# Modeling and Simulation of Diurnal Biological Processes in Cyanobacteria

Thanura R. Elvitigala, Himadri B. Pakrasi and Bijoy K. Ghosh

*Abstract*— Life on earth is strongly regulated by the day-night cycle, widely known as *diurnal cycle*. Accordingly, many organisms have developed an internal time keeping mechanism; *circadian clock*, in order to maintain these rhythms even with short term modification of the external light patterns. In previous studies, different biological processes have been identified as either diurnal or circadian controlled. In order to understand the interactions between these processes and the effect of external light input on the individual rhythms, it is important to develop a mathematical model which can capture the essential dynamics of these rhythms.

In this work, we propose a simple interacting oscillatory network model, which is sufficient to capture the different behavioral patterns in the biological processes. We show that this model is capable of reproducing the actual gene behaviors observed under different light input patterns. Also, the model proposed is shown to be resilient to noise. We use the model to understand the synchronization between different processes; the modulation of internal clock by the external light input; the changes expected in circadian clock and other peripheral processes under different light patterns, etc. We relate some of the simulation results with already available biological knowledge. We discuss effects of different network topologies which can be investigated by the future experiments.

## I. INTRODUCTION

Many physiological and metabolic processes of living organisms show a rhythmic behavior synchronized with the day/night cycle of the earth. These rhythmic patterns are known as the diurnal rhythms and have been identified in a wide range of organisms from unicellular oxygenic photosynthetic bacteria to humans [1]. It is widely believed that many of the diurnal rhythms are predominantly controlled by an internal clock, a time keeping circuit that maintains its rhythm even when the external pattern of light is altered for a short duration. Such rhythms would be described as 'circadian controlled rhythms'. Existence of a robust internal circadian clock in unicellular cyanobacteria is now well established [2]. This circuit is entrained by external cues such as light and dark pattern impinging on the cell body [3].

T. R. Elvitigala is with Department of Electrical and Systems Engineering, Washington University in St Louis, MO. tre1@ese.wustl.edu

H. B. Pakrasi is with the Department of Biology, Washington University in St Louis, MO. pakrasi@wustl.edu

B. K. Ghosh is with the Department of Mathematics and Statistics, Texas Tech University, Lubbock, TX. bijoy.ghosh@ttu.edu

Phase oscillators were originally used for modeling oscillatory systems having large number of weakly interacting oscillators ([4],[5]). Phase oscillator models are appropriate for modeling circadian rhythms, as they directly model the phase dynamics, which is the most important factor in understanding circadian rhythms. This model was used in [6] to represent the circadian clock of cyanobacteria and to establish that the interaction between cyanobacteria cells are negligible. In [7], a coupled phase oscillator network was proposed for modeling the circadian-controlled genes in cyanobacteria.

*Cyanothece* sp ATCC 51142 (*Cyanothece* hereafter), a unicellular cyanobacterium, shows a strong diurnal rhythm [8]. In [9], changes in gene behavior under altered light input patterns were discussed. Both experiments were performed under the same conditions, and utilized the same microarray chip design. In [8], cells were grown under 12h/12h Light and Dark (LD hereafter) conditions starting with dark phase and the samples were extracted every 4h for two days. In [9], cells were first kept under 12h/12h LD conditions for 24h, starting from light phase and later transferred to constant light (LL hereafter) conditions for additional 24h. Samples were obtained every 4h under both LD and LL conditions. Initial data processing steps are detailed in the corresponding work. The data from these two experiment data was analyzed by [10] using Fourier Transform-based methods. The genes were categorized into two main groups namely 'circadian controlled' and 'light responding', depending on whether they maintain their cyclic behavior under constant light (LL) or not. It was also shown that genes from some of the biological processes clearly fall into one of these behaviors. A good mathematical model that can simulate the important dynamics of these gene behaviors is important to understand the interactions between different processes.

In this work, we propose a simple interacting phase oscillator network to understand circadian controlled and light responding behaviors. We show that this model is sufficient to reproduce the actual gene behaviors observed under different light input patterns. We use it to understand the synchronization between different processes; modulation of the internal clock by the external light input; the changes expected in the circadian clock and other peripheral processes under different light patterns, etc. We relate some of the simulation results with already available biological knowledge. We discuss the effects of having different network topologies which can be investigated by future experiments.

## II. MODELING BIOLOGICAL PROCESSES AS AN OSCILLATOR NETWORK

In [10], genes identified to be diurnal, were separated as Circadian Controlled Genes (CCGs) and Light Responding Genes (LRGs). It was also found that genes belonging to some of the biological processes are clustered together in terms of their phases. This suggests the possibility of approximating each of such processes using a single oscillator. We picked two six-oscillator networks to represent the 24h CCGs and LRGs. This was due to two observations made in [10], namely:

1) Distribution of phases of genes belonging to well clustered biological processes are mostly localized within a 4h period;

2) The gene regulatory network, generated using a linear dynamical model, indicated that, for the majority of the genes in the network, the phase difference between the target and regulator was 4h. We can capture this relationship using 6 oscillators with approximately $\pi/3$ phase difference.

### A. Oscillator Network

The coupled oscillator model proposed here has a structure as given in Fig. 1. The model consists of a total of 14 oscillators. Two oscillators present in the centers of the two rings correspond to the light sensor and the circadian oscillator. Ring of 6-oscillators connected to light sensor represent 24h LRGs while those connected to circadian oscillator are associated with 24h CCGs. We assume each of the central oscillators provides a reference phase to the oscillators in its ring. For the oscillators in the ring we assume unidirectional interactions, representing a regulator-target relationship. In addition, the coupling between light sensor and the circadian oscillator ensures the entrainment of the circadian clock by the external light.
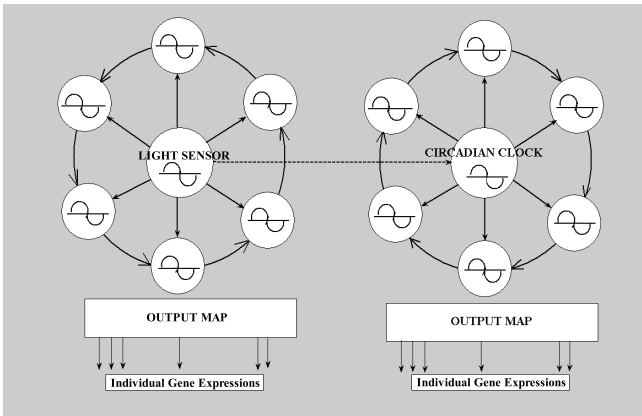


Fig. 1. Coupled oscillator model representing 24h LRGs and CCGs. Central oscillators, correspond to light sensor and the circadian oscillator, provide reference phases for their ring oscillators representing 24h LRGs and CCGs respectively. Individual gene expressions are obtained as a linear map of the oscillator outputs.

### B. Phase Oscillator Model

In the model presented here, each of the oscillators is modeled as a phase oscillator. Due to the lack of knowledge on the light sensor and the output channel of the circadian clock in the cyanobacteria, central oscillators were assumed to be harmonic oscillators and modeled as,

$$\dot{\phi}_{lc} = \omega_{lc_0}, \tag{1}$$

$$\dot{\phi}_{cc} = \omega_{cc_0} + \varepsilon_1 \sin(\phi_{lc} - \phi_{cc}), \tag{2}$$

where $\phi_{lc}$ and $\phi_{cc}$ are phases of light sensor and the circadian clock respectively and $\omega_{lc_0}$ and $\omega_{cc_0}$ are their corresponding Eigen frequencies, set to $2\pi/24$ corresponding to 24h oscillatory period.

Oscillators in the rings are non-harmonic oscillators, modeled to reproduce the gene expressions they represent. Their behavior was modeled as,

$$\dot{\phi}_{l_i} = \omega_{l_i} + \sum_{k=1}^{N} \varepsilon_{l_ik} \sin(k\phi_{l_i} + \delta_{l_ik})$$
$$+ \varepsilon_2 \sin(\phi_{lc} - \phi_{l_i} - \xi_{l_i}) + \varepsilon_3 \sin(\phi_{ri} - \phi_{l_i} - v_{l_i}), \tag{3}$$

$$\dot{\phi}_{c_j} = \omega_{c_j} + \sum_{k=1}^{N} \varepsilon_{c_jk} \sin(k\phi_{c_j} + \delta_{c_jk})$$
$$+ \varepsilon_4 \sin(\phi_{cc} - \phi_{c_j} - \xi_{c_j}) + \varepsilon_5 \sin(\phi_{rj} - \phi_{c_k} - v_{c_k}), \tag{4}$$

where $\phi_{li}$ and $\phi_{ri}$ are phases of the $i^{th}$ oscillator for LRGs and the oscillator preceding $i^{th}$ oscillator respectively. Analogously $\phi_{cj}$, $\phi_{cc}$ and $\phi_{rj}$ correspond to phases of the $j^{th}$ oscillator for CCGs, the circadian clock and the ring oscillator preceding $j^{th}$ oscillator respectively.

### C. Determining Coupling Strengths

The network consists of four types of coupling between oscillators, namely the light sensor–circadian clock ($\varepsilon_1$), the light sensor–ring oscillator ($\varepsilon_2$), the circadian clock–ring oscillator ($\varepsilon_4$), and the ring oscillator–ring oscillator ($\varepsilon_3, \varepsilon_5$). Values of these coupling coefficients were determined considering several features that the model needs to produce, including:

1) Faster Entrainment: The cyanobacterium circadian clock is capable of being rapidly entrained/phase reset by the external light ([11]). In order to obtain a faster entrainment, we would like to have a strong coupling strength between the light sensor and the circadian clock. However, since the circadian clock should be able to maintain its oscillations under changing light inputs, we need to ensure $\dot{\phi}_{cc} > 0$ for any phase differences between the light sensor and the circadian clock. Considering these two factors we picked $\varepsilon_1 = 0.1$

2) Cessation of process oscillations: Diurnal biological processes, responding to light pattern, stop their oscillations under constant light conditions. These changes in behavior are noticeable soon after the change in light input pattern, within the first few hours, as observed in [10]. In addition, the circadian clock mutants show changes in oscillation periods and arrhythmic behaviors their biological processes ([12]). In order to achieve these behaviors we pick $\varepsilon_2 = 0.3$ and $\varepsilon_4 = 0.3$.

3) Phase relationship between biological processes: Though clock plays an important role in coordinating other biological processes, regulator-target interactions between genes are also a key determinant on transcriptome levels of a cell. These interactions are taken into account by the coupling between ring oscillators. We picked a relatively weak coupling strengths for these connections and set $\varepsilon_3 = 0.05$ and $\varepsilon_5 = 0.05$.
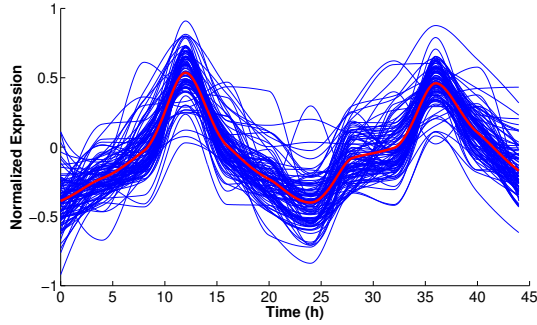
Fig. 2. Normalized expressions of genes with close phase relationship and their mean expression. Individual oscillators were designed to reproduce these mean expressions.
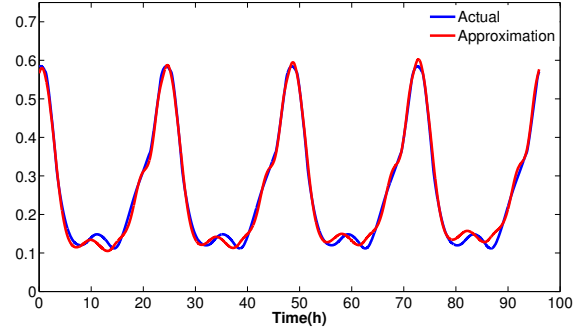


Fig. 3. Approximation of a phase derivative using the phase model. The proposed oscillator model is sufficient to get a good reconstruction of the actual phase dynamics.

### D. Parameter Identification

Each of the oscillators in the rings was modeled to capture the average expression of the genes it represents. For this purpose we grouped together genes having a close phase relationship and their mean expressions were calculated. In order to have the same contribution from each gene towards the mean, the original expressions were scaled and shifted. Fig. 2 shows the normalized expressions for one groups of genes and their mean expression.

Once the mean curve is obtained, it was concatenated several times to get an expression for multiple cycles. The resulting curve was smoothed using cubic interpolation to remove discontinuities. The phase was defined as the angle of a rotating vector, whose projection on the real axis would give the actual mean expression. The phase curve was also smoothed using zeroth order Savitzky-Golay FIR filter with a frame size of 41 [13] since any sudden changes in the slope would produce jumps in the phase derivative. The phase derivative was calculate using two point approximation. For all oscillations, these calculations were done using the gene expressions obtained from the first experiment.

Optimal values for parameters $\omega_{l_i}, \omega_{c_j}, \varepsilon_{l_i k}, \varepsilon_{c_j k}, \delta_{l_i k}$ and $\delta_{c_j k}$ in (3) and (4) were found by the least square optimization method minimizing the errors, given by

$$E_{li} = \| \dot{\phi}_{l_i} - \varepsilon_2 \sin\left(\phi_{lc} - \phi_{l_i} - \xi_{l_i}\right) - \varepsilon_3 \sin\left(\phi_{ri} - \phi_{l_i} - \upsilon_{l_i}\right)$$
$$-\omega_{l_i} - \sum_{k=1}^{N} \varepsilon_{l_i k} \sin\left(k\phi_{l_i} + \delta_{l_i k}\right) \| \tag{5}$$

and

$$E_{cj} = \| \dot{\phi}_{c_j} - \varepsilon_4 \sin\left(\phi_{cc} - \phi_{c_j} - \xi_{c_j}\right) - \varepsilon_5 \sin\left(\phi_{rj} - \phi_{c_j} - \upsilon_{c_j}\right)$$
$$-\omega_{c_j} - \sum_{k=1}^{N} \varepsilon_{c_j k} \sin\left(k\phi_{c_j} + \delta_{c_j k}\right) \| . \tag{6}$$

We picked $N$ to be 5 in order to get a good reconstruction. Fig. 3 shows the approximation of the phase derivative for one of the oscillators. It is clear with $N = 5$, phase model can approximate the phase derivatives with good accuracy. With this choice, the error of reconstructing the phase derivative was $\leq 8\%$ for all the oscillations in the system.

Parameters $\xi_{xi}$ and $\upsilon_{xi}$ correspond to the average phase difference in the phase of the current oscillator from that of the center and previous ring oscillator respectively.

In order to get the oscillator output under constant light conditions, we set the Eigen frequency of the light sensor to zero during the subjective dark regime (last 12h period in the second experiment). This makes the phase of light sensor a constant during this period and the other oscillators show a transient behavior due to change. Fig. 4 shows the outputs of oscillators corresponding
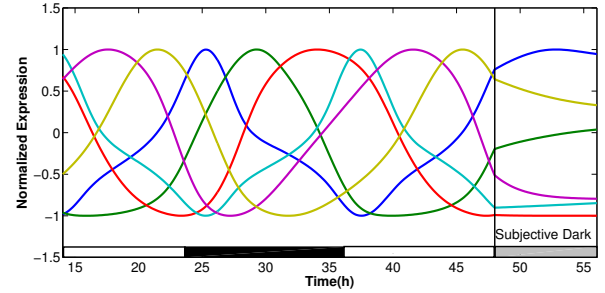


Fig. 4. Output of the 6 ring oscillators corresponding to LRGs, simulated under transient light conditions. During last 12h, the light sensor was kept at constant phase, which forced the ring oscillators to stop their oscillations also.

to light responding genes, under transient light input pattern in the second experiment.

## III. USE OF OSCILLATOR MODEL FOR STUDYING GENE BEHAVIOR

The oscillator model presented here can be used for various purposes. It can be used as a method of filtering and categorizing genes into groups. Oscillator outputs can be treated as a set of basis functions for this data set, which are better representatives of the actual gene expressions than sine/cosine functions. In addition, the model can be used to simulate gene behavior under various light conditions. It is also possible to study the effect of the oscillator output with changes in parameter values. Predictions from these simulations can be verified using experiments.

### A. Categorization of genes using Oscillator model

The actual gene expressions were projected onto the oscillator outputs in order to filter those which can be explained using the model. Each gene expression was explained using two closest oscillator outputs in terms of their phase. Goodness of fit was measured using the correlation between the approximation and the original expression.

A gene was picked only if it was well approximated using two oscillator outputs. We selected a correlation threshold of 0.8. In addition to a good approximation, we also required that the gene was explained by the same oscillators in both experiments. This ensures the extraction of genes with consistent behavior in two experiments. Fig. 5 shows the approximation of an actual gene expression using the two closest oscillator outputs.

Based on the calculations, 501 and 651 genes in the Circadian-controlled and the Light-responding processes respectively, were
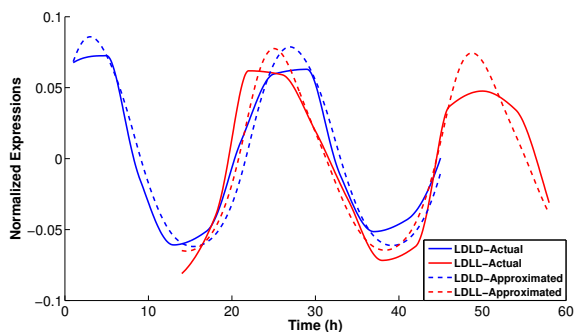
Fig. 5. Reconstruction of an gene expression using two oscillator outputs. Many diurnal gene expressions could be reconstructed as a linear map of two neighboring oscillators.
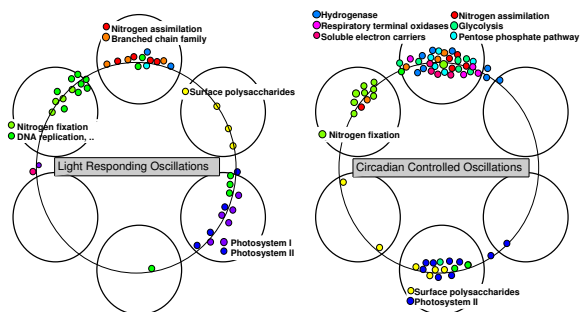


Fig. 6. Some of the processes which can be directly associated with the individual oscillators in the network. These include many of the vital processes needed for the survival of the cells.

well approximated by a linear combination of two oscillators. Among these, there were 345 genes which could be classified as both CCG and LRG. We assigned them to the group, which results in lower error in the approximation.

### B. Clustering of genes based on the projections

Those well-approximated genes were clustered based on the oscillators used to represent them. Fig. 6 contains the distribution of genes for some of the well clustered biological processes. One of the important observations made here is the tight co-regulation of genes belonging to processes which become active at the onset of light or dark phases. Also, compared to the middle of the night or day, more number of genes become active during these periods. This clearly shows the preparation of cells to adapt to the changing light conditions.

## IV. SIMULATION RESULTS

The oscillator network can be simulated under different conditions to make predictions on the behavior of the genes. These predictions can be verified by further experiments. Here we discuss some of the simulation results. We specifically focus on the effects on the circadian clock and its associated processes by changes in the light input. We relate some of the simulation results with actual observations in the literature.

### A. Different Network Topologies

The oscillator network was simulated after removing the clock-process, the process-process and both the clock-process and the process-process couplings, to study the effect of these changes on phases of the oscillators. For this part of simulation, we kept the strengths of both types of coupling at 0.05, so that the role of each type of coupling can be directly compared. The phase
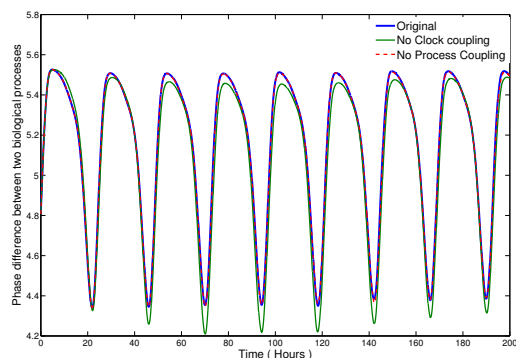


Fig. 7. Effects on phases of Circadian Controlled processes under different coupling topologies, measured as phase difference between two process. The effect of removing the coupling between the processes is negligible, compared to the effect of removing couplings between the clock and the processes.
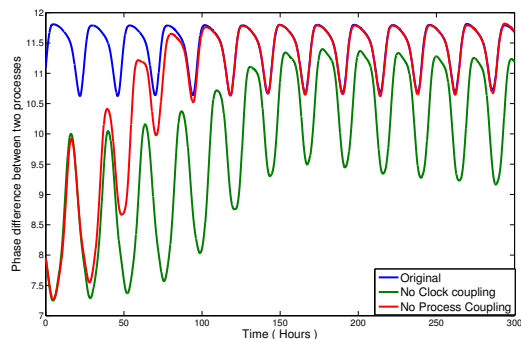


Fig. 8. Phase differed between two processes, resulting due to a phase shift of one, under different network topologies. With the connections to the clock, system recovers from the perturbation very quickly, with no significant effect on the other processes.

differences between two of the oscillator-outputs under different coupling configurations are shown in Fig. 7.

Based on the simulations, removal of the coupling between the center oscillator and the peripheral oscillators gives rise to a larger shift in the phase relations, compared to the removal of the coupling between the peripheral oscillators. This suggests that the circadian clock might have more significant role in maintaining the exact phase relationships between biological processes.

We also studied the transient behavior of different network topologies, once they were perturbed by shifting the phase of one of the processes (oscillator) by $\pi$ compared to its original phase. The perturbed oscillator returned to its original phase very quickly, when the coupling with the circadian clock was present and the other processes had little effect from the disturbance. However when the clock links were not present, the perturbed oscillator settled to a different phase, compared to its original. All the other processes were shifted in their phases as a result of the perturbation. Also under this configuration, a much longer period was required to regain the stationary phase behavior. Fig. 8 shows the simulation results. This again suggests the vital role, the circadian clock has in maintaining robust dynamics of the other biological processes.

These behaviors support the observation that the circadian clock is not essential for the survival of the cells but increases the competence of the cells by improving the coordination between different biological processes [14]. This has well been established
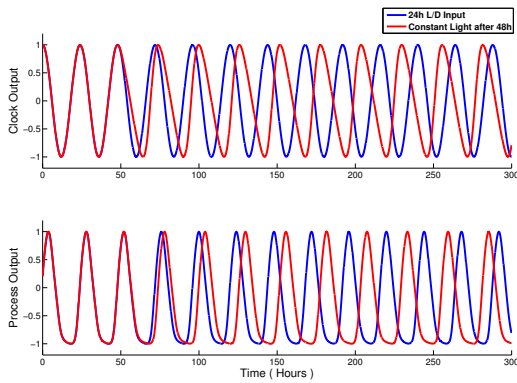
Fig. 9. Circadian Clock and One of the ring Oscillator Outputs under periodic and constant light input conditions. Effect of constant light is reflected in clock output immediately, but only observed in the processes after some delay.
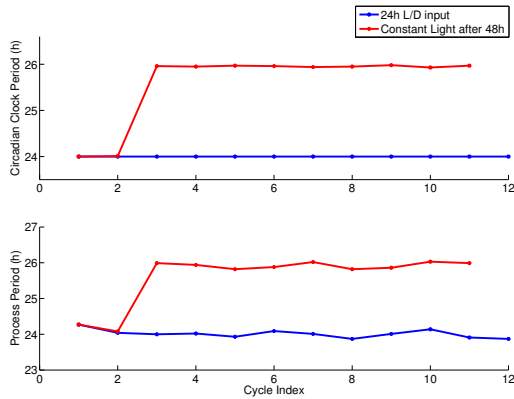


Fig. 10. Periods of Oscillators under 24h periodic and constant light input conditions
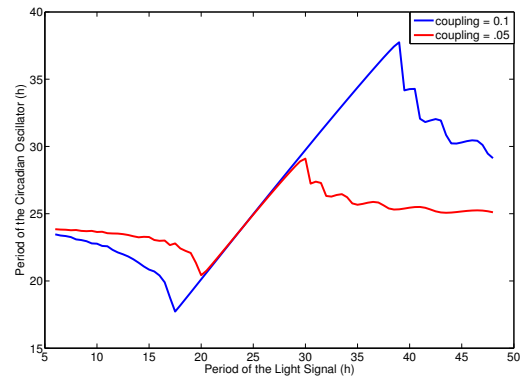


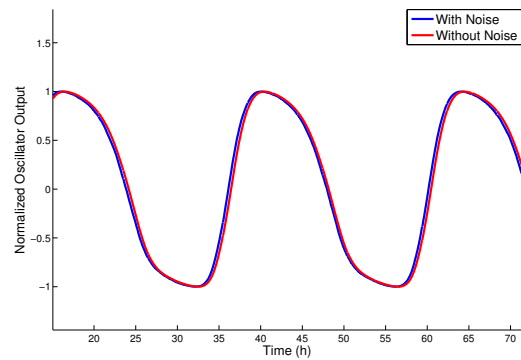Fig. 11. Adaptation of Circadian Clock to different periods of light input



Fig. 12. Output of a ring oscillator with and without noise. Effect of noise was negligible on the output of the oscillator. The robustness to the noise is an essential feature of the most of the biological systems.

for other organisms also, which include plants and humans.

### B. Effects of providing constant light input

In circadian control literature, it is known that the free running period of the clock is not exactly equal to 24h. Usually it can be slightly shorter or longer. Based on [2], *S. elongatus* has a free running period of around 25h. In order to see whether the model is capable of generating such a behavior, a simulation was run under constant light conditions. This was achieved by keeping the phase of light oscillator constant. The natural period of the circadian clock was kept at 24h. Fig. 9 shows the output of the circadian clock and one of the ring oscillators for periodic and constant light inputs. Fig. 10 shows the corresponding periods of oscillations. As a result of the coupling with the light oscillator, the circadian clock and the ring oscillator showed a period of around 26h. The free running period varied with the coupling strength. One other observation is that, while the circadian clock oscillations were immediately affected by the changes in light input, the processes under circadian-control were affected with some time delay. This is clear from Fig. 9.

### C. Adaptation to light patterns with different periods

The ability of the circadian clock to follow the different periods in the light input depends on the strength of the coupling between the circadian clock and the light sensor. Fig. 11 shows the period of oscillations of the circadian oscillator, under light cycles with

different periods, for two different coupling strengths. Clearly the circadian clock follows the light period in a wider range with an increased coupling strength between two oscillators. This observation can be used to determine the actual strength of coupling between the light sensor and the circadian clock.

### D. Effect of the noise

Most of the biological systems are robust to the noise inherent to them. As a result, any realistic model should be robust to fluctuations caused by noise. In order to test the resilience of the current model to the external noise, we added a noise component to the original model. We assumed, that the effect of noise changes the Eigen frequency of the oscillators. Therefore we replaced the $\omega$ terms with,

$$\omega_x = \omega_{x0}(1 + N_x), \tag{7}$$

where $N_x$ represents the White Gaussian noise. We limited the noise signal to be between -0.1 and 0.1 representing $10\%$ deviation of oscillator frequencies from their normal values. This is sufficient to capture the range of frequencies usually observed in the cyanobacteria circadian clock. Noise was added to all oscillators except the light sensor. Equations were solved using Eular method. We observed that the system was extremely robust and the effect of noise on the ring oscillators was negligible. Fig. 12 shows the simulation results for one of the ring oscillators with and without noise.

## V. CONCLUSIONS AND FUTURE WORKS

In this work, we proposed a simple coupled oscillator network to model the gene behaviors under different light input patterns. We showed that the model proposed here was capable of capturing important dynamics of the gene behaviors. The oscillator outputs were used to classify genes into different groups based on the phases of their expressions. We showed that some of the biological processes could directly be mapped to the relevant oscillators. Based on the simulation results, we argued that the circadian clock was more important for maintaining proper phase relationships between biological processes, compared to the interactions between individual processes. We also discovered that there was a noticeable time delay involved in the propagation of changes in light patterns to the circadian-controlled processes. Our model was able to reproduce some of the experimentally observed gene behaviors under altered light conditions. These included the changes in the natural period of circadian clock under constant light. In addition the model was shown to be resilient to noise, an essential feature in most of the biological systems.

It was shown that some behaviors of the network were mainly determined by the coupling strengths between oscillators. The current oscillator model can be improved by determining these coupling strengths using biological experiments.

## VI. ACKNOWLEDGMENTS

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