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by

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Teaser: In a recent paper by Igoshin *et al.* mathematical modelling meets biological intuition and provides an elegant solution on how a single signal in *M. xanthus* may induce two different spatio-temporal patterns of cells.

Keywords: Biological oscillations, pattern formation, negative feedback, mathematical modelling, intercellular signalling, chemosensory systems, *Myxococcus xanthus*, gliding motility.

Abstract

Starving *Myxococcus xanthus* cells organise into two strikingly different spatio-temporal patterns: rippling or aggregation of cells into fruiting bodies. Formation of both patterns depends on a cell surface-associated, non-diffusible signal, the C-signal. A key motility parameter modulated by the C-signal during pattern formation is the frequency at which cells reverse their gliding direction, with low and high levels of C-signalling causing an increase and a decrease in the reversal frequency, respectively. Recently, a simple yet elegant mathematical model was proposed to explain the mechanism underlying the non-linear dependence of the reversal frequency on C-signalling levels. The mathematical solution hinges on the introduction of a negative feedback loop into the biochemical circuit that regulates the reversal frequency. This system displays an oscillatory behaviour in which the oscillation frequency depends in a non-monotonic manner on the level of C-signalling. Thus, the biochemical oscillator recapitulates the effect of the C-signal on the reversal frequency. The challenge for biologists is now to test the mathematical model experimentally.

Spatio-temporal pattern formation in biological systems

Formation of spatio-temporal patterns of cells is a hallmark in many developmental processes. These patterns can be strikingly similar and even resemble patterns generated in chemical and physical systems. For instance, the rippling pattern generated by *Myxococcus xanthus* cells resembles rippling on a water surface, and the cAMP waves generated by *Dictyostelium discoideum* resemble the spiral pattern produced in a Belousov-Zhabotinsky reaction. However, the mechanisms underlying the formation of these patterns are fundamentally different [1]. Pattern formation in chemical and physical systems is the result of interactions that are exclusively based on the laws of thermodynamics and physics. Cells obviously also follow these laws. In addition, however, cells engage in specific interactions that mould the pattern being formed. Understanding how spatio-temporal patterns of cells are generated entails a detailed description of how cells interact and a clarification of the rules and the underlying molecular mechanisms that govern cellular behaviour. As shown in the recent publication by Igoshin *et al*. [2] on pattern formation in starving *M. xanthus* cells, experimentalists can benefit greatly from a mathematical modelling approach in their attempts to elucidate the mechanisms underlying pattern formation in biological systems.

Pattern formation in *M. xanthus*

Myxococcus xanthus cells create three types of spatio-temporal patterns (Fig. 1). In the presence of nutrients, colonies are formed in which cells spread co-ordinately outwards. Starvation initiates a developmental program that ultimately results in the formation of multicellular, spore-filled fruiting bodies. Before fruiting body formation is complete, cells create two distinct patterns, rippling and aggregation (also referred to as

streaming). During rippling, cells accumulate in equi-spaced ridge-like structures separated by troughs of low cell density. The ridge-like structures move co-ordinately and synchronously as travelling waves over the surface [3,4] (for a time lapse movie of rippling, see Ref. [5]). Microscopic examination of cell behaviour during rippling shows that individual cells essentially oscillate back and forth with no net-movement suggesting that colliding waves reflect each other [5,6]. Later, in the developmental program the wave structure disintegrates and cells begin to aggregate into the nascent fruiting bodies. During the aggregation process, cells are organised in elongated streams in which cells are arranged end-to-end and side-to-side [7]. Intriguingly, genetic analyses [8-10] and analyses of cell behaviour during rippling and aggregation [6,11] suggest that rippling and aggregation are induced by the same intercellular signal, the C-signal, which is non-diffusible and involves a contact dependent signalling mechanism.

Gliding motility in *M. xanthus*

Formation of the rippling and aggregation patterns, relies on the ability of *M. xanthus* cells to move and regulate their motility behaviour. *Myxococcus xanthus* cells move by gliding motility and, as cells glide over a surface, the speed is highly variable. Periodically, cells stop and then either resume gliding in the same direction or undergo a reversal in which the leading pole becomes the lagging pole [11-14]. The generation of the rippling and aggregation patterns crucially depends on the ability of cells to regulate their reversal frequency [6,11,13,15].

Myxococcus xanthus cells harbour two gliding motility motors. The S-motility motor consists of type IV pili (Tfp), which are present at one pole at a time [16]. Tfp-based motility likely relies on extension of Tfp from the pole, Tfp attachment to the

extracellular matrix on a nearby cell, followed by retraction of Tfp and, consequently, the forward movement of the Tfp-containing cell [17,18]. The A-motor has been proposed to rely on the extrusion of slime from polarly localised nozzle-like structures [19]. The opposing nature of the two gliding motors, i.e. pulling by Tfp and pushing by the A-motor, has led to the suggestion that the two motors are located at opposite poles and that a reversal depends on the coordinated polarity switching of the two motors [20,21].

The C-signal and pattern formation

Genetic evidence suggests that the effect of the C-signal on pattern formation depends on signalling levels with low levels of C-signalling inducing rippling and higher levels inducing aggregation [8-10]. Intriguingly, this dual effect of the C-signal on pattern formation, i.e. rippling or aggregation, is paralleled by a dual effect of the C-signal at the cellular level. Thus, analyses of the behaviour of individual cells suggest that low levels of C-signalling induce an increase in the reversal frequency [6] and higher levels of C-signalling induce a decrease in the reversal frequency [11]. Key questions in order to understand the pattern formation properties of the C-signal are how one signal may induce two opposite effects on the reversal frequency and how changes in the reversal frequency of individual cells may result in the generation of specific spatio-temporal patterns at the population level.

The starting point for the work of Igoshin *et al*. [2] is the model of the part of the C-signal transduction pathway that regulates the reversal frequency (for reviews of the C-signal transduction pathway, see Ref. [20,21]. Igoshin *et al*. point out two essential features in this model (Fig. 2). Firstly, in response to starvation, *csgA* expression

increases [8,22] resulting in accumulation of the full-length CsgA protein, which is subsequently proteolytically cleaved to the 17 kDa C-signal protein [9,23,24]. The increase in *csgA* expression depends on the *act* operon [25]. Igoshin *et al*. state that the increase in *csgA* expression depends on C-signalling and constitutes part of a positive feedback loop. However, conflicting data have been published on whether the increase in *csgA* expression depends on C-signalling [8] or whether it is independent of C-signalling [22]. In any case, the increase in *csgA* expression in response to starvation ensures that starving cells are continuously exposed to increasing levels of the signal. Secondly, the C-signal dependent activation of the response regulator FruA, which presumably involves phosphorylation of FruA, stimulates the Frz chemosensory system by inducing methylation of the MCP homologue FrzCD in a manner that depends on the FrzF methyltransferase (for a review of the Frz system, see Ref. [26]) [27,28]. FrzCD likely modulates the activity of the hybrid histidine protein kinase FrzE. FrzE, in turn, is thought to regulate the frequency of gliding reversals by regulating the activity of the hypothetical motor polarity-switching device. The details of how FruA interacts with the Frz system, how the Frz proteins interact, and how FrzE regulates motor polarity switching remain to be elucidated. Nevertheless, based on how other chemosensory systems operate [29], Igoshin *et al*. provide a conceptual framework for these interactions. Specifically, they suggest that FruA-P stimulates the conversion of the inactive form of FrzF to an active form, denoted FrzF*, which induces FrzCD methylation. Subsequently, the methylated form of FrzCD stimulates the FrzE autokinase, and FrzE-P stimulates motor polarity switching. The truly novel concept that Igoshin *et al*. introduce in the model of the pathway is a negative feedback loop in which FrzE-P is suggested to deactivate FrzF* (Fig. 2). By introducing this negative

feedback loop, the part of the pathway between FruA and FrzE-P is converted into a biochemical oscillator, the Frzilator [frizzylator].

The Frzilator

By modelling the dynamics of the fractions of total protein concentrations of FrzF*, FrzCD-CH_3 and FrzE-P over time and as a function of the level of C-signalling, Igoshin *et al.*'s model showed that in the absence of external stimulation, the concentrations of the three Frz proteins oscillate. The model parameters were tuned to make the oscillation period of the Frz proteins match the average reversal period (10 min) of *M. xanthus* cells. If a C-signalling pulse is modelled to be delivered to the biochemical oscillator during the rising phase of FrzF*, this results in a burst in the activation rate of FrzF, consequently, maximum FrzE-P occurs earlier and, thus, the oscillation period is shortened. On the other hand, a C-signalling pulse delivered during the falling phase of FrzF* does not speed up the oscillation cycle suggesting that the Frzilator has a built-in refractory period.

The Frzilator would be continuously oscillating if the level of C-signalling remained constant. However, as outlined above, the level of C-signalling that cells are exposed to increases during starvation. By simulating the oscillation frequency of the three Frz proteins at different levels of C-signalling, the model by Igoshin *et al.* predicted that the oscillation frequency depends in a non-monotonic manner on the level of C-signalling: At low to intermediate levels of C-signalling, the oscillation frequency increases as the signalling level increases; and, at higher levels of C-signalling, the oscillation frequency decreases as the signalling level increases. Ultimately, at the highest levels of C-signalling oscillations cease. The logic provided by Igoshin *et al.* to explain the non-

monotonic effect of the C-signal on the oscillation frequency is that at low levels of C-signalling, the rate-limiting step in the Frzilator is FrzF activation whereas at high levels of C-signalling the rate-limiting step is FrzF* deactivation. As the deactivation rate of FrzF* determines the oscillation frequency, oscillations cease at high levels of signalling.

Implications of and questions raised by the Frzilator

It is well established that mathematical models and biochemical systems, which involve a negative feedback loop can generate oscillations [30]. However, as pointed out by Goldbeter [30], it is rarely appreciated that these oscillations may not occur under all conditions. The Frzilator seems to be such a case. As pointed out by Igoshin *et al.*, the fascinating aspect of their model is that the oscillation frequency of the three Frz proteins in response to the C-signal recapitulates the effect of the C-signal on the cellular reversal frequency during rippling and aggregation [6,11]. Thus, the model provides a theoretical solution to the two opposite effects of the C-signal on the reversal frequency. However, the model also raises a number of questions.

A fundamental question raised by the Igoshin *et al*. model is the mechanism underlying the reduction in the oscillation frequency at high levels of C-signalling. Under these conditions, the concentration of FrzE-P is expected to be high and, therefore, the oscillation frequency is also expected to be high. Evidently, the mathematical model of Igoshin *et al*. predicts that under these conditions the reversal frequency is low. Thus, the concentration of FrzE-P under conditions of high C-signalling levels needs to be clarified.

A priori, one experimental observation seems to be at odds with the model. Certain FrzCD mutants contain FrzCD proteins that are constitutively signalling [31]. Presumably, the prediction from the Igoshin *et al*. model is that these mutants should be non-reversing, however, these mutants display a hyper reversing phenotype [13,31]. Unfortunately, the authors did not discuss the characteristics of these Frz mutants.

An interesting question is if and how the non-linear behaviour of the biochemical oscillator is linked to the switch from rippling to aggregation. Mathematically, this could be explored by coupling the biochemical oscillator to a model for cell motion, for instance the model previously presented by the authors [32], to test whether the transition from rippling to aggregation is observed at the oscillation frequencies presented in the current paper.

Experimentally, one of the most crucial issues is now to clarify the molecular chain of events that lead from C-signal reception to a gliding reversal including the biochemical circuits of the Frz proteins and the mechanism of polarity switching of the gliding motors. Moreover, it needs to be tested experimentally whether there is a negative feedback loop in the Frz pathway. Fortunately, the model provides readily testable hypotheses, e.g. does FruA-P stimulate FrzF methyltransferase activity? Does FrzE interact with FrzF? Does this interaction depend on the phosphorylation state of FrzE? And, is the FrzF methyltransferase activity negatively regulated by FrzE-P?

The Igoshin *et al.* model predicts the existence of a refractory period in the biochemical oscillator. If the properties of the biochemical oscillator explain regulation of the reversal frequency in response to the C-signal, the prediction is that a reversal period has

a minimal value. This prediction needs to be tested experimentally, for instance by monitoring cell behaviour using time-lapse microscopy in which the time interval between recordings is significantly shorter than the refractory period predicted by Igoshin *et al.*'s model.

Concluding remarks

The paper by Igoshin *et al*. is thought provoking and raises more questions than it answers. It illustrates the power of mathematical modelling in pinpointing potential interactions that may have escaped the experimentalist and in providing testable models. It also is a source of inspiration to experimentalists that rely solely on intuition when analysing their data.

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Legends to Figures

Figure 1. Cellular patterns formed by *Myxococcus xanthus* cells. In the rippling panel, black arrows point to the ridge-like accumulations of cells and the white arrows point to the troughs of low cell density. Scale bar: 50 μm.

Figure 2. Schematic model of the C-signal transduction pathway. Two cells engaged in C-signal transmission are depicted. In the model, the C-signalling event is the interaction between the 17 kDa cell surface-associated C-signal protein on one cell with a hypothetical C-signal receptor on an adjacent cell. The increased *csgA* transcription during starvation is indicated in red as a result of the putative positive feedback loop and in orange as independent of C-signalling. The cascade of covalent protein modifications suggested by Igoshin *et al*. is indicated in green. The negative feedback loop suggested by Igoshin *et al*. is indicated in blue.

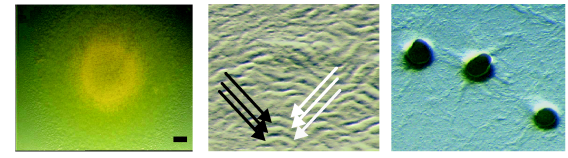

Figure 1: Stevens and Sogaard-Andersen

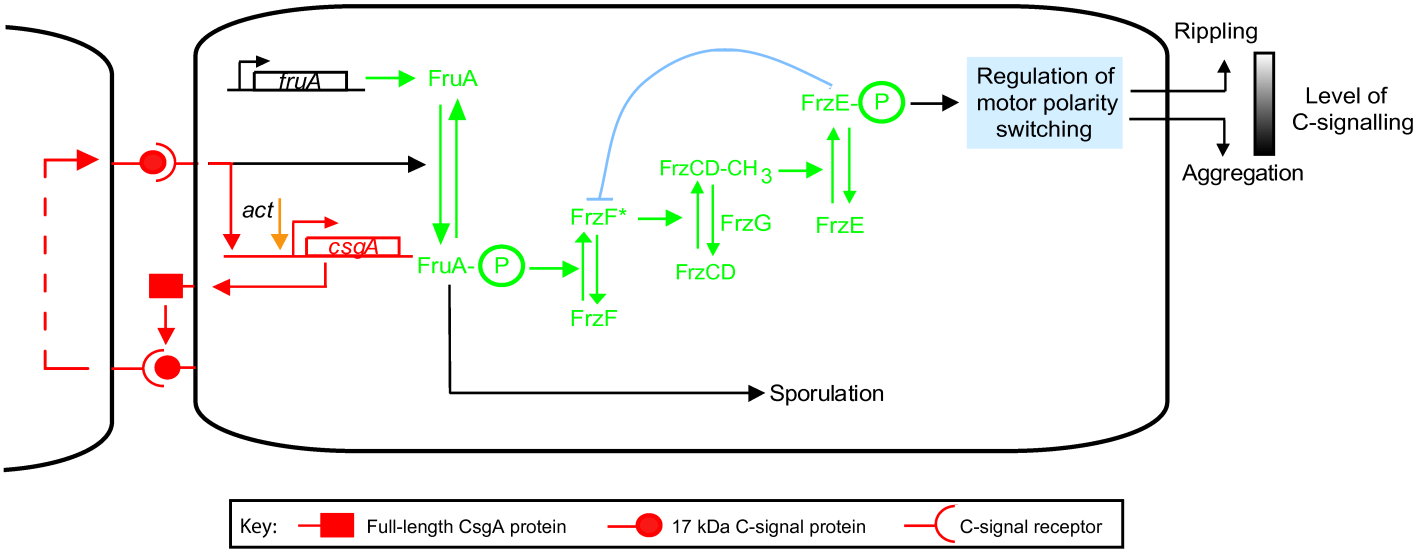

Figure 2: Stevens and Sogaard-Andersen