Modeling and Analysis of the Impacts of Jet Lag on Circadian Clock and its role in Cancer

By

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Dedicated

To my parents

Declaration

I, Azka Hassan, declare that this thesis entitled as "Modeling and Analysis of the Impacts of Jet Lag on Circadian Clock and its role in Cancer" submitted to Department of Computational Sciences, RCMS NUST, consists of original research work. Work taken from other studies has been duly cited. None of the work presented has been submitted either in this institute or any other degree awarding institution for any other degree or qualification. Although a journal article for this work has been submitted.

Azka Hassan

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Azka Hassan

Contents

List of Abbreviations

BMAL1 Brain and Muscle ARNT-Like 1 protein

BRN Biological regulatory network

CLOCK Circadian Locomoter Output Cycles protein Kaput

c-Myc Proto-Oncogene

CRYs Cryptochrome proteins

CTL Computational Tree Logic

DNA Deoxyribonucleic acid

LTL Linear temporal Logic

MYC Oncogenic Protein

PERs Period proteins

PN Petri Net

REV-ERBs Orphan nuclear receptor subfamily 1 group D

RORs Nuclear receptor subfamily 1 group F Retinoid-related Orphan Receptor

SCN Suprachiasmatic nucleus

SMBioNet Selection of Models of Biological Networks

SPN Standard Petri Net

THPN Timed Hybrid Petri Net

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Abstract

Circadian rhythms maintain a 24 hour oscillation pattern in metabolic, physiological and behavioral processes in all living organisms. Circadian rhythms are organized as biochemical networks located in hypothalamus and peripheral tissues. Rhythmicity in the expression of circadian clock genes plays a vital role in regulating the process of cell division and DNA damage control. The oncogenic protein, MYC and the tumor suppressor, p53 are directly influenced by the circadian clock. Jet lag and altered sleep/wake schedules prominently affect the expression of molecular clock genes. This study is focused on developing a Petri Net model to analyze the impacts of long term jet lag on the circadian clock and its probable role in tumor progression. The results depict that jet lag disrupts the normal rhythmic behavior and expression of the circadian clock proteins. This disruption leads to persistent expression of MYC and suppressed expression of p53. Thus, it can be inferred that jet lag altered circadian clock negatively affects the expression of cell cycle regulatory genes causing uncontrolled proliferation of tumor cells.

Chapter 1

Introduction

1.1 Background

The environment around us is under constant change. Some of these changes are unique but some repeat in a timely manner for example day and night, seasonal changes etc. To accommodate these diverse changes, an organism needs to be able to adapt and perform according to it in a rhythmic manner. This results in evolving a time keeping mechanism in organisms which allows them to habituate the external environmental changes. With every new day, human body resets all the biological and physiological activities for the daily routine and each process starts to oscillate in a timely manner [1]. This endogenous oscillation has been termed as "circadian rhythm", derived from a latin phrase meaning "about a day" [2–4]. Circadian oscillations are self-supported and endogenous time following systems. They oscillate in accordance with a 24 hours routine that enables organisms to survive environmental changes, thereby familiarizing their activities to the appropriate time of day [3–6]. Circadian oscillations require entrainment by the external environment without which they would dissociate from the natural cycles [2]. One of the most powerful stimulus is the light/dark cycle which not only regulates the sleep/wake cycle but

also controls other hormonal secretions and metabolic processes [2, 6, 7].

1.2 The Circadian Clock

Several studies [2, 8–12] have divided circadian clock into central and peripheral domains.

The master clock called Suprachiasmatic nucleus (SCN) is located in the anterior hypothalamus and orchestrates multiple autonomous oscillators in peripheral organs. SCN is a paired structure, where each part contains approximately 10,000 neurons, coupled together and oscillating in a coherent manner. SCN receives visual signals as external stimuli and other non photic signals through different hormonal and neuronal tracts. SCN as master clock or synchronizer has a duty to transmit timekeeping signals to other parts of the body [2, 8, 10].

Rhythmicity has been observed in the functions of organs, tissues and in the expressions of proteins and genes. This proves that apart of SCN, other organs, tissues and even cells persists an endogenous circadian oscillators. These are known as peripheral clocks. Peripheral clocks are present in organs like liver, kidney, pancreas, thyroid gland [9] etc. This autonomous clock has been found to be omnipresent maintaining a rhythmicity in its functions. Different experimental studies [11] have shown that each cell responds variably to entraining signals and controls different physiological outputs. The mitotic and gating activities during cell division also follow a rhythmic oscillatory manner.

1.3 Organization of Circadian Clock

To work effectively, biological clocks must work in a synchronized manner and adjust their timings according to the external cues. For this SCN is required to signal and regulate peripheral circadian clock. Any dis-

ruption in this will result in desynchronized rhythms. SCN uses a number of pathways to generate coherence between the core and peripheral clock. The peripheral clocks are entrained through different signaling mechanisms which involves circulating hormones, metabolites and neuronal signals [9, 13, 14]. However, there are a number of other external factors like daily feeding/fasting routine and temperature responsible for the entrainment [12]. Rhythmic oscillation of circadian clock regulate metabolic processes integrating these systems in a light independent manner. This regulation occurs at central and as well as peripheral level.

1.4 Problem Statement

Jet lag generates a circadian desynchrony in the human body. This disruption of circadian clock leads to abnormal proliferation of cells thus making the body prone to tumor.

1.5 Theme and structure of the study

After a comprehensive literature review describing the molecular mechanism of the Clock, connection to tumor proliferation and involvement of jet lag in circadian disruption, different computational techniques that were applied during this study have been discussed. Furthermore, the results obtained have been analyzed and discussed thoroughly followed by a summary concluding the study.

1.6 Aims and Objectives

The key objectives of this study are:

- Construction of a Biological Regulatory Network of the abstracted pathway of circadian clock and proteins involve in cell cycle.

- Construction of a Qualitative Model.
- Construction of a Petri Net Model.
- Analysis of results to study the impacts of jet lag on the circadian clock, effecting cell division cycle.

Chapter 2

Literature Review

2.1 Molecular Mechanism of Circadian Clock

At the molecular level, circadian clock mechanism in both core and the peripheral clocks is known to be analogous. This mechanism comprises of a complex system of translational and transcriptional feedback loops that oscillate in a 24 hour manner [15–17]. The mechanism revolves around two coupled protein complexes, the first one comprises of CLOCK (Circadian Locomotor Output Cycles protein Kaput) along with BMAL1 (Brain and Muscle ARNT like receptor 1) and the second consists of PER (Period) proteins with CRY (Cryptochrome) proteins. CLOCK-BMAL1 complex plays a part as positive limb i.e. as an activator and the second complex PER-CRY acts as the negative limb of the cycle i.e. as an inhibitor of the CLOCK-BMAL1 complex. This cycle works in such a manner that CLOCK activates the transcription of BMAL1 and then they heterodimerize leading to the formation of CLOCK-BMAL1 complex. This complex then activates the transcription of several genes out of which the two most important are *Pers* and *Crys*. PER and CRY proteins then heterodimerize and exert a negative impact on the CLOCK-BMAL1 complex thus suppressing its function and indirectly their own transcription. An additional

linked negative feedback loop consisting of transcriptional activation receptors, REV-ERBs and RORs, gets activated by CLOCK-BMAL1 complex. They work in a feedback loop where RORs regulate *Bmal1* transcription where REV-ERBs inhibit it. A pictorial example of the whole mechanism is shown in Figure 2.1. Every cycle takes about 24 hours, enabling the clock to regulate other metabolic and proteomic processes at specific times of the day [2, 17–20].

2.2 Cell Cycle and Repair Pathways

Another essential oscillatory system in our body is the cell cycle which allows a cell to efficiently and safely "reproduce". This cycle is divided into four main phases G1, G2, S and M, after which the cell splits into two daughter cells. Each cycle starts with the daughter cell going through different phases of enlargement and replication of DNA. The cycle starts with phase G1 in which the cell becomes larger, followed by the Synthesis phase (S phase) in which DNA replicates. In the next phase G2, the cell goes into rest and prepares itself for mitosis. The earlier stages in which the cell is being prepared for mitosis are known as "interphase". After the cell has been prepared, it enters the mitotic phase which further consists of four sub phases. This process involves condensation of chromosomes followed by their alignment on microtubules after which they are separated and cytokinesis occurs, thus dividing the cell into two daughter cells and the next cycle starts. Along with molecular checkpoints which regulate the progression of a cell through the cycle, there is another system of checkpoints which is interlinked and is activated upon identification of any DNA damage. DNA repair pathways ensures the activation of repair pathways in order to eliminate damages and prevent it from being passed onto the daughter cells [21, 22]. Two main proteins involved in proliferation of a cell are p53 and MYC. They play a vital role in cell cycle regulation, as

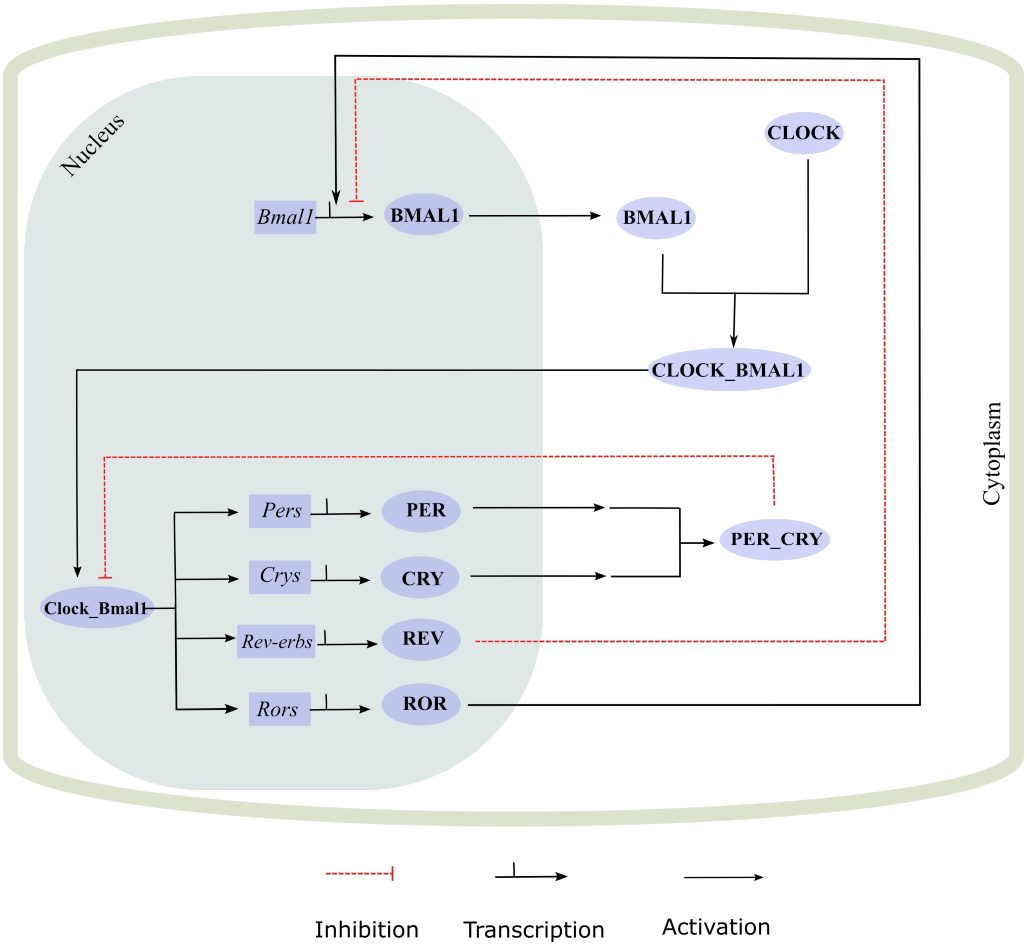

Figure 2.1: A simplified representation of molecular machinery of the circadian clock. The circadian clock consists of positive and negative interconnecting transcription/translational feedback loops controlling circadian timings. *Bmal1* transcription is initiated by CLOCK protein and both heterodimerize to form a complex . The CLOCK-BMAL1 complex (positive regulators) serves as the activator and prompts the transcription of *Per* and *Cry* genes. PER and CRY proteins then heterodimerize forming a PER-CRY complex. This complex inhibits the CLOCK-BMAL1 function thus leading to suppression of their own transcription. CLOCK-BMAL1 also controls the regulation of the nuclear receptors *RORs* and *Rev-erbs*, which help in regulating *Bmal1*'s transcription.

MYC is involved in G1 to S transition and p53 plays an important role as a tumor suppressor and is involved in G1-S checkpoint [23], inhibiting the progression of any damaged cell through the cycle [24].

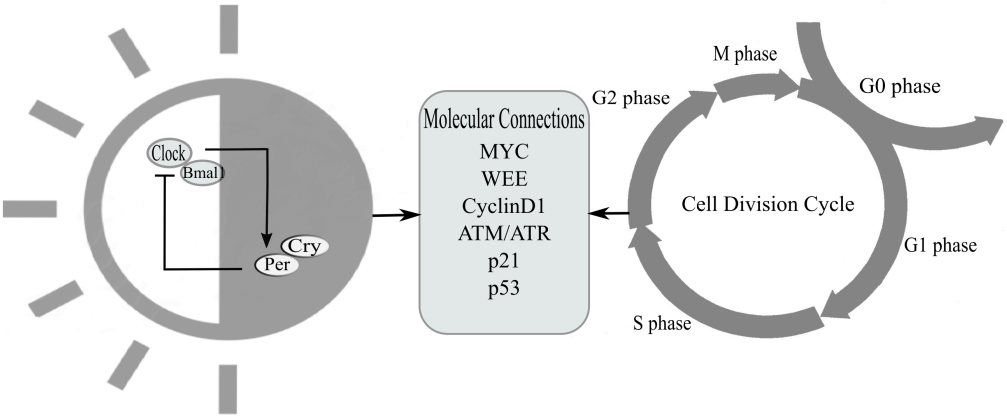

Figure 2.2: The figure represents proteins that are coupled between cell cycle and circadian clock. The circadian system is linked to the cell-division cycle through regulation of gene expression and post-translational mechanisms. Several cell cycle genes that regulate cell proliferation, such as WEE1, MYC and CYCLIN D1 and genes that are involved in different cell division checkpoints for initiating DNA repair pathways such as p21, p53 and ATM/ATR, are under circadian influence.

2.3 Coupling between Circadian Clock and Cell Cycle

Circadian clock and cell cycle are global oscillatory systems found in almost all the organisms and are interlinked with each other due to different molecular similarities [25]. Circadian Clock regulates cell cycle, this was first reported in the study by Franz Halberg [26]. Other experimental studies reporting that circadian genes are involved in the regulation of cell division are Greene [2], Savvidis and Koutsilieris [3], Masri et al. [5], Reddy

et al. [19], Rana and Mahmood [27], Li et al. [28]. It has been observed that CLOCK-BMAL1 complex can directly regulate cell cycle genes through E-box mediated reactions which is the binding site for CLOCK-BMAL1 complex and a number of cell cycle genes contain E-boxes in their promoter regions [27, 29]. Genes under direct influence of circadian clock involves *Wee1, Myc, Cyclin D1, p53* (see Figure 2.2). They play an important role at different checkpoints of cell division and are involved in cell proliferation and damage control [24, 29, 30].

Myc and p53 proteins playing a vital role in proliferation of a cell, are also under direct influence of the circadian clock. They usually appear to be ablated in cancer cells. According to the study of Fu et al. [24] CLOCK-BMAL1 complex directly inhibits the transcription of *c-Myc*. Disruption in PER2 reduces the level of BMAL1 thus reducing CLOCK-BMAL1 complex which directly effects the inhibition of Myc by the complex. Different studies [23, 31], states that circadian clock is one of the regulators of p53 tumor suppressor pathway. Oncogenic transformation initiated by MYC must be controlled [32] in which p53-mediated apoptosis plays an important part [33, 34]. Alteration of normal cells into tumors is facilitated by MYC which must be overcome by p53 through its apoptotic action [32–34].

2.4 Circadian Disruption due to Jet lag and its role in Cancer

One of the major life style changes involve abnormalities in sleep/wake cycle due to night shifts and frequent traveling, which generate the effect of jet lag [3]. Jet lag is known as circadian desynchrony, a sleep disorder caused by traveling across several time zones resulting in misalignment between internal circadian clock and the destination's local time. Inability of an individual to adapt to a sudden shift in these synchronizers causes

a desynchronization between the body and the external environment. Its severity depends on several variables, including the direction of travel and the time zones crossed. Studies [23, 35, 36] show that long term frequent time zone changes cause physiological and psychological health issues. Shift work also produces a similar effect as jet lag due to irregular sleep/wake routine and exposure to light at unusual times. Disruption of circadian rhythms due to these is associated with various forms of cancer in humans. Epidemiological studies [37–42] report that there is an increased incidence of cancers in pilots and flight attendants due to frequent transmeridian flights. These studies suggest that the consistent and long term changes light/dark and sleep/wake cycle lead to the disruption of circadian clock which can make body tumor prone. Similarly, people working on night shifts have a significantly greater risk of developing breast, colon, endometrial, prostate cancer and non-Hodgkin lymphoma. These studies [3, 6, 43–46] prove that chronic circadian rhythm disruption plays an important role in making the body cancer prone. A number of experimental studies [6, 23, 24, 47] on animal models proves jet lag disruption of circadian rhythms. This disruption facilitates tumor growth as circadian clock directly regulates cellular proliferation and repair. Cell division cycle due to disrupted rhythms prove to be less efficient is DNA repair and proliferate abnormally resulting in tumor (see Figure 2.3).

2.5 Computational Analysis on Clock disruption and Cancer

A system-level understanding of a biological network can give us various important insights regarding the system. Studying a network of gene interactions and biochemical pathways can help in understanding a system's behavior over time under various conditions. It can also help to determine

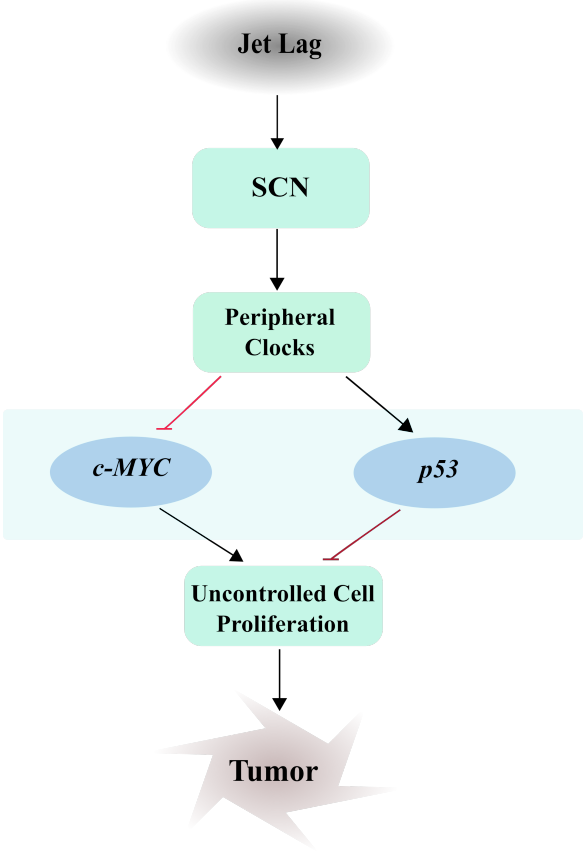

Figure 2.3: Jet lag disturbs sleep/wake and light/dark cycles which causes severe disruption in circadian clock leading to uncontrolled proliferation. Black solid line indicates the activation and red solid line represents inhibition.

the control mechanisms which can be used to minimize the malfunctions and can also provide potential therapeutic targets for the treatment of disease [48]. The basic understanding of the link between Circadian clock disruption, cell cycle proliferation and cancer has been established in several studies [6, 23]. But these studies are based on wet-lab experiments. In this study, computational systems biology techniques have been employed to study the mechanism of disruption in circadian rhythms supporting tumor growth. The system has been modeled in its abstracted form while preserving the wet-lab recognized behavior of the entities involved. Analysis of the resulting simulation graphs have been performed to validate the

required behavior of the circadian system and the role of its disruptions in tumor progression.

2.6 Our Contribution

To understand the mechanism and the oscillatory behavior of circadian clock, several models [4, 49–52], utilizing mathematical modeling technique and graph based modeling approach have been constructed. These models provide insight to help us understand the feedback mechanism and oscillatory behavior of the clock machinery. However the link between circadian clock and jet lag has not been modeled yet. In this study we have used graph based modeling technique to model the interaction between circadian clock and vital proteins myc and p53, involved in cellular proliferation. We have observed and analyzed the behavioral changes in the oscillations of circadian clock due to jet lag and the negative impacts of these changes on cellular proliferation causing tumor growth. This model can provide a better understanding of the system which can be useful in chronotherapies in future.

Chapter 3

Methodology

In this section the methodology employed in this study has been discussed. A schematic work flow of this methodology framework has been shown in figure 3.1.

3.1 René Thomas' Logic Formalism

In the late 1970s, logical formalism was presented by René Thomas [53]. This graph based formalism has its benefits over other boolean formalisms because of its ability to allow interaction threshold levels above "1". It is similar to differential equation based models but keeps the system less complex [53]. Moreover, this allows asynchronous dynamics to be modeled thus involving cyclic trajectories which are not present in the synchronous boolean formalism [54, 55]. Thomas' formalism relies on a modified graph theory, termed as the biological regulatory networks (BRNs). Components of BRN include entities and the interactions in between them. The concentration of an entity is shown by discrete levels and interactions are threshold dependent i.e. once the threshold is attained interaction takes place (see Figure 3.2). From different studies [56–59], the components and semantics of the formalism have been adapted which have been defined and explained.

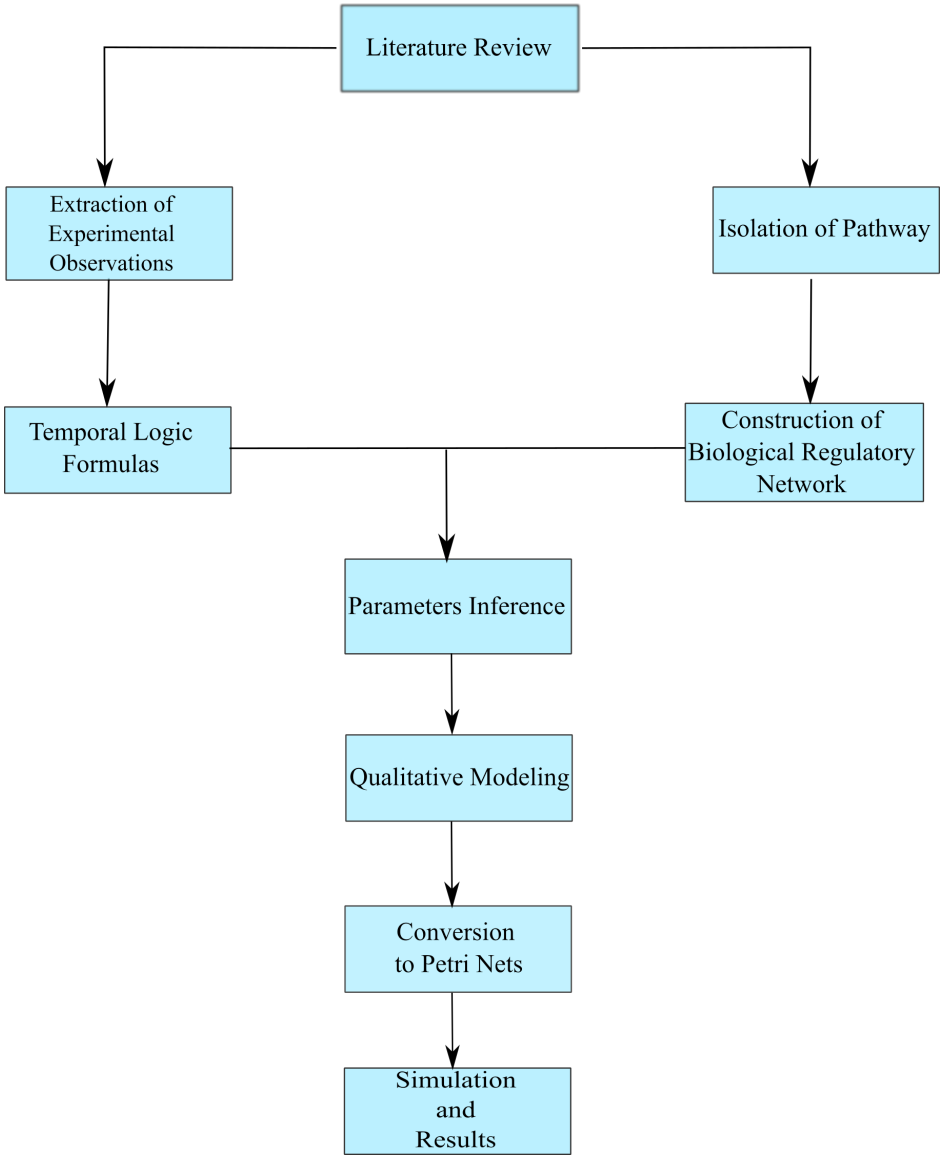

Figure 3.1: Work flow of the methodology employed in this study. Literature search was followed by network abstraction and BRN construction. Afterwards, the BRN along with the logical parameters was used for qualitative modeling. Finally, a Petri Net model was generated from the qualitative model.

Definition 1 (Biological Regulatory Network (BRN)): *A graph G = (V,E) is a biological regulatory network when,*

- *V represents sets of all vertices's,*
- *E represents ordered pair of edges defined as* $E \subseteq V \times V$,

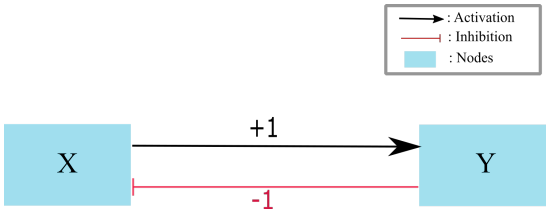

Figure 3.2: A BRN with two entities X and Y, where X is activating Y, shown by an edge labeled with +1 and Y inhibiting X, shown by an edge labeled -1.

All the edges are labeled according to the level and type of interaction (see Figure 3.2). Dynamics of the trajectories are determined by the state graph generated by BRN. Resource of an entity depends on the presence and absence of its activator or inhibitor. Each state determines the level of an entity evolving in the state space. A state space defines all the possible configurations of an entity represented by a state graph. State graph is generated against a particular set of logical parameters determining the behavior of entities in that particular state. Logical parameters are also known as K parameters [56–59].

3.1.1 Software utilized for BRN Construction

For BRN construction, software GINsim [60] was used. It implements the kinetic logical formalism through discrete automata. Two main types of graphs are generated with the help of GINsim. Logical Regulatory Graphs, which model regulatory networks and State Transition Graphs, which represent the dynamical behavior of entities.

3.2 Model Checking Approach for the Isolation of Parameters

Logical parameters are dependent on wet-lab experiments/observations. They help us understand the dynamics of a BRN. A new technique for the generation of parameters was mentioned in recent studies [61]. This technique known as model checking helps to generate desired logical parameter sets through the application of formal methods. To check whether a property is verified against a state space, model checking approach is applied. It is an exhaustive computational methodology that helps us in complete verification of the system against a set of required properties [62]. Temporal logic is used to verify properties in Model checking techniques. It can either be Linear-time Temporal Logic (LTL) or Computational Tree Logic (CTL). As CTL can cater the branching state space systems therefore it is preferred for biological networks. Wet-lab observations are encoded in CTL and then are verified against the BRN. State graphs are generated by all possible linear combinations of logical parameters. Only those parameter sets are selected which satisfy the CTL formula [63]. CTL formula involves path quantifiers and state quantifiers to represent properties of the system. The formula also supports complex forms like nesting of path-state quantifiers for verification of complex behaviors. These quantifiers are described here:

- Path Quantifiers: The two path quantifiers are $\exists$ and $\forall$, where $\forall$ specifies that the entire paths originating from a current state and $\exists$ states that at least one path initiating from the current state.
- State Quantifiers: The state quantifier '$\Box$' (globally) specifies that all the states along the specified path verify the property. The quantifier '$\Diamond$' (future) specifies that at least one future state along the specified path should hold the given property. The quantifier '$\bigcirc$' (next) speci-

fies the very next successor state(s) of the current state and '$\bigcup$' (until) specifies that the property p_1 holds until property p_2 is verified.

3.2.1 Software used for Model Checking

For the inference of parameters, Selection of Models of Biological Networks (SMBioNet) [61, 64] was utilized. It employs model checking approach to generate parameter sets satisfying the desired properties encoded in the form of CTL logic. The source code provided to SMBioNet consists of variables and interactions involved in the BRN.

3.3 Conversion of BRN to Petri Nets

Petri Nets were developed by Carl Adam Petri in 1962 for the analysis of the concurrent processes occurring in technical systems [65–67]. However, due to its simplicity and flexibility it has been successfully applied in other domains as well, such as chemical reactions, biochemical processes etc. This framework allows us to model discrete, continuous and hybrid systems by using kinetic logic formalism. Petri Nets have already been used for modeling numerous complex regulatory networks and pathways because of their versatility and ability to cater hybrid systems. Different types transcriptional, metabolic and protein-interactions can be modeled together as a single system [68–75].

GINsim allows to export the qualitative model into PN using the method describe by Chaouiya et al. [76]. The exported model is a standard PN model which can be converted into Hybrid PN for detailed analysis [76].

The frameworks, along with required definitions and properties are adapted from [77].

“

Definition 2 (Standard Petri Net): *A standard Petri Net is a tuple* $\langle$ *P, T,*

f, m_0 ⟩ where,

- *P is the finite set of places,*
- *T is the finite set of transitions,*
- *f : (P × T) ∪ (T × P) → $\mathbb{N}_{\geq 0}$ is the application of directed arcs, each having a positive integer weight.*
- *m_0 : P → $\mathbb{N}_{\geq 0}$ is the mapping function which assigns positive integers to the set of places as initial markings.*

Definition 3 (Timed Hybrid Petri Net (THPN)): *A Timed Hybrid Petri Net is a tuple ⟨ P, T, f, h, m_0, tempo ⟩ where,*

- *P is the finite set of places,*
- *T is the finite set of transitions,*
- *f: $(P \times T) \cup (T \times P) \rightarrow \mathbb{R}_{\geq 0}$ is the application of directed arcs, each having a positive integer weight,*
- *h: P ∪ T → {Disc, Cont} is the hybrid function assigning the type 'discrete' (P^D, T^D) or 'continuous' (P^C, T^C) to each vertex,*
- *m_0: P → $\mathbb{R}_{\geq 0}$ is the initial positive real value marking of places,*
- *tempo: T → $\{\mathbb{Q}_{\geq 0} | t \in T^D \cup \mathbb{Q} | t \in T^C\}$. This is an assignment function which assigns delays to discrete transitions and rates to continuous transitions.*

" (Page 9-11, [77]).

Example of a Timed Hybrid Petri Net is shown in Figure 3.3.

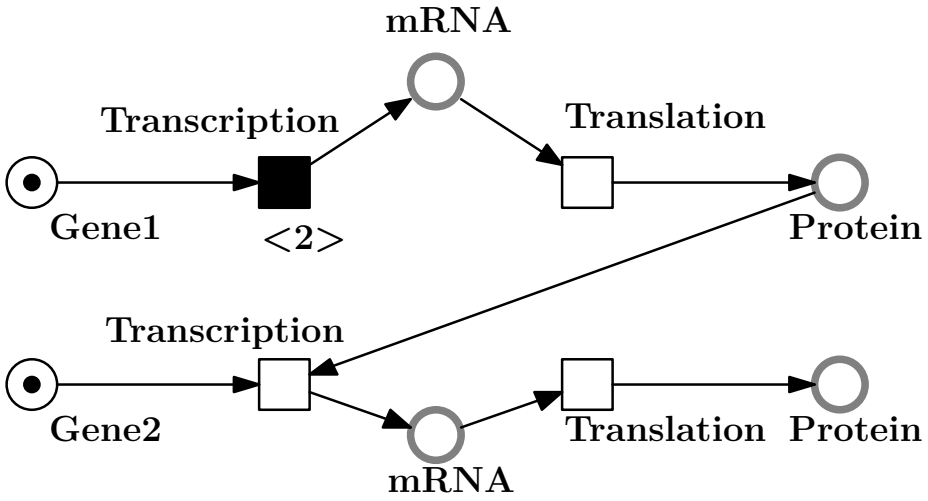

Figure 3.3: An example of Timed Hybrid Petri Net. '○' represents places. '□' represents transitions. Filled transitions are the 'Deterministic transitions', which controls the time delays. This figure demonstrates the central dogma of molecular biology.

3.3.1 Software used for Petri Net Construction:

Snoopy [78] was used in this study for PN construction. This tool utilities a Petri Net (PN) framework and is useful for modeling and it analyzes the system effectively. Models can be hierarchically structured, thus helping in modeling larger systems. It supports different forms of PN involving stochastic, continuous, discrete, hybrid etc.

Chapter 4

Results and Discussion

4.1 Results

This section discuses all the results, including construction of BRN, inference of parameters, conversion to PN and qualitative modeling. As this study focuses on the tumor growth due to disturbed circadian clock, only proteins that were involved in tumor proliferation were studied.

4.1.1 Construction of BRN

The pathway (see Figure 4.1) was reduced to BRN on the basis of a set of rules as mentioned in studies by Naldi et al. [60], Saadatpour et al. [79]. During this reduction it was tried to keep the nature of pathway preserved. In the next step a BRN for the reduced pathway was constructed using GINsim (see Figure 4.2). BRN consists of 6 entities, all the possible interactions and feedback loops. Edges are labeled with activity threshold levels for each entity. Inference of parameters for BRN was done using model checking [57].

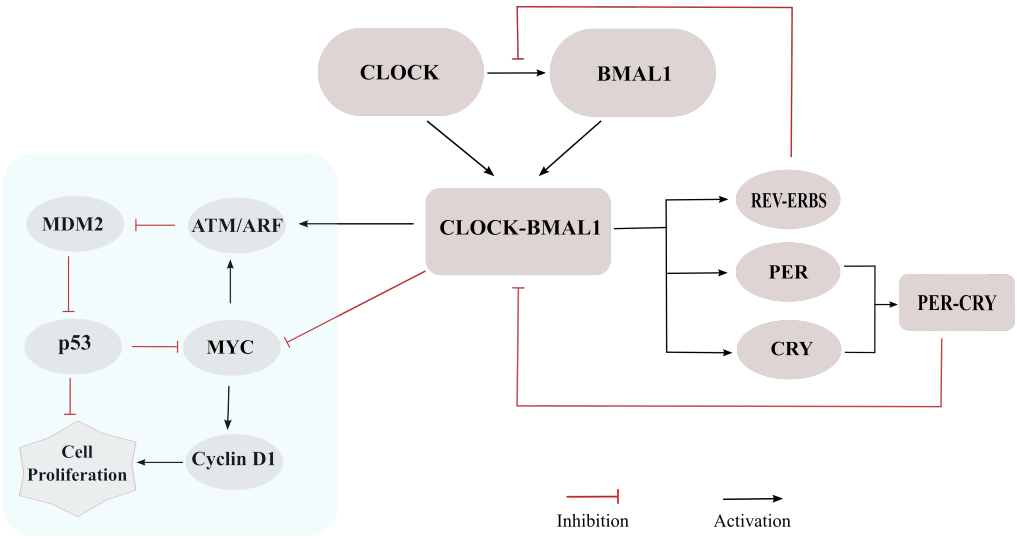

Figure 4.1: Network presented here depicts the linkage between Clock proteins, MYC and p53. Blue colored section show vital proteins involved in normal cell division.

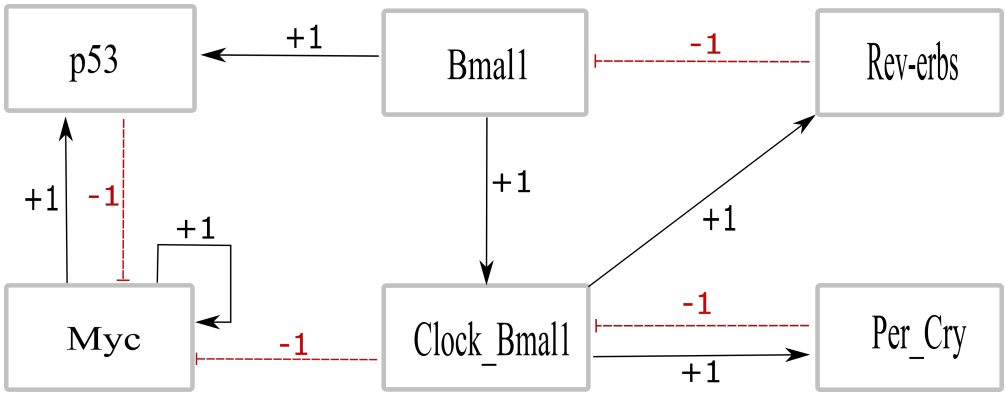

Figure 4.2: The Reduced BRN consists of six entities which involves; the core clock proteins and proteins that are involved in tumor growth i.e. p53 and MYC. There are four inhibitory interactions labeled with -1 and six activation interactions labeled with +1.

4.1.2 Inference of Parameters

For construction of a qualitative model for the BRN shown in Figure 4.2, logical parameters were estimated with the help of SMBioNet. Computational Tree Logic (CTL) was used to specify biological properties of the network. Formulas ψ_1 to ψ_4 were used in conjunction and the parameter sets satisfying these properties were selected. In the following formulas CB represents the CLOCK-BMAL1 complex, PC indicates the PER-CRY complex, R shows REV-ERBs.

$$\psi_1 = Init \Rightarrow \forall\bigcirc(\exists\Diamond(Init)) \tag{4.1}$$

$$\begin{aligned}\psi_2 = {} & \exists\Diamond(Bmal{=}0 \wedge CB = 0 \wedge PC = 0 \wedge R = 0) \Rightarrow \\ & \exists\bigcirc(\exists\Diamond(Bmal = 0 \wedge CB = 0 \wedge PC = 0 \wedge R = 0)) \\ & \wedge ((\exists\Diamond(\exists\Box Myc = 1 \wedge p53 = 0))\end{aligned} \tag{4.2}$$

$$\psi_3 = ((\exists\Diamond p53 = 1) \Rightarrow (\exists\Diamond Myc = 0) \Rightarrow (p53{=}0 \ \cup \ p53 = 1)) \tag{4.3}$$

$$\psi_4 = ((\exists\Diamond(\forall\Box \ Myc = 1)) \Rightarrow (\exists\Diamond(\forall\Box \ p53{=}0))) \tag{4.4}$$

Init (Initial state) in ψ_1 represents (CB=0 $\wedge$ PC=0 $\wedge$ R=0 $\wedge$ Myc=0 $\wedge$ p53=0). It states that all the entities that are initially at level '0' will evolve and come back to the initial state. This verifies the homeostatic nature of the system. ψ_2 states the condition where despite of all the core clock proteins oscillating in a homeostatic manner, MYC starts over expressing and p53 expression is suppressed. The third property ψ_3 states that expression of p53 at its normal level inhibits MYC's over expression. This property also states that the level of p53 expression will be either 0 or 1. ψ_4 states the condition where MYC starts over expressing due to compromised behavior of p53.

Due to the generation of a large number of models (≈ 20,000) by SMBioNet some of the parameter values were restricted based on the rules mentioned in the study of Bernot et al. [57]. The generated models were reduced down to 288 out of which 144 satisfied the CTL formula. Further reduction on the basis of biological observations reduced the number of models to 4. These 4 models differed in the parameter values for K_{CB} and K_{Myc}. Afterwards out of these 4 models a single parameter set was selected on the basis of biological knowledge for further analysis. The parameter sets generated and verified by SMBioNet and the one selected are mentioned in Table 4.1. The SMBioNet input and output code is provided in Supplementary Files 3 and 4 respectively. This parameter set along with BRN was used for the construction of a qualitative model (see Supplementary File 2).

4.1.3 Petri Net Modeling and Analysis

The qualitative model generated in GINsim was converted to a standard discrete Petri Net and finally into a Hybrid Petri Net (see Figure 4.3) using the method described by Chaouiya et al. [80]. Discrete places and transitions were converted into continuous places and transitions respectively. Each entity had two complementary places along with two transitions labeled as p and n, regulating its activation and inhibition respectively. Deterministic transitions were used to control the oscillatory timings of the system in a period of 24 hours. Rates of transitions for each entity were adjusted according to their sequence of their evolution in the system (see Table 4.2). The Petri Net model is provided as Supplementary File 1.

Case 1: Normal

This scenario depicts the usual behavior of circadian clock. Wet-lab experiments state that BMAL1 shows its maximum expression at around 0600hrs, PER-CRY at 1800hrs and REV at 0800hrs, with a repetition in expression

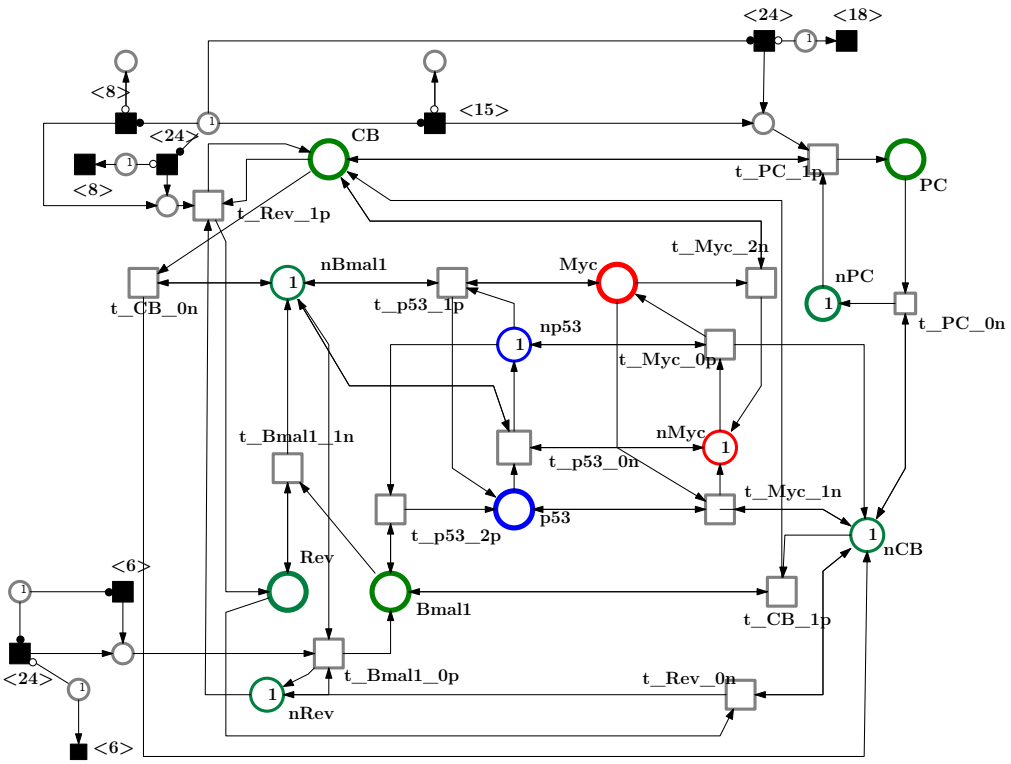

Figure 4.3: Hybrid Peri Net model for the system under consideration. Black filled transitions represent deterministic transitions used to control the oscillatory timings of Circadian clock proteins BMAL1, PER-CRY, CLOCK-BMAL1 and REV. Green '○' represent Circadian Clock proteins, blue '○' depicts p53 and red '○' represent MYC. Each place has a complementary place labeled with 'n' responsible for adding and removing tokens from that place. Transition names ending with 'p' are responsible for activation while transition names ending with 'n' are responsible for inhibition of each entity.

Table 4.1: Parameters, resource sets, parameter values provided to SMBioNet, parameter values generated by SMBioNet and finally the values selected for modeling.

Parameters	Resources	Values		
		Allowed	Generated	Selected
K_{Bmal}				
	{}	0	0	0
	{Rev}	1	1	1
K_{CB}				
	{}	0	0	0
	{Bmal}	0,1	0,1	1
	{PC}	0	0	0
	{Bmal,PC}	1	1	1
K_{PC}				
	{}	0	0	0
	{CB}	1	1	1
K_{REV}				
	{}	0	0	0
	{CB}	1	1	1
K_{p53}				
	{}	0	0	0
	{Bmal}	1	1	1
	{Myc}	1	1	1
	{Bmal,Myc}	1	1	1
K_{Myc}				
	{}	0	0	0
	{p53}	0	0	0
	{CB}	0	0	0
	{Myc}	0,1	0,1	0
	{p53,CB}	0,1	0,1	1
	{Myc,p53}	0	0	0
	{Myc,CB}	0	0	0
	{Myc,CB,p53}	1	1	1

after every 24 hours [81]. These clock entities are showing oscillations at approximately the same timings in the simulation results shown in Figure 4.4. Both the complexes CLOCK-BMAL1 and PER-CRY show oscillations in an opposite manner as the later suppresses the former. Same behavior is observed for REV-ERBs and BMAL1 proteins. MYC and p53 are known to express themselves in an opposite manner as p53 plays an important role in preventing MYC oncogenic expression [23, 82]. Expression patterns

Table 4.2: Table showing the rates used for continuous transitions. For each entity two types of transitions (p and n) are present, referred to as activation and inhibition, respectively.

Entities	**Normal**		**Mild**		**Chronic**	
	Activation	Inhibition	Activation	Inhibition	Activation	Inhibition
BMAL1	1	1	0.50	1.3	0.15	2
CLOCK-BMAL1	1	1	1	1	1	1
PER-CRY	0.97	1	0.40	1.3	0.20	2
REV	0.97	1	0.40	1	0.20	1
Myc	1	1	1	1	1	1
p53	1	1	1	1	1	1

of these proteins in the simulation results imitate this behavior (see Figure 4.4).

Case 2: Mild

Altered sleep/wake schedule due to traveling etc. can lead to mild jet lag effect which can cause disruptions in our circadian clock. In the model, rates of clock proteins were altered to generate a mild jet lag effect. These rates are shown in 'Mild' column of Table 4.2. Altered expression of circadian clock proteins consequently disturbed the MYC and p53 expression as shown in the simulation results (see Figure 4.5). The expression of p53 was suppressed along with reduced inhibition of MYC. This disruption is mild and readjustment of the circadian clock through proper meal timings and sleep/wake routine [43] might normalize MYC and p53 levels.

Case 3: Chronic

Chronic disruptions in the circadian clock can be due to long term jet lag and night shift work. Jet lag produces negative impacts on each clock protein by dampening its expression levels leading to disturbed expression pattern of MYC and p53 [43]. Rates of the clock proteins in the Petri Net model were changed to produce the effect of severe jet lag, as mentioned in column "Chronic" of Table 4.2. Simulation results shown in Figure 4.6

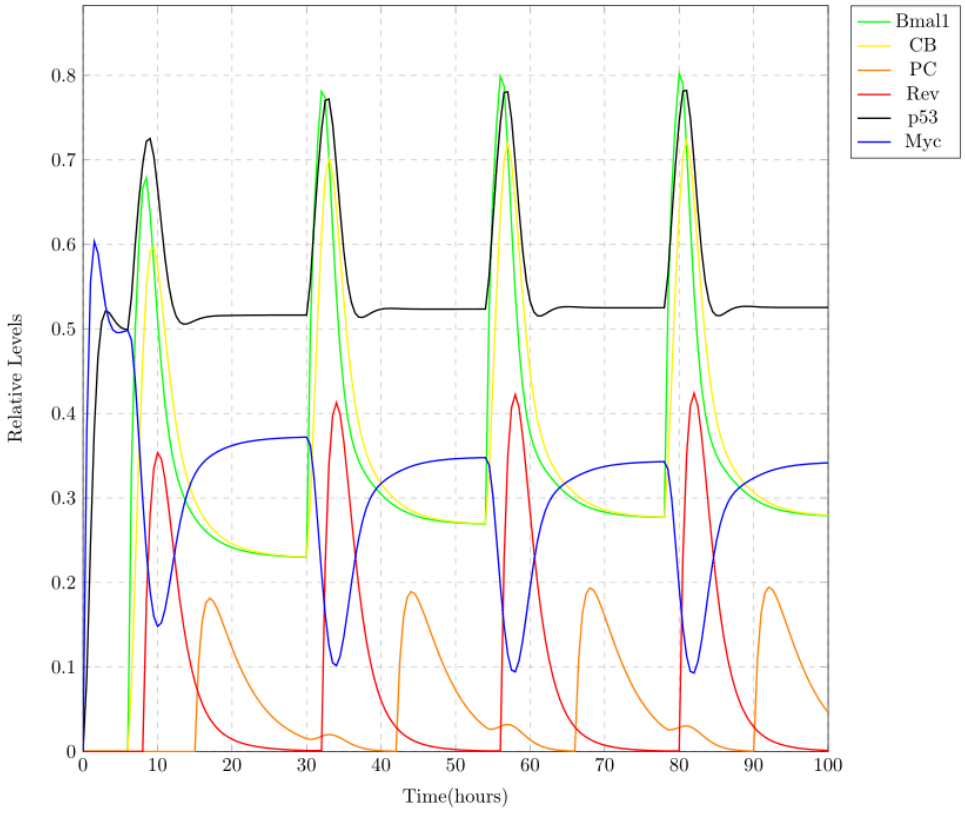

Figure 4.4: Simulation results depicting the normal oscillatory behavior of circadian clock proteins with each clock protein oscillating in a 24 hour periodic manner. The core clock proteins are directly influencing the expression of MYC and p53. p53 and MYC are showing opposite behavior i.e. with the increase in one of them, the other decreases and vice versa.

depict that under the effect of chronic jet lag, the circadian proteins suffer from a disruption in their expression levels. Due to this disturbance, p53 shows a suppressed pattern of expression and MYC starts expressing itself persistently (see Figure 4.6).

4.1.4 Comparison of Simulations

Comparison of the three cases described in section gives a clear picture of circadian disruptions effect on p53 and MYC. It can clearly be seen in

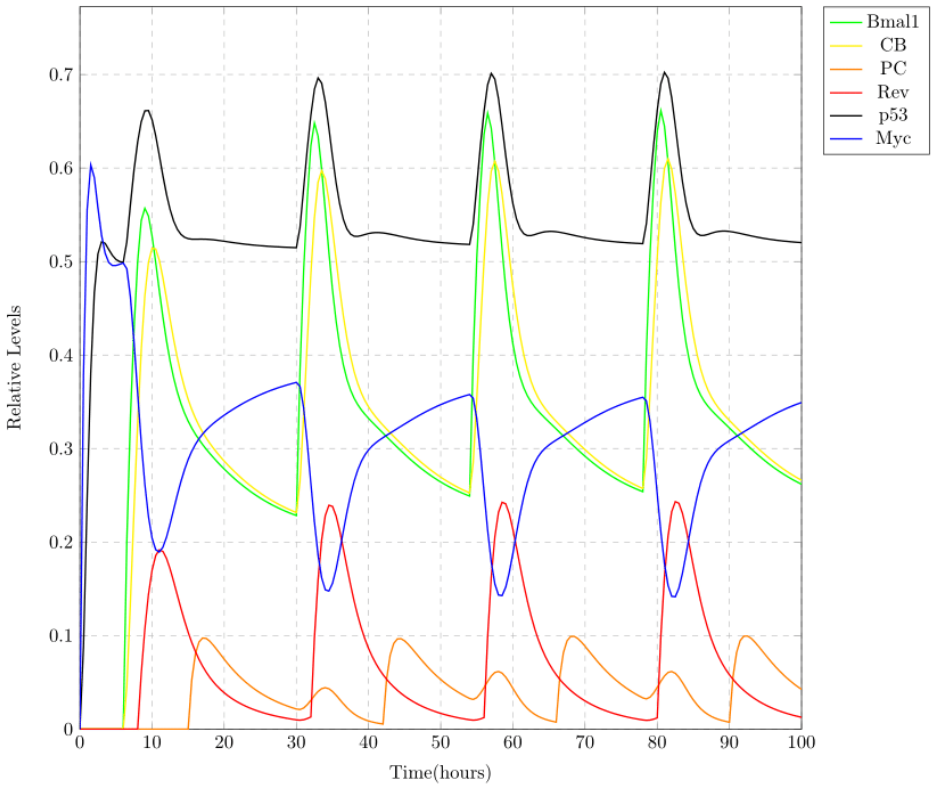

Figure 4.5: Simulation results showing mild effect of jet lag on circadian clock. Suppression in the relative expression levels of core clock proteins and p53 is clearly visible. On the other hand, MYC's average expression level is shown to increase. These simulation results represent that there is not much stress on the circadian system and it can be recovered.

Figure 4.7 that Myc starts expressing itself persistently with lower inhibition and its average expression level rises whereas, clock disruption causes suppression in p53 protein. Disrupted levels which can be seen in Figure 4.7 clearly suggests that clock disruption can cause DNA damages due to suppression of tumor suppressor protein, p53 and abnormal cellular proliferation due to MYC persistent expression.

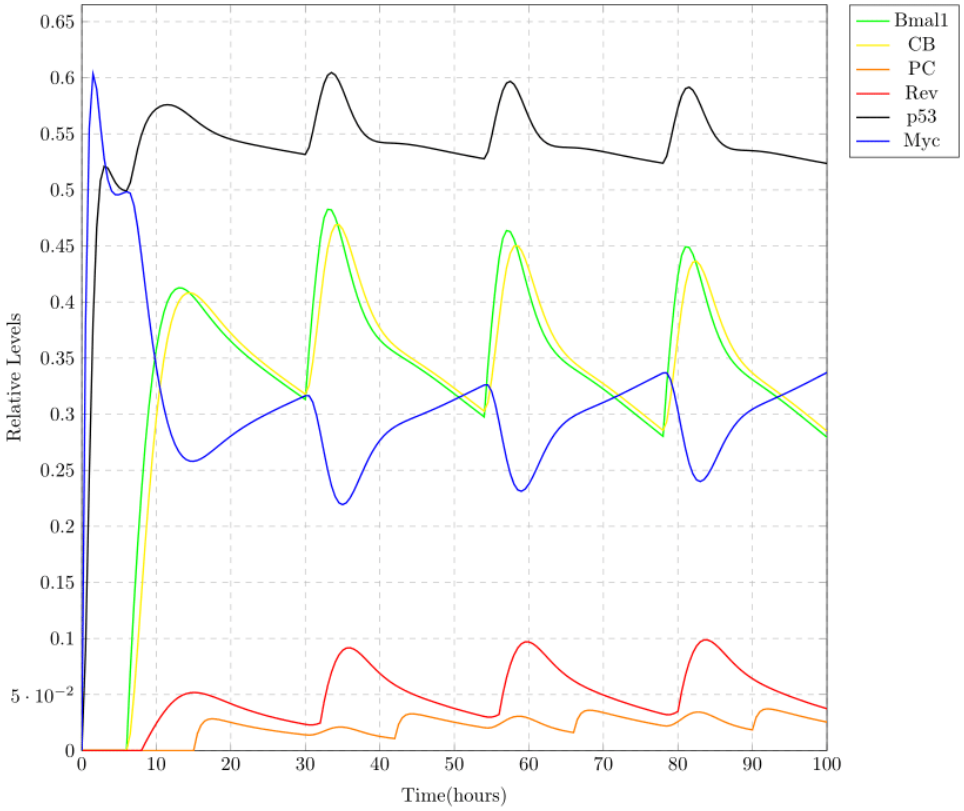

Figure 4.6: This case depicts chronic jet lag effect, where the relative expression levels of the core clock proteins are highly disturbed. This disruption lowers the inhibitory effects on MYC as Circadian clock and p53 levels are suppressed. The persistent expression of MYC can lead to growth of tumor.

4.2 Discussion

Short-term interruptions of circadian rhythms due to "jet lag" and "shift work" are known to cause metabolic and physiological disturbances. But these disruptions are reversible and clock can be readjusted to its normal timings [83]. Recently by the International Agency for Research on Cancer (IARC), long term shift work and chronic jet lag effect has been classified as a probable human carcinogen. This classification places jet lag and shift work in the same risk class as, ultraviolet radiation. Exposure to ar-

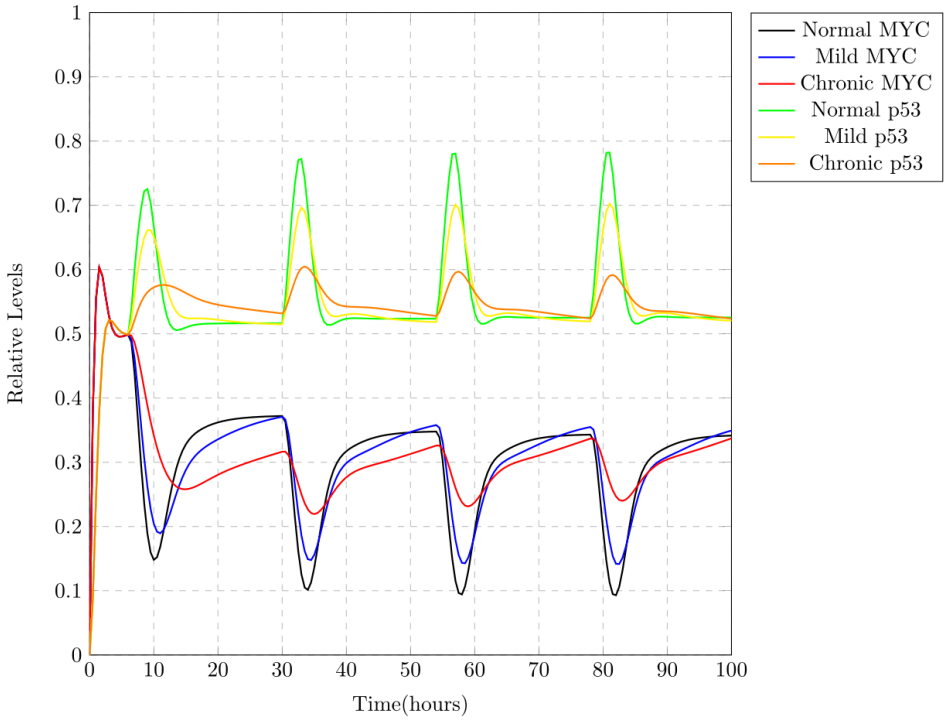

Figure 4.7: A comparison between normal, mild and chronic cases with respect to MYC and p53. Visible suppression in the relative expression levels of p53 and persistent expression of MYC protein due to jet lag can be observed.

tificial light conditions through shift work or jet lag, disrupts the bodyś capability to entrain efficiently to a 24hr time-frame, causing a phenomena known as "light at night". Exposure to light and darkness at unusual times leads to disruption of the normal sleep-wake rhythms. This causes desynchronization between central and the peripheral clocks. Subsequently circadian clock outputs, which have dominant downstream effects, become disrupted. The circadian clock oscillates cellular functions over 24 hours, including cell division cycle. The cell cycle and circadian clock work together at the molecular level of genes, proteins, and biochemical signals. The cell division cycle is synchronized with the circadian clock which also helps in maintaining the integrity of the genome [3, 6].

In several studies [16, 20, 24, 43, 84] artificial jet lag was imposed on mice and its effect on circadian genes was observed. Jet lag caused suppressed and irregular circadian clock gene expression. As some genes between circadian clock and cell division are coupled, the alteration in circadian clock proteins directly affected the proteins involved in cell division cycle. Disruption in the expressions of circadian clock proteins lead to the abnormal division of a cell. Two main proteins found deregulated in tumors are MYC (proto-oncogene protein) and p53 (tumor suppressor). These proteins play an important part in cellular proliferation and DNA damage control. These studies show over expression of MYC and p53 suppression due to circadian clock disruption. This alteration lead to the proliferation of damaged cells as MYC is an oncogene and facilitates the growth of tumor. In addition, circadian disruption compromises the behavior of p53 thus affecting its DNA repair process. [24, 43, 84].

In this study, the connection of circadian clock with MYC and p53 was modeled using Petri Net framework (Figure 4.3). Simulation results shown in figures 4.4, 4.5 and 4.6 depict three different case studies of jet lag disrupted circadian clock. These results are in accordance with the above mentioned observations. The first case (see Figure 4.4) shows the normal behavior of an undisrupted clock with the usual oscillatory behavior of each protein. These results show that an undisrupted clock will oscillate in its usual manner and consequently the coupled proteins MYC and p53 also oscillate in their specific periodic manner. The second model (Figure 4.5) describes a situation where circadian clock proteins are experiencing a slight suppression which is due to a mild jet lag effect. Suppression of clock proteins slightly affected MYC and p53 expression pattern. The last case (Figure 4.6) describes the chronic effect of jet lag i.e. jet lag for long period of time as occurs in the case of frequent travelers or night shift workers. Clock proteins are disrupted giving an effect of chronic jet lag on the basis on experimental observations mentioned in the study of Filipski

et al. [43]. Resulting simulations clearly show over expression of MYC and suppression of p53 due to disruptions in clock proteins. Disturbances in the expression pattern of these vital cell cycle proteins can effect the normal cell cycle division. Suppression of p53 leads to the failure of its DNA repair activity causing abnormality in the cells and persistent expression of MYC supports the proliferation of abnormal cells [43, 84].

Chapter 5

Conclusion

Circadian genes are involved in the regulation of different vital metabolic processes, cell division being one of them. Longterm jet leg effect can disrupt the circadian clock organization, thus causing deregulated cellular proliferation and tumor growth. The model presented in this study depicts that circadian clock plays a dual role in cell cycle progression. On one hand, it controls the expression of oncogenic protein MYC, while on the other hand, it suppresses proliferation of damaged cells by regulating the activation of p53. Keeping in view the simulation results obtained after modeling the effects of jet lag on circadian clock, it can be stated that alterations in sleep/wake and light/dark cycles cause circadian disruption. This disruption negatively impacts the expression of vital cell cycle genes MYC and p53. The expression levels of p53 are suppressed resulting in consistent expression of MYC. This condition favors the proliferation of tumor cells. Therefore, it is concluded that a properly functioning circadian clock is necessary for ensuring a tumor free system.

Bibliography

[1] Satchidananda Panda, John B Hogenesch, and Steve A Kay. Circadian rhythms from flies to human. *Nature*, 417(6886):329–335, 2002.

[2] Michael W Greene. Circadian rhythms and tumor growth. *Cancer letters*, 318(2):115–123, 2012.

[3] Christos Savvidis and Michael Koutsilieris. Circadian rhythm disruption in cancer biology. *Molecular medicine*, 18(1):1249, 2012.

[4] Jean-Christophe Leloup and Albert Goldbeter. Modeling the mammalian circadian clock: sensitivity analysis and multiplicity of oscillatory mechanisms. *Journal of theoretical biology*, 230(4):541–562, 2004.

[5] Selma Masri, Marlene Cervantes, and Paolo Sassone-Corsi. The circadian clock and cell cycle: interconnected biological circuits. *Current opinion in cell biology*, 25(6):730–734, 2013.

[6] Saurabh Sahar and Paolo Sassone-Corsi. Metabolism and cancer: the circadian clock connection. *Nature reviews. Cancer*, 9(12):886, 2009.

[7] Diego A Golombek and Ruth E Rosenstein. Physiology of circadian entrainment. *Physiological reviews*, 90(3):1063–1102, 2010.

[8] Charna Dibner, Ueli Schibler, and Urs Albrecht. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annual review of physiology*, 72:517–549, 2010.

[9] Takuro Yamamoto, Yasukazu Nakahata, Haruhiko Soma, Makoto Akashi, Takayoshi Mamine, and Toru Takumi. Transcriptional oscillation of canonical clock genes in mouse peripheral tissues. *BMC molecular biology*, 5(1):18, 2004.

[10] Andries Kalsbeek, Frank A Scheer, Stephanie Perreau-Lenz, Susanne E La Fleur, Chun-Xia Yi, Eric Fliers, and Ruud M Buijs. Circadian disruption and scn control of energy metabolism. *FEBS letters*, 585(10):1412–1426, 2011.

[11] Jennifer A Mohawk, Carla B Green, and Joseph S Takahashi. Central and peripheral circadian clocks in mammals. *Annual review of neuroscience*, 35:445–462, 2012.

[12] Francesca Damiola, Nguyet Le Minh, Nicolas Preitner, Benoıt Kornmann, Fabienne Fleury-Olela, and Ueli Schibler. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes & development*, 14 (23):2950–2961, 2000.

[13] Shin Yamazaki, Rika Numano, Michikazu Abe, Akiko Hida, Riichi Takahashi, Masatsugu Ueda, Gene D Block, Yoshiyuki Sakaki, Michael Menaker, and Hajime Tei. Resetting central and peripheral circadian oscillators in transgenic rats. *Science*, 288(5466):682–685, 2000.

[14] Henrik Oster, Sebastian Damerow, Silke Kiessling, Vladimira Jakubcakova, Diya Abraham, Jiong Tian, Matthias W Hoffmann, and Gregor Eichele. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell metabolism*, 4(2):163–173, 2006.

[15] Steven M Reppert and David R Weaver. Coordination of circadian timing in mammals. *Nature*, 418(6901):935, 2002.

[16] Choogon Lee, Jean-Pierre Etchegaray, Felino RA Cagampang, Andrew SI Loudon, and Steven M Reppert. Posttranslational mechanisms regulate the mammalian circadian clock. *Cell*, 107(7):855–867, 2001.

[17] Lauren P Shearman, Sathyanarayanan Sriram, David R Weaver, Elizabeth S Maywood, Ines Chaves, Binhai Zheng, Kazuhiko Kume, Cheng Chi Lee, Michael H Hastings, Steven M Reppert, et al. Interacting molecular loops in the mammalian circadian clock. *Science*, 288(5468):1013–1019, 2000.

[18] Caroline H Ko and Joseph S Takahashi. Molecular components of the mammalian circadian clock. *Human molecular genetics*, 15(suppl_2): R271–R277, 2006.

[19] Akhilesh B Reddy, Gabriel KY Wong, John O'Neill, Elizabeth S Maywood, and Michael H Hastings. Circadian clocks: neural and peripheral pacemakers that impact upon the cell division cycle. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 574 (1):76–91, 2005.

[20] Xiaoming Yang, Patricia A Wood, Eun-Young Oh, Jovelyn Du-Quiton, Christine M Ansell, and William JM Hrushesky. Down regulation of circadian clock gene period 2 accelerates breast cancer growth by altering its daily growth rhythm. *Breast cancer research and treatment*, 117(2):423–431, 2009.

[21] Ueli Schibler. Liver regeneration clocks on. *Science*, 302(5643):234–235, 2003.

[22] Stephen R Bolsover, Elizabeth A Shephard, Hugh A White, and Jeremy S Hyams. *Cell biology: a short course*. John Wiley & Sons, 2011.

[23] Susie Lee, Lawrence A Donehower, Alan J Herron, David D Moore, and Loning Fu. Disrupting circadian homeostasis of sympathetic signaling promotes tumor development in mice. *PloS one*, 5(6):e10995, 2010.

[24] Loning Fu, Helene Pelicano, Jinsong Liu, Peng Huang, and Cheng Chi Lee. The circadian gene period2 plays an important role in tumor suppression and dna damage response in vivo. *cell*, 111(1):41–50, 2002.

[25] Tim Hunt and Paolo Sassone-Corsi. Riding tandem: circadian clocks and the cell cycle. *Cell*, 129(3):461–464, 2007.

[26] Franz Halberg, Helmut A Zander, Marmion W Houglum, and Hans R Mühlemann. Daily variations in tissue mitoses, blood eosinophils and rectal temperatures of rats. *American Journal of Physiology–Legacy Content*, 177(3):361–366, 1954.

[27] Sobia Rana and Saqib Mahmood. Circadian rhythm and its role in malignancy. *Journal of circadian rhythms*, 8(1):3, 2010.

[28] Shujing Li, Xiang Ao, and Huijian Wu. The role of circadian rhythm in breast cancer. *Chinese Journal of Cancer Research*, 25(4):442, 2013.

[29] Takuya Matsuo, Shun Yamaguchi, Shigeru Mitsui, Aki Emi, Fukuko Shimoda, and Hitoshi Okamura. Control mechanism of the circadian clock for timing of cell division in vivo. *Science*, 302(5643):255–259, 2003.

[30] Katsutaka Oishi, Koyomi Miyazaki, Koji Kadota, Reiko Kikuno, Takahiro Nagase, Gen-ichi Atsumi, Naoki Ohkura, Takashi Azama,

Miho Mesaki, Shima Yukimasa, et al. Genome-wide expression analysis of mouse liver reveals clock-regulated circadian output genes. *Journal of Biological Chemistry*, 278(42):41519–41527, 2003.

[31] Jasper Mullenders, Armida WM Fabius, Mandy Madiredjo, René Bernards, and Roderick L Beijersbergen. A large scale shrna barcode screen identifies the circadian clock component arntl as putative regulator of the p53 tumor suppressor pathway. *PloS one*, 4(3):e4798, 2009.

[32] Zongbing You, Daniel Saims, Shaoqiong Chen, Zhaocheng Zhang, Denis C Guttridge, Kun-liang Guan, Ormond A MacDougald, Anthony MC Brown, Gerard Evan, Jan Kitajewski, et al. Wnt signaling promotes oncogenic transformation by inhibiting c-myc–induced apoptosis. *J Cell Biol*, 157(3):429–440, 2002.

[33] Gerald I Evan and Karen H Vousden. Proliferation, cell cycle and apoptosis in cancer. *nature*, 411(6835):342, 2001.

[34] Omid Vafa, Mark Wade, Suzanne Kern, Michelle Beeche, Tej K Pandita, Garret M Hampton, and Geoffrey M Wahl. c-myc can induce dna damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Molecular cell*, 9(5):1031–1044, 2002.

[35] Phyllis C Zee, Hrayr Attarian, and Aleksandar Videnovic. Circadian rhythm abnormalities. *Continuum: Lifelong Learning in Neurology*, 19(1 Sleep Disorders):132, 2013.

[36] Mary Choy and Rebecca L Salbu. Jet lag: current and potential therapies. *Pharmacy and Therapeutics*, 36(4):221, 2011.

[37] Scott Davis and Dana K Mirick. Circadian disruption, shift work and

the risk of cancer: a summary of the evidence and studies in seattle. *Cancer causes & control*, 17(4):539–545, 2006.

[38] Michael Conlon, Nancy Lightfoot, and Nancy Kreiger. Rotating shift work and risk of prostate cancer. *Epidemiology*, 18(1):182–183, 2007.

[39] Pierre R Band, Nhu D Le, Raymond Fang, Michéle Deschamps, Andrew J Coldman, Richard P Gallagher, and Joanne Moody. Cohort study of air canada pilots: mortality, cancer incidence, and leukemia risk. *American Journal of Epidemiology*, 143(2):137–143, 1996.

[40] Johnni Hansen. Risk of breast cancer after night-and shift work: current evidence and ongoing studies in denmark. *Cancer Causes & Control*, 17(4):531–537, 2006.

[41] Louis G Keith, Jaroslaw J Oleszczuk, and Martin Laguens. Circadian rhythm chaos: a new breast cancer marker. *International journal of fertility and women's medicine*, 46(5):238–247, 2001.

[42] Eero Pukkala, Rafael Aspholm, Anssi Auvinen, Harald Eliasch, Maryanne Gundestrup, Tor Haldorsen, Niklas Hammar, Jon Hrafnkelsson, Pentti Kyyrönen, Anette Linnersjö, et al. Cancer incidence among 10,211 airline pilots: a nordic study. *Aviation, space, and environmental medicine*, 74(7):699–706, 2003.

[43] Elisabeth Filipski, Franck Delaunay, Verdun M King, Ming-Wei Wu, Bruno Claustrat, Aline Gréchez-Cassiau, Catherine Guettier, Michael H Hastings, and Lévi Francis. Effects of chronic jet lag on tumor progression in mice. *Cancer research*, 64(21):7879–7885, 2004.

[44] Yong Zhu, Richard G Stevens, Derek Leaderer, Aaron Hoffman, Theodore Holford, Yawei Zhang, Heather N Brown, and Tongzhang Zheng. Non-synonymous polymorphisms in the circadian gene npas2

and breast cancer risk. *Breast cancer research and treatment*, 107(3): 421–425, 2008.

[45] Aaron E Hoffman, Tongzhang Zheng, Richard G Stevens, Yue Ba, Yawei Zhang, Derek Leaderer, Chunhui Yi, Theodore R Holford, and Yong Zhu. Clock-cancer connection in non–hodgkin's lymphoma: A genetic association study and pathway analysis of the circadian gene cryptochrome 2. *Cancer research*, 69(8):3605–3613, 2009.

[46] LW Chu, Y Zhu, K Yu, T Zheng, H Yu, Y Zhang, I Sesterhenn, AP Chokkalingam, KN Danforth, MC Shen, et al. Variants in circadian genes and prostate cancer risk: a population-based study in china. *Prostate cancer and prostatic diseases*, 11(4):342, 2008.

[47] Elisabeth Filipski and Francis Lévi. Circadian disruption in experimental cancer processes. *Integrative Cancer Therapies*, 8(4):298–302, 2009.

[48] Hidde De Jong. Modeling and simulation of genetic regulatory systems: a literature review. *Journal of computational biology*, 9(1):67–103, 2002.

[49] Sabine Becker-Weimann, Jana Wolf, Hanspeter Herzel, and Achim Kramer. Modeling feedback loops of the mammalian circadian oscillator. *Biophysical journal*, 87(5):3023–3034, 2004.

[50] Verner Paetkau, Roderick Edwards, and Reinhard Illner. A model for generating circadian rhythm by coupling ultradian oscillators. *Theoretical biology and medical modelling*, 3(1):12, 2006.

[51] Steven H Strogatz. Human sleep and circadian rhythms: a simple model based on two coupled oscillators.

[52] K Sriram, Gilles Bernot, and François Képès. Discrete delay model for the mammalian circadian clock. *Complexus*, 3(4):185–199, 2006.

[53] René Thomas. Regulatory networks seen as asynchronous automata: a logical description. *Journal of theoretical biology*, 153(1):1–23, 1991.

[54] Stuart A Kauffman. Metabolic stability and epigenesis in randomly constructed genetic nets. *Journal of theoretical biology*, 22(3):437–467, 1969.

[55] Katsumi Inoue. Logic programming for boolean networks. In *IJCAI Proceedings-International Joint Conference on Artificial Intelligence*, volume 22, page 924, 2011.

[56] Jamil Ahmad, Umar Niazi, Sajid Mansoor, Umair Siddique, and Jaclyn Bibby. Formal modeling and analysis of the mal-associated biological regulatory network: insight into cerebral malaria. *PloS one*, 7 (3):e33532, 2012.

[57] Gilles Bernot, Jean-Paul Comet, Adrien Richard, and Janine Guespin. Application of formal methods to biological regulatory networks: extending thomas' asynchronous logical approach with temporal logic. *Journal of theoretical biology*, 229(3):339–347, 2004.

[58] René Thomas. *Kinetic logic: a Boolean approach to the analysis of complex regulatory systems: proceedings of the EMBO course "formal analysis of genetic regulation", held in Brussels, September 6–16, 1977*, volume 29. Springer Science & Business Media, 2013.

[59] Jamil Ahmad, Adrien Richard, Gilles Bernot, Jean-Paul Comet, and Olivier Roux. Delays in biological regulatory networks (brn). *Computational Science–ICCS 2006*, pages 887–894, 2006.

[60] Aurélien Naldi, Duncan Berenguier, Adrien Fauré, Fabrice Lopez, Denis Thieffry, and Claudine Chaouiya. Logical modelling of regulatory networks with ginsim 2.3. *Biosystems*, 97(2):134–139, 2009.

[61] Zohra Khalis, Jean-Paul Comet, Adrien Richard, and Gilles Bernot. The smbionet method for discovering models of gene regulatory networks. *Genes, genomes and genomics*, 3(1):15–22, 2009.

[62] Christel Baier, Joost-Pieter Katoen, and Kim Guldstrand Larsen. *Principles of model checking*. MIT press, 2008.

[63] Edmund M Clarke, Orna Grumberg, and Doron Peled. *Model checking*. MIT press, 1999.

[64] Adrien Richard, Jean-Paul Comet, and Gilles Bernot. Formal methods for modeling biological regulatory networks. *Modern Formal Methods and Applications*, 5:83–122, 2006.

[65] René David and Hassane Alla. *Discrete, continuous, and hybrid Petri nets*. Springer Science & Business Media, 2010.

[66] Wilfried Brauer and Wolfgang Reisig. Carl adam petri and "petri nets". *Fundamental Concepts in Computer Science*, 3(5):129, 2009.

[67] M Blätke, Monica Heiner, and Wolfgang Marwan. Tutorial—petri nets in systems biology. *August*, 2011.

[68] Stephen D Scott, Abhishek Majumdar, Jitender S Deogun, and Steven D Harris. Yeast pheromone pathway modeling using petri nets. 2014.

[69] Fei Liu and Monika Heiner. Modeling membrane systems using colored stochastic petri nets. *Natural Computing*, 12(4):617–629, 2013.

[70] Lingxi Li and Hiroki Yokota. Application of petri nets in bone remodeling. *Gene regulation and systems biology*, 3:105, 2009.

[71] Claudine Chaouiya, Elisabeth Remy, and Denis Thieffry. Petri net modelling of biological regulatory networks. *Journal of Discrete Algorithms*, 6(2):165–177, 2008.

[72] Dorota Formanowicz, Andrea Sackmann, Piotr Formanowicz, and Jacek Błażewicz. Petri net based model of the body iron homeostasis. *Journal of biomedical informatics*, 40(5):476–485, 2007.

[73] E Simao, Elisabeth Remy, Denis Thieffry, and Claudine Chaouiya. Qualitative modelling of regulated metabolic pathways: application to the tryptophan biosynthesis in e. coli. *Bioinformatics*, 21(suppl_2): ii190–ii196, 2005.

[74] Monika Heiner, Ina Koch, and Jürgen Will. Model validation of biological pathways using petri nets—demonstrated for apoptosis. *Biosystems*, 75(1):15–28, 2004.

[75] Claudine Chaouiya. Petri net modelling of biological networks. *Briefings in bioinformatics*, 8(4):210–219, 2007.

[76] Claudine Chaouiya, Aurelien Naldi, and Denis Thieffry. Logical modelling of gene regulatory networks with ginsim. *Bacterial Molecular Networks: Methods and Protocols*, pages 463–479, 2012.

[77] Samar Hayat Khan Tareen and Jamil Ahmad. Modelling and analysis of the feeding regimen induced entrainment of hepatocyte circadian oscillators using petri nets. *PloS one*, 10(3):e0117519, 2015.

[78] Monika Heiner, Mostafa Herajy, Fei Liu, Christian Rohr, and Martin Schwarick. Snoopy–a unifying petri net tool. *Application and Theory of Petri Nets*, pages 398–407, 2012.

[79] Assieh Saadatpour, Réka Albert, and Timothy C Reluga. A reduction method for boolean network models proven to conserve attractors. *SIAM Journal on Applied Dynamical Systems*, 12(4):1997–2011, 2013.

[80] Claudine Chaouiya, Elisabeth Remy, and Denis Thieffry. Qualitative petri net modelling of genetic networks. *Lecture Notes in Computer Science*, 4220:95, 2006.

[81] Angela Relógio, Pal O Westermark, Thomas Wallach, Katja Schellenberg, Achim Kramer, and Hanspeter Herzel. Tuning the mammalian circadian clock: robust synergy of two loops. *PLoS computational biology*, 7(12):e1002309, 2011.

[82] Chi V Dang, Kathryn A O'Donnell, and Tarja Juopperi. The great myc escape in tumorigenesis. *Cancer cell*, 8(3):177–178, 2005.

[83] Thomas C Erren, Puran Falaturi, Peter Morfeld, Peter Knauth, Russel J Reiter, and Claus Piekarski. Shift work and cancer: the evidence and the challenge. *Deutsches Ärzteblatt International*, 107(38):657, 2010.

[84] Elisabeth Filipski, Pasquale F Innominato, MingWei Wu, Xiao-Mei Li, Stefano Iacobelli, Li-Jian Xian, and Francis Lévi. Effects of light and food schedules on liver and tumor molecular clocks in mice. *Journal of the National Cancer Institute*, 97(7):507–517, 2005.

Appendices

Appendix A: SMBioNet Source Code

VAR

Bmal = 0 1 ;
PC = 0 1 ;
myc = 0 1 ;
p53 = 0 1 ;
CB = 0 1 ;
Rev = 0 1 ;

REG

Rev [(Rev<1)]=> Bmal ;
CB [(CB>=1)]=> PC ;
myc [(myc>=1)]=> myc ;
CB [(CB<1)]=> myc ;
p53 [(p53<1)]=> myc ;
myc [(myc>=1)]=> p53 ;
Bmal [(Bmal>=1)]=> p53 ;
Bmal [(Bmal>=1)]=> CB ;
PC [(PC<1)]=> CB ;

CB [(CB>=1)]=> Rev ;

PARA

Parameters for Bmal

K_Bmal = 0 ;
K_Bmal+Rev = 1 ;

Parameters for PC

K_PC = 0 ;
K_PC+CB = 1 ;

Parameters for myc

K_myc = 0 ;
K_myc+CB = 0 ;
K_myc+myc = 0 1 ;
K_myc+p53 = 0 ;
K_myc+CB+myc = 0 ;
K_myc+myc+p53 = 0 ;
K_myc+CB+p53 = 0 1 ;
K_myc+CB+myc+p53 = 1 ;

Parameters for p53

K_p53 = 0 ;
K_p53+Bmal = 1 ;
K_p53+myc = 1 ;

K_p53+Bmal+myc = 1 ;

Parameters for CB

K_CB = 0 ;
K_CB+Bmal = 0 1 ;
K_CB+PC = 0 ;
K_CB+Bmal+PC = 1 ;

Parameters for Rev

K_Rev = 0 ;
K_Rev+CB = 1 ;

CTL

(AG((Bmal=0&CB=0&PC=0&Rev=0&myc=0&p53=0))->
AX(EF(Bmal=0&CB=0&PC=0&Rev=0&myc=0&p53=0))&

(EF(Bmal=0&CB=0&PC=1&Rev=1))->
(EX(EF(Bmal=0&CB=0&PC=1&Rev=1))&EF(EG(myc=1&p53=0)))&

(EF(p53=1))->EF(myc=0)->(E[(p53=0)U(p53=1)])&

EF(AG(myc=1))&EF(AG(p53=0)))

Appendix B: SMBioNet Output

MODEL 1

K_Bmal = 0
K_Bmal+Rev = 1

K_PC = 0
K_PC+CB = 1

K_myc = 0
K_myc+CB = 0
K_myc+myc = 0
K_myc+p53 = 0
K_myc+CB+myc = 0
K_myc+myc+p53 = 0
K_myc+CB+p53 = 0
K_myc+CB+myc+p53 = 1

K_p53 = 0
K_p53+Bmal = 1
K_p53+myc = 1
K_p53+Bmal+myc = 1

K_CB = 0
K_CB+Bmal = 0
K_CB+PC = 0
K_CB+Bmal+PC = 1

K_Rev = 0
K_Rev+CB = 1

MODEL 2

K_Bmal = 0
K_Bmal+Rev = 1

K_PC = 0
K_PC+CB = 1

K_myc = 0
K_myc+CB = 0
K_myc+myc = 0
K_myc+p53 = 0
K_myc+CB+myc = 0
K_myc+myc+p53 = 0
K_myc+CB+p53 = 1
K_myc+CB+myc+p53 = 1

K_p53 = 0
K_p53+Bmal = 1
K_p53+myc = 1
K_p53+Bmal+myc = 1

K_CB = 0
K_CB+Bmal = 0
K_CB+PC = 0
K_CB+Bmal+PC = 1

K_Rev = 0
K_Rev+CB = 1

MODEL 3

K_Bmal = 0

K_Bmal+Rev = 1

K_PC = 0

K_PC+CB = 1

K_myc = 0

K_myc+CB = 0

K_myc+myc = 0

K_myc+p53 = 0

K_myc+CB+myc = 0

K_myc+myc+p53 = 0

K_myc+CB+p53 = 0

K_myc+CB+myc+p53 = 1

K_p53 = 0

K_p53+Bmal = 1

K_p53+myc = 1

K_p53+Bmal+myc = 1

K_CB = 0

K_CB+Bmal = 1

K_CB+PC = 0

K_CB+Bmal+PC = 1

K_Rev = 0

K_Rev+CB = 1

MODEL 4

K_Bmal = 0
K_Bmal+Rev = 1

K_PC = 0
K_PC+CB = 1

K_myc = 0
K_myc+CB = 0
K_myc+myc = 0
K_myc+p53 = 0
K_myc+CB+myc = 0
K_myc+myc+p53 = 0
K_myc+CB+p53 = 1
K_myc+CB+myc+p53 = 1

K_p53 = 0
K_p53+Bmal = 1
K_p53+myc = 1
K_p53+Bmal+myc = 1

K_CB = 0
K_CB+Bmal = 1
K_CB+PC = 0
K_CB+Bmal+PC = 1

K_Rev = 0
K_Rev+CB = 1

SELECTED MODELS / CHECKED MODELS = 4 / 4 (137ms)