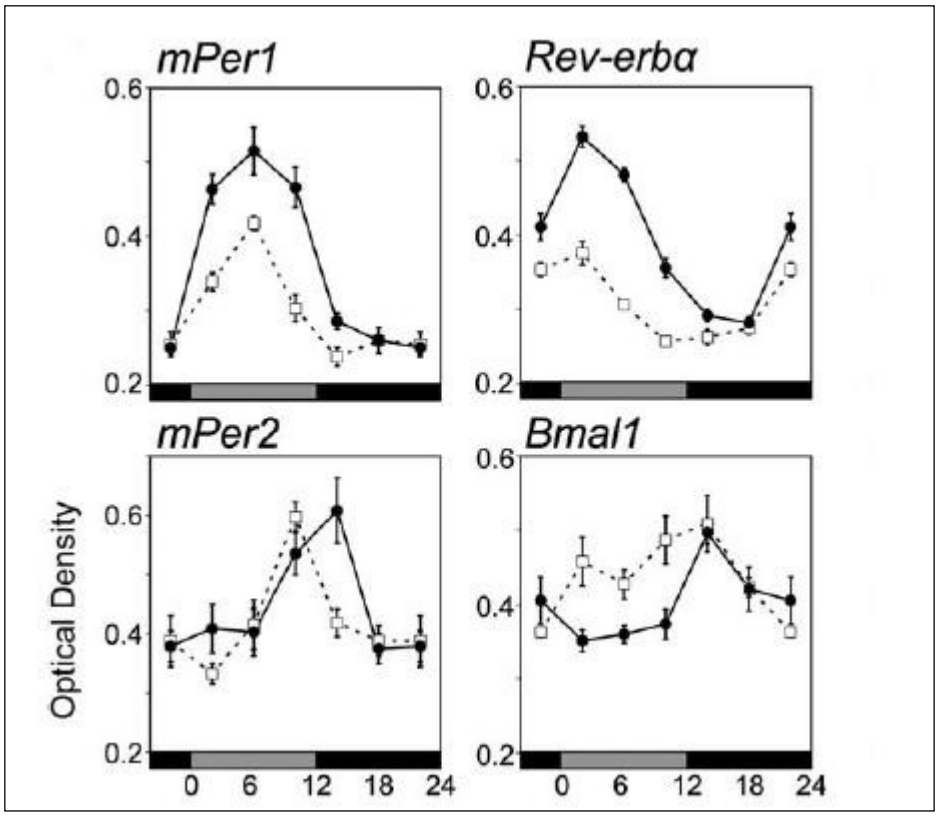

Figure 2-8 The effect of Knocked-out Clock gene: Phase shifts and level changes are observed [56]

In Figure 2.8, it is seen that the disruption of Clock gene has also significant effect on the mRNA concentrations of circadian clock proteins. It causes a decline in both mPer1 and mRev-Erbα concentration levels. In addition, disruption creates phase shifts in mPer2 and mBmal1 concentration profiles.

Chapter 3

MATERIAL AND METHODS

3.1 Modeling of Biological Systems

3.1.1 The General Signaling Network

In the last few decades, molecular biology has been especially successful in explaining the basic molecular-network nature of all life. Organisms are sophisticated molecular systems which are composed of biochemical reaction networks. Those networks show great diversity, however the basic principles do not vary so much. Feedback mechanisms are the core mechanism for many biochemical networks. The circadian clock mechanism is one the systems that obey the feedback loop principle [57].

The central dogma of molecular biology states that information is stored in DNA, transcribed to messenger RNA (mRNA), and then translated into proteins. This picture is significantly augmented with the action of certain proteins regulates transcription. These transcription factors provide a feedback pathway by which genes can regulate one another's expression as Mrna and then as protein [58].

The central dogma of molecular biology can be conceptualized as a genetic feedback network in which information flows from gene activity patterns through a cascade of inter- and intercellular signaling functions back to the regulation of gene expression. Gene expression is regulated by a circuit of signaling function. Information flow is the key for building up the model for biochemical networks. This flow begins with the triggering of the gene, continues with Mrna transcription, then by translation, formation of proteins is

responsible for the flow, and finally, by transcription factors, triggering or inhibiting of gene activation closes the loop for information flow.

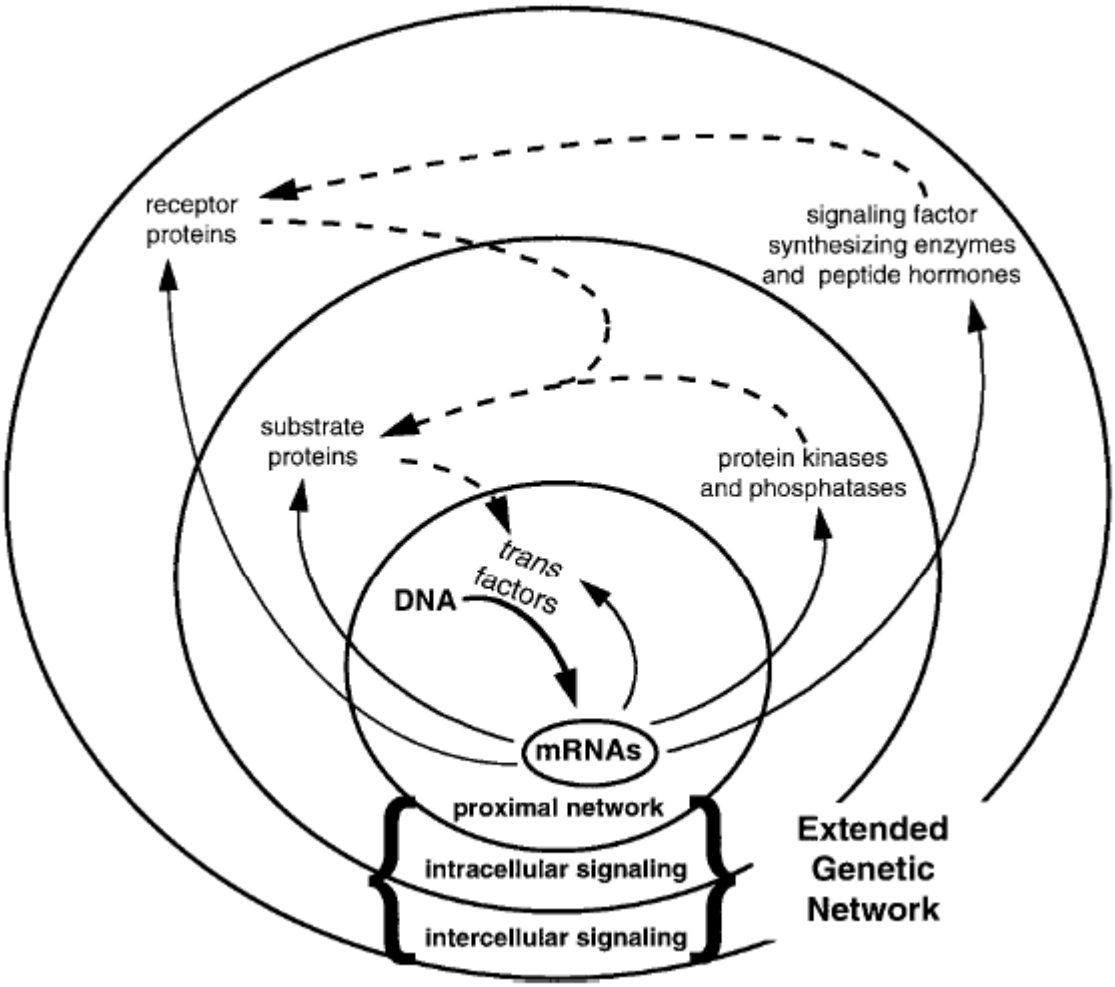

Figure 3-1 The general signaling network [57]

In Figure 3.1, the general signaling network can be seen. In the system, the gene is exposed to two different signaling, intracellular and intercellular. Intercellular effect can vary; it can

be light, temperature, or any chemicals. Intracellular signaling results from the activities of proteins. After Mrna transcription, translation and post-translational modifications result in substrate proteins, and those substrate proteins are responsible for creating the intracellular signaling.

3.1.2 The Circadian Clock Model Based on Signaling Network

The circadian clock mechanism is modeled based on the intracellular and intercellular signaling. By following this signaling, the model becomes accordant with the central dogma of molecular biology.

The model was implemented in The General Algebraic Modeling System (GAMS) as a system of 77 ordinary differential equations (ODEs). The model incorporates mass action kinetics. Because little experimentally derived kinetic data are available for this system, the model parameters are estimated by GAMS solver. The General Algebraic Modeling System (GAMS) is a high-level modeling system for mathematical programming and optimization. It consists of a language compiler and a stable of integrated high-performance solvers. GAMS is tailored for complex, large scale modeling applications, and allows to build large maintainable models that can be adapted quickly to new situations.

In this study, the model built by Forger and Peskin [4] is taken as basis. As stated before, the most detailed model is built in this study; every post translational modification is included in the system. Here, the new proteins that are known to be related with the circadian clock mechanism are added to the systems which are DEC1 and DEC2 proteins.

Since the reaction rate kinetics for most of the biological systems are unknown, every biological reaction is accepted as first order reactions. In other words, the change in the reaction rate is proportional to the first order of the concentration of a certain protein, or Mrna. In real case, the reaction rate can be (and usually is) proportional to different orders

of the concentrations of reactants. Even, the external effects are important in the reaction rate kinetic calculations.

In the model, the transcription of Per1, Per2, Cry1, Cry2, Rev-Erbα, Dec1 and Dec2 mRNAs are controlled. For Period proteins, the promoters are taken as the same, in other words the equations related to the transcription of those mRNAs are modeled in the same manner. The same method is also applied to Cryptochrome proteins. This approach is also followed in modeling DEC protein transcription, too.

The negative feedback loop between transcription and translation is governed by a parameter called G. G is used as a probability parameter, and naturally it oscillates between 0 and 1. The value of G is determined by the total concentration of Cryptochrome proteins bound to a binding site on a promoter. Like the core feedback loop mechanism, the same procedure is also applied to Rev-Erbα and Dec genes. The probability parameters for those proteins are Grv, and GD, respectively.

Hour is taken as basic time step for the modeling. It is also the only independent variable for the ordinary differential equations. All concentrations are defined with respect to the volume of cytoplasm where cytoplasmic reactions occur. That is, the concentration of any molecular species is the number of moles of that species divided by the cytoplasmic volume [4]. It means that the concentration of a same protein or a heterodimer in the cytoplasm and nucleus are represented by different ordinary differential equation.

Casein kinase is a very important enzyme for the circadian clock mechanism, as it catalyzes the phosphorylation of Period proteins. The concentration of kinase may vary throughout the day in the cell, but this variation does not affect the phosphorylation of Period proteins in the model, so the kinase concentration is set constant in the circadian day. Phosphorylation rate is modeled as a kinetic parameter.

Light effect in the model is applied to PER1, PER2 and DEC1 transcription rates. Light is added as a parameter to the differential equations, in where it increases the transcription rates.

Orthogonal collocation method is used to solve the differential equations. This method is a polynomial approximation method, and it can be easily applied to different systems. The initial conditions for the differential equations are set by using the real time PCR results which are found from the literature. If there is no available data for the specific differential equation, the initial condition is set as 0, since GAMS solver results are so robust in the sense of initial conditions, taking these values as 0 do not affect the system significantly.

The kinetic parameters are estimated with respect to real data, which increases the consistency with biological facts. The real time PCR data are given to the solver, and GAMS does successive iterations to find the optimum parameters that result in the oscillating concentration profiles of proteins and their Mrna's.

3.2 Mathematical Representation of Biological Clock Mechanism

In Forger-Peskin model and also in this model, every biological reaction in the cell is represented by a differential equation. All reactions are governed directly by mass action except transcription, whose rate is the activated transcription rate multiplied by the probability that the gene is activated. For instance, transcription on the PER1 promoter is $trPo*(1-G)^5$, because there are five sites where CRY could bind with probability G, and because all sites must be unoccupied for transcription to occur [4]. From the simplest model for drosophila to the detailed model for mammalians, every system uses differential equations.

The concentration change in the amount of PER1 protein is represented as follows:

$$\frac{Po}{dt} = tlp \times MPo - ac \times Po \times C + dc \times PoC - upu \times Po$$

In this equation, MPo represents the concentration profile of mRNA of Per1. Tlp represents the translation rate of mPer1, ac stands for enzymatic reaction of casein kinases and PER1 protein, dc represents the unbinding of PER1 to kinases, and lastly upu stands for the degradation of unphosphorylated PER1 protein unbound to CRY in the cell.

$$\frac{D2}{dt} = trlD2 \times MD2 - degD2 \times D2 - nl \times D2 + ne \times D2n$$

In another equation, which is representing the concentration change in DEC2 protein, trlD2 stands for the translation rate of Dec2 Mrna, MD2 represents the concentration of Mrna of DEC2, nl is the nuclear localization rate of DEC2, ne is the nuclear export of DEC2 in the cell nucleus.

In the model, the differential equations are the change in the concentrations of mRNAs and proteins, and the constants are the biological rates. Below some other biological rates are given in Table 3.2. The numerical values that are seen on Table 3.2 are the optimized parameters by GAMS. All the other biological rates, differential equations, and initial conditions are given in Appendix part.

Table 3.1 Examples of biological rate constants (Optimized by GAMS solver)

Symbol	Explanation	1/Ms
tlp	Translation of PER1 andPER2	0.60
tlr	Translation of CRY1 and CRY2	0.26
tlRv	Translation of REV-ERBα	2.53
up	Degradation of phosphorylated PER unbound to CRY	0.96
upu	Degradation of unphosphorylated	0.49

In this study, a different method is used to solve the equations, which allows the user to optimize the equations with respect to a reference point. This method is called orthogonal collocation method, which is included in the topic of collocation methods.

3.2.1 Collocation Method

Collocation method is a method for the numerical solution of ordinary differential equations, partial differential equations and integral equations. It has a very wide usage. The idea is to choose a finite-dimensional space of candidate solutions (usually, polynomials up to a certain degree) and a number of points in the domain (called collocation points), and to select that solution which satisfies the given equation at the collocation points [59].

Suppose that the ordinary differential equation

$y'(t) = f(t ; y(t)), \qquad y(t_0) = y_0,$

is to be solved over the interval $[t_0, t_0 + h]$. Choose $0 \leq c_1 < c_2 < \ldots < c_n \leq 1$.

The corresponding (polynomial) collocation method approximates the solution y by the polynomial p of degree n which satisfies the initial condition $p(t_0) = y_0$, and the differential equation $p'(t) = f(t,p(t))$ at all points, called the collocation points, $t = t_0 + c_k h$ where $k = 1, \ldots, n$. This gives $n + 1$ conditions, which matches the $n + 1$ parameters needed to specify a polynomial of degree n.

3.2.1.1 Orthogonal Collocation Method

Unlike finite difference ODE solvers, orthogonal collocation applies a polynomial approximation to the differential equation and requires satisfaction of the equation at discrete collocation points, the zeros of orthogonal polynomials [60]. In collocation method, the solution is approximated by a linear combination of basis functions, which are determined by requiring that the ODE is satisfied at each of a discrete mesh points, and the

boundary conditions should be satisfied. The method uses series expansion based on orthogonal basis function; the coefficients of the functions are determined by minimization of some criteria. The collocation methods are elegant in their simplicity and efficient in their application. They have several important advantages over the other discretization methods. It provides a high order of convergence, gives a continuous approximate solutions, and easily handles general boundary conditions while still being simple to program.

In orthogonal collocation a domain is divided into small subdomains, which are called finite elements. The orthogonal collocation is applied within the elements and the function and its first order derivative should be continuous at the boundaries between elements. The orthogonal collocation on finite elements divides the domain, for example 0 to 1, into finite elements, and sets the residual to zero at the collocation points interior to the elements.

Table 3.2 First Order Derivative Collocation Matrix

0.19681547722366	0.39442431473909	0.37640306270047
-0.06553542585020	0.29207341166523	0.51248582618842
0.02377097434822	-0.04154875212600	0.11111111111111

The function and its first derivative should be continuous at the boundaries between the elements. This is achieved with the help of the first order derivative collocation matrix, which is given in Table 3.1.

If the number of collocation points are increased, differential equation are required to be satisfied at more locations, and a more accurate solution is obtained.

3.2.1.1.1 Orthogonal Polynomials

Two functions $g_n(x)$ and $g_m(x)$ selected from a family of related functions $g_k(x)$ are said to be orthogonal with respect to a weighting function w(x) on the interval [a, b] if

$$\int_a^b w(x)g_n(x)g_m(x)\,dx = 0, \qquad n \neq m$$

$$\int_a^b w(x)[g_n(x)^2]\,dx \neq 0, \tag{3.1}$$

In general, c depends on n. if these relationships hold for all n, the family of functions $g_k(x)$ constitutes a set of orthogonal functions. Some common families of orthogonal functions are the sets sinkx and coskx. Orthogonality can be viewed as a generalization of the perpendicular property for two vectors in n dimensional space where n becomes very large and the elements (coordinates) of the vectors can be represented as continuous functions of some independent variable. The functions 1, x ,x^2,x^3, …, $x^n$are not orthogonal. However, several families of well-known polynomials do possess orthogonality. Four sets are Legendre, Laguerre, Chebyshev, and Hermite polynomials.

3.2.1.1.2 Choosing Collocation Points

The main idea of orthogonal collocation method is to approximate a function by passing a polynomial through values of the function at selected points. In this study, the differential equations, which are representing concentrations, are the functions. Approximation is done on each unit time interval. In orthogonal collocation method, different numbers of collocation points can be created, like 1, 2, 3, 4, 5, 9, 14. By increasing the collocation point, the accuracy of the approximation increases. However, as the collocation points increase, the system becomes larger and larger. This makes the system hard to be solved by computational calculations. So an optimization should also be done on deciding the number of collocation points. In circadian clock mechanism, the aim is to optimize the certain differential equations with respect to real time PCR results of

certain mRNAs. These concentration profiles show Gaussian like behavior, so increasing the number of collocation points do not bring significant advantage after 4 points. The equations are first order ordinary differential equation, so choosing 2, 3 or 4 collocation points is sufficient to have a close approximation. In this study 3 collocation points are used for optimizing the differential equations.

3.2.2 Running the Circadian Clock Model

After creating the ordinary differential equations, collocation method should be applied, and by setting the initial conditions, the system is ready to run. This study includes two steps, optimization and simulation, respectively. In optimization part, estimating a wild type circadian system's kinetic parameters is targeted. And in simulation part, observing the effect of individual proteins on overall circadian clock mechanism by gene knock-out method is the main goal.

3.2.2.1 Optimization

This method is applied for the specific system as follows: At first, the concentration profiles, which are taken from the RT-PCR results, are given to the model by entering a concentration datum for each hour. The main clock proteins; PER1-2, CRY1-2 and their Mrna concentrations are targeted in the study, since their oscillating concentrations are responsible for setting the circadian clock. Then each differential equation in the model is defined as 3 equations. The first equation is composed of the mathematical relation between the variables. The second equation divides the equation's time interval (set as 1 hour) into 3 parts, so the discrete collocation points mentioned above are obtained. And lastly, the third equation is responsible for continuity. The optimization part of the modeling is done by minimizing the difference between the data given to the model and the

results obtained from the equations. In order to achieve this goal, GAMS solver does successive iterations by changing the mass action kinetics constants. When the objective function is minimized, iterations stop. Since the equations in the system are non-linear, some parameters are needed to be fixed. In the specific case, the transcriptions rates of PER and CRY proteins are set as fixed. Those fixed values are taken from Forger-Peskin model.

3.2.2.2 Simulation

The second part of the study is based on simulation. Here, the simulation is done by knocking out the DEC genes. Knocking procedure is done as follows: Firstly, the kinetic parameters that are estimated from previous optimization are recorded to be used for simulation. Then the equations belonging to DEC's are removed from the model, and equations related with the concentration of DEC proteins are converted back in the model in where there are no DEC effect. Since the model needs an objective function, an arbitrary function is created, and the model is forced to optimize this arbitrary equation. At the same time, the parameters estimated from optimization part are fixed in this model; finally simulation is run with those values. Hence, overall system is free of DEC proteins, but all the kinetic parameters are accordant with real case, so the simulation is biologically meaningful. Then the same procedure is followed for DEC1 protein only. One of the main goals of this study is to see the effect of DEC proteins on overall circadian clock mechanism, so knock-out procedure is applied on these proteins firstly. After observing the DEC effect on the system, other proteins are knocked out from the system, too. Each protein, PERIOD proteins, CRYPTOCHROME proteins, ROR protein and REV-ERBα protein are deleted from the system one by one. Effect of each protein is also observed and graphically shown in the results part of this study.

The uniqueness of the model comes from its accuracy, since the best parameters that fit the experimental data are obtained by optimization, but not by trial and error

method. Another advantage of the model is that it can be applied easily for data obtained from different tissues of human body just by changing the data given to the model at the beginning. By doing this, new kinetic parameters for the specific tissues can be obtained since software will try to fit the concentration values of differential equations to the new data.

CHAPTER 4

RESULTS AND DISCUSSION

Circadian clock is based on a negative loop between mRNAs and their proteins. Period and Cryptochrome proteins inhibit their own transcription by inhibiting their own gene. Dec proteins act negatively on Per and Cry transcriptions too.

While modeling the biological pathway of circadian clock mechanism, the aim is to obtain Real-Time PCR concentration profiles for core mRNAs. It is observed that the concentration profiles of Per1, Per2, Cry1 and Cry2 mRNAs shows a Gaussian like behavior, and after a time lag, their protein concentration profiles show a similar behavior. In the following circadian day, a similar trend can be observed, in other words, the concentrations of proteins show oscillations, with very close magnitudes each day.

The circadian clock mechanism can be summarized as follows: after the transcription of Period and Cryptochrome mRNAs, they aretransported for translation. With the help of post translational modifications (like phosphorylation) Period and Cryptochrome proteins form heterodimers, and with the help of a second phosphorylation step, they are translocate into nucleus along with casein kinase eopsilonI. They form complex with Clock and Bmal1 dimers on E-box promoter, and BMAL1/CLOCK driven transcription, and the transcription is inhibited.

The model is built on GAMS solver, and the results are shown graphically. The aim is to see a similarity between the model results and real time data. The real time PCR results give detailed information about the behavior of wild type cells and knocked out cells. The

biological clock model is firstly forced for optimization to find the kinetic parameters that result in Gaussian like concentration behaviors. Then each gene is knocked out to see their individual effects, and those results are compared with real data. At last, gene dosing is performed on the system, and these results are discussed.

In RT-PCR results, the concentration of the certain mRNA is calculated based on a standard. For instance if there is 10 times the amount of signal in the experimental sample compared to the control sample for the target gene, this means that expression of the gene has increased 10-fold in the experimental cells or it could mean that there is simply 10 times as much RNA in the experimental lane. To check for this a 'Loading Control' in which the blot is probed for expression of a gene, which does not change, is used. And if this gene is expressed 2 times, this means that the real change in the target gene is 10/2 = 5 fold. So in this study, in every graphical representation, relative concentration profiles are used, without any units.

Commonly used standards are:

- Glyceraldehyde-3-phosphate dehydrogenase mRNA
- Beta actin mRNA
- MHC I (major histocompatibility complex I) mRNA
- Cyclophilin mRNA

4.1 Expression of Clock Genes in Wild Type Cells

, The experimental concentrations of mRNAs of clock genes are obtained from the literature [7,46,55]. In the optimization part of this project, differential equations are forced to show gene oscillations that are gained from experiments.

4.1.1 Osicillation of Clock Genes

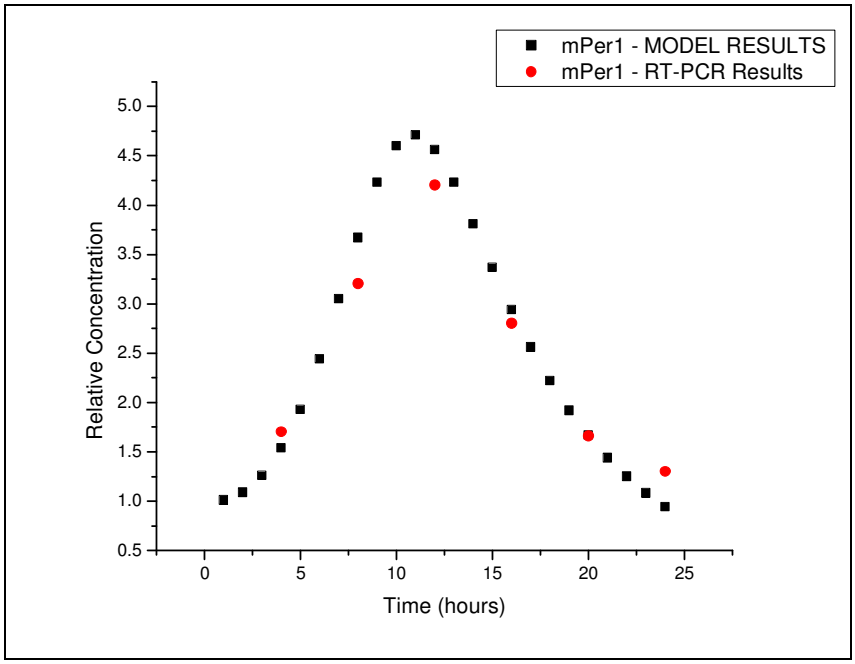

Figure 4-1 Circadian expression of Per1 gene in wild type cell: mPer1 relative abundance shows a 24 hour circadian behavior. The model is capable of predicting the oscillatory characteristic.

The expression of mPer1 peaks (Figure 4.1) around midday and a circadian expression behavior is observed. The model results are in agreement with RT-PCR values [55]. The software gives three points for each hour. In here, only the values corresponding to each hour are seen. Since the optimization is done for a 24 hour period, the oscillatory behavior of circadian clock mechanism at the next circadian day cannot be observed with this graphic.

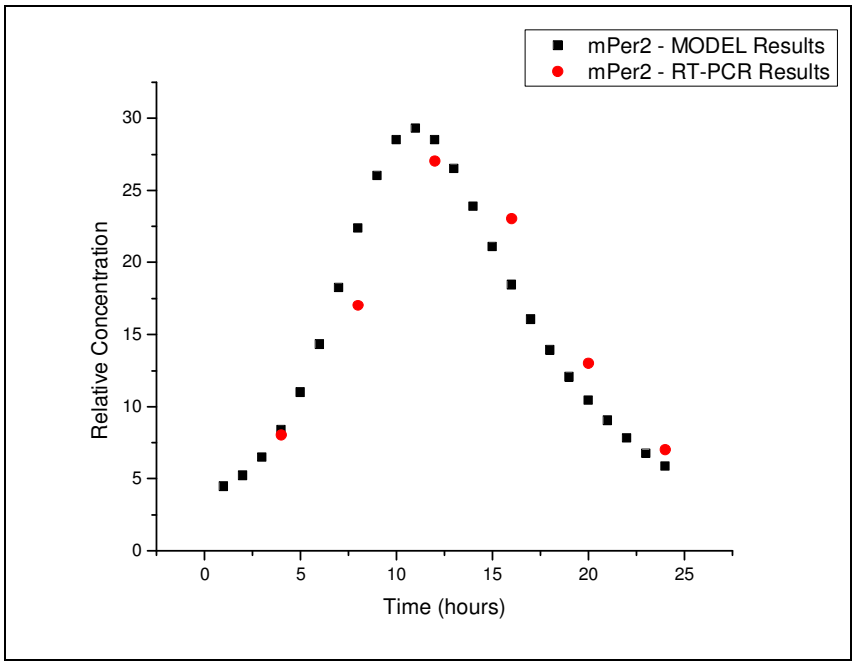

Figure 4-2 Rhythmic Expression of Per2 gene in wild type cell: The model is capable of predicting the gaussian behavior of mRNA concentration of mPer2.

Oscillation of Per2 gene shows a similar behavior with Per1 gene (Figure 4.2). The concentration peaks around midday, and a circadian behavior can be observed. In RT-PCR analysis, data are collected every 4 hours, so in the figures, RT-PCR result points are less than GAMS model results points.

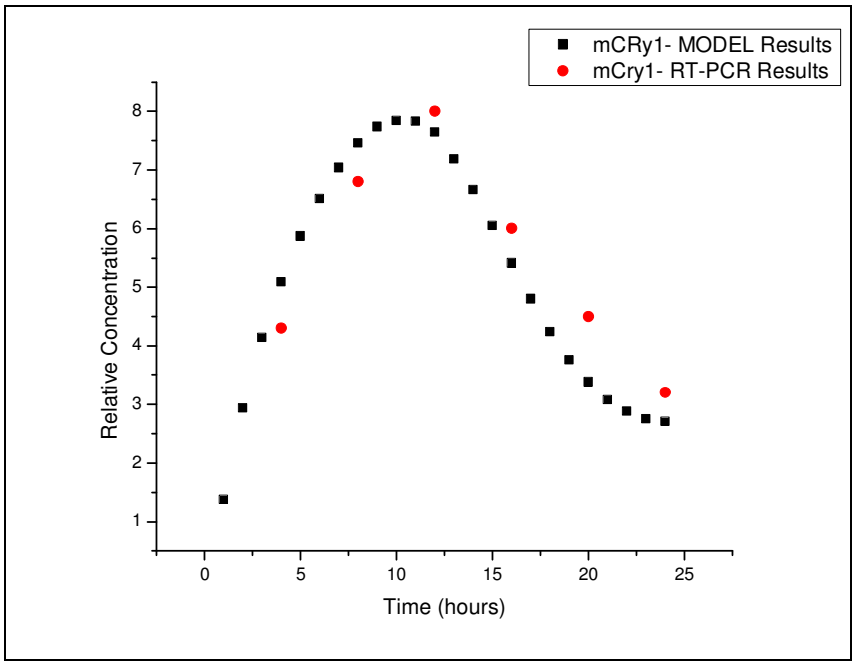

Figure 4-3 The expression of Cry1 gene in wild type cell: Both model and RT-PCR results are circadian, the model is capable of predicting this behavior

Cry1 gene expression results which are obtained from GAMS solver are in agreement with RT-PCR results behavior [62], the profile peaks around midday (Figure 4.3). Light is added to the system as a parameter, it increases the transcription rate of certain genes. In real case, light is responsible for triggering the clock mechanism. But in the model we do not have the triggering effect of light, so the concentration profiles represent the behavior of the internal circadian clock mechanism.

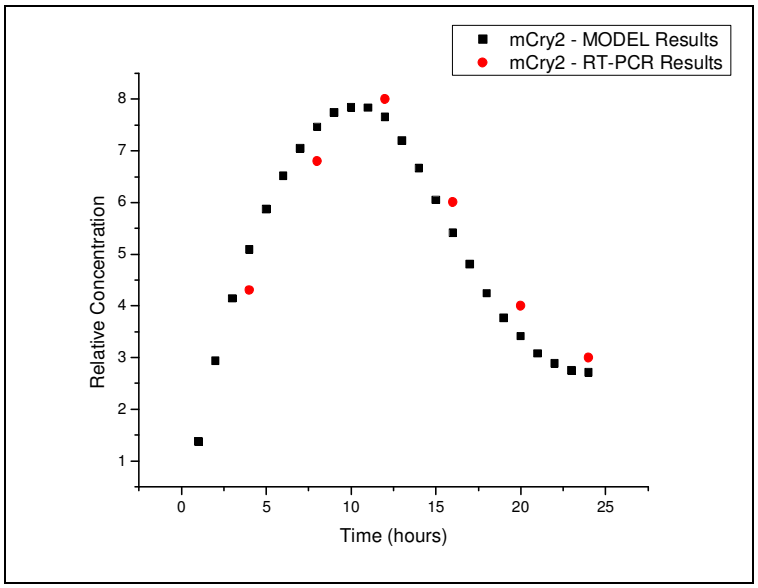

Figure 4-4 Rhytmic Expression of mCry2 in wild type cell: Model and RT-PCR results are in agreement, 24 hours circadian expression fashion is observed.

In Figure 4.4, mCry2 transcription level peak in midday, too. The model results and real data are in agreement [63].

4.1.2 Oscillating Concentrations of Proteins

In mammalian cells, after transcription, translation is not only the step for synthesis of proteins, after translation, post translational modifications are needed. The time lag between the translation and transcription is significant in mammalian cells. This time lag can be observed with the peak time of concentration profiles. The model is capable of showing the time lag.

The concentration profiles of Periods and Cryptochromes proteins are plotted with mRNA profiles in order to show the time lag. The concentration profiles of proteins peak after mRNA profiles peak; this is due to the time lag between transcription and translation, and the effect of post-translational modifications. (Figure 4-5-6-7).

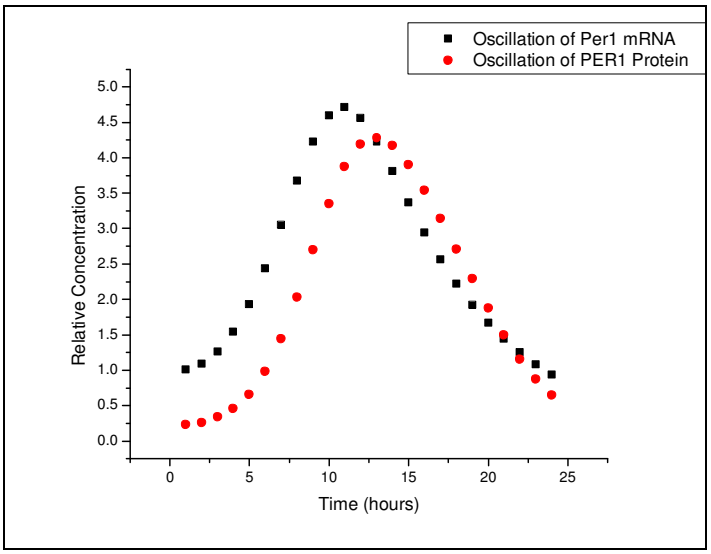

Figure 4-5 Daily synthesis of PER1 protein in wild type cell: The time lag between the peaks of protein and mRNA concentrations is observed.

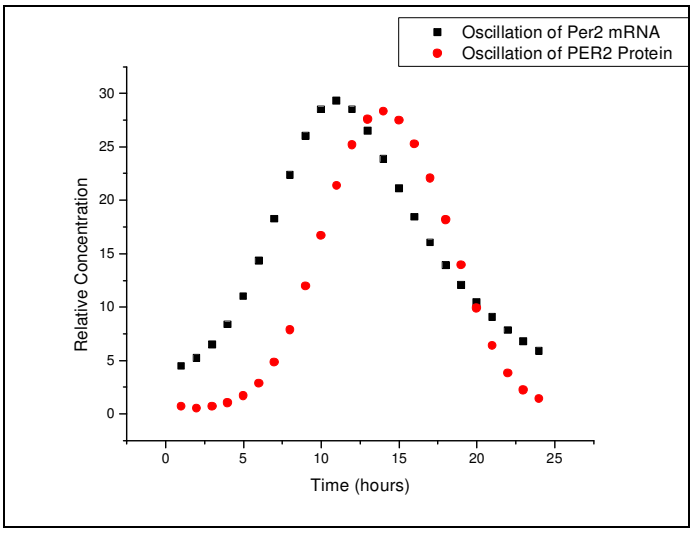

Figure 4-6 Oscillation of of PER2 protein in wild type cell: The time lag between the peaks of protein concentration and mRNA concentration is observed.

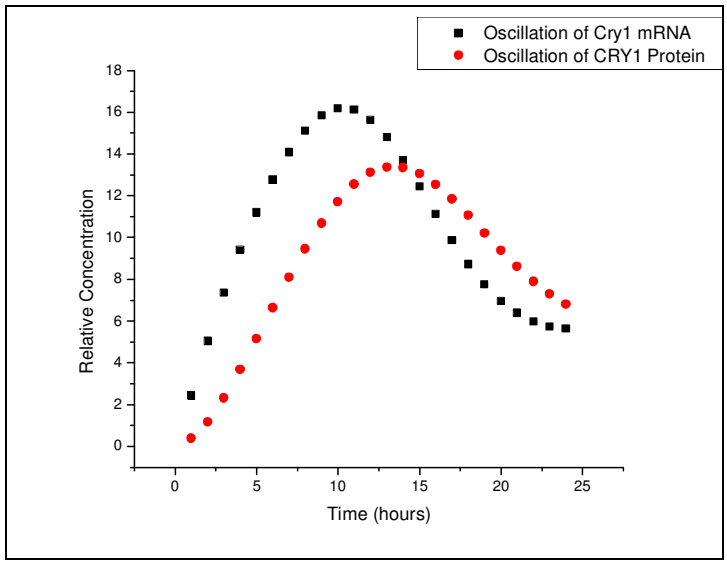

Figure 4-7 Rhytmic Synthesis of CRY1 protein in wild type cell: Protein concentration peaks 4 hours later than mRNA concentration peaks

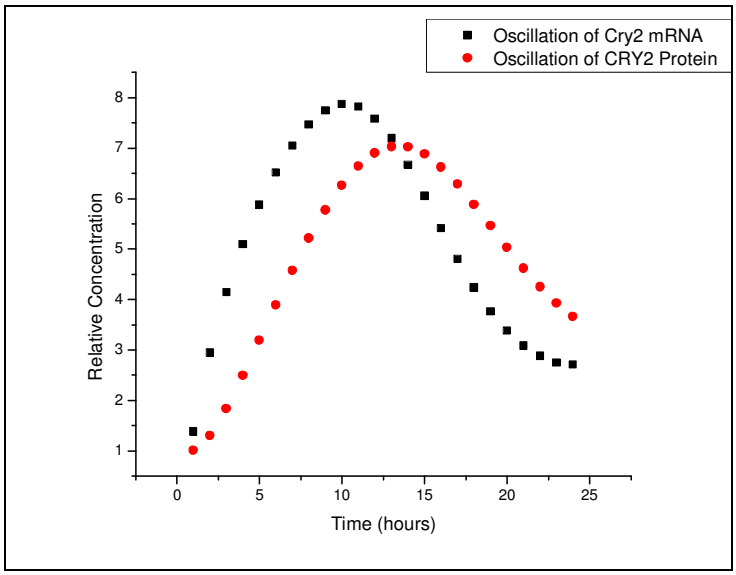

Figure 4-8 Rhytmic Synthesis of CRY2 protein in wild type cell: The time lag between the protein concentration and mRNA concentration is observed, this is in agreement with experimental results.

4.2 Individual Effect of Proteins on Circadian Clock Mechanism

4.2.1 Knock-Out Procedure

Individual effect of proteins on overall circadian clock mechanism can be observed by knockout procedure. In computational calculations, the differential equations that are related to certain protein are omitted from the system, and the mechanism is simulated with the kinetic parameters obtained from optimization part. The aim is to obtain a similar trend with experimental results. In this study, every gene in the system is knocked out individually, and then pairs of Dec genes are omitted.

4.2.1.1 Knock-out of Per1 Gene

Period1 protein is one of the 4 core elements of circadian clock mechanism. Below, the profiles of mPer2, mCry1 and mCry2, in the case of knocked out Per1 gene, are plotted with wild type results. Wild type data mPer1, mPer2, mCry1 and mCry2 are tabulated in Table A.3 in Appendix.

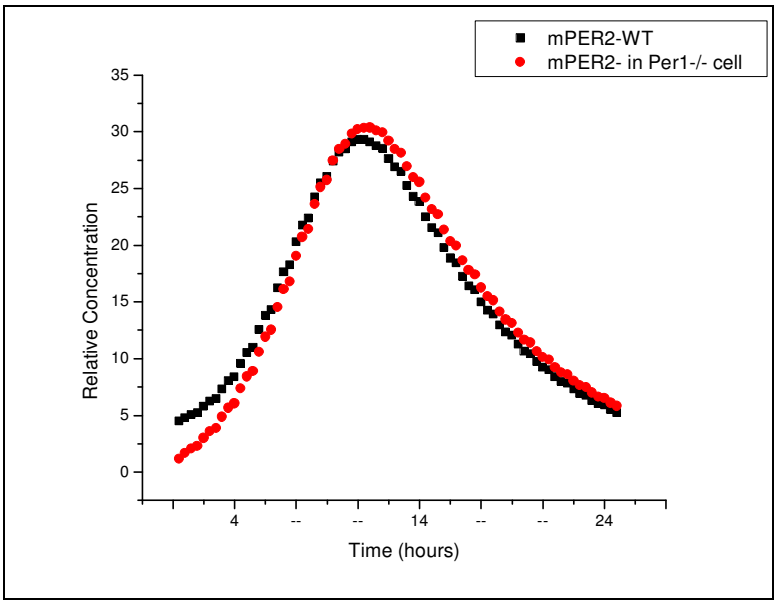

Figure 4-9 Expression of Per2 gene in $Per1^{-/-}$ cell: Per1 is dispensable for rhythmic expression of Per2 gene, expression of Per2 gene does not change.

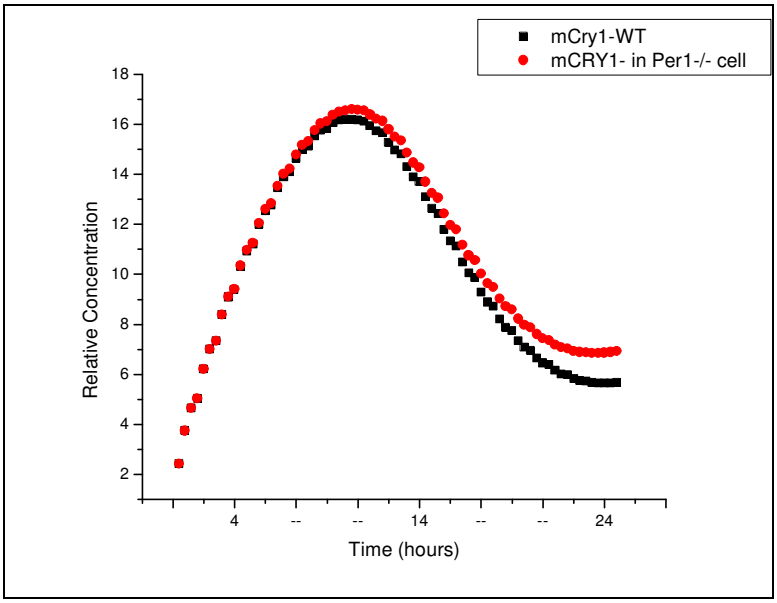

Figure 4-10 Oscillation of Cry1 gene in $Per1^{-/-}$ cell: Per1 is dispensable for rhythmic expression of Cry1 gene, the oscillatory behavior is kept with the same concentration level.

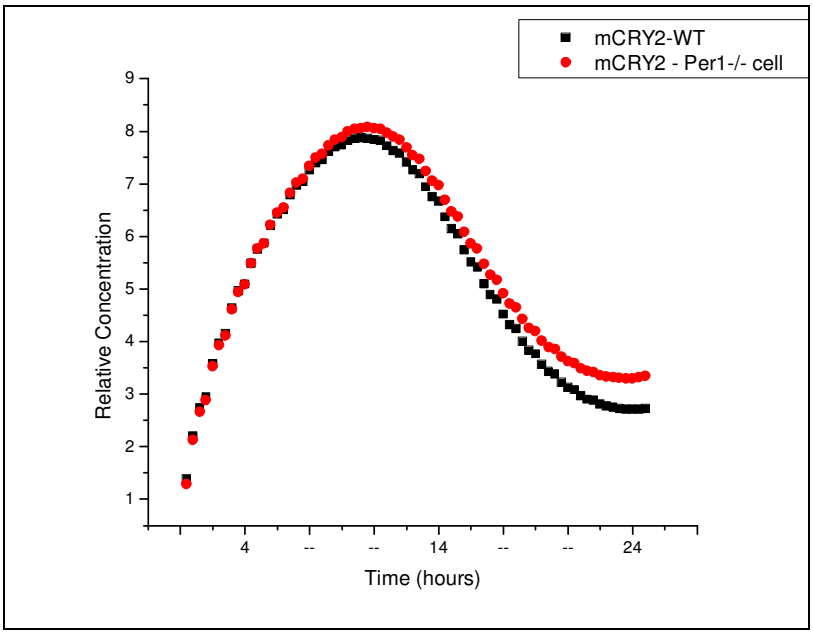

Figure 4-11 Rhytmic Expression of mCry2 in Per1$^{-/-}$ cell: Per1 is dispensable for rhythmic expression of Cry2 gene; the expression pattern is slightly affected.

The knockout of Per1 gene does not have a significant effect on the concentration profiles of mPer2, mCry1, and mCry2. All three mRNA concentrations increase in a very little amount and a small phase shift is observed for all three of them. Experimentally, the knockout of Per1, inDD period, does not have a significant effect on the overall system for the first circadian day [48]. In Figure 4.9, the concentration profile of mPer2 is seen. The wild type and mutant concentration profiles do not differ so much. There is a small change in the concentration level, but the profile is still circadian. The reason for the limited effect of Per1 is that it can be compensated by Per2 protein. Partial compensation of homolog genes are observed in experimental results [48]. Knockout of Per1 gene has a slight effect on the concentration profiles of Cryptochrome mRNAs. In Figure 4.10 and 4.11, the profiles of mCrys are seen. In both profiles, there are small changes in the concentration

amounts, but the concentrations are still rhythmic. As a result, it can be said that the model is capable of predicting the knockout of Per1 gene.

4.2.1.2 Knock-out of Per2 Gene

Knockout of Per2 gene has caused the largest change in circadian clock mechanism. Relative abundance of mPer1 increased dramatically, and the circadian behavior is almost lost despite the concentration drop at the end of the circadian day. Experimentally, it is known that Period proteins can compensate each other [48]. Since the relative concentration of mPer2 is larger than mPer1 in RT-PCR results, the system may be forced to fill the absence of Per2 protein, and this can lead to sudden increase in the expression of Per1 gene in the model. However, in real case, the concentraion profiles of mCry1, mCry2 and mPer1 decrease with the knockout of Per2 gene [48].

Like mPer1, Cryptochrome mRNA levels are also affected by knockout of Per2 gene. Oscillatory behavior of mRNA seems to be disturbed; the concentration drop at the end of the circadian day is not enough to say that the Gaussian like behavior is kept. Both Cry mRNA concentration levels increase significantly.

In Figure 4.12, the effect of knocked out Per2 gene on Per1 gene expression is plotted. The difference between the wild type cell and the mutant cell is significant. The concentration of mPer1 increases rapidly, and the circadian rhytmicity is not fully observed. The gene expressions of Crys are affected dramatically, too. The transcription of mRNAs increases to a very high level with compared to wild type cells. In Figure 4.13 and 4.14, the profiles of mCry1 and mCry2 can be seen, respectively. In both mCry profiles, rhytmicity cannot be observed as like in wild type cells. There is a relative abundances decrease in mCrys around the end of the day; however, this is not as sharp as the decrease in the wild type cells.

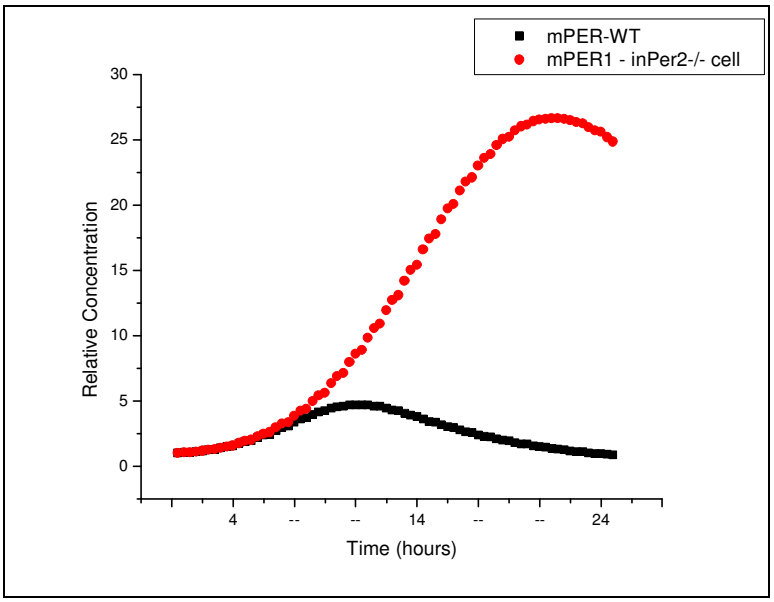

Figure 4-12 Loss of Rhytmicity of mPer1 in $Per2^{-/-}$ cell: Per2 gene function is indispensable for rhytmic expression of circadian genes

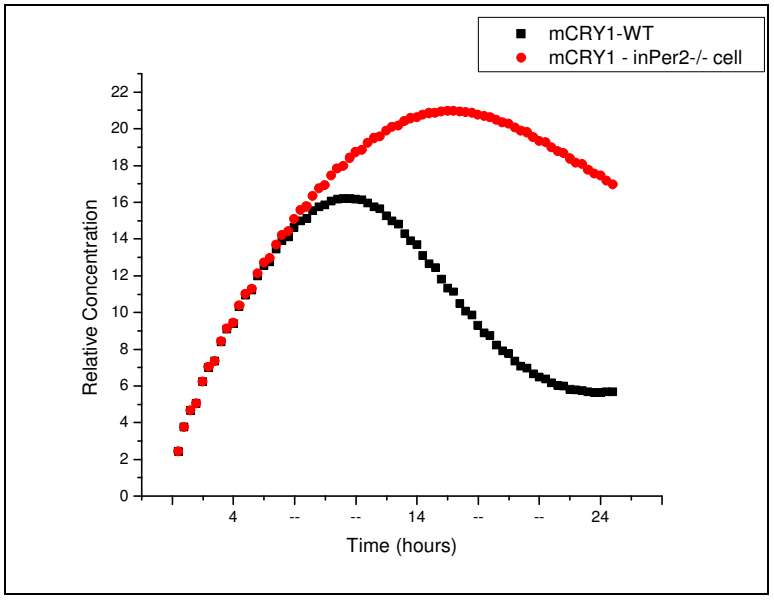

Figure 4-13 Loss of Rhytmicity of mCry1 in$Per2^{-/-}$ cell: Per2 gene is curicial for the oscillation of circadian genes

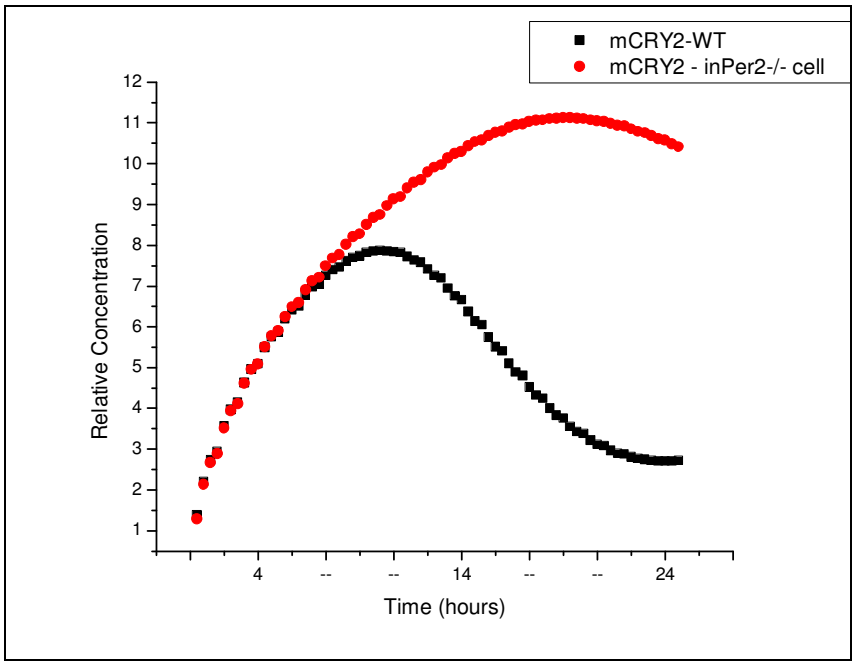

Figure 4-14 Arrhytmic expression of Cry2 gene in $Per2^{-/-}$ cell: Loss of Per2 gene results in a non-circadian behavior of Cry2 gene expression

When both genes are knocked out, the ryhtmicity of circadian clock mechanism is disturbed. The circadian expression of Cry mRNAs cannot be observed. This is accordant with the real results [51]. In Figure 4.15 and Figure 4.16, it is observed that the model is capable of predicting these experimental results.

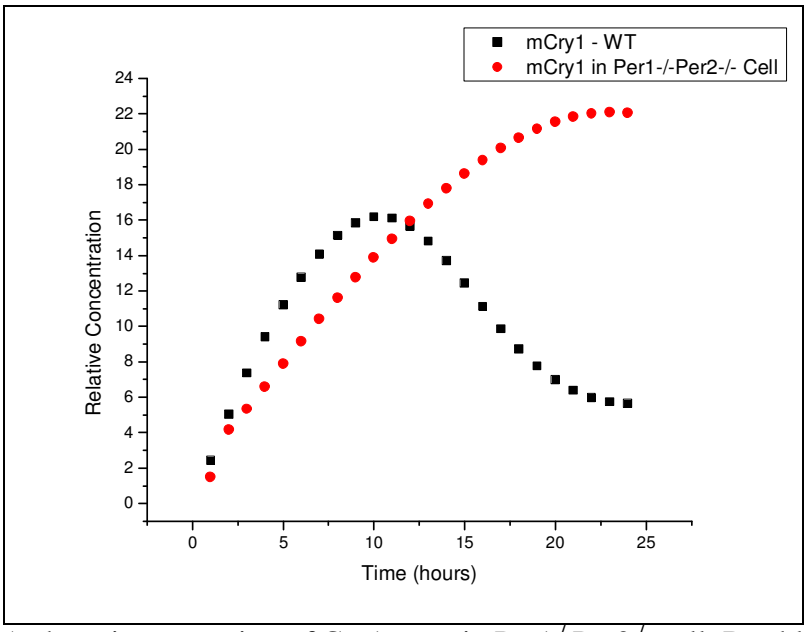

Figure 4-15 Arrhytmic expression of Cry1 gene in Per1$^{-/-}$Per2$^{-/-}$ cell: Double Per mutant cells show non-circadian expression pattern for Cry1 gene

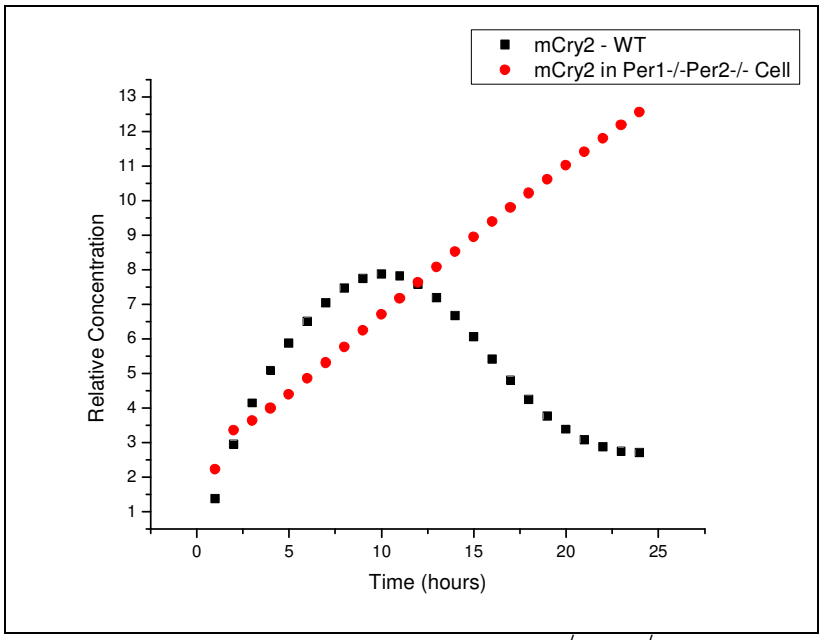

Figure 4-16 Arrhytmic expression of Cry2 gene in Per1$^{-/-}$Per2$^{-/-}$ cell: Double Per mutant cells show a disturbed expression pattern for Cry2 gene

4.2.1.3 Knock-out of Cry1 Gene

Knockout of Cry1 gene has a larger effect than Per1 on circadian clock mechanism, but it is not capable of disturbing the circadian expression of genes. In real case, the knockout of Cry1 does not change the circadian rhytmicity, however it changes the circadian period length. When Cry1 is knocked out, the concentration profile of mPer2 increases, however the concentration level of Per1 decreases. In both mPer profiles, phase shifts are observed [47].

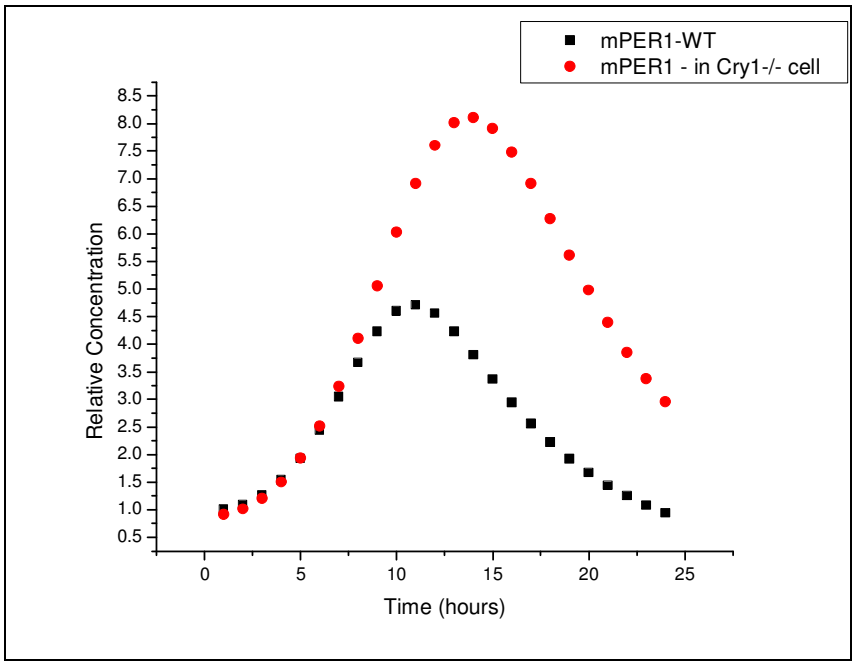

Figure 4-17 Conserved Rhytmicity of Per1 gene expression in $Cry1^{-/-}$ cell: Circadian expression is kept with relative concentration level changes and phase shifts.

Knockout of Cry1 has a large effect on the relative abundance of mPer1 (Figure 4.17). The concentration of mPer1 doubles, and there is a phase shift in the profile, the concentration peaks 2 hours later than the wild type cell. But the Gaussian behavior is kept the same. Except the concentration increase, the model is capable of simulating the mCry1 gene knockout on mPer1 concentration profile.

A similar effect is observed on Per2 gene expression, the concentration doubles, and a 2 hours phase shift is seen (Figure 4.18). Circadian behavior is conserved. mCry2 is not affected as much as Period mRNAs did. The concentration level increases, but the difference is not large, when compared to the wild type cell. There is an obvious phase shift, the concentration peaks later than the normal phenotype. The model perfectly predicts the expression changes of mPer2 when Cry1 gene is knocked out. The relative abundance increases, there is an obvious phase shift, and the circadian expression is kept. Since Cry1 inhibits the transcription of mCry2, the concentration is also observed on mCry2 profile in Figure 4.19.

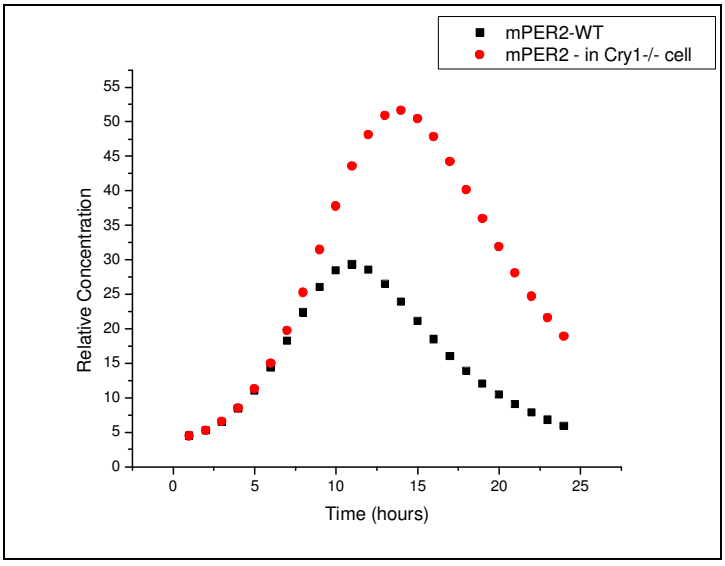

Figure 4-18 Conserved Oscillation of Per2 gene in $Cry1^{-/-}$ cell: Similiar to Per1 expression pattern, circadian expression is kept with concentration level changes and phase shifts for Per2 mRNA.

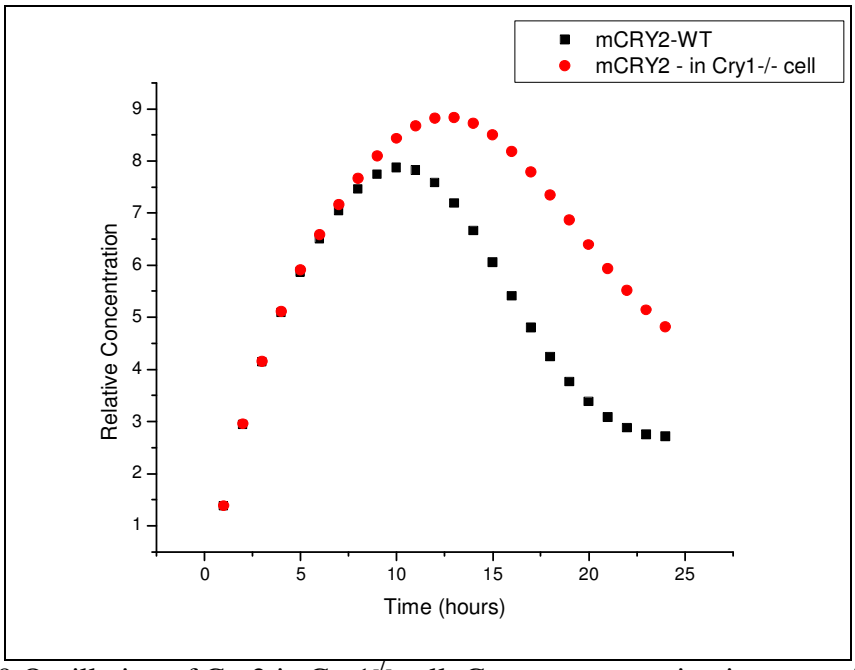

Figure 4-19 Oscillation of Cry2 in Cry1$^{-/-}$ cell: Gene compensation is responsible for the change in relative abundance of mCry2 in cell.

4.2.1.4 Knock-out of Cry2 Gene

Knockout Cry2 gene has a limited effect on circadian clock mechanism when compared to its homolog pair, Cry1. However, the trend is similar. The circadian expression is kept in every mRNA profiles. There is an increase in the relative abundance of mPer2, however mPer1 level decreased. There is an increase in mCry1 level, too. And in every profile, phase shifts are observed [47].

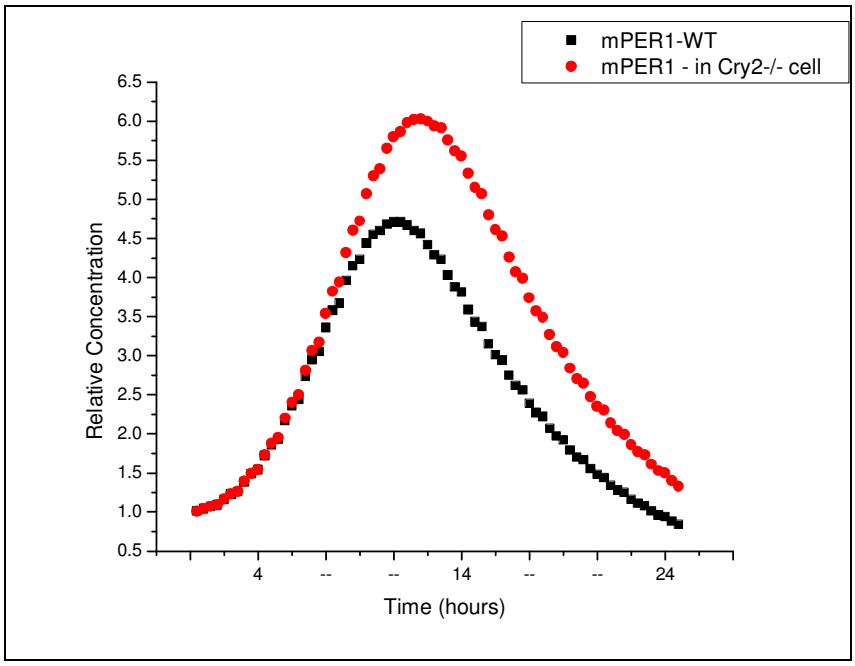

Figure 4-20 Rhytmic expression of Per1 gene in Cry2$^{-/-}$ cell: Oscillatory behaviour is kept, with phase shifts and concentration level changes for mPer1

The concentration of Per1 increases as Cry2 gene is knocked out. There is about %30 increase in the abundance of mPer1 in the cell (Figure 4.20). Also concentration peaks later, a phase shift is observed. Except the increase in the concentration profile, the model is capable of predicting the effect of knocked out Cry2 gene on mPer1 profile.

In the following two figures, the effect of knocked out Cry2 gene on mPer2 and mCry1 are plotted (Figure 4.21, Figure 4.22). The trend in similar with mPer1 expression, an obvious phase shift is observed with a higher peak. This is totally in agreement with real data. However, Cry1 mRNA concentration is not affected as much as Period mRNAs did. There is a small increase in the relative concentration of mCry1, with a phase shift.

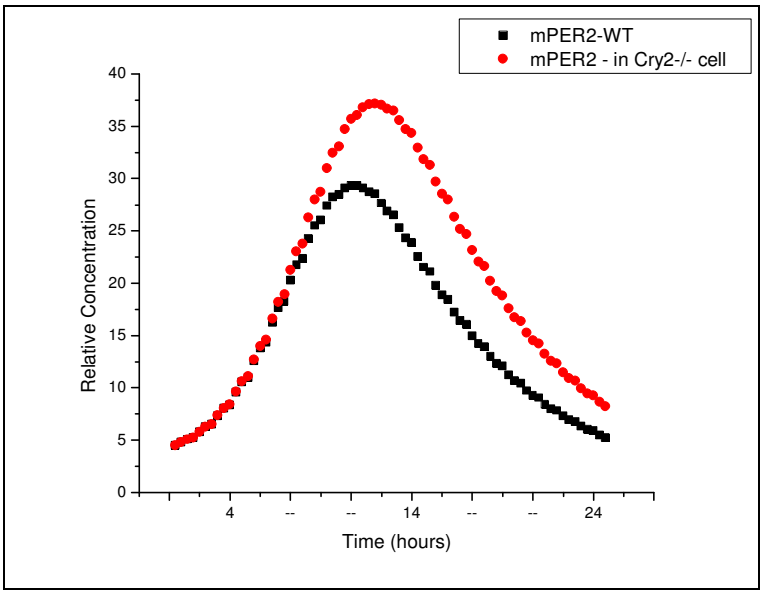

Figure 4-21 Rhytmic Expression of Per2 in Cry2$^{-/-}$ cell: Cry2 perturbation did not disturb the circadian rhythmicity of Per2 gene.

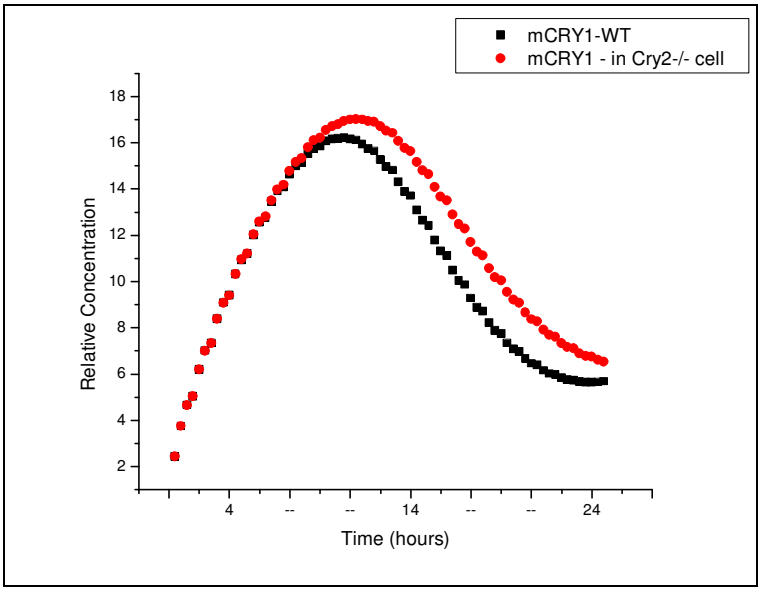

Figure 4-22 Oscillation of mCry1 in Cry2$^{-/-}$ cell: Gene compensation is responsible for the changes in circadian behavior of Cry1 mRNA concentration

When both genes are knocked out, it is observed that the rhythmic expression of Per1 and Per2 genes stops [19]. The model predicts this behavior. In Figure 4.23 and 4.24, the concentration profiles of mPer1 and mPer2 are plotted. As seen on the figures, the circadian expression can no longer be observed in the case of double knockout of Cryptochrome genes.

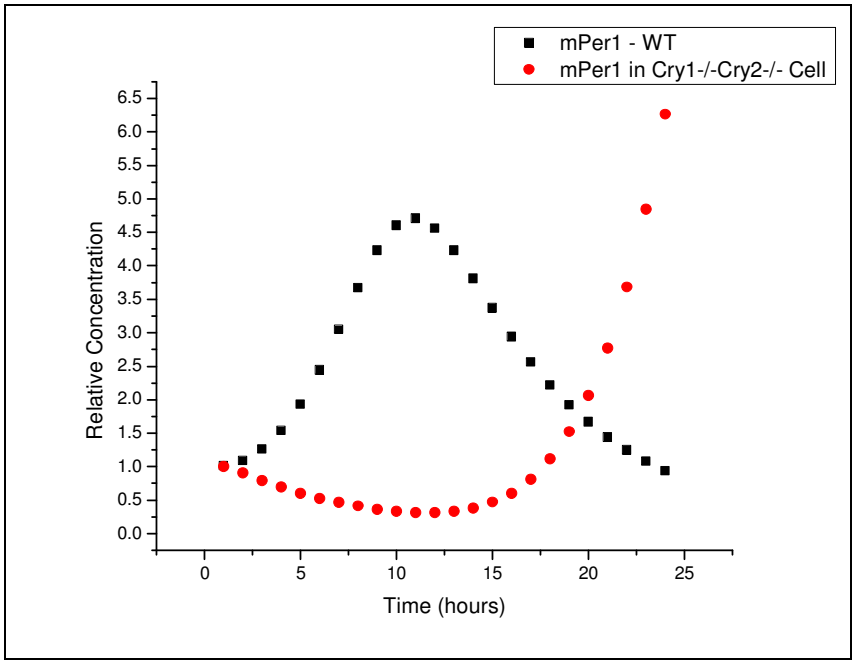

Figure 4-23 Arrhytmic expression of Per1 gene in $Cry1^{-/-}Cry2^{-/-}$ cell: Similiar to double Per mutant cell, double Cry mutant cell is arrhythmic, too.

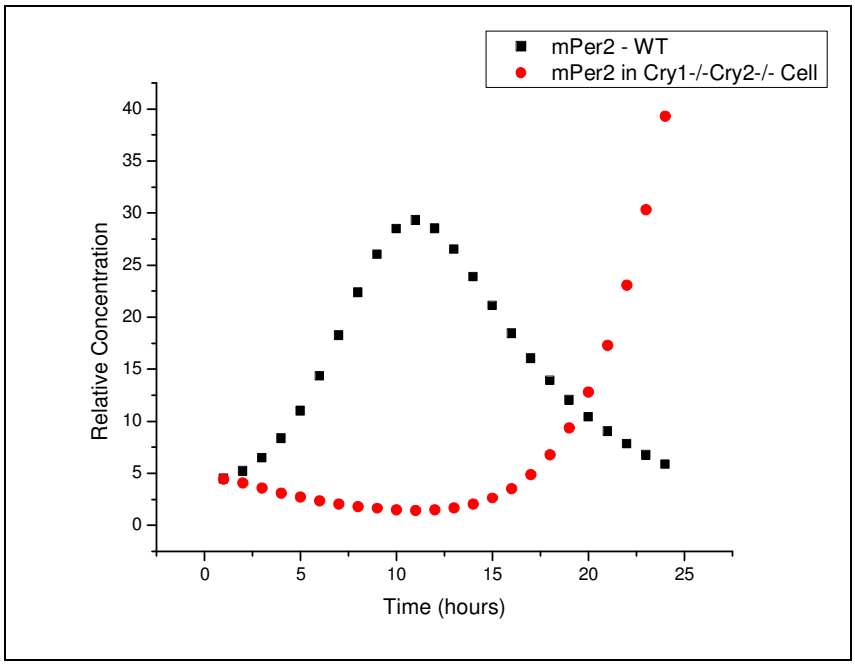

Figure 4-24 Arrhytmic expression of Per2 gene in $Cry1^{-/-}Cry2^{-/-}$ cell: Per2 gene expression shows no circadian behavior, double mutant has no oscillatory behavior.

4.2.1.5 The Effect of Knockout of Dec Genes on Circadian Clock Mechanism

Dec proteins are newly found when compared to other clock component. Their function in circadian clock mechanism is observed by knockout their genes in the cell. Dec proteins are known to be negatively affecting the activity of Per1 gene. Inhibiting its gene activity is found to increase the concentration level of mPer1 in the cell. As stated before, one of the novelties of this study is to add the Dec proteins to overall circadian clock mechanism. So, to see the effect of this newly added protein on circadian clock model, a knocking out procedure is also applied to its gene.

In Figure 4.25, it is seen that the mPer1 concentration is increased when Dec genes are knocked out. 10% increase is significant, so it can be said that DEC proteins has an important role in regulating the gene activity of Per1. Other mRNA concentrations of core

clock proteins are not affected by this knockout procedure (Figure 4.26, Figure 4.27, Figure 4.28). This is in agreement with the experimental results [7].

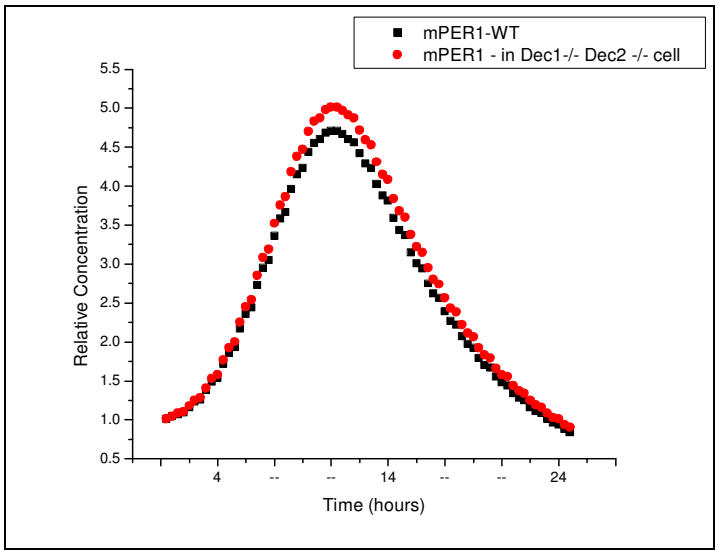

Figure 4-25 Oscillation of Per1 gene in Dec1$^{-/-}$ Dec2 $^{-/-}$ cell: Dec proteins has slight effect on the transcription behavior of Per1 mRNA.

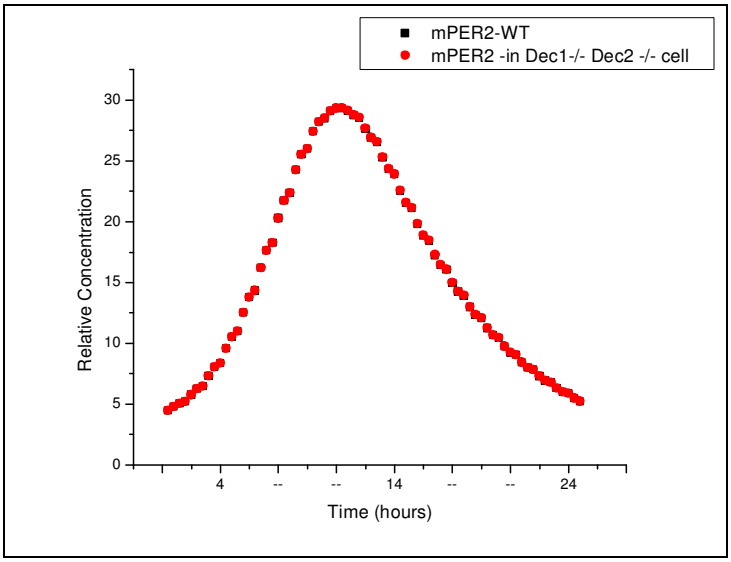

Figure 4-26 Conserved Oscilation of Per2 gene in Dec1$^{-/-}$ Dec2 $^{-/-}$ cell: Dec proteins has no effect on the transcription behavior of Per2 mRNA.

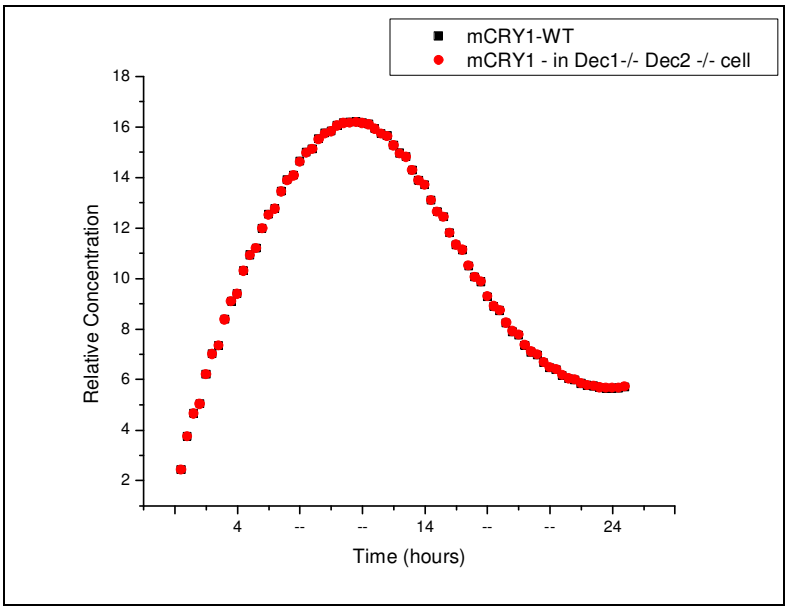

Figure 4-27 Rhytmic expression of Cry1 gene in Dec1$^{-/-}$ Dec2 $^{-/-}$ cell: Dec proteins have no effect on the transcription behavior of Cry1 mRNA.

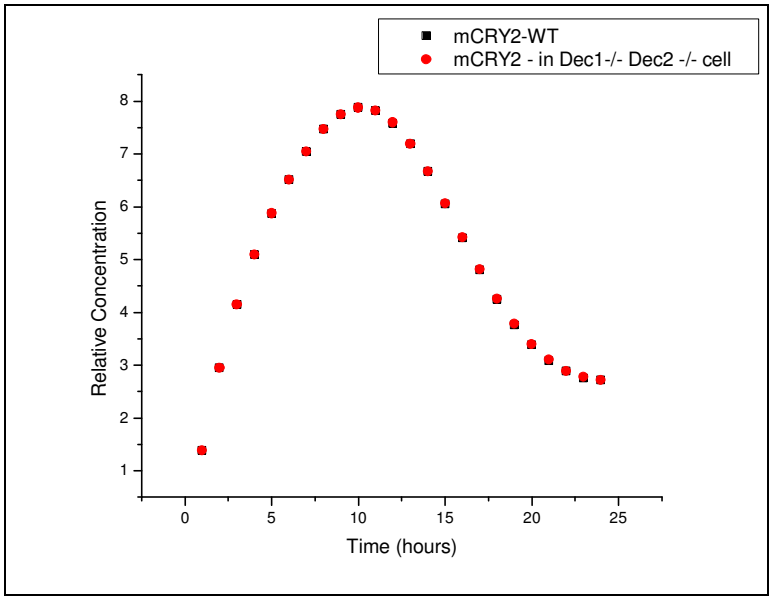

Figure 4-28 Oscillation of Cry2 gene in Dec1$^{-/-}$ Dec2 $^{-/-}$ cell (Dec KO): There is no change in the oscillatory behavior of Cry2 gene expression in Dec1$^{-/-}$ Dec2 $^{-/-}$ cell

4.2.2 Effect of Gene Dose of clock genes on Circadian Mechanism

An operating gene can be made inoperative by inhibiting its activity, but also increasing or decreasing the transcriptional rate of a gene is possible, too. In this study, the genes responsible for transcription of Per, Per2, Cry1 and Cry2 proteins are subjected to dosing. The wild type concentration profiles of mRNAs and the dosing results are plotted together in order to see the effect of dosing clearer.

The method used in gene dosing is as follows: The kinetic parameter that corresponds to the transcriptional rate of the certain gene is multiplied or divided by a certain number. Then the system is simulated with all the other parameters obtained from the optimization part. The concentration profile of the certain gene is not precisely 3 times higher than the wild type, because increasing the magnitude of the concentration also influences its own transcriptional rate by inhibiting itself. Also there can be phase shifts in the profile.

4.2.2.1 Gene Dose of Per1 on other Clock Genes Expressions

To see the effect of the clock gene transcript changes on amplitude and period length of clock, we performed gene dosage calculations. Simulating the overall system with a 3 times larger transcriptional rate of Per1 gene resulted in roughly 3 times bigger concentration profile for Per1, with a small phase shift. The concentration peaks later than the wild type cell.

However, this important change in the concentration level of mPer1 does not trigger a significant change in the profiles of other mRNAs. It has almost no effect on mPer2 transcription; Cryptochrome gene expressions differ from wild type cells only at the end of the circadian day, with a small increase. Like knockout of Per1 gene, dosing also does not affect the overall circadian clock mechanism significantly. This is accordant with the idea that Period proteins are the carriers of Cry proteins to the nucleus, and they can compensate

each other. The following four figures stand for the expressions of core clock genes when transcription rate of Per1 gene is tripled. In Figure 4.29, the effect of tripling the transcription rate of Per1 gene on its own mRNA. It is seen that the concentration level triples with the tripled rate. The concentration profiles of mPer2, mCry1 and mCry2 are not affected significantly with the gene dose of mPer1 (Figure 4.30, Figure 4.31 and Figure 4.32). There are small changes in the concentration profiles of those 3 mRNAs.

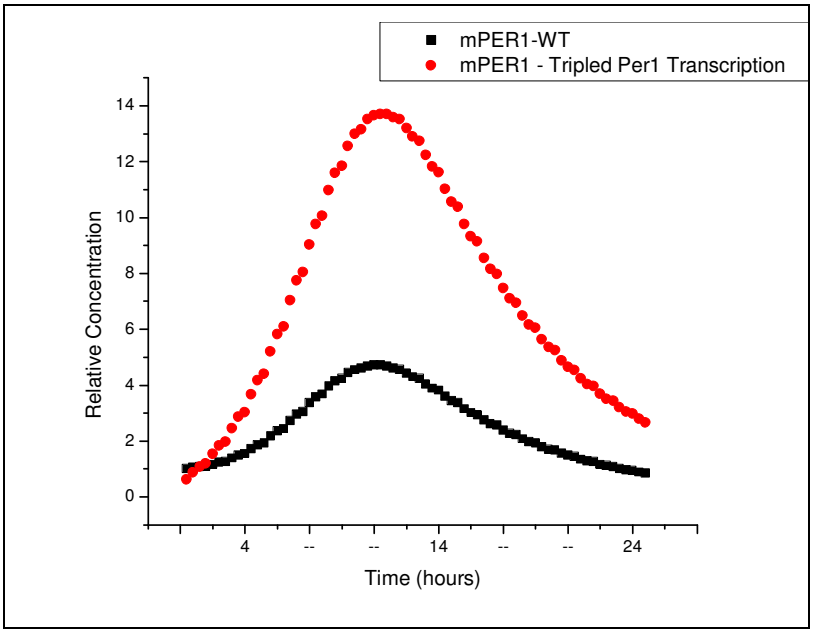

Figure 4-29 Oscillation of Per1 gene: Tripling Per1 transcription rate increased the relative abundance of Per1 mRNA in cell

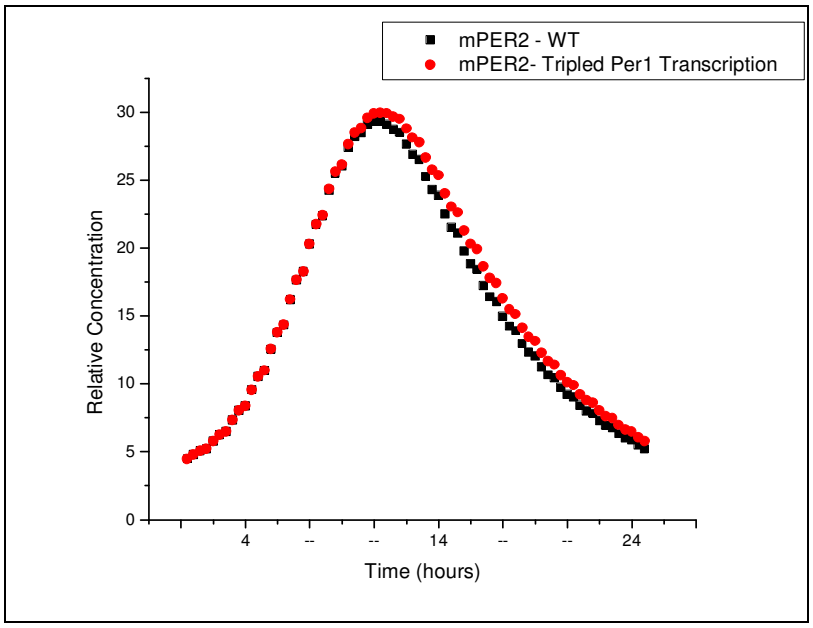

Figure 4-30 Rhytmic Expression of Per2: Tripled Per1 transcription rate has no effect on the expression of Per2 gene.

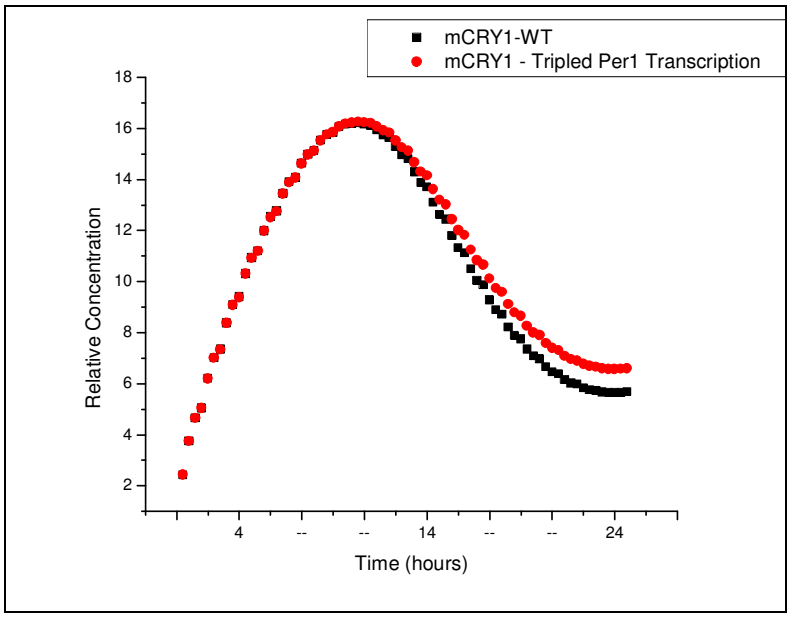

Figure 4-31 Oscillation of Cry1 gene: Tripling the transcription rate of Per1 gene has slight effect on the transcription of mCry1

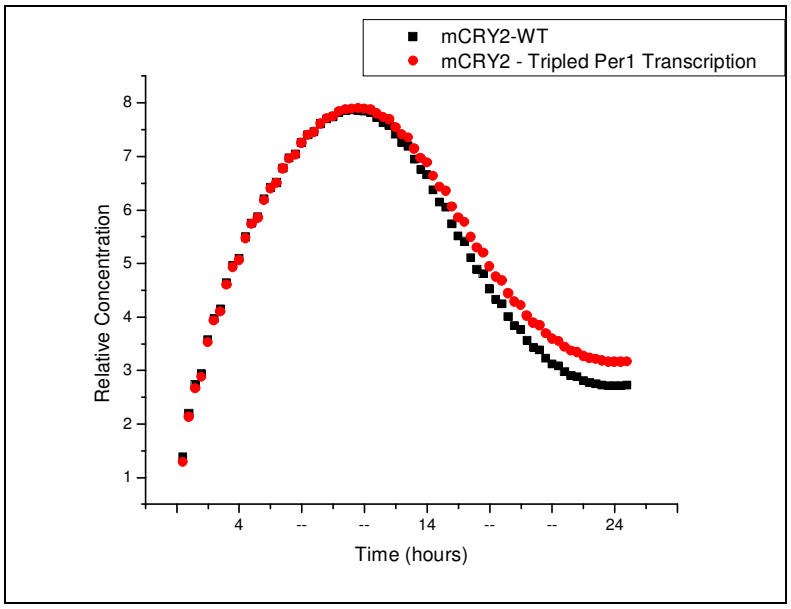

Figure 4-32 Expression of of Cry2 gene: Similiar to Cry1 gene expression, Cry2 gene is slightly affected by the change in transcription rate of Per1 gene.

Dividing the transcription rate of Per1 did not change the overall system, it only lowers the concentration profile of its own mRNA, but the rest of the system stays the same.

4.2.2.2 The effect of Gene Dose of Per2 on Circadian Clock Mechanism

The transcription rate of mPer2 is doubled, and then the expressions of genes are plotted below. Doubling the transcription rate increased the relative abundance of Per2 about 50%.There is no phase shift in the concentration profile (Figure 4.33). And in Figure 4.34, mPer1 abundance around 40% with no phase shift.

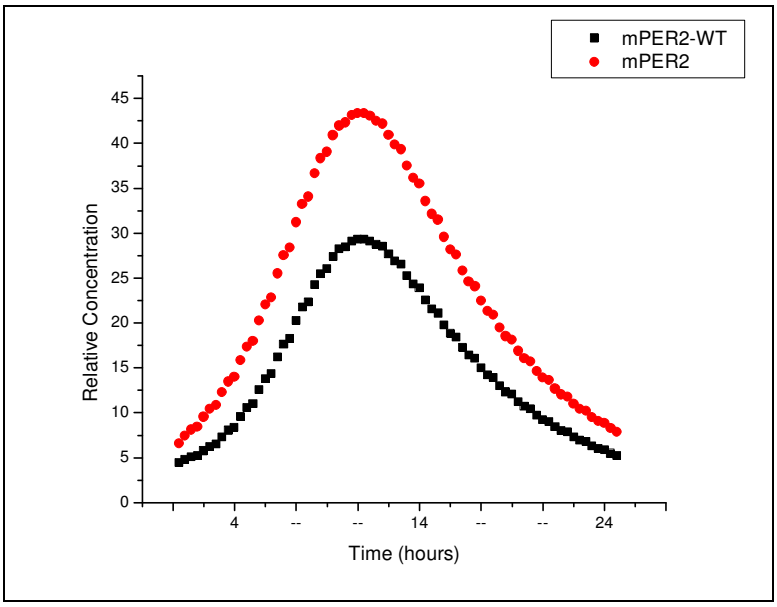

Figure 4-33 Expression of Per2 gene: Doubling the transciption rate of Per2 gene increased the relative concentration of its own mRNA, however, the concentration did not increased 2 times.

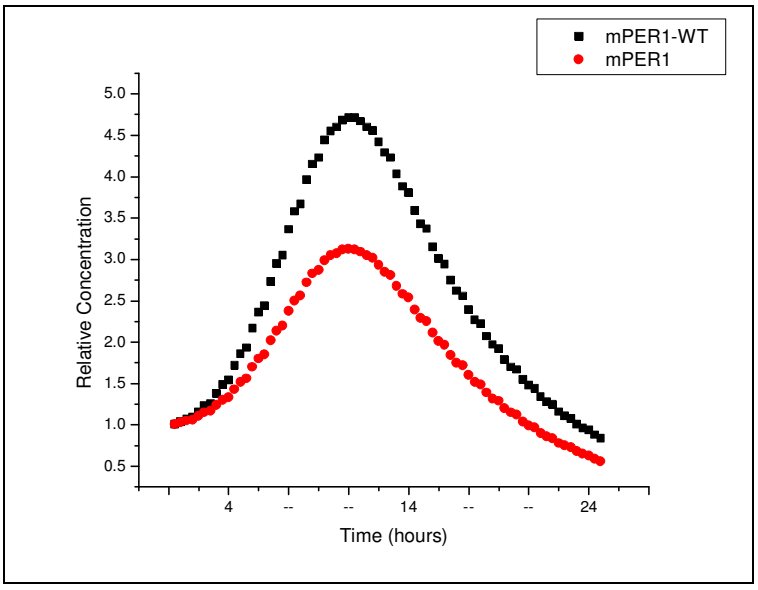

Figure 4-34 Oscillation of Per1 gene: The mPer1 concentration increases with no phase shift with a doubled Per2 transcription rate.

A similar behavior with mPer1 is observed in Cryptochrome mRNA concentrations, with a smaller decrease in the concentration level. In Figure 4.35 and Figure 4.36, a 15% drop with no phase shift is seen on the concentration profiles of both mCry1 and mCry2.

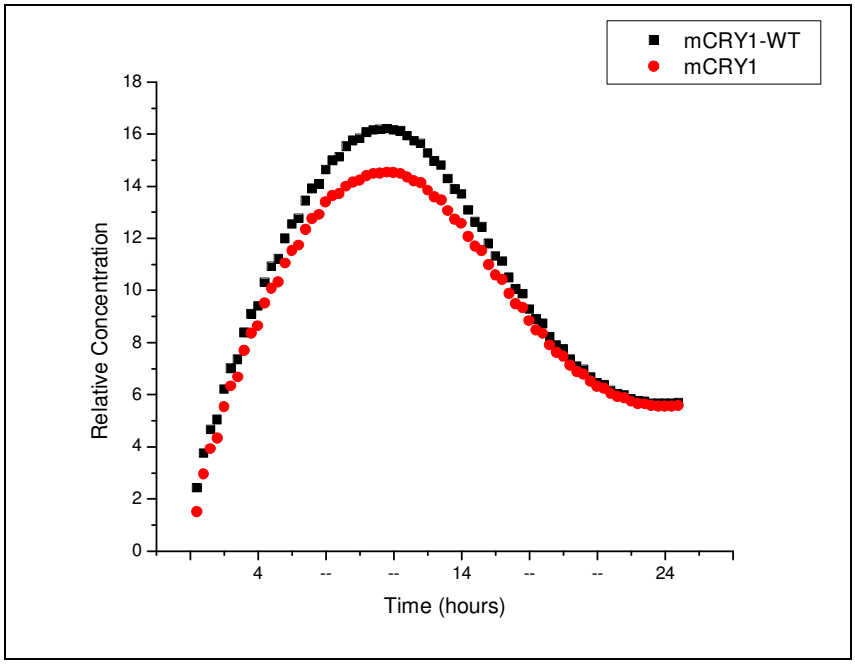

Figure 4-35 Oscillation of Cry1 gene: Doubled Per2 transcription rate has a slight effect on the relative aboundance of mCry1

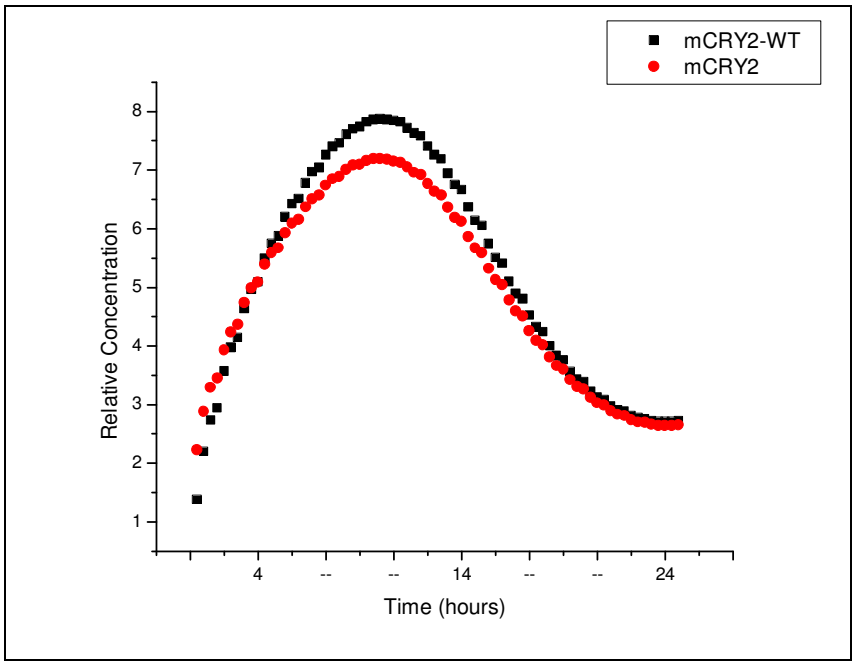

Figure 4-36 Rhytmic Expression of Cry2 with Doubled Per2 Transcription Rate: The increase in the mPer2 concentration has a slight effect on Cry2 gene expression.

Dividing the transcription rate of Per2 by 2 gives just the opposite results of increased transcription rate. The concentration levels of all mRNAs, except mPer2, increase.

4.2.2.3 The effect of various CRY transcripts on clock.

A similar procedure is followed for the transcriptional rate of Cry1 gene. Knockout procedure showed that the negative feedback behavior of circadian clock mechanism is strongly regulated by the activity of Cryptochrome proteins. A same behavior is also seen in gene dosing results; changes in the ranscriptional rates of Cry genes have important effects on circadian clock mechanism.

Simulating the system with a 3 times larger Cry1 transcription rate has made great changes on the oscillations of all core clock mRNAs. In Figure 4.37, the rhythmic expression level of of mCry1 triples, but a phase shift is not observed. A sharp drop in the

concentration level of mPer1 and mPer2 is observed in Figure 4.38 and Figure 4.39 respectively. There is 50% decrease in both Period proteins with an obvious phase shifts. This shows that Cry1 gene has a very important role in circadian clock mechanism, tripling its tanscription rate resulted in lowered concentration profiles of other core clock mRNAs, mPer1, mPer2, mCry2.

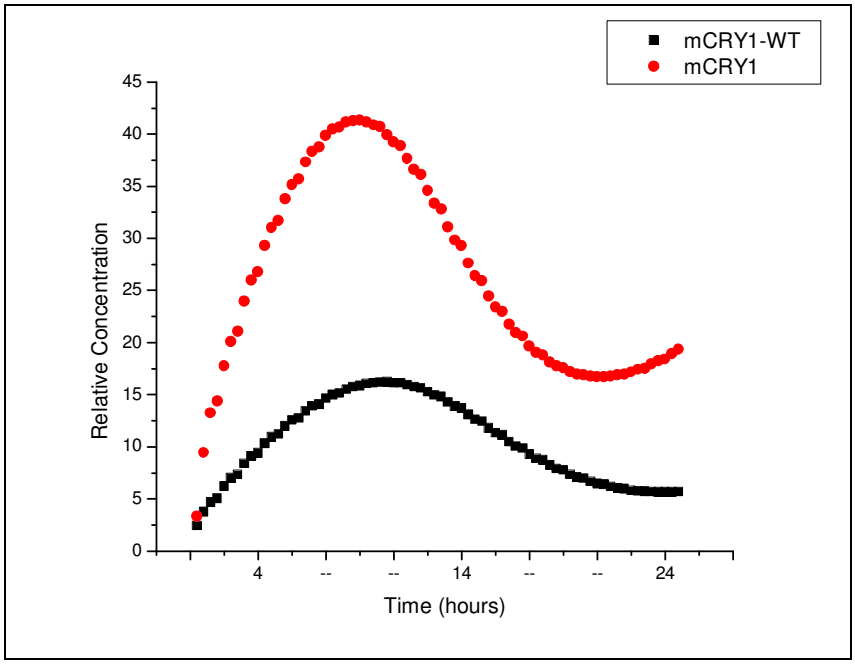

Figure 4-37 Oscillation of Cry1 gene: Tripled Cry1 transcription rate changes the expression level of its own gene, relative concentration of mCry1 nearly triples.

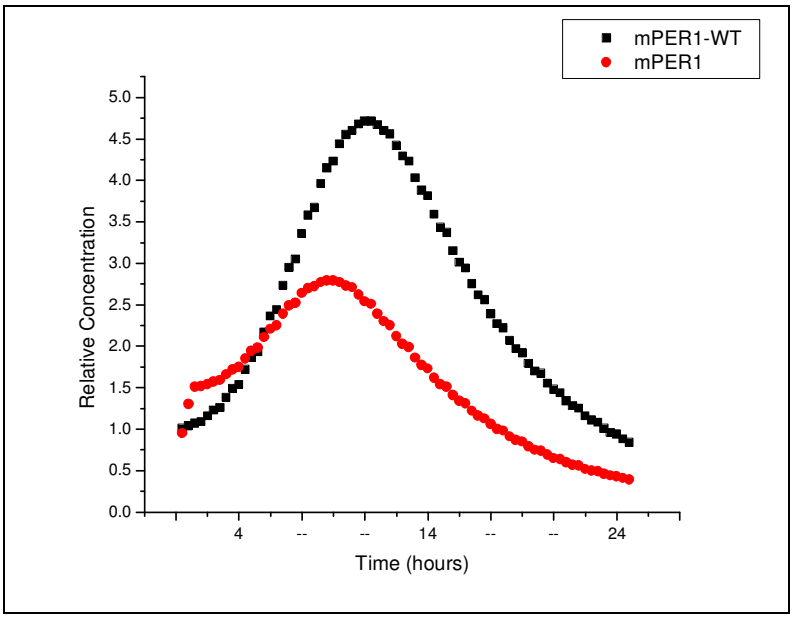

Figure 4-38 Rhytmic Expression of Per1 gene: Phase shifts and concentration level changes are observed in mPer1 profile with mCry1 abundance.

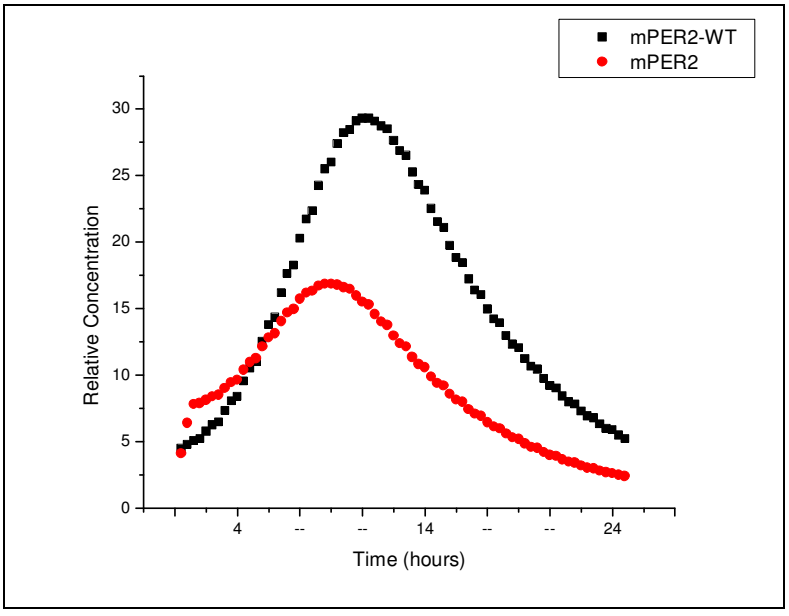

Figure 4-39 Conserved Rhytmicity of mPer2: Similiar to mPer1 relative concentration, tripled Cry1 transcription rate affects mPer2 level significantly.

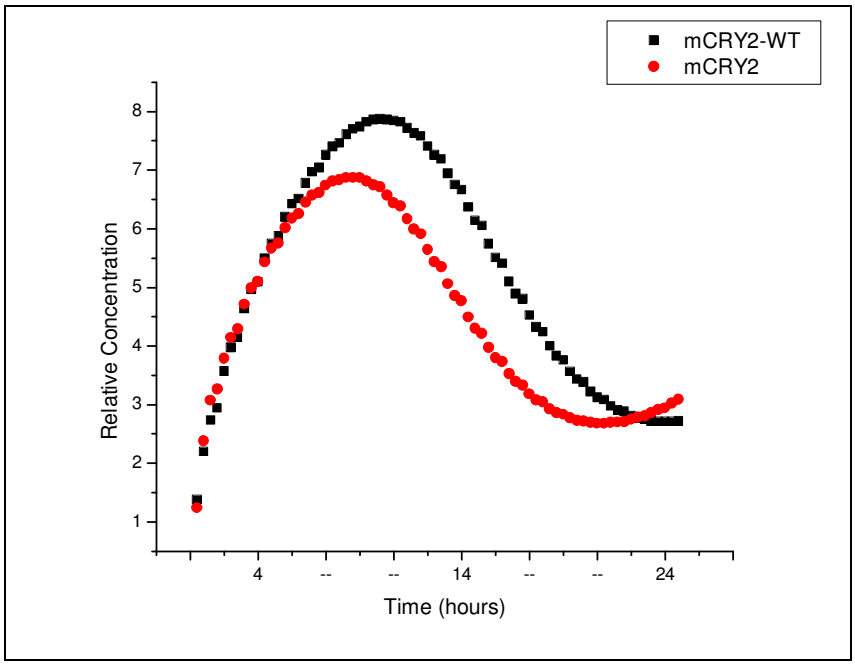

Figure 4-40 Change in the oscillatory behavior of Cry2 with Tripled Cry1 Transcription Rate

In Figure 40, it is seen that tripled transcription rate of Cry1 does not affect the concentration profile of Cry2 as much as it did Period mRNAs. However, a significant change is obvious. Gene dosage of Cry1 results in 15% decrease in the concentration level of Cry2 in cell, in addition to this, a phase shift is observed.

Decreasing the transcription rate of mCry1 results in higher concentration level values for mPer1, mPer2 and mCry2, which is in agreement with real case, since CRY1 inhibits the transcription of the rest clock mRNAs. There is compensation of homolog pairs, too [61].

4.2.2.4 The Effect of Gene Dosing of Cry2

Like its homolog pair, Cry2 is strongly regulating the overall circadian mechanism, and knockout of Cry2 gene from the system changes the behavior of circadian clock dramatically. Tripling the magnitude of Cry2 transcription affects the system so strongly, too.

Firstly, the concentration level of Cry2 increased more than expected. Other mRNA levels of core proteins tripled with a tripled transcription rate, but Cry2 level increased even more (Figure 4.41). Phase shifting is another result of gene dosage. But its effect is limited when compared to Cry1. In Figure 4.42 and Figure 4.43, the concentration levels of Per1 and Per2 proteins drop roughly 35%. A decrease in the concentration level of mCry1 is also observed in Figure 4.44. Similiar to Cry1 gene dosage, a phase shift is seen for all mRNA profiles. The increase in the concentration level of mCry2 fastens the inhibition of Per1, Per2 and Cry1 transcription, so they peak earlier than the wild type cell.

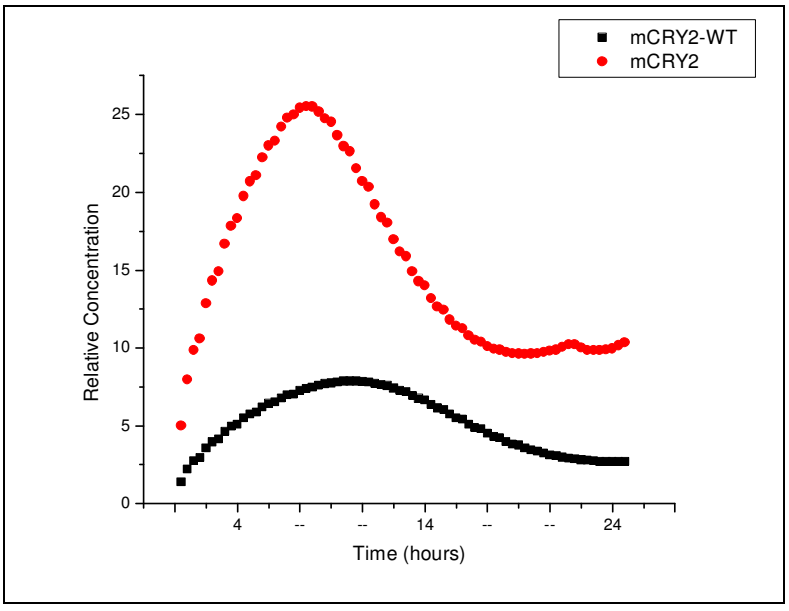

Figure 4-41 Change in expression level of mCry2 with tripled Cry2 transcription rate: Relative concentration of mCry2 nearly triples.

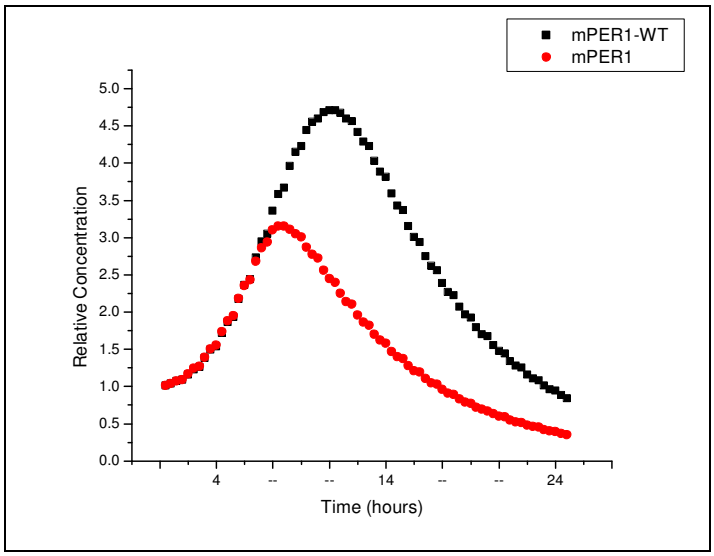

Figure 4-42 Change in expression level of mCry2 results in phase shifts and relative abundance changes in mPer1 oscillatory behavior.

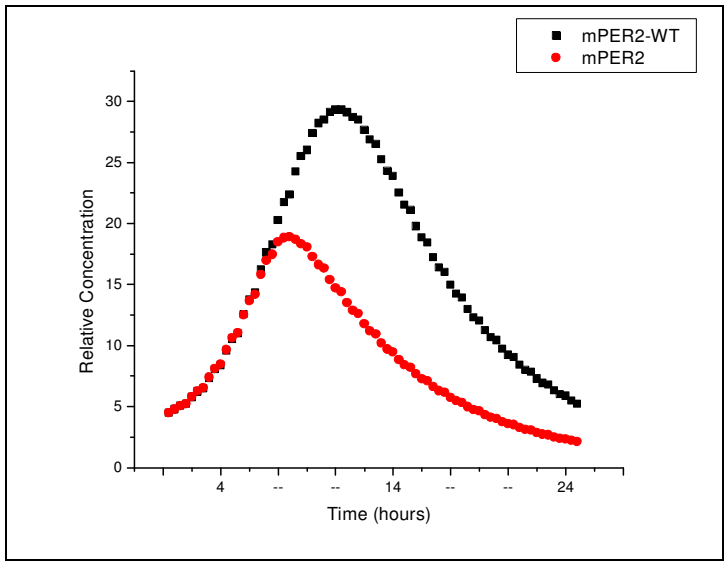

Figure 4-43 Change in expression level of mPer2: Tripled Cry2 transcription rate has a similiar effect on Per2 gene expression with Per1 gene, phase shifts and concentration level changes are observed.

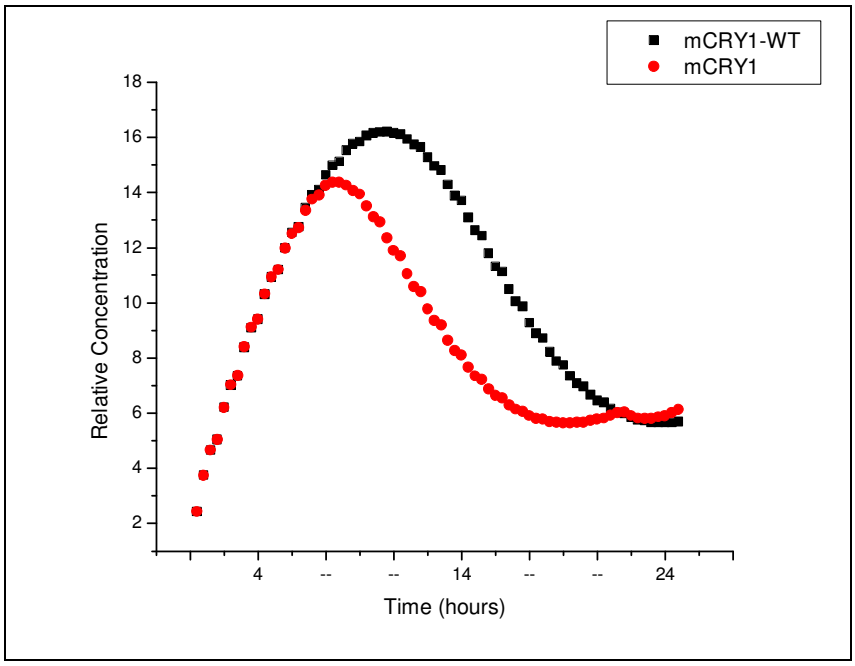

Figure 4-44 Oscillation of Cry1 gene: Tripled Cry2 transcription rate results in phase shifts and relative abundance level changes for Cry1 expression pattern.

Cry1 concentration profile is also affected with the gene dosing of Cry2. The concentration level drops 15%. Also a phase shift is observed. The overall behavior of Cry1 and Cry2 genes to dosing resembles to each other, which shows that they belong to the same family of proteins.

Decreasing the transcription rate of mCry2 results in higher concentration profile values for all the rest 3 mRNAs of circadian clock mechanism just like it did for Cry1. CRY1 and CRY2 proteins have similar roles in the system, so these results are in accordance with real biological facts.

CHAPTER 5

CONCLUSION

The aim of this study is to build a detailed, predictive model for mammalian circadian clock. To achieve this, a mathematical model, based on differential equations, is constructed, and the system is run in GAMS solver, based on real time PCR data. And then by knocking out, effect of each gene in the system is observed, and lastly by gene dosing, the effect of transcription rate on overall circadian clock mechanism is seen.

Firstly, it is seen that the model capable of simulating the first 24 hour of circadian mechanism. The model is constructed based on 24 hours, and the concentration profiles of Per1, Per2, Cry1 and Cry2 are showing Gaussian behavior perfectly, which in a accordance with RT-PCR data [7-55-62-63]

By knocking out in the light day cycle, the effect of each protein on the overall mechanism is observed. Based on model results, it is seen that the effect of DEC proteins is simulated very accurately. In real case DEC proteins have no effect on the concentration profiles of mPer2, mCry1 and mCry2 concentration profiles. But it increases the amplitude of the concentration profile of mPer1 [55]. This behavior is seen perfectly in the model results.

For the CRY results, in real case, the oscillation is not disrupted with single knock out of CRY1 or CRY2 proteins, this is achieved by the model, and the model is capable of predicting the amplitude increase in mPer2 concentration. However, the amplitude of mPer1 is different from the RT-PCR data, however it still remains Gaussian, and this is seen accurately [47].

In real case, knock out of Per1 gene does not have a significant effect on the circadian clock mechanism, and this is seen in model results accurately [50]. However, knocking out Per2 gene resulted an unexpected way in the model, this is not accordant with the real data.

Gene dosing has very important effects on the circadian clock mechanism. Doubling the Per2 gene transcription rate resulted in lowering concentration profiles of mPer1, mCry1 and mCry2. However, the effect of tripled Per1 gene transcription did not have a significant effect on the system. But tripling the rate of Cry1 gene resulted in lowered amplitude concentration profiles for mPer1, mPer2 and mCry2 with obvious phase shifts. The same result can be seen for the tripled transcription rate for mCry2. mPer1, mPer2 and mCry1 concentration profiles are lowered, and a phase shift is observed. The opposite behavior is observed for all 4 clock components when the transcription rates are divided by 2.

Overall, the model is capable of simulating the first circadian day, with Gaussian like concentration profiles. Moreover, the model shows accordance with real time data when knock out procedure is applied, with the exception of Per2 knock out.

APPENDIX

Appendix A. Model Equations

Terms are color coded as transcription (green), translation (blue), nuclear transport (purple), and binding/unbinding (brown). For complexes in the nucleus of the cell, an n appears after every molecule in the complex (e.g., PopCn in the text is written here as PopnCn). The equations in orange are omitted from the system when the corresponding gene is knocked-out.

L = Light Level

$$\frac{dG}{dt} = bin \times Rn *\times (1 - G) - unbin \times G$$

$$\frac{dGRv}{dt} = binRv \times RvnRvn \times (1 - GRv) - unbinRv \times GRv$$

$$\frac{dMRo}{dt} = trRo \times (1 - G) \times (1 - GRv) \times (1 - GRv) \times (1 - GRv) - umR \times MRo$$

$$\frac{MRt}{dt} = trRt \times (1 - G) - umR \times MRt$$

$$\frac{MPo}{dt} = trPo \times (1 - G) \times (1 - G) \times (1 - G) \times (1 - G) \times (1 - G) \times (1 - GD) + L - umPo \times MPo$$

$$\frac{MPt}{dt} = trPt \times (1 - G) \times (1 - G) \times (1 - G) \times (1 - G) \times (1 - G) + L - umPt \times MPt$$

$$\frac{MRv}{dt} = trRv \times (1 - G) \times (1 - G) \times (1 - G) - umRv \times MRv;$$

$$\frac{MD1}{dt} = trcD1 \times (1 - GD) + L - degMD \times MD1;$$

$$\frac{D1}{dt} = trlD1 \times MD1 - degD1 \times D1 - nl \times D1 + ne \times D1n;$$

$$\frac{D1n}{dt} = nl \times D1 - ne \times D1n - B1 \times D1n - box \times D1n;$$

$$\frac{MD2}{dt} = trcD2 \times (1 - GD) - degMD \times MD2;$$

$$\frac{D2}{dt} = trlD2 \times MD2 - degD2 \times D2 - nl \times D2 + nl \times D2n;$$

$$\frac{D2n}{dt} = nl \times D2 - ne \times D2 - B2 \times D2n - box \times D2n;$$

$$\frac{GD}{dt} = binD \times (D1n + D2n) \times (1 - GD) - unbinD \times GD;$$

$$\frac{dRv}{dt} = tlrv \times MRv - 2 \times arv \times Rv \times Rv + 2 \times drv \times RvRv - nl \times Rv + ne \times Rvn - uRv \times Rv$$

$$\frac{dRvn}{dt} = -2 \times Nf \times arv \times Rvn \times Rvn + 2 \times drv \times RvnRvn + nl \times Rv - ne \times Rvn - uRv \times Rvn$$

$$\frac{dRvRv}{dt} = arv \times Rv \times Rv - drv \times RvRv - nl \times RvRv + ne \times RvnRvn - 2 \times uRv \times RvRv$$

$$\frac{dRvnRvn}{dt} = Nf \times arv \times Rvn \times Rvn - drv \times RvnRvn + nl \times RvRv - ne \times RvnRvn - 2 \times uRv \times RvnRvn$$

$$\frac{dPo}{dt} = tlp \times MPo - ac \times Po \times C + dc \times PoC - upu \times Po$$

$$\frac{dPt}{dt} = tlp \times MPt - ac \times Pt \times C + dc \times PtC - upu \times Pt$$

$$\frac{dPoC}{dt} = ac \times Po \times C - dc \times PoC - hoo \times PoC - upu \times PoC$$

$$\frac{dPtC}{dt} = ac \times Pt \times C - dc \times PtC - hot \times PtC - upu \times PtC$$

$$\frac{dPopC}{dt} = hoo \times PoC + ac \times Pop \times C - dc \times PopC - up \times PopC - ht \times PopC - nl \times PopC + ne \times PonpCn - ar \times PopC * Ro + dr \times PopCRo - ar \times PopC \times Rt + dr \times PopCRt$$

$$\frac{dPtpC}{dt} = hot \times PtC + ac \times Ptp \times C - dc \times PtpC - up \times PtpC - ht \times PtpC - nl \times PtpC + ne \times PtnpCn\, ar \times PtpC \times Ro + dr \times PtpCRo - ar \times PtpC \times Rt + dr \times PtpCRt$$

$$\frac{dPop}{dt} = -ac \times Pop \times C + dc \times PopC - up \times Pop - ar \times Pop \times Ro + dr \times PopRo - ar \times Pop \times Rt + dr \times PopRt - nl \times Pop + ne \times Ponp$$

$$\frac{dPtp}{dt} = -ac \times Ptp \times C + dc \times PtpC - up \times Ptp - ar \times Ptp \times Ro + dr \times PtpRo - ar \times Ptp \times Rt + dr \times PtpRt - nl \times Ptp + ne \times Ptnp$$

$$\frac{dPoppC}{dt} = hto \times PopC - up \times PoppC + ac \times Popp \times C - dc \times PoppC + ne \times PonppCn - ar \times PoppC \times Ro + dr \times PoppCRo - ar \times PoppC \times Rt + dr \times PoppCRt$$

$$\frac{dPtppC}{dt} = ht \times PtpC - up \times PtppC + ac \times Ptpp * C - dc \times PtppC + ne \times PtnppCn - ar \times PtppC \times Ro + dr \times PtppCRo - ar \times PtppC \times Rt + dr \times PtppCRt$$

$$\frac{dPopRo}{dt} = ar \times Pop \times Ro - dr \times PopRo - ac \times PopRo \times C + dc \times PopCRo - nl \times PopRo + ne \times PonpRon$$

$$\frac{dPtpRo}{dt} = ar \times Ptp \times Ro - dr \times PtpRo - ac \times PtpRo \times C + dc \times PtpCRo - nl \times PtpRo + ne \times PtnpRon$$

$$\frac{dPopRt}{dt} = ar \times Pop \times Rt - dr \times PopRt - ac \times PopRt \times C + dc \times PopCRt - nl \times PopRt + ne \times PonpRtn$$

$$\frac{dPtpRt}{dt} = ar \times Ptp \times Rt - dr \times PtpRt - ac \times PtpRt \times C + dc \times PtpCRt - nl \times PtpRt + ne \times PtnpRtn$$

$$\frac{dPoppRo}{dt} = ar \times Popp \times Ro - dr \times PoppRo - ac \times PoppRo \times C + dc \times PoppCRo + ne \times PonppRon$$

$$\frac{dPoppRt}{dt} = ar \times Popp \times Rt - dr \times PoppRt - ac \times PoppRt \times C + dc \times PoppCRt + ne \times PonppRtn$$

$$\frac{dPtppRo}{dt} = ar \times Ptpp \times Ro - dr \times PtppRo - ac \times PtppRo \times C + dc \times PtppCRo + ne \times PtnppRon$$

$$\frac{dPtppRt}{dt} = ar \times Ptpp \times Rt - dr \times PtppRt - ac \times PtppRt \times C + dc \times PtppCRt + ne \times PtnppRtn$$

$$\frac{dPopp}{dt} = -ac \times Popp \times C + dc \times PoppC + ne \times Ponpp - ar \times Popp \times Ro + dr \times PoppRo - ar \times Popp \times Rt + dr \times PoppRt - up \times Popp$$

$$\frac{dPtpp}{dt} = -ac \times Ptpp \times C + dc \times PtppC + ne \times Ptnpp - ar \times Ptpp \times Ro + dr \times PtppRo - ar \times Ptpp \times Rt + dr \times PtppRt - up \times Ptpp$$

$$\frac{dPopCRo}{dt} = ar \times PopC \times Ro - dr \times PopCRo + ac \times PopRo \times C - dc \times PopCRo - nl \times PopCRo + ne \times PonpCnRon - hto \times PopCRo$$

$$\frac{dPtpCRo}{dt} = ar \times PtpC \times Ro - dr \times PtpCRo + ac \times PtpRo \times C - dc \times PtpCRo - nl \times PtpCRo + ne \times PtnpCnRon - ht \times PtpCRo$$

$$\frac{dPopCRt}{dt} = ar \times PopC \times Rt - dr \times PopCRt + ac \times PopRt \times C - dc \times PopCRt - nl \times PopCRt + ne \times PonpCnRtn - hto \times PopCRt$$

$$\frac{dPtpCRt}{dt} = ar \times PtpC \times Rt - dr \times PtpCRt + ac \times PtpRt \times C - dc \times PtpCRt - nl \times PtpCRt + ne \times PtnpCnRtn - ht \times PtpCRt$$

$$\frac{dPoppCRo}{dt} = ar \times PoppC \times Ro - dr \times PoppCRo + ac \times PoppRo \times C - dc \times PoppCRo + ne \times PonppCnRon + hto \times PopCRo$$

$$\frac{dPtppCRo}{dt} = ar \times PtppC \times Ro - dr \times PtppCRo + ac \times PtppRo * C - dc \times PtppCR + ne \times PtnppCnRon + ht \times PtpCRo$$

$$\frac{dPoppCRt}{dt} = ar \times PoppC \times Rt - dr \times PoppCRt + ac \times PoppRt \times C - dc \times PoppCRt + ne \times PonppCnRtn + hot \times PopCRt$$

$$\frac{dPtppCRt}{dt} = ar \times PtppC \times Rt - dr \times PtppCRt + ac \times PtppRt \times C - dc * PtppCRt + ne \times PtnppCnRtn + ht \times PtpCRt$$

$$\frac{dRo}{dt} = -ar \times Ro \times Pop - ar \times Ro \times Popp - ar \times Ro \times PopC - ar \times Ro \times PoppC + dr \times PopRo + dr \times PoppRo + dr \times PopCRo + dr \times PoppCRo - ar \times Ro \times Ptp - ar \times Ro \times Ptpp - ar \times Ro \times PtpC - ar \times Ro \times PtppC + dr \times PtpRo + dr \times PtppRo + dr \times PtpCRo + dr \times PtppCRo + tlr \times MRo - uro \times Ro$$

$$\frac{dRt}{dt} = -ar \times Rt \times Pop - ar \times Rt \times Popp - ar \times Rt \times PopC - ar \times Rt \times PoppC + dr \times PopRt + dr \times PoppRt + dr \times PopCRt + dr \times PoppCRt - ar \times Rt \times Ptp - ar \times Rt \times Ptpp - ar \times Rt \times PtpC - ar \times Rt \times PtppC + dr \times PtpRt + dr \times PtppRt + dr \times PtpCRt + dr \times PtppCRt + tlr \times MRt - urt \times Rt$$

$$\frac{dPonpCn}{dt} = ac \times Nf \times Ponp \times Cn - dc \times PonpCn - hto \times PonpCn + nl \times PopC - ne \times PonpCn - ar \times Nf \times PonpCn \times Ron + dr \times PonpCnRon - ar \times Nf \times PonpCn \times Rtn + dr \times PonpCnRtn - up \times PonpCn$$

$$\frac{dPtnpCn}{dt} = ac \times Nf \times Ptnp \times Cn - dc \times PtnpCn - ht \times PtnpCn + nl \times PtpC - ne \times PtnpCn - ar \times Nf \times PtnpCn \times Ron + dr \times PtnpCnRon - ar \times Nf \times PtnpCn \times Rtn + dr \times PtnpCnRtn - up \times PtnpCn$$

$$\frac{dPonp}{dt} = -ac \times Nf \times Ponp \times Cn + dc \times PonpCn - ar \times Nf \times Ponp \times Ron + dr \times PonpRon - ar \times Nf \times Ponp \times Rtn + dr \times PonpRtn + nl \times Pop - ne \times Ponp - up \times Ponp$$

$$\frac{dPtnp}{dt} = -ac \times Nf \times Ptnp \times Cn + dc \times PtnpCn - ar \times Nf \times Ptnp \times Ron + dr \times PtnpRon - ar \times Nf \times Ptnp \times Rtn + dr \times PtnpRtn + nl \times Ptp - ne \times Ptnp - up \times Ptnp$$

$$\frac{dPonppCn}{dt} = hto \times PonpCn + ac \times Nf \times Ponpp \times Cn - dc \times PonppCn - ne \times PonppCn - ar \times Nf \times PonppCn \times Ron + dr \times PonppCnRon - ar \times Nf \times PonppCn \times Rtn + dr \times PonppCnRtn - up \times PonppCn$$

$$\frac{dPtnppCn}{dt} = ht \times PtnpCn + ac \times Nf \times Ptnpp \times Cn - dc \times PtnppCn - ne \times PtnppCn - ar \times Nf \times PtnppCn \times Ron + dr \times PtnppCnRon - ar \times Nf \times PtnppCn \times Rtn + dr \times PtnppCnRtn - up \times PtnppCn$$

$$\frac{dPonpRon}{dt} = ar \times Nf \times Ponp \times Ron - dr \times PonpRon - ac \times Nf \times PonpRon \times Cn + dc \times PonpCnRon + nl \times PopRo - ne \times PonpRon$$

$$\frac{dPtnpRon}{dt} = ar \times Nf \times Ptnp \times Ron - dr \times PtnpRon - ac \times Nf \times PtnpRon \times Cn + dc \times PtnpCnRon + nl \times PtpRo - ne \times PtnpRon$$

$$\frac{dPonpRtn}{dt} = ar \times Nf \times Ponp \times Rtn - dr \times PonpRtn - ac \times Nf \times PonpRtn \times Cn + dc \times PonpCnRtn + nl \times PopRt - ne \times PonpRtn$$

$$\frac{dPtnpRtn}{dt} = ar \times Nf \times Ptnp \times Rtn - dr \times PtnpRtn - ac \times Nf \times PtnpRtn \times Cn + dc \times PtnpCnRtn + nl \times PtpRt - ne \times PtnpRtn$$

$$\frac{dPonppRon}{dt} = ar \times Nf \times Ponpp \times Ron - dr \times PonppRon - ac \times Nf \times PonppRon \times Cn + dc \times PonppCnRon - ne \times PonppRon$$

$$\frac{dPtnppRon}{dt} = ar \times Nf \times Ptnpp \times Ron - dr \times PtnppRon - ac \times Nf \times PtnppRon \times Cn + dc \times PtnppCnRon - ne \times PtnppRon$$

$$\frac{dPonppRtn}{dt} = ar \times Nf \times Ponpp \times Rtn - dr \times PonppRtn - ac \times Nf \times PonppRtn \times Cn + dc \times PonppCnRtn - ne \times PonppRtn$$

$$\frac{dPtnppRtn}{dt} = ar \times Nf \times Ptnpp \times Rtn - dr \times PtnppRtn - ac \times Nf \times PtnppRtn * Cn + dc \times PtnppCnRtn - ne \times PtnppRtn$$

$$\frac{dPonpp}{dt} = -ac \times Nf \times Ponpp \times Cn + dc \times PonppCn - ne \times Ponpp - ar \times Nf \times Ponpp \times Ron + dr \times PonppRon - ar \times Nf \times Ponpp \times Rtn + dr \times PonppRtn - up \times Ponpp$$

$$\frac{dPtnpp}{dt} = -ac \times Nf \times Ptnpp \times Cn + dc \times PtnppCn - ne \times Ptnpp - ar \times Nf \times Ptnpp \times Ron + dr \times PtnppRon - ar \times Nf \times Ptnpp \times Rtn + dr \times PtnppRtn - up \times Ptnpp$$

$$\frac{dPonpCnRon}{dt} = ar \times Nf \times PonpCn \times Ron - dr \times PonpCnRon + ac \times Nf \times PonpRon \times Cn - dc \times PonpCnRon + nl \times PopCRo - ne \times PonpCnRon - hto \times PonpCnRon$$

$$\frac{dPtnpCnRon}{dt} = ar \times Nf \times PtnpCn \times Ron - dr \times PtnpCnRon + ac \times Nf \times PtnpRon \times Cn - dc \times PtnpCnRon + nl \times PtpCRo - ne \times PtnpCnRon - ht \times PtnpCnRon$$

$$\frac{dPonpCnRtn}{dt} = ar \times Nf \times PonpCn \times Rtn - dr \times PonpCnRtn + ac \times Nf \times PonpRtn \times Cn - dc \times PonpCnRtn + nl \times PopCRt - ne \times PonpCnRtn - hto \times PonpCnRtn$$

$$\frac{dPtnpCnRtn}{dt} = ar \times Nf \times PtnpCn \times Rtn - dr \times PtnpCnRtn + ac \times Nf \times PtnpRtn \times Cn - dc \times PtnpCnRtn + nl \times PtpCRt - ne \times PtnpCnRtn - ht \times PtnpCnRtn$$

$$\frac{dPonppCnRon}{dt} = ar \times Nf \times PonppCn \times Ron - dr \times PonppCnRon + ac \times Nf \times PonppRon \times Cn - dc \times PonppCnRon - ne \times PonppCnRon + hto \times PonpCnRon$$

$$\frac{dPtnppCnRon}{dt} = ar \times NfPtnppCn \times Ron - dr \times PtnppCnRon + ac \times Nf \times PtnppRon \times Cn - dc \times PtnppCnRon - ne \times PtnppCnRon + ht \times PtnpCnRon$$

$$\frac{dPonppCnRtn}{dt} = ar \times Nf \times PonppCn \times Rtn - dr \times PonppCnRtn + ac \times Nf \times PonppRtn \times Cn - dc \times PonppCnRtn - ne \times PonppCnRtn + hto \times PonpCnRtn$$

$$\frac{dPtnppCnRtn}{dt} = ar \times Nf \times PtnppCn \times Rtn - dr \times PtnppCnRtn + ac \times Nf \times PtnppRtn \times Cn - dc \times PtnppCnRtn - ne \times PtnppCnRtn + ht \times PtnpCnRtn$$

$$\frac{dRon}{dt} = -ar \times Nf \times Ron \times Ponp - ar \times Nf \times Ron \times Ponpp - ar \times Nf \times Ron \times PonpCn - ar \times Nf \times Ron \times PonppCn + dr \times PonpRon + dr \times PonppRon + dr \times PonpCnRo + dr \times PonppCnRon - ar \times Nf \times Ron \times Ptnp - ar \times Nf \times Ron \times Ptnpp - ar \times Nf \times Ron \times PtnpCn - ar \times Nf \times Ron \times PtnppCn + dr \times PtnpRon + dr \times PtnppRon + dr \times PtnpCnRon + dr \times PtnppCnRtn - uro \times Ron$$

$$\frac{dRtn}{dt} = -ar \times Nf \times Rtn \times Ponp - ar \times Nf \times Rtn \times Ponpp - ar \times Nf \times Rtn \times PonpCn - ar \times Nf \times Rtn \times PonppCn + dr \times PonpRtn + dr \times PonppRtn + dr \times PonpCnRtn + dr \times PonppCnRtn - ar \times Nf \times Rtn \times Ptnp - ar \times Nf \times Rtn \times Ptnpp - ar \times Nf \times Rtn \times PtnpCn - ar \times Nf \times Rtn \times PtnppCn + dr \times PtnpRtn + dr \times PtnppRtn + dr \times PtnpCnRtn + dr \times PtnppCnRtn - urt \times Rtn$$

$$\frac{dCn}{dt} = -ac \times Nf \times\times Cn \times Ponp - ac \times Nf \times Cn \times Ponpp - ac \times Nf \times Cn \times PonpRon - ac \times Nf \times Cn \times PonppRon + dc \times PonpCn + dc \times PonppCn + dc \times PonpCnRon + dc \times PonppCnRon - ac \times Nf \times Cn \times Ptnp - ac \times Nf \times Cn \times Ptnpp - ac \times Nf \times Cn \times PtnpRon - ac \times Nf \times Cn \times PtnppRon + dc \times PtnpCn + dc \times PtnppCn + dc \times PtnpCnRon + dc \times PtnppCnRon - ac \times Nf \times Cn \times \mathrm{PonpRtn} - ac \times Nf \times Cn \times PonppRtn + dc \times PonpCnRtn + dc \times PonppCnRtn - ac \times Nf \times Cn \times PtnpRtn - ac \times Nf \times PtnppRtn + dc \times PtnpCnRtn + dc \times PtnppCnRtn + up \times PonpCn + up \times PonppCn + up \times PtnpCn + up \times PtnppCn$$

$$C = Ct - (PoC + PtC + PopC + PtpC + PoppC + PtppC + PopCRo + PopCRt + PtpCRo + PtpCRt + PoppCRo + PoppCRt + PtppCRo + PtppCRt + PonpCn + PtnpCn + PonppCn + PtnppCn + PonpCnRon + PonpCnRtn + PtnpCnRon + PtnpCnRtn + PonppCnRon + PonppCnRtn + PtnppCnRon + PtnppCnRtn + Cn)$$

$$Rn = (Ron + PonpRon + PonppRon + PonpCnRon + PonppCnRon + PtnpRon + PtnppRon + PtnpCnRon + PtnppCnRon + Rtn + PonpRtn + PonppRtn + PonpCnRtn + PonppCnRtn + PtnpRtn + PtnppRtn + PtnpCnRtn + PtnppCnRtn$$

Table A.1 Kinetic Parameters Optimized by GAMS Solver

Symbol	Explanation	1/Ms
tlp	Translation of PER1 andPER2	0.60
tlr	Translation of CRY1 and CRY2	0.26
tlRv	Translation of REV-ERBα	2.53
up	Degradation of phosphorylated PER unbound to CRY	0.96
upu	Degradation of unphosphorylated PER unbound to CRY	0.49
hot	Initial phosphorylation of PER2	5
uro	Degradation of CRY1 unbound to PER	0.28
urt	Degradation of CRY2 unbound to	0.26
ac	Binding of PER1 and PER2 to kinases	2.22
dc	Unbinding of PER1 and PER2 to kinases	5.09
ar	Binding of PER1 and PER2 to CRY1 and CRY2	0.0003
dr	Unbinding of PER1 and PER2 to CRY1 and CRY2	3.08
nl	Nuclear localization of initially phosphorylated PER and bound proteins	0.75
ne	Nuclear export of PER bound Proteins	0.75
hoo	Initial phosphorylation of PER1	0.29
hto	Phosphorylation that stops nuclear localization of PER1 and bound proteins	1.45
ht	Phosphorylation that stops nuclear localization of PER2 and bound proteins	2.53
Ct	Total kinase concentration	7.71
bin	Binding of CRY to CLK:BMAL1 in nucleus	20
unbin	Unbinding of CRY to CLK:BMAL1 in nucleus	0.02

trPo	Transcription of PER1	1558.73
trPt	Transcription of PER2	9184.56
trRo	Transcription of CRY1	30.44
trRt	Transcription of CRY2	14.21
trRv	Transcription of REV-ERBα	187.08
arv	Dimerization of REV-ERBα	0.21
drv	Undimerization of REV-ERBα	3.62
binRv	Normalized binding of nuclear REV-ERBα to RORE element	1.50
unbinRv	Normalized unbinding of nuclear REV-ERBα to RORE element	0.40
uRv	Degradation of REV-ERBα	16.25
umPo	Degradation of PER1 mRNA	0.15
umPt	Degradation of PER2 mRNA	0.15
umR	Degradation of CRY1,2 mRNA	0.41
umRv	Degradation of REV-ERBα mRNA	0.09
L	Strength of light stimulus	0.0003
Nf	Ratio of nuclear to cytoplasmic compartment volume	115.76
trcD1	Transcription of DEC1	4.79
trcD2	Transcription of DEC2	0.05
degD1	Degredation of DEC1 protein	19.41
degD2	Degredation of DEC2 protein	0.77
degMD	Degredation of DEC mRNA	0.25
trlD1	Translation of DEC1	2
trlD2	Translation of DEC2	2.01
box	Binding of DEC proteins to E-Box	0.0001
B	Binding of DEC proteins to BMAL1	0.0001

Table A.2 Boundary Conditions

G_0.fx('1')	0.836
GRv_0.fx('1')	0.1
MRo_0.fx('1')	2
MRt_0.fx('1')	1.1
MPo_0.fx('1')	1
MPt_0.fx('1')	4.4
MRv_0.fx('1')	1
Rv_0.fx('1')	1
Rvn_0.fx('1')	0
RvRv_0.fx('1')	0
RvnRvn_0.fx('1')	0
Po_0.fx('1')	0.5
Pt_0.fx('1')	1.5
PoC_0.fx('1')	0
PtC_0.fx('1')	0
PopC_0.fx('1')	0
PtpC_0.fx('1')	0
Pop_0.fx('1')	0
Ptp_0.fx('1')	0
PoppC_0.fx('1')	0
PtppC_0.fx('1')	0
PopRo_0.fx('1')	0
PtpRo_0.fx('1')	0
PopRt_0.fx('1')	0
PtpRt_0.fx('1')	0
PoppRo_0.fx('1')	0
PoppRt_0.fx('1')	0
PtppRo_0.fx('1')	0
PtppRt_0.fx('1')	0
Popp_0.fx('1')	0
Ptpp_0.fx('1')	0
PopCRo_0.fx('1')	0
PtpCRo_0.fx('1')	0
PopCRt_0.fx('1')	0
PtpCRt_0.fx('1')	0

PoppCRo_0.fx('1')	0
PtppCRo_0.fx('1')	0
PoppCRt_0.fx('1')	0
PtppCRt_0.fx('1')	0
Ro_0.fx('1')	0.3
Rt_0.fx('1')	1
PonpCn_0.fx('1')	0
PtnpCn_0.fx('1')	0
Ponp_0.fx('1')	0
Ptnp_0.fx('1')	0
PonppCn_0.fx('1')	0
PtnppCn_0.fx('1')	0
PonpRon_0.fx('1')	0
PtnpRon_0.fx('1')	0
PonpRtn_0.fx('1')	0
PtnpRtn_0.fx('1')	0
PonppRon_0.fx('1')	0
PtnppRon_0.fx('1')	0
PonppRtn_0.fx('1')	0
PtnppRtn_0.fx('1')	0
Ponpp_0.fx('1')	0
Ptnpp_0.fx('1')	0
PonpCnRon_0.fx('1')	0
PtnpCnRon_0.fx('1')	0
PonpCnRtn_0.fx('1')	0
PtnpCnRtn_0.fx('1')	0
PtnppCnRtn_0.fx('1')	0
Ron_0.fx('1')	0
Rtn_0.fx('1')	0
Cn_0.fx('1')	0.18
GD_0.fx('1')	0.05

Table A.3 RT-PCR Values of Circadian Clock mRNAs

Time	mPeriod1	mPeriod2	mCryptchrome1	mCryptochrome2
4	1.7	8	8	4.3
8	3.2	17	13.3	6.8
12	4.2	27	14.6	8
16	2.8	23	9.5	6
20	1.65	13	4	4.1
24	1.3	7	5.2	3

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