A Systems Biology perspective of Dynamical Patterning Modules in the transition to multicellularity: lessons from an aggregative bacteria

Alejandra Guzmán-Herrera[1,5], Juan A. Arias Del Angel[2,3,4], Natsuko Rivera-Yoshida[2,3,4], Mariana Benítez[2,3], Alessio Franci[1,*]

[1] Departamento de Matemáticas, Facultad de Ciencias, Universidad Nacional Autónoma de México
[2] Laboratorio Nacional de Ciencias de la Sostenibilidad, Instituto de Ecología, Universidad Nacional Autónoma de México
[3] Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México
[4] Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México
[5] Current address: MRC Laboratory for Molecular Cell Biology, University College London, London, UK
[*] Correspondence: afranci@ciencias.unam.mx

Abstract

Development and evolution are dynamical processes under the continuous control of organismal and environmental factors. Generic physical processes associated with biological materials and to certain genes and molecules provide a morphological template for the Evo-Devo of organism forms. Generic dynamical behaviors provide a temporal template for biological regulation and coordination. The role of generic physical processes and their associated molecules in development is the topic of the Dynamical Patterning Module (DPM) framework. The role of generic dynamical behaviors (realized by "network motifs") in biological regulation is the topic of Systems Biology (SB). We focus on a multicellular aggregative bacteria, *Myxococcus xanthus*, to pursue a joint DPM-SB perspective on the transition to multicellularity. Understanding *M. xanthus* development as a dynamical process embedded in a physical substrate provides novel insights on the interaction between developmental regulatory networks and generic physical processes in the evolutionary transition to multicellularity.

1. Introduction

Lifestyle transition from unicellularity to multicellularity has been one of the major evolutionary events, occurring independently multiple times and among different lineages (Maynard-Smith & Szathmáry, 1995, Grosberg & Strathmann, 2007, Bonner, 2016). The repeated occurrence of multicellularity enables the comparative and integrative study of potentially general molecular components, principles and mechanisms in development (Bonner, 2000). Such comparative studies may involve

whole genomes, specific gene sequences, expression patterns and morphogenetic processes (e.g. Nanjundiah et al., 2018; del Campo et al., 2014; Zhu et al., 2010). However, these studies have been carried out mostly for organisms that develop multicellular structures by incomplete cell division, or "staying together" (mainly plants and animals), whereas aggregative organisms exhibiting another ubiquitous trajectory to multicellularity, "coming together" (some groups of fungi and bacteria), have been much less studied (Figure 1).

Studying aggregative development in bacteria might be particularly illuminating since it takes place at the spatial and temporal scale at which evolutionary transitions to multicellularity may have occurred (Bonner, 2000; Rivera-Yoshida, et al., 2018). Among bacteria, *Myxococcus xanthus* is a delta-proteobacterium that develops into three-dimensional multicellular structures called fruiting bodies, which occur with low nutrient availability of the medium. Due to its stereotypical developmental process, *M. xanthus* has been considered as a model organism to approach the transition to multicellularity (Yang & Higgs et al., 2014; Arias Del Angel et al., 2017). At least three different cell fates are observed during its development: peripheral cells, stress-resistant spores and cells undergoing programmed cell death (Higgs et al., 2014).

Both the regulatory network and the molecular mechanisms involved in the development of *M. xanthus* have been extensively studied (Kroos, 2007; Escalante et al., 2012; Higgs et al., 2014; Bretl and Kirby, 2016). The analysis of the structural and dynamical properties of this network has contributed to the understanding of the mechanisms underlying cell-fate determination in this bacterium (Arias Del Angel et al., 2018, 2019). *M. xanthus* development has also been partially explained by physical and chemical morphogenetic processes affecting living and non-living matter, such as surface-tension-driven coarsening and substrate-mediated interactions (Bahar et al., 2014; Rivera-Yoshida et al, 2019). This multiplicity of factors and processes involved in *M. xanthus* aggregative development calls for integrative approaches that consider molecular network dynamics in association with morphogenetic processes.

An important step in this direction has been the postulation of Dynamical Patterning Modules (DPMs), which provide a theoretical framework that considers both the role of

specific molecules and, in the spirit of early theories of development (Thompson, 1942; Turing, 1952), the generic physical and chemical processes acting on living matter (Newman and Bhat 2008, 2009). While this framework has been fruitfully used to study morphogenetic principles in plants and animals (e.g. Zhu et al., 2010, Hernández-Hernández, 2012; Benítez et al., 2018), it tends to simplify and collapse the associated molecular regulatory network (MRN). Moreover, to the best of our knowledge, aggregative development has not been studied within this framework. On the other hand, the core of Systems Biology theory revolves around gene and molecular regulatory networks and the rich temporal dynamics that underlie their complex cellular functions (Hardin et al., 1990; Skotheim et al., 2008, Xiong & Ferrell, 2003; MacArthur et al., 2009). A core concept of Systems Biology in dissecting complex MRNs is that of "Network Motifs" (Alon, 2007). Systems biology ideas have been successfully applied to developmental MRNs. See (Davidson and Levin, 2005; Prill et al., 2005) for representative examples. However, the morphogenetic aspect of development is often ignored in those works. A "systems biology of morphogenesis" is still to be consolidated.

In the present study, we integrate the DPM and the Systems Biology frameworks to study *M. xanthus* multicellular development both in its generic physical morphogenetic aspects and its generic MRN dynamic aspects. This allowed us to further understand the mechanisms and dynamics underlying this organism's development and to discuss the potential role of specific molecular components in its development and evolution. Because of their generic nature, not grounded in any specific molecular or lineage identity, our analysis may shed light into the evolution of multicellularity itself.

We stress that we do not postulate any hierarchy between generic physical processes (engaged by DPMs) and generic network dynamical processes (described by Network Motifs). We rather postulate a synergy between the two: the morphogenetic aspect of development is mainly mediated by DPMs; the temporal coordination of the various morphogenetic processes, including timely and robust responses to environmental cues, is mainly mediated by Network Motifs. Crucially, in our description, the gene-centric viewpoint of development and evolution is weakened on both sides: what matters are the generic physical and network dynamical properties, rather than the specific molecular identity of the actors in play.

The rest of the paper is organized as follows. In Section 2 we briefly review the main strength and drawback of DPM and Systems Biology. In Section 3 we merge the two theories for the analysis of *M. xanthus* developmental gene regulatory network. In particular, we highlight the tight synergy between the two in understanding multicellularity. In Section 4 we summarize the key points of our analysis and stress their potential relevance for evolutionary-developmental biology in general.

2. Dynamic Patterning Modules theory and Systems Biology: two complementary theoretical approaches to developmental gene regulatory networks

2.1 Dynamic Patterning Modules theory links GRNs and generic morphogenetic processes

Stuart Newman and co-workers put forward the conceptual framework of "dynamic patterning modules" (DPMs) (Newman and Bhat, 2008, 2009). DPMs are defined as sets of gene products and other molecules in conjunction with the physical morphogenetic and patterning processes they mobilize in the context of multicellularity. This framework emphasizes how developing organisms are subject to physical and chemical processes (Newman and Bhat, 2008; Hernández-Hernández et al., 2012) (Figure 1, right). The DPM framework has been helpful to provide explanations of how cell masses develop into the characteristic morphologies of chemically and mechanically excitable mesoscopic materials (e.g., hollow, multilayered, elongated, segmented forms; Newman and Bhat, 2009). Furthermore, the DPM proposal recognizes organisms as physical entities that are also repositories of genetic information, and thus subject to evolutionary processes. A description grounded in the DPM framework has been previously presented for animal and plant development (Newman and Bhat, 2008, 2009; Hernández-Hernández et al., 2012; Benítez et al., 2018). Nevertheless, this framework has not been used for the study of any aggregative multicellular system.

Newman and co-workers employed the term “module” to emphasize the semi-autonomous behavior of a DPM but, in principle, DPMs interact with each other to form a large network generating a complex “patterning code”. However, the DPM framework does not focus on the molecular network dynamics of the constituent nodes of a module, nor on how different modules interact to achieve a given temporal coordination of DPM action. We argue that the “motif” concept of systems biology can shed further light on the dynamic properties of patterning modules in terms of their intrinsic nonlinear, molecular dynamics.

2.2. Systems Biology links GRNs and generic dynamical processes

Systems biology arose during the last century as an interdisciplinary field aiming at understanding the complexity of living systems in general, and gene regulatory networks (GRNs) in particular, from a mathematical and theoretical perspective. A key conceptual step of systems biology was to understand cell functions as regulated nonlinear dynamical processes, instead of as static mappings from genotypes to phenotypes (Monod & Jacob, 1961). At the end of the century, the advent of nonlinear dynamical systems theory and large-scale computer simulations laid the ground for a general systems biology theory. Complex cell functions, like the robust maintenance of periodic cellular cycles (Hardin et al., 1990; Skotheim et al., 2008) and cellular decision making (Xiong & Ferrell, 2003; MacArthur et al., 2009), started to be understood in terms of simple and generic *feedforward and feedback motifs* (Figure 1, left). These motifs provide a dictionary of prototypical nonlinear dynamical behaviors (Milo et al., 2002) that are shared across lineages. Synthetic biology has been fundamental in testing and developing systems biology principles. It showed many times that generic molecular behaviours can be implemented by designing the associated nonlinear dynamical behavior into network motifs (Elowitz & Leibler, 2000; Pomerening et al., 2003; Prochazka et al., 2017).

The idea that biological morphogenesis can be understood as a generic dynamical process laid the foundation of DPM theory (see above), while the main focus of classical systems and synthetic biology is still on single-cell behaviors and functions (see for instance the recent book (Del Vecchio & Murray, 2015)). Some recent studies

in synthetic biology designed MRNs to achieve the formation of desired multicellular morphologies (Toda et al., 2018; Glass & Riedel-Kruse, 2018). The idea underlying these recent works is to suitably regulate, intracellularly in time and intercellularly in space, the expression of genes associated with the synthesis of molecules that possess generic morphogenetic functions. Although not explicitly acknowledged by the authors, the resulting "morphogenetic toolboxes" are synthetic DPMs. The success of these recent approaches further suggest a general synergy between systems biology and DPM theory.

3. Dissection of *M. Xanthus* developmental GRN uncovers the key role of network motifs for DPM coordination

3.1 Patterning nodes, motifs nodes and coupling nodes of DPMs

Figure 2 reproduces the MRN associated with the multicellular development in *M. Xanthus* (Arias Del Angel 2018). Analogously to what has been done for plant and animal systems, we postulate a set of DPMs involved in *M. xanthus* development (Table 1) and identify the MRN network components, highlighted with different colors in Figure 2, associated with these DPMs. We introduce a distinction in the nodes integrating a DPM. *Patterning Nodes* are those that have an experimentally annotated developmental/morphogenetic function. They determine the identity of the DPM they belong to and are marked as fully colored nodes in the network. *Motif Nodes* are the ones that create dynamical motifs, in the sense of Systems Biology, with Patterning Nodes. They are outlined with a red dashed border and highlighted with the color of the DPM they are part of. *Coupling Nodes* are those that couple two or more DPMs and coordinate their dynamics. Coupling nodes do not belong to a specific DPM but are characterized by the DPMs that they connect. We hereby list the identified DPM Patterning Nodes and associated Motif Nodes in *M. xanthus*.

Adhesion (ADH) and Differential Adhesion (DAD). Adhesion (ADH) and differential adhesion (DAD) are the first and best described DPMs, both in animals and plants development (Newman & Bhat, 2009; Hernández-Hernández et al., 2012). Adhesion between neighboring cell is an essential property of multicellularity (Abedin & King, 2008). We identified a single patterning node (CsgA) belonging to ADH and DAD. CsgA

is a polarised membrane protein that is involved in adhesion and intercellular communication (Lobedanz & Søgaard-Andersen, 2003). Two motif nodes were also identified because they form a positive feedback loop (direct via RelA and indirect via SocE; Figure 2 and Arias Del Angel, 2018) with CsgA. This motif, also known as bistable or toggle switch, is the basic motif for bistability, that is, the coexistence of two possible stable equilibrium states corresponding to robust cell decisions (Ferrell & Machleder, 1998; Gardner et al., 2000).

Morphogen (MOR). Diffusive molecules allow intercellular communication that can affect cell identity or behaviour (Turing 1952; Green & Sharpe 2015). Their regulatory effect acts in a concentration dependent manner. Concentration gradients that emerge from passive or active mechanisms underlie the way in which diffusive molecules can create ordered spatial structures (“morphogenesis" (Turing 1952)) in multicellular organisms. Two patterning nodes of *M. xanthus* network were classified into this DPM: A-signal and Trypsin. A-signal is a cell density signal, consisting of small diffusive peptides cleaved by the protease Trypsin (Kuspa et al., 1992), that indicates when to start forming fruiting bodies. The MOR Motif Nodes (AsgE, Nla6 and Nla28; Figure 2 and Arias Del Angel, 2018) form an indirect positive feedback loop with MOR Patterning Nodes, creating a toggle switch and thus bistability.

Extracellular Matrix (ECM). The extracellular matrix is a complex macromolecular network that surrounds cell aggregates and forms part of their microenvironment. ECM composition can drastically vary through time and between different aggregates, determining their viscoelasticity and cohesivity. It keeps cells together, even in the absence of direct cell adhesion, and it can influence the shape, pattern, polarity and behaviour of multicellular structures (Daley et al. 2008; Theocharis et al. 2016; Mouw et al. 2017). The Patterning Node for this DPM is the Exo locus, which contains *M. xanthus* genes that export polysaccharides to the cell surface to coat the spores within the fruiting body and allow them to stick to each other (Müller et al., 2012). We did not identify any motif node for this Patterning Node, probably due to the lack of experimental characterization of its transcription factors.

Programmed Cell Death (PCD). Programmed cell death is a fundamental biological strategy of multicellular structures since it can change their mass, density and shape

without altering the identity of remaining cells (Jacobson et al. 1997), as observed in the formation of fingers and other extremities during morphogenesis (Jacobsen et al. 1996, Milligan et al. 1995). It also allows the elimination of dysfunctional individuals (*e.g.* cancerous cells) and ensures survival of the population (Jacobson et al. 1997). MazF is an endonuclease that is implicated in programmed cell death during fruiting body development of some *M. xanthus* strains (Nariya & Inouye, 2008). Thus, it is included as a PCD Patterning Node. Similar to ECM, we were not able to identify any MN for PCD. However, MazF itself is a connecting node between MOR and TIM (see below) DPMs (Figure 2).

All the DPM types mentioned above were previously identified as necessary for both animal and plant development (Newman & Bhat 2008, 2009, Hernández-Hernández et al. 2012). However, some fundamental genes of the *M. xanthus* developmental GRN could not be framed in any existing DPM. Therefore, we define two new DPMs based on their role during *M. xanthus* development: *Timing* and *Active Movement Coordination*. Similarly to the OSC DPM (Newman et al., 2008), the temporal more than the stationary, nature of these DPMs is what largely determines their functions.

Timing (TIM). We define this DPM as the regulator of developmental timing in *M. xanthus*. It is responsible for regulating when a developmental process starts and/or ends, affecting the final organisation and patterning of multicellular structures. In *M. xanthus*, knock-out experiments of various TIM Patterning Nodes have been shown to alter the onset of fruiting body formation after sensing the starvation signal that triggers multicellular development. In turn, this affects fruiting body size, spore number and viability, and the spatial pattern exhibited by the fruiting body population (Escalante et al., 2012; Rivera-Yoshida et al., 2019). All the Patterning Nodes in this DPM are post-translational regulators of the transcription factor MrpC2 (Motif Node), which is involved in cell fate decision making during development (Nariya & Inouye, 2006). The timing role of this DPM is reflected by its dynamical structure, formed by two very well known motifs: an incoherent feedforward loop (Milo et al., 2002) and an excitable fast positive/slow negative feedback loop (Izhikevich & FitzHugh, 2006; Tsai et al., 2008, Franci et al., 2018). The former involves the input cascades from NUT to MkapA passing respectively through PktD1 and PktD9 (observe that these two parallel paths have overall opposite sign). The latter involves all the nodes in the feedback loops

around the Mrpc2 motif node. We describe in detail the coupled dynamics of the two motifs appearing in the TIM DPM in the next section.

Active movement coordination (AMC). This DPM regulates and coordinates the direction of active motion of single cells in space. It induces a quasi-periodic reversal in the direction of motion by periodical switching *M. xanthus* molecular motor polarity. Because coupled to intercellular signaling pathways (C-signal), this periodic switch is synchronized between different cells and mutant lacking this movement-regulating synchronous oscillatory clock (by knocking out the Frz genes FrzCD, FrzE and FrzF; Figure 2) fail to develop (Igoshin et al., 2004). *M. xanthus* development is indeed preceded by a collective motion phase characterized by the emergence of highly structured traveling and standing waves (Sager & Kaiser, 1994). Unlike passive, diffusion-mediated, pattern formation, the resulting spatial distribution of cells needs the active, oscillatory movement reversal mediated by this DPM. In particular, it is not mediated by any morphogenetic gradient (Igoshin et al., 2004). In *M. xanthus*, the three Patterning Nodes together with FruA (the Motif Node) form the same fast positive/slow negative feedback loop observed in TIM. As already suggested by Igoshin et al., 2004 and as detailed in the next section, this motif can also produce sustained oscillations, responsible for movement behavior alternation.

3.2 The rich dynamics of DPMs

Beside their semi-autonomous morphogenetic role, DPMs are embedded in complex MRNs and participate in the rich network dynamics. A key observation of our dissection of the *M. xanthus* developmental MRN is that most DPMs possess a clear dynamic motif structure. The three motifs we identified are: bistable switch and multi-stable switch (ADH/DAD and MOR); fast positive/slow negative feedback loop (TIM and AMC); and incoherent feedforward loop (TIM). In all cases, except the incoherent feedforward, these motifs involve at least a Motif Node, that is, a node without any annotated morphogenetic function (in the classical DPM theory sense). Motif Nodes, and the connections they create with the Patterning Nodes, are crucial to generate and maintain a robust nonlinear DPM dynamical behavior. Removal of these nodes would disrupt the DPM functioning by disrupting its endogenous and exogenous dynamical behavior inside the network. These points are illustrated in Figures 3 and 4.

The heart of any bistable or multistable switch is positive feedback (Figure 3a). Positive feedback can arise by both mutual excitation or mutual inhibition. In the latter case, the bistable switch is usually referred to as "toggle switch" (Del Vecchio & Murray, 2015) (Section 5.3). Both mechanisms are present in the ADH/DAD DPM: CsgA creates a direct mutual excitation loop with RelA and an indirect mutual inhibition loop with SocE. The MOR DPM contains various direct and indirect mutual excitation loops. For simplicity, we illustrate the basic behavior of a bistable switch in the mutual excitation case. The mutual inhibition case follows qualitatively identical dynamical mechanisms. Mutual excitation is maximal in the intermediate activation region where the node activation functions are steepest. For sufficiently large coupling, any equilibrium in those regions is unstable, as sketched in the phase plane in the right plot. New stable equilibria appear in regions where activation functions are flatter and mutual excitation is week, that is, where node states are close to their maximal or minimal allowed values. As a consequence, any sufficiently small input, independently of its exact magnitude, pushes the states toward their minimal values and any sufficiently large inputs pushes the state toward their maximal values (center plot). The system is bistable and responds to continuous inputs with discrete, binary transitions. Bistability is crucial for robust cell regulation (Xiong & Ferrell, 2003; MacArthur et al., 2009). Removing positive feedback by removing either of the excitation branches makes the system respond linearly to incoming inputs (Figure 4a). Such linear response is weak and usually insufficient to robustly broadcast incoming inputs to the rest of the network. The role of the toggle switch motif in lateral-inhibition mediated morphogenesis, the LAT DPM in Newman & Bhat, 2008, is well known. The role of positive feedback motifs described here is related to signaling properties in general: beside morphogenesis, bistability is crucial to engender large, robust, binary-like responses to incoming signals.

Figure 3b reproduces the behavior of a generic realization of the fast positive/slow negative loop of the TIM DPM under the effect of the transient excitation provided by the DPM incoherent feedforward (IFF) loop (sketched in light green). The IFF loop response can be understood as follows. When NUT shuts off, the negative-sign, short path of the IFF loop shuts off, too. This leads to a rapid excitation of MkapA (represented as the node *y* in Figure 3a). The excitation ends as soon as the longer,

positive-sign path of the IFF loop turns on and inhibits MkapA. This transient excitation is the hallmark of the IFF motif, which responds linearly, but transiently to incoming inputs. The fast positive / slow negative loop response reflects its two interlinked components. The fast positive loop amplifies the transient excitation by generating a switch-like all-or-none response, graphically represented by the bistable x-nullcline in the right plot. This large, nonlinear response is only transient due to presence of the slow negative feedback, which slowly brings the system back to rest. The resulting transient nonlinear response (center plot) is usually referred to as “excitability”. It can easily be visualized in the phase plane projection in the right plot. It is a key signaling mechanism in neural systems (Izhikevich, 2007) and likely in MRNs, too (Süel et al., 2006; Tomlin & Axelrod, 2007). Either removing the fast positive loop or making the negative loop shorter (and therefore faster) destroys the excitable behavior (Figure 4b). In this case, the motif responds linearly to the transient excitation, which would again engender too weak a response to robustly broadcast to the rest of the network.

The AMC DPM exhibits the same qualitative topology as the TIM DPM (Figure 3c). The two DPM differ in their inputs. Beside the transient excitation mediated by IFF loop, the TIM DPM is under constant inhibition. Conversely, the AMC DPM does not receive any sustained inhibitory input. In the absence of sustained inhibition, excitability turns into sustained, relaxation-like oscillations by moving the motif equilibrium point on the unstable branch of the bistable x-nullcline (Izhikevich & FitzHugh, 2006). As opposed to the purely inhibitory oscillator described in (Igoshin et al., 2004), we conjecture that the resulting biochemical oscillator is of relaxation type because it arises from interlinking fast positive and slow negative feedback. This class of oscillators is known for exhibiting better robustness and tunability properties than purely negative feedback oscillators (Tsai et al., 2008; Franci et al., 2017). Similarly to the TIM DPM, either removing the fast positive loop or making the negative loop shorter (and therefore faster) destroys the sustained oscillatory behavior (Figure 4c). The DPM function would in this case be completely compromised.

3.3 A dynamical network of DPMs

Coupling nodes are responsible for interconnecting two or more DPMs and, in this way, generating richer network dynamics and eventually richer morphogenetic outputs.

Figure 5a reproduces the collective dynamics of a part of the DPM network: the TIM, AMC, and ADH/DAD DPM network. Nodes in each DPMs are modeled with the same simplified ordinary differential equation models used in Figures 3 and 4. These DPMs are interconnected accordingly to the network structure in Figure 2: Mrpc2 excites FruA, DevRST, and MazF; DevRST excites PktA2 and FruA; CsgA excites FruA (see the Julia Notebook in the Code section for details). We selected this subnetwork to illustrate the modeling power of the proposed approach while maintaining the model simple.

Although highly simplified, the interconnected network reproduces a series of experimental evidences, most notably, the transient activation of MrpC2 (TIM) (Lee et al., 2012), and the developmental decision-making role of MazF (PCD) (Nariya & Inouye, 2008). Also, it agrees with previous modelling efforts suggesting the transition from oscillatory to steady state behaviour of FruA posttranslational state (AMC) (Igoshin et al., 2004). The transient activation of TIM DPM creates a temporal window in which rich dynamical phenomena happen. It is in this window that the AMC DPM oscillation slowly turns off under the joint effect of DevRST and CsgA. In this temporal window DevRST is still transitioning from inactive to active, which could allow for a transition back to the vegetative state if nutrients are restored in this time span or if other environmental conditions change. Finally, during the transient activation of MrpC2, the PCD DPM (node MazF) can turn on permanently or not. This decision depends on tiny differences in the model parameters. In a multicellular setting, these differences could be induced by spatially coordinated intercellular communication. As opposed to other modeling setting, like boolean network modeling (Arias Del Angel et al., 2018), it is the continuous variation of biologically controlled parameters, and not the MRN initial conditions, that determines the cell fate in a multi-attractor setting. We summarize this information in Figure 5b and push further the biological implication of our analysis by predicting the response of the ECM DPM to PCD DPM decision. The PCD DPM indirectly inhibit the ECM DPM via the Nla6 coupling. In line with the biological role of these DPMs, activation of the PCD DPM would disfavor activation of the ECM DPM. Vice-versa, inactivation of the PCD DPM would favor activation of the ECM DPM. In other words, a cell which had entered development, can either commit to programmed cell death and not becoming a spore, or vice-versa, in a mutually exclusive manner. Finally, our simplified continuous-time dynamical model further

predicts the experimentally observed developmental anticipation effect of PtkD9 knockout (Escalante et al., 2012) (Supplementary Figure S1).

4. Final Remarks

In line with what has been previously proposed for plant and animal systems, we have postulated a set of DPMs associated to the behaviour of *M. xanthus* development which, being an aggregative multicellular microorganism, opens new avenues for wide comparative analyses among multicellular lineages (Table 1, Figure 1). We singled out a subset of DPMs that are common to most plants, animals and this multicellular bacterium, namely, Adhesion, Differential Adhesion, Morphogen, Extracellular Matrix and Programmed Cell Death (referred to as APO in previous work, but renamed here after a more general process, Newman and Baht, 2008, 2009). We also identified DPMs that could be characteristic of aggregative development, Timing and Active Movement Coordination. It is worth noting, however, that in contrast to what has been previously done for plants and animals, in this case DPMs have been inferred from a single model organism. It would therefore be important to test the occurrence of these DPMs, particularly TIM and AMC, in eukaryotic aggregative organisms, such as dyctiostelids, in synthetic multicellular aggregates, or even in animal or plant structures that organize by aggregation at a colonial scale.

While the DPM framework is now well-established and has led to the detailed characterization of specific and semi-autonomous DPMs (e.g. Zhu et al., 2010; Hernández-Hernández, 2018), little has been said about the node dynamics of these modules, how these modules link to each other in developmental processes, and how they transduce environmental signals. Besides characterizing DPMs for the aggregative bacteria under study, one of the objectives of this work was to use Systems Biology tools and concepts to explore the input-output dynamics of the molecular elements associated to each DPM and among DPMs. In turn, this would enable a much needed connection between the molecular scale of Systems Biology and the organismic scale of morphogenetic processes. This approach revealed some of the dynamic motifs and mechanisms that might, at least partially, underlie the robust behaviour, dynamic richness and semi-autonomy of DPMs. For instance, we suggest that the AMC DPM may involve a relaxation-like oscillator arising from interlinking fast

positive and slow negative feedback, which exhibits better robustness and tunability properties than purely negative feedback oscillators. Our analysis leads to the identification of what we called Motif Nodes, underlying DPM nonlinear dynamics, and Coupling Node, underlying DPM temporal coordination.

The identification of Motif Nodes allowed to single out molecules that enable particular dynamical behaviours. Variation in these Motif Nodes may underlie developmental qualitative variation of potential evolutionary relevance, not because some intrinsic property but because of their role as part of complex motifs and DPMs. Similarly, the presence of, absence of or variation in coupling nodes may underlie significant developmental, and thus morphological, changes. Again, the identification of this type of element narrows down the search for conserved or specific molecules in large comparative and Evo-Devo studies. The functional classification of network nodes into patterning, motif and coupling nodes may therefore be particularly suitable to pursue broad comparative studies in which developmental-system drift obscures comparative analyses based on the molecular nature or the phylogeny of the nodes (e.g. Arias Del Angel et al., 2017).

We conclude that the articulation of the DPM framework and Systems Biology can lead to a more nuanced understanding of the processes underlying development and morphogenesis and integrate knowledge and models from different scales, from the molecular networks associated to single DPMs to the organism level. In this case, our approach allowed to underpin some of the dynamic features behind the aggregative development of myxobacterial fruiting bodies, thus contributing to a better understanding of both specific and common aspects of the different paths to multicellularity.

Acknowledgements

N.R.-Y. and J.A.D.A. are doctoral students from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM) and received fellowship 580236 and 619808, respectively, from CONACYT. Authors thank Stuart

Newman valuable feedback. A.F. and M.B. received funding from UNAM-DGAPA-PAPIIT IA105518/RA105518.

Code

The code and equations used to generate Figure 3 and 4 is available at https://github.com/NeurAlessio/Myxo-DPM-SB/blob/master/TIM-MOV-SWI-Isolated.ipynb

The code used to generate Figure 5 is available at https://github.com/NeurAlessio/Myxo-DPM-SB/blob/master/TIM-MOV-SWI.ipynb

The code used to generate Figure S1 is available at https://github.com/NeurAlessio/Myxo-DPM-SB/blob/master/TIM-MOV-SWI-DeltaPktD9.ipynb

REFERENCES

1. Abedin, M., & King, N. (2008). The premetazoan ancestry of cadherins. *Science*, *319*, 946-948. doi: 10.1126/science.1151084.

2. Alon, U. (2007). Network motifs: theory and experimental approaches. Nature Reviews Genetics, 8, 450. doi:10.1038/nrg2102

3. Arias Del Angel, J. A., Escalante, A. E., Martínez-Castilla, L. P., & Benítez, M. (2017). An Evo-Devo Perspective on Multicellular Development of Myxobacteria. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, *328*, 165-178. doi: 10.1002/jez.b.22727

4. Arias Del Angel, J. A., Escalante, A. E., Martínez-Castilla, L. P., & Benítez, M. (2018). Cell-fate determination in Myxococcus xanthus development: Network dynamics and novel predictions. *Development, Growth & Differentiation*, *60*, 121-129. doi:10.1111/dgd.12424

5. Arias Del Angel, J. A., Rivera-Yoshida, N., Escalante, A. E., Martínez-Castilla, L. P., Benítez, M. (2019). Role of aggregate size, multistability and communication in determining cell fate and patterning in *M. xanthus.* bioRxiv 627703; doi: https://doi.org/10.1101/627703

6. Bahar, F., Pratt-Szeliga, P. C., Angus, S., Guo, J., & Welch, R. D. (2014). Describing Myxococcus xanthus aggregation using Ostwald ripening equations for thin liquid films. *Scientific reports*, *4*, 6376. doi: 10.1038/srep06376.

7. Benítez, M., Hernández-Hernández, V., Newman, S. A., & Niklas, K. J. (2018). Dynamical Patterning Modules, Biogeneric Materials, and the Evolution of Multicellular Plants. *Frontiers in plant science*, *9*. doi:10.3389/fpls.2018.00871

8. Bonner J: First Signals: The Evolution of Multicellular Development. Princeton University Press; 2000.

9. Bonner J: Multicellularity: Origins and Evolution. Edited by Niklas K, Newman S. The MIT Press; 2016.

10. Bretl, D. J., & Kirby, J. R. (2016). Molecular mechanisms of signaling in Myxococcus xanthus development. *Journal of molecular biology*, *428*, 3805-3830. doi:10.1016/j.jmb.2016.07.008

11. del Campo, J., Sieracki, M. E., Molestina, R., Keeling, P., Massana, R., & Ruiz-Trillo, I. (2014). The others: our biased perspective of eukaryotic genomes. *Trends in ecology & evolution*, *29*, 252-259. doi:10.1016/j.tree.2014.03.006

12. Daley, W. P., Peters, S. B., & Larsen, M. (2008). Extracellular matrix dynamics in development and regenerative medicine. *Journal of cell science*, *121*, 255-264. doi: 10.1242/jcs.006064

13. Davidson, E., & Levin, M. (2005). Gene regulatory networks. Proceedings of the National Academy of Sciences, 102, 4935-4935. doi:10.1073/pnas.0502024102

14. Elowitz, M.B. and Leibler, S., 2000. A synthetic oscillatory network of transcriptional regulators. *Nature*, *403*, p.335.

15. Escalante, A. E., Inouye, S., & Travisano, M. (2012). A spectrum of pleiotropic consequences in development due to changes in a regulatory pathway. *PloS one*, *7*, e43413. doi:10.1371/journal.pone.0043413

16. Ferrell, J.E. and Machleder, E.M., 1998. The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes. *Science*, *280*, pp.895-898. doi:10.1126/science.280.5365.895

17. Franci, A., Drion, G. and Sepulchre, R., 2017. Robust and tunable bursting requires slow positive feedback. *Journal of neurophysiology*, *119*, pp.1222-1234. doi: 10.1152/jn.00804.2017

18. Gardner, T.S., Cantor, C.R. and Collins, J.J., 2000. Construction of a genetic toggle switch in Escherichia coli. *Nature*, *403*, p.339.

19. Giglio, K. M., Zhu, C., Klunder, C., Kummer, S., & Garza, A. G. (2015). The enhancer binding protein Nla6 regulates developmental genes that are important for *Myxococcus xanthus* sporulation. *Journal of Bacteriology*, JB-02408. doi:10.1128/JB.02408-14

20. Glass, D.S. and Riedel-Kruse, I.H., 2018. A synthetic bacterial cell-cell adhesion toolbox for programming multicellular morphologies and patterns. *Cell*, *174*, pp.649-658. doi:10.1016/j.cell.2018.06.041

21. Green, J. B., & Sharpe, J. (2015). Positional information and reaction-diffusion: two big ideas in developmental biology combine. *Development*, *142*, 1203-1211. doi: 10.1242/dev.114991

22. Grosberg RK, Strathmann RR: The evolution of multicellularity: a minor major transition? Annu Rev Ecol Evol Syst 2007, 38:621- 654. doi:10.1146/annurev.ecolsys.36.102403.114735

23. Hanlon, W. A., Inouye, M., & Inouye, S. (1997). Pkn9, a Ser/Thr protein kinase involved in the development of *Myxococcus xanthus*. *Molecular Microbiology*, 23, 459-471.

24. Hardin, P.E., Hall, J.C. and Rosbash, M., 1990. Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. *Nature*, *343*, p.536. doi:10.1038/343536a0

25. Hernández-Hernández, V., Niklas, K. J., Newman, S. A., & Benítez, M. (2012). Dynamical patterning modules in plant development and evolution. *International Journal of Developmental Biology*, *56*, 661-674. doi: 10.1387/ijdb.120027mb

26. Hernández-Hernández, V., Barrio, R. A., Benítez, M., Nakayama, N., Romero-Arias, J. R., & Villarreal, C. (2018). A physico-genetic module for the polarisation of auxin efflux carriers pin-formed (pin). *Physical biology*, *15*, 036002. doi:10.1088/1478-3975/aaac99

27. *Higgs, P.I., Hartzell, P.L., Holkenbrink, C., Hoiczyk, E. Myxococcus xanthus* Vegetative and Developmental Cell Heterogeneity. In Rajagopalan, R., Sarwar, Z., Garza, A. G., & Kroos, L. (2014). Myxobacteria: genomics, cellular and molecular biology.

28. Igoshin, O. A., Goldbeter, A., Kaiser, D., & Oster, G. (2004). A biochemical oscillator explains several aspects of *Myxococcus xanthus* behavior during development. *Proceedings of the National Academy of Sciences*, *101*, 15760-15765. doi:10.1073/pnas.0407111101

29. Igoshin, O. A., Welch, R., Kaiser, D., & Oster, G. (2004). Waves and aggregation patterns in myxobacteria. *Proceedings of the National Academy of Sciences*, *101*(12), 4256-4261. doi:10.1073 pnas.0400704101

30. Izhikevich, E.M. and FitzHugh, R., 2006. Fitzhugh-nagumo model. *Scholarpedia*, *1*, p.1349. doi:10.4249/scholarpedia.1349

31. Izhikevich, E.M., 2007. *Dynamical systems in neuroscience*. MIT press.

32. Jacobsen, M. D., Weil, M., & Raff, M. C. (1996). Role of Ced-3/ICE-family proteases in staurosporine-induced programmed cell death. *The Journal of cell biology*, *133*, 1041-1051.

33. Jacobson, M. D., Weil, M., & Raff, M. C. (1997). Programmed cell death in animal development. *cell*, *88*, 347-354. doi:10.1016/S0092-8674(00)81873-5

34. Kroos, L. (2007). The Bacillus and Myxococcus developmental networks and their transcriptional regulators. *Annu. Rev. Genet.*, *41*, 13-39. doi:10.1146/annurev.genet.41.110306.130400

35. Kurosaka, S., & Kashina, A. (2008). Cell biology of embryonic migration. *Birth Defects Research Part C: Embryo Today: Reviews*, *84*, 102-122. doi:10.1002/bdrc.20125

36. Kuspa, A., Plamann, L., & Kaiser, D. (1992). A-signalling and the cell density requirement for *Myxococcus xanthus* development. *Journal of Bacteriology*, 174, 7360-7369. doi:10.1128/jb.174.22.7360-7369.1992

37. Lee, B., Holkenbrink, C., Treuner-Lange, A., & Higgs, P. I. (2012). *Myxococcus xanthus* developmental cell fate production: heterogeneous accumulation of developmental regulatory proteins and reexamination of the role of MazF in developmental lysis. *Journal of bacteriology*, *194*, 3058-3068. doi:10.1128/JB.06756-11

38. Lobedanz, S., & Søgaard-Andersen, L. (2003). Identification of the C-signal, a contact-dependent morphogen coordinating multiple developmental responses in *Myxococcus xanthus*. *Genes & development*, *17*, 2151-2161. doi:10.1101/gad.274203

39. Locascio, A., & Nieto, M. A. (2001). Cell movements during vertebrate development: integrated tissue behaviour versus individual cell migration. *Current opinion in genetics & development*, *11*, 464-469. doi:10.1016/S0959-437X(00)00218-5

40. MacArthur, B.D., Ma'ayan, A. and Lemischka, I.R., 2009. Systems biology of stem cell fate and cellular reprogramming. *Nature reviews Molecular cell biology*, *10*, p.672. doi:10.1038/nrm2766

41. Maynard-Smith J, Szathmáry E: The Major Transitions in Evolution. W.H. Freeman Spektrum; 1995.

42. Milligan, C. E., Prevette, D., Yaginuma, H., Homma, S., Cardwellt, C., Fritz, L. C., ... & Schwartz, L. M. (1995). Peptide inhibitors of the ICE protease family arrest programmed cell death of motoneurons in vivo and in vitro. *Neuron*, *15*, 385-393. doi:10.1016/0896-6273(95)90042-X

43. Milo, R., Shen-Orr, S., Itzkovitz, S., Kashtan, N., Chklovskii, D. and Alon, U., 2002. Network motifs: simple building blocks of complex networks. *Science*, *298*, pp.824-827. doi:10.1126/science.298.5594.824

44. Monod, J. and Jacob, F., 1961, January. General conclusions: teleonomic mechanisms in cellular metabolism, growth, and differentiation. In *Cold Spring Harbor symposia on quantitative biology* (Vol. 26, pp. 389-401). Cold Spring Harbor Laboratory Press.

45. Mouw, J. K., Ou, G., & Weaver, V. M. (2014). Extracellular matrix assembly: a multiscale deconstruction. *Nature reviews Molecular cell biology*, *15*, 771. doi:10.1038/nrm3902

46. Müller, F. D., Schink, C. W., Hoiczyk, E., Cserti, E., & Higgs, P. I. (2012). Spore formation in *Myxococcus xanthus* is tied to cytoskeleton functions and polysaccharide spore coat deposition. *Molecular Microbiology*, *83*, 486-505. doi:10.1111/j.1365-2958.2011.07944.x

47. Nanjundiah, V., Ruiz-Trillo, I., & Kirk, D. (2018). 4 Protists and Multiple Routes to the Evolution of Multicellularity. *Cells in Evolutionary Biology: Translating Genotypes into Phenotypes-Past, Present, Future*, 71. doi:10.1201/9781315155968

48. Nariya, H., & Inouye, S. (2005). Identification of a protein Ser/Thr kinase cascade that regulates essential transcriptional activators in *Myxococcus xanthus* development. *Molecular Microbiology*, 58, 367-379. doi:10.1111/j.1365-2958.2005.04826.x

49. Nariya, H., & Inouye, S. (2006). A protein Ser/Thr kinase cascade negatively regulates the DNA-binding activity of MrpC, a smaller form of which may be necessary for the *Myxococcus xanthus development*. *Molecular Microbiology*, 60, 1205-1217. doi:10.1111/j.1365-2958.2006.05178.x

50. Nariya, H., & Inouye, M. (2008). MazF, an mRNA interferase, mediates programmed cell death during multicellular *Myxococcus* development. Cell, 132, 55-66. doi:10.1016/j.cell.2007.11.044

51. Newman, S. A., & Bhat, R. (2008). Dynamical patterning modules: physico-genetic determinants of morphological development and evolution. *Physical Biology*, *5*, 015008. doi:10.1088/1478-3975/5/1/015008

52. Newman, S. A., & Bhat, R. (2009). Dynamical patterning modules: a" pattern language" for development and evolution of multicellular form. *International Journal of Developmental Biology*, *53*, 693-705. doi: 10.1387/ijdb.072481sn

53. Ogawa, M., Fujitani, S., Mao, X., Inouye, S., & Komano, T. (1996). FruA, a putative transcription factor essential for the development of *Myxococcus xanthus*. *Molecular Microbiology*, *22*, 757-767. 10.1046/j.1365-2958.1996.d01-1725.x

54. Plamann, L., Kuspa, A., & Kaiser, D. (1992). Proteins that rescue A-signal-defective mutants of *Myxococcus xanthus*. *Journal of Bacteriology*, 174, 3311-3318. doi:10.1128/jb.174.10.3311-3318.1992

55. Pomerening, J.R., Sontag, E.D. and Ferrell Jr, J.E., 2003. Building a cell cycle oscillator: hysteresis and bistability in the activation of Cdc2. *Nature cell biology*, *5*, p.346. doi:10.1038/ncb954

56. Prill, R. J., Iglesias, P. A., & Levchenko, A. (2005). Dynamic properties of network motifs contribute to biological network organization. PLoS biology, 3, e343. doi:10.1371/journal.pbio.0030343

57. Prochazka, L., Benenson, Y. and Zandstra, P.W., 2017. Synthetic gene circuits and cellular decision-making in human pluripotent stem cells. *Current Opinion in Systems Biology*, *5*, pp.93-103. doi:10.1016/j.coisb.2017.09.003

58. Rivera-Yoshida, N., Del Angel, J. A. A., & Benitez, M. (2018). Microbial multicellular development: mechanical forces in action. *Current opinion in genetics & development*, *51*, 37-45. doi:10.1016/j.gde.2018.05.006

59. Rivera-Yoshida, N., Arzola, A. V., Arias Del Angel, J. A., Franci, A., Travisano, M., Escalante, A. E., & Benitez, M. (2019). Plastic multicellular development of Myxococcus xanthus: genotype–environment interactions in a physical gradient. *Royal Society Open Science*, *6*, 181730. doi:10.1098/rsos.181730

60. Rolbetzki, A., Ammon, M., Jakovljevic, V., Konovalova, A., & Søgaard-Andersen, L. (2008). Regulated secretion of a protease activates intercellular signaling during fruiting body formation in *M. xanthus*. *Developmental Cell*, 15, 627-634. doi:10.1016/j.devcel.2008.08.002

61. Sager, B., & Kaiser, D. (1994). Intercellular C-signaling and the traveling waves of Myxococcus. *Genes & Development*, *8*, 2793-2804. doi:10.1101/gad.8.23.2793

62. Shimkets, L. J., & Rafiee, H. (1990). CsgA, an extracellular protein essential for *Myxococcus xanthus* development. *Journal of Bacteriology*, *172*, 5299-5306. doi:10.1128/jb.172.9.5299-5306.1990

63. Singer, M., & Kaiser, D. (1995). Ectopic production of guanosine penta-and tetraphosphate can initiate early developmental gene expression in *Myxococcus xanthus*. *Genes & Development*, *9*, 1633-1644. doi:10.1101/gad.9.13.1633

64. Skotheim, J.M., Di Talia, S., Siggia, E.D. and Cross, F.R., 2008. Positive feedback of G1 cyclins ensures coherent cell cycle entry. *Nature*, *454*, p.291. doi:10.1038/nature07118

65. Süel, G.M., Garcia-Ojalvo, J., Liberman, L.M. and Elowitz, M.B., 2006. An excitable gene regulatory circuit induces transient cellular differentiation. *Nature*, *440*, p.545. doi:10.1038/nature04588

66. Theocharis, A. D., Skandalis, S. S., Gialeli, C., & Karamanos, N. K. (2016). Extracellular matrix structure. *Advanced drug delivery reviews*, *97*, 4-27. doi:10.1016/j.addr.2015.11.001

67. Thompson, D.W., 1942. *On growth and form.* Cambridge University Press.

68. Thöny-Meyer, L., & Kaiser, D. A. L. E. (1993). *devRS*, an autoregulated and essential genetic locus for fruiting body development in *Myxococcus xanthus*. *Journal of Bacteriology*, *175*, 7450-7462. doi:10.1128/jb.175.22.7450-7462.1993

69. Toda, S., Blauch, L.R., Tang, S.K., Morsut, L. and Lim, W.A., 2018. Programming self-organizing multicellular structures with synthetic cell-cell signaling. *Science*, *361*, pp.156-162. doi:10.1126/science.aat0271

70. Tomlin, C.J. and Axelrod, J.D., 2007. Biology by numbers: mathematical modelling in developmental biology. *Nature reviews genetics*, *8*, p.331. doi:10.1038/nrg2098

71. Tsai, T.Y.C., Choi, Y.S., Ma, W., Pomerening, J.R., Tang, C. and Ferrell, J.E., 2008. Robust, tunable biological oscillations from interlinked positive and negative feedback loops. *Science*, *321*, pp.126-129. doi:10.1126/science.1156951

72. Turing, A.M., 1952. The chemical basis of morphogenesis. *Bulletin of mathematical biology*, *52*, pp.153-197.

73. Udo, H., Munoz-Dorado, J., Inouye, M., & Inouye, S. (1995). *Myxococcus xanthus*, a gram-negative bacterium, contains a transmembrane protein serine/threonine kinase that blocks the secretion of beta-lactamase by phosphorylation. *Genes & Development*, 9, 972-983. doi:10.1101/gad.9.8.972

74. Ueki, T., & Inouye, S. (2003). Identification of an activator protein required for the induction of *fruA*, a gene essential for fruiting body development in *Myxococcus xanthus*. *Proceedings of the National Academy of Sciences*, 100, 8782-8787. doi:10.1073/pnas.1533026100

75. Del Vecchio, D. and Murray, R.M., 2015. *Biomolecular feedback systems*. Princeton, NJ: Princeton University Press.

76. Xiong, W. and Ferrell Jr, J.E., 2003. A positive-feedback-based bistable 'memory module' that governs a cell fate decision. *Nature*, *426*, p.460. doi:10.1038/nature02089

77. Yang Z, Higgs P: Myxobacteria: Genomics, Cellular and Molecular Biology. Caister Academic Press; 2014.

78. Zhang, W., Munoz-Dorado, J., Inouye, M., & Inouye, S. (1992). Identification of a putative eukaryotic-like protein kinase family in the developmental bacterium

Myxococcus xanthus. *Journal of Bacteriology*, 174, 5450-5453. doi:10.1128/jb.174.16.5450-5453.1992

79. Zhu, J., Zhang, Y. T., Alber, M. S., & Newman, S. A. (2010). Bare bones pattern formation: a core regulatory network in varying geometries reproduces major features of vertebrate limb development and evolution. *PLoS One*, *5*, e10892. doi:10.1371/journal.pone.0010892

Table 1. DPM assignment for the molecular components involved in *M. xanthus* multicellular fruiting bodies development. Differentiation (*DIF*), adhesion (*ADH*), differential adhesion (*DAD*), programmed cell death (*PCD*), extracellular matrix (*ECM*), developmental transcription factor (*DTF*), timing (*TIM*), movement (*AMC*) and *DPM*-linking node (*DLN*).

DPM	Molecular component	Role in *M. xanthus* development	EvoDevo role	Reference
DIF	Trypsin	Protein degradation and A-signal biosynthesis	Intercellular communication	Plamann *et al.*, 1992
	A-signal	Cell density sensing and intercellular communication		Kuspa *et al.*, 1992
ADH / *DAD*	CsgA	Short-range intercellular communication	Multicellularity, cell sorting and layering	Shimkets & Rafiee,1990
PCD	MazF	mRNA interferase and programmed cell death	Differential cell loss	Nariya & Inouye, 2008)
ECM	Exo	Polysaccharides export and biosynthesis of spore coat	Adhesion and conglomerate integrity	Müller et al., 2012
TIM	PktA2 (Pkn1)	Indirect post-translational regulation of the transcription factor MrpC	Control of developmental timing	Zhang *et al.*, 1992
	PktA4 (Pkn9)	Indirect post-translational regulation of the transcription factor MrpC		Hanlon *et al.*, 1997
	PktC2 (Pkn8)	Indirect post-translational regulation of the transcription factor MrpC		Nariya & Inouye, 2006
	PknD1 (Pkn4)	Indirect post-translational regulation of the transcription factor MrpC		Nariya & Inouye, 2005
	PktD9 (Pkn2)	Indirect post-translational regulation of the transcription factor MrpC		Udo et al., 1995

	PskA5 (Pkn14)	Phosphorylation-dependent regulation of MrpC		Nariya & Inouye, 2006
	MkapA	Indirect post-translational regulation of the transcription factor MrpC		Nariya & Inouye, 2005
	MkapB	Indirect post-translational regulation of the transcription factor MrpC		Nariya & Inouye, 2005
AMC	FrzF	Regulation of cellular movement patterns	Active movement, aggregation and migration	Igoshin *et al.*, 2004
	FrzCD	Regulation of cellular movement patterns		Igoshin *et al.*, 2004
	FrzE	Regulation of cellular movement patterns		Igoshin *et al.*, 2004
Motif Nodes	AsgE		DPM metadynamics	
	Nla6	Regulation of cell-fate determination		Giglio et al., 2015
	Nla28			
	RelA	Trigger stringent response		Singer & Kaiser, 1995
	SocE			
	MazF	mRNA interferase and programmed cell death		Nariya & Inouye, 2008)
	MrpC	Regulation of cell-fate determination		Ueki & Inouye, 2003
	FruA	Regulation of cell-fate determination		Ogawa *et al.*, 1996
Coupling Nodes	*DevTRS* operon	Transcription of cell-fate specific genes	DPM coupling	Thöny-Meyer *et al.*, 1993
	Nla4			
	Nla18			
	AsgAB			
	ActABCD			

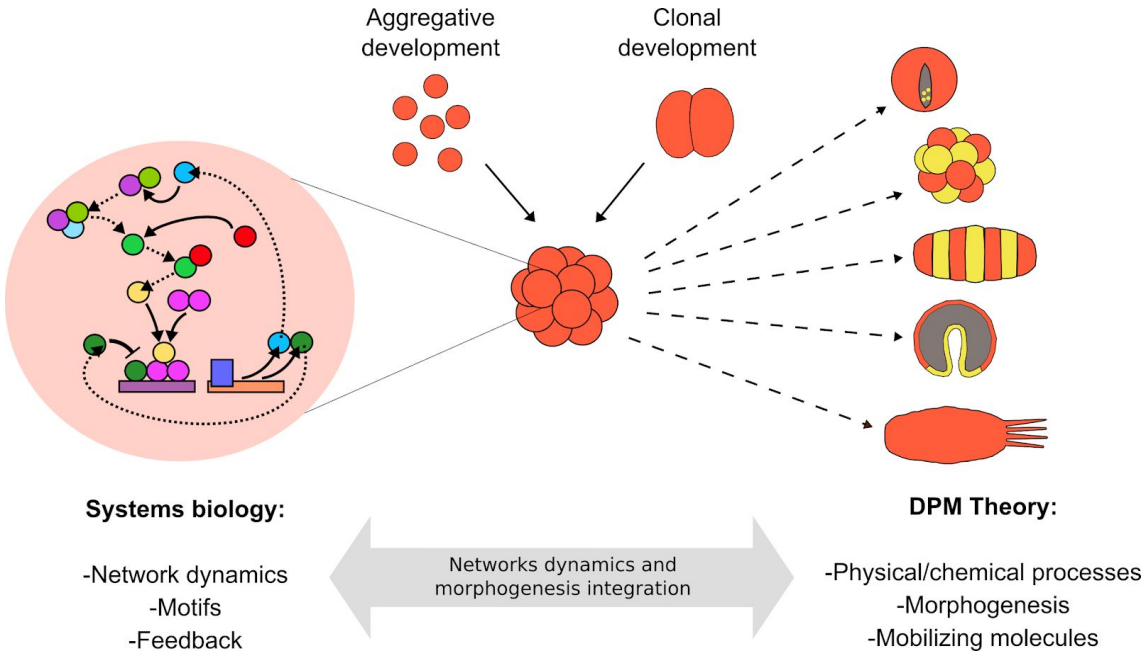

Figure 1. Independently of its origin (clonal or aggregative), the development of multicellularity led many times to the evolution of multicellularity. Two theories shed light on this evolutionary-developmental phenomenon: DPM theory and Systems Biology. The two theories can be integrated to shed a more complete across-scale picture of the transition to multicellularity.

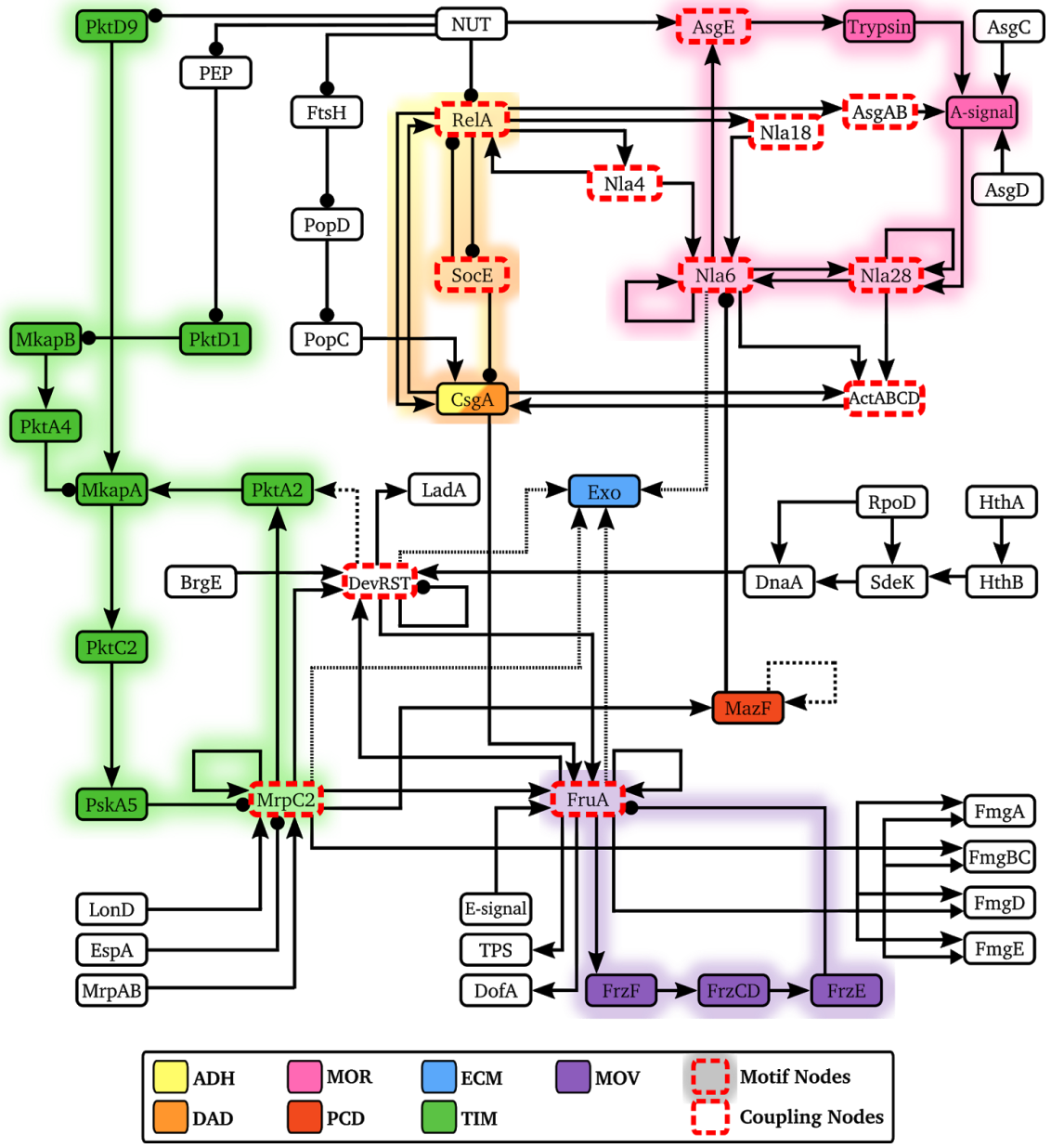

Figure 2. Dissection of *M. xanthus* developmental regulatory networks.

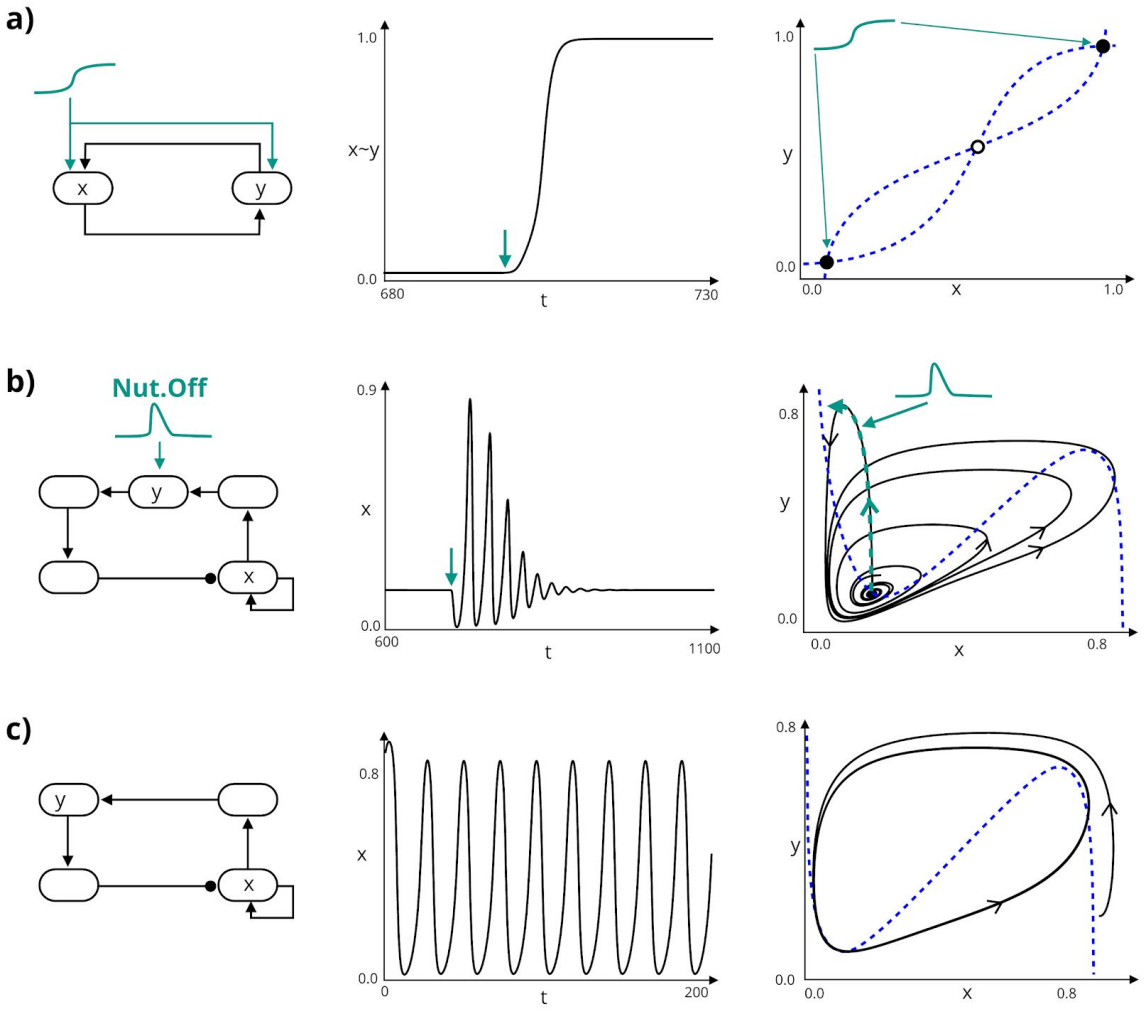

Figure 3. The identified network motif dynamics. Inputs are indicated in light green. Nullclines in the right plots are indicated in dashed blue. Pointed arrows indicate excitation; rounded arrows inhibition.

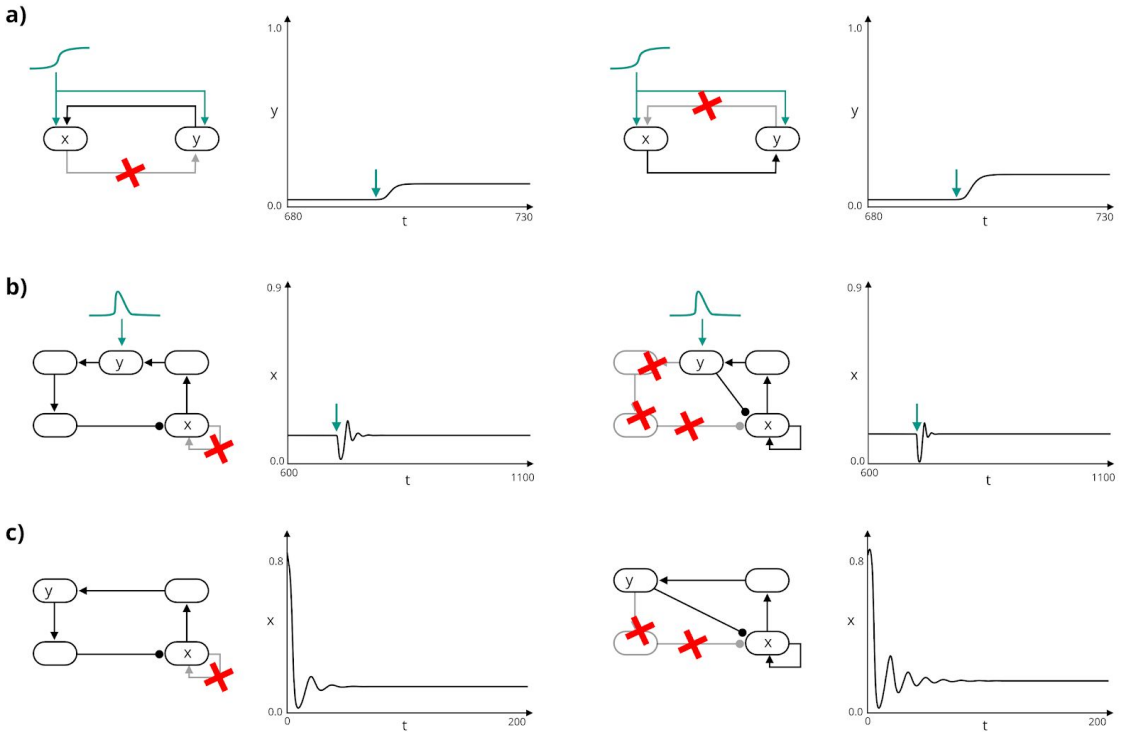

Figure 4. Perturbed dynamics of the identified network motifs.

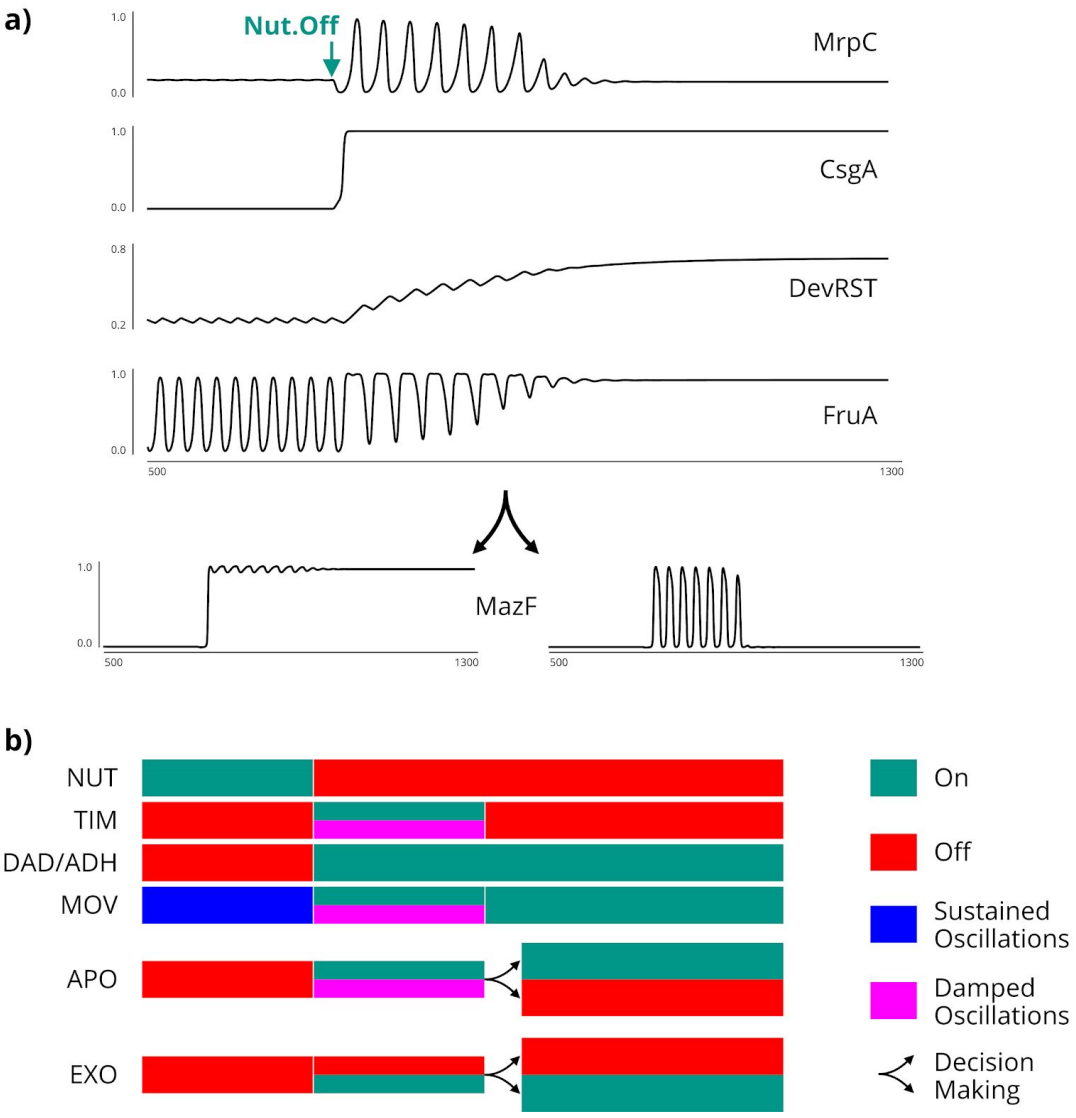

Figure 5. The coupled DPM dynamics predict biologically relevant developmental transitions.

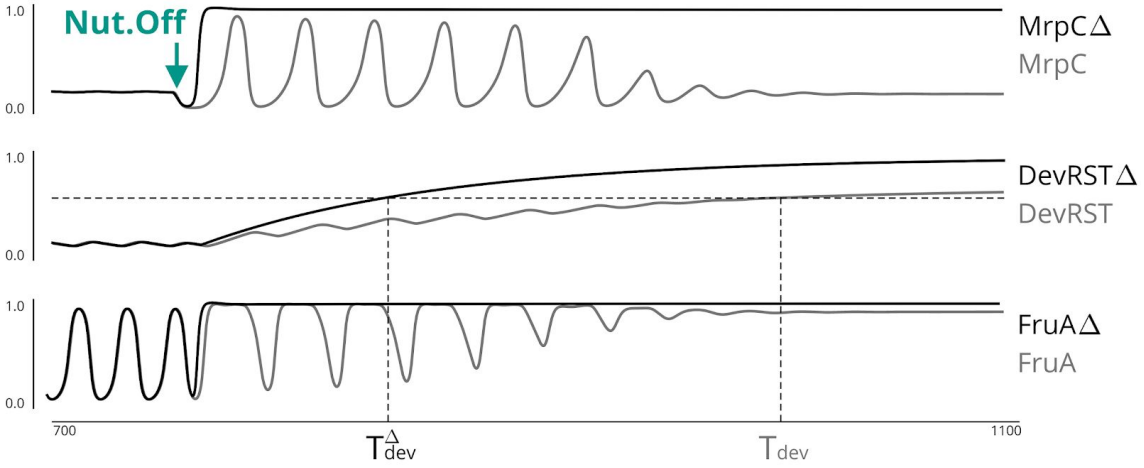

Figure S1. Knocking-out PktD9 in the coupled DPM network predicts experimentally observed developmental anticipation.