Chapter 55
Modeling of Spatially Extended Delay-Induced Circadian Oscillations Synchronized by Cell-to-Cell Communications

Dmitry A. Bratsun and Andrey P. Zakharov

Abstract We propose a spatially extended deterministic model with time delay for the circadian oscillations of protein concentrations. Our model is based on the nonlinear interplay between two proteins forming a time-delayed feedback loop comprised both positive and negative elements. In order to study spatio-temporal dynamics of the system, a novel algorithm of the numerical simulation of time-delayed reaction-diffusion systems is proposed. The algorithm based on finite difference method involves storing in a computer memory not all, but some selected nodal data, and the subsequent interpolation to determine intermediate values. Spatio-temporal protein patterns excited in complete darkness are studied numerically. It is shown that the synchronization of biorhythms can be produced by either of two mechanisms: (i) basal transcription factors and (ii) cell-to-cell communication.

Keywords Time-delay · Circadian rhythms · Pattern formation

Circadian rhythms are biological rhythms that are common to almost all living organisms. A remarkable feature of these rhythms is that they are not simply a response to 24 hours environmental cycles imposed by the Earth's rotation, but instead are generated internally by cell autonomous biological clocks. After the decades of research, the genetic mechanism of circadian oscillations has been widely recognized as a core of this phenomenon. Thus, the transcription/translation processes should be taken into account seriously when one starts to model the circadian rhythms. As it is known now, a feedback influence of protein on its own expression can be delayed which leads to non-Markovian phenomena in this system [1]. It is evident that the delay prevents the system from achieving equilibrium, and results instead in the familiar limit cycle oscillations. The deterministic and stochastic properties of gene regulation taking into account the non-Markovian character of gene transcription/translation was studied in [2, 3]. We have shown that time delay in the protein production or degradation may change the behavior of the system from sta-

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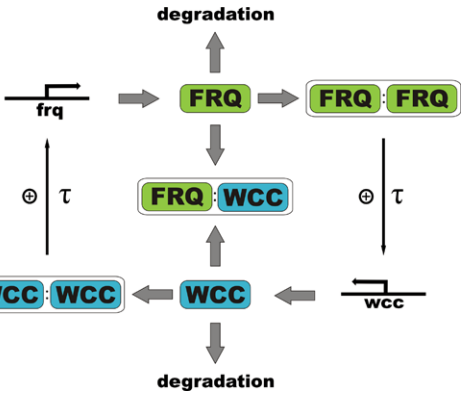

Fig. 55.1 Network architecture of the circadian rhythm molecular components in *N.crassa*

tionary to oscillatory even when a deterministic counterpart of the stochastic system exhibits no oscillations.

The filamentous fungus Neurospora crassa is an excellent model system for investigating the mechanism of circadian rhythmicity because of the wealth of genetic and biochemical techniques available. It is easy to grow and has a haploid life cycle that makes genetic analysis simple since recessive traits will show up in the offspring. The genome of N. crassa was recently reported as completely sequenced and all data are freely available online. The genome is about 43 megabases long organized in 7 chromosomes and includes approximately 10000 genes. With advances in molecular biology, understanding of the Neurospora circadian clock has improved, and main genetic components of this clock have been determined. Please see Fig. 55.1 for a simplified graphical depiction of this network architecture.

The primary molecular components of the circadian oscillator are the frequency and white collar genes (white collar 1 (wc-1) and white collar 2 (wc-2)) which form a feedback loop comprised of both positive and negative elements [4]. The corresponding white-collars proteins WC-1 and WC-2 are transcription factors which form a heterodimeric complex known as the white collar complex WCC. The WCC acts as a positive regulator of FRQ by activating its transcription in the dark and in response to blue light (WC-1 is a photoreceptor), while the frequency protein dimerizes and then acts as a negative regulatory element by binding to and inhibiting the function of WCC. As the circadian cycle progresses the FRQ protein is phosphorylated and degraded which allows the cycle to begin anew. Also, these species can be removed by association with WCC to form FRQ/WCC complexes. Furthermore the production of WC-1 and FRQ proteins are subject to a delay on the order of several hours after the mRNAs are made again mediated through an unknown post transcriptional mechanism. The previous experimental efforts have highlighted also the importance of degradation of the core clock components, particularly that of FRQ, plays in establishing the period of the circadian rhythm. However quantitative information about the magnitude of the degradation rates are still lacking for this core clock component.

In this work, we propose the model of temporal circadian dynamics of the Neurospora which is work is a further simplification of models proposed by Smolen

et al. [4] and Sriram and Gopinathan [5]. The main difference to earlier approaches is that we derive the dynamic equations directly from a set of biochemical reactions. In the part of spatially extended delay-induced circadian oscillations in Neurospora our modeling seems to be first in the literature. Perhaps this can be explained by a prevailing tradition in the study of circadian oscillations. It is possible also that this is due to computational difficulties that arise when studying spatially extended reaction-diffusion systems with time delay. To overcome these difficulties, we propose a new method for the numerical study of such systems and focus on the deterministic spatio-temporal dynamics neglecting the stochasticity of the system.

In the model of the N. crassa oscillator we assume there are two primary components: the heterodimeric WCC complex and the FRQ protein. We start our analysis from a set of biochemical reactions constituting the mechanism of bioclock and finally arrive to the following two-variable spatially extended systems [3]:

$$\frac{\partial F}{\partial t} = \frac{1}{1+4K_1^F F}\left(A_F + k_F \frac{K_1^W K_2^F \varphi(t) W^2(t-\tau)}{1+K_1^W K_2^F \varphi(t) W^2(t-\tau)} - B_F F - kFW\right) + D\left(\frac{\partial^2 F}{\partial x^2} + \frac{\partial^2 F}{\partial y^2}\right), \tag{55.1}$$

$$\frac{\partial W}{\partial t} = \frac{1}{1+4K_1^W W}\left(A_W + k_W \frac{K_1^F K_2^W F^2(t-\tau)}{1+K_1^F K_2^W F^2(t-\tau)} - B_W W - kFW\right) + D\left(\frac{\partial^2 W}{\partial x^2} + \frac{\partial^2 W}{\partial y^2}\right). \tag{55.2}$$

Here F and W stand for number of isolated monomers of FRQ and WCC respectively and D is the coefficient of protein diffusion in the cell. For simplicity, we assume that the diffusion coefficients of FRQ and WCC proteins are equal. Even supposing that the delay is defined by the length of the path traveled by RNA polymerase along the gene, one obtains different values since the wc-1 gene (it is part of the locus NCU02356.5) is a one and a half times longer than the frq gene (it is in the locus NCU02265). But the exact values of the delays are currently unknown, and for simplicity we assume that time delays have the same values: $\tau_F = \tau_W = \tau$. The model (55.1)–(55.2) includes a positive feedback loop in which activation of FRQ production by WCC increases the level of FRQ, leading to an increase in the level of WCC itself. The negative feedback loop in which FRQ represses the frq gene transcription by binding to the WCC is also modeled.

As it is known, the Neurospora not only has the advantage that powerful genetics and molecular techniques are able to be performed on it, but it has another advantage—circadian rhythms of conidiation that is easily monitored on Petri dishes. To observe the phenotypic expression of the Neurospora clock, conidia are inoculated at some place of a Petri dish. After growth for a day in a constant light, the position of the growth front is marked and the culture is transferred to constant dark. The light-dark transfer synchronizes the cells in the culture and sets the clock running from subjective dusk. Following transfer, the growth front is marked every 24 hours with the aid of the red light, which has no effect on the clock. Growth rate

is constant and the positions of the readily visualized orange conidial bands (separated by undifferentiated mycelia) allow determination of both period and phase of the rhythm. Thus, the computational domain $\Sigma \in (x, y)$ where the protein fields are solved numerically can be interpreted as a flat area of two-dimensional physical space of a Petri dish occupied by the mycelium of Neurospora. In fact, N. crassa is multicellular organism, and the translation of proteins occurs within individual cells. But we can consider the mycelium of the fungus as a whole due to the important feature of Neurospora: the mycelium of the organism consists of branched hyphae which show apical polar growth. The fungal hyphae are typically composed of multiple cells or compartments demarcated by septa with the central pore sometimes up to 0.5 microns in diameter. Thus, the protein produced in the separate cells of Neurospora seems to be able to cross the intercellular walls, and we can assume an existence of joint molecular cloud of protein inside a whole organism.

In order to perform two-dimensional simulations of delayed-induced circadian oscillations governed by Eqs. (55.1)–(55.2), we define a two-dimensional domain $\Sigma : (0 < x < 200, 0 < y < 200)$ with the following conditions for concentrations of FRQ and WCC proteins imposed at the boundary of the domain:

$$\left.\frac{\partial F}{\partial x}\right|_{x=0,200} = 0, \qquad \left.\frac{\partial F}{\partial y}\right|_{y=0,200} = 0, \qquad \left.\frac{\partial W}{\partial x}\right|_{x=0,200} = 0, \qquad \left.\frac{\partial W}{\partial y}\right|_{y=0,200} = 0. \tag{55.3}$$

The initial-boundary value problem (55.1)–(55.3) has been solved by finite difference method described in the previous section. The explicit scheme was adopted to discretize equations. The equations and boundary conditions have been approximated on a rectangular uniform mesh 400×400 using a second order approximation for the spatial coordinates. In some calculations, we introduce the boundary Γ separating the region where the Reactions (55.1)–(55.2) take place and the nonreactive area.

If the protein can easily pass through this boundary, we consider it as a free interface between immiscible fluids. Then the concentration of reactant which diffuses through the surface subjects to the boundary condition

$$F_1|_\Gamma = F_2|_\Gamma, \qquad D_1 \left.\frac{\partial F_1}{\partial \mathbf{n}}\right|_\Gamma = D_2 \left.\frac{\partial F_2}{\partial \mathbf{n}}\right|_\Gamma, \tag{55.4}$$

where $\mathbf{n}$ is the vector normal to the interface, $D_{1,2}$ are the coefficients of protein diffusion in the areas on opposite sides of Γ.

The spatial phase synchroniation is the process when spatially distributed cyclic signals tend to oscillate with a repeating sequence of relative phase angles. We have noticed above that some external stimuli can synchronize the spatio-temporal behaviour of the system. The external control of this active medium can be performed, for example, via the basal transcription factors. We found the system is particularly sensitive to the basal transcription of the WCC protein governed by the parameter A_W. With increase of WCC produced via the basal transcription machinery, the spatio-temporal structure of the system becomes more ordered. In contrast to the distinct chaotic pattern at $A_F = A_W = 0$ formed due to break up of spiral waves,

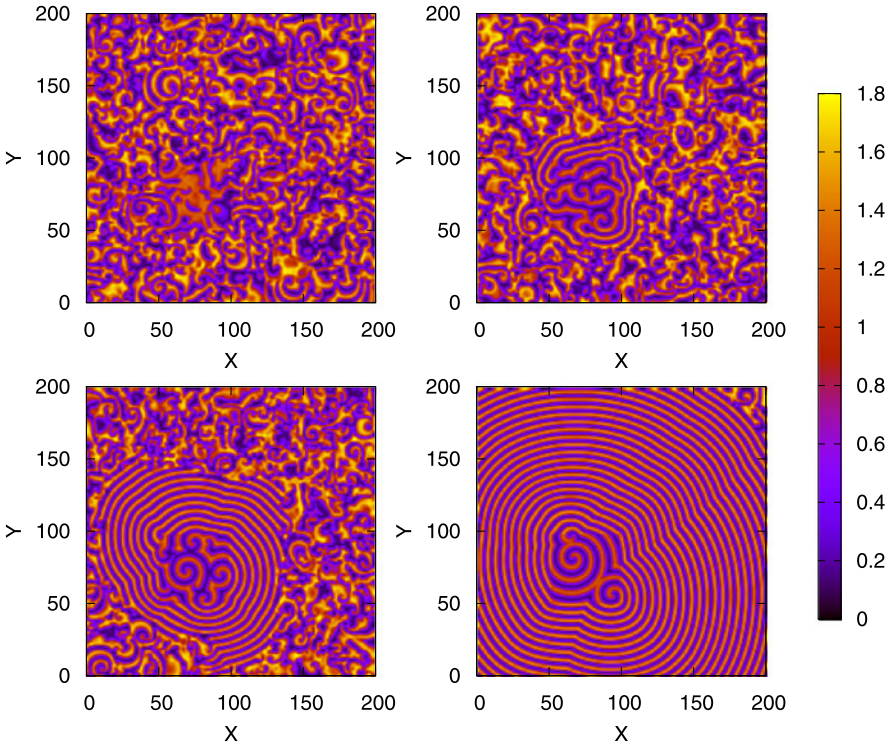

Fig. 55.2 The spatial synchronization of oscillations of the FRQ protein in the system with the basal transcription machinery, acting locally in the square area $50 < x < 100$, $50 < y < 100$ in the left lower quadrant of the domain Σ. In the rest of the domain the basal transcription is inhibited. *The frames* from *left* to *right* and from *up* to *down* correspond to times $t = 5000$, 7200, 11000, 20000 respectively. The coefficient of diffusion is $D = 0.01$

the structures for large values A_W looks more ordered. Since the basal transcription can be repressed, it can be switched on only in some places of the organism. So it would be interesting to see what happens when the basal transcription factors have been activated locally. Figure 55.2 gives an example of such numerical simulation. It is assumed that the basal transcription machinery ($A_F = 0$, $A_W = 2$) acts only in the area $50 < x < 100$, $50 < y < 100$ in the left lower quadrant of the domain Σ. It becomes effective at time $t = 5000$. We see that local basal transcription results in the global effect: it produces spatially synchronized oscillations over the whole domain Σ. From the point of view of nonlinear dynamics, the spiral traveling wave has recovered its structure. So, the process shown in figure is reverse to the core break up of spiral wave discussed above. It should be noted that the process of self-organization goes far beyond the area where the basal transcription works.

Finally we present the results of the numerical simulations showing that the synchronization of the circadian oscillations can occur due to intercellular communications via chemical signals (Figs. 55.3 and 55.4). We have attempted to simulate this

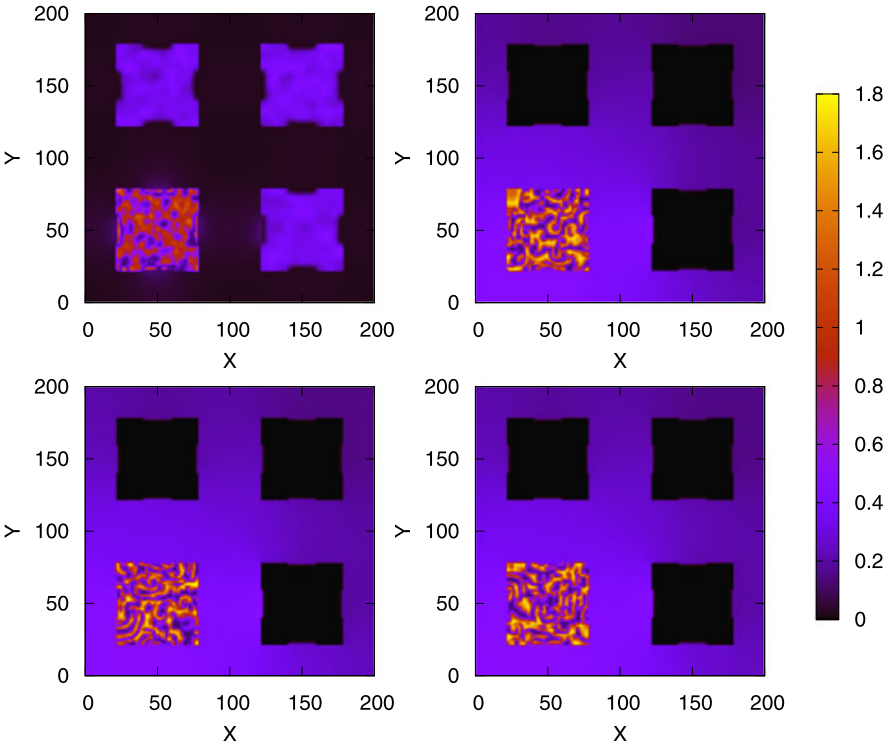

Fig. 55.3 Evolution of the concentration of the FRQ protein in complete darkness. There are four "cells" in the domain of integration, but only one of them is switched on. *The frames* from *left* to *right* and from *up* to *down* correspond to times $t = 200$, 5000, 10000, 20000 respectively

process by considering the four square areas of 60 to 60 referred to below as "cells", within which the system of Eqs. (55.1)–(55.2) for circadian oscillations has been integrated. The mixed boundary conditions have been imposed on the border of the area. There are four segments of the open border ("membranes") with the condition (55.4) of free diffusive penetration of the reagent species through fluid-fluid interface. The rest of the border is considered to be impermeable in accordance with the formula (55.3). Thus, each closed area is a rough model of living cell with its own circadian oscillations occurring inside the cell. Each area has four membranes and can communicate with the extracellular world via exchange of a certain protein.

We have supposed that such carrier of signals connecting the cell to the extracellular world is the FRQ protein. At the same time, we assume that the complex WCC cannot overcome the membrane and exists only inside the cell. Being in the extracellular space the protein does not react, but it can diffuse. Its dynamics obeys the standard diffusion equation.

Figure 55.3 presents the evolution of the concentration of FRQ in complete darkness and zero basal transcription, when only one cell (#3) is switched on. At these parameters the spiral wave develops into phase turbulence due to mechanism of the

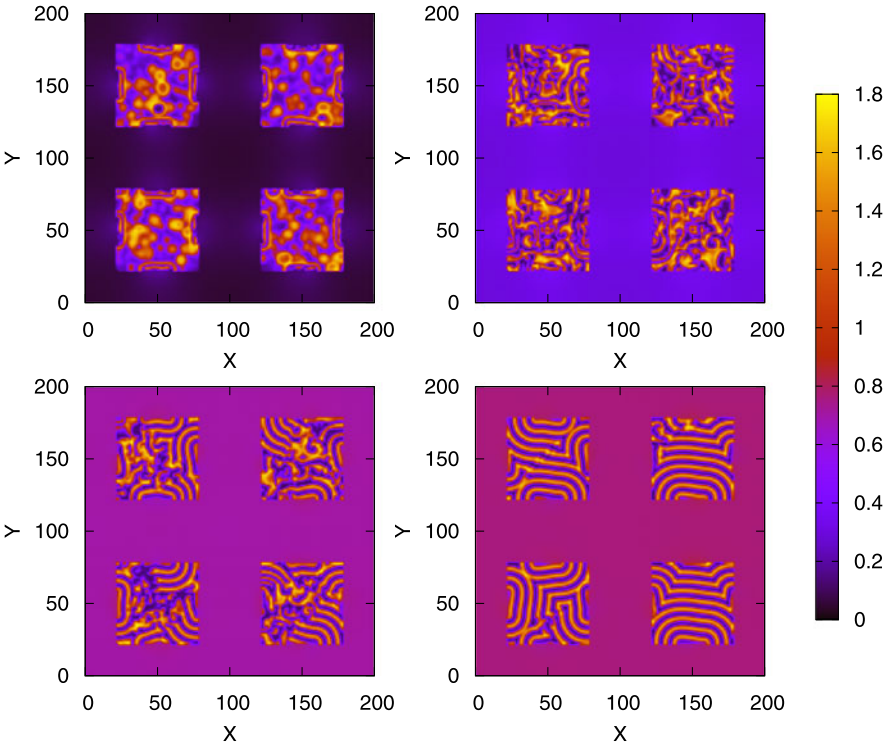

Fig. 55.4 The spatial synchronization of oscillations of the FRQ protein due to intercellular communications. *The frames* from *left* to *right* and from *up* to *down* correspond to times $t = 200$, 1000, 5000, 15000 respectively

core breakup. If the system does not receive any external signals, the chaotic state can be maintained for indefinitely long time periods. In the case shown in Fig. 55.3, there is the flow of FRQ from the cell #3 outward. The protein gradually fills the extracellular space due to diffusion. As long as the cell is the only cell in the system, a state of chaos continues to persist. The spatial synchronization of circadian oscillations occurs when there are a whole group of functioning cells in the system (Fig. 55.4). We found that the state of spatially synchronized bioclocks working in the different cells is achieved, when the concentration of transmitted protein in the extracellular space reaches a certain average value. The oscillations in the cell group are synchronized, when the level of concentration is about $F \approx 0.8$. Thus, in the group consisting of a large number of cells, the spatial synchronization seems to be attained more quickly between the cells that intensively produce the protein and exchange more vigorously the signals with their neighbors. This conclusion is supported by the recent experimental findings demonstrating spatio-temporal synchronization of circadian oscillations in a population of E. Coli [6]. The authors have noticed that oscillations arise because the acyl-homoserine lactone (this is a small molecule that can diffuse across the cell membrane) has a dual role, both en-

abling activation of the genes necessary for intracellular oscillations and mediating the coupling between cells.

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