MODELING THE SYNCHRONIZATION OF BIOCHEMICALLY COUPLED CIRCADIAN OSCILLATORS

Tsz-Leung To and Michael A. Henson*
Department of Chemical Engineering
University of Massachusetts
Amherst, MA 01003-3110

Frances J. Doyle III
Department of Chemical Engineering
University of California
Santa Barbara, CA 93106-5080

Abstract

The master clock responsible for mammalian circadian rhythm generation is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. The clock consists of about 10,000 pacemaker cells that collectively generate the circadian rhythm. Oscillations at the individual cell level have been attributed to autoregulatory negative feedback loops involving the Per and Cry genes, as well as positive feedback via the Bmal1 gene. Considerably less is known about the cellular mechanisms involved in the synchronization of individual oscillators such that a coherent circadian rhythm is generated at the cell population level. The objective of this contribution is to develop a conceptual model of circadian rhythm generation and synchronization by constructing large populations of biochemically coupled circadian oscillators. A previously developed model of gene regulation is used to represent a single pacemaker cell. A generic neurotransmitter is assumed to be rhythmically produced by each cell. The local neurotransmitter concentration observed by each cell is determined by summing the non-uniform contributions of adjacent cells. The effects of neurotransmitter binding and intracellular signaling are modeled by assuming that the local neurotransmitter concentration modulates the maximum transcription rate of Per mRNA in each pacemaker cell. Simulation results are presented to illustrate the effects of neurotransmitter release and modulation on synchronization dynamics.

Keywords

Cellular oscillations, circadian rhythm, cell population modeling

Introduction

The suprachiasmatic nuclei (SCN) of the anterior hypothalamus govern circadian rhythms in physiology and behavior in mammals (Klein et al., 1991). This master clock is entrained to the 24-hour period by the daily light-dark cycle, which acts via direct and indirect retina-to-SCN neural pathways. The SCN is composed of multiple, single-cell circadian oscillators that synchronize and produce coordinated circadian outputs that ultimately regulate overt rhythms (Welsh et al., 1995)

The mammalian clock consists of an autoregulatory transcriptional/translational feedback loop at the molecular level (Reppert 1998). The positive elements of this loop are two basic transcription factors, CLOCK and BMAL1, which appear to drive the transcription of three Period (mPer1-3) genes and one cryptochrome gene (mCry1)

(Gekakis et al., 1998). The mPER and mCRY proteins form heterodimers that are capable of negatively regulating their own transcription (Griffin et al., 1999). The same elements that regulate the core feedback loop also directly regulate the rhythmic transcription of some clock-controlled genes (Jin et al., 1999). Proteins encoded by these genes function as output signals to certain effector systems. Light input appears to reset the core loop by activating mPer1 and mPer2 gene expression (Albrecht et al., 1997).

Synchronization of individual oscillators produces a coherent circadian rhythm at the cell population level. The precise nature of the SCN coupling interactions that mediate synchronization remains unknown. The inhibitory transmitter γ-aminobutyric acid (GABA) has been postulated as a coupling agent for SCN neurons (Liu et al., 1997). Vasoactive intestinal peptide (VIP) also has been hypothesized to be responsible for intercellular communication among SCN neurons (Hasting et al., 2004).

In the paper a conceptual model of circadian rhythm generation and synchronization is presented. A small ensemble of individual circadian oscillators is assumed to be coupled through the action of a generic neurotransmitter such as GABA and VIP. We have previously used the cell ensemble modeling approach to investigate the synchronization of glycolytic oscillations (Henson et al., 2002) and respiratory oscillations (Henson, 2004) in yeast. While simpler models of circadian cell synchronization have been proposed (Kunz et al, 2003; Petri et al., 2001; Ueda et al., 2002), our model is more biologically plausible due to the single cell model used and the inclusion of neurotransmitter binding and signaling effects.

Model Formulations

A previously developed model of gene regulation (Leloup & Goldbeter, 1998) is used to represent a single pacemaker cell. The model describes the circadian oscillations of the Period (PER) and Timeless (TIM) proteins in *Drosophila*. The core elements are multiple phosphorylations of PER and TIM and the negative feedback exerted by a nuclear PER-TIM complex on the transcription of the *per* and *tim* genes. The single cell model produces sustained oscillations in conditions corresponding to continuous darkness or to entrainment by light-dark cycles.

We augment this single cell model with additional equations to represent intercellular communication. A generic neurotransmitter is assumed to be rhythmically produced with a predetermined phase relationship with the PER mRNA. An ensemble of individual cells is placed on a two-dimensional grid to mimic the spatial organization of circadian pacemakers. Rather than explicitly modeling neurotransmitter diffusion, the local neurotransmitter concentration observed by each cell is determined by summing the contributions of adjacent cells. Diffusion limitations are assumed to restrict the domain of influence such that only neighbors and next nearest neighbors affect a particular cell. The associated weight factors are chosen using the approach suggested by Kunz and Achermann (2003). The weight factors for each cell are constrained to sum to zero such that cells in a perfectly synchronized population have the same oscillation period and amplitude as an uncoupled cell.

Neurotransmitter binding and intracellular signaling are modeled by assuming that the local neurotransmitter concentration modulates the maximum transcription rate of PER mRNA in each pacemaker cell. Neurotransmitter binding is assumed to follow standard saturation kinetics. The following coupling equations are added to the Leloup and Goldbeter (1998) model to represent the *i*-th cell in the ensemble:

$$\rho_i(t) = aM_{P,i}(t-\theta) + b \tag{1}$$

$$\gamma_i(t) = \sum_{j=1}^{N} \alpha_{ij}\rho_j(t) \tag{2}$$

$$v_{sP,i}(t) = v_{sP0,i} + \frac{\Delta v_{sP}\gamma_i(t)}{K_M + \gamma_i(t)} \tag{3}$$

where ρ is the concentration of the neurotransmitter produced by the cell, M_P is the PER mRNA concentration, γ is the local neurotransmitter concentration observed by the cell, α are the weight factors, *N* is the total number of cells in the ensemble, v_{sP} is the transcription rate of PER mRNA, v_{sP0} is the basal transcription rate in the absence of neurotransmitter, Δv_{sP} is the maximum effect of the neurotransmitter on the PER mRNA concentration and K_m is the saturation constant of receptors. For simplicity the phase lag θ and the baseline deviation *b* are assumed to be zero such that neurotransmitter production is proportional to Per mRNA.

Nominal parameters values for the gene regulation model produce sustained oscillations with period of 24 hours (Leloup and Goldbeter, 1998). The parameter values used for the coupling model are presented with the simulation results. A dynamic simulation code was developed in MATLAB using the ordinary differential-algebraic equation solver ode23t. All the simulation results presented below were generated with nine oscillators.

Results and Discussions

Dynamic simulation tests were performed to evaluate the hypothesized synchronization mechanism. Figure 1 depicts nine uncoupled pacemaker cells which produce unsynchronized oscillations with a 24 hour period. Figure 2 depicts the synchronization of nine coupled cells with the same initial conditions as in Figure 1. The same weight factors are used for each cell: $\alpha_{ii} = -1$ for the cell itself and $\alpha_{ij} = +1/8$ for each of the eight surrounding cells. The other model parameters are chosen as $K_m = 2$, $\Delta v_{sP} = v_{sP}$ and $a = 0.5$. Figure 2 shows that synchronization is achieved after three oscillation periods with these parameter values. We found that the value used for the

weight factor α_{ii} has a substantial impact on the population dynamics. A value of zero leads to very slow synchronization, while positive values produce an unsynchronized population. These results are difficult to reconcile with the envisioned coupling mechanism since neurotransmitter produced by any cell is expected to trigger the same receptor binding and intracellular signaling events. The coupling model is based on the assumption that a pacemaker cell has an inhibitory effect on itself, while neurotransmitter released from neighboring cells is excitatory. The mathematical necessity and biological plausibility of this self inhibitory effect require further investigation.

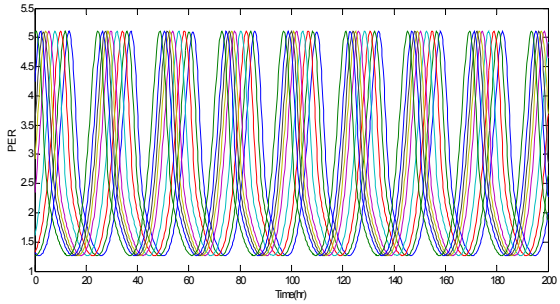

Figure1. Uncoupled oscillators with nominal parameter values suggested by Leloup and Goldbeter (1998).

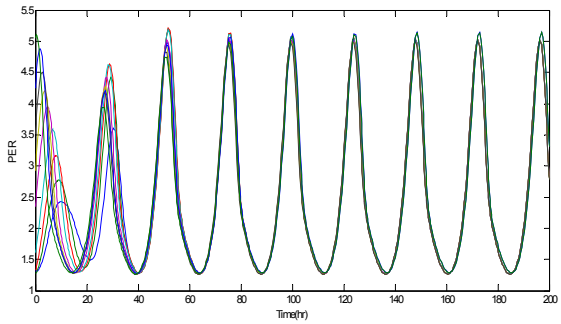

Figure 2. Synchronization dynamics of nine coupled pacemaker cells with K_m = 2, Δv_{sP} = 1, a = 0.5 and identical weight values for each cell.

Figure 3 shows the effect of increasing the saturation constant for receptor binding K_m. The synchronization dynamics are much slower than for the nominal K_m value depicted in Figure 2. Although not shown in Figure 3, the population eventually becomes completely synchronized. This result is physically reasonable as a larger K_m value results in slower receptor saturation and therefore a longer time for synchronization. However, the individual cells become unstable for low K_m. Thus the dynamics of receptor binding must be maintained within a certain range to ensure proper single cell function and rapid population synchronization. Our future work will include delineating such stability ranges for key model parameters.

The effect of increasing the neurotransmitter coupling parameter Δv_{sP} is shown in Figure 4. Surprisingly the asymptotic solution consists of two unsynchronized subpopulation that oscillate 180 degrees out of phase. While each subpopulation maintains a 24 period, much smaller oscillation amplitudes are obtained compare to the completely synchronized case in Figure 2. As expected no synchronization is observed when a very small value of Δv_{sP} is used. There appears to be a fairly limited range of Δv_{sP} values that produce a completely synchronized population.

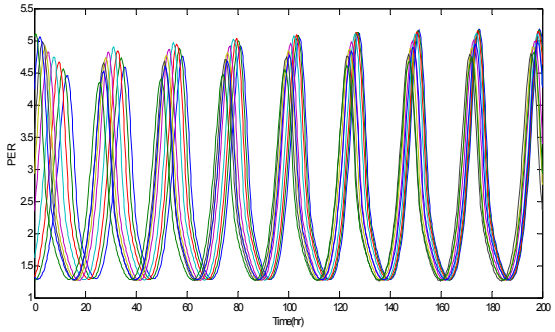

Figure 3. Synchronization dynamics of nine coupled pacemaker cells with K_m = 10, Δv_{sP} = 1, a = 0.5, and identical weight values for each cell.

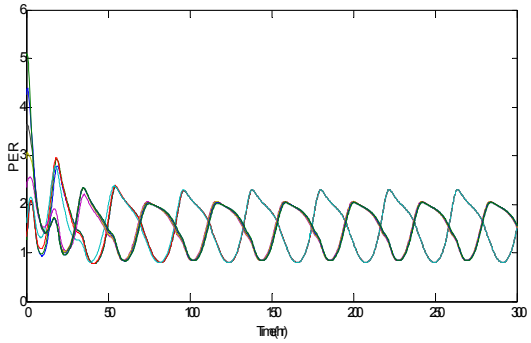

Figure 4. Synchronization dynamics of nine coupled pacemaker cells with K_m = 2, Δv_{sP} = 10, a = 0.5, and identical weight values for each cell.

The previous simulations were performed using identical weight values for each cell: α_{ii} = -1 for the cell itself and α_{ij} = +1/8 for each of the eight surrounding cells. This scheme implicitly assumes that the two-dimensional grid actually represents a type of spheroid where cells located along an edge are adjacent to cells on other edges. This approximation is not valid along the boundaries of the domain due to non-uniform neurotransmitter diffusion. The impact of such edge effects is investigated by modifying the weight specifications such that each cell is not uniformly affected by other cells. Each corner cell is only affected by three surrounding cells such that α_{ij} = +1/3. Each cell located along an edge but not in a corner is affected by five surrounding cells such that α_{ij} = +1/5. Figure 6 depicts the synchronization dynamics for these non-uniform weightings. Due to reduced overall coupling between pacemaker cells, more time is required to achieve a completely synchronized population compared to

uniform weightings (see Figure 2). However, the impact of edge effects is expected to be less significant for large populations with a smaller percentage of the total cells located along the domain boundary.

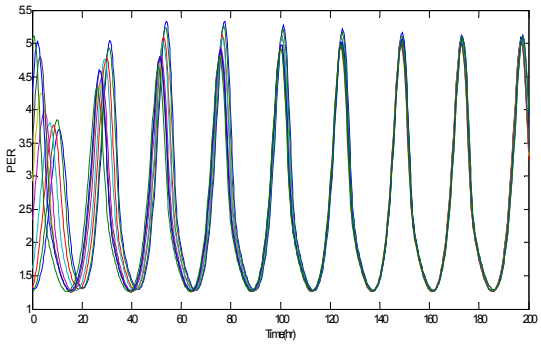

Figure 5. Synchronization dynamics of nine coupled pacemaker cells with $K_m = 2$, $\Delta v_{sP} = 1$, $a = 0.5$ *and non-uniform weight values..*

Conclusions and Future Work

A conceptual model was developed to investigate a proposed mechanism for synchronization of circadian pacemaker cells. Each cell was represented by the gene regulatory model of Leloup and Goldbeter (1998). An ensemble of nine cells placed on a two-dimensional grid was assumed to be coupled through the diffusion and binding of a generic neurotransmitter rhythmically produced by each cell. The population model was shown to produce synchronization of individual cell oscillations for a range of coupling parameters.

This paper represents our initial research on modeling circadian rhythm generation and synchronization. Our short-term objectives for future work focus on further refinement and analysis of the proposed coupling mechanism. This work will include: (1) the derivation of stability conditions for the coupling weights and other key model parameters such as K_m and Δv_{sP}; (2) the inclusion of light input and analysis of its effect on population synchronization and entrainment; and (3) the simulation of much larger ensembles with thousands of pacemaker cells. Our long-term goal is the development of experimentally based models of gene regulation, intracellular signaling and intercellular coupling that provides unique insights into mammalian circadian function.

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