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Flow-driven two-dimensional waves in colonies of *Dictyostelium discoideum*

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Abstract

*Dictyostelium discoideum* (*D. d.*) is a valuable model organism to study self-organization and pattern formation in biology. Recently we reported flow-driven waves in experiments with uniformly distributed populations of signaling amobae, *D. d.*, and carried out a theoretical study in a one-dimensional model. In this work, we perform two-dimensional numerical simulations using the well-known Martiel–Golbeter model to study the effect of the flow profile and intrinsic noise on the flow-driven waves. We show that, in the presence of flow, a persistence noise due to spontaneous cell firing events can lead to sustained structures that fill the whole length of the system. We also show that external periodic stimuli of cyclic adenosine monophosphate can induce 1:1 and 2:1 entrainments which are in agreement with our experimental observations.

1. Introduction

Negative environmental conditions induce the aggregation of social amoebae *Dictyostelium discoideum* (*D. d.*) to multicellular aggregates and eventually to millimeter sized fruiting bodies. This ‘social’ behavior has made *D. d.* an important model system for the study of pattern formation at the multicellular level. Randomly distributed *D. d.* cells form cooperative territories by signaling to each other with the chemical attractant cyclic adenosine monophosphate (*cAMP*). Cells initiate the process by sending out spontaneous cAMP pulses which propagate over macroscopic distances as self-sustained reaction–diffusion waves. As a result circular and spiral patterns self-organize throughout the entire cell population [1–5]. In the natural environment, *D. d.* cells can be exposed to an external fluid flow which would be expected to significantly change the wave generation processes. Recently, we observed flow-driven waves in a homogeneous field of signaling *D. d.* cells [6]. In these experiments, *D. d.* cells are attached to the substrate of a microfluidic channel and naturally secreted cAMP is advected along the imposed fluid flow. This transport anisotropy induces macroscopic wave trains that propagate in the flow direction and have a unique flow-independent period. These flow-driven waves are similar to the differential flow induced chemical instability waves observed previously in the Belousov–Zhabotinsky (BZ) reaction [7–11].

Flow-driven waves in the Martiel–Goldbeter (MG) model have been investigated by the linear stability analysis and one-dimensional numerical simulations in [12]. In this recent work we have shown that, in the convectively unstable regime, small perturbations in the concentration of cAMP are amplified and generate a wave train propagating strictly in the flow direction. We have also shown that the presence of a cell-free inlet area, similar to our experimental setup, played an important role in triggering sink-driven-oscillations (SDOs) in the vicinity of the inlet. These oscillations act as continuous time-dependent inlet perturbations and induce flow-driven waves that eventually extend over the whole length of the channel. Without SDOs and in the presence of one single initial inlet perturbation, a package of flow-driven waves is advected downstream and after it exits the channel, the system progressively returns back to the steady state. The latter behavior is not consistent with our experimental
observations where flow driven waves extend throughout the channel. Therefore the formation of SDOs is one possible mechanism to consistently explain our experimental observations.

In this paper, we perform two-dimensional numerical simulations of the MG model [13] to investigate the effect of a non-planar flow profile on flow-induced waves. The simulations show that both the flow profile and the boundary condition at the inlet area of the microfluidic setup greatly affect the extent and the persistence of the flow-driven waves. We also show that the natural noise due to the spontaneous cAMP pulses of *D. d.* cells plays a constructive role in sustaining flow-driven waves. Moreover, in our simulations with external periodic cAMP stimuli we observe 1:1 and 2:1 entrainments, which are in agreement with our experimental observations [6]. Lastly we explore the wave dynamics in the excitable regime (not convectively unstable) and observe interesting dynamics including the formation of regions with spontaneous activity that generate outward propagating cAMP pulses.

2. Numerical simulations of the two-component MG model in two-dimensions

We extend our one-dimensional numerical simulations in [12] to study the role of the flow-profile on flow-induced instabilities in two-dimensions. The two-component MG model [13–16] in the presence of diffusion and external flow obeys the following set of equations

\[ \partial_t \gamma = \epsilon_1 \nabla^2 \gamma + \nu \cdot \nabla \gamma + \frac{1}{\epsilon_1} \left[ \rho \Phi(\rho, \gamma) - \gamma \right], \tag{1a} \]

\[ \partial_t \rho = -f_1(\gamma) \rho + f_2(\gamma)(1 - \rho), \tag{1b} \]

with

\[ f_1(\gamma) = \frac{1 + \kappa \gamma}{1 + \gamma}, \quad f_2(\gamma) = \frac{L_1 + \kappa L_2 \sigma \gamma}{1 + \gamma}, \]

\[ \Phi(\rho, \gamma) = \frac{\lambda_1 + Y^2}{\lambda_2 + Y^2}, \quad Y = \frac{\rho \gamma}{1 + \gamma}, \]

where \( \rho \) is the fraction of active receptors on the membrane, \( \gamma \) is the dimensionless extracellular cAMP concentration, \( \epsilon_1 = k_f/k_r \) and \( \kappa = \frac{q f}{k_r} \). Here \( k_f \) is the desensitization rate of active receptors, \( k_r \) is the degradation rate of extracellular cAMP, \( s = \frac{\rho q f \sigma}{k_f (s + k_h(1 + \sigma))} \) is proportional to \( \sigma \) which is the production rate of intracellular cAMP.

The dimensionless time and space variables are defined as \( t = t' \times k_f \) and \( x, y = \frac{x'}{\sqrt{k_f D}} \times x', y' \) where \( t' \) and \( (x', y') \) are dimensional time and space variables, respectively. Moreover, \( D = 0.024 \text{ mm}^2 \text{ min}^{-1} \) [14] is the diffusion coefficient of cAMP. The dimensionless flow velocity \( \nu = V_f/k_r D \) where \( V_f \) is the dimensional velocity (see [12–14] for detailed description of the MG model and the definitions of the other parameters).

The linear stability analysis of equation (1a) was reported in details in [12]. The production rate of intercellular cAMP (\( \sigma \)) and degradation rate of extracellular cAMP (\( k_r \)) are among important parameters governing the system dynamics, as shown in figure 1. The phase diagram show regions of convective instability (CU) and absolute instability (AU). In the CU regime, if the flow velocity is larger than a critical value [12], small perturbations in the concentration of cAMP grow in time and generate a wave train moving only in the flow direction. The flow-driven waves are also observed in the AU regime of the phase diagram where they are advected downstream and interact with uniform bulk oscillations at the lower part of the channel and sweep them away [12]. We performed simulations for the set of parameters inside the CU region of the phase diagram to demonstrate the development of flow-driven waves (figure 2). This point is marked with star in figure 1 and is located close to the transition line to the AU regime. In the case of a planar flow profile, the simulations are essentially one-dimensional and the resulting flow-driven wave patterns are similar to the ones reported in [12]. The first 2.5 mm length of the channel corresponds to the inlet of the microfluidic setup in the experiments [6] and is free of cells. In addition, we assume that the initial concentration of cAMP in this part of the channel is zero. In the rest of the channel, the system is set to the steady state concentration and the perturbation is applied initially by slightly increasing the value of \( \gamma \) in the close vicinity of the inlet (figure 2(a)). An example of flow-driven waves with a uniform flow velocity of \( V_f = 1.5 \text{ mm min}^{-1} \) is shown in figure 2(b). For this flow velocity, a wave packet developed and each new wave front was formed further downstream than the previous one. This is different from experiments where the propagating flow-induced waves appear to fill the whole length of microfluidic channel [6]. However, for the smaller flow velocity of \( V_f = 1 \text{ mm min}^{-1} \), SDOs close to the inlet play an important role [12]. The points close to the inlet lose cAMP by advection and diffusion but gain only a little from the upstream area of the channel. This negative flux of cAMP drives the system into oscillatory state, if the flow velocity is larger than a minimum velocity \( V_{\text{min}} \) but smaller than a maximum velocity \( V_{\text{max}} \). SDOs act as a
permanent source of activity close to the boundary and therefore, as shown in figure 2(c), waves extend throughout the channel.

In the experiments, however, the flow profile inside the microfluidic channel is not planar. Flow velocity drops to zero close to the top and bottom (no-slip) boundaries following a parabolic law. The development of flow-driven waves with a parabolic flow profile is shown in figure 3. For the maximum flow velocity of 1.5 mm min$^{-1}$ at the middle of the channel, it decreases towards the top and bottom boundaries until it reaches to the values smaller than $v_{\text{max}}$ but larger than $v_{\text{min}}$. As mentioned above, SDOs increase periodically the concentration of cAMP in the vicinity of the inlet. This is indeed equivalent to continuous external pulses of cAMP with alternating amplitude. The pulses continuously trigger flow-driven waves which have parabolic fronts and cover the entire length of the channel. Remarkably, the cAMP concentration is higher at the middle of the wave front and decreases towards the boundaries where the flow velocity drops to zero. Note that in the absence of SDOs, only part of the channel is filled by a package of flow-driven waves that eventually ‘blows out’ of the system so that no permanent wave pattern is produced.

Another plausible mechanism to generate flow-driven waves throughout the channel is linked to random cAMP pulses secreted by one or more cells in a field of signaling amoebae. The spontaneous release of cAMP can be modeled by assigning a probability $p$ per time interval that an individual cell (or groups of cells) will pulse in each element of the grid (25 μm × 25 μm) [16]. Thus, the simulations are based on the plausible assumption that a group of about nine cells in each lattice unit behaves synchronously and fires at the same time. This weak but persistent noise source generates sustained wave patterns that are similar to our experimental observations in [6]. An example of flow-driven waves for the same set of parameters as in figure 2(b) but with stochastic firing events show that the wave pattern indeed extends almost over the entire length of the channel (see figure 4). Note
that in the simulations with a parabolic flow profile, in addition to cell firing events, SDOs are another source of perturbation in the system. Therefore in the absence of spontaneous cAMP pulses, SDOs act as a periodic source of cAMP stimuli in the area close to the inlet and the wave pattern persists. However with a planar flow profile, if the stochastic firing events are stopped after some time (e.g. 10 min) by setting $p = 0$, then the wave pattern is not persistent and is advected downstream of the channel and eventually out of the system.

3. Flow-driven waves in the presence of external pulses of cAMP

In our experiments in [6], we perturbed flow-driven waves via external periodic pulses of cAMP and observed M:N frequency responses, reminiscent of Arnold tongues in forced oscillatory systems. We simulated external

![Figure 3. Color map of the dimensionless concentration $\gamma$ at $t = 0.00$, 2.0, 2.5, 2.75, 4.0, 5.5 and 50 min illustrating the development of a convective instability for the parameter values marked with * in figure 1. The maximum flow velocity at the middle of the channel is $V_f = 1.5 \text{ mm min}^{-1}$.](image)

![Figure 4. The flow-driven wave patterns are sustained by noise originating from randomly firing cells. Spontaneous cAMP pulses extend the waves over the whole length of a channel with (a) planar flow profile and (b) parabolic flow profile. Probability of cell firing at each grid point is $p = 10^{-4}$ and all the cells in one grid point secret cAMP in synchrony for 0.6 s. All the parameters are the same as figure 2 and the flow velocity is 1.5 mm min$^{-1}$.](image)
cAMP perturbations by periodic increase of cAMP concentration at a region of 0.75 mm length which is located 25 mm away from the inlet. Figures 5(a) and (b) show examples of the spatiotemporal responses for CAMP injection periods of $T_1 = 1$ min and $T_2 = 2$ min, respectively. At $T_1 = 1$ min, first a 1:1 response is observed which propagates approximately 5 to 30 mm along the channel. At the distal end of the channel far from the injection site, flow-driven waves develop and a transition to 2:1 response occurs (see figure 6). Note that the 1:1 response waves either change the speed to catch up with flow-driven waves or they fail to propagate further downstream. After 15 min, the periodic CAMP stimuli interact also with upstream developed flow-driven waves. The high concentration of cAMP close to the inlet is due to SDOs, which are combined with flow-driven waves and interact with CAMP perturbations 25 mm downstream of the channel. As $T_2$ is increased to 2 min, the CAMP periodic stimuli are in synchrony with flow-driven waves and a 1:1 response propagates over the entire length of the channel (see figures 5(b) and 6(b)).

4. Flow-driven waves in the excitable regime

To study the role of flow profile, we performed extensive numerical simulations in the excitable part of the phase diagram. Although there are no experimental realizations of these simulation results, they are an interesting target for future studies. In the excitable regime, if the perturbation close to the inlet area is larger than a threshold value, it will be amplified and only one single excitation wave front propagates downstream in the channel. Note that since the inlet area is empty of cells, no excitation wave can propagate backward. However, if the perturbation is applied near the middle of the channel, there is a backward traveling wave in addition to the downstream propagating wave front. Both waves propagate with a velocity of approximately 1.5 mm min$^{-1}$ and have the same width and amplitude. The corresponding space–time plot is shown in figure 7(a) where the CAMP concentration profile at the middle of the channel is sequentially stacked. Accordingly, the speed of the excitation fronts is inversely proportional to the slope of the bands. At $t = 5$ min the flow with a planar profile is switched on and stays on for the rest of the simulation. As a result, the forward moving wave front speeds up to 2.6 mm min$^{-1}$ and the band with high concentration of CAMP expands slightly. However the backward propagating excitation wave slows down to 0.28 mm min$^{-1}$ and the CAMP concentration drops significantly. Similar to the results in [17] with an electric field, we observe the formation of regions of spontaneous activity, which permanently generate trains of CAMP pulses with a period of 3 min (figure 7(a)). Only the first spontaneously generated wave front (marked with * in the image) has no corresponding backward traveling wave. These pacemaker regions show a slow drift in the flow direction and the amplitude of spontaneously generated CAMP pulses decay quickly with time. The nucleation of new pulses of CAMP by an imposed external flow also occurs for parabolic flow profiles although it does not persist (figure 7(b)). Interestingly, the speed of

Figure 5. Observation of (a) 2:1 response and (b) 1:1 response in simulations with repetitive periodic stimuli of CAMP applied at $x = 25$ mm with time period of (a) 1 min and (b) 2 min. The CAMP concentration plots versus time in figure 6 are plotted along the red and blue lines in the corresponding space–time plots. The flow velocity is $V_f = 1.5$ mm min$^{-1}$.
forward propagating waves (2.37 mm min$^{-1}$) is slightly slower than the wave fronts with a planar flow profile. The backward propagating waves travel with the speed of 0.8 mm min$^{-1}$ which is faster compared to the case with planar flow profile. Moreover, the pacemakers appear every 3.8 min and terminate after 22 min.

In the next set of simulations, we alternatively switched the flow direction every 5 min and observed the wave dynamics. Figures 7(c) and (d) show the corresponding space–time plots for planar and parabolic flow profiles, respectively. Upon an alternating switch in the flow direction, multiple regions of spontaneous excitation are

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**Figure 6.** Various types of oscillations in response to repetitive periodic stimuli of cAMP shown as the black dashed lines. The red and blue colors correspond to the cAMP concentration plots along the red and blue lines in figure 5. The cAMP stimulus interval are (a) 1 min and (b) 2 min.

**Figure 7.** Space–time plots of spontaneous excitation of cAMP pulses triggered by external flow switched on at $t = 5$ min for (a) planar and (b) parabolic flow profiles. (c), (d) Wave dynamics observed showing the formation of multiple regions with spontaneous activity upon switching the flow direction with planar and parabolic flow profiles, respectively. The rightward flow with the magnitude 1 mm min$^{-1}$ is switched on after 5 min and the flow direction is changed every 5 min. The parameters are $k_e = 6$ min$^{-1}$ and $\sigma = 0.375$ min$^{-1}$. 
formed which emit new pulses of cAMP that soon cover the whole length of the channel. The newly emitted pulses collide with other incoming pulses and are annihilated. The two-dimensional color map of the wave patterns at different times are presented in figure 8 where a parabolic flow profile is applied. A region of spontaneous excitation appears in figure 8(d) which is located behind the initial propagating pulse and generates two new pulses that propagate outwards from this region. The number of pacemaker regions with spontaneous activity increases as the flow direction is switched multiple times and the excitations’ waves cover the whole length of the channel.

5. Discussion

The two-dimensional numerical simulations of the MG model described above show that the fluid flow profile has a significant effect on the corresponding flow-driven waves in a homogeneous field of signaling D.d. cells. In a microfluidic channel where the imposed flow has a parabolic profile, the flow velocity decreases towards the side walls and, in the vicinity of the wall drops below the critical velocity $v_{\text{max}}$ but remains larger than $v_{\text{min}}$ to trigger SDOs [12]. These induced oscillations act as a continuous source of activity in the vicinity of the inlet area and trigger flow-driven waves close to the inlet and are relayed throughout the channel. It is important to note that the inlet of the channel, which is free of cells, plays an important role to drive the instability. However, if the imposed flow has a planar profile and the flow velocity is not in the range to trigger SDOs, then a single package of flow-driven waves propagates downstream and eventually exits from the system. Only in the presence of time-dependent inlet perturbations or random cell firing events is an extension of the wave package over the whole length of the channel possible. Random stochastic pulses of cAMP are indeed a natural source of continuous noise in a field of signaling D.d. cells and play a constructive role in supporting flow-driven waves. This study shows that a combination of SDOs and intrinsic noise originating from cell firing events are responsible for the observation of flow-driven waves that always start in the vicinity of inlet and cover the whole length of the channel.
Moreover, the simulations of the MG model with external periodic stimuli of cAMP show 1:1 and 2:1 entrainments, reminiscent of Arnold tongues in forced oscillatory systems. We plan to perform extensive numerical simulations to explore the regions of parameters where the system response is locked to the driving frequency and find the transition lines between different regions. The two parameters of the Arnold diagram in our system will be the injection period of the external cAMP pulses and concentration of cAMP to control the strength of the external perturbation.

Our simulations also reveal interesting wave dynamics in the excitable regime of the phase diagram shown in figure 1. We observe the formation of regions with spontaneous activity that, in the presence of a planar flow profile, permanently generate forward and backward propagating cAMP pulses. However, if the imposed flow has a parabolic profile, there are two trains of forward and backward propagating cAMP pulses and the number of pulses in each train depends on the imposed flow velocity. This effect is partially similar to the dynamics in BZ system [18–22], where an external electric field can induce formation of only one train propagating in the opposite direction of the ‘mother’ pulse and the number of pulses in the train depends on the intensity of the applied electric field. The spontaneous formation of active regions in the presence of an imposed flow was not observed in our previous experiments with signaling D.d. cells [6]. Further experimental investigations of these theoretical predictions are currently underway in our laboratory where we perturb the system with various concentrations of cAMP and apply the external flow with different magnitudes and alternating directions.

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