

Deterministic Modeling Spatio-Temporal Dynamics of Delay-Induced Circadian Oscillations in *Neurospora crassa*

Dmitry Bratsun and Andrey Zakharov

Abstract We propose a spatially extended deterministic model with time delay for the circadian oscillations in the fungal species *Neurospora crassa*. The temporal behavior of the system is governed by a two variable model based on the nonlinear interplay between the *FRQ* and *WCC* proteins which are products of transcription of *frequency* and *white collar* genes. We show numerically that the model accounts for various features observed in experiments. Spatio-temporal protein patterns excited in *Neurospora* in complete darkness are studied for different initial conditions. It is shown that basal activation of transcription factors has a strong effect on pattern formation.

Keywords Time-delay · Circadian rhythms · Neurospora · Pattern formation

1 Introduction

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 h 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. 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

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Fig. 1 Conidiation of *Neurospora* growing on solid agar medium



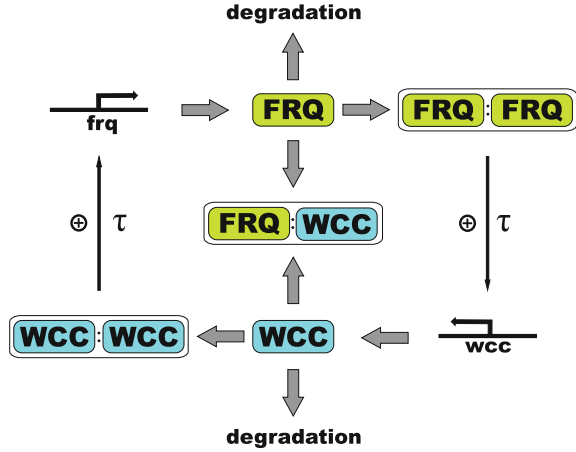
and stochastic properties of gene regulation taking into account the non-Markovian character of gene transcription/translation were studied in [2, 3].

In fact, a filamentous fungus *Neurospora crassa* (hereinafter *N.c.*) is an excellent model system for investigating the mechanism of circadian rhythmicity because of the wealth of genetic and biochemical techniques available. *N.c.*'s rhythms of asexual spore-formation produces easily assayed bands of conidia spores in cultures growing on solid agar medium (Fig. 1). A number of mutations are available that affect circadian rhythmicity, and molecular analyses of some of these genes have contributed to models for the circadian oscillators that are currently thought to be applicable to many other organisms. No wonder that since the rhythm was discovered in the *N.c.* [4], this organism has become a research polygon in the declared area.

With advances in molecular biology, understanding of *N.c.*'s circadian clock has improved, and main genetic components of this clock have been determined [5].

In this work, we propose the model of circadian oscillations in the *N.c.* which is a further simplification of models proposed by Smolen et al. [6] and Sriram and Gopinathan [7]. In this paper we have used the deterministic description of the system focusing on its spatial dynamics. In the part of spatially extended delay-induced circadian oscillations 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 reaction-diffusion systems with time delay. To overcome these difficulties, we have proposed a new method for the numerical study of such systems [8].

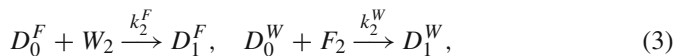
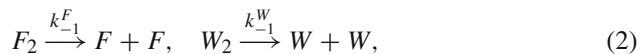
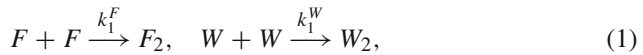
Fig. 2 Network architecture of the circadian rhythm molecular components in *Neurospora*

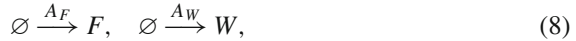
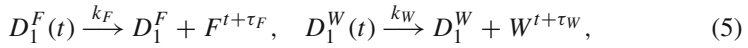
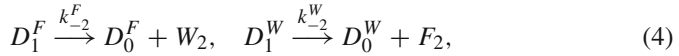


2 Two Variable Model of Circadian Rhythms in Neurospora

The simplified graphical depiction of protein network architecture responsible for circadian rhythms is presented in Fig. 2. The primary molecular components of the circadian oscillator are the *frequency* (*frq*) and *white collar* genes (*wc-1* and *wc-2*) which form a feedback loop comprised of both positive and negative elements [5]. The white-collar proteins *WC-1* and *WC-2* are transcription factors which form a heterodimeric complex known as the *WCC* complex. The *WCC* acts as a positive regulator of *FRQ* by activating its transcription in the dark, 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. 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.

In our model 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





where F and W stand for number of isolated monomers of FRQ and WCC respectively. In (1)–(8) the reactions of dimerization and dedimerization are given by (1) and (2), the transitions between operator-site states for each protein due to binding and unbinding some dimer to the promoter of corresponding gene are reflected by (3) and (4), the time-delayed productions of corresponding proteins is given by (5), the linear and non-linear degradation are described by (6) and (7), the basal transcriptions are given by (8) respectively. By supposing that reactions of dimerization (1)–(2) and binding/unbinding (3)–(4) are fast in comparison with the processes of production/degradation of proteins (5)–(8), we can conclude that their dynamics quickly enters into a local equilibrium and arrive finally to the following two-variable spatially extended system (the procedure for derivation of the equations is not presented here, see [9] for more details):

$$\frac{\partial F}{\partial t} = \frac{1}{1 + 4K_1^F F} \left(A_F + k_F \frac{K_1^W K_2^F W^2 (t - \tau)}{1 + K_1^W K_2^F W^2 (t - \tau)} - B_F F - kFW \right) + D \left(\frac{\partial^2 F}{\partial x^2} + \frac{\partial^2 F}{\partial y^2} \right), \quad (9)$$

$$\frac{\partial W}{\partial t} = \frac{1}{1 + 4K_1^W W} \left(A_W + k_W \frac{K_1^F K_2^W F^2 (t - \tau)}{1 + K_1^F K_2^W F^2 (t - \tau)} - B_W W - kFW \right) + D \left(\frac{\partial^2 W}{\partial x^2} + \frac{\partial^2 W}{\partial y^2} \right). \quad (10)$$

Here 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 are equal to the same value τ . As one can see, the model (9)–(10) 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. Generally, the temporal part of model (9)–(10) is a further simplification of models proposed by Smolen et al. [6]

and Sriram and Gopinathan [7]. Despite of its simplicity, the equations (9)–(10) still reflect all the characteristic features of circadian rhythms in *N.c.*

In (9)–(10) we also have taken into account a basal transcription governed by rates A (8). In eukaryotes, an important class of transcription factors called basal or general transcription factors (GTFs) are necessary for transcription to occur. Many of these GTFs do not actually bind DNA but are a part of the large transcription preinitiation complex that interacts with RNA polymerase directly.

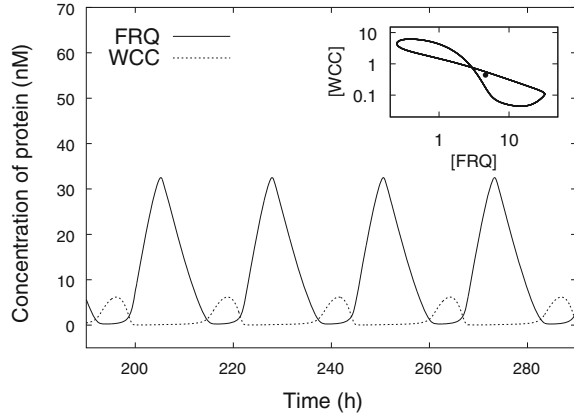
As it is known, the *N.c.* not only has the advantage that powerful genetics and molecular techniques are able to be performed on it, but it has another advantage—the circadian rhythms of conidiation are easily monitored on Petri dishes. To observe the phenotypic expression of the clock, conidia are 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. The 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 *N.c.* In fact, *N.c.* is a 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 *N.c.*: 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 $0.5 \mu\text{m}$ in diameter. Thus, the protein produced in the separate cells of *N.c.* 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 circadian oscillations governed by Eqs. (9) and (10), we define the domain $\Sigma: (0 < x < 200, 0 < y < 200)$ with zero-flux boundary conditions for concentrations of *FRQ* and *WCC* proteins. The initial-boundary value problem (9)–(10) has been solved by a finite difference method as described in detail in [8]. The explicit scheme was adopted to discretize equations. The equations have been approximated on a rectangular uniform mesh 400×400 using a second order approximation for the spatial coordinates.

3 Spatio-Temporal Nonlinear Dynamics

In the numerical calculations we have used the following fixed values of parameters: $K_1^F = 5$, $k_F = 8 \text{ nM/h}$, $K_1^W = 5$, $k_W = 4 \text{ nM/h}$, $K_2^F = 5$, $B_F = 0.3 \text{ h}^{-1}$, $K_2^W = 5$, $B_W = 0.4 \text{ h}^{-1}$, $k = 30 \text{ nM}^{-1} \text{ h}^{-1}$, $\tau = 6 \text{ h}$, $D = 0.01 \text{ m}^2 \text{ s}^{-1}$. These values have been taken from [6], plus we add some our own, e.g. for dimerization and diffusion.

Fig. 3 The time series and phase portrait (*inset*) of FRQ (solid line) and WCC (dashed line)



In Fig. 3, we plot the time series and phase portrait of the total number of FRQ and WCC proteins obtained by solving nonlinear equations (9)–(10) for zero basal transcription. The period of oscillations is about 22.65 h. Above the Hopf bifurcation point, in the phase space there is an unstable stationary point bounded by a stable limit cycle (Fig. 3, *inset*). All trajectories are attracted to the periodic solution. No subcritical oscillations have been found, and the transition seems to occur smoothly.

Let us discuss now the results of numerical simulation of spatially extended model (9)–(10). Two different cases of initial conditions will be considered. In the first case shown in Fig. 4, the initial state of the system was the random distribution of the FRQ and WCC proteins over the entire area of integration. It corresponds to a situation where each node initially has its own phase of oscillations. At time $t = 0$, all these rhythms are independent, but in the process of evolution they enter into the nonlinear interaction, which results in different types of spatio-temporal behavior. From the point of view of biology, a random distribution of initial phases is somewhat artificial, since a synchronization of biorhythms in cells occurs at the stage of embryonic development of the organism. Nevertheless, the numerical study of the system with random initial conditions allows developing a deeper understanding of the potential of the system to self-organize and to evaluate its probable forms of pattern formation.

Since FRQ and WCC are always in anti-phase, we can choose only one of them to illustrate the system dynamics. Figure 4 presents the patterns formed by the concentration of FRQ protein shown for four consecutive points of time. As it can be seen from figure, the nonlinear dynamics of spatially extended system consists of two distinct oscillatory modes. One is the oscillations occurring in spatially ordered interacting cells (Fig. 4, $t = 500$). The characteristic size of the cells increases slowly as time goes on. Thus, this is a typical quasi-standing wave pattern. The second oscillatory mode is a spiral traveling-wave pattern (Fig. 4, $t = 3000$). These waves arise from selected initial disturbances (Fig. 4, $t = 500$). Each spiral wave travels outward in all directions from its source. It continues until the spiral wave pattern occupies the entire domain Σ . Note that if the front of wave looks more or less orderly, by

entering deeply inside the secondary instability area there have appeared numerous secondary centers of the excitation of the spiral waves. The nonlinear interaction between them leads to the formation of chaotic pattern (Fig. 4, $t = 4100$).

Thus, the evolution of the system passes through two stages: first, a slowly time-varying cellular structure has appeared, which synchronizes the oscillations of different nodes. Since the system is far away from equilibrium, the fluctuations give rise to several spiral traveling waves which immediately break up in the core inducing the spatiotemporal chaos. Even though the level of the protein oscillates randomly in space, the system is in standby mode. We will show below that some external or internal stimuli can synchronize the system in space and time.

Let us consider the second case of initial conditions, when evolution of the system starts from a small local perturbation of *FRQ* (or *WCC*) against zero field in the remaining part of the domain Σ . It was established experimentally that the front of mycelium propagates radially in all directions with the constant velocity starting from the location where it was placed initially. It should be emphasized that the local phase of the oscillations caused by the regular concentric traveling wave is determined by the phase of the initial perturbation in the bud, and the spatio-temporal pattern as a whole is formed during the morphogenesis of the organism.

Thus, the oscillations in the bud can be referred to as a “global clock” of *Neurospora*. Figure 5 presents the pattern after 125 hours of the evolution (left) and the one-dimensional cross-section of the protein field (right). We see that *N.c.*'s conidia have formed six bands that correspond to the period of oscillations about 22.65 h (compare with spatial pattern shown in Fig. 1).

4 Effect of Basal Transcription on Pattern Formation

The interesting properties of the circadian rhythms reflected in our model, are the synchronization and disappearance of oscillations with an increase of the basal production rate of *FRQ* or *WCC*, which occur, for example, under constant light conditions. Figure 6 shows the various dynamic regimes in the parameter plane spanned by two rates of basal transcription. The Hopf bifurcation curve divides the parameter plane into the part where the stable periodic solution exists (inside the balloon), and the part where there is stable steady state (outside the balloon). One can see that when the rate exceeds some critical value, the amplitude of oscillations becomes zero (Fig. 6, *inset*).

It should be noted that our model predicts that the period of oscillations remains robust to increases in A while the amplitude of oscillations evidently does not. It is interesting to note that this behavior directly contradicts the predictions made by some conceptual models directly correlating period length to the amplitude of oscillations in the core molecular clock components. This fact could be crucial for the experimental proof which of mechanisms is really responsible for oscillations in the *N.c.*

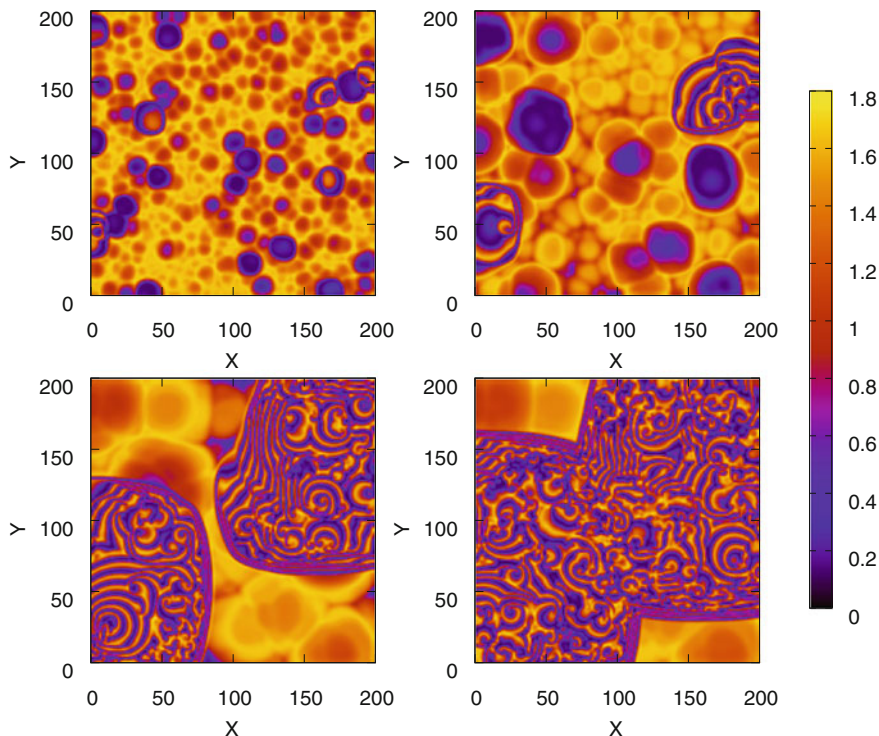


Fig. 4 The evolution of FRQ concentration without the basal transcription. The frames from *left* to *right* and from *up* to *down* correspond to times $t = 500, 1200, 3000, 4100$ respectively

The spatial phase synchronization 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 behavior of the system. The external control of this active medium can be performed, for example, via the basal transcription factors. We found that system dynamics is particularly sensitive to the basal transcription of the WCC protein. Figure 7 presents the density plots of FRQ concentration at time $t = 5000$ for four different values of A_W and $A_F = 0$. 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 $A_F = A_W = 0$ (Fig. 7a) formed due to break up of spiral waves, the structures shown in Fig. 7b–d for $A_W = 1; 2; 2.8$ respectively are frozen in space, i.e. each point oscillates periodically in accordance with its phase imposed by spatial pattern. The period of oscillations increases slightly with growth of the rate of basal transcription A_W . We found that further increase of A_W causes a sharp rise in FRQ synthesis and the termination of oscillations. It corresponds to a crossing of the Hopf bifurcation curve indicated in Fig. 6.

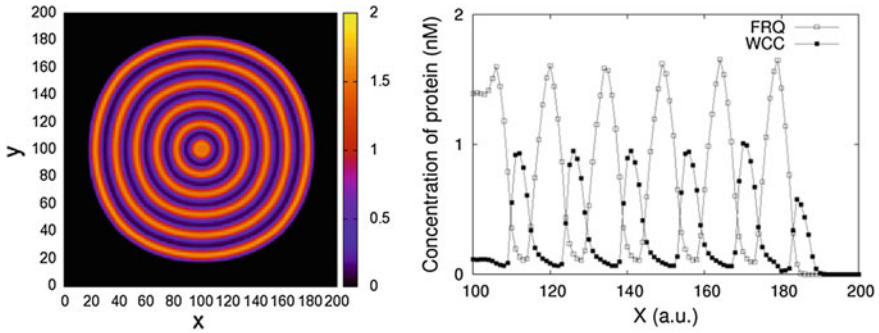
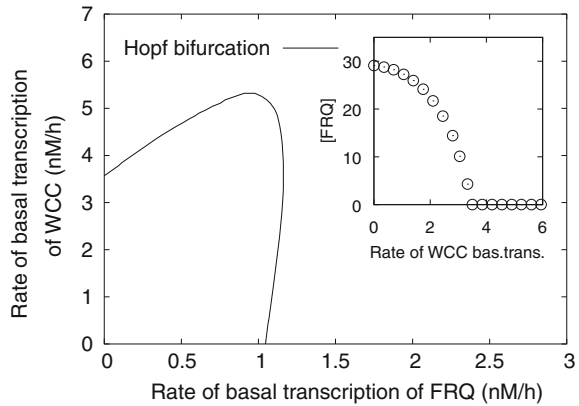


Fig. 5 The density plot of *FRQ* protein after 125h of evolution developed from the initial disturbance in the center (*left*); transverse profiles of *FRQ* (*open squares*) and *WCC* (*black squares*) proteins at time $t = 125$ (*right*)

Fig. 6 The neutral curve for the Hopf bifurcation (*solid line*) shown in the parameter plane spanned by two rates of basal transcription. The bifurcation diagram showing the amplitude of oscillations of *FRQ* versus A_W for $A_F = 0$ is plotted in the *inset*



5 Conclusions

The spatially extended deterministic model for delay-induced circadian oscillations in the fungal species *Neurospora crassa* has been proposed in the paper. The model is suitable to analyze both temporal and spatial dynamics of this organism. The core of the model describing the temporal behavior of the system is based on the interplay between two dynamical variables, concentrations of the *FRQ* and *WCC* proteins. Time delay in the processes of transcription/translation is, perhaps, the easiest source of oscillations in the genetic systems. It exists, apparently, because of the slow movement of RNA polymerase along the gene. It is important to note also that the gene processes are not just slow but also are compound multistage reactions involving the sequential assembly of long molecules. Thus, these processes should obey Gaussian statistics with a certain characteristic mean delay time.

We have shown that our model of circadian oscillations based on the time delay mechanism in transcriptional regulation accounts for various features observed in

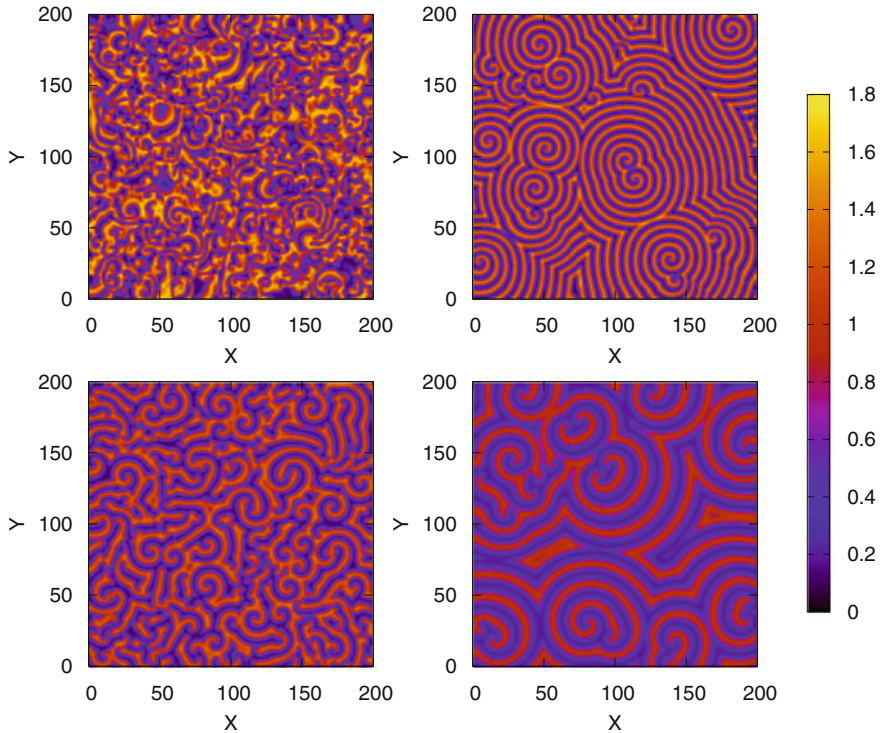


Fig. 7 Density plots of the concentration of the *FRQ* protein at time $t = 5000$ for $A_F = 0$ and different values of A_W : **a** 0; **b** 1; **c** 2; **d** 2.8

experiments such as effect of basal transcription factors, spatio-temporal synchronization and robustness to parameter variation.

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