Turing Patterns Inside Cells

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Concentration gradients inside cells are involved in key processes such as cell division and morphogenesis. Here we show that a model of the enzymatic step catalyzed by phosphofructokinase (PFK), a step which is responsible for the appearance of homogeneous oscillations in the glycolytic pathway, displays Turing patterns with an intrinsic length-scale that is smaller than a typical cell size. All the parameter values are fully consistent with classic experiments on glycolytic oscillations and equal diffusion coefficients are assumed for ATP and ADP. We identify the enzyme concentration and the glycolytic flux as the a priori given similarities between ATP and ADP. More recently, Hasslacher et al. [16] presented numerical simulations of Turing pattern formation in a closely related model [17] that were obtained assuming that ATP diffused 25 times faster than ADP. The authors gave a qualitative justification for their choice of diffusivities making an analogy between the role of enzymes in the glycolytic pathway and that of the immobile color indicators used for visualization in open chemical reactors [7,8] which effectively reduce the transport rate of one of the species involved in the reactions [18]. However, the argument that applied to the latter case cannot be translated to the case of the glycolytic pathway without a deeper analysis. Firstly, enzymes affect both the dynamics of ATP and ADP. Thus, the rescaling of diffusion coefficients may occur in such a way so as to prevent the formation of patterns. Second, when the 2-variable reaction-diffusion model is obtained by the adiabatic reduction of the larger set of equations in which the enzyme dynamics is explicitly considered [19], the elimination of the fast variables (the enzymes) introduces changes of the same order of magnitude in both the diffusion and the reaction terms [20]. Thus, both changes need to be considered simultaneously in order to analyze Turing pattern formation in this setting. Finally, this theoretical discussion remains meaningless unless reasonably sized patterns can be shown to exist for realistic values of the reaction rates and of the free diffusivities.

In this paper we overcome the drawbacks of these previous works by showing that the full 5-variable Selkov model describing the PFK-controlled steps of the glycolytic pathway supports Turing patterns for realistic reaction rates and equal diffusivities of ATP and ADP. We show that Turing structures of subcellular size (10 μm) may be found by increasing the glycolytic flux and the enzyme concentration while keeping fixed the set of kinetic constants that give good agreement with the classic experimental results on homogeneous oscillations in open chemical reactors [7,8].

INTRODUCTION

Concentration gradients inside the cytosol are a vital piece of the cell’s machinery. They underlie morphogenesis [1], and are involved in cell migration [2], cell growth [3], cell division [4], and the mechanical responses that follow fertilization, among other functions [5]. Stationary concentration gradients can spontaneously emerge from a homogeneous background in the presence of instabilities. In 1952 Alan Turing proposed a hypothetical sequential route for cell differentiation, based on a reaction-diffusion process [6]. Starting from a spatially uniform state he proved that stable inhomogeneities in “morphogen” concentration could spontaneously emerge through a diffusion-driven symmetry-breaking instability. His highly idealized model stimulated a large amount of work which eventually led to the observation of Turing patterns in open chemical reactors [7,8]. Nevertheless, the occurrence of Turing patterns has not been unequivocally proven for a biochemical reaction in which reaction rates, concentrations and diffusion coefficients are within realistic physiological values. We do so in this paper. More specifically, we show the existence of cell-sized Turing patterns in a model of glycolysis, using realistic parameter values and equal diffusion coefficients of ATP and ADP.

The general concept behind Turing patterns involves a combination of short-range activation and long-range inhibition [9]. Several reaction-diffusion models have been proposed in the literature to explain, among others, coat patterns in mammals [9], skin patterns in fish [10] or shell patterns in mollusks [11]. However, none of these models identifies the “morphogens” involved and some of the reaction schemes lack a biological motivation. This gives almost unrestricted freedom to choose kinetic parameters and diffusion ratios. Thus, the astonishing visual similarities often observed between the predicted and the real patterns might be unrelated to the actual biological phenomena.

One of the early attempts to look for an example of a symmetry breaking mechanism in biology was due to L. Prigogine et al. [12]. They analyzed a spatially extended version of the (adiabatically reduced) 2-variable Selkov model of glycolysis, derived in [13] (see also [14,15]). Namely, they added diffusion terms to each of the two differential equations describing the time evolution of the concentrations of ATP and ADP. It followed from their study that Turing patterns could only exist provided that ATP and ADP diffused at sufficiently unequal rates. This condition seems difficult to be met a priori, given the similarities between ATP and ADP. More recently, Hasslacher et al. [16] presented numerical simulations of Turing pattern formation in a closely related model [17] that were obtained assuming that ATP diffused 25 times faster than ADP. The authors gave a qualitative justification for their choice of diffusivities making an analogy between the role of enzymes in the glycolytic pathway and that of the immobile color indicators used for visualization in open chemical reactors [7,8] which effectively reduce the transport rate of one of the species involved in the reactions [18]. However, the argument that applied to the latter case cannot be translated to the case of the glycolytic pathway without a deeper analysis. Firstly, enzymes affect both the dynamics of ATP and ADP. Thus, the rescaling of diffusion coefficients may occur in such a way so as to prevent the formation of patterns. Second, when the 2-variable reaction-diffusion model is obtained by the adiabatic reduction of the larger set of equations in which the enzyme dynamics is explicitly considered [19], the elimination of the fast variables (the enzymes) introduces changes of the same order of magnitude in both the diffusion and the reaction terms [20]. Thus, both changes need to be considered simultaneously in order to analyze Turing pattern formation in this setting. Finally, this theoretical discussion remains meaningless unless reasonably sized patterns can be shown to exist for realistic values of the reaction rates and of the free diffusivities.

In this paper we overcome the drawbacks of these previous works by showing that the full 5-variable Selkov model describing the PFK-controlled steps of the glycolytic pathway supports Turing patterns for realistic reaction rates and equal diffusivities of ATP and ADP. We show that Turing structures of subcellular size (10 μm) may be found by increasing the glycolytic flux and the enzyme concentration while keeping fixed the set of kinetic constants that give good agreement with the classic experimental results on homogeneous oscillations in open chemical reactors [7,8].

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yields, yeast extracts [21,22,23,24,14]. The emergence of these patterns can be traced back to the differential interactions of ATP and ADP with PFK and its complexes. We then conclude that the key enzymatic step responsible for glycolytic oscillations may also provide a robust mechanism for the formation of steady state inhomogeneities in the concentration of ATP and ADP at the cellular and supracellular level.

RESULTS

The 5-variable Selkov model reads [13]:

\[ \begin{align*}
\frac{dv_1}{dt} &= \nu - (1 + K_1)\sigma_1 u_1 + K_1 u_2 + d_1 V^2 \sigma_1, \\
\frac{dv_2}{dt} &= v [u_2 - \gamma K_2 \sigma_2 u_3 + \gamma K_1 u_1] - \eta \sigma_2 + d_2 V^2 \sigma_2, \\
\frac{dv_3}{dt} &= u_2 - \sigma_1 u_1 + \frac{K_2}{K_1 + K_2} (\sigma_2 u_3 - u_1), \\
\frac{dv_4}{dt} &= \sigma_1 u_1 - u_2,
\end{align*} \]

where \( E \) is the free enzyme (PFK), which can form the complexes, \( ES_1^1 \) and \( ES_2^2 \); the substrate, \( S_1 \) (ATP) is supplied by an external source at the rate, \( \nu_1 \), and the product, \( S_2 \) (ADP) is removed by a first order reaction at rate \( \nu_2, S_2 \). From scheme (1) it is clear that the enzyme is inactive unless it has \( \gamma \) product molecules bound, forming the complex \( ES_2^2 \). As done in [13], we set \( \gamma = 2 \) all throughout this paper. It is this activating step of the allosteric enzyme which provides the mechanism responsible for the instabilities. We assume that ATP and ADP diffuse while the enzyme and its complexes are immobile due to the larger mass. We also assume that, initially, all concentrations are spatially uniform. Using mass action kinetics, the reaction-diffusion equations describing this model can be written in dimensionless form, as:

\[ \begin{align*}
\frac{d\tilde{v}_1}{dt} &= \nu - (1 + K_1)\sigma_1 \tilde{u}_1 + K_1 \tilde{u}_2 + \tilde{d}_1 \tilde{V}^2 \sigma_1, \\
\frac{d\tilde{v}_2}{dt} &= \nu [\tilde{u}_2 - \gamma K_2 \sigma_2 \tilde{u}_3 + \gamma K_1 \tilde{u}_1] - \eta \sigma_2 + \tilde{d}_2 \tilde{V}^2 \sigma_2, \\
\frac{d\tilde{v}_3}{dt} &= \tilde{u}_2 - \sigma_1 \tilde{u}_1 + \frac{K_2}{1 + K_2} \left( \sigma_2 \tilde{u}_3 - \tilde{u}_1 \right), \\
\frac{d\tilde{v}_4}{dt} &= \sigma_1 \tilde{u}_1 - \tilde{u}_2,
\end{align*} \]

(2)

where the dimensionless concentrations are:

\[ \begin{align*}
\sigma_1 &= k_1 + [S_1] \left( k_2 + k_{-1} \right), \\
\sigma_2 &= (k_3 + k_{-3})^{1/2} [S_2], \\
\tilde{u}_1 &= [ES_1^1] / \epsilon_0, \\
\tilde{u}_2 &= [ES_2^2] / \epsilon_0, \\
\tilde{u}_3 &= 1 - (\tilde{u}_1 + \tilde{u}_2),
\end{align*} \]

(3)

with \( \epsilon_0 \), the total concentration of enzyme which remains constant and uniform throughout the evolution. The other quantities are:

\[ \begin{align*}
\eta &= \frac{k_2 + k_{-1}}{\epsilon_0 k_{+1} k_{+2} v_2}, \\
\nu &= \frac{\nu_1}{k_{+1} \epsilon_0}, \\
\epsilon &= \frac{\epsilon_0 k_{+1} k_{+2}}{(k_{+1} + k_{-1})^2}, \\
\gamma &= \frac{k_{+1} + k_{-1}}{k_{+1} k_{+2}} \left( \frac{k_1 + k_3}{k_{+1}} \right)^{1/2}, \\
K_1 &= \frac{k_{-1}}{k_{+1}}, \\
K_3 &= \frac{k_3}{k_{+1}}.
\end{align*} \]

(4)

The dimensionless space and time coordinates are obtained by dividing the dimensional ones by an arbitrary length-scale, \( L \), and a linear growth rate, \( g \), of the unstable modes as a function of the square of the (dimensionless) wavenumber, \( k \), for \( \nu = 0.03, \eta = 1.215, \epsilon = 0.0003 \). There is a bounded band of unstable

respectively. Assuming that the diffusion coefficients of \( ATP(D_1) \) and \( ADP(D_2) \) are equal \( (D_1 = D_2) \), we take \( L = 10(DT)^{1/2} \). Using the diffusion coefficients in vivo, \( D \approx 150 \mu \text{m}^2 \text{s}^{-1} \) [25], we obtain \( L = 122 \mu \text{m} (T s)^{1/2} \), while the dimensionless diffusion coefficients are \( d_1 = d_2 = 0.01 \). The relatively small amount of enzyme used in experiments implies that \( \epsilon \ll 1 \). For this reason, a quasi-steady state approximation of Eqs. (2) was analyzed in [13] considering, furthermore, spatially uniform concentrations.

We now constrain the values of the various parameters of Eqs. (2) according to previous estimations and measurements [13,15,26,21,22,23]. Under the experimental conditions of [22], in which experiments are done using yeast extracts, the values of \( v_1 \) and \( v_2 \) at which the oscillations start are \( v_1^* \approx 5.8 \mu \text{m} \text{s}^{-1} \) and \( v_2^* \approx 0.04 \mu \text{m} \text{s}^{-1} \) [15]. The enzyme is very diluted in these experiments, with \( \epsilon_0 \) between 3 and 10 \( \mu \text{M} \), the average concentrations of \( ATP \) and \( ADP \) are \( [S_1]^* = 630 \mu \text{m} \) and \( [S_2]^* = 150 \mu \text{m} \), respectively, while the period of the oscillations, \( T \), is between 3 and 5 min [15]. It has been shown unequivocally that the transition to oscillatory behavior in glycolysis is due to a Hopf bifurcation [24]. This means that the transition can be generically encountered by varying only one parameter. Equations (2) have a single homogeneous fixed point solution that depends on several parameters, which indeed undergoes a Hopf bifurcation. Thus, there are various ways by which this bifurcation can be reached. Among them, we choose the set of parameter values that are compatible with the observed frequency of oscillations and ATP and ADP concentrations. In particular, we find that at \( \eta = \eta^* = 0.15, \nu = \nu^* = 0.004, \gamma = \gamma^* = 10^{-6}, \sigma = 15, K_1 = 1500, K_3 = 1 \), a Hopf bifurcation occurs for the set of Eqs. (2) in the spatially homogeneous case. Using the definitions of these dimensionless quantities in terms of dimensional ones and the experimentally determined values, \( v_1^* \approx 5.8 \mu \text{m} \text{s}^{-1} \) and \( v_2^* \approx 0.04 \mu \text{m} \text{s}^{-1} \), we obtain that the total amount of enzyme at the Hopf bifurcation is \( \epsilon_0 = \epsilon_0^* \approx 7.9 \mu \text{m} \) and that the various reaction rates satisfy

\[ k_{+2} \approx 178 \mu \text{m} \text{s}^{-1}, \quad \frac{1 + K_1}{k_{+1}} \approx 30 \mu \text{Ms}, \]

(5)

(6)

These numbers imply that, at the Hopf bifurcation, the stationary homogeneous solution of Eqs. (2) occurs at \( [S_1]^* - 150 \mu \text{m} \) and \( [S_2]^* \approx 145 \mu \text{m} \), and that the dimensional period of the oscillations is \( T^* \approx 2.7 \) min, which agrees with the experimentally determined values. We show the oscillatory behavior of \([ATP]\) and \([ADP]\) close to this bifurcation in Fig. 1(a).

Given these previous estimates, we explore the behavior of Eqs. (2), in the spatially extended case, varying the parameters \( v, \eta, \epsilon \) and keeping the purely kinetic constants,\( \sigma, K_1, \) and \( K_3 \) fixed at the previously mentioned values. We obtain the set of parameter values for which the homogeneous stationary solution of Eqs. (1) is unstable against spatially inhomogeneous perturbations (i.e., the Turing space) by analyzing the dispersion relation of the linearized evolution equations. We show in Fig. 1(b) the (dimensionless) linear growth rate, \( g \), of the unstable modes as a function of the square of the (dimensionless) wavenumber, \( k \), for \( \nu = 0.03, \eta = 1.215, \epsilon = 0.0003 \). There is a bounded band of unstable
Figure 1. Behaviors predicted by the S-variable Selkov model for different parameter values. (a) Glycolytic oscillations in $S_1$ (solid curve) and $S_2$ (dashed curve) for $\eta = 0.15$, $\nu = 0.00345$, $\varepsilon = 10^{-6}$, $\zeta = 15$, $K_1 = 1500$, $K_2 = 1$. (b) Linear growth rate of the unstable modes as a function of the square of the wavenumber, $k^2$, for $\eta = 1.215$, $\nu = 0.03$, $\varepsilon = 0.0003$, $\zeta = 15$, $K_1 = 1500$, $K_2 = 1$, and $d_1 = d_2 = 0.01$. Inset: Evolution of $[S_1]$ in the spatially homogeneous case for the same parameter values. (c) Turing space (shadowed domain) as a function of the dimensionless input and output rates of ATP ($\nu$) and ADP ($\eta$), for the same parameter values as in (b). (d) Predicted value of the wave-length of the most unstable mode at each point in the Turing space of (c).

DISCUSSION

Conclusions

In this paper we have provided the first closed example of Turing pattern formation in a model of a vital piece of a cell's real biochemistry, with a built-in mechanism for the change of the morphogens diffusion length, and with parameter values that are compatible with experiments. Our results suggest that the pattern of enzyme regulation that gives rise to the glycolytic oscillations may also provide the basis for the formation of stationary spatial structures both at the cellular and supracellular level. The model we have used is highly idealized and cannot account for certain observations. However, we think that some of its basic dynamical features should be common to those of more sophisticated models [22,28] and of the real system. In particular, it does reproduce the oscillations that are observed for dilute enzyme concentrations [22,23], and has helped the finding of rotating spirals in vitro [29]. There are studies that show that the Turing and Hopf bifurcations are intimately related in systems with immobile species [30]. Thus, we expect all these models to share the existence of a Turing bifurcation at large enough (immobile) enzyme concentrations and a Hopf bifurcation at lower ones. Our study has shown that the interactions involved in the PFK catalyzed step of the glycolytic pathway change the “effective” diffusion coefficients of ATP and ADP in the necessary direction for Turing pattern formation. These results could be tested in the type of open reactors that have especially been conceived for the investigation of spatio-temporal dynamics in glycolysis [31].

We have also found that the patterns can fit inside a typical cell and that the time it takes for the patterns to form is relatively short (of the order of minutes). The formation of Turing patterns in this biochemical pathway could then be related to organizing centers in eukaryotic cells, playing a role during cell division [4]. The fact that the Turing patterns have an intrinsic length-scale implies that there could be zero, one or several spots of high [ATP] inside the cell, depending on the relationship between the cell and the pattern sizes (see Fig. 2). In particular, there are two properties
which imply that the number of high ATP spots could change during the cell division cycle. First, the pattern size decreases as the strength of the glycolytic flux increases. This flux must increase during interphase for biosynthesis and growth. Thus, the pattern size might become small enough to fit inside the cell only after the glycolytic flux has increased sufficiently. The change of the cell size also acts in the same direction, allowing more “room” for more high ATP spots to fit as the cell grows during interphase. In this way, the change in the number of high ATP spots as the cell grows could provide a check-point that combines mechanical information (cell size) and biochemistry to trigger the subsequent chain of events in the cell division process. If a key stage of the replicating machine of every eukaryotic cell strongly relies on a metabolic pathway, it would be unlikely that such pathway had evolved after the appearance of the first eukaryotic cell. The pathway should be old and highly conserved. The glycolytic pathway shares both properties.

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**Author Contributions**

Conceived and designed the experiments: SP. Performed the experiments: DS. Analyzed the data: DS. Wrote the paper: SP DS.